JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Stabilization of Beef Meat by a New Active Packaging Containing Natural Antioxidants

CRISTINA NERÍN,*,† LAURA TOVAR,† DJAMEL DJENANE,§,# JAVIER CAMO,§ JESÚS SALAFRANCA,† JOSÉ ANTONIO BELTRÁN,§ AND PEDRO RONCALÉS§

Department of Analytical Chemistry, Aragon Institute of Engineering Research I3A, CPS-University of Zaragoza, Torres Quevedo Building, C/María de Luna 3, E-50018 Zaragoza, Spain, and Laboratory of Food Technology, Department of Animal Production and Food Science, Faculty of Veterinary Sciences, University of Zaragoza, C/Miguel Servet 177, E-50013 Zaragoza, Spain

A new antioxidant active packaging material for food has been designed and developed, consisting of a polypropylene film in which some natural antioxidants have been immobilized. The antioxidant properties of the new material have been tested by using both pure myoglobin and fresh beef steaks. Two different cell configurations (glass vial and Petri dish) and four different myoglobin concentrations—according to the common content of this compound in fresh meat and meat derivatives (1080, 1995, 3332, and 4414 μg g^{-1}, respectively)—have been evaluated in oxidation studies. Furthermore, three different concentrations of natural antioxidants in the film (designated as PR1, PR2, and PR3) were evaluated. Once myoglobin samples and the active films were introduced in the cell, they were exposed to cool white fluorescent light to accelerate oxidation for a period of time ranging from 5 to 30 days. Remaining myoglobin concentration was measured by molecular absorption UV−vis spectrophotometry at 409 nm. Organoleptic properties and color, texture, and physical characteristics of fresh meat packaged with the new active film have also been measured to evaluate the shelf life of the packaged meat. Results showed that, compared to normal polypropylene, the active film containing natural antioxidants efficiently enhanced the stability of both myoglobin and fresh meat against oxidation processes, thus being a promising way to extend the shelf life of fresh meat.

KEYWORDS: Active packaging; natural antioxidant; rosemary; polypropylene film; meat color; myoglobin

INTRODUCTION

During production, processing, distribution, and storage, food undergoes deterioration from chemical and microbiological processes. Typically, oxidative deterioration of meat and meat products is caused by degradation reactions of fats and pigments (*1*). Several factors affect consumer decisions to purchase meat, but probably the most important one is the quality perception (*1*, *2*). A bright red color for meat is perceived by consumers as being indicative of freshness, wholesomeness, and good eating quality (*3*). Meat color depends upon several individual factors and their interactions, the most representative ones being the animal species as well as the chemical stability of the meat pigment, myoglobin (*4*, *5*). Meat surface discoloration largely depends on the oxidation rate of the red oxymyoglobin onto metmyoglobin, which gives meat an unattractive brown color (*3*, *6*, *7*). This reaction generally proceeds in parallel to that of rancidity caused by fat degradation (*8*−*10*). The most important factors influencing pigment oxidation are temperature, relative humidity, oxygen partial pressure, light, and lipid oxidation (*11*−*15*).

Both traditional food packaging and producers resolve food oxidation by adding synthetic antioxidants to the food products during processing or at the end of the production (*16*, *17*), thus protecting lipids from oxidation and stabilizing oxymyoglobin (*18*). Although they have been widely used to extend the shelf life of meat derivatives, their addition to fresh meat is not permitted, as the food could no longer be considered to be a "fresh" product. Besides, the use of synthetic antioxidants is becoming increasingly unacceptable to consumers because of their health risks and toxicity (*19*−*22*). In this sense, a preferable option is the use of natural antioxidants (*23*−*25*).

Among the different possibilities, one of the most studied by a large number of authors is rosemary (*Rosmarinus officinalis* L.), which shows a very favorable effect on slowing oxidation in meat (*23*, *26*, *27*), because it provides a major source of natural antioxidants commercially used in foods (*28*−*34*).

Active packaging is an interesting alternative to the traditional use of the package above-described, one of the most innovative food packaging concepts being introduced to the continuous changes in current consumer demands and market trends (*35*).

* Corresponding author (e-mail cnerin@unizar.es; telephone +34-976-761873; fax +34-976-762388).
† Department of Analytical Chemistry, CPS-University of Zaragoza.
§ Laboratory of Food Technology, University of Zaragoza.
# Present address: Department of Microbiology and Biochemistry. Faculty of Biological and Agricultural Sciences. Mouloud Maameri University. 15000 Tizi-Ouzou, Algeria.

