Free Radical Research, 2002 Vol. 36 (7), pp. 803–810

Taylor & Francis
healthsciences

Effects of Food Processing on Flavonoids and Lycopene Status in a Mediterranean Tomato Variety

ROBERTA RE[a], PETER M. BRAMLEY[b] and CATHERINE RICE-EVANS[a,*]

[a]*Wolfson Centre for Age-Related Diseases, Guy's, King's and St. Thomas' School of Biomedical Sciences, King's College, Hodgkin Building, Guy's Campus, Guy's Hospital, St. Thomas Street, London Se1 9RT, UK;* [b]*School of Biological Sciences, Royal Holloway, University of London, Egham Surrey, TW20 0EX, UK*

Accepted by Professor B. Halliwell

(Received 26 June 2001; In revised form 2 January 2002)

This research is focused on the antioxidant properties of dietary components, in particular phenolics and carotenoids and the assessment of the contribution of the combined antioxidants to the total antioxidant activity (TAA) of tomato fruit. The aim of this study was to analyse the effects of processing on the antioxidant properties of tomato. The effects of three different methods of processing fresh tomatoes into tomato sauce were investigated with respect to the antioxidant properties of the fruit. Identification and quantification of the main carotenoids and flavonoids present in tomatoes was achieved by HPLC analysis and the effect on the concentration and availability of these compounds was investigated at different stages of the processing.

The processing affected mainly naringenin causing a reduction in the concentration. Conversely, levels of chlorogenic acid were increased suggesting an improvement in availability of this compound to extraction. The concentration of all-*trans*-lycopene was also increased following processing. Less than 10% isomerisation of all-*trans*-lycopene to the *cis* form was detected for all the methods analysed.

The effects of processing on the overall antioxidant activity support the theory of a general improvement in availability of individual antioxidants. For both hydrophilic and lipophilic extracts TAA values were increased.

Keywords: Food processing; Tomato; Carotenoids; Flavonoids; TEAC

INTRODUCTION

Epidemiological data provide evidence that consumption of diets rich in fruit and vegetables is associated with a reduction in risk of coronary heart disease and certain cancers.[1] Fruit and vegetables are rich sources of carotenoids, flavonoids and an array of other phytochemicals. Recently, interest has focused on the association between intake of diets containing tomato-based products and the decreased risk of a variety of cancers.[2–5]

Only a small amount of tomato fruit is consumed in its raw state, while most of it is eaten after processing, mainly into sauces. Consumption of processed tomatoes represents one of the main sources of this fruit in diet. On this basis the evaluation of the influence of food processing on naturally occurring antioxidants is a key factor in finding the conditions necessary to preserve or improve the activity and bioavailability of these compounds. This is, from a nutritional point of view, an important step in the correct interpretation and evaluation of studies regarding dietary habits and human health.[6]

Processing methods are generally believed to be responsible for a depletion of naturally occurring antioxidants in food. Therefore processed fruit and vegetables could be expected to have lower health-protecting capacities than fresh ones. This view is based on the analysis on unstable antioxidants, such

*Corresponding author. Tel.: +44-0-207-848-6141. Fax: +44-0-207-848-6143. E-mail: catherine.rice-evans@kcl.ac.uk

ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10715760290032584

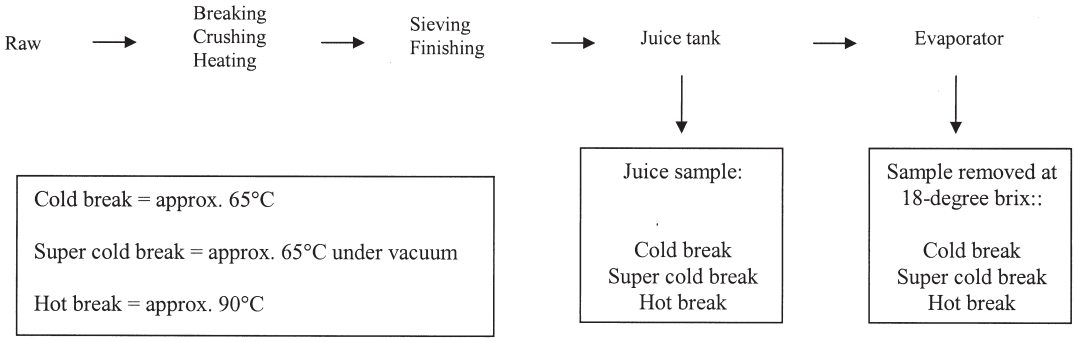

FIGURE 1 Scheme of the processing of the Sorrento tomatoes.

