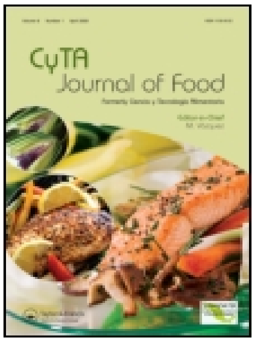

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Efficacy of high hydrostatic pressure as a quarantine treatment to improve the quality of mango fruits infested by the Mexican fruit fly *Anastrepha ludens*

Eficacia de la alta presión hidrostática como tratamiento cuarentenario para mejorar la calidad de los frutos del mango infestados por la mosca de la fruta mexicana *Anastrepha ludens*

H.E. Candelario-Rodríguez[a], M. Hurtado-González[b,c], J. Morales-Castro[b], G. Velazquez[a]*, J.A. Ramírez[a], J. Loera-Gallardo[d] and M. Vázquez[a,e]

[a]*Departamento de Ciencia y Tecnología de Alimentos, UAM Reynosa Aztlán, Universidad Autónoma de Tamaulipas, Calle 16 y Lago de Chapala s/n, Col. Aztlán, Reynosa, Tamaulipas 88740, Mexico;* [b]*Instituto Tecnológico de Durango, Posgrado en Ingeniería Bioquímica Blvd, Felipe Pescador 1830 Ote, Durango, Durango 34080, México;* [c]*Instituto de Ecología Aplicada, Universidad Autónoma de Tamaulipas División del Golfo No. 356, Col. Libertad. Cd, Victoria, Tamaulipas 87019, México;* [d]*Campo Experimental Rio Bravo, CIR Noreste, INIFAP., Apdo 172, Río Bravo 88900, Tamaulipas, Mexico;* [e]*Departament of Analytical Chemistry, Faculty of Veterinary, University of Santiago de Compostela, 27002-Lugo, Spain*

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High hydrostatic pressure (HPP) has been reported as an alternative quarantine process in fruits infested by *Ceratitis capitata* Wiedemann, *Rhagoletis indifferens* Curran and *Cydia pomonella* (L.). In Mexico and other Latin-American countries, the Mexican fruit fly *Anastrepha ludens* Loew is one of the most important insects infesting mangoes, citrus, and other fruits. The present study aimed to determine the effect of pressure level and time on the survival of eggs and larvae of the Mexican fruit fly. Eggs and larvae were pressurized at 25, 50, 75, 100, or 150 MPa for 0, 5, 10 or 20 min at 25 °C. Ripe and green mangoes were also pressurized under the same conditions. On pressurized eggs of 1, 2, 3, and 4 days old, their ability to hatch was recorded. On pressurized first, second and third instars, the percentage of survival was registered. Furthermore, third instars were studied for their ability to pupate and to develop adults. The results showed that although most of eggs and larvae died at pressures lower than 100 MPa, some of them were able to survive even at 150 MPa, and a few third instars were able to pupate and to develop to adulthood. Green mangoes were affected by pressures above 75 MPa but they were more resistant than ripe mangoes. HPP treatments seem to be feasible as a quarantine process for mangoes; however, more studies, such as combining HPP with temperature treatments, are needed in order to decrease the pressure level to avoid fruit damage.

Keywords: Mexican fruit fly; high hydrostatic pressure; eggs; larvae; quarantine; mango fruits

La alta presión hidrostática ha sido descrita como un proceso de cuarentena alternativa en frutas infestadas por *Ceratitis capitata* Wiedemann, *Rhagoletis indifferens* Curran y *Cydia pomonella* (L.). En México y otros países Latinoamericanos, la mosca de la fruta mexicana *Anastrepha ludens* Loew es uno de los más importantes insectos que infestan los mangos, cítricos y otras frutas. El presente estudio tuvo como meta determinar el efecto del nivel de presión y tiempo sobre la supervivencia de huevos y larvas de la mosca de la fruta mexicana. Huevos y larvas se presurizaron a 25, 50, 75, 100, y 150 MPa durante 0, 5, 10, y 20 min a 25 °C. Mangos maduros y verdes también se presurizaron bajo las mismas condiciones. En huevos presurizados de uno, dos, tres y cuatro días de vida, se registró su capacidad para eclosionar. En primera, segunda y tercera etapa larvaria presurizada, el porcentaje de supervivencia también se registró. Además, en la tercera etapa larvaria se estudió su capacidad para salir de la crisálida y desarrollar adultos. Los resultados mostraron que aunque la mayoría de los huevos y larvas murieron a presiones por debajo de 100 MPa, algunos de ellos fueron capaces de sobrevivir aun a 150 MPa, y unas pocas larvas de tercera etapa fueron capaces de salir de la crisálida y desarrollar adultos. Los mangos verdes se vieron afectados por las presiones por encima de 75 MPa pero fueron más resistentes que los mangos maduros. Los tratamientos de alta presión hidrostática parecen ser un proceso viable como método cuarentenario para mangos, sin embargo, se necesitan más estudios tales como la combinación de alta presión hidrostática junto con tratamientos térmicos para disminuir los niveles de presión y evitar daños en la fruta.

