



Effect of Rice Beer and Phyto-ingredients on Microbiological Qualities of Vacuum and Aerobically Packaged Marinated Duck Meat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research was carried out to investigate the effect of incorporating rice beer and phyto-ingredients on the microbiological qualities of vacuum packaged marinated duck meat. Ducks aged between 9 and 12 months were procured from a local market and hygienically slaughtered and dressed. Five batches of marinated duck meat were prepared, each consisting of four different treatments. Following slaughter and dressing, the ducks were marinated using four altered

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formulations: a control group (meat + spice paste), T₁ (meat + rice beer + spice paste), T₂ (meat+ phyto-ingredients + spice paste), and T₃ (meat + rice beer + phyto-ingredients + spice paste). These marinated meat samples were then vacuum-packaged and stored under refrigeration for 24 hours. Subsequent quality assessments were carried out on the samples at 5 days intervals for the vacuum-packed samples. The microbiological quality studies revealed that there is a significant increase ($P < 0.01$) in control samples compared to treated samples during the entire storage periods in both the packaging systems, whereas, total psychrophilic count could not be detected on 1st and 5th day of storage in vacuum packaging but on 10th and 15th day it increases in the storage periods. Similarly for aerobically packaging systems, Total viable mesophilic plate count (TVMPC) were not detected on 1st day of storage but on 3rd, 5th and 7th day of storage it increases in the storage periods. The yeast and mould count and *Coliform* count were absent in all the storage periods in both the packaging systems. The sulphite reducing *Clostridial* organisms and *Salmonella* were also found to be absent in all the samples for both the packaging systems.

Keywords: Meat; marination; phyto-ingredients; fermented beverages.

1. INTRODUCTION

“Meat is one of the most nutrient-dense foods. It provides most of the energy and essential nutrients, including high-quality protein, micronutrients (such as readily bioavailable iron, zinc, and selenium), vitamins (B6, B12, and folic acid), and bioactive compounds (taurine, carnitine, carnosine, ubiquinone, glutathione, and creatine) needed for various metabolic functions of human body” (Stadnik J, 2024).

These components have a specific function to our body. The excellent digestibility and well balanced composition of essential amino acids also make the meat a universally acceptable food commodity. Meat in India can be produced from the recognized domesticated meat animals and poultry. Poultry meat contributes about 50% of the total meat production, thus, poultry meat is considered to be an important meat producing animal species in India. Duck within the broader term of poultry produce second highest quantity of poultry meat. Out of total 3.74 million tonnes of poultry meat produced in India, the share of duck meat was 43941 tonnes (FAO STAT, 2019).

The region's agricultural and livestock productivity is significantly influenced by the fertile land resources and favourable climatic conditions of Assam. Throughout the ages, ducks have been a significant factor in the socio-economic landscape of rural Assam. Assam is a region in northeastern India that is renowned for the diverse array of indigenous waterfowl that are raised by farmers under traditional systems. The desi ducks account for 86.5% of the state's total duck population, which is 4.99 million. Duck farming is a primary element of the integrated farming system and is instrumental in the

empowerment of women and the improvement of the socio-economic status of the agricultural community in Assam. The people of Assam are primarily depicted as being highly interested in duck husbandry in order to satisfy their needs for meat and eggs as a source of animal protein (Dutta et.al., 2022). Out of seven distinct popular varieties namely Khaki Campbell, Nageswari, White Pekin, Muscovy, Charachemballi, Rajhanh and Pati, Pati (*Anas platyrhynchos domesticus*) duck which once considered as a non-descript indigenous duck is recently recognized as a new breed in Assam which constitutes about 85.6% of total duck population in Assam (Islam et al., 2002).

Duck meat which falls in between the red and white meat contains higher red fibre in breast and leg parts compared to chicken. It also contains high level of phospholipids, monounsaturated fatty acids especially oleic and linoleic acids (George et al., 2014) which constitutes about 60% of total fatty acids. Thus, consumption of duck meat is gaining more attention because of its status of intermediate category of meat. “Despite the higher demand for poultry meat compared to other animals, duck meat is significantly less consumed than chicken meat. Duck meat has a stronger gamey flavour and more fat (13.8 per cent) than chicken, therefore, it may be less popular among the consumers” (Biswas et al., 2019). Marination is a step with inside the pre remedy of a huge variety of meals merchandise. In the case of meat, it quickens the manner of maturation and it softens and tenderizes the meat and provides particular flavour to the very last product. This is feasible with the aid of using which includes acidic additives withinside the marinade which loosens the shape of the meat. Marination reasons

