SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 20(3) (2022) 232-243, DOI: 10.24323/akademik-gida.1186928

Research Paper / Araştırma Makalesi

Comparative Effects of Probiotic, Prebiotic, L-Arginine, and Fenugreek on Some Quality Criteria of Fermented Red Meat Pâtè

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Received (Geliş Tarihi): 01.08.2022, Accepted (Kabul Tarihi): 02.10.2022
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ABSTRACT

The different combinations of bioactive compounds, probiotics (*Streptococcus thermophilus* ATCC 19258 and *Lactobacillus bulgaricus* BAA-2844), prebiotic (fructooligosaccharides, FOS), fenugreek, and L-arginine, were added to the pâtè meat. Some pâtè meats were contaminated with *Salmonella Typhimurium* ATCC 14028 and *Listeria monocytogenes* ATCC 7644. Fermentation was applied only to batches containing 'probiotic' or 'probiotic and prebiotic' at two different temperatures, 22 and 37°C. Although fermentation at 37°C in combination with the additions of 'probiotics, prebiotic and arginine' and 'probiotics, prebiotic and fenugreek' resulted in 2.51 and 2.36 log (cfu/g) reductions on total mesophile aerobic bacterial (TMAB) counts, respectively, these treatments lowered the pH values of pâtè below 4.20 on the 22nd day of storage and caused an uncontrolled fermentation with a sourish taste. On the other hand, the combined additions of 'probiotics, prebiotic, fenugreek and arginine' or 'probiotics, prebiotic and arginine' or 'probiotic, prebiotic and fenugreek' in combination with fermentation at 22°C, caused reductions on TMAB counts between 1.01-1.09 log (cfu/g) with a constant bacteriostatic effect, and extended shelf life 10 days while improving the sensory quality. The addition of fenugreek inhibited *Listeria monocytogenes* more whereas the antimicrobial effect of L-arginine was more significant on *Salmonella Typhimurium*. The antimicrobial effect of adding the bioactive compounds in combination with fermentation at 22°C could eliminate the pathogens in the contaminated pâtè meat batches, causing 5.91 and 6.11 log (cfu/g) reductions on the counts of *Salmonella Typhimurium* and *Listeria monocytogenes,* respectively.

Keywords: Probiotic, Prebiotic, Red meat pâtè, Bioactive food compound, Microbial quality, Sensory quality

Probiyotik, Prebiyotik, L-Arginin ve Çemen Otunun Ezme Kırmızı Etin Bazı Kalite Kriterleri Üzerine Olan Karşılaştırmalı Etkileri

ÖZ

Araştırmada ezme (pâtè) sığır eti gruplarına probiyotik mikroorganizmalar; *Streptococcus thermophilus* ATCC 19258 ve *Lactobacillus bulgaricus* BAA-2844, prebiyotik (fruktooligosakkarit, FOS), toz halde çemen otu tohumu (*Trigonella foenum graecum*, fenugreek) ve L-arginin amino asidinin farklı kombinasyonları ilave edilmiş, takiben bu ezme et gruplarından "probiyotik" veya "probiyotik ve prebiyotik" ilave edilmiş olanlar 22 ve 37°C'de olmak üzere iki farklı sıcaklıkta 72 saat süre ile fermentasyon işlemine alınmıştır. "Probiyotik, prebiyotik ve arginine" ve "probiyotik, prebiyotik ve çemen out" ilaveleri, 37°C'deki fermentasyonla birleştirildiğinde, 4°C'de soğutma koşullarında muhafazaya alınan ezme etinin depolamanın 22. gününde, toplam mezofilik aerobik bakteri (TMAB) sayısını, sırasıyla 2.51 ve 2.36 log (kob/g) düzeylerinde baskılamıştır. Ancak aynı işlemler pH düzeyini 4.20 düzeyinin altına düşürerek ezme etinde aşırı ekşi tada neden olmuştur. "Probiyotik, prebiyotik, çemen otu ve arginine" veya "probiyotik, prebiyotik ve arginine" veya "probiyotik, prebiyotik ve çemen out" ilaveleri, 22°C'deki fermentasyonla birleştiğinde ise TMAB yükünü 1.01-1.09 log (kob/g) şeklinde çok daha az düzeyde baskılamış, buna karşılık bu uygulamaların duyusal

kaliteyi korurken daha kontrollü bir fermentasyona ve sürekliliği olan mikrobiyostatik bir etkiye sahip olduğu belirlenmiştir. Ezme ete çemen otu ilavesinin *Listeria monocytogenes* üzerinde çok daha baskılayıcı etkiye sahip olduğu, arginin ilavesinin ise *Salmonella Typhimurium* üzerindeki baskılayıcı etkisinin daha fazla olduğu tespit edilmiştir. Probiyotik, prebiyotik, çemen otu ve arginin'in farklı kombinasyonlarının ezme kırmızı ete ilavelerinin, sıcaklık fark etmeksizin fermentasyon işlemi ile birleştirildiğinde, *Salmonella Typhimurium* ve *Listeria monocytogenes* bakterilerini sırası ile 5.91 ve 6.11 log (kob/g) düzeylerinde baskıladığı ortaya koyulmuştur.

Anahtar Kelimeler: Probiyotik, Prebiyotik, Ezme kırmızı et, Biyoaktif gıda bileşeni, Mikrobiyel kalite, Duyusal kalite

INTRODUCTION

The pâtė meat products are launched on the market after cooking and pasteurization. The rich nutrient and water contents, higher a_w, 0.99, grinding, and cooking make the product susceptible to microbial contamination. The most important critical point is the low cooking temperatures like ≤ 63°C during production [1]. The higher temperatures than 63°C would lead to a tough and dry texture which prevents spreading and the creamy structure. As a result, the low temperatures during the cooking process and higher a_w, often lead to lower microbial quality. Confirmingly, the pork pâtė meat has a short shelf life which is only between 4 and 5 days under cooling storage conditions between 2 to 8°C [2]. Therefore, fermentation was applied for the first time in this study to extend the very short shelf life of the red meat pâtė with the sole and/or different combined additions of the food bioactive compounds; L-Arginine (L-Arg, A), Fenugreek (F), the probiotics (Lactic Acid Bacteria, LAB, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and the prebiotic (fructooligosaccharide, FOS, Pr).

Meat has always brought concerns about safety and economic loses together due to its fat, susceptibility to spoilage and food poisonings [3]. *Salmonella Typhimurium* and *Listeria monocytogenes*, the pathogenic organisms as the causative agents of nontyphoidal salmonellosis and listeriosis, which are characterized by gastroenteritis, bacteremia, meningitis, and abortus, are great trouble for particularly a wide range of immunocompromised individuals [4, 5]. The foods with highest risk for public health is the processed, 'ready to eat' food products under cooling stoage for long periods [6]. Among the foods which have the highest risk of contaminating these pathogens is pâtė meat since both of the pathogenic organisms tend to grow at ≥ 9°C, 1-2% salt concentrations, and 7.0 pH [7] which already exists in pâtė meat.

