BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Effect of multilayer nylon packages on the oxidative damage of minimally processed yam

Efeito da embalagem de nylon multicamada no dano oxidativo de inhame minimamente processado

Adriano do Nascimento Simões[1]*, Daniel Gomes Coelho[1], Kelem Silva Fonseca[1], Aurélio Paes Barros Júnior[2], Sergio Luiz Ferreira-Silva[1], Clarissa Soares Freire[1], Edson Fabio da Silva[1]

[1]Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada, Departamento de Produção Vegetal, Serra Talhada/PE - Brasil
[2]Universidade Federal Rural do Semiárido (UFERSA), Departamento de Ciências Vegetais, Mossoró/RN - Brasil

***Corresponding Author:** Adriano do Nascimento Simões, Universidade Federal Rural de Pernambuco (UFRPE), Departamento de Produção Vegetal, Unidade Acadêmica de Serra Talhada, CEP: 56909-535, Serra Talhada/PE - Brasil, e-mail: adriano.simoes@ufrpe.br

Cite as: Simões, A. N., Coelho, D. G., Fonseca, K. S., Barros Júnior, A. P., Ferreira-Silva, S. L., Freire, C. S., & Silva, E. F. (2019). Effect of multilayer nylon packages on the oxidative damage of minimally processed yam. *Brazilian Journal of Food Technology, 22*, e2017090. https://doi.org/10.1590/1981-6723.09017

Abstract

Appropriate storage packaging is an important aspect to minimize physiological deterioration and enhance the shelf-life of minimally processed products. Thus, the goal of this study was to evaluate the physiological and biochemical changes associated with the quality loss of minimally processed yam, maintained in two different packages at 5 ± 2 °C. The yam roots were selected, washed and conserved at 8 ± 2 °C for 24 hours. They were then peeled and cut into slices about 3 cm thick and minimally processed. Approximately 300 g of minimally processed yam roots were packed in polypropylene (PP, 4 µm thick) and multilayer nylon (NY, 15 µm thick) 15 cm wide x 20 cm long bags, which were stored at 5 ± 2 °C for 14 days. The minimally processed yam conserved in PP packaging presented fluorescence on the surface of the segments, characteristic symptoms of *Pseudomonas* spp., and showed higher peroxidase and catalase activities. The nylon packaging was more efficient in reducing oxidative damage and also inhibited polyphenol oxidase activity and decreased the accumulation of soluble proteins, resulting in decreased deterioration during storage. Thus, the quality of the minimally processed yam maintained in NY packaging was conserved for 14 days at 5 ± 2 °C.

Keywords: Oxidative stress; Enzymatic browning; Catalase; H_2O_2; *Dioscorea* spp.

Resumo

A adequação da embalagem de armazenamento é um aspecto importante para minimizar a deterioração fisiológica e melhorar a conservação de produtos minimamente processados. Dessa forma, o objetivo deste estudo foi avaliar as alterações fisiológicas e bioquímicas associadas à perda de qualidade de inhame minimamente processado, mantido em duas embalagens a 5 ± 2 °C. As raízes de inhame foram selecionadas, lavadas e mantidas a 8 ± 2 °C durante 24 horas. Em seguida, foram descascadas e cortadas em fatias com cerca de 3 cm de espessura, e minimamente processadas. Aproximadamente 300 g de raízes minimamente processadas

de inhame foram embaladas em sacos de polipropileno (PP, 4 µm de espessura) e nylon multicamada (NY, 15 µm de espessura), com 15 cm de largura e 20 cm de comprimento, e mantidos a 5 ± 2 °C durante 14 dias. As embalagens de polipropileno resultaram em fluorescência na superfície dos pedaços, sintomas característicos de *Pseudomonas* spp. e apresentaram maiores atividades de Catalase e Peroxidase. A embalagem de nylon multicamada foi mais eficiente em reduzir os danos por oxidação. Esta embalagem também inibiu a atividade de Polifenoloxidase e diminuiu o acúmulo de proteínas solúveis, resultando em redução nos sintomas de deterioração durante o armazenamento. Assim, a qualidade do inhame minimamente processado mantido em embalagem de nylon foi conservada até 14 dias a 5 ± 2 °C.

Palavras-chave: Estresse oxidativo; Escurecimento enzimático; Catalase; H_2O_2; *Dioscorea* spp.

1 Introduction

Yam (*Dioscorea* spp.) is a tuberous plant grown and consumed by millions of people in tropical countries (Weerarathne et al., 2017). The edible part of this plant is a tuber, an underground organ, much appreciated in diets for presenting nutritional and medicinal qualities. A 100 g portion of yam contains approximately 4.1 g fibre; 17.1 mg vitamin C; 0.293 mg vitamin B-6 and 7 µg of vitamin A (United States Department of Agriculture, 2013). In most commercial establishments yams are traded as whole tubers with no processing or cleaning, which results in low added value and contributes to post-harvest losses.

The main goal of minimal processing is to add value to plant products. The visual attributes are improved since the shells, peels and other inedible parts are discarded, leaving it ready for consumption or cooking, ensuring greater food security to the consumer (Barrett et al., 2010). This technique is presented as an important technological advance, but despite its practicality, convenience and market valuation, it has some drawbacks, since the handling and manipulation of the plant material during all steps is stress-inducing, leading to physiological responses similar to those of plants under stress (Brecht, 1995; Hong & Kim, 2001).

The damage caused by cutting and inadequate storage conditions induces fresh weight loss in cassava (Junqueira et al., 2014) and increases post-harvest physiological deterioration in fresh-cut potato (Silveira et al., 2017) and fresh-cut yam, with a rapid darkening of surface tissues, initially with brownish spots soon after peeling and cutting (Simões et al., 2016). In cassava roots, there is an accumulation of reactive oxygen species when the tissue darkens (ROS) (Xu et al., 2013). Excessive ROS are harmful to the plant cell metabolism because they are highly reactive, reacting with proteins, and causing lipid peroxidation and protein denaturation (Imlay, 2003). The damage caused by an excess of ROS creates adequate conditions for phenol oxidation, related to polyphenol oxidase (EC 1.10.3.1; PPO) and peroxidase (EC 1.11.7; POD) activities and resulting in enzymatic browning (Tomás-Barberán & Espín, 2001).

Under oxidative stress, plants have elaborate defence mechanisms to eliminate the excess of ROS in the cells (Freire et al. 2015). ROS-scavenging involves enzymatic pathways, including catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (G-POD) and ascorbate peroxidase (APX) activities, as well as non-enzymatic compounds such as ascorbate (AsA), glutathione (GSH), carotenoids and tocopherols (Demidchik, 2015). Catalase (EC 1.11.1.6, CAT) is an enzyme that appears to be involved in physiological disorders of cassava roots, acting in the removal of hydrogen peroxide (H_2O_2) and minimizing damage caused by the excess of ROS (Xu et al., 2013). This suggests that CAT activity may be an indicator of oxidative stress tolerance.

Appropriate storage conditions are important factors to minimize deterioration and improve the conservation of fresh-cut vegetables (Belay et al., 2016). The use of some kind of packaging has been reported as an effective alternative to minimize the physiological changes in whole and fresh-cut vegetables, by modifying the atmospheric conditions around the product, thus limiting oxygen availability for the polyphenol oxidations (Abbasi et al., 2016; Wilson et al., 2019; Zhang et al., 2015).

