

Final Project Completion Report

Project Title: Non-thermal processing of Assam lemon juice using ozone processing

Project Sanction: ASTEC/S&T/1614/12/2020-21/138-144 Dt. 01.02.22

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1. Introduction

Assam lemon (Kaji Nemu) stands as a vital fruit crop within the tapestry of northeastern India. Its distinctive essence, merging fragrance, flavor, and nutritional richness, has not only enchanted locals but has also gained recognition beyond regional borders. Scientific name is *Citrus limon Burm. f* and belonging to the citrus family Rutaceae, with a chromosome number (2n) of 18 (Rathour et al., 2023a), Assam lemon's allure transcends mere taste and cultural significance. Its nutritional profile, rich in vitamin C, immune system bolstering antioxidant and supports skin health and overall well-being. Furthermore, it harbors essential minerals such as potassium, calcium, and magnesium, vital for maintaining bodily functions. Beyond its culinary prowess, Assam lemon finds utility in indigenous healing practices, where its juice and peel aid digestion, alleviate cold symptoms, and soothe throat irritations. Its robust vitamin C content fortifies the immune system, serving as a shield against illnesses. Yet, Assam lemon's versatility extends even further. Its essential oils (Chetia et al., 2023) and zest are harnessed in aromatherapy and cosmetics, lending a rejuvenating touch to products spanning perfumes to skincare formulations. In essence, Assam lemon symbolizes not only the biodiversity but also the cultural richness of northeastern India.

In Assam, the land dedicated to cultivating Assam lemon measured 15.90 hectares, yielding a harvest of 157.32 metric tons during the period of 2021-2022, as per NHB records. This indigenous lemon variety, revered for its distinctive aroma and potent antioxidant properties, earned the esteemed title of state fruit in February 2024, bestowed upon it by the Assam government. Among the varieties, Kaji Nemu stands out with its elongated, oblong shape and seedless nature, attaining GI certification (T. P. Rathour et al., 2024). Rooted in the verdant lands of Assam, nestled amidst the eastern Himalayas and the Brahmaputra River basin, Assam lemon boasts a rich heritage. Beyond its role as a mere fruit, it holds a significant place in the culinary and cultural tapestry of Assam and its neighbouring regions. Its tangy zest enhances a myriad of dishes, elevating flavours and imparting a refreshing tang to beverages and condiments. Renowned for its acidic punch, it finds its way into vegetable dishes, fish, meat, and salads, as well as in the preparation of invigorating cold drinks, cordials, and preserves (Mukhim et al., 2015). The zest and juice of this lemon are prized ingredients in traditional Assamese cuisine, enhancing everything from curries to desserts, forming an integral part of Assamese meals. While its cultivation sprawls across various

northeastern states like Manipur, Mizoram, and Tripura, Assam remains the primary producer, followed closely by Manipur (Rathour et al., 2022b).

1.1 Thermal and non-thermal processing of juice

Consumers today are increasingly seeking out products that are not just safe, but also natural and packed with nutrition. Achieving this delicate balance involves meticulous control over the processes that alter the nutritional and quality attributes of food products. For instance, when it comes to fruit juices, conventional methods like heat treatment are often employed to eliminate foodborne microorganisms and deactivate enzymes like pectin methyl esterase (PME). However, such methods come with a trade-off; while they enhance safety, they can compromise the taste and nutritional value of the juice (Iqbal et al., 2022). To address this challenge, the industry commonly employs pasteurization, a process that rapidly heats and cools the juice to ensure microbial safety. Nevertheless, the quest for safer and more nutritious juice has spurred the exploration of alternative pasteurization methods that do not rely on high temperatures. Some of these innovative techniques include ozone treatment, ultraviolet light exposure, application of electric fields, and high-pressure processing. To meet regulatory standards set by authorities like the U.S. Food and Drug Administration (USFDA), these methods must demonstrate their efficacy in reducing microorganisms while preserving the quality and nutritional content of the juice (Shah et al., 2019 and Fundo et al., 2018). Exploring innovative methods in juice processing, ozone treatment emerges as a promising alternative, bypassing the need for heat or chemical additives. Back in 2001, the FDA's green light for ozone's direct use in food sparked considerable interest within the industry. This led to a surge in its adoption by juice producers in the US and Europe, prompting the FDA to issue industry guidelines in 2004. With its GRAS status, ozone stands out as a potent oxidant, widely utilized in both gaseous and aqueous forms for disinfection purposes in food safety protocols (Porto et al., 2020).

Ozone, a molecule comprising three oxygen atoms and is colorless with a bitter smell, exhibits potent antimicrobial properties, making it effective in destroying a wide range of microorganisms, including bacteria, viruses, and protozoa (Iqbal et al., 2022). Notably, ozone's environmentally friendly nature, as it decomposes into harmless by-products, further enhances its appeal as a food disinfectant. However, the effectiveness of ozone treatment can vary based on factors such as dosage, juice acidity, temperature, humidity, and composition. Additionally, it's crucial to recognize that ozone has a relatively short lifespan and requires careful handling to maximize its efficacy (Shah et al., 2019). The half-life of aqueous ozone

is only 20 min at 20°C, which is considered highly unstable and quickly degrades into oxygen (Miller et al., 2013). Its efficacy extends to various microorganisms, encompassing fungi, Gram-positive and Gram-negative bacteria, protozoa, and viruses (Cullen et al., 2009). Notably, ozone finds application in diverse juice sectors like apple, orange, and tomato juice processing without altering crucial parameters like pH, acidity, or enzymatic browning (Shah et al., 2019) [42]. Ozonation treatment, particularly for 15 minutes, reduced microbial activity to acceptable levels and enhanced the overall quality characteristics of the juice (Iqbal et al., 2022). Ozone processing of Kinnow juice resulted in changes in various physicochemical characteristics, such as total soluble solids, acidity, total phenolics, total flavonoids, total antioxidant activity, and DPPH radical scavenging activity, which are important factors in determining the shelf life of the juice (Iqbal et al., 2023). Furthermore, research on sugarcane juice demonstrates ozone's ability to enhance antimicrobial properties and minimize enzymatic activity, preserving taste and nutrition (Panigrahi et al., 2020). Yet, while its potential benefits are evident across different juice types, its impact on beverages like Assam lemon remains largely unexplored. Hence, ongoing research endeavors seek to unravel ozone's effects on the nutritional content, microbiology, enzyme activity, and shelf life of Assam lemon beverages at $4 \pm 2^\circ\text{C}$, $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ storage conditions for 28 days.

1.2 Ozone generation

Ozone naturally occurs in the lower atmosphere at relatively low concentrations. It predominantly resides in the stratosphere, reaching levels of up to 6 parts per million (ppm). This natural production of ozone primarily stems from two sources: lightning and ultraviolet (UV) radiation. Ozone, characterized by its high reactivity and instability, is typically generated on-site for various commercial applications. Its lifespan in distilled water at 20°C ranges between 20 to 30 minutes (Miller et al., 2013). The process of ozone generation involves the rearrangement of atoms, particularly the breaking of strong O-O bonds by significant energy input, resulting in the formation of free oxygen radicals. Commercially, ozone production relies on four recognized methods: electrical or corona discharge, electrochemical, UV, and radiochemical methods. From these methods we are using electrical or corona discharge method, often referred to as "silent discharge" operates by ionizing molecular oxygen through high-power alternating current. In this method, dried air or oxygen passes through an electric field generated between two high-voltage electrodes, separated by a dielectric material, commonly glass. Initially, the electrical current causes the splitting of oxygen molecules (O_2) into oxygen atoms (O); subsequently, these atoms combine with the

remaining oxygen molecules to form ozone (O_3). During this process, approximately 80% of the applied energy transforms into heat, necessitating immediate removal to prevent ozone decomposition into oxygen molecules and atoms. Typically, the gas discharged from an ozonator yields between 3% to 6% ozone when high-purity oxygen serves as the feed gas, whereas dry air yields only 1% to 3% ozone (Tiwari et al., 2008). The advantages of this method include the efficient generation of high ozone concentrations, the durability of the corona cell in comparison to UV lamps, and its cost-effectiveness, particularly for large-scale installations.

1.3 Kinetic modelling

In the realm of food science, understanding the kinetics of changes is crucial for ensuring product quality and safety. Kinetic modelling provides a powerful tool for predicting and managing these changes over time. Kinetic modelling in food science, focusing on models utilized to predict quality changes. Kinetic modelling involves the mathematical representation of chemical and biological processes occurring in food products over time. It enables researchers and industry professionals to predict the rate of changes such as microbial growth, enzyme activity, and chemical reactions, thereby aiding in the development of effective preservation strategies and shelf-life extension techniques. By incorporating factors such as storage temperature, packaging materials, and oxygen exposure into mathematical models, researchers can gain a deeper understanding of the kinetics underlying these changes and develop effective strategies for ensuring product quality and safety. Microbial activity plays a significant role in food spoilage and safety. Various mathematical models have been developed to predict the growth kinetics of microorganisms under different storage conditions. These models, such as the primary and secondary models, take into account factors like temperature, pH, and available nutrients to estimate microbial growth rates accurately. Enzymatic activity can lead to undesirable changes in food quality, such as texture loss and flavor deterioration. Kinetic models for enzymatic reactions help in understanding and predicting the changes of enzymes present in food matrices. These models consider factors such as substrate concentration, temperature, and enzyme activity to simulate the rate of enzymatic degradation accurately. For predicting shelf life, the study employed the Arrhenius equation to simulate the kinetic prediction across all quality parameters and shelf-life durations. The Arrhenius model, specifically proved effective in describing the temperature-dependent reaction rate constant by calculating the activation energy (E_a). This

approach applies to food products, allowing for the elucidation of each quality change based on reaction rate constants and temperature variations (Choosuk et al., 2022).

Ozone treatment for Assam lemon juice is not well-researched yet, this study wants to find out how it affects the juice's qualities like its physico-chemical properties, nutrients, microorganisms, and enzyme activity. Researchers have already tested ozone on other fruit juices like pomegranate, grapes, orange, and watermelon, but there's not much information about Assam Lemon juice.

Objectives

1. To study the influence of ozone treatment on physicochemical, nutritional, microbiological and enzymes in liquid food (Assam lemon juice)
2. To study the nutrient degradation kinetics in liquid food (Assam lemon juice) after ozone processing
3. To standardize the process parameters and study storage stability of liquid food (Assam lemon juice) treated with ozone processing in refrigerated storage

2. Methodology

2.1 Materials and Chemicals

Fresh Assam lemon (Kaji nemu), Plastic containers, Sodium hydroxide (NaOH), Phenolphthalein indicator, Sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent, Gallic acid, Metaphosphoric acid, L-ascorbic acid, 2,6-dichlorophenol-indophenol, Sodium bicarbonate (NaHCO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Methanol, Pectin, Nutrient agar, Potato dextrose agar, Petri dish, Cotton.

