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Technological Valorization of Spent Laying Hen Meat by Producing a Functional Pastirma Fermented with *Lactobacillus plantarum*.

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ABSTRACT

Spent laying hen meat it was found to be low value byproducts in the poultry industry; this is mainly because of unfavorable tough texture caused by high level of collagen content. This reduces the direct consumption of this and causes a great loss of economy. The purpose of the study was to technologically enrich spent laying hen meat through the processing of the meat into a functional Pastirma, and the impact of the Lactobacillus plantarum as a starter culture on the quality, safety, and sensory properties of the final product. T1 (control, spontaneous fermentation) and T2 (inoculated with 1 × 10 6 CFU/g L. plantarum) and T3 (inoculated with 1 × 10 7 CFU/g L. plantarum) were used to process spent hen meat into Pastirma. Samples were analyzed during ripening for microbiological safety (Total Viable Counts, Yeasts and Molds), physicochemical (pH, lipid oxidation through MDA), proteolysis index and sensory (color, aroma, tenderness, general acceptability). Inoculation with L. plantarum (T2 and T3) was found to greatly reduce the acidification process and reached a lower final pH (4.1-4.3) than the control (4.7) which was considered to be effective in reducing spoilage microorganisms. The antioxidant activity was also seen in the starter culture, which decreased the lipid oxidation (low MDA values). More importantly, T3 had the highest proteolysis index, which was directly correlated with a significant improvement in meat tenderness. Accordingly, T2 and T3 scored enormously higher on sensory tenderness, aroma, and overall acceptability scores compared to the control. Lactobacillus plantarum (and especially at the concentration of 1 × 107 CFU/g) is a very effective biotechnological method to address the textural challenges of spent hen meat. This process is successful to convert a low-value byproduct that is underutilized to a safe, stable and sensorially appealing value-added functional food which is a sustainable solution to the poultry industry.

1- Introduction

The poultry industry, and in particular, the production of table-eggs, this is because of cornerstones of the global food security framework, due to its effective role in the supply of animal protein of high quality at a reasonable price [1]. Nevertheless, spent laying hen meat is a major and long-term economic and environmental challenge in this industry When the production cycle (usually 72-80 weeks) is complete, the laying hens are culled, and the egg productivity reduces [2].

This meat is a low-value product commercially. This is mainly because of its unpleasant physical qualities, i.e. tough texture brought about by a high percentage of heat stable cross-linked collagen and low percentage of intramuscular fat [3]. The meat contains such qualities that it is not consumable directly, and its application is restricted to consumers. This meat is, therefore, frequently turned into very low-value byproducts, including animal feed, which is a grave economic loss and a waste of a precious protein resource [4].

In this perspective, the idea of technological valorization is introduced as a new strategy to use this low-value raw material to high-value-added food products [5]. One of the best possible solutions is the production of processed and fermented meat, including Pastirma. Not only does the fermentation and drying process preserve the meat, but enzymatic proteolysis and lipolysis are decisive in improving the sensory properties particularly tenderness and flavor of the meat, thereby facilitating its preservation [6].

Historically, the production of Pastirma has been focused on spontaneous (natural) fermentation that is an unregulated system that may result in changes in the quality of the final product and the hazard associated with microbiological safety [7]. Here the contribution of modern biotechnology, i.e., the use of microbial starter culture, comes into the limelight. Lactobacillus plantarum is regarded as one of the most significant starters in fermented-meat production [8]. This is explained by its strong capability to withstand adverse conditions (high salt, low pH), its competitive antagonistic activity with pathogenic microorganisms (biopreservation), and its enzymatic one, especially its proteolytic activity, which actively participates in meat tenderization and the production of specific flavor compounds [9].

