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RESEARCH ARTICLE

Biogenic amines as freshness index of meat wrapped in a new active packaging system formulated with essential oils of *Rosmarinus officinalis*

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Abstract

Biogenic amines (BAs) are considered as an important indicator of freshness and quality of food. In this work, a new active packaging (AP) system for meat that, incorporating essential oil of *Rosmarinus officinalis* at 4% (w/w), inhibits the increase of BAs and the bacteria involved into their production was developed. BAs were analyzed by a SPE-HPLC-DAD method during the storage time of meat (0–7 d, 4 °C). Results showed that, in each monitored day, Biogenic Amine Index (BAI) expressed in mg kg^{-1} is lower in meat wrapped in AP with respect to that packed in polycoupled packaging (PP) (from 19% to 62%). A strong correlation was found between the inhibition of increase of putrescine, cadaverine, histamine and their bacteria producers such as *Enterobacteriaceae, Pseudomonas* spp. and *Brocothrix thermospacta.* By exploiting antimicrobial and antioxidant action of essential oil of *R. officinalis*, the new APs contribute to increase the shelf life of fresh meat and to preserve its important nutrients.

Keywords

Active packaging, bacteria, biogenic amines, essential oils

History

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Introduction

Biogenic amines (BAs) are basic nitrogenous compounds present in living organisms and, hence, in foods (Ruiz-Capillas & Jimenez-Colmenero, 2004). Based on their chemical structures, they are classified into three categories: (1) aromatic, as histamine, tryptamine, tyramine, 2-phenylethylamine, (2) aliphatic diamines, as cadaverine and putrescine and (3) aliphatic polyamines, as spermine and spermidine (Smith, 1980). BAs are produced in food of proteic origin by three possible mechanisms: (a) decarboxylation of amino acids (catalyzed by the decarboxylases enzymes contained in various microrganisms) (Karovicova & Kohaidová, 2005), (b) normal cellular metabolism of tissues (Bardócz, 1995), (c) amination or transamination of aldehydes and ketones (Karovicova & Kohaidová, 2005). BAs are important from a toxicological point of view, because the intake of food with high concentrations of BAs can cause migraine, headaches, gastric and intestinal problems, and pseudo-allergic responses. Histamine poisoning produces effects on cardiovascular system such as low blood pressure (Stratton et al., 1991), while tyramine causes allergic skin reactions and increasing of blood pressure by releasing noradrenaline from the sympathetic nervous system (Bardócz, 1995). Due to the importance of their toxicological aspects, BAs are considered as an important indicator of freshness and quality of food. Various indices can be used for evaluating the quality of fresh food, firstly Biogenic Amine Index (BAI) that is the sum (mg kg^{-1}) of putrescine, cadaverine, histamine and tyramine, then the ratio between spermidine and spermine (SPD/SPM) (Silva & Glória, 2002) and the total sum of the analyzed BAs. From a nutritional point of view, meat represents an excellent source of macro and micronutrients, but it is perishable and very susceptible to chemical and physical changes. The diverse nutrients composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens. In particular, both red meat (bovine) and white meat (chicken) are particularly susceptible to protein degradation that takes place under appropriate conditions, and increasing, in this way, the level of BAs during the time of storage (Vinci & Antonelli, 2002). Many *Enterobacteriaceae*, *Pseudomonas* spp., and certain *Lactobacillus*, *Enterococcus* and *Staphylococcus* spp. are particularly involved in BAs formation in meat (Galgano et al., 2009). It is therefore essential that adequate preservation technologies are applied to maintain meat safety and quality (Aymerich et al., 2008). The most investigated new preservation technologies for fresh meat are new packaging systems such as modified atmosphere packaging (MAP) and active packaging (AP), which use natural antimicrobials for biopreservation (Zhou et al., 2010). AP is the incorporation of specific compounds into packaging systems that interact with the contents environment to maintain or increase product quality and shelf life (Kerry et al., 2006). AP functions and technologies include moisture control, O_2 scavengers or absorbers, CO_2 controllers, odor controllers, antimicrobial and antioxidant agents, either natural or synthetic (Vermeiren et al., 2002). By preserving the important nutrients of fresh meat such as proteins, essential amino acids, vitamins of

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group B and others, AP technology can be useful for enhancing the intake of these nutrients too.

Natural extracts and essential oils of plants are extraordinary sources of bioactive molecules mixtures, having important antioxidant and antimicrobial actions. The use of natural extracts, such as essential oils and their constituents, categorized as flavorings by the European Union (EU) and generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA), represents an interesting approach in AP technology (Gutiérrez et al., 2009). Considerable results, especially from a biological point of view, have been carried out on the assessment of the antioxidant activity of many herbs and spices of *Labiatae* family such as rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*) and others (Camo et al., 2008) incorporated in an active packaging film. The inhibition of growth of some BAs in Turkish Sucuk by the action of the extracts of *Salvia officinalis* L. and *R. officinalis* L. was reported (Karabacak & Bozkurt, 2008). The reduced production of putrescine in Turkish dry-fermented sausage, by the action of Green tea extract and *Thymbra spicata* oil, was reported too (Bozkurt, 2006). To date, no reports were published about the inhibitory action that natural extracts, incorporated in an AP system, have on the growth of BAs in fresh meat. Due to their low volatility and the lack of chromophores, BAs in food are mostly analyzed by liquid chromatography (LC) with pre- and post-column derivatization and UV-visible or fluorescence detection, or by using liquid chromatography-mass spectrometry system (LC-MS) without derivatization step (Sagratini et al., 2012).

