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Analytical Methods

Suitability of the TBA method for assessing lipid oxidation in a meat system with added phenolic-rich materials

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ABSTRACT

Numerous protocols and modifications of the thiobarbituric acid (TBA) test are available in the literature. The present paper compares the effectiveness of different TBA tests in minimizing the interferences caused by the addition of phenolic-rich materials (wild fruits) as antioxidants in cooked burger patties. The aqueous acid extraction procedure (EM) and a modified distilation TBA method (DM) were tested with different conditions of incubation – boiling (B) vs. room temperature (RT) – for monitoring lipid oxidation in cooked burger patties during refrigerated storage. DM-B and DM-RT were more suitable than EM procedures for assessing TBA-reactive substances (TBA-RS) in meat samples containing compounds such as anthocyanins, with similar spectral properties than that of the TBA–malondialdehyde (MDA) adduct. Additionally, interferences caused by browning development during incubation were avoided by DM procedures or by performing RT incubations. Correlations between TBA-RS numbers and hexanal contents in cooked pork burger patties were calculated in order to corroborate the suitability of the tested TBA procedures. The DM-RT procedure showed the highest correlation with hexanal content ($R^2 = 0.90$; $p < 0.001$).

1. Introduction

The thiobarbituric acid (TBA) test for malondialdehyde (MDA) determination is the most frequently used method for the assessment of lipid oxidation in muscle foods owing its sensitivity and relatively simple procedure (Raharjo & Sofos, 1993). TBA test involves the reaction between TBA and MDA produced from lipid hydroperoxide decomposition to form a pink complex with maximum absorbance at 532 nm (Tarladgis, Pearson, & Dugan, 1964). Nowadays, numerous protocols and modifications of this method have been reported in the literature. Four basic approaches have been employed to conduct the TBA test on food samples: (i) application of direct heating on the food samples with a TBA solution and extracting the resulting TBA–MDA adduct with butanol (Tarladgis et al., 1964; Williams, Field, Miller, & Welke, 1983), (ii) MDA determination on the extracted lipid portion of food samples (Pikul, Leszczynski, & Kummerow, 1983, 1989; Younathan & Watts, 1960), (iii) MDA determination on aqueous acid extracts of food samples (Pikul et al., 1983; Salih, Smith, Price, & Dawson, 1987; Tarladgis et al., 1964), and (iv) MDA determination on a portion of the steam distillate from food samples (Hoyland & Taylor, 1989; Ke, Cervantes, & Robles-Martinez, 1984; Tarladgis, Watts, Younathan, & Dugan, 1960). The two latter – the aqueous acid extraction method (EM) and the distilation method (DM) – are generally the most popular TBA tests amongst meat researchers (Estévez, Morcuende, & Ventanas, 2009). Amongst these two procedures, the EM may be considered as the most suitable method for estimating the MDA content in meat samples, because the meat itself is not exposed to heat treatment (Raharjo & Sofos, 1993). It is faster and easier to perform than the distilation method and is particularly recommended whenever a large number of samples need to be analysed rapidly (Estévez et al., 2009).

However, various drawbacks have been described for this procedure. For instance, certain compounds interfere with the reaction between TBA and MDA and/or hinder a proper spectrophotometric measurement. In order to avoid the interfering effect of water-soluble proteins, peptides (Schmedes & Holmes, 1989; Shamberger, Shamberger, & Willis, 1977), other aldehydes different to MDA (Kosugi, Kato, & Kikugawa, 1988), pigments (Bird & Draper, 1984; Shamberger et al., 1977), amino acids, additives and fat droplets (Hoyland & Taylor, 1991), the meat extract requires to be further filtrated, with this additional step being tedious and sometimes not completely effective. Some other compounds with similar spectral properties to that of the TBA–MDA adduct, may be present in the meat extracts which can lead to interferences with the spectrophotometric measurement at around 532 nm and hence, cause an overestimation of the results (Rey, Hopia, Kivikari, & Kahkonen, 2005). For such problematic samples, the DM may be a reliable option to assess TBA-RS numbers (Raharjo & Sofos, 1993). Whereas

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the DM could avoid the interference caused by non-volatile impurities, heating the samples during distilation generally leads to higher TBA-RS numbers as high temperatures enhances the formation of additional MDA, even in the presence of metal chelators and phenolic antioxidants (Raharjo & Sofos, 1993). Regardless of the method employed, some additional reactions and interferences may also take place during incubation of samples as long as heating is applied to promote TBA–MDA formation. In this sense, some authors recommended incubation at room temperature extending the incubation time from 30–45 min (at boiling temperature) to 15–20 h (at room temperature) (Tarladgis et al., 1960; Wang, Pace, Dessai, Bovel-Benjamin, & Philips, 2002). Raharjo, Sofos, and Schmidt, 1993 reported that filtering the extract by solid-phase cartridges (C18) before TBA–MDA reaction, improved speed, specificity and limit of detection of aqueous acid extraction TBA test in ground and cooked meat.

