



Effect of incorporation of green tea extract in icing medium on the quality and shelf life of *Nemipterus japonicus* (Bloch, 1791) in chilled storage

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ABSTRACT

The present study was aimed at investigating the effect of inclusion of green tea leaf (GTE) extract (at 3 and 6% levels) in the icing medium employed during the chilled storage ($2\pm 1^\circ\text{C}$) of Japanese threadfin bream *Nemipterus japonicus*. Fish stored in normal ice (NI) was treated as control. Changes in microbiological, biochemical and sensory quality characteristics were monitored during the period of storage. Multivariate comparison was performed using principal component analysis (PCA) for the mean sensory, microbiological and chemical attributes. Samples stored in normal ice (NI) had only 8 days shelf life whereas both 3% GTE and 6% GTE stored samples had a shelf life of 16 days. From an economic point of view, 3% GTE can be adopted for preservation of fish and there was no significant difference ($p > 0.05$) in the parameters between 3 and 6% GTE levels. The present study indicated that the application of GTE in ice (GTEI) is a promising technique to increase the shelf life of *N. japonicus* in chilled condition and hence the technique can be commercially exploited.

Keywords: Chilled storage, Green tea leaf extract, *Nemipterus japonicus*, Principal component analysis, Shelf life

Introduction

Fish is often referred to as 'rich food for poor people' and provides quality proteins, fats, vitamins and minerals. The nutrient intake of populations from fish is directly proportional to the amount of fish consumed (Gormley, 2013) but fresh seafood has a short shelf life, which causes substantial practical problems for its distribution. Improvements in shelf life can have an important economic impact by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets (Rhodehamel, 1992).

Food borne illnesses are still a major threat globally, including even the developed countries. In this context, it is imperative to research and develop application of substances with antibacterial and antifungal properties, to which pathogens and spoilage organisms do not develop resistance and that would be harmless to human beings (Bilska *et al.*, 2012). Consumers increasingly demand safe, healthy seafood, free of harmful additives and contaminants. The use of natural preservatives to increase the shelf life of seafood products is a promising opportunity since many organic substances have antioxidant and antimicrobial properties (Negro *et al.*, 2003).

Camellia sinensis (tea plant) is described as a potent natural source for promoting good health and longevity, as well as in treating many ailments, from indigestion to common cold. Green tea is higher in catechins than

black tea. Catechins are polyphenolic compounds present in the unfermented leaves of tea. Biological and pharmacological activities of green tea leaf extract (anti-inflammatory, anti-microbial, anti-tumour, anti-oxidative and anti-ageing) have been attributed to the presence of catechins and the crude green tea extract exhibits higher antimicrobial activity than that of isolated catechins. Shi *et al.* (1994) and Fan *et al.* (2008) reported that the tea extract has beneficial antibacterial and anti-oxidative activities, which demonstrates potential for their use as preservatives and antioxidants in food industry especially in the field of preservation of manufactured meat.

The present study was aimed at investigating the effect of incorporating green tea leaf extract in the icing medium used during chilled storage ($2\pm 1^\circ\text{C}$) of pink perch *Nemipterus japonicus*, one of the most commonly caught marine, demersal fish species, abundant in Indian coastal waters which has a significant, commercial value owing to its unique meat quality and taste.

Materials and methods

Preparation of frozen green tea extract

Green tea was extracted at 3 and 6% (w/v) levels in 5000 ml distilled water for 1 min at 90°C . The extract was allowed to cool, filtered and then ice cubes (GTEI) were prepared from both the extracts. Control ice cubes were prepared with potable water.

Sample preparation

Fresh pink perch *N. japonicus* (average weight varying from 60 to 80 g) procured from Cochin Fisheries Harbour, Ernakulam District, Kerala were iced immediately in 1:1 ratio and brought to the laboratory in insulated boxes within 1 h of procurement. It was de-iced, washed with potable water, divided into three batches and re-iced. The first batch was chilled with ice prepared from potable water (control, NI) and the other two batches were iced with GTEI having 3 and 6% concentrations of GTE, at fish:ice ratio of 1:1 (w/w). All samples were then kept in polystyrene boxes for 20 days with replenishment of NI and GTEI daily. Periodical samplings at every 4 days were done for quality assessment using biochemical, microbiological and sensory methods. All analyses were performed in triplicate on 0, 4, 8, 12, 16 and 20 days of storage.

Sensory evaluation

Sensory evaluation of the sampled pink perch fillets was done by a panel of 10 experts at each sampling time during the entire storage period of 20 days. Using a well-structured questionnaire, the panelists independently assessed the coded samples in small porcelain dishes. Panelists' gave scores for sensory characteristics, such as appearance, odour, flavour, texture and overall acceptability using a 9 point descriptive scale (Peryam and Pilgrims, 1957). On this scale, score 9.0 indicated liked extremely; score 1 indicated disliked extremely and score 5 was the limit of acceptability.

Chemical analyses

Total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) were determined by the Conway micro diffusion method of Beatty and Gibbons (1937). Peroxide value (PV) and free fatty acids (FFA) were determined according to AOCS (1989). Thiobarbituric acid value (TBA) was determined using AOAC (2000) method. The pH was measured using digital pH meter (AOAC, 2000). Extraction of sarcoplasmic and myofibrillar protein content was carried out by the method of Sankar (2000).

