Evaluation of Antioxidant Activity of Active Film Prepared with Chitosan and Sacha Inchi (*Plukenetia volubilis*) Leave Extract

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A thesis submitted in fulfilment of the requirement for the degree of Bachelor of Applied Science (Product Development Technology) With Honours

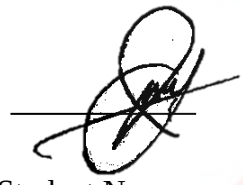
Faculty of Agro-Based Industry

Universiti Malaysia Kelantan

2022

DECLARATION

I hereby declare that the work embodied in this report is the result of the original research except for the excerpts and summaries which I have just described the source.



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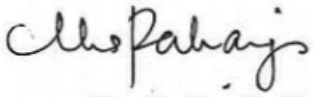

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ACKNOWLEDGEMENT

This study partially supported by Faculty of Agro Based Industry. I thank to Sir Azman as my final year project coordinator for Product Development Technology course for give the instruction to do the research. Next, I thank to my supervisor Dr. Ikarastika Rahayu who had guided me and gave me some knowledge for this research. Moreover, they had approved and supported me to do this research and ensured this study had been done successfully. I also would like to thank to my parent because allowed me to do this study and supported me by giving a spirit to finish this study.

I also like to show my gratitude to all my friends that helped me to give a moral support and share any information in order to finish this study.

Last but not least, I would like to thanks to all laboratory assistances that always had time and space to guide me during doing this study and using the instruments in the laboratory. Without all of the people, maybe this study cannot be done until now.

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ABSTRACT

Evaluation of Antioxidant Activity of Active Film Prepared With Chitosan and Sacha Inchi (*Plukenetia volubilis*) Leave Extract

Sacha inchi (*Plukenetia volubilis L.)* leaves were popular used to make tea product but there is no research on the sacha inchi incorporated with chitosan film. Hence, the purpose of this study was to develop novel active films with antioxidant activity based on chitosan with the incorporation of sacha inchi leaves extract for food packaging applications as well as determined the DPPH antioxidant, total phenolic content (TPC) and total flavonoid content. The in vitro antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, folin ciocalteu for total flavonoid content and aluminium chloride for total flavonoid content. The IC_{50} value was presented where the value is decrease if the concentration of sacha inchi where added. Hence, 1.5% sacha inchi extract have the lowest of IC_{50} value. Total phenolic content (TPC) test is also showed great value since $p<0.05$ and the highest concentration on gallic acid standard curve is 1.5% sacha inchi Extract (155.789 ± 4.218 GAE/mg). Other than that, total flavonoids content (TFC) also showed great concentration value since their value are also $p<0.05$ and the highest concentration on gallic acid standard curve is 1.5% sacha Inchi Extract (60.238 ± 1.5099 QE/mg). These findings offered a suggestion that the prepared chitosan films incorporated with sacha inchi extract can be applied as novel film in food packaging industry.

Keyword: DPPH radical scavenging, total phenolic content, total flavonoid content, sacha inchi extract, chitosan film, active packaging

ABSTRAK

Penilaian Aktiviti Antioksidan Filem Aktif Sediakan Dengan Chitosan dan Ekstrak Daun Sacha Inchi (Plukenetia Volubilis)

Daun Sacha inchi (*Plukenetia volubilis L.*) telah popular digunakan untuk membuat produk teh tetapi tiada kajian mengenai sacha inchi yang digabungkan dengan filem kitosan. Oleh itu, tujuan kajian ini adalah untuk membangunkan filem aktif novel dengan aktiviti antioksidan berasaskan kitosan dengan penggabungan ekstrak daun sacha inchi untuk aplikasi pembungkusan makanan serta menentukan antioksidan DPPH, jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid. Aktiviti antioksidan in vitro dinilai oleh aktiviti penghapusan radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH), folin ciocalteu untuk jumlah kandungan flavonoid dan aluminium klorida untuk jumlah kandungan flavonoid. Nilai IC_{50} ditunjukkan di mana nilainya berkurangan jika kepekatan sacha inchi ditambah. Oleh itu, 1.5% ekstrak sacha inchi mempunyai nilai IC_{50} yang paling rendah. Ujian jumlah kandungan fenolik (TPC) juga menunjukkan nilai yang tinggi kerana $p<0.05$ dan kepekatan tertinggi pada lengkung piawai asid gallik ialah 1.5% Ekstrak sacha inchi (155.789 ± 4.218 GAE/mg). Selain itu, jumlah kandungan flavonoid (TFC) juga menunjukkan nilai kepekatan yang tinggi kerana nilainya juga $p<0.05$ dan kepekatan tertinggi pada lengkung piawai asid gallic ialah 1.5% Ekstrak Sacha Inchi (60.238 ± 1.5099 QE/mg). Penemuan ini menawarkan cadangan bahawa filem kitosan yang disediakan yang digabungkan dengan ekstrak sacha inchi boleh digunakan sebagai filem baru dalam industri pembungkusan makanan.

Kata kunci: Penghapusan radikal DPPH, jumlah kandungan fenolik, jumlah kandungan flavonoid, ekstrak sacha inchi, filem kitosan

CHAPTER 1

INTRODUCTION

Since ancient time, polymers have always been an important part of commodities. Fibres (cellulose), wool and linen cotton are used to produce clothing, and paper tubes (cellulose) are used for papermaking which it is an example of the founder used the raw materials that contain polymers. Rubber tree (natural rubber) glue was introduced to Europe from South America in the 16th century. As early as Olmec, Maya and Aztecs used the polymer for ball making, containers and waterproof fabrics (Hurley, 1981).

Initially, the behaviour of polymers was rationalized based on the theory proposed by Thomas Graham, which treated them as colloidal aggregates of small molecules held together by unknown forces. Today, the synthetic polymer was used widely in the daily use. Without them, it would hit different on the modern society. The use of polymers has its unique properties such as low density, low cost, and good thermal insulation. Performance, high corrosion resistance, low energy consumption polymer production and the ability to be easily processed into final products. For specific applications, it can be combined with other materials (such as composite materials) to adjust or improve the properties of the polymer. The application can save energy, packaging and else.

1.1 Research Background

Sacha inchi (*Plukenetia volubilis*) falls under Euphorbiaceae family which is a perennial type of plant that having little trichomes on its leaves. It is native to quite a bit of tropical South America just as a portion of the Windward Islands in the Caribbean (Kew World Checklist of Selected Plant Families). Sacha inchi plant is cultivated economically in South East Asia, most amazingly in Thailand. Due to the concerns over the disposal of conventional synthetic plastic materials derived from petroleum, the growing interest in consumable films has increased mainly lately. The needs of plastic degradation quite a while and the vast majority of them wind up overburdening on landfill. Then again, the renewable agricultural items of edible films not exclusively debased promptly after their removal, yet additionally can expand. Among different accessible edible film materials, extensive consideration has been given to chitosan on account of its exceptional properties (Goosen, 1996). As a matter of first importance, bountiful business supplies are accessible.

Antioxidants are added substances ordinarily utilized in the polymer industry to prevent the thermal degradation of polymers during preparing and act as additives that are regularly added to packaged food to scavenge the oxygen radicals. A natural extract which is wealthy in antioxidant compounds ought to be consolidated into natural polymeric materials such as chitosan. All in all, this examination intends to research the capability of Sacha inchi leaf extract that act as a natural antioxidant agent in bio-based polymers such as chitosan for food packaging application.

The sacha inchi leaves extract was picked in this study is due to its high substance in phenolic mixtures like phenols, flavonoids, tannin, cardiac glycosides, steroids, terpenoids also compounds for polyphenolic and tocopherols, which are very

well-known in antioxidants. It is obligated for antioxidant agent action through the arrangement of chelating with metals, restriction of the protein activity of lipoxygenase, and probably as a free limit scrounger. Flavonoid has a positive correlation with DPPH radical scavenging activity. These might be proposed that the sacha inchi leaves antioxidant activity may be attributed to the presence of flavonoid compounds. In this manner, sacha inchi leaves extracts could be considered as a decent source of antioxidant.

In this study, chitosan film is picked because of its incredible ability to shape film, non-harmful, biodegradable, and inexhaustible in nature. Recent years, this exertion has delivered a different scope of film types using a combination of antimicrobial compound on the development and testing of antimicrobial films for wrapping or covering food varieties. Furthermore, chitosan has been proved being nontoxic, biodegradable, biofunctional, biocompatible and have the antimicrobial qualities (Wang, 1992). As contrasted of biobased food packaging materials, chitosan brings out their benefit where it able to incorporate the functional substances like minerals or nutrients and has antibacterial activity (Jeon,Kamil, & Shahidi, 2002). Along these lines, chitosan has been utilized as a material of the packaging for the quality protection of an assortment of food (Suyatma, Tighzert, & Copinet, 2005). One of the reasons for the antimicrobial character of chitosan is its decidedly charged amino group which interacts with negatively charged microbial cell membranes, prompting the spillage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi, Arachchi, & Jeon, 1999).

1.2 Problem Statement

Among of the natural polymers that is used for biodegradable packaging improvement, chitosan stands apart in view of its extraordinary ability to frame film, high mechanical strength, non-poisonous and incredible deterrent breaking point. Notwithstanding, the packaging film requires the film's suitable antioxidant properties to evade food biodeterioration due to a couple factor, chiefly the oxidation. To supplant the usage of manufactured antioxidant agent, a characteristic concentrate that rich with antioxidant compounds ought to be joined into chitosan film to maintain the food quality and prolong their shelf life. A recent study exhibited that sacha inchi (*Plukenetia volubilis*) leave extracts in antioxidant compound like phenols, flavonoids, tannin, cardiovascular glycosides, steroids, and terpenoids, and could be considered as an excellent antioxidant source and a promising possibility to be consolidated in packaging and would be helpful to the food industries. Considering the high antioxidant capacity of sacha inchi leaf extract, consequently, the point of this project is to create novel active film with antioxidant activity based on chitosan with the joining of sacha inchi leave extract for food packaging applications.

1.3 Objective

1. To develop novel active films with the incorporation of sacha inchi leaves extract for food packaging applications.

2. To evaluate the antioxidant activity of chitosan film and sacha inchi extract using DPPH assay.

3. To evaluate the total phenolic content and total flavonoid of extract and chitosan film that incorporated with sacha inchi extract.

