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A multi-step optimisation approach to extend burger shelf life

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Abstract

This research presents a multi-step approach to extend the shelf life of a new burger made of 60% dairy cow, 20% turkey and 20% chicken meat. In particular, antimicrobial compounds and a natural colour (cochineal) were combined with Modified Atmosphere Packaging (MAP). The work was divided into four subsequent experimental trials: the first trial was aimed to select the antimicrobial compounds; for this purpose, 0.02%, 0.05% and 0.07% potassium sorbate, 0.02% and 0.03% thymol and 1.98%, 3.60% and 5.40% sodium lactate were added to the burger samples. The second trial was aimed to apply modified atmosphere. Afterwards, the selected compounds and MAP were combined. Finally, chosen combination was combined with the cochineal. Microbiological, pH, gas composition and sensory changes were monitored during the storage at 4°C for 10 days. Results demonstrated that the proper combination of sodium lactate (3.60%), cochineal (0.30%) and MAP (30% O₂, 70% CO₂) assured a shelf life of 9.85 days, compared to the control burger which remained acceptable for less than 4 days.

Keywords: dairy cow, turkey and chicken burger, shelf life, sodium lactate, thymol, potassium sorbate, cochineal and MAP.

INTRODUCTION

Fresh meat products are usually marketed at refrigerated temperatures (2-5°C) because meat typically spoils due to two major causes, microbial growth and lipid oxidation (Sebranek et al., 2005). The use of antimicrobial ingredients is one of the widely used methods to maintain microbiological quality and prolong the shelf life of products (Bingol and Bostan, 2007).

Sodium lactate (SL), produced by microbial fermentation, is the sodium salt of the natural lactic acid (L+) and it is a normal component of muscle tissue (Choi and Chin, 2003). Antimicrobial effects of lactates have been investigated in meat and meat products (Bingol and Bostan, 2007; Choi and Chin, 2003; Kenawi et al., 2009; Lemay et al., 2002; Bingol et al., 2013). In addition to their antimicrobial effects, lactates are also shown to improve sensory characteristics of the products such as colour, texture, and flavour, and they are also shown to exert antioxidant effects (Bingol and Bostan, 2007; Jensen et al., 2003; Weiss et al., 2010). This salt acts as an un-dissociated acid, passing through the microbial membrane to acidify the cell (Carpenter and Broadbent, 2009). As a result, intracellular pH and cell metabolism may decline rapidly as organelles denature, and cell death may occur. Sorbic acid and its salts have several advantages as antimicrobial compounds. At first these compounds were used for their anti-mytotic activity, but
Cochineal, annatto, turmeric and saffron are the main appearance, colour, shape, size and surface defects. Consumers select food products primarily based on increasing popularity in the food industry because life can be reached.

Spraying applications (González-Fandos and Dominguez, previously selected best combination. were individually screened; in the third one, the best into four experimental trials: in the first two steps, chicken and turkey. To the aim, the study was organized into four experimental trials: in the first two steps, different antimicrobial compounds and MAP conditions were individually screened; in the third one, the best active compounds and MAP were combined and in the last one, the cochineal mixture was also added to the previously selected best combination.

The use of natural colorants has also become increasingly popular in the food industry because consumers select food products primarily based on appearance, colour, shape, size and surface defects. Cochineal, annatto, turmeric and saffron are the main natural colour compounds. Among them, cochineal is the most diffused; it has a red colour and it is obtained by crushing the female bodies of the cactus-eating insect *Dactylopiuscoccus*. Cochineal or carmine is found in many foods, especially sweets, ice lollies, fruit yoghurt, some processed meats, jam and fruit juice (Skypala, 2009).

Given this framework, this work aims to study various technological options to prolong the shelf life of a new meat burger made up of a combination of dairy cattle, chicken and turkey. To the aim, the study was organized into four experimental trials: in the first two steps, different antimicrobial compounds and MAP conditions were individually screened; in the third one, the best active compounds and MAP were combined and in the last one, the cochineal mixture was also added to the previously selected best combination.

