

Original Research Article

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Comparative Analysis of Bacterial Isolates from Fresh and Frozen Momos Using Phenotypic and Antibiotic Susceptibility Profiles

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ABSTRACT

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Momos are a common ready-to-eat meal item that can be eaten either freshly made or after being frozen. The purpose of this study was to compare the microbiological traits of fresh and frozen momos and evaluate the variations in their bacterial traits and microbial burden. Momo samples, both frozen and fresh, were gathered aseptically from certain sources and prepared for microbiological analysis. After determining the viable bacterial load using serial dilution and spread plate procedures, isolated colonies were obtained by streaking. Gram staining and colony morphology were used to describe the isolated organisms. Catalase and a few other biochemical assays were used for additional identification. To evaluate the bacterial isolates' susceptibility pattern to particular antibiotics, antibiotic susceptibility testing (AST) was carried out. Microbial load, bacterial diversity, biochemical traits, and patterns of antibiotic susceptibility between fresh and frozen samples were used to compare the outcomes. The study stresses the significance of appropriate preparation, handling, storage, and sanitary methods in preserving the microbiological safety of ready-to-eat foods and gives an overview of the microbiological quality of fresh versus frozen momos.

Introduction

Microbiology is being recognized as the scientific study of microorganisms, which are small, primarily single-celled or acellular life forms that are remaining invisible to the unaided eye. (Sattley and Madigan, 2015). It is dealing with various branches including Food microbiology, it defines microorganisms that are having positive or negative effects on food safety and quality

and is therefore being considered a matter of public health concern.

Street vendors in public spaces produce or sell ready-to-eat foods known as street meals. Without additional preparation, they are meant to be consumed right away or later (FAO, 2013). They are being widely appreciated because they offer distinctive Flavors, affordability, convenience, and cultural and social significance. In that

way Momos are Tibetan traditional dumplings as street food. Momo is an excellent source of dietary fibre, proteins, minerals, vitamins, and carbs (Chen et al., 2012). Their nutritional content ranges from 30 to 60 kcal per piece, depending on how they are filled and prepared (Singh and Singh, 2024).

A freezing technique known as Individual Quick Freezing (IQF), quickly freezes each food item separately, This procedure prolongs the shelf life of frozen foods while preserving their nutritional value and sensory appeal. Freezing and storing below -18°C prolongs shelf life while preserving the product's sensory and nutritional qualities. When the contaminated street food can cause Foodborne infections, or illnesses, that diseases or toxins by nature, and they are brought on by substances that enter the body through tainted food (Notermans and Hoogenboom-Verdegaal, 1992). Common foodborne pathogen such as *Salmonella spp.*, *Campylobacter spp.*, *E. coli*, etc. When the meat get spoiled certain microbes can occupy the food such as *Pseudomonas spp.*, *Lactobacillus*, *Enterobacter spp.*, and this will cause the pathogenic diseases such as Botulism, Salmonellosis, Campylobacteriosis.

The present study focuses on the comparative analysis of fresh momos and frozen momos microbial population in microbiological levels by isolating and identifying the bacteria present in the food sample.

Materials and Methods

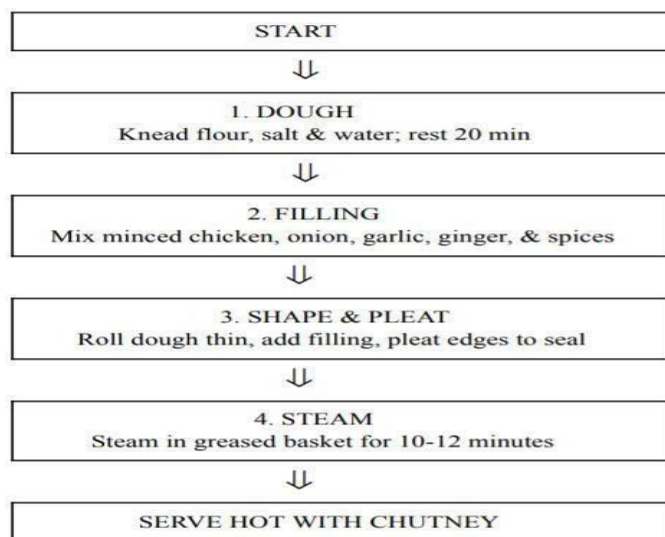
Collection and Preparation of Sample

Raw ingredients for momos preparation, including all-purpose flour, chicken, onion, coriander leaves, spring onion, ginger, garlic, salt, and sauces, are being collected from the Chrompet market (Rodriguez-Blanco, G, 2017). The ingredients are being washed with running tap water and are being dried using a cotton cloth to remove excess moisture. They are then being chopped or ground as required for the stuffing. The dough is being prepared to a soft consistency and is being rested for 10–15 minutes. The stuffing is being prepared by adding a small amount of oil, ginger, garlic, and other ingredients, and the chicken is being cooked thoroughly (Mira. N, 2019). The momos dough is being kneaded and shaped into thin wrappers. The prepared stuffing is being placed inside the wrappers, which are being folded into the desired shape (Guglick, M.A., 2001). Finally, the momos are

being steamed for 10–15 minutes until fully cooked. When the frozen momos buying near supermarket of chrompet. Here two samples were taken A & B

