



The Effect of Glycerol on the Properties of Biodegradable Cassava Starch (Saai Dieow Cultivar) Films for Plastic Plant Bag Application

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Abstract

This paper reports the effect of glycerol on the properties of biodegradable cassava starch films for plastic plant bag application. The film was prepared by dissolving cassava starch in water to the concentration of 5 wt%. To study the effect of the glycerol content on these properties, the amount of glycerol (plasticizer) was added during the formulation varied from 0 to 45 %w/w (0, 15, 25, 35 and 45 %w/w). The films were cast in a mold and dried at 60 °C for 24 h. The thickness of the films was in the range of 0.10–0.21 mm. The a_w (water activity) of films ranged from 0.40 to 0.53. The water solubility, water vapor permeability and softness of the film increased with the increasing of glycerol content. The percent elongation of the starch films increased with the decrease of the glycerol content and, in contrast, the tensile and puncture strength decreased. After the films were buried under the ground (8–10 cm depth) for 3 weeks, they were degraded by 100%. Degree of the degradation increased with the increasing of glycerol content. The biodegradable film plant bags were used to the transplanting of marigold seedlings production period (6 weeks). The cassava starch film with 35 wt% glycerol showed the best overall properties. These results indicated that cassava starch biodegradable film could be used for plastic plant bag application. In addition, this made-of-natural film is biodegradable, which can help to reduce the amount of non-biodegradable packaging waste.

Keywords: cassava starch, glycerol, plasticizer, biodegradable film

Introduction

The use of plastics in the world has been increased, worsening the environmental pollution problem of waste disposal. A lot of efforts have been taken to prepare biodegradable films and films made from renewable and natural polymer such biopolymers include starch, cellulose derivative, chitosan/chitin, gelatin, protein (animal or plant-based) and lipid (Abdou & Sorour, 2014; Wang, Wang, & Ye, 2017; González, Gastelú, Barrera, Ribotta, & Igarzabal, 2019). Biodegradable films are not meant to totally replace synthetic packaging films. However, they do have potential to replace the conventional packaging in some applications. These biodegradable films can be completely degraded by micro-organisms without emission of toxic gases.

Starch is one of the most studied natural polymers for the development of biodegradable films because it presents a low-cost carbohydrate polymer, easy to obtain and good ability to form films (Srikulkit, Bangyekan, & Aht-Ong, 2006; Rios, Assis, Lopes, Costa, & Flores, 2017). It primarily consists of branched and linear chains of glucose molecules. Two kinds of the chains presenting in starch are amylopectin and amylose. Several studies have been done to analyze starch films properties (Bertuzzi, Vidaurre, Armada, & Gottifredi, 2007). Starch is potential packaging materials in the agriculture, medicine and packaging industries. Cassava (Saai Dieow cultivar) is a good source of starch for film production from Lop Buri of Thailand because cassava starch is cheap, abundant and biodegradable. Cassava starch has good homogeneity, transparency and biodegradability (Vargas, Valencia-Sullca, Atares, & Chiralt, 2018). For this reason, this polymer is promising for the development of biodegradable films.



In the absence of the additive, films are formed purely polymeric ingredients they tend to be brittle. To solve this problem, plasticizers are added to the polymeric mixture in order to modify the flexibility and extensibility of the polymer film. In this case, the plasticizers and the polymer matrixes must be compatible. The plasticizer functions by increasing the mobility of the polymer chain and by reducing intermolecular forces and increase the mobility of polymer chains. Plasticizers such as glycerol and sorbitol are commonly used for modification of the hydrophilic film formations (Grossmann, Mali, Garcia, Martino, & Zaritzky, 2002). Saiah, Sreekumar, Leblanc, Castandet, & Saiter, (2009) show that mixture of water and glycerol as well as monoglyceride has greater influence and no significant effect on the structure and morphology of cassava starch film (Sreekumar, Leblanc, & Saiter, 2013; Saiah, Sreekumar, Leblanc, Castandet, & Saiter, 2007).

