

Use of Antimicrobial Treatments and Modified Atmosphere to Extend the Shelf Life of Fresh Sausages

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Abstract

The application of technologies, dipping treatments and packaging under modified atmosphere conditions, to prolong the shelf life of fresh pork sausages were assessed. The work was divided into two subsequent experimental trials. The first trial was aimed to select the concentration of essential oils. For this purpose, bay with nutmeg oils and fennel with black pepper oils were properly combined to dip of meat according to Two Central Composite Designs (CCD). The second trial was aimed to combine the meat dipping with the optimal concentrations of oils and sodium lactate solution (60%). All samples were packaged under modified atmosphere (MAP: 30% CO₂ 70% N₂). Total Aerobic Bacteria and sensory quality were monitored during the refrigerated storage and the shelf life was calculated as the lowest value between microbiological acceptability limit (MAL) and sensory acceptability limit (SAL). Results of two steps showed that an increase of sausage shelf life could be obtained combining more technologies. In particular, the shelf life of about 18 days was obtained combining the dipping of meat first in sodium lactate solution then in optimal concentrations of essential oils (1.25% fennel and 2.5% black pepper; 2.5% bay and 1.25% nutmeg), compared to the untreated samples that recorded a shelf life of two days.

Practical application: The shelf life extension of fresh meat products like sausages that generally have a short shelf life, represents a challenge for food companies. Short shelf life causes problems to food distribution, increases the food loss with relevant impact also on the environment. Therefore, the use of technological options to be used during processing and packaging could have important economic feedback.

Keywords: Fresh meat sausage; Sodium lactate; Essential oils; MAP; Shelf life

Introduction

Fresh sausage is a perishable food and requires protection from spoilage during its preparation, storage and distribution to give it the desired shelf life. One approach to extend the shelf life of fresh meat is to introduce antimicrobials, preferably natural compounds [1- 6]. Because the microbial contamination of meat products occurs primarily at the surface due to post-processing handling, dipping treatments with antimicrobial preservatives could be promising, thus helping to prevent the growth of spoilage microorganism [7, 8].

Essential oils (EO) are well-known inhibitors of microorganisms [9] and numerous workers reported their antimicrobial activities [10, 11]. Fennel (*Foeniculum vulgare*), with a sweet, earthy flavour, which belongs to the family Apiaceae has long been used as herbal remedy. Medicinally, fennel is used as analgesic, antioxidant, antispasmodic, anti-inflammatory, carminative and diuretic [12-15]. Black peppercorn (*Piper nigrum*) is a commonly used spice. The essential oil of black peppercorn has been shown to possess antimicrobial activity [16]. Bay (*Laurus nobilis*) is an evergreen tree native to the Mediterranean region. Laurel leaves have been used as flavouring ingredients and they have been traditionally added to meat, fish and poultry meals [17, 18]. Several studies have screened the potential capacity of laurel essential oil as antimicrobial agent [9] and antioxidant compounds [17]. Nutmeg (*Myristica fragrans*) is a widely used spice and flavouring ingredient in food products, with possible health beneficial effects, such as anti-inflammatory activity [21-23]. Sodium salts of organic acids, such as lactic, acetic and citric, have been used to control microbial growth, improve sensory attributes and extend the shelf life of various food systems [24] including meat [25- 28], poultry [29], and fish [30-32]. Furthermore, these salts are widely available at low costs [33]. Sodium lactate, produced by microbial fermentation, is the sodium salt of natural lactic acid and is a normal component of muscle tissue. Since

1989, the potential benefits of sodium lactate as antimicrobial agent spurred interest in research of meat products [26].

MAP is also considered as an effective method for food preservation. It is well known as a method for extending the shelf life of a variety of foods, including fresh meat and poultry [39-41]. The application of MAP has grown greatly in recent years, but optimization of gas composition is still critical to ensure both quality and safety of food [42]. The application of carbon dioxide to inhibit bacterial growth and nitrogen to avoid oxidation of fats in poultry meat packages can generate good preservation effects [42].

