



## **EFFECT OF SODIUM ALGINATE COATING WITH ASCORBIC ACID ON SHELF LIFE OF RAW PORK MEAT**

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### **ABSTRACT**

*The focus of this study was to evaluate the effect of sodium alginate coating loaded with ascorbic acid on shelf life of raw pork meat slices, used to prepare skewers. To the aim, the meat samples were first dipped in the sodium lactate solution (40% w/w) and then divided into 3 treatment groups: (i) control samples (dipped meat), (ii) coated meat, (iii) coated meat containing ascorbic acid (i.e. 500, 1000 and 1500 ppm). All samples were packaged under modified atmosphere packaging (50%O<sub>2</sub>/30%CO<sub>2</sub>/20%N<sub>2</sub>) and stored at 4±2°C. The samples were analysed for sensory and microbiological characteristics. The combination of dipping, active coating and MAP improved the sensory quality of packaged skewers. In contrast active coating does not considerable effect in slowing down the growth of spoilage microorganisms. Sample coated with 1500 ppm ascorbic acid displayed the longest shelf life, equal to 8.9 days, which was about 60% longer than the control.*

**Keywords:** Processed meat, Coating with ascorbic acid, Shelf life, Dipping.

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### **Contribution/ Originality**

The paper's primary contribution is finding that edible active coatings can improve the meat quality by retarding sensorial and microbiological deterioration and functioning as carriers of active compounds. The results suggest that combined the dipping and the active coating with modified atmosphere packaging the freshness of meat is comparatively prolonged.

## **1. INTRODUCTION**

Consumers worldwide favour ready-to-eat products, prone to rapid spoilage; therefore, food industries are nowadays seeking technologies to increase its shelf life. In the preparation of those products it is crucial importance to take into account all measure to ensure the quality during the entire shelf life. The developing methods in the packaging represent a major task of the meat processing industry. Edible coating or biodegradable packaging is a technology of controlled-

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release of active molecules in food system that has been introduced in food processing in order to obtain products with longer shelf life. Several applications for meat, poultry and seafood have been reviewed by [Gennadios \*et al.\* \(1997\)](#) with particular emphasis on the reduction of lipid oxidation, weight loss, moisture loss, microbial load, and volatile flavour loss. The coating can also serve as a carrier for antimicrobial compounds in order to maintain high concentrations of preservatives on the surface of foods ([Lacroix \*et al.\*, 2004](#)). For meat packaging, the coating was also suggested as carrier of antioxidants able to delay meat rancidity and discoloration ([Khan \*et al.\*, 2013](#)). Among antioxidant compounds, ascorbic acid is used to prevent oxidative rancidity and improve colour stability of fresh or processed meat. It is a reducing agent, which inhibits myoglobin oxidation and brown colour development in non-irradiated beef ([Sanchez-Escalante \*et al.\*, 2001](#); [Ahn and Nam, 2004](#)). Ascorbic acid can act as antioxidant or pro-oxidant depending on its concentration, presence of metal ions and tocopherol content ([Schaefer \*et al.\*, 1995](#)).

In addition to active coating, several antimicrobial solutions have been tested for their effects on carcasses and most of them have been reported to reduce the bacterial number under such experimental conditions ([Gill and Badoni, 2004](#); [Del Rìo \*et al.\*, 2007](#)). Among the antimicrobial compounds, sodium lactate, the sodium salt of lactic acid, has a bacteriostatic effect ([Shelef, 1994](#)). Although the most scientific reports highlight the antimicrobial effects of lactate ([Papadopoulos \*et al.\*, 1991](#); [Kuo \*et al.\*, 1994](#); [Wang, 2000](#); [Sallam and Samejima, 2004](#)) the treated by dipping is not widely used ([Gammariello \*et al.\*, 2014a](#)).

