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Total reducing capacity of fresh sweet peppers and five different Italian pepper recipes

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Abstract

Processing and preparation methods are generally believed to result in a depletion of naturally occurring antioxidants in food. To evaluate the antioxidant properties of fresh sweet peppers (*Capsicum annuum*) and five different Italian recipes based on sweet peppers (pickled; ''peperonata''; grilled; in sour–sweet condiment; salted), water- and lipid-soluble extracts from fresh and processed peppers were analysed using high-performance liquid chromatography coupled with an electrochemical detector. Total reducing capacity (TRC) and contributions of hydrophilic reducing capacity (HRC) and lipophilic reducing capacity (LRC) to the TRC were determined in all the samples. Three important antioxidant compounds were measured: ascorbic acid, β-carotene and lycopene. The contribution of these individual compounds to TRC was estimated.

Fresh pepper had the highest TRC, the highest HRC and the greatest content of ascorbic acid. HRC and ascorbic acid content decreased with processing, whilst LRC was generally increased. Ascorbic acid was the major component of HRC in all samples (ranging from 72% in peperonata to 88% in fresh pepper), confirming the high content of this vitamin in peppers. Lycopene was detected only in peperonata. Many liposoluble compounds present in the lipophilic extract were not identified (only 6–20% of LRC was β-carotene).

Keywords: HPLC; Electrochemical detector; Canned food; Mediterranean diet

1. Introduction

Epidemiological and clinical studies have shown that the intake of fruit and vegetables plays an important role in the protection of human health, reducing the risk of several age-related diseases including cardiovascular diseases, common cancers, cataract and macular degeneration, and particularly arteriosclerosis (Block, Patterson, & Subar, 1992; Hertog, Feskens, Hollmann, Katan, & Kromhout, 1993; Jacques, Hartz, Chylack, McGandy, & Sadowski, 1988; Kritchevsky et al., 1998; Law & Morris, 1998; Sarma, Brunner, Evans, & Wormald, 1994; Steinmetz & Potter, 1996; Varma, Devamanoharan, & Morris, 1995). These protective effects are mainly attributed to various antioxidants such as vitamins, phenolics, carotenoids and other phytochemicals (Rice-Evans & Miller, 1996; Szeto, Thomlinson, & Benzie, 2002).

Organisms are continuously exposed to free radicals, aggressive and highly reactive chemical species produced from the intermediate metabolism of oxygen. In order to counteract the harmful effects of free radicals, the human organism has developed a defence system, using a number of substances with antioxidant activity, both endogenous (enzymatic systems and glutathione) and exogenous (present in food). These compounds act by reducing free radicals (Guo, Cao, Sofic, & Prior, 1997). Due to this role, antioxidant compounds naturally present in food become extremely important. In recent years, some foods particularly rich in antioxidants have been termed *nutraceuticals* and *functional food*, highlighting their ability to promote human health.

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It is well known that naturally occurring antioxidants could be significantly lost as a consequence of food processing and storage (Kalt, Forney, Martin, & Prior, 1999; Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997). Recent research has established that food processing may also have some positive roles, improving the quality and healthy properties of food (Dewanto, Wu, & Liu, 2002a; Dewanto, Wu, Adom, & Liu, 2002b). This aspect is of great importance as only small amounts of vegetables are consumed raw, whilst most must be processed for quality, safety and economic reasons.

Fresh pepper (*Capsicum annuum*) contains more physiologically active compounds than other vegetables (Asilbekova, 2003). It has a high content of vitamin C (400–500 mg/100 g d.w. in the red and yellow variety, respectively) and β-carotene (350–700 mg/100 g d.w. in the red and yellow variety, respectively) (Tchiegang, Fewou, & Noutchougoue, 1999) both of which are important natural antioxidants. Ascorbate is involved in oxidation–reduction reactions with metal ions associated with metal-enzymes, it is a free radical scavenger in animal and plant tissues and acts against "bad" cholesterol preventing oxidation of LDL cholesterol, a process that contributes to plaque build up in the arteries (Foyer, 1993; Padayatty et al., 2003). β-Carotene and carotenoids are quenchers of reactive oxygen species and act as antioxidants at low oxygen pressure (Kiokias & Gordon, 2004).

As in the rest of south Europe, sweet peppers are produced and consumed on a large scale in Italy. In traditional Italian cooking peppers are prepared using a wide variety of methods and different recipes. In this research, we considered five of these recipes (peperonata; pickled; salted; sour–sweet; grilled) based on the two most consumed varieties of sweet peppers: variety California (red) and variety Heldor (yellow).