Although numerous studies have been done to evaluate the antimicrobial activity of the above-mentioned oils, there are only few studies on the combination of essential oils and sodium lactate in meat products [44]. Therefore, the present study was undertaken to investigate the effect of essential oils, sodium lactate and MAP for the improvement of fresh sausage shelf life during storage at 4°C. To the aim, the study was organized into two experimental trials: in the first one, the essential oils (i.e. bay with nutmeg and fennel with black pepper) were combined and two Central Composite Designs were performed. In the second trial, the best concentrations of essential oils were used in combination with dipping in sodium lactate and packaging under MAP.

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Materials and Methods

Sausages production

Pork meat was kindly provided by a meat processing company, Dodaro (Dodaro spa, Spezzano Albanese, Cs, Italy). The composition of sausages, given by the company above-mentioned, was: minced meat (60%), animal fat (20%), salt (1.5%), dextrose (0.5%), spices (10.8%), sodium salt of ascorbic acid, E301 (0.2%). The minced meat was mixed with all ingredients in a mixer to obtain sausage batter. The batter was then stuffed into natural casings of diameter 2.5 cm, using a mechanical bagging and the stuffed sausages were linked.

Trial 1

During the first trial, the concentration of essential oils varied according to a two-factor, three-level Central Composite Design (CCD) [45] and two experimental plans were performed to combine fennel oil with black pepper oil and bay oil with nutmeg oil. The coded levels and the combinations of the experimental design are reported in Table 1 (a, b). 10 Kg batch of raw meat, for each CCD, was divided into 1 kg parts to which the appropriate antimicrobial treatment was added. For each run (see table 1b), the meat pieces were dipped into 100 g extra virgin olive oil solution (EVO) enriched of essential oils (EO) and after dipping they were ground. After 10 min of dipping, meat pieces were ground by a mincing machine (Everest, Sberlati & C. s.n.c., Rimini, Italy) equipped with an 8 mm grinding plate. The solution left from dipping were added to the obtained minced meat and mixed with the ingredients above mentioned. Twenty sausages of 50g for each run were obtained. Each combination was repeated twice, and untreated sausages were used as the control. The sausages were packed in air and stored at 4°C for 8 days. 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 of 2.64 ± 0.12 cc/m² day, carbon dioxide transmission rate of 2.5 ± 0 cc/m² day at 23°C and 0% RH and water vapour transmission rate (WVTR) of 0.69 ± 0.01 g/m² day at 23°C and 90% RH, thickness of 90 µm, kindly provided by Di Mauro (Officine Grafiche spa, Salerno, Italy).

run	Experimental factors		Concentration (%)	
			fennel or bay	black pepper or nutmeg
Cnt	-1	-1	0	0
2	-1	1	0	0.25
3	1	-1	2.5	0
4	1	1	2.5	2.5
5	-1	0	0	1.25
6	1	0	2.5	1.25
7	0	-1	1.25	0
8	0	1	1.25	2.5
9	0	0	1.25	1.25
10	0	0	1.25	1.25

Table 1a: Run, experimental factors and concentrations of Central Composite Design (CCD).

Level	fennel or bay	black pepper or nutmeg
-1	0	0
0	1.25	1.25
1	2.5	2.5

Table 1b: Coded values of experimental factors.