Furthermore, the use of selected *L. plantarum* strains opens the door to producing *functional foods.* The starter culture itself can potentially act as a *probiotic*, thereby adding a health value to the final product that transcends its basic nutritional content [10]. Therefore, this research aims to fill the current knowledge gap by investigating the possibility of *technological valorization* of spent laying-hen meat (*the problem*) through the production of *functional Pastirma* (*the product*), using *Lactobacillus plantarum* (*the biotechnological tool*) as a starter culture. The research will focus on evaluating the impact of this starter culture on the microbiological safety, physicochemical properties (especially tenderness and pH), and the sensory profile of the produced *Pastirma.*

2- Materials and Methods

2.1. Raw Materials and Starter Culture

2.1.1. Meat and Other Ingredients

Breast meat (Pectoralis major) from spent laying hens (total 10 kg) of the spent laying hen aged 78 weeks at 4 h postmortem in a commercial poultry processing plant at Kut City, Iraq was collected. The meat was shipped (under a chilled environment (4 ± 1 o C) to the Meat Technology Laboratory, Department of Agricultural Biotechnology. It was on arrival that the samples were cut off any apparent fat and connective tissue and surface contamination. The proximate content of the untreated meat before processing was established based on AOAC (2019) protocols and included the following (mean ± SD): moisture 73.4 ± 0.8, crude protein 21.6 ± 0.6, crude fat 2.4 ± 0.3, and ash 1.1 ± 0.1% [11]. The ingredients in all curing and seasoning were bought at a certified local supplier. Salt (NaCl), sucrose and sodium nitrite were of analytical grade. The components of spices and çemen mixture ground fenugreek, fresh garlic, and paprika were checked by means of microbial safety and compositional uniformity.

2.1.2. Microbial Culture

Lactobacillus plantarum (ATCC 14917) was procured as a pure culture of this bacterium. It was chosen because it has a strong acidification capability, is proteolytic, and it has been utilized as a starter culture in the production of fermented meat products [12]. Maintaining de Man, Rogosa and Sharpe (MRS) agar slants at 4 o C and subculturing every month helped to maintain strain. Microscopically and streaking on MRS agar purity of the culture has been checked and uniform colony morphology has been observed to verify purity of the culture before use.

2.2. Preparation and Activation of the Starter Culture

L. plantarum was lyophilized and reactivated with two consecutive transfers (1% inoculum) into MRS broth (HiMedia, India) and incubated under anaerobic conditions at 37 o C in 24 h. Growth on bacteria was measured spectrophotometrically at 600 nm until the optical density (OD 600) attained 1.2-1.4 which translates to about 1 × 10 9 CFU/mL as confirmed by plate counting. They were refrigerated centrifuged (5000 × g, 15 min, 4 o c), washed twice in sterile physiological saline (0.85% NaCl), and resuspended in the same. The required inoculation levels of the experimental treatments were obtained using a standardized stock suspension (1 × 10 -1 CFU/mL) [13].

2.3. Experimental Design and Treatments

Ten kilograms of breast meat were randomly divided into three treatment groups, each processed in triplicate (n=3): T1 (Control): Pastirma was made using spontaneous fermentation without the addition of a starter culture. T2 (Low L. plantarum): This was inoculated to a final titre of 1 × 10 6 CFU/g. T3 (High L. plantarum): Inoculated to obtain a final concentration of 1 × 10 7 CFU/g. The treatment batches (3.4 kg/ batch) were mixed thoroughly so that there is consistent distribution of inoculum. The design of the experiment was entirely randomized and all batches were processed individually in terms of: curing, fermentation as well as ripening.

2.4. Processing of Pastirma

The general process was modified and followed after Aksu and Kaya (2002) with the introduction of adjustments to this process to facilitate starter culture

inoculation and controlled fermentation [14].

(a) Dry Curing: Pieces of meat (approximately 300 g in weight) were wiped with a dry curing mixture of 2.5 percent NaCl, 0.5 percent sucrose, and 120ppm sodium nitrite (w/w). The occurred samples were put in sterile polypropylene bottles and stored at 4 o C and allowed to soak the samples 48 h to allow diffusion of salt and partial drying of the sample.