Sample A -Fresh momos

Sample B – Frozen momos



Serial Dilution

Both sample A and sample B were undergo serial dilution to numerate the bacterial load in the sample (Davidson, C.E. 2014). When the momos sample was given a 9:1 ratio which means 9ml of distilled water and 1gm of momos sample were taken and mixed nicely, allow them for 2-3 mins and take the 1ml of Surprenant. This will be added in the test tube marked as 10^{-1} , 10^{-2} , and 10^{-3} respectively, each test tube was filled with 9ml of saline and 1ml of diluted sample was added into the 1st test tube and then 1ml of sample was taken into the 1st test transfer into the 2nd test tube followed by the third tube (Blodgett, R.J. 2005). Hence the bacterial load will be different in each test tube, it will help to recognize the bacterial load of the sample after doing serial dilution (Aislabie. J, 2005).

Spread Plate

The spread plate technique is being used as the most standard microbiological method to isolate and identify microbes in a liquid sample (Wise, K., 2006). It is helping aerobic and facultative aerobic microorganisms grow easily. When 20 mL of the basal medium like nutrient medium is being poured into a Petri plate, it is being

allowed to solidify (Sanders, E.R., 2012). Then, 0.1 mL of the serially diluted sample is being added in the respective plates 10^{-1} , 10^{-2} , 10^{-3} and it is being spread throughout the plate using an L-shaped rod. The plates are then being incubated for 24 hours.

Streaking Method

A microbiological technique called "streaking for isolation" uses an inoculating loop to separate specific bacterial colonies from a mixed community on agar plates. Because of this isolation, individual colonies may be examined and identified using their unique physical traits.

Individual species are categorized so they can survive in their ideal environment rather than working with the mixed culture. Many species are suppressed and just a few dominant species will grow in a harsh and competitive environment that is not ideal for the growth of all species in a mixed culture. Here the plates are inoculated with simple streaking (Katz, D.S., 2008).

Gram's Staining

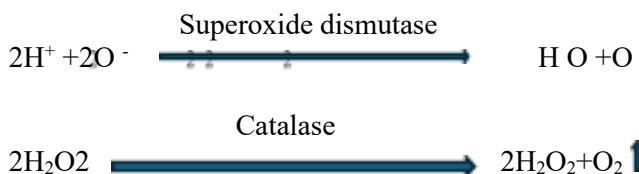
Gram staining is the fundamental technique in microbiology, A sterile glass slide was taken one drop of saline was added and one full of culture was taken inoculate into the glass slide make smear by heat fixing and the procedure containing mainly three chemical reagents such as primary, mordant, decolourizer, counterstain (Beveridge, T.J., 2001). When the crystal violet acting as a primary stain which will coated with the cell wall and Gram's iodine acting as mordant, it will replace the hydrogen bond in the crystal violet and form crystal violet -iodine complex (Hopps, H.C., 1973).

The next step of adding decolourizer acetone and this will decolorize the primary stain, if the gram-negative bacteria present because gram-negative bacteria lack with peptidoglycan but rich in lipopolysaccharides whereas the gram-positive bacteria rich in peptidoglycan but lacks will lipopolysaccharides. When the counterstain such as safran in has been added, which organism loss the primary stain it will uptake the counterstain.

Grams-positive bacteria appeared in purple/ violet colour and gram -negative bacteria appeared in pink colour (Bartholomew, J.W. and Mittler).

Catalase and Oxidase Test

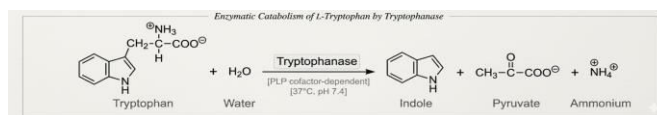
The aerobic respiration microorganism produces certain potent cellular toxins such as hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-). Aerobic and aerotolerant bacteria produce enzymes capable of detoxifying these compounds. Superoxide dismutase catalyses conversion of superoxide radicals to hydrogen peroxide. Catalase converts hydrogen peroxide into water and gaseous oxygen (O'Brien, P.J., 2006).



