CHAPTER 5

To study the shelf life of atmospheric cold plasma (ACP) treated orange juice during storage

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5.1 Introduction

Orange (cv. Wakro), an important citrus fruit of Arunachal Pradesh origin in Northeast India, is known for its rich source of vitamin C (ascorbic acid), phenolic compounds, minerals, taste, and flavor (Datta et al., 2021). It is highly vulnerable to post-harvest variations of harvested oranges during further processing, storage, and transportation. These quality changes are associated with alterations in colour, flavour, texture, nutritional value, and spoilage (Leizerson and Shimoni, 2005). The chemical changes in orange juice that occur during storage are a breakdown of sugars and organic acids, oxidation of ascorbic acid and other phytochemicals, which are often accelerated by temperature, light, oxygen level, packaging materials, and enzymatic and microbial activity, which may contribute to degradation and spoilage (Martí et al., 2009; Iturralde-García et al., 2022). However, the spoilage of juice during storage can be linked to the presence of aerobic mesophiles, yeasts, and molds, all of which can affect the juice's flavour, safety, and overall acceptability (Sadler et al., 1992; Aneja et al., 2014; Hernández-Carranza et al., 2021).

To assure the stability, safety, and quality of untreated and ACP-treated orange juice, it is vital to explore how different storage conditions, such as temperature, packaging, and storage time, interact with quality parameters and microbial populations. With this information, researchers and producers can optimize the storage conditions, practices, and shelf life while maintaining the nutritional and sensorial characteristics of the fruit juice. Therefore, this study focuses on the quality changes and microbial dynamics of untreated and ACP (optimized condition of objective-1) treated orange (cv. Wakro) juice during storage.

5.2 Materials and Methods

5.2.1 Raw materials and chemicals

Oranges (cv. Wakro) of Arunachal Pradesh, Northeast India, were procured from the local vendor of the Tezpur area, Assam, India. Phenolphthalein, 2,2-diphenyl-1-picrylhydrazyl, NaCl, $NaHCO_3$ 2,6-dichloroindophenol salt, methanol, NaOH, Folin-

Cocteau reagent (FCR), Na_2CO_3, nutrient agar (NA), potato dextrose agar (PDA), and distilled water were purchased from HiMedia Laboratories Pvt. Ltd., India, Sisco Research Laboratories Pvt. Ltd., India, and Merck Specialities Pvt. Ltd., India.

5.2.2 Juice preparation and plasma treatment

The collected oranges were washed with mild hot water, divided into two parts by a sterile knife, and manually squeezed using a sterile Dynore stainless steel juicer. The juice was then filtered through a sterilized double-layer muslin cloth in a sterile environment of the laminar airflow chamber (IIC 124-1A, Icon Instruments Company, India). All materials used for juice squeezing and analysis were sterilized in an autoclave at 121°C for 20 min. The juice had a total soluble solids (TSS) content of 12.20 ± 0.10 °Brix and a titratable acidity (TA) of 0.41 ± 0.02%. During atmospheric cold plasma (ACP) treatment, the gap between the electrodes was maintained at 15 mm. The orange (cv. Wakro) juice samples were treated under optimal conditions, including a juice depth of 4.6 mm, a voltage of 19 kV, and a treatment time of 2.6 min (obtained from Objective-1). The treated juice was then immediately transferred to sterile glass vials for storage study. Subsequently, the untreated and ACP-treated juice was stored in a refrigerator (10 ºC) and BOD biochemical oxygen demand (BOD) incubator (25 ºC) (IIC 109, Icon Instruments Company, India) at the Unit Operation Laboratory, Department of Food Engineering and Technology, Tezpur University. The changes in physicochemical properties, total viable counts (TVC), and yeast and mold counts (YMC) were analyzed every 3 days at intervals. The experimental plan for physicochemical and microbial analysis during storage is shown in **Fig. 5.2**. This study was carried out without using any preservatives in Wakro orange juice.

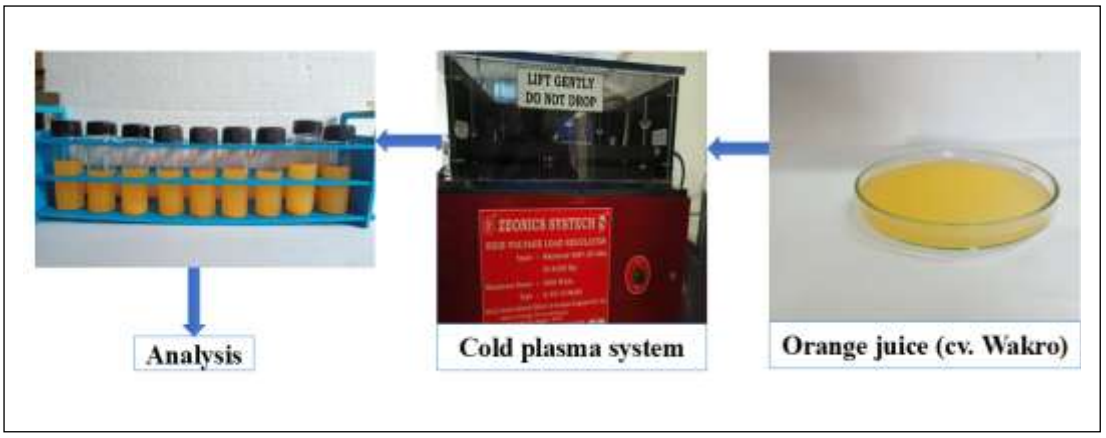

Fig. 5.1 Atmospheric cold plasma (ACP) treatment on orange (cv. Wakro) juice for storage study.