(b) Inoculation and Fermentation: After curing, the meat was washed with sterile cold water, dried under a towel and inoculated as per treatment. The samples inoculated and controls were put in a climate-regulated chamber (Binder KBWF 240, Germany) at 22 o C and 90 relative humidity (RH) where they were allowed to stay 3 days. The process of fermentation was tracked through a decrease in the pH (Initial pH 6.1 to final pH 5.2-4.9**).**

(c) Initial Ripening Fermented pieces were packed into natural beef casings (sterilized and dried at 60C, 10 minutes) suspended at 15 o C and 75 percent RH during 14 days. This phase helped in slow dehydration, stabilization of meat color and formation of texture.

(d) Coating (Cement Application): Ground fenugreek (50 percent) was mixed with crushed garlic (15 percent) and paprika (5 percent) and sterile water (30 percent) to make a homogenous coating. The dried pastirma pieces were re-coated (23 mm thick) and re-hanged at 15 o C and 75 percentage humidity during 7 days and finally allowed to dry.

(e) Packaging and Storage: For further analyses, finished products were vacuum-packed in oxygen-impermeable polyethylene bags and kept at 4 o C. The complete procedure of the curing till the final product took about 24 days.

2.5. Sampling and Analytical Procedures

2.5.1. Sampling Schedule

At four stipulated points (1) raw meat (before processing), (2) after fermentation (3 days), (3) after initial ripening (14 days), and (4) final product (21 days), samples were taken. Three different replicates of each treatment were used at every stage.

2.5.2. Physicochemical Analyses

pH: Ph measured with a digital **pH meter (Hanna Instruments HI 5221)** set at pH 4.0 and 7.0. **Water activity (a 0):** The value was measured using a dew-point water activity meter (Aqua Lab 4TE, USA).

2.5.3. Biochemical Analyses

Lipid oxidation: Determined as thiobarbituric acid reactive substances (TBARS) in the modified procedure of Botsoglou et al. (1994), and the results were in mg malondialdehyde (MDA)/kg of sample [15]. Proteolysis index: The ratio of non-protein nitrogen (NPN) and total nitrogen (TN) was determined through the trichloroacetic acid (TCA) (precipitation) method [16].

2.5.4. Microbiological Analyses

Microbial enumeration was done using the steps of the American Public Health Association [17]: Total viable counts (TVC): Plate Count Agar (PCA) 30 C after 48 h. Coliforms: Violet Red Bile Agar (VRBA) at 37 ºC by 24 h. *Lactobacillus counts*: MRS agar anaerobic incubation: at 30o C (GasPak system) 48 h. Yeasts and molds: Potato Dextrose Agar (PDA) acidified to pH 3.5 incubated at 25 ºC over 5 days. All were counted in log 10 CFU/g of sample.

2.5.5. Sensory Evaluation

This was last analyzed using pastirma samples, where 25 semi-trained panelists (12 male and 13 females, between the age of 22 and 45 years) at the Department of Agricultural Biotechnology tested it. The evaluation of the senses was done in white light and at individual booths at 22 o C. The samples were stored at room temperature and using random three digits. The sensory analysis was performed at the end of the pastirma production process, that is, in the 21 st day of the storage. A hedonic scale of 9 points was used to rank attributes such as color, aroma, tenderness, juiciness and acceptability in general (Peryam, 1990) with 1 meaning dislike most and 9 like most [18].

2.6. Statistical Analysis

All data were analyzed using data that was collected as an independent replicate of three samples in each treatment. Mean ± standard deviation (SD) was the expression of results. There was a two-way analysis of variance (ANOVA) done on the SPSS v25.0 (IBM Corp., Armonk, NY, USA) in a completely randomized design (CRD). The primary ones were storage period and treatment concentration. Normality of data and homogeneity of variances were checked with the help of the Shapiro Wilk test and the Levene test, respectively. In case any meaningful difference ($P < 0.05$) was observed, the means were compared using the Post hoc test which is Tukey Honest Significant Difference (HSD). Moreover, the data obtained through sensory evaluation were compared with one-way ANOVA to determine the effect of concentrations of treatment and Tukey HSD test was applied in case of significant differences.

