Research Article

SCI

Received: 21 March 2016 Revised: 21 June 2016 Accepted article published: 19 July 2016 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.7910

Carotenoids in fresh and processed tomato (*Solanum lycopersicum*) fruits protect cells from oxidative stress injury

Rita Del Giudice,[a,†] Ganna Petruk,[a,†] Assunta Raiola,[b] Amalia Barone,[b] Daria Maria Monti[a*] and Maria Manuela Rigano[b*]

Abstract

BACKGROUND: Lipophilic antioxidants in tomato (*Solanum lycopersicum*) fruits exert important functions in reducing the risk of human diseases. Here the effect of thermal processing on the antioxidant activity of lipophilic extracts from the commercial tomato hybrid 'Zebrino' was analysed. Carotenoid content and lipophilic antioxidant activity were determined and the ability of tomato extracts in rescuing cells from oxidative stress was assessed.

RESULTS: Lipophilic antioxidant activity was completely retained after heat treatment and extracts were able to mitigate the detrimental effect induced by oxidative stress on different cell lines. Lycopene alone was able to rescue cells from oxidative stress, even if to a lower extent compared with tomato extracts. These results were probably due to the synergistic effect of tomato compounds in protecting cells from oxidative stress injury.

CONCLUSION: The current study provides valuable insights into the health effect of the dietary carotenoids present in fresh and processed tomato fruits.

Keywords: antioxidant power; carotenoids; cytotoxicity; lycopene; oxidative stress; *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most consumed vegetables worldwide and nowadays its consumption has further increased owing to the development of many processing products such as soups, juices, purees and sauces. It has been estimated that about 75% of tomatoes are consumed in processed form.[1] Among these processed tomatoes, 35% are consumed as sauces, 18% as tomato paste, 17% as canned tomatoes, 15% as juices and 15% as catsup.[1] Epidemiological studies suggest that consumption of tomato and tomato-based products reduces the risk of chronic diseases such as cardiovascular disease and cancer.[2] In particular, intake of tomato and tomato-based products has been consistently associated with lower risk of prostate, lung and stomach cancers.[3] Typically, this protective action is attributed to antioxidant components such as carotenoids, phenols and polyphenols as well as synergistic interactions among them.[4–7] Tomato fruits are considered one of the main sources of dietary antioxidants such as carotenoids, in particular α-carotene, β-carotene, lycopene, lutein and cryptoxanthin.[8] Indeed, tomato fruits contain 8–40 mg lycopene kg^{-1} fresh weight (FW), which corresponds to about 80% of the total dietary intake of this carotenoid.[9,10] Carotenoids are lipophilic pigmented molecules responsible for the different colours of fruits and vegetables and are synthesized by plants and microorganisms but not by animals. In plants, carotenoids protect against photo-damage and contribute to the photosynthetic machinery.[11,12] Carotenoids are important dietary sources of vitamin A, after β-carotene retinol bioconversion into pro-vitamin A.[13]

Oxidative stress, induced by the imbalance between the generation of reactive oxygen species (ROS) and the cellular capacity to detoxify these species with antioxidant molecules, has been implicated in the causation and development of several chronic diseases.[7] ROS can be generated by normal metabolic activity as well as by lifestyle factors such as smoking and diet. Many recent studies have investigated the role of dietary antioxidants that can mitigate the damaging effects of ROS,[8,14] and carotenoids have been proposed as good antioxidants able to act as free radical scavengers owing to their chemical structure.[15] Indeed, recently, it has been demonstrated that tomato lipophilic extracts were able to prevent hydrogen peroxide (H_2O_2)-induced cell death in a cell-based model system using rat cardiac H9c2 cells.[7] Generally, the carotenoid content of foods is not significantly modified by common household cooking methods such

* *Correspondence to: DM Monti, Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, Via Cinthia 4, I-80126 Naples, Italy, E-mail: mdmonti@unina.it; or MM Rigano, Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, I-80055 Portici (Naples), Italy, E-mail: mrigano@unina.it*

† *These authors contributed equally to the paper*

a *Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, Via Cinthia 4, I-80126, Naples, Italy*

b *Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, I-80055, Portici, Naples, Italy*

as microwave cooking, steaming and boiling, although extreme and extended heat treatments could cause oxidative degradation of carotenoids.[16,17] Moreover, the carotenoid lycopene has been demonstrated to be absorbed more efficiently from processed tomato products than from raw tomatoes.[18] However, only a few works have focused on the impact of processing on the general nutritional quality and antioxidant activities of tomato fruits.

In this study, the carotenoid content, lipophilic antioxidant activity and protective effects of tomato lipophilic extracts from the commercial tomato hybrid 'Zebrino' were examined. This cultivar has been selected since, during an on-going breeding program carried out at the Department of Agricultural Sciences, it was demonstrated that the fruits of this hybrid have a high content of bioactive compounds (carotenoids, phenolic compounds, vitamin C) endowed with antioxidant activity.[19] The protective effects of lycopene alone have also been evaluated in this study.

The current work seeks to evaluate the health-promoting properties of tomato fruits by evaluating both the antioxidant and protective effects of lipophilic extracts obtained from unprocessed and processed fruits. Consumers could benefit from the results reported in this paper by increasing their awareness of the health benefits of fresh and processed tomato fruits; moreover, these results could be used by fresh and industrial tomato producers for the development of tomato-based functional foods.

MATERIALS AND METHODS

Chemical reagents

Standards and reagents were purchased from Sigma (St Louis, MO, USA), while solvents were from Fluka (Buchs, Switzerland).

Plant material

Plant material consisted of the tomato genotype Zebrino (ZBR) (De Ruiters, Monsanto, St Louis, MO, USA). Plants were cultivated according to a randomized design with three replicates (ten plants per replicate) in an experimental field located in Acerra (Naples, Italy) in the year 2014. Each sample consisted of 20 pooled fruits per plot. The samples were harvested at the full ripe stage as used in the industry. Fruits were chopped, ground to a fine powder in liquid nitrogen using a Fimar FRI150 blender (Rimini, Italy) and kept at −80 °C until analyses. The studied genotype was processed according to a classical thermal treatment. Briefly, after washing for 5 min in water, tomatoes were treated for 10 min at 92 °C. An aliquot of treated tomatoes was passed through a pulper in order to obtain a puree. Glass cans were filled with 60% of treated whole tomatoes and 40% of puree and successively vacuum sealed. Then the filled jars were pasteurized at 100 °C for 30 min and cooled by water. The processed samples were homogenized using a Fimar FRI150 blender and kept at −80 °C until analyses. Three cans for each sample were collected and analysed.