# MATERIALS AND METHODS

## Burger preparation

Dairy cow, chicken and turkey meat were purchased from wholesale meat market (S.I.C.A. srl, Vernole, Italy). For each experimental trial, the portions were cut and then ground separately into mincing machine (Everest srl, Rimini, Italy) equipped with a 4 mm grinding plate. The formulation was prepared mixing 20% turkey, 20% chicken and 60% dairy cow meat. This formulation was selected by a panel test on the basis of a previous sensory evaluation in which odour, taste and texture of cooked patties were evaluated. Salt (1%), potato flakes (4%) and rosemary (0.5%) were added to the mixture meat. All ingredients were mixed with a spiral dough hook at medium speed (80 rpm) for 4-5 minutes and a homogeneous emulsion-type raw batter was obtained. Burgers were formed using a conventional burger-maker (~50 g/patty), to give average dimensions of 5 cm diameter and 1 cm thickness.

The burger dough was subdivided into 9 equal groups. Due to the large number of samples to be investigated, the first experimental trial (Trial I) was further split into two sub-trials (i.e., Trial I-1 and Trial I-2). The Trial I-1 was used to assess the effectiveness of potassium sorbate and thymol, whereas in the Trial I-2 sodium lactate was tested. Concerning Trial I-1, the first group was kept as the control (Cnt). To three groups,0.02%, 0.05% and 0.07% (w/w) potassium sorbate (PS, Farmalabor, Canosa di Puglia, Italy) were added, respectively. Finally, to two groups, 0.02% and 0.03% (w/w) thymol (T, Sigma-Aldrich, Milano, Italy) were added, respectively. The rosemary was eliminated in the burger added with thymol. In the Trial I-2, to three groups, 1.98%, 3.60% and 5.40% (w/w)sodium lactate (60% SL, Giusto Faravelli spa, Milano, Italy) were added, respectively. The concentrations of potassium sorbate, thymol and sodium lactate have been chosen basing on the studies of Hsu and Sun, (2006), Mastromatteo et al., (2011) and Kenawi et al., (2009) carried out also on meat products. Meat patties were mixed for about 3 min to have a homogeneous distribution of compounds. All samples were packaged in bags hermetically sealed and stored at 4±1°C. The bags (210 x 320 mm long, thickness 90 μm) were kindly provided by Di Mauro (Officine Grafiche spa, Salerno, Italy). They were an anti-fog high-barrier multilayer film made up of Polyethylene terephthalate (PET), ethylene-vinyl alcohol (EVOH) and Polyethylene (PE) with an oxygen transmission rate of 2.64 ± 0.12 cm³/m² day, a carbon dioxide transmission rate of 2.5 ± 0 cm³/m² day and a water vapour transmission rate of 0.69 ± 0.01 g/m² day.

In the subsequent experimental trial (Trial II), all burgers were divided into 5 equal groups. The first group comprised additive-free sample sealed by means of S100-Tecnovac equipment (Tecnovac, Bergamo, Italy)
under normal atmosphere (named as OA). In the other groups, the samples were packaged under MAP conditions (named as MA). To realize modified headspace conditions, the following gas concentrations were used: MA1: 30% CO₂, 50% O₂, 20% N₂; MA2: 30% CO₂, 70% O₂; MA3: 70% CO₂, 30% O₂; MA4: 30% CO₂, 30% O₂, 40% N₂. After packaging the samples were stored at refrigeration temperature (4±1°C).

In the third trial (Trial III) thymol and sodium lactate were combined with MAP. In particular, the burger dough was subdivided into 4 equal groups. To two groups, 0.02% T and 3.60% SL were added, respectively, and the samples were packaged under MA3; another group was made up of burgers with both active compounds packaged under MA3 (named as 0.02% T-3.60% SL-MA3). Additive-free samples under the same MAP conditions were as the control group. All samples were stored at 4±1°C.

Finally (Trial IV), the burger dough was subdivided into 3 equal groups. The first two groups were kept as the controls. In particular, additive-free burgers and burgers with the sole natural colour (Cnt-0.30% E) were prepared and packaged under MA3. The third group included 3.60% SL samples loaded with 0.30% (w/w) colorant and packaged under MA3, named as 3.60% SL-0.30% E-MA3. Colorant is a cochineal mixture, Eurovigor 15/N (Europrodotti spa, Concorezzo, MB, Italy). A panel tests aimed to evaluate only the sensory changes of meat during an appropriate refrigerated storage period previously determined the proper amount of cochineal. The mixture was made up of dextrose; E500, Sodium hydrogen carbonate; E300, ascorbic acid; E301, Sodium ascorbate; natural flavours and E120, cochineal. All samples were stored at refrigeration temperature.

In each experimental step, two samples of burgers per each treatment were randomly removed from the refrigerator at different storage time for determinations of microbial count, pH, headspace gas composition and sensory evaluation. Figure 1 shows a flowchart of the trials carried out to better describe the how the samples were studied.

