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Effect of Ascorbic Acid Addition to Peppers on Paprika Quality

Micaela Carvajal,[1] Maria-Remedios Martínez,[1] Francisco Martínez-Sánchez[2] and Carlos F Alcaraz[1]*

[1] Centro de Edafología y Biología Aplicada del Segura-CSIC, PO Box 4195, 30080 Murcia, Spain
[2] Departamento de Tecnología de Alimentos, Escuela Politécnica Superior de Ingenieros Agrónomos, Orihuela, Universidad Politécnica de Valencia, Spain

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Abstract: The use of ascorbic acid addition to foods to protect pigments from oxidation has been widely applied. Control or ascorbic acid-added paprika peppers were processed in a similar way as for paprika manufacture. During the process, lipoxygenase activity, total colour, red/yellow pigment ratio and ascorbic acid levels were measured. In general, an increase of total colour and red pigments was observed in ascorbic acid-treated samples. The lipoxygenase activity was depressed in ascorbic acid-treated fruits on the first day of processing. However, the activity was increased again, at second day, when the ascorbic acid was oxidised, showing a close relationship between enzyme activity and the antioxidant. After the process, ascorbic acid was also added to half of the paprika from control peppers and its quality and stability of the pigments against light or heat was compared to the paprika from ascorbic acid-added peppers. At the end of the treatment a better quality was observed in paprika obtained from the ascorbic acid-added peppers.

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INTRODUCTION

Peppers are a good source of ascorbic acid (AA) which is a very important dietary antioxidant. However, the levels of this compound are very variable and may be affected by maturity, genotype and processing (Howard *et al* 1994). This vitamin acts as a protector of pigments preserving them from chemical and biochemical oxidation. During paprika manufacture, there are some steps which produce a decrease of pigments and ascorbic acid content, implying an important reduction of quality. The stability of the paprika pigments has been attributed to a number of factors, including cultivar (Alcaraz *et al* 1991; Martínez-Sánchez *et al* 1991), plant nutrition (Martínez-Sánchez *et al* 1993), moisture content, stage of ripeness at harvest (Kanner *et al* 1979) and antioxidant content (Biacs *et al* 1992). However, lipid oxidation has a major effect on fruit colour quality.

There are two distinct groups of lipoxygenase (EC 1.13.11.12; linoleate : oxygen oxidoreductase) enzymes described in plants as types 1 and 2. Type 1 lipoxygenase has been reported in relatively few plants and has an optimum activity at pH 9 with a slight tendency to cause cooxidation of other lipids during the reaction. Type 2 lipoxygenase occurs widely with optimum activity at pH 6·5–7·0 with a strong tendency to catalyse the cooxidation of other compounds. Chlorophyll, carotenoids, cholesterol, cytochrome *c*, and thiols are among the substances reported to suffer cooxidation (Eskin *et al* 1977). The formation of the carotenoid pigments in fruits has also seen related to lipoxygenase activity (Eskin *et al* 1977; Grosch *et al* 1977) and to the content of antioxidants which act as competitive inhibitors of this enzyme. AA is one such antioxidant which acts by scavenging active oxygen and so protects double bonds.

In this paper we studied the possibility of increasing paprika quality by reducing the pigment oxidation during the manufacture by ascorbic acid addition to the

* To whom correspondence should be addressed.

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fruits. In addition we compared the thermo- and photostability of the pigments in AA-added paprika.

EXPERIMENTAL

Capsicum annuum L plants, cv Bunejo, were obtained from JC Costa (CRIA, Murcia, Spain) and were grown under greenhouse conditions. Fertiliser, water and phytosanitary treatments were applied by a drip irrigation system.

