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Effects of cutting and maturity on antioxidant activity of fresh-cut tomatoes

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Abstract

To investigate the changes in total antioxidant activity of fresh-cut tomato during storage, tomato fruits harvested at three different stages of maturity were cut into 7-mm thick slices and stored at 5 °C. Intact fruits were stored in the same conditions as a control. The antioxidant activity was evaluated as the capacity to scavenge the radical $ABTS^{\cdot+}$ in both hydrophilic (HAA) and lipophilic (LAA) extracts. Cutting resulted in a decrease in the HAA compared to the control fruits and did not influence significantly the LAA. Changes in LAA during storage were described by a simple exponential model developing towards an asymptotic end value. The HAA also decreased exponentially in the beginning of the storage time but increased again afterwards. For both hydrophilic and lipophilic antioxidant activity the riper the fruit the higher was the antioxidant activity. Since no relevant interaction was found between time of storage and stage of maturity, the major factor determining the level of antioxidants in tomatoes seems to be the initial level of antioxidant activity at the moment of harvest. The levels of HAA did not differ significantly due to ripening during storage, while the LAA increased with ripening. These results indicate a potential effect of processing to decrease the antioxidant activity in vivo. This would represent a decrease in the value of cut tomatoes as a source of hydrophilic antioxidants in the diet compared with the fruit stored intact. In another experiment, realized at the same conditions, the total antioxidant activity was evaluated using a lipid peroxidation inhibition assay. Antioxidant activity could be measured in methanol extracts, but not in THF extracts. Aqueous extracts showed pro-oxidant activity. No effect of cutting or storage time was observed.

Keywords: Minimally processed; Fresh-cut tomato; Radical scavenging capacity; Lipid peroxidation inhibition; *Lycopersicum esculentum*; Modelling

1. Introduction

The consumption of a diet rich in fresh fruits and vegetables has been associated with a number of health benefits including the prevention of chronic diseases (Klerk, Veer, & Kok, 1998; WHO, 2003). This beneficial effect is believed to be due, at least partially, to the action of antioxidant compounds, which reduce oxidative damage in the body. While the prescription of supplements containing antioxidants has resulted in contradictory results upon human health, the results from epidemiological studies comparing populations with different diets show a clear trend in reduction of chronic diseases when there is an increase in the consumption of fruits and vegetables (Klerk et al., 1998). Because of that, campaigns to increase the consumption of these products have been launched in many countries (http://www.5aday.com/html/international/intmembers.php).

The fresh-cut industry claims their product is a convenient and health alternative to fulfil the dietary needs for fresh food and many fast food companies are diversifying their menu in order to offer a range of ready-to-eat salads to their clients. However, the

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many changes that happen in fruits and vegetables during harvesting, handling and processing can affect antioxidant status (Lindley, 1998). Data on content and retention of bioactive compounds in minimally processed fruits and vegetables are sparse (Lindley, 1998). Fresh cut tissues are primarily submitted to oxidative stress, presumably causing membrane damage and altering the composition and content of antioxidant compounds, resulting in changes in the total antioxidant activity of the tissue.

Decrease in the antioxidant activity after processing was reported for fresh-cut spinach (Gil, Ferreres, & Tomas-Barberan, 1999) and fresh-cut mandarin (Piga, Agabbio, Gambella, & Nicoli, 2002). In both cases, the antioxidant activity was measured as DPPH (2,2-diphenyl-1-picrylhydrazyl)-radical scavenging activity of methanol extracts. Wounding caused an increase in the antioxidant activity of Iceberg and Romaine lettuce measured as DPPH-radical scavenging activity (methanol extracts), or as FRAP-ferric-reducing antioxidant power assay (both methanol and phosphate buffer extracts) (Kang & Saltveit, 2002). The DPPH-radical scavenging activity of fresh-cut cactus fruits did not change during storage at 4 °C for up to 9 days (Piga, Caro, Pinna, & Agabbio, 2003). No reports on the antioxidant activity of fresh-cut tomatoes are known.

The content and composition of antioxidant compounds change during the ripening of tomato fruits and this is reflected in changes in the antioxidant activity (Cano, Acosta, & Arnao, 2003; Jimenez et al., 2002). If minimal processing operations result in changes in antioxidant activity, it is expected this will depend on the stage of ripeness of the fruit since the chemical composition of the fruit will be different.

One of the greatest difficulties in assessing the antioxidant activity in food products is the choice of the assay to be used. Antioxidants can act by different mechanisms namely inhibiting the generation of reactive species, directly scavenging them or raising the levels of endogenous antioxidant defences by upregulating gene expression (Halliwell, 2002). Radical scavenging methods are the most widely used because they are sensitive and easy to perform. However, the results must be interpreted cautiously since the ability of antioxidants to scavenge an artificial radical may not reflect the antioxidant activity in vivo nor its action against physiologically relevant radicals (Frankel & Meyer, 2000). Nevertheless, in vitro tests can indicate whether a given compound or food extract has potential to act as an antioxidant in vivo or alternatively that a direct antioxidant action is unlikely (Halliwell, 2002). The antioxidant activity of food or plant extracts can also be evaluated using biological model systems that mimic an in vivo situation, using biological membranes (Sluis, Dekker, Verkerk, & Jongen, 2000) and substrates present in vivo (Chang, Tan, Frankel, & Barrett, 2000).

