Decontamination of herbs and spices by gamma irradiation and low-energy electron beam treatments and influence on product characteristics upon storage

Felix Schottroff\textsuperscript{ab}, Thomas Lasarus\textsuperscript{c}, Michal Stupak\textsuperscript{d}, Jana Hajslova\textsuperscript{e}, Thomas Fauster\textsuperscript{ab} and Henry Jäger\textsuperscript{a}

\textsuperscript{a}Institute of Food Technology, Department of Food Science and Technology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria; \textsuperscript{b}Core Facility Food \& Bio Processing, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria; \textsuperscript{c}Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague, Czech Republic; \textsuperscript{d}FFoQSI GmbH, Austrian Competence Centre for Feed and Food Quality, Tulln, Austria

\textbf{ABSTRACT}
Culinary herbs and spices are an important sector of the food industry worldwide, but are often characterized by high levels of microbial contaminations. Therefore, irradiation is often applied to control the microbial burden. In this study, two spice decontamination technologies were compared: the established gamma irradiation as well as a newly developed low energy electron beam (LEEP) treatment. The aim of the study was to evaluate the efficacy of microbial surface inactivation for both treatments and their influence on quality attributes. Allspice berries, caraway seeds, oregano, and rosemary leaves were used as model matrices. Both treatments were shown to effectively reduce Enterococcus faecium counts below the detection limit (>3.3–5.5 log\textsubscript{10} CFU/g). No differences in color, water activity, chlorophyll, and carotenoid contents, as well as 31 terpenic compounds, were determined between LEEP and gamma treatments in comparison to the untreated reference, for storage times of up to 105 days. Untargeted fingerprinting (SPME-GC-HRMS) showed a clustering of LEEP-treated rosemary samples, but no significant differences for the other samples.

\textbf{KEYWORDS}
Herbs; spices; gamma irradiation; low energy electron beam (LEEP); microbial inactivation; quality evaluation

1. Introduction
Culinary herbs and spices are defined as dried, edible plant parts of a distinctly aromatic nature (ESA, 2018; Small, 2006). While the term herb commonly refers to leaves and blossoms, spices usually include all further edible parts of a plant (Small, 2006). For the sake of simplicity, culinary herbs and spices are both referred to as spices in the present manuscript.

Spices are widely used in food preparations and play an important role in diets worldwide (Peter, 2012). With a compound annual growth rate of 3.9\% and an estimated volume of 20.46 Bn US-$, the market for spices represents an important sector of the food industry (Bloomberg, 2019).

In general, spices are gently processed and dried, in order to preserve volatile aromatic constituents to a greatest possible extent. Moreover, herbs and spices are commonly produced in open environments, e.g. by sun drying, resulting in high levels of microbial contamination (Peter, 2006; Wojtowicz et al., 2007). As the rehydration of dried spices, e.g. in a food matrix, can result in possible revitalization and growth of pathogenic microorganisms, the presence of high microbial concentrations in spices may create a potential health risk for consumers (Mathot et al., 2021).

In addition to controlled hygienic production and storage conditions, spices are typically decontaminated, in order to reduce the microbial load (Atungulu & Pan, 2012). For this purpose, steam treatment and irradiation are most widespread processes on a commercial scale (Pinkas & Keller, 2014). While steam treatment is the most common method in the European Union (EU) and the United States of America (USA), it has been estimated that 33\% (Eustice, 2018) of dried spices used in the USA are decontaminated by irradiation. However, this technology is characterized by low consumer acceptance and legal restrictions in a variety of countries (Mahe rani et al., 2016; Wojtowicz et al., 2007).

Moreover, irradiation technologies suffer from several disadvantages: steam treatment is a thermal process, which may be associated with heat-induced degradation of product quality, including color, aroma, nutrients, or the formation of undesired reactions like caramelization (Schweigert et al., 2007). On the other hand, irradiation, is a non-thermal process. However, besides the already mentioned disadvantages considering consumer acceptance and legal issues, irradiation treatments are often carried out by toll manufacturers, thus leading to pronounced additional costs and more
complex logistics for spice manufacturers, therefore limiting flexibility of processing (EFSA, 2011; Kotilainen et al., 2019).

Thus, novel decontamination treatments become of increasing interest for the spice industry (Qiu et al., 2020). Since for low-moisture foods, such as spices and grains, most of the microorganisms reside on the surface, the inner parts of the food do not necessarily have to be exposed to a decontamination treatment (Baba et al., 2004; Hertwig et al., 2018; Martinez et al., 2015; Sabillón et al., 2016). Thus, surface decontamination treatments may be sufficient. Additionally, they may bear the potential for an improved product quality retention, by limiting penetration depth of the antimicrobial agent (Baba et al., 2004).

Therefore, this application study is investigating the effects of a newly developed low-energy electron beam (LEEB) equipment on industrial scale, developed as a surface treatment. For LEEB treatments, low-energy electrons (<300 keV) with a low penetration depth are introduced to food products (Hayashi et al., 1998; Kotilainen et al., 2019). The aim of this study was to compare surface decontamination of spices by the LEEB equipment with the established gamma irradiation, as a treatment known to be able to fully penetrate the products (WHO, 1988), using comparable surface doses for both processes. Due to the different sources of the radiation, i.e. gamma rays from $^{60}$Co compared to electron beams generated by a lamp, the effects of the treatments on microbial inactivation as well as food quality are assumed to be different (Kyun et al., 2019) and were therefore further evaluated. Moreover, due to the relatively novel nature of the LEEB technology, studies on its efficacy against different forms of microbial life (Butot et al., 2021; Deng et al., 2020; Henz et al., 2020) and especially its impact on quality attributes of different foods (Gryczka et al., 2020; Hayashi & Todorki-Suzuki, 2001; Mousavi Khaneghah et al., 2020) are still limited, as further elaborated by Hertwig et al. (2018). For this purpose, four different dried spices common to the European cuisine were used as samples (allspice berries, Pimenta officinalis L.; caraway seeds, Carum carvi L.; oregano leaves, Origanum vulgare L.; and rosemary leaves, Rosmarinus officinalis L.), all of which exhibited different shapes and consistencies. Spices were inoculated with Enterococcus faecium – as a nonpathogenic surrogate for Salmonella. In this regard, the antimicrobial effectiveness as well as influences on product quality were analyzed, for storage points of 21 days to 105 days after the treatment. In order to obtain a broad overview on possible quality changes caused by the two treatments, a variety of different product parameters were assessed, including potential changes in color, water activity, antioxidant capacity, chlorophyll, and carotenoid contents, as well as volatile compounds.

