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Research Article

Biofilm packaging development and characterization from underutilized Anchote starch and Aloe vera extracts

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Aloe vera gel, Anchote starch, biofilm, tensile strength, mechanical properties, under-utilized plant.

Abstract

Biofilms are the most feasible packing alternative for reducing postharvest losses and increasing the shelf life of agricultural products. The aim of this study was to develop and characterize biofilm using Anchote starch and Aloe vera gel extract. Biofilms were developed using different concentrations of Anchote starch, Aloe vera gel and glycerol. The physicochemical and mechanical properties of the developed biofilms were tested. It was found that different ratios of Anchote starch, Aleo vera gel, and glycerol had considerable effects on water solubility, moisture content, thickness, permeability and mechanical properties of the developed biofilms. The tensile strength of biofilm developed from 100% Anchote starch (control) was 59.75 MPa, while the biofilms developed with 5% Anchote starch, 0.4% Aloe vera gel and 0.4% glycerol as well as 4% Anchote starch, 0.4% Aloe vera and 0.5% glycerol had the lowest tensile strength, 16.98 MPa and 17.55 MPa, respectively. The water vapor permeability of the biofilm developed from Anchote starch was minimized when it was composited with Aloe vera gel and glycerol. The preferred physical and mechanical properties of the biofilm packaging were achieved by combining Anchote starch, Aloe vera gel and glycerol at the ratio of 5%, 0.4% and 0.4%, respectively.

1. Introduction

Preserving the quality, nutritional value and taste of fruits and vegetables after harvesting is a challenge. Studies show that there are significant postharvest losses (up to 50%) of fruits and vegetables [1]. Therefore, it is a pressing global issue to minimize these losses using appropriate techniques like bioplastic packaging. However, consumers have become increasingly concerned about chemical preservatives and plastic packaging with their effect on nutritional value change [2]. On the other hand, industries are using packaging made from polyethylene terephthalate, polyvinylchloride, polyethylene, polypropylene, polystyrene and polyamide due to the affordability, availability, flexibility, excellent mechanical performance, barrier to gases and heat stability [3, 4].

However, due to their poor biodegradability and intermolecular migration risk, plastic packaging is not preferred instead encourages the utilization of renewable, biodegradable and compostable packaging [5]. In addition, plastic packaging had negative environmental effects such as ground water pollution and dangers to the health of aquatic life [6]. Thus, replacing plastic packaging with renewable, biodegradable and eco-friendly packaging material is required [7]. Due to these reasons, research has been increasing to find new natural, degradable and safe food-grade materials. One of the alternative packaging would be biofilms which are made from food-grade natural materials to protect the food products from deterioration. Biofilms could provide a semipermeable barrier against oxygen, carbon dioxide, moisture and solute movement, which suppresses respiration and reduces water loss and oxidation [8].

Natural polymers like starch from potatoes, sweet potatoes and corn which are made from long chains of molecules can be used as biofilm packaging [9]. Since Anchote starch has such characteristics it may be used to develop biofilm [10]. Therefore, the utilization of this underutilized plant, Anchote, using Anchote starch to develop biofilm packaging could be one of the alternative materials for packaging development as well as maximizing the utilization of Anchote. Therefore, the aim of this study was to develop and characterize biofilm developed by mixing Anchote starch and Aloe vera gel extract.

2. Materials and methods

2.1 Sample collection

Anchote (*Coccinia abyssinica*) Tuber (4 kg) was collected from Debre Zeit Agricultural Research Center, and Aloe vera (*Aloe barbadensis*) (20 leaves) from Wondo Genet Agricultural Research Center, Ethiopia. Glycerol (100 mL) was purchased from the local market. All materials were transported to the Food Process Laboratory, Ambo University, Ethiopia. Aleo vera was stored at 4°C while Anchote and glycerol were stored at room temperature.