**Microbiological analyses**

At each sampling time, 10 grams of meat patties were homogenized with 90 ml of sterile peptone solution (Oxoid, Milan, Italy) in a stomacher LAB Blender 400 (Pbi International, Milan, Italy) for 3 min. Decimal dilutions were made in sterile saline. Total aerobic bacteria were determined using Plate Count Agar (PCA, Oxoid), after incubation at 30°C for 24–48 h. *Pseudomonas* spp. was enumerated on Pseudomonas Agar Base, with SR103E selective supplement (Oxoid), incubated at 25°C for 48 h. For *Enterobacteriaceae*, Violet Red Bile Glucose Agar (VRBGA, Oxoid) was used and plates were incubated at 37°C for 18–24 h. Lactic acid bacteria (LAB) were plated on de Man Rogosa Sharpe agar (MRS, Oxoid), supplemented with cycloheximide (100 mg/L, Sigma), incubated under anaerobiosis (Oxoid) at 30°C for 2–4 days. Dichloran Rose-Bengal Chloramphenicol Agar (Oxoid), with chloramphenicol selective supplement (SR0078, Oxoid), incubated at 30°C for 48 h was for yeasts and moulds. Two replicates of at least three appropriate dilutions depending on the sampling day were enumerated. After incubation, plates having 25–250 colonies (cfu) were counted and multiplied by the dilution factor to determine cfu/g of meat.

In order to quantitatively determine the efficiency of active compounds, a re-parameterized version of the Gompertz equation was fitted to the total aerobic bacteria, according to a similar approach also used in
previous works dealing with shelf life of meat (Mastromatteo et al., 2011; Del Nobile et al., 2009). The equation allowed calculating the microbiological acceptability limit (MAL), defined as the time at which the microbial loads reached the maximum value permitted. For total aerobic bacteria the threshold was set to $10^7$ cfu/g (Senter et al., 2000; Cannarsi et al., 2005).

**pH determination**

The pH measurements were performed on the first homogenized dilution of samples during storage (Mastromatteo et al., 2011). All measurements were made in duplicate.

**Gas composition of package headspace**

Prior to opening the bags, headspace gas composition was determined by a Checkmate 9900 gas analyzer (PBI Dansensor, Ringsted, Denmark). The gas composition was measured twice (Mastromatteo et al., 2011).

**Sensory evaluation**

A panel of seven trained assessors was used to evaluate colour, odour and overall quality of burgers on an 8-point scale (Das et al., 2008). Samples with a score equal or higher than 4 were considered acceptable. A re-parameterized Gompertz equation was fitted to the sensory data (Mastromatteo et al., 2011; Del Nobile et al., 2009) to allow calculating the sensory acceptability limit (SAL), defined as the time at which the overall quality of meat reached the threshold (score = 4).

**Shelf life calculation**

Considering that the shelf life of a product is the time at which one of its quality sub-indices reaches the threshold, in the current work the shelf life was calculated as the lowest value between MAL and SAL (Mastromatteo et al., 2011).

**Statistical analysis**

Data obtained from fitting procedure (MAL and SAL) were analyzed by analysis of variance (ANOVA) to determine if treatments were significantly different. A Duncan’s multiple range tests with the option of homogeneous groups (p< 0.05) was used to determine significance between samples. Statistica 7.1 for Windows (Stat-Soft Inc., Tulsa, OK) was used for this purpose.

**RESULTS AND DISCUSSION**

As reported above, a multi-step approach was used to prolong the shelf life of a new meat burger. In particular, both formulation and packaging conditions were optimized to slowdown meat quality decay. The study was divided into four sequential experimental trials. The first two were aimed to give information on the antimicrobial preservatives and MAP to be used. In the trial III, preservatives and MAP were combined. Finally, a cochineal mixture was also added to the optimized combination. In each trial the main quality indices (microbial and sensory) were monitored in order to determine the effectiveness of the selected preservation strategies. It is worth noting that burger formulation was carried out in different periods along 1 year, thus expecting a different quality of meat. Therefore, to overcome this problem, the shelf life values of tested samples were compared with the related control samples prepared in the same experimental trial.

