Research article

Starch-based edible films of improved cassava varieties Yavo and TMS reinforced with microcrystalline cellulose

Adjouman Yao Désiré a,b,*, Nindjin Charlemagne a,b, Kouadio Degbeu Claver a, Tetchi Fabrice Achille a, Sindic Marianne c

a Université Nongui Abrogoua, UFR des Sciences et Technologies des Aliments, 02 BP 801 Abidjan 02, Côte d'Ivoire
b Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, CSRS-CI, 01 BP 1303 Abidjan 01, Côte d'Ivoire
c Université de Liège, Gembloux Agro-Bio Tech, Laboratoire Qualité et Sécurité des Produits Agroalimentaires, Passage des Déportés, 2, 5030 Gembloux, Belgium

ARTICLE INFO

Keywords:
Yavo and TMS starch
Edible films
Microcrystalline cellulose
Films properties

ABSTRACT

The results of a recent study on starch-based films of improved cassava varieties show that these films have poor barrier properties and lower mechanical strength. Thus, for some applications, improving their resistance to breaking forces is a key factor in making their use possible and sustainable. In this study, to the starch of two improved varieties of cassava (Yavo and TMS), combined with peanut oil, soybean lecithin, glycerol was added microcrystalline cellulose (MCC) at 0, 7, 15 and 30 %. The addition of microcrystalline cellulose has resulted in an increase in the opacity (223.91 nm.UA to 425.33 nm.UA for Yavo and 251.42 nm.UA to 434.51 nm.UA for TMS), tensile strength (7.15 MPa-10.99 MPa for Yavo and 7.77 MPa-13.18 MPa for TMS), and Young's modulus (331.29 MPa-1351.08 for Yavo and 343.79 MPa-1476.08 MPa for TMS) of films. However, MCC induced a decrease in moisture content (15.99 %–11.43 % for Yavo and 14.24 %–10.66 % for TMS), water solubility (24.84 %–20.61 % for Yavo and 24.15 %–19.36 % for TMS), elongation at break (22.75 %–1.31 % for Yavo and 21.25 %–1.19 % for TMS) and water vapour permeability (WVP) (1.98 × 10⁻¹¹ to 1.39 × 10⁻¹¹ g Pa⁻¹s⁻¹m⁻² for Yavo and 1.93 × 10⁻¹¹ to 1.29 × 10⁻¹¹ g Pa⁻¹s⁻¹m⁻² for TMS). The MCC has also produced yellowish-coloured films. MCC has been shown to be effective in improving starch-based films of improved cassava varieties Yavo and TMS. These two varieties can be used in combination with MCC to produce food packaging.

1. Introduction

Cassava (Manihot esculenta Crantz) is one of the most important food crops in the humid tropical zone and the second most important food crop in Côte d’Ivoire after yams, with an estimated production of 5.367 million tonnes in 2017 (FAO, 2018). In the past, cassava cultivation in Côte d’Ivoire was mainly based on the use of cultivars with low production potential (less than 15 t/ha), susceptible to diseases and pests (N’Zué et al., 2003). In order to improve production, new improved varieties with greater disease resistance and higher production yield (35 t/ha) were developed (Bakayoko et al., 2012; Ebah-Djedji et al., 2012; Kouadio et al., 2014). Although these improved varieties have a high production yield, they are not being widely used in the production of “attiéki”, the national staple food for which the majority of cassava produced is destined (CSRS, 2017). The main reason given by producers for not using improved varieties of cassava for the production of attiéké is that these improved varieties have a high water content and would not lend themselves to its production. In order to promote these improved varieties of cassava in other fields, the starches of these varieties produced in Côte d’Ivoire have been studied on their functional parameters with a view to their industrial use (Ebah-Djedji et al., 2013; Doue et al., 2014). The study concluded that these starches could be exploited in the pharmaceutical industry as disintegrants for tablets because of their high water absorption capacity, which was far superior to that of potato and maize starch. In addition, they could be used in the chemical industry for the manufacture of glues and detergents because of their high swelling power and solubility, induced by their high amylose content. Also, taking into account the high proportion of large grains, they could also be used in the plastics industry for the production of biodegradable plastic films. Thus, studies have been carried out on biodegradable starch-based packaging of some of these improved cassava varieties (Nindjin et al., 2015; Adjouman et al., 2017, 2018a, 2018b). The results of these studies indicate that starches from improved varieties can be used in the production of edible films and coatings (Adjouman et al., 2017, 2018a,

* Corresponding author.
E-mail address: desyadjouman@gmail.com (A. Yao Désiré).

https://doi.org/10.1016/j.heliyon.2021.e06804
Received 8 July 2020; Received in revised form 26 November 2020; Accepted 10 April 2021
2405-8440/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
2018b). However, as with other starches used to produce biodegradable films, starch-based films of improved varieties of cassava grown in Côte d'Ivoire have poor water vapour barrier properties and low mechanical strength. The latter poses a major problem for applications as packaging material (Müller et al., 2009). Improving their resistance to breaking forces is therefore a key factor in enabling their sustainable use in packaging (Müller et al., 2009). To improve material properties, certain elements such as inorganic fillers and cellulose fibres have been added to starch-based films. Lignocellulosic materials were found to be a promising additive owing to their renewability, biodegradability, wide variety, low energy consumption and low cost and their density and high strength. To this end, Müller et al. (2009) have incorporated cellulose fibers into starch-based films plasticized with glycerol. They showed that the addition of cellulose fibers increases the stability of starch-based films subjected to variations in relative humidity and therefore identified a potential means to obtain stronger and more stable films. In addition, Panaitescu et al. (2015) showed that starch films with 7 % cellulose nanofibers had a mechanical strength and stiffness that come close to that of polyolefins and can therefore be considered an inexpensive alternative for applications in food. Microcrystalline cellulose (MCC), one of the derivatives of cellulose fibers, has been used in some studies as starch reinforcement for the production of biodegradable composite films (Ma et al., 2008; Rico et al., 2016; Reis et al., 2017; Syafri et al., 2018a, 2018b; Chen et al., 2020) and was found to be effective in improving the properties of starch-based films. The results of the study by Chen et al. (2020) on the improvement of mechanical and hydrophobic properties of composite starch films of cassava with modified MCC and nanocellulose as strength agent showed that these materials can improve the mechanical and hydrophobic properties of cassava starch films. Indeed, the levels of 0.5 % and 2 % MCC respectively allowed for the increase in the mechanical strength of the films and an improvement in the hydrophobic property of the films. Also, Coelho et al. (2017) indicated that the incorporation of MCC into polysaccharide-based films can be an approach to modifying film properties. The objective of this work was to study the effect of MCC incorporation on the properties of Yavo and TMS starch-based edible films, two improved cassava varieties.

