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Research Article

EFFECT OF REFRIGERATION ON QUALITY AND STABILITY OF FRESH FISH AND POULTRY MUSCLES

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The present study investigated the chemical, microbiological and sensory changes of poultry and fish (*Wallago attu)* muscle in refrigerated storage (4°±1°C). pH, Thiobarbituric acid and reactive substances (TBARS) value, Peroxide value (PV), Free fatty acid (FFA) value, Tyrosine value (TV), and microbiological study viz. Total plate count (TPC), Total psychrophillic count (TPSC), yeast & mould counts and coliform counts were estimated. Sensory analysis of cooked poultry meat curry and fish curry were done by 8 point hedonic scale by semi- trained sensory panelists. pH, TBARS values, PV, FFA values, TV, TPC and TPSC values were increased significantly ($p<0.05$) throughout the storage. Yeast and mould counts and coliform counts were not detected up to 3rd day of storage in fish meat and 7th day of storage in poultry meat. The study concluded that both the meats were accepted up to 7 days as per sensory analysis and the upper limit of safety optima was up to 10th day of refrigerated storage period as per chemical and microbiological quality basis.

Key words: Microbiological safety, Refrigeration preservation, Sensory analysis, *Wallago attu*

Meat is defined as the flesh of animals used as food. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens. Meat is a rich nutrient matrix that provides a suitable environment for proliferation of meat spoilage microorganisms and common food-borne pathogens, therefore adequate

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preservation technologies must be applied in order to preserve its safety and quality. Food safety is a top priority for authorities and consumers worldwide. Food safety objectives, hazard analysis and critical control point are being introduced worldwide. It is therefore essential that adequate preservation technologies are applied to maintain food safety and quality (Aymerich *et al*., 2008). A number of interrelated factors influence the shelf life and keeping quality of meat, specifically holding temperature, atmospheric oxygen (O_2), endogenous enzymes, moisture (dehydration), light and most importantly, micro-organisms (Biswas *et al*., 2007 and 2011). All of these factors, either alone or in combination, can result in detrimental changes in the colour (Faustman and Cassens, 1990), odour, texture and flavour of meat. Although deterioration of meat (proteolysis, lipolysis and oxidation) can occur in the absence of micro-organisms but microbial growth is by far the most important factor in relation to the keeping quality of fresh meat (Lambert *et al*., 1991).

Marine and fresh water products are known to easily deteriorate during post-mortem storage and processing as a result of different damage mechanisms such as autolytic degradation, microbiological spoilage and lipid oxidation (Whittle *et al*., 1990 and Olafsdottir *et al*., 1997).

Cold preservation techniques (chilling, freezing etc.) are important methods for the preservation of fish and poultry muscle. Although many damage pathways are inhibited by such processes, undesirable reactions associated with lipids and proteins have shown to occur, leading to detrimental changes in nutritional and sensory properties (Sikorski and Kolakowska, 1994; Erickson, 1997 and Kandeepan and Biswas, 2005). Addition Ferula assa-foetida aqueous extract in fish nuggets extended the shelf life of the product and protected it from oxidative deterioration even in aerobically packaged refrigerated storage (Das *et al*., 2017). Effect of ambient storage on the quality characteristics of aerobically packaged fish curls incorporated with different flours was studied by Raja *et al*.(2014). Biswas *et al*.(2016) again studied shelf life on restructured fish nuggets from *Wallago attu* extended with soy protein isolate.

Slaughter, evisceration and dressing operations inevitably produce microbial contamination in depth and especially on the surface of nutrient rich meat, through contact with equipment, tools, hands and clothes, despite all precautions. Again, micro-organism growth is a temperature dependent process. To avoid it, it is absolutely essential to reduce the temperature of the meat, especially on the surface, immediately after dressing. Cooling must therefore be carried out in the slaughterhouse itself. This operation is known as primary chilling. Meat loses weight through surface evaporation. This process depends on differences in temperature and relative humidity between

the meat and the environment. The carcass itself incorporates mainly muscles, bones, fat and connective tissue. The offal includes some edible organs, while some glands are used in pharmaceutical preparations. These different parts must be subjected to varying cooling conditions according to their susceptibility to microbial growth, to temperature effects and to the risk of surface dehydration (FAO, 1991).

To prevent deterioration process, particularly microorganism development, chilling has to be carried out quickly after carcass dousing at the end of the slaughter process and the chilled state has to be maintained until the meat is processed for consumption. For fresh meat, refrigeration, including storage above or below the freezing point, has been the traditional preservation method. Super chilling technology, which stores meat just above the freezing point, has been used with success (Nowlan *et al*., 1974 and Beaufort *et al*., 2009).

