International Food Research Journal 19(4): 1471-1477 (2012)
Journal homepage: *http://www.ifrj.upm.edu.my*

A DNA-based approach to discriminate between quince and apple in quince jams

[1]Arleo, M., [1]Ruibal, F., [1]Pereyra, J., [1, 2]Miquel, E., [1]Fernández, M. and [1,*]Martínez, C.

[1]LaTraMA ::: Laboratorio de Trazabilidad Molecular Alimentaria. Sección Bioquímica. Facultad de Ciencias, Universidad de la República (UdelaR). Iguá 4225, CP 11400, Montevideo, Uruguay

[2]Departamento de Histología y Embriología, Facultad de Medicina, UdelaR. Av. Gral. Flores 2125. CP: 11800. Montevideo, Uruguay

Article history

Received: 5 January 2012
Received in revised form: 12 April 2012
Accepted:12 April 2012

Keywords

Isolation
identification
apple
quince jams

Abstract

Authentication of food products is of primary importance for consumers and industries. They expect that product labeling represents its true identity. However, in many cases, either accidental or fraudulent substitution occurs. Molecular biology based methodologies are acquiring great interest for their applicability to track a given item at any stage along the food supply chain. PCR analysis allows the identification of DNA traces that may remain in a given food matrix from the principal component and/or from contaminants. This work describes a novel experimental protocol to extract, amplify and identify apple DNA from commercially available quince jams. Aiming to extract DNA from this complex matrix, a DNA extraction method was developed, based in reagents such as CTAB and PTB, that minimize the amount of contaminants co-precipitating with DNA and thus reducing PCR inhibition. In order to identify apple DNA in quince jam, two molecular methods based on the amplification by PCR and RFLP analysis of the highly conserved chloroplast gene *maturase-k* (matk), were developed. The results obtained show the presence of two distinct species *Cydonia oblonga* (quince) and *Malus* sp. (apple) in all commercial quince jams analyzed. This study represents an advance in DNA methodologies for the detection of fraud and/or contaminants in this complex food matrix.

© All Rights Reserved

Introduction

The consumer expects that product labeling represents its true identity. However, in some cases, either accidental or fraudulent substitution occurs. The former may occur as a result of inadequate cleaning following the changeover of products during manufacture and may be symptomatic of poor manufacturing practices. Fraudulent substitution is also a serious matter, representing deliberate extension of products with cheaper additives. (Ortola-Vidal *et al*., 2007).

Authentication of food products is of primary importance for both consumers and industries, at all levels of the production process, from raw materials to finished products. From the legislative point of view, quality standards have been established through the requirement of quality labels that specify the composition of each product. From the economic point of view, product authentication is essential to avoid unfair competition that can create a destabilized market and disrupt the regional economy and even the national economy. All food products targeted for adulteration are high-commercial-value products and/or produced in high tonnage around the world (Cordella *et al*., 2002).

The requirement of internal traceability procedures in food production has stirred also a certain level of technological implementation. Methodologies based on genetic and molecular biology are acquiring great interest for their applicability to track a given item at any stage along the food supply chain, from ‘‘farm to the fork” (Di Bernardo *et al*., 2005).

PCR analysis allows the identification of traces of DNA that may residue in a food matrix from the principal component and/or from contaminants (Lockley *et al*., 2000). DNA extraction methods can affect the PCR based analysis by: (i) the presence of PCR inhibitors in the food matrices, (ii) the excessive fragmentation of the DNA molecules, and (iii) the short average length of DNA fragments. Quantity and quality of the extracted DNA are extremely sample-dependent. In fact the food matrix production and its chemico-physical composition can introduce many degrees of variability into the DNA extraction methods and in the efficacy of the DNA amplification (Turci *et al*., 2010).

Quince (*Cydonia oblonga*) belongs to Rosaceae

*Corresponding author.
Email: *clamim@gmail.com*

family, sub family Maloideae along with pears (*Pyrus* spp.) and apples (*Malus* spp.). The quince is native to southern Europe and Minor Asia. (Yamamoto *et al*., 2004). It is a hard fruit, astringent and sour to eat raw. It is used to make jam, fresh fruit compote, jellies and wines (Laureiro *et al*., 2009). In Uruguay, most of the quince production is destined to the internal market. Some recurrent reports, coming from local industries, alerted us about the possible presence (adulteration or contamination) of apple in some specific quince jam brands. Both quince and apple DNA sequences are very similar, thus difficulting the design of an easy procedure (i.e. by using universal primers as in Taberlet *et al.*, 1992) for their identificaction and discrimination based only on PCR amplification.