as ascorbic acid, as indicators of processing damage. Data on the adverse effects of light, oxygen and heat on the oxidation of polyphenols and tocopherols and the loss of vitamin A activity, as a consequence of β-carotene isomerisation, have also been reported.[7,8]

We have evaluated the effects of food processing on tomato antioxidants by analysing modifications to the concentrations of the main phenolic components and the effect on the total antioxidant activity (TAA). Tomatoes and tomato products are also the main source of dietary lycopene. The issue is also whether the processing influences the concentration of lycopene and how the antioxidant activity is affected. Lycopene has been shown to be a strong antioxidant *in vitro*[9,10] and is amongst the major carotenoids in human serum.[11–13] Lycopene is more bioavailable from processed tomatoes than from raw tomatoes[14,15] and the relevance of the matrix in which the lycopene is present has also been investigated.[16,17]

MATERIALS AND METHODS

An Italian tomato variety (Sorrento) was used for processing and three different methods of processing were evaluated by procedures indicated in Fig. 1: hot break (HB)-processed at 90°C, cold break (CB)-processed at 65°C and super cold break (SCB)-processed at 65°C under vacuum. Whole fruit, before processing, were used as a control. Two stages for each process were investigated, sauce before the evaporation (S) and the paste obtained after evaporation (P).

Freeze Drying and Extraction of Tomato Fruit

Whole fruit was diced in small pieces and the wet weight was recorded. The fruit was freeze-dried and the resulting material was weighed before being powdered under liquid nitrogen and then divided into individual samples to be stored at −70°. The same procedure was followed for the processed tomatoes.

Carotenoid Extractions

Freeze dried samples were extracted with dichloromethane (DCM)/water 1:1 (v/v), vortex-mixed for two minutes and centrifuged at 1000 rpm for 15 min in a MSE Mistral 6000 centrifuge (MSE Ltd., Crowley, UK) to enhance the separation of the two phases. The organic layer was removed and the extraction process repeated by adding a further aliquot of DCM to the water layer. The two extracts were combined, passed through filter paper (Whatman, Maidstone, UK) and subjected to rotary evaporation at 30° to remove the organic solvent. The extract was reconstituted in a known amount of DCM and aliquoted. Samples were evaporated under nitrogen and stored at −70°C.

Flavonoid Extractions

Freeze dried powder was combined with 50% methanol/water and refluxed for 30 min. The refluxed sample was then centrifuged at 2400 rpm for 15 min in a MSE Mistral 6000 centrifuge. The supernatant was rotary evaporated under vacuum at 40°C to remove the methanol and the resulting aqueous extract aliquoted and stored at −70°C.

Analysis of Lycopene by Reverse Phase HPLC

Reverse phase analysis was employed for analysis on a Hewlett-Packard system 1100. All-*trans* lycopene was quantified by reference to standard curves constructed using solutions of crystalline standard AASP (Antioxidants Analysts Supplies and Consultants, Stanton Industrial Estate, Southampton, UK). Diode array detection was at 470 nm, the flow rate was 1 ml/min and the column temperature was maintained at 25°C. Lycopene isomers were analysed using a 250 × 4.6 mm C_{30} HPLC column (YNC Inc., Wilmington, NC USA) coupled to a 20 × 4.6 mm C_{30} guard. Solvent A was methanol, solvent B was methanol/water 80:20 (v/v) containing 0.2% (w/v) ammonium acetate, and solvent C was *tert*-butyl-methyl ether. The following gradient elution pro-

