PAPAYA PULP GELLING : IS IT PREMATURE RIPENING OR PROBLEMS OF WATER ACCUMULATION IN THE APOPLAST?

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ABSTRACT - Gelled aspect in papaya fruit is typically confused with premature ripening. This research reports the characterization of this physiological disorder in the pulp of papaya fruit by measuring electrolyte leakage, Pi content, lipid peroxidation, pulp firmness, mineral contents (Ca, Mg and K - in pulp and seed tissues), and histological analysis of pulp tissue. The results showed that the gelled aspect of the papaya fruit pulp is not associated with tissue premature ripening. Data indicate a reduction of the vacuole water intake as the principal cause of the loss of cellular turgor; while the waterlogged aspect of the tissue may be due to water accumulation in the apoplast.
Index terms: ultrastructural alterations, cell turgor, fruit quality, physiological disturbances, biochemical alterations.

GELEIFICAÇÃO DA POLPA DE MAMÃO: AMADURECIMENTO PREMATURO OU PROBLEMAS NO ACÚMULO DE ÁGUA NO APOPLASTO?

RESUMO - O aspecto geleificado da polpa de mamão é constantemente confundido com amadurecimento prematuro. Este trabalho caracterizou esse distúrbio fisiológico na polpa de frutos de mamão através de medidas de liberação de eletrólitos, conteúdo de Pi, peroxidação lipídica, firmeza da polpa, condudo mineral (Ca, Mg e K - na polpa e semente) e análises histológicas da polpa. Os resultados mostram que o aspecto geleificado da polpa de mamão não está associado com o amadurecimento prematuro. Os resultados indicam uma redução da entrada de água no vacúolo como a principal causa da perda de turgor celular, enquanto o aspecto encharcado da polpa pode ser devido ao acúmulo de água no apoplasto.
Termos para indexação: alterações ultra estruturas, turgor celular, qualidade de frutos, distúrbios fisiológicos, alteraçõs bioquímicas.

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INTRODUCTION

In the orchards of Linhares region (19°39'W; 40°07'S), Brazil, farmers and technicians have observed the occurrence of physiological disorder (PD) affecting fruit quality of Solo-type papaya (*Carica papaya* L. 'Golden'). This disturbance seems to occur seasonally (between May and July – season of moderate temperature – average of 22 °C) and is characterized by a translucent pulp tissue that appears gel-like. The gelling symptoms do not necessarily occur in all fruits from the same tree and healthy fruits are harvested as well as the gelling fruit. In these fruits, the gelling tissue develops from the endocarp to the epicarp, similarly to pulp ripening (ROSETTO et al., 2008). In contrast to fruit ripening, an unequal distribution of the PD symptoms in the fruit flesh may be found as a result of the asymmetric progression of gelling. Also, accumulation of liquid is usually found in the seed cavity, observed when the gelled fruit is sectioned, suggesting problems in cell water entrance.

The lack of characterisation of these PDs in the literature may lead to a confused evaluation of the fruit due to the visual resemblance with mechanical injury. However, the symptoms of mechanical injury originate from the outer tissue, next to the epicarp (point of impact) and develop to the endocarp. In this case, the cell wall-degradation leads to a softening of the tissue (CHUNG et al., 2006), as a result of increase in ethylene biosynthesis (DE VRIES et al., 1999, STOW et al., 2000, ROSETTO et al., 2008). These symptoms are not observed in papaya gelling flesh; instead, the gelling aspect is usually observed as soon as the fruit are carefully detached from the papaya tree. Consequently, the aspect of the papaya mesocarp gel-like without doubt may not be caused by mechanical injury.

Studies of the anatomy and physiology of fruit crops may help to understand how the sequence of the metabolic events leads to the development of PDs. Until development of methods for the prevention of these disorders can be ensured, an understanding of the physiological factors associated with their development is required.

In order to characterize the gelled PD and to confirm that this disturb is not a premature ripening in papaya fruit flesh, measurements of electrolyte leakage, Pi content, lipid peroxidation, pulp firmness were carried out; mineral contents (Ca, Mg e K) in pulp and seed tissue were quantified; and histological analysis in pulp tissue of gelled papaya fruit were accomplished.