Work has been performed for the determination of adulteration in such quince products, using analytical techniques such as HPLC, HPLC-DAD, HPLC-MS, among others (Andrade *et al*., 1998; Silva *et al.*, 2000; Luykx and van Ruth, 2008). However, there are no reported molecular studies of plant species present in supposedly pure quince products yet.

The chloroplastid coding gene *maturase-k* (matk) has been proposed as a molecular marker able to act as an universal land plant barcode (Fazekas *et al*., 2008). MatK is one of the most rapidly evolving plastid coding regions and its use as a barcoding system has consistently showed high levels of discrimination among angiosperm species (CBOL Plant Working group, 2009).

This study describes a novel experimental protocol to extract, amplify and identify fruit (quince and apple) DNA from commercially available quince jams. Different available DNA extraction procedures, adapted to plant tissues were tested. In order to assess the presence of apple DNA in quince jams, two molecular methods were developed, which include: DNA extraction and PCR amplification of two regions of matK gene, followed by digestion with specific restriction enzymes (Polymerase chain reaction followed by restriction fragment length polymorphism analysis -PCR-RFLP).

Materials and Methods

Quince Jams

Whole genomic DNA was extracted from the following samples: 1. Quince jams supplied by a local industry (100% quince and 50% quince-50% apple, prepared under controlled conditions with 700 g of sugar per kilogram of quince, named "control samples"); 2. Quince jams obtained from retail outlets ("problem samples I to V"); 3. Fresh leaves of *Malus domestica* and *Cydonia oblonga* (a kind gift from DIGEGRA-MGAP, Dirección Nacional de la Granja, Ministerio de Ganadería, Agricultura y Pesca, Uruguay, named as "standard samples").

DNA extraction

It was performed using commercially available DNA extraction kits, specifically developed to eliminate PCR inhibitors present in complex samples and/or plant tissues, following manufacturer´s instructions. It was also developed a modified CTAB protocol (initially described by Doyle & Doyle, 1987, see also Weising *et al*., 2005; Di Pinto *et al*., 2007) suitable for plant DNA with high polysaccharide content as expected to be found in quince jams samples.

Standard samples. Fresh leaves (0.1 g) were ground to powder in liquid nitrogen. The powder was dissolved in 1 ml homogenization buffer (1.5 M NaCl, 3% w/v hexadecyltrimethyl- ammonium bromide (CTAB), 100 mM Tris-HCl pH 8.0, 50 mM ethylene di amino tetra acetic acid (EDTA) pH 8.0, 0.2% 2-mercaptoethanol, 1% polyvinylpyrrolidone (PVP), 10% N-phenacyltiazolium bromide (PTB)) and incubated at 65 °C for 1h. The mixture was then extracted twice with an equal volume of chloroform-Isoamyl alcohol (24:1), and precipitated with absolute EtOH. The resulting DNA pellet was dissolved in 100 µl of milli Q^{TM} water, and kept at -20ºC until use.

To obtain DNA from jam (control and problem samples, 10 g) the same procedure was utilized with a key change: the liquid nitrogen was not used, and the jam was previously dissolved in 40 ml prewarmed TEN buffer (Tris 10mM, EDTA 10mM, NaCl 0.1 M) at 60°C, and filtrated through a blutex-like filter in order to enrich the sample in fruit tissue contents and to wash out sugars. Then, 0.2 g of filtrate were processed as described for standard samples. When necessary, an additional chloroform-Isoamyl alcohol (24:1) extraction was performed.

DNA quantification

Total isolated DNA was quantified and its purity determined by measurement of sample absorbance at 260 and 280 nm with a NanoDrop microvolume Spectrophotometer.

PCR amplification

Presence of inhibitors

In order to reveal the presence of PCR inhibitors, an internal positive control (IPC) was introduced, which should not be present in the extract, and that is amplified with a specific primers pair. The IPC amplification demonstrates the absence of PCR inhibitors.

