Chapter

Case Study of Bacterial Decontamination of an Aromatic and Medicinal Plant: Decontamination of Thymus Satureioides by Gamma Radiation at Low Doses and Impact on Hygienic and Physicochemical Quality

Mahrouz Mostafa, Lahnine Lamyae, Mghazli Safa, Mourad Ouhammou, Mohammed Mouhib and Moulay Ali Misdaq

Abstract

The purpose of our study is to verify the usefulness of gamma irradiation treatment at low doses (0.25, 0.5 and 1 kGy) combined to vacuum packaging on commercial teas of Thymus satureioides deliberately contaminated with *Escherichia coli*. The efficiency and the influence of the process on contamination level and the shelf life of the product were studied. The phenolic composition and concentration were identified in the unirradiated and irradiated thyme. The total phenolic content (TPC) was assayed by the Folin-Ciocalteu method, the individual phenolic compounds were determined by high liquid chromatography (HPLC) and the essential oil was characterized by gas chromatography coupled to mass spectroscopy (GC-MS). The plant was observed by scanning electrons microscopy and the radioactivity effect was analyzed. The results show a complete decontamination of thyme depending to the dose and the storage time. Privileged hygienic quality was found in the irradiated thyme with the highest concentrations of polyphenols. The process showed the conservation of thyme quality without any alteration of its characteristics or radioactivity effect.

Keywords: decontamination, gamma irradiation, polyphenol, radioactivity, thymus satureioides, quality

1. Introduction to the ionization process

The irradiation of food (also called ionization) is a process using radiation (photons, electrons, X-rays) of sufficient energy. It ensures optimal hygiene quality

or prolongs the shelf life and marketing of food, reduces losses during storage (destruction of insects) or substitutes for chemical substances, to present an alternative solution to autoclaving for sterile feeding (cosmonauts, immunocompromised patients, ...) or other applications for example of a technological nature (improvement of extraction yield in fruit juice ...). The term "radapertisation" is used to describe the ionization applied to prepackaged foods whose enzymes have been inactivated, at specified doses so that no alteration or toxicity occurs regardless of the conditions and duration of storage (in the absence post-trial contamination) [1, 2].

The interest of ionization in agribusiness is twofold. It essentially lies in the penetration of radiation into the heart of the foodstuff through the packaging (thus avoiding any recontamination) and without raising the temperature (fresh or frozen products). Unfortunately this process is poorly known and is considered with suspicion by some consumers in whom it evokes in a completely unfounded and irrational way a "radioactive danger" and its possible risks [3–8].

To treat foodstuffs by ionizing radiation consists in subjecting them to the direct action of certain electromagnetic, electronic or photonic radiations, of sufficient energy, so that they can be preserved more or less long while keeping to their best qualities organoleptic, hygienic and nutritional.

Ionization is now the term used, on the one hand to distinguish it from other irradiation treatments and on the other hand, because the term "irradiation" is misleading, in the sense that it inevitably leads to false associations of ideas such as: "irradiated products are radioactive" [9].

For the decontamination of food powders, three ionizing sources are used:

- The γ radiation corresponds to the spontaneous emission of photons by the nucleus of a radioactive isotope (γ-rays of cobalt-60, more rarely those of cesium-137 (for research) (authorized dose between 2 and 10 kGy) [1, 10].

- X-rays are electromagnetic waves emitted by the deep electronic layers of atoms. Their wavelength is between 0.01 and 10 nm (authorized dose: 5 MeV) [1].

- Accelerated electron beams or beta radiation refer to a displacement of electrons created from a source (authorized dose: 10 MeV).

These types of radiation have been chosen because they produce the desired effects on the food, without inducing radioactivity within the product or packaging and allow industrial use in terms of quantity and cost of operation [11].

The irradiation technology has undeniable advantages for the decontamination treatments of heat-sensitive products, rich in active ingredients such as herbs and spices.

In agrifood, the most used irradiation is ionization by gamma radiation. Gamma treatment has been approved by international bodies, namely FAO, IAEA and WHO. The report of the Joint Expert Committee, published in 1980, recommended irradiation as a safe process to achieve hygienization without causing nutritional, microbiological or toxicological concerns [12–14].

Gamma emission is produced by the decay of radioactive nuclides. The isotopes 60 of cobalt and 137 of cesium were chosen because the energy of the radiations they emit is 10 times lower than the minimum threshold allowed. As a result, they are not responsible for any induced radioactivity.

For this type of radiation, the dose is usually expressed in Gray. This dose is equivalent to the absorption per kg of ionized food, with an energy of 1 Joule (1 J/kg). The dose of radiation must be adapted to each case:

- The minimum radiation dose is the amount needed to achieve the desired effect on the food product [10].

- The maximum dose is given by the regulations in force and depends on the food.

- The key parameter of irradiation is the absorbed dose, which determines disinfection (<1 kGy), pasteurization (1–10 kGy) or sterilization (>10 kGy) [15].

The Directive 1999/3/EC [16] has declared a list of foods and food ingredients that can be processed by gamma radiation. The maximum overall average dose that can be absorbed is 10 kGy for dried aromatic herbs, spices and vegetable seasonings. The Food and Drug Administration (FDA) has limited the dose to 30 kGy for culinary herbs, seeds, spices, vegetable seasoning, and mixtures of these aromatic vegetable substances [17].

For aromatic plants, an ionization between 1 and 10 kGy provides a total decontamination of germs. However, some species just require low doses of the order of 1–2 kGy [12, 15].

These types of radiation have been chosen because they produce the desired effects on the food, without inducing radioactivity within the product or packaging and allow industrial use in terms of quantity and cost of operation [18]. Thus, irradiation is used in agri-food for various applications such as: inhibition of germination, disinsectisation, lengthening the shelf life of perishable foods, delaying the maturation and aging of fruits and vegetables, the destruction of parasites and the fight against food poisoning [2, 3, 8, 13, 14, 18].

The effects of ionizing radiation result from a transfer of energy to the material that ionizes or excites molecules and atoms. Among the molecules that undergo these effects, two have a particular importance: the water molecule, given its abundance, and the DNA molecule (deoxyribonucleic acid), because of its major biological function (expression of the genes that govern the functioning of the cell and duplication of the entire genetic heritage during cell division) and its unique presence in the nucleus of each cell. Ionizing radiation can induce various modifications especially in the structure of DNA:

- Oxidation of deoxyribose and release of a base by hydrolysis,

- Bridging between bases of the same strand or between bases belonging to two different strands,

- Hydroxylation of bases and in particular of thymine, with formation of peroxides in the presence of oxygen,

- Single-strand breaks between the base and the sugar of a nucleotide, essentially produced by the action of hydroxyl radicals,

- Alterations of bases and destruction of sugars.

The action of ionizing radiation corresponds to a damage to the membranes and nucleic acids of the microorganisms while preserving the integrity of the constituent atoms molecules of living products [19]. Ionization virtually eliminates pathogenic microorganisms (including sporulated forms) and does not alter the nutritional properties and organoleptic qualities of ionized products [18, 20].

