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PII: S0309-1740(14)00042-4
DOI: doi: 10.1016/j.meatsci.2014.02.006
Reference: MESC 6365

To appear in: *Meat Science*

Received date: 23 August 2013
Revised date: 31 January 2014
Accepted date: 4 February 2014

Please cite this article as: Barbosa-Pereira, L., Aurrekoetxea, G.P., Angulo, I., Paseiro-Losada, P. & Cruz, J.M., Development of new active packaging films coated with natural phenolic compounds to improve the oxidative stability of beef, *Meat Science* (2014), doi: 10.1016/j.meatsci.2014.02.006

DEVELOPMENT OF NEW ACTIVE PACKAGING FILMS COATED WITH NATURAL PHENOLIC COMPOUNDS TO IMPROVE THE OXIDATIVE STABILITY OF BEEF

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Abstract

The aim to develop active packaging films containing natural antioxidants and to evaluate their capacity to enhance the oxidative stability of beef during refrigeration. The antioxidant activity of a natural extract obtained from a brewery residual waste was evaluated and compared with that of a commercial rosemary extract and two synthetic antioxidants (BHT and propyl gallate). Different concentrations of each antioxidant were also added directly to beef samples, resulting in a reduction in lipid oxidation of up to 70-80 % relative to the control. Active antioxidant films coated with PVPP-WS extract reduced lipid oxidation by up to 80 %, relative to the control, during cold storage. The use of active packaging films containing natural extracts could improve the oxidative stability of meat products and should therefore be of great interest in the food industry.

Keywords: Lipid oxidation, beef, natural antioxidants, coating, active packaging.

1. Introduction

Lipid peroxidation is a major cause of deterioration of meat quality during processing, distribution and refrigeration, thereby reducing shelf stability and acceptability. Lipid oxidation can produce changes in meat quality parameters, such as organoleptic properties and nutritional value, and it leads to the generation and accumulation of compounds that may pose risks to human health (Gray, Gomaa, & Buckley, 1996).

The oxidative stability of meat can be extended by using antioxidants and proper packaging materials (Zhou, Xu, & Liu, 2010).

Synthetic antioxidants have long been used in the food industry to prevent or minimize lipid oxidation in food products. Because of growing concerns about the potential health hazards associated with synthetic antioxidants commonly used in the food industry (e.g. BHT, BHA and PG) and increased consumer demand for natural products, there is a growing interest in the use of naturally occurring antioxidants for use in food processing (Shahidi, & Zhong, 2010).

Residual waste substances obtained from agro-industrial by-products offer a practical and economic source of potent antioxidants that could replace synthetic preservatives (Balasundram, Sundram, & Samman, 2006).

Polyvinylpolypyrrolidone washing solution (PVPP-WS) extract is a natural extract obtained from a brewery waste stream. During storage of beer, colloidal haze can develop as a result of the formation of complexes between polypeptides and polyphenols (Siebert, 1999). The negative impact of polyphenols on haze stability is minimized by using polyvinylpolypyrrolidone resin (PVPP) to stabilize beer and extend its shelf life. PVPP stabilization removes a substantial part of the polyphenols in beer (both haze and non-haze active polyphenols), which can be recovered from the PVPP by an alkaline treatment (Mitchell, Hong, May, Wright, & Bamforth 2005).

➢The high antioxidant activity of the extract has been demonstrated in different in vitro experiments (Barbosa-Pereira, Angulo, Paseiro-Losada, & Cruz, 2013a). The antioxidant activity of PVPP-WS extract is related to the high concentrations of phenolic compounds, such as flavonols (catechin, gallocatechin and epigallocatechin) and hydroxycinnamic and hydroxybenzoic acids (gallic acid, caffeic acid, p-coumaric acid and ferulic acid), which act as free radical acceptors and chain breakers and are therefore responsible for the high free radical scavenging activity of the extract (Barbosa-Pereira et al, 2013a; Barbosa-Pereira, Pocheville, Angulo, Paseiro-Losada, & Cruz, 2013b).

Natural extracts from many herbs and spices have been studied and used to extend the shelf life of foods (Balasundram et al., 2006; Fernández-Lopez, Zhi, Aleson-Carbonell, Pérez-Alvarez, & Kuri, 2005; Sánchez-Escalante, Djenane, Torrescano, Beltrán, Roncales, 2003). Rosemary extract has been shown to possess strong antioxidant activity because of the high contents of phenolic diterpenes (e.g. carnosic acid, carnosol and rosmanol) and phenolic acids (e.g. rosmarinic acid), which act as oxidative chain breakers via electron donation (Zheng, & Wang, 2001).

