PHYSICOCHEMICAL CHANGES OF ‘GOLDEN’ PAPAYA STORED UNDER CONTROLLED ATMOSPHERE

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ABSTRACT

Changes in atmosphere composition make possible the increasing in postharvest life of fruits, but under extreme conditions it can alter the product quality. This research work investigates the physicochemical changes of ‘Golden’ papaya stored at 13 °C in different controlled atmospheres. It was evaluated peel colour, mass loss, pulp firmness, titratable acidity, pH, and soluble solids content in fruits stored under atmospheres with lower O₂ (1, 3 and 5%) and higher CO₂ concentrations (2, 6 and 10%). Fruits stored in 1% O₂ showed detrimental green colour changes, however the ones stored in O₂ levels above 3% presented small changes in colour. Furthermore, the colour changes were also delayed by increasing CO₂ levels to 10%. Pulp hardness and atypical acidity were perceived at 1% O₂ and 10% CO₂ levels. The O₂ level of at least 3% is essential to avoid physical and chemical disorders. By increasing the CO₂ levels to 10% effectively slowed down the ripening process, but sensory analysis should be conducted to ascertain the quality of the product.

Keywords: Carica papaya, cold storage, atmosphere composition, pulp quality, physiological disorders

TRANSFORMAÇÕES FISICO-QUÍMICAS DO MAMÃO ‘GOLDEN’ ESTOCADO SOB ATMOSFERA CONTROLADA

RESUMO

A mudança na composição da atmosfera de estocagem torna possível o aumento da vida útil dos frutos, mas sob condições extremas pode ocorrer alteração da qualidade do produto. Este trabalho investiga as alterações físicas e químicas do mamão ‘Golden’ estocado a 13 ºC e em diferentes composições de atmosfera controlada. Foram avaliados os parâmetros de cor da casca, perda de massa, firmeza da polpa, acidez titulável, pH e conteúdo de sólidos solúveis totais em frutos estocados nas composições de atmosfera com baixo teor de O₂ (1%, 3%, 5%) combinadas com teores crescentes de CO₂ (2%, 6% 10%). Os frutos estocados em 1% de O₂ não mostraram evolução da cor verde, no entanto os frutos estocados em atmosferas de 3% e 5% de O₂ apresentaram alteração da cor verde. Além disso, essa mudança de cor verde foi minimizada pelo aumento da concentração de CO₂ até 10%. Observou-se também um enrijecimiento de polpa e uma acidez atípica nos frutos mantidos em 1% O₂ e 10% de CO₂. Conclui-se que a concentração de O₂ de no mínimo 3% é essencial para evitar desordens físicas e químicas nos frutos. O aumento do teor de CO₂ até 10% efetivamente retardou o processo de amadurecimento, mas uma análise sensorial deveria ser realizada para certificar a qualidade do produto.

Palavras-chave: Carica papaya, estocagem refrigerada, composição de atmosfera, qualidade da polpa, desordens fisiológicas

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INTRODUCTION

In tropical climates, papaya looses its quality rapidly and its postharvest life is limited to seven days under environmental conditions (Paull et al., 1997). Despite their postharvest problems, papaya must be shipped to distant markets, usually by air or sea. For example, shipping time by sea can require at least 20 d from Brazil to Europe. Therefore, it is essential to assure a long postharvest life in order to send the fruit to distant markets (Yahia, 1998).

The combined techniques of cold storage and controlled atmosphere have presented successful results. However, the main problem is that there is no general rule for the best atmospheric composition, and different varieties of papaya present unpredictable physiological responses to the same O₂ or CO₂ concentrations (Saltveit, 2003). Off-flavour can develop in any fresh fruit or vegetable if it is exposed to O₂ and/or CO₂ levels that result in anaerobic respiration and the formation of ethanol and acetaldehyde. At low concentrations of O₂, physiological disorders may occur, such as impaired ripening of climacteric fruit, internal browning, external brown discoloration, and surface pitting (Kader, 1986).

