Antioxidant properties of tomato juice as affected by heating

Monica Anese,[1] Lara Manzocco,[2]* Maria Cristina Nicoli,[3] and Carlo R Lerici[2]

[1] Istituto di Produzioni e Preparazioni Alimentari, University of Bari, Via Napoli 25 – 71100 Foggia, Italy

[2] Dipartimento di Scienze degli Alimenti, University of Udine, Via Marangoni 97 – 33100 Udine, Italy

[3] Dipartimento de Scienze Ambientali Agrarie e Biotecnologie Agro- Alimentari, University of Sassari, Viale Italia 39-07100 Sassari, Italy

Abstract: The changes in the overall antioxidant properties of tomato juice samples and model solutions as a consequence of heat treatments were studied. The antioxidant properties were evaluated both through the measurement of the chain breaking and the oxygen scavenging activities. While a decrease in the antioxidant potential was found for short heat treatments, a recovery of these properties was measured by prolonging heating times. Results suggested that the initial reduction in the overall antioxidant activity can be attributed not only to the thermal degradation of naturally occurring antioxidants but also to the formation of early Maillard reaction products (MRP) with prooxidant properties. The gain in antioxidant activity coincided with the formation of brown MRP.

Keywords: antioxidant properties; Maillard reaction; tomato juice; heat treatment

INTRODUCTION

In recent years increasing attention has been paid to the role of antioxidants in human health. Such compounds, particularly those of natural origin, are recognised as factors in food preservation but are now believed to be important health protecting factors. In fact, they can act both by reducing the content of toxic components in foods and by supplying the human body with exogenous antioxidants.[1–8] For these reasons, information on the overall antioxidant properties of foods is becoming relevant in the fields of nutrition and food technology.

Most of the data now available generally relate solely to the measurement of the natural antioxidant content or to the evaluation of its ability to slow down lipid oxidation in accelerated stability tests.[9] They do not provide comprehensive information on the overall antioxidant potential of foods arising from the action of compounds via various mechanisms, mostly chain breaking, oxygen scavenging and metal chelation. The evaluation of the total antioxidant properties of foods should take into account the measurement of these properties and their relative importance in the food matrix.

It is well known that naturally occurring antioxidants could be significantly lost as a consequence of processing and storage. In particular, thermal treatments are generally believed to be the main cause of the depletion in natural antioxidants.[10,11] Thus, considering the important role of these compounds as health protecting factors, the original antioxidant properties of raw materials or foods should be maintained through the use of optimised food processing conditions. On the other hand, heating can also induce the formation of further compounds with antioxidant properties, as occurs during the development of the Maillard reaction.[12–15]

In the last decade, extensive work has been carried out on the antioxidant properties of Maillard reaction products (MRP). Experiments, mainly carried out on simple model systems, have shown that MRP exhibit antioxidant properties, particularly chain breaking and oxygen scavenging activities. Lipid oxidation rates were significantly slowed down when MRP were added or formed during heating.[16–28] Despite these efforts, few data are available on the changes in the overall antioxidant properties of foods as a consequence either of the depletion of natural antioxidants during processing and/or storage or, in the case of heat treatments, of the contemporary formation of MRP with antioxidant properties.[29] Observations carried out on coffee beverages, for example, showed that their overall antioxidant properties were greatly increased with increasing degree of roasting.[30]

In this study the changes in the overall antioxidant properties of tomato juice samples as a consequence of heat treatments are studied. Simple model systems, simulating the composition of tomato juice, are also considered. The antioxidant properties are evaluated both through the measurement of the chain breaking and the oxygen scavenging activities.

* Correspondence to: Lara Manzocco, Dipartimento di Scienze degli Alimenti, University of Udine, Via Marangoni 97-33100 Udine, Italy.