extensive degradation of structural proteins of the beef tissue, e.g., heavy chains of myosin, hastens the discharge of lysosomal enzymes into the cytosol, will increase the proteolysis of cathepsin, and reduces the warmth balance of perimysial collagen (elevated quantity of collagen to gelatin conversion at low pH all through cooking). Marination process improves tenderness, juiciness, flavour and water holding capacity and enhances shelf life of meat (Latoch et al. 2023). Fermented beverages which incorporate alcohol and natural acids can be an awesome supply for renovation of ingredients which includes meat. "The findings of an earlier study suggest the unfiltered beer-based marinades were effective in reducing lipid oxidation and Maillard reaction compounds formation, while improving the nutritional quality, safety and sensory preference of grilled ruminant meats." (Prieto et al., 2019). Traditional rice beer of North-east India is an integral part of tribal life-style connected culturally and religiously with them. The technique they use is located to be precise in lots of senses from the relaxation of the world. The strong point now no longer most effective lies within the starter way of life preparation, that is the top supply of yeast however additionally within the different elements like indigenous plant species and rice types used as substrate ensuing version in flavour and aroma. "One of the most popular ethnic drinks among these tribes of North East region is rice wine. When it comes to establishing the starter culture for rice wine fermentation, each tribe follows its own unique set of ancient rituals and beliefs" (Nath et al., 2019).

"Natural antioxidants are group of substances when present at low concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves" (Elejalde et al., 2022). "Meat quality can be improved by incorporating natural antioxidants to animal diets, adding these compounds onto the meat surface, or using active packaging. The use of antioxidants in food products is controlled by regulatory laws of a country or international standards" (Karre et al., 2013). Hence, the present meat technologists extracted new natural antioxidants from spices and herbs which are the best alternative to synthetics.

Considering the advantages of phyto-ingredients and rice beer in the form of a marinade, the present study was carried out with the following objective to study microbiological and to evaluate

the shelf-life of the product under different packaging systems at refrigeration ($4\pm1^{\circ}\text{C}$) storage temperature.

2. MATERIALS AND METHODS

2.1 Duck

Pati Duck of the age group of 9-12 months, irrespective of their sexes, were procured from the local Beltola market of Guwahati city. The ducks were slaughtered in a semi-mechanized poultry dressing unit of the Department of Livestock Products Technology and appropriately dressed hygienically. After slaughter, the carcasses were packed in medium-density food-grade polythene bags and kept in a refrigerator at $4\pm1^{\circ}\text{C}$ for 1-2 days and within that period the samples were prepared.

2.2 Collection of Indigenous Rice Beer

Locally made rice beer was collected from the Bodo community and prepared at the Amtola village, Raha, District Nagaon. The rice beer is known as "Jou" in the local Bodo language. Glutinous Rice (Bora saul) is boiled first, then cooled and allowed to dry up partially. A blended starter culture made from dry cake, commonly referred to as "Angkhu," is incorporated into rice, mixed thoroughly, and left overnight at ambient temperature. The rice mixture is then placed in earthen pots with a small amount of water and allowed to ferment for 3-4 days at room temperature. The distinct "alcoholic" aroma signifies that the mixture has matured. The semi-liquid "alcoholic mass" is strained to produce the beverage known as "Rice beer" in this process. The Rice beer is then transferred into air-tight amber glass bottles and taken to the laboratory, where it is stored at refrigeration temperatures ($4\pm1^{\circ}\text{C}$) until it is needed.

2.3 Collection of Spices

Good quality spices (Cumin, Coriander, Turmeric, Black pepper, Paprika) were collected from the local market, washed, and dried. The spices were made into powder using a conventional grinder mixer and packed in food-grade MDPE packets at room temperature until use.

2.4 Collection of Condiments

Fresh condiments (Garlic and Ginger) were collected from the local markets. Raw

condiments were washed, cut into small pieces, and ground into a paste using a conventional grinder mixer. The paste was collected in a beaker and stored in a refrigerator at $4\pm1^{\circ}\text{C}$ until use.

2.5 Marination and Packaging of Meat Samples

The wholesale breast cut was used for the present study. The cut was separated from the whole duck carcass. Marinades were prepared and applied to the breast meat samples. The marinated meat samples were then grouped as follows:

Control: Meat + Spice paste.

Treatment 1: Meat + rice beer+ Spice paste.