Palabras clave: mosca de la fruta mexicana; alta presión hidrostática; huevos; larvas; cuarentena; frutos del mango

Introduction

The Mexican fruit fly *Anastrepha ludens* Loew is a pest of economical importance distributed from the Rio Grande Valley in the south of Texas (USA) to Costa Rica. In Mexico, it is found all over the country; however, some northern states are classified as being

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*Corresponding author. Email: gvelazquez@uat.edu.mx

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free or having low prevalence of this pest. *A. ludens* has been reported infesting 23 species of plants, but prefers mangoes, citrus, and two rutaceae native plants from Mexico: yellow chapote (*Sargentia greggi*, S. Watson) and white sapote (*Casimiroa edulis*, Llave and Lex) (Hernández-Ortíz, 2007).

Effective quarantine treatments for fruits are required to prevent the spread of exotic pests through marketing channels to areas where they do not normally occur. These treatments must be not harmful to either the fruits or to people coming in contact with or consuming the commodity.

Fumigation with toxic compounds such as ethylene dibromide and methyl bromide is no longer the first quarantine treatment option due to human health and environmental concerns (Armstrong & Mangan, 2007). Fumigation has been replaced by irradiation and physical treatments including refrigeration (or cold) treatment, vapour heat and forced hot-air treatment, and hot-water immersion treatment (Armstrong & Mangan, 2007). Quarantine treatments are required to meet a prescribed degree of statistically probability that the treatment will kill over 99.9968% of the biological target (Probit 9) in USA, which means that no more than three individuals from a population of 100,000 will survive the treatment. Other countries have other requirements. For example, Japan requires no surviving individuals from a population of 30,000 (Jacobi, Macrae, & Hetherington, 2001).

The potential for high hydrostatic pressure (HPP) as an alternative method of quarantine in fruit has been reported previously. Butz and Tauscher (1995) indicated that eggs of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, withstood a pressure of 100 MPa for 20 min at 25 or 32.5 °C but they were inhibited to hatch when pressures of 125 to 600 MPa were used independently of the time of pressurization (0 to 20 min). Neven, Follet, and Raghubeer (2007) found that eggs of the codling moth, *Cydia pomonella* (L.) were unable to hatch when pressures higher than 207 MPa for 1 min at 16–18 °C were used. However, eggs and larvae of the western cherry fruit fly, *Rhagoletis indifferens* Curran, showed 100% mortality at pressures higher than 172.4 MPa for 1 min at 16–18 °C. These authors reported that eggs of both insects (fruit fly and moth) were more tolerant to HPP treatment than larvae.

The effect of HPP processing on mangoes infested by the Mexican fruit fly has not been studied. This tropical fruit is one of the most popular and widespread in the world. It is consumed preferentially fresh, although some nonfibrous pulpy mango varieties are used for processing (Boynton, Sims, Sargent, Balaban, & Marshall, 2002). Postharvest disinfestations of fresh mango fruit in the USA, Central and South America is performed preferentially by the hot water immersion treatment. This process consists of immersing fruits in water held at 43–46 °C for 65–90 min, depending on fruit size. It has been reported that it induces a range of external and internal heat injuries in a number of cultivars, including skin scalding, lenticel damage, cavitation and retention of unripe, starchy areas in the mesocarp as the fruit ripens (Jacobi et al., 2001).

The use of HPP processing (300–600 MPa) has been reported as an alternative method to preserve the quality and stability of precut mangoes (Boynton et al., 2002) and as a method to improve quality during freezing by using the high-pressure-shift freezing technique which reduced problems derived from thermal gradients, such as freeze-cracking and differences in ice crystal sizes at the surface and the centre of the samples (Otero, Martino, Zaritzky, Solas, & Sanz, 2000).

The objective of this work was to determine the effect of time and level of HPP processing at 25 °C on eggs hatching and survivorship and pupation of larvae of the Mexican fruit fly, *A. Ludens*.

