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Gamma radiation enhances the bioactivity of fresh parsley (*Petroselinum crispum* (Mill.) Fuss Var. Neapolitanum)

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ABSTRACT

Research showed the effects of drying, freezing, and irradiation on the bioactivity of parsley, but overlooked comparing them. In the present study, the effect of minimal processing paired with gamma irradiation (0.7–2.7 kGy) was evaluated in respect to the content of ascorbic acid, polyphenols, antiradical capacity and antibacterial activity of parsley methanolic extracts. The results were compared with natural drying (20–22 °C) and rapid freezing (−20 °C). Absorbed doses of 0.7–1.4 kGy can be recommended to no treatment from a nutritional point of view because the content of vitamin C was better preserved and a significant increase of polyphenols content was observed when compared with control samples. Drying can be recommended only in terms of vitamin C and polyphenols content per consumed serving, as it doubles the content of vitamin C and triples polyphenols compared to the same mass of fresh product. Plant extracts with optimal antioxidant and antimicrobial can be obtained from fresh herbs stored at refrigeration temperature as little as possible. Extracts from dried or frozen herbs preserved for long periods of time are not recommended. An absorbed dose of 2.7 kGy may be applied to parsley in order to increase its antibacterial against Gram-negative bacteria such as *E. coli* and *S. Typhimurium*.

1. Introduction

The demand for natural sources of preservatives is growing because consumers tend to prefer these products (Farah et al., 2015). Many antioxidant compounds (polyphenols, vitamins and pigments), naturally occurring in plants, are used widely to protect food from oxidation because they are radical or active oxygen scavengers (Berrington and Lall, 2012; Chaves et al., 2011; Zheng and Wang, 2001). Another concern is directed to food spoilage and pathogenic microorganisms. Thus, there is a growing interest to investigate the antimicrobial properties of phytocompounds that could enhance food safety (Farah et al., 2015; Seow et al., 2014; Solórzano-Santos and Miranda-Novales, 2012).

Parsley is the most consumed aromatic herbs in Europe (CBI, 2016) mainly because of its flavor. It is rich in phenols (Farah et al., 2015; Marín et al., 2016; Zheng and Wang, 2001) and presents significant antioxidant, antibacterial and antifungal activities (Berrington and Lall, 2012; Farah et al., 2015; Farzaei et al., 2013). The degradation of polyphenols a widely used as marker of the severity of processing treatments (Machhour et al., 2011).

Many studies show the efficiency of ionizing radiation to extend the shelf life of spices, aromatic herbs and leafy vegetables with good results in respect to quality and phytonutrients content (Fan and Sokorai, 2005; Jamshidi et al., 2015; Machhour et al., 2011). The effect of gamma radiation on the bioactive phytochemicals of parsely were relatively little studied (Fan and Sokorai, 2005).

However, few studies have considered to evaluate the effect of gamma radiation in comparison with other conventional preserving methods such as drying or freezing. Thus, this study aimed to assess the content of antioxidants, antiradical capacity and antibacterial activity of parsley processed in the three ways. The effect of minimal processing paired with gamma irradiation (0.7–2.7 kGy) was evaluated in respect to the content of ascorbic acid, polyphenols, antiradical capacity and antibacterial activity of parsley methanolic extracts. The results were compared with natural drying (20–22 °C) and rapid freezing (−20 °C).

Abbreviations: AAI, the antioxidant activity index; AEAC, ascorbic acid equivalent antioxidant capacity; DIZ, diameter of inhibition zone; FW, fresh weight; GAE, gallic acid equivalents; GEAA, gentamicin equivalent antimicrobial activity; I%, percentile radical scavenging activity; IC_{50}, 50% free radical scavenging activity; RDA, recommended dietary allowance; RSA, radical scavenging activity

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2. Materials and methods

2.1. Preparation of samples

Fresh parsley (*Petroselinum crispum* (Mill.) Fuss var. neapolitanum) was purchased in June from a local market in Bucharest and divided in 2 batches: one was minimally processed and the other was dried. A previously described minimally processing procedure was employed (Cătunescu et al., 2012). A total of 111 samples of 50 g were obtained from the first: 72 were irradiated, 36 were stored as such (the controls), and 3 were frozen at −20 °C. The second batch was washed, drained, and air-dried at shade for 7 days at 20–22 °C. After drying, the lot was divided in 10 g samples and thermo-sealed.

