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Fresh cut yam stored under different temperatures

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ABSTRACT

The objective of this study was to evaluate the microbiological, physiological and qualitative changes in fresh cut yam (*Dioscorea alata*) stored at different temperatures. The experimental design was completely randomized in factorial scheme 4x4, with four storage temperatures (5, 10, 15 and 20ºC) and four evaluation periods (0, 3, 6 and 9 days after processing) with four replications. The microbiological analyzes were performed at 0, 3 and 9 days after processing. Fresh cut yam stored at 5ºC kept respiration rate, ethylene production at low levels similar to those found in yam not processed and stored at 20ºC. The weight loss, lightness and psicotrophic bacteria counting in fresh cut yam stored at 5ºC were kept within the trading patterns until the third day.

Keywords: *Dioscorea alata*, respiration, ethylene, microbiology.

RESUMO

Inhame minimamente processado armazenado sob diferentes temperaturas

O objetivo deste trabalho foi avaliar as alterações microbiológicas, fisiológicas e qualitativas de inhame (*Dioscorea alata*) minimamente processado e armazenado em diferentes temperaturas. O delineamento experimental foi inteiramente casualizado em esquema fatorial 4x4, sendo, quatro temperaturas de armazenamento (5, 10, 15 e 20°C) e quatro períodos de avaliação (0, 3, 6 e 9 dias após o processamento), com quatro repetições. As análises microbiológicas foram realizadas aos 0, 3 e 9 dias após o processamento. A conservação do inhame minimamente processado armazenado a 5°C, manteve taxa respiratória e produção de etileno a níveis baixos semelhantes aos valores encontrados em inhame não processado e armazenado a 20°C. A perda de massa, luminosidade e contagem de bactérias psicotróficas do inhame mantido a 5°C esteve dentro dos padrões comerciais até o terceiro dia.

Palavras-chave: *Dioscorea alata*, respiração, etileno, microbiologia.

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The yam (*Dioscorea alata*) belongs to the Dioscoreaceae family, which has about 600 species, and 14 produce tubers used as food. The economic importance of this species is related to cultivation for human consumption due to high content of vitamins, minerals and carbohydrates (Bressan *et al.*, 2005). The small starch grains are responsible for the high digestibility, favoring the use of this food in the diet of children and elderly (Santos & Macedo, 2002; Mascarenhas & Resende, 2002).

Despite its prominence as a food crop, the yam is among the underutilized vegetables. This is due, besides other facts, to the change of the population culture that tends to spend less time cooking the meals and prefers to purchase more practical food to consume. The minimal processing of yam can be a factor of incentive in the increasing of the product demand. Other oleraceous with characteristics similar to yam (root vegetables and tubers) are already available in the market; stand out in this market niche, the cassava, the carrot, the beet root and the sweet potato.

The availability of the product ready to prepare is not synonymous of consumer acceptance, who looks for products that are easy to prepare and that keep the characteristics of fresh product *in natura.* The preservation of fresh cut products become critical because of mechanical injuries caused in tissues, which leads to a decrease in shelf-life of the food, considering that the damaged product becomes more sensitive to micro-organism attack which cause deterioration, besides increasing the proliferation of pathogenic agents (Durigan *et al*., 2002).

Among the techniques used to increase the shelf life, storage at low temperatures, the use of modified atmospheres and application of preservatives stand out. The use of low temperature is essential not only for the preparation but also for the maintenance of the fresh cut products. In this environment, metabolism and respiration rate are reduced, besides other physiological, biochemical and microbiological processes, which cause deterioration. However, it is common to observe that several times the fresh cut products are exposed to temperatures higher than those recommended as optimal, which increases the deterioration and risks to human health.

Considering the lack of information about minimally processing of yam, this study aimed to evaluate the physiological, microbiological and qualitative alterations of the fresh cut yam stored at different temperatures.