Most products require a minimum of a 1% to 3% O₂ level to prevent the occurrence of anaerobic metabolism (Imahori et al., 2004). Ideal atmospheric conditions have not yet been fully defined for papaya fruit, but range between 2-5 kPa O₂ and 5-8 kPa CO₂. It is unknown if modified and controlled atmosphere have a potential application for papaya. Studies are still required to establish the beneficial applications and adequate atmospheric conditions (Yahia & Singh, 2009).

The ‘Golden’ papaya is a new cultivar obtained in the 1990s by means of mass selection in Sunrise Solo orchards of the Empresa Caliman Agrícola (ES-Brazil). With an average weight of 450g, this papaya is widely accepted in the international market due to its excellent appearance and delicious flavour (Costa & Pacova, 2003).

This work investigated the storage of Golden papaya under a range of controlled atmospheric compositions, with the objective of identifying the storage conditions that would be the most appropriate for the preservation of its quality and extension of its storage time. The approach adopted focused on analyzing the fruit’s chemical composition, as well as pulp firmness, weight loss and skin colour changes.

MATERIAL AND METHODS

Papaya fruits cv. Golden were obtained from a commercial field in Linhares, ES (19°15’S, 39°51’70”W), and selected on the packaging line of Empresa Caliman Agrícola S.A. (ES-Brazil). The experiment was conducted with eighty fruits in each atmosphere composition. They were harvested with 10 to 15% peel yellowish and an average weight of 450g, being treated in a hydrothermal bath (48°C for 20 min) and immersed in fungicidal solution (Thiabendazole - 2g L⁻¹ or Prochloraz - 0.25mL L⁻¹) for 2 minutes. Afterwards, they were transported under refrigeration (10°C) to the laboratory. Thus, the storage experiment was started 36 h after the fruits harvest at the orchard.

Ten chambers measuring 0.14 m³, equipped with controlled atmosphere devices, were used to store eighty fruit in each at 13°C and 85-95% RH. The gas levels were set at 1%, 3%, and 5% O₂ combined with 2%, 6%, and 10% CO₂ respectively, while the control group was kept at 21% O₂ and 0.03% CO₂. Moreover, the chambers were flushed with gas mixtures on several intervals to maintain the controlled atmospheres. The O₂ and CO₂ levels were established by flushing the chambers with nitrogen gas and adding CO₂ at 15 L min⁻¹. The set up levels were maintained constant by scrubbing excess CO₂ in addition to adding air to increase the O₂ levels. Gas concentrations were monitored daily using computerized analyzers with paramagnetic (O₂) and infrared (CO₂) detection.

Ethylene adsorption equipment was used to minimize the self-catalytic effect on ripeness. Air was pumped for 15 minutes through a permanganate column. This procedure was repeated seven times a day to assure ethylene absence in the chamber.

The skin colour was determined using a Spectrophotometer (Hunterlab MiniScan XE Plus) calibrated on a standard white and black reflective plate. A D65 illuminant and a 10° standard observer were employed. The measurements were taken at two equidistant points on the equatorial region of the fruit’s sun-exposed side and were characterized by the Hunter L̴ parameter, which indicated the history of yellow colour throughout its storage.
Pulp firmness was measured with a digital penetrometer equipped with an 8 mm diameter plunger. The fruit were cut transversally through the equatorial region and the measurements were taken using a conical holder at two equidistant points located in the external half of the mesocarp of the fruit’s sun-exposed side. The results were expressed as the force in Newtons (N) required to push 11 mm of the probe into the pulp.

The mass loss during the storage was quantified using an electronic balance and the results were calculated based on the difference between the fruit’s initial weight and the weight recorded at each sampling, and normalized by 100 g.

For the physicochemical analysis, samples of pulp were removed from the outer half of the mesocarp on the fruit’s sun-exposed side, using a stainless steel knife. The samples were wrapped in aluminium foil, placed in plastic bags and stored in a commercial freezer at -20°C. Before conducting the analyses, the samples were unfrozen under tap water, pulped and homogenized at 18,000 rpm.

