QUALITY OF MINIMALLY PROCESSED YAM (*Dioscorea* sp.) STORED AT TWO DIFFERENT TEMPERATURES

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**ABSTRACT** – This work studied the physical, chemical and biochemical alterations in minimally processed yam stored at two different temperatures, as well as the incidence of bacteria of the genus *Pseudomonas*. The experimental design was completely randomised in a 2x8 factorial design, with two storage temperatures (5 and 10°C) and eight storage times (0, 2, 4, 6, 8, 10, 12 and 14 days). Experiments were in triplicate. Yam was selected, peeled and cut into slices of approximately 3 cm thickness. The slices were rinsed with water, sanitised and then drained in kitchen strainers. Approximately 300 g of the processed product were packed in nylon multilayers 15 µm thick, 15 cm wide and 20 cm long. The packs were sealed, weighed and kept at 5 and 10 ± 2°C for 14 days. Fresh weight loss, baking time, enzymatic activity of polyphenol oxidases, peroxidases and catalases, total soluble phenol content, and antioxidant capacity were evaluated, as well as visual analysis and incidence of *Pseudomonas* sp. Means of temperatures were compared by Tukey’s test at 5% significance. Yam storage at 5°C reduced weight loss and kept visual quality for longer; it also reduced cooking time and the activity of the enzymes polyphenol oxidase and peroxidase. In contrast, it promoted higher content of total soluble phenols, as well as a higher catalase activity and antioxidant capacity. During the storage time, there was no incidence of *Pseudomonas* sp. Minimally processed yam stored at 10°C may be sold for up to six days, and yam stored at 5ºC for up to 14 days.

**Keywords**: Enzymatic activity. Post-harvest conservation. *Dioscorea* sp.. *Pseudomonas* sp.

QUALIDADE DO INHAME (*Dioscorea* sp.) MINIMAMENTE PROCESSADO CONSERVADO EM DUAS TEMPERATURAS

RESUMO – O objetivo deste trabalho foi estudar as alterações físico-químicas, bioquímicas e incidência de *Pseudomonas* sp. em inhame minimamente processado conservado em duas temperaturas. O delineamento experimental utilizado foi o inteiramente casualizado em esquema fatorial 2x8, referente as temperaturas (5 e 10 ºC) e tempos de conservação refrigerada (0, 2, 4, 6, 8, 10, 12 e 14 dias), com três repetições. O inhame foi selecionado, descascado e cortado em rodelas de aproximadamente 3 cm de espessura. Essas rodelas foram enxaguadas em água, sanitizadas e drenadas em escorredores de cozinha. Aproximadamente 300 g do produto processado foram embalados em Nylon multicamadas de 15 µm de espessura, com 15 cm de largura por 20 cm de comprimento. As embalagens foram seladas, pesadas e mantidas a 5 e 10 ± 2 ºC por 14 dias. Avaliou-se a perda de massa, tempo de cocção, atividade enzimática das polifenoloxidases e peroxidases, conteúdo de fenóis solúveis totais, capacidade antioxidante, análise visual e incidência de *Pseudomonas* sp. As médias entre as temperaturas foram comparadas pelo teste de Tukey a 5 % de significância. A temperatura de 5 ºC reduziu a perda de massa fresca e da qualidade visual, o tempo de cocção e a atividade das enzimas polifenoloxidases e peroxidases. Por outro lado, proporcionou maior conteúdo de fenóis solúveis totais e capacidade antioxidante. Ao longo da conservação, não foi observada incidência de *Pseudomonas* sp. O inhame minimamente processado, sob temperatura de 10 ºC, pode ser comercializado por até 6 dias. A 5ºC, por até 14 dias.


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INTRODUCTION

In Brazil, the yam production (Dioscorea sp.) is about 244,000 tons annually, distributed over 25,000 hectares (FAOSTAT, 2011). The North-East has the largest production - about 90% - mainly in the states of Paraíba, Bahia, Alagoas, Sergipe, Pernambuco and Maranhão (SANTOS et al., 2007).

