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Effect of Immersion Time in Osmosis and Ultrasound on Papaya Cell Structure during Dehydration

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The effect of ultrasound-assisted osmotic dehydration applied at atmospheric pressure for different lengths of time on papaya tissue structure was evaluated. Ultrasound induced the loss of cellular adhesion, formation of large cell interspaces, and light rupture of the cell walls. The changes in the tissue structure caused by ultrasound application increased sugar loss, water loss, and effective water diffusivity. Ultrasound-assisted osmotic dehydration induced a gradual distortion in the shape of the cells, loss of cellular adhesion, and the formation of large channels caused by rupture of the cell walls. The changes caused by the application of osmotic dehydration resulted in high water loss and sugar gain.

Keywords Drying; Image analysis; Osmotic dehydration; Papaya; Ultrasound

INTRODUCTION

Drying is one of the most common methods for food conservation. It is an energy-intensive process and cost reduction can generally be attained by reducing air-drying time.[1] Osmotic dehydration and ultrasound application are pretreatments that can help reduce air-drying time and consequently reduce processing cost.

Osmotic dehydration is the most commonly reported pretreatment used before air drying. The technique consists in immersing the fruit in a hypertonic solution to remove part of the initial water content of the fruit. The driving force for water removal is the difference in osmotic pressure between the fruit and the osmotic solution. The complex cellular structure of the fruit acts as a semipermeable membrane in this process. $[2,3]$ Application of osmotic dehydration changes the fruit texture, especially because of pectin dissolution and breakdown of cells, as has been demonstrated for strawberries.[4–8]

Ultrasonic waves cause a rapid series of alternative compressions and expansions, in a similar way to a sponge when it is squeezed and released repeatedly (sponge effect). The forces involved in this mechanism can create microscopic channels in the fruit tissue, which may ease moisture removal. In addition, ultrasound produces cavitation, which may be helpful to remove strongly attached moisture. The sponge effect caused by ultrasound application may be responsible for the creation of microscopic channels and the enlargement of cell interspaces.^[9–11] These microchannels were shown in micrographs by Fernandes et al.^[12] in melon tissue after ultrasound application.

Studies on osmotic dehydration, ultrasound, and ultrasound-assisted osmotic dehydration have shown that different fruits respond differently to the application of these drying pretreatments.^[13–24] The aim of this study was to evaluate the effect of ultrasound-assisted osmotic dehydration on papaya tissue. The evolution of cellular structure was studied by light microscopy and the structural modifications resulting from osmotic dehydration and ultrasonic waves were analyzed.

MATERIALS AND METHODS

Preparation of Samples

Papayas, Formosa cultivar, were bought from the producer (Fortaleza, Brazil). Papaya were cut and sliced to obtain cubes of same dimensions $(2.0 \times 2.0 \times 2.0 \text{ cm})$. The moisture content was determined by heating in a drying oven (Marconi model MA-085) at 60° C for 48 h according to AOAC method 934.06.^[25] The initial concentration of solute (°Brix) was determined by refractometry.

Pretreatments

An experimental set of four papaya samples was immersed in distilled water and subjected to ultrasonic waves for 10, 20, and 30 min. Experiments were also carried

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out immersing four papaya samples in osmotic solution, which were subjected to ultrasonic waves for the same time intervals. The osmotic solution was prepared by mixing food-grade sucrose with distilled water to give a concentration of 25°Brix. The weight ratio of fruit to liquid medium was set at 1:4 to avoid dilution of the liquid medium.^[26]

The experiments with the ultrasonic treatment were carried out in separate 250-mL Erlenmeyer flasks to avoid interference between the samples and the runs. The experiments were carried out under ambient temperature (30°C) in an ultrasonic bath (Marconi model Unique USC, Piracicaba, Brazil; internal dimensions: $24 \times 14 \times 9$ cm; volume: 2.7 L) without mechanical agitation. The ultrasound frequency was 25 kHz and the intensity was $4870 \,\mathrm{W/m^2}$. The ultrasound intensity was determined by the calorimetric method. $[27]$

After removal from the solution, the samples were drained and blotted with absorbent paper to remove excess solution. Each assay was made in triplicate. From each replicate, one random sample was used in microscopy image analysis studies and three samples were air dried.

