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Effect of Chilling on Microbiological, Biochemical and Sensory Attributes of Whole Aquacultured Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792)

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Abstract

The effect of chilling (0-2ºC) on the quality deterioration of whole ungutted aquacultured rainbow trout (*Oncorhynchus mykiss*, Walbaum,1792) was studied by integrated evaluations of microbiological, biochemical, and sensory attributes. The counts of aerobic mesophilic, psychrotrophic bacteria and *Pseudomonas* increased exponentially. An initial lag phase was noticed for H2S producing bacteria, *Aeromonas* and Enterobacteriaceae. Presence of pathogens such as *Aeromonas hydrophila* and *A. sobria* are of concern in the case of delay in icing or temperature abuse during storage. The pH values increased from an initial value of 6.74 to 7.13. PV showed fluctuations. Of the chemical indicators of spoilage, Thiobarbituric acid (TBA) values increased very slowly reaching final value of 16.56 µg MA g^{-1}. Total Volatile Base Nitrogen (TVB-N) values exceeded 27. 87 mg N 100 g^{-1} on day 14 when the psychrotrophic counts exceeded 10^7 cfu g^{-1} indicating that this value may be useful as a measure of degree of freshness for whole ungutted rainbow trout. Based on the TVB-N and microbiological limits, the shelf life of trout at 0-2°C was 9-12 days.

Keywords: Rainbow trout; Chilled storage; Spoilage bacteria, H$_2$S producing bacteria; Volatile bases; Quality; Shelf life

Introduction

Several species of fresh water fish including common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*) and rainbow trout (*Oncorhynchus mykiss*) are being farmed in India in order to meet the increasing demand for fresh fish. Among the fresh water fishes, rainbow trout is farmed in many parts of India. Worldwide, farmed trout production is increasing. The demand for trout in the Country is estimated to be 800 T per annum [1]. Rainbow trout (*Oncorhynchus mykiss* Walbaum), an exotic cold water species, is considered to be a highly priced fish in India. The fish is marketed mainly as fresh in chilled condition. Recently it was found its way to supermarkets in metro cities as an exotic food item which has demand among the urban population. Trout fillets in the form of smoked and vacuum packaged products have high demand in northern European countries. Trout is a preferred table fish in the U.S and Europe. The increasing demand for high quality fresh seafood has intensified the search for methods and technologies for better utilization of fresh fish.

The quality of fresh fish is a major concern to the consumers and industry. Fish is extremely perishable and the shelf life of such products is limited in the presence of normal air by the chemical effect of atmospheric oxygen and the growth of aerobic spoilage microorganisms. It is generally accepted that the environment can influence the microflora associated with the skin, gills and intestines of finfish [2]. Therefore, fish grown in different aquaculture systems may harbour different numbers and species of bacteria. The culture practices such as pond fertilization, supplementary feeding with slaughter house waste or agricultural byproducts imposes a high probability of contamination on the aquacultured fish. Types and levels of bacterial populations associated with farmed fish are useful indicators of quality and safety of fish. Interaction between microbial metabolism and physic-chemical reactions accelerate fish quality deterioration as amines formation, lipid, nucleotide and protein degradation contributes to off odours, off flavours and texture softening [3,4] *Pseudomonas spp*. and *S. putrefaciens* are the specific spoilage bacteria of marine and freshwater tropical fish stored in ice [5].

Although quality attributes of farmed rainbow trout from temperate counties were evaluated by many workers [6–11], few carried out quality assessment of tropical freshwater fish species [12]. Dawood et al. [13] reported a rapid deterioration in quality of headed and gutted rainbow trout (*Salmo gairdneri*) over a 14 day period of storage when fish had been held at high ambient temperature (30°C) for 6 h. Studies indicated the shelf life of rainbow trout as two weeks for gutted fish in ice [8], 15–16 days for whole ungutted fish, 10–12 days for fillets stored in ice [12] and 6 days for gutted vacuum packed and refrigerated samples [14].

With the increase in aquaculture of this species in India, it is important to study the storage capacity of fish under refrigerated condition following harvest. Due to the perishable nature of fish, there is an obvious need for development of efficient preservation methods, which allow shelf life extension of these products. However, collective works on various quality aspects (chemical, microbiological, textural and sensory) of trout of tropical region stored in ice are scarce. The objective of this study was to assess the quality of farmed rainbow rout stored in ice and kept at chilled condition by integrated evaluations of sensory, microbiological and biochemical attributes and to understand

the spoilage microflora to develop efficient preservation methods which allow extension of shelf life.

