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Heat-induced chemical, physical and functional changes during grape must cooking

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Abstract

Cooked must is a product that could be used in food formulations, directly or after fermentation, to obtain many traditional foods. Must cooking was conducted in boilers of different materials (copper and stainless steel) for different times in order to obtain differently concentrated products.

The concentration of many constituents (sugars, organic acids, nitrogen compounds, metal ions and polyphenols) was observed upon cooking, together with the increase of neo-formation compounds, such as hydroxymethylfurfural and melanoidins, which give, to the musts, the typical brown colour and caramel-like odour. The concentration of metal ions, in particular, determined high levels of lead and copper (in the case of use of copper boilers) in the final products.

Polyphenol heat concentration determined the degradation of simple phenolics, such as catechins, and the formation of condensed tannins, which determined a loss of the antioxidant activity of the phenolic fraction, whereas the formation of melanoidins improved the total antioxidant activity of the product.

Keywords: Grape must; Heat concentration; Browning; Hydroxymethylfurfural; Melanoidins; Polyphenols; Antioxidant activity

1. Introduction

Grape must cooking is an ancestral practice in Mediterranean countries and it is required for the production of many traditional Italian foods and beverages, such as traditional balsamic vinegars of Modena and Reggio Emilia, "vino cotto" (namely cooked wine), a liquor wine from the Marche and Abruzzo regions, Marsala wines from Sicily and mosto cotto (cooked must), from the Apulia region, which is used as a filling in bakery formulations (Repubblica Italiana, 2006). Apart from Italy, cooked must is also used for Spanish sweet wines (Rivero-Pérez, Peréz Magariño, & Gonzàles-San José, 2002).

Cooked must is mainly produced from white grapes (e.g. Trebbiano cultivar) and is obtained by direct heating of must over an open fire in uncovered boilers or pans which are traditionally made of copper. The must is cooked until its volume is reduced by 10–60% according to the production technology and, once cooled down, it could either be fermented in barrels or used as is, depending on the product to be obtained.

Must cooking is traditionally carried out below boiling temperatures (80–95 °C) and could require quite long processing times (up to 48 h), depending on the capacity of the cooking pan. During cooking the must becomes dark and dense and many chemical changes occur due to the prolonged thermal treatment.

Product cooking determines the concentration of naturally occurring chemicals in must, among which are sugars and acids. Under acidic conditions, as occurring in fresh

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and cooked musts, sugars undergo thermal degradation with the formation of furan derivatives (Belitz & Grosch, 1999). Some authors (Antonelli, Chinnici, & Masino, 2004) investigated furans in cooked musts and attributed their formation to thermal degradation of sugars under acid conditions, even though furans such as 5-hydroxymethylfurfural (HMF) could be formed also in the Maillard reaction. Moreover, alpha-amino acids are present in grape musts and these compounds could undergo thermal condensation with sugars to form Maillard reaction products (MRPs) (Belitz & Grosch, 1999; Martins, Jongen, & van Boekel, 2001).

MRPs, such as HMF and melanoidins, were determined in sweet wines obtained from cooked musts (Mastrocola, Sacchetti, Di Mattia, Seghetti, & Piva, 2006; Rivero-Pérez et al., 2002) and the most concentrated and browned the product, the higher was the concentration of these compounds. If furanic compounds, and HMF in particular, are supposed to have negative effects on human health, compounds formed in the last phases of the Maillard reaction, such as melanoidins, are gaining attention due to their antioxidant potential (Lee & Shibamoto, 2002; Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001). MRPs were shown to contribute to the antioxidant activity of products from cooked musts (Mastrocola, Di Mattia, Seghetti, & Sacchetti, 2005; Mastrocola et al., 2006) but the relative contribution of these compounds to the total antioxidant activity of cooked musts is still unknown.

This study is aimed to understand the most important chemical and physical changes undergone by musts during cooking and to evaluate the effect of these changes on product quality and potential health properties. To this end, cooking was conducted for different times to give products of different concentrations and in boilers of different materials: copper and stainless steel.

2. Materials and methods

2.1. Materials

White grapes of Trebbiano d'Abruzzo were removed from stalks and pressed. The obtained must was divided into aliquots of 100 l and concentrated in copper and stainless steel boilers of 120 l capacity to about 65, 40 and 30 l. The masses were rapidly heated to boiling point; then the temperature was set at 95 °C for the whole process (up to 18 h). Each concentration process was duplicated. Samples for organic acid and physical analyses were taken at the end of the processes and treated with sodium azide to avoid further fermentation. Samples to be analyzed for sugar, amino acids, metal ions, polyphenols content, MRPs content and antioxidant activity were frozen and stored at −40 °C to reduce the rate of both chemical oxidation and Maillard reaction. Three different subsamples were taken and submitted to analysis.

2.2. Physical determinations

2.2.1. Density

This was measured at 20 °C using the official EU community methods for the analysis of musts and wines (EEC, 1990).