Trial 2

The second trial focused on the combined effects of dipping in two solution, sodium lactate 60% (NaL) and EVO enriched of essential oils, and packaging under modified atmosphere. In particular, 6 Kg of raw meat was subdivided into 6 equal groups. To the 1st group, sausage samples without treatments (Cnt); in the 2nd one, the samples dipped in sodium lactate solution (L). Thus, the meat pieces, prior to grinding, were dipped in pre-chilled (4°C) solution of sodium salt of lactic acid (60% w/w syrup) (Giusto Faravelli Spa, Milan, Italy). After 10 min of dipping, meat pieces were allowed to drain for 5 min on sterile stainless wire mesh screen at ambient temperature (18°C). In the 3rd group, sample were treated with the EVO solution enriched of fennel and black pepper oil (FP) and in the 4th one, sample treated with the EVO solution enriched of bay and nutmeg oil (BN). In particular, meat pieces, prior to grinding, were dipped into 100 g EVO solution enriched of best combinations of essential oils selected from the two CCD described in the previous trial and after dipping they were ground. In the last two groups, samples were obtained by combining the two treatments. Thus, in the 5th group, the raw meat was dipped into solution of sodium lactate and subsequently into EVO solution enriched of fennel and black pepper oil; in the 6th batch, the meat was dipped into solution of sodium lactate and subsequently into EVO solution enriched of bay and nutmeg oil. The same series of operations that were performed previously were undertaken also in this trial and twenty sausages of 50g for each group were obtained. The first 4th groups were used as controls. All sausages were packaged under MAP (30% CO₂ 70% N₂) and stored at 4°C for 20 days.

Total Aerobic Bacteria (TAB)

Twenty grams of sample were diluted in 180 ml of peptone water and homogenized for 1 min in a blender (Stomacher, International PBI, Milan, Italy). Serial dilutions of sausage homogenates were plated in Plate Count Agar (Oxoid, Milan, Italy), incubated at 30°C for 48h.

In order to determine the microbiological acceptability limit (MAL; i.e., the storage time at which the viable cell concentration reaches its threshold value), the re-parameterized version of the Gompertz equation was fitted to the total aerobic bacterial data of the second trial (the threshold value was set to 10⁷ cfu/g), according to Mastromatteo et al. [46] and Del Nobile et al. [47].

Sensory Analysis

Seven experienced judges, staff of the Department of Agricultural Sciences, Food and Environment, evaluated the sausage samples. Sensory evaluation was based on an 8-point scale [48] to determine colour (8, no discoloration; 1, extreme discoloration) and odour (8, extremely desirable; 1, extremely unacceptable/off-odours). On the basis of the above-mentioned attributes, panelist was also asked to score the overall quality of sausage using the same scale. Samples score equal or higher than 4 were considered acceptable. The same above-mentioned re-parameterized Gompertz equation was also used to fit the sensorial data of the second trial and allowed calculating the sensory acceptability limit (SAL), defined as the time at which the overall quality of the product reached the threshold (score = 4) [47].

Shelf life calculation

The shelf life (SL) was calculated as the lowest value between MAL and SAL values of the second trial. In fact, it is the time at which one of the meat quality sub-indices reach the threshold acceptability [46].

Statistical Analysis

Differences between the calculated MAL, SAL and SL values were compared by one-way variance analysis (ANOVA). A Duncan's multiple range tests with the option of homogeneous groups ($P < 0.05$), was used to determine significance between means. STATISTICA 7.1 for Windows (Stat-Soft, Inc, Tulsa, OK, USA) was used for this purpose.

The same statistical software was also used to fit a second-degree polynomial function to the independent variables using the following Eq. (1):

$$Y = \sum (B_{i1} * x_i) + \sum (B_{ii} * x_i^2) + \sum (B_{ij} * x_i * x_j)$$

Where: Y is the dependent variable (log reduction value), B_i , B_{ii} and B_{ij} are regression coefficients of the model, and x_i and x_j are the independent variables in coded values. Using this model, it was possible to determine the effects of linear, quadratic and interactive terms of the independent variables on the dependent one.

