2025 S.T. Yau High School Science Award (Asia)
Hong Kong, China

Heep Yunn School (Bio-006)

Utilizing Oxygen-absorbing Ability of Avocado Seeds to Synthesize Oxygen-absorber Sachets and Food Packaging

CHEUNG MAISIE CHEUK YU

CHAN HEI CHING

CHAN KA KIU

SUPERVISOR: MR. CHEUNG WING CHEONG

2025 S.T. Yau High School Science Award (Asia)

Research Report

<u>The Team</u>

Registration Number: **<u>Bio-006</u>**

Name of team member: **<u>Cheung Maisie Cheuk Yu</u>**
School: **<u>Heep Yunn School</u>**
Country: **<u>Hong Kong, China</u>**

Name of team member: **<u>Chan Hei Ching</u>**
School: **<u>Heep Yunn School</u>**
Country: **<u>Hong Kong, China</u>**

Name of team member: **<u>Chan Ka Kiu</u>**
School: **<u>Heep Yunn School</u>**
Country: **<u>Hong Kong, China</u>**

Name of supervising teacher: **<u>Mr. Cheung Wing Cheong</u>**
Job Title: **<u>Panel Head, Department of Junior Science</u>**
School: **<u>Heep Yunn School</u>**
Country: **<u>Hong Kong, China</u>**

<u>Title of Research Report</u>

Utilizing Oxygen-absorbing Ability of Avocado Seeds to Synthesize Oxygen-absorber Sachets and Food Packaging

Date : 14 August 2025

Utilizing Oxygen-absorbing Ability of Avocado Seeds to Synthesize Oxygen-absorber Sachets and Food Packaging

Cheung Maisie Cheuk Yu, Chan Hei Ching, Chan Ka Kiu

Abstract

This project aims to explore the use of avocado waste – avocado seeds as an ingredient for active packaging of food, in particular to serve as a material for oxygen-absorber sachets and food wrapping.

In the first part of the project, antioxidizing property and oxygen-absorbing ability of avocado seed (Hass and Shepard avocados) were assessed. By using DPPH test, it was found that all distilled water and ethanolic extracts of avocado seeds demonstrated significantly high antioxidizing property. In the case of 2 g of Shepard avocado seed and 1.33 g of Hass avocado extract by using 95% ethanol, the light absorbance of DPPH solution at 517 nm was reduced by 97.38% and 96.32% respectively. For the oxygen-absorbing ability, 20 g of Hass seed powders absorbed the greatest amount of oxygen (17.9%) in an enclosed environment of 137 cm^3, resulting in the final oxygen concentration of 3.0 %. All performances were enhanced with the addition of sodium ascorbate, green tea and turmeric powder. With seed to powder mass ratios of 1:1, 2:1 and 4:1, all oxygen levels were reduced to 0% in a volume of 137 cm^3. It was found that the capacity of avocado seed/sodium ascorbate mixture to absorb all oxygen in an enclosed flask was between 34.3 cm^3/g to 45.6 cm^3/g. The powders were then used for testing with bananas in an air-tight box of 990 cm^3. It was found that with the presence of 20 g mixture of seed powders and the aforementioned powder, the ripening process of bananas were delayed

In the second part of the project, starch was successfully extracted from Hass avocado seeds with the percentage yield ranging from 3.4% to 5.5%. Films were synthesized by using the seed starch mixing with different amount of chitosan to explore the effect on mechanical strength of the films (notated by ***AC10*** to ***AC01***, in which the number presenting the mass ratio of seed to chitosan). It was found that all films demonstrated similar tensile strength. ***AC10*** and ***AC01*** pure film exhibited the highest bending strength. All films made from avocado seeds had insignificant water uptake with the lowest value of 3.5% in the case of ***AC12***. All films showed a much higher water vapour permeability compared with the commercial oxygen sachet material. ***AC10*** present the value 18.5 times higher than that of the commercial one. ***AC10*** film also showed the highest oxygen permeability. At last, all films were proven to be biodegradable with reducing masses during a two-week soil burial test. ***AC21*** to ***AC01*** films were totally decomposed after 14 days. This is recommended that ***AC10*** and ***AC21*** are more suitable candidates for making sachets for oxygen-absorber and food wrapping film respectively.

Keywords: Anti-oxidant, Avocado seeds, Food packaging, Oxygen-absorbing, Starch

Acknowledgement

We would like to express our deepest gratitude to the school and our principal, Miss Bella Leung, for their support, which has made this research possible. I would also like to thank Mr. Cheung Wing Cheong, our research supervisor, for his patient guidance, encouragement, and valuable critiques of this work. My heartfelt thanks are also extended to the technicians of the Junior Science Department and the Chemistry Department for their support in providing the chemicals and necessary equipment. Lastly, we wish to thank our parents for their unwavering support throughout our study.

Commitments on Academic Honesty and Integrity

We hereby declare that we

1. are fully committed to the principle of honesty, integrity and fair play throughout the competition.
2. actually perform the research work ourselves and thus truly understand the content of the work.
3. observe the common standard of academic integrity adopted by most journals and degree theses.
4. have declared all the assistance and contribution we have received from any personnel, agency, institution, etc. for the research work.
5. undertake to avoid getting in touch with assessment panel members in a way that may lead to direct or indirect conflict of interest.
6. undertake to avoid any interaction with assessment panel members that would undermine the neutrality of the panel member and fairness of the assessment process.
7. observe the safety regulations of the laboratory(ies) where the we conduct the experiment(s), if applicable.
8. observe all rules and regulations of the competition.
9. agree that the decision of YHSA(Asia) is final in all matters related to the competition.

We understand and agree that failure to honour the above commitments may lead to disqualification from the competition and/or removal of reward, if applicable; that any unethical deeds, if found, will be disclosed to the school principal of team member(s) and relevant parties if deemed necessary; and that the decision of YHSA(Asia) is final and no appeal will be accepted.

Name of team member: **Cheung Maisie Cheuk Yu**

Name of team member: **Chan Hei Ching**

Name of team member: **Chan Ka Kiu**

Name of supervising teacher: **Mr. Cheung Wing Cheong**

Noted and endorsed by

Name of school principal: **Miss Bella Leung**

Table of Contents

1. INTRODUCTION

1.1. Avocado Seeds as Food Waste

Avocados (*Persea Americana Mill.*) have been a popular "super food" in the recent decades due to their high nutritional value. It was reported that the global avocado consumption has tripled from 6 billion pounds in 2000 to 18 billion pounds in 2020 [1]. With the increasing demand for the fruit, the disposal of avocado seeds is becoming a significant issue.

Fig.1. Different parts of an avocado [6]

An avocado seed, in the case of Hass avocado, contributes around 16% to 26% of the total mass of the fruit on average [2, 3]. It is estimated that there were about 600 thousand tons of avocado seeds disposed as waste in 2013. [4]. Not only does this exacerbate the pressure of landfill space, it will also increase the emission of greenhouse gases upon degradation [5].

Therefore, it would be beneficial both environmentally and economically to explore other potential uses of avocado seed waste.

1.2. Ripening of Fruits in the Presence of Oxygen

Deterioration of food is often caused by oxidative attack by the atmospheric oxygen. Not only does this affect the shelf life of the food but it also reduces its nutritional value.

In particular for fruits, aerobic respiration is mainly responsible for fruit ripening and deterioration. This leads to a decrease in shelf life and quality. It has been reported by various studies that higher rate of aerobic respiration leads to faster fruit ripening and subsequent deterioration (senescence) [7].
The effect is particularly prominent on climacteric fruits (like apples and bananas), in which the fruits exhibit a burst of respiration rate in the late ripening stage and lead to production of a hormonal stimulant – ethylene gas that speeds up the ripening process and leads to ultimate rotting (***Fig. 2***) [8].

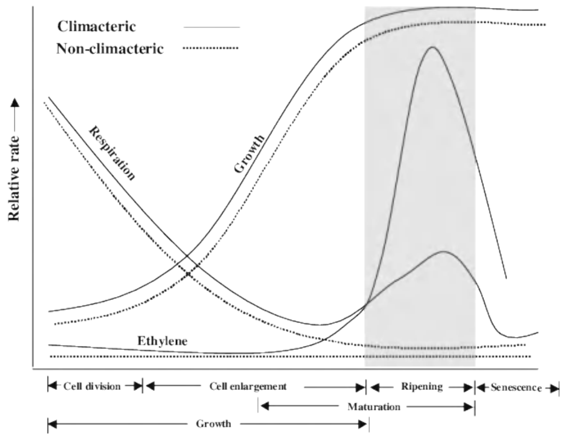


Fig.2. Rate of ethylene production and respiration during different stages of fruit development [9]

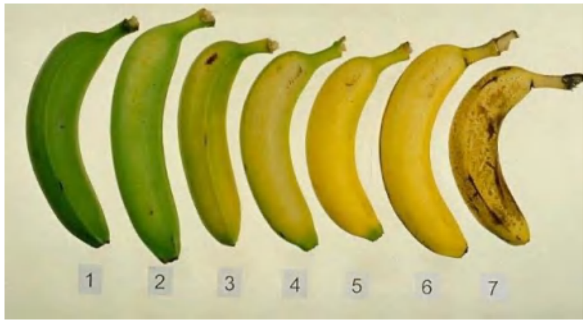


Fig.3. Ripening of a banana over a week [10]

Therefore, a low oxygen environment is favourable for storing fruits and maintaining their quality and freshness.

1.3. Problems with Current Oxygen-Absorber Sachets

Food often deteriorates and loses its colour during prolonged delivery and storage . One of the most prominent reasons is the occurrence of oxidation with the atmospheric oxygen inside the package. To deal with this issue, oxygen-absorber sachets are most commonly used to preserve daily packaged food.

There are various types of oxygen absorbers utilizing different principles to scavenge oxygen in a sealed environment (***Fig. 4***). Among all, ferrous oxygen-absorbers are the most commonly used. The absorbers are often made of a porous sachet package of paper and polyethylene that is permeable to oxygen [11]. The active ingredient inside includes iron powder, in the presence of sodium chloride and activated charcoal. The iron reacts with the oxygen to form iron oxide, therefore consuming the atmospheric oxygen (***Fig. 5.***).

Fig.4. Different brands of oxygen absorbers

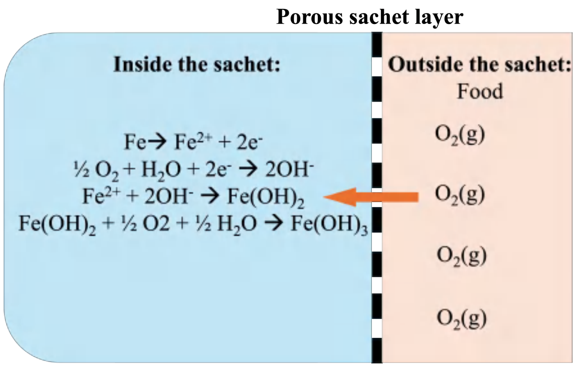


Fig.5. Reaction mechanism of scavenging oxygen by ferrous oxygen-absorber

However, the sachets are often made of polyethylene terephthalate (PET) and polyethylene (PE). Not only are these materials non-biodegradable, but they are made from petroleum. This increases the reliance of the society on this non-renewable source. The process of manufacture and disposal also poses negative impact on the environment. Furthermore, the use of metal powder also further exhausts the scarcity of the non-renewable resources.

1.4. Inspirations from Daily Life: Utilizing "Browning" of Avocado Seeds to Absorb Oxygen

It was often observed that a cut avocado seed turns orange gradually upon exposure to air (***Fig. 6***). The observation is similar to the common phenomenon of the "browning" of apples. From the literature review, the development of red-orange colour in the seeds in the air is caused by a chemical reaction catalysed by polyphenol oxidase present, producing a pigment called perseorangin [12] (***Fig. 7***).

Fig.6. Development of red-orange colour on a cut avocado seed after exposure to air

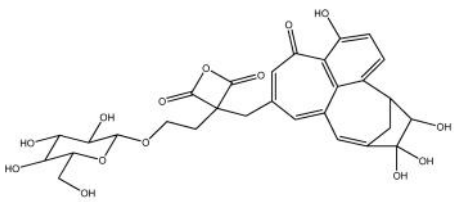


Fig.7. Chemical structure of perseorangin [13]

The reaction might seem undesirable, but it is of interest to us to explore the possibility of utilizing it for its antioxidizing properties and oxygen-absorbing capabilities.

1.5. Avocado Seeds as a Potential Source of Starch and Antioxidants

Avocado seeds are disposed as waste right after detaching from the fruit pulp. However, they contain various types of compounds that can be further extracted and utilised rather than being a mere food waste left in landfills.

It is found out that avocado seeds contain a considerable amount (about 29%) of starch [14]. In particular, avocado seeds contain a higher content (ranging from 35 to 48%) of amylose (a linear form of starch) than other sources [15]. Starch from the seeds can be used for various purposes, including forming gels to synthesize biofilms.

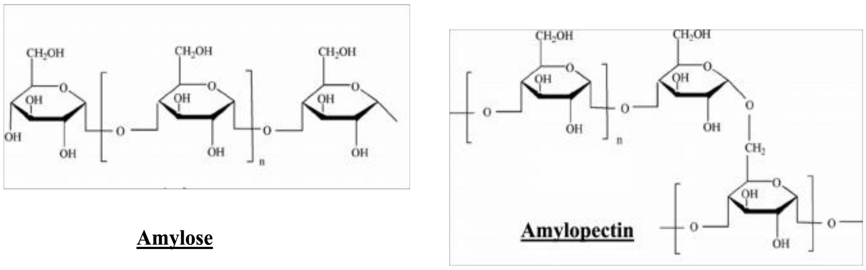


Fig.8. Chemical structures of two forms of starch: amylose and amylopectin [16]

Compound	Content (mg per 100 g)
Procyanidins	152 to 5560
Catechin	24.3 to 2000
Caffeic acid	13.7 to 22.5

Table 1. A list of three major polyphenol compounds identified from an avocado seed [17,18]

It has been identified that the seeds contain a significant level of polyphenol compounds (for example catechin, procyanidins (***Table 1***)) which gives rise to its high antioxidizing property [17,18]. To be particular, the seeds are responsible for about 57% of the total antioxidizing capacity of the whole fruit [19]. Therefore, avocado seeds have the potential as an alternative source of starch and provide anti-oxidizing properties to offer protection against oxidative attack.

1.6. Objectives and Scope of the Project

Packaging plays a crucial role in maintaining food quality for longer periods during delivery and storage. Instead of using non-renewable materials like iron and petroleum-based plastic to create oxygen-absorber sachets and packaging materials, there is a need to find environmentally friendly and sustainable alternatives.

Therefore, the aim of this project is to utilize avocado seeds, a cost-free food waste, to replace existing oxygen-absorbing materials to extend the shelf life and quality of stored food (fruits were the main testing subject in this project). Additionally, the possibility of extracting starch from the seeds to synthesize biodegradable films for making oxygen-absorber sachets and food packaging was also explored. This approach aims to provide more environmentally sustainable alternatives to achieve active packaging and slow down ripening and deterioration of fruits due to oxidative damage.

2. PREPARATION OF AVOCADO SEED SAMPLES

2.1. Choices and Sources of Avocados

Two cultivars of avocados – Hass and Shepard avocadoes (*Persea americana*.) were selected for investigation in this project (***Fig.9, 10***).

Fig.9. Hass avocados used in this project

Fig.10. A Shepard avocado used in this project

They were chosen as they were the two most commonly available in local wet markets. The avocados used in doing the following experiments were purchased from Yau Ma Tei Wholesale Fruit Market with the brands of H.J.K. Farms Pty Ltd. (Hass) and Sunnybank Grove (Shepard) from Australia respectively.

2.2. Pretreatment of Avocado Seeds

All avocados bought were cut open and the seeds were extracted. The seeds were then washed gently under running tap water with their endocarps attached. The seeds were dried with towel papers (***Fig.11, 12***) and were stored in a sealed vacuumed plastic bag at 4 ^{o}C in a refrigerator for later use.

Fig.11. A seed (with endocarp) from a Hass avocado

Fig.12. A seed (with endocarp) from a Shepard avocado

3. PART 1: USE OF AVOCADO SEEDS AS POTENTIAL FOOD PRESERVATIVE INGREDIENTS

3.1. Anti-oxidizing Property of Avocado Seeds

As mentioned in Section 1.5, avocado seeds consist of a considerable amount of natural phenolic compounds (like (+)-catechin, (−)-epicatechin, and 3-leucoanthocyanidins) [20] that contribute to their high anti-oxidizing power. Therefore, the following experiment was conducted to study their anti-oxidizing property and hence their potential to protect food against oxidation.

3.1.1. Preparation of Avocado Seed Extracts

To extract any natural anti-oxidizing compounds from avocado seeds (like phenolic compounds), solvent extraction with different solvents was applied.

3.1.1.1. Choice of Solvents

Distilled water and 95% ethanol were used to extract active compounds from the avocado seeds. The choice of ethanol is based on research studies that show many antioxidizing polyphenol compounds are fairly soluble in ethanol [21]. Distilled water was selected to explore the feasibility of using the safest and the most available solvent to extract active compounds.

3.1.1.2. Method, Results and Discussion

<u>**Method**</u>

1. Remove the endocarp from the avocado seeds.
2. Cut the seeds with a knife into tiny lumps.
3. Grind the lumps into powder.

Fig.13. The sieve used to standardise the diameter of powder particles

Fig.14. The filtration set-up

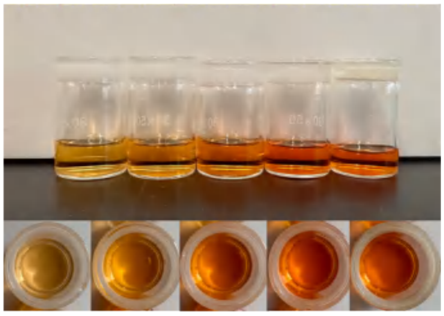
4. Sieve through the seed powder to obtain a size of 0.23 mm diameter (***Fig. 13***).
5. Place a measured mass (0.075 g, 0.15g, 0.33g, 0.67g, 1.33g, 2g) of seed powder into a beaker.
6. Pour 10 mL of solvent (distilled water or 95% ethanol) into the beaker.
7. Allow the mixture to stand for 24 hours.
8. Filter the mixture to obtain the filtrate as the extract (***Fig. 14***).

Results

Appearance of extracts

(a) Extraction with **distilled water**

(i) **Hass** avocado



(ii) **Shepard** avocado

Fig.15. Avocado seed extracts by using distilled water (i) Hass avocado (ii) Shepard avocado

(b) Extraction with **95% ethanol**

(i) **Hass** avocado

(ii) **Shepard** avocado

Fig.16. Avocado seed extracts by using 95% ethanol (i) Hass avocado (ii) Shepard avocado

Discussion

From the results in ***Fig. 15*** and ***Fig. 16*** , it can be observed that all extracts developed certain degrees of yellow or orange colour. The greater the mass of avocado seeds used, the higher the colour intensity of the extracts. It is noted that the colour of ethanolic extracts by using Shepard avocado seeds were much lighter in colour than their corresponding distilled water extracts. The development of yellow/orange colour can be attributed to the formation of a natural pigment perseorangin by the oxidation reaction as mentioned in Section 1.4.

In order to compare the anti-oxidizing properties of the extracts in a more quantitative manner, DPPH solution was used in the subsequent experiment.

3.1.2. Testing Method – Use of DPPH Solution

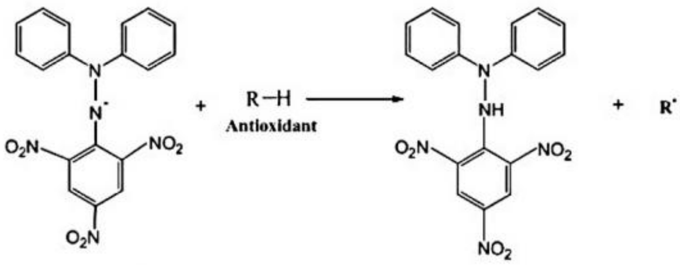
DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable organic compound with a nitrogen radical. It exists in the form of dark purple crystalline solid (***Fig. 17***) under room conditions. When it is dissolved in absolute ethanol, the solution is deep purple in colour (***Fig. 18***) with a light absorbance at around 517 nm.

Fig.17. DPPH solid used

Fig.18. DPPH ethanolic solution prepared

Fig. 19. DPPH solutions after mixing with ascorbic acid at different concentrations (25-125 µg/ml)[22]

A chemical reaction occurs when the DPPH solution is mixed with anti-oxidizing compounds (***Equation 1***). During the reaction, the free radical of the nitrogen atom in a DPPH molecule will receive an electron from the antioxidant, pairing off the odd electron. The resulting product is yellow in colour (***Fig. 19***).



Equation 1. Reaction of DPPH with antioxidant [23]

DPPH can be used to illustrate the action of avocado seed extracts on free radicals, thus indicating their ability to offer protection against oxidative attack from the surroundings. By measuring the change in light absorbance at 517 nm after mixing with the avocado seed extracts, the anti-oxidizing properties of the extracts can be compared. The higher the percentage decrease in the light absorbance at 517 nm (intensity of purple colour) of the final mixture, the higher the anti-oxidizing power of the sample.

In this study, 0.2 mM DPPH solution was used which was prepared by directly dissolving the crystals in absolute ethanol. The flask containing the solution was wrapped with aluminium foil and stored in a refrigerator at 4^{o}C to prevent side reactions of the free radicals. The solution was freshly prepared right before each experiment to minimize self-degradation of DPPH solution upon prolonged storage.

