Antioxidant effects of supercritical fluid garlic extracts in canned artichokes

E. Bravi¹ · O. Marconi¹ · V. Sileoni¹ · M. R. Rollo¹ · G. Perretti¹

Revised: 22 September 2016 / Accepted: 26 September 2016 / Published online: 20 October 2016
© Association of Food Scientists & Technologists (India) 2016

Abstract The effects of adding supercritical carbon dioxide extracts of garlic (at two different concentrations of allicin) on select chemical indices in extra-virgin olive oil used to canned artichokes were studied. Tests were performed after processing and over a storage period of 1 year. A sensorial test was also conducted on the canned artichokes to establish the impact on flavor (in particular perceptions of rancidity and garlic flavor). Acidity, peroxide levels and *p*-anisidine values were measured as quality analytical parameters. Radical scavenging activity was also evaluated using the DPPH assay. The samples containing supercritical garlic extracts were compared with several other formulations, including control sample (prepared by mixing artichokes with powdered chili pepper and fresh garlic), artichokes with only garlic or only chili pepper, and artichokes treated with the synthetic antioxidant BHT. The results suggested that the allicin extract may be superior, or at least comparable, with BHT in preserving canned artichokes as demonstrated by its positive effects on oxidative stability and sensory profile.

Keywords Antioxidants · Canned artichokes · Extra-virgin olive oil · Garlic · Radical scavenging activity · Supercritical fluid extraction

Introduction

Foods are subject to microbiological, enzymatic and chemical degradation processes over time. It follows then that most food storage processes are based on the inhibition of microorganism growth, the inactivation of enzymes, or on the control of chemical reactions (Caponio et al. 2003).

For the preservation of vegetables, oil is often used as covering liquid medium (such as in-oil artichokes, dried tomatoes, eggplants, mushrooms, etc.). The preserving effect of oil consists of separating the product from the air (i.e., obtaining <2 % oxygen in the final product) rather than its bacteriostatic or bactericidal action. Additionally vegetables may be blanched which reduces the number of contaminating microorganisms, inactivates enzymes, removes trapped air and modifies texture while mostly preserving color, flavor and nutritional value.

The quality of in-oil canned vegetables depends on the interactions between the traits of the vegetables and those of the covering oils. Spices and aromatic herbs may also be added to the oil, which not only contribute flavor but also containing compounds with documented antimicrobial, antioxidant and anti-inflammatory activities and can benefit the quality of the canned product (Baiano et al. 2005a; Bravi et al. 2015). In particular the antioxidant capacity of aromatic herbs and spices has been well-documented (Hinneburg et al. 2006).

During processing and storage both the vegetables and oils can undergo modifications because of mechanical, thermal, hydrolytic and oxidative degradation, affecting the quality of the oil and preserved food (Choe and Min 2006; Ragaert et al. 2007). Several studies have investigated the oxidative and hydrolytic reactions that occur in the covering oil during storage, and the influence of different oils as a covering medium on vegetable preservation (Baiano

✉ E. Bravi
e_bravi@yahoo.it

¹ Department of Agricultural, Food and Environmental Sciences, University of Perugia, Via S. Costanzo, n.c.n., 06126 Perugia, Italy

et al. 2005a, b, c; Bravi et al. 2015). Apart from oils, herbs, spices, other plant materials, and their extracts are of increasing interest in the food industry because their antioxidant compounds help to retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of preserved foods (Hinneburg et al. 2006; Nedyalka et al. 2006). Alternatively, synthetic antioxidants have been employed to protect oily foods, however they have facing friction towards its utilization and consumption (IARC 1986; E.U.-D.G. Environment 2007; U.S. National Library of Medicine 2010).

To date numerous studies have examined the antioxidant effects of spices and aromatic herbs added to oils. For example, Bandoniene et al. (2000) studied the antioxidant activity of acetone extracts of sage, sweet grass, sea buckthorn, costmary, Roman chamomile, and tansy on rapeseed oil. Bhale et al. (2007) tested the capabilities of methanol extracts from oregano and rosemary to prevent oxidation of long-chain polyunsaturated fatty acids in *Brevoortia tyrannus* oil. Kamkar et al. (2010) evaluated the antioxidant activities of the essential oil of *Mentha pulegium* L. as well as its methanol and water extracts in sunflower oil. Baiano et al. (2009) studied the chemical characteristics, phenolic content and antioxidant activity of olive oils flavored with garlic, lemon, oregano, hot pepper, and rosemary during 9 months of storage. Despite significant role of herbs and spices in food preservation, only a few studies have investigated the effects of oils in combination with herbs and spices in canned food (Baiano et al. 2005a, b, c; Bravi et al. 2015).

