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Effect of biopreservative cultures on the shelf life of modified atmosphere packaged chicken cocktail sausage

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Abstract: The present study was undertaken to extend the shelf life of modified atmosphere packaged chicken cocktail sausages by using biopreservative cultures (*Lactobacillus sakei* (B-2) and *Lactobacillus curvatus* (B-LC-48)). According to the results, cocktail sausages in control group stored either at 4 °C or 10 °C were spoiled as of day 28 due to a decrease in average flavor score and general acceptance score and increases in mesophilic and psychrotrophic colony counts ($p < 0.05$). In bioprotective cultures treated groups, no spoilage was detected throughout the 60-day storage at 4 °C, whereas those products stored at 10 °C spoiled as of day 42. Results of this study indicated that the bioprotective cultures tested were able to control the spoilage bacteria by establishing bacterial predominance starting from the first day of the shelf life ($p < 0.05$). It was concluded that these cultures can be useful in chicken cocktail sausages production, especially when a proper cold chain cannot be guaranteed during transportation and at retail.

Key words: Biopreservation, lactic acid bacteria, shelf life, chicken cocktail sausage, modified atmosphere packaging

1. Introduction

Chicken meat and meat products are one of the most popular products that become wide spread all around the world. However, these products can easily deteriorate due to microbial contaminations during processing and storage and lead to serious public health problems and economic losses [1]. In fresh or frozen chicken products, pathogenic microorganisms including *E. coli*, *Salmonella* spp., *Listeria monocytogenes*, *Enterococci*, and *Clostridium perfringens* have been found in emulsified meat products due to nonhygienic production practices and storage and transport under inappropriate conditions [1]. In previous studies, *Yersinia* [2], *Campylobacter* [3], *Staphylococcus aureus* [4], *Bacillus cereus* [5], *Salmonella* spp. [6], *Listeria monocytogenes* [7], and *E. coli* [8] have been reported in poultry and meat products.

Many decontamination methods are being used to reduce microbial risks in sausages for extending shelf life, preventing public health, and economic losses. These methods include spray washing, irradiation, modified atmosphere packaging and active packaging, thermal and non-thermal treatments, and chemicals [9]. However, in recent years, consumers' demands are lesser for processed food containing synthetic chemical additives. Therefore, it is stated that some new and natural methods are needed to improve the microbiological, chemical and sensorial quality of foods [10, 11]. Natural preservation methods are being used in sausages include using LAB and their metabolites (bacteriocins etc.), organic acids, plant-derived compounds (herbal extracts and essential oils) and animal-derived substances such as chitosan, lactoferrin, and lysozyme [12]. On the other hand, it is highlighted that each of these methods should be effectively optimized for each food production process [10].

Biopreservation is a natural method, which can be explained as providing food safety and prolonging the shelf life by using controlled or natural microbiota and their antimicrobial products [13]. Controlled and natural microbiota has been widely used as starter cultures in fermented products. In addition, in recent years, it has been successful in raw foods or processed foods except for fermentation. It has been shown in different studies that biopreservation methods inhibited the saprophyte and pathogenic flora in vegetables and fruits, red meat, raw fish meat, and heat-treated meat products without causing sensory changes [14–17]. The most widely used species of lactic acid bacteria (LAB) are *Aerococcus, Bifidobacterium, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Melisococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weisella*

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[18]. LAB are also used as starter culture and probiotic strains besides as a bioprotective culture in the food industry [19]. In addition, bacteriocins (peptide or protein structure) among the natural antimicrobial agents produced by LAB are used in the food industry. LAB species that are used as probiotics are generally indicated to be species belonging to *Lactobacillus* and *Bifidobacterium* [20].

Processed meat products are exposed to various contaminations during production. As a result of these contaminations, both public health problems and economic losses usually occur. Various preservation methods are applied to avoid these problems. The use of bioprotective cultures is a natural conservation method that has increasingly become important. To the best of our knowledge, there is little information in the literature about the effectiveness of the bioprotective cultures on the shelf life of ready-to-eat meat products such as sausages. Therefore, the aim of this study was to determine the effect of bioprotective cultures on the shelf life of modified atmosphere packaged chicken sausages.

2. Material and methods

2.1. Preparations of sausages

This study was carried out in the further-processing department of a commercial broiler slaughterhouse. Chicken sausages were produced using the formulation and cooking processes used by the company and were kept at 4 °C for 1 day before packaging. After peeling off the casing using the casing-peeling machine, the sausages were weighed in 350 g portions and then filled into plastic containers to be packaged with MAP. The treatment applications were applied for sausages before the packing process. There were not any treatment applied for the control group. *Lactobacillus sakei* (B-2 SafePro, Chr-Hansen, Kopenhagen, Denmark) and *Lactobacillus curvatus* (B-LC-48, SafePro, Chr-Hansen, Kopenhagen, Denmark) were applied respectively for each treatment group. Immediately, after bioprotective culture treatments, sausages were packaged with modified atmosphere packaging using 70% N_2, 30% CO_2 gas mixture [21]. Packaged sausages were stored at 2 different temperatures, 4 and 10 °C. Microbiological analyses, sensory attributes and pH value measurements were made in sausages during the storage period. The study was performed as 2 independent replications.