At harvest, peduncles and seeds were detached and samples of the fruit pericarp were washed with 10 g litre^{-1} BRIJ 35 solution (non-ionic detergent) and then rinsed three times with deionised water. Half of the fruits were soaked for 1 h in a 2 mg litre^{-1} AA solution in 4% *meta*-phosphoric acid (+AA) and the other half in 40 g litre^{-1} *meta*-phosphoric acid (control). The pericarps were dried in an air oven at 50°C for 2 days. In the industry, when peppers are dried in an oven, the temperature normally used is 65°C. In our experiments we decreased the temperature to 50°C for reducing the pigment oxidation and degradation. Several temperatures were assayed (40, 50, 60 and 70°C) and 50°C produced the minimal degradation of colour in the peppers of cv Bunejo (data not shown). Measurements of colour, lipoxygenase activity and AA concentration were done every 24 h. After the measurements, the dried pericarps where ground simulating the paprika manufacture. AA, 2 mg litre^{-1} in 40 g litre^{-1} *meta*-phosphoric acid, was added to half of the paprika obtained from control peppers (control + AA), the equivalent amount of *meta*-phosphoric acid was added to the other half (control) and to all the paprika obtained from +AA peppers (+AA). Paprika from each treatments were divided in two parts and introduced in an oven at 50°C or into a chamber illuminated with UV light for 5 days. The same measurements than previously with pericarp, were done every 24 h.

Lipoxygenase activity (LOX)

Crude extract

The fruit pericarp (3 g) was cut into short segments and 3 g of paprika were weighed. Both pericarp and paprika were homogenised at 4°C in 9 ml 50 mM phosphate buffer, pH 7·0, containing 1 g litre^{-1} TritonX-100, filtered through four layers of nylon cloth and centrifuged at 15 000 × *g* for 15 min.

Enzyme assay

Pure linoleic acid (10 μl) was suspended in 25 ml of 0·1 M sodium tetraborate containing 0·1% Tween 20 by sonication (Sekhar and Reddy 1972). The substrate (0·1 ml) was shaken vigorously with 2·9 ml of 0·1 M phosphate buffer, pH 4–5, in a spectrophotometer cuvette. The reaction was started by adding 0·1 ml of enzyme extract, and the increase in absorbance at 234 nm was measured (Daood *et al* 1988). A unit of enzyme was defined as the amount which produced an absorbance change of 0·001 AU s^{-1} at 234 nm.

Ascorbic acid

Capsicum pericarp and paprika (1 g) were homogenised with a mortar and a pestle in 10 ml 40 g litre^{-1} *meta*-phosphoric acid contained 250 mg PVP (polyvinyl-pyrrolidone) to absorb the interfering pigments, and left stirring for 1 h at 4°C in the dark. The homogenised samples were centrifuged at 5000 × *g* for 5 min and the supernatant filtered through a sep-Pack C_{18} cartridge. The eluate was directly injected in HPLC for ascorbic acid determination.

HPLC analysis

The column used was Chromsil C_{18} (10 μm), 25 × 0·4 cm. The mobile phase was 20 g litre^{-1} *di*-ammonium hydrogen phosphate (w/v) pH 2·8 adjusted with *ortho*-phosphoric acid. The flow rate was 0·4 ml min^{-1}. The detection was performed at 210 nm.

Colour (ASTA 1968)

Fresh pericarp cut in small pieces and paprika (0·5 g) was extracted with 100 ml acetone for 24 h in the dark. Supernatant (5 ml) was diluted to 50 ml with acetone and the absorbance was read at 460 nm against an acetone blank. The colour was expressed in ASTA units:

$$\text{ASTA} = A\ 164\ If\ w^{-1}$$

where A is sample absorbance, If is the deviation factor of the spectrophotometer, which was calculated using a standard 2030 NBS filter, that indicates the relation between the theoretical (A_t) and real (A_r) absorbances at 460 nm, 164 is the molar extinction coefficient of 1% capsanthin solution in acetone and w is the sample weight.

Red/yellow pigments ratio

The pericarp was extracted as for the ASTA determination. The absorbances were measured at 470 nm for red pigments and at 455 nm for yellow (Navarro and Costa 1993).