**Trial I**

Figures 2a and 3a show the viable cell concentration of total aerobic bacteria (TAB) plotted as a function of storage time for samples stored in normal atmosphere. The initial count (day 0) ranged from $4.81 \log$ cfu/g in loaded samples to $5.20 \log$ cfu/g in Cnt sample (Figure 2a). Starting from the second day of storage, the cell loads of all the samples exceeded the maximal recommended limit of $7 \log$ cfu/g for TAB in raw meat (Cannarsi et al., 2005), thus indicating a MAL value of about 2 days (Table 1). The MAL values were statistically equal, however, the cell load of samples loaded with PS and T were lower than those of the Cnt along the entire storage period. Results highlight that among the investigated preservatives, the thymol at both tested concentrations, exerted an inhibitory effect on growth of TAB, followed by the PS at 500 and 700 mg/Kg. The activity of thymol is generally attributed to the antimicrobial effects of the phenolic components. Generally, essential oils exhibit strong antibacterial properties against food-borne pathogens and spoilage microorganisms as a result of high percentage of carvacrol, thymol, p-cymene, γ-terpinene (Mahmoud et al., 2000). Thymol and carvacrol are the major component of oregano essential oil and are proved the two main compounds responsible for the antimicrobial activity (Juliano et al., 2000). Tsigarida et al. (2000) also reported that the addition of 0.8% oregano oil in vacuum-packaged beef muscle reduced the total viable count population of $2–3 \log$ cfu/g. The antimicrobial potential of PS has been more often evaluated in processed meat than in fresh meat (Friedrich et al., 2008). Vacuum packaged turkey bologna (Wederquist et al., 1995), sliced
pork bologna (Samelis et al., 2005), frankfurter and "pariza type" meat products (Drosinos et al., 2006) are the main examples of PS applications to processed meat products. As regards SL tested in Trial I-2, the initial TAB load ranged from 6.21 log cfu/g in loaded samples to 6.60 log cfu/g in the Cnt sample (Figure 3a). In most of the samples added with SL, the viable cell concentration did not overcome the threshold during the entire observation period, suggesting a MAL value higher than five days (Table 1). Only the Cnt and 1.98% SL samples went beyond the threshold (10^7 cfu/g) after the 2nd and 4th day, respectively and the MAL values were statistically different. The addition of SL has been also reported to produce a significant reduction of TAB growth in refrigerated ground beef (Sallam and Samejima, 2004) and ground pork (O'Connor et al., 1993; Brewer et al., 1995), as well as in cooked beef products (Miller and Acuff, 1994; Maca et al., 1999). More recently, Tan and Shelef (2002) also found that the microbial stability of ground pork stored at 2°C was enhanced by the combination of 2% SL or potassium lactate with NaCl, and the shelf life of meat was prolonged from 7 to 14 days. The results of the current work were also supported by the study of Sallam and Samejima (2004), who experienced the synergistic effect of sodium lactate and NaCl in refrigerated ground beef.

The preservatives used in the meat burger formulations especially influenced Enterobacteriaceae growth (Figures 2b and 3b). The PS and T samples brought a slight decrease in the viable cell concentration compared to the Cnt, from day 1 of storage; in contrast, SL sample showed this behaviour immediately.

Concerning Pseudomonas spp. (Figures 2c and 3c), data related to all the additive-free samples showed a short lag phase, followed by an increase in the viable cell concentration until the stationary phase was reached. Among the preservatives tested in the present study, the SL, together with T, proved to be the most effective against Pseudomonas spp. In particular, from day 1 until the last time of storage, a decrease in cell populations was observed in comparison to the Cnt sample. In contrast, PS did not greatly affect their growth compared to the Cnt.

The rods lactic acid bacteria (LAB) grew during the refrigerated storage (Figures 2d and 3d). There were no marked differences between the control sample and those containing PS and T (Trial I-1). Different behaviour

Figure 2. Evolution of total aerobic bacteria(a), Enterobacteriaceae(b), Pseudomonas spp. (c) and lactic acid bacteria (d) counts in meat burger during storage at 4°C. The curves are the best fit of the re-parameterized Gompertz equation to the experimental data (Trial I-1 a).
Table 1. Shelf life (d) of meat burger samples evaluated as the lowest value between the microbial acceptability limit (MAL) and the sensorial acceptability limit (SAL).