Andrade et al. (2012) evaluated the storage of yam pieces in three types of packaging: low density polyethylene with linear-low density polyethylene (LDPE/LLDPE), bioriented polypropylene laminated with low density polyethylene (BOPP/LDPE) and polystyrene (PS) associated with different O_2, CO_2 and N_2 concentrations at 5 °C. The authors found that the BOPP/LDPE package provided higher respiratory rates in pieces of yam for twelve days. However, the use of these types of package can be expensive due to the higher cost of gas injection. Donegá et al. (2013) studied polyvinyl chloride (PVC), but product conservation lasted less than 3 days at 5 °C. Although the PVC package is widely used commercially, it does not provide conservation with appropriate quality. Thus, the study of alternatives to PVC or LDPE packaging could result in satisfactory benefits for fresh-cut or minimally processed yam.

In general, studies with alternative packaging such as polypropylene and multilayer nylon could be a suitable approach for the preservation of minimally processed yam and might also be used for other roots subject to browning, such as cassava (Junqueira et al., 2014), arracacha (Menolli et al., 2008) and potato (Junqueira et al., 2009). Thus, the objective of this study was to evaluate the physicochemical and biochemical changes in minimally processed yam stored in two types of packaging and conserved at 5 ± 2 °C.

2 Materials and methods

2.1 Plant material

Commercial yam roots were purchased from producers in Petrolina, State of Pernambuco, Brazil, and taken to the Universidade Federal Rural de Pernambuco/Unidade Acadêmica in Serra Talhada, where they were selected for size (~20 cm) and integrity, the damaged ones being discarded. They were washed to remove excess soil and stored at 8 °C for 24 hours.

2.2 Minimal processing and conservation

Minimal processing was adapted according to Simões et al. (2016). The yam roots were peeled and cut into 3cm thick slices. The slices were immersed in water at 5 ± 2 °C for 5 minutes for an initial rinse, sanitized by immersing approximately 2000 g of slices in chlorinated water with 200 mg L^{-1} of active chlorine (sodium dichloroisocyanurate) for ten minutes, and finally immersed in a chlorinated solution with 5 mg L^{-1} of active chlorine for five minutes for a final rinse. The slices were drained in kitchen strainers for ten minutes. Approximately 300 g of drained slices were packed into each of three polypropylene (PP, 4 µm thick) and multilayer Nylon (NY, 15 µm thick) bags, all with dimensions of 15 cm wide and 20 cm long, and stored at 5 ± 2 °C for 14 days.

2.3 Analyses

2.3.1 Visual analysis and emission of fluorescence

The visual analysis was carried out by a trained panel based on a subjective rating scale ranging from 5 to 1 according to Simões et al. (2016). Rate 5: Slices with characteristic white surfaces, no hint of brownish spots, excellent appearance and odour for consumption; 4: Slices with changes in colour tones occurring during the first few days, but with quality for trade; 3: Slices with up to 10% of their surfaces showing brownish spots of moderate intensity. Acceptance limit; 2: Slices with approximately 50% of their surface areas showing a brownish colour, unfit for consumption and with package blown-up with probable accumulation of gases and, finally, 1: Slices with all the above described symptoms plus an alcoholic odour; totally unfit for consumption.

In addition, the emission of fluorescence was visualized from two yam slices per repetition, representing a treatment. They were separated to take photos using a semi-professional digital camera model Nykon D3100 (14.2 megapixels) in a darkroom (CN-6; Vilber Lourmat) with a 1 × 6 Watts filter, 1 × 6 Watts and 220 V 50/60 Hz (VL- 6.l; Vilber Lourmat).

2.3.2 Weight Loss (WL)

The fresh weight loss was determined gravimetrically according to the following Equation (1):

$$\%\ FWL = \left[\left(FWi - FWf\right) * 100\right] / FWi \quad (1)$$

Where: FWL = fresh weight loss; FWi = initial fresh weight; FWf = final fresh weight.

2.3.3 Extraction and quantification of Total Soluble Phenolics (TSP)

The extraction and quantification of TSP were carried out according to Reyes et al. (2007), with modifications. Extraction was carried out by macerating 1 g of yam slices with dimensions of about 10 × 10 × 5 mm at the slice equatorial region in a mortar with 10 mL of methanol. The macerated samples were allowed to rest for 24 hours in the dark at 4 °C and then centrifuged at 9,000 g and 2 °C for 21 minutes. To prepare the calibration curve, suitable concentrations of gallic acid were achieved by diluting suitable volumes of the stock solution of gallic acid (2 mM) and adding methanol (PA), distilled water, dilute Folin-Ciocalteau reagent [1000 µL of Folin reagent (2N) in 9 mL of distilled water] and sodium carbonate solution (1N) in test tubes with stoppers. For the samples, the extracts were mixed with methanol, the diluted Folin-Ciocalteau solution and the sodium carbonate solution. The tubes were shaken for 20 seconds and then stored in the dark at room temperature for 2 hours. After this period, the reading was taken using a spectrophotometer (Libra S8; Biochorom) at a wavelength of 725 nm and the results expressed on a fresh weight basis as mmol of gallic acid equivalent (GAE) kg^{-1}.

2.3.4 Extraction and assays of polyphenoloxidase (EC 1.14.18.1; PPO) and peroxidase (EC 1.11.1.7; POD) activities

The extraction and PPO and POD assays followed the methodology described by Freire et al. (2015), based on the removal of 0.25 g (approximately 5 mm) of the thick-equatorial side of the yam slice. This was macerated in a mortar with 6 mL phosphate buffer (0.2 M, pH 6.0) previously maintained at 4 °C. The homogenate was centrifuged at 9,000 g for 21 minutes at 4 °C. The PPO assay was carried out using a mixture of 1 mL of phosphate buffer (0.2 M, pH 6.0) with 1.5 mL of catechol (0.2 M) as the substrate and 50 µL of the enzyme extract (supernatant) added. The readings were taken at 425 nm for 2 minutes at 30 second intervals and the PPO activity estimated using a molar extinction coefficient of 1,300 mM^{-1} cm^{-1}. To prepare the blank, the enzyme extract was boiled for 10 minutes in a water bath (Model TE-054/te-056 © TECNAL). The results were expressed as µmol catechol g^{-1} min^{-1} on a fresh weight basis.

The POD assay was carried out using a mixture of 1 mL of 0.2 M phosphate buffer, pH 6, and 10 mL of enzyme extract. Aliquots of 100 µL of guaiacol (0.5%) and 100 µL of hydrogen peroxide (0.08%) were added to the mixture as the substrate and readings taken in a spectrophotometer at 470 nm for two minutes at 30 second intervals. The POD activity was estimated using a molar extinction coefficient of 36.8 mM^{-1} cm^{-1} and the results expressed as µmol guaiacol g^{-1} min^{-1} on a fresh weight basis.

2.3.5 Catalase extraction and assay (EC 1.11.1.6, CAT)

Catalase was extracted and its activity determined according to the methodology described by Beers Junior & Sizer (1952). For the extraction, a 0.25 g sample was removed from the equatorial side of the yam slice, macerated in a mortar containing 6 mL of phosphate buffer (pH 7) and centrifuged at 9,000 g for

21 minutes at 4 °C in order to obtain the extract. The CAT assay was carried out using a 50 µL aliquot of the extract, which was added to a solution containing 2.95 ml of 50 mM potassium phosphate buffer (pH 7.0) and H_2O_2 (20 mM). The absorbance was read at 240 nm and 30 °C every 30 seconds for 3 minutes, and the enzyme activity calculated based on the molar extinction coefficient of 36 M^{-1} cm^{-1} for H_2O_2. The results were expressed as nmol H_2O_2 g^{-1} min^{-1} on a fresh weight basis.

