PHYSIOLOGICAL, QUALITATIVE AND MICROBIOLOGICAL CHANGES OF MINIMALLY PROCESSED SQUASH STORED AT DIFFERENT TEMPERATURES

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Key words: Cucurbita moschata; carotenoids; respiratory rate; psychrotrophic bacteria

ABSTRACT

The purpose of this work was to study the physiological, qualitative and microbiological alterations in minimally processed squash stored at 1°C, 5°C and 10°C, and at 85-90% RH. Fruit showed higher respiratory rates (34.27mg CO_2 Kg^{-1} h^{-1}) and ethylene production rates (24.49µg C_2H_4 Kg^{-1} h^{-1}) at 10°C. At such temperature, an increase in mass loss, and reduction in soluble solids, ascorbic acid and total carotenoids was observed. There was also a greater development of psychrotrophic bacteria at 10°C. Minimally processed squash stored at 1°C showed lower respiratory rates (9.66mg CO_2 Kg^{-1} h^{-1}) and ethylene production (<0.03µg C_2H_4 Kg^{-1} h^{-1}). Besides, they showed lower losses in soluble solids, ascorbic acid and total carotenoids. Psychrotrophic bacteria count at 1°C remained within the allowed limits until the last day of storage, which represents a reduction of two logarithmic cycles in relation to the 10°C count. No *Salmonella*, total coliforms and coliforms at 45°C were observed during storage in any temperature.

ALTERACIONES FISIOLÓGICAS, MICROBIOLÓGICAS Y DE CALIDAD DE ZAPALLOS MÍNIMAMENTE PROCESADOS Y ALMACENADOS BAJO DIFERENTES TEMPERATURAS

Palabras claves: Cucurbita moschata; carotenos; tasa de respiración; bacterias psicotróficos

RESUMEN

La propuesta de este trabajo fue el de estudiar las alteraciones fisiológicas, microbiológicas y de calidad de zapallos mínimamente procesados (cortados en fresco) y almacenados a 1ºC, 5ºC y 10ºC, y 85-90% HR. Los frutos presentaron altas tasas de respiración (34,27mg CO_2 Kg^{-1} h^{-1}) y de producción de etileno (24,49µg C_2H_4 Kg^{-1} h^{-1}) a 10ºC. Con o aumento de la temperatura, también aumento la pérdida de masa fresca; y se observó reducciones de los sólidos solubles, ácido ascórbico y carotenos totales. Fue observado un significativo desarrollo de bacterias psicotróficos a 10ºC. Los zapallos cortados en fresco y almacenados a 1ºC presentaron tazas de respiración bajas (9,66mg CO_2 Kg^{-1} h^{-1}) y de producción de etileno (<0,03µg C_2H_4 Kg^{-1} h^{-1}). Asimismo los zapallos a 1ºC presentaron bajas perdidas de sólidos solubles, ácido ascórbico y carotenos totales. El contaje de bacterias psicrotróficos a 1ºC fueron bajos hasta el último día del experimento, estos zapallos cortados en fresco y almacenados a 1ºC presentaron reducciones de dos ciclos logarítmicos en comparación de los zapallos cortados en fresco y almacenados a 10ºC. No fueron detectados *Salmonella*, coliformes totales y coliformes a 45ºC a lo largo del experimento y en ninguna de las temperaturas probadas.

INTRODUCTION

Minimal processing is defined as any physical alteration in fruit and vegetables that preserves their fresh nature. It involves selecting, washing, cutting, sanitizing, centrifuging, packing, storage and marketing operations.

As observed for all fruits and vegetables, minimally processed products deteriorate after being processed, due to their own physiological action and microbial activity (Varoquaux & Wiley, 1997). The stress caused by processing leads to an increase in respiratory and ethylene production rates, which increases the activity of enzymes

responsible for browning, off-flavors and softening (Cantwell, 1992; Wiley, 1997).

Cold storage plays an important role in food preservation, as decreased temperatures slow the changes triggered by biochemical reactions and decrease bacterial and fungal development (Chitarra & Chitarra, 2005). Therefore, temperature control is the most important factor to minimize the effects of tissue injuries in minimally processed products (Brecht, 1995). Many authors recommend temperatures around 0°C to store minimally processed products, although temperatures around 5°C have been commonly used and, sometimes, 10°C (Schlimme, 1995).