Biochemical Test

Indole Test

The indole test is based on the capacity of specific bacteria to produce the enzyme tryptophanase, which converts tryptophan in the medium into ammonia, pyruvic acid, and indole. By adding Kovac's reagents (dimethylaminobenzaldehyde) which interacts with indole to generate a red- colored formation, indole synthesis can be detected (Jasim, A.H., 2021).



Peptone broth was made, sterilized, and transferred into sterile test tubes for the experiment. Aseptic conditions were used to inoculate the bacterial isolates into the broth, which was then incubated for 24 hours at 37°C (Collee, J.G., 1971). After incubation, each tube was carefully filled with about 0.5ml of Kovac's reagent. A favorable indole response was shown

Methyl Red

The Methyl red test is designed to detect organisms capable of overcoming mixed acid fermentation, Methyl red broth containing peptone, glucose and phosphate buffer. After inoculating culture in the test tubes were incubated for 24 hours then the next day methyl red was added and red colour formation truly indicates the pH change leads to positive results.

Voges Proskauer

The Voges-Proskauer test was designed for organisms that ferment glucose but quickly convert their acid products to acetoin 2,3 butanediol. Addition of VP broth into the culture tubes reagent to the medium oxidizes the acetoin to diacetyl, which in turn reacts with guanidine from peptone to produce a red colour. A positive VP result is red. Copper colour is a result of interaction between the reagents and it is negative (*McDevitt, S., 2009*)

Citrate Utilization

Simmons citrate agar is a defined medium that contains sodium citrate as the sole carbon source and ammonium phosphate as the sole nitrogen source. Bromothymol blue dye is green at pH 6.9 and blue at pH 7.6, is added as a pH indicator. Bacteria that survive in the medium utilize citrate and also convert the ammonium phosphate to ammonia and ammonium hydroxide both of which tend to alkalize the medium. As the pH goes up, the media changes from green to Prussian blue. Thus, conversion of medium to Prussian blue indicates a positive result.

Antibiotic SE McDevitt, S., 2009 Ntivity Test

When the growth of a microorganism is inhibited by the concentration of antibiotic plays in the Mueller Hinton agar plate containing bacterial culture, it is considered that microorganism is susceptible to that antibiotic employed.

When there is no zone formation is considered as antibiotic resistant to the bacterial culture (*Kamat, N.M., 2011*)

Results and Discussion

Spread Plate

The spread plate analysis showed the presence of yellow bacterial colonies in both fresh and frozen momos samples. However, the frozen momos sample exhibited a higher number of yellow colonies than the fresh momos sample, indicating a greater microbial load.

Streak Plate

The streak plate method showed the presence of yellow bacterial colonies in both fresh and frozen momos samples. Both samples exhibited a similar number of yellow colonies, indicating a comparable microbial load.

Gram's Staining

Gram staining showed Gram-negative cocci in fresh momos and Gram-positive bacilli frozen momos samples. The cells appeared spherical and cocci under the microscope.

Catalase Test

The catalase test was Positive for fresh momos and Negative for frozen momos samples. Bubble formation was observed after adding hydrogen peroxide, indicating the presence of catalase-producing bacteria.