Gram- bacteria are even more sensitive than Gram + and spores ever more resistant than vegetative cells, although some species have proved very resistant in their vegetative form. Viruses are very resistant to radiation [21]. A log reduction of 2 units on mold was observed on coffee beans with a dose of 5 kGy. Thayer et al. [22] determined that a treatment of 2.8 kGy on alfalfa seed with a source of Cesium 137 at a dose of 0.1 kGy/min allowed a reduction of more than 3 log cycles of Salmonella Mbandaka. They evaluate a destruction of 5 logarithmic cycles for a dose of 4.05 kGy. Many factors strongly influence the effectiveness of these treatments: the composition of the medium, the activity of the water, the temperature during the treatment, the presence of oxygen (which increases the sensitivity) and the freezing (the dose necessary for inactivation of 90% of the population is 2–3 times higher in frozen foods than in fresh foods). Doses of 5–7 kGy have been shown to be effective against yeasts, molds and spores [23].

Researchers have shown the effect of gamma radiation on some plants, especially the effect on the phenol composition. This research has approved the increase in phenols in plants after irradiation which leads to the improvement of the phenolic compounds content of these plants and therefore the increase of antioxidant power [2, 3, 9, 14, 20, 24].

Researchers have shown a significant quantitative and non-qualitative increase in the composition of the polyphenols of *Thymus vulgaris* and Menthapulegium compared with a non-irradiated control, following the application of relatively high doses of 10, 20 and 30 kGy [25]. However, other researchers have confirmed a slight increase in total polyphenol content in cumin seeds by applying doses of 1, 3 and 5 kGy. Thus, they showed no significant change in phenolic content by comparing non-irradiated Nigellastaiva seeds with those irradiated at doses of 2–16 kGy [26]. Mahrouz et al. observed a significant increase in the phenolic compounds of Citrus clementina and this is due to the maximal activity and stimulation of phenylalanine ammonia-lyase (PAL) following the application of a low dose of 0.3 kGy [27].

Often ionization alone cannot produce the desired results [18, 27–29]. In particular, the necessary dose is unsupportable by the product, the cost is very high and limits the ionization [30]. Thus, researchers have combined irradiation with other preservation methods:

a. **Ionization and refrigeration**: The protocol is based on the use of medium doses combined with a low temperature preservation of 0–5°C. This process is used for the preservation of fresh fruits and vegetables. Mahrouz et al. Improved the storage conditions of Moroccan clementine by combining low doses and cold at 5°C [6].

b. **Ionization and treatment in heat**: she allows to reduce the high doses of ionization harmful to the organoleptic quality of the product and to reduce the cost. The inactivation of enzymes autolytiques by the ionization of the meat requires a high dose of 200 kGy while associated with heat in 80°C, only a dose? 10 kGy is sufficient [15].

c. **Ionization and conditioning** (packaging): generally, the ionization is combined (organized) in the vacuum packing to avoid the contact between the food and the oxygen. Specially, food rich in fat is very sensitive (perceptible) to the oxygen. However, the study elaborated by Mahrouz et al. emphasized the preservation of the aromatic and medicinal plants " Menthapiperita " by gamma radiations in small doses of 1 kGy combined (organized) in the vacuum packing [31].

d. **Ionization and chemical treatment** (processing): the use of the chemical conservatives (curators) can be reduced by combination (overall) with the ionization. Nitrites used in the delicatessen, to warn (prevent) Clostridium Botulinium and which being able to be carcinogenic if they are transformed nitrosamines there, can be so reduced by the use combined (organized) by the ionization Gamma.

However, the ionization of certain foods may affect the chemical profile of these foods, either an improvement or a degradation [6, 27]. Mahrouz et al. [6] noted that irradiation at low doses of 1 kGy order of Moroccan clementine improved the organoleptic quality: sweeter taste, orange color more intense, phenolic content significantly higher than the control, ... and this during a live shelf of more than 4 weeks.

Radiation degradation products such as formaldehyde, butanone and cyclobutanone are released and can be attributed to chemical reactions responsible for the development of bad flavors and flavors during storage. The addition of essential oils of aromatic plants (rosemary, oregano, thyme, ...) or spices, combined with ionization, can reduce the dose to the extent that the active ingredients of these natural plants have an antimicrobial effect therefore improve the radiosensitivity of pathogenic bacteria and can also help improve the flavor of food. However, ionization decontamination has three major disadvantages:

- Ionization requires the implementation of very restrictive safety rules and therefore very expensive (lead envelope, security lock ...) and purchase costs are high.

- In contact with oxygen in the air, ionizing treatments cause the formation of very reactive free radicals that can cause the oxidation or hydroxylation of certain essential oils and aromatic compounds most often responsible for the organoleptic qualities of spices.

- Despite its proven effectiveness, irradiation faces the reluctance of consumers who see only a complicated and abstract technique involving nuclear energy. Faced with this psychological barrier and the obligation to label, manufacturers of powdered foods, especially spices and herbs, have chosen to opt for other techniques, which today limits the use of irradiation for the decontamination of food products [1, 19, 28].

2. Case study of bacterial decontamination of an aromatic and medicinal plant

2.1 Decontamination of thymus satureioides by gamma radiation at low doses and impact on hygienic and physicochemical quality

In the agro-food industry, the type of conservation treatment to be adopted is conditioned by the intended use of the product. Generally, Thymus satureioides is traditionally used in Morocco as herbal tea to prepare tea, which decreases the risk of contamination given the high temperature applied. However, in the industry, we cannot limit production to this single use, but we should prevent its application as flavoring already prepared foods such as salads or others. This operation could introduce cross-contamination by aromatic plants. As a result, the treatment and decontamination of the product has become an obligation based on its use.

The decontamination processes of aromatic and medicinal plants are essentially based on more or less destructive thermal processes. In addition, plants are a delicate product to handle during storage because of their richness in polyphenols. Several decontamination procedures have been developed and their effectiveness is most often at the expense of the overall quality of the product. As a result, plant conservation is ensured by athermal treatments such as fumigation, UV irradiation and hydrostatic pressures [12, 32, 33]. However, radiation decontamination remains the most used process for its bactericidal efficacy. Gamma irradiation is known as a process of decontaminating plants, materials and food products. This process is widely used in the world for the decontamination of aromatic plants, condiments and dried vegetables with a rate of 46% [34]. It allows the reduction of micro-organisms and ensures the stability of the product [18, 35].

The interest of ionization in agribusiness is twofold. It essentially lies in the penetration of radiation into the heart of the foodstuff through the packaging (thus avoiding any recontamination) and without raising the temperature (fresh or frozen products) [1, 18, 36].

The aim of our study is to evaluate the effect of low-dose gamma radiation storage combined with vacuum packaging on the hygienic and physicochemical quality of Thymus satureioides. Thus, we focused on the application of doses below 1 kGy. The treatment was carried out at the National Institute for Agronomic Research (INRA), Regional Center for Agricultural Research in Tangier, Research Unit on Nuclear Techniques Environment and Quality (URTNEQ). First, we tested the efficacy of a low dose (1, 0.5 and 0.25 kGy) on microbiological quality by studying the abatement of microorganisms after voluntary *E. coli* contamination as major contaminant of plants [12]. Second, we analyzed the impact of the process on the physico-chemical quality specifically of bioactive substances such as essential oils and phenolic compounds. In addition, we evaluated the impact of the dose on the overall quality of thyme: color, phenolic content by UV/visible spectrophotometry and HPLC, composition of essential oils by GC/MS and radioactivity. The quality of irradiated thyme was monitored during storage.

