Chapter 7

To study the effect of extracted essential oil from pomelo peel on storage stability using developed sensor

7.1. Introduction

The *Citrus grandis L.* Osbeck fruit widely recognized as Pomelo is a native plant of Southern Asia, which is locally available in China, Japan, Vietnam, Malaysia, India, and Thailand (Gonz´alez-Mas et al., 2011; Tocmo et al., 2020). It is believed to be the primary origin of grapefruit and a member of the Rutaceae family. Pomelo, along with orange, mandarin, lemon, and grapefruit, is currently one of the most frequently grown and consumed citrus fruits in Southeast Asia and other parts of the world (Cheong et al., 2012). The fruit of the pomelo is commonly consumed fresh or in the form of juice while the peels, seeds, and other parts of the plant are generally discarded as waste. The plant's various parts, including the leaf, pulp, and peel, have been used in traditional medicine for centuries because they have been shown to have therapeutic potential and are safe for human consumption (Mostafa et al., 2018; Tocmo et al., 2020). The leaves of the *Citrus grandis* plant and its oil are used in folk medicine to cure skin conditions, headaches, and stomach pain, respectively. *Citrus grandis* fruits are not just utilized for consumption. Traditional remedies frequently treat cough, edema, epilepsy, and other ailments with fruit peels in addition to using them for cosmetic purposes (Ruberto et al., 2000). The citrus species are the major source of essential oil, and the oils derived from citrus peel have a strong desirable aroma with a refreshing effect. There has been increasing in recent years as a result the commercial importance is growing. Essential oils are naturally derived metabolites including terpenes, sesquiterpenes, terpenoids, and aromatic compounds with different groups of aliphatic hydrocarbons, aldehydes, acids, alcohols, phenols esters, oxides, lactones, and ethers (Chen et al., 2016). Essential oils containing such compounds are well known to have antimicrobial and antioxidant properties and serve as an alternative to synthetic additives with the moving interest in natural products (Bordoloi et al., 1999; Gonz´alez-Mas et al., 2011). Studies have shown that the active components that exist in citrus essential oils, such as limonene, pinene, and terpinolene, exhibit a wide range of antimicrobials, antifungal, anti-inflammatory, and antioxidant activity (Huang et al., 2018; Van Haute et al., 2016). Besides, citrus essential oil has been classified as generally recognized as safe (GRAS) due to its great nutraceuticals and economic importance (Huang et al., 2018). Several studies have shown that essential oils have the potential to extend the shelf life and maintain the quality of fish and meat products (Bakkali et al., 2008; Choi et al., 2010; Rezaei et al., 2018; Dehghani et al., 2018).

Fish is widely recognised for its superior quality protein, inherent supply of polyunsaturated fatty acids (namely, docosahexaenoic and eicosapentaenoic acid), vitamin B_2 and D, and an abundance of minerals like calcium, salt, potassium, and iron. (Hasan et al., 2015, Rezaei et al., 2018, Tilami et al., 2018). However, because of their high moisture content, low acidity, reactive endogenous enzymes, and enhanced nutritional value, fresh fish are particularly vulnerable to microbial deterioration and biological alterations (Cai et al., 2015, Yu et al., 2020). Rigorous mortis, autolysis, bacterial invasion, and putrefaction are the steps in the spoiling process that lead to the development of volatile amines and an unpleasant off-odor caused by a rise in the population of microorganisms (Hao et al., 2021). Because of the low temperature, fish kept in cold storage may retain some of its flavour, texture, and freshness. However, the fast development of psychrophilic microbes degrades fish quality, resulting in an off-odor and shorter shelf life (Yu et al., 2020). Prior research has demonstrated that the growth and microbial compositions of fish can be controlled and their shelf life prolonged by the application of chitosan coating, oregano oil, cinnamon bark oil, salting, a gum-based coating that contains essential oil of thyme and clove, and occasionally in conjunction with other preservative methods (Hung et al., 2018, Yu e al., 2017, Zhang et al., 2015, Van Haute et al., 2016, Huang et al., 2017, Dehghani et al., 2018). In another study, nanoemulsion was prepared using D-limonene and found effective against pathogenic strains (Sonu et al., 2018). Pomelo fruit peel is one of the major processing by-products of pomelo fruit. To our best knowledge characteristics and functional properties of the essential oil of pomelo peel are still not properly addressed. The antibacterial properties of pomelo peel are not fully utilized to enhance the storage stability of fish fillets. The integration of smartphones with specialized sensors and software has become a powerful tool for detecting spoilage in real-time (Meng et al., 2017; Xu et. al., 2017; Doğan et al., 2024). Evaluating fish freshness is challenging due to its perishable nature, and current advanced techniques are expensive, bulky, and time-consuming, limiting their use by the fishermen/ retailer/ buyer.

Therefore, in the present study, the essential oil was extracted from pomelo peel and characterized and the efficacy of essential oil as a bio-preservative on the storage stability of fresh fish fillets was evaluated using PANI label and the developed smartphone sensor. Locally available freshwater fish were used since they are among the

major preferred fish. The outcome of the present study will not only help extend the storage stability of fish fillets but also increase the demand for underutilized pomelo fruit in the Northeastern region of India.

7.2. Materials and methods

Fresh fish (rohu (**Fig. 7.1a**), bahu (**Fig. 7.1b**), and silver carp (**Fig. 7.1c**) were purchased from the local market of Tezpur, Assam. Pomelo fruits of optimum maturity were procured from the horticultural department of Tezpur University, Assam, India. The chemicals used in this investigation namely 2,2-diphenyl-1-picrylhydrazyl, nutrient agar, ethanol, and anhydrous sodium sulfate were acquired from Merck-Sigma, India, and were of analytical quality.

7.2.1. *Extraction of essential oil*

Fresh pomelo was collected from the horticulture department of Tezpur University for their peel. The fresh peel after removal from the albedo was washed and blended in a grinder. The essential oil from the freshly ground pomelo peels was extracted by hydro-distillation method using a Clevenger-type apparatus for 3-4 h until all the oils had been extracted (Uysal et al., 2011). 30 g of peel and 400 mL of distilled water was added to the round bottom flask. The essential oil extracted was collected, dehydrated with the anhydrous sodium sulfate, and stored at 4 °C for further analysis. The essential oil yield was determined by the given formula (**Eq. 7.1**):

$$\%\, Yeild\ of\ essential\ oil\ = \frac{Volume\ of\ essential\ oil\ (mL)}{Volume\ of\ sample\ (mL)} \times 100 \qquad (7.1)$$

7.2.2. *Characterization of extracted essential oil*

The chemical compounds of the extracted essential oil were identified by Gas Chromatography equipped with Mass Spectrometry (GC-MS) 7890A GC system (Agilent Technologies Palo Alto, CA, USA) coupled with 240 ion trap MS (Agilent Technologies Palo Alto, CA, USA) that was equipped with Agilent 19091J- 413 HP 5 capillary column (30 m × 320 µm × 0.25 µm) and the column material composed of 5 % phenyl methyl siloxane stationary phase with helium as carrier gas at the flow rate of 1 mL/min. The essential oil was diluted 10 times in ethanol, and 1 µL sample was injected in split-less mode manually. The relative percentage of flavoring components was

calculated by peak areas and the components were identified using the database from NIST library V 2.0 (Huang et al., 2018).

7.2.3. Total phenolic content

The Folin-Ciocalteu method was used for total phenolic content (Abudayeh et al., 2019). The solution was made by mixing 2.5 mL of Folin-Ciocalteu (10 %) reagent with 0.5 mL of methanolic extracted oil sample (1 mL/mL). The mixture was vigorously mixed and allowed to incubate for 5 minutes at room temperature. Following the addition of 2 mL of 7.5 % Na_2CO_2, the absorbance at 765 nm was measured after another 90 minutes of dark incubation. Gallic acid concentrations of (0.2, 0.4, 0.6, 0.8, and 1) µg were used to draw the standard curve. Gallic acid equivalent (µg GAE/g extract) was used to express the sample's total phenolic content.