2.3.6 Hydrogen peroxide (H_2O_2) content

The hydrogen peroxide (H_2O_2) content was determined according to the methodology proposed by Cheeseman (2006). A 0.1 g sample of yam was removed from the equatorial side of the slice and macerated for 3 minutes in an ice bath with TCA (5%), and 0.75 mL of 100 mM potassium phosphate buffer pH 6.4 then added to the macerate. The extract was centrifuged at 9,000 g for 20 minutes at 4 °C and 0.1 ml aliquots of the supernatant added to test tubes together with 0.9 ml of reaction medium containing 100 µM of $FeSO_4$, 250 µM of $(NH4)_2SO_4$, 25 mM of H_2SO_4, 100 µM of xylenol orange and 99 mM sorbitol. The mixture was incubated at 25 °C for 30 min and the absorbance then read at 560 nm. The hydrogen peroxide concentrations were expressed as µmol g^{-1} on a fresh weight basis.

2.3.7 Lipid peroxidation (TBARS)

Lipid peroxidation was estimated based on the content of thiobarbituric acid reactive substances (TBARS), according to Heath & Packer (1968). For the extraction, a 0.1 g sample of yam was withdrawn from the equatorial side of the slice and macerated in a mortar in the presence of ice. An aliquot of 1.0 mL of 6% trichloroacetic acid (TCA) was then added to the samples and macerated for a further 3 minutes. The extract was centrifuged at 9,000 g and 4 °C for 15 minutes and 0.5 mL aliquots of the supernatant then added to 2.0 mL of reaction medium containing 20% TCA (w/v) and 0.5% (w/v) thiobarbituric acid (TBA).

The reaction mixture was heated in a water bath at 95 °C for 1h and was then cooled in an ice bath. After temperature stabilization, the absorbance was read in a spectrophotometer at wavelengths of 532 and 660 nm, and the absorbance values obtained at 532 nm subtracted from the absorbance values obtained at 660 nm. The TBARS content was estimated using a molar extinction coefficient of 155 mM^{-1} cm^{-1} and the results expressed as nmol g^{-1} on a fresh weight basis.

2.3.8 Soluble Proteins (SP)

The total soluble proteins were extracted according to Zimmermann et al. (2006), as modified by Ferreira-Silva et al. (2011). Yam samples of 0.1 g were macerated in a mortar in the presence of ice, with the addition of 100 mM Tris-HCl (pH 8.0) buffer containing 30 mM of DTT, 20% glycerol and 3% PEG-6000. After extraction, the extracts were centrifuged at 9,000 g for 30 minutes and 4 °C. The total soluble protein content was determined according to Bradford (1976), estimated using a standard curve prepared with bovine serum albumin (BSA). The results were expressed as $mg.g^{-1}$ on a fresh weight basis.

2.4 Experimental design and statistical analysis

The experimental design was completely randomized using a 2 × 8 factorial scheme, in which the first factor corresponded to the two packaging materials (polypropylene and multilayer nylon) and the second factor corresponded to the conservation times (0; 2; 4; 6; 8; 10; 12 and 14 days at 5 ± 2 °C), considering a visual analysis, weight loss, PPO activity and TSP contents, with three repetitions, where each 300 g package corresponded to one repetition. The POD and CAT activities, lipid peroxidation, soluble proteins and hydrogen peroxide were analysed on days 0; 7 and 14; and hence the factorial scheme for these analyses was 2 × 3: two packs and three conservation times, with three repetitions. All the data were

subjected to an analysis of variance using SISVAR 5.6 software (Ferreira, 2011), and the means and standard errors presented.

3 Results and discussion

In a previous study carried out by the same research group, the relationship between storage temperature and oxidative stress, causing the enzymatic browning of fresh-cut yam was investigated (Simões et al., 2016). This report showed that conservation at 5 °C promoted a high accumulation of soluble phenols and CAT activity and maintained the visual quality of the yam samples for 14 days.

In the present study, the influence of packaging on the visual quality, oxidative damage and antioxidant pathways of minimally processed yam was evaluated. In this report, evidence was provided that the appropriate packaging promoted high oxidative protection and consequently improved the conservation time of minimally processed yam with improved visual quality. It was found that the nylon multilayer (NY) package increased the storage time of yam, obtaining improved visual quality as compared to the polypropylene (PP) package, these results being related to the POD and CAT activities and the accumulation of soluble phenols, resulting in lower lipid peroxidation.

The statistical analyses (Table 1) showed significant and non-significant effects of the packaging and conservation times on the physiological and biochemical parameters analysed. The packaging influenced the visual analysis, fresh weight loss, PPO and CAT activities, TBARS and soluble protein content, whereas the conservation time provided significant effects on all the parameters analysed (Table 1). There was an interaction effect between the PPO activity, total soluble phenolic compounds, TBARS and soluble proteins (Table 1).

Table 1. Summary of the two-way analysis of variance (ANOVA) for the effects of packaging (nylon multilayer and polypropylene) and conservation time (0; 2, 4; 6; 8; 10 or 14 days or 0; 7 and 14 days) and their interactions with respect to the visual analysis (VA), fresh weight loss (FWL), polyphenoloxidase activity (PPO), total soluble phenolics content (TSP), peroxidase (POD) and catalase activities (CAT), hydrogen peroxide content (H_2O_2), lipid peroxidation (TBARS) and soluble protein contents (SP).

SV	df	Mean Square				
		VA	FWL	PPO	TSP	-
Packaging (P)	1	2.6759*	0.0822*	10083.68*	0.0118^{ns}	
Conservation times (CT)	7	10.2156*	0.0676*	1922.39*	0.3088*	
P * CT	7	0.2473^{ns}	0.0045^{ns}	609.20*	0.0800*	
Error	32	0.1527	0.0118	27.00	0.0073	
Total	**47**					
SV	**df**	**Mean Square**				
		POD	**CAT**	**H_2O_2**	**TBARS**	**SP**
Packaging (P)	1	0.2449^{ns}	4609.77*	0.0003^{ns}	712.2267*	42.6386*
Conservation times (CT)	2	0.3621*	30874.40*	0.2384*	776.8702*	6604.25*
P* CT	2	0.0835^{ns}	1152.98^{ns}	0.0122^{ns}	296.0977*	10.5693*
Error	12	0.0683	409.74	0.0571	31.5238	1.9867
Total	**17**					

*$p \leq 0.05$; ns = not significant ($p > 0.05$). Source of Variation; df: degree of freedom.

3.1 Effect of packages on the visual quality of minimally processed yam

The visual quality of minimally processed yams was reduced while fresh weight loss increased during conservation (Figure 1A, B). The decrease in visual scores was more significant in the PP package than in the NY package, where, after 12 and 14 days, the scores were less than 3 (Figure 1A). On the other hand, the scores of the minimally processed yams stored in nylon (NY) were always above 3 (Figure 1A), which can be related to the lower PPO activity (Figure 2B). PPO-triggered phenol oxidation produce substances (melanins) that promote surface browning of fruits and vegetables. In addition to its negative effect, PPO is

also believed to be involved in the oxidative degradation of ascorbate, a non-enzymatic antioxidant (Terefe et al., 2014). Thus, increased PPO activity causes direct effects on browning and contributes to an increase in ROS, such that the maintenance of its activity at lower levels in the yam samples in the NY packages (Figure 2B) must have been decisive in reducing browning. Also, fluorescence emission was not pronounced in NY-stored yam (Figure 3), which can have contributed to the maintenance of visual quality.