Materials and Methods

Material

Rainbow trout (*O.mykiss*) of average weight 250g and average length 278mm were obtained from aquaculture farm located at Rajamallay near Munnar in the High Ranges in Southern India. It was harvested by aggregating into a corner of the pond and scooped by two persons using a drag net. The fish were killed by immersing in ice-cold water (hypothermia), and transported to the laboratory within 6 h of harvesting, in insulated polystyrene boxes containing ice. On reaching the laboratory the whole fish samples were repacked with flake ice (ice/fish ratio 1:1) in polystyrene boxes, provided with outlets for water drainage and stored in a chilled room at a temperature of 0 to 2°C. The ice/fish ratio was maintained constant throughout the experiment. Ten randomly chosen fish were removed from ice after 0, 3, 6, 9, 12, 14 and 15 days for analysis

Microbiological analysis

Twenty five gram muscle with skin were aseptically weighed and homogenized with 225 ml sterile physiological saline for 60s. in a stomacher (Lab Blender 400; Seward Medical, Norfolk, IP24, IXB, UK). The homogenates were serially diluted and 0.5 ml of appropriate serial dilutions was plated on the surface of appropriate media in duplicate by spread plate method and then incubated. For mesophilic and psychrotrophic bacteria, tryptic soy agar plates (TSA, Oxoid, U.K.) were used and plates were incubated at 37 and 7°C for 2 and 10 days respectively [15,16]. *Pseudomonas* were counted on Cetrimide-Fusidin-Cephaloridine (CFC) agar (Oxoid code CM 559, supplemented with SR 103; Oxoid U.K.) after 3 days incubation at 20°C. [17,18]. *Aeromonas* spp. were counted on starch ampicillin (SA) agar (Hi Media, India) containing 10ug/ml of ampicillin incubated at 28°C fir 48 h. [19]. *Brochothrix thermosphacta* was determined on Streptomycin sulfate-Thallous acetate – Actidione Agar (STAA Hi Media, India) after incubation at 20°C for 4days [20].

Enterobacteriaceae and H_2S-producing bacteria (including *Shewanella putrefaciens*) were counted on violet red bile glucose agar (VRBGA, Oxoid code CM 485) and Iron Agar (IA, Oxoid code CM 867), respectively by pour plate method and plates were incubated respectively at 30°C for 24 h. [21] and 20°C for 5 days [22].

Faecal *Streptococci* and *Staphylococcus aureus* counts were determined respectively on KF Streptococci Agar (Oxoid code CM 701) after incubation at 37°C for 2 days and on Baird Parker Agar (Oxoid code CM 275) incubated at 37°C for 2 days and typical colonies were confirmed [23]. Total coliforms, faecal coliforms and *Escherichia coli* were estimated by the three tube MPN method [24].

The dominant aerobic microflora at the final sampling points was determined by isolating and identifying 20% of the colonies from PCA (30 and 7°C) plates. 20-25 colonies were randomly selected from PCA plates. A total of 112 bacterial cultures were isolated from fresh and ice stored trout. Fifty two colonies were picked from PCA plates (30°C) sampled from the fresh prawn and identified. To identify spoilage flora, 60 colonies were randomly selected from PCA (7°C) plates poured on the day fish were considered unacceptable (sensory score 6) and characterized morphologically and bio-chemically. A 5-8 black colonies each were isolated and characterized from IA, VRBGA and SA agar plates at the final sampling points. They were then grouped

according to the taxonomic schemes proposed by several authors for identification (Dainty et al. [25]; Molin and Ternstorm [17]; Krieg and Holt [26]; Sneath et al. [27]; Kirov [28]; Brenner et al. [29]). The isolated cultures were identified and confirmed using API 20NE and API 20NE system (Biomerieux, France).

Biochemical analysis

Moisture, ash and total nitrogen and total fat content were determined using AOAC methods N. 950.46B, 920.153, 928.08 and 960.39 of AOAC, [16] respectively. pH was measured in fish homogenates (10g of fish per 10ml of distilled water) with a Cyberscan 510 pH meter (Eutech Instruments, Singapore). Thiobarbituric acid (TBA) value was determined according to the method of Tarladgis et al. [30] by mixing 10g of fish meat with 100 ml. 0.2 N HCl. TBA value was calculated and expressed in µg malonaldehyde / g of fish sample. Total Volatile Base Nitrogen (TVBN) and Trimethyl amine (TMA) was determined in triplicate by the micro diffusion method [31] from the trichloro acetic acid extract of the muscle. TMA-N and TVB-N was calculated and expressed in mg/ 100g of the sample. Peroxide Value (PV) and Free Fatty Acid (FFA) were determined according to Jacobs [32] and AOCS [33] respectively.