Data analysis

All the measurements were repeated at least three times and for comparing the results from fresh and dry

samples, the percentage humidity was taken into account for the calculations. The data were analysed using unpaired *t*-tests.

RESULTS AND DISCUSSION

The total colour of the peppers was analysed during the drying process (Fig 1) and expressed as the official ASTA units. These units are generally used for determining paprika quality in manufacturing and trade. It has been observed that there is a very strong influence of heat (50°C) on the degradation process of the pigments, reducing the initial values by half after 2 days. However, the peppers to which AA were added presented significant higher concentration of total pigment after the first day in the oven. As the ASTA units are an average of total pigments in paprika, the measurement of red/yellow pigments ratio gives a better idea of which type of pigments is contributing in a higher proportion to total colour. The red/yellow pigments ratio (Fig 2) showed a general decrease in the first day into the oven, but a slight increase appeared during the 2nd day. There were no significant differences between the ratio of control and treated peppers after the first day, although the AA-treated peppers showed higher amount of red pigments as an increase of the ratio means. It can be appreciated that error bars are significantly tighter for the 48 sampling than for 0 and 24 h in Figs 1 and 2. This is simply due to when the pericarp is fresh it is more difficult to obtain an homogenous sample in terms of humidity and a lesser amount of pigments are contributing to the measurement.

The determination of the LOX activity gives very useful information about the potential degradation of the carotenoids by this enzyme. As shown in Fig 3 the activity in control samples was higher than the +AA samples at the beginning and after 1 day in the oven. However, after 2 days there was no difference between the LOX activity of control and AA-treated.

The AA content (Table 1), as expected, was much higher in peppers to which AA was added. After 24 h in the oven at 50°C, a great decrease was observed in both control and + AA peppers, and after 48 h similar values were found for both samples.

The cooxidation of carotenoids by LOX has been widely reported (Weber and Grosch 1976; Faubion and Hoseney 1981; Barimalaa and Gordon 1988). The cooxidation of molecules during the LOX-catalysed oxidation of linoleic acid has been related to the fact that a

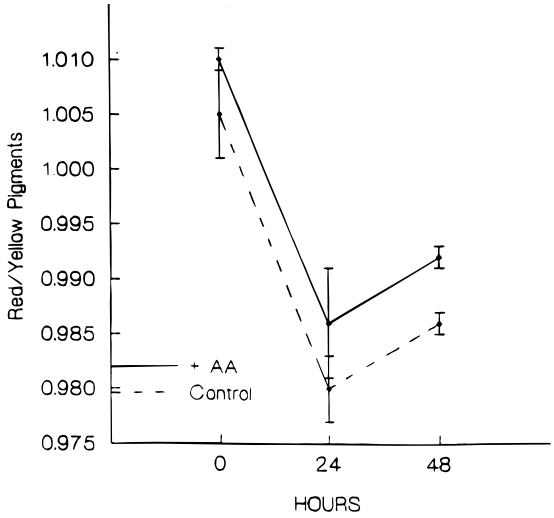

Fig 2. Effect of ascorbic acid addition on the red/yellow pigments ratio of pepper at the initial time and after 1 and 2 days in the oven at 50°C. Means ± SE, $n = 5$.

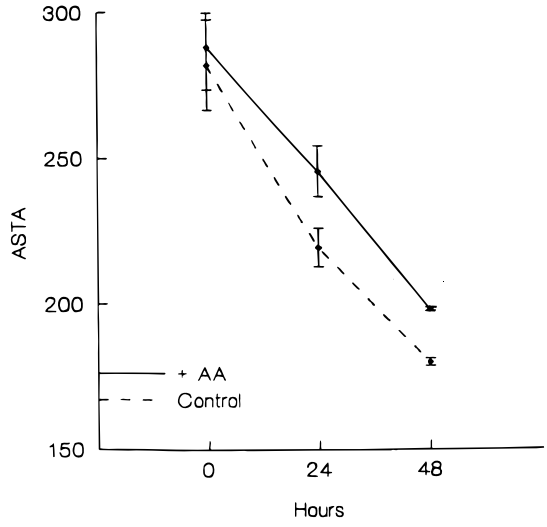

Fig 1. Effect of ascorbic acid addition on total colour (ASTA) of pepper at the initial time and after 1 and 2 days in the oven at 50°C. Means ± SE, $n = 5$.

