Food Chemistry 141 (2013) 1398–1405

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Effect of ionising radiation on polyphenolic content and antioxidant potential of parathion-treated sage (*Salvia officinalis*) leaves

Issam Ben Salem [a,b,c,*,1], Sana Fekih [a,b], Haitham Sghaier [b], Mehrez Bousselmi [a,b], Mouldi Saidi [b], Ahmed Landoulsi [c], Sami Fattouch [a,1]

[a] Laboratory of Protein Engineering and Bioactive Molecules (LIP-MB), National Institute of Applied Sciences and Technology (INSAT), University of Carthage, Tunis, Tunisia
[b] Research Unit, Application of Nuclear Techniques in the Fields of Health, Agriculture, and Environment, National Centre for Nuclear Science and Technology (CNSTN), Sidi Thabet Technopark, 2020 Ariana, Tunisia
[c] Biochemistry and Molecular Biology Laboratory, Faculty of Science of Bizerte, University of Carthage, Tunis, Tunisia

ARTICLE INFO

Article history:
Received 4 February 2013
Received in revised form 3 April 2013
Accepted 6 April 2013
Available online 18 April 2013

Keywords:
Antioxidant activity
Ionising radiation
Medicinal plant
Parathion
Polyphenols
Salvia officinalis

ABSTRACT

The γ-irradiation effects on polyphenolic content and antioxidant capacity of parathion-pretreated leaves of *Salvia officinalis* plant were investigated. The analysis of phenolic extracts of sage without parathion showed that irradiation decreased polyphenolic content significantly ($p < 0.05$) by 30% and 45% at 2 and 4 kGy, respectively, compared to non-irradiated samples. The same trend was observed for the Trolox equivalent antioxidant capacity (TEAC), as assessed by the anionic DPPH and cationic ABTS radical-scavenging assays. The antioxidant potential decreased significantly ($p < 0.01$) at 2 and 4 kGy, by 11–20% and 40–44%, respectively. The results obtained with a pure chlorogenic acid solution confirmed the degradation of phenols; however, its TEAC was significantly ($p < 0.01$) increased following irradiation. Degradation products of parathion formed by irradiation seem to protect against a decline of antioxidant capacity and reduce polyphenolic loss. Ionising radiation was found to be useful in breaking down pesticide residues without inducing significant losses in polyphenols.

1. Introduction

Ionising radiation has been demonstrated to be very effective for pathogen inactivation in both raw and cooked foods (Farkas, 1998). The International Consultative Group of Food Irradiation (ICGFI) concluded that irradiation of food at a dose level of 10 kGy or below was toxicologically safe and nutritionally adequate (WHO, 1981). During the last decade, several studies have shown varying sensitivities of particular food ingredients and nutrients to radiation treatment (Sommer, Schwartz, Solar, & Sontag, 2009). The phenolic content of rosemary was significantly altered following irradiation >10 kGy (Koseki et al., 2002), whereas, the capsaicinoids increased significantly, by about 10%, in sun-dried and dehydrated paprika samples irradiated at a dose of 10 kGy (Topuz & Ozdemir, 2003).

In addition to the occurrence of degradative biochemical reactions and spoilage microorganisms in food products, the presence of environmental toxicants, particularly pesticides, in freshly harvested material has attracted great attention in scientists. In agricultural phytosanitary practises, a small fraction of the used pesticide amount is directly involved in the pesticide action, and most of these chemicals remain as "residues", and may exert adverse effects on both target and non-target organisms (Fattouch et al., 2010). To our knowledge, despite several studies addressing the problems of pesticide residues toxicity in food, limited works have investigated the interactions between hazardous residues and health-promoting phytochemicals, particularly plant polyphenols (Rung & Schwack, 2005). The protective effect of the latter compounds is chiefly attributed to their antioxidant potential by scavenging free radicals, chelating metals in foods, activating antioxidant enzymes and inhibiting enzymes that cause oxidation reactions (Heim, Tagliaferro, & Bobilya, 2002).