MATERIAL AND METHODS

Papaya (*C. papaya* L. 'Golden') fruits were utilised in all the experiments at the three-quarters yellow stage of maturity (fruit with 51-75 % of yellow skin), according to Bron and Jacomino (2006). The number of replications was, at least, ten healthy and ten gelled fruit. The intact gelled fruits were identified in the Caliman Agrícola S/A packinghouse (Linhares, ES, Brazil) by sinking them in the water tank due to their higher density. The fruits were transported to the laboratory in refrigerated container. The time between sample collection and laboratory analysis was approximately 7 hours. In the laboratory, all of the fruits were weighed and cut in the middle, in a longitudinal section. The seeds were separated from the pulp by a spoon, weighed and counted. After firmness measurements, mesocarp samples were excised from the equatorial region of each fruit for soluble solids, electrolyte leakage, phosphate content, lipid peroxidation, anatomical and mineral analysis. After weighing and counting, the seeds were washed to eliminate the mucilage and mineral analysis was carried out. Healthy fruit were mechanically injured by dropping from a height of approximately 1.0 m to contrast with the gelling fruit for histological analyses.

The pulp firmness was determined by means of pulp penetration resistance measurements according to Fontes et al. (2008). Initially, each fruit was longitudinally sectioned in two faces. By using a bench penetrometer model 53205 (Fruit Pressure Tester, Italy) with an adaptor of 8 x 8 mm (height x diameter), the firmness was determined at three equidistant points of each face, 5 mm inwards from the exocarp. The results were expressed in Newton (N) unit.

Soluble solids content were determined from juice pressed by hand and the percentage of soluble solids was determined with an Atago ATC-1 refractometer (Atago, Co., Tokyo, Japan).

The methodology to electrolyte leakage was conducted according to Vasquez-Tello et al. (1990) with some modifications. Five cylindrical samples (10 mm x 10 mm) of each fruit were withdrawn from the fruit pulp to accomplish the electrolyte leakage measurements. After three washes in deionised water, the samples were immersed in 31 cm^3 of ultra-pure water (Milli-Q Biocel/A10, Millipore, USA) and maintained at 12 °C for 24 h. The free electrolyte leakage (FE) of the resultant solution was determined by means of a conductometer HI 8820N (Hanna Instruments, USA). The samples were heated at 80 °C for 1 h followed by 16 h at 12 °C to obtain the

total electrolyte content (TE). All the measurements of conductivity were performed when solutions achieved room temperature (25 °C).

A 0.5 cm^3 aliquot of each electrolytic solution was used for phosphate (Pi) release content determination, according to Fiske and Subbarow (1925).

About 500 mg of fruit pulp tissue were used for lipid peroxidation measurements, according to Alonso et al. (1997). Lipid peroxidation was determined through the malondialdeyde (MDA) content in pulp tissue extracts. MDA is a subproduct of lipid peroxidation that, when it reacts with thiobarbituric acid (TBA), forms a red colour complex. MDA content was calculated by using an extinction coefficient (ξ) of 155 $mM^{-1}.cm^{-1}$.

Pulp and seed tissues were sampled for mineral analysis. A total of fifteen replications were carried out for each fruit conditions (healthy and gelled). The samples were dried at 40 °C for 15 days. After drying, the seeds were ground in a Wiley-type mill with a 20 mesh sieve and maintained in hermetically-closed flasks. From the ground tissue, a sample of 500 mg was analysed for calcium (Ca), magnesium (Mg) and potassium (K) mineral content. The determination followed the methodology of Jones et al. (1991). Potassium was quantified by flame emission spectrophotometry, whereas calcium and magnesium were determined by atomic absorption spectrophotometry, after a nitro-perchloric digestion (HNO_3 and $HClO_4$).

To light microscopy, samples of fruit pulp tissue were fixed under low vacuum in Karnowsky solution (KARNOWSKY, 1965) for 2 h, at room temperature. Dehydration was carried out in a graded ethanol series, followed by infiltration and embedding in *JB-4* resin. Sections (5-6 μm) were obtained in a manual microtome, and stained with toluidine blue (0.05 %) in phosphate buffer, pH 4.7), for 5 min, rinsed in distilled water and air-dried. The sections were permanently mounted in *Permount*, and observed and documented using an upright *AxioPlan* (Zeiss, Jena, Germany) light microscope.

Samples of fruit pulp tissue were submitted to fixation and inclusion, following the standard preparation for electronic microscopy. The fruit fragments were fixed in 2.5 % glutaraldehyde and 2.0 % paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2 and post-fixed in 2.0 % OsO_4 during 2 h. Samples were dehydrated in acetone, and critical-point-dried using CO_2 in a Balzers CPD 080 apparatus and covered with 20 nm of gold (SCD 050 Bal-Tec). Afterwards, the samples were examined under an electron microscope scanning (DSEM ZEISS 962).