2.2 Experimental set up

2.2.1 Sample preparation

Fresh Assam lemons (Kaji nemu) were collected from the citrus garden, Tezpur University, Assam and were cleaned with Saryu vegetable and fruit cleaning powder to remove any pesticides and dirt. After that fruit peels were taken out properly with the help of sharp knife and juice was extracted by using SZC Portable juice extractor (Model no: B0BYYPKQ3G and worked at 3.7 Volts). The extracted juice was then filtered using muslin cloth and store the juice in sterile container for further use.

2.2.2 Preparation of Assam lemon beverage

Four different lemon juice concentrations were prepared: 25% lemon juice and 75% water (sample 1), 20% lemon juice and 80% water (sample 2), 15% lemon juice and

85% water (sample 3), and 10% lemon juice and 90% water (sample 4). To each concentration, sugar and salt were added in same quantities. The resulting beverages were then evaluated by a panel of tasters to assess their flavor balance, sweetness, sourness, and saltiness. This experiment aimed to provide insights into the optimal combination of lemon juice concentration, sugar and salt levels to create a well-balanced and palatable Assam lemon beverage.

2.2.3 Sensory evaluation

The resulting beverages were then evaluated by a panel of tasters to assess their flavor balance, sweetness, sourness, and saltiness. Sensory evaluation was conducted, employing a panel of semi-trained individuals, to assess four distinct lemon concentration levels in an Assam lemon beverage using Hedonic scale. The panelists preferred sample 4 over the other samples, signifying a higher level of overall satisfaction with its sensory qualities. This experiment aimed to provide insights into the optimal combination of lemon juice concentration, sugar and salt levels to create a well-balanced and palatable Assam lemon beverage. The selected sample will be used for further storage study.

2.2.4 Ozone treatment of Assam lemon beverage

Ozone gas is generated by using a water ozonizer (Ozone Air Sterilizer/ Disinfector, Model OZoZap, OSE-A1). This device was used to directly inject ozone gas into a sample. Both the treatment temperature (ambient) and the gas flow rate were fixed. The lemon beverage of 100 mL was treated with ozone gas for the periods of 10, 20 and 30 seconds in a closed environment (Fume hood) and ozone concentration for each treatment period was assessed using the Checker Disk Method (Hanna instruments HI38054 Ozone test kit). Values for standardized concentrations were recorded and tabulated. Untreated Assam lemon beverage (control = 0 seconds of ozone exposure time) and treated Assam lemon beverage (10, 20, 30 seconds ozone exposure time) were stored at $4 \pm 2^{\circ}\text{C}$ (Refrigerator), $15 \pm 2^{\circ}\text{C}$ (Matrix eco solutions B.O.D incubator Model: MES-1168) and $25 \pm 2^{\circ}\text{C}$ (Mettler incubator IN110) in sterile plastic containers for 28 days and mentioned parameters were examined for an interval of 7 days.

2.2.5 Nutritional analysis

2.2.5.1 Determination of pH

Using a digital pH meter (Eutech pH Meter, Model No: pH700), the beverage pH was determined. First, the pH meter was calibrated using commercial buffer solution at pH

4.0 and pH 7.0. Samples of lemon beverage were placed in a beaker and stirred with a magnetic stirrer while the temperature was allowed to reach room temperature. Before and after the treatment, measurements were made in triplicate.

2.2.5.2 Titratable acidity (TA)

The beverage samples titratable acidity was measured according to Tiwari et al., (2008). Samples that had been diluted with distilled water were titrated up to the phenolphthalein end point (pH 8.2) using standardized 0.1 N NaOH. Next, the formula was used to compute TA by converting the volume of NaOH to grams of citric acid per 100 mL of beverage.

$$TA = \frac{V \times 0.1N \text{ NaOH} \times 0.067 \times 100}{m}$$

Where, V is the volume (mL) of NaOH and m is the mass of lemon beverage.

2.2.5.3 Total phenolic content

The procedure that was used to determine the beverage total phenolic content was explained by Shah et al., (2019). The beverage sample were centrifuged for 5 minutes at 5000 rpm with a temperature of 4°C using a centrifuge (Eppendorf Centrifuge 5430 R, 230 V/50–60 Hz). 1 mL of centrifuged beverage sample, 1 mL of Folin-Ciocalteu reagent, and 10 mL of 20% sodium carbonate (Na₂CO₃) solution were placed in the 15mL centrifuge tube. The mixture was thoroughly mixed and allowed to incubate for one hour at room temperature in the dark (Khera incubator model no. KI-182). After that, using a UV Visible Spectrophotometer (Thermo Scientific Orion AquaMate Vis and UV-Vis Spectrophotometer 8100), the mixtures absorbance was measured at 765 nm. Data for total gallic acid concentrations between 0 and 500 mg/L were obtained from the calibration curve.

2.2.5.4 Ascorbic acid content

In accordance with the S Ranganna handbook of analysis and quality control, the ascorbic acid level of the lemon juice was determined.

Standardization of dye: 5 mL of a standard ascorbic acid solution was taken and mixed with 5 mL of 3% metaphosphoric acid (HPO₃). The mixture was titrated with 2,6-dichlorophenol-indophenol dye until a pink tint was visible for at least 15 sec. Then, the dye factor i.e., mg of ascorbic acid per mL of the dye, was then calculated using the following formula:

$$Dye\ factor = \frac{0.5}{titre}$$

Sample preparation: After measuring out 10–20 mL of lemon juice, 3% of HPO_3 was added to make 100 mL. First, a 2–10 mL aliquot of the sample HPO_3 extract was taken, and it was titrated with the standard dye to a pink end-point that should persist for at least 15sec. The results were expressed as mg of ascorbic acid per 100g or mL using the following formula:

$$\text{Asorbic acid} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume make up} \times 100}{\text{Aliquot of extract taken} \times \text{w. t or volume of sample taken}}$$

Note: Calculate the ascorbic acid content for the 10mL of juice present in the 100mL of lemon beverage (mass balance)

2.2.5.5 Antioxidant capacity

Shah et al., 2019 described the method for determining the lemon beverage radical scavenging activity against stable 2,2-diphenyl-1-picrylhydrazyl. To prepare a 0.1 mM stock solution, 4 mg of accurately weighed DPPH and 100 mL of pure methanol were dissolved and kept for future use at $20 \pm 1^\circ\text{C}$. 5 mL of the lemon beverage was taken and carefully mixed with 5mL of 80% methanol. After a thorough mixing, the mixture was placed in an incubator (Khera model no. KI-182) for 30 min. After that, the mixture was centrifuged for 10 min at 3000 rpm using a centrifuge (Eppendorf Centrifuge 5430 R, 230 V/50–60 Hz). Next, using a Vortex Mixer (Borosol Labquest 2800 rpm) 200 μL of centrifuged samples were vortexed and combined with 1 mL of the DPPH methanol solution in the dark after 30 min at room temperature. The absorbance of mixture was then measured at 515nm using UV Visible Spectrophotometer (Thermo Scientific Orion AquaMate Vis and UV-Vis Spectrophotometer 8100). The results were expressed as the percentage of decline of the absorbance using the following equation:

$$\text{DPPH}\% = \frac{(\text{A Control} - \text{A Sample})}{\text{A Control}} \times 100$$

Where, A control = Absorbance of DPPH solution and A sample = Absorbance of the sample

2.2.6 Enzymatic analysis

2.2.6.1 Pectin methyl esterase

Kimball et al., 1991 gave the method used to measure PME activity. The PME activity was expressed as PME $\mu\text{u/mL}$ which represents μ -equivalent of acid liberated per min per mL of sample at pH 7.7 and 30°C . where 40 mL of 1% pectin solution were mixed with 10 mL of lemon beverage. when the temperature of the mixture reached 30°C

using a pH-meter (Eutech pH Meter, Model No: pH700), the pectin-beverage mixture pH had been adjusted to 7.0 with 2.0 N NaOH and readjusted to 7.7 with 0.05 N NaOH. Subsequently, 0.1 mL of 0.05 N NaOH was added until pH of solution returned to 7.7 again, during which time was measured. PME activity was measured using the following equation and expressed as pectin methyl esterase units per mL of lemon beverage. A single unit of PME activity was determined to be the quantity of enzyme that releases 1.0 μ acid equivalent per minute under the assay conditions.

$$PME\ units/mL = \frac{NaOH \times V_{NaOH} \times 1000}{V_{juice} \times t}$$

Where NaOH is the NaOH concentration (0.05 N), V_{NaOH} is the volume of 0.1 mL of the NaOH solution used (0.05 N), V_{juice} is the volume of beverage (10 mL), and t is the time in minutes required for the solution to reach a pH of 7.7 after the addition of NaOH.

2.2.7 Microbial analysis

2.2.7.1 Total plate count

The plate count determination was carried out as per FDA's standard procedure of Bacteriological Analytical Manual described by FDA. In a laminar air flow (Genaxy scientific pvt ltd horizontal laminar air flow) 1 mL juice was placed in a test tube and adding a 9 mL saline solution was used for dilution. Diluents were added and shaken for 1-2 minutes at a low speed until the sample was fully cloudy. Dilutions of 10 mL were made by relocating 1 mL of the initial dilution to 9 mL of diluents. All dilutions were thoroughly agitated. Then, 12 to 15 mL of autoclaved (Equitron standard portable autoclave #7407ST, 2.0 kW) Nutrient agar was transferred into the petri dish (Disposable), after it solidifies add 1 mL of sample dilution and spread overall the plate. The plates were inverted over and were placed in an incubator (Khera bacteriological incubator model no. KI-182) for 48 hours at 37°C. After 2 days of incubation, plate counts were measured in every petri dish using digital colony counter (B.D. instrumentation digital colony counter Model: BDI-E51). The number of bacterial colonies in the sample was determined and multiplied by the reciprocal, and the findings were stated as log colony-forming units/mL (log CFU/mL) of lemon beverage. All analysis were carried out in duplicate.

