RESEARCH ARTICLE

# Microbiological Stability of Fermented Chicken Meat Spread Under Refrigerated (4±1ºC) Storage

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## Abstract

Chicken meat spread was prepared with malted sorghum flour as substrate and *Lactobacillus acidophilus* (LAB) @ 1 million CFU/g as a fermenting probiotic organism. The products were stored under refrigeration (4±1ºC) up to 16 days for microbial stability. Different formulations were evaluated: C (meat spread only, control), C1 (meat spread + LAB), T1 (meat spread with 2% malted sorghum flour + LAB), T2 (meat spread with 4% malted sorghum flour + LAB), and T3 (meat spread with 6% malted sorghum flour + LAB). The products were microbiologically stable during the study period of 16 days. Total plate count and Yeast and mold count increased significantly ($p \leq 0.05$) towards the end of the storage period; however, the values were within the safe limits for the meat products. Coliforms were absent during the entire storage study. The count of *Lactobacillus acidophilus,* observed in T2 and T3 on 0 day suggests that considering the serving size of product as 100 g per day the required minimum number of viable probiotic organism was $\geq 10^8$ CFU prescribed for a probiotic product.

**Key words:** Chicken meat spread, Fermented product, *Lactobacillus acidophilus*, Probiotics, Sorghum flour.

*Ind J Vet Sci and Biotech* (2024): 10.48165/ijvsbt.20.4.28

## Introduction

In modern times, meat and its products are not just following convenience trends; but they are also acknowledged as the cornerstone of the meat industry. Meat spread, a cooked spreadable product made with a combination of meat and non-meat ingredients, epitomizes this convenience aspect (Arya *et al*., 2017). With the increased knowledge of fermented meat products, the use of starter cultures has become a suitable strategy for improving processing and quality control (Laranjo *et al*., 2019). Live microorganisms in probiotic food, when consumed in sufficient quantities have health-promoting effects and can alter the gut microbiome (Binda *et al*., 2020). Studies have shown that LAB (lactic acid bacteria) e.g. *Lactobacillus, Pediococcus* etc. can be used as starter cultures in meat products (Bintsis, 2018). LAB can inhibit the growth and other activities of meat spoilage bacteria and can exert antifungal activity (Balciunas *et al*., 2013) offering better opportunities as natural and efficient food preservatives and as excellent alternatives to chemicals (Imade *et al*., 2021).

The advantages of including non-meat ingredients in meat products increased flavor, provide substrate and better storage stability due to lactic acid production in case of fermented products (Barbut, 2015). The most grown millet of India*, i.e.,* sorghum, is an excellent source of rich nutrients like protein, iron, zinc and vitamin B complex (Hariprasanna, 2023). The process of malting affects both the quantity and composition of phytochemicals present in sorghum grain, thereby influencing the potential health benefits of the final product. Sorghum phytochemicals, particularly anthocyanins and other flavonoids, are believed to possess various health benefits, including anti-inflammatory, antidiabetic, anticancer, antilipidemic, immuno-modulatory, antianaemic, neuroprotective, antidiarrheal, antimicrobial, and anthelminthic properties (Khoddami *et al*., 2017). Hence the present study was planned to develop a probiotic chicken meat spread using sorghum flour and LAB with enhanced microbial stability.

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**How to cite this article:** Agrawal, N., Singh, P. K., Yadav, S., Gupta, S., Singh, R., & Gupta, B. (2024). Microbiological Stability of Fermented Chicken Meat Spread Under Refrigerated (4±1ºC) Storage. Ind J Vet Sci and Biotech. 20(4), 134-138.

**Source of support:** Nil

**Conflict of interest**: None.

**Submitted** 03/03/2024 **Accepted** 29/04/2024 **Published** 10/07/2024

## Materials and Methods

The present study was conducted in the Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, Jabalpur (India). Dressed

chicken carcass was obtained from authorized meat shops of Jabalpur. The carcasses were deboned and stored in a deep freezer at -18°C until further use. Additives like table salt and soyabean oil were procured from the local market. Condiments like fresh onion, garlic and ginger were procured from the local market, separately peeled and a fine paste was prepared in domestic grinder. The condiment mix was prepared by mixing onion, garlic and ginger paste in 3:1:1 ratio and packed in LDPE bags and stored at -18±1°C until further use. Spice mix was prepared after cleaning, oven drying the ingredients at 45±1°C for 2 h and grinding in domestic grinder. Sorghum was procured from the local market of Jabalpur to prepare malted sorghum flour in the laboratory. The required starter culture of *Lactobacillus acidophilus* was procured from the market and was utilized for product preparation. Thermo-rigid, airtight PET (polyethylene terephthalate) containers were acquired from the local market and pre-sterilized using ultraviolet light for 30 min before use. The product was studied under refrigeration storage for a period of 16 days to assess the microbial quality including the growth and viability of *Lactobacillus acidophilus* organism which was required to be maintained at a certain level.