The hydrogen-bond and ionic interactions of the guanidinium group of L-Arginine (A) which is one of the bioactive food compounds and the amino acid in the study, enhance membrane permeability and cause an antimicrobial effect [8]. The main health benefit of the amino acid comes from its crucial role in the production of nitric oxide (NO) which reduces high blood pressure.

One of the bioactive compounds in the study is the seed of the medicinal plant *Trigonella foenum-graecum L.* (commonly known as Fenugreek, F) with its rich contents of ascorbic acid and various phenols. The ethanol extract of the seed (400 µg/mL) inhibits breast cancer cells, MCF-7 [9].

When the probiotics which inhibit pathogenic microorganisms and induce fermentation in pâtė meat in this study are considered, *Str. thermophilus* poses a curing effect against rotavirus and antibiotic-induced diarrhea and reduces cholesterol while the other probiotic, *L. bulgaricus,* inhibits the secretion of inflammatory cytokines [10]. One of the other study materials, Pr (the prebiotic which is a fructooligosaccharide, FOS) has medicinal functions in the prevention of gastritis, peptic ulcer, and obesity via their stimulating effect on the probiotics. Its recommended daily safe consumption amount is between 2 and 12 g [11].

The study aims to determine the effect of fermentation, based on the additions of the probiotics and/or prebiotics in pâtė meat, on *S. Typhimurium* and *L. monocytogenes,* shelf life, and overall quality of the pâtė meat.

MATERIALS and METHODS

In the study, 24 pâtė meat batches were treated with the sole and various combinations of the LAB (*Str. Thermophilus* and *L. bulgaricus),* A, F, and Pr. The other 24 batches in which the same treatments were made were artificially contaminated by *S. Typhimurium* and *L. monocytogenes*. The ones with the 'LAB' or 'LAB and Pr' additions in the non-contaminated and contaminated batches were taken to fermentation either at 22 or 37°C for 72 hours. The batches which contained neither 'LAB' nor 'LAB and Pr' were directly transferred to the cooling storage under 4°C since they would not have been fermented.

Preparation of Red Meat Pâtė

Tenderloin (*Musculus psoas major*) of the male beef meat at 3 years (31 months) was selected. The internal meat temperature of 30 kg of meat, followed by its trimming from surface fat and connective tissue, was lowered to 18°C in the 11th hour and then below 5°C gradually in the first 48 hours to prevent cold shortening. The meat was transferred to the final cooling at 7°C, 85% relative humidity, and air ventilation at 1 m/sec for 72 hours. It was cooked in water in the scalding vat (Brokelmann, 6080500/120 L; 400V, Bemak Mechanics, Istanbul) for 25 minutes until the internal temperature reached 65°C. 24 kg from the cooked meat was separated and transferred into the grinder (YKF 130/Capacity: 1000 kg/h, 7.5 kW, 2 blades and 2 mm

grinding plate, YKF Meat Process Machines, Istanbul). Because of the higher melting point, the ratio of the tail fat was kept to only 10%, and another 10% was added from tallow fat (6 kg fat+24 kg meat). The tail and tallow fat were frozen at –18°C for 24 h. 24 kg cooled, and cooked sirloin was chopped and ground with the chunks of 6 kg of the frozen inner and tail fat mixture in the grinder. The grinding process was repeated 4 times. The cooked ground meat with fat was taken into the mixing vat (SMG KR250, 4.5 kW, capacity: 150 kg, Seymag, Hungary). 11.5% water, 2% salt, 2% milk powder, 1% sodium caseinate, 0.5% potassium phosphate, 0.05% sodium nitrite, and 0.025% sodium ascorbate, the additives whose amounts which were previously described [12], were added. Also, red pepper (0.7%), black pepper (0.7%), cumin (0.7%), and rendered garlic (2%), were added to give the aroma and taste of Turkish sausage. The meat, fat, and other ingredients were mixed for 5 minutes and the mixed dough was taken into the grinder. In the grinder, an additional two circulations of grindings were made.

Control and Experimental Pâtė Meat Batches

The pâtė meat was divided into 48 groups, each of which contained 500 g of pâtė meat, separately. Sterilized aluminum foils, gloves, disposable sterilized spatulas, and knives were used. Then, each 500 g of pâtė meat was placed separately into the glass jars with aluminum screws on the sides with a vacuum cover. Before their use, glass jars were sterilized in an autoclave at 120°C for 15 min. The 24 pâtė meat batches were treated with the sole and various combinations of the LAB; *Str. thermophilus* and *L. bulgaricus,* A, F, and Pr. The other 24 batches in which the same treatments were made were inoculated by *S. Typhimurium* and *L. monocytogenes*. The ones with the 'LAB' or 'LAB and Pr' additions in the non-contaminated and contaminated batches were fermented in the incubator (MCO-5AC-PE IncuSafe, Nijverhelsweg, The Netherlands), as in the glass jars with aluminum screws as tightly closed. Anaerobic fermentation was applied. The non-fermented ones without any 'LAB' or 'LAB and Pr' were directly transferred to the cooling storage under 4°C. Fermentation was held at two different temperatures at 22 and 37°C for 72 h. The surface of each of the pâtė meat batches was covered with 120 mL olive oil (120 mL/500g) to avoid oxygen. The time and temperature combinations (22 or 37°C/72 h) applied for the fermentation in this study are very close to the time and temperature combination in one of the previous studies [13], which was between 16 and 25°C/24-48 h. After placing the pâtė meats into the jars (500 g/jar), the LAB containing batches, 10 mL from the pure culture of *Str. thermophilus*, which contains 10^{11} cfu/mL of the bacteria, and 10 mL from the pure culture of *L. bulgaricus*, which contains 10^{11} cfu/mL of the bacteria, were inoculated into the 500 g of pâtė meat in the jar ($2x10^9$ cfu/g *Str. thermophilus* and $2x10^9$ cfu/g *L. bulgaricus,* in 500 g of pâtė meat). After the inoculation of the LAB and/or the bioactive compounds, the pâtė meat was mixed homogenously by using a sterilized spatula and disposable knife. The addition of the probiotics in the probiotic (LAB) added batches was coded as 'LAB'. In the F-containing batches, F was added as 35 g and its addition was coded as 'F'. In the A-containing batches, A was added as 20 g and its addition was coded as 'A'. The Pr (Fructo-oligosaccharide, FOS) amount was 8 g (16mg/g, 1.6%) in the Pr-containing batches and the coding was made as 'Pr'. All of the codings were made accordingly, like in the example of 'LABPrFA', which means 'the treatment with the addition of LAB, Pr, F, and A'. The other 24 pâtė meat batches in which the same treatments were made, were contaminated with a 10 mL inoculum from the pure culture of *S. Typhimurium* which contained 10^7 cfu/mL *S. Typhimurium* (10^8 cfu/500 g, $2x10^5$ cfu/g). Then, these contaminated pâtė meat batches were also contaminated with a 10 mL from the pure culture of *L. monocytogenes* which contained 10^7 cfu/mL *L. monocytogenes* (10^8 cfu/500 g, $2x10^5$ cfu/g). The contamination was coded as 'SL' in the *Salmonella* and *Listeria* contaminated batches. Finally, one of the batches contained only pâtė meat and was kept as control without any contamination or bioactive compound (the code, C). The fermented pâtė meat batches were transferred to the cooling storage under 4°C after 72 h of fermentation. The pâtė meats that were taken to fermentation at 37°C were marked with the black down-pointing triangle,'▼', e.g., 'LAB▼', to differentiate them from the ones which were fermented at 22°C. Totally 24 non-contaminated treatment batches are shown in Table 1, and the 24 contaminated ones take place in Figure 1. Also, the pâtė meats above which were treated with the various combined additions of the bioactive food compounds and fermented at different temperatures were classified separately below to make it more comprehensible.