The plant lot was divided into three types of samples according to the analyzes to be carried out:

- Physico-chemical and therapeutic analyzes: preparation of 60 sachets of 25 g for each dose of irradiation
- Microorganism abatement study after deliberate contamination: preparation of 60 sachets of 25 g for each irradiation dose (3 different doses 0.25, 0.5 and 1 kGy) in addition to contaminated but unirradiated samples to identify the load bacterial.
- Uncontaminated and non-irradiated samples are used as a control for the analyzes.

2.2 Ionization protocol

The irradiation was carried out by gamma radiation with a source of cobalt (60Co) in the Boukhalef ionization station (INRA) of Tangier. After dosimetry determination, the flow rate is 1.05 Gy/min. We treated the non-contaminated samples and the contaminated samples in different doses. For each irradiation, we placed a batch of 20 samples in the irradiator. The doses chosen for the treatment are 1, 0.5 and 0.25 kGy. The treatment time was set according to the desired dose (**Table 1**).

Debit (Gy/min)	Dose (kGy)	Duration (min)
1.05	0.25	238 = 3 h 58 min
	0.5	476 = 7 h 56 min
	1	952 = 15 h 52 min

Table 1.
Ionization treatment conditions.

2.3 Observation by scanning electron microscope after irradiation

Samples were prepared for SEM observation either for unirradiated or irradiated thyme at 1 kGy. The samples are placed in the scanning electron microscope chamber (JEOL JSM 5500 LV), observed at 5 kV and photographed at different amplitudes.

2.4 Measurement of radioactivity of thyme

Les échantillons de thym irradiés ont été mis séparément dans une capsule en plastique cylindrique bien fermée en contact direct avec les deux détecteurs solides de traces nucléaires utilisés (**Figure 1**).

Since the irradiation cell is well closed, there is no escape of radon and thoron gases and the exposure time is 30 days. A secular radioactive equilibrium is established between uranium-238, thorium-232 and each of their respective progeny. The global trace densities ($cm^{-2}\ s^{-1}$) due to the alpha particles emitted by the uranium-238 and thorium-232 series recorded on the CR-39 and LR-115 type II detectors are given respectively by the following expressions:

$$\rho_G^{CR} = \frac{\pi q^2}{2\,S_d} C(U)\, d_s \left[A_U \sum_{j=1}^{8} k_j \varepsilon_j^{CR} R_j + \frac{C(Th)}{C(U)} A_{Th} \sum_{j=1}^{7} k'_j \varepsilon_j'^{CR} R'_j \right] \quad (1)$$

and

$$\rho_G^{LR} = \frac{\pi q^2}{2\,S'_d} C(U)\, d_s \left[A_U \sum_{j=1}^{8} k_j \varepsilon_j^{LR} R_j + \frac{C(Th)}{C(U)} A_{Th} \sum_{j=1}^{7} k'_j \varepsilon_j'^{LR} R'_j \right] \quad (2)$$

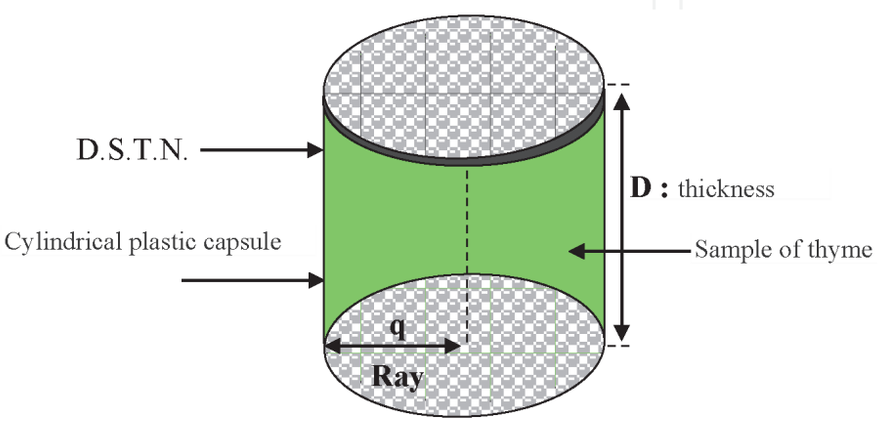

Figure 1.
Experimental device of the irradiation cell.

where:

- A_U ($Bq.g^{-1}$) et A_{Th} ($Bq.g^{-1}$) are the sample-specific activities for 1 ppm uranium-238 and 1 ppm thorium-232 respectively.
- d_s is the sample density of thyme studied ($g\ cm^{-3}$).
- S_d and S'_d are the scanned surfaces of detectors CR-39 and LR-115 type II respectively.
- R_j and R'_j are the alpha particle pathways within the studied thyme sample emitted by the families of l'^{238}U and of ^{232}Th.
- q is the radius of the capsule from which the films are irradiated.
- k_j et k'_j respectively, are the branching ratios corresponding respectively to the decay of the radionuclides of the series of l'^{238}U and of ^{232}Th.
- ε_j^{CR}, $\varepsilon_j'^{CR}$, ε_j^{LR} and $\varepsilon_j'^{LR}$ are the film detection efficiencies CR-39 and LR-115 type II for the alpha particles emitted by the two families of ^{238}U and of ^{232}Th, respectively.
- Pathways of α-particles in thyme and DSTN were evaluated using TRIM software.

By combining Eqs. (1) and (2), we obtain a relationship between the overall trace densities and the ratio of thorium contents to that of uranium, for a sample of thyme. Thus, we have:

$$\frac{C(Th)}{C(U)} = \frac{A_U}{A_{Th}} \frac{\left(\frac{S'_d}{S_d}\right)\sum_{j=1}^{8} k_j\ \varepsilon_j^{CR} R_j - \left(\frac{\rho_G^{CR}}{\rho_G^{LR}}\right)\sum_{j=1}^{8} k_j\ \varepsilon_j^{LR} R'_j}{\left(\frac{\rho_G^{CR}}{\rho_G^{LR}}\right)\sum_{j=1}^{7} k_j\ \varepsilon_j^{CR} R_j - \left(\frac{S'_d}{S_d}\right)\sum_{j=1}^{7} k_j\ \varepsilon_j^{LR} R'_j} \tag{3}$$

et

$$C(U) = \frac{2\ S'_d\ \ \rho_G^{LR}}{d_s \pi q^2 \left[A_U \sum_{j=1}^{8} k_j\ \ \varepsilon_j^{LR} R'_j + \left(\frac{C(Th)}{C(U)}\right)\ A_{Th} \sum_{j=1}^{7} k_j \varepsilon_j^{LR} R'_j\right]} \tag{4}$$

By calculating the detection efficiencies of solid nuclear track detectors CR-39 (ε_j^{CR}, $\varepsilon_j'^{CR}$) and LR-115 type II (ε_j^{LR}, $\varepsilon_j'^{LR}$) using the code "SSNTDEαM", and counting the densities of traces recorded on (ρ_G^{CR}, ρ_G^{LR}), we can determine the ratio C(Th)/C(U) and then the uranium and thorium contents contained in the samples of thyme studied [37].

3. Results and discussion

3.1 Microbiological or hygienic quality

Analysis of food safety indicator germs "total aerobic mesophilic flora FMAT, yeasts and molds, *E. coli*, ASR anaerobic sulfiturizer and salmonella" revealed that our lot is not contaminated and can be used to our study [38–43].