In the last years, the increasing interest in replacing synthetic antioxidants by natural ones has led to the evaluation of the antioxidant potential of numerous plant materials. Most research studies currently accomplished on antioxidant action focuses on phenolic compounds such as flavonoids (Heinonen, 2007). According to previous studies (Ganhão, Estévez, Kylli, Heinonen, & Morcuende, 2010; Rey et al., 2005), the inclusion of phenolic-rich plant materials in meat systems would inhibit lipid oxidation whereas the TBA test might not always reflect that antioxidant effect. Flavonoids from plants and fruits, such as anthocyanins, are natural pigments, display intense antioxidant actions and, according to preliminary tests, cause serious interferences in TBA measurements. With regard to this fact, the objective of this work was to compare the effectiveness of different TBA tests in minimizing the interferences caused by the addition of phenolic-rich materials as antioxidants in cooked burger patties. The effect of the incubation conditions on the TBA-RS measurements was also studied.

2. Material and methods

2.1. Chemicals

All chemicals and reagents used for the present work were AAS grade and purchased from Panreac (Panreac Química, S. A., Barcelona, Spain), Merck (Merck, Darmstadt, Germany) and Sigma Chemicals (Sigma–Aldrich, Steinheim, Germany).

2.2. Fruits

Samples of strawberry tree (*Arbutus unedo* L., AU), common hawthorn (*Crataegus monogyna* L., CM), dog rose (*Rosa canina* L., RC) and elm-leaf blackberry (*Rubus ulmifolius* Schott., RU) cultivars were collected at the stage of full ripeness in Cáceres region, Spain (altitude = 450 m) during the summer and autumn of 2007. After hand-harvest, the samples were immediately transferred to the laboratory, cleaned and sorted to eliminate damaged and shrivelled fruits and then frozen at −80 °C.

2.3. Extraction of fruit phenolics

Fruits (30 g), including peel and pulp, were cut into pieces while the seeds were carefully removed. Fruit was ground, dispensed in a falcon tube and homogenised with 10 volumes (w/v) of absolute ethanol. The homogenates were centrifuged at 4000 rpm for 10 min at 6 °C. The supernatants were collected and the residue was re-extracted once more following the procedure previously described. The two supernatants were combined, evaporated using rotary evaporator and re-dissolved using 250 g of distilled water. Water solutions from each fruit were prepared and stored in refrigeration until used for the manufacture of porcine burger patties (less than 24 h) as described below. Neither insoluble fragments nor residues were observed in the water solutions.

2.4. Manufacture of porcine burger patties

Six types of porcine burger patties were prepared depending on the addition of different fruit extracts (AU, CM, RC, RU) including negative (no added extract, C) and positive control (added quercetin; 230 mg/kg, Q) groups. In the basic formulation, the ingredients per kg of burger patty were as follows: 725 g meat (porcine *longissimus dorsi* muscle), 250 g distilled water, and 25 g sodium chloride. In the formulation of the treated patties, the 250 g of distilled water were replaced by 250 g of a water solution containing the corresponding fruit extracts or the quercetin. All ingredients were minced in cutter until a homogeneous raw batter was obtained. Eight burger patties per batch were prepared in two independent manufacturing processes (four patties per batch each time). Burger patties were formed using a conventional burger-maker (100 g/patty), to give average dimensions of 10 cm diameter and 1 cm thickness. Preliminary cooking trials were performed to establish the cooking conditions required to achieve a meat core temperature of 73 °C. Porcine burgers were placed on trays and cooked at 170 °C for 18 min in a forced-air oven. The cooking loss of burger patties was calculated as follows: Cooking loss = $[(W_b - W_a)/W_b] \times 100$ where W_b and W_a are the weights of the burger patties before and after cooking, respectively. The cooked burger patties were dispensed in polypropylene trays, wrapped with PVC film and subsequently stored for 12 days at +2 °C in a refrigerator under white fluorescent light (620 lux), simulating retail display conditions. Sampling was carried out on days 1 and 12 for TBA-RS and hexanal content, with day 1 being the day after that of the manufacture. After each refrigeration stage (4 patties per batch and sampling day) burgers were frozen (−80 °C) until analytical experiments were carried out (less than 2 weeks).

