PE8.37 Analysis of the oxidative status of meat systems through the TBA-RS method: minimizing the overestimating effect of added phenolic-rich materials 403.00

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Abstract—many protocols and modifications have been reported in the literature on how to perform tiobarbituric acid (TBA) test in meat and meat products. This work compares the effectiveness of different TBA tests in minimizing the interferences caused by the addition of phenolic-rich materials as antioxidants in cooked meat systems. Extraction vs. distillation methods and boiling vs. room temperature incubation temperature were tested. TBA distillation methods were more suitable for meat samples containing substances which can interfere with TBA-Malonaldehyde adduct measurements. Additionally, browning development interferences were avoided by distillations methods or when the incubation of the samples in the extractive TBA method was conducted at room temperature.

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Index Terms—Lipid Oxidation, Meat systems, Phenolics, TBA

I. INTRODUCTION

THE TBA-RS test for the measurement of lipid oxidation of meat and meat products was proposed over 60 year ago. Nowadays, many protocols and modifications have been reported in the literature on how to perform TBA test in meat and meat products. Three basic approaches have been employed to conduct the TBA test on foods [1]. It can be performed (1) on aqueous acid extracts of food samples [2, 3] (2) on an aliquot of a steam distillate of food samples [4], and (3) on the extracted lipid portion of food samples [5]. The chromophore formed by the reaction of malonaldehyde (MDA) with TBA is then quantified spectrophotometrically at around 532 nm. Of these three basic approaches, the aqueous acid extraction may be considered as the best method for estimating the MDA content in meat samples, because the meat itself is not exposed to heat treatment [6]. It is faster and easier to perform than the distillation method and is particularly recommended whenever a large number of samples need to be analyzed rapidly. Sometimes, some impurities may still be present in the meat extracts and this can lead to interferences with the red pigment formation. The presence of water-soluble proteins, peptides, pigments, amino acids, additives and fat droplets in the extract requires a filtration step that could be tedious and sometimes not completely effective. For such problematic samples, the distillation technique may be the only option available to assess TBA values [6]. Whereas the distillation method avoid the interference caused by impurities, heating the samples during distillation promotes further oxidation, leading to the formation of additional MDA and other TBA reactive substances [6].

Alternatively, some authors recommended incubation at room temperature to avoid potential interference reported when using high temperatures during TBA-MDA reaction [4, 7]. In these cases, a longer incubation period (15-20h) is highly recommendable. On the other hand, in the last years, the increasing interest in replacing synthetic antioxidants by natural ones has led to the antioxidant evaluation of numerous plant materials. Most of research currently accomplished on antioxidant action focuses on phenolic compounds such as flavonoids. According to previous studies (unpublished data from preliminary works), colored substances from plants used as potential antioxidant products, such as anthocyanins or other pigments, can produce 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 meat systems.

II. MATERIAL AND METHODS

A. Extraction of fruit phenolics 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 from Cáceres region, Spain. Fruits were homogenized with distilled water using an Omni-mixer homogenizer. The homogenates were centrifuged and then supernatants were collected and the residue was re-extracted once more following the procedure previously described. This extract was used for manufacturing meat systems.

B. Meat system manufacture Depending on the addition of different antioxidant extracts, six meat systems were prepared, including negative control (no added antioxidant) and positive control (added quercetin; 230mg/kg) groups. In the basic formulation, the ingredients per 100 g of manufactured meat system were as follows: 73 g meat (raw pork loin), 25 g distilled water, and 2.5 g sodium chloride. Depending on the experimental batch, different antioxidants were added to the aforementioned formula: RC, CM, RU, AU and Q (quercetin). Percentages of added fruit extract were calculated based on the total amount of product (3% of total meat system weight). Eight replicates of each meat system were prepared. Meat system were formed using a conventional burger-maker (100g/patty), to give average dimensions of 10 cm diameter and 1.5 cm thickness. Meat systems were placed on trays and cooked at 170 ºC for 18 min in a forced-air oven. The cooked meat systems were subsequently stored for 12 days in a refrigerator illuminated with white fluorescent light (620 lux), simulating retail display conditions. Sampling was carried out on days 1 and 12 for TBA-RS numbers, with day 1 being the day after of the manufacture. After each refrigeration stage, all burgers were frozen (-80 ºC) until analytical experiments were carried out.

