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Original Contribution

EFFECT OF INOCULATION OF *PEDIOCOCCUS ACIDILACTICI* METABOLITES ON THE MICROBIAL PROFILE OF TURKEY MEAT STORED AT DIFFERENT LOW TEMPERATURE RANGE

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ABSTRACT

Studies were carried out to investigate the effect of *Pediococcus acidilactici* culture on turkey meat product stored at different low temperatures range (refrigeration, chilling, freezing and blast freezing). Six lactic acid bacteria species were isolated from fresh turkey meat sample under different low storage conditions. They were identified as *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus buchneri*, *Lactobacillus sake*, *Lactobacillus curvatus*, *Lactobacillus brevis*. Based on percentage occurrence, *Pediiococcus acidilactici* was selected for further studies in this work. The result obtained from the inoculation of cell suspension of *P. acidilactici* into the turkey meat sample stored at blast freezing, freezing, chilling and refrigeration conditions revealed that the microbial load decreased in count from 0 day to 28-th day in all the conditions. The blast freezing had the least while refrigeration had the highest count. Further identification of these micororganisms in the inoculated meat sample under study showed that *Staphyloccus aureus* occurred most frequently (14%) and the least was *Esherichia coli with 4*%. The result of nutritional analysis of the turkey meat sample inoculated with *P. acidilactici cell* suspension and stored for 96hr showed that the crude protein and thiobarbituric acid decreased by 37.25% and 54.17% respectively while the free fatty acid increased by 118%. In conclusion, the turkey meat sample under blast freezing showed the the least microbial load which implies longest shelf life. The blast freezing temperature appears to be the most relatively efficient storage temperature in this study.

Key words: *Pediococcus acidilactici*, blast freezing, freezing, refrigeration, chilling, turkey meat

INTRODUCTION

Economic losses due to spoilage of meat and meat product constitute major concern to retailers and consumers in Nigeria. The use of lactic acid bacteria for food processing is not only based on their characteristics flavor enhancing nature but more on their ability to lower pH and to produce antimicrobial agents such as lactic acid, acetic acid, ethanol, diacetyl, hydrogen perioxide, reutrin and bacteriocins (1, 2, 3, 4). In addition to preservation, a number of nutritional, technological and health benefits are associated with the use of LAB fermentation. (5)

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Lucke (6) reported that this property of Lactic acid bacteria had enhanced stable and safer fermented end- product. Food and feed fermented with lactic acid bacteria have been reported to contain higher vitamins, predigested protein and lactose, absence of phytate and glucosoinolates**,** anticarcinogenic and hypochlolesterimic activities (7, 8).

Meat has long been considered a highly desirable and nutritious food, but unfortunately it is also highly perishable because it provides the nutrients needed to support the growth of many types of microorganisms (9, 10) Apart from microbial spoilage some chemical and biochemical changes do occur during prolonged storage and these may reduce the shelf life of the meat (9). Owning to the spoilage potential of meat, many varieties of preservation techniques

are being employed in improving its keeping quality and shelf life. Such techniques include heating, drying, curing, salting, sugar addition, irradiation and cold temperature storage (9).

Factors affecting meat spoilage include intrinsic (e.g. pH, a_w, composition, type and extent of initial contamination} and extrinsic parameters (e.g temperature and packaging atmosphere). Among these, temperature is considered the most important factor because temperatures approaching 0^0C or lower retard the growth and metabolic activities of microorganisms (11, 5). Today meat preservation is associated with refrigerator, the deep freezer, canning process and blast freezing which is not popularly used in Nigeria (12). Modern refrigeration and freezing equipments have made it possible to transport and store perishable foods for long period of time (5). One of the concern in current food preservation research is the replacement of chemical preservatives due to their harmful side-effects with more natural biopreservative method such as the use of organic acids or the application of fermentation process (13, 14, 15). Also modern methods include the use of enzyme lysosomes from chicken eggs and membrane–active bacteriocin and nisin (16). Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity and susceptibility to genetic mutation (17). However, microbes are preferred source of enzymes due to their rapid growth, limited space required for cultivation, ready accessibility to genetic mutation (17). The utilization of freezing, chilling and refrigeration techniques is highly documented. However there is paucity of information regarding the application of blast freezing technique in meat preservation in Nigeria. Therefore, this present work is aimed at studying the efficiency of blast freezing technique in meat storage and preservation.