2. Materials and methods

2.1. Sterilization by gamma irradiation and LEEB

Raw, non-pretreated spices (Figure 1) with a low microbial burden were obtained in bulk packages from a commercial wholesaler (Almi GmbH, Oftering, Austria), and afterward divided into smaller units of 3 kg (for quality studies) or 300 g (for microbiological studies). Samples were packaged in polyethylene bags, air was manually excluded, and bags were heat-sealed. For the gamma treatment, samples were sent to Germany for commercial, industrial-scale irradiation within the packaging. Treatment was carried out by a certified provider (BGS Beta-Gamma-Service GmbH & Co. KG, Wiehl, Germany). In this regard, $^{60}$Co was used as a source for the gamma rays and a homogeneous surface dose of 12 kGy was applied. The treatment was carried out at ambient temperature and humidity ($-20^\circ\text{C}, -40\%$), by placing the samples on pallets, with three boxes of spices in one layer per pallet. By this, shielding of the spices by tight stacking and consequent inhomogeneity of the treatment was avoided. Pallets were circulated around the radiation source for approximately 2 h, until the desired dose was reached (BGS, 2020). The applied dose was controlled by radiochromic dosimetry films (B3 film, GEX Corp., Centennial, CO, USA), which were evenly distributed at a distance of 20 cm on the outside of the boxes. Films were analyzed using a spectrophotometer (Spectronic Genesys 5, Milton Roy, Rochester, NY, USA). By this, a maximum dose uniformity ratio of 1.2 was determined for the treatment.

The LEEB treatment was conducted in Switzerland (Bühler AG, Uzwil, Switzerland), using a continuous out-of-pack treatment. The used equipment was a newly developed production-scale machine, composed of two opposing lamps in the center (‘treatment zone’), generating the electron beam. Inside the lamps, electrons are accelerated in vacuum and emitted toward the product. Spices are introduced to the treatment zone by free fall, which enables separation and a homogeneous irradiation of each particle (Kotilainen et al., 2019). Comparability of the free-fall behavior of the different spices was verified by high speed video recordings, showing nearly identical residence times inside the treatment zone in the millisecond-range for all spices (data not shown). Therefore, comparable LEEB doses were achieved. For all trials, the voltage was set to 250 keV, and the current was adjusted to reach a dose of 12 kGy on the surface of the products. Applied doses were validated using radiochromic dosimetry films (B3 film, GEX Corp., Centennial, CO, USA) with a thickness of 18 μm. The procedures were carried out as advised by
FDA (2011), Anderson (n.d.), and ISO 14470:2011. For each treatment, the product was continuously conveyed toward the treatment zone. The products were introduced to radiation from both lamps in a free fall. The LEEB system has been characterized according to ISO/ASTM 51818:2013 by the manufacturer.

In order to apply comparable treatment conditions, a dose of 12 kGy was chosen for both technologies. This surface dose was selected due to technical reasons related to the LEEB equipment. The EU Directive 1999/3/EC of the European Parliament and of the Council limits the use of ionizing radiation to a maximum of 10 kGy. However, this refers to an overall average value, while in contrast the applied surface dose was 12 kGy, graduating to zero. Therefore, the average absorbed dose of 10 kGy would most likely not be exceeded. Moreover, in the USA, the maximum permitted dose is 30 kGy (CFR, 2019). Therefore, the used dose represents an intermediate value in this regard.

Gamma and LEEB treatments were carried out on the same day in duplicate (as process replicates). After each treatment, samples were returned to Austria (University of Natural Resources and Life Sciences, Vienna) for quality evaluation, and to a commercial laboratory in Switzerland (Labor Veritas AG, Zurich, Switzerland) for microbiological testing. Therefore, the first analyses were carried out 21 days after the treatment, including an untreated transport control. All samples were stored for a duration of 105 days at 20°C and analyzed regularly during this period.

### 2.2. Microbiological procedures

The spices designated for the microbiological studies were surface-inoculated with *Enterococcus faecium* NRRL B-2354, as a nonpathogenic surrogate for *Salmonella enterica* (Arias-Rios et al., 2019). The procedure was carried out according to a protocol described elsewhere (ABC, 2014). Briefly, batches of 50 g were thoroughly mixed with *E. faecium* suspension inside sterile bags and pooled afterward.

Subsequent to the treatments, 25 g of each sample were vigorously mixed with 50 mL tryptic soy broth (TSB), and serially diluted in Butterfield’s phosphate buffer. 1 mL of microbial suspension was pour-plated with tryptic soy agar and subsequently incubated at 35°C for 48 h (ABC, 2014). Colony forming units (CFU) were manually counted and expressed as log₁₀ (CFU/g). Initial counts of the *E. faecium* suspension used for inoculation were in the range of 10⁷ CFU/mL. All samples were analyzed in triplicate. Colony forming units were evaluated for the first (day 21) as well as the last (day 105) storage point. Both, inoculation as well as

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**Figure 1.** Pictures of the spices used for the experiments, i.e. allspice berries (top left), caraway seeds (top right), dried oregano (bottom left), and dried rosemary (bottom right).
determination of microbial counts were carried out by an accredited commercial laboratory (Labor Veritas AG, Zurich, Switzerland).

2.3. Assessment of product quality

Quality attributes of the LEEB and gamma treated spices were periodically analyzed and compared to the untreated reference, until a final storage time of 105 d. The target of these analyses was a broad quality evaluation, focusing particularly on phytochemicals, at it is known that some of their beneficial attributes, such as pigmentation, antioxidation, or flavor, may be influenced by irradiation treatments (Alothman et al., 2009).

2.3.1. Color

Color measurements were accomplished using a digital color assessment system (DigiEye, VeriVide Ltd., Enderby, UK), equipped with a standardized lighting chamber and a high-resolution digital camera, together with a corresponding image evaluation software (DigiEye 2.7.2, VeriVide Ltd., Enderby, UK). For the measurements, a petri dish was evenly filled with the respective spice, excluding empty spaces between the particles, as well as foreign bodies. L*a*b*-values (EN ISO 11664–4) were extracted from the acquired images in the software. All measurements were repeated in triplicate. From these values, color differences ΔE_L were calculated, comparing treated (L*a*b_i) and untreated (L*a*b_u) samples (Equation 1.1). Starting at a ΔE of 2.3, a slightly noticeable difference between the samples was assumed (Sharma & Bala, 2017).

\[ \Delta E_L = \sqrt{(L_i - L_u)^2 + (a_i - a_u)^2 + (b_i - b_u)^2} \] (1.1)

2.3.2. Water activity

Water activity (a_w) of the samples was determined at 23°C, using an automatic humidity temperature indicator (Rotronic HW3 Hygrolab, Rotronic AG, Bassersdorf, Switzerland), according to the manufacturer’s specifications. All measurements were repeated in duplicate.

