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EVALUATION OF DNA INTEGRITY IN PROCESSED TOMATO FOOD TO DETECT TRANSGENIC GENES

EVALUACIÓN DE LA INTEGRIDAD DEL DNA EN ALIMENTOS PROCESADOS DEL TOMATE PARA LA DETECCIÓN DE GENES TRANSGÉNICOS

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Abstract
Biotechnology helps to alleviate problems of insufficient food by introducing transgenic foodstuff. The existence of transgenic food in the market and the lack of information in food labeling indicating the product's action, certification of origin, and the quality of foodstuff have required the development of powerful tools to detect the transgenic organisms in food. In this study the DNA's integrity processed tomato food was evaluated. Preparation of tomato puree and ketchup were required to evaluate the influence of additives on the integrity, quality, and amplificability of genomic DNA of tomato to detect transgenic genes. Samples of fresh tomato were analyzed as positive control. The accD, LAT52, and PG amplicons indicated the presence of transgenic tomato fruits. The ingredients or acid additives and storage time were the most important factors that affected the detection of transgenes. The results confirmed that quality and integrity of the DNA extracts are very important to develop the molecular techniques for the detection of transgenes and food traceability.
Keywords: DNA integrity, transgenes, processed food, traceability, tomato.

Resumen
La Biotecnología contribuye con el desarrollo de alimentos transgénicos para resolver problemas de insuficiencia alimentaria. La existencia de alimentos transgénicos en el mercado y la falta de información en el etiquetado sobre la producción, certificación del origen y la calidad de los productos alimenticios han requerido de poderosas herramientas para la detección de alimentos transgénicos. En este estudio se evaluó la integridad del ADN en alimentos procesados de tomate; la elaboración de puré de tomate y cátsup fue necesaria para evaluar la influencia de los aditivos y el tiempo sobre la integridad y amplificabilidad del ADN genómico de tomate. Muestras de tomate fresco se analizaron como control positivo. Los amplicones accD, LAT52 y PG indicaron la presencia de tomates trangénicos. Los ingredientes o aditivos ácidos y el tiempo fueron los factores más importantes que afectan la detección de transgenes. Por lo tanto, la calidad y la integridad de los extractos de ADN son muy importantes para el desarrollo de las técnicas moleculares en la detección de transgenes y la trazabilidad de los alimentos.
Palabras clave: integridad del ADN, transgenes, alimento procesado, trazabilidad, tomate.

1 Introduction

The Food and Agriculture Organization of de United Nations (FAO) estimates that agriculture will have to feed a human population of 8 billion for 2020, of which 840 million people live in food poverty conditions. Biotechnology has developed genetic modified organisms (GMO), particularity in plants, which allows an increasing on the production of food in the Agricola sector. So, the adoption of these new technologies in the alimentary chain leads to consider the food quality and safety within a global context that is constantly evolving in terms of normative regulations and requirements. Globalization in food has implications on the development of a better alimentary chain and its safety (FAO, 2003), i.e.

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internal traceability has been indicated as a production action to improve reliability of labeling, to certify the origin and quality of products on the market, and to prevent fraudulent or deceptive labeling (Golan *et al*., 2002; López *et al*., 2003). However, there are no indications on the traceability of the product in many foods, and there is no caption in the food label indicating whether the food is of transgenic origin. In the general population it is common to talk about existence of the GMO foods, but no actual knowledge is available that indicates their traceability and consumption in GMO foods (Golan *et al*., 2002).

Moreover, a number of plant species have been genetically modified, including tomato, which is one of the most important and popular vegetable in the world, and is an ingredient of a great variety of dishes on our tables (Saeed *et al*., 2014). Tomato (*Solanum lycopersicum* Mill.) is a highly perishable vegetable fruit of the family *Solanaceae*, which is considered native to South America, but different wild varieties of tomato have been found in Mexico that is a center of fruit domestication (Savo Sandero *et al*., 2013). Tomato is considered as source of energy mainly due to its fructose and glucose content, it is also a source of folic acid, with high concentrations of vitamins A, C, E, some minerals like potassium, calcium; it contributes with a very important antioxidant, i.e. lycopene, which has importance in the diet (Candelas-Cadillo *et al*., 2005; Galicia *et al*., 2008, Domínguez-Hernandez *et al*., 2016). Consumption of tomato may prevent cardiovascular diseases and leads to a lower prostate cancer risk, possibly due to lycopene's action on cancer mechanisms, but the research is still poor (Davies and Hobson, 1981, Rai *et al*., 2012). In addition, in the food processing industry, tomato is one of the most important vegetables in this industrial sector, such as production of chopped tomato puree, ketchup, sauces and soups (Liedl *et al*., 2013). For this reason, there is a large demand of tomato worldwide, but information on internal traceability is not available, and molecular studies for the detection of GMOs have been focused on corn, beans, cotton, etc. (Marmiroli *et al*., 2008).

