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ORIGINAL ARTICLE

Influence of vacuum-ageing duration of whole beef on retail shelf life of steaks packaged with oregano (*Origanum vulgare* L.) active film under high O_2

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Abstract Beef *Longissimus lumborum* (LL) was no aged (LL0), aged for 7 days (LL7) and 14 days (LL14) under vacuum at 1 ± 1 °C. The obtained beefsteaks were packaged in high oxygen (Hi-O_2) with active packaging (AP) during 13–21 days at 1 ± 1 °C. Redness (CIE a* values), metmyoglobin percentage (MetMb%), total flora (PCA), thiobarbituric acid-reactive substances (TBA-RS), instrumental tenderness (Warner–Bratzler shear force: WBSF), and sensory analyses were performed. The various variables differed amongst the ageing times and packaging systems (AP vs. control). Three and ten additional days of retail shelf life were observed for steaks from LL7 and LL14, respectively. AP increased efficiently the retail shelf life of beefsteaks, but did not affect meat tenderness. The extended ageing from 7 to 14 days also induced higher tenderness in beefsteaks and did not show any affect negative effect on other quality parameters. Innovative technology referring to ageing under vacuum combined with Hi-O_2 MA/AP would be desirable for beefsteaks during display and constituted a good alternative for meat supermarkets.

Keywords Beef · Vacuum-ageing · High O_2 · Active packaging · Shelf life

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Introduction

In recent decades, the meat industry has registered major change to adapt to trends and consumer habits, as well as market demands. The retail display of beef meats in supermarkets was subjected to problems of consumer perception and resulted in a product quality highly variable. Commonly, ageing without packaging is widely used to improve tenderness, providing a more palatable and acceptable product to consumers. However, ageing meat without packaging requires greater environmental control practices to achieve a consistent product quality.

The purple colour of beef and the visible purge loss in the vacuum bag are thought to be unattractive to consumers, which vacuum packaging is not frequently used for retail display of red meat. Retail packaging of beef in modified atmospheres (MA) under high O_2 (60–80%) provide a stable red meat colour, which is attractive to the consumer (Djenane et al. 2000). Another advantage of MA packaged meat is the absence of off-odour when opening packs. The odour that may be perceived when opening vacuum packs has been described as sour, acid or cheesy (Dainty et al. 1979). The colour of fresh meat is not well correlated with the eating quality; however, is a visible quality guide for the consumers in the supermarkets. However, tenderness of beef is the most important for the eating satisfaction for consumers. The main inconvenient of high-O_2 modified atmosphere packaging (MAP) that the increase in protein oxidation might have a negative effect on the tenderness of meat. However, why beefsteak gets tougher when packaged directly in high-O_2 MAP has not yet been fully clarified. Recently, a significant trend was recorded for new technologies that can be used to improve the quality and stability of food. Amongst these technologies, active packaging (AP) systems based on the

incorporation of bioactive agents in the package are being developed (Djenane et al. 2009; Lorenzo et al. 2014). Several contributions on the use of oregano (*Origanum vulgare* L.) as a natural food preservative have been yet published (Govaris et al. 2011). Successful combinations of oregano with other preservation techniques, such as MAP, have also been described (Sánchez-Escalante et al. 2003). Nevertheless, most of the contributions reported involve the direct addition of bioactive agents to the packaged food (Djenane et al. 2011a, b).

The Spanish meat retailers use MA to provide fresh meat cuts with a short ageing time or no ageing at all. Commonly, the success in food innovation depends critically on consumers' perceptions and response to the technologies (Rollin et al. 2011). The Spanish supermarkets have raised three questions about beefsteaks quality. (1) Might supermarkets kept beefsteaks directly in vacuum to avoid repackaging? (2) Could supermarkets retail directly beefsteaks under high oxygen (Hi-O_2) when obtained from *Longissimus lumborum* (LL) without ageing? (3) Could supermarkets kept the whole LL ageing under vacuum followed by retail display of beefsteaks under high O_2 MA? We designed a project to investigate these questions and studied the effect of AP on beefsteaks quality.

Materials and methods

Preparation of samples

The beefs used for this trial were cared in accordance with the guidelines from the Spanish Ministry of Agriculture (Boletín Oficial del Estado 2007).

Six Spanish *Pirenaica* breed were slaughtered on the same day according to standard commercial slaughterhouse. All animals from the same farm received the same feeding regime throughout the period *ante-mortem*. The carcasses were suspended by the Achilles tendon and kept in a chilling room at 2 ± 1 °C. The strip loins (LL) were removed from six carcasses (48 h *post-mortem*), and trimmed of external fat. Four of LL muscles were placed individually in plastic bag (coextruded polyethylene: PE and polyamide: PA; EUROBAG & Film, S.L. Malaga, Spain) with high barrier properties (H_2O vapour transmission rate of 2.8 g/m^2 per day at 23 ± 1 °C and 85 ± 2% of relative humidity (RH) and O_2 transmission rate of 40 cm^3/m^2 * atm per day at 23 ± 2 °C and 75% of RH), and vacuum packaged (EV-15/CN, Tecnotrip, Terrassa, Spain). Two bags were stored for 7 days and the others for 14 days at 1 ± 1 °C.

Obtained beefsteaks were randomly divided into three groups according to Table 1. The 1st group (steaks obtained directly from no aged *Longissimus lumborum*: LL0) contained 24 steaks distributed on 6 selected times of sampling and analysis (1, 5, 9, 13, 17 and 21st day of display); the 2nd and 3rd group (steaks obtained from aged *Longissimus lumborum* during 7 days: LL7 and aged *Longissimus lumborum* during 14 days: LL14, respectively) and each contained 16 samples for 4 selected times of sampling and analysis (1, 5, 9 and 13th day of display). A total, 56 steaks were aseptically cut. Each steak (10 cm long × 2 cm thick; ∼100 g of weight) was individually packed into a polystyrene tray (15.5 × 21.5 × 2.5 cm).

The active film was prepared by covering a polypropylene (PP) film (thickness: 20 μm, density: 18.93 ± 0.02 g/m^2) with a layer of varnish containing the oregano extract (4%) (Garcés et al. 2003). The PP film covers ∼80% of the upper internal surface of plastic bag (PE/PA) in a way that the headspace separates the varnish layer containing oregano and samples.

All trays contained samples were completely surrounded with a PE: 80 μm/PA: 20 μm coextruded films supplied by Irma, Zaragoza (Spain) (density of 87.35 g/m^2; H_2O vapour transmission rate of 5–7 g/m^2 per day at 25 °C and 85% of RH and O_2 transmission rate of 40–50 cm^3/m^2 * atm per day at 25 °C and 85% of RH) and filled with a gas mixture of 80% O_2 + 20% CO_2. The volume injected into the headspace was approximately 2 L. The gas to meat volume ratio was ∼2/1. All samples were displayed in a refrigerated cabinet under illumination at 1 ± 1 °C, simulating real supermarket conditions. All illuminated trays were exposed continuously at 800–1000 lx fluorescent light (low-UV, colour-balanced lamp, Promolux® Platinum L36 w; Market Group Ventures Inc., Shawnigan Lake, B.C., Canada). The trays were rotated every 24 h to minimize a possible abuse temperature and light intensity differences at the surface of samples. The PE/PA laminate used for packaging allowed transmission of about 80% of the visible light and 60–70% of the UV light from 330 to 380 nm; transmission was about 0% at 275 nm. Four trays were taken randomly at each selected time. Two of them were used for sensory, red index (CIE a*), metmyoglobin percentage (MetMb%) and Warner–Bratzler shear force (WBSF) analyses, and two were used for microbial analysis and thereafter for TBA-RS analysis. Three repetitions of each of the attribute were carried out, with a minimum of three replicates per repetition.

