THE EFFECT OF RED WINE OR OLIVE EXTRACTS AS NATURAL ANTIOXIDANTS ON OMEGA-3 FATTY ACIDS ENRICHED LAMB PATTIES

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Abstract – The oxidative stability of omega-3 fatty acids (n3) enriched lamb patties with added red wine extract (RWE) or olive extract (OE) in a concentrate of 200 mg gallic acid equivalents (GAE)/kg meat, or 100 mg of α-tocopherol/kg meat (VE) was evaluated in terms of colour, lipid and protein oxidation, and long chain (LC) fatty acids content. The patties were packed in high oxygen modified atmosphere (MAP) and stored up to 9 days (4 °C). The patties from the OE group showed higher antioxidant capacity measured by ORAC and FRAP assays and resulted in lower TBARS and protein carbonyl formation than in the patties from RWE and VE groups. However, patties from RWE group showed less discolouration rate at the end of storage period, with the patties from VE presenting greater LC fatty acids content over storage. Taking into account the results in the current trial, olive extract would result in the more interesting natural antioxidant for the meat industry.

Key Words – Lipid oxidation, Polyphenols, Protein oxidation

I. INTRODUCTION

Recently, an increased interest in healthier meat products has been observed. Special attention is focused on the omega-6/omega-3 relation, with diverse meat products being enriched in n3 fatty acids to obtain a healthier relation. However, the labile double bonds in these fatty acids make these products highly susceptible to oxidation processes. As lipid oxidation is one of the main processes that results in loss of meat quality, the use of antioxidants to protect n3 enriched products is required. The meat industry has used synthetic antioxidants as an effective method to prevent oxidative processes during the storage period of the meat and meat products. Nevertheless, restrictions regarding food safety and the increased demand of natural compounds by consumers have led to questions regarding synthetic antioxidants. Due to this fact, the natural compounds, such as polyphenols, are currently of great interest as natural antioxidants within the meat industry. Red wine and olives are sources of polyphenols, which exert a high antioxidant capacity and are being used as antioxidants to retard the oxidation in the meat [1, 2]. Hence, the objective of the current study was to evaluate the red wine and olive extracts as natural antioxidants on the oxidative stability of n3 enriched lamb patties stored in high oxygen MAP.

II. MATERIALS AND METHODS

A. Preparation, packaging and storage of lamb patties

Selected legs from lamb were purchased from a Spanish local supermarket. After deboning and removing the visual subcutaneous fat and connective tissue, the meat was chopped to 1 x 1 cm pieces, and divided into four batches of 2.200 kg. All batches were formulated with the appropriate deodorized fish oil (Algatrium plus, BRUDY TECHNOLOGY, Barcelona, Spain) rich in DHA, to produce 100 mg LC fatty acids/100 g meat. One batch was added red wine extract (mainly consists of catechin, epicatechin and proanthocyanidins; Provinols®, Seppic S.A., France) (RWE group) and other was added olive extract (mainly consists of tyrosol and hydroxytyrosol; Hytolive®, Genosa I+D, Málaga, Spain) (OE group) to obtain a final concentration of 200 mg GAE/kg meat. The third batch was added 100 mg α-tocopherol/kg meat (DSM

Nutritional Products Iberia S.A., Spain) (VE group), with the last batch without added antioxidant, being control sample (C group). Total phenolics content of the extracts was determined by the Folin-Ciocalteu method [3] and the results obtained were 55 mg GAE/100 mg product and 16 mg GAE/100 mg product for the red wine extract and olive extract, respectively. The meat was mixed in a food mixer, minced, and burger patties were made using a conventional burger mould (100 g/patty). Twelve patties per batch were prepared, and for each storage time, three patties were used for analysis. Patties were collected for day 0 samples, vacuum packed and stored at -80 °C until analysis. The rest of the patties were packed in high oxygen MAP (70% O_2/ 30% CO_2). The packages were stored in the dark at 4 °C for up to 9 days. Analyses were carried out at the following days: 0, 3, 6 and 9.