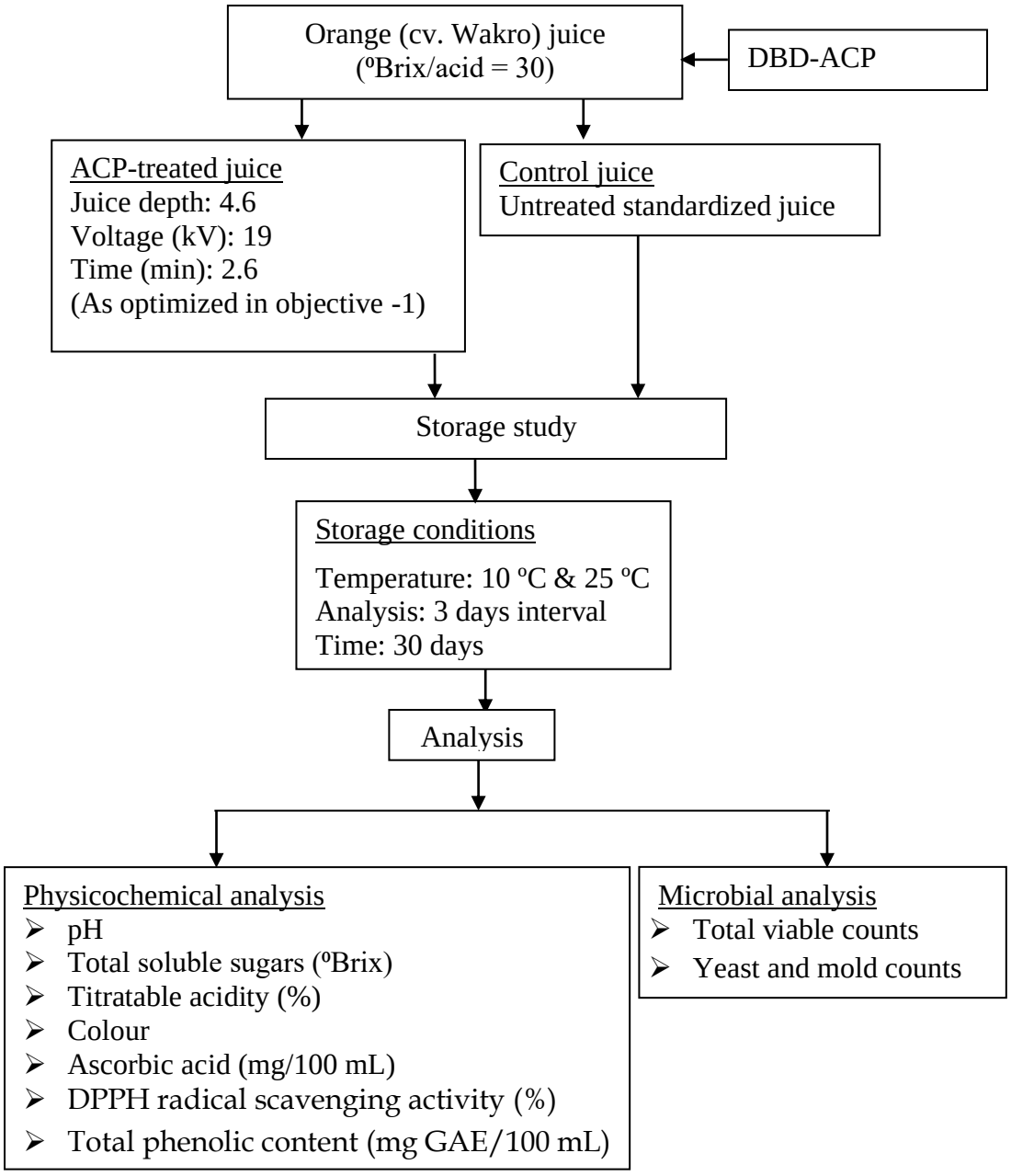

Fig. 5.2 Detailed work plan for Objective-4.

5.2.3 Total soluble solids and pH

The total soluble solids (TSS) and pH of untreated and ACP-treated orange (cv. Wakro) juice were measured after calibration (standard pH buffer: 4.0, 7.0, and 9.1) using a hand refractometer (range: 0–32 °Brix) (ERB-32, ERMA) and digital pH meter (pH 700, Eutech Instruments, Singapore).

5.2.4 Titratable acidity

The titratable acidity (TA) of the orange (cv. Wakro) juice was determined by the titration method (Ladanyia, 2010). For this analysis, 5 ml of filtered juice was mixed

with 20 ml of distilled water (1:4). Then, 5 ml of juice was removed from the mixture and titrated against 0.1 NaOH solution. A 1% phenolphthalein solution was used as an indicator. The TA on a percentage basis was calculated from **Eq. 5.1.**

$$Titratable\ acidity\ (\%) = \frac{Titre\ value \times 0.1\ N\ NaOH \times volume\ made\ up \times 64.04 \times 100}{Volume\ of\ juice\ taken\ for\ estimation \times volume\ of\ juice\ taken \times 1000} \quad (5.1)$$

5.2.5 Colour

The colour of untreated and ACP-treated juice was measured using Hunter Color Lab (Ultrascan VIS, Hunter Color Lab, USA) as to CIE parameters L^* (darkness to lightness), a^* (redness to greenness), and b^* (blueness to yellowness). The total colour change (ΔE) was computed from **Eq. 5.2** (Kumar et al., 2023).

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (5.2)$$

Where L_0^*, a_0^*, and b_0^* are the colour values of untreated juice; L^*, a^*, and $b^*$are the colour values of treated juice.

5.2.6 Ascorbic acid

The detailed procedure of ascorbic acid (AA) content determination in orange (cv. Wakro) juice is mentioned in **Section 3.2.5** of **Chapter 3**.

5.2.7 DPPH radical scavenging activity

The DPPH radical scavenging activity of orange (cv. Wakro) juice was evaluated according to the procedure adopted by Islam et al. (2019) and given in detail in **Section 3.2.8** of **Chapter 3**.

5.2.8 Total phenolic content

Folin–Ciocalteu Reagent (FCR) method was adopted for determination of total phenolic content of orange (cv. Wakro) juice (Illera et al., 2018) and the procedure is mentioned in **Section 3.2.9** of **Chapter 3**.

5.2.9 Microbial (total viable counts and yeast and mold counts) analysis

The microbial load in untreated and ACP-treated orange (cv. Wakro) juice samples was determined using the serial dilution-pour plate method (Swami Hulle et al., 2015; Guerrouj et al., 2016). Microbial populations in juice samples were evaluated in terms of total viable counts (TVC) and yeast and mold counts (YMC). Nutrient agar (NA)

and potato dextrose agar (PDA) were utilized as microbial growth media for total TVC and YMC, respectively. For analysis, 15–20 mL of sterile agar solution was poured into each sterile petri plate and allowed to solidify for 30 min at ambient temperature in the laminar airflow chamber. To prepare dilutions, 1 mL of juice sample was transferred to a sterile dilution tube containing 9 mL of sterile saline water (0.85 % NaCl). The dilution tubes were shaken for proper mixing. After that, 0.1 mL of each diluted solution was poured onto the surface of the previously agar-filled petri dish. The inoculated culture was spread throughout the surface of the agar medium for uniform distribution using a sterile spreader. Subsequently, plates were covered and immediately kept in the chamber of the BOD incubator. The incubation conditions for TVC and YMC were set at 37 ºC for 48 h and 25 ºC for 72 h, respectively. After incubation, the colonies formed in the plates were counted manually and measured as colony-forming units (CFU/mL) using **Eq. 5.3**. The analysis's detection limit was 10 CFU/mL (1 log CFU/mL) (Swami Hulle et al., 2015). An arbitrary 0.5 log10 CFU mL^{-1} value was chosen while colonies were not shown on the plates or less than the detection limit (Varela-Santos et al., 2012). The experiment was conducted in duplicate for microbial analysis, and the mean value of the $\log_{10}$ CFU/mL sample was recorded.

$$CFU/mL = \frac{Number\ of\ colonies \times total\ dilution\ factor}{Volume\ of\ culture\ plated\ in\ mL} \quad (5.3)$$

5.2.10 Statistical analysis

The data obtained were expressed as mean ± standard deviation (SD). The significance test at a 95% confidence interval was conducted using the Duncan multiple range test in the SPSS program (IBM SPSS Statistics, USA). A significant difference was taken as $p < 0.05$.