Weight, moisture content, and the sugar content of the fruit and the liquid medium were used to calculate water loss (WL) and solid gain (SG) of the samples, according to the equations:

$$
WL(^{0}_{0}) = \frac{(w_i \cdot X_i - w_f \cdot X_f)}{w_i} \cdot 100
$$
 (1)

$$
SG(\%) = \frac{w_f \cdot X_{sf} - w_i \cdot X_{si}}{w_i} \cdot 100
$$
 (2)

where X_i is the initial fruit moisture on wet basis (g water/g), X_f is the final fruit moisture on wet basis (g water/g), X_{si} is the initial fruit soluble solid content (g solid/g), X_{sf} is the final fruit soluble solid content (g solid/g), w_i is the initial fruit mass (g), and w_f is the final fruit mass (g).

Air Drying

The samples were set in a single layer in trays and were air dried in a forced circulating air-drying oven (Marconi model MA-085, Piracicaba, Brazil). The forced circulating air-drying oven was set at 60°C. Air was injected at the sides of the dryer at $0.5 \,\mathrm{m/s}$, flowing in parallel direction relative to the samples. The air moisture content was 16% and was determined by psychrometry. The fruit moisture (water content) during the air-drying period was measured weighting the fruit samples every 20 min for the first 5 h of drying and then every hour until constant weight.

The experimental data were used to calculate the effective water diffusivity of papayas during air drying according to Fick's Law of diffusion. The equation used for the falling-rate period of the drying process was based on the simplification of Fick's second law considering long processing period.^[1] The effective water diffusion parameter was adjusted using Eq. (3) with a parameter estimation procedure based on the minimization of the error sum of squares.

$$
\frac{dH}{dt} = -\frac{2\pi}{\delta^2} \cdot D \cdot (H - H_{eq})
$$
 (3)

where D is the effective water diffusivity (m^2/s) , H is the moisture content, H_{eq} is the equilibrium moisture content, t is the time (s), and δ is the thickness of the fruit (m).

Light Microscopic Analysis

After the end of each pretreatment the samples were carefully cut into cubes of 5 mm average side. The sample cubes were fixed with 4% solution of paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, and 1% glutaraldehyde for 24 h at ambient temperature.[28] The material was then dehydrated in a graded ethanol series and embedded in Historesin embedding kit (Jung). The tissue blocks were sectioned at 8 *m*m on a Leica RM 2065 microtome (Leica, Nussloch, Germany). The Periodic Acid-Schiff reagent (PAS) cytochemical reaction was employed for polysaccharide detection.[29] Photomicrographs of the cell structure were taken using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) with a digital image capture system.

RESULTS AND DISCUSSION

The effects of both pretreatments on water loss and sugar gain are presented in Table 1. The initial moisture content of the papayas was 0.883 ± 0.004 g water/g of fruit and the initial soluble solids content was $11.6 \pm 0.4^{\circ}$ Brix. Two experiments with identical operating conditions have been conducted to test the repeatability of the water loss data, sugar gain data, and moisture data during the pretreatment and the air-drying step. The percentage of reproducibility was calculated based on Eq. (4):

$$
\frac{P_1 - P_2}{P_1} \times 100\% \tag{4}
$$

where P presents the parameters WL, SG, and H (moisture content) and subscripts 1 and 2 present two consecutive data with similar drying conditions. Results obtained from the experiments have shown that we were able to reproduce WL, SG, and H within ± 3.2 , 6.5, and 8.5%, respectively.

The results show that water loss increased with time and also increased when an osmotic solution was employed. The increase in water loss because of increasing soluble solids concentration in the osmotic solution is consistent with the greater osmotic pressure of the system. When distilled water was used as the liquid medium the fruit transferred sugar to the liquid medium, losing 13.8% of sugar after 30 min of treatment. Using an osmotic solution of 25- brix (soluble solids content), the sugar gain increased