Squash show a great potential for the minimally processed market. Fruit are normally very large, weighing 12 to 25Kg and are marketed in pieces. This can lead to great losses of product. Minimal processing fruit can contribute to reduce losses, besides to aggregate value to this commodity.

Squash is constituted by 1.3% fibers and 96% water, and 100g of the fruit contains 4 calories, 28mg of vitamin A, 70mg of vitamin B5, 10mg of vitamin B2 and 5,5mg of vitamin B, besides salts, such as calcium, phosphorus, potassium, sodium, iron and sulfur (Luengo et al., 2000). Fruit can be consumed unripe or ripe. Unripe fruit are used in salted dishes and ripe ones in home-produced or industrialized sweets and salted recipes (Camargo Filho & Mazzei, 2002).

Many research approaches the metabolism of several minimally processed fruits and vegetables stored at different temperatures. However, there are few studies on minimally processed squash. Therefore, the purpose of this work was to evaluate the physiological, qualitative and microbial alterations in minimally processed squash stored at different temperatures.

MATERIAL AND METHODS

Plant material. Ripe squashes (*Cucurbita moschata* Duch. cv. Canhão) obtained from a producer in Tupã, SP, Brazil, were used for this study. Fruit was sorted for uniform appearance, size and absence of physical and pathological damage. After selection, fruit were first washed in running water and detergent, to remove rough impurities, and underwent an initial disinfection, in which fruit were immersed in a 200ppm active chlorine solution for ten minutes. Then, fruit were placed in a cold chamber at 10 $\pm$1°C and 90±5% RH for 16 hours to keep a low metabolic activity before processing.

Minimal processing. The minimal processing was carried out at 15°C. Initially, squashes were sliced into 3.0cm-thick round discs using a stainless steel sharp knife. After, seeds and spongy parts were removed using a spoon. Pieces were peeled and prepared cubes into 3 x 3 x 3cm. After cutting, pieces were washed in a 200 mg L^{-1} chlorine solution during 3 minutes and rinsed during 1 minute and were then placed in a common dish drainer to remove the excess water. Fresh-cut squash were placed in expanded polystyrene trays (300g by tray) measuring 14 x 20cm, wrapped in 12-micra thick polyvinyl chloride (PVC) films and stored in at 1±1°C, 5±1°C or 10±1°C and 90±5% RH during 12 days.

Assessments. The evaluations of respiratory rate and ethylene production were carried out daily and within the first four hours after processing, while for the other variables the evaluations were carried out every three days.

To determine respiratory rate, 150g of the minimally processed product were placed in 600mL glass flasks and hermetically sealed for 1 hour at different temperatures. A silicone septum was fitted in the flask lids to allow the collection of a 1mL aliquot from the flask internal atmosphere and readings were carried out in a gas chromatograph (Thermoffinigan, model Trace 2000 GC). Results in % CO_2 were used in the calculation of the respiratory rate, which considered the

flask volume, the radish mass and the time the flask were kept closed. The respiratory rate was determined within 4 hours after minimal processing, by means of 4 readings, the first of which (time zero) was carried out 1 hour after processing. Posterior readings were conducted daily for 12 days. Results were expressed in mg CO_2 kg^{-1} h^{-1}.

The procedures to determine ethylene production were the same used in respiratory rate trials, except that flaks were closed for 2 hours. Internal atmosphere readings were carried out in a gas chromatograph (Thermoffinigan, model Trace 2000 GC) equipped with flame ionization detector (FID). The injector, column, and detector temperatures were 100, 100 and 250ºC, respectively, and hydrogenous carrier gas flow was 0.40 mL s^{-1}. Results were expressed in μg C_2H_4 kg^{-1} h^{-1}.