The aim of this work was to set up a new AP for fresh meat that, incorporating essential oils of *R. officinalis*, inhibits the increase of BAs and the bacteria (*Enterobacteriaceae*, *Pseudomonas* etc.) involved into their production. Moreover, the antioxidant action of new AP system was tested by monitoring hexanal as marker of lipidic oxidation of fresh meat.

Methods

Packaging and meat samples

The AP used in this study was realized by spraying essential oils on the internal surface of the packaging samples kindly provided by ESSEOQUATTRO s.p.a. industry (Carmignano di Brenta, Italy) and protected by European Patent EP 1584464 A1. The base packaging was a sheet comprising a first layer made of paper coupled to a second layer made of high density (HD) polyethylene (PE) with a third metallic layer fixed between them (Figure 1). Essential oil of *R. officinalis* was obtained directly from aerial part of the plant by hydrodistillation process using a Clevenger apparatus. Principal components of the obtained essential oil, as 1,8-cineol (36.2%), camphor (16.4%), α-pinene (11.7%), camphene (4.7%), β-pinene (2.2%), borneol (3.1%), were determined by GC-MS analysis. Afterwards, the obtained essential oil was diluted with ethanol (30% essential oil/70% ethanol) and then sprayed on the internal surface of packaging (polyethylene layer) at different concentrations ranging from 0.1% to 4% w/w. The meat was wrapped in the prepared AP and then inserted in a specific bag realized according to EP 1584464 A1 that was sealed for entrapping the volatiles of essential oil, without vacuum. For studying the capacity of the new AP to inhibit the growth of BAs and to enhance the shelf life of meat, experiments were realized by testing APs in comparison with the more common poly-coupled packagings (PP) for fresh meat having a cellulose layer coupled to a PE HD film.

Meat samples (chicken), purchased from a local butcher's shop of Camerino community, were bought and immediately brought into the laboratory. Each slice of meat was wrapped in AP and in PP, and stored in refrigerator at 4 °C. The experiments were performed in triplicate. The chemical and microbiological analysis were realized at day 0, 2, 4 and 7 by opening each day a different and whole packaging containing the slices of meat.

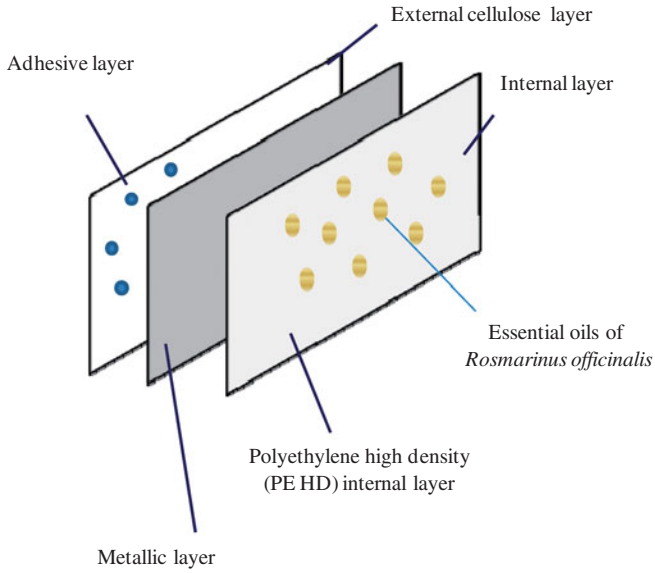

Figure 1. Technical structure of the used active packaging.

Materials and standards

BAs, namely spermine tetrahydrochloride (SPE, $C_{10}H_{26}N_4 \cdot 4HCl$, >98%, CAS No. 306-67-2), spermidine trihydrochloride (SPD, $C_7H_{17}N_3$ 3HCl, >98%, CAS No. 334-50-9), cadaverine dihydrochloride (CAD, $C_5H_{14}N_2$ 2HCl, >98%, CAS No. 1476-39-7), putrescine dihydrochloride (PUT, $C_4H_{12}N_2$ 2HCl, >98%, CAS No. 333-93-7), histamine dihydrochloride (HIS, $C_5H_9N_3 \cdot 2HCl$, >99%, CAS No. 56-92-8), tyramine hydrochloride (TYR, $C_8H_{11}NO \cdot HCl$, >98%, CAS No. 60-19-5), 2-phenylethylamine hydrochloride (PHE, $C_8H_{11}N \cdot HCl$, >98%, CAS No. 156-28-5), tryptamine hydrochloride (TRY, $C_{10}H_{12}N_2 \cdot HCl$, >99%, CAS No. 343-94-2), 1,7-diaminoheptane (internal standard) (I.S., $C_7H_{18}N_2$, >98%, CAS No. 646-19-5) and dansyl chloride ($C_{12}H_{12}ClNO_2S$, >99%, CAS No. 605-65-2) were supplied by Sigma-Aldrich (Milano, Italy). Hexanal and 2-methyl pentanal (internal standard) were supplied by Sigma-Aldrich (Milano, Italy). Trichloroacetic acid (TCA) for analysis was from Sigma-Aldrich (Milano, Italy). Individual stock solutions of BAs were prepared by dissolving 10 mg of each compound in 10 mL of HCl 0.1 M (Merck, Darmstadt, Germany) and stored in glass-stopper bottles at 4 °C. Individual stock solutions of hexanal and 2-methyl pentanal were prepared by dissolving 100 mg of each compound in 100 mL of water. Standard working solutions, at various concentrations, were daily prepared by appropriate dilution of different aliquots of the stock solutions with water. HPLC-grade methanol and sodium sulphate >99% were supplied by Sigma-Aldrich (Milano, Italy). Deionized water (<8 MΩ cm resistivity) was obtained from the Milli-Q SP Reagent Water System (Millipore, Bedford, MA). All the solvents and solutions were filtered through a 0.45-µm PTFE filter from Supelco (Bellefonte, PA) before use. HPLC-grade acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Cartridges Discovery SPE DSC-18 Silica Tube 6 mL, 1 g, were purchased from Supelco (Bellefonte, PA). Stomacher Easy Mix, model SC20, was supplied from Aes Lab (Cedex, France). The fibers used for solid phase microextraction (SPME) analysis (Supelco, Bellofonte, PA) were Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) 65 µm.