MATERIAL AND METHODS

Tubers of yam (*Dioscorea alata*) were purchased in the retail trade in the city of São Paulo, Brazil, transported to the laboratory USP-ESALQ and selected for the absence of mechanical

damage defects, visible infections and uniformity in size of the tubers. The procedure was initially with detergent and running water at 5ºC in order to remove the field heat. The tubers were sanitized by immersion in a solution containing active chlorine (200 mg L^{-1}) for five minutes. Then they were peeled manually, followed by withdrawal of its apex and its base. Cuts were made in slices (2.5 cm thick), using a sharp blade made of stainless steel. After cutting, the yams were immersed in water containing active chlorine (200 mg L^{-1}) and immediately centrifuged for 3 minutes, for water drainage. The product was weighed and packaged in polystyrene tray covered with PVC film (12 µm). In each container were placed about 500 g of the product. The containers were stored at 5, 10, 15 and 20^{0}C, with relative humidity (RH) at 90±5% for 9 days.

Physiochemical and anatomic evaluations were performed at 0, 3, 6 and 9 days of storage, while microbiological analyses were performed at 0, 3, and 9 days after the processing. The evaluations of gas composition were performed daily until the fourth day and, then, every two days.

The variables evaluated were: a) Respiratory rate: Whole yams and approximately 450 g of the fresh cut product were placed in glass jars with capacity of 600 mL, remaining sealed for one hour. The gas sample was withdrawn from the jars with a syringe (5 mL), through a silicone septum attached to the lid, and injected into the gas chromatograph (Thermoquest GC Trace 2000) with a flame ionization detector (FID), with hydrogen as drag at a flow of 25 mL min^{-1}. The temperatures were 80^{0}C in column, 100^{0}C in gun, 250^{0}C in detector and 350^{0}C in methanator. The results expressed as ppm CO_2 were converted to % of CO_2 and used to calculate the respiratory rate, the free volume of the jar, the time it remained closed and the mass of yams being taken into account. The readings were held daily up to four days, and then, six, eight and nine days. The results were expressed in mL CO_2/kg/h; b) Ethylene production: The procedures to determine the rate of ethylene production were similar to those used to determine the respiratory rate, as regards collection of the gas sample. The sample was injected into gas chromatograph (Thermoquest GC Trace 2000) with flame ionization detector (FID), with hydrogen as drag at a flow of 25 mL min^{-1}, the temperatures of column; gun and detector were at 80^{0}C, 100^{0}C, 100^{0}C, respectively. The results were expressed in µL C_2H_4 kg^{-1} h^{-1}; c) Mass loss: Calculated by difference, in percentage, between the initial mass of the package content and mass at the time of evaluation; d) Firmness: It was determined using a manual penetrometer of 8 mm diameter. The results were expressed in Newton; e) Coloration: It was used a colorimeter Minolta CR-300, with illuminant D65, carrying out readings of L (lightness). Each replication was composed by three samples, in which readings were carried out; f) Soluble solids content (SS): The samples were ground and one drop was placed on digital refractometer (Atago PR-101), with automatic correction of temperature for 20^{0}C. The results were expressed in ºBrix; g) Titratable acidity: 10 g of samples were weighed and placed in 90 mL of distilled water. Potentiometric titration was performed with 0.1N sodium hydroxide to pH 8.10. The results were expressed in % citric acid.

For ultra structural analysis by scanning, four portions of the outer surface of cuts from each treatment were removed, fixed in Karnosvsky's solution (Karnosvsky, 1965), placed in the vacuum pump, and dehydrated in an ethanol series to 100% of ethanol. Then the samples were dried by the method of critical point of CO_2, mounted on aluminum brackets and covered with a 30-40 nm thin layer of gold 30-40 nm with Balzers MED 010 metallizer. The analyses and electron micrographs were carried out at NAP/MEPA - Núcleo de Apoio à Pesquisa em Microscopia Eletrônica Aplicada à Agricultura, ESALQ-USP. It was used scanning electron microscope Zeiss Model DSM-940A, operated at 20 kV, with scales of electron micrographs printed directly on them.

The contaminant microorganisms of the fresh cut yam were evaluated by counting the total of psychrotrophic bacteria, the most probable number (MPN) of total and fecal coliforms and presence or absence of *Salmonella,* as established by Resolution RDC nº 12 of 01/02/2001, of the Agência Nacional de Vigilância Sanitária (ANVISA).