2.2.7.2 Total fungal count

The fungal count was determined by the FDA standard procedure of the Bacteriological Analytical Manual, as explained by FDA. In a laminar air flow

(Genaxy scientific pvt ltd horizontal laminar air flow) 1 mL of juice sample was taken in a test tube. It was added 9 mL saline water and agitated for 1-2 minutes until the cloudy solution. 10 mL dilution was prepared; then, 1 mL was transferred in the previous 9 mL dilution. All dilutions were mixed well. Then, 12 to 15 mL of autoclaved (Equitron standard portable autoclave #7407ST, 2.0 kW) potato dextrose agar was poured into a petri dish (Disposable), after it solidifies add 1 mL of sample dilution and spread overall the plate. All potato dextrose agar plates were incubated (Mettler incubator IN110) at 20–25°C. After 2 days of incubation, mold counts were measured in every petri dish using digital colony counter (B.D. instrumentation digital colony counter Model: BDI-E51), and the findings were stated as log colony-forming units/mL (log CFU/mL) of lemon beverage. All analysis were carried out in duplicate.

3.3 Kinetic modelling

The data obtained from objective 1 will be used to study the kinetics changes of microbiological and enzymatic activity. Data (A) was plotted across Storage time (days) and different kinetic models (Table 1) will be applied and best model will be selected based on the adequacy of the fit and other statistical parameters (Table 2). The modelling was performed using the Origin pro 2024 software.

Table 1: Different models with respective kinetic equations

S.no	Model	Equation	Reference
1	Zero order	$A = A_0 + kt$	wibowo et al., 2015
2	First order	$A = A_0 \exp(kt)$	
3	Second order	$A = \frac{A_0}{1 + A_0 kt}$	

Note: A represents activity at time t, A_0 is the initial activity, k is the reaction rate constant at a given temperature (day^{-1})

Table 2: Different error functions and its equations

S.no	Error function	Expression
1	R^2 (Coefficient of determination)	$1 - \frac{\sum_{i=1}^n (Y_{pre} - Y_{exp})}{\sum_{i=1}^n (Y_m - Y_{exp})}$
2	χ^2 (Chi-square)	$\frac{\sum_{i=1}^n (Y_{pre} - Y_{exp})^2}{Y_{exp}}$
3	RMSE (Root mean square error)	$\sqrt{\frac{\sum_{i=1}^n (Y_{pre} - Y_{exp})}{n}}$

Note: Y_{pre} is the predicted value obtained from modelling, Y_{exp} is the observed value, Y_m is the average value and n is the no. of experiments.

2.4 Activation energy

The reaction rate constant (k) obtained from best model will be used to calculate the activation energy. By using Arrhenius equation (eq.1) temperature (kelvin) was plotted across reaction rate constant (k) for calculating activation energy (E_a).

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \dots\dots\dots \text{eq.1}$$

where E_a is activation energy (KJ/mol), R is the gas constant (8.314 J mol⁻¹), T is the temperature (K)

Results

4.1 Effect of ozone treatment and storage time on physico-chemical properties of Assam lemon beverage

4.1.1 pH

Compare with control, no significant changes were detected with in the treatments during $4 \pm 2^\circ\text{C}$, $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ storage conditions. This may be because the juice contains more citric acid in the form of free acid, and it contains inorganic salts and other substances to form a buffer, so the pH value did not change significantly (Yuan Ma et al., 2022). Similar to our results, other studies on ozone-treated cantaloupe melon juice found no significant differences in thermal and ozone-treated juices in storage [sroy et al., 2019]. Juices like grape (Tiwari et al., 2009a), orange (Tiwari et al., 2008), tomato (Tiwari et al., 2009), apple cider (Choi et al., 2005), strawberry (Tiwari et al., 2009 and Alexandre et al., 2012) ozonized juices have no significant change in pH. Combined treatment of cold plasma and ozone prebiotic orange juice also showed no changes in pH (Almeida et al., 2015). During those three storage conditions, there was an increase in pH from the 0 to 7th day, but after that there is no significant change until the end of the storage period. Similarly, other studies found that pH of kiwifruit pulp beverage treated with high hydrostatic pressure increased significantly during storage from 1st day to 10th day (Chen et al., 2020). Unfortunately, after 14th day, the beverage samples placed in the $25 \pm 2^\circ\text{C}$ storage were spoiled therefore 21 & 28 days has not analysed or discarded. Similarly, other studies found that there are no changes in pH of pomegranate and *Prunus mume* juices treated with pasteurization and high hydrostatic pressure during ambient storage (Vegara et al., 2013 and Yuan Ma et al., 2022). Non-thermal treatments did not influence the pH, consistent with findings in numerous prior studies (Chen

et al., 2020, Yuan Ma et al., 2022, Vegara et al., 2013, Alexandre et al., 2012 and Liu et al., 2013). Comparing the three storage conditions, samples stored at $4 \pm 2^\circ\text{C}$ exhibited lower acidity compared to those stored at $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ as indicated in Fig 2.

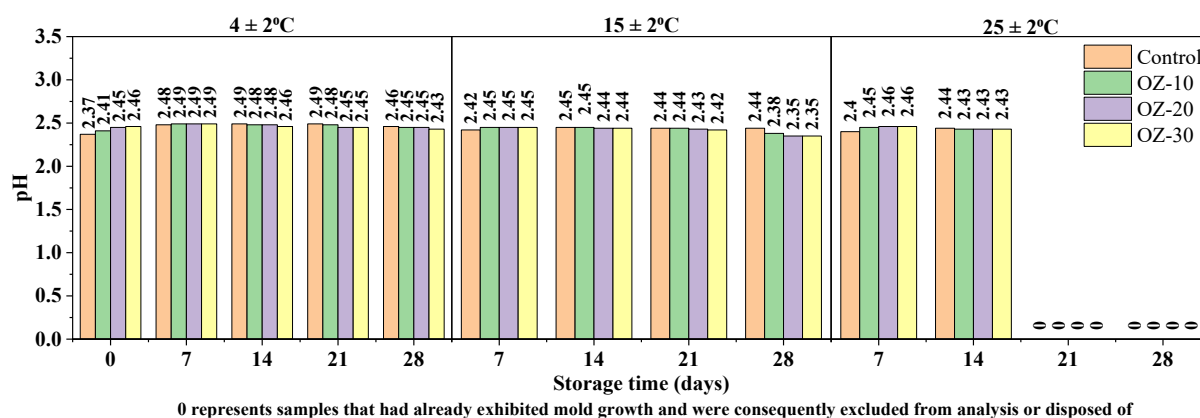


Fig 2: pH of control and ozone treated beverages during storage

4.1.2 Titratable acidity

It was noticed that the ozone processing was a significant alteration in the titratable acidity of Assam lemon beverage during $4 \pm 2^\circ\text{C}$, $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ storage. The titratable acidity was not significantly affected by the ozone treatment of lemon beverage with in the treatments, and only slight differences were observed between the control samples and those treated with ozone. Similar patterns were observed in ozonated bananas and oranges when they were treated with ozone (Ullrich et al., 2023). Other studies on ozone-treated apple cider, orange juice, prebiotic orange juice, tomato juice, strawberry juice found no significant difference of titratable acidity (Tiwari et al., 2008, Tiwari et al., 2009, Almeida et al., 2015 and Choi et al., 2005). During those three storage conditions, the titratable acidity went up in both control and treated samples up to 7 days, but after that it starts reduced up to 21 days and starts increased again until the end of the storage period. On the final day of refrigerated samples, titratable acid levels were lower or similar to the 0 day. Similar patterns were observed in ozonated apricots when they were treated and stored under refrigeration for 16 days (Panou et al., 2018) and also in ozonated bananas and oranges when they were treated and stored under ambient condition for 11 days (Ullrich et al., 2023). Unfortunately, after 14th day, the beverage samples placed in the $25 \pm 2^\circ\text{C}$ storage were spoiled therefore 21 & 28 days has not analysed or discarded. Comparing the three storage conditions, samples stored at $4 \pm 2^\circ\text{C}$ exhibited lower acidity compared to those stored at $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ as indicated in Fig 3.

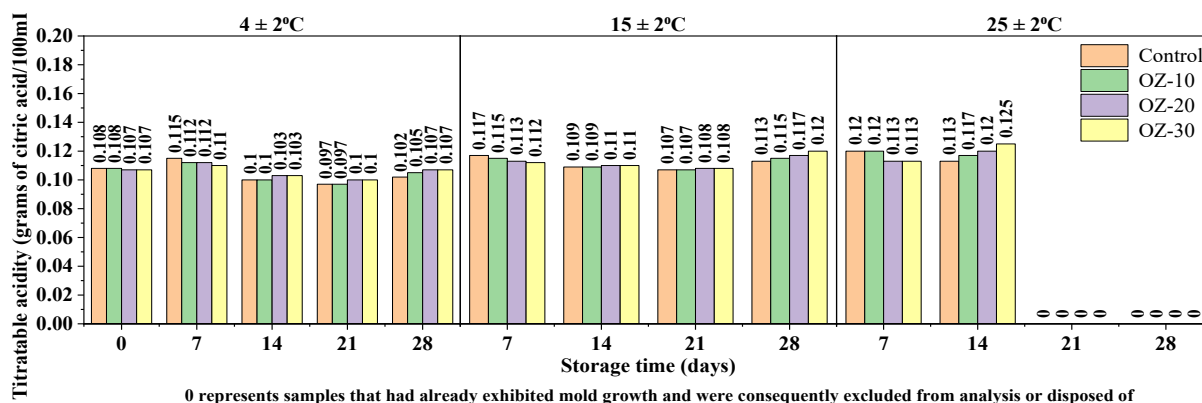


Fig 3: Titratable acidity of control and ozone treated beverages during storage

4.1.3 Total phenolic content

Ozonation made a big difference in how much phenolic content was in the Assam lemon beverage during $4 \pm 2^\circ\text{C}$, $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ storage. The phenolic content changed depending on how long we treated it with ozone. During $4 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ storage conditions total phenolic content went up after 0 to 10 seconds of ozone treatment and then quickly go down. Similar patterns were seen in other studies on orange juice, pummelo juice, the phenolic content rise after 0 to 10 minutes of ozone treatment and then quickly go down, just like what we saw (Shah et al., 2019). They think the rise in phenolic content might be because ozone changed the cell walls, freeing up some phenolic content. Ozone makes lots of free radicals, and these might help create more of these phenolic compounds (Fundo et al., 2018). The increase in the phenolic content of the fruit also might have been caused by cell wall modification that occurred during ozone exposure. Cell wall modification may release some of the conjugated phenolic compounds in the cell. The auto-decomposition of ozone is accompanied by the production of numerous free radicals. Therefore, the by-products of ozone decomposition were scavenged by the phenolic compounds in the fruits, to different extents, which might have led to the reduction of phenolic contents (Ali et al., 2014 and Shah et al., 2019). When they did similar things with banana and pineapple for 20 minutes, the phenolic and flavonoid stuff went up a lot. But sometimes, these phenolic and flavonoid things might go down because of some free radicals produced during the process (Alothman et al., 2010). Similar patterns were found in juices like lemon, lime, orange has decreased the phenolic content of each juice by 50% after 20 min of ozonation time (Shah et al., 2019). Whereas in $15 \pm 2^\circ\text{C}$ storage, the total phenolic content quickly goes down from 0 to 30 seconds with in the treatments. During those three storage conditions, the phenolic content went up in both control and treated samples until the end of the storage period. Similar trends

were detected in pummelo juice where increasing trend of phenolic content observed from 0 to 8th week (Shah et al., 2019a). Similar patterns were observed in ozone pre-conditioned papaya fruit where phenolics increased significantly for 10 days during ambient storage (Ali et al., 2014). Storing the beverage made the phenolic content went up over time. Unfortunately, after 14th day, the beverage samples placed in the $25 \pm 2^{\circ}\text{C}$ storage were spoiled therefore 21 & 28 days has not analysed or discarded. Comparing the three storage conditions, samples stored at $15 \pm 2^{\circ}\text{C}$ and $25 \pm 2^{\circ}\text{C}$ have more phenolic content than $4 \pm 2^{\circ}\text{C}$ samples as indicated in Fig 4.