Finally, modified atmosphere packaging (MAP) is well known technique, applied with success for extending the shelf life of a variety of foods, including fresh meat and poultry ([Chouliara and Kontominas, 2006](#); [McMillin, 2008](#); [Lorenzo and Gómez, 2012](#)). Meat researchers ([Mastromatteo \*et al.\*, 2010](#); [Mastromatteo \*et al.\*, 2011](#); [Gammariello \*et al.\*, 2014b](#)) have found that combination of active compounds with MAP can prolong the shelf life of processed meat.

Due to the lack of research on preservation strategies to be applied to ready-to-eat meat products, the aim of this study was to investigate the combination of dipping, active coating and MAP on fresh meat skewers. In particular, the effects of dipping with sodium lactate, coating with ascorbic acid and MAP on quality decay of pork meat skewers stored at 4°C were assessed.

## **2. MATERIALS AND METHODS**

### **2.1. Preparation of Sodium Alginate Solution**

Sodium alginate (Farmalabor, Canosa di Puglia, Ba, Italy) was added to sterile distilled water at approximately 50°C to allow the alginate powder to dissolve into solution. A 2% solution (g alginate/g water) along with 2% glycerol was prepared. Different amounts of ascorbic acid (500, 1000 and 1500 ppm) were added to the alginate solution to develop the active coating.

### **2.2. Skewer Sample Preparation and Packaging**

The meat was kindly provided by a meat processing company (Dodaro spa, Spezzano Albanese, Cs, Italy) and it was transported to the laboratory in polystyrene boxes covered with

ice. Walnut pork meat was cut into small cubes of dimensions ca. 2x2x2 cm. Meat samples were treated by dipping for 10 min in pre-chilled (4°C) aqueous solution of sodium lactate (Faravelli spa, Milano, Italy) at the concentration of 40% (w/w). After dipping, the meat pieces were allowed to drain for 5 min on a sterile stainless wire mesh screen at the ambient temperature (18°C). The samples was divided into three separate groups: (i) control dipped meat (Cnt), (ii) meat dipped and coated with sodium alginate solution (Coa), (iii) meat dipped and coated with sodium alginate solution loaded with different concentration of ascorbic acid (Coa500, Coa1000 and Coa1500). The coated samples were immersed in the coating solutions for 30 sec and then drained for 30 s, followed by a dipping in 0.5% aqueous calcium chloride solution (CaCl<sub>2</sub>) for 30 s and again a draining for 30 s. The pieces of meat were inserted in the stick.

All skewers were sealed by means of S100-Tecnovac equipment (Tecnovac, Bergamo, Italy) under MAP (50% O<sub>2</sub>, 30% CO<sub>2</sub>, 20% N<sub>2</sub>) and stored at 4±2°C for 12 days. The bags, 220 × 320 mm long, were handle-made by an anti-fog high-barrier multilayer film kindly provided by Di Mauro (Officine Grafiche spa, Salerno, Italy). The film was made up of polyethylene terephthalate (PET), ethylene-vinyl alcohol (EVOH) and polyethylene (PE) with an oxygen transmission rate of 2.64 ± 0.12 cc/m<sup>2</sup> day, a carbon dioxide transmission rate of 2.5 ± 0 cc/m<sup>2</sup> day and a water vapour transmission rate of 0.69 ± 0.01 g/m<sup>2</sup> day. Sensory evaluations and microbial counts were carried out before packaging and after 1, 2, 5, 6, 7, 8, 9 and 12 days of storage.

### 2.3. Sensory Analysis

Seven experienced judges, staff of the department of Agricultural Sciences, Food and Environment, on each sampling day were asked to evaluate the samples on an 8-point scale to determine colour discoloration (8, no discoloration; 1, extreme discoloration), odour (8, extremely desirable; 1, extremely unacceptable/off-odours) and overall quality (8, extremely desirable; 1, extremely unacceptable) of the samples (Das *et al.*, 2008). Acceptable criteria assumed that rejection would occur when the sensory attributes declined below 4.

In order to determine the Sensory Acceptability Limit, SAL (i.e., the storage time at which overall quality of product reached its threshold value), the re-parameterized version of the Gompertz equation was fitted to sensory data, as also reported in another work also dealing with fresh meat products (Del Nobile *et al.*, 2009).