Sensory analysis

Sensory analysis of whole trout stored in ice were performed during storage by a ten member trained sensory panel composed of the staff from the laboratory. While drawing the samples for sensory evaluation, special attention was given to check any change in colour or odour. The panel assessed different attributes like appearance, odour, flavour and texture. The overall acceptance was determined by evaluating the attributes like odor, taste and texture of whole cooked fish (cooking steaks in boiling water containing 2% salt for two minutes) Each sample of the lot was classified using a 10 point hedonic scale, 4 being the acceptability limit.

Sensory analysis of whole trout was performed during iced storage according to the European Community (EC) grading scheme by ten trained panelists [34]. The panel assessed different attributes like appearance, odour, flavour and texture. The appearance of the skin, eyes, gills and internal organs, surface slime, and the odor and texture of each fish (whole) was assessed into four quality grades - excellent quality (perfect condition, E), high quality (slight loss of excellent characteristics, A), good quality (some deterioration, but fit for sale, B) and unfit for sale (C). Color analysis was performed with a Hunter lab Miniscan ® XE plus spectrocolorimeter (Hunter Associates Laboratory, Inc. Reston, Virginia, USA). Measurements were recorded using the L* a* b* colour scale [35]. Chroma (C*) and Hue (h*) also were calculated from the L* a* b* values. Three repetitions of the different colour parameters were recorded.

Statistical analysis

Experiments were replicated twice on different occasions with fish samples from the same farm. Results are presented as mean ± standard deviation and significance of the differences between the mean values was determined by One-way Analysis of Variance (ANOVA), followed by Duncan's test using SPSS software (version 10.0) for Windows. *p*-value lower than 0.05 was considered statistically significant.

Results and Discussion

Aerobic mesophilic and psychrotrophic bacteria grew exponentially from an initial load of 3-5 $\log_{10}$cfu g^{-1} reaching 7.6 $\log_{10}$cfu g^{-1} on day 15 (Figure 1). The initial mesophilic bacterial load of 4.7 $\log_{10}$cfu g^{-1}
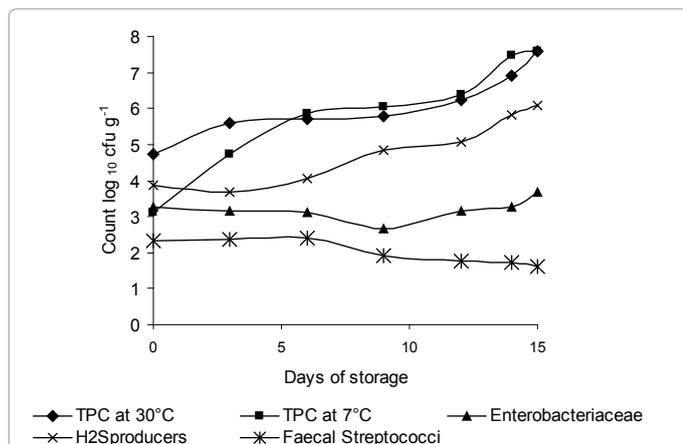
Figure 1: Changes in the population of aerobic mesophilic, bacteria, psychrophic bacteria, H2S producing bacteria, faecal streptococci and Enterobacteriaceae in trout stored at 0-2°C.

indicated good quality of trout as it ranged from 2-6 $\log_{10}$cfu g^{-1}. [36]. These values obtained in this study are close to the values reported earlier for aquacultured fresh trout from Spain [37,41], Greece [38, 39] and U.K [40]. It is widely accepted that the initial microbial load of fresh water varies depending on water conditions and temperature. The initial and final aerobic mesophilic bacterial associations of trout stored in ice were found to be similar to those reported in the literature for trout stored aerobically.

In this study, mesophilic and psychrotrophic counts in fresh fish tissues were close to or lower than the m value (5x105 cfu/g) recommended by the International Commission of Microbiological Specification for Foods [42] for whole fresh water fish. Taking the 107 cfu g^{-1} psychrotrophic count as the spoilage level, the shelf life of chilled stored trout in this study was 9-12 days as reported earlier by Fik and Surówka [43] and Rezaei et al. [44]. For fresh fish, the microbiological limit (M) for human consumption proposed by ICMSF [42] is 107cfu g^{-1}.