2. Materials and methods

2.1. Material

Native starch was obtained by cold extraction from the improved cassava varieties Yavo and TMS. The cassava roots were harvested at maturity after 12 months of growth in an experimental field at the Brinagro research station of the Swiss Scientific Research Centre in Côte d'Ivoire (Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, CSRS). After harvesting in Brinagro, the fresh roots were transported to the chemistry laboratory of the CSRS at the main site in Adiopodoumé, Abidjan. Glycerol bidistilled at 99.5 % purity was used as a plasticizer, and soy lecithin was added as an emulsifying agent. MCC was used to strengthen the starch-based films. The water vapour permeability of the films was determined by adding anhydrous calcium chloride (CaCl₂). All chemicals (MCC and others) were supplied by VWR prolabo Chemicals (Leuven, Belgium). The CORA peanut oil (Gemboux, Belgium) used in this study was purchased over a large area in Belgium.

2.2. Cassava native starches extraction

The extraction of native starches was carried out in the laboratory. The freshly harvested cassava roots were peeled, cut, and then crushed using a hammer mill and then diluted in water. The paste obtained was taken up in a solution of sodium chloride (4% W/V) to separate the proteins from the starch and then passed through a series of sieves with a mesh size of 500, 250, 100 μm, respectively. The liquid obtained was subjected to alternating decantation and washing (at least 4 times). The pellet was separated from the supernatant and washed several times. The deposit obtained was drained and then spread on an aluminum foil and allowed to dry at 45 °C. For 48 h in an oven. The product obtained was ground to have starch powder (Amani et al., 2004).

2.3. Physical parameters of starches from improved cassava varieties

Thermal properties of the starches were determined by differential scanning colorimetry according to the method described by Lopez et al. (2008). A Polymer Laboratories colorimeter (Rheometric Scientific Survey, UK) with PI-V5.41 software and a heating program of 10 °C/min from 10 to 120 °C was used. Aqueous suspensions of starch (20 % w/w) were prepared. The samples were weighed into aluminum pans and sealed. An empty pan was used as a reference. Thermograms (heat flow as a function of the heating temperature) were recorded. These curves gave the following information: start temperature (To), peak temperature (Tp) and area below the peak (ΔH). To is the start of the loss of birefringence of the granule; Tp is the temperature at which birefringence is lost (gelatinization temperature) and ΔH is the energy required for the transition.

Pasting behaviour was determined by the method described by Malumba et al. (2009). The Brabender visco-amylograph (Duisberg, Germany) was used to obtain the pasting characteristics of starches from Yavo and TMS, two improved varieties of cassava. To 10 g of starch (dry basis) was added 100 g of deionized water and the mixture obtained at room temperature was subjected to gradual heating (6.5 °C min⁻¹) from 30 to 95 °C. This temperature was maintained during 10 min followed by a gradual cooling step (-4.5 °C min⁻¹) from 95 to 50 °C. This temperature was maintained for 5 min before the end of pasting. The relevant values obtained from the pasting profile were: the peak viscosity (the maximum viscosity during pasting), set-back viscosity (the difference between the viscosity at the end of cooling and the viscosity at the end of the holding phase) and final viscosity (the viscosity at the end of cooling). All measurements were performed in triplicate and viscosities were expressed in BU (Brabender units).

The apparent amylose content of the starch samples was determined by colorimetric reaction and subsequent measurement of the absorbance of the blue amylose-iodine complex formed according to the method described by Morrison and Laignelet (1983).

The paste clarity of the starches was determined as previously described by Craig et al. (1989). Approximately 0.11 g of starch was weighed into quartz screw tubes. The mass was supplemented to 10 g with distilled water. The closed tube with the well homogenized content was left in a boiling water bath at 100 °C for 30 min with uniform stirring. The solution obtained was cooled and the paste clarity or percent transmittance (%) was determined using a Shimadzu UV-2401-PC (Kyoto-Japan) spectrophotometer at 650 nm against a blank sample containing distilled water.

2.4. Preparation of films reinforced with MCC

The preparation of starch-based films reinforced with MCC was conducted in three steps. The first step was done according to the method described by Moraes et al. (2014). The MCC powder was adjusted to the starch mass (4.0 g) (0, 7, 15 and 30 wt.% dry starch basis) used and mixed in 200 ml of distilled water for 24 h under constant stirring at 750 rpm. The solution obtained was homogenized at 13,000 rpm for 10 min using an Ultra Turrax T25 Basic homogenizer (IKA-WERKE, Staufen-Germany). The second stage followed the method previously described (Adjouman et al., 2017, 2018b). First, native starch powder (4.0 g) and glycerol (25 wt. % dry starch basis) (Table 1) were added to the solution. The mixture was then heated gradually from 30 °C to 75 °C for 20 min, under constant stirring (750 rpm) on an IKA® C-MAG hot plate (HS7). The mixture of oil (5 wt.% based on starch weight), soya lecithin (5 wt.% based on oil weight) and 100 mL distilled water was also heated from 30 °C to 75 °C for 20 min under constant stirring (750 rpm). This mixture was homogenized at 24,000 rpm for 2 min using the Ultra Turrax
Table 1. Dry matter, amylose content, pasting properties and thermal transition characteristics of starches of improved cassava varieties.

| Starch | Dry matter (%) | Amylose content (%) | Peak V (BU) | Final V (BU) | Setback (BU) | T0 (°C) | Tp (°C) | Te (°C) | ΔH (J/g) |
|--------|---------------|---------------------|------------|-------------|-------------|--------|--------|--------|---------|
| Yavo   | 87.63         | 18.37               | 61.33c     | 499         | 377         | 207    | 68.28  | 72.55  | 94.38   | 14.11   |
| TMS    | 88.37         | 20.36d              | 48.86d     | 478         | 384         | 210    | 68.92  | 73.25  | 89.26   | 13.33   |

% T: percentage de transmittance, To: onset temperature, Tp: peak temperature, Te conclusion temperatures, Peak V: peak viscosity, Final V: final viscosity, BU: Brabender Units.