Chemical analyses

Analysis of biogenic amines

The analytical procedure is based on a previously published method, slightly modified (Sagratini et al., 2012). Each slice of meat (chicken) was grinded with a blender, then 5 g of sample were homogenized for two minutes by using an Ultra-Turrax S 18N-10 G (IKA-Werke Gmbh & Co., Staufen, Germany) with 25 mL of 5% TCA, centrifuged at 5000 rpm for 10 min. An aliquot of supernatant acid solution (1 mL) was mixed with 300 µL of a saturated $NaHCO_3$ solution, 200 µL of NaOH 2 M and 2 mL of dansyl chloride (10 mg/mL acetone). The dansylation reaction was conducted in the dark at 45 °C for 45 min. After that, the residual dansyl chloride was destroyed by adding 100 µL of 28% NH_4OH. The mixture was evaporated to 1.5 mL under flow of N_2.

The aqueous residue was purified on a Strata C_{18}-E cartridge (6 mL, 1 g). The cartridge was activated with 2×2 mL of acetonitrile, conditioned with 2×2 mL of water, then the aqueous residue was loaded onto the cartridge at a flow rate lower than 0.5 mL min^{-1}; the cartridge was then washed with 2×2 mL of water, thoroughly dried, and then elution was performed using 4 mL of acetonitrile. The eluate was filtered on 0.45-µm PTFE filter and injected in HPLC-DAD.

HPLC analyses

HPLC/DAD studies were performed using a Hewlett Packard (Palo Alto, CA) HP-1090 Series II, made of an autosampler, a binary solvent pump, and a diode-array detector (DAD). The separation was performed on a C_{18} Gemini column (5 µm, 250×3 mm) equipped with a Gemini C_{18} guard column (5 mm, 4×3 mm), both from Phenomenex (Torrance, CA). The column was thermostated at 40 °C. The peaks were detected at 254 nm. The mobile phase for the HPLC-DAD analysis was: water (A) and methanol/acetonitrile (70:30, v/v) (B), at a flow rate of 0.5 mL min^{-1}. The gradient program was: 0 min 60% B, 0–10 min 70% B, 10–20 min 90% B, 20–26 min 100% B, 26–30 min 100% B. Finally, phase B was decreased to 60% from 30 to 35 min and held at 60% until the end of the run at 50 min. The injection volume was 20 µL.

pH

The determination of pH was assessed by using a pH meter (Jennway 3510, Dunmow, UK) equipped with a probe for solids (Hamilton Double pore S7/BNC Plug, Bonaduz, Switzerland).

Analysis of hexanal

The procedure for analyzing hexanal was slightly modified by that reported by Giuffrida et al. (2005). Each slice of meat (chicken) was grinded with a blender, then 5 g of sample were homogenized for 2 min by using an Ultra-Turrax system with 15 mL of water, then 20 mL of water were added to the extract and finally centrifuged at 5000 rpm for 10 min. An aliquot of supernatant solution (0.5 mL) was placed in 10 mL vials tightly capped with a PTFE-silicon septum, then 0.25 g of NaCl and 1.5 mL of water were added, and finally subjected to SPME analysis at 40 °C. After 10 min of conditioning with stirring at 1300 rpm, the SPME fiber was exposed to the sample headspace for 20 min, removed and inserted into the GC injection port. By using a 3 min desorption time, the extract was directly transferred to the analytical column for analysis. The SPME fibers were cleaned to prevent cross-contamination by inserting the fiber into the auxiliary injection port at 230 °C for 30 min and re-used. 2-Methyl pentanal was included as internal standard during homogenization process at concentration of 100 µg kg^{-1}

GC-MS analyses

A gas chromatograph/mass selective detector (GC/MSD) (Agilent, Santa Clara, CA, Agilent 6890N with Agilent 5973N) equipped with a DB-WAX column (30 m, 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific, Folsom, CA) was used. The system was run through an AgilentChem workstation, applying a flow rate (He) of 1 mL min^{-1}, split mode, and using an injector temperature of 230 °C for PDMS/DVB fiber. The temperature program was: 35 °C for 5 min, then from 35 °C to 230 °C at 15 °C min^{-1}, then 230 °C for 5 min. Data were acquired in the electron impact (EI) mode with an ionization voltage of 70 eV, using selected ion monitoring (SIM mode). The selected ions were: hexanal (*m/z* 41, 44, 56), 2-methyl pentanal (I.S., *m/z* 43, 58). Percent values were the mean of three replicates for each sample, analyzing data with MSD ChemStation software (Agilent, Version G1701DA D.01.00).

Microbiological analyses

Ten grams of each slice of meat (chicken) from AP and PP were homogenized in 90 mL of peptone solution (0.1%) in a Stomacher – Easy MIX (AES Laboratory, Bruz, France). A series of ten-fold dilutions (10^{-2} to 10^{-10}) was prepared and a given amount of each dilution was spread on several specific media: Plante Count Agar (PCA, Oxoid), Violet Red Bile Glucose Agar (VRBGA, Oxoid), Agar Base with selective supplements CFC, (PAB, Oxoid) and Streptomycin Thallous Acetate Actidione Agar (STAA, Oxoid) supplemented with streptomycin sulphate and thallous acetate for counting of mesophilics bacteria, *Enterobacteriaceae*, *Pseudomonas* spp. and *Brochotrix thermosphacta*, respectively.