In general, the phenolic compound contents were similar regardless of the packaging type (Figure 2A). Nevertheless, after two and four days of storage, the phenolic compound contents were higher in the multilayer nylon packages, with a tendency to decrease up to the 8th day, as compared to the first time point (Figure 2A), which can be explained by oxidation via enzymatic processes, mainly due to the action of PPO (Tomás-Barberán & Espín, 2001). On the other hand, as from the tenth day, synthesis of phenolic compounds occurred due to the increase in oxidative stress, as indicated by the POD and CAT activities and TBARS contents (Figures 4A, B and D). The phenolic compounds are mediators that minimize stress-related damage and reactive oxygen species homeostasis (Uarrota & Maraschin, 2015). Thus, the higher phenolic compound contents in the yam samples stored in polypropylene packing on the tenth and twelfth days could also be related to an enhanced oxidative stress.

Although the fresh weight loss from the minimally processed yam samples increased in both packages during conservation (Figure 1B), the yams stored in polypropylene (PP) packages showed greater dehydration as compared to those stored in nylon (NY) packages. After 14 days, the fresh weight loss from the PP-packed yams (0.35%) was approximately three times that from the NY-packed yams (0.11%) (Figure 1B). The higher loss of fresh mass from the PP-packed yams can be related to the structure and conformation of the source material, providing a reduced barrier to H_2O diffusion permeability in relation to the NY packages. PVC packages showed a weight loss above 1% in minimally processed yam, which resulted in white spots (Donegá et al., 2013).

Fluorescence emission appeared on the surface of the yam slices after from 10 to 14 days storage in the polypropylene packaging (Figure 3). Furthermore, the surface became sticky, which can be related to the incidence of *Pseudomonas* ssp. (Simões et al., 2016). However, this not was evidenced in the yam slices stored in NY packaging. The reduced thickness of the PP packages in comparison with the NY ones might have favoured greater O_2 permeability, which promoted the development of *Pseudomonas* sp., since they have an aerobic metabolism (Sillankorva et al., 2004). Thus, the PP packages provided an adequate atmosphere for the development of *Pseudomonas* sp. and the occurrence of oxidative damage in the cells at the final time point analysed.

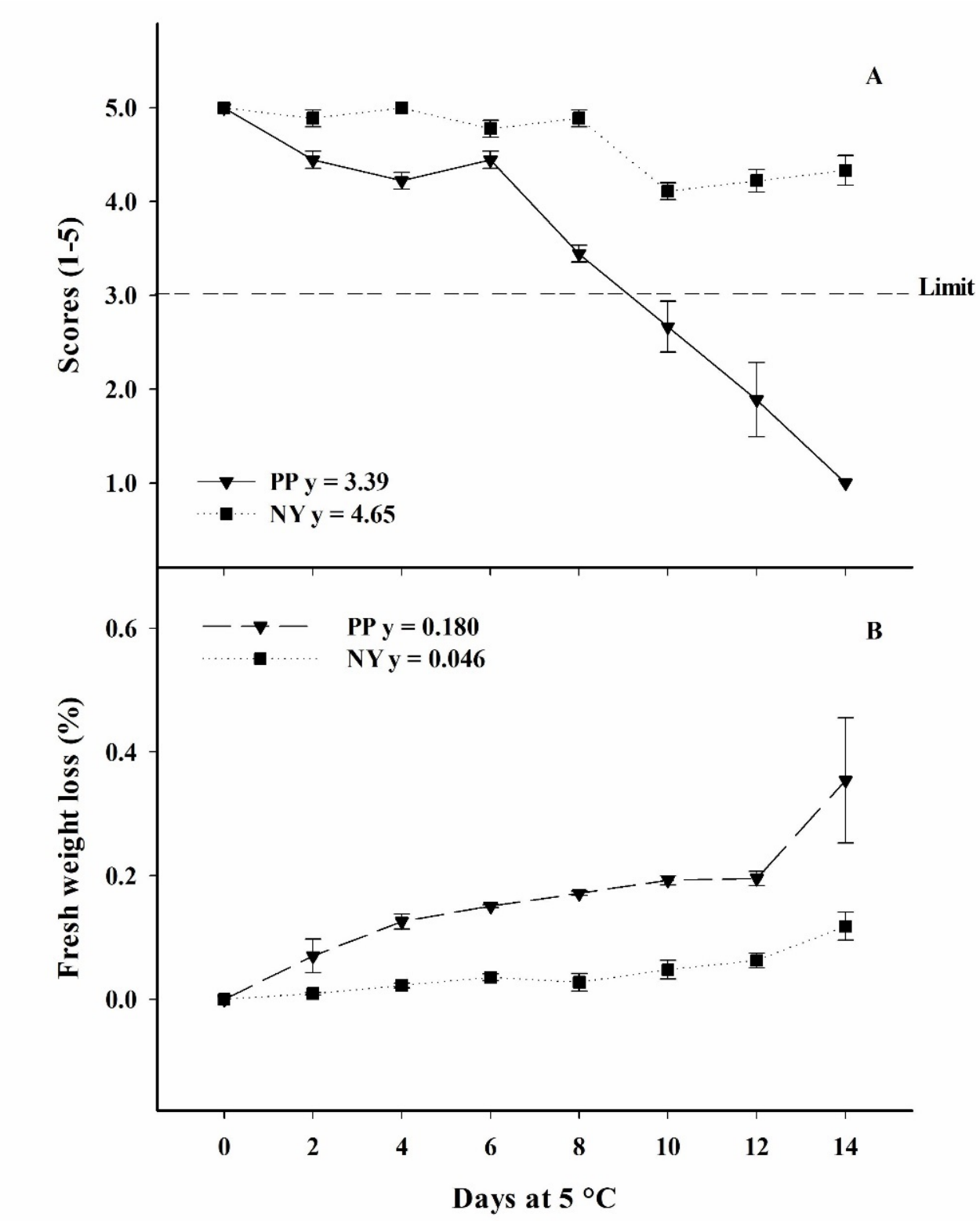

Figure 1. Visual analysis (A) and weight loss (B) in minimally processed yam stored in polypropylene and multilayer nylon packaging. The packages were stored at 5 ± 2 °C for 0; 2; 4; 6; 8; 10 or 14 days. The vertical bars represent the standard error. The segmented line (- - -) indicates the limit of acceptance of the final product, referring to note 3. Data from three replications.