Material and methods

Rearing of eggs and larvae

The experimental work was conducted in the facility of Moscafrut at Metapa de Domínguez, Chiapas, Mexico, where 220 millions of *A. ludens* are reared weekly. The eggs and larvae used in our study were supplied from this facility and they were reared under the usual procedures. The adults were in metal cages of 1.5 × 1.4 × 0.3 m^3. There were 70,000 specimens/cage and they were fed with an artificial diet containing protein and sugars in an 1:3 ratio (for composition see http://www.moscasdelafrutamexico.org.mx/moscafrut/crianaso.htm). Water was freely available in moistened filter papers. Flies were maintained at 26 °C, 60% RH, and a photoperiod of 13:11 (L:D) h. Eggs were laid on cloths and collected twice a day by soaking the exterior of the cloths to loose them. They were transferred to plastic containers of 20 L and kept at a concentration of 600 eggs/mL of water. Eggs were incubated during 4 days at 26 °C ± 1 °C and aeration was supplied by an air pump.

On the fourth day, guar gum was added to the liquid containing the eggs, to prepare a suspension able to maintain a uniform distribution of the eggs. Then, a sample of 2 mL was dispersed in 300 g of diet and placed in a rectangular 1 L plastic container which is covered with a cloth mesh. The temperature was held at 27 °C during the first instar (1–3 days), at 26 °C during the second instar development (4–6 days), and at 25 °C for the third instar development (7–9 days). The relative humidity was held at 75 ± 5% and the containers remained in darkness.

High hydrostatic pressure treatments

Pressure treatments were carried out by using a Cold Isostatic Press Model CIP42260 (Avure Autoclave

Systems, Columbus, OH), with a pressurizing chamber of 101.6 mm of inner diameter and 584.2 mm of length and operating pressure capacity of 60,000 psi (near to 412 MPa). A mixture of 5:1 water:anticorrosive lubricant (Hydrolubric 120-B. E.F. Houghton and Co., Valley Forge, PA) was used as the pressurizing fluid and the temperature of this mixture was adjusted at 25 °C before pressurizing.

Eggs and larvae were pressurized at 25, 50, 75, 100, or 150 MPa for 0, 5, 10, or 20 min at 25 °C. The time required to reach the final pressure was 8, 17, 44, 60 and 82 s, respectively. The release of pressure required was always less than 30 s. The temperature of the pressurizing fluid was never less than 23 °C at the end of the treatments.

High hydrostatic pressure treatment on eggs

To keep uniformity, only the eggs produced in the afternoon were used in this study. Eggs of 1, 2, 3 or 4 days old were processed independently. An aliquot of an egg/water solution containing 600 eggs/mL was taken from a 20 L plastic container to fill up completely eppendorf tubes, closing them immediately to avoid the presence of air bubbles. The isostatic pressurization was in the range of 25–150 MPa for 0–20 min at 25 °C ± 1 °C. Each eppendorf tube was used only once. After treatments, a brush was used to separate the treated eggs from the eppendorf tubes. Treated eggs were arranged in three rows of 100 eggs in Petri dishes using a stereoscope and incubated at 26 °C ± 1 °C during 7 days to record the egg hatch.

Obtaining and surviving of larvae

On the fourth day after oviposition, 0.1% guar gum (Tic Gums Inc, MD) was added to the liquid containing the eggs to prepare a suspension able to maintain a uniform distribution of the eggs. A 2 mL sample of the suspension that contained 600 eggs/mL was dispersed in 300 g of diet, and placed in a rectangular plastic container of 1 L, as mentioned. The containers were covered with a cloth mesh and the eggs were held at 27 °C for the first instar (1–3 days old), 26 °C for the second instar (4–6 days old), and 25 °C for the third instar (7–9 days old). Containers were maintained under complete darkness and 75 ± 5% RH and used for pressure treatments.

High hydrostatic pressure treatment on larvae

The high pressure treatments were in the range of 25–150 MPa for 0–20 min at 25 °C ± 1 °C. The treatments were applied to first, second and third instars, when they were 2, 5 and 8 days old, respectively. Each instar plus 300 g of diet were introduced into plastic containers of 200 mL per instar. The containers were hermetically closed immediately before the high pressure treatment. After the high pressure treatment, they were opened and larvae in diets were returned to a 1 L plastic container, dispersed smoothly and incubated for 9 days in the same previous conditions. The living larvae were recorded and transfer to petri dishes. Groups of 100 larvae were deposited in each one of three Petri dishes containing vermiculite to induce pupation. They were held at 20 °C, 80% RH in darkness for 48 h. After the holding period, the number of pupae formed was recorded. The survival or reproductive capacity of the flies was not registered.

High hydrostatic pressure treatment on mangoes

Ripe and green Tommy Atkins Mangoes obtained from a local market were utilized in our study. Three mangoes were processed for each treatment. They were introduced into the working chamber directly immersed in the pressurizing liquid at 25 °C and pressurized at 25, 50, 75, 100, 150, and 200 MPa for 0, 5, 10, and 20 min. After pressurizing, mangoes were stored at 20 °C for 9 days. Immediate assessment of mangoes was made, immediately after storage ended to check for fruit damage.