During the last decades, medicinal and aromatic plants have been extensively studied and found to be excellent sources of bioactive and health-promoting compounds. In addition to their aromatic and flavouring properties, medicinal and aromatic plants have been used as additives to prevent the oxidative deterioration and microbial proliferation in several perishable food products (Fattouch, Sadok, Raboudi-Fattouch, & Slama, 2008; Sarkardei & Howell, 2008). Sage, *Salvia officinalis*, is one of the most well-known aromatic herbs. Sentences such as "Why should a man die while sage grows in his garden?" reflect the importance of this plant in traditional medicine (Ramos, Azqueta, Pereira-Wilson, &

* Corresponding author at: National Centre for Nuclear Science and Technology (CNSTN), Sidi Thabet Technopark, 2020 Ariana, Tunisia. Tel.: +216 71 537410; fax: +216 71 537555.
E-mail address: issamcnstn@yahoo.fr (I. Ben Salem).
[1] These authors contributed equally to the work.

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.04.008

Collins, 2010). Native to southern Europe, it is largely cultivated in the Mediterranean countries, including Tunisia, where it is used as a common ornamental species as well as for its medicinal and aromatic properties. *Salvia* leaves are recognised as a source of beneficial phenolic compounds and natural potent antioxidants (Lu & Yeap Foo, 2001; Matsingou, Petrakis, Kapsokefalou, & Salifoglou, 2003; Ramos et al., 2010). In folk medicine, sage is used as an herbal tea and for healing wounds, as well as for alleviating stomach, liver, and rheumatic pain (Kelen & Tepe, 2008; Sokovic, Tzakou, Pitarokili, & Couladis, 2002). Moreover, this *Lamiaceae* has interesting pharmacological properties, such as antioxidant, anti-inflammatory, analgesic, antipyretic, homeostatic, hypoglyacemic, and antitumour activities (Fiore et al., 2006). While the phenolic composition of this plant has been already described, few works have focused on the irradiation of ground or powdered sage leaves (Brandstetter, Berthold, Isnardy, Solar, & Elmadfa, 2009; Nagy, Solar, Sontag, & Koenig, 2011; Pérez, Banek, & Croci, 2011) and no studies on the γ-irradiation effect on whole sage leaves have been reported. Thus, in the present study, the effect of ionising radiation of sage leaves on their total phenolic content and antioxidant potential was examined. In addition, parathion-pretreated leaves of this medicinal plant were investigated in comparison to control non-pretreated samples, in order to check the effectiveness of the irradiation treatment on parathion residues.

2. Materials and methods

2.1. Chemicals and reagents

Polyphenolic standards, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], Trolox (6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid), parathion, paraoxon, aminoparathion and *p*-aminophenol were from Sigma–Aldrich (St. Louis, MO). Folin–Ciocalteu reagent (100%), methanol and formic acid were from Fluka (St. Louis, MO). Stock solutions of analytical standards (100 ppm) were prepared in acetone and stored in darkness at −20 °C. The solvents used were of analytical grade. Water was distilled and filtered through a Milli-Q apparatus before use.

2.2. Biological material

Fresh young sage leaves harvested in February–March were kindly provided by the National Institute of Agriculture of Tunisia (INAT). The leaves were homogeneously sampled based on their weight and size with variation not exceeding 10%. The leaves were washed in distilled water for 3 min, dried between Whatman filter papers, and then divided into six batches (5 leaves each batch).

2.3. Pretreatment with parathion and γ irradiation

Three batches were treated by presoaking leaves in a freshly prepared parathion solution diluted in water at recommended concentration (1 ppm) for agricultural uses. The first and second batches were respectively irradiated at 2 and 4 kGy, while the third was non-irradiated and used as a control. The fourth and fifth (irradiated with 2 and 4 kGy, respectively) as well as the sixth (non-irradiated) batches were presoaked in distilled sterile water instead of parathion. Prior to irradiation, all batches were gravitationally drained for 1 h, and then transferred to labelled glass test tubes (one leaf per tube). During the irradiation step, the non-irradiated samples were kept at room temperature (27 ± 2 °C).