The objective of the present research was to investigate the changes in total antioxidant activity of fresh-cut tomato during storage, using two methods. One that measures the scavenging of the radical cation ABTS-2,2'-azinobis (3-ethyl-benzothiazoline-6-sulphonate) and the other that measures the inhibition of ascorbate/iron induced lipid peroxidation of rat liver microsomes. The ABTS assay was chosen among the radical-scavenging assays because it allows to assess both hydrophilic and lipophilic extracts and because it has been successfully used to measure the antioxidant activity in fresh tomato and tomato products (Arnao, Cano, & Acosta, 2001; Cano et al., 2003). The second method, that uses rat liver microsomes as oxidizable substrate, has the advantage of being close to an in vivo situation where both an aqueous and a lipid phase are present. This method has been used to measure antioxidant activity of methanol extracts of apple fruit, apple juice, polyphenol standards (Sluis et al., 2000, Sluis, Dekker, Jager, & Jongen, 2001) and methanol extracts of tomato products (Boxtel, 2002). In the present study, this method was used to measure also aqueous and lipophilic extracts of tomato.

2. Materials and methods

2.1. Chemicals

Ascorbic acid and iron (III) sulphate heptahydrate were obtained from Merck. Thiobarbituric acid (TBA) was obtained from BDH chemicals. 2,2-Azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as the crystallized diammonium salt was obtained from Biochemika. Horseradish peroxidase type VI (HRP), Trolox and trichloroacetic acid (TCA) were obtained from Sigma. All other chemicals were of analytical or HPLC grade purity.

2.2. Spectrophotometric measurements

Spectrophotometric measurements were recorded with a SLT spectrophotometer with a microtiterplate reader (lipid peroxidation assay) or with a USB 2000 Fiber Optic Spectrometer (ABTS assay), both interfaced on line with a PC-computer.

2.3. Experiment 1

2.3.1. Harvesting and processing

Tomato fruits (*Lycopersicum esculentum* cv. Belissimo) were harvested at Made (The Netherlands) in a single day in May 2003, in three colour stages, named here as I, II and III that corresponded, respectively, to stages 3, 5 and 9 in the tomato colour scale (kleur stadia-tomaten) of The Greenery (http://www.thegreenery.com).

After harvesting, the fruits were washed in tap water, immersed for 60 s in sodium hypochlorite solution (1 mg/l, pH 6.8) and then rinsed in tap water. Fruits similar in colour, shape and size were grouped in groups of 6. Three were stored intact and three were sliced in 7-mm thick transversal slices. The first and last slices from the stem end were discarded, while the following four were stacked in the same position they had in the fruit in a white polystyrene tray ($138 \times 138 \times 25$ mm) and covered with a plastic film (Magnetron). For each maturity × temperature × storage time combination, five replicates, corresponding to a tray with three fruits were analysed separately. Both sliced and intact tomatoes were stored at 5 ± 0.5 °C. After slicing, but before cooling, five replicates from each maturity stage were analysed. Fruits from stages I and II were analysed every two days and from stage III every day in a total of six evaluations per combination. Temperature data were recorded by an eight channel thermocouple with a personal computer interface.

2.3.2. Sample preparation

At the indicated intervals, the slices were removed from storage. One quarter of each slice was immediately frozen in liquid nitrogen. Intact fruits were cut into slices as described before and immediately frozen in liquid nitrogen. Samples were freeze-dried, ground and stored at −80 °C until analysed as described below.

2.3.3. Extraction procedure

During the whole extraction procedure, the samples were handled in darkness or low light conditions. To an exact amount between 0.1 and 0.2 g of tomato powder 3 ml of water was added. The samples were stirred in darkness for 30 min and centrifuged for 10 min at 7000 rpm (Hettich-Universal 16R). About 1 ml of the supernatant was transferred to Eppendorf tubes and centrifuged again for 10 min at 16,400 rpm at 10 °C (Eppendorf centrifuge 5417R). The supernatant was used in the assay (water extract). The same procedure was repeated after the addition of 3 ml methanol to the remaining pellet (methanol extract), followed by the extraction with 10 ml of tetrahydrofuran (THF extract). The THF extract was diluted four times with methanol to avoid damage to the plastic microtiter plates used in the antioxidant activity measurement. The obtained extracts were kept on ice in darkness until assayed (few hours).

2.3.4. Antioxidant assay

The rat liver microsomes were thawed at room temperature, diluted at least five times with Tris–HCl/KCl buffer (50 mM, pH 7.4) and centrifuged for 1 h at 28,000 rpm at 4 °C. The pellet was resuspended in Tris–HCl/KCl buffer (50 mM, pH 7.4) to obtain a final concentration of 18.8 mg/ml protein. The 48-wells plates containing 210 μl (for water and methanol extracts) or 240 μl (for the THF extract) of diluted microsome per well were pre-incubated for 5 min at 37 °C. After incubation, 60 μl (water and methanol extracts) or 30 μl (THF extract) of the test sample was added. The lipid peroxidation was induced by the addition of 15 μl ascorbic acid (4 mM) and 15 μl $FeSO_4$ (0.2 mM). After incubation for 60 min at 37 °C, the reaction was stopped by the addition of 0.5 ml TBA/TCA–HCl (16.8% w/v TCA in 0.125 N HCl) followed by incubation for 15 min at 80 °C and centrifugation for 15 min at 2500 rpm. A 250-μl aliquot of each sample was transferred to 96-wells plates and the absorption was measured at 540 nm (colour) and 620 nm (turbidity correction) with microtiter-plate reader.

Control reactions were prepared by adding the solvent (water, methanol or THF:methanol (1:3)) instead of the tomato extract. Trolox (700 μM) was used as a positive control and controls without the microsomes were used to account for the interference of pigments from tomato in the assay.