Commercialisation of yam is carried out without any beneficiation. Losses during storage are caused by insects, microorganisms and inadequate transport (PEIXOTO NETO et al., 2000). Moreover, the way the tubers are commercially kept, with soil and organic matter adhered to the surface, reduces their market value and adversely affects their storage life.

Minimal processing is an alternative to add value to the yam. Hereby, inedible parts are removed, leaving the product ready for consumption or preparation (HOWARD; GRIFFIN, 1993). However, the physical changes caused by minimal processing promote similar physiological responses as observed in plants under stress (BRECHT, 1995). There are reports of increased respiration rates, ethylene synthesis and enzymatic reactions (WATADA; ABE ; YAMUCHI, 1990), as well as dehydration intensification (BRECHT, 1995).

Yam darkens quickly after cutting. The first visible symptoms of deterioration are the presence of brownish stains on the surface. Dehydration in its tissues also results in starch deposition on the surface, causing a whitish appearance (DONEGÁ et al., 2013). In addition, there are changes in the attributes related to the quality of minimally processed foods, such as increased soluble solids, total soluble phenols, polyphenoloxidase activity and a pH reduction (FURTADO, 2013).

In another tuber, cassava (Manihot esculenta Crantz), quality loss occurs due to the increased production of reactive oxygen species (ROS), which promote the breakdown of cell membranes (XU et al., 2013). In its minimally processed form, cassava shows physiological changes during storage, such as a reduction in soluble solids (FREIRE et al., 2014) and total soluble phenols increased activity of polyphenoloxidases and peroxidases (FREIRE et al., 2015).

In recent studies on minimally processed yam, experiments with suitable packaging, edible coating, refrigeration temperatures (BRITO, 2011; ANDRADE et al., 2012; FURTADO, 2013; DONEGÁ et al., 2013), and alternative packaging were undertaken (SILVA, 2009).

Suitable storage temperatures can minimise the damage caused by cutting, and reducing storage temperature by 10°C leads to a two- or three-fold decrease in metabolic rates (BRECH, 1995). Controlling storage temperature can also minimise enzymatic activities (FATIBELLO-FILHO; VIEIRA, 2002). Refrigeration is a traditional way to reduce the activity of the enzyme polyphenoloxidase (SILVA et al., 2009), however, in supermarkets and commercial centres throughout Brazil, products are kept at temperatures above 5°C for economic reasons.

This work studied the physical, chemical and biochemical alterations of minimally processed yam stored at 5 and 10°C and the incidence of bacteria of the genus Pseudomonas.

MATERIALS AND METHODS

The study was conducted at the Universidade Federal Rural de Pernambuco/Unidade Acadêmica de Serra Talhada. Physiologically mature yam was purchased from producers in the region of Petrolina - PE. The tubers were selected for size, visual appearance, and integrity, and damaged tubers were discarded. They were then washed under running water and kept at 8°C for 24 h.

The experimental design was a completely randomised design (CRD) with two factors: storage temperature (5 and 10 ± 2 °C) and storage time after minimal processing (0, 2, 4, 6, 8, 10, 12 and 14 days).

We adopted the minimal processing methodology from Brito (2011). The tubers were peeled and cut into slices about 3 cm thick with stainless steel knives previously sanitised in chlorinated water with 200 mg L^{-1} of active chlorine (3% sodium dichloroisocyanurate dihydrate). For the initial rinse, slices were immersed in water at 5 ± 2°C for 5 min. Sanitisation was carried out by immersing about 2 kg of slices in chlorinated water with 200 mg L^{-1} of active chlorine for ten min. For the final rinse, slices were rinsed by immersing in chlorinated water with 5 mg L^{-1} of active chlorine for five min. Drainage was kept in dish strainers for ten minutes.