3- Results and discussion

3.1. Physicochemical Analyses

Table 1 and Table 2 show the changes in pH and water activity (a 1) of the pastirma manufactured by means of spent layer chicken meat during the 21 days of operation in relation to the inoculation levels (T1, T2, T3) of Lactobacillus plantarum. In relation to the pH (Table 1), there was a statistically significant ($P < 0.05$) decrease in the values of the pH, and the average of the values decreased to the final values of 6.67, 4. There was also a large treatment effect ($P < 0.05$). The average pH at treatment T1 (5.60a) was the highest with an average T2 (5.28b) and T3 (5.18c) giving average PHs of a significantly lower pH. In water activity (Table 2) effect, it was also found that there was a significant ($P < 0.05$) decrease over the processing time. The means of a 14 and 21 days (0.927b and 0.910b) were significantly less than the initial processing (0.983a) and Day 3 (0.953a). Conversely, no significant ($P > 0.05$) difference existed among the inoculation treatments (T1, T2, and T3) in terms of the mean water activity (0.943a, 0.940a and 0.948a respectively). The large decrease in pH during processing (Table 1) it was found to be most important indicators of the success of fermentation. Such a quick acidification, especially in the T2 and T3 treatments is a direct consequence of the metabolic activity of L. plantarum. These lactic acid bacteria use accessible sugars to produce lactic acid [19]. This observation is very close to the published literature; Kamiloğlu et al. (2020), in particular, also found that pH indeed decreased significantly when L. plantarum starter cultures were used to produce Turkish Sucuk [20]. This high-speed acidification is necessary to provide microbiological safety by suppressing acid-sensitive pathogens [21,22]. Equally, the fact that

water activity decreases significantly over time ($P < 0.05$) ensures the effectiveness of the physical processing stages (Table 2). This reduction is not associated with bacterial activity, as the significant differences between treatments (T1, T2, T3) are absent but is directly related to the salting and drying processes, which deprive free water [23]. This is in line with the results by Ceviker et al. (2025) and Apriliyani et al. (2025), who showed that a reduction in aw is dependent on the drying conditions and the loss of moisture, rather than the nature of starter culture [24,25]. Taken together, all these data reveal a successful and statistically justified application of the so-called Hurdle Technology. To provide stability and safety of the final product, the starter caused reduction of pH (microbiological hurdle) interacts synergistically with the reduction caused by processing (a 3) (physical hurdle), which was confirmed to be significant ($P < 0.05$).

Table 1. Effect of *Lactobacillus plantarum* inoculation levels on time-dependent changes in pH of pastirma produced from spent layer chicken meat (Mean ± SD).

Treatment	Time\Days				Average Treatment Effect
	Before Processing	3	14	21	
T1	6.7±0.01	5.8±0.07	5.2±0.01	4.7±0.13	5.60^{a}±0.70
T2	6.5±0.02	5.7±0.12	4.6±0.02	4.3±0.25	5.28^{b}±0.15
T3	6.8±0.11	5.4±0.09	4.4±0.17	4.1±0.08	5.18^{c}±0.12
Average Time Effect	6.67^{a}±0.54	5.63^{b}±0.05	4.73^{c}±0.33	4.37^{d}±0.16	

Values are expressed as mean ± SD (n = 3). Different letters within the same row or column indicate significant differences according to two-way ANOVA followed by Tukey's HSD test ($P < 0.05$).

Table 2. Effect of *Lactobacillus plantarum* inoculation levels on time-dependent changes in Water activity (a_v) of pastirma produced from spent layer chicken meat (Mean ± SD).

Treatment	Time\Days				Average Treatment Effect
	Before Processing	3	14	21	
T1	0.98±0.001	0.96±0.001	0.93±0.002	0.90±0.003	0.943^{a}±0.001
T2	0.98±0.001	0.95±0.003	0.92±0.001	0.91±0.001	0.940^{a}±0.001
T3	0.99±0.002	0.95±0.001	0.93±0.003	0.92±0.002	0.948^{a}±0.002
Average Time Effect	0.983^{a}±0.001	0.953^{a}±0.003	0.927^{b}±0.001	0.910^{b}±0.002	

Values are expressed as mean ± SD (n = 3). Different letters within the same row or column indicate significant differences according to two-way ANOVA followed by Tukey's HSD test ($P < 0.05$).

