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EFFECT OF DIFFERENT CONCENTRATION OF NATURAL ANTIOXIDANT ON THE QUALITY OF MAHI MAHI (*CORYPHAENA HIPPURUS* L.) STORED UNDER REFRIGERATED CONDITION

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ABSTRACT

The present study was carried out to evaluate the effect of moringa leaf extract (MLE) and different packaging method on the shelf life of the fish during refrigeration storage. Freshly caught fish was processed and treated with 2 different concentrations of moringa leaf extract (MLE) for the 5 minutes under refrigeration storage (4±1°C). Moringa leaf extract helps to slowdown the autoxidation and reduce the microbial activity. During the storage period TPC count, TMA-N and TVB-N were increased but the samples treated with the moringa leaf extract shows rate of spoilage less compared to the control. Samples treated with (1% and 3%) MLE were acceptable up to 15th day. Moringa act as antioxidant and antimicrobial help to extend the shelflife of the fish and enhance the product quality during the refrigeration storage.
***Keywords*:** Moringa leaf extract, refrigeration storage, *Coryphaena hippurus* (mahi mahi).

Introduction

Fish is one of the most important sources of animal protein worldwide, especially in tropical and coastal regions. It is also among the most perishable food products due to its high moisture content (approximately 75 to 80%), which creates a favorable environment for the growth of spoilage microorganisms and the activity of enzymes such as lipase and lipoxygenase. These enzymes contribute to lipid oxidation, resulting in off flavors, rancidity and the degradation of both nutritional and sensory quality (Zambou *et al*., 2024).

Traditional preservation techniques such as salting, sun-drying, and smoking are widely used in many developing countries. While these methods can extend shelf life, they also have the potential to accelerate the oxidation of fatty acids, which compromises food safety and nutritional integrity (Falowo *et al*., 2017). To prevent such degradation, synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been commonly used in the food industry. Despite their effectiveness, there is growing concern about the health risks associated with these chemical preservatives, including their possible carcinogenic effects (Abalaka *et al*., 2010).

Natural antioxidants derived from plants are increasingly favored due to their safety, multi functionality and bioactivity. These compounds, including flavonoids, phenolic acids, and carotenoids, can donate hydrogen atoms and neutralize free radicals, thereby inhibiting lipid peroxidation and slowing spoilage (Falowo *et al*., 2017). One such plant gaining significant attention is *Moringa oleifera Lam*. widely known as the drumstick tree, horseradish tree. *Moringa oleifera* belongs to the family *Moringaceae*, a single-genus family with 13 recognized species, and is native to the sub-Himalayan regions of north western India. It has since become naturalized in tropical and subtropical areas of Africa, Asia, the Caribbean, and South America (Fahey, 2005; Mahmood *et al*., 2010).

Moringa oleifera is often referred to as the "miracle tree" due to its diverse applications in nutrition and medicine. Almost all parts of the plant leaves, seeds, roots, flowers and pods are edible and contain bioactive compounds that contribute to its

therapeutic and nutritional value. The leaves, in particular, are rich in essential nutrients such as vitamins A and C, calcium, potassium, iron and protein. They also contain powerful antioxidants and antimicrobial agents including flavonoids, phenolic compounds, and carotenoids (Mezhoudi *et al*., 2022). Recently, there has been increasing interest in using plant extracts as natural antioxidants and preservatives in the food industry. Their appeal lies in their potential to extend shelf life and enhance consumer health. As natural antioxidants, plant extracts can donate hydrogen ions, thereby inhibiting the formation of free radicals and interrupting the process of autoxidation in muscle foods. (Falowo *et al*., 2017).

Material and Methods

Fresh mahi mahi (*Coryphaena hippurus*) measuring 74.5±1.87cm an average length and 1.08±0.04 kg in average weight were caught off the west coast of gujrat using a trawl net. It was transported in an insulated box in iced condition (0-2°C) from the Veraval fish landing center to the Fish Processing Laboratory at the College of Fisheries Science, Kamdhenu University, Veraval. The fish was washed, cleaned and dressed using a knife to remove the head, fins, scales, gills and gut, followed by washing with potable fresh water. Pure and certified quality moringa leaf powder used in the present study was procured from My Herb Nutrasun Lifecare LLP.

