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Antioxidant activity of tomato lipophilic extracts and interactions between carotenoids and α-tocopherol in synthetic mixtures

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ABSTRACT

In the present study we assayed the antioxidant activity of lipophilic extracts obtained from different tomato varieties. The results showed that cherry tomatoes, characterized by a high carotenoid content, had the highest antioxidant activity. A quantitative analysis of lycopene, β-carotene, lutein and α-tocopherol was also performed and the correlation between the antioxidant content and the antioxidant activity was estimated. The highest correlation coefficient was found for lycopene ($R^2 = 0.9236$, $P \leq 0.001$). The analysis of two-component mixtures containing α-tocopherol and carotenoids showed that significant synergism occurred for all the combinations which contained α-tocopherol and β-carotene mixed together. The highest synergistic effects were detected for α-tocopherol-lycopene mixtures, which were the most efficient combinations tested in the present study. The analysis of the carotenoid combinations indicated that synergism occurred for lycopene-β-carotene, lycopene-lutein and lutein-β-carotene mixtures. The analysis of four-component mixtures did not show statistically significant synergistic effects.

1. Introduction

The great interest in studying antioxidant compounds is due to their ability to neutralize active oxygen species and free radicals that play an important role in the pathogenesis of such degenerative diseases. A large number of epidemiological and clinical studies have associated a lower incidence of some cancer types (Riboli & Norat, 2003; Steinmetz & Potter, 1996), cardiovascular diseases (Joshipura et al., 2001; Ness & Powles, 1997) etc with a high antioxidant dietary intake. The positive correlation between vegetable intake and cancer prevention has been attributed to the presence of antioxidant compounds (Machlin, 1995; Ziegler, 1991).

Many studies have shown the beneficial effects produced by carotenoids and α-tocopherol on human health. Their biological roles have been described in many papers. The role of different carotenoids in the prevention of degenerative diseases has been attributed to their antioxidant properties (Conn, Schlach, & Truscott, 1991; Di Mascio, Kaiser, & Sies, 1989; Landrum & Bone, 2001; Rao & Agarwal, 1999; Sundquist, Briviba, & Sies, 1994). Other studies have described α-tocopherol (vitamin E) as one of the most important lipid-soluble radical scavenging antioxidant in membranes and in plasma (Burton, Joyce, & Ingold, 1983).

Tomatoes are important dietary sources of carotenoids, especially lycopene. The lipophilic fractions of these vegetables are a mixture of components including lycopene and other minor compounds such as β-carotene, lutein and α-tocopherol (Abushita, Daood, & Biacs, 2000;Abushita, Hebshi, Daood, & Biacs, 1997; Leonardi et al., 2000). Tomatoes are considered functional foods because of their high contents of physiologically active compounds.

The antioxidant properties of single carotenoids and α-tocopherol were tested in many studies by using different approaches. However, limited information is available on the antioxidant properties of these compounds when they are combined in synthetic mixtures and contradictory data have been presented about synergistic effects. Carotenoid mixtures were shown to be more effective than the individual compounds in TBARS assay and the synergistic effects were particularly evident for mixtures containing lycopene or lutein (Stahl et al., 1998). Synergistic effects were also observed for lycopene-α-tocopherol combinations by using different experimental approaches such as LAME and AVMN model systems (Shi et al., 2007) or the inhibition of the LDL oxidation (Fuhrman, Volkova, Rosenblat, & Aviram, 2000). Other authors tested the scavenging activity of lycopene-α-tocopherol-β-carotene mixtures on the DPPH free radical and observed synergistic effects for lycopene-β-carotene and lycopene-α-tocopherol mixtures (Liu, Shi, Colina Ibarra, Kakuda, & Jun Xue, 2008). No synergism was observed for mixtures containing the same compounds when the percent inhibition of spontaneous oxidation

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in rat brain homogenate was used as model (Castro, Moraes Barros, Lanfer Marquez, Montizuki, & Higashi Sawada, 2005).

Several studies on the antioxidant properties of the single compounds have been performed by using different experimental models. A recent review summarized the complex aspects of antioxidant reactions and analyzed the chemical principles of antioxidant capacity assays (Huang, Ou, & Prior, 2005). The mechanisms implicated in the interactions among antioxidant compounds have not been completely clarified. It seemed that the type of compounds, the concentration and the ratio at which they are mixed are important variables in defining the antioxidant capacity. A mechanism has been proposed to explain the interactions between carotenoids and α-tocopherol (Böhm, Edge, Land, McGarvery, & Truscott, 1997). In this case the synergistic effects have been shown as a consequence of the transfer of electrons from the carotenoid to the α-tocopherolxyl radical to regenerate α-tocopherol. Similar considerations have been suggested to explain the interactions between vitamin C, carotenoids and α-tocopherol (Böhm et al., 1997; Niki, Noguchi, Tsuchihashi, & Gotoh, 1995).