2.2.2. Viscosity

Viscosity was measured at 25 °C by a Stress Tech Rheometer (Reologica Instruments AB, Sweden) equipped with a coaxial cylinder measuring system (CC25). Analyses were carried out by varying the flow rate in a range between 0.33 and 87.67 s^{-1}. Data were fitted by the power law equation ($X = K\gamma^n$) for the calculation of K and n.

2.2.3. Colour

Reflectance analyses were carried out by a Minolta (Tokyo, Japan) CM-500 spectrophotometer using the CIE illuminant D65 and 10° standard observer conditions (CIE, 1986). Before analysis, the instrument was calibrated on a white standard (L^*: 96.58; a^*: −0.09; b^*: −0.05 on SCE modality). Samples were poured into white beakers (L^*: 68.08; a^*: −0.54; b^*: −0.52 on SCE modality) of 1 cm height and were covered with a transparent glass in order to set the optical path constant. Transparent glass showed no significant influence on colour determinations (data not reported). Colour measurements were carried out in the SCE (specular component excluded) modality as the glass determines a high specular reflection.

2.3. Chemical determinations

2.3.1. General measures

Total dry extract, density, refractometric index, tritable acidity, pH, total nitrogen and hydroxymethylfurfural (HMF) content were determined using the official EU community methods for the analysis of wines (EEC, 1990). For HMF determination, samples were diluted 1:10 with 10% ethanol solution to enter the linear range. 5-(Hydroxymethyl)-furfural (Sigma, Steinheim, Germany) was used for calibration curves. Spectrophotometric determinations were carried out with a Perkin–Elmer (Boston, MA) Lamba Bio 20 spectrophotometer.

2.3.2. Sugar determination

Musts were purified using C18 SPE cartridges (500 mg, 6 ml) (International Sorbent Technology, UK); 500 μl of sample were loaded into the column previously conditioned with 2.5 ml of methanol and 5 ml of water. Sugars were eluted with 5 ml of 0.1 N sulphuric acid, made up to 5 ml final volume and analysed by HPLC. Isocratic separation of analytes was carried out at 35 °C using an Aminex HPX-87C (Bio-Rad, Watford, UK) carbohydrate column (300 × 7.8 mm) equipped with a Bio-Rad Carbo-C precolumn (30 mm × 4.6 mm). The system consisted of a Spectra System (ThermoFinningan, Rodano, Italy) P4000 pump, Spectra System AS3000 autosampler, a degasser and a

Spectra System RI detector. Integration and data elaboration were performed by the ChromQuest software (ThermoFinningan). The mobile phase was 0.1 N sulphuric acid with a flow rate of 0.5 ml min^{-1}. Twenty microliters of filtered samples (0.45 μm membrane filter) were injected with separation in 20 min. Fructose and glucose (Sigma, Steinheim, Germany) were used as external standards for identification and quantification.

2.3.3. Organic acids determination

Tartaric acid was determined by the colorimetric method proposed by Vidal and Blouin (1979) which is based on the reaction of the analyte with vanadic acid to give an orange colour which was measured at 500 nm. Malic and citric acids were determined by the enzymatic methods of Moellering (1985) and Moellering and Gruber (1966), respectively, using Diffchamb Enzyplus kits (Raisio Diagnostic, Turku, Finland).

2.3.4. Determination of metal ions

Copper and lead were determined by the anodic stripping method proposed by Palchetti, Laschi, and Mascini (2005). The method is based on the deposition of the analytes from a solution under constant stirring on a sensor and their further determination by square wave voltammetry in quiescence conditions. The sensor was obtained by modifying screen-printed electrodes with a solution containing 100 g of mercury acetate and 100 ml of acetic acid in 10 ml of bidistilled water. The solution (1.5 ml) was treated with 3.5 ml of an aqueous Methocel (The Dow Chemical Company, Midland, MI) solution, from 0.02 to 25 mg/ml. The sensors were pretreated by applying a potential of −1.1 V for 300 s. A potentiostat Autolab PGSTAT 12 (Eco Chemie, Utrecht, The Netherlands) equipped with GPES (General Purpose Electrochemical System) software (4.8 version) was used for analysis. Operative conditions were as follows: conditioning potential (−0.3 V for 60 s), deposition potential (−1.1 V for 120 s), square wave width (28 mV), accumulation potential (3 mV at 15 Hz). The quantification was carried out by an external standard calibration curve and results were expressed in mg/l.

2.3.5. Solid phase extraction of the phenolic and non-phenolic fractions

Commercially available octadecyl C_{18} cartridges (1 g, 6 ml) (International Sorbent Technology, UK) were used for the extraction of the phenolic fraction according to the following protocol: 1 ml of sample diluted 1:10 in 0.1 N sulphuric acid was loaded onto the column previously conditioned with 2 ml of methanol and 5 ml of water. The column was eluted with 4 × 10 ml of 0.1 N sulphuric acid to eliminate all the water-soluble compounds and the recovered solution was designated the non-phenolic extract (NPE). The compounds retained by the column were recovered by eluting with 4 × 10 ml of 60% methanol solution and the collected solution was designated the phenolic extract (PE). Trapping and release of the phenolic fraction from the C_{18} solid phase was performed according to Di Stefano and Guidoni (1989).