1.4 Hypothesis

H° = The active film has no antioxidant activity, flavonoid and phenolic content.

H^{1} = The active film shows good antioxidant activity, total flavonoid and phenolic content.

1.5 Scope of Study

This study is focus on the development of better antioxidant on active packaging. The sacha inchi leave will be extracted and its antioxidant compounds particularly phenolic and flavonoids content will be elucidated prior to the incorporation with chitosan film. It was expected that, the sacha inchi leave extract is rich with total phenolic (TPC) and flavonoids contents (TFC) hence resulted to the high antioxidant activity. Upon incorporation with chitosan matrix, the effect of the addition of different concentration of the sacha inchi leave extract will be investigated. The films will be evaluated for their antioxidant activity, physicochemical and mechanical properties. It was hypothesized that the incorporation of sacha inchi leave extract into chitosan film would improve the antioxidant properties of the film and this could be positively related to their TPC and TFC with redox properties. The incorporation of sacha inchi leave extract would also improve the physico-mechanical film's properties. The films that will develop in this study are feasible since the polymer matrix i.e chitosan used in its development is renewable and naturally abundant, biodegradable and non-toxic. The addition of sacha inchi leave extract with high antioxidant properties making the film even more fascinating.

1.6 Significance of Study

Undeniably, food packaging from plastic is the most widely used ubiquitous in developed economies where plastic is carrying various benefits. Into food industries, the plastic packaging will be functioned as food protection from the oxygen, water vapour ultraviolet light and contamination from both chemical and microbiological. Therefore, it contributes to the extension of the shelf-life and maintenance of food product packaging. Apart from degradation caused by microorganisms, spoilage is mainly caused by presence of oxygen and product of chemical reactions, not only that, the oxidation considerably limits the shelf-life of food products, it also could cause a loss of both sensorial and nutritional quality of food and may even lead to the formation of toxic aldehydes.

In this experiment, the development of biodegradable food packaging from the leave extraction was a vital movement on getting a better film. Thus, it could be a great factor to satisfy the consumer to experience the best antioxidant film as active packaging and would be an opportunity to promote sacha inchi as a better antioxidant product since it been reported from several research and attracted their attention due to environmental pollution cause by conventional biodegradable plastic.

CHAPTER 2

LITERATURE REVIEW

2.1 Sacha Inchi (*Plukenetia Volubilis*)

2.1.1 Characteristics of Sacha Inchi

In the Amazon field, sacha inchi, is a climbing shrub plant from the Euphorbiaceae. Its seeds are widely discovered for as a source of proteins and omega-3 and 6. However, exploration of the potential use of sacha inchi leave and pomace is considerably limited. Nitty gritty details on ecology and cultivation of this plant are essential for the use in a sustainable manner. Disregarding the way that its crude seeds and leaves contain poisons, these parts that are good for usage resulting to searing (Srichamnong, *et al*., 2018). Other types of the *Plukenetia*'s genus incorporated are *P. brachybotrya, P. polyadenia, P. loretensis,* and *P. huayllabambana*. Their morphological and physicochemical properties vary from *P. volubilis* also called as sacha inchi (Chirinos, Pedreschi, Domínguez, & Campos, 2015; Chirinos, Zorrilla, *et al*., 2016; Rodríguez *et al*., 2011a). It also be created in other world's part such as east Asia in view of its incredible potential as an economic crop (Chandrasekaran & Liu, 2015; Gutiérrez, Segura, Sanchez-Reinoso, Díaz, & Abril, 2017). Most of the reported studies relating to sacha inchi were from the origin country of sacha inchi (Peru & Brazil) and some part of Southeast Asia such as Thailand and Vietnam, that have turned this plant as an economic plant. Inca peanut has a star-formed organic product container (3–5 cm). As the natural product develops, the shading abandons green to blackish

earthy colored. The organic product cases contain eatable dim earthy colored oval seeds (1.5–2 cm) (Fu *et al*., 2014; Sathe, Hamaker, Sze-Tao, and Venkatachalam, 2002). Normally, on ideal temperature somewhere in the range of 25 and 30 °C, the inca peanut seeds can be cultivate (Da Silva, Vieira, Boneti, Melo, and Martins, 2016).

Most of the studies were focusing on the nutritional and physicochemical properties of sacha inchi oil. To the best of our knowledge, study that related to sacha inchi either on nutritional and physicochemical properties are not much and their incorporation with other bio-based polymer was reported in Malaysia. Though the nutritional and physicochemical properties of sacha inchi were well reported abroad, most of the studies were focused on the sacha inchi oil. The study on the antioxidant activity of the sacha inchi leave extract is very limited. The research on the antioxidant properties was first reported by Wuttisin *et al*. in February 2021 which found that the sacha inchi extract rich in polyphenol compound. Although Incha leave extract of bio-based polymer films, it also fascinating properties that may have beneficial effects on bio-based polymer films, the related information or publications about producing active composite films are not found. To our knowledge, no research studies of chitosan film incorporated with sacha inchi leaves extract been carried out on the antioxidant activity. This is the first study that explores the potential use of sacha inchi leaves extract as an antioxidant agent in food packaging.

This project provided comprehensive study on the antioxidant compounds in sacha inchi act and their antioxidant activity before and upon incorporation with chitosan film. This is the first study that will provide information on the appropriateness and suitability of using the sacha inchi leave extract as a low-cost and natural antioxidant agent for the development of edible film in food packaging. The results

from this study are not only beneficial in food industry but also in other applications that require the addition of natural antioxidants to prevent the oxidative deterioration. Furthermore, the process proposed in this research is simple, effective and inexpensive because it implies the addition of organic, non-toxic and naturally available extracts and reducing cost and time. Improving the antioxidant properties of conventional chitosan film is important because it allows this polymer film to become more effective against the oxidation of lipids in food.

Chitosan is derived from chitin, which is the second most abundant polysaccharide on earth close to cellulose and is accessible from side effects in the shellfish industry (Alishahi, & Aïder. 2012) which it is a sugar extracted from the hard external skeleton from crustacean and mollusc. In diluted acidic aqueous solutions, the structure of chitosan could provide protonated solubility in the solution due to the presence of amino groups. A few remarkable properties of chitosan bring out unique opportunities to the advancement applications in biomedical. The chitosan medical and pharmaceutical interest would be their component that prompt a superior comprehension and likewise can identified the existence of the positive charges on chitosan backbone while the haemostatic of chitosan. Thus, chitosan also can prompt redesign and an opening of the tight intersection proteins, clarifying the penetration upgrading property of this polysaccharide on the negative part of cells membrane.

Figure 1: Sacha Inchi Plant

Figure 2: Sacha Inchi Fruit

Figure 3: Sacha Inchi Leaf

2.1.2 Geographical Distribution

Sacha inchi can be found all throughout the world, but mainly in Central and South America, which from the Antilles to Bolivia. Sacha inchi (Plukenetia volubilis Linneo) is a Peruvian jungle native that belongs to the Europhorbiaceae family of plants, includes 300 genera and 7500 species (Williams, 2001). Their localization are as showed on table 1. It is grown at altitudes ranging from 200 to 2000 metres above sea level (Guillén *et al*., 2003), and its growth is influenced by a variety of geoclimatic factors (Table 2).

PLUKENETIA GENUS	
Species	Localization
Branchybotyra	Bolivia, Ecuador, Brazil
Lehmanniana	Ecuador, Colombia
Huayllabambana	Peru
Mulglandulosa	Venezuela
Lorestensis	Columbia, Guyana, Venezuela, Bolivia, Brazil
Penninervia	Costa Rica, Belice, Guatemala, Mexico, Venezuela, Nicaragua
Serrata	Brazil
Polyadenia	Guyana, Venezuela, Ecuador, Brazil, Bolivia
Supraglandulosa	Surinam, Guyana Francesca, Brazil
Stipellata	Colombia, Mexico, Panama, Guatemala, Nicaragua, Costa Rica
Verrucosa	Guyana, Brazil, Surinam
Vollubilis	Colombia, Surinam, Antilles Menores, Venezuela, Ecuador, Brazil, Bolivia

Table 1: *Plukenetia* localization

Factor	Feature
Altitude	It grows from 200 m above sea level in the low jungle and 2000 m above sea in high jungle
Temperature	It grows and behaves well at various temperatures (10°C min, 36°C). High temperatures are unfavorables, causing the fall of the flowers and fruits, mainly the fresh ones.
Water	This crop requires permanent availability of water in order to achieve a sustainable development, exhibiting better growth if the rainy season is uniform throughout the year (850 to 1000mm). Irrigation is essential in dry months. Relatively long periods of drought or low temperature cause slow and difficult growth.
Light	The crop needs more days to complete its vegetative cycle when it is exposed to low light intensities. In situations when the shade is very intense, the flowering decreases.
Soil	This plant has a wide adaption to different types of soil; it grows in acid soils as well as in land with high concentration of aluminium. It is necessary to choose the ground that makes possible proper development and productivity. It develop in clay soils, sandy loam and tolerate acid soils.
Drainage	This crop requires and adequate drainage I order to eliminate excess water both superficially and deeply. For good drainage the texture of the soil must be considered.

Table 2: Geoclimatic factor

Plukenetia genus distribution in Latin America and crop characteristics of the volubilis species. Due to the expansion of the Amazon jungle and hence the biodiversity of this region, Brazil, Venezuela, and Colombia are the countries with the most plant genera. One of the most striking aspects of this species of plant is its capacity to thrive in a variety of soils, albeit it does require specific conditions for healthy growth, one of which being regular access to water sources (Bussmann *et al*., 2009).

