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Chitosan-Based Coatings for Shelf-Life Elongation of Cassava Root Tubers

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Abstract: Cassava is a major staple crop for millions of people in Africa and most developing countries. It is also a good raw material for bioethanol production. However, it suffers a major limitation to serve its full potential due to its high susceptibility to postharvest physiological deterioration (PPD). The aim of this study is to explore the use of formulated chitosan-antioxidant coatings for exogenous application on cassava cultivars for shelf-life elongation. The formulated films were derived from chitosan and two antioxidants namely ascorbic acid and quercetin. The films were found to have pronounced effects on PPD responses in the different cassava cultivars used in this study. Chitosan-quercetin film recorded up to 81% decline in the production of hydrogen peroxide (H_2O_2), a predominant reactive oxygen species that contributes to cassava PPD. Chitosan-ascorbic acid and chitosan films recorded 67% and 52% decline respectively, in the level of H_2O_2 produced in the cassava cultivars. The chitosan-antioxidant film formulations applied in this study proved effective in attenuating the PPD in the cassava samples, with evidence on the drastic reduction of H_2O_2 produced in the coated samples compared with the uncoated cultivars. The cyanide content and moisture content of the cassava cultivars on harvest may have contributed to the rate of PPD as presented in this study.

Keywords: *Cassava shelf-life elongation, chitosan-antioxidant coating, hydrogen peroxide, ascorbic acid, quercetin.*

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a starch-rich treasured root tuber of great importance. It is cultivated in different tropical and subtropical areas of the world where it plays an important role in global food security (Kolawole *et al*., 2010). It is the world's fourth most vital staple crop besides rice, wheat, and maize, delivering the necessary food energy requirement for nearly 1 billion people in 105 nations (FAOSTAT, 2021). It is also used as animal feed and is a good raw material for bioethanol production (Fathima *et al*., 2023). The global production level of cassava is 304 million tons (FAOSTAT, 2021).

Cassava is one of the most drought-tolerant crops and can grow well under a variety of climates, where other tuber crops will not thrive without additional external inputs (More *et al*., 2023). The carbohydrate yield is high ranging from 32% to 35% on a fresh weight basis and from 80% to 91% on a dry matter basis (Montagnac *et al*., 2009). It is capable of delivering the daily required dietary intake, as the root tubers contain significant levels of nutrients, including vitamins such as thiamin, riboflavin, niacin, and ascorbic acid (Bayata, 2019). Despite its high agronomic potential, cassava root tubers have the disadvantage of low protein content, presence of high amounts of linamarin, a cyanogenic

glucoside, and rapid postharvest physiological deterioration (PPD), which leads to distribution limitation and under-utilization of the tuber. PPD is an abiotic response to the damage caused on cassava roots during harvesting due to the oxidation of phenolic compounds, scopoletin (hydroxycoumarin) involved in plant defense by reactive oxygen species (ROS). It occurs in two stages that involve the primary deterioration, which is physiological, and secondary deterioration caused by microbial infection that ultimately leads to the softening of the root tissue (Luna *et al*., 2021).

During the primary deterioration, a visible sign of blue/black or brown discoloration appears in the vascular parenchyma at the cut or broken surfaces of the tuber. This discoloration quickly spreads to the entire tuber within 2-3 days, leading to structural changes in the stored starch and causing the tuber to be unsuitable for consumption and industrial applications. The perishable nature of cassava tuber limits its market potential and discourages all stakeholders in the value chain (Howeler *et al*., 2013). Losses arising from stockpiling at market place in Ethiopia is estimated at 30-50% (Parmar *et al*., 2018); while at the global level, losses of 19% have been reported (FAO & IFAD, 2000).

PPD of cassava is impacted by several factors which include environmental conditions, genotypes, dry weight/moisture content, harvest timing, soil preparation, and plant physiology (Rahmawati *et al*., 2022). Several studies on cassava PPD process have identified the development of reactive oxygen species (oxidative burst) as a primary phase in the deterioration process. This oxidative burst is caused by the exposure of the root to oxygen during harvest (Zidenga *et al*., 2012; Ma *et al*., 2016; Gomez *et al*., 2019; Wu *et al*., 2022). Hydrogen peroxide (H_2O_2) has been classified as one of the predominant ROS driving the PPD process. The interaction of oxidative burst and cyanogenesis during the process has also been documented (Reilly *et al*., 2004; Zidenga *et al*., 2012).

During the PPD process, there are changes in gene expression, and buildup of secondary metabolites, some of which possess antioxidant capabilities that deactivate cellular free radicals. The increased activities of the enzymes: catalase, peroxidase, and superoxide dismutase that modulate ROS levels, have been reported (Reilly *et al*., 2004). Aspartic protease which helps to regulate ROS production in cassava and phenolic compounds has been shown to be involved in the PPD process (Fernando *et al*., 2002). Metallothionein, a group of conjugated proteins has also been reported to play a substantial role in the regulation of ROS (Ma *et al*., 2023).

Reducing postharvest losses (PHLs) of food crops is crucial for increased agricultural productivity and sustainability. Shelf-life extension of cassava root tubers to beyond a week could resolve 90% of the deterioration constraints associated with the crop and enhance its economic potential. The benefits of cassava genotype with delayed PPD in Nigeria, Ghana, and Uganda have been estimated as US $ 2.9 billion, $ 855 million, and $ 280 million, respectively, over a 20-year time period (Rudi *et al*., 2010).

Many PHL interventions on cassava have been reported (Hu *et al*., 2016 ; Ma *et al*., 2016 ; Atenio *et al*., 2018 ; Liu *et al*., 2019 ; Djabou *et al*., 2023 ; Wehengbam *et al*., 2023). These include efforts to breed cassava cultivars with delayed PPD (Li *et al*., 2017), polybag storage of cassava root tubers with fungicides, applications of ethanol (> 20%), sodium sulphite (10%), sodium dithiocarbamate (10 %), saturated sodium chloride, benomyl (500 ppm) and dicloran (1000 ppm) as coatings on fresh root tubers (Zainuddin *et al*., 2018). Consumer concerns about the safety of these chemicals with toxic health effects call for an alternative greener approach.

Methods of controlling cassava PPD through oxygen exclusion are currently attracting much research interests. Cassava genotypes with high antioxidant contents have been reported to have a longer shelf life (Sánchez *et al*., 2006), and the application of exogenous melatonin on cassava root tubers has been reported to show a significant reduction in the levels H_2O_2 produced and attenuated the

blue-black discoloration caused by vascular streaking (Ma *et al*., 2016). The introgression of antioxidant activity into cassava root tuber has been shown as an effective technique for extending the shelf-life of fresh root tubers (Nduwumuremyi *et al*., 2016).

Edible film coatings have gained significant interest in recent decades due to their ability to fortify the natural layers of plants while regulating the exchange of coating gasses and preventing loss of moisture and other important components. These coatings improve the quality of food products by creating a moisture/oxygen barrier which helps to increase its shelf-life (Janjarasskul & Krochta, 2010). Chitosan and its derivatives are known as bioactive molecules that have recently been found in various applications, especially in the medical, water treatment and anticorrosion fields (Xia *et al*., 2022; Akartasse *et al*., 2022; Gomes de Menezes *et al*., 2021; Arroub & El Harfi, 2020; El Mouden *et al*., 2018). The synergistic capabilities of chitosan loaded with other antioxidants in combating loss caused by PPD in fruits have been reported (Lo'ay & Dawood, 2017; Malik *et al*., 2020; Yadav *et al*., 2020). This interaction is believed to create reinforcement in the viability of chitosan to delay post-harvest decay. The present study reports the use of chitosan-antioxidant film coatings for cassava shelf-life elongation.