3.1.3. Anti-oxidizing Property of Different Avocado Seed Extracts

Method

1. Prepare control solution by mixing 4 mL 0.2 mM DPPH solution (in absolute ethanol) with 1 mL solvent (distilled water or 95% ethanol) and shake well.
2. Prepare a blank solution by mixing 1 mL avocado seed extract with 4 mL absolute ethanol and shake well.
3. Add 4 mL DPPH solution into 1 mL avocado seed extract and shake well.
4. Place the solutions in the dark for 30 minutes to allow the reaction to take place.
5. Measure the light absorbance of each solution at 517 nm by using a colorimeter with a green light filter.
6. Repeat the steps with all other extracts.

Results

1. Colours of the **control solutions**

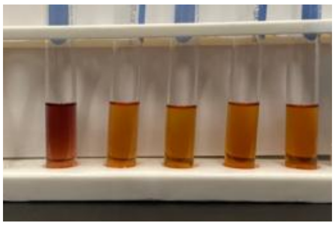
   A mixture of 5 mL DPPH solution with **distilled water (the left)** and **95% ethanol (the right)**

Fig.20. Colours of DPPH solution mixed with distilled water (left) and 95% ethanol (right)

2. Colours of the DPPH/Avocado seed extract mixtures after 30 minutes

(a) **Distilled water extracts**

(i) **Hass** (ii) **Shepard**



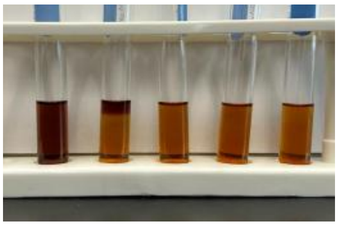
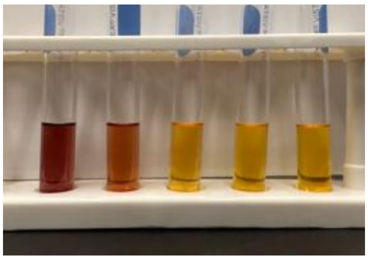


Fig.21. Final colour of the mixture of DPPH solution and different distilled water extracts after 30 minutes

(b) **Ethanol extracts**

(i) **Hass** (ii) **Shepard**



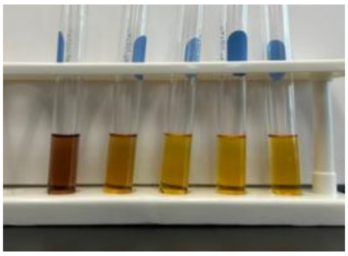


Fig.22. Final colour of the mixture of DPPH solution and different ethanol extracts after 30 minutes

3. Measurement of **Anti-oxidizing Power of Seed Extracts**

The anti-oxidizing power of the extracts, in terms of free radical scavenging power, was compared by calculating the percentage decrease of light absorbance at 517 nm by using the following ***Equation 2*** [24]:

Anti-oxidizing power of avocado seed extract

(percentage decrease in light absorbance at 517 nm)

$$= \left(1 - \frac{A_{sample} - A_{blank}}{A_{control}}\right) \times 100\%$$

Equation 2. Percentage decrease in light absorbance at 517 nm

where A_{sample}, A_{blank} and $A_{control}$ refer to the light absorbance of sample solution, blank solution and control solution at 517 nm

Anti-oxidizing Power (Percentage decrease in light absorbance at 517 nm)

(a) Data arranged according to **types of avocado seeds** used

(for raw data, please refer to Appendix on P.66)

(i) **Hass** (ii) **Shepard**

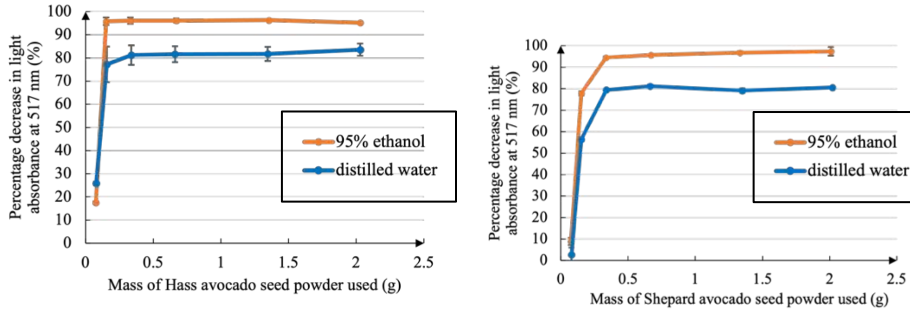


Fig.23. Comparison of percentage decrease in light absorbance at 517 nm after mixing DPPH solution with different avocado seed extracts

(b) Data arranged according to **types of solvent** *(for raw data, please refer to Appendix on P.66)*

(i) **Distilled water** (ii) **Ethanol**

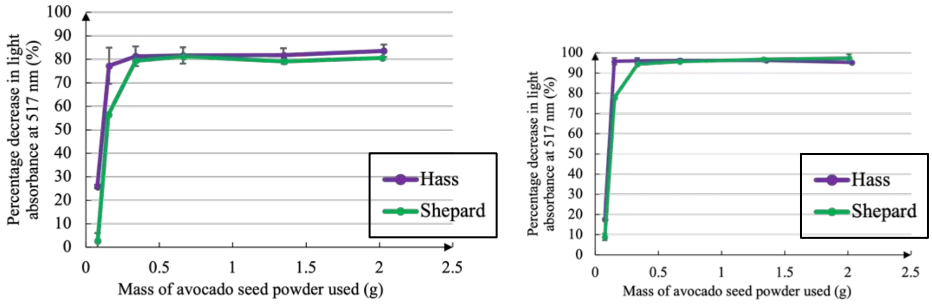


Fig.24. Comparison of percentage decrease in light absorbance at 517 nm after mixing DPPH solution with avocado seed extracts by using different solvents

Discussion

According to the final colours of mixture of DPPH solution and avocado seed extracts shown in ***Fig. 21*** and ***Fig. 22***, all of them demonstrated observable decreases in purple intensities of the DPPH solutions. These results preliminarily indicated that both distilled water and ethanol extracts of Hass and Shepard avocado seeds demonstrated significant antioxidizing properties. The conclusion was further supported by the measurements of light absorbance at 517 nm.

From the results in ***Fig. 23***, all extracts prepared from 0.33 g or more of the seeds showed at least 79.38% decrease in light absorbance at 517 nm. In particular, the highest performance of 97.38% and 96.32% were obtained when 0.2 g/cm^3 Shepard seed extract (2 g of Shepard avocado seed used in 10 mL solvent) and 0.133 g/cm^{3}of Hass seed extract with ethanol.

To compare the extraction efficiency of the solvent, 95% ethanol is a better choice than distilled water for both types of avocado seeds. In most of the cases, ethanol extracts led to larger decrease in light absorbance at 517 nm when compared with water extracts using the same mass of seeds. The largest difference (21.5%) was observed when 0.15 g of Hass seeds were used.

From the results in ***Fig. 24***, all extracts showed significant antioxidizing power when 0.15 g of seed was used in extraction with 10 mL solvents (0.015 g/cm^3). Both Hass and Shepard avocado seeds exhibited similar degree of antioxidizing power when larger masses (0.5 g or more) of seeds were used in both solvents. However, when a small amount (0.15 g and 0.33 g) of the seeds was used, Hass avocado seed extracts showed a significantly better performance than Shepard seed extracts (23.16% higher than Shepard in the case of 0.075 g of seeds extracted with distilled water and 17.95% higher than Shepard in the case of 0.15 g of seeds extracted with ethanol).

With the promising antioxidizing power shown in the experiments, the following experiments were carried out to explore whether we can utilise this property to reduce the oxygen content in a confined area, thus achieve the purpose of food preservation.

3.2. Oxygen-Absorbing Property of Avocado Seeds

Since both Hass and Shepard avocado seeds demonstrated significant antioxidizing power in Section 3.1, it is of interest to us that whether the seeds can even absorb or remove oxygen in a confined area by oxidation reaction. Therefore, the oxygen-absorbing properties of both types of seeds were studied and compared with the commercial oxygen-absorber materials.

3.2.1. Testing Method and Experimental Set-up

To test the oxygen-absorbing ability of a sample, an experimental set-up was designed and set up.

Fig.25. The set-up used to measure oxygen-absorbing ability of a sample

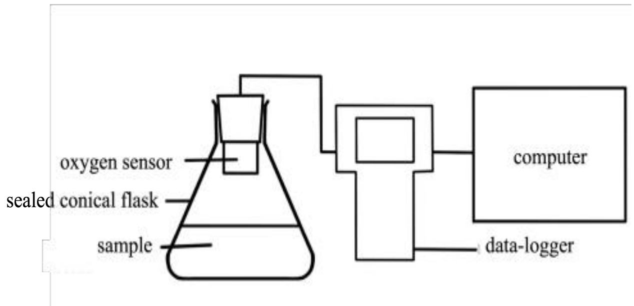


Fig.26. The design diagram of the set-up

A sealed conical flask with 137 mL volume was embedded with oxygen sensor (*PASPort TM*) connected to a data-logger (*PASCO PS-2002*) and computer. The stopper was smeared with Vaseline and the mouth was wrapped with parafilm to ensure an enclosed environment. The data-logger and computer monitored and recorded the change in oxygen gas concentration in the conical flask real-time.

3.2.2. Commercial Oxygen Absorbers for Comparison

To compare the effectiveness of removing oxygen in an enclosed space, commercial oxygen absorber sachets were purchased from Sugar House Baking Ingredients Store. The major active components of the oxygen absorber are fine iron powder, activated charcoal and sodium chloride (***Fig. 27***). Iron removes oxygen from air by oxidation while activated charcoal adsorbs the gas. The content of the sachets was taken out and its oxygen-absorbing ability was measured with the above experimental set-up.

Fig.27. Commercial oxygen absorber sachet with its content

Method

1. Calibrate the oxygen sensor in the conical flask of the above experimental set-up to 20.9%.
2. Run the programme on the computer to monitor the change in oxygen concentration in the air present in the flask (10 seconds per measurement) (***Fig. 28***).
3. Take out the content from the oxygen absorber sachet.
4. Measure the mass (0.5 g, 2 g, 4 g and 6 g) of content quickly.
5. Put the content into a conical flask with air volume of 137 cm^3 and assemble the above set-up.
6. Stop the measurement until there is no further change of oxygen concentration for at least 10 minutes.

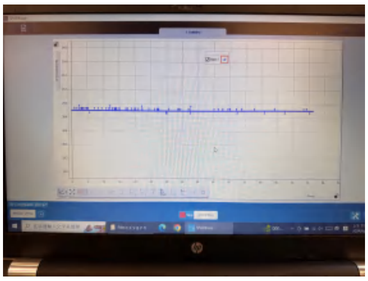


Fig.28. The computer programme recording the change of oxygen gas concentration in a control.

Results

(a) **Appearance** of the content:

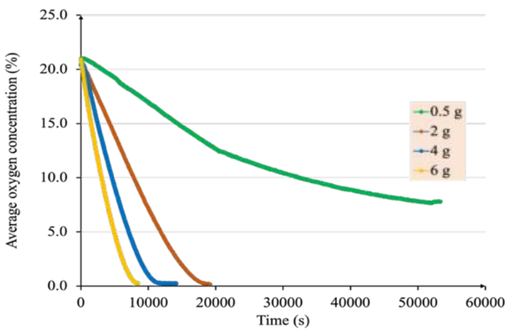
(i) Freshly-taken out (ii) After the experiment

Fig.29. Appearance of commercial oxygen absorber content (i) before exposing to air (ii) after exposing to air about 2 hours

(b) **Oxygen-absorbing Ability of Commercial Oxygen Absorber Content**

(for raw data, please refer to Appendix on P.67)

(i) Decline in oxygen concentration



(ii) Time needed to remove all oxygen

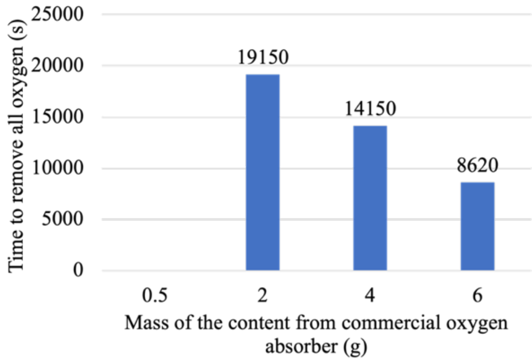


Fig.30. (i) A graph showing the change of oxygen gas concentration; (ii) time needed to remove all oxygen from the flask (137 cm^3) with different masses of content from the commercial oxygen absorber used;

Discussion

From the results in ***Fig. 30(i)***, all oxygen in the flask was removed when 2 g or more of the content from the commercial oxygen absorber. For the case of 6 g of the content, the shortest time of about 2 hours (8620 seconds) were needed to absorb all oxygen. However, for the case of 0.5 g of the content, the final oxygen gas concentration only dropped to 7.7% after about 14 hours (53340 seconds) without approaching 0% for the working volume of 137 cm^3.

3.2.3. Oxygen-absorbing Ability of Avocado Seed Powders

<u>Method</u>

1. Calibrate the oxygen sensor in the conical flask of the above experimental set-up to 20.9%.
2. Run the programme on the computer to monitor the change in oxygen concentration in the air present in the flask (10 seconds per measurement).
3. Remove the endocarp from the avocado seeds.
4. Cut the seeds with a knife into tiny lumps.
5. Grind the lumps into powder by using a household blender ***(Fig. 31)***.

Fig.31. The household blender used

6. Sieve through the seed powder to obtain powder of the size of 0.23 mm diameter.
7. Measure the mass (6 g, 8 g, 12 g and 20 g) of content quickly.
8. Put the powder into a conical flask with air volume of 137 cm^3 and assemble the above set-up.
9. Stop the measurement until there is no further change of oxygen concentration for at least 10 minutes.

<u>Results</u>

(a) Appearance of **Hass** avocado seeds:

(i) Before experiment:
Pale pink solid

(ii) After experiment:
Red-orange solid

Fig.32. Hass avocado seed powder before and after experiment.

(b) Appearance of **Shepard** avocado seeds:

(i) Before experiment

Pale brown solid

(ii) After experiment

Red-orange solid

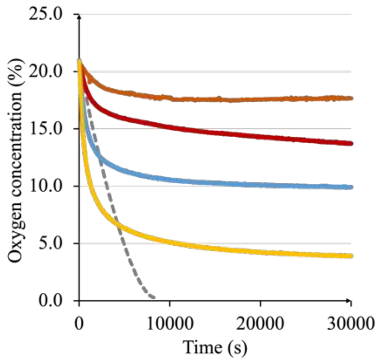
Fig.33. Shepard avocado seed powder before and after experiment.

(c) Oxygen-absorbing Ability of Avocado Seeds

(i) Data arranged according to **types of avocado seeds** used

(for raw data, please refer to Appendix on P.68)

I. Hass



II. Shepard

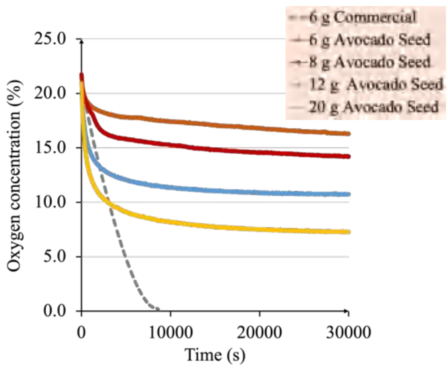
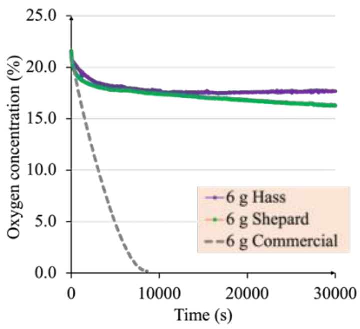


Fig.34. A graph showing the change of oxygen gas concentration with different masses of (I) Hass avocado and (II) Shepard avocado seed powders used

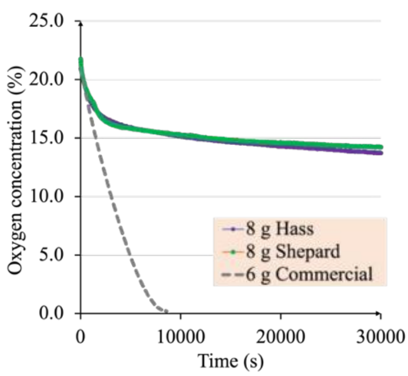
(ii) Data arranged according to **masses of avocado seeds** used

(for raw data, please refer to Appendix on P.68)

I. 6 g



II. 8 g



III. 12 g **IV. 20 g**

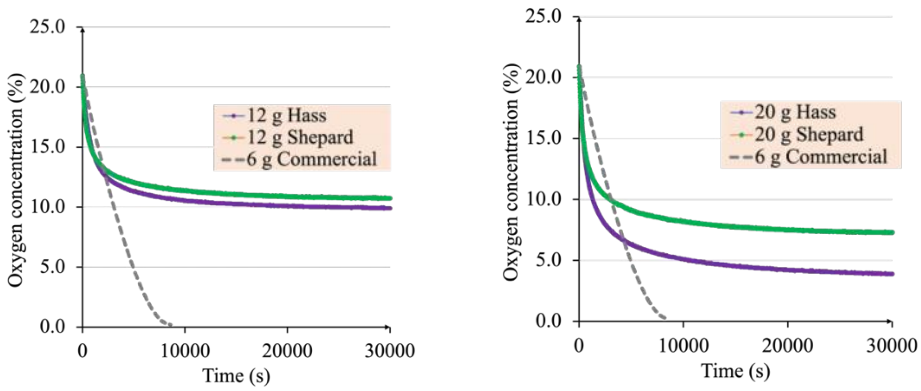


Fig.35. A graph showing the change of oxygen gas concentration with (I) 6 g (II) 8 g (III) 12 g and (IV) 20 g of avocado seeds used

Discussion

From the results in ***Fig. 32*** and ***Fig. 33***, it was shown that the avocado seed powders turned orange-red in colour in about 10 minutes after exposing to the air. It can be explained by the formation of coloured pigment perseorangin due to oxidation with the atmospheric oxygen [25].

From the graphs in ***Fig. 34***, both Hass and Shepard avocado seeds of different masses exhibited a considerable degree of oxygen-absorbing ability. The more seed powders used, the more oxygen was removed. In particular, 20 g of Hass avocado seed powders absorbed the greatest amount of oxygen (17.9%), resulting in the final oxygen concentration of 3.0 %.

To compare the two types of avocados, both seeds showed similar oxygen-absorbing abilities when less than 8 g was used. However, in the cases of 12 g and 20 g, Hass avocado seeds exhibited an increasingly better performance than Shepard seeds of the same masses. In the case of 20 g of seed used, Hass avocado seeds reduced the oxygen concentration in 137 mL space to a final level of 3.0% after about one day. This value was 4% lower than that of Shepard seeds (7.0%).

These experimental results ensure the potential of using avocado seeds as a material for absorbing oxygen. However, as compared with the 6 g commercial oxygen absorber content (mainly iron powder and activated charcoal) in the dotted lines in ***Fig. 34*** and ***Fig. 35***, the performances of avocado seeds were still not as good. Therefore, the following experiments were conducted to further enhance the oxygen-absorbing ability of the seed powders.

3.2.4. Enhancement of Oxygen-Absorbing Property of Avocado Seeds

To enhance the ability of avocado seeds to remove oxygen from a confined space, some common highly antioxidizing powders were added to the seed powders to study their effects on the oxygen-absorbing property.

3.2.4.1. Choice of Avocado Seeds

As Hass avocado seeds removed the greatest amount of oxygen in the results shown in ***Fig. 35***, they were used instead of Shepard avocado seeds in the subsequent experiments.

3.2.4.2. Choice of Antioxidizing Powders

Three commonly known powders with high antioxidizing performance were mixed with the avocado seeds in attempt to improve their oxygen-absorbing ability. The powders selected include sodium ascorbate powder, green tea powder and turmeric powder (***Fig. 36***). The following table (***Table 2***) shows the source of each type of powders used in this project:

Powder	Source
Sodium ascorbate	NutriBiotic
Green tea	Ten Ren's Tea
Turmeric	GBT Trading Sdn. Bhd.

Table 2. A table showing the sources of sodium ascorbate, green tea and turmeric powder used in this project

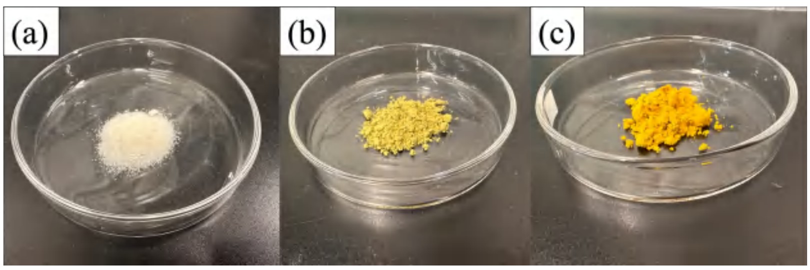


Fig.36. Photographs of the sodium ascorbate, green tea and turmeric powder used.

3.2.4.3. Antioxidizing Properties of the Powders

To further demonstrate and compare the antioxidizing properties of the selected powders, DPPH test described in Section 3.1.2 on p.6 was carried out.