Pâtė Meats Fermented at 37°C

The following treatment batches; LAB▼ (only Lactic Acid Bacteria added), LABA▼ (Lactic Acid Bacteria and L-Arginine added), LABF▼ (Lactic Acid Bacteria and Fenugreek added), LABFA ▼ (Lactic Acid Bacteria, Fenugreek and L-Arginine added), LABPr▼ (Lactic Acid Bacteria and Prebiotic added), LABPrA▼(Lactic Acid Bacteria, Prebiotic and L-Arginine added), LABPrF▼(Lactic Acid Bacteria, Prebiotic and Fenugreek added), and LABPrFA▼(Lactic Acid Bacteria, Prebiotic, Fenugreek, and L-Arginine added), were taken to the fermentation at 37 °C.

Pâtė Meats Fermented at 22°C

The counterparts of the batches above in which the same bio-active compounds were added, are as follows; LAB (only Lactic Acid Bacteria added), LABA (Lactic Acid Bacteria and L-Arginine added), LABF (Lactic Acid Bacteria and Fenugreek added), LABFA (Lactic Acid Bacteria, Fenugreek, and L-Arginine added), LABPr (Lactic Acid Bacteria and Prebiotic added), LABPrA (Lactic Acid Bacteria, Prebiotic and L-Arginine added), LABPrF (Lactic Acid Bacteria, Prebiotic and Fenugreek added), and LABPrFA (Lactic Acid Bacteria, Prebiotic, Fenugreek, and L-Arginine added). Differently, these pâtė meats were taken to fermentation at 22°C. So, the pâtė meats which contained 'LAB' or 'LAB and Pr' were

fermented either at 37°C or 22°C to be able to compare the effect of temperature on the pâtė meats.

Non-Fermented Pâtė Meats

The following pâtė meat batches which contained neither 'LAB' nor 'LAB and Pr' are as follows; Pr (only Prebiotic added), PrA (Prebiotic and L-Arginine added), PrF (Prebiotic and Fenugreek added), PrFA (Prebiotic, Fenugreek, and L-Arginine added), A (only L-Arginine added), C (the pâtė meat without any of the bioactive food compound), F (only Fenugreek added) and FA (Fenugreek and L-Arginine added). These pâtė meats were directly transferred to the cooling storage under 4°C.

Contaminated Pâtė Meats Fermented at 37°C

The following contaminated pâtė meats were fermented at 37°C. These are; SLLABPr▼ (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria and Prebiotic added), SLLABPrA▼ (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria, Prebiotic and L-Arginine added), SLLABPrF▼ (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria, Prebiotic and Fenugreek added), SLLABPrFA▼ (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria, Prebiotic, Fenugreek and L-Arginine added), SLLAB▼ (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria added), SLLABA▼ (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria and L-Arginine added), SLLABF▼ (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria and Fenugreek added), SLLABFA▼(*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria, Fenugreek and L-Arginine added).

Contaminated Pâtė Meats Fermented at 22°C

The following pâtė meat batches were fermented at 22°C**.** These are as follows; SLLABPr (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria and Prebiotic added), SLLABPrA (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria, Prebiotic and L-Arginine added), SLLABPrF (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria, Prebiotic and Fenugreek added), SLLABPrFA (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria, Prebiotic, Fenugreek and L-Arginine added), SLLAB (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria added), SLLABA (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria and L-Arginine added), SLLABF (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria and Fenugreek added), SLLABFA (*S. Typhimurium* and *L. monocytogenes* inoculated, Lactic Acid Bacteria, Fenugreek and L-Arginine added).

Contaminated and Non-Fermented Pâtė Meats

The following contaminated pâtė meat batches which contained neither 'LAB' nor 'LAB and Pr' are as follows; SL (*S. Typhimurium* and *L. monocytogenes* inoculated), SLFA (*S. Typhimurium* and *L. monocytogenes* inoculated, Fenugreek and L-Arginine added), SLPrA (*S. Typhimurium* and *L. monocytogenes* inoculated, Prebiotic and L-Arginine added), SLPrFA (*S. Typhimurium* and *L. monocytogenes* inoculated, Prebiotic, Fenugreek, and L-Arginine added), SLF (*S. Typhimurium* and *L. monocytogenes* inoculated, Fenugreek added), SLPr (*S. Typhimurium* and *L. monocytogenes* inoculated, Prebiotic added), SLPrF (*S. Typhimurium* and *L. monocytogenes* inoculated, Prebiotic and Fenugreek added) and SLA (*S. Typhimurium* and *L. monocytogenes* inoculated, and L-Arginine added). These pâtė meats were directly transferred to the cooling storage under 4°C. So, 24 non-contaminated and 24 contaminated pate meats (Table 1 and Figure 1) were produced.

All of the batches were taken for analysis after the completion of the 3 days of fermentation. The analyses were made on the 3rd. 5th., 10th., 15th. and 22nd day of the shelf life under 4°C. *Str. thermophilus* ATCC 19258, *L. bulgaricus* BAA-2844, *S. Typhimurium* ATCC 14028, and *L. monocytogenes* ATCC 7644 had been purchased from Hıfzısıhha Refik Saydam Research Center (Culture Collection. Ministry of Health. Ankara. Turkey) as freeze-dried long before the study.

pH Measurement

pH was measured in a pH meter (PeakTech 530. China, resolution: 0.01 pH, range: 0-14) with 10 mL supernatant containing 5 g of pâtė meat and 90 mL distilled water for each batch of the pâtė meat.

Microbial Analysis

25 g of pâtė meat (as 12.5 g from the surface and 12.5 g from the 4 cm depth in the glass jar) was taken and added to 225 mL of distilled water. The solution was placed in the stomacher and mixed for 30 seconds. Then, 10 mL of supernatant by using a 10 mL sterilized pipette was transferred into a 10 mL sterilized test tube. The dilutions up to 10^{-15} were prepared by using sterilized 1mL pipettes. All of the microbial counts were made by pour plating. Plate Count Agar (PCA. Oxoid. UK), Rogosa Agar (Oxoid, UK), *Salmonella Shigella* Agar (Oxoid, UK), and Chromogenic *Listeria* Agar (Oxoid, UK) were used in the enumerations of Total Mesophile Aerobic Bacteria (TMAB), LAB, *S.Typhimurium* and *L. monocytogenes*, respectively [14, 15, 16, 17]. The agar solutions were poured into the sterilized Petri dishes. Because *Lactobacilli* and *L. monocytogenes* prefer a microaerophilic atmosphere, in the enumeration of LAB, a second layer from the Rogosa Agar and in the enumeration of *L. monocytogenes*, a second layer from the Chromogenic *Listeria* Agar, was overlayed. The Petri dishes were incubated at 35°C for 3 days and 2 days for the colony formation and enumeration of LAB and TMAB, respectively. In the enumeration of *S. Typhimurium* and *L. monocytogenes*, the Petri dishes were incubated at 37°C for 24-48 h.