### Preparation of Malted Sorghum Flour

Malted sorghum flour was prepared by soaking whole sorghum grains in water (1:4 ratio) for 4-5 days at 18-20$^{o}$C (Igyor *et al.,* 2001; Emendack *et al.,* 2021) and after observing visible sprouts, grains were dried under sunlight followed by hot air oven drying at 50$^{o}$C for 30 min. Dried grains were grounded in the mixer grinder to make malted sorghum flour.

### Development of the Product

Modified method of Khanam *et al*. (2020) was followed for the preparation of chicken meat spread. Lean chicken meat was cut into small pieces and minced using meat mincer. The minced meat was chopped in a bowl chopper to prepare a fine emulsion where salt, spices, condiments and oil were added in the sequence mentioned. The ingredients were thoroughly mixed and the emulsion was then mixed with malted sorghum flour at 0, 2, 4 and 6% levels (10, 20 and 30% respectively as rehydration was done with water in the ratio of 1:4) for treatment C1, T1, T2, and T3, respectively, while chicken meat spread alone served as control (C). The prepared emulsion was steam cooked for 35 min without pressure and the cooked mixture (after cooling to room temperature) was then blended for 2 min after adding the starter culture (*Lactobacillus acidophilus* @ 1 million CFU/g meat emulsion) in treatment C1, T1, T2, and T3 to achieve a fine paste-like consistency, followed by fermentation for 12 h. Fermentation was optimized at 20°C with a relative humidity of 90±5%. Finally, it was packaged in PET jars followed by storage at refrigeration temperature (4±1$^{o}$C) for evaluation at 0, 4, 8, 12 and 16 days of storage. The control group (C) where LAB was not added was stored under refrigeration after blending without subjecting it to fermentation. Table 1 depicts the formulation used for preparation of chicken meat spread.

**Table 1:** Formulation used for preparation of chicken meat spread

| Ingredients | Percentage (%) | | | | |
|---|---|---|---|---|---|
| | C | C1 | T1 | T2 | T3 |
| Chicken meat | 86 | 86 | 76 | 66 | 56 |
| Soyabean oil | 6 | 6 | 6 | 6 | 6 |
| Condiments | 3 | 3 | 3 | 3 | 3 |
| Spice mix | 3 | 3 | 3 | 3 | 3 |
| Salt | 2 | 2 | 2 | 2 | 2 |
| Water | - | - | 8 | 16 | 24 |
| Malted sorghum flour | - | - | 2 | 4 | 6 |
| **Total** | **100** | **100** | **100** | **100** | **100** |

### Microbiological Quality Evaluation

Products were stored at refrigeration temperature (4±1$^{o}$C) and samples were analyzed from 0 to 16 days or spoilage, whichever was earlier, at a regular interval of 4 days for the microbial analysis (APHA, 1992) with respect to total plate count (TPC), coliform count, yeast and mould count and *Lactobacillus acidophilus* count.

### Statistical Analysis

Data was analyzed statistically on 'SPSS-22.0' (SPSS Inc., Chicago, Il USA) software package as per standard methods (Snedecor and Cochran, 1994). The average values were reported along with standard deviation. The statistical significance was estimated at 5% level ($p \leq 0.05$).