Method

1. Measure a series of masses of sodium ascorbate powder (0.02 g, 0.04 g, 0.08 g, 0.15 g, 0.33 g and 0.6 g).
2. Stir the powder well in 10 mL of distilled water (***Fig. 37***).
3. Filter the extract to remove any suspending powder to obtain a clear filtrate.
4. Carry out the steps listed mentioned in Section 3.1.3 on p.6.
5. Repeat the experiment with green tea powder and turmeric powder.

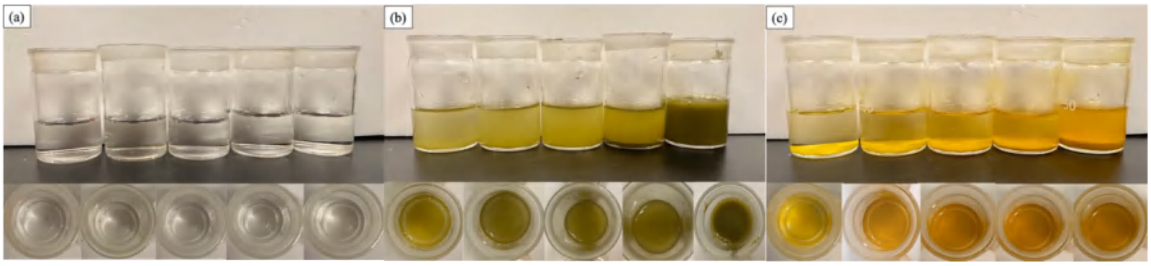


Fig.37. Solutions of (a) sodium ascorbate (b) green tea powder and (c) turmeric powder in distilled water

Results

Anti-oxidizing Power (Percentage decrease in light absorbance at 517 nm)

(for raw data, please refer to Appendix on P.71)

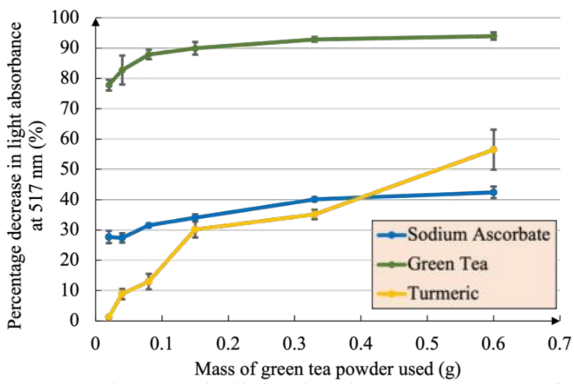


Fig.38. Comparison of percentage decrease in light absorbance at 517 nm after mixing DPPH solution with sodium ascorbate, green tea and turmeric solutions

Discussion

According to the result shown in ***Fig.38***, all solutions of sodium ascorbate, green tea and turmeric demonstrated considerable antioxidizing powers except the solution prepared with 0.02 g of turmeric powder, ranging from 8.86% to 93.98%. In particular, green tea solutions exhibited the highest antioxidizing power among others in all masses. Solution prepared from 0.6 g of green tea powder reduced light absorbance at 517 nm by 93.98%, demonstrating its promising power in cleaving free radicals in DPPH solution.

3.2.4.4. Effects of the Powders on the Oxygen-Absorbing Ability of Avocado Seeds

Due to the promising antioxidizing power of the powders in Section 3.2.4.3, they were blended and mixed with Hass avocado seeds and the oxygen-absorbing abilities of the mixture were measured.

Method

1. Calibrate the oxygen sensor in the conical flask of the above experimental set-up to 20.9%.
2. Run the programme on the computer to monitor the change in oxygen concentration in the air present in the flask (10 seconds per measurement).
3. Remove the endocarp from the avocado seeds.
4. Cut the seeds with a knife into tiny lumps.
5. Measure the mass of the seed lumps
6. Mix the seed lumps with sodium ascorbate powder, green tea powder and turmeric powder according to the following mass ratio:

Mass of Hass avocado seed (g)	0	10	13.3	16
Mass of Enhancer powder (g)	20	10	6.67	4
Mass ratio of avocado seed to powder	**0 : 1**	**1 : 1**	**2 : 1**	**4 : 1**

7. Grind the solid mixture into powder by using a household bean blender.
8. Sieve through the powder to obtain a size of 0.23 mm diameter.
9. Place 20 g of powder into a conical flask with air volume of 137 cm^3.

10. Stopper the flask (sealed with Vaseline) with the probe of a calibrated oxygen sensor connected to a data-logger and a computer.
11. Run the programme on the computer to monitor the change in oxygen concentration in the air present in the flask (10 seconds per measurement).
12. Stop the measurement until there is no further change of oxygen concentration for at least 10 minutes.

Results

(a) Appearances of **mixtures of avocado seed and different powders**:

(i) **Sodium ascorbate**

Mass ratio of avocado seed to powder	Time of exposure to air (hour)		
	0	12	24
1 : 1			
2 : 1			
4 : 1			

Table 3. Appearances of mixture of avocado seed and sodium ascorbate after different time of exposure to air

(ii) **Green tea powder**

Mass ratio of avocado seed to powder	Time of exposure to air (hour)		
	0	12	24
1 : 1			
2 : 1			
4 : 1			

Table 4. Appearances of mixture of avocado seed and green tea powder after different time of exposure to air

(iii) **Turmeric powder**

Mass ratio of avocado seed to powder	Time of exposure to air (hour)		
	0	12	24
1 : 1			
2 : 1			
4 : 1			

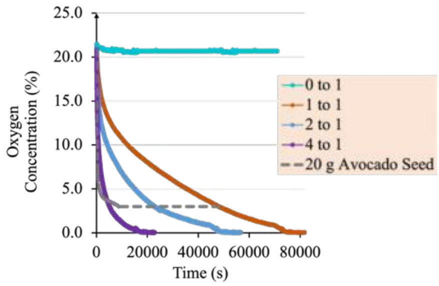
Table 5. Appearances of mixture of avocado seed and turmeric powder after different time of exposure to air

(b) Oxygen-absorbing Ability of the Mixtures

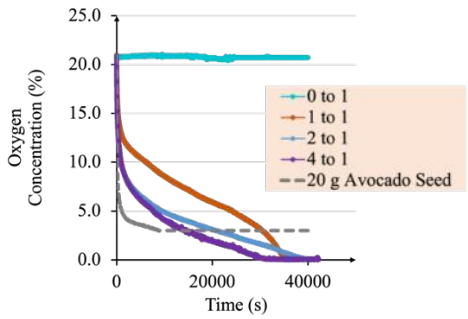
(i) Data arranged according to **types of powders** used

(for raw data, please refer to Appendix on P.72)

(I) Sodium Ascorbate



(II) Green Tea



(III) Turmeric Powder

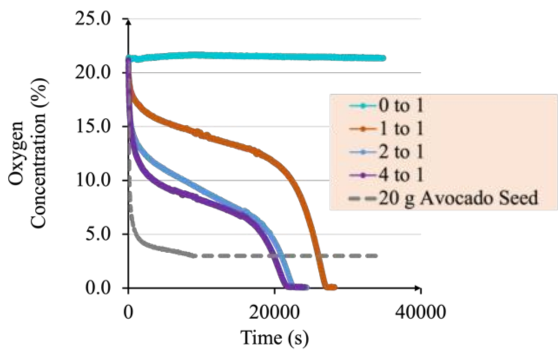
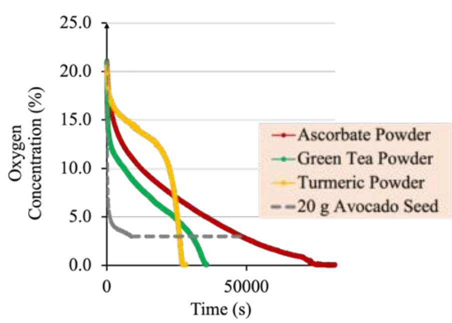


Fig.39. A graph showing the change of oxygen gas concentration with mixtures of different mass ratio of avocado seed to (I) sodium ascorbate; (II) green tea and (III) turmeric powders used

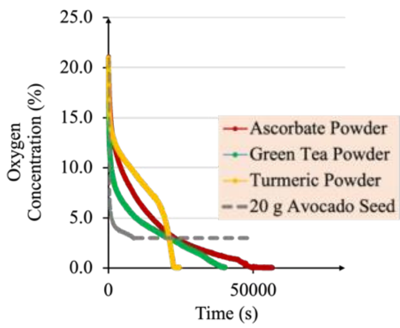
(ii) Data arranged according to **mass ratio of avocado seed to the powder** used

(for raw data, please refer to Appendix on P.72)

(I) 1 : 1



(II) 2 : 1



(III) 4 : 1

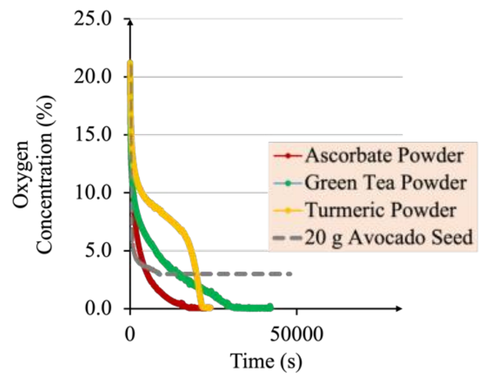


Fig.40. A graph showing the change of oxygen gas concentration with mixtures of avocado seed and the powders of mass ratio of (I) 1 : 1; (II) 2 : 1; and (III) 4 : 1.

Discussion

From the results in ***Fig. 39***, all avocado seeds mixed with sodium ascorbate, green tea and turmeric powders reduced oxygen gas concentration in the flask of 137 cm^3 to 0%. Comparing with only using 20 g seed powder without adding any additives, the mixture further reduced the oxygen content by 3%.

For all cases with different powders added, mass ratio of avocado seed to powder 4:1 took the shortest time to remove all the oxygen in the flask of volume 137 cm^3(***Fig. 40***). In particular, mixture with sodium ascorbate took the shortest time among all cases to remove all oxygen.

However, results from using solely the powders without mixing the avocado seeds did not show any signs of oxygen absorption. This is suggested that the powders might couple with avocado seeds and introduce a new mechanism to absorb oxygen. The oxygen-absorbing performance of the seed was significantly enhanced. One of the proposed reasons is that when the seed powder reacted with oxygen, the antioxidizing powder present protected the seed, and induced a more rapid reaction between oxygen and the powder.

The result was supported by the appearance of the avocado seeds after mixing with different powders. From ***Table 3*** to ***Table 5*** on p.15, it was observed that the development of red-orange colour (as an indication of formation of oxidation product) was tampered with, as compared with the final colour of the pure seed after exposing to air in ***Fig. 32*** and ***Fig.33*** on p.11.

3.2.5. Oxygen-absorbing Capacity of Avocado Seeds

From the previous sections, it was proved that avocado seeds have a significant ability to absorb oxygen from an enclosed environment. In this section, experiments were carried out to study the capacity of the seed/ascorbate mixture to absorb oxygen.

<u>Method</u>

1. Add various masses (10 g, 5 g, 3 g) of seed/sodium ascorbate mixture in 4 : 1 mass ratio to a flask of 137 cm^3.
2. Enclose the flask with a stopper embedded with an oxygen sensor.
3. Seal the flask securely.
4. Monitor the change in oxygen content in the flask by a data-logger connected to the software on a computer.

<u>Results</u>

Decline in oxygen concentration *(for raw data, please refer to Appendix on P.74)*

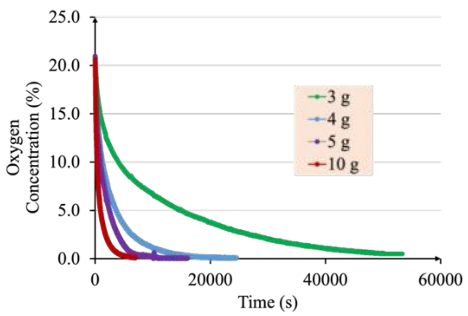


Fig.41. A graph showing the change of oxygen gas concentration using different masses of avocado seed/sodium ascorbate mixture in 4:1 mass ratio.

Discussion

From ***Fig. 41***, it can be seen that the minimum mass of avocado seed/sodium ascorbate mixture in 4:1 mass ratio needed to remove all oxygen in a flask of 137 cm^3 was between 3 g to 4 g. Therefore, it was estimated that the oxygen-absorbing capacity of the avocado seed /sodium ascorbate mixture is between 34.3 cm^3/g to 45.6 cm^3/g (volume of air per gram). Comparing to the value of the commercial oxygen-absorber sachets which is 109 cm^3/g, the performance of the avocado seed oxygen absorber was not as good, however, it is still a considerable alternative considering its much reduced cost and impact on the environment.

4. PART 2: DELAY RIPENING OF FRUITS

To test for the effectiveness of using avocado seeds as an oxygen absorber to preserve food, a certain fruit were selected for the following experiments.

4.1. Indicator for Level of Ripening of Fruits/Vegetables

In order to assess the level of ripening of fruits over a period of time without interfering with the experimental set-ups, the following observable aspects were evaluated:

- **Colour of the peels and pulps**

  The colours of peels and pulps of the fruit were compared daily. Development of any dark spots or rotten spots was noticed.

- **Firmness of the Surface**

  As the fruits ripen, the inner content will soften and cell walls will start to deteriorate [26], resulting in a decline in firmness and smoothness of the outer surface.

4.2. Choice of Fruits and Vegetables

To illustrate the effect of oxygen absorption by the avocado seeds on ripening and deterioration of fruits (as mentioned in Section 1.2 on p.1), a climacteric fruit –bananas *(Musa paradisiaca)* were studied in the experiments (***Fig. 42***).

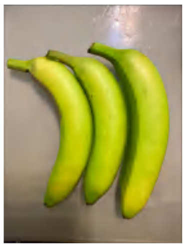


Fig.42. Bananas used

The bananas used in the following experiments were purchased from local Yau Ma Tei Wholesale Fruit Market. Immature bananas of the same batch with smooth surface and similar colour were selected for doing the experiment.

4.3. Testing with Bananas

Method

1. Prepare the following powder mixture and put them into a tea bag sachet:

	Set-up				
	1	2	3	4	5
Content apart from the fruits	Nil (control)	20 g avocado seed powder	20 g of mixture of seed and **sodium ascorbate** in 4:1 mass ratio	20 g of mixture of seed and **green tea powder** in 4:1 mass ratio	20 g of mixture of seed and **turmeric powder** in 4:1 mass ratio

2. Put one banana into an airtight box of dimension 18 cm (L) x 11 cm (W) x 5 cm (H) (volume of 990 cm^3) together with one of the prepared sachets. Bananas came from the same batch with similar initial pale green colour of the peels. The boxes were placed in the same area under room conditions.
3. Close the box and record the appearance of the banana in each box.

Results

Appearance of bananas

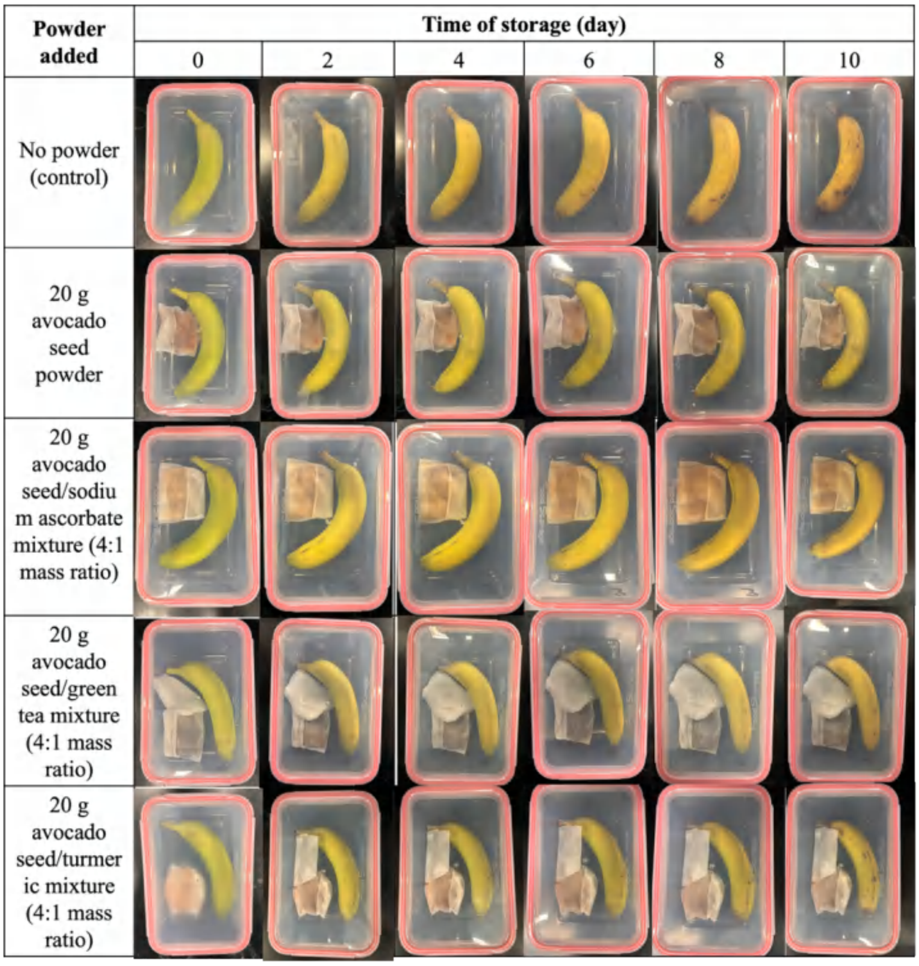


Fig.43. Photographs showing the change in degree of ripeness of the bananas

Discussion

From ***Fig. 43***, the peels of all bananas turned yellow during the course of the experiment. However, the banana in the control turned most yellow with most dark spots on Day 10 of the experiment (***Fig. 45***). For set-ups 2 to 6, traces of green colour, which is an indicator of immaturity, could still be found. For the pulps, the control one showed a dark brown colour while that of setup 2 remains yellowish white.

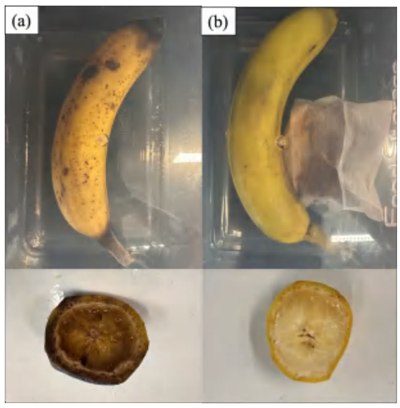


Fig.45. The banana in (a) control (b) setup 2 with avocado seed powder after 10 days

Besides, many water droplets could be seen on the inner wall of the container of the control while this was not observed in other setups (***Fig. 46***). This indicated a higher rate of respiration for the banana inside comparing with other set-ups. As mentioned in Section 1.2, a higher rate of respiration would lead to a higher rate of ripening and deterioration of fruits.

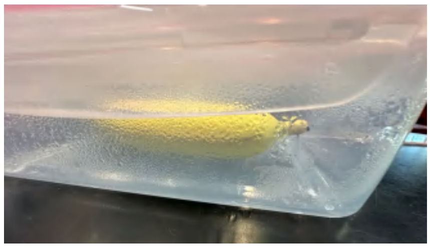


Fig.46. Water droplets observed in the control on Day 1

The above result serves as a preliminary finding to demonstrate that the avocado seed powders have an noticeable effect in slowing down ripening of fruits by removing the atmospheric oxygen.

5. PART 3: SYNTHESIS OF AVOCADO SEED PACKAGING MATERIALS

To even better utilise the avocado seeds, it was our second focus to synthesize packaging materials by extracting starch from the seeds, either for holding food/fruit or the oxygen-absorber for food preservation to replace the existing petroleum-based materials of polyethylene terephthalate (PET) and polyethylene (PE). Therefore, the following experiments were conducted to explore the potential of using avocado starch to make packaging materials.

5.1. Extraction of Avocado Seed Starch

According to literature review, an average Hass avocado seed consists of about 29% starch [27]. Therefore, the following experiment aimed to extract starch from the seeds.

Method

1. Measure the mass of avocado seeds and cut them into small pieces.
2. Immerse the pieces with 250 mL 0.2% sodium metabisulphite solution for 24 hours to prevent any undesired oxidation reaction (***Fig. 47***).
3. Grind the pieces into powder with the sodium metabisulphite solution by using a vacuum blender (Trombe, 50 W) for 1 minute (***Fig. 48***).
4. Filter the homogenised mixture by using muslin cloth and wash the residue with distilled water (***Fig. 49***).


Fig.47. Lumps of avocado seeds soaked with 0.2% sodium metabisulphite solution

5. Leave the mixture to stand for 24 hours in refrigerator (4 ºC) for decantation (***Fig. 50***).
6. Discard the slurry orange upper layer.
7. Wash the residue with distilled water.
8. Centrifuge residue suspension at 1200 rpm for 10 minutes till clear suspension is observed (***Fig. 51***).
9. Discard any upper supernatant slurry orange layers (***Fig. 52***).
10. Dry the residue in an oven at 40ºC and measure the mass.