The weight loss was determined as being the difference between the initial and the final mass of each repetition and the results were expressed in % of weight loss. The soluble solids concentration (SS) was determined by direct reading of centrifuged fresh cut in a digital refractometer (Atago PR-101, Atago Co. Ltd., Tokyo, Japan) with the results expressed in percentage (%). Ascorbic acid content was determined by titration according Carvalho et al. (1990) and the results expressed in mg of ascorbic acid per 100g of sample. Lightness (L^*), chromaticity (C*) and hue angle (h^o) were determined using a colorimeter (Minolta CR-300, Osaka, Japan). Pulp firmness was determined with a manual penetrometer with an 8mm-diameter probe, with results expressed in Newton.

To determine total carotenoids amount 3g of the sample were triturated in a mortar together with 3g of Hyflosupercel and 50mL of cold acetone. The content was filtered with suction through a sintered glass funnel (or Buchner funnel with filter paper). The filtered sample was placed in a separator funnel with polytetrafluoroethylene stop-cock containing 40mL of petroleum ether and added 300mL of distilled water slowly, letting it flow along the walls of the funnel. The aqueous phase was then discarded and one more part of the filtered sample was added, and the same procedure was carried out with 200mL of distilled water from 3 to 4 fold. After the total removal of the acetone, the petroleum ether phase was filtered through a glass funnel containing 15g of anhydrous sodium sulfate and placed in a volumetric flask (50 mL). The volume was completed to 50 mL with petroleum ether. Readings were carried out at 450nm in a spectrophotometer and values were used to determine the total carotenoids amount, using the following formula: [A x volume (mL) x 10^4]/[$A^{1\%}_{1cm}$ x sample weight (g)], where A=absorbance; volume=sample volume (50mL); $A^{1\%}_{1cm}$ = β-carotene absorbance coefficient in petroleum ether (2592). Results were expressed in μg g^{-1} (Rodriguez-Amaya & Kimura, 2004).

Microbiological analysis. Microbiological analyses were conducted on the processing day (day 0), on the 6^{th} and 12^{th} days of storage.

The contaminant microflora of the minimally processed squash was evaluated by total psychrotrophic bacteria count, the most probable number (MPN) of total and fecal coliforms and the presence or absence of *Salmonella*, according to what is determined by the Regulation n^o 12, 02/01/2001, *Agência Nacional de Vigilância Sanitária* (ANVISA), from Brazil.

For the psychrotrophic bacteria and coliforms count, 50g of minimally processed squash were used in each replicate. Samples were aseptically weighed and placed into sterilized blenders containing 450mL of sterile peptonated water (0.1%) for homogenization, constituting a 10^{-1}dilution. From the 10^{-1} dilution, a 10^{-2} dilution was obtained by pipetting 10mL of the solution 10^{-1} and mixing it with 90mL of sterile peptonated water

(0.1%). From the 10^{-2} dilution, a 10^{-3} dilution was obtained by the same process.

For the psychrotrophic bacteria count, samples were deep plated using 1mL of each dilution in duplicate and 20mL of Plate Count Agar (PCA). After this procedure, plates rested until complete solidification of the cultivation medium, when they were inverted and incubated at 7°C for 10 days. Results were expressed in CFU g^{-1}.

A psychrotrophic bacteria count was carried out. Once squash were cold stored and, under cold temperatures, the absence of psychrotrophic bacteria growth implies no mesofilic bacteria growth.

Total coliforms and coliforms at 45°C were determined by the most probable number (MPN) method, using the Multiple Tubes Technique. This technique has two distinct stages: i) presumption test, which determines the presence of lactose-fermentative microorganisms and in which injured cells can be recovered; ii) confirmative test, which allows the determination of the actual total and fecal coliforms population. For the presumption test, three series of five test tubes with 10 mL lactose broth equipped with an inverted Durham tube were used. The tubes were incubated in a thermostatic oven at 35-37°C for 24-48 hours. Positive tubes showed empty Durham tubes due to the production of gas by the coliforms bacteria group during lactose fermentation.

If the presumption test was positive, aliquots from positive tubes were used in the confirmative test in order to verify whether the bacteria really belonged to the coliform group. Positive samples were inoculated into tubes containing Brilliant Green Lactose Bile Broth (CVBLB) and into tubes containing EC Broth. CVBLB tubes were inoculated in a thermostatic oven at 35-37°C for 24-28h and, after that, it was verified whether there was presence of gas in the Durham tubes. The production of gas in CVBLB confirms the presence of bacteria belonging to the coliform group, whether from fecal origin or not, called total coliforms. EC tubes were thermostatically incubated at 45°C for 24 hours. After the incubation period, the presence of gas confirmed the presence of coliforms at 45°C. Considering the number of positive tubes in each series using both media, and locating their corresponding numbers in a table of the Compendium of Methods for the Microbiological Examination of Foods (Downes, 2001), it was possible to obtain the MPN of total coliforms and coliforms at 45°C per gram of minimally processed squash.