The IPC added to the PCR reaction mix corresponds to a DNA isolated from cattle, and the specific primers used in this assay were:5´-GACCCCCAGCTCCATCAAACAT CTCATCTTGATGAAA-3´ (F-SIM) and 5´-AGAAAAGTGTAAGACCCGTAATATAAG-3´ (R-K), that amplify a cyt b gene 274 bp fragment. (Matsunaga *et al*., 1999). This PCR assay was performed in a final volume of 25 µl containing: 1 U Top *Taq* DNA Polymerase (Bioron), 2.5µl Buffer 10x Complete (Bioron), 0.25 µl 25mM dNTPs, 1 µl of each primer 10 µM, and 100 ng of jam samples DNA with the addition of 100 ng of cattle DNA as IPC. Amplification reactions were run under the following conditions: DNA denaturation at 94°C for 4min, followed by 30 cycles with: 20s at 94 °C; 20s at 64°C; 20s at 72°C and finally a primer thermal extension at 72°C for 7 min. Amplification products were resolved by 6% polyacrylamide gel-electrophoresis and were posteriorly stained with silver solution (Sanguinetti *et al*., 1994).

Chloroplast matk PCR for plant identification

Publicly available fruit matk DNA sequences were obtained from databases and aligned using Clustal W algorithm (Thompson *et al*., 1994). Two methods based on the amplification by PCR of this highly conserved chloroplast gene, and suitable for plant species identification in quince jam were developed during the course of this study. Here, the two strategies for the identification and differentiation of *Cydonia oblonga* (quince) and *Malus* sp. (apple) in this matrix are described.

The first strategy utilizes the forward primer matk AF (5´-TCTTACCGAATAGGTCCAAAAC-3´) and reverse primer matk AR (5´-GGCTCTACCCATTTATTCACTC- 3´), that amplify a 97 bp fragment (named "A region") from *Malus* sp. and another 88 bp fragment from *Cydonia oblonga* (Figure 1A). The second strategy utilizes the forward primer matk BF (5´-CTTCTCATTTACGATTAACC-3´) and reverse primermatkBR(5´-CCTTGAAGAACCATAAGATG -3´), that amplify a 129 bp (named "B region") fragment in both species (Figure 1B). PCR assays were performed in a final volume of 25 µl containing: 1 U Top *Taq* DNA Polymerase (Bioron), 2.5µl Buffer 10x Complete (Bioron), 0.25 µl 25 mM dNTPs, 1 µl of each primer 10 µM, and 100 ng of DNA.

Amplification reactions were run under the following conditions: DNA denaturation at 94°C for 2min, followed by 34 cycles with: 30s at 94 °C; 30s at 50°C; 1min at 72°C and finally a primer thermal extension at 72°C for 10 min. Amplification products were analyzed as in 4.1, with the following modification: a 12% polyacrylamide gel was utilized instead of 6%.

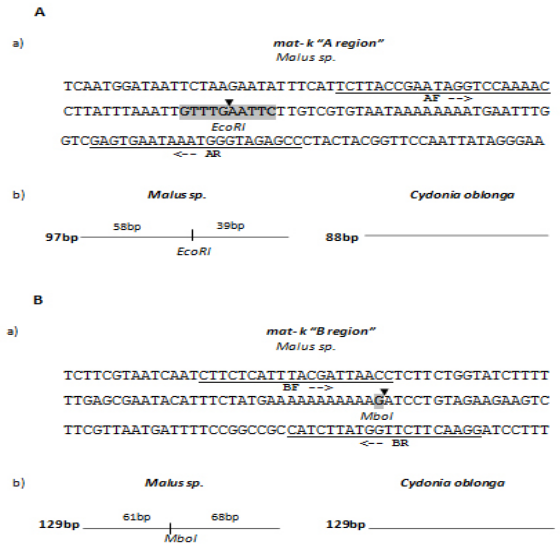

Figure 1. A a) Schematic representation of the A region of mat-k gene (GenBank: DQ860466.1) from *Malus* sp. PCR primers are indicated with an horizontal arrow, and specific restriction sites are shown with a vertical arrow. Gap in corresponding Cidonya oblonga DNA sequence is shown in grey. b) Predicted EcoRI restriction pattern of chloroplast mat-k A region in apple (left), and quince (right). PCR amplicons are 97 and 88 bp long for apple and quince respectively. EcoRI restriction site and its relative position is indicated above the *Malus* sp. DNA fragment. *Cydonia oblonga*, mat-k A region does not have an EcoRI restriction site " I " indicates restriction site location. B a) Schematic representation of the B region of mat-k gene from *Malus* sp. PCR primers and gap present only in *Cidonya oblonga* are indicated as in A. b) Predicted MboI, restriction pattern of chloroplast mat-k B region. The full length of the PCR product is 129bp for both species. Restriction site and their relative position, is indicated above the *Malus* sp. DNA fragment. Cydonia oblonga, mat-k B region does not have an MboI restriction site " I " indicates the location of the restriction sites.

