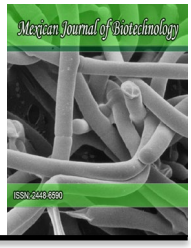
Mexican Journal of Biotechnology 2023, 8(2):1-16

Journal homepage:www.mexjbiotechnol.com

ISSN:2448-6590



ORIGINAL RESEARCH

Changes in quality and gene transcript levels of soursop (*Annona muricata* L.) fruits during ripening

Cambios en la calidad y niveles de transcripción de genes de frutos de guanábana (*Annona muricata* L.) durante maduración

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Article history:
Received: 5 December 2022 / Received in revised form: 25 February 2023 / Accepted: 25 February 2023 / Published online: 1 April 2023.
https://doi.org/10.29267/mxjb.2023.8.2.1

ABSTRACT

Rapid softening is one of the main problems of soursop (*Annona muricata* L.) fruits. The physicochemical parameters and gene expression response of cell-wall-related genes in soursop fruits were evaluated. The temperature of 15 ± 2 °C delayed the physicochemical composition of soursop fruits by three days and significant down-regulated the expression of cell-wall related genes. Pectin methylesterase (*PME1*), pectate lyase (*PL22*), polygalacturonase (*PG*), Endoglucanase (*EG*), and expansins (*EXP13* and *EXP7*) genes showed a down-regulation of their expression during ripening at 28 ± 2 °C and 15 ± 2 °C. The bioinformatic analysis demonstrated two conserved

domains associated with degradation of cell wall. Higher gene level of the *EXP7* gene was detected on Day 6 of storage at 28 ± 2 °C, showing a 3.5-fold increase in the gene expression compared to Day 0. Furthermore, *PME1, PL22, PG, EG*, and *EXP13* expression was down-regulated by the temperature of 15 ± 2 °C ($p < 0.05$). Gene expression was highly correlated in the fruits stored at 28 ± 2 °C. On the other hand, a high correlation between the *EXP7, PME1, and PL22* genes was recorded on the fruits stored at 15 ± 2 °C. The multivariate analysis demonstrated that acidity is an important variable during ripening and the two *EXP* genes, and *EG* are highly correlated with acidity and negatively correlated with firmness. In conclusion, the findings of this research suggest that these genes are down-regulated by the combination of days of storage and temperature.

Keywords: bioinformatics; plant cell wall; refrigeration; softening; transcriptional response

RESUMEN

El rápido ablandamiento es uno de los principales problemas de los frutos de guanábana (*Annona muricata* L.). Se evaluaron los parámetros fisicoquímicos y la respuesta transcripcional de genes relacionados con la pared celular en frutos de guanábana. La temperatura de 15 ± 2 °C retrasó tres días la composición fisicoquímica de los frutos de guanábana y disminuyó significativamente la expresión de genes relacionados con la pared celular. Los genes de pectinmetilesterasa (*PME1*), pectato liasa (*PL22*), poligalacturonasa (*PG*), endoglucanasa (*EG*) y expansinas (*EXP13* y *EXP7*) mostraron una baja expresión durante la maduración a 28 ± 2 °C y 15 ± 2 ºC. Se identificaron dos dominios conservados asociados con la degradación de la pared celular. Se detectó una mayor expresión del gen *EXP7* al Día 6 a 28 ± 2 °C, mostrando un aumento de 3.5 veces en la expresión génica comparado con el Día 0. La expresión de *PME1, PL22, PG, EG* y *EXP13* estuvo reprimida por la temperatura de 15 ± 2 °C ($p < 0.05$). La expresión génica estuvo altamente correlacionada en los frutos almacenados a 28 ± 2 °C. Por otro lado, existió una alta correlación entre los genes *EXP7, PME1* y *PL22* en los frutos almacenados a 15 ± 2 °C. El análisis multivariado demostró que la acidez es una variable importante durante la maduración y los dos genes de *EXP* y *EG* están altamente correlacionados con la acidez y negativamente correlacionados con la firmeza. En conclusión, los transcritos están reprimidos por la combinación de días de almacenamiento y temperatura.

Palabras clave: bioinformática; pared celular; refrigeración; ablandamiento; respuesta transcripcional

1. INTRODUCTION

Nayarit, Mexico is the principal producer of soursop (*Annona muricata* L.) around the world, constituting nearly 81% of the whole production at the national level (Anaya-Dyck *et al.,* 2021; SIAP, 2019). Particularly, this crop possesses a wide variety of bioactive

compounds including antioxidant, antimicrobial, anthelmintic, antihypertensive, anti-inflammatory, antidiarrhea, antimalarial, and anticancer activities, among others (Mutakin *et al.,* 2022). However, soursop fruits have a short postharvest shelf life, being highly perishable and susceptible to postharvest decay due to the rapid loss of firmness. Soursop fruit is classified as climacteric due to its high respiration rate and the peak of ethylene production after five or six days of harvest (Berumen-Varela *et al.,* 2019; Jiménez-Zurita *et al.,* 2017). Fruit ripening involves several enzymes related to pectin, a polysaccharide that acts in the plant cell architecture, and when its content increase, can cause the cell wall to dissemble (Paniagua *et al.,* 2014). Pectin is degraded by pectinolytic enzymes, such as pectin methylesterase (PME), polygalacturonase (PG), and pectate lyase (PL), among others (Kohli *et al.,* 2015).

PME and PG are related to the de-methylation of pectin to pectate and the hydrolysis of pectate by cleaving the glycosidic bonds, respectively (Shin *et al.,* 2021). On the other hand, it has been reported that high levels of gene expression or enzymatic activity are related to cell wall degradation including the PME, PG, endoglucanases (EG), and expansins (EXP) enzymes. EG degrades the cellulose linkage, which is one of the most abundant components of the cell wall. Furthermore, EG is associated with the cell wall and cellulose biosynthesis such as changing the porosity of the cell wall (Jara *et al.,* 2019; Shani *et al.,* 2006). Additionally, in cherimoya fruits, it has also been reported that expansins (*EXP*) are associated with cell wall disorganization during ripening under postharvest storage (Berumen-Varela *et al.,* 2020a). Temperature is one of the most important factors that directly affect soursop quality. The use of refrigeration has been demonstrated to prolong the shelf life of soursop fruits without affecting their quality, reporting storage temperatures between 15-18 °C to delay ripening (Espinosa *et al.,* 2013; Jiménez-Zurita *et al.,* 2017). Taking this into account, our research group sequenced and annotated the first soursop fruit transcriptome under two postharvest storage conditions, identifying genes related to the modification of cell wall (mainly in the pectin) such as pectin methylesterase (*PME*), pectate lyase (*PL*), polygalacturonase (*PG*), rhamnogalacturonan lyase (*RGL*), among others (Palomino-Hermosillo *et al.,* 2022). Indeed, the refrigeration temperature delayed ripening and prolong the postharvest shelf life of the fruits by three days (Berumen-Varela *et al.,* 2020a; Palomino-Hermosillo *et al.,* 2022). Changes in gene expression can occur due to modifications in the cell wall composition during postharvest storage. Therefore, investigating these changes at the molecular level can help to elucidate molecular mechanisms to increase the shelf life of this crop and allow its commercialization. Take into consideration, the objective of this study was to evaluate the physicochemical parameters and the gene expression of cell-wall-related genes in soursop fruits under postharvest storage.