2.3.5. Calculations

The antioxidant activity was expressed as IC_{50}: the concentration of the tomato extract at which the oxidation was inhibited in 50% in a relation to a blank. This value was determined from the inhibition versus concentration curves by a fitting procedure described by (Sluis et al., 2000).

2.4. Experiment 2

2.4.1. Harvesting, processing and storage

Tomatoes (cv. Belissimo) grown in a greenhouse in Made (The Netherlands) were harvested in a single day in April 2004 in the following colour stages: I, II and III corresponding to the following grades of the tomato colour scale of The Greenery: I = grade 3–4; II = grade 5; III = grade 9. Selection of the fruits followed by sanitisation, processing, packaging and storage were done as described for Experiment 1. The first measurement was done one day after processing and then every other day in a total of six evaluations.

2.4.2. Extraction

At the indicated intervals, the slices were removed from storage. Intact fruits were prepared in a manner similar to that described for cut fruits. Extraction was based on the procedure described by (Arnao et al., 2001). The tomato slices were homogenised using a domestic homogeniser Tristar for about 20 s. An exact weight of the homogenate (around 1 g) was immediately mixed with 2 ml of Na-phosphate buffer (pH 7.5) and 5 ml of ethyl acetate and crushed in a homogeniser Heidolph Diax 900 for 1 min. The homogenate was centrifuged at 6000 rpm for 5 min at 5 °C (Hettich-Universal

16R). About 1 ml of each phase was pipetted, transferred to an Eppendorf tube and centrifuged at 16,400 rpm for 5 min at 5 °C (Eppendorf centrifuge 5417R). The hydrophilic extract was analysed immediately. The lipophilic extract was stored at −80 °C overnight and analysed in the next morning.

2.4.3. Antioxidant activity assay

Antioxidant activity was measured using a modified version of the ABTS/HRP decolouration method as described in (Arnao et al., 2001). For the hydrophilic antioxidant activity (HAA), the reaction mixture contained 2 mM ABTS, 20 nM HPR, 60 μM H_2O_2, in 50 mM Na-phosphate buffer (pH 7.5) in a total volume of 2 ml. The reaction was monitored at 730 nm for 1 min to check whether the absorbance was stable. Then, 60 μl of the aqueous extract was added to the reaction medium and the decrease in absorbance, which is proportional to the amount of $ABTS^{\cdot+}$ quenched, was determined at intervals of 1 min, for 5 min. For lipophilic antioxidant activity (LAA), the reaction medium contained 0.7 mM ABTS, 200 nM HRP and 60 μM H_2O_2, in pure ethanol acidified with phosphoric acid (0.6 μl/ml) in a total volume of 2 ml. The absorbance decrease was determined from the difference between the A_{730} values before and 5 min after sample addition. Antioxidant activity was calculated as moles of $ABTS^{\cdot+}$ quenched by 1 mol of Trolox, based on the stœchiometric relationship that 1 mol of ABTS quenches 2 mol of Trolox (Arnao et al., 2001). In both cases, the antioxidant activity was expressed as Trolox equivalents per 100 g of tomato fresh weigh. Total antioxidant activity (TAA) was obtained by the sum of HAA and LAA.

2.5. Internal package atmosphere

To assure that the cut and intact fruits were stored in the same atmospheric conditions, a very permeable film (recommended for use in microwave cooking) was used. In preliminary tests, this film protected the fruits and slices against dehydration without changing significantly the internal atmosphere in the package. After 1 week storage at 5 °C, the O_2 concentration changed from 19.70 ± 0.21% to 21.37 ± 0.22% in the package containing intact fruits and from 19.71 ± 0.13% to 21.26 ± 0.46% in the package containing sliced fruits. The CO_2 concentration changed from 0.083 ± 0.010% to 0.065 ± 0.010% in the package containing intact fruits and from 0.065 ± 0.004% to 0.091 ± 0.01% in the package containing sliced fruits.

2.6. Statistical analysis

Data were first analysed by Analysis of Variance using PROC GLM from SAS (Statistical Analysis System) software considering stage of maturity, treatment (processing or not) and storage time as sources of variance. Later a non-linear regression analysis was conducted in order to study the changes on time, taking into consideration those factors and interactions that were significant in the analysis of variance. The model was developed as described in the next section and calibrated using the nonlinear regression procedure in GENSTAT (Rothamsted, UK).

2.7. Model development

The changes in the antioxidant activity (AA) of fresh-cut tomato slices was described and analysed with a simple exponential model (first-order kinetics) developing towards an asymptotic end value. The AA was considered to be built up by a variable part that changes according to a first-order mechanism and a fixed part that is invariable or is in equilibrium at the circumstances under study.

This resulted in the basic first-order model as described in

$$AA = (AA_0 - AA_{fix}) \cdot e^{-k \cdot t} + AA_{fix}, \quad (1)$$

where AA is the antioxidant activity at time t after harvest; AA_0 is the initial antioxidant activity at harvest; AA_{fix} is the invariable part of the antioxidant activity; k is the reaction rate constant (at storage temperatures); t is the time (in days), counting from the moment of harvest.