2.2 Anchote starch extraction

Anchote starch extraction was done based on the method described by Babu et al. [11]. The Anchote tuber was thoroughly washed to remove debris and peeled. The peeled tuber was cut into small pieces using a mechanical cutter. Then, 1 kg of it was taken and soaked with sodium chloride solution at room temperature and left overnight. Then after, the soaked tuber was taken out and wet-milled into a slurry using a laboratory mixer for 5 min and dispersed into the respective solution at a ratio of 1:2 (v/v) until smooth slurry was formed. Then, the dispersed Anchote paste was filtered through a muslin cloth. The residue left on the muslin cloth was washed repeatedly with distilled water and kept overnight to sediment. Then after, the supernatant was decanted and the left sediment was washed with distilled water to remove residual solvent and non-starch compounds. The washing process was done repeatedly and filtering until the washed water became clear and free of suspended materials. Finally, the collected starch was dried in an oven at 40°C for 48 h. Then, it was ground with mortar and pestle, sieved through a 250 µm sieve, packed in a polyethylene bag, and stored at room temperature until further analysis.

2.3 Aloe vera gel extraction

Aloe vera gel was extracted and prepared according to Rasouli et al. [12]. First, aloe vera leaves were washed with tap water and rinsed with distilled water. The green-colored outer cortex layers were removed and the colorless gel matrix was collected using a spoon. Then, the collected gel was mixed for 3 min using a juice blender (SK-157, China). The blended gel was filtered to remove impurities and glycerol was added to improve the plasticizer properties.

2.4 Experimental design

The film was prepared by mixing different ratios of Anchote starch (4%, 5%, and 6%), Aloe vera gel (0.3%, 0.4%, 0.5%), and glycerol (0.3%, 0.4%, 0.5%) using Box-Behnken experimental design to get the formulation shown in Table 1.

Table 1. Ratio of Anchote starch, Aloe vera gel and glycerol for biofilm development.

Formulation Ratios	Anchote (%)	Aloe vera (%)	Glycerol (%)
F1	4	0.4	0.3
F2	5	0.4	0.4
F3	4	0.3	0.4
F4	4	0.4	0.5
F5	5	0.5	0.5
F6	5	0.3	0.5
F7	6	0.4	0.3
F8	6	0.5	0.4
F9	5	0.3	0.3
F10	6	0.4	0.5
F11	4	0.5	0.4
F12	5	0.5	0.3
F13	6	0.3	0.4
F14 (Control)	5	0	0

F: Formulation

2.5 Preparation of the biofilm

The biofilm was developed using the casting method as described by Gutiérrez and González [13]. Then, distilled water was added to make a 100 mL solution.

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The mixture was boiled at 98°C for 30 min in a water bath with constant shaking for starch gelatinization and inactivation of enzymes in the mixture. Finally, it was poured into the Petri-dish until its bottom part was covered and dried in an oven at 50°C for 16 h. The biofilms were carefully removed from the casting Petri-dish.

2.6 *Physiochemical characteristics of the biofilm*

2.6.1 *Film thickness*

The film thickness was measured using a digital caliper as mentioned by Abera et al. [14] with the accuracy and precision of 0.001 mm. The measurements were taken randomly from six different points of the film samples and their average values were taken.

2.6.2 *Water solubility*

The solubility of the film was determined following the method of Li et al. [15] with minor modifications. The weight (Wi) of the film sample was measured directly and soaked in 40 mL of distilled water at room temperature (25°C) for 24 h which was occasionally agitated. The remained film was dried in the oven at 105°C until a constant was weight obtained. Then, water solubility (WS) was calculated using Eq. 1.

$$WS\ (\%) = \frac{Wi-Wf}{Wi} * 100 \qquad \text{Eq. 1}$$

Where, WS is water solubility, W_i and W_f are the initial and final weights of the dried samples, respectively.

2.6.3 *Color measurement*

The color of the biofilm was evaluated using the method of Khoshgozaran-Abras et al. [16]. The color parameters (L*, a*, and b*) were measured with a CR-400 colorimeter (Konica Minolta Co., Ltd., Japan). The total color difference (ΔE) of the biofilms was calculated using Eq. 2.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \qquad \text{Eq. 2}$$

Where, $\Delta L = L^*_{standard} - L^*_{sample}$, $\Delta a = a^*_{standard} - a^*_{sample}$, and $\Delta b = b^*_{standard} - b^*_{sample}$ and the white plate color standard was used as the background.