<table>
<thead>
<tr>
<th>Sample</th>
<th>MAL</th>
<th>SAL</th>
<th>Shelf life</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial I-1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cnt</td>
<td>1.80±0.30</td>
<td>4.73±2.63</td>
<td>1.80±0.30</td>
</tr>
<tr>
<td>0.02%PS</td>
<td>2.26±0.06</td>
<td>3.84±0.12</td>
<td>2.26±0.06</td>
</tr>
<tr>
<td>0.05%PS</td>
<td>2.03±0.69</td>
<td>4.04±0.79</td>
<td>2.03±0.69</td>
</tr>
<tr>
<td>0.07%PS</td>
<td>2.08±0.41</td>
<td>4.02±0.21</td>
<td>2.08±0.41</td>
</tr>
<tr>
<td>0.02%T</td>
<td>2.41±0.31</td>
<td>&gt;4</td>
<td>2.41±0.31</td>
</tr>
<tr>
<td>0.03%T</td>
<td>2.60±0.34</td>
<td>4.04±2.07</td>
<td>2.60±0.34</td>
</tr>
<tr>
<td><strong>Trial I-2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cnt</td>
<td>1.59±0.02</td>
<td>1.41±0.80</td>
<td>1.41±0.80</td>
</tr>
<tr>
<td>1.98%SL</td>
<td>3.91±0.38</td>
<td>1.72±0.44</td>
<td>1.72±0.44</td>
</tr>
<tr>
<td>3.60%SL</td>
<td>&gt;5</td>
<td>1.66±0.43</td>
<td>1.66±0.43</td>
</tr>
<tr>
<td>5.40%SL</td>
<td>&gt;5</td>
<td>1.66±0.43</td>
<td>1.66±0.43</td>
</tr>
<tr>
<td><strong>Trial II</strong></td>
<td></td>
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</tr>
<tr>
<td>Cnt</td>
<td>4.10±0.46</td>
<td>4.84±0.19</td>
<td>4.10±0.46</td>
</tr>
<tr>
<td>MA1</td>
<td>4.62±0.49</td>
<td>7.07±1.61</td>
<td>4.62±0.49</td>
</tr>
<tr>
<td>MA2</td>
<td>5.70±0.35</td>
<td>&gt;7</td>
<td>5.70±0.35</td>
</tr>
<tr>
<td>MA3</td>
<td>6.30±0.35</td>
<td>6.10±0.83</td>
<td>6.10±0.83</td>
</tr>
<tr>
<td>MA4</td>
<td>4.66±0.46</td>
<td>4.79±1.58</td>
<td>4.66±0.46</td>
</tr>
<tr>
<td><strong>Trial III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cnt-MA3</td>
<td>2.48±0.61</td>
<td>3.87±1.35</td>
<td>2.48±0.61</td>
</tr>
<tr>
<td>0.02%T-MA3</td>
<td>2.26±0.53</td>
<td>3.48±0.31</td>
<td>2.26±0.53</td>
</tr>
<tr>
<td>3.60%SL-MA3</td>
<td>&gt;6</td>
<td>1.05±0.28</td>
<td>1.05±0.28</td>
</tr>
<tr>
<td>0.02%T-3.60%SL-MA3</td>
<td>&gt;6</td>
<td>2.20±0.49</td>
<td>2.20±0.49</td>
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<tr>
<td><strong>Trial IV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cnt-MA3</td>
<td>3.73±0.33</td>
<td>7.37±0.43</td>
<td>3.73±0.33</td>
</tr>
<tr>
<td>Cnt-0.30%E-MA3</td>
<td>4.19±0.38</td>
<td>9.94±0.68</td>
<td>4.19±0.38</td>
</tr>
<tr>
<td>3.60%SL-0.30%E-MA3</td>
<td>&gt;10</td>
<td>9.85±0.13</td>
<td>9.85±0.13</td>
</tr>
</tbody>
</table>

Data in column with different letters are significantly different (P<0.05).
Values are means ± Standard error for n=2.

**Trial I-1**: Cnt (additive-free meat burger), 0.02%PS (meat burger loaded with 0.02% potassium sorbate), 0.05%PS (meat burger loaded with 0.05% potassium sorbate), 0.07%PS (meat burger loaded with 0.07% potassium sorbate), 0.02%T (meat burger loaded with 0.02% thymol), 0.03%T (meat burger loaded with 0.03% thymol).

**Trial I-2**: Cnt (additive-free meat burger), 1.98%SL (meat burger loaded with 1.98% sodium lactate), 3.60%SL (meat burger loaded with 3.60% sodium lactate) and 5.40%SL (meat burger loaded with 5.40% sodium lactate).

**Trial II**: Cnt (additive-free meat burger packaged in ordinary atmosphere), MA1: 30% CO2, 50% O2, 20% N2; MA2: 30% CO2, 70% O2; MA3: 70% CO2; MA4: 30% CO2; 30% O2; 40% N2.