2.2. Preparation and application of bioprotective cultures

Among the LAB, *Lactobacillus* species are the most commonly used cultures for bioprotection [22]. Therefore, two different *Lactobacillus* species were preferred in this study. The bioprotective cultures were obtained as 25 g of lyophilized packages from the Crh-Hansen (Kopenhagen, Denmark) and stored at –18 °C until use. A total of 25 g lyophilized culture dissolved in 500 mL tap water and applied by spraying. For this purpose, 2 mL bioprotective culture sprayed onto the 350 g chicken sausage filled packages, and then sausages were automatically packaged. Subsequently, packages were shaken manually for 2 min to homogenously distribute the bioprotective culture on the whole sausage surfaces. By this way, approximately 5–6 $\log_{10}$ CFU/g bioprotective culture concentration was obtained onto the sausages surfaces.

2.3. Microbiological analyses

Microbiological analyses were carried out on 0, 14, 28, 42 and 60th days with 2 samples (2 different packages). Each sample package was opened under aseptic conditions and 25 g of the sample were weighed into the stomacher bag and 225 ml of 0.1% sterile peptone water (Merck, Darmstadt, Germany) added to the bags, then homogenized for 2 min (Stomacher 400, France). Total viable count (TVC), psychrotrophic bacteria, yeast-molds, *Lactobacillus-Leuconostoc-Pediococcus*, and coliform counts were determined. All microbiological analyses were carried out in duplicate.

Plate Count Agar (PCA) (Merck, Darmstadt, Germany) was used for the total viable counts and psychrotrophic bacterial counts, pour plating method was used and plates were incubated at 35 ± 1 °C 24–48 h and 5–7 °C 7–10 days, respectively [23]. Violet Red Bile Agar (Merck, Darmstadt/Germany) was used for the coliform bacteria count, pour plating method was used, and plates were incubated at 37 ± 1 °C for 24 h [24]. Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Oxoid, UK) was used by spread plating for the yeast-mold count, and the plates were incubated at 25 ± 1 °C for 5 days [25]. For the *Lactobacillus-Leuconostoc-Pediococcus* count, de Man Rogosa Sharpe Agar (MRS) (Biokar BK089HA, France) was used by pour plating, and the plates were incubated at 30 ± 1°C 72 h [26].

2.4. Sensory and pH analyses

Ten grams of samples were weighed into the sampling bag and 90 mL disttilled water was added onto the bag, then homogenized and pH values (25 ± 1°C) were determined by using digital pH meter (HI, 11310, Hanna Instruments, USA). As it is known, since sausages are ready-to-use products, sensory analyses were performed directly without any heat treatment. Sensory analyses were evaluated by the 10 trained panelists (10) on 0, 14, 28, 42 and 60th days of the storage period in terms of flavor, color, odor, texture, juiciness, sliminess, brittleness, appearance, and general acceptability parameters by using Hedonic scale range between 0 and 9 points. 5 point was selected as the lowest acceptable level [27].

2.5. Statistical analyses

All statistical analyzes were carried out by using SPSS package program version 21.0 [28]. Microbiological data

were converted to $\log_{10}$ CFU/g and subjected to statistical analyzes. The mean values of microbiological, sensory and pH data among groups and sampling days were compared by using analysis of variance (ANOVA) post-hoc Tukey's test. Statistical significance level was accepted as $p < 0.05$.

3. Results

3.1. Total viable count

Total viable counts were given in Table 1. Although, in the control group stored at 4 and 10 °C, TVC were found as 3.23 $\log_{10}$ CFU/g on day 0, these counts were continuously increased during the storage period and reached to 5.68 and 7.18 $\log_{10}$ CFU/g at 60th day, respectively ($p < 0.05$). Total viable counts in the B2 and B-LC-48 groups stored at 4 °C were found around 6–7 $\log_{10}$ CFU/g on day 0 due to inoculation of bioprotective cultures. During the storage period, the differences between the initial and 60th days were found insignificant in both groups ($p > 0.05$), except B2 stored at 10 °C.

In the B2 group stored at 10 °C, TVC significantly increased during the storage period. The count was found as 8.7 $\log_{10}$ on the 28th day, and it was significantly higher than 0 and 14th days ($p < 0.05$). For the B-LC-48 group, microbial stability at 10 °C was maintained, and the differences among storage days were not significant ($p > 0.05$), except between 42nd and 60th days. TVC in the B2 and B-LC-48 groups were found generally higher than the control group in the first 3 weeks of the storage. However, these differences were gradually decreased from 4 weeks and disappeared in some groups (Table 1).

3.2. Psychrotrophic bacteria

There was a significant increase in the number of psychrotrophic bacteria in the control group at 4 °C between 0 to 60 days (2.58–4.95 $\log_{10}$ CFU/g) ($p < 0.05$). Psychrotrophic bacteria counts were decreased in B2 and B-LC-48 groups (6.78–4.85 $\log_{10}$ CFU/g) following a trend contrary to the control group, but these decreases were not significant, except for B2 at 4 °C (Table 2).

Psychrotrophic bacteria counts in the control group at 10 °C, which represents the poor storage conditions, increased rapidly from the initial number of 2.58 $\log_{10}$ CFU/g to 4.38 within 2 weeks and then gradually increased during storage and reached to 6.48 $\log_{10}$ CFU/g in 60 days ($p < 0.05$). It was found that the number of psychrotrophic bacteria in bioprotective culture treated groups were lower than the control groups in both storage temperature at the end of the storage period. Although there were no differences among the sampling days in the B2 group at 10 °C ($p > 0.05$), there was a significant decrease only between day 0 and day 60 in the B-LC-48 group ($p < 0.05$). When the differences among the groups were taken into consideration during the sampling days, no significant differences were found for both storage temperatures, except for day 0 ($p < 0.05$).