Treatment 2: Meat + Phyto-ingredients+ Spice paste.

Treatment 3: Meat + Rice beer+ Phyto-ingredients + Spice paste.

2.6 Packaging

The marinated samples were vacuum and aerobically packed. The packets were marked and stored at refrigeration temperature ($4\pm1^{\circ}\text{C}$) for 24 hours. After this period, the samples were subjected to various quality assessments. Proximate composition, texture profile, colour profile and sensory evaluation were analyzed in bit longer intervals of 1st, 5th, 10th and 15th days for vacuum-packed samples.

3. MICROBIOLOGICAL QUALITY

3.1 Total Viable Mesophilic Plate Count (TVMPC)

Enumeration of total viable plate count of the treated samples were done in standard plate count agar medium, pH 7.0 ± 0.1 by following pour plate technique as described by AOAC (2005). The plates were incubated at 37°C for up to 72 h.

3.2 Total Psychrophilic Count

The same method of plating and counting as done for mesophilic counts was also followed for psychrophilic counts. The plates were incubated at $6\pm1^{\circ}\text{C}$ for 3 days and then colonies were counted as described by AOAC (2005).

3.3 Coliform Count

Coliform counts of the meat samples were made at similar time intervals as that of the total viable

counts by inoculating the appropriate dilution of the sample on Endo Agar Base pH 7.4 ± 0.1 and on incubating at 37°C up to 24-72h (AOAC, 2005). Counting was done by using a bacteriological colony counter and their numbers were counted and expressed as \log_{10} CFU/g of sample.

3.4 Yeast and Mould Counts

Yeast and moulds count of the meat samples was done as per the method of AOAC (2005) using potato dextrose agar medium.

3.5 Presence or Absence Test for *Staphylococcus aureus*

Enumeration of *Staphylococcus aureus* of the treated samples was done in Mannitol Salt Agar (MSA) medium, pH 7.4 ± 0.2 by following pour plate technique.

3.6 Presence or Absence Test for Sulphide Reducing *Clostridia* organisms

Enumeration of sulphide-reducing *Clostridia* was done by following the method as described by AOAC (2005).

3.7 Presence or Absence Test for *Salmonella* organisms

Enumeration of *Salmonella* was done by following the method as described by AOAC (2005).

4. RESULTS AND DISCUSSION

4.1 Total Viable Mesophilic Counts

4.1.1 Vacuum packaged products

The result of the TVMC of marinated duck meat on different storage periods at refrigeration temperature for vacuum packaged samples is presented in Table 2.

It was observed that the TVMC showed significantly increasing trend ($P<0.01$) both in control and treated samples on progression of storage periods at refrigeration temperature. It was also observed that the treatment 2 and 3 samples recorded lowest TVMC than those of Treatment 1 and control samples. This might be attributed to the antimicrobial effect of phyto-

extracts used in the experimental samples (Hijazeen, 2022). A similar increasing trend in total mesophilic plate count during the storage periods were also recorded by Reddy et al. (2017). Nath et al. (2016) in chicken meat sausages and Lishianawati et al. (2021) in black garlic added duck nuggets. Gogoi et al. (2020) also recorded increasing trend in total mesophilic plate count during the storage days in pork nugget incorporated with natural antioxidants.

4.1.2 Aerobic packaging methods

The result of the TVMC of marinated duck meat samples on different storage periods at refrigeration temperature for aerobically packaged samples are presented in Table 3.

Similar trend in gradual increase of bacterial loads were also observed like that of vacuum packaging method. Similar reasons of bacteriostatic / bactericidal effect could also be attributed for aerobically packaged samples. The vacuum packaged samples recorded significantly lower ($P<0.01$) TVMC counts than its aerobic counterparts throughout the storage period. Absence of air in the vacuum packaged samples might have inhibited the growth of aerobic bacterial resulting in lower TVMC. Thus, Vacuum

packaging system for marinated duck meat product during storage period may be a better option for packaging of meat.

4.2 Yeast and Mould Count

Yeast and mould count were not detected in any of the samples included in the present study are presented in Table 4. This might be due to the adoption of hygienic processing practices. The results were in agreement with the reports of Reddy et al. (2017) who carried out their studies in chicken meat patties, Chandralekha (2010) in refrigerated chicken meat balls added with natural and synthetic antioxidants.

