

# EVALUATION OF GRAPE SEED EXTRACT AS ANTIOXIDANT IN HAMBURGUERS

González-Rodríguez, R.M., Temperán, S., Lorenzo, J.M., Montes, R., Bermúdez, R., and Franco, D.\*

Meat Technology Centre of Galicia, Street Galicia No. 4, Technology Park of Galicia, San Cibrao das Viñas, E- 32900 Ourense, Spain  
\*e-mail: danielifranco@ceteca.net

**Abstract**— The aim of this study was to evaluate the capacity of a grape seed extract to delay colour deterioration, lipid oxidation and microbial spoilage in hamburgers. Five batches of pork patties were manufactured: control (C), control without salt (CS), and meat (identical to the control C) supplemented with 200 mg/kg of BHT, BHA or grape seed extract (G). Hamburgers were stored at 4 °C in two modified atmosphere packages (MAP): M1 [30% O<sub>2</sub>-70% CO<sub>2</sub>] and M2 [80% O<sub>2</sub>-20% CO<sub>2</sub>]. pH, colour parameters (L\*, a\*, b\*), lipid oxidation (TBARS formation) and microbial spoilage (lactic acid bacteria (LAB) and *Pseudomonas*) were determined at 0, 2, 6, 8, 13 and 15 days of storage time. L\*, a\* and b\* decreased in all batches, except L\* values in C and CS patties. The best final values of a\* were found for the grape extract in both packages (M1 and M2). Display time had a significant effect (P<0.001) on TBARS values; hamburgers treated with BHT, BHA and G showed TBARS index means significantly lower than the controls (C and CS) in the final sampling point (0.17, 0.25, 0.34 < 0.51 and 1.36, P<0.05 for BHA, BHT, G, C and CS for M1, respectively). M2 packaged samples showed a higher microbial contamination than M1 samples and the addition of the grape extract reduced the growth of BAL and *pseudomonas*, respect to the control C, in both MAP evaluated.

**Keywords**— pork patties, TBARS, spoilage

## I. INTRODUCTION

Shelf-life and quality of fresh red meat can be extended by using modified atmosphere packaging (MAP) coupled with refrigerated storage [1]. The basic of MAP is the elimination of air in the container and replaced by a gas or gas mixture (N<sub>2</sub>, CO<sub>2</sub> and O<sub>2</sub>) in different concentrations. The packaging of fresh red meat is carried out to avoid contamination, delay the spoilage, permit some enzymatic activity to improve tenderness, reduce weight loss, and where applicable, to ensure a cherry-red colour in red meats at retail or consumer level [2]. In fact, muscle colour at the point of purchase is an indicator of freshness [3] and quality

is equated to a bright red colour and surface discoloration is the ultimate criteria that the consumers use to reject meat. The presence of O<sub>2</sub> maintains the meat pigment myoglobin in its oxygenated form, oxymyoglobin, which gives fresh meat its bright red colour. However, high concentration of oxygen has been shown to increase lipid oxidation in meat and meat products causing rancidity [4–5].

Another alternative against oxidation is often countered through endogenous or exogenous inclusion of antioxidants. The best antioxidants should not affect product organoleptic quality and remain effective at low concentrations. Synthetic antioxidants such as tert-butyl-4-hydroxyanisol (BHA) and tert-butyl-4-hydroxytoluene (BHT) have proved effective as inhibitors of lipid oxidation [6], although concerns relating to possible associated toxicity has led to desire for their replacement with antioxidants from natural sources [7]. Several grape seed extracts have showed both antioxidant and antimicrobial activities on meat and meat products [8–11].

This study examines the effect of including a natural antioxidant (grape seed extract) on colour deterioration, lipid oxidation and aerobic bacterial growth in pork hamburgers.

## II. MATERIALS AND METHODS

### A. Preparation of samples and package conditions

Five batches of patties of ground meat (hamburgers) [control (C), control without salt (CS), BHT, BHA and grape seed extract (G)] were manufactured in our pilot plant. Hamburgers of 100 g (n=2 per batch and display time) were manufactured using the primal cuts of shoulder and loin from pig. Meat was ground using a 6 mm plate in a refrigerator mincer machine (La Minerva, Bologna, Italy). The meat was mixed and compressed by hand; a 7.5 g of NaCl per kg of meat, 0.5 g/kg of white pepper, 0.25 g/kg of oregano and 200 mg/kg of BHT, BHA and grape seed extract was also added in BHT, BHA and G batch, respectively.

