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Effect of Chlorine Dioxide Gas on the Properties of Packaging Materials and Effect of Chlorine Dioxide Gas to Maintain the Quality of Fresh Strawberries

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EFFECT OF CHLORINE DIOXIDE GAS ON THE PROPERTIES OF PACKAGING MATERIALS AND EFFECT OF CHLORINE DIOXIDE GAS TO MAINTAIN THE QUALITY OF FRESH STRAWBERRIES

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Food, Nutrition, and Culinary Sciences

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by
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Accepted by:
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ABSTRACT

ClO_2 as an antimicrobial gas in the headspace of produce package is a relatively novel approach. Gaseous ClO_2 is more effective than aqueous ClO_2 and can be used in the headspace of fresh food packages. ClO_2 gas can diffuse into product surfaces and films. As an oxidizer, it can react with and change polymeric package components, possibly affecting the product's shelflife.

This research studied effects of ClO_2 gas treatments on produce packaging materials (APET, two PE types, Nylon). The treatment group (with ClO_2) and the control group (without ClO_2) of packaging materials were stored at room temperature and at three different relative humidities (49%, 84%, 99%) in sealed chambers. Dart drop, tensile tests, T_g, T_m, T_c, and water vapor transmission rate of materials were performed at six times over a 21-day period. A low dose (<0.1 ppm) of ClO_2 was a suitable application in these produce packaging materials. This research allows further experimenting with the literature on varying RH's for the general application (<1 ppm) of ClO_2 on each polymer used for common fresh produce applications.

This research also studied the use of ClO_2 gas sachets to determine the effects on the sensory properties of strawberries. Conditions included typical strawberry storage systems (open pallet system under cold conditions (0-2 ^{0}C) and a controlled atmosphere storage system (99% RH, 0-2 ^{0}C). Both conditions were tested for 21 days with and without ClO_2.

ClO_2 gas preserved strawberry quality with or without maximum RH. In the absence of 99% RH, ClO_2 treated strawberries exhibited better quality than the untreated berries. High RH (99%) alone has significant quality retention of strawberries in a chamber system with or without ClO_2. Either ClO_2 or high (99%) RH plays a significant role in strawberry preservation in each other's absence.

DEDICATION

I would like to dedicate my dissertation work to my family in the USA-husband Manjula Senanayake, and my three sons Anuja Tharinda, Anuka Nethun, and Aken Anutya, who were behind me in my ups and downs throughout my academic career. My special gratitude is to my elder son Anuja Tharinda, who understood me well during my hard time and supported me a lot during my Ph.D. journey. My husband was the one who encouraged me every single day to achieve my academic dream.

I also would like to dedicate my work to my parents and siblings, who were a strength in my life, to become who I am today. My special gratitude to my mother, Premarathna Manike, for being a part of my academic journey. All your love and strong support made me who I am today. Thank you!

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CHAPTER ONE

INTRODUCTION

Globally, more than one-third of all food on our planet is never consumed (Ishangulyyev et al., 2019; IPCC, 2018; Bond et al., 2013). Eight hundred seventy (870) million people worldwide suffer from chronic undernourishment (Ishangulyyev et al., 2019). Forty (40) million people live in a food-insecure household in the United States, which is one in eight people. One in six children in the USA lives with hunger (Bond, 2013). One of the primary contributions to this tragedy is food waste.

Fresh agricultural produce is one of the primary food sources in the daily food supply chain. Approximately half (52%) of all produce is unconsumed in the US market, which is the number one of the primary sources of food waste (The Sonoco Institute, 2018). The spoilage of fresh produce is worth $15.6 billion at the retail level (The Sonoco Institute, how packaging can help solve our food waste problem). One of the primary solutions to this food waste strategy is food preservation by increasing the shelflife. The value of shelflife improvement of fresh produce by one day is $1.8 billion to the agricultural industry (The Sonoco Institute, 2018).

Strawberries are the most commonly purchased and widely appreciated fruits enjoyed by consumers in the United States. This is due to the characteristic bright red color, a strong sense of aroma, juicy texture, and sweetness. Strawberries have a $ 3.5 billion market share, which is 82% of all fresh fruits in 2017 (AgMRC, 2021). World production of strawberries (mainly from China and the United States) was around 8.9 million tons in

2019. In 2017, 1.6 billion pounds of strawberries, which are high-value agricultural commodities, were produced in the United States, with a value of $3.5 billion (AgMRC, 2021). In 2020, approximately 35% of $ 2.2 billion in US strawberry production was wasted due to the very fragile nature of strawberries. Due to the enormous economic value of strawberries, shelf life extension by numerous technologies has the potential to address an aspect of food sustainability.

Chlorine dioxide (ClO_2) is FDA-approved to use as a disinfectant/sanitizer in fresh produce. It is commonly used to disinfect fresh produce and extend the shelf life of fresh produce in food industries. It has a strong oxidizing capability, 2.5 times that of chlorine (Singh et al., 2021; Sun et al., 2019). Due to the sensitive/delicate nature of strawberries, the use of traditional aqueous chlorine dioxide is not practically effective, as washing the strawberry will produce bruising, which decreases the shelflife of strawberries. Many recent papers provide evidence that ClO_2 is more effective as an antimicrobial gas when used in the headspace of produce packages, increasing the safety and quality of the product (Chiabrando et al., 2018; Sun et al., 2014; Popa et al., 2007). ClO_2 gas is more effective due to a higher absorption rate than aqueous chlorine dioxide (Han et al., 2001).

Improving the packaging design with an antimicrobial compound or adding a packaging technology such as active packaging could help to maintain sustainable products. Antimicrobial packaging (e.g., ClO_2 gas generating) could be utilized to extend the shelf life and improve the microbial safety of fresh produce. Many studies have also reported the antimicrobial activity of ClO_2 against different microorganisms (Kuruwita, Chapter 2; Chiabrando et al., 2018; Cho et al., 2017). Also, chlorine dioxide in-package sachet treatments can extend food distribution systems from the point of packaging through retail. High demand in the market for such antimicrobial packaging for food has been reported (Singh et al., 2019; Gaikwad et al., 2019).

The incorporation of precursors (dry compounds) directly into the polymeric film or the use of a ClO_2 gas releasing sachet in-package system or even ClO_2 gas treatment outside a permeable packaging system throughout the storage period (continuous treatment) is a relatively novel method for food packaging. Very few research papers have been published on the “use of ClO_2 sachets to increase the shelflife of fresh produce” (Kuruwita, Chapter 2). Although there has been some research, ClO_2 as an antimicrobial gas in the headspace of produce packages (using sachets) is still unavailable in a commercial application. There is a need to study how such a packaging system could help extend the shelflife / maintain the freshness of strawberries. This is the first study that researched the effect of ClO_2 gas-producing sachets to preserve the sensory parameters of fresh strawberries in a pallet storage system, including a humidity-controlled (99%RH) ClO_2 gas treating closed chamber system for over 14 days. Studies have been done on some aspects of this study about the shelf-life extension of fresh strawberries packaged in clamshells with ClO_2 generating sachets (Kessler, 2020; Chiabrando et al., 2018; Wang et al., 2014). The objective of this study was to determine the effect of controlled-release ClO_2 gas treatments (using sachets) on the sensory level of strawberries (qualitatively and quantitatively) that may affect the shelf life of strawberries. The study targeted a practical strawberry storage system of an open pallet system under refrigerated conditions (0-2 ^{0}C) compared to a laboratory-controlled closed chamber system under optimum conditions of high relative humidity (99%) and (0-2 ^{0}C) refrigerated temperature.

If strawberries or other produce were treated with ClO_2 gas, the polymers of produce packages would be in contact with the fresh produce and the headspace. Polymer chains may react with this strong oxidizing agent (ClO_2), depending upon the polymer structure (functional groups, crystallinity, amorphous regions of semi-crystalline polymers, etc.). Changes in polymer properties after exposure to ClO_2 may include chain scission, crosslinking, depolymerization, formation of conjugated double bonds, formation of carbonyl groups, etc. Polymer properties such as mechanical properties (tensile strength, tensile modulus, etc.), thermal properties (melting temperature, glass transition temperature, etc.), and barrier properties (oxygen permeability, water vapor permeability, etc.) are unique characteristics of each polymer. If these polymers and their properties were changed permanently, possible outcomes could include loss of package integrity, resulting in a reduction of shelf life of the packaged product, as well as loss of safety or quality. Current knowledge and literature regarding the compatibility of ClO_2 gas with polymer packaging materials used to contain food are limited. Therefore, the second objective of this research is to determine the effect of ClO_2 gas treatments on the properties of selected produce packaging materials that may affect the shelflife of products.

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CHAPTER TWO

CHLORINE DIOXIDE GAS SACHET IN STRAWBERRY (INCLUDING ALL BERRIES) PRESERVATION: A SYSTEMATIC REVIEW OF THE LITERATURE

INTRODUCTION

Strawberries are the most popular and consumer-attractive summer fruit in the United States, with an increasing market value (USDA, 2019). Strawberry has a unique flavor profile due to flavonoids-anthocyanins pigments, which vary widely between each fruit. It is also rich in healthy nutrients like vitamins, minerals, amino acids, and is a natural source of phenolic compounds with antioxidant and anti-inflammatory properties. However, the strawberry is a highly perishable fruit that has a short shelf life due to rapid weight loss, softening, and mold formation.

Strawberry waste is 533 million pounds per year in the US market, which causes between $0.73 and $1.17 billion-dollars loss. Almost 10 percent of all harvested strawberry crops were wasted during 2015 (Nina, 2017). Strawberries have a shelf-life of 7-10 days at 0 ºC for maximum quality retention and less at higher temperatures (Agricultural Research Service, 2016). Strawberries should be stored at 0-2 ºC with relative humidity (RH) between 90-99%. Cold temperature and high humidity decrease respiration and water loss of strawberries. Proper handling and storage are also necessary to avoid physical damage and to delay microbial deterioration.

Extension of shelflife of such a high-value agricultural products is of high importance. Extending the shelf-life of strawberries by one day will save around $1.8

billion for the agricultural industry (Sonoco Institute, 2018). Shelf-life extension of strawberries during post-harvest storage can be achieved by proper storage conditions (RH between 90-99%, temperature 0-2ºC), and by controlling yeast/mold growth using antimicrobial treatments such as edible coating application, calcium dipping, UV radiation, ultrasonic treatment (Aday & Caner, 2011; Peano et al., 2014). Modified atmosphere packaging is also helpful for maintaining a proper environment for an extended shelf-life.

Gaseous chlorine dioxide (ClO_2) is increasingly used for disinfection of food such as fresh meat, meat products, and other produce within the packaging system because ClO_2 has a strong antimicrobial effect with minimal impact on food safety and/or on the package. Aqueous ClO_2 is most commonly used to disinfect various food products, yet gaseous ClO_2 displays several advantages, such as mixing more easily with the package atmosphere, dispersing more rapidly, and diffusing more quickly into product surfaces and films. ClO_2 gas is more effective than an aqueous form at the same concentration due to its greater diffusivity into the tissues (Han et al., 2001).

Chlorine dioxide gas is delivered to treat produce in many ways, including by generators, by mixing chemicals in a large open container that releases the gas, or by mixing chemicals in a sachet which then emits the gas. In-package chlorine dioxide releasing sachets can distribute a low dose over time and have proven effectiveness (Han et al., 2001; Lee et al., 2004).

After an exhaustive search, no review studies were found on the effectiveness of ClO_2 gas releasing sachet in berry preservation (shelf-life extension of berries). The objective of this literature review was to answer the following research questions:

1. Would chlorine dioxide gas sachets extend the shelflife of berries?
2. What types of berries are preserved using ClO_2 gas sachets?
3. What are the best chlorine dioxide gas releasing rates and humidity levels that have been used for maximum shelflife of berries?

The primary objective of this search was to systematically review the literature to determine the literature gap in the use of ClO_2 sachet to improve the shelflife of berries by summarizing the literature on the topic for future research.

METHODS

Search strategy

A systematic literature review was performed to identify and analyze the current research regarding “chlorine dioxide gas in produce (specially berries) preservations.” The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) principle was used as the guidance in our literature search (Liberati et al., 2009). Five databases/search engines were used to perform the search:

- EBSCO (Academic Search Complete and Food Science and Technology)
- Web of Science
- Science Direct
- Academic One File
- Engineering Village

We conducted our database search using a search phrase that was comprised of search terms (Table 2.1) from three categories: Preservative -related, Food items -related, and Preservation method-related terms (Table 2.1) using the inclusion criteria.

Table 2.1: Literature Search Items

Terms-Preservative		Term-Food items		Terms-Preservation way
“Chlorine dioxide” OR ClO_2	AND	Food OR Fruit OR Vegetable OR Produce OR Berry (strawberry, blue berry,	AND	Preserv* OR prot* OR antimicrobial OR retard* OR oxidant* OR shelflife OR shelf-life OR spoilage OR “food safety”

Inclusion criteria:

- Language-English
- Publication years-1990–2018
- Geographical area-worldwide
- Publication type-peer reviewed journal articles

The reference lists of all review articles and eligible articles were manually searched to identify additional published articles that might have been missed during database searching.

Study selection

We conducted our initial search to include all the peer-reviewed journal articles between 1990 and 2018 in English and published in all geographic areas. After compiling results from across databases, duplicates were removed. This is shown in Figure 1 in the identification section.

Next, the titles and abstracts of the remaining documents were reviewed using our eligibility criteria. All the documents published in English between 1990 and 2019 that describe the three general categorized terms (Table 2.1) were "screened" by title and abstract. Only those documents that were relevant to the keywords and topic were kept as eligible. This is shown in Figure 1 in the screening section. This is shown in Figure 1 in the Screening section.

The full texts of eligible articles that were selected after screening were reviewed for final inclusion. The reference lists of these documents were checked to identify additional articles that may be relevant. According to PRISMA methodology, reference lists of any review articles are to be searched for additional eligible articles (in this search, none were found). This is shown in Figure 1 in the Eligibility section.

The final step was a full-text review of the remaining eligible articles. To be included after this step, an article had to describe the use of chlorine dioxide gas sachets to improve the shelflife of (fruits and vegetables-produce), be peer-reviewed articles, and published in English between 1990 and 2018. Studies were included regardless of geographic area. According to PRISMA methodology, review articles are to be excluded from analysis (in this search, none were found). To be included, a study should be focused on at least one type of berry with or without other produce. This is shown in Figure 1 in the Included section.

RESULTS

Study selection

A total of 771 documents were found through initial database searching at the identification (Figure 1). After removing duplicate records (N=457), 314 records were selected for the initial screening of titles and abstracts. In the initial screening, 239 records were excluded because the title and abstract did not meet the inclusion criteria. Finally, 47 eligible articles were identified for full-text review. After reviewing the full texts of the 47 potentially eligible records, 39 records were excluded for the following reasons:

Studies on the use of ClO_2 releasing sachets on produce (fruits and vegetables other than berries) preservation (N=7).

Studies on the use of ClO_2 releasing sachets on meat preservation (N=2).

Studies (N=3) that use ClO_2 gas to preserve the berries, but not a sachet (such as using generators).

Studies (N=22) that use ClO_2 gas to preserve other produce, but not a sachet (such as using generators).

Studies (N=3) that use ClO_2 gas to preserve food other than produce, but not a sachet (such as using generators).

Studies of ClO_2 gas releasing films for produce (N=2).

A total of 8 studies met our inclusion criteria which directly target the subject of chlorine dioxide gas sachet in berries preservations.

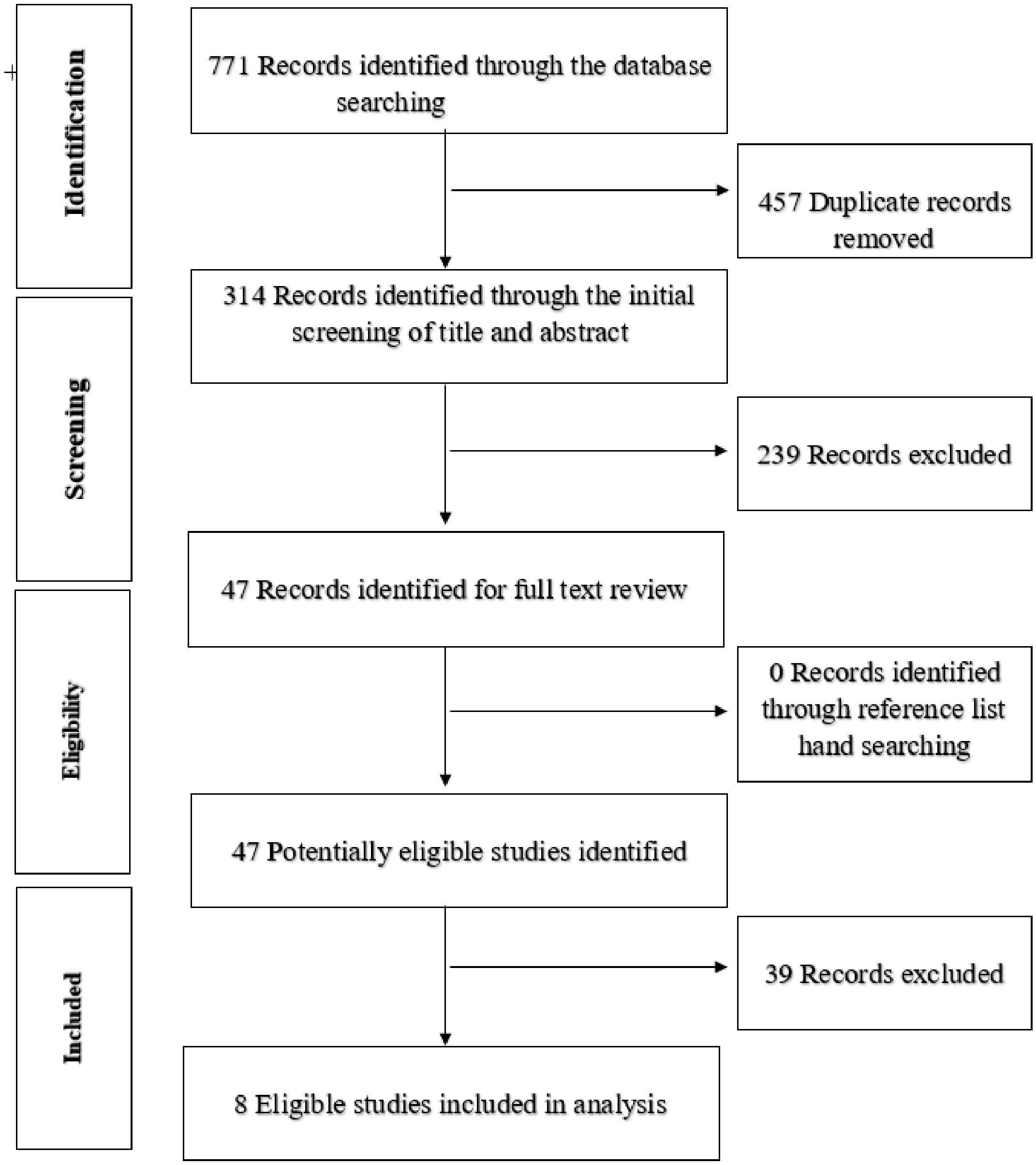

Figure 2.1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), 2009 Flow Chart Describing the Literature Search Procedure.

Study characteristics

All 8 studies were published in English between 1990 and 2018 and were peer-review articles. Several types of berries, including some other produce, have been studied. Four studies were for strawberries (N=4), five studies were about blueberries (N=5), and one study was conducted on raspberries, strawberries, and blueberries (N=1) (Table 2.2).

The studies showed a wide range of controlled release ClO_2 gas concentrations (1-50 ppm) and one study did not define a concentration. The study time/ ClO_2 gas exposure time ranges from a short time (30, 60, 120 minutes to 2.5, 5, 12 hours) to a long time (9-12 days).

Study temperatures included refrigerated conditions at 1, 2, 3, 4, 6, and 10 °C and 20 °C (N=4), and 23 ^{0}C (room temperature) (N=4). Only three studies mentioned the maintenance of RH (75-90%, 99%, 50%) at room temperature.

The target quality after treatment with ClO_2 gas was different between studies. Most (N=6) of the studies were designed to look at the reduction of microorganisms for safety and quality. The remainder of the studies (N=2) had a primary target of evaluating the sensory quality of products. However, some (N=2) of the six anti-microbial studies also evaluated at least one sensory aspect of the products and one (N=1) of the product quality studies also looked at the reduction of yeast and mold.

The papers that included studies of micro-organisms included some (N=2) that studied the reduction of only salmonella, some (N=2) that studied three bacteria (*E. coli*, *salmonella*, and *L. monocytogenes*), and some (N=5) studied control of yeast and mold.

Table 2.2: Summary of Studies Reporting Chlorine Dioxide Gas Sachet in Berry Preservations

First Author, Year	ClO_2 (g) concentrations	Conditions used	Product/s used	Target	Test results/Key findings
Bridges, 2018	0.03, 0.06, and 0.12 mg ClO_2/g produce for a 2.5-hour exposure, 0.04, 0.07, and 0.15 mg ClO_2/g produce for a 5.0-hour exposure time	Exposure time 2.5 or 5.0 hours at room temperature (23 ^{0}C)	Baby-cut carrots, lowbush blueberries, and beefsteak tomatoes in polypropylene clamshells (Using a scaled-up closed-circulation treatment system)	Target bacteria- (Shiga toxin-producing *Escherichia coli* (STEC), serovars of *Salmonella enterica*, and *Listeria monocytogenes*	0.15 mg ClO_2/g, 5h- Maximum STEC reductions of >7 logs observed on carrots and tomatoes, 4.9 and 5.5 for Salmonella and L. monocytogenes, respectively 3.7 STEC, 2.7 *Salmonella*, and 2.1 log *L. monocytogenes* reduction on blueberries left minimal residue levels (<3ppm)
Chiabrando, 2018	ClO_2 generating pads formulated with chemical mixtures (controlled release)	Short storage time (3 days at 4 ^{0}C and 3+2 days at 20 ^{0}C), Long storage time (12 days at 2 ^{0}C)- 4, 8, 12 days	Strawberry (*Fragaria X ananassa* Duch.)	Quality parameters by instruments-color, titratable acidity, total soluble solids, anthocyanins, antioxidant capacity, and weight loss, vitamin C, Total yeast, and mold. Sensory descriptors- presence of visible mold, presence of moisture, presence of water in the box, presence of anomalous odors, the color of skin and leaf, global appearance and aroma.	Short storage- Sensory evaluation – positively acceptable. No differences Maintained better quality parameters Long storage- Preserved the quality parameters. No degradation of pigments (red- a* value) after ClO_2 gas treatment. a* value decreased significantly. No sig. Changes of L*. Reduced the weight loss and microbial proliferation- A weight loss of treated strawberries was significantly lower than control after 8 and 12 days. ClO_2 gas reduced significant levels of yeast and mold count (Ex: 3.1 to 2.3 log CFU/g after 8 days). Sensory evaluation- Negative scores on skin and leaf color-Skin whitening, Low overall appearance in terms of color Concl: Suitable to preserve for 8 days at 2 ^{0}C. Alternative easy sanitizer to control yeast and mold than product washing.

First Author, Year	ClO_2 (g) concentrations	Conditions used	Product/s used	Target	Test results/Key findings
Zhang, 2014	ClO_2 gas (4 ppm) using three 3-kg sachets	20 ^{0}C freezer under different conditions where the berries reached a temperature of 3 ^{0}C after 3 h (quick-frozen), 2 days (intermediate-frozen), and 5 days (slow-frozen). Sampling 6 months	Blueberries	MAB yeast and mold populations.	ClO_2 gassing followed by quick freezing (after 3 h) provided effective microbial control compared to intermediate (2 days) and slow freezing (5 days). The bacterial and yeast mold count after 6 months of frozen storage of treated and controlled blueberries was reduced by 2 and 1 log CFU/g, respectively. ClO_2-gassed and un-gassed fruit, with MAB, yeast, and mold populations increasing ~1 log CFU/g during quick freezing to 3 ^{0}C and ~2 log CFU/g during intermediate and slow freezing to 3 ^{0}C.
Wang, 2014	Controlled release of 0.5 g of crystalline ClO_2 in a pad (in a 90–95% RH chamber, the pad releases ~ 60–70 % ClO_2 gas in 5 days at 4 °C)	Refrigerated conditions at 1, 6, and 10 °C and 20°C Experiment 1- 1. 1 °C for 10 days 2. 10 °C for 6 days 3. 20 °C for 3 days Experiment 2- day 0 test 1. 6 °C for days 6, 8, and 13 3. 20 °C for days 1, 2, and 3 Experiment 3- day 0 test 6 °C for 4, 8, and 10 days	Strawberry fruit cv. 'Festival' in perforated commercial clamshells	Experiments 1, 2, 3, 4 - Weight loss, firmness, surface color, other quality, and physiological parameters- soluble solids content (SSC), titratable acidity (TA), and volatile compounds. Fruit surface stomate activity. Experiments 2, 3 - Decay incident assessment Experiments 4 - Surface stomata (opened or closed) by a light microscope	Strawberries in perforated commercial clamshells with ClO_2 treatment (0.5 g ClO_2) as a slow-releasing pack, (1-6 ^{0}C for 14 days) slowed weight loss, softening, and reduced the decay incidence. Weight loss for ClO_2-treated fruit was 81–208 % less than in control when storage temperature was ≤10 °C. 50 % of stomata in ClO_2-treated fruit were closed after 7 days of storage at 6 °C, whereas all stomata were open in control fruit. ClO_2 prevented opening stomata, reduced weight loss, and maintained firmness. Soluble solids content, TA, and surface color values were not significantly affected by ClO_2 treatment, storage temperature, or storage time. Fruit volatile profiles were discriminated by storage temperature and time, but not by ClO_2 treatment

First Author, Year	ClO_2 (g) concentrations	Conditions used	Product/s used	Target	Test results/Key findings
Sy, 2005	Sachet formulated to release gaseous ClO_2 at concentrations of 4.1, 6.2, and 8.0 mg/liter	Within 30, 60, and 120 min, respectively, at 23± 1^{0}C. 75 to 90% RH-lethality	Blueberries (Vaccinium corymbosum L.), Strawberries (Fragaria ananassa Duchesne) and Red Raspberries (Rubus idaeus L.)	Salmonella (five serotypes of *Salmonella enterica*), Yeasts, and Molds Sensory (appearance, color, and aroma)	1. Treatment with 8.0 mg/liter of ClO_2, Salmonella on blueberries was significantly reduced by 2.4 to 3.7 log CFU/g. Salmonella on strawberries was reduced by 3.8 to 4.4 log CFU/g. Salmonella on raspberries was achieved a significant reduction of 1.5 log CFU/g of. 2. Treatment with 4.1 to 8.0 mg/liter of ClO_2, reductions of yeast and molds on blueberries, strawberries, and raspberries were 1.4 to 2.5, 1.4 to 4.2, and 2.6 to 3.0 log CFU/g, respectively. Significant reductions in Salmonella 1.9 to 3.7, 2.2 to 4.4, and 0.5 to 1.5 log CFU/g of blueberries, strawberries, and raspberries, respectively. Treatment with 4.1 mg/liter of ClO_2, did not markedly affect the sensory quality (appearance, color, and aroma) of fruits stored for up to 10 days at 8^{0}C.
Yuk, 2006	100 mg 23 °C ± 2 °C, approximate 50% relative humidity	23 °C ± 2 °C, approximate 50% relative humidity	Strawberry (Bell Pepper, Cucumber)	5-serovar cocktail of Salmonella	ClO_2 treatment decreased counts to undetectable levels on all inoculation sites on cucumber and on strawberry smooth surfaces, but failed to eliminate Salmonella from bell pepper and the stem scar and the puncture wounds of strawberries. ClO_2 treatments effectively reduced Salmonella cells inoculated on the smooth surface and stem scar of strawberries compared with un-sanitized control.

First Author, Year	ClO_2 (g) concentrations	Conditions used	Product/s used	Target	Test results/Key findings
Popa, 2007	Setup 1- 4 mg/liter, 0.16 mg/g 12 h in a sealed 20-liter container (99.9% relative humidity) at 22 ^{0}C Setup 2- 18 mg/liter (0.13 mg/g) for 12 h Pilot study - Sensory 0.19 mg of ClO_2 gas per g of fruit for 12 h at 22 ^{0}C and 99% RH	Very short storage for 12 h at 22 ^{0}C and 99% RH	(Frozen) blueberry Setup 1- 100 g inoculated blueberries. Setup 2- 30 lugs (~9.1 kg per lug) of uninoculated blueberries on 1.2 by 1.2-m pallets (5 lugs per level x six levels)	Target bacteria- *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* O157:H7, as well as five yeasts and molds	Setup 1- Reductions of 3.94, 3.62, 4.25, 3.10, and 3.17 log CFU/g for *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, yeasts, and molds, respectively. Setup 2- Reductions of 2.33, 1.47, 0.52, 1.63, and 0.48 log CFU/g were seen for mesophilic aerobic bacteria, coliforms, E. coli, yeasts, and molds, respectively. No sig. differences in microbial inactivation between lug levels and, with one exception (mesophilic aerobic bacteria), between the bottom and top surface of individual lugs Pilot study - Sensory No significant changes in sensory attributes (appearance, aroma, texture, flavor, and overall acceptability) compared to control.

DISCUSSION

Antimicrobial gas treatment such as ClO_2 gas on post-harvest foods as well as packaged foods through antimicrobial packaging systems (active packaging) or antimicrobial gas treatment through perforated packaging systems to food is paramount important for product safety and shelflife extension. Chlorine dioxide (ClO_2) in its gaseous form has been used in numerous studies for vapor-phase decontamination, both in treating produce before packaging and decontaminating the products inside their packages. ClO_2 releasing rate/dose and exposure time have been widely studied, but the influence of treatment apparatus (such as the use of sachets) has not been widely studied. The literature provided evidence that berries close to a ClO_2-emitting sachet received large doses, and berries located at more distance received significantly less ClO_2 exposure (Ellis, Cooksey, Dawson, Han, and Verano, 2006).

Chlorine dioxide gas sachets are suitable to maintain freshness and quality for a short period of storage (<10 days) rather than extending the shelflife of berries. However, a significant microbial reduction in both pathogenic bacteria (increased food safety) and yeast and mold (main deterioration of berries) has been improved with the use of ClO_2 sachets. The sensory quality (especially the firmness of berries and other parameters such as color, aroma, and appearance), which is the consumer attractive factor to determine the berry freshness, has been improved significantly. Most importantly, the water content of berries has been preserved by reducing water loss (Chiabrando, 2018; Wang, 2014). Therefore, improvement of shelflife of berries by the sensory quality and microbial quality using ClO_2 sachets have been discussed in this section.

Sensory evaluation

ClO_2 exposed strawberries have proven to have better quality maintenance (color, appearance, overall acceptability) after ClO_2 treatment using sachets and also no adverse effect on fresh sensory qualities, aroma, and flavors (Chiabrando, 2018; Wang, 2014; Sy, 2005). Long time exposure (>10 days) to ClO_2 preserved the quality parameters of strawberries while short time exposure maintains a better freshness and quality of strawberries. No degradation of a* color (pigments) was evident. Weight loss of strawberries is the primary cause of quality deterioration and is a significant factor to determine the final quality of strawberries for shelflife maintenance. According to Chiabrando (2018), weight loss of ClO_2 treated strawberries was significantly lower than control after 8 and 12 days. ClO_2-treated strawberries were found to be preserved for 8 days at 2 °C (due to exhaustion of ClO_2 generating pads) (Chiabrando, 2018). According to Wang (2014), strawberries in perforated commercial clamshells with ClO_2 treatment as a slow-releasing pack (1-6 ^{0}C for 14 days) demonstrated less weight loss, less softening, and reduced decaying incidence, as compared to a control with no ClO_2. Weight loss for ClO_2-treated fruit was 81–208 % less than in control when storage temperature was ≤10 °C. 50 % of stomata, (which are present in strawberry skin as pores that open and close to let oxygen, carbon dioxide, and water vapor in and out) in ClO_2-treated fruit were closed after 7 days of storage at 6 °C, whereas all stomata were open in the control fruit (Wang, 2014).

Overall, ClO_2 prevented the opening of the stomata, reduced weight loss, and maintained firmness. Soluble solids content, total acidity (TA), and surface color values were not significantly affected by ClO_2 treatment, storage temperature, or storage time. Fruit volatile compounds were changed by storage temperature and time but not by ClO_2 treatment (Wang, 2014).

Microbial reduction

Chlorine dioxide (ClO_2) is a highly oxidizing antimicrobial gas that reacts with the proteins of cell membranes in microorganisms and destroys them through cell wall oxidation. In consideration of microbial perforation (quality deterioration by microbial activity), yeast and mold are the most common causes of quality and safety deterioration of strawberries. ClO_2 treatment caused a significant reduction in yeast and mold count in berries (Popa, 2007), while other studies also showed a considerable reduction in yeast and mold, which is summarized in Table 2 (Sun, 2014; Zhang, 2014; Sy, 2005). Higher reductions of yeast and mold (approximately >2 logs CFU/g) were observed depending on the environmental conditions (temperature and relative humidity) used and ClO_2 concentration. The literature provides evidence that a higher ClO_2 dose combination with lower temperature reduced the growth of yeast and mold count (Zhang, 2014).

When considering bacteria, *Salmonella* is the main bacterium that causes foodborne diseases and thus reduces the safety and quality of berries. Many studies were targeted at reducing *Salmonella* from ClO2-treated berries (Bridges, 2018; Popa, 2007; Yuk, 2006; Sy, 2005). Two studies focused on reducing 3 common bacteria, including salmonella (*E. coli*, *salmonella*, and *L. monocytogenes*), which are the three most common causes of foodborne outbreaks in berries (Bridges, 2018; Popa, 2007). The use of ClO_2 treatment effectively reduced these microbial populations. According to Sy (2005), a significant reduction of *Salmonella* in blueberries, strawberries, and raspberries occurred with a higher dose of ClO_2 (8ppm), compared to a lower dose (4 ppm).

LIMITATIONS

Additional studies related to this subject might be published that were not covered in our inclusion criteria, such as articles published in other databases, articles published in other languages, and those published before the year 1990 and after 2018. In addition, some studies might have been excluded due to our keyword limitation. The identification of bias may be important for future research on this topic of quality assessment if someone needs to publish a review paper.

CONCLUSIONS

- Controlled release ClO_2 gas is a suitable antimicrobial gas in storage settings or commercial clamshell packaging to extend the shelflife of produce.
- The ClO_2 gas sachet is a simple, economical, effective, and practical method to enhance microbiological shelflife extension and safety.
- ClO_2 gassing followed by quick freezing provides effective microbiological control.
- The lower reduction of bacterial count on blueberries compared to other produce was due to higher surface area.
- The ClO_2 gas sachet is an easy-to-use alternative sanitizer to control yeast and mold as compared to product washing with aqueous ClO_2.

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CHAPTER THREE

EFFECT OF CHLORINE DIOXIDE GAS ON POLYMERS: A SYSTEMATIC REVIEW OF THE LITERATURE

ABSTRACT

Gaseous chlorine dioxide (ClO_2) is a more effective disinfectant (rapid diffusion to product and packaging surfaces) as an antimicrobial gas in the headspace of fresh food packages compared to aqueous chlorine dioxide. ClO_2 gas can diffuse into product surfaces and films. Since ClO_2 is a strong oxidizing agent, it can react with polymer packages and change the polymer properties and performance that may in turn affect the product's shelflife. In this paper, we systematically review the literature to determine the effect of ClO_2 gas treatments on polymeric packaging materials that may affect the shelf life of fresh foods. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) principles method was used. The search was performed using five search engines/databases. Seven studies were identified for inclusion. Many polymer properties, mainly tensile strength (TS), oxygen permeability, and chemical structure were changed significantly ($p \leq 0.05$) after exposure to ClO_2 gas depending on the polymer used, gas concentration and contact time, relative humidity (RH), and temperature. This paper is a summary of all literature to determine ClO_2 gas exposure on the effect of polymer properties. This summary allows researching further on varying RH and temperatures for the general application (0-10 ppm) of ClO_2 gas on each polymer used for common fresh product applications. It defines the literature gaps, such as the need for further

experimentation on RH and temperature effects when using ClO_2 gas (0-10 ppm) on polymers used for common fresh product applications. This paper may also be used as a guide for future researchers who investigate the use of ClO_2 antimicrobial packaging for fresh foods to select the optimum conditions for product integrity and design a packaging system suitable for fresh products.

KEYWORDS: chlorine dioxide; effect; polymers; properties; produce; packaging; shelflife

INTRODUCTION

Chlorine dioxide (ClO_2) is a widely used disinfectant in food industries, competing with (Cl^-), and it is currently approved by the U.S. Food and Drug Administration (FDA) as an antimicrobial gas inside the packages of meat, poultry, and seafood products USFDA. (2001). ClO_2 can be used to disinfect and maintain the quality of fresh produce in food industries. Chlorine dioxide can be used as a fumigant treatment to sanitize fresh fruits such as blueberries, raspberries, and strawberries to keep them fresh. It has a strong oxidizing capability, 2.5 times that of chlorine. Several recent papers provide evidence that ClO_2 is effective as an antimicrobial gas when used in the headspace of produce packages, increasing the safety and quality of the product (Netramai et al., 2016; Sun et al., 2014; Ray et al., 2013).

Although there has been some research, ClO_2 as an antimicrobial gas in the headspace of produce packages is still a relatively novel approach. Produce packages are often made of polymers such as polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE). These polymers are often constantly in contact with the fresh produce, as well as being in contact with the headspace. Therefore, polymer chains may react with this strong oxidizing agent ClO_2, depending upon the polymer structure and chemistry (functional groups, crystallinity, amorphous regions of semi-crystalline polymers, etc.). Changes in polymer properties after exposure to ClO_2 also depend on details related to the application, such as gas concentration and environmental conditions (relative humidity (RH), temperature).

The most common changes of polymers after exposure to a strong oxidizing agent such as ClO_2 include chain scission, crosslinking, depolymerization, formation of conjugated double bonds, formation of carbonyl groups, etc. Polymer properties such as mechanical (tensile strength (TS), elongation at break (EB), etc.), thermal properties (melting temperature (T_m), glass transition temperature (T_g), etc.), and barrier properties (oxygen permeability, water vapor permeability, etc.) can be changed permanently or temporarily due to prolonged exposure to ClO_2 gas. If these polymers are changed permanently, possible outcomes could include loss of package integrity resulting in a reduction of shelf life of the packaged product and even loss of safety or quality. Current knowledge and literature regarding the compatibility of ClO_2 gas with polymer packaging materials that are used to contain food are limited.

After an exhaustive search, no published systematic literature review of studies examining the effect of ClO_2 gas on packaging materials were found. The objective of our literature review was to answer the following research questions: 1. Would ClO_2 gas change the polymer properties? 2. What type of polymers are affected by ClO_2 gas? 3. What polymer properties were changed by ClO_2 gas? 4. Is there a relationship between ClO_2 gas concentration and the degree of change of the polymer properties? 5. Do other factors (such as RH and temperature) change the effect of ClO_2 gas on polymers?

The primary objective of this search was to systematically review the literature to determine the literature gap in the effect of ClO_2 gas treatments on packaging materials that may affect the shelf life of products. (Another objective is to summarize the literature on the topic and to apply the literature gap to design an experiment on the effect of ClO_2 gas exposure on the properties of berry packaging clamshells and stretch hoods). This review will also provide perspective to future research on the use of ClO_2 gas as an antimicrobial compound for food packaging applications (active packaging system).

METHODS

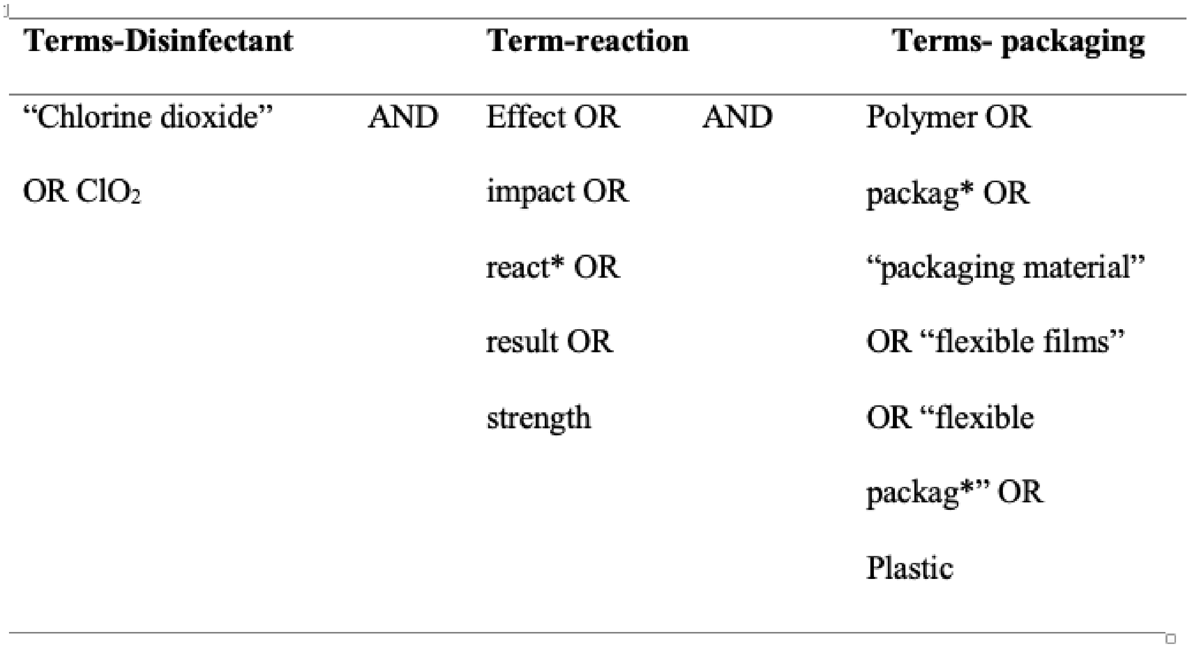
Search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) principles guided the literature search (Moher et al., 2009) to identify studies published on determining changes of polymer properties after exposure to ClO_2 gas. The search was performed using five search engines/databases – EBSCO (databases: Academic Search Complete, Food Science and Technology), Web of Science, Science Direct, Academic One File, and Engineering Village using the search terms shown in Table 3.1.

At the initial readings of the relevant topics and from the guidance of academic packaging professionals, we identified three general categorized terms. We conducted our database search using a search phrase that was comprised of search terms from these three categories: Disinfectant-related, reaction-related, and packaging material-related terms (Table 3.1).

The reference lists of review articles and included documents were also manually searched to identify additional published documents that might have been missed during the database search.

Table 3.1: Literature Search Items



Terms-Disinfectant		Term-reaction		Terms- packaging
"Chlorine dioxide" OR ClO_2	AND	Effect OR impact OR react* OR result OR strength	AND	Polymer OR packag* OR "packaging material" OR "flexible films" OR "flexible packag*" OR Plastic

Study selection

We conducted our initial search to include all documents, including peer-reviewed articles published between 1990 and 2019 in English and published in all geographic areas. After compiling results from across databases, duplicates were removed. This is shown in Figure 1 in the identification section.

Next, the titles and abstracts of the remaining documents were reviewed using our eligibility criteria. All the documents published in English between 1990 and 2019 that describe the three general categorized terms (Table 5.1) were "screened" by title and abstract. Only those documents that were relevant to the keywords and topic were kept as eligible. This is shown in Figure 1 in the screening section.

Full texts of the documents remaining after screening were then obtained. The reference lists of these documents were checked to identify additional articles that may be relevant. Review articles were excluded from eligibility. However, after searching the reference lists of both review articles and other eligible documents, additional eligible articles were included. This is shown in Figure 1 in the eligibility section.

The final step was a full-text review of the remaining articles. To be included after this step, a document/article had to describe the ClO_2 gas effects on polymers, be published in English, and be published between 1990 and 2019. Studies were included regardless of geographic area. This is shown in Figure 1 in the included section.

RESULTS

Study selection

A total of 414 documents were found through initial database searching at the identification (Figure 1). After removing duplicate records (N=149), 265 records were selected for the initial screening of titles and abstracts. In the initial screening, 235 records were excluded because the title and abstract did not meet the inclusion criteria. Finally, 30 eligible articles were identified for full-text review, and 2 records were included through manual searching of reference lists. After reviewing the full texts of the 32 potentially eligible records, 25 records were excluded for the following reasons: studies that do not include the full text (N=1), review studies (N=1), studies (N=14) about ClO_2 gas incorporated packaging material (as antimicrobial packaging to treat packaged products but not about the study of ClO_2 gas that affects packaging), studies (N=9) that reported use of ClO_2 to treat food such as meat and produce before packaging (to see any effect on the product safety). A total of 7 studies met our inclusion criteria which directly target the subject of ClO_2 gas effects on polymers.

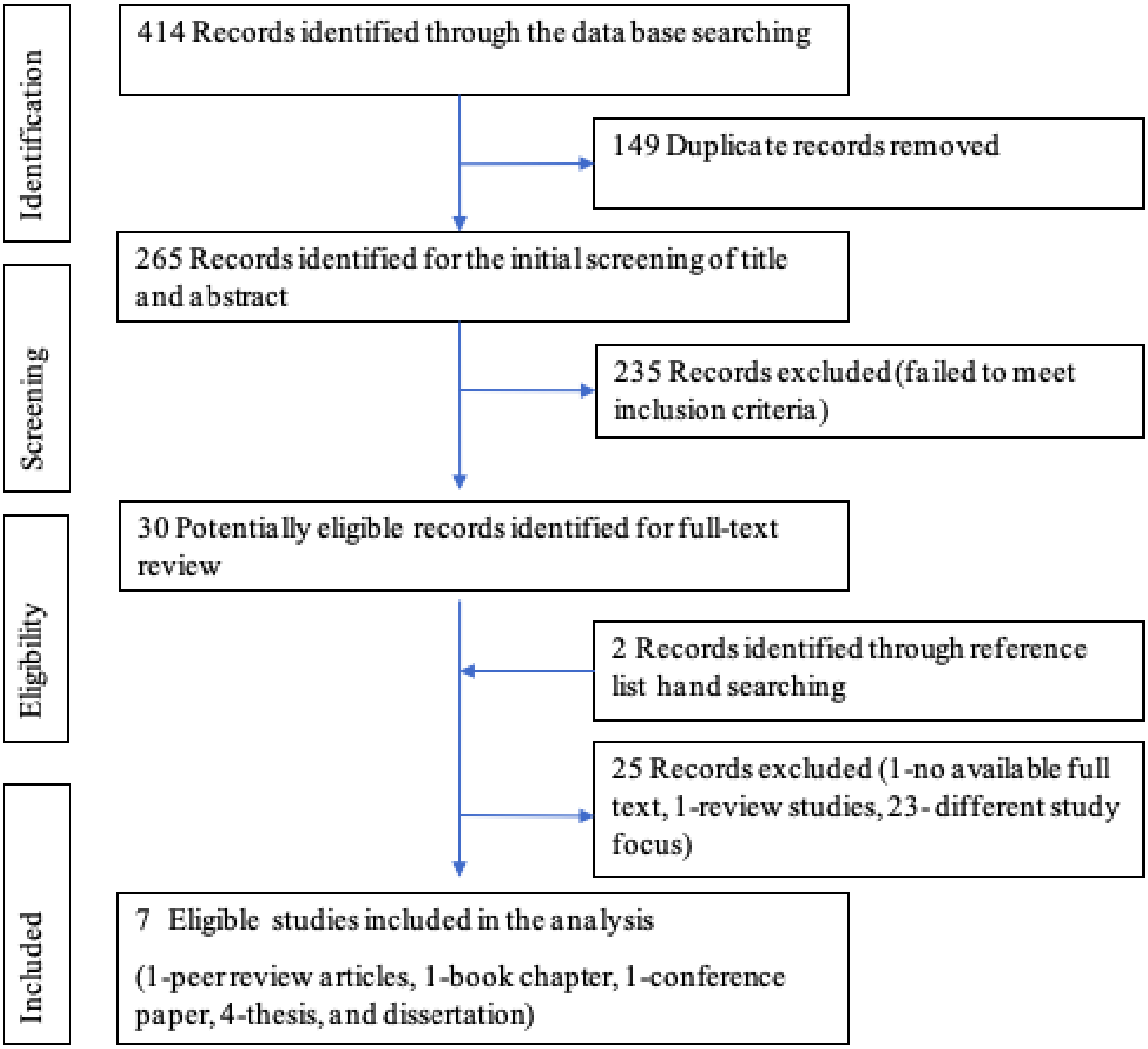


Figure 3.1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), 2009 Flow Chart Describing the Literature Search Procedure.

Study characteristics

All 7 studies were published in English between 1990 and 2019. One was a peer-review article, one was a book chapter, one was a conference paper, and the remaining four were thesis and dissertations. Rubino (2010) is a peer-reviewed paper that originated from the thesis and dissertations of Netramai (2011). Netramai (2012) is also a book chapter published about this study (part of the data presented from the original study). However, we included them as three published documents. Shin (2006) is a conference paper and presented only part of the data reported by Shin (2007), so these were included as two documents. (A summary analysis of these studies is provided in Table 2).