The color of the film was defined as the area under the curve and is expressed in units of absorbance x nanometers (nm.UA).

With S, solubility; P0, initial dry weight; Pf, final dry weight. The measurements were made in triplicate for each film.

2.9. Determination of the mechanical properties of films

Film opacity was determined using standard operating procedures (BSI, 1968) modified by Gontard et al. (1992) and applied by others (Malli et al., 2004; Lopez et al., 2008). Film samples were cut to 1 × 3 cm and placed on the inside of a spectrophotometer cell (Shimadzu UV-2401-PC Kyoto, Japan) to record the absorbance spectrum between 400 nm and 700 nm. The opacity of the film is defined as the area under the curve and is expressed in units of absorbance x nanometers (nm.UA). The measurements were made in triplicate for each film.

2.11. Determination of film colour

The color of the film was determined by the method described by Bourtoom and Chinann (2008) and also by Pires et al. (2018). This determination was made using an international Datacolor spectrophotometer D65/10 (Miniscan XE Virginia, USA). L* represents the luminance (from white to black), (a*) the chromatic index from green to red and (b*) that from yellow to blue. From these data, the color difference (ΔE° ab) of starch-based composite films and films reinforced with MCC was calculated with respect to the color of a white reference surface according to the following expression (Eq. (4)):

$S (%) = \frac{|(P_0 - P_f)|}{P_0} \times 100$  

(3)

With S, solubility; P0, initial dry weight; Pf, final dry weight. The measurements were made in triplicate for each film.

2.10. Determination of film opacity

Film opacity was determined using standard operating procedures (BSI, 1968) modified by Gontard et al. (1992) and applied by others (Malli et al., 2004; Lopez et al., 2008). Film samples were cut to 1 × 3 cm and placed on the inside of a spectrophotometer cell (Shimadzu UV-2401-PC Kyoto, Japan) to record the absorbance spectrum between 400 nm and 700 nm. The opacity of the film is defined as the area under the curve and is expressed in units of absorbance x nanometers (nm.UA). The measurements were made in triplicate for each film.

2.11. Determination of film colour

The color of the film was determined by the method described by Bourtoom and Chinann (2008) and also by Pires et al. (2018). This determination was made using an international Datacolor spectrophotometer D65/10 (Miniscan XE Virginia, USA). L* represents the luminance (from white to black), (a*) the chromatic index from green to red and (b*) that from yellow to blue. From these data, the color difference (ΔE° ab) of starch-based composite films and films reinforced with MCC was calculated with respect to the color of a white reference surface according to the following expression (Eq. (4)):

$S (%) = \frac{|(P_0 - P_f)|}{P_0} \times 100$  

(3)

With S, solubility; P0, initial dry weight; Pf, final dry weight. The measurements were made in triplicate for each film.

2.9. Determination of the mechanical properties of films

Film opacity was determined using standard operating procedures (BSI, 1968) modified by Gontard et al. (1992) and applied by others (Malli et al., 2004; Lopez et al., 2008). Film samples were cut to 1 × 3 cm and placed on the inside of a spectrophotometer cell (Shimadzu UV-2401-PC Kyoto, Japan) to record the absorbance spectrum between 400 nm and 700 nm. The opacity of the film is defined as the area under the curve and is expressed in units of absorbance x nanometers (nm.UA). The measurements were made in triplicate for each film.

2.11. Determination of film colour

The color of the film was determined by the method described by Bourtoom and Chinann (2008) and also by Pires et al. (2018). This determination was made using an international Datacolor spectrophotometer D65/10 (Miniscan XE Virginia, USA). L* represents the luminance (from white to black), (a*) the chromatic index from green to red and (b*) that from yellow to blue. From these data, the color difference (ΔE° ab) of starch-based composite films and films reinforced with MCC was calculated with respect to the color of a white reference surface according to the following expression (Eq. (4)):

$S (%) = \frac{|(P_0 - P_f)|}{P_0} \times 100$  

(3)

With S, solubility; P0, initial dry weight; Pf, final dry weight. The measurements were made in triplicate for each film.

2.9. Determination of the mechanical properties of films

Film opacity was determined using standard operating procedures (BSI, 1968) modified by Gontard et al. (1992) and applied by others (Malli et al., 2004; Lopez et al., 2008). Film samples were cut to 1 × 3 cm and placed on the inside of a spectrophotometer cell (Shimadzu UV-2401-PC Kyoto, Japan) to record the absorbance spectrum between 400 nm and 700 nm. The opacity of the film is defined as the area under the curve and is expressed in units of absorbance x nanometers (nm.UA). The measurements were made in triplicate for each film.

2.11. Determination of film colour

The color of the film was determined by the method described by Bourtoom and Chinann (2008) and also by Pires et al. (2018). This determination was made using an international Datacolor spectrophotometer D65/10 (Miniscan XE Virginia, USA). L* represents the luminance (from white to black), (a*) the chromatic index from green to red and (b*) that from yellow to blue. From these data, the color difference (ΔE° ab) of starch-based composite films and films reinforced with MCC was calculated with respect to the color of a white reference surface according to the following expression (Eq. (4)):

$S (%) = \frac{|(P_0 - P_f)|}{P_0} \times 100$  

(3)

With S, solubility; P0, initial dry weight; Pf, final dry weight. The measurements were made in triplicate for each film.

2.9. Determination of the mechanical properties of films

Film opacity was determined using standard operating procedures (BSI, 1968) modified by Gontard et al. (1992) and applied by others (Malli et al., 2004; Lopez et al., 2008). Film samples were cut to 1 × 3 cm and placed on the inside of a spectrophotometer cell (Shimadzu UV-2401-PC Kyoto, Japan) to record the absorbance spectrum between 400 nm and 700 nm. The opacity of the film is defined as the area under the curve and is expressed in units of absorbance x nanometers (nm.UA). The measurements were made in triplicate for each film.

