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Biochemical relationships and browning index for assessing the storage suitability of artichoke genotypes

Maria Cefola [1], Isabella D'Antuono [1], Bernardo Pace [1], Nicola Calabrese, Antonia Carito, Vito Linsalata, Angela Cardinali *

Institute of Sciences of Food Production, CNR — National Research Council of Italy Via G. Amendola, 122/O, 70126 Bari, Italy

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ABSTRACT

Selection of artichoke cultivars with specific physical, physiological and biochemical characteristics is required by processors and retailers in order to commercialize artichokes as fresh or fresh-cut products. In this work six artichoke cultivars were evaluated for their cold storage suitability by following the variations in their main physical (browning index), physiological (respiration rate) and biochemical (antioxidant activity, total phenol content, polyphenol oxidase and peroxidase activity) parameters at 1 °C for 12 days. In addition, linear regressions among antioxidant activity, total phenols and enzymatic activities were assessed with the aim of selecting the most suitable cultivar for storage and processing. The low values for the physical, physiological and biochemical parameters measured in Romolo led us to indicate this cultivar as the best for storage. On the other hand, it may be preferable to designate cultivars, with higher values for the same parameters for fresh consumption, partly due to their high antioxidant content.

1. Introduction

Artichoke (*Cynara cardunculus* (L.) subsp. *scolymus* Hayek) is an important component of the Mediterranean diet, its annual per capita consumption is about 8.0 kg in Italy (leading producer and consumer) while, for other Mediterranean countries, South and North America, the values of consumption are lower (Bianco & Calabrese, 2009).

Nutritional and pharmaceutical properties of artichoke heads are linked to their special chemical composition, which includes high levels of polyphenolic compounds and inulin. Caffeic acid derivatives are the main phenolic compounds in artichoke heads and leaves, with a wide range of caffeoylquinic acid derivatives with 5-O-caffeoylquinic acid (chlorogenic acid) as the most important of these derivatives. Other phenolics such as the flavonoids apigenin and luteolin (both present as glucosides and rutinosides) as well as different cyanidin caffeoylglucoside derivatives have been identified in artichoke tissues (Fratianni, Tucci, De Palma, Pepe, & Nazzaro, 2007; Lattanzio, Cardinali, Di Venere, Linsalata, & Palmieri, 1994; Llorach, Espin, Tomas-Barneran, & Ferreres, 2002; Orlovskaya, Luneva, & Chelombit'ko, 2007). In addition, artichoke has shown to have *in vitro* hepatoprotective, anticarcinogenic, antioxidative, antibacterial, anti-HIV, bile-expelling, and diuretic properties as well as the ability to inhibit cholesterol biosynthesis and low density lipoprotein (LDL) oxidation (Lattanzio, Kroon, Linsalata, & Cardinali, 2009).

In order to select cultivars for processing as fresh-cut products, the genotype is the first and one of the most important pre-harvest factors to be evaluated. Since cultivars differ in their genetic make-up, the fresh product varies in quality parameters such as size, color, flavor, texture, nutrition, pest resistance, processing ability, eating quality and yield (Beverly, Latimer, & Smittle, 1993). In particular, artichoke heads, should have some quality traits (fullness, safety, freshness, cleanness) defined by the European legislation (United Nations Economic Commission, 2010). Generally, cultivars characterized by physiological and biochemical parameters which improve storage are much sought after by processors and retailers in order to commercialize artichoke as a fresh or fresh-cut product. In fact, several authors have already focused their research on the screening of artichoke cultivars that are more suitable for processing (Bonasia, Conversa, Lazzizera, Gambacorta, & Elia, 2010; Cabezas-Serrano, Amodio, Cornacchia, Rinaldi, & Colelli, 2009).

