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# Characteristics and Biodegradability of Oxidized Starch Bioplastics from Agricultural Biomass

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**ABSTRACT**

*Conventional petrochemical plastics are mechanically and chemically stable but are often not biodegradable. What's more, depending on their synthetic chemical composition, these plastics can also pose a health risk by contaminating trophic and food chains. Plastics based on biodegradable biopolymers are an alternative to non-biodegradable fossil plastics. To improve bioplastics derived from local agrobiomass, two starch stocks were extracted and purified from cassava tubers. One was chemically oxidized and the other non-oxidized. Plastic biofilms were synthesized from these two stocks. Physicochemical properties such as thickness, density, water content, water uptake, opacity and water solubility of these biofilms were studied. SEM analysis coupled with an energy dispersive X-ray spectrometer was used to determine the morphology and chemical composition of the biofilms. Biodegradability was assessed by burial in soil. The total starch oxidation rate was approximately 5%. Starch oxidation resulted in a significant decrease in biofilm solubility from 16.496% ± 0.372 to 1.428% ± 0.718. However, the process resulted in a 0.502 unit increase in biofilm density due to starch oxidation. Other physicochemical properties showed that these biofilms are suitable for a wide range of applications. The biofilm obtained after starch modification is less biodegradable.*

**Keywords:** *Biopolymer, Oxidation, Bioplastics, Biofilm, Biodegradation*

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## INTRODUCTION

Population growth in African cities has been accompanied by an increase in solid waste, including petrochemical plastic waste. Common plastics are typically polymers that are derived from fossil fuels, and most of them are not biodegradable (Ross *et al*., 2017). The non-biodegradable nature of petrochemical plastics gives them a long-life cycle. When they are released into the environment after use, their waste remains persistent, posing a real health and environmental problem. The proliferation of plastic waste impacts the quality of ecosystems, and harms human health as well as that of wild and domestic animals. The incineration of plastic waste may enable energy recovery but is likely to increase net $CO_2$ emissions and generate huge quantities of ash and slag containing dangerous and toxic compounds (Ali *et al*., 2023). In Côte d'Ivoire, few data are available on the extent of environmental pollution by plastic waste. However, Jambeck *et al*. (2018) estimate that 100,000 tons of plastic waste are poorly managed, i.e. 0.80 kg/person/day, and predict that there will be 500,000 tons by 2025. This plastic waste is discarded unsorted and ends up in sewage channels, landfills, bays, lagoons and marine waters (Koumi *et al*., 2021). According to Koumi *et al*. (2021), the capital Abidjan produces over 288 tons of plastic waste a day, only 5% of which is recycled. This has led the political authorities to restrict the import, marketing and use of petrochemical plastic packaging, while encouraging the use of biodegradable packaging (law n°2013-327 of May 22, 2013). Biodegradable plastics can be obtained from natural biological resources and are known by the terminology bioplastics (Ross *et al*., 2017). Biodegradable bioplastics represent a real alternative to petrochemical plastics and can be obtained from local bioresources. In general, bioplastics have a lower carbon footprint than petrochemical plastics (Palencia *et al*., 2021). This could solve the problem of the cost-availability mismatch of plastic packaging and help limit the amount of plastic waste in the environment. Bioplastics can be synthesized from agropolymers such as polysaccharides and proteins (Martins *et al*., 2022; Perez-Puyana *et al*., 2024), from substances produced by micro-organisms, or from polymers such as polylactic acid (PLA), polybutylene succinate (PBS) and polyhydroxyalkanoate (PHA) (Ross *et al*., 2017). In recent years, research on biodegradable bioplastics has progressed considerably, from the individual use of different biopolymers (Faizan Muneer *et al*., 2021; Kawaguchi *et al*., 2022; Khouri *et al*., 2024) to the production of composite bioplastics (Montoya-Anaya *et al*., 2023; Noviagel *et al*., 2024) or bioplastics derived from microorganisms (Six *et al*., 2024). This research involves bioplastics using a conventional or modified bioresource, or in composite form, through a blend of conventional and/or modified bioresources (Ross *et al*., 2017; Pérez-Pacheco *et al*.,