TABLE I Phenolic concentrations at different stages of processing

		Hot break		Cold break		Super cold break	
mg/kg dry wt	Whole fruit	Pre-evaporated	Evaporated	Pre-evaporated	Evaporated	Pre-evaporated	Evaporated
Chlorogenic acid	36.71 ± 2.2	86.45 ± 11.5	92.11 ± 19.7	31.03 ± 2.9	101.77 ± 1.9	25.66 ± 7.0	73.23 ± 16.7
Caffeic acid	77.33 ± 7.9	96.13 ± 18.9	99.33 ± 22.0	108.26 ± 10.4	93.37 ± 11.7	114.85 ± 25.1	138.26 ± 19.3
p-Coumaric acid	78.11 ± 12.3	83.88 ± 12.7	88.75 ± 14.8	78.50 ± 5.82	78.41 ± 7.6	111.57 ± 28.9	108.95 ± 20.1
Ferulic acid	39.88 ± 6.6	31.99 ± 10.4	38.29 ± 12.5	31.21 ± 9.3	37.60 ± 4.0	56.73 ± 20.0	54.95 ± 15.5
Rutin	319.29 ± 47.6	240.44 ± 45.6	253.86 ± 64.3	154.47 ± 19.8	239.81 ± 20.8	227.36 ± 47.0	279.64 ± 52.3
Naringenin	224.82 ± 59.3	22.07 ± 4.0	44.80 ± 19.5	20.13 ± 3.9	34.24 ± 9.5	19.76 ± 7.4	19.61 ± 4.2

Mean ± SD, $n = 3$.

gram was followed (60 min in total): 95A/5B/0C maintained for 6 min, changed linearly to 80A/5B/15C over 1 min, maintained for 5 min, changed linearly to 30A/5B/65C over 18 min, maintained for 18 min returned linearly to 95A/5B/0C over 2 min. The column was equilibrated for 10 min between injections. Assignment of isomers was made by reference to the retention time and the absorption spectra by photodiode array according to Holloway *et al.*[18]

Preparation of Standards

All flavonoid standards were prepared in methanol at 1 mg/ml apart from rutin, which was prepared at 0.5 mg/ml. For flavonoid analyses, 500 μl of extract was incubated at 37°C either with or without 0.5 mg β-glucosidase for 2 h. The internal standard, salicylic acid (10 μg/ml), was added prior to incubation. Mobile phase A (see below) was added to all extracts to a final volume of 1 ml. All samples were passed through a 5 μm filter prior to HPLC analysis. Stock solutions of all-*trans* lycopene were prepared in dichloromethane (DCM) and concentrations determined using the extinction coefficient. Standard curves were produced by evaporation of known amounts of stock solution and reconstituting in ethyl acetate for HPLC analysis. Canthaxanthin (Hoffman-La Roche, Basel, Switzerland) was used as internal standard.

Analysis of Flavonoids by Reverse Phase HPLC

HPLC analysis was carried out on a Waters system with Millennium 32 software (Milford, USA). Analyses were performed using a 250 × 4.6 mm C_{18} Nova-Pak column maintained at 30°C. A linear gradient at a flow rate of 0.6 ml/min was employed. Mobile phase A consisted of methanol/water/5 M HCl (80:20:0.1, v/v) and solvent B was acetonitrile. The following gradient was run for 60 min in total: 95A:5B from 0–10 min, decreasing linearly to 50:50 by 35 min, held for a further 5 min and back to 95A:5B at 45 min for the following 15 min.[19] A ten minute period was allowed between injections to ensure re-equilibration of the column. All solvents were passed through an on-line degasser. Data were collected at a monitoring wavelength of 320 nm. Peak identification was by retention time, spectral characteristics and spiking with authentic standards.

Total Antioxidant Activity Assay

Trolox (™Hoffman-La Roche) (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid, Aldrich Chemical Co., The Old Brickyard, Gillingham, Dorset SP8 4BR, UK) was used as an antioxidant standard. It was prepared in ethanol at 2.5 mM, for