2.6.4 *Moisture content of biofilm*

The moisture content of biofilms was determined according to the method of Farahnaky et al. [17]. The initial weight of the biofilm was recorded as Wi. It was dried in the oven at 105°C for 24 h and then, the dried biofilm sample was weighed (Wf) and the final moisture content was calculated using Eq. 3.

$$\text{Moisture content}\ (\%) = \frac{Wi-Wf}{Wi} * 100 \qquad \text{Eq. 3}$$

Where, W_i and W_f are the initial and final weights of the dried samples, respectively

2.6.5 *Swelling index*

The swelling index of the biofilm sample was measured according to Gutiérrez and González [13]. A weighed film sample (W_{dry}) was put in distilled water at room temperature (23 ± 2°C). The biofilm was periodically removed from the water and reweighted until constant weight was attained ($W_{swollen}$). The water uptake was determined using Eq. 4.

$$\text{Swelling index} = \frac{Mswollen-Mdry}{Mdry} \qquad \text{Eq. 4}$$

Where, M_{Dry} and $M_{Swollen}$ represent initial and swollen masses of the film, respectively.

2.7 *Mechanical properties*

The mechanical properties such as tensile strength, elongation at break and elastic modulus of the biofilms were measured using a texture analyzer (LR30K, LLOYD Instruments Ltd., England). The specimens corresponded to type five and the test method was ASTM D638–14 standard test method for tensile properties of plastics [14]. The films were cut using a double blade cutter. Before testing, the thickness of the filmstrip and the width in the thinner dimension of the filmstrip were measured using a micrometer. The filmstrips were clamped in the testing machine which was operated at an initial gap separation of 30 mm with a cross head speed of 100 mm/min and an extension rate of 100 mm/min by applying preload or stress 5.6 N. The tensile strength, elongation at break, and Young's modulus were determined. Five measurements were performed for each sample. The TS results were expressed in Mega Pascal (MPa), Elongation at break (%) and Young's modules in percent (MPa).

$$TS\ (MPa) = \frac{F}{S} \qquad \text{Eq. 5}$$

Where, TS-Tensile strength in MPa, F-The maximum tensile force when the sample breaks in Newton (N), S- Cross-sectional area of specimen in m^2.

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Table 2. Moisture content, solubility and swelling index of the developed biofilms.

Sample ID	Moisture content (%)	Water solubility (%)	Swelling index
F1	11.36 ± 0.13[a]	31.72 ± 0.14[b]	197.87 ± 16.40[c]
F2	9.57 ± 0.34[c]	28.76 ± 0.54[c]	189.12 ± 6.67[d]
F3	7.36 ± 0.04[de]	32.98 ± 0.03[b]	195.28 ± 18.42[c]
F4	7.59 ± 0.15[de]	34.49 ± 0.20[a]	221.86 ± 22.93[a]
F5	8.76 ± 0.11[cd]	32.51 ± 0.10[b]	216.35 ± 39.04[b]
F6	5.49 ± 0.40[f]	29.13 ± 0.05[c]	185.16 ± 14.50[e]
F7	10.09 ± 0.51[b]	25.53 ± 0.00[e]	156.74 ± 79.45[i]
F8	7.90 ± 0.03[d]	29.94 ± 0.10[c]	174.96 ± 18.98[g]
F9	7.60 ± 0.19[de]	24.27 ± 0.07[f]	183.38 ± 24.21[ef]
F10	6.67 ± 0.24[e]	28.34 ± 0.24[cd]	176.88 ± 58.98[g]
F11	8.23 ± 0.12[d]	28.33 ± 0.08[cd]	218.79 ± 16.49[ab]
F12	9.91 ± 0.03[bc]	27.24 ± 0.10[d]	182.45 ±50.02[f]
F13	7.43 ± 0.04[de]	26.83 ± 0.35[de]	168.78 ± 46.06[h]
F14	4.55 ± 0.00[g]	20.77 ± 0.47[g]	143.55 ± 9.76[j]

Values in a column with different letters show significant difference ($p<0.05$).

2.8 Water vapor transmission rate

The water vapor transmission rate (WVP) of the biofilm was determined gravimetrically at 25 °C under 50% of relative humidity using water vapor transmission measuring cups with the ASTM E96-95 standard method [18]. The weight loss of each cup was measured every 1 h interval for 8 h. Each representative sample was measured three times. Then, the WVP of the films was calculated using Eq. 6.

$$WVP = \frac{WVTR * n}{\triangle P} \quad \text{Eq. 6}$$

Where, WVP- Water vapor transmission coefficient, 10^{-9}g*m /(m^2*Pa*s); WVTR- the amount of water vapor transmitted through the instrument measured, g/(m*d); n- Film thickness, mm; △P- the output pressure of the gas is 0.20 MPa.