## Results and Discussion

### Total Plate Count (TPC)

The results of the study presented in Table 2 reveal that there was significant ($p \leq 0.05$) change in total plate count (TPC) on subsequent days of storage. Our findings corroborated with Erkkilä *et al.* (2001), Kumar *et al*. (2015) and Pradhan (2019). Moreover, Slima *et al*. (2018) suggested that addition of probiotic strains improved their potential as a bioprotective tool to extend shelf-life. It was observed that all the fermented samples under study had significantly ($p \leq 0.05$) higher TPC from the starting point of storage till the last day of study. The increased TPC values in all the fermented groups throughout the storage might be due to active multiplication of *Lactobacillus acidophilus* and forming colonies during the fermentation process which increased the total microbial count in the product. The study also showed highest TPC in T3 group, whereas comparatively lower TPC value was observed in C1 group. The TPC of control sample suggests that it was safe for consumption even after 16 days of refrigerated storage. Kumar *et al*. (2015) stated that meat spread could be considered safe for refrigerated storage up to 21 days. Regarding the fermented samples the higher TPC due to LAB fermentation is again safe for consumption as in

fermented meat products higher TPC is observed and as TPC limit criteria is not applicable in fermented meat products (FSSAI, 2008).

### Yeast and Mould Count (YMC)

The yeast and mould count of chicken meat spread during storage was observed from day 12 in C, C1, T1, T2 and T3, which gradually increased in all groups with the progress of study period. The study showed that there was significant ($p \leq 0.05$) increase in yeast and mould count with the subsequent day of storage. These observations aligned with the study conducted by Yadav (2017) and Pradhan (2019). The yeast and mould growth when compared between different trials was found to be significantly ($p \leq 0.05$) different in treatments for the fermented and non-fermented samples. Maximum yeast and mould growth was observed in C group with change from day 12 yeast and mould growth from 1.68 to 1.91 CFU/g on day 16, whereas comparatively lesser change in yeast and mould growth was observed in T3 group with 1.39 CFU/g on day 12 to 1.63 CFU/g on day 16 (Table 2). T3 containing 6% malted sorghum flour + LAB showed good results due to its strong antimicrobial properties. Similar impact of lactobacillus and malted barley flour was reported by Pradhan (2019). LAB can exert antifungal activity and inhibit its growth in product (Balciunas *et al*., 2013).

### *Lactobacillus acidophilus* Count

The *Lactobacillus acidophilus* counts gradually enhanced in all the samples under study, however the observations were significantly ($p \leq 0.05$) different in trials from the 8$^{th}$ day onwards when C1 was compared with T2 and T3, suggesting the role of malted sorghum flour as a substrate for *Lactobacillus acidophilus*. The study showed that highest *Lactobacillus acidophilus* count was noticed in T3 group with values as 7.11 CFU/g on day 16, whereas comparatively lower value was observed in C group (without sorghum flour), 5.78 CFU/g on day 16 (Table 2). The increased *Lactobacillus acidophilus* values in all the groups throughout the storage might be due to active multiplication of *Lactobacillus acidophilus* during the fermentation process which increased the LAB count in the product. The organism was not detectable in the C samples as it was not added and no fermentation happened there. The study showed that there was significant ($p \leq 0.05$) change in *Lactobacillus acidophilus* on subsequent days of storage. These findings were in accordance with the study conducted by Kumar *et al*.