The objective of the present work was to investigate the effects of glycerol on the physical chemical and mechanical properties of biodegradable cassava starch films. They were fabricated for use as plastic plant bags for transplanting of marigold seedlings production and also to study the biodegradation behaviour of films.

Methods and Materials

Preparation of cassava starch

Fresh cassava tubers (Saai Dieow cultivar), with uniform size and shape, were obtained in a local farm (Khok Samrong, Lop Buri, Thailand). The cassava tubers were cleaned with water and chopped. The chopped samples were homogenized in a blender, sieved and left for decantation. After 3 h, the supernatant was discarded and the precipitate was degreased by re-suspension with water and decanted again. The remaining starch precipitated at the bottom of the container. Finally, the obtained starch was dried at 60 °C for 24 h and sieved through a 150 mesh. The dried starch was stored in a desiccator (Torres, Troncoso, Torres, Díaz, & Amaya, 2011).

Film preparation and casting

Starch films were prepared by a casting method. Dried starch was diluted in distilled water to form a 5% (w/w) starch solution. The solution of cassava starch was gelatinized at 90 °C 20 min under constant stirring. Glycerol was added as plasticizer at concentrations of 0, 15, 25, 35 and 45 % by the starch weight. Starch and glycerol were mixed to make batches with a total weight of 400 g. When the starch solution temperature was around 50 °C, gelatinized suspensions were cast onto flat, leveled, non-stick Teflon plates (36 cm x 27 cm x 1.4 cm) then the plates were dried at 60 °C for 18 h in a hot air oven. All the dried films were peeled and stored in a desiccator at room temperature for 48 h before analysis (Basiak, Lenart, & Debeaufort, 2018).

Film thickness measurement

The film thicknesses were determined using a digital micrometer (Mitutoyo Co., Japan), with precision 0.01 mm and resolution 0 mm–25 mm. Five measurements were randomly taken at different locations for each specimen and the mean value was used.

Solubility in water

The water solubility of the films was determined according to the method described by Gontard, Guilbert, and Cuq (1992), with some modifications. The samples were cut into discs with 2 cm diameter, weighed and subjected to drying at 105 °C for 3 h. After drying, the samples were weighed and immersed in 100 mL of



distilled water and the whole was subjected to mechanical stirring at 25 ° C for 24 h. After this period, the samples not solubilized were subjected to drying at 105 ° C for 3 h. The water solubility of the films was obtained from the equation:

$$S (\%) = \left(\frac{W_i - W_f}{W_i} \right) \times 100$$

where W_i is the initial dry weight of the sample (g), and W_f is the final dry weight (g).

Color

Measurements were performed in a Hunter Lab colorimeter (Color Flex Z2, U.S.A.) using an aperture of 1.5 cm-diameter. The exposed area was sufficiently great relative to the illuminated area to avoid any light trapping effect. Color parameters range from $L^* = 0$ (black) to $L^* = 100$ (white). Standard values considered were those of the white background (Nogueira, Soares, Cavasini, Fakhouri, & Oliveira, 2019).

Water Activity (a_w)

The a_w values were measured at 25 ° C using a water activity meter (Aqualab model series 4, Decagon device Inc, U.S.A). The a_w value is defined as the ratio of partial pressure of water vapor in the product to that in the presence of pure water. Most enzymes are inactivated at <0.85 . At less than 0.75 bacterial growth is inhibited. At less than 0.6 all growth is inhibited (Abdullah, Nawawi, & Othman, 2000).