5.3 Results and Discussion

5.3.1 Total soluble solids

The TSS is a key indicator of the juice's sweetness, flavour, and overall quality (Magwaza and Opara, 2015). The initial TSS of squeezed orange (cv. Wakro) juice was 12.2 ± 0.10 °Brix, and ACP treated at optimum conditions (juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min) was 12.10 ± 0.10 °Brix. In a sample

stored at 10 ºC, the TSS of untreated juice was found to have an insignificant effect from 0-6 days of storage period ($p > 0.05$) (**Fig. 5.3**). Storage at 25 ºC, TSS of untreated juice was also found to be significantly different from 3 to 6 days ($p > 0.05$); however, compared with the 0^{th} day, it showed a minor change which is significant ($p < 0.05$) (**Fig. 5.3**). In this study, untreated juice samples stored at 10 ºC and 25 ºC were discarded after 9 days due to the development of foul odor. On the other hand, the ACP-treated juice was stored continuously, and the data was recorded after each interval (3 days) of up to 30 days. At 10 ºC, the TSS of ACP-treated juice had no significant variation from 12.10 ± 0.10 to 12.23 ± 0.06 ºBrix at the end of 6^{th} day ($p < 0.05$). Further, there was a slight reduction in TSS by the 12^{th} day (**Fig. 5.3**). At 25 ºC, the TSS of ACP-treated juice showed insignificant variation from 0 to 3 days; after that, minor changes were observed with significant differences ($p < 0.05$) at the end of the 6^{th} day. However, there was no significant difference ($p > 0.05$) in TSS from the 12^{th} to the 16^{th} day (**Fig. 5.3**). The rise of TSS may be due to the conversion of starch into simple sugars (Duque et al., 1999) and decreased probably due to re-conversion of sugars into polysaccharides (Wills et al., 1980). As a result, the TSS of orange juice underwent a cyclic change with minor variations under the storage period of 30 days (**Fig. 5.3**). However, changes in TSS of untreated and ACP-treated juice during storage are influenced by temperature and hydrolysis processes which can lead to both decreases and increase in TSS over the storage time. ACP-treated juice maintained its TSS with only slight fluctuations, likely due to enzymatic processes like starch conversion to sugars and vice versa, without significant degradation in quality (Bisht et al., 2021). Kumar et al. (2024) observed that the TSS in ACP-treated kiwifruit juice showed insignificant variations with slight changes throughout the storage period.

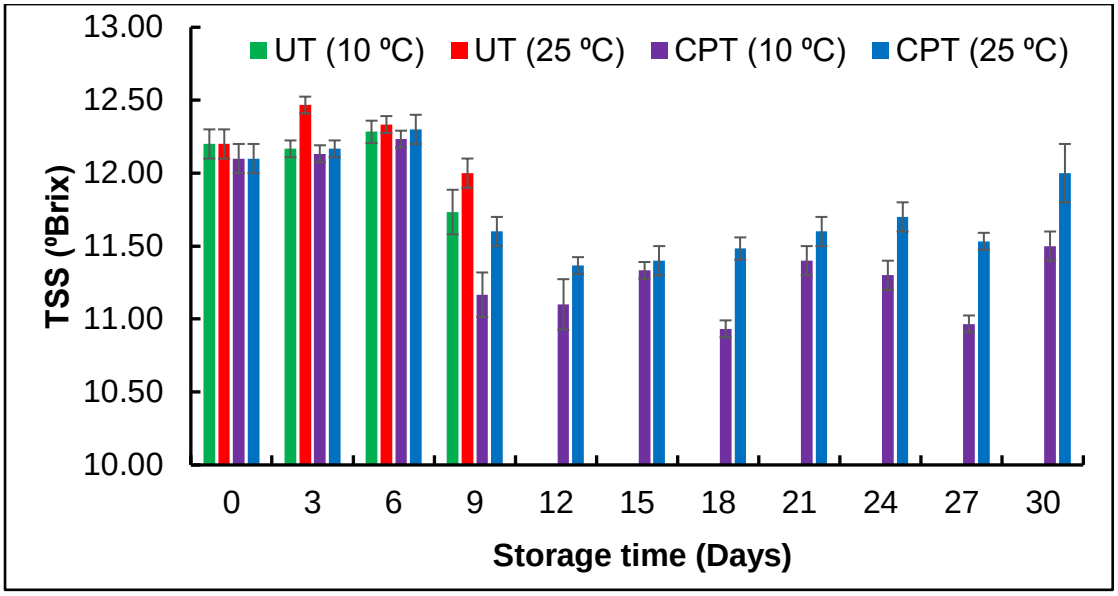

Fig. 5.3 Variation of total soluble solids (TSS) in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.3.2 pH

The pH of juice during storage is generally influenced by storage temperature, acidity, and ascorbic acid degradation (Tabikha et al., 2010). The initial pH of untreated and ACP (juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min) treated orange (cv. Wakro) juice was 3.49 ± 0.02 and 3.48 ± 0.01, respectively, indicating there is no significant difference ($p > 0.05$). Juice stored at 25 ºC, the pH of untreated juice was found to have no significant variation ($p > 0.05$) from 3 to 6 days, whereas a statistically significant change was observed at the end of the 9th day ($p < 0.05$). Also, at 10 ºC, pH of untreated juice was observed to have a minor change that ranged from 3.49 ± 0.02 to 3.56 ± 0.01 over 9th day (**Fig. 5.4**). Samples stored at 25 ºC, the pH of ACP-treated juice was found to be both statistically significant ($p < 0.05$) and non-significant ($p > 0.05$) change at different periods over 30 days (**Fig. 5.4**). **Fig. 5.4** shows that pH of ACP-treated juice was gradually changed from 3.49 ± 0.02 to 3.64 ± 0.01 at the end of a 30th day, storage at 10 ºC. The minor change in the juice pH may be due to the hydrolysis of polysaccharides to disaccharides and monosaccharides (Rehman et al., 2014). The active acidity in the ACP-treated juice was remained insignificant or somewhat increased with the storage period. The study's results indicate that storage temperature and acidity had a significant impact on the pH. However, the slight rise in pH probably does not impact the overall quality of the juice. The findings of the study are similar with Starek-Wójcicka et al. (2022), who

observed an increase in pH of CP-treated tomato juice during storage period of 10 days. Research by Kumar et al. (2024), who observed there was no significant effect on pH of ACP-treated juice.

