Food Research International 51 (2013) 141–147

Contents lists available at SciVerse ScienceDirect

Food Research International

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

In vitro antioxidant activity of dietary polyamines

Natalia Toro-Funes, Joan Bosch-Fusté, M. Teresa Veciana-Nogués, Maria Izquierdo-Pulido, M. Carmen Vidal-Carou *

Department of Nutrition and Food Science-XaRTA, Campus de l'Alimentació Torribera, Faculty of Pharmacy, University of Barcelona, Av. Prat de la Riba 171, 08921, Santa Coloma de Gramenet, Spain

ARTICLE INFO

Article history:
Received 25 September 2012
Accepted 28 November 2012

Keywords:
Spermine
Spermidine
Antioxidant
Dietary polyamines
Radical scavenging
Metal chelating

ABSTRACT

The polyamines spermidine and spermine are molecules naturally present in food. The effect of polyamines on oxidative stability was studied by means of *in vitro* experimental models. The potential antioxidant activity of polyamines compared to other antioxidants was investigated in both lipophilic and hydrophilic media by the DPPH method. Although antioxidant capacity was observed in both media, it was stronger in the lipophilic matrix. Thus, soybean oil was used as an *in vitro* model to study the concentration-dependent response of polyamines, which showed a strong antioxidant capacity at a wide range of concentrations (from 30 to 1250 μg/mL). The antioxidant activity of polyamines was also studied in comparison with other antioxidants (octyl galate, α-tocopherol and palmitoyl ascorbate) at different points of the oxidation process: formation of peroxides, conjugated compounds, carbonylic and volatile compounds. Both spermidine and spermine were able to delay the oxidation at every step of the process, being as protective as octyl galate and more so than α-tocopherol and palmitoyl ascorbate. Finally, the antioxidant activity of polyamines was studied in extreme oxidation conditions of high temperature, oxygen and the addition of Fe^{2+} and was found to be related to their ability to chelate metals.

1. Introduction

N,N′-bis(3-aminopropyl)-1,4-diaminobutane or spermine (SPM) and N-(3-aminopropyl)-1,4-diaminobutane or spermidine (SPD) are natural polyamines formed during metabolic processes in every living cell, especially in tissues with a high cellular turnover, being essential to cell survival. Thus, polyamines are naturally present in not only tissues and physiological fluids but also in foods at variable concentrations (Nishibori, Fujihara, & Akatuki, 2007). The main source of exogenous polyamines is dietary, from food of both animal and vegetable origin, and human milk. They are rapidly and completely absorbed in the duodenum and first portions of the jejunum. The absorption mechanisms include carriers and paracellular absorption and the compounds undergo intensive metabolization before reaching the systemic circulation (Larqué, Sabater-Molina, & Zamora, 2007).

Polyamines are aliphatic molecules with amine groups distributed along their structure. They occur as free bases in native form but, due to their structure, can also be water-soluble. These compounds can establish hydrogen bridges with hydroxylic solvents such as water or alcohol (Larqué et al., 2007).

Polyamines have many biological functions and it is well known that they are involved in cellular replication and differentiation, metabolism regulation, and stabilization of membranes and nucleic acids. They have also been reported to act as secondary messengers and growth factors, but their mode of action remains a matter of speculation (Larqué et al., 2007; Moinard, Cynober, & de Bandt, 2005).

Among their biological functions, SPM and SPD also have the ability to act as antioxidants not only in biological systems, mainly in their membrane constituents, but also in proteins and nucleic acids. Some authors have described different antioxidant mechanisms for polyamines in biological systems, such as free radical scavenging and/or transition metal chelation (Løvaas, 1997; Das & Misra, 2004; Groppa, Tomaro, & Benavides, 2007). Other mechanisms include the ability to modulate the membrane surface charges, to inhibit enzymes or to mechanically protect the substrate (Løvaas, 1997; Tadolini, 1988; Douki, Bretonniere, & Cadet, 2000). However, the specific chemical mechanism of the antioxidant effects of polyamines is still not established.