Preparation of The Moringa Leaf Extract & treatment procedure

1% Extract preparation: The aqueous Moringa extract will be by mixing 1 gram of Moringa leaf powder with 99 ml of deionized water heated to between 70°C and 90°C for 20 minutes. The extract underwent purification using strainer paper, and the resulting filtered solution was then refrigerated at 6°C for subsequent use. 3% Extract preparation: The aqueous Moringa extract will be made by mixing 3 grams of Moringa leaf powder with 97 ml of deionized water heated to between 70°C and 90°C for 20 minutes. The extract underwent purification using strainer paper, and the resulting filtered solution was then refrigerated at 6°C for subsequent use (Abel *et al*. 2021). The fish were then manually gutted, removing the head, fins, gills and entrails to eliminate blood and other impurities, the gutted fish were washed with chilled running water. After processing, the mahi mahi weighed and were subsequently divided into 3 equal lots each.

In treatment T1 (control), the fish sample will be pack in LDPE pouch without any treatment. In treatments T2 and T3 samples will treat with 1% (w/v) and 3% (w/v) moringa leaf extract and left for 5 minutes under refrigeration conditions (Chakraborty *et al*. 2017). The treated mahi mahi chunk were packed according to weight in Low density polyethylene plastic bag (LDPE) (200 gauge). All the packed samples were stored under refrigerated conditions at 0-40C. The physical, biochemical, microbial, and sensory analysis will be done every 5-day interval during storage.

Biochemical Analysis

Determination of Trimethylamine Nitrogen (TMA-N)

Preparation of trichloro acetic acid (TCA) extract: A 10 g fish sample was extracted using 30 ml of 20% trichloroacetic acid (TCA) by grinding it in a mortar and pestle. The mixture was then filtered quantitatively through Whatman filter paper No. 1 and diluted to 50 ml with distilled water before being stored in refrigeration. The TCA extract was utilized to measure trimethylamine and total volatile base nitrogen.

Trimethylamine nitrogen (TMA-N) was determined using the micro diffusion method outlined by Beatty and Gibbons (1937). In the inner chamber of the diffusion unit, 1 ml of standard N/100 sulfuric acid was placed. In the outer chamber, 1 ml of the TCA extract was added, followed by 0.5 ml of neutralized formaldehyde and 1 ml of saturated potassium carbonate. The unit was then sealed with a glass lid and left undisturbed overnight. The solution in the inner chamber was titrated with standard N/100 sodium hydroxide using Tashiro's indicator. A blank test was also conducted without a sample. TMA-N was calculated and expressed in mg/100 g of the sample.

$$\text{TMA-N (mg/100gm)} = \frac{(Vs\text{-}Vb)\times 0.14 \times \text{Volume of extract}}{\text{Vol. of sample taken} \times \text{Weight of sample}} \times 100$$

Where,

Vs = Titre value of 0.01 N NaOH for the sample (ml),

Vb = Titre value of 0.01 N NaOH for the blank (ml).

Determination of Total Volatile Base Nitrogen (TVB-N)

The preparation of the TCA extract was identical to that used for TMA-N. Total volatile base nitrogen (TVB-N) in the sample was measured using the micro diffusion method described by Beatty and Gibbons (1937). In the inner chamber of the diffusion unit, 1 ml of standard N/100 sulfuric acid was placed. In the outer chamber, 1 ml of the TCA extract was added, along with 1 ml of saturated potassium carbonate. The unit was then sealed with a glass lid and left undisturbed overnight. The unreacted acid in the inner chamber was quantified by titrating with standard N/100 sodium hydroxide using Tashiro's indicator. A blank test was also performed without a sample. TVB-N was calculated and expressed in mg/100 g of the samples.

$$\text{TVB-N (mg/100gm)} = \frac{(Vs\text{-}Vb)\times 0.14 \times \text{Volume of extract}}{\text{Vol. of sample taken} \times \text{Weight of sample}} \times 100$$