In the present experiment, the tomato fruits were harvested from the same greenhouse at the same growing conditions but at different stages of ripeness. Unlike the development of colour (Lana, Tijskens, & Kooten, 2004) and firmness (Lana, Tijskens, & Kooten, 2005), the level of antioxidants apparently starts to decay only after harvest, especially after processing. The hydrophilic antioxidant activity increased again at the later stages of storage. A linear increase was, therefore, added to the simple exponential model (Eq. (2)). The increase in activity at the later stages of storage was dependent on the treatment. A separate value of k_l was, therefore, allowed for both treatments. To accommodate for the differences in the general level of antioxidant activity between the treatments (cut-whole) and the stage of development (breaker-pink-red), the asymptotic end value was made dependent on these factors.

$$AA = (AA_0 - AA_{fix}) \cdot e^{-k \cdot t} + AA_{fix} + \Delta AA_{stage} + \Delta AA_{treat} + k_{l,\,treat} \cdot t, \quad (2)$$

where ΔAA_{stage} is the shift in AA depending on the stage of ripeness; ΔAA_{treat} is the shift in AA depending on the treatment; $k_{l,\,treat}$ is the rate constant for the linear increase in AA (at storage temperatures), depending on the treatment applied;

2.7.1. Data analysis

Based on Eq. (2), a non-linear regression analysis was performed (Genstat Rothamsted, UK). The data averaged over the five replicates were analysed without further transformation using ripening stage, treatment (cut or whole fruit) and time simultaneously as explaining variables (multi-variate non-linear regression analysis). The kinetic parameter (k), the invariable part of AA_{fix} and the initial value of AA_{var} were estimated in common for all the slices. All other parameters were estimated separately for each stage of maturity of treatment.

3. Results and discussion

3.1. Inhibition of lipid peroxidation

The water extract showed pro-oxidant activity in the lipid peroxidation assay without a clear effect of sample concentration. The THF extracts showed very flat responses curves and no accurate calculation of IC_{50} could be performed. Therefore, the results presented in Fig. 1 are those obtained with methanol extracts without a previous water extraction.

The antioxidant activity of tomato extracts, expressed as IC_{50} (Fig. 1) was affected by the ripening stage of the fruit ($Pr > F = 0.0107$) and storage time ($Pr > F = 0.0160$) but did not differ between cut and intact fruits ($Pr > F = 0.8649$). No interaction between these factors was significant. The averaged antioxidant activity increased from stage I to II and decreased again at stage III (Table 1). The significant effect of time was probably related with the shorter storage time for fruits at stage III (5 days) compared to stages I and II (9 days). When the time interval was considered as number of sampling dates (6 for all stages), and not as days after processing, the effect of time was considerably smaller ($Pr > F = 0.0613$). However, even in the first analysis, the effect of stage was higher than that of storage time, with F values of 4.69 and 2.57, respectively.

Despite of the significant effects indicated by the ANOVA it, was impossible to adjust any model to the data. The variation was quite large (Fig. 1) and no clear trend could be established especially in relation to the effects of storage time and stage of maturity.

A pro-oxidant action of hydrophilic extracts from tomato samples was reported by Lavelli, Peri, and Rizzolo (2000) who used a copper catalysed linoleic acid oxidation assay. However, aqueous extracts of fresh tomatoes showed antioxidant action in a copper catalysed phosphatidylcholine lyposome oxidation assay but no activity when the same extracts were used in a linoleic acid emulsion oxidation induced by endoperoxide (Takeoka et al., 2001). The pro-oxidant action reported by Lavelli et al. (2000) was considered to be due to the presence of ascorbic acid and its action activating metal-ion catalysed reactions, which probably also happened under the conditions of the present assay, where ascorbic acid is used as oxidative inducer together with iron sulphate.

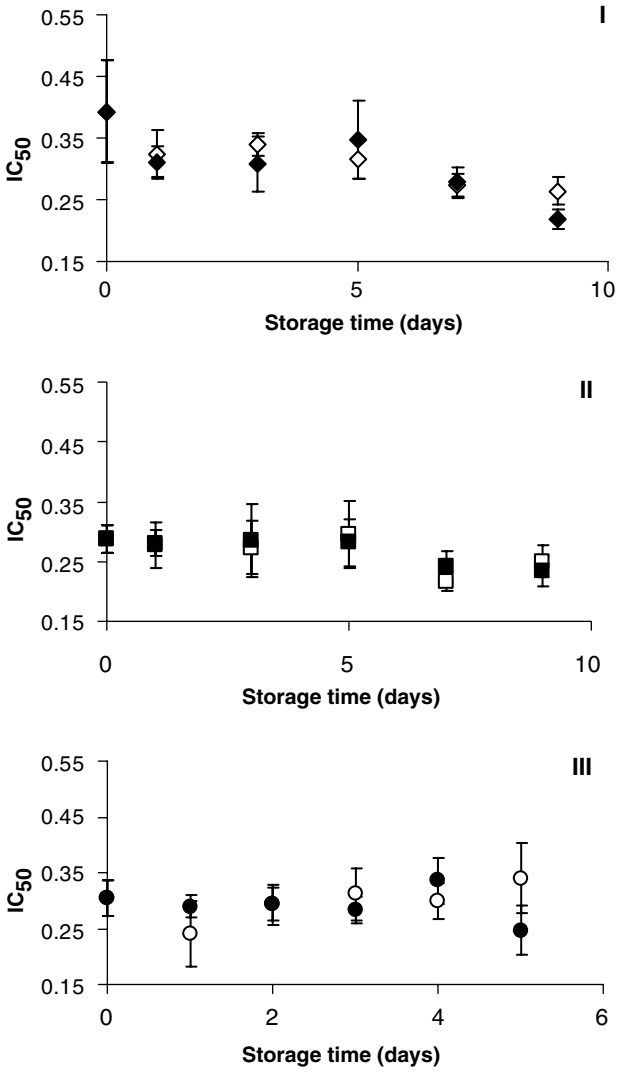

Fig. 1. Antioxidant activity of methanol extracts of sliced tomato harvested at stages I, II and III as indicated. The antioxidant activity is expressed as IC_{50}, which is the concentration in μg tomato dry weight/ml extractor that causes 50% inhibition of lipid peroxidation compared to the control. Open symbols refers to intact fruits and closed symbols to cut fruits. The values are the means of five replicates ± SE.