Of the bacterial groups examined in the present study, H2S-producing bacteria had the highest counts followed by *Pseudomonas* spp., Aeromonas, *B. thermosphacta* and Enterobacteriaceae (Figures 1 & 2). H2S producing bacterial counts constituted <5% of the total flora in fresh trout (3.8 $\log_{10}$ cfu g^{-1}), their levels increased significantly (P <0.01) during storage and its proportion in the total flora reached 10-15% at the end of storage indicating their role in the spoilage. H2S producing bacteria were identified as *Shewanella* and Aeromonas. *Pseudomonas* displayed the typical growth pattern of psychrotrophic bacteria without a lag phase (Figure 1) increasing from initial counts of 3.0 to 5.02 $\log_{10}$ cfu g^{-1} on day 15. These two bacterial groups were found to be the specific spoilage organism (SSO) in fish from temperature and tropical waters [5]. *Shewanella* spoilage is characterized by TMA and sulphides (H2S) whereas the *Pseudomonas* spoilage is characterized by absence of these compounds and occurrence of sweet, rotten sulphydryl odours. *Pseudomonas* and *Shewanella* isolates produced large amounts of TVBN, secreted huge amounts of proteolytic enzymes and intense off-odours and were identified as strong spoiling bacteria. Chytiri et al. [12] also reported dominance of *Pseudomonas*, H2S producing bacteria (including *Shewanella* putrefaciens) and *B. thermosphacta* in the spoilage microflora of whole ungutted and filleted trout over an 18-day storage period in ice.

Aeromonas was also found to be members of the microflora of farmed trout with an initial load of 3.18 $\log_{10}$ cfu g^{-1} as reported earlier by Nam and Joh [45]. A reduction in bacterial load was noticed during the first week of iced storage and growth was resumed after 12 days (Figure 2). Aeromonas isolates were identified as A. hydrophila and A. sobria. These bacterial species produced proteinases, reduced TMAO and produced off-odours indicating their spoilage potential. Although these bacteria are capable of growth at chill temperatures, they required a period of adaptation (i.e., the lag phase and slow growth phase) in this study. Lee et al.[46] isolated A. hydrophila from diseased trout from Korea and this bacterium is responsible for hemorrphagic septicemia, a disease affecting a wide variety of freshwater and marine fish [47]. Epizootic Ulcerative syndrome caused by A. sobria resulted in great damage to fish farms in Bangladesh and India [48]. Gonzalez et al.[49] noticed the strong potential spoilage activity of aeromonads in wild and aquacultured iced freshwater fish. A. hydrophila, A. veronii biovar veronii and A. veronii biovar sobria are the strains more often associated with gastroenteritis in humans and the enteropathogenic potential of these strains were comparatively high when grown at low temperatures than at 37oC[50,51]. The occurrence of A. hydrophila and A. sobria in trout farms and ice stored trout must be taken into consideration because it can cause gastroenteritis and wound infections.The ability of these organisms to grow at refrigeration temperatures indicates the potential food safety issues from such foods.

Brochothrix thermosphacta population in ice stored trout increased from an initial count of 2.32 $\log_{10}$ cfu g^{-1} to 3.9 $\log_{10}$ cfu g^{-1} (Figure 3). Similar counts for whole ungutted trout were reported by Chytiri et al. [12] on day 15 in iced storage.

Enterobacteriaceae were also part of the microflora of farmed rainbow trout which is in agreement with the findings of Arashisar et al. [10], Chytiri et al. [12] and Nerantzaki et al.[39] . Enterobacteriaceae counts decreased during the first week of storage from an initial value of 3.2 $\log_{10}$ cfu g^{-1}. At the end of storage, a count of 3.66 $\log_{10}$cfu g^{-1} was noticed. The dominant species identified in this study were Citrobacter freundii, Hafnia alvei and Pantoea agglomerans. In this study, Enterobacteriaceae were found in high numbers in fresh trout and their
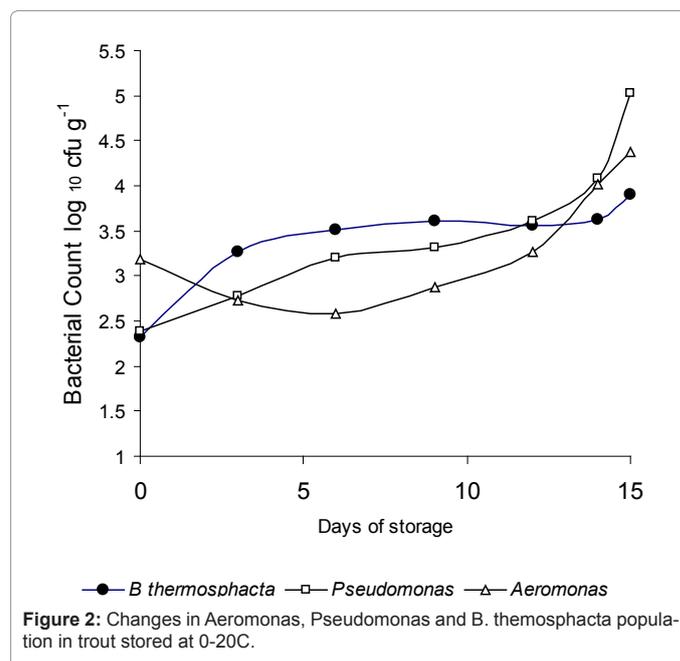

Figure 2: Changes in Aeromonas, Pseudomonas and B. themosphacta population in trout stored at 0-20C.

abundance decreased during ice storage, possibly because of their lower growth rate than that of other Gram-negative psychrotrophic spoilers. The contribution of Enterobacteriaceae to the microflora of trout and its potential to cause spoilage must be taken into consideration in case of delay in chilling after catch or temperature abuse during storage.