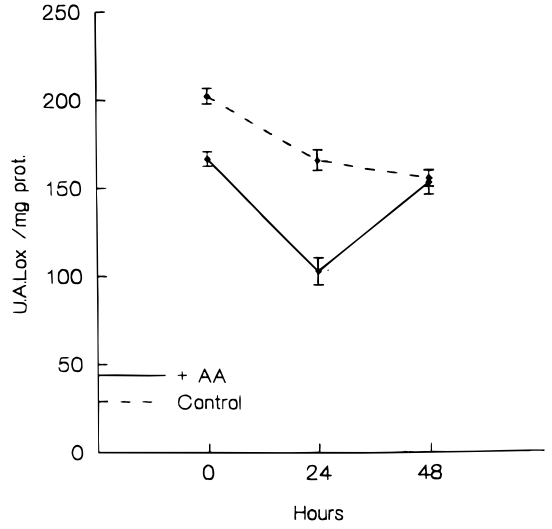

Fig 3. Effect of ascorbic acid addition on lipoxygenase activity (UA LOX per mg protein) of pepper at the initial time and after 1 and 2 days in the oven at 50°C. Means ± SE, $n = 5$.

TABLE 1
Effect of ascorbic acid addition on ascorbic acid composition (mg g^{-1} FW) of pepper at the initial time and after 1 and 2 days in the oven at 50°C (Means ± SE, $n = 5$)

	Control	+AA
0 h	0·33 ± 0·05	0·92 ± 0·08
24 h	0·11 ± 0·01	0·50 ± 0·09
48 h	0·05 ± 0·03	0·06 ± 0·02

large proportion of peroxyl radicals is not directly converted to hyroperoxide by the enzyme (Weber and Grosch 1976). The LOX inhibitors, eg AA, decreased carotenoid destruction in beans (Nicolas *et al* 1981). Also, the addition of linoleic acid mixed with O_2 resulted in considerable carotenoid loss, whereas lack of substrate or heat inactivation of LOX, prevents carotenoid loss (Matsuo *et al* 1970). In our samples it could be appreciated that heat affect LOX activity, but degradation of carotenoids occurred. It has been reported that mixing pasta with AA (Walsh *et al* 1970) prevents carotenoid oxidation during the heating process because ascorbic acid was a competitive inhibitor of LOX. From our results it can be observed that LOX activity is higher in control peppers likely as a consequence of the lower AA content. Furthermore, this higher LOX activity is related to the greater reduction in pigments concentration, because although both control and AA-added peppers showed strong carotenoid degradation during the manufacture due to the heat and oxygen, this degradation is lower in AA-treated fruits. Unfortunately, AA in foods is very labile and offers only a temporary antioxidant effect, being affected, for example, by pH, temperature, enzyme activity, oxygen and light (Ponting and Joslyn 1948; Liao and Seib 1987; Wills and Silalahi 1990). The fact of that LOX and red/yellow pigments rate show a similar pattern, lead us to think that a relation between both determinations is possible. If the increase of LOX activity (48 h) after the decrease (from 0 to 24 h) could be explained by the degradation of AA, the increase of the red/yellow pigments ratio only can be due to an increase of yellow pigments degradation. However, a further interpretation from the limited data obtained would be unwise.