2.11. Determination of film colour

The color of the film was determined by the method described by Bourtoom and Chinann (2008) and also by Pires et al. (2018). This determination was made using an international Datacolor spectrophotometer D65/10 (Miniscan XE Virginia, USA). L* represents the luminance (from white to black), (a*) the chromatic index from green to red and (b*) that from yellow to blue. From these data, the color difference (ΔE° ab) of starch-based composite films and films reinforced with MCC was calculated with respect to the color of a white reference surface according to the following expression (Eq. (4)):

$S (%) = \frac{|(P_0 - P_f)|}{P_0} \times 100$  

(3)

With S, solubility; P0, initial dry weight; Pf, final dry weight. The measurements were made in triplicate for each film.

2.9. Determination of the mechanical properties of films

Film opacity was determined using standard operating procedures (BSI, 1968) modified by Gontard et al. (1992) and applied by others (Malli et al., 2004; Lopez et al., 2008). Film samples were cut to 1 × 3 cm and placed on the inside of a spectrophotometer cell (Shimadzu UV-2401-PC Kyoto, Japan) to record the absorbance spectrum between 400 nm and 700 nm. The opacity of the film is defined as the area under the curve and is expressed in units of absorbance x nanometers (nm.UA). The measurements were made in triplicate for each film.

2.11. Determination of film colour

The color of the film was determined by the method described by Bourtoom and Chinann (2008) and also by Pires et al. (2018). This determination was made using an international Datacolor spectrophotometer D65/10 (Miniscan XE Virginia, USA). L* represents the luminance (from white to black), (a*) the chromatic index from green to red and (b*) that from yellow to blue. From these data, the color difference (ΔE° ab) of starch-based composite films and films reinforced with MCC was calculated with respect to the color of a white reference surface according to the following expression (Eq. (4)):

$S (%) = \frac{|(P_0 - P_f)|}{P_0} \times 100$  

(3)

With S, solubility; P0, initial dry weight; Pf, final dry weight. The measurements were made in triplicate for each film.
\[ \Delta E = \sqrt{(L^2 - L_0^2)} + (a' - a_0') + (b' - b_0') \]  

(4)

2.12. Statistical analyses

Single-factor ANOVA variance analysis was performed on the mechanical, optical and barrier properties of starch-based films, reinforced with MCC at different rates, using STATISTICA 7.1 software. The DUNCAN test at a 5% threshold was applied to detect differences between sample sets. Student's t-test made it possible to compare the means obtained with the starches of the two improved varieties of cassava.

3. Results and discussion

3.1. Physical parameters of starches from improved cassava varieties

The physical parameters of the starches from Yavo and TMS, the improved cassava varieties used in this study are presented in Table 1. The highest amylose value was obtained with the TMS variety and the clearest starch gel was obtained with the Yavo variety. Starch with low amylose content has a high gel clarity. Amylose contents are known to influence the clarity of starch gels. A high amylose content can result in more opaque starch doughs (Schmitz et al., 2006). The maximum viscosity of Yavo and TMS starch gels as characterized by Brabender Visco-amylograph was 499 and 478 Brabender Analysis Units (BU) for Yavo and TMS respectively (Table 1). The final viscosity of the starch gel of the TMS variety (384 BU) was higher than that of the Yavo variety (377 BU). The difference between the viscosity at the end of cooling and the viscosity at the end of the set-back phase was 207 BU and 210 BU for Yavo and TMS, respectively. Thus for these two varieties, for a low maximum viscosity, the final viscosity and SetBack were high and for a high maximum viscosity, the final viscosity and SetBack were low. Such observations have been made by some authors (Song and Jane, 2000; Blazek and Copeland, 2008). According to these authors, a high amylose content would lead to a decrease in the maximum viscosity of starch gels, whereas the final viscosity would increase with the increase in amylose content. The gelatinization temperatures (start To, peak Tp and conclusion Tc) increased respectively for the starch of the two varieties Yavo and TMS as shown in Table 1. Enthalpy (AH) was 14.11 J/g and 13.33 J/g for Yavo and TMS respectively.

3.2. Moisture content and water solubility of reinforced cassava starch films

Table 2 shows the moisture content and water solubility values of MCC-reinforced cassava starch-based composite films. At 0 % MCC, the moisture content values of the films were 15.97 % and 14.24 % for Yavo and TMS, respectively. When 30 % MCC was added, the moisture content decreased to reach 11.43 % for Yavo and 10.66 % for TMS. As the MCC concentration increases, the moisture content decreases. Concerning the water solubility of the films, at 0 % the values were 24.84 % and 24.15 % respectively for Yavo and TMS. When the concentration went from 0 % to 30 %, solubility was reduced to 20.61 % for Yavo and 19.36 %. The analysis of variance showed that the MCC concentration significantly influenced the moisture content and solubility of cassava starch composite films (p < 0.05). The ANOVA analysis of variance showed a significant difference between the moisture content and water solubility of the Yavo and TMS starches films (p < 0.05). Student's T-test (t: -8.0207; dl: 4; P: 0.001) showed a significant difference (P < 0.05) between the Yavo and TMS varieties in the moisture content of 0 % MCC films. On the other hand, at 7, 15 and 30 % no significant difference was observed as indicated by the p-values (Table 2). Regarding the water solubility of the films, Student's T-test (t: -3.8045; dl: 4; P: 0.019) showed a significant difference (P < 0.05) between the Yavo and TMS varieties at 0 % MCC. As with the moisture content of the films, no significant difference was observed at 7, 15 and 30 % MCC. Yavo and TMS films reinforced with MCC had equal moisture content and solubility in water. The increase in MCC levels in cassava starch-based composite films has resulted in a significant decrease in the moisture content and water solubility of the films. These results showed that cellulose increases the crystallinity of composite films. This may be due to the more crystalline nature of cellulose as indicated by some authors (Amash and Zugenmaier, 2000; Ma et al., 2005). The crystalline cellulose in the film promotes interactions between the OH groups of the two polysaccharides. This allows the production of more crystalline zones that reduce solubility in water (Müller et al., 2009; Ma et al., 2008; Amash and Zugenmaier, 2000; De Azeredo, 2009). Cellulose is also less hydrophilic than starch owing to its compact microfibrillar arrangement and higher crystallinity, rendering it more hydrophobic (Curvelo et al., 2001; Bras et al., 2010). It has a stabilizing effect on the starched polymer matrix, mainly on amylose molecules that undergo reduced mobility (Ma et al., 2005). This observed decrease would also indicate an intermolecular interaction between starch and MCC in the starch composite film as it has been previously observed on cassava starch and carboxymethylcellulose films (Li et al., 2008; Tongdeesootorn et al., 2011). The intermolecular interaction between starch and MCC that has taken place leads to a reduction in water content and water solubility, which may be due to the reduction in starch concentration in the film matrix (Tongdeesootorn et al., 2011). The addition of 7 % MCC did not improve the water solubility of composite films. The fact that this concentration has not promoted a major increase in crystalline zones in composite films may be one explanatory factor (Ailla-Suárez et al., 2013). However, it may also be due to improper incorporation of the added MCC level into the matrix of the composite film.

