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Investigating the effect of Trachyspermum essential oil on the physicochemical properties of sago starch films

Maryam Sayahnezhad
Department of Chemical engineering majoring in food industry
Shahrood branch. Islamic Azad University, Shahrood, Iran

Abstract
The present study investigates the effect of Trachyspermum essential oil on the physicochemical properties of sago starch films. In this study, starch films supported with Trachyspermum essential oil at concentrations of 0, 5%, 10%, and 15% were prepared using the casting method. All physicochemical properties were performed according to the American Society for Testing and Materials (ASTM) method. To prepare the film with essential oil, the dispersion of the film was first prepared. Starch, plasticizer, distilled water, and Trachyspermum essential oil (5%, 10%, and 15%) were used to prepare the film-forming solution. To rate of permeability to film oxygen was examined using the mocon oxtran (Minneapolis, USA 2/21ASTM) in Win perm TM software and ASTM D3985-50 standard method. To calculate permeability to water vapor, the curve of the mass of water passing through the film per unit of time was first drawn and the slope of the curve was calculated in the linear part of the curve ($\Delta m/\Delta t$). Then, the obtained value was divided by the area of the film, which is the same as WVTR or the water vapor transmission rate. Physicochemical properties such as water absorption, water solubility, and moisture content decreased significantly with increasing the amount of essential oil ($p<0.05$). Generally, based on the examinations, starch films containing essential oils of Trachyspermum can be used as active packaging in the food industry.

Keywords; Trachyspermum essential oil, Sago starch, Antimicrobial properties, Active packaging, Edible film.

Introduction

Environmental pollution is considered one of the most serious problems of the current century for humans. Food packaging waste is one of the major sources of environmental pollution [1, 2]. Most of these wastes are synthetic materials of petroleum origin that take a long time to decompose and they remain in nature [3]. Biopolymer is a natural polymer [4, 5]. A biodegradable biopolymer means a natural polymer that can be decomposed with the help of microorganisms and converted into natural products such as water, carbon dioxide, etc [6, 7]. These compounds can be edible and consumed with food if only permitted edible compounds are used in their preparation [8]. The use of biodegradable biopolymers for food packaging has attracted the attention of researchers and scientists for years [3]. These biopolymers can return to nature and are decomposed by microorganisms. Their return to nature prevents environmental pollution [3]. Edible films increase the shelf life of food. It also meets the consumer's need to consume more natural foods and has less harm to the environment. The goal is not to replace edible films with existing synthetic films, but they can be produced together with synthetic packaging to reduce synthetic waste. These packages can control the permeability of gases such as oxygen, carbon dioxide, and water vapor, and the migration of lipids.

They can also protect the nutritional compounds and additives in the food [9]. Sago is a starch found in Southeast Asia and obtained from the sago palm. Sago's palm is a significant source for people in rural areas since it has several applications, especially in the production of starch or sago flour. Sago starch has been used for a long time, especially in Southeast Asia (for the production of vermicelli, bread, crackers, and many traditional foods). However, limited studies have been conducted on its physicochemical properties and characteristics. It is cheap and has significant characteristics such as easy gelatinization and high viscosity [10]. The traditional methods for extracting sago starch can be classified into two levels, the domestic level and the small-scale plant processing level. The modern extraction method includes some changes in the small processing plant [11].

It is known as Zanian in the Persian language. It is an aromatic and annual plant that grows in Iran, India, Pakistan, and Egypt. It has white flowers and small brown fruits. It is a popular and traditional flavoring spice used in traditional Iranian medicine thanks to its therapeutic effects. Its fruits have many therapeutic effects, including anti-flatulent, anti-nausea, anti-flatulent, diuretic, uterus cleaner, etc. Its essence is widely used to eliminate children's flu symptoms in traditional medicine. Ripe seeds of this plant include 2-4% essence, which is rich in thymol. Aromatic essences have antimicrobial, antifungal, and antiviral effects and are less toxic than chemical drugs. Several mechanisms involved in the emergence of its antiviral effects include preventing the formation of viral DNA and RNA or preventing the activity of viral replication [11]. The composition of its essence has been reported very differently in different studies. In this study, the significant compounds of its essence include thymol (50%, 54%), terpinene (26.10%), and cymene (22.10%) [11].