3.3. Lactobacillus-Leuconostoc-Pediococcus

Findings on the numbers of *Lactobacillus-Leuconostoc-Pediococcus* were given in Table 3. In the control group, it was found that the number of bacteria increased continuously during the storage period, and the differences were significant at both storage temperatures ($p < 0.05$). In the B2 and B-LC-48 groups, statistical difference was not detected at 4 °C, while it was detected at 10 °C ($p < 0.05$). It was found that the differences among the groups disappeared on the 42nd day at 4 °C and 10 °C ($p > 0.05$).

3.4. Yeast-mold and coliform

The numbers of yeast-molds during storage in all groups were shown in Table 4. Yeast-mold counts were found to be approximately 2.0 $\log_{10}$ CFU/g in all groups on the initial

Table 1. Changes in total viable count (TVC) numbers in chicken cocktail sausages during the storage period ($\log_{10}$ CFU/g±SD).

Storage Temperature	Groups	Sampling days				
		0	14	28	42	60
4 °C	Control	3.23 ± 0.21^{Cy}	3.73 ± 0.69^{BCy}	4.93 ± 0.73^{ABz}	5.0 ± 0^{Ay}	5.68 ± 0.29^{Ay}
	B2	6.83 ± 0.09^{ABx}	6.40 ± 0.18^{ABx}	5.9 ± 0.72^{Byz}	7.63 ± 0.94^{ABx}	8.18 ± 1.77^{Ax}
	B-LC-48	7.1 ± 0.27^{x}	8.85 ± 0.19^{x}	6.53 ± 0.35^{yz}	6.25 ± 0.65^{xy}	7.15 ± 1.17^{xy}
10 °C	Control	$3.23 \pm 0,21^{Cy}$	4.6 ± 1.3^{BCy}	5.65 ± 1.22^{AByz}	6.33 ± 1.42^{ABxy}	7.18 ± 0.36^{Axy}
	B2	6.83 ± 0.09^{BCx}	6.15 ± 0.21^{Cx}	8.7 ± 0.63^{Ax}	7.63 ± 0.9^{ABx}	8.05 ± 0.3^{Ax}
	B-LC-48	7.1 ± 0.27^{ABx}	7.05 ± 0.3^{ABx}	6.87 ± 0.15^{ABy}	6.48 ± 0.75^{Bxy}	7.83 ± 0.61^{Ax}

$^{A-C}$: Values with different letters in the same line are statistically different ($p < 0.05$). **$^{x-z}$:** Values with different symbols in the same column are statistically different ($p < 0.05$). Control: without any treatment; B-2: *Lactobacillus sakei* treated sausage samples; B-LC-48: *Lactobacillus curvatus* treated sausage samples.

Table 2. Changes in the number of psychrotrophic bacteria numbers in chicken cocktail sausages during storage (log_{10} CFU/g±SD).

Storage Temperature	Groups	Sampling days				
		0	14	28	42	60
4 °C	Control	2.58 ± 0.59^{By}	4.27 ± 1.19^{AB}	4.35 ± 0.26^{A}	4.80 ± 0.79^{A}	4.95 ± 0.82^{A}
	B2	6.78 ± 0.15^{Ax}	5.80 ± 0.14^{B}	4.78 ± 0.63^{B}	5.93 ± 0.53^{AB}	4.87 ± 1.50^{B}
	B-LC-48	6.98 ± 0.05^{Ax}	4.0 ± 0.14^{B}	3.93 ± 0.82^{B}	5.08 ± 1.45^{AB}	4.85 ± 0.75^{AB}
10 °C	Control	2.58 ± 0.59^{Cy}	4.38 ± 0.36^{B}	5.50 ± 0.85^{AB}	5.90 ± 0.71^{A}	6.48 ± 0.61^{A}
	B2	6.78 ± 0.15^{x}	5.90 ± 0.14	6.55 ± 2.18	5.23 ± 1.59	4.88 ± 1.35
	B-LC-48	6.98 ± 0.05^{Ax}	6.10 ± 1.13^{AB}	5.08 ± 1.26^{AB}	5.68 ± 0.92^{AB}	4.0 ± 1.29^{B}

$^{A-C}$: Values with different letters in the same line are statistically different ($p < 0.05$). **$^{x-z}$:** Values with different symbols in the same column are statistically different ($p < 0.05$). Control: without any treatment; B-2: *Lactobacillus sakei* treated sausage samples; B-LC-48: *Lactobacillus curvatus* treated sausage samples.

Table 3. Changes in *Lactobacillus-Leuconostoc-Pediococcus* numbers in chicken cocktail sausages during the storage period (log_{10} CFU/g±SD).

Storage Temperature	Groups	Sampling days				
		0	14	28	42	60
4 °C	Control	2.93 ± 0.73^{By}	3.08 ± 0.6^{Bz}	4.38 ± 0.29^{Az}	5.08 ± 0.5^{Ay}	5.1 ± 0.64^{Az}
	B2	6.78 ± 0.1^{x}	6.33 ± 0.15^{x}	6.38 ± 0.26^{xy}	6.18 ± 0.64^{xy}	6.63 ± 0.68^{xy}
	B-LC-48	6.18 ± 1.16^{x}	5.85 ± 0.6^{x}	6.33 ± 0.39^{xy}	5.55 ± 1.05^{y}	5.53 ± 0.4^{yz}
10 °C	Control	2.93 ± 0.73^{Cy}	4.63 ± 0.56^{BCy}	5.63 ± 1.07^{AByz}	6.15 ± 1.09^{ABxy}	7.03 ± 0.47^{Awx}
	B2	6.78 ± 0.1^{ABx}	6.13 ± 0.21^{Bx}	7.6 ± 0.9^{Ax}	7.58 ± 1.13^{Ax}	8.08 ± 0.15^{Aw}
	B-LC-48	6.18 ± 1.16^{Bx}	6.0 ± 0.18^{Bx}	6.5 ± 0.77^{ABxy}	7.6 ± 0.71^{ABx}	7.93 ± 0.47^{Aw}

$^{A-C}$: Values with different letters in the same line are statistically different ($p < 0.05$). **$^{w-z}$:** Values with different symbols in the same column are statistically different ($p < 0.05$). Control: without any treatment; B-2: *Lactobacillus sakei* treated sausage samples; B-LC-48: *Lactobacillus curvatus* treated sausage samples.