Results and Discussion

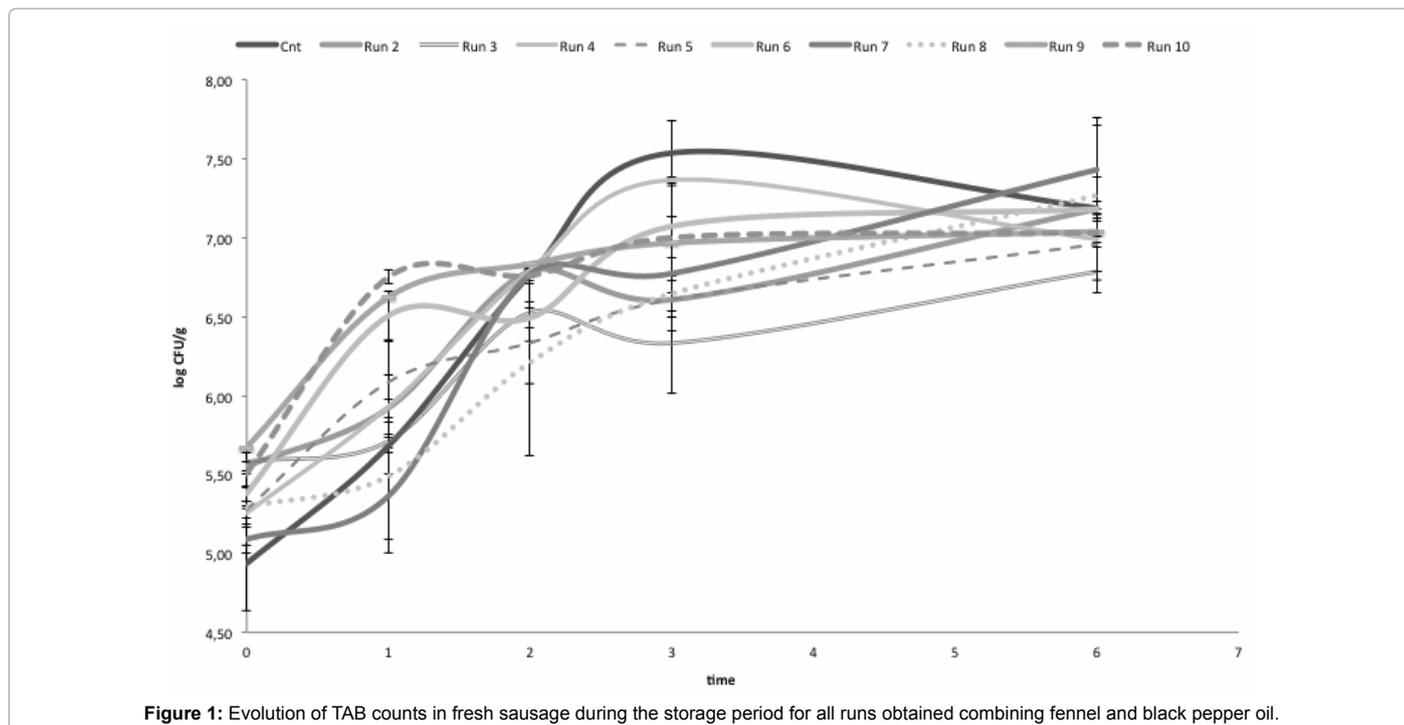
Microbial and sensory qualities were used to determine the shelf life of sausages. In particular, the study was divided into two sequential experimental trials. The first one was aimed to give an insight into the synergic effect of different essential oils to slow down microbial proliferation, and find the optimal essential oil concentration. The second trial was aimed to combine more preservative technologies to MAP. It is worth noting that sausage formulation was carried out in different periods along 1 year, thus different quality of meat was recorded. Therefore, to overcome this issue, the sausage shelf life values were compared with the related control sample prepared in the same experimental batch.

Effects of active compounds on sausages quality

Figure 1 illustrates the evolution of TAB viable cell concentration during the refrigerated storage for all runs obtained combining fennel