MATERIALS AND METHODS

Collection of Samples

Turkey meat samples were procured from Ekiti, Oyo and Lagos States in South Western Nigeria. The samples were brought to the laboratory in sterile containers for immediate use.

Isolation procedure

Five grams of the fresh turkey meat sample was weighed and transferred into 100 ml of sterile distilled water in 250 ml conical flask and this was vigorously shaken to dislodged the microorganisms present in the sample into the sterile distilled water. Serial dilution of the water extract from the meat sample was carried out according to the method described by Harrigan and McCane (18) and 0.5 ml of dilution 10^4 was transferred into sterile Petri-dishes containing Rogosa and Sharpe (MRS) agar and incubated anaerobically at 37^0C for 48h.The plates were examined for growth and the isolates observed were subcultured repeatedly until pure cultures were obtained and stored on MRS slants in Mac Canthney bottles and kept in the refrigerator. The remaining portion of the turkey meat sample was divided into three portions and were subjected separately to blast freezing treatments using blast freezer (50 horse power Presco Model), Freezing using Deep freezer (Ariston Model No 153), refrigeration and chilling using (Haier Thermocool Model No HR - 137) for 28days.

Isolation was carried out again on the turkey meat samples subjected to different low temperature treatments as described earlier at 7days interval for 28days using MRS agar.

Identification procedure

Pure cultures of the isolate were identified using API 50CH and 50 CHL medium. (API system, Montalieu, Vericeu, France) and percentage occurrence of the LAB isolates was calculated (This procedure had been used in an earlier publication by me)

Preparation of inoculum

A loop full of a twenty four hours old culture of the LAB isolate with the highest % of occurrence was transferred into 150 ml Erlenmeyer flask containing 50 ml of sterile MRS broth which was incubated anaerobically at 37^0C for 48h.One ml of the bacterial cells suspension was aseptically inoculated into sterile plate containing MRS agar and incubated anaerobically for 24h.The number of colonies observed on plate were counted and this serves as the CFU/ML

Inoculation of turkey meat sample

Another three set of fresh turkey meat samples were obtained and differently inoculated with 1ml of culture suspension (1.70×10^4) of the LAB isolate showing the highest % occurrence. The inoculated turkey meat samples were subjected separately to blast freezing, freezing, refrigeration and chilling for 28days.

Isolation technique
Isolation of microorganisms from the inoculated turkey meat sample with 1ml of culture suspension (1.70×10^4) of the LAB isolate showing the highest % occurrence and subjected separately to blast freezing, freezing, refrigeration and chilling for 28days was carried out as described earlier at 7days interval but Nutrient Agar(NA), Mac Conkey Agar (MC) and Potato Dextrose Agar (PDA) were used and incubation was done aerobically at 30^0C while incubation periods of 48h and 7days were employed and observed microorganisms on plate were enumerated.

Identification procedure
The bacterial isolates obtained from the turkey meat samples inoculated with 1ml of culture suspension (1.70×10^4) of the LAB isolate showing the highest % occurrence and stored at different low temperatures were identified using API 20E and API 20NE, while the fungal isolates were identified with reference to compendium of soil fungi. (19)

Microbial Treatment of turkey meat sample
A fresh turkey meat sample (5gms) was obtained and inoculated with 1ml of culture suspension (1.70×10^4) of the LAB isolate showing the highest % occurrence and kept at blast freezing temperature for 5days.

Nutritional Analysis
Determination of Crude Protein Content, Free Fatty Acid (FFA) and Thiobarbituric Acid (TBA) of fresh turkey meat sample inoculated with 1ml with of culture suspension (1.70×10^4) of the LAB isolate showing the highest % occurrence and stored at blast freezing temperature for 5 days were determined according to the method of AOAC (20)

RESULTS
Table 1 shows percentage occurrence of Lactic acid bacteria isolated from turkey meat samples at different low temperature storage while **Table 2** shows microbial load of fresh turkey sample inoculated with culture suspension of *Pediococcus acidilactici (log cfu)* and stored at different low temperature storage. **Table 3** shows the frequency of occurrence of different isolates from turkey meat sample inoculated with *Pediococcus acidilactici and* stored at different low temperatures. **Table 4** shows nutritional analysis of turkey meat sample inoculated with 1ml of *Pediococcus acidilactici cell* suspension and stored at blast freezing temperature for 5 days.