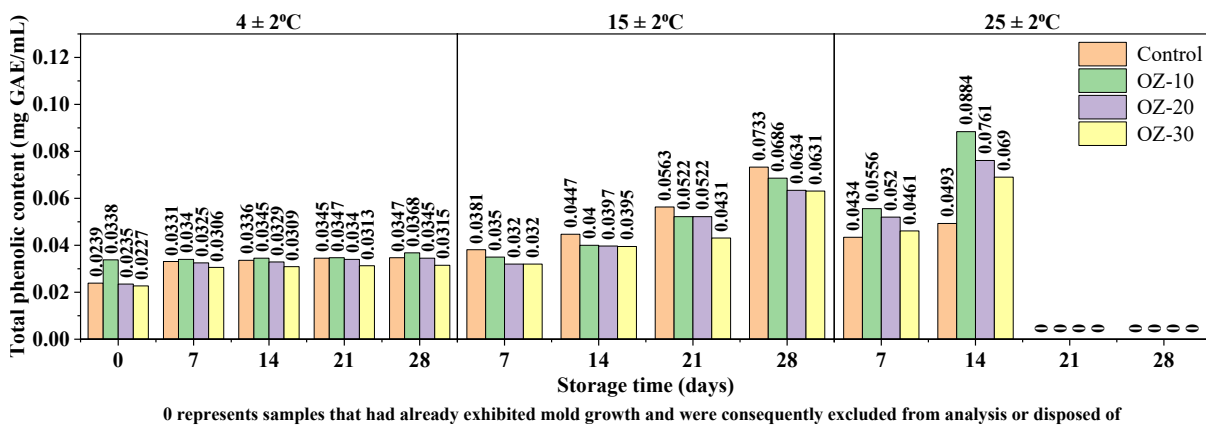


Fig 4: Total phenolic content of control and ozone treated beverages during storage

4.1.4 Ascorbic acid (AA) content

The ascorbic acid content in Assam lemon beverage is significantly influenced by ozone-processing time. After treating Assam lemon beverage with ozone, the decreasing of AA was detected with in the treatments in $4 \pm 2^{\circ}\text{C}$, $15 \pm 2^{\circ}\text{C}$ and $25 \pm 2^{\circ}\text{C}$ storage conditions, dropping from 0.714 to 0.285 mg/100mL in 0 day, 0.642 to 0.214 in 7th day, 0.571 to 0.142 in 14th day, 0.428 to 0.142 in 21st day, 0.285 to 0.142 in 28th day. Similar trends were detected in ozone treated tomato juice (Tiwari et al., 2009), orange juice (Tiwari et al 2008), pummelo juice (Shah et al., 2019a), watermelon juice (Lee et al., 2022) where ascorbic acid content significantly decreased with increasing treatment time and ozone concentration. Other juices like lemon juice, lime juice and orange juice degraded significantly after ozone treatment (600 mg/h) with processing time of 0–30 min. They explained that ozone, with its high oxidation power, breaks down organic stuff like AA and phenolic compounds. Ozone reacts directly with AA or with other free radicals, making different compounds that don't last long. This process creates unstable compounds that fall apart and form other compounds, causing AA to degrade. This breakdown happens because of highly reactive free radicals that are

made when ozone breaks down on its own. These radicals react strongly with aromatic compounds, changing them by adding or removing parts with high electron density. Also, another enzyme called ascorbate oxidase, found in some fruits, might help break down AA under ozone stress (Shah et al., 2019a and Shah et al., 2019). Similarly, during the storage AA dropping from 0.714 to 0.285 mg/100mL in control, 0.571 to 0.142 in OZ-10, 0.428 to 0.142 in OZ-20, 0.285 to 0.142 in OZ-30 until the end of the storage period. Similar trends were detected during 12 weeks storage of pummelo juice there is significant degradation in ascorbic acid content for both unfiltered and filtered ozone-treated pummelo juice (Shah et al., 2019a). Other studies on garlic found that increasing in storage time decreases the garlic acids so vitamin C also decreased (Liu et al., 2021). Unfortunately, after 14th day, the beverage samples placed in the $25 \pm 2^\circ\text{C}$ storage were spoiled therefore 21 & 28 days has not analysed or discarded. Authors found ozone treatment helps ascorbic acid content decrease more slowly than other non-thermal processing technologies. Comparing the three storage conditions, equivalent levels of ascorbic content were observed across all conditions as indicated in Fig 5.

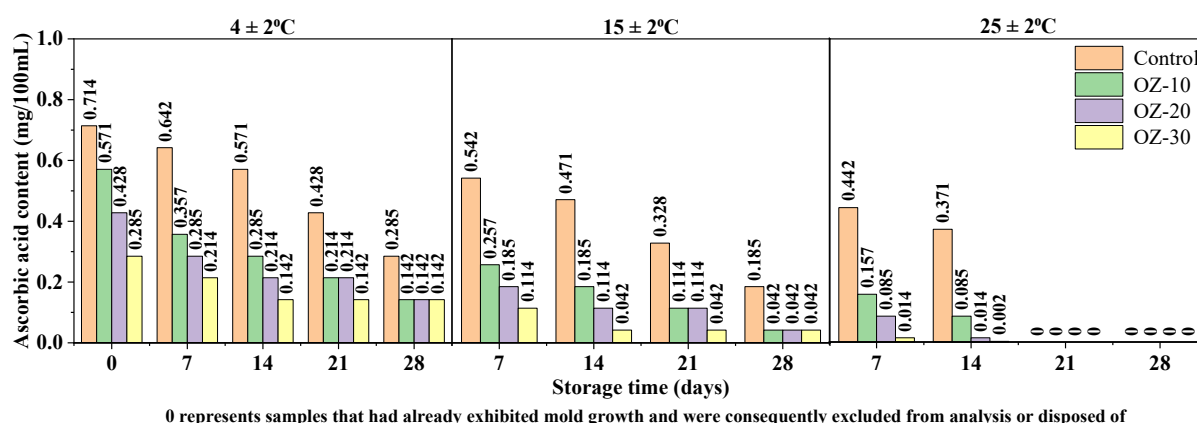


Fig 5: Ascorbic acid content of control and ozone treated beverages during storage

4.1.5 Antioxidant capacity

The findings revealed that the treatments and storage time significantly impacted the antioxidant capacity of Assam lemon beverage during $4 \pm 2^\circ\text{C}$, $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ storage conditions. A decrease in the antioxidant activity with the increase in treatment time. Similar trends were detected in ozone treated cantaloupe melon juice, fresh-cut guava (Fundu et al., 2018 and Alothman et al., 2010). Other juices like lemon juice, lime juice and orange juice degraded antioxidants after ozone treatment. They explained that due to scavenging activity of the total phenolic content within the juice the antioxidants reduced (Tiwari et al., 2008 and Shah et al., 2019). During $4 \pm 2^\circ\text{C}$ and $15 \pm 2^\circ\text{C}$ storage conditions, the antioxidant capacity

went up in both control and treated samples up to 14 days, but after that it starts reduced until the end of the storage period. Similar patterns were found in ozone treated fresh-cut pitaya during $8 \pm 2^{\circ}\text{C}$ storage, the antioxidant capacity increased maximum value after 2 days of storage but after it starts reduced (Li et al., 2022). Whereas in $25 \pm 2^{\circ}\text{C}$ storage, the antioxidant capacity went up in both control and treated samples up to 7 days, but after that it starts reduced. Unfortunately, after 14th day, the beverage samples placed in the $25 \pm 2^{\circ}\text{C}$ storage were spoiled therefore 21 & 28 days has not analysed or discarded. Similar patterns were found in ozone treated papaya fruit under ambient condition, a significant increase of antioxidant capacity was measured at day 10 in the 2.5 ppm treated fruit but after day 10 it starts reduced (Ali et al., 2014). Comparing the three storage conditions, samples stored at $15 \pm 2^{\circ}\text{C}$ have more antioxidant capacity than $4 \pm 2^{\circ}\text{C}$ and $25 \pm 2^{\circ}\text{C}$ samples as indicated in Fig 6.