Restriction enzyme digestion (PCR-RFLP)

When necessary, the resulting PCR products were digested with appropriate restriction enzymes, and fragments were resolved in a polyacrilamide- gel electrophoresis. The restriction enzymes only digest the PCR product from apple (*Malus* spp) allowing its differentiation from quince (*Cydonia oblonga*) (Figures 1A and 1B).

10 µl of PCR product (obtained with primers AF-AR), were digested with 1.5 units of EcoRI (Fermentas) in 20 µl final volume for 1 h at 37°C. The same volume of PCR product (obtained with primers BF-BR) was digested with 1.5 units of MboI (Fermentas) under the same conditions.

Results

DNA extraction

Many commercial kits were assayed to obtain DNA from the different quince jam samples, and the one that yielded better results was: QIAamp DNA

Table 1. DNA spectrophotometric analysis. Data of absorbance at 260 and 280nm and calculated DNA concentrations from some representative samples (standard leaves and problem jams). Fresh fruit leaves yielded the highest amounts of DNA, whereas jams (with the highest degree of processing) yielded the lowest amounts of DNA. The modified CTAB method yielded better results in either quantity or quality in all analyzed samples, with the exception of problem quince jam II, where QIAamp DNA Stool Minikit showed a better performance

Sample	*CTAB/PTB*			*Stool Minikit*		
	Abs260nm	**[ADN] ng/µl**	**Abs 260/280**	**Abs260nm**	**[ADN] ng/µl**	**Abs 260/280**
Problem quince jam (I)	0.889	44.4	2.0	0.609	30.47	1.06
Problem quince jam (II)	1.066	53.3	1.54	1.097	54.8	1.45
Apple leaves (Apple Standard)	3.412	170.5	2.0	2.099	105	1.32
Quince leaves (Quince Standard)	2.406	120.2	1.95	0.874	43.6	1.2

Stool Minikit (QIAGEN, Milano, Italy). In turn, this commercial kit was compared with the modified CTAB method (Doyle and Doyle, 1987) in relation to both yield and quality/amplificability.

Quantity and quality of DNAs obtained from fruit leaves and jams were evaluated by spectrophotometric analysis. Data of DNA concentration obtained by the different methods evaluated are shown in Table 1. As expected, the fresh fruit leaves yielded the highest amounts of DNA, whereas jams with the highest degree of processing, yielded the lowest amounts of DNA. The modified CTAB method yielded better results in either quantity or quality in all analyzed samples, with the exception of problem quince jam II, where QIAamp DNA Stool Minikit showed a better performance.

Amplificability and presence of inhibitors

The presence of inhibitors in jams was verified using cattle total DNA as an internal PCR control (IPC), that was amplified with a primer pair designed to target the mitochondrial cytb gene (Matsunaga *et al*., 1999). Inhibition tests showed IPC amplification in fresh leaves samples notwithstanding the method used for its extraction. Concerning the DNA extracted from the different jams, the modified CTAB protocol produced better results in PCR reactions (Figure 2).

"A region" amplification with matk AF-AR primers and EcoRI digestion

PCR amplifications performed on DNA from quince and apple fresh leaves (standard samples and positive PCR controls), yielded the two expected bands, 88 and 97 bp, respectively. PCR reactions in quince and apple jam DNA produced the same amplicon sizes, but with lowest performance.