2024). Despite this vitality of research, biodegradable plastic materials are still inaccessible to many sections of the population in many developing countries, due to their high cost compared with their petrochemical counterparts. In such a context, starch, with its high polysaccharide content (de Andrade *et al*., 2022) its wide availability in Côte d'Ivoire and its low production cost, appears to be a promising biopolymer for the development of biodegradable bioplastics. However, starch alone is rarely used as a bioresource in the bioplastics production process, due to its hydrophilic nature and the low physicochemical properties of the resulting plastic (Pérez-Pacheco *et al*., 2024). Processes such as the hydroxylation (Colivet & Carvalho, 2017) and acetylation (Bai *et al*., 2024) of starch by substituting ester or ether groups for hydroxyls is an effective way of improving the processing and mechanical properties of the product. Because of its multi-purpose nature, bioplastic must not only be able to degrade rapidly, but also have good physicochemical properties. Polysaccharide bioplastics contain numerous reactive groups such as hydroxyl, amino and carboxyl groups. These groups form a dense network of hydrogen bonds and are a limiting factor in their processing and forming capabilities (Wang *et al*., 2024). Modifying the polysaccharide at the beginning of the synthesis process can improve the quality of the resulting biofilm. The literature reports that additives such as titanium dioxide (Noviagel *et al*., 2024) and sulfuric acid (Roy *et al*., 2020) are sometimes added during the elaboration process of certain bioplastics. The result of these additives is a bioplastic with improved properties. The present study aims to contribute to the improvement of some physico-chemical properties of oxidized starch-based bioplastics, while seeking to guarantee good biodegradability. Two types of bioplastics will be developed from native starch extracted from a local cassava variety and then oxidized. The biodegradability of these bioplastics will be assessed through soil burial tests carried out under controlled conditions.

## MATERIALS AND METHODS

*Native starch extraction*

Starch was extracted from cassava tubers of the species TMS 30572 (Manihot esculenta crantzmanioc TMS 30572) (Akpingny *et al*., 2017), available throughout Côte d'Ivoire. Cassava tubers were skinned, cut into small pieces, and carefully washed in distilled water. The washed pieces were then ground in a laboratory grinder (Silver Crest 5500, France). The resulting grindings were diluted in distilled water to obtain a paste-like solution, the supernatant of which was filtered through a 100 µm mesh sieve. The filtrate obtained is left to settle for 2 h at room temperature. The supernatant is again carefully collected, and the residual pasty matrix representing the crude starch is purified using the method described by Zoungranan *et al*. (2020). After purification, the final residue obtained represents the starch required for the rest of the study. This native starch is oven-dried at 40° for 72 h.

*Starch oxidation*

For the oxidation process, a 100 g mass of native starch was placed in a 1 L Erlenmeyer flask. A 500 mL volume of 0.5 M potassium dichromate ($K_2Cr_2O_7$) was then added, and the pH was adjusted to 7. The Erlenmeyer flask is covered with aluminum foil to reduce the effects of photolysis, and then placed on a vibrating shaker (Heidolph Vibramax 100, Germany) for shaking at 750 rpm, for 24 hrs. The supernatant is removed, and the residue is washed twenty times with distilled water by quenching followed by decantation. The washed residue, which represents the oxidized starch, is oven-dried at 40°C for 72 hours.

*Yield and moisture content*

Starch yields were determined using the following formul

$$Yield\ (\%) = \frac{starch\ mass}{mass\ of\ ground\ cassava} \times 100 \qquad (1)$$

To determine starch moisture content, a sample mass $m_1$ is weighed into a crucible of known mass, and then placed in an oven at 105°C for 24 hours. After cooling to laboratory temperature, the mass $m_2$ of the sample is determined by subtracting the mass of the crucible from that of the whole (crucible + sample). The humidity H (%) is determined using the following equation:

$$H(\%) = \frac{m_1 - m_2}{m_1} \times 100 \qquad (2)$$

With

- $m_1$ (g): The initial sample mass
- $m_2$ (g): The oven-dried sample mass (g)