2.0 Methodology

2.1 Sourcing of cassava samples

20 tubers each of four different cultivars of cassava storage roots were collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. They were all harvested on the same day. The samples were sorted according to cultivars into transparent polythene bags. They were listed as follows: IBA980581 (IITA release year, 2006), White root; IBA961632 (IITA release year, 2006), White root; TME 419 (IITA release year, 2005), White root; IBA070593 (IITA release year, 2014), Yellow root. They were assigned codes shown in **Table 1.**

Table 1. Sample codes of cassava cultivars obtained from IITA, Ibadan, Nigeria

S/N	Cultivar Name	Sample Code
1	IBA980581 - White root	BLUE
2	IBA961632- White root	GREEN
3	IBA070593- Yellow root	RED
4	TME 419- White root	BLACK

2.2 Experiments

Isolation of chitin

Chitin was isolated from crab shells collected from a local seafood market, Makoko, Lagos State, Nigeria.

1 kg of crab shells were washed with water to remove dirt and body tissues before they were sun-dried, and crushed into flakes. The dried flakes were pretreated with 0.85 M hydrochloric acid to remove fractions of the organics, after which it was air-dried. 330 g of the dried flakes were milled into a powdery form and treated with 1 M HCl for 18 hours at 30 ^{o}C to remove the minerals. This was followed by treatment with 0.75 M NaOH for 24 hours to remove the proteins. The final product, chitin, a coarse pink-colored substance (Trung *et al*., 2020) was dried in an oven at 60 ^{o}C until it gave a constant weight.

Preparation of chitosan

Chitosan (CS) was prepared from chitin by deacetylation as earlier reported (Min *et al*., 2004). 30 g of chitin was introduced into 100 mL concentrated NaOH (40 %) in a round-bottomed flask containing propan-2-ol (150 mL). The mixture was allowed to reflux for 18 hours to give a cream-white, fluffy product which was allowed to cool and decanted. It was washed thrice with distilled water containing 5 % EDTA to remove trace metals, and oven-dried at 60 ^{o}C to obtain chitosan which was characterized with a Bruker ATR-FTIR Spectrometer (Benchtop) running on OPUS 7.1 software).

Preparation of chitosan-ascorbic acid film coating

This was prepared according to the method earlier reported (Lo'ay & Dawood, 2017). 10 % poly-vinyl alcohol (PVA) solution was prepared by dissolving of 10 g of PVA in 100 mL ultrapure Milli-Q® water under magnetic stirring at 80 ^{o}C for 4 hours. 10 % chitosan, prepared by dissolving 10 g of chitosan in 100 mL of 2 % acetic acid solution, was introduced into the PVA solution by magnetic stirring for 2 hours. This was followed by the introduction of 10 % ascorbic acid into the CS/PVA mixture and stirring was continued at ambient temperature for 12 hours to give 10 % CS/PVA/AA solution which was stored at 4 ^{o}C before use.

Preparation of chitosan-quercetin film coating

10 g of quercetin was dissolved in 1 mL ethanol before mixing with 100 mL 10 % CS/PVA to give CS/PVA/Q film.

Chitosan-mediated synthesis of silver nanoparticles

This was carried out following the procedure earlier reported (Tran *et al*., 2010). 1 g of chitosan was dissolved in 1% acetic acid (100 mL) to give a resulting mixture of 1% w/w chitosan/acetic acid solution in a round-bottomed flask. 25 mL of 0.1 M $AgNO_3$ was added to the solution, and refluxed for 6 hours at a temperature of 90 ^{o}C. A dark orange colloidal solution of silver nanoparticles (AgNPs) was obtained and centrifuged at 5,000 rpm for 1 hour. The supernatant obtained was a clear orange solution, making up the CS/AgNPs stock.

Application of chitosan-based materials on harvested cassava cultivars

Each set of cassava root tubers, sorted according to cultivar types was coated with the respective chitosan formulations. The treated cassava samples were placed on a drain in the laboratory at room temperature, and the films allowed to dry over the tubers on the same day.

Electrochemical determination of hydrogen peroxide standards

Electrochemical determinations of hydrogen peroxide (H_2O_2) standard solutions were carried out with a BASI-Epsilon potentiostat/galvanostat, obtained from Bioanalytical Systems Inc. (West Lafayette, IN, USA). Standard solutions of H_2O_2 (2, 4, 6, 8, and 10 mM) were prepared in 0.1 M phosphate buffer (pH 7.0). 10 mL of each solution was transferred to the electrochemical cell, and purged with nitrogen gas for 10 min. The potential of the solution was scanned between -100 and -1000 mV using a scan rate of 50 mV/s. The working electrode was AgNPs immobilized on a glassy carbon electrode (3 mm), reference electrode (Ag/AgCl) and counter electrode (platinum disk electrode)**.** Cathodic peak currents for the reduction of H_2O_2 were recorded.

Electrochemical determinations of H_2O_2 in cassava cultivars

The method described in our earlier study (Akitoye *et al*., 2020) was employed. 1 cm slice of the cassava tuber was cut from the proximal and distal ends respectively. 5 g of the cut tuber was blended with 20 mL phosphate buffer pH 7.0. The resulting slurry was filtered with a Whatman No. 1 filter paper and cotton wool to obtain a clear filtrate. 10 mL of the filtrate was transferred to the electrochemical cell and purged with nitrogen for 10 min before scanning the potential as described for the H_2O_2 standards. The cathodic peak currents for the reduction of hydrogen peroxide were also recorded.

Determination of dry matter contents of cassava cultivars

The dry matter contents of the cassava cultivars were determined using the method of Atieno *et al*., 2018). 20 g of each cultivar was dried at 105 ^oC for 24 hours until a constant weight was obtained. Dry matter content was calculated from the following equation:

$$\%\ \text{Dry Matter Content} = \frac{(100 - (\text{Sample weight after drying})}{(\text{Sample weight before drying})} * 100$$

Electrochemical determination of cyanide standards

The conventional three-electrode configuration was used in the determinations. The working electrode (3 mm) was made of glassy carbon, while a platinum electrode (1.6 mm) served as the counter electrode. The reference electrode was Ag/AgCl. The working electrode was polished with alumina powder to obtain a mirror-like image, washed with de-ionized water and dried. The electrochemical method for cyanide detection earlier described by Noroozifar *et al*., 2011 was used with minor modification. Standard solutions of potassium cyanide (2, 4, 6, 8, and 10 mM) were prepared in 0.1 M phosphate buffer pH 7.0. These solutions were used for calibration. 5 mL of each solution was added to 5 mL 0.1 M potassium ferricyanide. The potential of each solution was scanned between +1000 and -1000 mV using a scan rate of 50 mV/s.

Electrochemical determination of cyanide levels in cassava cultivars

1 cm slice of the cassava tuber was cut from the proximal and distal ends of each cultivar respectively. 5 g of each was blended with 20 mL of 0.1 M phosphate buffer pH 7.0 and filtered. 5 mL of the filtrate was transferred to the electrochemical cell containing 0.1 M potassium ferricyanide. The potential of the solution was scanned as described for the standard cyanide solutions.

2.3 Characterization of silver nanoparticles

10 mL of the CS/AgNPs stock solution was made up to 100 mL with deionized water in a standard flask. The absorbance of the solution was measured with a Schimadzu UV-Vis spectrophotometer (UV2600 series ver 1.07) to characterize the particles.

3. Results and Discussion

3.1 Characterization of chitin and chitosan

The IR spectral of chitin and chitosan are shown in **figures 1 a & b**. Analysis of the two spectral shows that chitin was successfully deacetylated to chitosan. **Figure 1a** shows that the OH stretching band was observed at 3439.04 cm^{-1}, close to the standard absorption band at 3448 cm^{-1}. The NH stretching band at 3253 cm^{-1} and 3100 cm^{-1} are close to 3253 cm^{-1} and 3107 cm^{-1} earlier reported (Kumari and Kishor, 2020) for α chitin. These bands nearly disappeared in chitosan as seen in **figure**

1b. The CH absorption bands were seen 2874.82 cm^{-1}. The band intensity at 1661 cm^{-1} and 1619 cm^{-1} in chitin (amide I) decreased in chitosan and shifted to 1639.66 cm^{-1} and 1587.07 cm^{-1}. The amide 1 band is indicative of a hydrogen bond between a carbonyl and hydroxyl group of chitin. The band intensity at 1651 cm^{-1} corresponding to the stretching of –C=O and CO-NH- in amide was found to decrease in chitosan and shifted to 1639.65 cm^{-1}. The absorption band at 1552 cm^{-1} and 1307 cm^{-1} corresponds to amide II (N-H bending) and amide III (C-N stretching). The amide II band is attributable to N-H bending, a feature of the chitin acetamide group. It is seen in **figure 1b** that after the deacetylation, the band at 1552 cm^{-1} disappeared and a new band at 1595 cm^{-1} (NH_2 bending) was observed in chitosan. The absorption bands due to the amide group and CH stretching in chitin was observed at 1428.88 cm-1 and 1375/1307.45 cm^{-1} respectively. The minor bands at 1076 and 1055 cm^{-1} corresponds to C-O stretching, and absorption between 1349 and 1421 cm^{-1} can be attributed to the amide groups in the glucosamine structure (Sugiyanti *et al.*, 2018).