Where,
Vs = Titre value of 0.01 N NaOH for the sample (ml)
Vb = Titre value of 0.01 N NaOH for the blank (ml)

Microbiological Analysis

Preparation of Sample

A 10 g fish sample was placed in a sterile sample dish and then transferred to a sterile plastic bag. The sample was homogenized with 90 ml of sterile phosphate buffer or normal saline solution. The homogenate was then blended in a stomacher and diluted tenfold to create a 10^{-1} dilution. For additional dilutions, 1 ml from the 10^{-1} dilution was combined with 9 ml of the appropriate medium for plating, resulting in 10^{-2}, 10^{-3}, and 10^{-4} dilutions.

Total Plate Count

The microbiological characteristics of the fish sample were assessed following the standard method recommended by (Mailoa *et al*. 2017; AOAC. 2006). Plate Count Agar (PCA) was utilized to enumerate the total plate count. For each dilution, 1 ml was transferred into separate petri dishes in duplicate. Sterile Plate Count Agar (PCA) (18-20 ml) was added to the petri dishes, mixed well, and allowed to solidify. The petri dishes were then incubated at 37 ± 1 °C for 48 hours. After the incubation period, the colonies were counted.

TPC (CFU/g)=Average No. of colonies x Dilution factor

Sensory Characteristics

Sensory characteristics of the fish samples were assessed using a 9-point Hedonic scale, as outlined by Peryan & Pilgrim (1957). Selected samples were analyzed, and five panelists evaluated the ranks for texture, color, appearance, odor, and overall acceptability using the same scale. Scores for each attribute were recorded, with a score of 6 or above indicating good quality, while a score of 5 marked the limit of acceptability. The evaluation form used for the sensory assessment is provided in Appendix A.

Statistical Analysis

Statistical analysis was based on triplicate analysis for each sample at each specific storage time. ANOVA (Analysis of variance) statistical technique was used to find out the significant difference (Mean ± S.D) in samples between the treatments as per the standard statistical methods. (Snedecor & Cochran, 2014).

Result and Discussion

The proximate composition of fresh mahi mahi, as shown in Table (1), aligns closely with the findings reported by Assana *et al*. (2020). The fish were captured in September 2024. The raw material had a moisture content of 76.32±0.21, a crude protein content level of 18.17±0.05, a crude fat level of 2.15±0.03 and an ash content of 1.14±0.03, with all values (Mean ± SD). The quality of fish is influenced by several chemical parameters, including pH, trimethylamine nitrogen (TMA-N), and total volatile basic nitrogen (TVB-N). In the present study, all measured parameters for the selected fish species, *Coryphaena hippurus*, were found to be within acceptable limits, as summarized in Table 1. The pH of the fish was recorded at 6.28 ± 0.04 (mean ± standard deviation), which falls within the normal range for fresh fish, suggesting minimal post-mortem degradation. Trimethylamine nitrogen (TMA-N) is a widely recognized indicator of seafood freshness. It is formed through the microbial and enzymatic reduction of trimethylamine oxide (TMAO), and its accumulation is associated with spoilage and a decline in sensory quality. According to (FSSAI. 2017). TMA-N levels exceeding 10-12 mg N/100g are indicative of spoilage and are considered unsuitable for consumption. In this study, the TMA-N content was measured at 1.23 ± 0.02 mg N/100g, confirming the freshness of the raw material. Total volatile basic nitrogen (TVB-N) is another key marker of fish spoilage, primarily associated with the microbial degradation of proteins and the subsequent formation of volatile compounds such as ammonia and primary amines. TVB-N values exceeding 33-35 mg N/100g are typically indicative of advanced spoilage (FSSAI. 2017). The TVB-N content of the fish used in this study was determined to be 9.58 ± 0.04 mg N/100g, further affirming that the fish was of high quality at the time of analysis.

Table 1 : Raw material characteristics, Values are in (mean±SD).

