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Water Vapor Permeability of Edible Films Based on Improved Cassava (*Manihot esculenta* Crantz) Native Starches

Adjouman YD[1,2,3]*, Nindjin C[1,2], Tetchi FA[1], Dalcq AC[3], Amani NG[1], and Sindic M[3]

[1]*Université Nangui Abrogoua, UFR des Sciences et Technologies des Aliments, Abidjan, Côte d'Ivoire, West Africa*
[2]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, CSRS-CI, Abidjan, Côte d'Ivoire, West Africa*
[3]*Université de Liège, Gembloux Agro-Bio Tech, Gembloux, Belgique, Belgium*

Abstract

Starch is used in the production of edible biodegradable packaging as an attractive alternative to synthetic polymers because it is a natural biopolymer of low cost and high availability. Many studies have been carried out on films based on cassava starch and the results show that these have good flexibility and low water vapor permeability. This present research was conducted to analyse the effect of glycerol, peanut oil and soybean lecithin on the water vapor permeability (WVP) of edible films based on improved cassava (*Manihot esculenta* Crantz) native starches from Côte d'Ivoire. The films were prepared using 4 g cassava starch, 25% and 30% glycerol (w/w), 5% and 10% peanut oil (w/w) and 0% to 5% soybean lecithin (w/w oil) in Petri dishes. The WVP of the films was determined at 25°C and 75% relative humidity. The moisture content of the films was determined in an oven at 105°C and the film thickness was determined manually using a micrometer. The resulting films were homogeneous, transparent and crack-free. WVP, moisture content and thickness of the films increased with increasing glycerol concentration. Increasing the concentrations of the peanut oil and soybean lecithin decreased the thickness and increased the moisture content of the films. The combination of glycerol and peanut oil increased the WVP of the films, whereas addition of soybean lecithin had no effect. The lowest WVP values were obtained using 25% glycerol, 5% peanut oil and 5% soybean lecithin. Starch obtained from improved cassava varieties cultivated in Côte d'Ivoire can be a potential ingredient in the production of food packaging.

Keywords: Cassava starch; Water vapor permeability; Emulsified films; Glycerol; Peanut oil; Soybean lecithin

Abbreviations: WVTR: Water Vapor Transmission Rate; WVP: Water Vapor Permeability; FIRCA: Interprofessional Fund for Agricultural Research and Council; W/W: Weight per Weight

Introduction

Increasing pressure from society and legislation to minimise synthetic non-degradable packaging has encouraged research directed towards the production of biodegradable alternatives prepared from natural biopolymers. Consequently, the literature contains a plethora of studies that aim to either improve the properties of materials already used or to use new materials to attempt to produce packages with similar properties to their synthetic counterparts. In particular, the development of biodegradable films and edible materials from eco-friendly biopolymers, products and by-products of agro-materials and renewable sources is growing due to an increased environmental awareness [1,2]. For edible films and coatings, the materials and additives in their formulations are restricted to food-grade compounds. Thus, the main component of these thin and flexible materials capable of forming a continuous matrix is a biopolymer [2]. Among the natural polymers, starch is considered one of the most promising materials due to its combination of low-cost, performance [3,4], such as flexibility, transparency and thermoplasticity [5], and high natural abundance [6]. According to the European Starch Industry Association, the global starch market was valued at over USD 56 million in 2014 with an estimated production output of 76 million tonnes in the same year [7]. China and Brazil are the main contributors, with a total increase of 10% per year, while other countries have observed 1%-2% per annum [8]. Cassava (*Manihot esculenta* Crantz) roots are one of the most important sources of starch worldwide. The global cassava production in 2015 was estimated at 281.1 million tonnes (fresh root equivalent) with approximately 54% produced in Africa [9]. Cassava is the second major food crop after yam in Côte d'Ivoire, which produced an estimated 5.1 million tonnes of cassava in 2015 [9].