2.3.3. Antioxidant capacity

Antioxidant activity was determined by a modified version of the DPPH (2,2-diphenyl-1-picylhydrazyl) method (Brand-Williams et al., 1995; Mishra et al., 2012; Sharma & Bhat, 2009). For this, extraction of the spices was carried out as described by Pérez et al. (2011). Briefly, ground spices were mixed with 50 mL of methanol (analytical grade; Merck KGaA, Darmstadt, Germany) in an overhead shaker (IKA-Werke GmbH & CO. KG, Staufen, Germany) in the dark for 2 h. Subsequently, samples were centrifuged at 3100 × g (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany) at ambient temperature for 10 min. Sample mass ranges were dependent on the expected antioxidant capacity and adjusted during the storage (Table 1).

Analyzed were performed in a 96 well plate, as described by Prieto (2012). For this, 0.2 mM DPPH (Sigma Aldrich Corp., St. Louis, MO, USA) were added to a serial dilution of the sample. An ascorbic acid standard (0.3 g/L Sigma Aldrich Corp.) was additionally measured. Plates were incubated in the dark at ambient temperature for 30 min, then photometrically measured at 515 nm in a microplate reader (Infinite 200 Pro, Tecan Group AG, Maennedorf, Switzerland). All measurements were repeated in duplicate.

Scavenging activity [%] was calculated from the obtained absorbance levels of the sample A_sample and the pure solvent A_control (Equation 2).

\[ \text{Scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \] (2)

Plotting the scavenging activity over the different concentration levels obtained by the serial dilution of the sample allowed the calculation of the EC50-value [mg/mL] from a linear regression of the initial part of the obtained curve. In this context, EC50 is defined as the sample concentration scavenging 50% of the initial DPPH radical (Chen et al., 2013). Additionally, ascorbic acid equivalents (AAE) [mg/g] were calculated by combination of above-mentioned measurements with an ascorbic acid standard curve.

2.3.4. Chlorophyll and carotenoid content

For the measurements, spices were ground and respective sample masses (Table 1) were mixed with 10 mL methanol. Samples were placed in an overhead shaker (IKA-Werke GmbH & CO. KG, Staufen, Germany) in the dark for 2 h. Extracts were centrifuged at 3100 × g for 10 min at ambient temperature. The supernatant was syringe-filtered (ROTILABO CME, 0.45 μm, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), filled into a 3.5 mL quartz cuvette (10 mm Suprasil quartz cell with PTFE lid, Shimadzu Corp., Kyoto, Japan) and measured photometrically (UV-1800 UV-Vis Spectrophotometer, Shimadzu Corp., Kyoto, Japan). Absorption spectra were determined in the range of

![Table 1. Sample mass ranges used for the DPPH measurement of antioxidant activity and for chlorophyll and carotenoid analyses.](image-url)
780–410 nm. Concentrations of chlorophylls a, \(c_a\) [\(\mu g/g\)], and b, \(c_b\) [\(\mu g/g\)] as well as the total carotenoid concentration, \(c_{total}\) [\(\mu g/g\)] were calculated (Equations 3–5) from the absorbance at different wavelengths \(A\lambda\), as specified elsewhere (Dudek et al., 2014; Lichtenthaler, 1987; Lichtenthaler & Buschmann, 2001).

\[
c_a = 16.72A_{665.2} - 9.16A_{652.4} \tag{3}
\]

\[
c_b = 34.09A_{652.4} - 15.28A_{665.2} \tag{4}
\]

\[
c_{total} = \frac{1000A_{470} - 1.91c_a - 95.15c_b}{225} \tag{5}
\]

2.3.5. Analysis of volatile compounds

Samples from the first, middle, and last storage points (21, 63, and 105 d) were selected for instrumental analysis. The individual samples were placed in a gas-tight container, replacing the headspace with nitrogen. Samples were shock frozen at \(-30^\circ\)C. Frozen samples were sent to University of Chemistry and Technology, Prague, where they were kept frozen until further analysis.

2.3.5.1. Chemicals and reagents. Certified standards of Terpene Mix 18 (α-cedrene; α-terpinene; bornol; fenchol; fenchone; γ-terpinene; geraniol; guaiol; limonene; α-pinene; β-pinene; pulegone; sabinene; sabinene hydrate; terpineol-mix isomers; trans-nerolidol; α-humulene; α-terpinolene) with the concentration range from 2476 to 2548 mg/L in hexane and purities ≥ 78% as well as Terpene Mix 21 (3-carene, β-ocimen, bisabolol, camphene, camphor, caryophyllene oxide, cedrol, cineol, cis-nerolidol, menthol, geranyl acetate, isoborneol, p-cymene, isopulegol, linalool, myrcene, nerol, trans-caryophyllene, valencene, α-phellandrene) with the concentration range from 2484 to 2536 mg/L in hexane and purities ≥ 80% were obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). A mix of saturated alkanes C7–C30 in hexane, with a concentration of 1000 \(\mu g/mL\) each were obtained from Sigma-Aldrich GmbH (Steinheim, Germany). The alkane C7–C30 mix was prepared at a concentration level of 10,000 ng/mL in isoctane and used as an internal standard. Calibration solutions of all terpenic compounds mentioned above at concentrations of 2.5, 12.5, 25, 125, 250, 1250, and 2500 ng/mL were prepared in isoctane and stored at \(-18^\circ\)C. Each calibration level contained an internal standard of alkane mix C7–C30 at 100 ng/mL.

Analytical grade ethyl acetate and isoctane were purchased from Merck KGaA (Darmstadt, Germany) and sodium chloride (99.9% purity) was obtained from Penta s.r.o. (Prague, Czech Republic). Purified water (Milli-Q®, Merck KGaA, Darmstadt, Germany) was used for all instrumental analyses. Two strategies for the analysis of the samples were applied, as described below.

2.3.5.2. Target screening analysis of terpenic compounds. 50 mg of each sample were mixed with 10 mL of ethyl acetate and vigorously shaken for 2 min. 1 mL of ethyl acetate and 0.1 mL of internal standard were added to 8.9 mL isoctane. For the samples with target analyte concentrations above the calibration range, 0.1 mL of the ethyl acetate extract and 0.1 mL of internal standard were added to 9.8 mL of isoctane.

For validation of the measurements, commercial samples of allspice berries, caraway seeds, oregano, and rosemary were obtained from a Czech retail market. The spiking levels were chosen at concentrations of 50 mg/kg for the samples. For all matrices, six replicates were conducted. Samples were spiked and left at room temperature for 30 minutes before analysis. Recovery (expressed as REC %) of the analytes naturally occurring in the samples was set to 100%, as a repeatable extraction (n = 3) was performed and the analytes in the second and third extract were below the limit of detection. Repeatability (expressed as RSD %) of the method was calculated from six replicate analyses of each sample. REC and RSD values for all tested matrices were in the following ranges: rosemary – REC considered as 100% (all target analytes naturally occurred in the sample), RSD 1–16%; caraway – REC 54–104%, RSD 4–24%; oregano – REC 98–104%, RSD 4–24%; allspice – REC 62–111%, RSD 3–20%.