Table 1. The 3., 5., 10., 15. and 22. day pH levels, TMAB and LAB counts of the non-contaminated pate meat batches ich were evaluated in the discrimitaion test

			pH				TMAB*					LAB**			
LAB▼	5.42	5.41	5.38	5.37	5.23	4.53	4.79	5.14	5.29	7.19	4.79	5.23	5.91	6.34	7.29
LABA ▼	5.43	5.41	5.35	5.33	5.2	4.37	4.69	5.04	5.11	6.23	4.94	5.83	6.39	6.58	7.33
LABF ▼	5.44	5.43	5.35	5.34	5.22	4.38	4.78	5.06	5.23	6.39	4.65	5.33	5.35	5.39	5.81
LABFA▼	5.4	5.25	4.59	4.23	4.09	4.35	4.54	4.88	5.54	6.83	4.64	5.81	6.41	6.79	7.49
LABPr▼	5.51	5.42	5.41	5.28	5.2	4.34	4.2	4.87	5.72	6.9	4.8	5.74	6.81	7.44	8.81
LABPrA▼	5.38	5.21	4.39	4.21	4.02	4.31	4.41	4.75	4.87	5.31	4.9	5.41	6.39	6.95	7.34
LABPrF▼	5.39	5.13	4.6	4.34	4,19	4.4	4.74	4.83	4.99	5.46	4.95	5.37	6.37	6.49	7.17
LABPrFA▼	5.41	5.3	4.7	4.4	4.15	4.27	4.31	5.29	5.11	6.67	4.71	5.6	6.45	7.34	8.74
LAB	5.61	5.57	5.52	5.45	5.22	4.45	4.9	5.24	5.96	7.3	4.24	4.3	4.97	5.33	5.81
LABA	5.64	5.6	5.53	5.4	5.2	4.5	4.7	5.08	5.72	7.04	4.2	4.16	5.01	5.46	6.09
LABF	5.62	5.6	5.49	5.41	5.2	4.51	4.76	5.18	5.8	7.14	4.18	4.86	5.17	5.52	6.17
LABFA	5.6	5.51	5.44	5.32	5.12	4.46	4.63	4.92	5.61	6.91	4.21	4.26	5.06	5.68	6.18
LABPr	5.51	5.32	5.28	5.18	4.91	4.13	4.93	4.87	5.72	6.9	4.24	4.21	5.01	5.27	6.34
LABPrA	5.32	5.24	5.17	5.02	4.81	4.28	4.62	4.73	5.34	6.73	4.38	4.61	5.12	5.87	6.82
LABPrF	5.36	5.28	5.2	5.11	4.83	4.16	4.42	4.73	5.37	6.81	4.41	4.74	5.71	6.74	7.45
LABPrFA	5.27	5.46	5.01	4.91	4.71	4.07	4.87	4.62	5.51	6.76	4.47	4.96	5,84	6.92	7.94
Pr	5.6	5.59	5,55	5.5	5.4	4.71	4.86	5.32	6.04	7.34	4.01	4.11	4.38	4.94	5.31
PrA	5.62	5.51	5.44	5.33	5.21	4.55	4.71	5.22	5.88	7.18	4.21	4.29	4.71	5.1	5.55
PrF	5.65	5.57	5.48	5.33	5.19	4.59	4.74	5.2	5.8	7.17	3.04	3.41	3.88	4.24	4.7
PrFA	5.59	5.47	5.34	5.27	5.17	4.51	4.67	5.04	5.73	7.08	3.18	3.55	4.07	4.41	4.91
A	5.62	5.61	5.57	5.47	5.31	4.61	4.79	5.19	5.81	7.24	4.61	4.79	5.19	5.81	7.24
C	5.85	6.09	6.34	6.44	6.56	4.72	6.07	6.48	6.97	7.82	2.58	2.77	3.51	4.04	4.52
F	5.61	5.57	5.56	5.52	5.42	4.79	4.93	5.44	6.21	7.44	3.91	4.02	4.24	4.68	5.11
FA	5.68	5.69	5.74	5.81	5.94	4.7	4.84	5.37	5.96	7.21	2.55	2.72	2.9	3.56	4.33
Day	3	5	10	15	22	3	5	10	15	22	3	5	10	15	22

*: TMAB: Total Mesophile Aerobic Bacteria, **: LAB: Lactic Acid Bacteria, ***: d: day

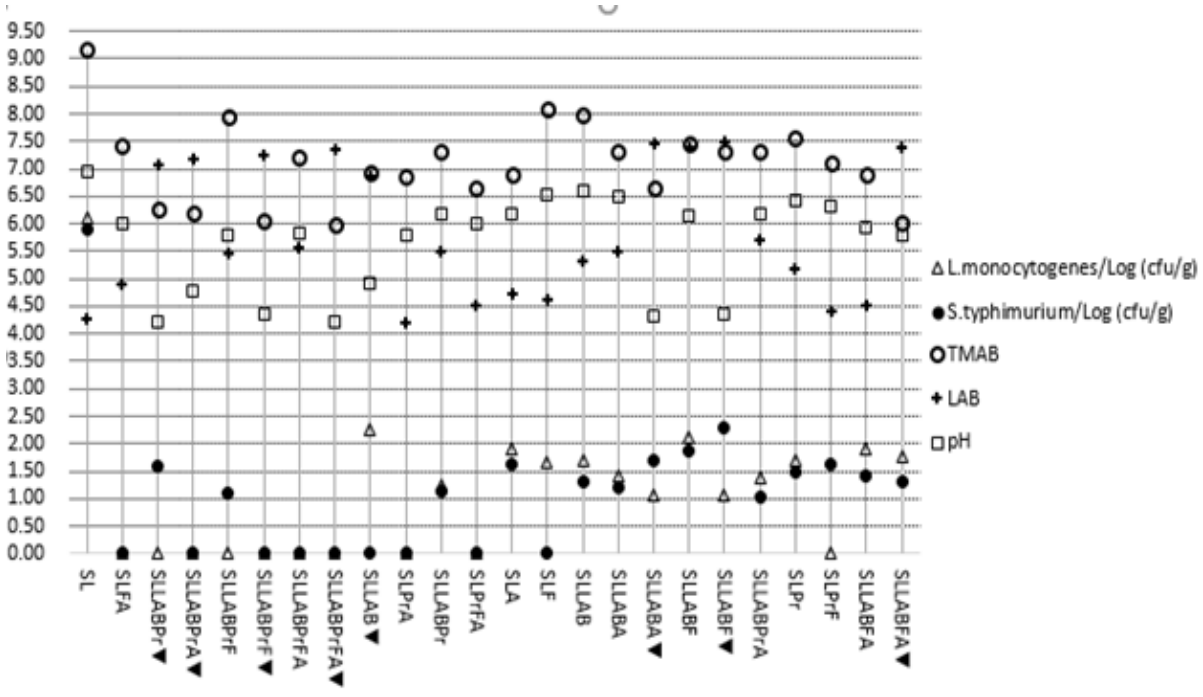


Figure 1. The most significant treatments which have the highest inhibitions on *S. Typhimurium* and *L. monocytogenes*. LAB and TMAB counts and pH levels on the 22nd day