### 2.4. Microbiological Analyses

Samples (20 g) in duplicate from the coated and uncoated meat skewers were aseptically homogenized for 3 min with sterile peptone solution (Oxoid, Milan, Italy) (180 mL) in a Stomacher LAB Blender 400 (Pbi International, Milan, Italy). Appropriate serial dilutions of the homogenate were carried out. Total aerobic bacteria (TAB) were determined using Plate Count Agar incubated at 30°C 48 h. *Pseudomonas* spp. were enumerated on Pseudomonas Agar Base supplemented with CFC supplements, incubated at 25°C for 48 h. *Enterobacteriaceae* counts were enumerated on Violet Red Bile Glucose Agar after incubation at 37°C for 24 h. Lactic acid

bacteria (LAB) were plated on de Man Rogosa Sharpe agar incubated under anaerobiosis at 30°C for 48h. Media and supplements for the microbiological analyses were purchased from Oxoid (Milan, Italy). The results were expressed as the logarithm of the colony forming units per gram (log cfu/g).

To quantitatively determine the efficiency of the strategies proposed in terms of microbiological quality, the same equation was also fitted to the microbiological data and the Microbiological Acceptability Limit (MAL), defined as the time at which the viable cell concentration reached its threshold, was calculated (Del Nobile *et al.*, 2009; Mastromatteo *et al.*, 2011). The threshold was set to 7 log cfu/g for total aerobic bacteria (ICMSF (International Commission on Microbiological Specifications for Foods) Microorganisms in Foods, 1986).

### 2.5. Shelf Life Calculation

The shelf life value was calculated as the lowest value between SAL and MAL, thus representing the time at which one of the quality indices of the product reaches its threshold (Del Nobile *et al.*, 2009; Mastromatteo *et al.*, 2011).

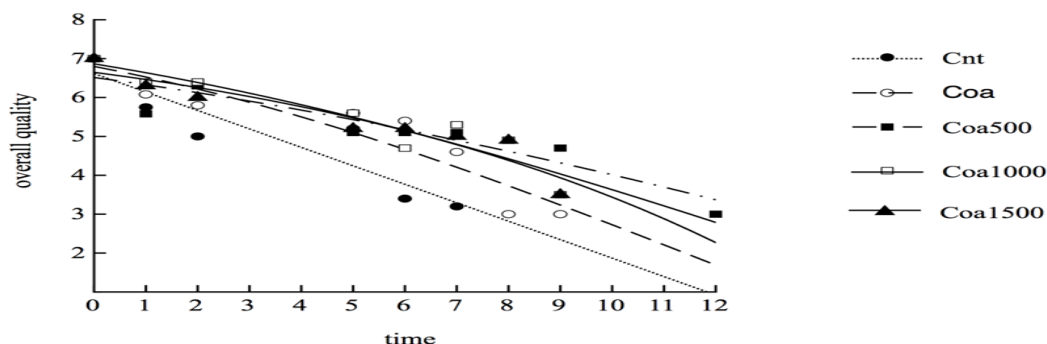
### 2.6. Statistical Analysis

Data obtained from fitting procedure (SAL and MAL) as well as the shelf life values were compared by one-way analysis of variance. 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.

## 3. RESULTS AND DISCUSSION

### 3.1. Sensory

In this study pork meat skewers quality was monitored during 12 days of refrigerated storage. Sensory and microbial quality was used to assess the product shelf life.



**Fig-1.** Overall quality of coated meat during storage at 4°C. The curves are the best fit of the re-parameterized Gompertz equation to the experimental data.

**Source:** Cnt (●) = dipped; Coa (○) = dipped and coated with alginate; Coa500 (■) = dipped and coated with alginate loaded with 500 ppm of ascorbic acid; Coa1000 (□) = dipped and coated with alginate loaded with 1000 ppm of ascorbic acid; Coa1500 (▲) = dipped and coated with alginate loaded with 1500 ppm of ascorbic acid.