Operating condition	Treatment period (min)	Sugar $loss^a$ $(\%)$	Water loss (%)	Water effective diffusivity ^b (m^2/s)
No pretreatment (air-drying only)				$6.50 \cdot 10^{-9} \pm 0.09 \cdot 10^{-9}$
Distilled water	10	9.89 ± 0.64	3.15 ± 0.63	$6.69 \cdot 10^{-9} \pm 0.57 \cdot 10^{-9}$
Distilled water	20	10.66 ± 0.18	7.72 ± 1.92	$8.37 \cdot 10^{-9} \pm 0.12 \cdot 10^{-9}$
Distilled water	30	13.77 ± 1.09	9.70 ± 1.45	$8.07 \cdot 10^{-9} \pm 0.54 \cdot 10^{-9}$
Osmotic solution $(25^{\circ}Brix)$	10	-3.05 ± 0.49	12.11 ± 1.53	$5.42 \cdot 10^{-9} \pm 0.44 \cdot 10^{-9}$
Osmotic solution $(25^{\circ}Brix)$	20	-4.45 ± 2.92	13.42 ± 0.43	$5.91 \cdot 10^{-9} \pm 0.46 \cdot 10^{-9}$
Osmotic solution $(25^{\circ}Brix)$	30	-10.26 ± 1.16	16.37 ± 1.97	$7.32 \cdot 10^{-9} \pm 0.53 \cdot 10^{-9}$

TABLE 1 Water loss and sugar gain after pretreatment and water diffusivity during air drying

^aNegative numbers represent sugar gain instead of sugar loss.

^bRegression R^2 for all water effective diffusivity curves were higher than 0.985.

with time and showed a steep increase between 20 and 30 min of treatment.

The microscopic image analysis of the fresh fruit showed typical thin-walled cells with normal morphology and no visible intercellular spaces. The tissue of the fruit showed a high degree of disruption of cells, creating several large cell interspaces, during the first 10 min under ultrasound application when distilled water was used as the liquid medium. The disruption of the cell has contributed to the high sugar loss observed during ultrasound application (Fig. 1). At the end of the ultrasonic pretreatment the fruit lost 9.7% of water, which may be related to the sponge effect of the ultrasonic waves and to the changes on the fruit tissue. After 30 min of ultrasonic treatment, the cells became slightly distorted and some cells began to breakdown (Fig. 2A). The disruption of cells continued and more microscopic channels were formed (Fig. 2B). Microscopic voids in papayas were mostly formed by disruption of contiguous cells, which produced large cell interspaces. The formation of microscopic voids in papayas differed from the mechanism observed in melons, where microscopic channels were formed by flattening and elongation of cells. The formation of microscopic channels in papayas, however, was similar to the formation of channels in pineapples where smaller cell interspaces were formed by disruption of cells.[17]

The sugar loss observed for papayas was lower than the sugar loss observed for other fruits such as banana, pineapples, and malay apples, which have lost 21.3, 23.2, and 17.0%, respectively, after 30 min under the same conditions.^[13,17,30]

The use of ultrasound increased the effective water diffusivity by 28.7% during the air-drying process (Table 1). The increase in effective water diffusivity reduced the total time required for drying. This result confirmed the observations of Fuente-Blanco et al.^[9] that the ultrasonic pretreatment affects the fruit tissue, making it easier for the water to diffuse through the tissue of the fruit during the air-drying process. It also showed that the microscopic channels contribute toward increasing the effective water diffusivity.

The increase in the effective water diffusivity was lower than the values found for melons and pineapples where an increase by 39.3 and 64.3%, respectively, was observed.^[17] The lower increase in water diffusivity may be related to the size of the cell interspaces formed in papaya tissue that were smaller in length than those observed in pineapples and melons. Although a greater number of large cell

FIG. 1. Photomicrographs of papaya cubes after 10 min of ultrasound pretreatment. (A) Region with small cell interspaces; and (B) region with large cell interspaces. Arrow indicates the cell interspaces. Magnification of $380\times$.

FIG. 2. Photomicrographs of papaya cubes after 30 min of ultrasound pretreatment. (A) Region with cell breakdown; and (B) region with cell interspaces. Magnification of $380\times$.

interspaces were formed in papayas, these interspaces were separated by cells, which increased the resistance for water to permeate through the tissue.

Significant differences were observed when an osmotic solution was employed in the pretreatment. Longer microchannels were formed by disruption of cells and also by breakdown of cells. After 10-min immersion in an osmotic solution (25°Brix), the cells became more distorted (Fig. 3A) and several cell interspaces formed by disruption of cells and channels formed by breakdown of cell walls appeared (Fig. 3B). Breakdown of the cells was observed in some regions, and produced large spaces that may be formed by solubilizing of chelator-soluble pectin of the middle lamella. Chelator-soluble pectin is the substance that most contributes to cell adhesion and firmness and according to the microscopic images may solubilize at the early stages of osmotic dehydration and ultrasound application.