Methods

Measurements of pH

The pH of steaks was measured using a micro pH-meter model 2001 (Crison Instruments, Barcelona, Spain) after

Table 1 Experimental design

Ageing systems (whole *Longissimus lumborum* muscle)	Ageing times (days)	Number of beefsteaks		Packaging systems		Retail display periods (days)	Total *post mortem* periods (days)
		No ageing LL	Prior vacuum ageing LL	Control	Treated samples		
No ageing	0	24	–	MAP[a]	MAP/AP[b]	21	23
Vacuum ageing	7	–	16	MAP	MAP/AP	13	22
Vacuum ageing	14	–	16	MAP	MAP/AP	13	29

[a] *MAP* modified atmospheres packaging

[b] *MAP/AP* modified atmospheres packaging with oregano active packaging

homogenizing 3 g of sample in 27 mL of distilled water for 10 s at 1300 rpm with an Ultra-Turrax T25 macevator (Janke & Kunkel, Staufen, Germany).

Instrumental surface colour and metmyoglobin determination

Instrumental surface colour (CIE a*) of each steak was measured using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan) 30 min after pack opening, in order to allow colour stabilization on air exposure (CIE 1978). The average value for each steak was the mean of ten determinations, avoiding the zones with excessive fat or blood. The MetMb% of the total myoglobin (Mb) perceptible at the steak surface was estimated spectrophotometrically, according to Stewart et al. (1965) by measuring steak surface reflectance at 525 and 572 nm (Minolta CM-2002; Osaka, Japan). The maximum value of the ratios of $(K/S)_{572}$ to $(K/S)_{525}$ at the beginning of the experiment was fixed as 0% MetMb; K and S were the absorption and the scattering coefficients, respectively, and K/S ratios were calculated from reflectivity ($R\infty$) values using the Kubelka–Munk equation. The value of 100% MetMb was obtained following the same procedure after oxidising a sample in a 1% (w/v) solution of potassium ferricyanid (Ledward 1970).

Lipid oxidation analysis

Ten grams of ground meat were weighed in a 50 mL test tube and homogenized in 20 mL of trichloroacetic acid (TCA 10%, Biochem, Chemopharma, Spain) with an Ultra Turrax Mixer T25 (Janke & Kunkel, Staufen, Germany), and centrifuged at 4000 rpm for 15 min using a Megafuge 1.0 centrifuge (Heraeus Sepatech, Spain). Fresh solution (20 mM) of thiobarbituric acid (TBA, Sigma, Aldrich, Germany) was prepared. The supernatant was filtered through a Whatman No. 1 filter paper. 2 mL of the supernatant were collected and 2 mL of TBA (20 mM) solution were added in test tubes. The tubes were heated in a water bath (B. Braun, Melsungen, Germany) at 100 °C for 15 min and then cooled for 20 min. The absorbance of the solution was measured at 531 nm using a Shimadzu UV–Visible Spectrophotometer (UV-1603, Shimadzu, Kyoto, Japan) against a blank containing 2 mL of deionized H_2O and 2 mL of TBA (Djenane, 2015). The results were expressed as mg of MDA/kg of meat and calculated using a standard curve prepared with 1,1,3,3-tetramethoxypropane (Sigma Aldrich Corporation, St. Louis, MO, USA).

Microbiological analysis

For psychrotrophic aerobic flora analysis, two sterile cotton wool swabs moistened with 0.10% peptone water were used to swab 10 cm^2 of the steak surface delimited by a sterile, stainless steel template. Swabs were stirred in 10 mL of 0.10% peptone water. Serial ten-fold dilutions were prepared by diluting 1 mL in 9 mL of 0.10% peptone water. Three plates were prepared from each dilution by pouring 1 mL into appropriate agar (Plate Count Agar, Merck-España, Madrid). The counts were expressed as the $\log_{10}$ of colony forming units per cm^2 ($\log_{10}$ cfu/cm^2).

Warner–Bratzler shear force

Instrumental tenderness was measured using the Warner–Bratzler (WB) method as described by Honikel (1998) with minor modifications. Steaks were placed in individual plastic bags and cooked in a water bath (Model B21, Fisher Scientific GmbH, Schwerte, Germany) for 45 min at 80 °C to reach approximately an internal temperature of 75 °C. After cooking, samples were overwrapped in polyvinyl chloride (PVC) film; vacuum packaged and cooled for 24 h at 2 °C before coring (AMSA 1995). Prior to texture analysis, samples were kept at room temperature for 3 h. At least six cores (1 cm diameters × 3 cm of longitude) from each steak were removed parallel to the longitudinal

orientation of the muscle fibers. The cores were sheared perpendicular to the muscle fibers orientation using an Alliance RT/5 (MTS Systems Corp., Eden Prairie, MN, USA) with a WB shear device and crosshead speed set at 2 mm/s. Results were expressed as load in kg.

Sensory evaluation

Steaks were evaluated by trained members according to the method of Cross et al. (1978). Panellists were selected amongst staff of the Department of Food Science (Faculty of Veterinary, University of Zaragoza. Spain). Three open-discussion sessions were held to familiarize panellists with the attributes and the scale to be used. The attributes studied were: "red colour", "discoloration", and "off-odour". They were rated using a 5-point descriptive scale, according to Djenane et al. (2001), using a paper scorecard. The attribute "red colour" was scored using an intensity of 5-point scale, in which 1 denoted extremely brilliant meat and 5 denoted extremely faded meat. Scores for "discoloration" referred to percentage of discoloured surface: 1 = none, 2 = 0–10%, 3 = 11–20%, 4 = 21–60%, and 5 = 61–100%. Scores for "off-odour" referred to the intensity of odours associated to meat oxidation: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme. A score ≤ 3 in any of the attributes denoted that meat was acceptable for sale and consumption.

Statistical analysis

Data analysis was performed using the SPSS statistical package (SPSS Inc., Chigaco, IL). The significance of differences amongst the treatments at each day of display were determined by analysis of variance (ANOVA) using the least square difference (LSD) method of the General Linear Model procedure of SPSS. Differences were considered significant at the $p < 0.05$ level.

Results and discussion

pH measurements

The different ageing times of beef LL muscle did not affect ($p > 0.05$) the pH (mean values of 5.64–5.66). Moreover, packaging types also did not affect the pH of beefsteaks during display. Samples had mean pH values of 5.68 and 5.78 ($p > 0.05$). The pH_u value was reached after 24 h *post-mortem* and then remains stable. These results were similar to previous studies. Djenane et al. (2003a) reported that pH values were not affected by storage conditions.