7.2.4. *DPPH* radical scavenging activity*

2,2-diphenyl-1-picrylhdrazyl free radical (DPPH*) scavenging activity of essential oil was determined as per Blois et al. (2018). Essential oil (0.5 mL) was mixed with DPPH* (0.1 mM, 3.5 mL) solution. The mixture was mixed vigorously and kept in the dark for 30 min. Then, the absorbance was recorded at 517 nm using a UV visible spectrophotometer (Cary 60 UV–Vis Spectrophotometer, Agilent Technologies, Germany). The DPPH* scavenging activity was determined using the following equation (**Eq. 7.2**):

$$\%\ Inhibition = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100 \qquad (7.2)$$

7.2.5. *Antimicrobial activity of essential oil*

The antimicrobial activity of pomelo peel essential oil was determined using the disc diffusion method (Yazgan et al., 2019). Bacterial cultures viz., *Yersinia pestis*, *Escherichia coli* (MTCC 40), *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160), *Mycobacterium smegmatis* (MTCC 14468), *Listeria monocytogenes* (MTCC 839), *Pseudomonas aeruginosa* (MTCC 2297), and fungal culture of *Candida albicans* (MTCC 183) were grown overnight in Luria Broth (LB) and Potato Dextrose Broth (PDB), respectively. The cultures (100 µL) were spread on the prepared Luria

Broth Agar (LBA) and Potato Dextrose Agar (PDA) plates, and a disk was placed on those plates. The amount of 3 μL of pomelo peel essential oil was added onto each disk and incubated overnight at 37 ℃ for bacteria and 28 ℃ for fungus. After incubation, the plates were evaluated for antimicrobial activity and the zone of inhibition (ZOI) was measured accordingly.

7.2.6. *Preparation of samples*

Fresh fish was brought alive from the market to the laboratory. The fish were slaughtered, descaled, degutted, filleted, and washed with sterile cold running water. During filleting, skin, and parts of bones remained. After washing, the filleted fish (weighing 430 g ± 2 g) were left to drain extra water in the sterile steel mesh for 4-5 min. The oil mixer was prepared at a concentration of 0.8 % and 1.2 % essential oil with sesame oil under sterile conditions at room temperature. An oil mixture (1 mL) was applied to the fish fillet (30 g) and stored in an airtight glass container, the fillet without pretreatment with essential oil was considered as control. The treated and control fillets were stored at refrigeration temperature and microbial analyses were conducted for 15 days with an interval of 3 days to examine the antimicrobial effect of pomelo peel on the fish fillets. **Fig. 7.2.** illustrates the graphical presentation of the pre-treatment of fish with pomelo peel essential oil.

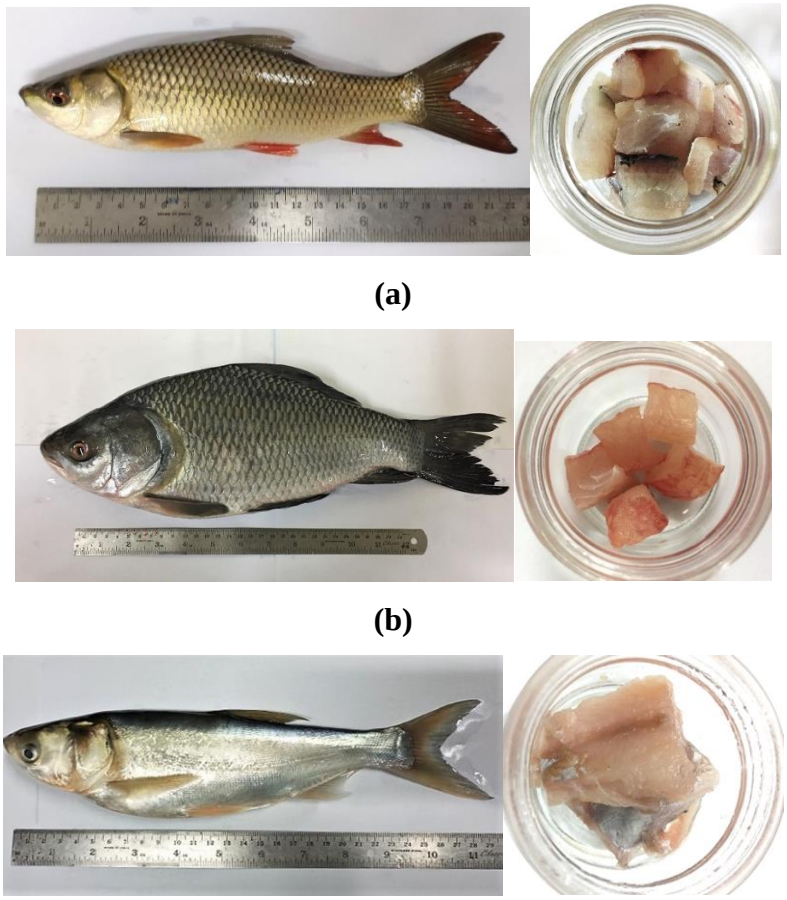


Figure 7.1. (a) Rohu (*Labeo rohita)*, **(b)** Bahu (*Labeo calbahu*), and **(c)** Silver carp (*Hypophthalmichthys molitrix*).

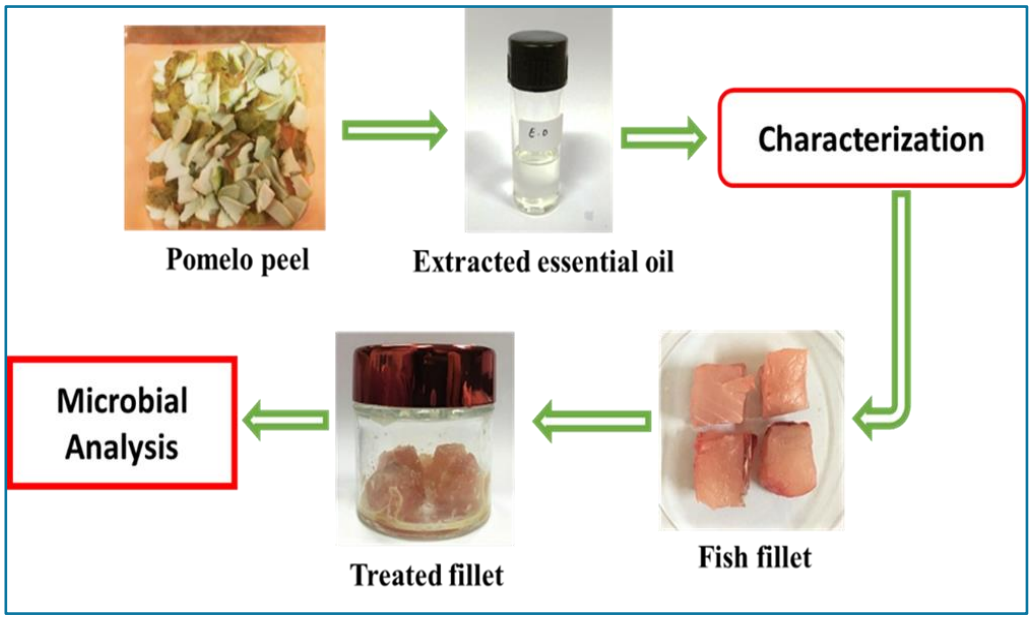


Figure 7.2. Pre-treatment of fish with pomelo peel essential oil.

7.2.7. *Performance evaluation using developed smartphone sensor*

In this experiment, both essential oil-treated fish fillets and control fillets (without essential oil treatment) were tested using PANI labels that were attached inward to the containers. These containers were then stored at refrigeration temperature. The purpose was to assess the response of the PANI labels over time. The response of the PANI labels was evaluated at 3-day intervals over a period of 15 days using a developed smartphone sensor. The results obtained from the smartphone sensor were validated using a UV-visible spectrophotometer and other conventional techniques. By using both smartphone-based and conventional spectrophotometry methods, the reliability and accuracy of the sensor response could be confirmed. The evaluation aimed to demonstrate the effectiveness of PANI labels in detecting spoilage changes in fish fillets, particularly in response to treatments with essential oils.

7.2.8. *Measurement of TVB-N and pH*

Total volatile basic nitrogen (TVB-N), which represents volatile amines in fish samples, was measured with an ion-selective electrode designed for ammonia (NH_4^+ ISE). 5 g of the fish sample was partially dissolved and dissociated in an aqueous phase, allowing the concentration of ammonia (NH_4^+) ions in the water to be determined. The signal output from the electrode was converted to mg/100 g using the method described by Kuswandi et al. (2012). A pH electrode was used to determine the pH of the fish sample. These findings were used to establish correlations with fish quality while in storage.