The '1-2 Test' Kit (Biocontrol/USA) was used for the *Salmonella* detection. This is an official test approved by the AOAC that can be used with all kinds of food. The presence of *Salmonella* is characterized by the formation of an immune band on the upper half of the gel in the motility chamber. It is a U-shaped white band formed by the agglutination of bacterial cells with the antibody solution.

Data analysis. Analysis of variance was performed using SAS software according to a completely randomized experimental design in a 3 x 5 factorial arrangement (three temperatures and five storage periods). Four replicates of 300g of minimally processed squash were used per treatment. The data were subjected to analysis of variance and the least significant differences were calculated. Differences between any two treatments greater than the sum of two standard deviations were always significant (P>0.05).

RESULTS AND DISCUSSION

The respiratory rates of minimally processed squashes decreased during the first hours of storage, regardless of the storage temperature (Figure 1). Results were similar to those found by Smyth et al. (1998) for minimally processed lettuce, which showed a decrease in the CO_2 production rate from approximately 470pmol.g^{-1} s^{-1} (1 hour after processing) to 125pmol.g^{-1} s^{-1} (24 hours after

cutting). Saavedra del Aguila et al (2006) also had found similar behavior in minimally processed radishes stored in different temperatures. The high initial respiratory rate is due to the stress caused by cutting, which promotes cell de-compartmentalization and is directly controlled by the cell repair mechanism, favoring the decrease in respiratory rate (Saltveit, 2003).

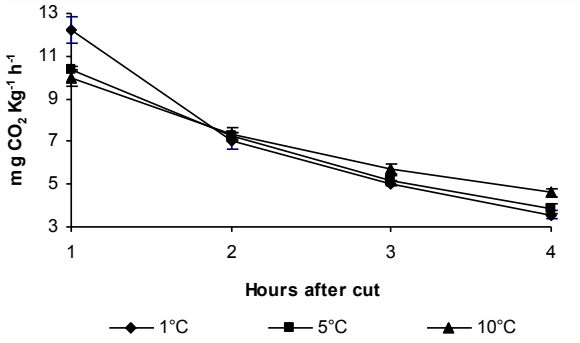

Figure 1. Respiration rate of evolved CO_2 (mg Kg^{-1} h^{-1}), after 1-4hs of the processing, in minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n= 5*).

Minimally processed squashes stored at 10°C showed higher respiratory rates during storage period when compared to those stored at 1°C and 5°C (Figure 2). Squashes stored at 10°C reached a peak of 34.27mg CO_2 Kg^{-1} h^{-1} on the 3rd day of storage, while those kept at 1°C and 5°C reached of 9.66mg CO_2 Kg^{-1} h^{-1} and 8.58mL CO_2 Kg^{-1} h^{-1}, respectively, on the same day. There was no significant difference in respiratory rate for minimally processed squash stored at 1°C and 5°C.

The respiratory peak of minimally processed squashes stored at 10°C is probably due to the stress cause by processing, which is responsible for cell de-compartmentalization leading to the metabolism substrates receiving in contact with enzymatic complexes, increasing the respiratory rate. The subsequent decrease in the respiratory rate, from the 5th day, is not well explained, but it is possible that there is a self-regulatory mechanism of the respiratory activity in the tissues due to the high ATP production (Purvis, 1997). Also, such reduction in the respiratory rate can be due to respiratory substrates not reacting with enzymes present in the cut surface cells anymore.