The present study has been designed to investigate the quality and stability in fish and poultry muscles under refrigerated storage (4°±1°C) and to identify the safe storage optima for both the meat under refrigeration temperature.

MATERIALS AND METHODS

Raw fish and poultry meat processing: Live poultry birds of 6 weeks age and average weight of 1.5 kg were procured from local market at Belgachia, Kolkata. Slaughtering of live birds was carried out under standard humane procedure as outlined by the University ethical committee for animals and birds. Dressing and deboning of poultry carcasses were done in the department and dressed poultry meat was minced by meat mincer (Sirman®, Italy, Model Buffalo TC 32) fitted with 6 mm plate.

Boal fish (*Wallago attu*) was procured from local market at Belgachia, Kolkata. They were deboned manually in the department and minced in meat mincer (Sirman®, Italy, Model Buffalo TC 32) fitted with 6 mm plate.

Both the minced meat samples were wrapped in UV sterilized LDPE bag and kept at refrigeration temperature (4°±1°C) for future use. Along with the minced meats, required amount of un-minced dressed and cut meats from both samples were kept under storage for sensory evaluation.

Analyses : The initial quality and stability of the samples were investigated on day 0, and repeated on day 3, 7, 10 and 14. Quality was estimated in terms of pH, TBARS (Thio-barbituric acid & reactive substances) value, Peroxide value (PV), Free fatty acids (FFA) value and Tyrosine value (TV). Microbiological parameters covered were Total plate count (TPC), Total psychrophillic count (TPSC), yeast and mould count and coliform count to check the stability.

Sensory evaluation of poultry meat curry and fish curry was estimated on each day of storage and general appearance, flavor, juiciness, texture and overall acceptability scores were computed in an 8 point hedonic scale score card.

All the parameters were performed in triplicate and data obtained were carried out using ANOVA technique according to the method described by Snedecor and Coachran (1989) by Randomized block design (RBD).

pH: pH of the samples were estimated by the method described by Egbert *et al.*(1992)

Thiobarbituric acid reactive substances (TBARS): Thiobarbituric acid and reactives substances value was estimated as per procedure given by Tarladgis *et al.* (1960).

Peroxide value: Peroxide value was estimated as per procedure given by AOCS (1992) with slight modifications.

Free fatty acid: Free fatty acid value was determined by modified AOCS method (Koniecko, 1979).

Tyrosine value: For assessing of tyrosine value, the procedure of Strange *et al.*, (1977) was followed with some modifications.

Microbiological analysis: Samples were prepared according to APHA (1992). The yeast and mould counts were determined as per the procedure described in APHA (1992). The coliform count was determined as per the procedure described in APHA (1992) using melted violet red bile agar. The average number of colonies was multiplied with dilution factor to obtain total count as colony forming unit (CFU) per gm of the sample. This count was then converted to coliform count of log CFU/g of sample.

Sensory evaluation: The poultry and fish curry samples were served warm to panelists for sensory evaluation on 0, 3rd, 7th, 10th and 14th day. Sensory evaluation method using an eight-point descriptive scale (Keeton, 1983) was followed with modifications, where 8 = excellent; 1 = extremely poor. Water was provided to rinse mouth between the samples. The panelists judged the samples for their general appearance, flavor, juiciness, texture and overall acceptability.

Statistical analysis: Statistical analysis of the data obtained (arithmetic mean of triplicate observations) were subjected to two-way analysis of variance on the basis of 2 (treatments) × 5 (storage days) × 6 (replications). Further to test the significance among each group, Post-hoc test (Tukey's HSD) has been carried out by SPSS- 20 software package at 5% level of significance.

RESULTS

pH: The pH values (Mean± SE) of minced poultry and fish meat were presented in Table 1. It was evident from the results that

pH values of both the samples increased over the time significantly ($p<0.05$) and pH of fish muscle always remained slightly higher than the poultry muscles. However, beyond 3rd day of storage, immediately the pH of fish muscle entered into the alkalinity level.

Thiobarbituric acid reactive substances (TBARS): The mean TBARS values and standard deviations of poultry and fish meat samples at different storage period were presented in Table 1. Up to 3rd day of refrigerated storage, no significant increment ($p>0.05$) had been noticed in TBARS values of poultry meat, but beyond 3rd day, it grew with a significant ($p<0.05$) steep. Whereas, the TBARS values of fish meat samples immediately started to increase significantly ($p<0.05$) and this increment was noticed up to 14th day of storage.