Mangoes were analyzed by an untrained group of 10 panelists who declared themselves to be usual mango consumers. The mangoes were evaluated according to various parameters established by the panelists, to check for evident changes between untreated and pressurized mangoes. The parameters were: external uniformity, opacity (lost of lightness in the external surface), discoloration, exudation by the peduncle, texture (tested by compressing the mangoes with fingers), and the overall appearance. Values for parameters were based on a scale of one to five, giving the value of 1 to untreated mangoes.

Results

Effect of high hydrostatic pressure on eggs

Table 1 shows that eggs of all ages were highly resistant to pressurizing treatments in the range of 25 to 75 MPa for 0 to 20 min.

At a pressure of 100 MPa, the 1 day old eggs were not affected by pressurizing treatment at 100 MPa for 0 min, but hatch was reduced when the time of pressurizing increased from 5 to 20 min. Eggs 2, 3 and 4 days old, pressurized for 0 to 10 min, were highly resistant to treatment, but hatching was reduced when the treatment lasted 20 min. This was less noticeable in 3 days old eggs.

Pressurizing at 150 MPa for 5 to 20 min inhibited hatching of all eggs 2 and 3 days old. One day old eggs did not hatch after 20 min. However, some eggs were able to hatch after 5 and 10 min. In 4 days old eggs, hatching was never inhibited but low hatching rates were observed.

Table 1. Effect of time and level of pressurizing at 25 °C on *A. ludens* egg hatch.

Tabla 1. Efecto del tiempo y nivel de presión a 25 °C sobre la eclosión de huevos de *A. ludens*.

Pressure (MPa)	Time (min)	Age			
		1 day old	2 days old	3 days old	4 days old
25	0	88.3 ± 4.7	90.0 ± 1.0	90.7 ± 4.9	78.0 ± 2.65
	5	93.3 ± 3.2	90.3 ± 1.2	93.7 ± 2.3	93.3 ± 6.03
	10	87.3 ± 2.1	92.3 ± 3.8	92.3 ± 3.1	91.0 ± 3.0
	20	91.7 ± 1.5	94.7 ± 1.5	88.7 ± 4.6	89.3 ± 2.5
50	0	100.0 ± 0.0	92.0 ± 2.7	91.3 ± 4.6	90.0 ± 1.0
	5	91.0 ± 1.0	89.3 ± 3.8	96.0 ± 2.6	76.7 ± 3.5
	10	91.3 ± 2.9	91.7 ± 3.5	91.0 ± 2.6	85.0 ± 1.0
	20	86.3 ± 4.6	90.3 ± 3.8	90.0 ± 5.2	84.0 ± 4.6
75	0	100.0 ± 0.0	91.3 ± 5.5	89.3 ± 1.5	100.0 ± 0.0
	5	91.3 ± 1.5	100.0 ± 0.0	92.7 ± 2.5	98.0 ± 1.0
	10	84.3 ± 6.1	93.0 ± 2.0	89.0 ± 6.1	88.3 ± 1.6
	20	88.0 ± 5.6	91.7 ± 2.5	88.0 ± 2.6	87.3 ± 2.7
100	0	85.3 ± 1.2	91.7 ± 3.5	94.7 ± 2.6	84.7 ± 1.5
	5	54.7 ± 2.3	91.3 ± 1.5	93.0 ± 4.5	85.7 ± 2.5
	10	54.3 ± 1.53	80.7 ± 1.5	85.0 ± 3.0	80.0 ± 1.1
	20	11.0 ± 0.6	43.3 ± 1.2	72.3 ± 2.6	49.7 ± 1.73
150	0	61.7 ± 1.2	48.7 ± 0.2	80.3 ± 3.5	86.0 ± 4.5
	5	1.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 0.1
	10	5.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.2
	20	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 0.1

Mean values and standard deviation of three replicates.

Promedio y desviación estándas de tres réplicas.

Effect of high hydrostatic pressure treatment on larvae

The first instars were the most sensitive to HPP treatments. They exhibited mortality rates higher than 98% in all treatments, independent of the pressure level and time (Table 2). Although 100% mortality was observed under 75 MPa and 100 MPa for 20 min, there was a 0.3% survival rate when 150 MPa for 20 min, and 0.1% at 50 MPa for 20 min.

Figure 1 shows a photograph of second and third instars of *A. ludens* before and after pressurizing at 150 MPa for 20 min.