C. TBA-RS measurements Thiobarbituric acid-reactive substances (TBA-RS) were evaluated using both distillation method and the extraction method. The effect of incubation conditions for TBA-RS (room temperature: 24ºC/ 20 hours vs boiling: 100ºC/ 45 minutes) was also studied. Aqueous acid Extraction Method: the procedure for TBA extraction was performed as described by Tarladgis [2] except that perchloric acid was used in place of trichloroacetic acid as recommended by Salih [3]. Distillation Method: The TBA distillation method was performed as described by Tarladgis [4] with some modifications, consisting in a previous filtrate step before distillation, for avoiding a direct heating of the samples during distillation process. Recovery of MDA was evaluated by addition of known amounts of 1,1,3,3 tetramethoxytetraethoxypropane (TEP) to the sample extracts. The extraction recovery of TEP was 71% and 76% for the extraction and distillation method, respectively.

III. RESULTS AND DISCUSSION

Natural antioxidants present in plants have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects. In the present work, some Mediterranean wild fruit have been tested as potential antioxidants sources.

In ours previous studies some problems, related with TBA-RS measurements in meat systems, appeared as a consequence of interferences with compounds existing in plants or plant extract used as antioxidants. Among these substances, sugars and other compound which lead to browning development during boiling TBA-MDA incubation, and anthocyanins and other natural pigments, which already display red-colorations similar to TBA-MDA adduct, should be considered. This fact is shown in Figure 1, where the absorbance at 532nm (at which TBA-RS are measured) of perchloric acid extracts of meat systems is displayed. These data reveal a considerable absorbance peak at 532nm previous to the TBA addition which leads to an overestimation of TBA-RS result.

Among these results, meat systems elaborated with blackberry (Rubus ulmifolius Schott.) presented a high 532nm absorbance which greatly interfere TBA-RS measurement (see Figure 2). In this case, this fact can be attributed to the high amounts of anthocyanin in RU (373.1 mg/100g of fruit d.w.; unpublished data). Regarding this interferences in TBA test, some authors have reported that interfering compounds can be successfully removed by filtering the extract by solid-phase extraction [1].

However, to our knowledge, we still have not found a filtering method which easily eliminates interferences

from either plant pigments or substances which produce browning during TBA-MDA incubation. In this sense, this study has compared the effectiveness of different TBA tests, namely: extraction vs. distillation methods (with previous filtration before distillation) and boiling vs. room temperature incubation temperature, in minimizing the interferences derived from the addition of Mediterranean wild fruit extracts in cooked meat systems subjected to refrigerated storage. Results are summarized in Figure 3.

In general terms, TBA tests showed an expected oxidation pattern of cooked meat systems: refrigerated storage increased TBA-RS numbers in Control meat systems, whereas the addition of fruit extract reduced the extent of lipid oxidation during refrigerated storage of samples. However, considerable differences were found when different TBA tests were compared. Extraction methods obtained higher and contradictory TBA-RS results in meat systems with added fruit extracts compared to distillation methods, especially, when RU and AU meat systems were analyzed.

Based on our previous findings, these results can be attributed to the presence of red-colored pigments and precursors of browning development during boiling TBA-MDA incubation. These interferences were eliminated using both distillation methods, or partially reduced for browning development, when extraction TBA method was conducted with room temperature incubation instead boiling incubation.

This finding can be related to the fact that distillation method involves collecting an aqueous distillate of an acidified food sample and direct interference by non-lipid components is minimized [6]. Moreover, the differences observed between boiling and room temperature incubation for extraction TBA tests can be also attributed to the effect of temperature in brown-pigment formation (probably from sugars or other saccharides). Thus, previous research have found that reaction between MDA and TBA is more specific at room temperature than at boiling temperature because the generation of interfering colored compounds is minimized at room temperature [7].