A	**Physical Characteristics**		
	1	Total length (cm)	74.50±1.87
	2	Weight of fish (kg)	2.27±0.65
B	**Proximate Composition**		
	1	Moisture (%)	76.32±0.21
	2	Total Protein (%)	18.17±0.05
	3	Total Fat (%)	2.15±0.03
	4	Total Ash (%)	1.14±0.03
C	**Chemical Characteristics**		
	1	pH	6.28±0.04
	2	TMA-N (mg/100g)	1.23±0.02
	3	TVB-N (mg/100g)	9.58±0.04
C	**Microbiological Characteristics**		
	1.	TPC (logCFU/gm)	2.26 ± 0.05

Table 2 : Change in Trimethylamine Nitrogen (TMA-N) during refrigeration storage

Storage days	T1	T2	T3
0	2.86±0.11^{a}	1.96±0.14^{ab}	1.23±0.10^{b}
5	5.16±0.13^{a}	4.92±0.12^{a}	3.94±0.13^{b}
10	7.89±0.13^{a}	7.07±0.14^{ab}	6.66±0.11^{b}
15	12.24±0.10^{a}	9.7±0.12^{b}	9.26±0.11^{b}
20	16.29±0.12^{a}	14.02±0.11^{b}	13.06±0.12^{b}

*Values are in (mean±SD), n=3. ^{a,b,c} Values with the different subscripts in a column for each parameter differ significantly ($p<0.05$).

TMAO (trimethylamine oxide) is found in mostly all the naturally available fish. TMA is one of the most important indicators of spoilage in the fish. Due to microbial and bacterial degradation the fish loses its freshness. Value of the TMA-N exceeding 10mg/100gm is considered as a spoiled (FSSAI. 2017). Table 2 indicates how the TMA-N content of each sample gradually increased with extended the storage time. In the present research, it was revealed that the control T1 was acceptable up to 10 days, on 15th day T0(c) was not acceptable for the consumption, while, treated samples were accepted up to 15 days and loose the freshness on 20th day. Incresing trend was observed in all the treatments, with values T1(c) (2.86±0.11 to 16.29±0.12), T2 (1.96±0.14 to 14.02±0.11) and T3 (1.23±0.10 to 13.06±0.12), highest rate of the increasing was observed in control compared to T2 and T3. TMA-N is widely recognized as a key indicator of fish freshness, with its level increasing over time in both control and treated samples, but the rate of increase in the value was different in treated and control groups. Moringa has antioxidant properties that helps to reduce the rate of the spoilage. Chakraborty *et al*. (2017) found the similar result that samples which treated with moringa were accepted for a longer time as compared to control groups, which indicates that moringa helps to reduce the rate of spoilage.

Table 3 : Change in Total Volatile Basic Nitrogen (TVB-N) during refrigeration storage

Storage days	T1	T2	T3
0	9.58±0.04^{a}	8.56±0.13^{b}	8.13±0.10^{b}
5	18.22±0.02^{a}	17.65±0.03^{a}	16.49±0.04^{b}
10	28.28±0.05^{a}	23.24±0.09^{b}	21.89±0.04±^{c}
15	37.25±0.08^{a}	28.23±0.09^{b}	26.85±0.11^{c}
20	45.42±0.05^{a}	37.96±0.16^{b}	36.95±0.07^{c}

*Values are in (mean±SD), n=3. ^{a,b,c} Values with the different subscripts in a column for each parameter differ significantly ($p<0.05$).

TVB-N is also one of the most important spoilage indicators in fish and fishery products. Due to microbial and enzymatic activity, fish lose their freshness. A TVB-N value above 33-35 mg/100 gm is considered spoiled (FSSAI. 2017). The value in Table 3 shows the increasing rate of the TVB-N in all the treatments. As compared to the control, the treated groups showing the good result, the rate of the increasing of the TVB-N value is slow; in between the treated groups. Results show that the moringa leaf extract helps to extend the shelf life of the fish. Refrigeration storage helps to slow down the rate of spoilage and helps to reduce autoxidation. Initially all the samples were fresh and acceptable, as the storage period increases spoilage also increases. Up to 10TH day control was acceptable and treated groups were acceptable up to 15th day. Jadhav & Anal (2018) found the similar result that MLP treated samples maintain lower TVB-N values as compared to untreated samples, showing that moringa helps to reduce the growth of bacteria and reduces the spoilage of fish. According to mezhoudi *et al*. (2022), due to microbial and enzymatic activity, the TVB-N value increases over the storage period. The antimicrobial property of the coating ingredient delays the spoilage of the fish and extends the shelf life of the fish.