Table 1
Antioxidant activity of tomato methanol extracts expressed as IC_{50}, which is the concentration in μg tomato dry weight/ml extractor that causes 50% inhibition of lipid peroxidation compared to the control

Stage of maturity	IC_{50}
II	0.288^{a}
III	0.304^{ab}
I	0.393^{b}

The values are the means of five replicates, two treatments and six storage times.
Means followed by the different letters are significantly different at $P = F < 0.05$ by LSD test.

The lack of antioxidant activity in the THF extract in the lipid peroxidation assay was highly unexpected since this extract contains lycopene, which is known to be an efficient scavenger of peroxyl radicals (Woodall, Lee, Weesie, Jackson, & Britton, 1997). However, Chen and Djuric (2001) questioned whether carotenoids can act as antioxidants in biological membranes and suggested that, although carotenoids are sensitive to degradation by free radicals, they do not protect against lipid peroxidation. The importance of the lipid substrate should also be taken into consideration as depicted from the results of Takeoka et al. (2001). The lycopene-containing hexane extract of fresh-tomatoes exhibited greater antioxidant activity than the aqueous and methanol extracts when phosphatidylcholine liposome was used as substrate for oxidation, but about half the activity of methanol extracts when linoleic acid was used as substrate.

Another possibility is that the dilution of the samples in methanol used here resulted in a concentration of antioxidant compounds too low to be effective in the assay. Wilborts (2003) reported problems when measuring the antioxidant activity of lycopene and β-carotene solutions using the same assay as used here. Lycopene, at concentration high enough to exhibit activity, was only soluble in pure THF, which dissolved the plastic wells in which the assay was performed. When diluted in THF-methanol the concentration of these compounds was insufficient to show activity in the assay. When hexane and ethyl acetate were used in the place of THF in the present work no activity could be measured also, even when the hexane extract was not diluted (data not shown).

3.2. $ABTS^{\bullet+}$ scavenging capacity

3.2.1. Analysis of variance

The results obtained for antioxidant activity using the ABTS assay are presented in Figs. 2 and 3. Minimal processing decreased the TAA (data not shown) and HAA ($Pr > F = < 0.0001$) but had no effect on the LAA ($Pr > F = 0.1660$). This indicates that the observed decrease in total antioxidant activity (TAA) during storage was mainly due to the decrease in HAA.

Changes in LAA during storage were dependent on the stage of maturity of the fruit, while the changes in HAA depended both on processing and on fruit maturity stage (Table 2). A traditional approach to study the changes in antioxidant activity with time would involve the decomposition of the interactions stage * time and treat * time for HAA and stage * time for LAA; that is the study of the effect of treatment and stage of maturity for each sampling date. Using this approach, and given the fact that the sampling time is different in the individual series, makes it difficult to obtain a more general picture of the behaviour with time and how it depends on the other factors under study (in the present case stage of maturity and processing). Instead, the data were analysed using non-linear regression.

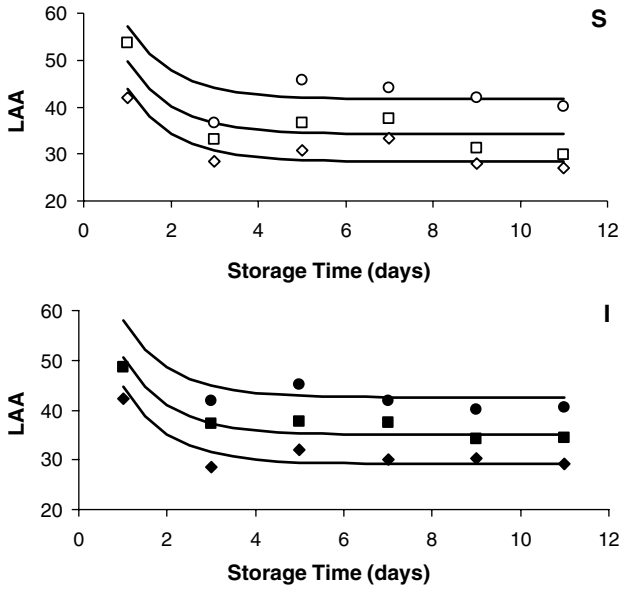

Fig. 2. Lipophilic antioxidant activity of tomato fruits harvested at stages I (◆), II (■) or III (●) and stored sliced (S) (open symbols) or intact (I) (closed symbols) at 5 °C. Points are measured data (means of five replicates) and lines are simulated values according to Eq. (1).

3.2.2. Modelling

The first regression analysis was conducted for each treatment (cut and intact) separately (Eq. (1)). Later, the effect of treatment was incorporated (Eq. (2)) so that the combined analysis included simultaneously the effect of time, treatment and stage. Non-linear regression is an iterative procedure and, therefore, strongly relies on good initial values for the parameters to be estimated. These values were obtained in a sequence of analyses, each step increasing in complexity in relation to the previous one, while building up the number of reliable estimates. Even in the final analysis (see results, Table 3) not every parameter could be estimated simultaneously. This is the case, for example, of AA_{var} for LAA. The reason is that in the time range where a rapid decay occurs, too few measuring points (about 3) were taken. That made it impossible to estimate the initial value and the asymptotic end value in the same analysis.