Guilbert (1986) showed that the use of edible films in the packaging of food and medicine has received increasing attention due to the high biodegradability of these films, their good resistance against gases, fat and aromatic and flavor compounds that can help maintain the quality of food. Mortenson (1999) showed that edible films are produced as a thin layer before being used in food packaging, and then they are used for packaging like synthetic polymers [12]. Utara et al. (2002) showed that covering food with edible films has several advantages, including health, sensory, and economic characteristics. The coating itself also has nutritional value, prevents spoilage and microbial contamination, and increases the strength and integrity of food [13]. Norziah M.H (2012) examined the physical and mechanical properties of a composite edible film based on sago starch, fish gelatin with glycerol, and sorbitol softeners (25% w/w). Mixed edible film (1:0, 2:1, 3:1, 4:1, 5:1) was prepared. The results revealed the significant effect of adding fish gelatin in starch solution ($p<0.05$), which was associated with lower tensile strength and higher water vapor permeability [14]. Mohammadi et al. (2012) examined the effect of rich zinc on the microbial and physical properties of sago starch edible film. The nano-enriched zinc oxide was homogenized and combined with sago starch through ultrasound. A decrease in water vapor permeability and a decrease in moisture were observed. However, the contact angle increased. Also, the sago starch film combined with nano-zinc oxide has excellent antimicrobial activity against Staphylococcus aureus. These properties show that nano zinc oxide can potentially be used as filler in starch-based films and as an active packaging material in the pharmaceutical and food industries [10].

In another study performed by Azam Arabi et al., wheat gluten protein was used to produce edible film. The effect of 1.5, 2.5, and 3.5 g of glycerol on the film tensile properties was investigated. The results revealed that by increasing the amount of glycerol in the film preparation formulation, their solubility in water increases [15]. Tang Desun Turn et al. examined the effect of carboxymethyl cellulose concentration on the physical properties of cassava starch-based films. Carboxymethyl cellulose was added as a plasticizer to the structure of cassava film to improve the mechanical properties of these films since cassava-based films are brittle and weak. Then, its mechanical properties were examined. The results revealed that adding carboxymethyl cellulose increases the tensile strength, reduces the elongation to the breaking point, and reduces the solubility in water [12]. Customers demand more durable and higher-quality, and high recyclability food. These demands have increased the interest in edible and biodegradable films or materials used to increase the life of food and improve quality. These films can reduce the amount of conventional non-renewable synthetic polymer packaging materials and can be prepared from proteins, polysaccharides, lipids, or a combination of these materials. Due to the hydrophilic properties of starch polymers, starch films are a little barrier against it [16]. The primary goal of the present study is to investigate the effect of Trachyspermum essence on the physicochemical properties of sago starch films.

Materials and Methods

Preparation of plasticizer

Glycerol and sorbitol were used to prepare the plasticizer. Glycerol and sorbitol were prepared in a ratio of 3:1. Selecting this ratio as a plasticizer was based on the study conducted by Mohammadi et al., which showed the highest resistance to thermal stitching in this ratio [17].

Control film production

To prepare the film, the dispersion forming the film was prepared. To prepare the film that forms the solution, starch, plasticizer, and distilled water were used. First, a 4% (w/w) solution of sago starch was prepared. Then, 45% of plasticizer (w/w) was added to the starch, and the necessary amount of distilled water was added until the solution reached 100 g. The solution was kept for 45 minutes at a temperature of about 85°C to complete the starch gelatinization process while stirring constantly.