In consequence, the existence of GMOs in the market and the lack of information for the consumer has required the development of methodologies based on genetic and molecular biology to track a given item at any stage along the food supply chain, from "farm to the table" (Di Bernardo *et al*, 2005; Savo Sander *et al*., 2013). Thus, molecular techniques are a powerful tool to detect transgenic organisms. The correct detection of GMOs in the laboratory starts with the selection of the molecular technique and isolation of the molecules to be analyzed. DNA is the preferred molecule for almost any kind of sample, including processed foods, because DNA is a more stable molecule than RNA or proteins, therefore, molecular methods are based mainly on the use of DNA (López *et al*., 2003; Turci *et al*., 2010). Among these, polymerase chain reaction (PCR) technology has become one of the main tools of molecular biology and is widely used for the detection and identification of GMOs due its high sensitivity. Depending on the purpose of the analysis (qualitative or quantitative), DNA amplification can be developed through real-time PCR or end-point PCR (Holst-Jense *et al*., 2003; Smith, 2007).

The genomic DNA extraction method can affect the PCR-based analysis by (i) the presence of PCR inhibitors in the food matrices, (ii) the excessive fragmentation of the DNA (Marmiroli *et al*., 2003). Therefore, the selection of the genomic DNA extraction method is one of the key steps for the detection of GMOs. The most appropriate method for genomic DNA extraction is the one that allows isolating DNA with the best efficiency and integrity, and permits the removal of substances, such as polyphenols, polysaccharides, among others, which can inhibit its amplification (Holst-Jensen *et al*, 2003; Meyer, 1999; Smith *et al*., 2007). Although, genomic DNA can be fragmented during food processing, this does not imply that the DNA cannot be detected. During heat sterilization in the food canning process, it is possible to obtain genomic DNA in small fragments with a sufficient length to differentiate between closely related species or to detect the transgenes (López, 2003).

In this study, the DNA integrity in processed tomato food was evaluated to detect the presence of transgenic genes mainly in tomato puree and ketchup. The presence of additives in the processed food was considered in the DNA integrity assessment for the detection of transgenic genes. Samples of fresh tomato were analyzed as positive control. The analyzed samples of tomato puree and ketchup were chosen based on their complexity and the technological treatment to which they were subjected from fresh tomato to tomato sauce. Samples of these processed products were bought from a supermarket, considering the major national brands. Preparation of tomato puree and ketchup were required to evaluate the influence of additives on the isolation, integrity, quality, and amplificability of the genomic DNA of tomato and to detect transgenic genes.

2 Materials and methods

2.1 Detection of transgenic genes

2.1.1 Sample collection and total genomic DNA extraction

Ten fresh tomato fruits were collected for each of the main varieties found in the Mexican market: *S. lycopersicum* var. Roma (saladette), *S. lycopersicum* var. cerasiforme (cherry), and *S. lycopersicum* var. Floradade (round tomatoes) on different days of the month.

All the samples tomato fruits from each variety were used for total genomic DNA extraction and subsequent molecular analyses. Prior to DNA extraction, all fruits were cut in four parts and placed in clean zip-lock bags. Then, samples were placed in dry ice for 10 min and one part for each sample was homogenized using a sterilized mortar. Total genomic DNA extraction was done using approximately 100 mg of homogenized sample, and 400 μl of extraction buffer (10 mM Tris-HCl, pH 8; 150 M NaCl; 2 mM EDTA; 1% SDS) was added and mixed by vortexing for 5 s. The samples were incubated at 65 °C for 20 min and shaken very 10 min in a vortexer. DNA was purified by extracting with 200 μL phenol:chloroform:isoamyl alcohol (24:25:1), followed by centrifugation at 10,000 g for 10 min at room temperature; the supernatant was transferred to a new micro-tube and DNA was precipitated with -20 °C absolute ethanol and washed with 500 μL of 70% ethanol. The DNA was air-dried and re-suspended in 30 μl of TE buffer (10 mM Tris-HCl, pH 8; 1 mM EDTA) (Turci *et al*., 2010). DNA degradation and quality was checked by electrophoresis on a 0.8% agarose gel in 1×TAE (45 mM Tris-acetate, 1 mM EDTA, pH8) followed by staining with ethidium bromide (1-mg/mL) and visualized under UV light in a Benchtop Variable Transilluminator (UVP Inc, Upland, CA, USA).