In our study, we applied low doses on a bacterial load of the order of 1.1106 CFU/g. The monitoring of the microbiological quality of the irradiated thyme (**Figure 2**) demonstrates a decontamination of the order of 98, 96 and 90% after irradiation with the doses of 1, 0.5 and 0.25 kGy respectively and these percentages tend to 100% during storage. Following this treatment, we obtained a complete elimination of *E. coli* bacteria in our samples. Such results show the effectiveness of ionization even at low doses on the debacterization of thyme contaminated with *E. coli*.

In addition, the irradiation dose of 1 kGy was more lethal with a reduction rate of 98% just after irradiation and 100% after 7 days of storage. This dose appears to be the lowest effective dose for E decontamination. Coli. These results show the effectiveness of ionization, even at low doses, on *E. coli*-contaminated thyme.

Similar effects have been observed [28, 44]. The study on freshly cut celery showed a decrease in the number of bacteria and fungi of the order of 102 and 101, respectively, with 1 kGy of irradiation and the number of *E. coli* decreased to less than 30 CFU/g [13]. Surviving colony analysis (**Figure 3**) shows the effect of irradiation doses on thyme compared with unirradiated inoculated thyme and control thyme. It is remarkable that the contamination decreases during storage even for non-irradiated inoculated thyme. This result can be explained by a synergy of effects between the irradiation treatment and the plant itself. Right after treatment, there is a direct influence of the process on the bacteria. During storage, on the one hand there is the effect of the plant's compounds including the essential oil and its compounds could be in contact with bacteria showing a bactericidal effect. On the other hand, the influence of irradiation on the metabolism of the plant by the activation of its compounds during storage.

Each point is the average of three repetitions. The analysis of the differences is made by the bidirectional analysis of the variances (ANOVA and Tukey), the main effects are the time and the dose. The same letters indicate a lack of significance at $P > 0.05$.

In the literature, the effect of irradiation has been attributed to ionizing radiation acting directly or indirectly on DNA and inducing local modifications of the double helix. The interaction with the DNA leads to ionizations and excitations that produce direct modifications of the molecule. In addition, the interaction of ionizing radiation with water molecules causes the radiolysis of water which results in the formation of free radicals. These products, very unstable, in turn, form other radical species or molecules (H_2O_2) that have a high reactivity with any nearby biological molecule

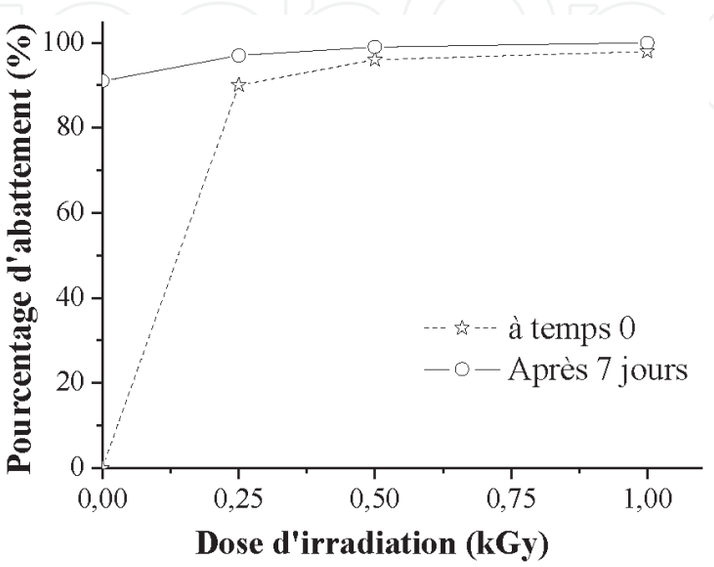

Figure 2.
Abatement of E. coli *as a function of ionization dose and storage duration.*

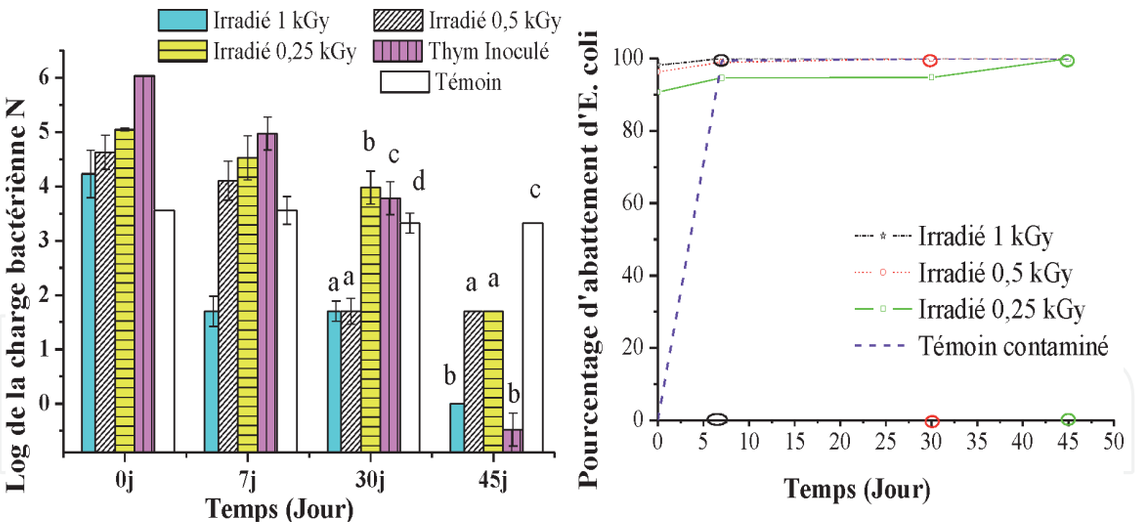

Figure 3.
Monitoring of the bacterial load according to irradiation doses and storage.

(water, protein, DNA). Their interactions with DNA produce chemical modifications of the polymer, such as oxidation [18, 45]. In addition, the increase in the treatment dose causes the decontamination effect. Similar results are presented in the case of plant decontamination (Menthaepip., Cynaraescol., Valerianae rad., Lepidiumsativum, Brassicanigra L. Koch and lemon leaves) [28, 46]. In addition, the microbicidal activity, induced by polyphenols and stressful conditions due to vacuum storage, is observed in unirradiated inoculated control thyme. Burt has demonstrated the effect of the essential oil on the cell membrane of bacteria. Due to its hydrophobic character, it penetrates cell membranes and mitochondria making them permeable and leads to leakage of cell contents [47].