2.1.3 Ethnobotanical Uses of Sacha Inchi

Sacha inchi is familiar on cholesterol control and cardiovascular health improvements and is credited with various health benefits. Because of its monounsaturated and saturated fatty acids, it is nutritionally beneficial (Chirinos *et al*. 2013). Critical ω-3 and ω-6 fatty acids, with α-linolenic acid (C18:3 n-3, ω-3), accounting for 35.2–50.8 percent of the total lipid fraction, and linoleic acid (C18:2 n-6, -6) accounting for 33.4–41.0 percent is made up by the polyunsaturated fatty acid fraction (Guillén *et al*. 2003). They are also rich with protein and have excellent antioxidant properties (Gutiérrez *et al*. 2011). They are being known since prehispanic times, and it is thought to have been cultivated by the Incas between 3,000 and 5,000 years ago, it been proved by pottery depicting sacha inchi vines and nuts discovered in Incan graves along the coast of Peru (Bernal & Correa 1992; Brack 1999) it been found in case populated by several different ethnic groups such as Mayoruna, Campa, Nomatsiguenga, & others. Other than that, it also be used as a food source and a poultice to soften, revitalise, and rejuvenate the skin (Flores 2010; Flores and Lock 2013; Hamaker *et al*. 1992). In Perú, the plant is also used in a variety of traditional dishes, most of which can also be made with peanuts (*Arachis hypogaea L*) such as salt roasted seeds, or butter, or the leaves can be fried and other traditional foods that carry by different culture (Flores 2010). The ethnobotanical surveys of the plant are rare or non-existent even the nutritional value and growing interest in the crop. Thus, awareness of uses and cultivation practises in the plant's native range elucidate in order to help in the plant's sustainable use while also benefiting the local community are on the current study.

2.1.4 Phytochemistry Studies of Sacha Inchi

The primary classes of naturally occurring toxins of plant origin (Phytotoxins) are alkaloids, lectins, and saponins, which are secondary metabolites found in many parts of plants, including seeds, leaves, roots, and bark. Secondary metabolites are important defence mechanisms for plants against predators and pathogens. Some plants can cause mild to severe toxicity in wildlife, cattle, and people through direct or indirect toxicity when consumed (Speijers *et al*., 2010).

Plants, fungi, bacteria, and animals all have secondary metabolite molecules called alkaloids (Dolan, Matulka, & Burdock, 2010). On the basis of structure, they can be categorised into three parts which are the real alkaloid (heterocyclic with nitrogen inside), pro-alkaloid (heterocyclic without nitrogen), and pseudoalkaloid (made from amino acid) (Aniszewski, 2007). Alkaloids have several functions in plants, including fighting against herbivores, serving as a growth hormone, and allowing plant cells to communicate (Aniszewski, 2007). Plant alkaloids can be extracted from any section of the plant. Alkaloids' toxicity manifests itself in a variety of ways, including gastrointestinal toxicity, kidney toxicity, and genotoxicity. Alkaloids, such as pyrrolizidine alkaloids, can also be transformed through biotransformation and interact with DNA, causing mutation and cancer (Bode & Dong, 2015).

Carbohydrates binding lectins are abundant in legume seeds such as green beans, red kidney beans, and white kidney beans. In humans, it been linked to hemagglutination, nausea, and gastroenteritis (Dolan *et al*., 2010). Lectins bind to mucosal cells of the colon, preventing nutrients from being absorbed. When lectins

from black beans and soybeans were provided to rats at 0.5 percent and 1 percent of their diet, respectively, they impeded their growth. After two weeks of feeding, rats received a fatal dose of isolated lectins from kidney beans at 0.5 percent of their diet (Omaye, 2004). However, appropriate on cooking, such as heating, could lessen the toxicity of some lectins (Grant, More, McKenzie, & Pusztai, 1982). Higher plants contain saponins, which are natural glycosides. Saponins are made up of hydrophobic (fat-soluble), sapogenin, and hydrophilic (water-soluble) sugar components (carbohydrate moiety). Saponins are important in plants for a variety of reasons, including pathogen defence and growth hormone. There have been reports of health advantages such as anti-inflammation, immunomodulation, and anti-cancer, as well as side effects such as hemolysis induction (Podolak, Galanty, & Sobolewska, 2010). Furthermore, steroidal saponins isolated from Narthecium ossifragum were found to cause renal tubular cell (LLC-PK1) toxicity in a prior work (Uhlig, Wisloff, & Petersen, 2007).

Some of study had proven the composition of sacha inchi have phenolic compounds, tocopherol, and phytosterol, among other health-promoting compounds contain in it (Chirinos *et al*. 2013). The study also proves that total phenolic content (TPC) of the seeds is higher to be compared with macadamia and almonds (Kornsteiner, Wagner, & Elmadfa, 2006). They are also having a most abundant phytosterol present in the seeds is β-sitosterol, followed by stigmasterol and camp sterol. But sacha inchi is a poor source of carotenoids due to their low total carotene content which is 0.07–0.09 mg of carotene equivalent/100 g seeds. They are also well known as oil from the cold-pressed seeds for being healthy because of its high essential fatty acid levels (Fanali *et al*., 2011). In addition, as opposed to olive, soy, corn, and sunflower oils, oil

from the sacha inchi has the largest volume of (Hanssen & SchmitzHübsch, 2011). Inca peanut oil has been shown to not only be healthy, but also to increase (high-density lipopreotein) HDL cholesterol in humans (Gonzales & Gonzales, 2014). Thus, the oil which is now available as a dietary supplement and edible oil in supermarkets, has a high economic value. They has also been imported for to Southeast Asia for cultivation due to nearly similar tropical weather conditions. Aside from the production of oil, their seeds can be lightly roasted and salted as a snack, similar to salted peanuts, and the dried leaves can be used to make a tea which it has been becoming increasingly popular in Thailand, despite consumer concerns about the safety of Inca peanut-related products. Other than that, sacha inchi seeds and leaves contain significant levels of alkaloids, saponins, and lectins when raw, which can be hazardous if eaten before heating, but which are destroyed by roasting. Polyunsaturated fatty acids can be found in the edible seed oil.

Additionally, the chitosan analgesic effects are also clarified the polycationic nature on chitosan. Presently, polymer bearing of group of amino and polysaccharide are also containing fragile glycosidic bond in order to clarify the biodegradability. Some of the Chitosan's proteases, and basically lysozyme is really debased in vivo. Other than that, due to its capacity to tie with cholesterol, fats, proteins, and metal ion, they can also be utilized as chelating agent (Bhardwaj & Kundu, 2010). In addition, the numerous appealing properties like biodegradability, natural origin and also reactivity on numerous areas of their utilization.

2.1.5 Pharmacological Studies

The production of sacha inchi is small but contain a very high protein and other nutrients. Sacha inchi, which has recently been dubbed a superfood, has a plethora of health benefits and becoming more common as a plant-based protein source (Wang *et al*, 2019). Other than that, it is commonly used in plant-based protein powders and smoothies (Lonnie *et a*l 2020). There are various ways to consume the inca peanut but the protein powder that offers a sustainable, plant-based source of protein that contains all nine essential amino acids are the most common processed. Sacha inchi-based protein powder is a perfect way to consume this superfood, whether more protein has been added to diet or just want to enjoy the many benefits of sacha inchi. Additionally, sacha inchi protein is grain-free, gluten-free, and soy-free, making it ideal for people seeking to follow a plant-based diet while still managing food sensitivities.

Sacha inchi is a nutrient-dense nut for such a small nut. Fatty acids, like omega-3 fatty acids, omega-9 fatty acids, and omega-6 fatty acids, are abundant in sacha inchi (Asif, 2011). On a plant-based diet, it is a perfect source of fatty acids because it contains all three on. Unlike some other plant-based protein sources, sacha inchi is a "complete" protein since it contains all nine necessary amino acid.

Although most animal-based protein sources are "complete," many plant-based proteins sources only contain a portion of the essential amino acids which it on a plant-based diet, it's important to eat a diverse variety of protein sources. Fortunately, sacha inchi is a perfect animal protein substitute that makes having all of the nutrients you need on a plant-based diet a breeze (Quinlan & Quinlan, 2006). When compared to

other popular protein sources such as meat or dairy, sacha inchi is a more sustainable source of protein.

South of American has grown the sacha inchi more than a decade. Growing sacha inchi plant as a crop can help to promote reforestation and protect the rain forest. According to research, sacha inchi can help to prevent cardiovascular disease (Maurer *et al*, 2012). Fatty acid profile of sacha inchi is close to that of flaxseed oil, another "superfood" plant that us consuming sacha inchi oil can lower blood pressure and cholesterol levels by referring to a recent report (Gonzales & Gonzales, 2014).

Consumer that applied sacha inchi oil for four months had lower "negative" cholesterol levels and lower arterial blood pressure also can aid in insulin sensitivity improvement. Sacha inchi particularly had showed insulin sensitivity improvement after a meal high in saturated fat. This means that after a heavy meal, sacha inchi will assist you in digesting your food and absorbing essential nutrients (Alayón, Avila, & Jiménez, 2018).

Vitamins A and E are also abundant in sacha inchi is a great source of protein on a plant-based diet that can help with overall health because it contains these beneficial nutrients. Sacha inchi is high in essential fatty acids, which can help with inflammation and pain relief (Torres Sánchez, Hernández-Ledesma, & Gutiérrez, 2021). Inflammation and discomfort caused by chronic inflammatory disorders may be reduced with sacha inchi. Sacha inchi is a delicious, organic snack (Villacorta, & Shaw, 2013). Apart from that, it has a slew of other health advantages. With all of these advantages and more, sacha inchi is an excellent addition to a vegan or vegetarian diet.

2.2 Use of Polymer Worldwide

The common packaging materials that have been used in the food industry is plastics also known synthetic polymers that made of petroleum. These polymers include polyethylene terephthalate (PET), high-density and low-density polyethylene (LDPE and HDPE, respectively), polypropylene (PP), polyvinyl chloride (PVC) and polystyrene (PS) (Housewirth, 2017). The global production of synthetic polymers is dominated by polyolefins or also known as polyethylene and polypropylene then followed by other types of polymers such as LDPE, HDPE, PVC and others (Ritchie & Roser, M, 2018). The reason why is because they can be made with relatively cheap natural gas and also they are the lightest synthetic polymers produced on a large scale. Other than that, polyolefins can resist damage from water, air, grease and detergents. After all, they can easily shape into products, but strong. However, these materials have serious downsides. Their degradation rate is very slow, which means that polyolefins can remain in the environment for decades to hundreds of years. At the same time, the effects of climate change will mechanically abrade them creating microparticles that can be ingested by fish and animals, making their way up the food chain toward people.

2.3 Polymer Film From Natural Resources As Food Packaging

Natural polymers are widely used in various biomedical applications like drugs, tissue regeneration scaffolds and drug delivery agents. In wound care, they are used as dressings and regeneration templates for acute or chronic wounds. A wide range of sources can be derived from a wide variety of sources such as plants, animals and microorganisms. The similarity between the natural polymer-based skeleton and the extracellular matrix, mechanical adaptability, high biocompatibility and high water are retention capacity attract skin regeneration.