Among the indicator organisms, faecal streptococcal population was 2.3 log 10 cfu g^{-1} initially and at the end of storage the count was ca. 1.6 log 10 cfu g^{-1} (Figure 1). S. aureus numbers were low (1.3 log 10 cfu g^{-1}) in fresh trout and were within the acceptable limit. S. aureus were not detected in trout during iced storage. Icing affected populations of total coliforms, Faecal coliforms and E. coli levels (Figure 3) and 1-2 log reduction was noticed . E. coli counts in trout on day 3 (< 6 g^{-1}) was below the m limit (11 g^{-1}) recommended by the ICMSF [42]) for good quality fish. High levels of faecal coliforms were previously reported for fish farms in India [52-54].

A total of 52 strains were isolated from 30^C PCA plates and identified. In fresh trout, majority of the isolates (70%) were gram-negative rods. The main bacterial groups identified among the 52 isolates randomly selected from TSA plates were i. Gram-negative aerobic coccobacilli and rods (Moraxella, Acinetobacter, Flavobacterium), ii. Gram-negative aerobic motile rods (*Pseudomonas*), iii.Enterobacteriaceae (Enterobacter, Citrobacter, Hafnia, Klebsiella), iv. Aeromonadaceae (Aeromonas), v. Micrococcaceae (Kocuria, Staphylococcus) andvi.Gram-positive spore forming bacteria (Bacillus). González et al. [41] reported predominance of Acinetobacter, *Pseudomonas*, Staphylococcus, Enterococcus and Bacillus in rainbow trout from Spain. On icing, the abundance of Enterobacteriaceae decreased. A total of 56 strains were characterized from 30^C PCA plates. The majority (>60%) of the trout isolates after 15 days in ice belonged to genera Moraxella, Acinetobacter, *Pseudomonas*, *Shewanella*, Aeromonas and Flavobacterium indicating that spoilage of fresh trout stored aerobically is due to the activity of more than one specific spoilage organism. Flavobacterium have been found in other farmed fish species such as catfish and some are also the causative agent of bacterial cold water disease and rainbow trout fry syndrome [55,56]. Several investigations have concluded that Gram- negative rod-shaped bacteria (e.g., *Pseudomonas*, Moraxella and Acinetobacter) dominate on many fish caught in tropical waters [36,57].

Results of proximate analysis of whole ungutted trout stored at 0-2 0C during the 15-day storage are given in Table 1. Raw trout is a fish with a fat content of 1.60 ± 0.12 g/100 g edible meat, a protein content of 19.80 ± 0.65 g/100g edible meat and ash content of 0.61±0.23 g/100g edible meat. The levels of fat and ash were low compared to that reported for trout from other regions [58-60,41]. During storage in ice, no significant variations in the composition of major constituents viz. moisture, protein and fat were observed. A slight increase of 3.33% moisture content was observed after 15 days in chilled storage. The retention of good texture of fish muscle during chilled storage can be attributed to the minimum leaching of the major constituents and low water penetration into the flesh.

The changes in TVB-N and TMA levels in whole trout throughout the storage in ice are shown in Table 2. The results of this study confirmed the earlier studies of Rodriguez et al. [61] and Chytiri et al. [12] who reported TMA values of ≤ 1.0 mg/100g for whole fresh trout and values of ≤ 3.0 mg/100g on day 15 for ice stored fish indicating the low level of trimethylamine oxide (TMAO) in the flesh of this fish species. TMAO quantity in fish varies with the species and the environment. A wide range of TMA-N values have been reported by several investigators as acceptability limit i.e., 1-5mg N/100 g [62- 65]. Sikorski et al. [66] and Dalgaard et al. [67] reported that a population of 108-109 cfu/g of *S. putrefaciens* was considered crucial for TMA production. In this study, the count of H2S- producing bacteria (including *S. putrefaciens*) was low (6.07 log cfu/g) at the end of iced storage which could be the reason for low levels of TMA.