The analyses of psychrotrophic bacteria were carried out by the method of plating in depth, being incubated at 7^{0}C for 10 days. Dilutions, from 10^{-1} to 10^{-3}, in duplicate were selected. The counting of the plates was done with the aid of a Quebec colony counter and the results were expressed in UFC g^{-1}. The counting of coliforms at 35^{0}C and coliforms at 45^{0}C was determined using the most probable number method (MPNN) and, from each sample, decimal dilutions, 10^{-1}, 10^{-2} and 10^{-3}, were prepared by the technique of multiple tubes. For the detection of *Salmonella*, Kit "1-2 test" was used (BioControl/USA). Official method approved by AOAC (Association of Official Analytical Chemists International).

The experimental design was completely randomized in 4x4 factorial scheme, and the studied factors were storage temperature (at four levels: 5, 10, 15 and 20°C) and the evaluation period (at four levels: 0, 3, 6 and 9 after processing). Four replications of 500 g of the fresh cut product were used and approximately the same mass quantity for the whole product. The data were subjected to analysis of variance and the average compared by the minimum significant difference test ($p \leq 0.05$), in which the differences between two treatments greater than the sum of two standard errors were considered significant.

RESULTS AND DISCUSSION

Whole and fresh cut yams stored at 5^{0}C kept a low respiration rate during storage, with values ranging between 10 and 20 mL CO_2 kg^{-1} h^{-1} (Figure 1A). These two treatments could be evaluated until the ninth day. For fresh cut yams and stored at 10 and 15^{0}C, a peak of respiration rate was verified on the fourth day of storage, reaching values of 169 and 123 mL CO_2/kg/h, respectively.