3.3. Water vapour permeability of reinforced cassava starch films

The water vapour permeability values of MCC-reinforced cassava starch composite films are presented in Table 2. Values ranged from 1.98 × 10⁻¹¹ to 1.39 × 10⁻¹¹ (g Pa⁻¹ s⁻¹ m⁻¹) for Yavo at 0 % and 30 % respectively. For the TMS variety, water vapour permeability ranges from 1.93 × 10⁻¹¹ to 1.29 × 10⁻¹¹ (g Pa⁻¹ s⁻¹ m⁻¹) at 0 % and 30 % MCC, respectively. The variance analysis showed that the MCC content had an effect on the WVP of cassava starch composite films (p < 0.05). However, the ANOVA variance analysis did not show any significant difference between the WVP of the Yavo and TMS starches films (p > 0.05). Also, the

| MCC content (%) (w/w of starch) | Moisture content (%) | Solubility (%) | WVP (g Pa⁻¹ s⁻¹ m⁻¹) x10⁻¹¹ |
|---------------------------------|---------------------|--------------|------------------|
| Yavo                            | TMS                 | P value      | Yavo             | TMS             | P value      |
| 0                               | 15.97 ± 0.2a        | 14.24 ± 0.3ab| 24.84 ± 0.1aa    | 24.15 ± 0.3ab   | 0.001        | 1.98 ± 0.1ab  | 1.93 ± 0.1ab  | 0.438        |
| 7                               | 14.38 ± 0.3ac       | 13.55 ± 1.1abc| 24.71 ± 0.3ac   | 24.31 ± 0.3ac   | 0.353        | 2.13 ± 0.1bc  | 2.01 ± 0.1bc  | 0.071        |
| 15                              | 13.08 ± 0.2cd       | 12.22 ± 0.5ab| 22.42 ± 0.8bc   | 22.05 ± 0.8bc   | 0.059        | 1.93 ± 0.1ac  | 1.83 ± 0.1bc  | 0.173        |
| 30                              | 11.43 ± 0.2de       | 10.66 ± 0.9cd| 20.61 ± 0.9de   | 19.36 ± 0.8de   | 0.230        | 1.39 ± 0.1bc  | 1.29 ± 0.2bc  | 0.309        |

Values assigned different lowercase letters in the same column are significantly different at p < 0.05. Values assigned of different capital letters on the same line are significantly different at p < 0.05.
Student’s T-test showed no significant difference (P > 0.05) between the Yavo and TMS varieties at 0, 7, 15 and 30 % MCC as indicated by the p-values (Table 2). The low levels of MCC (7 and 15 %) incorporated in the starch of improved cassava varieties have not improved the WVP of the films. These results are similar to those obtained when 0, 4, 7 and 10 % MCC was added to starch in the study on the influence of MCC in blown thermoplastic starch and polyester films (Reis et al., 2017). However, in this study, the addition of MCC at 30 % in starch-based films resulted in a decrease in the WVP of the films. This decrease is related to the crystallinity of the cellulose. Cellulose has a higher crystallinity and a compact microfibrillar arrangement. The decrease in WVP after the addition of cellulose has been observed by some authors (Psomiadou et al., 1996; Alaia-Suárez et al., 2013). Their studies focused on corn starch and MCC films on the one hand and potato starch and chayote tuber films combined with cellulose nanoparticles on the other. Also, this improvement in WVP to 30 % would be related to the fact that the presence of high concentrations of MCC probably introduced a tortuous path, which made it more difficult for water to diffuse through the film matrix (Kristo and Biliaderis, 2007).

3.4. Mechanical properties of reinforced cassava starch films

The tensile strength, elongation at break and Young’s modulus values of MCC reinforced cassava starch composite films are presented in Table 3. The tensile strength values range between 7.15-10.99 MPa and between 7.77-13.18 MPa for the varieties Yavo and TMS, respectively. Young’s modulus also ranges between 331.29-1351.08 MPa and between 343.79-1476.08 MPa for the varieties Yavo and TMS, respectively. Elongation at break varies from 22.75 % to 1.31 % and from 21.25 % to 1.19 % for the Yavo and TMS varieties, respectively. The TMS variety provided the highest tensile strength and Young’s modulus values and the lowest elongation values compared to the values obtained with Yavo. The analysis of variance showed that the level of MCC added to cassava starch composite films had a significant effect on tensile strength, Young’s modulus, and elongation at break (p < 0.05). The 30 % MCC rate has led to more rigid and less flexible films. The analysis of variance showed a significant difference between the tensile strength, Young’s modulus, and elongation at break of cassava starch films reinforced by MCC (p < 0.05). This difference is at the level of 0, 7 and 30 % of MCC added. Thus, the elongation at break of cassava starch films reinforced by MCC (p < 0.05). This difference can be seen in Figure 1, with the films at 0 % MCC (a) and 30 % MCC (b) respectively. Also, Student’s T test at 0 % (t: 5.1996; df: 4; P: 0.006) and 7 % (t: 4.0159; df: 4; P: 0.016) showed a significant difference between Yavo and TMS films. Unlike 15 and 30 % where no significant difference was observed. The increased opacity of MCC-reinforced composite films may be related to the original color of the MCC. The opacity of the films increases with the increase in the added MCC rate. In addition, this increase would be due to the average particle size of MCC, which is much larger than the size of the interstitial space in a matrix film (Shi et al., 2013) and that, when light passes through these films in which MCC is present in high quantities, the amount of light transmitted by the film is much less (Coelho et al., 2017). The results of this study are similar to those of Moraes et al. (2014), who produced composite films based on cassava starch incorporated with cellulose fiber by the tape-casting method, and Coelho et al. (2017) who worked on the effect of moderate electric fields on the properties of MCC reinforced starch and chitosan films. The difference between the opacity of the Yavo and TMS films at 0 and 7 % MCC would be related to the base clarity of the starches. Yavo’s starch had shown a clearer gel than TMS’s.