The second instars showed lower mortality rates than third instars at pressure levels of 25 MPa, independent of time of pressurization. In general, the survival rate of second instars decreased as the level of pressurization increased. There was also a trend of decreasing survival as the time of pressurization increased in each pressure level.

The third instars exhibited the highest survival rate compared to first or second instars when pressurized at 50, 100, and 150 MPa for all treatment times. A decrease in the survival rate was observed by increasing the pressure level and by increasing the time of process at each level of pressurizing. Several larvae survived even at 100 MPa for 20 min. Pressurizing at 150 MPa for 5 to 20 min resulted in 100% mortality of larvae.

The high pressure treatments affected the ability of most larvae to pupate and subsequently to develop as adults (Table 3). Although only a few first instars survived most of the pressurizing treatments, almost all of them were able to pupate even at 150 MPa for 20 min.

Table 2. Effect of time and level of pressurizing at 25 °C on survival of *A. ludens* larvae.

Tabla 2. Efecto del tiempo y nivel de presión a 25 °C sobre la supervivencia de larvas de *A. ludens*.

Pressure (MPa)	Time (min)	Survival (%)		
		First instar	Second instar	Third instar
25	0	0.5	22.1	12.9
	5	1.7	19.2	13.0
	10	0.5	16.7	5.7
	20	0.5	12.4	5.2
50	0	0.8	8.5	10.4
	5	0.9	0.1	21.3
	10	0.5	2.4	25.9
	20	0.1	1.2	11.2
75	0	0.1	5.4	12.1
	5	0.5	4.2	13.5
	10	0.5	0.9	4.5
	20	0.0	0.2	4.5
100	0	0.9	7.4	13.2
	5	0.3	0.1	4.3
	10	0.0	0.2	2.4
	20	0.0	0.0	0.4
150	0	0.7	0.7	3.5
	5	0.2	0.1	0.0
	10	0.0	0.2	0.0
	20	0.3	0.0	0.0

The values means the results of just one analysis.

Los valores representan el promedio de un solo analisis.

Second instars exhibited a higher capacity to survive pressurizing treatments (Table 2), but most of

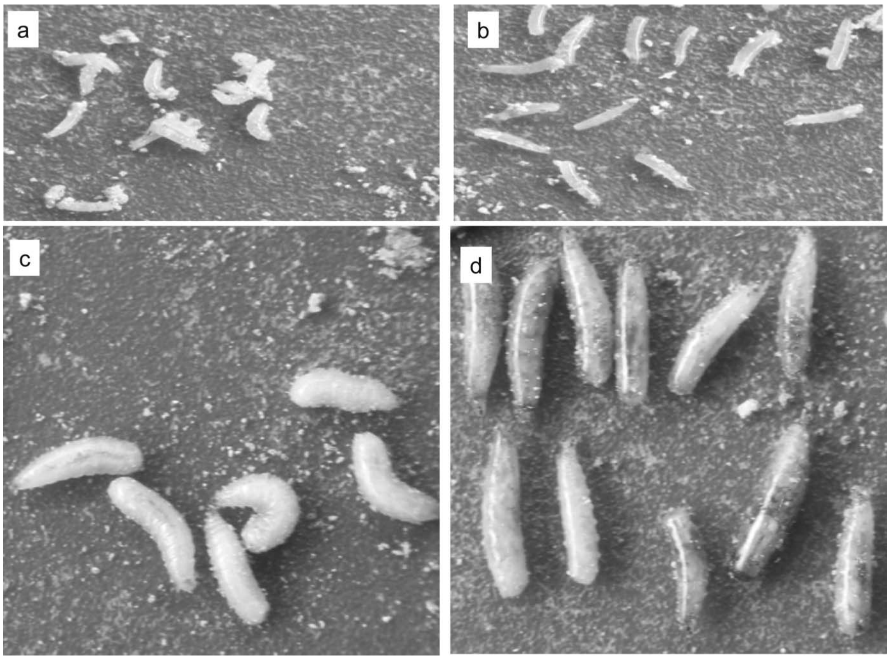

Figure 1. *A. ludens* larvae: (a) second instar untreated; (b) second instar pressurized at 150 MPa for 20 min; (c) third instar untreated; (d) third instar pressurized at 150 MPa for 20 min.

Figura 1. Larvas de *A. Ludens*: (a) Larvas de segunda etapa sin tratamiento; (b) larvas de segunda etapa presurizadas a 150 MPa durante 20 min; (c) larvas de tercera etapa sin tratamiento; (d) larvas de tercera etapa presurizadas a 150 MPa durante 20 min.

Table 3. Effect of time and level of pressurizing at 25 °C on pupation of surviving *A. ludens* larvae.

