



**EXPANDING THE RANGE OF FOOD CHOICE FOR IMMUNOSUPPRESSED
PATIENTS BY IRRADIATION**

Thesis

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INTRODUCTION

In the past decades, there is an increase worldwide in the consumer demand towards minimally processed high quality and yet microbiologically safe foods. Nevertheless, the issue of increasing shelf life is of growing importance today. There is a worldwide struggle against foodborne infections and contaminants, to prevent the food loss due to insect and microbial spoilage. The growing tendency of foodborne illnesses caused by pathogenic bacteria also poses a major problem. Immunocompromised patients are especially vulnerable to food-borne infections, because their natural immune system is operating below the expected level, therefore, the range of foodstuffs that can be safely consumed is significantly of a reduced versatility. Among the emerging new food preservation technologies, food irradiation has been successfully applied in the food industry but this procedure is not widely used. Ionizing radiation has the potential to ensure safety and quality of food, control food deterioration and food-borne pathogenic microorganisms. In contrast to sterilization with autoclaving - which also significantly reduces nutritional value of the food - this treatment obviously provides a more gentle approach and thus the quality values – both nutritional and sensory - approximate the quality parameters of the original state. With ionization treatment the range of foodstuffs can be increased significantly for immunocompromised patients. It is of utmost importance that the meals should also provide gastronomic pleasure in the most difficult days for those patients who are deprived by consuming currently used heat treatment technologies. Nevertheless the deterioration of foodstuffs preserved by irradiation depends on the used radiation doses. Also, irradiation over a certain dose can worsen the chemical parameters and sensory properties in food. Thus, investigation should be carried out product by product to test the effects of different dosages. Furthermore, this procedure can play a significant role in reducing of food losses and reduce the need to use chemical preservatives.

BACKGROUND OF THE RESEARCH, AIMS

In healthcare practice there is no generally accepted microbiological limit for non-sterile but "reduced microbial", "hygienic" foods that can be safely offered for consumption for immunocompromised patients, therefore the limit values of microbiological parameters for the diet of such patients were determined by experts. These recommendations were the following:

Mesophilic aerobic and facultative anaerobic microbial counts <500 CFU/g

Listeria spp. not detected in 25 g

Salmonella spp. not detected in 25 g

Yeast and mold <50 CFU/g

Coliform count <10 CFU/g

Staphylococcus aureus count <10 CFU/g

Aerobic spore count <10 CFU/g

Anaerob spore count <10 CFU/g

Based on a survey performed in Hungarian hospitals and on dietary consultants, the range of foodstuffs served for consumption by patients was determined based of their nutritional value and organoleptic properties but the special requirements presented by immunosuppressed patients were not necessarily taken into account.

Thus, I formulated the following questions and sought answers during my research:

- What is the optimum radiation dose that renders the pre-packaged foodstuffs safe to consume by immunocompromised patients based on the recommended microbiological criteria, and at the same time the nutritional and sensory properties are not altered significantly?
- How does irradiation affect the composition of the original microbial communities of the products and how does it change during storage?
- Is there any differences in the chemical parameters of fruits, vegetables and desserts following irradiation be detected, compared with the results of untreated samples?
- Does the physical properties of the fruit change following treatment (color, texture)?
- Does the radiation treatment change the organoleptic properties of experimental samples?
- *Listeria monocytogenes* is very important in the food industry due to its wide spectrum of resistance to environmental factors: the ability to grow in low pH, low temperature and high salt concentration medium. Considering these properties, what effect does gamma irradiation have on *Listeria monocytogenes* (Scott A, ATO-DLO, Wageningen,

Netherlands) and *Listeria innocua* (OHKI, Budapest) as model microorganisms on the inoculated fresh vegetables, fruits and curd desserts?

MATERIALS AND METHODS

The model foodstuffs selected included pre-cut apple (cultivars Golden, Idared and Granny Smith), orange, banana, tomato, carrot, Túró Rudi (cottage cheese dessert), curd cheese cream; as well as sour cherry, raspberry and chestnut puree and cake.

Experiments were performed using *Listeria monocytogenes* and *Listeria innocua* test microorganisms.