2.3.6. Polyphenols analysis

Total polyphenols content was determined by the colorimetric reaction with the Folin Ciocalteau reagent (Singleton & Rossi, 1965). Before analysis, phenolic compounds were extracted by solid phase extraction because substances, such as reducing sugars, alcohol and tartaric acid, as well as antioxidant compounds (e.g. Maillard reaction products), could interfere in the determination of polyphenols with the Folin Ciocalteau reagent (Naczk & Shahidi, 2004).

Catechins were determined on the phenolic fraction by the colorimetric reaction with 4-dimethyl-amino-cinnamaldehyde (DAC), as described by Zironi, Buiatti, and Celotti (1992). Samples were diluted 1:10 to 1:50 with a 10% ethanol solution to enter the linear range. Must (1 ml) filtered with 0.45 μm nylon filters was treated with 5 ml of DAC reagent and the spectrophotometric analysis was carried out at 640 nm. The quantification was carried out by an external standard calibration curve and results were expressed in mg/l of (+) catechin equivalent.

Condensed tannins (proanthocyanidins) were determined on the phenolic fraction by the colorimetric method described by Bate-Smith (1975). Sample were diluted 1:50 with a 10% ethanol solution to enter the linear range. Must (2 ml) was treated with 6 ml of reaction reagent (HCl/*n*-butanol/Fe_2SO_4) and the colorimetric reaction was carried out at 100 °C for 30 min. The spectrophotometric analysis of anthocyanidins was carried out at 550 nm. The quantification was carried out by an external standard calibration curve and results were expressed in mg/l of leucocyanidin equivalents.

2.3.7. Primary amino acids determination

Primary amino acids were quantitatively determined by the ninhydrin spectrophotometric method. Samples (1 ml) were centrifuged at 8000*g* and the surnatant was treated with 1 ml of 10% trichloroacetic acid, vortexed for 1 min and stored at 8 °C for 15 min. The solutions were centrifuged and filtered on Whatman 541 filters. Clarified solutions were properly diluted and analysed using the procedure reported by Troll and Cannan (1953). The quantification was carried out by an external standard calibration curve and results were expressed in mg/l of *L*-leucine.

2.3.8. Melanoidins

Melanodins were determined by spectrophotometric determination (Rivero-Pérez et al., 2002) with slight modifications. Before analysis, musts were dialysed using cellulose dialysis tubing (Sigma) that retains most molecules ⩾12,000 Da. 15 ml of must were put into the dialysis tube and were placed in a glass vessel with 1 l of water. This solution was maintained at 5 °C with stirring for 12 h. This procedure was repeated twice. The volume of must which remained in the dialysis tube was diluted to a known

volume with bidistilled water. Melanoidins content was determined by reading the absorption value at a wavelength of 280 nm.

2.4. Antioxidant activity determination

The radical-scavenging activity was determined by the $ABTS^{+\cdot}$ radical cation decolorization assay, as described by Re et al. (1999). The bleaching rate of $ABTS^{+\cdot}$ in the presence of the sample was monitored at 734 nm. A volume of 2.97 ml of $ABTS^{+\cdot}$ solution (Abs = 0.7 ± 0.02) was used. The reaction was started by the addition of 30 μl of either musts diluted up to 1:100 or extract samples diluted up to 1:10. $ABTS^{+\cdot}$ bleaching was monitored at 25 °C for at least 60 min and the decoloration after 5 min was used as the measure of antioxidant activity. In this dilution range, the $ABTS^{+\cdot}$ bleaching was proportional to the concentration of the sample added to the medium and the dose–response curve was fitted by a linear model. Antioxidant activity was calculated by the ratio of the regression coefficient of the dose–response curve of the sample and the regression coefficient of the dose–response curve of Trolox (hydrophilic homologue of tocopherol) and was expressed as μmoles of Trolox equivalents per ml of sample.

2.5. Statistical analysis

Analytical data were reported as means and standard deviations calculated on replicate treatments. Data were further analysed by ANOVA and significant differences between means were tested by LSD test. Linear and non-linear regression analyses were carried out on data and the goodness of fit was checked by the determination coefficient R^2. Data were processed using the STATISTICA for Windows (StatSoft™, Tulsa, OK) package.

3. Results and discussion

Must concentration upon cooking to about 65%, 40% and 30% of the initial volume determined an increase of the total solids content (Table 1). The concentration factor of different musts was calculated on the basis of their total solids content and shown in Table 1 as total solids concentration factor (TSCF).

The concentration of musts resulted in an increase of product density and viscosity (Table 1) which showed a strictly linear correlation with TSCF ($y = 0.073x + 1.008$; $R^2 = 0.992$ and $y = 0.007x - 0.005$; $R^2 = 0.999$, respectively). The use of cooking boilers of different materials did not affect either the total solids concentration or the product density and viscosity.