and black pepper oil. This combination was chosen because fennel seeds and black peppercorns are usually used in the formulation of fresh sausages [49]. Aerobic plate counts often are chosen as an indicator of the effectiveness of hazard analysis critical control point (HACCP) plans, because data for all aerobic bacteria are more easily collected than data for pathogens of concern or other indicator organisms [50]. There was almost the same trend in all sausage samples; in fact a sharp increase occurred over the storage period. However, the cell load of most of the investigated runs was lower than those of the control (Cnt) along the entire storage period and there are significant differences. Recently there have been considerable studies involving essential oils and extracts of spices and herbs on inhibiting the growth of microbes [51], even though there are limited numbers of studies on food products [52-54]. During application of antimicrobial to foods, interactions between phenolic compounds and some food components can occur [55,56] and their antimicrobial efficacy may be reduced by certain food components [57-59]. Ozcan et al. [14] found that fennel essential oils exhibit an inhibitory effect against a wide range of *Bacillus* species. Mimica-Dukic et al. [13] also reported that the essential oils of fennel are active against *Aspergillus* species. The essential oil of black peppercorn has been shown to possess antimicrobial activity [16, 60-62] also describes the antimicrobial activity of volatile oils of black pepper against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. Finally, Dorman and Deans [16] examined the antimicrobial activity of the volatile oils of black pepper, clove, geranium, nutmeg, oregano and thyme against 25 different genera of bacteria. All the bacterial strains tested showed some degree of sensitivity to volatile oils. The oil with the widest spectrum of antibacterial activity was found to be the one obtained from thyme, followed by oils from oregano, clove, nutmeg, black pepper and geranium.

To compare the antimicrobial effectiveness of different combinations against the TAB group, growth data were expressed as log reduction (Δ), calculated as follows [63]:



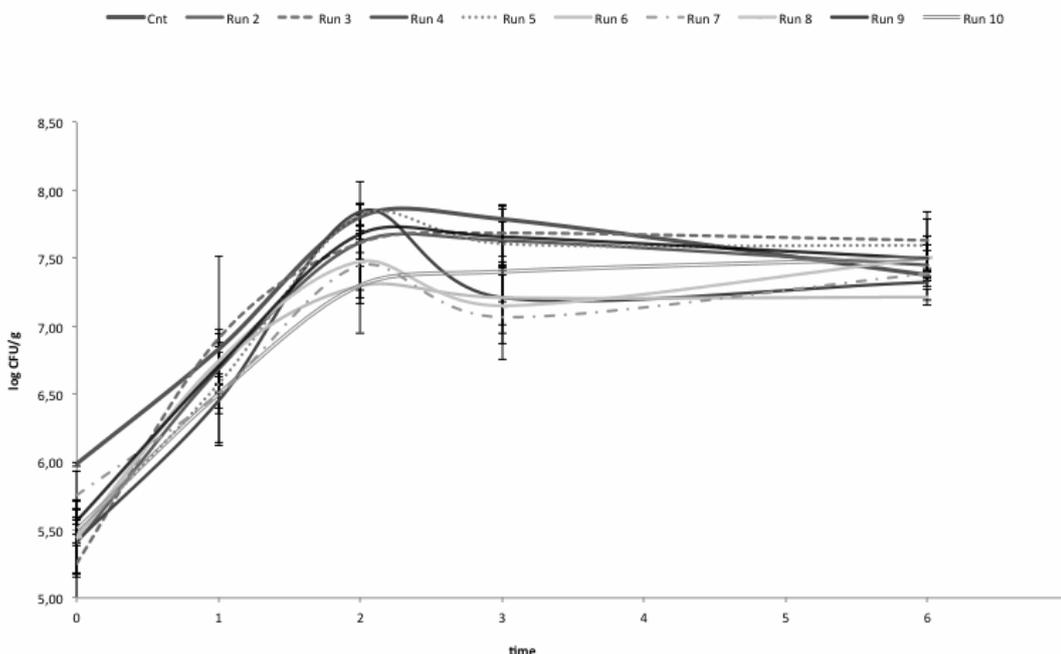


Figure 2: Evolution of TAB counts in fresh sausage during the storage period for all runs obtained combining bay and nutmeg oil.

$$\Delta = \log \text{CFU}_{c,t} - \log \text{CFU}_{a,t}$$

Where $\log \text{CFU}_{c,t}$ is the cell load of TAB in Cnt sample (without oils) at time t and $\log \text{CFU}_{a,t}$ is the cell load of the samples treated with the oils at the same storage time.