A wide range of packaging polymers was evaluated in these studies. Many studies mainly used polyethylene types (low-density polyethylene-LDPE, linear low-density polyethylene-LLDPE) as well as polystyrene (PS), polypropylene (PP), and nylon. The study published in 2010 Rubino evaluated more polymers such as the “mainstream” PE types (LDPE, LLDPE, high-density polyethylene-HDPE), biaxially oriented polypropylene (BOPP), PS, poly(vinyl chloride)-PVC, polyethylene terephthalate (PET), poly(lactic acid), nylon. Rubino[6] also utilized a multilayer structure of ethylene-vinyl acetate (EVA) and ethylene vinyl alcohol (EVOH) -(EVA/EVOH/EVA).

The maximum exposure time of the ClO_2 treatment of polymers in these studies was 14 days (N=3). Three studies tested ClO_2 treatment of polymers in short frequencies, testing over multiple intervals within a day (N=1) and up to 3 days (N=2). One study experimented with effect of ClO_2 treatment on polymers for 11 days (N=1). All 7 studies conducted mechanical tests: TS and EB. Other tests that were conducted in some studies were tests for glass transition temperature (T_g) and melting temperature (T_m) using differential scanning calorimetry (DSC) (N=4), permeability tests oxygen transmission rate (OTR), and water vapor transmission rate (WVTR) (N=5), color tests evaluating the overall lightness/darkness (L*), redness/greenness (a*), yellowness/blueness (b*) values (N=3), and chemical tests using the Fourier Transform Infrared (FTIR) (N=3).

ClO_2 dose as a factor associated to change the polymer properties

These studies used a range of ClO_2 concentrations, from very low (0.1-1 ppm) to a considerably higher concentration (100-2000 ppm). Four studies used medium levels of ClO_2 (low dose-6 mg/L to high dose-10 mg/L). One of the studies used the lowest level, and the two studies used the higher levels.

Relative humidity and temperature as a factor associated to change of polymer properties

Variable temperature was not used as a factor in most of the studies on the effect of ClO_2 on polymer properties. Six studies used room temperature (23 °C). One study used both the minimum (5 °C) and the maximum (35 °C). It is possible in some regions that the 35 °C temperature could be the temperature under which the produce is handled and distributed even inadvertently temperature abused during handling and distribution.

Relative humidities used ranged from medium levels (45%) to higher levels (100%). None of the studies used lower levels of RH. Three studies have been conducted at 50% RH (N=3), two were at 100% (N=2), the other two studies were used at 45 and 65% (N=1), and 45%, 65%, and 85% (N=1).

Table 3.2: Summary of Studies Reporting the Effect of Chlorine Dioxide Gas on Polymers.

First Author, Year	Type of Material	Reported ClO_2 (g) Concentrations	Experimental Conditions	Material Tests	Key Findings
Netramai, 2012 (Book Chapter)	PE-LDPE, LLDPE, HDPE, BOPP, PS, PVC, PET, poly(lactic acid), nylon, EVA/EVOH/EVA	10 mg/L (3600 ppmV ClO_2)	23^0C, 50% RH for 24 H (1 D), 168 H (7 D), and 336 H (14 D)	Chemical (IR) Barrier (WVTR, CO_2TR, OTR)	Chemical changes- permanent structural changes (all polymers) after 24 H of ClO_2 exposure, nylon changed regardless of time, reduction of rate of structural change with time. (Polar polymer-changes in hydroxyl (-OH) and C-N groups, the formation of carbonyl groups (-CO-); non-polar polymer-partial chlorination, main chain degradation). Barrier changes- nylon-improvement in the barrier to O_2, PE, PET, EVA/EVOH/EVA-degradation in barriers to moisture, oxygen, carbon dioxide, HDPE- higher degradation.

First Author, Year	Type of Material	Reported ClO_2 (g) Concentrations	Experimental Conditions	Material Tests	Key Findings
Netramai, 2011 (Dissertation)	PE-LDPE, LLDPE, HDPE, BOPP, PS, PVC, PET, poly(lactic acid), nylon, EVA/EVOH/EVA	10 mg ClO_2/L of gas (3600 ppmV ClO_2)	23^0C, 50% RH for 1 D, 7 D, and 14 D	Physical - (T_g, T_m, enthalpy of fusion by DSC), Mechanical - (TS at yield or break point (Nm-2) and MoE (N m-2), Barrier - (WVTR, CO_2TR, OTR) and Color - (L*, a*, b*) Chemical - infrared (IR) spectra	Chemical changes- permanent structural changes after 24 H of ClO_2 exposure, reduction of rate of structural change with time. nylon changed regardless of time, PS-no changes, PET-very slight changes (changes of C-H bonds). (Polar polymer-changes in hydroxyl (-OH) and C-N groups, the formation of (-CO-); non-polar- partial chlorination, main chain degradation). Barrier changes- WV barrier- PET, PVC significantly degradation PE, PET, EVA/EVOH/EVA – degradation of barrier to moisture, oxygen, carbon dioxide (main chain scission), Nylon-improved barrier to O_2 (partial chlorination increased polarity), All materials CO_2TR > OTR-highest for HDPE. Physical changes- T_g-PS, PET, and T_m-EVOH, nylon significantly reduced, nylon-heat of fusion increased-crystallinity increased. Mechanical changes- TS, MOE-significantly reduced in PE-oxidative degradation, with no changes in other polymers. Color-L* increased in LLDPE, PVC, PS, PET nylon, with significant overall color change in PVC, PS.

First Author, Year	Type of Material	Reported ClO_2 (g) Concentrations	Experimental Conditions	Material Tests	Key Findings
Rubino, 2009 (Paper)	PE-LDPE, LLDPE, HDPE, BOPP, PS, PVC, PET, poly(lactic acid), nylon, EVA/EVOH/EVA	10 mg ClO_2/L of gas (3600 ppmV ClO_2)	23^{0}C, 50% RH for 24 H (1 D), 168 H (7 D), and 336 H (14 D)	Physical - (T_g, T_m, enthalpy of fusion by DSC), Mechanical - (TS at yield or break point (Nm-2) and MoE (N m-2), Barrier - (WVTR, CO_2TR, OTR) and Color - (L*, a*, b*) Chemical - infrared (IR) spectra	Chemical changes- permanent structural changes after 24 H of ClO_2 exposure, reduction of rate of structural change with time. nylon changed regardless of time, PS-no changes, PET-very slight changes (changes of C-H bonds). (Polar polymer-changes in hydroxyl (-OH) and C-N groups, the formation of (-CO-); non-polar- partial chlorination, main chain degradation). Barrier changes- WV barrier- PET, PVC significantly degradation PE, PET, EVA/EVOH/EVA – degradation of barrier to moisture, oxygen, carbon dioxide (main chain scission), Nylon-improved barrier to O_2 (partial chlorination increased polarity), All materials CO_2TR > OTR-highest for HDPE. Physical changes- T_g-PS, PET, and T_m-EVOH, nylon significantly reduced, nylon-heat of fusion increased-crystallinity increased. Mechanical changes- TS, MOE-significantly reduced in PE-oxidative degradation, with no changes in other polymers. Color-L* increased in LLDPE, PVC, PS, PET nylon, with significant overall color change in PVC, PS.

First Author, Year	Type of Material	Reported ClO_2 (g) Concentrations	Experimental Conditions	Material Tests	Key Findings
Shin, 2007 (Dissertation)	LDPE, PS, PVC	100-2000 ppm	23°C and RH 100%, at 1, 12, 24, 48, and 72 H	Mechanical (TS and EB) Barrier (OTR), Color	Mechanical- Significant change of TS & EB, PS- TS significantly increased, EB% decreased by 50% and TS increased at >250 ppm ClO_2, PS became more brittle, PVC, LDPE-no significant change, LDPE, Cyovac 1050-a significant impact of EB at 2000 ppm, (>500 ppm ClO_2 caused degradation of polymer chains, formation of polar groups). Barrier- PE- at (>500 ppm $ClO_{2,}$ O_2 permeability is significantly lower than controls, increased crystallinity, PS-barrier improved, PVC, LDPE-no significant permeability changes. Color changed significantly only in PS at 1000 ppm. (L* value decreased by 4.55 and b* increased by 2 .8 6).
Shin, 2006 (Conference paper)	PE, PP, PS, nylon, and Cyovac 1050	Different concentrations in headspace (10-1000) ppm, 10 ppm for 1M 0, 500, 1000, 2000 ppm	23°C and RH 100%, (pre-conditioned at 23±2°C, 50 ± 5% RH), at 1 M	Mechanical (TS, EB) Barrier- (OTR) Thermal/Physical- (mentioned DSC-Tm, Tg)	Physical- no significant changes of T_g, T_m, at 10 ppm/1m of any polymers. Mechanical- PS, nylon- EB significantly decreased at >500ppm $ClO_{2.}$ Barrier- PS-degraded consistently with increased ClO_2 No changes in other polymers.

First Author, Year	Type of Material	Reported ClO_2 (g) Concentrations	Experimental Conditions	Material Tests	Key Findings
Stufflebeam, 2006 (Thesis)	LDPE, OPP, BON, and EVOH	6 mg/L (6 ppm)	45 and 65% RH, at 0, 1, 2, 5, 8, and 11 D. (Preconditioning prior to treatment 23°C and 35% RH)	Mechanical (TS and EB), Barrier- (OTR)	Mechanical- BON-ClO_2 and both RHs affected the TS and EB (only the MD), but not for OPP, LDPE. (TS of nylon increased significantly with ClO_2+ 65% RH than the treatment between 45% and 65% RHs), (a significant difference in TS of nylon (ClO_2+ 65% RH nylon < 65%RH control), EB of nylon ClO_2+65%RH < ClO_2+ 45%RH, TS, EB-time of ClO_2 exposure has a significant decrease on both MD, CD for 48 H, RH (65%RH>45%RH) has a greater effect on TS, EB) PP- a significant increase in TS for 48h then decreased, EB Significantly increased only at 11D (both MD, CD) . Barrier- ClO_2 Significantly affect the OTR of BON, but not EVOH (sig. greater effect at 65% than 45%). nylon- OTR decreased within 1D (cross-linking), then increased (bond hydrolysis). RH had a significant effect on the OTR, both RH had significantly higher OTR than the same film exposed to ClO_2, effect of OPP > nylon, effect is within 24h, and temporary.

First Author, Year	Type of Material	ReportedClO_2 (g) Concentrations	Experimental Conditions	Material Tests	Key Findings
Ozen, 2000 (Dissertation)	LLDPE, LDPE, OPP and BON	ClO_2 concentration 0.1-1 mg/L (0.1, 0.55, 1 ppm)	RH (45-85%)-45, 65 and 85%, T (5- 35°C)-5, 20, 35°C, at 5, 10, 15, 24 H	Mechanical properties (TS and EB) Physical/thermal- differential scanning calorimetry (DSC), and Barrier- (WVTR, OTR)	Mechanical- considerably affected both TS (~50% decrease) and EB of OPP, decrease of TS in LLDPE, LDPE and increase of TS in nylon – (none statistically significant) T was a significant factor for TS of LDPE. RH had a significant influence in OPP. effect of ClO_2 decreased with increasing RH. effect of RH on TS, EB high at high T. Thermal- treatment with ClO_2 did not cause any significant changes in OPP, BON. Barrier- A small increase of OTR in LLDPE, ClO_2 concentration, treatment time (not statistically significant) BON- OTR decreased to 23.5% (not significant).

Note: RH: relative humidity; T: temperature; D: day; H: hour; M: month; PE: polyethylene; PP: polypropylene; LDPE: low-density polyethylene; LLDPE: linear low-density polyethylene; HDPE: high-density polyethylene; PP: polypropylene; BOPP: biaxially oriented polypropylene; BON: biaxially oriented nylon; PS: polystyrene; PVC: poly(vinyl chloride); PET: polyethylene terephthalate; EVA: ethylene vinyl acetate; EVOH: ethylene vinyl alcohol; IR: infrared; WVTR: water vapor transmission rate; CO_2TR: carbondioxide transmission rate; OTR: oxygen transmission rate; TS: tensile strength at break; EB: elongation at break; MoE: modulus of elasticity; DSC: differential scanning colorimeter; Tm: melting temperature; Tg: glass transition temperature; L*: lightness/darkness; a*: redness/greenness; b*: yellowness/blueness; MD: machine direction; CD: cross direction.;

DISCUSSION

The intent of this paper was to summarize and systematically review the documents published on the topic of “the effect of ClO_2 gas on polymers.” Answering the research questions, the literature provides evidence that ClO_2 gas treatment can alter many polymer properties, including physical, chemical, and mechanical properties depending on the environmental conditions used and the type of polymer. Polymer properties in some polymers changed even with low levels of ClO_2 gas concentrations, as low as <10 ppm. Moreover, the highest level of ClO_2 gas concentrations (>100 ppm) resulted in greater degrees of degradation due to higher oxidation capability, which will also be discussed.

Factors affecting the changes of polymer properties:

Polymer type and its properties/characteristics

The polymer type – the physical and chemical structure (and resultant properties such as polarity and crystallinity) are important factors that impact changes when exposed to ClO_2 gas. Studies have been mainly focused on common food packaging polymers such as PE types (LDPE, LLDPE, HDPE), PP, PET, nylon, and PS. All the polymers include carbon-hydrogen and carbon-carbon bonds, and some of them have other bonded elements. When we consider a non-polar polymer like PE, it has three main types-LDPE, LLDPE, and HDPE, which can be categorized with density and branches. Some other polymers have elements and bonding arrangements that make them polar, such as PET and nylon used in some studies. These different functional groups (PET has carboxyl groups which show different properties compared to nylon which has amino groups) provide unique characteristics to polar polymers.

The reactive component – $ClO_2^\bullet$ (free radical of the ClO_2 gas) may react with polar polymers more readily than with nonpolar polymers, especially with the nitrogen on the nylon polymer, which appears to be more reactive with – $ClO_2^\bullet$ than the carboxyl group of PET (Rubino et al., 2010). Only nylon showed a significant change in chemical structure (an increase of crystallinity, changes in T_m, changes in C-N bonds by partial chlorination) due to ClO_2 gas exposure. No significant evidence for the structural changes in PET was found except for slight changes in peak intensities of IR spectra, which might be due to a slight change in the C-H bond or methylene group. However, the moisture barrier of PET decreased significantly. This may be due to oxidative degradation as a result of the increase in the polymer's chain mobility and the decrease in its intermolecular forces (Rubino et al., 2010).

Nonpolar polymers may go through partial chlorination and main chain degradation after prolonged exposure to ClO_2 gas. Polar materials are expected to change in -OH groups and C-N, as well as the formation of C=O groups during continuous exposure to ClO_2 Netramai et al., 2012). These structural changes of polymers occur not only with ClO_2 but also with other strong oxidizing agents and activities such as O_3, and UV radiation (Ozen, 2000).

Chlorine dioxide gas concentration over time

Many studies have used ClO_2 gas levels (6-10 ppm) for use with food to look for an effect on the polymer properties. The small amount of ClO_2 gas (0.1-1 ppm) used did not show any effect on polymers, while the medium level of ClO_2 gas has resulted in changes in some of the polymer properties. Higher doses of ClO_2 gas (100-2000 ppm) demonstrated more pronounced effects on the polymer properties or made significant changes in polymer properties (Shin, 2007). The use of a higher level of ClO_2 gas is not practical for food and packaging because its strong oxidative behavior deteriorates packaging materials and bleaches fresh food, leaving more than the FDA-approved residual level (3 mg/L) (NCBI, 2005; USFDA, 2022). Although the use of a higher level of ClO_2 gas is not applicable in the food and packaging industries, it is useful to know the relationship between ClO_2 gas concentration and changes in polymer properties. The relationship between ClO_2 concentration for sanitization of foods in packages and the possible modification of physical properties of packages is a consideration. It is important to know the practical limit of ClO_2 use as a sanitizer in packaging applications. ClO_2 concentration does have an impact on polymer properties. The effect of ClO_2 concentration was only considered in the studies of Ozen (2000) and Shin (2007). ClO_2 concentration was a significant factor for the TS of LDPE, LLDPE, PP, and nylon. Shin (2007) is the only study that used a high level of ClO_2 concentration and found that high ClO_2 concentration (>100 ppm) resulted in a decrease in the OTR of PS. (This may be due to the degradation of polymer chains of PS when exposed to ClO_2). The changes of each polymer's properties after being exposed to ClO_2 will be discussed further in each section below.

Chlorine dioxide gas exposure time

The effect of ClO_2 gas on polymers also depends on the ClO_2 exposure time. Short-term exposures have caused temporary changes, and long-term exposures have shown to result in permanent changes. However, when the ClO_2 concentration was increased to a higher level (>100 ppm), changes in polymer properties were permanent regardless of exposure time. Rubino (2010) evaluated the longest exposure time (14 days) and observed that all types of film samples were affected (polymer structure). The changes in the peak intensities of IR spectra after 14 days of exposure were the most dramatic, followed by those after 7 days and then those after short-term exposure. However, peak intensity changes were at a slower rate with longer exposure times. ClO_2 release rate and concentration reduce over time in any system, whether it is a closed system or an open system. This is because ClO_2 can both react with and diffuse into the substrate surfaces. A slower rate of change in polymers for longer exposure times is due to low available ClO_2 or low available functional groups of the polymer. Rubino (2010) also explained that this slower rate of change could be attributed to reduction of availability on the film surface of functional groups such as amino ($-NH_2$), carboxyl (-COOH), and carbonyl (-CO-), etc. with which the ClO_2 can react. Oxidative degradation, which is usually a surface phenomenon, would cause the numbers of these reactive sites to be reduced.

Environmental factor-temperature

Environmental factors, such as temperature and RH, have a direct effect on polymer properties with or without the presence of ClO_2 gas in the packaging system, depending on the polymer type. Many studies have used room temperature combined with different RH's. One study used cold/refrigerated temperature, which is important in the food industry. In addition, 35°C has been considered, which is an expected increase to room temperature in some product storage systems. Changing the temperature to a higher level is not a practical application for food packaging polymers during storage conditions. This is because the temperatures above refrigeration typically have a detrimental effect on product quality and shelflife, and refrigeration temperatures are often well controlled in food industries when using ClO_2 incorporated packaging or ClO_2 gas treatment on produce in perforated packages.

Only Ozen (2000) considered the temperature as a variable. The temperature was the only significant factor for TS of ClO_2 treated LDPE films. However, both treatment temperature and gas concentration had a significant effect on the TS of LLDPE films. Therefore, the storage temperature was the most significant factor affecting the mechanical properties of LLDPE, LDPE, and OPP exposed to ClO_2 Ozen (2000). Temperature, RH, and ClO_2 concentration have a significant effect on the mechanical properties of OPP. The reason for temperature being a significant factor is that temperature accelerates the reaction of polymer chain degradation by ClO_2, and it also decreases TS (due to the higher degree of motion of polymer chains).

Environmental factor-relative humidity

Relative humidity played a significant effect in changing the properties of ClO_2 treated polymers. Only the papers by Ozen (2000) and Stufflebeam (2006) varied RH (more than other studies). Relative humidity caused a significant effect on the TS of OPP, where the high RH caused a considerable decrease in TS and an increase in EB of OPP Ozen (2000). RH was also a significant factor for TS of LLDPE, causing a decrease in TS after 15 h of ClO_2 treatment Ozen (2000). However, according to Ozen (2000), the interaction of TS of LDPE after 10 h treatment with ClO_2 shows that ClO_2 concentration became a more important factor with decreasing RH.

Properties of nylon films are greatly affected by the humidity due to the hydrogen bonds in the nylon's structure. The biaxially oriented nylon (BON) film samples exposed to ClO_2 gas at 65% RH showed a significant ($p \leq 0.05$) decrease in EB when compared to the BON film sample exposed to ClO_2 at 45% RH.[12] The N-H bond of nylon is highly reactive, and nylon may make hydrogen bonds with water faster than ClO_2 at high RH due to the presence of excess water. Alternatively, the reaction of ClO_2 may facilitate water attaching to nylon via hydrogen bonds. Stufflebeam (2006) explains that since BON is polar, it is hydrophilic, which means it tends to absorb moisture. The theory is that the sorption of water vapor leads to a decrease in TS and an increase in EB. This phenomenon is known as plasticization, which occurs when small molecules enter a polymeric matrix causing an increase in free volume within the polymer structure and a decrease in the T_g. Therefore, all the factors (RH, temperature, and ClO_2) play in an interactive manner on the changes of polymer properties.

Changes in chemical properties of polymers

Intensities of IR spectra can be used to reveal chemical changes such as the formation of polar groups in the polyolefins, changes in functional groups, main chain scission degradation, and possible chlorination of several materials. The shifting of peaks in the IR spectra reveals the possible presence of C-Cl bonds. ClO_2 exposure may cause molecular reordering in some polymers (for example, nylon increases in crystallinity). Oxidative changes occur in PE due to chain degradation and polar group formation. None of the studies reported significant evidence on the formation of any functional groups under the practical level of ClO_2 gas exposure (<10 ppm). Therefore, no adverse impact on food safety is expected.

Rubino (2010) has studied if the chemical changes of polymers are permanent or temporary depending on the ClO_2 exposure time. Post-exposure film conditioning (at 23 °C and 50% RH) suggested that the changes in the FTIR scans were temporary when ClO_2 exposure was short-term. After conditioning, the absorbance intensities of short time exposed samples were equivalent to those of the respective control samples. However, after 1 day of ClO_2 exposure, most of the chemical changes observed in the FTIR spectra tended to be permanent. Nylon showed considerable changes in IR intensities. Nylon changed permanently (chemical structure through chlorination, increased crystallinity, etc.) when exposed to ClO_2 gas, regardless of exposure time (Netramai et al., 2012). The similar IR spectra of HDPE, LDPE, and LLDPE showed minor changes in the intensities of the IR. This may be due to changes in the C-H bonds of the methylene group (Rubino et al., 2010). PVC and BOPP did not change significantly. Shifts in some of the peaks to higher wavenumbers in the IR spectra of the different types of PEs and PS indicate the possible presence of a C-Cl bond. Rubino (2010) suggests that degradation of the polymer's main chain and the formation of polar groups could be the reason for this.

The increased intensities in the IR of nylon and EVA/EVOH/EVA films could be related to the polymers' polarity. This may cause an increased barrier to gases for materials such as nylon and EVOH. An increase in the intensities of IR in the multilayer EVA/EVOH/EVA film indicates a change of the hydroxyl group in the EVOH layer and the formation of carbonyl groups (Rubino et al., 2010). The increases in absorbance intensities of IR of PLA and nylon could be due to changes in the hydroxyl groups and N-H bonds, respectively. According to the study of Rubino (2010) and Netramai (2011), there was no significant formation of any groups in the ClO_2-exposed PET films. Therefore, PET could be a reasonable option for the safety of the food product.

Changes in barrier properties of polymers

A barrier to CO_2, O_2, and water vapor is a significant parameter in determining/selecting an appropriate packaging material for a product. When considering produce packaging, loss of the O_2 barrier due to ClO_2 treatment may affect the respiration rate of fresh produce, which is a significant cause of over-ripening and deterioration of produce.

Significant changes in barrier properties were observed in some films. After exposure to ClO_2 gas, the O_2 barrier of the nylon increased (Netramai et al., 2012). Nylon has a low available amorphous region where permeability occurs. The partial chlorination of nylon increased the polarity after being exposed to ClO_2 gas, reduced the available amorphous region, and thus increased the barrier. In other polymer materials, the barrier properties to O_2 and CO_2 tended to decrease after ClO_2 exposure. In comparison, on day 0 and after 14 days of ClO_2 gas treatment, the most notable permeability change (increase) was observed in HDPE film. CO_2TR of HDPE, PS, and EVA/ EVOH/EVA multilayer film was decreased. The moisture barrier of PET was significantly reduced while that of other polymers did not change. Overall, polyamide (nylon) showed many interesting changes, including increased OTR, increased heat of fusion, and a possible increase in crystallinity. According to Shin (2007), high ClO_2 concentration (>100 ppm) consistently affected barrier properties of PS (OTR decreased). This may be due to the degradation of polymer chains. Rubino[6] also evaluated the effect of ClO_2 gas on barrier properties (oxygen, carbon dioxide, and water vapor permeabilities) of different polymers and observed an alteration in permeability when the polymer was exposed to a higher ClO_2 gas concentration (3600 ppmV) for 14 days, which is the mean shelf life for several modified atmospheric packaging (MAP) products. Ozen[8] also found a minor increase in the OTR of LDPE, LLDPE, and OPP when exposed to ClO_2 gas as opposed to nylon-based packaging material, which decreased the OTR. The study of Ozen (2000) observed similar findings on the effects of ozone (O_3) in the packaging system and its effect on biaxially oriented nylon. When an increased OTR was found, this change was possibly due to the main chain scission in the polymer matrix, which is responsible for increased chain mobility and allows the permeation of O_2 and CO_2 through the polymer matrix (Kulshreshtha and Awasthi 1999; Selke et al., 2004).

Changes in mechanical properties of polymers

Most of the seven included studies showed that ClO_2 had an effect on TS in at least some polymers. In some studies, TS and modulus of elasticity (MoE) of ClO_2 treated PE’s decreased significantly. This may be due to oxidative changes of PE, such as degradation of polymer chains (formation of the partially polar group, etc.). These changes could lower the mechanical properties of ClO_2 treated PE material (Rubino et al., 2010; Netramai, 2011). Ozen (2000) and Shin (2007) have mentioned that (>500 ppm) higher concentration of ClO_2 may cause degradation of polymeric chains and strengthen the intermolecular forces resulting from the formation of polar groups. It has been reported that, although polymers' oxidation impairs mechanical properties, it improves the molecular ordering resulting in increased crystallinity. Oxidation of PE has been shown to increase the crystallinity and MoE of PE films (Tsobkallo et al., 1988). The TS of ClO_2 treated BOPP, PS, PVC, PET, poly(lactic acid), nylon, and EVA/EVOH/EVA did not significantly change. However, the formation of polar groups was present in IR spectra. An increase in polarity can cause an increase in TS due to an increase of intermolecular forces (Rubino et al., 2010, Netramai , 2011).

Shin (2006) reported that the EB of PS decreased by approximately 50% at a 250 ppm or higher level of ClO_2 (an increase in treatment concentration resulted in an increase in TS strength). PVC and LDPE films had no significant ($p \leq 0.05$) changes in mechanical properties (Shin, 2007). The percent change in (EB) of PS and nylon were significantly ($p \leq 0.05$) decreased by the high dose of ClO_2 treatment (>500 ppm) (Shin, 2006). The percent change in TS of nylon decreased gradually with the increasing use of ClO_2 (0 to 2000 ppm). This could be due to changes in bond strength of polar materials with the reaction of polar ClO_2 (free radicals). The opposite was found for nylon compared to the other polymers could have been due to the plasticization of nylon at high RH (100%) (Shin, 2006; Stufflebeam, 2016). However, Ozen (2000) observed no significant changes ($p \leq 0.05$) of TS at a low ClO_2 dose (0.1-1 ppm) in all polymers considered - LLDPE, LDPE, OPP, and even with BON. A minor decrease in TS (and increase in EB) of LDPE, LLDPE was observed after treatment with ClO_2 (regardless of any conditions used). TS of ClO_2 treated BON films increased while a decrease was observed in TS of OPP (Ozen, 2000). Stuffelebeam (2006) also observed that ClO_2 gas (6 ppm) had no significant effect ($p \leq$

0.05) on the TS of OPP and LDPE films. Chlorine dioxide gas and RH affected the TS and EB of BON film in only the machine direction (MD). The TS of ClO_2 treated BON film showed a significant ($p \leq 0.05$) increase between 45% and 65% RH. Chlorine dioxide alone influenced the TS of BON at 65% RH significantly ($p \leq 0.05$) (Compared to the control at 65% RH). The BON film samples exposed to ClO_2 gas at 65% RH showed a significant ($p \leq 0.05$) decrease in EB when compared to the BON film sample exposed to ClO_2 at 45% RH. TS of BON in both machine and cross-directions significantly ($p \leq 0.05$) decreased within 48 hours of exposure to ClO_2 gas but began to increase gradually after 48 hours. This could be due to the entrapment of free radicals within the polymer structure, which could, over time, cause some cross-linking within the film. The "rebuilding" of macromolecules and therefore, increased molecular bonding could be the reason for the gradual increase (Stufflebeam, 2006).

Changes in thermal properties of polymers

No significant changes in thermal properties of ClO_2 treated polymers were identified in DSC data of T_m and T_g of many polymers except nylon (Rubino et al., 2010; Netramai, 2011; Ozen, 2000; Shin, 2006). Only Rubino (2010) and Netramai (2011) observed a significant increase in the heat of fusion (a shift in T_m) of nylon. This could be a result of an increase in crystallinity of the exposed nylon sample with molecular reordering.

Ozen (2000) reported that exposure to ClO_2 did not result in any significant changes in the thermal properties of OPP and BON, except a slight decrease in melting enthalpy (ΔH_m) of OPP compared to control. T_m and T_g of polymers did not demonstrate any significant changes of properties with any concentration (10-1000 ppm) of ClO_2 gas, which suggests that there is no formation of a functional group or change in the structure of other polymers. Shin (2006) has mentioned that no significant difference was observed between non-treated and ClO_2 treated samples.

Changes in optical properties of polymers-Changes in color/appearance

Overall, no significant color changes (L*, a*, b* values) were observed in the polymers at the practical level of ClO_2 concentrations (<10ppm). Shin (2007) has observed no changes of colors in LDPE and PVC films at any concentration of CIO_2 gas, but PS, after exposure to a high level of CIO_2 gas, showed a change. PS was shown to have a significant color change at 1000 ppm (when exceeding the practical limit of 10 ppm) of CIO_2 (L* value decreased by 4.55 and b* increased by 2.86) (Shin, 2007). This may be due to the oxidation of PS, which is more susceptible to oxidation at a higher level of ClO_2 concentrations and change the color. Rubino (2010) and Netramai (2011) observed significant overall color differences (ΔE*) in ClO_2 treated PVC and PS. Lightness (L*) of the ClO_2 exposed LDPE, PVC, PS, PET, and nylon films increased. The b* values of the films also changed to more yellow until 7 days and then shifted to more blue until day 14. This change of color from opaque white/transparent to a dull yellowish may be due to the degradation reactions, such as the formation of conjugated double bonds and the oxidation of additives (Rubino et al., 2010).

LIMITATIONS

Additional studies related to this subject might be published that were not covered in our inclusion criteria, such as articles published in other databases, articles published in other languages, and those published before the year 1990 and after 2019. In addition, some studies might have been excluded due to our keywords limit. This paper does not emphasize the quality of the published papers/documents based on a quality assessment tool – a meta-analysis. The identification of bias may be important for future research on this topic.

CONCLUSIONS

Overall, minimum changes in physical and mechanical properties were observed in many exposed materials for the application of practical level (< 10 ppm) of ClO_2 as an antimicrobial gas in the package of food or headspace of the product or to treat from the outside of the product package. The comparisons to control films showed decreases in barrier properties (or increases in permeation) of several exposed polymers (with a significant decrease in PET), except for the improvement of barrier to O_2 in nylon film. When selecting a packaging material for a particular food with the appropriate ClO2 gas concentration, loss of barrier properties should be a concern. However, it has been mentioned in the literature that any adverse impact on food safety is not expected under a practical level of ClO_2 gas exposure (<10 ppm).

Based on this extensive search, no published systematic reviews of the changes of polymer properties after exposure to ClO_2 gas exist. Current knowledge of the compatibility of ClO_2 gas with packaging materials for foods is very limited. Therefore, investigations on “the use of ClO_2 gas on the shelf-life extension of packaged food” is justified. In addition, this summary can be used to develop appropriate strategies for designing ClO_2 incorporated antimicrobial gas generating packaging material to contain fresh produce. The future focus should be on the effects of different environmental conditions, such as RH and temperature, on the integrities of packaging material, to further determine the potential use of an acceptable level of ClO_2 gas (<10 mg/L) as an antimicrobial gas in the headspace of packaged products.

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CHAPTER FOUR

EFFECT OF CHLORINE DIOXIDE GAS EXPOSURE (USING SACHETS) ON THE PROPERTIES OF PACKAGING MATERIALS USED TO CONTAIN PRODUCE

INTRODUCTION

Fresh agricultural produce is one of the primary food sources in the food supply chain. Approximately half (52%) of all produce (fruits and vegetables) are unconsumed in the US market, which is the number one source of food waste (The Sonoco Institute, 2018). Packaging of fresh produce is essential to protect the product after harvesting. Popular polymers such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) are commonly used in the food packaging industry as they have many suitable manufacturing properties and product protective properties (Singh et al., 2020; Gaikwad et al., 2018).

Further protection of produce is possible with the use of an antimicrobial product system to prevent mold and yeast, as well as other microbes. ClO_2 is a strong oxidizing agent against bacterial, viral, and protozoan pathogens and is stronger than chlorine on a mass-dose basis (2.5x chlorine) (Singh et al., 2021; Sun et al., 2019). The FDA has approved to use of ClO_2 to treat fresh produce with a residual level of ≤ 3 mg/L (FDA, 2001). Packaging with antimicrobial gas such as ClO_2 preserves produce quality but does not enhance the quality (Singh et al., 2021; Sun et al., 2019).

Controlled release ClO_2 gas is a suitable antimicrobial agent that can be used in storage settings or in commercial clamshell packaging to extend the shelflife of produce.

Moreover, ClO_2 can be incorporated into packaging material (antimicrobial packaging), creating an “active package” to perform the desired function of an antimicrobial activity while maintaining the freshness and quality of the product. However, ClO_2 gas fumigation at the initial stage of produce storage (before packaging or after packaging right before loading to cool room) is the most common way of disinfecting produce (Singh et al., 2021; Sun et al., 2019).

ClO_2 gas releasing sachets could offer a simple, economical, effective, and practical method to enhance microbiological shelflife extension and safety of produce (Popa, 2007). However, continuous treatment of ClO_2 in a storage setting and retail display of produce is not widely available. The use of ClO_2 sachets inside the product package to treat produce is not yet commercially available. Due to the strong oxidizing capability and very reactive nature of ClO_2 gas, when used as part of a packaging system, the ClO_2 (free radicals) could change the chemical structure of polymeric packaging materials. The effect of ClO_2 gas on polymers may vary with many factors, such as the polymer structure and chemistry (functional groups, crystallinity, amorphous regions of semi-crystalline polymers, etc.) and the parameters of the application of ClO_2 gas, such as gas concentration and environmental conditions (relative humidity, temperature) (Kuruwita, Chapter 2; Kuruwita, chapter 3).

Polymer properties, such as mechanical properties (tensile strength, elongation at break, impact strength, etc.), thermal properties (melting temperature, glass transition temperature, crystallization temperature, etc.), and barrier properties (oxygen permeability, water vapor permeability, etc.) could also be affected due to prolonged exposure to ClO_2 gas. It is possible that the alteration of packaging materials may influence the package’s integrity and performance, resulting in reduced shelf life of food (Singh et al., 2020; Saengnil et al., 2014).

Current knowledge and literature regarding the compatibility of ClO_2 gas with polymer packaging materials used to contain food are limited. Based on a systematic literature review on the "effect of chlorine dioxide gas on polymers: a systematic review of the literature," minimal changes in physical and mechanical properties were observed in many polymers under the low-medium level of ClO_2 gas concentration (Kuruwita, Chapter 3). However, the barrier properties of several exposed polymers degraded (with a significant decrease in PET), except for the improvement of the barrier to O_2 in nylon-6 film (Kuruwita, Chapter 3). When selecting a packaging material for a particular food with the appropriate ClO_2 gas concentration, loss of barrier properties should be a concern. Any adverse impact on food safety was not reported in the literature under the low-medium level of ClO_2 gas exposure (<10 ppm).

The objective of this research is to determine the effect of controlled-release ClO_2 gas treatments (using sachets) on produce packaging materials under various relative humidities (49%, 84%, 99%) for a long exposure time (21 days). This will provide information to fill part of the literature gap identified in the literature search mentioned above. This study will also provide further guidance and perspective for future research on using ClO_2 gas as an antimicrobial compound in the headspace of food packaging systems (active packaging systems).

METHOD

Materials

Packaging materials

Amorphous polyethylene terephthalate (APET), grade Pentafood rigid APET FD-E630F01, a thickness of 20±0.2 mils was provided by Klockner Pentaplast of America, Inc., (Charlottesville, VA). Nylon-6, grade Capran 1500RT, with a thickness of 0.63±0.2 mils was supplied by Honeywell Films (now Advansix), (Pottsville, PA). These were the two polar materials used in this study. Nylon-6, which showed changes of its properties with ClO_2 in previous experiments (Kuruwita, Chapter 3), was used in the experiment as a "negative control". Biaxially oriented polyethylene stretch films, AmTopp OPE grade 1 (ELU) and grade 2 (ELB), with a thickness of 0.72±0.2 mils were provided by Inteplast Group, (Charlotte, NC). These were the non-polar materials used.

All four materials (APET, OPE's - grade 1, grade 2, and nylon-6) were cut into the sizes of 6 X 12 inches. The materials were supplied in roll form, so one side was "wound out" and the other was "wound in". Each sample taken from the rolls was labeled with marks that allowed the researchers to keep track of the two sides of the material. Before each trial, samples were stored under 20-25 ^{0}C room temperature and 45-55% RH in the room. Room temperature and RH were recorded throughout the experiment.

Chlorine dioxide sachet

Chlorine dioxide sachets were donated from ICA TriNova (Newman, GA). The sachets were placed into a normal environment, the inner mixture of chemicals reacts with moisture from the environment to produce and release the ClO_2 gas. Chlorine dioxide sachets were designed to provide a dose of continuous slow-release chlorine dioxide rate of 0.05 mg/day at 20ºC.

Relative humidity

A saturated salt solution of KNO_3 (Alfa Aesar, 30 Shore Road, Heysham, LA3 2XY, England) was prepared in a small beaker to maintain 90-99% relative humidity. Other humidity's (49% RH and 80-84%RH) were maintained using commercially available 2-way humidity control packs (Boveda, Boveda Inc., 10237 Yellow Circle Drive, Minnetonka, MN 55343, U.S.)

Closed sealed chambers

Six aquaria (glass fish tanks) 508 mm X 355 mm (20'' X 14'') were utilized as chambers for the experiment. Three of these glass chambers were used for the treatment groups and another three for the control groups. A sealable lid was made of heavy gauge aluminum foil (facing the interior of the chamber) and corrugated board to support the foil, to provide rigidity, and fit tightly into the aquaria tops. This assured a closed chamber set up for each group.

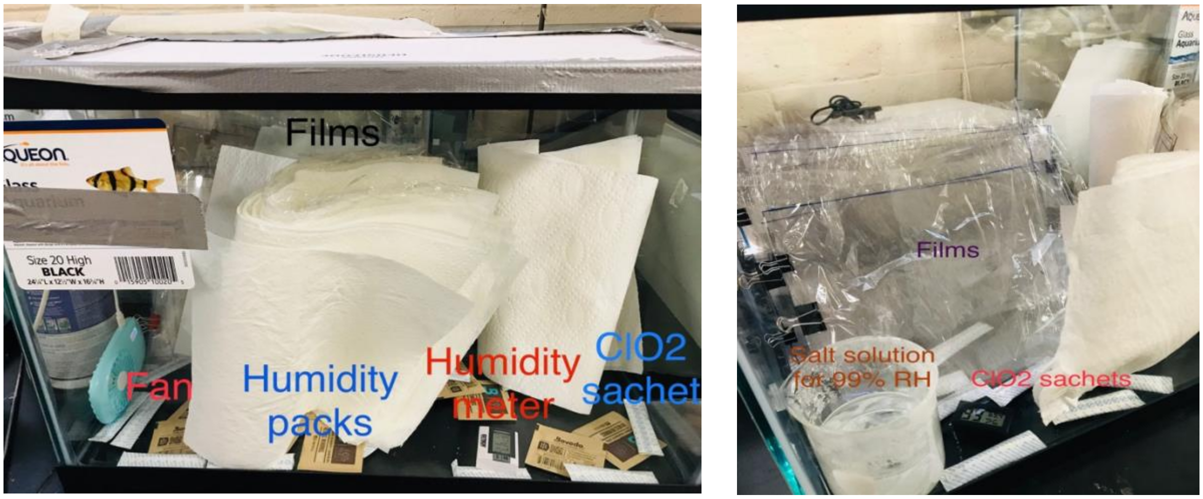

Figure 4.1, 4.2: Experimental Apparatus – Sealed Glass chamber with films exposed to the slow release of ClO_2 gas sachets (low dose long time of release) under controlled humidity at RT

Procedure

Day zero (D_0) measurements were conducted on all four material types to identify each material's properties. This included physical testing (Differential Scanning Calorimetry, DSC for melting point, T_m), mechanical testing (dart drop, tensile strength), barrier testing (water vapor transmission rate, WVTR). This is shown graphically in Figure 3. These tests are described in more detail below.

Treatment groups

Three glass chambers were set up, one for each RH. A saturated KNO_3 salt solution was used to make 99% RH in one chamber. The other two chambers were prepared using humidity control packs of 84% and 49% RH, respectively. Temperature-humidity meters (Fisher Scientific, Pittsburgh, PA) were kept inside each chamber, and RH's were monitored until they reached the desired level. Six ClO_2 sachets were put inside each treatment chamber.

For each of the four materials, two sets of 30 samples each were put inside each chamber. A fast-medium speed (fast mode was on) small portable fan (AEOSBIK, Shenzhen, China, GB4706.1.2005) was set up to circulate the atmosphere inside each chamber. Temperature-humidity meters (Fisher Scientific, Pittsburgh, PA) were kept inside each chamber to monitor the temperature and relative humidity every day (An example setup is displayed in Figure 1, and 2).

Five samples of each material from each experimental set were collected on the testing days (D_1, D_2, D_5, D_9, D_{14}, and D_{21}) for each test, DSC for T_m, mechanical tests-dart drop, and tensile strength, barrier tests-WVTR described in Figure 3.

Control groups

Three glass chambers were set up, one for each RH. A saturated KNO_3 salt solution was used to make 99% RH in one chamber. The other two chambers were prepared using humidity control packs of 84% and 49% RH, respectively. Temperature-humidity meters (Fisher Scientific, Pittsburgh, PA) were kept inside each chamber, and RH's were monitored until they reached the desired level. Temperature-humidity meters (Fisher Scientific, Pittsburgh, PA) were kept inside each chamber to monitor the temperature and relative humidity (Figure 3).

Sampling and testing on the control groups were conducted in the same manner as the treatment groups (outlined above in Section -*Treatment groups*).

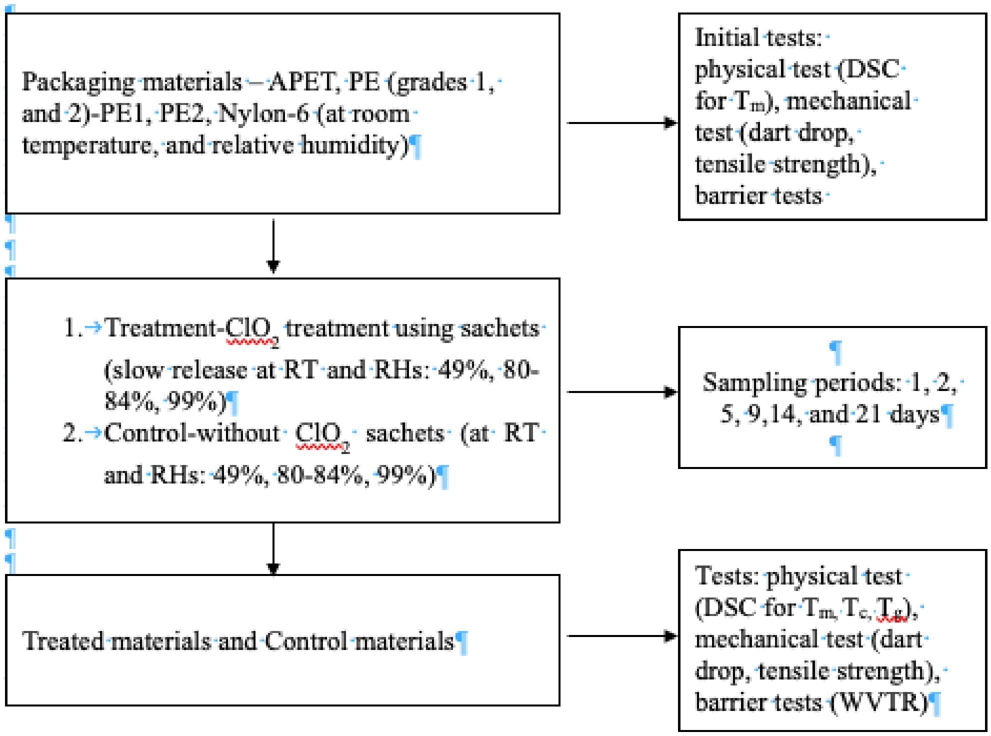

Figure 4.3. Description/Summary of Experimental Method and Instrumental Tests for Materials

Mechanical tests-dart drop

The impact strength (IS) of materials is measured to determine the total energy impact of materials by measuring the kinetic energy lost by a free-falling dart that passes through the film. A dart drop impact tester (Dynisco polymer test, DDI, MA, USA) was used to measure the IS of polymer materials according to ASTM D4272M-15)/ISO7765-2. Five measurements were taken for each material, and the mean and standard deviation given on the machine screen was recorded. Many previous studies looked at tensile strength (Kuruwita, Chapter 3), which is a low rate-of-strain test. However, polymeric materials may exhibit different behaviors depending upon the rate of strain. This test was included to look at high strain rate testing (Figure B.1).

Dart drop conditions were as follows:

Dart weight = 270 x 4 g (Max)

Dart diameter = ~3.5mm,

Drop height = 26 inches (Min)

were setup for each run.

Mechanical tests-tensile strength

Tensile strength, TS (MPa), and modulus of elasticity, MoE (MPa), in both machine direction (MD) and cross direction (CD)/transverse direction were measured using an Instron^{TM} 5967 universal tensile tester machine (INSTRON, Norwood, MA, USA). This was conducted for both the control and ClO2-treated polymeric films. The TS at the breakpoint and the MoE were considered for all the polymers. The ASTM procedure used was ASTM D638. The Bluehill Universal software (INSTRON bluehill Universal, Norwood, MA, USA) was used to run the tensile tests in the Instron machine (Figure C.1).

Parameters used were:

Sample width: ½ inches

Sample length: ~6 inches

Sample thickness: Measured for each sample, an average of three measurements

Initial Jaw Separation: 2 inches

Jaw separation rate: 20 inches/min

Elongation: Until the sample broke, or the machine length was reached

DSC for T_g, T_m, T_c

The melting (T_m), and crystallization (T_c) temperatures (°C) of the control and ClO_2 exposed polymeric films were determined using a differential scanning calorimeter (DSC 2920, TA Instruments, New Castle, DE) according to ASTM E-698 and E-1231. T_g was also measured for the nylon-6 and PET materials (The T_g of PE's is not within the capabilities of the machine. The analyses were done using Universal Analysis Software (UAS Version 3.9A, TA Instruments, New Castle, DE). Triplicates of each sample were run. Maximum temperatures varied for the test based upon the expected T_m for each material.

The conditions used were:

1. Ramp 1.00 ^{0}C/min to 25.00 ^{0}C
2. Isothermal for 1.00 min
3. Ramp 10.00 ^{0}C/min to 300.00 ^{0}C
4. Isothermal for 1.00 min
5. Ramp 10.00 ^{0}C/min to 25.00 ^{0}C
6. Isothermal for 1.00 min
7. Ramp 10.00 ^{0}C/min to 300.00 ^{0}C

(The temperatures used for numbers 3 and 7 were for APET, and nylon-6. It was 200.00 ^{0}C for PEs).

Water vapor transmission rate (WVTR)

Water vapor transmission rate is considered a barrier characteristic of polymer packages. Results were recorded for most films, but the WVTR tests run for nylon-6 did not work properly, due to high wettability because of plasticization of nylon-6 under high RH (90%) conditions.

For the PE grades, sample masking (reduced area - 5 cm^2) was done using Aluminum masks in order to perform the test correctly. For other samples, film area was 50 cm^2. WVTR of all ClO_2 treated and control polymeric films were determined in accordance with ASTM E96/E96M Test. WVTR was evaluated using Mocon (Minneapolis, MN) water vapor permeability analyzers (Permatran-WVR modules 3/31, machine 1 (module 1 S/N 0195D092), machine 2 (module 3 S/N 0699AD299). The permeation lab was also being used for other research, so machine availability dictated the use of two machines. Two samples of the same film were run simultaneously, and two replicates of each polymer material were measured. Therefore, four total measurements were taken for each material and averaged for the mean.

Statistical analysis

JMP pro-15 (SAS Institute, Inc., Cary, NC, USA) was used to calculate mean values and standard deviations of DSC data for T_g, T_m, T_c, dart drop-IS, tensile strength, and WVTR. The values were analyzed with analysis of variance (ANOVA) using JMP pro-15 (SAS Institute, Inc., Cary, NC, USA). Significant differences in each test were detected using the least significant differences (LSD), at the confidence level of 95% ($p \leq 0.05$). When ANOVA detected a significant difference, the student's t-test was used to determine the relationship between pairs of measurements at a significant level ($\alpha = 0.05$).

RESULTS

Mechanical tests-dart drop

APET (thickness ~ 20 mils) did not break in the dart drop test (with or without ClO_2). It was much thicker than the typical materials tested on the machine, which is designed for flexible packaging rather than semi-rigid.