TVBN, including trimethylamine, dimethylamine, ammonia and other volatile basic nitrogen compounds, was produced mainly by bacterial decomposition of fish flesh. In this study, TVB-N levels increased to 31.25 mg N 100g^{-1} on day 14. The values exceeded the limit of acceptability of 25 mg N 100g^{-1} proposed by Stansby [68]. However, fish were acceptable based on sensory score and microbiological counts. Critical limits of 25, 30 and 35 mg N 100g^{-1} of TVB-N were established for different groups of fishes [69]. However, no limit for acceptability has been established for rainbow trout. Hence, based on the TVB-N levels, microbiological counts and spoilage indicators, TVB-N limit of 27 mg N 100g^{-1} may be proposed for rainbow trout as acceptable limit. As TMA production was low, ammonia probably accounted for the major portion of volatile bases. Giménez et al. [9] observed TVB-N value of 35 mg N 100g^{-1} in trout fillet stored under air on day 8 and reported good correlation with bacterial counts (108 cfu g^{-1}). In contrast, Arashisar et al. [10] reported values of 40 mg N 100g^{-1} in filleted trout at the end of 14 days storage. However, the values were < 20 mg N 100g^{-1} when psychrotrophic bacterial levels exceeded 107cfu g^{-1} on day 6 at 4 ± 1˚C. There is good correlation between TVB-N and microbiological parameters in the present study as reported earlier by Katikou et al. [70]. The results also suggest that since TVBN values exceeded limit of acceptability on day 14 when fish had a stale order, it may be useful as a measure of degree of freshness; although some reports differ in this context [71, 12].

While evaluating the spoilage potential of nine bacterial groups isolated from cold smoked salmon, Stohr et al. [72] have reported that Gram negative bacterial strains such as Aeromonas, *Shewanella* and Serratia produced TMA in concentrations ranging from 11.0 to 13.1 mg
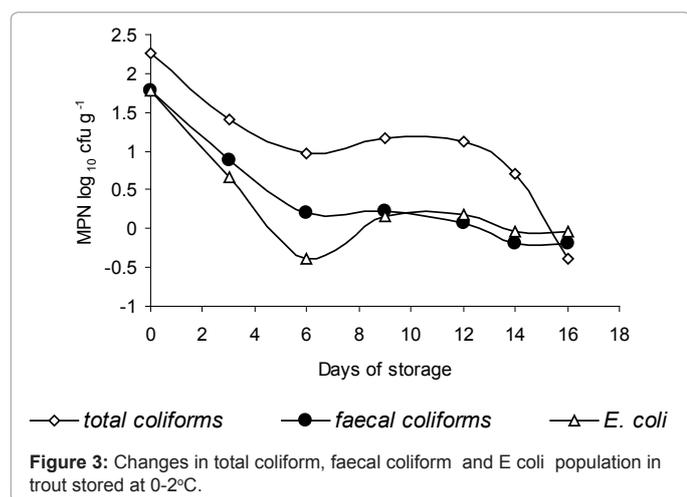

Figure 3: Changes in total coliform, faecal coliform and E coli population in trout stored at 0-2°C.

DAYS	Moisture (%)	Protein (%)	Fat (%)(dwb)	Ash (%)
0	78.04 ± 0.31	19.80± 0.65	1.60±0.12	0.61±0.23
3	79.06 ± 0.41	19.18±0.82	1.56 ±0.05	0.66 ±0.04
6	79.12± 0.23	19.06± 0.56	1.54 ± 0.06	0.64 ± 0.07
9	79.41± 0.32	18.48 ± 0.77	1.57± 0.14	0.66 ± 0.04
12	79.73± 0.45	18.09 ±0.54	1.47 ± 0.09	0.72 ± 0.06
14	80.24±0.42	18.06±0.56	1.45 ±0.04	0.65 ±0.04
15	80.64 ± 0.11	17.97 ±0.28	1.42 ± 0.04	0.61 ±0.03

Table 1: Proximate composition of trout stored at 0-20C(n= 3 ×2).

Days	PV (meq O_2/Kg)	TBA (µg MA/g)	FFA (mg % oleic acid)	TMA (mg /100 g)	TVB-N (mg N/100 g)	pH
0	ND	4.97±0.27 [a]	3.28±0.26 [a]	1.23±0.11 [a]	14.6±0.26 [a]	6.77 ±0.03 [a]
3	9.11±0.08 [a]	4.5±0.1 [b]	2.92±0.04 [b]	1.5±0.1 [b]	19.67±0.29 [b]	6.75 ±0.02 [a]
6	5.34±0.42 [b]	9.55±0.11 [c]	2.33±0.09 [c]	1.6±0.2 [bc]	19.73±0.46 [b]	7.03±0.04 [b]
9	3.48±0.35 [c]	14.14±0.16 [d]	2.95±0.01 [b]	1.71±0.03 [bc]	21.08±0.16 [c]	7.12±0.01 [c]
12	4.62±0.17 [d]	15.47±0.06 [e]	3.4±0.36 [a]	1.68±0.03 [bc]	27.87 ±0.26 [d]	7.14 ±0.03 [c]
14	5.82±0.28 [b]	16.2±0.36 [f]	2.81±0.10 [b]	1.79±0.07 [c]	31.25±0.15 [e]	7.14 ±0.02 [c]
15	8.4 ±0.35 [e]	16.56±0.47 [f]	3.93±0.05 [d]	3.32 ± 0.13 [d]	31.20±0.44 [e]	7.13 ±0.03 [c]

*Within each column, means with the same superscript do not differ significantly ($p > 0.05$).