2.1.2 PCR amplification

DNA extracted from tomato fruit samples was tested for PCR amplificability with the SSR LEat002, targeting the Cleb1P20 locus (Gene Bank accession number AI778183). The SSR (simple sequence repeat) or microsatellite marker is currently the preferred molecular marker by its importance for variety identification of tomato, it due to their property of genetic codominance, high reproducibility, and multiallelic variation, also the results are faster and clearer cut (He *et al*., 2003; Turci *et al*., 2010). PCR amplificability of DNA isolated from samples was tested using specific primers (Table 1); to standardize the procedure, the same master mix was utilized to amplify the molecular marker (SSR LEat002) from all extracted DNAs. PCR assay was performed in a final volume of 20 μL containing: 1 U of Go Taq Flexi DNA-Polymerase (Promega, USA), 0.25 mM dNTP mix (Promega), 0.25 pM of each primer, 3.5 mM $MgCl_2$, 1×reaction buffer (Promega), and 100 ng of genomic DNA. Amplification reactions were run under the following conditions: DNA denaturation at 94 °C for 1 min, followed by 40 cycles of 45 s at 94 °C; 45 s at 59 °C; 2 min 30 s at 72 °C, and finally a primer thermal extension at 72 °C for 10 min. All PCR reactions were carried out using a thermocycler (Benchtop Gradient, Labnet International, USA). Amplification products were separated by electrophoresis using a 2% (w/v) agarose in TAE 1× (Sambrook *et al*., 1989), stained with 1-mg/mL of ethidium bromide solution and visualized with a Benchtop Variable Transilluminator (UVP Inc, Upland, CA, USA).

2.1.3 Amplification of transgenic genes

The amplification of transgenic genes was done by PCR. The used protocol was as explained above, adjusting the annealing temperature according to the Tm of the specific primers for the transgenic genes reported for tomato (Table 1). The sizes of all the obtained amplicons were identified using a molecular weight marker (100 bp ladder) by agarose gel-electrophoresis.

2.2 Amplification of transgenic genes in processed food of tomato

For detection of the transgenic genes in processed tomato food, tomato puree and ketchup were used. Samples of these products were bought in a supermarket; processed products were chosen within the product range of major national brands, seasoned and natural tomato puree and tomato ketchup. Then, the genomic DNA was extracted as described above. To standardize the procedure, the same master mix was utilized to amplify the nucleic acids extracted from the processed food matrices.

Table 1. Transgenic genes and specific primers to detect by PCR transgenes in tomato fruits and processed food

Primer	Origen organism	Characteristic	Sequence	Tm (°C)	Amplicon length (bp)	Reference
LEat002*	*Solanum lycopersicum*	Simple sequence repeats market ($SSR_{(AT)_9}$). Specific of tomato.	F: 5'- ACTGCATTTCAGGTACATACTCTC-3' R: 5'-ATAAACTCGTAGACCATACCCTC-3'	59	200	He *et al*. 2003; Turci *et al*. (2010)
accD	*Pseudomonas chlororaphis*	Gene encodes1 –amino-cyclopropane-1-carboxylic acid deaminase, it may help control ethylene levels.	F: 5'-ACGAGGGTTCCAGTAGACGA-3' R: 5'-AGGCATTGGGTTCGGCAATA-3'	57	705	Plett *et al*. (2009)
CaMV 35S	Cauliflower Mosaic Virus	Gene interruptor; pathogen resistant.	F: 5'-GCTCCTACAAATGCCATCA-3' R: 5'-GATAGTGGGATTGTGCGTCA-3'	54	195	Wolf *et al*. (2000)
LAT52	*Solanum lycopersicum*	Gene encodes a heat-stable, glycosylated, cysteine-rich, it is necessary for pollen development, requires further tests.	F: 5'-AAAACTCAGCGAGAACTT-3' R: 5'-GGAACTTATCATCCCATA-3'	60	946	Yang *et al*. (2005)
TNOS-ntpII	*Escherichia coli*	Kanamycin resistance gene.	F: 5'-GAATCCTGTTGCCGGTCTTG-3' R: 5'-TTATCCTAGTTTGCGCGCTA-3'	54	180	Van den Eede *et al*. (2010)
PG	*Solanum lycopersicum*	Polygalacturonase (PG) gene, it reduced translation of the endogenous PG messenger RNA.	F: 5'- GGATCCTTAGAAGCATCTAGT-3 R: 5'-CGTTGGTGCATCCCTGCATGG-3'	60	384	Van den Eede *et al*. (2010)

***** DNA extracted from tomato was tested for PCR amplificability with the SSR LEat002.

2.3 Evaluation of genomic DNA integrity in processed tomato food

The effect of the additives used in the tomato puree and ketchup were evaluated on the genomic DNA extraction to detect transgenic genes. Therefore, tomato puree and ketchup were elaborated using the Roma tomatoes, previously analyzed with respect to transgenic genes.

2.3.1 Elaboration of tomato puree and ketchup

Tomato puree and ketchup were prepared in lab-scale following a standard protocol with several modifications based on the formulation of the label for commercial products (Ranganna, 1977). Three Roma tomatoes were weighed before starting the elaboration of the products. Tomatoes were blanched in boiling water for 5 min or until the skins broke up, they were withdrawn from the water; then the skin and peduncle were removed from the fruit. The remnant tomato tissue was weighed and divided into equal parts to prepare seasoned tomato puree and ketchup. Seasoned tomato puree was prepared with 187.5 g of tissue, which was liquefied and strained to remove seeds, then adding 1-g garlic, 8 g onion, 1-g sodium chloride, 2.5 g monosodium glutamate (MSG), 0.46 g citric acid, and 14 g starch, and the mixture was homogenized and vacuum packed into a glass jar. A second portion of tomato tissue was liquefied to preparer tomato ketchup, the liquefied tomato was strained and the obtained puree was cooked over low heat, and 20 g refined sugar, 2.5 g ginger, 0.4 g lemon tea, 7 g honey, 7 mL sherry vinegar, 2.5 g monosodium glutamate (MSG), 0.16 g and 0.8 g pequin chili and five Chinese spice powders (in equal amounts: fennel seed, star anise, black pepper, clove and cinnamon powders) were added. Once finished, the sauce was placed in a glass jar and vacuum sealed.