One popular spice, garlic (*Allium sativum* L.), is well known for its antioxidant capabilities (Nuutila et al. 2003; Bravi et al. 2015). Garlic's antioxidant properties have been attributed to a variety of sulphur-containing compounds (thiosulfinates) and their precursors (Chung 2006; Okada et al. 2005) and in vivo tests have indicated that allicin (the most abundant thiosulfinate in garlic) is one of the primary antioxidant compounds when used at moderate concentrations (Gazzani et al. 1998; Bakir et al. 2015). Beyond flavoring and preserving food, garlic has also been used as a nutraceutical or phytopharmaceutical for the prevention and treatment of various human diseases, including cancer (Banerjee et al. 2003; Del Valle et al. 2008).

Garlic extracts, thanks to their antimicrobial activity and antioxidant potential, can extend the shelf life of unprocessed or processed foods by reducing the microbial growth rate or viability and are particularly useful for food preservation applications. However different extraction techniques lead to different extract compositions and effects because of their yield and capacity for selectivity. Supercritical fluids have been shown to be efficient solvents with better transport properties (diffusivity, mass transfer coefficient, penetration ability) than many liquid

organic solvents (Brunner 2005). In particular, carbon dioxide (CO₂) shows high selectivity for valuable micro-constituents in natural products and a complete separation of solvent traces from the extract and treated matrix can be achieved (Del Valle et al. 2008). Furthermore, CO₂ is non-flammable, relatively non-toxic, and relatively inert (King et al. 1989; Brunner 2005). Thus, supercritical CO₂ (SC-CO₂) produces a garlic extract free of solvent residues that is suitable for use as a natural antioxidant in food stabilization (Del Valle et al. 2008). A further advantage to the quality of extracts produced from oxidation-prone substances (such as allicin) is that they are exposed to neither oxygen nor high temperatures during extraction with SC-CO₂ (Brunner 2005).

In the current study, the effectiveness of SC-CO₂ garlic extracts (at two different concentrations of allicin) were assessed in stabilizing artichokes canned in extra-virgin olive oil (EVO) used as covering oil during a shelf life test. After storage, the quality of the covering oil was studied to verify the effect of the garlic extracts during processing and storage. Hydrolytic and oxidative quality parameters were measured and a sensory analysis was performed.

Materials and methods

HPLC-grade n-hexane, 2-propanol, perbenzoic acid ($\geq 77\%$), and allyl disulfide ($\geq 80\%$) were purchased from Sigma-Aldrich (Milan, Italy). All other reagents were of analytical grade. Commercial EVO and artichokes were provided by Vizzino “Orto Buono” (Minervino di Lecce, Italy).

Artichokes samples preparation

In-oil canned artichokes were prepared with EVO as a covering oil, and were provided by Vizzino “Orto Buono”. All the samples were produced on the same day and were analyzed immediately after pasteurization (T0) or after 3, 6, 9, or 12 months (T1, T2, T3, and T4 respectively) of storage at room temperature.

HPLC–DAD analysis

Allicin content in the supercritical fluid garlic oil extracts was determined by HPLC coupled with a diode array detector (DAD). The following equipment was used for the HPLC analysis: a Jasco Inc (Easton, MD, USA) Pu-2089 plus pump, a Rheodyne 7725 injector, equipped with a 20 μ l injection loop, an Agilent 1100 DAD and an Agilent 1100 thermostat for HPLC columns. The system was managed by an Agilent Chem-Station for LC 3D System (Agilent Technology, Santa Clara, CA, USA).

The chromatographic separation was achieved at 20 °C using a Waters μ Porasil column (3.9 mm ID $\times$ 300 mm, 10 μ m; Waters Corporation, Milford, MA, USA). All procedures were carried out according to the methods of Psomiadou and Tsimidou (1998) modified by using an isocratic separation with n-hexane/2-propanol (99:1, v/v) as the eluent. The flow rate was set at 1.2 mL/min. The three wavelengths for the determination of allicin content were 210, 254, and 324 nm.