3.2. Biochemical Analyses

Table 3 and Table 4 demonstrate the effect of inoculation on lipid oxidation (MDA) and proteolysis index. To determine the effect of lipid oxidation (Table 3), the values of MDA were significantly ($P < 0.05$) raised during the 21 days, and the mean of the value rose by 0.48a to 0.93d. With respect to the effect of treatment, T1 had a stronger average value of MDA (0.79b) than T2 (0.69a) and T3 (0.63a), though T2 and T3 did not differ significantly. The increase of the proteolysis index (Table 4) was also found to be significant ($P < 0.05$) over time as the average of the values (6.50d and 11.19a) was noted to increase. The treatment effect was very significant ($P < 0.05$) with the highest mean value of proteolysis of T3 (9.58a) followed by T2 (9.01b) and T1

(7.82c) with significant difference among different values of the treatment.

The increase in lipid oxidation (MDA) with time (Table 3) is quite natural when processing and drying meat. Nevertheless, the much lower values of the MDA in treatments T2 and T3 in comparison with T1 is a strong indication of a protective antioxidant effect of the L. plantarum starter cultures. The literature is largely consistent with this observation; in studies like Luo et al. (2024), the researchers proved that sausages inoculated with L. plantarum exhibit considerably lower thiobarbituric acid reactive substances (TBARS) levels (the level measured by MDA) than those used as control [26]. This secondary effect of antioxidant action is an important one, delays the onset of rancidity and is important to the overall product quality and shelf-life.

Moreover, the data indicate a major effect of the starter culture on proteolysis (Table 4) which is important in the development of the characteristic flavor and texture of fermented meats. Proteolysis index was found to be much higher in T3, T2 and T1 respectively. Such a tendency is an ideal reflection of the acidification outcomes (Table 1, the decreasing order of T3, T2, T1 in pH). Such a close correlation is very well documented; two synergistic factors, as it was confirmed by Nie et al. (2014), cause protein degradation in fermented products; (1) the activation of endogenous muscle enzymes (such as cathepsins) that are most effectively active in the acidic environment formed by the starter culture, and (2) the intrinsic proteolytic enzymes produced by the L. plantarum strains themselves [27]. This increased rate of proteolysis results in increased concentration of free amino acids and small peptides, which are the desirable precursors of desired flavor compounds in the end product [28].

All these data lead to the conclusion that the L. plantarum inoculation (and especially T3 and T2) is a multifunctional quality enhancer. It does not only create a critical microbiological hurdle (low pH, Table 1), but also positively affects the quality of the product by conferring significant lipid oxidation (Table 3) inhibition and the desired proteolysis necessary to form flavors (Table 4).

Table 3. Effect of *Lactobacillus plantarum* inoculation levels on time-dependent changes in Lipid oxidation (MDA)/kg**) of pastirma produced from spent layer chicken meat (Mean ± SD).**

Treatment	Time\Days				Average Treatment Effect
	Before Processing	3	14	21	
T1	0.48±0.002	0.63±0.005	0.94±0.007	1.12±0.002	0.79^{b}±0.001
T2	0.51±0.001	0.57±0.002	0.82±0.001	0.88±0.001	0.69^{a}±0.005
T3	0.47±0.004	0.51±0.001	0.73±0.002	0.81±0.004	0.63^{a}±0.003
Average Time Effect	0.48^{a}±0.001	0.57^{b}±0.006	0.83^{c}±0.005	0.93^{d}±0.001	

Values are expressed as mean ± SD (n = 3). Different letters within the same row or column indicate significant differences according to two-way ANOVA followed by Tukey's HSD test (P < 0.05).