Before chemical extraction, the dry matter content of all samples was determined by vacuum drying the samples for at least 12 h at 60 °C to constant weight. The moisture content of both processed and unprocessed samples was found to be around 900 g kg^{-1}. In particular, the mean dry matter content of fresh tomato was 100 g kg^{-1}, while that of processed tomato was 96 g kg^{-1}.

Extraction of lipophilic compounds

To obtain the lipophilic extract, 1 g of sample was extracted with 16 mL of acetone/hexane (40:60 v/v) using an IKA T 25 Ultra-Turrax High-Speed Homogenizer 115VAC (Cole-Parmer, Vernon Hills, IL, USA).[20] The mixture was centrifuged at 3500 × *g* for 5 min at 4 °C according to a modified procedure reported by Rigano *et al.*[21] Supernatants were collected and stored at −20 °C until analyses.

Carotenoid determination

For carotenoid determination, the absorbance of lipophilic extracts was read at 663, 645, 505 and 453 nm. *β*-Carotene and *cis*- and *trans*-lycopene isomer levels were calculated according to the equations reported by Zouari *et al.*[20] Total carotenoids were calculated by reading the absorbance at 480, 648 and 666 nm according to the formula reported by Wellburn.[22] Results were then converted into g kg^{-1} FW.

Antioxidant activity determination

Lipophilic antioxidant activity (LAA) was evaluated using the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test.[23] The ABTS assay was performed as described by Rigano *et al.*[21] Briefly, 100 µL of supernatant, obtained from the extraction reported above, was added to 1 mL of $ABTS^{\bullet+}$, the mixture was incubated for 2.5 min and the absorbance was read at 734 nm. The standard curve was linear between 0 and 20 µmol L^{-1} Trolox and results were expressed as mmol Trolox equivalent (TE) kg^{-1} FW.

MTT test

Human HeLa adenocarcinoma cells, human hepatic carcinoma HepG2 cells, human breast adenocarcinoma MCF-7 cells and murine BALB/c 3 T3 and SV-T2 fibroblasts (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St Louis, MO, USA) supplemented with 100 mL L^{-1} foetal bovine serum (HyClone, Logan, UT, USA), 2 mmol L^{-1} L-glutamine and antibiotics. Cells were grown in a 5% CO_2 humidified atmosphere at 37 °C and seeded in 96-well plates at a density of 2×10^3 cells per well. Lipophilic tomato extracts, obtained as reported above, were dried by rotovapor (R-210, Buchi), re-dissolved in dimethyl sulfoxide (DMSO) and then added to the cells 24 h after seeding for dose-dependent cytotoxicity assays. After 48 h incubation, cell viability was assessed by the MTT assay as described in Galano *et al.*[24] Briefly, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent dissolved in DMEM in the absence of phenol red (Sigma-Aldrich) was added to the cells (0.5 mg mL^{-1} final concentration). Following 4 h incubation at 37 °C, the culture medium was removed and the resulting formazan salts were dissolved by adding isopropanol containing 0.01 mol L^{-1} HCl (100 µL per well). Absorbance values were determined at 570 nm using an automatic plate reader (Microbeta Wallac 1420, PerkinElmer, Waltham, MA, USA). Cell survival was expressed as percentage of viable cells in the presence of the tomato extract under test compared with control cells grown in the absence of the extract. Three separate analyses were carried out with each sample. Control experiments were performed either by growing cells in the absence of the extract or by adding to the cell cultures identical volumes of DMSO. The method used avoids any possibility of a DMSO effect on the final results.

Oxidative stress analyses

To evaluate the protective effect of lipophilic extracts against oxidative stress, cells were plated at a density of 4×10^4 cells cm^{-2}, incubated in the presence of 0.6 mg mL^{-1} lipophilic extracts or 0.4 µg mL^{-1} commercial *trans*-lycopene (Sigma) for 48 h and then treated with 300 µmol L^{-1} sodium arsenite (SA) for 2 h. At the end of incubation, cell viability was assessed by the MTT assay

as reported above, ROS production was measured by the DCFDA assay, glutathione (GSH) level was measured by the DTNB assay and lipid peroxidation was assessed by the TBARS method.

DCFDA assay

To determine ROS levels within the cytosol, cells were incubated with the cell-permeable redox-sensitive fluorophore 2′,7′-dichlorodihydrofluorescein diacetate (H_2-DCFDA) (Sigma-Aldrich) at a concentration of 25 µmol L^{-1} for 30 min at 37 °C. Cells were then washed twice with warm phosphate-buffered saline (PBS) supplemented with 1 mmol L^{-1} $CaCl_2$, 0.5 mmol L^{-1} $MgCl_2$ and 30 mmol L^{-1} glucose (PBS plus), detached by trypsin, centrifuged at 1000 × *g* for 10 min and re-suspended in PBS plus at a density of 1×10^5 cells mL^{-1}. H_2-DCFDA is non-fluorescent until, in the presence of ROS, it is hydrolysed by intracellular esterases and readily oxidized to the highly fluorescent 2′,7′-dichlorofluorescein (DCF). DCF fluorescence intensity was measured at an emission wavelength of 525 nm and an excitation wavelength of 488 nm using a PerkinElmer LS50 spectrofluorimeter. Emission spectra were acquired at a scanning speed of 300 nm min^{-1}, with five slit widths for excitation and emission. ROS production was expressed as percentage of DCF fluorescence intensity of the sample under test compared with the untreated sample. Three separate analyses were carried out with each extract. Control experiments were performed by supplementing cell cultures with identical volumes of DMSO.