(*Received 7 July 1998; revised version received 24 July 1998; accepted 7 October 1998*)

MATERIALS AND METHODS

Sample preparation

A 6°Brix tomato puree and an aqueous solution containing 10 g kg^{-1} glucose, 15 g kg^{-1} fructose and 2.4 g kg^{-1} L-glutamic acid (Carlo Erba Analitycals, Milano, Italy) were used. The model solution was adjusted to pH 4.5 with 10 M NaOH. No dilution effects were observed. Aliquots of tomato puree or of model solution were introduced into 100 ml screw capped bottles and heated in an oil bath at 95°C or 70°C for up to 50 h. After heating, samples were rapidly cooled in cold running water. The tomato samples were centrifuged at 5000 × g for 20 min (Heraeus, Sepatech Megafuge 1.0, Osterode, Germany). Model solutions and tomato juice samples were then stored at −20°C. All the experiments were carried out on the thawed tomato aqueous fractions and model solutions. Preliminary trials showed that freezing and thawing did not affect the antioxidant activity of the samples.

Analytical determinations

Total solid content

The determination of the total solid content of the tomato aqueous fraction was carried out according to AOAC.[31] Measurements were made in duplicate and the difference between the two determinations, carried out on the same sample, did not exceed 0.3 g kg^{-1} solid content.

Optical density

Absorbance at 420 nm was measured using a Varian DMS 80 UV–Vis spectrophotometer.[32] Solutions were diluted with distilled water to give absorbance signals on scale. Measurements were carried out in triplicate. Coefficients of variation, expressed as the percentage ratio between the standard deviation and the mean value, were found to be less than 5%.

Ascorbic acid

The ascorbic acid content was measured using an enzymatic assay (Boeringher Mannheim, Mannheim, Germany).

Rancimat test

The Rancimat test was carried out according to a method described previously,[33] using a model 679 Metrohom Rancimat (Metrohom, Herisau, Switzerland). Aliquots of 3 g virgin olive oil stratified over 5 g tomato aqueous fraction were put in the reaction tubes and heated at 95°C in the presence of a 20 litres h^{-1} air flow. The air flow through the sample caused a turbulence so that an intimate contact between the solid components of tomato and the oil matrix was achieved. As water affects oil oxidation rate, a virgin olive oil–water sample was tested as a reference. Results were expressed as a performance ratio between the induction time of the oil–tomato and of the oil–water mixtures. Measurements were carried out in duplicate.

Oxygen uptake

The oxygen consumption of the tomato aqueous fraction was measured according to the methodology of Ref 34. A YSI model 53 oxygen monitor (Yellow Springs Instruments Co Inc, Yellow Springs, OH, USA) equipped with an oxygen electrode was used. The airtight reaction vessel was filled with 3 ml of tomato juice samples conditioned at 25°C. The oxygen consumption was recorded using a Bromma recorder (LKB Producter, Bromma, Sweden). Distilled water was used as a control. The rate of the oxygen uptake was calculated from the initial linear portion of the curve obtained. The oxygen consumption activity was expressed as μmoles oxygen consumed min^{-1} g^{-1} dry matter, assuming that the oxygen concentration in air-saturated water at 25°C is 237 μmol litre^{-1}. Five measurements were carried out on each sample and the coefficient of variation was $<12\%$.

Chain breaking activity

The procedure for the determination of the chain breaking activity has previously been described.[8] In particular, the ability of a compound or of a mixture of compounds to quench peroxyl radicals produced by a 103.8 mM aqueous solution of ABAP (2,2′-azo-bis(2-amidinopropane)dihydrochloride) (Wako Chemicals GmbH, Neuss, Germany) was measured spectrophotometrically (Kontron Istruments, Uvikon 860, Milano, Italy) at 443 nm by analysing the first order rates of crocin bleaching.

The competition kinetics follow the equation:

$$\Delta A_o/\Delta A = V_o/V = (kc[C] + ka[A])/kc[C]$$
$$= 1 + (ka/kc)([A]/[C])$$

where ΔA_o and ΔA are the absorbance variations in the absence or in the presence of antioxidants; V_o and V are the bleaching rates in the absence or in the presence of antioxidants; kc and ka are the rate constants of the crocin bleaching in the absence or in the presence of antioxidants; $[A]$ and $[C]$ are the concentration of antioxidant and crocin. Crocin was isolated from saffron (Sigma Chemical Co, Sant Louis, MO, USA) by methanol extraction after repeated washing with ethyl ether. The crocin solution was diluted with methanol in order to obtain a 0.30 M crocin solution (the absorption coefficient of crocin at 443 nm is 1.33 × 10^5 mol^{-1} cm^{-1}). Analyses were carried out at 49°C in 2 ml incubation medium containing 0.1 M phosphate buffer pH 7.0, 9.11 μM crocin and increasing amounts of tomato juice samples or sugar-amino acid model systems. The reaction was started by adding 50 μl ABAP aqueous solution. Duplicate measurements were made for

each sample. All the dry matter of samples was assumed to have antioxidant properties.