Microbiological analysis

For microbiological analysis, 50 g of the fish flesh was transferred to a stomacher bag (Seward, London, UK), 450 ml of Butterfield's phosphate buffer was added and blended for 2 min with a stomacher (Lab blender 400, Seward, Medical, London, UK) to obtain the original homogenate fluid (10^{-1} dilution). From the 10^{-1} dilution, other serial decimal dilutions were prepared. Three replicates of at least three appropriate dilutions were enumerated. Plate count agar (Difco 247940) was used

for mesophilic plate count which was made according to the American Public Health Association standard (APHA, 2002) and the inoculated plates were incubated for 48 ± 2 h at 35°C for aerobic plate count (BAM, 2001) and for 7 days at 5°C for psychrotrophic count. Streptomycin-thallus-acetate-actidioneagar (STAA) (Oxoid CM0881) supplemented with STA supplement was used for the isolation and quantitative enumeration of *Brochothrix thermosphacta* (Gardner, 1996). Hydrogen sulphide producers were cultured on peptone iron agar (Gram *et al.*, 1990). White or semi-transparent convex colonies on STAA plates and black colonies formed on peptone iron agar (Himedia M440) plates were enumerated after 5 days of incubation at 20°C . *Pseudomonas* colonies were enumerated from Kings B agar medium (Himedia M1544) after 2 days of incubation at 20°C (King *et al.*, 1954). Violet red bile glucose agar media (VRBGA) (Difco 218661) was used for *Enterobacteriaceae* enumeration and characteristic large colonies with purple haloes were counted from VRBGA plates after 24 ± 2 h of incubation at 35°C (Mossel *et al.*, 1979). Microbial data were transformed into logarithms of the number of colony forming units (CFU) per gram. Averages and standard deviations of the transformed values were then estimated, to take the variability in bacterial cell counts into consideration.

Principal component analysis

Principal component analysis is one of the widely used statistical techniques for reducing the number of variables to a smaller set (called factors) based on patterns of correlation among the original variables (Lawless and Heymann, 1998). In order to describe the changes in microbial and chemical parameters for *N. japonicus* samples and to obtain an overall view of the main variations among 3 and, 6% GTEI stored samples and control samples, the experimental data was subjected to PCA using SAS 9.3 and variability explained by each principal component was estimated. The principal components with maximum variation were taken for clustering the treatments.

Results and discussion

Changes in sensory attributes

Sensory assessment has always played a key role in quality and freshness evaluation in the fish industry. The most important methods to evaluate freshness of seafood are the sensory methods (Bonilla *et al.*, 2007). The various sensory characteristics, such as outer appearance, odour and colour are still very important in the quality assessment in the fish processing industry. Loss of freshness in seafood is the result of post-mortem biochemical,

physicochemical and microbiological processes characteristic of each species and influenced by handling onboard and on land and by technological processing. These changes are perceivable and can be evaluated in sensory terms by sight, touch, smell and taste. Sensory panel evaluations for the fresh fish in the present study indicated that all fish samples on the initial day rated as good (average score 8.79). On the initial day, the fish samples had fresh “seaweed” odour; firm and elastic texture and bright, shining colour and appearance. Changes in the sensory score of fish samples over the entire storage period are shown in Fig. 1a-e. Sensory scores for all the three samples significantly reduced ($p < 0.01$) with the days of storage but even the control samples retained its sensory characteristics for a period of 12 days. In this study, fish stored in GTEI exhibited slight grey discoloration on the skin and scales but no discoloration was observed in the

fish muscle. The grey colour in the skin could be attributed to the leaching of some components like tea catechins in the GTE. The study also indicated that this colour has not leached onto the flesh during the entire storage period. Similar to this study, Mitsumoto *et al.* (2005) found that tea catechins caused a certain grey discoloration in cooked beef and chicken patties. Jo *et al.* (2003) found that GTE improved colour and did not affect the odour, flavour or tenderness of cooked pork patties. Present study exhibited significant variation in organoleptic quality between samples stored in GTEI and control samples. It was obvious that storing fish samples in GTEI exhibited higher sensory scores compared to control samples, which could be explained on the basis of the antioxidant properties of the tea catechins. Fish spoilage gave rise to the subsequent development of strongly fishy, rancid and putrid odour in fish stored in NI (control sample), but the