For the analysis of the soluble solids content, the homogenized material was sieved through cotton fabric and the readings were taken using a hand-held digital refractometer, with automatic temperature compensation, and expressed in °Brix.

The titratable acidity was quantified by titrating 5 g of homogenized pulp, diluted in 150 mL of distilled water with a 0.01 mol L^-1 NaOH solution, to a pH end-point of 8.2, with the result being expressed as the percentage of citric acid. The pH measurements were taken with a pH meter by means of direct immersion of the electrode in the homogenized solution.

The experiment was conducted in a [(3x3)+1] x 8 factorial arrangement, which refers to three CO₂ levels, three O₂ levels and one ambient atmosphere for the control group (1% O₂ plus 2% CO₂; 1% O₂ plus 6% CO₂; 1% O₂ plus 10% CO₂; 3% O₂ plus 2% CO₂; 3% O₂ plus 6% CO₂; 3% O₂ plus 10% CO₂; 5% O₂ plus 2% CO₂; 5% O₂ plus 6% CO₂; 5% O₂ plus 10% CO₂; and 21% O₂ plus 0.03% CO₂ in addition to eight sampling times (6, 12, 18, 24, 30, 36, 42 and 48 days). The experimental design was completely randomized for each combination of factors. Each treatment involved ten replicates of one fruit for the physicochemical analysis.

Statistical software (SAEG Software, Brazil) was employed to analyze the results by data regression. Subsequently, the results were compared through model identity using a level of statistical significance of \( p \leq 0.05 \). Before adjusting the models, the data were subjected to a variance analysis (ANOVA) considering the model of principal factors and the interaction among these factors. Sampling dimensioning was also applied, using a 10% deviation level around the average data of each variable, ensuring the application of the models to an infinite population of Golden papaya.

Data regression was carried out to compare the results by means of model identity at a 95% probability level. The dataset for the complete factorial system, encompassing the nine atmospheres, was adjusted by an orthogonal function using binary variables, as stated by Little et al. (1991). The regression model is as follows:

\[ Y = a_0 + a_1 t + a_2 t^2 + a_3 D_1 + a_4 D_2 + a_5 D_1 + a_6 D_1 t + a_7 D_2 + a_8 D_2 t + e \]

The adjustment equation of the data for each atmospheric condition was obtained by the substitution of the corresponding binary values. The regression equation for the ambient atmospheric condition (control) was adjusted separately.

**RESULTS AND DISCUSSION**

In this paper, a unique general model was applied for the data regression of seven hundred and twenty fruit samples, stored in different atmospheric conditions. Therefore, the coefficient of determination is unique and represents the total mean square root of the data adjusted by the data regression equation. For all of the variables, the deviation of the regression was not significant using \( p \leq 0.05 \).

Figures 1.a, 1.b and 1.c show measurements of the skin colour of the fruit stored at 1%, 3%, and 5 % O₂ combined with 2%, 6%, and 10% CO₂, respectively, whilst the control group was kept at 21% O₂ and 0.03% CO₂. The curves indicate the development of the yellow colour on the sun-exposed side of the fruit stored during 48 days (d) at 13°C and 85-95% RH. The curve fitting for 5% O₂ was similar to that found at 3% O₂, both treatments indicating a small changing in colour during the storage of fruits, being minimized proportionally to the increasing CO₂ levels.
Figure 1. Hunter $b$ parameter at the sun-exposed side of Golden papaya stored in 1% (a), 3% (b), and 5% (c) O$_2$, combined with 2%, 6%, 10% CO$_2$ and control (21% O$_2$ plus 0.03% CO$_2$) during 48 d at 13
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°C and 85-95% RH (R^2 - multiple range test for the regression model, Rc^2 - coefficient of determination for the control regression model).

Fruit stored in atmospheres with a 1% O_2 level (Figure 1.a) presented impaired ripening, in view of the fact that the Hunter b values remain unchanged during the first 24 days of storage. The increment in the average values, between 24 and 30 d of storage, resulted from the fruit being exposed to the outside environment when the doors of the storage chamber were opened in order to repair the motherboard responsible for the control, analysis, injection and adsorption of gases inside the chamber. After that, the Hunter b average data remained unchanged, therefore supporting the notion that these atmospheric conditions impair the development of the yellowness index. Although the opening of the chamber’s doors caused an increase in the Hunter b values, data regression showed that the inhibition of the ripening process is remarkable at the lower level of O_2 (1%).