**Trial III**: Cnt-MA3 (additive-free meat burger packaged in MA3); 0.02%T-MA3 (meat burger loaded with 0.02% thymol packaged in MA3); 3.60%SL-MA3 (meat burger loaded with 3.60% sodium lactate packaged in MA3); 0.02%T-3.60%SL-MA3 (meat burger loaded with 0.02% thymol and 3.60% sodium lactate packaged in MA3).

**Trial IV**: Cnt-MA3 (additive-free meat burger packaged in MA3); Cnt-0.30%E-MA3 (meat burger loaded with 0.30% cochineal mixture packaged in MA3); 3.60%SL-0.30%E-MA3 (meat burger loaded with 3.60% sodium lactate and 0.30% cochineal mixture packaged in MA3).

was recorded for SL samples; in fact, the LAB count was lower in the treated meat than in the Cnt prepared in the Trial I-2.
No moulds were detected in the samples during the storage period. With regard to the yeasts (data not shown), the addition of preservatives into burger showed a slight decrease in the cell load compared with the additive-free samples in both two sub-trials; in particular, SL was more efficient than PS and T in inhibiting yeast.
Similar pH trends were recorded over the 6-day storage period in all the sample of the first trial (data not shown). There was a slight decline in pH values for all the samples of both sub-trials; values ranged from about 5.60 (Cnt sample) to 6.25 (5.40% SL samples) without significant differences between the samples. This finding is in agreement with results of Mastromatteo et al., (2009) and Friedrich et al., (2008), who showed that SP and T had no influence on pH. Bloukas et al., (1997), Lin and Lin, (2002) and Choi and Chin (2003) also found that SL did not affect pH values of low-fat frankfurters and sausages.

The initial values of CO\textsubscript{2} and O\textsubscript{2} are 0.03% and 20%, respectively. The O\textsubscript{2} concentration of samples slightly decreased and then reached a value of about 17% at the end of storage (data not shown). The CO\textsubscript{2} concentration, instead, increased gradually, reaching a value of about 7%. In general, a decrease of O\textsubscript{2} and an increase of CO\textsubscript{2} concentration can be expected due to the microbial activity (Knock et al. 2006a; 2006b).

In the second column of Table 1, the SAL values of packaged burger products are listed. As can be seen, the SAL values were statistically similar; in particular, the SAL value was strongly affected by 0.02% T, in fact a SAL greater than 4 was recorded. For the others samples, SAL values slightly higher or similar to the control were observed. Although the application of PS resulted in a red colour of meat burgers, an undesirable odour was noticed. This observation was in accordance with results of Friedrich et al. (2008) and Kondaiah et al. (1985), who also found that PS was also responsible for slight but strange odours. Papadopoulos et al. (1991) showed that use of SL slowed the decrease of “on-notes” associated with fresh beef during storage. Mastromatteo et al. (2011) also recorded the high sensory acceptability of sausages containing thymol.

Burger shelf life values are also listed in the third column of Table 1. As can be seen, for most of the samples the shelf life coincides with MAL values, whereas the sensory quality represented the limiting factor for the acceptability of sample with SL. Results obtained from the first two sub-trials showed that the addition of the preservatives alone did not prolong to a great extent the shelf life of burger compared with the control samples. Among the tested antimicrobial
compounds, 0.02% T and 3.60% SL were chosen and used in the subsequent experimental steps.

**Trial II**

In the Table 1, the MAL values of burgers packaged under MAP are listed. As can be seen, the lowest MAL value was recorded for the Cnt sample (4 days), whereas among the burgers under MAP, the samples packaged under MA3 obtained a MAL value of about 6 days and these values were statistically different. There was a steady increase in the amount of TAB during the storage period (Figure 4a). Among the modified atmospheres, MA3 was the most effective for the inhibition of TAB, may be due to the inhibitory effect of CO₂ on microbial growth. In contrast, MA1 and MA4 showed a gradual decline in count and MA2 had a rather irregular trend. The graphs for Enterobacteriaceae (Figure 4b) and Pseudomonas spp. (Figure 4c) counts show certain similarities. There was almost the same trend in the Cnt sample with a steady increase along the storage period. The MA samples had a significantly slower growth rate compared to the Cnt. In addition, there were several notable differences between the other modified atmospheres.