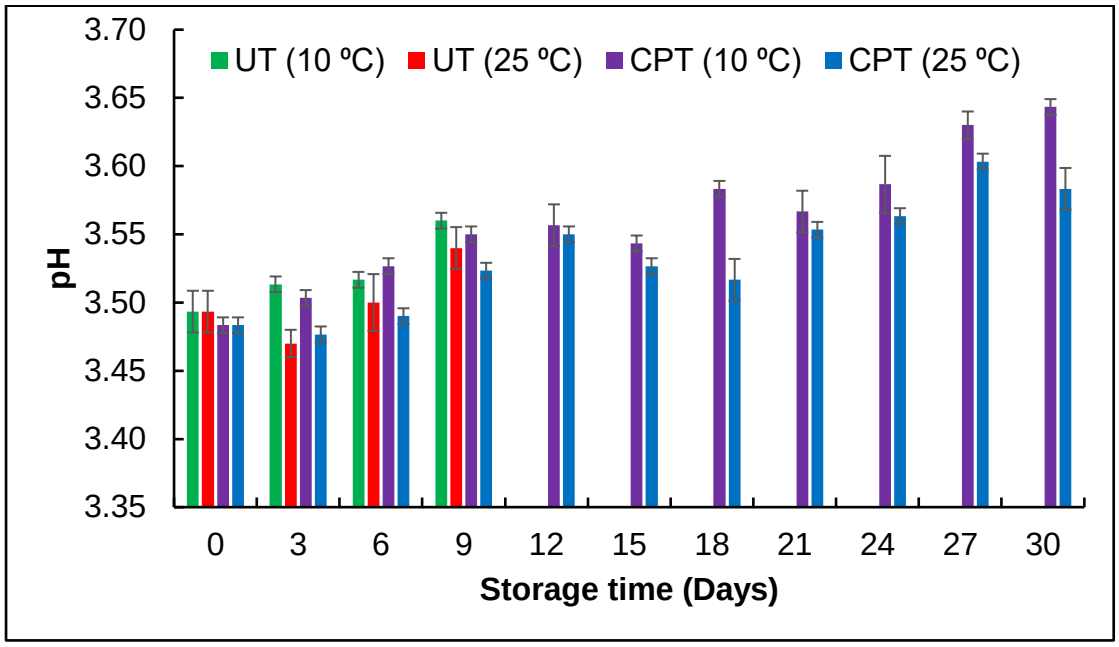

Fig. 5.4 Variation of pH in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.3.3 Titratable acidity

The TA is an important parameter for evaluating fruit juices' freshness, quality, stability, and safety during storage. It reflects the insight of chemical and microbial stability, flavor maintenance, and overall preservation of fruit juice (Kaddumukasa et al., 2017; Punia Bangar et al., 2022). The initial TA of untreated and ACP (juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min) treated orange (cv. Wakro) juice was found to be 0.41 ± 0.02% and 0.42 ± 0.03%, respectively, indicating an insignificant effect ($p > 0.05$). Sample storage at 25 ºC, the TA of untreated (UT) juice ranged from 0.41 ± 0.02% to 0.37 ± 0.02% at the end of the 6th day under storage, showing insignificant variation ($p > 0.05$). At 10 ºC, TA in the juice was observed to have insignificant variation over the 9th day ($p > 0.05$) (**Fig. 5.5**). It was observed that the TA in ACP-treated juice was minorly improved from 6 days to 15 days and then gradually decreased till 30th day under storage at 25 ºC (**Fig. 5.5**). TA in ACP-treated juice was observed as 0.45 ± 0.03% at the 15th day. However, after 15 days, it gradually decreased at the end of 30 days with a value of 0.34 ± 0.02% under storage temperature at 10 ºC (**Fig. 5.5**). TA can be inversely correlated

with the pH of juice as the titratable acidity decreased while pH increased. Rehman et al. (2014) suggested that the hydrolysis of polysaccharides into sugars may also increase acidity. On the other hand, a decrease in TA could be attributed to the growth of microbial populations and enzymatic activity, which may metabolize organic acids in the juice, leading to a decrease in acidity (Kaddumukasa et al., 2017; Sindhu and Khatkar et al., 2018). However, an increase or decrease of TA of juice within the storage period may also be influenced by temperature, time, and some of the complex biochemical reactions.

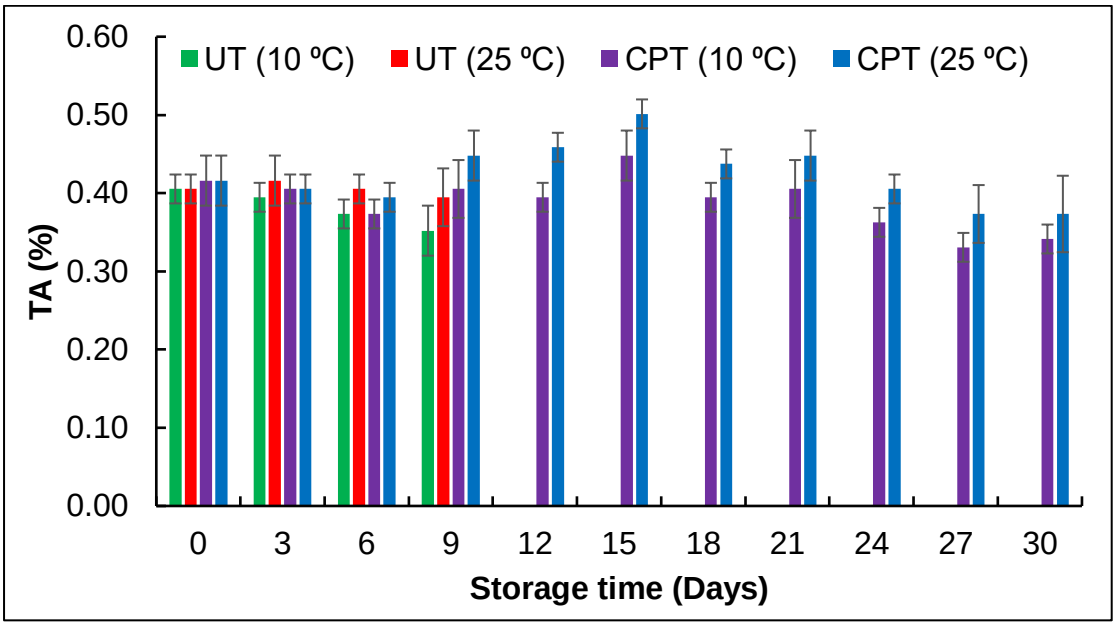

Fig. 5.5 Variation of titratable acidity (TA) in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.3.4 Total colour change