2.3.2 Total genomic DNA extraction and amplification of transgenic genes

Different samples of each puree and ketchup processing step were taken and stored in refrigeration for genetic material extracting. Total DNA was extracted at 0, 3, and 11 days of food elaboration to evaluate the effect of the ingredients and storage time. DNA extraction was performed with 500 mg of the sample used for the DNA extraction protocol described above. Evaluation of DNA extracts was performed by electrophoresis in agarose gel. The extracted DNA of the sample obtained at each step of the elaboration of processed food was used to amplify the transgenic genes. The PCR to amplify the transgenic genes was developed as described above.

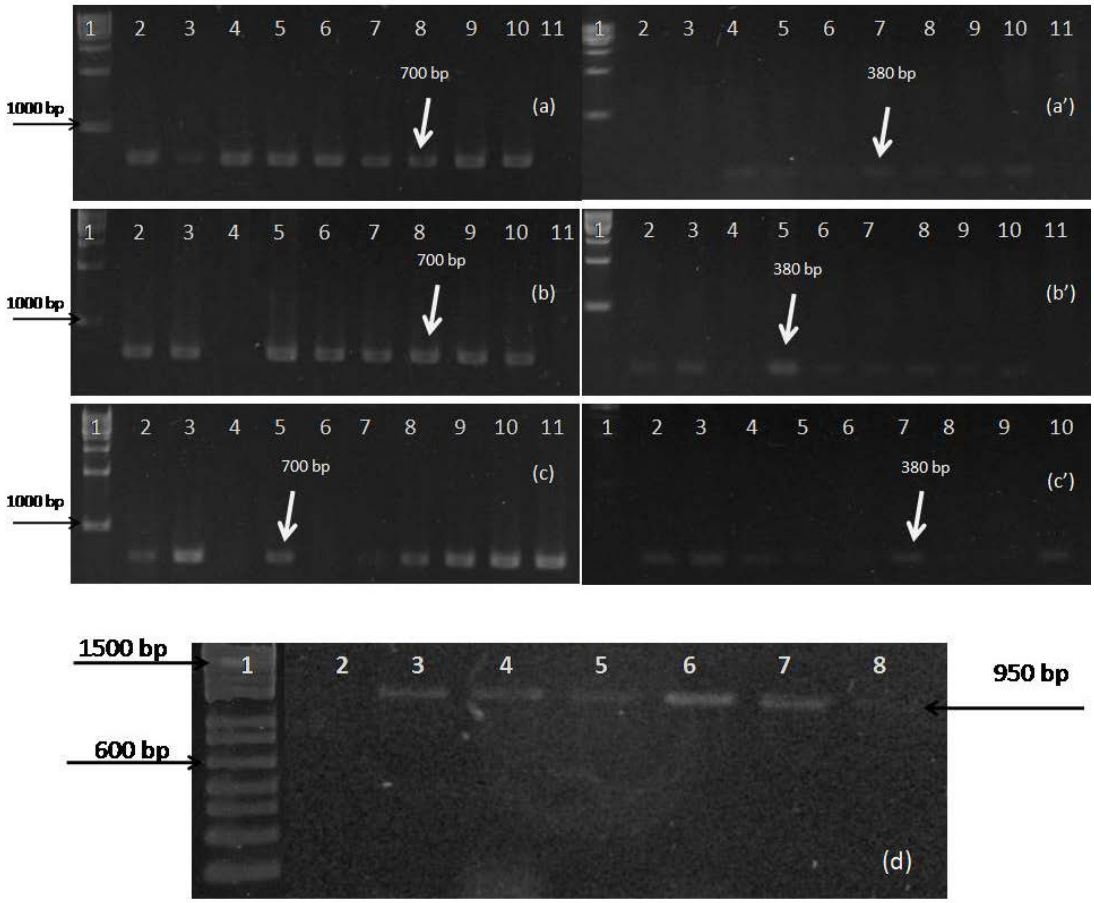

Fig. 1. Amplification of transgenes by PCR performed with DNA template of different tomato varieties. The left pictures correspond to the accD amplicons, expected size 700: (a) Roman tomato; (b) Floradade tomato; (c) Cerasiforme tomato. The right pictures correspond to the PG amplicons, expected size 380 pb. (a') Roman tomato; (b') Floradade tomato; (c') Cerasiforme tomato. Well (1) 1-kb DNA step ladder (Promega, USA); (2-11) amplicons obtained of 10 samples of genomic DNA. (d) LAT52 amplicons obtained only of genomic DNA from the Roman tomato, expected size 946 pb. Well: (1) 100 bp DNA ladder (Invitrogen, USA); (2-8) amplified product.