3.5. Opacity of reinforced cassava starch films

Table 4 shows the opacity values of cassava starch composite films reinforced with MCC. At 0 % MCC, the opacity values were 223.91 nm.UA for the Yavo variety and 251.42 nm.UA for the TMS variety. After adding 7, 15 and 30 % MCC, opacity increased to 260.80; 334.90 and 425.33 nm.UA respectively for the Yavo variety. For the TMS variety, opacity increased to 279.21; 344.34 and 434.51 nm.UA respectively. The ANOVA analysis of variance showed a significant difference between the opacity of the Yavo and TMS starch films (p < 0.05). This difference can be seen in Figure 1, with the films at 0 % MCC (a) and 30 % MCC (b) respectively. Also, Student’s T test at 0 % (t: 5.1996; df: 4; P: 0.006) and 7 % (t: 4.0159; df: 4; P: 0.016) showed a significant difference between Yavo and TMS. Unlike 15 and 30 % where no significant difference was observed. The increased opacity of MCC-reinforced composite films may be related to the original color of the MCC. The opacity of the films increases with the increase in the added MCC rate. In addition, this increase would be due to the average particle size of MCC, which is much larger than the size of the interstitial space in a matrix film (Shi et al., 2013) and that, when light passes through these films in which MCC is present in high quantities, the amount of light transmitted by the film is much less (Coelho et al., 2017). The results of this study are similar to those of Moraes et al. (2014), who produced composite films based on cassava starch incorporated with cellulose fiber by the tape-casting method, and Coelho et al. (2017) who worked on the effect of moderate electric fields on the properties of MCC reinforced starch and chitosan films. The difference between the opacity of the Yavo and TMS films at 0 and 7 % MCC would be related to the base clarity of the starches. Yavo’s starch had shown a clearer gel than TMS’s.

Table 3. Tensile strength, elongation at break and Young’s modulus values of reinforced films.

| MCC content (%) (w/w of starch) | TS (MPa) | EB (%) | YM (MPa) |
|---------------------------------|---------|--------|---------|
|                                 | Yavo    | TMS    | P value | Yavo    | TMS    | P value | Yavo    | TMS    | P value |
| 0                               | 7.15 ± 0.6^{±} | 7.77 ± 0.4^{±} | 0.027   | 22.75 ± 2.34^{±} | 21.25 ± 2.61^{±} | 0.246   | 331.29 ± 93.8^{±} | 343.79 ± 95.9^{±} | 0.796   |
| 7                               | 8.19 ± 0.9^{±c} | 8.52 ± 0.7^{±b} | 0.475   | 19.23 ± 2.23^{±c} | 18.23 ± 1.96^{±b} | 0.360   | 563.84 ± 71.9^{±b} | 575.09 ± 70.8^{±c} | 0.756   |
| 15                              | 9.91 ± 0.7^{±c} | 10.04 ± 0.9^{±b} | 0.491   | 5.85 ± 1.43^{±c}  | 4.63 ± 1.01^{±c}  | 0.068   | 962.91 ± 45.9^{±c} | 980.15 ± 39.7^{±c} | 0.435   |
| 30                              | 10.99 ± 0.5^{±c} | 13.18 ± 0.5^{±b} | 0.003   | 1.31 ± 0.25^{±c}  | 1.19 ± 0.21^{±c}  | 0.309   | 1351.08 ± 28.9^{±c} | 1476.08 ± 73.8^{±c} | 0.001   |

MCC: Microcrystalline cellulose; TS: Tensile Strength; EB: Elongation at Break; YM: Young’s modulus. Values assigned different lowercase letters in the same column are significantly different at p < 0.05. Values assigned different capital letters on the same line are significantly different at p < 0.05.
3.6. Colors of reinforced starch films

Table 5 shows the color parameters of cassava starch films reinforced with MCC. Luminance values $L^*$ ranged from 92.20 to 93.57 and from 91.92 to 93.35 for the varieties Yavo and TMS, respectively. The values of the $b^*$ index ranged from 2.66 to 3.28 and from 2.47 to 2.88 for Yavo and TMS. The rate of MCC incorporated in cassava starch composite films had a significant effect (p < 0.05) on $L^*$ luminance, $b^*$ index and color difference $\Delta E^{*}_{ab}$. The increase in the MCC rate resulted in an increase in the luminance $L^*$, the $b^*$ index of the resulting films. The $a^*$ index did not really change with the increase in MCC rate for any variety and the color difference $\Delta E^{*}_{ab}$ varied with the change in the $b^*$ index. Compliant results were obtained when MCC was incorporated into the agar matrix (Shankar and Rhim, 2016) and starch and chitosan films (Coelho et al., 2017). The increase in the values $b^*$ and $L^*$ indicate that the added MCC has led to an intensification of yellowing of the starch film, possibly by the colour of MCC (Coelho et al., 2017).

4. Conclusion

This study highlighted the influence of microcrystalline cellulose on the properties of starch-based composite films of improved cassava varieties. MCC (0–30 %) has improved the properties of starch-based films of improved cassava varieties by increasing their strength and rigidity and reducing water vapour permeability and water content. The 30 % MCC added resulted in films that are much stiffer and less permeable to water vapour. MCC has been shown to be effective in improving starch-based films of improved varieties of cassava grown in Côte d’Ivoire. Also, the results obtained did not show a big difference between Yavo’s starch-based films and TMS. Thus, both varieties can be well used for film production with MCC. However, future analyses such as FTIR, DRX analyses will be considered to better understand the incorporation of low levels of MCC in the starch matrix of the improved cassava varieties in this study. The starch-based films of improved cassava varieties Yavo and TMS with MCC can be used as packaging materials for fruits and vegetables replacing the mainly used plastic packaging materials.

Declarations

Author contribution statement

Adjouman Yao Désiré: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Nindjin Charlemagne: Conceived and designed the experiments.