10.1021/jf060775c CCC: $33.50
Published on Web 09/02/2006

A clear proof of its promising possibilities is the development of specific legislation, such as EC Regulation 1935/2004 (*36*). Active packaging performs some desired role other than providing an inert barrier between the product and external environment. Among different active packaging concepts, the incorporation of active agents to the polymeric packaging is an exciting development, which could preserve the quality of packaged foods through an extended period of time, by releasing in a controlled way the active agents to the surface or the inner atmosphere of the product or just by scavenging radicals responsible for deleterious effects in food quality. In this work a new active packaging has been designed and developed, consisting of a polypropylene film—polyethylene is also being now considered for this purpose—in which a rosemary extract containing natural antioxidants is immobilized (*37*). In this way the active plastic layer acts as an antioxidant and protects the food inside the package.

The antioxidant performance of the innovative active film has been evaluated as a function of the active compound's concentration in the polymer and the contact system between the active polymer and the food by using pure myoglobin (*38*) as well as fresh beef meat. Therefore, the usefulness of the present work is obvious and finds immediate application in the food industry.

The objective of the present research was to determine the efficiency of the new active material on the inhibition of oxidation and consequently on the extension of the shelf life while maintaining the quality characteristics of meat and meat products. Then, the oxidation rate, color, and organoleptic properties of fresh meat have been evaluated in the presence of the new active film.

The design of the whole study and the analytical requirements to get an efficient new active packaging, as well as the results obtained, are shown and discussed.

MATERIALS AND METHODS

Chemicals. Horse heart myoglobin (90%, CAS Registry No. 00684-32-0), 2-thiobarbituric acid (TBA, 98%, CAS Registry No. 504-17-6), and trichloroacetic acid (99.0%, CAS Registry No. 76-03-9) were purchased from Aldrich (Madrid, Spain); malondialdehyde tetrabutylammonium salt (98%, CAS Registry No.l 100683-54-3) was from Fluka (Madrid, Spain); Amexol, a commercially available natural extract of rosemary (*R. officinalis* L.) registered like a spice, mainly for meat products, was provided by Laboratorios Amerex S.A. (Madrid, Spain); sodium chloride (99.5%, CAS Registry No. 7647-14-5), sodium sulfate (99%, CAS Registry No. 7757-82-6), and potassium hexacyanoferrate-(III) (99.0%, CAS Registry No. 13746-66-2) were from Panreac (Barcelona, Spain); quantitative filter paper MN 640W was from Macherey-Nagel GmbH & Co. KG (Düren, Germany); water was obtained from an Elix 5 water purification system from Millipore (Madrid, Spain).

Stock solutions of myoglobin were prepared by appropriate dilution of the pure compound in aqueous sodium chloride (0.9% w/v). Their concentrations were selected according to the common content of myoglobin in fresh meat and meat derivatives (*39*), thus ranging between 1080 and 4414 μg g^{-1}. All of the solutions used in this study have been systematically controlled by weight to improve accuracy.

Instruments. Myoglobin concentration was measured on a UV−vis spectrophotometer Unicam Helios λ (Cambridge, U.K.) with 10 mm light path Hellma quartz cuvettes (Müllheim, Germany). An orbital shaker P. Selecta Rotaterm (Barcelona, Spain) was used for homogenization of samples.

Color changes in the surface of beef steaks were measured by a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan) with D65 illuminant and 10° standard observer position by recording CIE L^* (lightness), a^* (redness), and b^* (yellowness) values.

Active Packaging Samples. Plastic films intended for use in active packaging were based on 20 μm thickness coextruded bioriented polypropylene film made by Poligal, S.A. (Narón, Spain), with a three-layer structure as follows: external layers (5%) of polypropylene−3% ethylene copolymer from BP (Lillo, Belgium) including silica at parts per million level as antiblocking agent and an inner layer (95%) of isotactic polypropylene homopolymer containing erucamide and quaternary amines at parts per million level from Repsol (Tarragona, Spain). Three different experimental samples designated PR1, PR2, and PR3, containing each one double concentration relative to the former Amexol (0.1−8.0% w/w in plastic film) were laboratory-produced by Artibal S.A. (Sabiñánigo, Spain) via an innovative process protected by European Patent EP1477519-A1. Polypropylene (PP) was also used as blank sample.

Myoglobin Oxidation: Test Cells. Two different cell configurations were used for the oxidation studies with myoglobin. One of them was a 20 mL glass vial, and the other one was a glass Petri dish (10 cm diameter). In the first case, 3 g of myoglobin solution and a 12 cm^2 (3 × 4 cm) piece of each plastic sample were placed in a 20 mL glass vial, without being in direct contact with each other in order to simulate real use conditions, because this is usually the case with meat products. Then, vials were hermetically closed with silicone/PTFE-lined septa and aluminum crimp caps and exposed to cool white fluorescent light to accelerate the oxidation process for different periods of time. In the Petri dish, the myoglobin solution was covered with a 144 cm^2 (12 × 12 cm) piece of the active film, avoiding direct contact between them, and the film was tightened with a nylon tie, sealed with high-vacuum grease (Dow Corning, Barcelona, Spain), and exposed to fluorescent light. In both cases (vial and Petri dish) air tightness was always checked just after its closure, and a set of samples was analyzed in quadruplicate after each established period of time.