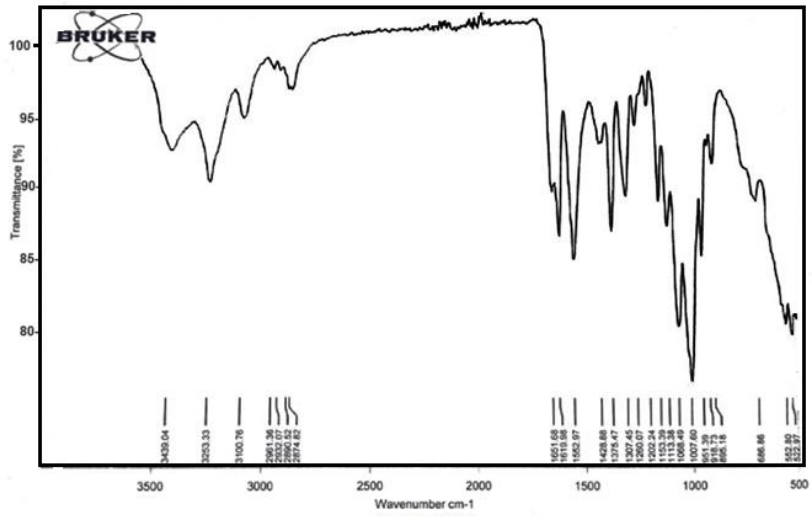

Figure 1a. IR spectrum of chitin

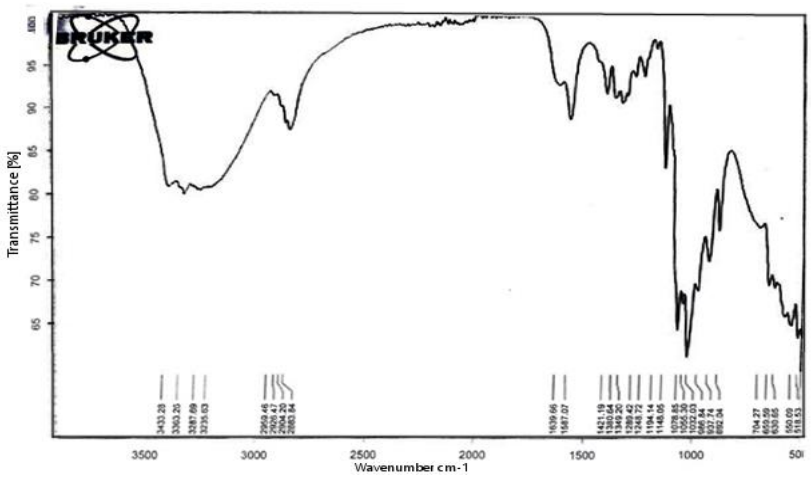

Figure 1b. IR spectrum of chitosan

3.2 ***Characterization of Silver Nanoparticles***

The physicochemical properties of nanoparticles are important for their behavior, safety, and distribution. This necessitates the characterization of the prepared AgNPs. A simple technique commonly employed for the characterization is the UV-Visible spectroscopy. The formation of the

silver nanoparticles was accompanied by a colour change from a colourless solution of the silver nitrate to a dark yellow hue indicating the reduction of Ag+ in the silver nitrate precursor solution to Ag° in the synthesized nanoparticles. The absorbance spectrum of the AgNPs was obtained in the range of 300–700 nm (**Figure 2**). The AgNPs showed maximum absorption at 400 nm, indicating the conversion of the silver ions from $AgNO_3$ to AgNP. It reflects the interphase interaction of the chitosan with the silver nanoparticle. Typically, a strong, broad peak shown around 400 nm is as a result of the surface plasmon vibrations of the excited silver nanoparticles. The bands of poly-dispersed AgNPs are often influenced by factors such as shape, size, morphology, composition and dielectric environment. This agrees with previous reports in the literature (Tran *et al*., 2016), (Kumar *et al*., 2019).

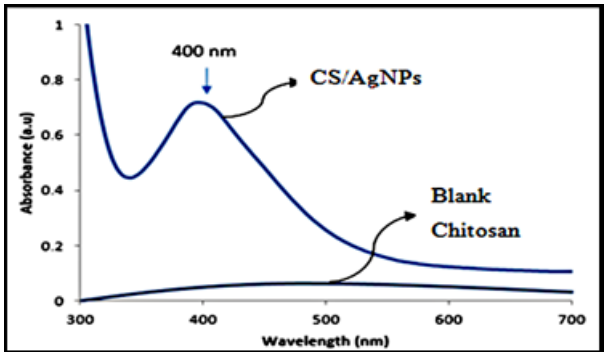

Figure 2. UV-Visible absorption of silver nanoparticles.

3.3 Electrochemical determination of H_2O_2 standards with AgNPs

The electrochemical determination of hydrogen peroxide standards was carried out with AgNPs immobilized on a glassy carbon electrode. The results are shown in the overlay of the voltammograms in **Figure 3**.

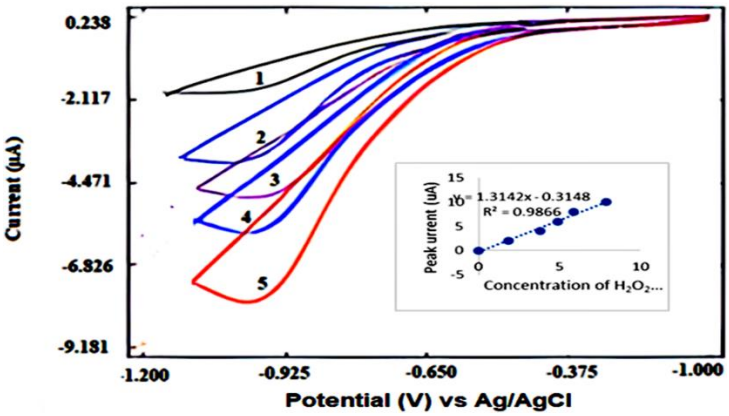

Figure 3. Overlay of the voltammograms for determination of H_2O_2 standards in 0.1 M phosphate buffer, pH 7.0 (1: 2 mM H_2O_2; 2: 4 mM H_2O_2; 3: 6 mM H_2O_2; 4: 8 mM H_2O_2; 5: 10 mM H_2O_2

It is seen in **Figure 3** that the cathodic peak current for the reduction of H_2O_2 which occurred at -950 mV, varied linearly (inset) with the concentration of the hydrogen peroxide in the concentration range (2 mM to 10 mM) studied. This shows the suitability of the electrochemical technique for quantitative measurements of different concentrations of H_2O_2 in real samples.

3.4 Electrochemical determination of H_2O_2 concentrations in the cassava cultivars

The concentrations of hydrogen peroxide in the cassava cultivars were electrochemically determined as with the standards. **Figure 4 a & b** show the voltammograms for the determination of

H_2O_2 levels in the tubers. For days 1 and 2 after harvest (**Figure 4a**), the voltammograms did not reveal any appreciable differences in their H_2O_2 concentrations. This suggests that all the cultivars were yet to experience any significant PPD. However, from day 3, the levels of H_2O_2 produced in the cultivars were significant enough to differentiate between them electrochemically as shown in **Figure 4b**. The black cultivar produced the highest level of hydrogen peroxide (851.79 mg/100g FW). This result shows that this cultivar is more susceptible to PPD, while the red cultivar is least susceptible to PPD as it produced the lowest level of H_2O_2 (352.99 mg/100g FW).