Meat colour

Conventional unwrapped and over-wrapped meat during retail display has lower colour shelf life. However, fresh red meat cannot be packaged without Hi-O_2 when retailed in supermarkets. Untreated steaks (LL0) showed a decrease in CIE a*, and reached value of $\sim$9.50 even at 13th day of display, which was representative of loss of red colour (Fig. 1). CIE a* value below 10, indicating a short life for meat colour. There was an increase in CIE a* values (14.19–16.29) from 0 to 9 days of display in untreated steaks (LL14) while decreasing afterwards, especially at 13th of display. Untreated steaks (LL7) showed shelf life limit of redness at 9th day of display, however this limit was not reach even at the end of display for steaks (LL14), corresponding at 16–27 days of colour shelf life, respectively.

Meat redness was also affected by packaging systems ($p < 0.05$). Our results demonstrated that combination of Hi-O_2 MA with AP during display period was effective for colour stability compared to steaks packaged only under Hi-O_2. It was noted that for untreated steaks (LL0), a limit value of red index was reached at 13th day of display while for treated samples; this limit was reached at 17th day of display period, corresponding at 4 additional days for red colour stability. At the final of display period, the treated steaks (LL7) and LL14 had higher CIE a* value than treated steaks (LL0).

The lack of the bright red colour of packaged beef was regarded as a possible disadvantage in marketing. This effect has been reported in several other studies (Camo et al. 2011; Djenane et al. 2001).

At retail sale, colour stability is the most considerable attribute of meat quality. A number of commercial approaches have been developed to meet consumer expectations in the search of an attractive bright red colour along with a good eating quality and a long shelf life. For economic reasons, the meat supermarket works to minimise all types of colour loss during display. Chamorro et al. (2012) studied trends in meat consumption in Spain. According to them, there should be a clear tendence in consumers' purchase decision criteria to move away from external appearance. Regarding the packaging materials, design and functionality, the panellists assigned positive evaluations to any innovation identified in previous studies. In this study, it was noted that the difference in colour stability between steaks from no aged, short and long ageing times of LL muscle was also evident from other quality parameters as indicated by MetMb% and TBA-RS variations during display since the decrease in CIE a* value has been related to the appearance of brown colour and

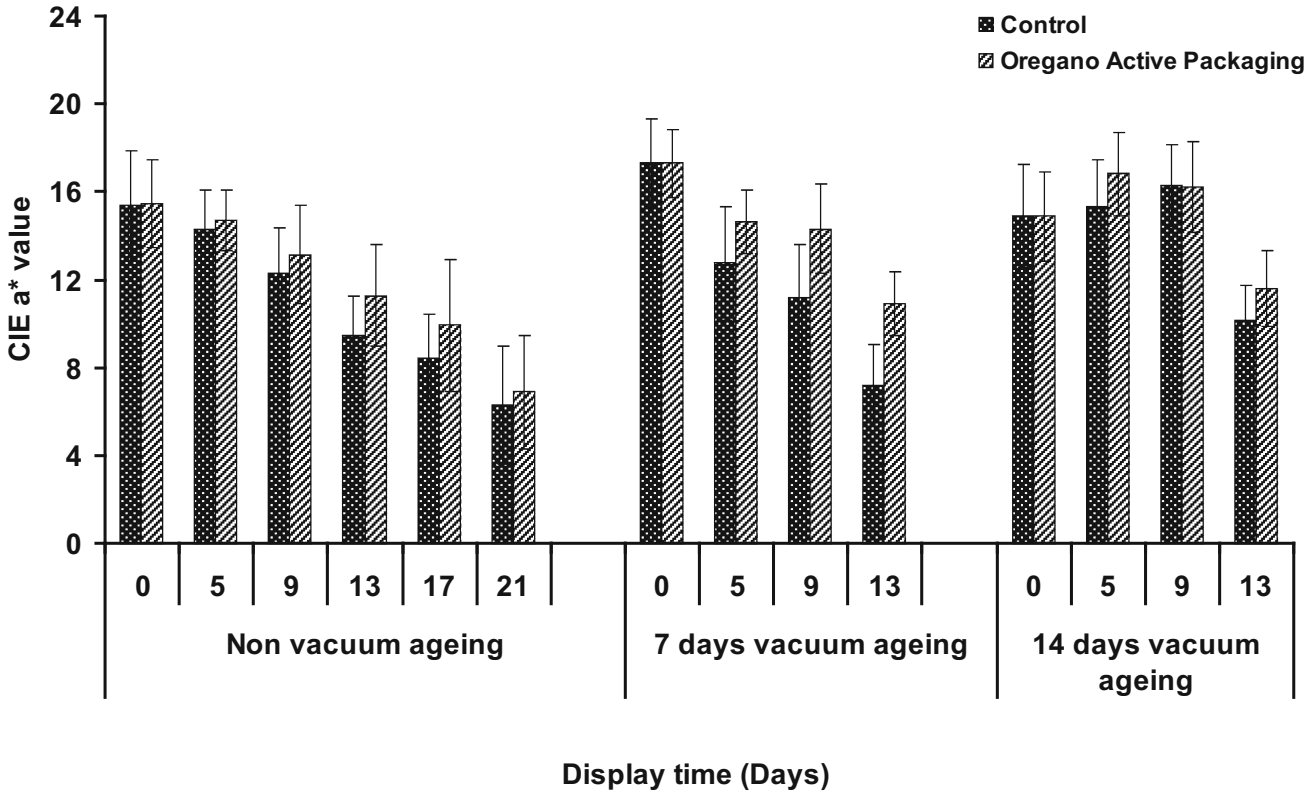

Fig. 1 Effect of vacuum-ageing times and packaging systems on the CIE a* values of beefsteaks during display at 1 ± 1 °C. *Error bar* represents the standard deviation

undesirable odour, respectively. This result agreed partially with that reported by Franco et al. (2009), who detected no significant changes in meat redness by using long vacuum-ageing times; they referred those results to a low Mb oxidation in prior vacuum packaged meat. Lagerstedt et al. (2011) found an increase in redness due to ageing when comparing with non-aged meat as they studied the effect of different ageing times under vacuum on the instrumental colour of beef LL. Vitale et al. (2014) observed higher initial CIE a* in aged meat than in non-aged meat which was referred to a higher blooming capacity of vacuum aged steaks. Vieira et al. (2006), studying the effect of different ageing times of beef *Longissimus thoracis* muscle, and found no significant ($p > 0.05$) effect of the ageing period on the redness values.

Metmyoglobin analysis

The relative content of MetMb was significantly increased during display for all samples (Fig. 2). It was evident that, the presence of the active film containing oregano protects Mb against oxidation processes ($p < 0.05$). As can be seen, there is a clear evidence of the influence of vacuum-ageing times in pigment oxidation. On the one hand, the steaks (LL14) showed a fewer oxidation rates. On the other hand, the protection against oxidation was efficient in the presence of active film, the most likely reason being that relatively high amount of oregano present in the active film (4%). This fact suggests that the concentration of active compounds in the film should be optimized by taking into account the final application for which the AP is projected. Surface MetMb increased steadily throughout display for untreated steaks (LL0), reaching a value of 77% at 21st of display. However, surface MetMb not reached 34.50% for treated steaks (LL14) even at the end of display (29 days *post-mortem*). The intermediate ageing time (LL7) was also efficient. At the 9th day of display (18 days *post-mortem*), the total MetMb for treated steaks, reached a similar content (30.80%) than untreated steaks (LL0) at the same day of display (9 days *post-mortem*). Most important is the fact that MetMb% value >40 was obtained at 13th day of display for untreated steaks (LL0); this percentage has been demonstrated to be the limit between red and brown perception by sensory panellists (Sánchez-Escalante et al. 2003).