B. Analytical procedures

Antioxidant capacity of the meat was assessment by means of oxygen radical absorbance capacity (ORAC) [4] and by the ferric ion reducing antioxidant parameter (FRAP) [5]. Discolouration rate was determinated by spectrophotometry as $A_{580} - A_{630}$ [6]. Lipid oxidation was measured by means of the Thiobarbituric acid reactive substances (TBARS) test [7]. Protein oxidation was evaluated on the basis of the formation of carbonyl groups [8]. Fatty acids were analysed following the method proposed by Lee *et al*. [9] and the fatty acid methyl esters were then analysed by gas chromatography coupled to a flame ionization detector.

C. Statistical analysis

Statistical analysis was performed using StatView® version 5.0 (SAS Institute, Cary, NC). Data of antioxidant capacity were subjected to a one-way analysis of variance to analyse differences between treatments. The rest of the data were analysed by a two-ways analysis of variance where the treatment (T) and the storage period (SP) were included as fixed effects. A Tukey/Kramer test was used for mean comparisons ($p<0.05$) when the interaction between both effects (T x SP) was significant.

Table 1 Antioxidant capacity of patties added α-tocopherol (VE), red wine extract (RWE), olive extract (OE), or without antioxidant (C) measured by ORAC (μM Trolox equivalents/g meat) and FRAP (μM Fe^{2+}/g meat) assays

	C	VE	RWE	OE	SEM[(1)]	Significance[(2)]
ORAC	0.54^{b}	0.44^{c}	0.51^{b}	0.68^{a}	0.02	***
FRAP	28.8^{b}	23.8^{b}	24.2^{b}	52.5^{a}	1.75	***

[(1)] Standard error mean; [(2)] ***, $p<0.001$; [a,b,c] Different superscript letters within the same row indicate significant difference ($p<0.05$)

III. RESULTS AND DISCUSSION

When studying the antioxidant capacity of a sample, it is important to test different assays, since each of them provide different information regarding the chemical reactions in which an antioxidant is involved. In this sense, depending upon the reactions involved, these assays can be classified into two types: assays based on hydrogen atom transfer reactions (i.e. ORAC assay) and assays based on electron transfer (i.e. FRAP assay). Taking into account the results obtained in the current study (Table 1), it seems that only the olive extract exerted antioxidant capacity measured by both assays. However, the existence of radical scavenge activity by α-tocopherol due to its ability to transfer hydrogen atoms is widely known [10]. Likewise, the polyphenols present in the red wine, such as proanthocyanidins and their monomers, have an important antioxidant capacity [11].

This antioxidant capacity was observed in the percentage of discolouration rate on the patties stored in MAP, in which an interaction between storage period and treatment was observed ($p<0.001$) (Figure 1A). At the beginning of storage period (day 0), there were differences between treatments, where the RWE group showed a lower value than the VE and C groups. Possibly, due to the red wine extract having a dark red colour, this may have interfered with the colour of the meat. Nevertheless, at the end of storage period (day 9), C group showed the highest percentage of discolouration, as assessed by the lowest value of the A_{580}-A_{630} parameter, being in the following order: C> VE ≥ OE ≥ RWE.

With respect to the TBARS values, there was an interaction between treatment and storage period ($p<0.001$) (Figure 1B). Patties from the VE group showed the same formation rate as the C group, and both exceeded the acceptability limit of 2 mg MDA/kg meat proposed by Campo *et al*. [12] from day 3 and onward. Patties from RWE group were below this limit during the first 6 days of storage period, and the patties from the OE group presented TBARS values below 2 mg MDA/kg meat over the storage period, resulting in this being accepted by the consumer even on day 9. These results agree with those from Jongberg et al. [1] and Cofrades et al. [2], who observed lipid oxidative stability in meat products added with white grape extract or hydroxytyrosol, respectively. Adding mixed tocopherols on n3 fortified beef patties did not minimise lipid oxidation over a 6 day storage period [13], which is in line with the result obtained in the current study.

An interaction (T x SP) was observed ($p<0.001$) for the protein carbonyl formation (Figure 1C). On day 6 of storage period, a lower formation rate took place in the patties from the RWE and OE groups in comparison with the C and VE groups, but, at the end of storage period (day 9), the formation of carbonyl groups was lower in patties with added antioxidants than in the patties from the C group, shown as followed: OE ≤ RWE ≤ VE < C. The protein carbonyl formation has also been reported to be lower in beef patties with added white grape extract [1]. The lower protein carbonyl content in the patties from the RWE and OE groups could be due to a less extent of TBARS formation in the meat (Figure 1B), since the secondary lipid oxidation products react with proteins to cause damage. However, it is not clear whether lipid oxidation initiates protein oxidation or whether protein oxidation initiates lipid oxidation or if the two types of oxidation are coupled [14]. In an *in vitro* study [15], it was observed that the α-tocopherol displayed intense antioxidant activity against protein carbonyl gain, which is consistent with our results.