Then, the solution was cooled to a temperature of about 28-30°C, and 92 g of it was placed on plates of Polymethyl methacrylate material (with the brand name of plexiglass) at the dimensions of 16 x 16 cm and thickness of 2 mm was poured and dried for about 24 hours in laboratory conditions (temperature of 25°C and relative moisture of 50%). Then, they were separated from the plates and the films were stored in a desiccator where saturated magnesium nitrite solution was poured into its bottom. The thickness of the prepared films was randomly measured at 5 points of the film after separating the films from the plate. Then, the films were cut into desired sizes for further tests.

Preparation of films with the essence of Trachyspermum

The dispersion of the film was first prepared to prepare the film with essence. To prepare the film-forming solution, starch, plasticizer, distilled water, and Trachyspermum essence (5%, 10%, and 15%) were used. First, a 4% (w/w) solution of sago starch was prepared. Then, (45%) (weight-weight) plasticizer was added to starch, and the required amount of distilled water was added until the solution reached 100 g considering the essence weight. The solution was kept at a temperature of about 85°C for 45 minutes to complete the starch gelatinization process while it was constantly stirred. Then, the solution was cooled to a temperature of about 28-30°C. During the solution cooling, when its temperature is about 50°C, the desired amount of essence was added and it was constantly stirred until its temperature reached about 28-30°C.

Then, 92 g of it was poured on a polymethyl methacrylate plate with dimensions of 16 x 16 cm and dried for about 24 hours in laboratory conditions (at a temperature of 25°C and relative moisture of 50%). Then, they were separated from the plates, and the films were kept in a desiccator where saturated magnesium nitrite solution was poured into the bottom. After separating the films from the plate, the thickness of the prepared films was randomly determined at 5 points using a caliper (Insize model) with a resolution of 0.02 mm, and their mean was used for calculations. The films were cut into the desired sizes for further tests.

Physicochemical tests

1. Oxygen permeability test

Given the significance of oxygen and carbon dioxide gases in food preservation, the permeability of edible films is crucial to these gases. Gases, water vapor, and soluble substances can pass through the packaging film in two ways:

1-Small gaps and holes in the wall of the polymer coating

- Diffusion of gas from polymer membrane: Polymer membrane is made up of spiral and entwined filaments. There are empty spaces and holes between these filaments. The diffusion of gases, water vapor, and other materials takes place through these holes. To perform the oxygen permeability test, the samples were cut in repetitions in the desired sizes according to the device and packed in plastic envelopes and sent to Damghan laboratory, where the necessary tests were performed. The results were presented in the form of graphs and analyzed.

The film oxygen permeability was measured using mocon oxtran (Minneapolis, USA 2/21) in Win perm TM software using the ASTM D3985-50 standard method and specific colorimetric sensors (Coulox®). The film was installed with aluminum foils coated with an open surface of 5 cm3 in diffusion cells. The test was performed at 28 ° C and atmospheric pressure and relative moisture of 21% and 50% oxygen test gas. The oxygen passed through the film with N_2 and H_2 carrier gas was directed to Colometric sensors. Measurements were performed continuously for 1 hour until they reached an equilibrium point (ASTM 2005a) [18].

2. Water vapor permeability test (wvp)

When the water is absorbed by food, water activity (aw) increases, and the possibility of microbial, chemical, and enzymatic spoilage increases. Loss of water in some foods, such as grains, reduces their weight. As a result, the resistance of polymer films against moisture is crucial. The water vapor permeability was measured using the modified ASTM E96-05 standard. To perform this experiment, glass cups with a diameter of 5.7 cm and a height of 3.5 cm were used. In this regard, 10 cc of deionized water was poured into each cup, which could create 100% moisture in the space inside the cup so the moisture inside the cup is higher than the moisture outside the cup. First, pieces of the film were cut from the healthy parts at the size of the outer opening of the cup. Three replicates were prepared from each sample. Then, 10 cc of deionized water was poured into each cup and the films were placed on the outer opening of the cup and sealed with the help of parafilm, and the film was placed fixed on the cup. There was a gap between the films and the water level inside the cup.