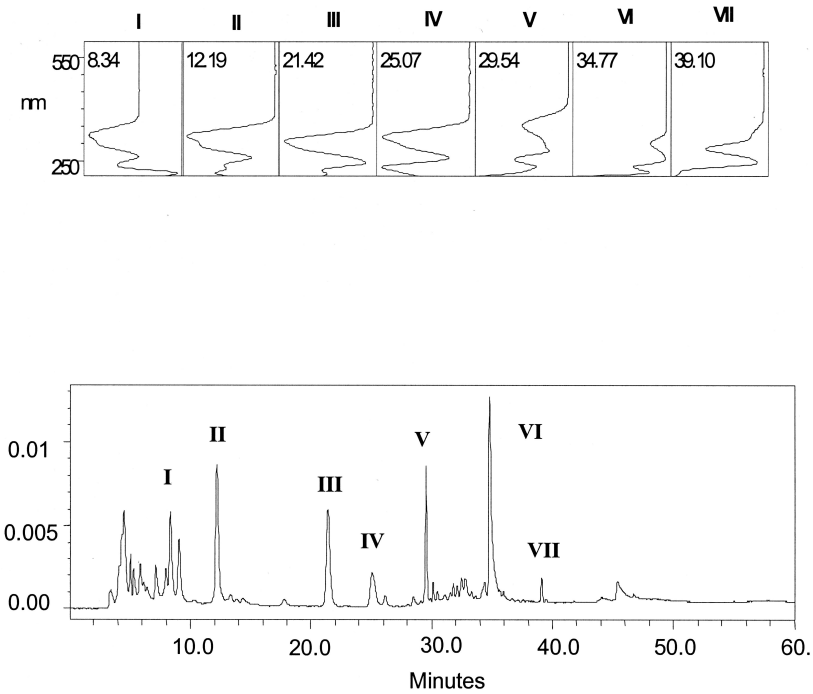

FIGURE 2 Representative chromatogram of enzyme-treated phenolic sample of Sorrento tomatoes. (I) Chlorogenic Acid (quinic acid ester), (II) Caffeic Acid, (III) *p*-Coumaric Acid, (IV) Ferulic Acid, (V) Rutin (diglycoside), (VI) Salicylic Acid (IS) and (VII) Naringenin.

use as a stock standard. Fresh working standards were prepared daily. ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt, and potassium persulphate (di-potassium peroxdisulphate) were obtained from Sigma-Aldrich (Fancy Road, Poole, Dorset BH12 4QH, UK) and HPLC grade ethanol from Rathburn Chemicals Ltd. (Caberston Road, Walkerburn, Peebleshire, Scotland EH43 6AU).

Flavonoids and hydroxycinnamates were obtained from Extrasynthese (Extrasynthese, B.P. 62, Z.I. Lyon-Nord, 69726 Genay Cedex, France). Stock solutions of the carotenoids were prepared in dichloromethane and concentrations confirmed using extinction coefficients. Stock solutions of flavonoids and hydroxycinnamates were prepared in ethanol and subsequently diluted in ethanol for introduction into the assay system at concentrations within the activity range of the assay (1.5–15 μM