Table 4 : Change in Total Plate Count (TPC) during refrigeration storage

Storage days	T1	T2	T3
0	2.07±0.11[a]	1.93±0.12[a]	1.41±0.15[a]
5	3.55±0.11[a]	2.61±0.14[b]	2.27±0.10[b]
10	4.44±0.12[a]	3.68±0.10[ab]	3.23±0.07[b]
15	5.13±0.11[a]	4.59±0.12[ab]	4.05±0.08[b]
20	6.63±0.07[a]	5.91±0.13[ab]	5.35±0.11[b]

*Values are in (mean±SD), n=3. [a,b,c] Values with the different subscripts in a column for each parameter differ significantly ($p<0.05$).

Bacterial load was Measured in colony-forming units per gram (CFU/gm). TPC indicates the bacterial load in the sample. The acceptable limit for the TPC in chilled fish is 5 x 10^5CFU/gm, above the limit, it is considered spoiled and unacceptable for human consumption because that product is hazardous (FSSAI. 2017). The change in the TPC value during the refrigeration storage is shown in Table 4. Significant difference was observed in TPC value during the storage period. Initially TPC count for the control was 2.07±0.11, after the 20th day TPC value for the control group was 6.63±0.07. control group was accepted up to 10 days. Treated samples were accepted up to 15th day, from day 0 to day 20th TPC count was increases in both the treatments but the rate was different, TPC count for the T2 from 0 to 20th day was (1.93±0.12 to 5.91±0.13), for the T3 (1.41±0.15 to 5.35±0.11). Result indicate that the antimicrobial effect of the moringa inhibits the growth of the bacteria. Greeshma *et al*. (2019) found the similar result that samples treated with moringa extract have shown good result compared to untreated samples. Due to the antimicrobial effect of the moringa, fish can be stored for more days compared to untreated samples.

Change in sensory characteristics during refrigeration storage

Changes in sensory score was observed in all the treatments. Almost similar sensory score was observed in starting of the experiment. During the refrigeration storage condition score value was decreasing in all the treatments in all the sensory characteristics. The sample treated with the 3% moringa extract got the highest appearance score. Mohan *et al*. (2012) found that samples treated with chitosan got higher sensory value compared to control samples. I colour and odour similarly decresig trend was observed during the refrigeration storage. Similar result was observed by Greeshma *et al*. (2019) found a decrease in the colour and odour score of the silver carp fillets treated with the moringa extract. Similarly, Mezhoudi *et al*. (2022) highest score for the colour and odour in fish fillets treated with a combination of moringa and gelatin as compared to the control and gelatin alone. Microbial and enzymatically activity is also one of the factors for the loss of texture value, but the antimicrobial effect of the moringa extract helps to maintain the textural profile. According to Putri *et al*. 2023, samples treated with the moringa extract have shown good texture scores compared to untreated sample. According to Greeshma *et al*. (2019) samples treated with moringa extract gave the highest score of overall acceptability compared to the control. Moringa is effective for the preservation of the fish. Figure 1 indicates appearance score, figure 2 indicates colour score, figure 3 indicates odour score, figure 4 indicates texture score

and figure 5 indicates overall acceptability score. Samples treated with the 3% moringa leaf extract gives better score in sensory characteristics compares to control.

Conclusion

The present study suggested that moringa leaf extract helped to preserve the quality of the fish. Natural antioxidant helped to replace the synthetic antioxidants. Moringa leaf extract had significant impact on the biochemical, microbiological and sensory attributes of fresh mahi mahi. an antibacterial, antimicrobial and antioxidant properties present in moringa leaf extract maintained the fish quality favorable for consumption-wise and fetching good market price by reducing the fish wastage. This research concluded that up to 15 days, 3% Moringa leaf extract under refrigeration storage had significantly improved the quality, freshness, shelf life and made more profitable as compared to other treatments.