CS batch was elaborated only with meat. Mass was maintained under refrigeration for 20 hours and, after this time, hamburgers were produced in moulds with a diameter of 10 cm and a height of 1 cm in a burger-maker (Gaser, A-2000, Girona, Spain).

Hamburgers were packed in 300 mm thick trays of polystyrene sealed with polyethylene film for gas mixtures of 74 mm thick and with a permeability less than 2 mL/m<sup>2</sup>/ 24 h/ bar. The packaging was carried out using a heat sealer LARI3/Pn T-VG-R-SKIN (Ca.Ve.Co., Palazzolo, Italy). The compositions of the atmospheres used were: M1 [30% O<sub>2</sub>-70% CO<sub>2</sub>] and M2 [80% O<sub>2</sub>-20% CO<sub>2</sub>]. The trays were stored at 4 °C on a refrigerated chamber with light, trying to simulate real supermarket conditions. Analyses were carried out for 0, 2, 6, 8, 13 and 15 days of storage time. In every sample point, pH, colour parameters, TBARS index and microbial spoilage were determined by duplicate.

### B. Analytical methods

The pH of samples was measured using a pH-meter (HI 99163, Hanna Instruments, Eibar, Spain) equipped with a glass probe for penetration. A CR-400 portable colorimeter (Konica Minolta, Osaka, Japan) was used to measure meat colour in the CIELAB space (Lightness, L\*; redness, a\*; yellowness, b\*) [12]. Lipid stability, expressed by TBARS index, was evaluated using the method proposed by Vyncke [13]; results were expressed as milligrams of malonaldehyde (MDA) per kilogram of meat.

To microbial analyses, 10 g of sample was aseptically taken and homogenised with 90 mL of sterile 0.1% peptone water in a masticator blender (IUL Instruments, Barcelona, Spain) for 2 min at room temperature. Successive decimal dilutions were prepared by mixing 1 mL of the previous dilution with 9 mL of 0.1% peptone water and plated out following standard methodologies. *Pseudomonas* was determined in *Pseudomonas* Selective Agar (Merck, Darmstadt, Germany) incubated at 37 °C for 48 hours. Lactic acid bacteria (LAB) counts were determined on MRS Agar (Oxoid, Unipath Ltd., Basingstoke, UK) (pH 5.6) incubated at 30 °C for 5 days. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. Microbiological data were

transformed into logarithms of the number of colony forming units (cfu/g).

## III. RESULTS AND DISCUSSION

During display days, no significant differences ( $P>0.05$ ) were observed for pH values among hamburger batches. Average values for pH measured in the hamburgers ranged from 5.83 to 6.27 for all display period. When we compared mean pH values, during all storage period, between MAP package a significant difference ( $P<0.001$ ) was found (5.95 vs. 6.05 for 30% O<sub>2</sub> and 80% O<sub>2</sub>, respectively). The pH appeared to be related to CO<sub>2</sub> concentration; in fact, the higher the CO<sub>2</sub> concentration the lower was the pH value and are agree with reported previously [5, 14]. This effect has been related to the absorption of CO<sub>2</sub> by meat which results in the production of carbonic acid [15].

Storage time had a significant effect, hence luminosity, redness and yellowness decreased in all batches with the exception of luminosity values for C and CS. Similar results were found in beef steaks [16]. Comparing MAP, there were no significant differences ( $P>0.05$ ) for luminosity and yellowness in the last sampling point. As the bright red colour in meat is one of the most important quality attributes influencing the consumer's decision to purchase, evolution of redness is shown in Fig. 1 for M1 and M2. Redness decreased for all batches during display days and for both MAP. Redness values for M2 package were significant ( $P<0.05$ ) lesser than for M1 reaching at 16 days higher values in M1 (10.90 vs. 9.46 for M1 and M2, respectively). In M1 and M2 package, the best final values for redness were found for grape seed extract (12.90 and 10.40).