Myoglobin Oxidation: Experimental Conditions. In glass vials, PP, PR1, PR2, and PR3 behavior was evaluated at four different myoglobin concentrations (1080, 1995, 3332, and 4414 μg g^{-1}) after 10, 15, 20, 25, and 30 storage days. Remaining myoglobin concentration was measured by taking 1 g of the myoglobin solutions from vials and diluting up to 10 g (or 20 g when required) with 0.9% (w/v) aqueous sodium chloride. Then, absorbance was read at 409 nm according to the method of Sykes et al. (*39*). All of the analyses were performed in triplicate.

In Petri dish series the main difference with the former tests was the higher surface of the myoglobin solution, which varies the active film/solution surface ratio, the rest of the conditions being unchanged. Two different experiments were carried out: (a) *without solid substrate*, where 3 g of a 1080 μg g^{-1} myoglobin solution was placed into each Petri dish and exposed to fluorescent light for 11 and 24 days, respectively, at room temperature, the analytical method being unchanged with respect to glass vials; (b) *with solid substrate*, where an inert solid was placed in the Petri dish and impregnated with the myoglobin solution. According to their a priori null or negligible chemical reactivity with other materials present in the cells, three different solids were sequentially used: sodium sulfate, sodium chloride, and filter paper. With sodium salts, 0.5 g of myoglobin solution (6000 μg g^{-1}) was added to 2 g of the inert substrate, and then cells were closed and exposed to fluorescent light from 11 to 20 days. The analytical method was slightly modified, as it was necessary to extract myoglobin from the substrate. As extraction agent, 0.9% (w/v) aqueous sodium chloride was used, and finally its absorbance was measured. All of the samples were analyzed in triplicate.

When using filter paper, the relationship between the myoglobin content in the surface of a fresh meat fillet and the involved area of the active film was calculated, resulting in ≈10 μg of myoglobin cm^{-2} of film. For this reason, the solution concentration used in this case was 820 μg g^{-1}, from which 0.3 g was added to filter paper. Samples were exposed to fluorescent light for 1, 2, 4, 8, 16, 24, 48, 96, and 192 h. After exposure time, myoglobin was extracted from filter paper, which was cut in small pieces and placed into glass test tubes, 3 mL of aqueous sodium chloride was added, and the mixture was homogenized with the orbital shaker at 230 rpm for 5 min at room temperature. The supernatant was removed and introduced in a test tube. The extraction process was repeated twice, and the aliquots of each sample

were collected together in the same tube. Finally, absorbance was read at 409 nm. Three replicates of each test were analyzed.

Preparation and Packaging of Beef Samples. The muscle longissimus dorsi was removed from a beef carcass at 48 h postslaughter and trimmed of external fat. Steaks (1.5 cm thick and ≈100 g in weight) were aseptically cut and exposed to air during 1 h at 1 °C to allow blooming. After blooming, each steak was placed on a polystyrene tray of size 15.5 × 21.5 × 2.5 cm. The trays with the steaks were introduced in pouches made of a polyethylene−polyamide laminate of 5−7 g/m^2/24 h water vapor permeability at 23 °C and 40−50 mL/m^2/24 h oxygen permeability at 23 °C (Sidlaw Packaging-Soplaril, Barcelona, Spain). An antioxidant active film piece of 11.6 × 16 cm, which totally covers all of the steak surface, was fixed between the upper part of the tray and the laminate within each pouch, avoiding direct contact with the steaks, because this situation is representative of most real use conditions in meat packagings. The pouches were filled with a gas mixture of 70% O_2 + 20% CO_2 + 10% N_2 supplied by Abelló Linde S.A. (Barcelona, Spain) and sealed. They were displayed at 2 ± 1 °C in a cabinet illuminated with a standard supermarket fluorescent light. Three packages were opened at 0, 5, 9, 12, and 14 days of display for analysis of surface color (CIE *a**), metmyoglobin percentage, and sensory evaluation of discoloration.

Color Measurement: CIE *a and Metmyoglobin Formation.** Measurements were carried out 30 min after package opening. Each value was the mean of 30 determinations, avoiding the zones with excessive fat to achieve measurements that were representative of the real lean color of beef steaks.

