Research Article

Effect of Freeze-Thaw Cycle on Microbiological Quality of Chicken Meat

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Abstract

The objective of this study was to know the effect of freeze-thaw cycles of chicken meat on microbiological assessment. The effects of three successive freeze-thaw cycles on chicken meat were investigated comparing with fresh chicken meat for 30 day by keeping at -18 ± 2° C. The freeze-thaw cycles were subjected to two thawing methods and carried out to know the best one. Chilled chicken meat is one of the most important item in retail meat industry. Sometimes chicken meat is exposed to warm and fluctuating temperature due to break in cold chain which have great impact on the quality and acceptability of chicken meat. Chicken meat samples collected from retailer shop packed in HDPE were stored at -18^{o} C and analyzed for changes in microbiological quality after thawing for 12 hr at refrigeration temperature and 1 hr in hot water (40^{o} C). Microbiological quality revealed that SPC, *E.coli* and *Psychrophilic* count decreased considerably during frozen storage, indicating that frozen chicken meat after thawing at refrigeration temperature and in hot water (40^{o} C) could be safely stored for 30 days without any deterioration in microbiological quality. None of chicken meat sample revealed the presence of *salmonella*.

Keywords: Chicken meat, Freeze-thaw cycle, Microbiological quality, *Salmonella*

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Introduction

Chicken meat occupies a significant place in the overall agriculture economy of the country. For processed meat and poultry industry in India, the demand- drivers are hygiene, quality and food safety. However, significant investment is required to achieve high standards. Rising per capita consumption in domestic market and increase in global demand open us new opportunities to Indian meat, fisheries and poultry industry to adopt modern technologies in production, preservation, processing, value addition and consumer packaging, apart from more investments in this sector. India ranks ninth in the world for poultry market. The domestic poultry industry is the fastest growing segment with a compound growth rate of 15%. Being the most popular meat in India, poultry meat has been receiving significant boost through investments. Poultry meat production in India is estimated at about 2.47 million tones and exports of poultry products are currently worth about Rs 457.8 corers [1]. India ranks fifth in broiler meat production next to China and Europe. Annual production of broiler meat is more than 3725 thousand MT, which contribute to 7.97% of total broiler meat production in the world [2]. Chicken meat is consumed in all the segments of the society without any religious and social taboos; hence it serves as main source of meat in the country. Further the export potential of chicken meat is increasing day by day. At present, India has established good marketing network for chicken meat in the countries like Saudi Arabia, UAE, Bahrain, Jordan, Kuwait, Sri-Lanka, Qatar and Omana occupies a major share in the export market.

Chicken meat is generally being exported in the frozen state. Further delay in shipment and fluctuations in market rates necessitate the storage of consignment for longer period. Sometimes due to break in cold chain during transport or storage meat may pass through freeze thaw cycle. Under such circumstances, the meat is exposed to warm and fluctuating temperature for a period varying from 2-3 hours and sometimes even longer depending on the situation. Such frozen meat usually fetches lower market price than fresh meat. As a routine practice, considerable quantity of frozen meat is thawed in meat shops and sold as unfrozen meat without being labeled. In many countries, domestically produced meat is usually sold at a higher price than that of imported frozen meat. Some of the meat sellers may take the advantage of such situation and present thawed frozen meat as fresh, since the latter fetches higher price. In order to protect the consumer and to curb the practice of domestic meat producers, it is necessary to differentiate the quality of fresh meat with that of thawed frozen meat sold in the market.

Material and Methods

Source of raw materials

Fresh chicken meat were collected aseptically from local market of Mumbai city in a sterilized polythene pouch and transferred in ice packs. Meat samples were shifted in a refrigerator maintained at 4 ± 1^0 C for overnight and subsequently shifted to deep freezer maintained at -18 ± 2^0 C. Maximum care was taken to avoid contamination during collection and transport. .

Processing of meat samples

Chicken meat samples were immediately washed with clean water and kept in a chiller (at 4 ± 1^0C) for ageing. The samples were deboned and separable fat and connective tissues were removed. The samples were then cut into small pieces of approximate 2 cm x 1 cm x 1 cm size and analyzed for physico-chemical parameters.