The aim of the study was to evaluate the antioxidant activity of sweet peppers, raw and processed following traditional Italian recipes, expressed as total reducing capacity (TRC), using HPLC coupled with an electrochemical detector. The individual contributions of three important components (ascorbic acid, β-carotene and lycopene) to total, hydro- and lipo-philic reducing capacity were also assessed.

This method, developed and widely used in our laboratory for the estimation of the reducing capacity of other plant food (Riccio, Del Re, Ferrari, & Trevisan, in preparation), has showed to be very sensitive to all reducing compounds, both hydro- and lipo-soluble, present in the extracts. It was chosen because allows the direct analysis of electroactive primary antioxidants (both hydro- and lipo-philic) by applying a potential similar to physiological conditions, thus mimicking the cellular environment, without the addition of reducing substrates or free radicals and using a single extraction.

2. Materials and methods

2.1. Chemicals and samples

L-Ascorbic acid, β-carotene, lycopene, and Trolox were obtained from Sigma Chemical CO. (St Louis, MO). All other chemicals were obtained from Carlo Erba Reagents (Milan, Italy). Water was purified using a MilliQ filtration system (Millipore CO., Bedford, Massachusetts). The experiments were performed using Spanish red peppers (variety California) and yellow peppers (variety Heldor) in the proportion 2:1, as required by the recipes. Peppers from the same production lot were processed and provided by an Italian canning factory. The processing scheme is reported in Fig. 1. The samples analysed were: fresh (raw peppers from the same harvest with seeds and stems removed and frozen at −20 °C until analysis); pickled; "peperonata"; grilled; in sour–sweet condiment; salted.

Peperonata, sour–sweet, and salted peppers were packaged in 2.5 kg cans, pickled peppers were packaged in 4 kg cans and grilled peppers in glass jars of 280 g.

Immediately after opening the can or jar, samples of 200 g were removed, homogenized by using a blender (Waring, USA) and stored in plastic bags at −20 °C until the end of the experiment.

2.2. Extraction

Twenty grams of homogenized sample were extracted for 30 min with 100 mL of a dichloromethane/methanol solution (1/1, v/v). The extract was filtered using a glass microfibre filter (Whatman International, UK). The filter was washed with 20 mL of dichloromethane. To aid separation of the phases, 100 mL of acidified water (5 mL L^{-1} of H_3PO_4 84%, v/v) were added. Two phases resulted from this process: an intensely coloured phase containing lipophilic compounds and a water–methanol phase containing hydrophilic compounds. If necessary, the emulsion was dispersed by adding 30 mL of NaCl saturated solution.

The organic phase was eluted, washed twice with distilled water, dehydrated with sodium anhydrous sulphate, filtered and evaporated in a Rotavapor® Büchi (Flavil, Switzerland). The dry residue was dissolved in 10 mL of toluene and analysed using HPLC to determine LRC and lycopene and β-carotene reducing capacity.

The water–methanol phase was washed with 50 mL of dichloromethane (which was then retrieved and added to the organic phase). The volume of the extract was adjusted to 200 mL by the addition of acidified water. The resulting water phase was used to determine HRC and ascorbate reducing capacity.

All extractions were performed in triplicate, under subdued light and in nitrogen saturated containers. All extracts were analysed as soon as possible and stored (if unavoidable) at −20 °C for a maximum of 3 days.

2.3. Reducing capacity by HPLC analysis

Extracts were analysed using a high-performance liquid chromatograph system, consisting of an HP 1090 Liquid Chromatograph (Hewlett Packard) and an electrochemical detector (ESA analytical Coulochem model 5100A). The detector was equipped with an analytical cell (model