The metmyoglobin percentage of the total myoglobin perceptible at the beef steaks' surface was estimated spectrophotometrically according to the procedure of Stewart et al. (*40*), by measuring reflectance at 525 and 572 nm. The average value of the ratios of $(K/S)_{572}$ to $(K/S)_{525}$ at the beginning of the experiment was fixed as 0% metmyoglobin (MetMb). The value of 100% MetMb was obtained after oxidizing a sample in a 1% (w/v) solution of potassium hexacyanoferrate(III) (*41*). The obtained value for each steak was the average of 30 determinations.

Lipid Oxidation Analysis. Malondialdehyde is generated during the oxidative degradation of lipids. This compound reacts with 2-thiobarbituric acid, giving a red compound that is spectrophotometrically determined by measuring absorbance at 531 nm (*14*). Meat samples of 10 g were taken and mixed with 10% trichloroacetic acid using an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany). Samples were centrifuged at 2300 rpm for 30 min at 5 °C; supernatants were filtered through quantitative filter paper. Two milliliters of the filtrate was taken and mixed with 2 mL of TBA (20 mM); tubes were homogenized and incubated at 97 °C for 20 min and then cooled to room temperature, and the absorbance was measured at 531 nm against a reference blank containing the TBA reagent. The concentration of samples was calculated using a calibration curve, covering the range between 1 and 20 mmol L^{-1}. TBARS values were expressed as milligrams of malondialdehyde per kilogram of sample.

Sensory Discoloration Evaluation by a Trained Panel. Beef steaks were evaluated by a six-member trained panel (two females and four males, ranging in age from 25 to 55 years) of laboratory coworkers. They were habitual with meat and taste panels. Panelists received additional training prior to analysis according to the method described by Cross et al. (*42*). Surface discoloration of the beef steaks was evaluated 30 min after opening using a 5-point scale according to the method of Djenane et al. (*43*): 1 = none, 2 = 0−10%, 3 = 11−20%, 4 = 21−60%, and 5 = 61−100%.

The samples were presented in polystyrene trays identified with three-digit random numbers. Each panelist received one sample of each treatment randomly numbered and served. All samples were evaluated under cool white fluorescent lighting, positioned so that it provided 800 lx at the counter surface. Samples for evaluation were presented at room temperature (≈25 °C).

RESULTS AND DISCUSSION

Myoglobin Oxidation Test on Vials. First, the analytical characteristics of the spectrophotometric method for the determination of myoglobin were calculated. Data are shown in **Table 1**.

Table 1. Analytical Characteristics of Spectrophotometric Determination of Myoglobin

detection limit (μg g^{-1})	quantitation limit (μg g^{-1})	linear range (μg g^{-1})	linearity (R^2)	precision (% RSD, n = 3)
1.54	1.71	1.54 227.20	0.9992	1.6

Table 2. Myoglobin Concentration (Micrograms per Gram) in Vial Test (Confidence Intervals: 95%, n = 4)

time (days)	PP (1080)	PR1 (1080)	PR2 (1080)	PR3 (1080)
0	1080.1 ± 27.7	1080.1 ± 28.4	1080.1 ± 26.4	1080.1 ± 31.2
10	1040.9 ± 143.7	1043.5 ± 141.7	1044.2 ± 106.5	1033.1 ± 94.9
20	995.4 ± 77.7	1014.0 ± 95.2	1029.2 ± 36.7	1021.6 ± 136.6
25	973.4 ± 119.2	971.9 ± 52.7	973.6 ± 127.7	987.2 ± 127.6
30	976.6 ± 115.6	978.9 ± 128.2	946.9 ± 42.3	956.2 ± 72.7

time (days)	PP (1995)	PR1 (1995)	PR2 (1995)	PR3 (1995)
0	1994.9 ± 52.3	1994.9 ± 53.5	1994.9 ± 56.1	1994.9 ± 49.6
10	1680.7 ± 161.3	1866.9 ± 144.1	1853.3 ± 243.4	1966.4 ± 148.0
15	1490.6 ± 49.9	1675.2 ± 198.1	1677.7 ± 217.7	1629.5 ± 85.9
20	1263.4 ± 68.3	1414.6 ± 60.8	1663.8 ± 92.7	1729.0 ± 128.6
25	1308.9 ± 50.1	1375.7 ± 134.4	1375.5 ± 43.3	1579.8 ± 100.9
30	999.7 ± 34.7	968.5 ± 121.9	1041.4 ± 44.2	1057.7 ± 72.6

time (days)	PP (3332)	PR1 (3332)	PR2 (3332)	PR3 (3332)
0	3331.7 ± 84.4	3331.7 ± 89.7	3331.7 ± 81.5	3331.7 ± 87.8
10	3054.2 ± 229.5	3279.1 ± 267.1	3281.1 ± 133.0	3329.4 ± 172.0
15	2731.4 ± 329.0	3090.9 ± 123.2	3167.5 ± 390.4	3277.8 ± 363.2
20	2571.8 ± 276.9	2950.3 ± 298.3	3069.5 ± 271.3	3187.8 ± 457.2
25	2323.9 ± 161.7	2809.0 ± 334.9	3112.5 ± 318.8	3114.2 ± 98.6
30	2015.7 ± 101.1	2764.4 ± 317.5	3052.9 ± 309.1	3022.6 ± 437.1