Colour is one of the most significant qualitative characteristics of fresh and processed foods influencing consumers' preferences (Tuly et al., 2023). The total colour change (ΔE) in orange (cv. Wakro) juice after ACP treatment and during storage has been calculated using **Eq. 5.2**. The change of colour, ΔE, was compared between untreated and ACP-treated juice at 10 ºC and 25 ºC during storage. After ACP treatment at optimum conditions (obtained from objective-1 as juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min), ΔE (0.35 ± 0.06) showed a significant effect ($p < 0.05$). As shown in **Fig. 5.6**, when stored at 10 ºC, ΔE of untreated juice was increased after the 6th day. On the other hand, storage at 25 ºC, ΔE of untreated juice was increased after 3rd day (**Fig. 5.6**). At 10 ºC, the ΔE in ACP-treated juice was

slightly increased from 0.35 to 0.40 up to 6 days, and after 12 days it was further increased more. At the end of the 30th day of storage, the value was 1.95 ± 0.05. At 25 ºC, the Δ*E* of ACP-treated juice ranged from 0.39 ± 0.04 to 3.21 ± 0.02, as shown in **Fig. 5.6**. The colour change involves may be due to several biochemical reactions, namely phenolic oxidation, non-enzymatic browning, polymerization and AA degradation during storage, respectively, leading to a change in colour in juice (Tuly et al., 2023; Kumar et al., 2024). The ΔE in ACP-treated juice was slower, with a more gradual increase in color differences compared to untreated juice. This indicates that ACP treatment helped preserve the juice's visual quality, making it more appealing to consumers during storage.

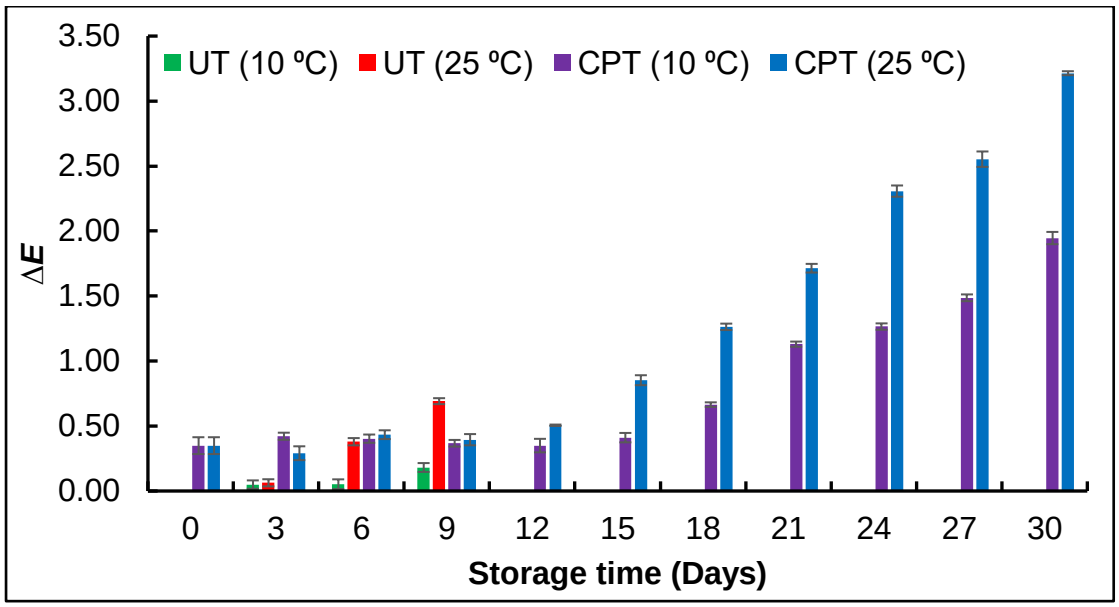

Fig. 5.6 Variation of total color difference (Δ*E*) in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.3.5 Ascorbic acid

The AA is an essential bioactive compound in orange juice's nutritional composition. On the 0th day of storage, the AA content of untreated and ACP (juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min) treated juice was 36.35 ± 1.34 mg/100 mL and 27.02 ± 1.27 mg/100 mL, respectively. The AA content of both untreated and ACP-treated juice was significantly reduced with increasing the storage days and temperature ($p < 0.05$), as shown in **Fig. 5.7**. At 25 ºC, the degradation of AA in untreated juice ranged from 36.35 ± 1.34 to 3.04 ± 0.66 mg/100 mL over 9 days; while at 10 ºC, it ranged from 36.35 ± 1.34 to 7.02 ± 1.17 mg/100 mL till 9 days of

storage. This revealed that the AA reduction in stored juice at 25 ºC was more than the degradation that occurred at 10 ºC (**Fig. 5.7**). At 25 ºC, AA concentration in ACP-treated juice reduced from 27.02 ± 1.27 to 3.51 ± 1.17 mg/100 mL till end of 9th days. In contrast, at 10 ºC, the concentration decreased from 27.02 ± 1.27 to 1.56 ± 0.68 mg/100 mL till the end of the 12th day period (**Fig. 5.7**). The slower reduction in antioxidant activity in ACP-treated juice at 10 °C indicates reduced oxidative damage during storage, helping preserve juice quality and nutritional value longer. However, this degradation of AA in juice during storage could be attributed to several factors such as temperature, light, oxygen exposure, storage duration, respectively (Huelin et al., 1953). However, storage temperature plays an important role in AA reduction. Since AA is highly sensitive to heat, degradation caused both its anaerobic and aerobic breakdown to be accelerated at higher temperatures, such as 25°C (Amaro et al., 2024). The study's results revealed that the storage temperature and time significantly affected the AA content in juice during storage. Although both untreated and ACP-treated juices showed a reduction in AA content over time, the ACP-treated juice demonstrated slower degradation of AA, particularly at lower temperatures (10°C). Souza et al. (2023) and Elez-Martínez et al. (2006) reported similar observations in untreated and ACP-treated juice.