4.3 Coliform Counts

Coliform counts not detected throughout the study period in all the treated samples are presented in Table 4. It reflects the strict hygienic conditions followed during their preparation of samples as well as high treatment employed during the cooking process (Kumar and Sharma, 2004), observations were also reported by Banon et al. (2007) in refrigerated low sulphite beef patties added with ascorbate, green tea and grape seed extracts.

Table 1. Formulation of marinated duck meat

SL. No.	Ingredients	Control (%)	Treatment 1 (%)	Treatment 2 (%)	Treatment 3 (%)
1.	Duck Breast meat	93	90	88	85
2.	Assamese rice beer	-	3	-	3%
3.	Phyto-ingredients (Tea extract, Pomegranate, Lemon, Bamboo shoot extract)	-	-	5	5
4.	Salt	1.5	1.5	1.5	1.5
5.	Sodium tripolyphosphate	0.5	0.5	0.5	0.5
6.	Spices (Cumin, Coriander, Turmeric, Black pepper, Paprika)	1.5	1.5	1.5	1.5
7.	Condiments (Garlic, Ginger)	3.5	3.5	3.5	3.5
	Total	100	100	100	100

Table 2. Effect of rice beer and phyto-ingredients of marinated vacuum packaged duck meat on total viable mesophilic bacterial count (\log_{10} CFU/g) (Mean \pm SE) at refrigeration temperature

Storage periods in days	Treatment groups			
	C	T1	T2	T3
1	4.62 \pm 0.00 ^{Aa}	4.54 \pm 0.01 ^{Ab}	4.18 \pm 0.01 ^{Ac}	4.05 \pm 0.02 ^{Ad}
5	5.22 \pm 0.01 ^{Ba}	5.09 \pm 0.02 ^{Bb}	3.63 \pm 0.01 ^{Bc}	3.19 \pm 0.01 ^{Bd}
10	5.48 \pm 0.01 ^{Ca}	5.33 \pm 0.03 ^{Cb}	3.80 \pm 0.01 ^{Cc}	3.70 \pm 0.01 ^{Cd}
15	5.80 \pm 0.02 ^{Da}	5.62 \pm 0.00 ^{Db}	4.25 \pm 0.01 ^{Dc}	4.04 \pm 0.01 ^{Ad}

$n=5$

Means with different superscripts within a column (capital letters) and within a row (small letter) differ significantly

Table 3. Effect of rice beer and phyto-ingredients of marinated aerobically packaged duck meat on total mesophilic bacterial counts (\log_{10} CFU/g) (Mean \pm SE) at refrigeration temperature

Storage periods in days	Treatment groups			
	C	T1	T2	T3
1	4.70 \pm 0.01 ^{Aa}	4.62 \pm 0.01 ^{Ab}	4.34 \pm 0.02 ^{Ac}	4.15 \pm 0.02 ^{Ad}
3	5.32 \pm 0.01 ^{Ba}	5.18 \pm 0.01 ^{Bb}	3.77 \pm 0.03 ^{Bc}	3.21 \pm 0.02 ^{Bb}
5	5.55 \pm 0.02 ^{Ca}	5.46 \pm 0.02 ^{Cb}	3.87 \pm 0.01 ^{Cc}	3.76 \pm 0.02 ^{Cd}
7	5.94 \pm 0.01 ^{Da}	5.77 \pm 0.02 ^{Db}	4.38 \pm 0.02 ^{Dc}	4.16 \pm 0.02 ^{Dd}

n=5

Different superscripts within a column (capital letters) and within a row (small letter) differ significantly

4.4 Presence or Absence of *Staphylococcus* organisms antimicrobial activity of the phyto-ingredients (Reddy et al. 2017).

Staphylococci were found to be absent in all the control and treated samples throughout the storage period are presented in Table 4. The absence of Staphylococci count might be attributed to hygienic handling and processing of the product. Antimicrobial and antifungal activity of phyto-ingredients (Gupta et al. 2004), cooking time and temperature combination employed and packaging methods followed. Similar findings were also reported by (Gogoi et al. 2020) on pork nuggets by using different humectants and antioxidants.

4.5 Presence or Absence of Sulphite reducing clostridial organisms

Sulphite reducing clostridium counts not detected through out the study period in all the treated samples are presented in Table 4. It reflects the strict hygienic conditions followed by

4.6 Presence or Absence of *Salmonella* organisms

The absence of Salmonella count were found to be absent in all the control and treated samples throughout the storage periods are presented in Table 4. The absence of salmonella count might be attributed to hygienic processing, packaging and antimicrobial activity of phyto-ingredients (Gupta et al., 2004; Reddy et al., 2017).