Allicin was synthesized as a standard for the set-up of the HPLC–DAD method. The synthesis of allicin was based on a previous study (Bocchini et al. 2001). Twenty ml of a 0.1 M solution of perbenzoic acid in dichloromethane was slowly added to 100 ml of a 0.1 M solution of allyl disulfide in dichloromethane under rapid magnetic agitation and cooled to -10 °C. The reaction mixture was allowed to stand at room temperature for 1 h. Excess acid was removed by washing the mixture with a sodium bicarbonate solution. The dichloromethane solution was rinsed with distilled water, dried over sodium sulfate, and the solvent was removed by rotary-evaporation. The dried product was weighed, and standard solutions in diethylether were prepared. Analysis by HPLC–DAD of the solution showed that allicin accounted for >70 % of the standard.

Preparation of EVO enriched with a supercritical fluid extract of garlic

EVO was enriched with a SC-CO₂ garlic extract to be used as a covering oil. The garlic extraction was performed with SC-CO₂ at a pressure of 24 MPa, separator temperature of 307 K, and extractor temperature of 309 K in a Muller Extract Company GmbH (Koburg, Germany) pilot plant. Approximately 100 g of garlic was put in the 500-mL extractor vessel. The SC-CO₂ flow-rate was 170 g/min. The extraction time was 180 min. Collection was performed in 500 mL of EVO, loaded in the separator before the extraction, at atmospheric pressure.

The garlic-enriched EVO was diluted with diethyl ether, and the relative concentrations of allicin were verified by HPLC–DAD.

Preparation of in-oil canned artichokes

Cleaned artichokes (25 kg) were blanched for 10 min in boiling water with citric acid to prevent discoloring, then marinated with a common marinade (1:1 water: white wine vinegar with 1 g/L citric and ascorbic acids) for 12 h. After marination the artichokes were drained, washed with water, centrifuged and cut into quarters. The artichokes were canned in EVO following a recipe that included the addition of chili pepper (0.245 g/kg of total product; moisture

13 %) and fresh garlic (0.272 g/kg of total product; moisture 70 %). Seven variations of the recipe were used to produce the different study samples (Table 1). Each sample was prepared by mixing artichokes with different ingredients, depending on the formulation, and a dose of 280 g was put in transparent glass vessels that were then filled in EVO (2:1 vegetables: EVO, w/w) and hermetically sealed with metal caps. The BHT concentration was chosen at its maximum legal limit (0.02 %, BHT) (Iqbal and Bhanger 2007).

All the canned artichokes samples were pasteurized at 97 °C for 10 min, and then quickly cooled to room temperature. After separation from the vegetable matrix, the covering oil samples were filtered with anhydrous sodium sulfate and analyzed after different storage times. Storage study was carried out for 12 months in the dark at room temperature (10–30 °C).

Chemical analyses

The ongoing hydrolysis and oxidation of the covering oils were monitored by measurements of the acidity, expressed as g of oleic acid per 100 g of oil (E.U. Reg. 1348/2013), peroxide values, expressed as milliequivalents (mEq) of active oxygen per kg of oil (E.U. Reg. 1348/2013), and *p*-anisidine values (AOCS 2013).

Radical trapping potential was determined by the DPPH (2,2-diphenylpicrylhydrazyl) assay. The DPPH assay is one of the most popular methods used to evaluate the antioxidant capacity of foods (Pyrzyska and Pekal 2013). The scavenging ability of the DPPH radical was measured by the method described by Nencini et al. (2007), slightly modified. Briefly, 1000 μ L of 10^{-4} M DPPH methanol solution was added to 100 μ L of oil sample diluted in hexane/diethyl ether (50/50; v/v). Each mixture was then shaken and kept for 60 min in the dark at room temperature. The decrease in absorbance at 515 nm was evaluated using a spectrophotometer. Each test was carried out in triplicate.

Sensory evaluation

Sensory evaluation was conducted to evaluate the palatability of three different formulations of canned artichokes (CTRL, A1, and A2). Nine trained panelists (7 women and 2 men between the ages of 25–55) evaluated the canned vegetables using the quantitative descriptive analysis technique reported in Bravi et al. (2015). The panelists were trained over 10 sessions, using standard food products, to identify and determine descriptors relating to taste and texture.