time (days)	PP (4414)	PR1 (4414)	PR2 (4414)	PR3 (4414)
0	4413.7 ± 108.2	4413.7 ± 123.2	4413.7 ± 115.5	4413.7 ± 126.9
10	3963.5 ± 171.6	4132.6 ± 526.7	4215.1 ± 322.6	4235.4 ± 304.5
15	3626.3 ± 119.8	4028.4 ± 485.7	3591.5 ± 339.6	3928.2 ± 373.7
20	3444.5 ± 439.4	3647.5 ± 384.5	3555.7 ± 171.0	3670.0 ± 321.4
25	3031.4 ± 387.5	3086.5 ± 122.8	3153.6 ± 228.4	3192.0 ± 359.9
30	3054.3 ± 254.0	2859.2 ± 140.1	2913.5 ± 317.0	2643.8 ± 223.1

The results obtained with the first set of experiments showed that the presence of the active film containing natural antioxidants efficiently protected myoglobin against oxidation processes, although the myoglobin concentration had a critical influence. **Table 2** shows the remaining concentration of myoglobin after the exposure time of solutions to the active film in vials. Data shown correspond to the average of four independent determinations and include the confidence intervals.

As can be seen, there is a clear evidence of the influence of myoglobin concentration inside the cells. On the one hand, the samples containing the lowest concentration of myoglobin showed a faster oxidation rate, with a moderate delay in the presence of the active film. On the other hand, for the highest myoglobin concentration, the protection against oxidation was not efficient, the most likely reason being that its concentration was too high compared with the amount of antioxidant present in the active film strip. 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 active package is intended.

In greater detail, in the case of 1080 μg g^{-1}, no significant differences among PP and any of the active films were observed

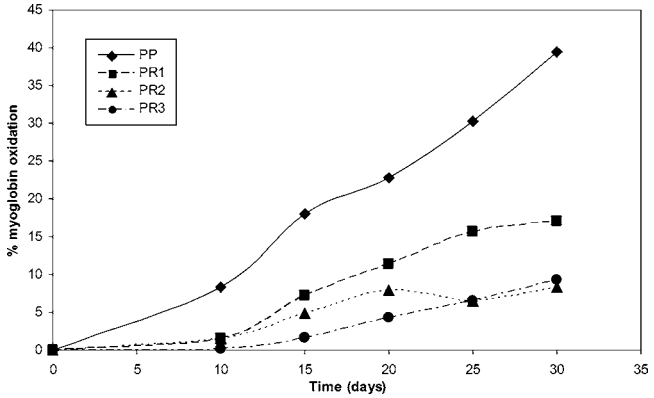

Figure 1. Effect of different concentrations of natural antioxidants in an active film on the oxidation processes using 3332 μg g^{-1} myoglobin solutions in vial test.

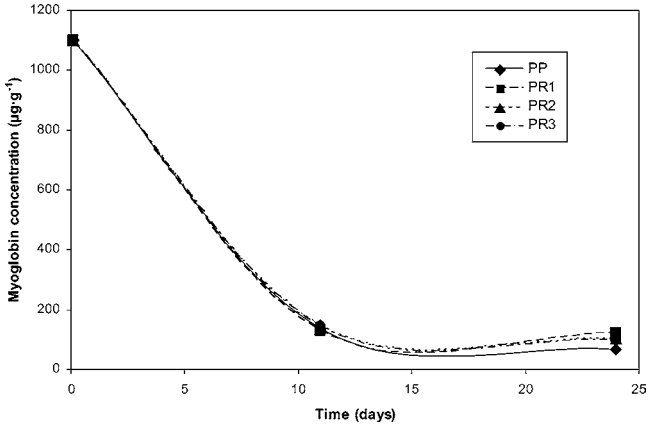

Figure 2. Effect of different concentrations of natural antioxidants in an active film on the oxidation process using a 1080 μg g^{-1} myoglobin solution in a Petri dish without substrate.