Figure 1 gives overall quality plotted as a function of storage time for all samples. The curves shown in the figure were obtained by following the same fitting procedure reported by [Del Nobile et al. \(2009\)](#) and [Mastromatteo et al. \(2011\)](#) the SAL values are listed in Table 1.

**Table-1.** Shelf life of coated meat samples evaluated as the lowest value between the sensory acceptability limit (SAL) and the microbial acceptability limit (MAL).

Samples	SAL [day]	MAL [day]	Shelf life [day]
Cnt	5.5±1.00 <sup>a</sup>	> 7	5.5±1.00 <sup>a</sup>
Coa	7.4±0.7 <sup>b</sup>	7.2±0.2 <sup>a</sup>	7.2±0.2 <sup>bc</sup>
Coa500	10.0±0.9 <sup>c</sup>	6.5±0.2 <sup>a</sup>	6.5±0.2 <sup>ab</sup>
Coa1000	9.1±0.9 <sup>c</sup>	8.2±0.8 <sup>b</sup>	8.2±0.8 <sup>cd</sup>
Coa1500	8.9±0.8 <sup>c</sup>	11.2±1.1 <sup>c</sup>	8.9±0.8 <sup>d</sup>

**Source:** Cnt, dipped; Coa, dipped and coated with alginate; Coa500, dipped and coated with alginate loaded with 500 ppm of ascorbic acid; Coa1000, dipped and coated with alginate loaded with 1000 ppm of ascorbic acid; Coa1500, dipped and coated with alginate loaded with 1500 ppm of ascorbic acid.

Data in each column with different letters are significantly different ( $P<0.05$ ).

As expected, the overall quality scores of tested samples gradually decreased during time, regardless of the strategy adopted. An extension of 10 days was achieved by using the active coating, i.e. Coa500 sample. The appearance, colour, odour and overall acceptability of coated meat, with and without the active compound, were significantly different from the sole dipped meat ( $P<0.05$ ) (Table 2). In particular, the uncoated samples presented a slimy appearance and the colour deterioration started from the 6th day of storage. In particular, the uncoated samples had a slimy appearance, and it had colour deterioration at about 6 days of storage. In comparison, Coa1500, Coa1000 and Coa500 samples had an acceptable sensory score even after 8.9, 9.1 and 10 days of storage, respectively. This is probably attributed to the combination of adopted strategies; in particular the use of sodium lactate limited the formation of undesirable off-odour. [Brewer et al. \(1991\)](#) reported that the addition of sodium lactate to fresh pork sausage delayed the development of sour- and off-flavours. In addition, studies on the application of alginate coating to treated muscle foods reported improvements of moisture retention and texture, reduction in shrink, enhancement of juiciness, colour and odour ([Cutter and Sumner, 2002](#); [Cutter, 2006](#)). Another study found that coating improved the overall appearance, the colour, the juiciness, the flavour, the texture and the overall palatability of meat patties ([Chidanandaiah et al., 2009](#)). In fact, the samples coated with sodium alginate (Coa) had a SAL value of 7 days. Also the antioxidant effect of ascorbic acid minimized colour deterioration, prolonging the sensory acceptability of meat. In fact ascorbic acid is used to prevent oxidative rancidity and improve colour stability in fresh or further processed meat. It is a reducing agent, which inhibits myoglobin oxidation and brown colour development in non-irradiated beef ([Sanchez-Escalante et al., 2001](#); [Ahn and Nam, 2004](#)).

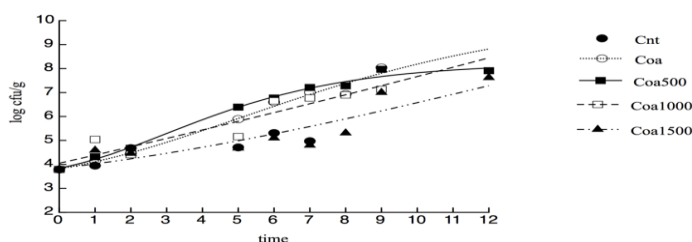
**Table-2.** Effect of active coating on the sensory attributes of meat during storage at 4°C.