Table 2: Changes in Peroxide Value (PV), Thiobarbituric Acid value (TBA), Trimethyl amine (TMA), Free Fatty Acid value (FFA), Total Volatile Nitrogen (TVB-N) and pH of trout stored at 0-20C*(n= 3 ×2).

Days	Skin	Eyes	Gills	Flesh colour	Outer slime	EC Grade
0	Bright, shining ; firm	Translucent cornea; convex; absence mucus	Fresh odor; red color	Pinkish white	thin; transparent	E
3	Bright shining; firm	Translucent cornea; convex; absence mucus	Fresh odor; red color	Pinkish white	thin; transparent	E
6	Bright shining; firm	Translucent cornea; convex; absence mucus	Fresh odor; red color	Pinkish white	thin; transparent	E
9	Waxy; slight loss of shine; soft	Opalescent cornea; plane; moderate mucus	Fishy odor; red color	Whitish	Aqueous; transparent	A
12	Waxy; slight loss of shine; soft	Opalescent cornea; plane; moderate mucus	Stale odor; dark red color	Whitish	Opaque; thick; slight milky	B
14	Dull; some bleaching	Opalescent cornea; plane; excessive mucus	Stale odor; dark red color	Whitish with slight yellow stain	Opaque; thick; slight milky	B
15	Dull; some bleaching	Opalescent cornea; sunken; excessive mucus	Spoiled odor; dark red color	Whitish with slight yellow stain	Thick;Milky	C

Table 3: Sensory assessment of trout stored at 0-2°C.

N 100g^{-1} and high TVBN production was generally correlated with high TMA production. In this study, even with low TMA levels, high TVBN values of 31.25 mg N 100g^{-1} were obtained and this may be attributed to ammonia production.

pH values for whole ungutted trout samples increased with storage time from an initial value of 6.74 (Table 2) indicating bacterial growth and production of volatile basic compounds such as ammonia by fish spoilage bacteria. Increase in pH due to accumulation of alkaline compounds through autolytic activities and microbial metabolism has been reported in earlier studies [65,73,74]. Many microbes including *Pseudomonas* produce ammonia during amino acid metabolism.
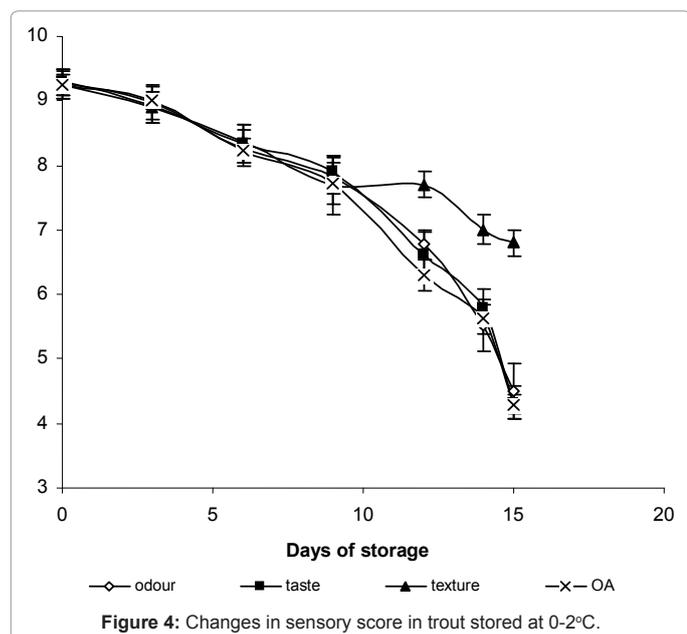
Changes in PV, TBA and FFA for whole ungutted trout stored at 0-2° C during the 15-day storage are shown in Table 2. PV and TBA are indices to measure the first and second stages of oxidative rancidity respectively. PV measures peroxides and hydroperoxides and a value of above 10 -20 is an indication of rancidity [75]. In this study PV showed fluctuations during the chilled storage, but the values were very low to cause rancidity or off flavour at any point of time during the study.