Table 4. Opacity of cassava starch-based films reinforced with MCC.

| MCC Rate (%) (w/w starch) | Opacity (nm.UA) |
|---------------------------|----------------|
|                           | Yavo           | TMS           | P value |
| 0                         | 223.91 ± 3.4A   | 251.42 ± 8.5B | 0.006   |
| 7                         | 260.82 ± 6.6B   | 279.21 ± 4.4C | 0.016   |
| 15                        | 334.90 ± 5.6C   | 344.34 ± 4.3D | 0.372   |
| 30                        | 425.33 ± 3.4D   | 434.51 ± 6.5D | 0.133   |

Values assigned different lowercase letters in the same column are significantly different at p < 0.05. Values assigned of different capital letters on the same line are significantly different at p < 0.05.

Table 5. Values of the color parameters of starch-based films reinforced with MCC.

| Taux de CMC (%) | Yavo          | TMS           |
|-----------------|---------------|---------------|
|                 | $L^*$         | $a^*$         | $b^*$ | $\Delta E^{*}_{ab}$ | $L^*$         | $a^*$         | $b^*$ | $\Delta E^{*}_{ab}$ |
| 0               | 92.20 ± 0.2A  | -0.37 ± 0.1A  | 2.66 ± 0.1A | 2.86 ± 0.1A | 91.92 ± 0.2A  | -0.44 ± 0.1A  | 2.47 ± 0.1A | 2.84 ± 0.1A |
| 7               | 92.70 ± 0.1B  | -0.32 ± 0.2B  | 2.86 ± 0.2B | 2.87 ± 0.2B | 92.62 ± 0.2B  | -0.38 ± 0.1B  | 2.63 ± 0.1B | 2.67 ± 0.2B |
| 15              | 93.18 ± 0.1C  | -0.41 ± 0.1C  | 3.25 ± 0.1C | 3.15 ± 0.1C | 93.09 ± 0.1C  | -0.38 ± 0.1C  | 2.78 ± 0.2C | 2.71 ± 0.2C |
| 30              | 93.57 ± 0.2D  | -0.34 ± 0.1D  | 3.28 ± 0.1D | 3.17 ± 0.1D | 93.35 ± 0.1D  | -0.40 ± 0.1D  | 2.88 ± 0.1D | 2.77 ± 0.1D |

Values assigned different lowercase letters in the same column are significantly different at p < 0.05. Values assigned of different capital letters on the same line are significantly different at p < 0.05.

3.6. Colors of reinforced starch films

The table shows the color parameters of cassava starch films reinforced by the MCC. Luminance values $L^*$ ranged from 92.20 to 93.57 and from 91.92 to 93.35 for the varieties Yavo and TMS, respectively. The values of the $b^*$ index ranged from 2.66 to 3.28 and from 2.47 to 2.88 for Yavo and TMS. The rate of MCC incorporated in cassava starch composite films had a significant effect (p < 0.05) on $L^*$ luminance, $b^*$ index and color difference $\Delta E^{*}_{ab}$. The increase in the MCC rate resulted in an increase in the luminance $L^*$, the $b^*$ index of the resulting films. The $a^*$ index did not really change with the increase in MCC rate for any variety and the color difference $\Delta E^{*}_{ab}$ varied with the change in the $b^*$ index. Compliant results were obtained when MCC was incorporated into the agar matrix (Shankar and Rhim, 2016) and starch and chitosan films (Coelho et al., 2017). The increase in the values $b^*$ and $L^*$ indicate that the added MCC has led to an intensification of yellowing of the starch film, possibly by the colour of MCC (Coelho et al., 2017).

4. Conclusion

This study highlighted the influence of microcrystalline cellulose on the properties of starch-based composite films of improved cassava varieties. MCC (0–30 %) has improved the properties of starch-based films of improved cassava varieties by increasing their strength and rigidity and reducing water vapour permeability and water content. The 30 % MCC added resulted in films that are much stiffer and less permeable to water vapour. MCC has been shown to be effective in improving starch-based films of improved varieties of cassava grown in Côte d’Ivoire. Also, the results obtained did not show a big difference between Yavo's starch-based films and TMS. Thus, both varieties can be well used for film production with MCC. However, future analyses such as FTIR, DRX analyses will be considered to better understand the incorporation of low levels of MCC in the starch matrix of the improved cassava varieties in this study. The starch-based films of improved cassava varieties Yavo and TMS with MCC can be used as packaging materials for fruits and vegetables replacing the mainly used plastic packaging materials.

Declarations

Author contribution statement

Adjouman Yao Désiré: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Nindjin Charlemagne: Conceived and designed the experiments.
Koudiao Degbeu Claver: Analyzed and interpreted the data.
Tetchi Fabrice Achille, Sindj Mbarian: Contributed reagents, materials, analysis tools or data.

Funding statement
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement
Data included in article supplementary material/referenced in article.

Declaration of interests statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

Acknowledgements
The authors would like to thank Thomas Bertrand for all the technical support in the laboratory. They also would like to thank Jasmina Saric from the Project and Grant Service Unit of the Swiss Tropical and Public Health Institute for revision of the manuscript.