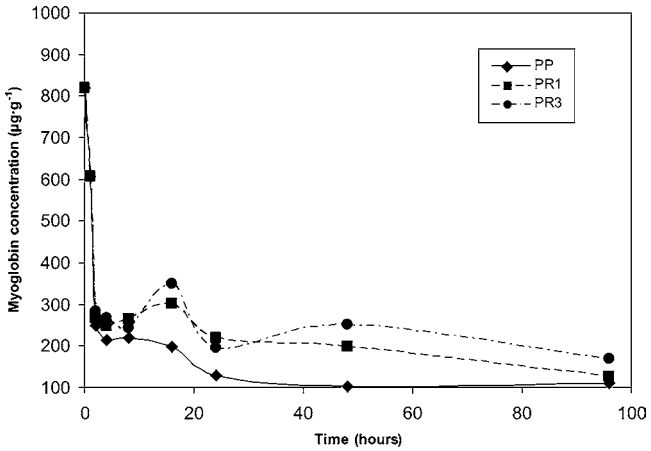

Figure 3. Effect of different concentrations of natural antioxidants in an active film on the oxidation processes using an 820 μg g^{-1} myoglobin solution in a Petri dish with filter paper as solid substrate.

in statistical terms (one-way ANOVA and two-sample *t* test assuming unequal variance or heteroscedastic *t* test, both at 95% confidence level).

Studies carried out with 1995 μg g^{-1} myoglobin solution showed a clear protection behavior against oxidation provided by the active films. The active film containing the highest concentration of natural antioxidants (PR3) was the less efficient against oxidation during all of the experimental series, followed by PR2 and PR1, respectively, with protection enhancement ranging from 11.1 to 36.9% in the first 20 days. This achievement does not agree with the expected behavior, very probably because the air-to-food ratio is ≈100 times higher than usual in a common packaged meat. Another explanation could be the pro-oxidant effect that some antioxidants show at high concentration versus oxidizable compounds, as mentioned by other authors (*44*, *45*). Nevertheless, the expected a priori behavior, namely, higher protection with higher concentration of active compounds in the film, was noted when 3332 μg g^{-1} myoglobin solution samples were analyzed, as can be seen in **Figure 1**. In this case, the protective effect ranged from 7.4% (PR1) to 51.5% (PR3) during the 30 days of the test. Oxidation percentage instead of remaining myoglobin concentration is plotted versus time to enhance visualization of the protective effect.

With respect to 4414 μg g^{-1}, myoglobin concentration remained almost constant during the first 10 days, followed by a faster oxidation rate between 10 and 30 days. No significant antioxidant protection (one-way ANOVA and paired *t* test, 95% confidence level) was observed, including several erratic results.

All of the samples were exposed to fluorescent light to accelerate the oxidation process. An undesired effect derived from the obtained data analysis was the evidence that after 20 days the myoglobin can decompose itself, this fact being the most likely reason of the unpredictable profile obtained in all of the experiments after 23 days under experimental conditions.

Myoglobin Oxidation in Petri Dishes. Samples without solid substrate showed a very high oxidation rate during the first 5 days, after which myoglobin concentration remained almost constant, as shown in **Figure 2**, where a slight protection effect was observed in all samples containing antioxidants. Nevertheless, no significant differences (95% confidence level) from the statistical point of view were observed by either ANOVA or *t* test.

When the experimental setup with inert solid substrates was considered, the obtained results showed different behavior, evidencing a clear influence of the solid substrate used. Due to the reduced solubility of sodium sulfate in aqueous sodium chloride, myoglobin was not quantitatively extracted from the solid, and anomalous results were obtained. In this sense, sodium chloride was the second choice because its solubility in water is high. The problem in this case was that myoglobin forms colloids, which disturbed the absorbance measurements. Finally, filter paper led to successful results. According to previous experimental setups, the faster oxidation rate took place at short periods of time, and because of this the time of exposure ranged in this case from 1 to 96 h, as **Figure 3** shows. As could be expected, a slight protection (not statistically significant, 95%) was achieved by using the active films in comparison with PP. The foreseeable behavior was in general observed, obtaining the maximum protective effect with the most concentrated active film (PR3). It must be mentioned that the intermediate concentration (PR2) was not tested in this study.

Fresh Beef Steaks Study: Metmyoglobin Formation. **Figure 4** shows the results of surface metmyoglobin formation, expressed as percentage of total myoglobin, on fresh beef steaks packaged in modified atmosphere and displayed in an illuminated cabinet at 2 ± 1 °C in the presence of active films with increasing concentrations of rosemary extract. Surface metmyoglobin increased steadily throughout display in control samples, reaching a value of ≈90% after 14 days. Most important is the fact that values >40% were obtained at day 12; this percentage has been demonstrated to be the limit between red and brown perception by sensory panelists (*3*). This behavior is in good agreement with the report of Djenane et al.