2.5. TBA methods

Thiobarbituric acid-reactive substances (TBA-RS) were assessed in burger patties using both an aqueous acid extraction method (EM) and a distilation method (DM). The effect of incubation conditions for TBA-RS (room temperature (RT): 24 °C/20 h vs boiling (B): 100 °C/45 min) was also studied. Results were expressed as mg malondialdehyde (MDA)/kg burger patty. Analysis was performed in quadruplicate.

2.6. Aqueous acid extraction method (EM)

Oxidative stability of cooked burger patties was evaluated by measuring the formation of thiobarbituric acid-reactive substances (TBA-RS) as described by Tarladgis et al. (1964) except that perchloric acid was used in place of trichloroacetic acid as recommended by Salih et al. (1987). Briefly, 12 g of each burger patty was dispensed in cone plastic tubes and homogenised with 35 mL of 3.86% perchloric acid, using a Omni-mixer homogeniser for 1 min. The homogenate blended was centrifuged (3000 rpm for 3 min) and filtered through Whatman No. 54 filter paper. The filtrate was adjusted to 50 mL with perchloric acid (3.86%). Next, 2 mL aliquots of the filtrate were transferred to separate test tubes (in duplicate) and mixed with 2 mL of 0.02 M TBA in perchloric acid (3.86%). The mixture was vigorously agitated in a vortex and one half the test tubes and the tubes from the standard curve were incubate at room temperature (24 °C) in the dark for 20 h (EM-RT), while the other half was heated in a boiling water bath (100 °C) for 45 min (EM-B) to develop the pink colour. After cooling the reaction mixture under running water and centrifuging (3000 rpm for

2 min), the absorbance was determined at 532 nm using a Hitachi U-2000 spectrophotometer against a blank containing 2 mL of perchloric acid (3.86%) and 2 mL of TBA reagent. The TBA value used to express the results were calculated from standard curves and known dilutions of tetraethoxypropane (TEP) and the results were expressed as TBA-RS numbers, mg malondialdehyde (MDA)/kg burger patty.

2.7. Distilation method (DM)

The TBA distilation method was performed as described by Tarladgis et al. (1960) with some modifications, consisting in a previous filtrate step before distilation, for avoiding a direct heating of the samples during distilation process. Briefly, 12 g of each burger patty was homogenised with 35 mL of 3.86% perchloric acid, using an Omni-mixer homogeniser for 1 min. The homogenate blended was centrifuged (3000 rpm for 3 min) and filtered through Whatman No. 54 filter paper. The filtrate was adjusted to 50 mL by adding perchloric acid (3.86%) and then the samples were distilled by mean of a Büchi distilation Unit-324 and the first 50 mL of distillate collected. Next, 2 mL aliquot of the distillate was mixed with 2 mL of 0.02 M TBA in perchloric acid (3.86%) in test tubes (duplicate). The test tubes were vigorously vortexed and one half of these were incubated at room temperature (24 °C) in the dark for 20 h (DM-RT), while the other half of samples were heated in a boiling (100 °C) water bath for 45 min (DM-B) in order to develop the colour reaction. In both cases, the tubes from the corresponding standard curves were identically processed. All test tubes were cooled under running water and centrifuged (3000 rpm for 2 min). The absorbance was measured at 532 nm using a Hitachi U-2000 spectrophotometer against a blank containing 2 mL of distillate water and 2 mL of TBA reagent. The results from the samples were plotted against a standard curve prepared with known concentrations of tetraethoxypropane (TEP). The results (TBA-RS) were expressed as mg malondialdehyde (MDA)/kg burger patty.

2.8. Determination of the TBA-RS numbers

The TBA-RS numbers were calculated by multiplying the absorbance values at 532 nm by a constant coefficient *K*. This value was calculated from standard curves and known dilutions as follows:

$$K = [(\text{mol MDA}/5\text{ mL})/A_{532} \times \text{MDA mol. wt.} \times \text{DF} \times 10^6 \times (100/\%\text{recovery})]/\text{m}$$

where (mol MDA/5 mL)/A_{532} is represented by l/slope of the standard curve, the mol. wt. of malonaldehyde is 72.03 g/mol, DF is the dilution factor, l0^6 converts the units so that results can be expressed as mg malonaldehyde/kg sample,% recovery (see below) and m is the sample mass (in grams).

Recovery of MDA was evaluated by addition of known amounts of 1,1,3,3 tetraethoxytetraethoxypropane (TEP) to the sample extracts. The percent recovery is calculated as follows:

$$\%\text{recovery} = 100 \times A_{SP}/A_{TEP}$$

where A_{SP} is the absorbance of the spiked food sample (corrected for endogenous malonaldehyde) and A_{TEP} is the absorbance of the corresponding TEP dilution. The extraction recovery of TEP was 71% and 76% for the extraction and distilation methods, respectively.