The concentration of the naturally occurring substances and the supply of thermal energy upon cooking determined the formation of brown pigments which was monitored by colour changes. The cooking process determined a loss of lightness and a shift of the hue angle ($h°$) from yellow to red (Table 1) due to the occurrence of browning reactions (MacDougall & Granov, 1998). Both L^* and $h°$ values were linearly correlated with the concentration factor ($R^2 = 0.978$ and 0.974, respectively).

On the a^*b^* chromaticity plane (Fig. 1), it is noticeable that, upon must concentration of about 35%, the yellow chromaticity coordinate (b^*) showed a dramatic decrease, which could be explained by the thermal degradation of naturally occurring yellow pigments. With further must concentration, the red chromaticity coordinate (a^*) increased with the increasing of the cooking extent. The use of stainless steel boilers instead of copper ones limited the product browning.

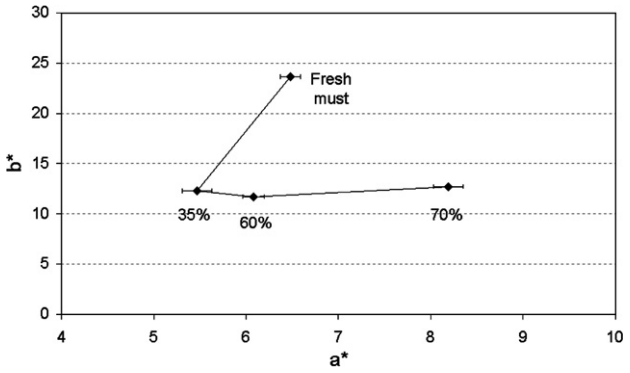

Fig. 1. Colour changes of must concentrated of 35%, 60% and 70% on the a^*b^* chromaticity plane.

Table 1
Total solids and physical properties (mean ± s.d.) of must concentrated to different extent and in different boilers

Sample	Total solids (g/l)	TSCF (g/g)	Density (kg/l)	Viscosity (K × 10^2)	Colour	
					L^*	$h°$
Fresh must	213.8 ± 16.5 d	1	1.077 ± 0.004 d	0.19 ± 0.01 d	28.77 ± 0.16a	70.12 ± 0.56a
Cooked musts						
Copper boiler						
35% concentration	335.6 ± 19.8c	1.6	1.126 ± 0.006c	0.61 ± 0.01c	23.40 ± 0.13b	58.68 ± 0.84b
60% concentration	556.3 ± 23.4b	2.6	1.205 ± 0.005b	1.31 ± 0.18b	18.98 ± 0.06d	52.59 ± 1.52d
70% concentration	671.9 ± 24.0a	3.1	1.230 ± 0.007a	1.70 ± 0.20a	14.30 ± 0.23e	46.81 ± 0.82e
Steel boiler						
60% concentration	555.4 ± 34.1b	2.6	1.199 ± 0.006b	1.03 ± 0.15b	20.26 ± 0.05c	57.11 ± 0.41c

Means with the same letters within the same column are not significantly different at a $p < 0.01$ value.

The formation of brown pigmented compounds upon must cooking could be ascribed to non-enzymatic browning (NEB) reactions. Caramelisation is unlikely to occur in this product because it generally occurs with higher sugar concentrations (Antonelli et al., 2004) and temperatures of about 140 °C (Kroh, 1994). The chemical changes responsible for browning were investigated by analysing the concentrations of chemicals (Table 2).

The concentration of chemicals upon cooking was analysed by the regression coefficient of the linear response curve obtained by plotting the concentration factor (CF) of each single compound as a function of TSCF. A regression coefficient lower or higher than one indicates the loss or the formation of that compound during cooking, respectively.

Glucose and fructose concentration factors were strictly correlated with TSCF ($R^2 = 0.998$ and 0.996, respectively) with regression coefficients of 0.966 and 0.926, to indicate a less than proportional concentration of these compounds. This result was observed also by Antonelli et al. (2004) who attributed it to sugar thermal degradation. In particular, a concentration factor of fructose lower than that of glucose confirms the greater reactivity of fructose in acid media. The type of boilers (copper or stainless steel) did not affect the sugar concentration. Antonelli et al. (2004) attributed the thermal degradation of sugars under acid conditions to enolization reactions with further formation of furanic compounds. The hypothesis of sugar consumption through the Maillard reaction pathways should not be rejected, since aminic nitrogen in must is mainly present in alpha-amino acids (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 1998).

Nitrogen concentration factor showed a negative quadratic correlation with TSCF which describes a progressive loss of nitrogen compounds during cooking (Fig. 2). Nitrogen in must is mainly present in alpha-amino acids (Ribéreau-Gayon et al., 1998) which, upon heating, could undergo Maillard reaction and form water-insoluble high molecular weight melanoidins. Moreover, proteic nitrogen could be easily precipitated upon cooking, due to coagulation and formation of complexes with tannins (Ribéreau-Gayon et al., 1998).