Fig.1 S1



Fig.2 S2



Fig.3 S1



Fig.4 S2

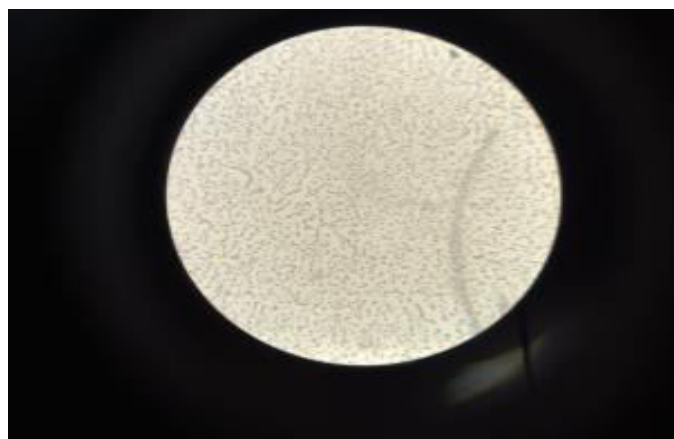


Fig.5 S1

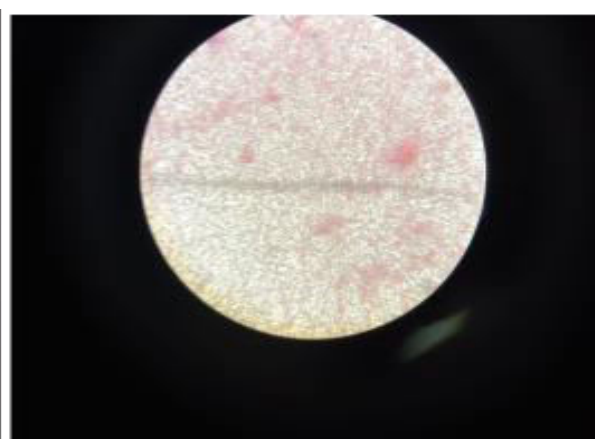


Fig.6 S2



Fig.7 S1

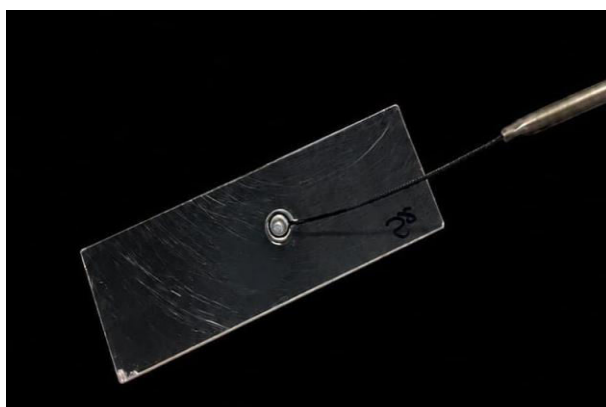


Fig.8 S2

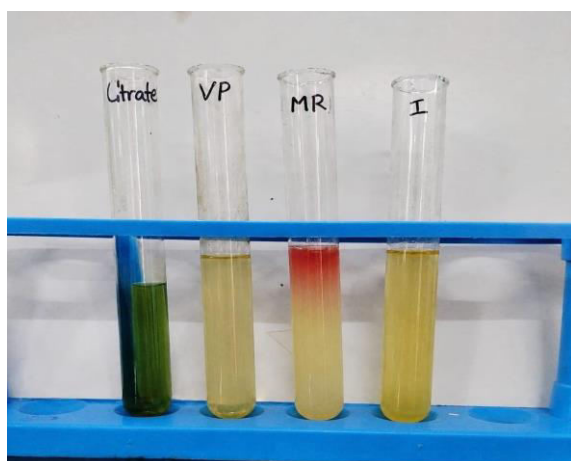


Fig.9 S1

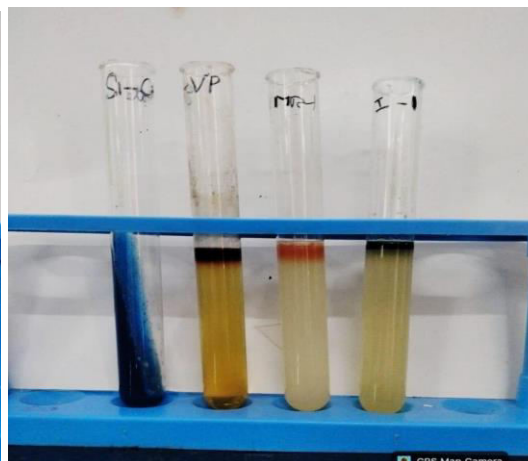


Fig.10 S2



Fig.11 S1

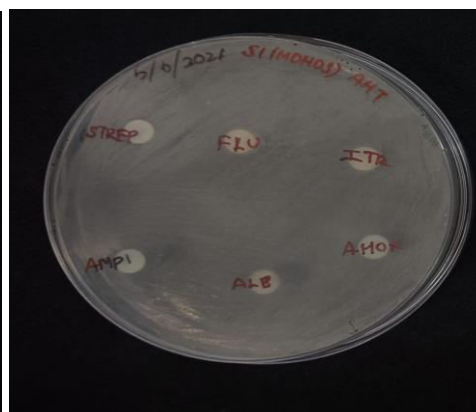


Fig.12 S2

Table.1 Biochemical Test

Test	Frozen Momos	Fresh Momos
Indole	Negative	Negative
Methyl red	Positive	Negative
Voges poskauer	Negative	Positive
Citrate	Negative	Positive
Catalase	Negative	Positive

Antibiotic Sensitivity Test

While the frozen momos sample displayed bacterial growth with clear zones of inhibition surrounding the tested disks, antibiotic susceptibility testing revealed no discernible bacterial growth in the fresh momos sample.

In conclusion, the bacterial isolate Based on preliminary identification tests, the study found *Lactobacillus* in the frozen momos sample and *Moraxella catarrhalis* in the

fresh momos sample. These results show that fresh and frozen momos samples have different bacterial profiles. Further confirmatory biochemical or molecular tests are required for accurate species identification.

Author Contributions

Yogalakshmi. N: Equal contribution of collecting data, analysis, writing. Swetha. I: Equal contribution of collecting data, analysis, writing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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