Tabla 3. Efecto del tiempo y nivel de presión a 25 °C sobre la pupación de larvas supervivientes de *A. ludens*.

		Pupating (%)			Developing adults (%)
Pressure (MPa)	Time (min)	First Instar	Second Instar	Third Instar	Third Instar
25	0	0.5	1.8	0.8	0.0
	5	1.5	0.5	2.2	0.1
	10	0.5	1.0	1.3	0.0
	20	0.4	0.3	1.1	0.0
50	0	0.5	0.3	0.7	0.0
	5	0.7	0.1	0.2	0.0
	10	0.4	0.2	0.9	0.0
	20	0.1	0.0	0.5	0.0
75	0	0.1	0.5	0.5	0.0
	5	0.5	0.1	0.3	0.0
	10	0.5	0.0	0.0	0.0
	20	0.0	0.0	0.2	0.1
100	0	0.7	0.3	1.1	0.2
	5	0.3	0.0	0.0	0.0
	10	0.0	0.0	0.1	0.0
	20	0.0	0.0	0.0	0.0
150	0	0.5	0.1	0.0	0.0
	5	0.2	0.1	0.0	0.0
	10	0.0	0.2	0.0	0.0
	20	0.2	0.0	0.0	0.0

The values means the result of just one analysis.

Los valores representan el promedio de un solo analisis.

them did not pupate (Table 3). However, several were able to pupate after being pressurized at 150 MPa for 10 min.

Third instars exhibited a higher ability to survive pressurizing treatments above 50 MPa (Table 2) than first or second instars. However, they showed a lower ability to pupate (Table 3). No third instar was able to pupate after being pressurized at 150 MPa for 0 min and most of those that survived this treatment and pupate were unable to develop to adults. Several larvae pressurized at 100 MPa for 0 min were able to develop to adulthood, but none were able to develop to adulthood at 100 MPa for 5 to 20 min or at 150 MPa, independently of time of pressurizing.

Effect of high hydrostatic pressure treatment on mangoes

The effect of HPP on mature mangoes is shown in Figure 2. Untreated control mangoes were evaluated as 1 in a 5 point scale. The higher the value assigned by the panelists, the lower the quality of the mango, with 5 being the worst. All ripe mangoes were sensitive to the pressurizing treatments as a function of the level and time of pressurization. Mangoes pressurized at 200 MPa for 20 min lost inner structure and consistency, obtaining the appearance of an inflated balloon and a soft consistency when pressed with fingers (Figure 2). The peel showed high opacity (loss of lightness) and an extended discoloration. The peduncle exhibited an excessive exudation of liquid caused by the pressurizing. The general appearance of these mangoes was rated very negatively and they were considered not appropriate to consume.

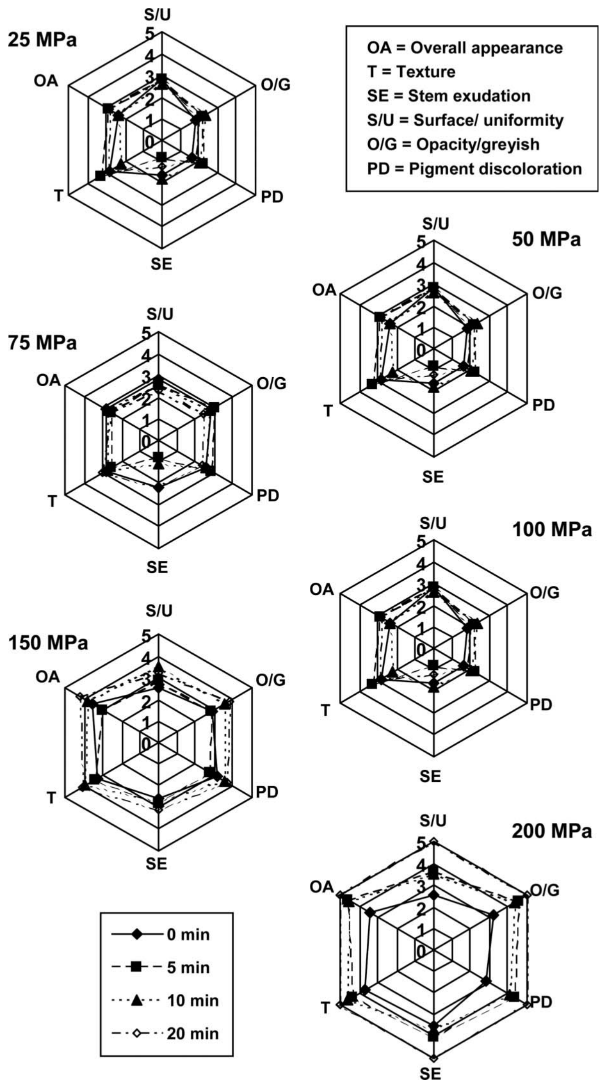

Figure 2. Sensory evaluation of mangoes pressurized at 25, 50, 75, 100, 150, or 200 MPa. Values for parameters were based in a scale of 1 to 5, giving the value of 1 to untreated mangoes. Untreated controls would be represented as a star with points in the inner circle (level 1).