The IS of nylon-6, PE (grade 1), and PE (grade 2) were recorded and analyzed using JMP. The data are presented respectively in Figure 4.1.a, Figure 4.2.a, and Figure 4.3.a. in the JMP graph builder. The IS of all materials is presented in Figure 4.4 with 95% confidence intervals (R^2 for linearity of data was provided). Excel graphs for each material are also provided respectively in Figure 4.1.b, Figure 4.2.b, and Figure 4.3.b.

The IS of nylon-6 was significantly different from the IS of PE (grade 1) and PE (grade 2). The IS of PE (grade 1) was not significantly different from IS of PE (grade 2). This can be seen in Figure 4.4.

The IS of the two ClO_2 treated PE's (grade 1, grade 2) showed no significant difference ($\alpha < 0.05$) at any relative humidity used (49-99%) for 21 days (Figures 4.2.a, 4.2.b, and 4.3.a, 4.3.b).The IS of ClO_2 treated nylon-6 decreased (the change was close enough to the significant level ($p = 0.0532$) at 99% RH on each sampling day with ClO_2 treatment. (The combined effect of ClO_2 and 99% RH decreased the IS of nylon-6 (Figure 4.1.a, Figure 4.1.b). All of the nylons (at all RHs) appear to have a general downward trend in IS until Day 9, but only the 99% RH sample maintained this good linear trend.

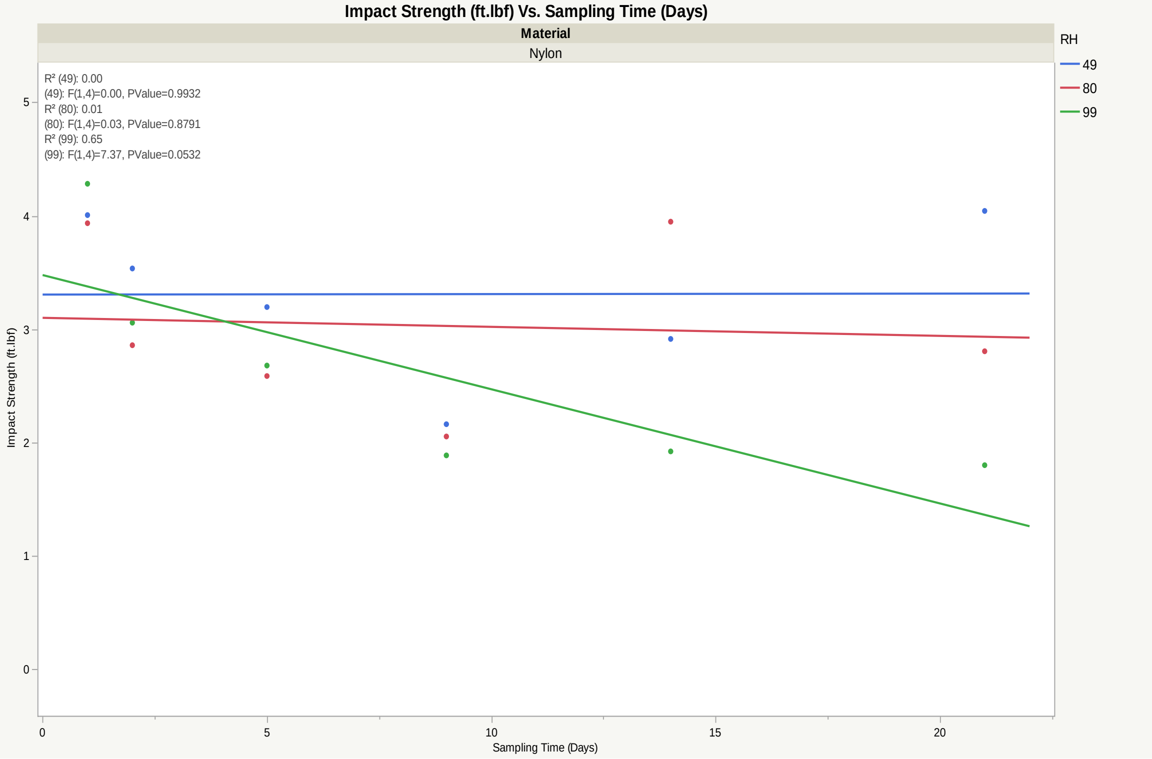

Figure 4.4.1.a: JMP Graph for Impact strength/ toughness (ft/lbf) of ClO_2 Treated Nylon-6 at 99, 80, and 49% Relative Humidities by Time (Days) with p-value.

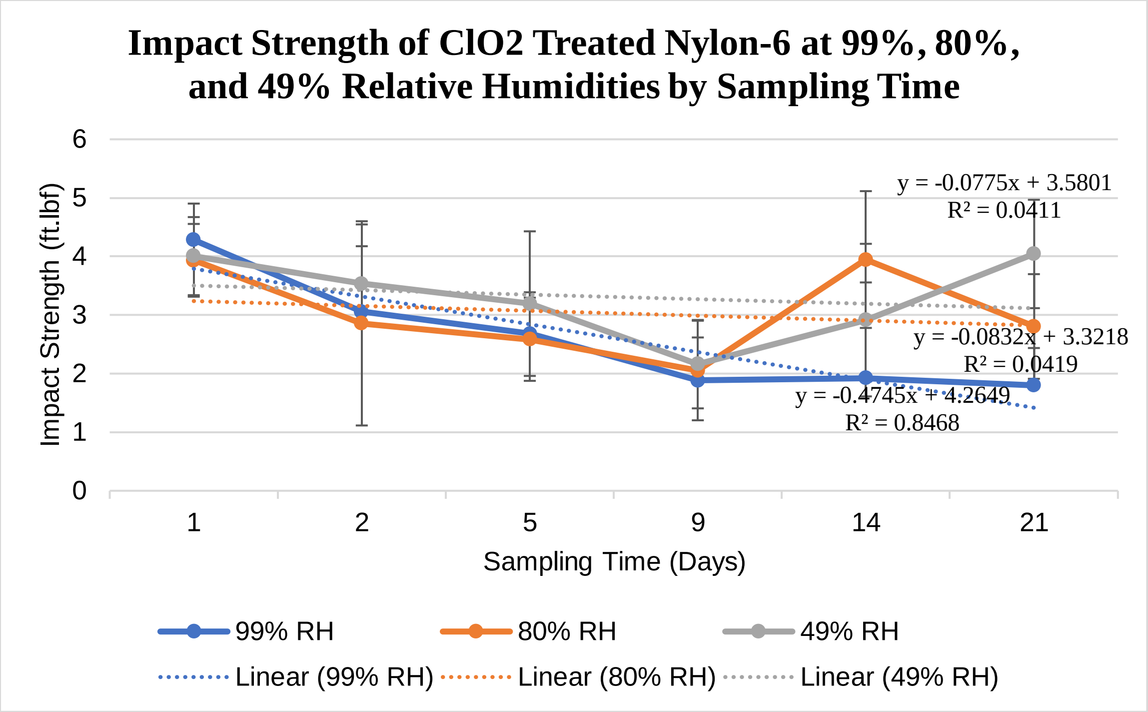

Figure 4.4.1.b: Impact strength/ toughness (ft/lbf) of ClO_2 Treated Nylon-6 at 99, 80, and 49% Relative Humidities by Time (Days).

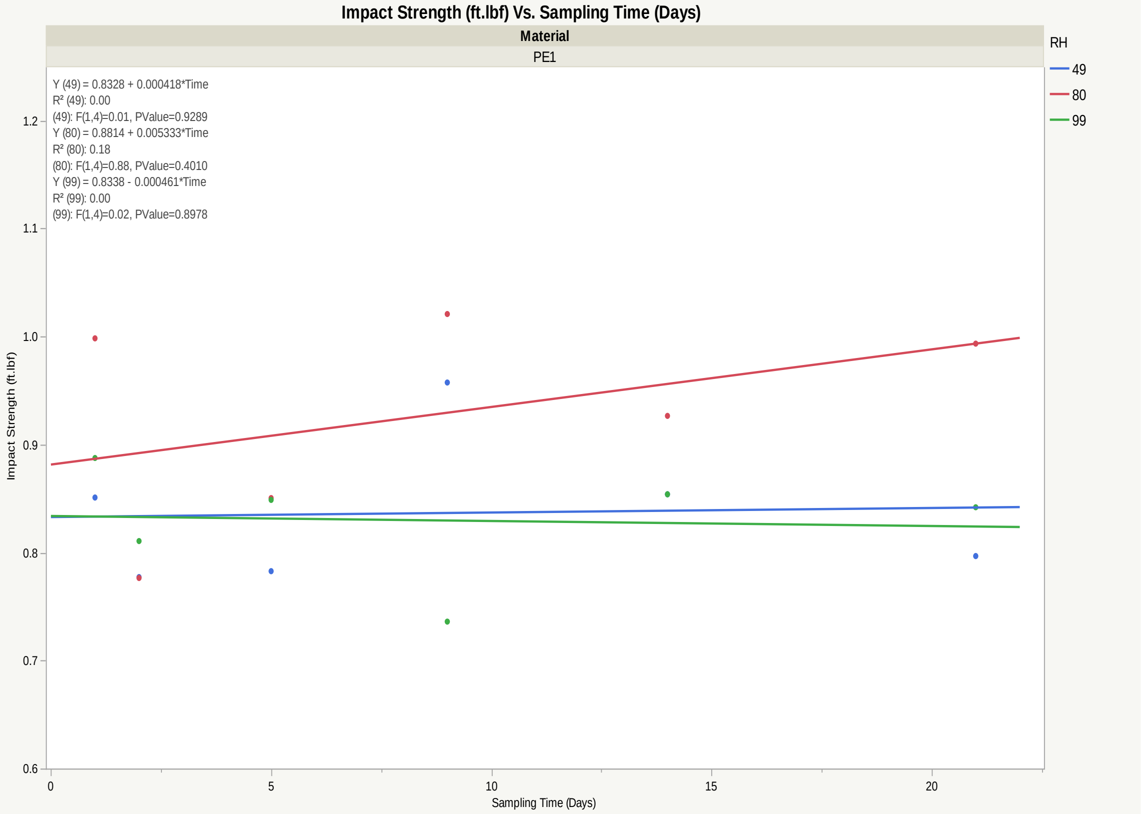

Figure 4.4.2.a: JMP Graph for Impact strength/ toughness (ft/lbf) of Treated Oriented PE (Type 1) at 99, 80, and 49% Relative Humidities by Time (Days) with p-value.

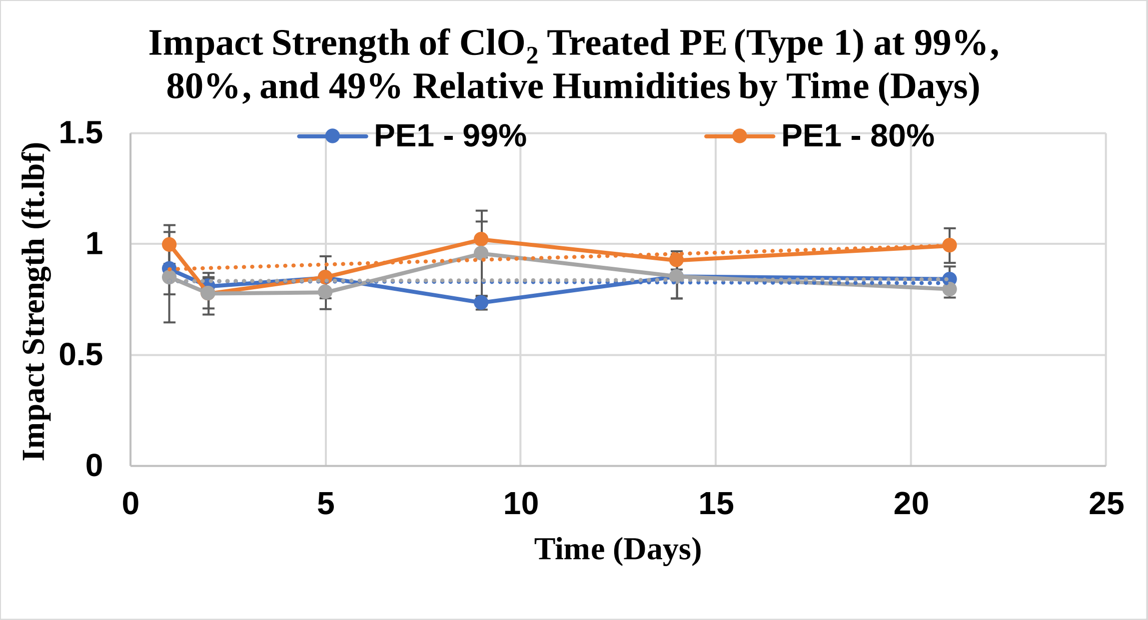

Figure 4.4.2.b: Impact strength/ toughness (ft/lbf) of Treated Oriented PE (Type 1) at 99, 80, and 49% Relative Humidities by Time (Days).

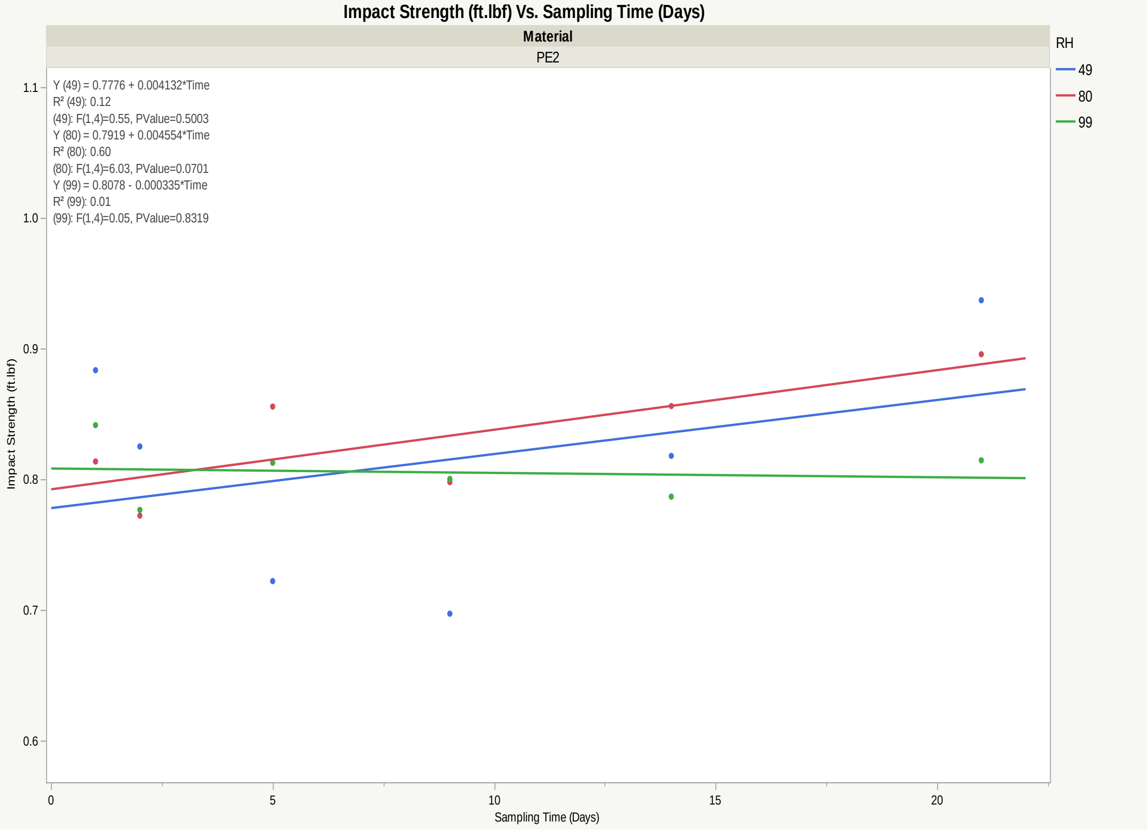

Figure 4.4.3.a: JMP Graph for Impact strength/ toughness (ft/lbf) of Treated Oriented PE (Type 2) at 99, 80, and 49% Relative Humidities by Time (Day) with p-value.

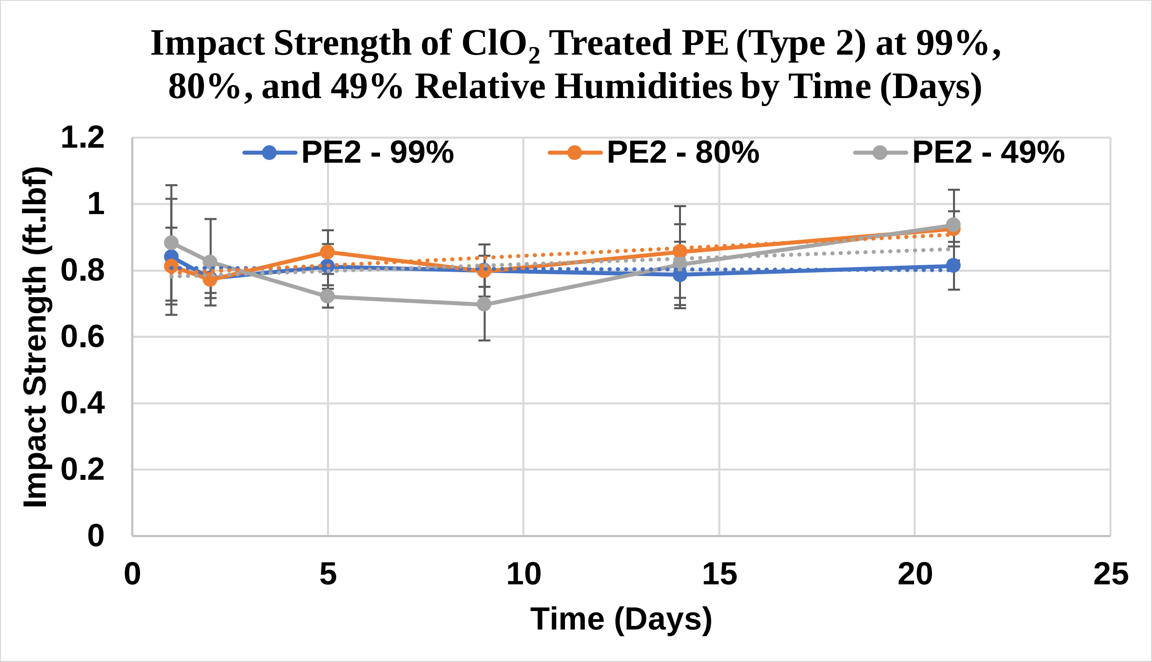

Figure 4.4.3.b: Impact strength/ toughness (ft/lbf) of Treated Oriented PE (Type 2) at 99, 80, and 49% Relative Humidities by Time (Day).

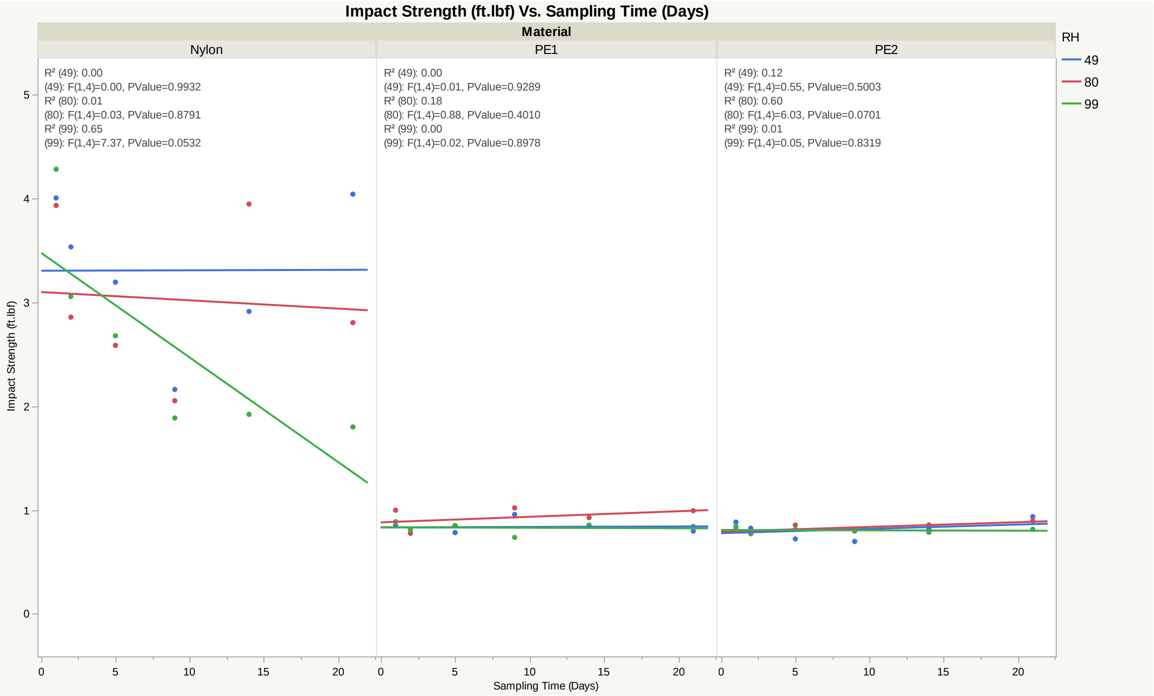

Figure 4.4.4: JMP Graph for Impact strength/ toughness (ft/lbf) of Treated APET, Nylon-6, and PE (type 1, type 2) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) with p-value.

Mechanical tests-tensile strength at break and tensile modulus

The TS graphs for individual materials are presented in Figures 5, 6, 7, and 8 with 95% confidence intervals. The tensile modulus graphs for individual materials are presented in Figures 10, 11, 12, and 13 using Excel. TS at break and tensile modulus of all materials are presented in Figures 9 and 14.TS at break of nylon-6 is significantly different from the TS at break of all other materials (Figure 4.6).

Tensile moduli of APET and nylon-6 are significantly different from each other, and both are significantly different from the PE’s (Figure 10-14). Tensile moduli of the two PE’s are not significantly different (Figures 12, 13). Tensile strength at break and tensile moduli for all of the films tested showed no significant differences with respect to treatment time or RH. Data are presented in Figures 9, and 14 using the JMP graph builder with 95% confidence intervals (R^2 for linearity of data is also provided). For all materials, TS at break and tensile moduli did not show a significant difference with respect to material direction (MD vs. CD) and material direction*RH was a significant factor for TS at break (Figures 5-8, and Figure 10-13). Therefore, ClO_2 affected the TS at the break of nylon-6 in MD directions at 99% RH only on day 1 compared to the other RHs (Figure 4.6).

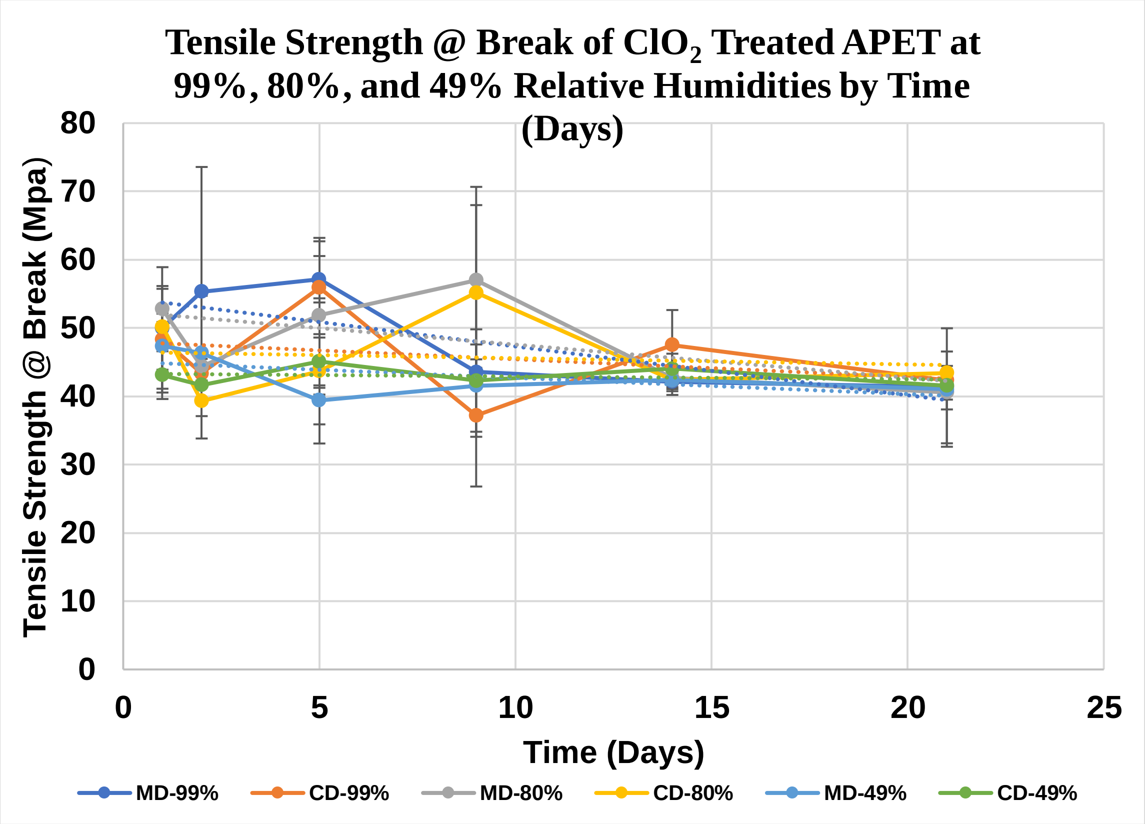

Figure 4.5: Tensile strength at Break (Mpa) of Treated APET at 99, 80, and 49% Relative Humidities by Time (Day)

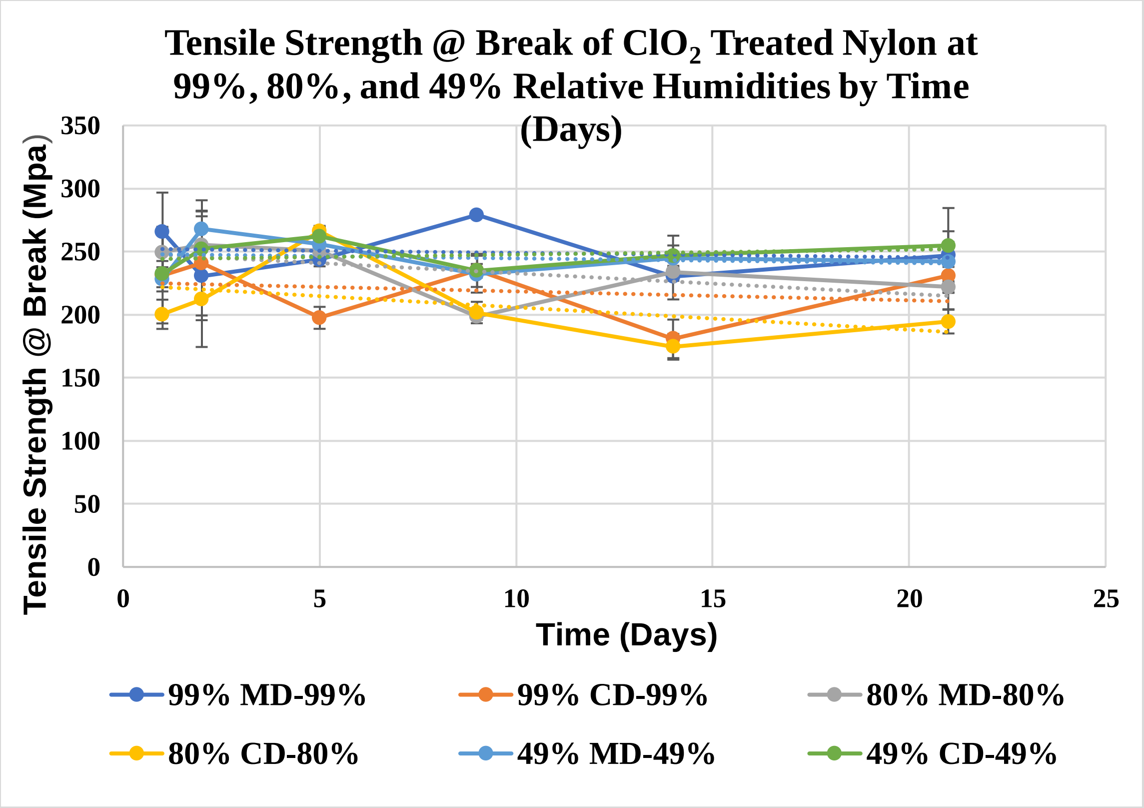

Figure 4.6: Tensile strength at Break (Mpa) of Treated Nylon-6 at 99, 80, and 49% Relative Humidities by Time (Day)

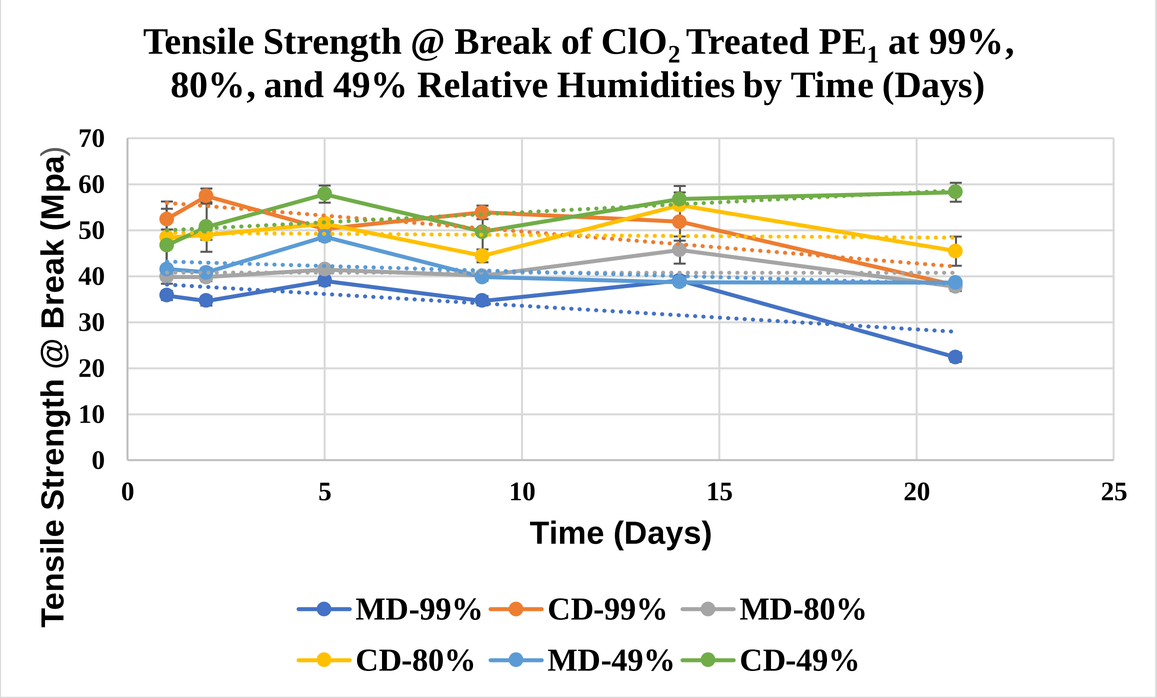

Figure 4.7: Tensile strength at Break (Mpa) of Treated PE_1 at 99, 80, and 49% Relative Humidities by Time (Day).

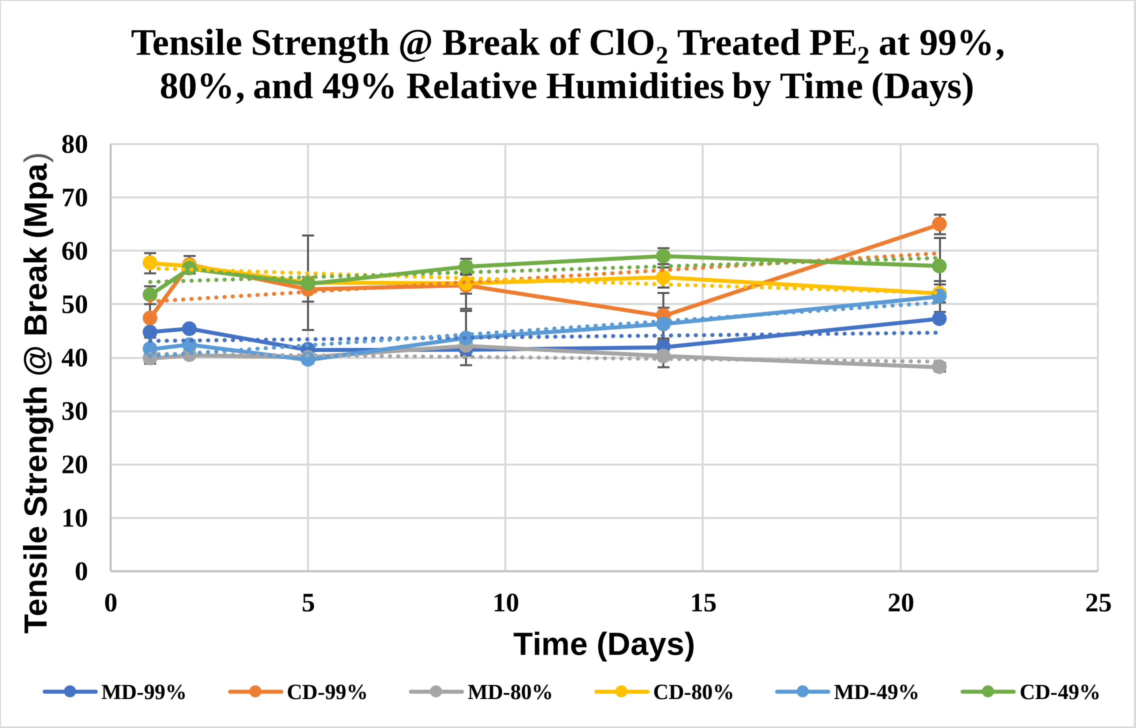

Figure 4.8: Tensile strength at Break (Mpa) of Treated PE2 at 99, 80, and 49% Relative Humidities by Time (Day).

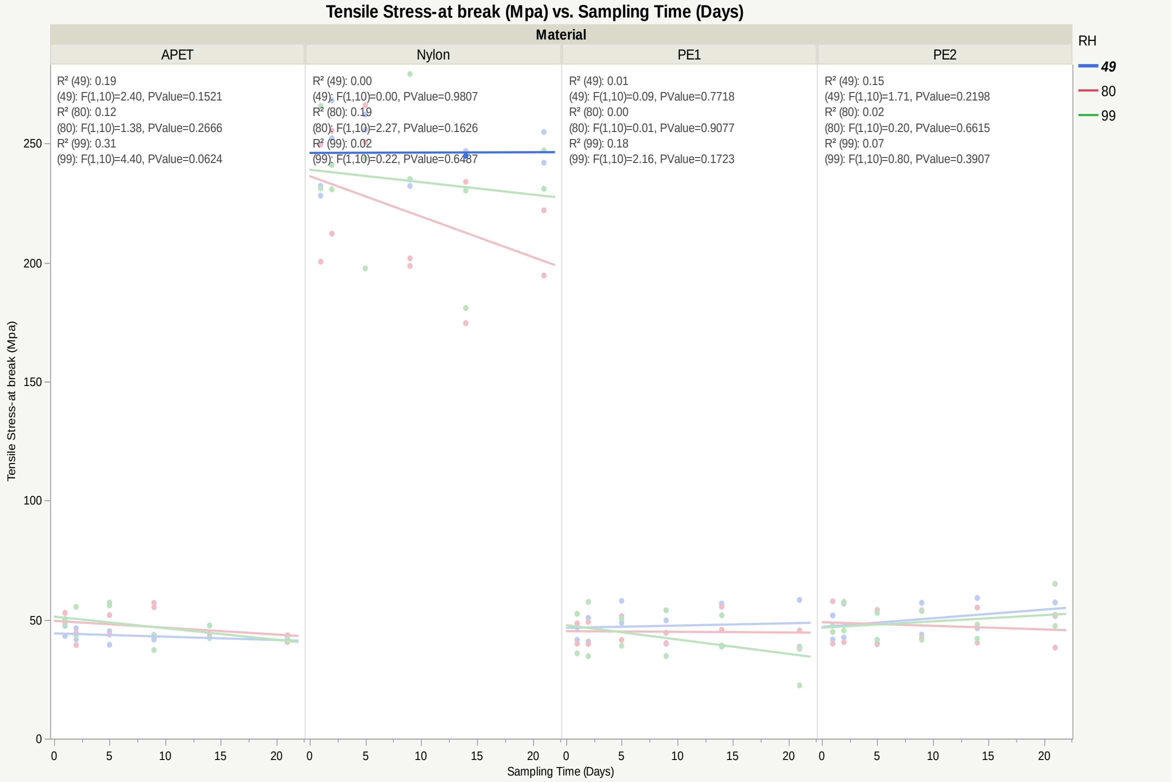

Figure 4.9: JMP Graph for Tensile strength at Break (Mpa) of Treated APET, Nylon-6, PE1, PE2 at 99, 80, and 49% Relative Humidities by Time (Day).

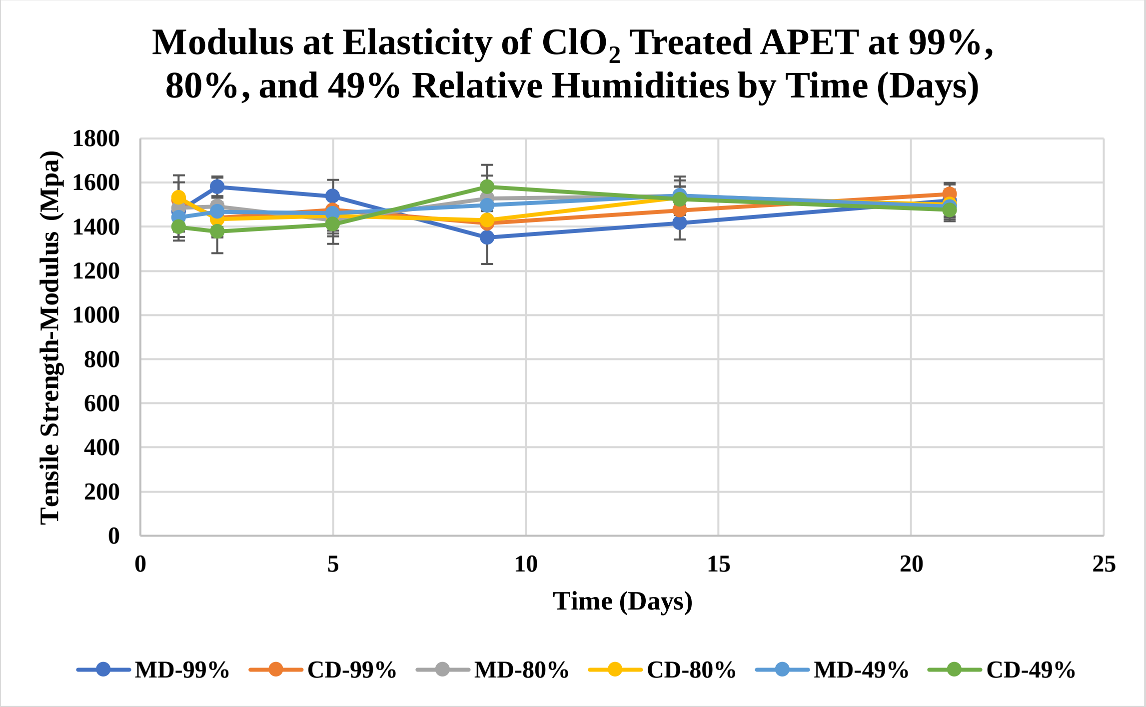

Figure 4.10: Modulus at Elasticity (Mpa) of Treated APET at 99, 80, and 49% Relative Humidities by Time (Day)

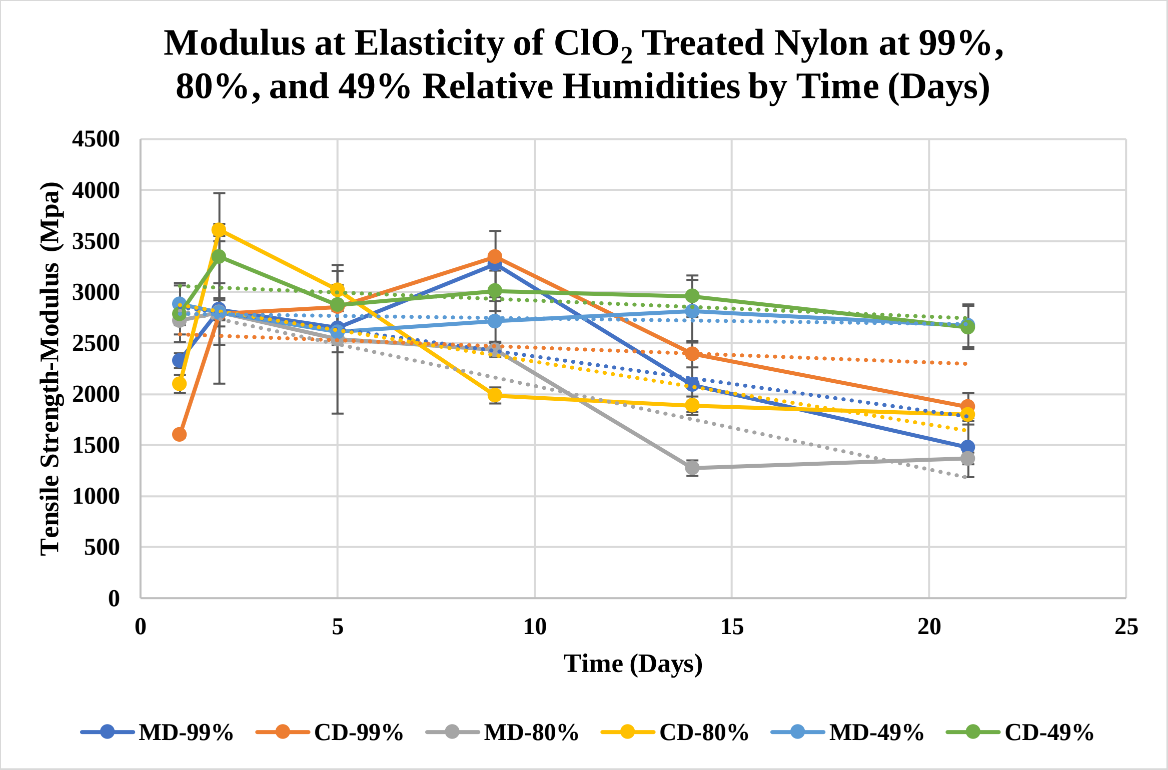

Figure 4.11: Modulus at Elasticity (Mpa) of Treated Nylon-6 at 99, 80, and 49% Relative Humidities by Time (Day)

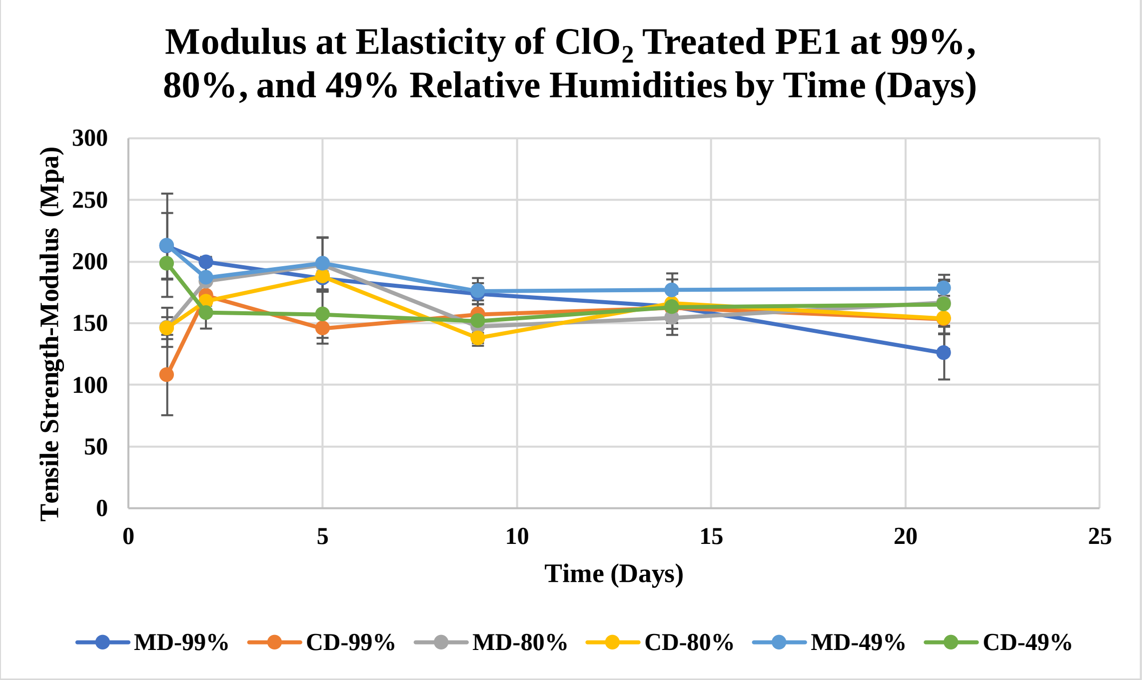

Figure 4.12: Modulus at Elasticity (Mpa) of Treated PE1 at 99, 80, and 49% Relative Humidities by Time (Day)

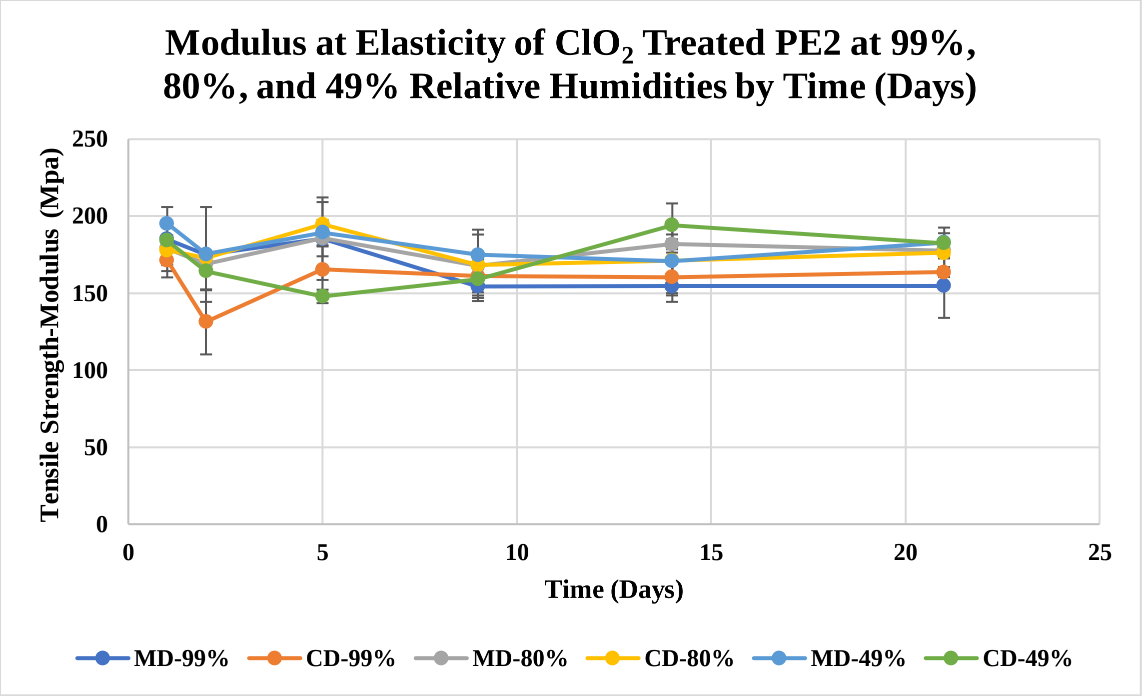

Figure 4.13: Modulus at Elasticity (Mpa) of Treated PE2 at 99, 80, and 49% Relative Humidities by Time (Day)

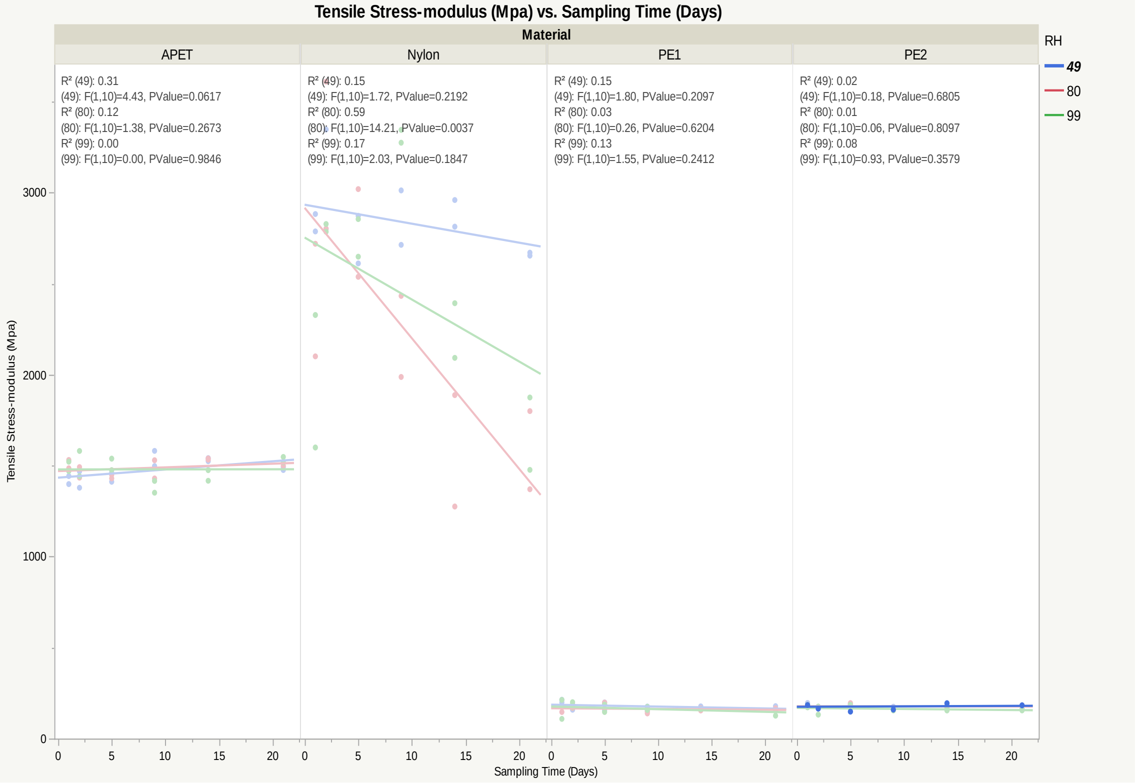

Figure 4.14: JMP Graph for Modulus at Elasticity (Mpa) of Treated APET, Nylon-6, PE1, PE2 at 99, 80, and 49% Relative Humidities by Time (Day)

DSC

DSC data for the T_m of all materials are presented in Figure 24, and also in the graphs of T_m vs. time for individual materials are presented in Figures 16, 18, 20, and 22 with 95% confidence intervals (R^2 for linearity of data were provided). According to the ANOVA using the least square significant differences (LSD) and with respect to these linear regression graphs (Figure 24), T_m values were significantly different ($p \leq 0.05$) only with respect to 'material'. In further comparison, each material pair was analyzed with the student's t-test, where the ($\alpha = 0.05$) was considered significantly different.