3 Results and discussion

3.1 Detection of transgenic genes in fresh tomato fruits

The molecular techniques based on DNA analyses were used to evaluate the integrity of genomic DNA of tomato (*S. lycopersicum* L.), particularity in the elaboration of tomato puree and ketchup. Quality and amplificability of the extracted DNA are needed to detect transgenic gene in processed food.

An important point of the present study was detecting transgenic genes in the tomato fruits of the main varieties found in the Mexican market, Roma, cherry, and round tomatoes. The extracted DNA presented good quality and quantity (Figure not shown), demonstrating that the procedure was good for the extraction of genomic DNA of matrix processed food, as it was reported previously (Turci *et al*., 2010). A PCR product of LEat002 was obtained from the extracted DNA of each tomato with an approximate size of 200 bp (data not shown). This fact indicates that the extracted DNA has the quality required to be used in the PCR method and to amplify a specific DNA region.

Using the specific primers for *acc*D, CaMV 35S, *LAT52, TNOS-ntpII*, and *PG* genes, only *acc*D, *LAT52*, and *PG* genes were amplified (Fig. 1). Thus, the obtained results indicate the presence of transgenic tomato fruits in the Mexican market. This result poses

the next question: Is it possible to detect transgenic genes in processed tomato food?

3.2 Extraction of DNA from processed tomato foods

In the present study genomic DNA isolated from seasoned and natural tomato puree and tomato ketchup of the major national brands presented very fragmented DNA with impurity that the PCR for the SSR LEaT002 molecular marker did not show an amplicon (data not shown). Then, seasoned tomato puree and ketchup were prepared, and a sample of each step of their elaboration was taken to extract genomic DNA. Extraction of genomic DNA was done on the same day of the elaboration of processed food; to evaluate the influence of the additives and the storage time, the genomic DNA was extracted 3 and 11 days after their elaboration. The results obtained for tomato puree showed that the DNA extracts did not show degradation when extracted on the same day of elaboration (Fig. 2a). On the third day after the preparation of puree, the DNA extracts exhibited degradation when citric acid, MSG, and starch were added (Fig. 2b).