Water vapor permeability (WVP)

The films were fixed in aluminum permeation cell (inner diameter: 45 mm, height: 32 mm) according to the method ASTM 96-95 (ASTM, 2000) with some modifications (Pagno et al., 2015), previously filled with silica gel (0 % RH) and hermetically sealed. Test cups were replaced in a desiccator cabinet maintained at a temperature (28 ± 2 ° C) and relative humidity of 75 ± 2 %. The samples weight during the water vapor absorption process was measured after 24 h and WVP was calculated on the basis of equation:

$$WVP = \frac{w.L}{A.t.\Delta p}$$

where w is the weight of water permeated through the film (g), L is the thickness of the film (mm), A is the permeation area (m^2), t is the time of permeation (hr), and Δp is the water vapor pressure difference between the two sides of the film (KPa).

Fourier Transform Infrared (FT-IR)

Infrared spectra of films were recorded using a Frontier spectrometer (Perkin Elmer, U.S.A). The measurements were performed at room temperature, after removal from the desiccator. A total of 32 scans was performed at 4 cm^{-1} resolution. The measurements were recorded between $4,000$ and 400 cm^{-1} .

Mechanical properties

Tensile and elongation (% E) properties were determined using a TA.XT2 Stable Micro Systems texture analyzer (Surrey, England) in accordance with ASTM D-882-91 method (ASTM, 1996). The films (100 mm x 25 mm) were clamped between two tensile grips. Force (N) and deformation (mm) were recorded during extension at 50 mm min^{-1} , with an initial distance between the grips of 50 mm. These tests were performed at room temperature ($20-25$ °C). Tensile strength (N/mm) was measured.



Puncture tests were made to determine puncture strength (N/mm) and deformation using the same equipment. Samples with diameters of 100 mm were fixed on the plate of the equipment with a hole of 20 mm diameter with help of a tape (3M Scotch, Thailand). A HDP/TPB Tortilla/Pastry Burst Rig probe of 5 mm diameter was moved perpendicularly to the film surface at a constant speed of 1 mm/s until the probe passed through the film. At rupture point force and deformation were determined (Alves, Mali, Bele' ia, & Grossmann, 2007).

Biodegradation test of starch films

Starch films were cut into a rectangle shape (2.5 cm × 5.0 cm) and dried until constant weight. The samples were added in plastic boxes (20 cm x 48 cm x 12.5 cm). Then the samples were buried at the depth of 8 cm from the surface of the soil. The plastic boxes containing samples were incubated at room temperature (28–35°C) and humidity of the soil was maintained at 20–40%. The experiment was carried out for 4 weeks. Samples were weighed every week by taking from the soil and washing it gently with distilled water to remove the soil. Weight loss (WL) during soil burial was measured according to Sivakumar, Maran, Thirugnanasambandham, and Sridhar (2014). The mass of each sample was weighed before and after degradations and weight loss of each film sample was obtained using the equation of Martucci and Ruseckaite (2009).

$$WL (\%) = \left(\frac{M_0 - M_1}{M_0} \right) \times 100$$

where M_0 is the pre-degraded dried weight of the sample and M_1 is the dried weight of the sample after degradation.

Plant materials and growth conditions

The American marigold marky gold seedlings were procured from a local plant located from the Lop Buri of Thailand. Marigold plants were grown as seedlings for 7 days to an average plant height of 5–6 cm, respectively. The rectangular biodegradable plant bags of 5-inch length and 3-inch width were made out of the films from cassava starch at different concentrations of glycerol. All of the plant were transplanted to plastic pots (24.5 cm x 19.0 cm) filled with soil for 6 weeks. Polyethylene film was considered as a control (Bilck, Olivato, Yamashita, & de Souza, 2014).

Statistical analysis

Different formulations of plasticized cassava starch films were compared to each other, and control samples without glycerol were tested to evaluate the effect of this plasticizer on some studied characteristics. All experiments were replicated at least three times. Analysis of the variance (ANOVA) and Duncan's multiple range test comparisons were carried out using a level of 95% confidence ($p \leq 0.05$). Data were presented as mean ± standard deviation.