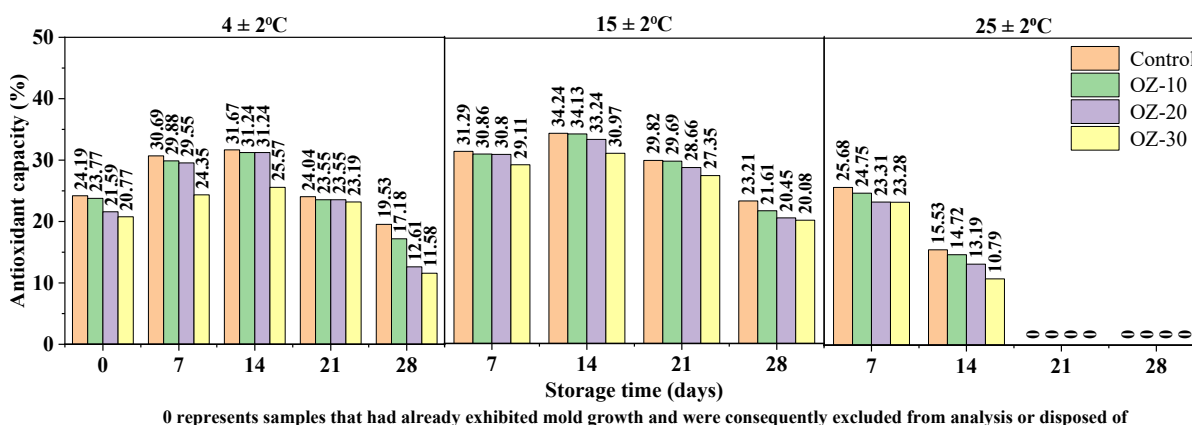


Fig 6: Antioxidant capacity of control and ozone treated beverages during storage

4.2 Effect of ozone treatment and storage time on enzymatic properties of Assam lemon beverage

4.2.1 Pectin methyl esterase (PME)

The findings revealed that the treatments and storage time significantly impacted the PME of Assam lemon beverage during $4 \pm 2^{\circ}\text{C}$, $15 \pm 2^{\circ}\text{C}$ and $25 \pm 2^{\circ}\text{C}$ storage conditions. A decrease in the PME activity with the increase in treatment time. Similar trends were observed in ozone treated lime, lemon, orange juices, where PME decreased by 50% by increasing treatment time (Shah et al., 2019). During those three storage conditions, the PME activity went up in both control and treated samples until the end of the storage period. Similar patterns were detected in ozone treated fresh-cut lettuce during 4°C storage, a PME activity significantly increased in all treatments (Rico et al., 2006). Other studies on pummelo juice at $4 \pm 1^{\circ}\text{C}$ storage show the increment trend of PME activity throughout the 12 weeks for

unfiltered as well as filtered juice (Shah et al., 2019a). By the end of storage, control had the highest and the OZ-30 had the lowest PME activity. Unfortunately, after 14th day, the beverage samples placed in the $25 \pm 2^\circ\text{C}$ storage were spoiled therefore 21 & 28 days has not analysed or discarded. The unfavourable changes in Assam lemon beverage like thickness, cloudiness, taste, and color mostly happen because of pectin enzymes. If we can control or lessen the activity of one of these enzymes called PME, we can stop pectin substances from breaking down too much. This helps keep the beverage thick and stable. Among these pectic enzymes, the PME residual level was chosen as a reference value to evaluate the effectiveness of ozone treatment. During storage, the PME activity in the beverage went up every day in all three conditions. This increase might be because PME was trying to neutralize the free radicals made by ozone. Comparing the three storage conditions, samples stored at $4 \pm 2^\circ\text{C}$ have more PME inactivation than $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ samples as indicated in Fig 7.

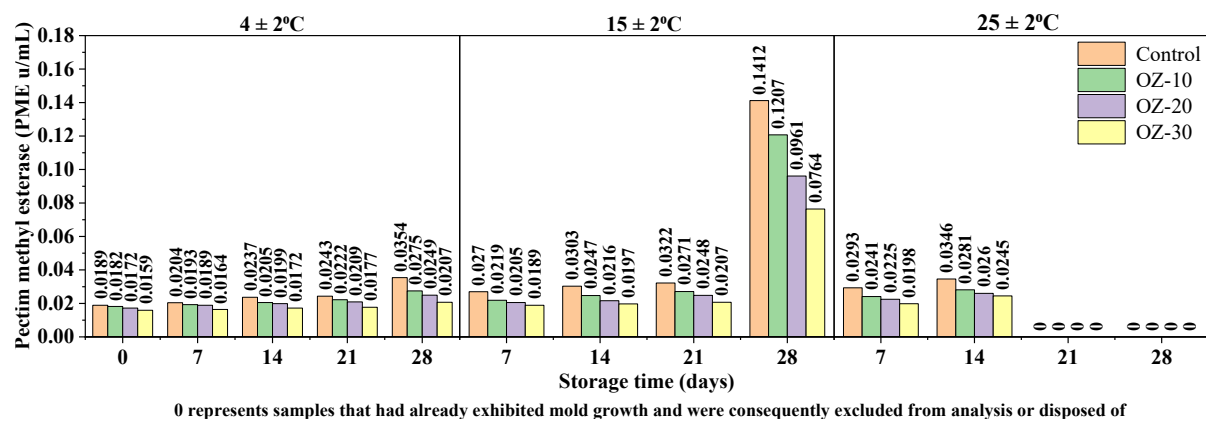


Fig 7: PME activity of control and ozone treated beverages during storage

4.3 Effect of ozone treatment and storage time on microbial properties of Assam lemon beverage

4.3.1 Total plate count (TPC)

The treatments and storage time results were presented as highly significant on the total plate count of Assam lemon beverage during $4 \pm 2^\circ\text{C}$, $15 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ storage conditions. At 0 day, highest value of total plate count was in the control and the least were in OZ-30. The total plate count went down with the increase in treatment time. Similar trends were found in watermelon juice and sugar cane juice where the plate count reduced by increase in treatment time. Ozone might weaken and break the walls and reduce their population (Lee et al., 2021, Panigrahi et al., 2020 and Miller et al., 2013). Other studies found that ozone treatment can decrease plate count in fresh-cut papaya and soy milk (Yeoh et al., 2014 and Anam et al.,

2020). During those three storage conditions, the total plate count went up in both control and treated samples until the end of the storage period. Other studies on sugar cane, pummelo juice, fresh peeled garlic treated with ozone, the total plate count increased during storage (Garud et al., 2018, Shah et al., 2019a and Liu et al., 2021). Unfortunately, after 14th day, the beverage samples placed in the $25 \pm 2^{\circ}\text{C}$ storage were spoiled therefore 21 & 28 days has not analysed or discarded. During storage, the total plate count went up every day in all three conditions. Comparing the three storage conditions, samples stored at $4 \pm 2^{\circ}\text{C}$ have less plate count than $15 \pm 2^{\circ}\text{C}$ and $25 \pm 2^{\circ}\text{C}$ samples as indicated in Fig 8.

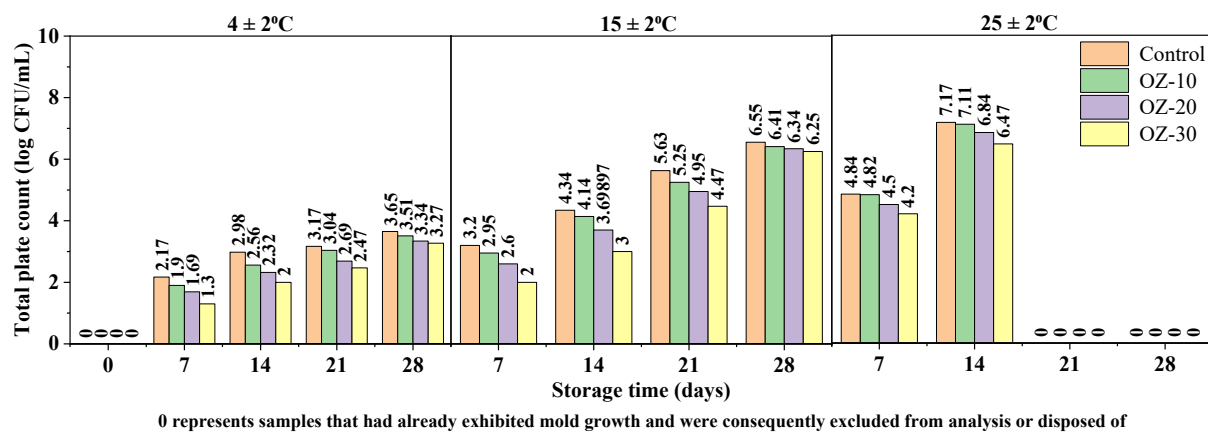


Fig 8: Total plate count of control and ozone treated beverages during storage

4.3.2 Total fungal count (TFC)

The treatments and storage time results were presented as highly significant on the total fungal count of Assam lemon beverage during $4 \pm 2^{\circ}\text{C}$, $15 \pm 2^{\circ}\text{C}$ and $25 \pm 2^{\circ}\text{C}$ storage conditions. At 0 day, highest value of total fungal count was in the control and the least were in OZ-30. The total fungal count went down with the increase in treatment time. Similar patterns were found in watermelon juice, sugar cane juice, pummelo juice, date fruits where the yeast and mold reduced by increase in treatment time (Lee et al., 2021, Panigrahi et al., 2020, Shah et al., 2019a and Najafi et al., 2009). During those three storage conditions, the fungal plate count went up in both control and treated samples until the end of the storage period. Unfortunately, after 14th day, the beverage samples placed in the $25 \pm 2^{\circ}\text{C}$ storage were spoiled therefore 21 & 28 days has not analysed or discarded. During storage, the total fungal count went up every day in all three conditions. Comparing the three storage conditions, samples stored at $4 \pm 2^{\circ}\text{C}$ have less fungal count than $15 \pm 2^{\circ}\text{C}$ and $25 \pm 2^{\circ}\text{C}$ samples as indicated in Fig 9.

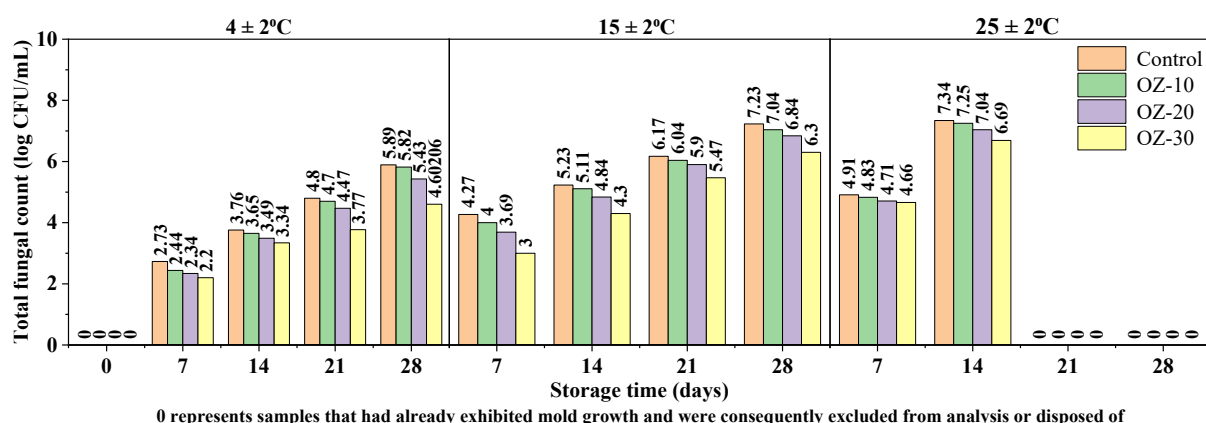


Fig 9: Total fungal count of control and ozone treated beverages during storage

4.4 Kinetic study

4.4.1 Kinetic modelling of changes in microbiological and enzyme activity

Tables 3 and 4 depict the application of zero-order, first-order, and second-order models to analyze the experimental data of PME activity in Assam lemon beverages, including control, OZ-10, OZ-20, and OZ-30 samples, respectively. Average values for R^2 , RMSE, and Chi-square were calculated across all models. Based on these averages, the maximum R^2 and lowest RMSE and Chi-square values were selected. Notably, both zero-order and first-order models exhibited a strong agreement between experimental and predicted values for PME and microbiological activity respectively. Specifically, the PME activity demonstrated a better fit with zero-order reaction models, showcasing higher R^2 values ranging from 0.9015 to 0.9971, lowest RMSE values (9.48×10^{-5} to 0.00226) and Chi-square values (1.06×10^{-7} to 4.33×10^{-6}). This suggests a linear activation pattern of PME in Assam lemon beverages during storage. Moreover, the kinetic rate constants of PME were observed to be slower at 4°C and 15°C compared to 25°C. Regarding total plate count and fungal count changes, the first-order model proved to be the most suitable, displaying R^2 values between 0.9409 and 0.9998 for total plate count and 0.9718 and 0.9998 for fungal count, depending on the storage temperature. Similar to these other studies on lychee juice subjected to UV radiation found first-order has a best fitted model (visuthiwan et al., 2021). Notably, the kinetic rate reaction differed notably at 25°C compared to lower temperatures. Overall, the experimental findings underscored a clear increase in PME, total plate, and fungal counts at higher temperatures.