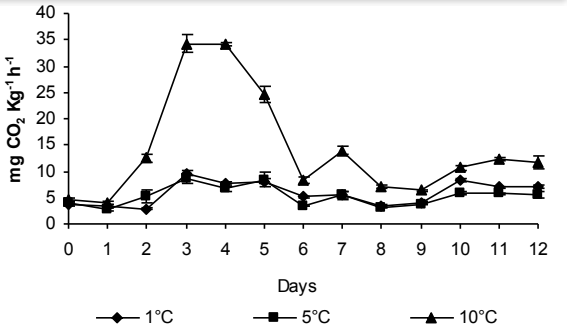

Figure 2. Respiration rate of evolved CO_2 (mg Kg^{-1} h^{-1}) in minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n = 5*).

There are many studies about the influence of temperature on the metabolic activity of minimally processed vegetables. Habibunnisa et al. (2001), studying minimally processed pumpkins (*Cucurbita maxima*), observed a reduction from 157.54mg CO_2 Kg^{-1} h^{-1} at 28°C to 25.26mg CO_2 Kg^{-1} h^{-1} at 5°C.

Hong & Kim (2001), studying the effect of temperature on the respiratory rate of minimally processed shallot (10cm-long) observed 9.19mL CO_2 Kg^{-1} h^{-1}; 27.02mL CO_2 Kg^{-1} h^{-1} and 78.74mL CO_2 Kg^{-1} h^{-1} at 0°C, 10°C and 20°C, respectively. Watada et al. (1996) verified an increase in the respiratory rate from 5.7 to 9.4 and 13.0mg CO_2 Kg^{-1} h^{-1} in squash stored at 0°C, 5°C and 10°C, respectively.

Ethylene production was no detected during the first 4 hours after cutting under any temperature. However, during storage at 10°C, minimally processed squash showed ethylene production peak on the 3rd day, reaching 24.49µg C_2H_4 Kg^{-1} h^{-1}, which was 6 fold higher than values observed for squash stored at 5°C (Figure 3). After the 3rd day of storage, the ethylene production rate of squashes kept at 10°C started a decrease, reaching values near

those observed for squash stored at 5°C. Minimally processed squashes kept at 5°C reached ethylene production peak on the 4th day of storage, while those stored at 1°C showed no ethylene production during the storage period. Mertens & Tranggono (1989) observed that cauliflowers stored at 1°C produced less than 0.03µL C_2H_4 Kg^{-1} h^{-1} and almost 0.1µL C_2H_4 Kg^{-1} h^{-1}, when kept at 10°C.

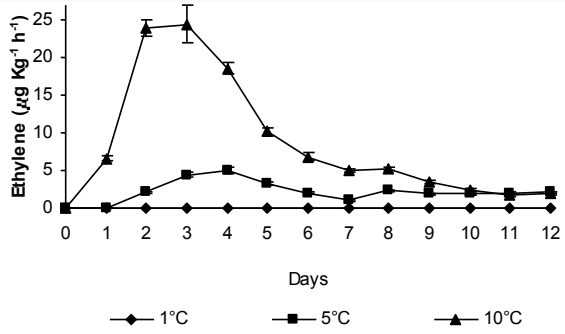

Figure 3. Ethylene production rate (µg Kg^{-1} h^{-1}) in minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n= 5*).

According to Sakr et al. (1997), the increase in the ethylene production may be a hormonal and biochemical response to the stress caused by cutting, as it first affects the plasmatic membrane, which in turn changes its physical characteristics to compensate the disturbances and to try to repair the damages during processing. Brecht (1995) has described that the increase in the respiratory rate in tissues physically injured may be a consequence of the increase in ethylene production, which stimulates respiration. Therefore, the respiratory peak observed in squash stored at 10°C can be a consequence of the increase in the ethylene production. One of the mechanisms of the ethylene action is to increase the expression of enzymes associated with respiration and with their own production (Hyodo et al., 1985; Brecht, 1995).

There was an increase in mass loss during the storage period, regardless of the temperature (Figure 4). Pre-processed squash stored at 10°C showed higher mass loss during the storage period, reaching 1.41%. Squashes stored at 1°C presented lower mass loss and those kept at 5°C showed intermediate values. This relatively small mass loss may be attributed to the protection offered by packing.