Table 1. *The Percentage occurrence of Lactic acid bacteria isolated from turkey meat samples*

Isolates	Percentage occurrence
Pediococcus acidilactici	47.2
Lactobacillus plantarum	35.9
Lactobacillus buchneri	14.3
Lactobacillus sake	10.7
Lactobacillus curvatus	8.3
Lactobacillus brevis	7.4

The result of isolation showed that the highest occurring microorganisms was *Pediococcus acidilactici* showing (47.2%), followed by *Lactobacillus plantarum* (35.9%) while the least was *Lactobacillus curvatus* (7.4 %) a refrigeration, b chilling, c freezing, d blast freezing.

TEK location 1, Ekiti state, TIB location 2,Oyo state, Lagos state TIB location 3,Oyo state.
The turkey meat sample stored at the blast freezing temperature contained the least number of microorganisms, 3.90 at the 28th day while the highest microbial load 5.16 was recorded in turkey meat sample stored at refrigeration temperature at the 28th day.

Further identification of the microbial load to species level in the turkey meat samples under different low temperatures storage (blast freezing, freezing, refrigeration and chilling) showed that *Staphyloccus aureus* recorded the highest frequency of occurrence with (14%) while the least occurring microorganism was observed to be *Escherichia coli* with 4%.

Table 2. *The Microbial load of fresh turkey sample inoculated with 1ml culture suspension of Pediococcus acidilactici (log cfu) and stored at different low temperatures storage.*

Duration	LOCATION											
(Days)	TEK[a]	TLA[a]	TIB[a]	TEK[b]	TLA[b]	TIB[b]	TEK[c]	TLA[c]	TIB[c]	TEK[d]	TLA[d]	TIB[d]
0	5.30	5.20	5.14	5.20	4.80	4.80	4.98	5.14	5.12	5.23	5.24	5.30
7	5.44	5.14	5.14	4.84	4.92	4.72	5.14	5.05	5.10	5.22	5.16	4.93
14	5.32	5.26	5.19	4.85	4.83	4.83	5.10	5.10	5.06	5.20	5.19	4.64
21	5.17	5.15	5.16	4.73	4.73	4.75	5.05	5.05	5.04	5.18	5.27	4.25
28	5.16	5.09	5.08	4.70	4.61	4.18	4.96	4.92	4.90	4.05	3.90	4.11

Table 3. *The Frequency of occurrence of different isolates from turkey meat sample inoculated with 1ml cell suspension of Pediococcus acidilactici and stored at different low temperatures.*

Microorganisms	Frequency of occurrence
Enterobacteriacea	
Pseudomonas putida	7
Pseudomonas flourescens	7
Klebsiella pneumoniae	11
Enterobacter aerogenes	6
Eschericia coli	4
Proteus mirabilis	9
Gram Positive Organisms	
Micrococcus species	9
Staphylococcus species	14
Bacillus substilis	7
Bacillus cereus	5
Fungi	
Aspergillus niger	11
Aspergillus flavus	6
Rhizopus stolononifer	6
Penicillium notatum	5

Table 4. *Nutritional analysis of turkey meat sample inoculated with 1ml of Pediococcus acidilactici cell suspension and stored at blast freezing temperature for 5days.*

Storage Period (h)	**CP(%)**	**FFA(%)**	**TBA(mg/malonaldehyde/kg)**
0	70.44	0.30	0.48
24	65.60	0.42	0.42
48	63.10	0.50	0.36
72	52.50	0.50	0.32
96	51.40	0.54	0.28
120	44.20	0.60	0.22

The proximate analysis of the turkey sample inoculated with 1ml cell suspension of *Pediococcus acidilactici* and stored under blast freezing temperature for 5days is shown in **Table 4.** The crude protein content was higher in the control when compared with the treated turkey meat sample. It was observed that it decreased from 70.44 to 44.00 at the 5day, while fatty acid content increased from 0.30 to 0.60 within the same period. However the thiobabituric acid showed a decrease of 118% after 120hr.