RESULTS AND DISCUSSION

The overall antioxidant properties of tomato samples heated at 95°C for up to thirty hours were qualitatively assessed measuring the effectiveness in prolonging the induction period prior to oxidation of an oil subjected to an accelerated stability test. Table 1 shows the performance ratio of the heated tomato samples. Results indicated that after an initial decrease, observed for the 2 h and 4 h heated samples, a recovery of the original antioxidant potential can be achieved by prolonging the heating time.

Since the accelerated stability test does not give any information about the mechanisms involved in the antioxidant activity of the naturally occurring and/or the heat-induced compounds present in the product, the chain breaking and the oxygen scavenging properties were evaluated.

Figure 1 shows the changes in the chain breaking activity of tomato samples subjected to heat treatment at 95°C for various times. Corresponding values of optical density at 420 nm are also reported. A slight reduction in the chain breaking activity during the first 3 h heating was detected. This variation did not correspond to any appreciable change in optical density. By prolonging heating times, a recovery and a further increase in the chain breaking activity was observed. Samples heated for 15 h and longer, showed chain breaking activity values progressively higher than those measured for the unheated tomato sample. These variations were associated with the notable increase in optical density.

Similar experiments, carried out at 70°C, confirmed the presence of an initial decrease in the chain breaking activity within the first 5 h of heating. By prolonging heating times, a recovery in the chain breaking activity was observed but only up to values close to those measured for the unheated tomato samples. In this case no changes in optical density were observed (Fig 2).

The variations in the chain breaking activity of tomato juice samples as affected by heating could be the consequence of different reactions, which involve the natural antioxidants and/or the development of MRP. Natural antioxidants present in the tomato aqueous phase (mainly ascorbic acid) can easily undergo thermal degradation and/or consumption in the Maillard reaction pathway.[35,36] In fact, the reduction of the ascorbic acid concentration in the tomato samples was close to 90% after only 4 h of heating (data not shown). Nevertheless, the formation of compounds with pro-oxidant properties during the early stages of the Maillard reaction may also be an explanation.

In order to evaluate the contribution of the MRP only, the chain breaking activity of heated model systems containing glucose, fructose and glutamic acid was assessed. Data referred to experiments carried out at 70°C and 95°C are reported in Fig 3. It is interesting to observe that an initial decrease in the chain breaking activity was detected even in the case of the model systems. In addition, as previously observed for the tomato juice samples, the recovery and the further increase in the chain breaking activity of the model system was accompanied by an increase in optical density when heated at 95°C, as shown in Table 2.

Table 1. Performance ratio of tomato sample heated at 95°C for up 30 h

Heating time (h)	*Performance ratio*[a]
0	1.22 ± 0.02
2	1.13 ± 0.03
4	1.17 ± 0.02
15	1.26 ± 0.06
30	1.31 ± 0.03

[a] Calculated as the ratio between the induction time of the oil–tomato samples and of an oil–water mixture, taken as a reference; each measurement is an average of two replications ± standard deviation.

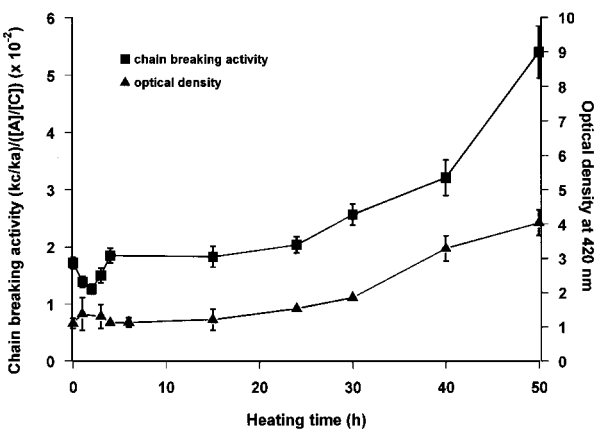

Figure 1. Changes in the chain breaking activity and in the optical density at 420 nm of tomato juice samples heated at 95°C.