The antioxidant activity of both the hydrophilic and lipophilic extract showed the same exponential decay starting after processing, with a reference rate constant of 0.949 ± 0.091 for LAA and 0.281 ± 0.065 for HAA. Later on, the HAA increased with storage time, while the LAA remained practically unchanged. The estimated values for the rate constant of increase ($k_{l,\,treat}$) were consequently significantly positive for HAA (Table 3) while close to zero for LAA (not shown). Changes in LAA, therefore, could well be explained by Eq. (1),

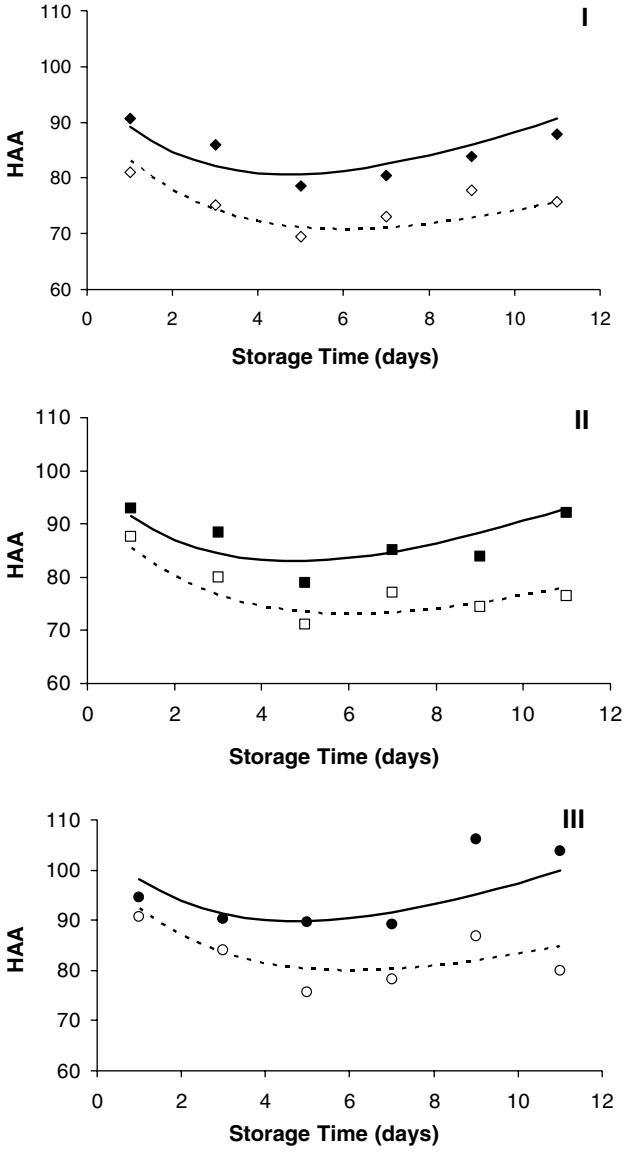

Fig. 3. Hydrophilic antioxidant activity of tomato fruits harvested at stages I, II or III and stored sliced (open symbols and dotted line) or intact (closed symbols and solid lines) at 5 °C. Points are measured data (means of 5 replicates) and lines are simulated values according to Eq. (1).

without the incorporation of the linear increase. The scatterplot for LAA (Fig. 4) and for HAA (Fig. 5) indicates a good fit of the model to the data.

The pattern of change of HAA during storage was the same whether the fruits were intact or cut, but the antioxidant activity was systematically lower for cut fruits (negative value for ΔAA_{cut} when ΔAA_{whole} was fixed to zero). A similar pattern of change was reported for fresh-cut mandarin where the antioxidant activity decreased in the beginning of the storage period and later increased (Piga et al., 2002). This was considered to be due to changes in the antioxidant activity of polyphenols, which when undergoing enzymatic or chemical oxidation exhibit increased antioxidant efficiency when present in an intermediary state. The rate constant for this linear increase depended on treatment (Table 3) and was higher for intact fruits. This indicates that the significance of the interaction treatment * time was mainly related with differences in the magnitude of the

Table 2
Results of analysis of variance for the lipophilic (LAA) and hydrophilic (HAA) antioxidant activity of sliced tomato

Extract	Source of variation	*F* value	*Pr* > *F*
LAA	Stage of maturity	177.76	<0.0001
	Treatment	1.94	0.1660
	Storage time	90.87	<0.0001
	Stage * treatment	0.19	0.8299
	Stage * time	2.37	0.0125
	Treatment * time	1.75	0.1263
	Stage * treatment * time	1.19	0.3006
HAA	Stage of maturity	49.27	<0.0001
	Treatment	174.14	<0.0001
	Storage time	20.15	<0.0001
	Stage * treatment	2.67	0.0732
	Stage * time	2.79	0.0035
	Treatment * time	3.73	0.0034
	Stage * treatment * time	1.48	0.1514

Table 3
Result of statistical non-linear regression based on Eq. (2) for HAA (hydrophilic antioxidant activity) and on Eq. (2) without a linear increase ($k_{l,\,treat} = 0$) for LAA (lipophilic antioxidant activity)