Fig.48. The vacuum blender with avocado seed extract

Fig.49. Filtration of avocado seed extract

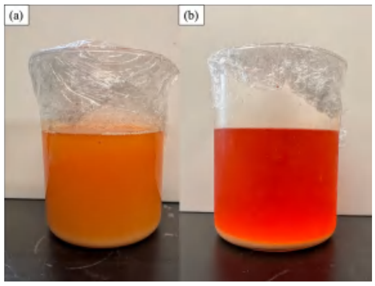


Fig.50. Avocado seed extraction (a) before decantation (b) after decantation

Fig.51. The centrifuge machine

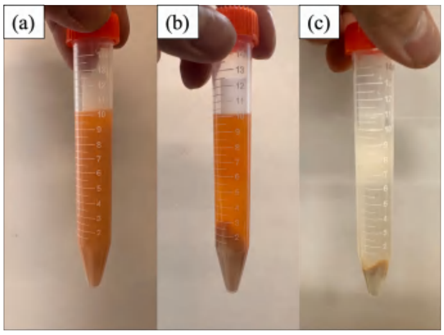


Fig.52. Avocado seed extract after (a) one (b) two and (c) three times of centrifugation

Results

(a) Appearance of crude starch and purified starch

(i) Crude starch **without being centrifuged**: **Red-orange** brittle solid

(ii) Purified starch **after being centrifuged**: **White brittle** solid

Fig.53. Appearance of dried (i) crude starch without being centrifuged and (ii) starch after being centrifuged

(b) **Percentage Yield** of Starch from Avocado Seeds *(for raw data, please refer to Appendix on P.75)*

The percentage yield of starch obtained by using different masses of Hass avocado seeds are calculated by using ***Equation 3***:

$$\textit{Percentage yield of avocado starch} = \frac{\textit{Mass of dried and purified starch } (M_S)}{\textit{Mass of avocado seed used } (M_A)} \times 100\%$$

Equation 3. Percentage yield of avocado seed starch

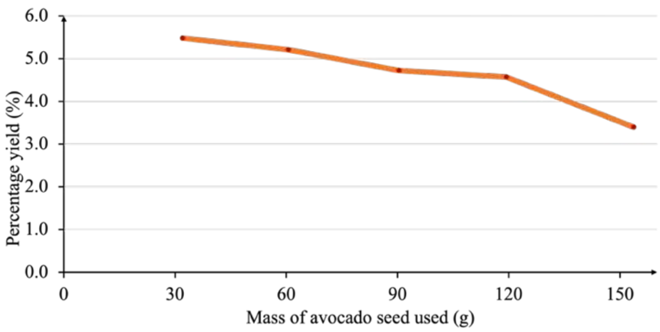


Fig.54. A graph showing the variation of percentage yield of starch obtained when different masses of avocado seeds were used.

Discussion

From ***Fig. 53***, the crude starch without purification is bright orange in colour. The colour indicates the presence of much perseorangin, a pigment formed by enzyme-catalyzed oxidation with the atmospheric oxygen. However, the centrifugation was effective in removing much of the pigment, resulting in pure white solid residue.

The percentage yield of starch obtained was significantly lower than the literature value (29%) [28]. The highest yield of 5.5% was obtained when 31.993 g of avocado seed was used. From ***Fig.54***, the percentage yield of extraction of starch decreased gradually with increasing mass of seed used. One of the possible reasons is that increasing amount of starch was discarded with the orange slurry supernatant layer during the purification. The layer was bright orange in colour, indicating the presence of most perseorangin. As the layer was slurry and sticky in nature, increasing amount of starch would be expected to trap inside and being removed during purification.

To further confirm the identity and purity of the product, two more follow-up experiments were conducted in the upcoming Sections 5.2 and 5.3.

5.2. Simple Qualitive Analysis of the Purified Starch

To test for the identity of the purified product, iodine test was conducted as a simple and preliminary analysis.

Method

1. Place a spatula of purified starch into a petri dish.
2. Add a drop of iodine solution to the solid.
3. Observe any colour change of the iodine solution.

Results

Appearance of purified product

(a) Before iodine test:
White solid powder

(b) After iodine test:
Blue-black colour formed

Fig.55. the appearance of the purified product (a) before and (b) after iodine test

Discussion

From ***Fig. 55***, the iodine solution instantly turned from reddish brown to blue-black upon contacting with the product. This preliminarily indicated that the product contained a significant content of starch.

5.3. Synthesis of Avocado Seed Starch Films

To utilize the starch extracted from the avocado seeds in Section 5.1, the following procedure was conducted to synthesize films to explore the possibility of using them for food packaging.

5.3.1. Gelatinization of Avocado Seed Starch

Avocado seed starch was reported to gelatinise between 60°C to 80°C [29]. During the process, intermolecular attractions between starch granule molecules are broken and new hydrogen bondings are formed with the water molecules [30]. The resulting mixture becomes gel-like.

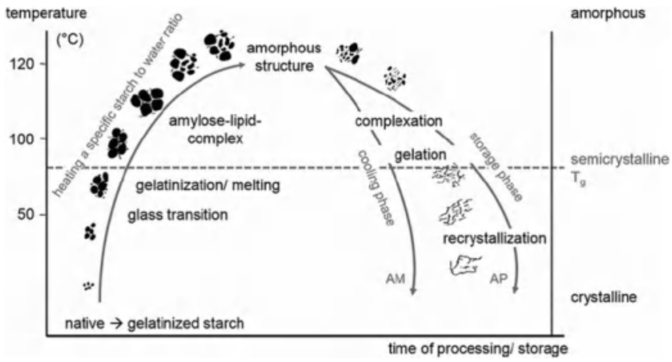


Fig.56. A graph illustrating the change in structure of starch upon heating [31]

5.3.2. Use of Additives

5.3.2.1. Glycerol

To improve the flexibility and hydrophilicity of the starch films made, glycerol (***Fig. 57***) was added [32]. Glycerol acts as a plasticizer during the formation of the film and its molecules can interact with the amylose molecules by forming hydrogen bonds. Thus, increasing the flexibility of the resulting films. The glycerol used in this project was food-grade and purchased from Artemis, Malaysia (***Fig. 58***).

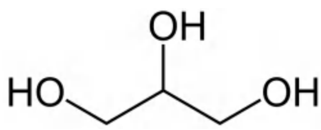
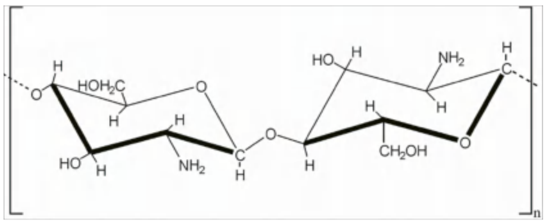


Fig.57. Chemical structure of glycerol

Fig.58. Glycerol used in this project

5.3.2.2. Chitosan

Chitosan is a biopolymer made from chitin extracted from exoskeleton of organisms like insects and shrimps (***Fig. 59***). It was reported that its amino groups and hydroxyl groups forming interaction with the hydroxyl group in starch molecules, forming a polymer with a higher tensile strength [33]. To improve tensile strength of avocado seed starch films, chitosan solution was mixed with avocado seed starch solution in different proportions in the following experiments.



chitosan

Fig.59. Chemical structure of chitosan

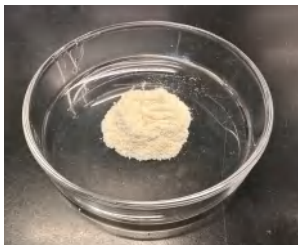


Fig.60. Chitosan powder used in this project

The chitosan powder used in this project was bought from Madar Corporation Ltd., United Kingdom (***Fig. 60***).

5.3.3. Formation of Avocado Seed Starch Films

<u>Method</u>

A. Preparation of Avocado Seed Starch Solution

1. Mix avocado seed starch with distilled water with the mass (g) to volume (mL) ratio of 1:35.
2. Warm the mixture at about 70ºC (not exceeding 80ºC) with stirring for 5 minutes until gelatinization occurs (***Fig. 61***).
3. Add glycerol to the mixture with the volume ratio of distilled water to glycerol = 140 : 3.
4. Keep stirring the mixture for another 30 minutes with the heater turned off.

B. Preparation of Chitosan Solution

1. Mix chitosan powder with 1 % acetic acid with the mass (g) to volume (mL) ratio of 1 : 25, and stir well.
2. Add glycerol into the mixture with the volume ratio of acid to glycerol = 50 : 3 and stir well.
3. Warm the mixture at about 70°C for about 35 minutes (***Fig. 62***).

C. Mixing of Solutions

1. Mix the avocado seed starch and chitosan solutions in the following ratio:

Abbreviation of the film formed	***AC10***	***AC21***	***AC11***	***AC12***	***AC01***
Volume ratio of avocado seed starch to chitosan solution	1:0	2:1	1:1	1:2	0:1

2. Heat the mixture at 70°C for further 20 minutes.
3. Pour 25 mL the mixture into a 10 cm x 6 cm silicone mould.
4. Dry it in 50°C oven until it is dried and the film is detached from the mould (***Fig. 63***).

Fig.61. Avocado seed starch solution after gelatinisation

Fig.62. Chitosan solution

Fig.63. Different gel mixtures in the silicone moulds put in the oven

Results

Appearances and textures of the films

AC10	***AC21***	***AC11***	***AC12***	***AC01***
				
Soft, semi-transparent	Rigid, semi-transparent with slight trace of yellow	Rigid, semi-transparent with slight trace of yellow	Rigid, opaque with pale yellow	Hard, semi-transparent, yellow

Table 6. Comparison of appearances (colour, transparency, rigidity) of the films

Discussion

Fig.64. Side view of ***AC01*** film

From ***Table 6***, pure avocado seed starch film (***AC10***) was soft with much flexibility with the lightest colour.

As the chitosan content increased, rigidity and yellow colour intensity of the films increased gradually. In particular, the rigidity and hardness of ***AC01*** film were so sufficient that it was the only film that maintained a specific fixed shape that it adopted during the drying process (***Fig. 64***).

To compare the suitability of the films for food packaging, a number of properties were assessed in the following experiments.

5.4. Various Properties of Avocado Seed Starch/Chitosan Films

To investigate the feasibility of utilizing avocado seed starch films for food packaging purposes, it is essential to evaluate a range of film properties. The specific criteria to be addressed vary depending on the intended applications. However, this project primarily aims to identify suitable films that can serve as alternatives to petroleum-based polyethylene for producing (1) sachets used in oxygen absorbers; and (2) wrapping packaging materials for food or its containers.

5.4.1. Tensile Strength and Bending Strength

To cater for different purposes in food packaging, the films might need to withstand stress of two types: stretching and bending. For the ability to stretch, the tensile strength should be measured. Tensile strength is defined as maximum stress the film can withstand without breaking when it is being stretched [34].

Some packaging films might need to wrap and bend, therefore bending strength should also be measured. Bending strength is defined as the maximum stress the film can withstand without rapturing when it reaches a specific angle of curvature.

Both strengths of the films were assessed in the following experiment.

5.4.1.1. Design of Experimental Set-up

To test for the tensile strength, the following set-up was devised and set up:

Fig.65. The experimental set-up used to assess tensile strength of the films.

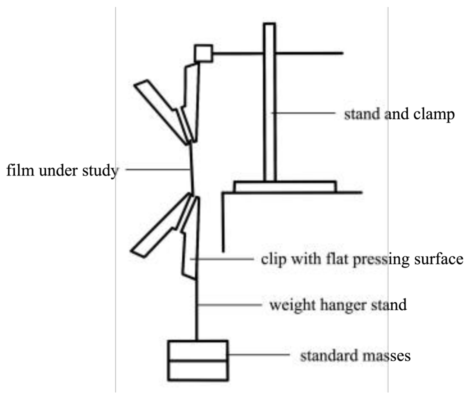


Fig.66. A design diagram of the set-up used

The film under study was held vertically by the clips (***Fig. 65***), and standard masses were hanging to the lower clips sequentially until the film broke. The more masses the film can withstand, the higher its tensile strength.

To test for the bending strength, the following set-up was devised and assembled:

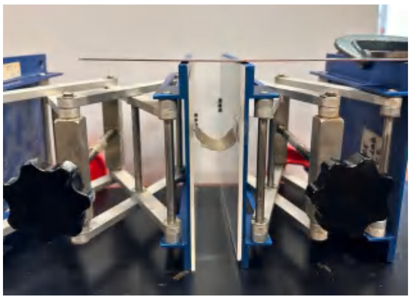


Fig.67. The experimental set-up used to assess bending strength of

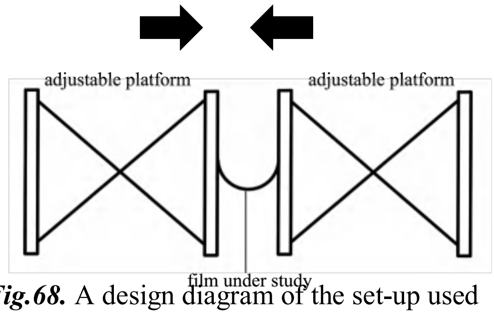


Fig.68. A design diagram of the set-up used

The film under study was hold between two plates with adhesive. The two plates were squeezed towards each other slowly until the film broke. The angle of curvature of the film was monitored (***Fig. 69***).

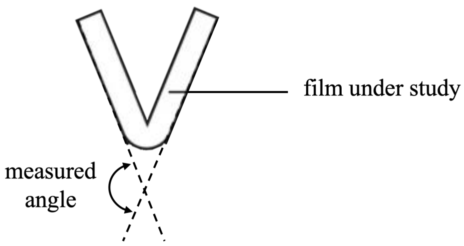


Fig.69. Measured angle to assess the bending strength

The greater the angle curvature before the film breaks, the greater its bending strength.

5.4.1.2. Measurement of Tensile and Bending Strengths

<u>Method</u>

A. Tensile Strength

1. Cut the film to 8 cm x 5 cm.
2. Measure the thickness at 5 spots of the film by using a micrometer.
3. Clip 1 cm of both ends of the film with flat-pressing clips.
4. Measure the initial length of the film between the film.
5. Hang 10g of standard mass to the lower clip one by one.
6. Record the length of the after stabilisation.
7. Record the maximum mass used before the film breaks.

B. Bending Strength

1. Cut the film to 3 cm x 5 cm.
2. Measure the thickness at 5 spots of the film by using a micrometer.
3. Secure both ends of the film to the plate of the set-up by using adhesive.
4. Squeeze the plates towards each other gradually.
5. Record the curvature of the film before it breaks.

<u>Results</u>

A. Tensile Strength *(for raw data, please refer to Appendix on P.76)*

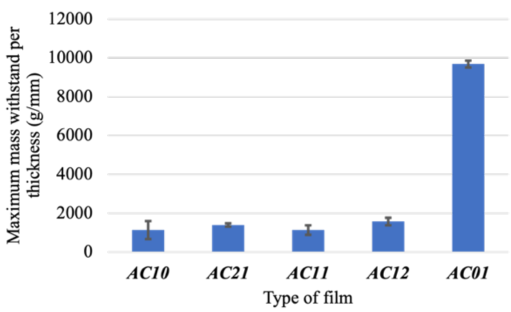


Fig.70. A graph showing the maximum mass per unit thickness each film can withstand during the test

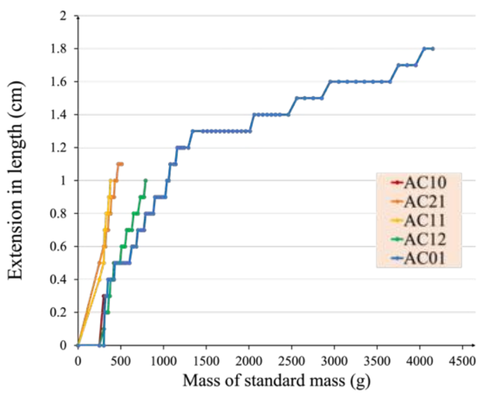


Fig.71. A graph showing the elongation of films against mass they withstood

B. Bending Strength

Angle of curvature of the films before they ruptured:

AC10	***AC21***	***AC11***	***AC12***	***AC01***
No rupture	180^o	180^o	180^o	No rupture

Table 7. Comparison of bending angles of different films

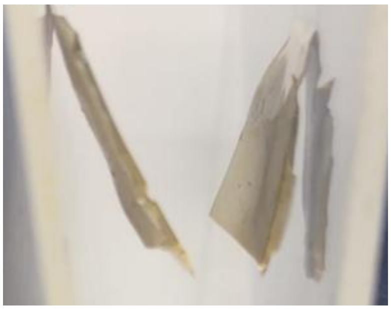


Fig.72. Rupture of ***AC21*** film after the bending strength test

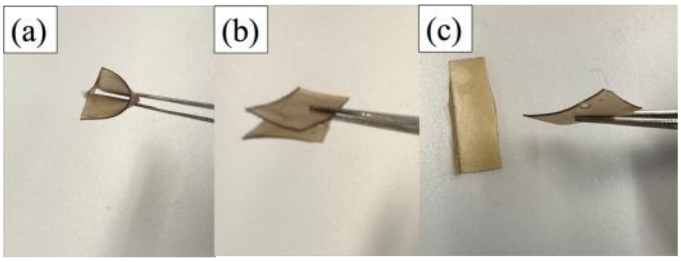


Fig.73. Rupture of ***AC11*** film after bending with a pair of forceps: (a) initial form; (b) right after rupturing; (c) break section of the film

<u>**Discussion**</u>

From ***Fig. 70***, all films with avocado seeds exhibited similar tensile strength. However, pure chitosan film (***AC01***) demonstrated the highest strength.

From ***Table 7***, it can be noted that both pure films ***AC10*** and ***AC01*** demonstrated the highest bending strength. Moreover, all other films formed by mixing avocado seed starch and chitosan could withstand a large bending angle. However, they eventually ruptured once they were entirely folded (bending angle of 180^o). From ***Fig. 72*** and ***Fig. 73***, the ruptured section of the film was very clear.

From the results, it can be concluded that film ***AC10*** and ***AC01*** are more recommended for packaging that requires wrapping and bending, which better fits as a material for oxygen-absorber sachets.

5.4.2. Water Uptake Percentage

Studying the water uptake percentage of biofilm in packaging is essential to ensure the material's durability and effectiveness in packaging content of different dryness. High water uptake can compromise the integrity of the packaging, leading to potential product spoilage and reduced shelf life.

Method

1. Cut the films to 2 cm x 3 cm.
2. Measure the initial mass of the films.
3. Put the films into a static beaker of 50 mL of distilled water at 25°C for 2 hours respectively (***Fig. 74***).
4. Take out the films from the water after 2 hours.
5. Swipe the surface of the films gently with kitchen towel to absorb the water on the surface.
6. Measure the masses of the films.

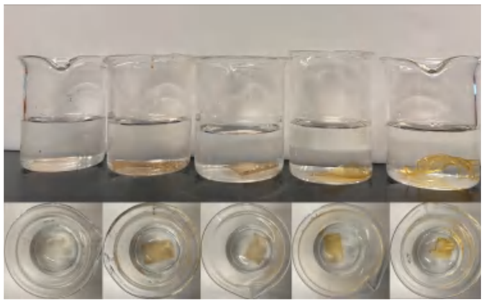


Fig.74. Beakers containing the films for water uptake test (from the left: ***AC10***, ***AC21***, ***AC11***, ***AC12***, ***AC01***)

Results

The water uptake percentage was calculated by using ***Equation 4***:

$$\textbf{\textit{Water uptake percentage}} = \frac{M_f - M_i}{M_i} \times 100\%$$

where
M_i is the initial mass of the film;
M_f is the final wet mass of the film after immersing into water for 2 hours

Equation 4. Water uptake percentage of films **from water immersion**

(a) **Appearance after soaking in distilled water for 2 hours**

All films retained their original size (2 cm x 3 cm) except for ***AC01***. The final dimension of ***AC01*** film was 3 cm x 5 cm.

AC10	***AC21***	***AC11***	***AC12***	***AC01***
				

Table 8. Comparison of sizes of films after soaking in distilled water for 2 hours under room conditions.

(b) **Water uptake percentage** *(for raw data, please refer to Appendix on P.76)*

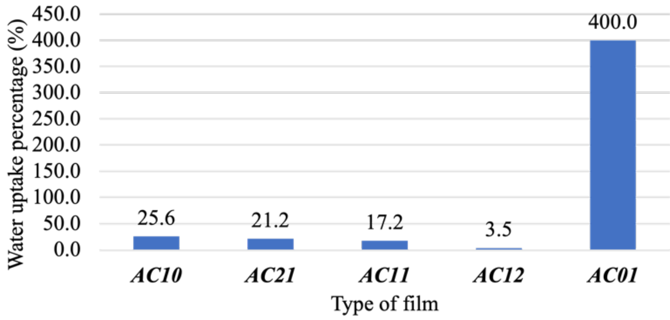


Fig.75. A graph showing the water uptake percentage of different films

Discussion

From ***Fig. 75***, all films exhibited different degrees of water uptake after soaking in distilled water for 2 hours. Apart from pure chitosan film ***AC01***, the water uptake percentage decreased with an increasing chitosan content without changes in dimension (***Table 8***). The overall water uptake was not significant for films ***AC10*** to ***AC12***, in which ***AC12*** only demonstrated 3.5 % water uptake. This is a favourable trait for being oxygen-absorber sachets.

However, it is worth noting that ***AC01*** film absorbed the greatest amount of water during the test. It demonstrated 400% increase in mass. This result was further supported by the drastic increase in area of the film (***Table 8***). The area increased from (2 cm x 3 cm) 6 cm^2 to (3 cm x 5 cm) to 15 cm^2, corresponding to 150% increase.

5.4.3. Water Vapour Permeability

As transmission of water vapour through the film might affect its performance in holding oxygen-absorbing materials or as a protective coating to preserve food, the following experiment was conducted to study the water vapour permeability of the films.