2.9 Biodegradability test of biofilm

The biodegradability of the biofilm was evaluated using the soil burial method as described by Ruggero et al. [19]. The weighed (W1) biofilm sample (2cm × 2cm) was buried at a depth of 2cm for 22 days. The sample was taken and weighed (W2) every 2 days. The biodegradability of the biofilm was calculated using Eq. 7.

$$\text{Degradation (\%)} = \frac{Wi - Wf}{Wi} * 100 \quad \text{Eq. 7}$$

Where, W_i - Initial weight of the biofilm, W_f - Final weight of the biofilm

2.10 Statistical analysis

All the data were analyzed using ANOVA using JMP software (Pro 13 software, 2013). The results were expressed as mean ± standard deviation. The means were compared using Tukey's test at a significance level of 5%.

3. Results and discussion

3.1 Moisture content of biofilm

The moisture content of a biofilm was determined by the amount of moisture present in the biofilm matrix. As shown in Table 2, the moisture content of the biofilms varied from 4.55% to 11.36%. The F14 (100% Anchote starch, Control) had the lowest value, whereas F1 (4% Anchote starch, 0.4% Aloe vera and 0.3% Glycerol) had the highest moisture content. This showed that biofilms developed from Anchote starch with Aloe vera had a higher moisture content than biofilms developed from Anchote starch alone. In addition, the increment of Aloe vera gel concentration in the formulation increased the moisture content in the biofilm. This might be due to the relatively high moisture content of Aloe vera gel. This is in agreement with studies that reported an increase in moisture content to sodium alginate film with the addition of Aloe vera gel [20] and fish gelatin and Aloe vera gel composite film [21]. The increment of moisture content in the biofilm may improve the hydrophilic character of the composited biofilm.

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On the other hand, the film developed from Anchote starch with glycerol had lower moisture content (Table 2). A study showed that a film developed from starch flour and glycerol composite has lower moisture content due to strong starch-glycerol interactions [13], reducing water absorption from the atmosphere. This reduced moisture content lowers the possibility of mold growth, which could affect the biofilm's appearance and mechanical properties. Therefore, the biofilm developed from 5% Anchote starch, 0.4% Aloe vera and 0.4% glycerol had intermediate moisture content, making it suitable for coating.

3.2 Water solubility

The water solubility of the biofilms ranged from 20.77% to 34.49% (Table 2). The lowest water solubility (20.77%) was found from F14 (the biofilm made from 100% Anchote starch), whereas the highest water solubility (34.49%) was found from F4 (4% Anchote starch, 0.4% Aleo vera and 0.5% glycerol) biofilm. This result showed that while the concentration of Anchote starch increased and the concentration of glycerol decreased in the biofilm composite, the solubility decreased, and vice versa. It was also found that a high percentage of starch, and a low percentage of glycerol and Aleo vera gel reduce the solubility of the film in water, whereas a low percentage of starch with a high percentage of glycerol increases the solubility. Similarly, the film plasticized using 1-ethyl-3-methylimidazolium acetate or sorbitol exhibited high water solubility as concentration increased [14]. In addition, the solubility of the alginate and Aloe vera composite film was increased as the Aloe vera ratio was increased [20]. This might be owing to the presence of higher water-soluble components in Aloe vera gel, like sugars, organic acids, and amino acids, which make the film easily solubilized [22]. When the solubility of the biofilm is high, it is difficult to control in wet areas and humid environments, since the biofilm is dissolved in excessive wet areas that may expose the packed food product. On the contrary, if it is insoluble in water, it degrades poorly in water, which could give relatively a higher resistance to keep the packed product in wet area. In addition, the water solubility of a biofilm is one of the important parameters, which determines the biofilm's application. Less soluble biofilm is suitable for storage purposes, whereas biofilms with a high solubility might be used for instant food [23]. Therefore, based on this study, the film developed from the composite of 5% Anchote, 0.4% Aloe vera and 0.4% glycerol exhibited intermediate water solubility characteristics.