7.2.9. *Microbial Analysis*

Microbial analysis of fish fillets was conducted as per the method of Giarratana et al. (2016) where fish flesh (5 g) was homogenized with 45 ml of peptone water (0.1 %). Serially ten-fold dilution in 0.1 % peptone water was prepared and aliquots of 0.1 mL of diluted sample were spread on the nutrient agar plates with control for microbial enumeration. After incubation of the plates at 37 °C for 48 h, the total viable count (TVC) was calculated as given below, and all counts were reported as log Cfu/g.

$$\text{Log CFU/g} = \frac{\text{No. of colony} \times \text{Dilution factor}}{\text{Volume of sample}} \qquad (7.3)$$

7.2.10. *Texture Analysis*

A texture analyzer (TA-XD Plus, Stable Microsystems, UK) with a 30 kg load cell was used to analyze the texture of 3 cm x 3 cm fish fillets. A p/5 cylindrical probe was used to compress the fish fillets for the texture profile analysis (TPA) test. Each fillet was compressed twice, at a speed of 1.0 mm/s, to 30 % of its original height. The pre-test and post-test speeds were set to 5 and 10 mm/s respectively. Six replicates of each textural parameter were tested on each sample from the same batch, and the average value was recorded. The textural parameters measured were hardness, springiness, cohesiveness, and gumminess, following the method described by Wiles and Green (Wiles et al., 2004).

7.2.11. *Statistical analysis*

All experiments were performed in triplicates (n=3). The statistical results were obtained through one-way analysis of variance (ANOVA) through Duncan's multiple range test ($p<0.05$) using package IBM SPSS Statistics Version 20.0, Armonk, NY: IBM Corporation software. All the data were presented as the mean with the standard deviation (SD) and Origin 2021 was used to plot the graphs.

7.3. Results and discussion

7.3.1. Extraction yield of pomelo peel essential oil

The yield of essential oil extracted from fresh pomelo peel using hydrodistillation was found to be 2.66 %. However, a comparatively lower yield was reported in the study of Chen et al. (2016) and Liu et al. (2017). According to Tuan et al. (2019) experiments, the yield of pomelo peel oil is about 1 % on a fresh weight basis. Likely, the greater yield of pomelo peel essential oil found in the present study may be due to the fruit's origin, location, and genotype, as studies have reported that the variation in yield may be because the essential oil produced from pomelo peel depends on the origin, genotype, season, and cultivators (Tuan et al., 2019). The study showed that the pomelo peel grown in the North-Eastern region of India has a significant amount of essential oil which may be utilized for several industrial applications.

7.3.2. *Characterization of pomelo peel essential oil*

Essential oil from any plant source is responsible for certain flavors and aromatic activities like intense green, waxy, bitter, or pungent. Citrus essential oils are classified into major five categories including hydrocarbon, acid, alcohol, aldehydes, and esters which account for insecticidal, rancid odor, and certain ailments including immune function, cardiovascular disease, and inflammation. Major compounds identified in extracted oil are presented in **Table 7.1**. Among the twelve major compounds found, the monoterpene hydrocarbon D-limonene (89-90 %) was the most abundant, followed by terpinyl acetate (2.8 %), α-pinene (2.3 %), β-pinene (2.2 %), and terpinolene (0.3 %). The results agreed with the finding of Bordoloi et al. (1999) that the peel of pomelo cultivated in the Northeastern region of India has limonene as the major constituent. Limonene was found to be the major compound in grapefruit peel essential oils about 88.6-91.5 % in the study of Uysal et al. (2011) and showed antimicrobial activities of grapefruit essential oil against spoilage microorganisms. In another study, 67.78 % D-limonene from the essential oil of the combination of *Citrus maxima* and *Citrus sinensis* was reported by Singh et al. (2010). Aldehydes are an important component that contributes to the properties of citrus flavor and D-limonene is the major component of citrus's essential oils (Perez-Cacho et al., 2008; Fernández-Vázquez et al., 2013). Saturated aliphatic aldehydes (octanal and decanal) were also detected, which are

renowned for peel and citrus-like aromas and are probable citrus flavor candidates. The sample also contained neral and geranial (monoterpene aldehydes). These chemicals distinguish pomelo from other citrus types (Gonz´alez-Mas et al., 2011). Linalool (a monocyclic terpenoid) is responsible for citrus' pleasant aroma. Essential oils rich in monoterpenes are recognized as food preservatives (Ruberto et. al., 2000) and are considered natural antioxidants (Yanishlieva et al., 1999). The identified volatile components are the major components of citrus essential oil and the most typical terpenes that are well known to have some physiological, antimicrobial, and chemo-preventive properties.

Table 7.1. Chemical compounds of pomelo peel essential oil

Identified compound	Retention time	% Flavoring of compound
α-pinene	8.39	2.35
D-limonene	11.05	89.23
Terpinolene	12.43	0.30
1,2-Dihydropyridine	12.88	0.58
5-Caranol	15.22	0.95
Terpinyl acetate	15.72	2.87
Citral	18.08	0.19
Cyclohexane,1-methylene-4-(1-methylethylidene)	20.53	0.96
(-)-Aristolene	21.57	0.04
Isoledene	22.95	1.75
Copane	22.39	0.23
α-muurolene	24.77	0.54

7.3.3. *DPPH* scavenging activity and total phenolic content of pomelo peel essential oil*

The DPPH* scavenging activity of pomelo peel essential oil was observed to be 65 % and is related to the composition of extracted oil (due to synergistic effects) where the major compounds were limonene, pinene, citral, etc. Choi reported that the

antioxidant activity ranged from 17.7 % to 64 % of 34 varieties of citrus essential oils (Choi et al., 2010). The range in percentage of antioxidant properties of essential oils was due to the different chemical compositions of the essential oils (Lan-Phi et al., 2015). Another study also reported the relationship between chemical composition and the antioxidative properties of oil (Himed et al., 2019; Wang et al., 2008). Pomelo is a good source of antioxidants such as flavonoids, polyphenols, and ascorbic acid which are effective free radical neutralizers. Polyphenols are one of these antioxidants that are crucial in reducing oxidative damage (Tsai et al., 2017). The oxidative stress caused by free radicals is greatly reduced by the polyphenolic compounds, flavonoids, and the ascorbic acid found in pomelo, which works to balance out the free reactive radicals (Anmol et al., 2021). The antioxidant activity of D-limonene, one of the main components in oil, may be responsible for the high percentage of natural antioxidants in the essential oil in the present study in terms of free radical scavenging. The DPPH* was selected to assess the antioxidant properties of pomelo peel oil and its potential role in extending the shelf life of fish during storage. This method was chosen because it directly measures the ability of the oil to neutralize free radicals, which is relevant to inhibiting oxidation in stored fish. Alternative methods, such as ORAC (oxygen radical absorbance capacity), were not utilized as they were not required for this specific purpose.

The phenolic content of the pomelo peel essential oil was observed to be 6.68 mg GAE/g. The extracted essential oil has a high yield of phenolic which is relevant to the study of Bordoloi et al. (1999) that the natural by-product of the pomelo fruit is nutritious and has therapeutic properties. The flavanones found in pomelo peel are mostly in the form of aglycones and glycosides, with the most detected flavanone aglycones being hesperetin, naringenin, and eriodictyol. Earlier reports also stated that the concentration of total phenolic content ranged from 9.33 to 54.56 mg GAE/g in the pomelo plant's peels (Ding et al., 2013; Pandey et al., 2019). Flavonoids, Vitamin C, and carotenoids make up most of the pomelo peel, and they are essential for delivering various biological functions like antioxidant, anti-inflammatory, and anti-atherogenic (Abudayeh et al., 2016, Vijayalakshmi et al., 2016). The phenolic and antioxidant properties of pomelo peel essential oil possess a synergetic effect on controlling the microbial population during fish fillet storage.

7.3.4. *Antimicrobial activity of pomelo peel essential oil*

The antimicrobial activity of oil against eight common pathogenic microorganisms using gentamicin as positive control is shown in **Fig. 7.3**. It was noticed that the pomelo peel oil prevented the growth of all the tested organisms. The oil has maximum activity against *C. albicans* followed by *B. cereus* with a zone of inhibition (ZOI) of 23 mm and 21 mm, respectively. The activity against *L. monocytogenes* was found to be more than gentamicin. The ZOI for *Yersinia pestis*, *E. coli, S. aureus, M. smegmatis, and P. aeruginosa* was 14 mm, 11 mm, 14 mm, 14 mm, and 10 mm, respectively (**Table 7.2**). Similar results were reported by Uysal et al. (2011) in the study of the antibacterial activity of grapefruit essential oil. Studies have reported that the biological activity of various essential oils has shown antimicrobial effects against certain pathogens (Tepe et al., 2005; Uysal et al., 2011; Deng et al., 2020). Besides that, the antibacterial action of pomelo peel essential oil may be related to the oil's composition, and the peel oil is attributed to limonene, the oil's main component. Giarratana et al. (2016) reported on the effect of limonene on spoilage organisms. Terpene alcohol also has high antibacterial activity against bacterial species, most prominently *S. aureus* and *E. coli* (Knobloch et al., 1989). In addition, Lis-Balchin et al. (1999) found that α-pinene, β-pinene, sabinene, and limonene exhibit potent antibacterial activity by compromising the integrity of bacterial or fungal membranes and inhibiting ion transport procedures and respiration. The pomelo peel essential oil in the study showed strong activity against pathogenic strains and therefore is applied to control and prevent spoilage of fresh fish fillet. The strains selected in the present study have great importance in food spoilage and safety (Mostafa et al., 2018).