Results and Discussion

The films with glycerol concentration 0% and 15% by weight were brittle. On the other hand, the films obtained with 45% by weight of glycerol were very sticky. However, films obtained with 25% and 35% by weight of glycerol are flexible, homogeneous and transparent, as shown in Figure 1. However, glycerol can



form hydrogen bonds with the hydroxyl sites of the glucose. This phenomenon increases the free volume that also increases the mobility of the polymer chains. As a result, the flexibility of the film was increased. (Riku, Harry, & Yrjo, 2007).

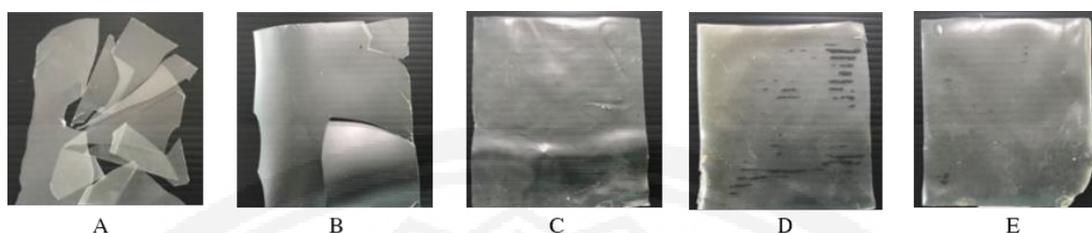


Figure 1 Appearance of cassava starch films at different concentrations of glycerol A) 0% B) 15% C) 25% D) 35% and E) 45%

The thickness of the films ranged from 0.10 mm to 0.21 mm, with significant differences ($p \leq 0.05$) between the formulations (Table 1). Increasing the concentration of glycerol led to increased thickness of the films which resulted in more hydrophilic groups susceptible to interact with water, leading to higher thickness of the films.

The lightness (L^*) values of the film with glycerol concentration of 0% and 15% were higher than those of the 25%, 35% and 45% films. The low glycerol content allowed the amylose chains in the starch film to crystallize resulting in opaque white film. As the glycerol content in the film was increased, the L^* values decreased as the glycerol acted as a plasticizer which reduced the starch crystallization, resulting in less opaque film (Muscat, Adhikari, Adhikari, & Chaudhary, 2012).

Water activity (a_w) is a critical factor affecting the sensory quality of food products. The microbial growth proceed at the rates highly associated with water activity. The samples showed the a_w content between 0.40 and 0.53 (Table 1), indicating that Microbial growth can not be expected, The optimum a_w range microbial growth is between 0.70 and 0.99% (Veiga, 2007). This could be explained by the plasticizing and hygroscopic properties of the glycerol, which increased mobile region on the film (Mitchell, 1998) and subsequently increased the water uptake (Cheryan, Gennadios, Woller, & Chinachoti, 1995).

The water vapor permeability (WVP) values of the 25%, 35% and 45% glycerol containing films were significantly different ($p \leq 0.05$) as shown in the Table 1. However, the films with 0% and 15% glycerol content were too brittle, the fracture on the film caused the fluctuation of the WVP values, for the evaluation for WVP. The WVP of the cassava starch films increased with the increasing of the glycerol content, which could be related to the increase of the free hydroxyl groups in the films. This could enhance the water vapor transmission through the films (Bertuzzi et al. , 2007; Mali, 2002; Mali, Sakanaka, Yamashita, & Grossmann, 2005).

Table 1 shows that water solubility of cassava starch films increases for increasing glycerol concentrations ($p \leq 0.05$). The water solubility of the films ranges from 10% to 36 %. The films with glycerol concentration 45% showed the highest solubility. These results may be related to the hygroscopic property of glycerol and with the increase of O-H bonds on cassava starch films with the increase of glycerol concentration, that is more available to interact with the water molecules. This hypothesis could be cofimed by the FTIR analyses.