For the calculation of the contents of terpenic compounds in the samples, MassHunter Quantitative Analysis 10.1 software (Agilent Technologies Inc., Palo Alto, CA, USA) was used.

2.3.5.3. Non-target analysis. A solid phase microextraction (SPME) fiber with a dimension of 50/30 \(\mu m\), consisting of divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was supplied by Supelco Inc. (Bellentreffe, PA, USA). Prior to use, the fiber was conditioned following the manufacturers’ recommendations. Incubation time (10 min) and temperature (40°C), as well as extraction time (10 min) and temperature (40°C), and desorption time (2 min) were found to be the optimal conditions for each type of sample. For the SPME analysis, 50 mg of the sample were mixed with 2 mL of saturated NaCl solution.

MassHunter Unknowns Analysis software B.10.1 (Agilent Technologies Inc., Palo Alto, CA, USA) was employed for processing of the raw data. Following spectral deconvolution, all detected signals were exported to Mass Profiler Professional Software 15.0.
(Agilent Technologies Inc., Palo Alto, CA, USA), where peak alignment according to mass (±10 ppm) and retention time (±0.1 min) together with filtration according to frequency of occurrence was performed. In the next step, the data set obtained by filtration was exported to MassHunter Quantitative Analysis Software 10.1 (Agilent Technologies Inc., Palo Alto, CA, USA) for recursive analysis. In the final phase, the normalized data (relative intensities of each signal obtained by division of the sum of all signals) was investigated by multivariate chemometric analysis. SIMCA 16 software (Umetrics, MKS Instruments AB, Umeå, Sweden) was employed for unsupervised principal component analysis (PCA).

Marker compounds were identified and verified using NIST17 library, isotopic pattern, exact mass (mass error <5 ppm) and Kovats retention index.

2.3.5.4. Gas chromatography–high resolution mass spectrometry (GC-HRMS).

An Agilent 7200b system consisting of an Agilent 7890B gas chromatograph equipped with a multimode inlet, a PAL RSI 85 sampler (CTC Analytics AG, Zwingen, Switzerland) for automated headspace solid-phase microextraction (HS-SPME) and direct injection, as well as a quadrupole-time-of-flight mass spectrometer (Q-TOF; Agilent Technologies, Inc., Palo Alto, CA, USA), was employed. For the instrument control and data acquisition, MassHunter GC/MS Acquisition software 10.0.384.1 (Agilent Technologies, Inc., Palo Alto, CA, USA) was used.

For the analysis of terpenoid compounds, samples were injected in split mode (split ratio 1:5) at an inlet temperature of 250°C. Helium was used as a carrier gas at a flow rate of 1 mL/min. The oven temperature program was set as follows: 40°C initial temperature, held constant for 1 min, followed by a heating rate of 10°C/min until 150°C, then a heating rate of 5°C/min until 190°C, then a heating rate of 20°C/min until 310°C, which was then held constant for 5 min. Sample components were separated on a 30 m HP-5 MS UI capillary column (d = 0.25 mm, film thickness: 0.25 μm; Agilent Technologies Inc., Palo Alto, CA, USA). To prevent deviations of the retention times for target compounds, retention time locking was applied. For the calibration and locking of the GC method, α-humulene was used at a retention time of 14.402 min with a flow rate of 1 mL/min.

Samples prepared by SPME were injected in splitless mode (splitless period of 1 min) at 220°C and the oven temperature program was set to 40°C (for 2 min), followed by a heating rate of 5°C/min until 230°C, which was then held constant for 5 min. Sample components were separated on a 50 m HP-INNOWax capillary column (d = 0.2 mm, film thickness: 0.2 μm; Agilent Technologies Inc., Palo Alto, CA, USA).

The mass spectrometric detector was operated in electron ionization mode. The temperature of the ion source was 230°C. The mass range was 40–550 m/z and the resolution of the mass analyzer was set to >12,500 full width at half maximum (FWHM).

2.4. Data analysis and visualization

Calculations and analyses of the obtained data were carried out using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed in Statgraphics Centurion 18 (Statgraphics Technologies, Inc., The Plains, VA, USA), using Student’s t-test. Data visualization was realized by using Sigma Plot 14 (Systat Software, Inc., San Jose, CA, USA).

3. Results and discussion

3.1. Microbial inoculum and inactivation levels

Spice inoculation with E. faecium suspensions was carried out as specified by corresponding validation protocols for industrial use (ABC, 2014). This procedure lead to different initial microbial counts for the individual spices (Figure 2). This may be due to the fact that the different spices exhibited different surface structures and porosities as well as surface to volume ratios. Therefore, their ability to absorb microbial suspensions may be different. Moreover, the constituents of some spices may exert a limited antimicrobial efficacy, leading to a partial reduction of CFU on the surface of the spices (Charles, 2012; Peter, 2004). Therefore, the higher initial counts of caraway seeds may be related to their weak antimicrobial potential, as described in literature (Nemeth, 1999; Seidler-Łożykowska et al., 2013).

![Figure 2](image)

Figure 2. Bacterial counts, expressed as log_{10} of the number of colony forming units (CFU), N, of spices inoculated with E. faecium, for untreated as well as LEEB and gamma treated spices at the beginning (21 d) well as at the end (105 d) of the monitoring period. Data points depict mean values, error bars refer to standard deviation. Limit of detection: 10 CFU/g.
Both decontamination methods showed a distinct inactivation effect on the bacteria, i.e. both treatments reduced the CFU counts below the detection limit (<10 CFU/g) for all spices (Figure 2). Therefore, inactivation levels in the range of >3.3 log\(_{10}\) to >5.5 log\(_{10}\) were achieved for LEEB and gamma irradiation. Comparable results were found by Gryczka et al. (2018), who reported equivalent inactivation levels of the native microflora on dried black pepper, onion flakes, and bay leaves, comparing high energy electron beam (10 MeV) with LEEB decontamination (200 and 300 keV). Similar findings were reported for black pepper, white pepper, and allspice by the same group, using LEEB at 300 keV (Gryczka et al., 2020). Bacterial counts were analyzed again at the end of the storage period (105 d) and it could be shown that no re-growth occurred, i.e. all counts remained below the detection limit (<10 CFU/g). This implies that no sublethal injury or viable but nonculturable state with subsequent resuscitation was induced by the two treatments (Schottroff et al., 2018). Therefore, it can be concluded that LEEB treatment and gamma irradiation are equally effective against \(E.\ faecium\) surface contaminations. As it is reported that \(E.\ faecium\) may serve as a suitable nonpathogenic surrogate for \(Salmonella\ enterica\) in radiation processing (Arias-Rios et al., 2019), the effectiveness of both treatments against \(Salmonella\) surface contaminations can therefore also be assumed. However, this should be further evaluated, also taking into account other \(Salmonella\) serovars, as well as the microflora naturally occurring on spices.