5.4.3.1. Use of Dry Cup Device

With reference to the method suggested by a research [35], a dry cup was used. The dry cup was designed to contain some solid drying agent. The mouth of the cup was covered with a ring which can be locked to the cup using screws (***Fig. 76***). To conduct the experiment, the sample film was secured between the ring and the cup. Side of the mouth of the cup was sealed by wrapping it with parafilm.

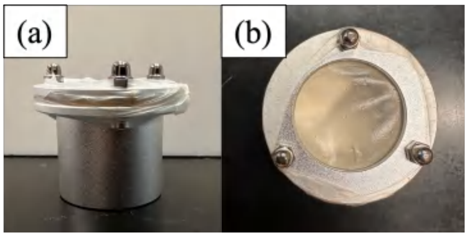


Fig.76. (a) Side view of the dry cup (b) Top view of the dry cup with the film ***AC10*** inserted

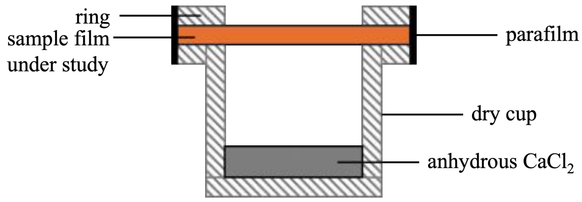


Fig.77. A design diagram of the dry cup device

The device was then put inside an airtight container with 20 mL saturated brine sodium chloride solution to ensure the initial relative humidity inside the container was around 75% to 77%. A portable humidity meter was placed inside the container to monitor the change in humidity.

Fig.78. The set-up for measuring water vapour permeability in air-tight box

When the water vapour passes through the film, it is then absorbed by the drying agent inside with an increase in mass. Therefore, the change in mass of the cup with its content was monitored to study the permeability of water vapour through the film under study.

5.4.3.2. Measurement of Water Vapour Permeability

Method

1. Put a cup of 20 cm^3 saturated sodium chloride solution in an airtight container.
2. Measure the relative humidity inside the box until the reading is steady
3. Measure the thickness of the film by using a micrometer.
4. Put about 2 g of anhydrous calcium chloride into the dry cup.
5. Secure the sample film between ring and the cup (with the diameter of the opening of 3.5 cm).
6. Wrap the side of the cup with parafilm.
7. Measure the initial mass of the cup and its content, as well as the initial relative humidity inside.
8. Put the dry cup into an airtight container box.
9. Seal the airtight box with Vaseline and parafilm
10. Measure the mass of the cup every 30 minute up to 210 minutes.

Results

To calculate the water vapour permeability of each film, the following equation (***Equation 5***) was used:

$$\textbf{\textit{Water Vapour Permeability}} = \frac{G \times d}{S(R_1\text{-}R_2)A} \text{ [35]}$$

Equation 5. Water Vapour Permeability of films

where
G is rate of mass flow (g s^{-1});
d is average thickness of the film (m);
S is the saturation vapour pressure at the tested temperature (Pa);
R_1 and R_2 are relative humidity inside the dry cup and outside the dry cup in the airtight container (%); and
A is transfer area of the film to the dry cup (m^2)

By using the data from the experiment, a graph of mass of dry cup against time was plotted for each film. Then, the rate of mass flow of the dry cup was calculated by the slope of the line obtained from linear regression (r^2>0.99) of the mass-time graph.

(for raw data, please refer to Appendix on P.76)

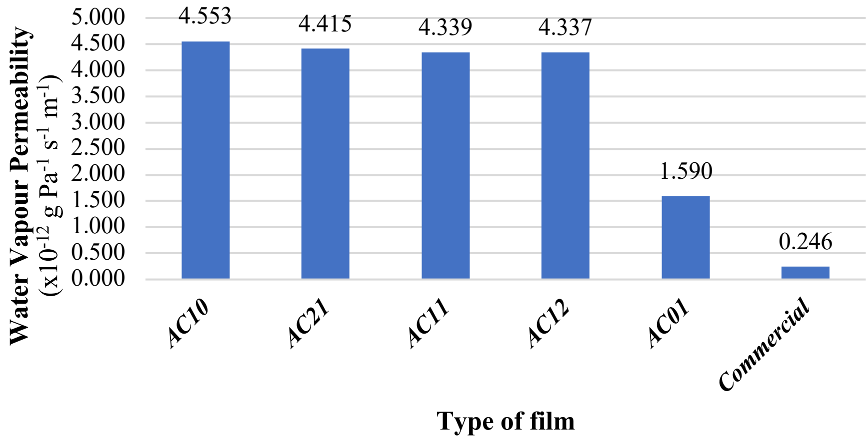


Fig.79. A graph showing the water vapour permeability of the films.

Discussion

From the result shown in ***Fig. 79***, the water vapour permeability for film ***AC10*** to ***AC12*** was significantly higher than that of pure chitosan film ***AC01***. The highest discrepancy between ***AC10*** film and ***AC01*** film was 2.9 times. This indicates that film ***AC01*** could prevent the greatest amount of moisture in air from passing through.

However, if compared with the commercial one, all films synthesized had higher water vapour permeability. In the case of ***AC10***, the water vapour permeability was higher than that of the commercial sachet by 18.5 times.

5.4.4. Oxygen Permeability

As a material for making the sachet for oxygen absorber, high oxygen permeability is an essential property. However, as a protective coating material on food, oxygen permeability is not a desirable trait. Therefore, the following experiment was conducted to compare the oxygen permeability of the films.

5.4.4.1. Design of Experimental Set-up

The following experimental set-up (***Fig. 80***) was designed and assembled with a special glass jar.

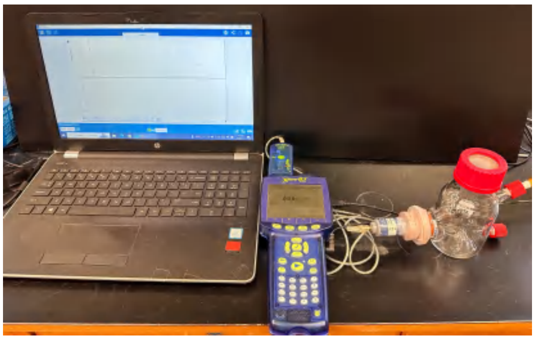


Fig.80. The set-up for measuring oxygen permeability.

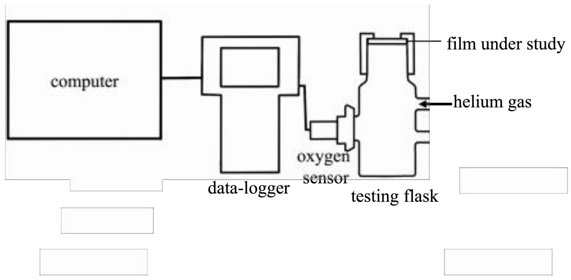


Fig.81. The design draft of the set-up.

The glass jar has two openings of 3.3 cm diameter, together with two tubing outlets. An oxygen sensor connected to data-logger and computer was inserted into opening A (***Fig.82(a)).*** A ring-shaped lid embedded with the sample film was used at opening B (***Fig.82(b)***).

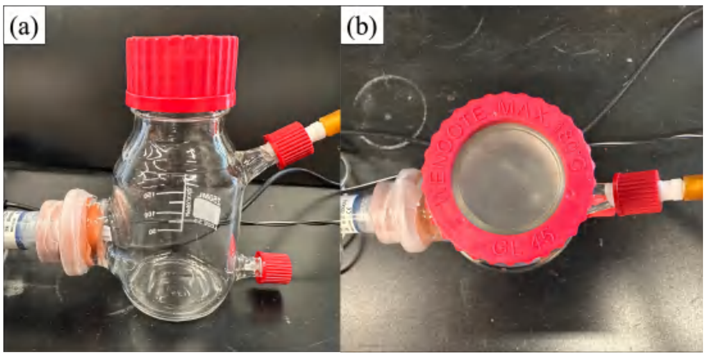


Fig.82. (a) Side view of the flask (b) Top view of the flask with the film ***AC10*** embedded

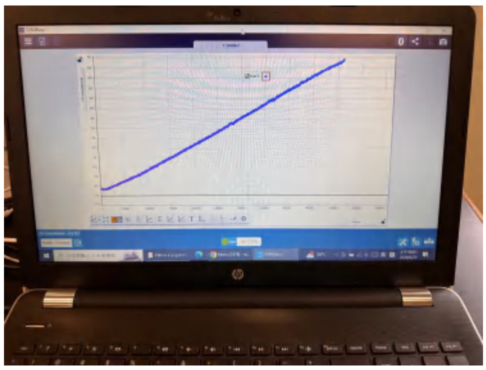


Fig.83. Computer software to monitor and record the oxygen concentration detected in the testing flask

The oxygen inside the flask was first displaced out by pumping helium gas into the flask from the upper outlet. The software on the computer was used to monitor the real-time oxygen concentration inside the flask (***Fig. 83).***

To start the experiment, all outlets of the flasks were covered and sealed with parafilm. The film was the only entry point for the oxygen to enter the flask. The change in oxygen gas concentration inside the flask was monitored by the computer, which indicates the oxygen permeability of the film.

5.4.4.2. Measurement of Oxygen Permeability

<u>Method</u>

1. Cut the film into a circle with 3.5 cm diameter.
2. Embed the film into the ring lid of the measurement flask. (***Fig. 82(b))***
3. Start recording the oxygen concentration by using the oxygen sensor.
4. Connect the upper outlet of the flask with a supply of helium gas.
5. Pump in the helium gas into the flask until the oxygen concentration drops to 1.2%.
6. Monitor the change in oxygen concentration from the software in the computer.

<u>Results</u>

(for raw data, please refer to Appendix on P.79)

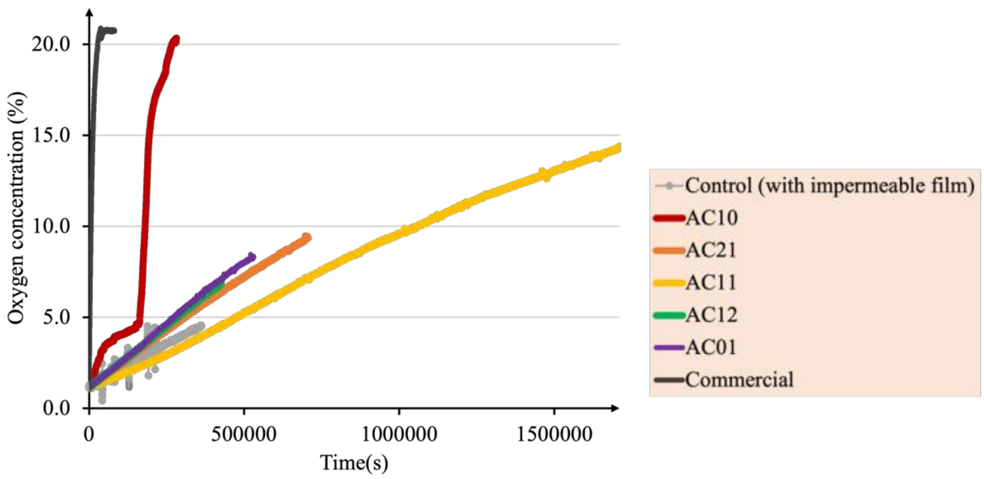


Fig.84. A graph showing the oxygen permeability of the films.

Discussion

From ***Fig. 84***, pure avocado seed film ***AC10*** possessed the highest oxygen permeability compared with all other films synthesised. On the other hand, pure chitosan film ***AC11*** exhibited the lowest oxygen permeability. For other films with mixture of avocado seeds and chitosan, their results were similar. The result indicates that the addition of chitosan lowers the permeability of avocado seed starch films. As a high oxygen permeability is a crucial property of the oxygen-absorber sachet materials, ***AC10*** is deemed to be the most suitable candidate in this aspect.

5.4.5. Light Transmittance

5.4.5.1. Design of Experimental Set-up

To measure the light transmittance of the films, a well-controlled environment was set up by using photography light box (***Fig. 85***). This is to ensure an environment with controlled light supplies with constant light intensity.

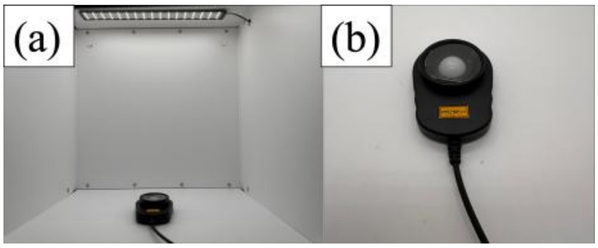


Fig.85. (a) The light box used for measuring light transmittance; (b) film ***AC10*** covering the sensor

Fig.86. The digital lux meter used

A digital lux meter was used (***Fig. 86***). The sensor was put inside the light box with the meter outside. The film under study was placed right on top of the sensor and illuminances of light after passing through the films were measured by the meter.

5.4.5.2. Measurement of Light Transmittance

Method

1. Turn on the light in the light box.
2. Calibrate the lux meter.
3. Place the film under study on the sensor. Ensure the surface blocking the sensor is flat and horizontal.
4. Cover the box with a light-proof cloth.
5. Record the reading from the meter.

Results

(a) Observing texts through the films

AC10	*AC21*	*AC11*	*AC12*	*AC01*
				

Table 9. Comparison of texts observed through the films.

(b) Illuminance per unit thickness *(for raw data, please refer to Appendix on P.80)*

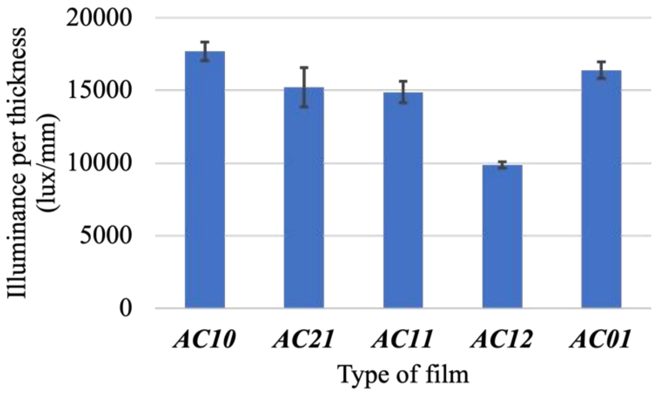


Fig.87. Comparison of illuminance per unit thickness of film

Discussion

The results in ***Fig. 87*** indicate that both pure avocado seed starch film (***AC10***) and pure chitosan film (***AC01***) possessed the highest light transmittance. However, all films formed by mixed content had poorer light transmittance. The value dropped as the chitosan content increased.

5.4.6. Biodegradability

As the films were made from biopolymers, they were expected to be biodegradable and environmentally friendly. Therefore, a burial soil test was conducted to test for their biodegradability.

5.4.6.1. Preparation of Soil Bed

A soil bed was prepared with composite potting soil. 3 cm thick of soil was added in a perforated plastic container (***Fig. 88***). Tap water was sprinkled onto the soil bed at the beginning of the experiment to keep the soil moist. After covering the film samples into the soil bed, the container was covered with a plastic wrap to prevent extensive loss of water from the soil during the course of the experiment (***Fig. 89***). The following table (***Table 10***) shows the information of the soil and environment of the experiment.

Fig.88. Soil bed with the samples before burial

Fig.89. Soil bed with the samples covered with plastic wrap

Parameter	**Content**
Moisture	30-40%
pH	6.3
Content	P_2O_5 0.1% K_2O 0.4% Organic matter 52% Nitrogen 0.5%
Ambient temperature	24.6°C

Table 10. Parameters of the soil

5.4.6.2. Measurement of Biodegradability

The samples were taken out from the soil bed daily to examine their masses as well as areas. For the measurement of the area, *"SketchAndCalc Area²"* programme on computer was used (***Fig. 90***)

Fig.90. Measurement of the area of AC10 film after 2 days using *"SketchAndCalc Area²"*

<u>**Method**</u>

1. Cut the film to the dimension of 5 cm x 3 cm.
2. Bury the film samples into the soil completely.
3. Wrap the perforated plastic container with a plastic wrap.
4. Take out the samples carefully on daily basis.
5. Remove the soil on the surfaces of the films and press them gently with a kitchen towel to absorb excess moisture on the surfaces.
6. Measure the mass of the films.
7. Take a photograph of each film beside a ruler (as a standard reference using the software).
8. Measure the area of each film by using *"SketchAndCalc Area²"* with the photographs (***Fig. 90***).

Results

(for raw data, please refer to Appendix on P.80)

(a) Change in **mass** of the film (b) Change in **area** of the film

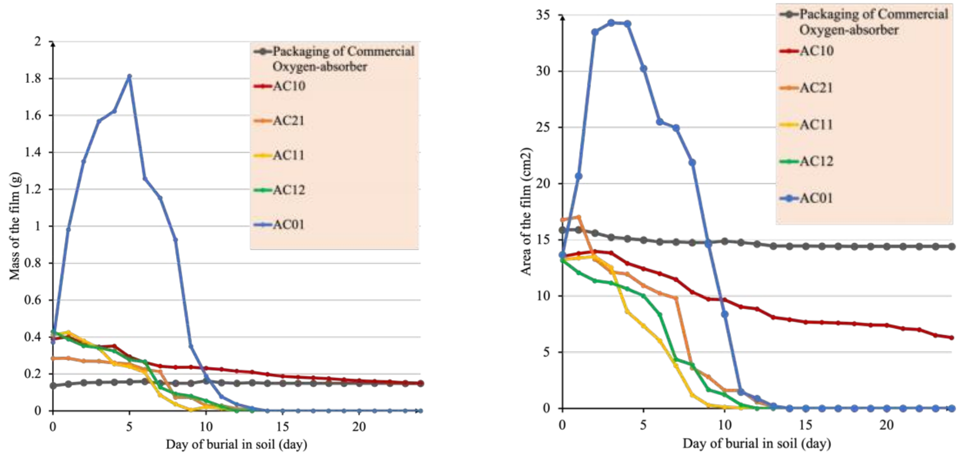


Fig.91. A graph showing (a) drop in masses (b) drop in areas of the films during soil burial test

For the appearances of the films during the Soil Burial Test, please refer to Appendix on P.82.

Discussion

From ***Fig. 91(a)***, masses of all films made in this project dropped to 0 g during the soil burial test after 14 days, except for ***AC10*** film. However, ***AC10*** film also showed a trend of decreasing mass in the test. In particular, films made from mixture of avocado seed starch and chitosan showed the quickest decline in mass comparing to pure films ***AC10*** and ***AC01***. In particular, ***AC11***disintegrated with the faster rate. On the other hand, the mass of the commercial oxygen-absorber sachet remained almost unchanged. This clearly indicated that the films made in this project were biodegradable.

It is also worth noting that the masses and areas of ***AC11***, ***AC12*** and ***AC01*** films increased during the first 4 days. In the case of pure chitosan film ***AC01***, its mass on day 4 was 4.67 times that of its initial mass. This phenomenon was supported by the drastic increase in area shown in ***Fig.91(b)*** as well as in ***Table 8*** on p.27. One possible reason is that chitosan in the films demonstrated good water-absorbing and water-retaining properties that they absorbed much water from the soil at the beginning of the test. However, the films started to decompose in the latter days, resulting in a drop in masses.

5.4.7. Melting Point Range of Purified Starch

Melting point range of the starch was measured by using a Thiele tube to better understand its thermo-property so as to explore its potential uses.

5.4.7.1. Use of Thiele tube

A Thiele tube (***Fig. 92***) was used to determine the melting point range of the avocado seed starch extracted.

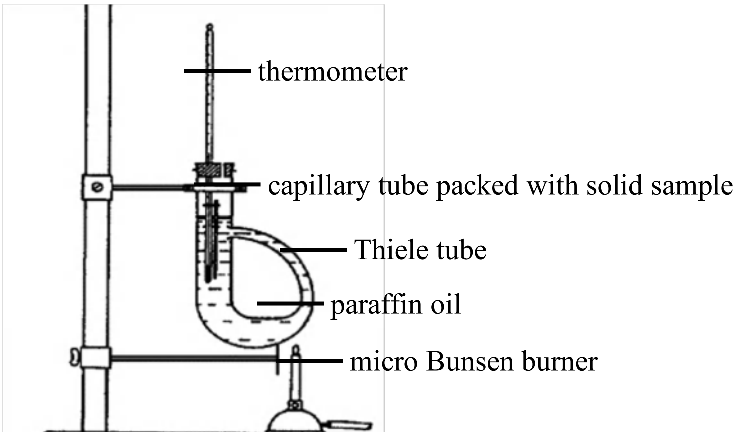


Fig.92. A Thiele tube for measuring melting point range of a solid sample [36]

The temperatures at which the sample in the capillary tube starts to melt and has just melted completely were recorded during the experiments. The two temperatures indicate the melting point range of the sample.

Measurement of melting points

<u>Method</u>

1. Pack the solid sample into a sealed glass capillary tube.
2. Attach the packed capillary tube to a thermometer by using a rubber band.
3. Insert the capillary with the thermometer into a Thiele tube filled with paraffin oil (***Fig. 93***).
4. Heat the side arm of the Thiele tube with a Bunsen flame in a fume cupboard.
5. Observe the physical state of the solid wax sample in the capillary tube.
6. Record the temperature (T_1) from the thermometer when the solid first starts to melt.