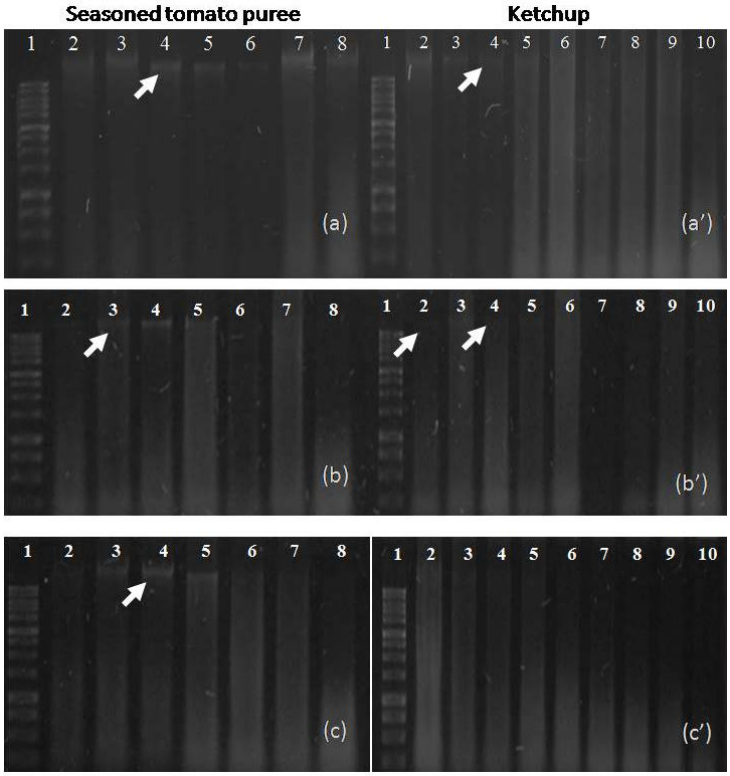

Fig. 2. Gel electrophoresis of the DNA extracts from the tomato puree and ketchup elaborated in this work. (a, a') DNA extracts from puree and ketchup at time zero of processing; (b, b') DNA extracts from puree and ketchup 3 days after processing; (c, c') DNA extracts from puree and ketchup after 10 days of processing. Tomato puree, well:(1) 1kb DNA ladder (Promega, USA); (2) liquefied raw tomato; (3) liquefied tomato and garlic; (4) liquefied tomato, garlic, and onion; (5) liquefied tomato, garlic, onion, and NaCl; (6) liquefied tomato, garlic, onion; NaCl, and sodium glutamate; (7) liquefied tomato, garlic, onion; NaCl, sodium glutamate, and citric acid; (8) liquefied tomato, garlic, onion; NaCl, sodium glutamate, starch, and citric acid. Ketchup, well: (1) 1kb DNA ladder (Promega, USA); (2) mashed simmered tomato (3) tomato paste and refined sugar; (4) tomato puree, refined sugar, and ginger; (5) tomato puree, refined sugar, ginger, and lemon tea (6) tomato puree, refined sugar, ginger, lemon tea, and honey; (7) tomato puree, refined sugar, ginger, lemon tea, honey, and sherry vinegar; (8) tomato puree, refined sugar, ginger, lemon tea, honey, sherry vinegar, and MSG; (9) tomato puree, refined sugar, ginger, lemon tea, honey, sherry vinegar, MSG, and chili powder; (10) tomato paste, refined sugar, ginger, lemon tea, honey, sherry vinegar, MSG, chili powder and Chinese dust.

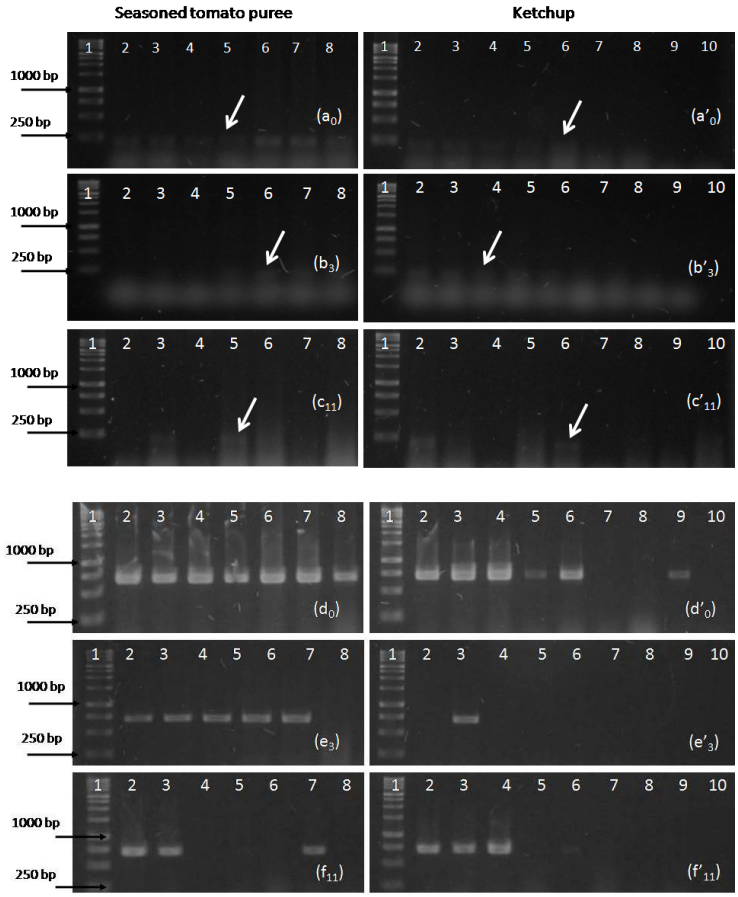

Fig. 3. Electrophoresis gel of the amplicons obtained from processed food. (a-c, a'-c') SSR LEat002 amplicons, expected size 200 bp; (d-f, d'-f') accD amplification, expected size 700 pb. The subindex indicates storage time (0, 3, and 11 days). Tomato puree, well:(1) 1kb DNA ladder (Promega, USA); (2) liquefied raw tomato; (3) liquefied tomato and garlic; (4) liquefied tomato, garlic, and onion; (5) liquefied tomato, garlic, onion, and NaCl; (6) liquefied tomato, garlic, onion; NaCl, and sodium glutamate; (7) liquefied tomato, garlic, onion; NaCl, sodium glutamate, and citric acid; (8) liquefied tomato, garlic, onion; NaCl, sodium glutamate, starch, and citric acid. Ketchup, well: (1) 1-kb DNA ladder (Promega, USA); (2) mashed simmered tomato (3) Tomato paste and refined sugar; (4) tomato puree, refined sugar, and ginger; (5) tomato puree, refined sugar, ginger, and lemon tea (6) tomato puree, refined sugar, ginger, lemon tea, and honey; (7) tomato puree, refined sugar, ginger, lemon tea, honey, and sherry vinegar; (8) tomato puree, refined sugar, ginger, lemon tea, honey, sherry vinegar, and MSG; (9) tomato puree, refined sugar, ginger, lemon tea, honey, sherry vinegar, MSG, and chili powder; (10) tomato paste, refined sugar, ginger, lemon tea, honey, sherry vinegar, MSG, chili powder and Chinese spices.