Modern technology aims to maintain the freshness and integrity of food and provide companies with a cost-effective way to package their products (Spitz 1996). Although this method is effective, technological advances have led to much higher prices for food packaging and at the same time lowered prices for enterprises (Kerry 2006). To maintain mechanical strength, biodegradable polymers for food packaging are being studied with increasing attention to the environment (Avella 2005, Del Nobile 2009). Moreover, to address growing concerns about more sustainable food packaging, new recycling programs accompanied by new technological changes are also underway (Santos 2005, Subramanian 2000).

2.4 Active Packaging

The phrases active packaging, intelligent packaging or smart packaging consult with packaging structures used with foods, pharmaceuticals, and also on numerous different kinds of products. It also may be described as a system of the packaging that intentionally consists of additives that release or absorb materials into or from the food packaging or the environmental surrounding the foods to prolong their shelf-life or to preserve or enhance the circumstance of the food packaging (Regulation (CE) No. 450/2009 (29/05/2009)). Therefore, active packaging does something more than simply present as a barrier to external detrimental factors because the packaging system performs an active role in food preservation and a standard during the advertising process (Pereira de Abreu, Cruz, & Paseiro Losada, 2012).

2.5 Chitosan

Chitosan-based products have been created in recent years due to their unique chemical characteristics. Because of the presence of amine groups, chitosan is extremely soluble in acidic solutions (often below pH = 6.0). It is also a weak basic (pKa = 6.3). Because protonation makes the amine groups positively charged at low pH, chitosan can be a water-soluble cationic polyelectrolyte. When the pH of chitosan residues rises above 6, the amine groups are deprotonated, and the biopolymer loses its charge, resulting in an insoluble polymer (Yi *et al*., 2005). It's found in the exoskeletons of mollusks and crustaceans, as well as fungus and insect cuticles (Kaur *et al*., 2014). Because of its natural abundance, it can produce more than 1000 tonnes per year, with around 70% of that coming from marine species (Islam *et al*., 2017).

Out of a chemical standpoint, chitin is a poly(-(1-4)-N-acetyl-d-glucosamine) with β(1→4) linkages (Kaur *et al*., 2014), thought to have a cellulose-like structure with an acetamido group in the C2 position. Chitin has 3 polymorphs, labelled as α, β and γ, depending on the orientation of polysaccharide chains 3. The α-type is the most common among them in shellfish shells. This polymorph has an antiparallel structure, which each chain forms strong hydrogen bonds with the one next to it, resulting in great thermochemical stability and insolubility (Khoushab & Yamabhai, 2010). As a result, chitin is a highly insoluble polymer that is difficult to degrade. Chitin can only be degraded by chinitanase enzymes, which are abundantly distributed in nature (Elieh-Ali-Komi & Hamblin, 2016).

In the industry today, there is a well-established methodology for extracting chitin from shellfish wastes that comprises the steps of demineralization, deproteinization, and decolorization (Shahidi & Synowiecki, 1991). An alkaline treatment, in which lipids and proteins are hydrolyzed, is used to deproteinize the meat. The demineralization stage is usually done using acids, but the decolorization stage necessitates an oxidative treatment. Finally, chitin can be deacetylated by a basic treatment, yielding chitosan, a soluble polymer in an acidic aqueous media. The degree of deacetylation varies depending on the production technique and species employed, but at least 85 percent deacetylation is necessary for good chitosan solubility (No & Meyers, 1995).

2.6 Antioxidants

Antioxidants are substances that can prevent or delay free radicals, unstable molecules produced by the body due to environmental influences. Most of polymer industry are commonly used antioxidant to prevent the thermal degradation of polymers while processing. Generally, polyphenols, organophosphates and thioether compounds, which are synthetic antioxidants compounds are widely use even it had been questioned due to their potential toxicity associated with their transmission to food. Afterward, there are some reports to delivered to oxygen-sensitive food and improving their chemical stability by release of antioxidants that added to the packaging films. However, due to potential risks and the need to take strong legislative actions, people question the availability of synthetic antioxidants in food. Another method that has been extensively studied is the use of tocopherols, plant extracts and essential oils from spices and herbs which is a natural antioxidant that are widely used (Almalaika, Ashley & Issenhuth, 1994). It is also to mention that industrial food waste can be used as a source of antioxidants (Barbosa-Pereira, Angulo, Paseiro Losada & Cruz, 2013). Food packaging that been release of the natural antioxidant in the food market is a great process to interest the food technicians as it can reduce lipid oxidation (Barbosa-Pereira, Cruz *et al*., 2013), as well as increase the nutritional value of food.

2.7 Antiproliferative and Antitumor Activity

Sacha inchi leaf extracts activity is intriguing because of it contain 5.34-10.85% polyphenols (especially the chloroform leaf extract) (Lima Nascimento *et al.*, 2013), compounds found in Euphorbiaceae plants, such as Euphorbia (Duarte *et al.*, 2010), that have in vitro antiproliferative activity in the digestive tract, despite being well-known for their antioxidant activity (as reducing agents) and even as metal chelators. This similar feature, related to lipid peroxidation inhibition, is thought to protect against the start of cancer, given that flavonoids are the most abundant polyphenols discovered in P. volubilis L. (Crivineanu *et al.*, 2009). The primary biological activities detected in sacha inchi components are listed in Table 3.

Biological activity	**Plant part**	**Country**	**Assay outcome**	**Reference**
	Seed	Peru	Seeds of 16 cultivars were assessed searching for different phytochemicals; a high variability was found in the content of the evaluated compounds. The hydrophilic and lipophilic antioxidant capacities were correlated with total phenolic and total carotenoid contents, respectively. This study positions the seed as source of polyunsaturated fatty acids, tocopherols, phytosterols and phenolic compounds with antioxidant capacity.	(Chirinos, 2013)
	Seed (oil)	Peru	The antioxidant activity of	(Muñoz

			the lipophilic and hydrophilic extracts of the oil was measured *in vitro* by ABTS and DPPH assays. Lipophilic extract showed greater antioxidant activity using the DPPH assay than hydrophilic extract, which showed greater activity using the ABTS method.	Jáuregui *et al*., 2010)
	Seed (raw and honey-coated)	Peru	Several approaches (open boiling, pressure boiling, low and high temperature roasting and honey roasting) were applied to kernels to assess the variations in the total phenolic content. The result of the DPPH assay was influenced by process temperature and water activity of the seeds.	(Štěrbová *et al*., 2017)
	Leaf (leaf extract and leaf extract-based silver nanoparticles)	Ecuador	The antioxidant effect of AgNPs (silver nanoparticles) was higher than of leaf extracts against the DPPH radicals. Maximum radical scavenging activity was 22.5% in 0.6 mL of AgNPs whereas 19% in 1.0 mL of leaf extracts.	(Kumar *et al*., 2014)
Antidyslipidemic	Seed (roasted)	Peru	The effect of the intake of 30 g sacha inchi seeds per day for 6 weeks was assessed on 28 volunteers. The control group received 30 g confit wheat (*Triticum aestivum*). A reduction in cholesterol, triglycerides and LDL levels was observed, as	(Huamán Saavedra *et al*., 2012)

			well as an increase in HDL levels.	
	Seed (oil)	Peru	This experimental work sought to know the effect, effective dose and side effects of sacha inchi oil in the lipid profile of 24 patients with hypercholesterolaemia. The participants were randomized to receive 5 or 10 mL of an oil suspension for four months. Intake of the oil resulted in a drop in mean total cholesterol and non-esterified fatty acid values with c-HDL elevation in both groups.	(Din *et al.*, 2017)
Antitumoral	Leaf (leaf extracts)	Brazil	HeLa (cervix) and A549 (lung) tumor cell lines were treated with several leaf extracts. The methanol and hexane compounds were able to reduce the proliferation of HeLa cells up to 54.3 and 48.5%, respectively.	(Lima Nascimento *et al.*, 2013)
	Seed (oil)	Peru	Seed oil was shown to have potential anticancer activity in Walker 256 tumor-bearing rats. A sacha inchi oil-based diet (1 g/kg body mass, daily, for 4 weeks) reduced tumor mass and proliferation of Walker 256 tumor cells *ex vivo*. This assay also identified an increased lipoperoxidation in Walker 256 tumor tissues as well a reduction of the	(Schiessel *et al.*, 2015)

			glycaemia, triglycerides and inflammatory cytokine plasma levels.	

Table 3: Biological activities of sacha inchi

Table 3 show the summarises experimental trials aimed at proving three therapeutic or biological applications of specific sacha inchi plant sections. In terms of the crop's origin, it's clear that the majority of the research comes from South America, notably Peru. ABTS stands for 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) and DPPH stands for 2,2-diphenyl-1-picryl-hydrazyl-hydrate.

CHAPTER 3

METHODOLOGY

3.1 Plant Material

The sacha inchi (*Plukenetia Volubilis*) sample is obtained from a farmer in Bachok, Kelantan. We received 300 g of sacha inchi crude leaves part.

3.2 Extraction of Sacha Inchi Leaves

The method of extraction of sacha inchi leaves as follow Qin, 2015, the technique contains the accompanying advances were receiving the leaves as crude materials then cut manually and performing drying process under the sunlight for 3 days. Then, the leaves were dried in an air circulatory tray drier at 50 ℃ for 72 hours. After that the leaves was soaked in distilled water for 72 hours. The leaves extract was filtered then freeze-dried and stored at 4 ℃ in the chiller until use.

3.3 Preparation of Chitosan Film

All material was taken from UPKEM. Chitosan film was prepared by following Abdurrahim, 2019 method with slight modification. Table 4 showed the optimization of chitosan quantity in the film.

Chitosan Mass (%)	Texture Before Heat	After Heat
0.5 (the original formula of the journal	Too liquid	Does not dry and form a film
1.0	Too liquid	Does not dry and form a film
2.0	Not so thick and not too liquid	Form film but still wet
3.0	Too thick	Form film but too hard and brittle

Table 4: Optimization of Chitosan Quantity

The method was started by dissolving 2.5% w/w Aldrich's Sigma chitosan, 1% w/v glycerol, 1% v/v acetic acid in a beaker. Heat using hot plate and continuous stir using magnetic stirrer for 15 minutes. The solution then measured to 15 ml per film and put into the petri dish. The petri dish then heated in the oven with 70 C for 2 hours.