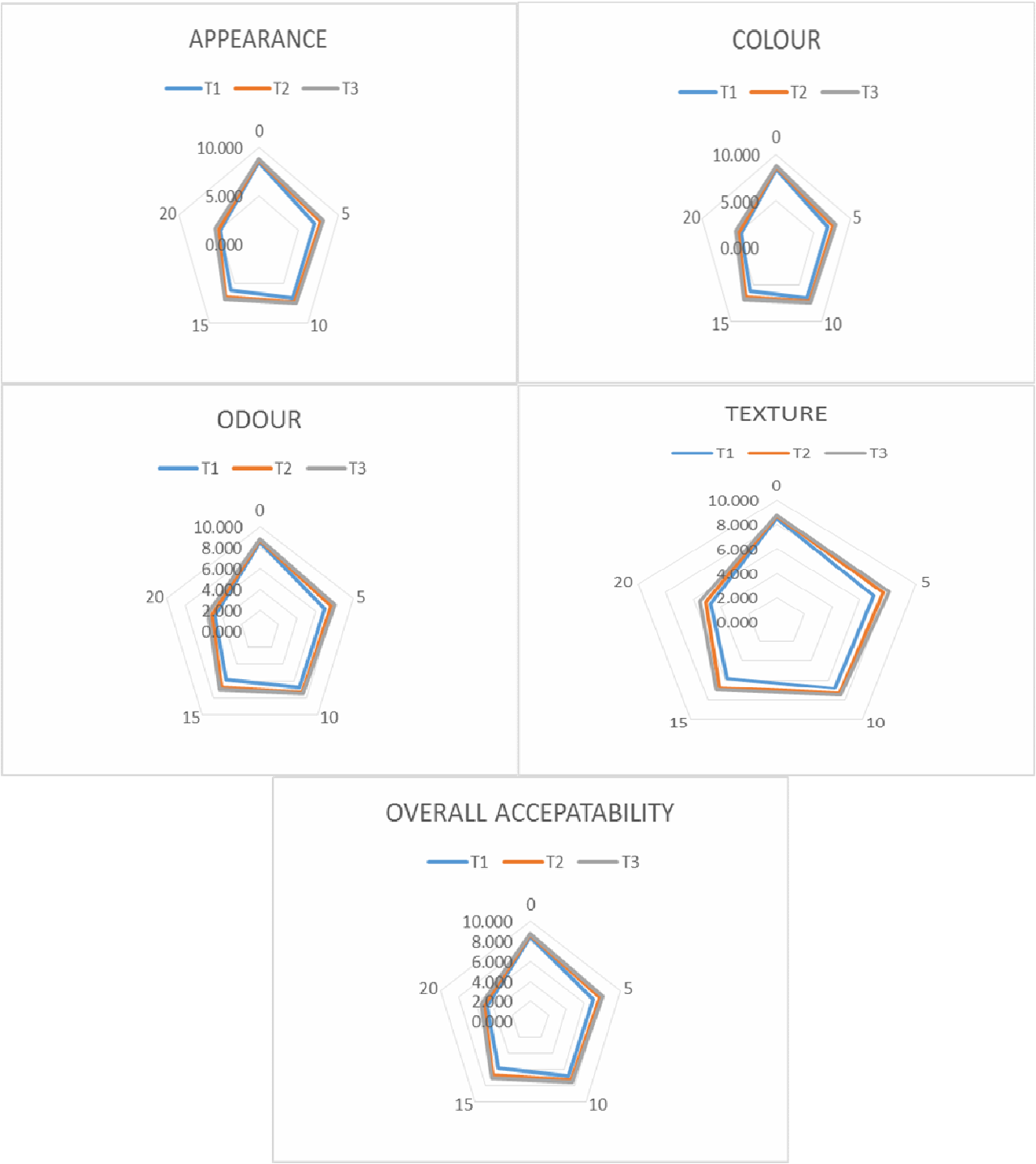

Fig. 1: Change in different sensory characteristics during refrigeration

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