Among the physiological and biochemical parameters which can affect the cultivar's suitability for storage and processing, the most significant are the respiration rate and the activity of the key enzymes involved in the browning and senescence processes. Kader (2002) classified artichoke as a vegetable with a very high respiration rate and a relatively high perishability in cold conditions; since its visual and sensory qualities deteriorate rapidly, and its shelf-life is limited. One of the main problems related to artichoke storage is the high

Abbreviations: PPO, polyphenol oxidase; POD, peroxidase; H_2O_2, hydrogen peroxide; fw, fresh weight; DPPH, 2,2-diphenyl-1-picrylhydrazyl; MeOH, methanol; AcOH, glacial acetic acid; EtOH, ethanol; PVP, polyvinylpyrrolidone; HPLC, high-performance liquid chromatography; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; VP, Violet du Provence; LDPE, low density polyethylene; BI, browning index; SNK, Student–Newman–Keuls.

* Corresponding author at: ISPA-CNR, Via G. Amendola, 122/O, 70126 Bari, Italy. Tel.: +39 080 5929303; fax: +39 080 5929374.
E-mail address: angela.cardinali@ispa.cnr.it (A. Cardinali).

[1] These authors contributed equally to this work.

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browning rate of the receptacle and bracts caused by oxidation of phenolics catalyzed by PPO enzymes, with subsequent formation of dark compounds (Cabezas-Serrano et al., 2009; Lattanzio et al., 1994; Tomás-Barberán & Espin, 2001). This phenomenon regularly restricted the shelf life of fresh artichokes, mainly after cutting, since it provokes a loss in visual quality undesirable by consumers. Generally, to determine the change in vegetable visual quality, color measurement might be used by food industry as an objective method, quite faster and simpler than chemical analysis. In artichoke processing, in which enzymatic and non-enzymatic browning might take place, browning index (BI) represents the real brown color and it could be considered an important technological parameter. This index was already used by different authors to measure browning development in different fruits and vegetables (Bal, Kar, Santosh, & Naik, 2011; Maskan, 2000).

The main enzymes responsible of the browning in artichokes are PPO and POD. The first, mainly located in plastids (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994), catalyzes the formation of *o*-quinones with two different reactions: the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones (Sanchez-Ferrer, Rodriguez-Lopez, & Garcia-Carmona, 1995). POD, instead, performs single electron oxidation on a wide variety of compounds in the presence of hydrogen peroxide (Dunford & Stillman, 1976). Although PODs are widely distributed in plants, their role in the enzymatic browning of foods and vegetables is still under discussion, because the internal level of hydrogen peroxide in plants limits POD activity. However, their involvement in slow processes such as internal browning is possible because, during the oxidation of phenolic compounds in PPO-catalyzed reactions, a hydrogen peroxide generation can be occurred (Richard-Forget & Gauillard, 1997). All considering, the main agent responsible for enzymatic browning in fruits and vegetables is PPO, although a possible synergistic effect between PPO and POD cannot be excluded (Nicolas et al., 1994; Tomás-Barberán & Espin, 2001). Many authors have already addressed the problem of PPO inhibition by removing one or more components necessary for its reaction (i.e. O_2, enzyme, Cu^{2+} contained on its active site, or substrate) (Lambrecht, 1995; Richardson & Hyslop, 1985), or by mechanical or chemical methods (Garcia & Barrett, 2002), or delaying the post-cutting browning of fresh-cut artichokes, or comparing the effectiveness of a number of anti-browning agents (Amodio, Cabezas-Serrano, Peri, & Colelli, 2011).

On the basis of these findings, in this work we studied the susceptibility to browning of six artichoke cultivars during cold storage, by measuring their browning index (BI), as a technological parameter. In addition to evaluate the suitability of these cultivars for storage and processing, the BI was related to the main quality parameters responsible for browning: PPO, POD, polyphenol content, antioxidant activity, and respiration rate. Finally, a linear regression among antioxidant compounds, phenols and enzymatic activities was assessed with the aim of finding a cultivar with specific characteristics that might make it more suitable for storage and processing.