Approximately 300 g of slices were packed in multilayer nylon bags 15 µm thick (NY 15), 15 cm wide and 20 cm long. The bags were sealed with a handmade sealer (40 cm pedal; plasmatic), weighted on a semi-analytical balance (ARD110; OHAUS®), and kept in vertical displays with forced air circulation (VB40W, Metalfrio) at 5 and 10 ± 2°C for 14 days. The following parameters were evaluated:

a) Fresh weight loss was determined gravimetrically by weighing the samples before storage and after different storage times on a semi-analytical balance (ARD110; OHAUS®). The percentage of fresh weight loss was estimated from the difference between the initial and the final weight.

b) Baking time was estimated according to Andrade (2013). A stainless steel container with a vent and 2 L capacity was filled with 1 L water.
which was kept simmering on a cooking stove. When the water reached the boiling point, 100 g of yam slices were added and the container capped. Baking time of yam was defined as the time in minutes needed to soften the tissue so that a stainless fork can penetrate the centre of the slice without resistance.

c) Extraction and determination of polyphenoloxidase activity (PPO, EC 1.14.18.1) were carried out as described by Silva (1981) and adapted by Simões et al. (2015). A sample of 0.25 g with a height of 1,000 mm, 1000 mm width, and 5 mm thickness from the surface tissue located on the equatorial side of the yam slice. The sample was macerated in a mortar with 6 mL phosphate buffer (0.2 M, pH 6.0) previously kept at 4°C. The extract was then centrifuged (Universal 320 R; Hettich) with 9,000 g for 21 min at 4°C. Determination of polyphenoloxidase activity was carried out following the methodology described by Coelho (2001). In brief, we used a mixture of 1 mL phosphate buffer (0.2 M, pH 6.0) and 1.5 mL catechol (0.2 M) as substrate. We then added 50 µL of the enzymatic extract (supernatant). For the white one, we used the enzymatic extract boiled for 10 min in water bath (TE-054/te-056; Tecnal, SP, Brazil), while the other analytical procedures remained unchanged. Samples were spectrophotometrically measured (Libra S8; Biochrom) at 425 nm for 2 min with successive readings every 30 sec. Polyphenoloxidase activity was calculated using the molar extinction coefficient of 2.95 mM potassium phosphate buffer with hydrogen peroxide (20 mM) and 0.05 mL of the extract (supernatant). For the white one, we used 3 mL of potassium phosphate buffer, while the other analytical procedures remained unchanged. Samples were spectrophotometrically measured (Libra S8; Biochrom) at 240 nm for 3 min with successive readings every 30 sec; temperature was kept at 30°C. Enzyme activity was calculated using the molar extinction coefficient of 36 M⁻¹ cm⁻¹ and results were expressed as nmol H₂O₂ per gram fresh weight per minute.

d) Extraction and determination of the peroxidase activity (POD, EC 1.11.1.7) were carried out following the methodology described by Silva (1981) and adapted by Simões et al. (2015). We used a sample of 0.25 g with a height of 1,000 mm, a width of 1,000 mm, and a thickness of 5 mm from the surface tissue located on the equatorial side of the yam slice. The sample was macerated in a mortar with 6 mL phosphate buffer (0.2 M, pH 6.0) previously kept at 4°C. The extract was then centrifuged (Universal 320 R; Hettich) with 9,000 g for 21 min at 4°C. Determination of peroxidase activity was performed using a mixture containing 1300 mM potassium phosphate buffer (50 mM, pH 7.0). The extract was then centrifuged (Universal 320 R; Hettich) with 9,000 g for 21 min at 4°C. Determination of catalase activity was performed using a mixture containing 2.95 mM potassium phosphate buffer with hydrogen peroxide (20 mM) and 0.05 mL of the extract (supernatant). For the white one, we used 10 mL of potassium phosphate buffer, while the other analytical procedures remained unchanged. Samples were spectrophotometrically measured (Libra S8; Biochrom) at 725 nm based on the calibration curve obtained with gallic acid. Results were expressed as µmol per gram fresh weight (FW) per minute.

e) Extraction and determination of catalase activity (CAT, EC: 1.11.1.6) was carried out according to the methodology described by Beers Júnior and Sizer (1952). We used a sample of 0.25 g with a height of 1,000 mm, 1000 mm width, and 5 mm in thickness from the surface tissue located on the equatorial side of the yam slice. The sample was macerated in a mortar with 6 mL potassium phosphate buffer (50 mM, pH 7.0). The extract was then centrifuged (Universal 320 R; Hettich) with 9,000 g for 21 min at 4°C. Determination of catalase activity was performed using a mixture containing 2.95 mM potassium phosphate buffer with hydrogen peroxide (20 mM) and 0.05 mL of the extract (supernatant). For the white one, we used 3 mL of potassium phosphate buffer, while the other analytical procedures remained unchanged. Samples were spectrophotometrically measured (Libra S8; Biochrom) at 725 nm based on the calibration curve obtained with gallic acid. Results were expressed as mmol H₂O₂ per gram fresh weight per minute.