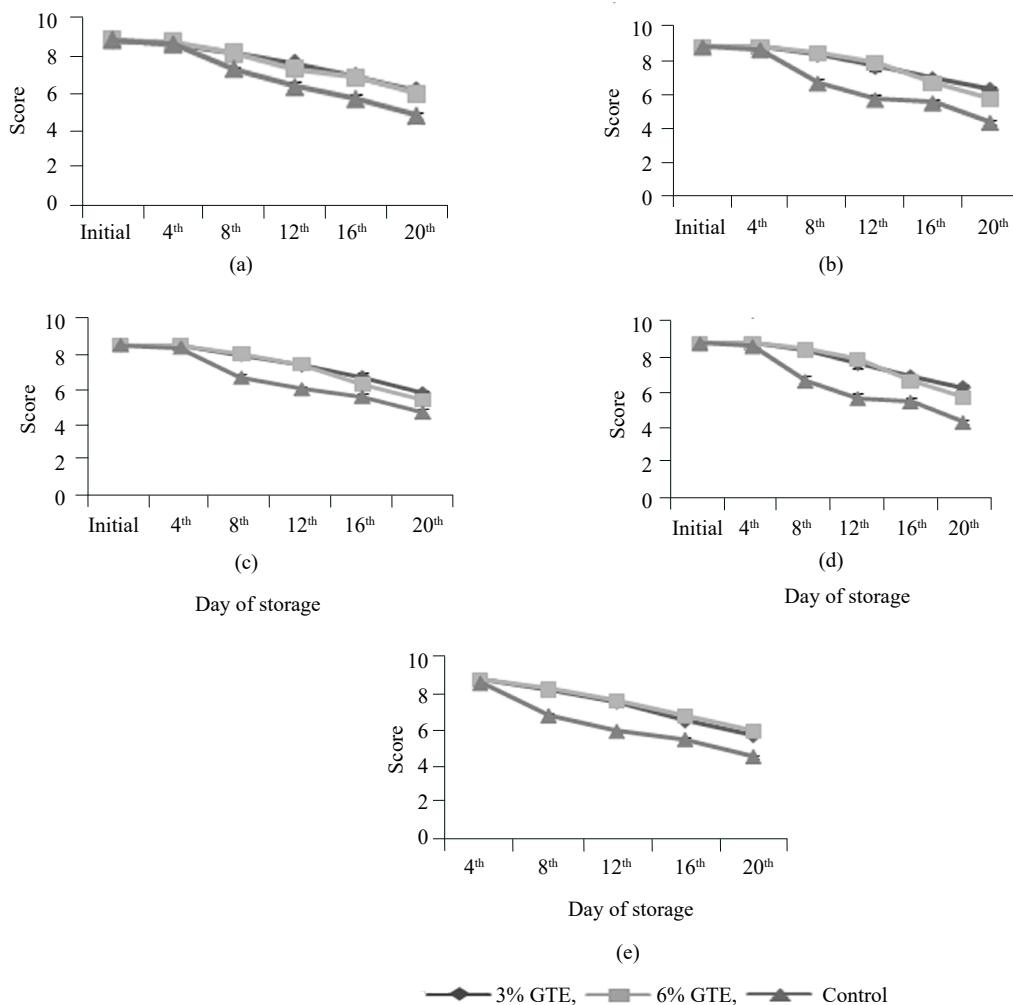


Fig.1. Changes in sensory characteristics during chilled storage of *N. japonicus*. (a) Colour values, (b) Texture, (c) Appearance, (d) Texture and (e) Overall acceptability

sensory parameters of the fish samples stored in GTEI were within the acceptance limit till the last day of storage. Fish is more susceptible to oxidation and tea catechins present in the GTE can inhibit lipid oxidation in raw fish. The tea catechins are potent natural antioxidants and exhibit greater antioxidant efficacy. Hence the flavour and taste of fish stored in GTEI is protected to an extent.

Changes in chemical parameters

TVB-N levels were monitored as a good index of fish muscle freshness, because its increase is related to spoilage by the activity of endogenous enzymes and bacterial growth. TVB-N is produced by the degradation of proteins and non-protein nitrogenous compounds into simpler substances mainly ammonia, trimethylamine, creatine, purine bases and free amino acids, which is caused by microbial activity. Connell (1995) reported that a level of 30-35 mg N 100 g⁻¹ is considered to be the upper limit, beyond which fishery products are considered unfit for human consumption. Scherer *et al.* (2006) noted that TVB-N levels were more suitable for spoilage assessment in marine than in freshwater fish. The level of TVB-N in freshly caught fish is generally between 5 and 20 mg N₂ per 100 g muscle. During storage, a progressive increase in the TVB-N content is reported for chilled, vacuum packed, modified atmosphere packed and active packed refrigerated fish products (Mohan *et al.*, 2008). In the present study, at the beginning of storage, the TVB-N value was 11.116 mg 100 g⁻¹ of fish flesh. This value decreased to 7.02 and 6.346 mg TVB-N 100 g⁻¹ for 3 and 6% GTEI stored samples respectively on 4th day of storage, thereafter significantly ($p < 0.05$) increased to 35.41 and 35.026 mg TVB-N 100 g⁻¹ respectively by day 20 (Fig. 2a). Control sample exhibited an increasing trend in the TVB-N value from the first day onwards and crossed the maximum permissible limit on 12th day of storage. Since TVB-N is produced mainly by bacterial decomposition of fish flesh, the higher mesophilic count of control samples compared to other samples throughout storage could account for the higher TVB-N values of control samples. This could also be attributed to rapid reduction of bacterial population or decreasing the capacity of bacteria for oxidative deamination of non-protein nitrogen compounds.

The spoiled fishy odour of marine fish is often linked with trimethylamine (TMA), which is formed due to the action of the bacterial enzyme on trimethyl amine oxide (TMAO) reductase on TMAO. Bacteria like *Aeromonas* spp. *Shewanella putrefaciens*, *Photobacterium phosphoreum*, psychrotolerant *Enterobacteriaceae* and *Vibrio* spp. are known to possess the ability to reduce TMAO (Gram *et al.*, 1990). Chytiri *et al.* (2004) have shown that TMA levels increased during chilled storage of whole fish and fillets of rainbow trout in ice, due to

an increase in specific spoilage bacteria. TMAO is mainly present in marine fish and absent in freshwater fish. In the current study TMA on initial day was zero and during storage, the levels increased gradually and on the 12th day of storage, control sample (NI) recorded TMA-N value of 15.26 mg 100 g⁻¹ (Fig. 2b) which exceeded the limits of acceptability of 10-15 mg 100 g⁻¹ (Connell, 1995). TMA-N production for GTEI stored samples were within the acceptable limits upto 16th day of storage. This could be attributed to rapid reduction of bacterial population or decrease in the capacity of bacteria for oxidative deamination of non-protein nitrogen compounds due to the effect of phenolic compounds in GTE. Bahmani *et al.* (2011) reported 0.64 mg TMA-N per 100 g flesh of golden grey mullet, after 16 days of iced storage.

Lipid oxidation is a major quality problem, which leads to the development of off-flavour and off-odours in edible oils and fat-containing foods and is known as oxidative rancidity. The primary product of lipid oxidation is the fatty acid hydroperoxide, which is measured as peroxide value (PV). The peroxide value (PV) was 3.36 milli equivalents kg⁻¹ in the initial sample. Initial PV was found to be 0.8-1.2 for herring (Smith *et al.*, 1980), 1.21 for seerfish steaks (Mohan *et al.*, 2008) and 27.6 for fresh sardine (Cho *et al.*, 1989). PV increased progressively with the storage in all the samples but the control samples exhibited significantly higher values than the other samples (Fig. 2c). Antioxidants inhibit lipid oxidation by acting as hydrogen or electron donors and interfere with the radical chain reaction by forming non-radical compounds that will not propagate further radical reaction which may have accounted for the lower values in the case of GTEI stored samples. In this study, PV values were significantly affected ($p < 0.05$) by holding fish samples in different concentrations of GTEI.