Table 1 Physicochemical properties of cassava starch films using different glycerol concentration

Amount of glycerol (%w/w)	Thickness (mm)	Color L*	a_w	WVP (g/m ² .kPa.hr)	Water Solubility (%)
0	0.10 ± 0.03 ^c	23.94 ± 1.13 ^a	0.40 ± 0.02 ^c	-	10.21 ± 2.18 ^d
15	0.15 ± 0.03 ^b	22.96 ± 2.07 ^a	0.41 ± 0.01 ^c	-	21.72 ± 0.46 ^c
25	0.18 ± 0.01 ^{ab}	18.96 ± 1.59 ^b	0.43 ± 0.02 ^c	0.12 ± 0.01 ^a	32.29 ± 0.33 ^b
35	0.20 ± 0.01 ^a	18.81 ± 0.45 ^b	0.48 ± 0.02 ^b	0.15 ± 0.01 ^b	34.02 ± 0.10 ^b
45	0.21 ± 0.01 ^a	17.72 ± 1.08 ^b	0.53 ± 0.01 ^a	0.19 ± 0.01 ^c	36.71 ± 0.25 ^a

Remark Mean of three replicates ± standard deviation, ^{a-d} Means in the same column with different lowercase superscripts indicate significant differences ($p \leq 0.05$)

The effect of glycerol was initially evaluated by FTIR analyses. Figure 2 shows the FTIR spectra of the cassava starch (Figure 2A), glycerol (Figure 2B), cassava starch films with different concentrations of glycerol 0% (Figure 2C), 15% (Figure 2D), 25% (Figure 2E), 35% (Figure 2F) and 45% (Figure 2G). Figure 2A shows the FTIR spectrum of cassava starch powder. The broad band ranging between 3500 and 3100 cm⁻¹ is attributed to O-H stretching. The broad band between 2800 and 3000 cm⁻¹ is attributed to C-H stretching and 996 cm⁻¹ and 928 cm⁻¹ (C-OH bending). The FTIR spectrum of glycerol is shown in Figure 2B. Typical absorption bands of glycerol are located in the region 3200 cm⁻¹, the peak due to the O-H stretching of hydroxyl groups. The FT-IR spectrum of cassava starch films and glycerol plasticizer are shown in Figure 2C-2G. The addition of 0%, 15%, 35% and 45% of glycerol to cassava starch had shifted the O-H stretching peak at 3279 cm⁻¹ to higher wave numbers, 3293 cm⁻¹. This shift was caused by a decrease in the intermolecular force of the hydroxyl groups between the glycerol-starch, starch-water and water-glycerol interactions (Zhang & Han, 2006). This shifting was caused by the completion between the starch and glycerol for forming hydrogen bonds with water molecules. As a result, the wave number was shifted to the higher frequency, which was also found by Park, Im, Kim, and Kim (2000).

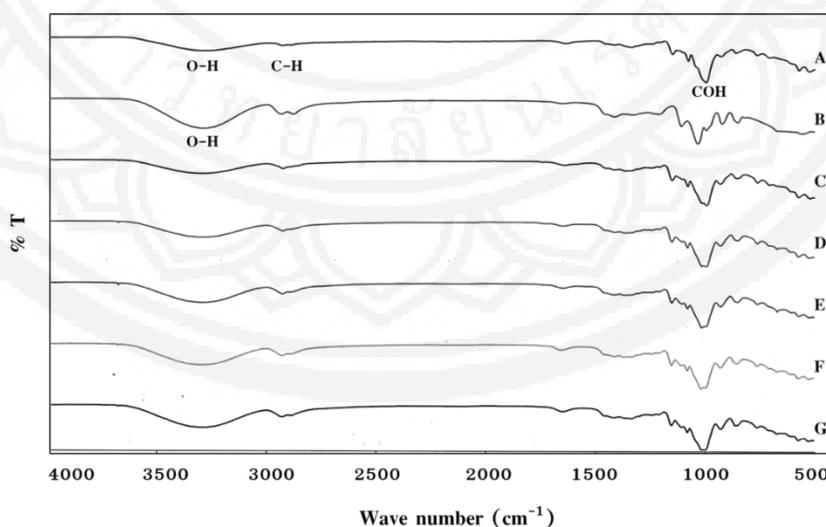


Figure 2 FT-IR spectra of (A) cassava starch, (B) glycerol, (C) cassava starch films with different concentrations of glycerol 0%, (D) 15%, (E) 25%, (F) 35% and (G) 45%