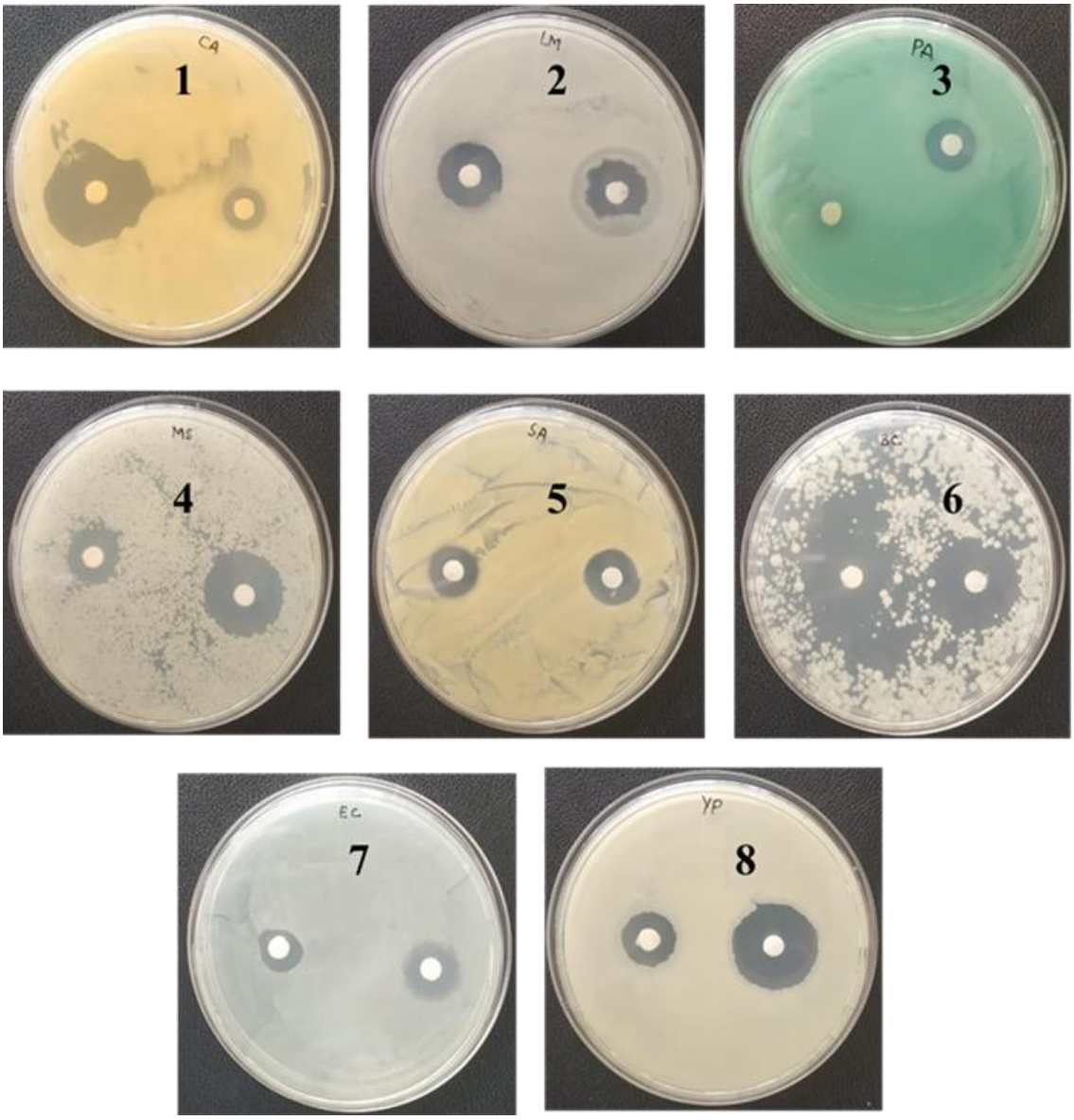


Figure 7.3. Antimicrobial properties of Essential oil (From 1 to 8: *Candida albicans, Listeria monocytogenes, Pseudomonas aeruginosa, Mycobacterium smegmatis, Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and *Yersinia pestis*).

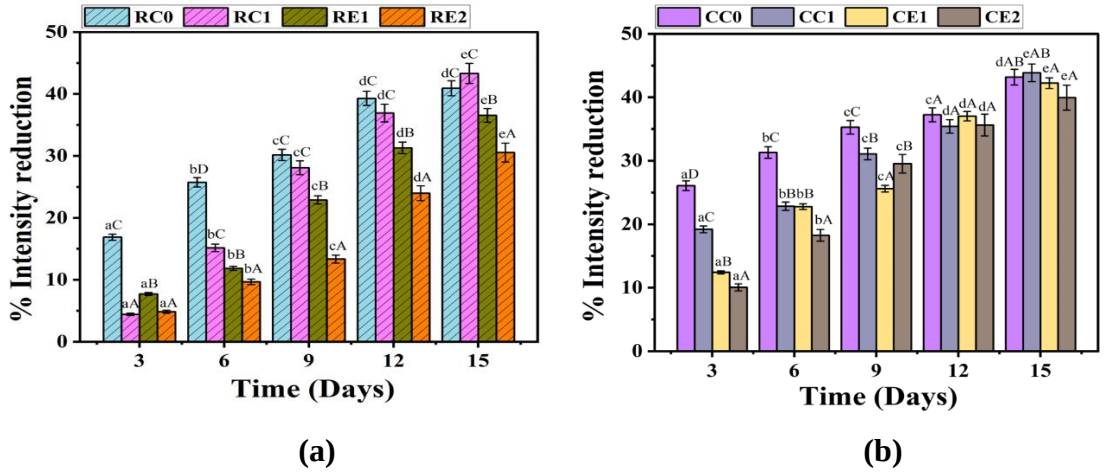
Table 7.2. Zone of inhibition

Bacteria/ Yeast	Two-disc oil sample (mm)	One disc Gentamycin (mm)
Listeria monocytogenes	16	15
Pseudomonas aeruginosa	0	13
Mycobacterium smegmatis	14	21
Staphylococcus aureus	14	15
Bacillus cereus	21	22
Escherichia coli	11	14
Candida albicans	23	12

7.3.5. *Study of the effect of essential oil as a preservative using the developed smartphone sensor*

The performance of the smartphone sensor equipped with the PANI label was evaluated using essential oil treated rohu and common carp fish fillets. It was found that the freshness of essential oil-treated fish fillets (RE1, RE2, CE1, and CE2) was maintained for up to 12-15 days during storage at refrigeration temperature (**Fig. 7.4 a** and **b**). In contrast, the untreated fillets (RC0 and CC0) of both fish varieties showed above 25 % intensity reduction at 6 days, which exceeds the acceptable limit established in Chapter 6, section **6.3.2**. The intensity reduction continued to increase with prolonged storage time. The essential oil-treated fillets maintained their freshness longer, resulting in a slower increase in percentage intensity reduction compared to untreated fillets. The response of the PANI label remained effective even after the fillets were treated with essential oil, corresponding to the findings of the conventional methods. Additionally, sesame oil-treated fillets confirmed some effectiveness in inhibiting microbial activity in both varieties of fish. In the rohu fish samples, the percentage intensity reduction for fillets treated with 0.8 % (RE1) and 1.2 % (RE2) essential oil was 22 % and 13 % respectively at 9 days. By 12 days, the intensity reduction reached 30 % for RE1, and by 15 days, it reached 29 % for RE2 (**Fig. 7.4a**). This demonstrates that increasing the concentration of essential oil reduces the degree of spoilage, as evidenced by the lower % of intensity reduction. Similarly, the essential oil prolongs the shelf life of common

carp fish fillets as compared to the untreated fillets which could be effectively evaluated by the developed PANI-based smartphone sensor (**Fig. 7.4b**).