3.3 Swelling index

A higher swelling capacity of the biofilm is related to higher moisture absorption. The swelling index of the developed film ranged from 143.77 to 221.86 (Table 2). This study indicated that increasing the concentration of Aloe vera gel was associated with an increase in the swelling index. This is in agreement with Hadi et al. [20] findings that increasing the concentration of Aloe vera gel increases swelling capacity. This might be attributed to the hydrophilic nature of Aloe vera gel, which enhances the swelling index of the biofilm. On the other hand, as the Anchote starch ratio was increased in the biofilm composite, the swelling index was decreased. This could be due to the highly ordered crystalline structure of the Anchote starch [10]. According to the study, the swelling capacity of the native starch is lowered because of a highly ordered crystalline structure of the starch chains which resists swelling [22].

3.4 Color

This study showed that the L* value ranged from 75.53 to 89.21, with the highest value recorded on F14 biofilm (100% Anchote starch,control) and the lowest value was observed on F8 film (4% Anchote, 0.5% Aloe vera, and 0.4% glycerol) (Table 3). These data showed that when the concentration of Anchote starch increased, the lightness of the biofilm was increased. The color difference might be due to film thickness, the thicker films being more opaque, as well as differences in transparency and opalescence at different ratios [22]. In addition, the color of films could be affected by the type, nature and concentration of film composites [9].

According to Chin et al. [21], the increment of the Aloe vera gel ratio did not show a considerable effect in the lightness of the film developed from Aloe gel and fish gelatin composite since both are transparent solutions. On the other hand, the gelatin film containing 9% Aloe gel was significantly darker than other films, possibly due to the presence of more solid particles in the Aloe vera gel, which may cause cloudier film. In addition, Riquelme et al. [24] showed that there were color

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Table 3. Color values of the developed biofilms

Sample ID	L-value	b-value	a-value	ΔE
F1	83.16 ± 0.06^{cd}	0.00 ± 0.00^{c}	-1.13± 0.06^{h}	83.06 ± 0.0^{bc}
F2	84.11 ± 0.47^{c}	0.22 ± 0.04^{a}	-0.33 ± 0.02^{c}	83.07 ± 0.32^{bc}
F3	83.50 ± 0.10^{c}	0.27 ± 0.06^{a}	0.00 ±0.0 0^{b}	83.63 ±0.45^{bc}
F4	80.70 ± 0.20^{d}	0.13 ± 0.06^{b}	0.00 ± 0.00^{b}	84.74 ± 0.16^{b}
F5	77.53 ± 0.15^{e}	-0.53 ± 0.06^{e}	-0.53 ± 0.06^{d}	88.41 ± 0.06^{a}
F6	82.80 ± 0.10^{cd}	-0.60 ± 0.00^{f}	-0.63 ± 0.06^{e}	78.77 ± 1.43^{k}
F7	86.80 ± 0.17^{b}	0.00 ± 0.00^{c}	-0.77 ± 0.06^{fg}	86.52 ± 0.54^{ab}
F8	85.23 ± 0.25^{bc}	-0.23 ± 0.06^{d}	-0.47 ± 0.06^{cd}	83.04 ± 0.08^{bc}
F9	87.10 ± 0.10^{b}	0.10 ± 0.10^{b}	-1.13 ± 0.06^{h}	87.68 ± 0.08^{a}
F10	85.83 ± 0.06^{bc}	-0.60 ± 0.10^{f}	-0.83 ± 0.06^{f}	85.55 ± 0.53^{ab}
F11	75.53 ± 0.06^{f}	-0.83 ± 0.06^{g}	-0.73 ± 0.06^{g}	83.41 ± 0.25^{bc}
F12	78.73 ± 0.20^{e}	-0.50 ± 0.10^{e}	-1.03 ± 0.06^{gh}	82.21 ± 0.39^{c}
F13	87.73 ± 0.06^{b}	-0.27 ± 0.06^{d}	-0.80 ± 0.00^{f}	82.42 ± 0.26^{c}
F14	89.21 ± 0.10^{a}	-0.27 ± 0.06^{d}	0.40 ± 0.00^{a}	84.09 ± 0.09^{b}

Values in a column with different letters show significant difference ($p<0.05$).