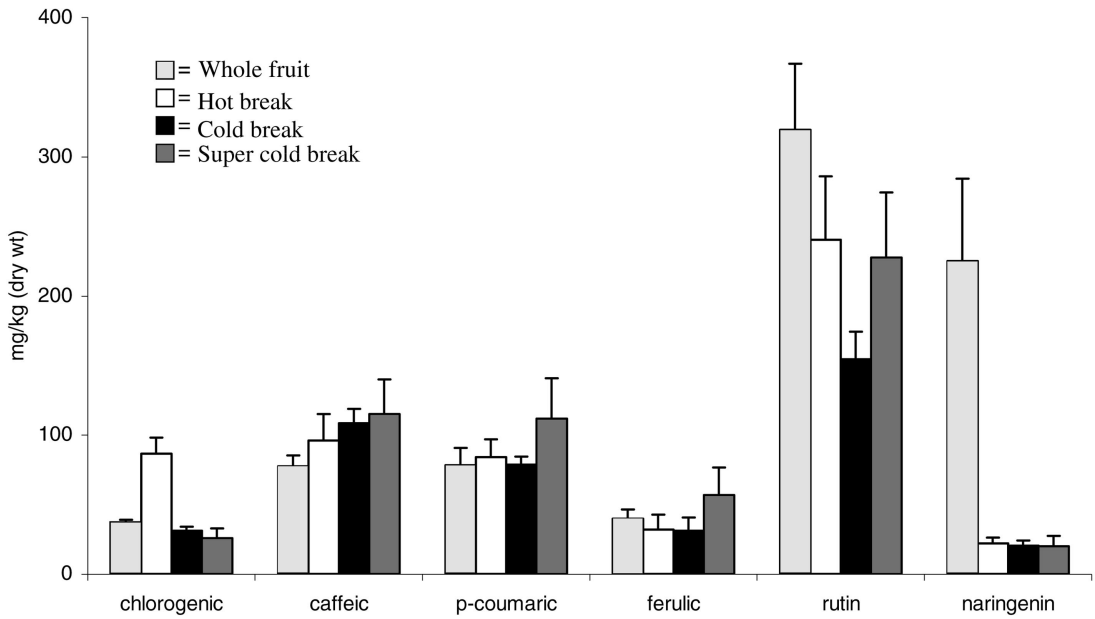

FIGURE 3 Changes to the phenolic concentrations after processing (Mean ± SD, $n = 3$).

TABLE II Total Antioxidant Activity (TAA) of phenolic extracts at different stages of processing

Phenolic extracts		Dry weight	Wet weight
Whole fruit		16.15 ± 0.1	1.14 ± 0.0
Hot break	Pre-evaporated	22.93 ± 1.4	0.03 ± 0.1
	Evaporated	23.98 ± 1.2	4.85 ± 0.2
Cold break	Pre-evaporated	19.37 ± 0.1	0.96 ± 0.0
	Evaporated	20.00 ± 0.4	4.35 ± 0.1
Super cold break	Pre-evaporated	19.69 ± 0.4	1.02 ± 0.0
	Evaporated	20.32 ± 0.8	4.9 ± 0.2

Mean ± SD, $n = 3$.

final concentration). None of the solvents interfered in the assay. The antioxidant activity was assessed according to Re *et al.*[20] using a 4 min time point. Experiments were performed on the Hewlett-Packard spectrophotometer model HP 8453 (Heathside Park Road, Cheadle Heath, Stockport Cheshire SK3 0RB, UK) fitted with peltier temperature control.

RESULTS

Hydrophilic Extract

After extraction, the phenolic constituents were separated by HPLC analysis and the TAA assessed. Six flavonoids were quantified, three of which were the hydroxycinnamates, caffeic acid, *p*-coumaric acid and ferulic acid (Table I). Identification of the hydroxycinnamic acids was possible following cleavage of glucose moieties with β-glucosidase. Chlorogenic acid (a quinic ester), rutin (a rhamnoglucoside) and naringenin were unaffected by