The Tunisian gamma irradiation facility (at Sidi Thabet) is designed for the preservation of foodstuff and sterilisation of medical devices. The source consists of eight encapsulated ^{60}Co pencils with a diameter of 9.7 mm and an overall length of 452 mm. The starting activity of the source was 99.162 kCi. The installation is equipped with a stainless steel telescopic source rack that allows obtaining a linear source of approximately 900 mm height. The source pencils are distributed circularly on a diameter of 140 mm for the upper source rack and of 80 mm for a lower one. The source rack comprises 20 housings allowing sources loading for several years. These sources are stored in dry conditions in a cylindrical shield container in which they were transported. *Salvia* leaf samples were exposed to gamma radiation to an overall average range of doses between 0 and 4 kGy at a dose rate of 22.21 Gy/min and at room temperature (27 ± 2 °C).

2.4. Irradiation of parathion and chlorogenic acid standards

Parathion standard preparation (1 ppm) was obtained by diluting stock solutions in water. Chlorogenic acid was prepared in water at the equivalent concentration (540 ppm) of the polyphenolic extract of the sage leaves neither pretreated with parathion nor irradiated (0 kGy). Samples were exposed to gamma radiation at dose levels of 2 and 4 kGy. Non-irradiated (0 kGy) solutions were kept at room temperature and used as a control for comparative analysis. Each experiment was done in triplicate. In order to avoid compounds hydrolysis, the samples were immediately used for analytical investigation.

2.5. Polyphenols extraction and content estimation

One gram of sage leaf was ground in the presence of 10 ml of cold acetone/water (3:1 v/v, kept at −20 °C). The mixture was sonicated for 20 min, and then centrifuged at 4000 rpm for 15 min at room temperature. The supernatant was collected, and acetone was evaporated at 40 °C on a rotary evaporator (Fattouch et al., 2007). To prevent oxidation of the polyphenols, extraction was achieved rapidly and extracts were immediately used or conserved in darkness at −20 °C until further use.

Prior to HPLC analysis, the total phenolic content (TPC) of the *Salvia* leaves extracts was estimated spectrometrically by the Folin–Ciocalteu method, as described by Dhaouadi et al. (2013) with slight modifications. Briefly, 100 μl of diluted sample were added to 400 μl of 1:10 diluted Folin–Ciocalteu reagent. After 5 min, 500 μl of 10% (w/v) sodium carbonate solution were added. Following 1 h of incubation at room temperature, the absorbance at 765 nm was measured in triplicate. TPC was calculated from the equation determined from linear regression after plotting known solutions of gallic acid (10–100 ppm). Results are expressed in mg of gallic acid equivalent (GAE) per gram of fresh weight (fw) of plant material.

2.6. Assessment of antioxidant capacity

The antioxidant activity of the polyphenolic extracts was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical (Najjaa, Zerria, Fattouch, Ammar, & Neffati, 2011). The DPPH scavenging reaction was performed in polypropylene tubes at room temperature. One millilitre of a 4×10^{-5} M methanolic solution of DPPH was added to 25 μl of the sample. The mixture was shaken vigorously and left in the dark at room temperature for 60 min. The absorbance of the resulting solution was measured at 517 nm. Methanol was used as a blank solution, and DPPH solution added to 25 μl of distilled water served as control. The antiradical activity was also assessed using a second functional test based on the ABTS scavenging capacity as described by Dhaouadi et al. (2013). The absorbance of the reactive mixture was measured at 734 nm and compared to the antioxidant potency of Trolox used as a reference. The results were expressed in terms of Trolox