Studies carried out under model system with Mb solution showed a clear protection behaviour against oxidation provided by the active films (manner dependent of concentration) (Nerín et al. 2006). The active film containing the highest concentration of oregano was the less efficient

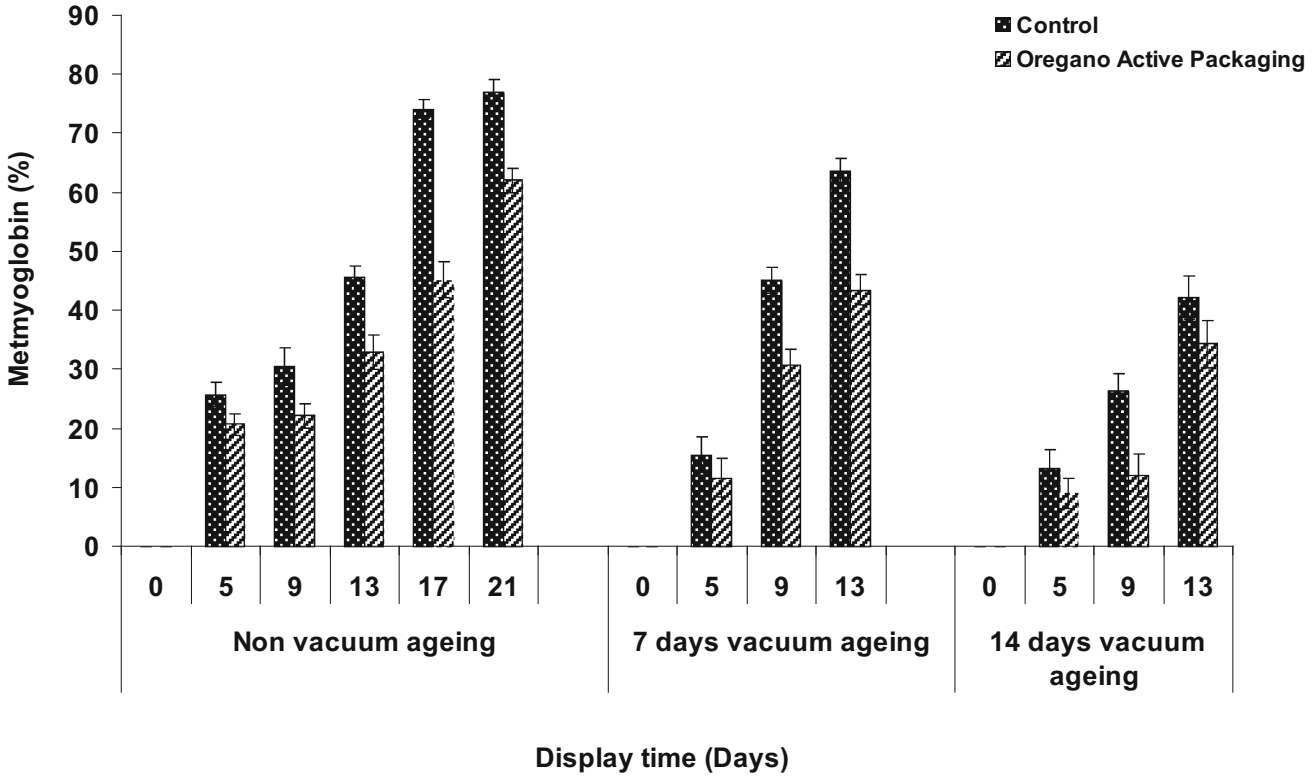

Fig. 2 Effect of vacuum-ageing times and packaging systems on the MetMb% of beefsteaks during display at 1 ± 1 °C. *Error bar* represents the standard deviation

against oxidation during all of the experimental series. This achievement does not agree with the expected behaviour, very probably because of the pro-oxidant effect that some antioxidants show at high concentration under model systems. Camo et al. (2011) found that oregano extract did not delay both lipid oxidation and pigment oxidation and, consequently, stabilizes the colour of fresh meat.

Lipid oxidation

The initial TBA-RS values did not differ amongst samples ($p > 0.05$); however, the increase in lipid oxidation over time was higher for untreated than treated steaks (Fig. 3). The development of antioxidant AP systems is attracting emerging technologies for reducing the incidence of lipid peroxidation and constituted a good alternative for food industry. TBA-RS value is consistently correlated to storage time. No meat science research has assessed the effects of vacuum-ageing times of whole LL and subsequent retail display of LL cuts under Hi-O_2 MA/AP on meat retail shelf life.

In the current study, the time of ageing and packaging systems significantly affected TBA-RS ($p < 0.05$). The TBA-RS values were significantly higher for untreated steaks (LL0) compared with treated steaks (LL7 and LL14). At 9th and 13th day of display (18 and 29 days *post-mortem,* respectively), the TBA-RS values were ~2 mg MDA/kg for untreated steaks (LL7 and LL14), while for untreated steaks (LL0), TBA-RS value showed ~2 mg MDA/kg at 13th day of display, indicating that steaks would result in rancid odour after only 13 days of display (15 days *post-mortem*).

The effect of ageing on lipid oxidation controversial. Popova et al. (2009) reported that ageing under vacuum for 14 days affected lipid oxidation of beef throughout storage since it was more intense than in the control, being the difference in TBA-RS significant ($p < 0.05$) on the 1st and the 6th days of storage. Yang et al. (2002) found no effect of beef vacuum-ageing for 47 days on initial TBA-RS values under aerobic storage compared with no aged beef. However, after 7 days of aerobic storage, TBA-RS of aged beef increased significantly while the increase in non-aged beef was small. Clausen et al. (2009), focusing on the effect of vacuum and MA packaging on lipid oxidation, reported very low TBA-RS values for meat stored only in vacuum for 23 days while they increased largely in samples stored 6 days in MAP after vacuum. Oregano has been shown to inhibit lipid oxidation in cooked ground beef, pork and raw beef (Rojas and Brewer 2008; Sánchez-Escalante et al. 2003). The protective effect against lipid oxidation of AP system with oregano extract had been