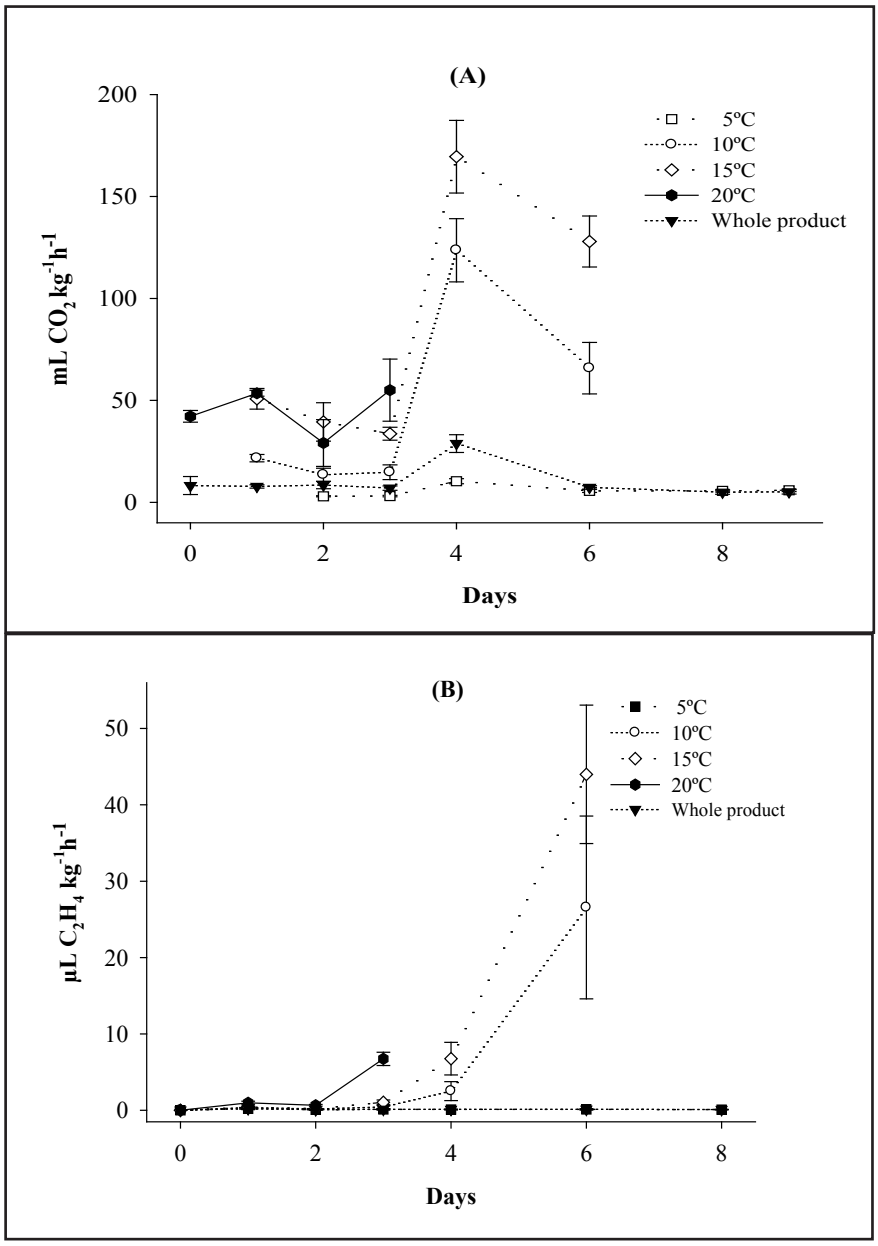

Figure 1. Respiratory rate (A); ethylene production (B) in yam stored at 20ºC and fresh cut yams stored in different temperatures. *Vertical bars represent the standard error of mean (taxa respiratória (A) e produção de etileno (B) em inhame inteiro a 20ºC e inhame minimamente processado armazenados em diferentes temperaturas. *Barras verticais representam o erro padrão da média). Piracicaba, ESALQ, 2011.

These two treatments were discarded on the sixth day due to the high state of deterioration. Fresh cut product stored at 20^{0}C was discarded on the third day due to high state of deterioration. It is known that the temperature plays a fundamental role in plant respiration, and its reduction decrease the respiration rate (Watada *et al*., 1990; Brecht, 1995; Porte & Maia, 2001). In this work, the fresh cut yams stored at 5^{0}C presented a respiration rate in the same order of magnitude as that observed for the raw yam stored at 20^{0}C (Figure 1A). It is common to observe peaks of respiration in fresh cut products, which come from the stress caused by the processing. This stress causes loss of cellular compartmentalization (Figure 3, A-L) and, the respiratory substrates are easier access to enzyme complexes, accelerating the respiration rate (Vitti *et al*., 2003).

Fresh cut yams, stored at 10 and 15°C had the ethylene production increased from the third day on, reaching values of 27 and 44 µL C_2H_4 kg^{-1}, respectively. Continuous increase in ethylene production was observed at temperatures of 10 and 15°C, even with the decrease of respiration rate (Figures 1, A-B). This increase may be associated with the exponential development of microorganisms during the storage period (Table 1). The ethylene production for the whole yam stored at 20^{0}C and for the fresh cut one stored at 5^{0}C remained low and constant throughout the storage period, with values lower than 1.0 µL C_2H_4/kg/h. The temperature of 5^{0}C was efficient for the reduction of the metabolism of the fresh cut yams. Vitti *et al*. (2004) also observed low respiration rates (0.90 µL C_2H_4/kg/h) in fresh cut beet roots stored at 5^{0}C.

No significant differences were observed for pulp firmness in different temperatures tested, however the increase in yam firmness was observed during the storage period (Figure 2A). This increase in firmness was accompanied by an increase in mass loss (Figure 2B), which consequently caused the wilting of the fresh cut yam and this caused difficulty in penetration of the penetrometer tip.

Sasaki *et al*. (2006) observed an increase in firmness of fresh cut pumpkins, due to their dehydration and consequently wilting of the pieces, which also originated greater readings of firmness.

The mass loss increased during the storage period, however, with lower intensity in stored pieces at 5^{0}C which reached less than 1.0% at the end of nine days of storage. For the temperatures of 10 and 15^{0}C, this characteristic reached 1.5% after 6-day storage (Figure 2B).

