Ready-to-cook fresh meal: study for shelf life prolongation

A. L. Incoronato¹ · D. Gammariello¹ · A. Conte¹ · M. A. Del Nobile¹

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Abstract In this study, to preserve the quality of a fresh meal based on a mix of meatballs and semi-dry vegetables, two main strategies were combined: during process the meat before grinding was dipped in sodium lactate and during packaging different modified atmospheres were applied (30:70 CO₂:N₂, 70:30 CO₂:O₂, 5:5:90 O₂:CO₂:N₂). The parameters monitored during the storage at 4 °C were the headspace gas composition, the microbial loads (Total viable count, *Pseudomonas* spp., lactic acid bacteria and *Enterobacteriaceae*), the pH and the sensory quality. Overall, the results indicate that the sodium lactate acted in synergy with MAP improving above all the microbial quality. A significant shelf life prolongation was obtained with 30 % CO₂ in the package, corresponding to a 168 % shelf life increase compared to the control sample in air.

Keywords Ready-to-cook fresh meal · Sodium lactate · Meat · MAP · Shelf life

Introduction

The demand for ready-to-eat or ready-to-cook food is rising due to changing in lifestyle. To cater this need, a range of chilled food products is being introduced in the market continually. Ready-to-cook products are developed by food companies to answer the need of fast preparation; consumers

desire high quality, health-conscious products, quickly prepared meal to enjoy at home or office. However, maintenance of quality of complex food dishes is of key importance for success of this sector (Adetunji and Odetokun 2012). These products are generally characterized by short shelf life, being good substrates for microbial proliferation. Bacterial growth, color changes and lipid oxidation are to date well-recognized factors responsible for fresh meat and poultry acceptance (Zhao et al. 1994). Such parameters may be successfully controlled by modified atmosphere packaging (MAP), being considered by meat industries a valid solution to keep the quality characteristics of meat and meat-derived products. Among microorganisms, MAP affects above-all aerobic Gram-negative bacteria (Gill and Tan 1980; Deak and Beuchat 1996) but generally, for meat-based products the best effects of MAP on spoilage were recorded when it was combined with active substances applied during process (Gammariello et al. 2014; Irkin and Esmer 2010; Karabagias et al. 2011; Mastromatteo et al. 2011; Muhlisin Sun et al. 2013).

The use of natural antibacterial compounds, such as extracts of spices and herbs, essential oils, organic acids, salts and bacteriocins is widely reported in the literature to improve food shelf life (Jałosińska and Wilczak 2009; Jamilah et al. 2008). Since microbial growth occurs primarily on meat surface, many attempts have been made to delay spoilage with organic acids applied by spraying or dipping (Dickson and Anderson 1992; Kochevar et al. 1997; Hardin et al. 1995). Organic acids and their salts are GRAS compounds and approved for use as acidifiers in various meat products (USDA-Food Safety and Inspection Service 1995). The acid penetrates the cell of microorganism by means of diffusion and then dissociates and acidifies the cell interior, thus interfering with cellular metabolism or decreasing the biological activity due to pH changes of cell environment (Cherrington et al. 1991). Among the acids, many researches have been carried out on

✉ M. A. Del Nobile
matteo.delnobile@unifg.it

¹ Services Center of Applied Research, University of Foggia, via Napoli, 25, 71122 Foggia, Italy

lactic acid and their salts (lactates) because it is proved that sodium lactate could improve flavor, color, tenderness, juiciness and cooking yields of ground beef and other meat products (Maca et al. 1997). Sodium lactate has been also shown to delay spoilage (Maca et al. 1999; Vasavada et al. 2003) and pathogens (Mbandi and Shelef 2001) in meat.

Considering that sanitation of meat surface prior to grinding or prior to the production of batters may improve microbial control, the objective of this research was to evaluate the influence of sodium lactate applied before processing the meatballs on microbial and sensory quality of a ready-to-cook meal prepared with meat and semi-dry vegetables, then stored under MAP conditions.