2.9. Hexanal content

Hexanal was assessed in the headspace from cooked porcine burger patties by solid-phase micro extraction (SPME) and gas chromatography/mass spectrometry (GC/MS) using a gas chromatograph Hewlett–Packard 6890 series coupled to a mass selective detector Hewlett–Packard HP-5793. The method developed by Estévez, Ventanas, Ramírez, and Cava, 2004, was employed with minor modifications as follows: The SPME fibre, coated with divinylbenzene-carboxen-poly(dimethylxilosane) (DVB/CAR/PDMS) 50/30 μm, was preconditioned prior to analysis at 220 °C during 45 min. One gram of minced sample was placed in a 4 mL SPME vial and sealed with a silicone septum. The sample was allowed to equilibrate during 30 min while immersed in water at 37 °C. During the extraction, the SPME fibre was inserted through the septum and exposed to the headspace of the vial. After extraction, the SPME fibre was immediately transferred to the injector of the chromatograph which was in splitless mode at 270 °C. Hexanal was separated using a 5% phenyl-95% dimethyl polysiloxane column (Restek, USA) (30 m × 0.25 mm id., 1.05 μm film thickness). The GC/MS conditions were as follows: the carrier gas was helium at 18.5 psi, resulting in a flow of 1.6 mL min^{-1} at 40 °C. The SPME fibre was desorbed and maintained in the injection port at 220 °C during the whole chromatography run. The temperature program was isothermal for 10 min at 40 °C and then raised at the rate of 7 °C min^{-1} to 250 °C and held for 5 min. Transfer line to the mass spectrometer was maintained at 270 °C. The mass spectrometer operated in the electron impact mode with electron energy of 70 eV and a multiplier voltage of 1650 V, collecting data at a rate of 1 scan s^{-1} over a range of *m*/*z* 40–300. Hexanal was positively identified by comparing its mass spectra and retention time with those displayed by the standard compound. The area of each peak was integrated using ChemStation software (Hewlett–Packard Co.) and the total peak area was used as an indicator of hexanal generated from the samples. Results from the hexanal analysis were provided in area units (AU). Analysis was performed in quadruplicate.

2.10. Statistical analysis

All analyses were conducted in burger patties in duplicate (4 patties per batch and sampling day × 2 analyses; $n = 8$). All data were expressed as means ± standard deviation of eight measurements and analysed by SPSS for Windows (v. 15.0). Analysis of variance (ANOVA) and Tukey tests were carried out to study significant differences on the measured parameters. Differences were considered significant at $p < 0.05$. Relationships among measured parameters were calculated using the Pearson's correlation coefficient.

3. Results and discussion

Plant and fruit phenolics have recently attracted considerable interest because of their presumed safety and their potential antioxidant, nutritional and therapeutic effects. In the present work, some Mediterranean wild fruits were evaluated as inhibitors of lipid oxidation in cooked burger patties using several TBA tests. Fig. 1 shows the TBA-RS numbers obtained from samples subjected to two different procedures – the aqueous acid extraction method (EM) and the distilation method (DM) – and two different incubation conditions – 24 °C for 20 h (RT) and 100 °C for 45 min (B). In order to corroborate the reliability of the different TBA methods applied in the present study, the TBA-RS numbers obtained from these procedures were systematically compared with the corresponding hexanal counts. The results obtained from the hexanal analysis are not affected by the presence of compounds with interfering potential on the MDA–TBA adduct and hence, were assumed to reflect more precisely the oxidative status of the burger patties. As a reliable indicator of lipid oxidation (Shahidi & Wanasundara, 2002), hexanal has been widely used to follow the development of oxidative rancidity in muscle foods (Dupuy, Bailey, St. Angelo,