To transmission electron microscopy, samples of fruit pulp tissue were fixed in an aqueous solution containing 2.5 % glutaraldehyde and 4.0 % paraformaldehyde in 0.05 M cacodylate buffer (pH 7.2), under a low vacuum for 2 h. Subsequently, the samples were rinsed and post-fixed in 2.0 % OsO_4 during 2 h. The post-fixed samples were dehydrated in a graded series of acetone solutions and embedded in epoxi resin (*Polybed*). The ultra-thin sections (80 nm) were collected on copper grids (300 mesh), and stained with 1.0 % uranyl acetate followed by 5.0 % lead citrate. Sections were observed at 80 kV using a transmission electron microscope (ZEISS EM 900).

Data were subjected to analyses of variance and Tukey test were used for mean separation when the *F* test was significant at $P < 0.05$.

RESULTS AND DISCUSSION

Papaya fruit showing a gelling aspect are characterised by a disturbance in mesocarp regions of the fruit, resembling a water-soaked appearance of the flesh tissue that is not externally visible until it has reached very advanced stages of development (Figures 1A and B). This pulp disturbance is thought to develop from the ovarian cavity to the exocarp direction with variable degrees and, when the entire pulp is gelled, the fruit is commercially disqualified. Figures 1C and D show healthy fruit after mechanical injury with pulp damage from the exocarp to the ovarian cavity, contrasting to pulp disturbance.

Table 1 shows significant decreases ($P < 0.05$) in soluble solids, weight, total electrolyte content, total release of Pi, Mg and K contents in gelled fruit. This shows a physico-chemical alteration of fruit however suggesting cell integrity of membranes of the gelled fruit, which are able to maintain the osmotic gradient between the cytosol and the apoplast, as also supported by microscopy analysis (Figures 2B, 3B, D and F). The maintenance of the physical integrity of membranes is an important factor related to stress tolerance, such as water stress (ROY-MACAULEY et al., 1992), chilling injury (MARANGONI et al., 1996) and plant senescence (PALLIYATH; DROILLARD 1992, MARANGONI et al., 1996). When comparing lipid peroxidation (Table 1 - estimated by MDA content), no significant differences ($P < 0.05$) were observed between the healthy and gelling fruit, reinforcing the hypothesis of the maintenance of the cellular integrity. Positive correlations between cellular integrity, lipid peroxidation levels and electrolyte leakage have also been reported by Alonso et al., (1997) in coffee seedlings under stress (chilling)

conditions. Itzhaki et al. (1998) reported a decrease in phospholipid content as a major change in the membrane of the senescent rose petals.

According to Figure 2, morphological alterations were observed in gelling tissues, but without evident alterations in cell structure in healthy (A), gelling (B) and mechanically injured (C) treatments. However, these images revealed that, in the tissue of the healthy and mechanically injured papaya (Figures 2A and C), cells were entirely turgid and with little intercellular space. Gelled flesh tissue showed plasmolysed cells and large spaces between cells (Figure 2B), probably with water in the apoplast.

Light microscopy analysis corroborated the presence of more conspicuous intercellular spaces in the gelled flesh tissue (Figure 3D, arrow) when compared to healthy flesh tissue (Figure 3C). Transmission electron microscopy (Figures 3E and F) demonstrated the integrity of both cells walls (healthy and gelled). However, the cells walls of gelled fruit showed deformity, with cellulose microfibril loosening (Figure 3F). These characteristics are typical of flesh tissues during fruit ripening (ALI; LAZAN, 1998), however soluble solids (Table 1) are not found, in discordance with the hypothesis of precocious ripening. The soluble solids content in ripe papaya is at least 11° Brix (SOUZA et al., 2009).

In nectarines, the woolliness disturbance is related to the loss of cell membrane integrity when the cell fluids are released into the intercellular spaces and bind with pectin (VON MOLLENDORFF et al., 1992). In mango fruit with internal breakdown, a reduction in total pectins and pectinesterase activity were observed as well as a reduction of the firmness and the total soluble solids (TORRES & SAÚCO, 2004). Pectic substances are capable of binding water, forming gel complexes implicated in the development of woolliness in peaches (Ben-Arie & Lavee, 1971) and in plum (TAYLOR et al., 1995). However, woolliness is associated with loss of juiciness (BEN-ARIE; LAVEE, 1971; TAYLOR et al., 1995), which was not observed in gelled as well as in healthy fruit. In addition, the results of the free electrolyte leakage (Table 1) and images (Figures 2 and 3) showed the physical integrity of the cellular membranes.