The alpha amino acids (AA) concentration factor was slightly lower than that of nitrogen in the first stage of heating, when the TSCF was less than 1.6, but eventually, with

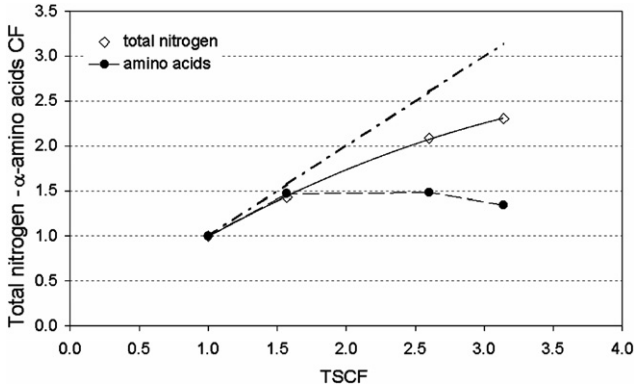

Fig. 2. Total nitrogen and α-amino acids concentration factor as a function of the total solids concentration factor. The bold dashed line indicates the theoretical concentration line. Any value below or above the theoretical concentration line indicates the consumption or the formation of the analyte, respectively.

Table 2
Chemical composition (mean ± s.d.) of must concentrated to different extent and in different boilers

Sample	TSCF (g/g)	Glucose (g/l)	Fructose (g/l)	Tartaric acid	Malic acid (g/l)	Citric acid (g/l)	pH (g/l)	Total nitrogen (mg/l)
Fresh must	1	105 ± 21.4d	98.4 ± 5.4d	4.22 ± 0.07d	2.82 ± 0.06e	0.38 ± 0.04d	3.08 ± 0.03a	352 ± 2e
Cooked musts								
Copper boiler								
35% concentration	1.6	165 ± 18.7c	153 ± 11.3c	5.69 ± 0.21c	3.86 ± 0.14d	0.61 ± 0.07c	2.96 ± 0.02b	503 ± 2d
60% concentration	2.6	275 ± 22.4b	254 ± 13.4b	9.58 ± 0.14b	6.38 ± 0.21c	0.76 ± 0.09b	2.76 ± 0.03c	735 ± 5b
70% concentration	3.1	320 ± 28.5a	290 ± 28.5a	11.3 ± 0.35a	7.88 ± 0.32a	0.92 ± 0.13a	2.72 ± 0.02cd	811 ± 5a
Steel boiler								
60% concentration	2.6	264 ± 26.2b	245 ± 12.2b	11.1 ± 0.39a	7.19 ± 0.26b	0.82 ± 0.09a	2.69 ± 0.03d	654 ± 2c

Sample	Amino acids (mg/l)	Copper (mg/l)	Lead (μg/l)	Total phenolics (ppmGAE)	Catechins (mg/l)	Tannins (mg/l)	HMF (mg/l)	Melanoidins (Abs_{280})
Fresh must	267 ± 14.6d	0.46 ± 0.03d	18 ± 2d	27.1 ± 0.1e	23.9 ± 0.2a	34.4 ± 3.1e	15 ± 1e	0.081 ± 0.003e
Cooked musts								
Copper boiler								
35% concentration	392 ± 10.8a	0.94 ± 0.07c	26 ± 5c	423 ± 15.3d	13.9 ± 0.2b	311 ± 11d	216 ± 16d	0.463 ± 0.002d
60% concentration	343 ± 5.0c	1.33 ± 0.16b	35 ± 7b	781 ± 16.9c	6.1 ± 0.2d	850 ± 34c	391 ± 18c	0.606 ± 0.003b
70% concentration	359 ± 4.0b	1.79 ± 0.21a	48 ± 10a	1259 ± 56.7a	3.1 ± 0.2e	1531 ± 87a	592 ± 29a	0.901 ± 0.004a
Steel boiler								
60% concentration	395 ± 6.5a	0.91 ± 0.06c	31 ± 5bc	924 ± 31.9b	8.2 ± 0.1c	921 ± 37b	448 ± 17b	0.541 ± 0.006c

Means with the same letters within the same column are not significantly different at a $p < 0.01$ value.

the increasing of solute concentration, the concentration factor of AA decreased progressively (Fig. 2) to indicate a consumption of AA during the second stage of heating. During this stage, the browning development was particularly evident (Fig. 1) and this suggests a possible consumption of AA through Maillard reaction pathways to give brown-coloured melanoidins. Even if the low pH values of musts does not favour the Maillard condensation due to protonation of the nucleophilic amino nitrogen, the protonation of carbonyl compound should enhance its reactivity with the nucleophilic agent (Martins et al., 2001). Browning in heat-treated acid media (pH = 3.0) was also studied by other authors (Shinoda, Komura, Homma, & Murata, 2005) and amino acids showed a stimulation effect on browning, thus confirming their involvement in NEB reactions, even at low pH.