The fruit stored in atmospheres of 3% O_2 showed a minimal increase in the Hunter b parameter in the first 24 d of storage, in comparison to the control group (Figures 1.b). After 30 d of storage, an increase in the yellowness index can be observed, however the Hunter b values declined at the end of the storage period due to an increase in freckling of the skin, which is characterized by the formation of larger brownish areas. This effect was more predominant on the fruit belonging to the control group, which exhibited the highest yellowness index. Nonetheless, it was noted that the different atmospheres did not affect the extent of freckling during the storage since they appeared spread on fruit peel in all treatments, reducing the colour index at the end of the storage period.

An increase in the CO_2 level from 2% to 10% minimized the yellowness index (Hunter b), and consequently the ripening process of the fruit stored in atmospheres containing 3% or 5% O_2 levels.

To evaluate the consequences of the suppression of the O_2 level on the skin’s yellowness, the results were compared taking into account a 10% CO_2 level, given that it presented the highest synergic effect on the inhibition of ripening (Figure 2). Data regression showed that an atmosphere composed of 1% O_2 promoted a sensible effect on the inhibition of the increment of Hunter b values when compared to 3% and 5% O_2 levels, whose colour record presented the smallest difference between them.

![Figure 2](image)

**Figure 2.** Hunter b parameter at the sun-exposed side of Golden papaya stored in 1%, 3%, and 5% O_2 combined with 10% CO_2 during 48 d at 13°C and 85-95% RH (R^2-multiple range test for the regression model).
According to Kader (1986), changes in skin colour are slowed down in fruit kept in controlled atmospheres. Lowering the O₂ level around fresh fruit and vegetables reduces their respiratory rate, but depending on the product, a minimum of about 1-3% O₂ is required to prevent a shift from aerobic to anaerobic respiration. Elevated CO₂ concentrations also reduce the respiration rate, but depending on the commodity and the O₂ concentration, CO₂ can cause the accumulation of ethanol and acetaldehyde within the tissues. Also, the minimum O₂ level in the atmosphere is necessary to warrant a concentration gradient that promotes the gas diffusion to inner tissue.

Reduced O₂ levels decrease ethylene production. Under anaerobic conditions, the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene is inhibited (Kader, 1986). Elevated CO₂ levels also affect the production of ethylene (de Wild et al., 2003). Moreover, CO₂ is an essential co-factor for ACC oxidase (Dong et al., 1992); however, in elevated concentrations, it inhibits the self-catalytic production of ethylene (Chavez-Franco & Kader, 1993).

The CO₂ concentration can stimulate or inhibit ethylene synthesis, depending on the internal level of ACC (Rothan & Nicolas, 1994). At high ACC levels, CO₂ stimulates its biosynthesis, but at the levels found in fruit, it inhibits ethylene production. The CO₂ effect depends on its concentration, exposure time, temperature, and variety (Mathooko, 1996).

The fruit’s firmness data are shown in Figures 3.a, 3.b and 3.c for atmospheres containing 1%, 3% and 5% O₂, respectively. The fruit in the control group, which were exposed to 21% O₂ and 0.03% CO₂, displayed low values throughout the storage time. This finding differs from that described by Almeida et al. (2005), who reported a sharp decline in firmness at the onset of ripening of the Golden papaya, reaching the same magnitude as that observed in the present study, during six days of storage at 13°C. The lower initial values of firmness found in this study were probably due to the fact that the fruits were placed in controlled atmospheres only 36 h after they were harvested, when probably has developed the loss of initial firmness.