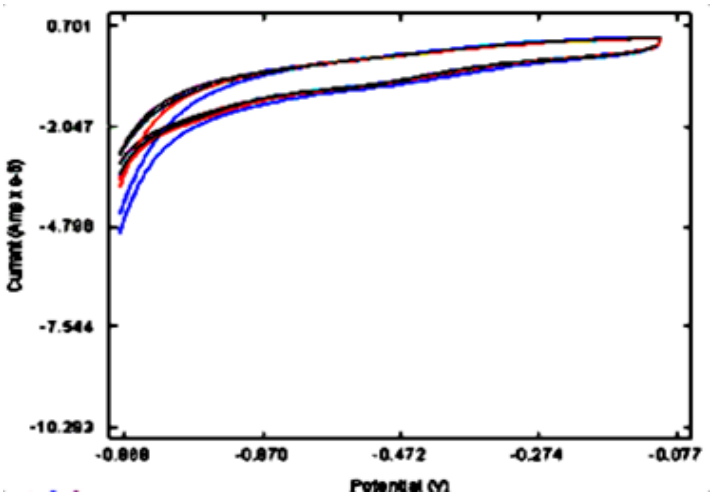

Figure 4a. Overlay voltammograms for H_2O_2 production (Day 1& 2 after harvest)

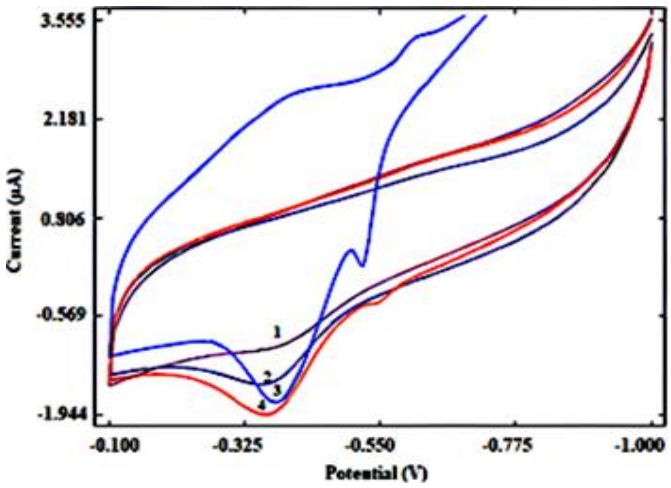

Figure 4b. Voltammogram overlay for H_2O_2 production (Day three 1: "Red cultivar"; 2: "Green cultivar"; 3: "Blue cultivar"; 4: "Black cultivar")

A number of studies have placed the generation of ROS, predominantly H_2O_2 as a route for the postharvest deterioration of cassava (Zidenga *et al*., 2012), (Uarrota *et al*., 2014), (Zainuddin *et al*., 2018). This has been shown to occur within the first few hours after harvest and gets more pronounced on the third day as we earlier reported (Akitoye *et al*., 2020). Previous reports in the literature on H_2O_2 detection in cassava employed 3,3'-diaminobenzidine (DAB) to localize and detect this reactive oxygen species (Daudi & A O'Brien, 2012), (Liu *et al*., 2014). A major drawback in the use of DAB is that the staining technique is not quantitative. This study employed voltammetry for the detection and quantification of H_2O_2 associated with cassava PPD.

Several plants, including cassava, produce reactive oxygen species (ROS) due to biological metabolic activities including photosynthesis and respiration (Berni *et al*., 2019). ROS overproduction can be initiated by stress or pathogenic attacks that occur during harvest. H_2O_2 is a predominant ROS produced during cassava PPD. According to Vranova and Inze (2002), plants have either enzymatic or non-enzymatic defense mechanisms for neutralizing ROS toxicity and preventing oxidative damage. Ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase make up the enzymatic scavenging system, whereas ascorbic acid, glutathione, and proline make up the non-enzymatic system. Plants produce more ROS and SOD in response to environmental stress because they act as the first line of defense and are crucial for stress tolerance (Apel and Hirt, 2004).

3.5 Effect of chitosan-antioxidant formulations on hydrogen peroxide production rate in the cassava cultivars

A remarkable decrease in the concentration of hydrogen peroxide was observed on coating the cassava root tubers with respective chitosan-oxidant formulations, compared to the uncoated counterparts. In the uncoated cassava root tubers, the Black cultivar was the most susceptible to PPD while the Red was least susceptible to PPD. The chitosan-quercetin film combination was found to be most effective in controlling PPD in the cultivars, exhibiting 81 % decrease in H_2O_2 concentration for the Blue cultivar. H_2O_2 concentration in the uncoated Blue cultivar was 772.62 mg/100 g FW. This value decreased to 148.09 mg/100 g Fresh Weight (FW) after coating with CS-Q. In the other cultivars, coating with CS-Q decreased the H_2O_2 production by 70 %, 50 %, and 42 % in the Black, Green and Red respectively. The level of H_2O_2 in the uncoated Black cultivar was 851.79 mg/100 g FW; as against 258.82 mg/100 g FW) when coated with CS-Q. For the Green cultivar, the uncoated had 607.56 mg/100 g FW of H_2O_2, while the CS-Q coated form had 304.87 mg/100 g FW) of H_2O_2. The H_2O_2 level in the Red cultivar was also found to decrease after coating with CS-Q. The level before coating was 352.99 mg/100 g FW while the level after coating was 205.52 mg/100 g FW. It is noteworthy that while the Red cultivar had the lowest level of H_2O_2 amongst the uncoated cultivars studied, it recorded the least decrease in H_2O_2 production after coating with CS-Q.

Coating of the four cassava cultivars with CS-AA was also found to decrease the production of H_2O_2. It resulted in a decrease of H_2O_2 production by 67 % in the Red cultivar (H_2O_2 in uncoated: 352.99 mg/100 g FW; H_2O_2 in Red cultivar coated with CS-AA: 115.49 mg/100 g FW). In the Black cultivar, a decrease of 55 % was observed. (H_2O_2 in uncoated Black: 851.79 mg/100 g FW; H_2O_2 in Black cultivar coated with CS-AA: 379.99 mg/100 g FW). In the Blue cultivar, the decline recorded in the level of H_2O_2 was 49 % (H_2O_2 in uncoated cultivar: 772.62 mg/100 g FW, while H_2O_2 level in Blue cultivar coated with CS-AA was 395.42 mg/100 g FW). In the Green cultivar, coating was found to reduce the rate of H_2O_2 production by 32 % (H_2O_2 in uncoated cultivar: 607.56 mg/100 g FW while the level in the Green cultivar coated with CS-AA was 332.29 mg/100 g FW).

The unblended chitosan film, also showed a decrease in the rate of production of H_2O_2 in the cassava cultivars, but it was not as effective as its antioxidant-loaded counterparts. The chitosan film coating was found to perform best in the Blue cultivar, showing a decrease of 52 % **(**H_2O_2 level in the uncoated: 772.62 mg/100 g FW while the level decreased to 372.65 mg/100 g FW of Cassava). The other cultivars, after coating with CS, recorded decreases of 49 %, 34 % and 29 % for Black, Green and Red respectively. These results are shown in the voltammograms in **Figures 5a to 5e** and **Table 2. Figure 5f** is a chart showing the summary of H_2O_2 levels in the cassava cultivars studied.