Table 4. Changes in the number of yeast-mold in chicken cocktail sausages during the storage period (log_{10} CFU/g±SD).

Storage Temperature	Groups	Sampling days				
		0	14	28	42	60
4 °C	Control	1.98 ± 0.94^{C}	3.45 ± 0.62^{Bxy}	3.95 ± 0.31^{AB}	4.8 ± 0.36^{Axy}	4.85 ± 0.52^{Ax}
	B2	1.95 ± 0.82^{D}	3.08 ± 0.22^{Cy}	4.1 ± 0.22^{AB}	4.75 ± 0.26^{Axy}	3.7 ± 0^{Bcy}
	B-LC-48	1.68 ± 0.26^{B}	3.28 ± 0.38^{Ay}	3.85 ± 0.73^{A}	4.28 ± 0.5^{Ay}	3.7 ± 0.23^{Ay}
10 °C	Control	1.98 ± 0.94^{B}	3.93 ± 0.69^{Axy}	4.38 ± 0.4^{A}	5.15 ± 0.3^{Ax}	4.55 ± 0.06^{Ax}
	B2	1.95 ± 0.82^{C}	4.4 ± 0.22^{ABx}	4.93 ± 0.52^{A}	4.28 ± 0.43^{ABy}	3.45 ± 0.29^{By}
	B-LC-48	1.68 ± 0.26^{C}	4.03 ± 0.41^{ABxy}	4.53 ± 0.74^{A}	4.35 ± 0.29^{ABxy}	3.6 ± 0.12^{By}

$^{A-D}$: Values with different letters in the same line are statistically different ($p < 0.05$). **$^{x-z}$:** Values with different symbols in the same column are statistically different ($p < 0.05$). Control: without any treatment; B-2: *Lactobacillus sakei* treated sausage samples; B-LC-48: *Lactobacillus curvatus* treated sausage samples.

day. However, control group samples showed a continuous increase at both temperatures (4 and 10 °C) during the storage period ($p < 0.05$).

In the B2 and B-LC-48 groups, a decrease was observed after 42 days at 4 °C, and after 28 days at 10 °C, and these differences were found significant ($p < 0.05$). In general, there were no statistical differences between the different storage temperatures of all the groups ($p > 0.05$). In addition, there were no statistical differences among the groups kept at the same storage temperature during the whole storage period ($p > 0.05$), except for the 60th day at 4 °C. Coliform bacteria were not detected in all groups and on analysis days.

3.5. Sensory analysis and pH value

Appearance and general acceptability scores were shown in Tables 5 and 6. It was determined that all the groups were taken 8.4 points out of 9 on the initial day in terms of the appearance. In the B2 and B-LC-48 groups on the 60th day (7.7 and 6.3), while the scores of the groups decreased, they were still acceptable at 4 °C. In the control group, at 42nd days there was no evaluation made because of the visual deterioration. In the B2 and B-LC-48 groups which stored at 10 °C, no evaluation was performed since signs of visual deterioration were detected on 60th day. However, there were no statistical differences among the groups and the storage temperatures ($p > 0.05$).

It was found that the control, B2, and B-LC-48 groups were taken 8.6, 8.1 and 8.1 points at 4 °C storage temperature, respectively. In the B2 and B-LC-48 groups scored 8.0 and 8.3 points at 60th days and no statistically significant differences were found ($p > 0.05$). Since all the groups displayed evident visual deterioration signs at 10 °C, no evaluation was performed on 60th day. There were no statistical differences found among the groups in the days which evaluations were performed ($p > 0.05$) (Table 6). In addition, it was determined that there were no statistical differences in flavor, color, odor, texture, juiciness, sliminess, and brittleness among the groups (p

Table 5. Appearance scores of the chicken cocktail sausages during the storage (Mean±SD).

Storage Temperature	Groups	Sampling days				
		0	14	28	42	60
4 °C	Control	8.4 ± 0.79	8.7 ± 0.58	7.7 ± 0.52	-*	-*
	B2	8.4 ± 0.79	8.3 ± 1.15	7.0 ± 1.67	6.7 ± 0.58	7.7 ± 1.50
	B-LC-48	8.4 ± 0.79	8.0 ± 1.0	7.3 ± 0.82	7.7 ± 0.58	6.3 ± 2.83
10 °C	Control	8.4 ± 0.79	8.7 ± 0.58	6.2 ± 2.48	-*	-*
	B2	8.4 ± 0.79	8.0 ± 1.0	6.5 ± 1.87	7.7 ± 0.58	-*
	B-LC-48	8.4 ± 0.79	8.7 ± 0.58	7.5 ± 0.84	7.3 ± 0.58	-*

* Sensory analyses were not performed because of the signs of deterioration detected. Control: without any treatment; B-2: *Lactobacillus sakei* treated sausage samples; B-LC-48: *Lactobacillus curvatus* treated sausage samples.