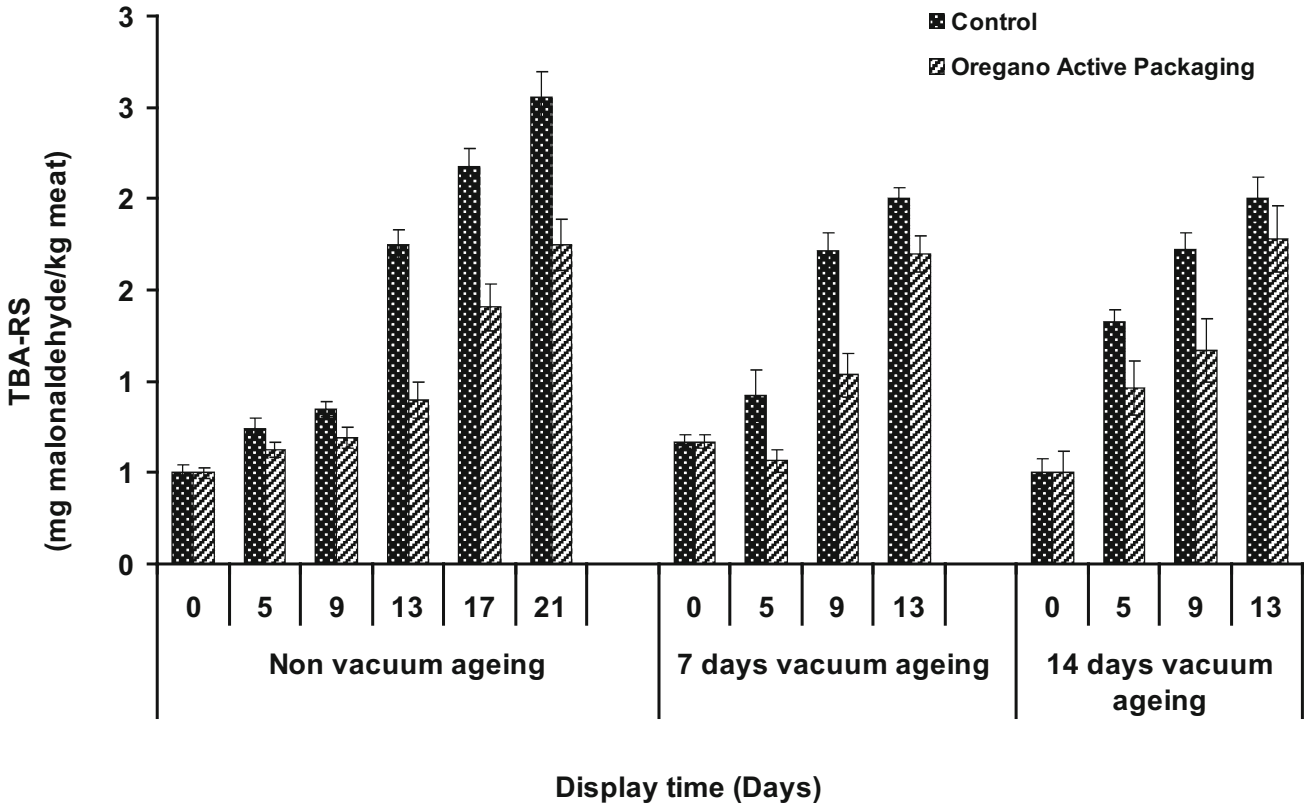

Fig. 3 Effect of vacuum-ageing times and packaging systems on the TBA-RS values of beefsteaks during display at 1 ± 1 °C. *Error bar* represents the standard deviation

already reported by Nerín et al. (2006). In fact, oregano was most active antioxidants in AP, in accordance with Camo et al. (2011) who reported that AP significantly ($p < 0.05$) enhanced oxidative stability of beefsteaks, depending on the oregano concentration of the active film.

Conventionally, TBA-RS has been used as an index of lipid oxidation in meat and has been well correlated with its perception by consumers. This appears to be of great significance from a sensory point of view. Many authors have proposed threshold TBA-RS values for relating the degree of lipid oxidation and the perception of rancid odour by panellists. Martínez et al. (2006) reported that TBA-RS value above 1 had unacceptable off-odour. Djenane et al. (2001) found that TBA-RS value of about 2 mg MDA/kg was required for consumer detection of rancidity. In fact, treated steaks reach a critical value only at the end of the display, resulting in an antioxidant AP ability between of 12.50–52.40% compared to untreated steaks; therefore, they would not present perceptible off-odours during display period, what is in accordance with sensory results. The results of the present study show that adding oregano (phenolic-rich extracts) in PP film protects steaks against lipid oxidation when displayed under Hi-O_2 MA. Jouki et al. (2014) found that active films exhibited a higher level of radical scavenging activity with values of 45.09, 56.65 and 61.03% for 1, 1.5 and 2% of oregano containing films, respectively. Generally, it was found that the herbal extracts possess high antioxidant activity, even higher than some synthetic antioxidants like butylated hydroxyatoluene or butylated hydroxyanisole (Radha Krishnan et al. 2013). Carvacrol, thymol, c-therpinene and p-cymene are the most active constituents of oregano extract, with a wide spectrum of antimicrobial and antioxidant properties (Rocha-Guzman et al. 2007).

It has been postulated that pigment oxidation is promoted by free radicals formed following lipid oxidation; therefore, the obtained results suggest that active film used promote a maximum inhibition of both lipid and Mb oxidation. In support of this hypothesis, the inhibitory effect of the active film was crucial. Also in the work of McKenna et al. (2005) on beef, muscles of lower colour stability were characterized by higher oxidative rancidity. This relationship between lipid and Mb oxidation is well established for beef (Djenane et al. 2003b). MetMb% had a negative correlation with beef redness (CIE a*) which agrees with Martínez et al. (2006) who reported a highly significant and negative correlation between both parameters.