3.1.1 Preparation of Chitosan Films Solution

As described by Tan *et al*., 2019, the film sample prepared (1 percent w/v) was sliced into the smallest feasible bits and then immersed in deionized water. After 24 hours in a room environment, each combination was centrifuged at 3000 rpm for 20 minutes to remove insoluble residual film and be filtered. The supernatant were used as a stock solution for determining antioxidant activity, at concentrations of 10 mg/mL.

3.1.2 Preparation of Sacha Inchi leaf extract solution

The extract was weighed and then added to distiled water to a final concentration of 10 mg/mL (extract stock solution) (Nascimento *et al*., 2013).

3.4 Antioxidant Analysis Using DPPH Assay

To predict the antioxidant activity of sacha inchi leaf extract, the method was adopted form Nascimento, 2013 with slightly modification. DPPH (standard 2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay was used by the mechanism which lipid antioxidants act to inhibit free radical scavenging capacity and scavenging of DPPH radical. The method was widely used due to relatively short time required for the analysis. Thus, ascorbic acid was used as the positives with DPPH method. Briefly, the extract solutions of film samples were diluted with deionized water at 0.075, 0.15, 0.30, 0.60 and 1.20 mg/mL. Afterwards, 1.0 mL of diluted solution was thoroughly mixed with 2.0 mL of DPPH solution (180 μM, dissolved in absolute ethanol) followed by shaking for 10 s. The reaction mixture was then incubated in dark condition for 20 min before triple-measuring the absorbance of each solution using UV–vis

spectrophotometer at 517 nm. And deionized water displaced sample was used as blank. The scavenging effect against DPPH radical was assessed using equation:

$$DPPH\ Scavenging\ Effect\ (\%) = \frac{Abs_{DPPH} - Abs_{extract}}{Abs_{DPPH}} \times 100$$

where Abs_{DPPH} is the absorbance value at 517 nm of the methanolic solution of DPPH and $Abs_{extract}$ is the absorbance value at 517 nm for the sample extracts. The step was repeated for DPPH test of chitosan film and also chitosan film that incorporated with sacha Inchi. The data was expressed by determined the IC_{50} value. To calculate IC_{50}, the simplest estimate of IC_{50} is to plot x-y and fit the data with a straight line (linear regression). IC_{50} value is then estimated using the fitted line, where:

$$Y = ax + b$$

$$IC50 = \frac{0.5-\mathrm{b}}{a}$$

3.5 Evaluation of Total Flavanoid Content and Total Phenolic Content of Both Extract and Film

3.5.1 Determination of Total Phenolic Content (TPC)

The amount of TPC is determined after some modifications according to the method described by Waterman and Mole (1994). The sacha inchi leaf extract was dissolved in distilled water (2 mg/ml)1. The standard curve was set to 0,1,2,3,5 and 10 ml of the phenol solution into 100 ml volumetric flask diluted with 100 mL distilled water. The solution then was mixed 20 µL, 1.58 mL water, 100µL of Folin-Ciocalteu reagent, 300µL of sodium carbonate solution and left at 40°C for 30 min. After the incubation period, the optical density was measured at 765 nm UV–vis spectrophotometer. Gallic acid was used as a reference compound. The result is expressed as microgram gallic acid equivalent per milligram of extract (microgram GAE/mg).

3.5.2 Determination of Total Flavanoid Content (TFC)

Total flavonoid content was determined following a method by Park *et al* (2008). In a 10 ml test tube, 0.3 ml of sacha Inchi extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO2 (0.5 M) and 0.15 ml of AlCl3.6H2O (0.3 M) were mixed. After 5 min, 1 ml of NaOH (1 M) was added. The solution was mixed well, and the absorbance was measured against the reagent blank and the optical density was measured at 415nm using UV–vis spectrophotometer at 506 nm. The standard curve for total flavonoids was made using rutin standard solution. The flavonoid concentration is expressed as microgram quercetin equivalent per milligram of extract (microgram QE/mg).

3.6 Statistical Analysis

The data was collected intriplicate and expressed as mean±standard deviation (SD). After that, the data was analysed by using of One-Way Anova Test and by using Microsoft Excel application for windows and the P value less than 0.05 considered as significant.

CHAPTER 4

RESULT AND DISCUSSION

Flavonoids are the largest group of plant phenols, while phenol is the simplest family of phenolic chemicals (Saxena *et al*., 2013). Nascimento *et al*. (2013) also published the same finding which it described the presence of phenolic chemicals and flavonoids in sacha inchi leaves. These phytochemicals are recognised to have therapeutic properties such as antioxidants, enzymatic activity modulation, cellular proliferation suppression, antibacterial, cardioprotective agents and anti-inflammation having positive benefits in the human diet.

4.1 Antioxidant activity by DPPH scavenging activity

DPPH radical-scavenging ability relatively stable organic radical, DPPH, has been widely used in the determination of antioxidant activity of single compounds, different plant extracts and film extracts. DPPH is an extremely stable organic free radical with a deep violet hue exhibiting absorption peaks in the range of 515 to 528 nm. The ability of antioxidants to donate hydrogen is assumed to be the reason for their action on DPPH (Baumann, 1979). Free radical scavenging actions are critical for preventing the harmful effects of free radicals in a variety of illnesses, including cancer. The DPPH free radical scavenging method is widely used to test the antioxidant properties of plant extracts. In the DPPH test, a violet-coloured DPPH solution is converted to a yellow-coloured product upon receiving proton from phenolics, it loses it chromophore, by adding the extract in a concentration-dependent manner, as shown in

figure 1. Because of the short time necessary for analysis, this approach has been widely utilised to predict antioxidant activity. The whole system allows a large number of samples to be tested in a short time (Arabshahi-Delouee and Urooj 2007).

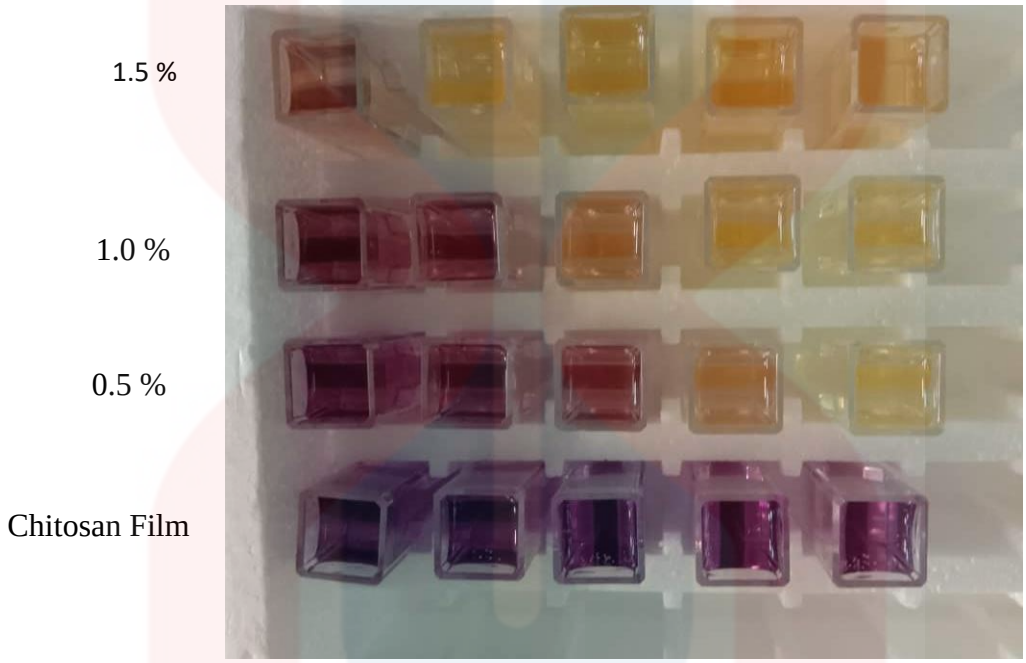

Figure 4: Concentration (mg/mL) of chitosan film incorporated with sacha inchi in prepared serial dilution

Figure 5: Concentration (mg/mL) of sacha inchi extract in prepare serial dilution

Ascorbic acid is a water-soluble vitamin that acts as a potent reducing and antioxidant agent in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries. The IC50 value of ascorbic acid was 0.012 mg/mL. As showed in table 5, the higher the weigh bring lower IC50 values indicate higher antioxidant activity

Concentration	Control	Sample (mg/mL)	%RSA
0.075	0.536	0.27	49.627
0.15	0.536	0.213	56.903
0.3	0.536	0.163	69.5896
0.6	0.536	0.11	79.478
1.2	0.536	0.021	96.082

Table 5: Calculation of % Radical Scavenging from DPPH Assay of Ascorbic Acid

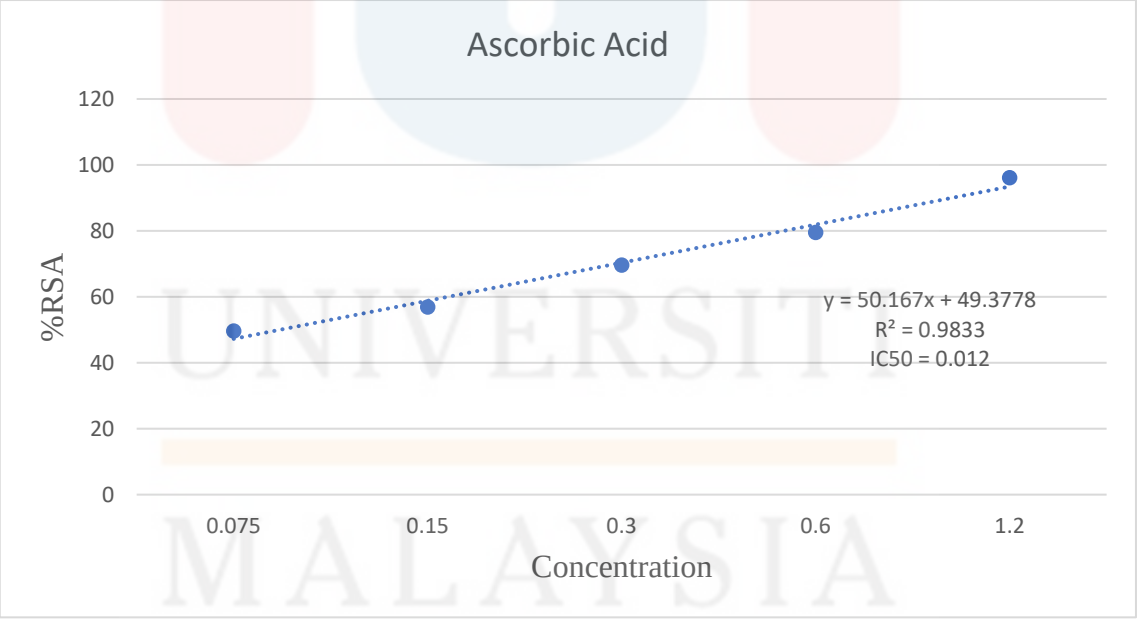

Figure 6: Ascorbic Acid Standard Curve

Calculation of % Radical Scavenging from DPPH Assay (0.5% SI extract)			
Absorbance Measurement Data			
Concentration	Control	Sample	%RSA
0.075	0.536	0.406	24.253731
0.15	0.536	0.366	31.716418
0.3	0.536	0.311	41.977612
0.6	0.536	0.149	72.201493
1.2	0.536	0.054	89.925373