The oxidation of lipids plays a central role in the occurrence of numerous chronic diseases, such as atherogenesis, and contributes to the diverse vascular sequelae of diabetes and aging (Fang, Yang, & Wu, 2002). Moreover, it is known that an increased polyamine intake is involved in decreasing several chronic diseases (Bardocz, 1995). Lagishetty and Naik (2008) concluded that the antioxidant effect of polyamines could play a major role in the prevention of inflammatory chronic diseases. Both *in vitro* and *in vivo* studies have demonstrated that polyamines suppress inflammatory mediators, such as pro-inflammatory cytokines (Soda et al., 2005). This potential anti-inflammatory effect has led Soda (2010) to hypothesize that polyamine intake may help with cardiovascular

* Corresponding author. Tel.: +34 934033785; fax: +34 934035931.
E-mail address: mcvidal@ub.edu (M.C. Vidal-Carou).

0963-9969/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2012.11.036

diseases. It has also been reported that an increased polyamine intake may decrease *in vitro* age-associated pathology and promotes longevity (Minois, Carmona-Gutierrez, & Madeo, 2011).

In comparison with other antioxidants, polyamines have been scarcely studied (Løvaas, 1991). Several rapid screening methods can be used for their evaluation, such as the Ferric Reducing Antioxidant Power (FRAP), Total Radical-trapping Antioxidant Parameter (TRAP), Oxygen-Radical Absorbance Capacity (ORAC), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) methods, which all measure radical scavenging capacity (Frankel & Meyer, 2000; Sánchez-Moreno, 2002; Khanna et al., 2011). Each one has its *pros* and *cons* but most of them cover only some parts of the whole oxidation process, so the results obtained may be inconsistent and difficult to compare (Frankel & Meyer, 2000; Sun & Tanumihardjo, 2007; Frankel & Finley, 2008; Badarinath et al., 2010). To avoid these problems, several authors have proposed general guidelines to evaluate antioxidant capacity, advocating the measurement of antioxidant activity at different points of the chain reaction instead of employing a total antioxidant capacity test (Sun & Tanumihardjo, 2007; Huang, Ou, & Prior, 2005; Prior, Wu, & Schaich, 2005). Thus, it is possible to reduce false positives (e.g., measuring a low peroxide value in a reaction that instead of accumulating peroxides, decomposes them quickly in volatile oxidation products) and false negatives (e.g., finding a low radical scavenging capacity in a sample containing an antioxidant that acts mainly by preventing radical formation) (Huang et al., 2005).

Both SPD and SPM have been reported as efficient antioxidants in several experimental biological systems but there is no consensus on the mechanism by which polyamines act as antioxidants. Therefore, the aim of this work was to explore the antioxidant activity of SPM and SPD, in comparison with other well recognized antioxidants, in *in vitro* models of both lipophilic and hydrophilic media. The antioxidant capacity was measured by different tests and at different steps of the lipid oxidation process, as recommended by the general guidelines (Fig. 1) (Frankel & Finley, 2008).

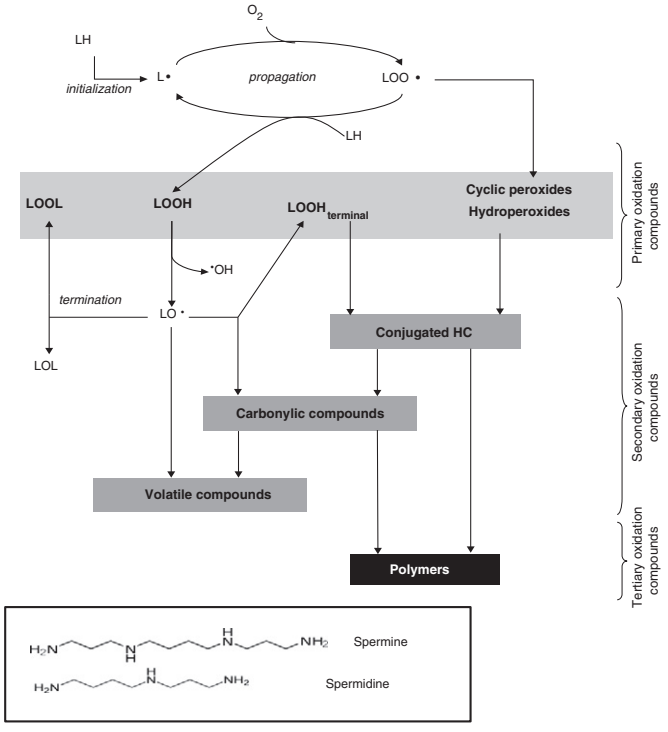

Fig. 1. Simplified scheme of the main steps involved in lipid oxidation process.