Then, the cups were placed in a desiccator containing activated silica gel. The initial weighing was done before placing the cups in the desiccator and the initial weight was recorded. Then, the cups were placed in a desiccator and weighed every 2 hours. Accordingly, 5 weights were recorded for each cup. After completing the work, the thickness of each film was accurately measured at 5 random points and used for calculations. The amount of cup weight loss is equivalent to the amount of water passing through the film. To calculate the water vapor permeability, the curve of the mass of water passing through the film per unit of time was first drawn and the slope of the curve was calculated in the linear part of the curve ($\Delta m/\Delta t$). Then, the obtained value was divided by the area of the film, which is WVTR or the water vapor transmission rate [18].

Equation (1)

Slope of the curve = water vapor transmission rate

$WVTR=((\Delta m/\Delta t))/(A)-1$

To obtain WVP based on Equation 1, the value of WVTR is multiplied by the average thickness of the film (Ɣ) and the resulting value is divided by the difference in water vapor pressure on both sides of the film.

Equation 2

$wvp=(WVTR\times Ɣ)/\Delta p$

Equation 3

$wvp= (\Delta m/\Delta t)\times Ɣ)/(A \times\Delta p)$

Equation 4

$wvp= (\Delta m/\Delta t)\times Ɣ)/(A \times\Delta p)$

Δm: mass changes

A: Film area

Δt: changes in time

Δp: partial pressure difference of water vapor on both sides of the film

($\Delta m/\Delta t$): the slope of the curve

Ɣ: film thickness

3- Water solubility test

To measure the water solubility, about 400 to 600 mg of each film sample was first weighed and placed in a glass and then accurately weighed by a digital scale. Then, the films were poured for 1 hour inside the beaker in which 80 ml of deionized water was poured. Then, it was stirred gently every 20 minutes. Then, the solution was smoothed through a filter. Then, the filter paper with the film was kept in the oven at 40 °C for 24 hours and weighed again [12].

Equation 5

The weight of the filter paper - the weight of the film with the filter paper after placing the oven = the weight of the film after placing the oven

Equation 6

The weight of the film after placing oven - the weight of the initial film = the weight of the dissolved film

Equation 7

$\frac{\text{dry weight of film} - \text{final dry weight of film}}{\text{initial dry weight of film}} = \text{solubility}$

Figure 1: Solubility test

4-Density test
To measure the density, the films were cut at the size of 2x3 cm and the samples were weighed with a scale with an accuracy of 0.001. Their thickness was measured with a micrometer. Then, we placed them in the following formula.
Equation (8(
Density= film weight/thickness x area
5-Moisture test
To examine the moisture level, we first weigh about 400 to 600 mg of each film sample using a scale with an accuracy of 0.001 m1 and placed it in a desiccator containing calcium chloride for 24 hours. Then, we weigh the films again. Then, we obtain the moisture content using the following formula.
Equation 9
$$\text{Moisture} = \frac{m_1 - m_2}{m_1} \times 100$$

6- Colorimetry
The film surface color was determined using colorimeters (Minolta 3500 spectrophotometer, Osaka-Japan), and L*, a*, and b* values were reported. Before analysis, the instrument was calibrated with 100% and 0% passing objects. Experiments were performed in three stages using a computer system in Magic Spectra software (Version 2/11, Cyber Chrome Minolta, Japan).
The value of color was measured in three random positions, which includes the center of the film sample. The maximum value is 100 and the minimum value is 0. Positive a* was defined as red and negative a* as green, while positive b* was defined as yellow and negative b* was defined as blue [10].
7-Water absorption capacity (WAC)
To examine the water absorption capacity level, some pieces of the film at the size of 400 to 600 mg were weighed by a scale and placed in a desiccator containing calcium chloride (to reduce the moisture to zero) for 24 hours. The samples were weighed with a scale with an accuracy of 0.001. Then, we placed the films in a beaker containing 80 ml of deionized water for one hour and stirred it every 20 minutes. Then, it was powdered and dried. Then, we weighed it and placed it in the following formula [10].
Equation 10