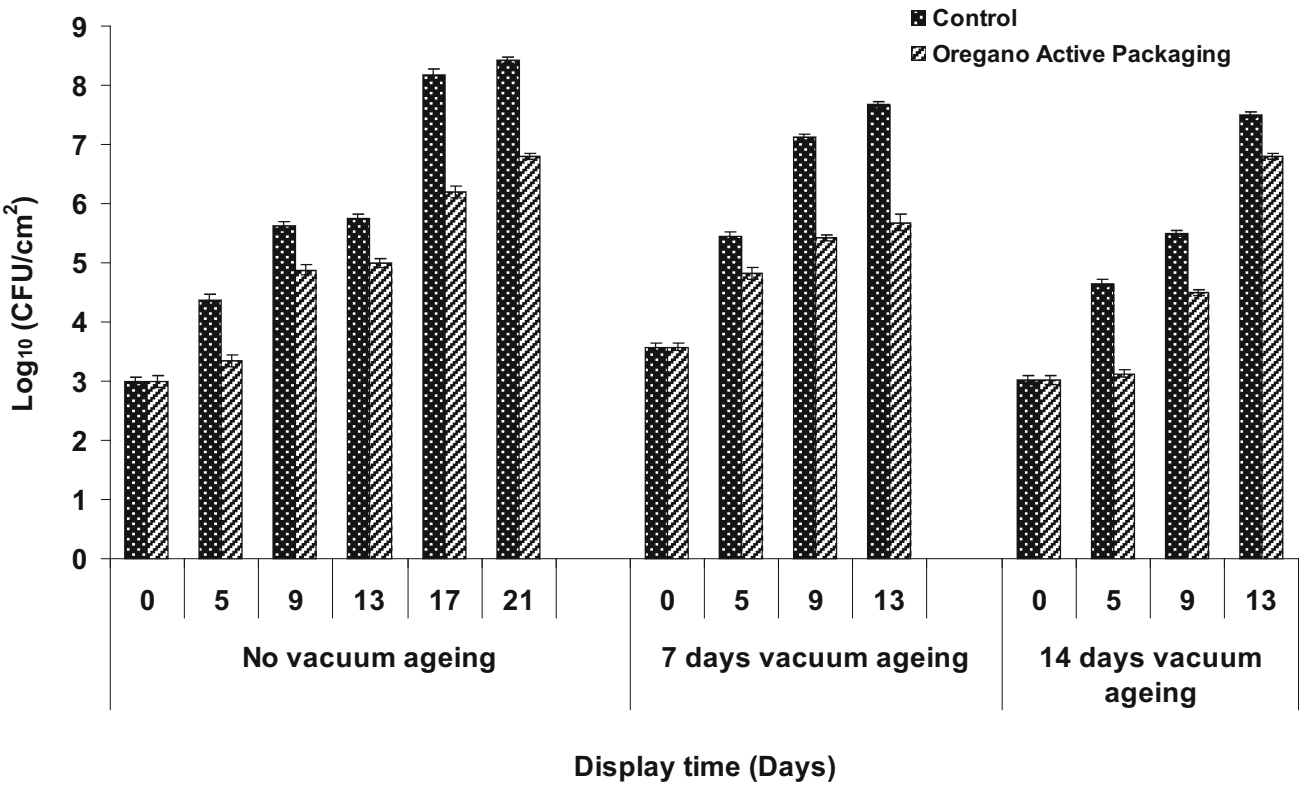

Fig. 4 Effect of vacuum-ageing times and packaging systems on the bacterial counts in beefsteaks during display at 1 ± 1 °C. *Error bar* represents the standard deviation

Microbial analysis

The ageing and packaging systems significantly affected the counts of psychrotrophic aerobic flora ($p < 0.05$). After 2 weeks of display, the number of bacteria in untreated steaks (LL0) was 8.20 $\log_{10}$ cfu/cm^2 (Fig. 4). However, the treated steaks did not achieve 7 $\log_{10}$ cfu/cm^2 even at the end of display. There was a clear evidence of the influence of vacuum-ageing times on psychrotrophic aerobic flora. The foreseeable behaviour was in general observed in all steaks, showing the maximum protective effect with ageing times (7 and 14 days). AP was showed significant antimicrobial activity compared to untreated samples ($p < 0.05$), without reaching the limit value ($\sim$7–8 $\log_{10}$ cfu/cm^2), generally associated to slime and off-odours formation.

The effectiveness of antimicrobial AP was greater compared to direct addition of preservative agents into food (Muriel-Galet et al. 2012). Several researchers have reported the antibacterial effect of oregano. Skandamis and Nychas (2002) studied the combined effect of oregano essential oil (EO) with MA, vacuum and aerobic storage conditions on the microbiological attribute of fresh beef stored at 5 and 15 °C. Higher shelf life was observed in samples stored under MAP and treated with oregano EO. The same results were observed by Kapetanakou et al. (2014), who indicated that meat exposed to active vapours of EO combined with MAP is a promising alternative antimicrobial packaging technology for extending shelf-life during storage at chill or abuse temperatures. Dadalioglu and Evrendilek (2004) found that oregano EO showed a higher antibacterial activity. Its strong inhibitory effect appeared to be due to the higher content of carvacrol (68.23%), defined as a strong antimicrobial agent.

Oregano was chosen as antimicrobial agent in AP since. It is a natural compound with antimicrobial activity against a broad range of bacteria and is categorized as Generally Recognized as Safe (GRAS). Its inhibitory effect on the growth of various microorganisms is well documented. Jouki et al. (2014) found that AP with oregano EO (1–2%) had a great potential for its application as a natural agent to preserve foods in antioxidant or antimicrobial films. A number of studies have demonstrated that antimicrobial agents incorporated onto packaging films could be very effective in reducing the levels of food borne microorganisms. Emiroğlu et al. (2010) evaluated the antibacterial activity of edible films incorporated with oregano EO against *Escherichia coli* O157: H7, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Lactobacillus plantarum* on fresh ground beef during refrigerated storage (4 °C). However, oregano based film did not had significant effect

Table 2 Effect of beef *Longissimus lumborum* (LL) vacuum-ageing times on Warner–Bratzler shear force values (mean ± SD) during display period

Vacuum-ageing (days)	WBSF (kg)					
	Days of display					
	1	5	9	13	17	21
0	4.72 ± 0.14^{aA}	3.71 ± 0.04^{bA}	3.70 ± 0.07^{bA}	3.24 ± 0.16^{cA}	3.20 ± 0.18^{c}	3.13 ± 0.12^{c}
7	4.00 ± 0.11^{aB}	3.63 ± 0.10^{bA}	3.04 ± 0.08^{cB}	2.99 ± 0.10^{cB}	–	–
14	2.79 ± 0.13^{aC}	2.83 ± 0.17^{aB}	2.28 ± 0.08^{bC}	2.17 ± 0.16^{bC}	–	–

$^{a–c}$ Means of the same row (between days of display) with different letters differ significantly ($p < 0.05$)

$^{A–C}$ Means of the same column (between ageing times) with different letters differ significantly ($p < 0.05$)

on total viable counts (TVC) when applied on ground beef patties. This might be due to the predominance of lactic acid bacteria (LAB) counted within the TVC. LAB were known as predominant microflora in MAP packaged fresh meats and are resistant to antimicrobial treatments.

Warner–Bratzler shear force

The effect of ageing on the instrumental texture is shown in Table 2. The ageing times had a significant effect on WBSF. At initial day of display, a decrease in shear force was observed for steaks (LL7 and LL14) compared to steaks (LL0). However, at 5, 9 and 13 days of display, the steaks (LL14) were significantly more tender ($p < 0.05$) than steaks (LL0), reducing up to 33% WBSF at 13th day of display. The effect of ageing on meat instrumental texture has been extensively studied. Marino et al. (2013) found that the proteolysis is the major factor that contributed to the variation in shear force tenderness amongst different samples meat. One of the most common causes of unacceptability in meat quality is toughness. It is attributed to various factors including the amount of intramuscular connective tissue, intramuscular fat, and the length of the sarcomere (Kemp et al. 2010). Hou et al. (2014) found that pelvic suspension increased sarcomere length. In addition, ageing time had a significant effect on pH, meat colour, WBSF, and myofibril fragmentation index of LL muscle. Lorenzen et al. (2003) a low correlation of WBS values with "in home" consumer judgments; they partially attributed the low relationship to the high variability in steak culinary preparation in "in home" consumer studies. Correlations of WBSF with the sensory assessment of tenderness are known to be highly variable, showing r-values ranging from −0.32 to 0.94 (Caine et al. 2003). Our study, demonstrated that the shear force for steaks (LL0) was higher compared to steaks from aged LL during all period of display.

Huffman et al. (1996) proposed for beef loin shear force values of 4.10 kg as a threshold of acceptable eating quality to consumers. Beefsteaks from LL0 resulted unusually tough with relatively lower shear force values (4.72 kg) than those reported by other researchers for beef. However, Pérez-Juan et al. (2012) and Vitale et al. (2014) observed higher values of shear force (5.10 kg) for non-aged meat proceeding from mature cows.