2. MATERIALS AND METHODS

2.1 Plant material

Soursop fruits ‘GUANAY-1’ were harvested at physiological maturity according to fruit shape, peel color, and size from an orchard located in Venustiano Carranza, Nayarit, Mexico (21° 32'2.77" N, 104° 58'39.73" W) as reported by Berumen-Varela *et al.,*

(2020b). A total of 50 fruits without mechanical and/or pathogenic damage were selected, disinfected with 2.0% sodium hypochlorite, and then washed with water.

2.2 Postharvest storage and physicochemical analysis

Soursop fruits were stored at 28 ± 2 °C and 15 ± 2 °C in a controlled temperature chamber (Climacell® CLC-B2V-M/CLC404-TV, Angelbachtal, Germany) until reaching senescence. The temperature of 15 ± 2 °C prolonged the soursop shelf life by three days, delaying the onset of senescence up to the Day 9 of storage. Hence, the physicochemical analysis was performed in the stages of physiological maturity, maturity of consumption and onset of senescence (0, 3, and 6 days at 28 ± 2 °C and 3, 6 and 9 days at 15 ± 2 °C, respectively). The physicochemical parameters measured were firmness with a digital penetrometer (SSEYL GY-4 Digital Fruit Penetrometer) in three areas of three fruits and reported in Newtons (N), the total soluble solids (TSS) with a digital refractometer (Hanna HI 96801) and the pH with a potentiometer (Hanna Instruments HI2210). Titratable acidity was also determined according to volumetric titration with 0.01 N of NaOH and phenolphthalein as an indicator following the official method (AOAC, 2005). Soursop mesocarp from three fruits was collected in the same days of storage aforementioned and immediately placed in RNAlater solution (Sigma-Aldrich), frozen in liquid nitrogen, and stored at -80ºC as reported by Berumen-Varela *et al.,* (2020b).

2.3 Conserved domain analysis

Previously, our research group assembled and annotated the soursop fruit transcriptome (Palomino-Hermosillo *et al.,* 2022). Then, within this information, we searched for conserved domains in all the sequences tested using CDD/SPARCLE with the default parameters (Lu *et al.,* 2020).

2.4 RNA extraction and first-strand cDNA synthesis

Total RNA was extracted (75 mg) with the Spectrum Plant Total RNA kit (Sigma-Aldrich) following the manufacturer's instructions of each day of storage analyzed. The RNA was analyzed by NanoDrop ND-1000 UV-Vis spectrophotometer at 260 nm (Nano Drop Technologies Inc., Wilmington, DE, USA) and agarose gel electrophoresis. Then, the first-strand cDNA was synthesized from 1 µg of total RNA using the SuperScriptIII reverse transcriptase kit according to the manufacturer's instructions.

2.5 Real time-quantitative PCR (RT-qPCR)

Based on the *EXP*, *PG*, *PME*, *EG*, and *PL* genes sequences annotated by BLASTx and/or BLASTp in the soursop transcriptome (Palomino-Hermosillo *et al.,* 2022). Primers and probes were designed using the software Primer3 (Untergasser *et al.,* 2012). The sequences, amplicon size and gene names are displayed in Table 1. The specificity of the primers was analyzed by the Primer-Blast tool and then verified by conventional PCR. Subsequently, RT-qPCR was performed using a StepOnePlus™ Real-time PCR System (Applied Biosystems Inc, Foster City, CA, USA) with a final

volume of 20 µL containing 1X TaqMan Fast Advanced Master Mix (10 µL), 0.4 µM (0.8 µL) of forward and reverse primer, 0.18 µM (0.72 µL) of the probe and 40 ng de cDNA (2 µL). The RT-qPCR reactions were run in three technical replicates with three biological replicates. Amplification conditions were one cycle of 95°C for 5 min followed by 45 cycles of 95°C for 1 min and 55°C for 1 min with a signal acquisition in the FAM channel at the end of the annealing/extension step. Non-template controls were also included. Relative gene expression was calculated with the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) using *Ubiquitin* (*UBC*) as the reference gene (Berumen-Varela *et al.,* 2020b; González-Agüero *et al.,* 2016). Day 0 was considered the calibrator sample to calculate de final values. Relative gene expression values were reported as Fold change and plotted in Rstudio using the ggplot2 package. Fold change larger than 1 was considered that the gene was up-regulated, and down-regulated when the fold change was lower than 1.

Table 1. Primer's sequence used to amplify the genes of this study. Fw and Rv mean forward and reverse primers, respectively

Gene name	Sequence (5'-3')	Amplicon size (bp)
EXP7	Fw: TTTTGCAGCCACTTCAACCG	
	Rv: AGTGGGAAAGGGTGTGTGTG	104
	Probe: GCCTTACAACTTCTTCCCCC	
EXP13	Fw: CATGGTGGAGTCAGGGTCAC	
	Rv: GAATCGATCCACTCCCACCC	85
	Probe: CGGCCGGGATTATTTTGAGC	
EG	Fw: TGACAAGAGAGCGGGGACTA	
	Rv: TTGGCCTGGAAAGTTGGTGT	138
	Probe: CTCTGCTCTCTAATCCCGGG	
PG	Fw: CTCCTGCCTCGTTCTTCTCC	
	Rv: ATCACCGGAACCAGCTTGAG	145
	Probe: ATCCAGAGGTTAGTGGTGCC	
PME1	Fw: ACTGCATGATCACCGTCCTC	
	Rv: CGCGACGACCCTAATCAGAA	145
	Probe: GGCCCAGGAAGGTTTTGAAA	
PL22	Fw: ACTGAATGGGGATGCATGGG	
	Rv: GATCATCTTCGCCCGTGACA	125
	Probe: CCGTCGATCGTCTTGTAGGA	
UBC	Fw: CTCCAGCAAGATCCTCCAGC	
	Rv: TCCTCCGTCCCATGGTGTAT	108
	Probe: GGAACGCTGTCATCTTTGGG	