2.1.1. Irradiation

A number of 72 samples of minimally processed parsley were gamma irradiated at the IRASM - Multipurpose Industrial Irradiator at the Horia Hulubei National Institute of Physics and Nuclear Engineering, Măgurele, România. The irradiator was a SVST Co-60/B type loaded with 100 kCi. The actual absorbed doses were: 0.7 ± 0.1 kGy; 1.4 ± 0.1 kGy; 2.0 ± 0.2 kGy; 2.7 ± 0.3 kGy, determined for 2.7 kGy dose with an ethanol-chlorobenzene (ECB) dosimeter. The samples were irradiated in batches of 18 bags per dose at room temperature.

2.1.2. Storage

The control and irradiated parsley was stored at 4 °C for 4 days before assessment, while frozen samples at −20 °C and the dried batch at room temperature, at shade, both for 3 months.

2.2. Determination of water content

Humidity was determined gravimetrically: 5 g of parsley leaves and stems were dried in a drying oven (Memmer Thermoreglable) to constant weight at 105 °C and a pressure below 100 mm Hg (3 h). Weight loss was estimated as water content.

2.3. Methanol extraction procedure

The extraction procedure proposed by Cătunescu et al. (2012) was followed: 1 g of parsley was extracted with 10 ml of acidified methanol (99.09:0.01 v/v, MeOH:HCl) (reagent grade pure methanol Chempur) in a mortar at shade until total discoloration of tissues. The extracts were filtered (Whatman no. 1 filter paper) and dried in a vacuum rotary evaporator at 37 °C (Ojala, 2000; Wong and Kitts, 2006). The dry residues were redissolved in 5 ml of pure methanol and kept at −20 °C in opaque containers until further analysis.

2.4. Ascorbic acid separation, identification and dosage

The methanolic extracts were filtered (Teknokroma Syringe Nylon Filters 0.45 μm; 13 mm diameter) prior to HPLC injection. The ascorbic acid in the extracts was separated, identified and dosed in a HPLC Agilent 1200 system coupled with UV–VIS detector (DAD) (Cătunescu et al., 2012; Roman et al., 2013). An Eclipse XDB-C18 column (5 μm; 150×4.6) was eluated isocratically by a water/acetonitrile/formic acid (94/5/1; v/v/v) mobile phase at a flow rate of 0.5 ml · min^{-1}. The chromatograms were registered at 240 nm. Standard L-ascorbic acid (Sigma 99% standard L-ascorbic acid) was used for identification. A calibration curve was plotted using 5 solutions of standard L-ascorbic acid and bidistilled water: 0.8 mg · ml^{-1}; 0.7 mg · ml^{-1}; 0.6 mg · ml^{-1}; 0.5 mg · ml^{-1}; 0.2 mg · ml^{-1}. The curve was used to dose the ascorbic acid in the samples. The results were expressed in mg · 0.01 g^{-1} fresh weight (FW).

2.5. Total polyphenols determination

The content of total phenols in the methanolic extracts was determined spectrophotometrically following Folin-Ciocalteu method (Roman et al., 2013). A 1 mg · ml^{-1} stock solution of gallic acid in 40% ethanol was prepared. The solution was further diluted to obtain 5 concentrations of gallic acid (1 mg · 0.01 ml^{-1}; 0.5 mg · 0.01 ml^{-1}; 0.25 mg · 0.01 ml^{-1}; 0.125 mg · 0.01 ml^{-1} and 0.0625 mg · 0.01 ml^{-1}) to plot a calibration curve. One ml of stock solution, 5 ml of Folin-Ciocalteu reagent and 60 ml of distilled water were mixed. A volume of 15 ml of 7.5% Na_2CO_3 solution were added after 1 min and the solution was stirred thoroughly. The absorbance was measured after 2 h at 750 nm (Biotek multidetector UV–Vis spectrometer). The same procedure was followed for the remaining dilutions, while the blank was 1 ml of 40% ethanol.