Table 3: Kinetic modelling of PME activity

Sample	Model	Model parameters & error functions	4 ± 2°C	15 ± 2°C	25 ± 2°C
		A ₀	0.0189	0.0206	0.0197

Control	Zero-order	k	0.00027	0.00061	0.00112
		R^2	0.93993	0.90157	0.96602
		χ^2	6.07×10^{-7}	5.09×10^{-6}	4.33×10^{-6}
		RMSE	0.00077	0.00226	0.00208
	First-order	A_0	0.0189	0.0205	0.0197
		k	0.01291	0.02448	0.04319
		R^2	0.93951	0.86139	0.93675
		χ^2	0.00131	0.01181	0.01235
		RMSE	0.03626	0.10869	0.11111
	Second-order	A_0	0.0189	0.0204	0.0197
		k	-0.60141	-0.99423	-1.71488
		R^2	0.93828	0.81979	0.90398
		χ^2	2.91474	26.61835	30.61126
		RMSE	1.70726	5.1593	5.53274
OZ-10	Zero-order	A_0	0.01807	0.01855	0.01852
		k	0.00018	0.00042	0.00070
		R^2	0.98888	0.9901	0.98787
		χ^2	4.9×10^{-8}	2.17×10^{-7}	6.01×10^{-7}
		RMSE	0.00022	0.00046	0.00077
	First-order	A_0	0.0181	0.0186	0.0185
		k	0.00938	0.01878	0.03102
		R^2	0.99419	0.97517	0.97219
		χ^2	6.28×10^{-5}	0.0011	0.0027
		RMSE	0.00793	0.03317	0.05194
	Second-order	A_0	0.0181	0.0187	0.0186
		k	-0.46761	-0.84729	-1.3827
		R^2	0.9977	0.95425	0.95181
		χ^2	0.06162	4.21592	9.48706
		RMSE	0.24824	2.05327	3.08011
OZ-20	Zero-order	A_0	0.01741	0.01744	0.0175
		k	0.00017	0.00034	0.00062
		R^2	0.98031	0.96856	0.98625
		χ^2	7.35×10^{-8}	4.63×10^{-7}	5.4×10^{-7}
		RMSE	0.00027	0.00068	0.00073
	First-order	A_0	0.0174	0.0175	0.0175
		k	0.00909	0.01643	0.02951
		R^2	0.97157	0.96307	0.97084
		χ^2	0.00029	0.00127	0.00256
		RMSE	0.0172	0.03561	0.05063
	Second-order	A_0	0.0174	0.0176	0.0175
		k	-0.4791	-0.79907	-1.40557
		R^2	0.96131	0.94998	0.95129
		χ^2	1.13179	4.11807	9.91296
		RMSE	1.06385	2.0293	3.14849
	Zero-order	A_0	0.01587	0.01632	0.01577
		k	8.85×10^{-5}	0.00026	0.00061
		R^2	0.99072	0.95239	0.99712
		χ^2	9×10^{-9}	4.14×10^{-7}	1.06×10^{-7}

OZ-30	First-order	RMSE	9.48×10^{-5}	0.00064	0.00032
		A_0	0.0158	0.0163	0.0159
		k	0.00528	0.01392	0.03088
		R^2	0.99058	0.93702	0.99993
		χ^2	3.24×10^{-5}	0.0016	6.76×10^{-6}
	Second-order	RMSE	0.00569	0.03994	0.0026
		A_0	0.0158	0.0163	0.0160
		k	-0.31463	-0.75113	-1.57691
		R^2	0.99012	0.91764	0.99504
		χ^2	0.12101	6.20273	1.21438
		RMSE	0.34786	2.49053	1.10199

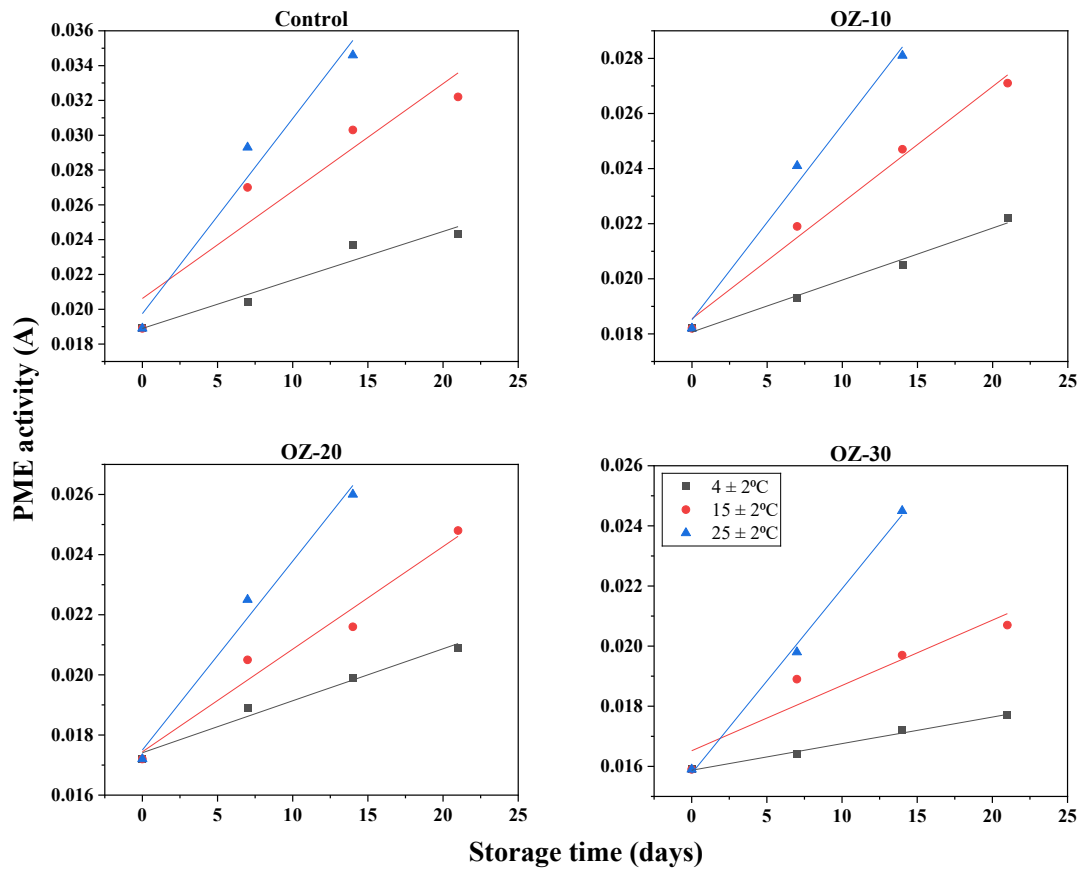


Fig. Best fit achieved by the zero-order model for pectin methyl esterase (PME) activity

Table 4: Kinetic modelling of total plate count

Sample	Model	Model parameters & error functions	$4 \pm 2^\circ\text{C}$	$15 \pm 2^\circ\text{C}$	$25 \pm 2^\circ\text{C}$
	Zero-order	A_0	-261.02	-667080	-4.8×10^{10}
		k	194.14	160045.71	2.07×10^9
		R^2	0.85456	0.6944	0.75039
		χ^2	785835	1.38×10^{12}	1.39×10^{20}
		RMSE	886.47	1175134.84	1.18×10^{10}

Control	First-order	A_0	200.97	1705.86	48515.57
		k	0.15214	0.37327	0.92388
		R^2	0.9409	0.9961	0.99045
		χ^2	0.17809	0.06674	0.8064
		RMSE	0.42201	0.25834	0.89802
	Second-order	A_0	196	2190	83800
		k	-0.00028	-2.73×10^{-5}	-1.02×10^{-6}
		R^2	0.70505	0.65774	0.7535
		χ^2	4.06×10^{-6}	4.78×10^{-8}	3.33×10^{-11}
		RMSE	0.00202	0.00021	5.77×10^{-6}
OZ-10	Zero-order	A_0	-346.06	-495770	-3.5×10^9
		k	148.42	113761.42	1.5×10^9
		R^2	0.84832	0.65514	0.75046
		χ^2	482535	8.34×10^{11}	7.33×10^{19}
		RMSE	694.64	913515.99	8.56×10^9
	First-order	A_0	91.17	931.02	47061.47
		k	0.17498	0.378	0.90395
		R^2	0.99246	0.99983	0.99074
		χ^2	0.02851	0.00304	0.74858
		RMSE	0.16884	0.05515	0.86521
	Second-order	A_0	101	1230	80200
		k	-0.00054	-4.85×10^{-5}	-1.06×10^{-6}
		R^2	0.7586	0.6512	0.75386
		χ^2	1.17×10^{-5}	1.54×10^{-7}	3.63×10^{-11}
		RMSE	0.00342	0.00039	6.03×10^{-6}
OZ-20	Zero-order	A_0	-271.04	-428720	-2.7×10^9
		k	96.28	95482.85	1.14×10^9
		R^2	0.77095	0.6326	0.75033
		χ^2	337410	6.48×10^{11}	4.25×10^{19}
		RMSE	580.87	805372.81	6.52×10^9
	First-order	A_0	52.42	337.26	21642.19
		k	0.17457	0.4104	0.93731
		R^2	0.99066	0.99728	0.98945
		χ^2	0.03518	0.05631	0.91768
		RMSE	0.18756	0.2373	0.95796
	Second-order	A_0	62.5	546	38300
		k	-0.00087	-0.00010	-2.23×10^{-6}
		R^2	0.77984	0.66373	0.75343
		χ^2	2.66×10^{-5}	7.48×10^{-7}	1.59×10^{-10}
		RMSE	0.00516	8.65×10^{-4}	1.26×10^{-5}
OZ-30	Zero-order	A_0	-296.06	-356530	-5×10^8
		k	83.42	77552.85	2.14×10^8
		R^2	0.72111	0.61329	0.75075
		χ^2	329760	4.64×10^{11}	1.49×10^{18}
		RMSE	574.24	681590.06	1.22×10^9
	First-order	A_0	20.07	62.61	12104.47
		k	0.21086	0.46851	0.86725
		R^2	0.99153	0.98515	0.9937