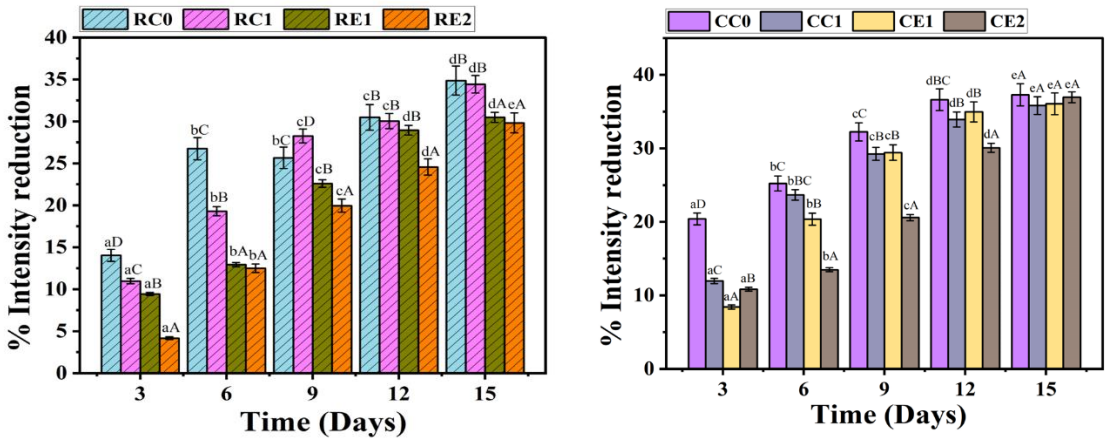
(a) **(b)**

Figure 7.4. Percent (%) intensity reduction of smartphone sensor response, **(a)** Rohu fish fillet, and **(b)** Common carp fish fillet. **(Note: RC0 –** Untreated rohu fish fillet, **RC1 –** Sesame oil treated rohu fish fillet, **RE1 –** 0.8 % essential oil treated rohu fish fillet, **RE2 –** 1.2 % essential oil treated rohu fish fillet, **CC0 –** Untreated common carp fish fillet, **CC1 –** Sesame oil treated common carp fish fillet, **CE1 –** 0.8 % essential oil treated common carp fish fillet, **CE2 –** 1.2 % essential oil treated common carp fish fillet). Small letters indicate significant differences ($p < 0.05$) in the same fish sample during storage time, and capital letters indicate significant differences ($p < 0.05$) with essential oil-treated fish samples at a particular storage time.

7.3.6. *Validation of the finding of smartphone senor using UV-visible spectrophotometer*

The response of the PANI label for essential oil treated and untreated fish fillets was evaluated using a UV-visible spectrophotometer (Cary 60 UV–Vis Spectrophotometer, Agilent Technologies, Germany). As the storage time increased, a shift in the spectra resulted in a reduction in transmittance intensity, indicating the degree of spoilage in both varieties of fish (**Fig. 7.5 a** and **b**). The essential oil-treated fillets remained within acceptable limits, as established in Chapter 3, for 12-15 days for rohu fish fillets (**Fig. 7.5a**) and 9-12 days for common carp fish fillets (**Fig. 7.5b**) during refrigeration storage. The PANI label response using the UV-visible spectrophotometer corresponded with the readings observed using the smartphone-based sensor. Slight

deviations in values may be attributed to the in-built optical filter embedded in the CMOS camera sensor of the smartphone. However, the results obtained from both the smartphone sensor and the spectrophotometer were consistent, demonstrating that ammonia concentration in the fish increased with the storage time and that the pomelo peel essential oil prolongs the shelf life of the fish to some extent. As fish tissues are degraded by microorganisms, the odorless compound trimethylamine oxide in the fish is converted into trimethylamine (TMA) and dimethylamine (DMA). Concurrently, ammonia is produced by the decomposition of urea and amino acids by bacteria. These basic volatile amines are good indicators of fish freshness. It was observed that a sharp intensity reduction in transmittance at 6 days (**Fig. 7.5**) aligned with the intensity reduction observed by the smartphone sensor after 5-6 days (**Fig. 7.4**). The variation in the degree of spoilage between the fish varieties could be attributed to differences in their composition, habitat, temperature, and the conditions during and after harvesting, all which impact fish freshness. Relative correlation between the smartphone sensor and the spectrophotometer indicates that the PANI label, when paired with the smartphone sensing system, is effective for detecting fish spoilage and can also effectively detect the efficacy of essential oil as a preservative in fish fillets during storage.



(a) **(b)**

Figure 7.5. Percent (%) intensity reduction of spectrophotometer response**, (a)** Rohu fish fillet, and **(b)** Common carp fish fillet. **(Note: RC0 –** Untreated rohu fish fillet, **RC1 –** Sesame oil treated rohu fish fillet, **RE1 –** 0.8 % essential oil treated rohu fish fillet, **RE2 –** 1.2 % essential oil treated rohu fish fillet, **CC0 –** Untreated common carp fish fillet, **CC1 –** Sesame oil treated common carp fish fillet, **CE1 –** 0.8 % essential oil treated common carp fish fillet, **CE2 –** 1.2 % essential oil treated common carp fish fillet).

Small letters indicate significant differences ($p < 0.05$) in the same fish sample during storage time and capital letters indicate significant differences ($p < 0.05$) with essential oil treated fish samples at a particular storage time.

7.3.7. *Measurement of TVB-N and pH values*

The TVB-N (Total Volatile Basic Nitrogen) levels in both essential oil treated and untreated fish fillets of both varieties stored at refrigeration temperatures were measured as shown in **Fig. 7.6 a** and **b.** Initially, the TVB-N value was below 25 mg/100 g for the first 0-3 days of storage but increased significantly after 3 days. For untreated rohu fish fillets, the TVB-N value crossed 35 mg/100 g by day 6, indicating spoilage (**Fig. 7.6a**). In contrast, the TBV-N levels for essential oil-treated rohu fillets (RE2) remained below 30 mg/100 g until day 9, although they exceeded the recommended safe level (as per the European Union Regulation (EC) no. 2074/2005) after 12 days of storage. For essential oil treated common carp fillets (CE2), the TVB-N value was 28 mg/100 g at 6 days and rose to 30-33 mg/100 g between 9-12 days, reaching 35 mg/100 g at 15 days of storage (**Fig. 7.6b**). The TVB-N values increased significantly with the storage time, showing a close correlation with the readings from the developed smartphone sensor. The essential oil treatment effectively showed a rise in the TVB-N values compared to untreated fillets.

The pH values of essential oil treated and untreated rohu and common carp fish fillets were also measured, as shown in **Fig 7.6 c** and **d** respectively. Initially, the pH values were low but gradually increased during storage. However, the increase in the pH was significantly slower in the essential oil-treated fillets, likely due to the antimicrobial effect of the pomelo peel essential oil, which inhibits spoilage activity. The relationship between pH and the degree of spoilage suggests that the rise in pH is due to alkaline chemicals produced by microbial activity during fish deterioration, correlating with the increase in TVB-N. These findings confirm that the developed smartphone sensor aligns well with the TVB-N and pH values of the fish, effectively indicating spoilage.