Peroxide value (PV) : The peroxide value is a useful method to determine the early stages of fat oxidation and the product is considered rancid when PV of 20-40 meq / kg is reached (Economou *et al*., 1991). The mean peroxide values and standard deviations of poultry and fish meat samples at different storage periods were presented in Table 1. Both the poultry and fish meat samples showed a gradual significant ($p<0.05$) increase in peroxide value with advancement of storage period. The increase in peroxide values of poultry meat samples was not significant ($p>0.05$) up to 3rd day.

Free fatty acid value (FFA) (as % oleic acid): Free fatty acids are the products of enzymatic or microbial degradation of lipids. Determination of FFA gives information about stability of fat during storage (Das *et al*., 2008). The mean ± SE values of the free fatty acids (as % oleic acid) of minced poultry and fish meat samples increased significantly ($p<0.05$) with the advancement of storage period (Table 1).

Tyrosine value: The degree of autolysis and bacterial proteolysis in meat could be measured as tyrosine value which actually determined the quantity of amino acid - tyrosine and tryptophan present in an extract of meat. Significant effect ($p<0.05$) in tyrosine value was noticed throughout the storage period (Table 1). However, poultry meats did not exhibit any significant effect up to 3rd day of storage, but initial tyrosine value differed significantly ($p<0.05$) with 7th day mean.

Microbiological study: The mean ± SE values of TPC, TPSC, yeast and mould and coliform count were expressed as log $cfug^{-1}$ and presented in Table 2. Up to 3rd day of refrigerated storage, no significant effect had been detected in TPC and TPSC counts of poultry meat samples. The yeast and mould counts and coliform counts were not detected up to 3rd and 7th day of storage respectively. In fish meat samples, coliforms were detected on 7th day. The increment in TPC values of fish muscles

Table 1. Changes in quality of minced fish and poultry muscles under refrigeration storage (4°±1°C) (Mean ± SE)

Sample	Day	pH	TBARS value	Peroxide value	FFA value	Tyrosine Value
Poultry meat sample	0	6.13 ± 0.24^{a}	0.345 ± 0.007^{a}	1.287 ± 0.032^{a}	0.218 ± 0.043^{a}	0.153 ± 0.005^{a}
	3	6.35 ± 0.14^{ab}	0.417 ± 0.042^{a}	1.634 ± 0.024^{a}	2.324 ± 0.045^{b}	0.214 ± 0.016^{ab}
	7	6.45 ± 0.21^{b}	0.633 ± 0.028^{b}	2.543 ± 0.052^{b}	3.876 ± 0.586^{c}	0.289 ± 0.026^{b}
	10	6.69 ± 0.51^{c}	0.826 ± 0.062^{c}	4.543 ± 0.127^{c}	4.143 ± 0.038^{d}	0.368 ± 0.074^{c}
	14	6.88 ± 0.81^{d}	1.294 ± 0.102^{d}	5.783 ± 0.083^{d}	4.943 ± 0.069^{d}	0.495 ± 0.051^{d}
Fish meat sample	0	6.74 ± 0.42^{a}	0.402 ± 0.023^{a}	1.843 ± 0.038^{a}	0.541 ± 0.348^{a}	0.169 ± 0.009^{a}
	3	6.93 ± 0.77^{ab}	0.635 ± 0.051^{b}	2.543 ± 0.072^{b}	2.167±0.214^{b}	0.238 ± 0.120^{ab}
	7	7.06 ± 0.17^{b}	0.768 ± 0.071^{c}	4.073 ± 0.231^{c}	3.267 ± 0.059^{c}	0.316 ± 0.051^{b}
	10	7.13 ± 0.18^{b}	0.928 ± 0.074^{d}	6.843 ± 0.094^{d}	5.268 ± 0.126^{d}	0.486 ± 0.081^{c}
	14	7.21 ± 0.32^{c}	1.273 ± 0.187^{e}	7.023 ± 0.034^{d}	6.867 ± 0.241^{e}	0.591 ± 0.064^{d}

*Means bearing different superscripts (a, b, c, d, e, etc.) within a column differ significantly ($P < 0.05$); n = 6

Table 2. Changes in microbiological quality of minced poultry and fish muscles under refrigeration storage (4°±1°C) (Mean ± SE)