**Table 2:** Microbiological quality of chicken meat product during refrigeration storage (Mean ± SD, n=6)

| Treatment | Storage days | | | | |
|---|---|---|---|---|---|
| | 0 | 4 | 8 | 12 | 16 |
| **Total plate count (CFU/g)** | | | | | |
| C | $2.63^{Aa}$±0.10 | $2.68^{Aab}$±0.08 | $2.75^{Abc}$±0.09 | $2.81^{Ac}$±0.05 | $2.84^{Ac}$±0.04 |
| C1 | $4.58^{Ba}$±0.07 | $4.64^{Bab}$±0.05 | $4.70^{Babc}$±0.13 | $4.74^{Bbc}$±0.11 | $4.78^{Bc}$±0.12 |
| T1 | $4.59^{Ba}$±0.07 | $4.64^{Ba}$±0.13 | $4.69^{Bab}$±0.06 | $4.76^{Bb}$±0.10 | $4.79^{Bb}$±0.04 |
| T2 | $4.65^{Ba}$±0.17 | $4.70^{Ba}$±0.11 | $4.74^{Ba}$±0.15 | $4.76^{Ba}$±0.11 | $4.81^{Ba}$±0.08 |
| T3 | $4.69^{Ba}$±0.18 | $4.72^{Bab}$±0.15 | $4.79^{Bab}$±0.12 | $4.81^{Bab}$±0.05 | $4.87^{Bb}$±0.07 |
| **Yeast and mould count (CFU/g)** | | | | | |
| C | ND | ND | ND | $1.68^{Cb}$±0.13 | $1.91^{Cc}$±0.07 |
| C1 | ND | ND | ND | $1.56^{BCb}$±0.09 | $1.79^{BCc}$±0.12 |
| T1 | ND | ND | ND | $1.53^{ABb}$±0.14 | $1.75^{ABc}$±0.11 |
| T2 | ND | ND | ND | $1.41^{ABb}$±0.13 | $1.64^{Ac}$±0.12 |
| T3 | ND | ND | ND | $1.39^{Ab}$±0.10 | $1.63^{Ac}$±0.15 |
| ***Lactobacillus acidophilus* count (CFU/g)** | | | | | |
| C | ND | ND | ND | ND | ND |
| C1 | $4.45^{Ba}$±0.10 | $4.87^{Bb}$±0.12 | $5.33^{Bc}$±0.20 | $5.61^{Bd}$±0.22 | $5.78^{Bd}$±0.12 |
| T1 | $5.69^{Ca}$±0.15 | $5.77^{Cab}$±0.14 | $5.86^{Cbc}$±0.16 | $5.95^{Cc}$±0.04 | $6.11^{Cd}$±0.08 |
| T2 | $6.74^{Da}$±0.14 | $6.78^{Dab}$±0.12 | $6.85^{Dab}$±0.17 | $6.94^{Dbc}$±0.07 | $7.06^{Dc}$±0.14 |
| T3 | $6.78^{Da}$±0.15 | $6.84^{Dab}$±0.18 | $6.93^{Dab}$±0.11 | $6.99^{Dbc}$±0.04 | $7.11^{Dc}$±0.13 |

**Coliform count (CFU/g) of the chicken meat spread:**
ND, It was not detected in any of the treatment or period of storage.

Means with different superscripts in upper case in a column and lower case in a row differ significantly ($p \leq 0.05$). C = Control, C1= 0% malted sorghum flour + LAB, T1, T2 and T3 = 2, 4 and 6% malted sorghum flour + LAB.

(2015) and de Marins *et al*. (2022), where *Lactobacillus acidophilus* counts increased during storage. Addition of *Lactobacillus acidophilus* to food products not only enhances their shelf-life as a preservative but also acts as a probiotic (Gomes and Malcata, 1999). Due to their therapeutic potential in maintaining intestinal microbial balance, strains from the *L. acidophilus* group have been extensively used as starter cultures for different food products. Considering the serving size of product as 100 g, the count of probiotic organism (*Lactobacillus acidophilus*) in the developed products (T2 and T3) observed on day 0 suggests that minimum number of viable probiotic organism prescribed for probiotic food was $\geq 10^8$ CFU in the product. The viable number of added probiotic organisms in food shall be $\geq 10^8$ CFU in the recommended serving size per day (FSSAI, 2021). As probiotic food contains live organisms for potential health benefits, it was added in the present study after proper cooking and before fermentation taking all the hygienic precautions to avoid any contamination. Probiotics are sensitive to high temperatures, like those used in cooking, which might make it harder to incorporate them in meat products that are prepared at home (Munekata *et al*., 2022).

### Coliform Count (CFC)

The coliform count (CFC) was not detected in any of the groups during the entire storage period. This could be due to good hygienic practices during product preparation as well as high heat treatment during cooking for chicken meat spread preparation. The coliforms were also not recorded by Kumar *et al*. (2015), Yadav (2017) and Pradhan (2019) throughout the period of storage, possibly due to stringent hygienic practices and higher cooking temperatures. Yadav (2017) also noticed the absence of coliforms after cooking across all groups and observed that heat processing eliminated most bacteria present in the fermented chicken sausages.

## Conclusions

The present study indicates that total plate count, yeast and mould count and *Lactobacillus acidophilus* count increased significantly ($p \leq 0.05$) with the increase in storage period; however, coliforms were absent during the entire study. The lactobacillus count indicated the effective role of malted sorghum flour as a substrate for the growth of organism, and results of T2 and T3 indicated that even on the 0 day it had the feature of a probiotic food considering a 100 g serving size.

## Acknowledgement

The office of Dean, College of Veterinary Science and Animal Husbandry, Jabalpur is acknowledged for financial assistance to conduct this study. The support provided by different departments is also duly acknowledged.

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