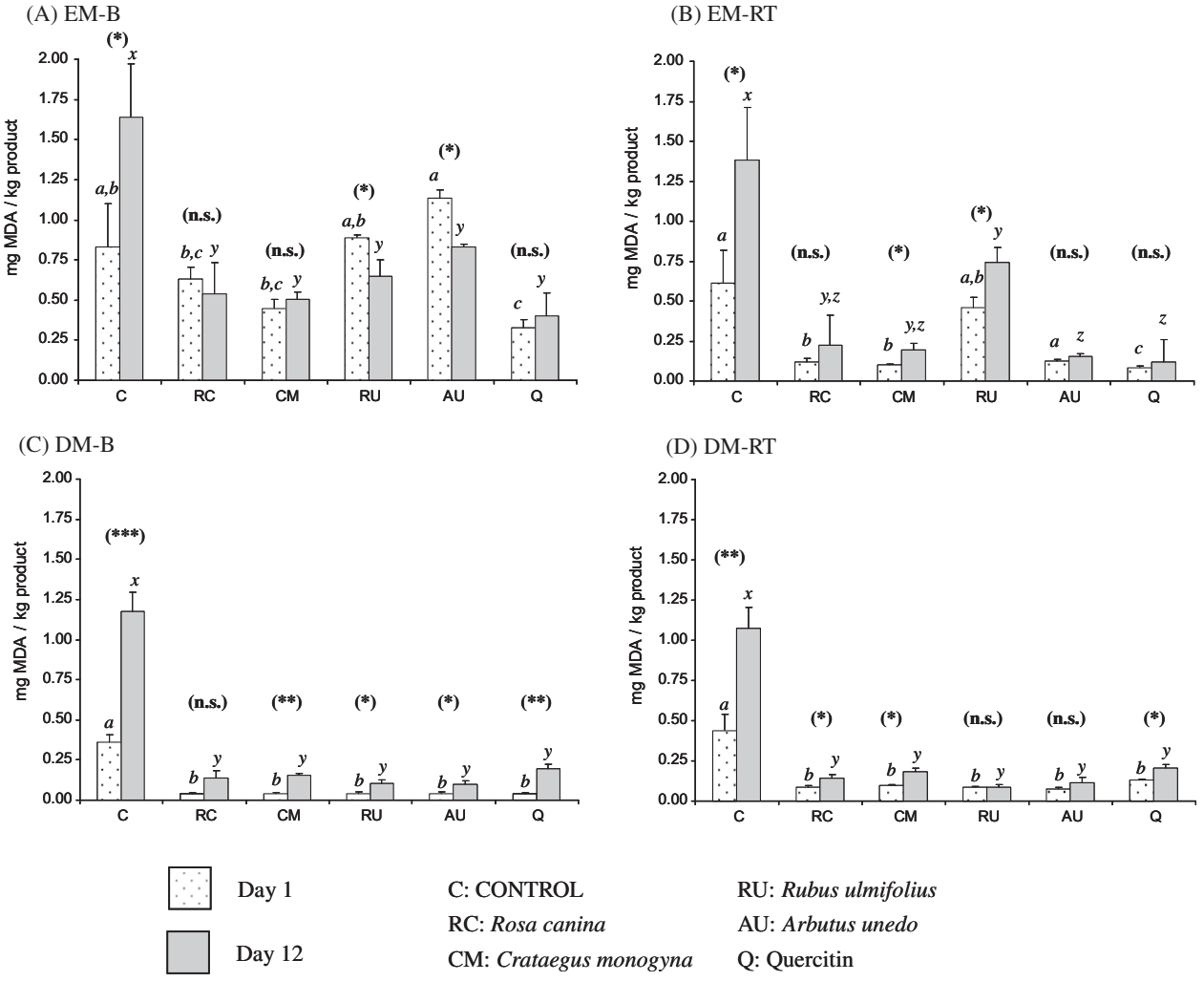

Fig. 1. Comparison of different TBA assays in the assessment of evolution of TBA-RS numbers during refrigerated storage of cooked burgers patties with added fruit extracts. Significant differences between days of refrigerated storage are denoted by asterisks: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; n.s., non significant. Significant differences, $p < 0.05$, between meat system batches within a day of storage are denoted by different letters on top of error bars.

Legendre, & Vercelotti, 1987). Considerable differences were found while comparing results obtained from the different TBA tests.

Being considered the most convenient and basic procedure for TBA-RS assessment (Pegg, 2005), the extraction method with boiling incubation temperature (EM-B) was firstly applied (Fig. 1A). Unexpected and contradictory TBA-RS results were obtained for cooked burgers patties with added fruit extracts when this procedure was employed. Whereas most fruit extracts seemed to reduce lipid oxidation, the TBA-RS numbers for the treated burgers were surprisingly high, particularly at day 1. An increase of the TBA-RS numbers is generally expected to occur during refrigerated storage of cooked burger patties as a result of the onset of oxidative reactions following cooking. Whereas this increase was observed in control patties, an unexpected lack of significance was found between day 1 and day 12 for TBA-RS numbers in patties treated with RC, CM and Q. Furthermore, a surprising significant decrease of TBA-RS numbers was observed during chill storage of burger patties treated with RU and AU. The unexpected results obtained from the EM-B TBA test could be ascribed to the presence of interfering compounds, mainly; coloured pigments from tested fruits. Among these, anthocyanins and other natural pigments, which naturally display similar red-colourations to that of the TBA–MDA adduct, should be considered as main responsible. Fig. 2, which displays the absorbance of perchloric acid extracts of RU at 532 nm (at which TBA-RS are measured), illustrates this interference. According to this figure, the acid extracts of RU display, in the absence of TBA, a considerably high absorbance peak at 532 nm which would