Rosemary extracts have been widely used in the food industry, and many authors have reported their effectiveness in reducing lipid oxidation in meat products. Nevertheless, most studies reported involve the direct addition of rosemary to the packaged food, and relatively few deal with the inclusion of rosemary in the packaging material (Sánchez-Escalante et al., 2003; McBride, Hogan, & Kerry, 2007; Nerín et al., 2006).

Active packaging is currently one of the most dynamic technologies used to preserve the quality of food via the release of active agents from the packaging film. Release of the active agents can be controlled over an extended period of time to maintain or

extend the quality and shelf-life of products, without the need for direct addition of any substances to the foodstuff (Zhou et al., 2010; Lee, 2010).

Some studies have evaluated how antioxidants (such as BHT, BHA, alpha-tocopherol and natural extracts) incorporated in packaging film migrate out of the film and retard lipid oxidation in the stored foodstuff (Moore, Han, Acton, Ogale, Barmore, &Dawson, 2000; Barbosa-Pereira et al., 2013c). The incorporation of rosemary extract in active packaging film has been described by Nerín et al. (2006), with promising results in relation to extending the shelf life of beef.

Another concept in active packaging is the addition of bioactive substances by a coating process. The development of coatings with antimicrobial capacity has been studied in relation to preservation of meat products (Zhou et al., 2010; Lee, 2010; Kerry, O'Grady, & Hogan, 2006; Bonilla, Atarés, Vargas, & Chiralt, 2012). Antioxidant additives can also be incorporated by coating them onto food packaging materials to control spoilage by oxidation and to preserve food quality (Vermeiren, Dvelieghere, van Beest, & de Kruijf, Debevere, 1999; Lee, An, Lee, Park, & Lee, 2003).

The aim of this study was to evaluate and compare the antioxidant effect of two natural extracts (PVPP-WS and rosemary extract) and two synthetic antioxidants (BHT and PG) on the oxidative stability of beef during cold storage. The natural extracts were used to coat active films with antioxidant properties. Finally, the effectiveness of the new antioxidant active packaging films in delaying lipid oxidation of beef during cold storage was evaluated in samples in which the packaging was with and without direct contact with the food sample.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT) (99.0%, CAS No. [128-37-0]), sodium azide (99.0%, CAS No. [26628-22-8]), 2-thiobarbituric acid (TBA) (≥98%, CAS No. [504-17-6]) and trichloroacetic acid (TCA) (puriss. p.a. 99.5%, CAS No. [76-03-9]) were purchased from Sigma-Aldrich (Steinheim, Germany). Propyl gallate (PG) (98%, CAS No. [121-79-9]), 1,1,3,3-tetraethoxypropane (TEP) (purum ≥95% (GC), CAS No. [122-31-6]) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (TECHN ≥85%, CAS No. [1898-66-4]) were supplied by Fluka Chemie AG (Buchs, Switzerland). Methanol (GC≥99.9%, CAS No. [67-56-1]), orthophosphoric acid (85% GR for analysis, CAS No. [7664-38-2]) and ethanol (absolute for analysis, CAS No. [64-17-5]) were provided by Merck (Darmstadt, Germany).

2.2. Natural extracts

PVPP-WS extract: this extract, which contains natural antioxidants, was obtained from a residual stream generated during the PVPP cleaning process in the brewing industry by a process described by Barbosa-Pereira et al., (2014). In beer production, a clarification step is essential to improve beer stability. As a result of this process, a PVPP sludge is obtained. The PVPP sludge loaded with polyphenolic compounds was washed with a NaOH solution (2% w/w) at room temperature. After the NaOH-PVPP was filtered, a clean PVPP resin and a PVPP washing solution (PVPP-WS) containing phenolic compounds were obtained.

The PVPP washing solution (PVPP-WS) was acidified to pH 1.5 with HCl (37%), and polyphenolic compounds were extracted with ethyl acetate by stirring for 30 minutes at room temperature. Organic and aqueous phases were separated by decantation and the organic phase was collected and evaporated to dryness at 40 ºC. Residual water was removed from the extract by lyophilisation to yield the dry crude extract (Barbosa-

Pereira, Angulo, Paseiro-Losada, & Cruz, 2013a). The content of phenolic compounds is shown in Table 1a (Barbosa-Pereira et al., 2013b).

Rosemary extract: commercial rosemary extract (MS-198-08 GIN 601331 Rosemary Extract P) was supplied in powder form by INGRENAT (Ingredientes naturales SL., Murcia, Spain). The composition of the rosemary extract is shown in Table 1b.

2.3. Instruments

The concentration of 2-thiobarbituric acid reactive substances (TBARs) and the absorbance in the DPPH method were determined in a dual-beam spectrophotometer (Uvikon XL, Bio-Tek Instruments, Milan, Italy).

Secondary oxidation compounds were extracted from beef by use of a T 25 ULTRA-TURRAX digital homogenizer and an MS2 Mini Vortex Shaker (both from IKA).