Taking in account the results obtain in this work, TBA-RS distillation methods are more suitable for meat samples containing substances which can interfere TBA-MDA adduct measurements. However, TBA distillation methods have been criticized because of the overestimation caused during distillation, which promotes further oxidation and gives higher measurements compare to extraction methods. Nevertheless, according to these findings, this drawback could be apparently diminished by means of a previous filtration step before distillation.

IV. CONCLUSION

Pigments from fruit extracts as well as browning development during incubation of TBA-MDA, significantly affect TBA-RS measurements in meat systems. TBA distillation methods are more suitable for meat samples containing substances interfering with TBA-MDA adduct measurements. Additionally, browning development interferences can be avoided by using distillation methods or by performing incubation of samples in the extractive TBA method at room temperature.

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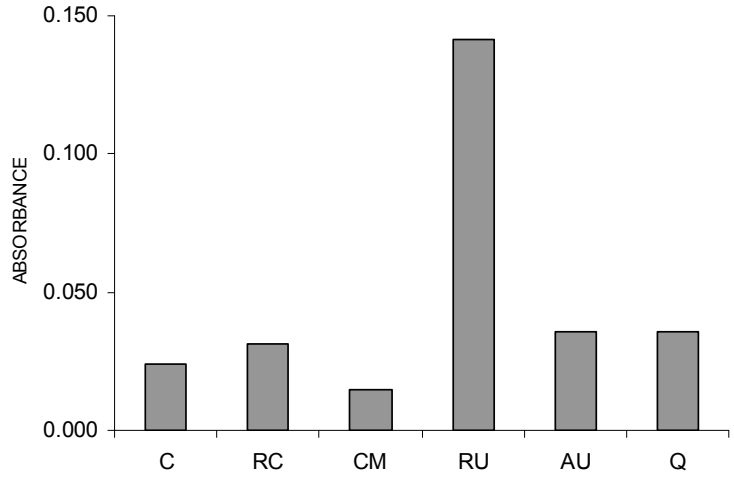

Figure 1.- Absorbance at 532nm of a perchloric acid extract obtain from phenolic-added meat systems before adding TBA (see figure 3 for key)

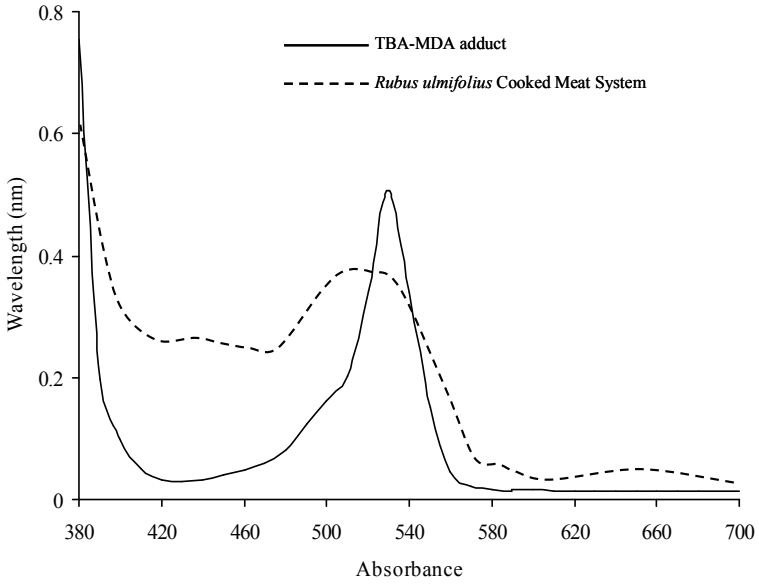

Figure 2.- Absorbance scans of perchloric acid extracts obtained from cooked meat system with added *Rubus ulmifolius* and MDA-TBA adduct.