The canned artichokes were tested at room temperature. The samples were evaluated for sensory attributes

Table 1 Seven different formulations of study samples

Formulation	Artichokes	EVO oil	Chili pepper	Garlic	Allicin	BHT
Study samples						
CTRL	✓	✓	✓	✓		
WS	✓	✓				
OG	✓	✓		✓		
OCP	✓	✓	✓			
A1	✓	✓			✓	
A2	✓	✓			✓	
BHT	✓	✓				✓

CTRL control sample, WS sample without spices, OG sample with only garlic, OCP sample with only chili pepper, A1 sample with 0.007 % of allicin, A2 sample with 0.017 % of allicin, BHT sample with 0.02 % of BHT

including flavor: (1) rancid, sour milk, fatty, oxidized, having a rank, unpleasant taste or smell characteristic of oil and fats when no longer fresh; (2) garlic, of crushed garlic cloves and (3) artichokes, smell and taste typical of artichokes and texture: (1) hardness and (2) chewiness. A bipolar numerical 10-point scale (from 0 to 9) was used for hardness (0 = extremely soft to 9 = extremely hard), whereas a unipolar numerical 10-point scale (from 0 to 9) was used for chewiness (0 = none to 9 = extremely gummy) and the flavors rancid, garlic, and artichoke (0 = none to 9 = extremely strong).

Statistical analysis

All chemical analyses were performed in triplicate and the data were analyzed using MATLAB Statistics Toolbox (version 7.6.0, The Mathworks Inc., Natick, USA) to perform the appropriate statistical tests (one-way and two-ways ANOVA). Values were considered significantly different at $P < 0.05$.

Results and discussion

Quality parameters

Three quality parameters (acidity, peroxide values, and *p*-anisidine values) were evaluated in the seven canned artichokes formulations. Acidity, which is a measure of free fatty acid content, is an important tool for the assessment of oil degradation because it estimates the extent of hydrolysis a sample has undergone. In the current study acidity increased in all samples during storage (Table 2). The samples flavored with garlic and/or chili pepper (CTRL, OG, OCP) displayed the highest values, statistically significant at the end of shelf-life trial, indicating greater hydrolytic degradation. These differences could be due to the presence of the spices,

which may have affected the composition of the canned artichokes (e.g. introducing water and hydrolytic enzymes). Despite being in contact with a moist vegetable matrix, acidity values even after 12 months of storage were still under the maximum limit for extra-virgin olive oil (EU Reg. 61/2011).

The analyses of peroxide values in the samples are shown in Table 2. Peroxide content decreased during storage in all the formulations. This observation may be explained by the breakdown of hydroperoxides to secondary products of oxidation responsible for rancid off-flavors. At the beginning of the shelf-life trial (T0) the samples flavored with garlic and/or chili pepper as well as the sample without spices (CTRL, OG, OCP, WS) showed the highest peroxide values (14.53, 14.75, 14.71, and 14.25 mEq O₂/Kg of oil, respectively). Nonetheless, all values were lower than the European legal limits set for extra-virgin olive oil (EU Reg. 61/2011). It may be that the garlic extracts and the BHT protected the samples A1, A2 and BHT already during the processing, resulting in a less peroxide formation. During storage, the highest statistical significant values of peroxides were observed in WS (13.16 mEq O₂/Kg of oil at T1, 10.33 at T2, 8.14 at T3 and 6.60 at T4), whereas the lowest statistical significant values were found in A2 (8.61 at T1, 5.81 at T2, 5.57 at T3, and 3.90 at T4). The CTRL, BHT and A1 samples showed similar, intermediate peroxide numbers. This effect is probably due to the different antioxidants affecting peroxide formation and subsequent secondary oxidation thereby reducing the peroxide content. Increase in *p*-anisidine values usually correspond to decreases in peroxide contents (Bravi et al. 2015).

The results showed that secondary products of oxidation increased in all formulations (Table 2). The lowest values (statistically significant from T0), were observed in the BHT (1.58 at T0, 2.56 at T1, 2.78 at T2, 3.25 at T3, and 4.13 at T4) and A2 samples (2.03 at T0, 2.59 at T1, 2.83 at T2, 3.58 at T3, and 3.81 at T4), followed by A1 (2.36 at T0,

Table 2 Acidity, peroxide levels, and *p*-Anisidine value of covering oils from canned artichokes as a function of storage time