2.4. Antioxidant activity of natural extracts – DPPH method

The antioxidant activity of the natural extracts was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method described by von Gadow, Joubert, and Hansmann (1997), with slight modifications.

Standard solutions of the different antioxidants and of two synthetic compounds with antioxidant properties usually used in the food industry, PG and BHT, were prepared in methanol. An aliquot of antioxidant (50 µL) was added to 2 mL of DPPH radical methanolic solution (3.6×10^{-5} M), and the solution was shaken vigorously on a vortex shaker and left to stand in the dark, for 16 min at room temperature. The absorbance of the solution was then determined at 515 nm. All determinations were performed in triplicate. The decrease in absorbance was converted to DPPH inhibition percentage (IP), according to the following equation:

$$\mathrm{IP} = \frac{\mathrm{A_0} - \mathrm{A_{16}}}{\mathrm{A_0}} \times 100$$

where

A_0 is the absorbance of the control at initial time;

A_{16} is the absorbance of the sample after incubation for 16 minutes.

The concentration of antioxidant compound or natural extract required to achieve 50 % inhibition of the radical DPPH (Equivalent concentration=EC_{50}) was determined from the linear regression curve (i.e. the different concentrations of antioxidant [within the range 0.1 to 5 g L^{-1}] plotted against the percentage of free radical scavenging activity [IP %]).

2.5. Processing of active packaging samples by coating

Several formulations of the antioxidant compounds plus a low density polyethylene (LDPE) matrix were prepared. Active films were prepared with different antioxidant contents and percentages of compounds to study the antioxidant effectiveness of the polymer formulations in the beef. All coatings were applied with a rod of 40 microns (TR40) at a speed of 90 mm/sec. The final weight was similar to that used in the packaging industry (3.2 g m^{-2}). The formulations are shown in Table 2. The concentrations employed to formulate the active films (expressed in %) refer to the weight of polyvinylic resin used in the process of coating, so that the amount of natural extract used to produce these films is lower than in the assay to which the extract was directly added.

2.6. Evaluation of antioxidant activity in food

2.6.1. Beef samples

Freshly cut beef, of thickness 1 cm, was purchased in a local retail store. The meat was divided into small pieces of 50 ± 1 g and of surface area 8.5 × 8.5 cm. A small

amount of sodium azide (approx. 1mg) was applied to the surface of the beef samples to prevent microbial spoilage during storage.

2.6.2. Evaluation of antioxidant effect of direct addition of the antioxidant to beef

In this preliminary test, different concentrations of natural extracts (PVPP-WS and rosemary) and synthetic antioxidants (BHT and PG) were added to different pieces of beef. Two different concentrations of each antioxidant were used (0.05% and 3%, w/w). Addition of an intermediate concentration of the PVPP-WS extract (0.5%) to the surface of beef was also tested. The effect of the different antioxidants in the headspace of the container enclosing the beef sample was also tested, at the highest concentrations tested for direct contact with sample (3% for the synthetic antioxidants and rosemary extract and 0.5 and 3% for PVPP-WS extract). Samples without antioxidant compounds were used as controls.

The beef samples to which the different antioxidant compounds were added were stored at 4 ºC for 17 days. Samples were collected after 3, 7, 10, 14 and 17 days for TBARs determination.

2.6.3. Effectiveness of active packaging films on lipid stability of beef

Beef samples were wrapped in films coated with the natural extracts (PVPP-WS and rosemary extracts), which covered the entire meat surface. Three concentrations of extracts were used to coat the films (3, 10 and 20 %, w/w). The effect of the natural extracts was also evaluated in the headspace of the container enclosing the beef sample, using the highest concentration tested for direct contact (20 %). Two control tests were performed in this assay: a control with film but without the addition of natural extracts and a control of meat not covered with film. The meat samples were placed in a glass dish and stored at 4 °C for 16 days. Lipid oxidation of samples was evaluated periodically (0, 3, 6, 9, 13 and 16 days).

2.6.4. Lipid oxidation analysis – TBARs

Lipid oxidation of beef was determined by analysis of 2-thiobarbituric acid reactive substances (TBARs), according to a slightly modified version of the procedure described by Witte, Krause, and Bailey (1970).

Five grams of beef, to which BHT was added (0.2 mg mL^{-1}), were extracted with 45.5 mL of extraction solution containing 10 % trichloroacetic acid (TCA) in 0.02 M orthophosphoric acid to a final volume of 50.0 mL. Samples were mixed in an Ultra-Turrax homogenizer for 30 seconds and then filtered through a nylon membrane filter (0.45 µm). Five mL of extracted solution and 5 mL of TBA solution (0.02 M) were added to a test tube, which was then sealed with a screw cap, homogenized on a vortex shaker and then incubated in an oven at 100 °C for 40 minutes. Finally, the samples were cooled at room temperature, and the absorbance measured at 530 nm against a blank containing 5 mL of TCA solution and 5 mL of TBA reagent.