f) Total soluble phenols were extracted and quantified according to the methodology described by REYES et al. (2007), with modifications. We used a sample of 0.25 g with a height of 1,000 mm, 1,000 mm width, and 5 mm in thickness from the surface tissue located on the equatorial side of the yam slice. The sample was macerated in a mortar with 10 mL pure methanol and kept in the dark at 4°C for 24 h. The extract was then centrifuged (Universal 320 R; Hettich) at 9,000 g for 21 minutes at 2°C. For the quantification of total soluble phenols we used 150 µL of the extract (supernatant) diluted in 2,400 µL distilled water and added 150 µL Folin Cioucauteu (0.25 N). The mixture was homogenised in a tube shaker (Biomixer; model QL-901) for 3 min and 300 µL of calcium carbonate (1 N) were added. The mixture was then stirred in a tube shaker (QL-901; Biomixer) and kept at room temperature for 2 h. The preparation of the white one consisted in replacing the supernatant by 150 µL of pure methanol while the other analytical procedures remained unchanged. Samples were spectrophotometrically measured (Libra S8; Biochrom) at 725 nm based on the calibration curve obtained with gallic acid. Results were expressed as mg per gram fresh weight.

g) Antioxidant capacity was determined in the same methanolic extract used to quantify total soluble phenols, following the methodology described by Brand-Williams et al. (1995) and modified by Sánchez-Moreno (2002). Determination of the antioxidant capacity was performed using a mixture containing 3,800 µL of a 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution at 60 µM with 200 µL of the extract (supernatant). The mixture was stirred in a tube shaker (QL-901; Biomixer) and kept in the dark for 30 min. Samples were
satisfactorily measured (Libra S8; Biochrom) at 515 nm. As a control, we used a mixture containing 3,800 µL of DPPH at 60 µM and 200 µL methanol. Antioxidant capacity was determined based on the calibration curve obtained with DPPH. Results were expressed as percentage of the radical scavenging activity (% RSA).

h) Visual analysis was performed by a trained panel based on a subjective rating scale ranging from five to one. Five referred to slices with a characteristic white surface, no indication of brownish spots, and appearance and odour suitable for consumption. Four referred to slices with colour changes in relation to the initial day, but with a quality suitable for commercialisation. Three referred to slices with up to 10% of the surface consisting of brownish stains with moderate intensity; this was defined as the acceptance limit. Two referred to slices with approximately 50% of the surface consisting of brownish stains, unfit for human consumption and with gas build-up in the package. One referred to slices showing all the symptoms described above and alcoholic odour, totally unfit for human consumption (SILVA, 2014).

i) Incidence of Pseudomonas sp was determined as follows: two representative yam slices of each treatment were selected and photographed using a semi-professional digital camera (Nikon; D3100 14.2 megapixels) coupled with a darkroom (CN-6; Vilber Lourmat) under ultraviolet light at 365 nm with a 1 x 6 Watts filter 1 x 6 Watts and 220 V 50/60 Hz (VL-6.1; Vilber Lourmat).

Analysis of variance (ANOVA) was carried out to test for differences among treatments. Mean values between the two temperatures were compared by Tukey’s test (p<0.05) using Sisvar® (version 5.0). Storage times were adjusted, when possible, to the regression equation using Table Curve® (2D version 5.1) (JANDEL SCIENTIFIC, 1991). Graphs were plotted using SigmaPlot® (version 10.0).

RESULTS AND DISCUSSION

We detected interactions between temperature and storage time for the parameters fresh weight loss, baking time, polyphenoloxidase and peroxidase activity, and antioxidant capacity. Still, it was observed effects of the isolated factors for visual analysis, catalase activity and content of total soluble phenols. Fresh weight loss was increased in minimally processed yam kept at 5 and 10°C. However, fresh weight loss in samples kept at 10°C was more intense with 0.35% and significantly different from day four of storage (Figure 1).
The storage temperature of 10°C may have intensified fresh weight loss due to increased metabolic activity. Increased weight loss was expected in minimally processed products as a result of increased water loss following cutting (BRECHT, 1995).