### 3.2. Color changes of spices over storage time

In general, color measurement revealed product colors of all untreated samples tending toward the brown-range, although some spices like oregano or rosemary appear greenish to the bare eye (Figure 1). With values of \(a^*\) in the positive range for oregano (4.9 ± 0.4) and rosemary (1.2 ± 0.3), these colors were rather in the red than in the green range. Moreover, the yellow proportion (+b\(^*\)) of oregano (26.1 ± 0.3) and rosemary (24.3 ± 0.3) was relatively high, therefore implying a yellow-brown color. Allspice, on the other hand, showed an \(a^*\)-value of 11.3 ± 1.7 and a \(b^*\)-value of 4.7 ± 3.0, indicating a red-brown color. Caraway seeds exhibited values of \(a^*\) and \(b^*\) of 10.0 ± 1.0 and 13.1 ± 1.2, respectively. Therefore, this spice is characterized by a relatively plain brown.

For the LEEB and gamma treated samples, each storage point was compared to the untreated reference by means of ΔE-values calculated from the measured \(L^*\)-\(a^*\)-\(b^*\) values (Equation 1). Results showed no significant (\(p < 0.05\)) color differences between the two individual treatments compared to the reference (Figure 3), for each respective storage point. However, most data points were fluctuating around ΔE values in the range of 1–2. Similar, but slightly lower color changes (ΔE of up to 1.24) were reported by Woldemariam et al. (2021) for red pepper decontaminated with high energy electron beam (5 MeV, 30 kGy). For the same spice, Kyung et al. (2019) found maximum color changes characterized by ΔE-values of 3.68 and 1.81 for treatments with an absorbed dose of 3 kGy, using high energy electron beam and

![Figure 3. Color differences, expressed in ΔE-values, of LEEB and gamma treated allspice (A), caraway (B), oregano (C), and rosemary (D) samples over storage time. The untreated samples of the same storage point were used as the reference. Data points depict mean values, error bars refer to standard deviation.](image-url)
gamma irradiation, respectively. Therefore, the obtained values for the LEEB treatment are in line with the published literature on radiation of spices by high energy electron beam and gamma irradiation, showing minor changes in the optical appearance of spices, independent of the respective treatment.

The values of the first storage point seemed distinctly higher than the data points of the further measurements for most spices, with mean ∆E values of up to 6.7 (allspice), which would be associated with a pronounced alteration of spice color. This, however, was not noticeable by visual inspection by the bare eye. Depending on the respective spice, color measurements showed a distinct fluctuation, which is due to the irregular shapes and inconsistent coloring of the individual berries, seeds, or leaves (Figure 1). Horváthová, Suhaj, Polovka et al. (2007a) reported distinct changes (increases as well as decreases) of the redness value (a*), as measured in methanol extracts, directly after gamma irradiation (5–30 kGy) for black pepper, oregano, and allspice. In comparison to the irradiation itself, changes were reported to be more pronounced during the 4-month storage for allspice and oregano, but not for black pepper. Moreover, the difference in redness between treated and untreated allspice and oregano became negligible during the storage. This is also reflected in the results of the present study, as the color remained mostly unchanged after the first storage point (> 21 d) for all analyzed spices.

In general, independent of the individual treatment, some color variation was found during storage, which can be explained by fluctuation of the measurement, but no clear trend could be observed (Figure 3), therefore implying relative stability of spice color upon storage.

### 3.3. Alterations in water activity \( a_w \)

The \( a_w \)-values of the untreated spices were in the range of 0.4–0.6, with values being the lowest for caraway (0.416 ± 0.013) and the highest for allspice (0.544 ± 0.019). This is below the levels enabling microbial growth, however, osmotolerant microorganisms and spores present on the surface might survive for longer durations, therefore posing a risk to food safety (Troller & Christian, 1978).

The measured \( a_w \)-values showed no significant difference (\( p > 0.05 \)) between the individual treatments and the control for oregano and rosemary (Figure 4). The values of LEEB treated allspice berries were significantly lower (\( p < 0.05 \)) than the gamma and control values. However, the difference was only of a minor nature, i.e. in the range of less than 0.03 \( a_w \)-units. This is in agreement with a study on LEEB treatment of black pepper and coriander, where a slight reduction in moisture content was shown for both spices (Kotilainen et al., 2021). In the case of caraway seeds, water activity of LEEB treated samples was significantly higher (\( p < 0.05 \)) than for gamma irradiated and untreated samples, however, also with minor differences of less than 0.02 \( a_w \)-units. These findings are in partial agreement with the published literature. Duncan et al. (2017) reported a significant reduction in water activity of irradiated peppercorn, cumin seeds, and onion powder, but a slight increase for the \( a_w \) of

![Figure 4. Water activity values (\( a_w \)) of untreated references, as well as LEEB and gamma treated allspice (A), caraway (B), oregano (C), and rosemary (D) samples over storage time. Data points depict mean values, error bars refer to standard deviation.](image-url)
oregano subsequent to gamma irradiation (8 kGy). A reason for this could be the fact that irradiation took place within paper bags, therefore enabling a distinct transportation of water through the packaging, especially at elevated temperatures. However, the treatment temperature was not specified. Kyung et al. (2018), on the other hand, reported an increase of the water activity of red pepper subsequent to both high energy electron beam (10 MeV) and gamma irradiation at 3 kGy, from 0.54 to 0.59 and 0.60, respectively. Thus, the influence of different irradiation treatments on water activity and the relation with the structure and composition of different spices is still not fully elucidated yet and further research is necessary.

In general, fluctuations of the $a_w$ values might be explained by the different handling during the treatments. LEEB is an out-of-pack process, whereas gamma irradiation is carried out using packaged samples. This may explain the occurrence of sample-dependent slightly altered water activity levels after LEEB treatment, due to potential interaction with the surrounding air. Another possible explanation for the reduced water content after LEEB treatment would be a slight surface temperature increase due to LEEB, which may allow for limited evaporation in an open environment. Moreover, it may be possible that electrons or free radicals created by interactions of electrons with molecules of the spices further interact with compounds and break down water molecules or release OH$^-$ groups out of them. Thus, they may later interact with each other and form water molecules (Tahergorabi et al., 2012). Furthermore, slight deviations may also be due to inhomogeneity of spice samples in general, slightly different environmental influences during transportation, or storage, as well as fluctuations and uncertainty of the measurement.

Independent of the treatment, no significant changes (p > 0.05) of the water activity were found during storage for allspice, caraway, and rosemary. Only oregano showed a slight decrease in $a_w$ with increasing storage time, up to 0.04–0.06 units. However, no difference between control, gamma treatment and LEEB was found for this storage point. A potential reason for this could be the relatively large surface area of the oregano samples, compared to the other spices used in this study (Figure 1).