3.2 Organoleptic quality: color analysis

Plant color analysis by UV/Visible spectrophotometry demonstrated maximum absorbance of thyme pigments in the wavelength of 340 nm and a slight absorbance at 630 nm and 664 nm from the absorbance of chlorophyll (**Figure 4**). The control

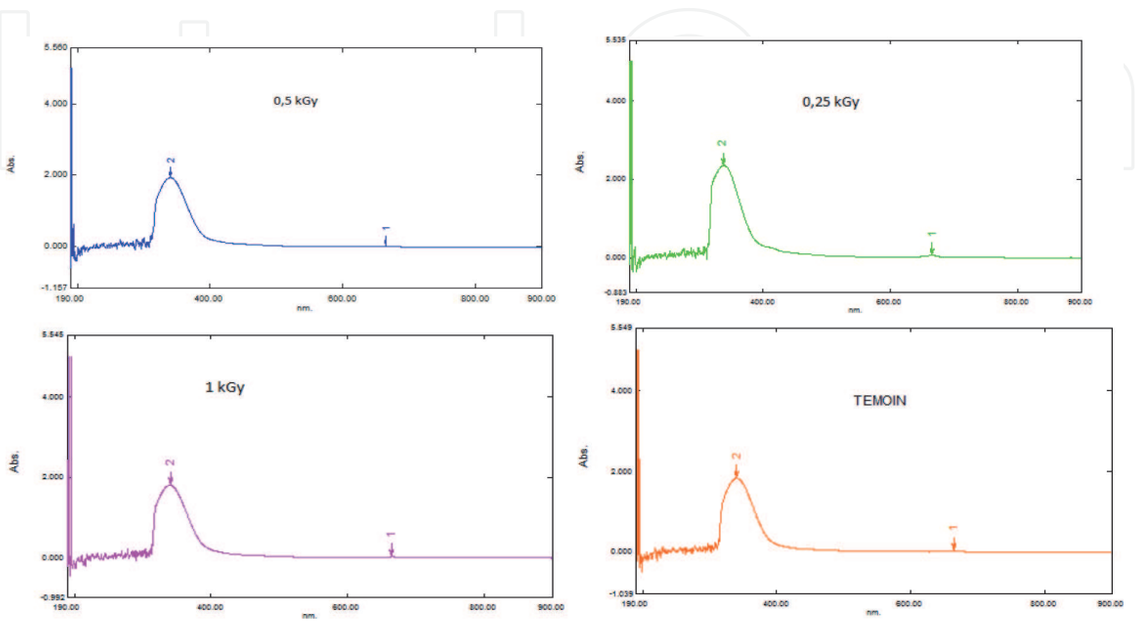

Figure 4.
Absorption spectrum of control and irradiated thyme pigments.

and irradiation thyroid absorption spectra have the same pace. Thus, we find that athermal treatment with low dose gamma radiation has no impact on the degradation of total pigments.

Similar results were reported and correlated the effect observed on the pigments at the irradiation dose. Machhour et al. [31] illustrated the absence of difference between the pigment spectra of peppermint before and after irradiation at 1 kGy. Similarly, Pinela et al. [48] have shown that gamma irradiation treatment at a dose of 1–10 kGy has no influence on the T. lignosa color parameter. Next door, Koseki et al. [35] showed that there is no evidence that radiation caused significant degradation of total β-carotene in basil, artichoke and rosemary samples irradiated at 0, 10, 20 and 30 kGy However, the irradiated Vignaradiata (L.) study of 10–100 Gy showed that gamma irradiation significantly affects photosynthetic pigments. Thus, the chlorophyll a and b content decreases with increasing gamma exposure [49].

3.3 Morphological observation of the texture by the MEB

The surface of thyme leaves before and after irradiation is shown in **Figure 5**. The glands observed on the surface of non-irradiated thyme are modified after treatment. Irradiation can cause structural abnormalities in the cell wall.

Some studies have linked this effect to sample preparation. Hammond and Mahlberg [50] associated the modification and structural alteration of *Cannabis sativa* on the one hand with fixation and dehydration. On the other hand, they noted the effect of freeze-sublimation treatment for the difference between fresh and prepared samples. Similarly, Nayak et al. [51] demonstrated a correlation between irradiation exposure of 3–12 kGy and increased cell wall permeability and changes in histological properties.

4. Quality of antioxidants: characterization of phenolic compounds

The analysis of the essential oil and phenolic compounds is used as an indicator of the quality of aromatic plants. Therefore, the composition of thyme before and after irradiation has been studied. The determination of total polyphenols (PPT) is shown in **Figure 6**. At the beginning of the treatment, the total phenol compounds content in the irradiated plant remains similar to that of the control. However, PPTs increase during storage as a function of radiation dose. This increase may be related to the effect of irradiation, which affects the chemical bonds and therefore induces the discharge of soluble phenols of low molecular weight.

Values are expressed as mean ± standard deviation (n = 3). Vertical bars represent standard deviations. Values with the same letters are not significantly different ($P > 0.05$).

The effect of irradiation has been reported for gamma irradiated plants at different doses. Khattak [5] showed a significant increase in the composition of the total polyphenols of Fagonia arabica compared to the non-irradiated control after doses between 1 and 10 kGy. Similar results were confirmed by Cheng and Breen and Mahrouz et al. [6, 52]. Similarly, Mahrouz et al. [28] correlated this change to the activation of phenylalanineammoniac lyase (PAL) biosynthesis as the first enzyme involved in the synthesis of phenolic compounds. However, Pinela et al. [29] showed a decrease in the concentration of phenolic compounds in irradiated versus non-irradiated samples. Also, Banerjee et al. [3] demonstrated the inhibition of PAL activity in irradiated shriveled cabbage. In the case of this product, the PAL enzyme influences the inhibition of browning. This finding was confirmed by a decrease in PAL gene expression just after irradiation.

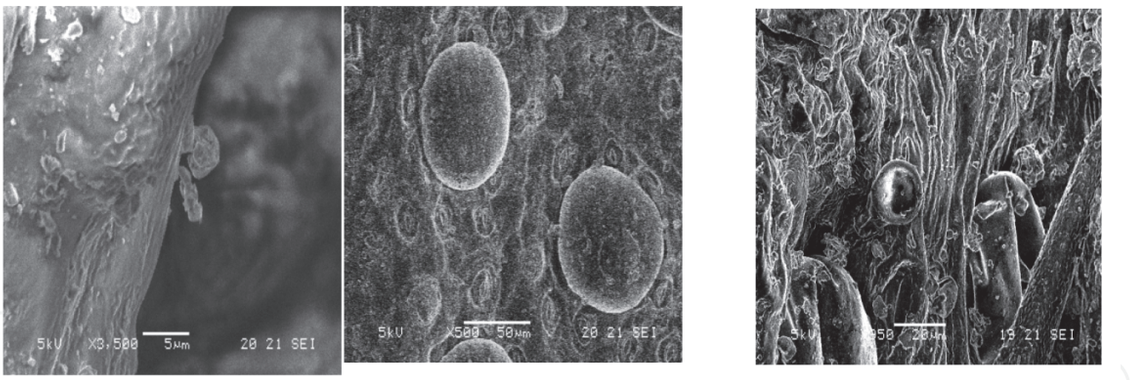

Figure 5.
Observation by scanning electron microscope of control and irradiated thyme.