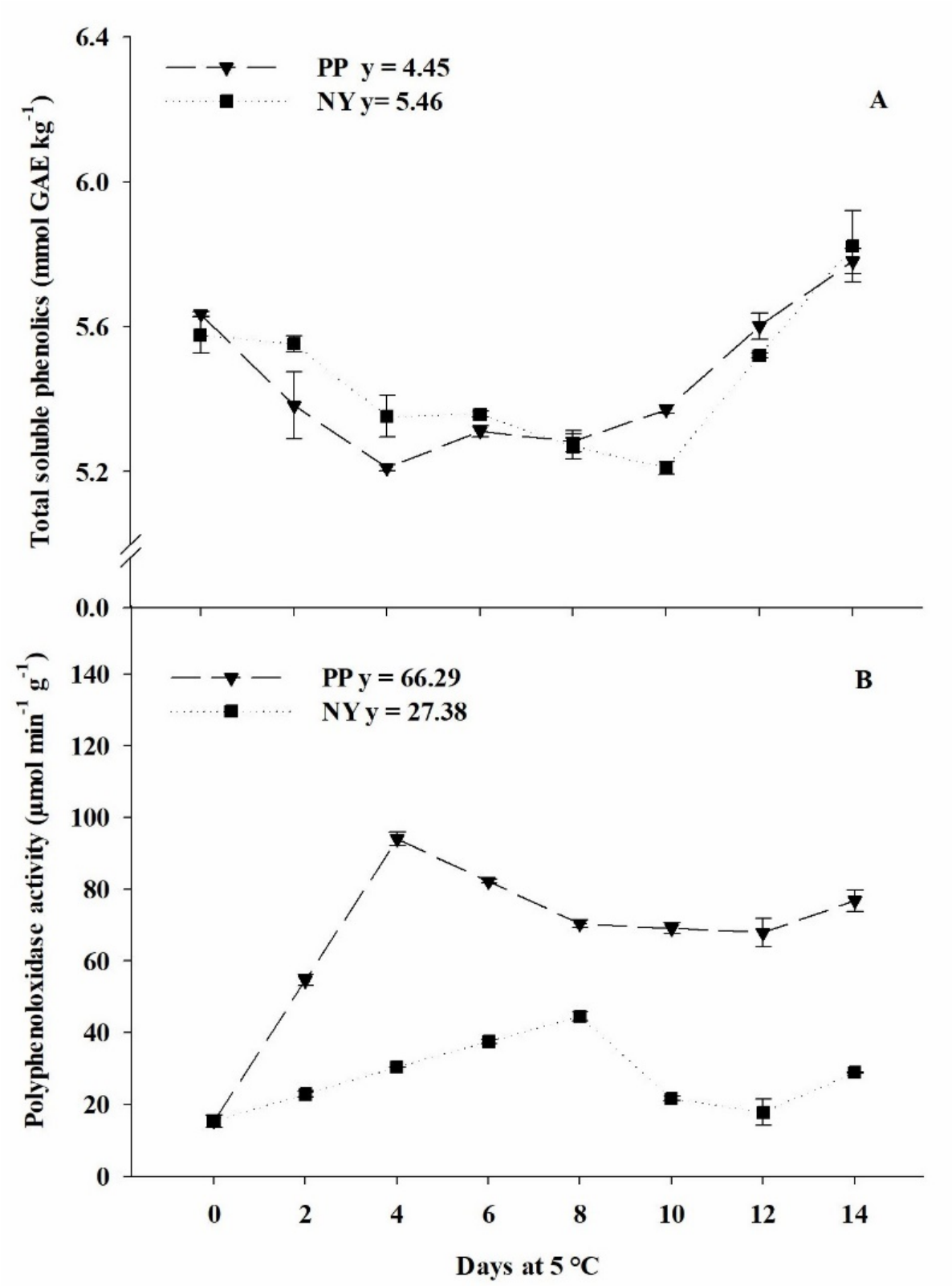

Figure 2. Total soluble phenolic compounds (A) and polyphenoloxidase activity (B) in minimally processed yam slices stored in polypropylene and multilayer nylon packaging. The packages were stored at 5 ± 2 °C for 0; 2, 4; 6; 8; 10 or 14 days. The vertical bars represent the standard error.

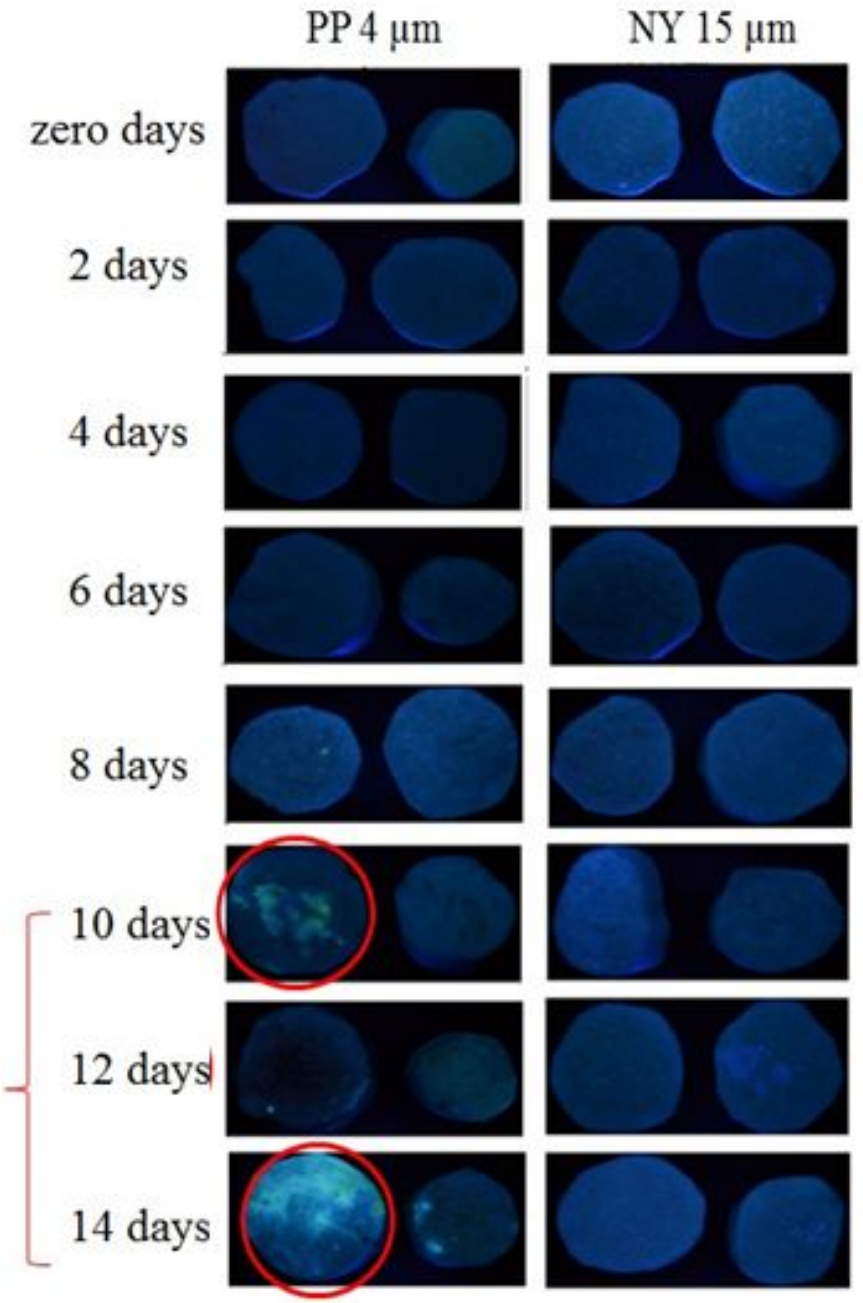

Figure 3. Fluorescence emission in minimally processed yam slices stored in polypropylene and multilayer nylon packaging. The packages were stored at 5 ± 2 °C for 0; 2; 4; 6; 8; 10 and 14 days. The red circles indicate fluorescence emission.