Sensory Quality Determination

Among 48 batches, only the non-contaminated batches with TMAB counts below the maximum tolerable amount of TMAB. 6.00 Log (cfu/g), on the 15th day, was taken to the discrimination test. So, F and Pr batches that had 6.21 and 6.04 Log (cfu/g) TMAB counts, respectively, on the 15th. day, were eliminated and not included in the discrimination test. After the completion of the microbial counting and pH analysis, the pâtė meat batches and control, except F and Pr, were produced as fresh once again for the sensory analysis. These batches with their TMAB counts, pH levels on the 15th day and the panelist scores in the discrimination and descriptive tests are shown in Table 2.

Table 2. The total acceptance numbers from the panelists in the discrimination test and the scores for each criteria in the descriptive test

OAD[2]		Log**	pH	D[1] texture	D[1] taste	D[1] color	D[1] aroma	Σ	Di*Σ taste	Di*Σ color	Di*Σ texture	Di*Σ[3] aroma
391	LABPr▼	5.51	5.28	15	13	12	11	51	104	105	93	89
348	LAB	5.96	5.45	15	12	12	11	50	91	91	85	81
359	PrA	5.88	5.33	15	13	12	11	51	100	92	87	80
472	LABPrF	5.37	5.11	15	14	13	12	54	117	122	92	97
400	PrFA	5.73	5.27	15	13	12	12	52	104	107	140	121
515	LABPrFA	5.51	4.91	15	14	14	14	57	126	128	138	118
501	LABPrA	5.34	5.02	15	14	12	14	55	118	127	108	92
429	LABPr	5.72	5.18	15	14	13	12	54	113	116	113	104
445	FA	5.96	5.81	15	13	12	13	53	111	117	87	87
379	PrF	5.8	5.33	15	13	11	12	51	103	102	94	87
377	LABFA	5.61	5.32	15	13	12	11	51	100	96	91	84
372	LABF▼	5.23	5.34	15	13	12	11	51	102	95	94	85
392	LABA	5.72	5.4	15	13	12	12	52	107	106	86	84
352	LABF	5.8	5.41	15	12	11	12	50	96	86	101	92
406	LAB▼	5.29	5.37	15	13	13	12	53	105	108	109	104
440	LABA▼	5.11	5.33	15	13	13	12	53	113	114	82	91
	A	5.81	5.47	14	11	10	9	44				
	LABFA▼	5.54	4.23	14	8	10	11	43				
	LABPrA▼	4.87	4.21	13	7	10	10	40				
	LABPrF▼	4.99	4.34	14	8	12	11	45				
322	C	6.97	6.44	10	11	9	9	39	85	85	74	78
	LABPrFA▼	5.11	4.46	14	8	12	12	46				

**: TMAB Log (cfu/g), D[1]: Discrimination, Di*: Descriptive, [2]: Overall score of the points from each criterion in the descriptive test, [3]: Total

Discrimination of the pâtė meat batches was evaluated as 'accepted' and 'rejected' from the point of ‘taste’, ‘color’, ‘texture’, and ‘aroma’ [18]. When the number of acceptance of the panelists is considered, the maximum acceptable score for any of the sensory quality criteria of control in the discrimination test is ‘11’ of the 17 panelists. This acceptance level corresponds to 64.7% of preference. Based on this preference level, all of the treatments of the pâtė meat batches which had lower than 11 acceptances for any of the sensory quality criteria were excluded and not transferred to the descriptive test. These treatments are A, LABFA▼, LABPrA▼, LABPrF▼, and LABPrFA▼(Table 2).

Each of the four treatments. LABFA▼, LABPrA▼, LABPrF▼ , and LABPrFA▼, were accepted by the fewest out of the 17 panelists, 8, 7, 8, and 8, respectively, in the sensory quality criteria, ‘taste’. Also, A took 10 acceptances in color and 9 acceptances in aroma (Table 2). So, A, LABFA▼, LABPrA▼, LABPrF▼, LABPrFA▼, Pr, and F were not transferred to the descriptive test and were excluded. The 17 panelists for the descriptive test were trained before the test about each of the quality criteria of the pâtė meat for 2h each day for 4 day-sensory sessions. Using the 10-point scale [19], each quality criteria of each of the 17 batches including control was compared in the descriptive test. The gradings of each of the quality criteria are as follows; color (1:brown/pink/blue/green and 10: brown dark red and their combinations), taste (1: rotten. extremely sourish, or moldy, 10: meaty, tasty, reasonably and pleasantly sourish with garlicky and spicy, but no sweetness), texture (1: firm. tough. 10: slippery, emulsified, greasy, creamy, uniform) and aroma (1: H_2S/sulfide, moldy, putrefied, 10: no unpleasant smell like H_2S/sulfide, moldy or putrefied).