The level of lipid oxidation of meat was estimated on base of TBARS index (Fig. 2). All samples started with very low values of about 0.13 mg MDA/kg in fresh meat. Store time was a significant ( $P<0.001$ ) factor for TBARS values increase. For both MAP, samples remained stable during the first 13 days and showed no significant differences ( $P>0.05$ ) between antioxidant extract. Samples from M1 showed means of MDA concentration higher than M2, although M2 was higher oxygen content.

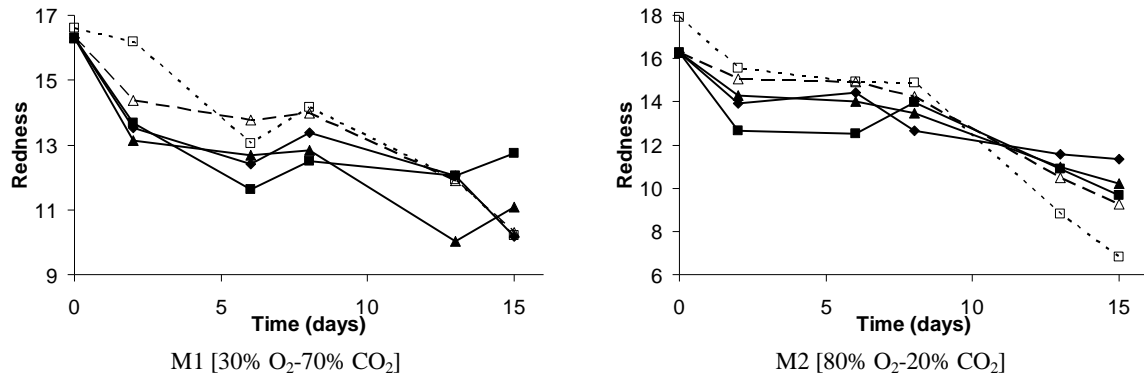


Fig. 1 Effect of antioxidant and packaging on redness during shelf life [(□) C, (Δ) CS, (▲) BHA; (◆) BHT and (■) G]

It is well known that lipid oxidation is directly correlated with haeminic pigment oxidation in beef [17]. In this study, applying Pearson correlation, we could observe that redness index and TBARS index are correlated ( $r=-0.40$   $P<0.01$  and  $r=0.72$ ,  $P<0.01$ , for M1 and M2, respectively). Hamburgers from treatments BHT, BHA and G showed means of TBARS index significantly lower than that for controls at the last sampling point (0.16, 0.25, 0.34 < 0.51 and 1.35,  $P<0.05$ , for BHT, BHA, G, CS and C, respectively). BHA and BHT batches provided the best protection against lipid oxidation of meat in M1 and M2, respectively at the end of storage time, whereas grape extract showed higher TBARS values than synthetic antioxidant but lower than control treatments. The addition of antioxidants to meat had an effect positive, because it was observed a significant higher TBARS value mean for treatments “control” than those for treatments “grape”.

For both MAP, BAL and *Pseudomonas* population increased significantly ( $P<0.05$ ) during storage time (see Fig. 3 and Fig. 4). A higher BAL and

*Pseudomonas* spoilage for M2 samples than those for M1 packaging was observed. The grape extract treatment reduced the growth of BAL and *Pseudomonas* compared with control, so this grape extract had a moderate antimicrobial effect.

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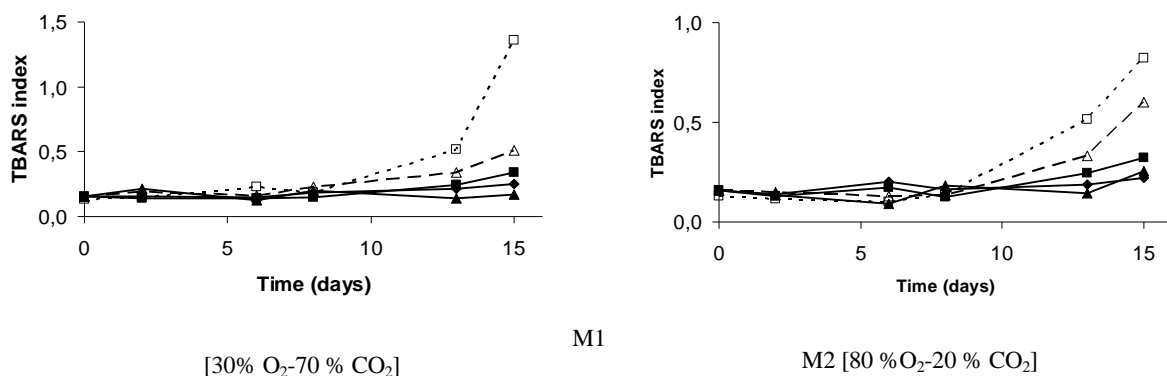