The physical integrity of the membrane per se does not reveal its functional integrity. The primary role of most membrane proteins is transport; such proteins include the H^+-ATPases and Ca^{+2}-ATPases pumps. The H^+-ATPases work as electrogenic pumps and are able to utilise the energy associated with an electrochemical proton gradient across the membrane to drive the synthesis or hydrolysis of ATP (RATAJCZAK, 2000). In the vacuole, a V-type H^+-ATPase creates a $\mu H^{\pm}$ gradient across the tonoplast and this power is switched with ion transport, especially K, into the vacuole. Therefore, any factor affecting this proton pump activity may interfere in the K-stimulated osmotic gradient generated across the tonoplast. According to Dietz et al. (1998), Mg deficiency may provoke osmotic changes in the vacuole as this ion is a cofactor and an important factor of regulation of the V-type H^+-ATPase. As a consequence of lower K entry, the direction of the driving force might be altered and water is consequently forced out into intercellular spaces, resulting in the loss of the cellular tonus. In pineapple, it has been suggested that the decreased apoplastic osmotic potential, due the presence of sucrose in apoplast, and the subsequent increase of the water transfer into apoplast may favour the occurrence of pineapple translucency, a PD (CHEN; PAULL, 2000).

The Ca, Mg and K contents in seeds (Table 1), was not different ($P<0.05$) among healthy and gelling fruit, indicating that there is no competition between pulp fruit and seed for these minerals in both healthy and gelling fruit. The lower values ($P<0.05$) to flesh K and Mg content and cell ionic content (as demonstrated by the total electrolyte content values) (Table 1) verified in gelled fruit, seem to be in agreement with the hypothesis of the water soaked appearance of the tissue. In addition, the penetration resistance did not differ significantly ($P<0.05$) between the gelled and healthy fruit. These data suggest that the fruit gelling aspect is neither associated a premature nor advanced ripening of the tissue, but to cell sap saturation in the flesh tissue caused primarily by its accumulation in the apoplast, rather than the vacuole.

It is generally accepted that fruit ripening is accompanied by the softening of the flesh tissue by enzymatic cell wall-degradation (BRADY, 1987, PAULL et al., 1999). The Ca bound to pectin in the cell wall has been associated with maintenance of the firmness of fruit (QIU et al., 1995), and Ca is the most commonly reported nutrient associated with postharvest disorders (FERGUSON; WATKINS, 1989). These statements, in addition to the detected values for the flesh Ca contents (Table 1), suggest that fruit gelling is neither due to premature ripening nor loss of cell wall-bound Ca. The difference in Ca contents between healthy and gelling flesh tissue was not significant ($P<0.05$); these levels were higher than the critical Ca concentration for papaya (1.3

mg.g^{-1} DW) proposed by Qiu et al. (1995). Studies in apples with application of Ca reveal that this element inhibits the solubilization process of the cell wall polyuronids, delaying the pulp softening process (GLENN; POOVAIAH, 1990).

The other physiological disorder with symptoms like as gelling is water-soaking in melon fruit (CHATENET et al., 2000). In these PD, a higher water mobility associated with a higher density was observed in water-soaked areas, as compared to sound tissues indicates an increase in the level of free water. These authors (CHATENET et al., 2000) also observed the presence of large intercellular spaces, as seen in gelling papaya fruit (Figures 2B and 3D). However, the melon tissue presented disorganisation of the cell wall, probably due, according to the authors, to the depletion of cell wall Ca. The Ca has been directly involved in texture changes and the adequate Ca content of fruit at harvest is known to be crucial in maintaining fruit quality (SAMS, 1999). Ours data suggest that Ca does not participate in the development of the gelling disturbance.

In future studies, new approaches will be considered, such as field analyses of the climatic variables, besides the participation of H^+-ATPases and the availability of energetic molecules in the beginning of the gelling disturbance. In addition, a better understanding of the cellular mechanisms related to gelling in papaya fruit may result in new subsidies that could be applied to the crop management avoiding, or at least minimising, the harmful effects of the PD.

TABLE 1 - Analysis of the characteristics of flesh and seeds of papaya (*Carica papaya* L. cv. Golden) healthy and gelled fruit.