Table 6. General acceptability scores of the chicken cocktail sausages during the storage (Mean±SD).

Storage Temperature	Groups	Sampling days				
		0	14	28	42	60
4 °C	Control	8.6 ± 0.53	8.3 ± 0.58	7.8 ± 0.98	-*	-*
	B2	8.1 ± 0.69	7.7 ± 1.53	7.3 ± 0.82	7.0 ± 1.0	8.0 ± 0.5
	B-LC-48	8.1 ± 1.07	8.3 ± 0.58	7.7 ± 1.03	7.3 ± 1.15	8.3 ± 0.82
10 °C	Control	8.6 ± 0.53	8.0 ± 1.0	6.7 ± 1.75	-*	-*
	B2	8.1 ± 0.69	8.0 ± 1.0	7.2 ± 0.75	7.7 ± 0.58	-*
	B-LC-48	8.1 ± 1.07	8.3 ± 0.58	7.5 ± 0.55	8.0 ± 0,	-*

* Sensory analyses were not performed because of the signs of deterioration detected. Control: without any treatment; B-2: *Lactobacillus sakei* treated sausage samples; B-LC-48: *Lactobacillus curvatus* treated sausage samples.

> 0.05) (data not shown). As to pH value, there was no statistical differences among the groups and sampling days ($p > 0.05$). Regardless of the storage temperature, and treatment groups, pH values ranged from 6.24 to 6.91 between day 0 and day 60.

4. Discussion

The aim of this study was to investigate the effect of *Lactobacillus sakei* (B-2) and *Lactobacillus curvatus* (B-LC-48) cultures on the shelf life of chicken sausages with modified atmosphere packaging at 4 °C and 10 °C. The results showed that both protective cultures generally increased shelf life without adversely affecting the sensory properties of the products compared with the control group. According to the sensory quality parameters, the products in the control group were deteriorated after 28 days at both storage temperatures, while the protective cultures treated groups spoiled after 60 days at 4°C and after 42 days at 10 °C.

Although the use of different LAB for extending the shelf life of foods and/or to provide inhibition of important pathogens has long been the subject of scientific research, it wasn't long before these cultures have become commercial products. Antimicrobial effects of bioprotective cultures are explained by the producing the organic acids, peroxides, carbon dioxide, bacteriocins, decreasing the Eh value, the superior competitive properties, and the synergistic effects among these factors [29, 30]. Most of the researches are related to the preservation of raw meats (beef, poultry, seafood, hamburgers, frozen meatballs, minced meat) under various atmospheric conditions [14, 17, 31–38]. In addition, the studies which used bioprotective cultures in ready-to-eat meat products were mainly focused on the inhibition of *Listeria monocytogenes*. It has been reported that *L. sakei* showed an inhibitory effect due to produce anti-listerial effective bacteriocin in heat-treated bacon [39], heat-treated sausages [34, 40] and fermented sausages [41]. The effect of *L. curvatus* on extending the shelf life was mostly investigated in raw red meat and chicken meat [34, 35] and frozen meatballs [38]. The cocktail sausage is a heat-treated emulsified and ready to eat meat product. Meat source (bovine, poultry, pork, etc.), spice mix, product size (long, short), packaging type (vacuum package, MAP) may vary according to consumer demands.

Although it is generally heat-treated product above 70 °C, shelf life problems are very common. This product is generally exposed to a significant amount of externally microbial contamination in the production stages. *Listeria* spp. and lactic acid bacteria are the most common species among the microorganisms that cause deterioration and threaten the health of consumers. Güngör & Gökoğlu (2010) [42] reported that determining the sources of contamination in a commercial sausage production line, the highest source of contamination points were raw material (7.04 $\log_{10}$ CFU/g) and spice mix (7.84 $\log_{10}$ CFU/g) and followed by the staff and the surfaces of the equipment. In the same study, it was also stated that the peeling of the sausages after heat treatment did not cause a significant difference in the bacterial load.

In the literature review, no study was found about the chicken sausages used in this study. However, there is a limited number of studies on sausages made from beef or pork meat [43, 44]. Łaszkiewicz et al. (45] applied the *Lactiplantibacillus plantarum* SCH1 strain (7.0 $\log_{10}$ CFU/g) into the cooked sausages produced from mechanically separated poultry meat, and stored for 3 weeks in cold storage. It was found that microbial quality of *Lb. plantarum* treated samples were better than that of control samples after 3 weeks of storage. In addition, Milani et al. [43] applied the *Lactobacillus alimentarius* to the surface of the sausage with 10^7 CFU/cm^2 to examine the effect on shelf life in frankfurter-type sausages and stored them in vacuum packaged for 8 weeks at 5 °C and 10 °C. In this study, it was reported that during the 56-day storage period the number of TMAB in the *Lactobacillus alimentarius* inoculated group was lower than control group 2.0 and 1.0 $\log_{10}$ CFU/g at 5 and 10 °C, respectively. A similar difference was also found for psychrotrophic bacteria. In terms of sensory qualities, the control group was deteriorated earlier than other groups. This study has the most similar design, and these findings are the most similar ones in the literature to our study. However, the culture and packaging type are different. Milani et al. [43] reported that vacuum packaging deteriorated due to irrigation and gas production. This type of deterioration does not occur in the MAP. Therefore, it is considered advantageous to use the bioprotective cultures applied in the present study in combination with MAP conditions.