The bacterial counts were made after 24–48 h of aerobic incubation at 25 °C both for *Pseudomonas* spp. and *B. thermosphacta*, after 24–48 h of aerobic incubation at 37 °C both for mesophilic bacteria and for *Enterobacteriaceae*. Gram stain, morphological and biochemical analysis were performed on selected colonies isolated from each sample in order to confirm the strain identity.

Statistical analysis

All the microbiological experiments were carried out in triplicate, and each sample was analyzed in duplicate. The results are expressed as mean ± standard deviation (SD). Statistical significance of the differences between the meat wrapped in AP and that packed in PP as control was analyzed using Student's *t*-test. Differences were considered significant at the $t < 0.05$ level.

Results and discussions

Biogenic amines

In this study, eight BAs (the most common and analyzed) were monitored in meat samples: tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine. BAs were analyzed by a SPE-HPLC-DAD method that uses the derivatization process with dansyl chloride and internal standard (1,7-diaminoheptane). Figure 2(A) shows an overlapping of two chromatograms referred to a chicken meat sample analyzed at day 0 and to a BAs mix standard at concentration of 5 mg L^{-1}. As it is possible to observe, from a chromatographic point of view, the analytes (BAs peaks 1–9) were perfectly separated and this permits to perform a good quantification at low level of concentration of BAs. The insertion in the analytical method of a SPE C_{18} purification step was fundamental to remove interfering polar analytes and to obtain cleaner extracts that gave good HPLC-DAD chromatograms.

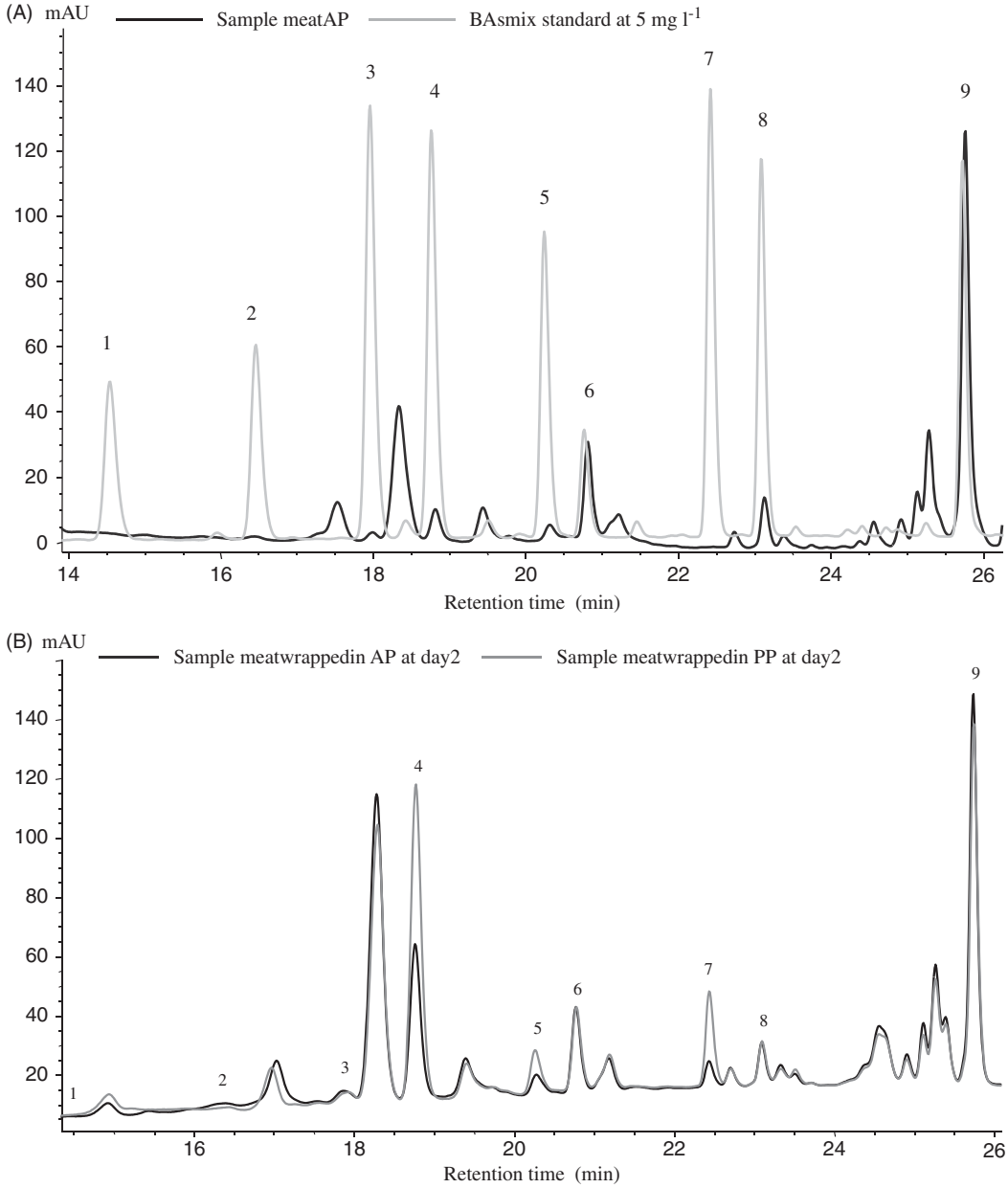

Figure 2. Overlapping of (A) two chromatograms referred to a meat (chicken) sample wrapped in an AP analyzed at day 0 and to a BAs mix standard at concentration of 5 mg L^{-1} (B) two chromatograms referred to a sample (chicken) meat wrapped in AP and to another wrapped in PC both analyzed at day 2. Legend: (1) tryptamine (TRY), (2) 2-phenylethylamine (PHE), (3) putrescine (PUT), (4) cadaverine (CAD), (5) histamine (HIS), (6) 1,7-diaminoheptane (I.S.), (7) tyramine (TYR), (8) spermidine (SPD), (9) spermine (SPM).