WAC= weight of absorbed water/dry weight of film

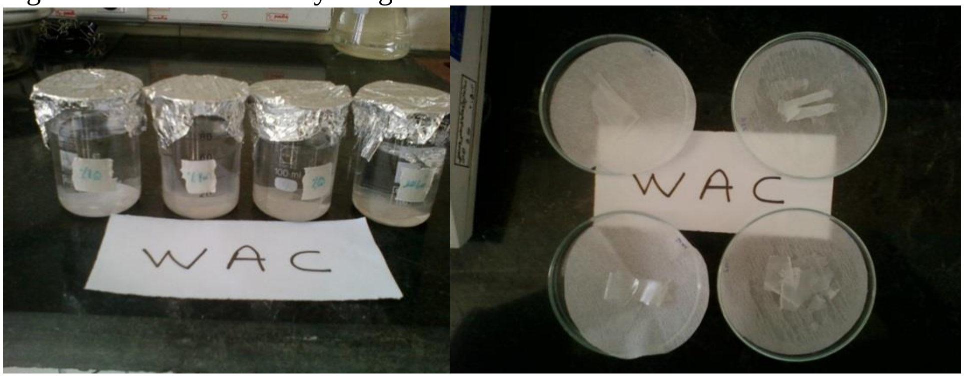

Figure 2: Absorption test

One-way analysis of variance test was used to determine the existence of a significant difference at the 5% probability level using GraphPad Prism 6 software.

Results

Measurement of solubility and moisture content

Charts 3 and 4 show the results of the solubility and moisture content of the obtained films. As shown, the solubility of the films decreases significantly from 41.24 to 17.92% when the concentration of essences of sago in the sago starch film increases from 0% to 15% ($p<0.05$).

The moisture content of these films also decreased from 3.9 to 3.44 percent, while it was not significant. The reduction in the solubility and moisture content of the films is due to the hydrophobicity of the essences used. Intra-molecular linking of polysaccharides with phenolic compounds of high molecular weight results in breaking some parts of the film during solubility. The molecular properties of phenolic compounds affect the elasticity of the film matrix.

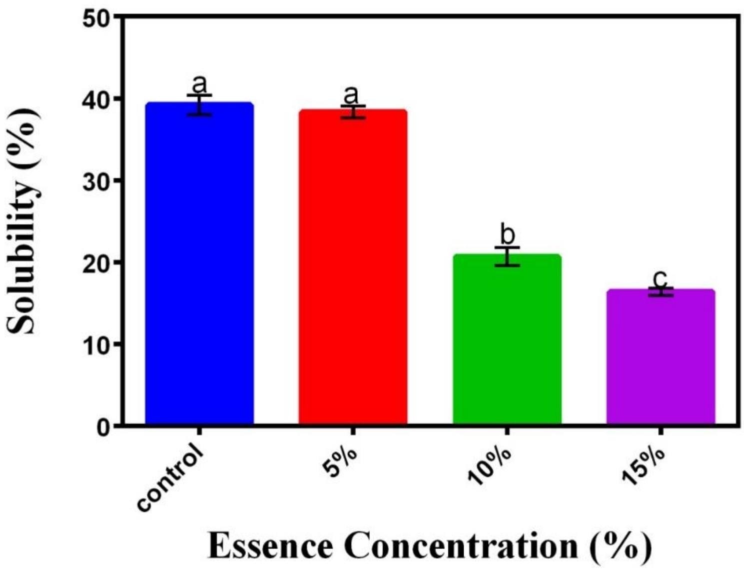

Chart 3: The effect of the Trachyspermum essence concentration on the solubility of sago starch films
Different Latin letters on the columns represent a significant difference at the 5% probability level ($p>0.05$).