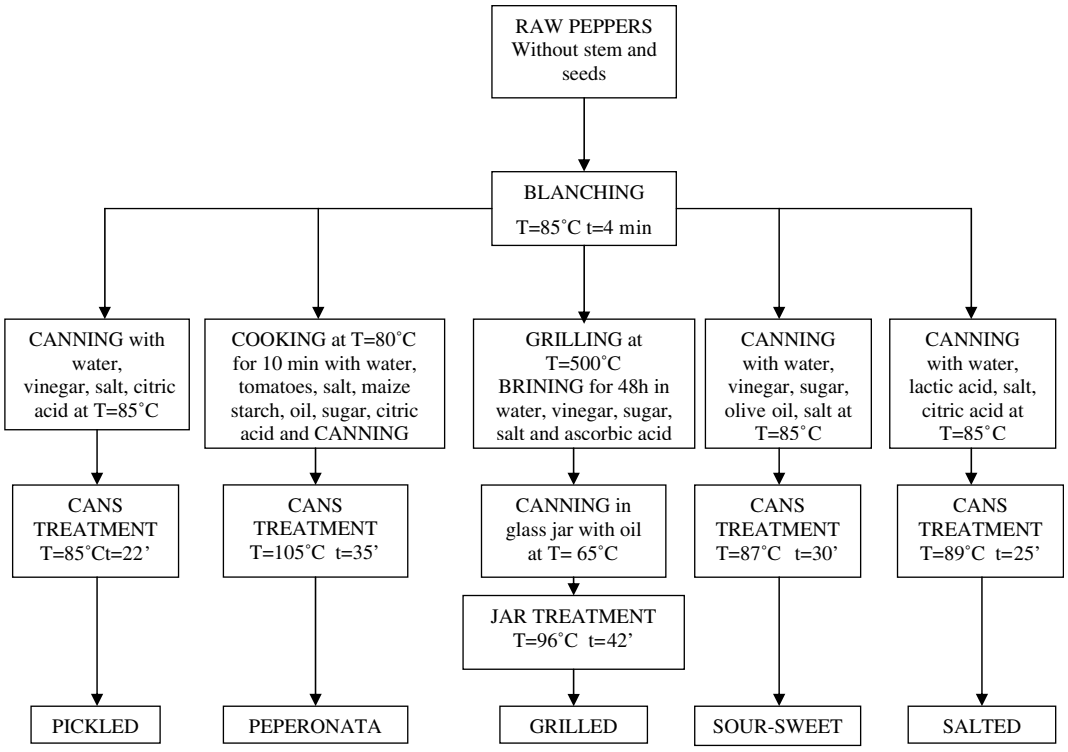

Fig. 1. Processing scheme for the preparation of the different recipes. After the blanching peppers were processed by cooking, grilling, adding of additives and/or other ingredients, depending on the prescriptions of the original recipe. All the preparations were canned.

5010) and set to a redox potential of +400 mV. Electronic data acquisition and peak integration were performed using a 4400 Integrator Varian. Chromatographic separation was performed on a SUPELCOSIL ABZ plus (Supelco, Bellefonte, USA) column (25 cm × 4.6 mm i.d.; particle size, 5 μm), with a flow rate of 1 mL min^{-1} and isocratic elution. HRC was assessed using a mobile phase 0.05 M NaH_2PO_4 (at pH 3, acidified with H_3PO_4)/CH_3OH (50/50, v/v). LRC was assessed using a mobile phase of acetonitrile/methanol/dicloromethane (65:20:15, v/v/v). All extracts were injected in duplicate. The volume injected was 10 μL.

HRCs and LRCs were calculated as the sum of the peak areas of each redox species. Peak areas were calibrated using the standard antioxidant Trolox. TRCs for each sample were calculated as the sum of HRC and LRC.

Ascorbic acid, β-carotene and lycopene peaks were identified by comparison with chromatograms of their standard solutions. In order to calculate their contributions to the TRC, HRC and LRC, ascorbic acid, β-carotene and lycopene contents were expressed as Trolox equivalents per gram of sample. Trolox, an analogue of vitamin E, can be dissolved in both aqueous and organic media, and was used as a reference substance to express hydrophilic and lipophilic compounds with the same unit of measurement.

The linearity of response ranked from 5 to 60 μmol L^{-1} of Trolox ($r^2 = 0.99$) for HRC and from 5 to 120 μmol L^{-1} of Trolox for LRC ($r^2 = 0.98$). The limit of detection (LOD) and the limit of quantification (LOQ) were 1.5 and 5 μmol L^{-1} of Trolox, respectively, for both methods. Following the guidelines from International Conference of Harmonization, the LOD was set considering a peak with a resolution with a signal-to-noise ratio, up to 3:1 in the samples (Shabir, 2003).

2.4. Statistical analysis

Data were tested by two-way ANOVA using SAS, version 8.2 (SAS Institute Inc., Cary, North Carolina), followed by the Tukey test to assess differences between group means.

Differences at $p < 0.05$ were considered significant, using the type of processing treatment as the classification factor (six levels) with three replicates (leaving one error with 12 d.o.f.).

3. Results

TRC (the sum of HRC and LRC), LRC and HRC of all samples are shown in Fig. 2.