Post-harvest fresh mass loss is related to the losses of water content and reserve material through transpiration and respiration, being proportional to the increase in temperature (Carvalho & Lima, 2002). The decrease of the quality of the fresh cut product with loss of water through transpiration is generally intensified, considering that the cut increases the exposure surface to the surrounding air. In the present

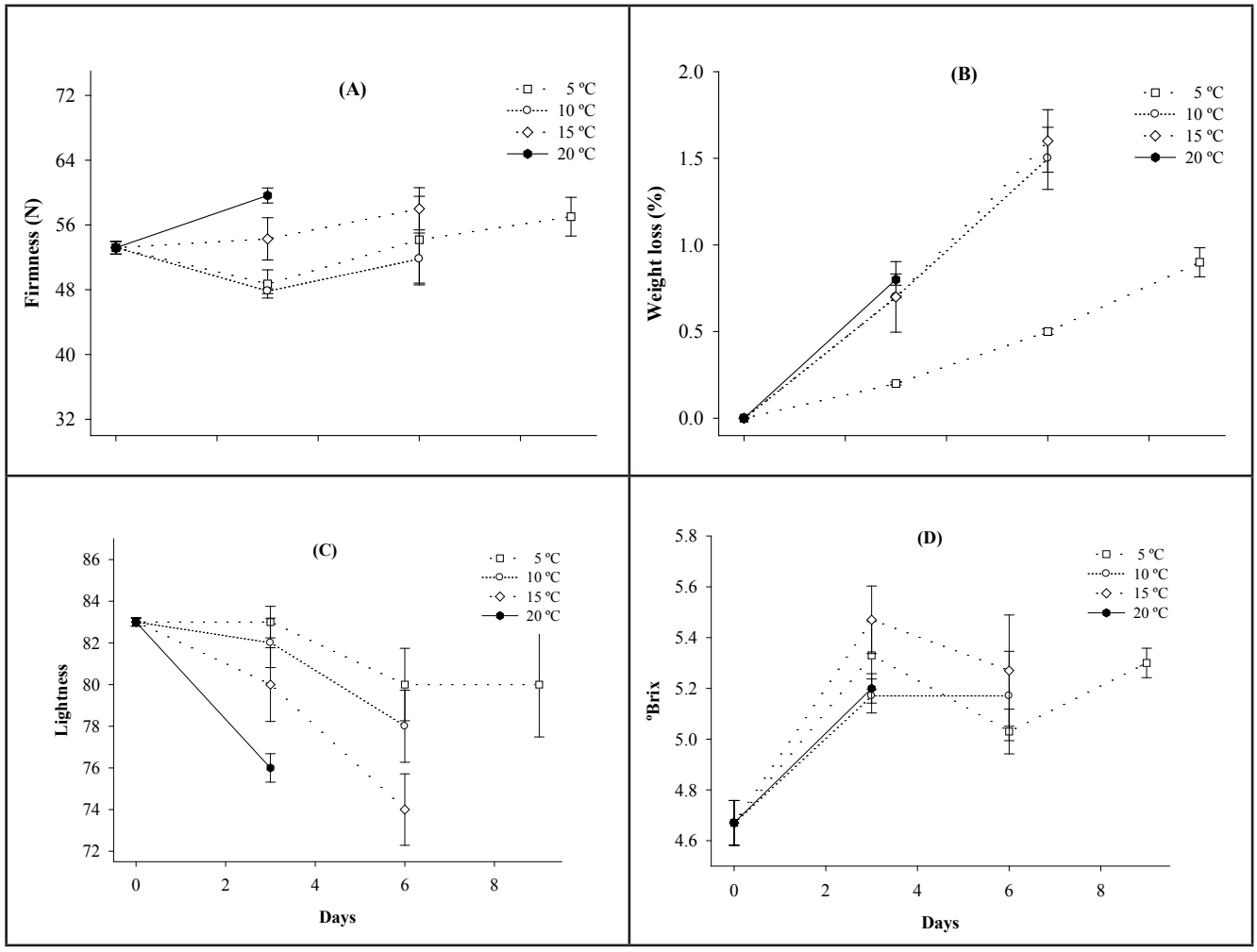

Figure 2. Firmness (A); weight loss (B); lightness (C); soluble solids content (D); in fresh cut yams stored in different temperatures. *Vertical bars represent the standard error of mean (firmeza (A); perda de massa (B); luminosidade (C); teor de sólidos solúveis (D); em inhames minimamente processados, armazenados em diferentes temperaturas. *Barras verticais representam o erro padrão da média). Piracicaba, ESALQ, 2011.