Apart from these primary and secondary oxidation products, glycerides, glycolipids and phospholipids are hydrolysed by lipases to free fatty acids (FFA), which then undergo further oxidation to produce low molecular weight compounds, such as aldehydes and ketones. These compounds are also responsible for off-flavour; off-odour and taste of fish (Toyomizu *et al.*, 1981). The presence of FFA is due to the oxidation and hydrolysis of lipids and is undesirable since the fatty acids may be converted to odorous volatiles. The initial FFA content of pink perch flesh was low, which increased with storage period. Fig. 2d depicts the FFA values of fish in control sample (NI) and GTEI in threadfin bream during 20 days of chilled storage. During chilled storage, initially FFA formation was due to the result of endogenous enzyme activity. Later on, microbial activity overtook and FFA formation was mostly as a result of bacterial enzyme activity. A partial

inhibitory effect of the GTE on the endogenous enzyme activity results in lowering FFA formation in the fish muscle. Barassi *et al.* (1987) reported a direct relationship between FFA release and loss of fish freshness for hake (*Merluccius hubbsi*). Ozyurt *et al.* (2009) reported an increase in FFA content with storage period for red mullet (*Mullus barbatus*) and gold band goat fish (*Upeneus moluccensis*) during storage in ice. FFA may be involved in reactions with myofibrillar proteins and promote protein aggregation (Pacheco-Aguilar *et al.*, 2000).

Changes in pH value of fish stored in GTEI and NI is depicted in Fig. 2e. Nam *et al.* (2002) reported that pH value was a crucial factor for determination of meat quality. In the present study, a gradual increase in the pH value of fish was observed in control sample during 20 days of storage and reached a maximum of 6.77 at the end of sampling period (day 20). This increase in the pH may be due to the enzymatic degradation of the fish muscles and the production of volatile basic components (*e.g.*, ammonia and trimethylamine) by spoilage bacteria. From the same figure, it could also be noted that the pH of GTEI stored samples did not significantly increase ($p > 0.05$), however, slight increase was recorded for samples stored in 3 and 6% GTEI after 8 days of storage. It can be concluded that the lower pH of GTE-treated samples enhanced microbial inhibition and contributed to extending shelf life of fish samples by inhibiting the activity of endogenous proteases on total protein.

Thiobarbituric acid is used as an indicator of lipid oxidation. The presence of TBA-reactive substances is due to the second stage auto-oxidation during which peroxides are oxidised to aldehydes and ketones. Fig. 2f depicts TBA values of fish in control sample (NI) and GTEI in threadfin bream during 20 days of chilled storage. The level of tissue aldehydes, the secondary degradation products of lipid oxidation as a result of peroxide breakdown into smaller molecules, is often assessed in biological systems (Khayat and Schwall, 1983). TBA measurements showed that there was an increase in lipid oxidation in control sample and those stored in GTEI. The increase in TBA value during iced storage may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids. However, the increase in TBA values of samples stored in GTEI was lower than that of control samples throughout the period of storage as shown in Fig. 2f. This indicated that the formation of malonaldehydes was significantly retarded in samples stored in GTEI. Upto 8th day of storage, significant ($p < 0.05$) differences were observed in the TBARS content of the treatments at all sampling time except for samples stored in 6% GTEI. There were no significant differences among treatments 3 and 6% GTEI. However, a significant difference ($p < 0.05$)

was observed between samples stored in 6% GTEI at the end of storage time. On 8th day of storage, the lowest amount of malonaldehyde was observed in chilled fish stored in 6% GTEI. The minimum TBARS value for fish stored in higher concentration of GTEI, suggested positive correlation between antioxidant properties of GTE extract to prevent or retard the formation of malonaldehydes. In an investigation by Tang *et al.* (2001), tea catechins added at concentration greater than 300 mg kg⁻¹ were necessary to reduce lipid oxidation in mackerel patties as indicated by significant decrease ($p < 0.05$) in TBARS content. However, previous report by Van Het Hof *et al.* (1997) revealed that green tea extract applications has no adverse influence on lipid deterioration in terms of TBARS analysis. The data from the present study revealed that GTE can extend the shelf life of fish by inhibiting lipid oxidation in fish. This indicates that GTE has beneficial antibacterial and antioxidative activities, which demonstrated potential for their use as preservatives and antioxidants in seafood industry especially to enhance shelf life on storage.

Fig. 2g and h depict myofibrillar and sarcoplasmic protein values respectively of threadfin bream under chilled storage in control sample (NI) and GTEI. Myofibrillar and sarcoplasmic protein contents showed a gradual decrease till 20th day of storage in all samples. Here a negative correlation between FFA formation and protein extractability was observed. FFAs attach themselves hydrophobically or hydrophilically to the appropriate sites on the protein surface, creating a hydrophobic environment which results in decrease in protein solubility. According to Leake and Karel (1985), decrease in protein content has been attributed to oxidised lipid-protein interactions that lead to losses in labile amino acids. Comparison among the control and treated samples, clearly indicated effect of the treatments on protein digestibility. Protein content in both NI and GTEI stored fish showed an inverse correlation with microbial load and degree of oxidation (TBA value) during chilled frozen storage. The lower protein content on chilled storage may be due to increased microbial growth resulted from higher water activity (*aw*) and enzymatic autolysis. Moreover on chilled storage, the protein content decreased due to protein denaturation and proteolysis induced by enzymatic activities of psychrotrophic microbial growth.