Malondialdehyde (MDA) is one of the most abundant relatively stable end products generated during secondary lipid oxidation. The concentration of MDA was calculated from the calibration curve obtained using 1,1,3,3-tetraethoxypropane (TEP), a precursor of MDA, at concentrations between 0 and 5 mg L^{-1}. Results were expressed as milligrams of malondialdehyde per kilogram of sample (mg MDA kg^{-1} of beef).

2.7. Statistical analysis

Data were analysed by analysis of variance (ANOVA) and significant differences were assessed by the Duncan's multiple range test at $P < 0.05$ using the IBM SPSS Statistics 20.0.0 statistical software package. Data are presented as the mean ± standard deviation.

3. Results and discussion

Initial screening was carried out to determine the potential of an extract obtained from the brewery waste stream as a natural antioxidant. This was done by use of an indirect method that determines the in vitro antioxidant ability of antioxidant compounds and natural extracts as free radical scavengers of the radical DPPH. Thereafter, a direct method (TBARs) was used to evaluate the effectiveness of the antioxidants in preventing oxidative degradation of lipids with the production of compounds such as conjugated hydroperoxides and aldehydes. A preliminary test was performed, prior to formulation of the active films, to evaluate the effectiveness of the natural extracts by adding different concentrations of each directly to the surface of the beef samples. A second test was carried out to evaluate how effective the active films were in preventing lipid oxidation in beef samples packed with and without direct contact with active films.

3.1. Antioxidant properties of natural antioxidants

The capacity of natural extracts and synthetic antioxidants to scavenge free radicals was determined using the radical DPPH. Synthetic antioxidants commonly used in the food industry, BHT and PG, were used as reference compounds for comparative purposes. The commercial rosemary extract was used as a reference natural extract, since it has been extensively studied and its antioxidant effectiveness demonstrated (Erkan, Ayranci, & Ayranci, 2008; Romano, Abadi, Repetto, Vojnov, & Moreno, 2009). The results of the comparisons are shown in Table 3. The EC_{50} value is reciprocally related to the antioxidant activity of the sample.

Natural extracts exhibited higher radical scavenging activity than the synthetic antioxidant BHT. The best results were obtained with PG (EC_{50} = 0.046 g L^{-1}), which is consistent with data obtained in other studies (Martinez-Tome, Jimenez, Ruggieri, Frega, Strabbioli, & Murcia, 2001). The natural extract obtained from the brewery

waste stream (PVPP-WS extract) exhibited a high level of antioxidant activity (EC_{50} = 0.346 g L^{-1}), which was seven times higher than that of BHT and six times higher than that of the rosemary extract. The EC_{50} value was lower for the rosemary extract (2.10 g L^{-1}) than for BHT, which is consistent with previously reported data (Romano et al., 2009; Almela, Sánchez-Muñoz, Fernández-López, Roca, & Rabe, 2006). However it should be noted that the rosemary extract used in this study is a commercial one that contains excipients and therefore is not a pure extract (Table 1b).

3.2. Antioxidant effectiveness of natural products added directly to the surface of beef

The antioxidant effect of the different antioxidants was determined in beef samples by the TBARs method. The data obtained for the reference antioxidants (synthetic antioxidants and rosemary extract) applied at 2 different concentrations (low: 0.05% and high: 3%) and the PVPP-WS extract applied at 3 concentrations (low: 0.05%, medium: 0.5% and high: 3%) to the surface of beef samples, and also tested in the headspace of the containers, are shown in Table 4. Lipid oxidation of beef samples increased during storage at 4ºC to different degrees and the control (without addition of antioxidant) yielded high TBARs values. All the antioxidants reduced lipid oxidation in beef relative to the control. At the lowest concentration tested (0.05 % (w/w) (L)), the highest effect was obtained with the PG, in which lipid oxidation was inhibited by up to 90% during entire storage time. At 17th day of storage the TBARs values were reduced by 93.2%. The other antioxidants reduced lipid oxidation relative to the control in the following order: PVPP-WS extract > rosemary extract > BHT, to a lower degree than PG. These results are consistent with those obtained withthe DPPH method that showed the same order of antioxidant activity for the antioxidants. The PVPP-WS and rosemary extracts displayed a similar effect against lipid oxidation with a reduction of around 40-