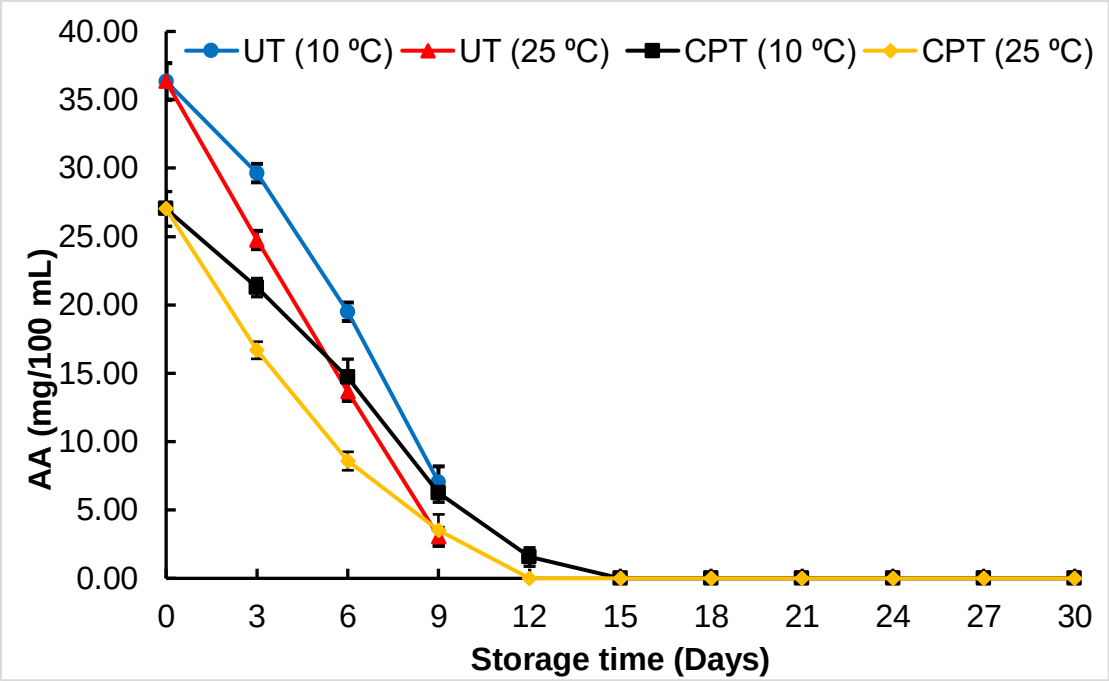

Fig. 5.7 Ascorbic acid (AA) reduction in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.3.6 DPPH radical scavenging activity

DPPH radical scavenging activity assesses the antioxidant capacity of various substances in fruit juices (Baliyan et al., 2022). The DPPH scavenging activity of both untreated and ACP-treated orange (cv. Wakro) juice was significantly reduced and influenced by storage conditions (**Fig. 5.8**). The initial DPPH radical scavenging activity of the untreated and ACP (juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min) treated juice was 69.74 ± 69% and 51.99 ± 0.59%, respectively, which was decreased while storage and high temperature. DPPH radical scavenging activity of untreated juice in glass vials bottles reduced from 69.74 ± 69% to 33.20 ± 0.58% at 10 ºC and 69.74 ± 69% to 25.29 ± 0.70% at 25 ºC till the end of the 9^{th} days period (**Fig. 5.8**). On the other hand, DPPH radical scavenging activity of ACP treated orange juice degraded from 51.99 ± 0.59% to 1.49 ± 0.40% at 10 ºC over 21 days and 51.99 ± 0.59% to 4. 41 ± 1.07% at 25 ºC over the storage period of 15 days (**Fig. 5.8**). Storage at 10°C resulted in less degradation of DPPH radical scavenging activity in ACP-treated juice compared to higher temperatures over the storage period, leading to reduced oxidative damage. This indicates storage temperature was significantly influenced on juice. However, the reduction of DPPH radical scavenging activity during storage could be attributed to mainly due to heat, duration of storage, and AA degradation (Kim et al., 2020). The observations of ACP-treated orange (cv. Wakro) juice were consistent with the investigation conducted by Illera et al. (2019), who reported that DPPH radical scavenging activity decreased during storage and was affected by time and temperature.

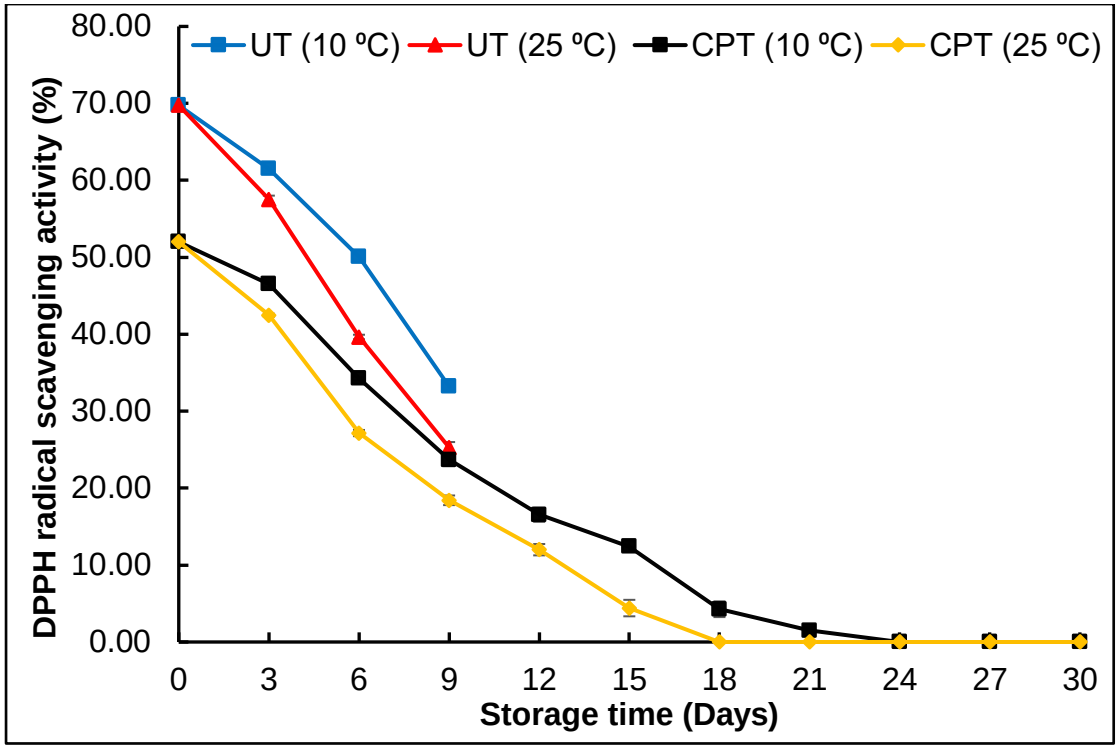

Fig. 5.8 DPPH radical scavenging activity reduction in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.3.7 Total phenolic content