Under laboratory conditions fresh fruits and vegetables were washed, peeled and then cut into pieces. Frozen whole cherry and raspberry were thawed at room temperature then pureed using a blender. From pre-packed sponge cake and chestnut puree sterile cubes (4x4 cm) were cut. From each sample 100 ± 10 grams were measured into a 125 ml commercial sterile polyethylene box for irradiation. Túró Rudi and curd cheese cream were treated in their original packaging. The 24 hour slant agar strains were washed with 5 ml of sterile dilution solution for the activation of the used *Listeria* test microorganism for the challenge test of the fruit, vegetables and cottage cheese. From this suspension 1 ml was transferred into 100 ml sterile BHI (Brain Heart Infusion, MERCK 1.10493) and cultured at 30°C for 24 hours in a shaking water bath. Then, 15 ml of the suspension was transferred into 2 liters of sterile distilled water. Following homogenization, the prepared fruits and vegetables samples were immersed into it and left for 10 minutes. The inoculation of curd cream followed the same procedure up to culturing in BHI infusion. Following that, 350 ml of curd cream was inoculated with 0.1 ml of suspension and homogenized in a sterile dish. One part of the inoculated dairy cream was frozen and the other part was chilled.

The irradiation dosage applied, the storage temperatures and durations are summarized in table below:

Sample	Radiation dose (kGy)	Product/storage temperature (°C)	Storage time (days)	Test days
Apple (Golden)	0; 0.5; 1.0; 1.5; 2.0	5°C/5°C	8	0; 2; 6; 8
Apple (Idared)	0; 0.5; 1.0; 1.5; 2.0	5°C/5°C	8	0; 2; 6; 8
Apple (Granny Smith)	0; 0.5; 1.0; 1.5; 2.0	5°C/5°C	8	0; 2; 6; 8
Orange	0; 0.5; 1.0; 1.5; 2.0	5°C/5°C	7	0; 2; 5; 7
Banana	0; 0.5; 1.0; 1.5; 2.0	5°C/5°C	7	0; 2; 5; 7
Tomato	0; 0.5; 1.0; 1.5; 2.0	5°C/5°C	8	0; 2; 6; 8
Carrot	0; 0.5; 1.0; 1.5; 2.0	5°C/5°C	8	0; 2; 6; 8
Túró Rudi	0; 2.0; 2.5; 3.0	-18°C/5°C	8	0; 2; 6; 8
Curd cream	0; 2.0; 2.5; 3.0	-18°C/5°C	8	0; 2; 6; 8
Sour cherry puree	0; 1.0; 2.0; 3.0	-18°C/ -18°C	7	0; 2; 4; 7
Raspberry puree	0; 1.0; 2.0; 3.0	-18°C/ -18°C	7	0; 2; 4; 7
Chestnut puree	0; 1.0; 2.0; 3.0	-18°C/ -18°C	6	0; 2; 4; 6
Cake	0; 1.0; 2.0; 3.0	-18°C/ -18°C	6	0; 2; 4; 6

Radiation treatment of the samples was carried out with ⁶⁰Co radiation source at the Agroster Zrt. facilities in Budapest.

The mesophilic aerobic and facultative anaerobic microorganism number was determined according to MSZ EN ISO 4833:2003. In the determination of the mesophilic aerobic spore count, KFI Microbiological Methods Manual, Part II, Method 12 - 19.08.2003 and KFI Microbiological Methods Manual, Part II, Method 20 - 19.08.2003, were used for the determination of mesophilic anaerobic spore counts. Chromocult Coliform Agar (MERCK 1.10426) was used for the determination of the coliform number. Thus 2 x 0.5 ml sample suspensions were surface plated on the prepared and solid chromogenic agar. The yeast and mold number were carried out in accordance with the ISO 21527-1:2008 norm. The *Staphylococcus aureus* number was determined according to MSZ EN ISO 6888-1:1999. For the determination of the lactic acid bacterial count the MSZ ISO 15214:2005 method was used. The detection of *Salmonella* spp. was carried out in accordance with the MSZ EN ISO 6579: 2006 norm. Determination the presence of *L. monocytogenes* in ISO 11290-1:2004, while the *L. monocytogenes* number determination was based on MSZ EN ISO 11290-2:2012.