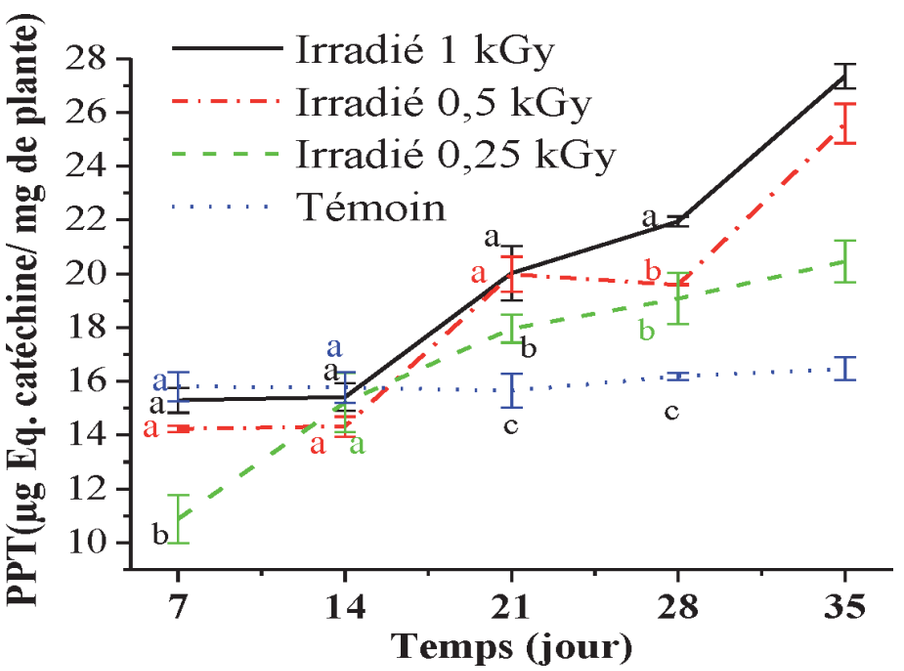

Figure 6.
The total polyphenols of thyme control and irradiated during storage.

The phenolic material extracted was analyzed by HPLC. The chromatographs of the control and irradiated thyme have been illustrated at various wavelengths. The samples showed similar profiles where at least seven peaks are observed. Rosmarinic acid, luteolin 7-O-glucoside and luteolin 3-O-glucoside predominate in all samples. In addition, caffeic acid and catechin were identified before and after irradiation. However, the other peaks are not identified. Nevertheless, previous research has reported the presence of other phenolic compounds. Ramchoun et al. [53] identified caffeic acid and catechin in Thymus satureioides and flavonoids such as rosmarinic acid, luteolin 7-O-glucoside, apigenin-7-glycoside and hesperetin. Similarly, Ismaili et al. [54] illustrated the high level of total phenols in the polar fraction of the methanolic extract of T. satureioides and correlated with the main isolated compounds: rosmarinic acid, luteolin aglycones, eriodictyol, thymonine and three flavonoids which are luteolin-3'-O-glucuronide, luteolin-7-O-glucoside and eriodictyol-7-O-glucoside. In the Van Den Broucke [55] study, three flavones were reported: cirsilineol, 8-methoxycirsilineol and thymonine. In addition, the composition obtained after irradiation is approximately similar for the different

doses compared to the control. This demonstrates that storage by gamma irradiation has no significant change in the qualitative phenolic composition.

4.1 Physico-chemical quality: analysis of the essential oil of thyme by CPG-MS

The essential oil of Thymus satureioides is characterized by its variable composition (**Table 2**). The plant studied is characterized by its richness in carvacrol (45.86%), borneol (9.89%), thymol (2.14%) and β-caryophyllene (6.88%) and a low concentration of camphene (0.28%), terpineol α- (1.87%), α-pinene (0.12%) and linalool (0.43%). This composition is quite different from the composition of the essential oils previously reported. The change in thyme composition is a function of harvest period, extraction technique and environmental conditions, including area, season and fertilization [56–58]. Some studies have shown a concentration of borneol as a stronger characteristic compound than carvacrol. As a result, the amounts of the compounds became different: borneol (23–32%), thymol (4–16%),

N°	TR	IR	Compound	Percentage (%)			
				Witness	1 kGy	0.5 kGy	0.25 kGy
1	5.58	1060	α-Thujene	0.04	0.03	0.04	0.04
2	5.75	1072	α-Pinène	0.12	0.12	0.18	0.28
3	6	1089	Camphène	0.28	0.26	0.4	0.62
4	7	1055	α-terpinènes	0.02	0.02	0.02	0.03
5	7.13	1064	*p*-cymène	0.21	0.16	0.19	0.44
6	7.53	1090	Limonène	0.04	0.04	0.03	0.05
7	7.65	1098	γ-Terpinènes	0.12	0.09	0.09	0.17
8	7.8	1008	(E)-Sabinene hydrate	0.08	0.06	0.06	0.07
9	8.23	1038	Linalol	0.43	0.51	0.53	0.47
10	9.03	1092	Camphor	1.74	0.4	0.53	0.24
11	9.38	1017	Bornéol	9.89	15.02	14.01	8.92
12	9.68	1039	α-Terpinéol	1.87	2.52	0.25	2.32
13	10.1	1067	Bornyl acétate	0.01	0.01	2.58	0.05
14	10.5	1097	NI	10.3	1.66	0.21	0.25
15	11	1038	Thymol	2.14	2.48	2.33	2.3
16	11.2	1053	Carvacrol	45.86	40.81	42.17	36.05
17	11.4	1067	NI	4.53	6.45	7.09	6.38
18	12.4	1042	Copaène	0.23	0.36	0.29	0.48
19	13.1	1000	β-caryophyllène	6.88	8.59	8.09	6.85
20	13.4	1021	NI	0.09	0.21	0.22	0.26
21	14.2	1087	NI	1.2	1.43	1.7	1.39
22	15	1062	Caryophyllène oxyde	0.64	0.93	0.95	1.03
23	15.6	1017	NI	1.47	2.84	2.36	3.76
			Total	88.19	85	84.32	72.45

NI: non identified; TR: retention time (min); IR: Kovats retention index.

Table 2.
Composition of essential oils extracted from control and irradiated thyme.

Samples	ρ_G^{LR} (10^{-5} cm^{-2} s^{-1})	ρ_G^{CR} (10^{-5} cm^{-2} s^{-1})	C (^{238}U) (10^{-6} g/g)	C (^{232}Th) (10^{-6} g/g)
Non irradiated	1.3 ± 0.05	5.62 ± 0.22	0.36 ± 0.01	0.63 ± 0.03
Irradiated at 1 kGy	1.35 ± 0.07	5.84 ± 0.29	0.41 ± 0.02	0.72 ± 0.04
Irradiated at 0.5 kGy	1.32 ± 0.08	5.73 ± 0.34	0.39 ± 0.02	0.68 ± 0.04

Table 3.
Concentration of uranium C (238U) and thorium (232Th) of control and irradiated thyme.

camphene (7–27.4%), α-terpineol (6–11%), α-pinenes (17.5%) and linalool (6.3%). In addition, the concentration of certain compounds is modified after treatment with gamma irradiation. This effect is observed depending on the dose applied specifically 1 and 0.5 kGy. The effect of irradiation is investigated by the increase of borneol (15.02%) and β-caryophyllene (8.59%). A slight effect was observed on thymol (2.48%) and caryophyllene oxide (0.93%). However, the amount of some compounds decreased, especially carvacrol (40.81%), p-cymene (0.16%) and camphor (0.4%). The results showed that the content of borneol increased significantly while carvacrol decreased. Similarly, the influence of irradiation has been observed on other unidentified compounds.

By analogy, the investigation of the biosynthesis of phenolic compounds in fresh coriander seedlings (culantro) showed a significant increase of flavonoids, anthocyanins and flavonone in the plant irradiated at a dose of 40 Gy compared to the control. However, flavonols decreased by increasing the dose of irradiation [29]. Similarly, an analysis by Farag et al. [59] demonstrated the change of monoterpene hydrocarbons to terpene alcohol in the essential oil of irradiated black pepper.