Table ٤. Effect of *Lactobacillus plantarum* inoculation levels on time-dependent changes in Proteolysis index (%) of pastirma produced from spent layer chicken meat (Mean ± SD).

Treatment	Time\Days				Average Treatment Effect
	Before Processing	3	14	21	

T1	6.45±0.070	7.63±0.270	8.04±0.010	9.17±2.050	7.82^{c}±1.020
T2	6.57±0.220	7.87±0.250	9.82±0.500	11.80±0.100	9.01^{b}±1.003
T3	6.49±0.320	8.51±0.330	10.73±1.120	12.61±0.200	9.58^{a}±0.088
Average Time Effect	6.50^{d}±0.011	8.00^{c}±1.090	9.53^{b}±1.000	11.19^{a}±2.044	

Values are expressed as mean ± SD (n = 3). Different letters within the same row or column indicate significant differences according to two-way ANOVA followed by Tukey's HSD test (P < 0.05).

3.3. Microbiological Analyses

Tables 5, 6 and 7 of the microbiological changes through the 21 days of processing are given. Table 5 (Total Viable Counts, or TVC) was a diverging effect in relation to the treatment. TVC in the control (T1) changed significantly ($P < 0.05$) during processing, reaching the levels of 5.64 to 8.08 log10 CFU/g. By sharp contrast, the counts of the inoculated treatments (T2 and T3) declined sharply after Day 3, and reached much lower counts (5.11 and 5.04 log10 CFU/g, respectively). Therefore, the mean effect of treatment on T1 (7.51c) was considerably greater than that of T2 (5.57b) and T3 (5.53a).

Table 6 of Lactobacillus counts revealed a significant ($P < 0.05$) increase throughout the time in all treatments. The treatment (T2 and T3) that was inoculated with starter showed a fast proliferation with an ultimate count of 9.11 log10 CFU/g and 9.18 log10 CFU/g, respectively. These were highly significant ($P < 0.05$) compared to the end result in the control treatment T1 (7.04 log10 CFU/g). The mean control effect validated that T3 (8.58a) and T2 (8.52b) possessed considerably bigger populations of Lactobacillus as compared to T1 (6.47c).

Yeast and Mold counts (Table 7) were in a similar trend as TVC. In T1 counts were relatively constant and high during the 21 days (terminated at 3.40 log10 CFU/g). But in T2 and T3, the counts decreased considerably ($P<0.05$) beyond Day 3 to end up as 1.70 and 1.60 log10 CFU/g. The mean treatment effect indicated that the treatment level of T1 (3.56c) was substantially higher than the treatment level of T2 (3.26b) and T3 (3.23a).

This is clearly shown in the microbiological data which shows the successful dominance and protective effect of the Lactobacillus plantarum starter culture. The main aim of inoculation is to make sure that the targeted microorganism outcompetes the indigenous flora which was effectively accomplished. The Lactobacillus was quickly dominant and the counts of T2 and T3 (Table 6) went to more than 9 log. This is in line with other research studies by Yilmaz et al. (2024), who found that inoculated Lactobacillus strains rapidly dominated the microflora in the fermented sausages [29].

This dominance exerted an extensive and substantial antimicrobial and antifungal effect as observed in Tables 5 and 7. This fact of both Total Viable Counts (Table 5) and Yeasts and Molds (Table 7) inhibition in the T2 and T3 treatments is an essential observation.

This is explained by two major processes: Competitive Exclusion and Acidification: The high rate of growth of L. plantarum (Table 6) and the resultant high rate of pH decrease (as observed in Table 1) provides a very hostile environment that deters most of the spoilage bacteria (TVC) and fungi (Yeasts/Molds). This is similar to the study by Mao et al. (2024), who