2.6 Statistical analysis

Data were analyzed under a randomized complete block design (days of storage as block). The statistical analysis and plots were carried out in Rstudio v.4.2.1 using the agricolae, ggplot2 and ggpubr packages. Shapiro–Wilk test and Bartlett test were used to all variables to examine the normality and the homogeneity of variances, respectively. These data were analyzed by analysis of variance (ANOVA) with $p < 0.05$ significance level. Tukey's HSD test (Honestly Significant Differences) between temperatures was performed when ANOVA showed significant differences. Pearson correlation analysis was done to analyze the association between the genes for each temperature using the pairwise complete observation of the corrplot package. Furthermore, principal component analysis (PCA) was performed from a scale correlation matrix (scaled to unit variance) to evaluate the importance of each variable using the factoMineR, and factoextra packages.

3. RESULTS

3.1 Physicochemical parameters

The soursop fruits stored at 15 ± 2 °C delayed ripening, impacting in the firmness, TSS, pH and acidity of the fruits compared to the fruits stored at 28 ± 2 °C ($p < 0.05$) as shown in Figure 1. Soursop stored at 28 ± 2 °C showed a constant decrease of firmness across the days of storage as shown in Figure 1A. In this regard, a 7.5-fold and 2.1-fold significant decline from Day 0 to Day 6 and from Day 3 to Day 6 was recorded, respectively. The TSS increased from Day 0 to Day 3 followed by a decrease on Day 6 in the fruits stored at 28 ± 2 °C as shown in Figure 1B. Conversely, pH decreased from Day 0 to Day 3 followed by an increase on Day 6 (Fig. 1C). On the other hand, the titratable acidity increased according to the days of storage, from the beginning to the end of storage (Fig. 1D). On the other hand, the temperature of 15 ± 2 °C delayed the physicochemical parameters by three days (Fig. 1A). Nevertheless, the firmness of the fruits decay from Day 3 to Day 6 and from Day 6 to Day 9 as shown in Figure 1A. On the contrary, the TSS increased from Day 0 to Day 6 and Day 9 as shown in Figure 1B. In the case of pH, the levels maintain constant between Day 0 and Day 3 of storage followed by decrease on day six and an increase on day nine. The acidity values increased throughout the days of storage, finding the highest values on day nine of storage compared to Day 0 and Day 3 as shown in Figure 1D.

3.2. Domain analysis

Conserved domain analysis identified that *EXP13* gene have four domains: PLN00193, which encodes for Expansin-A, DPBB_EXPA_N that is a N-terminal double fold domain for the alpha-expansin subfamily, DPBB_1 that codes for a Rlp-A lipoprotein and Polloen_allerg_1 domain which is related to the pollen allergens in human. Indeed, *EXP7* showed two domains: PLN00050 which is associated with a Expansin-A and as well as the *EXP13*, the Pollen_allerg_1 domain. Moreover, we identified four pectate lyase domains in the *PL22* gene (Amb_all, PelB, PL-6, Pec_lyase_C), all related with

the activity of pectate lyase. Indeed, Pectinesterase, PLN02313 and PemB domains were identified in the *PME1* gene. Furthermore, two polygalacturonase domains (PLN02155, COG5434/pgu1) and one glycosyl-hydrose domain (pfam00295). Finally, we found domains associated with glyco-hydralse (Glyco_hydro_9) and endoglucanase (PLN02266) in the *EG* gene.