Procurement of media, Chemicals and reagents

Chemicals and reagents required for experiment (Trichloroacetic acid, TBA reagent, follin and Ciacalteu's reagent etc.) were procured from standard manufacturers. All the chemicals of analytical grade, required for various analyses were procured from standard firms viz., Himedia Chemicals Pvt Ltd, and S.D Fine Chemicals Pvt Ltd, etc.

Procurement of glassware and plastics

Glassware required for experiment (pH, TBA, Tyrosine, ERV, Drip loss, Moisture, Protein, Fat and Microbiological examination) were procured from standard manufacturers like Himedia Chemicals Pvt Ltd, and S.D Fine Chemicals Pvt Ltd, etc.

Measurement of quality parameters

Microbiological procedures

All the microbiological analysis was done as per the standard method of [3], serial dilutions for inoculation were prepared according to [4].

Preparation of serial dilutions

10 gm of meat sample was made into paste in a sterile mortar and fine suspension was prepared by adding 90 ml of sterile 0.1 % peptone water using a sterile pestle for 2 min to get 10^{-1} dilution. One ml of this dilution was transferred to 9 ml of sterile 0.1 % peptone water in a test tube and mixed uniformly to get 10^2 dilutions. Subsequent dilutions were made as per the requirement following the same procedure.

Standard plate count (SPC)

0.1 ml of appropriate dilution was transferred in a sterilized petridish to which about 15-20 ml of pre sterilized melted plate count agar was poured. Plates were allowed to set, incubated at 37^{o}C temperature for 48 hrs and colonies were counted. The average count was multiplied by a dilution factor and expressed as log cfu/g of sample.

Psychrophilic count

The procedure similar to SPC was used for determination of psychrophilic count, except incubation of plates at 4 ± 1 ^{0}C for 7 days instead of 37°C. Colonies were counted and expressed as log 10 cfu/g of sample

E. coli count

0.1 ml of appropriate dilution was transferred into a sterilized petridish. About 15-20 ml presterilized melted Eosin methylene blue (EMB) agar was pour plated. Plates were allowed to set, incubated at 37 ^{0}C for 48 hrs and colonies were counted and expressed as log cfu/g of sample.

Salmonella count

Pre-enrichment: Buffered peptone water is generally used for pre-enrichment incubate meat sample in the ratio of

1:10 (25 g in 225 ml) in buffered peptone water and incubate for 16-20 hr at 37 ^{0}C.

Enrichment: Transfer sufficient inoculums (1:10) to Salmonella enrichment Broth and incubate for 18-24 hrs at 42 ^{0}C. The control of temperature is critical.

Selective plating

0.1 ml of appropriate dilution was transferred in a sterilized Petri dish. About 15-20 ml of presterilized melted Salmonella Shigella agar (SS) was pour plated. Plates were allowed to set and incubated at 37 ^{0}C for 48 hrs.

Results and Discussion

Microbiological quality

SPC

The initial value of SPC for fresh chicken meat was 5.67 log cfu/ g, indicating good chicken meat. SPC was affected significantly ($p<0.02$) by thawing methods within cycles and among the cycles (**Table 1**). SPC was decreased in cycle 1 but increased in cycle 2 and 3 subsequently in different thawing process which did not cross the initial count. SPC was significantly ($p<0.05$) changed in different interactions of cycles and thawing methods. Thawing at 40°C in cycle 3 had the lowest and thawing at 4°C in cycle 1 had the highest SPC among the interactions (Table 1). [5] and [6] also reported decline in SPC during freezing of buffalo meat. The decrease in count may be attributed to sudden frozen shock to microorganisms during freezing of meat. It is further established that freezing extends the lag phase of microbial population [7].

Table 1 Effect of thawing on SPC during frozen storage

Type of thawing	Storage period (days)			
	0	**10**	**20**	**30**
Control	$5.67^{c}\pm0.02$	$5.67^{c}\pm0.02$	$5.67^{a}\pm0.02$	$5.67^{a}\pm0.02$
T_1	$5.70^{B}\pm0.02$	$5.92^{Aa}\pm0.04$	$5.35^{Cb}\pm0.03$	$5.19^{Db}\pm0.02$
T_2	$5.71^{B}\pm0.02$	$5.83^{Ab}\pm0.05$	$5.19^{Cc}\pm0.04$	$4.97^{Dc}\pm0.01$

Means with the superscript with capital letters in same column and small letters in same row indicate significant difference ($p<0.01$)
Note - T_1 –Thawing of frozen chicken meat at 4^{o}C for 12 hr.
T_2 – Thawing of frozen chicken meat at 40^{o}C for 1 hr.