The total antioxidant capacity of the examined fresh and frozen fruits was determined by Benzie and Strain (1996). The total polyphenol content was measured by Singleton and Rossi (1965). The determination of carotenoid, tocopherol, and vitamin C content of fresh vegetables was determined using a liquid chromatography HPLC. The fat content was determined (Túró Rudi and curd cream) with the gravimetric Soxhlet method (MSZ 20900-2:1987) and the fatty acid composition was determined by gas chromatography (Supelco Fame Mix 37-component standard MSZ ISO 5508:1992). During the sensory evaluation, minimum of 10 evaluators (both qualified and unqualified evaluators) expressed their opinion about the samples. During the preference test, the evaluators could give up to 9 points (4 = none, 9 = excellent) to the 4 sensory properties (flavor, color, odour, texture) then the scores were evaluated using Kramer ranking. The traditional hardness measurement were measured by Stable Micro System TA-XT2 table precision penetrometer. The color of the irradiated fruits (L^* , a^* , b^*) was investigated using a ColorLite sph850 handheld spectrophotometer. The pH measurements of the samples were used a pH measuring instrument OP 211/2. For the statistical evaluation of the results, PAST 2.15 version was used.

RESULTS

In the storage experiment where sliced apple was irradiated, only the Idared variety results complied with the microbiological parameters set by the expert team for the special diet of immunocompromised patients. The product can be stored safely at 5°C for up to 6 days if the highest dose of 2.0 kGy has been applied. On the two other varieties (Golden, Granny Smith) it was the number of radiation resistant yeasts that caused the non-conformity.

On orange samples, although the yeasts at this relatively low pH were initially present in small numbers they were able to grow in cool conditions during the 7-day storage period. Furthermore, even the 2.0 kGy dose could not decrease their number below the microbiological limit of 50 CFU/g which is important for food safety. In the case of bananas, the dose of 0.5 kGy was enough so banana can be stored safely at 5°C for 7 days. The dose of 1.5 kGy for tomatoes and 1.0 kGy for carrots was enough to reach the microbiological criteria proposed by the experts. As a summary, the vegetables after appropriate radiation dose treatment can be included in the diet of immunocompromised patients.

The outcome of the dessert studies has shown that the mesophilic aerobic and anaerobic spore counts were the critical parameters in the microbiota of curd desserts (Túró Rudi) and their count could be reduced to a safe level when a dose of 2.5 kGy was applied. With this dose all

microbiological parameters of curd desserts declined to the acceptable range, which could be preserved throughout the cold storage duration of 8 days.

The dose of 3.0 kGy seemed to be successful in reducing the level of mesophilic aerobic and facultative anaerobic, and lactic acid bacteria as well as molds in frozen cherry puree. However, in this case a dose higher than 3.0 kGy had been necessary to reduce the yeast to an acceptable level. As a conclusion, cherry puree harboured extremely radiation resistant yeasts. To the contrary, the use of the 3.0 kGy dose on frozen raspberry puree was enough to provide a low number of microbes. Also for this product the dominant part of microbiota were yeasts, but did not show such resistance to irradiation as in case of cherry puree. The initial mesophilic aerobic and facultative anaerobic microbial counts of frozen chestnuts were high, but with a 3.0 kGy dose, their number could be reduced below the level recommended by the expert committee. The microbial counts of the sponge cake were already low so it can be recommended in this special diet.

Though the fruits included in the studies did not fully satisfy the microbiological requirements set by the expert panel, not including them in the daily diet of immunocompromised patients would not be advisable. The suggestion would be to process the fruits and check the microbiological compliance with set criteria.

In the study yeasts showed greater radiation resistance than the bacteria. From the irradiated (1, 2 and 3 kGy) curd crème and frozen raspberry samples yeasts were isolated and identified by molecular biological methods. *Candida inconspicua*, *Candida lusitanae* and *Trichosporon* sp. - opportunistic pathogenic species have been isolated and identified. *Candida inconspicua* is one of the most often isolated species with clinical relevance, however, this was identified only from the control samples, not from the irradiated samples (Pomázi et al., 2015).

The results of the challenge tests have shown that both *L. monocytogenes* and *L. innocua* survived and in the case of bananas it could even grow in the control vegetables, fruit samples and cottage cheese desserts under refrigerated conditions. The acidic pH of the orange did not affect the survival of the tested microorganisms. The inoculation experiments carried out on carrots also confirmed that fresh carrots have inhibitory effect on *Listeria* proliferation.

Radiation caused in all cases a several magnitude decrease in both *L. monocytogenes* and *L. innocua* numbers, which shows their sensitivity to radiation treatment. The decrease in the *Listeria* counts in different foodstuffs varied considerably from each other because the effect of irradiation on microbes depends greatly on the composition and chemical parameters of foodstuffs, such as pH, water activity, vitamin content etc. – these parameters all affecting

microbial tolerance to irradiation. Refrigerated products require a higher dose of radiation to achieve the desired effect compared to a refrigerated product. Additionally, the D_{10} values of *L. monocytogenes* in sliced, inoculated and irradiated tomatoes are approximately the same as the D_{10} value of *L. innocua* under the same conditions.