Acidity (g oleic acid/100 g of oil)		Peroxide content (mEq O ₂ /Kg of oil)										<i>p</i> -Anisidine values									
Months		Months										Months									
0	3	6	9	12	0	3	6	9	12	0	3	6	9	12	0	3	6	9	12	0	3
CTRL	0.39 ^a	0.43 ^{aB}	0.47 ^{bAB}	0.56 ^{cAB}	0.72 ^{dD}	14.53 ^{eB}	10.70 ^{dB}	8.03 ^{eB}	6.07 ^{bAB}	5.08 ^{aBC}	2.30 ^{aC}	3.68 ^{bC}	4.55 ^{cD}	4.72 ^{dC}	4.91 ^{eB}	2.30 ^{aC}	3.68 ^{bC}	4.55 ^{cD}	4.72 ^{dC}	4.91 ^{eB}	2.30 ^{aC}
WS	0.37 ^{aA}	0.45 ^{bAB}	0.49 ^{bB}	0.52 ^{cA}	0.59 ^{dA}	14.25 ^{eB}	13.16 ^{dD}	10.33 ^{eD}	8.14 ^{bD}	6.60 ^{aD}	2.89 ^{aE}	4.19 ^{bD}	4.59 ^{cD}	4.81 ^{dC}	5.42 ^{eC}	2.89 ^{aE}	4.19 ^{bD}	4.59 ^{cD}	4.81 ^{dC}	5.42 ^{eC}	2.89 ^{aE}
OG	0.36 ^{aA}	0.41 ^{bA}	0.43 ^{bA}	0.57 ^{cB}	0.71 ^{dCD}	14.75 ^{eB}	12.29 ^{dC}	7.76 ^{eB}	7.23 ^{bC}	5.10 ^{aBC}	2.99 ^{aE}	4.11 ^{bCD}	4.08 ^{cC}	5.01 ^{dC}	5.11 ^{eC}	2.99 ^{aE}	4.11 ^{bCD}	4.08 ^{cC}	5.01 ^{dC}	5.11 ^{eC}	2.99 ^{aE}
OCP	0.37 ^{aA}	0.50 ^{bB}	0.50 ^{bB}	0.59 ^{cB}	0.71 ^{dCD}	14.71 ^{eB}	12.42 ^{dC}	9.34 ^{cC}	8.07 ^{bD}	4.93 ^{aB}	2.63 ^{aD}	3.63 ^{bC}	4.46 ^{cCD}	4.68 ^{dC}	5.55 ^{eC}	2.63 ^{aD}	3.63 ^{bC}	4.46 ^{cCD}	4.68 ^{dC}	5.55 ^{eC}	2.63 ^{aD}
A1	0.39 ^{aA}	0.46 ^{bAB}	0.45 ^{bAB}	0.52 ^{cA}	0.66 ^{dBC}	12.74 ^{eA}	9.19 ^{dA}	7.73 ^{eB}	6.98 ^{bC}	5.12 ^{aBC}	2.36 ^{aC}	2.74 ^{bA}	3.36 ^{cB}	3.87 ^{dB}	4.52 ^{eB}	2.36 ^{aC}	2.74 ^{bA}	3.36 ^{cB}	3.87 ^{dB}	4.52 ^{eB}	2.36 ^{aC}
A2	0.35 ^{aA}	0.46 ^{bAB}	0.46 ^{bAB}	0.57 ^{cB}	0.64 ^{dAB}	12.07 ^{eA}	8.61 ^{dA}	5.81 ^{cA}	5.57 ^{bA}	3.90 ^{aA}	2.03 ^{aB}	2.59 ^{bA}	2.83 ^{cA}	3.58 ^{dAB}	3.81 ^{eA}	2.03 ^{aB}	2.59 ^{bA}	2.83 ^{cA}	3.58 ^{dAB}	3.81 ^{eA}	2.03 ^{aB}
BHT	0.35 ^{aA}	0.46 ^{bAB}	0.46 ^{bAB}	0.60 ^{eB}	0.67 ^{dBC}	12.31 ^{eA}	8.60 ^{dA}	7.08 ^{eB}	6.6 ^{bBC}	5.72 ^{aC}	1.58 ^{aA}	2.56 ^{bA}	2.78 ^{cA}	3.25 ^{dA}	4.13 ^{eAB}	1.58 ^{aA}	2.56 ^{bA}	2.78 ^{cA}	3.25 ^{dA}	4.13 ^{eAB}	1.58 ^{aA}

n = 3; values in the same row followed by different superscript lowercase letters are statistically different, while values in the same column followed by different superscript uppercase letters are statistically different ($P < 0.05$)

CTRL control sample with garlic and chili pepper, WS sample without spices, OG sample with only garlic, OCP sample with only chili pepper, A1 sample with 0.007 % of allicin, A2 sample with 0.017 % of allicin, BHT sample with 0.02 % of BHT

2.74 at T1, 3.36 at T2, 3.87 at T3, and 4.52 at T4; statistically significant from T1). The highest *p*-anisidine values were observed in WS, OG and OCP. The greater resistance to oxidative degradation displayed by A2, which contained the highest dose of garlic extract, highlights the protective effect of the supercritical garlic extract. A similar result was found previously by the same authors in a study on vegetable oil (Bravi et al. 2016).