In the present study, *Lactobacillus-Leuconostoc-Pediodoccus* spp. (LLP) counts, due to the inoculated cultures, in the B2 and B-LC-48 groups (6.18-678 $\log_{10}$ CFU/g) were found higher than the control group (2.93 $\log_{10}$ CFU/g) at day 0. However, the LLP count in the control group was rapidly increased and reached the same count as treatment groups. The differences were disappeared on the 28th day at 4 °C and the 14th day at 10 °C between the control and treatment groups. Although there was a rapid change in the control group, the LLP numbers in the B2 and B-LC-48 groups did not change for 60 days. However, there was a 1 $\log_{10}$ increase in treatment groups stored at 10 °C (Table 3). This shows that the bioprotectives cultures differ from the endogenous LLP in the control group, do not grow during the storage period and suppress the reproduction of other flora members. It also has been reported in other studies that high levels of an inoculated

number of bioprotective cultures remain unchanged on the food surface for a long time is desirable [43]. If the number of biocultures was low levels and they grew like endogenous flora, it would be inevitable that the product would be degraded. The main reason for the deterioration in the control group since 28 days was thought to be the inability to stop the reproduction of LLPs.

The current study has confirmed once again that the protective effects of bioprotective cultures are closely related to storage temperature [43, 44]. In the B2 and B-LC-48 groups, there was no deterioration for 60 days at 4 °C representing ''proper cold chain temperature", whereas deterioration started after 42 days at 10 °C representing ''poor refrigerator conditions". In the literature, some information relating to the shelf life of the products, especially meat products, is available with the number of TVC or LAB. Adams et al. [46] reported that sausages of can be considered as spoiled when TVC number reached 10^6 CFU/g. Baumgart et al. [47] also reported that the highest acceptable LAB count in vacuum packaged sausages as 10^6 CFU/g. However, these values may not always be a definite limit. In the present study, the deterioration was determined according to the sensory properties in the control group stored at 4 °C and on the 42nd day. This day the number of TVC and LAB were found as of 5.0 $\log_{10}$ and LLP 5.08 $\log_{10}$ CFU/g, respectively. In the sausages stored at 10 °C, the days of deterioration were the same, but the TVC and LLP numbers were above the 6.0 $\log_{10}$ CFU/g. In the B2 and B-LC-48 groups, deterioration was not observed in sensory properties, although it was well above the limit of degradation due to inoculated microorganisms. Kara [48] reported in a study that deterioration days were determined for TVC numbers as 17–19 days in vacuum packaging sausage samples of two different companies. The course of psychrotrophic bacterial counts, although at lower levels, was similar to that of TVC. It can be assumed that psychrotrophic flora, such as *Pseudomonas* spp., which is the most important bacteria in the deterioration of the products stored at low temperatures, can be suppressed or inhibited by the bioprotective cultures.

No significant difference was observed in yeast and molds counts among the groups during the storage period in both storage temperatures. Although numbers of yeast and molds in control groups in both storage temperatures were continuously increased, in the treatment groups gradually increased until day 28 at 10 °C and until day 42 at 4 °C, and then exhibited a trend to decrease until day 60. However, this trend was not significant except for the B2 group. In the B2 group, the decrease from day 28 to day 60 at 10 °C was found to be significant. It can be interpreted based on these data that *L. sakei* and *L. curvatus* have no effect on the number of yeast-mold at 4 °C, and they have limited effect at 10 °C. There are numerous data on the inhibitory effect of lactic acid bacteria on yeast and molds [49, 50]. The possible reason why this effect was not seen obviously in the present study could be explained by the lack/absence of antifungal effects of commercially available *L. sakei* and *L. curvatus* strains or by interactions in MAP conditions. In the present study, pH values at 4 °C and 10 °C during the storage period started at around 6.7–6.9 and changed slightly throughout the storage period in all groups. This situation may be due to the use of additives with high buffers such as polyphosphates in the production process and the high buffering capacity of chicken breast meat.

5. Conclusion

As a result, it has been shown that *L. sakei* and *L. curvatus* strains used in this study dominated initial microflora from day 0 of production of chicken sausages and controlled the degrading bacteria even under poor refrigeration conditions. It is particularly concluded that the use of these cultures would be beneficial in the production of chicken sausages and in transport and retail applications in which proper cold chain conditions are hard to be met.

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References

1. Rouger A, Tresse O, Zagorec M. Bacterial contaminants of poultry meat: sources, species, and dynamics. Microorganisms 2017; 5 (3): 50. doi: 10.3390/microorganisms5030050

2. Peng Z, Zou M, Li M, Liu D, Guan W et al. Prevalence, antimicrobial resistance and phylogenetic characterization of *Yersinia enterocolitica* in retail poultry meat and swine feces in parts of China. Food Control 2018; 93: 121–128. doi: org/10.1016/j.foodcont.2018.05.048

3. Pillay S, Amoako DG, Abia ALK, Somboro AM, Shobo CO et al. Characterisation of *Campylobacter* spp. isolated from poultry in KwaZulu-Natal, South Africa. Antibiotics 2020; 9 (2): 42. doi:10.3390/antibiotics9020042

4. Li Q, Li Y, Tang Y, Meng C, Ingmer H, et al. Prevalence and characterization of *Staphylococcus aureus* and *Staphylococcus argenteus* in chicken from retail markets in China. Food Control 2019; 96: 158-164. doi:10.1016/j.foodcont.2018.08.030

5. Tahmasebi H, Talebi R, Zarif BR. Isolated of *Bacillus Cereus* in chicken meat and investigation β-lactamase antibiotic-resistant in Bacillus Cereus from chicken meat. Advances in Life Sciences 2014; 4 (4): 200-206. doi: 10.5923/j.als.20140404.03