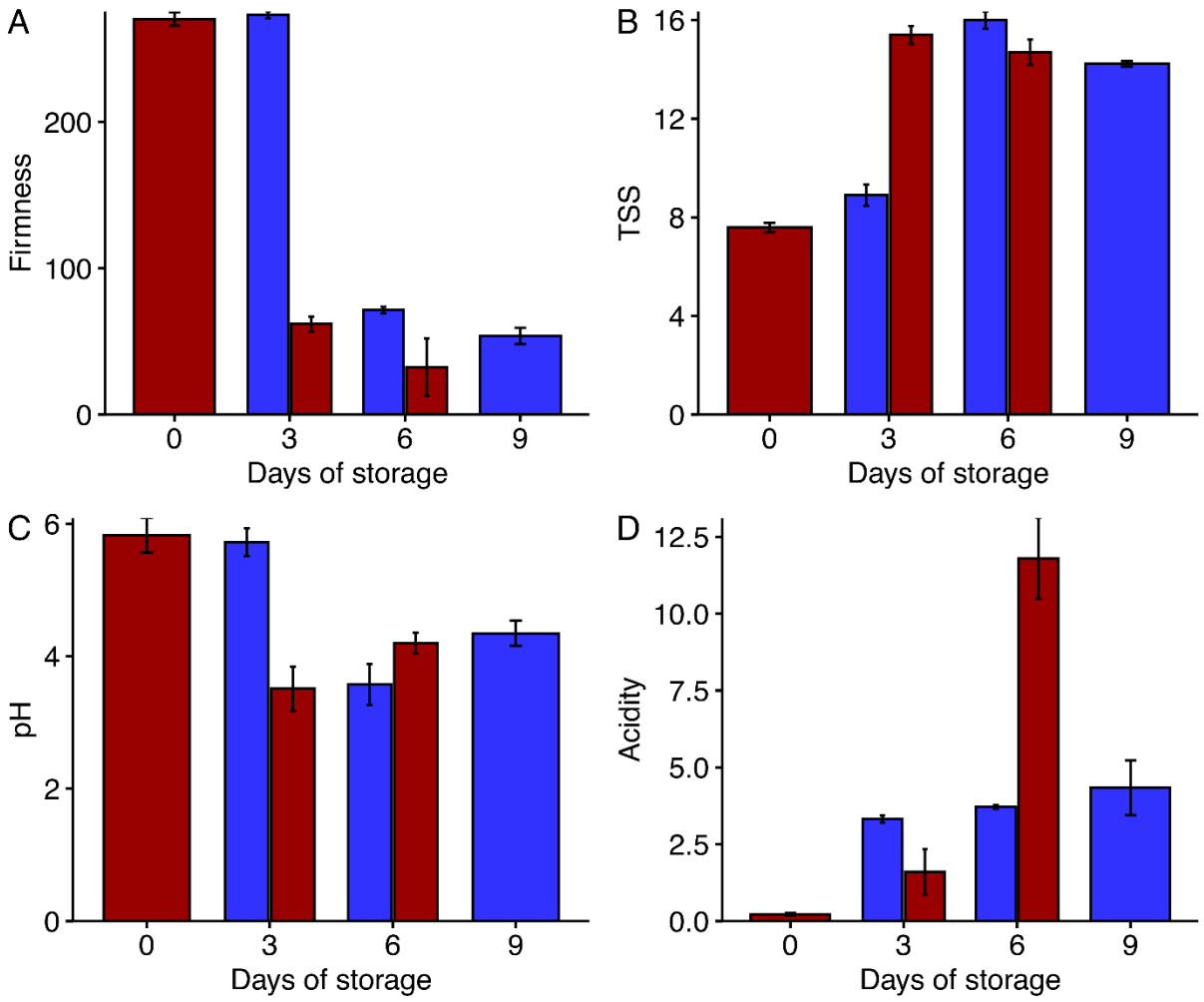

Fig. 1. Physicochemical parameters of fruits stored at 28 ± 2 °C (red) and 15 ± 2 °C (blue). Firmness (A), TSS (B), pH (C), and Titratable acidity (D). Significant difference between temperatures was found in all the variables evaluated with a $p < 0.05$ according to Tukey's HSD test.

3.3. Gene expression patterns of *EXP3* and *EXP7*

The expression of expansins genes at 28 ± 2 °C and 15 ± 2 ºC is shown in Figure 2. The temperature of 15 ± 2 ºC significantly affect the expression of the expansins genes compared to temperature of 28 ± 2°C ($p < 0.05$). On the *EXP13*, a down-regulation of the gene was observed across the days of storage at both temperatures (Fig. 2A).

EXP13 gene at 28 ± 2 °C showed the lowest expression levels in the Day 3 of storage followed by the Day 6 as shown in Figure 2A. On the other hand, *EXP7* showed the highest significant expression on day six of storage at 28 ± 2 °C, with a 3.65-fold increase compared to day zero as shown in Figure 2B.
Moreover, a decreasing pattern in gene expression of *EXP13* at 15 ± 2 °C was recorded, finding the lowest down-regulation after nine days of storage *EXP13* as shown in Figure 2A. Further, the *EXP7* gene was down-regulated in all days of storage at 15 ± 2 ºC (Fig. 2B). The lowest value was recorded on Day 3 of storage in all conditions tested in both *EXP* genes.

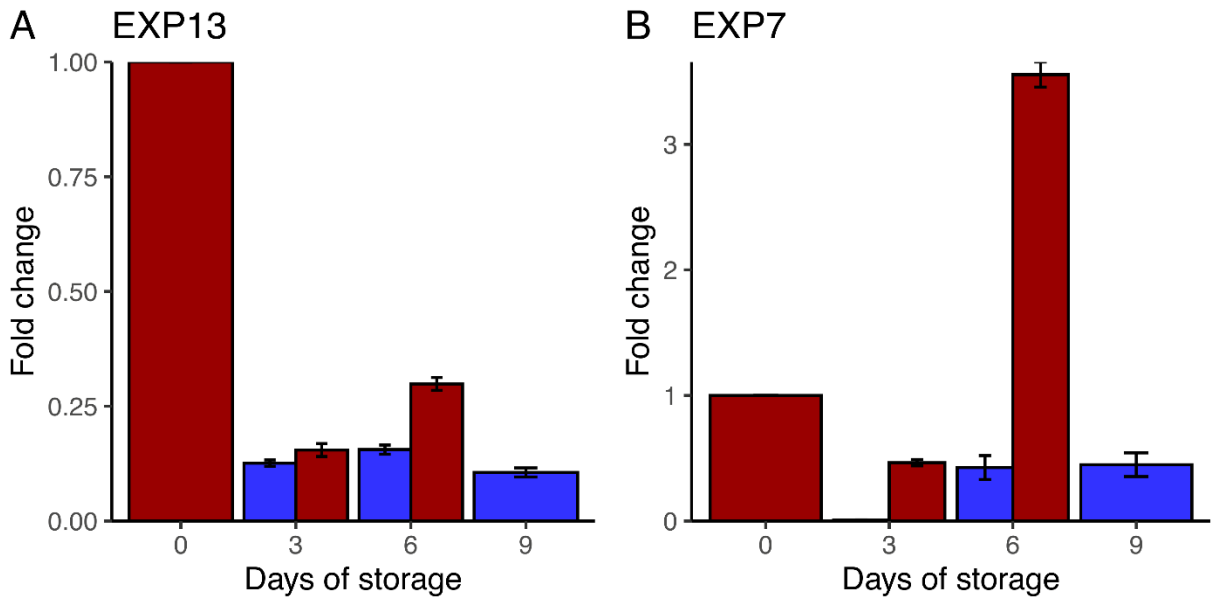

Fig. 2. Expansins gene expression at 28 ± 2 °C (red) and 15 ± 2 °C (blue). *EXP13* (A), and *EXP7* (B). Vertical lines indicate the standard deviation of the means from nine data (three technical replicates and three biological replicates). Significant difference between temperatures was found in the gene expression with a $p < 0.05$ according to Tukey's HSD test.

3.4. Gene expression patterns of *PL22, PME1, PG and EG*

The expression of cell-wall related genes at 28 ± 2 °C and 15 ± 2 ºC is shown in Figure 3. The temperature of 15 ± 2 ºC significant down-regulated the gene expression of *PL22*, *PME1*, *PG* and *EG* ($p < 0.05$). *PL22* and *PME1* showed a similar trend, observing a decrease on Day 3 of storage followed by an increase on Day 6 of storage (Figs. 3A-3B). The expression levels of *PG* were significantly down-regulated on each day of storage compared to Day 0 as shown in Figure 3C. Indeed, the *EG* gene was also down-regulated, reaching the lowest expression level on Day 3 of storage (Fig. 3D). In agreement with the results previously described, *PL22* and *PME1* genes showed a down-regulation of gene expression, recording the lowest values on Day 3 of storage. Both *PL22* and *PME1* showed a decrease on Day 3 of storage followed by an increase

on Day 6 of storage at both temperatures (Figs. 3A-3B). The expression levels of *PG* were down-regulated on each day of storage compared to Day 0 (Fig. 3C). Indeed, the *EG* gene was also down-regulated, reaching the lowest expression level on Day 3 of storage (Fig. 3D).