Statistical Analysis

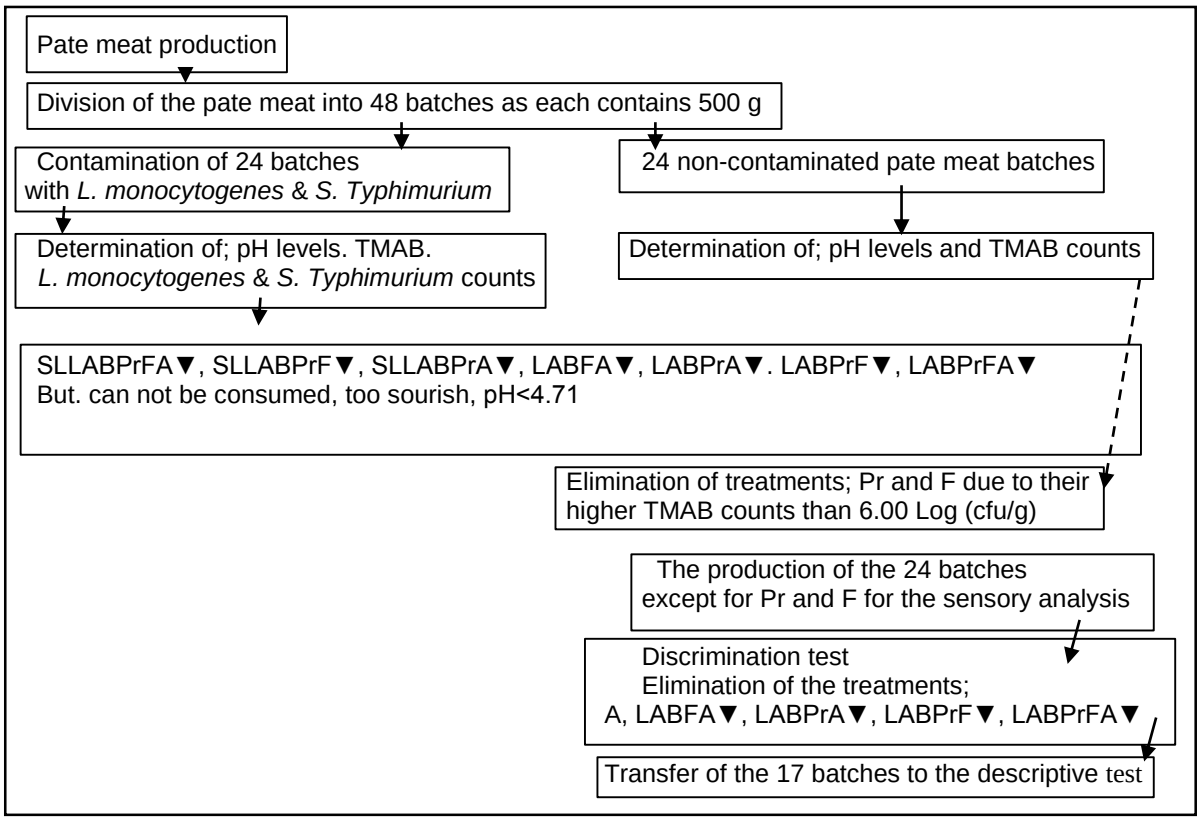
The pH measurements and microbial countings were made by using three replicates for each treatment batch. The average of the replicates was taken for the statistical analysis. A one-way analysis of variance (ANOVA) and Duncan’s Multiple Range Test [20], were performed to analyze in order to evaluate the effects on the treatments and the storage periods by SPSS for Windows (SPSS version 15.0 for Windows). The critical difference was determined at the 5% level.

RESULTS and DISCUSSION:

Effect of Sole and Different Combinations of Fermentation, LAB, Pr, F, and A on Sensory Quality

The batches. LABPrFA, LABPrA, and LABPrF, have the highest points in the descriptive test in 'taste' with 515, 501, and 472, respectively (Table 2). The difference between the points given to LABPrFA, LABPrA, and LABPrF and other treatments in taste was found significant ($p<0.01$). The first significant reason for the higher 'taste' scores of these pâtė meat batches might be the better microbial quality and controlled fermentation with a lower temperature, 22°C. On the other hand, the lowest TMAB counts and pH levels belong to the fermented batches when compared with the other batches and control (Table 1) ($p<0.05$). Moreover, the higher pH levels on the 22nd day, 4.71, 4.81, and 4.83, which belong to LABPrFA, LABPrA, and LABPrF, respectively (Table 1), indicate that the consumer accepts the sourish taste of 'fermentation' if only pH is not lower than '4.71'. Otherwise, the treatments, LABFA, LABPrA▼, LABPrF▼ , and LABPrFA▼, all of which have lower TMAB counts and pH levels lower than 4.71 the same day ($p<0.01$) (Table 1 and Table 3), would have been accepted in the discrimination test.

Table 3. The determination of the superior treatments with the optimum microbial and sensorial criteria



A clear explanation for their rejection might be the higher temperature during fermentation in these treatments, 37°C, which results in excessive and uncontrolled fermentation with an unacceptable sourish taste. On the other hand, the seeds of F have a bitter taste and pungent aroma because of their saponins and tannins, such as protodioscin [21]. This bitter taste and the pungent aroma of the compound might have been congruent with the sourish and bitter taste and aroma of fermentation which may be a reason for the preference of the F-added pâtė meats. The LAB counts of the eliminated batches, LABFA▼, LABPrA▼, LABPrF▼, and LABPrFA▼, on the 22nd day, are higher (Table 3) ($p<0.01$). So, the other common attribute of LABFA▼, LABPrA▼, LABPrF▼, and LABPrFA▼, the 'fermentation at 37°C', might have induced the growth of LAB, leading to pH decrease and 'sourishness' which may be crucial in their elimination in the sensory analysis. The inducing effect of the combined additions of the probiotics, arginine, fenugreek, and the fermentation at 37°C, on the growth and count of the lactic acid bacteria, may be one of the initial reasons which lead to the elimination of the same treatments in the discrimination test. Nearly, all of the treatments which include the various combined additions of the bioactive compounds as combined with the fermentation at 37°C increased the LAB counts to ≥7.17≤8.74 Log (cfu/g) on the 22nd day while others had <6.82 Log (cfu/g) ($p<0.01$) (Table 1). These findings are also important since they indicate that the effect of the fermentation, particularly at the higher heat level (37°C), has been completed on the 22nd day of the shelf life of the pâtė meat if that is fermented at 37°C and added the various combined additions of the bioactive compounds. On the 22nd day, these treatments arrived at the limit of the spoilage in LAB origin since the counts of LAB

cause spoilage when their amounts are between 7.59 and 8.25 Log (cfu/g) [22]. Confirming this, in both the discrimination and descriptive test results, the difference between the batches which were fermented at 22ºC and which were fermented at 37ºC is significant ($p<0.01$) (Table 4).

Table 4. Results of ANOVA and Duncan analyses

	ANOVA Significance	Duncan (from the most superior to the less)
Taste	<0.01	LABPrFA, LABPrA
Aroma	<0.01	PrFA, LABPrFA
Color	<0.01	LABPrFA, LABPrA
Texture	<0.05	PrFA, LABPrFA
LAB*day	<0.01	LABPr▼, LABPrFA▼
LAB*treatment	<0.01	LABPr▼, LABPrFA▼
LAB*heat	<0.01	LABPr▼, LABPrFA▼
LAB*pH	<0.01	LABPrFA▼, LABFA▼
LIS*treatment*pH	<0.01	SLLABPrFA▼, SLLABPrF▼, SLLABPrA▼/ SL**
LIS*heat	<0.01	37°C, 22°C
SAL*treatment*pH	<0.01	SLLABPrFA▼, SLLABPrF▼, SLLABPrA▼/ SL**
SAL*heat	<0.01	37°C, 22°C
SAL*heat*day	<0.05	SLLABPrFA▼, SLLABPrF▼, SLLABPrA▼/ SL**
SAL*day	<0.05	SLLABPrFA▼, SLLABPrF▼, SLLABPrA▼/ SL**
TMAB*treatment	<0.01	LABPrA▼, LABPrF▼, SLABPrFA▼, LABA▼/ C**
TMAB*heat	<0.01	LABPrA▼, LABPrF▼, SLABPrFA▼, LABA▼/ C**
TMAB*heat*day	<0.01	LABPrA▼, LABPrF▼, SLABPrFA▼, LABA▼/ C**
TMAB*day	<0.01	LABPrA▼, LABPrF▼, SLLABPrFA▼, LABA▼/C**
pH*treatment	<0.01	LABPrA▼, LABFA▼, LABPrFA▼, LABPrF▼, SLLABPrFA▼/ SL**
pH*heat	<0.01	37°C, 22°C