Figura 2. Evaluación sensorial de mangos presurizados a 25, 50, 75, 100, 150, y 200 MPa. Los valores para los parámetros se basan en una escala de uno a cinco, dando el valor de uno a los mangos no tratados. Los controles no tratados podrían representarse como una estrella con los puntos en el circulo interior (nivel 1).

Preliminary study conducted with green mangoes indicated that these fruits were more resistant than mature mangoes to HPP (data not shown). Green mangoes pressurized at 200 MPa for 20 min showed extensive softening immediately after processing but changes were too much less evident than those observed in ripe mangoes. After 3 days, several changes in the external appearance were observed, including discoloration of the skin, and an extraneous appearance of putrefaction.

Mangoes pressurized at 100 or 150 MPa for 20 min were softer than control as a function of the pressure level. Mangoes under 75 MPa were little softer at contact than control. Mangoes treated at 25 and 50 MPa did not show external changes or softness when compared with control.

The higher the pressure and the time the higher the damage, and consequently a lowering in quality was detected.

Discussion

The time of pressurizing at 25, 50, and 75 MPa showed little effect on eggs hatch, but did appear to inhibit the ability of hatching of eggs at levels of 100 and 150 MPa: the higher the pressure level, the higher the impact of time of pressurizing.

Egg hatch of the Mediterranean fruit fly (*C. capitata*) was inhibited by pressurizing at 50 to 100 MPa at 25 °C for 5 to 20 min. However, hatch was inhibited at pressures above of 125 MPa for 5 min (Butz & Tauscher, 1995). Hatching of codling moth (*C. pomonella*) eggs was unaffected when pressurized at 179.3 MPa for 1 min at 16–18 °C but the eggs of western fruit fly (*R. indifferens*) did not hatch after being pressurized at 172.4 MPa for 1 min at similar temperatures (Neven et al., 2007). Pressurizing treatments above 200 MPa inhibited codling moth egg hatch. These results agree with our study, indicating that inhibiting eggs to hatch requires both high levels of pressure and the maintenance of such pressure for several minutes.

The resistance of insect eggs to pressurizing treatments varied among species but it seems to require at least 150 MPa to inhibit hatch. Similar behavior has been reported in microorganisms (Galazka, Ledward, Dickinson, & Langley, 1995). Baroduric organisms survive HPP treatments between 50 and 200 MPa but cannot grow in such pressure levels. Organisms vary in their response to the HPP; these differences are not only for species, are also for strains of a same species (Galazka et al., 1995).

Protein denaturation/aggregation induced by high pressure depends on pressure level, pressure holding time (Uresti, Velazquez, Ramírez, Vázquez, & Torres, 2004) and temperature (Borderías, Pérez-Mateos, & Solas, 1997). Protein resistance to HPP denaturation seems to be species dependent. Myosin from beef and four fish species treated at 200 MPa for 20 min at 5 °C exhibited different level of decrease in their ATPase activity depending on the species (Ashie, Lanier, & MacDonald, 1999). Another factor which might be considered while comparing the resistance to pressurizing treatments of different insect eggs is that presence of sugars and polyols at 8–12% has a stabilizing effect on proteins against pressurizing (Ashie et al., 1999; Uresti, Velazquez, Vazquez, Ramírez, & Torres, 2005). The studies reported determining eggs resistance to pressurizing using fruit (Neven et al., 2007) or water (Butz & Tauscher, 1995).

The adverse effect of HPP on the physiology of insect eggs hatching has not been reported. Eggs are a unique biological system with a globular form containing proteins dissolved or suspended in the intracellular liquid and enclosed by external membrane and internal membranes. Egg proteins suffer constant changes while hatching. The adverse effect of HPP on egg hatching might be associated with negative effect on the structure and permeability of external and internal membranes, negative effect on proteins or a combination of both factors.

HPP affects unicellular organisms in different ways, including changes in the morphology of the cell which might be reversible when pressure levels lower than 200 MPa are used, denaturation of the proteins and changes in the permeability of the membrane cell, inhibition of the energy producing reactions and denaturation of the enzymes essentials for growth and development of the cell (Farr, 1990; Pothakamury, Barbosa-Cánovas, & Swanson, 1995). Other negative effects include separation of the cell membrane from the cell wall, contraction of the wall cell with forming of porous, modifications of the citoskeletal, nucleus and cell organeles, coagulation of cytoplasmic protein, expulsion of intracellular components through wall cell and biochemical and genetic changes by inhibiting of enzymes involved in the DNA replication and transcription (Téllez-Luis, Ramírez, Pérez-Lamela, Vázquez, & Simal-Gándara, 2001).