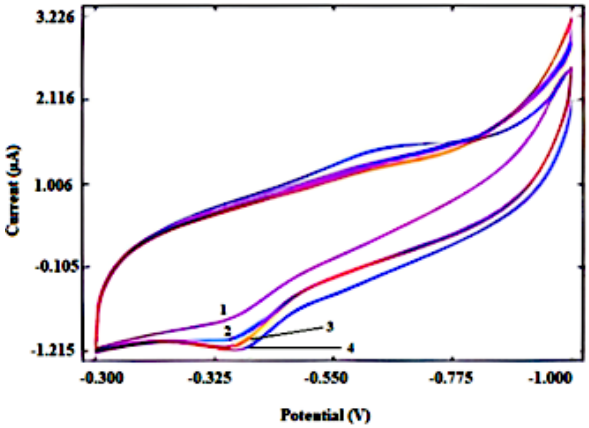

Figure 5a: Voltammograms showing H_2O_2 levels in BLACK cultivar- Day 10 (1: CS-Q coated; 2: CS-AA coated; 3: CS coated; 4: uncoated)

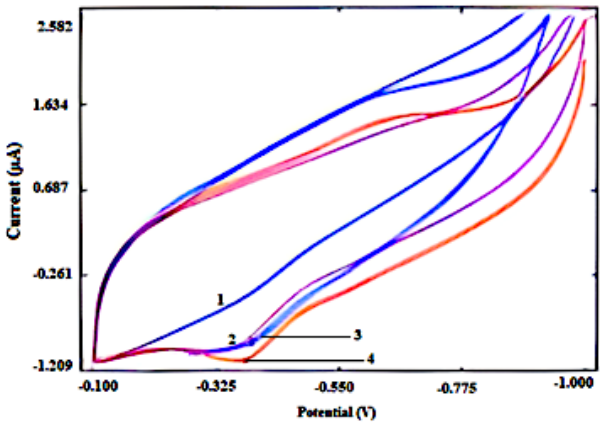

Figure 5b: Voltammograms showing H_2O_2 levels in BLUE cultivar- Day 10 (1: CS-Q coated; 2: CS coated; 3: CS-AA coated; 4: uncoated)

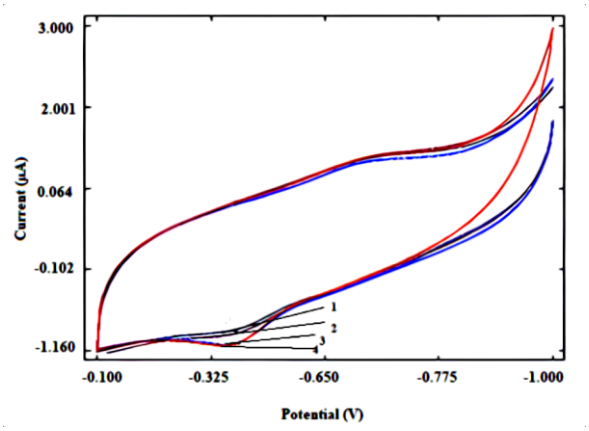

Figure 5c: Voltammograms showing H_2O_2 levels in GREEN cultivar- Day 10 (1: CS-Q coated; 2: CS-AA coated; 3:CS coated; 4: uncoated)

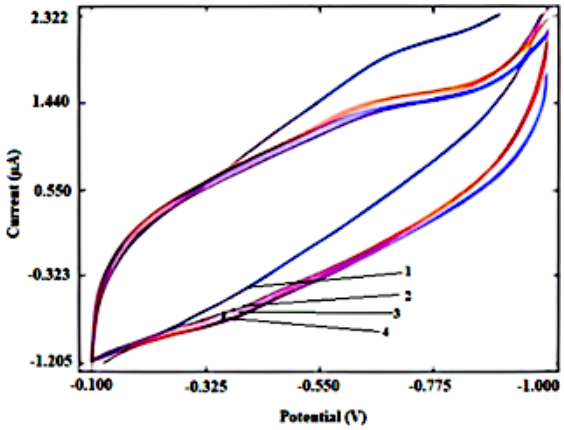

Figure 5d: Voltammograms showing H_2O_2 levels in RED cultivar- Day 10 (1: CS-AA coated; 2: CS-Q coated; 3:CS coated ; 4:uncoated)

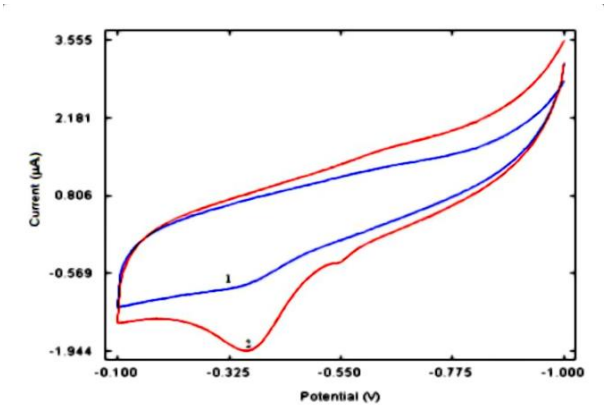

Figure 5e: H2O2 levels in (1) chitosan-coated Red cultivar, (2) uncoated Red cultivar

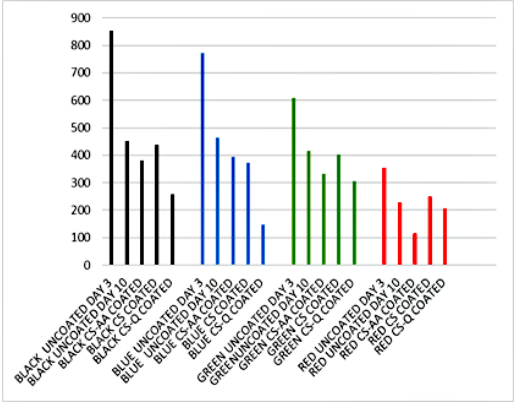

Figure 5f: Summary chart of H_2O_2 levels in cassava cultivars analyzed.

The above results show that chitosan-based formulations rich in antioxidants are very effective in mitigating cassava PPD. For the chitosan formulations studied, the order of efficiency is chitosan-quercetin > chitosan-ascorbic acid > unblended chitosan. Generally, the red cultivar, which is the beta-carotene species, demonstrated the best performance amongst the four cultivars studied in terms of H_2O_2 production and delay in the onset of PPD. The coatings used in this study are edible, made from chitosan a biopolymer.

Table 2. Hydrogen peroxide produced in different cultivars coated with chitosan formulations.

S/N	Cultivar/Film Applied	Current (μA) @ 380 mV	H_2O_2 Concentration (mg/100 g FW of cassava)
1	Black (uncoated), day 3	1.961	851.79
2	Black coated with CS-AA Film, day 10	1.049	379.89
3	Black coated with CS Film, day 10	1.159	436.81
4	Black CS-Q Film, day 10	0.815	258.82
5	Blue (uncoated) day 3	1.808	772.62
6	Blue coated with CS-AA Film, day 10	1.079	395.42
7	Blue coated with CS Film, day 10	1.035	372.65
8	Blue coated with CS-Q Film, day 10	0.601	148.09
9	Green (uncoated) day 3	1.489	607.56
10	Green coated with CS-AA Film, day 10	0.957	332.29
11	Green CS Film, day 10	1.09	401.11
12	Green CS-Q Film, day 10	0.904	304.87
13	Red (uncoated) day 3	0.997	352.99
14	Red coated with CS-AA Film, day 10	0.538	115.49
15	Red coated with CS Film, day 10	0.796	248.98
16	Red coated with CS-Q film, day 10	0.580	205.52

Chitosan functions as an exogenous defense, boosting the activity of endogenous defense-related enzymes, including peroxidase, phenylalanine ammonia-lyase (PAL), polyphenol oxidase, catalase, and SOD activity (Obianom *et al*., 2019). Formulations based on chitosan can also extensively be used to induce the formation of phytoalexins that are part of a plant defense arsenal against pathogenic attacks. During PPD process in cassava, the action of PAL results in the synthesis of low molecular weight metabolites such as coumarins and catechins which act as anti-microbial and anti-oxidants as part of the plant defense system. The chitosan formulations used in this study possibly acted as antimicrobial and antioxidant agents in mitigating the PPD in the cassava cultivars. The rate of H_2O_2 production summarized in the chart (**Figure 5F**) show that the chitosan-antioxidant films were effective exogenous coatings for the delay of PPD in cassava. This supports the finding in an earlier report (Uarrota *et al*., 2014) where cassava tubers with lower H_2O_2 production exhibited delayed PPD.

3.6 *Dry matter contents of cultivars*

The dry matter contents of the four cassava cultivars collected from IITA were determined. The results are shown in **Table 3**.