Edible films may be heterogeneous in nature, consisting of polysaccharides, proteins and lipids, or their combinations. This approach allows using the separate functional characteristics of each film-forming component to improve the film properties [10-12]. For instance, gelatin has been incorporated into starch [13] and hydroxypropyl starch films [14]. More recently Acosta et al. [15] combined cassava starch, gelatin and fatty acid ester (E-471) to improve cassava starch film properties. Perazzo et al. [16] produced active packaging from cassava starch and an aqueous extract of green tea and palm oil extracts. In other studies, composite films have been prepared based on cassava starch and apple pectin with the addition of laurel oil and oleic acid [17] and cassava starch, peanut oil and sucrose [18]. Rodriguez et al. [19] found a synergistic behaviour (lower tensile strength, higher elongation and higher water vapour permeability, WVP) between glycerol (as plasticiser) and emulsifiers, such as Tween 20, Span 80 and soybean lecithin, in potato starch films. Finally, Dias et al. [20] used glycerol as the plasticiser in gelatin-based films containing *Yucca schidigera* extract and lecithin, respectively. They obtained solid, flexible films with low WVP and concluded that addition of other emulsifiers commonly associated with plasticisers typically used in the production of edible and biodegradable films should be explored.

In this context, this study aimed to analyse the effect of glycerol,

***Corresponding author:** Adjouman YD, Université Nangui Abrogoua, UFR des Sciences et Technologies des Aliments, 02 B.P 801 Abidjan, Côte d'Ivoire, Tel: +225-08642119; E-mail: desyadjouman@gmail.com

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peanut oil and soybean lecithin on the WVP of films based on improved cassava native starches produced in Côte d'Ivoire.

Materials and Methods

Materials

The native starch used was obtained by cold water extraction from cassava (*M. esculenta* Crantz) varieties Bocou 2, Yavo and TMS, belonging to the National Agricultural Research Centre in Côte d'Ivoire genotype collection. The cassava plant was harvested at maturity, 12 months after plantation. Glycerol (bidistilled, 99.5% purity) and soybean lecithin were purchased from VWR Prolabo Chemicals (Leuven, Belgium). The CORA brand peanut oil used in this study was purchased at a supermarket in Belgium.

Methods

Film preparations and thickness measurements: The emulsified films were prepared in two steps. First, 4 g (w/w, starch) cassava starch was mixed with glycerol (1-1.2 g based on the starch weight) with two-thirds of distilled water and the final mixture heated from 30°C to 75°C for 20 min. Peanut oil (w/w, based on the starch weight), soybean lecithin (w/w based on the amount of added oil) and distilled water (a third of the total mixture) (Table 1) was also heated together for 20 min from 30°C to 75°C. Both solutions were heated with constant stirring at 750 rpm/min. Thereafter, the solution of peanut oil, soybean lecithin and distilled water was homogenised at 24000 rpm for 2 min using an Ultra-Turrax T25 basic (IKA Werke, Staufen /Germany). Next, the homogenised solution was mixed with the starch and glycerol and then heated from 75°C to 95°C at 750 rpm for 25 min. A 20-g aliquot of the final solution obtained was cast on the surface of a Petri dish and oven-dried in a ventilated model (Memmert UF-110, Schwabach / Germany) at 35°C for 24 h. The dried films were removed and stored at 55% moisture (saturated ammonium nitrate) in a desiccator at 25°C for 48 h, before testing. Films with only starch and glycerol were prepared by heating from 30°C to 95°C at 750 rpm/min for 45 min before a 20-g aliquot was cast, dried and stored under the same conditions as the emulsified films. The thickness of all films was determined using a hand micrometer (NSK, Japan) at 10 random positions on the films. Three observations are available per variety and formulation.

Starch 4% (g)	Oil 5-10% (g/g starch basis)	Glycerol 25% and 30% (g/g starch basis)	Soy lecithin 0% and 5% (mg/g oil incorporated)	Potassium sorbate (0.2 g)
4.0	0.2	1.0	30.0	0.2
	0.2	1.0		
	0.2	1.2	30.0	
	0.2	1.2		
	0.4	1.0	60.0	
	0.4	1.0		
	0.4	1.2	60.0	
	0.4	1.2		

Table 1: Film formulations.