Pressurizing induces a protein denaturation/aggregation different from that resulting from freezing, drying or heating where denaturation is immediately followed by irreversible aggregation affecting negatively the biological properties of proteins. Although pressure treatments at low temperature cause protein denaturation by breaking intermolecular bonds inducing major changes in protein conformation favoring a protein aggregation reaction (Fernández-Martín, Perez-Mateos, & Montero, 1998), pressurizing at temperature under the temperature of protein denaturation seems to induce a protein aggregation dominated by side-to-side interactions of proteins with a low degree of denaturation and not by aggregation of proteins with large changes in molecular conformation (Gilleland, Lanier, & Hamann, 1997; Uresti et al., 2005).

Resistance to HPP by eggs must be influenced by special properties in the nature or composition of the resistant eggs, because the HPP effect is instantaneous and uniform in all the closed system inside the working chamber reducing the volume in different levels depending on the amount of pressure (Téllez-Luis et al., 2001; Torres & Velazquez, 2005).

Larvae were less resistant to HPP treatments than eggs, exhibiting higher mortality even at the low pressure treatment of 25 MPa. The time of pressurizing had an important effect on larvae mortality especially at higher pressure levels.

First instars generally showed lower resistance to HPP treatments and most of them died during the treatment or during incubation. However, a few larvae that resisted HPP treatments even at 150 MPa for 20 min, preserved their capacity to pupate.

Second instars were more resistant to 25 MPa than first and third instars. Although the resistance of the second instar to HPP decreased by increasing the pressure, several specimens survived even at 150 MPa for 10 min and maintained the ability to pupate.

Third instars decreased their resistance to HPP as the pressure increased, and all the specimens died at 150 MPa for 5 to 20 min. Several larvae which survived 100 MPa at 5 to 20 min maintained the ability to pupate but were not able to develop imagos. The highest pressure under which imagos were able to form was 100 MPa for 0 min.

Western cherry fruit fly larvae showed distinct resistance to HPP treatments at the different stages decreasing in the following order: third, first, and second instar. All larvae of western cherry fruit fly died after pressurizing at 172.4 MPa, but a pressure of 208.9 MPa was required to reach a total mortality of codling moth (Neven et al., 2007).

Results from these studies and others reported in literature indicate that high pressure at 16 to 25 °C as a quarantine method depends on the insect species. The Mediterranean fly fruit showed lower resistance at 100 MPa than the Mexican fruit fly fruit to HPP at 150 MPa. The Western cherry fruit fly (172.4 MPa) and the codling moth (208.9 MPa) were more resistant to HPP treatments.

The fruit affected for these insects showed low resistance to HPP treatments. Golden delicious apple, which is attacked by codling moth, was considerably affected by pressurizing at 96.6 MPa for 2 min. Sweet cherry was affected by pressurizing at 69 MPa. In our study, ripe mangoes were affected even at 50 MPa during storage at 20 °C. However, green mangoes were more resistant to pressurizing treatments showing few external changes when pressurized at 100 MPa and not evident changes when treated under 75 MPa. The higher resistance might be associated with the firmer structure of the green mango allowing the cell wall to resist the mechanical damage of the pressurizing. The tolerance of the Mediterranean fruit fly eggs diminished when HPP was conducted at 0 or 45 °C and the time or the level of pressurizing required was decreased (Butz & Tauscher, 1995).

Conclusions

HPP treatments were able to inhibit the hatch of most of the eggs and to kill most of the larvae of the Mexican fruit fly. Holding time of pressurizing improved the efficiency of HPP treatments. Eggs showed higher resistance than larvae to pressure. However, several specimens of eggs and larvae were able to resist even 150 MPa, preserving the ability to hatch. Several larvae were able to survive and pupate even after being pressurized at 150 MPa and a few third instars were able to pupate and develop to adulthood after being pressurized at 100 MPa. Pressurizing treatment above 150 MPa at 25 °C was required to destroy all the eggs and larvae of the Mexican fruit fly but raw mangoes and other fruits are not able to support such processing.

HPP processing is shown to be a potential alternative quarantine treatment for the Mexican fruit fly; however, more studies are required to determine if combining temperature with HPP is feasible to increase the mortality of the larvae and the inhibition of eggs hatch, while minimizing the damage to the fruits.

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