*Carboxyl and carbonyl oxidation rate determination*

The method described by (Smith, 1967) and (Chattopadhyay *et al*., 1997) with some modifications was used to determine the rate of carbonyl and carboxyl in oxidized starch. To determine the carboxyl content, 1 g of oxidized starch was dispersed in a beaker containing 50 mL of distilled water, then heated in a water bath at 80°C with manual stirring, until the onset of gelatinization. After cooling, the solution is titrated with NaOH solution (0.1 M) to the phenolphthalein point. The experiment is repeated under the same conditions, with 1 g native starch in a 50 mL beaker to serve as a baseline. The carboxyl content of the oxidized starch is given by Eq. 3:

$$\%COOH = \frac{0.045 \times C \times (V_{OX} - V_{NA})}{m} \times 100 \qquad (3)$$

With

- $V_{OX}$ (mL): NaOH volume required to achieve oxidized starch conversion.
- $V_{NA}$ (mL): NaOH volume required to achieve conversion with native starch
- m (g): mass of starch (native or oxidized)
- C (mol/L): NaOH solution concentration

To determine the carboxyl content, a hydroxylamine solution was first prepared in a volumetric flask by dissolving 7 g of hydroxylamine chloride in water, followed by the addition of 100 mL of 0.5 M NaOH solution. The mixture is then made up to 500 mL with distilled water. In a beaker containing 50 mL of distilled water, 0.5 g of oxidized starch was dispersed, and the mixture was heated to 80°C in a water bath for 30 minutes, with

stirring, until gelatinization began. After cooling, the pH was adjusted to 3.8 with 0.1 M HCl. A 15 mL volume of hydroxylamine chloride solution was then added, and the beaker was covered with plastic film and left to stand for 2 hours. The mixture is then rapidly titrated with HCl solution (0.1M) to pH= 3.8.

$$\%CO = \frac{0.028 \times C \times (V_{OX} - V_{NA})}{m} \times 100 \quad (4)$$

With

- $V_{OX}$ (mL): HCl volume required to return to pH = 3.8 with oxidized starch,
- $V_{NA}$ (mL): HCl volume required to return to pH = 3.8 with native starch,
- m (g): the mass of starch,
- C (mol/L): HCl concentration

*Bioplastics processing*

The method used to produce bioplastics is based on that described by (Amin *et al*., 2019). For each type of starch (native or oxidized), a mass of 2.5 g is placed in a 250 mL beaker. A volume of 20 mL distilled water is added, then successively 2 mL glycerol and 3 mL HCl (0.1 M) are added. The mixture is stirred with a glass rod for 10 min, then heated to 80°C in a water bath, while maintaining stirring until a colloidal solution form that is difficult to stir. 3 mL NaOH (0.1 M) is added to reduce the viscosity of the mixture. The alcohol derived from the fermentable sugars in the starch reacts with hydrochloric acid to form an alkyl halide. The addition of NaOH, after the removal of NaCl, leads to the formation of a less viscous unsaturated compound, which in turn reacts with another monomer unit to form the bioplastic. The beaker is removed from the water bath and its contents spread out hot, without a spreader bar, onto a glass plate for cooling. The glass plate is then placed in an oven at 40°C for 24 hours. The plastic film is carefully removed from the glass plate when cold.

*Biofilms thickness and density*

Each type of bioplastic film obtained was peeled off the glass plate and cut into 1.5 cm × 1.5 cm pieces. For each type of bioplastic, the thickness X of the biofilm was measured using a Horex D-NR vernier caliper 0404124171 (Germany). Measurements were taken at different points on each biofilm. The thickness of each biofilm was taken as the arithmetic mean of these measurements. The mass and dimensions of the biofilms obtained were used to calculate the density ρ (g/cm$^3$) of the sample.

$$\rho = \frac{m}{S \times X} \quad (5)$$

Where

- ρ (g/cm$^3$) biofilm density
- m (g): the biofilm mass
- S (cm$^2$): surface area of the biofilm
- X (cm): biofilm thickness

*Biofilms humidity*

To determine the moisture content of bioplastics, samples are dried in an oven at 105°C for 12 hours. Moisture content is determined using Eq. 2.