Moisture content: The moisture content of the films was determined gravimetrically by oven drying at 105°C until constant weight (dry sample weight) [1]. The results were expressed as a percentage of the initial film weight, according to equation (1). At least triplicate analyses were performed per variety and formulation.

$$Humidity(\%) = \frac{(InitialWeight - 1)}{FinalWeight} \times 100 \quad (1)$$

WVP determination: The WVP of the films was conducted according to the ASTM method [21] with some modifications. Circular film samples were mounted and sealed on the open mouth of cylindrical cups containing 50 g of anhydrous calcium chloride (0% relative humidity, RH) to maintain an RH difference of 75% through the film. The assembly was placed in a desiccator at 25°C containing a saturated solution of sodium chloride (75% RH). After the steady-state conditions were reached (2 h), eight weight measurements were taken over 8 h. Changes in the weight of the cups were recorded to the nearest 0.0001 g and plotted as a function of time. The slope of each line was calculated by linear regression (r^2>0.99), and the water vapor transmission rate (WVTR) was calculated from the slope of the straight line (g/s) divided by the cell area (m^2). The exposed area was 0.00246 m^2. After the permeation tests, the film thickness was measured and the WVP (g m^{-1} s^{-1} Pa^{-1}) was calculated as follows:

$$WVP = [WVTR / S(R_1 - R_2) \times D] \quad (2)$$

Where S is the saturation vapor pressure of water (Pa) at the test temperature (25°C), R_1 is the RH in the desiccator, R_2 is the RH in the permeation cell and D is the film thickness (m). Duplicate analyses were performed per variety and formulation.

Statistical analysis: A two-way analysis of variance (ANOVA) was performed to study the effect of the cassava varieties and the film formulations on the continuous variables, namely WVP, thickness and moisture content (level of significance = 0.05). The significance of the interaction between the cassava varieties and film formulations was studied. The normality of the distribution of the observations and the homoscedasticity were checked a posteriori, on the residuals of the model. The Newman–Keuls and the contrasts methods were used for the comparison of the means, which were significantly different. The statistical analysis was performed using SAS software version 9.4.

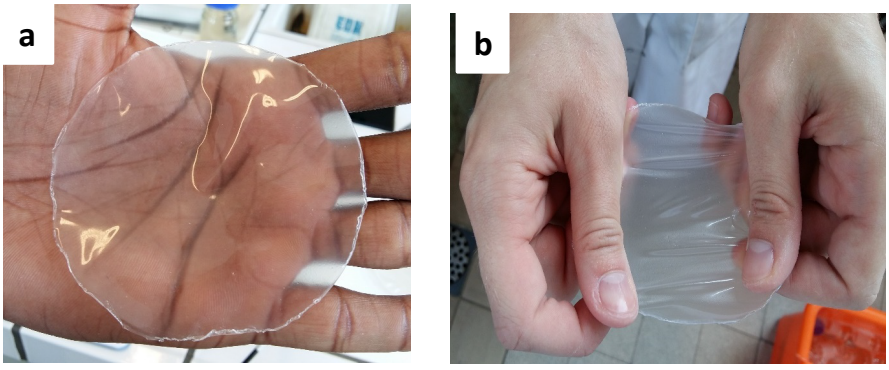

Figure 1: Cassava starch based emulsified film: a) Appearance of the film, b) Flexibility of the film.

Results and Discussion

Films obtained with the various improved cassava starch varieties were homogeneous, transparent and did not exhibit any cracks (Figure 1a). Furthermore, they were flexible and tenable in the hand, as shown in Figure 1b. Films prepared from 30% glycerol and 10% oil, with or without soybean lecithin, were very sticky and often difficult to handle. Films produced with only glycerol (30%) in the formulations also presented these behaviours.