All the material pairs showed a significant difference from each other at each sampling time from day 1 to day 21 except the material pair of two grades of PE's (Figure 16, 18, 20, 22).

T_m of all the materials did not change significantly ($p \leq 0.05$) with time (Day 1-21) at all RH's (49-99%) (Figure 16, 18, 20, 22 and Figure 24). For all materials, T_m vs. time at each RH did not change significantly (Figures 16, 18, 20, 22).

T_c values were significantly different ($p \leq 0.05$) only with respect to 'material' (Figure 25). T_c of the materials did not have any significant difference upon time or RH except for nylon-6. When considering T_c in nylon-6 (LSD), Time* RH was significantly different (Figure 17, 19, 21, 23, and Figure 25).

T_c vs. time at each RH for individual materials did not change significantly except for the significant difference in T_c of nylon-6 at 49% RH on day 21 (Figure 19).

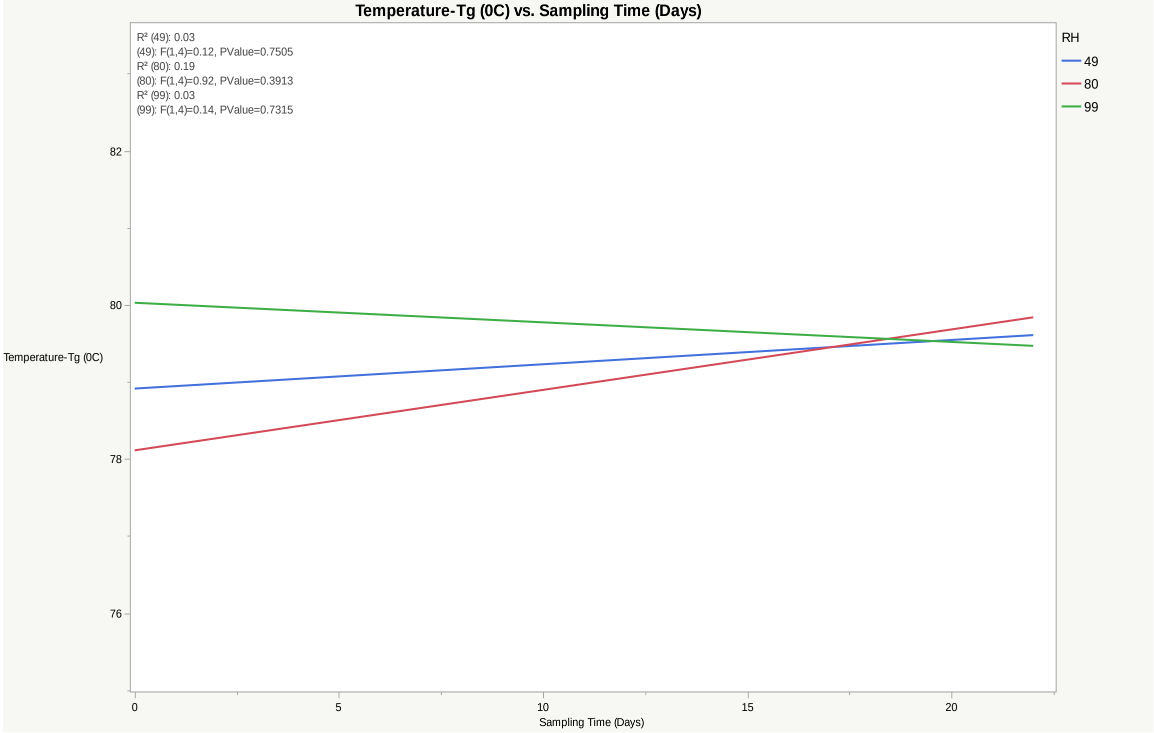

Figure 4.15: JMP Graph for Glass Transition Temperature of ClO_2 Treated APET (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

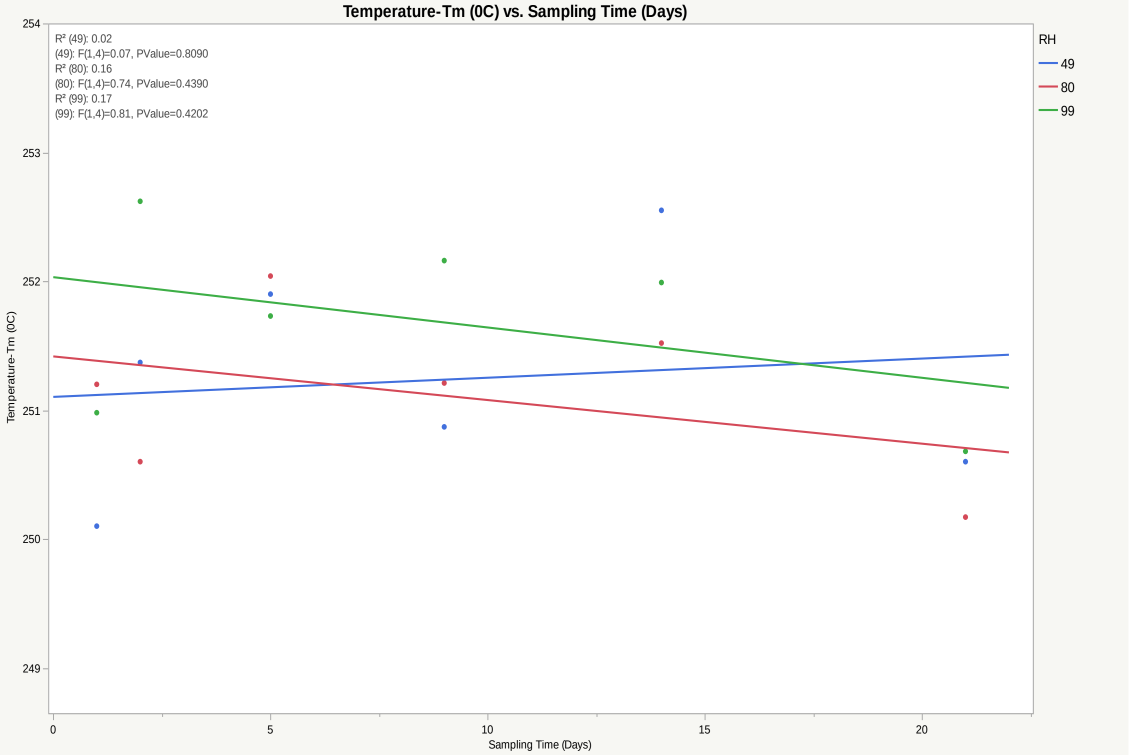

Figure 4.16: JMP Graph for Melting Temperature of ClO_2 Treated APET (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

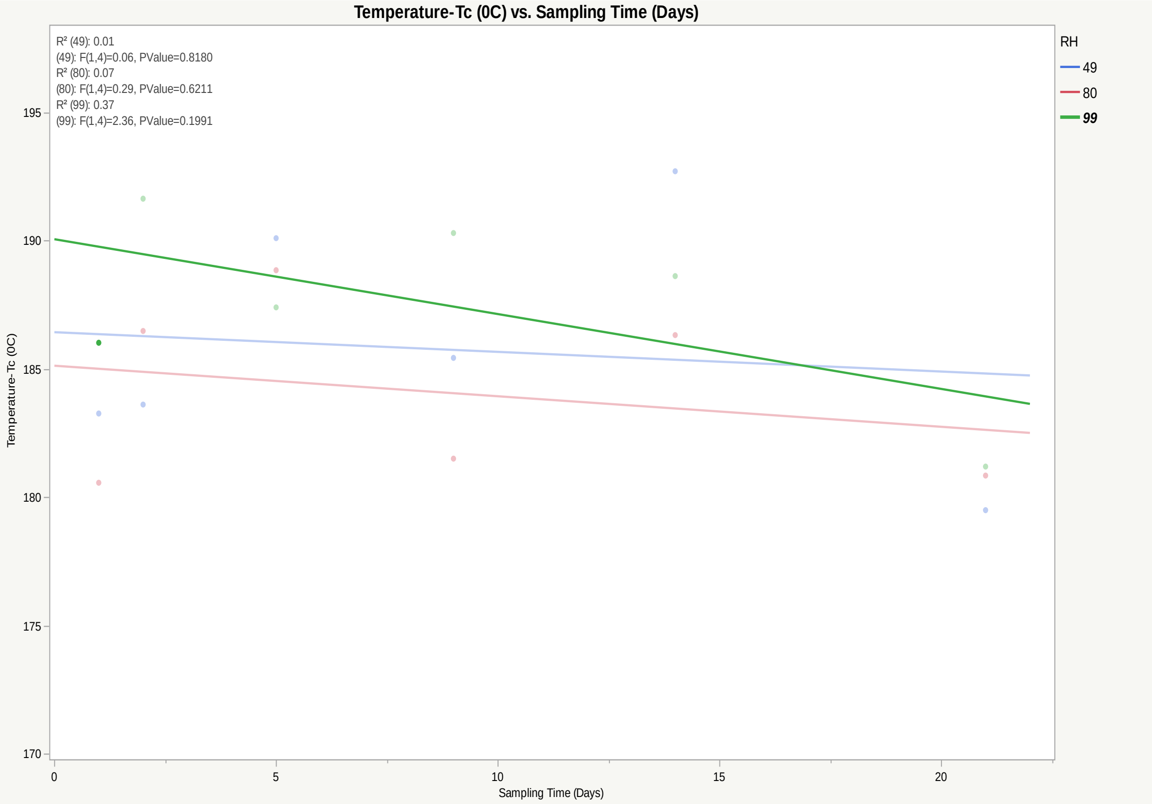

Figure 4.17: JMP Graph for Crystallization Temperature (Cold) of ClO_2 Treated APET (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

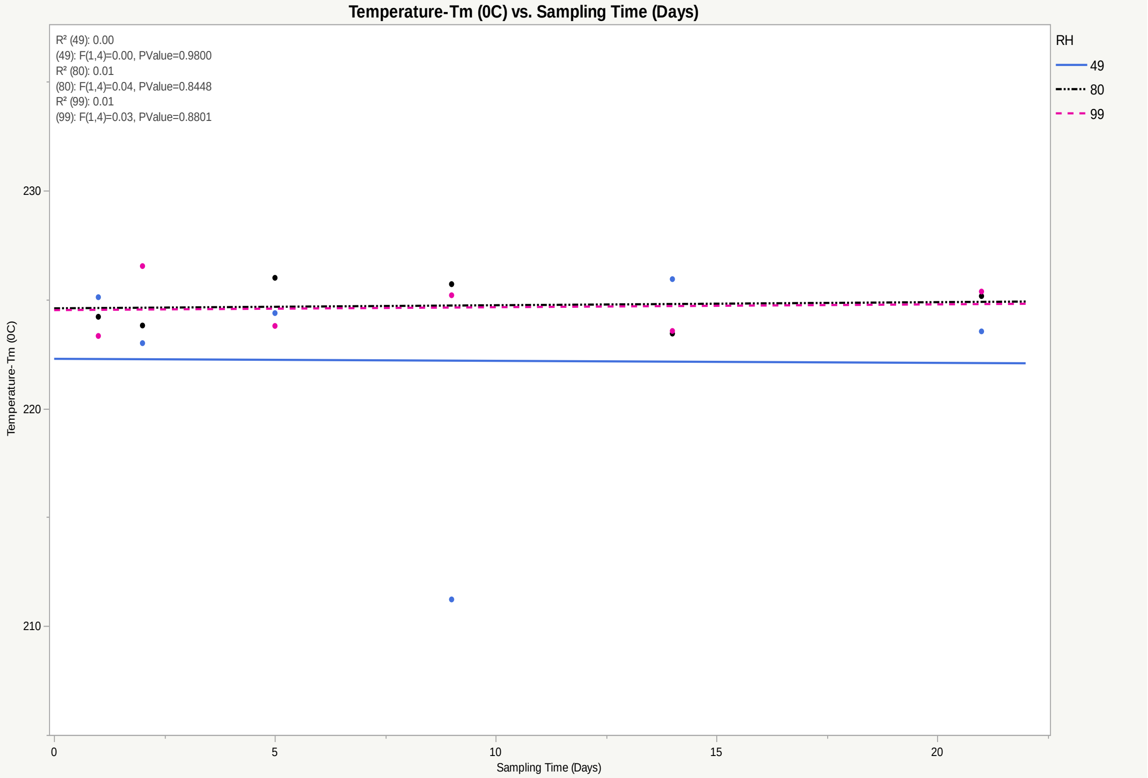

Figure 4.18: JMP Graph for Melting Temperature of ClO$_2$ Treated Nylon-6 (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

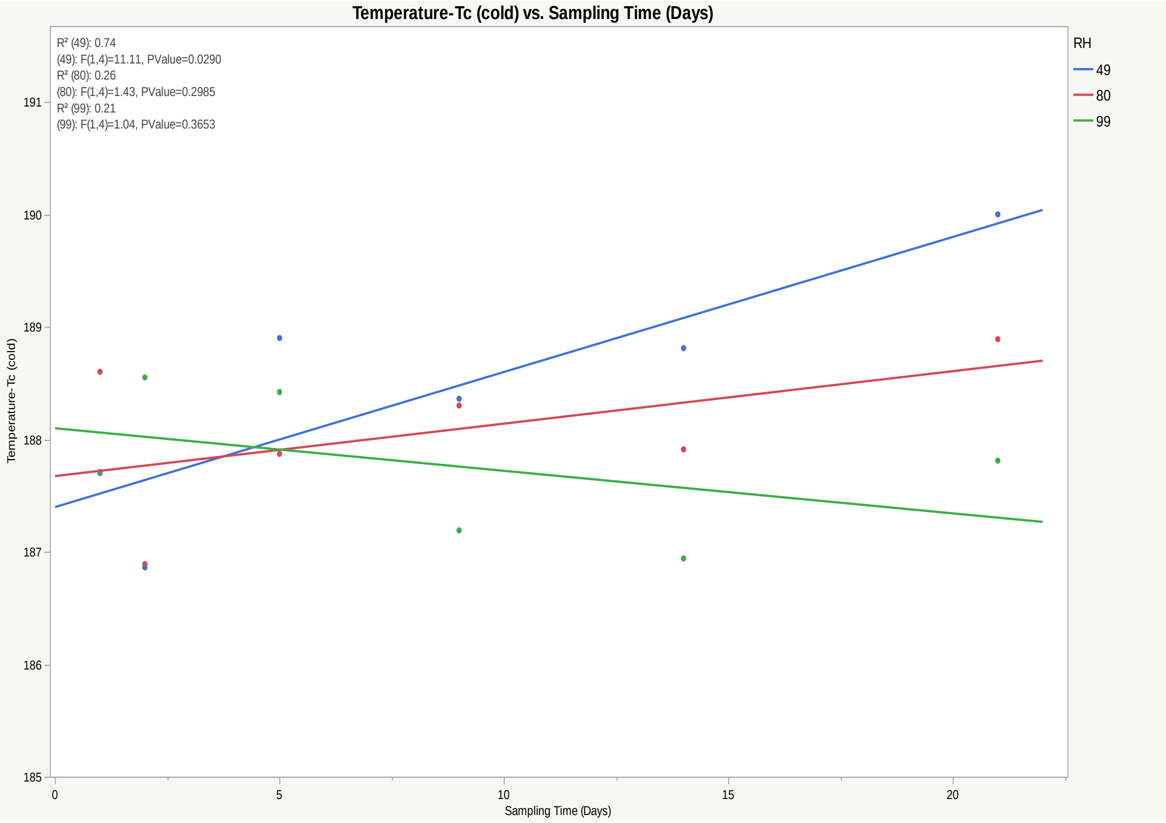

Figure 4.19: JMP Graph for Crystallization Temperature of ClO$_2$ Treated Nylon-6 (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

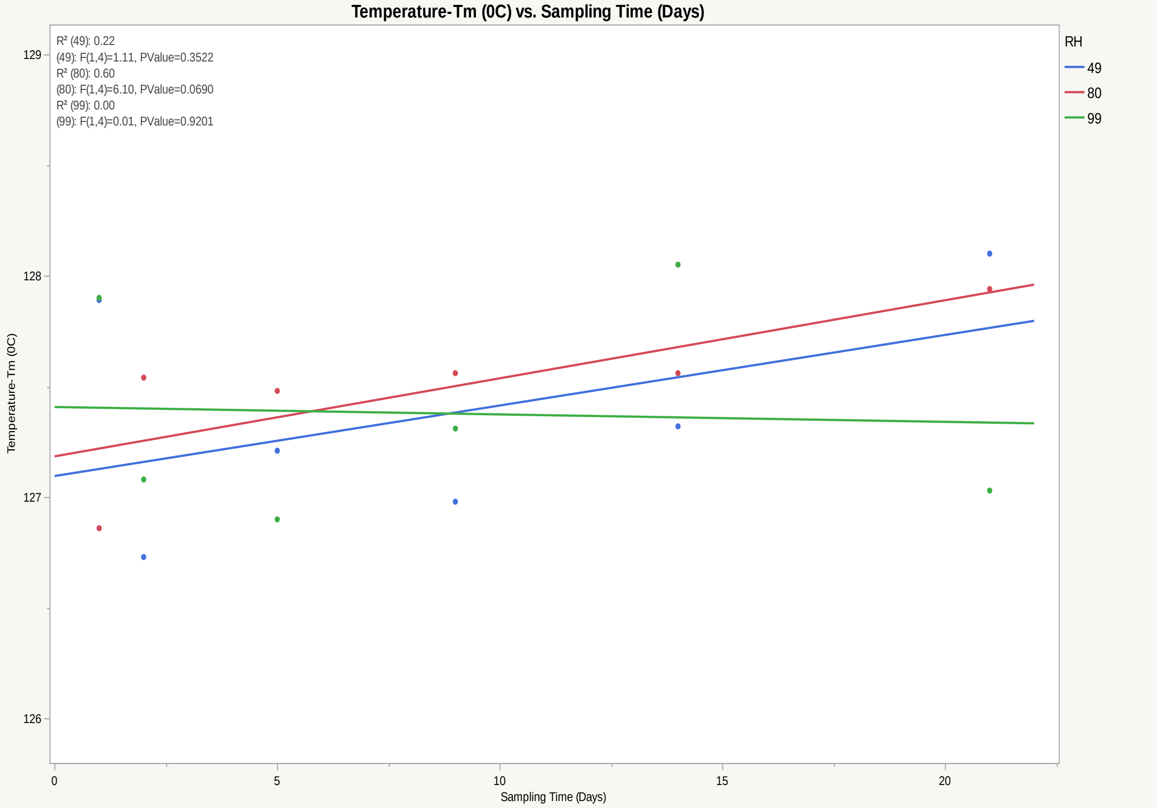

Figure 4.20: JMP Graph for Melting Temperature of ClO_2 Treated PE (type 1) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

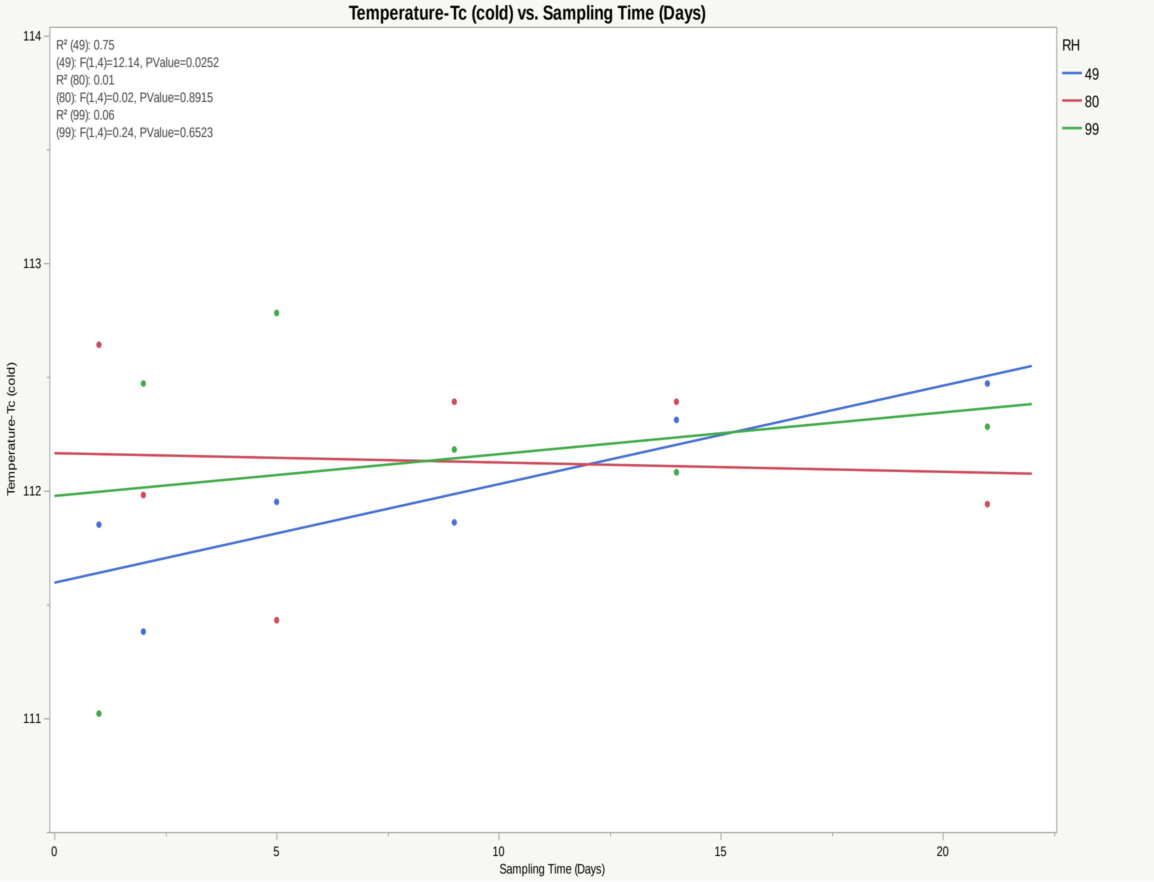

Figure 4.21: JMP Graph for Crystallization Temperature of ClO_2 Treated PE (type 1) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

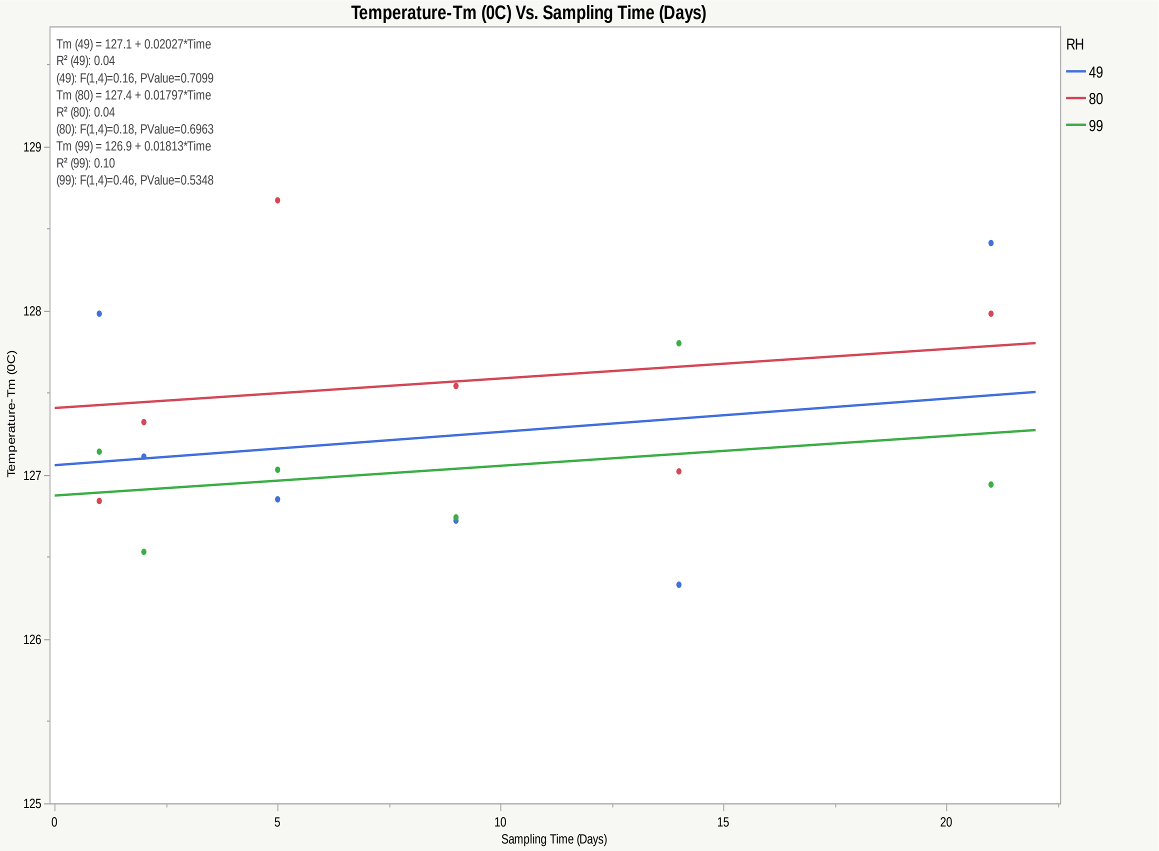

Figure 4.22: JMP Graph for Melting Temperature of ClO_2 Treated PE (type 2) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

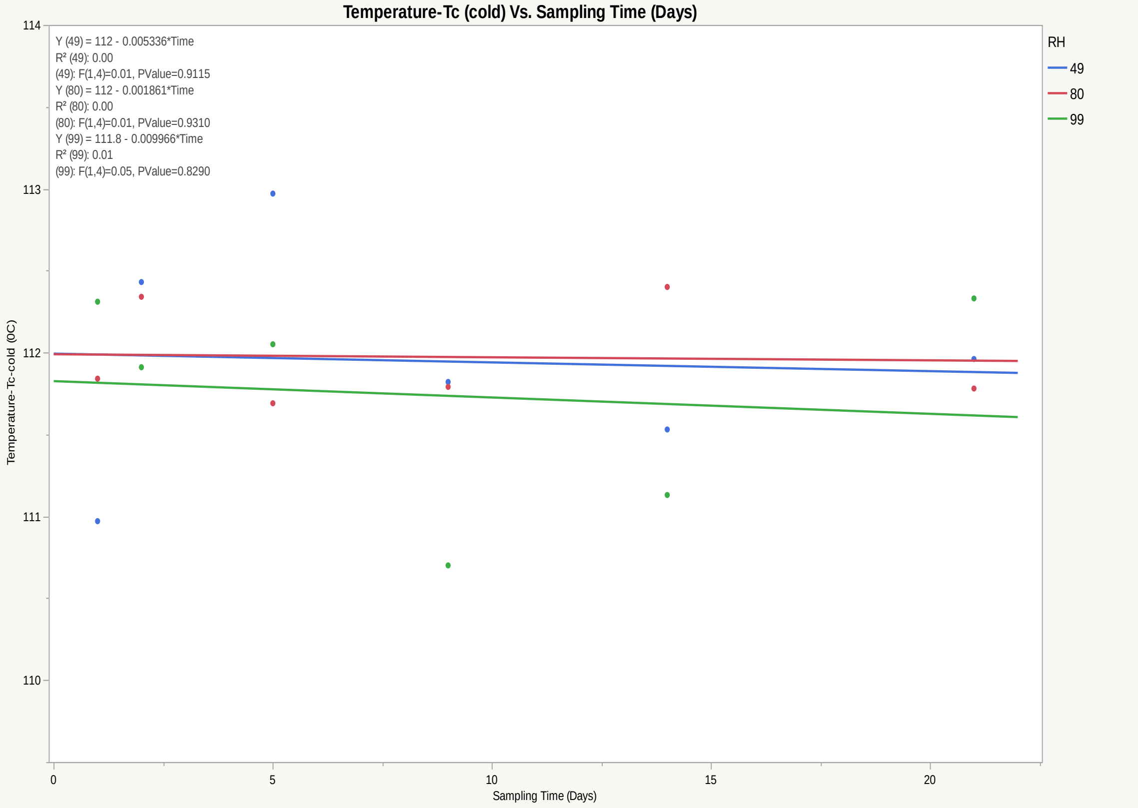

Figure 4.23: JMP Graph for Crystallization Temperature of ClO_2 Treated PE (type 2) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days) With p-value.

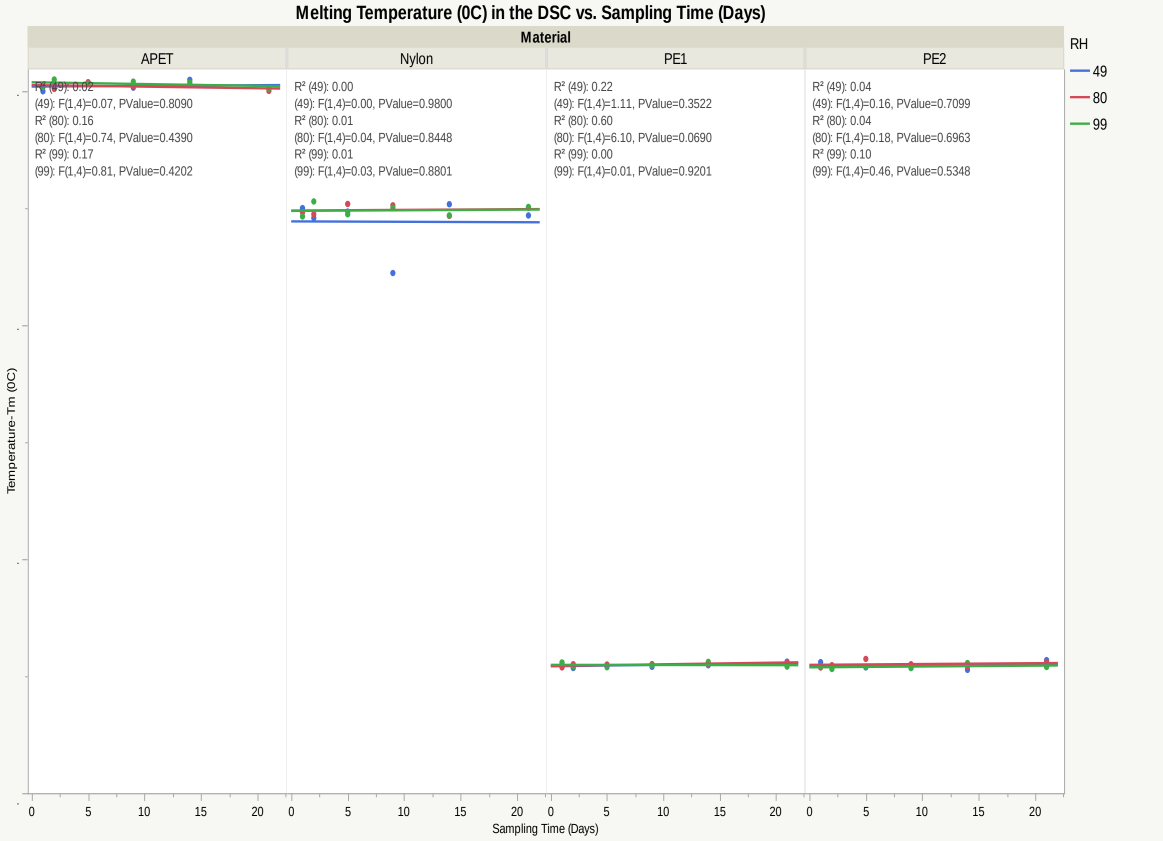

Figure 4.24: JMP Graph for Melting Temperature of ClO_2 Treated APET, Nylon-6, and PE (type 1, type 2) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days).

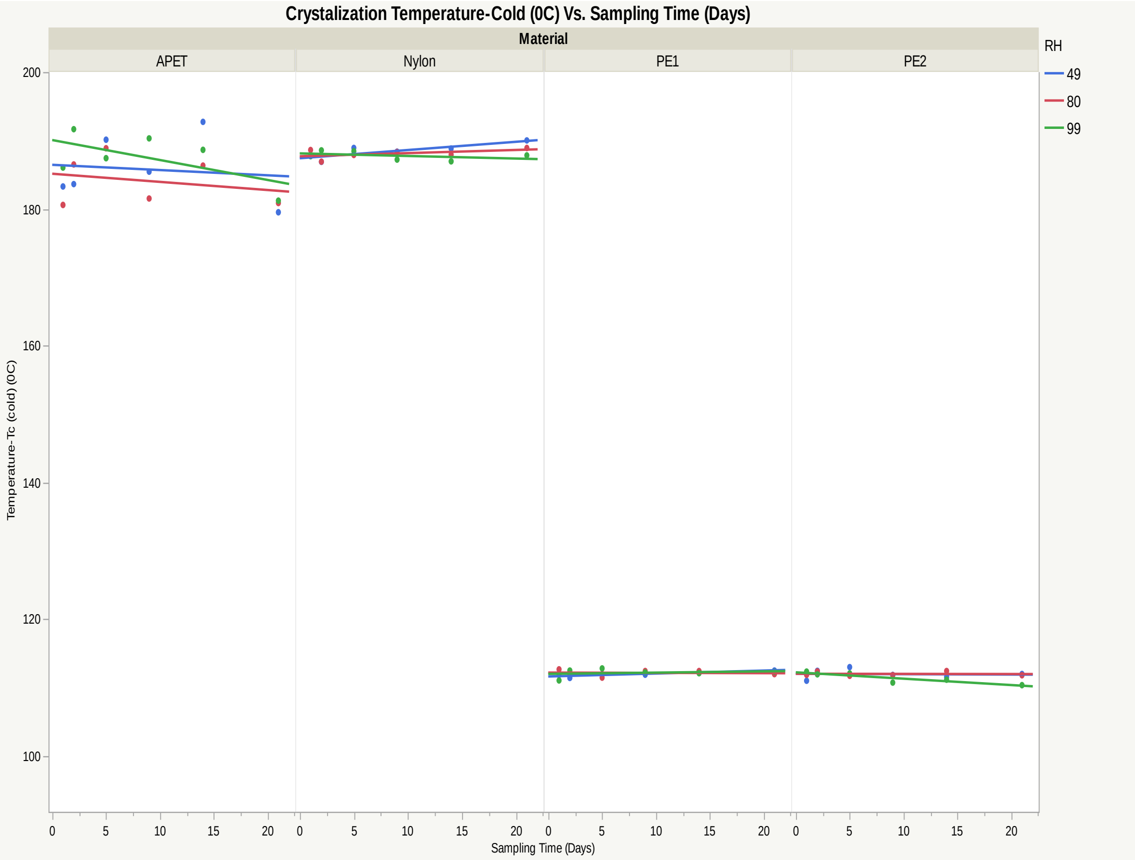

Figure 4.25: JMP Graph for Crystallization Temperature of ClO_2 Treated APET, Nylon-6, and PE (type 1, type 2) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days).

Water vapor transmission rate (WVTR)

Water Vapor Permeation (WVP) data of all materials are presented in Figure 29. Separate graphs for individual materials are provided in Figures 26, 27, and 28 with 95% confidence intervals, R^2, and P-values for significant differences were provided. According to the statistical data using the least square significant differences (LSD), WVP was significantly different ($p \leq 0.05$) with respect to ‘material’. The student’s t-test was performed for further analysis.

The material pairs APET-PE1, and APET-PE2 showed a significant difference at each sampling time from day 1 to day 21 with ClO_2 for all RH's. PE1-PE2 did not show a significant difference from day 1 to day 21 at 49, 80, and 99% RH's with ClO_2 (Figure 29). All the materials showed no significant changes throughout the sampling period (Figure 26-28).

WV-Permeation of APET vs. RH showed a significant difference on day 14 at 99% RH compared to the other 2 RHs. (Figure 26).

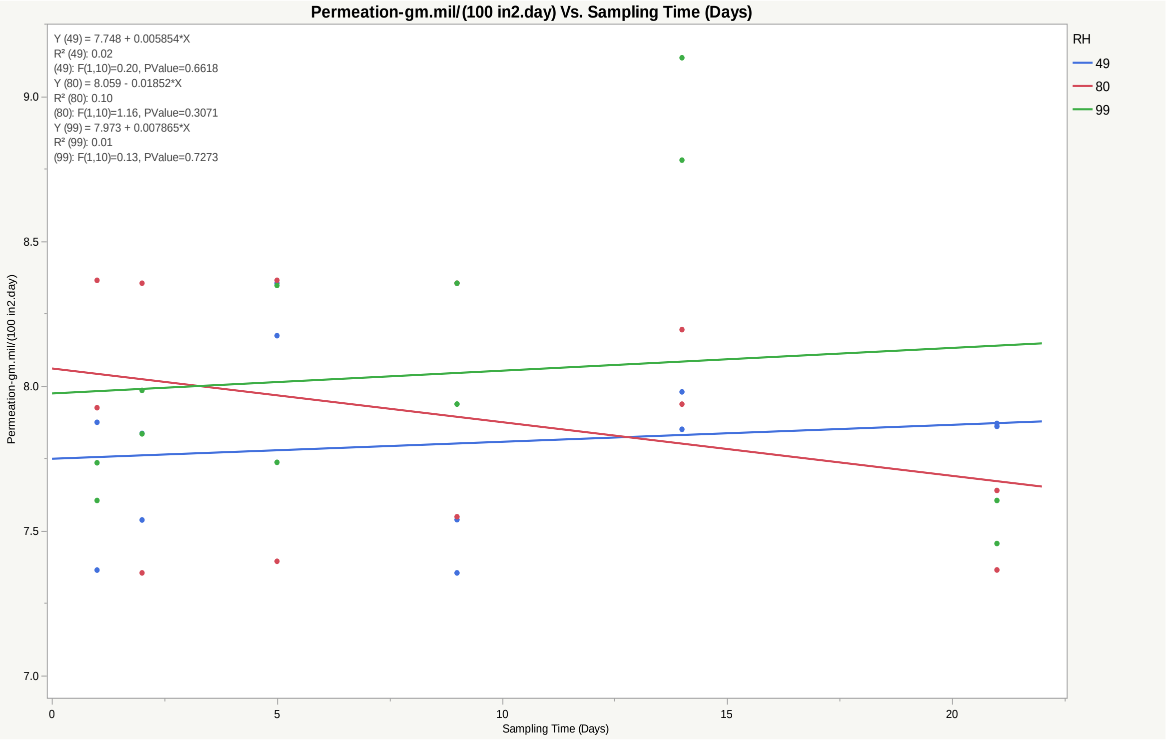

Figure 4.26: Water Vapor Permeation of ClO_2 Treated APET (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days).

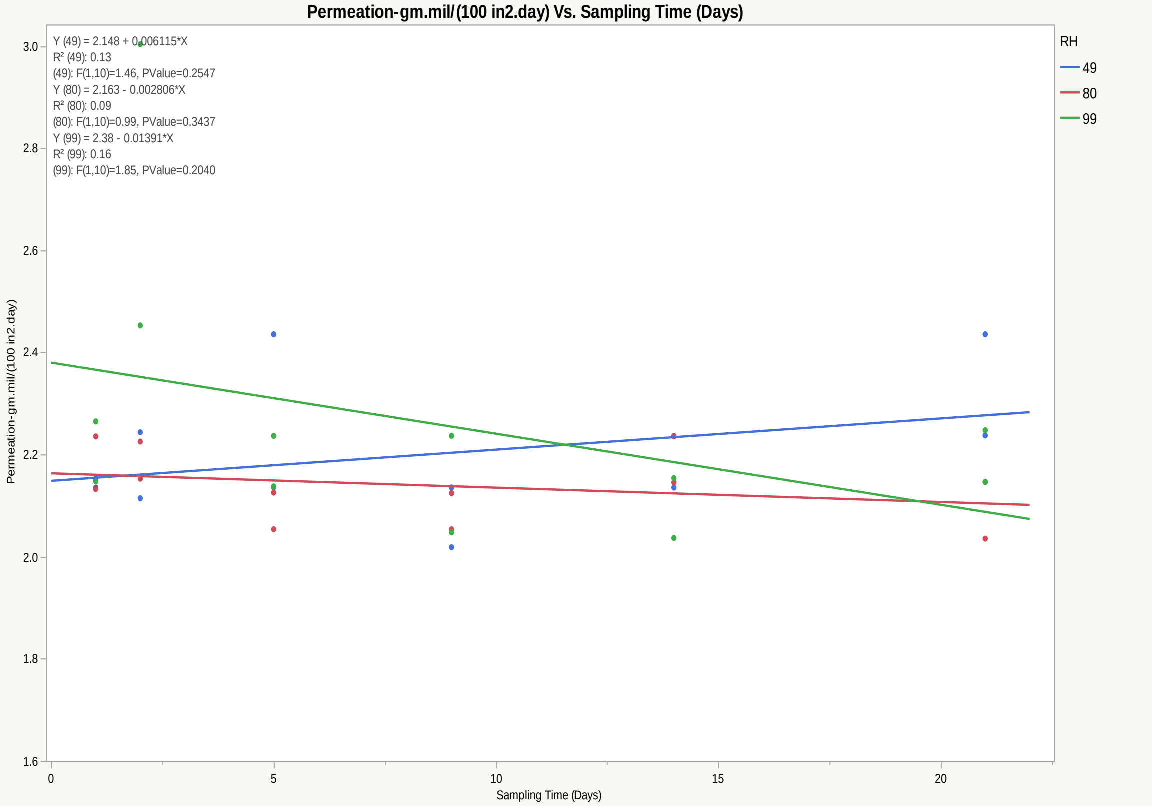

Figure 4.27: Water Vapor Permeation of ClO_2 Treated PE (type 1) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days).

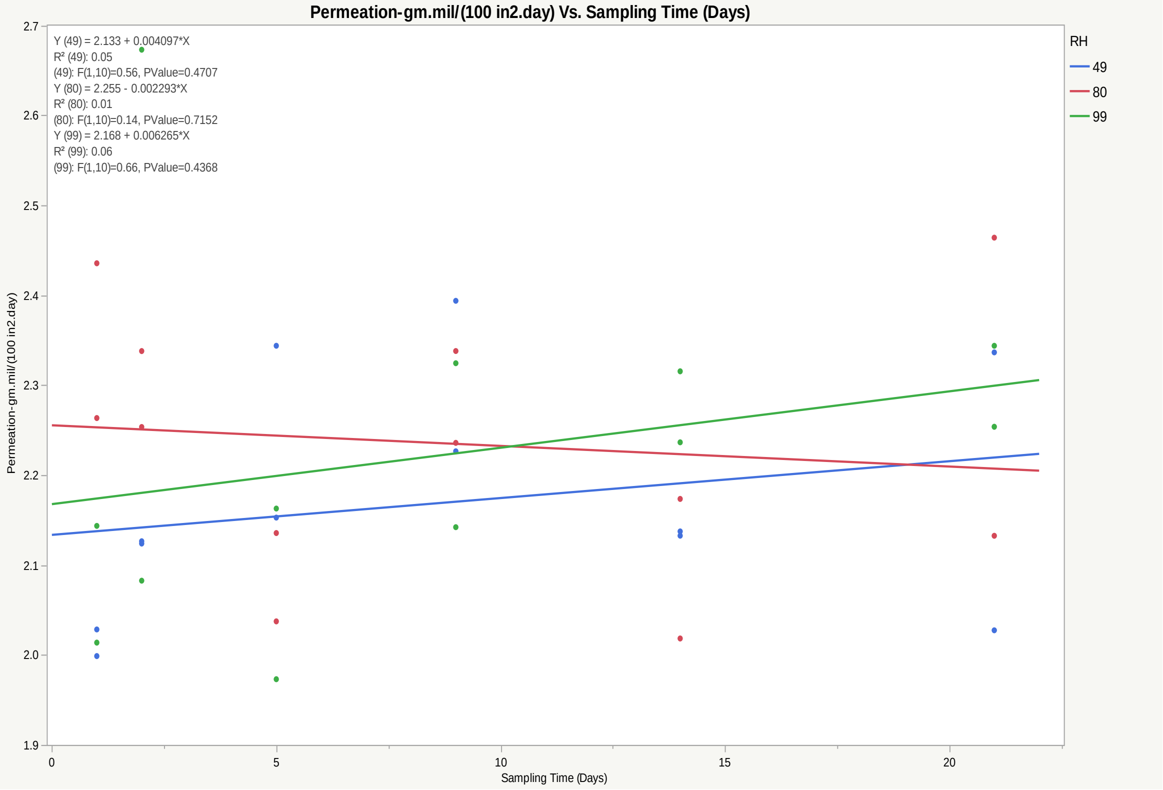

Figure 4.28: Water Vapor Permeation of ClO_2 Treated PE (type 2) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days).

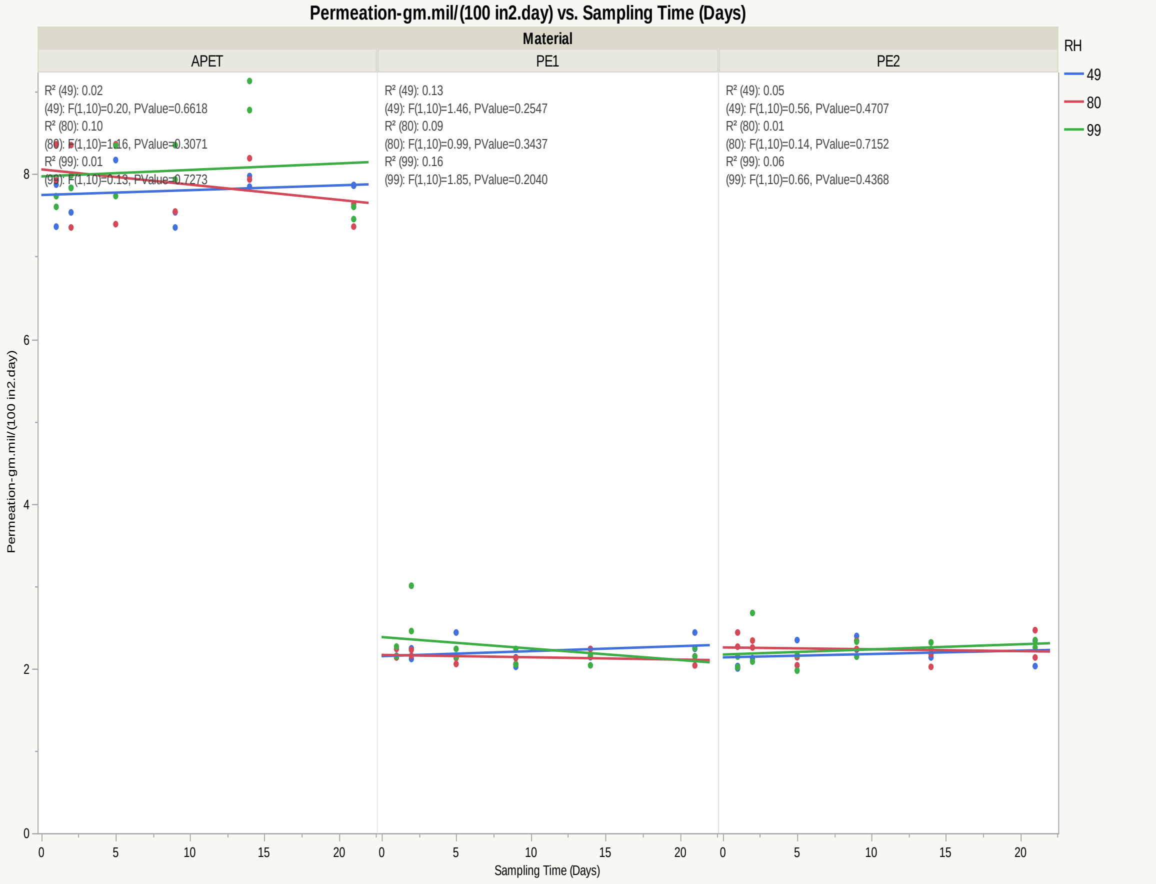

Figure 4.29: Water Vapor Permeation of ClO_2 Treated APET, and PE (type 1, type 2) (^{0}C) at 99, 80, and 49% Relative Humidities by Time (Days).