An unknown peak, that increases during the storage time of meat wrapped both in AP and in PP, was observed at 18.5 min between the peaks of cadaverine and putrescine. By analyzing the UV-Vis spectrum of the compound, which showed a maximum of absorbance at 330 nm, a dansylated product was hypothesized.

Figure 2(B) reports an overlapping of two chromatograms referred to a sample meat wrapped in AP and to another wrapped in PP packaging, both analyzed at day 2. It is possible to observe that the chromatographic profile of both samples is similar, but the meat wrapped in AP shows a content of BAs lower than that packed in PP. In particular, this fact is evident for cadaverine (peak 4), histamine (peak 5) and tyramine (peak 7). Concentration of each BA is reported in Table 1.

The performances of the new active packaging system, enriched with essential oils of *R. officinalis* at 4% w/w, were tested by monitoring its capacity to inhibit the growth of BAs in meat during time, and increasing in this way the shelf life of wrapped food. Three indices relative to BAs were considered: (a) Biogenic Amine Index (BAI) that is the sum (mg kg^{-1}) of putrescine, cadaverine, histamine and tyramine, (b) the ratio between the content of spermidine and spermine (SPD/SPM), (c) the total content of the monitored BAs. Figure 3 reports the shelf life study of chicken meat packed in AP in comparison with chicken meat packed in a classic PP, taking into account the three BAs indices described above. BAI and total BAs indices were significant in each monitored day ($t < 0.01$), while the ratio SPD/SPM index was significant ($t < 0.05$) in each monitored day except day 2 (Figure 3).

Concerning BAI index (Figure 3A), in each monitored day the sum of putrescine, cadaverine, histamine and tyramine expressed in mg kg^{-1} is lower in meat wrapped in AP with respect to that packed in PP. In particular, the differences in limiting the increase of BAIs in favor of AP ranged from 19% (at day 7) to 62% (at day 2). Concerning SPD/SPM index (Figure 3B), after 2 d

Table 1. Level of BAs expressed in mg kg^{-1} (mean ± SD) and pH in chicken meat wrapped in active packaging (AP) and polycoupled packaging (PP) during shelf life study. Each experiment was realized in triplicate ($n = 3$) with a RSD <9%.

	Storage time (days) and packaging materials						
		2		4		7	
Biogenic amines	0	AP	PP	AP	PP	AP	PP
TRY	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
PHE	0.25 ± 0.01	0.70 ± 0.01	0.10 ± 0.01	6.71 ± 0.35**	9.50 ± 0.49**	33.40 ± 2.94*	17.20 ± 0.06*
PUT	0.73 ± 0.02	1.50 ± 0.01	3.30 ± 0.03	7.10 ± 0.80**	26.90 ± 0.46**	33.10 ± 0.30**	62.31 ± 0.48**
CAD	14.02 ± 0.01	50.20 ± 3.65**	118.50 ± 7.40**	300.50 ± 1.45**	388.21 ± 10.98**	513.90 ± 13.94**	652.80 ± 3.06**
HIS	6.58 ± 0.01	9.30 ± 0.41**	24.30 ± 1.70**	48.80 ± 0.57**	117.20 ± 4.56**	86.51 ± 0.10**	144.10 ± 0.90**
TYR	0.00 ± 0.00	7.01 ± 0.14**	29.20 ± 2.56**	182.50 ± 1.08*	177.21 ± 4.44*	269.90 ± 6.11*	256.20 ± 1.27*
SPD	17.60 ± 0.20	17.00 ± 1.02	18.20 ± 0.49	17.11 ± 0.06	16.50 ± 0.64	17.60 ± 0.60	16.31 ± 0.52
SPM	109.16 ± 1.20	116.70 ± 10.14	120.82 ± 1.97	121.20 ± 1.69**	107.22 ± 0.30**	111.21 ± 0.59**	84.30 ± 0.22**
pH	5.94 ± 0.01	5.99 ± 0.01	6.01 ± 0.03	6.03 ± 0.01**	6.12 ± 0.01**	6.12 ± 0.02**	6.53 ± 0.02**

TRY (tryptamine), PHE (2-phenylethylamine), PUT (putrescine), CAD (cadaverine), HIS (histamine), TYR (tyramine), SPD (spermidine), SPE (spermine). *: data were significant for $t < 0.05$; **: data were significant for $t < 0.01$.

there is slight decrease for both AP and PP meats, but in each monitored day this index is lower for AP meat with respect to PP meat. The ratio SPD/SPM could be considered one of the most important index for evaluating the quality of meat, especially for chicken meat. It is well known that different microorganisms have the ability to produce different amounts of specific BAs (Shalaby, 1996); in this way, the level and the type of BAs are influenced by the specific bacteria flora that lives in food, influencing the BAs quality indices as BAI. Instead, the levels of polyamines (spermine and spermidine) are affected by microbial growth, but are independent of the type of flora (Hernández-Jover et al., 1997). Taking into account the total content of the analyzed BAs (Figure 3C), the inhibitory action of AP enriched with essential oil of *R. officinalis* at 4% w/w on the growth of BAs is confirmed. In fact, in each monitored day, the total content of BAs is lower in meat wrapped in AP with respect to that packed in PP, where the differences ranged from 14% (day 7) to 36% (day 2).