The Table 2 shows the thickness of the cassava starch-based films including 25% or 30% glycerol, 0%, 5% or 10% peanut oil and 0% or 5% soybean lecithin. For the films containing only glycerol in the formulations, the highest thicknesses were obtained with 30% glycerol (P<0.001). The thickness increased significantly as the glycerol concentration was increased, in agreement with previous authors [22-26]. For films containing 25% glycerol, increasing the oil concentration from 5 to 10%, significantly increased the film thickness for the variety TMS (P<0.001). In contrast, varieties Yavo and Bocou 2 did not show this trend (P>0.05). Adding 5% soybean lecithin and 5 or 10% oil to the starch slurry containing 25% glycerol, significantly increased the thickness of the resulting films made from Bocou 2 and Yavo varieties (P<0.001). At 30% glycerol concentration, increasing the oil concentration from 5 to 10% significantly decreased the film thickness made from Bocou 2 and Yavo varieties (P<0.001), which contrasted with the observations for 25% glycerol. At 30% glycerol concentration, the addition of soybean lecithin in the presence of oil, significantly increased film thickness of Yavo variety (P<0.01) but not with TMS and Bocou 2 varieties (P>0.05). These results disagreed with those of Nindjin et al. [18] and Nilsuwan et al. [27], which demonstrated that a combination of oil and plasticiser increased the film thickness. The highest film thickness were 98.5 µm, 92.5 µm and 91.7 µm, respectively for varieties Bocou 2, Yavo and TMS with 30% glycerol formulation lacking of peanut oil and soybean lecithin (Figure 2).

Film formulations			Film thickness (µm)		
Glycerol (%)	Oil (%)	Soy lecithin (%)	Bocou 2	Yavo	TMS
25	0	0	79.5 ± 0.50^a E	82.0 ± 1.00^a D	83.5 ± 1.80^a BC
30	0	0	98.5 ± 0.50 A	92.5 ± 1.00 A	91.7 ± 0.75 A
25	5	0	92.0 ± 2.00 BC	85.7 ± 0.25 C	82.5 ± 2.00 BC
25	5	5	86.5 ± 3.50 CD	88.7± 0.25 BC	78.5 ± 1.00 D
25	10	0	79.0 ± 0.50 E	75.4 ± 0.50 E	85.0 ± 1.00 B
25	10	5	87.3 ± 1.25 CD	80.5 ± 0.50 D	79.7 ± 1.25 CD
30	5	0	97.5 ± 1.50 AB	86.5 ± 0.50 C	84.5 ± 0.50 B
30	5	5	95.5 ± 4.50 AB	91.0 ± 2.00 AB	73.0 ± 0.50 E
30	10	0	80.2 ± 0.75 E	81.5 ± 1.50 D	76.0 ± 1.00 DE
30	10	5	83.0 ± 0.50 DE	82.0 ± 2.00 D	85.0 ± 2.00 B

Lowercase superscript "a" indicates the standard deviations of three measurements.
Values assigned different uppercase letters in columns are significantly different.

Table 2: Thickness of films based on improved cassava starch varieties.

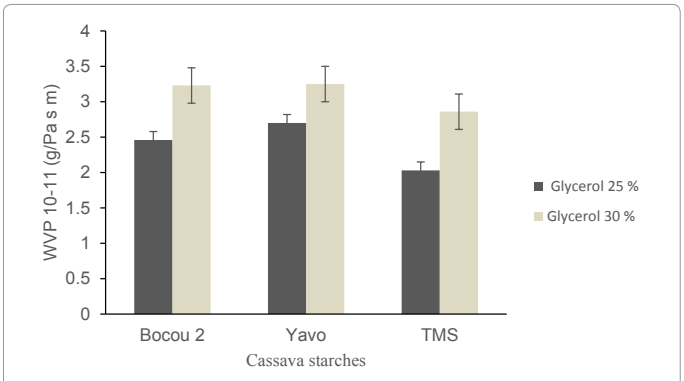

Figure 2: Effect of glycerol content on water vapor permeability of cassava starch-based films.

Film formulations			Moisture content (%)		
Glycerol (%)	Oil (%)	Soy lecithin (%)	Bocou 2	Yavo	TMS
25	0	0	16.57 ± 0.06^a F	17.15 ± 0.47^a B	16.35 ± 0.11^a DE
30	0	0	21.83 ± 0.01 C	21.53 ± 0.37 A	21.92 ± 0.03 A
25	5	0	11.79 ± 0.08 J	10.95 ± 0.07 D	11.13 ± 0.03 H
25	5	5	12.78 ± 0.02 I	11.74 ± 0.12 D	12.26 ± 0.03 G
25	10	0	18.06 ± 0.02 E	17.39 ± 1.12 B	17.07 ± 0.65 D
25	10	5	18.73 ± 0.05 D	17.58 ± 0.13 B	18.28 ± 0.12 C
30	5	0	15.71 ± 0.1 H	13.88 ± 0.03 C	14.86 ± 0.12 F
30	5	5	16.38 ± 0.05 F	14.83 ± 0.05 C	15.75 ± 0.04 E
30	10	0	23.99 ± 0.03 B	20.49 ± 0.12 A	20.98 ± 0.4 B
30	10	5	24.34 ± 0.04 A	20.87 ± 0.03 A	21.03 ± 0.24 B

Lowercase superscript "a" indicates the standard deviations of three measurements.
Values assigned different uppercase letters are significantly different.