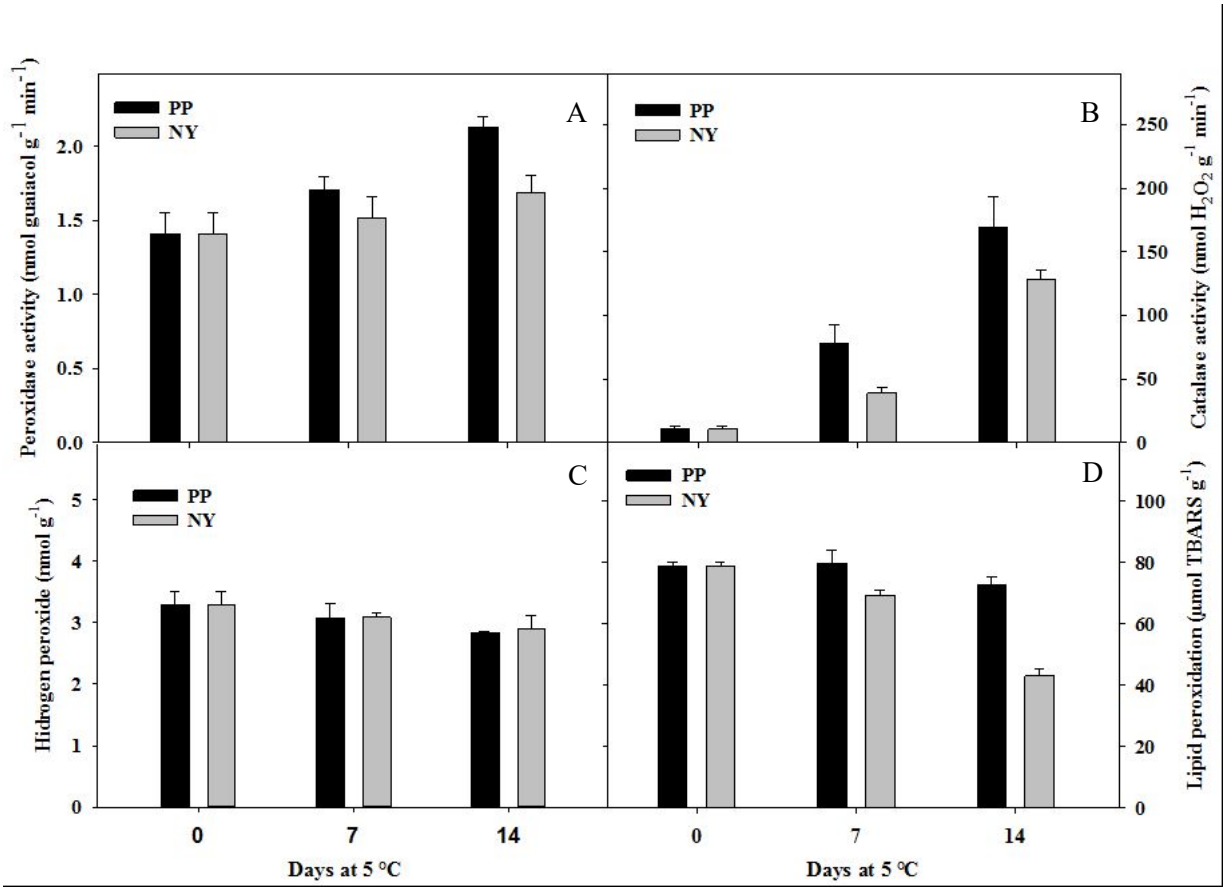

Figure 4. Peroxidase (A) and catalase (B) activities, hydrogen peroxide content (C) and lipid peroxidation (D) in minimally processed yam slices stored in polypropylene and multilayer nylon packaging. The packages were stored at 5 ± 2 °C for 0; 7 and 14 days. The vertical bars represent the standard error.

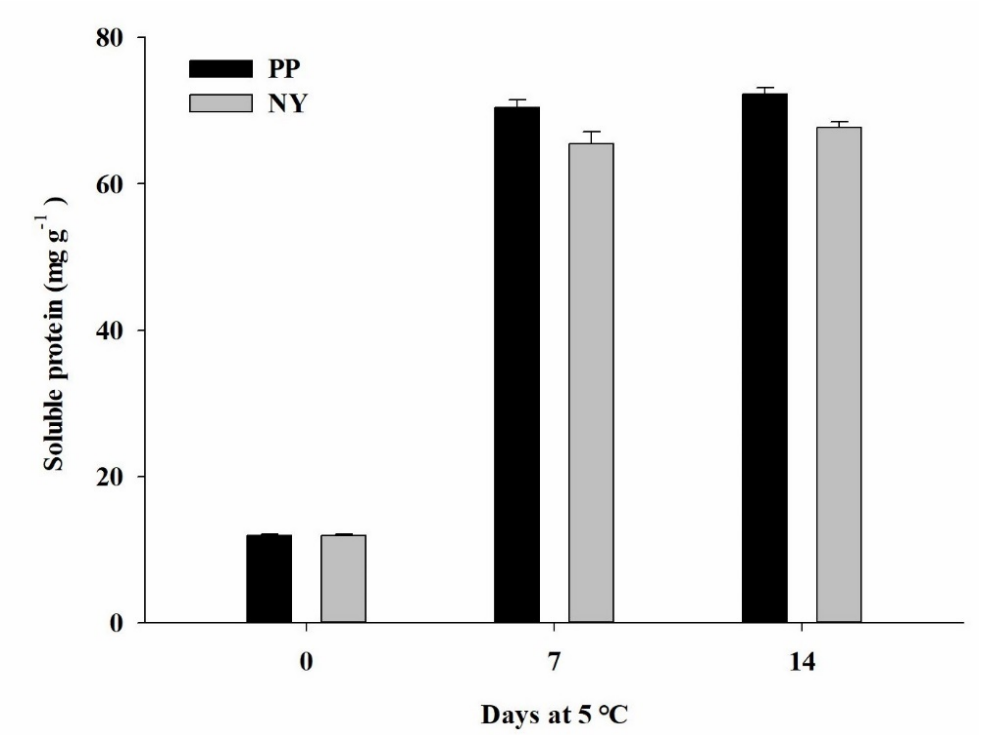

Figure 5. Soluble protein in minimally processed yam slices stored in polypropylene and multilayer nylon packaging. The packages were stored at 5 ± 2 °C for 0; 7 and 14 days. The vertical bars represent the standard error.

3.2 The influence of the packaging on the biochemical markers related to oxidative stress

The peroxidase (POD) activity increased by 33.8 and 16.2% in the PP and NY packages, respectively, after 14 days, in comparison with zero time (day 0) (Figure 4A). This also highlights the involvement of POD in the browning of the yam tissue stored in PP packaging, mediated by POD and PPO, as also reported by Omidiji et al. (2006). This indicates that yams stored in PP packaging were submitted to greater oxidative damage, resulting in browning of the tissue. POD establishes a strict correlation between the generation of ROS and enzymatic browning, considering that it uses H_2O_2 as the substrate, helping to maintain ionic homeostasis, but at the same time producing tetraguaiacol quinone, a brown substance. This indicates that both the levels of ROS and browning were lower in yams stored in multilayer nylon packages (Figures 1A, 4A and 4C).