According to literature (Vinci & Antonelli, 2002), in red meat the BAs increases slower than in white meat and the total concentration of BAs was lower during time study. Chicken muscles contain shorter fibers that can be easily attacked by proteolytic enzymes, by producing in this way an higher quantity of BAs with respect to red meat. Preliminary experiments were carried out by using lower concentration of essential oil of *R. officinalis*, i.e. 0.1%, 1%, 2%, but not evident differences were not obtained both in terms of BAs and microorganisms.

Concerning the concentration of single BAs during the shelf life study (Table 1), no relevant differences between meat in AP and PP were reported for tryptamine, phenylethilamine and spermidine. The concentration of tyramine at days 4 and 7 is higher in AP meat with respect to PP meat, while, according to the article of Vinci (Vinci & Antonelli, 2002), the concentration of spermine reaches a maximum value and then tends to decrease with time more quickly in PP meat than in AP meat. Concerning putrescine, cadaverine and histamine, relevant differences in terms of concentration and in favor of AP meat were registered in each monitored day, i.e. 0, 2, 4, 7.

In regard to pH, Silva & Glória (2002) noted that during 7 days of storage the concentration of alkaline compounds in meat, including BAs, increased; during our shelf life study the pH of chicken meat increased both for AP and PP (from 5.94 to 6.12 and from 5.94 to 6.53, respectively), but reaching a higher value for PP meat. Moreover, significant differences ($t < 0.01$) in favor of AP were recorded at days 4 and 7. We can conclude that AP maintained the values of pH more stable for chicken meat with respect to PP that produced a faster increase connected to a higher level of BAs.

Microbiological analysis

BAs are produced in food by various mechanisms, but one of the most important is the decarboxylation via decarboxylase enzymes contained in some specific bacteria. Literature reports that *Enterobacteriaceae* and *Pseudomonas* spp. in meat produce cadaverine and putrescine, respectively (Ruiz-Capillas & Jimenez-Colmenero, 2004; Shalaby, 1996) and *Brochotrix thermosphacta* in pork meat produces cadaverine. *Enterobacteriaceae* were involved in the production of tyramine (Ruiz-Capillas & Jimenez-Colmenero, 2004).

Camo et al. (2008) report the antimicrobial and antioxidant action of AP enriched with extracts of *R. officinalis* on packed lamb steaks. Essential oils of *R. officinalis* contain monoterpene hydrocarbons as α-pinene, camphene and mircene, oxygenated monoterpenes as linalool, camphor and borneol, sesquiterpene hydrocarbons as octadecenoid acid that demonstrated to possess an antibacterial activity (Okoh et al., 2010).

In this work two selected bacterial species, i.e. *Pseudomonas* spp. and *Brochotrix thermosphacta*, *Enterobacteriaceae* and mesophilic bacteria in meat samples (chicken) packed in AP, enriched with essential oils of *R. officinalis* at 4% w/w, and in PP were studied. Results of microbiological study are reported in Table 2. Significant differences were obtained for $t < 0.05$. As it is possible to observe, for mesophilic bacteria and *Pseudomonas* spp., in each monitored day the level of microorganisms in white meat (chicken) is lower in AP with respect to that packed in PP. Moreover, significant results were registered in each monitored day except for those of day 7 relative to *Pseudomonas* spp. The analysis of *Brochotrix thermosphacta* confirms the inhibitory action of AP on the bacterial growth in each monitored day. Positive results were obtained for *Enterobacteriaceae* too, especially at day 2, in which the level of bacteria is significantly lower in meat wrapped in AP with respect to that packed in PP.

From microbiological analyses of red meat (bovine), similar considerations about the positive action of AP on shelf life of wrapped food can be made.

Interesting considerations can be made analyzing the results reported in Table 1, relative to concentration of single BA expressed in mg kg^{-1} found in chicken meat wrapped in AP and PP during the shelf life study. Concerning putrescine, a lower concentration in AP meat with respect to PP meat in each