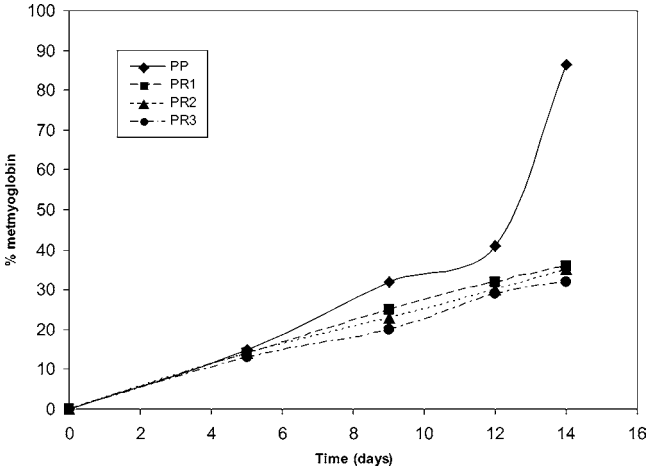

Figure 4. Surface metmyoglobin formation (percent on total myoglobin) on beef steaks packaged in modified atmosphere with active films and displayed at 2 ± 1 °C under conventional illumination.

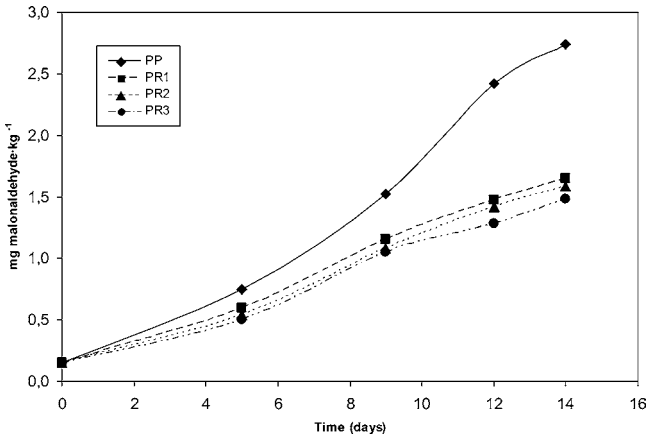

Figure 5. TBARS values on beef steaks packaged in modified atmosphere with active films and displayed at 2 ± 1 °C under conventional illumination.

(*43*), who compared myoglobin oxidation in beef steaks either displayed under light or stored in the dark.

Beef steaks displayed in packs containing the antioxidant films had significantly lower values than the control ($p < 0.05$) from the eighth day of display. In fact, they did not reach 40% metmyoglobin even at the end of the display period. However, although there was a trend to lower values with higher rosemary concentration in the active films, no significant differences ($p > 0.05$) were evident among them; that is, the effect was not dependent on the concentration used. This appeared to be somehow contradictory with the results of model experiments of this study, as well as with the results of Mielnik et al. (*46*), who reported that the antioxidant properties of several commercial rosemary extracts directly added to mechanically deboned turkey meat were concentration-dependent. This might be related to the fact that the inhibitory effect on lipid oxidation (TBARS formation, **Figure 5**) was not dependent on the concentration. It has been postulated that myoglobin oxidation is promoted by free radicals formed following lipid oxidation; therefore, the obtained results suggest that any of the three concentrations used reached a maximum inhibition of both lipid and myoglobin oxidation. In support of this hypothesis, the inhibitory effect of the active films with any of the rosemary concentrations was comparable to that of direct spraying of the rosemary extract (1 g L^{-1}) on beef surface (*3*).

Fresh Beef Steaks Study: Redness Index (CIE *a). Figure 6** shows the evolution of the CIE redness index *a** throughout

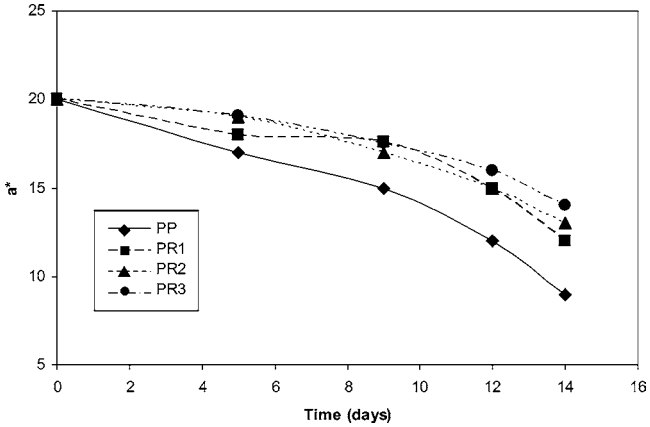

Figure 6. Surface CIE *a** values on beef steaks packaged in modified atmosphere with active films and displayed at 2 ± 1 °C under conventional illumination.