Many studies have been undertaken in order to find threshold values values of WBS for sensory tenderness acceptability. Destefanis et al. (2008) reported that beef with WBS values higher than 5.37 kg and lower than 4.37 kg were perceived by most panellists as tough and tender, respectively. In this case, 62.30% of the consumers were able to distinguish tough from intermediate and tender meat. Miller et al. (2001) and Shackelford et al. (1991) indicated values ranged from 4.31 to 5.99 kg. Our results indicate that WBS values were lower than those obtained in previous studies. Lowe et al. (2004) found 7.24 kg WBSF values for beefsteaks obtained from Friesian cows for 20 h ageing times. Allen et al. (2009) found 4.93 kg values for Holstein cows even after 14 days of ageing. According to Huffman et al. (1996) a change of 1 kg, or more, was necessary in order to find a noticeable difference between texture samples. Lagerstedt et al. (2011) observed that the combination of vacuum with MAP resulted in higher shear force compared with steaks stored only in vacuum for the same time. Other authors have observed the effect of Hi-O_2 MAP on fresh meat shear force. Lagerstedt et al. (2011) found that direct Hi-O_2 MAP systems for beefsteaks should not be recommended for either the meat industry or the retail display because Hi-O_2 affected negatively both shear force and water holding capacity, as well as sensory tenderness, flavour and juiciness. They suggested that cross-linking/aggregation of myosin might be responsible for the worsening of sensory quality of beefsteaks packaged in Hi-O_2 MA.

Sensory analysis

Ageing and the different packaging systems had an effect ($p < 0.05$) on all attributes sensory scores (Table 3). A score ≤3 in any of the attributes denoted that steaks were

Table 3 Sensory scores (Mean ± SD) for red colour[1], discoloration[2] and off-odour[3] of beef steaks packaged under Hi-O_2 MA with active films during display

Parameter	*Longissimus lumborum* ageing (days)	Beef steaks treatment	Days of display					
			0	5	9	13	17	21
Red colour[1]	No ageing (LL0)	MA*	1 ± 0.0^{aA}	1 ± 0.0^{aA}	1 ± 0.0^{aA}	2.83 ± 0.41^{bA}	3.17 ± 0.41^{bA}	4.83 ± 0.41^{cA}
		MA + AP**	1 ± 0.0^{aA}	1 ± 0.0^{aA}	1 ± 0.0^{aA}	2 ± 0.0^{bB}	2.83 ± 0.41^{cB}	5 ± 0.0^{dA}
	7 (LL7)	MA	1 ± 0.0^{aA}	1 ± 0.0^{aA}	2.83 ± 0.41^{bB}	4.83 ± 0.41^{cC}	–	–
		MA + AP	1 ± 0.0^{aA}	1 ± 0.0^{aA}	2.17 ± 0.41^{bC}	4.67 ± 0.52^{cC}	–	–
	14 (LL14)	MA	1 ± 0.0^{aA}	1 ± 0.0^{aA}	2.83 ± 0.41^{bB}	3.17 ± 0.41^{bA}	–	–
		MA + AP	1 ± 0.0^{aA}	1 ± 0.0^{aA}	2 ± 0.0^{bC}	2.17 ± 0.41^{bB}	–	–
Discoloration[2]	No ageing (LL0)	MA	1 ± 0.0^{aD}	1 ± 0.0^{aD}	2 ± 0.0^{bD}	3.83 ± 0.41^{cD}	4 ± 0.0^{cD}	5 ± 0.0^{dD}
		MA + AP	1 ± 0.0^{aD}	1 ± 0.0^{aD}	2 ± 0.0^{bD}	2.17 ± 0.41^{bE}	3.17 ± 0.41^{cE}	5 ± 0.0^{dD}
	7 (LL7)	MA	1 ± 0.0^{aD}	1 ± 0.0^{aD}	3.83 ± 0.41^{bE}	5 ± 0.0^{cF}	–	–
		MA + AP	1 ± 0.0^{aD}	1 ± 0.0^{aD}	2 ± 0.0^{bD}	5 ± 0.0^{cF}	–	–
	14 (LL14)	MA	1 ± 0.0^{aD}	1 ± 0.0^{aD}	2.83 ± 0.41^{bF}	4 ± 0.0^{cD}	–	–
		MA + AP	1 ± 0.0^{aD}	1 ± 0.0^{aD}	2 ± 0.0^{bD}	3 ± 0.0^{cG}	–	–
Off-odour[3]	No ageing (LL0)	MA	1 ± 0.0^{aw}	1 ± 0.0^{aw}	2 ± 0.0^{bw}	3 ± 0.0^{cw}	4 ± 0.0^{dw}	5 ± 0.0^{ew}
		MA + AP	1 ± 0.0^{aw}	1 ± 0.0^{aw}	1 ± 0.0^{ax}	1.17 ± 0.41^{ax}	3 ± 0.0^{bx}	4 ± 0.0^{cx}
	7 (LL7)	MA	1 ± 0.0^{aw}	2 ± 0.0^{bx}	3 ± 0.0^{cy}	5 ± 0.0^{dy}	–	–
		MA + AP	1 ± 0.0^{aw}	1 ± 0.0^{aw}	2 ± 0.0^{bw}	4.67 ± 0.52^{cy}	–	–
	14 (LL14)	MA	1 ± 0.0^{aw}	2 ± 0.0^{bx}	2.83 ± 0.41^{cz}	4 ± 0.0^{cz}	–	–
		MA + AP	1 ± 0.0^{aw}	1 ± 0.0^{aw}	1.83 ± 0.41^{bw}	3 ± 0.0^{cw}	–	–

A score ≤ 3 in any of the parameters denoted that meat was acceptable for sale or consumption

* MA = modified atmosphere (80% O_2 + 20% CO_2)

** AP = Active packaging (4% of oregano extract)

[a–e] Means of the same row (between days of display) with different letters differ significantly ($p < 0.05$)

[A–C] Means for red colour of the same column (between ageing and treatments) with different letters differ significantly ($p < 0.05$)

[D–G] Means for discoloration of the same column (between ageing and treatments) with different letters differ significantly ($p < 0.05$)

[w–z] Means for off-odour of the same column (between ageing and treatments) with different letters differ significantly ($p < 0.05$)

[1] Beef colour intensity was scored on a 5-point scale (1 = extremely brilliant, 5 = extremely faded)

[2] Discoloration: referred to percentage of discoloured surface: 1 = none, 2 = 0–10%, 3 = 11–20%, 4 = 21–60%, and 5 = 61–100%

[3] Off-odour: referred to the intensity of odours associated to meat oxidation: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme

acceptable for sale or consumption. At 13th day of display, the untreated steaks (LL0) had higher ($p < 0.05$) off-odour scores than treated steaks (3 vs. 1.17). Increased off-odour of untreated steaks was probably due to oxidative rancidity by increased lipid oxidation as shown by the TBARS values (Fig. 3), which could be easily perceived by the panellists. Meat surface discoloration occurred faster when steaks were packaged in MAP without active film than when steaks were packed with active film. Although few works are available on the effect of active film on colour change of beef, our results are similar to those reported by Park et al. (2010) and Camo et al. (2011) who studied, respectively, the effect of the antioxidant film and MAP on colour change of beefsteaks. The sensory results agree with colour data determined instrumentally, indicating that ageing for long times (14 days) affected positively colour stability as assessed by trained panellists compared with no aged beef (LL0) or beef aged for shorter times (LL7), particularly LL0 which had the lowest colour sensory scores (lowest colour stability) from 13th day of display (15 days *post-mortem*).