The values of log reduction for different times of all monitoring runs were calculated. Some runs were found unsuitable to slow down the load of microorganisms investigated during the storage (data not shown). Overall, a log reduction (Δ) of TAB of about 1 log cycle was observed for the runs 1, 2, 4 and 7 after 3 days of storage; this reduction has become smaller, in some cases it vanished, at the end of storage (6 days) in ordinary conditions.

In general, antimicrobial activity of essential oils depends on both their major constituents and their concentration [64]. The small amounts of minor components might also contribute the antimicrobial activity of the oils [65]. Moreover, their antimicrobial properties depend on genus, species and method of drying, extraction and geographical area (climatic factors) of spices [66]. To date, there are not researches that use the combination of fennel and black pepper oil to extend the shelf life of fresh sausage or in general meat products.

Figure 2 shows the evolution of TAB viable cell concentration during refrigerated storage of samples treated with the combinations of bay and nutmeg oils. This combination was chosen as an alternative to the previous one because the laurel and the nutmeg are sometimes used in the preparation of dishes made of pork meat. Data related to the control show the following trend: an increase in the viable cell concentration until the stationary phase is reached. As can be inferred from data shown in the figure, starting from the one day of storage, there was a strong increase in the cell loads of all samples, even though an antimicrobial effectiveness seems to be exerted in samples treated with essential oil. It is worth noting that in some cases the EO activity decreased considerably when added to a complex food system. For example, Firouzi et al. [67] found that oregano and nutmeg were

effective against *E. coli* O157:H7 in a broth system, but had no effect in ready-to-cook chicken. The presence of high amount of protein and fat requires higher amount of either spices or their essential oils [68], and fat content might prevent penetration of these substances into the cells forming a coating layer on the surface of the cells [69]. In addition, antimicrobial activity against microorganisms involves different modes of action depending on major components of the oils. In general, the oils with high levels of eugenol (clove, bay and cinnamon leaf), cinnamic aldehyde (cinnamon bark, cassia oil) and citral are usually strong antimicrobials [70,71]. The efficacy of runs on the growth of TAB was also determined (data not shown). In particular, run 3, 5, 6 and 7 caused a slight and steady log reduction during refrigerated storage; among these runs, the highest Δ , about 0.7 log cycle, was observed for the run 7 after 3 days of storage. In contrast, no inhibition was obtained in the case of run 1, 2, 4 and 8 during the observed period of storage. Bay oil was found to be effective in the control of *Listeria monocytogenes* in ground chicken breast meat [72]. Similarly, Smith-Palmer et al. [73] determined the bactericidal effects of MIC (Minimum Inhibitory Concentration) values for *L. monocytogenes* and *E. coli* with bay oil of 0.04 and 0.1% (v/v) respectively and they found the bay the most effective one against tested bacteria. Ameen [74] and Jaber [75] found that nutmeg oil had an antibacterial activity against *Streptococcus pyogenes*, *Staph. aureus*, *Pseudomonas aeruginosa* and *E. coli*. To date, there are not studies that use the combination of bay and nutmeg oil to extend the shelf life of fresh sausage.

Dependent variable	Best fit equations	R*	F°	SE§
$\Delta t=3$	$0.464859 [\text{fennel}] - 0.472859 [\text{black pepper}] - 0.350598 [\text{fennel}] [\text{black Pepper}]$	0.97	38.97	0.21
$\Delta t=3$	$0.207 [\text{bay}]$	0.79	15.27	0.26

* Regression coefficient

° Fisher test value

§ Standard error

Table 2: Best fit equations describing the main interactive and quadratic effect of essential oils on log reduction (Δ) values.