Characteristics analyzed	Healthy fruit[1]	Gelled fruit
Pulp Firmness (N)	1.85 ± 0.27 **a**	2.08 ± 0.30 **a**
Number of seeds	494.83 ± 37.09 **a**	461.50 ± 32.72 **a**
Weight (g)	333.20 ± 10.78 **a**	310.37 ± 15.70 **b**
Soluble solids (°Brix)	10.00 ± 0.11 **a**	12.4 ± 0.60 **b**
Free electrolyte leakage *	57.30 ± 3.88 **a**	29.90 ± 1.12 **a**
Total electrolyte content*	363.40 ± 12.32 **a**	265.90 ± 34.83 **b**
Free release of Pi**	0.004 ± 0.001 **a**	0.002 ± 0.001 **a**
Total release of Pi**	0.018 ± 0.001 **a**	0.014 ± 0.001 **b**
Lipid peroxidation***	0.031 ± 0.008 **a**	0.028 ± 0.007 **a**
Pulp Ca content ****	2.67 ± 0.43 **a**	1.63 ± 0.31 **a**
Seeds Ca content ****	7.52 ± 0.50 **a**	8.68 ± 0.42 **a**
Pulp Mg content ****	3.43 ± 0.44 **a**	1.95 ± 0.17 **b**
Seeds Mg content ****	4.28 ± 0.34 **a**	4.35 ± 0.35 **a**
Pulp K content****	26.17 ± 1.95 **a**	15.83 ± 0.47 **b**
Seeds K content****	22.00 ± 0.70 **a**	23.00 ± 0.63 **a**

[1] The data refer to the average (± se) of at least ten fruit, picked at random from a sample of fruit of the orchard. The averages followed by different letters indicate significant differences at 5%, by the F test. FW – fresh weight, DW – Dry weight, * µS, ** mmol.dm^{-3}, *** µmol MDA.mg^{-1} FW, ****g.kg^{-1} DW.

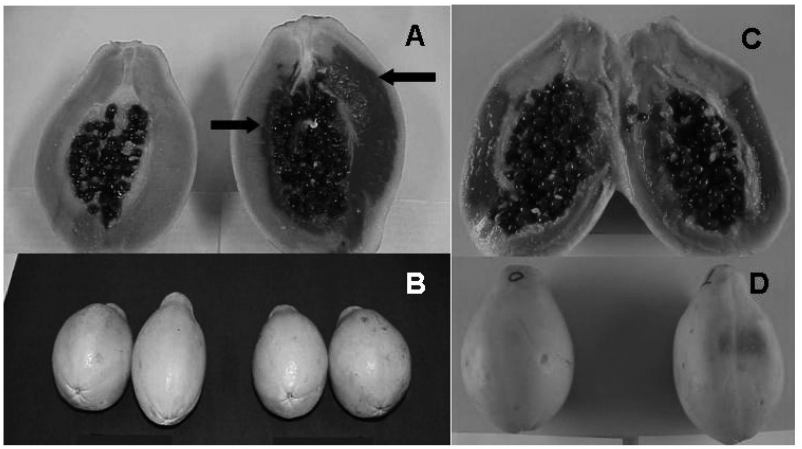

FIGURE 1 - (A) Photograph of the longitudinal sections of healthy (left) and gelled papaya fruit (right). Note arrows indicating the asymmetric symptoms in the gelling fruit pulp. (B) Photograph showing the normal external aspect in both healthy (left) and gelled (right) fruit. (C) Photograph of the longitudinal section of papaya submitted to mechanical injury. (D) Photographs show the normal external aspect of both healthy (left) and submitted to mechanical injury (right) fruit.

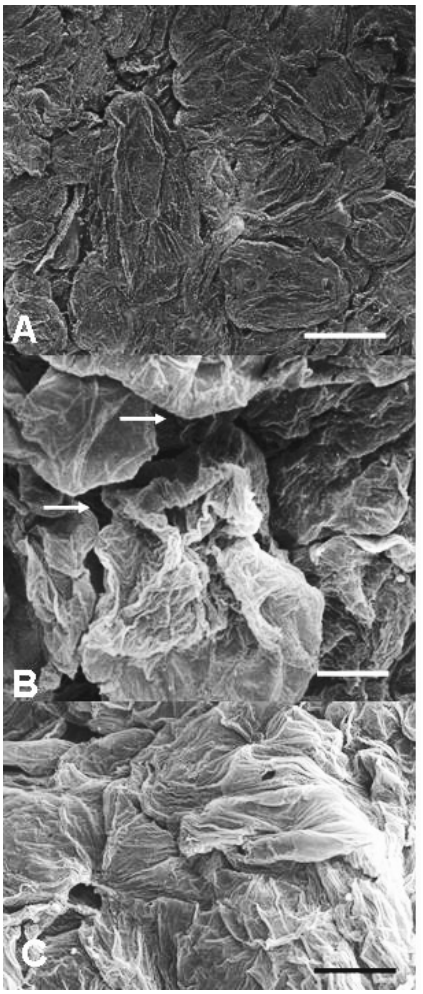

FIGURE 2 - Scanning electron micrographs of tissues of the healthy mesocarp (A), Bar = 100 μm; gelled mesocarp (B), Bar = 20 μm and mechanically-injured papaya fruit (C), Bar = 20 μm. Note arrows indicating the intercellular spaces in B.