DTNB assay

The interaction of the sulfhydryl group of GSH with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) produces a yellow-coloured compound, 5-thio-2-nitrobenzoic acid (TNB), whose intensity can be measured at 412 nm. Thus the rate of TNB production is directly proportional to the concentration of GSH in the sample. To estimate intracellular GSH levels, cells were detached by trypsin, centrifuged at 1000 × *g* for 10 min and re-suspendend in lysis buffer (300 mmol L^{-1} NaCl and 5 mL L^{-1} NP-40 in 100 mM Tris–HCl, pH7.4) containing protease inhibitors. After 30 min incubation on ice, lysates were centrifuged at 14 000 × *g* for 30 min at 4 °C. Supernatant protein concentration was determined by the Bradford assay. Then 50 µg of proteins were incubated with 3 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) and 144 µmol L^{-1} DTNB in 30 mmol L^{-1} Tris HCl (pH 8.2) and centrifuged at 14 000 × *g* for 5 min at room temperature. Finally, the absorbance of the supernatant was measured at 412 nm using a multiplate reader (BioRad, Hercules, CA, USA). GSH levels were expressed as percentage of TNB absorbance of the sample under test compared with the untreated sample. Three separate analyses were carried out with each extract. Control experiments were performed by supplementing cell cultures with identical volumes of DMSO.

Measurement of lipid peroxidation

Levels of lipid peroxidation were determined using the thiobarbituric acid (TBA)-reactive substance (TBARS) assay.[25] Cells were detached by trypsin and centrifuged at 1000 × *g* for 10 min, then 5×10^5 cells were re-suspendend in ice-cold PBS and mixed with 6.7 mL L^{-1} TBA and an equal volume of 200 mL L^{-1} trichloroacetic acid (TCA). Samples were then heated at 95 °C for 30 min, incubated on ice for 10 min and centrifuged at 3000 × *g* for 5 min at 4 °C. TBA reacts with oxidative degradation products of lipids, and complexes absorb at 532 nm. Lipid peroxidation levels were expressed as percentage of absorbance at 532 nm of the sample under test compared with the untreated sample. Three separate analyses were carried out with each extract. Control experiments were performed by supplementing cell cultures with identical volumes of DMSO.

Statistical analyses

Biological replicates of samples were analysed in triplicate. Quantitative parameters were expressed as mean ± standard deviation (SD). Differences among unprocessed and processed samples were determined using SPSS 6, Version 15.0 (SPSS Inc., Chicago, IL, USA). Significance was assessed by Student's *t* test at a significance level of 0.05.

RESULTS AND DISCUSSION

In this study, the antioxidant and protective activities of lipophilic extracts obtained from unprocessed and processed commercial tomato hybrid 'Zebrino' (ZBR) were analysed. This cultivar is characterized by a skin colour changing from dark green to dark brown/red, with green tiger stripes when fully ripe. Carotenoid content and LAA were examined and the ability of tomato lipophilic extracts and lycopene alone in rescuing cells from oxidative stress was assessed.

Carotenoid content and antioxidant activity

Figure 1A shows the change in the carotenoid content of ZBR extracts before and after heat treatment. The mean amount of total carotenoids in ZBR was 0.095 g kg^{-1} FW, and this value did not change significantly after thermal processing. Lycopene and

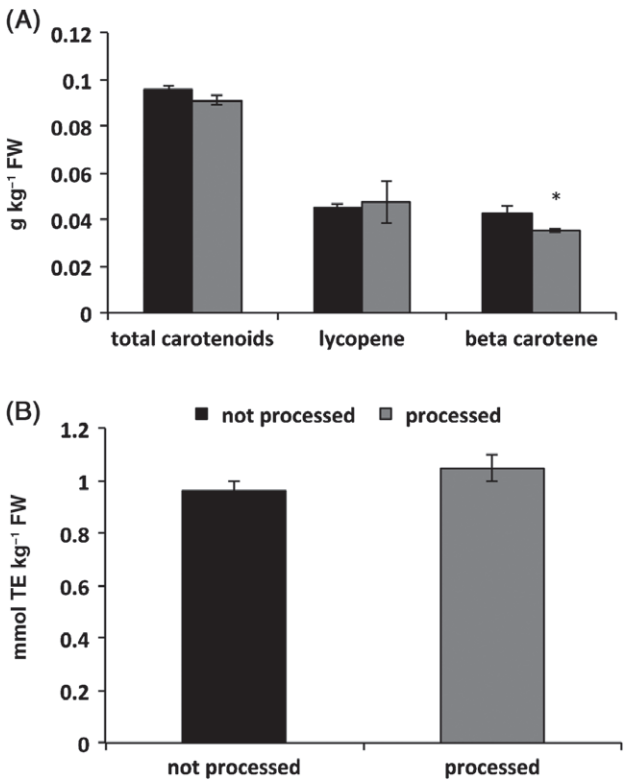

Figure 1. *In vitro* characterization of carotenoid tomato extracts. (A) Carotenoid content (g kg^{-1} FW) and (B) lipophilic antioxidant activity (mmol TE kg^{-1} FW) of ZBR lipophilic extracts before (unprocessed, black bars) and after thermal processing (processed, grey bars).

β-carotene represented about 47 and 45% of total carotenoids in unprocessed samples respectively. Frusciante *et al.*[26] and Rigano *et al.*[21] found that lycopene constituted about 90% of total carotenoids in different varieties of red tomatoes. On the contrary, the ZBR genotype shows a different lycopene/total carotenoid ratio and a higher content of β-carotene compared with common red tomatoes. These values correlate well with the presence of green tiger stripes on the skin and the deep green/brown flesh of the fully ripe fruit. After processing, the amount of lycopene did not change significantly, whereas a significant ($P < 0.05$) decrease of 17% in β-carotene level was recorded. In the literature, controversial data on carotenoid stability during thermal processing are reported. In accordance with our data, it has been reported that carotenoid content was stable in processed tomatoes.[16] Capanoglu *et al.*[27] reported a significant decrease in both lycopene (32%) and β-carotene (36%) levels, whereas Re *et al.*[28] reported an increase in lycopene levels in several tomato products. These contrasting results may depend on the different genotypes evaluated and on the temperature and time adopted in the processing methods.

Figure 1B shows the LAA in ZBR extracts before and after thermal processing. The mean LAA was 0.96 mmol TE kg^{-1} FW before processing and did not change after heat treatment. The lipophilic tomato extract may contain other antioxidant phytochemicals such as vitamin E. Our results, together with results from Li *et al.*,[7] demonstrated that carotenoids constitute the major fraction of lipophilic compounds in tomato fruits and contribute significantly to their overall antioxidant activity. The LAA reported in this study for the ZBR cultivar was higher than those reported for other tomato cultivars. Indeed, Cano *et al.*[29] measured an antioxidant activity of 0.81 mmol TE kg – 1 FW in tomato mature red fruit, whereas Toor and Savage[30] found mean levels of LAA equal to 0.18, 0.07 and 0.09 mmol TE kg^{-1} FW in the skin, pulp and seeds respectively of several tomato commercial cultivars.