Firstly, the MA3 was the most effective for the inhibition of Enterobacteriaceae and Pseudomonas spp., being bacteria sensitive to CO₂ (Jay et al., 2005). Secondly, the MA1 tied with MA2 in Pseudomonas spp. counts, while the evolution of Enterobacteriaceae in the MA2 was rather erratic. In addition, the antimicrobial effect of MA4 on Pseudomonas spp. evolution at the beginning was weak with respect to the other MAP, and then became more pronounced at the end of the storage. Several authors reported a similar behaviour in MAP packages where CO₂ was used at high levels, resulting in a limited growth of Enterobacteriaceae (Bingol and Ergun 2011; Chouliara et al., 2007; Esmer et al., 2011; Karabagias et al., 2011).

LAB constitutes a substantial part of the natural microflora of MAP meats (Chouliara et al., 2007). Burger samples stored under MAP showed a steady rise over the storage time (Figure 4d) and the cell load was slightly lower with respect to that of sample packaged in normal atmosphere.

With regard to yeasts (data not shown), the trend was similar in all the MA samples with a gradual increase in the counts over the time, in contrast a considerable rise from the 3rd day of storage was observed in the Cnt sample. The effectiveness of MAP in reducing growth of
moulds and yeasts, compared to packaging in normal atmosphere, was also observed by various authors in the literature (Bingol and Ergun, 2011; Chouliara et al., 2007). The pH of packaged burger ranged more or less between 6.38 and 6.07 in all the tested samples with slight differences that were not statistically significant (data not shown). Headspace atmosphere did not undergo changes in composition throughout the storage period (data not shown). The high concentrations of O$_2$, ranged from 70% to 50% used in MA1 and MA2, allowed maintaining the meat pigment myoglobin in the oxygenated state as oxymyoglobin to confer the meat the desirable bright red colour (Friedrich et al., 2008). The SAL values are listed in Table 1. As expected, the quality of tested burgers steadily decreased, regardless of the packaging strategy adopted. It is worth noting that MA2 sample did not overcome the threshold for the entire observation period, suggesting that the SAL is higher than 7 days. This result is in agreement with what found by Luño et al. (1998) who indicated that the presence of O$_2$ in the packaging headspace allowed preserving the characteristic red colour of fresh meat by O$_2$ binding to ferrous heme.

As regard the shelf life values, also listed in Table 1, a somewhat prolongation was recorded for sample packaged under MA3 that obtained a shelf life value of about 6 days. In particular, a positive effect on both microbiological and sensory quality was observed in comparison to the other active MAPs and control samples. Hence, in the subsequent experimental trials the burgers were packaged under MA3.

**Trial III**

The MAL values are also listed in table 1. As can be seen, the Cnt-MA3 together with the 0.02% T-MA3 went beyond the threshold ($10^7$cfu/g) within 3 days of storage. On the other hand, the MAL values for 3.60% SL-MA3 and 0.02% T-3.60%SL-MA3 samples exceeded the monitored storage period (i.e., 6 days). Data suggest that MAP combined with the preservatives enhanced microbial stability by inhibiting the proliferation of TBA (Figure 5a). The initial quality of meat used in this trial was not very good, as indicated by the high number of bacteria (about 6 log cfu/g) before adding the active compounds. The initial TAB in loaded burgers ranged from 5.27 in 3.60% SL-MA3 to 5.50 log cfu/g in 0.02% T-3.60% SL-MA3. This indicated that the addition of SL and T into formulation immediately reduced the cell loads.

**Figure 5.** Evolution of total aerobic bacteria (a), *Enterobacteriaceae* (b), *Pseudomonas* spp. (c) and lactic acid bacteria (d) counts in meat burgers during storage at 4°C (Trial III). The curves are the best fit of the re-parameterized Gompertz equation to the experimental data.
Figure 6. Evolution of total aerobic bacteria (a), Enterobacteriaceae (b), Pseudomonas spp. (c) and lactic acid bacteria (d) counts in meat burgers during storage at 4°C, (Trial IV). The curves are the best fit of the re-parameterized Gompertz equation to the experimental data.

The effectiveness of MAP and T on total viable count was also demonstrated in the literature (Mastromatteo et al., 2007). With respect to Enterobacteriaceae (Figure 5b), the Cnt-MA3 shows a period of stability at the beginning, followed by a slight increase at the end of the storage, whereas for the others samples there was a rather irregular trend, hitting a peak in the middle of the storage period.

As far as the Pseudomonas spp. is concerned, they were found in similar amounts in all the samples with a steady increase in cell load during the storage (Figure 5c).