	LAA		HAA	
Parameter	Estimate	S.E.	Estimate	S.E.
$\Delta AA_{breaker}$	0	Fixed	0	Fixed
ΔAA_{pink}	5.87	1.26	2.34	1.51
ΔAA_{red}	13.38	1.26	9.15	1.51
K	0.949	0.091	0.281	0.066
AA_{var}	40.0	Fixed	40.09	6.55
AA_{fix}	29.16	1.09	55.83	3.69
ΔAA_{whole}	0	Fixed	0	Fixed
ΔAA_{cut}	−0.81	1.03	−5.06	2.49
$k_{l,\,whole}$	–		3.000	0.282
$k_{l,\,cut}$	–		2.106	0.282
N_{obs}	36		36	
R^2_{adj}	87.5		80.7	

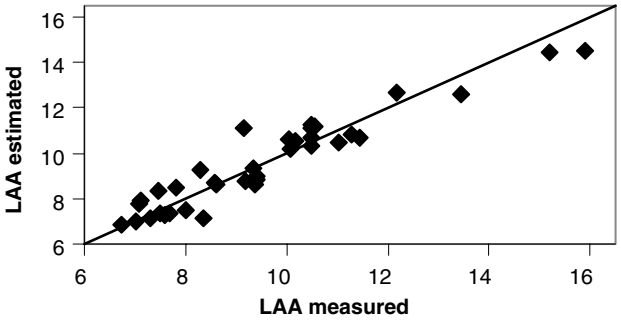

Fig. 4. Scatter plot for mean data of lipophilic antioxidant activity.

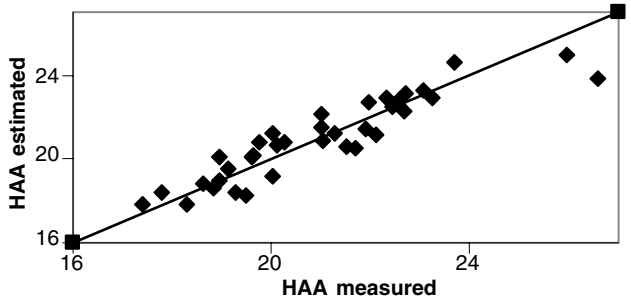

Fig. 5. Scatter plot for mean data of hydrophilic antioxidant activity.

linear increase, since the reaction rate for the exponential decay could be estimated in common for both cut and intact fruit.

Significant changes in LAA were observed only in the first three days of storage but could not be ascribed to cutting since the same phenomenon occurred in intact fruits. The estimated value of $\Delta AA_{cut,}$ although negative, was rather small and could not be reliably estimated (Table 3). The reasons for this decrease in LAA could not be determined in the present work. Probably, the physiological response to the stress induced by harvesting consumed a major part of the available antioxidant activity. Reports on the lipophilic antioxidant activity as a response to cutting are unknown by the authors.

The radical scavenging capacity increased with ripening for both lipophilic and hydrophilic fractions. The increase in LAA with ripening was also reported by Cano et al. (2003), and related to changes in lycopene content. Contrary to those authors, a significant increase in HAA with ripening was found in the present work. The higher values for the estimates ΔAA_{pink} and ΔAA_{red} for LAA, indicate that the effect of stage of maturity was more pronounced in LAA than HAA, confirming the information obtained from the ANOVA where F values of 177.76 and 49.27 were obtained, respectively, for LAA and HAA.

The same pattern of changes during storage was observed for all stages. That means that differences between fruits at different stages during storage were mainly due to differences in the initial antioxidant activity when the fruits were processed.

3.3. Relation between both assays

The effect of cutting, fruit maturity stage and storage time on the antioxidant activity of tomato extracts was not the same in the two different antioxidant assays used in the present work. Although different extractors were used in each assay, part of the compounds extracted by the Na-P buffer should be present in the water and methanol extracts used in the lipid peroxidation assay. Similarly, part of the compounds extracted by ethyl acetate should be present in methanol and THF extracts.

Contrasting results between radical scavenging methods and in vitro lipid peroxidation methods were discussed in an extensive review by Frankel and Meyer (2000) and reported by Garcia-Alonso, de Pascual-Teresa, Santos-Buelga, and Rivas-Gonzalo (2004). This apparently contradictory behaviour occurs because the effectiveness of antioxidants depends on the test system used (Frankel & Meyer, 2000). The lipid peroxidation method includes other mechanisms of antioxidant action besides the radical scavenging, the only mechanism evaluated in the $ABTS^{+}$ assay. On the other hand, partitioning and interfacial properties are not taken into consideration in the ABTS assay, while they represent an important factor in biological assays.

3.4. Significance of the decrease in HAA

The present results indicate that processing can induce a reduction of radical scavenging capacity, which is only one of the mechanisms by which antioxidants can prevent oxidative damage. The fact that an artificial radical was used should be kept in mind while extrapolating the results to an in vivo situation.

Although a minor decrease in antioxidant activity was measured as a response to processing, at the moment it is not possible to know the impact of this decrease in the value of the food as source of antioxidant in the diet. In spite of the agreement about the health benefits of antioxidants, there is no dietary prescription of recommended daily consumption as it is the case for nutrients such as vitamins and proteins.

4. Conclusions

The processing of tomato fruit into transversal slices induced a decrease in the radical scavenging capacity of hydrophilic extracts towards the ABTS radical. This indicates a potential effect of processing to decrease the antioxidant activity in vivo. This would represent a decrease in the value of cut tomatoes as a source of antioxidants in the diet compared with the fruit stored intact. The observed increase in the hydrophilic antioxidant activity at the end of the storage period indicates that some repair or recycling mechanism is operative. This same mechanism is present in intact fruits since they showed the same pattern of change as sliced fruits. Nevertheless, even with this later increase, the level of antioxidant activity in cut fruits hardly reached the level present in intact fruits.