Table 4. Thickness and mechanical properties of biofilms

Sample ID	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
F1	0.01 ±0.03^{c}	18.86 ± 0.78^{e}	32.58 ± 0.56^{bc}	46.29 ± 0.04^{c}
F2	0.03 ± 0.01^{bc}	16.98 ± 0.91^{f}	33.62 ± 0.29^{b}	32.13 ± 0.39^{g}
F3	0.02 ± 0.01^{c}	18.30 ± 0.39^{e}	31.47 ± 0.76^{c}	41.10 ± 1.37^{f}
F4	0.02 ± 0.00^{c}	17.55 ± 0.83^{f}	33.16 ± 0.85^{b}	31.14 ± 1.1^{g}
F5	0.02 ± 0.00^{c}	19.90 ± 0.20^{e}	26.68 ± 0.40^{d}	29.23 ± 0.87^{h}
F6	0.03 ± 0.00^{bc}	21.57 ± 0.41^{d}	52.91 ± 0.91^{a}	36.63 ± 1.01^{f}
F7	0.05 ± 0.00^{ab}	27.66 ± 0.98^{b}	23.20 ± 0.14^{ef}	56.19 ± 2.38^{b}
F8	0.05 ± 0.00^{ab}	24.28 ± 0.99^{c}	21.20 ± 0.24^{f}	46.40 ± 1.49^{c}
F9	0.04 ± 0.00^{b}	22.31 ± 0.20^{d}	19.74 ± 1.15^{g}	41.19 ± 0.91^{d}
F10	0.04± 0.01^{b}	23.82 ± 0.25^{cd}	16.95 ± 0.37^{h}	42.82 ± 0.86^{d}
F11	0.02 ± 0.00^{c}	21.74 ± 0.26^{d}	32.08 ± 0.10^{bc}	31.67 ± 0.65^{g}
F12	0.03± 0.00^{bc}	23.46 ± 0.64^{cd}	24.60 ± 0.75^{e}	38.90 ± 1.24^{e}
F13	0.04 ± 0.00^{b}	26.88 ± 0.28^{b}	16.43 ± 0.63^{h}	56.97 ± 2.01^{b}
F14	0.06 ± 0.03^{a}	29.81 ± 0.56^{a}	14.25 ± 0.25^{i}	59.75 ± 0.93^{a}

Values in a column with different letters show significant difference ($p<0.05$).

differences among gelatin films occurred due to the Maillard reaction and gelatin browning when the films were dried at certain temperature.

3.5 Film thickness

The thicknesses of the biofilms ranged from 0.01 mm to 0.06 mm (Table 4). The F1 film (4% Anchote starch, 0.4% Aloe vera gel and 0.3% glycerol) and F14 film (100% Anchote starch) were the thinnest and thickest, respectively. This indicates that increasing the percentage of starch resulted in considerably ($p<0.05$) thicker films. Similarly, adding Aloe vera gel and glycerol significantly reduced the thickness of the biofilm. The trend was reported for Aleo vera on chitosan-based film [16]. This thicker film might have resulted from the agglomeration of Anchote starch. However, the agglomeration of starch suggests the poor interfacial adhesion between starch and Aloe vera gel due to the high amount of water present in Aloe vera gel. Karim et al. [25] observed that rapid crystallization resulted in a high residual content in the film. Thus, poor particle size distribution in the form of starch agglomeration might occur. A similar result was reported by Kassa et al. [26] who found that increasing the concentration of Anchote cellulose

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nanocrystals enhanced the thickness of a film. As stated by Kaur et al. [23], a thick film may affect the texture of a food product, whereas a thinner film is easier to apply and may provide less protection. In addition, the film thickness is also an important factor since it affects the density and mechanical properties of the biofilm. Therefore, this study chose the formulation with the optimal thickness to make it practicable for effective film. As a result of this study, the biofilm developed from 5% Anchote, 0.4% Aloe vera, and 0.4% glycerol had a suitable thickness for coatings since it was opaque and more transparent compared to other film composites.

