Radiation Physics and Chemistry 80 (2011) 604–607

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Technical Note

Microbial decontamination by low dose gamma irradiation and its impact on the physico-chemical quality of peppermint (*Mentha piperita*)

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ARTICLE INFO

Article history:
Received 7 March 2010
Accepted 3 November 2010
Available online 10 November 2010

Keywords:
Peppermint
Ionizing radiation
Decontamination
Escherichia coli
Essential oils
Polyphenols

ABSTRACT

Peppermint was inoculated with *Escherichia coli* and its decontamination was carried out by gamma irradiation at low irradiation doses (0.5, 1.0 and 2.66 kGy). The efficiency of this decontamination method was evaluated and its impact on the quality parameters of peppermint, such as the color and ash content, as well as the effect on fingerprint components such as phenols and essential oils, was studied. Gas chromatography coupled to mass spectrometry (GC/MS) and High Performance Liquid Chromatography (HPLC) were used to characterize essential oils and phenolic compounds, respectively. The results indicated a complete decontamination of peppermint after the low dose gamma irradiation without a significant loss in quality attributes.

1. Introduction

Mentha piperita (Lamiaceae), well known as peppermint, is one of the herbal plants that has garnered a tremendous level of attention as a herbal remedy used for a variety of symptoms and diseases (McKay and Blumberg, 2006). This herbal plant, highly produced and consumed in Morocco, is susceptible to chemical and microbial contaminations due to the environmental conditions where it is grown as well as the practices of harvesting, handling, storage and processing. Besides being a health hazard to the consumer, the contaminations can cause the spoilage and deterioration of the food it is added to. The decontamination of peppermint is then vital to make it suitable for human consumption and commercialization.

Several decontamination techniques have been reported for medicinal and aromatic plants or plant materials in general, for example some are thermal processes such as vapor treatments, ohmic heating, thermal shock, controlled sudden decompression or pulsed light (Fine and Gervais, 2003). These processes may cause partial deterioration of the sensory attributes of plant materials. Techniques such as fumigation or irradiation are occasionally used as non-thermal alternatives to overcome such issues. However, fumigation, with gaseous ethylene oxide or methyl bromide, is actually prohibited or restricted in several countries for health or environmental issues. On the other hand, ionizing irradiation methods such as electron beam accelerators and gamma irradiation are being used. Processing by ionizing radiation has attracted a great deal of interest as an environment-friendly, versatile and effective technology that can control a variety of microorganisms and thus extend the shelflife of food. Gamma irradiation is a physical technique based on the use of radiation to destroy microorganisms by inhibiting their physiological processes. Gamma irradiation improves the hygienic quality of various foods and herbal materials and reduces losses due to microbial contamination and insect damage (Farkas, 1998). Additionally, gamma irradiation shows advantages in improving the color and the antioxidant activity (Byun et al., 1999; Jo et al., 2003b; Kim et al., 2006). In the food industry, gamma irradiation can be used for different purposes depending on the absorbed dose. Simple food disinfection can be obtained at doses lower than 1 kGy and radicidation can be performed with doses ranging between 1 and 10 kGy; however sterilization is achieved at doses greater than 10 kGy.

The aim of this work is to study the *E. coli* decontamination of peppermint with gamma irradiation using a relatively low dose. The choice of *E. coli*, which recurringly contaminates peppermint plants, was made because it is commonly found in the intestinal tract of warm-blooded organisms. The effect of this process on the

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[1] The authors wish to dedicated this paper to the memory of Dr. Ismail Hadrami, a wonderful and creative colleague who passed away at the beginning of a very promising career.

0969-806X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.radphyschem.2010.11.002

quality aspects of some extracts, such as essential oils and phenolic compounds, was thoroughly investigated.

2. Materials and methods

2.1. Samples

Sun-dried specimens of peppermint (*Mentha piperita*) were obtained from Société Impériale des Thés et Infusions (SITI; Marrakesh, Morocco) and used without any sorting or cleaning treatment. Samples were cut in small pieces and packed in vacuum sealed plastic bags until experimentation.

2.2. Microbial loading and analyses

Samples were inoculated with *Escherichia coli* (*E. coli*) type CCMM B4 taken from a culture medium containing nutrient agar. The suspension of *E. coli* was diluted with sterile water to the desired volume and pulverized on peppermint samples.

For microbial analyses, the counts were determined by the surface plate agar method. 25 g of inoculated irradiated or non-irradiated peppermint samples was added to 250 ml of sterile water and mixed thoroughly. Each suspension was diluted up to 10^5 times with the same sterile water, and then 0.1 ml aliquots were spread on the surface of McConkey agar plate. Total colonies were counted after 24 h incubation at 44 °C and counts were recorded in colony-forming units per gram (cfu/g). The results were the average of three replicates for each sample.