TBA values for whole ungutted trout samples increased steadily from an initial value of 4.9 ± 0. 3 µg MA/g and reached a value of 14.1 µg MA/g on day 9 when fish retained high quality as per EC grade and the value was 16.6 µg MA/g when fish spoiled on day 15 (Table 2). The results of this study confirmed the earlier finding that oxidative rancidity remained relatively low in aquacultured whole ungutted trout throughout the entire period of storage in ice and oxidative rancidity

indices viz., PV and TBA are poor indicators of quality since lipids in trout are relatively stable during chilled storage[12]. The low level of lipid oxidation products suggest that whole ungutted rainbow trout stored at 0-2 0 C has some intrinsic factors to prevent oxidation. The lipids were found to better stable in whole than in gutted or filleted trout possibly due to the fact that it is harder for oxygen to penetrate into whole fish and there may be a higher accumulation of proteolysis products, acting as antioxidants, with time of storage [76]. Because the rainbow trout studied showed no increased lipid oxidation during the first week of storage (a decrease in PV relative to the initial level being observed), it may be suggested that the muscle tissue of rainbow trout, particularly in the whole fish, was predominantly a site of antioxidant over pro-oxidant activity at that time [77]. Fifteen days in chilled conditions has accumulated only 4% of FFA which indicates that rainbow trout has very low level of lipase activity during chilled storage.

Changes in sensory attributes of the whole ungutted trout in chilled storage were given in descriptive terms as recorded by given by the panelists (Table 3). Whole ungutted trout was in excellent condition up to six days in chilled conditions and the high quality was retained for nine days. The fish was still considered to be of good quality and fit for sale between 12–14 days of chilled storage. The fish samples were spoiled on 15 th day.

The overall acceptability scores for whole ungutted trout remained in the range of seven points up to nine days indicating that there was no significant loss of sensory attributes viz., odour, taste and texture.

Figure 4: Changes in sensory score in trout stored at 0-2°C.

Days	L*	a*	b*	Hue	Chroma
0	47.26 ±.05	2.41 ± 0.15	9.32 ± 0.07	75.49 ± 0.97	9.63 ± 0.03
3	47.72 ± 0.14	2.23 ± 0.03	9.82 ± 0.02	77.19 ± 0.16	10.07 ± 0.03
6	48.52 ± 0.03	2.09 ± 0.02	10.23 ± 0.04	78.42 ± 0.09	10.44 ± 0.04
9	49.75 ± 0.23	1.95 ± 0.18	9.84 ± 0.64	78.79 ± 0.37	10.04 ± 0.66
12	50.89 ± 0.56	1.51 ± 0.07	10.42 ± 0.19	81.77 ± 0.37	10.53 ± 0.19
14	53.02 ± 0.19	1.25 ± 0.03	10.61 ± 0.09	83.29 ± 0.22	10.68 ± 0.09
15	55.65 ± 0.45	1.28 ± 0.07	10.79 ± 0.11	83.23 ± 0.32	10.86 ± 0.12

L* value (Lightness), a* (red) value, b*(yellowness)

Table 4: Changes in colour parameters of the mince of trout stored at 0-2°C (n= 3 ×2).

The limit of acceptability for odour and taste was reached by the 14 th day in chilled condition (Figure 4). However the chilled fish retained the texture with slight changes and the limit of acceptability was not reached for this attribute during the entire chilled storage period.

The sensory changes observed in rainbow trout during storage in ice were in agreement with the descriptions presented by other authors [11,66,78,79]. The quality deterioration in trout was correlated with reduced tastefulness of the cooked fish. The reduction in tastefulness was caused primarily probably by nucleotide decomposition [80,81]. Based on the sensory score, trout had a shelf life of 12-14 days in ice.

Change in colour parameters of mince prepared from chill stored rainbow trout is given in Table 4. During chill storage there is a gradual increase in L* value (Lightness) with a corresponding decrease in a* (red) value. The b*(yellowness) increased during this period. The instrumental colour values correlated well with the sensory observation of flesh given in Table1. Fresh sample (0 day) has pinkish white flesh which progressively turned paler during storage and has yellow stained white colour at the end of storage period. This finding indicated that freshness of trout, in relation to sensory analysis, was lost after total aerobic bacterial count reached limit count.

Conclusion

The results of the study indicate that the shelf life of whole gutted trout stored in ice as determined by the chemical and microbiological quality is 9-11 days. The whole ungutted rainbow trout stored in ice remained in excellent condition up to six days and retained high quality up to nine days. Based on sensory score, trout had a shelf life of 12-14 days in ice. The results obtained in the present study for trout tend to confirm the earlier observations that TMA, PV and TBA are of questionable use as quality indices. TVBN values exceeded the limit of acceptability when fish had a stale order and microbial count exceeded 3×10^6 cfu g^{-1} indicating that TVBN may be useful as a measure of degree of freshness. Detailed sensory evaluation is the effective and practical method to assess the freshness of chill stored whole ungutted rainbow trout.

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