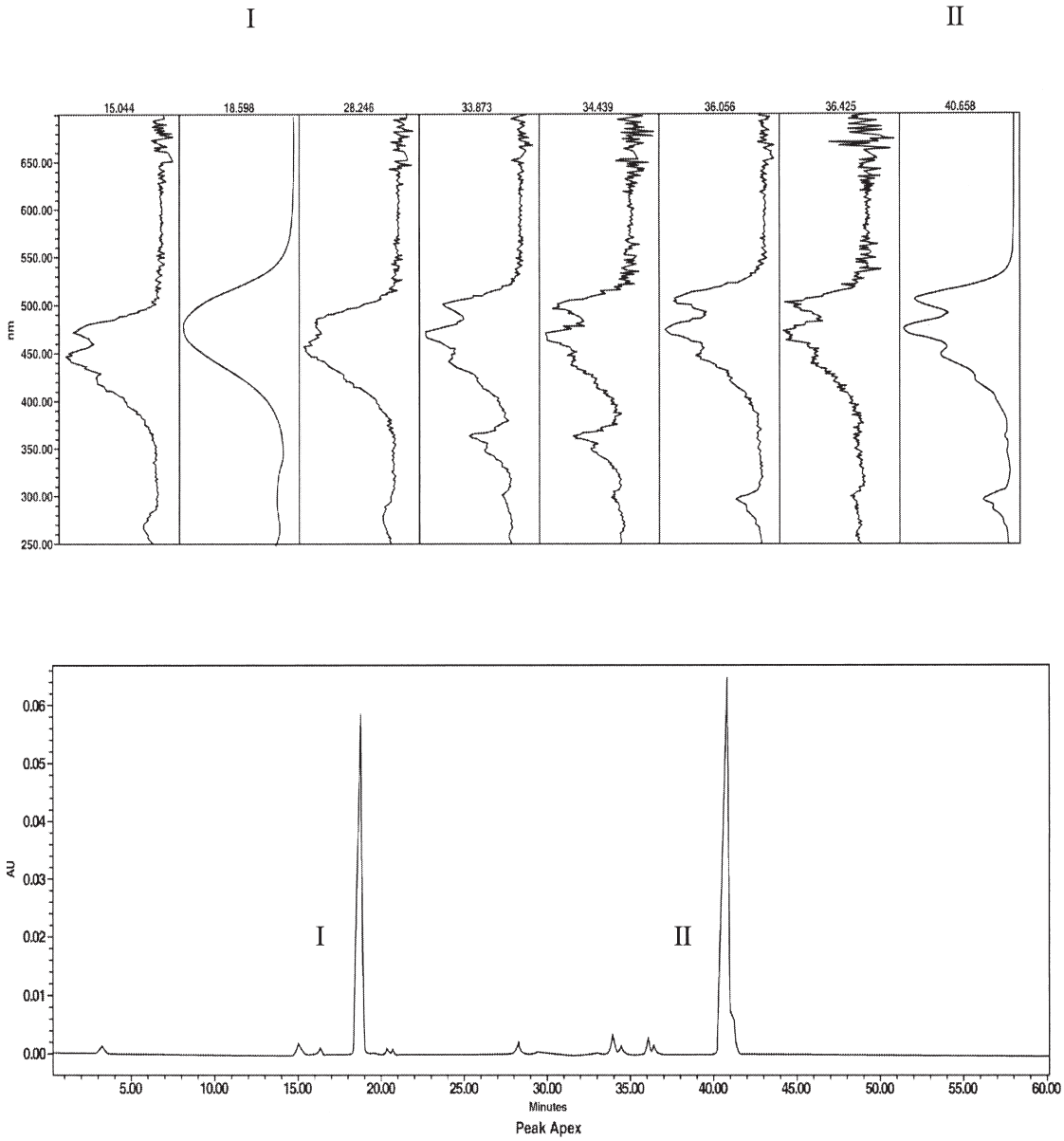

FIGURE 4 Representative chromatogram of the lipophilic extract from Sorrento tomatoes. (I) Canthaxanthin (IS) and (II) *trans*-Lycopene.

TABLE III All-*trans* lycopene concentration at different stages of processing

		Trans lycopene	*Cis*-lycopene	Total lycopene
Whole fruit		91.73 ± 9.3	7.68 ± 0.4	99.41 ± 9.0
Hot break	Pre-evaporated	264.42 ± 15.0	16.90 ± 3.0	281.32 ± 16.7
	Evaporated	201.41 ± 11.1	11.63 ± 0.5	213.04 ± 11.3
Cold break	Pre-evaporated ($n = 2$)	242.06	16.4	258.4
	Evaporated	331.22 ± 14.6	18.18 ± 2.5	349.40 ± 17.2
Super Cold Break	Pre-Evaporated	144.87 ± 13.2	12.08 ± 0.4	156.95 ± 13.6
	Evaporated	148.74 ± 10.7	8.14 ± 0.6	156.88 ± 11.3

Mean ± SD, $n = 3$.

the enzyme treatment as they are not present in the tomato fruit as glycosides. Figure 2is a representative chromatogram of enzyme treated phenolic sample.

When the whole fruit was analysed, rutin was the flavonoid present at the highest level, followed by naringenin. Chlorogenic and ferulic acid were the lowest (Table I). Caffeic and *p*-coumaric acid were present at similar levels. The effect of the three different processing methods, hot break (HB), cold break (CB) and super cold break (SCB) on the concentration of each flavonoid was analysed. Naringenin was the most affected by processing. Its concentration dropped by about 90% after processing ($p < 0.001$). Rutin was mainly affected by the CB ($p < 0.01$), where the concentration dropped by approximately 50%. The three hydroxycinnamates showed a similarity whereby SCB produced an increase in their concentration by about 30%. Chlorogenic acid also showed an increase (approx. 60%) but in this case after HB. CB and SCB did not cause any change (Fig. 3).