DISCUSSION

Determination of percentage occurrence of Lactic acid bacteria isolated from turkey meat samples at different low temperature storage, determination of microbial load of fresh turkey samples inoculated with culture suspension of *Pediococcus acidilactici* and stored at different low temperature storage, determination of the frequency of occurrence of different isolates from turkey meat sample inoculated with *Pediococcus acidilactici and* stored at different low temperature and determination of nutritional analysis of turkey meat sample inoculated with 1ml of *Pediococcus acidilactici cell* suspension and stored at blast freezing temperature for 5days were carried out. Table1 shows percentage occurrence of Lactic acid bacteria isolated from turkey meat samples at different low temperature storage. The LAB species isolated from fresh turkey meat samples under low different temperature storage were identified as *Pediococcus acidilactici*, *Lactobacillus plantarum*, *Lactobacillus buchneri, Lactobacillus sake, Lactobacillus curvatus and Lactobacillus brevis,* Hamasaki *et al* (21) and Kato *et al* (22) had previously reported the isolation of *L. plantarum* and *P. acidilactici* from spoiling cooked meat stored at 10^{o}C while Stiles (9) had identified aciduric *Lactobacillus spp* including *L. sake*, *L. curvatus* and *L. plantarum* from fresh meat samples.. The survival of LAB especially in cold turkey meat sample could be due to the substrate uptake, cell permeability, enzyme systems and synthetic pathway which are able to function at psychrophilic temperature (23). However, the nutritious nature of meat makes it to be susceptible to microbial growth and proliferation because it provides the nutrients needed to support the growth of many types of microorganisms (9)

Table 2 shows microbial load of fresh turkey sample inoculated with 1ml culture suspension of *Pediococcus acidilactici* and stored at different low temperatures storage. The decrease in microbial load observed in the turkey meat samples inoculated with culture suspension of P acidilactici may be due to the production of antimicrobial compounds such lactic acid, diacetyl, hydrogen peroxide and bacteriocin which had been reported to be secreted by the administered microorganism and these create a high inhibitory ability to other invading microorganism (1).

However, the viable counts of microorganisms at blast freezing temperature was observed to be the least while the refrigeration temperature recorded the highest. This observation explains the spoilage of meat vis-à-vis growth of microorganisms. During refrigeration treatment, increase in viable count led to meat spoilage. Brackett (24) reported that refrigeration treatment is primarily a surface phenomena resulting in the formation of slime and off odour. Spoilage of fresh meat at refrigeration temperature is a problem of aesthetics, product quality and economics (25).

Table 3 shows the frequency of occurrence of different isolates from turkey meat sample inoculated with 1ml cell suspension of *Pediococcus acidilactici* and stored at different low temperatures. The presence of spoilage microorganisms in meat preserved under cold storage conditions had earlier been reported (22).

The presence of *Klebsiella pneumoniae, Proteus mirabilis, Enterobacter aerogenes, Escherichia coli, Pseudomonas putida, Pseudomonas flourescens, Bacillus subtilis, Bacillus cereus, Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus and Penicillium notatum* in turkey meat samples inoculated with culture suspension of *P.acidilactici* might be caused by the selective limitation of the antibactericidal and antifungicidial activities of the metabolites produced by *P.acidilactici* and this might be responsible for the existence of these microorganisms with different % of occurrence. In addition, the differences in the level of the microbial load of different turkey meat samples stored under the same temperature regime might be caused by contamination of raw product, sanitation in the processing plant and the speed and care within which the product was processed in their different locations of procurement (26)

Table 4 represents nutritional analysis of turkey meat sample inoculated with 1ml of *Pediococcus acidilactici* cell suspension and stored at blast freezing temperature for 5days. The decrease in crude protein content of the turkey meat sample could be due to proteolytic activities of the inoculated *P. acidilactici* and other microbes during storage (9), while the increase in free fatty acid could be explained as a result of citrate metabolism by some species of LAB thus enhancing FFA production (27). However the decrease in thiobarbituric acid production is an indication of microbial safety/stability of the meat product. It can therefore be deduced that turkey meat sample will be most microbiologically stable under blast freezing temperature than other temperature storage reported in this study.

CONCLUSION

From this investigation blast freezing storage coupled with the application of *Pediococcus acidilactici* metabolite seemed to be the most efficient technique in preserving meat product.

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