The concentration factors of organic acids showed the same linear trend as observed for sugars, even if their regression coefficients with TSCF were 0.80, 0.85 and 0.62 ($R^2 = 0.996$, 0.996 and 0.962), respectively, for tartaric, malic and citric acids. The low values of the CF of organic acids could be explained by heat-induced reactions between nitrogen-free carboxylic acids and sugars (Lewis, Esselen, & Fellers, 1949). Organic acids, such as citric, tartaric and malic acids, could all react with sugars, through a condensation reaction, followed by CO_2 production, to give brown compounds (Lewis et al., 1949; Livingston, 1952). Citric is the most reactive acid (Lewis et al., 1949) and this could explain the lower regression coefficient of its CF with TSCF. The sample concentrated in stainless steel boilers showed higher values of organic acids than did those in copper boilers. During cooking in copper boilers, there is a migration of cations, such as copper ions (Table 2), which could further promote the rate of the sugar-acid browning reaction (Haas & Stadtman, 1949). Citric acid showed lower CF/TSCF values than other acids also upon cooking in stainless steel; this suggest a higher consumption of citric acid, independently of the container, which is probably due to the number of carboxylic functions. The reaction between nitrogen-free carboxylic acids and sugars may be a cause of browning in foodstuffs, and considering that these acids are much more abundant in must than are amino acids, their importance in the browning reaction should not be overlooked.

Even if organic acid concentration showed a dramatic increase during concentration, the must pH did not change a lot and this is in accordance with the results obtained by Antonelli et al. (2004). The hydrogen ions concentration factor was correlated with TSCF, with a regression coefficient of 0.63 ($R^2 = 0.99$), which could be explained by the fact that hydrogen ions could take part in many chemical reactions, such as protonation of aminic and weak acid functions at low pH (Cheftel, Cheftel, & Besançon, 1986) and, upon heating, also in Maillard reaction and sugar degradation under acid conditions (Belitz & Grosch, 1999). The sample cooked in stainless steel, due to its higher concentration of acids, also showed a hydrogen ions concentration higher than the analogous sample cooked in copper (Table 2).

The concentration of naturally occurring compounds upon cooking also determined the concentration of metals that could be present in musts as environmental and technological contaminants, such as heavy metals and copper. Lead content underwent a concentration almost proportional to TSCF (regression coefficient: 0.972; $R^2 = 0.998$) and the must concentration to 70% increased lead content from 0.017 to 0.048 mg/kg. The material of the boiler did not affect the lead content. Lead content in foods is regulated by EU law (EEC, 2001): for concentrated fruit juices (such as cooked must) it is fixed at 0.05 mg/kg; otherwise, for wines, just in the case of cooked must fermentation to traditional sweet wines, the limit of copper is fixed at 0.2 mg/kg. Thus the quantification of contaminants becomes of utmost importance for the analysis of musts about to be subjected to concentration.

The cooking process conducted in copper boilers determined a more than proportional increase of copper content which showed a CF strictly correlated with TSCF ($R^2 = 0.998$) with a regression coefficient of 1.25, to indicate a copper loss from the boiler. Copper limits in foods such as concentrated musts are not fixed by EU regulations but, in the case of cooked must fermentation to give traditional sweet wines, the final concentration of copper in must should not be greater than 1 mg/kg which is the maximum limit fixed for wines by Italian law (Repubblica Italiana, 1987). The must cooked in stainless steel boilers showed a significant lower copper content than did musts cooked in copper boilers and underwent a copper concentration lower than TSCF (CF = 0.77); nonetheless, must concentration to 70% could determine a critical copper content for cooked musts about to be fermented to give sweet wines.

Phenolic compounds showed different behaviours upon cooking and concentration. Total polyphenols (TPP), determined by reaction with the FC reagent, showed a CF higher than TSCF that is unlikely to happen during concentration; this result could be explained by taking into account the behaviour of the two phenolic fractions considered in this study: catechins and proanthocyanidins (Fig. 3). Cathechins showed a progressive decrease with the increasing of TSCF which could be explained by the thermal degradation and oxidation of phenolic compounds upon heat treatment, and the more concentrated the must, the longer is the heating time. Condensed tannins, such as proanthocyanidins, which were present in low concentration in fresh must, showed an increasing trend with the increase of cooking due to polymerization of single polyphenols upon oxidation and further condensation reactions. The rate of these reactions, which normally occur in wines during aging (Ribéreau-Gayon et al., 1998), could be accelerated by a high temperature of heating. As could be observed in Fig. 3, the TPP content increased with increase of condensed tannins and thus a CF of TPP higher than the TSCF could be explained by the accumulation of