		Storage Time							
		1	2	5	6	7	8	9	12
Cnt	Appearance	5.6± 0.7	4.8± 0.3	5.1± 0.4	3.5± 0.0	3.5± 0.0	NA	NA	NA
	Colour	5.3± 1.0	4.0± 0.0	5.2± 0.4	3.3± 0.3	3.0± 0.5	NA	NA	NA
	Odour	7.0± 0.0	5.5± 0.0	5.2± 0.3	3.9± 0.4	3.7± 0.3	NA	NA	NA
Coa	Appearance	5.3± 1.1	4.6± 0.4	5.3± 1.0	5.7± 0.3	4.5± 0.0	3.3± 0.3	NA	NA
	Colour	6.3± 0.3	4.9± 0.4	5.4± 0.9	5.4± 0.4	4.2± 0.4	3.0± 0.0	NA	NA
	Odour	6.7± 0.5	5.9± 0.2	5.6± 0.4	5.1± 0.2	5.0± 0.0	3.6± 0.4	NA	NA
Coa500	Appearance	5.7± 0.3	6.5± 0.0	5.3± 0.6	5.2± 0.4	5.2± 0.4	4.9± 0.4	4.0± 0.0	3.0± 0.0
	Colour	5.6± 0.4	6.4± 0.6	5.3± 0.8	5.3± 0.8	5.3± 0.3	4.9± 0.4	4.0± 0.0	3.0± 0.0
	Odour	5.0± 0.7	6.4± 0.2	5.3± 0.3	5.3± 0.3	5.5± 0.3	5.0± 0.5	5.0± 0.0	3.0± 0.0
Coa1000	Appearance	6.4± 0.2	6.4± 0.2	6.0± 0.0	4.7± 0.4	4.8± 0.3	4.8± 0.3	3.5± 0.0	NA
	Colour	6.4± 0.2	6.4± 0.2	5.6± 0.5	4.7± 0.4	5.3± 0.4	5.0± 0.3	3.5± 0.0	NA
	Odour	6.9± 0.2	6.9± 0.2	5.6± 0.5	5.0± 0.0	5.2± 0.4	4.9± 0.2	3.5± 0.0	NA
Coa1500	Appearance	6.7± 0.4	6.0± 0.0	4.8± 0.8	5.2± 0.3	5.0± 0.3	4.8± 0.3	3.5± 0.0	NA
	Colour	6.5± 0.5	6.0± 0.0	5.4± 0.8	5.2± 0.3	5.2± 0.7	5.0± 0.5	3.5± 0.0	NA
	Odour	6.9± 0.2	6.5± 0.0	5.6± 0.4	5.2± 0.3	4.6± 0.4	4.7± 0.6	3.5± 0.0	NA

NA =sample not analysed due to unacceptability.

Cnt, dipped; Coa, dipped and coated with alginate; Coa500, dipped and coated with alginate loaded with 500 ppm of ascorbic acid; Coa1000, dipped and coated with alginate loaded with 1000 ppm of ascorbic acid; Coa1500, dipped and coated with alginate loaded with 1500 ppm of ascorbic acid.

### 3.2. Microbiology



**Fig-2.** Evolution of total aerobic bacteria in coated meat during storage at 4°C. The curves are the best fit of the re-parameterized Gompertz equation to the experimental data.

Data in column with different letters are significantly different ( $P < 0.05$ ).

Values are means ± Standard error for  $n=2$ .

**Source:** Cnt (●) = dipped; Coa (○) = dipped and coated with alginate; Coa500 (■) = dipped and coated with alginate loaded with 500 ppm of ascorbic acid; Coa1000 (□) = dipped and coated with alginate loaded with 1000 ppm of ascorbic acid; Coa1500 (▲) = dipped and coated with alginate loaded with 1500 ppm of ascorbic acid.