The radical scavenging capacity of lipophilic extracts decreased sharply in the first two days of storage. Since no significant effect of cutting was observed, this decrease in activity is more likely to be related with stress caused by harvesting or low temperature storage.

For both hydrophilic and lipophilic antioxidant activity, the riper the fruit the higher was the antioxidant activity. Since no relevant interaction was found between time of storage and stage of maturity, the major factor determining the level of antioxidants in tomatoes seems to be the initial level of antioxidant activity at the moment of harvest.

The information obtained from analysis of variance could effectively be used in developing dynamic models. Although the behaviour of antioxidant level in whole and cut tomatoes seems to be rather complex, the dynamics of change could be modelled in a surprisingly simple way.

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References

Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry, 73*, 239–244.

Boxtel, E. van. (2002). Polyphenols in tomato and tomato-based products. Research Project Report (p. 98). Wageningen University, Wageningen.

Cano, A., Acosta, M., & Arnao, M. B. (2003). Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum* Mill). *Postharvest Biology and Technology, 28*, 59–65.

Chang, S., Tan, C., Frankel, E. N., & Barrett, D. M. (2000). Low-density lipoprotein antioxidant activity of phenolic compounds and polyphenol oxidase activity in selected clingstone peach cultivars. *Journal of Agriculture and Food Chemistry, 48*, 147–151.

Chen, G., & Djuric, Z. (2001). Carotenoids are degraded by free radicals but do not affect lipid peroxidation in unilamellar liposomes under different oxygen tensions. *FEBS Letters, 505*, 151–154.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*, 1925–1941.

Garcia-Alonso, M., de Pascual-Teresa, S., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2004). Evaluation of the antioxidant properties of fruits. *Food Chemistry, 84*, 13–18.

Gil, M. I., Ferreres, F., & Tomas-Barberan, F. A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry, 47*, 2213–2217.

Halliwell, B. (2002). Food derived antioxidants: How to evaluate their importance in food and in vivo. In E. Cadenas & L. Packer (Eds.), *Handbook of antioxidants* (pp. 1–45). New York: Marcel Dekker Inc.

Jimenez, A., Creissen, G., Kular, B., Firmin, J., Robinson, S., Verhoeyen, M., et al. (2002). Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Plant, 214*, 751–758.

Kang, H. M., & Saltveit, M. E. (2002). Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agricultural and Food Chemistry, 50*, 7536–7541.

Klerk, M., Veer, P. van 't, & Kok, F. J. F. J. (1998). *Fruits and vegetables in chronic disease prevention* (p. 86). Wageningen: LUW, Wageningen.

Lana, M. M., Tijskens, L. M. M., Kooten, O. van. (2004). Fresh-cut tomatoes: effects of temperature and maturity at harvest on the RGB colour. In *Programme and book of abstracts of the 2nd Central European congress on food* (p. 276). Budapeste.

Lana, M. M., Tijskens, L. M. M., & Kooten, O. van. (2005). Effects of storage temperature and fruit ripening on firmness of fresh-cut tomatoes. *Postharvest Biology and Technology, 35*(1), 87–95.

Lavelli, V., Peri, C., & Rizzolo, A. (2000). Antioxidant activity of tomato products as studied by model reactions using xanthine oxidase, myeloperoxidase, and copper-induced lipid peroxidation. *Journal of Agricultural and Food Chemistry, 48*, 1442–1448.

Lindley, M. G. (1998). The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. *Trends in Food Science and Technology, 9*, 336–340.

Piga, A., Agabbio, M., Gambella, F., & Nicoli, M. C. (2002). Retention of antioxidant activity in minimally processed mandarin and satsuma fruits. *Lebensmittel-Wissenschaft und-Technologie, 35*, 344–347.

Piga, A., Caro, A. D., Pinna, I., & Agabbio, M. (2003). Changes in ascorbic acid, polyphenol content and antioxidant activity in minimally processed cactus pear fruits. *Lebensmittel-Wissenschaft und-Technologie, 36*, 257–262.

Sluis, A. A. van der, Dekker, M., Verkerk, R., & Jongen, W. M. F. (2000). An improved, rapid in vitro method to measure antioxidant activity. Application on selected flavonoids and apple juice. *Journal of Agricultural and Food Chemistry, 48*, 4116–4122.

Sluis, A. A. van der, Dekker, M., Jager, A. de, & Jongen, W. M. F. (2001). Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *Journal of Agricultural and Food Chemistry, 49*, 3606–3613.

Takeoka, G. R., Dao, L., Flessa, S., Gillespie, D. M., Jewell, W. T., Huebner, B., et al. (2001). Processing effects on lycopene content and antioxidant activity of tomatoes. *Journal of Agricultural and Food Chemistry, 49*, 3713–3717.

WHO. (2003). Diet, nutrition and the prevention of chronic diseases. World Health Organization, ISSN 0512-3054; 916. Technical Report Series (p. 149). Geneva: World Health Organization.

Wilborts, M. (2003). Evaluation of different antioxidant assays. Research Project Report (p. 50). Wageningen: Wageningen University.

Woodall, A. A., Lee, S. W., Weesie, R. J., Jackson, M. J., & Britton, G. (1997). Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Biochimica et Biophysica Acta (BBA) – General Subjects, 1336*, 33–42.