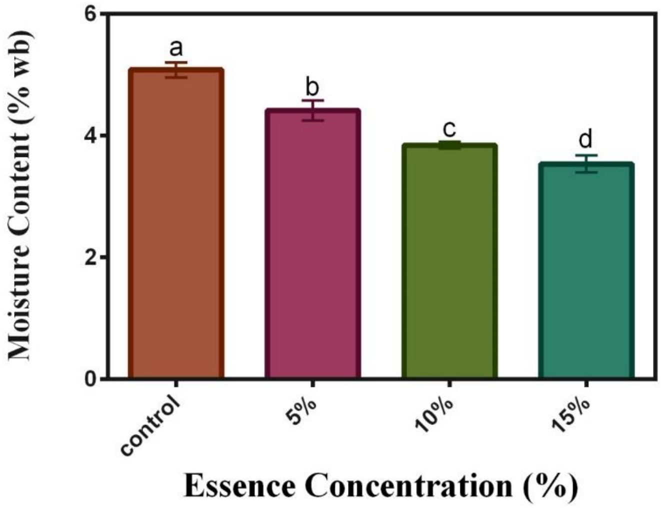

Chart 4: The effect of Trachyspermum essence concentration on the moisture content of sago starch films

Different Latin letters on the columns represent a significant difference at the 5% probability level ($p>0.05$).

Measurement of water absorption capacity (WAC) and density

Chart 5 shows the results of the water absorption capacity of the obtained films. Based on this chart, when the concentration of Trachyspermum essence in the sago starch film increased from 0% to 15%, the water absorption capacity of these films decreased significantly from 3.81 to 1.59 (gram of water per gram of dry matter) ($p<0.05$). The moisture absorption is due to the hydroxyl groups in starch that bond with water. In this study, by adding hydrophobic essence to the biopolymer matrix, the accessible hydroxyl groups for water molecules decreased. Thus, they reduced the hydrophilic properties of starch films.

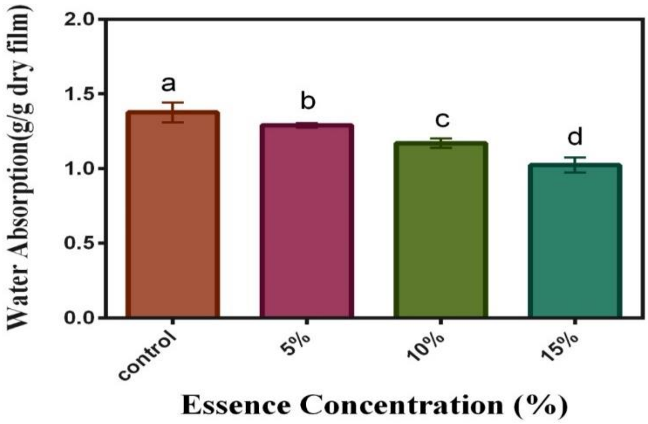

Chart 5: The effect of Trachyspermum essence concentration on the water absorption capacity of sago starch films

Different Latin letters on the columns represent a significant difference at the 5% probability level ($p>0.05$).

The density of the films did not have a significant effect on the density of the films since the percentage of essence is very small compared to the total solid matter of the film and did not have a significant effect on the moisture content of the films. On average, it was 0.56 g/cm3 for all films. The presence of moisture and water vapor is one of the most significant reasons for spoilage reactions in food. Water vapor permeability (WVP) is one of the most significant characteristics of food packaging polymers, especially biopolymers. Chart 6 shows the results of the water vapor permeability of the obtained films.