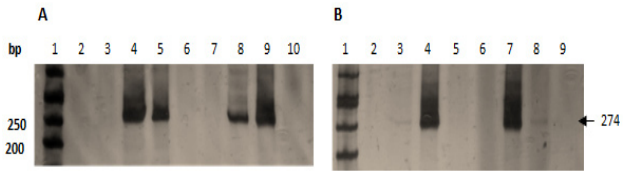

Figure 2. IPC PCR reactions for samples extracted with the modified CTAB protocol (left) and Stool MiniKIt (right). Silver stained polyacrylamide 6% gels A: lane 1- 100bp molecular ladder, lane 2- (not loaded), lane 3- Control quince jam (100% quince), lane 4- Problem quince jam I, lane 5- Control quince jam, (50% quince-50%apple), lane 6- Problem quince jam II, lane 7- Problem quince jam III, lane 8- Problem quince jam IV, lane 9- PCR positive control (IPC only, no jam) , lane 10- negative PCR control. B: lane 1- 100bp molecular ladder, lane 2- Control quince jam, (100% quince), lane 3- Problem quince jam I, lane 4- Control quince jam, (50% quince-50% apple), lane 5- Problem quince jam II, lane 6- Problem quince jam III, lane 7- Positive control (IPC only, no jam), lane 8- Problem quince jam IV, lane 9- negative PCR control. The amplified fragment for Internal Positive Control is 274 bp long

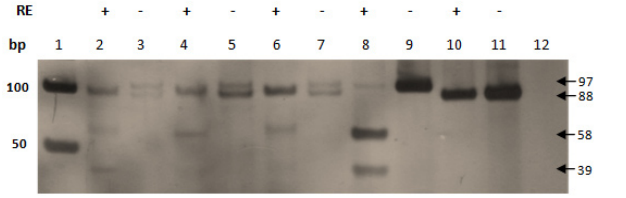

Figure 3. PCR-RFLP patterns from mat-k A region, obtained after amplicon digestions with EcoRI: lane 1- 50 bp molecular ladder, lane 2,3- Control quince jam (50% quince-50%apple), lane 4,5- Problem quince jam I, lane 6,7- Problem quince jam IV, lane 8,9- apple standard (apple positive control), lane 10,11- quince standard (quince positive control), lane 12- negative PCR control. The amplified fragment in quince is 88 bp long, while in apple is 97 bp. The digestion reaction is specific for *Malus* sp, and generates two smaller fragments 58 and 39 bp long. + : EcoRI digestion. - : no digestion

Posterior amplicon digestions with EcoRI restriction enzyme clearly showed the expected two smaller size DNA fragments (58 and 39 bp) in problem jam samples and apple leaves (Figure 3).

"B region" amplification with matk BF-BR primers and MboI digestion

A single fragment of 129 bp was clearly visible in gels loaded with PCR amplifications performed with DNA extracted from the same samples. Gel analysis of MboI digested amplicons confirmed the presence of the expected DNA fragments: two fragments, 61 and 68 bp long (Figure 4). It was observed a single band of about 129 bp similar to the resultant positive control. However, not all MboI digestions revealed the two bands pattern with smaller molecular sizes. In lane 2, where the resulting digestion of the amplicon obtained from DNA extracted from a control quince jam, with known composition (100% quince) was loaded, there was only one 129 bp band. In contrast, in lanes corresponding to the digestion of problem jams (I, IV and V), as in the quince jam with known composition 50% apple – 50% quince, it was observed the 129 bp band and two lower molecular

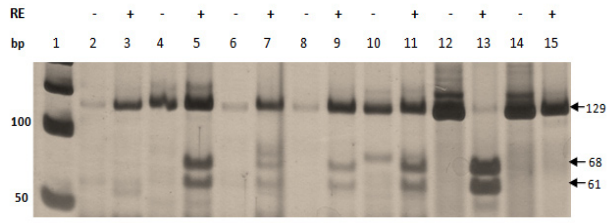

Figure 4. PCR-RFLP patterns for B region in mat-k, obtained after amplicon digestion with MboI: lane 1- 50 bp molecular marker, lane 2,3- Control quince jam (100% quince), lane 4,5- Control quince jam (50% quince-50%apple) PCR, lane 6,7- Problem quince jam I, lane 8,9- Problem quince jam IV, lane 10,11- Problem quince jam V, lane 12,13- apple standard (apple positive control), lane 14,15- quince standard (quince positive control). The amplification in both species results in 129 bp. The digestion reaction is specific for *Malus* sp., and generates two (61 and 68 bp long) smaller fragments. +: MboI digestion. -: no digestion

weight bands (68 and 61 bp), that would correspond to the product of digestion of the amplified fragment for apple species.

To further confirm the presumptive species present in problem jams, a cloning and sequencing of amplicon DNAs from these samples was performed. DNA sequencing data confirmed the presence of apple DNA in problem quince jams (data not shown).