3.6 Mechanical properties of biofilm

This study found that among the developed biofilms, F15 (100% Anchote starch) had the highest tensile strength and Young's modulus with values of 29.81 MPa and 59.75 MPa, respectively. The minimum tensile strength was found in F2 (5% Anchote, 0.4% Aloe vera and 0.4% glycerol and F4 (4% Anchote, 0.4% Aleo vera and 0.5% glycerol) with the values of 16.98 MPa and 17.55 MPa, respectively (Table 4). A similar trend was also observed for Young's modulus as the Aleo vera and glycerol concentrations were increased. These findings demonstrated that the increased concentration of Aloe vera and glycerol resulted in decreased tensile strength and Young's modulus values. In addition, increasing the concentration of Aloe vera gel and glycerol in the biofilm composites from 0.3 to 0.5 g significantly ($p<0.05$) increased the elongation at the break of the biofilm when it was compared to the control (100% Anchote starch) (Table 4). This could be attributed to the high moisture content of Aloe vera gel and the impact of glycerol, which enables the starch chain slide and lowers secondary linking forces between polymer chains [25]. The tensile strength of the biofilm reflects the maximum stress developed during tensile stretching. Therefore, a high plasticizer concentration increases the flexibility of the film, which leads to a lower tensile strength and elasticity modulus [14].

Young's modulus also indicates the stiffness of the film, hence the higher Young's modulus, the stiffer the film is. The mechanical properties of a film are determined by the type of plasticizer and the concentration of ingredients used to develop the biofilm [14].

The inclusion of a plasticizers lowers polymers' contact and chain cohesion, hence the presence of glycerol influences biofilm elongation at break [27]. Therefore, this property was increased when the glycerol level in the biofilm composite was increased from 0.3 to 0.5% (Table 4). This might be due to the occurrence of stronger interactions between plasticizer and biopolymer that induce loss of macromolecular mobility. On the other hand, the plasticizer reduces the strong intramolecular attraction between the starch chains, resulting in a reduction of the stiffening effect of starch granules and consequently a loss in film tensile strength and increased elongation at break [28].

According to this study, increasing the Aloe vera gel ratio in the biofilm composites resulted in reduced tensile strength and higher elongation at break (Table 4). A similar trend was also observed in the value of elongation at break, which was found to be increased from 0.9% (sodium alginate) to 2.73% (sodium alginate-Aloe vera gel) [20]. It was also observed that the mechanical properties of biofilms varied depending on the concentration of sodium alginate and Aloe vera gel used. It was found that from the addition of 10-50% of Aloe vera gel, the maximum value of tensile strength was recorded at 10% Aloe vera gel concentration, with the tensile strength increased from 20.84 N/mm^2 to 25.72 N/mm^2. However, increasing the concentration of Aloe vera gel reduced the tensile strength of the film [20]. As a result of this study, the biofilm developed from 5% Anchote, 0.4% Aloe vera, and 0.4% glycerol was more flexible, durable and stiffer than the other biofilm samples, making it more suitable for coating.

3.7 Water vapour permeability (WVP)

Water vapour permeability is the amount of moisture that passes through per unit area per time. A low WVP value shows that the products have a longer shelf life [23]. The permeability values of the developed biofilms ranged from 48.25 to 47.79 g/m^2h (Fig. 1). The result showed that the permeability of the biofilm was low. This might be due to the high moisture content of Aloe vera gel, which reduces water to move through it. Thus, adding Aloe vera gel significantly ($p<0.05$) decreased the water vapor transmission rate (WVTR). The permeability of film is highly dependent on the film composition,

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particularly the ratio of hydrophilic to non-hydrophilic groups in the film, which determines the film's water interaction [29].

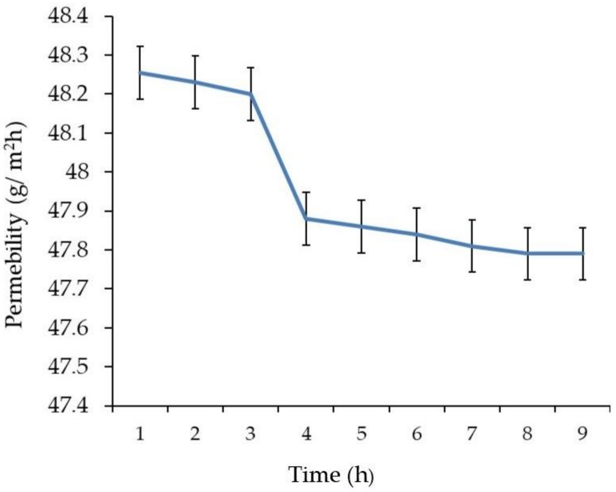

Figure 1. Permeability of biofilm developed from Anchote starch, Aleo vera and glycerol with the ratio of 5%, 0.4% and 0.4%, respectively.