In contrast, the DNA extracts obtained at 11 days of preparation presented a greater degradation and no DNA was obtained when sodium citrate was added (Fig. 2c). This fact indicates that the sodium citrate was the most aggressive additive that induces degradation of genomic DNA in a short time. For ketchup, the DNA extracts presented some degree of degradation at the time of adding honey, and this was increased with the addition of sherry vinegar and MSG. This phenomenon was observed starting at the moment of ketchup elaboration (Fig. 2a', b'); but 11 days after, the extracted DNA was totally degraded, which can be observed as a sweeping along the electrophoresis gel (Fig. 2c'). In the ketchup, DNA degradation was more notorious because in its preparation ingredients as honey and sherry vinegar were required, which have acid pH 3.4 and 2.92, respectively; therefore, the final pH of ketchup was 3.57, which affected the integrity of genomic DNA linked to the warming process during preparation

of the product. This effect may be caused by the exposure to heat, enzymatic degradation by nucleases, temperature change, ionic strength, chemical agents, and pH values. The mechanism of DNA destruction by heat is based on depurination or deamination. At temperatures above 100 °C, a significant strand scission and irreversible loss of secondary structure occurs. The influence of pH may be limited due to cell wall structure protecting the DNA from cleavage. Moreover, the initiation of cell lysis and preliminary DNA destruction is accelerated at low pH, suggesting a quicker destruction of DNA. In food, the rates of the DNA degradation are strongly affected by matrix properties, as well as processing and storage conditions.

3.2.1 PCR amplification

As mentioned above, genomic DNA can be fragmented during food processing but does not necessarily imply that the DNA cannot be detected. Hence, the extracted genomic DNAs from the different samples were used to amplify the SSR LEat002 molecular marker for tomato puree and ketchup elaborated in this work. From all the tomato puree samples of the first day of preparation a 200 bp amplicon was obtained (Fig. 3_{a0}, b_3, c_{11}). However, the correspondent band at SSR LEat002 molecular marker lost clearness and integrity over time. For ketchup samples, the SSR LEat002 molecular marker on the first five genomic DNA samples were amplified only; a band can be clearly observed but integrity is lost, this indicates that the presence of acidic ingredients as sherry vinegar and additives (MSG) affect DNA amplification (Fig. $3a'_0$, b'_3, c'_{11}). Also, storage time influences the amplification of genomic DNA as it is degraded, and the ingredients as citric acid, honey, and sherry vinegar increase the DNA degradation of the tomato matrix (Fig. 2).

The amplification of the *acc*D, LAT52, and PG transgenes was consistent with respect to results obtained in fresh fruits (Figs. 3 and 4). The obtained amplicons indicated that in both processed products, the acid ingredients or additives and time are big enemies for the detection of GMO, as well as the absence of amplicons of genomic DNA in the commercial product samples. Although, in commercial products the reported minimal percentage of tomato is 10%, a quality analysis of different tomato purees made by the Mexican consumer protection agency (PROFECO, for its acronym in Spanish) reported a less percentage of tomato pulp and with a larger content of additives, particularly thickening, coloring, and flavoring agents (Tirado-Gallegos *et al*., 2016); thus, these ingredients and storage time contributed to the poor genomic DNA extraction and lack of molecular results that could help to detect transgenes in processed foods.

A genetically modified organism is characterized by containing a fraction of DNA from another organism in their DNA. As a result, the transgenic organism usually gains a new function of commercial interest. Tomato has been engineered to produce large fruits, but genetically modified crops are not well accepted yet by consumers. In this analysis, the obtaining of amplicons *acc*D, LAT52, and PG is an indication of the presence of possible transgenes in tomato. PCR protocols used for GMO screening are based on the detection of the 35S promoter in the cauliflower mosaic virus (CaMV) and the *Agrobacterium tumefaciens* nopaline synthase (nos) terminator sequences, and in some cases, the NptII terminator (Xu *et al*., 2006; Wolf *et al*., 2000). These three genetic elements are presented in numerous but not in all GMOs, as in this case these elements did not amplify.

In transgenic tomato fruit, the gene encoding the ACC deaminase is expressed, showing a degree of ethylene inhibition and delay in the progression of ripening. Visually a transgenic fruit that is stored at room temperature depicts a significant reduction in softening, and a firm fruit is observed for up to 5 months (Plett *et al*., 2009, Klee *et al*., 1991). Degradation of ACC inhibits ethylene synthesis but does not interfere with the fruit's ability to perceive ethylene, because transgenic fruit exposed to exogenous ethylene ripens normally (Plett *et al*., 2009). All of these factors indicate that the expression of ACC deaminase should result in a greatly extended shelf life for tomatoes and other climacteric fruits and vegetables (Chernin and Glick, 2012). The existence of transgenic tomato fruits is also confirmed with the presence of the LAT52 amplicon, this gene acts as an endogenous reference gene in GM tomato detection, which is normally used as the internal control for qualitative and quantitative PCR analysis (Yang *et al*., 2005). Finally, the insertion of an additional copy of the PG encoding gene in the "antisense" orientation was the first strategy to develop the FLAVR SAVR^{TM} tomato, with reduced translation of the endogenous PG messenger RNA (mRNA). The PG enzyme is the chief mechanism of pectin degradation in tomato fruit leading to fruit softening.