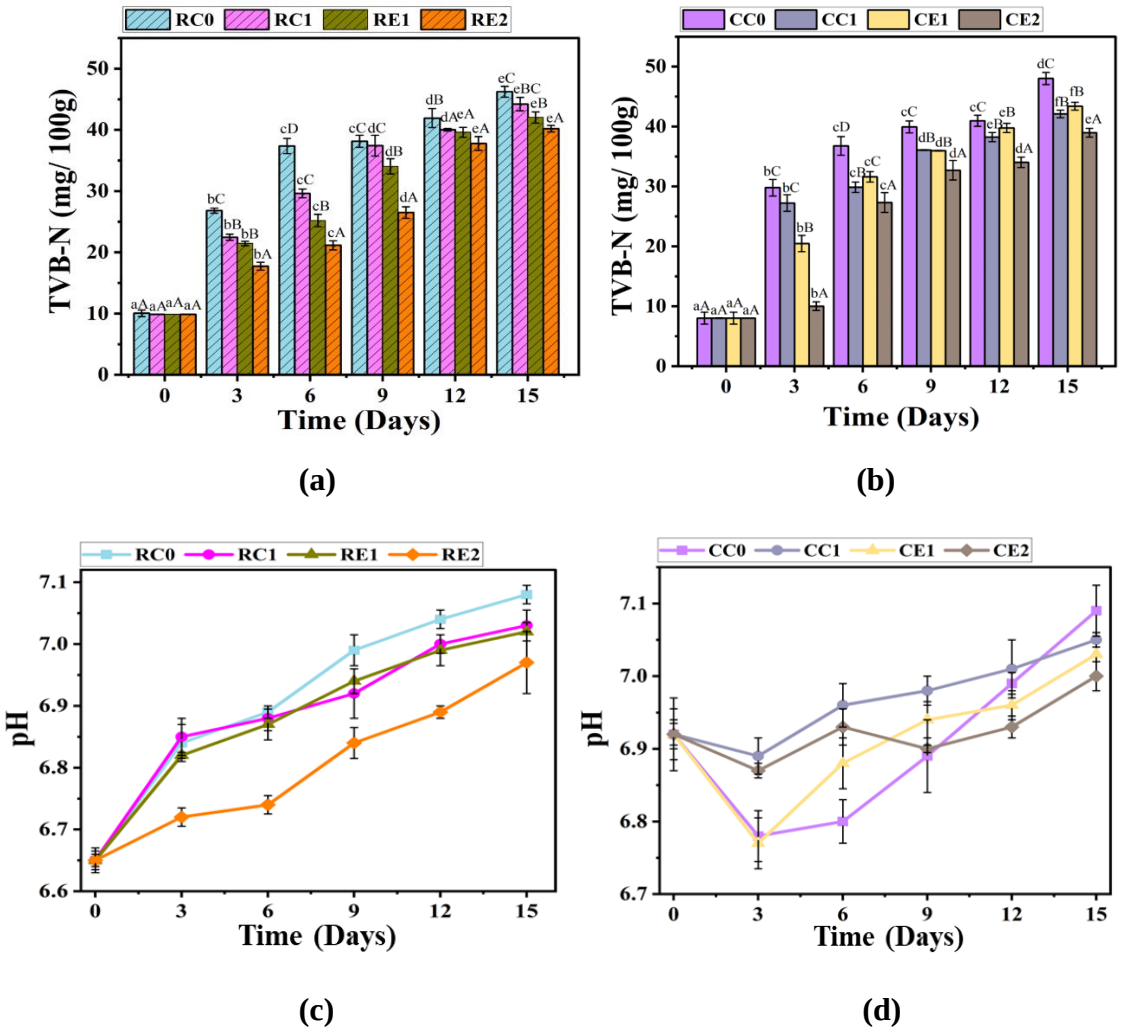


Figure 7.6. (a) TVB-N value of essential oil treated and untreated rohu fish fillet, **(b)** TVB-N value of essential oil treated and untreated common carp fish fillet (Small letters indicate significant differences ($p < 0.05$) in the same fish sample during storage time and capital letters indicate significant differences ($p < 0.05$) with essential oil-treated fish samples at a particular storage time), **(c)** pH value of essential oil treated and untreated rohu fish filet, and **(d)** pH value of essential oil treated and untreated common carp fish fillet. **(Note: RC0 –** Untreated rohu fish fillet, **RC1 –** Sesame oil treated rohu fish fillet, **RE1 –** 0.8 % essential oil treated rohu fish fillet, **RE2 –** 1.2 % essential oil treated rohu fish fillet, **CC0 –** Untreated common carp fish fillet, **CC1 –** Sesame oil treated common carp fish fillet, **CE1 –** 0.8 % essential oil treated common carp fish fillet, **CE2 –** 1.2 % essential oil treated common carp fish fillet).

7.3.8. *Microbial analysis*

The total viable count and the *pseudomonas* count of the tested fish fillets are shown in **Fig. 7.7 a, b, c,** and **d**. During microbial analysis, the pomelo peel essential oil-treated fish fillets were found significantly effective as compared to untreated (control) fillets from 0 days to 15 days. Initially, the total viable count (TVC) of the untreated (control) sample was 5 log CFU/g, 5 log CFU/g, 0 log CFU/g, and 5 log CFU/g for rohu, bahu, silver carp, and common carp respectively, and grew throughout storage. During 12–15 days, it exceeded the value of 7 log CFU/g, which was determined to be above the fresh fish fillet acceptability level. The limit for total aerobic plate counts in fresh and frozen fish, according to the International Commission on Microbiological Specifications (ICMS), is 6.5-7 log CFU/g. The oil-treated fillets (0.8 % and 1.2 %) had a significantly lower count in all three varieties of fish. Statistically, significant differences ($p \leq 0.05$) were observed during the period of storage (**Table 7.3**). The microbial count of RE1 and CE1 was observed to be 6.5 log CFU/g and 6.55 log CFU/g, respectively in 15 days, which resulted in a comparatively lower than the untreated (controlled) fish sample (**Fig. 7.7 a** and **b**). The microbial load of RC0 and CC0 crossed 6.5 log CFU/g at 6 days. Also, a corresponding decrease in the microbial load was observed with the increase in the concentration of essential oil. This could be attributed to the main components of pomelo peel essential oil, where studies have revealed that chemicals such as limonene, pinene, and terpinolene have antibacterial and antioxidant effects (Uysal et al., 2011). Also, the findings are in line with the % intensity reduction obtained using the smartphone-based sensor proving to be effective even after the application of essential oils as natural preservatives. The gradual increase in the *pseudomonas* count was also observed with a slower rate in essential oil treated fillets than untreated fillets in both the rohu and common carp fish fillets (**Fig. 7.7 c** and **d**).

Table 7.3. Log CFU/g for 15 days of storage of essential oil treated rohu, bahu, and silver carp fish fillets

Sample	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
RC0	5.00 ± 0.00^{bA}	5.54 ± 0.06^{dB}	5.74 ± 0.04^{efC}	6.04 ± 0.04^{fgD}	6.95 ± 0.00^{eE}	7.24 ± 0.00^{fF}
RC1	0.00 ± 0.00^{aA}	5.15 ± 0.15^{bcB}	5.39 ± 0.09^{cdB}	5.69 ± 0.09^{cdeC}	6.40 ± 0.03^{cdD}	6.70 ± 0.01^{dE}
RE1	0.00 ± 0.00^{aA}	5.00 ± 0.00^{bB}	5.30 ± 0.00^{bcC}	5.45 ± 0.15^{bcCD}	5.65 ± 0.05^{aDE}	5.88 ± 0.03^{bE}
RE2	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	5.00 ± 0.00^{aB}	5.00 ± 0.00^{aB}	5.45 ± 0.15^{aC}	5.67 ± 0.18^{aC}
BC0	5.00 ± 0.00^{bA}	5.39 ± 0.09^{cdB}	5.82 ± 0.03^{efC}	6.37 ± 0.08^{hD}	7.06 ± 0.02^{eE}	7.24 ± 0.04^{fE}
BC1	0.00 ± 0.00^{aA}	5.30 ± 0.00^{cdB}	5.78 ± 0.07^{efC}	5.89 ± 0.11^{efC}	6.49 ± 0.01^{dD}	6.95 ± 0.03^{eE}
BE1	0.00 ± 0.00^{aA}	5.00 ± 0.00^{bB}	5.39 ± 0.09^{cdC}	5.74 ± 0.04^{deD}	6.03 ± 0.08^{bE}	6.36 ± 0.04^{cF}
BE2	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	5.00 ± 0.00^{aB}	5.30 ± 0.00^{bC}	5.54 ± 0.06^{aD}	5.84 ± 0.06^{abE}
SC0	0.00 ± 0.00^{aA}	5.39 ± 0.09^{cdB}	5.90 ± 0.05^{fC}	6.22 ± 0.04^{ghD}	6.95 ± 0.00^{eE}	7.25 ± 0.02^{fF}
SC1	0.00 ± 0.00^{aA}	5.15 ± 0.15^{bcB}	5.59 ± 0.11^{deC}	6.07 ± 0.11^{fgD}	6.26 ± 0.02^{cD}	6.81 ± 0.05^{deE}
SE1	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	5.15 ± 0.15^{aB}	5.60 ± 0.00^{cdC}	5.95 ± 0.03^{bD}	6.27 ± 0.04^{cE}
SE2	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	5.00 ± 0.00^{aB}	5.30 ± 0.00^{bC}	5.59 ± 0.11^{aD}	5.95 ± 0.05^{bE}

*Values are represented as mean ± standard deviation of three determination (n=3). Values followed by different superscripts are significantly different ($p < 0.05$) within rows (small letters) and columns (capital letters). **RC_0**: Untreated rohu fillet; **RC_1**: rohu fillet treated with oil only; **RE_1**: Rohu fillet treated with 0.8 % essential oil; **RE_2**: Rohu fillet treated with 1.2 % essential oil; **BC_0**: Untreated bahu fillet; **BC_1**: Bahu fillet treated with oil only; **BE_1**: Bahu fillet treated with 0.8 % essential oil; **BE_2**: Bahu fillet treated with 1.2 % essential oil; **SC_0**: Untreated silver carp fillet; **SC_1**: Silver carp fillet treated with oil only; **SE_1**: Silver carp fillet treated with 0.8 % essential oil; **SE_2**: Silver carp fillet treated with 1.2 % essential oil.