### 3.4. Changes in antioxidant capacity

The DPPH analysis revealed distinctly different values of EC$_{50}$ and ascorbic acid equivalents of the different untreated spices, when compared to each other (Figure 5). While allspice and rosemary showed relatively high antioxidant capacities, the values for caraway seeds were distinctly low. EC$_{50}$ and AAE values of oregano were in-between this range. The variation of the values was expected, as the different composition of the used spices is likely to cause different antioxidant capacities. A comparison of the obtained values with literature data is difficult, however, as the antioxidant capacity distinctly depends on factors like origin of the plants and the used drying method, among others (Hossain et al., 2010; Baiano & Previtali, 2018). However, tendentially, Hossain et al. (2008) determined higher antioxidant activities of allspice, compared to oregano and rosemary, and Shan et al.

![Figure 5](image_url). Antioxidant capacity, i.e. ascorbic acid equivalents (AAE) and EC$_{50}$ values of untreated references, as well as LEEB and gamma treated allspice (A), caraway (B), oregano (C) and rosemary (D) samples over storage time. Data points depict mean values, error bars refer to standard deviation.
reported an antioxidant capacity of caraway seeds distinctly lower compared to oregano and rosemary. Therefore, the spices analyzed in this study followed a similar ranking.

Comparison of LEEB treated, gamma irradiated, and untreated spices showed no distinct differences between the samples for each individual matrix (Figure 5). Depending on the respective storage point, for allspice the EC₅₀ value of the untreated samples was significantly (p < 0.05) lower than that of the treated samples. However, the values were in a rather low range and this could only reflect analytical fluctuations due to the proximity of the values to the limit of quantification. This may further be substantiated by the fact that the AAE values showed no significant difference.

Evaluation of antioxidant capacity over the storage period showed some fluctuations between the storage points for most samples, independent of the treatment. For allspice and rosemary, there appeared to be a slight increase of the EC₅₀ value, corresponding to a decrease in antioxidant activity, over the storage time. However, a clear trend, was not noticeable. Instead, this may rather be justified by uncertainty of the measurement of ~20%, especially, with most fluctuations being in the range of the standard deviation.

Considering the effects of gamma irradiation on antioxidant activity of spices, the obtained results are in accordance with literature data. In terms of allspice (Polovka et al., 2007), caraway (Fatemi et al., 2011; Polovka & Suhaj, 2010a), and oregano (Horváthová, Suhaj, Polovka et al., 2007b; Polovka et al., 2007; Sádecká & Polovka, 2008), no differences in DPPH radical scavenging activity subsequent to gamma treatments up to 30 kGy have been reported. For rosemary, a slight enhancement of antioxidant activity has been reported (Pérez et al., 2007). In terms of high energy electron beam (5 MeV), Weldemariam et al. (2021) reported a significant reduction of the antioxidant activity (7%) in red pepper powder only for the highest dose of the studied range (0–30 kGy). Due to being a relatively novel technology, no pronounced literature data on the influence of LEEB on antioxidant activity of spices are available. Therefore, this study could show that the behavior of LEEB and gamma irradiated samples appears to be similar in terms of antioxidant activity.

3.5. Chlorophyll and carotenoid contents

Chlorophyll and carotenoid concentrations of the individual untreated spices, as well as the ratios of chlorophyll a to chlorophyll b and total chlorophylls to total carotenoids differed distinctly (Figure 6). This reflects the different nature of the used samples. As expected, chlorophyll concentrations were highest in oregano and rosemary, as they originate from leaves. Moreover, these two spices also showed the highest carotenoid concentrations. Chlorophyll and carotenoid contents of allspice were distinctly lower, and caraway showed the lowest concentrations by far, as expected (Peter, 2004, 2006, 2012).

A comparison of the effects of LEEB treatment and gamma irradiation did not reveal significant differences (p > 0.05) in relation to the untreated references, for all of the used spices (Figure 6). Literature data on the influence of gamma treatment on spice quality suggest that radiolysis and oxidation caused by irradiation can lead to degradation of carotenoids, however, depending on the respective spice (Polovka & Suhaj, 2010a; Sebastião et al., 2002; Topuz & Ozdemir, 2003). Calucci et al. (2003) reported the influence of gamma irradiation (10 kGy) on quality attributes of nine different spices and found a significant reduction of carotenoids in 57% of the samples. Relating to the samples of the present study, lutein and zeaxanthin contents were reported to be reduced by 12.6% and 11.4%, as well as 37.7% and 37.0% for oregano and rosemary, respectively. This could not be confirmed by the present study. Reasons for these different findings could be the use of different spice varieties, which potentially underwent different drying procedures. This shows that quality alterations in irradiated spices distinctly differ. Therefore, the acquisition of further data is necessary in the future to gain deeper insights.

In the present case, no such changes were detectable for gamma treated spices. For high energy electron beam (5 MeV) treatment of red pepper, up to a dose of 10 kGy no pronounced influence on carotenoids was reported in the literature, whereas up to 13% of carotenoids were degraded at 30 kGy (Woldemariam et al., 2021). In case of LEEB treatment, no corresponding data are available to date. However, results suggest that LEEB and gamma treatments perform equally well considering the retention of carotenoids and chlorophylls.

Independent of the respective treatment, minor storage-specific alterations in chlorophyll and carotenoid contents could be observed for individual spices. Especially for allspice and caraway, a slight downward trend was detected, whereas values of oregano and rosemary remained relatively steady until the end of the storage period. This may be explained by the granular structure of the two former spices (Figure 1), leading to an increased void volume between the individual seeds and berries, therefore potentially causing a greater exposure to oxygen and light (Mercadante, 2007), followed by a certain degradation of chlorophylls and carotenoids.
3.6. Influence of treatments and storage on terpenoid contents of spices

Contents of terpenoid compounds for untreated, LEEB and gamma treated spices were determined by GC-HRMS. Due to the different nature of the spices, values differed distinctly. While for caraway seeds, all concentrations of terpenoid compounds were determined to be below the detection limit, levels in allspice and oregano were in the range of several hundred milligrams per kilogram, whereas for rosemary, terpenoid concentrations of several thousand milligrams per kilogram were quantified. Moreover, it was shown that neither values for LEEB nor gamma treated spices were distinctly different from the untreated reference for each storage point (Figure 7), as values of all determined terpenoid compounds were within the fluctuation of the measurement (8–28%). Considering changes over storage time, independent of the individual treatment, an obvious decline in the contents of terpenoid compounds was found for allspice (Figure 7A), as well as a minor decrease in values for oregano (Figure 7B), whereas for rosemary (Figure 7C) no such changes were detected, given the fluctuation of the measurement.