The stability of the paprika pigments from control and AA-added peppers (control and +AA) was compared to that of paprika control AA-added (control + AA). The UV light catalysed the degradation of the pigment content due to an increase in oxidation (Philip and Francis 1971), therefore, a decrease of the total colour was observed in control and treated paprika (Fig 4a). The slope is much higher from the beginning of the second day and then a stabilising until the fifth day. There was always a major pigment content in paprika from AA-added peppers and the differences

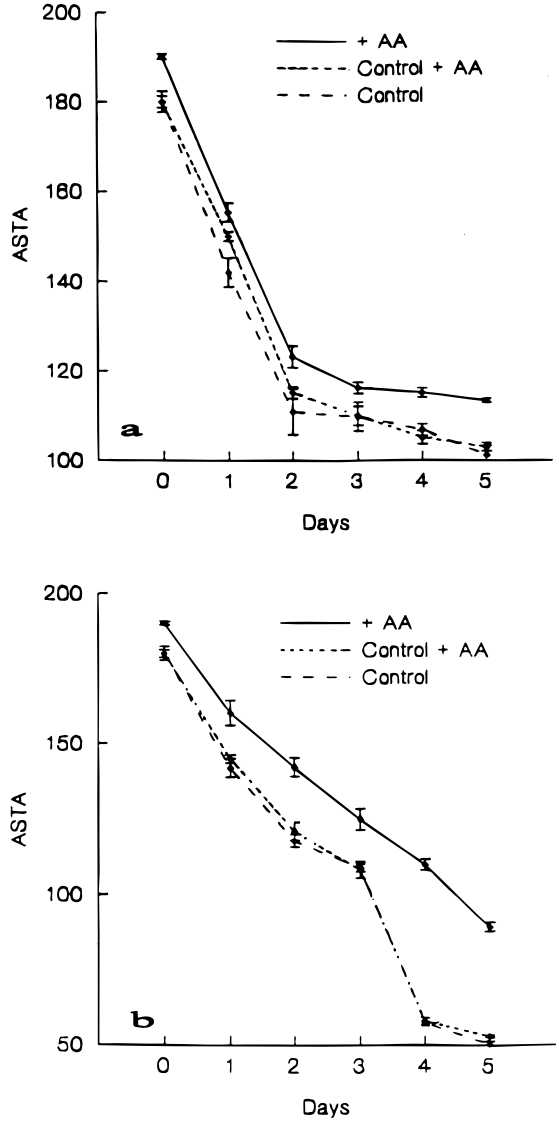

Fig 4. Stability of the total colour (ASTA) against (a) UV light and (b) heat (50°C) treatments of: paprika from control peppers, AA-added paprika from control peppers and paprika from fruits previously AA-added. Means ± SE, $n = 5$.

were even higher at the 4th and 5th day. The quality of that paprika was extra at the end of the experiment and of first class with the other two treatments. Similar behaviour was observed in the stability against temperature (Fig 4b) showing paprika control + AA no significant differences with control. As in the above experiment, the paprika from AA-added peppers had more pigment concentration than the other two (control and control − AA). Although in this case the quality obtained at the end of the experiment was lower than in the assay with UV light, paprika from AA-added paprika was first class, meanwhile in the other two (control and control + AA) were second class.

The AA content and LOX activity of paprika were also measured (data not shown). Both disappeared by the 2nd day of sampling and it seems that they have a

very low influence on pigment oxidation, having more effect the initial values of total colour.

Even though the stability of AA is low, its effect before its degradation is strong enough to protect pigments during paprika processing. Therefore, we obtained more colour quality in paprika from AA-treated fruits. This protection during paprika processing gives better results in this paprika (+AA) even after the temperature and light treatments than in control + AA paprika which enables us to advise that it would be better to choose pepper with higher quality or increase the AA concentration of the peppers than try to protect the pigments with AA after the paprika manufacture. Addition of AA could have some positive influence on nutritional quality of the product, but its effect on the stability of paprika could be considered to be a more important effect.

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