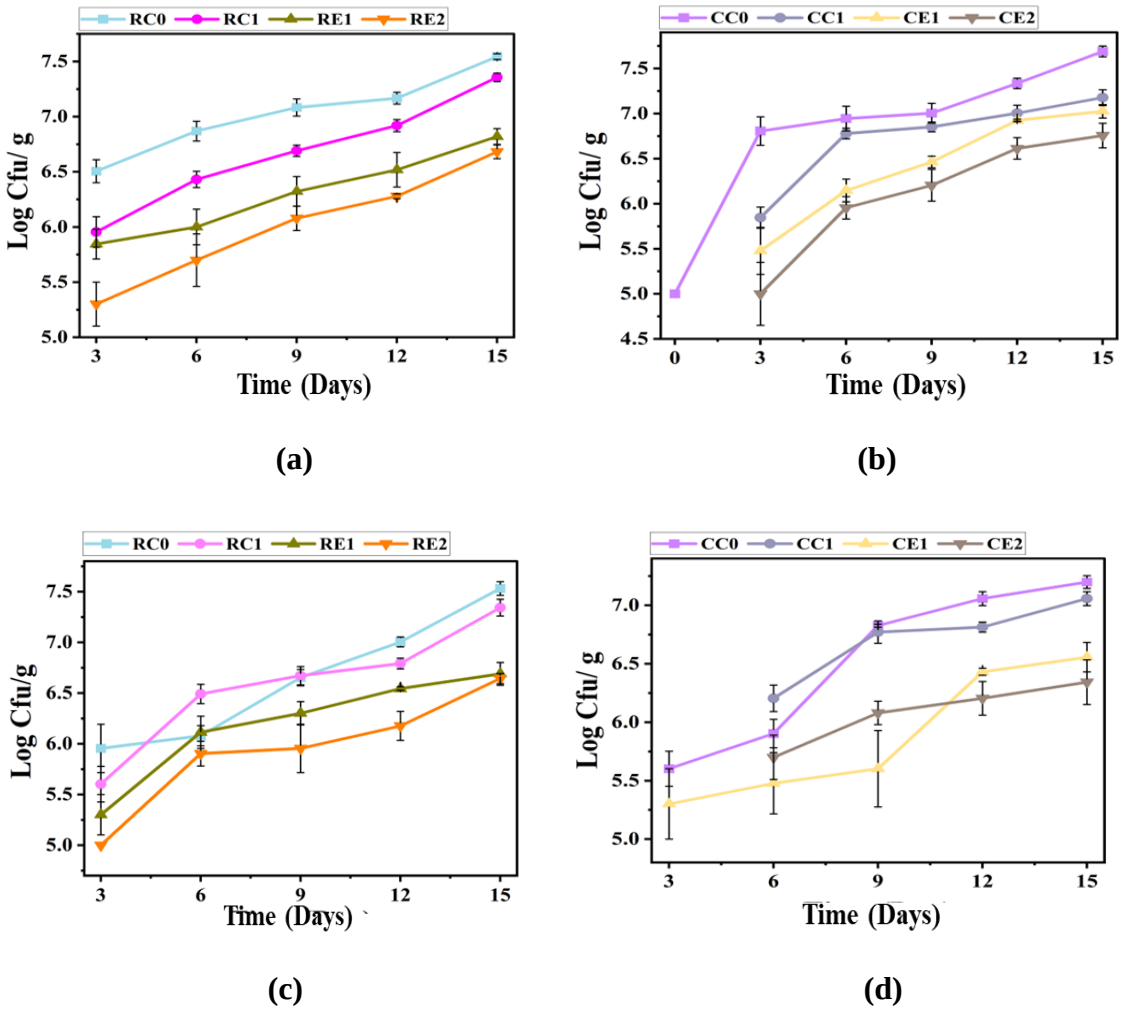


Figure 7.7. Total viable count (TVC) of essential oil treated and untreated **(a)** Rohu fish fillet, **(b)** Common carp fish fillet, **(c)** *Pseudomonas* count of treated and untreated rohu fish fillet, and **(d)** *Pseudomonas* count of treated and untreated common carp fish fillet. **(Note: RC0 –** Untreated rohu fish fillet, **RC1 –** Sesame oil treated rohu fish fillet, **RE1 –** 0.8 % essential oil treated rohu fish fillet, **RE2 –** 1.2 % essential oil treated rohu fish fillet, **CC0 –** Untreated common carp fish fillet, **CC1 –** Sesame oil treated common carp fish fillet, **CE1 –** 0.8 % essential oil treated common carp fish fillet, **CE2 –** 1.2 % essential oil treated common carp fish fillet).

7.3.9. *Texture analysis*

The internal cross-linking of connective tissue and the detachment of fibers are key indicators of the freshness and quality of fish muscle, as these attributes depend heavily on the texture and structure of the fish. Texture parameters for both essential oil-treated and untreated rohu and common carp fish are illustrated in **Fig. 7.8** and **Fig. 7.9**. For untreated rohu fish fillets, the hardness index decreased from 5.6 N to 3.5 N over 15 days of storage (**Fig. 7.8 a**), while untreated common carp fillets showed a decrease from 5.78 N to 2.4 N (**Fig. 7.9a**). Essential oil treatment and low temperatures significantly eased the overall textural changes in the fillets during storage. Overall, significant differences in hardness, springiness, cohesiveness, and gumminess were observed as the storage period progressed. Fish tissue is generally more perishable than other animal tissues, even under refrigeration. The bacteria that live on cold-blooded fish at low ocean temperatures are well-adapted to cold and continue to grow under typical refrigeration conditions, leading to a gradual decline in connective tissue structure and impacting the textural properties of the fish (Cheng et al., 2014). Fish muscle is particularly prone to softening during postmortem conditions, which adversely affects its textural quality. After the fish dies, autolysis caused by collagenases and other proteases alters the collagen structure and breaks down muscle tissue, leading to the release of total volatile basic nitrogen (TVB-N) (ICMS, 1986).