TRCs ranged from 4.20 to 9.92 μmol g^{-1} fresh weight. This range is in agreement with a previous study using 22 different vegetable types (Cao, Sofic, & Prior, 1996).

Fresh pepper had the highest TRC followed by grilled peppers. Other samples did not differ significantly.

As shown in Fig. 2, most TRC (ranging from 65% in peperonata to 91% in fresh peppers) was represented by

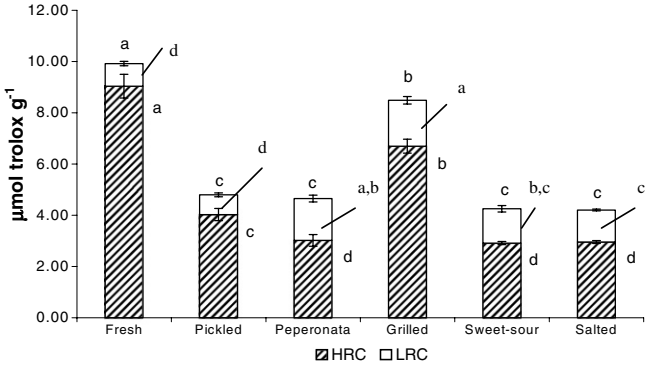

Fig. 2. Comparison of TRC, HRC and LRC of fresh and canned peppers and contribution of HRC and LRC to TRC. Columns not marked with the same letter are significantly different ($p < 0.05$).

HRC. HRC ranged from 2.91 to 9.04 μmol g^{-1} fresh weight in sour–sweet peppers and fresh peppers, respectively. HRCs were ranked in the same order as TRCs (fresh > grilled > pickled > salted ≅ sour–sweet ≅ peperonata; differences were statistically significant). Fresh pepper had the highest content of hydrophilic antioxidant compounds. Most HRC was represented by ascorbate (ranging from 72% in peperonata to 88% in fresh peppers). Unknown water soluble compounds represented 11% (salted) to 28% (peperonata) of HRC (Table 1). HRC, TRC and ascorbic acid decreased with processing. As reported in Table 2, processing significantly reduced HRC, and resulted in a loss of ascorbate of up to 73% (peperonata).

LRCs ranged from 0.77 to 1.79 μmol g^{-1} fresh weight in pickled and grilled peppers, respectively, in contrast to HRCs. Grilled peppers and peperonata had the highest LRC, whilst fresh and pickled peppers had the lowest (grilled ≅ peperonata ⩾ sweet–sour ⩾ salted > fresh ≅ pickled; differences were statistically significant). Only a small amount of LRC (ranging from 6% in peperonata to 28% in fresh peppers) was represented by β-carotene. Most LRC was due to unknown lipophilic reducing compounds. Lycopene was detectable only in peperonata, in which it represented 40% of LRC. LRC was positively affected by processing with an increase of up to 103% in grilled peppers. By contrast, β-carotene content was higher in fresh peppers and declined with cooking, with a decrease ranging from 16% in grilled peppers to 60% in peperonata (Table 2). Unknown reducing compounds ranged from 54% of LRC in peperonata (in which most of the LRC was identified as lycopene) to 88% in grilled peppers (Table 1).

4. Discussion

The antioxidant activity of vegetables depends on their composition, agronomic techniques and methods of conservation and preparation. Processing of raw fruits and vegetables represents a critical point in determining the final antioxidant properties of the product. On this basis, the evaluation of processing methods on the content of natural antioxidants in vegetables is critical in optimising

Table 1
Hydro and lipo-reducing capacity expressed as Trolox equivalents

	HRC		LRC		
	Ascorbic acid	Unknown water-soluble reducing capacity	β-Carotene	Lycopene	Unknown lipid-soluble reducing capacity
Fresh	7.95 ± 0.33a (88%)	1.09 ± 0.13a (12%)	0.25 ± 0.01a,b (28%)	n.d.	0.63 ± 0.08d (72%)
Pickled	3.13 ± 0.25c (78%)	0.90 ± 0.21a (22%)	0.13 ± 0.01c,d (17%)	n.d.	0.64 ± 0.08d (83%)
Peperonata	2.18 ± 0.12d (72%)	0.84 ± 0.11a (28%)	0.10 ± 0.01d (6%)	0.66 ± 0.04 (40%)	0.87 ± 0.11c,d (54%)
Grilled	5.70 ± 0.19b (85%)	1.00 ± 0.05a (15%)	0.21 ± 0.02b (12%)	n.d.	1.58 ± 0.16a (88%)
Sweet–sour	2.50 ± 0.13d (86%)	0.41 ± 0.07b (14%)	0.17 ± 0.01c (13%)	n.d.	1.17 ± 0.08b (87%)
Salted	2.64 ± 0.14c,d (89%)	0.32 ± 0.06b (11%)	0.28 ± 0.02a (22%)	n.d.	0.97 ± 0.02b,c (78%)