4.7 Total Viable Psychrophilic Count

4.7.1 Vacuum packaging products

The mean values of TVPBC of marinated duck meat samples for both the control and treated samples of vacuum packaged samples at different storage period are presented in Table 5.

Table 4. Effect of packaging methods on yeast and mould counts (CFU PER GRAM), Coliform counts (MPN/GRAM) and presence or absence of *Staphylococcus* organisms, *Salmonella*, sulphite reducing clostridial organisms

Microbiological parameters	
Yeast and Mold Count	Not detected
Coliform count	
<i>Staphylococcus aureus</i>	
<i>Salmonella</i>	
Sulphite reducing clostridia	

Table 5. Effect of rice beer and phyto-ingredients of marinated vacuum packaged duck meat on total psychrophilic bacterial counts (\log_{10} CFU/g) (Mean \pm SE) at refrigeration temperature

Storage periods in days	Treatment groups			
	C	T1	T2	T3
1	ND	ND	ND	ND
5	ND	ND	ND	ND
10	1.74 \pm 0.02 ^{Aa}	1.63 \pm 0.01 ^{Ab}	1.56 \pm 0.01 ^{Ab}	1.56 \pm 0.09 ^{Ab}
15	2.73 \pm 0.02 ^{Ba}	2.64 \pm 0.01 ^{Ba}	2.53 \pm 0.01 ^{Bb}	2.43 \pm 0.01 ^{Bc}

n=5, ND= Not Detected,

Different superscripts within a column (capital letters) and within a row (small letter) differ significantly

Table 6. Effect of rice beer and phyto-ingredients on total psychrophilic bacterial counts (\log_{10} CFU/g) of marinated aerobically packaged duck meat (Mean \pm SE) at refrigeration temperature

Storage periods in days	Treatment groups			
	C	T1	T2	T3
1	ND	ND	ND	ND
3	1.64 \pm 0.01 ^{Aa}	1.45 \pm 0.01 ^{Ab}	1.35 \pm 0.01 ^{Ac}	1.23 \pm 0.00 ^{Ad}
5	1.87 \pm 0.02 ^{Ba}	1.72 \pm 0.01 ^{Bb}	1.70 \pm 0.01 ^{Bb}	1.64 \pm 0.01 ^{Bc}
7	2.96 \pm 0.01 ^{Ca}	2.77 \pm 0.01 ^{Bb}	2.63 \pm 0.01 ^{Cc}	2.53 \pm 0.02 ^{Cd}

n=5, ND= Not Detected

Different superscripts within a column (capital letters) and within a row (small letter) differ significantly

In the present study TVPBC for all the control and treated samples were not detected on 1th to 5th day of storage for vacuum packaging. This might be due to the absence of oxygen which discouraged the growth of aerobic psychrophilic bacteria and there was a gradual increase in the count of viable psychrophilic microorganisms with the increase in the storage period. It might be due to growth and multiplication of psychrophilic organisms which comes in contact during handling and storage. The present findings may be corroborated well with the reports of Biswas et al. (2011) who also reported significant increase in TVPBC of duck patties with increase in storage period. There results were in agreement with Cholan (2008) in low fat chicken nuggets, Chandralekha (2010) in refrigerated chicken meat balls added with natural and synthetic antioxidants.

4.7.2 Aerobically packaged products

The mean values of TVPBC of marinated duck breast meat samples for both the control and treated samples of aerobically packed samples at different storage period are presented in Table 6. Similar trend for increasing order in TVPBC during storage period was observed like that of vacuum packaging method. Similar reasons as mentioned in vacuum packaging method of increasing the TVPBC in duck meat products could also be attributed for aerobically packaging method.

5. CONCLUSION

A study was conducted to evaluate the effect of rice beer and phyto-ingredients on microbiological qualities of vacuum packaged duck meat products. The rice beer and phyto-ingredients selected for the study were incorporated in various marinating combinations into four different treatments viz., Control (Meat and spice paste), Treatment 1 (Rice beer and

spice paste), Treatment 2 (phyto-ingredients and spice paste), Treatment 3 (Rice beer, phyto-ingredients and spice paste). It was clearly observed that the marinade prepared combining all the ingredients had significant effect on improving microbiological quality of duck meat products. The shelf life could be extended up to 7 days by aerobic packaging and up to 15 days by vacuum packaging method at refrigeration temperature.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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