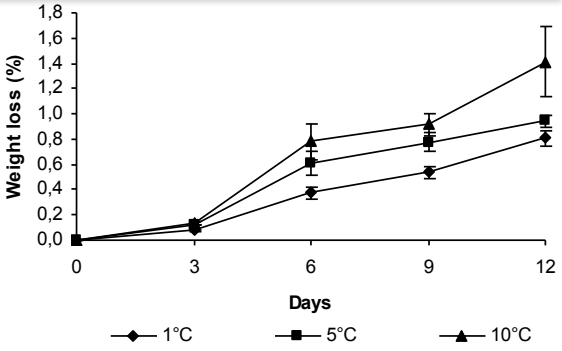

Figure 4. Weight loss (%) of minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n = 5*).

There were no significant variations in pulp firmness under the temperatures studied. The values ranging from 70 to 80N (Figure 5).

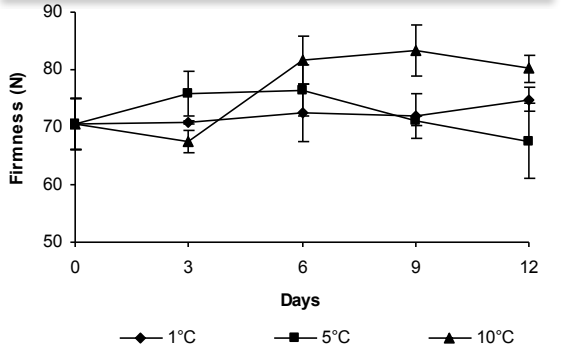

Figure 5. Firmness (N) of minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n = 5*).

There was a decrease in the soluble solids during storage in all temperatures. The most significant variation occurred at 10°C (Figure 6) and it was probably associated to a higher consumption of carbohydrates during respiration under such temperature.

There was a reduction in the ascorbic acid amount throughout the storage period for the three temperatures, mainly at 10°C (Figure 7). According to Franco (2008), the reduction in

the vitamin C amounts is due to the degradation of the ascorbic acid caused by heat, oxidation and storage, exposure to cold temperature or alkalinity. In addition, the damage caused by the minimal processing stimulates the defense antioxidative reactions, which may have consumed the ascorbic acid.

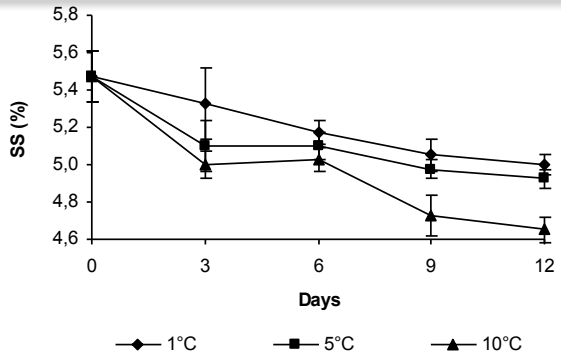

Figure 6. Soluble solids (%) of minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n = 5*).

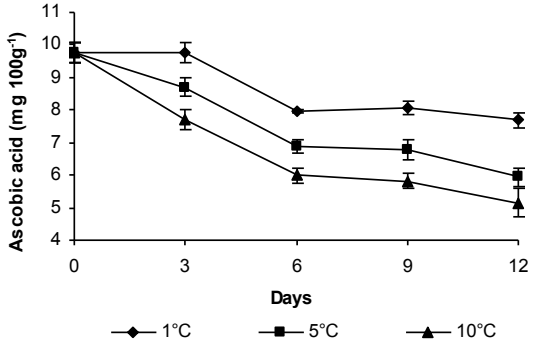

Figure 7. Ascorbic acid (mg $100g^{-1}$) of minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n= 5*).

Regardless of the temperature, there was a reduction in the total carotenoids amounts from the processing day (day 0) to the 3rd day of storage. After the 3rd day, values remained constant and the highest ones were observed at 1°C and 5°C (Figure 8). This was probably due to the reduction in metabolism under lower temperatures, which also decreases the process of carotenoids degradation. According to Rodriguez-Amaya (2001), the main carotenoids found in squashes are α-carotene and β-carotene. The same author states that carotenoids are susceptible to isomerization and oxidation during processing and storage. The practical consequences of this fact are loss of color, reduction in biological activity and the formation of volatile compounds that promote desirable or undesirable scents in food. Therefore, the reduction in the total carotenoids amounts may interfere with the skin color in squashes.