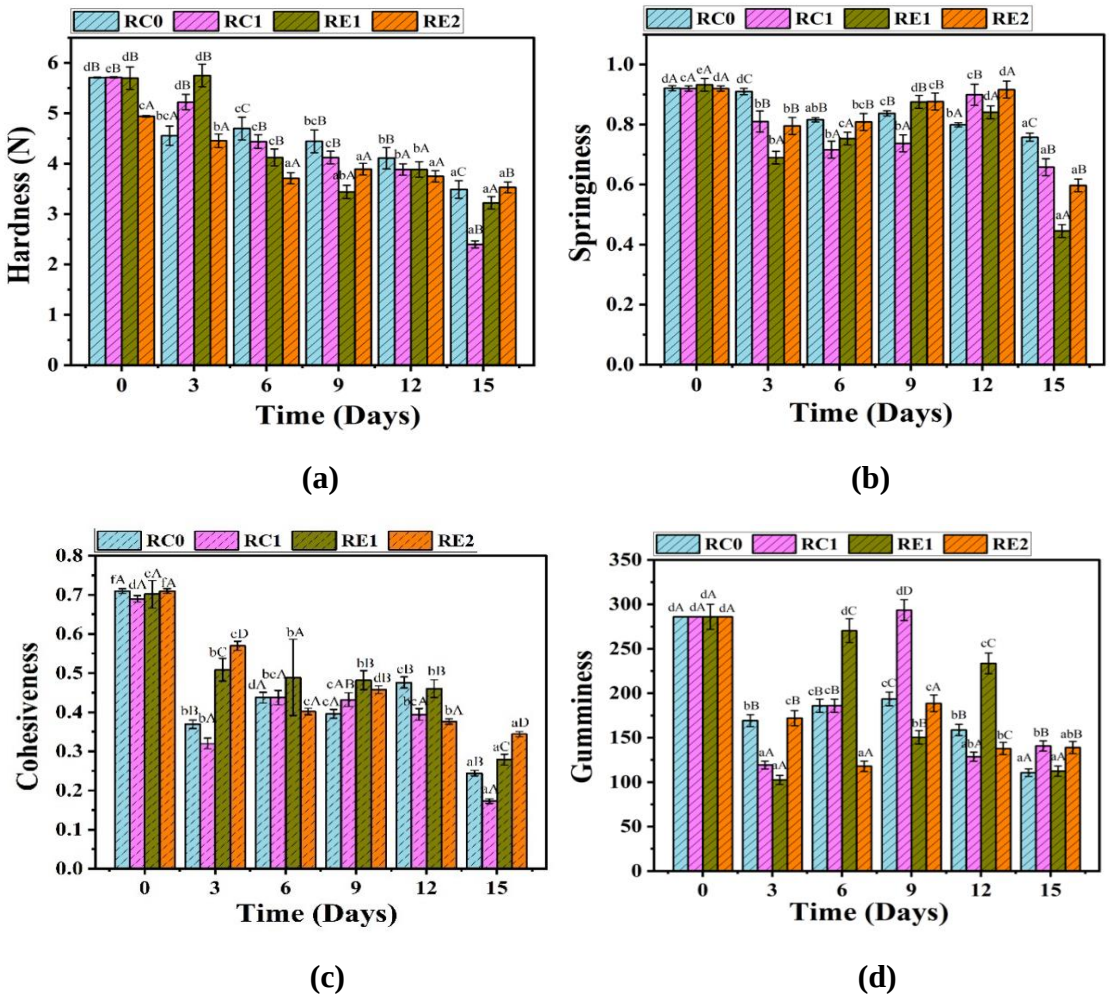


Figure 7.8. Texture profile analysis of **(a)** Hardness, **(b)** Springiness, **(c)** Cohesiveness, and **(d)** Gumminess of essential oil treated and untreated rohu fish fillets during storage at refrigeration temperature. **(Note: RC0 –** Untreated rohu fish fillet, **RC1 –** Sesame oil treated rohu fish fillet, **RE1 –** 0.8 % essential oil treated rohu fish fillet, **RE2 –** 1.2 % essential oil treated rohu fish fillet). Small letters indicate significant differences ($p < 0.05$) in the same fish sample during storage time and capital letters indicate significant differences ($p < 0.05$) with essential oil treated fish samples at a particular storage time.

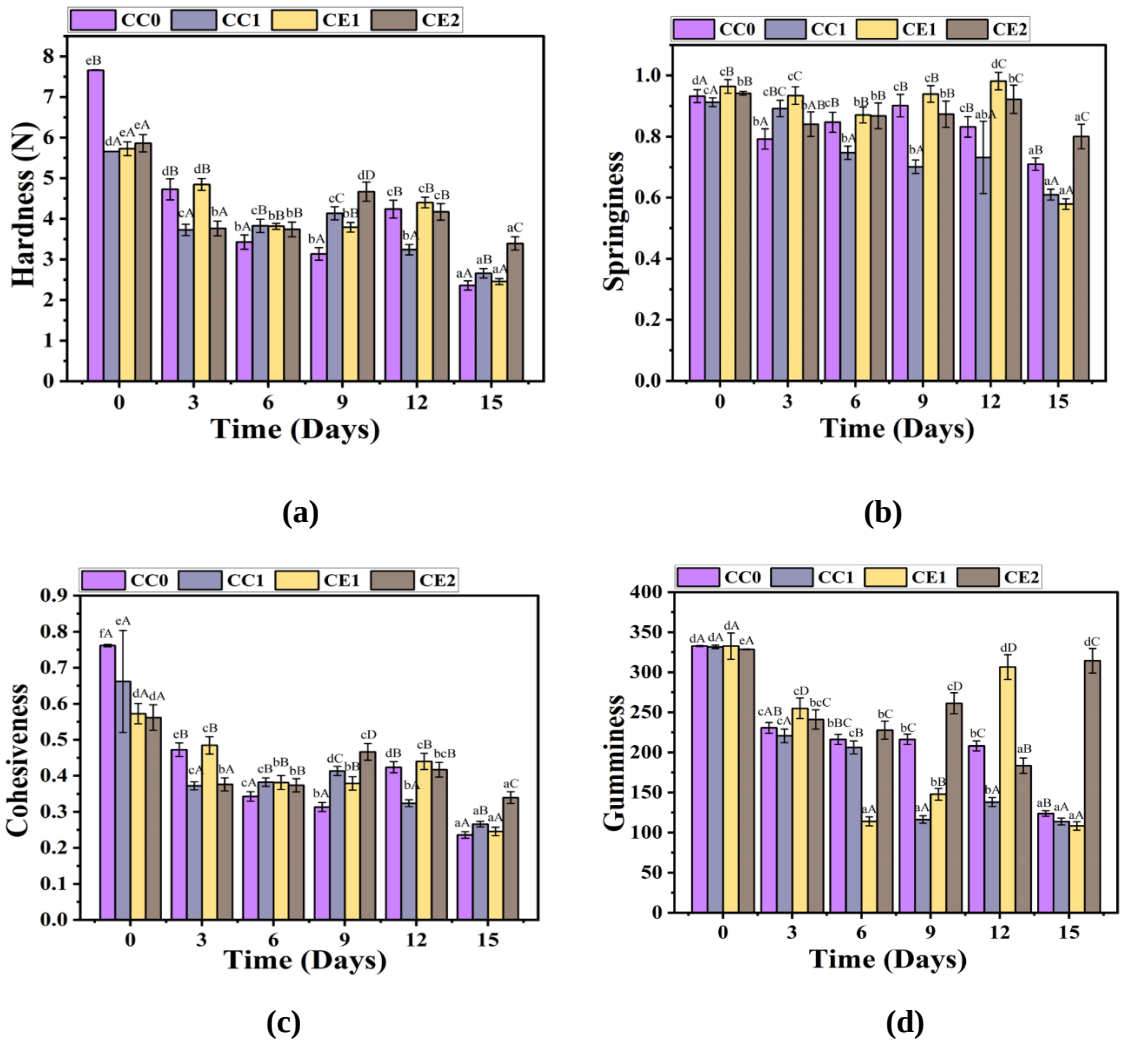


Figure 7.9. Texture profile analysis of **(a)** Hardness, **(b)** Springiness, **(c)** Cohesiveness, and **(d)** Gumminess of essential oil treated and untreated common carp fish fillets during storage at refrigeration temperature. (**Note: CC0 –** Untreated common carp fish fillet, **CC1 –** Sesame oil treated common carp fish fillet, **CE1 –** 0.8 % essential oil treated common carp fish fillet, **CE2 –** 1.2 % essential oil treated common carp fish fillet). Small letters indicate significant differences ($p < 0.05$) in the same fish sample during storage time and capital letters indicate significant differences ($p < 0.05$) with essential oil treated fish samples at a particular storage time.

7.4. Conclusion

The result of the present study indicates the effectiveness of essential oil against pathogenic strains, originating as a natural alternative to hazardous chemical antimicrobials. The present study successfully extracted and characterized the essential oil from pomelo peels resulting in a yield of 2.66 % using hydro-distillation. The composition of the oil showed twelve major compounds, with D-limonene being the predominant component at 89-90 %, followed by terpinyl acetate, α-pinene, β-pinene, and terpinolene. This essential oil exhibited potent antioxidant activity, marked by a DPPH* scavenging activity of 65 %. The oil demonstrated antimicrobial effects against various organisms, showing maximum activity against *C. albicans* and *B. cereus* with inhibition zones of 23 mm and 21 mm respectively. It notably surpassed the activity of gentamicin against *L. monocytogenes*. The oil also displayed moderate activity against *Yersinia pestis, E. coli, S. aureus,* and *P. aeruginosa*. In fish storage, the oil-treated fillets proved significantly more effective than untreated ones throughout 15 days, showcasing substantial differences ($p \leq 0.05$) during storage. The performance of the developed sensor was successfully tested on fish fillets treated with essential oil at refrigeration temperature. Notably, the sensor effectively detected the fish rejection threshold during storage (10^7 CFU/g microbial population and 30 mg/100 g TVB-N value) in pomelo peel essential oil treated fillet.

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