2. Materials and methods

2.1. Samples

Soybean oil of European Pharmacopoieia grade was obtained from Roig Farma-Grupo Fagron (Barcelona, Spain). Soybean oil was chosen since it is an easily oxidized substrate due to its high proportion of unsaturated fatty acids (about 58% according to the USDA Nutrient Database for Standard Reference). The oil was stripped of all its natural antioxidants before use, following the method of Kim, Hamhm, and Min (2007). Briefly, the oil was bleached through a glass column packed with three layers: 1) silicic acid, 2) a mixture of activated silicic charcoal and Celite®, and 3) a mixture of powdered sugar and Celite®, kept under nitrogen and protected from light to avoid oxidation.

2.2. Chemicals

Spermine (SPM), spermidine (SPD), (±)-α-tocopherol (AT), 6-O-palmitoyl-L-ascorbic acid (PA), ascorbic acid (AA) and octyl gallate (OG) were obtained from Fluka/Riedel-de Haën (Buchs, Switzerland).

Iron (II) sulfate heptahydrate, barium chloride-2-hydrate and Celite® 577 were obtained from Fluka/Riedel-de Haën (Buchs, Switzerland). Methanol, 1-buthanol, ethyl acetate, trichloromethane, cyclohexane, hydrochloric acid 35%, ammonium thiocyanate, silicic charcoal and powdered sugar were from Panreac (Barcelona, Spain). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97% purity) and silicic acid of 60 mesh (Davisil® grade 635) were purchased from Aldrich (Steinheim, Germany) and 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

2.3. Methods

2.3.1. DPPH radical scavenging activity

Radical scavenging capacity was determined using the DPPH method as described by Odriozola-Serrano, Soliva-Fortuny, and Martín-Belloso (2008). The absorbance of the mixture was measured at 515 nm in a UV–vis spectrophotometer UV-160A (Shimadzu, Kyoto, Japan), the results were expressed as mM of Trolox Equivalents (TE).

2.3.2. Peroxide value determination

A spectrophotometric method based on the ability of peroxides to oxidize ferrous ions to ferric ions was used to determine the peroxide value (PV) (Shantha & Decker, 1994). The absorbance was spectrophotometrically measured at 500 nm and the results were expressed as peroxide values (PV) (mEq of peroxide/kg of sample).

2.3.3. K_{232}, K_{270} and K_{280} specific extinction coefficients

Specific extinction coefficients to estimate the presence of conjugated dienes, trienes and carbonyls were determined by the official EU method EEC/2568/91 for olive oil adapted to soybean oil in our laboratory (European Commision Regulation EEC/2568/91). Briefly, an aliquot of 0.05–0.1 g of the oil was diluted to a final volume of 20 mL with cyclohexane. The absorbance was measured at 232 nm for conjugated dienes (K_{232}), 270 nm for conjugated trienes (K_{270}), and 280 nm for conjugated carbonyls (K_{280}).

2.3.4. TBARS value

The AOCS official method Cd 19–90 was used to measure thiobarbituric acid reactive species (TBARS) (AOCS. American Oil Chemists Society, 2009a). Briefly, an aliquot of 0.05–0.1 g of sample was diluted to 25 mL in 1-butanol. 5 mL of the diluted sample and 5 mL of the 2-thiobarbituric acid solution (200 mg in 100 mL of 1-buthanol) were transferred to a screw-cap test tube, which was placed in a thermostatic bath at 95 °C for 120 min. Finally, the absorbance was