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The Effect of Packaging Methods on the Shelf-life of Iron Fortified Mozzarella Cheese

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ABSTRACT

Background: The present study was undertaken to study the effect of different packaging methods on the shelf-life of iron fortified mozzarella cheese prepared from cow milk, goat milk and mixed milk using kiwi fruit extract.

Methods: Mozzarella cheese samples were packed both aerobically and under vacuum packaging condition and microbiological load (Total viable count, *Escherichia coli, Salmonella, Shigella, Staphylococcus aureus,* yeast and moulds and spores count) were monitored throughout the storage period in order to determine its shelf-life.

Result: The total viable count (TVC) increased gradually from 0 day till 15 days of refrigerated storage for all the samples of iron fortified mozzarella cheese under both aerobic and vacuum packaging conditions irrespective of the types of milk and enzymes used. Under aerobic packaging condition, highest total viable count was observed for goat's milk sample in both control and treatment groups all throughout the storage period. Mixed milk iron fortified mozzarella cheese exhibited least count in both the groups. The TVC of all the cheese samples were higher in aerobic packaging compared to vacuum packaging condition. The shelf-life or best before use of the product could judged as 15d from the date of manufacturing.

Key words: Aerobic and vacuum packaging, Microbial load, Mozzarella cheese, Shelf-life.

INTRODUCTION

Mozzarella cheese is a soft, unripened, white cheese belonging to the Pasta-filata family. It is manufactured mainly for its functional properties rather than for its flavour and is used by the food sector as an ingredient (Hammad *et al.* 2017) in pizza, cheese burger, cheese based salads, pasta, *etc*. (Vogt *et al.* 2015 and Alam *et al.* 2016). The demand for Mozzarella cheese is increasing due to expansion of pizza parlours and fast food chains (Bhattarai and Acharya, 2010). Traditionally mozzarella cheese is made from water buffalo's milk in Italy which is found to be organoleptically superior and nutritionally better (Jana and Mandal, 2011 and Vogt *et al.* 2015), however, cow's milk (Ghosh *et al.* 1990), ewe's milk and goat's milk (Darwish, 1977) have also been reported to be used in the preparation of mozzarella cheese in some European countries, USA and Middle East countries with proper modifications.

Rennet has been used as the main milk coagulating enzyme meant for cheese production since time immemorial. However, in recent years, FSSAI (2009) has prohibited the use of animal rennet and as such microbial enzymes and recombinant chymosin are extensively used in manufacture of mozzarella cheese. Similarly, food scientists have started to find other alternative sources of coagulating enzymes derived from plants having proteolytic activity like actinidin (*Actinidia deliciosa*), ficin (*Ficus carica*) and papain (*Carica papaya*), *etc* that has the property to coagulate milk protein.

Iron is an essential trace element in human nutrition and its deficiency usually occurs due to either insufficient dietary intake, poor utilization of iron from ingested food or a combination of these two. Iron deficiency is the most prevalent nutritional problem in the world affecting approximately 1.3 billion individuals leading to anemia, thus making it one of the most important public health issues (Savita *et al.* 2013). Direct addition of iron to milk and milk products might be an effective way of increasing dietary intake of iron by the general population. Cheese is one such dairy product which is an excellent source of calcium and protein but is deficient in iron (Blanc, 1981). Iron fortification of cheese thus can add nutritional value to dairy products which have a relatively high iron bioavailability (Woestyne *et al.* 1991).

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Today several preservation techniques have been put forward to extend the shelf-life of food products, among which packaging is the most promising one. The packaging process offers several basic roles such as preventing microbial and chemical deterioration and enhancing the handling and marketing of packaged products. The present study was undertaken to investigate the effect of packaging methods on the shelf-life of iron fortified mozzarella cheese by monitoring the microbiological load throughout the storage period.

MATERIALS AND METHODS

The study on the effect of packaging methods on the shelf-life of iron fortified mozzarella cheese was undertaken in the Department of Livestock Products Technology and All India Coordinated Research Project on Post Harvest Engineering Technology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati during the period from August, 2016 to December, 2018.