Sample	Day	Total Plate Count (log cfu g^{-1})	Total Psychrophillic Count (log cfu g^{-1})	Yeast &Mould Count (log cfu g^{-1})	Coliform count (log cfu g^{-1})
Poultry meat sample	0	2.35 ± 0.132^{a}	0.89 ± 0.024^{a}	N.D.	N.D.
	3	2.86 ± 0.154^{a}	1.06 ± 0.036^{a}	N.D.	N.D.
	7	3.46 ± 0.210^{b}	1.45 ± 0.0421^{b}	0.653 ± 0.064^{a}	N.D.
	10	5.21 ± 0.134^{c}	1.78 ± 0.112^{b}	1.123 ± 0.075^{b}	1.042 ±0.105^{a}
	14	6.35 ± 0.167^{d}	2.561 ± 0.104^{c}	1.642 ± 0.101^{c}	1.349 ± 0.114^{b}
Fish meat sample	0	2.83 ± 0.421^{a}	1.05 ± 0.062^{a}	N.D.	N.D.
	3	3.12 ± 0.238^{b}	1.29 ± 0.053^{ab}	N.D.	N.D.
	7	4.01 ± 0.206^{c}	1.71 ± 0.067^{b}	0.686 ± 0.126^{a}	1.134 ± 0.152^{a}
	10	5.71 ± 0.513^{d}	2.25 ± 0.105^{c}	1.963 ±0.130^{b}	1.463 ± 0.069^{b}
	14	6.89 ± 0.157^{e}	2.86 ± 0.119^{d}	2.256 ± 0.149^{c}	2.121 ± 0.196^{c}

*Means bearing different superscripts (a, b, c, d, e, etc.) within a column differ significantly ($P < 0.05$); n=6

Table 3. Sensory analysis of poultry meat curry and fish curry under refrigeration storage (4°±1°C) (Mean ± SE)

Sample	Day	Appearance	Flavor	Juiciness	Texture	Overall acceptability
Poultry meat sample	0	6.89 ± 0.235^{a}	6.84 ± 0.167^{a}	6.63 ± 0.307^{a}	6.54 ± 0.967^{a}	6.44 ± 0.926^{a}
	3	6.79 ± 0.635^{ab}	6.73 ± 0.412^{ab}	6.59 ± 0.692^{ab}	6.13 ± 0.812^{b}	6.13 ± 0.892^{ab}
	7	6.12 ± 1.103^{b}	6.33 ± 0.428^{b}	6.12 ± 0.968^{b}	6.03 ± 0.928^{b}	5.93 ± 0.478^{b}
	10	5.54 ± 1.120^{c}	5.86 ± 0.662^{c}	5.69 ± 1.022^{c}	5.56 ± 0.962^{c}	5.14 ± 0.697^{c}
	14	5.01 ± 0.781^{d}	5.13 ± 0.822^{d}	5.05 ± 0.942^{d}	5.03 ± 0.932^{d}	4.28 ± 0.894^{d}
Fish meat sample	0	6.84 ± 0.421^{a}	6.96 ± 0.623^{a}	6.59 ± 0.697^{a}	6.61 ± 0.897^{a}	6.72 ± 0.906^{a}
	3	6.72 ± 0.278^{ab}	6.35 ± 0.051^{b}	6.35 ± 0.792^{ab}	6.43 ± 0.942^{ab}	6.11 ± 0.807^{ab}
	7	6.10 ± 0.845^{b}	5.86 ± 0.436^{c}	6.06 ± 0.918^{b}	6.13 ± 0.888^{b}	5.73 ± 0.956^{b}
	10	5.33 ± 0.618^{c}	5.10 ± 0.254^{d}	5.26 ± 0.972^{c}	5.37 ± 0.902^{c}	5.16 ± 0.692^{c}
	14	4.81 ± 0.372^{d}	4.86 ± 0.687^{e}	4.83 ± 0.882^{d}	5.04 ± 0.937^{d}	4.23 ± 0.926^{d}

*Means bearing different superscripts (a, b, c, d, e, etc.) within a column differ significantly ($P < 0.05$); n = 6

were also noticed to be significant ($p<0.05$) on 3rd day when compared with the initial counts. Microbiological studies indicated that all the parameters showed a significant ($P<0.05$) increasing trend throughout the storage period.

Sensory analysis: The mean sensory parameters and SD values of cooked poultry meat curry and fish curry samples on 0th, 3rd, 7th, 10th and 14th day of refrigerated storage were presented in Table 3. All the parameters computed got sufficiently higher scores up to 7th day of storage, after which they decreased significantly ($p<0.05$) throughout the storage. Seeing the results of overall acceptability scores, it might be summarized that both the samples were liked by the panelists up to 7th day after which the scores declined towards disliking.