DISCUSSION

The study objective was to determine the effect of the controlled and continued release of ClO_2 gas (a dose of 0.05 mg/day at 20 ºC) treatment on selected packaging materials (APET, Nylon-6, two grades of PE - PE1, PE2) under various relative humidities (49%, 84%, 99%) for a long period until day 21. When considering the mechanical properties of these selected materials, the results indicate that both impact strength and tensile strengths of some materials have minor effects under given ClO_2 treatment conditions due to the oxidative effect of ClO_2 gas, which will be discussed. ClO_2 treatment did not affect the thermal properties of materials, except for a minimal change to nylon-6 (a significant increase of T_c at 49% RH on day 21), WV permeability of APET has shown an increase.

Mechanical properties of films such as impact strength and tensile strength are important measurements and evaluations in the flexible packaging industry to determine the suitability of materials for packaging applications. This is the first study that measures the changes in impact strength of ClO_2 gas exposed materials under different RHs. The tensile strength is a good test for evaluating material behaviors under low strain rates. However, materials may exhibit different (often more brittle) properties at high strain rates. Impact strength (dart drop test) uses a high strain rate.

The findings of this study indicated that the impact strength of nylon-6 film had a considerably higher reduction at 99% RH compared to the other RHs during ClO_2 gas treatment for 21 days (Figure 4.1.a, and Figure 4.1.b). It is possible that the ClO_2 gas would encounter all the water it needs at 99% RH to create a maximum number of ClO_2 free radicals, which could then act to degrade the properties of the nylon-6. Alternatively, the plasticization of nylon-6 may provide spaces for ClO_2 free radicals to enter into the nylon-6 structure. This may cause changes in the bond strength of the polar nylon-6 with the reaction of polar ClO_2 free radicals. Plasticization continues even when the nylon-6 matrix is fully saturated (Reuvers et al., 2015). Although there is evidence of plasticization of nylon-6 with water (Stufflebeam, 2006), which could result in changes in this experiment, the impact strength of nylon-6 showed no change with the 99% RH control but did show (higher) change with 99% RH and ClO_2 gas.

The findings of this study also suggest that neither PE grade reacted (oxidative effect or polarity effect) with free radicals of ClO_2 enough to change the bond strength of the materials. It is possible that this result could instead be due to the fact that the PEs were oriented. This is the first study to utilize oriented PE’s, so there are no other studies to use in comparison. Alternatively, it could be that PE’s, oriented or unoriented, are stable in tests at low and high strain rates in the presence of humidity and ClO_2. Previous studies (Stufflebeam, 2006; Ozen, 2000) have shown the low rate-of-strain strength properties of unoriented polyethylenes to be fairly stable with respect to (low dose of) ClO_2 treatments, so it is also possible that the PE’s suffer limited strength property degradation at high strain rates also.

Comparing each material, as was expected, MoE and TS at break of materials demonstrate significant differences between the materials (nylon-6 vs. APET, nylon-6 vs. PE, etc.) (Figure 5 -Figure 14). This is due to the differences between the chemical structures of the materials. The tensile strength at break and tensile modulus showed no changes with respect to ClO_2 gas, RH’s, or a combination of the two over the 21 days test period (Figure 5- Figure 14). This could be due to the low level of ClO_2 dose that was utilized. Such a dosage may not change materials’ strength to break (TS) or their resistance to initial deformation (MoE). These findings are aligned with some of the literature (Stufflebeam, 2006; Ozen, 2000). Ozen observed no significant changes ($p \leq 0.05$) of TS at a low ClO_2 dose (0.1-1 ppm) in all polymers including nylon-6 and Low-density PE. This partially supports our finding for PE’s under low ClO_2 dose (0.05 ppm). At higher ClO_2 dosages (>10ppm), other researchers found that PE’s tend to change their mechanical properties - TS and modulus of elasticity (MoE) of ClO_2 treated PE's decreased significantly, which could be due to oxidation and partial polarity of PE, such as degradation of polymer chains (Kuruwita, Chapter 3; Netramai, 2011; Rubino et al., 2010; Shin, 2007). The ClO_2 dosage used for this research was found to be sufficient to preserve the shelflife of strawberries (and probably other produce) in another part of this research project (Kuruwita, Chapter 5), so this low dose could be applicable for an active antimicrobial packaging system.

Stufflebeam (2006) found that the tensile strength of BON significantly ($p \leq 0.05$) decreased within 48 hours of exposure to ClO_2 gas and increased gradually after 48 hours. The reason could be due to the entrapment of ClO_2 free radicals within the polymer structure, which over time could cause some cross-linking within the film. The "rebuilding" of macromolecules and therefore, increased molecular bonding could be the reason for the gradual increase (Stufflebeam, 2006). According to a literature search conducted by the authors, the literature provides evidence that the plasticization of nylon-6 at high RH caused opposite results of TS (increase in TS of nylon-6 and decrease of TS of other materials) compared to other polymers (Kuruwita, Chapter 3) although RH did not affect significantly the mechanical properties measured in the current study.

Figure 5 appears to show a decreasing pattern of the TS at break of ClO_2 treated APET throughout the storage time of 21 days at any RH. However, this was not determined to be statistically different. If this pattern were true (in spite of the statistics), this decreasing pattern could be due to a reaction of ClO_2 free radicals with the APET. These minor structural changes could cause a reduction of TS at break of APET over time. It is possible that the ClO_2 would affect the tensile strength of APET in a longer time span than 21 days. The lack of statistical significance could be related to the fact that the APET was very thick (20 mil), and the ClO_2 would be likely to act initially on surfaces and slowly diffuse into the body of the sheet. The surface oxidations for PET are reported to be complex and can lead to the formation of many functional groups, e.g., carboxylic acid, terminal vinyl groups, and phenols (Walzak et al., 1995). However, this low dose of ClO_2 gas may not be enough to make a significant structural change or formation of such groups in the ClO_2 treated APET samples under these testing conditions.

As stated previously, tensile testing was conducted in both MD and CD directions. For the nylon-6, the material direction was not a significant factor for MoE. According to the ANOVA, the material direction of the TS at break was not a significant factor, but the material direction*RH was. Therefore, in the student t-test, a significant difference between MD and CD (a significantly higher TS at break in MD than CD) was found for the TS at break of MD of nylon-6 at 99% under ClO_2 gas treatment. Stufflebeam, (2006) observed some similar findings of ClO_2 affecting the TS at break of nylon-6 in MD.

From the DSC test, the T_m and T_c of all the materials (except the two grades of PE's) showed a significant difference from other materials in all conditions from day 1 to day 21 (Figure 13-23). This was expected since all of the materials involved are known to have repeatable melting and crystallization properties. This DSC data for Tm and Tc further provide evidence of the similarities between the two grades of PE's. There were no significant differences in the Tm and Tc based upon RH, time, or presence/absence of ClO_2.

Other literature also provides evidence that no significant changes occur in T_m (and T_g) of ClO_2 treated polymers (Netramai, 2011; Rubino et al., 2010; Ozen, 2000). However, Rubino (2010) and Netramai (2011) observed a significant increase in the heat of fusion with a (non-significant) ($p > 0.05$) shift in T_m of IR spectra of nylon. The heat of fusion was not measured in the current research. As explained above, nylon-6 behaved differently from other polymers in the presence of ClO_2 gas treatment, and Rubino's shift in heat of fusion could be a result of an increase in crystallinity after the formation of polar groups and molecular reordering at the end of the treatment period.

However, many of the studies reported that exposure to ClO_2 (low to very high concentration)- (10-1000 ppm) did not significantly change the thermal properties of polymers in general compared to the control without ClO_2 treatment. These DSC data (Figure 15-25) for T_m, T_g, and T_c suggested that there is no formation of a functional group, polarity changes, crystallinity changes, etc. (Xu et al., 2020; Shin, 2006; Ozen, 2002). The T_c of nylon-6 at 49% RH with ClO_2 was statistically different (significantly higher) from those at other RH values on day 21 (49% RH: 190 ^{0}C, 84% RH: 188.89 ^{0}C, 99% RH: 186.81^{0}C) (Figure 19). Although it is statistically different from the other two, this may or may not be practically different. Since this is the only study to look at T_c, it cannot be compared to other studies.

The barrier to water vapor (water vapor permeability) of a material is an important parameter in selecting an appropriate packaging material for a product. As in other property comparisons, the water vapor permeabilities of the two PEs were similar with or without ClO_2 treatment (Figure 27, 28). This is due to using essentially the same material, with some subtle differences (such as density and an extra peak in PE1 on the DSC, suggesting that one is a blend, etc.). Nylon-6 did not work properly for the WVTR test due to its high water absorption capacity and subsequent plasticization. Plasticization of nylon-6 was also evident in the experiments on the effect of ClO_2 treatment on polymers properties under high relative humidity conditions, which causes increased crystallization of nylon and changes to the final material properties (Kuruwita, Chapter 3; Stufflebeam, 2006).

Permeation to WV of APET had a statistically significant difference from all other materials (APET-PE1, APET-PE2) with ClO_2 gas treatment (Figure 29). This is due to the fact that permeability is a unique property for each material. However, APET showed a significant difference (increase) in permeability to WV at 99% RH after day 14 compared to other RHs (Figure 26). Literature also supports our finding of significantly increased permeation of moisture (a significant deterioration of moisture barrier) in APET material. According to Rubino's, 2010 findings, the oxidative degradation of the material after ClO_2 exposure could lead to an increase in the polymer's chain mobility and a decrease in its intermolecular forces.

The materials evaluated in this study that is most often used in berry packaging are APET (clamshells) and polyethylene (stretch hoods). These materials demonstrated relatively few changes in the presence of ClO_2. APET's permeability got worse; however, clamshells used in berry packaging are not designed to be hermetic. (In fact, most clamshells have openings to allow the atmosphere to move about). Given the limited effect of the gas on the materials, the use of ClO_2 gas for berry preservation (ClO_2 as an antimicrobial gas in the headspace to treat berries) should be an acceptable method from the packaging standpoint.

CONCLUSION

IS

- The impact strength of nylon-6, PE1, and PE2 are significantly different from one another.The combined effect of ClO_2 and high RH (99%) has a significant effect on the impact strength of nylon-6 that may cause changes in the polarity. RH alone did not significantly affect the impact strength of nylon-6. The use of ClO_2 gas at high RH in nylon-6 packaging products should be a concern from a mechanical standpoint.
- The decreasing pattern of impact strength of treated nylon-6 at all relative humidities (RH) during long term ClO_2 exposure (until day 9) may be a result of chemical changes (oxidative degradation) of the polymer (Figure 4.1.a, 4.1.b).
- The impact strengths of PE1 and PE2 were not affected by relative humidity or ClO_2 gas treatment over 21 days. This means that PE could be a good material that can be used as a food packaging material under a low level of ClO_2 gas treatment without any mechanical impact on the product.

TS

- No significant change of TS of any materials occurred at this low concentration of ClO_2 gas.
- The material direction was a significant factor for TS at break (MD direction only) of nylon-6 at 99% RH with ClO_2 gas treatment. Therefore, selecting nylon-6 as an

antimicrobial packaging with ClO_2 gas at high relative humidity (99%) packaging is a concern. However, the change of TS at break of nylon-6 in both directions (MD and CD), should be a factor for further consideration in experimenting to determine how nylon-6 is affected by different doses of ClO_2 gas under different levels of RH.

Barrier to water vapor

- When selecting a packaging material for a particular food with the appropriate ClO_2 gas concentration, loss of barrier properties could be a concern.
- APET films showed a significant decrease in barrier properties to water vapor (or increases in permeation) after exposure to ClO_2 gas. This is an important concern in food packaging with the type of application is a hermetically sealed package. In this case, because the packaging system is a non-hermetic clamshell, WVP is less important.
- PE under low ClO_2 gas treatment is a good material to use in food packaging applications where the moisture barrier is a concern.

DSC

- The T_m of all the materials did not change significantly after exposed ClO_2 gas and this suggests that the formation of functional groups or change in the structure of polymers may not be seen or may not be significant with this low level of ClO_2 treatment.

LIMITATIONS

- This research was already underway when Clemson university enacted the Covid-19 lockdown. The researchers had restrictions on being in the lab. During this time, some data were lost for the O_2TR test, and experiments that the researchers wanted to do, such as IR, contact angle tests were not available.

- The APET used was a thermoformable grade used for clamshells. However, it was tested in sheet form. This made it impossible to measure impact strength. The overall thickness may have also caused less effect in the presence of ClO_2 than might have been seen in thinner sheets of APET.

FUTURE SUGGESTIONS

- The DSC did not show any formation of functional groups of structural change of each polymer, However, ClO_2 probably begins working on the surface first, so the peaks from the bulk of the polymer could mask changes on the surface. For future research, perhaps someone could measure for surface changes, such as contact angle measurements using polar and nonpolar liquids or ATR. However, Rubino, 2010 has proved that no changes in the chemical structures of any polymers used under 10 ppm ClO_2 treatment.
- Since this is the only study to look at T_c, the significant difference between nylon-6 at 49% RH with ClO_2 from other RHs needs to be further studied.
- The change of TS at break of nylon-6 in both directions (MD and CD), should be a factor for further consideration in experimenting to determine how nylon-6 material direction affects at different doses of ClO_2 gas under different levels of RHs.
- Studying the effect of different concentrations of ClO_2 gas or for longer periods of treatment than 21 days may be of interest. A range of ClO_2 doses of 1-5 ppm, or somewhere under 10 ppm was suggested from the literature on the ClO_2 effect on

polymer properties (Kuruwita, Chapter 3). Bleaching of fresh produce would likely be a limit (Kuruwita, Chapter 2).

- Studying more produce packaging materials (including OPP, any biobased, and recycling materials) may be of interest.
- This study looked at 49, 80, and 99 % RH. Studying the impact of other values of RH (including lower) may help to fill the remaining literature gap.
- When considering produce packaging, barrier protection to O_2 is of paramount importance. Loss of the O_2 barrier due to ClO_2 treatment may affect the respiration rate of fresh produce, which is a significant cause of ripening and deterioration of produce. Therefore, further testing of permeabilities (O_2, CO_2, etc.) at this ClO_2 dose would be desirable.

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CHAPTER FIVE

USE OF CHLORINE DIOXIDE GAS-PRODUCING SACHETS TO EXTEND THE FRESHNESS OF STRAWBERRIES DETERMINED BY SENSORY EVALUATION

INTRODUCTION

Globally, more than one-third of all food on our planet never reaches a table (is never consumed) (FAOUN, 2021). 870 million people are suffering from chronic undernourishment (UN News, 2012). One of the primary contributions to this tragedy is food waste. Preservation (Shelflife extension) by maintaining the quality of fresh produce (fruits and vegetables) is a possible solution for reducing produce waste. Approximately half (52%) of all produce is unconsumed in the US market, and produce is the number one source of food waste. The spoilage of fresh produce is worth $15.6 billion at the retail level (Sonoco Food Waste). The value of shelflife improvement by one day is $1.8 billion to the agricultural industry (The Sonoco Institute, 2018).

In the US market, consumers prefer strawberries over all other fresh fruit products. Strawberries have a $ 3.5 billion market share, which is 82% of all fresh fruits in 2017 (Sun et al., 2014). The production of strawberries in the world was around 8.9 million tons in 2019 (mainly from China, the US, and Mexico). In 2020, approximately 35% of $ 2.2 billion US strawberry production was unconsumed (wasted) due to the very sensitive and very fragile nature of strawberries. The value of a one-day shelflife increase for strawberries is around $84 million annually.

Chlorine dioxide is the most widely used to disinfect drinking water. However, it is commonly used to disinfect fresh produce compared with (Cl^-) and to extend the shelf life of fresh produce in food industries. Chlorine dioxide can be used as a fumigant treatment to sanitize fresh fruits such as blueberries, raspberries, and strawberries to keep them fresh. It has a strong oxidizing capability, 2.5 times that of chlorine gas. Chlorine dioxide leaves little to no solid residue on treated foods. Chlorine dioxide is also approved by FDA and EPA as an antimicrobial gas with a residual level of 3 mg/L. Many recent papers provide evidence that chlorine dioxide is effective as an antimicrobial gas when used in the headspace of fresh produce packages, increasing the safety and quality of the fresh product (Sun et al., 2014; Wang et al., 2014; Popa et al., 2007). Chlorine dioxide gas is delivered to treat fresh produce in many ways, including generators, mixing chemicals in a large open container that releases gas, and using sachets. In this research, sachets were used. Chlorine dioxide gas releasing sachets can be placed in an enclosed container (bucket, basket, etc.) or in a treatment cabinet to treat produce in an experimental setup (with an air circulating fan). Also, sachets can be placed in clamshells or pallet systems. The use of a ClO_2 gas sachet is a simple, economical, effective, and practical method to enhance microbiological shelflife extension and safety. Chlorine dioxide gas treatment can be used in many ways- high dose/short exposure vs. low dose/long exposure, single event vs. continuous-release, bulk fumigation vs. localized release. These choices are made depending on how producers and grocers want to treat the product and how efficiently the product should be treated.

Berries are commonly washed in tanks of chlorine dioxide dissolved in water (50 - 100 ppm) as a treatment to reduce the presence of yeast and mold. However, regrowth of surviving yeast and mold in batch freeze storage can be expected (Popa et al., 2007). The current application of ClO_2 fumigant treatment is not widely used yet, although it is more effective compared to the liquid form. It is used to sanitize" and “preserve” fruits such as blueberries, raspberries, and strawberries, reducing bacteria, yeast, and mold growth (Popa et al., 2007). Very few research papers have been published on the topic of the “use of chlorine dioxide sachets to increase the shelflife (extend the freshness of produce) of fresh produce” (Kuruwita, Chapter 2). Although there has been some research, ClO_2 as an antimicrobial gas in the headspace of fresh produce packages (using sachets) is still a relatively novel approach, and commercial application is not widely used yet. This is the first study to research the effect of ClO_2 gas-producing sachets to extend the shelflife of fresh strawberries in a pallet storage system, including a humidity-controlled (99%) ClO_2 gas treating closed chamber system for over 14 days. A recent study done by Kessler, 2020 studied some aspects of this in “Shelf-Life Extension of Fresh Strawberries Packaged in Clamshells with Chlorine Dioxide Generating Sachets”. The focus of that research was mainly on the study of the distribution of chlorine dioxide over the strawberry packaging system, shelflife extension with the sachets inside clamshells, etc.). The objective of this study was to determine the effect of controlled-release ClO_2 gas treatments (using sachets) on the sensory properties of strawberries (qualitatively and quantitatively) that may affect the shelf life of strawberries targeting practical strawberry storage system of an open pallet system under cold conditions (0-2 ^{0}C).

METHOD

Material

Fresh strawberries

Fresh strawberries (*Fragaria x ananassa*) picked and packaged by "ALWAYS FRESH FARMS" (Winter Haven, FL 33881, USA) were distributed in a cool truck to Ingles market, (Seneca, SC-29678, USA), and the samples for our experiment were directly bought from the refrigerated truck when unloading at the Ingles loading dock and transferring to the warehouse. The truck temperature was maintained at 40-45 °F during the distribution from Florida to Seneca. A total of 6 flats (A "flat" is a corrugated crate that includes 8 strawberry clamshells) were used for the experiment, with a net weight of 454 g (1lb) for each strawberry clamshell having a total of 21.792 Kg (48 lb). Strawberry samples were delivered to Clemson university and immediately put into cool storage at 0-4 °C.

Chlorine dioxide sachet

Chlorine dioxide sachets were donated from ICA TriNova (Newman, GA). These fruitgard® sachets are prepared by placing two components into the pouch, (parts A and B), which react together to produce ClO_2. The sachet was designed to provide continuous fast release mixing part A 5 times (31.25 g for the pallet system, 5g for the chamber system) and part B 1 time (6.25 g for the pallet system, 1g for the chamber system) a dose of 0.04-0.06 mg/day at 0-4 C.

Figure 5.1 summarizes the entire experiment including both the sensory evaluation test (sensory analysis) and the instrumental tests (instrumental analysis). The same strawberry samples that were used for the sensory evaluation attributes (color, aroma, firmness, overall acceptability) were also used for the instrumental tests including color test, texture analysis, and total soluble solids (TSS).

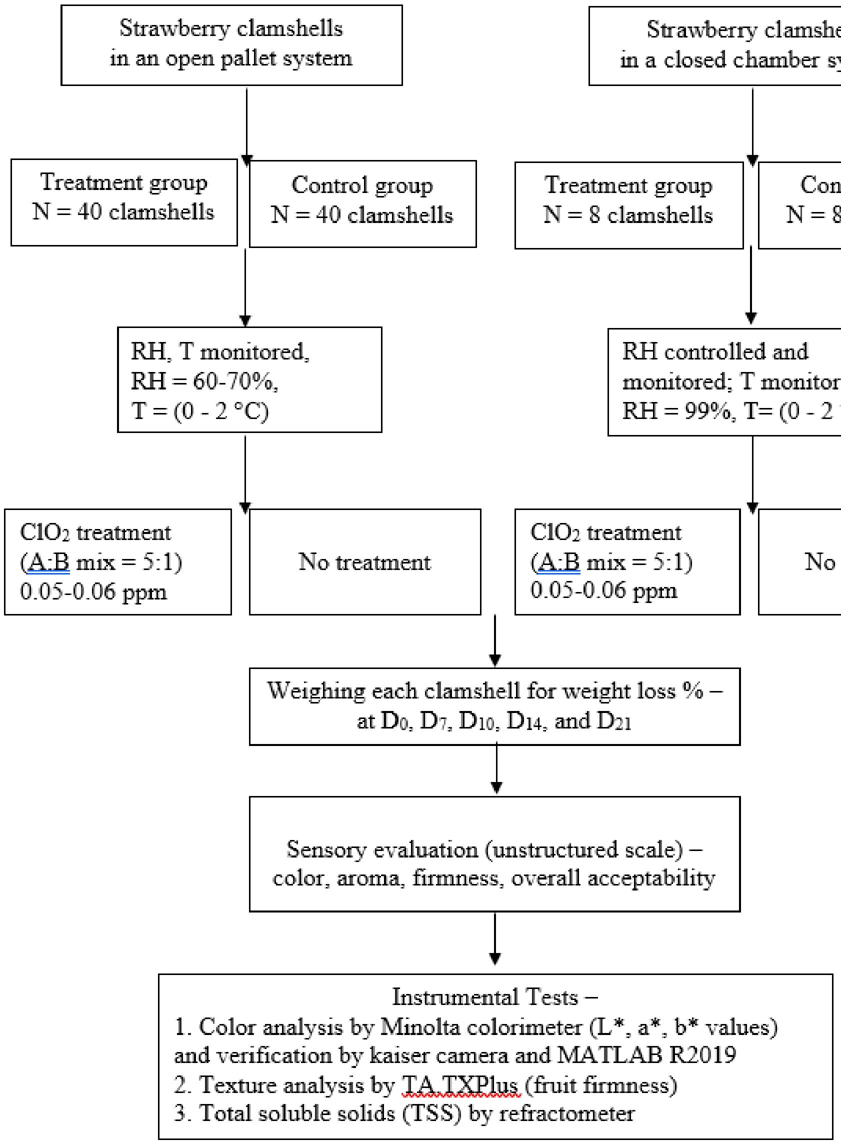


Figure 5.1: Summary of Experimental Setups, Sensory Analysis, and Instrumental Analysis of Fresh Strawberry. (T = temperature, RH = relative humidity, N = number).

Experimental design

Sample preparation for the experimental setups

All 48 strawberry clamshells were visually checked for initial quality and freshness (color, firmness, appearance-presence of bruises, dryness, any mold growth, etc.). Any fruit that failed acceptance in the initial screening (a score of only 0 was acceptable) was manually removed by the researcher (Table 5.1). Therefore, the sensory parameters of all the strawberries were scored as 0 at day zero (D_0) by the researcher (Table 5.1). To avoid tight storage in each container, half of the strawberries in each clamshell were removed and placed into empty clamshells. This resulted in 96 clamshells (12 flats) and 21.792 Kg (48 lb).

Treatment group pallet system

Five flats of newly arranged strawberry clamshells were labeled for the treatment group pallet system (treatment, pallet, clamshell number as T-P-1, … T-P-40, etc.). Two pallets ~ 609 mm X 508 mm (24’’ X 20’’) were placed into a laboratory-scale two-door Symphony refrigerator (VWR, Atlanta, GA, USA). Three flats of the rearranged strawberry clamshells were stored on each pallet. Four fast-medium speed small portable fans (AEOSBIK, Shenzhen, China, GB4706.1.2005) were set up to circulate air inside the refrigerator (2 fans on each side). A TRACEABLE fisherbrand temperature-humidity meter (Fisher Scientific, Pittsburgh, PA) was kept inside for daily monitoring of the temperature and relative humidity. A ClO_2 sachet was kept inside the refrigerator facing the pallet system, as shown in Figures 5.2.1 and 5.2.2.

Figure 5.2.1 and 5.2.2: Experimental Apparatus – Pallet Strawberry Storage Exposed to a Slow Release of ClO_2 Gas Sachets (Low Dose, Continuous Release) at (60-70%) Humidity at (0 - 2 °C).

Control group pallet system

Five flats of newly arranged strawberry clamshells were labeled for the control group pallet system (control, pallet, clamshell number as C-P-1, … C-P-38, etc.). All five flats of strawberries were arranged on a pallet 508 mm X 355 mm (24’’ X 20’’) in a laboratory-scale refrigerator. A temperature-humidity meter (Fisher Scientific, Pittsburgh, PA) was kept inside for daily monitoring of the temperature and relative humidity.

Treatment group closed chamber system.

The refrigerator used was not designed to provide high humidity (95-99 % optimum level for strawberries) but the optimum temperature (0-2 ^{0}C) for the pallet system. However, it may be of interest to see the freshness of the ClO_2 treated strawberry at optimum relative humidity (99 %), which may be found in storage in any commercial setting or on some strawberry farms. However, Forced-air cooling is the standard method for cooling fresh strawberries in commercial storage cold rooms at 90-95% RH, which is considered optimum RH in the literature (23, 24, 25). The following system was used to provide a high humidity evaluation.

A glass fish tank 508 mm X 355 mm (20’’ X 14’’) was used with a sealable lid to have a closed chamber set up. 99% relative humidity was maintained inside the chamber using KCO_3 (Alfa Aesar, 30 Bond Street, Ward Hill, MA 01835) saturated salt solution in a small beaker. One flat of newly arranged strawberries (8 clamshells) was labeled for the treatment group chamber system (treatment, chamber, clamshell number as T-C-1, … T-P-8, etc.). Eight strawberry clamshells were arranged in a way to display 4 layers with two clamshells on top of each, as shown in Figure 5.3. A ClO_2 sachet was kept inside the glass chamber and sealed tightly to avoid movement of any gas in or out. This setup was placed in the same treatment refrigerator on the upper shelf (Figure 5.3). A temperature-humidity meter (Fisher Scientific, Pittsburgh, PA) was kept inside for daily monitoring of the temperature and relative humidity.

Figure 5.3: Experimental Apparatus – Chamber Strawberry Storage exposed to slow release of ClO_2 gas sachets (low dose long time of release) at (99%) humidity at (0 - 2 °C).

Control group closed chamber system

An aquarium for high humidity measurement (99%) of control (non-ClO_2) was set up in the same way as described above. Treatment group closed chamber system), except that no ClO_2 sachets were included. Eight clamshells in the control group chamber system were labeled as (control, chamber, clamshell number as C-C-1, … C-C-8, etc.). This setup was placed in the same control refrigerator on the upper shelf.

Sensory analysis

Selection of participants (Recruitment of participants)

Clemson University Institutional Review Board (IRB) approval was received for the "Sensory Evaluation of ClO_2 Gas Treated Strawberries" study. Qualtrics ^{XM} (Qualtrics

International, Seattle, WA) web-based platform was used to select participants through an initial screening survey (Appendix-1) which was emailed to the Food, Nutrition, and Packaging Sciences Department. At the initial screening through Qualtrics, 35 people were eligible to participate in the sensory panel for the sensory evaluation of the fresh strawberries study. The eligible participants were informed about the schedule of each session. Based on availability, 25 subjects participated in the sensory panel.

Familiarization session/training

In the familiarization session, the sensory panel was trained on sensory parameters (color, aroma, firmness, overall acceptability for intent to consume) of strawberries. Panel members were also familiarized with how to score (evaluate on a scale of 0-15 cm) each parameter on the unstructured scale in the sensory ballot (Appendix-H). This was performed based on a given description of parameters (Table 5.1).

Table 5.1: Freshness quality scores for sensory parameters with 6 scoring categories from 0-15 cm.

Sensory Parameters	Sensory Scores					
	0	3	6	9	12	15
Color	Completely red (dark/light) for fully ripen or pink-red for partially ripen, small part of white, shiny	Red and Seeds starts to be prominent, slightly shiny	Slightly opaque, Seeds more prominent	Slightly opaque, Seeds more prominent	Brown, opaque	Brown, opaque
Aroma	Strong and pleasing strawberry odor	Moderate strawberry odor	Slight strawberry odor	Slight strawberry odor or Slight off odor (Starts mold)	Strong off odor (Mold and fermentation)	Strong off odor Mold and fermentation
Firmness	Very firm	Moderately firm	Slightly firm	Slightly soft, possible juice oozing out from bruises	Soft and/or mushy	Very soft and/or mushy
Overall Acceptability	Super good	Really good	Good	Acceptable (trim, cook)	Bad, throw away	Very bad, throw away

After a score of 6, which has minimum acceptable sensory qualities of strawberries is considered the end of shelflife.

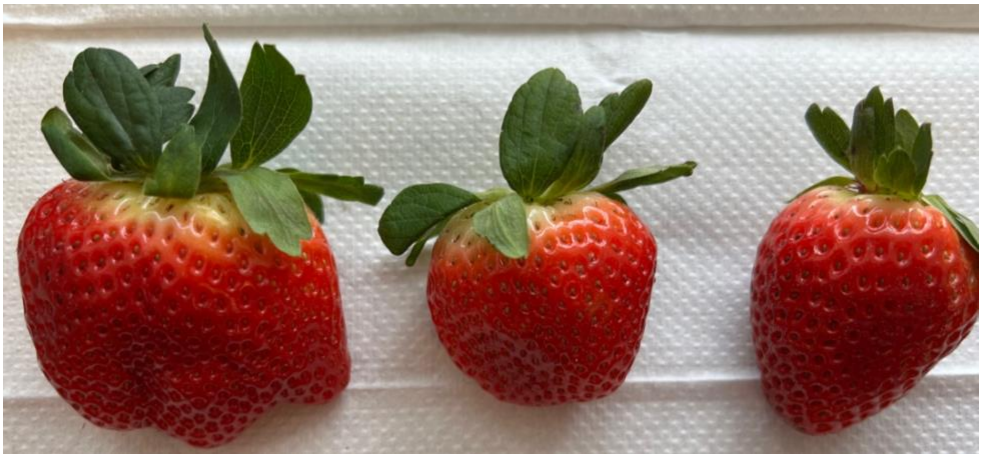
Sensory evaluation sessions

Three individual sensory stations were arranged under equivalent lighting conditions in a laboratory at Clemson University. Two random strawberries of similar size were collected from each experimental group (four groups - 2 treatments, 2 controls) to arrange a sensory station for visual evaluation. The same procedure was conducted to arrange all 3 sensory stations for each sensory session (D_7, D_{10}, D_{14}, and D_{21} of the study period). Each of the 3 stations consisted of 4 sets of samples with 3-digit sample codes (101-treatment pallet system, 202-treatment chamber system, 303-control pallet system, 404-control chamber system) as shown in Figure 5.4. While sampling for evaluation, strawberries with mold were also removed on each sampling day to prevent the spread of mold. Throughout the test, the weights of strawberries removed were tracked for each sampling day.

sory rameters	Sensory Scores					
	0	3	6	9	12	15
lor	Completely red (dark/light) for fully ripen or pink-red for partially ripen, small part of white, shiny	Red, pink and Seeds starts to be prominent, slightly shiny	Slightly opaque, Seeds more prominent	Slightly opaque, Seeds more prominent	Brown, opaque	Dark brown-cover whole fruit, opaque
ma	Strong & pleasing strawberry odor	Moderate strawberry odor	Slight strawberry odor	Slight strawberry odor + Slight off odor (Starts mold)	Strong off odor (mold &/or fermentation)	Strong off odor (mold &/or fermentation)
ness	Very firm	Moderately firm	Slightly firm	Slightly soft, possible juice oozing out from bruises	Soft and/or mushy	Very soft and/or mushy
ability to eat	Super good	Really good	Good	Acceptable (trim, cook)	Bad, throw away	Very bad, throw away

Figure 5.4. Sensory Evaluation Station 1 on Day 10 represents samples 101, 202, 303, and 404 from left to right, and table 5.1 for sensory score and strawberry reference picture for the color score.

The sensory panel subjects signed the consent form and received an individual project code number to use on the ballot on the first day of the sensory sessions. The sensory panel members were instructed to touch the strawberries with gloves and handle the fruit gently to keep them fresh until the end of the experiment. One person at one station at one time was allocated on each sensory evaluation day. The day zero (D_0) strawberry samples were evaluated for all four sensory parameters by the researcher and scored as zero scores (Figure 5.5).



D_0-Sample 1 D_0-Sample 2 D_0-Sample 3

Figure 5.5: D_0 strawberry samples for the sensory parameters and the instrumental tests

Data were collected on an MS Excel® 2016 (Microsoft Corporation, Redmond, WA) sheet separately for each parameter on each sensory session.

Instrumental analysis

Weighing for water loss

All the strawberry clamshells under the labeled name were weighed on D_0 before the first day of storage and on each sensory evaluation day (D_7, D_{10}, D_{14}, and D_{21}) as the weight of clamshells before taking samples for sensory evaluation and instrumental test and the weight of clamshells after taking samples for tests using the laboratory scale analytical balance with an accuracy of 10^{-2} (Max:1000g, d=0.01g) (Adam, ADAM EQUIPMENT Co. Ltd., Milton Keynes, U.K.) (Figure 5.6). These data were used to calculate the weight loss % of strawberries until the end of the experimental period. Weight loss was calculated as a percentage (%) using the formula described by Akhtar, Abbasi, and Hussain (2010).

$$\text{Weight loss \%} = \frac{\text{Initial fruit weight} - \text{Final fruit weight}}{\text{Initial fruit weight}} \text{ X } 100$$

Figure 5.6: Weight Measuring of a Sample of Strawberry Clamshell Using an Analytical Balance.

The weight loss % at each storage day/sampling day is calculated as the total weight loss % on the day (D_7, D_{10}, D_{14}, and D_{21}) compared to the D_0 weight but not the changes in weight loss % between sampling days. Because some of the tests were destructive, each testing day started with less total strawberries available than the previous day. To find weight loss related to D_0, it was assumed that the strawberries removed for destructive testing (or for mold) would have lost weight at the same rate as the remaining strawberries.

Color measurements

The surface color - L* (lightness-darkness), a* (redness-greenness), and b* (yellowness-blueness) values of strawberries (3 locations around the equator of each strawberry X 3 samples) were measured using a Minolta colorimeter (CHROMA METER CR-400, Minolta CO., LTD, Japan), and the data were recorded by sample group name and sampling date. The same procedure was done for the D_0 samples (Figure 5.7).

Figure 5.7: L*, a*, and b* Values of a Strawberry Sample Using Minolta Colorimeter.

Verification of strawberry samples on each sensory day were done using kaiser camera and MATLAB R2019 for L*, a*, and b* values of strawberries not for data analysis but for observation due to limited available facilities at the time of the experimental run.

Texture Analyses

A texture analyzer, TA.XT.plus, (Stable Micro Systems Ltd., Surrey, UK), along with the P/5S (2 mm dia cylinder stainless steel measuring probe), were used for texture determination. The system was equipped with texture profile analysis (TPA). Firmness was measured as the maximum penetration force (g) reached during tissue breakage for a 5 mm distance. The maximum force (g) required to penetrate a probe 5 mm into strawberries is practically similar to the crunchiness of strawberries (the maximum effort to chew) in consumers' mouths. The maximum penetration force was recorded manually in the graph and also compared with the data for the maximum force given by the machine itself. The measurable parameters set were pretest speed (1 mm $s^{-1)}$; test speed (1 mm s^{-1}) and penetrating distance (5 mm into the fruit). The maximum force required for sample compression was calculated as an average of 8 measurements for 1 fruit around the equator. This was done for 3 samples (triplicate) for each experimental group/sampling day, as well as for the first day samples (Figure 5.8).

Figure 5.8: TA.TXPlus (Fruit Firmness Analyzer) for a Strawberry Sample

Total Soluble solids content (TSS)

The soluble solids content was obtained by measuring the Refractive Index of the strawberry juice using a digital hand-held pocket digital refractometer (SPER SCIENTIFIC 300053). Initial calibration was made with deionized water provided with the instrument. A drop (~1 ml) of the juice was placed on the lens, and the reading was taken in degree Brix (◦Bx). This reading gives the % of soluble solids content (% SSC) in the fruit. The lens was carefully rinsed with deionized water between each sample. Three measurements were taken for each fruit, and data were recorded for each sampling day under the sample code/label. The same procedure was done for the first-day samples (Figure 5.9.1, and 5.9.2).

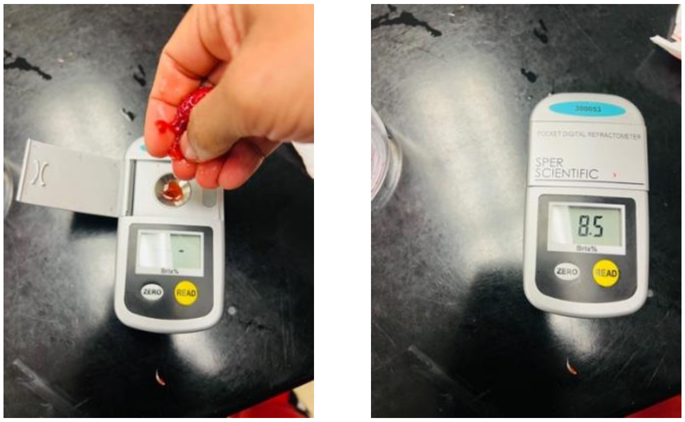


Figure 5.9.1, 5.9.2: TSS (Brix^0) of a Sample of Strawberry Using Refractometer

Statistical analysis:

MS Excel® 2016 (Microsoft Corporation, Redmond, WA) was used to calculate mean values and standard deviations of replications of each instrumental analysis: weight loss (%), L*, a*, b* colors and delta E, total soluble solids, maximum penetration force, and sensory parameters (color, aroma, texture, overall acceptability). The mean values were analyzed for the analysis of variance (two factor ANOVA) using JMP pro-15 (SAS Institute, Inc., Cary, NC, USA). Significant differences in treatments and controls were detected using the least significant differences (LSD), $\alpha < 0.05$ was considered significantly different, and the student's t-test was used to model the relationship between two variables at a significant level ($\alpha < 0.05$). Since the sample size of two experimental setups (pallet system and chamber system) is different only for weight loss %, weight loss data were analyzed using all pair Turkey method. MS Excel® 2016 was also used for regression tests when looking for time-based trends.

RESULTS

Instrumental parameters:

Weight loss (%)

Weight loss as a percentage of the original weight was recorded for the strawberries. These data are presented in Figures 10.1 and 10.2. Figure 10.2 is provided because the error bars overlap in Figure 10.1. The error bars throughout this section are the size of ± one standard deviation. Weight loss percentage values were significantly different ($p \leq 0.05$) with respect to sampling time, the experimental group (treatment vs. control), and the interaction of sampling time*experimental group.

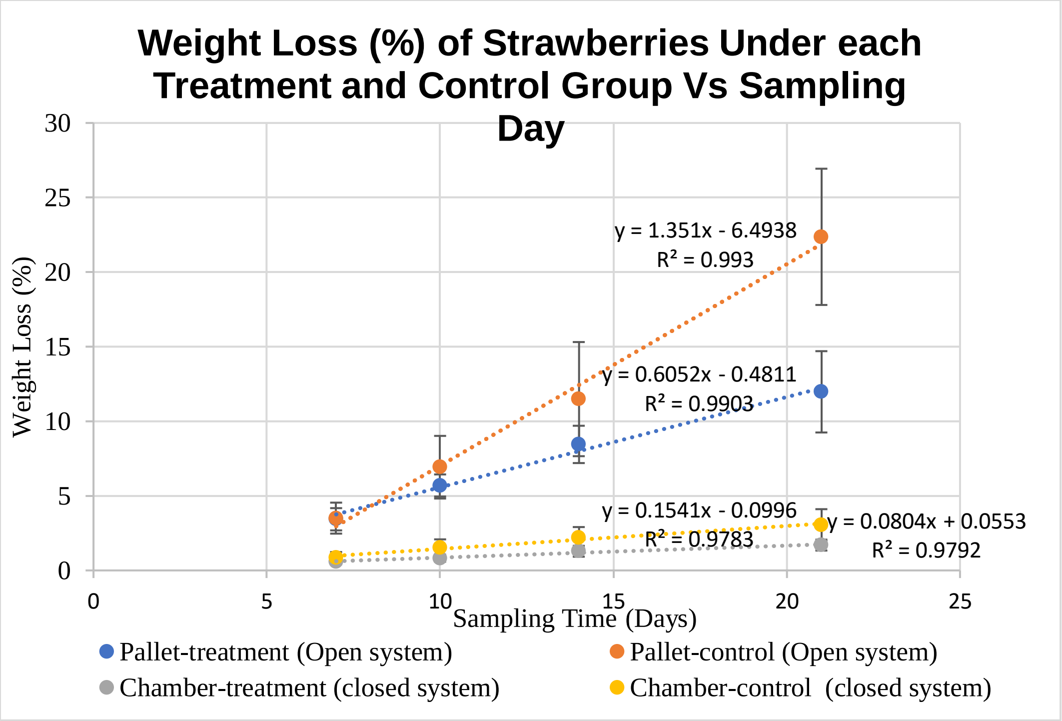


Figure 5.10.1: Weight loss (%) of Strawberries on each Sampling Day for each Sample Group

On day 7, all groups were significantly different from each other except groups 404-202 and 303-101. Similarly, on days 10, 14, and 21, all groups are significantly different from the other groups except the 404-202 pair. It appeared that weight loss % data for all groups have a linear relationship throughout the period (R^2 in Figure 10.2 is very close to 1).

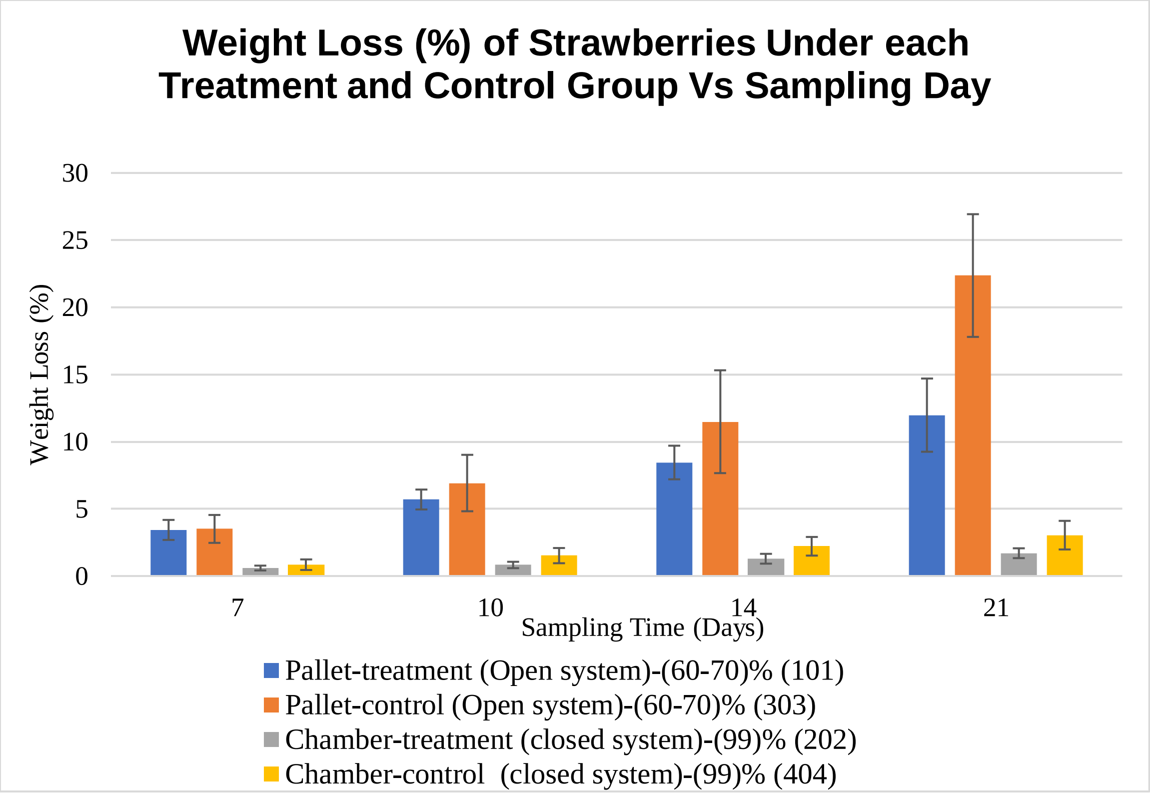


Figure 5.10.2: Comparison of Weight loss (%) of Strawberries for each experimental group Vs. Sampling Day.

A column graph (Figure 10.2) was used to compare each experimental group on each sampling day, and also the results of the statistical analysis (t-test) will be discussed under this graph. All groups are significantly different from each group (303-202, 303-404, 101-202, 101-404, 303-101) except 404 and 202 on all sampling days.

Color

A Minolta colorimeter was used to measure the colors of the strawberries. L* (the lightness to darkness factor) is depicted in Figure 11.1. L* showed significant differences with respect to sampling time and experimental group (treatment vs. control). The interaction of sampling time*experimental group did not show a significant difference ($p \leq 0.05$).

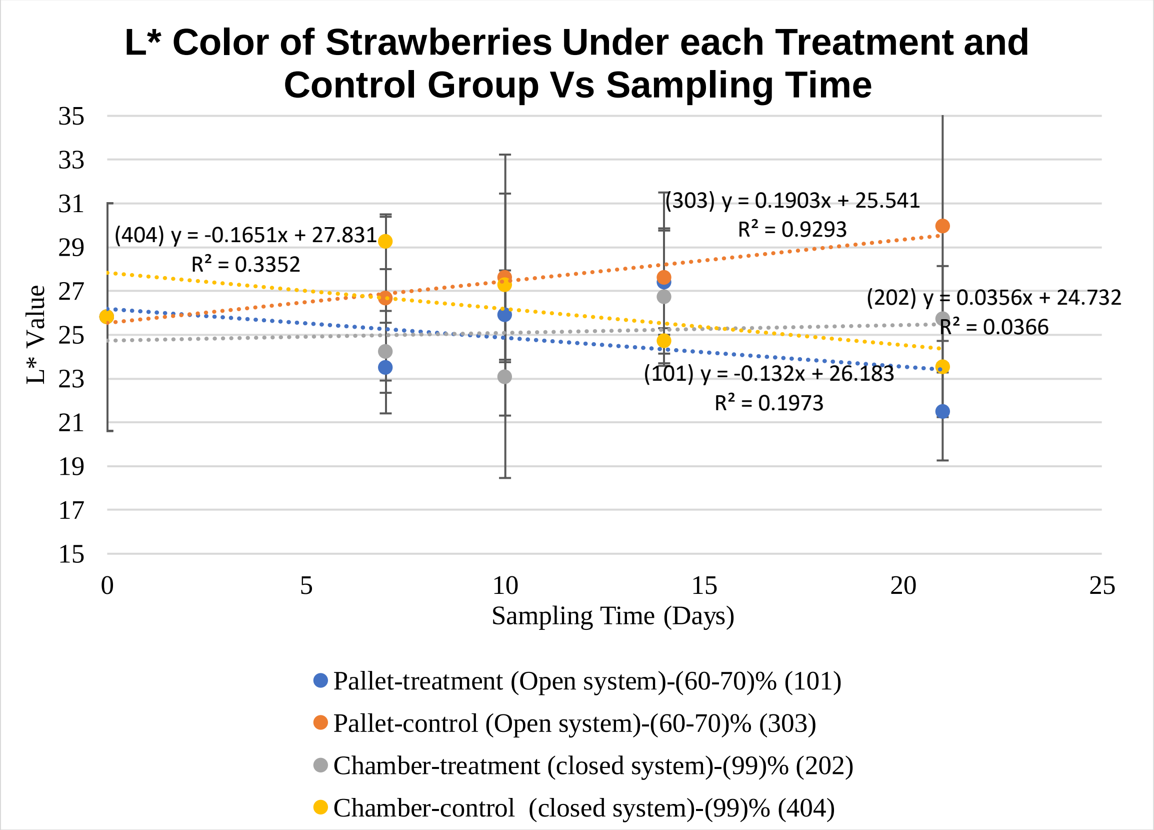


Figure 5.11.1: L* Color of Strawberries on each Sampling Day for each Sample Group.