Table 3: Moisture content of films based on improved cassava starch varieties.

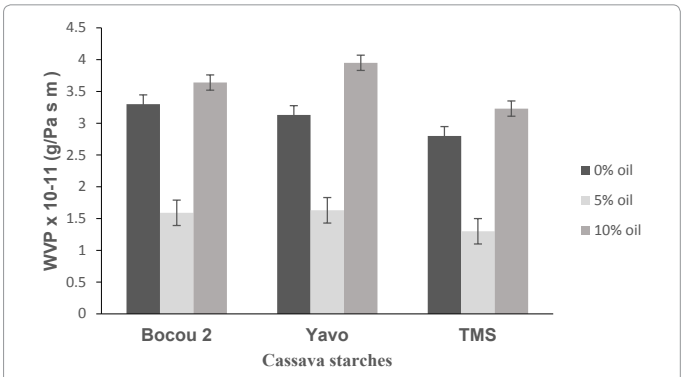

Figure 3: Effect of oil content on water vapor permeability of cassava starch-based films.

The glycerol concentration had a significant effect (P<0.001) on moisture content of the films without oil and soybean lecithin in the formulations. Moisture content increased significantly with increasing glycerol concentration, irrespective of the cassava variety (Table 3). This result concurred with Alves et al. [28] and Lopez et al. [5], which observed an increase in moisture content of the starch-based films following an increase in the concentration of glycerol. This phenomenon is linked to the hydrophilic properties of glycerol. Adding 5% oil to the formulations significantly decreased the moisture content of the films (P<0.001) but increasing the oil concentration to 10% significantly increased the moisture content (P<0.001), irrespective of the cassava starch variety. Addition of soybean lecithin significantly influenced the moisture content of the films (P<0.001). Some lipids have a low affinity with the ingredients in which they are used to produce films. In our study, the affinity was not investigated. Nindjin et al. [18] reported a less affinity between vegetable oil and sucrose, when studying the effects of these components on the properties of cassava starch-based edible films. Thus, the increase in moisture content on addition of soybean lecithin and its consequent interaction with glycerol could be explained by the hydrophilicity of lecithin. Rodriguez et al. [19] investigated

the combined effect of plasticisers and emulsifiers on the properties of starch-based edible films and found that films with glycerol and a high surfactant concentration behaved similar to films with a relatively larger plasticiser content. In the current study, the lowest moisture contents were found for the formulations containing 25% glycerol, 5% oil without lecithin (Figure 3). The values were of 10.95%, 11.13% and 11.79% for Yavo, TMS and Bocou 2, respectively (Table 3).

Using soybean lecithin as emulsifier whatever the presence or absence of glycerol, significantly influenced the WVP of the films made from starch of the variety Yavo ($p<0.05$) but not those from the varieties Bocou 2 and TMS ($p>0.05$). The WVP of the starch-based films made from the three improved cassava varieties with glycerol at 25 and 30% concentrations, showed a significant difference (Table 4). The WVP values obtained for Bocou 2, Yavo and TMS increased from 2.51×10^{-11} g/m s Pa, 2.43×10^{-11} g/m s Pa and 2.06×10^{-11} g/m s Pa to 4.09×10^{-11} g/m s Pa, 3.83×10^{-11} g/m s Pa and 3.55×10^{-11} g/m s Pa, at 25% and 30% glycerol, respectively. Similarly, many previous authors have also observed an increase in WVP with an increase in glycerol content of starch-based films [22,28-33]. This phenomenon has been explained by a decrease in intermolecular forces between polymer chains as the plasticiser concentration is increased, thus, increasing the free volume and movement of molecular chains [34], which promotes the dissemination of water molecules through the film. Park and Chinnan [35] explained the effect as a result of swelling of the hydrophilic matrix due to different structural changes in films with different thicknesses. For starch films plasticised with 15% to 45% glycerol, Sanyang et al. [30] also found that increasing the glycerol concentration increased the WVP, with the lowest concentration of glycerol corresponding to the lowest values of WVP. These authors attributed this behaviour to the strong interaction of starch molecules, which dominate the matrix at the lowest plasticiser concentrations, leading to a dense network of starch and a more compact structure. Conversely, the increase in plasticiser concentration promotes mobility and flexibility of the starch chains due to the structural modification of the molecular starch-starch interactions in a loose network. Therefore, the matrices become less dense and the WVP of the film increases.