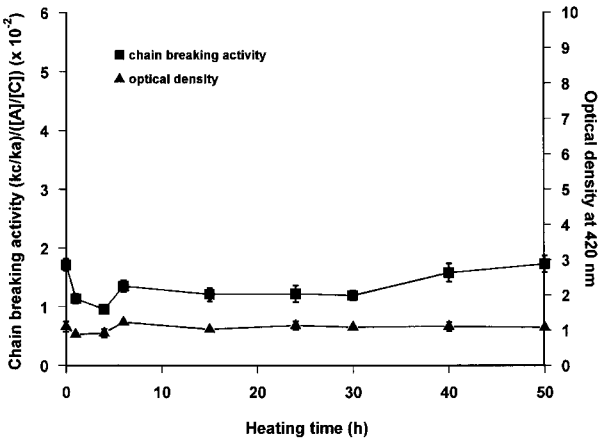

Figure 2. Changes in the chain breaking activity and in the optical density at 420 nm of tomato juice samples heated at 70°C.

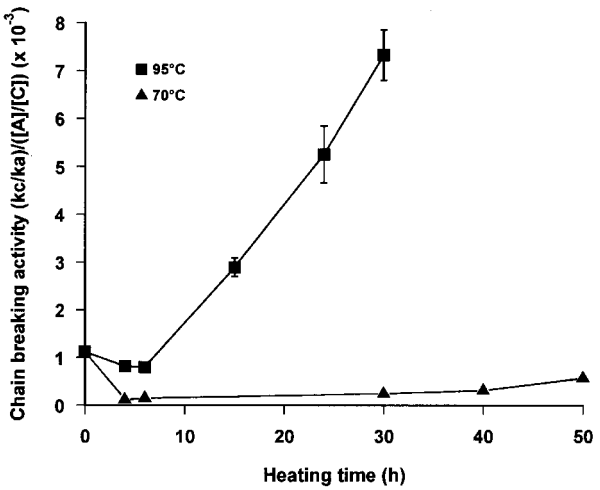

Figure 3. Changes in the chain breaking activity of a 10 g kg^{-1} glucose, 15 g kg^{-1} fructose and 2.4 g kg^{-1} L-glutamic acid aqueous solution heated at 70°C and 95°C.

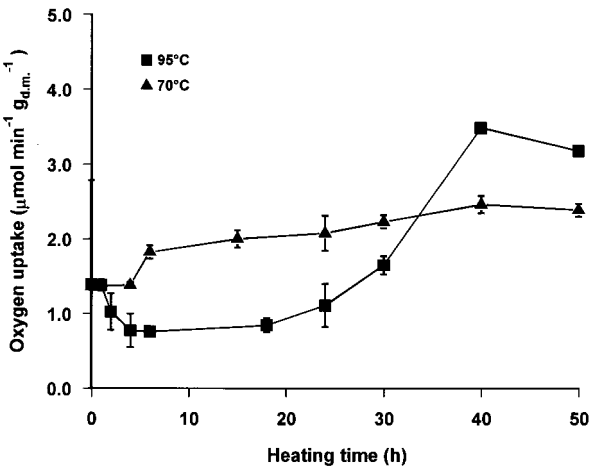

Figure 4. Oxygen uptake of tomato juice samples heated at 70°C and 95°C.

The formation of free radicals during the early stages of the Maillard reaction was previously proposed[37] for a glucose–alanine model system. These authors observed that highly reactive radicals were formed just prior to the Amadori rearrangement and that their disappearance was accompanied by a gradual development of browning.

It is likely that the pro-oxidant properties, found both for the tomato samples and for the model solutions subjected to short heat treatments, were due to the reaction of the MRP radicals with crocin. Thus, the observed increase in the crocin bleaching rate is the result of the action of both peroxyl radicals produced by ABAP and MRP radicals.

Figure 4 shows the oxygen consumption rates of the tomato samples as a function of heating time at 70°C and 95°C. As regards samples heated at 95°C, the changes in the oxygen scavenging properties were similar to those found for the chain breaking activity. In fact, an initial decrease and a further recovery of the oxygen consumption rate were observed, while heat treatments carried out at 70°C caused only a slight increase in the oxygen consumption rate.