*Biofilms opacity*

A UV-visible photospectrometer (WFJ-752, China) was used to measure the opacity of the bioplastic film. For each type of bioplastic, a 1.5 cm x 1.5 cm piece was carefully placed in the spectrophotometer cuvette for optical density reading at 600 nm. The measurement is carried out in triplicate, and opacity is determined by:

$$Opacity\ (\%) = \frac{A_{600nm}}{X} \times 100 \quad (6)$$

With

- $A_{600}$nm: absorbance at 600 nm
- X: biofilm thickness (mm)

*Bioplastics water absorption*

The dry mass (ms) of bioplastics was determined after 3 h drying at 105°C in the oven. After cooling, the bioplastics were immersed in water for 24 h. To determine the mass (msat) after soaking in water. The water absorption capacity or swelling was determined as follows:

$$\text{Water Absorption}\ (\%) = \frac{m_{sat} - m_s}{m_{sat}} \times 100 \quad (7)$$

Where

- $m_s$ (g): the dry biofilm mass of
- $m_{sat}$(g): the mass after biofilm immersion in water

*Biofilms water solubility*

The 1.5 cm x 1.5 cm bioplastic film, mass m, is oven-dried for 3 h, then immersed in a beaker containing a volume V= 50 mL of distilled water. A vibrating shaker (Heidolph Vibramax 100, Germany) is used to stir the solution for 24 h at 450 rpm. The solution is filtered through a Whatman No. 4 filter of known initial mass $m_0$. The residual solid is removed, and the filter paper is dried for 3 hours. After cooling, the mass $m_1$ is recorded. The experiment was carried out in triplicate and the percentage solubility was determined as follows:

$$Solubility\ (\%) = \frac{m_1 - m_0}{m} \times 100 \quad (8)$$

With

- $m_0$ (g): mass of dry filter paper
- $m_1$ (g): is the mass of filter paper after supernatant filtration
- m (g): the biofilm (1.5 cm x 1.5 cm) mass

*SEM – EDS analysis*

The morphology and chemical composition of the processed plastics were analyzed using a Hiro SH 400 M SEM (Limonest, France) coupled to a Bruker QUANTAX EDS microprobe using a 6/30 X Flash detector.

*Biodegradation estimation*

The soil used for burial was taken from the botanical forest of Nangui Abrogoua University (Côte d'Ivoire). To determine soil moisture content, an initial mass ($m_1$) of sieved soil was dried at 105°C in the oven for 48 hours to obtain a constant final mass ($m_2$). The moisture content is given by Eq. 2. The water retention capacity of the raw soil was determined by Eq. 7. The pH of the raw soil was measured by the Forster (1995) method using a pH meter (Hanna HI 9813-5, Bucharest, Romania). Biodegradability tests by burial were carried out in a sterile control soil and a soil enriched with microorganisms. Both types of soil, with a particle size of 2 mm, were placed in polyethylene bottles beforehand. The sterile soil was obtained by calcining the raw soil in a muffle furnace (Nabertherm GmbH, Bremen, Germany) at 500°C for 4 hours. The soil was enriched with microorganisms using sheep dung collected from a nearby farm. The dung was dried at room temperature for 72 h, then sieved to obtain a dry dung with a grain size of 2 mm or less.