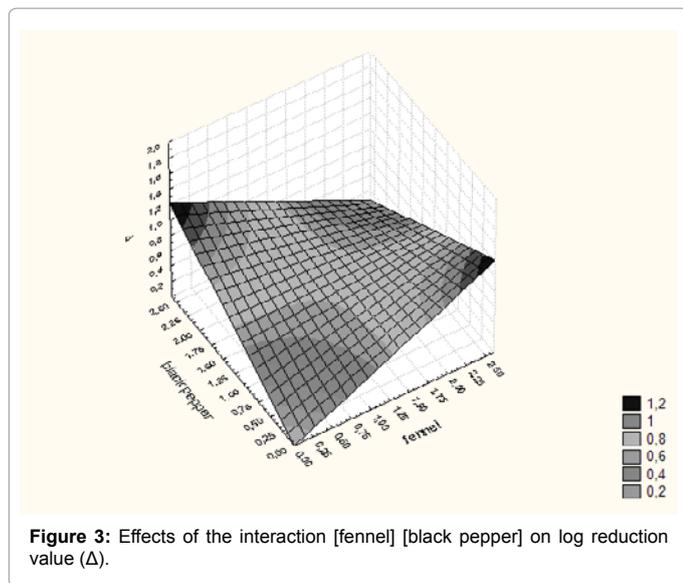


Figure 3: Effects of the interaction [fennel] [black pepper] on log reduction value (Δ).

	MAL	SAL	SL
Cnt-MA	1.91 ± 0.53 ^b	>3	1.91 ± 0.53 ^b
L-MA	>17	15.07 ± 0.48	15.07 ± 0.48 ^d
FP-MA	5.31 ± 1.21 ^a	>7	5.31 ± 1.21 ^a
BN-MA	4.99 ± 0.66 ^a	>6	4.99 ± 0.66 ^a
L-FP-MA	13.75 ± 0.69 ^c	>15	13.75 ± 0.69 ^c
L-BN-MA	18.32 ± 0.58 ^d	>20	18.32 ± 0.58 ^a

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

Table 3: Shelf life (SL) of sausage samples evaluated as the lowest value between the microbial acceptability limit (MAL) and the sensorial acceptability limit (SAL).

Surface methodology was employed to investigate the synergic effect of the selected natural compounds on the quality decay of fresh sausage. The obtained polynomial functions describing these effects are reported in Table 2. The R values indicate the adequacy of the models proposed; the F values indicate a high level of significance ($P < 0.0001$). A three-dimensional surface plot can be advantageously used to assess the influence of the independent variables on Δ values. In particular, 3-D graphs can be obtained by plotting the Δ values against two investigated independent variables (fennel and black pepper oils) (Figure 3). As can be inferred from the figure, a maximum is present when the two essential oils are used individually at the highest concentration or when one of them is at high and the other are used at low concentrations. In contrast, an antagonistic effect was found when the essential oils were used all at the lowest concentration. Therefore, combination of oils may lead to additive, synergistic or antagonistic effects, as stated by Delaquis et al. [76] and Fu et al. [77]. The figure 4 shows the 3-D graph for Δ values of the combination of bay and nutmeg oils. It is interesting to note that a marked log reduction is present when the bay oil is at high concentration and any concentration of nutmeg oil. Unfortunately, a comparison between the selected antimicrobial compounds with published data is very difficult, as the outcome of a test is affected by numerous factors, such as the food matrix and, of course, the source of antimicrobial compounds used [78].

The TAB cell load of the two CCD was experimentally analyzed and the results were statistically compared with the predicted values of the mathematical model. In addition, it has been possible to identify

the optimal concentration to be used for the subsequent trial using the error calculation. In particular, the best combinations are the run 7 made up of 1.25% fennel and 2.5% black pepper and the run 5 made up of 2.5% bay and 1.25% nutmeg.