Antioxidant activity

To evaluate the antioxidant activity of the samples, the free radical scavenging activity was determined using the DPPH test. The results are expressed as the concentration of sample that inhibited 50 % of radical activity (IC50 value). Lower values indicate increasing antioxidant activity, and the results are presented in Table 3. The sample A2 showed the highest antioxidant activity (0.11 at T0, 0.20 at T1, 0.18 at T2, 0.21 T3, and 0.28 at T4), followed by BHT and A1, during all storage time (for BHT the differences were significant at T0, T2 and T4). The control sample showed intermediate values (statistically significant at T2, T3 and T4). The lowest antioxidant activities were observed in decreasing order in OG, OCP and WS. These results suggested that supercritical garlic extracts containing 0.017 % allicin may be able to neutralize free radicals in oil by donating electrons (Galano and Francisco-Marquez 2009).

Sensorial analysis

Starting at T0 a sensory test was run on the samples containing supercritical garlic extract compared with the control in order to evaluate the sensory impact of canning with the different oils on the artichokes. Table 4 shows the results for the quantitative descriptive analysis comparing the CTRL, A1 and A2 at T0, T1, T2, T3 and T4. The sensorial test identified significant differences between the samples in their levels of garlic flavor (flavor associated with the essential oils released by crushed garlic cloves) and rancidity (the taste associated with hydrolyzed/oxidized fats.) The artichokes flavor (smell and taste typical of cooked vegetables), hardness (by steadily compressing the vegetable between the molars, the force required for compression), and chewiness (the length of the time required to masticate the vegetable to a state of swallow-ing) were not significant (data not reported).

Garlic flavor and rancidity are important parameters to evaluate the quality of the formulations of canned artichokes. In particular, garlic flavor for the consumer compliance and rancidity for the stability of the formulations. Regarding garlic flavor, the CTRL sample was significantly lower ($P < 0.05$) than A1 and A2 at T0 which was

Table 3 IC50 values, obtained by the DPPH test, in covering oils from canned artichokes as a function of storage time

	0 Months	3 Months	6 Months	9 Months	12 Months
DPPH (IC50)					
CTRL	0.28 ^{aD}	0.29 ^{bB}	0.31 ^{cC}	0.36 ^{dB}	0.58 ^{eC}
WS	0.40 ^{aG}	0.52 ^{bE}	0.58 ^{cF}	0.60 ^{dC}	0.74 ^{eE}
OG	0.30 ^{aE}	0.32 ^{bB}	0.41 ^{cD}	0.57 ^{dC}	0.71 ^{eE}
OCP	0.36 ^{aF}	0.39 ^{bD}	0.54 ^{cE}	0.60 ^{dC}	0.64 ^{cD}
A1	0.18 ^{aB}	0.22 ^{bA}	0.25 ^{cB}	0.28 ^{dA}	0.35 ^{eB}
A2	0.11 ^{aA}	0.20 ^{bA}	0.18 ^{cA}	0.21 ^{dA}	0.28 ^{eA}
BHT	0.20 ^{aC}	0.22 ^{bA}	0.23 ^{bB}	0.24 ^{cA}	0.29 ^{dAB}

n = 3; values in the same row followed by different superscript lowercase letters are statistically different, while values in the same column followed by different superscript uppercase letters are statistically different ($P < 0.05$)

CTRL control sample with garlic and chili pepper, WS sample without spices, OG sample with only garlic, OCP sample with only chili pepper, A1 sample with 0.007 % of allicin, A2 sample with 0.017 % of allicin, BHT sample with 0.02 % of BHT

Table 4 Sensory evaluation scores of the CTRL, A1 and A2 samples as a function of storage time

	0 Months	3 Months	6 Months	9 Months	12 Months
Garlic flavor					
CTRL	3 ^{aA}	6 ^{bB}	6 ^{bB}	6 ^{bB}	6 ^{bC}
A1	5 ^{aB}	5 ^{aA}	5 ^{aA}	5 ^{aA}	5 ^{aB}
A2	7 ^{cC}	7 ^{cC}	6 ^{bB}	5 ^{bA}	4 ^{aA}
Rancidity					
CTRL	1 ^{aA}	2 ^{bB}	2 ^{bB}	2 ^{bA}	5 ^{cB}
A1	1 ^{aA}	2 ^{aB}	2 ^{aAB}	2 ^{aA}	1 ^{aA}
A2	1 ^{aA}	1 ^{aA}	1 ^{aA}	2 ^{bA}	1 ^{aA}

n = 9; values in the same row followed by different superscript lowercase letters are statistically different, while values in the same column followed by different superscript uppercase letters are statistically different ($P < 0.05$)