Free radical scavenger activity of lipophilic tomato extracts

Afterwards, the maximum concentration of lipophilic extracts at which no significant cytotoxic effect is observed was evaluated. For this purpose, one normal cell line (immortalized murine fibroblast cell line BALB/c 3 T3) and four cancer cell lines were selected: BALB/c 3 T3 murine fibroblasts transformed with SV40 virus (SVT2), human adenocarcinoma cells (HeLa), human hepatic carcinoma cells (HepG2) and human breast adenocarcinoma cells (MCF-7). The viability of cells treated for 48 h with increasing amounts of tomato extracts was tested by the MTT reduction assay as an indicator of metabolically active cells.

The results of dose–response experiments are shown in Fig. 2. The values are the average of three independent experiments, each carried out with triplicate determinations. It was observed that 0.24 and 0.6 mg mL^{-1} lipophilic tomato extracts did not affect cell viability significantly, whereas at 1.2 mg mL^{-1} a cytotoxic effect was observed on all cell lines analysed. The concentration at which a cytotoxic effect was observed (1.2 mg mL – 1) was lower than that reported on rat cardiomyoblasts,[7] but in that study a different cell viability assay and incubation time (24 h) were used. Interestingly, both the extracts from unprocessed and processed fruits showed a similar effect on cell survival; however, on MCF-7 cells, a cytotoxic effect of the lipophilic extract obtained from fresh fruits was observed even at the lowest extract concentration. Based on these experiments, 0.6 mg mL^{-1} lipophilic tomato extract was selected as the optimal concentration to analyse the free radical scavenger activity against oxidative stress induced by

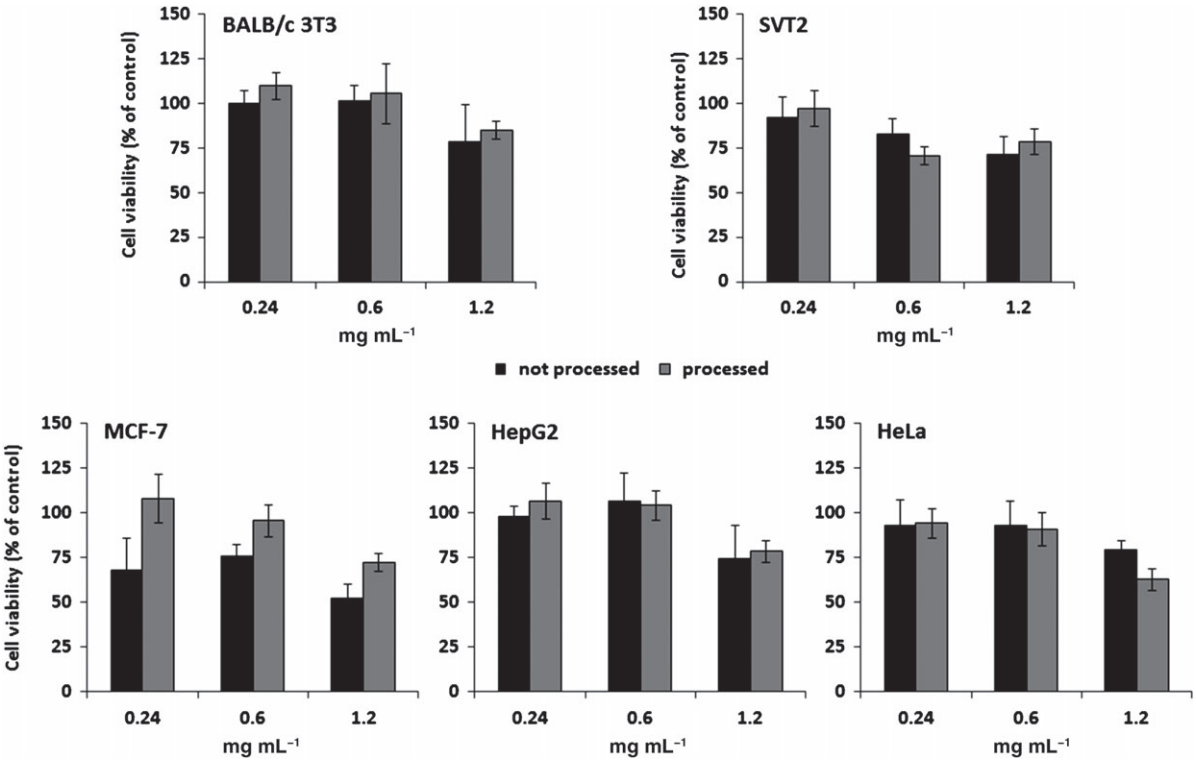

Figure 2. Effects of lipophilic tomato extracts on normal and cancer cells. BALB/c 3 T3, SVT2, MCF-7, HepG2 and HeLa cells were treated with increasing concentrations of lipophilic tomato extracts from unprocessed (black bars) and processed (grey bars) samples for 48 h. Cell viability was assessed by the MTT assay and expressed as described in the 'Materials and Methods' section. All values are given as mean ± SD ($n \geq 3$).