7. Record the temperature (T_2) from the thermometer when the solid has just melted completely.

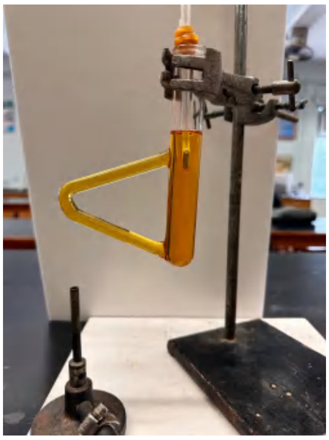


Fig.93. The Thiele tube used

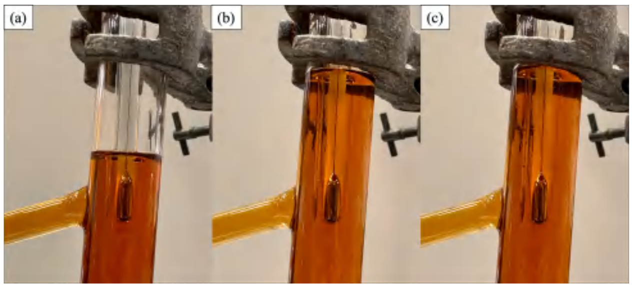


Fig.94 (a) original appearance of the starch before experiment; (b) the moment when first drop of liquid formed; and (c) the moment when all solid was melted

Results

	Trials					Average value	Standard deviation
	1	2	3	4	5		
T_1 (°C)	175	174	175	177	178	175.8	1.6
T_2 (°C)	180	180	180	183	182	181	1.4
Difference in temperature (°C)	5	6	5	6	4	5.2	0.8

Table 11. Melting point range of purified avocado seed starch extracted

Average melting point range of the purified starch: 175.8 °C to 181°C.

Discussion

From the above results in ***Table 11***, the average melting point range of the purified starch: 175.8°C to 181°C. The difference between the upper and lower limit of the range was 5.2°C, which indicated that the extracted starch was not highly pure as the melting range of pure solid should be as narrow as within 1 to 2 °C. Further better purification method should be explored as future work.

However, the results offered an insight of producing a sachet for oxygen-absorbers by sealing the film ***AC10*** with high temperature. ***Fig.95*** showed the sealing of film ***AC10*** with a thermal hair straightener to produce a sachet. All other films (***AC21*** to ***AC01***) cannot be sealed by using the same method.

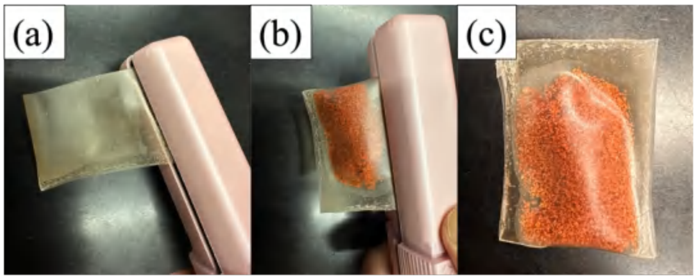


Fig.95 The process of sealing a ***AC10*** film with a thermal hair straightener to form a sachet.

6. PRACTICAILITY OF AVOCADO SEED OXYGEN-ABSORBERS AND STARCH FILMS

6.1. Test for Microbial Activities of Seed Powder and Starch Films

6.1.1. Use of Microbial Test Papers

Three types of microbial test papers were purchased from Food Safety Tech. They aim to test for the growth of general microbial colonies, fungal colonies and coliform colonies. The test papers contain cultural medium with specific dye indicator and water-absorbing gel.

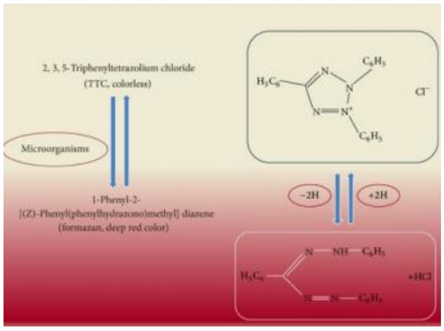


Fig. 96 Reaction of TTC with microorganisms [37]

For general microbial papers, they contain a dye called 2, 3, 5-triphenyltetrazolium chloride (TTC). The dye is colourless when oxidized, but it will be reduced by microorganisims upon contact to form a red comopind formazan. ***Fig.96*** shows the reaction mechanism.

6.1.2. Testing of Microbial, Fungal andColiform Activity

To test whether the avocado seed powder and its starch films produced would encourage the growth of micro-organisms or bacteria during storage, the following experiments were conducted:

Method

1. Add 0.1 g of the sample into 20 mL distilled water in a disinfected beaker.
2. Mix the sample well with the solution.
3. Add 1 ml of each solution onto a test paper for microbial activity, fungal activity and coliform activity.
4. Cover all test papers with their own cover plates.
5. Put each paper into an individual sealed airtight bag.
6. Put the papers into an oven of around 36^{o}C for 24 hours.
7. Count the number of colony-forming unit (CFU) on each paper.
8. Conduct a control with only distilled water on each type of paper.

Results

After two-week of storage

(a) Avocado Seed Powder

	Microbial Activity	Fungal Activity	Coliform Activity
Control			
CFU	68	55	60
Avocado Seed Sample			
CFU	60	10	30

Fig. 97. Appearance of test papers on microbial, fungal and coliform activity after adding extract of avocado seed powder after 2-week storage under room condition

(b) Avocado Seed Starch Film

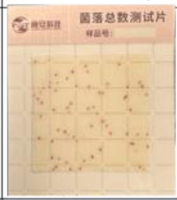
	Microbial Activity	**Fungal Activity**	**Coliform Activity**
Control			
CFU	68	55	60
Avocado Seed Starch Film Sample			
CFU	54	25	29

Fig. 98. Appearance of test papers on microbial, fungal and coliform activity after adding extract of avocado seed starch films after 2-week storage under room condition

<u>Discussion</u>

From ***Fig. 97*** and ***Fig. 98***, it can be observed that colonies were observced on the control sheets. However, both avocado seed powder and starch films showed less colonies. It might be due to the presence of acetogenins that leads to anti-microbial properties of the seeds as reported in some research [38].

6.2. Consideration of Cost

To evaluate the feasibility of applying our result in real life, the cost of producing the avocado seed oxygen-absorbers and avocado starch seed films were estimated in the following section.

6.2.1. Cost of Individual Ingredients

	Sodium Ascorbate Powder	Green Tea Powder	Turmeric Powder	Chitosan Powder	Distilled water	Acetic Acid (1%)	Glycerol
Brand	NutriBiotic	Ten Ren's Tea	GBT Trading Sdn. Bhd.	Madar Corporation Ltd.	Watsons	HK Labware Co. Ltd	椰樹牌
Supplier	iHerb	HKTV mall	Me-market	Amazon	Watsons	HK Labware Co. Ltd	HKTV mall
Amount	454 g	120 g	200 g	100 g	18000 mL	294500 mL	250 g
Cost (HKD)	\$145	\$42	\$59	\$50	\$81	\$1327.5	\$68
Cost (HKD) per gram or per mL	\$0.319	\$0.35	\$0.295	\$0.5	\$0.0045	\$0.0045	\$0.272

Table 12. Cost of individual ingredients for making avocado seed oxygen-absorbers and starch films

6.2.2. Avocado Seed Oxygen-absorbers

	Types of Avocado Seed Oxygen-absorbers			
Type of cost	Pure Avocado Seed (20 g)	Avocado Seed + Sodium Ascorbate (20 g, 4:1 mass ratio)	Avocado Seed + Green Tea Powder (20 g, 4:1 mass ratio)	Avocado Seed + Turmeric Powder (20 g, 4:1 mass ratio)
Avocado Seed (HKD)	\$0	\$0	\$0	\$0
Other Powder (HKD)	N.A.	4 g sodium ascorbate: 0.319 × 4 = **\$1.28**	4 g green tea powder: 0.35 × 4 = **\$1.4**	4 g turmeric powder: 0.295 × 4= **\$1.18**
Electricity Cost (HKD) *(assume \$0.899 for each kWh energy used)*	Grinding by a 150-W electric household bean blender (2 minutes): 0.15 kW× 0.0333 h × 0.899 = **\$0.004491**			
Total cost (HKD) **per 20 g**	**\$0.004491**	**\$1.284**	**\$1.404**	**\$1.184**

Table 13. Calculation of cost for different types of avocado seed oxygen-absorbers

6.2.3. Avocado Seed Starch Films

Consider the preparation of solutions enough for making four biofilms of size 10 cm × 10 cm × ~ 0.22 mm:

	Types of Avocado Seed Starch Films	
Type of cost (HKD)	***AC10***	***AC21***
Avocado Seed	6 g: $0	4.286 g: $0
Chitosan	N.A.	2.8 g: 0.5 × 2.8 = **$1.4**
Solvent	210 mL distilled water: 0.0045 × 210 = **$0.945**	150 mL distilled water + 70 mL 1% acetic acid: 0.0045 × 150 + 0.0045 × 70 = **$0.99**
Glycerol	4.5 g: 0.272 × 4.5 = **$1.224**	7.5 g: 0.272 × 7.5 = **$2.04**
Electricity Cost *(assume $0.899 for each kWh energy used)*	(a) Grinding by a 500-W electric handheld blender (2 minutes): 0.5kW× 0.0333 h× 0.899 = **$0.01497** (b) Centrifugation by a 45-W machine (10 mins, 3 times): 0.045kW× 0.5 h× 0.899 = **$0.02023** (c) Heating by a 480-W hot plate (4 hours): 0.48 kW× 4h× 0.899 = **$1.726** (d) Drying by a 250-W drying cupboard (12 hours): 0.25 kW× 12h× 0.899 = **$2.697**	
Total cost of 4 films (10 cm × 10 cm × ~ 0.22 mm)	$6.627	$8.888
Total cost **per film** (10 cm × 10 cm × ~ 0.22 mm)	**$1.66**	**$2.22**

Table 14. Calculation of cost for different types of avocado seed starch films

Discussion

From ***Table 13***, 1 g of avocado seed/sodium ascorbate mixture (4:1 mass ratio) cost $0.06 while 1 g of commercial ferrous oxygen-absorber powder cost $0.14.

However, from Section 3.2.5, 1 g of avocado seed/sodium ascorbate mixture can remove oxygen in 34.3 cm^3 to 45.6 cm^3 air in an enclosed flask. If we compare the mass required to absorb oxygen from 1 cm^3 of air, 0.022 g to 0.029 g of avocado seed/sodium ascorbate mixture (4:1 mass ratio) is required. For the commercial one, 0.0092 g is needed. After considering the cost, $0.0014 to $0.0019 is needed to absorb all oxygen from 1 cm^3 of air for avocado seed/sodium ascorbate mixture while $0.001288 is needed for commercial one. The cost of the avocado seed oxygen absorber is slightly higher (8.7%) than that of the commercial one.

From ***Table 14***, assuming the thickness to be 0.22 mm, each unit area (cm^2) of ***AC10*** and ***AC21*** films cost \$0.0166 and \$0.0222 respectively. Together with their biodegradability and the use of sustainable and environmentally friendly raw materials, avocado seed starch films are still favouable alternatives to petroleum-based plastic wraps or packaging materials.

The above cost is expected to be reduced if the synthesis process is industrialised with mass purchase of raw materials.

7. CONCLUSION

With the rise in popularity of food delivery services, it has become crucial to employ active packaging that can maintain food freshness for an extended period.

In this project, significant antioxidant properties were observed in both seed extracts obtained using 95% ethanol or distilled water. 0.2 g/cm^3 ethanolic extract from Shepard seed and 0.133 g/cm^3 ethanolic extract from Hass seeds demonstrated reduction in light absorbance at 517 nm by 97.38% and 96.32% respectively.

Additionally, avocado seed powders proved to be effective in absorbing oxygen. When 20 grams of Hass avocado seed powder was employed, the oxygen gas concentration in a 137-mL space was effectively reduced from 20.9% to 3%. The presence of sodium ascorbate, green tea powder, or turmeric powders further enhanced this action. The combination of avocado seeds and powders reduced the oxygen gas concentration to 0%. Notably, the mixture of avocado seed powder with sodium ascorbate exhibited the fastest oxygen removal time. It was found that the capacity of avocado seed/sodium ascorbate mixture to absorb all oxygen in an enclosed flask was between 34.3 cm^3/g to 45.6 cm^3/g. This finding was validated using bananas, where the mixture of the seed with the three powders slowed down the ripening process of bananas.

Furthermore, to maximize the use of cost-free avocado seeds, starch was successfully extracted and purified from the seeds, with a percentage yield ranging from 3.4% to 5.5%. The obtained starch was utilized in synthesizing films with varying chitosan content to enhance mechanical properties. The table below provides a summary of the films prepared in this project.

Property	*AC10*	*AC21*	*AC11*	*AC12*
Tensile Strength	+	+ +	+	+ +
Bending Strength	+ + +	+	+	+
Water Uptake	+	+	+	+
Water Vapour Permeability	+ + +	+ +	+ +	+ +
Oxygen Permeability	+ + +	+ +	+	+ +
Light Transmittance	+ + +	+ +	+ +	+
Biodegradability	+ +	+ + +	+ + +	+ + +

Key
+: lowest
+ + +: highest

Fig.92. Summary and comparison of all tested properties of avocado seed starch films in this project

After conducting a series of experiments to evaluate different film properties, it was determined that pure avocado seed film (***AC10***) was more suitable for replacing polyethylene sachets in oxygen-absorbers due to its higher oxygen permeability and bending strength. However, for packaging materials or containers, ***AC21*** emerged as a more suitable candidate due to its lower oxygen and water vapour permeability while having a considerable tensile strength.

With further study, it is in our next agenda to increase the tensile strength of avocado seed starch films to synthesize a biodegradable material better suits for making food packaging wrap (*refer to Section 8.1*). From the findings in this project, it is hoped that avocado seeds, instead of being a mere food waste, could be better utilized as a renewable and sustainable materials in active food packaging.

8. FUTURE WORK

8.1. Improve Various Properties of Avocado Seed Films

It has been reported that alginate can cross-link with starch to create a biofilm with a higher mechanical strength [39] (***Fig. 93***). Therefore, it is our next goal to explore the use of alginate to improve various properties of the existing avocado seed films we studied in this project.

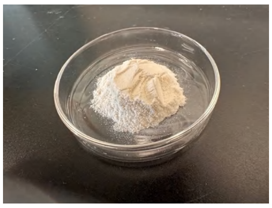


Fig.93. Sodium alginate used

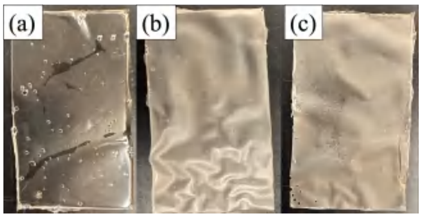


Fig.94. Avocado seed/alginate film synthesized with mass ratio: (a) 0 : 1 (b) 1 : 1 (c) 4 : 1

The preliminary testing has begun to test the properties of the films made from mixing sodium alginate with avocado seed starch (***Fig. 94***). It was found that avocado seed starch/alginate film could withstand up to 3099 g/mm, which is higher than those of ***AC10*** to ***AC12*** films.

8.2. Incorporate Ethylene-absorbing Coffee Ground with Avocado Seed Powder

Many climacteric fruits, like bananas and tomatoes, will ripe much faster due to self-production of ethylene gas that acts as a hormonal stimulant to promote fruit ripening [40]. This situation is especially prominent during the delivery and storage of fruits in an enclosed and packed environment.

As coffee ground (***Fig. 95***) is known to be a good smell adsorbent, a preliminary study has been launched to test for its ethylene-absorbing ability. ***Fig. 96*** shows the set-up

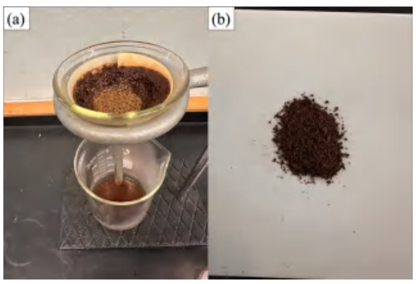


Fig.95. (a) preparation of coffee ground through filtration; (b) coffee ground obtained after drying

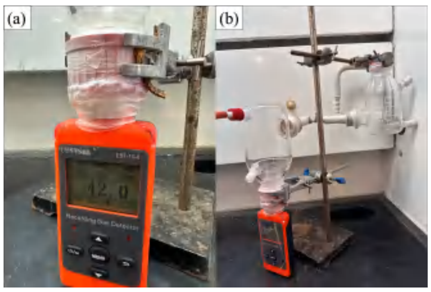


Fig.96. (a) Ethylene meter used; (b) The setup for measuring changes in ethylene concentration

designed and ***Fig. 97*** shows the change of ethylene gas concentration after exposing to different masses of coffee grounds.

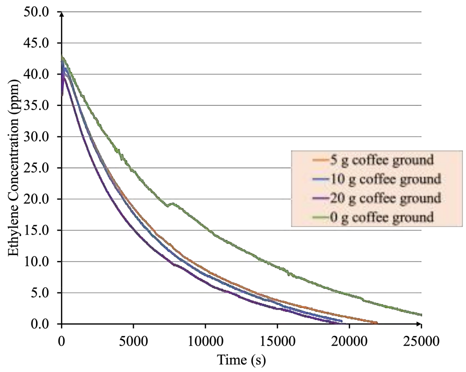


Fig.97. The result showing the effect of coffee grounds with different masses on the ethylene gas concentration

This is our ultimate goal to mix coffee grounds with avocado seed powder as a material for absorbing both ethylene and oxygen, which can further preserve fruits and slow down the ripening process.

8.3. Synthesis of Composite Films with Oxygen-Absorbing Ability

To prepare a better environmentally friendly biofilm packaging, it is in our agenda to synthesize a composite wrapping film which possesses intrinsic oxygen-absorbing ability by using avocado seeds or other plant materials (***Fig. 98***). This is in our initial design that the outer layer does not allow oxygen to permeate while the inner layer includes biomaterials (e.g. avocado seeds) to absorb oxygen inside the food package and offer antioxidizing protection against oxidative attack.

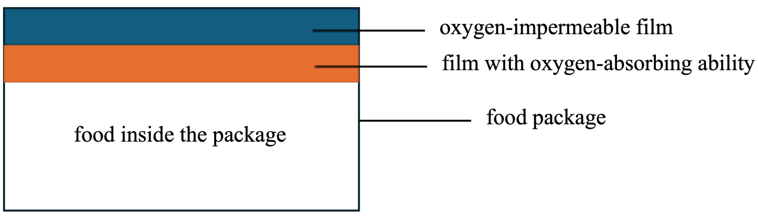


Fig.98. A preliminary design of a composite film with oxygen-absorbing ability

9. REFERENCES

[1] Huang, Kuan-Ming & Guan, Zhengfei & Blare, Trent & Hammami, A.Malek. (2023). Global Avocado Boom.

[2] Diana, Yepes & Carlos, Marquez & Edith, Cadena. (2018). Effect of Maturity State of Avocado (Persea Americana Mill. cv. Hass) on Seed Characteristics. Advance Journal of Food Science and Technology. 16. 301-306. 10.19026/ajfst.16.5971.

[3] Domínguez, M.P., Araus, K., Bonert, P., Sánchez, F., San Miguel, G., Toledo, M. (2014). The Avocado and Its Waste: An Approach of Fuel Potential/Application. In: Lefebvre, G., Jiménez, E., Cabañas, B. (eds) Environment, Energy and Climate Change II. The Handbook of Environmental Chemistry, vol 34. Springer, Cham. https://doi.org/10.1007/698_2014_291

[4] Tanweer Ahmad, Mohammed Danish, A review of avocado waste-derived adsorbents: Characterizations, adsorption characteristics, and surface mechanism, Chemosphere, Volume 296, 2022,134036, ISSN 0045-6535, https://doi.org/10.1016/j.chemosphere.2022.134036.

[5] Sandoval-Contreras, T.; González Chávez, F.; Poonia, A.; Iñiguez-Moreno, M.; Aguirre-Güitrón, L. Avocado Waste Biorefinery: Towards Sustainable Development. *Recycling* 2023, *8*, 81. https://doi.org/10.3390/recycling8050081

[6] García-Vargas, M.C.; Contreras, M.d.M.; Castro, E. Avocado-Derived Biomass as a Source of Bioenergy and Bioproducts. *Appl. Sci.* 2020, *10*, 8195. https://doi.org/10.3390/app10228195

[7] Paul V, Pandey R. Role of internal atmosphere on fruit ripening and storability-a review. J Food Sci Technol. 2014 Jul;51(7):1223-50. doi: 10.1007/s13197-011-0583-x. Epub 2011 Nov 26. PMID: 24966416; PMCID: PMC4062679.

[8] Busatto N, Tadiello A, Trainotti L, Costa F. Climacteric ripening of apple fruit is regulated by transcriptional circuits stimulated by cross-talks between ethylene and auxin. Plant Signal Behav. 2017 Jan 2;12(1):e1268312. doi: 10.1080/15592324.2016.1268312. PMID: 27935411; PMCID: PMC5289524.

[9] Paul, Vijay & Pandey, Rakesh & Srivastava, G.. (2012). The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene-An overview. Journal of food science and technology. 49. 1-21. 10.1007/s13197-011-0293-4.

[10] Krishnakumar, T.. (2021). STUDIES ON THE EFFECT OF 1-METHYLCYCLOPROPENE (1-MCP) ON SHELF LIFE AND QUALITY OF BANANA (Musa sp.). 10.13140/RG.2.2.36815.00166.

[11] Gupta P. Role of oxygen absorbers in food as packaging material, their characterization and applications. J Food Sci Technol. 2023 Feb 9;61(2):1-11. doi: 10.1007/s13197-023-05681-8. Epub ahead of print. PMID: 36785798; PMCID: PMC9908507.