Among the examined fruits orange had the highest total antioxidant capacity (275 $\mu\text{M AS/g}$ dry matter), followed by raspberry puree (271 $\mu\text{M AS/g}$ dry matter) and sour cherry puree (255 $\mu\text{M AS/g}$ dry matter) which were also outstanding values.

The different apple cultivars (255 $\mu\text{M GS/g}$ dry matter - 353 $\mu\text{M GS/g}$ dry matter) and orange (188 $\mu\text{M GS/g}$ dry matter) were rich in polyphenolic components mainly.

The ionizing radiation had a significant effect on the total antioxidant capacity (FRAP) of orange and frozen sour cherry puree, as well as in the total polyphenol content of orange and banana. The conclusion is not clear cut whether an increase or a decrease in these parameters is brought about by ionizing radiation treatment, because – also supported by literature data – the results varied widely with regard to fruit and irradiation dose in my case also. In most cases, the changes did not even correlate to the doses applied. Based on the statistical evaluation these differences were significant, but the underlying mechanisms are not well understood and thus any conclusion drawn should be handled carefully.

In the case of sliced tomatoes, α -tocopherol and some carotenoids are found to be the most sensitive to irradiation. The decrease in some cases is about half the original concentration resulting from the 2.0 kGy dose. The levels of α -, γ -tocopherols in carrots decreased considerably compared to the initial concentrations. Furthermore, a significant loss in the ascorbic acid content of both vegetables was detected. It is of prime importance, that despite the potential decrease of these compounds following treatment, patients should still get these foodstuffs with these physiologically important nutrients in natural form, not to mention the sensory value of the products.

There were no detectable oxidation changes in the curd part of Túró Rudi and curd cream due to 2.0, 2.5 or 3.0 kGy irradiation. Any difference was detectable only in the outer covering of Túró Rudi which showed the signs of starting oxidative processes. Curd based desserts which have been treated with low doses (≤ 2.5 kGy), rendered microbiological and organoleptically acceptable, safe regarding oxidative changes can be used to increase the variety of foodstuffs offered to immunosuppressed patients, thus the distribution of these food categories is highly recommended in hospital catering services provided the required storage conditions can be met.

Based on the results of the sensory tests carried out immediately after treatment, it could be stated, that in the case of low dose treatments (≤ 3.0 kGy), the sensory properties of the fruits, vegetables and desserts did not change markedly in most cases. But in some cases, such as 2.0 kGy treatment of carrots and 3.0 kGy treatment of raspberry puree and chestnut cake treated with the maximum dosage, the difference in the sensory parameters was statistically justified. Thus it can be stated that since the properties of the product might change, the choice of appropriate dosage plays a major role in the preservation of organoleptic properties.

According to texture analyses performed by an SMS TA-XT2 precision penetrometer, significant differences from the control sample were observed for the Golden (≥ 1.0 kGy) and Granny Smith (2.0 kGy) apple cultivars. In these cases, the treatment resulted in softening, the extent of which was proportional to radiation doses. There were only small changes after irradiation in the texture of the other fruits compared to untreated samples.

Based on the results of tristimulus color measurements of fruits and fruit purses treated at different doses it can be concluded that neither the clarity nor the coloring factors have changed significantly due to the treatments on the examined different apple varieties. Only the higher (2.0 and 3.0 kGy) doses caused significant changes in case of frozen fruit purees, orange and banana compared to controls. However, after visual inspection, it can be stated that human sensory perception could not differentiate between control and treated samples. During the eight-day refrigerated storage, enzymatic processes progressed both in the control and in the irradiated fruits resulting in changes in the individual color components, so it can be stated that the differences were not caused by the preservative treatment.