2.3. Irradiation treatment

The irradiation of inoculated peppermint samples was carried out at the Boukhalef Ionization Plant (Tangier, Morocco). The plant is equipped with a ^{60}Co radio-isotopic source. 10 samples, of 25 g each, of inoculated peppermint samples were loaded into the reactor. The dosimetry was performed using routine Fricke dosimeters and the most suitable process dose/rate ratio was around 1.85 Gy/min. Three dose levels, namely 0.5, 1.0 and 2.66 kGy, were used. Immediately after the irradiation, samples were submitted to microbiological analyses or were stored at −18 °C for further physico-chemical characterizations.

2.4. Color intensity analysis

Around 2 g of powdered peppermint samples were extracted with 20 ml of acetone at room temperature during 24 h and under magnetic stirring. The extraction system was maintained in the dark by wrapping it in aluminum foil to prevent any adventitious photolytic degradation. The chlorophylic extract was then filtered and adjusted to 50 ml by adding a mixture of acetone/water (80/20, v/v) and was then diluted to 1/20. The spectra were recorded with a UV–visible spectrophotometer type Anthelie (SECOMAM, France); this was performed in triplicate with each sample.

2.5. Extraction and analysis of essential oils

In order to avoid thermal degradation of the essential oils, the extraction was performed at a low temperature. Samples of powdered peppermint (10 g) were extracted with dichloromethane at 4 °C for 30 min and then filtered with Whattman paper filter (N2). The extracted essential oils were further analyzed by gas chromatography (GC) using a Finnigan Trace GC Ultra instrument coupled with a mass spectrometry detector (Polaris Q). The column (VB-5, 0.25 μm × 0.25 mm × 30 m) was used at programmable temperatures ranging from 40 to 300 °C with two plateaus at 40 and 300 °C while the temperature of the injector was set at 250 °C. Products were identified by comparing their retention times and mass spectra with standards when available.

2.6. Extraction and characterization of phenolic compounds

Phenolic contents were extracted according to the method reported by Radi et al. (2004). Briefly, 100 mg of peppermint samples were ground with cold methanol (80% vv) at 4 °C. The resulting material was centrifuged at a speed of 7000 rpm for 20 min at 4 °C. The supernatant was recovered and the extraction process was then repeated three times on the respective resulting residues in order to ensure the complete extraction of the phenolic products. The resulting supernatants were mixed together and stored at −20 °C until the completion of experiments.

Total phenolic contents were measured using the Folin–Ciocalteau colorimetric method (Swain and Goldstein, 1964). 50 μl of the extracted phenolic material was mixed with 1.7 ml of distilled water and 0.25 ml of Folin–Ciocalteau reagent. After rigorous agitation, 0.5 ml of sodium carbonate solution (20% wt/v) was added and the mixture was immediately incubated at 40 °C during 30 min. Absorbance at 765 nm was then recorded for the mixture and the total phenolic content was expressed as (+)—catechin equivalent (in mg) per 100 g of dry matter.

The analytical characterization of the phenolic compounds was performed with High Performance Liquid Chromatoghraphy (HPLC) using a Waters 600E chromatograph equipped with a photodiode array detector (Waters 990). An analytical reversed phase column type Interchrom C18 (4.6 × 150 mm, 5 μm, Macherey–Nagel, Duren, Germany) was used. The solvent system was a mixture of acetonitrile/water in which the pH was adjusted to 2.6 with addition of droplets of phosphoric acid. Elution was performed at a solvent flow rate of 1 ml/min by using a gradient of acetonitrile ranging from 5% to 80% in water. The chromatograms were recorded at 280, 320 and 350 nm and the different phenolic compounds were identified by comparing their retention times and UV spectra with available standards.

3. Results and discussion

The peppermint analyzed in the present study is traditionally used in Morocco to prepare hot tea drinks and occasionally due to its therapeutic properties. In this study, we are investigating the feasibility of gamma irradiation as a decontamination technique. The study was designed to decontaminate peppermint by using gamma irradiation at a very low dose and to determine the effect on the total phenolic content, the composition of essential oils and other quality attributes of peppermint in order to get a preliminary evaluation of its feasibility.

3.1. Microbial decontamination

Samples of peppermint were subjected to *E. coli* inoculation with a microbial loading of 2.8 10^6 UFC/g and irradiated at different doses, namely 0.5, 1.0 and 2.66 kGy. Interestingly, after sample irradiation, the survival of microbial colonies was insignificant even at the lower irradiation dose applied, ca 0.5 kGy. These results demonstrated the success of the complete decontamination of peppermint samples.

Furthermore, we were interested in the impact of the irradiation, applied for decontamination purposes, on the quality attributes of peppermint samples. Hereafter, we studied several quality indicators such as the ash content, color, essential oils and phenolic