The total TPC of untreated orange (cv. Wakro) juice in glass vials varied from 46.23 ± 0.69 to 22.66 ± 0.94 mg GAE/100 mL and 46.23 ± 0.69 to 16.36 ± 0.52 mg GAE/100 mL over the storage time of 9 days at 10 ºC and 25 ºC, indicating both storage period and the temperature had significantly affected the TPC ($p < 0.05$) (**Fig. 5.9**). On the other hand, ACP (juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min) treated was gradually decreased from 51.33 ± 0.45 to 43.97 ± 0.26 mg GAE/100 mL over 6 days at 10 ºC and then increased to 51.93 ± 0.69 at the end of 12th day. Subsequently, a decreasing trend was observed up to the end of the 30th day of the storage period (**Fig. 5.9**). At 25ºC, TPC in ACP-treated juice was significantly reduced from 51.33 ± 0.45 to 9.16 ± 0.94 mg GAE/100 mL over 30 days (**Fig 5.9**). The TPC reduction in the juice during storage may be influenced by temperature, light, storage, and time, respectively (Kim et al., 2020). However, an increase in TPC of ACP-treated juice during storage could be attributed to physiological stress cellular disruption leading to enhanced synthesis of phenolic compounds (Munekata et al., 2020). The findings of this investigation are in line with the observation of Souza et al. (2023), who also reported that the TPC increased in CP treated 'Lima' orange juice

from 8 to 12 days during storage at 2 ± 1 °C over 16 days. In this study, ACP-treated juice maintains higher TPC levels at 10°C and shows better resilience to temperature-induced degradation compared to untreated juice, where both storage time and temperature significantly impact TPC.

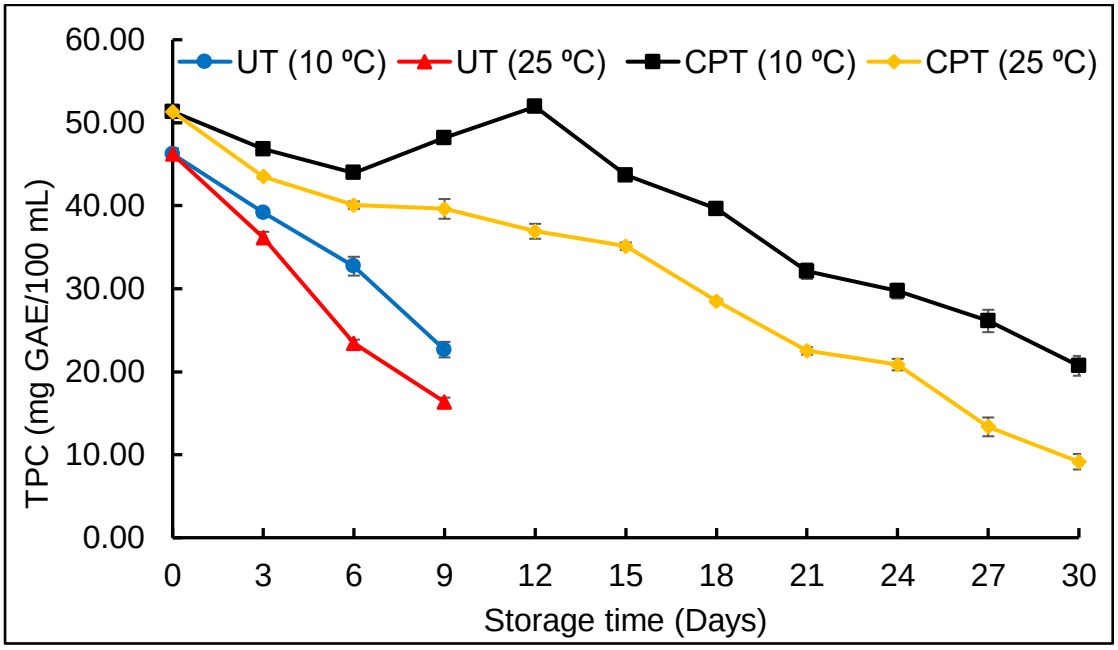

Fig. 5.9 Variation of total phenolic content (TPC) in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.3.8 Total viable counts

The growth of microbial populations influences the shelf life and organoleptic characteristics of juice. The TVC in both untreated and ACP (juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min) treated orange (cv. Wakro) juice was found to be less than the detection limit (1log CFU/mL) at 0^{th} day. In this study, the odour was developed in untreated samples and was discarded after 9 days. The TVC in untreated orange (cv.Wakro) juice was observed to be insignificant ($p > 0.05$) from 0-3 days period. However, after 3^{rd} day, the log CFU/mL value was significantly increased at 10 ºC ($p < 0.05$) (**Fig. 5.10**). At 25 ºC, TVC in juice was observed to be 3.20 ± 0.12 log CFU/mL at the end of 3^{rd} day of storage, which was significantly increased to 4.92 ± 0.04 log CFU/mL by 9 days (**Fig. 5.10**). As shown in **Fig. 5.10**, TVC in ACP-treated juice remained unchanged from 0 to 9 days storage period at 10 ºC, demonstrating excellent microbial stability. However, the log CFU/mL values were significantly increased after 9^{th} day to the end of 30^{th} day (**Fig. 5.10**). However,

at 25 ºC, TVC of ACP-treated juice was significantly high after 3rd day during storage (**Fig. 5.10**). These investigations are in agreement with the previous study, which demonstrated that ACP treatment reduces the microbial populations in kiwifruit and pineapple juice (Kumar et al., 2024; Pipliya et al., 2024). In this study, Off-odour in untreated samples led to product rejection after 9 days, while ACP treatment delayed this issue significantly by maintaining microbial control, reducing the likelihood of spoilage-related odours. ACP treatment effectively maintained low microbial counts at 10°C for an extended period. Although microbial growth increased at 25 °C after 3 days, ACP-treated juice still showed better microbial control than untreated juice under the same conditions.