According to Figure 11.1, group 303 has a good fit (R^2 near one) for a linear relationship, showing a slight increase in L*. None of the other groups showed a good fit for a linear trend. A significant difference in color L* was noted on day 21 between the groups 303 and 101 and also 303 with 404.

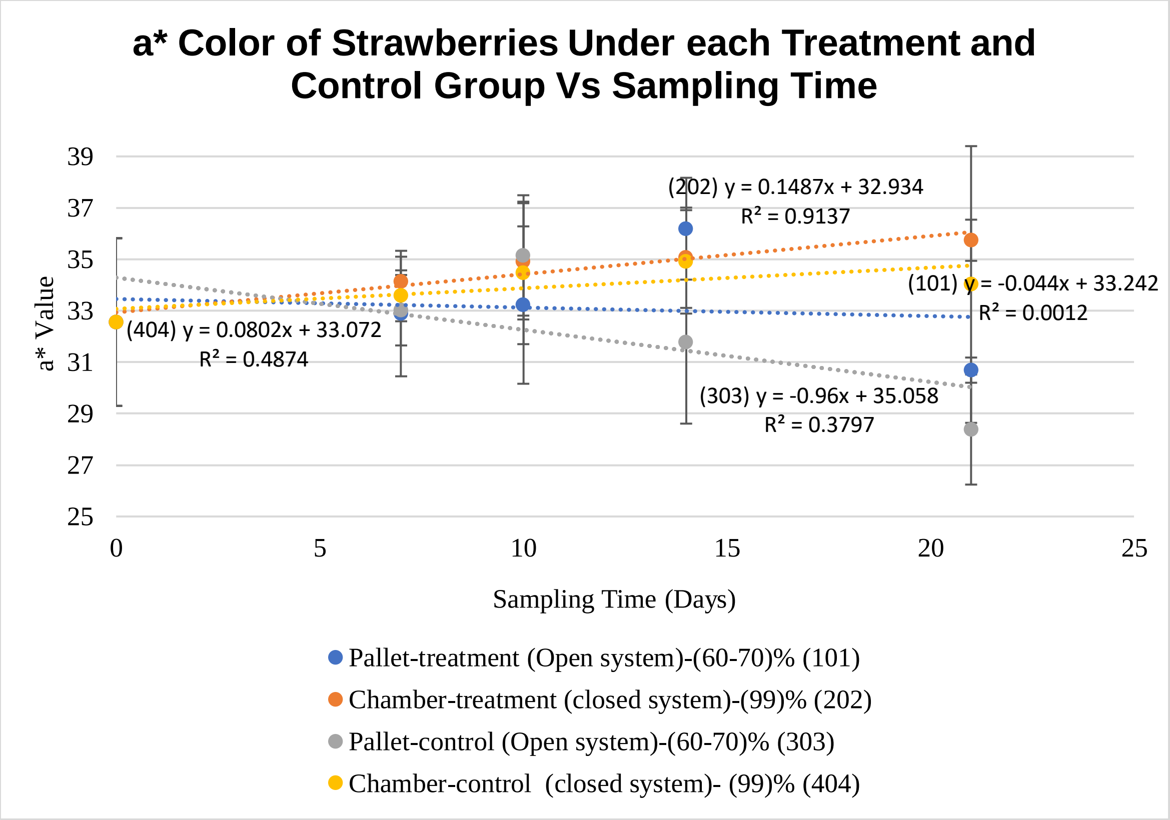


Figure 5.11.2: a* Color of Strawberries on each Sampling Day for each Sample Group.

Color a*, which is red to green, is shown in Figure 11.2. This parameter only showed a significant difference with respect to the experimental groups. Group 202 appeared to show a linear trend, although the ANOVA showed no dependence upon a time. None of the other groups showed a trend using regression. A significant difference in color a* existed on day 14 between the groups 303 and 202 and also between 101 and all 3 other groups.

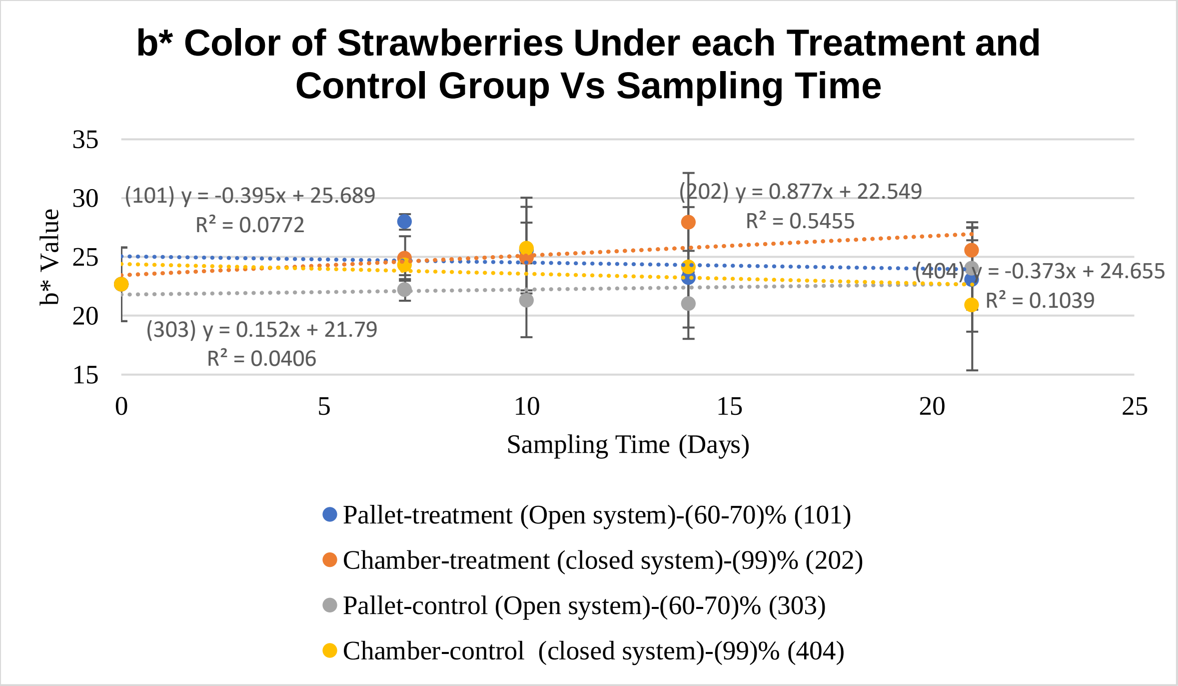


Figure 5.11.3: b* Color of Strawberries on each Sampling Day for each Sample Group.

The changes in color b* (blue to yellow) are depicted in Figure 11.3. This parameter demonstrated significant differences only between experimental groups, according to the ANOVA. As would be expected from that ANOVA, the data did not appear to show linear trends for 21 days in any of the groups.

A significant difference in color b* was evident on day 7 between groups 303 and 101. Color b* also showed significant differences on day 21 between groups 303 and 404, as well as between 303 and 202.

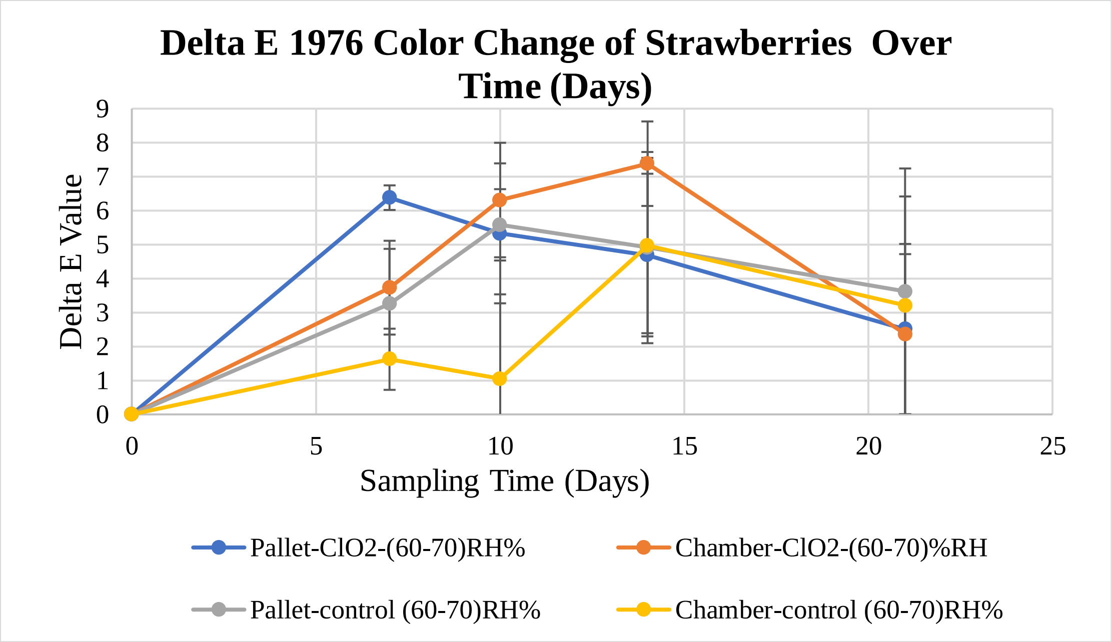


Figure 5.11.4: Delta E value for Color of Strawberries on each Sampling Day for each Sample Group.

The changes in delta E value are depicted in Figure 11.4. This parameter demonstrated significant differences only with sampling time for all experimental groups, according to the ANOVA. As would be expected from that ANOVA, the data did not appear to show linear trends for 21 days in any of the groups.

A significant difference in delta E was evident only on day 7 between groups 101-202, 101-303, and 101-404.

TSS

Total Soluble Solids (TSS) is a measure of solid components in the strawberry that will dissolve in water. The results of this test are shown in Figure 12.1. According to the ANOVA conducted, this parameter showed a significant difference only with experimental groups.

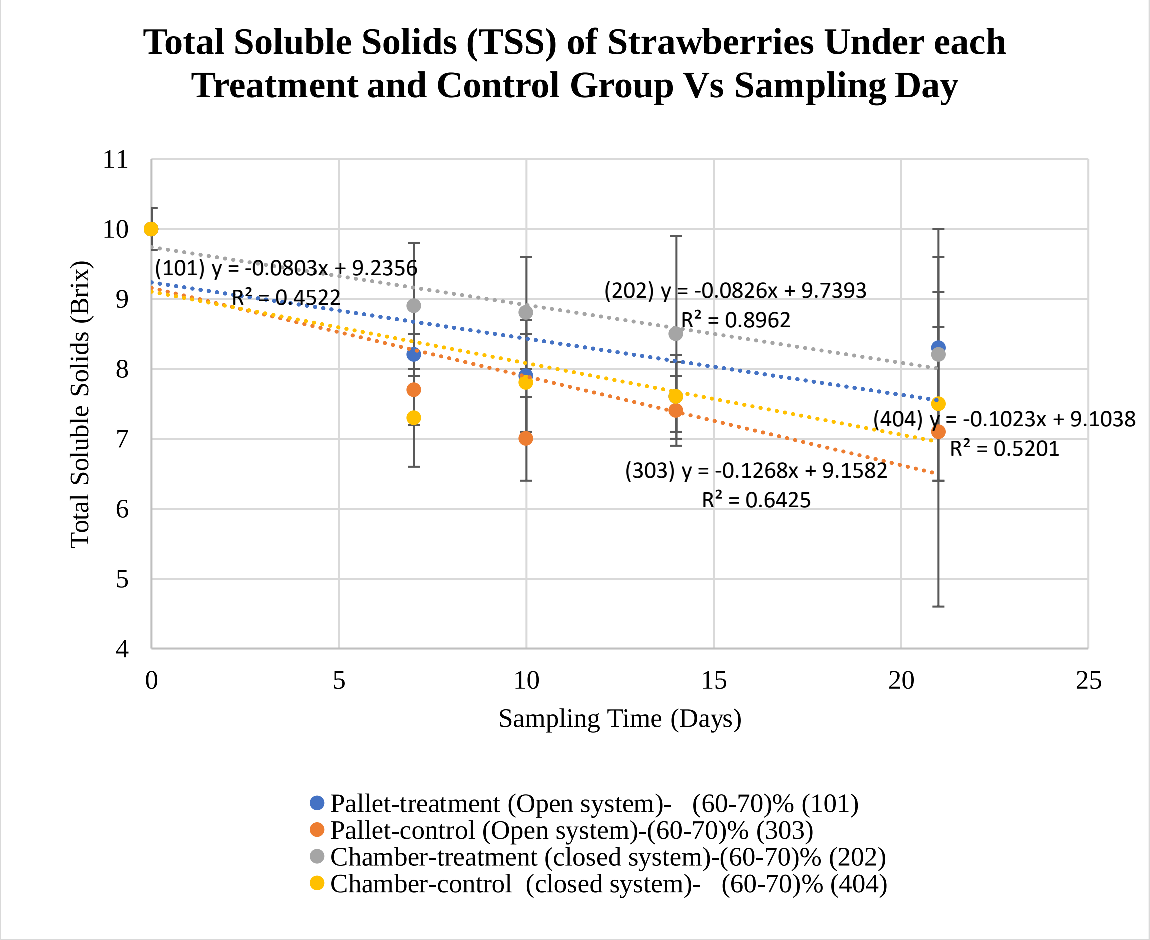


Figure 5.12.1: TSS of Strawberries on each Sampling Day for each Sample Group

Only group 202 appeared to show a linear trend for 21 days. The other groups did not appear to show a linear trend, as would be expected from the ANOVA. A significant difference in TSS was seen on day 7 between the groups 303 and 202 / 404, as well as between 101 and 404. A significant difference in TSS was noted on day 10 between groups 303 and 202 /404, between groups 101 with 202, and between groups 101 and 303. A significant difference in TSS also existed on day 14 between groups 303 and 202. In spite of all the significant differences, it is difficult to conclude much from these data except that group 202 degrades with time.

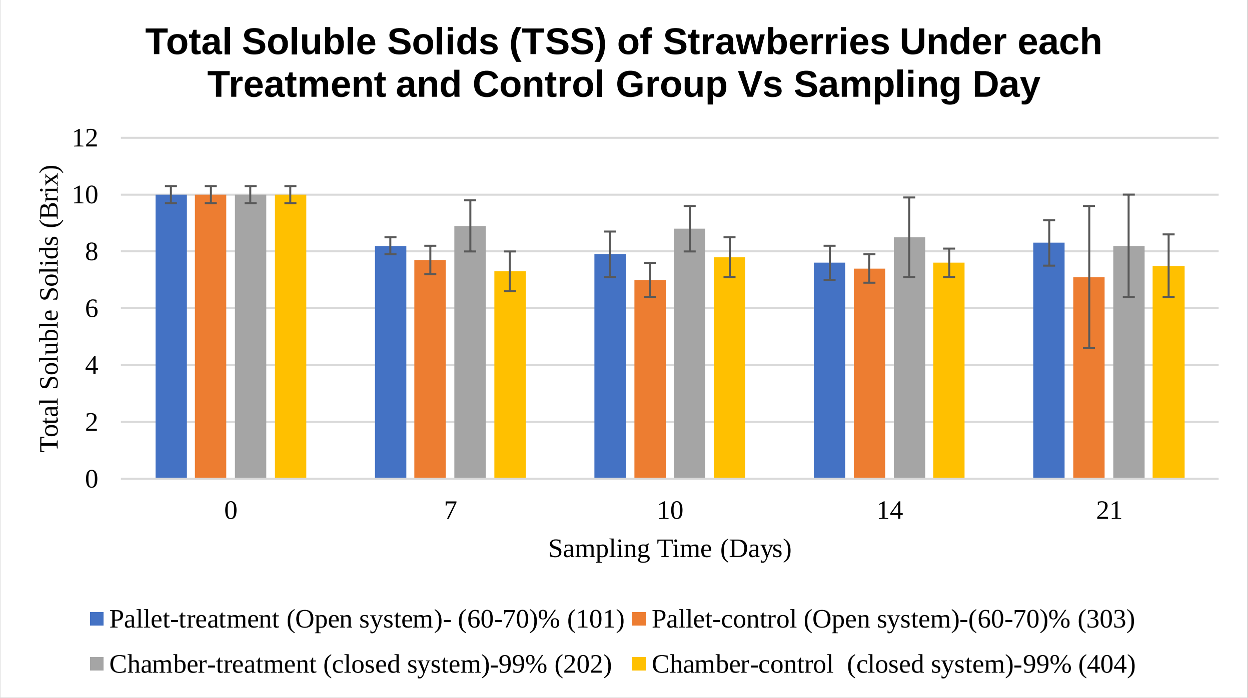


Figure 5.12.2: Comparison of TSS of Strawberries on each Sampling Day for each Sample Group

According to Figure 12.2, experimental groups were compared throughout the period of 21 days. All the group pairs gave a significant difference except (404-101 and 404-202) for TSS.

Maximum penetration force

Maximum penetration force, using texturometer was measured on the strawberries over the duration of the experiment. This can be seen in Figures 13.1 and 13.2. Figure 13.2 is provided because the error bars overlap in Figure 13.1. Penetration force showed significant differences with respect to sampling time and experimental group (treatment vs.

control), but the interaction between sampling time*experimental group did not show a significant difference ($p \leq 0.05$).

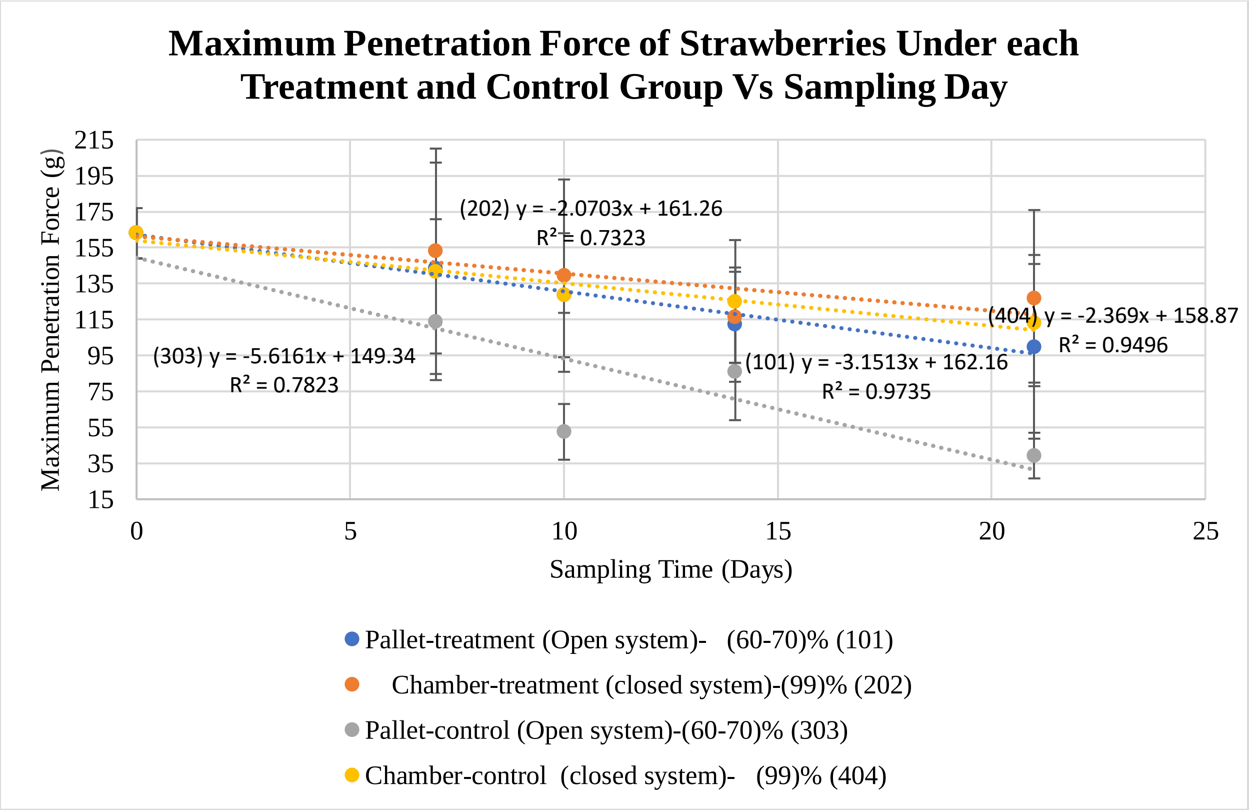


Figure 5.13.1: The Maximum Penetration Force of Strawberries on each Sampling Day for each Sample Group

According to Figure 13.1, all groups have a reasonably good fit for a linear trend over the 21 days. Group 303 was significantly different ($p \leq 0.05$) from all other groups at each sampling time except day 0. The other three (101, 202, and 404) groups did not show any significant difference ($p \leq 0.05$) between the three groups.

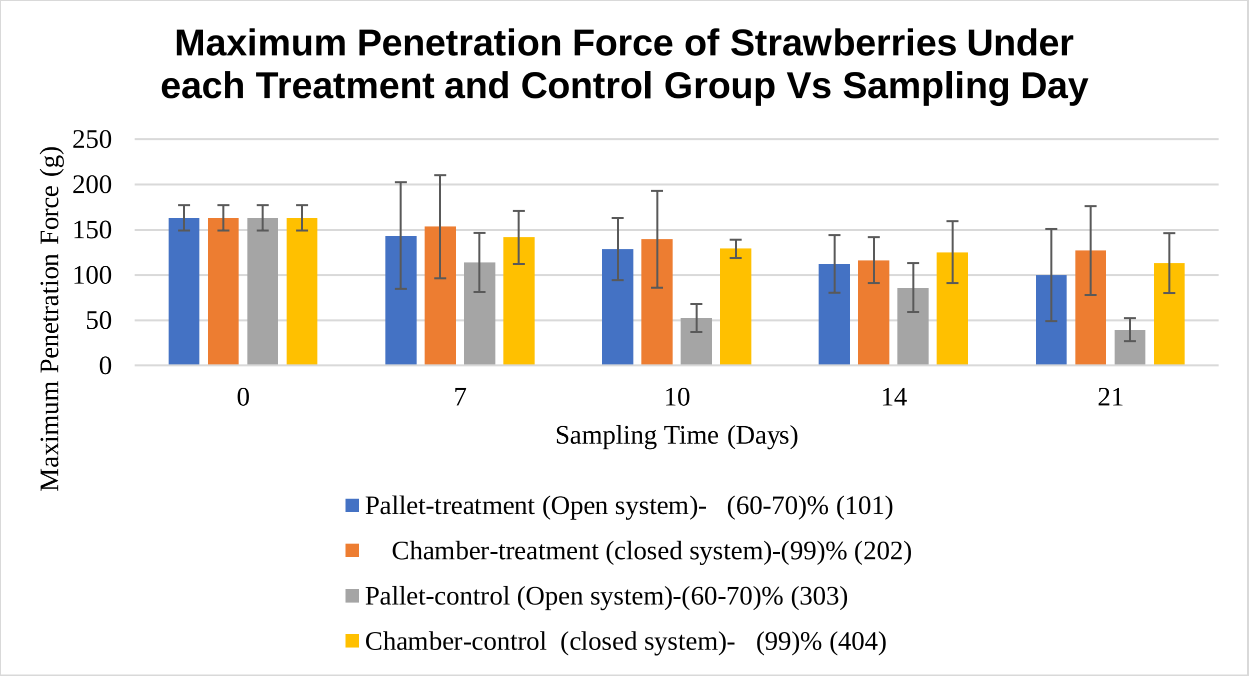


Figure 5.13.2: The Comparison of Maximum Penetration Force of Strawberries on each Sampling Day for each Sample Group

Sensory Analysis:

Color

The sensory panel rated color based on Table 5.1. The results are presented in Figure 14.1 and 14.2. The data showed significant differences ($p \leq 0.05$) with respect to sampling time, experimental group (treatment vs. control), and the interaction of sampling time*experimental group.

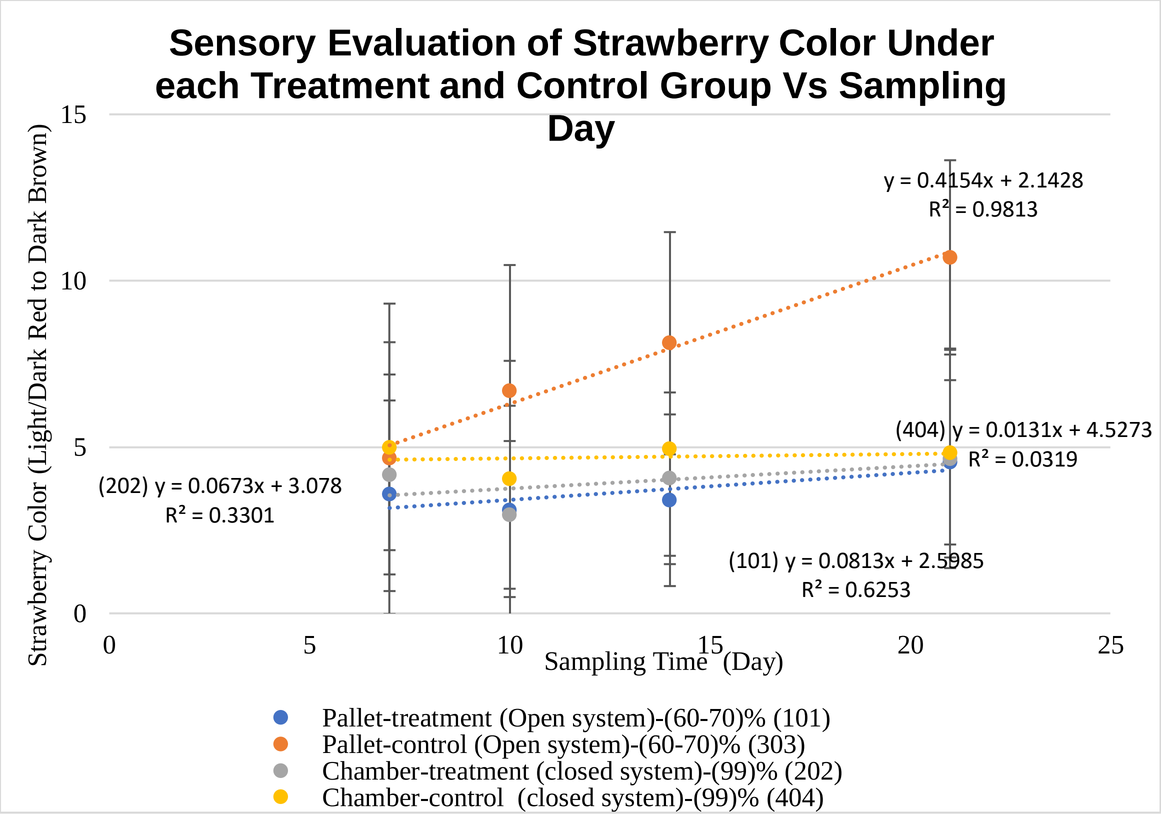


Figure 5.14.1: The Sensory Color Change of Strawberries (from red to brown) on each Sampling Day for each Sample Group

Only group 303 showed a good fit for a linear trend, showing that the panel members perceived degradation of color. None of the other groups showed a strong trend. According to the ANOVA completed on this parameter, there were no significant differences between the groups based upon color on day 7. On day 10, the panel perceived significant differences between group 303 and all of the other groups ($p \leq 0.05$). No other significant differences were seen on day 10. On days 14 and 21, group 303 was different from all other groups ($p \leq 0.05$) and the other groups were not significantly different from each other.

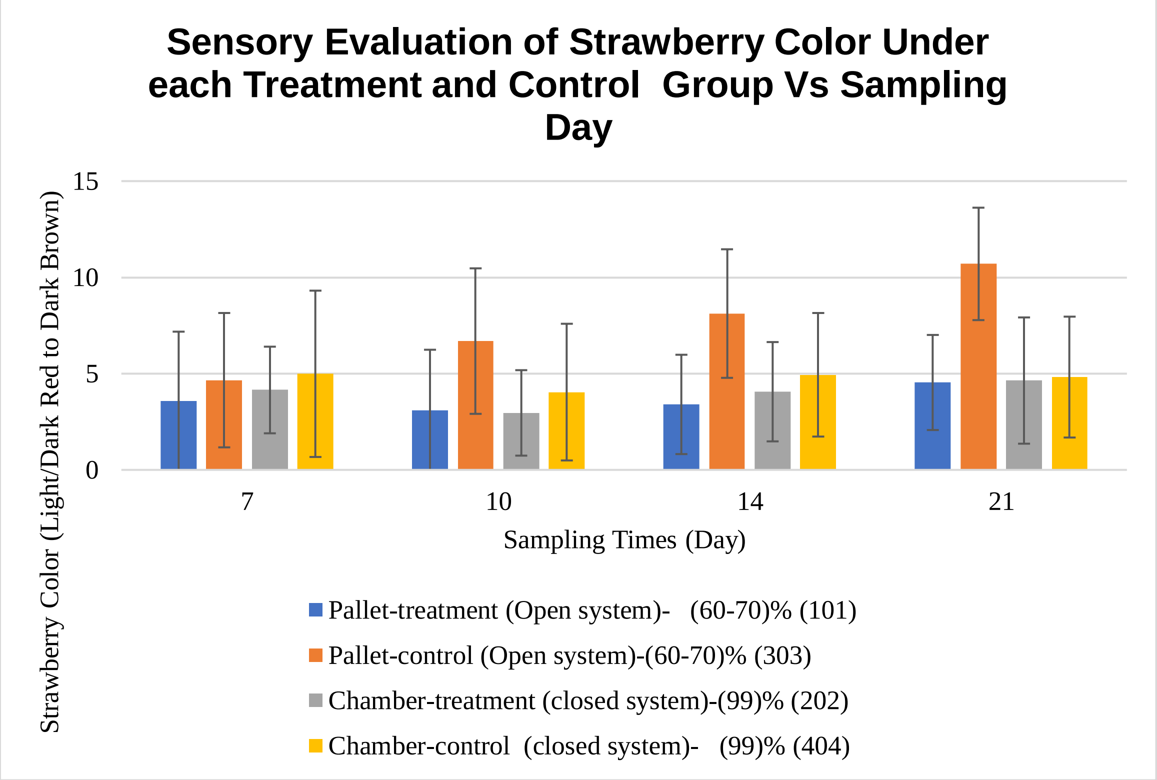


Figure 5.14.2: The Sensory Color Change of Strawberries (from red to brown) on each Sampling Day for each Sample Group

Aroma

The sensory panel rated the strawberries on aroma based upon Table 5.1. These data are presented in Figure 15. The aroma scores showed significant differences ($p \leq 0.05$) with respect to sampling time, experimental group (treatment vs. control), and the interaction of sampling time*experimental group.

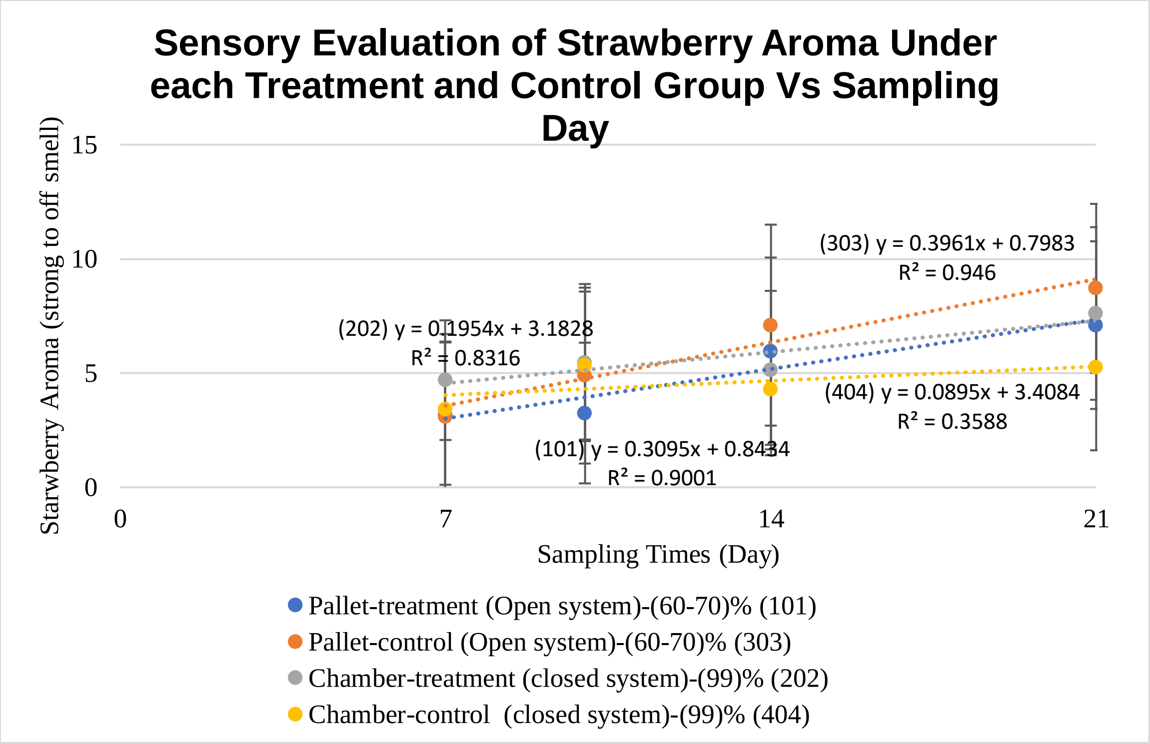


Figure 5.15.1: The Change of Aroma in Strawberries (from strong to off smell) on each Sampling Day for each Sample Group

Groups 101, 202, and 303 all show a reasonably good fit for a linear trend toward worsening aroma. Group 404 did not show a good fit. According to the ANOVA, no significant difference existed between any groups on day 7. Group 101 is significantly different from groups 202 / 404 on day 10. Group 303 showed a significant difference from group 404 on day 14. On day 21, a significant difference was observed between 202 and 404, as well as between 303 and 404.

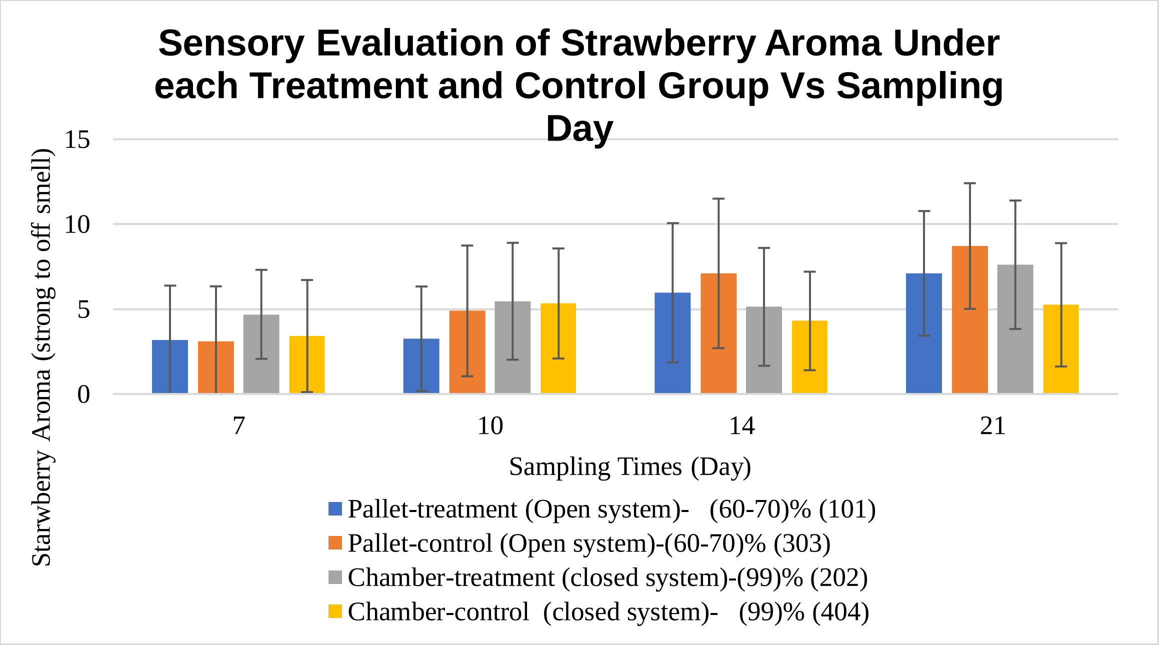


Figure 5.15.2: The Change of Aroma in Strawberries (from strong to off smell) on each Sampling Day for each Sample Group

Firmness

The sensory panel's ratings of firmness were evaluated based upon Table 5.1. The firmness data is shown in Figure 16.1 and 16.2. The firmness scores exhibited significant differences ($p \leq 0.05$) with respect to sampling time, experimental group (treatment vs. control), and the interaction of sampling time*experimental group.

The data in group 303 shows a good fit for a linear trend toward softening of the fruit. Other groups did not show a linear pattern or even did not show any considerable change.

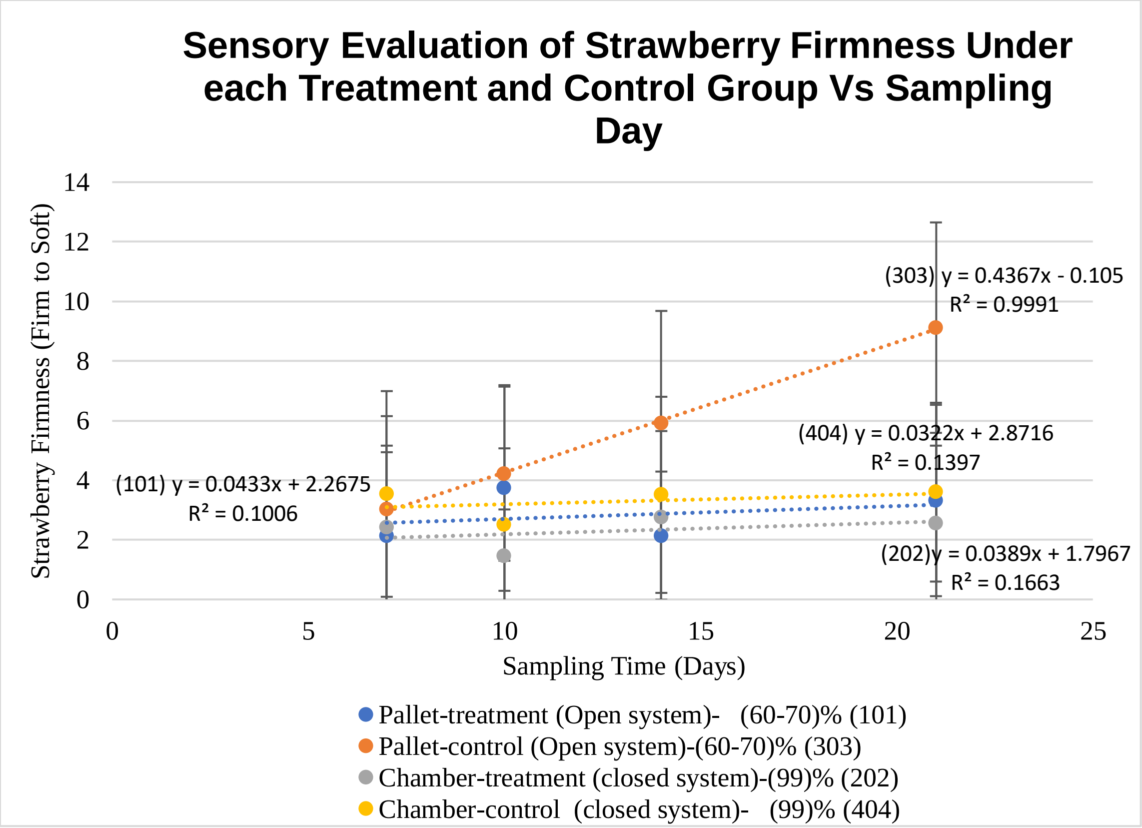


Figure 5.16.1: The Change of Firmness in Strawberries (from firm to soft) on each Sampling Day for each Sample Group

The ANOVA showed that no significant difference was seen on day 7 between any groups. Group 202 was only significantly different from groups 101 / 303 on sampling day 10. Group 303 showed significant differences from all the groups on days 14 and 21. The only significant differences between groups 303 and 404 were measured on day 10.

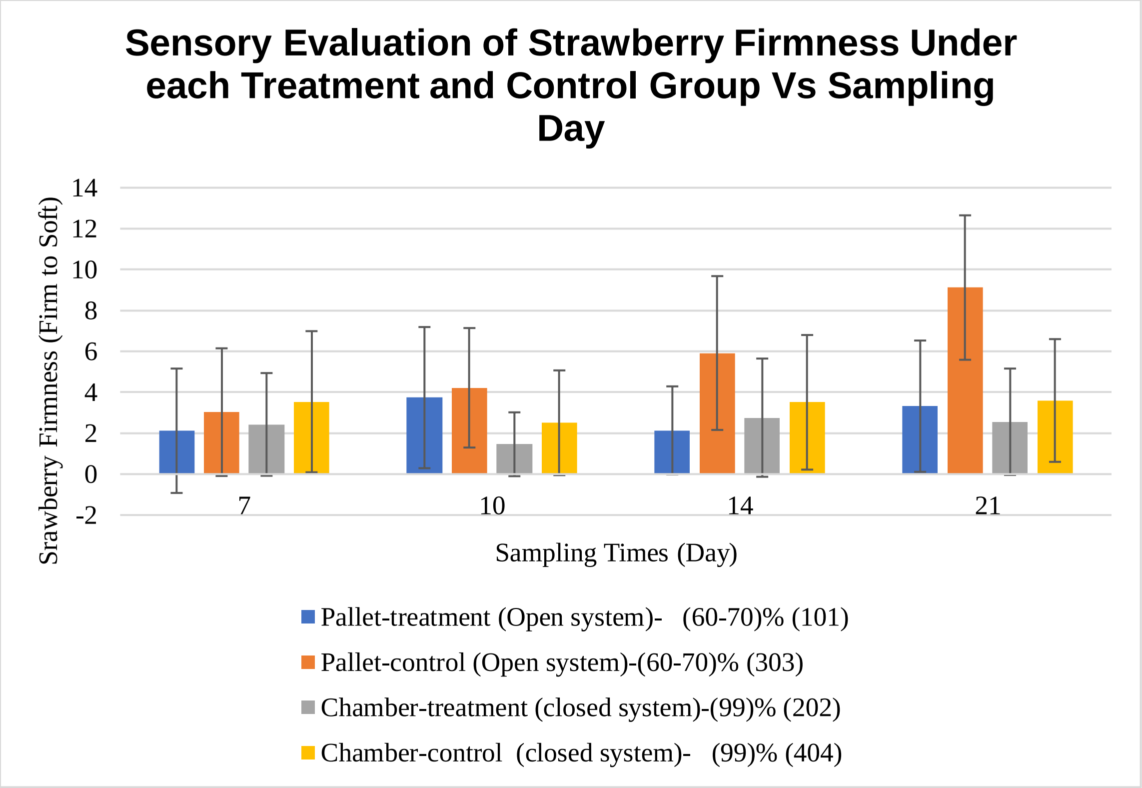


Figure 5.16.2: The Change of Firmness in Strawberries (from firm to soft) on each Sampling Day for each Sample Group

Intent to consume

Members of the sensory panel were asked to express their intent to consume the strawberries (overall acceptability without consuming) according to guidance in Table 5.1. Intent to consume scores showed significant differences ($p \leq 0.05$) with respect to sampling time, experimental group (treatment vs. control), and the interaction of sampling time*experimental group.

Except for group 404, all the groups displayed a reasonably good fit for linearity throughout the sampling period. Of the three that show a reasonably good fit, group 303 clearly shows the highest trend toward lower intent to consume.

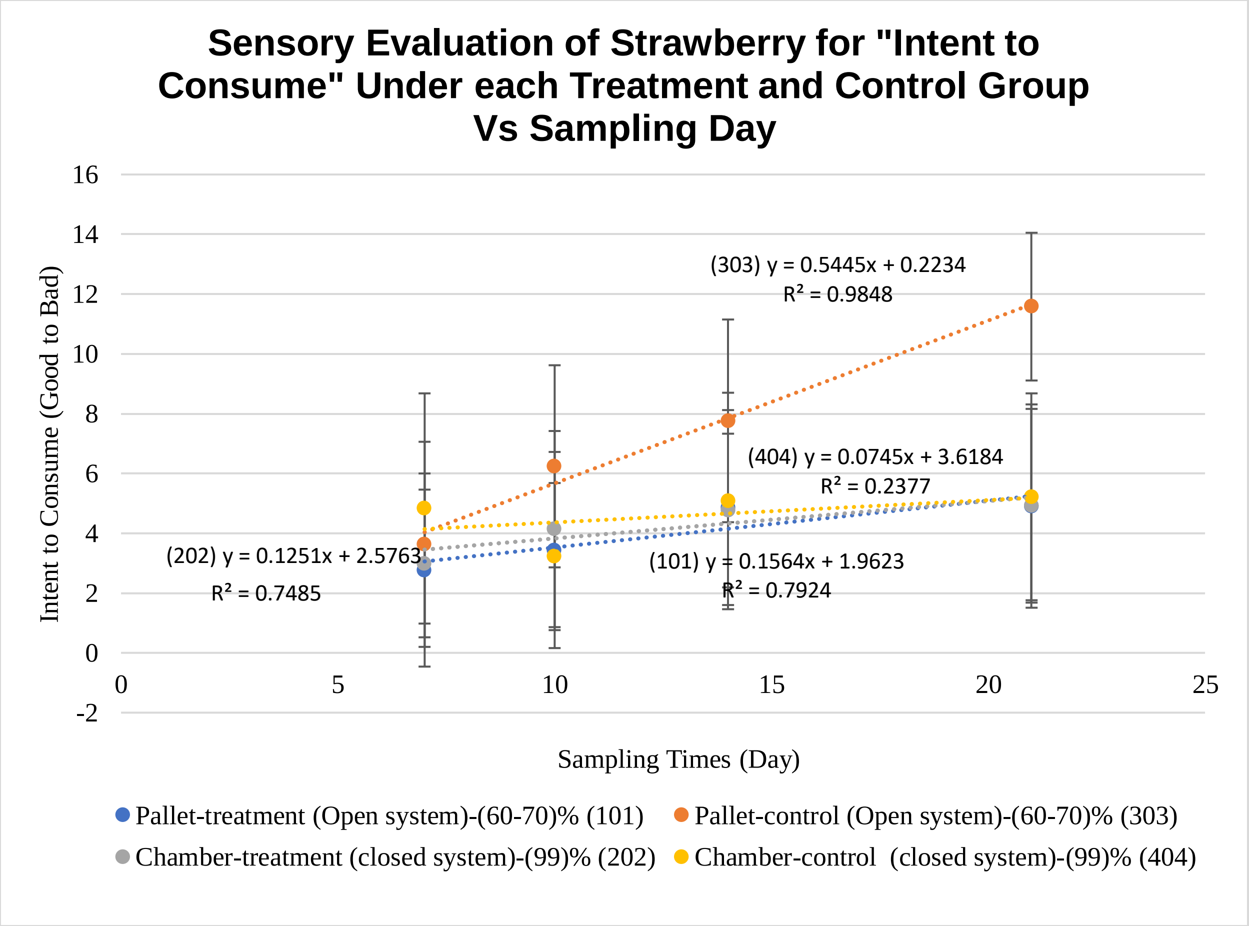


Figure 5.17.1: The Change of “Intent to Consume” in Strawberries (from good to bad) on each Sampling Day for each Sample Group

ANOVA showed that only group 303 was significantly different from all groups on days 10, 14, and day 21. However, group 303 did not show any significant difference on day 7. Only group 101 has a significant difference from group 404 on day 7.