Values are expressed as the mean in μmol g^{-1} fresh weight ± the standard error. Mean values not followed by the same letter are significantly different ($p < 0.05$). Numbers in parentheses are contributions (%) of single compounds to the water- or lipo-soluble reducing capacity, respectively.

Table 2
Percentage change in ascorbic acid content, β-carotene content and total reducing capacity of peppers following processing

	Ascorbic acid	β-Carotene	TRC	HRC	LRC
Pickled	−61	−48	−52	−55	−13
Peperonata	−73	−60	−53	−33	+85
Grilled	−28	−16	−14	−26	+103
Sweet−sour	−68	−32	−57	−68	+52
Salted	−67	+12	−58	−67	+42

processing conditions in order to preserve or increase their ability to promote health.

Sweet peppers (*C. annuum*) are consumed increasingly worldwide. In the present work, we considered five different Italian recipes based on red and yellow sweet peppers (peperonata; pickled; salted; sour–sweet; grilled) to determinate their TRCs and assess how preparation techniques affect TRC and the content of reducing compounds.

Fig. 3 show the chromatographic profiles of the water methanol phase and the organic phase of two different samples.

The chromatographic profile of the water methanol phase was very clear and regular for all samples. Most HRC was from ascorbic acid ($R_t = 2.9$ min).

By contrast, the chromatographic profile of the organic phase was difficult to analyse and only a partial characterization was attempted. It is noteworthy that a large proportion of HRC comprised unknown compounds. To identify lipophilic reducing compounds, different standards were injected and their retention times compared with peaks in the chromatographic profiles of samples of lipophilic extracts. Other studies have demonstrated peppers to be a good source of capsanthin, tocopherols, quercetin, zeaxanthin and lutein (Buratti, Pellegrini, Brenna, & Mannino, 2001; Marín, Ferreres, Tomás-Barberán, & Gil, 2004). However, it was not possible to identify any of these compounds in the chromatographic profiles of the samples reported herein.

On the basis of the data obtained from analysis of fresh and processed peppers, it is clear that there is some correlation between processing treatments, TRC, LRC, HRC and the content of compounds with reducing capacity. Differences between fresh and canned peppers were, for the most part, significant ($p < 0.05$). This is in agreement with previous studies on food processing and the bioavailability of nutrients and food antioxidants.

Fresh pepper had the highest TRC, the highest HRC, and the greatest content of ascorbic acid. HRC, ascorbic acid and therefore TRC (which is represented particularly by HRC) decrease with processing. This is in agreement with previous studies (Favell, 1998; Hunter & Fletcher, 2002; Lathrop & Leung, 1980) which report the loss of ascorbic acid and the depletion of antioxidant properties as a result of blanching, cooking, pasteurisation, sterilization, dehydration and freezing.

Despite the decrease in β-carotene, the reducing capacity of which could have been lost through the conversion of the *trans* form to *cis* forms, catalysed by thermal processing, as demonstrated in previous works (Chandler & Schwartz, 1987), LRCs increase in almost all the processing methods considered. The data obtained from the analysis of LRC agrees with observations made in other studies. During processing, natural antioxidant compounds may be compensated by the appearance of other antioxidants. In some cases, as reported by Dewanto et al. (2002a, 2002b) for tomatoes and sweet corn, processing can