		χ^2	0.04655	0.40541	0.46703
		RMSE	0.21576	0.63672	0.6834
	Second-order	A_0	25.5	135	19200
		k	-0.00222	-4.42×10^{-4}	-4.46×10^{-6}
		R^2	0.75547	0.6794	0.754
		χ^2	1.94×10^{-4}	1.13×10^{-5}	6.37×10^{-10}
		RMSE	0.01395	0.00336	2.52×10^{-5}

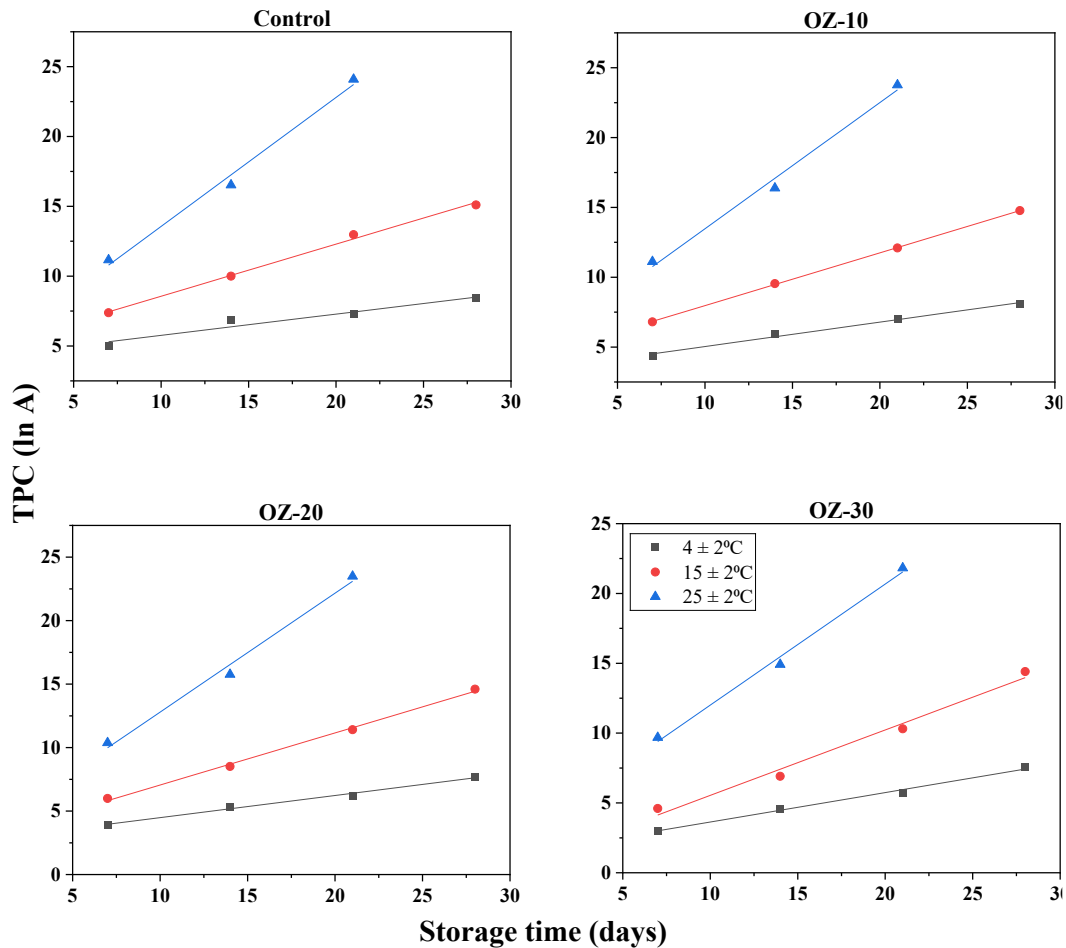


Fig. 3.10 Best fit achieved by the first-order model for total plate count (TPC)

Table 5: Kinetic modelling of total fungal count

Sample	Model	Model parameters & error functions	$4 \pm 2^\circ\text{C}$	$15 \pm 2^\circ\text{C}$	$25 \pm 2^\circ\text{C}$
	Zero-order	A_0	-146902	-3168700	-7.7×10^9
		k	34236.85	746757.14	3.28×10^9
		R^2	0.66514	0.66985	0.75036
		χ^2	7.22×10^{10}	3.36×10^{13}	3.51×10^{20}
		RMSE	268868.57	5802424.24	1.87×10^{10}

Control	First-order	A_0	525.11	18148.44	58236.06
		k	0.34611	0.32239	0.94553
		R^2	0.99986	0.99931	0.99204
		χ^2	0.00213	0.00873	0.70266
		RMSE	0.04612	0.09344	0.83825
	Second-order	A_0	730	25500	98300
		k	-8.15×10^{-5}	-2.32×10^{-6}	-8.71×10^{-7}
		R^2	0.67392	0.68854	0.75279
		χ^2	3.94×10^{-7}	3.0×10^{-10}	2.44×10^{-11}
		RMSE	6.27×10^{-4}	1.73×10^{-5}	4.94×10^{-6}
OZ-10	Zero-order	A_0	-126904	-2031000	-6.5×10^9
		k	29366.57	484857.14	2.78×10^9
		R^2	0.66062	0.67942	0.75034
		χ^2	5.42×10^{10}	1.35×10^{13}	2.53×10^{20}
		RMSE	232964.88	3686218.93	1.59×10^{10}
	First-order	A_0	301.88	11001.2	47899.48
		k	0.36812	0.33064	0.94711
		R^2	0.99932	0.9987	0.99169
		χ^2	0.01126	0.0175	0.73663
		RMSE	0.10609	0.13228	0.85827
	Second-order	A_0	386	13700	81500
		k	-1.55×10^{-4}	-4.37×10^{-6}	-1.05×10^{-6}
		R^2	0.64952	0.66101	0.75283
		χ^2	1.60×10^{-6}	1.20×10^{-9}	3.55×10^{-11}
		RMSE	0.00127	3.47×10^{-5}	5.95×10^{-6}
OZ-20	Zero-order	A_0	-49606	-1288500	-3.5×10^9
		k	11946.28	310214.28	1.5×10^9
		R^2	0.68816	0.69058	0.75039
		χ^2	7.92×10^9	5.28×10^{12}	7.33×10^{19}
		RMSE	89005.71	2298255.58	8.56×10^9
	First-order	A_0	249.85	5605.48	36039.11
		k	0.33725	0.34527	0.92206
		R^2	0.99804	0.99811	0.9904
		χ^2	0.02742	0.02772	0.8066
		RMSE	0.16559	0.16648	0.89814
	Second-order	A_0	302	8660	62300
		k	-1.98×10^{-4}	-8.75×10^{-6}	-1.37×10^{-6}
		R^2	0.65625	0.65664	0.75354
		χ^2	2.53×10^{-6}	4.90×10^{-9}	6.04×10^{-11}
		RMSE	0.00159	7.0×10^{-5}	7.77×10^{-6}
OZ-30	Zero-order	A_0	-6408.03	-361300	-8.3×10^8
		k	1761.71	89671.42	3.57×10^8
		R^2	0.71994	0.71804	0.75074
		χ^2	1.47×10^8	3.86×10^{11}	4.15×10^{18}
		RMSE	12161.45	621934.20	2.03×10^9
	First-order	A_0	217.40	1282.09	31778.65
		k	0.25097	0.36444	0.82831
		R^2	0.97188	0.9904	0.98794

		χ^2	0.2232	0.15771	0.82081
		RMSE	0.47245	0.39713	0.90599
	Second-order	A_0	219	1400	55000
		k	-2.70×10^{-4}	-4.35×10^{-5}	-1.55×10^{-6}
		R^2	0.65599	0.63963	0.75689
		χ^2	4.71×10^{-6}	1.30×10^{-7}	7.58×10^{-11}
		RMSE	0.00217	3.61×10^{-4}	8.71×10^{-6}

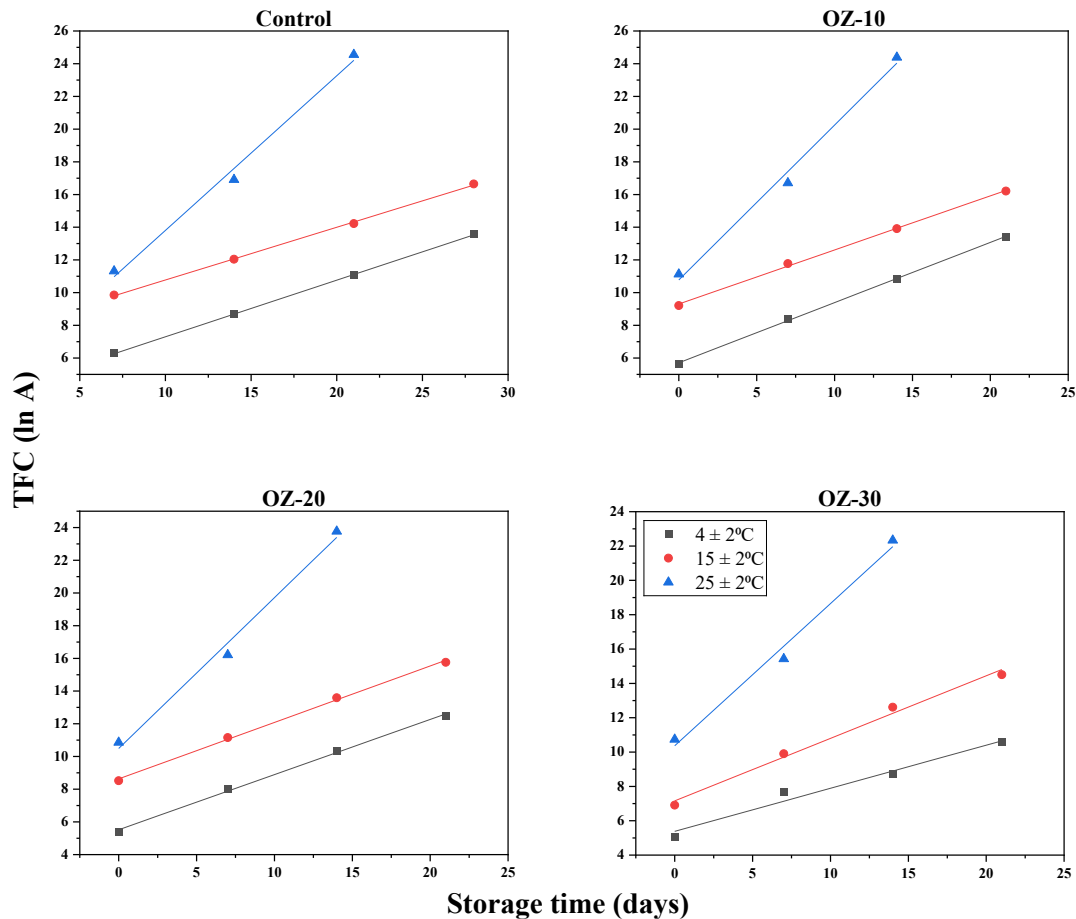


Fig. 3.11 Best fit achieved by the first-order model for total fungal count (TFC)