The activity of CAT increased about 16 times in the PP packages and was higher than in the NY packages (Figure 4B). It is believed that the increase in the activities of POD and CAT in the yams stored in PP packages may indicate enhanced oxidative damage as a result of the action of reactive oxygen species (ROS). This might have induced the increase in CAT activity to remove the H_2O_2 (Figure 4B). The H_2O_2 content in the tissues of the minimally processed yams stored in both packages, reduced slightly during storage, with no differentiation between the packages (Figure 4C).

The higher CAT and POD activities in the yam slices stored in the PP packages can be associated with the greater reduction in the H_2O_2 content during storage, since the enzymes use peroxide in their reactions (Foyer & Noctor, 2005). Of the protective enzymes, CAT was more efficient because it showed the greatest increases in activity during storage, which indicates that this enzyme is more active in the removal of ROS from minimally processed yam slices.

Abbasi et al. (2016) showed that different packaging promoted discrepant changes in the physiological and biochemical characteristics of potato, such as in the activityies of the PPO and POD enzymes. Hong & Krochta (2003) emphasized that good oxygen-barrier properties were critical to provide a long shelf life for packaged products. Therefore, the high oxidative stress in PP-packed yam slices could be due to the high

oxygen permeability of this packaging material, providing greater membrane peroxidation (Figure 4D) and high oxygen availability for polyphenol oxidations by PPO (Figure 2B).

Lipid peroxidation (TBARS), which has often been used as an indicator of oxidative membrane damage, decreased during storage, and was 14 and 41% lower in the yam slices stored in NY packages for 7 and 14 days, respectively (Figure 4D), probably due to protection by peroxidases and catalases (Figures 4A and 4B). Membrane damage is a key process in the occurrence of enzymatic browning because it releases enzymes that spread through the tissue, and come into contact with their substrates, previously found in distinct compartments (Oms-Oliu et al., 2010). Thus, the greater lipid peroxidation in yam slices stored in polypropylene packages (Figure 4D), contributed to the intensification of browning.

An increase in soluble proteins was verified after 7 and 14 days, as compared to day zero (Figure 5), maybe due to protein synthesis, rather than denaturation caused by ROS (Neill et al., 2002). ROS also play a key role in signalling and defence, as has been debated in several recent studies, contrary to the paradigm that ROS generation is negative and its elimination is positive (Foyer et al., 2017). However, in minimally processed roots, the oxidative burst due to the cutting and handling procedures and ROS production are closely linked to enzymatic browning (Zidenga et al., 2012), compromising the visual aspect, one of the first aspects to be observed by the consumer. Thus the use of effective alternatives to minimize the processes that cause browning, such as the use of suitable packages, is highly important.

The results of Simões et al. (2016) provided evidence that the conservation of fresh-cut yam at low temperature (5 °C) promoted an increase in CAT activity and preservation of the phenolic compound contents, reducing oxidative damage and enzymatic browning, as compared to those conserved at a higher temperature (10 °C). In the present study, the use of PP packaging resulted in greater oxidative damage, as evidenced by lipid peroxidation, although there was greater POD and CAT activity as compared to NY packaging. This shows that enzymatic and non-enzymatic protection is not sufficient to maintain the quality for a long time if the storage conditions continue to be favourable for oxidative damage. Thus it was evident that the packaging interfered with the ROS generation and elimination processes, and consequently, in the enzymatic browning of minimally processed yam.

4 Conclusions

Multilayer nylon packaging provided less dehydration and less evidence of cellular damage through lipid peroxidation of minimally processed yam, resulting in a greater accumulation of soluble phenols and greater protection by POD and CAT as compared to polypropylene packaging. Consequently, this resulted in less browning of the minimally processed yam slices. These evidences represent good physicochemical, biochemical and physiological markers of minimally processed yam slices and show that they maintained good quality for 14 days. Therefore, NY packaging is more appropriate for the conservation of minimally processed yam at 5 ± 2 °C.

Acknowledgements

The authors are grateful to the *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* (FACEPE), for funding the research project (APQ-1029-5.01/10; APQ-0795-5.01/16) for the fellowship (IBPG-0588-5.01/11) to the sixth author. And the They are also grateful to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for funding the research project Proc. 88881-159183/2017-01 and for awarding a fellowship to the seventh author.

References

Abbasi, K. S., Masud, T., Qayyum, A., Khan, S. U., Ahmad, A., Mehmood, A., Farid, A., Jenks, M. A. (2016). Transition in quality attributes of potato under different packaging systems during storage. *Journal of Applied Botany and Food Quality*, *89*(1), 1-11.

Andrade, R. D., Palacio, J. C., Pacheco, W. A., & Betin, R. A. (2012). Almacenamiento de trozos de ñame (*Dioscorea rotundata* Poir) en Atmósferas Modificadas. *Información Tecnológica*, *23*(4), 65-72. http://dx.doi.org/10.4067/S0718-07642012000400008

Barrett, D. M., Beaulieu, J. C., & Shewfelt, R. (2010). Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. *Critical Reviews in Food Science and Nutrition*, *50*(5), 369-389. PMid:20373184. http://dx.doi.org/10.1080/10408391003626322

Beers Junior, R. F., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *The Journal of Biological Chemistry*, *195*(1), 133-140. PMid:14938361.

Belay, Z. A., Caleb, O. J., & Opara, U. L. (2016). Modelling approaches for designing and evaluating the performance of modified atmosphere packaging (MAP) systems for fresh produce: a review. *Food Packaging and Shelf Life*, *10*(1), 1-15. http://dx.doi.org/10.1016/j.fpsl.2016.08.001

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. PMid:942051. http://dx.doi.org/10.1016/0003-2697(76)90527-3

Brecht, J. K. (1995). Physiology of lightly processed fruits and vegetables. *HortScience*, *30*(1), 18-22. http://dx.doi.org/10.21273/HORTSCI.30.1.18

Cheeseman, J. M. (2006). Hydrogen peroxide concentrations in leaves under natural conditions. *Journal of Experimental Botany*, *57*(10), 2435-2444. PMid:16766599. http://dx.doi.org/10.1093/jxb/erl004

Demidchik, V. (2015). Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. *Environmental and Experimental Botany*, *109*(1), 212-228. http://dx.doi.org/10.1016/j.envexpbot.2014.06.021

Donegá, M. A., Tessmer, M. A., Mooz, E. D., Dall'Orto, L. T., Sasaki, F. F., & Kluge, R. A. (2013). Fresh cut yam stored under different temperatures. *Horticultura Brasileira*, *31*(2), 248-254. http://dx.doi.org/10.1590/S0102-05362013000200012

Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, *35*(6), 1039-1042. http://dx.doi.org/10.1590/S1413-70542011000600001

Ferreira-Silva, S. L., Voigt, E. L., Silva, E. N., Maia, J. M., Fontenele, A. V., & Silveira, J. A. G. (2011). High temperature positively modulates oxidative protection in salt-stressed cashew plants. *Environmental and Experimental Botany*, *74*(1), 162-170. http://dx.doi.org/10.1016/j.envexpbot.2011.05.015

Foyer, C. H., & Noctor, G. (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant Cell*, *17*(7), 1866-1875. PMid:15987996. http://dx.doi.org/10.1105/tpc.105.033589