From the beginning of the experiment up to about 24 d, when the failure in the controlled atmosphere system occurred, the fruit stored in 10% CO₂ and lower O₂ levels presented higher firmness than that of fruits in the control group. Fruit stored in 1% O₂ and 10% CO₂ displayed an increase in firmness (>35 N), indicating the development of physiological disorders caused by elevated CO₂ and low O₂ levels (Figure 3.a). Jones (1984) stated that under stressful conditions, vegetal tissue suffer the biosynthesis of phenylalanine ammonia-lyase (PAL), which catalyzes the formation of lignin in the cell wall, explaining, probably, the increase in tissue firmness observed in the present work. An increase in PAL activity was identified by Silva et al. (2005) in Golden papaya stored under stressful conditions of chilling injury.

The fruit’s mass loss occurred progressively during storage in different atmospheres, as indicated by Figure 4. In fact, the Golden papaya shows a constant mass loss rate along the storage period due to the skin’s resistance to mass diffusion (Pinto et al., 2006). The control fruit showed an average mass loss of 3.6% after 48 d of storage. This small level of mass loss was due to high relative humidity inside the closed chamber, where the air humidity stays in equilibrium with the fruit humidity. The fungal growth did not influence the measurements once that only at the end of the storage were observed in small quantity.

O₂ suppression minimized the mass loss when compared with the atmosphere of the control group, but no differences were observed among the lowers O₂ levels (Figure 4).

Data regression indicated that the mass loss of the fruit stored in 1%, 3% and 5% O₂ levels was 3.3% after 48 d, indicating that for this range of O₂ levels the change in the ripening rate did not interfere in the mass loss. In the same way, increasing the CO₂ concentrations up to 10% did not affect the results. The same behaviour was noted in papaya fruit treated by irradiation technique, in which there was no effect of mass loss due to irradiation in the three maturation stages analyzed (Pimentel & Walder, 2004). There was only gradual mass loss of papaya as it ripened.

Figures 5.a, 5.b and 5.c show the acidity measurements of the fruit pulp stored at 1%, 3% and 5% O₂, respectively.
Figure 3. Firmness of the external half of the mesocarp of the sun-exposed side of ‘Golden’ papaya stored in 1% (a), 3% (b), and 5% O₂ (c) combined with 2%, 6%, 10% CO₂ and control (21% O₂ plus 0.03% CO₂) during 48 d at 13°C and 85-95% RH (R² - multiple range test of the regression model, R² - coefficient of determination for the control regression model).

Figure 4. Mass loss of ‘Golden’ papaya stored in lower O₂ levels combined with 2%, 6%, 10% CO₂, and control (21% O₂ plus 0.03% CO₂) during 48 d at 13°C and 85-95% RH (R² - multiple range test for the regression model, R² - coefficient of determination for the control regression model).
Figure 5. Total titratable acidity in the external half of the mesocarp of the sun-exposed side of ‘Golden’ papaya stored in 1% (a), 3% (b), and 5% O$_2$ (c) combined with 2%, 6%, 10% CO$_2$, and control (21% O$_2$ plus 0.03% CO$_2$) during 48 d at 13°C and 85-95% RH ($R^2$ - multiple range test for the regression model, $R^2_c$ - coefficient of determination for the control regression model).
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Under controlled environmental conditions, the fruit presented higher titratable acidity at the beginning of storage, possibly due to the hydrolysis of pectic substances in the cell wall. Subsequently, the acidity decreased until the 18th d of storage, probably as a result of the consumption of organic acids to support the ripening process, as reported by Almeida et al. (2006). From then on, the acidity increases until the end of the storage period, probably due to new synthesis of organic acids. The pH measurements revealed the opposite behaviour, showing the highest level on the 18th day. Pinto et al. (2006) reported a similar history of acidity for Golden papaya stored under refrigeration (control treatment without package), noting that fruits stored in packages of PEBD film presented smaller acidity changes along the storage due to the lower ripening rate.

Fruits stored in 1% O₂ and 2% or 6% CO₂ (Figure 5.a) showed lower average acidity values than the ones in the control group after 6 d of storage, but the average acidity increased considerably after 12 d and then declined sharply after 18 d. These oscillations in acidity possibly indicate the occurrence of metabolic disorders at low O₂ levels (1% O₂). However, the data regression curves did not characterize this behaviour by a second-degree fitting.