After 20 min, several cells showed loss of pectin and groups of cells (two or three) became contiguous with the solubilizing of part of the cell wall (Fig. 4). Water loss and sugar gain increased steeply between 20 and 30 min subjected to ultrasound-assisted osmotic dehydration. Figure 5 shows that after 30 min the tissue of papayas presented a high degree of cell breakdown, forming some very large spaces where water and sugar could flow more easily, which have contributed to the steep increase in water loss and sugar gain observed during this period. Effective water diffusivity also has increased steeply after this period (Table 1), influenced by the very large spaces formed in papaya tissue.

The values found in this work for the effective diffusivity of water for fresh papayas were slightly higher than the values reported by El-Aouar et al.^[31] $(4.78 \times 10^{-9} \text{ m/s})$, but the value reported by El-Aouar et al.^[31] was obtained

FIG. 3. Photomicrographs of papaya cubes after 10 min of ultrasoundassisted osmotic dehydration using a 25°Brix osmotic solution. (A) Region with formation of microscopic channels (arrow indicates the microscopic channel); and (B) region with breakdown of cells and severely distorted cells. Magnification of $380\times$.

FIG. 4. Photomicrographs of papaya cubes after 20 min of ultrasoundassisted osmotic dehydration using a 25°Brix osmotic solution. Region with severely distorted cells and channels formed by disruption of cells and by breakdown of contiguous cells. Magnification of $380\times$.

for a smaller variety of papaya. The values obtained in this work for the effective diffusivity of water for ultrasoundassisted osmotic dehydrated papayas were also higher than the values reported by El-Aouar et al.^[31] $(1.78 \times 10^{-9} \text{ m/s})$ for osmo-dehydrated papayas. The difference may be explained by the operating conditions applied in each study. In the present study, papayas were subjected to ultrasound and to an osmotic solution of 25°Brix, whereas in El-Aouar et al.'s^[31] study papayas were subjected to an osmotic solution of 70°Brix, which may have saturated the fruit with sucrose, creating an extra resistance for the diffusion of water.

From an economical point of view, the ultrasonic pretreatment is cost-effective. To process 1 kg of papaya, an ultrasonic bath requires 11.1 kJ/min of operation. This value required value per kilogram of fruit is higher than that required by a circulating oven or a tray dryer, which requires 4.5 kJ/min of operation. The higher power consumption of the ultrasonic process is compensated for by the reduction in the air-drying time. Calculating the total energetic cost at the best operating conditions, the fresh fruit will require 314 min to reduce its moisture content by 95% and will consume 1397 kJ/kg of fruit; and the ultrasonic process using distilled water as the liquid medium will require 20 min of ultrasound and 244 min of air-drying, consuming 1308 kJ/kg of fruit. On the other

FIG. 5. Photomicrographs of papaya cubes after 30 min of ultrasoundassisted osmotic dehydration using a 25°Brix osmotic solution. (A) Region with distorted cells and medium size cell interspaces; and (B) region with cells with severe cell breakdown. Magnification of $380\times$.

hand, the use of ultrasonic-assisted osmotic dehydration will require 30 min of ultrasound and 279 min of air drying, consuming 1574 kJ/kg of fruit. The latter result is higher because of the lower value of the effective diffusivity of water, which increases the required air-drying time. If the cost of energy is assumed to be US\$0.306/kWh (cost of electrical power in Brazil in June of 2008), the cost of the ultrasonic process would stand at US\$0.119/kg. The cost of using the air-drying process without pretreatment of the fruit would cost US\$0.134/kg. The results show that the ultrasonic process is economically viable, 11% less expensive than the air-drying process to dry papaya. It is important to notice that these values were calculated based on small-scale equipment and that lower operating costs may be expected for large-scale production.

CONCLUSION

Ultrasound and ultrasound-assisted osmotic dehydration induced changes on papaya cell structure. Channels were formed in the tissue structure and may be responsible for the increase in the effective water diffusivity because they offer lower resistance to water diffusion.

Formation of microscopic channels occurred mainly by disruption of cells when distilled water was used. When an osmotic solution was used, microscopic channels were formed by disruption of cells and by a high degree of breakdown of cells.

Good agreement was obtained between cellular structure change, water loss, sugar gain, and water diffusivity. The channels formed by disruption of cells contributed to increase water and sugar mass transfer between the fruit and the liquid medium. Water diffusivity increased when the treatment was carried out for more than 30 min because of the formation of microchannels and breakdown of cells, which lowered the resistance to water diffusion.

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