work, the mass loss caused qualitative losses of the fresh cut yam, being the wilting and powdery appearance the most evident characteristics, similar to what occurs to beetroot (Vitti *et al.*, 2003), and fresh cut carrot (Simões *et al*., 2010). Using the ultrastructural analysis of the cuts, it was observed that the external dehydration aspect is due, partly, to the starch grain deposition because of the cellular leakage giving a powdery texture and, partly, due to the tissue dehydration (Figure 3, A-L).

A reduction of the lightness of the product, at all the temperatures studied, was observed, with more evidence for the temperature of 20^{0}C (Figure 2C). Enzymatic processes and development of microorganisms in fresh cut yam changed the characteristics of the product and made it darker, losing some characteristics of the initial product. At 5^{0}C, the change in lightness was less pronounced during storage, although no difference in L values up to three days of storage was shown.

No significant differences were found between the treatments during the storage time for titratable acidity, with mean values of 0.14% of acidity (data not shown). No significant differences for soluble solids content (SS) were verified in the different temperatures tested. However, an increase in the SS content during storage was observed (Figure 2D). On the day of processing, the SS content was, in average, 4.7 ^{0}Brix, reaching a maximum content of 5.3 ^{0}Brix on the ninth day. Silva *et al.* (2003), working with fresh cut cassava, did not observe a significant increase in SS values, the authors found values between 4 to 6 °Brix. The accumulation of soluble solids during the shelf-life of fruit and vegetables can occur due to the conversion of starch into sugar or the water loss which improve the sugar concentration in the tissue during the storage (Vilas-Boas, 1999). In the present work, one of the reasons of the increase of SS content probably is the water loss observed through the mass loss (Figure 2B).

The total of psychrotrophic bacteria count showed values below the limit up to the third day of storage at 5ºC and 10ºC (Table 1), with count of 4.2x10^2 UFC (colony-forming unit) g^{-1} and 6.3x10^4, respectively. Because Brazil has no specific legislation to indicate limits for psychrotrophic bacteria count, the