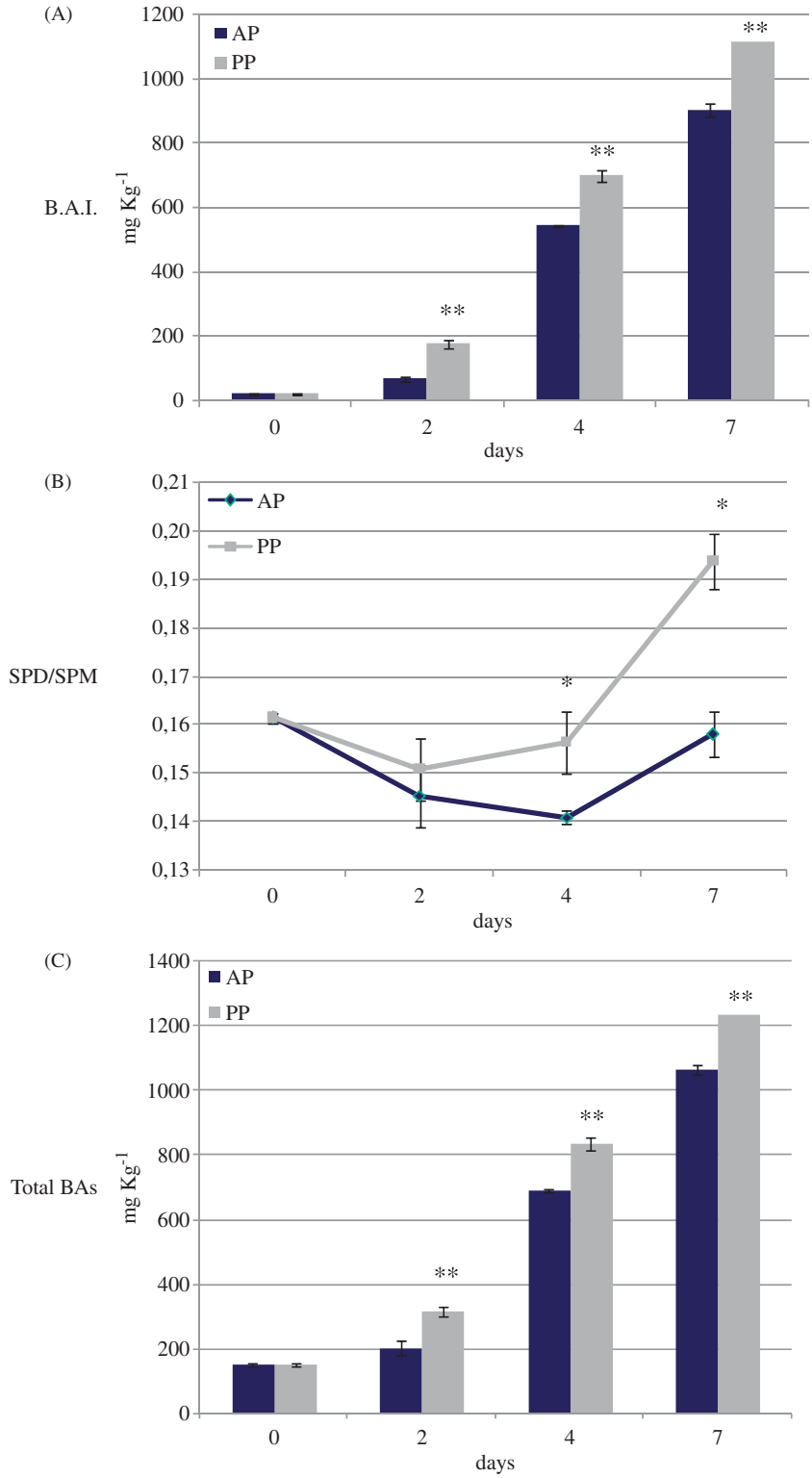

Figure 3. Shelf life study of chicken meat wrapped in AP in comparison with chicken meat wrapped in a classic PP, taking into account the three BAs indices: (A) biogenic amines index (BAI) expressed in mg kg^{-1}, (B) ratio SPD/SPM, (C) total BAs content expressed in mg kg^{-1}. *: data were significant for $t<0.05$; **: data were significant for $t<0.01$.

Table 2. Shelf life study of chicken meat wrapped in AP in comparison with chicken meat wrapped in a classical PP by monitoring: (A) Mesophilics, (B) *Pseudomonas* spp., (C) *Brochotrix thermospacta*, (D) *Enterobacteriaceae.*

	Storage time (days) and packaging materials						
		2		4		7	
Bacteria	0	AP	PP	AP	PP	AP	PP
Mesophilics (log cfu/g)	4.10 ± 0.04	4.60 ± 0.02*	5.30 ± 0.04*	6.90 ± 0.10*	7.20 ± 0.04*	8.90 ± 0.11*	9.20 ± 0.05*
Pseudomonas spp. (log cfu/g)	3.80 ± 0.07	4.60 ± 0.19*	5.20 ± 0.01*	6.80 ± 0.14*	7.10 ± 0.08*	8.84 ± 0.16	9.08 ± 0.05
Brochotrix thermospacta (log cfu/g)	3.20 ± 0.20	4.10 ± 0.06*	4.70 ± 0.02*	6.10 ± 0.05	6.20 ± 0.13	8.08 ± 0.09*	8.45 ± 0.02*
Enterobacteriaceae (log cfu/g)	2.18 ± 0.13	2.20 ± 0.32*	2.81 ± 0.14*	4.24 ± 0.10	4.10 ± 0.16	6.86 ± 0.10	6.79 ± 0.05

*: data were significant for $t<0.05$. Data are expressed as the mean of bacterial counts ± SD.

monitored day was reported, i.e. 1.5 versus 3.3 mg kg^{-1} (day 2), 7.1 versus 26.9 (day 4), 33.1 versus 62.3 mg kg^{-1} (day 7). As cited before, one of the most important bacterial species involved in the production of putrescine is *Pseudomonas* spp.; taking into account the microbiological analyses, a good correlation was found between the inhibition of increase of *Pseudomonas* spp. at day 2 and 4 (significant data) and the inhibition of growth of putrescine during the same monitored days. Similar considerations can be made taking into account cadaverine, that was found in lower concentration in AP meat with respect to PP meat in each monitored day, i.e. 50.2 versus 118.5 mg kg^{-1} (day 2), 300.5 versus 388.2 mg kg^{-1} (day 4), 513.9 versus 652.8 mg kg^{-1} (day 7). *Brochotrix thermosphacta* and *Enterobacteriaceae*, the main bacteria involved in the production of cadaverine in meat, showed at day 2 a lower level in AP meat with respect to PP meat (significant data), according to the concentration of cadaverine found in meat samples (Table 1). Moreover, for *Pseudomonas* spp. was confirmed the inhibitory action of AP on bacterial growth, strongly related to the lower level of cadaverine found in chicken meat at days 2 and 4 (significant data) with respect to meat packed in PP. Similar correlating considerations can be made for *Enterobacteriaceae* and histamine, that can be considered one of the most important BAs from a toxicological point of view; in fact, in each monitored day histamine shows a lower level in AP meat with respect to PP meat (from 1.6 to 2.6 times lower).