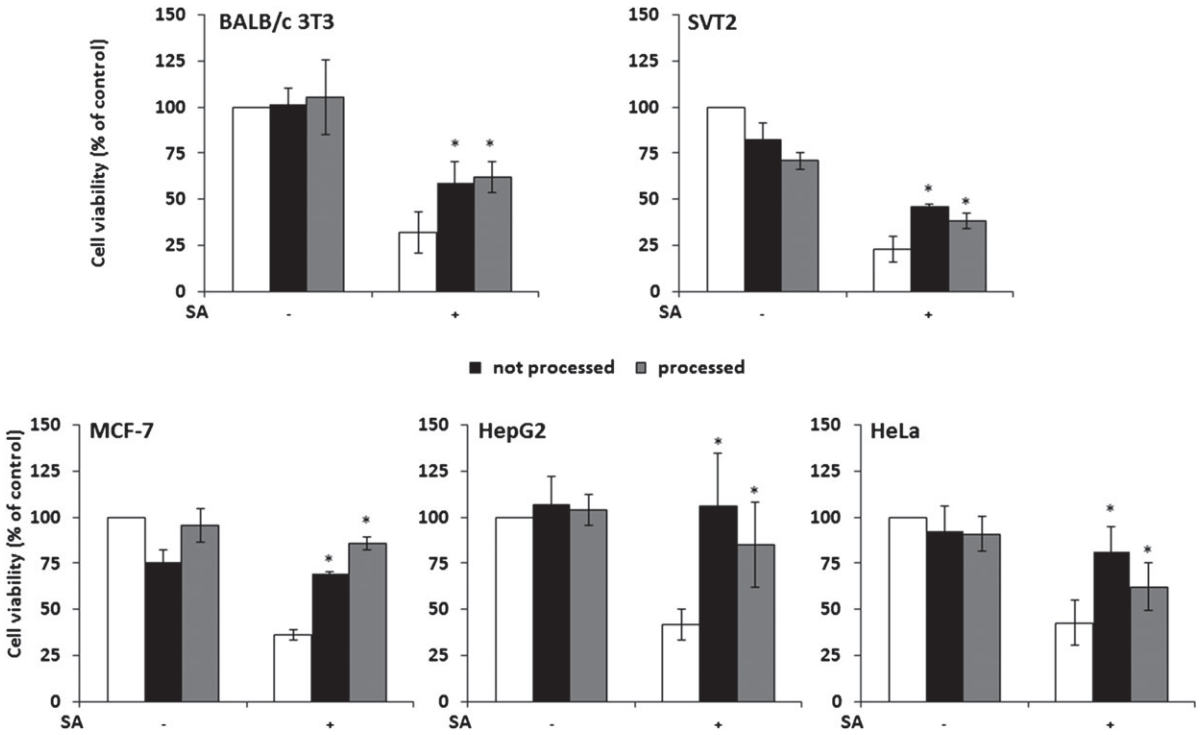

Figure 3. Effects of lipophilic tomato extracts on normal and cancer cells. BALB/c 3 T3, SVT2, MCF-7, HepG2 and HeLa cells were pre-incubated with 0.6 mg mL^{-1} lipophilic tomato extracts from unprocessed (black bars) and processed (grey bars) samples for 48 h and then treated with 300 µmol L^{-1} SA for 2 h. White bars refer to control cells, untreated (−) or treated with SA (+). Cell viability was assessed by the MTT assay and expressed as described in the 'Materials and Methods' section. All values are given as mean ± SD ($n \geq 3$). Asterisks (*) indicate values that are significantly different from SA-treated cells ($P < 0.01$) as determined by Student's *t* test.

sodium arsenite (SA). Among health hazards, inorganic arsenic, present in drinking water, is a major threat to human health, particularly in Asian countries (Bangladesh, Taiwan, Vietnam and India), Argentina, Chile and several states of the USA (Arizona, California and Nevada).[31,32] To inspect the ability of lipophilic tomato extracts to contrast SA-induced oxidative stress, cells were pre-incubated in the presence of lipophilic tomato extracts obtained from fruits processed by thermal treatment or from fresh fruits. Cells were then treated with SA as described in the 'Materials and Methods' section.

Cytoprotective activity of lipophilic tomato extracts

Cell viability was assessed by the MTT assay. As shown in Fig. 3, after SA treatment, cell viability was dramatically reduced compared with control cells in all cell lines analysed (white bars). Noteworthy, when cells were pre-incubated in the presence of lipophilic tomato extracts from either processed (grey bars) or unprocessed (black bars) samples, a strong protective effect was recorded. No significant differences were observed in the protective effect of extracts from unprocessed or processed fruits, thus suggesting that heat treatment does not affect the antioxidant power of tomato extracts. The recovery observed was about 50% in all cell lines analysed (Fig. 3, black and grey bars *versus* white bars). It is interesting to note that the unprocessed lipophilic tomato extract was able to significantly protect MCF-7 cells against oxidative stress even though, when tested in the absence of stress, it exerted a slight cytotoxic effect. The protective effect of the lipophilic extract observed in this study is in line with that reported on rat cardiomyoblasts in the presence of H_2O_2.[7]

Lipophilic tomato extracts mitigate SA-induced ROS production

We chose SVT2 cells as cancer cell line and BALB/c 3 T3 cells as normal cells to deeply inspect the effects of SA on cell redox homeostasis. Since perturbation in the cellular redox status is related to ROS production, we measured ROS levels in cells pre-treated with lipophilic tomato extracts, exposed to SA and treated with H_2-DCFDA. This cell-permeable oxidation-sensitive dye is converted to its fluorescent form in the presence of ROS. As shown in Fig. 4A, in normal cells, no significant ROS production was observed upon exposure to the lipophilic extracts, whereas SA induced an increase of 50% in ROS levels compared with control cells (white bars). Noteworthy, SA-induced ROS production was strongly decreased when cells were pre-treated with both unprocessed (58% decrease) and processed (48% decrease) lipophilic ZBR extracts. No significant difference was observed between unprocessed and processed tomato lipophilic extracts in protecting cells from ROS production. In cancer cells (Fig. 4B), instead, a slight increase in ROS levels was observed when cells were incubated with lipophilic extracts compared with untreated cells (20 and 40% increase for unprocessed and processed extracts, respectively). SA treatment resulted in a high increase in ROS levels (70%) compared with control cells, and pre-treatment with lipophilic extracts induced a significant reduction in ROS production (about 41 and 74% decrease for unprocessed and processed ZBR extracts, respectively). The unprocessed and processed extracts were able to protect cells from ROS production to the same extent. These results suggest that pre-treatment with lipophilic extracts is able to abolish ROS production induced by oxidative stress and that heat treatment does not affect the antioxidant activity of lipophilic extracts. This is particularly interesting considering that