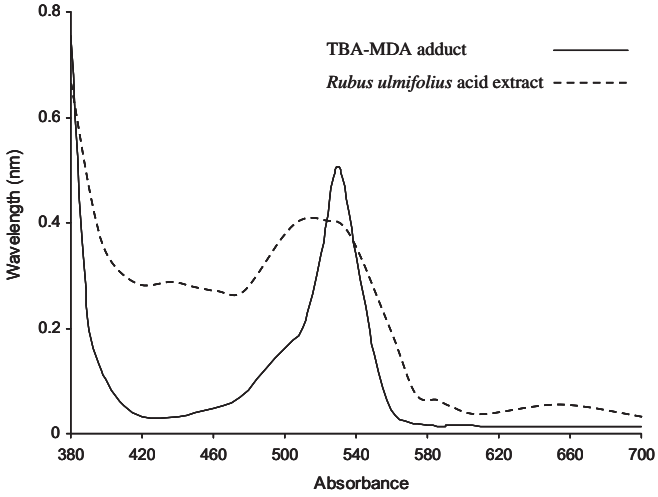

Fig. 2. Absorbance scans of the MDA–TBA adduct and perchloric acid extracts of RU.

eventually lead to an overestimation of the TBA-RS numbers. The other fruit extracts as well as Q absorbed light to a lesser extent in the same wavelength range which confirmed the interference of fruit phenolics on the TBA-RS measurements. When added to burger patties (Fig. 3), the fruits remained having their interfering influence, with this effect being particularly more intense in the patties with added RU extracts. In fact, the RU extract, literally dyed burger patties with a distinctive purplish colour (Ganhão, Morcuende, Estévez, & texture deterioration during chill storage. Meat Science, 2010). Anthocyanins are major pigment constituents of RU (373.1 mg/100 g of fruit d.w.) and would play a major role on these interfering effects. Numerous plant tissues contain endogenous pigments that absorb in the 532 nm region or form such pigments under the acidic conditions necessary for the MDA extraction and subsequent reaction with the TBA (Draper et al., 1993). The apparent decrease of TBA-RS numbers during chill storage (Fig. 1A) of RU and AU patties would more plausibly respond to the degradation of these sensitive pigments rather than an actual decrease of TBA-RS. In accordance to other authors, (Hoyland & Taylor, 1991; Schmedes & Holmes, 1989) meat components, such as pigments, proteins, peptides or lipid droplets could also have influenced the spectrophotometric measurements as aqueous acid extracts of the control patties also absorbed light at 532 nm (Fig. 3). While performing the TBA test, the absorbance values from these interfering compounds might be added to the absorbance from the TBA–MDA adduct, leading to the overestimated erroneous results previously described for the EM-B procedure. In contrast to the results obtained from the EM-B TBA test, the analysis of hexanal in these samples revealed significant increases of oxidation rates during refrigerated storage of cooked burger patties and in general, more intense antioxidant actions of added fruits (Fig. 4). The correlation between results from the EM-B TBA test and the hexanal measurements was significant but relatively low (R^2 = 0.64; Table 1).

In addition to the effect of fruit phenolics, some other compounds such as those formed from browning reactions during incubation at boiling temperatures, were suspected to interfere with the TBA-RS assessment. According to our observations, heating times longer than 30 min at 100 °C were found to result in intense browning development, particularly in extracts from patties with added AU. Other authors reported similar observations in aqueous acid extracts subjected to incubations with TBA at high temperatures (Botsoglou et al., 1994). Porcine burgers patties with added fruit extracts contain high amounts of precursors for browning reactions, namely aldehydes, reducing sugars, and amino groups. In order to evaluate the impact of incubation temperatures

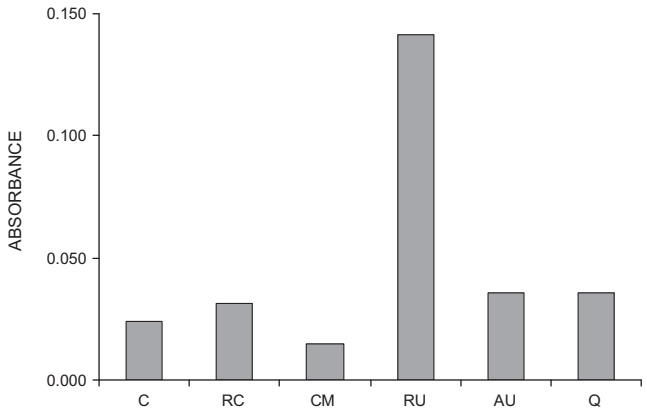

Fig. 3. Absorbance at 532 nm of a perchloric acid extract obtained from burger patties in the absence of TBA (see Fig. 1 for keys).