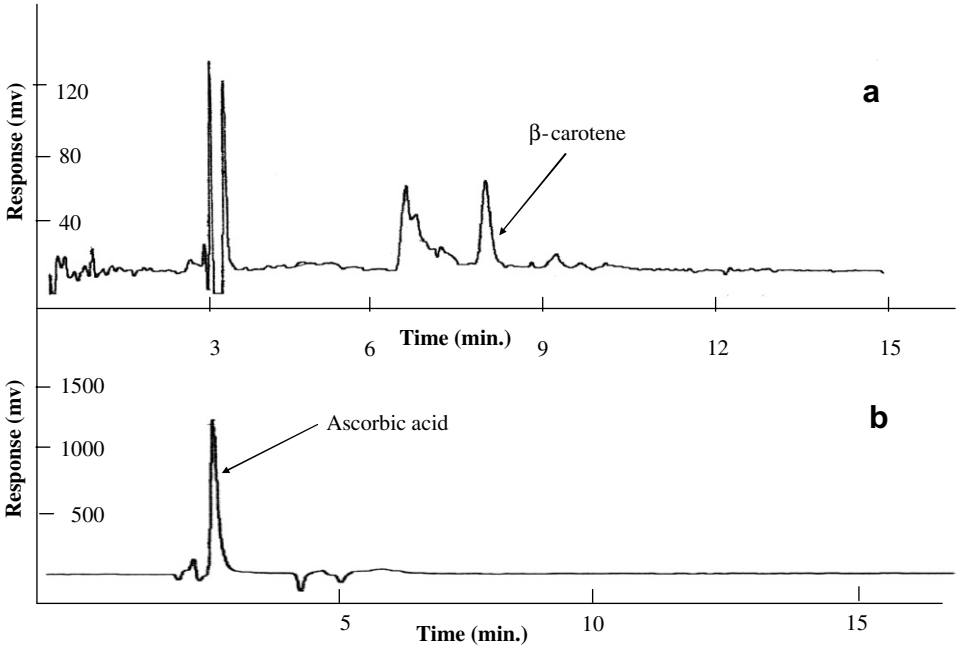

Fig. 3. Chromatographic profiles of the organic phase of peperonata (a) and the water methanol phase of fresh pepper (b).

enhance the antioxidant properties of the food. This is mainly attributed to the increased bioavailability of some antioxidants, and identified and unidentified products with antioxidant capacity. Specifically, several authors (Namiki, 1990; Yen & Tsai, 1993) suggest that Maillard reaction products (MRPs) might have both antioxidant and antimutagenic characteristics.

TRC, HRC and ascorbic acid decreased with processing, with the exception of grilled peppers. Despite being grilled at 500 °C and placed in pickling brine for 48 h (processing operations that usually result in a depletion in the antioxidant properties of vegetables (Nicoli, Anese, & Parpinel, 1999; Tchiegang et al., 1999)), grilled peppers exhibited good TRC and HRC, and a good content of vitamin C. This is mainly due to the addition of ascorbic acid as a preservative during canning. Moreover, this product was conserved in olive oil, a very good source of antioxidant compounds, able to preserve antioxidant properties (Gorinstein et al., 2003; Pellegrini, Visioli, Buratti, & Brighenti, 2001). The effect of olive oil is apparent in the LRC value for grilled peppers, the highest amongst the samples analysed.

Peperonata was the only sterilized product considered, and may be anticipated to have a lower TRC. However, the addition of other ingredients, including tomato, positively influenced TRC, contributing lycopene. Lycopene was not detected (n.d.), in the other samples analysed. Furthermore, peperonata had a relatively high content of LRC compared to HRC due to the addition of oil. However, the effects of sterilization may be reflected in the content of ascorbic acid and β-carotene. The contents of these compounds were lowest in peperonata compared to the other samples analysed.

Pickled, sour–sweet and salted peppers have similar methods of preparation and had comparable TRC. Particularly, sour–sweet peppers had a good LRC due to the addition of oil during preparation.

As for fresh peppers, pickled peppers contained the lowest amount of liposoluble antioxidants, probably because oil was not added during preparation. However, the higher LRC of salted peppers is surprising, given that oil is also absent from the recipe. This is due to the high content of β-carotene in this product, which contributes to the LRC to a greater extent than in other processed peppers. Salted, along with fresh peppers, have the highest content of β-carotene among all the samples. It maybe that the salt used in preparation has some positive influence in preserving β-carotene. It is also possible that the lot utilized in the preparation of the salted peppers contained fruits at a different stage of maturation, a parameter that has a significant influence on β-carotene content (Howard, Talcott, Brenes, & Villalon, 2000).

5. Conclusion

This work demonstrated that, although thermal processing caused a depletion of important vitamins such as β-carotene and ascorbic acid, this depletion could be readily compensated through careful selection of ingredients and methods of preparation. Through the use of modern technology in the canning process, in association with shrewdness in the formulation of 'ready to eat food', the food industry can improve the nutritional quality of food products present on the market. Furthermore, our research confirmed the importance of the Mediterranean diet, demonstrating that traditional recipes are nutritionally well formulated and able to preserve, or at least compensate, the loss of important natural antioxidants through the addition of other important sources of reducing compounds including olive oil.

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