50% with respect to the control during storage. Similar results were obtained at the highest (3 % (w/w) (H)) and lowest 0.05 % (L) concentrations tested (see Table 4). PG was the most active compound and had a slightly greater effect than observed at 0.05% (w/w) (L), at the 10th day of storage there was a reduction of 99% with respect to the control and a reduction of 94.5 % in MDA formation after 17 days of cold storage. The reductions with BHT and rosemary extract were similar to those obtained when applied at the lower concentration with TBARs values 48% lower than the control at the 10th day of storage. At the 17th day, the reduction of TBARs values was of 7.4% for BHT and 49.3% for rosemary extract. The results are consistent with data obtained by other authors in beef products, i.e. lower TBARs values obtained with the rosemary extract than with BHT at the same concentration (Bozkurt, & Belibağlı, 2008; McBride et al., 2007, Trindade, Mancini-Filho, & Villavicencio, 2010). However, differences were greater with a natural extract of PVPP-WS, with almost a complete reduction in lipid oxidation (89% at the 10th day of storage), similar to PG.

The effect of the antioxidants in scavenging of free radicals present in the headspace of the containers enclosing the meat samples were also evaluated, in tests with the highest concentration of antioxidant used (3 % (w/w) (H-HS)). The system inside the container was prepared so that the packaging film was not in direct contact with the meat sample.showed No significant effect on lipid oxidation was observed in the beef samples. Rosemary has been reported to be a promising antioxidant extract with positive effects as a scavenger of free radicals in the vapour phase in model systems (Nerín, Tovar, & Salafranca, 2008). It is possible that the concentrationin the present study was not high enough in the vapour phase to have a positive effect in reducing lipid oxidation.

The effect of the PVPP-WS extract on the lipid oxidation of beef was tested at different concentrations when in direct contact with the sample. The addition of the natural extract containing phenolic compounds enhanced the oxidative stability of the beef when compared with the control. The results show that higher concentrations of polyphenols had greater antioxidative effects. Samples in which the lowest concentration of antioxidant extracts (0.05 %) was used are those in which lipid oxidation was least reduced, although decreases of around 40% were obtained. Lipid oxidation was inhibited in samples in which the highest concentrations of antioxidant extract (0.5 % and 3 %) were used. At the intermediate concentration (0.5 %) the TBARs values were approximately 80% lower than in control samples after 17 days of storage, and at the high concentration (3 %), the TBARs values were approximately 85% lower than in the control sample (during cold storage). The results showed that the a marked reduction in TBARs values could be achieved using the intermediate concentration (around 80-84%)..

3.3. Evaluation of antioxidant effect of the active films coated with natural extracts

When incorporated into the polymer, antioxidant compounds will not be directly available (as they were in the previous assay, in which the extracts were added directly to the surface of the beef samples). In this test, films coated with 3, 10 and 20 % of the natural extracts ($w_{extract}/w_{polymer}$) were evaluated. The effect of these films in the headspace of the container was again tested with films to which the highest concentration of extractwas added (20%). Because the main aim of this work was to develop active films containing natural antioxidants, the antioxidant activity of the natural extracts (rosemary and PWPP-WS extracts) was tested. The antioxidant effect of active films was determined, using the TBARs method, as their capacity to inhibit lipid

oxidation in beef samples during the storage. The results, expressed as mg MDA Kg^{-1} of sample, are shown in Table 5. Two control tests were performed, a test without film to evaluate the oxidation of beef (control without film), and a test with LDPE film without antioxidant extract added ,to evaluate the effect of the film on the surface of the meat sample (control film). LDPE films spiked with rosemary extract at different concentrations (3, 10 and 20 % (w/w) yielded films R 3 %, R 10 % and R 20 % respectively (Table 2). Film R 20 % HS is the same as film R 20 %, except that it is not in direct contact with the samples, exerting its effect in the headspace of the container. The same nomenclature (Table 2) was used with the films incorporating the PVPP-WS extract at the same concentrations (PVPP-WS 3 %, 10 %, 20 % and 20 % HS). All the active films tested exhibited an effect that protected the beef from oxidation (Table 5). The rosemary extract-coated active films in contact with meat samples yielded a reduction of approximately 50 % in the TBARs values. At the 9^{th} day of storage the reduction of TBARs values was around 60% with respect to the control. Slight differences were observed for the three concentrations used in the reduction of lipid oxidation relative to the controlt. This effect increased with the amount of rosemary extract used to develop the active film in the order 20% > 10% > 3%. The PVPP-WS extract was more effective in reducing lipid oxidation than the rosemary extract, with reductions in TBARs values higher than 60% at the 9^{th} day of storage. This was more evident at the highest concentration applied (20 %), which delayed the onset of oxidation until the ninth day, after which slight oxidation was observed, but which was much lower than in the other samples, with a reduction of 93 % with respect to the control without film. However, all the active films substantially reduced lipid oxidation in beef. The control film in contact with the surface reduces lipid oxidation because less sample surface is in contact with the oxygen in the headspace of the container when