Fresh weight loss of yam kept at 5°C did not exceed 0.08%, which was well below the results observed by Donegá et al. (2013) who found a loss of 0.8%, in minimally processed yam packed in a polyvinyl chloride (PVC) film under the same temperature. We found that even considering the loss of fresh weight, storing yam at 5°C did not result in diminished quality as the starch deposition on the surface prevented the slices from drying-out. Donegá et al. (2013) observed similar results, however, PVC was used as packing material, which has lower thickness than our multilayer nylon packages and could have had influence on the findings.

Baking time of minimally processed yam decreased with storage time at both temperatures tested (Figure 2). Moreover, the reduction in baking time was more intense in yam stored at 5°C than in yam stored at 10°C. Significant differences in baking time between storage temperatures were observed from the fourth day of storage. Initial average baking time was 18.14 min. After 14 days of storage, baking time was reduced to 15 min for yam kept at 5°C and to 11.67 min for yam kept at 10°C.

Longer baking times of minimally processed yam stored at 10°C can be related to higher dehydration at this temperature (Fig 1).

In a similar experiment with beans (Phaseolus vulgaris L.), Coelho et al. (2008) found that the baking time was influenced by the hydration rate of the beans. Therefore, it is possible that yam stored at 10°C may have taken longer to absorb water during the baking process, therefore increasing the time required to reach the baking temperature. Furthermore, the temperature of 10°C may have boosted some defense mechanisms, causing increased lignin deposition in the walls of injured cells and therefore, hindering rehydration of the yam slices.

Polyphenoloxidases and peroxidase activities increased during yam storage at both temperatures (Figure 3). Slices stored at 10°C had significantly higher polyphenoloxidase and peroxidase activities from day two onwards. Menolli et al. (2008) observed similar results in baroa-potatoes (Arracacia xanthorrhiza). It might be possible that the slices stored at 10°C had higher respiration rates and therefore accumulated more CO₂, which leads to an increase in polyphenoloxidase activity.
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**Figure 3.** Polyphenol oxidase (A) and peroxidase activity (B) in roots of yam minimally processed stored at 5 ( ) and 10 ± 2 °C ( ) for 0, 2, 4, 6, 8, 10, 12 and 14 days. Serra Talhada – PE, UFRPE-UAST, 2014. The vertical bars in graphic B represent the standard deviation from the mean and the minimal significant difference (MSD) at 5 %. Data for three replications. Equal letters, that compare temperatures one the same day of conservation, they did not differ significantly by Tukey test at 5% probability.

In yam, peroxidase activities increase after peeling (OMIDIJI; OTUBU, 2006). In our study, the increased activities of polyphenoloxidase and peroxidase may be related to the darkening of the slices during storage (see figure 7).

Soluble phenol concentration of yam stored at...
5 and 10°C increased with storage, with significant differences from day six onwards (Figure 4).

Yam stored at 5°C showed higher mean values of total soluble phenol than yam stored at 5°C. Furtado (2013) found similar results in minimally processed yam stored at 8°C.

Baroa-potatoes stored at 5 and 10°C showed increased amount of total soluble phenols and a higher occurrence of dark spots, even with increase in polyphenoloxidase and peroxidase activity (MENOLLI et al., 2008). Medeiros (2009) associates increase in polyphenoloxidase activity with increased total soluble phenols. However, increased concentration of total soluble phenols in yam slices stored at 5°C (Figure 5), did not result in increased polyphenoloxidase and peroxidase activities in our study. These enzymes use total soluble phenols as substrate (WATADA; ABE; YAMUCHI, 1990), which may have led to higher oxidation rates in slices stored at 10°C; Freire et al. (2015) observed similar findings in minimally processed table cassava (Manihot esculenta). Furthermore, the relatively low temperature of 5°C may have promoted chilling injury and consequently a greater production of total soluble phenols; this was also observed by Menolli et al. (2008).