The total phenols in the methanolic extracts was determined using plates with 24 wells of 3 ml. Each well contained: 2.375 ml of distilled water; 0.025 ml of methanolic extracts; 0.150 ml of Folin-Ciocalteu reagent and 0.450 ml Na_2CO_3. The extracts were replaced with 0.025 ml of 40% ethanol for blanks. The absorbance was measured after 2 h at 750 nm (Biotek multidetector UV–Vis spectrometer). The total amount of polyphenols was expressed in mg GAE · 0.01 g^{-1} FW using the calibration curve.

2.6. Antioxidant activity

The antioxidant activity was assessed using DPPH radical scavenging method. Following Molyneux (2004) recommendations, the procedures proposed by Heilerova et al. (2003) and Farah et al. (2015) were adapted as follows. A stock methanolic DPPH solution with a concentration of 0.025 g · l^{-1} was prepared. The solution was further diluted to obtain 4 concentrations: 0.020 g · l^{-1}; 0.015 g · l^{-1}; 0.010 g · l^{-1}; 0.005 g · l^{-1}. The absorbance of 3.9 ml of each dilution and 0.1 ml of distilled water was measured at 515 nm (UV–Vis 1700 PharmaSpec Shimadzu). The results were plotted against the concentrations, resulting the DPPH calibration curve.

The methanolic extracts were diluted to obtain the following concentrations: 200 mg FW · ml^{-1}; 150 mg FW · ml^{-1}; 100 mg FW · ml^{-1}; 50 mg FW · ml^{-1} and 25 mg FW · ml^{-1}. Vitamin C (0.30 g · l^{-1}; 0.20 g · l^{-1}; 0.15 g · l^{-1}; 0.10 g · l^{-1}; 0.03 g · l^{-1}) and gallic acid (0.50 g · l^{-1}; 0.40 g · l^{-1}; 0.30 g · l^{-1}; 0.20 g · l^{-1}; 0.10 g · l^{-1}) were used as positive controls. A volume of 0.01 ml of sample was pipetted in a cuvette containing 3.9 ml stock DPPH solution and 0.09 ml of distilled water. The absorbance was measured at 515 nm after 30 min (UV–Vis 1700 PharmaSpec Shimadzu).

The percentile radical scavenging activity (I%) was computed as in Eq. (1).

$$I\% = (A_{blank} - A_{sample})/A_{blank} \bullet 100, (\%) \quad (1)$$

where: A_{blank} = absorbance of stock DPPH solution; A_{sample} = absorbance of sample

The values 50% free radical scavenging activity (IC_{50}) were graphically determined by plotting sample concentration against I% (Scherer and Godoy, 2009).

The mass of DPPH reduced by 100 g FW of sample represented the radical scavenging activity (RSA) defined by Molyneux (2004) was computed as in Eq. (2).

$$RSA = m_{DPPH}(IC_{50})/m_{sample}(IC_{50}) \bullet 10^5, (mg\ DPPH \bullet 0.01\ g^{-1}FW) \quad (2)$$

where: $m_{DPPH}(IC_{50})$ - mass of DPPH in cuvette at IC_{50}, (μg); $m_{sample}(IC_{50})$ - mass of sample in cuvette at IC_{50}, (μg)

The results were further expressed in ascorbic acid equivalent antioxidant capacity per 100 g FW (AEAC) (Lim et al., 2007) (Eq. (3)).

$$AEAC = IC_{50(AA)}/IC_{50(sample)} \bullet 10^5 (mg\ ascorbic\ acid \bullet 0.01\ g^{-1}FW) \quad (3)$$