Hexanal

Lipidic oxidation has long been recognized as a leading cause of quality deterioration in muscle foods and is often the decisive factor in determining food product storage life (Goodridge et al., 2003). Hexanal, one of the dominant volatile secondary products formed during the oxidation of linoleic acid, has become a popular indicator of lipidic oxidation in foods. The performances of the new active packaging system, enriched with essential oils of *R. officinalis* (4% w/w) having an antioxidant action, were tested, by monitoring its capacity to inhibit the growth of hexanal in meat during time. The SPME-GC-MS developed method was tested both in red (bovine) and in white (chicken) meat wrapped in AP and in PP for the time storage (7 days) at 4 °C. The PDMS/DVB fiber was choosen for this study after testing polydimethylsiloxane (PDMS) 100 µm and polyacrylate (PA) 85 µm fibers that gave lower results concerning sensitivity and reproducibility (data not shown).

Table 3 reports the levels of hexanal that were found in chicken meat during study. In each monitored day, the concentration of hexanal found in meat wrapped in AP was lower than that found in meat packed in PP. In particular, at day 2, the level of hexanal found in PP meat was the double of that found in AP meat (1857 versus 3634 µg kg^{-1}). These data confirm that, both with the inhibitory action on the increase of BAs and their bacteria producers (antimicrobial action), there is an antioxidant action on fresh meat by the components of essential oil of *R. officinalis* incorporated in the AP. For this reason, the new formulated AP can enhance the shelf life of fresh meat.

Table 3. Level of hexanal expressed in µg kg^{-1} (mean ± SD) in chicken meat wrapped in active packaging (AP) and polycoupled packaging (PP) during shelf life study. Each experiment was realized in triplicate ($n = 3$) with a RSD <7%.

	Hexanal level (µg kg^{-1}) during storage time (days)			
	0	2	4	7
AP	1480 ± 74	1857 ± 48*	3120 ± 201*	4500 ± 37*
PP	1480 ± 74	3634 ± 86*	5760 ± 143*	7670 ± 101*

*: data were significant for $t < 0.05$.

HPLC-DAD method validation

The analytical method for analyzing BAs in meat was validated by determining linearity, recovery at two fortification levels, run-to-run and day to day precision, limits of detection (LODs) and limits of quantification (LOQs). Calibration curves of the analyzed compounds were constructed injecting 20 µL of standard solutions at five different concentrations, namely 0.5, 1, 5, 10 and 25 mgL^{-1} using the HPLC/DAD technique. All the calibration curves of the analyzed BAs showed a correlation coefficient higher than 0.998. The recovery percentages, obtained spiking the matrix (meat) at concentrations of 1 mg kg^{-1} and 5 mg kg^{-1} with a standard mixture of eight BAs plus internal standard, were in the range 75%–95% and 73%–90%, respectively. Five replicates for each concentration of mix standard at 1 mg kg^{-1} were performed and the relative standard deviation (RSDs) ranged from 1.5% to 3.2% for run-to-run precision and from 1.2% to 10.8% for day-to-day precision. LOD and LOQ were estimated on the basis of 3:1 and 10:1 signal to noise (S/Ns). The LODs of BAs, obtained with standards containing the compounds being studied at low concentration levels, were in the range 0.02–0.1 mg kg^{-1}, while the LOQs were in the range 0.1–0.4 mg kg^{-1}.

SPME-GC-MS method validation

PDMS/DVB fiber was chosen for method validation, after testing PDMS and PA fibers that gave lower results in terms of sensitivity and reproducibility. According to the article by Giuffrida et al. (2005), the calibration curve in water by using internal standard was chosen for quantification purpose. For the recovery and the quantification studies the response factor (Rf) was determined, by calculating the ratio between the area of hexanal and the area of internal standard. Calibration curve of hexanal was constructed on five different concentrations, i.e. 1, 5, 10, 50 and 100 µg L^{-1} and showed a correlation coefficient of 0.997. Five replicates for each concentration were performed, and the relative standard deviations (RSDs) were 1.3% for run-to-run precision and 4.5% for day-to-day precision.

Conclusions

BAs are an important parameter for evaluating the quality of food, in particular for meat, through different indices as biogenic amines index (BAI), ratio SPD/SPM and total BAs content. In this work, a new active packaging (AP) system for meat, formulated with essential oil of *R. officinalis* that inhibits the growth of BAs during the storage time, was developed. The analysis of BAs through a SPE-HPLC-DAD method and microbiological analysis monitoring specific bacteria producers of BAs as *Enterobacteriaceae*, *Pseudomonas* spp. and *Brochotrix thermospacta*, have demonstrated that there is a good correlation between the inhibition of growth of BAs and of bacteria producers. The antibacterial and anti-BAs action was due to the biological action of volatile components of *R. officinalis* extracted by hydrodistillation from plant. Moreover, by analyzing hexanal through SPME-GC-MS procedure as a marker of lipidic oxidation of fresh meat, the antioxidant action of new APs was assessed.

The results obtained with this study, both from chemical and microbiological point of view, lead us to conclude that these new APs contribute to inhibit the growth of BAs and bacteria producers already after 2 days of storage, increasing in this way the shelf life of fresh meat. As a consequence, the preservation of nutrients of meat such as protein or essential amino acids for a longer time can enhance their intake.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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