DISCUSSION

pH: Reddy and Rao (1996); Papadima and Bloukas (1999); Singh and Verma (2000) and Nayak and Tanwar (2004) also reported similar increasing trend of pH during refrigerated storage of different meat products. Decomposition products such as volatile bases could lead to a pH rise during storage of fish mince (Debbarma and Majumdar, 2013).

Thiobarbituric acid reactive substances (TBARS): Bhat *et al.* (2011); Reddy and Rao (1996); Thompson *et al.* (1984) and Nag *et al.* (1998) also found similar increasing trend in TBARS content of different meat and meat products under

refrigerated storage condition. Concerning the permissible limit of TBA value in fish and fish products (4.5 mg MDA/kg) recommended by EOS (2005); none of the storage time group exceeded such limit in any occasion of examination even after showed objectionable odors and flavors; these results substantiate the hypothesis reported by Connell (1990) who stated that TBA values of 1–2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish will normally develop an objectionable odor and taste.

Peroxide value (PV): Numerically, all the values were far below of 20 meq /kg. The data were supported by the findings of Hassan and Fan (2005) and Soyer *et al*. (2010) who reported significant effect ($p<0.01$) of storage period on the PV of meat samples.

Free fatty acid value (FFA) (as % oleic acid): In fish, lipid oxidation can occur enzymatically or non-enzymatically. The enzymatic hydrolysis of fats by lipases is termed lipolysis (fat deterioration). During this process, lipases split the glycerides forming free fatty acids which are responsible for: (a) common off-flavor, frequently referred to as rancidity and (b) reducing the oil quality (Ghaly *et al*., 2010). The extent to which FFA value reaches after 14^{th} day of storage was found to be higher in fish muscles than in poultry muscles. Similar increasing trend in FFA values were noticed by Anand *et al*. (1991); Nayak and Tanwar (2004) and Nagamallika *et al*. (2006) in various meat products during refrigerated storage.

Tyrosine value: Increment in tyrosine value might be due to the enhanced microbial load, enhanced production of proteolytic enzymes in the late logarithmic phase of microbial growth which were altogether responsible for autolysis and bacterial proteolysis (Dainty *et al*. 1975). The results of the present study could also be collated with the observation of Das *et al*. (2014); Pearson (1968); Dainty *et al*. (1975) and Eyas (2001) where they also reported the similar effect of storage period on tyrosine value of different types of meat products.

Microbiological study: Das *et al*. (2008) observed similar increasing trend of Total plate count and Total psychrophilic count while studying on chevon nuggets in frozen storage. Nag *et al*. (1998) also observed a similar increasing trend in total plate count under refrigerated storage while studying on quality attributes and shelf life of chicken nuggets extended with rice flour. However, both the TPC and TPSC values of poultry and fish muscles had not exceeded the permissible limit up to 10^{th} day of storage, i.e. log 10^6cfu g^{-1} of sample for TPC (Jay, 1996) and 4.6 log cfu g^{-1} for TPSC values as reported by Cremer and Chipley (1977). But when coliform counts and yeast and mould counts were concerned, it might be advisable to the consumers that fresh hot chilled poultry meat and fish may be consumed preferably up to 7^{th} day to a maximum of 10^{th} day's

refrigerated storage to ensure microbial safety.

Sensory analysis: The decrease in appearance, color and flavor scores of both the samples with advancement of storage period might be due to pigment and lipid oxidation (Bhat *et al*., 2011) and increased TBA values of samples (Tarladgis *et al*., 1960). Decrease in juiciness score might be attributed to the fact due to some loss of moisture from the product during storage. The texture score of the poultry and fish meat also decreased significantly ($p<0.05$) which might be attributed to the loss of moisture and breakdown of fat and protein (Bhat *et al*., 2011). The decrease in overall acceptability might be the synergistic effect of declining of scores for all other sensory parameters.

Based on the above findings, it may be concluded that, refrigeration technique was able to retard meat spoilage and deterioration by off flavor formation, proteolysis, lipid oxidation or microbial degradation up to 7th day to 10th day of refrigerated storage. Although somewhere the preserved meat did not cross the maximum microbiological safety limit up to 10th day of storage, but sensory analysis did not permit to recommend for its consumption beyond 7th day of refrigerated storage. The study in future may be opted for investigating other meat species in different time temperature combination. Hurdle technology concept may be employed to retard the meat spoilage and to fetch the better shelf-life in different meat or meat products.

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