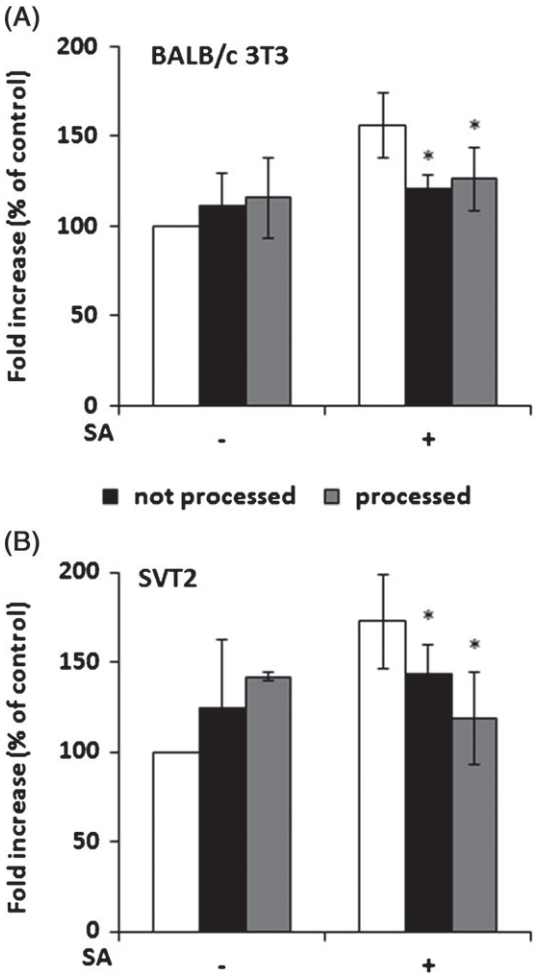

Figure 4. DCFDA assay for detection of intracellular ROS produced in response to oxidative stress in (A) normal and (B) cancer cells. Cells were pre-incubated with 0.6 mg mL^{-1} lipophilic tomato extracts from unprocessed (black bars) and processed (grey bars) samples for 48 h and then treated with 300 µmol L^{-1} SA for 2 h. White bars refer to control cells, untreated (–) or treated with SA (+). Values are expressed as fluorescence intensity compared with untreated cells. Asterisks (*) indicate values that are significantly different from SA-treated cells ($P < 0.01$) as determined by Student's *t* test.

carotenoids such as lycopene are mainly found in *trans* conformation in fresh tomato fruit, whereas they are normally transformed into the more bioactive *cis* form after food processing.[6]

Lipophilic tomato extracts prevent GSH depletion and lipid peroxidation

Since intracellular glutathione (GSH) is the most important antioxidant defence molecule and its depletion is the first hallmark of the progression of apoptosis, we evaluated GSH intracellular levels.

As shown in Fig. 5, pre-treatment of normal (Fig. 5A) and cancer (Fig. 5C) cells with lipophilic extracts had no significant effect on intracellular GSH levels. Oxidative stress induced by SA resulted in about 30% GSH depletion in both cell lines, but GSH intracellular levels were not affected when cells were pre-incubated with lipophilic extracts obtained from either processed or unprocessed samples.

GSH level is directly related to the degree of lipid peroxidation in the cell membrane,[33] as it participates in eliminating lipid peroxidation products by forming a GSH conjugate.[34] Therefore, we analysed lipid peroxidation levels in our experimental system by TBARS assay (Figs 5B and 5D). No lipid peroxidation was observed when cells were incubated in the presence of either unprocessed or processed lipophilic tomato extracts, whereas lipid peroxidation showed a 120 and 150% increase in normal and cancer cells respectively after SA treatment (Figs 5B and 5D). Noteworthy, pre-incubation of cells with lipophilic extracts completely abolished the effect of SA on lipid peroxidation, and no significant difference was observed between cells treated with extracts from processed or unprocessed samples. These results clearly indicate that treatment of cells with lipophilic extracts, either unprocessed or processed, is able to prevent the negative effect of SA-induced oxidative stress.

Lycopene role in protective effects of ZBR extracts

It has been hypothesized that lycopene was the compound responsible for the observed protective effect.

To verify the specific role of lycopene in protecting cells from oxidative stress, ROS production and intracellular GSH levels were analysed by following the same experimental procedure described above. The lycopene concentration used was 0.4 µg mL^{-1}, since this represents the amount of lycopene present in ZBR extracts when used at 0.6 mg mL^{-1}. As shown in Figs 6A and 6B, pre-treatment of cells with lycopene lowered ROS levels after SA treatment to 58% in normal cells and 33% in cancer cells. These values are in agreement with those reported for ZBR extracts. In Figs 6C and 6D, the results of intracellular GSH levels before and after SA treatment are reported. In cancer cells, a strong protective effect of lycopene from oxidative stress was found, as intracellular GSH levels were slightly higher than those of control cells in SA-treated samples. On the contrary, when normal cells were incubated in the presence of SA, no significant protective effect was observed after lycopene treatment. We hypothesize that the higher sensitivity of normal cells compared with cancer cells results in a lower ability of cells to rescue from stress. Altogether, these results demonstrated that other compounds are present in ZBR lipophilic extracts that could play a role in protecting cells, such as β-carotene and vitamin E, thus supporting the idea of a synergistic effect among structurally different lipophilic compounds.[35]

CONCLUSIONS

The results reported in this paper are particularly interesting since carotenoids are known to regulate different cellular pathways and functions,[8] but their bio-absorption is strongly influenced by several factors, including break-up of the food matrix and cooking temperatures. Here it was demonstrated that ZBR lipophilic extracts were able to counteract the detrimental effects induced by oxidative stress on different cell lines and that the high LAA exhibited by ZBR hybrid was mainly due to the presence of lycopene. However, it is noteworthy that ZBR extracts were more effective than lycopene alone in protecting cells, thus supporting the importance of a synergistic effect among antioxidants present in tomatoes.

To date, only a few reports are available on the protective effects of lipophilic tomato extracts on cells. A direct correlation between carotenoid content and antioxidant activity of tomatoes on rat cardiomyoblasts has been previously found.[7] In addition, it was demonstrated that tomato lipophilic and hydrophilic extracts protected cells from H_2O_2-induced cell death and that the antioxidant