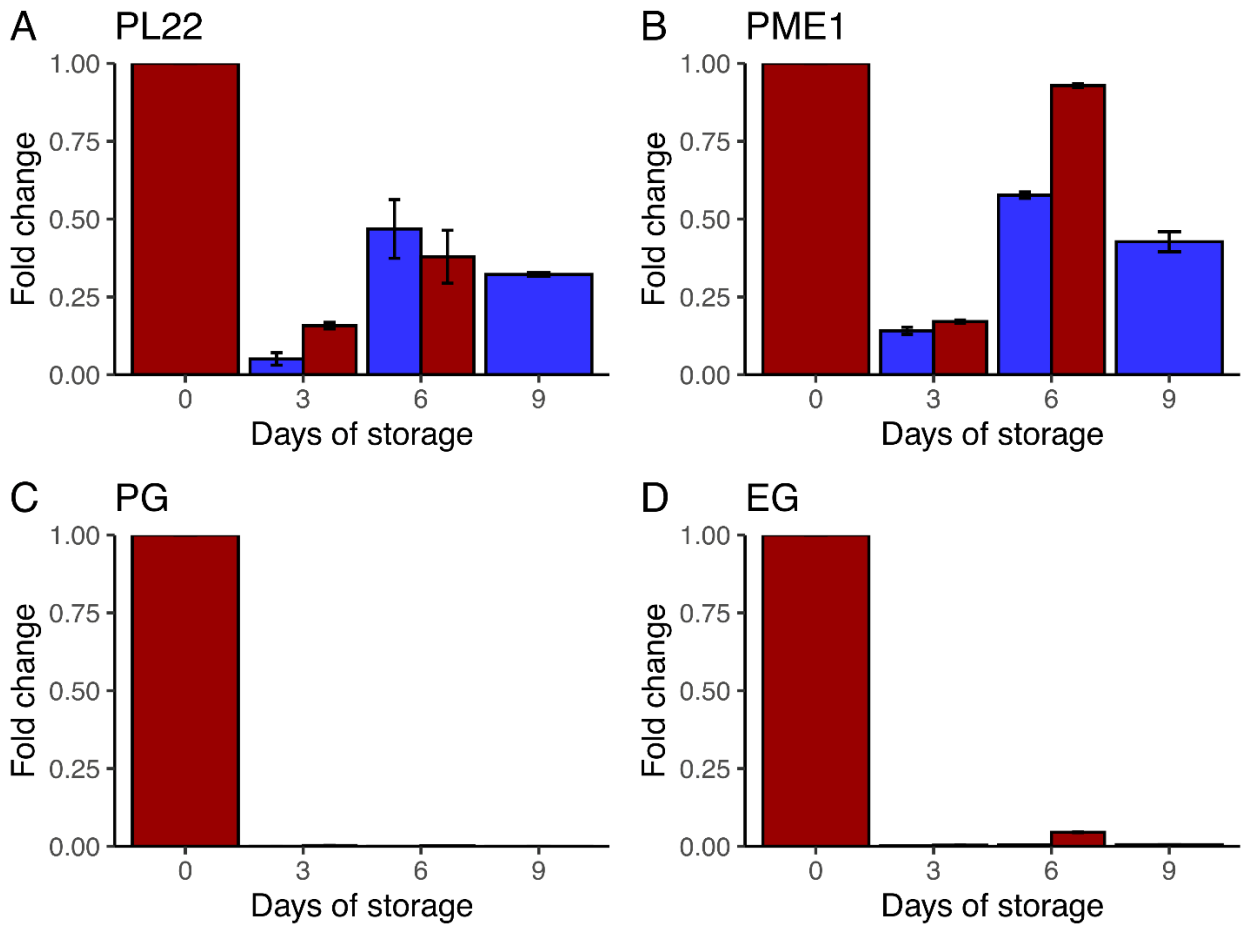

Fig. 3. Expression of cell-wall related genes at 28 ± 2 °C (red) and 15 ± 2 °C (blue). *PL22* (A), *PME* (B), *PG* (C), and *EG* (D). Vertical lines indicate the standard deviation of the means from nine data (three technical replicates and three biological replicates). Significant difference between temperatures was found in the gene expression with a $p < 0.05$ according to Tukey's HSD test.

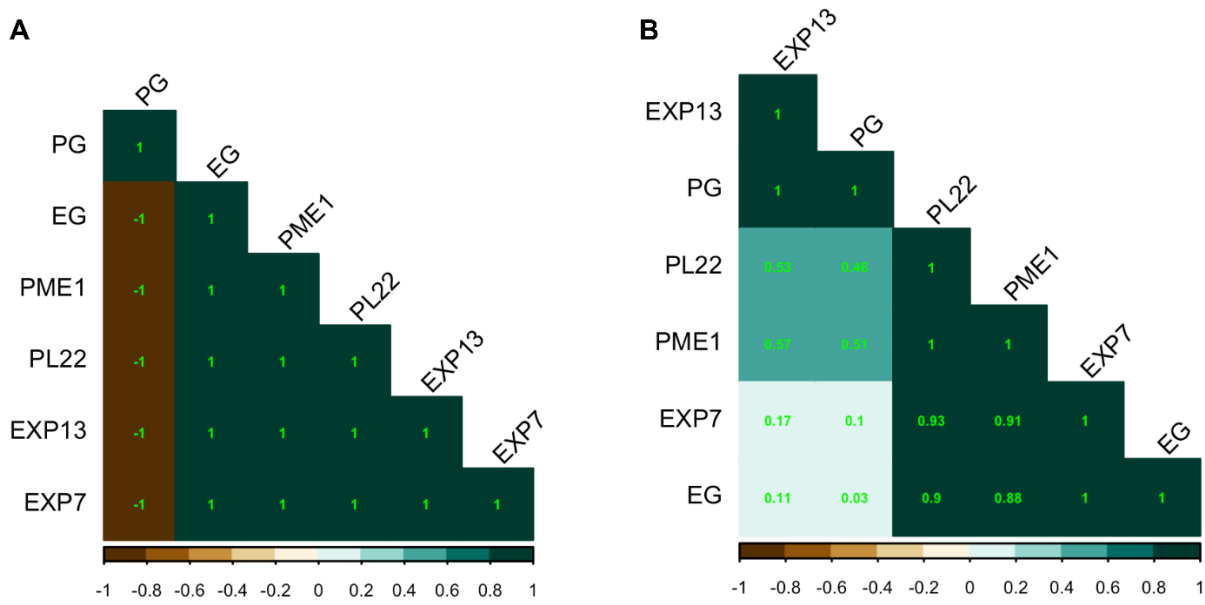

Fig. 4. Pearson correlation analysis between genes. Genes at 28 ± 2 ºC (A), and Genes at 15 ± 2 ºC (B). Positive correlations are displayed in green and negative in brown color. The coefficient ranged in values from -1 to +1.