TABLE 6: 0.5% SI Extract

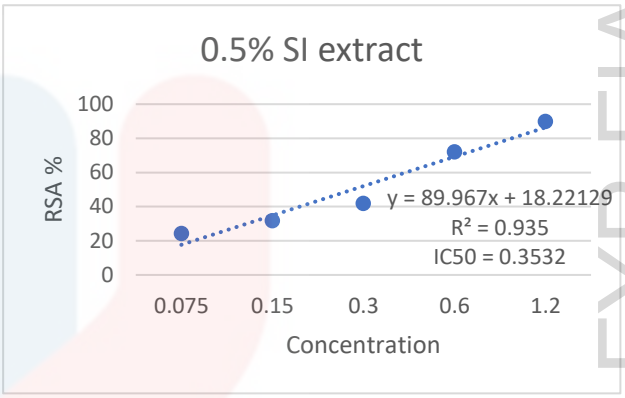

Figure 7: 0.5% extract

Calculation of % Radical Scavenging from DPPH Assay (1.0% SI extract)			
Absorbance Measurement Data			
Concentration	Control	Sample	%RSA
0.075	0.536	0.398	25.74626
0.15	0.536	0.333	37.873134
0.3	0.536	0.258	51.865672
0.6	0.536	0.158	70.522388
1.2	0.536	0.076	85.820896

TABLE 7: Calculation 1.0% SI Extract

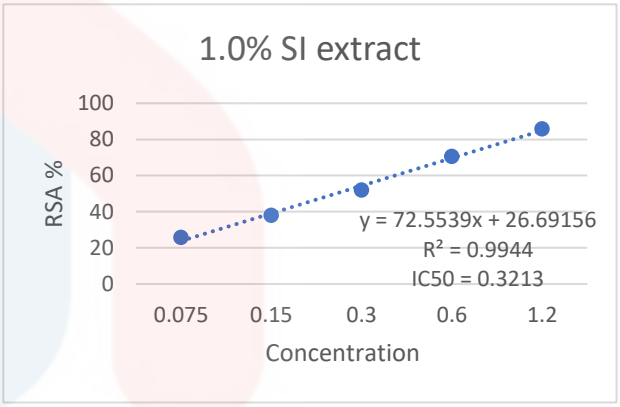

Figure 8: 1.0% SI extract

Calculation of % Radical Scavenging from DPPH Assay (1.5% SI extract)			
Absorbance Measurement Data			
Concentration	Control	Sample	%RSA
0.075	0.536	0.3	44.0298
0.15	0.536	0.253	52.7985
0.3	0.536	0.165	69.2164
0.6	0.536	0.115	78.5447
1.2	0.536	0.046	91.4179

TABLE 8: Calculation 1.5% extract

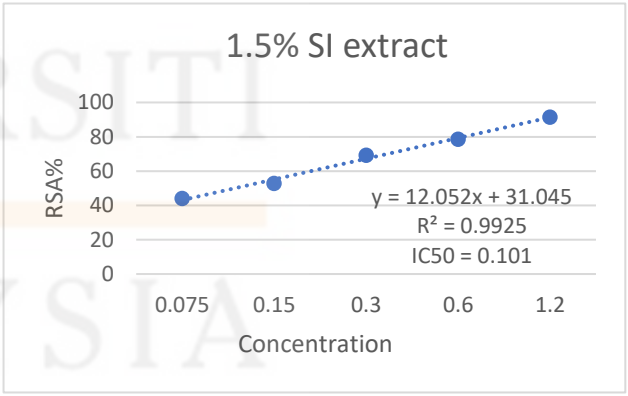

Figure 9: 1.5% SI extract

Calculation of % Radical Scavenging DPPH Assay (Chitosan + 0.5% SI extract)			
Absorbance Measurement Data			
Concentration	Control	Sample	%RSA
0.075	0.536	0.462	13.80597
0.15	0.536	0.457	14.738806
0.3	0.536	0.393	26.679104
0.6	0.536	0.226	57.835821
1.2	0.536	0.126	76.492537

TABLE 9: Calculation 0.5% + Chitosan

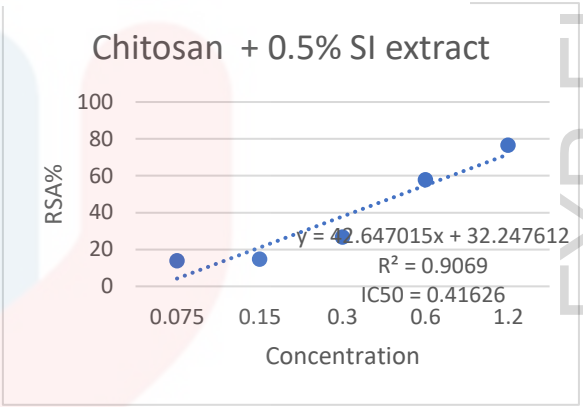

Figure 10: 0.5% SI extract+chitosan

Calculation of % Radical Scavenging DPPH Assay (Chitosan + 1.0% SI extract)			
Absorbance Measurement Data			
Concentration	Control	Sample	%RSA
0.075	0.536	0.495	7.6492537
0.15	0.536	0.358	33.208955
0.3	0.536	0.227	57.649254
0.6	0.536	0.097	81.902985
1.2	0.536	0.081	84.88806

TABLE 10: Calculation 1.0% + Chitosan

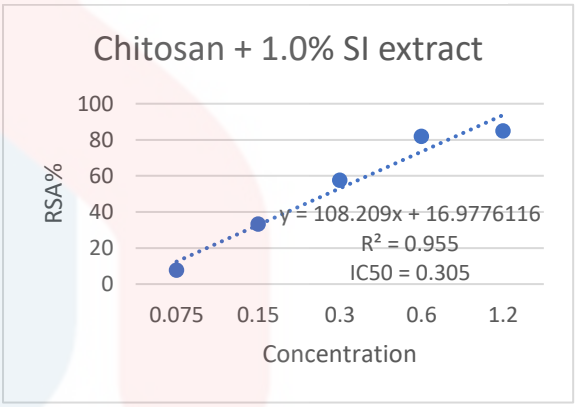

Figure 11: 1.0% SI extract+chitosan

Calculation of % Radical Scavenging DPPH Assay (Chitosan + 1.5% SI extract)			
Absorbance Measurement Data			
Concentration	Control	Sample	%RSA
0.075	0.536	0.5	6.71641
0.15	0.536	0.478	10.8208
0.3	0.536	0.321	40.1119
0.6	0.536	0.157	70.7089
1.2	0.536	0.094	82.4626

TABLE 11: Calculation 1.5% + Chitosan

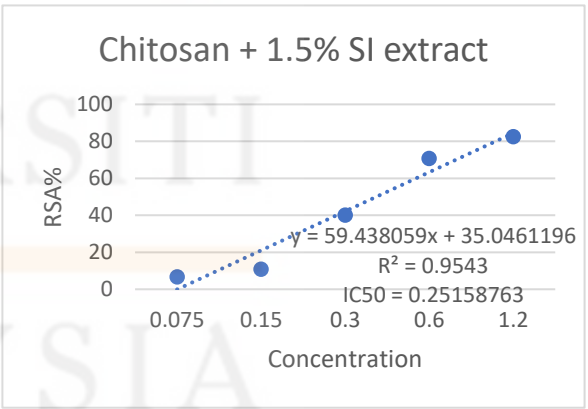

Figure 12: 1.5% SI extract+chitosan

Calculation of % Radical Scavenging DPPH Assay (Chitosan Film)			
Absorbance Measurement Data			
Concentration	Control	Sample	%RSA
0.075	0.536	0.521	2.7985075
0.15	0.536	0.514	4.1044776
0.3	0.536	0.471	12.126866
0.6	0.536	0.435	18.843284
1.2	0.536	0.371	30.783582

TABLE 12: Calculation Chitosan

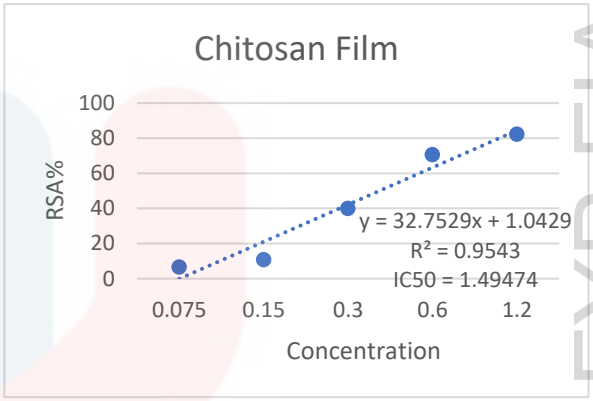

Figure 13: 1.5% SI extract

	DPPH (mg/mL)	IC_{50} (DPPH)
Chitosan Film	0.471 ± 0.0618	1.495
0.5% Sacha Inchi Extract	0.311 ± 0.1499	0.353
1.0% Sacha Inchi Extract	0.258 ± 0.1299	0.321
1.5% Sacha Inchi Extract	0.165 ± 0.103	0.101
Chitosan Film + 0.5% SI	0.393 ± 0.1499	0.416
Chitosan Film + 1.0% SI	0.277 ± 0.176	0.305
Chitosan Film + 1.5% SI	0.321 ± 0.183	0.252

Values were presented as mean ± SD of three independent measurements

Table 13: Values mean ± SD of IC_{50} DPPH

DPPH radical-scavenging ability of the chitosan-based films was shown in figure 12. Pure chitosan films showed 30.78 % DPPH radical-scavenging ability of 1.2 concentration (0.321 ± 0.183 mg/mL), which might be because that the capacity of residual free amino groups of chitosan to react with free radicals forming stable macromolecular radicals and ammonium groups (Xie *et al*. 2001). Meanwhile sacha inchi extract showed higher percentage of radical scavenging assay toward DPPH as shown on figure 4 to 6. The DPPH radical scavenging assay are significantly increased as sacha inchi extract concentration increased. Meanwhile the value of sacha inchi extract without chitosan film are not significant (p=0.744875). The film incorporated with 1.5% sacha inchi extract contained the highest percentage of radical scavenging effect, which it was found to be the most active radical scavenger. The interaction between phenolic compounds and chitosan molecules could influence the DPPH radical-scavenging ability of the films. Similar trend had been reported by Siripatrawan and Harte (2010).