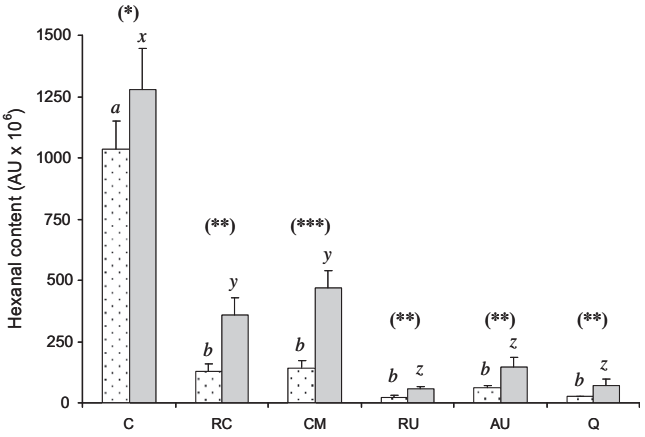

Fig. 4. Hexanal content in porcine burger patties with added fruit extracts. Significant differences between days of refrigerated storage are denoted by asterisks: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; n.s., non significant. Significant differences, $p < 0.05$, between meat system batches within a day of storage are denoted by different letters on top of error bars.

Table 1
Pearson's correlation coefficients (R^2) between hexanal content and TBA-RS numbers measured in burger patties with added fruit extracts through different procedures.

Procedure	R^2	p-Value
EM-B	0.64	<0.01
EM-RT	0.74	<0.01
DM-B	0.88	<0.001
DM-RT	0.90	<0.001

on TBA-RS measurements, aqueous acid extracts from burger patties were also incubated with TBA at room temperature (EM-RT). The TBA-RS numbers obtained from this procedure are shown in Fig. 1B. In general terms, the TBA-RS numbers obtained from the EM-RT procedure were lower than those obtained from the EM-B. These results confirmed that the formation of interfering browning compounds, whose formation is enhanced by boiling temperatures, is avoided when incubation is carried out at room temperature. Consistently, previous research have found that the generation of interfering coloured compounds is minimised at room temperature and the reaction between MDA and TBA is more specific (Wang et al., 2002). It was not surprising; hence, that burger patties with added AU were particularly affected by incubation temperature as this fruit contain more reducing sugars than the other tested fruits. In particular, sugars comprise around the 50% of the total dry weight of AU (Alarcão-E-Silva, Leitão, Azenheira, & Leitão, 2001). The EM-RT procedure avoided the interferences caused by high temperatures during incubation and provided an oxidation pattern more similar to that of the hexanal. In fact, the correlation between TBA-RS numbers obtained from the EM-RT procedure and the hexanal content was higher than that aforementioned for the EM-B procedure (R^2 = 0.74 vs. R^2 = 0.64; Table 1).

Whereas the interferences caused by the browning development were removed by performing the incubation at room temperature, the interferences caused by the natural fruit pigments seemed to remain in the EM-RT procedure. As long as the fruit pigments are extracted by perchloric acid, and remain in the extracts after incubation, they eventually affect the absorbance measurements at 532 and this situation is particularly evident in burger patties with added RU (Fig. 1B). According to the results from the present study, the EM procedure might not be suitable for performing the TBA test when samples contain high amounts of anthocyanins or other fruit pigments with similar spectral proper-