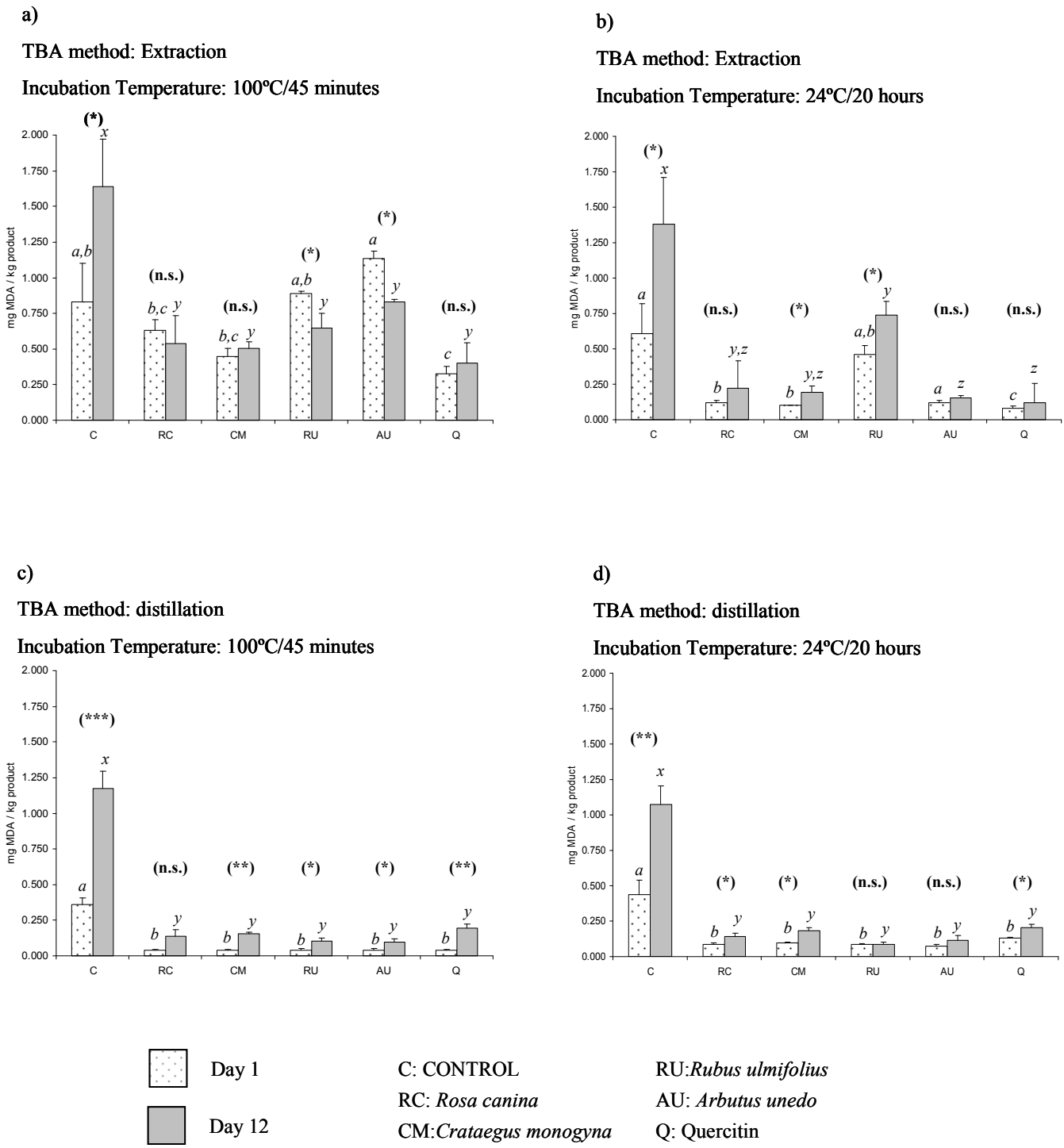

Figure 3.- Comparison of different TBA assays in the assessment of evolution of TBA-RS numbers during refrigerated storage of cooked meat systems with added fruit extracts. Significant differences between days of refrigerated storage are denoted by asterisks: *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$; 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.