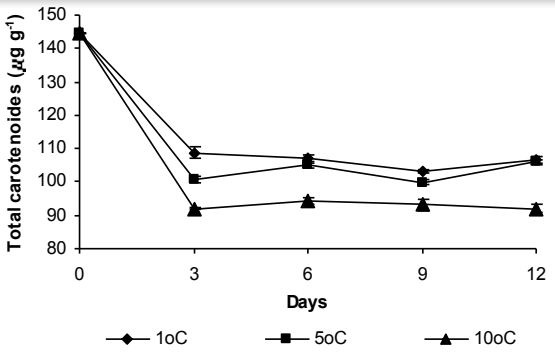

Figure 8. Total carotenoids (μg g^{-1}) of minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n= 5*).

There was an increase in the lightness for squashes stored at 10°C (Figure 9). At the same time, it could be observed the skin whitening in pumpkins under such temperature. A similar process called "white blush" is observed in minimally processed carrots (Bolin & Huxon, 1991; Tatsumi et al., 1991) and beet root (Vitti et al., 2005). This process is probably associated with dehydration and/or lignin formation (Cisneros-Zevallos et al., 1995).

Squash stored at 1°C presented nearly stable chromaticity (C*) values (between 53 and 54) throughout the storage period (Figure 10). Squashes stored at 10°C showed a decrease in C* values throughout the storage period, ranging from 53.27 to 45.81. In fact, squashes kept at 10°C lost their intense orange color.

There was no difference in hue angle (h°) between treatments on the days of analyses. Hue angle values were nearly constant

throughout the storage period (data not shown).

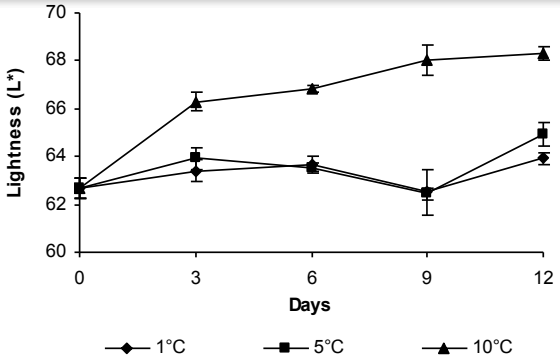

Figure 9. Lightness (L*) of minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n = 5*).

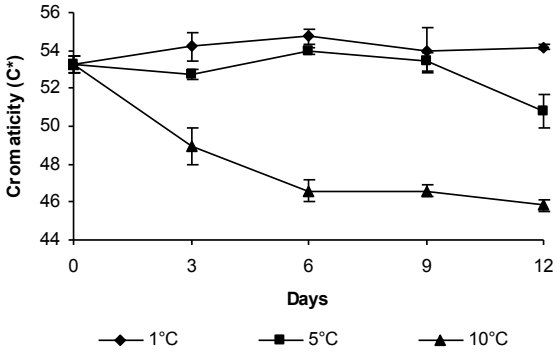

Figure 10. Cromaticity (C*) of minimally processed squash stored at different temperatures. Vertical bars represent ±S.D. (*n = 5*).

Color is one of the most important quality parameters, and the consumers associate the external coloration with the highest quality of the product. Thus, the orange color in squashes is associated with product quality and, the more intense the color, the higher quality is attributed by the consumer. Low storage temperatures used for minimally processed products decrease color degradation in the injured vegetable tissues (Wiley, 1997). In the present work it was possible to observe that the loss of color was proportional to the increase in storage temperature.

As there is no specific legislation concerning minimally processed products in Brazil, the parameters used were the ones established by Morton (2001), which enable the commercialization of frozen vegetables and similar products presenting total aerobic mesofilic bacteria count up to 10^5-10^6 CFU g^{-1}.The increase in the psychrotrophic bacteria population was slowed by the reduction in the storage temperature (Table 1). Minimally processed squash stored at 1°C showed a total psychrotrophic bacteria count within tolerated limits, reaching values of $5.0x10^5$ CFU g^{-1} on the 12th day of storage, which is below of the values established by Morton (2001). Squash already presented bacteria count of $4.0x10^5$ CFU g^{-1} on the 6th day of storage and of $8.0x10^6$ CFU g^{-1} on the 12th day. Although those values are within the acceptable limits, the consumption of a product presenting such bacteria count may be risky, as there is a greater chance that a pathogen microorganism is present. Squash stored at 10°C presented bacteria count of $4.5x10^6$ CFU g^{-1} on the 6th day of storage and of $7.2x10^7$ CFU g^{-1}on the 12th day, which is considered an extremely high rate, making the product improper for consumption.