Terpenoid compounds are important aroma compounds of many essential oils (Stephane & Jules, 2020). Therefore, a degradation of essential oils by a decontamination treatment would lead to the loss of the most characteristic quality attributes of spices. For the used spices, no influence of LEEB and gamma treatments on a variety of terpenoid compounds were determined, compared to the untreated reference. This is in accordance with studies investigating essential oil contents of gamma irradiated caraway seeds (Fatemi et al., 2011).
published to date, but it could be shown that the quality of LEEB treated spices resembled the one of gamma irradiated samples. However, since the penetration depth of LEEB treatment is limited to surfaces, the effect of higher doses is expected to be more limited in comparison to gamma treatment. The location of the aroma compounds in different spices (surface vs. core) might impact the level of potential alterations.

3.7. Untargeted analysis

Solid phase microextraction coupled to gas chromatography and high-resolution mass spectrometry (SPME-GC-HRMS) followed by unsupervised principal component analysis (PCA) were used for evaluation of data obtained from untreated, as well as LEEB and gamma irradiated spices.

This analytical strategy enabled the determination of specific volatile compounds characteristic for different groups of samples – according to the individual treatment (Figure 8) or storage time (supplementary figure S1).

For rosemary samples, a distinct separation according to the treatment technology was found. LEEB treated samples were well distinguishable from the samples treated by gamma irradiation and the untreated samples (Figure 8D). Thus, samples treated by gamma irradiation and untreated samples had more consistent profiles of volatile compounds compared to samples treated by LEEB. The most significant marker responsible for this separation was 3-carene (data not shown). However, further research on the interactions of the treatment and the individual compounds is necessary.

In case of allspice, caraway, and oregano samples, no clustering according to the treatment technology occurred (Figure 8A-C), i.e. comparable profiles of volatile compounds were found for untreated, LEEB, and gamma treated samples. On the other hand, a pronounced separation was found according to storage time (supplementary figure S1), when comparing the first storage point (21 d) with the last storage point (105 d). In this regard, the most significant markers are coumarin, thymol, and eugenol, for allspice, caraway, and oregano, respectively (data not shown). This is most likely associated to minor evaporation and diffusion effects of essential oils during storage time.

4. Conclusions and outlook

In summary, it could be shown that both processes, gamma irradiation as well as LEEB treatment, enable an efficient reduction of microbial counts. Simultaneously, both treatments could retain the product quality to a great extent.
Due to the heterogeneous nature of spices, it is difficult to generalize effects of decontamination treatments on their quality attributes. Thus, a broad data basis should be acquired, to be able to better understand the effects of antimicrobial technologies, especially for those just recently developed. Therefore, for LEEB treatment, further herbs and spices should be treated and potential negative effects on the product quality should be carefully evaluated. In this regard, further quality parameters should be evaluated, making use of advanced instrumental analyses, such as LC and GC, coupled with MS/MS, or QTOFMS.

Moreover, in terms of antimicrobial efficacy of the LEEB treatment, a few studies on the precise mechanism of action on microbial cells already exist in case of spores, but more detailed research on inactivation pathways is necessary. This should also include additional vegetative microbial species and bacterial endospores, as well as viruses and mold spores.

Declaration of interest statement
The authors declare no conflict of interest.

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References
ABC (2014). “Almond board of California (ABC) guidelines for using Enterococcus faecium nrrl b-2354 as a surrogate microorganism in almond process validation.” http://www.almonds.comprocessors/processing-safe-product/pasteurization Accessed: June 06, 2020
Allothman, M., Bhat, R., & Karim, A. A. (2009). Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends in Food Science & Technology, 20, 201–212. https://doi.org/10.1016/j.tifs.2009.02.003
Anderson, N. (n.d.). “FDA Regulations and Process Validation Considerations.” U.S. Food and Drug Administration. Retrieved March 01, 2021, from https://nifa.usda.gov/sites/default/files/resource/Overview%20of%20FDA%20Regulations%20and%20Process%20Validation%20Considerations.pdf
Araújo, C. R. R., Corrêa, G. M., Abreu, V. G. D. C., Silva, T. D. M., Osorio, A. M. B., Oliveira, P. M. D., & Alcântara, A. F. D. C. (2017). Effects of gamma radiation on essential oils: a review. new insights on gamma rays. A. M. Maghraby, 179–202. IntechOpen.
ISO 14470:2011 Food irradiation — Requirements for the development, validation and routine control of the process of irradiation using ionizing radiation for the treatment of food. Kirkin, C., Mitrevski, B., Gunes, G., & Marriott, P. J. (2014). Combined effects of gamma-irradiation and modified atmosphere packaging on quality of some spices. Food Chemistry, 154, 255–261. https://doi.org/10.1016/j.foodchem.2014.01.002

Kotilainen, H., Meneses, N., Laaksonen, O., & Yang, B. (2021). Effects of low-energy electron beam (LEE) treatment on physicochemical attributes of black pepper and coriander. In K. Knoerzer & K. Muthukumaran (Eds.), Innovative Food Processing Technologies (pp. 79–100). Elsevier.

Kotilainen, H., Meneses, N., Ibbotson, T., & Conde-Petit, B. (2019). “Laatu” White paper.” https://digital.buhlergroup.com/laatu/ Accessed: May 20, 2020. Bühler AG, Uzwil, Switzerland.

Kyung, H.-K., Ramakrishnan, S. R., & Kwon, J.-H. (2018). Evaluation of capsaicinoid profile and antioxidant properties in dried Korean red pepper (Capsicum annuum L.) as affected by variable dose rates of electron beam and gamma ray irradiation. Journal of Food Science and Technology, 55, 3902–3910. https://doi.org/10.1007/s13197-018-3313-9

Kyung, H.-K., Ramakrishnan, S. R., & Kwon, J.-H. (2019). Dose rates of electron beam and gamma ray irradiation affect microbial decontamination and quality changes in dried red pepper (Capsicum annuum L.) powder. Journal of the Science of Food and Agriculture, 99, 632–638. https://doi.org/10.1002/jsfa.9225

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology, Academic Press, 148, 350–382. https://doi.org/10.1016/0076-6897(87)4036-1

Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical Chemistry, 1, F4.3.1–F4.3.8. https://doi.org/10.1002/0471142913afa040301

Maherani, B., Hossain, F., Criado, P., Ben-Fadhel, Y., Salmieri, S., & Lacroix, M. (2016). World market development and consumer acceptance of irradiation technology. Foods, 5, 79. https://doi.org/10.3390/foods5040079

Martinez, B., Stratton, J., Bianchini, A., Wegulo, S., & Weaver, G. (2015). Transmission of Escherichia coli O157: H7 to internal tissues and its survival on flowering heads of wheat. Journal of Food Protection, 78, 518–524. https://doi.org/10.4315/0362-028X.JFP-14-298

Mathot, A. G., Postollec, F., & Leguernel, I. (2021). Bacterial spores in spices and dried herbs: The risks for processed food. Comprehensive Reviews in Food Science and Food Safety, 20, 840–862. https://doi.org/10.1111/1541-4337.12690

Mercadante, A. Z. (2007). Carotenoids in foods: sources and stability during processing and storage. Food colorants: chemical and functional properties. C. Socaciu, 213–235. CRC Press.

Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chemistry, 130, 1036–1043. https://doi.org/10.1016/j.foodchem.2011.07.127

Mosavi, K., Khanghah, A., Hashemi, Mosavi, M., Oliveira, C. A. F., Vanin, F., & Sant’Ana, A. S. (2020). Microbial beam irradiation to reduce the mycotoxin and microbial beam contaminations of cereal-based products: An overview. Food and Chemical Toxicology, 143, 111557. https://doi.org/10.1016/j.fct.2020.111557

Nemeth, E. (1999). Caraway: The Genus Carum. CRC Press.

Pérez, M. B., Banek, S. A., & Croci, C. A. (2011). Retention of antioxidant activity in gamma irradiated Argentinian sage and oregano. Food Chemistry, 126, 121–126. https://doi.org/10.1016/j.foodchem.2010.10.087

Pérez, M. B., Calderón, N. L., & Croci, C. A. (2007). Radiation-induced enhancement of antioxidant activity in extracts of rosemary (Rosmarinus officinalis L.). Food Chemistry, 104, 585–592. https://doi.org/10.1016/j.foodchem.2006.12.009

Peter, K. V. (2004). Handbook of herbs and spices (Vol. 2). Woodhead Publishing.

Peter, K. V. (2006). Handbook of herbs and spices (Vol. 3). Woodhead Publishing.

Pinker, J. M., & Keller, S. E. (2014). Spices. the microbiological safety of low water activity foods and spices. J. B. Gurtler, M. P. Doyle, & J. L. Kornacki, 99–114. Springer New York.

Polovka, M., Brezova, V., & Šimko, P. (2007). EPR spectroscopy: A tool to characterize gamma-irradiated foods. Journal of Food and Nutrition Research, 46, 75–83. www.vup.sk/en/download.php?bullID=40

Polovka, M., & Suhaj, M. (2001a). Detection of caraway and bay leaves irradiation based on their extracts’ antioxidant properties evaluation. Food Chemistry, 119, 391–401. https://doi.org/10.1016/j.foodchem.2009.07.005

Prieto, J. M. (2012). Preparation of DPPH Radical, and antioxidant scavenging essay. https://www.researchgate.net/profile/Yahya_Alsalih2/post/DPPH_96 микрометр_reader_essay_referenced/attachment/5a55a6bb53d2f0ba4a372f/AS5811261015818331515562667777/down load/PrietoDPPHprotocol.pdf Accessed: July 02, 2020.

Qiu, L., Zhang, M., Mujumdar, A. S., & Liu, Y. (2020). Recent developments in key processing techniques for oriental spices/herbs and condiments: A review. Food Reviews International, 1, 21–21. https://doi.org/10.1080/07559129.2020.1839492

Sabillo, L., Stratton, J., Rose, D. J., Regassa, T. H., & Bianchini, A. (2016). Microbial load of hard red winter wheat produced at three growing environments across Nebraska, USA. Journal of Food Protection, 79, 646–654. https://doi.org/10.4315/0362-028X.JFP-15-424

Sádecká, J., & Polovka, M. (2008). Multi-experimental study of γ-irradiation impact on oregano (Origanum vulgare L.). Journal of Food and Nutrition Research, 47, 85–91. https://www.vup.sk/en/download.php?bullID=68

Schottroff, F., Fröhling, A., Zunabovic-Pichler, M., Krottenthaler, A., Schützer, O., & Jäger, H. (2018). Sublethal injury and viable but non-culturable (vbnC) state in micro-organisms during preservation of food and biological materials by non-thermal processes. Frontiers in Microbiology, 9, 2773. https://doi.org/10.3389/fmicb.2018.02773

Schweigert, U., Carle, R., & Schieber, A. (2007). Conventional and alternative processes for spice production – A review. Trends in Food Science & Technology, 18, 260–268. https://doi.org/10.1016/j.tifs.2007.01.005

Sebstiáňa, K. I., Almeida-Muradian, L. G. B., Romaneli, M. F., Koseki, P. M., & Villavicencio, A. L. C. H. (2002). Effect of gamma-irradiation on the levels of total and cis/trans isomers of beta-carotene in dehydrated parsley. Radiation Physics and Chemistry, 63, 333–335. https://doi.org/10.1016/S0969-806X(01)00626-0

Seidler...
Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of Agricultural and Food Chemistry, 53, 7749–7759. https://doi.org/10.1021/jf051513y
Sharma, G., & Bala, R. (2017). Digital color imaging handbook. CRC Press.
Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. Food Chemistry, 113, 1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008
Small, E. (2006). Culinary Herbs. NRC Research Press.
Stephane, F. F. Y., & Jules, B. K. J. (2020). Terpenoids as important bioactive constituents of essential oils. In M. S. D. Oliveira, S. Silva, & W. A. D. Costa (Eds.), Essential oils - bioactive compounds, new perspectives and applications. IntechOpen.
Tahergorabi, R., Matak, K. E., & Jaczynski, J. (2012). Application of electron beam to inactivate Salmonella in food: Recent developments. Food Research International, 45, 685–694. https://doi.org/10.1016/j.foodres.2011.02.003
Topuz, A., & Ozdemir, F. (2003). Influences of γ-irradiation and storage on the carotenoids of sun-dried and dehydrated paprika. Journal of Agricultural and Food Chemistry, 51, 4972–4977. https://doi.org/10.1021/jf034177z
Troller, J. A., & Christian, J. H. B. (1978). Water Activity and Food. Academic Press.
WHO. (1988). World Health Organization (WHO) - Food irradiation: A technique for preserving and improving the safety of food. World Health Organization.
Wojtowicz, E., Zawirska-Wojtasiak, R., & Przygoński, K. (2007). Influence of steam water sterilization process on the content of volatile aroma compounds in marjoram (Origanum majorana L.) estimated with GC/MS and GC/O. Polish Journal of Food and Nutrition Sciences, 57, 151–155.
Woldemariam, H. W., Kießling, M., Emire, S. A., Teshome, P. G., Töpfl, S., & Aganovic, K. (2021). Influence of electron beam treatment on naturally contaminated red pepper (Capsicum annuum L.) powder: Kinetics of microbial inactivation and physicochemical quality changes. Innovative Food Science & Emerging Technologies, 67, 102588. https://doi.org/10.1016/j.ifset.2020.102588