Psychrophilic count

The psychrophilic count of chicken meat during frozen storage also followed a similar declining trend as like that of *E. coli*. Significant decrease in psychrophilic count was observed throughout the storage period of 30 days (**Table 2**). The psychrophilic count increased non-significantly ($P>0.02$) up to 1st freeze thaw cycle of chicken meat. Thereafter, the count increased significantly ($P<0.05$) in 2nd and 3rd freeze-thaw cycle. Similar declining trend in psychrophilic count was reported by [8] and [6] for buffalo meat during frozen storage. Reduction in count might be due to sudden shock on the bacterial population particularly during frozen storage which extends the lag phase of microbial proliferation [7]. The increased enzyme activity of psychotrophs at low temperature hugely contributed in deterioration of meat quality at the end of storage**.**

Table 2 Effect of thawing on *psychrophilic* count during frozen storage

Type of thawing	Storage period (days)			
	0	**10**	**20**	**30**
Control	0	0	0	0
T_1	0.63^{D}	$3.06^{Aa}\pm0.04$	$2.89^{Ba}\pm0.04$	$2.82^{Ca}\pm0.06$
T_2	0.53^{A}	$3.02^{A}\pm0.03$	$2.85^{A}\pm0.04$	$2.80^{A}\pm0.05$

Means with the superscript with capital letters in same column and small letters in same row indicate significant difference ($p<0.01$)
Note - T_1 –Thawing of frozen chicken meat at 4^o C for 12 hr.
T_2 – Thawing of frozen chicken meat at 40^o C for 1 hr.

E. coli

In the present study it was evident that fresh chicken had a *coli* count of 2.25±0.01 log10cfu/g which decreased in 3rd freeze thaw cycle significantly in both thawing method ($P<0.05$) indicating that these organisms could not sustain a cyclic freeze thaw exposure. The results corroborated with documented findings as [6] who also observed decreasing trend in *coliform count* in freezing temperature storage. Similar trend was also reported by [9], [8] and [10]. Storage of meat samples in freezer at -18°C might be having greater effect on ice formation on both sides as well as within the cells [11] which might have reduced the count during storage. *E. coli* was satisfactory in chicken meat before and after repeated freeze-thaw cycles. The initial *E. coli* for control was 2.25 log cfu/g. after freezing *E. coli* was less than 2 log cfu/g. Thawing methods did not affect ($p>0.05$) the *E. coli* within cycles and among the cycles. No interactive effects were observed on *E. coli* in this study (**Table 3**). Similar trend was observed by [12].

Table 3 Effect of thawing on *E.coli* count during frozen storage

Type of thawing	**Storage period (days)**			
	0	**10**	**20**	**30**
Control	$2.25^{Aa}\pm0.01$	$2.25^{Aa}\pm0.01$	$2.25^{Aa}\pm0.01$	$2.25^{Aa}\pm0.01$
T_1	$2.22^{A}\pm0.01$	$2.18^{Bb}\pm0.03$	$2.11^{Cb}\pm0.02$	$1.99^{Db}\pm0.05$
T_2	$2.20^{A}\pm0.01$	$2.19^{Bb}\pm0.02$	$2.15^{Bb}\pm0.05$	$2.01^{Cb}\pm0.05$

Means with the superscript with capital letters in same column and small letters in same row indicate significant difference ($p<0.01$)
Note - T_1 –Thawing of frozen chicken meat at 4^{o} C for 12 hr.
T_2 – Thawing of frozen chicken meat at 40^{o} C for 1 hr.

Salmonella Spp.

It is observed that, chicken meat sample were negative for *Salmonella* species. During frozen storage and thawing of 30 days, none of the meat samples revealed the presence of *Salmonella* spp. The findings of present investigation are in agreement with those reported by [13] and [14] for buffalo meat, [15] for chevon samples, [16] for goat meat and [17] for frozen buffalo meat and mutton samples.

Conclusion

Based on the above observations, it is concluded that frozen chicken meat after thawing could be safely stored up to 30 days at -18 ± 2^{0} C temperature without impairing the microbial quality.

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