Results reported above showed that heating caused an increase in the overall antioxidant potential of the tomato juice. This occurred as a consequence of the formation of melanoidins during the advanced steps of the Maillard reaction. In the light of these findings, stability and shelf life of tomato derivatives or food products containing tomato as an ingredient are expected to be improved when prolonged thermal treatments are applied. However, short heat treatments promoted an initial reduction in the original antioxidant potential of the product. These data suggest that compounds with pro-oxidant properties are formed during the early stages of the Maillard reaction. These results appear of considerable interest as regards short time heat treatments which could be responsible of a depletion of the overall antioxidant potential of the products even through the formation of compounds with pro-oxidant properties.

The effects of these substances on the shelf life of the product as well as on human health can easily be imagined and further investigations appear to be worthwhile.

Table 2. Changes in optical density at 420 nm of glucose–fructose–glutamic acid aqueous solutions heated at 70°C and 95°C for different lengths of time

Heating time (h)	*Optical Density*	
	70°C	*95*°C
0	ND[a]	ND
2	ND	ND
4	ND	ND
6	ND	ND
15	ND	0.08
24	ND	0.19
30	ND	0.29
40	ND	0.79
50	ND	0.90

[a] ND, not detectable.

REFERENCES

1 Pokorny J, Natural antioxidants for food use. *Trends Food Sci Technol* **2**:223–226 (1991).
2 Fereidoon S, Janitha PK and Wanasundara PD, Phenolic antioxidants. *Crit Rev Food Sci Nutr* **32**:67–103 (1992).
3 Krinsky NI, Plant carotenoids and related molecules: important dietary antioxidants, in *Proceeding of Biochemical Society Symposium No 61*, Ed by Rice-Evans C, Halliwell B and Lunt GG. Portland Press, London, UK, pp 117–126 (1994).
4 Block G and Langseth L, Antioxidant vitamins and disease prevention. *Food Technol* **48**:80–84 (1994).
5 Rice-Evans C, Plant polyphenols: free radical scavengers or chain-breaking antioxidants? in *Proceeding of Biochemical Society Symposium No 61*, Ed by Rice-Evans C, Halliwell B