4.2 Radioactive quality: analysis of thyme radioactivity

The results of the radioactivity are shown in **Table 3**. These results show that there was no difference in concentration of uranium (238U) and thorium (232Th) in unirradiated and irradiated thyme. As a result, there was no radioactivity following gamma radiation, except natural radioactivity.

Acknowledgements

This project is supported by the MESRSFC and the CNRST of the Kingdom of Morocco in THE PRIORITY AREAS OF RESEARCH SCIENTIFIC AND TECHNOLOGICAL VALORIZATION THROUGH THE INNOVATION OF MOROCCAN TERROIR PRODUCTS: AROMATIC AND MEDECINAL PLANTS IN IAA AND ICPC (R2BINNOVA) code: PPR-B-R2BINOV-Mahrouz-FS-UCA-Marrakesh.

DOI: http://dx.doi.org/10.5772/intechopen.93521

Author details

Mahrouz Mostafa[1*], Lahnine Lamyae[1,2], Mghazli Safa[1], Mourad Ouhammou[1], Mohammed Mouhib[3] and Moulay Ali Misdaq[4]

1 LICVEDDE (Research Laboratory of Innovation and Sustainable Development and Expertise in Green Chemistry), Department of Chemistry, FSSM, Cadi Ayyad University, Marrakesh, Morocco

2 Laboratory of Spectroscopy Applied and Environment LSCAE, Superior School of Technology, University My Slimane, Beni Mellal, Morocco

3 Unité de Recherche sur les Techniques Nucléaires l'Environnement et la Qualité (URTNEQ), Centre Régional de la Recherche Agronomique de Tanger, Tanger, Morocco

4 Laboratoire de Physique Nucléaire, Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech, Morocco

*Address all correspondence to: mahrouz@uca.ac.ma

References

[1] Farkas J, Mohacsi-Farkas C. History and future of food irradiation. Trends in Food Science and Technology. 2011;**22**: 121-126. DOI: 10.1016/j.tifs.2010.04.002

[2] Farkas J. Radiation decontamination of dry food ingredients and processing aids. Journal of Food Engineering. 1984; **3**:245-264

[3] Banerjee A, Penna S, Variyar PS, Sharma A. Gamma irradiation inhibits wound induced browning in shredded cabbage. Food Chemistry. 2015;**173**: 38-44. DOI: 10.1016/j.foodchem.2014.09.166

[4] Thongphasuk P, Thongphasuk J. Effects of g-irradiation on free radicals, active components and toxicity of turmeric rhizomes. Rangsit Journal of Arts and Sciences. 2013;**3**:169-177

[5] Khattak KF. Evaluation of microbial loads, physical characteristics, chemical constituents and biological properties of radiation processed *Fagonia arabica*. Radiation Physics and Chemistry. 2012; **81**:679-685. DOI: 10.1016/j.radphyschem.2012.02.012

[6] Mahrouz M, Lacroix M, Aprano GD, Oufedjikh H, Boubekri C. Shelf-life and quality evaluation of clementine following a combined treatment with g-irradiation. Radiation Physics and Chemistry. 2004;**71**:141-143. DOI: 10.1016/j.radphyschem.2004.04.048

[7] Antonio AL, Carocho M, Bento A, Quintana B, Botelho ML, Ferreira ICFR. Effects of gamma radiation on the biological, physico-chemical, nutritional and antioxidant parameters of chestnuts - a review. Food and Chemical Toxicology. 2012;**50**:3234-3242. DOI: 10.1016/j.fct.2012.06.024

[8] Tomac A, Horacio R, Isabel M. Modelling the effect of gamma irradiation on the inactivation and growth kinetics of psychrotrophic bacteria in squid rings during refrigerated storage. Shelf-life predictions. Journal of Food Engineering. 2013;**117**:211-216. DOI: 10.1016/j.jfoodeng.2013.02.021

[9] Sterigenics. Food safety. In: Oak Book. 2015

[10] Gominet M. Traitement des denrées alimentaires par rayonnements ionisants. Tech l'Ingénieur, Trait Agroaliment; 2001. pp. 1-4

[11] Hoover D, Metrick C, Papineau A, Farkas D, Knorr D. Biological effects of high pressure on food microorganisms. Food Technology. 1989:99-107

[12] Kumar S, Gautam S, Powar S, Sharma A. Microbial decontamination of medicinally important herbals using gamma radiation and their biochemical characterisation. Food Chemistry. 2010; **119**:328-335. DOI: 10.1016/j.foodchem.2009.06.034

[13] Lu Z, Yu Z, Gao X, Lu F, Zhang L. Preservation effects of gamma irradiation on fresh-cut celery. Journal of Food Engineering. 2005;**67**:347-351. DOI: 10.1016/j.jfoodeng.2004.04.038

[14] Supriya P, Sridhar KR, Ganesh S. Fungal decontamination and enhancement of shelf life of edible split beans of wild legume Canavalia maritima by the electron beam irradiation. Radiation Physics and Chemistry. 2013. DOI: 10.1016/j.radphyschem.2013.08.007

[15] IAEA. Radiation Processing for Safe, Shelf-Stable and Ready-to-Eat Food. Montreal, Canada; 2003

[16] Directive 1999/3/EC. Directive of the European Parliament and of the council of 22 February 1999 on the establishment of a community list of

foods and food ingredients treated with ionising radiation. Official Journal of the European Communities L66/24. 1999

[17] FDA. Code of Federal Regulation, Irradiation in the production, processing and handling of food. 21 CFR 179 Food Drugs; 2015. Available from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/

[18] Farkas J. Irradiation for better foods. Trends in Food Science and Technology. 2006;**17**:148-152. DOI: 10.1016/j.tifs.2005.12.003

[19] Mostafavi HA, Fathollahi H, Motamedi F, Mahyar S. Food irradiation : Applications , public acceptance and global trade. African Journal of Biotechnology. 2010;**9**. DOI: 10.5897/AJB09.1045

[20] Farkas J. Irradiation as a method for decontaminating food. A review. International Journal of Food Microbiology. 1998;**44**:189-204

[21] Farkas J. Physical methods of food preservation. Food Microbiology: Fundamentals and Frontiers. 2007: 685-712

[22] Thayer DW, Boyd G, Fett WF. Gamma-radiation decontamination of alfalfa seeds naturally contaminated with *Salmonella mbandaka*. Journal of Food Science. 2003;**68**:1777-1781

[23] Mittendorfer J, Bierbaumer HP, Gratzl F, Kellauer E. Decontamination of food packaging using electron beam - status and prospects. Radiation Physics and Chemistry. 2002;**63**:833-836

[24] Kouhila M, Belghit A, Daguenet M, Boutaleb BC. Experimental determination of the sorption isotherms of mint (*Mentha viridis*), sage (*Salvia o cinalis*) and verbena (*Lippia citriodora*). Journal of Engineering Science and Technology. 2001;**47**:281-287

[25] Zantar S, Haouzi R, Chabbi M, Laglaoui A, Mouhib M, Boujnah M, et al. Effect of gamma irradiation on chemical composition, antimicrobial and antioxidant activities of *Thymus vulgaris* and *Mentha pulegium* essential oils. Radiation Physics and Chemistry. 2015; **115**:6-11. DOI: 10.1016/j.radphyschem.2015.05.019