References
Adjeunam, Y.D., Nindjin, C., Tetchi, F.A., Dalqc, A.C., Amani, N.G., Sindj, M., 2017. Water vapor permeability of edible films based on improved cassava (Manihot esculenta Crantz) native starches. J. Food Process. Technol. 8, 665.
Adjeunam, Y.D., Nindjin, C., Kouakou, N.K., Tetchi, F.A., Amani, N.G., Sindj, M., 2018a. Effect of edible coating based on improved cassava starch on post-harvest quality of fresh tomatoes (Solanum lycopersicum L.). Int. J. Nutr. Sci. Food Tech. 4, 1–10.
Adjeunam, Y.D., Nindjin, C., Konan, R.B., Coulibaly, S., Amani, N.G., Sindj, M., Tetchi, F.A., 2018b. Effect of glycerol, peanut oil and soybean lecithin contents on the properties of biodegradable film of improved cassava starches from Côte d’Ivoire. Int. J. Environ. Agr. Biotech. 3, 1432–1446.
Aïla-Suarez, S., Palma-Rodriguez, H.M., Rodriguez-Hernández, A.I., Hernández-Uribe, J.P., Bello-Pérez, L.A., Vargas-Torres, A., 2013. Characterization of films made with chayote tuber and potato starches blending with cellulose nanospheres. Carbohydr. Polym. 98 (1), 102–107.
Almasi, H., Ghanbarzadeh, B., Entezami, A.A., 2010. Physical properties of edible modified starch/carboxymethyl cellulose films. Innov. Food Sci. Emerg. 11, 697–702.
Gontard, N., Goulbert, S., Coj, J.L., 1992. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. J. Food Sci. 57, 190–195.
Koudajo, K.K.H., Kitt, D.J.B., Bekayoko, S., Soro, D., Girardin, O., 2014. Variabilité physico-morphologique des racines tubéreuses de manioc (Manihot esculenta Crantz) cultivées sur ferralsol en zone de forêt d’Afrique de l’Ouest. J. Appl. Biol. 82, 7316–7325.
Kristo, E., Biliaderis, C.G., 2007. Physical properties of starch nanocrystal-reinforced pullulan films. Carbohydr. Polym. 68 (1), 146–158.
Li, Y., Shoemaker, C.F., Ma, J., Shen, X., Zhang, F., 2008. Paste viscosity of rice starches of different amylose content and carboxymethylcellulose formed by dry heating and the physical properties of their films. Food Chem. 109, 616–623.
Lopez, O.V., Garcia, M.A., Zaritzky, N.E., 2008. Film forming capacity of chemically modified corn starches. Carbohydr. Polym. 73, 573–580.
Ma, X., Yu, J., Kennedy, J.F., 2005. Studies on the properties of natural fibers-reinforced thermoplastic starch composites. Carbohydr. Polym. 62 (1), 19–24.
Ma, X., Chang, P.R., Yu, J., 2008. Properties of biodegradable thermoplastic starch/carboxymethyl cellulose and starch/microcellulose composite films. Carbohydr. Polym. 72 (3), 369–375.
Mali, S., Grossmann, M.V.E., Garcia, M.A., Martino, M.N., Zaritzky, N.E., 2004. Barrier, mechanical and optical properties of plasticized yam starch films. Carbohydr. Polym. 56, 129–135.
Malumbo, P., Massaux, C., Deroanne, C., Masimango, T., Béra, F., 2009. Influence of drying temperature on functional properties of wet-milled starch granules. Carbohydr. Polym. 75, 299–306.
Moraes, J.O., Schelbe, A.S., Sereno, A., Laurindo, J.B., 2014. Scale-up of the production of cassava based starch films using tape-casting. J. Food Eng. 119, 800–808.
Morrison, W.R., Laineget, B., 1983. An improved colorimetric procedure for determining total protein and total amylase in cereal and other starches. J. Cereal. Sci. 1, 9–20.
Müller, C.M.O., Laurindo, J.B., Yamashita, F., 2009. Effect of cellulose fibers addition on the mechanical properties and water vapor barrier of starch-based films. Food Hydrocolloids 23, 1328–1333.
Nindjin, C., Beyrer, M., Amani, G.N., 2015. Effects of sucrose and vegetable oil on properties of native cassava (Manihot esculenta Crantz) starch-based edible films. Afr. J. Food Agric. Nutr. Dev. 15, 9905–9921.
N’Zue, B., Dje, D., Ndabasherbye, J., Lobozie, G.P., 2003. Étude statistique sur la promotion de la filière manioc en Côte d’Ivoire. Rapport technique. Contrat cnra/ note d’Ivoire, p. 20.
Panaitescu, D.M., Frone, A.N., Ghiurea, M., Chiulan, I., 2015. Influence of bioactive components on food properties. Ind. Crop. Prod. 70, 170–177.
Pires, J.F.A., do Souza, V.G.A., Ceremundo, A.L., 2018. Chitin/monomorillonite bionanocomposites incorporated with rosemary and ginger essential oil as packaging for fresh poultry meat. Food packag. shelf life 142, 142–149.
Psomiadou, E., Arvanitoyannis, I., Yamamoto, N., 1996. Edible films made from natural resources: microcrystalline cellulose (MCC), methylcellulose (MC) and corn starch and polysols-Part 2. Carbohydr. Polym. 31, 193–204.
Reis, M.O., Olivato, J.B., Zanela, J., Yamashita, F., Grossmann, M.V.E., 2017. Influence of microcrystalline cellulose in thermoplastic starch/polyester blown films. Polimerores 12 (2), 129–135.
Rico, M., Rodríguez-Llamazares, S., Barral, L., Bouza, R., Montero, B., 2016. Processing and characterization of polys laminated-starch reinforced with microcrystalline cellulose. Carbohydr. Polym. 149, 83–93.
Schmitz, C.S., de Simas, K.N., Joao, J.J., de Mello Castanho Amboni, R.D., Amante, E.R., 2006. Cassava starch functional properties by etherification-hydroxypropylation. Int. J. Food Sci. Technol. 41, 681–687.
Shan, S., Shin, J.W., 2016. Preparation of nanocellulose from micro-crystalline cellulose: the effect on the performance and properties of agar-based composite films. Carbohydr. Polym. 135, 18–26.
Shi, A.M., Wang, L.J., Li, D., Adhikari, B., 2013. Characterization of starch films containing starch nanoparticles Part 1: physical and mechanical properties. Carbohydr. Polym. 96 (2), 593–601.

Song, Y., Jane, J.L., 2000. Characterization of barley starch of waxy, normal, and high amylose varieties. Carbohydr. Polym. 41 (4), 365–377.

Syafri, E., Kasim, A., Asben, A., Senthamaarakkannu, P., Sanjay, M.R., 2018a. Studies on Ramie cellulose microfibrils reinforced cassava starch composite: influence of microfibrils loading. J. Nat. Fibers 17, 122–131.

Syafri, E., Kasim, A., Abral, H., Sulungbudi, G.T., Sanjay, M.R., Sari, N.H., 2018b. Synthesis and characterization of cellulose nanofibers (CNF) ramie reinforced cassava starch hybrid composites. Int. J. Biol. Macromol. 120, 578–586.

Tongdeesoontorn, W., Mauer, L.J., Wongruong, S., Sriburi, P., Rachatanapun, P., 2011. Effect of carboxymethyl cellulose concentration on physical properties of biodegradable cassava starch-based films. Chem. Cent. J. 5 (1), 1–8.

Versino, F., Garcia, M.A., 2014. Cassava (Manihot esculenta Crantz) starch films reinforced with natural fibrous filler. Ind. Crop. Prod. 58, 305–314.