Mechanical properties (tensile and puncture strength) of the cassava starch films are presented in Table 2.

The results show that tensile strength decreased with increasing the glycerol concentration ($p \leq 0.05$). This was attributed to the reduction of intermolecular forces with glycerol and increasing polymer chains mobility. Due to the plasticizing effect, the free volume among the adjacent chains of polysaccharide increased and making the structure more fragile. This involved a decrease in tensile strength. Similar results have been previously reported for hydroxypropyl starch films (Arvanitoyannis & Biliaderis, 1998) and for potato and barley starch films (Koskinen, Suortti, Autio, Myllärinen, & Poutanen, 1996). The percent elongation of the film increased with the glycerol content in the films ($p \leq 0.05$). As glycerol reduces the starch crystallization, the films displayed an elastomeric behavior. This behavior was also reported by Flores et al. (2015). The puncture strength decreased with increase of glycerol content as observed in Table 2 ($p \leq 0.05$). As glycerol concentration increased from 15% to 45%, the film became less dense and under stress and thus the movements of polymer chains were facilitated (Sauid, Amin, Musa, & Hamid, 2017).

Table 2 Mechanical properties of cassava starch films using different glycerol concentrations

Amount of glycerol (%w/w)	Tensile Strength (N/m ²)	%Elongation	Puncture Strength (N/m ²)
0	10.65±1.12 ^a	1.84±0.21 ^d	75.15±1.05 ^a
15	9.42±0.02 ^b	10.70±1.12 ^c	62.40±1.11 ^b
25	7.14±0.01 ^c	15.20±2.50 ^b	51.26±1.01 ^c
35	6.07±0.02 ^d	18.05±2.23 ^b	35.90±1.75 ^d
45	4.19±0.01 ^e	26.22±2.53 ^a	26.87±1.58 ^e

The biodegradable of cassava starch films using different glycerol concentration were submitted to biodegradability analysis. The images of the films prior to their burial in soil and after 3 weeks of experiment are depicted in Table 3. After 3 weeks of the test, the film containing with increase in the proportion of glycerol show mass loss rate of the biodegradable films increased. The polyethylene bags showed no sign of degradation. The highest percentage (100%) of biodegradable was observed for cassava starch films containing 35% and 45% of glycerol, whereas the lowest percentage of biodegradable were 75.00% and 80.23% for cassava starch films containing 15% and 25% of glycerol, respectively. In contrast, the cassava starch films containing 0% of glycerol only loss around 28.50% of biodegradable in the same time period. This difference attributed to the high loss of mass during the process of biodegradation depended on the water absorption and solubility capacity. This also promoted the growth of microorganisms naturally present in the soil, which helped to enhance the microbial assisted biodegradation process.

Table 3 Biodegradable of cassava starch films using different glycerol concentration

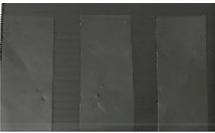
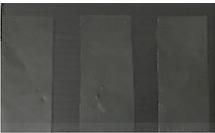
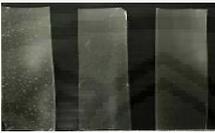
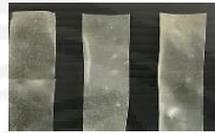
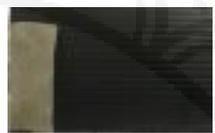
Film	Physical appearance		Biodegradation (%)
	0 week	3 weeks	
Polyethylene film (Control)			0.0
cassava starch films + glycerol 0%			28.50 ± 2.20
cassava starch films + glycerol 15%			75.00 ± 1.53
cassava starch films + glycerol 25%			80.23 ± 1.11
cassava starch films + glycerol 35%			100 ± 0.00
cassava starch films + glycerol 45%			100 ± 0.00