Fig. 2 Effect of antioxidant and packaging on TBARS index during shelf life [(□) C, (Δ) CS, (▲) BHA; (◆) BHT and (■) G]

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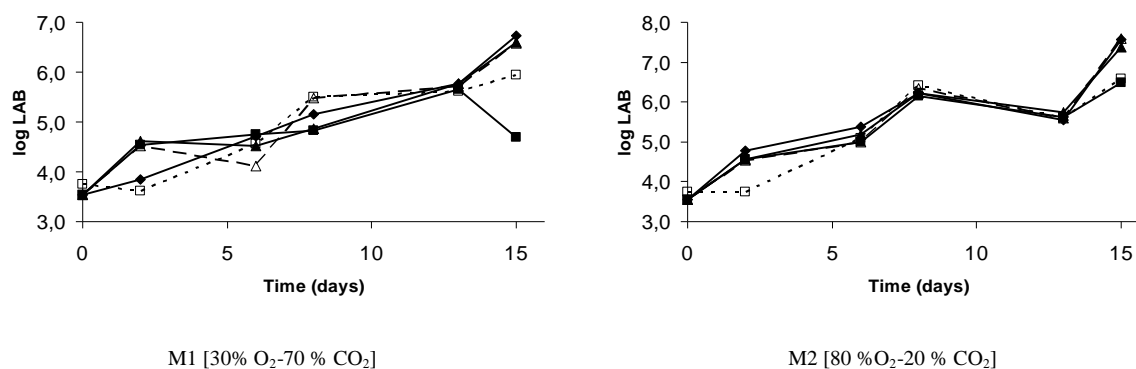


Fig. 3 Effect of antioxidant and packaging on BAL spoilage during shelf life [(□) C, (△) CS, (▲) BHA; (◆) BHT and (■) G]

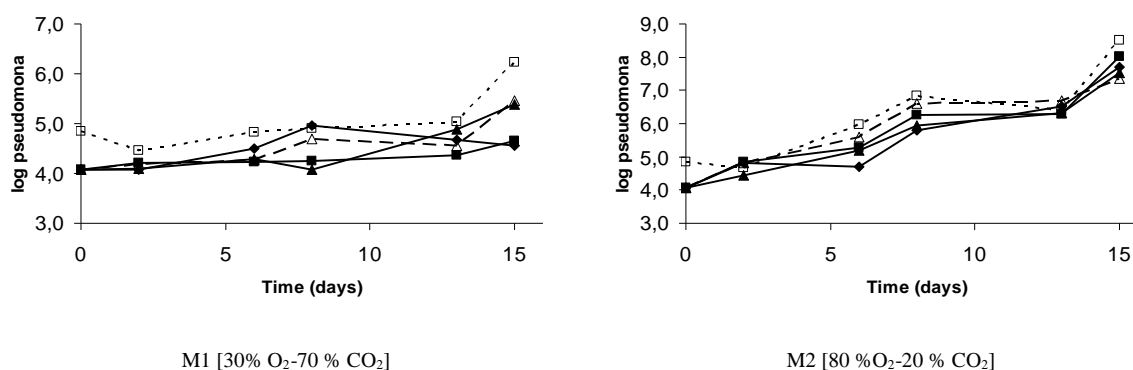


Fig. 4 Effect of antioxidant and packaging on *Pseudomonas* spoilage during shelf life [(□) C, (△) CS, (▲) BHA; (◆) BHT and (■) G]