Foyer, C. H., Ruban, A. V., & Noctor, G. (2017). Viewing oxidative stress through the lens of oxidative signaling rather than damage. *The Biochemical Journal*, *474*(6), 877-883. PMid:28270560. http://dx.doi.org/10.1042/BCJ20160814

Freire, C. S., Simões, A. N., Barros Júnior, A. P., Vieira, M. R. S., Ferreira-Silva, S. L., & Silva, E. F. (2015). Activity of oxidative enzymes involved in the browning of minimally processed sweet cassava (*Manihot esculenta* Crantz). *Australian Journal of Crop Science*, *9*(4), 296-302.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, *125*(1), 189-198. PMid:5655425. http://dx.doi.org/10.1016/0003-9861(68)90654-1

Hong, S. I., & Kim, D. M. (2001). Influence of oxygen concentration and temperature on respiratory characteristics of fresh-cut green onion. *International Journal of Food Science & Technology*, *36*(3), 283-289. http://dx.doi.org/10.1046/j.1365-2621.2001.00456.x

Hong, S. I., & Krochta, J. M. (2003). Oxygen barrier properties of whey protein isolate coatings on polypropylene films. *Journal of Food Science*, *68*(1), 224-228. http://dx.doi.org/10.1111/j.1365-2621.2003.tb14143.x

Imlay, J. A. (2003). Pathways of oxidative damage. *Annual Review of Microbiology*, *57*(1), 395-418. PMid:14527285. http://dx.doi.org/10.1146/annurev.micro.57.030502.090938

Junqueira, M. D. S., Soares, N. D. F. F., Reis, R. C., Carneiro, J. D. D. S., Benicio, R. T., & Yokota, S. R. C. (2009). Effect of active packages on enzymatic browning in fresh-cut potatoes (*Solanum tuberosum*). *Semina: Ciências Agrárias*, *30*(3), 613-618. http://dx.doi.org/10.5433/1679-0359.2009v30n3p613

Junqueira, M. S., Simões, A. N., Sediyama, T., Côrrea, P. C., & Puschmann, R. (2014). Biochemical and bioactive phytonutrients changes in tissues of two cultivars of fresh-cut cassava in stick form under refrigerated storage. *Ciência Rural*, *44*(7), 1284-1290. http://dx.doi.org/10.1590/0103-8478cr20120141

Menolli, L. N., Finger, F. L., Puiatti, M., Barbosa, J. M., & Barros, R. S. (2008). Role of oxidative enzymes on the darkening induced by chilling of arracacha roots. *Acta Scientiarum. Agronomy*, *30*(1), 57-63.

Neill, S. J., Desikan, R., Clarke, A., Hurst, R. D., & Hancock, J. T. (2002). Hydrogen peroxide and nitric oxide as signalling molecules in plants. *Journal of Experimental Botany*, *53*(372), 1237-1247. PMid:11997372. http://dx.doi.org/10.1093/jexbot/53.372.1237

Omidiji, O., Okpuzor, J. E., & Otubu, O. (2006). The contribution of an ionic peroxidase isozyme to enzyme-mediated browning in *Dioscorea esculenta* L. tubers. *Pakistan Journal of Nutrition*, *5*(5), 478-480. http://dx.doi.org/10.3923/pjn.2006.478.480

Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., Munuera, I. P., Fiszman, S., & Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. *Postharvest Biology and Technology*, *57*(3), 139-148. http://dx.doi.org/10.1016/j.postharvbio.2010.04.001

Reyes, L. F., Villarreal, J. E., & Cisneros-Zevallos, L. (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, *101*(3), 1254-1262. http://dx.doi.org/10.1016/j.foodchem.2006.03.032

Sillankorva, S., Oliveira, R., Vieira, M. J., Sutherland, I., & Azeredo, J. (2004). Bacteriophage Φ S1 infection of *Pseudomonas fluorescens* planktonic cells versus biofilms. *Biofouling*, *20*(3), 133-138. PMid:15545062. http://dx.doi.org/10.1080/08927010410001723834

Silveira, A. C., Oyarzún, D., Sepúlveda, A., & Escalona, V. (2017). Effect of genotype, raw-material storage time and cut type on native potato suitability for fresh-cut elaboration. *Postharvest Biology and Technology*, *128*(1), 1-10. http://dx.doi.org/10.1016/j.postharvbio.2017.01.011

Simões, A. N., Freire, C. S., Silva, E. F., Barros Júnior, A. P., & Ferreira-Silva, S. L. (2016). Quality of minimally processed yam (*Dioscorea* sp.) stored at two different temperatures. *Revista Caatinga*, *29*(1), 25-36. http://dx.doi.org/10.1590/1983-21252016v29n104rc

Terefe, N. S., Buckow, R., & Versteeg, C. (2014). Quality-related enzymes in fruit and vegetable products: effects of novel food processing technologies, part 1: high-pressure processing. *Critical Reviews in Food Science and Nutrition*, *54*(1), 24-63. PMid:24188232. http://dx.doi.org/10.1080/10408398.2011.566946

Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture*, *81*(9), 853-876. http://dx.doi.org/10.1002/jsfa.885

Uarrota, V. G., & Maraschin, M. (2015). Metabolomic, enzymatic, and histochemical analyzes of cassava roots during postharvest physiological deterioration. *BMC Research Notes*, *8*(1), 648-662. PMid:26541143. http://dx.doi.org/10.1186/s13104-015-1580-3

United States Department of Agriculture – USDA. (2013). *Nutrient values and weights are for edible portion*. Retrieved in 2016, November 28, from http://www.ars.usda.gov/Services/docs.htm?docid=8964

Weerarathne, L. V. Y., Marambe, B., & Chauhan, B. S. (2017). Intercropping as an effective component of integrated weed management in tropical root and tuber crops: A review. *Crop Protection*, *95*(1), 89-100. http://dx.doi.org/10.1016/j.cropro.2016.08.010

Wilson, M. D., Stanley, R. A., Eyles, A., & Ross, T. (2019). Innovative processes and technologies for modified atmosphere packaging of fresh and fresh-cut fruits and vegetables. *Critical reviews in food science and nutrition*, *59*(3), 411-422. PMid:28891686.

Xu, J., Duan, X., Yang, J., Beeching, J. R., & Zhang, P. (2013). Enhanced reactive oxygen species scavenging by overproduction of superoxide dismutase and catalase delays postharvest physiological deterioration of cassava storage roots. *Plant Physiology*, *161*(3), 1517-1528. PMid:23344905. http://dx.doi.org/10.1104/pp.112.212803

Zhang, M., Meng, X., Bhandari, B., Fang, Z., & Chen, H. (2015). Recent application of modified atmosphere packaging (MAP) in fresh and fresh-cut foods. *Food Reviews International*, *31*(2), 172-193. http://dx.doi.org/10.1080/87559129.2014.981826

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*(4), 1396-1407. PMid:22711743. http://dx.doi.org/10.1104/pp.112.200345

Zimmermann, P., Heinlein, C., Orendi, G., & Zentgraf, U. (2006). Senescence-specific regulation of catalases in Arabidopsis thaliana (L.) Heynh. *Plant, Cell & Environment*, *29*(6), 1049-1060.

Funding: FACEPE (APQ-APQ-1029-5.01/10; APQ-0795-5.01/16 IBPG-0588-5.01/11) and CAPES (Proc. 88881-159183/2017-01.

Received: June 28, 2017; Accepted: Jan. 03, 2019