To further explore the effect of the temperature of postharvest storage on the gene expression with the days of storage, a PCA analysis was performed using the response variables under all conditions tested. The two principal components (PC1 and PC2) represented 86.33% of the total variance (Fig. 5). In this regard, PC1 accounted the 60.94% of the variance and is strongly correlated with the *EXP13*, *EXP7, PME1*, *EG* genes, and acidity. On the other hand, PC2 explained 25.39% of the variance, with pH, TSS, and firmness contributing the most. Indeed, the variable that contribute the most was the TSS while the lowest was *PG*. Additionally, the two *EXP* genes and *EG* are highly correlated with Acidity and negatively correlated with firmness (Fig. 5).

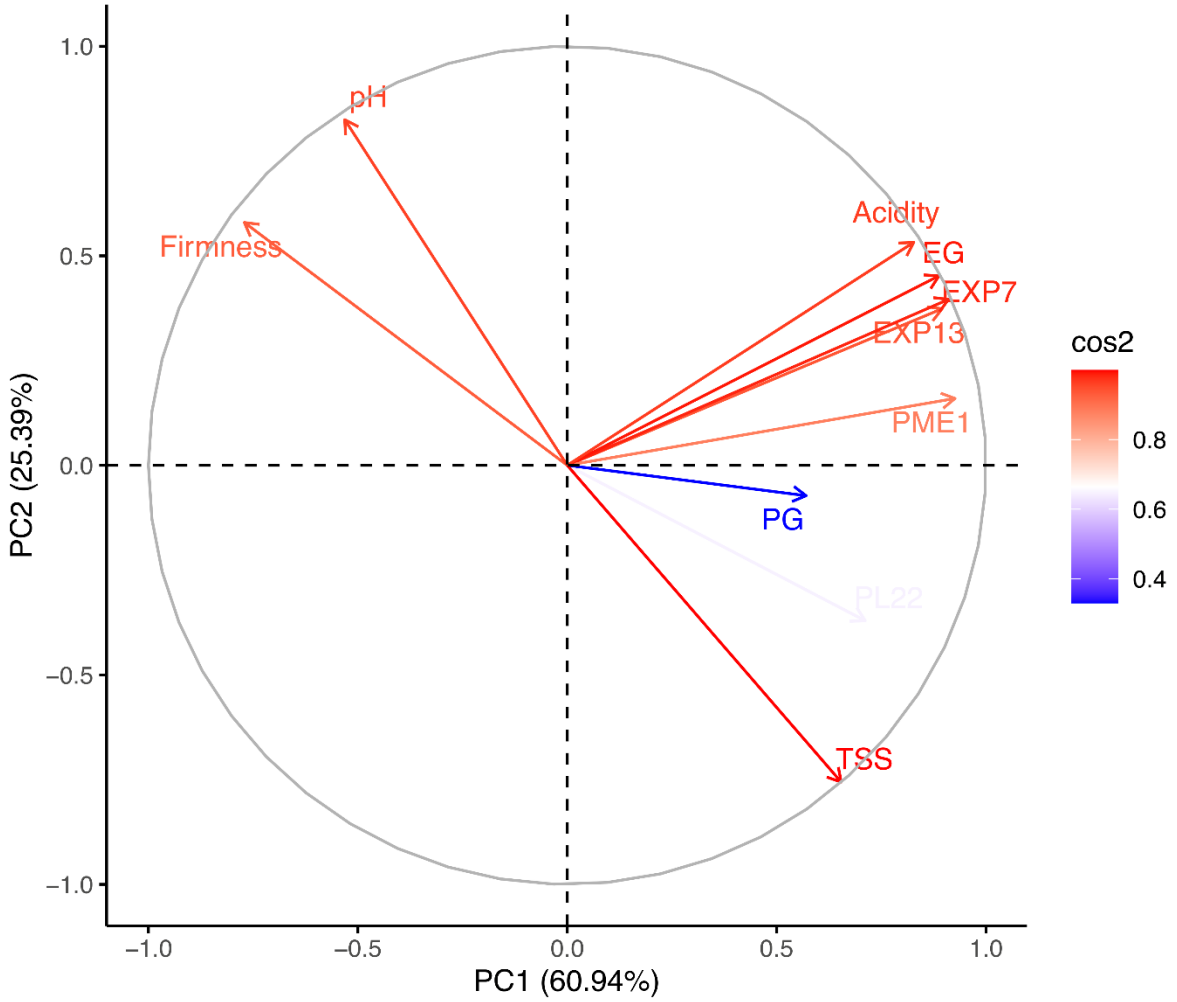

Fig. 5. PCA of all the variables evaluated. PCA shows the first (PC1) and second (PC2) components with the percentage of the variance in brackets. The color scale indicates the quality representation of the variables (cos2) between 0 and 1.

4. DISCUSSION

Several processes such as fruit ripening and softening are dependent on cell wall composition reconstruction (Carpita, 2000). The temperature of 15 ± 2 ºC delayed the soursop ripening for three days, showing similar results on Day 6 of storage at 28 ± 2 ºC and Day 3 of storage at 15 ± 2 ºC. Indeed, no phytopathogenic damage was observed in the fruits stored at 15 ± 2 ºC. These results agree with those found by Palomino-Hermosillo *et al.,* (2022); Berumen-Varela *et al.,* (2020a); Jiménez-Zurita *et al.,* (2017) reporting similar physicochemical behavior to those found in this investigation by applying low temperatures to soursop fruits. Ripening involves the action of several degrading cell wall enzymes such as PG, PL, PME, and EXP since these enzymes hydrolyze the bonds between the polymers that compose the cell wall. We found several

conserved domains in all the genes evaluated. Most of these domains, are closely related with the function of the enzymes in the plant cell wall.