explain that pH lowering is a major challenge when it comes to the inhibition of spoilage flora [30]. Antagonistic Compounds: L. plantarum is famous in the production of antimicrobial compounds, including bacteriocins (accounting the reduction in TVC) and antifungal compounds (accounting the reduction in Yeasts/Molds). These findings are highly indicative of the fact that the strains utilized in T2 and T3 were incredibly good producers of the given compounds, which actively eliminated the competition. This is in line with Güngoren (2025) who also observed that L. plantarum has the capacity to inhibit the growth of yeast and molds in pastirma [31]. Contrastingly, the risk of spontaneous fermentation is proven in the control treatment (T1). The native lactobacilli (Table 6, T1) grew significantly slower, and this gave time to the general spoilage microflora (TVC, Table 5) and fungi (Y&M, Table 7), which led to a much greater microbial loading. All these data support the conclusion that L. plantarum inoculation (T2 and T3) not only helps to standardize fermentation (Table 1) and enhances the quality (Tables 3 and 4) but also to provide products with safety by actively managing undesirable spoilage and pathogenic microorganisms (Tables 5 and 7).

Table 5. Effect of *Lactobacillus plantarum* inoculation levels on time-dependent changes in Total viable counts log $_{10}$ (CFU/g) of pastirma produced from spent layer chicken meat (Mean ± SD).

Treatment	Time\Days				Average Treatment Effect
	Before Processing	3	14	21	
T1	5.64±0.05	5.96±0.03	6.91±0.08	8.08±1.04	7.51^{c}±0.07
T2	5.65±0.01	5.91±0.03	4.99±0.03	5.11±0.11	5.57^{b}±0.11
T3	5.63±0.06	5.90±1.00	4.07±0.01	5.04±0.10	5.53^{a}±0.02
Average Time Effect	5.64^{a}±0.02	5.93^{b}±0.02	6.44^{c}±0.04	7.60^{d}±0.08	

Values are expressed as mean ± SD (n = 3). Different letters within the same row or column indicate significant differences according to two-way ANOVA followed by Tukey's HSD test (P < 0.05).

Table 6. Effect of *Lactobacillus plantarum* inoculation levels on time-dependent changes in Lactobacillus counts log$_{10}$ (CFU/g) of pastirma produced from spent layer chicken meat (Mean ± SD).

Treatment	Time\Days				Average Treatment Effect
	Before Processing	3	14	21	
T1	3.65±0.01	3.71±0.08	5.91±0.02	7.04±0.02	6.47^{c}±0.01
T2	3.67±0.05	3.89±0.01	6.99±0.04	9.11±0.07	8.52^{b}±0.03
T3	3.69±0.02	3.93±0.01	7.03±0.03	9.18±0.05	8.58^{a}±1.00
Average Time Effect	3.67^{c}±0.01	3.85^{c}±0.03	6.85^{b}±0.02	8.97^{a}±0.01	

Values are expressed as mean ± SD (n = 3). Different letters within the same row or column indicate significant differences according to two-way ANOVA followed by Tukey's HSD test (P < 0.05).

Table 7. Effect of *Lactobacillus plantarum* inoculation levels on time-dependent changes in Yeasts and molds log$_{10}$ (CFU/g) of pastirma produced from spent layer chicken meat (Mean ± SD).

Treatment	Time\Days				Average Treatment Effect
	Before Processing	3	14	21	

T1	3.57±0.40	3.61±0.33	3.62±0.02	3.40±0.01	3.56^{c}±0.25
T2	3.56±0.11	3.55±0.10	1.74±0.07	1.70±0.03	3.26^{b}±0.17
T3	3.54±0.13	3.51±0.12	1.65±0.19	1.60±0.02	3.23^{a}±0.11
Average Time Effect	3.56^{d}±0.14	3.56^{c}±0.07	3.16^{b}±0.15	2.94^{a}±0.22	

Values are expressed as mean ± SD (n = 3). Different letters within the same row or column indicate significant differences according to two-way ANOVA followed by Tukey's HSD test (P < 0.05).