and Lunt GG. Portland Press, London, UK, pp 103–116 (1994).
6 Halliwell B, Murcia MA, Chirico S and Aruoma OI, Free radicals and antioxidants in food and *in vivo*: what they do and how they work? *Crit Rev Food Sci Nutr* **35**:7–20 (1995).
7 Ramarathnam N, Osawa T, Ochi H and Kawakishi S, The contribution of plant food antioxidants to human health. *Trends Food Sci Technol* **6**:75–82 (1995).
8 Tubaro F, Micossi E and Ursini F, The antioxidant capacity of complex mixtures by kinetics analysis of crocin bleaching inhibition. *J Am Oil Chem Soc* **73**:173–179 (1996).
9 Rossell JB, in *Rancidity in Foods*, Ed by Allen JC and Hamilton RJ, Elsevier Applied Science, London, UK, pp 21–45 (1983).
10 Liao ML and Seib PA, Chemistry of L-ascorbic acid related to foods. *Food Chem* **30**:289–312 (1988).
11 Jonsson I, Thermal degradation of carotenoids and influence of their physiological functions, in *Nutrition and Toxicological Consequences of Food Processing*, Ed by Friedman M. Plenum Press, New York, NY, USA, pp 75–82 (1991).
12 Eichner K, Antioxidative effect of Maillard reaction intermediates. *Prog Food Nutr Sci* **5**:441–451 (1981).
13 Ames JM, The Maillard browning reaction—an update. *Chem Ind* **5**:558–561 (1988).
14 O'Brien GP and Morrissey PA, Nutritional and toxicological aspects of the Maillard reaction in food. *Crit Rev Food Sci Nutr* **28**:211–248 (1989).
15 Rizzi GP, The Maillard reaction in foods, in *Maillard Reaction in Chemistry, Food and Health*, Ed by Labuza TP, Reineccius GA, Monnier VM, O'Brien J and Baynes JW. Royal Society of Chemistry Publisher, Cambridge, UK, pp 11–19 (1994).
16 Griffith T and Johnson JA, Relation of the browning reaction to storage stability of sugars cookies. *Cer Chem* **34**:159–169 (1957).
17 Maleki M, The effect of non-enzymatic browning in the presence of glucose and glycine on the development of rancidity in corn oil. *Fette Seifen Anstrichmittel* **75**:103–104 (1973).
18 Kawashima K, Itoh H and Chibata I, Antioxidant activity of browning products prepared from low molecular carbonyl compounds and amino acids. *J Agric Food Chem* **25**:202–204 (1977).
19 Lingnert H and Lundgren T, Antioxidative Maillard reaction products IV. Application in sausage. *J Food Proc Preserv* **4**:235–246 (1980).
20 Won JT and Kim DH, Antioxidant activity of various solvents extracts obtained from a Maillard-type browning reaction mixture. *Korean J Food Sci Technol* **12**:235–241 (1980).
21 Maeng YS and Kim DH, Antioxidant activity of ethanol-extracts from a Maillard browning mixture and some antioxidants in soybean oil and soybean oil–water emulsion systems. *Korean J Food Sci Technol* **13**:273–382 (1981).
22 Tanaka M, Sugita S, Chiu WK, Nagashima Y and Taguchi T, Influence of water activity on the development of antioxidative effect during the Maillard reaction between histidine and glucose. *Bull Japan Soc Scient Fish* **56**:525–530 (1990).
23 Elizalde BE, Dalla Rosa M and Lerici CR, Effect of Maillard reaction volatile products on lipid oxidation. *J Am Oil Chem Soc* **68**:758–762 (1991).
24 Elizalde BE, Bressa F and Dalla Rosa M, Antioxidantive action of Maillard reaction volatiles: influence of Maillard solution browning level. *J Am Oil Chem Soc* **4**:331–334 (1992).
25 Lee HS, Antioxidative activity of browning reaction products isolated from storage-aged orange juice. *J Agric Food Chem* **40**:550–552 (1992).
26 Shyu SL and Yen GC, Nonenzymic browning reaction of roasted peanut kernels and its effect on oxidative stability of peanut oil. *J Chin Agric Chem Soc* **30**:59–70 (1992).
27 Severini C, Pittia P, Nicoli MC and Pinnavaia GG, Interaction between Maillard reaction products and lipid oxidation during the roasting process and storage of hazelnuts (*Corylus avellana*), in *Development in Food Engineering*, Ed by Yano T, Matsuno R and Nakamura K, Blackie Academic and Professional Press, Glasgow, UK, pp 289–291 (1994).
28 Bressa F, Tesson N, Dalla Rosa M, Sensidoni A and Tubaro F, Antioxidant effect of Maillard reaction products: application to a butter cookie of a competition kinetics analysis. *J Agric Food Chem* **44**:692–695 (1996).
29 Nicoli MC, Anese M, Parpinel MT, Franceschi S and Lerici CR, Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett* **114**:71–74 (1997).
30 Nicoli MC, Anese M, Manzocco L and Lerici CR, Antioxidant properties of coffee brews in relation to the roasting degree. *Lebensm Wiss u Technol* **30**:292–297 (1997).
31 AOAC, *Official Methods of Analysis*, Ed by Cunniff P, Association of Official Analytical Chemists International, Arlington, Virginia, USA (1995).
32 Nicoli MC, Anese M and Lerici CR, Influence of pH on the kinetics of non-enzymatic browning in heat-treated glucose–glycine model systems. *Ital J Food Sci* **6**:139–146 (1993).
33 Hasenhuettl G and Wan PJ, Temperature effects on the determination of oxydative stability with the Metrohom Rancimat. *J Am Oil Chem Soc* **69**:525–527 (1992).
34 Anese M, Pittia P and Nicoli MC, Oxygen consuming properties of heated glucose/glycine aqueous solutions. *It J Food Sci* **5**:75–79 (1993).
35 Robertson GL and Samaniego CM, Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning lemon juice during storage. *J Food Sci* **51**:184–187 (1986).
36 Davies CGA and Wedzicha BL, Ascorbic acid browning: the incorporation of C_1 from ascorbic acid into melanoidins. *Food Chem* **49**:165–167 (1994).
37 Namiki M and Hayashi T, A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation, in *The Maillard Reaction in Foods and Nutrition*, Ed by Waller GR and Feather MS. American Chemical Society, Washington DC, USA, pp 21–47 (1983).