During the processing the tomato sauce (S) is evaporated to obtain a paste (P). The effect of this process on the flavonoid concentration was investigated. No significant differences were detected in the HB process, between the pre and post evaporation. If anything, naringenin and rutin concentrations seemed to be slightly increased after the evaporation process, but the statistical analysis did not show significance.

In the CB process after evaporation, the chlorogenic acid concentration was significantly increased from 31.03 ± 2.9 to 101.77 ± 1.9 mg/kg dry weight ($p < 0.001$). The rutin concentration was also increased from 154.47 ± 19.8 to 239.81 ± 19.8 mg/kg dry weight, with a small increase in naringenin from 20.13 ± 3.9 to 34.24 ± 9.5 mg/kg dry weight after evaporation, the latter not reaching significance. In the SCB after evaporation, the chlorogenic acid concentration was significantly increased from 25.66 ± 7.0 to 73.23 ± 16.7 mg/kg dry weight ($p < 0.01$). Other changes were not significant. Naringenin, ferulic acid and *p*-coumaric acid did not show any modifications, but a small change was detected in the caffeic acid level.

The overall antioxidant activity of the hydrophilic extract was improved after processing. HB was significantly higher than CB and SCB ($p < 0.01$), but there was no significant difference between the CB and SCB methods. The TEAC value for the whole fruit was significantly lower than the values obtained for the tomato sauce, HB, CB, and SCB, but no significant increase was detected after evaporation in the paste for any of the processing methods investigated (Table II).

Lipophilic Extract

Lycopene constitutes the major carotenoid in tomato and tomato products. As it is light and temperature sensitive, this compound can potentially be destroyed by heat during processing. The results obtained in this study showed that the concentration of total lycopene was 99.4 ± 9.0 mg/kg dry weight in the whole fruit. Tomato contains up to 90% water so the equivalent concentration of lycopene in the fresh tomato based on its water content was about 7 mg/kg fresh weight. A representative HPLC chromatogram of the lipohilic extract is reported in Fig. 4.

For all the processing methods analysed, lycopene showed an increase in concentration (Table III). In particular, in the CB process, the lycopene concentration was 2.5 times higher compared to the whole fruit values ($p < 0.001$). This value was even higher after evaporation where the total lycopene concentration was increased by almost 30% in the paste. The lycopene concentration was almost doubled in the sauce after HB ($p < 0.001$) but no increase was detected after evaporation. The SCB was the method with the least modification of the lycopene concentration, but still a higher value was found compared to the unprocessed fruit ($p < 0.01$). As in the HB the evaporation process did not affect the lycopene. All the processing methods improved the availability of the lycopene where the mechanical destruction of the matrix, in which the lycopene is present, improves the availability of this compound.

Approximately 90% of lycopene present in nature is in the *all trans* form and isomerisation can be

TABLE IV TAA of the lipophilic extract

Carotenoid extract		Dry weight	Wet weight
Whole fruit		5.97 ± 0.6	0.42 ± 0.0
Hot break	Pre-evaporated	11.99 ± 1.4	0.54 ± 0.1
	Evaporated	11.98 ± 1.0	2.42 ± 0.2
Cold break	Pre-evaporated ($n = 2$)	15.02	0.75
	Evaporated	20.27 ± 0.4	4.41 ± 0.1
Super cold break	Pre-evaporated	12.31 ± 0.3	0.64 ± 0.0
	Evaporated	11.88 ± 0.4	2.87 ± 0.1

Mean ± SD, $n = 3$.

caused by exposure to light and high temperature. Analysis of the isomeric composition showed no change in the pattern in any of the methods investigated, before or after evaporation. Less than 10% of lycopene detected was in the *cis* form in the tomato fruit and the percentage of *cis* isomers did not increase in the sauce of the paste.

The TAA response was linear with the change in concentration of lycopene. The TEAC values for the CB samples, before and after evaporation, are 3–4 times higher than the whole fruit values and double the whole fruit for the HB ($p < 0.001$) and SCB ($p < 0.001$). No difference could be detected for these two methods after evaporation (Table IV).