* Interaction ** The highest count

More important was that the points in both evaluations were significantly higher in the batches fermented at 22ºC ($p<0.01$). Briefly, one of the crucial factors in the preference for the sensory quality of the fermented pâtė meat might have been the LAB counts. For example, the preference of the batches with lower LAB counts which were fermented at 22ºC was significantly more than that of the preference of the batches with higher LAB counts and which were fermented at 37ºC ($p<0.01$). The decrease in pH which formed extremely in the batches, LABFA▼, LABPrA▼, LABPrF▼, and LABPrFA▼, all of which were fermented at 37ºC, might have been due to the higher temperature, 37ºC. The increased heat level, 37°C as compared with 22°C might have induced the growth of LAB. So, the higher growth rate of LAB during fermentation at 37ºC, might have been an important reason for the sourishness in these four batches. Opposite, the most preferred treatments in the descriptive and discrimination tests, LABPrFA, LABPrA, and LABPrF, are the treatments that were fermented at 22°C. This lower heat level 22°C, might have maintained a much more controlled fermentation with less growth and activity of LAB (Table 1). The higher preference in the A- added pâtė meats may be the suppression of 'bitterness' due to the interaction of A with NaCl, which was previously informed [23]. Accordingly, the guanidinium group of A might have interacted with sodium channels in taste bud membranes. The improving effect of A was also inspected in 'color' and 'texture'. The addition of A enhances 'redness', which might be due to the inducing effect of A on myofibrillar proteins to form a more smooth, compact, and uniform gel matrix while decreasing 'brightness' and increasing 'red' tone [24]. Also, the highest score, 122, belongs to LABPrF, preceded by LABPrFA in color, which might be due to the same amino acid which naturally exists in F [25]. In consideration of 'texture', the difference between the higher scores belonging to PrFA, LABPrFA, LABPr (140, 138, and 113, respectively) and other treatments were significant ($p<0.05$) (Table 4). These findings indicate two important factors. First, 'the fermentation at 22°C' and second, 'the addition of F'. The preference of the A- added pâtė meats, LABPrFA, PrFA, and LABA, in texture might have been due to the decrease in water binding capacity by the decrease in pH and stimulating effect of A on fermentation. These might have formed a gel structure and closer integration of the myofibrillar proteins due to the decreasing effect of A on pH [26]. Finally, 'galactomannan' in F, a hydrocolloid that increases viscosity [27], might have been another reason for the preference of the F-added batches, PrFA and LABPrFA, in texture. A summary of the superior batches with their most likely reasons is also summarized in Table 5.

Table 5. The superior batches for each of the sensory quality criteria with possible reasons

Criteri	Superior batches	Possible reasons
Taste & Aroma	LABPrFA/LABPrA/LABPrF/ LAB▼/PrFA. LABPr	F (saponins. tannins. protodioscin. alkaloids. phenols-pungent. sourish aroma & taste / L-Arg (A) in F Retardation of spoilage The controlled fermentation at 22°C in the treatments. LABPrFA. LABPrA. LABPrF and PrFA L-Arg (A) (guanidinium and NaCl interaction)
Color	LABPrF/LABPrFA/lABPrA	Emulsification (the grinding. pH decrease due to fermentation and L-Arg (A) and/or F) The improving effect of a controlled fermentation at 22°C L-Arg (A) (It's inducing effect on fermentation). Increase of the red tone-induced by L-Arg (A) and fermentation. Decrease in water holding capacity
Texture	LABPrFA/PrFA	L-Arg (A) (It's inducing effect on fermentation). Increase in viscosity as a result of the solubilization of myofibrillar proteins by the decrease in pH-induced by L-Arg(A) and/or F and/or fermentation F (gel formation by galactomannan)

pH and Microbial Counts

All of the non-contaminated pâtė meat batches which were treated with any of the sole or different combinations of F, A, Pr, and LAB have lower pH levels and TMAB counts than the control (Table 1) ($p<0.01$). Control reached pH levels above 6 on the 5th day. On the other hand, the pH levels of all the other pâtė meat batches fermented at either 37 or 22°C and treated with the sole or different combinations of the bioactive food compounds, remained pH below 6 until the 22nd day (Table 1). Like 'treatment', the effect of time on pH, the counts of LAB, TMAB, and the pathogens were significant ($p<0.01$) (Table 4). pH and the counts of the pathogens decreased while the counts of LAB increased in the contaminated and non-contaminated fermented pâtė meat treatment batches through the shelf life ($p<0.01$) (Table 1 and Figure 1). On the other hand, the heat level during fermentation, 22 or 37°C, significantly affected the pH levels and the counts of TMAB, LAB, and *S.Typhimurium* ($p<0.01$). The pH level decreased as the heat level during fermentation was increased to 37°C and with time from the start to the 22nd day ($p<0.01$) (Table 1). The lowest pH levels and TMAB counts belong to LABPrA▼ in the non-contaminated pâtė meat batches and SLLABPrFA▼ in the contaminated pâtė meat batches (Table 1 and Table 4).

These findings indicate that the fermentation of the pâtė meat at 37°C by adding LAB, Pr, A, and F led to the lowest acidity loss ($p<0.01$) (Table 1). All these batches' attributes have in common is their fermentation at 37°C instead of 22°C. These findings underline the importance of the following factors to keep the acidity loss minimum in pâtė meat production. The first is the 'fermentation' itself, the second is the 'heat level during fermentation'. it was evident that 37°C maintains acidity much more as compared with 22°C ($p<0.01$) (Table 4). The reason might have been the stimulating effect of the higher temperature, 37°C, on LAB and, as a result, the increase in the acid production by LAB. Another factor might have been the activity of LAB to produce bacteriocin, which might have been more at 37°C as compared with 22°C, which was also informed [28]. The decrease in pH by the addition of Pr (FOS), which may be due to its inducing effect on the growth of LAB, is also in accordance with the literature findings, which inform a 0.5 and 1 decrease in pH in 1% FOS added yogurt [29]. Like Pr, the addition of A increased LAB counts in all of the pâtė meat batches ($p<0.05$) (Table 1). The interaction between the increase in LAB and the decrease in pH through the shelf life is significant ($p<0.01$) (Table 4). The interaction of TMAB with pH is also significant that pH decrease by A, Pr, LAB, and fermentation through the shelf life inhibited the TMAB growth ($p<0.01$) (Table 4). The addition of A exhibited synergism with F. This synergism was found more significant in the contaminated pâtė meat batches ($p<0.05$). In the only study related to the combined effect of F and A, the production of the main component of F, trigonelline in the fenugreek sprout was informed to be induced by A. 20 µM [30]. Confirming the synergism of A and F, the contaminated pâtė meat batches, SLLABFA▼, SLLABPrFA▼, SLLABPrF▼, did have significantly more antimicrobial effects with lower TMAB counts, 6.03, 5.98, and 6.04, respectively, on the 22nd day ($p<0.01$) (Figure 1). SLLABPrFA▼ has the lowest TMAB count with 5.98 Log on the 22nd day. This treatment made a 3.19 Log reduction in the TMAB count when compared with the TMAB count of SL, 9.17 Log (cfu/g), the same day, while LABPrA▼ among the non-contaminated pâtė meat batches did have the lowest TMAB count with 5.31 Log (cfu/g) (Table 1).