ties. According to other authors, some other interferences could be present in samples processed by EM procedures. The first interferences reported in the literature were due to the presence of a yellow chromagen (max. 450–460 nm) overlapping the pink peak (max. 530–537 nm) causing erroneously high values if it was of sufficient intensity (Crackel, Gray, Pearson, Booren, & Buckley, 1988). This yellow chromagen may be formed by a variety of aldehydic compounds reacting with TBA and could be ascribed to sugars or their degradation products (Baumgartner, Baker, Hill, & Wright, 1975; Salih et al., 1987). These authors suggested that EM should only be used if compounds producing the yellow pigment are absent, or present in sufficiently small quantities not to interfere. The EM may be also inadequate in cases of coloured samples and for high fat samples (>10%) where turbidity may occur in extracted samples (Hoyland & Taylor, 1991; Salih et al., 1987; Siu & Draper, 1978; Williams et al., 1983). Some authors (Draper et al., 1993; Raharjo et al., 1993) have reported that these interfering compounds can be successfully removed by filtering the extract by solid-phase extraction (C_{18} cartridges). Whereas this procedure could provide increased specificity and sensitivity to the TBA test, it is highly time-consuming and not practical for routine analysis. Moreover, to our knowledge, there are no suitable filtering methods for the efficient removal of interferences derive from fruit pigments or browning compounds formed during TBA-MDA incubation. For all these reasons, and regardless of the incubation procedure chosen, the EM appears to be an imprecise procedure when applied to cooked burger patties with added fruit extracts for measuring TBA-RS.

The distilation methods (DM) were employed to evaluate their ability to reduce the interferences found in the EM. The DM was whether coupled to incubations at boiling temperature (DM-B) or incubations at room temperature (DM-RT). The TBA-RS numbers obtained from these procedures are shown in Fig. 1C and D, respectively. The TBA-RS numbers obtained from both, DM-B and DM-RT procedures, were equivalent and led to coherent results. TBA-RS increased in all cooked burger patties after 12 days of refrigerated storage and treated patties had significantly smaller TBA-RS numbers which reflects the effective antioxidant action of fruit extracts against lipid oxidation. In contrast with the EM procedures, the DM counterparts led to considerably lower TBA-RS numbers, particularly in the patties with added fruit extracts. The results obtained from the DM procedures were in agreement with those obtained from the hexanal analysis (Fig. 4). In fact, the positive and significant correlations between TBA-RS numbers from both DM procedures and hexanal content ($R^2 = 0.88$ and $R^2 = 0.90$, respectively) were considerably higher than those obtained between hexanal and TBA-RS from EM procedures. These high correlations illustrate the large consistency between the DM procedures for TBA-RS assessment and the hexanal analysis. These results suggest that DM procedures, as well as the hexanal analysis, are not affected by the interferences caused by fruit and meat components and the reactions in which these compounds are involved. The lack of interferences was expected as DM involves collecting a clear aqueous distillate of an acidified food sample and direct interference by fruit and other meat components is minimised. In contrast with aqueous acid extracts from patties with added fruits (Fig. 3), the corresponding distillates displayed no spectral properties at 532 nm (data not shown) which confirms the absence of interferences when TBA-RS are assessed through DM procedures. The highest correlation with the hexanal content was obtained by the DM-RT, with this correlation value being consistent with those usually reported in the literature (Ahn, Grün, & Mustapha, 2007; Brunton, Cronin, & Monahan, 2001; Shahidi & Pegg, 1994; St. Angelo et al., 1987). According to our findings, this procedure was particularly reliable for the assessment of TBA-RS in cooked burgers patties with added fruit extracts.

Other authors compared the suitability of EM and DM procedures for the assessment of TBA-RS in meat systems. The results obtained in the present study contrast with those reported by Pikul et al. (1989), Witte, Krause, and Bailey (1970), Vyncke (1975), and Salih et al. (1987), who reported that DM procedures generally lead to higher TBA-RS numbers than EM. Rhee (1978) attributed the higher TBA-RS numbers from DM to the thermal decomposition of MDA precursors during distilation and its liberation by heat from its bound state with proteins. In those works, the distilation of MDA is performed on the meat samples, which generally leads to additional formation of MDA and an overestimation of TBA-RS numbers (Pikul et al., 1983; Siu & Draper, 1978; Witte et al., 1970). However, in the modified DM procedure employed in the present study, the meat is not directly exposed to heat as the distilation is performed on the filtrated meat extracts. Therefore, the formation of extra MDA from muscle lipids during distilation is not applicable in the present study. According to these findings, the overestimation of TBA-RS when performing DM procedures could be apparently diminished by means of a previous filtration step before distilation. In addition to this, the DM procedure likely avoided the overestimation of TBA-RS reported for the EM procedures and caused by fruit pigments and browning-derived compounds.

4. Conclusion

The presence of extracts from wild fruits in meat products causes intense interferences during TBA-RS assessment. Pigments from wild fruits, particularly from RU, and browning reactions developed during incubation of TBA-MDA, particularly in samples with added AU, greatly affect TBA-RS measurements in burger patties. TBA distilation methods are suitable for avoiding interferences caused by fruit pigments. Additionally, interferences caused by browning reactions can be avoided by using distilation methods or by performing incubation of samples in the extractive TBA method at room temperature.

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