The fruit stored in 3% and 5% O₂ levels showed a good correlation between their acidity content throughout the storage period (Figures 5.b and 5.c), which is congruent with the regression data of the control group (21% O₂ plus 0.03% CO₂). However, the increase in CO₂ levels caused acid accumulation at the beginning of storage, probably due to the solubility of CO₂ in the intracellular solution. According to Lencki et al. (2004), the amount of carbon dioxide dissolved in the tissue, at CO₂ levels typically found in modified atmosphere systems (7 kPa), can be as high as 38% of the total amount found in the void space of the package.

At the end of the storage, the lower values of acidity in the fruit stored at 3% and 5% O₂ levels combined with higher CO₂ levels (Figures 5.b and 5.c), indicated that the ripening process slowed down in relation to the fruit of control group (21% O₂ plus 0.03% CO₂).

The total soluble solids (TSS) content of the fruit stored in 1%, 3% and 5% O₂ levels did not differ among the treatments, but they presented a small changing in TSS content as the CO₂ was increased (Figures 6).

**Figure 6.** Soluble solids content in the external half of the mesocarp of the sun-exposed side of ‘Golden’ papaya stored in lower O₂ levels combined with 2%, 6%, 10% CO₂ and control (21% O₂ plus 0.03% CO₂) during 48 d at 13°C and 85-95% RH (R² - multiple range test for the regression model, Re² - coefficient of determination for the control regression model).
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Fruits in the control group (21% O₂ plus 0.03% CO₂) presented a smooth decrease in TSS content during storage at 13°C, with a tendency of an increase at the end. Almeida et al. (2006) and Pinto et al. (2006) noted a slight increase in the TSS content in the outer half of the mesocarp, only in the ripe stage of ‘Golden’ papaya. A possible explanation for this behaviour is that, in the present study, the ripening process was not totally completed after 48 d of storage due to the efficiency of the ethylene adsorption in slowing down the ripening process. Photographic records of the fruits used in the present work (data not shown) revealed small green areas on the poles of the fruit, reinforcing this observation.

In fact, papaya does not accumulate starch during its growth (Gómez et al., 1999). The relationship between acid invertase enzyme activity and the fruit’s sweetness was identified by Zhou et al. (2000). Gomez et al. (2002) found that galactose can be used to synthesize sucrose, whose levels in the cell wall decrease during the ripening. This can explain a tendency of increase the TSS content at the end of the storage in the present work, probably due to metabolic degrading of pectic substances of the cell wall and synthesis of organic acids and small amount of sugars. According to Gomez et al. (2002), the fact that ripe and semi-ripe papaya are sweeter than unripe ones, although their total soluble sugar content stay in the same level, may be attributed to changes in texture, which would result in the release of different levels of sugar from the papaya cells in the mouth during mastication.

CONCLUSIONS

Fruit stored in 1% O₂ showed impaired ripening. However, the fruit stored in 3% and 5% O₂ presented slow ripening, which was minimized as the CO₂ level was increased to 10%. However the fruit quality was not ensured at this higher level of CO₂. Suppression of the O₂ level minimized the mass loss, but the CO₂ concentration did not affect this phenomenon, which may have been influenced by saturation humidity inside the closed chamber.

Atmospheres containing 1% O₂ and 10% CO₂ levels caused hardness of the fruit’s pulp. The same tendency was noted in fruit stored in 3% and 5% O₂ levels. Atypical acidity histories were found in fruit stored in 1% O₂ levels. In atmospheres with 3% and 5% O₂ levels, the increase in the CO₂ concentration up to 10% slowed down the acidity changes in the pulp.

The lowest O₂ level suitable for avoiding physical and chemical disturbs, minimizing weigh loss and extending storage time of Golden papaya was at 3% O₂ level. An increase in the CO₂ level up to 10% effectively slowed down the yellowness index and acidity changing during the storage, but a sensorial analysis should be conducted to ascertain the presence of any unfavourable taste and flavour after ripening.

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