Effects of combined treatments on sausages quality

The above-mentioned data suggest that the dipping of meat surface with essential oils cannot assure the quality of fresh sausage for long time. Therefore, in this experimental trial, the dipping with EO has been combined with dipping in sodium lactate solution and packaging under MAP. In the Table 3, the MAL values of investigated samples are listed. As can be seen, the Cnt-MA went beyond the threshold after about 2 days of storage. About 5 days were found for samples treated with the optimum concentration of essential oils and packaged under MAP. Chouliara et al. [52] also found that MAP (70% CO 30% N₂) and oregano oil had inhibitory effects on total viable count in fresh chicken meats. On the other hand, the MAL values of sample treated with sodium lactate and packaged under MAP exceeded the monitored storage period (i.e., 17 days). The bacteriostatic property of sodium lactate may result from intracellular acidification [79,80]. Finally, the MAL value of LFP-MA and LBN-MA samples were 13.7 and 18.3 days, respectively. Data suggest that MAP combined with the dipping treatments enhanced microbial stability by inhibiting the proliferation of TAB.

The SAL values are listed in the second column of Table 3. The sensory quality of sausage steadily decreased, regardless of the strategies adopted. As can be inferred, most of the samples are acceptable for the entire sampling period; in contrast, the sample L-MA shows a SAL of about 15. It is interesting to note that the use of essential oils improved the sensory quality of sausage; therefore, a careful selection of them is required. In fact Mastromatteo et al. [46] found that lemon and thymol recorded the highest sensory score combining different MAPs with natural essential oils on shelf life of reduced pork back fat content sausages.

Data on shelf life of investigated sausages are also reported in the third column of the Table 3. As can be inferred from these data, the microbial quality is responsible for the unacceptability of the all samples, except for L-MA sample. However, the combination of dip

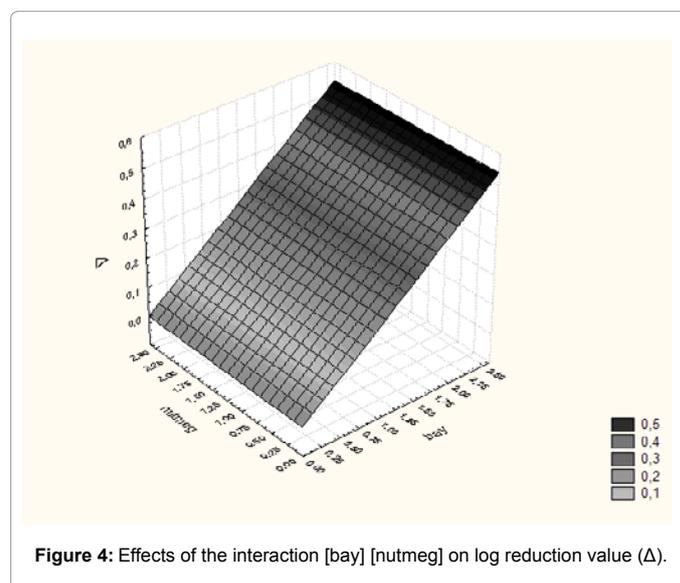


Figure 4: Effects of the interaction [bay] [nutmeg] on log reduction value (Δ).

in 60% sodium lactate, in the essential oils and the packaging under MAP are the most effective treatment in retaining good microbial characteristics. On the contrary, the sensory quality does not limit the shelf life of the investigated food products. In particular, the treatment with antimicrobial compounds improved texture and discoloration of sausages and inhibited off odour formation. In addition to its desirable effect on sensory attributes, sodium lactate has an antimicrobial effect.

Conclusion

These results are in agreement with those of Incoronato et al. [28] who studied the effect of meat dipping prior to grinding in combination with MAP on burgers. They are also in agreement with those reported by Gammariello et al. [27], who reported an increase of ready-to-cook meal shelf life combining dipping of meat in sodium lactate solution and packaging under MAP. Finally, hurdle technology which involves simultaneous multiple preservation approaches has generally met with success in controlling pathogens and maintaining food quality during storage [81]. For the sake of clarity, the percentage shelf life increase of each tested sample was also calculated in comparison with the Cnt-MAP sample; values of 620% and 859% were estimated for LFP-MA and LBN-MA samples, respectively.

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