The amount of antioxidant required to reduce the DPPH concentration by 50%, as expressed by interpolation from a linear regression analysis, is inversely proportional to a compound's antioxidant ability (Liu, et.al., 2008). A substance with a lower IC_{50} has a higher antioxidant activity. The free radical scavenging activities of the extracts were determined using DPPH scavenging assay and the results were displayed in Table 11 as mean ± standard deviation. With reference to the positive control of ascorbic acid, the scavenging ability of the sacha inchi leaves extracts, chitosan film and chitosan film incorporated with sacha inchi extract on DPPH was shown. The lowest IC_{50} value was from the highest sacha inchi extract which is 1.5% (IC_{50} = 0.101µg/mL) Table 11 displays the extracts' IC_{50} values in a DPPH radical scavenging activity experiment. The

predominant antioxidant components were phenolics, and their overall amount was proportionate to their antioxidant activity (Liu, et.al., 2008). Hence, from table 11 explained the IC_{50} value of chitosan film incorporated with sacha inchi extract where have an increasing absorbance reading among of them and decreasing of the IC_{50} value. Sacha inchi leaves contain flavonoids, flavonols and related polyphenols are able of donating a hydrogen atom to a free radical to neutralize it. The differences between the results of this study and those of other studies can be attributed to a variety of factors, including differences in plant matrix, different solvents used in extraction, and differences in the compositions and antioxidant activities of the extracts (an extract with a phenolic compound with a higher number of hydroxyl groups has a higher antioxidant activity), as well as the method and conditions of extraction (temperature and time) (Robards, 2003).

4.2 Determination of Total Phenolic Content (TPC)

TPC activity is the process to figure out the amount of phenolic content in the samples. Phenolic compounds that contained in the plants have redox properties, and the properties allow them acting as antioxidants. The colour will turn to bluish if there have a reaction like showed at figure 13 and 14.

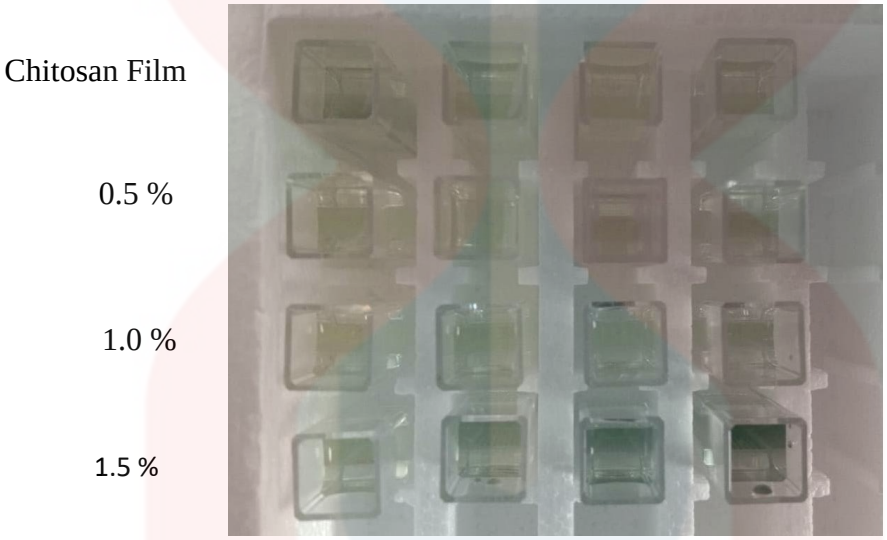

Figure 14: Concentration (mg/mL) of sacha inchi extract in prepared serial dilution on determined total phenolic content

Figure 15: Concentration (mg/mL) of chitosan film incorporated with sacha inchi in prepared serial dilution on determined total phenolic content

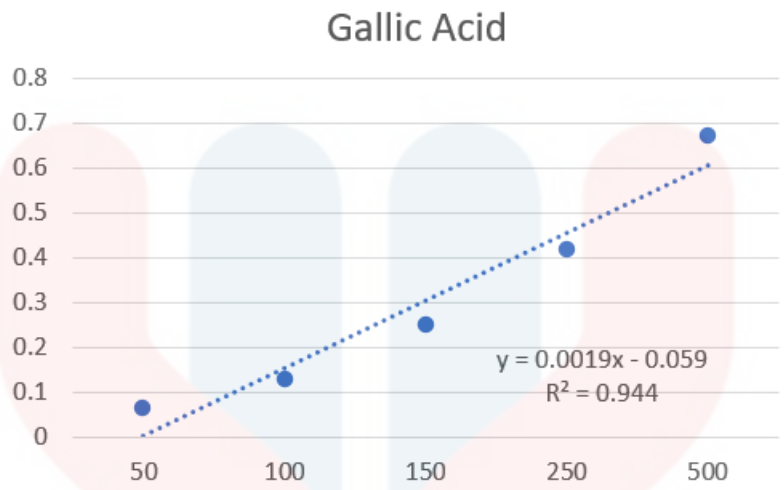

Figure 16: Gallic Acid standard curve

	Concentration (microgram GAE/mg)
Chitosan Film	42.632 ± 1.985
0.5% Sacha Inchi Extract	57.368 ± 0.656
1.0% Sacha Inchi Extract	69.474 ± 7.842
1.5% Sacha Inchi Extract	155.789 ± 4.218
Chitosan Film + 0.5% SI	55.789 ± 0.496
Chitosan Film + 1.0% SI	63.158 ± 0.248
Chitosan Film + 1.5% SI	90.000 ± 0.496

Table 14: Concentration of sacha inchi leaves in Gallic Acid

The table was calculated using intercept formula on gallic acid standard curves (figures 12) and the values were presented as mean ± SD of three independent measurements.

The total phenolic content of the chitosan-based films, chitosan film incorporated with sacha inchi and sacha inchi extract was evaluated by Folin–Ciocalteu method in gallic acid standard curve, was presented in figure 15. Aside from that, it is indicated that in the gallic acid standard curve, the greater the absorbance reading, the higher the concentration. The phenolic contents can be used as an important indicator of antioxidant capacity and be used as a preliminary screen for any product when intended as antioxidants in food packing materials (Viuda-Martos *et al*. 2011). The phenolic contents can be used as an important indicator of antioxidant capacity and be used as a preliminary screen for any product when intended as antioxidants in food packing materials (Viuda-Martos *et al*. 2011). In table 12, the results showed that total phenolic content of film extract significantly ($p < 0.05$) increased with increasing sacha inchi leaves extract concentration exhibited the lower values ($p=6.34x10^{-10}$) with higher value (90.000 ± 0.496 microgram GAE/mg) for the film incorporated with 1.5% sacha inchi extracted meanwhile the sacha inchi extract are higher ($p=3.49x10^{-6}$) with higher value (155.789 ± 4.218 microgram GAE/mg) for 1.5% sacha inchi extract.

There has been no previous research on the yields of sacha inchi leaf extracts in the literature. Phenolic compounds are the most abundant and extensively dispersed phytochemicals in the plant kingdom (Saxena *et al*., 2013). Flavonoids, phenolic acids, stilbenes, coumarins, and tannins make up the majority of them (Islam *et al*., 2015). Oxidation properties of phenolic compounds allow them to serve as antioxidants (Soobrattee *et al*., 2005). Their antioxidant capacity is aided by their hydroxyl groups, which scavenge or stabilise free radicals through hydrogenation or by their appearance in the presence of oxidising agents (Uddin *et al*., 2020). TPC could be utilised as a starting point for fast antioxidant activity testing. Kanatt *et al*. (2012) reported that the incorporation of natural plant extract with high phenolic content, such as mint extract

and pomegranate extract, in chitosan films enhanced antioxidant potential to the films. Mayachiew and Devahastin (2010) also indicated that the incorporation of gooseberry extract increased total phenolic content of chitosan films. There is a link between total phenolic content and antioxidant activity of Sacha Inchi extracts in this investigation. However, the presence of glycerol may affect the total phenolic content and antioxidant ability of films. This might be because that glycerol was an organic filler and showed antioxidant activity (Gutierrez *et al*. 2012).

4.3 Determination of Total Flavonoid Content (TFC)

The total flavonoid content of the extracts was determined using an aluminium chloride complex forming assay (Piyanete, *et al*., 2009). The flavonoid content was calculated using the quercetin equivalent as a standard. For this, a quercetin calibration curve was created. The serial dilution was set up to 10,25,50, 75, and 100 mg/L. One of the most commonly used bioflavonoids for the treatment of metabolic and inflammatory illnesses is the used of quercetin. As seen in figure 16 and 17, TFC was offered in a yellow colour also the figure showed that sacha inchi extract and chitosan film incorporated with sacha inchi extract in prepared serial dilution on determined total flavonoid content.