Changes in the microbiological attributes

Total microbial count is an important criterion for quality evaluation in fresh and frozen seafood products. The changes in the microbial flora of control fish samples and those stored in GTEI at various time intervals are depicted in Fig. 3a. As shown, initial mesophilic count of fresh fish was 5.23 log cfu g⁻¹, then a reduction to 4.8 log cfu g⁻¹ and 5.18 log cfu g⁻¹ was observed in GTEI

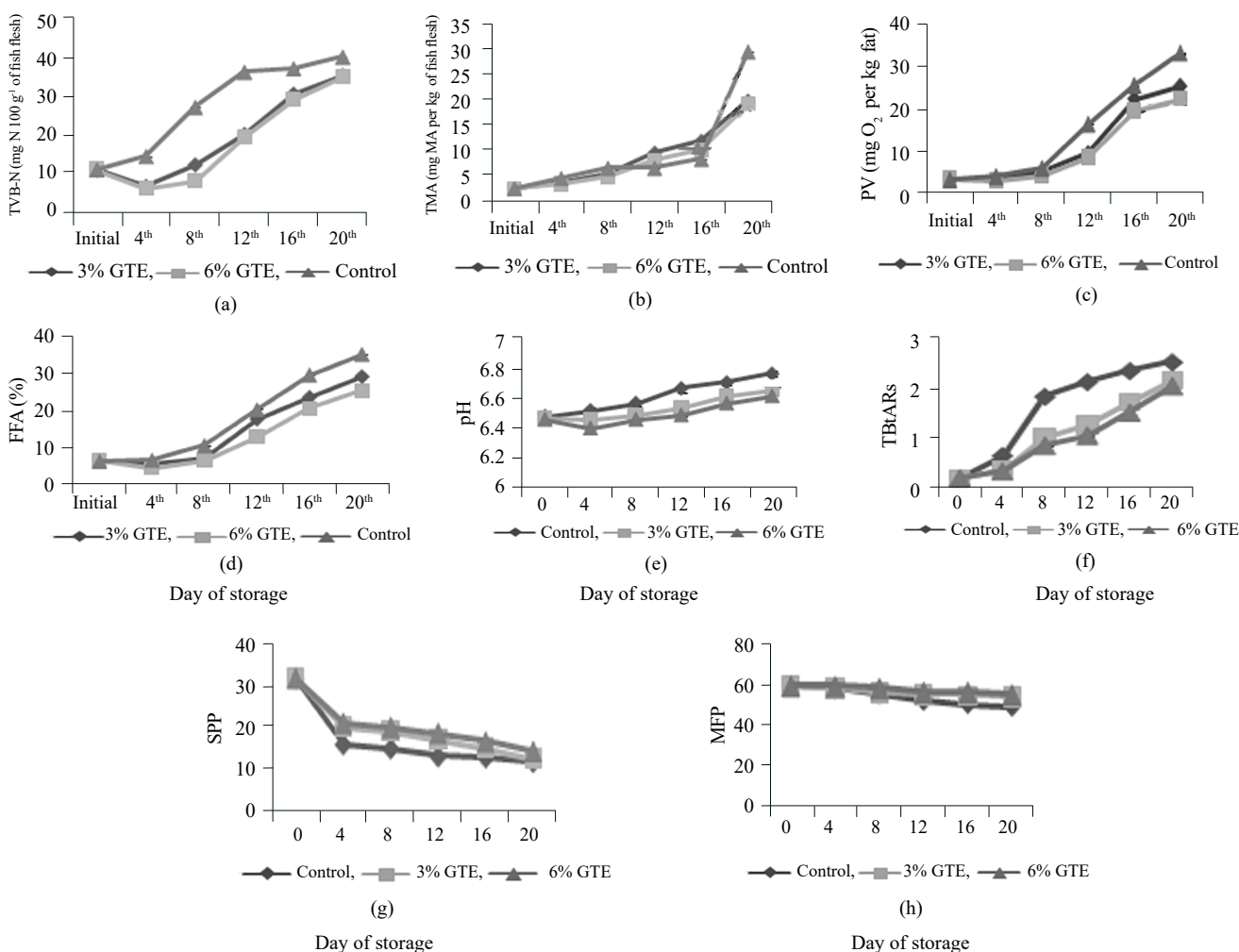


Fig. 2. Changes in chemical parameters during chilled storage of *N. japonicus*. (a) TVB-N; (b) TMAN; (c) PV; (d) FFA; (e) pH; (f) TBARS; (g) SPP and (h) myofibrillar protein

stored samples and control samples respectively. Then the mesophilic counts exhibited an increasing trend with time. The initial mesophilic \log_{10} values were similar to the values reported in cod (Dalgaard *et al.*, 1993). Lakshmanan *et al.* (2000) reported that the TPC levels above 10^7 cfu g⁻¹ resulted in visible spoilage of fish flesh. In the present study, mesophilic counts in the control samples, crossed the acceptable limit of $7 \log_{10}$ cfu g⁻¹ on 12th day of storage, whereas 3 and 6% GTEI stored samples did not cross the acceptance limit till the 20th day of storage. Shewan (1977) found that mesophilic Gram positives were dominant in fish from warmer waters such as the Indian Ocean. Bahmani *et al.* (2011) reported an initial 3 log total plate count (TPC), which reached 7 log on 16th day of storage.

As the time of storage increased, psychrophilic bacteria showed higher growth than the mesophilic

bacteria, dominating the flora at low temperatures. Psychrophilic count on the initial day was \log_{10} 4.26 cfu g⁻¹, which exhibited an increasing trend over time and reached upto \log_{10} 9.05 cfu g⁻¹ in control samples, \log_{10} 7.66 cfu g⁻¹ in 3% GTEI stored samples and \log_{10} 7.18 cfu g⁻¹ in 6% GTEI stored samples (Fig. 3b) on 20th day of chilled storage. Bahmani *et al.* (2011) reported an initial 2 log psychrotrophic count which reached 9 log on the 16th day of storage. Chytiri *et al.* (2004) reported a count of 7.0 log cfu cm⁻² for trout fillet after 10 days of storage.

With regard to *B. thermosphacta*, a bacterium more common in meat products, an initial value of \log_{10} 3.88 cfu g⁻¹ reached \log_{10} 8.24 cfu g⁻¹ in control samples on 20th day of storage (Fig. 3c). The conditions of fish in chilled storage selectively favour development of this organism due to its ability to grow at 1°C under oxygen depletion and in the presence of elevated carbon dioxide concentrations.

B. thermosphacta is one of the most abundant spoilage organisms of fresh and cured meats, fish and fish products, due to its tolerance to high-salt and low-pH conditions, its ability to grow at refrigeration temperatures and its production of organoleptically unpleasant compounds (Borch *et al.*, 1996).