[12] Emmanuel Hatzakis, Eugene P. Mazzola, Rachel M. Shegog, Gregory R. Ziegler, Joshua D. Lambert, Perseorangin: A natural pigment from avocado (Persea americana) seed, Food Chemistry, Volume 293, 2019, Pages 15-22, ISSN 0308-8146, https://doi.org/10.1016/j.foodchem.2019.04.064.

[13] García-Vargas, M.C.; Contreras, M.d.M.; Castro, E. Therapeutic Bio-Compounds from Avocado Residual Biomass. *Proceedings* 2021, *79*, 4. https://doi.org/10.3390/IECBM2020-08656

[14] Composition of Avocado Seed Dr. LeRoy S. Weatherby Chemistry Department, University of Southern California, Los Angeles, California Avocado Association 1934 Yearbook 19: 132-134

[15] Indarti, E & Nurlaila, & Muzaifa, Murna & Noviasari, Santi & Rozali, Z & Yusup, Eliza. (2022). Characteristics of avocado (Persea americana) and kluwih (Artocarpus camansi) seeds starch with different extraction methods. IOP Conference Series: Earth and Environmental Science. 951. 012095. 10.1088/1755-1315/951/1/012095.

[16] Ismail, Hanafi & Irani, Maryam & Ahmad, Zulkifli. (2013). Starch-Based Hydrogels: Present Status and Applications. International Journal of Polymeric Materials and Polymeric Biomaterials. 62. 411-420. 10.1080/00914037.2012.719141.

[17] Soong, Y.Y. and P.J. Barlow, 2004. Antioxidant activity and phenolic content of selected fruit seeds. Food Chem., 88(3): 411-417.

[18] Setyawan, Hendrix & Sukardi, Sukardi & Puriwangi, C. (2021). Phytochemicals properties of avocado seed: A review. IOP Conference Series: Earth and Environmental Science. 733. 012090. 10.1088/1755-1315/733/1/012090.

[19] Wang, W., T.R. Bostic and L. Gu, 2010. Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. Food Chem., 122(4): 1193-1198.

[20] Segovia, Francisco & Hidalgo, Gádor Indra & Villasante, Juliana & Ramis, Xavier & Almajano, María Pilar. (2018). Avocado Seed: A Comparative Study of Antioxidant Content and Capacity in Protecting Oil Models from Oxidation. Molecules. 23. 2421. 10.3390/molecules23102421.

[21] "The effects of different extraction conditions on the polyphenol, flavonoids components and antioxidant activity of Polyscias fruticosa roots" N Q Nguyen et al 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 022067

[22] Sija, S. & Athulya, A. & Mahima, M. & Vidhya, Ashok. (2019). Antioxidant and antimicrobial activity of different plant parts of Anacardium occidentale L. and Mangifera indica L.: a comparative study. International Journal of Pharmaceutical Sciences and Drug Research. 11. 10.25004/IJPSDR.2019.110401.

[23] Hairunisa, Indah & Mentari, Indri & Julianti, T & Wikantyasning, Erin & Cholisoh, Zakky & Ningsih, Caya & Muslim, M. Rifqi Fadillah. (2021). Antioxidant Activities in Different Parts of Pulasan (Nephelium mutabile Blume) from East Borneo. IOP Conference Series: Earth and Environmental Science. 736. 012018. 10.1088/1755-1315/736/1/012018.

[24] Braz Dent J (2012) 23(1): 22-27 Antioxidant Activity by DPPH Assay of Potential Solutions to be Applied on Bleached Teeth

[25] Emmanuel Hatzakis, Eugene P. Mazzola, Rachel M. Shegog, Gregory R. Ziegler, Joshua D. Lambert, Perseorangin: A natural pigment from avocado (Persea americana) seed, Food Chemistry, Volume 293, 2019, Pages 15-22, ISSN 0308-8146, https://doi.org/10.1016/j.foodchem.2019.04.064.

[26] Fruit Texture and the Science Behind It, University of Maryland Extension FS—1189, Nov 2021

[27] Juliani Buchveitz Pires, Starch extraction from avocado by-product and its use for encapsulation of ginger essential oil by electrospinning, International Journal of Biological Macromolecules, Volume 254, Part 1, 2024,127617, ISSN 0141-8130,https://doi.org/10.1016/j.ijbiomac.2023.127617.

[28] Harry G. Brittain, Richard D. Bruce, 2006. Chapter 4 Thermal analysis, Editor(s): S. Ahuja, N. Jespersen, Comprehensive Analytical Chemistry, Elsevier, Volume 47, Pages 63-109,ISSN 0166-526X, ISBN 9780444522597, DOI: https://doi.org/10.1016/S0166-526X(06)47004-5.

[29] Mariana D. Salazar-Irrazabal, Edson E. Ramirez-Tixe, Frank F. Velasquez-Barreto, Luis A. Bello-Pérez, Avocado seed starch: Effect of the variety on molecular, physicochemical, and digestibility characteristics, International Journal of Biological Macromolecules, Volume 247, 2023, 125746, ISSN 0141-8130,

[30] Ratnayake WS, Jackson DS. Starch gelatinization. Adv Food Nutr Res. 2009;55:221-68. doi: 10.1016/S1043-4526(08)00405-1. PMID: 18772106.

[31] Sethi, S., Choudhary, P., Nath, P., Chauhan, O.P. (2022). Starch Gelatinization and Modification. In: Chauhan, O.P. (eds) Advances in Food Chemistry. Springer, Singapore. https://doi.org/10.1007/978-981-19-4796-4_3

[32] Tarique, J., Sapuan, S.M. & Khalina, A. Effect of glycerol plasticizer loading on the physical, mechanical, thermal, and barrier properties of arrowroot (Maranta arundinacea) starch biopolymers. Sci Rep 11, 13900 (2021). https://doi.org/10.1038/s41598-021-93094-y

[33] Akter, Nousin & Khan, Ruhul & Tuhin, Mohammad & Haque, M. & Nurnabi, Md & Fahmida, Parvin & Islam, Mohammad Rafiqul. (2012). Thermomechanical, barrier, and morphological properties of Chitosan-reinforced starch-based biodegradable composite films. Journal of Thermoplastic Composite Materials. 27. 10.1177/0892705712461512.

[34] Tanvi Pal, Sumit Pramanik, Kapil Dev Verma, Shania Zehra Naqvi, P.K. Manna, Kamal K. Kar, 6 - Fly ash-reinforced polypropylene composites, Editor(s): Kamal K. Kar, Handbook of Fly Ash, Butterworth-Heinemann, 2022, Pages 243-270, ISBN 9780128176863, https://doi.org/10.1016/B978-0-12-817686-3.00021-9.

[35] Marco Giacinti Baschetti, Matteo Minelli, Test methods for the characterization of gas and vapor permeability in polymers for food packaging application: A review, Polymer Testing,Volume 89, 2020, 106606, ISSN 0142-9418, https://doi.org/10.1016/j.polymertesting.2020.106606.

[36] Harry G. Brittain, Richard D. Bruce, 2006. Chapter 4 Thermal analysis, Editor(s): S. Ahuja, N. Jespersen, Comprehensive Analytical Chemistry, Elsevier, Volume 47, Pages 63-109,ISSN 0166-526X, ISBN 9780444522597, DOI: https://doi.org/10.1016/S0166-526X(06)47004-5.

[37] Ain Nadirah Binti Romainor, Suk Fun Chin, Suh Cem Pang, Lesley Maurice Bilung, Preparation and Characterization of Chitosan Nanoparticles-Doped Cellulose Films with Antimicrobial Property, Journal of Nanomaterials, 10.1155/2014/710459, 2014, 1, (2014).

[38] Sneh Punia Bangar, Kyle Dunno, Sanju Bala Dhull, Anil Kumar Siroha, Sushil Changan, Sajid Maqsood, Alexandru Vasile Rusu, Avocado seed discoveries: Chemical composition, biological properties, and industrial food applications, Food Chemistry: X, Volume 16, 2022, 100507, ISSN 2590-1575, https://doi.org/10.1016/j.fochx.2022.100507.

[39] Onyido, Ikenna & Sha'Ato, Rufus & Nnamonu, Lami. (2012). Environmentally Friendly Formulations of Trifluralin Based on Alginate Modified Starch. Journal of Environmental Protection. 03. 10.4236/jep.2012.39127.

[40] Chen M, Gu H, Wang L, Shao Y, Li R, Li W. Exogenous Ethylene Promotes Peel Color Transformation by Regulating the Degradation of Chlorophyll and Synthesis of Anthocyanin in Postharvest Mango Fruit. Front Nutr. 2022 May 20;9:911542. doi: 10.3389/fnut.2022.911542. PMID: 35669069; PMCID: PMC9165547.

10. APPENDIX

Raw data for 3.1.3 Anti-oxidizing Power of Avocado Seed Extracts

(a) **Hass** Avocado Seed Extracts

(i) **Distilled water** as solvent

Control solution:

Light absorbance at 517 nm after passing through pure distilled water: 1.155

Mass of avocado seed powder used (g)	Trial 1			Trial 2			Trial 3			Average percentage decrease in absorbance (%)	Standard deviation
	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)		
0.075	0.002	0.867	25.1	0.002	0.85	26.6	0.002	0.858	25.9	25.86	0.74
0.150	0.002	0.211	81.9	0.002	0.216	81.5	0.002	0.368	68.3	77.23	7.73
0.333	0.002	0.245	79.0	0.002	0.247	78.8	0.002	0.162	86.1	81.30	4.20
0.667	0.025	0.258	79.8	0.025	0.263	79.4	0.002	0.169	85.5	81.59	3.43
1.333	0.046	0.274	80.3	0.046	0.279	79.8	0.002	0.173	85.2	81.76	2.98
2.000	0.068	0.276	82.0	0.068	0.274	82.2	0.028	0.182	86.7	83.61	2.65

(ii) **95% ethanol** as solvent

Control solution:

Light absorbance at 517 nm after passing through 95% ethanol: 1.105

Mass of avocado seed powder used (g)	Trial 1			Trial 2			Trial 3			Average percentage decrease in absorbance (%)	Standard deviation
	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)		
0.075	0.002	0.911	17.7	0.002	0.909	17.9	0.002	0.92	16.9	17.53	0.53
0.150	0.002	0.043	96.3	0.002	0.033	97.2	0.002	0.068	94.0	95.84	1.63
0.333	0.002	0.042	96.4	0.002	0.032	97.3	0.002	0.061	94.7	96.11	1.33
0.667	0.002	0.044	96.2	0.002	0.035	97.0	0.002	0.054	95.3	96.17	0.86
1.333	0.002	0.044	96.2	0.002	0.042	96.4	0.002	0.042	96.4	96.32	0.10
2.000	0.002	0.057	95.0	0.002	0.051	95.6	0.002	0.056	95.1	95.23	0.29

(b) **Shepard** Avocado Seed Extracts

(i) **Distilled water** as solvent

Control solution:

Light absorbance at 517 nm after passing through pure distilled water: 1.175

Mass of avocado seed powder used (g)	Trial 1			Trial 2			Trial 3			Average percentage decrease in absorbance (%)	Standard deviation
	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)		
0.075	0.002	1.175	0.2	0.002	1.101	6.5	0.002	1.160	1.4	2.70	3.33

0.150	0.002	0.515	56.3	0.002	0.503	57.4	0.002	0.524	55.6	56.43	0.90
0.333	0.002	0.247	79.1	0.002	0.236	80.1	0.002	0.250	78.9	79.38	0.63
0.667	0.002	0.225	81.0	0.002	0.214	82.0	0.002	0.231	80.5	81.16	0.73
1.333	0.029	0.261	80.3	0.03	0.279	78.8	0.003	0.259	78.2	79.09	1.05
2.000	0.041	0.274	80.2	0.033	0.259	80.8	0.036	0.263	80.7	80.54	0.32

(ii) **95% ethanol** as solvent

Control solution:

Light absorbance at 517 nm after passing through 95% ethanol: 1.096

Mass of avocado seed powder used (g)	Trial 1			Trial 2			Trial 3			Average percentage decrease in absorbance (%)	Standard deviation
	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)		
0.075	0.002	1.008	8.2	0.002	0.979	10.9	0.002	1.013	7.8	8.94	1.67
0.150	0.002	0.232	79.0	0.002	0.250	77.4	0.002	0.251	77.3	77.89	0.98
0.333	0.002	0.067	94.1	0.002	0.056	95.1	0.002	0.063	94.4	94.53	0.51
0.667	0.002	0.049	95.7	0.002	0.045	96.1	0.002	0.053	95.3	95.71	0.36
1.333	0.002	0.044	96.2	0.002	0.032	97.3	0.002	0.035	97.0	96.81	0.57
2.000	0.037	0.044	99.4	0.037	0.088	95.3	0.035	0.063	97.4	97.38	2.01

Raw data for 3.2.2 Oxygen-absorbing Property of Commercial Oxygen-Absorber Content

The original data was collected every 10 seconds. The following table only presents data collected every 1800 seconds (30 minutes) for simplicity reason.

	Mass of Commercial Oxygen Absorber Content			
	0.5 g	2 g	4 g	6 g
Time (s)	Average Percentage of Oxygen Gas Concentration (%)			
0	20.9	20.9	20.9	20.9
1800	20.5	18.4	16.8	14.0
3600	19.8	16.0	12.3	8.4
5400	19.0	13.4	8.3	4.0
7200	18.2	10.9	4.8	0.0
9000	17.4	8.5	2.1	No further change in data
10800	16.6	6.2	0.5	
12600	15.8	4.2	0.0	
14400	15.0	2.4	No further change in data	
16200	14.2	1.1		
18000	13.5	0.0		
19800	12.7	No further change in data		
21600	12.2			
23400	11.8			

25200	11.4			
27000	11.0			
28800	10.7			
30600	10.3			
32400	10.0			
34200	9.7			
36000	9.4			
37800	9.1			
39600	8.9			
41400	8.7			
43200	8.5			
45000	8.3			
46800	8.1			
48600	7.9			
50400	7.8			
52200	7.7			

Raw data for 3.2.3 Oxygen-absorbing Property of Avocado Seeds
The original data was collected every 10 seconds. The following table only presents data collected every 1800 seconds (30 minutes) for simplicity reason.

(a) Hass Avocado Seeds

	Mass of Hass Avocado Seed			
	6 g	8 g	12 g	20 g
Time (s)	Average Percentage of Oxygen Gas Concentration (%)			
0	20.9	20.9	20.9	20.9
1800	19.1	17.1	13.1	9.0
3600	18.3	16.2	11.8	7.0
5400	18.1	15.8	11.2	6.1
7200	17.9	15.5	10.9	5.6
9000	17.7	15.2	10.7	5.3
10800	17.5	15.0	10.5	5.0
12600	17.5	14.9	10.3	4.8
14400	17.5	14.7	10.3	4.6
16200	17.5	14.6	10.2	4.5
18000	17.5	14.4	10.2	4.4
19800	17.6	14.3	10.1	4.3
21600	17.6	14.2	10.0	4.1
23400	17.6	14.1	10.0	4.1
25200	17.7	14.0	10.0	4.0
27000	17.6	13.9	10.0	4.0
28800	17.7	13.8	9.9	3.9

30600	17.7	13.7	9.9	3.9
32400	17.8	13.6	9.9	3.8
34200	17.9	13.5	9.8	3.8
36000	17.8	13.4	9.8	3.8
37800	17.9	13.3	9.8	3.7
39600	17.9	13.3	9.8	3.7
41400	17.9	13.2	9.7	3.7
43200	17.9	13.1	9.7	3.7
45000	17.9	13.1	9.7	3.6
46800	17.9	13.0	9.6	3.6
48600	17.9	12.9	9.6	3.6
50400	17.9	12.8	9.6	3.6
52200	17.9	12.8	9.6	3.5
54000	17.9	12.8	9.6	3.5
55800	17.9	12.8	9.6	3.5
57600	17.9	12.7	9.6	3.5
59400	17.9	12.6	9.5	3.5
61200	17.9	12.6	9.6	3.4
63000	17.9	12.5	9.5	3.4
64800	17.9	12.8	9.6	3.4
66600	17.9	12.8	9.5	3.3
68400	17.9	12.5	9.5	3.3
70200	17.9	12.5	9.5	3.3
72000	17.9	12.4	9.5	3.3
73800	17.9	12.4	9.4	3.2
75600	17.9	12.1	9.5	3.2
77400	17.9	12.1	9.4	3.2
79200	17.9	12.1	9.4	3.2
81000	17.9	11.9	9.4	3.1
82800	17.9	11.9	9.4	3.1
84600	17.9	11.9	9.4	3.1
86400	17.9	11.9	9.3	3.0
88200	17.9	11.9	9.2	3.0

(b) Shepard Avocado Seeds

	Mass of Shepard Avocado Seed			
	6 g	8 g	12 g	20 g
Time (s)	Average Percentage of Oxygen Gas Concentration (%)			
0	20.9	20.9	20.9	20.9
1800	18.4	16.9	13.5	11.0
3600	18.0	16.0	12.4	9.7
5400	17.8	15.7	11.9	9.0

7200	17.7	15.5	11.7	8.6
9000	17.5	15.3	11.4	8.3
10800	17.4	15.2	11.3	8.1
12600	17.3	15.0	11.2	7.9
14400	17.1	14.8	11.1	7.8
16200	17.0	14.8	11.0	7.7
18000	16.9	14.7	10.9	7.6
19800	16.8	14.6	10.9	7.5
21600	16.7	14.5	10.8	7.5
23400	16.6	14.4	10.8	7.4
25200	16.5	14.4	10.8	7.3
27000	16.5	14.3	10.8	7.3
28800	16.3	14.3	10.7	7.3
30600	16.2	14.2	10.7	7.3
32400	16.2	14.1	10.7	7.3
34200	16.1	14.1	10.7	7.3
36000	16.1	14.0	10.7	7.2
37800	16.0	14.0	10.7	7.2
39600	16.0	13.9	10.7	7.2
41400	15.9	13.8	10.7	7.2
43200	15.9	13.8	10.7	7.2
45000	15.8	13.8	10.7	7.2
46800	15.8	13.7	10.6	7.2
48600	15.7	13.7	10.6	7.2
50400	15.7	13.6	10.6	7.2
52200	15.7	13.6	10.6	7.2
54000	15.7	13.6	10.6	7.2
55800	15.7	13.6	10.6	7.2
57600	15.6	13.5	10.6	7.1
59400	15.6	13.5	10.6	7.1
61200	15.6	13.5	10.6	7.1
63000	15.6	13.4	10.6	7.1
64800	15.5	13.4	10.6	7.1
66600	15.5	13.4	10.6	7.1
68400	15.5	13.3	10.6	7.1
70200	15.4	13.3	10.6	7.1
72000	15.4	13.3	10.6	7.1
73800	15.3	13.3	10.6	7.1
75600	15.4	13.3	10.5	7.1
77400	15.4	13.3	10.5	7.0
79200	15.3	13.3	10.5	7.0
81000	15.3	13.3	10.5	7.0

82800	15.3	13.4	10.5	7.0
84600	15.2	13.3	10.5	7.0
86400	15.2	13.2	10.5	7.0
88200	15.2	13.2	10.5	7.0

Raw data for 3.2.4.3 Anti-oxidizing Power of the Powders
Control solution:
Light absorbance at 517 nm after passing through pure distilled water: 1.362

(a) Sodium ascorbate

Mass of avocado seed powder used (g)	Trial 1			Trial 2			Trial 3			Average percentage decrease in absorbance (%)	Standard deviation
	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)		
0.02	0.002	0.978	28.3	0.002	1.019	25.3	0.002	0.966	29.2	27.63	2.04
0.04	0.002	0.979	28.3	0.002	1.015	25.6	0.002	0.980	28.2	27.36	1.51
0.08	0.002	0.926	32.2	0.002	0.937	31.4	0.002	0.942	31.0	31.50	0.60
0.15	0.002	0.904	33.8	0.002	0.884	35.2	0.002	0.914	33.0	34.02	1.12
0.33	0.002	0.828	39.4	0.002	0.81	40.7	0.002	0.817	40.2	40.06	0.67
0.6	0.002	0.856	40.5	0.002	0.789	42.2	0.002	0.759	44.4	42.38	1.97

(b) Green tea

Mass of avocado seed powder used (g)	Trial 1			Trial 2			Trial 3			Average percentage decrease in absorbance (%)	Standard deviation
	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)		
0.02	0.115	0.444	75.8	0.105	0.387	79.3	0.113	0.408	78.3	77.83	1.78
0.04	0.025	0.196	87.4	0.057	0.290	82.9	0.002	0.303	77.9	82.75	4.77
0.08	0.186	0.36	87.2	0.14	0.280	89.7	0.15	0.330	86.8	87.91	1.58
0.15	0.002	0.109	92.1	0.002	0.166	88.0	0.002	0.143	89.6	89.92	2.11
0.33	0.049	0.134	93.8	0.03	0.131	92.6	0.064	0.169	92.3	92.88	0.78
0.6	0.035	0.114	94.5	0.02	0.12	92.7	0.068	0.139	94.8	93.98	1.15

(c) Turmeric

Mass of avocado seed powder used (g)	Trial 1			Trial 2			Trial 3			Average percentage decrease in absorbance (%)	Standard deviation
	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)	Absorbance of blank	Absorbance of sample	Percentage decrease in absorbance (%)		
0.02	0.002	1.34	1.8	0.002	1.349	1.1	0.002	1.352	0.9	1.25	0.46
0.04	0.002	1.222	10.4	0.002	1.267	7.1	0.002	1.241	9.0	8.86	1.66

0.08	0.002	1.196	12.3	0.002	1.15	15.7	0.004	1.220	10.7	12.92	2.55
0.15	0.105	1.04	31.4	0.098	1.090	27.2	0.057	0.984	31.9	30.15	2.60
0.33	0.11	1.008	34.1	0.12	1.013	34.4	0.09	0.95	36.9	35.12	1.52
0.6	0.187	0.68	63.8	0.203	0.82	54.7	0.273	0.941	51.0	56.49	6.61

Raw data for 3.2.2.4 Oxygen-absorbing Property of Mixtures of Avocado Seed and the Powder

The original data was collected every 10 seconds. The following table only presents data collected every 28800 seconds (8 hours) for simplicity reason.