The content of long chain (LC) fatty acids was affected by the storage period ($p<0.001$) and treatment ($p<0.001$) and there was a trend of interaction between both factors ($p=0.0512$) (Figure 1D). The drastic reduction on the LC fatty acids content was observed on day 9 of storage period, where the lowest content was found in the patties from the C group. Within the patties with added antioxidants, on day 9, the VE group

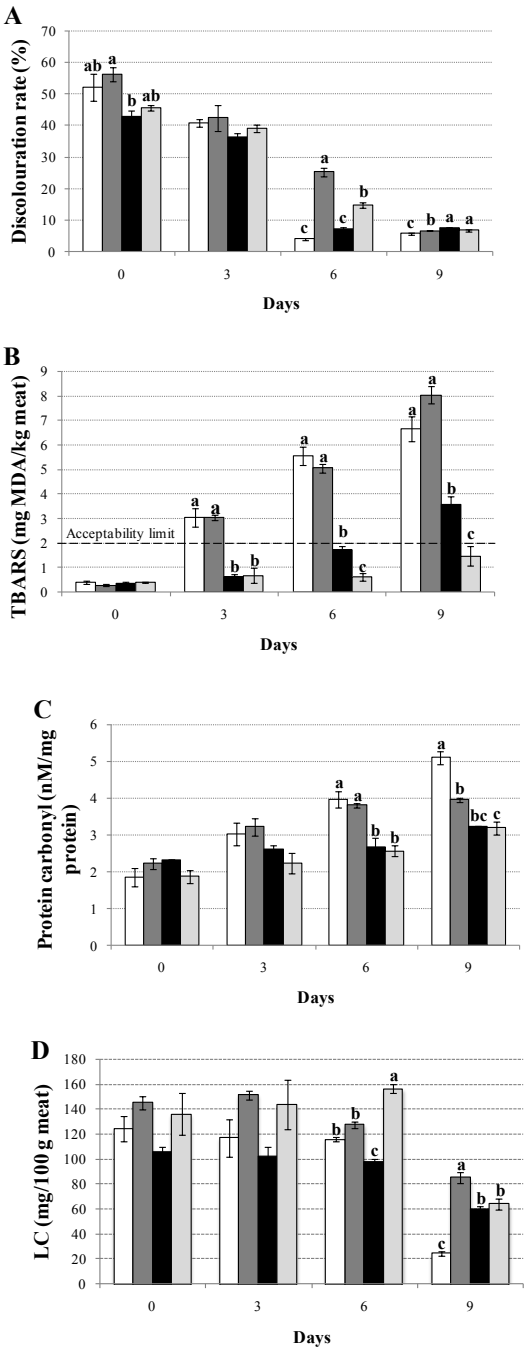

Figure 1. Oxidative stability of n3 enriched lamb patties added antioxidants: □ control, ■ α-tocopherol, ■ red wine extract, □ olive extract (OE). Values with different superscripts are significantly different ($p<0.05$).

showed a higher content than the RWE and OE groups. The higher LC fatty acids content in the patties from the RWE and OE groups in comparison with C group, agree with the results regarding the TBARS values observed (Figure 1B). However, this does not apply to the VE patties. This fact could be possible due to the LC fatty acids were added directly on the meat and they were not embedded into the membrane, hence, the α-tocopherol may interact with them, but we cannot confirm this hypothesis.

CONCLUSION

The olive extract added to patties was able to preserve meat from the lipid and protein oxidation over a 9 day storage period to a further extent than red wine extract or α-tocopherol. However, red wine extract and α-tocopherol showed less discolouration and higher LC fatty acids content at the end of storage, respectively. It should be mentioned the necessity to continue with sensory analysis to evaluate the possible interference of both olive and red wine extracts regarding the global evaluation of the lamb patties.

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