Discussion

A molecular analysis of two matK regions was carried out, in order to discriminate two closely related plant species, quince and apple, in a complex matrix as quince jam. From initial investigations of plastid regions (Kress *et al*., 2005; Chase *et al*., 2007), the coding gene matK has emerged as one of the leading candidates as a barcoding system in plants (Ledford, 2008), as this gene shows a rapid substitution rate compared to other chloroplast coding regions (Chase *et al*., 2007; Hollingsworth, 2008). A single nucleotide variable site and a 10 bp gap, present in the aligned DNA sequences, allowed a unique identification procedure for quince and apple species. This information was used to identify flanking conserved DNA sequences suitable for primer design (Figure 1).

Two different DNA extraction methods were compared: QIAamp DNA Stool Minikit (Qiagen), the commercial kit that in our hands yielded better results, and modified CTAB method (Doyle and Doyle, 1987) as a cheap alternative to the commercial kits (Table 1). The modified CTAB method yielded a higher DNA quantity when working with jam samples. The amount of DNA recovered clearly depended on the type of matrix and on the level of sample processing.

Low DNA yield and failed PCR amplifications by using the commercial kit could be due to the presence of Maillard products in the DNA extracts or impurities such as polyphenolics and polysaccharides, including pectins. DNA extraction with PTB cleaves glucose-derived protein cross-links and helps to release the DNA that might have been trapped within sugar-derived condensation products. (Di Bernardo *et al*., 2005). These methods seem to be most suitable for removing impurities and releasing DNA from quince jams, which is evident examining the results of DNA concentrations and purities obtained with both methods (Table 1).

It often happens that the sample to be analyzed is a complex matrix that can contain chemical compounds able to interfere with the DNA polymerase enzyme activity. Enzyme inhibition can thus bring about a false negative result. (Lauri *et al*., 2008). By means of the inhibition assay (Figure 2) could be demonstrated the efficiency/inefficiency of the DNA obtained using different protocols in relation to its amplificability. The inefficiency is directly related to the low integrity of DNA, due to the treatment during jam manufacturing and the possible presence of secondary compounds (i.e. pectins) that inhibit enzymatic reactions (Di Bernardo *et al.*, 2005; Turci *et al*., 2010).

All samples were subjected to this inhibition assay. The results showed that the control quince jam (100% quince), and the problem quince jams II and III, inhibited the PCR reaction. To overcome this problem, DNA from these samples was re-extracted with an additional chloroform-isoamylic alcohol step. This DNA samples then showed no inhibition, when analyzed by PCR using AF-AR and BF-BR primer sets and subsequent digestion with EcoRI and MboI restriction enzymes. This analysis showed the presence of apple species in problem samples II and III (data not shown).

In summary, two molecular methods based on the amplification by PCR and RFLP analysis of a highly conserved chloroplast gene, *maturase-k* (matk), suitable for apple identification in quince jam were developed during the course of this study. First, by using primers AF-AR and simply displaying PCR products in a 12% polyacrylamide gel (Figure 3), it was possible to detect the presence of two distinct species (quince and apple) in control and problem jams. For a complete analysis and confirmation a digestion step with EcoRI enzyme, which cleaves DNA coming only from one of the species (apple) was implemented. A similar result was obtained by PCR and RFLP analysis using primers BF-BR and the restriction enzyme MboI (Figure 4). A further confirmation and method validation for the presence of quince and apple species in quince jams was performed by amplicon cloning and recombinant

plasmid automated DNA sequencing (data not shown). All problem quince jams analyzed using this strategy showed the presence of the two species: *Cydonia oblonga* (quince) and *Malus* sp. (apple).

Conclusion and perspectives

The results obtained in this study represent an advance in DNA methodologies for the detection of fraud and/or contaminants in complex food matrices. It also a first attempt for the determination and identification of plant species in quince jams.

There are three essential factors that determine the success of the method of detection of species by PCR: quantity, quality, and purity of extracted DNA. These properties depend directly of the DNA extraction method. Through this work, it was found that conventional methods for DNA extraction from vegetable matrices are not useful for this type of complex matrix that involves a tough elaboration process. However, a DNA extraction method suitable for this type of food matrix was developed, using reagents such as PTB and CTAB, and followed (if necessary) by a re-extraction with chloroform-Isoamyl alcohol, which minimize the amount of contaminants that co-precipitate with DNA (i.e. pectins) and reduce the ability of PCR inhibition. This last point resulted to be most critical, because it was only with this latter technique that we achieved the detection, discrimination and identification of both quince and apple plant species contained in quince jams.