Table 1. Total psychrotrophic bacteria count, in minimally processed squash stored at different temperatures, using a conventional method (PCA)

Temperatures	Days after processing		
	0	6	12
1°C	5,0 x 10	$8,0 \times 10^3$	$5,0 \times 10^5$
5°C	6,0 x 10	$4,0 \times 10^5$	$8,0 \times 10^6$
10°C	4,0 x 10	$4,5 \times 10^6$	$7,2 \times 10^7$

**The results represent the arithmetic means of the UFC g^{-1} of minimally processed squash.*

Piagentini et al. (1997) observed an increase in the psychrotrophic microorganism population in minimally processed cabbage, from 10^2 to 10^5 CFU g^{-1} after four days of storage at 4°C. García-Gimeno & Zurera-Cosano (1997) observed an increase in the psychrotrophic microorganism count in salad samples made of minimally processed vegetables stored at 4°C.

Izumi & Watada (1994) studied the microbial growth in minimally processed

carrots stored at 0, 5 and 10°C. The authors observed that the total bacteria count was higher when the product was kept under the highest temperature. After 15 days of storage, the product showed a total mesofilic bacteria population of 7.0 log CFU g^{-1} at 0°C and above 8.01 log CFU g^{-1} at 5°C. Carrots stored at 10°C presented bacteria count of 8.01 log CFU g^{-1} on the 11th day of storage.

Habibunnisa et al. (2001) observed a mesophyll aerobic bacteria count of 3.2 x 10^5 CFU g^{-1} on the 25th day of storage in minimally processed pumpkins stored at 5°C.

Storage temperature is perhaps the most important factor affecting the growth of microorganisms in minimally processed products (Brackett, 1987; King Jr. & Bolin, 1989; Nguyen & Carlin, 1994).

Although there are no established standards for total psychrotrophic bacteria and total coliforms counts in the Brazilian legislation concerning the amount of microorganisms present in food, it can be stated that high microorganisms amounts (> 10^5-10^6 CFU g^{-1}) are undesirable due to the following reasons: risk of food spoilage, actual or potential loss of organoleptic qualities, and loss of desirable visual characteristics. The higher the microorganisms count in food, the higher the probabilities of pathogens and/or deteriorating organisms being present (Caruso & Camargo, 1984).

The presence of *Salmonella* was not detected, as well as the growth of total coliforms and of coliforms at 45°C under any storage temperatures throughout the studied period.

There is still no specific legislation concerning minimally processed fruit and vegetables. However, the results found in the present study are within the limits established by the RDC resolution number 12, January 2, 2001, from *Agência Nacional de Vigilância Sanitária* (ANVISA) of the Ministry of Health, which establishes the absence of *Salmonella* (in 25g of product) and allows a maximum of 10^2 MPN coliforms at 45°C/g for fresh vegetables, aiming the protection of public health.

According to this study, the storage temperature showed significant effects on the physiological and qualitative processes in minimally processed squash. It was observed that product presented higher respiratory and ethylene production rates at 10°C, when compared to squashes stored at 1°C or 5°C. Another consequence of storage at 10°C was the reduction in the soluble solids. Moreover, as the metabolism of squash was more intense at 10°C, there was a high consumption of anti-oxidative substances, such as ascorbic acid and total carotenoids, reducing the nutritional food quality. The microbial analyses, especially the psychrotrophic bacteria count, showed the importance of keeping a cold chain along the whole product processing and commercialization periods. As the presence of total coliforms, coliforms at 45°C and *Salmonella* was not observed in the samples analyzed, it was possible to verify the efficiency of the hygienic-sanitary procedures during the stages of product processing, which prepares the product to fulfill the microbial standards currently used in the country.

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