6. Ferrari RG, Rosario DK, Cunha-Neto A, Mano SB, Figueiredo E et al. Worldwide epidemiology of *Salmonella* serovars in animal-based foods: A meta-analysis. Applied and Environmental Microbiology 2019; 85 (14): e00591-19. doi: 10.1128/AEM.00591-19

7. Iannetti L, Schirone M, Neri D, Visciano P, Acciari V et al. *Listeria monocytogenes* in poultry: Detection and strain characterization along an integrated production chain in Italy. Food Microbiology 2020; 103533. doi:10.1016/j.fm.2020.103533

8. Cyoia PS, Koga VL, Nishio EK, Houle S, Dozois CM et al. Distribution of ExPEC virulence factors, blaCTX-M, fosA3, and mcr-1 in *Escherichia coli* isolated from commercialized chicken carcasses. Frontiers in Microbiology 2019; 9: 1-9. doi:10.3389/fmicb.2018.03254

9. Zhou GH, Xu XL, Liu Y. Preservation technologies for fresh meat–A review. Meat Science 2010; 86: 119–128. doi: 10.1016/j.meatsci.2010.04.033

10. Rosario DKA, Rodrigues BL, Bernardes PC, Conte-Junior CA. Principles and applications of non-thermal technologies and alternative chemical compounds in meat and fish. Critical Reviews in Food Science and Nutrition 2020; 1-21. doi: 10.1080/10408398.2020.1754755

11. Yin MC, Cheng WS. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Science 2003; 63: 23–28. doi:10.1016/S0309-1740(02)00047-5

12. Hugo CJ, Hugo A. Current trends in natural preservatives for fresh sausage products. Trends in Food Science & Technology 2015; 45: 12-23. doi: org/10.1016/j.tifs.2015.05.003

13. Gálvez A, Abriouel H, López RL, Omar NB. Bacteriocin-based strategies for food biopreservation. International Journal of Food Microbiology 2007; 120 (1-2): 51–70. doi: 10.1016/j.ijfoodmicro.2007.06.001

14. Trias R, Badosa E, Montesinos Bañeras EL. Bioprotective *Leuconostoc* strains against *Listeria monocytogenes* in fresh fruits and vegetables. International Journal of Food Microbiology 2008; 127 (1-2): 91-98. doi: 10.1016/j.ijfoodmicro.2008.06.011

15. Ghanbari M, Jami M, Domig KJ, Kneifel W. Seafood biopreservation by lactic acid bacteria: A review. LWT-Food Science and Technology 2013; 54 (2): 315-324 doi: 10.1016/j.lwt.2013.05.039

16. Favaro L, Penna ALB, Todorov SD. Bacteriocinogenic LAB from cheeses: Application in biopreservation?. Trends in Food Science & Technology 2015; 41 (1): 37-48. doi: 10.1016/j.tifs.2014.09.001

17. Miettinen MK, Palmu L, Björkroth KJ, Korkeala H. Prevalence of *Listeria monocytogenes* in broilers at the abattoir, processing plant, and retail level. Journal of Food Protection 2001; 64 (7): 994–999. doi: 10.4315/0362-028x-64.7.994

18. Alvarez-Sieiro P, Montalbán-López M, Mu D, Kuipers OP. Bacteriocins of lactic acid bacteria: Extending the family. Applied Microbiology and Biotechnology 2016; 100 (7): 2939–2951. doi: 10.1007/s00253-016-7343-9

19. Giraffa G, Chanishvili N, Widyastuti Y. Importance of *Lactobacilli* in food and feed biotechnology. Research in Microbiology 2010; 161 (6): 480-487. doi: 10.1016/j.resmic.2010.03.001

20. Campana R, van Hemert S, Baffone W. Strain-specific probiotic properties of lactic acid bacteria and their interference with human intestinal pathogens invasion. Gut Pathogens 2017; 9 (12): 1-12. doi: 10.1186/s13099-017-0162-4

21. Tovunac I, Galic K, Prpic T, Juric S. Effect of packaging conditions on the shelf-life of chicken frankfurters with and without lactate addition. Food Science and Technology International 2011; 17: 167–175. doi: 10.1177/1082013210381952

22. de Angelis M, Gobetti M. *Lactobacillus* spp. General characteristics. Reference module in food science. In:. Fuquay J W. Encyclopedia of dairy sciences. 2nd ed. Academic Press. 2011. pp. 78-90.

23. USDA/FSIS. 2011. Microbiology Laboratory Guidebook. Metot 3.01. Quantitative analysis of bacteria in foods as sanitary indicators.

24. ISO 4832:2006 (E). 2006. Microbiology of food and animal feding stuffs-horizontal method for the enumeration on coliforms-colony-count technique.

25. ISO 21527-1: 2008(en). 2008. Microbiology of food and animal feeding stuffs —horizontal method for the enumeration of yeasts and moulds — Part 1: colony count technique in products with water activity greater than 0,95.

26. ISO 15214:1998(en). 1998. Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of mesophilic lactic acid bacteria-colony-count technique at 30 degrees C.

27. Cardello AV. Measuring consumer expectations to improve food product development. In: Macfie H J H (Ed.), Consumer-led food product development. Cambridge: Woodhead publishing. 2007. pp. 223–261.