DISCUSSION

A number of factors are known to promote free radical-mediated reactions in food during processing and storage causing oxidative degradation.[7] In fruit and vegetables the loss of natural antioxidants is of great nutritional importance because these compounds are believed to inhibit *in vivo* the initiation and progression of a range of human diseases.[21] Previous studies dealing specifically with tomatoes have predominantly examined ascorbic acid, tocopherols and carotenoids.[8,14,17]

A study on heat-based processing of tomato[8] shows a loss in ascorbic acid of 38% from the raw fruit to the sauce and a further 16% after processing to produce a paste. These workers also observed a 20% loss of α-tocopherol during thermal processing of tomato paste and 33% of γ-tocopherol, although on the basis of the quantitative change (μg lost) the contribution of the two tocopherol forms in the antioxidant process is α-tocopherol > γ − tocopherol. When the carotenoid content was analysed, they found that the lycopene content was increased in the tomato paste by approximately 27%. The percentage of isomerisation was less than 5% and did not change during processing.

The present study has analysed the effect of three different processing methods on lycopene, the main carotenoid present in tomatoes, and the phenolic components. Of the flavonoids identified though HPLC separation, the processing affected mainly naringenin. All three methods caused a reduction in the concentration of naringenin. Conversely, levels of chlorogenic acid were increased after hot break and remained constant after evaporation. The same increase was detected after evaporation for the other two methods, CB and SCB, suggesting an improvement in availability of this compound to extraction. To a smaller extent, evaporation seems to improve the availability of rutin after cold and super cold break, but the levels extracted remain lower compared to the content of the whole fruit. The effects of processing on the overall antioxidant activity support the theory of a general improvement in availability of individual antioxidants. For both hydrophilic and lipophilic extracts, TAA values were increased. A 20% higher TAA was determined for the hydrophilic extract and up to a 70% increase in the lipophilic extract, after evaporation in the cold break method.

Lycopene levels are expected to be slightly lower in processed tomatoes due to the fact that the skin, which is high in lycopene, had been removed. However, the concentration of all-*trans*-lycopene was increased following processing. All processing methods analysed showed an increase in concentration of between 40 and 60% and more than a 70% increase in the tomato paste was obtained in the cold break method. Due to the high sensitivity of carotenoids to heat, isomerisation of all-*trans*-lycopene to the *cis*form was expected. Less than 10% isomerisation was detected for all the methods analysed.

The majority of studies published to date have compared the bioavailability of lycopene from different food matrices[14–17] and have indicated that lycopene is better absorbed from processed forms of tomato than form raw fruit. Stahl and Sies,[14] studied the uptake of lycopene from processed and unprocessed tomato juice showing that boiling the tomato juice for 1 h with 1% corn oil would increase the bioavailability. A similar response was observed when comparing raw tomatoes with tomato paste when supplementing a single dose equivalent to 23 mg of lycopene. Processing affects the uptake of carotenoids by

helping to destroy the matrix in which carotenoids are present and lipid aids the extraction into the lipophilic phase.[15] *Cis*-isomers of lycopene account for $>50\%$ of the total lycopene found in human serum and tissues,[14,22] yet in tomatoes and tomato-based food products, all-*trans* lycopene comprises 79–91% of total lycopene.[8] This has lead to the theory that *cis*-isomers of lycopene may be better absorbed and thus more bioavailable than the all-*trans* form.[14,23]

The matrix in which lycopene is present in food also seems to influence the level of isomerisation *in vitro*. The food matrix may contribute greatly to the stability of the all *trans* form of lycopene in the fruit. This is supported by the observation that when tomatoes are heat processed, less than 10% isomerisation is noted. Heat treatment improves the bioavailability of lycopene without significantly changing the isomer composition of the heat-treated food.[14,15]

In conclusion, the antioxidant properties of tomatoes are improved by processing. *In vitro* availability of both hydrophilic and lipophilic constituents is improved by processing, suggesting improved bioavailability. Consumption of tomato products, such as ketchup and tomato sauces, can be considered an important element for a healthy diet. The enhanced bioavailability of lycopene from processed tomato products, as seen in intervention trials,[14,15,18,24] can be justified by the improved availability of this compound from the matrix of the food. Detailed knowledge of the form of lycopene isomers present in plasma after supplementation with tomato products is still required to understand the bioactivity of this carotenoid *in vivo*.

Acknowledgements

Syngenta is acknowledged for its financial support.

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