Preparation of iron fortified mozzarella cheese

Iron fortified Mozzarella cheese was prepared from cow's milk (100 per cent), goat's milk (100 per cent) and mixed cow and goat milk (50:50) using chymax powder (control group) and kiwifruit crude extract (treatment group) as per standard technique laid down by Kanawjia *et al.* (2011) with slight modification. Immediately after procurement, the milk was subjected to filtration followed by standardization to 3-4% fat level. The standardized milk was heated at 72°C/ 2min followed by cooling to 4°C. $CaCl_2$ was added @ 0.02g $100ml^{-1}$ prior to acidification. Acidification was accomplished by the addition of 20% (w/v) citric acid to pH of 5.2–5.4 followed by addition of $FeCl_3.6H_20$ @1g/L. The temperature of the acidified milk was subsequently raised to 29°C and CHY-MAX Powder Extract NB was added @80 International Milk Clotting Unit/L of milk (control). For treatment groups, kiwifruit extract was used @150 µg/ml**.** The milk in both the group was allowed to set until complete coagulation of the milk could be observed. The curd mass was then cut with sterile cheese knives and allowed to stand undisturbed in whey for 5min. Slowly the temperature of the curd was raised from 29°C to 38°C within 45min along with continuous stirring of the coagulated mass for uniform cooking and then held at 38°C for 30min. Whey was drained off and the curd mass was collected and stretched in hot water (85°C/10min) and molded into a ball. The cheese balls were then immersed in 20% w/v chilled brine solution for 2h followed by surface drying under refrigerated condition (7-9°C/6h). The prepared cheese samples were packed under two different packaging conditions, aerobically in polyethylene pouches and vacuum packed in high density polyethylene (HDPE) films of 200gauge (12×10″) packaged in Sevana™ (Electrical Appliances Pvt. Ltd., Kerala, India) at a vacuum pressure of 720mm Hg and stored at refrigeration temperature throughout the entire study period.

Enumeration of microorganisms

Methods described by Harrigan and McCance (1976) were followed for enumerating the total viable count, *Escherichia coli, Salmonella, Shigella, Staphylococcus aureus,* yeast and moulds and spores in prepared cheese samples by using appropriate media and incubation conditions.

Total viable count

One millilitre of appropriate dilution was transferred in to sterilized petri dishes in duplicates. Twenty millilitres of pre-sterilized, molten and cooled Plate Count Agar (PCA, Hi-Media, India) medium was poured into the petri dishes. The inoculum was mixed thoroughly by rotating the plates several times in clockwise and anti-clockwise directions. After solidification of the agar, the plates were incubated at 37°C for 24h. Colonies grown were counted and the counts were expressed as $\log_{10}$cfu/g of sample.

Coliform count

Coliform count of the iron fortified mozzarella cheese were enumerated by inoculating 1ml of the diluent in Endo agar (Hi-Media, India) followed by incubation at 37°C for 24h. Average number of colonies counted was then expressed as presence or absence of coliforms in 0.1g of cheese samples.

Escherichia coli

E. coli count of iron fortified Mozzarella cheese was enumerated by inoculating 0.1g of the sample in Endo agar followed by incubation at 42°C for 24h. Results were interpreted as presence or absence of *E. coli* in 1g of sample.

Listeria monocytogenes

Presence or absence of *Listeria monocytogenes* of the iron fortified cheese sample was done by inoculating the required dilutions in Polymyxin-Acriflavin-Lithium Chloride-Ceftazidime-Aesculin-Mannitol (PALCAM, Hi-Media, India) agar and incubated at 37°C for 24h. Results were interpreted as presence or absence of *Listeria monocytogenes* in 25g of sample.

Staphylococcus aureus

For detecting the presence of *Staph. aureus* 1ml of appropriate dilution was transferred into sterilized Petri dishes in duplicates, in which 15-20ml of pre-sterilized and molten Mannitol Salt Agar (MSA, Hi-Media, India) medium was poured. The inoculum was mixed thoroughly by rotating the plates several times in clockwise and anti-clockwise directions. After solidification of the agar, the plates were incubated at 37°C for 24h. Results were inferred as presence or absence of *Staph. aureus* in 1g of cheese sample.

Salmonella and *Shigella*

Twenty five grams of iron fortified mozzarella cheese samples were resuscitated in 100ml of sterilized nutrient broth and incubated at 37°C for 24h. The resuscitated sample was streaked on previously poured, solidified and

sterility checked Salmonella-Shigella Agar (SSA, Hi-Media, India) plates and incubated at 37°C/24-72h. Results were interpreted as presence or absence of *Salmonella* and *Shigella* in 25g of cheese samples.

Yeasts and moulds count

Yeast and moulds counts of the sample was done by inoculating the required dilutions in Rose Bengal Chloramphenicol Agar (RBCA, Hi-Media, India) and incubated at 37°C for 72-120h or till the formation of visible colonies. Results were interpreted as presence or absence of yeast and moulds in 1g of sample.