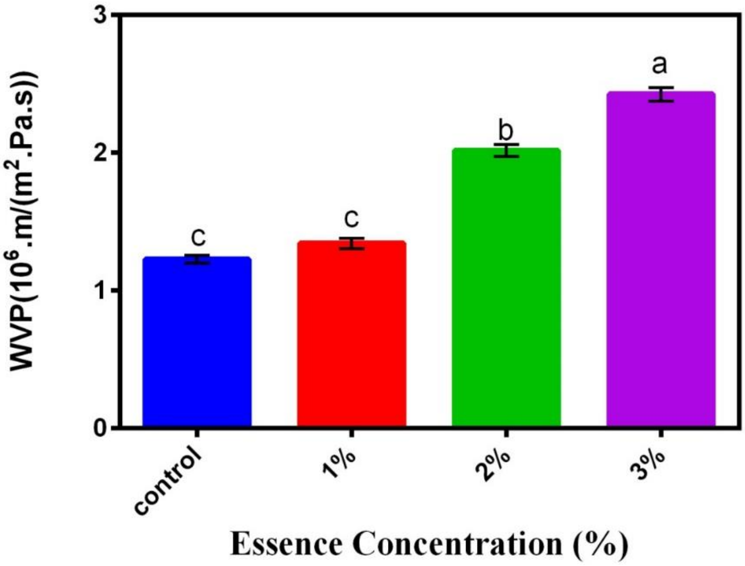

Chart 6: The effect of Trachyspermum essence on the permeability of sago starch films to water vapor.

Different Latin letters on the columns represent a significant difference at the 5% probability level ($p>0.05$).

Based on Chart 6, the results showed that increasing the concentration of Trachyspermum essence from 0% to 15% in the polymer matrix increased the permeability to water vapor from 1.24 to 2.35 (10-6). The compounds in Trachyspermum essence have disturbed the regular structure of polymer chains of sago films .This phenomenon increased the rate of water vapor passing through edible films significantly ($p>0.05$), although this increase was not significant. These results are in line with the results of past studies.

Color characteristics

Table 1 presents the results of the color evaluation. The results revealed that when the concentration of essence in sago starch film increases from 0% to 15%, the transparency (L* factor) of starch films containing essence decreased significantly from 97.01 to 94.37 by increasing essence concentration from 0% to 15% ($p > 0.05$). Moreover, the results revealed that the color of the starch films containing essence changed significantly ($p>0.05$) toward red (factor a*) and yellow (factor b*) by increasing the concentration of essence. However, these color changes cannot be easily detected by the normal eye and all the films look the same.

Table 1: Colorimetric parameters of starch film with different Trachyspermum essence concentrations

Essence percentage	L*	a*	b^*
0%	97.01 ± 0.45^{a}	0.67 ± 0.06^{b}	4.21 ± 0.42^{b}
5%	95.80 ± 0.45^{b}	0.72 ± 0.02^{b}	4.23 ± 0.42^{b}
10%	95.25 ± 0.45^{bc}	0.85 ± 0.08^{a}	5.49 ± 0.42^{a}
15%	94.37 ± 0.54^{c}	0.94 ± 0.07^{a}	5.78 ± 0.28^{a}

Conclusion

The results revealed that the water vapor permeability decreased by increasing essence concentration. It was due to the disruption of the regular structure of the polymer chains of sago films by the compounds in the essence. Investigating color characteristics revealed that the transparency decreased by increasing the concentration of essence and the color of sago starch films containing Trachyspermum essence changed to red and yellow with a slight increase in concentration. Solubility, water absorption, and moisture content of sago starch films containing Trachyspermum essence decreased by increasing the concentration of essence. Due to the good properties of Trachyspermum essence, it is recommended to compare different plant essences on the biophysical properties of biopolymers and to prepare and evaluate bio-composites according to other biopolymer bases such as polysaccharides, proteins, etc. Despite the appropriate characteristics of sago starch films, such as biodegradability and having plant sources, they have some limitations, such as the limitation of the ground plant during extraction and the lack of possibility to use 100% concentration of essence in the film.

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