CTRL control sample with garlic and chili pepper, A1 sample with 0.007 % of allicin, A2 sample with 0.017 % of allicin

expected because the latter two had the supercritical extract. The supercritical extract contains concentrated, isolated allicin levels, while the sliced garlic used in the control sample did not contain isolated allicin and it was in lower concentrations (Ghani 2010). Starting at T1, the garlic flavor perception significantly increased (from 3 to 6) in CTRL till the end of the storage. Garlic perception in the supercritical extract samples significantly decreased in sample A2 by T4 but was unchanged in A1. Therefore the garlic perception is reduced for both the extract added samples along the storage time (from the third month for A1 and from the ninth month for A2, until the end of storage). At the beginning of shelf life trial the addition of garlic extract causes a significant higher perception of garlic flavor, however this perception significantly decrease during the storage time (probably for the high volatility of the sulphur-containing compounds); the increasing of the garlic flavor in control sample during the storage time is probably due to the release of thiosulfinates from fresh

garlic causing a higher perception of garlic flavor in ctrl sample after 12 months of storage.

Regarding rancidity, sample A2 displayed significantly lower rancidity at T1, T2, and T4 compared with CTRL. In fact rancidity in A2 was even lower than A1 at T1 and T2. Rancidity values were stable until T2 after which a significant increase was observed. CTRL showed the highest rancidity at the end of storage, confirming the higher oxidative stability of the samples with supercritical garlic extract. The results supported the efficacy of supercritical garlic extracts in protecting in-oil canned vegetables from oxidative degradation.

Conclusion

The beneficial effects of supercritical garlic extract on quality parameters and antioxidant activity in canned artichokes were: (1) at the beginning of the shelf-life trial, the

lowest peroxide values in A1 and A2 underlined the antioxidant effect; (2) increasing amounts of supercritical garlic extract highlighted the dose-dependent efficiency of garlic extract in preserving in-oil canned artichokes from oxidative degradation during storage; (3) the sensorial investigation confirmed that the samples treated with supercritical garlic extracts had lower rancidity at the end of 12 month's storage. The supercritical garlic extract samples scores for garlic perception were different from the control. The findings from this study suggest that it is possible to produce canned vegetable products of higher quality by optimizing the use of traditional ingredients through selective food technologies.