Figure 17: Sacha inchi extract in prepared serial dilution on determined total flavonoid content

Figure 18: Concentration (mg/mL) of chitosan film incorporated with sacha inchi in prepared serial dilution on determined total flavonoid content

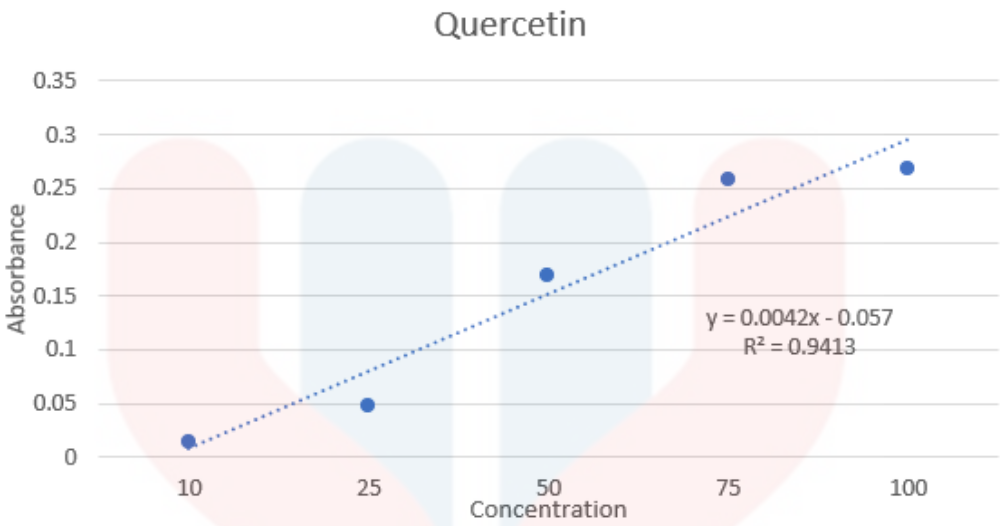

Figure 19: Quercetin Standard Curve

	Concentration (microgram QE/mg)
Chitosan Film	20 ± 1.468
0.5% Sacha Inchi Extract	32.857 ± 2.19
1.0% Sacha Inchi Extract	50.952 ± 3.322
1.5% Sacha Inchi Extract	60.238 ± 1.5099
Chitosan Film + 0.5% SI	21.429 ± 0.786
Chitosan Film + 1.0% SI	25 ± 1.105
Chitosan Film + 1.5% SI	42.619 ± 6.322

Table 15: Concentration of sacha inchi leaves in quercetin

The table was calculated using intercept formula on quercetin standard curves (figures 13) and the values were presented as mean ± SD of three independent measurements

The concentration of the film and sacha inchi leaves are as showed in table 13 and presented as mean ± standard deviation. The sample of film that incorporated with Sacha Inchi extract exhibited significant value of ($P<0.05$) ($p = 0.000267$) meanwhile the sacha inchi extract are also significant ($p = 0.000134$). Apart from that, table 13 showed the higher their absorbance reading, the higher the concentration in quercetin standard curve. Flavonoids are probably the most important natural phenolics. TFC in the extract was determined by the spectrophotometric method with aluminum chloride. The flavonoids combine with aluminum to form a complex flavonoid-aluminum that could be measured at 415 nm (Quettier, 2000). The most abundant flavonoid which has a good antioxidant property is quercetin. Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols, according to the basic concept of the aluminium chloride colorimetric method. It also forms acid labile compounds with the ortho-dihydroxyl groups in flavonoids' A- and B-rings. Flavonoids were measured using the aluminium chloride colorimetric method (Siddique, 2010). In this analysis, it show the good activity of concentration value when sacha inchi extract are increase.

4.4 Correlation between TPC, TFC and antioxidant activity

The correlation between the TPC, TFC and their antioxidant activities were done and displayed in correlation coefficients r^2 values from linear regression analysis in table 14. A positive correlation was observed between antioxidant activity and phenolic compound. The results showed that IC_{50} has a positive correlation with DPPH antioxidant activity (R^2 = 0.8149) while TPC have a positive correlation with TFC antioxidant activity (R^2 = 0.29488). The correlation indicated that the richness in phenolic compounds especially higher flavonoids contents lead to better DPPH scavenging activity (Felhi *et al*., 2016). The results suggested that the antioxidant activity of sacha inchi leaves might be attributed to the presence of flavonoids to react with DPPH, TPC and also TFC (Wuttisin and Boonsook, 2019). As a result, flavonoids can be utilised to predict sacha inchi leaves' antioxidant potential. The DPPH results would be strengthened by the favourable connection between antioxidant and IC_{50} value. This study demonstrates that increasing total phenolic components in extracts increases antioxidant activity, which is in line with earlier findings (Bakari *et al*., 2015) and increasing the concentration of sacha inchi extract in each test. Furthermore, the antioxidant activity of sacha inchi leaves could be attributed to the presence of non-phenolic compounds, as well as other synergistic or antagonistic compounds.

	ANTIOXIDANT	IC_{50}	TPC	TFC
ANTIOXIDANT	1			
IC_{50}	0.82149	1		
TPC	-0.77079	-0.53149	1	
TFC	-0.65093	-0.55979	0.29488	1

Table 16: Correlation matrix of TPC, TFC, IC_{50} and antioxidant value

CHAPTER 5

CONCLUSION

The presence of phytochemicals such as phenols, flavonoids, and DPPH antioxidants in Sacha Inchi leaf extracts was discovered in this study. The correlation between TPC, TFC, and their antioxidant activities was determined and displayed in correlation coefficients R^2 values using linear regression analysis on the graph, resulting in a positive correlation between them with an R^2 value approaching one. The redox properties of all of the tests may be justified by their chemical structure. For this reason, the high absorbance reading incorporated sacha inchi extracts may explain the high antioxidant activity of the extracts. Furthermore, the antioxidant activity of sancha inchi leaves could be attributed to the existence of non-phenolic chemicals, and the leaves could contain other synergistic or antagonistic substances. Hence, in this study also show phenolic and flavonoid are contributed to antioxidant. As showed on the result, the sacha inchi that incorporated with the film give significant reading. Other chemical components in sacha inchi leaves will need to be identified through further research. As a result, these unique active films have a lot of promise for being developed into food packaging materials, and they're a promising alternative to synthetic materials based on chitosan thanks to the addition of sacha inchi leaves extract.

4.2 Future Study

Further studies are required to identify other chemical components in sacha inchi leaves. The presence of flavonoids such as in quercetin and other phytochemicals are recommended to be characterized by several analysis such as HPLC, polyphenols and else. Furthermore, quercetin is suggested to use as a reference compound in DPPH and ABTS radical scavenging activity for prediction the antioxidant activity of sacha inchi leaves due to the positive correlation between flavonoid and antioxidant activity.

From the study, I also recommend for further research to perform durability testing which the performance testing technique used to determine the characteristics of the chitosan film incorporated with sacha inchi extract itself. It is also a film transit test that is also a simulation of events to the physical and climatic real life supply chain as well as hazards. Along with that, the shelf life of the film produced could determine if the film is suitable to be used as food packaging material. Lastly, I would suggest to university to provide a film laminator with heat for a better experience in process of developing active film neither chitosan nor other substances.

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APPENDIX A

Table A.1: Anova value of film + Sacha Inchi extract of DPPH

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
film 0.5	5	2.68	0.536	0
film 1.0	5	1.223	0.2446	0.016863
film 1.5	5	0.879	0.1758	0.010512
chitosan film	5	2.312	0.4624	0.003819

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.442981	3	0.14766	18.93488	1.63E-05	3.238872
Within Groups	0.124773	16	0.007798			
Total	0.567754	19				

Table A.2: Anova table of Sacha Inchi extract of DPPH

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0.5	5	1.664	0.3328	0.022479
1	5	1.258	0.2516	0.031044
1.5	5	1.55	0.31	0.033628

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.01754	2	0.00877	0.301889	0.744875	3.885294
Within Groups	0.3486	12	0.02905			
Total	0.36614	14				

Table A.3: Anova table of film + Sacha Inchi extract of TPC

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
chitosan film	3	132.1061	44.03537	5.908314
0.5% film	3	168.42	56.14	0.369603
1.0% film	3	188.948	62.98267	0.092225
1.5% film	3	268.947	89.649	0.369603

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3350.171	3	1116.724	662.769	6.34E-10	4.066181
Within Groups	13.47949	8	1.684936			
Total	3363.65	11				

Table A.4: Anova table of Sacha Inchi extract of TPC

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
0.5	3	172.631	57.54367	0.646454
1	3	222.632	74.21067	92.23886
1.5	3	458.42	152.8067	26.68294

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15530.16	2	7765.08	194.828	3.49E-06	5.143253
Within Groups	239.1365	6	39.85609			
Total	15769.3	8				

Table A.5: Anova table of film + Sacha Inchi extract of TFC

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
chitosan film	3	59.286	19.762	3.232279
0.5 film	3	64.762	21.58733	0.926058
0.1 film	3	73.809	24.603	1.832997
1.5 film	3	132.618	44.206	59.94571

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1146.939	3	382.313	23.19261	0.000267	4.066181
Within Groups	131.8741	8	16.48426			
Total	1278.813	11				

Table A.4: Anova table of Sacha Inchi extract of TFC

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
chitosan film	3	59.286	19.762	3.232279
0.5 film	3	64.762	21.58733	0.926058
0.1 film	3	73.809	24.603	1.832997
1.5 film	3	132.618	44.206	59.94571

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1146.939	3	382.313	23.19261	0.000267	4.066181
Within Groups	131.8741	8	16.48426			
Total	1278.813	11				

Table A.6: Absorbance Reading

Parameter	Antioxidant	IC_{50}	TPC	TFC
Chitosan Film	0.471	1.495	0.032	0.038
0.5% Sacha Inchi Extract	0.311	0.353	0.052	0.122
1.0% Sacha Inchi Extract	0.258	0.321	0.072	0.149
1.5% Sacha Inchi Extract	0.165	0.101	0.237	0.187
Chitosan Film + 0.5% SI	0.393	0.416	0.049	0.051
Chitosan Film + 1.0% SI	0.277	0.305	0.061	0.33
Chitosan Film + 1.5% SI	0.321	0.252	0.112	0.164

APPENDIX B

Figure B1: Pure Chitosan Film

Figure B2: Chitosan Film + 0.5 % Sacha Inchi ectract

Figure B3: Chitosan Film + 1.0 % Sacha Inchi ectract

Figure B4: Chitosan Film + 1.5 % Sacha Inchi ectract

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