The effect of the sole and combined additions of the bioactive compounds, time, and heat on *S. Typhimurium* and *L. monocytogenes* in the artificially contaminated pâtė meats is significant ($p<0.01$) (Figure 1) (Table 4). SLLABPrFA▼ made one of the most significant inhibitions on both of the pathogens while eliminating them on the 22nd day, indicating a synergism of the bioactive compounds to enhance the antimicrobial effect. The antimicrobial effect of F was found greater on *L. monocytogenes* than on *S. Typhimurium* ($p<0.05$),

whereas the antimicrobial effect of A became stronger on the growth of *S.Typhimurium* ($p<0.05$). The reason may be the penetration of scopoletin. It is coumarin and one of the main antimicrobial substances in F, which might have been blocked by the efflux pumps that only exist in the Gram-negative cell wall of *S. Typhimurium* [31]. This may explain the more significant antimicrobial effect of all of the F-added treatments on *L. monocytogenes* (Figure 1). On the opposite, the antimicrobial effect of A was found more significant on *S. Typhimurium* than on *L. monocytogenes* ($p<0.05$). The reason may be the difference in the cell wall structure and the interaction of this structure with the amino acid. A might have caused more damage by producing Nitric Oxide in the Gram-negative cell wall of *S.Typhimurium* since the cell wall of the bacteria contains more lipid content. On the other hand, no matter how both of the pathogens were eliminated by SLLABPrF▼ and SLLABPrFA▼ (Figure 1), these treatments can not be preferred because of their extreme sourishness. Their extremely low pH levels, which cause a sour taste, make their superior inhibitory effects on TMAB growth meanless, like in their non-contaminated counterparts. Onn the other hand, SLLABPrFA, SLFA, SLPrA, SLPrFA, and SLLABPrA▼, the treatments with pH levels higher than 4.71, which eliminated both of the pathogens on the 22 days of the shelf life, made 5.91 and Log 6.11 Log (cfu/g) inhibitions on *S.Typhimurium* and *L. monocytogenes*, respectively (Figure 1) ($p<0.01$).

One of the most significant findings is that too much increase in acidity inhibited the antimicrobial effect of A on *L. monocytogenes* ($p<0.05$). At low pH, the total negative charge of the peptidoglycan layer is reduced because of the positive charge of alanine. As a result, the peptidoglycan layer becomes neutral which limits the attraction between the peptidoglycan layer of the bacteria and A [32].

The main paradox in the findings is that TMAB counts and pH levels are not the only criteria in determining a food product's overall quality and marketing. For example, in this study, LABPrA▼, LABFA▼, LABPrFA▼ , and LABPrF▼ in the non-contaminated pâtė meats are the treatments with lower TMAB counts. However, their pH levels until the 22nd day, which were 4.02, 4.09, 4.15, and 4.19, respectively, were the lowest ones that caused extreme sourishness. The decrease in pH can often extend the shelf life while inhibiting microbial growth. However, the extremely low pH level is also the primary reason for their extreme sourishness and elimination in the discrimination test. The' sourishness' has long been preferred in traditional fermented meat products such as fermented sausage and fermented dry summer sausage with their dry texture. But, the material in this study, pâtė meat, had a much higher water content and water activity. Therefore, the sourishness which was caused by the fermentation, particularly at 37ºC, could not be tolerated enough in this product, most likely due to the higher water content and activity both of which might have acted as an acid carrier. On the other hand, LABPrFA, LABPrA, LABPrF, and PrFA, could inhibit TMAB only between 1.01-1.09 Log (cfu/g) but exhibited a constant and effective microbiostatic effect all through the shelf life while maintaining the sensory quality. These might be due to the inner biochemical dynamics in pâtė meat, which favor the formation of the microbiostatic effect. Though the inhibition is weak, the constant microbiostatic effect made these treatments capable of extending the shelf life 10 days more than the control without decreasing the sensory quality (Table 1 and Table 5). In the only study which applied fermentation, pork liver was used and fermentation lowered pH to 4 at 30°C, while subsequent cooking to 70°C followed by vacuum packaging had extended the shelf life more than 22 d at 4 to 10°C [33]. Nevertheless, it used fermentation only to lower pH before production. The product had been pasteurized and vacuumed, but was not a fermented one. In another study, cooking the chicken liver to an internal temperature of 60 to 73.9°C made a 1.9 to ≥ 6.4 Log (cfu/g) reduction in the total count of *Salmonella* spp. [34]. However, as indicated in the Introduction, higher heat levels than 63°C, can not be applied in pâtė meat production since it makes the texture dry while the texture should be creamy and soft. It should also be noted that the additions of A and F as combined, improved the microbial quality in both contaminated and non-contaminated pâtė meat batches, indicating the synergism between A and F. Though the association of the risk of Listeriosis with the pâtė meat is well known, there are no study findings to compare the *Listeria* counts in this study with.

CONCLUSION

The results are crucially important since they underline the importance and beneficial effect of the lower heat level during fermentation in the pâtė meat. The addition of all or most of the bio-active compounds, A, F, LAB, and Pr, and the combination of the additions of these bioactive compounds with the fermentation at 37ºC caused superior microbial quality. However, it unexpectedly increased the rate and speed of fermentation, decreased pH excessively, formed extreme sourishness, and made the product liable to the spoilage caused by LAB on the 22nd day of the shelf life of the fermented pâtė meat under 4°C. On the other hand, the lower heat level. 22°C, maintained a much more controlled fermentation without any adverse effect on the sensory quality. The addition of F made more inhibition on *L. monocytogenes,* whereas the antimicrobial effect of the addition of A was greater on *S. Typhimurium*. More important is that the different combined additions of the bioactive food compounds as combined with the fermentation either at 22°C or 37°C (e.g., SLLABPrF▼, SLLABPrFA▼, SLLABPrFA, SLFA, SLPrA, SLPrFA, and SLLABPrA▼) eliminated both pathogens on the 22. day, made 5.91 and Log 6.11 Log (cfu/g) inhibitions on *S.typhimurium* and *L. monocytogenes*, respectively. So, for the first time, the extremely short shelf life of the pâtė meat was extended by fermentation as combined with the various additions of the natural and functional food bioactive compounds. Although some of the batches which were fermented at 37°C as combined with the various additions of LAB, Pr, A, and F (the batches; LABPrA▼, LABFA▼,

LABPRFA▼, and LABPrF▼) extended the shelf life to 10 and 17 days, their extremely higher pH levels below 4.71 were not preferred by the panelist. Instead, some of the treatment batches which were fermented at 22°C as combined with the various additions of LAB, Pr, F, and A (The batches; LABPrFA, LABPrF, LABPrF, and PrFA) could extend the shelf life 10 days more as compared with control (which had only 3 days) without any adverse change in their sensory quality.

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