In 30 g of each type of matrix, a mass m of bioplastic was buried for 15 days. The bioplastic is wrapped in a fine plastic net with a mesh diameter of 200 µm and buried in the soil. The mass of the net was previously measured using a balance (Ohaus EX124, Guangzhou, China). After 72 h, the bioplastic was carefully removed with the net, and then dried for 1 h in an oven at 40° C. The assembly is weighed again. Subtracting the empty mass of the net gives the residual bioplastic mass. For each fixed factor, there are three times as many vials as the number of days defined for the study. This ensures three individual measurements and no disruption to the biodegradation process. Every 72 hours, the sample is taken and oven-dried for 30 minutes at 105°C to determine the mass m'. These different masses are taken using an electronic balance with a precision of 0.1 mg. Bioplastic biodegradation is estimated according to the equation: The biodegradation rate of the bioplastic is determined according to the equation:

$$Biodegradation\ (\%) = \frac{m - m'}{m} \times 100 \qquad (9)$$

With

- m: biofim mass before landfilling
- m': biofilm mass after burial

*Data processing*

All calculations were performed with Microsoft Office Excel 2016 Professional. Mean values and standard deviation were determined from three individual measurements.

## RESULTS AND DISCUSSION

*Starch oxidation rate*

The starch extraction and oxidation process resulted in a yield slightly above one-quarter with a moisture content of less than 15%. The total oxidation rate of oxidized starch is relatively low at about 5%, with a predominance of carboxyl groups **(Table 1)**.

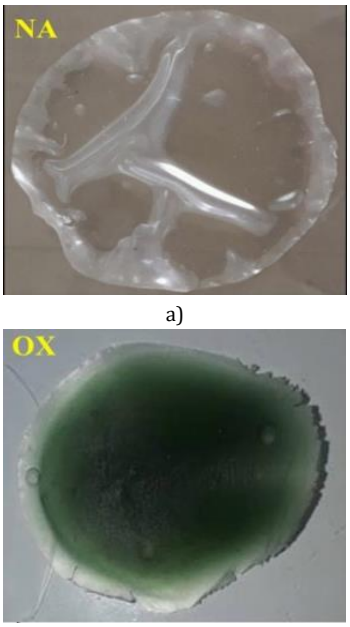
**Table 1.** Starch properties

| | Yield (%) | Moisture (%) | %CO | %COO |
|---|---|---|---|---|
| Native starch | 28.182 ± 0.012 | 14.774 ± 0.102 | - | - |
| Oxidized starch | 25.423 ± 0.023 | 12.156 ± 0.203 | 1.904 ± 0.069 | 3.015 ± 0.017 |

Starch is composed primarily of two polysaccharides, amylopectin and amylose. Unlike amylose, amylopectin is highly branched and accounts for 70-80% of starch. The relative dominance of the carboxyl group may be related to the predominance of amylopectin, whose more abundant primary alcohol functions contribute to the acidification of the mixture by reaction with potassium dichromate. This acidification induces acid hydrolysis (Gonzalez & Wang, 2023) of the starch, leading to its degradation and limiting the carbonyl content. According to Dias *et al.* (2011), who found similar values, pH, temperature and oxidant concentration had no significant effect on these different values. These authors also noted that modification of the starch during the process, the use of a catalyst, or even the acidic and basic nature of the reaction medium influence the carbonyl and carboxyl contents.

*Biofilms appearance and specific features*

The two types of biofilms obtained were easily demolded. These are biofilms based on native starch (NA) and biofilms based on oxidized starch (OX). **Figure 1** shows the visual appearance of the two types of bioplastic films obtained. The NA biofilm appears colorless, while the OX biofilm has a greenish appearance. This greenish appearance appears during the synthesis process, when the viscosity of the reaction mixture is reduced by adding HCl after the action of NaOH. It reflects the residuals acid solubilization of chromium III ions.




a)

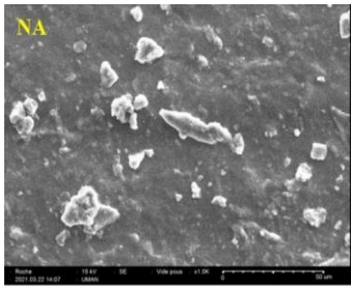
b)