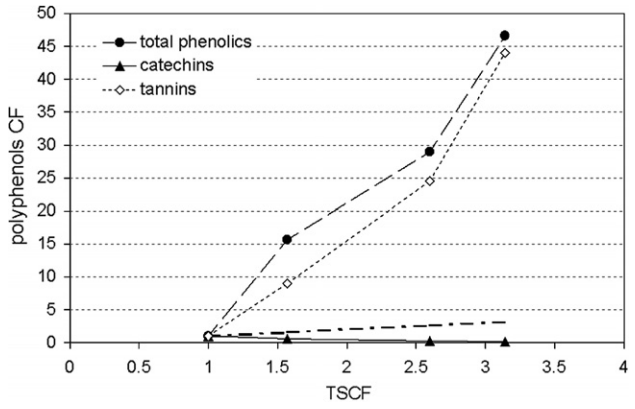

Fig. 3. Polyphenol concentration factor as a function of the total solids concentration factor. The bold dashed line indicates the theoretical concentration line. Any value below or above the theoretical concentration line indicates the consumption or the formation of the analyte, respectively.

neo-formation compounds. Overestimation of TPP by the methodology used in this study could occur, because the reaction of polyphenols with Folin-Ciocalteau reagent is non-specific and each single phenol shows a different response to this reagent (Naczk & Shahidi, 2004). Products cooked in stainless steel boilers showed higher values of catechins and TPP than did those cooked in copper boilers, which could be explained by the lower copper contents of these products; copper, in fact, is a pro-oxidant and could thus increase the rates of polyphenol oxidation and condensation reactions.

HMF content was linearly correlated with TSCF and its CF was higher than TSCF (Fig. 4) due to the formation of this compound during sugar thermal degradation. HMF, and other furanic compounds, account for the caramel-like odour of heated carbohydrates; although HMF has been reported to possess cytotoxic, genotoxic and mutagenic activity (Lee & Shibamoto, 2002), it should not pose any health risk under condition of use as a flavour ingredient (Adams et al., 1997).

Antonelli et al. (2004) attributed HMF formation to thermal degradation of sugars and not to Maillard reaction occurrence. This hypothesis is supported by the results obtained by Shinoda et al. (2005) which showed that, in an acid-sugar solution, AA did not have any stimulation effect on HMF or furanic compound formation upon heating. However, in the same work, AA were proved to stimulate browning reaction upon heating of the same solution, thus suggesting their possible role in NEB occurrence through the Maillard reaction pathways. The decrease of AA and the resulting browning development observed in this work suggest that HMF could also be produced during the Maillard reaction.

Melanoidins, polymeric nitrogen compounds formed in the final stage of the Maillard reaction, were detected in cooked must (Table 2). Other works have reported the occurrence of high molecular weight melanoidins in products from cooked must (Rivero-Pérez et al., 2002; Mastrocola et al., 2006). Melanoidin content showed a CF higher than TSCF due to the formation of these compounds upon heating (Fig. 4). The concentration of melanoidins was linearly correlated with TSCF but showed a lower coefficient of regression than that of HMF, indicating that, upon must cooking, the formation of furanic compounds occurs to a greater extent than that of brown polymeric pigments. The use of stainless steel boilers instead of copper ones reduced the melanoidins content of musts, thus limiting the product browning (Table 1), and increased the HMF content. Probably the higher acidity of the sample cooked in steel favours the formation of furan compounds more than that of melanoidins.

The antioxidant activity of must increased with concentration and, in particular, the antioxidant activity of the phenolic fraction decreased, whilst the antioxidant activity of the non-phenolic fraction increased (Fig. 5). The decrease of antioxidant activity of the phenolic fraction upon

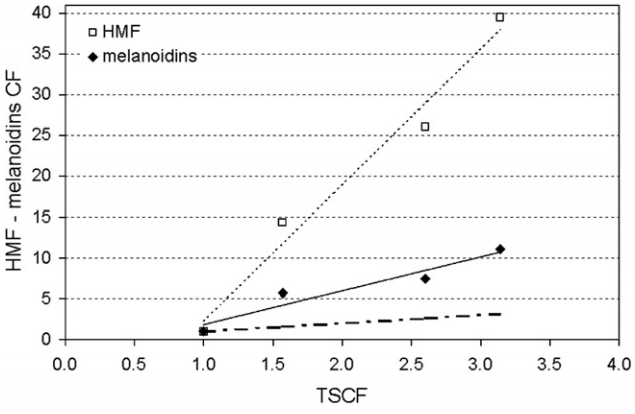

Fig. 4. Correlation between the concentration factor of HMF and melanoidins and the total solids concentration factor. The bold dashed line indicates the theoretical concentration line. Any value below or above the theoretical concentration line indicates the consumption or the formation of the analyte, respectively.

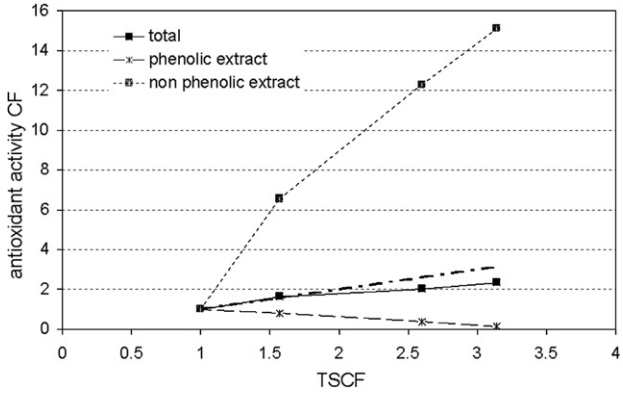

Fig. 5. Antioxidant activity concentration factor of musts, PE and NPE as a function of the total solids concentration factor. The bold dashed line indicates the theoretical concentration line. Any value below or above the theoretical concentration line indicates the decrease or the increase of the antioxidant activity corrected for volume.