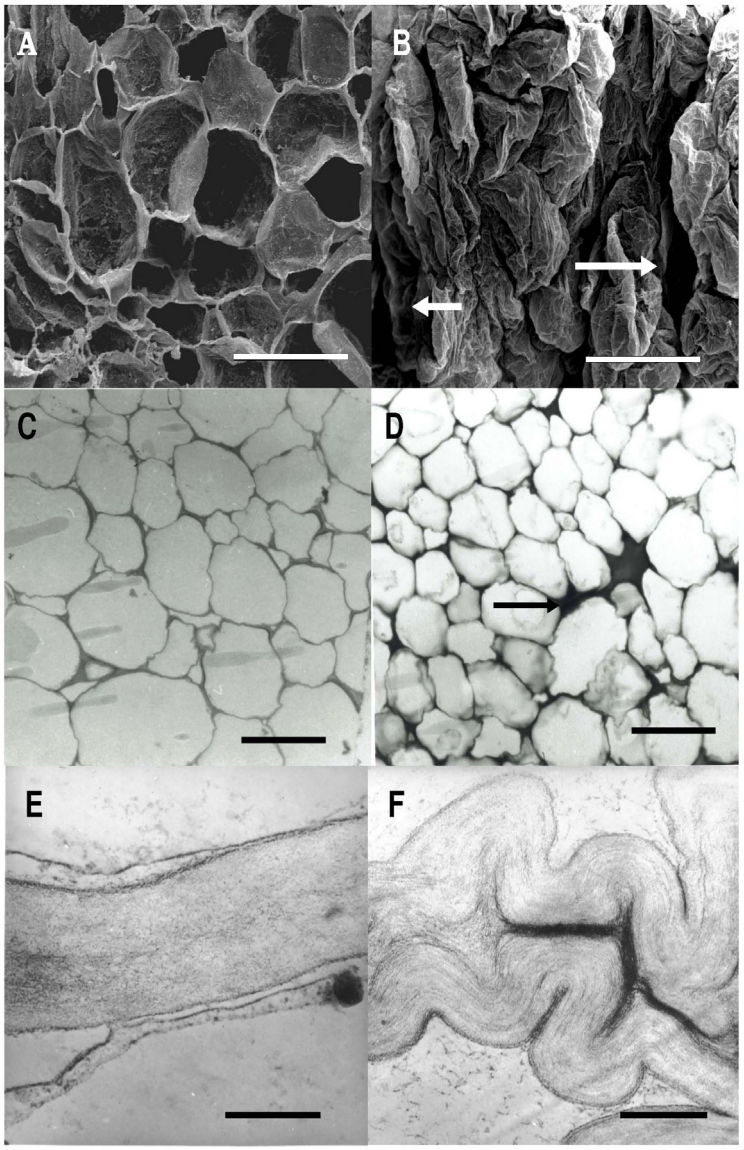

FIGURE 3 - Samples of healthy (A, C and E) and gelled (B, D and F) mesocarp papaya fruit. Scanning electron micrographs (A and B) show plasmolized cell and intercellular spaces in gelled fruit (arrows), Bars = 100 μm. Light microscopy (C and D) show the intercellular space in gelled fruit (arrow), Bars = 20 μm. Transmission electron microscopy from cell wall (E and F) showing disestablished cell wall in gelled fruit, Bars = 0.5 μm.

CONCLUSIONS

Our results suggest that the gelling aspect of papaya fruit is neither due to premature ripening nor mechanical injury of the flesh. Data suggest that the inhibition of water transport to the vacuole, followed by the loss of cellular turgor pressure is the main causes of the PD. Moreover, the water soaked appearance of the tissue is due to the accumulation of water in the apoplast. There is no evidence of the participation of Ca in this disturbance. In future studies, new approaches will be considered such as the participation of H^+ATPases and the availability of energetic molecules on the origin of this disturbance.

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