**Figure 1.** Visual appearance of study biofilms

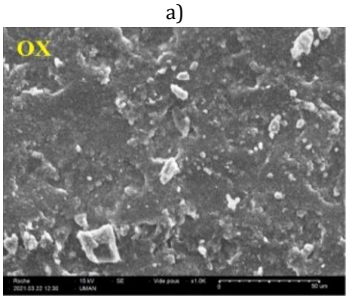
*Microstructure of the surface*

The surfaces of the NA and OX films **(Figure 2)** are irregular in appearance with the presence of abundant micro-residues depending on the type of starch. These residues indicate that the starch was not completely gelatinized during the bioplastic production process. These micro-residues on the surface result

from the swelling of certain insoluble granules during the plasticization phase (Amin *et al*., 2019) . According to Thakur *et al.* (2019), if swelling and solubilization are incomplete, there will be a decrease in viscosity which may affect network shrinkage and therefore film crystallinity in subsequent stages. An increase in surface roughness can be observed when moving from the NA film to the OX film, which has a more amorphous structure (Nguyen *et al*., 2021; Febrianti *et al*., 2023; Lobach *et al*., 2023; Puzovic *et al*., 2023).




a)

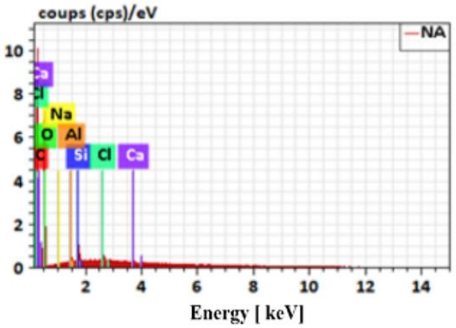



b)

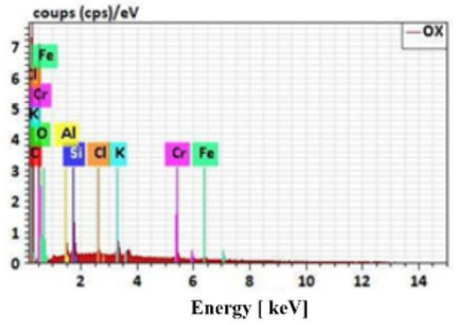
**Figure 2.** SEM of biofilms at 50 µm using Hirox SH 4000 M.

*Biofilms compositions*

The results of the chemical composition analysis of bioplastic films using the Flash 6/30 X-ray detector on Bruker's QUANTAX EDS microprobe are shown in **Figure 3**. As we move from NA to OX, the intensity of the X-rays emitted, in many shots per second, decreases. The element mapping clearly shows the presence of residual chromium and potassium in the OX biofilms, after multiple washing of the starch after oxidation.




a)




b)

**Figure 3.** Biofilms NA and OX composition spectrum, using QUANTAX EDS_X Flash 6/30 Bruker

*Biofilms physicochemical characteristics*

According to Dularia *et al.* (2019), amylose has a long, linear backbone with a crystalline motif that favors the formation of solid films due to strong network interactions. In contrast, amylopectin, with its short, branched chains, causes weak intermolecular bonding and imparts brittle properties to the resulting film. Starch oxidation could therefore improve the properties of the resulting film by reducing amylopectin to a greater extent. The results of the evaluation of specific film properties such as thickness, moisture content, water holding capacity, opacity, solubility and density are shown in the **Table 2** (Mai *et al*., 2021; Makhdoom *et al*., 2021; Hoang *et al*., 2022; Shahmars & Valiev, 2022).

**Table 2**. Physicochemical characteristics studied

| | Thickness (mm) | Moisture (%) | Water retention (%) | Opacity ($mm^{-1}$) | Solubility (%) | Density |
|---|---|---|---|---|---|---|
| NA | 0,064 ± 0,005 | 17,708 ± 0,591 | 19,769 ± 1,824 | 1,864 ± 0,372 | 16,496 ± 0,372 | 0,688 ± 0,011 |
| OX | 0,156 ± 0,012 | 13,266 ± 0,716 | 9,206 ± 0,736 | 7,062 ± 0,718 | 1,428 ± 0,718 | 1,190 ± 0,013 |