[26] Khattak KF, Simpson TJ, Ihasnullah. Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella staiva* seed. Food Chemistry. 2008;**110**:967–972. DOI:10.1016/j.foodchem.2008.03.003

[27] Oufedjikh H, Mahrouz M, Lacroix M, Amiot MJ, Taccini M. The influence of gamma irradiation on flavonoids content during storage of irradiated Clementina. Radiation Physics and Chemistry. 1998;**52**:107-112

[28] Musa HAA, Ahmed EEA, Osman GAM, Ali HA, Ludwig-Müller J. Microbial load and stability of some phytochemical components of selected sudanese medicinal plant materials as affected by gamma irradiation. International Journal of Science and Nature. 2011;**2**:204-209

[29] Aly AAE-H. Biosynthesis of phenolic compounds and water soluble vitamins in Culantro (*Eryngium foetidum* L.) plantlets as affected by low doses of gamma irradiation. Analele Universitatii din Oradea, Fascicula Biologie. 2010:356-361

[30] Idlimam A. Séchage des plantes aromatiques. Cadi ayyad; 2016

[31] Machhour H, El Hadrami I, Imziln B, Mouhib M, Mahrouz M. Microbial decontamination by low dose gamma irradiation and its impact on the physico-chemical quality of peppermint (*Mentha piperita*). Radiation Physics and Chemistry. 2011;**80**:604-607. DOI: 10.1016/j.radphyschem.2010.11.002

[32] Fine F, Gervais P. Efficiency of pulsed UV light for microbial decontamination of

food powders. Journal of Food Protection. 2004;**67**:787-792

[33] Chmielewski AG, Migdał W. Radiation decontamination of herbs and spices. Nukleonika. 2005;**50**:179-184

[34] Kume T, Furuta M, Todoriki S, Uenoyama N, Kobayashi Y. Status of food irradiation in the world. Radiation Physics and Chemistry. 2009;**78**: 222-226. DOI: 10.1016/j.radphyschem.2008.09.009

[35] Koseki PM, Lucia A, Villavicencio CH, Brito MS, Nahme ligia C, Sebastiao katia I, et al. Effects of irradiation in medicinal and eatable herbs. Radiation Measurements. 2002; **63**:681-684

[36] Sádecká J. Irradiation of spices-a review. Czech Journal of Food Sciences. 2007;**25**:231-242

[37] Misdaq M, Merzouki A, Elabboubi D, Sfairi T. A new method for studying the transport of gamma photons in various geological materials by combining the SSNTD technique with Monte Carlo simulations. Radiation Measurements. 2001;**33**:175-181

[38] Bonnefoy C, Guillet F, Leyral G, Verne-Bourdais É. Microbiologie et qualité dans les industries agroalimentaires. Guy: Leyral; 2002

[39] Vollhardt P-C. Traité de chimie organique. 2nd ed1995

[40] Brown E. Traité de chimie organique; 1999

[41] Delers F, Chambaz J, Lacorte J-M. Contrôle intestinal de l'absorption des lipides alimentaires par les polyphénols extraits de la pomme. 1er Colloq. Int. sur les polyphénols la santé pour Vieil. en bonne santé, Romain, 2003, p. 1–39.

[42] Scalbert A. Les polyphénols végétaux: des molécules aux atouts multiples. 1er Colloq. Int. sur les polyphénols la santé pour Vieil. en bonne santé, Romain, 2003, p. 1–39.

[43] CECMA. Ligne directrices et normes pour l'interprétation des résultats analytiques en microbiologie alimentaire. 5th ed. Québec, Canada; 2009

[44] Degryse A-C, Delpla I, Voinier M. Atelier santé environnement: risque et bénéfices possibles des huiles essentielles; 2008

[45] Aquino S. Gamma radiation against toxigenic fungi in food, medicinal and aromatic herbs. In: Méndez-Vilas A, editor. Science against Microbial Pathogens: Communicating Current Research and Technological Advances Formatex; 2011. pp. 272-281

[46] Razem D, Katusin-Razem B. Dose requirements for microbial decontamination of botanical materials by irradiation. Radiation Physics and Chemistry. 2002;**63**:697-701

[47] Béliveau R. Des polyphénols contre les maladies du cœur. In: Le J Montréal Votre Vie. 2010. p. 46

[48] AFSSA. Recommandations relatives aux critères de qualité des huiles essentielles, Contribution pour l'évaluation de la sécurité des produits cosmétiques contenant des huiles essentielles. France; 2008

[49] Sengupta M, Chakraborty A, Sen S. Ionizing radiation induced changes in phenotype, photosynthetic pigments and free polyamine levels in *Vigna radiata* (L.) Wilczek. Applied Radiation and Isotopes. 2013;**75**:44-49. DOI: 10.1016/j.apradiso.2013.01.036

[50] Hammond CT, Mahlberg PG. Morphology of glandular hairs of *Cannabis sativa* from scanning electron microscopy. American Journal of Botany. 1973;**60**:524-528

[51] Nayak CA, Suguna K, Narasimhamurthy K, Rastogi NK. Effect of gamma irradiation on histological and textural properties of carrot, potato and beetroot. Journal of Food Engineering. 2007;**79**:765-770. DOI: 10.1016/j.jfoodeng.2006.02.040

[52] Cheng GW, Breen PJ. Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. Journal of the American Society for Horticultural Science. 1991;**116**:865-869

[53] Ramchoun M, Sellam K, Harnafi H, Alem C, Benlyas M, Khallouki F, et al. Investigation of antioxidant and antihemolytic properties of *Thymus satureioides* collected from Tafilalet region, south-east of Morocco. Asian Pacific Journal of Tropical Biomedicine. 2015;**5**:93-100. DOI: 10.1016/S2221-1691(15)30151-9

[54] Chandak A, Somani SK, Dubey D. Design, development and testing of multieffect distiller/evaporator using Scheffler solar concentrators. Journal of Engineering Science and Technology. 2009;**4**:315-321

[55] Van Den Broucke C. The therapeutic value of thymus species. Fitoterapia. 1983;**4**:171-174

[56] Jaafari A, Ait Mouse H, Rakib EM, Ait M'barek L, Tilaoui M, Benbakhta C, et al. Chemical composition and antitumor activity of different wild varieties of Moroccan thyme. Brazilian Journal of Pharmacognosy. 2007;**17**:477-491

[57] El Bouzidi L, Alaoui Jamali C, Bekkouche K, Hassani L, Wohlmuth H, Leach D, et al. Chemical composition, antioxidant and antimicrobial activities of essential oils obtained from wild and cultivated Moroccan thymus species. Industrial Crops and Products. 2013;**43**:450-456. DOI: 10.1016/j.indcrop.2012.07.063

[58] Alaoui Jamali C, El Bouzidi L, Bekkouche K, Hassani L, Markouk M, Wohlmuth H, et al. Chemical composition and antioxidant and anticandidal activities of essential oils from different wild Moroccan thymus species. Chemistry & Biodiversity. 2012;**9**:1188-1197

[59] Farag ZSEA, Aziz NH, Ali A. Comparing effects of washing, thermal treatments and gamma irradiation on quality of spieces. Nahrung. 1996;**40**:32-36