Anaerobic spore count

Anaerobic spore count of the samples was done by streaking from appropriate dilution on a previously poured, sterilized, sterility checked, solidified Starch Milk Agar (SMA) and incubated anaerobically at 37°C/24h. Results were inferred as presence or absence of anaerobic spores in 1g of cheese samples.

Statistical analysis

The experiment was conducted in a factorial RBD, considering 2 enzymes and 3 types of milk. Two-way ANOVA was performed in the generalized linear model (Proc GLM) as mentioned below and pair-wise comparison between the means was done by following the method of least significant difference (LSD) with the help of SAS 9.3.

$$Y_{ijk} = \mu + E_i + M_j + ExM_{ij} + e_{ijk}$$

Where,

Y_{ijk} = Dependent variable (k^{th} observation in i^{th} enzyme and j^{th} milk).

μ = General effect.

E_i = Effect due to i^{th} enzyme (i=1,2).

M_j = Effect due to j^{th} milk (i=1,2,3).

ExM_{ij} = Interaction effect due to i^{th} enzyme and j^{th} milk.

e_{ijk} = Effect due to non-assignable causes.

RESULTS AND DISCUSSION

Total viable count

The results of TVC of iron fortified mozzarella cheese samples prepared from cow's milk, goat's milk and mixed milk in the control group (chymosin) and treatment groups (kiwi fruit crude extract) packaged under aerobic and vacuum packaging conditions and stored at refrigeration temperature are presented in (Table 1 and 2), respectively. The TVC of all the samples increased gradually from 0d till 15d of storage under both the packaging conditions irrespective of the types of milk and enzymes used.

Aerobic packaging

Under aerobic packaging condition, highest TVC was observed in mozzarella cheese prepared from goat's milk in both the treatment and control group compared to cow's milk and mixed milk cheese samples all throughout the storage period. The initial TVC of goat milk cheese samples

Table 1: Average (Mean±SE) of total viable count (log_{10}cfu/g)* of iron fortified mozzarella cheese under aerobic packaging condition.

Types of milk	0 Day		Average (n=10)	5th Day		Average (n=10)	10th Day		Average (n=10)	15th Day		Average (n=10)
	Control (n=5)	Treatment (n=5)		Control (n=5)	Treatment (n=5)		Control (n=5)	Treatment (n=5)		Control (n=5)	Treatment (n=5)	
Cow	2.33^{a}±0.07	2.50±0.03	2.42^{b}±0.05	2.67^{a}±0.10	3.19±0.08	2.93^{a}±0.11	3.17^{a}±0.10	3.35±0.05	3.26^{a}±0.06	3.76^{a}±0.16	3.75±0.033.	3.74^{a}±0.08
Goat	2.98^{b}±0.33	2.77±0.16	2.88^{b}±0.17	3.37^{b}±0.27	3.36±0.18	3.36^{b}±0.15	3.94^{b}±0.51	3.77±0.24	3.86^{b}±0.27	4.53^{b}±0.50	4.08±0.23	4.30^{b}±0.27
Mixed	2.23^{a}±0.08	2.55±0.04	2.39^{a}±0.07	2.63^{a}±0.10	2.98±0.02	2.81^{a}±0.07	3.12^{a}±0.15	3.24±0.02	3.17^{a}±0.08	3.69^{a}±0.05	3.78±0.07	3.74^{a}±0.04
P value	< 0.05	> 0.05	< 0.01	< 0.01	> 0.05	< 0.01	< 0.05	> 0.05	< 0.05	< 0.05	> 0.05	< 0.05

P<0.05 (highly significant); P<0.05 (significant); P>0.05 (non-significant).

Means with common superscript column wise does not differ significantly.

Table 2: Average (Mean±SE) of total viable count (log_{10}cfu/g)* of iron fortified mozzarella cheese under vacuum packaging condition.

Types of milk	0 Day		5th Day			10th Day			15th Day		
	Control	Treatment	Control (n=5)	Treatment (n=5)	Average (n=10)	Control (n=5)	Treatment (n=5)	Average (n=10)	Control (n=5)	Treatment (n=5)	Average (n=10)
Cow	-	-	2.25^{a}±0.12	2.75±0.01	2.50^{a}±0.10	2.69^{a}±0.12	3.10±0.01	2.89^{a}±0.09	3.00±0.16	3.40±0.03	3.20±0.10
Goat	-	-	3.12^{b}±0.28	2.75±0.08	2.93^{b}±0.15	3.48^{b}±0.37	3.17±0.10	3.33^{b}±0.19	4.00±0.55	3.45±0.13	3.73±0.28
Mixed	-	-	2.36^{a}±0.11	2.73±0.03	2.54^{a}±0.08	2.71^{a}±0.10	3.06±0.04	2.88^{a}±0.08	3.10±0.05	3.39±0.05	3.25±0.06
P value	-	-	< 0.01	> 0.05	< 0.01	< 0.05	> 0.05	< 0.05	> 0.05	> 0.05	> 0.05`