compared with the control without film. This may explain the effect of the active films in the headspace of the container, which showed less reduction in lipid oxidation than the control film. There was some effect of these natural extracts in the headspace of the container that could be due to their capacity to act as scavengers of oxygen and/or radicals responsible for the oxidation process. These results are in agreement with results for polypropylene films spiked with rosemary extract at a concentration of 1% when stored for 29 days (Nerín et al., 2008). The PVPP-WS extract was also effective, although to a lesser extent (reduction of 6.6% with respect to control without film). However, inhibition of oxidation was less than that obtained with a simple polymer in close contact with the sample without active compounds. It appears that close contact between the active film and the meat sample is required for a large reduction in lipid oxidation in meat samples.

The active films developed in the present study have an inhibitory effect on lipid oxidation comparable to that observed after direct addition of the antioxidants to the meat sample section 3.2). The results obtained with the PVPP-WS 20 % film (produced with the PVPP-WS extract) showed the effectiveness of the natural extract obtained from the brewery industry and confirm the possible use of the extract as a food antioxidant, as recognised for rosemary extract (Bozkurt et al., 2008; Trindade et al., 2010; Fernandez-Lopez et al., 2005). The results obtained with the food samples are consistent with those obtained with the DPPH method (section 3.1), which confirms that these natural extracts display a radical scavenging capacity and that this may be one of the mechanisms of action whereby the extracts reduce lipid oxidation in meat samples.

4. Conclusions

The PVPP-WS extract displays a higher degree of radical scavenging activity than rosemary extract and BHT, a synthetic antioxidant commonly used in the food industry.

Natural extracts proved to be effective antioxidants and reduced lipid oxidation when added directly to the surface of refrigerated beef samples and therefore the direct contact with foodstuff is mandatory to obtain a significant reduction in lipid oxidation of beef samples. Therefore, it may be possible to use these natural extracts to replace synthetic antioxidants in food. The PVPP-WS extract displays a similar antioxidant effect to PG in reducing lipid oxidation, and was more effective than BHT and rosemary extract. Active films containing natural extracts were successfully produced, and the use of these active films coated with natural antioxidants enhanced oxidative stability of beef relative to that obtained with the film control during cold storage. The best results were obtained with the extract obtained from the brewery residual stream (PVPP-WS), which reduced lipid oxidation by up to 90 %. These active films appear promising for the development of active antioxidant packaging for use with meat products.

Acknowledgements

This work was financially supported by the Ministry of Education and Science of Spain - CENIT Program - National Strategic Consortium in Technical Research (CENIT-2007-2016-FUTURAL). The authors are grateful to the Mahou-San Miguel Group and FUTURAL project (Ingenio Program – CDTI).

The authors express their sincere thanks to Ms. Patricia Blanco Carro, Ms. Cristina Casal Romero and Mr. Gonzalo Hermelo Vidal for their excellent technical assistance.

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Table 1a. Total and individual phenolic compounds (mg g^{-1}) in the PVPP-WS extract

Phenolic compound (mg g^{-1})					
Benzoic acid derivates	***38.1***	***Cinnamic acids***	***59.2***	***Flavonols***	***48.9***
Gallic acid	20.1	Caffeic acid	14.1	Isoquercetin	28.3
Protocatechuic acid	15.5	p-coumaric acid	11.4	Quercetin	14.5
4-hydroxybenzoic acid	2.54	Ferulic acid	33.7	Kaempferol	6.06
Flavan-3-ols	***214***	***Flavanones***	***14.6***	***Acetophenone derivates***	***14.6***
Gallocatechin	132	Naringenin		Acetosyringone	
Epigallocatechin	30.6				
Catechin	29.9	***Flavones***	***8.10***	***Stilbenoids***	***5.35***
Epicatechin	21.4	Apigenin		Resveratrol	
Total phenolic compounds present in the PVPP-WS extract, 403 **mg g^{-1}**					

Table 1b. MS-198-08 GIN 601331 Phenolic composition of the rosemary extract.

Compound (mg g^{-1})	
Phenolic diterpenes	***101.32***
Carnosic acid	87.18
Carnosol	14.14
Excipient	
Maltodextrin	898.68

Table 2. Formulations of the LDPE coatings (% (w/w)).

Formulations	Natural extracts	
	Rosemary	**PVPP-WS**
Film Control	-	-
Film R 3%	3 %	-
Film R 10%	10 %	-
Film R 20%	20 %	-
Film PVPP-WS 3%	-	3 %
Film PVPP-WS 10%	-	10 %
Film PVPP-WS 20%	-	20 %

Table 3. Free radical scavenging activity – DPPH

Antioxidant	**EC_{50} (g L^{-1})***
BHT	2.64±0.025[a]
PG	0.046±0.002[b]
Rosemary extract	2.10±0.013[c]
PVPP-WS extract	0.346 ±0.007[d]

* Values are means of three determinations. Different letters mean statistically significant differences at $P < 0.05$.