Table 3
Antioxidant activity (mean ± s.d.) of must concentrated to different extent and in different boilers

Sample	TSCF	Antioxidant activity (μmol Trolox equiv/ml)		
		Total	Phenolic extract	Non-phenolic extract
Fresh must	1	0.925 ± 0.013e	0.791 ± 0.011a	0.125 ± 0.007d
Cooked musts				
Copper boiler				
35% concentration	1.6	1.47 ± 0.057d	0.635 ± 0.021b	0.819 ± 0.037c
60% concentration	2.6	1.82 ± 0.064c	0.291 ± 0.057d	1.54 ± 0.061b
70% concentration	3.1	2.10 ± 0.107b	0.122 ± 0.035e	1.89 ± 0.075a
Steel boiler				
60% concentration	2.6	2.24 ± 0.061a	0.384 ± 0.017c	1.81 ± 0.075ba

Means with the same letters within the same column are not significantly different at a $p < 0.01$ value.

cooking is due to oxidation phenomena that determine the formation of high molecular weight condensed tannins with lower antioxidant activity than monomeric phenols (Saint-Cricq de Gaulejac, Vivas, Freitas, & Glories, 1999). On the other hand the increase of the antioxidant activity of the non-phenolic fraction upon cooking could be attributed to the formation of Maillard reaction products, such as melanoidins, which were proved to act as effective antioxidants (Manzocco et al., 2001; Manzocco, Mastrocola, & Nicoli, 1999; Nicoli, Anese, & Parpinel, 1999). The elution of a MRPs model system with the acidulated aqueous solution used in this study permits an 85% recovery of antioxidant activity (Mastrocola et al., 2006); thus MRPs could significantly contribute to the antioxidant activity of the non-phenolic fraction. However it is notable that the separation by SPE performed in this study is not quantitative because, even if SPE is a reliable technique for polyphenols extraction (Naczk & Shahidi, 2004), the retention of polyphenols by the solid phase is never complete (Table 3); moreover, in thermally treated foods, some polyphenols could remain entangled in the melanoidin polymeric structure. Additionally, as regards MRPs, some water-insoluble melanoidins could be eluted, together with the phenolic fraction, by methanol (Borrelli et al., 2003).

The increase of antioxidant activity due to cooking is reflected in a high antioxidant activity of the foods derived from cooked must, such as sweet wines (Manzocco et al., 1999; Mastrocola et al., 2005, 2006) and balsamic vinegars (Di Mattia, Giordano, Pittia, Rotilio, & Mastrocola, 2004). Since Maillard reaction could further proceed, even at ambient temperature, these products are traditionally stored for prolonged periods (over 30 years) without losing their sensory properties due to oxidative spoilage. The use of stainless steel boilers instead of copper boilers could further improve the antioxidant activity of these products by limiting the oxidation of polyphenols and also improving the antioxidant activity of the non-phenolic fraction (Table 3).

The phenolic fraction accounted for almost the total antioxidant activity in fresh must but, in concentrated musts, the antioxidant activity of the non-phenolic fraction overcame that of the phenolic fraction. This result is important because, if the bioavailability of polyphenols in human metabolism is proved (Manach, Williamson, Morand, Scalbert, & Ramasy, 2005; Williamson & Manach, 2005) and their bioefficacy is generally accepted, the metabolism of food-borne advanced MRPs is not yet completely elucidated and it is still an open question as to whether isolated melanoidin structures are bioavailable or undergo metabolic biotransformation and subsequently cause physiological effects *in vivo*. Moreover, MRPs have been proved to act both as carcinogens and as anticarcinogens compounds (Lee & Shibamoto, 2002; Manzocco et al., 2001), so a deeper understanding of possible toxicological implications of these functional compounds is needed. Also, in cooked must, the phenolic fraction is mainly represented by proanthocyanidins and the bioavailability of these compounds is still debated.

4. Conclusions

Concentration by heat determines several changes that could affect the chemical composition of musts and give the typical characteristics that allow their appreciation by the consumers; however, due to the concentration of environmental and technological contaminants, such as metal ions, much attention must be paid to the prime material composition.

Heat treatment also determines non-enzymatic browning reaction, with the formation of furanic compounds and melanoidins through different pathways. Melanoidins, in particular, enhance the antioxidant activity of the musts and confer a high resistance toward oxidation to the products derived from these musts. Furans, by contrast, have been reported to possess cytotoxic, genotoxic and mutagenic activity. However, the general low concentration of these compounds in foods and the limited consumption of cooked musts, in particular, should not pose any health risk under the conditions of use.

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