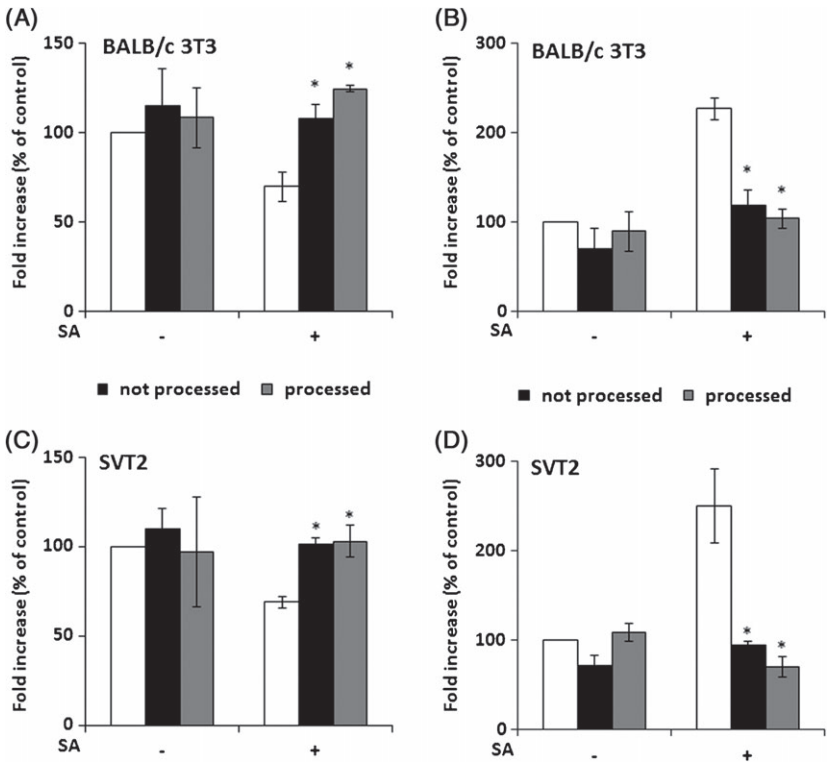

Figure 5. Determination of intracellular GSH and lipid peroxidation in (A, B) normal and (C, D) cancer cells. Cells were pre-incubated with 0.6 mg mL^{-1} lipophilic tomato extracts from unprocessed (black bars) and processed (grey bars) samples for 48 h and then treated with 300 μmol L^{-1} SA for 2 h. White bars refer to control cells, untreated (–) or treated with SA (+). A and C refer to intracellular GSH levels (DTNB assay) and B and D refer to lipid peroxidation levels (TBARS assay). In each experiment, values are expressed as fold increase compared with control (i.e. untreated) cells. Asterisks (*) indicate values that are significantly different from SA-treated cells ($P < 0.01$) as determined by Student's *t* test.

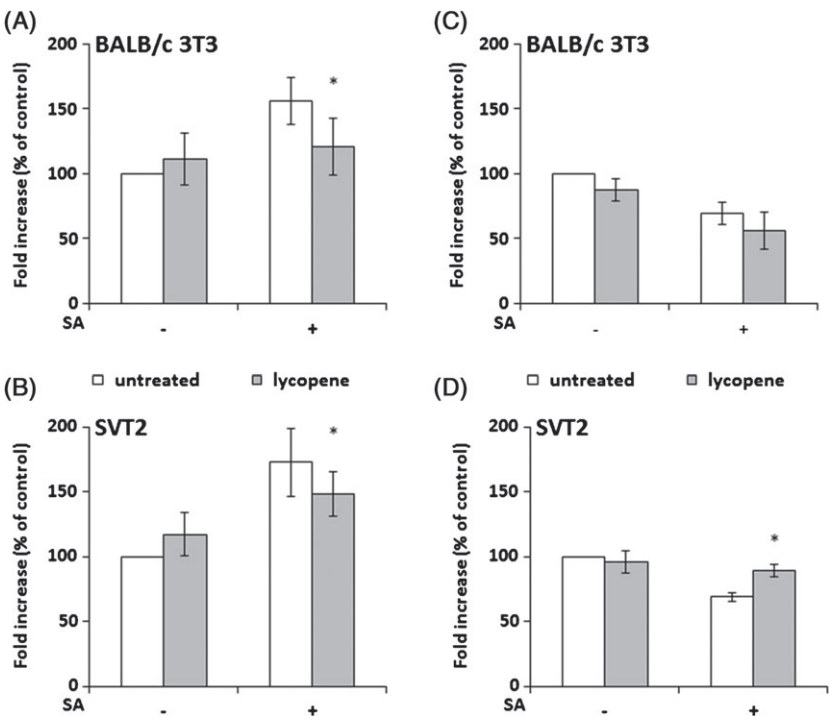

Figure 6. Protective effects of lycopene against oxidative stress in (A, C) normal and (B, D) cancer cells. Cells were pre-incubated with 0.4 μg mL^{-1} lycopene (light grey bars) for 48 h and then treated with 300 μmol L^{-1} SA for 2 h. White bars refer to control cells, untreated (–) or treated with SA (+). (A, B) DCFDA assay; (C, D) intracellular GSH levels. In both experiments, values are expressed as fold increase compared with control (i.e. untreated) cells. Asterisks (*) indicate values that are significantly different from untreated cells ($P < 0.05$) as determined by Student's *t* test.

activity did not change significantly after *in vitro* digestion, thus supporting the hypothesis that gastrointestinal digestion does not alter the antioxidant power of carotenoids.[36] Although many studies report the beneficial effects of lycopene and β-carotene on different cell lines after oxidative stress induction, as far as we know, this study is the first to demonstrate this ability by using lipophilic tomato extracts obtained from fruit before and after heat treatment. This work supports the beneficial role of carotenoids in ameliorating several chronic diseases in which oxidative stress can be considered a hallmark, thus suggesting a therapeutic potential for tomato-based products.

In the future, the study performed here on ZBR cultivar could be carried out also on other tomato genotypes characterized by high LAA in order to verify if they exhibit properties analogous to those exerted by ZBR on different cells. This analysis could be used by breeders to generate new hybrids characterized by higher nutritional levels.

ACKNOWLEDGEMENT

The authors thank GenoPOM-pro Project (PON02_00395_3082360) for the financial support of the activities reported in the present study.