As regards microbial quality, Figure 2 illustrates the evolution of Total Aerobic Bacteria for

all meat samples during storage, with the relative fitting curves. The initial TAB for the uncoated meat samples was of 3.79 log cfu/g, thus highlighting the antimicrobial effect of dipping treatment. The graph shows changes between the different meat samples over the 12-days period. In particular, the viable cell concentration of Cnt slowly increased until the 7th day of storage, when the test was stopped due to the sensory unacceptability, without reaching the microbiological limit. On the contrary, the microbial concentration in all the coated samples increased faster and exceeds the above-mentioned limit. Consequently, the MAL values reported in Table 1 show that the Cnt sample had the value higher than 7 days; among the coated meat, the Coa1500 sample obtained the highest MAL value, which was equal to 11 days. The dipping treatment with sodium lactate reduced the microbial proliferation; many scientific reports highlight the antimicrobial effects of sodium lactate (Papadopoulos *et al.*, 1991; Jensen *et al.*, 2003; Sallam and Samejima, 2004; Diez *et al.*, 2009) and the effectiveness of MAP in reducing the growth of aerobic microorganisms (Smith *et al.*, 1990; Jayasing *et al.*, 2002). In addition, as stated by Gammariello *et al.* (2014a) MAP combined with sodium lactate dipping enhanced the microbial stability of ready-to-cook fresh meal. Results obtained in this work suggest that there is only a slight effect of the ascorbic acid on microbial growth. In agreement with these results, no inhibitory effect of ascorbic acid on psychrotrophic growth in beef patties packaged under MAP was also described in the literature (Shivas *et al.*, 1984; Sanchez-Escalante *et al.*, 2001). Anyhow, these results are encouraging if they are compared to the data reported in literature on meat no dipped packaged in ordinary atmosphere, which have a MAL of about 3 days (Gammariello *et al.*, 2014a).

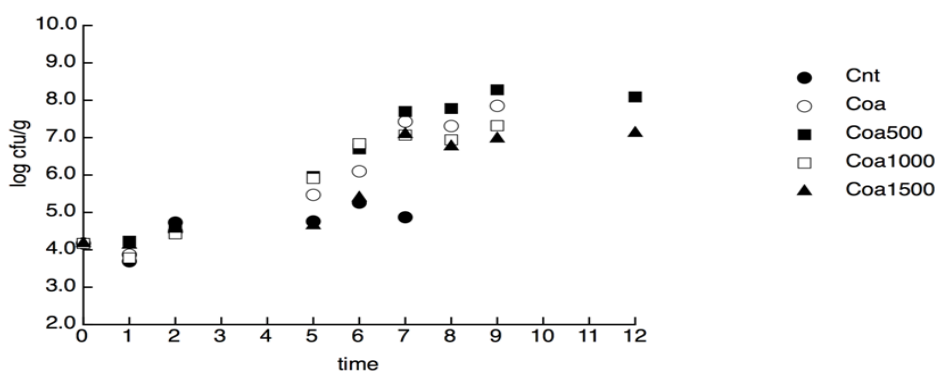


Fig-3. Evolution of *Pseudomonas* spp. counts in coated meat during storage at 4°C.

Source: Cnt (●) = dipped; Coa (○) = dipped and coated with alginate; Coa500 (■) = dipped and coated with alginate loaded with 500 ppm of ascorbic acid; Coa1000 (□) = dipped and coated with alginate loaded with 1000 ppm of ascorbic acid; Coa1500 (▲) = dipped and coated with alginate loaded with 1500 ppm of ascorbic acid.

At the beginning of the test the viable *Pseudomonas* spp. concentration, which is part of the microflora of the investigated product was 4.17 log cfu/g. Figure 3 shows the viable count of *Pseudomonas* spp. for all the investigated samples. As can be inferred from the figure, the Cnt sample shows a slight increase during the entire observation period, whereas the use of coating

did not seem to inhibit the growth of this microbial group because a period of stability, followed by a steady increase in the load cell, can be observed.

Concerning the trend of LAB it was found similar in all samples (data not shown); there was a moderate increase over the observation period and the lower load cell was recorded for the Cnt and Coa samples.