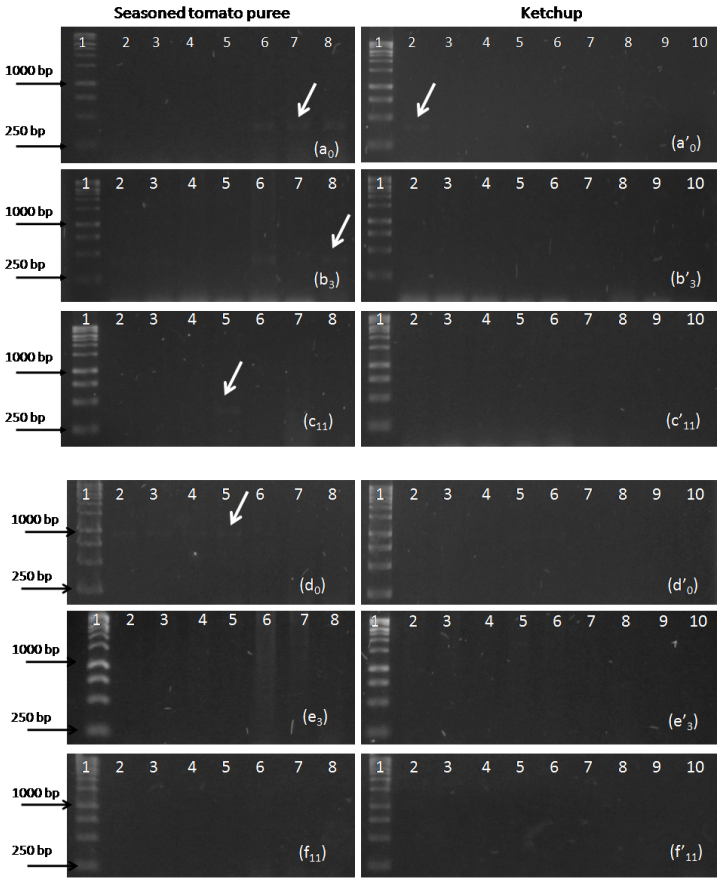

Fig. 4. Electrophoresis gel of the amplicons obtained from processed food. (a-c, a'-c') PG amplicons, expected size 384 bp; (d-f, d'-f') LAT52 amplicons, expected size 950 bp. The subindex indicates storage time (0, 3, and 11 days). Tomato puree, well:(1) 1kb DNA ladder (Promega, USA); (2) liquefied raw tomato; (3) liquefied tomato and garlic; (4) liquefied tomato, garlic, and onion; (5) liquefied tomato, garlic, onion, and NaCl; (6) liquefied tomato, garlic, onion; NaCl, and sodium glutamate; (7) liquefied tomato, garlic, onion; NaCl, sodium glutamate, and citric acid; (8) liquefied tomato, garlic, onion; NaCl, sodium glutamate, starch, and citric acid. Ketchup, well: (1) 1-kb DNA ladder (Promega, USA); (2) mashed simmered tomato (3) tomato paste and refined sugar; (4) tomato puree, refined sugar, and ginger; (5) tomato puree, refined sugar, ginger, and lemon tea (6) tomato puree, refined sugar, ginger, lemon tea, and honey; (7) tomato puree, refined sugar, ginger, lemon tea, honey, and sherry vinegar; (8) tomato puree, refined sugar, ginger, lemon tea, honeys herry vinegar, and MSG; (9) tomato puree, refined sugar, ginger, lemon tea, honey, sherry vinegar, MSG, and chili powder; (10) tomato paste, refined sugar, ginger, lemon tea, honey, sherry vinegar, monosodium glutamate, chili powder, and Chinese spices.

The transgenic variety ripens normally but experiences less pectin breakdown and, therefore, has increased thickness and consistency that benefits all stages of harvesting and processing (Smith *et al*., 1990).

Conclusions

The obtained results confirm that the quality and integrity of the extracted DNA are very important to develop the molecular techniques for the detection of transgenes in food traceability. In the tomato, it was possible to detect genes characterized as transgenic, but not in processed foods derived from tomato due to the absence of a DNA with the amount and quality required. However, DNA analysis made in freshly prepared food allowed detecting transgenes, but these prepared foods should not exceed 10 days of preparation, because the product ingredients damage the DNA. The *LAT52* and *PG* genes detected belong to the same tomato genome that has been manipulated

by genetic engineering to study the phenotype in tomato plants or to annul the expression of these genes to acquire certain advantages over wild crops. The gene *accD* is the only bacterial gene that confers the phenotype to delay fruit ripening for increased shelf life. Therefore, the tomato available at the national market is a transgenic fruit or also named a genetically modified organism (GMO).

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