Table 4. TBARs values in beef samples spiked with natural and synthetic antioxidants, stored at 4 ºC during 17 days.*

		TBARs (mg MDA kg^{-1} of beef)					
	Concentration	Storage time (days)					
Antioxidant	% (w/w)	0	3	7	10	14	17
Control	0	$0.280^{A}\pm0.021$	$5.37^{ab\ B}\pm0.761$	$9.82^{a\ C}\pm0.085$	$12.4^{ab\ CD}\pm0.182$	$13.9^{a\ D}\pm1.52$	$13.5^{ab\ D}\pm0.421$
BHT (L)	0.05	$0.280^{A}\pm0.021$	$3.25^{de\ B}\pm0.240$	$6.23^{b\ C}\pm0.324$	$8.31^{de\ D}\pm0.635$	$10.2^{bc\ E}\pm0.487$	$12.5^{abc\ F}\pm0.062$
BHT (H)	3	$0.280^{A}\pm0.021$	$3.41^{de\ B}\pm0.053$	$8.57^{c\ C}\pm0.327$	$7.97^{de\ C}\pm0.859$	$11.4^{b\ D}\pm0.500$	$12.5^{abc\ D}\pm0.1.77$
BHT (H-HS)	3 HS	$0.280^{A}\pm0.021$	$4.75^{b\ B}\pm0.939$	$8.72^{c\ C}\pm0.461$	$11.1^{ac\ D}\pm0.417$	$15.5^{de\ E}\pm0.684$	$12.6^{ac\ D}\pm1.04$
PG (L)	0.05	$0.280^{A}\pm0.021$	$0.483^{g\ B}\pm0.113$	$0.560^{d\ B}\pm0.087$	$0.207^{g\ A}\pm0.011$	$1.33^{g\ D}\pm0.064$	$0.914^{d\ C}\pm0.004$
PG (H)	3	$0.280^{AB}\pm0.021$	$0.506^{g\ BC}\pm0.020$	$0.\ 695^{d\ C}\pm0.090$	$0.190^{g\ A}\pm0.009$	$1.06^{g\ D}\pm0.239$	$0.716^{d\ C}\pm0.114$
PG (H-HS)	3 HS	$0.280^{A}\pm0.021$	$6.30^{c\ B}\pm0.324$	$9.29^{e\ C}\pm0.383$	$9.61^{cd\ C}\pm0.332$	$14.4^{a\ D}\pm1.03$	$15.8^{f\ E}\pm0.385$
Rosemary extract (L)	0.05	$0.280^{A}\pm0.021$	$3.62^{d\ B}\pm0.069$	$6.28^{b\ B}\pm0.025$	$7.03^{ef\ C}\pm2.02$	$9.08^{c\ D}\pm0.539$	$9.36^{g\ D}\pm1.00$
Rosemary extract (H)	3	$0.280^{A}\pm0.021$	$2.73^{ef\ B}\pm0.100$	$4.96^{f\ C}\pm0.444$	$6.54^{ef\ C}\pm0.1.48$	$9.50^{bc\ D}\pm0.451$	$6.84^{h\ C}\pm0.311$
Rosemary extract (H-HS)	3 HS	$0.280^{A}\pm0.021$	$5.94^{ac\ B}\pm0.131$	$11.0^{g\ C}\pm0.012$	$13.5^{b\ D}\pm0.415$	$18.3^{f\ E}\pm0.041$	$14.1^{b\ D}\pm0.364$
PVPP-WS extract (L)	0.05	$0.280^{A}\pm0.021$	$2.88^{def\ B}\pm0.018$	$5.36^{f\ C}\pm0.026$	$6.51^{ef\ C}\pm0.007$	$8.84^{c\ D}\pm1.36$	$8.40^{g\ E}\pm0.065$
PVPP-WS extract (M)	0.5	$0.280^{A}\pm0.021$	$2.37^{f\ BC}\pm0.072$	$2.28^{h\ BD}\pm0.082$	$2.01^{g\ D}\ \pm0.101$	$3.49^{h\ E}\pm0.075$	$2.59^{e\ C}\pm0.248$
PVPP-WS extract (H)	3	$0.280^{A}\pm0.021$	$2.41^{f\ B}\pm0.205$	$1.641^{i\ C}\pm0.145$	$1.36^{g\ C}\pm0.117$	$2.47^{gh\ B}\pm0.142$	$2.05^{de\ D}\pm0.042$
PVPP-WS extract (M-HS)	0.5 HS	$0.280^{A}\pm0.021$	$5.32^{ab\ B}\pm0.076$	$10.2^{a\ C}\pm0.299$	$12.7^{ab\ D}\pm0.338$	$16.8^{ef\ E}\pm0.716$	$15.8^{f\ E}\pm0.897$