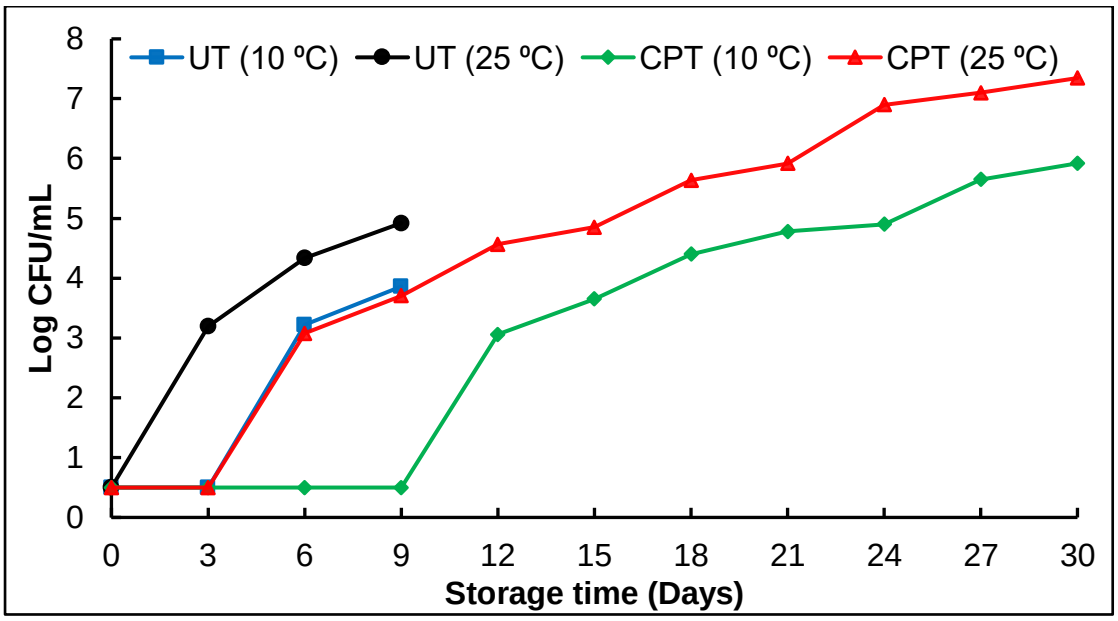

Fig. 5.10 Variation of total viable counts (TVC) in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.3.9 Yeast and mold counts

The YMC of both untreated and ACP (juice depth: 4.6 mm, voltage: 19 kV, and treatment time: 2.6 min) treated orange (cv. Wakro) juice were also observed to be less than the detection limit (1log CFU/mL) at 0th day of storage. YMC of untreated and ACP-treated juice was significantly increased with the increase in storage days and temperature ($p < 0.05$) (**Fig. 5.11**). As observed in **Fig. 5.11**, log CFU/mL values in untreated juice at 25 ºC exhibited higher, indicating that high temperature significantly impacted the YMC. At 10 ºC, YMC in ACP-treated juice showed an insignificant effect on the log CFU/mL increment from 0-6 of the day storage period.

In contrast, after this period, it significantly increased with the storage period of up to 30 days (**Fig. 5.11**). At 25 ºC, YMC in ACP treated was observed at 3.06 ± 0.08 log CFU/mL at the end of 3 days, which was then significantly increased to 8.25 ± 0.03 log CFU/mL over 30 days (**Fig. 5.11**). The lower YMC in ACP-treated juice might be due to the cell wall damage resulting from the ACP-generated reactive species, which may restrict the growth of microbes (Punia et al., 2022). The results of the study are in line with the findings of Pipilya et al. (2024). At 10°C, the YMC in ACP-treated juice showed insignificant growth from day 0 to 6, indicating excellent microbial control during this initial storage period. In contrast, the untreated juice exhibited significant YMC growth as early as the start of storage, demonstrating inferior microbial stability.

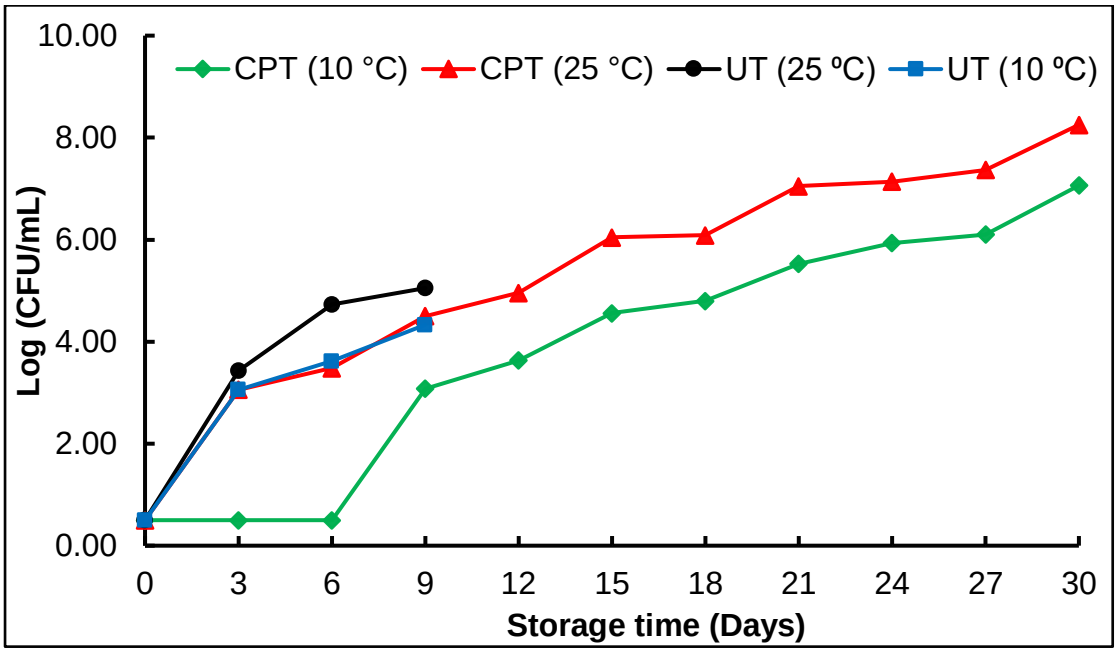

Fig. 5.11 Variation of yeast and mold counts (YMC) in untreated (UT) and cold plasma treated (CPT) orange (cv. Wakro) juice during storage.

5.4 Conclusion

The current study investigated the variation of physicochemical parameters (TSS, pH, TA, Δ*E*, AA, DPPH radical scavenging activity, TPC) and microbial counts (TVC and YMC) of untreated and ACP-treated orange (cv. Wakro) juice during storage 10 ºC and 25 ºC. The quality of orange (cv. Wakro) juice was significantly influenced by storage temperature, duration, and processing conditions. TSS, pH, and TA fluctuated with minor variation during the storage period of 30 days. Total color change, Δ*E* in

ACP-treated juice was significantly increased at the end of the 30th day with the values of 1.95 ± 0.05 and 3.21 ± 0.02 at 10 ºC and 25 ºC. However, AA, DPPH, and TPC in stored juice were decreased at both storage temperatures (10 ºC and 25 ºC). The higher degradation rate was observed at room temperature (25 ºC). The TVC in ACP-treated juice was significantly increased, though an insignificant variation (p > 0.05) was observed in 0 to 9 days at 10 ºC. The YMC in ACP-treated juice was observed to have an insignificant change (p > 0.05) at the end of the 6th day at 10 ºC. However, ACP treatment significantly reduced the growth of microbial populations, as shown by lower total viable counts (TVC) and yeast and mold counts (YMC) compared to untreated juice. This contributed to a longer shelf life and reduced the risk of spoilage.

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