28. IBM SPSS, IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: USA.

29. Moradi M, Kousheh SA, Almasi H, Alizadeh A, Guimarães JT et al. Postbiotics produced by lactic acid bacteria: The next frontier in food safety. Comprehensive Reviews in Food Science and Food Safety 2020; 19: 3390-3415. doi: 10.1111/1541-4337.12613

30. Castellano P, Belfiore C, Fadda S, Vignolo G. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. Meat Science 2008; 79: 483-499. doi: 10.1016/j.meatsci.2007.10.009

31. Reiter MG, Bueno CM, López C, Jordano R. Occurrence of *Campylobacter* and *Listeria monocytogenes* in a poultry processing plant. Journal of Food Protection 2005; 68 (9): 1903-6. doi: 10.4315/0362-028x-68.9.1903

32. Mor-Mur M, Yuste J. Emerging bacterial pathogens in meat and poultry: an overview. Food and Bioprocess Technology 2010; 3 (24). doi: 10.1007/s11947-009-0189-8

33. Melero B, Diez AM, Rajkovic A, Jaime I, Rovira J. Behaviour of non-stressed and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells on fresh chicken burger meat packaged under modified atmosphere and inoculated with protective culture. International Journal of Food Microbiology 2012; 158 (2): 107-12. doi: 10.1016/j.ijfoodmicro.2012.07.003

34. Maragkoudakis PA, Mountzouris KC, Psyrras D, Cremonese S, Fischer J et al. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella* Enteritidis. International Journal of Food Microbiology 2009; 130: 219–226. doi: 10.1016/j.ijfoodmicro.2009.01.027

35. Castellano P, Vignolo G. Inhibition of *Listeria innocua* and *Brochothrix thermosphacta* in vacuum-packaged meat by addition of bacteriocinogenic *Lactobacillus curvatus* CRL705 and its bacteriocins. Letters in Applied Microbiology 2006; 43 (2): 194–199. doi: 10.1111/j.1472-765X.2006.01933.x

36. Hu P, Xu XL, Zhou GH, Han YQ, Xu BC et al. Study of the *Lactobacillus sakei* protective effect towards spoilage bacteria in vacuum packed cooked ham analyzed by PCR–DGGE. Meat Science 2008; 80: 462–469. doi:10.1016/j.meatsci.2008.01.011

37. Schillinger U, Kaya M, Lücke FK. Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. Journal of Applied Bacteriology 1991; 70: 473-477. doi: 10.1111/j.1365-2672.1991.tb02743.x

38. Castellano P, Belfiore C, Vignolo G. Combination of bioprotective cultures with EDTA to reduce *Escherichia coli* O157:H7 in frozen ground-beef patties. Food Control 2011; 22 (8): 1461-1465. doi: 10.1016/j.foodcont.2011.02.018

39. Comi G, Andyanto D, Manzano M, Iacumin L. *Lactococcus lactis* and *Lactobacillus sakei* as bioprotective culture to eliminate *Leuconostoc mesenteroides* spoilage and improve the shelf life and sensorial characteristics of commercial cooked bacon. Food Microbiology 2016; 58: 16-22. doi: 10.1016/j.fm.2016.03.001

40. Vermeiren L, Devlieghere F, Vandekinderen I, Rajtak U, Debevere J. The sensory aceptability of cooked meat products treated with a protective culture depends on glucose content and buffering capacity: a case study with *Lactobacillus sakei* 10A. Meat Science 2006; 74 (3): 532-545. doi: 10.1016/j.meatsci.2006.05.003

41. Gao Y, Li D, Liu X. Bacteriocin-producing *Lactobacillus sakei* C2 as starter culture in fermented sausages. Food Control 2014; 35 (1): 1-6. doi: 10.1016/j.foodcont.2013.06.055

42. Güngör E, Gökoğlu N. Determination of microbial contamination sources at a frankfurter sausage processing line. Turkish Journal of Veterinary and Animal Science 2010; 34: 53-59. doi: 10.3906/vet-0805-28

43. Milani LIG, Fries LLM, Boeira LS, Bessa LS, Melo V et al. Bioprotection of frankfurter sausages. Acta Alimentaria 1998; 27: 221-229.

44. Andersen L. Biopreservation with Flora Carn L-2. Fleischwirtsch 1995; 75: 1327–1329.

45. Łaszkiewicz B, Szymanski P, Zielinska D, Kołozyn-Krajewska D. Application of *Lactiplantibacillus plantarum* SCH1 for the bioconservation of cooked sausage made from mechanically separated poultry meat. Applied Sciences 2021; 11: 1576. doi: 10.3390/app11041576

46. Adams MR, Baker T, Forrest CL. A note on shelf-life extension of British fresh sausage by vacuum packing. Journal of Applied Microbiology 1987; 63 (3): 227-231. doi: 10.1111/j.1365-2672.1987.tb04940.x

47. Baumgart J. 1990. In: Mikrobiologische Untersuchung von Lebensmitteln, Behr's Verlag, Hamburg.

48. Kara S. A research on the determination of shelf life of vacuum packaged sausages. Masters Thesis, Ankara University, Graduate School of Natural and Applied Sciences. 1994.

49. Sadiq FA, Yan B, Tian F, Zhao J, Zhang H et al.. Lactic acid bacteria as antifungal and anti-mycotoxigenic agents: A comprehensive review. Comprehensive Reviews in Food Science and Food Safety 2020; 18: 1403-1436. doi: 10.1111/1541-4337.12481

50. Brosnan B, Coffey A, Arendt EK, Furey A. Rapid identification, by use of the LTQ orbitrap hybrid FT mass spectrometer, of antifungal compounds produced by lactic acid bacteria. Analytical and Bioanalytical Chemistry 2012; 403 (10): 2983-2995. doi: 10.1007/s00216-012-5955-1