Materials and methods

Samples preparation

A ready-to-cook meal was made up at a laboratory scale with pork ham processed in raw meatballs and semi-dry vegetables (zucchini, peppers and tomatoes). Sodium-lactate (sodium salt of lactic acid, 60 % w/w) (Giusto Faravelli Spa, Milano, Italy) was used to pre-treat meat before processing and different MAP conditions were applied during packaging. Meat was provided by a meat company, Dodaro Spa (Spezzano Albanese, Cs, Italy) and transported to the laboratory in polystyrene boxes with an appropriate quantity of flaked ice. Semi-dry vegetables were purchased from a local company, Fiordelisi (Fiordelisi srl, Stornarella, Fg, Italy), and transported to the laboratory in boxes. Meat was cut into approximately 30 or 40 g pieces that were divided into five batches. The pieces were treated by dipping for 10 min in pre-chilled (4 °C) aqueous solution of sodium lactate. After 10 min of dipping, meat pieces were allowed to drain for a few seconds to remove excess of solution, on a sterile stainless wire mesh screen at ambient temperature. Untreated samples were dipped in sterile water and were used as the control. All the meat pieces from each batch were ground separately using a meat grinder (Everest, Sberlati & C. s.n.c., Rimini, Italy) equipped with a 4 mm grinding plate. Before use, all metal parts of the meat grinder were also dipped in aqueous solution of sodium lactate for 25 min. Sodium chloride (1 %), cheese (3 %), grated bread (3.5 %), garlic (0.03 %), parsley (2 %) and white bread (10 %) with the addition of milk (20 %), all purchased from a local market, were added to the meat mixture. All ingredients were mixed with a food processor (Multichef, Ariete, Firenze, Italy) for 4–5 min and a homogeneous emulsion-type raw batter was obtained. Subsequently, meatballs were prepared (10 ± 1 g) and then dipped in a batter with distilled water (145 ml water/100 g batter) (CZN0300WA, Spichem, Milan, Italy). The samples were allowed to drain

for 10 s to remove excess of batter and then coated in grated bread.

Semi-dry vegetables were seasoned with sodium chloride (0,72 % w/w), garlic (0,44 % w/w) and parsley (0,56 % w/w). The seasoned vegetables were left at room temperature for about 2 h in order to allow the uniform distribution of the ingredients. For packaging, 4 meatballs and 50 g of seasoned vegetable were placed into a tray and packaged in bags hermetically sealed and stored at 4 ± 1 °C. The bags were 220×320 mm long, and they are constituted by an anti-fog high-barrier multilayer film made up of polyethylene-terephthalate, ethylene-vinyl alcohol and polyethylene, with oxygen transmission rate (OTR) 2.64 ± 0.12 cm³/m² day, carbon dioxide transmission rate (CDTR) 2.5 ± 0.01 cm³/m² day at 23 °C and 0 % RH and water vapor transmission rate (WVTR) 0.69 ± 0.01 g/m² day at 23 °C and 90 % RH, thickness 90 µm, kindly provided by Di Mauro (Officine Grafiche spa, Salerno, Italy).

Three gas mixtures were used: 30:70 (CO₂:N₂) (SL-MAP30), 70:30 (CO₂:O₂) (SL-MAP70) and 5: 5:90 (O₂:CO₂:N₂) (SL-MAP5), by means of a gas mixer (PBI-Dansensor Model 9000, Ringsted, Denmark). Pouches were heat-sealed by means of a packaging machine (S100-Tecnovac equipment, Bergamo, Italy) connected to the gas mixer. NA-lactate-free samples packaged under ordinary atmosphere (CNT-OA), together with treated samples packaged in air (SL-OA) were also prepared as the references.

Two samples for each treatment were randomly removed from the refrigerator at different storage times and tested for microbiological quality, pH measurement, determination of headspace gas composition and sensory properties.

Gas composition of the package headspace

Prior to opening the bags, headspace gas composition was determined by using a Checkmate 9900 gas analyzer (PBI Dansensor, Ringsted, Denmark). The volume taken from the package headspace for gas analysis was about 10 cm³. To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for a single determination of the headspace gas composition.

Microbiological analyses and pH determination

Samples of 25 g (each consisting of equal amounts of both meat and vegetable pieces) were aseptically removed from the bags transferred into a Stomacher bag and homogenized for 3 min (Stomacher LAB Blender 400, Pbi International, Milan, Italy) containing 225 ml sterile peptone solution (Oxoid, Milan, Italy). Further decimal serial dilutions were prepared from this homogenate in the same sterile diluents and these were subsequently used for enumeration and differentiation of microorganisms. Total aerobic bacteria (TAB) were

determined using Plate Count Agar after incubation for 48 h at 30 °C. *Pseudomonas* spp. were enumerated on Pseudomonas Agar Base supplemented with cetrimide, fucidine and cephaloridine (CFC) supplements providing a selective isolation medium for *Pseudomonas* spp. Colonies were counted after 48 h at 25 °C. Lactic Acid Bacteria (LAB) were enumerated by the pour plating technique on de Man Rogosa Sharpe agar and incubated under anaerobiosis at 30 °C for 48 h. *Enterobacteriaceae* counts were enumerated by the pour plating technique on Violet Red Bile Glucose Agar after incubation at 37 °C for 24 h. Media and supplements for the microbiological analyses were procured from Oxoid (Milan, Italy). The microbiological analyses were carried out twice on two different batches. The results were expressed as the logarithm of the colony forming units for gram (log cfu/g).