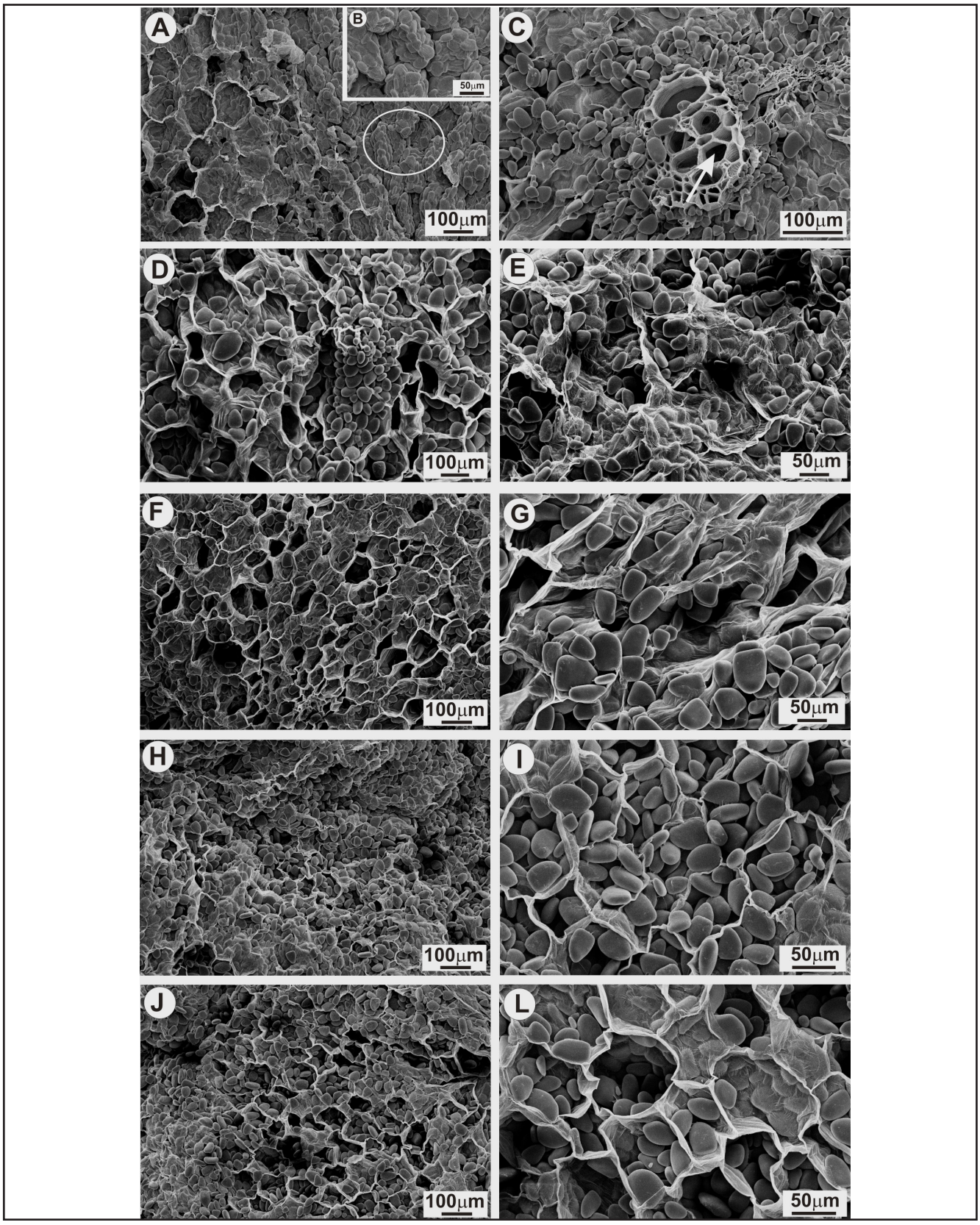

Figure 3. Scanning electromicrographies of yam tubers after minimal processing. A-C) immediately after cutting, D-E) 3-day storage at 20ºC, F-G) 6-day storage at 15ºC, H and I) 6-day storage at 10ºC, and J-L) 9-day storage at 5ºC. A) It is possible to see regions with whole cell (detail in B) and regions with ruptured cells due to the cutting, C: presence of vascular bundle, starch granules extravasated, D-L) cells ruptured with extravasation of starch granules during the storage period E-G) dehydration of the cell membrane due to high temperatures of storage (eletromicrografias de varredura em tubérculos de inhame após o processamento mínimo. A-C) imediatamente após o corte, D-E) 3 dias de conservação a 20°C, F-G) 6 dias de conservação a 15°C, H e I) 6 dias de conservação a 10°C, J-L) 9 dias de conservação a 5°C. A) podem ser evidenciadas regiões com células íntegras (detalhe em B) e regiões com células rompidas pelo corte, C) presença de feixe vascular e grânulos de amido extravasados, D-L) células rompidas com extravasamento dos grânulos de amido durante o período de conservação, E-G) desidratação da membrana celular devido a temperaturas de conservação elevadas). Piracicaba, ESALQ, 2011.

Table 1. Total count of psychrotrophic bacteria (BP), coliform at 35ºC (C35ºC) and coliforms at 45ºC (C45ºC) in fresh cut yams stored at different temperatures (contagem total de bactérias psicrotróficas (BP), de coliforme a 35°C (C35ºC) e a 45°C (C45ºC) em inhame minimamente processado, armazenado em diferentes temperaturas). Piracicaba, ESALQ, 2011.