Table 3. Sensory Scores of Discoloration[a] of Beef Steaks Packaged in Modified Atmosphere with Active Films and Displayed at 2 ± 1 °C under Conventional Illumination

	days of storage				
sample	0	5	9	12	14
control	1.0 ± 0.0	1.0 ± 0.0	2.1 ± 0.4	3.2 ± 0.3	4.1 ± 0.6
PR1	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	2.2 ± 0.5	3.3 ± 0.4
PR2	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	3.1 ± 0.2
PR3	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	2.2 ± 0.5	2.8 ± 0.4

[a] Percent discolored surface: 1 = none, 2 = 0–10%, 3 = 11–20%, 4 = 21–60%, and 5 = 61–100%.

display. Values of *a** decreased in all samples, but steaks packaged with the active films showed a slower decrease than did PP used as control. The latter reached values below 10 after 12 days of display, whereas samples with the active films did not reach a value of 10 even at the end of the display period. Therefore, results were totally coincident with those of metmyoglobin formation, in agreement with previous reports (*1*, *3*, *18*, *23*). Again, the protective effect on color was independent of the rosemary concentration in the active films ($p > 0.05$).

Fresh Beef Steaks Study: Lipid Oxidation (TBARS Values). As previously mentioned, **Figure 5** shows the evolution of the TBARS values of beef steaks during display. Results demonstrated that lipid oxidation increased in all samples throughout display, but with a different rate ($p < 0.05$). Control steaks reached final values of ≈2.74 mg of malondialdehyde kg^{-1}, whereas steaks packaged with the active film had final values of ≈1.50 mg of malondialdehyde kg^{-1}. No significant differences ($p > 0.05$) were found among the latter samples by effect of the antioxidant extract concentration in the films. This was in good agreement with the results of metmyoglobin formation and color evolution, although the effect on lipid oxidation was more intense; in fact, packaging with the active films resulted in a 43% inhibition of malondialdehyde formation at the 14th day of display. Furthermore, the inhibitory effect on lipid oxidation of the active films appeared to be comparable to that of direct addition of Amexol to meat samples (*1*, *3*, *18*, *23*, *43*).

Fresh Beef Steaks Study: Sensory Evaluation of Discoloration. Sensory scores for discoloration are given in **Table 3**. It must be first emphasized that a score of 3, corresponding to an evident discoloration area of ≈10−20%, is the limit for acceptance, according to Djenane (*3*). Control steaks had values >3 after 12 days of display. Steaks packaged with PR1 and

PR2 active films had values >3 at the 14th day of display, whereas samples with the PR3 film did not reach this limit at the same time. However, differences among the three samples with active films were not statistically significant ($p > 0.05$). This was in good agreement with the results of metmyoglobin formation and a^* values previously presented and means that 14 days may be considered the average display life of steaks packaged in the active antioxidant system. Therefore, active packaging was able to extend the shelf life of beef packaged in modified atmosphere and displayed under conventional illumination by ≈2 days, that is, 17%.

SAFETY

Hemoglobin has no specific data known with regard to effects of overdose in humans; no dangerous characteristics are to be anticipated, with the exceptions of allergic reaction and sensitization. Malondialdehyde tetrabutylammonium salt is corrosive and causes burns; it is hygroscopic and photosensitive. Amexol is registered like a spice, and no special care is required. Trichloroacetic acid causes severe eye, skin, and digestive and respiratory tract burns and irritation, and contact with metals, oxidizing materials, and bases must be avoided; it is hygroscopic and very toxic to aquatic organisms and may cause long-term damage in the environment; its LD_{50} (oral, rat) is >90 mL kg^{-1}. TBA is incompatible with strong oxidizing agents and is an irritant; its LD_{50} (mouse, intraperitoneal) is 600 mg kg^{-1}. Potassium hexacyanoferrate(III) is incompatible with acids, oxidant agents, ammonia, fluorine, hydrogen halides, nitrides, and CrO_3; it causes skin and eye irritation; its fLD_{50} (rat, oral) is 1600 mg kg^{-1}, and its decomposition originates very toxic products including hydrocyanic gas, which induces cellular respiratory blockage with possible cardiovascular disorders, breathing difficulties, loss of consciousness, and death. Sodium chloride is incompatible with strong oxidizing agents and may cause eye irritation; its LD_{50} (rat, oral) is 3000 mg kg^{-1}. Sodium sulfate is hygroscopic and is incompatible with strong acids, aluminum, magnesium, and strong bases; its LD_{50} (mouse, oral) is 5989 mg kg^{-1}.

ACKNOWLEDGMENT

Artibal S.A. (Sabiñánigo, Spain) is acknowledged for providing the active film samples.

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Received for review March 20, 2006. Revised manuscript received July 6, 2006. Accepted July 9, 2006. This research has been financed by Project COOPERA−Innovaragón 2003−2004 from Gobierno de Aragón (Spain) and by Project CAL03-080 from the National Institute of Agrofood Research INIA (National Institute of Agricultural and Food Research and Technology, Spain).

JF060775C