3.4. Sensory Evaluation Analyses

Table 8 shows the scores of sensory evaluations of pastirma treatments. Color, Aroma, Tenderness and Overall Acceptability were significantly (P < 0.05) influenced by the inoculation level. There was no significant difference (N.S) between the treatments used to measure Juiciness. In the case of Color, all the groups had significantly different scores, T3 (7.16a) scored the most and then T2 (7.04b), and T1 (6.11c). In the case of Aroma and Tenderness, the treatment was very different (P < 0.05). T3 scored the highest (8.76 and 7.92 respectively), and then T2 (8.51 and 7.66) and T1 (7.24 and 7.32) scored the lowest. The same trend was affirmed in Overall Acceptability with T3 (7.55a) and T2 (7.37b) rated significantly higher than T1 (7.10c).

The final confirmation of the physicochemical and microbiological data found in the earlier steps is the sensory evaluation (Table 8). The sensory panel was unanimously in favor of the inoculated treatments (T2 and T3), and these preferences are directly attributed to the faster proteolysis seen in Table 4.

Aroma, Tenderness and Overall Acceptability (Link to Table 4): The higher scores of Aromas, Tenderness and Overall Acceptability in T3 and T2 (Table 8) are explained directly by the faster rate of proteolysis in Table 4. T3 had the highest index of proteolysis, then T2, and finally T1. This enhanced protein digestion (proteolysis) can accomplish two objectives: (1) This breaks down the muscle fiber structure, which enhances tenderness, and (2) This produces a higher concentration of free amino acids and small peptides, which are the building blocks to desirable flavor and aroma compounds [32,33]. This difference was not only observed by the panel but was also desired.

Color (Link to Table 3): The quality of the color scores in T2 and T3 (Table 8) is also due to the activity of the starter culture. This is probably because of two reasons: (1) Lactobacillus strains were able to promote the curing process (nitrite reduction) resulting in a more stable and desirable occured red color (nitroso-myoglobin), and (2) The antioxidant effect in Table 3 (T2 and T3 had lower MDA values) prevented the oxidation of the meat pigments and thus prevented the appearance of the brownish, dull appearance in T1.

Juiciness (Link to Table 2): The absence of significance in the difference in the scores of Juiciness (Table 8) is an important aspect of consistency. It completely supports the results of Table 2 that indicated that the water activity (a 0) was not significantly varied in T1, T2 and T3 treatments. As water activity (aw), an indicator of free water, was identical, the sensory panel was able to discern that the juiciness of all the samples was the same as it should have been. Finally, it is proven

by sensory data that L. plantarum inoculation (T2 and T3) is not only a tool of preservation and safety (Tables 1, 5, 7) but the driving force of product quality resulting in a product with much improved color, aroma and tenderness which further leads to the greatest overall consumer acceptance.

Table 8. Effect of *Lactobacillus plantarum* inoculation levels on time-dependent changes in Sensory Evaluation of pastirma produced from spent layer chicken meat (Mean ± SD).

Treatment	color	aroma	tenderness	juiciness	overall acceptability
T1	6.11^{c}±0.05	7.24±0.13	7.32±0.11	6.19±0.22	7.10^{c}±0.66
T2	7.04^{b}±0.1	8.51±0.20	7.66±0.51	6.22±0.15	7.37^{b}±0.19
T3	7.16^{a}±0.17	8.67±0.04	7.92±0.12	6.25±0.18	7.55^{a}±0.11
Sig.	0.05	0.05	0.05	N. S	0.05

Values represent means of sensory scores (n = 25). Different letters within the same column indicate significant differences among treatments (one-way ANOVA, Tukey's HSD, P < 0.05).

4- Conclusion

The study has been able to establish the technological value of low value spent laying hen meat into high-quality Pastirma. Spontaneous fermentation (T1) was much inferior compared to using Lactobacillus plantarum as the starter culture (T2/T3) because it guaranteed microbiological safety due to rapid acidification and inhibited microorganisms that lead to spoilage. The culture was also shown to improve quality through inhibition of lipid oxidation and a significant reduction in the major defect in meat toughness by high proteolytic activity. Therefore, Pastirma inoculated with higher scores on tenderness, aroma and overall acceptability. In this biotechnological model the byproduct of an industry is successfully turned into a safe and desirable as well as value-added functional food.

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