P<0.05 (highly significant); P<0.05 (significant); P>0.05 (non-significant).
Means with common superscript column wise does not differ significantly.

were 2.98±0.33 (log_{10}cfu/g) and 2.77±0.16 (log_{10}cfu/g) on 0d and increased to 4.08±0.23 (log_{10}cfu/g) and 4.30±0.27 (log_{10}cfu/g) on 15d of storage period in both the control and treatment groups, respectively. Highest TVC in goat milk cheese samples might probably be due post pasteurization contamination of milk or contamination of the curd during the plasticizing process during cheese manufacturing. On the contrary, mixed milk iron fortified mozzarella cheese exhibited the least TVC throughout the storage period in both the control and treatment group except on 0d and 15d of storage period wherein cow's milk iron fortified mozzarella cheese samples exhibited the least value (2.50±0.03 and 3.75±0.03 log_{10}cfu/g) in the treatment group. Highly significant difference (p<0.01) was noted for the average TVC on 0d and 5d while significant difference (p<0.05) was observed on the 10d and 15d of storage period between the different types of milk used in the preparation of cheese samples. The effect of enzymes on TVC was found to be non-significant (p>0.05) in all the samples except for cow's milk iron fortified mozzarella cheese sample where significant difference (p<0.05) was noted on 5d of storage period.

Vacuum packaging

The TVC of all the cheese samples were lower in vacuum packaging compared to aerobic packaging due to anaerobic conditions created by complete removal of air from the packaging material. This resulted in disturbance of homeostasis mechanism of the organisms, resulting in lower bacterial count in vacuum packaged iron fortified mozzarella cheese samples. In vacuum packaging condition similar to aerobic packaging, goat milk cheese samples exhibited highest TVC in comparison to cow's milk and mixed milk cheese samples. The initial TVC of goat milk cheese samples were 3.12±0.28 (log_{10}cfu/g) and 2.75±0.08 (log_{10} cfu/g) on 5d and increased to 4.00±0.55 (log_{10}cfu/g) and 3.45±0.13 (log_{10}cfu/g) on 15d of storage period in both the control and treatment groups, respectively. This might probably be due post pasteurization contamination of milk or contamination of the curd during the plasticizing process during cheese manufacturing as stated earlier. Highly significant difference (p<0.01) was observed for the average TVC on the 5d and significant difference (p<0.05) was noted on the 10d of storage for the different types of milk used for the preparation of iron fortified mozzarella cheese, while non-significant (p>0.05) difference was observed on the 15d of storage period. The effect of enzymes on the TVC of iron fortified mozzarella cheese prepared from different types of milk did not differ significantly (p>0.05) in any of the cheese samples. Although all the products were below the max permissible limit for TVC in mozzarella cheese (max TVC 4.69 log_{10}cfu/g as per PFA standard), but based on the discolouration caused by oxidation of iron, the shelf-life or best before use could be judged as 15d from the date of manufacturing of the product. The results of the present study corroborates with the findings of Coppola *et al.* (1995) and Beshir (1999).

Nil counts for Yeast and moulds, *E. coli,* Coliform, *Staph. aureus, Salmonella*, *Shigella, Listeria monocytogenes* and anaerobic spore counts were noted for all the types of cheese samples under both the packaging conditions, all throughout the storage period, under refrigerated condition which corroborates to the findings of Kuchroo and Fox (1982); Esho *et al.* (2013) and Rehman *et al*. (2017).

CONCLUSION

The TVC increased gradually from 0d till 15d of refrigerated storage for all the samples of iron fortified mozzarella cheese under aerobic and vacuum packaging conditions irrespective of the types of milk and enzymes used. Goat milk cheese sample exhibited highest TVC under both aerobic and vacuum packaging condition, in the control and treatment groups, respectively all throughout the storage period. Mixed milk iron fortified mozzarella cheese exhibited least count in both the groups. The TVC of all the cheese samples were higher in aerobic packaging compared to vacuum packaging condition. Nil counts for Yeast and moulds, *E. coli,* Coliform, *Staph. aureus, Salmonella*, *Shigella, Listeria monocytogenes* and anaerobic spore counts were noted for all the types of cheese samples.

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