Figure 3 shows the biodegradable plant bags from cassava starch at different concentrations of glycerol 25%, 35% and 45%. The films with glycerol concentration of 0% and 15% were brittle, and again it was not possible to cut samples for packaging of cassava starch films. The use of biodegradable film with glycerol concentration 25%, 35% and 45% is an alternative for the production of bags for seedlings.

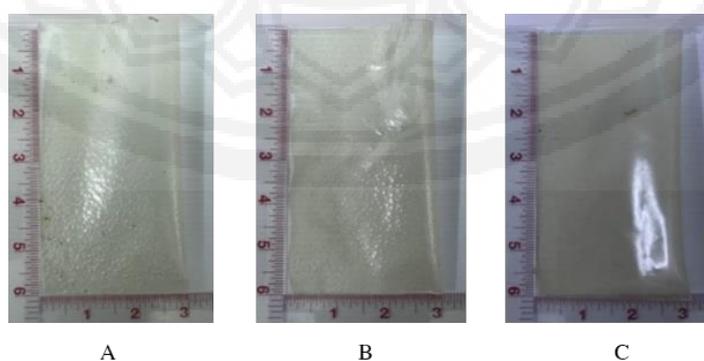


Figure 3 Biodegradable plant bags from cassava starch at different concentrations of glycerol A) 25% C) 35% and D) 45%

**Table 4** The growing marigold in the biodegradable plant bags from cassava starch at different concentrations of glycerol

Glycerol Conc.	Growing of marigold						
	week 0	week 1	week 2	week 3	week 4	week 5	week 6
Control							
25%							
35%							
45%							

The marigold seedlings would be economically attractive for commercial growers due to its faster growth. The marigold seedlings were procured from a local plant that came from the Lop Buri of Thailand. All the plants were of the same size and were transferred to polyethylene bags (PE), plant bags from cassava starch with glycerol 25%, 35% and 45%. The seedlings were buried at the State University of Thailand for 6 weeks and were watered daily with 80 mL of water. The growth of the seedlings after 6 weeks from transplanting is shown in Table 4. The growth and flowering of marigold plants was no significant difference between polyethylene bags and plant bags from cassava starch with glycerol 25%, 35% and 45% which have no phytotoxic effects on growth and productivity of plants. The use of biodegradable films from cassava starch is an alternative for the production of bags for seedlings, as these can then be transplanted directly into the soil without removing the bag, which help to reduce the risk of damage to the roots. It would be economically attractive for commercial growers.

Conclusion and Suggestions

Biodegradable cassava starch films containing different concentrations of glycerol (0%, 15%, 25%, 35% and 45 % w/w) were evaluated as to obtain promising biodegradable films to be used a plastic plant bag application. According to this study, biodegradable cassava starch film with glycerol 35% was the most recommended. The lower amount of plasticizer produced brittle films, while a higher content resulted in sticky films. Glycerol was used as a typical plasticizer in starch films; with the increased glycerol concentration, thickness, a_w , water vapor permeability, water solubility, percent elongation increased while tensile and puncture strength decreased. Biodegradable films with increasing glycerol exhibit hydrophilic character, present high loss of mass for 3 weeks during the process of biodegradation due to increased water promote the growth of microorganisms in the soil. The biodegradable film plant bags were used to the transplanting of marigold seedlings production period (6 weeks). The growth and flowering of marigold plants were insignificant difference between polyethylene bags and plant bags from cassava starch.



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