H₂S producing bacteria have been reported as the specific spoilage bacteria in fish from temperate and tropical waters (Gram and Huss, 1996) and fresh fish stored aerobically (Koutsoumanis and Nychas, 1999). H₂S producers release several sulphur-containing compounds, including hydrogen sulphide, methyl mercaptan and dimethyl sulphide leading to very intense and unpleasant off-odours and are capable of reducing TMAO to TMA, thus generating strong ammonia and foul smell (Stammen *et al.*, 1990). Initial hydrogen sulphide producing bacterial count obtained in the present study was 2.75 log cfu g⁻¹. The initial number of H₂S producing bacteria was much lower than the initial total viable count. This is probably due to the fact that in newly processed fresh fish, specific spoilage organisms are usually present in very low concentrations and constitute only a minor part of the total microflora. But, on advancement of storage, H₂S producing bacterial count reached log₁₀ 5.08, log₁₀ 4.7866 and log₁₀ 7.093 in 3% GTEI, 6% GTEI stored samples and control samples respectively (Fig. 3d).

Pseudomonas constituted the major fraction of the bacterial flora in the present study. This was in agreement with Gram *et al.* (1990), Gram and Huss (1996) and Liston (1980). *Pseudomonas* species is the main spoilage bacteria in fish stored in ice mainly because of their short generation time, which produces a number of volatile aldehydes, ketones and esters of short-chain fatty acids and non-hydrogen sulphides, imparting fruity, rotten and sulphhydryl odours and flavours. Liston (1980) reported that *Pseudomonas* was able to utilise a variety of compounds including NPN in the fish muscle juice quickly and efficiently as one of the characteristics to ensure dominance. Some species of *Pseudomonas* were responsible for the objectionable off-odours detected during the last days of storage. According to Liston (1980), this is due to the breakdown of S-containing compounds *e.g.* cysteine and methionine by *P. fluorescens*, *P. perolens*, *P. putida* and *P. putrefaciens*. However, in the present study, the initial count of *Pseudomonas* was found to be log₁₀ 4.46. Earlier studies reported an Initial *Pseudomonas* count of 1.3 log cfu g⁻¹ in seer fish steaks (Mohan *et al.*, 2008) and for trout (Chytiri *et al.*, 2004), which increased with storage, period in both samples. On the 4th day of storage, *Pseudomonas* count decreased in all samples from the initial value which

then showed an increasing trend over time and reached log₁₀ 6.76, log₁₀ 6.55 and log₁₀ 8.43 in 3% GTEI, 6% GTEI stored samples and control samples respectively, on the 20th day of storage (Fig. 3e). *Pseudomonas* count in this study was significantly lower than that of hydrogen sulphide producers, because when grown in co-culture on fish siderophore producing *Pseudomonas* inhibited *Shewanella putrefaciens* (Gram and Dalgaard, 2002). Significant lower counts (p<0.01) were exhibited by GTEI stored samples than the control samples which affirmed the preservative nature of green tea extract.

The presence of *Enterobacteriaceae* in fish and their spoilage potential are important when fish are obtained from polluted water or if there is a delay in chilling after catch (Bahmani *et al.*, 2011). In the present study, the initial *Enterobacteriaceae* count was log₁₀ 3.11, which was comparable to that reported for many tropical and temperate fishes (Chytiri *et al.*, 2004). On the 4th day of chilled storage, the total *Enterobacteriaceae* count in GTEI stored samples reduced from that of the initial value, while the control samples exhibited an increase in counts from the initial values. The reduction in microbial counts may be due to the inhibitory activity of green tea extracts. The *Enterobacteriaceae* population was lower than that obtained for other bacterial groups in this study, which was in agreement with the results reported for sea bream (Tejada and Huidobro, 2002). Total *Enterobacteriaceae* count for control and treated samples reached 5.53, 4.45 and 4.27 log₁₀ cfu g⁻¹ for control sample and samples stored on GTEI at 3 and 6% after 20 days of storage, respectively (Fig. 3f). Although this group can grow at low temperature, their abundance decreased during ice storage, possibly because of their slow growth rate than that of other Gram negative psychrotrophic spoilers and due to their inability to compete with the other Gram negative psychrotrophic spoilers.

It can be concluded that the use of GTE incorporated ice can extend the shelf life of fish samples by delaying the spoilage activity. The results of the treatment also indicated that 6% GTEI storage exhibited more activity in inhibiting spoilage bacteria and extending the iced storage life of fish samples to 16 days compared to 8 days for control sample. Hanafy *et al.* (2011) reported that 4% GTEI storage extended shelf life of *Oreochromis niloticus* samples to 14 days compared to 6 days for control fishes.

Principal component analysis (PCA) was performed on the mean values of 17 variables of microbiological and chemical parameters of the samples of pink perch. The first two principal components extracted, explained 85.93% of the variation in the data. The first 3 principal components explained 93.02% of the variance in the