In order to determine the microbiological acceptability limit (MAL defined as the time at which the microbial loads reached the maximum value permitted), the re-parameterized version of the Gompertz equation was fitted to the total aerobic bacterial data (TAB), 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). For TAB count the critical limit was set to 5×10^6 cfu/g referred to the 94/65 EC and 2073/2005 EC regulations (EC 1994; EC 2005).

The pH value was recorded on each sampling day using a pH meter (Crison Instruments model 2001, Barcelona, Spain). The pH determination, conducted twice, was performed on the first homogenized dilution of each sample.

Sensory analysis

A panel of seven experienced judges was used to evaluate color, odor and overall quality of raw samples 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 product reached the threshold (score = 4).

Shelf life calculation

The shelf life was calculated as the lowest value between MAL and SAL. In fact, it is the time at which one of the meat quality sub-indices reaches its threshold (Mastromatteo et al. 2011).

Statistical analysis

Experimental data were compared by one-way Anova analysis. A Duncan's multiple range test, with the option of

homogeneous groups ($P < 0.05$), was used to determine significance among differences. To this aim, Statistica 7.1 for Windows 152 (StatSoft Inc., Tulsa, OK, USA) was used.

Results and Discussion

Microbiological quality of ready-to-cook samples

The MAL values related to TAB for each sample are listed in the Table 1. Data emphasize that the microbial quality limited the shelf life of this ready-to-cook meal. As an example, Fig. 1 shows the evolution of TAB plotted as a function of storage time for all the tested samples. For the control samples, a steady increase in the viable cell concentration was observed during the storage period, thus reaching the threshold after about 2 days. Afterwards, cell counts remained practically constant, with a cell population accounting for about 8.62 log cfu/g. As can be inferred from data shown in the Fig. 1, the presence of the sodium lactate (SL-OA) slowed down TAB growth, if compared to the control sample. The addition of Na-lactate inhibited their growth of about 1 log cycle from the first day of storage; subsequently, this log reduction was more marked after the second day. In fact, treated samples recorded a MAL value of 4.76 days. After this period, the cell load increased and reached values higher than 8.6 log cfu/g at 7th day of storage similarly to the control. As can be seen in the Fig. 1, the combination of MAP and lactate had an inhibitor effect on TAB cell load. In fact, all investigated active samples showed a significant prolongation of the microbial lag phase, as well as a substantial reduction of the final loads attained in the stationary phase in comparison with the control sample. In particular, the best result was achieved by the SL-MAP30 sample, which showed a MAL value of 6.21 days, whereas SL-MAP70 and SL-MAP5 samples obtained a value of 6.14 and 6.03 days, respectively. Data obtained were in agreement with those of the literature, concerning the use of sodium lactate in some meat products. Papadopoulos et al. (1991) reported that in cooked, vacuum-packaged beef,

Table 1 Microbial acceptability limit (MAL), sensory acceptability limit (SAL) and shelf life (mean $\pm$ SD) of ready-to-cook meatballs mixed with semidry vegetables

Samples	MAL (day)	SAL (day)	Shelf life (day)
CNT-OA	2.31 $\pm$ 0.17 _a	7.46 $\pm$ 0.17 _a	2.31 $\pm$ 0.17 _a
SL-OA	4.76 $\pm$ 0.53 _b	12.66 $\pm$ 0.10 _c	4.76 $\pm$ 0.53 _b
SL-MAP30	6.21 $\pm$ 0.19 _c	12.90 $\pm$ 0.17 _c	6.21 $\pm$ 0.19 _c
SL-MAP70	6.14 $\pm$ 0.63 _c	6.71 $\pm$ 0.00 _a	6.14 $\pm$ 0.63 _c
SL-MAP5	6.02 $\pm$ 0.18 _c	10.54 $\pm$ 0.00 _b	6.02 $\pm$ 0.18 _c

^{a-c} Data in each column with different letters are significantly different ($P < 0.05$)