We needed to investigate the interactions between carotenoids and α-tocopherol because only few and contradictory data are available. Moreover, the need to study and to clarify these effects is strongly associated with the emerging use of nutritional supplements which contain mixtures of two or more antioxidant compounds. The aims of the present work were (1) to evaluate the antioxidant activity of tomato lipophilic extracts and to establish a correlation coefficient between the antioxidant capacity and the content of single compounds such as lycopene, β-carotene, lutein and α-tocopherol; (2) to assay the antioxidant activity of a synthetic mixture which simulated the tomato lipophilic composition; (3) to test synthetic mixtures in which the compounds were mixed at different concentration levels, with the aim to verify if synergistic effects occurred. To measure the antioxidant activity, we used the ABTS assay because no papers are available on synergistic interactions between carotenoids and α-tocopherol by using this experimental approach.

2. Material and methods

2.1. Reagents and standards

All solvents used were of HPLC grade from BHD (Poole, England). β-carotene and lycopene standards were produced by Sigma (St. Louis, USA); D-α-tocopherol and lutein standards were from ICN Biochemical Inc. (Ohio, USA). Ammonium persulfate was from Merk & Co. Inc. (Darmstadt, Germany) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) (ABTS) in the diammonium salt form was produced by Fluka Chemie (Buchs, Switzerland). Trolox (6 hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) was from Hoffman La Roche Aldrich Chem. Co. (Saint Louis, MO, USA).

2.2. Tomato sampling and sample preparation

The samples analyzed in the present study were from three different tomato varieties: cluster type (cv. Ikram F1), cherry type (cv. Naomi F1) and salad type (cv. Eroe F1). Naomi is a cherry type tomato with small and red skin fruits (approximately 8–12 g in weight); Ikram is a tomato variety with fruits larger than a cherry tomato, normally commercialized at full ripeness (approximately 80–100 g in weight); Eroe is a salad variety with fruits of medium-large size (8–10 cm diameter range and 150–200 g in weight). Tomatoes were purchased in a local supermarket at commercial maturity. On the day of purchase, the samples were homogenized (IKA Labortechnik, model T25 basic) and lyophilized for 24 h (Freeze Dryer Modulyo, Edwards equipped with a Motors BS 5000–11 pump, Edwards, England). The lyophilized samples were then stored at −20 °C until their analysis.

2.3. Quantitative analysis of tomato lipophilic extracts

2.3.1. Carotenoids extraction

Carotenoids were extracted using a procedure previously published (Setiawan, Sulaeman, Giraud, & Driskell, 2001) with small variations. A sample of 275 mg of homogenized freeze-dried tomatoes was extracted using 10 ml THF in presence of 0.01% butylated hydroxytoluene (BHT) and then centrifuged at 3000 rpm for 10 min. This extraction was performed twice until the pellet became colourless. The organic fractions were collected and evaporated to dryness under nitrogen. The residue was dissolved in 3 ml of chloroform and appropriately diluted with the mobile phase mixture (methanol: acetonitrile: dichloromethane 50:48:2); 1 ml was filtered (0.45 μm Minisart SRP 4 filter, Sartorius, Germany) and analyzed by using HPLC. Three replications were carried out to examine each sample.

2.3.2. -α-tocopherol extraction

α-tocopherol was extracted using base hydrolysis of 275 mg lyophilized tomatoes followed by the addition of 10 ml of $C_2H_4Cl_2$ (Raffo, La Malfa, Fogliano, Maiani, & Quaglia, 2006). The organic fractions were collected, evaporated to dryness under nitrogen and the remaining residue was resuspended in methanol and diluted with the mobile phase mixture (methanol:acetonitrile:dichloromethane 50:48:2). The HPLC analysis was performed to quantify α-tocopherol contents in tomato extracts. For each sample, three replications were carried out.

2.3.3. HPLC analyses

LC 410 Series Perkin-Elmer apparatus (Norwalk, Connecticut, USA) equipped with a UV/VIS LC295 Perkin-Elmer detector and with 1022 Plus integrator Perkin-Elmer was used. The column was a reversed-phase LiChrospher 100 RP 18 (5 μm, 125 × 4.6 mm) Merck. Elution was carried out using a mixture of methanol:acetonitrile:dichloromethane (50:48:2) at a flow rate of 1.0 ml/min and the run time was 35 min (Saleh & Tan, 1991). UV–VIS detector was set at 290 nm for the simultaneous detection of all investigated compounds. The quantitative analysis of lycopene, β-carotene, lutein and α-tocopherol was based on an external standard method.

2.4. Determination of antioxidant activity

2.4.1. Tomato lipophilic extracts

The extraction was carried out using a method described in a previous work (Raffo et al., 2006). A sample of 275 mg of homogenized freeze-dried tomatoes was extracted with 10 ml of CH_2Cl_2 and then centrifuged at 3000 rpm for 10 min. The extraction was performed twice and the supernatant fractions were collected and evaporated to dryness under nitrogen. The residue was dissolved in 3 ml of CH_2Cl_2 and analyzed.

The antioxidant activity was measured using ABTS radical cation ($ABTS^{\cdot+}$) decolorization assay (Pellegrini, Re, Yang, & Rice-Evans, 1999). In brief, 1 ml of the $ABTS^{\cdot+}$ solution was added to different volumes of the lipophilic extract (20, 40 or 60 μl) and diluted at a final volume of 2 ml using ethanol. The solution was vortexed for 10 s and the decolourization produced by the presence of antioxidants was measured at 751 nm (UV/Visible Lambda 2 spectrophotometer, Perkin-Elmer, Norwalk, Connecticut, USA), 10 min after initial mixing. Trolox was used to prepare the standard curve and