Among the samples, the lowest cell concentration was recorded for the 3.60% SL-MA3 sample.

The LAB trends were similar in all the samples with counts that gradually increased (Figure 5d). Only the 3.60% SL-MA3 sample showed a low contamination of LAB.

No mould was enumerated on samples. With regards to yeasts, an erratic trend similar to that found for Enterobacteriaceae was noted in all the investigated samples (data not shown), thus demonstrating the variability of raw material.

Similar pH trend were observed in the investigated samples over the 6-day observation period (data not shown). In particular, a slight decline toward the end of the storage time in all the samples was noted, reaching a value as low as 5.5 in Cnt-MA3 and 0.02% T-MA3 samples. However, no significant differences were detected among the burgers packaged under MAP.

Values of headspace composition in the MAP samples remained almost constant during storage, due to the good barrier properties of bags (data not shown). A slight shift was observed in the burger packaged under normal atmosphere; in particular, the $O_2$ in the headspace decreased from 20% to 16% and $CO_2$ increased from 0% to 6%.

In the Table 1, the SAL values of the packaged meat products are also listed. As can be observed, the SAL values were statistically different and strongly affected by the combination of SL and MAP; in fact, SAL values of about 1 and 2 days were recorded for 3.60% SL-MA3 and 0.02% T-3.60% SL-MA3 samples, respectively. Although the use of SL seemed to be beneficial for meat odour, this was counterbalanced by an undesirable burgers discolouration. In contrast, a SAL value of 3.87 and 3.48 days was observed in Cnt-MA3 and 0.02% T-MA3 samples, respectively. Unfortunately, to date there is no published data to support or to be used as reference.
for these last tested samples.

The shelf life values reported in Table 1 show that the combination of preservatives and MAP did not prolong significantly the burgers shelf life. In particular, the combination of SL and MA3 inhibited the growth of main spoilage microorganisms, with prolongation of MAL but, in contrast, it detrimentally affected the sensory quality with an undesirable effect on meat colour. Therefore, for the last experimental trial the sample 3.60% SL-MA3 was used in combination with a natural colorant able to improve meat colour.

**Trial IV**

As can be seen in Table 1 and Figure 6a, the combination of sodium lactate and MAP significantly delayed the rate of TBA. The 3.60% SL-0.30% E-MA3 recorded a MAL value greater than 10 days whereas, MAL values of 3.73 and 4.19 days were observed for Cnt-MA3 and Cnt-0.30% E-MA3 samples, respectively. No antimicrobial effect was demonstrated by the sole addition of cochineal mixture into minced meat (data not shown).

With regard to *Pseudomonas* spp., the addition of additives brought about a steady decrease in the cell load compared to the control sample (Figure 6b).

*Enterobacteriaceae* cell load (Figure 6c) was low over the entire storage period for all the investigated samples, thus demonstrating good hygienic quality of meat (Zeitoun et al., 1994).

The LAB trend was similar in all the samples (Figure 6d); after an initial period where the cell load was practically constant, there was a gradual increase in the LAB cell concentration from the 5th to the 10th day of storage.

Finally, an upward trend was observed for yeasts (data not shown); in all the burgers low yeast counts (ca. 2 log cfu/g) were recorded until the 10th day of storage. Beyond this period of time, there were small fluctuations in population of this aerobic specie, which reached a value of about 2.50 log cfu/g at the end of the observation period.

Data showed that the pH value did not change significantly in the samples (data not shown), only a slight decrease was noticed. The gas concentrations in this experimental step were similar to those recorded in the previous Trial (data not shown).

As can be seen from the Table 1, the addition of the cochineal mixture strongly influenced the SAL values; in fact, SAL of 9.94 and 9.85 days were recorded in Cnt-0.30% E-MA3 and 3.60% SL-0.30% E-MA3 samples, respectively. The application of the natural colorant slowed down the discolouration process of the burgers and limited the formation of undesirable off-odour. Considering the effects of SL on microbial quality, a significant prolongation of shelf life was recorded for treated samples investigated in this last trial. Burgers remained acceptable for 9.85 days compare to the Cnt-MA3 and Cnt-0.30% E-MA3 that reached a shelf life of 3.73 and 4.19 days, respectively.

Therefore, the use of sodium lactate with cochineal with packaging in MAP made up of 30% O₂, 70% CO₂ appears to be a valid technique to preserve the new burger made of 60% dairy cow, 20% turkey and 20% chicken meat, without adversely affecting the characteristic freshness of the meat product.

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