Table 3. Dry matter content of cassava cultivars

S/N	Cultivar	(A) Weight of fresh sample (g)	(B) Weight of sample after drying (g)	(C) Moisture Content (A-B) (g)	(D) % Moisture Content (C/A)*100	% Dry Matter (100-D)
1	GREEN	20	7.3	12.7	63.5	36.5
2	BLUE	20	6.3	13.7	68.5	31.5
3	BLACK	20	5.1	14.9	74.5	25.5
4	RED	20	5.0	15.0	75.0	25.0

The results in **Table 3** show that the moisture contents of the cultivars were 75%, 74.5%, 68.5%, and 63.5% for Red, Black, Blue, and Green respectively. High moisture content corresponds to low dry matter content. In a previous report by Sarkiyayi & Agar 2010, moisture content of cassava root tubers were shown to reduce with age of the root tuber and this could be another factor influencing the PPD process. However, the cultivars used in this study were harvested the same day.

Expectedly, the dry matter contents for the cultivars followed an inverse relationship with the moisture content as shown in **Table 3**. Amongst the cultivars studied, the Green had the highest dry matter (36.5%), followed by Blue (31.5%). Black and Red were close at 25.5% and 25.0% respectively. Information on the dry matter content of the cultivars is important because PPD of cassava is impacted by several factors, one of which is the dry matter content (Sanchez *et al*., 2006), (Sarkiyayi & Agar 2010), (Atieno *et al*., 2018), (Zainuddin *et al*., 2018). A study by Atieno *et al*., 2018, revealed that cassava varieties with higher dry matter content were more prone to PPD compared with varieties with lower dry matter content. While this observation holds true for the Red cultivar, with the lowest dry matter and least content of H_2O_2 obtained in this study, the other cultivars deviated as the PPD susceptibility for Blue, Black and Green cultivars did not follow the expected order of the dry matter contents. Dry matter is one of several factors that can affect PPD in cassava. Other factors include environmental conditions, genotypes, harvest timing, soil preparation, and plant physiological properties (Rahmawati *et al*., 2022).

3.7 Cyanide contents of the cultivars

The cyanide contents of the cassava cultivars were determined with a view to understanding how cyanogenesis process influences the rate of deterioration of the cassava cultivars. The determination was carried out by cyclic voltammetry. The overlay of the voltammograms for the determination of various concentrations of potassium cyanide (KCN) in 0.1M phosphate buffer, pH 7.0 is shown in **Figure 6a**. The anodic peak currents at 150 mV were inversely proportional to the concentrations of the cyanide over a concentration range of 2 mM - 10 mM (insert in **Figure 6a**). This shows that the electrochemical process is diffusion controlled which enabled a quantitative measurement of the different cyanide concentrations. The overlay of the voltammograms for the determination of cyanide levels in the cassava cultivars is shown in **Figure 6b,** while the results of the cyanide levels in the root tubers are shown in **Table 4**.

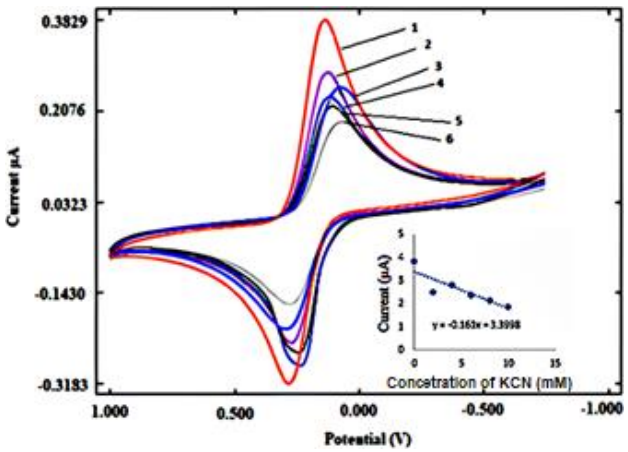

Figure 6a. Overlay of the voltammograms for various concentrations of KCN: (1=$K_3[Fe(CN_6)]$, 2= 2 mM KCN, 3= 4 mM KCN, 4= 6 mM KCN, 5=8 mM KCN, 6=10 mM KCN)

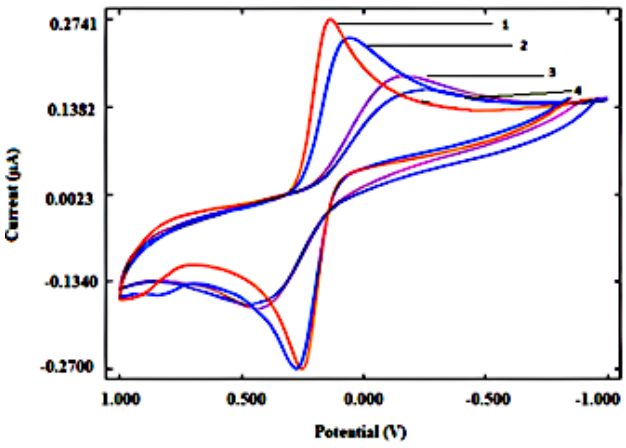

Figure 6b.: Overlay of the voltammograms for determination of cyanide levels in cassava cultivars (1:Red, 2: Blue, 3: Black, 4: Green)

Table 4. Results of electrochemical determination of cyanide levels in the cassava cultivars

S/N	Cultivar	CURRENT (μA) at 150 mV	Concentration of cyanide in Cassava cultivars (mM)
1	GREEN	1.5925	11.22547
2	BLUE	1.8589	9.570807
3	BLACK	2.4518	5.888199
4	RED	2.7246	4.193789

The results in **Table 4** show that the cyanide level in the Green cultivar was the highest while the level in the Red cultivar was the lowest. The Red cultivar is also known as vitamin A cassava. It is noteworthy that the Red cultivar also had the highest moisture content (lowest dry matter) among the cultivars studied. The total cyanide content of this cultivar is 167.75 mmol/100 g FW. A report by Sarkiyayi & Agar, 2010 showed that sweet cassava contained lower levels of total cyanide content and a higher level of moisture content, while bitter cassava (with a higher total cyanide level) contained a lower level of moisture content and was more susceptible to PPD. The results obtained in this study for the Red cultivar agrees with this observation. The cyanide levels in the Black, Blue and Red cultivars follow the expected trend in the order of susceptibility to PPD when compared to their levels of H_2O_2. Only the Green cultivar, with the highest cyanide level, deviated from the expected trend in order of susceptibility to PPD. The effect of cyanide content on cassava PPD has not been fully validated in literature as other studies have also linked high moisture content with a lower total cyanide (Akinwale *et al.*, 2010), (Rukundo *et al.*, 2013).

Cassava contains potentially toxic levels of cyanogenic glycosides, made up of linamarin (95%) and lotaustralin (5%). Linamarin is present in all cassava tissues. During harvest, these cyanogenic glycosides are exposed to the action of the enzyme linamarase leading to the cleavage of glucose from linamarin resulting in the formation of acetone cyanohydrin, a highly labile compound that degenerates into hydrogen cyanide and acetone at slightly high temperatures (Zidenga *et al.*, 2012), (Atieno *et al.*, 2018).

Conclusions

The results obtained in this study provide a clear correlation between PPD and the production of H_2O_2 in the cassava cultivars. The chitosan-antioxidant films were effective in delaying the initiation of PPD, leading to lower rates of H_2O_2 production, thereby prolonging the freshness of the tuber. The cyanide content of the root tubers correlated with the rate of production of H_2O_2, as uncoated tubers with high cyanide contents were found to be more susceptible to PPD. The effect of moisture content of the tuber on PPD is not clear as the results obtained were anomalous.

These results will be beneficial in developing solutions to cassava PPD with a view to enhancing the shelf life of the root tuber. It will also enable the extensive utilization of cassava for highly sought-after products such as starch, flour and glucose. The coated cassava samples which include Red CS-Q, Red CS-AA, Blue CS-Q, Black CS-AA, and Black CS-Q were observed to retain their freshness and quality for up to 30 days on the shelf.