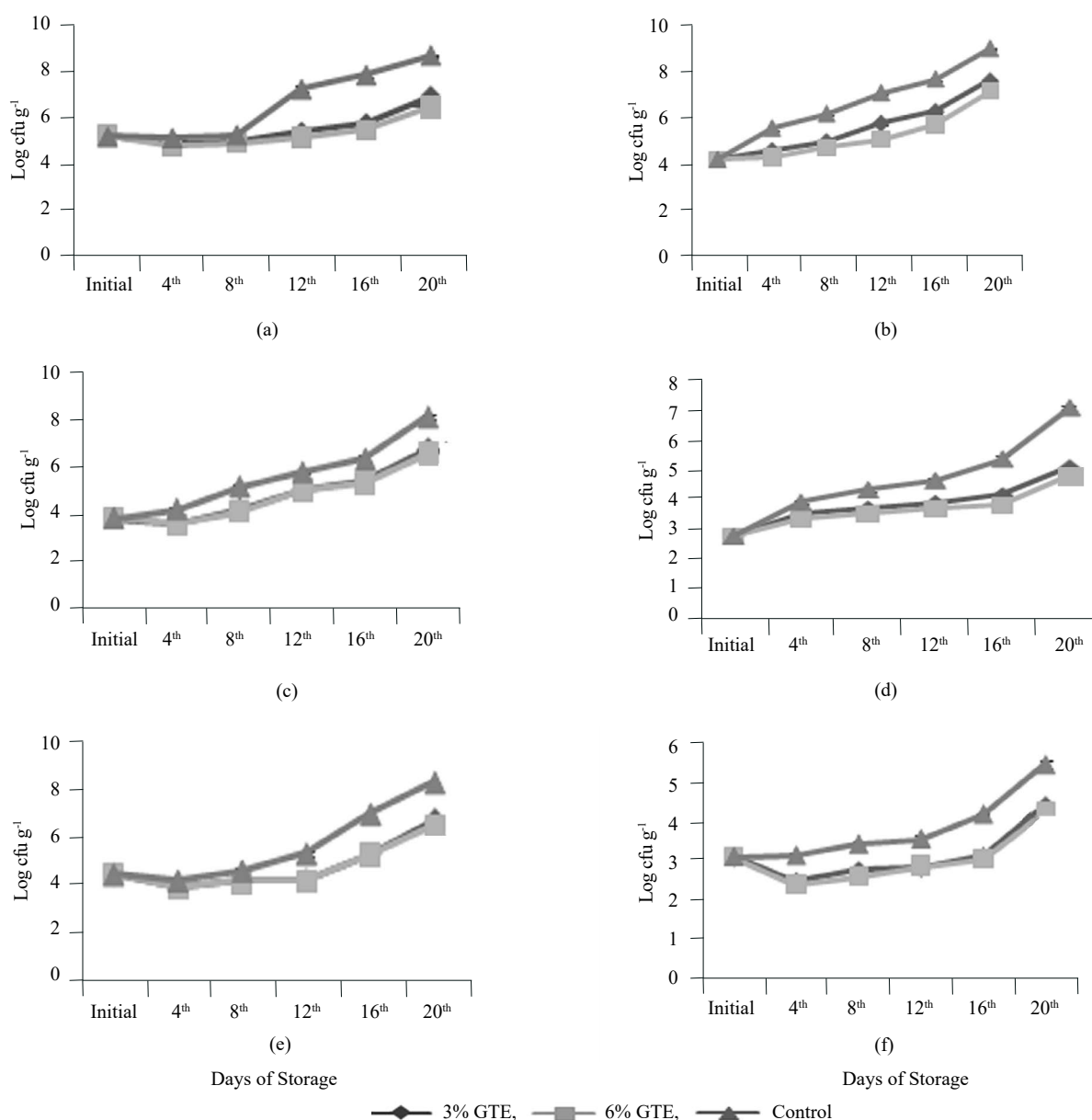


Fig. 3. Changes in the microbiological attributes during chilled storage of *N. japonicus*. (a) Mesophilic plate count; (b) Psychrophilic counts; (c) *B. thermosphacta* (d) Hydrogen Sulphide producers (e) *Pseudomonas* counts (f) Total Enterobacteriaceae count

data. The correlation loadings of the PCs showed high correlations of all the parameters studied. PCA plot clearly separated the investigated samples according to storage time and conditions, thus indicating the importance of the impact of GTEI stored samples. The storage period influenced the distribution of the samples along the PC 1 (Fig. 4a). From the PC score plots it is very clear that the control samples have exhibited the microbial, chemical and sensory attributes distinct from the treated samples. Also the samples treated with GTE which were 16 to 20 days old are depicted in 3rd quadrant of the PC score

graphs (Fig. 4b and c), whereas the samples which are 4-8 days old were plotted near the X-axis (Fig. 4a and c). Fig. 5 a-c present the biplots pertaining to the first three dimensions extracted viz., PC1 vs PC2, PC1 vs PC3 and PC2 vs PC3 respectively in respect of 6% GTEI treated samples. Around 96.6% of the variation in the data could be explained by these principal components. Similar trend was seen for the variables, sarcoplasmic proteins (SPP) and myofibrillar proteins (MFP) as in case of 3% GTEI as they were depicted away from rest of the variables (Fig. 5a) at a wider angle.