The analyses of skewers showed that *Enterobacteriaceae* were in the range of 3 log cfu/g at the beginning of the storage period; the initial counts were similar to those reported by numerous other authors that studied meat products (Seydim and Saricus, 2006; Chouliara *et al.*, 2007; Esmer *et al.*, 2011; Karabagias *et al.*, 2011).

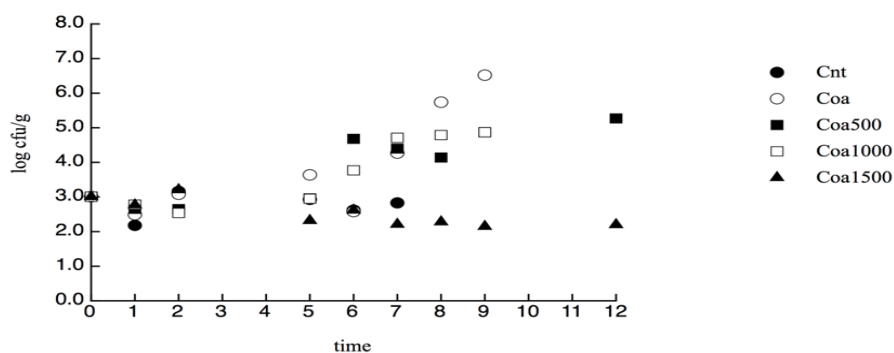


Fig-4. Evolution of *Enterobacteriaceae* counts in coated meat during storage at 4°C.

Source: Cnt (●) = dipped; Coa (○) = dipped and coated with alginate; Coa500 (■) = dipped and coated with alginate loaded with 500 ppm of ascorbic acid; Coa1000 (□) = dipped and coated with alginate loaded with 1000 ppm of ascorbic acid; Coa1500 (▲) = dipped and coated with alginate loaded with 1500 ppm of ascorbic acid.

The cell load of Coa1500 and Cnt samples did not increase throughout the storage (Figure 4). In contrast the trend in the other investigated samples was rather erratic, hitting a peak in the middle of the storage period. As a consequence, any variation in the sensory characteristics of meat pieces coated is not probably attributed to differences in microbial growth and it is most probably due to inhibition of chemical reactions.

### 3.3. Shelf Life

The shelf life of each sample was calculated as the lowest value between the MAL, evaluated on the basis of TBA viable count, and the SAL values, and it was also reported in the Table 1. As can be inferred from these data, the microbial quality is responsible for meat unacceptability of coated samples, except for Coa1500 sample. On the contrary, the sensory quality limited the shelf life of the Cnt sample. A significant shelf life prolongation was recorded for Coa1500 sample, which remained acceptable for about 9 days, if compared to the Cnt that reached a shelf life of 5.5 days. To date, the coatings extended the shelf life of food products (Earle and Snyder, 1966; Earle, 1968; Daniels, 1973; Earle and Mckee, 1976; Cutter and Sumner, 2002) but the findings of the current work give a new contribution to the field because demonstrated the combined effects of dipping, coating and MAP on meat skewers. In particular, while the incorporation of ascorbic acid



in the coating was useful to improve the sensory quality of meat, delaying the colour deterioration, the dipping controlled the microbial proliferation and limited the formation of undesirable off-odour.

#### 4. CONCLUSIONS

Ascorbic acid incorporated in a bio-based coating, in combination with dipping and MAP, were used to extend the shelf life of the raw pork meat slices, used to prepare skewers. Main quality attributes were monitored for about 12 d to determine the decline in product quality during chilled storage. Meat sample, coated without any active compound, or dipped with sodium lactate showed a shelf life limited to about 7 and 5 d, respectively. In contrast, the sample coated with 1500 ppm ascorbic acid allowed a significant prolongation of shelf life to 11 d. Considering that this strategy is simple, a dipping combined with coating, and packaging in MAP could be very beneficial and of commercial importance to the meat industry.

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Contributors/Acknowledgement: All authors contributed equally to the conception and design of the study.

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