Recent study on the effect of ageing times on tenderness, lipid and colour stability of beefsteaks packaged under Hi-O_2 MA during simulated retail display, demonstrated that allowing ageing for 6 or 8 days improved colour stability, similar to those of ageing for a shorter time (3 days) or non-aged beef. Ageing times of 14 and 21 days resulted in a tenderness similar to that obtained by meat aged for 8 days, though colour and lipid stability were affected negatively and, consequently, the shelf life was reduced (Vitale et al. 2014). Contini et al. (2014) showed that antioxidant AP is effective in reducing the lipid oxidation of meat during storage and in maintaining its sensory characteristics, particularly tenderness and overall

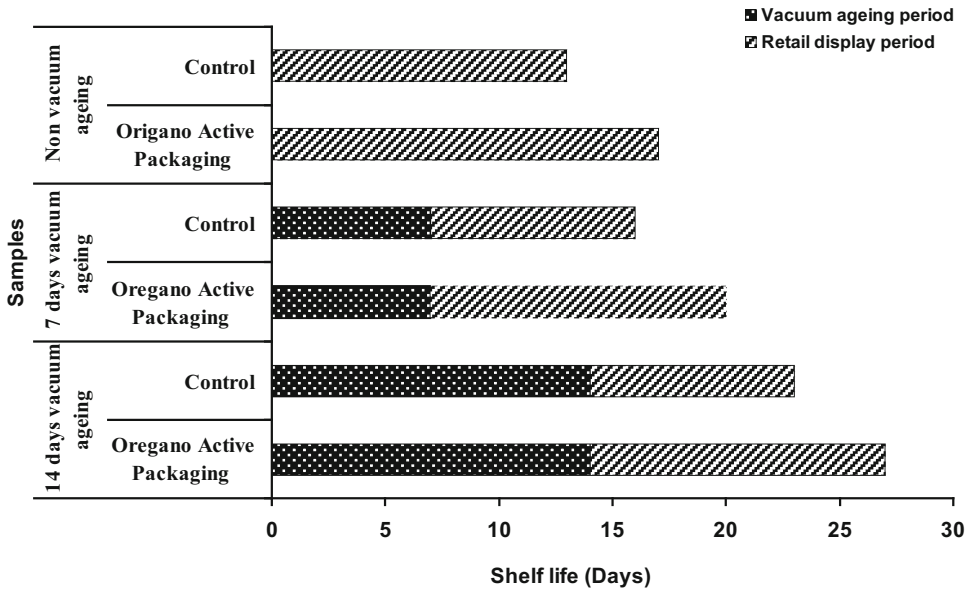

Fig. 5 Effect of vacuum-ageing times and packaging systems on the total retail shelf life (days) of beefsteaks during display at 1 ± 1 °C

acceptability. La Storia et al. (2012) found that the meat stored with AP scored the best rankings in the sensory evaluation.

Retail shelf life

To give for reader understanding scientific validity of our findings, the retail shelf life of steaks is determined based on sensory analysis (attribute scoring: A score ≤3 in any of the parameters denoted that meat was acceptable for sale or consumption), chemical (TBA-RS value: limit ~2 mg MDA/kg, and MetMb value: limit ~40%), physical (colour CIE a* value: limit ~10), and microbiological (psychrotrophic aerobic count: limit ~7 $\log_{10}$ cfu/cm^2) properties. We have defined the shelf life as "the period between animal slaughter and the retail sampling, during which the beefsteak is in a state of satisfactory quality in terms of chemical, physical, microbiological and sensory attributes". However, in commercial practice, this definition overlooks the fact that the consumer may store the product at home for some time before consumption.

The display of steaks packaged under Hi-O_2 MA demonstrated that oregano was needed for obtaining a significant increase of retail shelf life (Fig. 5). AP is an innovating technology that could improve quality of meat. Combination of AP with Hi-O_2 MA can provide improved methods of beef storage at retail allowing a prolonged shelf life of the products while preserving their quality. It was evident that the meat quality attributes during the display period depended on the ageing times and packaging systems. At the beginning of the display, the treated steaks (LL0) showed 4 additional days of shelf life compared to untreated steaks. However, steaks (LL7 and LL14), did not suffered a notable deterioration, as indicated by the high retail shelf life period that passed from 4 additional days (LL0 × AP) to 14 days (LL14 × AP). The different variables (sensory scores, CIE a*, MetMb, TBARS, total counts) differ amongst the different ageing times and the different packaging systems ($p < 0.05$). This clearly explains the difference in shelf life values between the different treatments. Maintaining temperatures at, or below, 7 °C throughout the cold chain from the abattoir to the retail customer is a requirement for the hygienic production and transportation of meat. In our experimental conditions, lower storage temperatures may be desirable to maximise the shelf life of beefsteaks.

Oregano extract has been shown to result in an extension of retail life by 1.6–5 days without compromising sensory quality (Camo et al. 2011). O'Sullivan et al. (2011) found that opening MAP retail packs 30 min before cooking has a benefit on the perceived sensory quality of the cooked meat. This appears to be of relevance for the meat industry since the fact of providing package guidance to optimise the flavour perceived by the consumer would improve the overall sensory quality of meat.

Conclusion

It can be concluded that purple colour together with a visible purge loss within the vacuum bag is unattractive to consumers, which explained why vacuum packaging is infrequently used for retail display. (2) Could supermarkets retail directly beefsteaks under Hi-O_2 when obtained from

whole muscle without ageing? It can be concluded that the retail shelf life of these cuts decreased subsequently. Beefsteaks from LL0 would result in rancid odour and discoloured after only 13 days of display. The Instrumental tenderness for these beefsteaks during display was lower if compared with beefsteaks from aged LL. However, AP does not affect meat tenderness, but affect positively the red colour, lipid oxidation, psychrotrophic aerobic count, and sensory properties. (3) Could supermarkets kept the whole muscle vacuum-ageing followed by cutting and subsequent retail display of cuts in Hi-O_2 MA? It can be concluded that the extended vacuum-ageing from 7 to 14 days at 1 ± 1 °C induced higher instrumental tenderness of beefsteaks, but did not affect negatively other quality parameters. Vacuum-ageing followed by Hi-O_2 MA increased retail shelf life of beefsteaks. Ageing for 14 days increased efficiently the retail shelf life of beefsteaks. AP plays an important role in keeping the meat quality, ensuring proper conditions during the display time. This technology could accelerate meat tenderization without any negative effect on commercial attributes, such as colour and odour of meat. Good correlation of quality parameters to sensory analysis attributes of packaged beefsteaks was observed. Overall, these data suggest that while storage and display propagate the deterioration of beefsteaks under Hi-O_2 MAP system, the stability of product can be enhanced with this technology.

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Compliance with ethical standards

Conflict of interest The authors declares that there is no conflict of interest.

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