PVPP-WS extract (H-HS)	3 HS	$0.280^{A}\pm0.021$	$5.22^{ab\ B}\pm0.281$	$10.14^{a\ C}\pm0.026$	$10.3^{cd\ CD}\pm1.25$	$15.7^{de\ E}\pm0.566$	$11.4^{c\ D}\pm0.258$

* Mean values (n=3) followed by different lower case letters, superindexes within the same time and capital letters superindexes within the same row denote statistically significant differences at $P < 0.05$.

Table 5. TBARs values obtained in beef samples packed with different active films and with controls, stored at 4 ºC during 16 days.*

		TBARs (mg MDA kg^{-1} of beef)					
		Storage time (days)					
Film name	Film description	0	3	6	9	13	16
Without film	Control without film	$0.576^{A}\pm0.077$	$6.01^{a\ B}\pm0.602$	$8.13^{a\ C}\pm0.352$	$10.7^{a\ D}\pm0.589$	$13.0^{a\ E}\pm1.11$	$12.1^{a\ F}\pm0.366$
Control	Control film	$0.576^{A}\pm0.077$	$3.50^{c\ B}\pm0.054$	$5.85^{b\ BC}\pm0.389$	$6.64^{b\ CD}\pm0.61$	$7.96^{b\ D}\pm0.41$	$9.25^{b\ D}\pm1.25$
R 3%	Rosemary 3%	$0.576^{A}\pm0.077$	$1.94^{d\ B}\pm0.152$	$2.44^{c\ C}\pm0.273$	$4.21^{c\ D}\pm0.271$	$5.51^{c\ E}\pm0.400$	$5.97^{c\ F}\pm0.761$
R 10%	Rosemary 10%	$0.576^{A}\pm0.077$	$0.941^{de\ A}\pm0.456$	$1.72^{cd\ B}\pm0.111$	$3.98^{c\ C}\pm1.38$	$4.78^{c\ D}\pm1.41$	$5.61^{c\ E}\pm0.351$
R 20%	Rosemary 20%	$0.576^{A}\pm0.077$	$1.05^{de\ A}\pm0.041$	$1.10^{cd\ A}\pm1.02$	$3.62^{c\ B}\pm0.602$	$4.92^{c\ C}\pm1.49$	$6.70^{c\ D}\pm0.238$
R 20% HS	Rosemary 20% HS	$0.576^{A}\pm0.077$	$4.36^{bc\ B}\pm0.844$	$7.03^{ab\ C}\pm1.58$	$7.60^{b\ C}\pm0.127$	$11.0^{d\ D}\pm1.10$	$12.3^{a\ D}\pm2.20$
PVPP-WS 3%	PVPP-WS 3%	$0.576^{A}\pm0.077$	$1.45^{de\ AB}\pm0.022$	$2.20^{c\ B}\pm0.220$	$4.15^{c\ C}\pm0.831$	$6.04^{c\ D}\pm0.688$	$6.43^{c\ D}\pm0.691$
PVPP-WS 10%	PVPP-WS 10%	$0.576^{A}\pm0.077$	$0.635^{e\ A}\pm0.024$	$1.42^{cd\ A}\pm0.511$	$4.09^{c\ B}\pm0.177$	$4.85^{c\ B}\pm0.580$	$5.35^{c\ B}\pm0.953$
PVPP-WS 20%	PVPP-WS 20%	$0.576^{A}\pm0.077$	$0.590^{e\ A}\pm0.017$	$0.630^{d\ A}\pm0.024$	$0.747^{d\ A}\pm0.294$	$2.48^{e\ B}\pm0.634$	$2.83^{d\ B}\pm0.550$
PVPP-WS 20% HS	PVPP-WS 20% HS	$0.576^{A}\pm0.077$	$4.94^{ab\ B}\pm0.310$	$7.87^{a\ C}\pm0.479$	$11.0^{a\ D}\pm0.020$	$11.0^{d\ D}\pm0.758$	$11.3^{ab\ D}\pm0.895$

* Mean values (n=3) followed by a different lower case letters, superindexes within the same time and capital letters superindexes within the same row denote statistically significant differences at $P < 0.05$.

Highlights

- Brewery residual stream extract displayed notable antioxidant properties
- Natural extract effectively reduced lipid oxidation when in direct contact with food
- Active packaging films enhanced oxidative stability of beef during refrigeration
- Active antioxidant films with natural antioxidants were developed by coating
- Natural extracts could be used in meat industry as natural antioxidants