(a) Mixture of Avocado Seed and Sodium Ascorbate

Time (s)	Mass ratio of avocado seed to powder			
	0 to 1	1 to 1	2 to 1	4 to 1
	Average Percentage of Oxygen Gas Concentration (%)			
0	20.9	20.9	20.9	20.9
28800	20.8	14.4	11.2	5.0
57600	20.7	12.4	9.0	2.6
86400	20.8	11.2	7.5	1.6
115200	20.6	10.2	6.2	0.8
144000	20.5	9.4	5.2	0.4
172800	20.7	8.7	4.3	0.1
201600	20.7	8.0	3.6	0.0
230400	20.7	7.4	3.0	No further change in data
259200	20.7	6.8	2.5	
288000	20.7	6.2	2.2	
316800	20.7	5.7	1.9	
345600	20.7	5.1	1.6	
374400	20.7	4.7	1.3	
403200	20.7	4.2	1.1	
432000	20.7	3.7	0.9	
460800	20.7	3.3	0.6	
489600	20.8	2.9	0.1	
518400	20.6	2.5	0.0	
547200	20.5	2.1	0.0	
576000	20.7	1.8	No further change in data	
604800	20.7	1.6		
633600	20.7	1.3		
662400	20.7	1.1		
691200	20.7	0.9		

720000	20.7	0.5		
748800	20.7	0.1		
777600	20.7	0.1		
806400	20.7	0.0		

(b) Mixture of Avocado Seed and Green Tea Powder

Time (s)	Mass ratio of avocado seed to powder			
	0 to 1	1 to 1	2 to 1	4 to 1
	Average Percentage of Oxygen Gas Concentration (%)			
0	20.9	20.9	20.9	20.9
28800	20.8	11.3	7.7	7.5
57600	20.9	10.2	6.2	5.8
86400	20.9	9.0	5.3	4.6
115200	20.9	8.1	4.6	3.7
144000	20.8	7.3	4.0	3.0
172800	20.8	6.5	3.5	2.4
201600	20.6	5.8	3.1	1.9
230400	20.6	5.3	2.7	1.5
259200	20.7	4.5	2.2	1.1
288000	20.7	3.6	1.8	0.4
316800	20.7	2.4	1.4	0.1
345600	20.7	0.5	0.9	0.1
374400	20.7	No further change in data	0.4	0.1
403200	20.7		No further change in data	0.1
432000	20.7			No further change in data
460800	20.7			
489600	20.7			
518400	20.7			
547200	20.7			

(c) Mixture of Avocado Seed and Turmeric Powder

Time (s)	Mass ratio of avocado seed to powder			
	0 to 1	1 to 1	2 to 1	4 to 1
	Average Percentage of Oxygen Gas Concentration (%)			
0	21.0	20.9	20.9	20.9
28800	21.4	16.1	12.0	10.3
57600	21.6	15.2	10.7	9.1
86400	21.6	14.6	9.6	8.5

115200	21.6	13.9	8.6	7.8
144000	21.6	13.3	7.6	7.0
172800	21.5	12.7	6.5	5.9
201600	21.5	11.6	4.1	2.5
230400	21.5	9.2	0.1	0.1
259200	21.5	3.2	No further change in data	No further change in data
288000	21.4	No further change in data		
316800	21.4			
345600	21.4			

Raw data for 3.2.5 Oxygen-absorbing Capacity of Avocado Seed/Sodium Ascorbate Mixture (4 :1 mass ratio)

The original data was collected every 10 seconds. The following table only presents data collected every 14400 seconds for simplicity reason.

Time (s)	Mass of avocado seed/sodium ascorbate mixture (in 4:1 mass ratio)			
	3 g	4 g	5 g	10 g
	Percentage of Oxygen Gas Concentration (%)			
0	20.9	20.8	20.9	20.6
14400	13.6	9.4	7.8	3.3
28800	11.2	6.1	4.1	1.1
43200	9.7	4.2	2.1	0.4
57600	8.6	2.9	0.9	0.0
72000	7.9	2.1	0.4	No further change in data
86400	7.2	1.5	0.3	
100800	6.7	1.1	0.1	
115200	6.1	0.8	0.1	
129600	5.6	0.6	0.0	
144000	5.1	0.4	0.0	
158400	4.7	0.3	0.0	
172800	4.3	0.2	No further change in data	
187200	4.1	0.1		
201600	3.7	0.1		
216000	3.4	0.1		
230400	3.1	0.1		
244800	2.8	0.0		
259200	2.7	No further change in data		
273600	2.4			
288000	2.2			
302400	2.0			
316800	1.8			
331200	1.6			

345600	1.5			
360000	1.4			
374400	1.3			
388800	1.1			
403200	1.0			
417600	1.0			
432000	0.9			
446400	0.8			
460800	0.7			
475200	0.6			
489600	0.6			
504000	0.5			
518400	0.5			
532800	0.5			

Raw data for 5.1 Percentage Yield of Starch from Avocado Seeds

Mass of avocado seed used *(M_A)* (g)	31.993	60.521	90.354	119.35	153.696
Mas of empty petri dish (g)	60.169	59.892	58.709	31.042	29.921
Mass of petri dish + avocado seed starch (g)	61.922	63.045	62.977	36.495	35.141
Percentage yield (%)	5.5	5.2	4.7	4.6	3.4

Raw data for 5.4.1.2 Tensile Strength of the Films

Type of film	Trial 1			Trial 2			Average maximum mass withstand per unit thickness (g/mm)	Standard deviation
	Average thickness (mm)	Maximum mass withstand (g)	Maximum mass withstand per unit thickness (g/mm)	Average thickness (mm)	Maximum mass withstand (g)	Maximum mass withstand per unit thickness (g/mm)		
AC10	0.228	300	1316	0.188	370	1968	1642	461
AC21	0.214	510	2383	0.238	540	2269	2326	81
AC11	0.246	380	1545	0.218	410	1881	1713	238
AC12	0.302	790	2616	0.29	680	2345	2480	192
AC01	0.268	4150	15485	0.302	4600	15232	15358	179

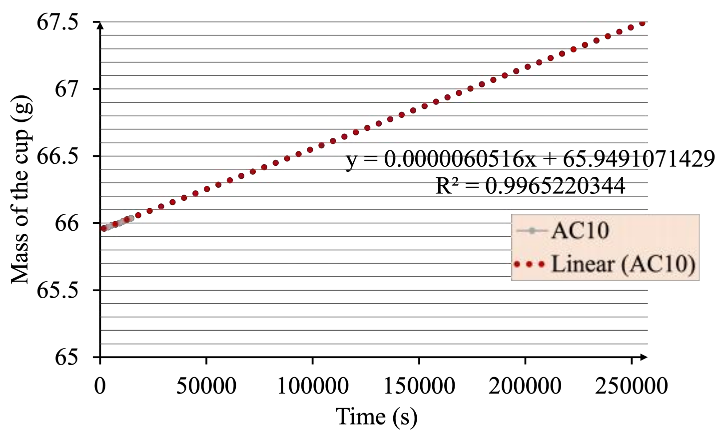
Raw data for 5.4.2 Water Uptake Percentage of Different Films

		Average thickness (mm)	Initial mass (g)	Final mass (g)	Water uptake (%)
Type of film	AC10	0.174	0.125	0.157	147
	AC21	0.388	0.255	0.309	55
	AC11	0.246	0.163	0.191	70
	AC12	0.26	0.172	0.178	13
	AC01	0.312	0.173	0.865	1282

Raw data for 5.4.3.2 Water Vapour Permeability Test

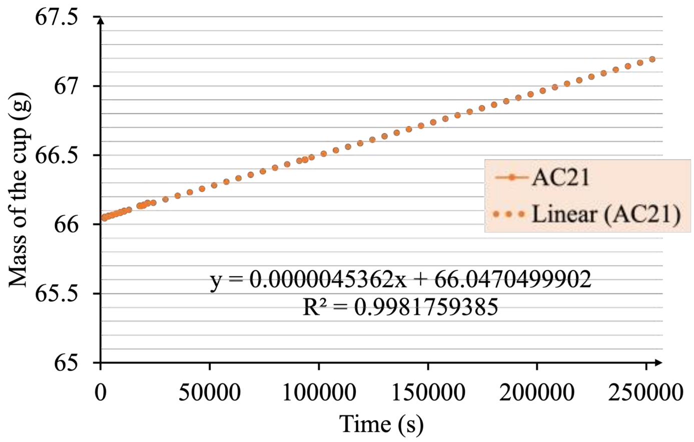
(a) ***AC10*** **film**

Average thickness (cm)	0.178
Starting humidity (%)	77.6
Time (s)	Mass of dry cup (g)
0	66.031
1800	66.04
3600	66.044
5400	66.047
9000	66.062
10800	66.065
14400	66.081
18000	66.091
54000	66.29



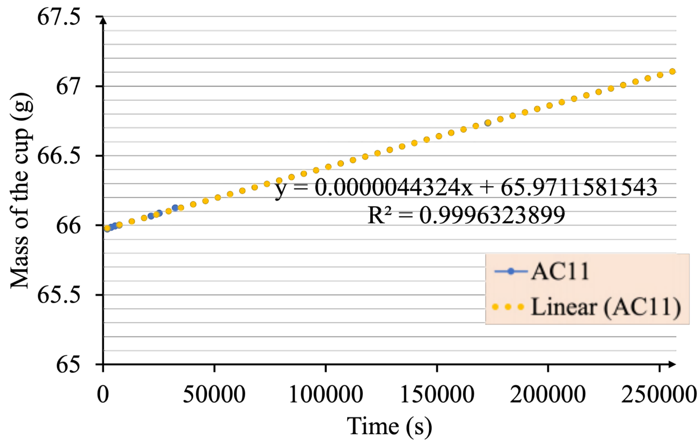
(b) ***AC21* film**

Average thickness (cm)	0.23
Starting humidity (%)	70.1
Time (s)	Mass of dry cup (g)
0	66.047
1800	66.061
3600	66.068
5400	66.078
7200	66.087
9000	66.097
16200	66.134
18000	66.141
19800	66.155
91800	66.468
257400	66.991



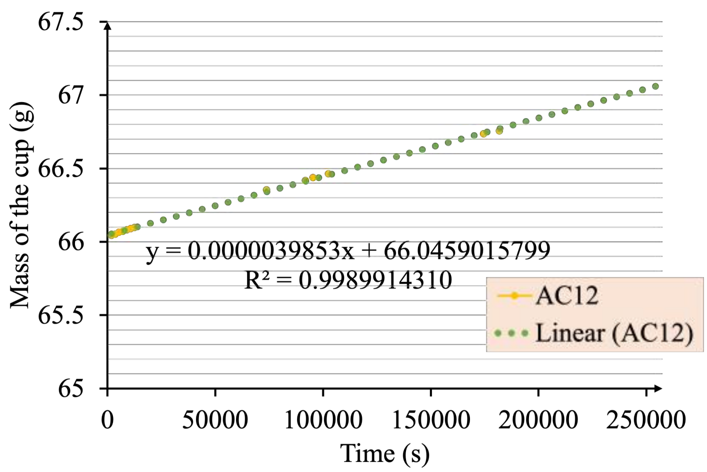
(c) ***AC11* film**

Average thickness (cm)	0.246
Starting humidity (%)	78.6
Time (s)	Mass of dry cup (g)
0	65.973
1800	65.985
3600	65.995
5400	66.001
19800	66.066
23400	66.086
30600	66.125
171000	66.735



(d) ***AC12* film**

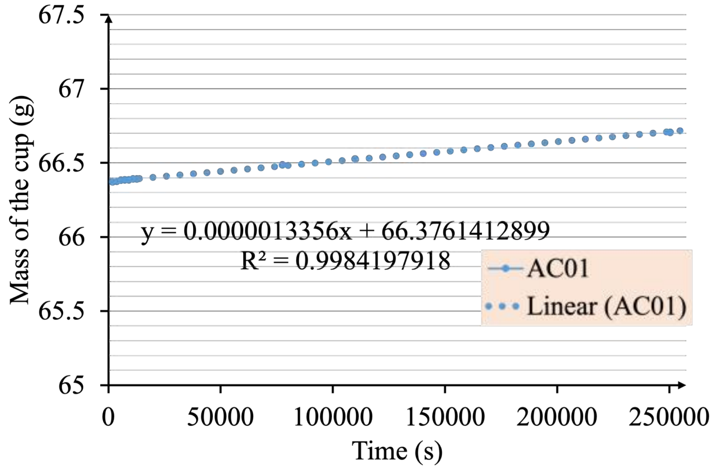
Average thickness (cm)	0.246
Starting humidity (%)	76.4
Time (s)	Mass of dry cup (g)
0	66.044
1800	66.052
3600	66.065
5400	66.071
7200	66.082
9000	66.09
10800	66.098



72000	66.354
90000	66.417
93600	66.437
100800	66.464
172800	66.737
180000	66.756

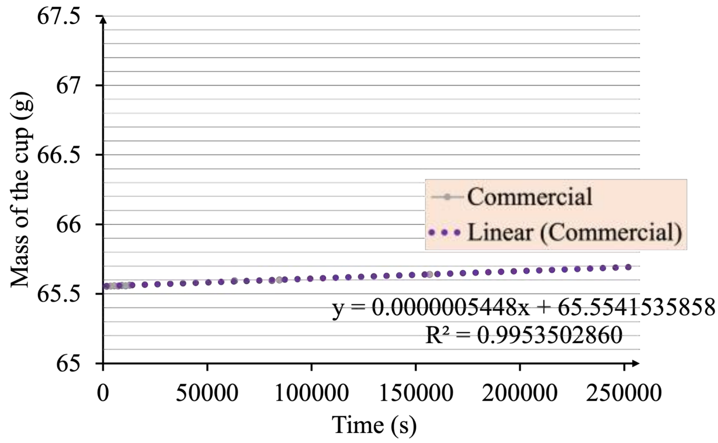
(e) ***AC01* film**

Average thickness (cm)	0.292
Starting humidity (%)	76.7
Time (s)	Mass of dry cup (g)
0	66.373
1800	66.377
3600	66.384
5400	66.387
7200	66.387
9000	66.392
10800	66.392
75600	66.488
108000	66.527
248400	66.7059



(f) **Commercial Oxygen-Absorber Sachet**

Average thickness (cm)	0.104
Starting humidity (%)	76.4
Time (s)	Mass of dry cup (g)
0	65.554
1800	65.556
3600	65.558
5400	65.557
7200	65.56
9000	65.558
10800	65.562
61200	65.593
79200	65.596
82800	65.6
154800	65.639



Sample	***AC10***	***AC21***	***AC11***	***AC12***	***AC01***	Commercial Oxygen-absorber sachet
Mass flow rate (g/s) (slope from the line)	6.0516 $\times10^{-06}$	4.5362 $\times10^{-06}$	4.4324 $\times10^{-06}$	3.9853 $\times10^{-06}$	1.3356 $\times10^{-06}$	5.448 $\times10^{-07}$
Transfer area (m^2)	0.0009621	0.0009621	0.0009621	0.0009621	0.0009621	0.0009621
d (m)	0.000178	0.00023	0.000246	0.000246	0.000292	0.000104
S (Pa)	3169	3504	3323	3075	3323	3131
R_1-R_2	77.6	70.1	78.6	76.4	76.7	76.4
Water Vapour Permeability ($\times10^{-12}$ g Pa^{-1} m^{-1} s^{-1})	**4.553**	**4.415**	**4.339**	**4.337**	**1.590**	**0.246**

Raw data for 5.4.4.2 Oxygen Permeability Test

Time (s)	Type of film						
	AC10	***AC21***	***AC11***	***AC12***	***AC01***	Control	Commercial
	Average Percentage of Oxygen Gas Concentration (%)						
0	1.2	1.2	1.2	1.2	1.2	1.2	1.2
85520	3.9	2.2	1.7	2.3	2.2	2.3	*the concentration reached 20.9% (average oxygen concentration)*
171040	6.8	3.3	2.3	3.4	3.5	2.8	
256560	19.4	4.4	3.0	4.6	4.7	3.6	
342080	*end of measurement*	5.4	3.7	5.8	6.0	4.3	
427600		6.4	4.5	*end of measurement*	7.1	*end of measurement*	
513120		7.4	5.3		8.1		
598640		8.3	6.0		*end of measurement*		
684160		9.1	7.0				
769680		*end of measurement*	7.7				
855200			8.4				
940720			9.1				
1026240			9.7				
1111760			10.4				
1197280			11.1				
1282800			11.7				
1368320			12.2				
1453840			12.7				
1539360			13.3				
1624880			13.8				
1710400			14.3				

Raw data for 5.4.5.1 Light Transmittance

Illuminance without any films in the light box (control) = 4436 lx

Type of film	Trial 1			Trial 2			Average drop in illuminance per unit thickness (lx mm^{-1})	Standard deviation
	Average thickness (mm)	Illuminance (lx)	Illuminance per unit thickness (lx mm^{-1})	Average thickness (mm)	Illuminance (lx)	Illuminance per unit thickness (lx mm^{-1})		
AC10	0.21	3621	17243	0.2	3630	18150	17696	641
AC21	0.21	3396	16171	0.22	3137	14259	15215	1352
AC11	0.2	3080	15400	0.21	3015	14357	14879	737
AC12	0.29	2815	9707	0.28	2807	10025	9866	225
AC01	0.2	3360	16800	0.21	3357	15986	16393	576

Raw data for 5.4.6.2 Biodegradability

(a) Change in Area

		Area of Film (cm^2)													
		Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day13
Packaging of Commercial Oxygen-absorber		15.89	15.9	15.61	15.23	15.1	14.98	14.83	14.82	14.76	14.76	14.89	14.77	14.64	14.45
Film	***AC10***	13.51	13.78	13.96	13.83	12.91	12.43	11.99	11.48	10.34	9.72	9.67	9.03	8.85	8.1
	AC21	16.78	17.02	13.27	12.13	11.95	10.92	10.23	9.81	3.6	2.8	1.6	1.6	0.56	0.13
	AC11	13.24	13.38	13.5	12.55	8.62	7.34	6.02	3.8	1.18	0.29	0.12	0.05	0	0
	AC12	13.17	12.08	11.35	11.17	10.64	10.01	8.34	4.36	3.88	1.66	1.23	0.32	0	0
	AC01	13.67	20.68	33.5	34.32	34.23	30.24	25.52	24.97	21.89	14.6	8.39	1.46	0.87	0.21

		Area of Film (cm^2)													
		Day14	Day15	Day16	Day17	Day18	Day19	Day20	Day21	Day22	Day23	Day24	Day25	Day26	Day27
Packaging of Commercial Oxygen-absorber		14.44	14.44	14.43	14.43	14.42	14.42	14.42	14.42	14.42	14.42	14.42	14.4	14.39	14.39
Film	**AC10**	7.9	7.68	7.65	7.6	7.54	7.42	7.4	7.1	7	6.5	6.3	6.3	6.15	6.12
	AC21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	AC11	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	AC12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	AC01	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(b) Change in Mass

		Mass of Film (g)													
		Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day13
Packaging of Commercial Oxygen-absorber		0.137	0.146	0.153	0.155	0.156	0.158	0.16	0.15	0.15	0.15	0.163	0.15	0.143	0.135
Film	***AC10***	0.389	0.402	0.364	0.347	0.351	0.293	0.263	0.242	0.236	0.237	0.231	0.225	0.215	0.21
	AC21	0.284	0.285	0.27	0.269	0.26	0.254	0.225	0.212	0.073	0.073	0.028	0.028	0.012	0.006
	AC11	0.411	0.425	0.381	0.34	0.254	0.24	0.208	0.085	0.036	0.005	0.02	0.013	0	0
	AC12	0.429	0.389	0.353	0.342	0.325	0.277	0.266	0.128	0.092	0.08	0.054	0.024	0	0
	AC01	0.372	0.982	1.35	1.569	1.623	1.813	1.258	1.153	0.927	0.35	0.189	0.077	0.035	0.014

		Mass of Film (g)													
		Day14	Day15	Day16	Day17	Day18	Day19	Day20	Day21	Day22	Day23	Day24	Day25	Day26	Day27
Packaging of Commercial Oxygen-absorber		0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.149	0.149	0.149	0.149	0.149
Film	**AC10**	0.198	0.188	0.183	0.179	0.175	0.169	0.164	0.16	0.158	0.153	0.151	0.15	0.148	0.145
	AC21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	AC11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	AC12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	AC01	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Photographs of the films in Soil Burial Test for Selected Days

Type of film	Time of Burial (Day)								
	0	3	5	8	11	14	17	21	24
Commercial Sachet Materials									
AC10									
AC21							*totally degraded*	*totally degraded*	*totally degraded*
AC11						*totally degraded*	*totally degraded*	*totally degraded*	*totally degraded*
AC12						*totally degraded*	*totally degraded*	*totally degraded*	*totally degraded*
AC01							*totally degraded*	*totally degraded*	*totally degraded*