The expression pattern of cell-wall-related genes under two postharvest storage conditions (28 ± 2 °C and 15 ± 2 ºC) was evaluated. The temperature of 15 ± 2 ºC down-regulated the expression of cell-wall related genes. Studies of soursop gene expression in different cell-wall enzymes have not been performed. In cherimoya (*Annona cherimola* Mill.) fruits, mRNA levels of genes coding *EXP* were evaluated under different storage temperatures through northern blot, identifying different expression patterns. In this context, the *AcExp1* mRNA was detected after six days of storage in fruits stored at 10°C, 15 °C, or 20 °C while the *AcEXP2* and *AcEXP3* mRNA levels were high in the four and five days of storage at 15 ºC (Shen *et al.,* 2009). In this study, we found that only the *EXP7* gene was up-regulated on Day 6 of storage at 28 ± 2 ºC, which is related to the start of softening and the peak of ethylene. In soursop fruits, Berumen-Varela *et al.,* (2020a) analyzed the gene expression of one *EXP* gene during ripening, demonstrating an increase in the gene expression at 28 ± 2 ºC on the Day 6 of storage, which coincides with the found in the *EXP7* at 28 ± 2 ºC. On the other hand, the same authors found a down-regulation of the *EXP* gene expression at 15 ± 2 ºC on the Day 6 and Day 9 of storage, which agree with the results found in this study with the *EXP13* gene. Altogether, our results strongly suggest that the genes encoding the same family play a different role in the cell. The PME, PL, and PG are characterized by catalyzing pectin modifications and cell wall disassembly due to their capacity to reduce the molecular size of polymers, which play a key role in many processes, including fruit ripening (Camacho-Cristóbal *et al.,* 2008). According to our results, *PME1* and *PL22* showed a strong down-regulation during ripening at 28 ± 2 ºC and 15 ± 2 ºC. Furthermore, in the early stages of ripening, there is no pectin depolymerization (Ghiani *et al.,* 2011). Therefore, it seems that these genes are not active in these stages, specifically on Day 3 of storage. An induction of *PL* and *PME* genes at the late stages of ripening in climacteric fruits such as soursop and banana, respectively (Palomino-Hermosillo *et al.,* 2022; Siddiqui *et al.,* 2020). A possible explanation is that fruit softening is a collective mechanism in which several cell wall enzymes participate, indicating that the evaluated here are not closely related to the depolymerization of the plant cell. Nevertheless, this mechanism is unclear and needs future studies. On the other hand, these genes were more down-regulated at 15 ± 2 ºC than 28 ± 2 ºC, suggesting that the down-regulation of these genes might help to reduce the bound pectin solubilization and depolymerization by reducing the gene expression of *PME1* and *PL22.* Finally, *PG* and *EG* also presented a down-regulation (almost null) across the days of storage at 28 ± 2 ºC and 15 ± 2 ºC. The expression levels of *PG* genes present a climacteric behavior during fruit softening in several fleshy fruits such as pear, and strawberry (Wei *et al.,* 2015). Likewise, *FaEG1* and *FaEG2* in strawberry fruits encoding *EG* genes have been highly expressed in fruits and associated with softening (Llop-Tous *et al.,* 1999; Trainotti *et al.,* 1999). Nevertheless, in this study, *EG* was down-regulated at 28 ± 2 ºC. A probable explanation of this result is that EG is a wide family, and not all the *EG* are induced and related to ripening. Therefore, future studies should be carried out with other isoenzymes or isoforms. On the other hand, the temperature of 15 ± 2 ºC demonstrated lower expression levels than the 28 ± 2 ºC, finding a down-regulation in *EG* and all the cell-wall genes evaluated. This result indicates that the temperature of 15 ± 2 ºC delays the metabolism of the degradation of the cell wall of the

fruits, impacting directly the gene expression of these enzymes. In fact, a high correlation was observed between the *EXP7*, *PME1*, and *PL22* genes, which was further demonstrated by PCA results. Indeed, expression of all the genes was negatively correlated with firmness, which agrees with the down-regulation recorded in most of all the conditions analyzed. In conclusion, we show that these genes are down-regulated by the combination of days of storage and temperature, providing novel insights into genes associated with the plant cell wall during soursop fruit ripening under postharvest storage.

ACKNOWLEDGMENTS

The authors thank CONACyT for the financial support by the grant Ciencia Básica y/o Ciencia de Frontera Modalidad Paradigmas y Controversias de la Ciencia, grant number 319996: ”Análisis integral de datos transcriptómicos y metabolómicos asociados a la calidad de los frutos de guanábana (*Annona muricata* L.) durante almacenamiento poscosecha” and the postdoctoral fellowship granted to the first author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

REFERENCES

Anaya-Dyck, J.M., Hernández-Oñate, M.Á., Tafolla-Arellano, J.C., Báez-Sañudo, R., Gutiérrez-Martínez, P. & Tiznado-Hernández, M.E. 2021. La cadena productiva de guanábana: una opción para el desarrollo económico en Compostela, Nayarit. Estudios sociales. Revista de alimentación contemporánea y desarrollo regional 31.

AOAC, A., 2005. Association of Official Analytical Chemists.

Berumen-Varela, G., Hernández-Oñate, M.A. & Tiznado-Hernández, M.E. 2019. Utilization of biotechnological tools in soursop (*Annona muricata* L.). Scientia Horticulturae 245, 269–273. https://doi.org/doi.org/10.1016/j.scienta.2018.10.028.

Berumen-Varela, G., Martínez-González, M.E., Palomino-Hermosillo, Y.A., Jiménez-Zurita, J.O., Peña-Sandoval, G.R. & Balois-Morales, R. 2020a. Expression profile of EXP, Succ-CoA and ALDH genes in soursop (*Annona muricata* L.) fruits during ripening in response to refrigeration conditions. Indian Journal of Biotechnollogy 9.

Berumen-Varela, G., Palomino-Hermosillo, Y.A., Bautista-Rosales, P.U., Peña-Sandoval, G.R., López-Gúzman, G.G. & Balois-Morales, R. 2020b. Identification of reference genes for quantitative real-time PCR in different developmental stages and under refrigeration conditions in soursop fruits (*Annona muricata* L.). Scientia Horticulturae 260, 108893. https://doi.org/doi.org/10.1016/j.scienta.2019.108893.

Camacho-Cristóbal, J.J., Herrera-Rodríguez, M.B., Beato, V.M., Rexach, J., Navarro-Gochicoa, M.T., Maldonado, J.M. & González-Fontes, A. 2008. The expression of several cell wall-related genes in Arabidopsis roots is down-regulated under boron deficiency. Environmental and Experimental Botany 63, 351–358. https://doi.org/10.1016/j.envexpbot.2007.12.004.