Fig 1 Evolution of total aerobic bacteria during storage. The curves are the best fit to the experimental data. CNT-OA (Na-lactate-free sample packaged under ordinary atmosphere); SL-OA (sample treated with Na-lactate 60 %, packaged in ordinary atmosphere); SL-MAP30 (sample treated with Na-lactate 60 %, packaged under MAP 30:70 $\text{CO}_2\text{:N}_2$); SL-MAP70 (sample treated with Na-lactate 60 %, packaged under MAP 70:30 $\text{CO}_2\text{:O}_2$); SL-MAP5 (sample treated with Na-lactate 60 %, packaged under MAP 5:5:90 $\text{O}_2\text{:CO}_2\text{:N}_2$)

increasing levels of lactate resulted in lower total bacteria counts of roasts. Unda et al. (1991) observed that survival was considerably reduced in vacuum-packed beef roast when treated with brine containing 2 % lactate. The effects of lactate in combination with MAP were superior to the dipping of lactate. In particular, the effect of MAP SL-MAP30 was more effective than other MAP on reducing TAB. The observations above are in agreement with the results reported by other authors. Irkin and Esmer (2010) reported that the use of MAP (20 % CO_2 - 80 % N_2) without oxygen and with/without bay oil was the most effective one in controlling growth of *Listeria monocytogenes* and *Escherichia coli* and also to extend the shelf life of naturally contaminated ground chicken meat. Muhlisin Sun et al. (2013) also proposed use of MAP (30 % CO_2 - 70 % N_2) in combination with additives mixture (calcium lactate, sodium acetate and rosemary extract) to reduce aerobic and anaerobic bacteria counts and was effective for maintaining the quality and extending the shelf-life of pre-cooked hamburger patties.

With regard to *Pseudomonas* spp., the bacteria were able to proliferate in the CNT-OA sample (data not shown). Their concentration increased from 10^3 to 10^7 cfu/g throughout the storage period. In general, for all the investigated samples with Na-lactate brought about a prolonged lag phase followed by an increase in cell numbers after 6 days of storage. Only for SL-MAP30 and SL-MAP70 samples, the cell load proliferated from 10^3 to 10^4 cfu/g during the 13 days monitoring period. Gram-negative bacteria are generally more sensitive to CO_2 than Gram-positive bacteria (Church 1994) because most Gram-positive bacteria are facultative or strict anaerobes (Gill and Tan 1980), but individual bacteria vary in sensitivity to CO_2 (Farber 1991). Lactic acid bacteria are facultative

anaerobes, able to grow under high concentrations of CO_2 (Chouliara et al. 2007). These microorganisms constitute a substantial part of the natural microflora of meats (Karabagias et al. 2011). In addition, it has been documented that lactic acid bacteria dominated the microbial flora in sodium lactate treated beef during vacuum-packaged storage at 0 °C (Papadopoulos et al. 1991) as well as in frankfurter-type sausage treated with Na-lactate and stored at 0–4 °C (Zivkovic et al. 2002). In fact, in this study, the samples treated with sodium lactate and stored under MAP showed a steady rise over the storage time and the cell load was slightly lower with respect to that of CNT-OA sample. Only the SL-MAP70 sample showed a low contamination of LAB (data not shown). With respect to *Enterobacteriaceae*, considered as a hygiene indicator (Zeitoun et al. 1994), the trend was similar in CNT-OA and SL-OA with a gradual increase in the counts over the time (data not shown). The initial value of *Enterobacteriaceae* in all sample was about 1 log cfu/g (day 0), indicative of good quality meat products; the counts increased progressively in the samples packaged under ordinary atmosphere, reaching a value of 6.69 log cfu/g in CNT-OA and 6.32 log cfu/g in SL-OA (day 7). Afterwards, cell counts remained relatively stable, suggesting that the stationary phase has been attained. On the contrary, the samples SL-MAP30 and SL-MAP70 showed a reduced proliferation that remained practically constant until the 7th days. After this period, the viable cell concentration in SL-MAP30 slightly increased over storage period (3.41 log cfu/g), whereas in SL-MAP70 remained unchanged (1.54 log cfu/g). In contrast, the samples SL-MAP5 showed a prolonged lag phase followed by a continued increase in the viable cell concentration after 3rd days of storage, and then a viability loss of about 1 log cycle compared with the samples packaged under ordinary atmosphere, reaching a maximum concentration of about 6.4 log cfu/g at the end of the observation period. This limited growth of *Enterobacteriaceae* is in accordance with several authors who also reported similar behaviors in MAP packages where CO_2 was used at high levels (Bingol and Ergun 2011; Chouliara et al. 2007; Esmer et al. 2011; Karabagias et al. 2011). In addition, with regard to MAP, results of the present study are in agreement with those of Santos et al. (2005) who reported that Gram negative bacteria such as the *Pseudomonads* and *Enterobacteriaceae* are more sensitive to CO_2 than Gram positive bacteria such as LAB.