Antioxidant capacity of minimally processed yam stored at 5 and 10°C decreased with storage time. Yam kept at 5°C showed reduction in antioxidant capacity by 47.7% compared to 50.29% reduction in yam stored at 10°C (Figure 5).

Yam stored at 5°C had higher antioxidant capacity from the day two of storage, but the difference in antioxidant capacity was only significant on days two and four (Figure 5).

Total soluble phenols are highly correlated with the antioxidant capacity of plant tissue, and total soluble phenols may have reacted with the reactive oxygen species (ROS) (FERRARI; TORRES, 2003) acting as antioxidant.

Catalase activity in yam stored at 5°C increased with storage time (Figure 6). Storage at 10°C resulted in lower catalase activities. However, in contrast to peroxidase activity, which was higher in yam stored at 10°C, we found no significant difference in catalase activity between the two temperatures (Figure 3). This indicates that peroxidases consumed more hydrogen peroxide (H$_2$O$_2$) at 10°C. This was not observed in the case of catalases, which also use H$_2$O$_2$ as a substrate.
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**Figure 5.** Antioxidant capacity in roots of yam minimally processed stored at 5 (-----) e 10 ± 2 °C (-----) for 0, 2, 4, 6, 8, 10, 12 and 14 days. Serra Talhada – PE, UFRPE-UAST, 2014. Equal letters, that compare temperatures one the same day of conservation, they did not differ significantly by Tukey test at 5% probability.

**Figure 6.** Catalase activity in roots of yam minimally processed stored at 5 (-----) e 10 ± 2 °C (-----) for 0, 2, 4, 6, 8, 10, 12 and 14 days. Serra Talhada – PE, UFRPE-UAST, 2014. The vertical bars in graphic B represent the standard deviation from the mean and the minimal significant difference (MSD) at 5 %. Data for three replications. Equal letters, that compare temperatures one the same day of conservation, they did not differ significantly by Tukey test at 5% probability.
In terms of visual analysis, yam stored at 5°C received the highest scores throughout storage time and did not exceed the acceptance limit (Figure 7). In contrast, yam stored at 10°C received lower scores throughout storage time and reached the acceptance limit after a storage time of 14 days (Figure 7).

![Figure 7. Visual analysis (sensory acceptance) in roots of yam minimally processed stored at 5 ( ) e 10 ± 2 °C ( ) for 0, 2, 4, 6, 8, 10, 12 and 14 days. Serra Talhada – PE, UFRPE-UAST, 2014. The vertical bars in graphic represent the standard deviation from the mean and the minimal significant difference (MSD) at 5 %. Data for three replications. Equal letters, that compare temperatures one the same day of conservation, they did not differ significantly by Tukey test at 5% probability. The horizontal dashed line represents the limit for marketing, it is related to note 3.]

The decreased quality in yam slices stored at both temperatures may be related to increased polyphenoloxidase and peroxidase activities (Figure 3), resulting in the production of insoluble and dark pigments, melamins, which lead to a darkening of the slices (LAMIKANRA, 2002). Additionally, yam stored at 10°C had gas accumulated in the packaging, which was not observed for yam stored at 5°C.

Although there was no significant difference in visual appearance, temperature is a factor that can influence the visual quality of minimally processed yam.

In all samples, there was no incidence of psicotrophic bacteria of the genus *Pseudomonas* (Table 1). This result may be related to the use of multilayer nylon packaging, which has a low permeability for O₂ and may therefore have provided inadequate atmosphere for *Pseudomonas*. Most likely, it has also led to lower occurance of oxidative damage in cells and tissues (SILLANKORVA, 2004).
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Table 1. Incidence of *Pseudomonas* sp. in roots of yam minimally processed stored at 5 e 10 ± 2 °C for 0, 2, 4, 6, 8, 10, 12 and 14 days. Serra Talhada – PE, UFRPE-UAST, 2014.

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CONCLUSIONS

In general, minimally processed yam stored in 15 µm thick multilayer nylon packaging at 10°C can be kept for up to six days. In case, yam needs to be stored for up to 14 days, a temperature of 5°C is more appropriate.

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