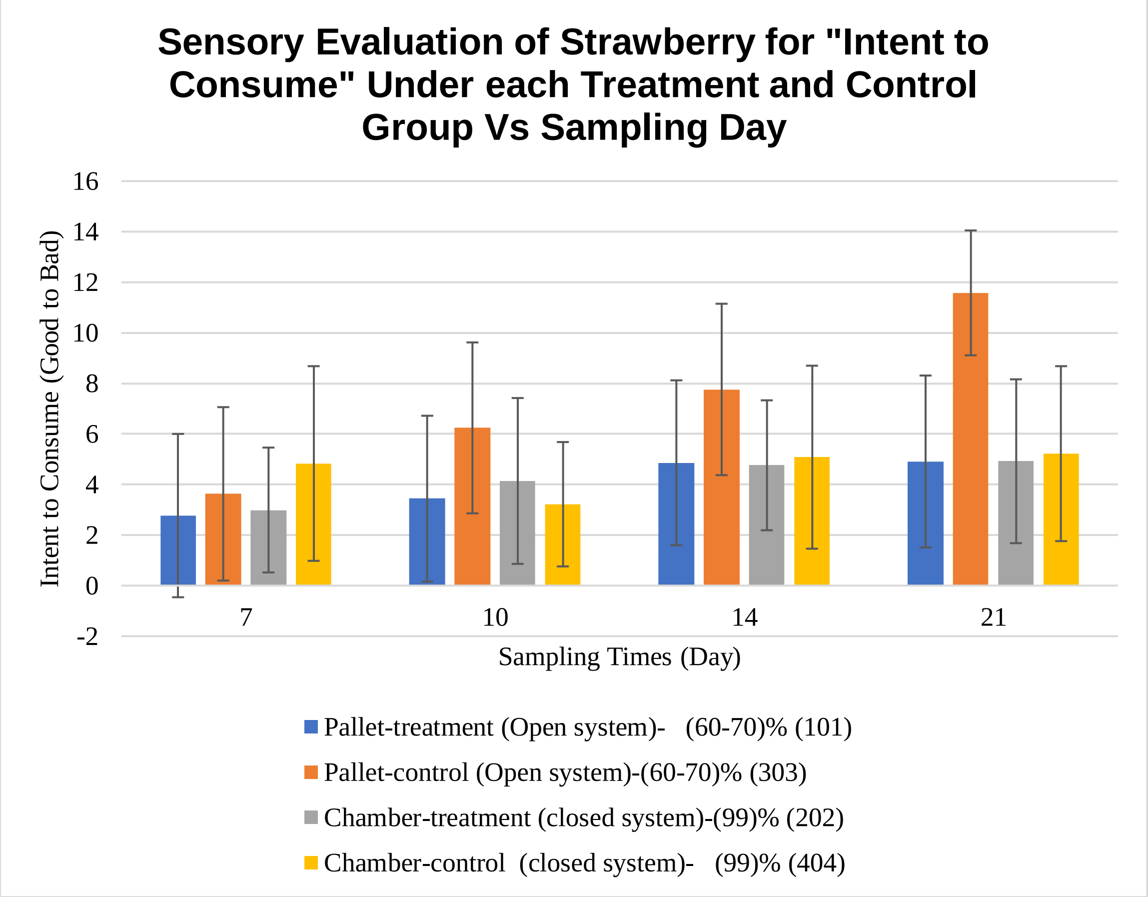


Figure 5.17.2: The Change of "Intent to Consume" in Strawberries (from good to bad) on each Sampling Day for each Sample Group

DISCUSSION

Generally, fresh strawberries have a short shelflife of 10-12 days and are susceptible to rapid weight loss, softening, bruising, and mold growth due to high water content and high metabolic activity (AMRC, 2019). This study demonstrated the objective of controlled release of ClO_2 gas sachets to enhance the shelflife of strawberries and to maintain the freshness of strawberries in an open pallet system under refrigerated conditions. One set of the experimental setup used was similar to industrial strawberry storage settings in the pallet system under refrigerated conditions (60-70%, 0 - 2 °C). The other set of condition was a close-air circulating chamber in a laboratory experimental setup that maintained the optimum RH (99%, 0 - 2 °C) for strawberries.

The finding of this research indicated that both optimum RH (99%) and ClO_2 gas effectively maintained the freshness of strawberries longer, depending on the quality parameter (color, firmness, etc.), when compared to control settings without optimum RH or ClO_2. Although the comparison of medium RH control and medium RH treatment (with ClO_2) shows a significant difference, it is possible that there are interactions between the available water in the atmosphere and the ClO_2. Therefore, it is most accurate to say that either the ClO_2 gas or the combined effect of ClO_2 gas with medium RH was shown to enhance the shelflife of fresh strawberries.

This was noted from a standpoint of quality (determined by instrumental analysis) and a standpoint of sensory perception (determined by sensory analysis). The results presented in the previous section will be discussed further in the sections below.

Instrumental Analysis

Weight loss %

According to Valero (2013), weight loss in fresh fruits like strawberries (which is the primary cause of quality deterioration) is mainly caused by transpiration and respiration (Valero et al., 2013). The main factor for quality deterioration and affecting the shelflife is weight loss. The weight of strawberries is primarily lost through the loss of water via surface stomata.

In this study, Figure 10.1, and 10.2 shows the weight loss of strawberries as a percentage of initial weight (D_0) at each storage period for 21 days under each experimental setup. As stated above, the four groups were: pallet system ClO_2 treatment group 60-70% RH-(101), pallet system control – no ClO_2 group 60-70% RH-(303), closed chamber system ClO_2 treatment group 99% RH-(202), closed chamber system control – no ClO_2 group 99% RH-(404). As expected, the weight of strawberries stored under each experimental setup gradually decreased during storage of 21 days. In other words, the weight loss % of strawberries in all experimental groups showed a continuous/gradual increase until 21 days of storage (Figures 10.1, and 10.2). However, the weight loss % of strawberries for 21 days in group 303 (the one that most closely duplicates the most common storage practice) is higher than in the other groups.

When considering the difference in weight loss % between sampling days, the increase of weight loss % was also higher in 303 compared to the other groups. For further explanation, this difference in weight loss % between each two sampling periods (D_7-D_{10}, D_{10}-D_{14}, D_{14}-D_{21}) of 303 is always higher than that of the previous sampling gap (D_{14}-D_{21} > D_{10}-D_{14} > D_7-D_{10}). The weight loss % of the last sampling period D_{14}-D_{21} was nearly double the weight loss % of the previous sampling period D_{10}-D_{14}. This indicates the maximum (the highest) weight loss was found after 14 days of normal strawberry storage/shelflife.

It can be seen from Figure 10.1 that the open pallet control system (303) is the worst performer, followed by the open pallet treatment (101). It can also be seen that the ClO_2 treatment dramatically reduces the weight loss in the pallet system since the weight loss trend (slope) is lower for pallet treatment 101. Weight loss in the 99% RH groups (chamber systems) (202 and 404) appears to show little changes, as would be expected.

When considering the weight loss % at the end of the entire storage period for 21 days, the maximum weight loss % was observed in the group of 303 (22.36%), followed by group 101 (11.97%). The minimum weight loss % at day 21 was found in chamber closed system 99% (treatment group)-202 (1.7%), followed by chamber closed system 99% (control group)-404 (3.04%).

Overall, the weight loss % of strawberries in the pallet experimental setup (both treatment and control groups) are significantly higher than in the chamber system (both treatment and control groups). This may be due to the rapid loss of moisture in the lower humidity refrigerated environment (groups 303 and 101) and the resultant loss of water (mass transfer) from strawberries. This is known as transpiration, which is known to be faster in a low humidity environment compared to the high humidity (99%) environment. Alternatively, some of this loss could be due to the higher respiration rate of strawberries in an open system than in a closed system. RH, ClO_2, or a combination of these factors was also seen to influence strawberry weight loss. The combination of relative humidity and the experimental setup - (open system or closed system) also played a significant effect on the weight loss % of strawberries. This is shown by the significantly higher weight loss % in both pallet systems compared to the chamber systems (with or without the effect of ClO_2). After 10 days, the weight loss % of treatment strawberries was significantly lower compared to the control. Similar results were observed (a significant decrease in weight loss % after 8-12 days compared to control) in the same conditions using ClO_2 pads on the top lids of clamshells (Chiabrando et al., 2018; Aday & Caner, 2011).

However, at medium (60-70) % RH, significantly higher weight loss (%) in the pallet treatment group (101) compared to the control (303) suggests that ClO_2 helps to reduce the weight loss (%) of strawberries. In the closed systems at 99% RH, the effect of ClO_2 on the weight loss % of strawberries (202) is not significantly different from that of the control (404). This suggests that ClO_2 is not the only factor that reduces the weight of strawberries in a closed system, and RH can also help in the reduction of weight loss of strawberries.

Literature provides evidence that stomate activity at the strawberry surface directly influences the water loss of strawberries (Wang et al., 2014; Valero et al., 2013). Valero (2013) studied the effect of ClO_2 treatment on stomata opening and closing (Valero et al., 2013). Valero found that 50% of stomata were closed in treated (ClO_2) strawberries, whereas all stomata were open in control strawberries after 7 days at 6 ^{0}C. Their research found a 6.38% weight loss after 10 days of storage at 1 ^{0}C. This is similar to our data, where weight loss was 6.92% under the same conditions (0-2 ^{0}C) on day 10, even though their treatment utilized ClO_2 pads inside each clamshell.

Firmness

The texture of strawberries was described and analyzed in terms of firmness (maximum penetration force) in the instrumental analysis. The firmness of strawberries was used to describe and measure the mechanical properties of fruit measured as (g or N) (Gunness et al., 2009). The firmness of strawberries in each experimental group decreased throughout the test period, which can be expected, given the weight loss data (Figure 13.2). The primary factor for the resultant lower firmness of strawberries is water loss % (6). As a result, the loss of water in strawberries caused a decline in firmness (Wang et al., 2014). A significant difference between the pallet control group (303) and all other groups for the firmness can be expected because the other groups were tested under at least one favorable condition (ClO_2, 99% RH) for strawberry firmness.

The graphs from the measured texture (Figures 13.1 and 13.2) were visually compared to the graphs from the sensory firmness data. It can be seen by comparing these graphs that there are similarities. For instance, Figure 13.1 shows a loss of the puncture force for group 303 over time, and the sensory panel found a loss of firmness in group 303 over the same time period.

Similar to the weight loss % of strawberries, the firmness of strawberries was significantly influenced by the combined effect of ClO_2 gas and relative humidity (Figure 13.2). However, ClO_2 has a higher effect on preserving the firmness of strawberries at medium relative humidity used (60-70%) in open pallet systems than that of the high relative humidity (99%) used in chamber closed systems. ClO_2 has a higher effect on reducing water loss (weight loss) and preserving strawberry firmness at medium RH (when the absence of a 99% factor), although at 99% RH, the firmness difference is not significantly different (Figure 13.2). After day 10 (which is the expected shelflife of normal strawberry storage), the firmness of group 303 significantly dropped.

TSS

Total soluble solids content is a measurable parameter that can predict the flavor/sweetness of strawberries. The recommended TSS for the best strawberry flavor is 7% (Agricultural Research Service, 2016). With the exception of group 202, TSS did not exhibit strong linearity with time within each group. Also, ANOVA showed significant differences between 303 and other groups on some sampling days but not consistently, and differences between other groups showed fluctuating outcomes. This could be due to the wide range of variability of strawberries. The TSS test is destructive, so it is not possible to measure the TSS of the same strawberry throughout the sampling period (21 days).

The interesting observation was the lack of significant differences in TSS between the treatment and control groups. This may be because ClO_2 did not affect the reduction or increase of TSS (flavor profile-including sweetness). Other researchers also found no significant differences in TSS between treatment and control groups where strawberries were packaged in a perforated clamshell with chlorine dioxide pads (Chiabrando et al., 2018; Wang et al., 2014).

Color

Color is a very important parameter to determine the quality and freshness of strawberries because many consumers judge the quality of strawberries primarily by visual appearance, not by the dates provided on the labels (Gunness et al., 2009). In this study, many fluctuations of color values (L*, a*, b*) in each group on each sampling time were observed. Significant differences of color in some groups only on a few sampling times may be due to wide variabilities among strawberries (from one strawberry to another and even within one strawberry). Although the red color of strawberries (a*) did not show much change throughout the sampling time and even between experimental groups, group 202 has a good positive trend for the fresh red color of strawberries. Although group 202 has an increasing trend of a* value, one cannot conclude that the freshness of strawberries remained only within this group. Because practically, this is not the only group that maintained the freshness of strawberries. A significant difference ($p \leq 0.05$) in color a* on day 14 existed between the groups 303 and 202 and also between 101 and all 3 other groups. No significant change in a* values in the chamber treatment group (202) at the end of the sampling period was observed. A higher a* (or no significant change of a*) is expected for color preservation for the shelflife extension of strawberries. This decreased pattern of a* value graph for group 303, was similar to some of the other instrumental data-firmness for group 303, which showed very low shelflife by sensory quality parameters (firmness, color, overall acceptability, etc.), with more similar sensory evaluation data. (303 showed a reduction of red (turned to brown) color faster compared to the other groups in both instrumental and sensory data).

Chiabrando (2018) studied a "short storage" scenario (in which strawberries were stored for 3 days at 4 ºC, then 2 days at 20 ºC) and in a "long distribution" scenario (in which the berries were held at 2 ºC for 12 days). In the short storage condition, no significant differences in color existed until after day 3, and the chlorine dioxide treated groups were lighter (higher L* values were observed due to the bleaching effect). For the long storage, a* decreased significantly during storage; however, no significant differences were observed as a result of the treatment (Chiabrando et al., 2018). Another study investigating strawberries packaged with chlorine dioxide-releasing sachets showed that during storage, the strawberries became darker over time (fresh color deterioration due to shrivel following weight loss), and ClO_2 gas treatment did not result in a significant change in color (Wang et al., 2014). However, Wang (2014) also observed that a large variability exists between the individual strawberries. Wang 2014 further supported our inconclusive data, and therefore, no conclusion was made to determine the shelflife of strawberries based on color.

Delta E of strawberries significantly changed throughout the storage time in all groups. Delta E has a similar trend with other colors (b* and L*). Delta E of strawberries in each group did not significantly differ between each group except pallet treatment group between all other groups on day 7. With all color values, the freshness of strawberries was better explained by red color “a”.

Sensory Analysis-

The literature suggests the use of descriptors such as aroma, sweetness, firmness, and juiciness as significant quality attributes to describe the overall quality of strawberries (Han et al., 2005). In our study, all these descriptors were used for the sensory panel except sweetness. The researchers did not use sweetness because verification of residual ClO_2 was not part of the experimental design.

Color

The characteristic color of strawberries is due to anthocyanins which are a class of polyphenolic pigments (Buendia et al., 2010). The red color degradation of strawberries is mainly due to the loss of water-soluble anthocyanin pigments via water loss and the increased respiration rate-enzymatic process (Dervisi, P., Lamb, J., & Zabetakis, I. (2001).

The color of strawberries is one of the most important sensory and quality factors that determine the freshness of strawberries for consumer preferences.

The fresh color of strawberries (red) in pallet-control (60-70) % (303) gradually reduced and turned into dark brown after 10 days (Figure 14.1, 14.2, and Table 5.1). If one considered only sensory color to determine the shelflife of strawberries, the shelflife of strawberries in the pallet-control (60-70) % (303) group was for 10 days, whereas all the other groups were >21 days (sensory score >6, Table 5.1). All other groups had good color (shelflife) maintenance until day 21.

However, the dosage of ClO_2 over time should be well controlled to avoid the bleaching effect, especially in chamber systems. In a preliminary study conducted while designing this experiment, bleaching was observed in the chamber-stored strawberries in the clamshells nearest to the ClO_2 sachets. The chlorine dioxide dosage is one of the major factors for strawberry discoloration (Arango et al., 2016). For the final experiment, the correct ratio of the chemicals compared to the preliminary experiment was mixed in the sachet to treat the amount of strawberries in each experimental setup. The dosage received by any given berry in the packaging system is dependent on 1) The release rate of the sachet, 2) the reaction rate of chlorine dioxide and the strawberry surface, 3) the surface area encountered before reaching the substrate, 4) distance from the source, and 5) exposure time (Kessler, 2020).

Both the treatment and control group at 99% RH appeared to preserve the color of strawberries. Therefore, high RH (99%) alone also has significant preservation of strawberry color to enhance the shelf-life from a sensory panel standpoint. This may be due to the preservation of water content at high RH to maintain the overall appearance (color, shininess, shrinking of skin, etc.) of berries. Control and treatment showed no real difference in the sensory perception of strawberry color at the high RH (99%) value.

The literature sensory data on color profile showed no significant difference in color change early in the shelflife (Popa et al., 2007). However, after 8-12 days of storage, whitening (bleaching) of the skin was noted on treated berries. This can be expected due to longer exposure to ClO_2 or higher dose of ClO_2 (Popa et al., 2007). A random few whitening samples were found in our experiment, although this was limited because of learnings from the pilot experiment.

Aroma

Consumers' attraction to strawberries is mainly with the surface color and aroma. Therefore, the aroma is one of the consumer preferences for purchasing strawberries. The strawberry aroma is a result of a complex mixture of aromatic components (esters, alcohols, and carbonyl compounds) and the interactions between those constituents. Thus, the nature of strawberry aroma is still poorly understood (Azodanlou, R. (2001). In past sensory studies, sweetness and aroma were considered significant factors to determine the overall appreciation of strawberries (Azodanlou, R. (2001).

In this experiment, ClO_2 did not positively or negatively affect the aroma of fresh strawberries, nor did the panel report detection of ClO_2 odor. The expectation was that higher (worse) aroma levels might be found in control groups compared to treatment groups. However, the results were mixed. This may be due to the high standard deviations (relative to the average scores for the aroma test, which in turn could be due to variability of fruits used or variability of the panel members since they were not a trained sensory panel. However, this sensory panel reflects/represents a normal consumer panel. After day 14 (On days 14 and 21), the pallet-control (303) showed the decay of aroma smell compared to the chamber-control (99%) (404).

The fresh strawberry aroma was maintained until day 7 without any treatment or RH under refrigerated conditions, and ClO_2 did not appear to affect on reduction/replacement of aroma smell by ClO_2 odor. A sensory study for microbiological safety and quality of blueberries also agreed with our findings of "no significant changes of aroma and overall quality during short storage of berries", however, a significant decrease in color and aroma was observed in long-term storage after 7-10 days (Popa et al., 2007). The chamber-control (99) % (404) showed good retention of aroma level compared to the other groups for 21 days, (except on day 10, which could be due to the above-mentioned variabilities in panel perspective and fruit), Sensory data for aroma revealed that "aroma" is not a good sensory profile to determine the shelflife of strawberries or to determine the freshness of strawberries.

This study also suggests that aroma may not be the best sensory parameter to determine the quality/shelflife of strawberries due to the wide range of variabilities in strawberries. There is a good correlation between strawberry flavor and aroma which always has an uptrend (Wang et al., 2014). When the flavor is good aroma is always good. Our findings were aligned with the literature about strawberry flavor profile being unaffected by ClO_2 treatment (Wang et al., 2014).

Aroma scores showed a lot of variability and fluctuation over time. Overall, the shelflife/freshness of strawberries using aroma profile in all groups except group 404 was for 14 days (sensory score >6, Table 5.1).

Firmness

The softening (deterioration of firmness) of strawberries is a primary factor to determine the quality of strawberries. Strawberry softening occurs due to biochemical reactions in the presence of enzymes (pectin enzymes, polygalacturonase, pectin methylesterase) in the cell and also due to loss of water (Wang et al., 2014; Velickova et al., 2013).

After day 7, the softness of strawberries in group 303 changed dramatically, with a significant increase for day 21 compared to other groups (The firmness of the pallet-control (303) fell toward the end of shelflife (day 14-21). All other groups did not show any pattern (although there were many fluctuations) or even a significant increase or decrease throughout the test of 21 days. Both ClO_2 and high RH positively affected the freshness of strawberries to preserve the firmness throughout the test. The significant difference between groups 202 and 404 (the increase of softness in group 404 compared to 202) could be due to the combined effect of both ClO_2 and high RH. However, all these groups except group 303 graphically did not show a significant difference (after day 14) between each group (Figure 16.1 and 16.2). This group 303 was significantly and also practically different from all other groups at the end of shelflife. Considering sensory and instrumental data for the firmness of strawberries for 21 days, the firmness of strawberries is a reasonable factor to determine the shelflife of strawberries.

For the shelflife estimation of strawberries using the sensory parameter-firmness, the shelflife of strawberries in the pallet-control (60-70) % (303) group was for 14 days, whereas all the other groups were >21 days (sensory score >6, Table 5.1). According to the sensory panel, all the groups except group 303 maintained good firmness until day 21.

Intent to consume

"Intent to consume" is also called "overall acceptability" or "overall appreciation," It is intended to cause the panelist to consider all of the above parameters/descriptors that have been evaluated and, from these, to determine the panelist's willingness to consume the strawberry.

The low level of the panelists' "Intent to consume" of the pallet-control (303) is "likely" (Table 5.1) due to lower scores from the panel on all the other sensory parameters of 303.

For the determination of shelflife of strawberries using the sensory parameter-intent to consume, the shelflife of strawberries in the pallet-control (303) group showed the end of shelflife starts at day 10. All the other groups showed good consumer acceptability for strawberries at the end of the 21 day test (sensory score >6, Table 5.1). The sensory panel was willing to consume strawberries from all groups for days 7-10. After this, the panel was willing to consume strawberries only from groups 101, 202, and 404 throughout the test. However, the preference gradually decreased throughout the end of day 21.

Ramin (2001) demonstrated that the overall appreciation, on the other hand, led to the conclusion that the two attributes "sweetness" and "aroma" are the determinant of the quality of strawberries (Azodanlou, 2001). In the current study, other parameters such as color, firmness were found to be related to intent to consume. The sensory panel instructions guided panelists to use all available parameters to determine intent to consume.

Implications and Consequences

This is the first study published about the use of ClO_2 gas-producing sachets to treat strawberries in a pallet system (placing the sachets outside of the clamshells). Studies are available on the use of ClO_2 gas-producing sachets inside strawberry clamshells. In the absence of one significant factor for strawberry shelflife (i.e., 99% RH), ClO_2 improves some shelf-life parameters at a medium RH. Therefore, if one cannot maintain very high RH at refrigerated temperature (0-2 ^{0}C), ClO_2 gas treatment using a sachet appears to be a viable approach to improve the shelflife of strawberries under such conditions in a pallet system. At 99% RH, (control group-202 and treatment group-404), the effect of ClO_2 did not make a significant difference in preserving strawberry quality (with the exception of sensory panel aroma on day 21). Both of these groups (202, and 404) maintained good strawberry quality parameters for enhancing shelflife. Moreover, we cannot compare two different systems, pallet (open) and chamber (closed) systems, which may not retain the same level of ClO_2 concentration throughout the test period.

LIMITATIONS

All fresh strawberry samples were purchased from the same store and were of the same variety. It was very difficult to get the samples with the exact same sample quality due to the nature of such fruits. Strawberries have a lot of variation in quality parameters, as well as in size and shape. The researchers randomly selected similar size, maturity, and quality samples for each sampling day for each test. These quality variables for each parameter have been considered in the data analysis. In spite of these efforts, the variability of the fruit is reflected in the large standard deviations seen in some of the sensory descriptors between samples/replications.

The same strawberry samples were used for both sensory and instrumental tests to reduce this variability between similar instrumental and sensory panel tests (e.g., sensory firmness and penetration force) on each sampling day. In the future, it might be helpful if someone does color tests using more advanced methods such as a “digital Nikon camera and copy lighting units” system that takes pictures and analyzes L*, a*, and b* values with MATLAB software for the same strawberry samples on each sampling day for each experimental group. For this, a slightly different sampling method should be used in contrast to this study. In this study, the strawberries observed by the panel were also used for all the instrumental tests, thus meaning the test was “destructive”. In conjunction with this improved camera would be to test the same strawberries (and points on the strawberries) during each sampling period. This is only applicable to color tests. The same strawberries cannot be used for other instrumental tests, which are destructive.

Also, hand-held Near-infrared spectroscopy (NIRS), which is a non-destructive test to determine the TSS of strawberries can be used for the verification of the data measured with a refractometer.

Temperature fluctuations could have occurred during the experimental handling of the strawberries. While moving strawberries from the grocery store to the laboratory cool room, the berries spent some time at room temperature. Subsequently, strawberries that had been damaged when shipping to the grocery store were removed from the testing pool.

This removal of damaged strawberries occurred at room temperature and could have also led to minor temperature abuse. Since all groups were treated the same way, the temperature abuse may not have affected the outcome of the experiment.

Water condensation occurs at a high relative humidity of 99% in the closed chamber systems on the surfaces of the chamber. In the preliminary experiment, mold growth was observed faster even though there was good quality retention of strawberries in chamber systems. In the final experiment, the water condensation on the surfaces was dried with tissues on each sampling day to prevent mold formation on the external surfaces of the clamshell.

Damaged/bad strawberries had to be removed and some wet containers had to be dried before storing them in each experimental group on the first day (D_0). This was done because the preliminary study showed a dramatic deterioration of the quality of damaged strawberries compared to the undamaged berries and faster mold growth that spread to good berries.

The quality of strawberries is also determined by flavor level since strawberries are rich in phytonutrients. However, this study did not measure any flavor profile. The flavor was not used as a sensory parameter due to ClO_2 treatment. Since we did not measure the residual level of strawberries, and for the safety of the sensory panel, the flavor profile was not counted in this study. However, the literature showed evidence that flavor was considered a factor to determine the sensory quality of treated strawberries. A preliminary study with a non-trained panel revealed no significant differences between treated and control strawberries on flavor profile (6).

FUTURE SUGGESTIONS:

- One can change the significant factors that affect the shelflife of strawberries, such as temperature (different temperatures including temperature abuse conditions), RH (low RH's <50%), and ClO_2 dose (medium 1-5 ppm, high >5ppm), as factors for strawberry shelflife in these two systems (one industrial pallet setting and another experimental chamber setting).
- In future research, someone could experiment with Controlled Atmosphere Packaging for strawberries in the pallet system, wrapping them with stretch hood materials to avoid air coming and out of the system in a more expanded experimental space like a cool room.

CONCLUSIONS

- High RH (99%) played a significant role in preserving the sensory quality of strawberries.
- ClO_2 gas at medium-high RH (60-70%) also played a significant role to preserve the sensory quality of strawberries.
- The firmness of strawberries by sensory evaluation (by lightly pressing) and instrumental evaluation (puncture) is a reasonable factor in determining the shelflife of strawberries.
- The Control pallet system at medium RH (60-70%) is the normal/standard retail display of strawberries with a minimum shelflife of 10 days, considering all the sensory parameters and instrumental data except the color (to some extent agreed with the low value of color a*).

- High RH (99%) in a closed chamber system (with or without ClO_2 treatment) is the best way to preserve strawberry quality; however, the method is not practically achievable in farm and retail settings.
- Under these two experimental setups for the shelflife of strawberries, the best practical improvement in a pallet system open system is ClO_2 treatment at medium-high (60-70%) RH, although the best practically achievable levels of RHs in a cold room/refrigerated condition need to have experimented with.
- In the pallet system, the effect of ClO_2 treatment for short storage periods (<10 days) is inconclusive with sensory and instrumental data compared to the control. Because the freshness of strawberries was good in both the treatment and control groups due to no significant difference and practical differences were observed between the control and treatment groups before day 10. However, for longer storage periods (>10 days), it appears to preserve strawberry quality (since a significant difference in all the parameters was observed after day 14 compared to the control).
- The aroma and TSS of strawberries concluded no relationship with ClO_2 or combined effect with RH. Therefore, the aroma, TSS, may not be a factor affected by ClO_2.

ACKNOWLEDGEMENT

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CHAPTER SIX

CONCLUSIONS AND FUTURE RECOMMENDATIONS

Based on the findings of this research following conclusions and future recommendations are suggested.

CONCLUSIONS

- High RH (99%) with refrigerated temperature (0-2 ^{0}C) is a significant factor ($p \leq 0.05$) in preserving the freshness and quality (weight loss %, firmness/texture, color) of strawberries.
- ClO_2 gas played a significant role ($p \leq 0.05$) to preserve the freshness and quality of strawberries at medium-high RH (60-70%).
- The firmness is a significant factor ($p \leq 0.05$) to determine the quality of strawberries and it is a reasonable factor in determining the shelflife of strawberries.
- According to the sensory panel, strawberries of the control pallet system without ClO_2 at medium RH (60-70%) have a shelflife of 10 days (except for the color measured by the colorimeter), which is the standard retail display of strawberries with a maximum shelflife of 10 days.
- High RH (99%) in a closed chamber system (with or without ClO_2 treatment) is the best way to preserve strawberry quality; however, the method is not practically achievable in farm and retail settings.
- Under these two experimental setups for the shelflife of strawberries, the best practical improvement in a pallet system-open system is ClO_2 treatment (at

medium-high (60-70%) RH), although the best practically achievable levels of RHs in a refrigerated condition (0-2 ^{0}C) need to have experimented with.

- In the pallet system, the effect of ClO_2 treatment for short storage periods (<10 days) is inconclusive with sensory and instrumental data compared to the control. Because the freshness of strawberries was good in both the treatment and control groups with no significant difference ($p \leq 0.05$) and practical differences between the control and treatment groups before day 10.
- In the pallet system, the effect of ClO_2 treatment for longer storage periods (>10 days), significantly ($p \leq 0.05$) preserved the strawberry quality (all the measured parameters) after day 14 compared to the control without ClO_2 treatment.
- The aroma and TSS of strawberries concluded no significant relationship with ClO_2 or combined effect with ClO_2 and RH. Therefore, the aroma, TSS, may not be a factor affected by ClO_2.
- In the absence of one significant factor for strawberry shelflife (i.e., 99% RH), ClO_2 improves some shelf-life parameters at a medium RH. Therefore, if one cannot maintain very high RH at refrigerated temperature (0-2 ^{0}C), ClO_2 gas treatment using a sachet appears to be a viable approach to improve the shelflife of strawberries under such conditions in a pallet system.
- At 99% RH, (control group-202 and treatment group-404), the effect of ClO_2 did not make a significant difference in preserving strawberry quality (except for sensory panel aroma on day 21). Both groups (202, and 404) maintained good strawberry quality parameters for enhancing shelflife. Moreover, we cannot

compare two different systems, pallet (open) and chamber (closed) systems, which may not retain the same level of ClO_2 concentration throughout the test period.

- The impact strength of nylon-6, PE1 and PE2 are significantly different in each material which is a unique film property.
- The combined effect of ClO_2 and high RH (99%) has a significant effect on the impact strength of nylon-6 that may cause changes in the polarity. RH alone did not significantly affect the impact strength of nylon-6. The use of ClO_2 gas at high RH in nylon-6 packaging products should be a concern from a mechanical standpoint.
- The decreasing pattern of impact strength of treated nylon-6 at all relative humidities (RH) during long term ClO_2 exposure (until day 9) may be a result of chemical changes (oxidative degradation) of the polymer.
- The impact strength of PE1 and PE2 was not affected by relative humidity or ClO_2 gas treatment over 21 days. This means that PE could be a good material that can be used as a food packaging material under a low level of ClO_2 gas treatment without any mechanical impact on the product.
- No significant changes of TS occurred on any materials at this low concentration of ClO_2 gas.
- The material direction was a significant factor only for TS at break of MD of nylon-6 at 99% with ClO_2 gas treatment. Therefore, selecting nylon-6 as an antimicrobial packaging with ClO_2 gas at high relative humidity (99%) packaging is a concern from a tensile strength standpoint. However, the change of TS at break of nylon-6 in both directions (MD and CD), should be a factor for further consideration in

experimenting to determine how nylon-6 is affected at different doses of ClO_2 gas under different levels of RHs.

- When selecting a packaging material for a particular food with the appropriate ClO_2 gas concentration, loss of barrier properties should be a concern. APET films showed a significant decrease in barrier properties to water vapor (or increases in permeation) after being exposed ClO_2 gas. This is an important concern in food packaging. This is an important concern in food packaging with the type of application (when the WVP is a significant factor to determine the quality of the product in a hermetically sealed package).
- PE under low ClO_2 gas treatment is a good material to use in food packaging applications where the moisture barrier is a concern.
- Nylon-6 material is not a suitable material for moisture barrier standpoint where the moisture barrier is a concern in the product packaging system.
- Nylon-6 material, while questionable from a strength standpoint, could be a suitable packaging material from an O_2 barrier standpoint when ClO_2 treatment is used and in cases where the barrier is a concern in the product packaging system.
- There was no significant change in T_m of any of the materials used. This suggests that the formation of functional groups or other changes in the structure of polymers did not occur or occurred to an insignificant degree with the low level of ClO_2 treatment used.

FUTURE RECOMMENDATIONS

- In the future, it might be helpful if someone does color tests using more advanced methods such as a “digital Nikon camera and copy lighting units” system that takes pictures and analyzes L*, a*, and b* values with MATLAB software for the same strawberry samples on each sampling day for each experimental group. For this, a slightly different sampling method should be used in contrast to this study. In this study, the strawberries used for puncture testing were also used for color, thus meaning the test was “destructive”. In conjunction with this improved camera would be to test the same strawberries (and points on the strawberries) during each sampling period. This is only applicable to color tests. However, the same strawberries cannot be used for other instrumental tests (which are destructive) and sensory tests for the panel to avoid undesired variability.
- Temperature abuse could have occurred during the experimental handling of the strawberries. While moving strawberries from the grocery store to the laboratory cool room, the berries spent some time at room temperature. It is estimated that this was 10 minutes of temperature abuse. Subsequently, strawberries that had been damaged when shipping to the grocery store were removed from the testing pool. This removal of damaged strawberries occurred at room temperature and could have also led to temperature abuse. (The time for this step was limited by removing only one clamshell at once from the storage.) Recommendation: A future researcher could arrange for berries to be directly shipped/delivered to the laboratory from the farm in a refrigerated truck to avoid temperature abuse during distribution.

- Water condensation occurs at high relative humidity of 99% in the closed chamber systems on the surfaces of the chamber. In the preliminary experiment, mold growth was observed faster even though there was otherwise good quality retention of strawberries in chamber systems. In the final experiment, the water condensation on the surfaces was dried with tissues on each sampling day, and extra attention was paid to avoid water dripping onto the top of the clamshells to preclude mold growth. Recommendation: Future researchers could keep a water absorbent inside each chamber to minimize condensed water on the chamber surfaces and to limit extra work to dry the water out during the experiment. This would also free up the researchers to spend more time concentrating on other aspects of the experiment on each sampling day.
- The quality of strawberries is also determined by flavor level since strawberries have a distinctive flavor profile that is enjoyed by many people. However, this study did not measure a flavor profile. Flavor was not used as a sensory parameter due to ClO_2 treatment. The researchers did not use sweetness because verification of residual ClO_2 was not part of the experimental design. And due to the perspective of the sensory panel on chemical treatment (their point of belief), the flavor profile was not a part in this study. However, the literature showed evidence that flavor was often considered a factor to determine the sensory quality of treated strawberries. A preliminary study from another researcher with a non-trained panel revealed no significant differences between treated and control strawberries on flavor profile (Wang, 2014). If researchers can get the FDA approval for the use of

fruitgard® ClO_2 gas-producing sachet to treat strawberries, with the residual level tests, etc., one can add tasing the flavor profile of treated strawberries compared to the control into the sensory evaluation checklist in future studies.

- One can change the significant factors that affect the shelflife of strawberries, such as temperature (different temperatures including temperature abuse conditions); RH (low RH's <50%); ClO_2 dose (medium 1-5 ppm, high >5ppm), which may depend on the weight of strawberries; treatment duration; and distance to treatment point as factors for strawberry shelflife in these two systems (one industrial pallet setting and another experimental chamber setting with ClO_2 treatment outside the package).
- In future research, someone could experiment with Modified Atmosphere Packaging (MAP) for strawberries in the pallet system, wrapping them with stretch hood materials to avoid air coming in and out of the system in a more expanded experimental space like a cool room.
- Since the DSC did not show any formation of functional groups of structural change of each polymer, someone could measure for surface changes of polymers such as contact angle measurements using polar and nonpolar liquids or ATR, and also someone can perform an IR test to see any chemical changes of polymers, However, Rubino, 2010 has reported that no changes of chemical structures of any polymers used.
- Due to a significant change of TS at break of ClO_2 gas treated nylon-6 in both directions (MD and CD) at 99% RH, humidity should be a factor for further

consideration in experimenting to determine how nylon-6 is affected by different doses of ClO_2 gas under different levels of RH. Selecting nylon-6 as an antimicrobial packaging with ClO_2 gas at high relative humidity (99%) packaging is a concern (from the mechanical standpoint).

- Nylon-6, while questionable from a strength standpoint, could be a suitable packaging material as an antimicrobial packaging with ClO_2. Nylon-6 may not be a suitable material (whether it is ClO_2 treated or not) where the moisture barrier is a concern in the product packaging system. Nylon-6 could be a suitable material where the oxygen barrier is necessary (Kuruwita, Chapter 3). However, when a water vapor barrier is a concern in product packaging, the use of APET should be carefully considered as its barrier is degraded. Future researchers could experiment in more detail on the effects of ClO_2 gas exposure on the water vapor barrier properties of APET.
- Studying the effect of different concentrations of ClO_2 gas, as well as studying longer periods of treatment may be of interest. A range of ClO_2 doses of 1-5 ppm (or somewhere under 10 ppm) was suggested from the literature on the ClO_2 effect on polymer properties (Kuruwita, Chapter 3). Bleaching of fresh produce would likely be a limit (Kuruwita, chapter 2).
- Studying more produce packaging materials (including OPP, HDPE, which can be used as an alternative berry packaging material that can make clamshells, any biobased materials that may be used, and recycled materials) may be of interest.

- This study looked at 49, 80, and 99 % RH. Studying the impact of other values of RH (including lower) may help to fill the remaining literature gap. If someone studies the effect of RHs on the changes of material properties after exposure to ClO_2 gas, very low RH levels also need to be considered too.
- When considering produce packaging, barrier protection to O_2 is also of paramount importance. Loss of the O_2 barrier due to ClO_2 treatment may affect the respiration rate of fresh produce, which is a significant cause of ripening and deterioration of produce. Therefore, further testing of permeabilities (O_2, CO_2, etc.) at this ClO_2 dosage would be desirable.
- Since only the MD direction of ClO_2 treated nylon-6 was a significant factor for TS at break at 99% RH with ClO_2 gas treatment, the change of TS at break of nylon-6 in both directions (MD and CD), should be a factor for further consideration in experimenting to determine how different doses of ClO_2 gas (at different levels of RH) affects directional strength properties of nylon-6.

APPENDICES

Appendix A

Dart Drop Impact Test

Figure A-1: Dart Drop Impact Tester (ASTM D4272M-15)/ISO7765-2).

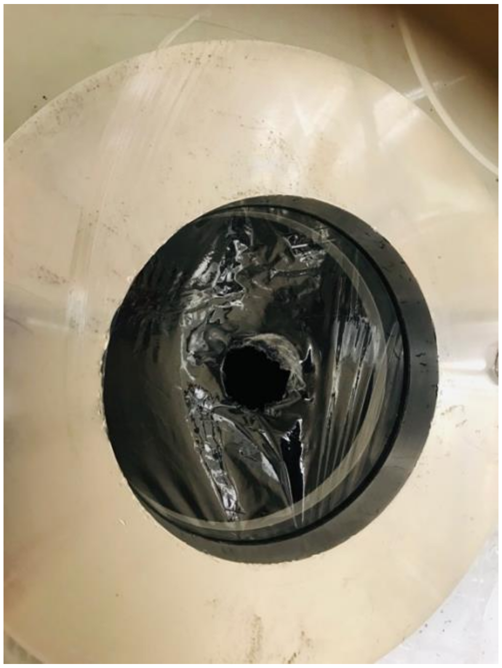
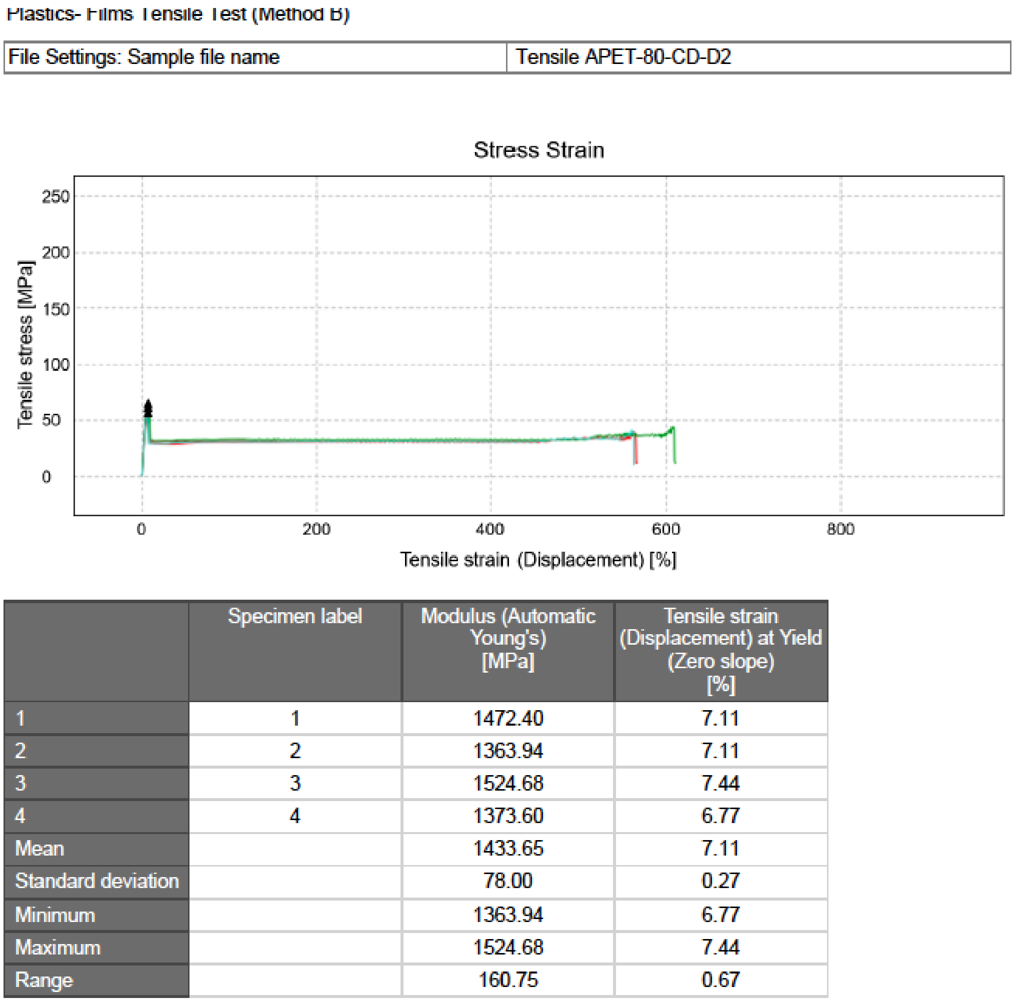


Figure A-2: Example Results of Ruptured (hole) PE Film after Dart Drop.

Appendix B

Tensile Test

Figure B-1: Example of Nylon Film Clamped Between Two Jaws of Tensile Tester (Instron).



Plastics- Films Tensile Test (Method B)

File Settings: Sample file name	Tensile APET-80-CD-D2

	Specimen label	Modulus (Automatic Young's) [MPa]	Tensile strain (Displacement) at Yield (Zero slope) [%]
1	1	1472.40	7.11
2	2	1363.94	7.11
3	3	1524.68	7.44
4	4	1373.60	6.77
Mean		1433.65	7.11
Standard deviation		78.00	0.27
Minimum		1363.94	6.77
Maximum		1524.68	7.44
Range		160.75	0.67

Figure B-2: Example Results of Stress-Strain Curve and Data for a Sample APET Film.

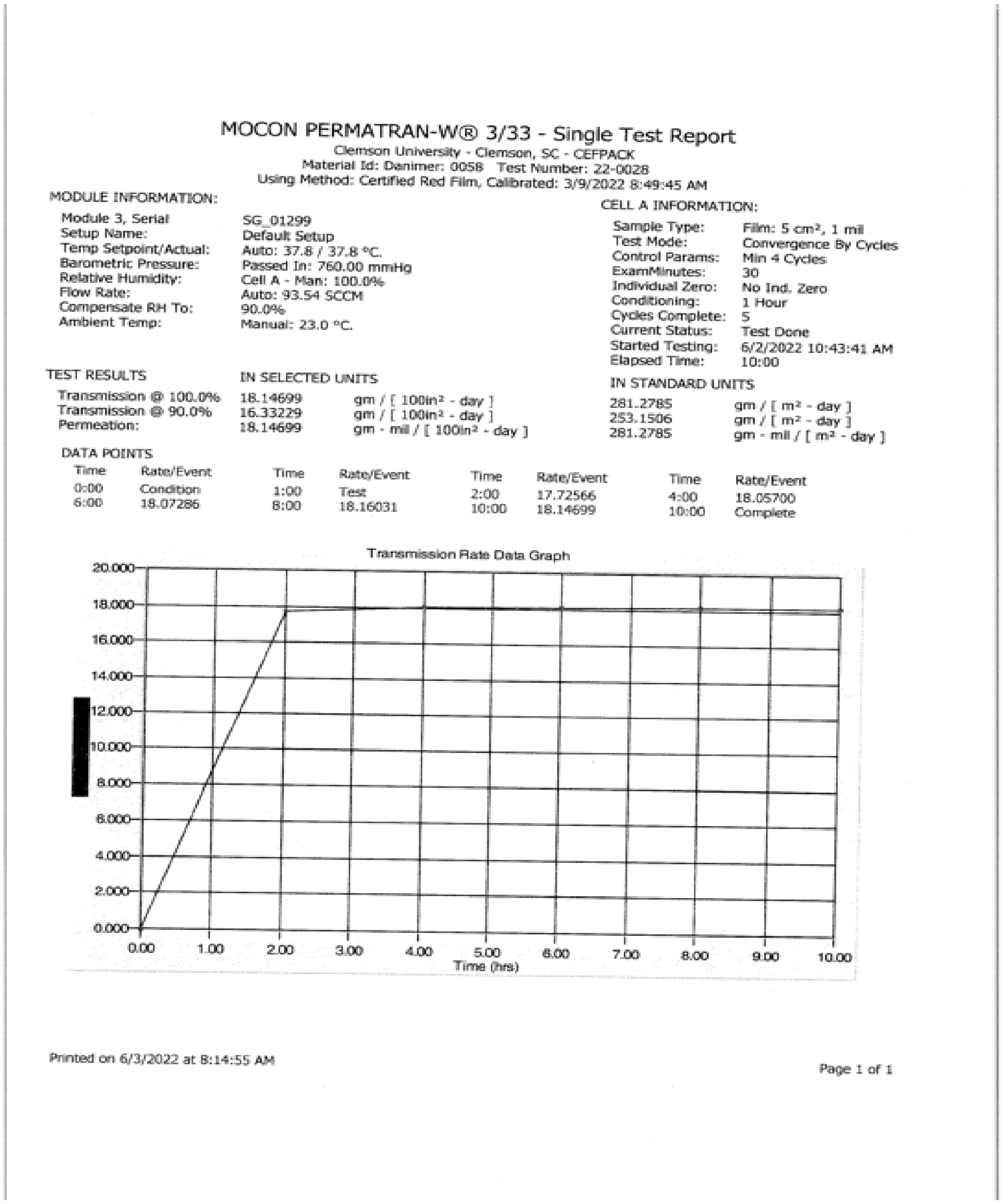
Appendix C

Water Vapor Permeability Test

Figure C.1: MOCON PERMATRAN-W 3/31, Module M-1 That Measured Water Vapor Permeability of Packaging Materials.

========= SECTION NAME:	HEADER INFORMATION =========
System Title of Report:	MOCON PERMATRAN-W® 3/33 - Single Test Report for Module Number 3, Cells , B
User Supplied Header Information:	Clemson University - Clemson, SC - CEFPACK
Exported on:	2/18/2020 5:16:54 PM
========= SECTION NAME:	MODULE 3 INFORMATION =========
Serial Number:	SG_01299
Setup Name:	Default Setup
Temp Setpoint/Actual:	Auto: 37.8 / 37.8 °C.
Barometric Pressure:	Manual: 760.00 mmHg
Flow Rate:	Manual: 0.00 SCCM
Compensate RH To:	90.0%
Ambient Temp:	Manual: 23.0 °C.
========= SECTION NAME:	CELL B INFORMATION =========
Test Number:	APET-D5
Material ID:	APET
Using Method:	Default Method
Sample Type:	Film: 50 cm², 20.63 mil
Test Mode:	Convergence By Cycles
Control Params:	Min 4 Cycles
Exam Minutes:	45
Individual Zero:	No Ind. Zero
Conditioning:	2 Hours
Cycles Complete:	5
Relative Humidity:	Cell B - Man: 100.0%
Current Status:	Finished
Started Testing:	2/14/2020 9:45:26 AM
Elapsed Time:	9:57
========= SECTION NAME:	TEST RESULTS FOR CELL B =========
	IN SELECTED UNITS
Transmission @ 100.0%	0.368576 gm / [100in² - day]
Transmission @ 90.0%	0.331718 gm / [100in² - day]
Permeation:	7.603720 gm - mil / [100in² - day]
	IN STANDARD UNITS
Transmission @ 100.0%	5.712928 gm / [m² - day]
Transmission @ 90.0%	5.141636 gm / [m² - day]
Permeation:	117.8577 gm - mil / [m² - day]
========= SECTION NAME:	DATA POINTS FROM CELL B =========

Figure C.2: Example Excel Datasheet of APET Sample on Day 5 on one of the Cells (Cell B) in the Permatran Machine



MOCON PERMATRAN-W® 3/33 - Single Test Report
Clemson University - Clemson, SC - CEFPACK
Material Id: Danimer: 0058   Test Number: 22-0028
Using Method: Certified Red Film, Calibrated: 3/9/2022 8:49:45 AM

MODULE INFORMATION:

Module 3, Serial	SG_01299
Setup Name:	Default Setup
Temp Setpoint/Actual:	Auto: 37.8 / 37.8 °C.
Barometric Pressure:	Passed In: 760.00 mmHg
Relative Humidity:	Cell A - Man: 100.0%
Flow Rate:	Auto: 93.54 SCCM
Compensate RH To:	90.0%
Ambient Temp:	Manual: 23.0 °C.