pH and headspace gas composition of ready-to-cook samples

The pH values did not change significantly during storage at 4 °C in all the active samples (data not shown). These results showed that, over the storage time, the addition of Na-lactate maintained the ready-to-cook meal at almost constant pH,

while the value of CNT-OA sample slightly decreased (5.82–5.19) without any significant differences. Sodium lactate was reported to stabilize pH during storage and it is used as a pH control agent (Bingol and Bostan 2007; Maca et al. 1997, 1999; Papadopoulos et al. 1991).

Headspace atmosphere did not undergo marked changes in composition throughout the storage period (data not shown), suggesting that the initial MAP conditions were protracted for the entire observation period, because of the high barrier properties of the packaging film.

Sensory quality of ready-to-cook samples

Figure 2 gives the overall quality plotted as a function of storage time for all the samples during storage at 4 °C. The curves shown in the figure were obtained by the fitting to the experimental data, whereas the horizontal solid line is the sensory threshold. The values of SAL parameter are also listed in the second column of Table 1. As can be observed, the SAL values were statistically different and strongly affected by the combination of sodium lactate and MAP. It should be noted that the highest score of overall quality was awarded to the SL-MAP30 samples becoming unacceptable after almost 12.90 days, whereas CNT-OA samples were refused after 7.46 days. In particular, the odor was the sensory attribute that limited the overall quality of CNT-OA. The detected off-odors were mainly putrefactive and sour. The other samples received an acceptable sensory score for more than 12.66 and 10.54 days for SL-OA and SL-MAP5, respectively. The value of SL-MAP70 is significantly

Fig 2 Evolution of the overall quality during storage. The curves are the best fit to the experimental data. CNT-OA (Na-lactate-free sample packaged under ordinary atmosphere); SL-OA (sample treated with Na-lactate 60 %, packaged in ordinary atmosphere); SL-MAP30 (sample treated with Na-lactate 60 %, packaged under MAP 30:70 CO₂:N₂); SL-MAP70 (sample treated with Na-lactate 60 %, packaged under MAP 70:30 CO₂:O₂); SL-MAP5 (sample treated with Na-lactate 60 %, packaged under MAP 5:5:90 O₂: CO₂:N₂)

lower than that of other treated samples, with a SAL value less more than 6 days.

Shelf life of ready-to-cook samples

Wherever the overall quality of a fresh food product depends on several quality sub-indices, its shelf life represents the time at which one of them reaches its threshold. In the case under investigation, the shelf life of each tested sample was calculated as the lowest value between the MAL, evaluated on the basis of TAB, and SAL related to the overall quality (Table 1). As can be inferred from data, the microbial quality was always responsible for the product unacceptability. In particular, for all samples packaged under MAP condition a shelf life value more than 6 days was obtained compared to other samples packaged under ordinary atmosphere for which a shelf life value of about 2 days (CNT-OA) and less more than 4 days (SL-OA) was recorded. It should be noted that the highest score of shelf life was awarded to the SL-MAP30 sample that correspond to an increase of 168 %, compared with the shelf life of the CNT-OA sample. This suggests that the dipping process combined with MAP gradually reduced the final viable cell load of the ready-to-cook meal. In fact, the presence of sodium lactate alone did not affect significantly the shelf life, while the dipping of Na-lactate in synergy with the MAP 30:70 (CO₂:N₂) inhibited microbial growth. Therefore, the treatment significantly extended the shelf life of meatballs with vegetables and considering the highly perishable character of processed meat the results could be of great relevance for the industrial sector of ready-to-cook fresh meals.

Conclusions

The mix of meatballs and semi-dry vegetables represents a new product, with high convenience quality and suitable to modern consumer satisfaction, being a strategic solution to overcome the barrier of time-consuming meal. Beyond the convenience, the work demonstrates a valid way to extend the shelf life of the ready-to-cook fresh meal by the use of sodium lactate in combination with MAP. Results highlighted the possibility to extend the microbial acceptability limit of the meal from less than 3 days to more than 6 days, thus confirming the effectiveness of treatments that are easy to be applied and not expensive for future industrial applications.

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