REFERENCES

1 US Department of Agriculture, Economic Research Service, *Tomatoes* [Online]. (2008). Available: http://www.ers.usda.gov/ [September 2015].
2 Giovannucci E, Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J Natl Cancer Inst* **91**: 317–331 (1999).
3 Hwang ES and Bowen PE, Effects of tomato paste extracts on cell proliferation, cell-cycle arrest and apoptosis in LNCaP human prostate cancer cells. *BioFactors* **23**:75–84 (2005).
4 Martínez-Valverde I, Periago MJ, Provan G and Chesson A, Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *J Agric Food Chem* **82**:323–330 (2002).
5 Maiani G, Caston MJ and Catasta G, Carotenoids: actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Mol Nutr Food Res* **53**:S194–S218 (2009).
6 Raiola A, Rigano MM, Calafiore R, Frusciante L and Barone A, Enhancing the health promoting effects of tomato fruit for biofortified food. *Mediators Inflamm* 2014:139873 (**2014**). DOI: 10.1155/2014/139873.
7 Li H, Deng Z, Liu R, Loewen S and Tsao R, Carotenoid compositions of coloured tomato cultivars and contribution to antioxidant activities and protection against H_2O_2-induced cell death in H9c2. *Food Chem* **136**:878–888 (2013).
8 Rao AV and Rao LG, Carotenoids and human health. *Pharmacol Res* **55**:207–216 (2007).
9 Kong KW and Ismail A, Lycopene content and lipophilic antioxidant capacity of by-products from *Psidium guajava* fruits produced during puree production industry. *Food Bioprod Process* **89**:53–61 (2011).
10 Renju GL, Kurup GM and Saritha Kumari CH, Effect of lycopene from *Chlorella marina* on high cholesterol-induced oxidative damage and inflammation in rats. *Inflammopharmacology* **22**:45–54 (2013).
11 Agarwal S and Rao AV, Carotenoids and chronic diseases. *Drug Metabol Drug Interact* **17**:189–210 (2000).
12 Johnson EJ, The role of carotenoids in human health. *Nutr Clin Care* **5**:47–49 (2002).
13 Tang G, Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. *Am J Clin Nutr* **91**:1468S–1473S (2010).
14 Halliwell B and Cross CE, Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect* **102**:5–12 (1994).
15 Gerster H, The potential role of lycopene for human health. *J Am Coll Nutr* **16**:109–126 (1997).
16 Graziani G, Pernice R, Lanzuise S, Vitaglione P, Anese M and Fogliano V, Effect of peeling and heating on carotenoid content and antioxidant activity of tomato and tomato-virgin olive oil systems. *Eur Food Res Technol* **216**:116–121 (2003).
17 Sanchez-Moreno C, Plaza L, de Ancos B and Cano MP, Impact of high pressure and traditional thermal processing of tomato puree on carotenoids, vitamin C and antioxidant activity. *J Sci Food Agric* **86**:171–179 (2006).
18 Friedman M, Anticarcinogenic, cardioprotective, and other health benefits of tomato compounds lycopene, α-tomatine, and tomatidine in pure form and in fresh and processed tomatoes. *J Agric Food Chem* **61**:9534–9550 (2013).
19 Ruggieri V, Francese G, Sacco A, D'Alessandro A, Rigano MM, Parisi M, *et al.*, An association mapping approach to identify favourable alleles for tomato fruit quality breeding. *BMC Plant Biol* **14**:337 (2014).
20 Zouari I, Salvioli A, Chialva M, Novero M, Miozzi L, Tenore GC, *et al.*, From root to fruit: RNA-Seq analysis shows that arbuscular mycorrhizal symbiosis may affect tomato fruit metabolism. *BMC Genomics* **15**:221 (2014).
21 Rigano MM, Raiola A, Tenore GC, Monti DM, Del Giudice R, Frusciante L, *et al.*, Quantitative trait loci pyramiding can improve the nutritional potential of tomato (*Solanum lycopersicum*) fruits. *J Agric Food Chem* **62**:11519–11527 (2014).
22 Wellburn AR, The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiol* **144**:307–313 (1994).
23 Miller JN and Rice-Evans CA, Factors influencing the antioxidant activity determined by the $ABTS^{\bullet+}$ radical cation assay. *Free Radic Res* **26**:195–199 (1997).
24 Galano E, Arciello A, Piccoli R, Monti DM and Amoresano A, A proteomic approach to investigate the effects of cadmium and lead on human primary renal cells. *Metallomics* **6**:587–597 (2014).
25 Camera E, Mastrofrancesco A, Fabbri C, Daubrawa F, Picardo M, Sies H, *et al.*, Astaxanthin, canthaxanthin and β-carotene differently affect UVA-induced oxidative damage and expression of oxidative stress-responsive enzymes. *Exp Dermatol* **18**:222–231 (2009).
26 Frusciante L, Carli P, Ercolano MR, Pernice R, Di Matteo A, Fogliano V, *et al.*, Antioxidant nutritional quality of tomato. *Mol Nutr Food Res* **51**:609–617 (2007).
27 Capanoglu E, Beekwilder J, Boyacioglu D, Hall R and de Vos R, Changes in antioxidant and metabolite profiles during production of tomato paste. *J Agric Food Chem* **56**:964–973 (2008).
28 Re R, Bramley PM and Rice-Evans C, Effects of food processing on flavonoids and lycopene status in a Mediterranean tomato variety. *Free Radic Res* **36**:803–810 (2002).
29 Cano A, Costa M and Arnao MB, Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum* Mill.). *Postharv Biol Technol* **28**:59–65 (2003).
30 Toor RK and Savage GP, Antioxidant activity in different fractions of tomatoes. *Food Res Int* **38**:487–494 (2005).
31 Smedley PL and Kinniburgh DG, A review of the source, behavior and distribution of arsenic in natural waters. *Appl Geochem* **17**:517–568 (2002).
32 Paul S, Chakraborty S, Ali MN and Ray DP, Arsenic distribution in environment and its bioremediation: a review. *Int J Agric Environ Biotechnol* **8**:189–204 (2015).
33 Schneider LA, Dissemond J, Brenneisen P, Hainzl A, Briviba K, Wlaschek M, *et al.*, Adaptive cellular protection against UVA-1-induced lipid peroxidation in human dermal fibroblasts shows donor-to-donor variability and is glutathione dependent. *Arch Dermatol Res* **297**:324–328 (2006).
34 Tarozzi A, Marchesi A, Hrelia S, Angeloni C, Andrisano V, Fiori J, *et al.*, Protective effects of cyanidin-3-*O*-β-glucopyranoside against UVA-induced oxidative stress in human keratinocytes. *Photochem Photobiol* **81**:623–629 (2005).
35 Fernández-García E, Photoprotection of human dermal fibroblasts against ultraviolet light by antioxidant combinations present in tomato. *Food Funct* **5**:285–290 (2014).
36 Li H, Deng Z, Liu R, Loewen S and Tsao R, Bioaccessibility, *in vitro* antioxidant activities and *in vivo* anti-inflammatory activities of a purple tomato (*Solanum lycopersicum* L.). *Food Chem* **159**:353–360 (2014).