The OX films appear thicker, less moist, denser and less water soluble. These films are also opaquer and have a higher water-holding capacity. Therefore, the water holding capacity increased with starch oxidation. Oxidation of native starch significantly reduced the solubility and water content of biofilms and increased their density and thickness. This improvement may contribute to an increase in the mechanical strength (Oluwasina *et al*., 2019) of OX-type biofilms. Oxidation of native starch reduced its solubility from 16.496% ± 0.372 to 1.428% ± 0.718. This is an indication of a low rate of release of chromium, an essential trace element, which is present at low levels in some foods (SFPA-SFEM, 2020). This decrease in solubility could be explained by the lower gelatinization temperature of oxidized starch (Romero-Bastida *et al*., 2005). The earlier gelatinization process of OX-starch lasts longer. Oxidation significantly reduces the solubility of the resulting biofilms. The study showed that NA biofilms had a higher water absorption rate, which could be explained by examining their surface microstructure. The surface of OX biofilms has more granules that are likely to retain water. Opacity results show that OX biofilms are the opaquest. This may be related to the formation of chromium chloride when HCl is added. In small amounts, chromium chloride is an essential trace element for the human body. This opacity may also be due to the formation

of cross-linked starch molecules, which cause very strong intermolecular, covalent and hydrogen bonds (Shah *et al*., 2016). The result is an increase in biofilm opacity and compactness. This compactness leads to a reduction in the transmission path. OX biofilms could be interesting for packaging photosensitive materials. OX films also have a greater capacity to absorb water, which could be explained by the formation of more hydrophilic groups between the starch polymers and the oxidant. According to Lawal *et al.* (2005), the new functional groups on oxidized starch could cause electrostatic repulsion between OX starch molecules and facilitate water access to starch matrices compared to NA starch.

*Biodegradability*

Biodegradation of biofilms is a process that involves three important stages: biodeterioration, bio-fragmentation and assimilation by the microorganisms that ingest the polymer and convert the oligomers and monomers into CO2, water and biomass (de Castro *et al*., 2022). The results obtained **(Figure 4)** from burial in sterile soil, by subtraction, enabled us to attribute the degradation phenomenon to the role of the microorganisms contained in the enriched soil.

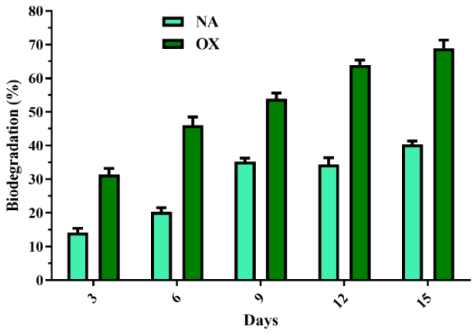



**Figure 4.** Comparative biodegradation of NA and OX biofilms

**Figure 4** shows the results of monitoring the degradation of native starch biofilms compared with oxidized starch biofilms over 15-day Overall, there is evidence that starch oxidation slows down the degradation process of the resulting OX biofilms. The formation of carboxylic and carbonyl functional groups after oxidation could also explain this result by limiting the number of microbial attack sites. In their study of the biodegradation behavior of starch bioplastics, Zain *et al.* (2018) observed a similar result. In other words, the overall biodegradability of the resulting plastic decreases as the content of the two biopolymer components in starch is reduced (Irhan & Oran, 2022; Carpio-Vargas *et al*., 2023; Triyono & Amijaya, 2023; Zeka *et al*., 2023).

## CONCLUSION

The study focused on the search for improved processes for the synthesis of bioplastics from manioc starch, a local biomass. The process presented involves modifying the molecular structure of the starch through an oxidation phase. The various characteristics of the biofilms resulting from the study show excellent properties that can be exploited in several fields of application for environmentally friendly plastic packaging. Biodegradability tests have shown that the material produced by the chemical modification of starch is less biodegradable than the native starch material. OX-type packaging therefore degrades more slowly. The study shows that it is possible to obtain plastics adapted to specific applications, which would contribute to the valorization of agricultural biomass and to a circular economy (Poornachitra & Maheswari, 2023; Botelho *et al*., 2024).

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