NEW SCIENTIFIC RESULTS

1. The examined 3 types of fruits, 2 types of vegetables and 4 kinds of desserts after irradiation treatment with good manufacturing and hygiene practices can be fitted into the meals of immunocompromised patients. The selected products which complied with the set microbiological criteria are as follows:

Sample	Radiation dose (kGy)	Storage temperature (°C)	Best before duration (days)
cuts apple	2.0	5°C	6
sliced banana	0.5	5°C	7
sliced tomato	1.5	5°C	8
sliced carrot	1.0	5°C	8
Túró Rudi	2.5	-18°C	min. 8
curd cream	3.0	-18°C	min. 8
chestnut cake with raspberry puree	3.0	-18°C	min. 6

2. It was confirmed that in order to eliminate the yeasts in case of salads made from apples and oranges more than 2.0 kGy, in frozen sour cherry puree more than 3.0 kGy irradiation dose is required. In addition, *C. inconspicua*, a clinically relevant species for immunosuppressed patients, was isolated from several untreated samples could not be detected in irradiated samples.

3. It was found that a significant reduction in tocopherol content of sliced tomatoes and carrots and in almost all parameters of carotenoid content compared to the control was observed following a 2.0 kGy dose irradiation.

4. The radiation dose of 3.0 kGy in the frozen Túró Rudi caused a significant reduction in the conjugated diene content in the covering part of this dessert, which resulted in a lower, but still acceptable sensory change.

5. The 1.5 kGy dose treatment reduced the cell number of *Listeria monocytogenes* and *Listeria innocua* by at least 5 orders of magnitude and did not change significantly for the remaining duration of the 8 days under chilled conditions. The challenge test also demonstrated that both *L. monocytogenes* and *L. innocua* test strains were more resistant to ionizing radiation treatment under frozen conditions (-18°C) than in the cooled state of the product (5°C).

FINDINGS AND SUGGESTIONS

1. In some fruits, the desired effect of decreasing the microbiological counts was masked by the growth of radiation-resistant yeast therefore the suggestion would be to continue the isolation and further study of such microorganisms that pose a risk to the safety of foodstuffs.
2. The microbiological assessment of each batch before irradiation is advisable to be conducted in order to determine the required dose or apply a combined treatment.
3. A sensory evaluation among patient would be preferable to carry out.
4. As a continuation of the study, based on surveys conducted in national hospitals, the radiation treatment assessment of green paprika, cucumber, lettuce, pears, strawberries, grapes, citrus fruits, diabetic desserts, oilseeds and other ready to eat meals would be most urgent, since these products or product categories were on the list of foodstuffs recommended and requested by dieticians and patients.
5. Furthermore, in my opinion, it is necessary to continue informing consumers, doctors, hospital workers and government officials to increase the knowledge and acceptance of this technology.
6. Following cost calculations, catering companies could also be contacted an informed about special needs catering markets and the opportunities provided by this technology.

LIST OF PUBLICATIONS REGARDING THE THESIS

In journals with impact factor

B. Fekete, G. Kiskó, É. Stefanovits-Bányai and CS. Mohácsi-Farkas: Use of irradiation to improve the microbiological safety of some fresh pre cut fruits. *Acta Alimentaria*,(2012) Vol. 41 (Suppl.), pp. 53–62.

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Hunagrian conference abstracts

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Fekete B., Kiskó G., Mohácsiné Farkas Cs. (2012. május) Gyümölcssaláták mikrobiológiai biztonságának növelése ionizáló sugárkezeléssel, TÁMOP-4.2.1/B-09/1/KMR-2010-0005 konferencia, Budapest.

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Nyirő-Fekete Brigitta, Pivaresi Edina, Mohácsiné Farkas Csilla, (2015. április 22.-23.) Desszertek mikrobiológiai biztonságának növelése ionizáló sugárkezeléssel, Hungalimentaria, Budapest.

International conference abstracts

Csilla Mohácsi-Farkas, **Brigitta Fekete**, Gabriella Kiskó (3-7 Sept. 2012): Use of Irradiation to Provide Wider Selection of Safe and Organoleptically Adequate Foods for Immuno-Compromised patients, FoodMicro conference, Istanbul.

Brigitta Fekete, Gabriella Kiskó, Csilla Mohácsi-Farkas (21-23 May 2012.) Effect of irradiation on *Listeria monocytogenes* inoculated on fresh-cut fruits, IAFP's European Symposium on Food Safety, Warsaw, Poland.

Brigitta Nyirő-Fekete, Gabriella Kiskó, Csaba Felkai, Andrea Lugasi, Csilla Mohácsi-Farkas (7-9 May 2014) Effect of gamma irradiation on the microbiological, chemical and sensory properties of cottage cheese dairy products, IAFP's European Symposium on Food Safety, Budapest, Hungary.

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