Temperature	Time (days)	BP (UFC g^{-1})	C35ºC (MPN g^{-1**})	C45ºC (MPN g^{-1**})
	0	<10	43	<3.0
5ºC	3	4.2 x 10^2	28	6.2
	9	Inc. *	460	23
10ºC	3	6.3 x 10^4	1,100	15
15ºC	3	1.83 x 10^5	>1,100	15
20ºC	3	Inc. *	>1,100	43

*Inc= uncountable; results are expressed as UFC (colony forming unit) g^{-1} product. **results are expressed in MPN (most probable number) g^{-1} product [*Inc= incontável; resultados expressos em UFC (unidade formadora de colonia) g^{-1} do produto. **resultados expressos em MPN (número mais provável) g^{-1} do produto].

parameters presented by Morton (2001) were used, which allow the marketing of frozen vegetables and similar with total count of aerobic mesophilic bacteria up to 10^5 UFC g^{-1}. In temperature of 15^{0}C the yams presented for psychrotrophic counts of 1.83x10^5 UFC g^{-1}, on the 3rd day of storage. With these counts the consumption of this product is not recommended, because the probability of having pathogenic microorganisms increases. Vitti *et al.* (2010) found an increase of psychrotrophic population in fresh cut potatoes, with values of 10^5 UFC g^{-1} on the fifth day of storage at 15^{0}C, and 7.9x10^7 UFC g^{-1} on the first day of storage at 25^{0}C, while Silva *et al*. (2003) observed, in fresh cut cassava, psychrotrophic bacteria count of 10^6 UFC g^{-1} after nine days at 10^{0}C. In this work, the number of UFC was high after the ninth day of storage at 5^{0}C, considering that at the storage at 10, 15 and 20ºC the fresh cut yam sample showed visible characteristics of deterioration, and they had not been submitted to microbiological analysis.

The count was high, not only for coliforms at 35°C but also for coliforms at 45^{0}C (Table 1), showing that the sanitizing process did not avoid contamination. The initial count of the coliforms at temperature of 5^{0}C was, in average, 43 MPN g^{-1} of the product, whereas after nine days of storage the count increased substantially, reaching values around 460 MPN g^{-1}. The sanitizing with chlorine compounds is cited as one of the most effective methods to prevent contamination; however, Ahvenainen (2003) alerts that such compounds are not, necessarily, effective on tubers. Moreover, the raw material can carry a very high microbial load from the natural soil microbiota. After the third day of storage at 10, 15 and 20ºC, the yam samples showed counts for coliforms at 35°C of 1100 to >1100 MPN g^{-1} of the product. Endo *et al*. (2008) also found high counts of fresh cut potatoes, 1100 and >2400 MPN g^{-1}, from the sixth day of storage at 8ºC. On the other hand, the coliforms at 45^{0}C, remained below the limits established in the Technical Regulation RDC No. 12 of January 2, 2001 of ANVISA, which recommends the maximum value of 10^3 MPN g^{-1}. At the storage temperature of 5ºC, the count was lower, 6.2 MPN g^{-1} of the product on the third day, and 23 MPN g^{-1} on the ninth day. On the third day of storage, the counts were of 15 MPN g^{-1} not only for the yam stored at 10ºC but also at 15ºC. At 20ºC, the count reached 43 MPN g^{-1} on the third day (Table 1).

The results obtained for determination of *Salmonella* in the fresh cut yam samples were according to the microbiological standards established in the Technical Regulation RDC No. 12 of January 2, 2001, ANVISA, which establishes the absence of *Salmonella* in 25 g of the product, on roots and tubers, aiming the preservation of human health.

In the present work it was observed that the temperatures of 10, 15 and 20°C are not efficient to preserve food quality and safety of fresh cut yam, and required the use of lower temperature. The temperature of 5^{0}C is cited as the most recommended for fresh cut products in general. In the case of yam, using this temperature remained low respiration rate and ethylene production. The mass loss, lightness and the count of psychrotrophic bacteria are within the recommended commercial standards up to the third day of storage at 5°C. Longer periods of storage are not recommended and new methods of preservation should be studied, especially alternative sanitizer treatments.

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