References

- AOCS (2013) Official methods and recommended practices. ISBN: 204 978-1-893997-74-5
- Baiano A, Gomes T, Caponio F (2005a) Hydrolysis and oxidation of covering oil in canned dried tomatoes as affected by pasteurization. *Grasas Aceites* 56:177–181
- Baiano A, Gomes T, Caponio F (2005b) A comparison between olive oil and extra-virgin olive oil used as covering liquids in canned dried tomatoes: hydrolytic and oxidative degradation. *Int J Food Sci Technol* 40:829–834
- Baiano A, Gomes T, Severini C (2005c) Effects of herbs on hydrolytic and oxidation degradation of olive oil in canned tomatoes. *JAOCs* 82:759–765
- Baiano A, Terracone C, Gambacorta G, La Notte E (2009) Changes in quality indices, phenolic content and antioxidant activity of flavored olive oils during storage. *JAOCs* 86:1083–1092
- Bakir T, Sonmezoglu I, Apak R (2015) Antioxidant/prooxidant effects of garlic on linoleic acid peroxidation induced by copper (II). *J Turk Chem Soc Sect A* 2:36–40
- Bandoniene D, Pukalskas A, Venskutonis PR, Gruzdiene D (2000) Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. *Food Res Int* 33:785–791
- Banerjee SK, Sood S, Dinda AK, Das TK, Maulik SK (2003) Chronic oral administration of raw garlic protects against isoproterenol-induced myocardial necrosis in rat. *Comp Biochem Physiol C Pharmacol* 136:377–386
- Bhale SD, Xu Z, Prinyawiwatkul W, King JM, Godber JS (2007) Oregano and rosemary extracts inhibit oxidation of long-chain n-3 fatty acids in menhaden oil. *J Food Sci* 72:504–508
- Bocchini P, Andalò C, Pozzi R, Galletti GC, Antonelli A (2001) Determination of diallyl thiosulfinate (allicin) in garlic (*Allium sativum* L.) by high-performance liquid chromatography with a post-column photochemical reactor. *Anal Chim Acta* 441:37–43
- Bravi E, Mangione A, Marconi O, Perretti G (2015) Comparative study of oxidation in canned foods with a combination of vegetables and covering oils. *Ital J Food Sci* 27:260–270
- Bravi E, Perretti G, Falconi C, Marconi O, Fantozzi P (2016) Antioxidant effects of supercritical fluid garlic extracts in sunflower oil. *J Sci Food Agric*. doi:10.1002/jsfa.7690
- Brunner G (2005) Supercritical fluids: technology and application to food processing. *J Food Eng* 67:21–33
- Caponio F, Gomes T, Summo C (2003) Quality assessment of edible vegetable oils used as liquid medium in canned tuna. *Eur Food Res Technol* 216:104–108
- Choe E, Min DB (2006) Mechanisms and factors for edible oil oxidation. *Compr Rev Food Sci Food Saf*. 5:169–186
- Chung LY (2006) The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. *J Med Food* 9(2):205–213
- Commission Regulation (EU) 61/2011, amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis
- Del Valle JM, Mena C, Budinich M (2008) Extraction of garlic with supercritical CO₂ and conventional solvents. *Braz J Chem Eng* 25(3):535–542
- E.U.-D.G. Environment (2007). Study on enhancing the endocrine disrupter priority list with a focus on low production volume chemicals. Revised report to DG Environment (Hersholm, Denmark: DHI water and environment) http://ec.europa.eu/environment/endocrine/documents/final_report_2007.pdf. Retrieved 25 Aug 2015
- E.U. Official Journal of the European Union, Commission Implementing Regulation 1348/2013 amending Regulation (EEC) 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis
- Galano A, Francisco-Marquez M (2009) Peroxyl-radical-scavenging activity of garlic: 2-propenesulfenic acid versus allicin. *J Phys Chem B* 113:16077–16081
- Gazzani G, Papetti A, Massolini G, Daglia M (1998) Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *J Agric Food Chem* 46(10):4118–4122
- Ghani MJ (2010) Determination of alliin and allicin in different types garlic using high performance liquid chromatography. *JUAPS* 4(2):16–23
- Hinneburg I, Dorman HJD, Hiltunen R (2006) Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem* 97:122–129
- IARC (1986). Monographs on the evaluation of carcinogenic risks o humans, vol 40. monographs.iarc.fr/ENG/Monographs/vol1-42/. Retrieved 25 Aug 2015
- Iqbal S, Bhanger MI (2007) Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chem* 100:246–254
- Kamkar A, Jebelli Javan A, Asadi F, Kamalinejad M (2010) The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. *Food Chem Toxicol* 48:1796–1800
- King JW, Johnson JH, Friedrich JP (1989) Extraction of fat tissue from meat products with supercritical carbon dioxide. *J Agric Food Chem* 37:951–954
- Nedyalka V, Yanishlieva NV, Marinova E, Pokorny J (2006) Natural antioxidants from herbs and spices. *Eur J Lipid Sci Technol* 108:776–793
- Nencini C, Cavallo F, Capasso A, Franchi GG, Giorgio G, Micheli L (2007) Evaluation of antioxidative properties of Allium species growing wild in Italy. *Phytother Res* 2:874–878
- Nuutila AM, Puupponen-Pimia R, Aarni M, Oksman-Caldentey KM (2003) Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem* 81:485–493
- Okada Y, Tanaka K, Fujita I, Sato E, Okajima H (2005) Antioxidant activity of thiosulfinates derived from garlic. *Redox Rep* 10:96–102
- Psomiadou E, Tsimidou M (1998) Simultaneous HPLC determination of tocopherols, carotenoids, and chlorophylls for monitoring their effect on virgin olive oil oxidation. *J Agric Food Chem* 46:5132–5138
- Pyrzynska K, Pekal A (2013) Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Anal Methods* 5:4288–4295

- Ragaert P, Devlieghere F, Debevere J (2007) Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. *Postharvest Biol Technol* 44:185–194
- U.S. National Library of Medicine (2010). Haz-map: occupational exposure to Hazardous agents, <http://hazmap.nlm.nih.gov>. Retrieved 25 Aug 2015