The main function of food packaging is often to avoid or at least reduce the moisture transfer between the food and the surrounding atmosphere, or between two components of a heterogeneous food product, thus, the WVP of the film should be as low as possible [36]. In the current work, the oil was added to obtain emulsified films with

Film formulations			WVP × 10^{-11} (g/m s Pa)		
Glycerol (%)	Oil (%)	Soy lecithin (%)	Bocou 2	Yavo	TMS
25	0	0	2.51 ± 0.12^{a} B	2.43 ± 0.105^{a} BC	2.06 ± 0.005^{a} BC
30	0	0	4.09 ± 0.235 A	3.83 ± 0.005 A	3.55 ± 0.05 A
25	5	0	1.42 ± 0.05 C	1.65 ± 0.015 CD	1.19 ± 0.11 D
25	5	5	1.4 ± 0.265 C	1.12 ± 0.06 D	1.06 ± 0.106 D
25	10	0	3.45 ± 0.09 A	3.76 ± 0.06 A	2.98 ± 0.035 A
25	10	5	3.48 ± 0.06 A	3.31 ± 0.025 AB	2.82 ± 0.505 AB
30	5	0	1.82 ± 0.005 BC	1.6 ± 0.05 CD	1.48 ± 0.115 CD
30	5	5	1.74 ± 0.08 C	2.18 ± 0.01 C	1.47 ± 0.025 CD
30	10	0	3.67 ± 0.025 A	4.32 ± 0.06 A	3.59 ± 0.1 A
30	10	5	3.96 ± 0.005 A	4.16 ± 0.025 A	3.53 ± 0.185 A

Lowercase superscript "a" indicates the standard deviations of three measurements.
Values assigned different uppercase letters are significantly different.

Table 4: Water vapour permeability of films based on improved cassava starch varieties.

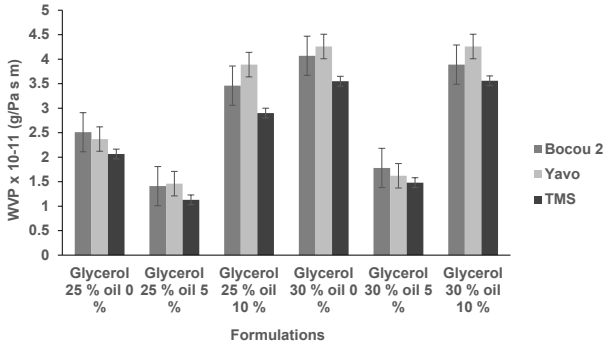

Figure 4: Combined effect of glycerol and oil contents on water vapor permeability of cassava starch-based films.

decreased WVP based on improved cassava starch. Accordingly, a significant decrease ($P<0.001$) was verified. Adding 5% oil decreased the WVP compared to combinations in which the oil was not added but the opposite effect was produced when 10% oil was incorporated ($P<0.001$). These results concur with those of Garcia et al. [37] for emulsified starch films containing corn and sunflower oil. A possible explanation for the increase in WVP of the emulsified film at 10% oil may be due to the oil (peanut) used. Both peanut and whole sunflower oil are rich in unsaturated fatty acids, such as oleic acid and linoleic acid. Compared to saturated fatty acids, unsaturated fatty acids are less effective at improving WVP because they are more polar [38,39]. The presence of one or more unsaturated bonds decreases the melting point of the lipid compound, which may explain the increase in WVP of the film because diffusion is 1000 times faster in liquids than in solids. Moreover, Gontard et al. [40] observed that due to its double bond, oleic acid has a degree of mobility, reducing the density of the macromolecular network and, thus, the effectiveness of the water. Moreover, increasing the number of carbon atoms (14 to 18), of water alcohols and fatty acids, improved the barrier effectiveness as the non-polar part of the molecule increases, reducing the solubility and, therefore, the transfer of water molecules through the film [41].