4.5 Activation energy

Table 6 shows the best fitted models for PME and Microbial activity is zero-order, first order models respectively in Assam lemon beverages and its activation energy calculation. The activation energy was highest in control sample and lowest in OZ-30 sample for PME, TPC, TFC respectively.

Table 6: Activation energy for best fitted models

Best fitted model	Parameters		Control	OZ-10	OZ-20	OZ-30
Zero-order	Pectin methyl esterase (PME)	E_a (KJ/mol)	46.62	44.63	42.31	40.44
		R^2	0.998	0.991	0.999	0.986

First-order	Total plate count	E_a	58.86	54.88	53.50	46.32
		R^2	0.997	0.992	0.998	0.999
	Total fungal count	E_a	31.75	29.78	31.95	38.56
		R^2	0.651	0.619	0.725	0.933

Conclusion

The motivation of the study was to investigate how ozone treatment challenged the physicochemical, enzymatic, and microbiological characteristics of Assam lemon beverages up to 28 days of storage at $4 \pm 2^\circ\text{C}$, $15 \pm 2^\circ\text{C}$, and $25 \pm 2^\circ\text{C}$. Based on the extensive experimentation and analysis conducted on the effect of ozone treatment and storage time on the physico-chemical, enzymatic, and microbiological properties of Assam lemon beverage, several significant conclusions can be drawn. Firstly, regarding the physico-chemical properties, it was observed that ozone treatment did not significantly alter the pH of the beverage across different storage conditions. This stability in pH suggests that the natural buffering capacity of the beverage, primarily attributed to its citric acid content, remained intact even after ozone treatment and storage. Additionally, the titratable acidity of the beverage showed minimal variation between ozone-treated and untreated samples, indicating that ozone treatment had negligible impact on this parameter. However, significant changes were noted in the total phenolic content and ascorbic acid (AA) content of the beverage following ozone treatment. Ozone exposure led to a transient increase in phenolic content, likely due to the liberation of phenolic compounds from cell walls, followed by a rapid decline. Similarly, the AA content exhibited a notable decrease post-treatment, attributed to the high oxidation power of ozone leading to the degradation of AA and phenolic compounds. Despite these changes, ozone treatment demonstrated a potential advantage in slowing down the degradation of AA compared to other non-thermal processing methods. Secondly, in terms of enzymatic properties, ozone treatment showed a significant impact on pectin methyl esterase (PME) activity. PME activity decreased with increasing ozone treatment time, suggesting a potential mechanism to preserve the beverage's texture and stability by limiting pectin breakdown. This finding underscores the importance of PME as a reference parameter for evaluating the efficacy of ozone treatment in preserving beverage quality. Lastly, concerning microbiological properties, ozone treatment exhibited notable antimicrobial effects, leading to a reduction in both total plate count (TPC) and total fungal count (TFC) with increasing treatment time. This antimicrobial action of ozone contributed to the preservation of beverage shelf-life by inhibiting microbial growth and proliferation.

However, it's important to note that spoilage occurred in samples stored at higher temperatures ($25 \pm 2^{\circ}\text{C}$), indicating the need for proper storage conditions to maintain beverage quality over time.

Through the application of zero-order, first-order, and second-order models, the data were analyzed on PME activity and microbiological counts (TPC, TFC). Zero-order and first-order models showed strong agreement between experimental and predicted values for PME activity. Zero-order models exhibited higher R^2 values and lower RMSE and Chi-square values, indicating a linear activation pattern of PME during storage. The kinetic rate constants of PME were slower at lower temperatures (4°C , 15°C) compared to 25°C . The first-order model was found to be the most suitable for modelling changes in total plate count and fungal count. This model displayed high R^2 values across different storage temperatures, indicating a good fit. The kinetic rate reaction differed notably at 25°C compared to lower temperatures, with a clear increase in reaction rate observed at higher temperatures.

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Dr. Nishant R. Swami Hulle

Principal Investigator



GFR 12 – A
[(See Rule 238 (1))]

FORM OF UTILIZATION CERTIFICATE
FOR AUTONOMOUS BODIES OF THE GRANTEE ORGANIZATION

UTILIZATION CERTIFICATE FOR THE YEAR 2022-2023 in respect of non-recurring

1. Name of the Scheme/project : Non-thermal processing of Assam lemon juice using ozone processing
2. Whether recurring or non-recurring grants : Non recurring
3. Grants position at the beginning of the Financial year : 2022
 - (i) Cash in Hand/Bank : Rs. NIL
 - (ii) Unadjusted advances : NIL
 - (iii) Total : NIL
4. Details of grants received, expenditure incurred and closing balances: (Actuals)

Unspent Balances of Grants received years [figure as at Sl. No. 3 (iii)]	Interest Earned the year	Interest deposited back to the Government	Grant received during the year			Total Available funds (1+2-3+4)	Expenditure incurred	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction No. (i)	Date (ii)	Amount (Rs.) (iii)	Amount (Rs.)	Amount (Rs.)	Amount (Rs.)
0.00	0.00	0.00	ASTEC/S&T/1614/12/2020-21/138-144	01/02/2022	159000.00	159000.00	79105.00	79895.00

Component wise utilization of grants:

Grant-in-aid-General	Grant-in-aid-Salary	Grant-in-aid-creation of capital assets	Total
11250.00	-	67855.00	79105.00

Details of grants position at the end of the year

- (i) Cash in Bank : 79895.00
- (ii) Unadjusted Advance : NIL
- (iii) Total. : 79895.00



GENERAL FINANCIAL RULES 2017
Ministry of Finance
Department of Expenditure

FORM GFR 12A

5 2017
Finance
Expenditure



Certified that I have satisfied myself that the conditions on which grants were sanctioned have been duly fulfilled/are being fulfilled and that I have exercised following checks to see that the money has been actually utilized for the purpose for which it was sanctioned:

- (i) The main accounts and other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors. The figures depicted above tally with the audited figures mentioned in financial statements/accounts.
- (ii) There exist internal controls for safeguarding public funds/assets, watching outcomes and achievements of physical targets against the financial inputs, ensuring quality in asset creation etc. & the periodic evaluation of internal controls is exercised to ensure their effectiveness.
- (iii) To the best of our knowledge and belief, no transactions have been entered that are in violation of relevant Act/Rules/standing instructions and scheme guidelines.
- (iv) The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms and are not general in nature.
- (v) The benefits were extended to the intended beneficiaries and only such areas/districts were covered where the scheme was intended to operate.
- (vi) The expenditure on various components of the scheme was in the proportions authorized as per the scheme guidelines and terms and conditions of the grants-in-aid.
- (vii) It has been ensured that the physical and financial performance under Non-thermal processing of Assam lemon juice using ozone processing (name of the scheme) has been according to the requirements, as prescribed in the guidelines issued by Govt. of India and the performance/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure - I duly enclosed.
- (viii) The utilization of the fund resulted in outcomes given at Annexure - II duly enclosed (to be formulated by the Ministry/Department concerned as per their requirements/specifications.)
- (ix) Details of various schemes executed by the agency through grants-in-aid received from the same Ministry or from other Ministries is enclosed at Annexure - II (to be formulated by the Ministry/Department concerned as per their requirements/specifications).

N. Swami

Dr. Swami Hulle Nishant Rachayya
(Principal Investigator)
Tezpur University

Associate Professor
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[Signature]

Dy. Finance & Accounts Officer
Tezpur University
प्रभारी वित्त अधिकारी
तेजपुर विश्वविद्यालय
Finance Officer I/c
Tezpur University

[Signature]

Registrar,
Tezpur University
Registrar
Tezpur University

STATEMENT OF EXPENDITURE for FY 2022-24:

(Audited)

Sr. No.	Sanctioned Budget Heads as per Sanction Order	Funds Allocated (sanctioned or revised) (In Rs.)	Funds Received	Expenditure Incurred		Total expenditure (in Rs.)	Balance (in Rs.)	Remarks
				1 st Year 22 Jun 2022 to 31 st March 2023 (In Rs.)	2 nd Year April 2023 to 31 st March 2024 next year (In Rs.)			
1.	Minor Equipment's	100000.00	159000.00	67855.00	31775.00	99630.00		
2.	Consumables	50000.00		-	22118.00	22118.00		
3.	Contingency	30000.00		-	17370.00	17370.00		
4.	Institutional Overhead charges	18000.00		11250.00	-	11250.00		
	Total	198000.00		79105.00	71263.00	150368.00	8632.00	
	Grand Total				-			

Name and Signature of Principal Investigator with date

(Swami Hulle Nishant Raibayya)

Assistant Professor

Department of Food Engg. & Technology

Tezpur University

Assam, India - 784029

Note:

- Expenditure under the sanctioned heads, at any point of time, should not exceed funds allocated under that head, without prior approval of DST.
- Utilization Certificate for each financial year ending 31st March has to be enclosed, along with a request for carry forward permission next year.
- *Please Add/Delete budget heads as per sanction order.
- Please indicate expenditure in exact rupees only.

Signature of Competent Financial Authority with date and Seal

प्रभारी वित्त अधिकारी

तेजपुर विश्वविद्यालय

Finance Officer I/c

Tezpur University