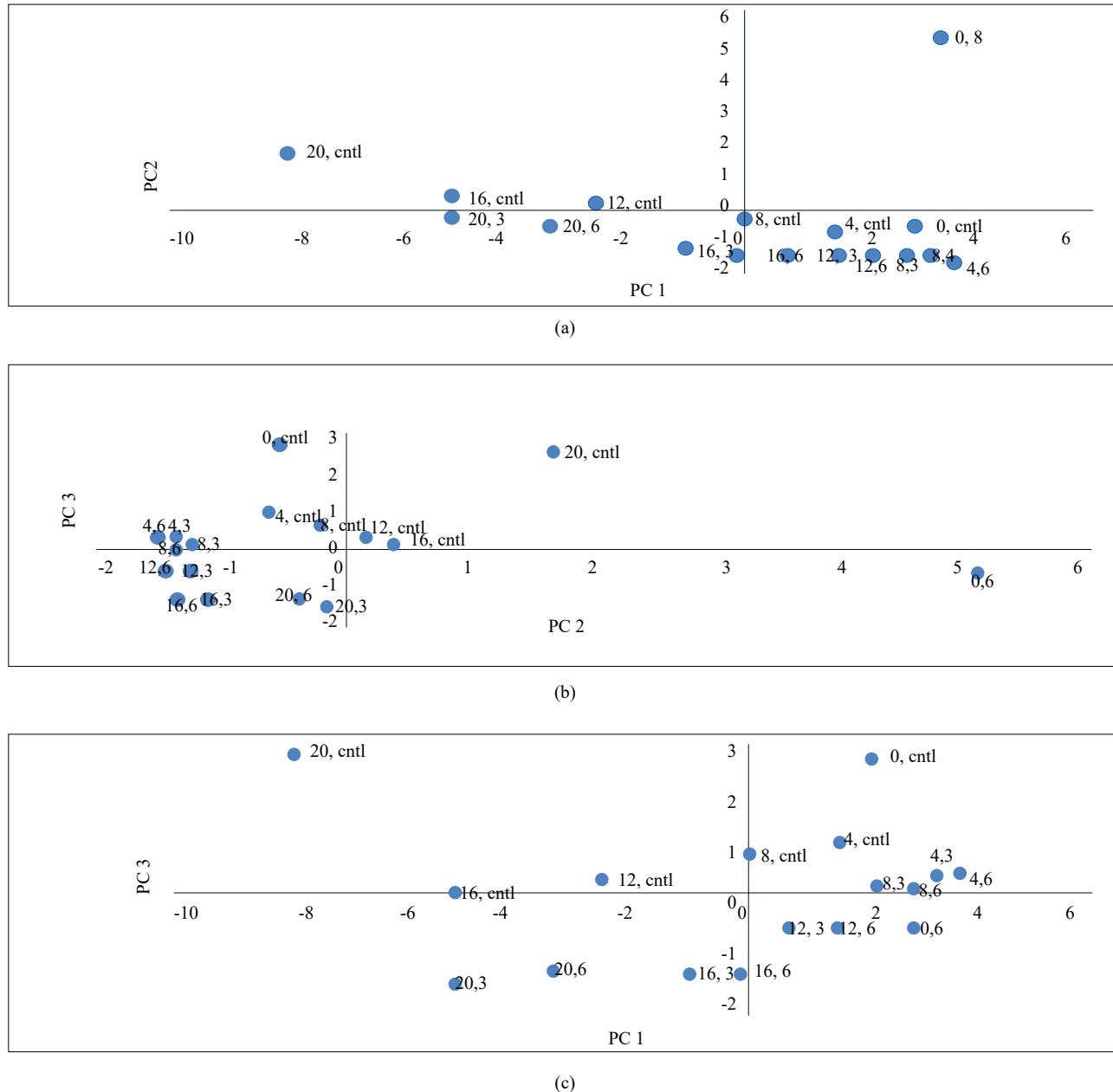


Fig. 4. Score plots of first three principal components on spoilage indicators during chilled storage of *N. japonicus*. (a) Principal components 1 vs 2; (b) Principal components 2 vs 3 and (c) Principal components 1 vs 3

Worldwide, people are moving towards 'green consumerism'. 'Going back to nature' will minimise the risks associated with synthetic preservatives. The use of frozen green tea extract is a promising food safety approach in the shelf life extension of fish. The present study has clearly shown that preservation of fish using GTEI resulted in a longer shelf life than normal ice. Extracts of leaves from the tea plant *Camellia sinensis* contain

polyphenolic components, which can inhibit the growth of a wide range of Gram positive and Gram negative bacteria with moderate potency. In this study 6% GTEI exhibited more activity than 3% GTEI, but the results obtained were comparable. So from an economic point of view, 3% GTEI can be adopted for preservation of fish. Moreover, as fish has good sensory quality, a proper preservation technique suggested in this study is highly relevant.

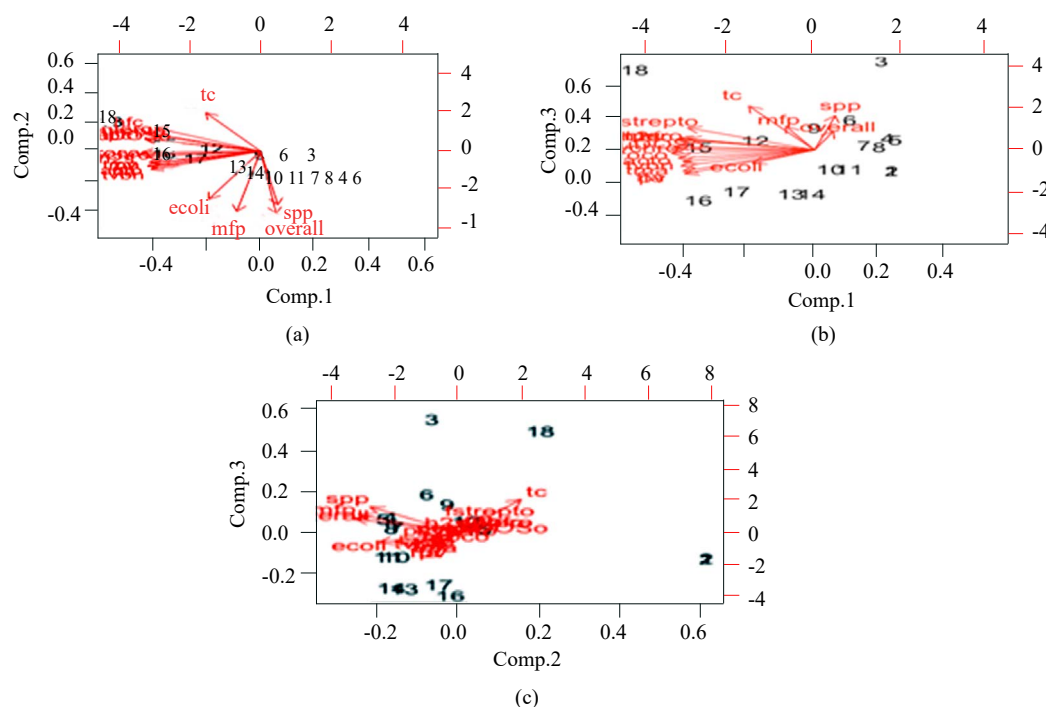


Fig. 5. Biplots of PCA on spoilage indicators during chilled storage of *N. japonicus*. (a) PC1 vs PC2; (b) PC1 vs PC3 and (c) PC2 vs PC3

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