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References

Akartasse N., Azzaoui K., Mejdoubi E., Elansari L. L., Hammouti B., Siaj M., Jodeh S., Hanbali G., Hamed R., Rhazi L. (2022) Chitosan-Hydroxyapatite Bio-Based Composite in film form: synthesis and application in Wastewater, *Polymers*, 14(20), 4265, https://doi.org/10.3390/polym14204265

Akinwale M. G. B., Aladesanwa R. D., Akinyele B. O., Dixon A. G. O., & Odiyi A. C., (2010) Inheritance of β-carotene in cassava (Manihot esculenta crantza, *International Journal of Genetics and Molecular Biology*, 2, 198–201.

Akitoye A. A., Ibrahim G. O., & Okiei W. O., (2020) Electrochemical quantification of the levels of hydrogen peroxide in cassava using glassy carbon electrode modified with chitosan/silver nano-hybrid. *Proceedings of the Nigerian Academy of Science*, 13, 2.

Apel K., & Hirt H. (2004) Reactive oxygen species: metabolism, oxidative stress, and signal Transduction, *Annual Review of Plant Biology*, 55(1), 373–399. doi.org/10.1146/annurev.arplant.55.031903.141701

Arroub H., El harfi A. (2020) Treatment of hot-dip galvanization by coagulation-flocculation, adsorption and by new hybrid process coagulation-flocculation/adsorption using lime-(chitosan-epichlorohydrin)/ activated carbon based on dates stones, *Mor. J. Chem.*, 8(1), 167-175. https://doi.org/10.48317/IMIST.PRSM/morjchem-v8i1.16074

Atieno L., Owino W., Ateka E. M., & Ambuko J., (2018) Effect of surface coatings on the shelf life and quality of cassava, *Journal of Food Research*, 7**,** 46–60. https://doi.org/10.5539/jfr.v7n1p46

Bayata A., (2019) Review on nutritional value of cassava for use as a staple food. *Science Journal of Analytical Chemistry*, **7**, 83–91. https://doi.org/10.11648/j.sjac.20190704.12

Berni R., Luyckx M., Xu X., Legay S., Sergeant K., Hausman J.F., Lutts S., Cai G., Guerriero G. (2019) Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism, *Environmental and Experimental Botany*, 161, 98–106. https://doi.org/10.1016/j.envexpbot.2018.10.017

Daudi A., & O'Brien A. J. (2012) Detection of hydrogen peroxide by DAB staining in Arabidopsis Leaves, *Bio Protocol*, 2(18), e263. PMCID: PMC4932902

Djabou A. S. M., Ewane H. P. K., Eyamo V. J. E., Ketchiemo F. T., Figueiredo P. G., & Niemenak N., (2023) Influence of harvest periods on cassava (*Manihot esculenta* Crantz) agronomic traits and physiological response to postharvest physiological deterioration, *American Journal of Plant Sciences*, 14(1), 89–103. https://doi.org/10.4236/ajps.2023.141007

El Mouaden K., El Ibrahimi B., Oukhrib R., Bazzi L., Hammouti B., Jbara O., Tara A., Chauhan D. S., Quraishi M.A. (2018) Chitosan polymer as a green corrosion inhibitor for copper in sulfide-containing synthetic seawater, *International Journal of Biological Macromolecules*, 119, 1311-1323.

FAO & IFAD. (2000) The world cassava economy, facts, trends and outlook. Food and Agriculture Organization and International Fund for Agricultural Development of the United Nations, Rome, Italy.

F.A.O.S.T.A.T. (2021) Food and agriculture organization of the United Nations. FAOSTAT statistical database. Rome: Italy.

Fathima A. A., Sanitha M., Tripathi L., & Muiruri S., (2023) Cassava (Manihot esculenta) dual use for food and bioenergy: A review, *Food and Energy Security*, 12, e380. https://doi.org/10.1002/fes3.380

Fernando C. D., Reilly K., Okogbenin E., Beeching J. R., Iglesias C., & Tohme J., (2002) Mapping wound-response genes involved in post-harvest physiological deterioration (PPD) of cassava (Manihot esculenta Crantz), *Euphytica* 128, 47-53. https://doi.org/10.1023/A:1020695719706

Gomes de Menezes, F.L., de Lima Leite, R.H., Gomes dos Santos, F.K., Aria, A.I., Aroucha, E.M.M. (2021) TiO2-enhanced chitosan/cassava starch biofilms for sustainable food packaging, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 6305, Article number 127661

Gomez M. A., Lin Z. D., Moll T., Chauhan R. D., Hayden L., Renninger K., & Bart R., (2019) Simultaneous CRISPR/Cas9-mediated editing of cassava eIF4E isoforms nCBP-1 andnCBP-2 reduces cassava brown streak disease symptom severity and incidence, *Plant Biotechnology Journal*, 17(2), 421–434. https://doi.org/10.1111/pbi.12987

Howeler R. L., Thomas N., Sanjuán K. H., Quirós H., Isebrands J., & Gonzále C., (2013) Save and Grow: Cassava: A Guide to sustainable production intensification, FAO, Roma Italia.

Hu W., Kong H., Guo Y., Zhang Y., Ding Z., Tie W., Yan Y., Huang Q., Peng M., Shi H., & Guo, A., (2016) Comparative physiological and transcriptomic analyses reveal the actions of melatonin in the delay of postharvest physiological deterioration of cassava, *Frontiers in Plant Science*, 7, 736. https://doi.org/10.3389/fpls.2016.00736

Janjarasskul T., & Krochta J. M., (2010) Edible packaging materials, *Annual review of food science and Technology*, 1, 415–448. https://doi.org/10.1146/annurev.food.080708.100836

Kolawole P. O., Agbetoye L., & Ogunlowo S. A., (2010) Sustaining World Food Security with improved cassava processing technology: The Nigeria Experience, *Sustainability*, 2, 3681-3694; https://doi.org/10.3390/su2123681

Kumar S., Ye F., Dobretsov S., & Dutta J., (2019) Chitosan nanocomposite coatings for food, paints, and water treatment applications, *Applied Sciences*, 9: 2409. https://doi.org/10.3390/app9122409

Kumari S., & Kishor R., (2020) Chitin and chitosan: origin, properties, and applications. *Handbook of Chitin and Chitosan*, 1–33. https://doi.org/10.1016/b978-0-12-817970-3.00001-8

Li X., Fan S., Hu W., Liu G., Wei Y., He C., & Shi H., (2017) Two cassava basic leucine zipper (bZIP) transcription factors (MebZIP3 and MebZIP5) confer disease resistance against cassava bacterial blight, *Frontiers in plant science* 8:2110. https://doi.org/10.3389/fpls.2017.02110

Liu G., Li B., Li X., Wei Y., Liu D., & Shi H., (2019) Comparative physiological analysis of methyl jasmonate in the delay of postharvest physiological deterioration and cell oxidative damage in cassava, *Biomolecules*, 9(9), 451. https://doi.org/10.3390/biom9090451

Liu Y. H., Offler C. E., & Ruan Y. L., (2014) A simple, rapid and reliable protocol to localize hydrogen peroxide in large plant organs by DAB-mediated tissue printing, *Frontiers in Plant Science*, 5. https://doi.org/10.3389/fpls.2014.00745

Lo'ay A. A., & Dawood H. D., (2017) Active chitosan/PVA with ascorbic acid and berry quality of "Superior seedless" grapes, *Scientia Horticulturae*, 224, 286–292. https://doi.org/10.1016/j.scienta.2017.06.043

Luna J., Dufour D., Tran T., Pizarro M., Calle F., Domínguez M. G., Hurtado I. M., Sanchez T., & Ceballos H., (2021) Post-harvest physiological deterioration in several cassava genotypes over sequential harvests and effect of pruning prior to harvest, *International Journal of Food Science and Technol*ogy, 56(3), 1322–1332. https://doi. org/10.1111/ijfs.14711

Ma Q., Zhang T., Zhang P., & Wang Z. Y., (2016) Melatonin attenuates postharvest physiological deterioration of cassava storage roots, *Journal of Pineal Research*, 60, 424–434. https://doi.org/10.1111/jpi.12325

Ma Y., Xue M., Zhang X., & Chen S., (2023) Genome-wide analysis of the metallothionein gene family in cassava reveals its role in response to physiological stress through the regulation of reactive oxygen species, *BMC Plant Biology*, 23, 227. https://doi.org/10.1186/s12870-023-04174-2