The combination between glycerol and oil was shown significant ($P<0.001$). At 5% oil concentration, an increase in the glycerol concentration induced a significant increase in the WVP of all films, irrespective of the cassava starch variety studied ($P = 0.05$). The same trend was observed at 10% oil concentration, except with the starch variety Yavo. Likewise, Nilsuwan et al. [27] demonstrated that at a constant concentration of palm oil, increasing the glycerol concentration (0%, 10%, 20% and 30%) increased the WVP of fish skin gelatin films. However, in the current study, the main purpose of adding glycerol, oil and lecithin in the formulations was to provide the lowest possible WVP. The results showed that the interaction between 25% glycerol and 5% oil gave the lowest WVP values of 1.4×10^{-11}, 1.12×10^{-11} and 1.06×10^{-11} g/m s Pa for varieties Bocou 2, Yavo and TMS, respectively. WVP is improved when the size of lipidic droplets present in the emulsified films is reduced and these fatty inclusions are more dispersed [42]. Soybean lecithin was used for this purpose in the current study but either the presence or absence of lecithin not significantly improved the WVP for the varieties Bocou 2 and TMS ($P>0.05$). The reductions in the WVP values of the films after addition of lecithin were minimal. This was not the case for the Yavo variety where the presence of lecithin significantly decreased WVP. The addition of an emulsifier may also have diminished the humidity content of the films, due to the interaction of hydrogen bridges between the hydrocolloid and the polar groups of the emulsifier, thus reducing the number of

polar groups available to interact with the water molecules [43]. This may be the case with the Yavo variety where WVP has experienced a significant decrease (Figure 4).

The WVP values of the films formulated with 25% glycerol, 5% peanut oil and 5% soybean lecithin (1.4×10^{-11} g/m s Pa, 1.12×10^{-11} g/m s Pa and 1.06×10^{-11} g/m s Pa, respectively, for varieties Bocou 2, Yavo and TMS) are lower than those of certain biodegradable plasticised films, such as wheat gluten with glycerol (7×10^{-10} g/m^{-1} s^{-1} Pa^{-1}) [44], corn starch plasticised with sorbitol (1.75×10^{-10} g/m s Pa) [37] and corn zein plasticised with glycerol (8.9×10^{-10} g/m s Pa) [45]. The values are also lower than some synthetic films, such as cellophane (8.4×10^{-10} g/m s Pa) [46], but higher than low-density polyethylene (9.14×10^{-13}g/m s Pa) and high-density polyethylene (2.31×10^{-13} g/m s Pa) [47]. The values are also low compared to certain emulsified films, for example, hydroxypropyl méthylcellulose with plasticiser and oil (1.90×10^{-11} g/m s Pa) [41], cassava starch and 15% glycerol with hydrogenated vegetable oil (2.94×10^{-11} g/m s Pa) and agar, 15% glycerol and hydrogenated vegetable oil (1.44×10^{-11} g/m s Pa) [48] but higher than those observed by Nilsuwan et al. [27] (7.92×10^{-12} g/m s Pa) for fish gelatin films with 30% glycerol and 75% palm oil.

Conclusion

This work was conducted to study the effect of incorporating glycerol, peanut oil and soybean lecithin on the WVP of films based on improved starch cassava varieties cultivated in Côte d'Ivoire and their potential for food packaging applications. The WVP, moisture content and film thickness increased with increasing glycerol concentration. In general, increasing the concentration of the peanut oil and soy lecithin decreased the thickness and increased the moisture content of the films. The combination of glycerol and peanut oil also increased the WVP of the films but the addition of soybean lecithin had no effect. The lowest WVP values were obtained using 25% glycerol, 5% peanut oil and 5% soy lecithin. Improved cassava starch varieties cultivated in Côte d'Ivoire may be a potential ingredient in food packaging.

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