Carpita, N., 2000. The cell wall. Biochemistry and molecular biology of plants 52–109.

Espinosa, I., Ortiz, R.I., Tovar, B., Mata, M. & Montalvo, E. 2013. Physiological and physicochemical behavior of soursop fruits refrigerated with 1-methylcyclopropene. Journal of Food Quality 36, 10–20. https://doi.org/doi.org/10.1111/jfq.12011.

Ghiani, A., Onelli, E., Aina, R., Cocucci, M. & Citterio, S. 2011. A comparative study of melting and non-melting flesh peach cultivars reveals that during fruit ripening endo-polygalacturonase (endo-PG) is mainly involved in pericarp textural changes, not in firmness reduction. Journal of Experimental Botany 62, 4043–4054. https://doi.org/10.1093/jxb/err109.

González-Agüero, M., Tejerina Pardo, L., Zamudio, M.S., Contreras, C., Undurraga, P. & Defilippi, B.G. 2016. The unusual acid-accumulating behavior during ripening of cherimoya (*Annona cherimola* Mill.) is linked to changes in transcription and enzyme activity related to citric and malic acid metabolism. Molecules 21, 398. https://doi.org/doi:10.3390/molecules21050398.

Jara, K., Castro, R.I., Ramos, P., Parra-Palma, C., Valenzuela-Riffo, F. & Morales-Quintana, L. 2019. Molecular insights into FaEG1, a strawberry endoglucanase enzyme expressed during strawberry fruit ripening. Plants 8, 140. https://doi.org/10.3390/plants8060140.

Jiménez-Zurita, J.O., Balois-Morales, R., Alia-Tejacal, I., Sánchez Herrera, L.M., Jiménez-Ruiz, E.I., Bello-Lara, J.E., García-Paredes, J.D. & Juárez-López, P. 2017. Cold Storage of Two Selections of Soursop (*Annona muricata* L.) in Nayarit, Mexico. Journal of Food Quality 2017, 1–9. https://doi.org/10.1155/2017/4517469.

Kohli, P., Kalia, M. & Gupta, R. 2015. Pectin methylesterases: a review. Journal of Bioprocessing & Biotechniques 5, 1. https://doi.org/10.4172/2155-9821.1000227.

Livak, K.J. & Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Llop-Tous, I., Domınguez-Puigjaner, E., Palomer, X. & Vendrell, M. 1999. Characterization of two divergent endo-β-1, 4-glucanase cDNA clones highly expressed in the nonclimacteric strawberry fruit. Plant Physiology 119, 1415–1422. https://doi.org/10.1104/pp.119.4.1415.

Lu, S., Wang, J., Chitsaz, F., Derbyshire, M.K., Geer, R.C., Gonzales, N.R., Gwadz, M., Hurwitz, D.I., Marchler, G.H. & Song, J.S. 2020. CDD/SPARCLE: the conserved domain database in 2020. Nucleic Acids Research 48, D265–D268. https://doi.org/10.1093/nar/gkz991.

Mutakin, M., Fauziati, R., Fadhilah, F.N., Zuhrotun, A., Amalia, R. & Hadisaputri, Y.E. 2022. Pharmacological Activities of Soursop (*Annona muricata* Lin.). Molecules 27, 1201. https://doi.org/10.3390/molecules27041201,

Palomino-Hermosillo, Y.A., Berumen-Varela, G., Ochoa-Jiménez, V.A., Balois-Morales, R., Jiménez-Zurita, J.O., Bautista-Rosales, P.U., Martínez-González, M.E., López-Guzmán, G.G., Cortés-Cruz, M.A. & Guzmán, L.F. 2022. Transcriptome Analysis of Soursop (*Annona muricata* L.) Fruit under Postharvest Storage Identifies Genes Families Involved in Ripening. Plants 11, 1798. https://doi.org/10.3390/plants11141798,

Paniagua, C., Posé, S., Morris, V.J., Kirby, A.R., Quesada, M.A. & Mercado, J.A. 2014. Fruit softening and pectin disassembly: an overview of nanostructural pectin modifications assessed by atomic force microscopy. Annals of Botany 114, 1375–1383. https://doi.org/doi.org/10.1093/aob/mcu149,

Shani, Z., Dekel, M., Roiz, L., Horowitz, M., Kolosovski, N., Lapidot, S., Alkan, S., Koltai, H., Tsabary, G. & Goren, R. 2006. Expression of endo-1, 4-β-glucanase (cel1) in *Arabidopsis thaliana* is associated with plant growth, xylem development and cell wall thickening. Plant Cell Reports 25, 1067–1074. https://doi.org/0.1007/s00299-006-0167-9,

Shen, W.-B., Li, C.-R., Chen, J.-Y., Xie, J.-H. & Lu, W.-J. 2009. Expansin gene expression in cherimoya fruit is correlated with flesh firmness during fruit ripening and softening. The Journal of Horticultural Science and Biotechnology 84, 333–339. https://doi.org/10.1080/14620316.2009.11512527,

Shin, Y., Chane, A., Jung, M. & Lee, Y. 2021. Recent advances in understanding the roles of pectin as an active participant in plant signaling networks. Plants 10, 1712. https://doi.org/10.3390/plants10081712,

SIAP. Servicio de Información Agroalimentaria y Pesquera., n.d. https://doi.org/Available online: https://nube.siap.gob.mx/cierreagricola/ (accessed on 27 June 2022).

Siddiqui, N.R., Muhammad, A., Khan, M.R., Ali, G.M., Mahmood, T., Shahzad, A. & Jabbar, S. 2020. Differential gene expression of pectin esterase and changes in pectin during development and ripening stages of fruit in selected cultivars of banana. Food Science and Technology 40, 827–831. https://doi.org/10.1590/fst.20719,

Trainotti, L., Spolaore, S., Pavanello, A., Baldan, B. & Casadoro, G. 1999. A novel E-type endo-β-1, 4-glucanase with a putative cellulose-binding domain is highly expressed in ripening strawberry fruits. Plant Molecular Biology 40, 323–332.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. & Rozen, S.G. 2012. Primer3—new capabilities and interfaces. Nucleic Acids Research 40, e115–e115. https://doi.org/10.1093/nar/gks596.

Wei, J., Qi, X., Cheng, Y. & Guan, J. 2015. Difference in activity and gene expression of pectin-degrading enzymes during softening process in two cultivars of Chinese pear fruit. Scientia Horticulturae 197, 434–440. https://doi.org/10.1016/j.scienta.2015.10.002.