2. Materials and methods

2.1. Plant material, processing and storage conditions

Artichoke heads (*C. cardunculus* (L.) subsp. *scolymus* Hayek) of six cultivars (five hybrids propagated by seeds, Concerto, Harmony, Opal, Romolo, Symphony and one vegetative propagated, Violet du Provence or VP), were used.

All cultivars were grown in the experimental farm of the Institute of Sciences of Food Production (E. Pantanelli) located in Policoro, southern Italy, harvested at optimal stage for fresh consumption (defined by the compactness of fully developed buds), and transported to the post-harvest laboratory. They were selected to remove damaged samples and processed on the same day by cutting the stem at 5 cm in length. For each nine packages (three replications × three storage duration, at harvest and after 6 and 12 days) were prepared by placing four artichoke heads in open low density polyethylene (LDPE) bags with high permeability. All samples were stored for up to 12 days in a cold room at 1 °C (±0.5). At harvest and at each storage periods, quality attributes were determined as described below.

2.2. Reagents and standards

- Extraction and chromatography solvents, MeOH, AcOH, EtOH, were of certified HPLC grade and pure standard of chlorogenic acid was obtained from Sigma-Aldrich (St. Louis, MO, USA) and apigenin-7-O-glucoside from PhytoLab GmbH & Co., KG (Vestenbergsgreuth Germany).
- For the chromatographic analyses HPLC-grade water was prepared using a Milli-Q system. DPPH radical and Trolox used for the antioxidant, were purchased from Sigma-Aldrich (St. Louis, MO, USA), Folin–Ciocalteu's phenol reagent was purchased from Merck (Germany). PVP and all reagents utilized for enzyme extraction were supplied by Sigma or Aldrich (St. Louis, MO, USA).

2.3. Respiration rate

The respiration rate of each artichoke cultivar was measured after 24 h from harvest, when the inside product temperature was 1 °C (±0.5), and after 6 and 12 days of cold storage, using a closed system (Kader, 2002). Fresh produce (about 300 g) for each artichoke cultivar, was put into 6 L sealed plastic-jars, where carbon dioxide was allowed to accumulate until the value of a standard gas mixture containing carbon dioxide and nitrogen (0.1%–99.9% Sapio, MI, Italy). Then, a 1 mL gas sample was taken from the head space through a rubber septum and injected into the gas chromatograph (p200 micro GC Agilent, Santa Clara, CA, USA) equipped with dual columns and thermal conductivity detector. Carbon dioxide was analyzed with a retention time of 16 s and total run time of 120 s on a 10 m PPU column at a constant temperature of 70 °C.

2.4. Color analysis and browning index

Twelve, for each cultivar, were used for the color analysis. At harvest and at each storage periods, each artichoke head was longitudinally cut and color parameters (L*, a* and b*) were immediately measured on 3 different points on the cut surface of the receptacle for a total of 36 measures for each cultivar. A colorimeter (CR-400, Konica Minolta, Osaka, Japan) equipped with the D65 illuminant, in the reflectance mode and in the CIE L* a* b* color scale was used. The colorimeter was calibrated with a standard reference having values of L*, a* and b* corresponding to 97.55, 1.32 and 1.41, respectively. The color parameters were used to calculate the BI using the following formula (Eqs. (1) and (2)), where a_0^*, is the initial color measurement of raw artichokes and L_t^*, $a_t^*$and b_t^* are the color measurements at each storage period (Bal et al., 2011).

$$BI = \frac{100(x-0.31)}{0.17} \tag{1}$$

$$x = \frac{a_t^* + 1.75L_t^*}{5.645L_t^* + a_0^* - 3.012b_t^*}. \tag{2}$$

2.5. Antioxidant activity and total phenol content

The following extraction procedure was used for both antioxidant activity and total phenol determinations. Five grams of artichoke head for each replication were homogenized in a methanol:water solution (80:20) for 1 min, and then centrifuged at 5 °C at 6440 × *g* for 5 min.