CELL A INFORMATION:

Sample Type:	Film: 5 cm^2, 1 mil
Test Mode:	Convergence By Cycles
Control Params:	Min 4 Cycles
ExamMinutes:	30
Individual Zero:	No Ind. Zero
Conditioning:	1 Hour
Cycles Complete:	5
Current Status:	Test Done
Started Testing:	6/2/2022 10:43:41 AM
Elapsed Time:	10:00

TEST RESULTS	IN SELECTED UNITS		IN STANDARD UNITS	
Transmission @ 100.0%	18.14699	gm / [100in^2 - day]	281.2785	gm / [m^2 - day]
Transmission @ 90.0%	16.33229	gm / [100in^2 - day]	253.1506	gm / [m^2 - day]
Permeation:	18.14699	gm - mil / [100in^2 - day]	281.2785	gm - mil / [m^2 - day]

DATA POINTS

Time	Rate/Event	Time	Rate/Event	Time	Rate/Event	Time	Rate/Event
0:00	Condition	1:00	Test	2:00	17.72566	4:00	18.05700
6:00	18.07286	8:00	18.16031	10:00	18.14699	10:00	Complete

Transmission Rate Data Graph

[redacted]

Printed on 6/3/2022 at 8:14:55 AM

Page 1 of 1

Figure C.3: Example Datasheet and Transmission Rate Data Graph of a Customer Sample on one of the Cells (Cell A) in the Permatran Machine.

Appendix D

Thermal Tests-DSC (T_m, T_c) Test

Figure D.1: DSC Machine.

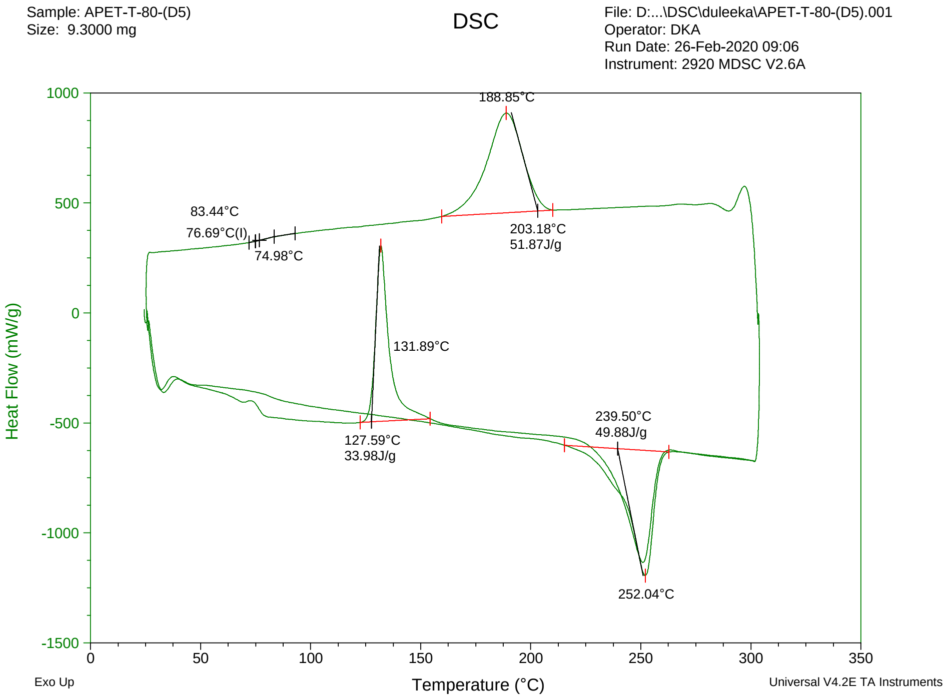


Figure D.2: Example Results of Differential Scanning Colorimeter (DSC) for a Sample of ClO_2 Gas Treated APET Film at 80% RH on Day 5 (APET-T-80-D5).

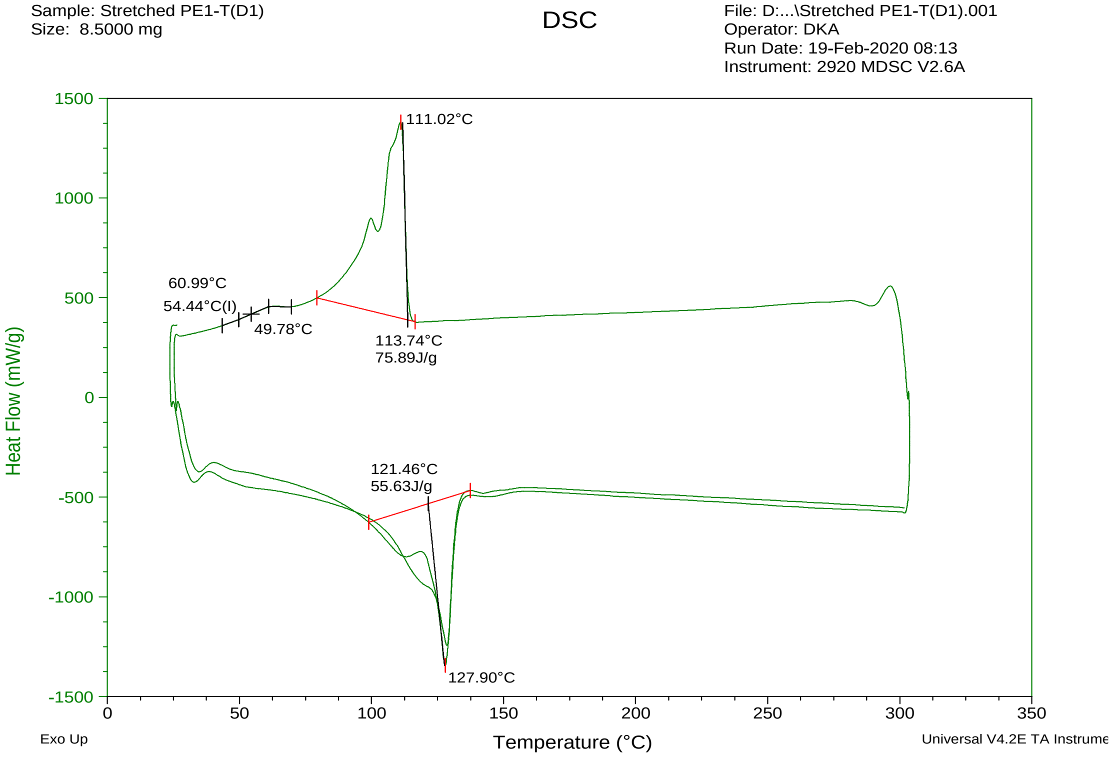


Figure D.3: Example Results of Differential Scanning Colorimeter (DSC) for a Sample of ClO$_2$ Gas Treated PE1 Film at 99% RH on Day 1 (PE1-T-99-D1).

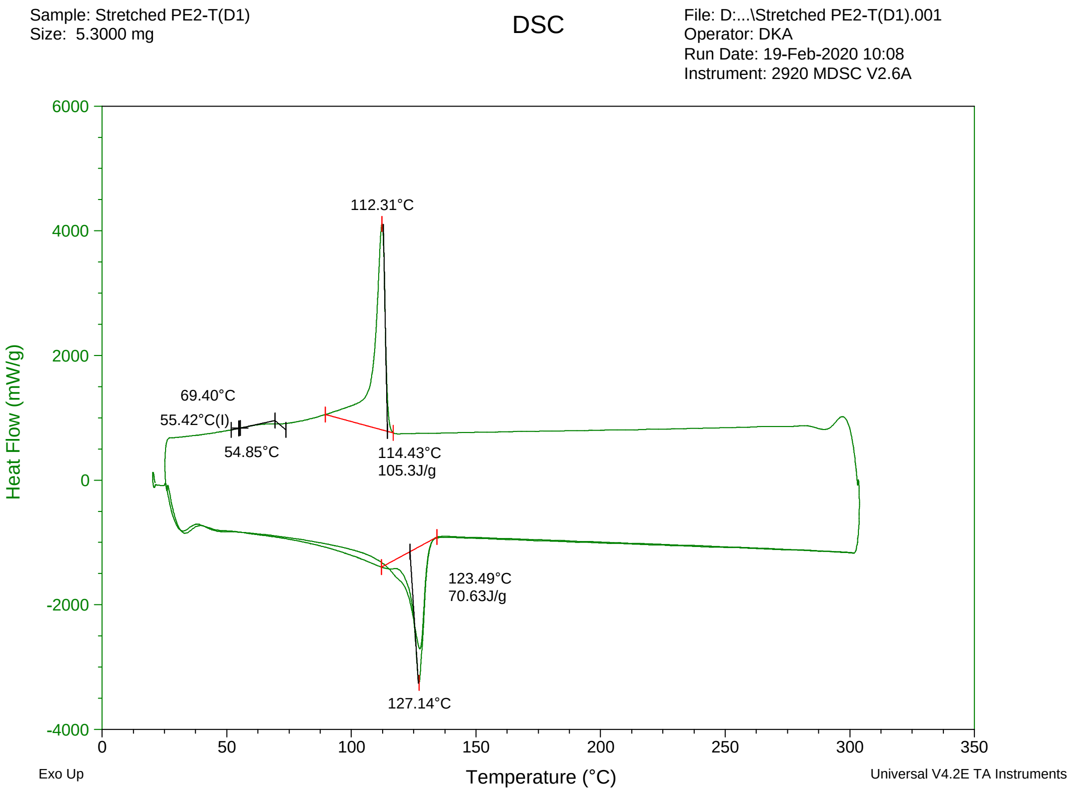
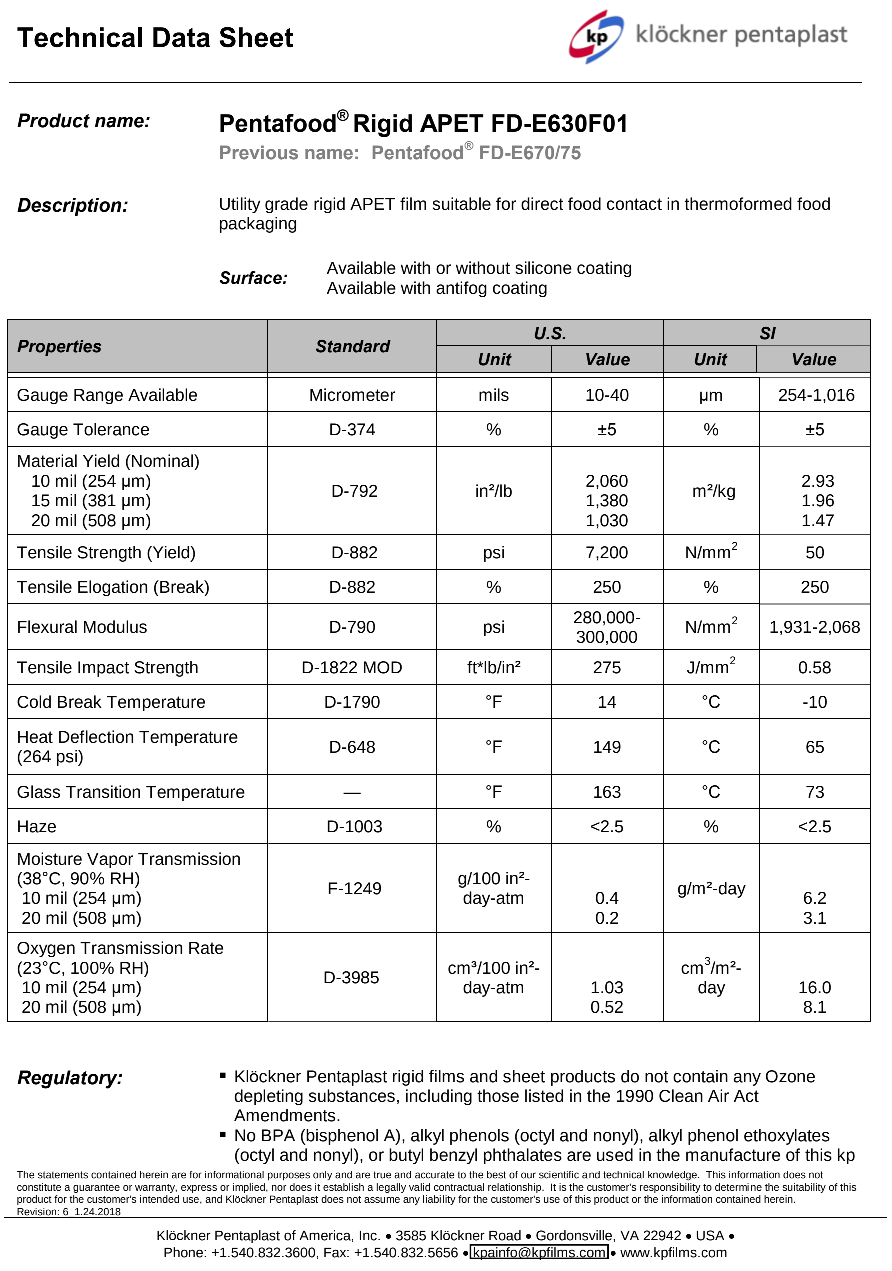


Figure D.4: Example Results of Differential Scanning Colorimeter (DSC) for a Sample of ClO_2 Gas Treated PE2 Film at 99% RH on Day 1 (PE2-T-99-D1).

Appendix E

MDS/Material Specifications



Technical Data Sheet

klöckner pentaplast

Product name: **Pentafood® Rigid APET FD-E630F01**
Previous name: Pentafood® FD-E670/75

Description: Utility grade rigid APET film suitable for direct food contact in thermoformed food packaging

Surface: Available with or without silicone coating
Available with antifog coating

Properties	***Standard***	***U.S.***		***SI***	
		Unit	***Value***	***Unit***	***Value***
Gauge Range Available	Micrometer	mils	10-40	μm	254-1,016
Gauge Tolerance	D-374	%	±5	%	±5
Material Yield (Nominal) 10 mil (254 μm) 15 mil (381 μm) 20 mil (508 μm)	D-792	in²/lb	2,060 1,380 1,030	m²/kg	2.93 1.96 1.47
Tensile Strength (Yield)	D-882	psi	7,200	N/mm^2	50
Tensile Elogation (Break)	D-882	%	250	%	250
Flexural Modulus	D-790	psi	280,000-300,000	N/mm^2	1,931-2,068
Tensile Impact Strength	D-1822 MOD	ft*lb/in²	275	J/mm^2	0.58
Cold Break Temperature	D-1790	°F	14	°C	-10
Heat Deflection Temperature (264 psi)	D-648	°F	149	°C	65
Glass Transition Temperature	—	°F	163	°C	73
Haze	D-1003	%	<2.5	%	<2.5
Moisture Vapor Transmission (38°C, 90% RH) 10 mil (254 μm) 20 mil (508 μm)	F-1249	g/100 in²-day-atm	0.4 0.2	g/m²-day	6.2 3.1
Oxygen Transmission Rate (23°C, 100% RH) 10 mil (254 μm) 20 mil (508 μm)	D-3985	cm³/100 in²-day-atm	1.03 0.52	cm^3/m²-day	16.0 8.1

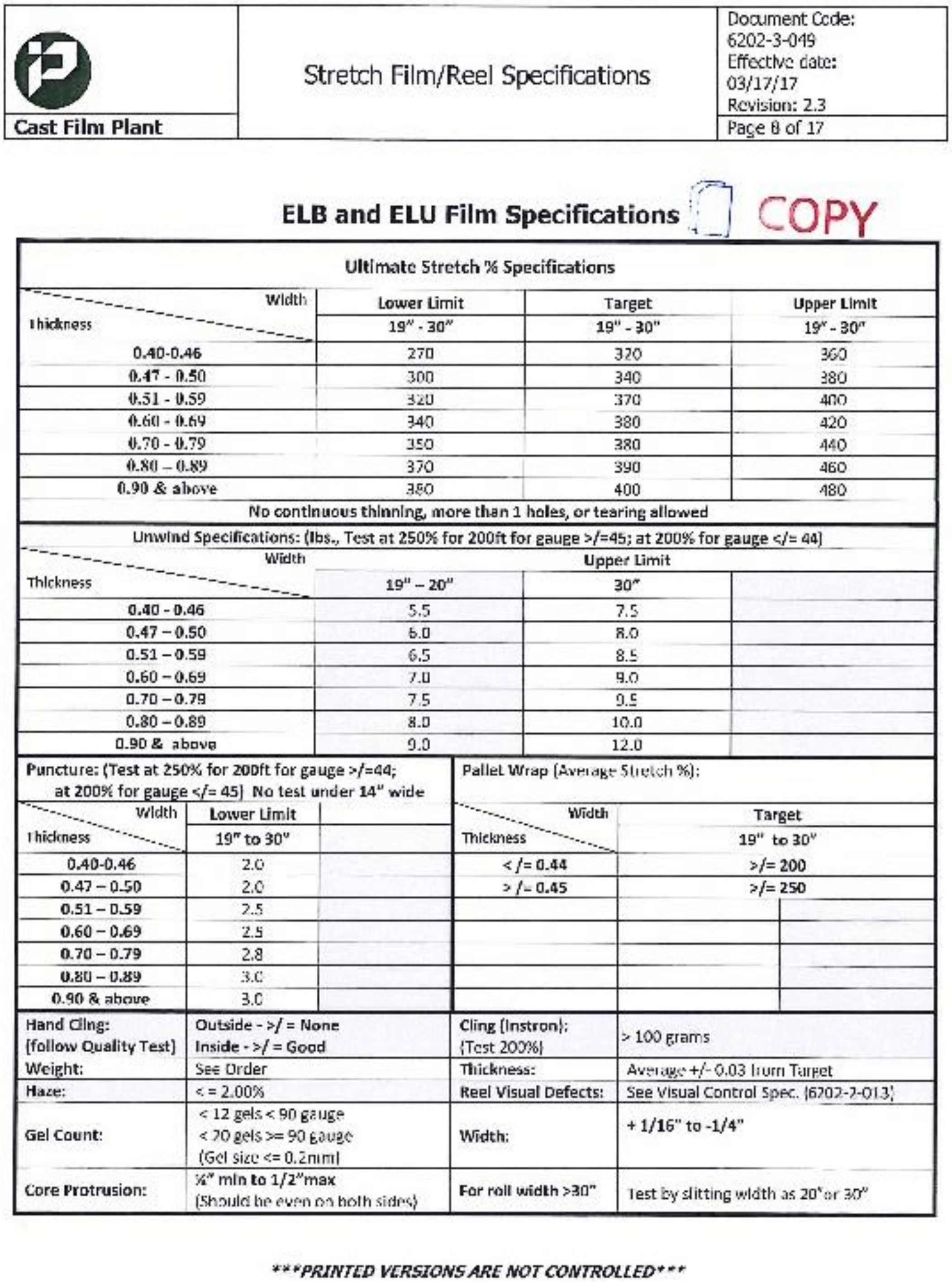
Regulatory:

- Klöckner Pentaplast rigid films and sheet products do not contain any Ozone depleting substances, including those listed in the 1990 Clean Air Act Amendments.
- No BPA (bisphenol A), alkyl phenols (octyl and nonyl), alkyl phenol ethoxylates (octyl and nonyl), or butyl benzyl phthalates are used in the manufacture of this kp

Revision: 6_1.24.2018

Klöckner Pentaplast of America, Inc. • 3585 Klöckner Road • Gordonsville, VA 22942 • USA •
Phone: +1.540.832.3600, Fax: +1.540.832.5656 • kpainfo@kpfilms.com • www.kpfilms.com

Figure E.1: MDS OF APET Used.



	Stretch Film/Reel Specifications	Document Code: 6202-3-049 Effective date: 03/17/17 Revision: 2.3
Cast Film Plant		Page 8 of 17

ELB and ELU Film Specifications COPY

Ultimate Stretch % Specifications

Width / Thickness	Lower Limit	Target	Upper Limit
	19" - 30"	19" - 30"	19" - 30"
0.40-0.46	270	320	360
0.47 - 0.50	300	340	380
0.51 - 0.59	320	370	400
0.60 - 0.69	340	380	420
0.70 - 0.79	350	380	440
0.80 – 0.89	370	390	460
0.90 & above	380	400	480

No continuous thinning, more than 1 holes, or tearing allowed

Unwind Specifications: (lbs., Test at 250% for 200ft for gauge >/=45; at 200% for gauge </= 44)

Width / Thickness	Upper Limit		
	19" – 20"	30"	
0.40 - 0.46	5.5	7.5	
0.47 – 0.50	6.0	8.0	
0.51 – 0.59	6.5	8.5	
0.60 – 0.69	7.0	9.0	
0.70 – 0.79	7.5	9.5	
0.80 – 0.89	8.0	10.0	
0.90 & above	9.0	12.0	

Puncture: (Test at 250% for 200ft for gauge >/=44; at 200% for gauge </= 45) No test under 14" wide

Width / Thickness	Lower Limit	
	19" to 30"	
0.40-0.46	2.0	
0.47 – 0.50	2.0	
0.51 – 0.59	2.5	
0.60 – 0.69	2.5	
0.70 – 0.79	2.8	
0.80 – 0.89	3.0	
0.90 & above	3.0	

Pallet Wrap (Average Stretch %):

Width / Thickness	Target
	19" to 30"
</= 0.44	>/= 200
> /= 0.45	>/= 250

Hand Cling: **(follow Quality Test)**	**Outside - >/ = None** **Inside - >/ = Good**	**Cling (Instron):** (Test 200%)	> 100 grams
Weight:	See Order	**Thickness:**	Average +/- 0.03 from Target
Haze:	< = 2.00%	**Reel Visual Defects:**	See Visual Control Spec. (6202-2-013)
Gel Count:	< 12 gels < 90 gauge < 70 gels >= 90 gauge (Gel size <= 0.2mm)	**Width:**	**+ 1/16" to -1/4"**
Core Protrusion:	**¼" min to 1/2"max** (Should be even on both sides)	**For roll width >30"**	Test by slitting width as 20" or 30"

******PRINTED VERSIONS ARE NOT CONTROLLED******

Figure E.2: MDS OF PE Type 1 And 2 Used.

AmToppWrap "Extreme" Series
Technical Data

		55	60	70	80	90	115
Tear (Gms./Sht)	MD	130	150	175	225	250	340
	TD	443	450	475	500	525	570
		1.4	1.6	1.9	2.1	2.3	2.6
%)		88	90	90	88	88	87
ensile (PSI)	MD	7321	7200	7000	6800	6500	6000
	TD	4400	4400	4400	4400	4400	4400
Elongation (%)	MD	549	550	575	600	625	650
	TD	780	800	850	875	900	925
Gms)		94	100	110	125	140	165
Wrapper Stretch	%	220	255	290	300	310	330
ng	gms	110 to 145 for all gauges and depend on stretch %					

Figure E.3: MDS OF PEs Used.

Appendix F

ClO_2 Releasing Curves

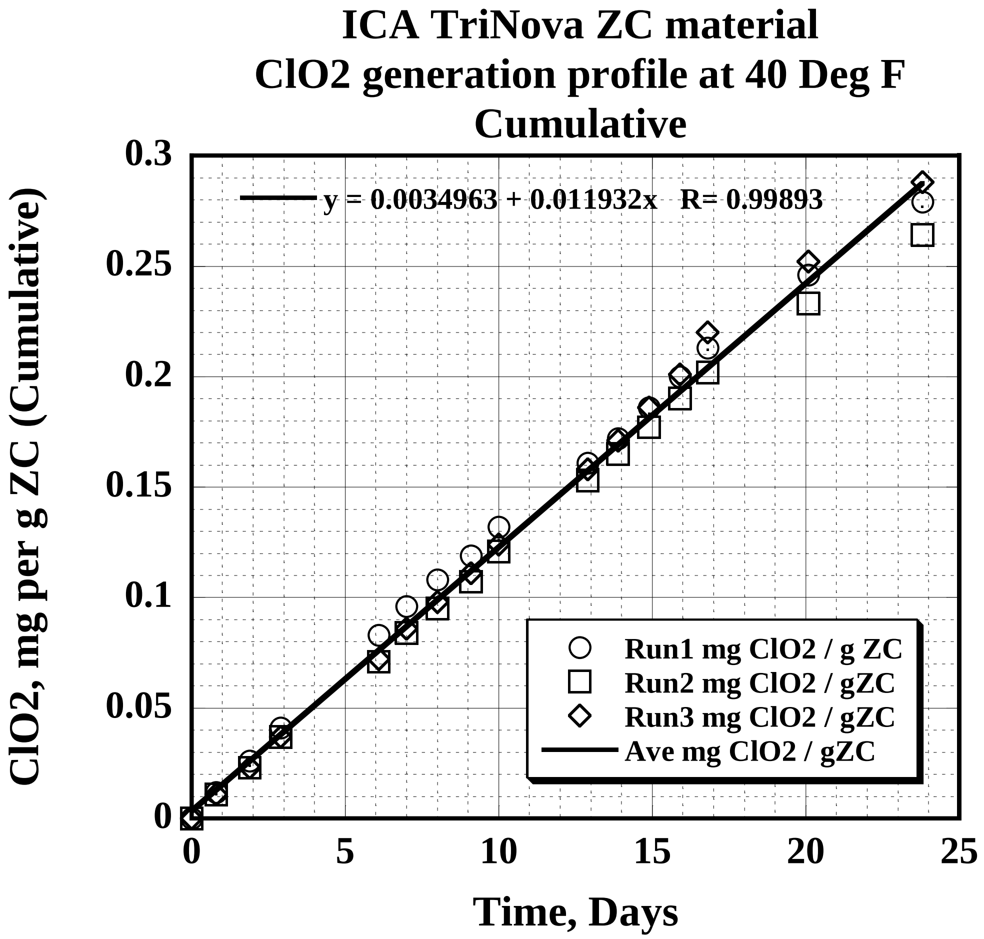
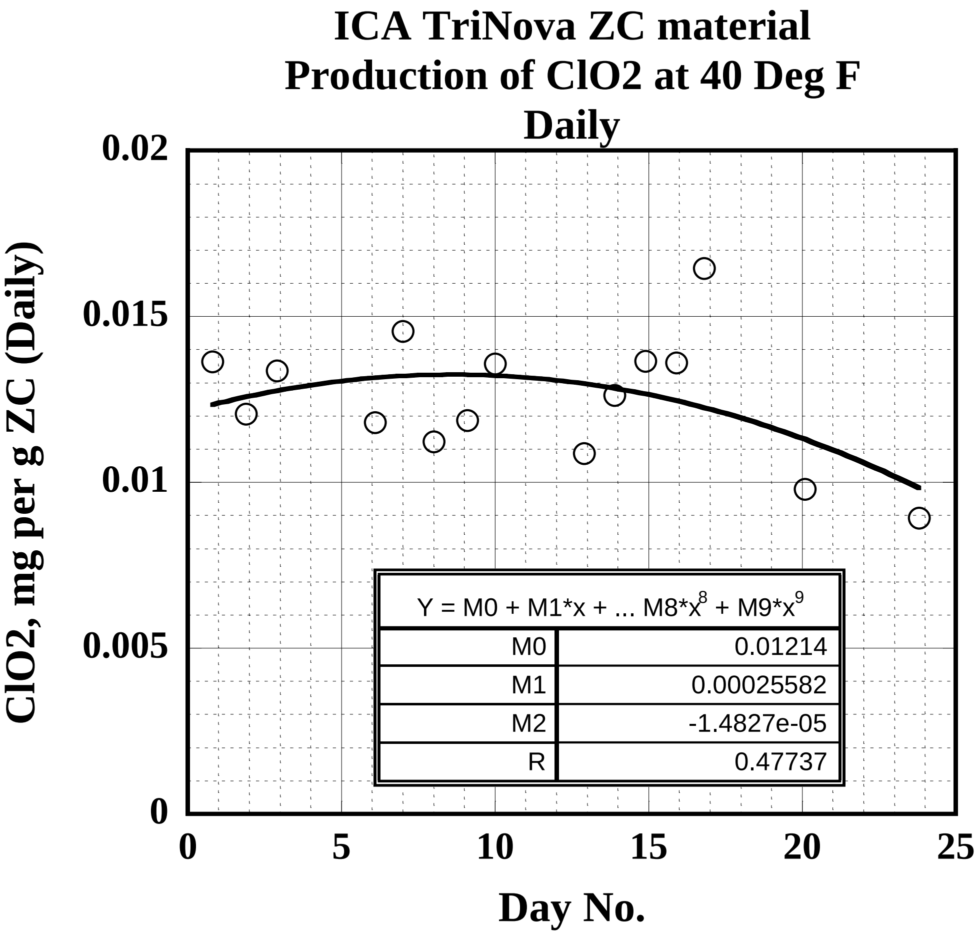


Figure F.1: Average mg ClO_2 per (g/ZC) Generation Profile at 40 °F By Days.

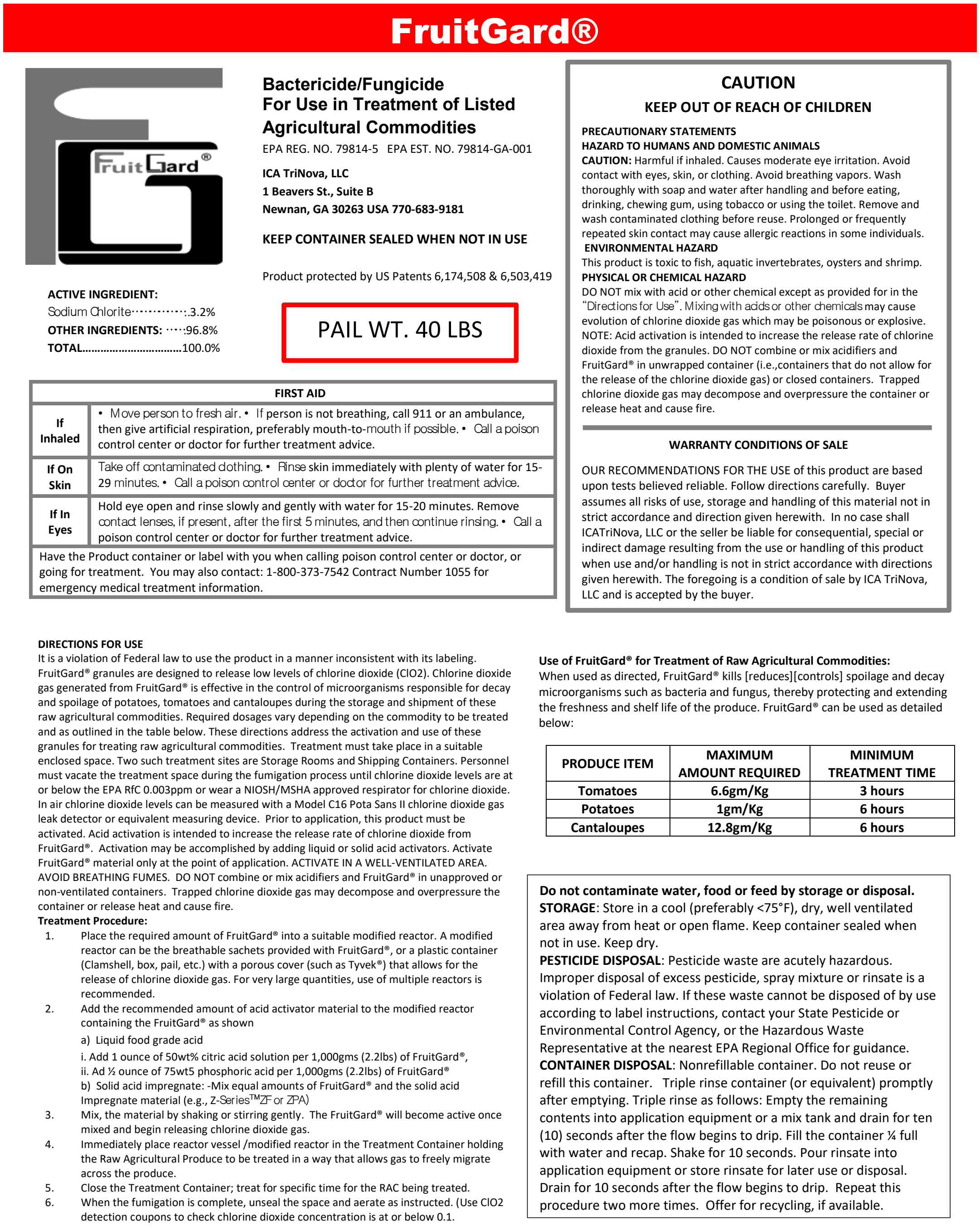


Y = M0 + M1*x + ... M8*x^8 + M9*x^9	
M0	0.01214
M1	0.00025582
M2	-1.4827e-05
R	0.47737

Figure F.2: Daily Release of mg ClO_2 per g ZC Vs Days.

Appendix G

EPA Approval for ClO2 Sachets



FruitGard®

Bactericide/Fungicide For Use in Treatment of Listed Agricultural Commodities

EPA REG. NO. 79814-5 EPA EST. NO. 79814-GA-001

ICA TriNova, LLC
1 Beavers St., Suite B
Newnan, GA 30263 USA 770-683-9181

KEEP CONTAINER SEALED WHEN NOT IN USE

Product protected by US Patents 6,174,508 & 6,503,419

ACTIVE INGREDIENT:
Sodium Chlorite…………:.3.2%
OTHER INGREDIENTS: ……:96.8%
TOTAL……………………………100.0%

PAIL WT. 40 LBS

FIRST AID	
If Inhaled	• Move person to fresh air. • If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. • Call a poison control center or doctor for further treatment advice.
If On Skin	Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-29 minutes. • Call a poison control center or doctor for further treatment advice.
If In Eyes	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, and then continue rinsing. • Call a poison control center or doctor for further treatment advice.

Have the Product container or label with you when calling poison control center or doctor, or going for treatment. You may also contact: 1-800-373-7542 Contract Number 1055 for emergency medical treatment information.

CAUTION

KEEP OUT OF REACH OF CHILDREN

PRECAUTIONARY STATEMENTS
HAZARD TO HUMANS AND DOMESTIC ANIMALS
CAUTION: Harmful if inhaled. Causes moderate eye irritation. Avoid contact with eyes, skin, or clothing. Avoid breathing vapors. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse. Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals.

ENVIRONMENTAL HAZARD
This product is toxic to fish, aquatic invertebrates, oysters and shrimp.

PHYSICAL OR CHEMICAL HAZARD
DO NOT mix with acid or other chemical except as provided for in the "Directions for Use". Mixing with acids or other chemicals may cause evolution of chlorine dioxide gas which may be poisonous or explosive. NOTE: Acid activation is intended to increase the release rate of chlorine dioxide from the granules. DO NOT combine or mix acidifiers and FruitGard® in unwrapped container (i.e.,containers that do not allow for the release of the chlorine dioxide gas) or closed containers. Trapped chlorine dioxide gas may decompose and overpressure the container or release heat and cause fire.

WARRANTY CONDITIONS OF SALE

OUR RECOMMENDATIONS FOR THE USE of this product are based upon tests believed reliable. Follow directions carefully. Buyer assumes all risks of use, storage and handling of this material not in strict accordance and direction given herewith. In no case shall ICATriNova, LLC or the seller be liable for consequential, special or indirect damage resulting from the use or handling of this product when use and/or handling is not in strict accordance with directions given herewith. The foregoing is a condition of sale by ICA TriNova, LLC and is accepted by the buyer.

DIRECTIONS FOR USE
It is a violation of Federal law to use the product in a manner inconsistent with its labeling. FruitGard® granules are designed to release low levels of chlorine dioxide (ClO2). Chlorine dioxide gas generated from FruitGard® is effective in the control of microorganisms responsible for decay and spoilage of potatoes, tomatoes and cantaloupes during the storage and shipment of these raw agricultural commodities. Required dosages vary depending on the commodity to be treated and as outlined in the table below. These directions address the activation and use of these granules for treating raw agricultural commodities. Treatment must take place in a suitable enclosed space. Two such treatment sites are Storage Rooms and Shipping Containers. Personnel must vacate the treatment space during the fumigation process until chlorine dioxide levels are at or below the EPA RfC 0.003ppm or wear a NIOSH/MSHA approved respirator for chlorine dioxide. In air chlorine dioxide levels can be measured with a Model C16 Pota Sans II chlorine dioxide gas leak detector or equivalent measuring device. Prior to application, this product must be activated. Acid activation is intended to increase the release rate of chlorine dioxide from FruitGard®. Activation may be accomplished by adding liquid or solid acid activators. Activate FruitGard® material only at the point of application. ACTIVATE IN A WELL-VENTILATED AREA. AVOID BREATHING FUMES. DO NOT combine or mix acidifiers and FruitGard® in unapproved or non-ventilated containers. Trapped chlorine dioxide gas may decompose and overpressure the container or release heat and cause fire.

Treatment Procedure:

1. Place the required amount of FruitGard® into a suitable modified reactor. A modified reactor can be the breathable sachets provided with FruitGard®, or a plastic container (Clamshell, box, pail, etc.) with a porous cover (such as Tyvek®) that allows for the release of chlorine dioxide gas. For very large quantities, use of multiple reactors is recommended.
2. Add the recommended amount of acid activator material to the modified reactor containing the FruitGard® as shown
   a) Liquid food grade acid
   i. Add 1 ounce of 50wt% citric acid solution per 1,000gms (2.2lbs) of FruitGard®,
   ii. Ad ½ ounce of 75wt5 phosphoric acid per 1,000gms (2.2lbs) of FruitGard®
   b) Solid acid impregnate: -Mix equal amounts of FruitGard® and the solid acid Impregnate material (e.g., Z-Series™ZF or ZPA)
3. Mix, the material by shaking or stirring gently. The FruitGard® will become active once mixed and begin releasing chlorine dioxide gas.
4. Immediately place reactor vessel /modified reactor in the Treatment Container holding the Raw Agricultural Produce to be treated in a way that allows gas to freely migrate across the produce.
5. Close the Treatment Container; treat for specific time for the RAC being treated.
6. When the fumigation is complete, unseal the space and aerate as instructed. (Use ClO2 detection coupons to check chlorine dioxide concentration is at or below 0.1.

Use of FruitGard® for Treatment of Raw Agricultural Commodities:
When used as directed, FruitGard® kills [reduces][controls] spoilage and decay microorganisms such as bacteria and fungus, thereby protecting and extending the freshness and shelf life of the produce. FruitGard® can be used as detailed below:

PRODUCE ITEM	MAXIMUM AMOUNT REQUIRED	MINIMUM TREATMENT TIME
Tomatoes	**6.6gm/Kg**	**3 hours**
Potatoes	**1gm/Kg**	**6 hours**
Cantaloupes	**12.8gm/Kg**	**6 hours**

Do not contaminate water, food or feed by storage or disposal.
STORAGE: Store in a cool (preferably <75°F), dry, well ventilated area away from heat or open flame. Keep container sealed when not in use. Keep dry.
PESTICIDE DISPOSAL: Pesticide waste are acutely hazardous. Improper disposal of excess pesticide, spray mixture or rinsate is a violation of Federal law. If these waste cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.
CONTAINER DISPOSAL: Nonrefillable container. Do not reuse or refill this container. Triple rinse container (or equivalent) promptly after emptying. Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for ten (10) seconds after the flow begins to drip. Fill the container ¼ full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. Offer for recycling, if available.

Appendix H

Sensory Evaluation Checklist

Scaling on unstructured scale/Questionnaire using unstructured scale

Project code number: Date:

(Please evaluate in the order of product sample codes: 101, 202, 303, and 404)

- Please evaluate the **color, aroma, firmness, and overall acceptability (intent to consume)** for 4 samples of strawberries.
- Evaluate all 4 samples on the same scale for each parameter.
- Make vertical lines on the horizontal line to indicate your rating of each sensory parameter of each sample. Label each vertical line with the code number of the sample it represents.

Sensory criteria 1, for samples 101, 202, 303, and 404

1. **Color**

0 cm 15 cm

Shiny light/dark red-pink Dark brown

Sensory criteria 2, for samples 101, 202, 303, and 404

2. **Aroma**

0 cm 15 cm

Very good aroma smells No aroma smell
(Strong off odor) (Strong strawberry odor)

Sensory criteria 3, for samples 101, 202, 303, and 404

3. **Firmness**

0 cm 15 cm

Very Firm Very soft and mushy

Sensory criteria 4, for samples 101, 202, 303, and 404

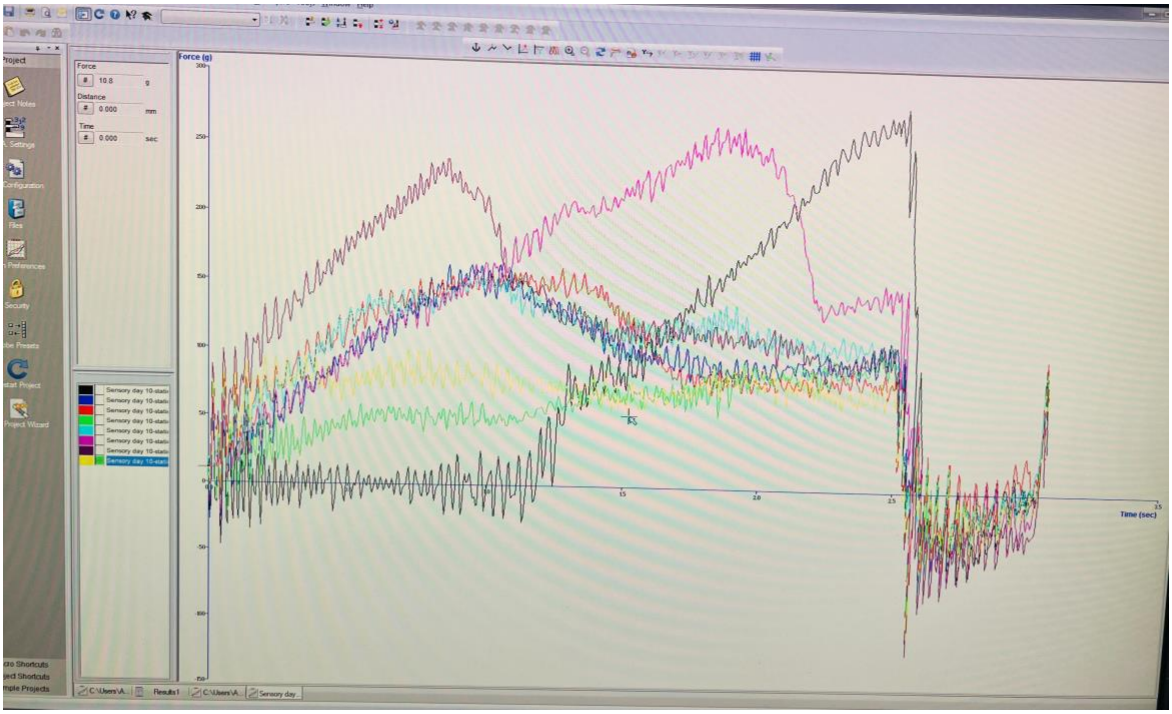
4. **Overall acceptability (Intent to consume)**

0 cm 15 cm

Super good to consume Bad to consume

Appendix I

TA.TXPlus (Fruit Firmness Analyzer) Test


Force 10.8 g

Distance 0.000 mm

Time 0.000 sec

Figure I: Penetration Force (Firmness) of a Sample of Strawberry Measured By TA.TXPlus (Fruit Firmness Analyzer).

Appendix J

Kaiser Camera for L*, a*, b* Colors And MATLAB R2019 Data Analysis

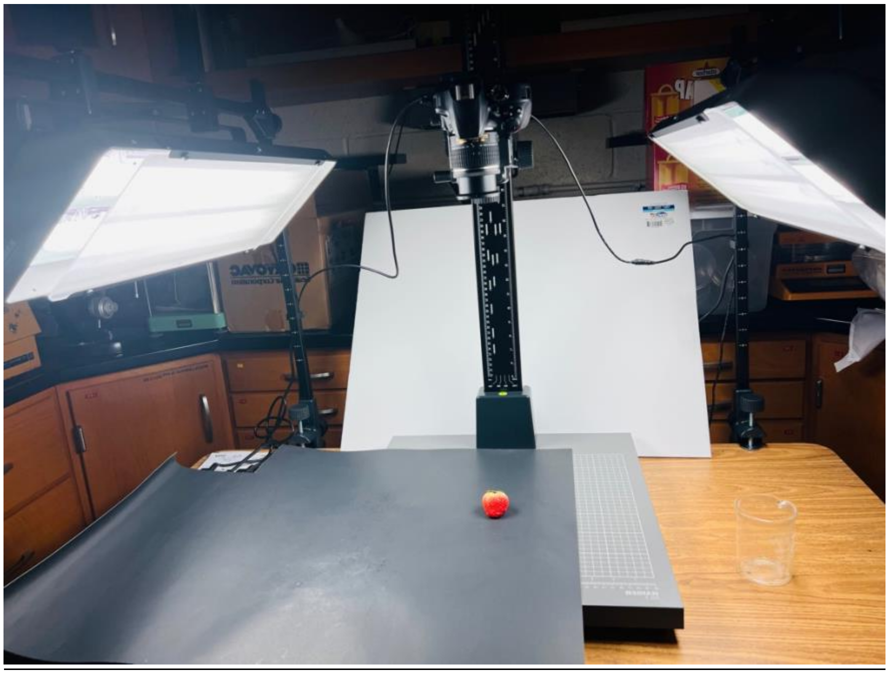


Figure J.1: Verification of Strawberry Color, Focusing Kaiser Camera to a Location of Strawberry Surface

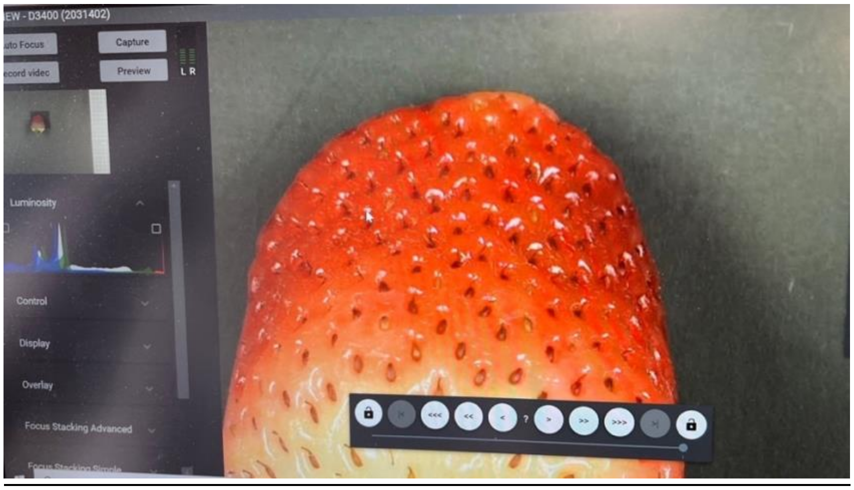
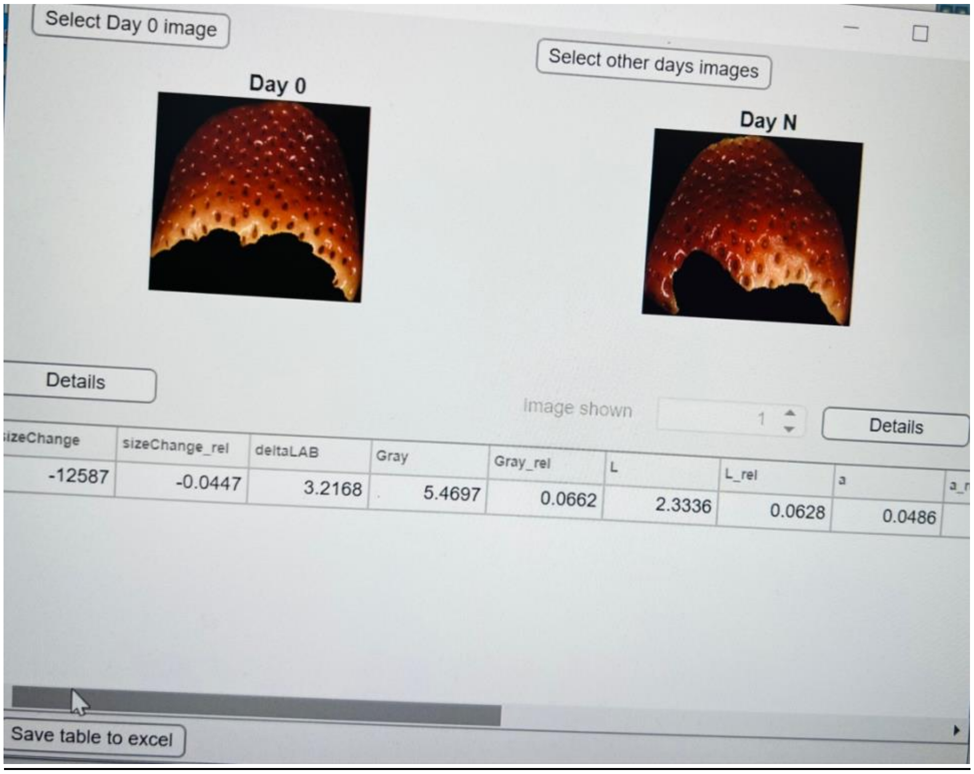


Figure J.2: Verification of Strawberry Color, A Focused Picture (D_0 Sample 1) Taken by Kaiser Camera and Saved for Color Measurements Using MATLAB R2019



izeChange	sizeChange_rel	deltaLAB	Gray	Gray_rel	L	L_rel	a	a_r
-12587	-0.0447	3.2168	5.4697	0.0662	2.3336	0.0628	0.0486	

Figure J.3: Color Measurements Data Including L*, a*, b* Values Using MATLAB R2019 Data Analyzer

Gray	R	G	B	L	a	b
87.22694965	155.6086374	62.67238117	35.25788484	39.31047986	36.63365851	36.31390145

Figure J.4: Example of D_0 Strawberry Sample 2 Color Measurements Data Including L*, a*, b* Values Saved in an Excel Sheet