However, in order to develop a modified atmosphere packaging for fruit, air and water vapor permeability (WVP) should be limited. A low WVP indicates that the biofilm can limit moisture transfer between the food and the environment, which extends the shelf life of the packed food products. Therefore, the biofilm developed from 5% Anchote, 0.4% Aloe vera, and 0.4 % glycerol would be more suited for packaging due to its reduced WVP (Fig. 1). This occurred as a result of the possible interaction between the Aloe vera gel components and Anchote starch molecules, which reduces the availability of the hydrophilic groups, resulting in a decrease in the WVTR of the film. Similarly, the hydrogen bond interactions between amylose and the functional group in a polymeric matrix form a more compact network, limiting gas and moisture diffusion and reducing its permeability [22, 30].

3.8 Biodegradability of biofilm

The biofilm developed from the composites of 5% Anchote starch, 0.4% Aloe vera gel and 0.4% glycerol was subjected to further investigation based on the physical and mechanical properties to evaluate the biodegradability. This investigation found that the degradation of the biofilm increased as the burial period increased (Fig. 2). The biofilm was degraded by more than 40% of its initial weight on the 14th day, followed by more than 65% on the 18th day (Fig. 2). Finally, 92% of the biofilm was degraded at 22nd day. The rate of biofilm degradation is determined by its chemical structure, molecular weight, water and surface area [31]. It was also mentioned that the addition of plasticizer enhances the biodegradation time of the bioplastics [3].

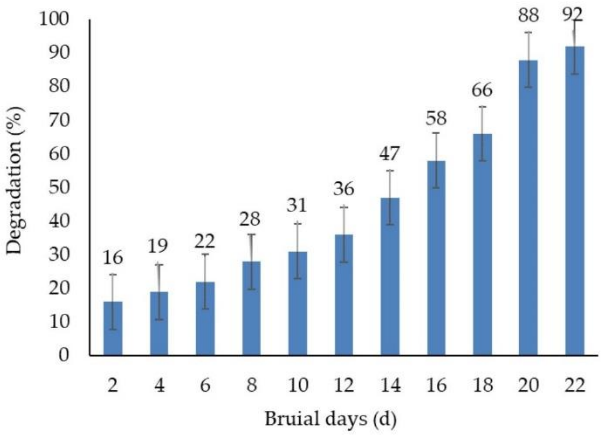

Figure 2. Biodegradability of the biofilm sample developed from Anchote starch, Aleo vera and glycerol with the ratio of 5%, 0.4% and 0.4%, respectively.

4. Conclusions

The physical and mechanical properties of biofilms developed from different concentrations of Anchote starch (4%, 5%, and 6%) and Aloe vera gel (0.3%, 0.4%, and 0.5%) were investigated. The tensile strength and Young's modules values of the biofilm developed from Anchote starch alone were higher than the biofilm developed from the composite of Anchote starch, Aloe vera and glycerol, which is vice versa for elongation at break. This shows that the mechanical properties of the biofilm developed from Anchote starch could be improved when Aloe vera and glycerol are incorporated. The water vapor permeability of biofilm was low, which could able to prevent the penetration of moisture into the packed food. In addition, this biofilm was degraded within 15-22 days, which makes it environmentally friendly. Therefore, the ratio of 5% Anchote strach, 0.4% Aleo vera gel, and 0.4% glycerol it the optimum combination to develop suitable biofilm packaging. In conclusion, the biofilm developed from the combination of Anchote starch, Aloe vera gel and glycerol has considerable importance as an alternative packaging material for agricultural products.

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Authors' contributions

Conceptualization and Methodology, S.G.A.; Software, S.G.A. and D.W.D.; Validation, S.G.A. and D.W.D.; Formal Analysis, investigation, resources, S.G.A and D.W.D.; Data Curation, S.G.A. and D.W.D.; Writing – Original Draft Preparation, S.G.A.; Writing– Review & Editing, D.W.D.; Visualization, S.G.A; Supervision, D.W.D; Funding Acquisition, A.G.S.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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