Malik A. I., Kongsil P., Nguyễn V. A., Ou W., Srean P., López-Lavalle L. A. B, et al., (2020) Cassava breeding and agronomy in Asia: 50 years of history and future directions, *Breeding Science*, 18180. https://doi.org/10.1270/jsbbs.18180

Min B. M., Lee S. W., Lim J. N., You Y., Lee T. S., Kang P. H, et al., (2004) Chitin and chitosan nanofibers: electrospinning of chitin and deacetylation of chitin nanofibers, *Polymer*, 45, 7137–7142. https://doi.org/10.1016/j.polymer.2004.08.048

Montagnac J. A., Davis C. R., & Tanumihardjo S. A., (2009) Nutritional value of cassava for use as a staple food and recent advances for improvement, *Comprehensive Reviews in Food Science and Food Safety*, 8(3), 181–194. https://doi.org/10.1111/j.1541-4337.2009.00077.x

More S. J., Bardhan K., Ravi V., Pasala R., Chaturvedi A. K., Lal M. K., & Siddique K. H. M., (2023) Morphophysiological responses and tolerance mechanisms in cassava (*Manihot esculenta* Crantz) under drought stress, *Journal of Soil Science and Plant Nutrition*, 23, 71–91. https://doi.org/10.1007/s42729-023-01127-4

Nduwumuremyi A., Melis R., Shanahan P., & Asiimwe T., (2016) Introgression of antioxidant activity into cassava (*Manihot esculenta* C.): An effective technique for extending fresh storage roots shelf life, *Plant Breeding*, 135, 1–8. https://doi.org/10.1111/pbr.12323

Noroozifar M., Khorasani-Motlagh M., & Taheri A., (2011) Determination of cyanide in wastewaters using modified glassy carbon electrode with immobilized silver hexacyanoferrate nanoparticles on multiwall carbon nanotube, *Journal of Hazardous Materials*, 185(1), 255–261 doi:10.1016/j.jhazmat.2010.09.02

Obianom C., Romanazzi G., & Sivakumar D. (2019) Effects of chitosan treatment on avocado postharvest diseases and expression of phenylalanine ammonia-lyase, chitinase and lipoxygenase genes, *Postharvest Biology and Technology*, 147, 214-221. https://doi.org/10.1016/j.postharvbio.2018.10.004

Parmar A., Fikre A., Sturm B., & Hensel O. (2018) Post-harvest management and associated food losses and by-products of cassava in southern Ethiopia, *Food Security*, 10(2), 419–435. https://doi.org/10.1007/s12571-018-0774-7

Rahmawati R. S., Khumaida N., Ardie S. W., & Sudarsono S., (2022) Effects of harvest period, storage and genotype on postharvest physiological deterioration responses in cassava, *BIODIVERSITAS*, 23(1), 100-109. https://doi.org/10.13057/biodiv/d230113

Reilly K., Gómez-Vásquez R., Buschmann H., Tohme J., & Beeching J., (2004) Oxidative stress responses during cassava post-harvest physiological deterioration, *Plant Molecular Biology*, 56, 625–641. https://doi.org/10.1023/B:PLAN.0000019076.76614.88

Rudi N., Norton G. W., Alwang J., Asumugha G. (2010) Economic impact analysis of marker-assisted breeding for resistance to pests and postharvest deterioration in cassava, *African Journal of Agricultural and Resource Economics*, 4, 110-122. https://doi.org/10.22004/ag.econ.93862

Rukundo P., Shimelis H., Laing M., & Gahakwa D., (2013) Storage root formation, dry matter synthesis, accumulation and genetics in sweet potato, *Australian Journal of Crop Science* 7, 2054–2061. http://www.cropj.com/rukundo_7_13_201

Sánchez T., Chávez A. L, Ceballos H., Rodriguez-Amaya D. B., Nestel P., & Ishitani M., (2006) Reduction or delay of post-harvest physiological deterioration in cassava roots with higher carotenoid content, *Journal of the Science of Food and Agriculture*, 86, 634–639. https://doi.org/10.1002/jsfa.2371

Sarkiyayi S., & Agar T. M., (2010) Comparative analysis on the nutritional and anti-nutritional contents of the sweet and bitter cassava varieties, *Advance Journal of Food Science and Technology*, 2, 328–334. https://doi.org/10.15744/2393-9060.3.302

Sugiyanti D., Darmadji P., Anggrahini S., Anwar C and Santoso U. (2018) Preparation and characterization of chitosan from Indonesian Tambak Lorok shrimp shell waste and crab shell waste, *Pakistan Journal of Nutrition*, 17(9), 446-453. https://doi.org/10.3923/pjn.2018.446.453

Tran H. V., Tran L. D., Ba C. T., Vu H. D., Nguyen T. N., Pham D. G., *et al.*, (2010) Synthesis, Characterization, antibacterial and antiproliferative activities of Monodisperse Chitosan-Based Silver Nanoparticles, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 360, 32–40. https://doi.org/10.1016/j.colsurfa.2010.02.007

Trung T. S., Van Tan N., Van Hoa N., Minh N. C., Loc P. T., & Stevens W. F., (2020) Improved method for production of chitin and chitosan from shrimp shells *Carbohydrate Research*, 489, 107913. https://doi.org/10.1016/j.carres.2020.107913

Uarrota V. G., Moresco R., Coelho B., Nunes E. C., Peruch L. A. M., Neubert E. O., Rocha M., & Maraschin M. (2014) Metabolomics combined with chemometric tools (PCA, HCA, PLS-DA and SVM) for screening cassava (Manihot esculenta Crantz) roots during postharvest physiological deterioration, *Food Chemistry*, 161, 67–78. https://doi.org/10.1016/j.foodchem.2014.03.110

Vranová E., Inzé D., & Breusegem F., (2002) Signal transduction during oxidative stress. *Journal of Experimental Botany*, 53(372), 1227–1236. https://doi.org/10.1093/jexbot/53.372.1227

Wahengbam E. D., Devi C. P., Sharma S. K., Roy S. S., Maibam A., Dasgupta M., Luikham S., Chongtham T., Ningombam A., Bhupenchandra I., Singh L. K., Devi Y. P., Thokchom S., Khaba C. I., Singh N. B., Rajashekar Y., Das S., Mohanty S & Sahoo M. R., (2023) Reactive oxygen species turnover, phenolics metabolism, and some key gene expressions modulate postharvest physiological deterioration in cassava tubers, *Frontiers in Microbiology*, 14, 1148464. https://doi.org/10.3389/fmicb.2023.1148464.

Wu X., Xu J., Ma Q., Ahmed S., Lu X., Ling E., & Zhang P., (2022) Lysozyme inhibits postharvest physiological deterioration of cassava, *Journal of Integrative Plant Biology*, 64(3), 621-624. https://doi.org/10.1111/jipb.13219

Yadav S., Mehrotra G. K., Bhartiya P., Singh A., & Dutta P. K., (2020) Preparation, physicochemical and biological evaluation of quercetin based chitosan-gelatin film for food packaging, *Carbohydrate Polymers*, 227, 115348. https://doi.org/10.1016/j.carbpol.2019.115348

Xia Y., Wang D., Liu D., Su J., Jin Y., Wang D., Han B., Jiang Z. and Liu B. (2022) Applications of Chitosan and its Derivatives in Skin and Soft Tissue Diseases. *Front. Bioeng. Biotechnol*. 10, 894667. https://doi.org/10.3389/fbioe.2022.894667

Zainuddin I. M., Fathonib A., Sudarmonowati E., Beeching J. R., Gruissema W., & Vanderschuren H. (2018) Cassava postharvest physiological deterioration: From triggers to symptoms. *Postharvest Biology and Technology*, 142,115–123. https://doi.org/10.1016/j.postharvbio.2017.09.004

Zidenga T, Leyva-Guerrero E., Moon H., Siritunga D., & Sayre R., (2012) Extending cassava root shelf life via reduction of reactive oxygen species production, *Plant Physiology*, 159, 1396–1407. https://doi.org/10.1104/pp.112.200345