All the strategies developed, involved a qualitative analysis of the plant species present in the analyzed food matrices. However, the next stage in the development and implementation of these methods of molecular traceability is the application of quantitative methodologies such as real-time PCR.

Acknowledgements

Biochemistry Lab at Faculty of Sciences, UdelaR; C.S.I.C., UdelaR; Laboratorio de Bromatología, I.M., DI.GE.GRA., M.G.A.P., Limay SRL.

References

Andrade, P. B., Carvalho, A. R. F., Seabra, R. M. and Ferreira, M. A. 1998. A previous study of phenolic profiles of quince, pear, and apple purees by HPLC diode array detection for the evaluation of quince puree genuineness. Journal of Agricultural and Food Chemistry 46: 968−972.

CBOL Plant Working group. 2009. A DNA barcode for land plants. Proceedings of the National Academy of Sciences of the United States of America 106-31: 12794–12797.

Chase, M.W., Cowan, R.S, Hollingsworth, P.M., Van den Berg, C., Madriñán, S., Petersen, G., Seberg, O., Jorgsensen, T., Cameron, K.M., Carine, M., Pedersen, N., Hedderson, T.A.J., Conrad, F., Salazar, G.A., Richardson, J. E., Hollingsworth, M.L., Barraclough, T. G., Kelly, L. and Wilkinson, M. 2007. A proposal for a standardised protocol to barcode all land plants. Taxon 56: 295–299.

Cordella, C., Moussa, I., Martel, A.C., Sbirrazzuoli, N. and Lizzani-Cuvelier, L. 2002. Recent developments in food characterization and adulteration detection: Technique-oriented perspectives. Journal of Agricultural and Food Chemistry 50: 1751-1764.

Di Bernardo, G., Galderisi, U., Cipollaro, M. and Cascino, A. 2005. Recent developments in food characterization and adulteration detection: Technique-oriented perspectives. Methods to improve the yield and quality of DNA from dried and processed figs. Biotechnology Progress 21: 546-549.

Di Pinto, A., Forte, V.T., Guastadisegni, M.C., Martino, C., Schena, F.P. and Tantillo, G. 2007. A comparison of DNA extraction methods for food analysis. Food Control 18: 76–80.

Doyle J. J. and Doyle J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Fazekas, A.J., Burgess, K.S., Kesanakurti, P.R., Graham, S.W. and Newmaster, S.G. 2008. Multiple multilocus DNA Barcodes from the plastid genome discriminate plant species equally well. Plos one 3 (7): e2802. doi:10.1371/journal.pone.0002802

Hollingsworth, P.M. 2008. DNA barcoding plants in biodiversity hot spots. Progress and outstanding questions. Heredity 101: 1–2.

Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A. and Janzen, D.H. 2005. Use of DNA barcodes to identify flowering plants. P.N.A.S. 102-23: 8369–8374

Laureiro, L., Giménez, J. and Balatti, A. 2009. Estado actual y perspectivas de la producción e industrialización del membrillo en Uruguay. Universidad de la República, MSc. thesis.

Lauri, A. and Mariani, P. O. 2008. Potentials and limitations of molecular diagnostic methods in food safety. Genes and Nutrition 4: 1–12,

Ledford, H. 2008. Botanical identities: DNA barcoding for plants comes a step closer. Nature 451: 616.

Lockley, A. K. and Bardsley, R. G. 2000. DNA-based methods for food authentication. Trends in Food Science and Technology, 11(2): 67–77.

Luykx, D.M.A.M and Van Ruth, S.M. 2008. An overview of analytical methods for determining the geographical origin of food products. Food Chemistry 107: 897–911

Matsunaga, T., Chikuni, K., Tanabe, R., Muroya, S., Shibata, K., Yamada, J. and Shinmura, Y. 1999. A quick and simple method for the identification of meat species and meat products by PCR assay, Meat Science 51: 143-148.

Ortola-Vidal, A., Schnerr, H., Rojmyr, M., Lysholm, F. and

Knight, A. 2007. Quantitative identification of plant genera in food products using PCR and Pyrosequencing technology. Food Control 18: 921–927.

Sanguinetti, C. J., Días Neto, E. and Simpson, A. J. 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17: 914-921.

Silva, B. M., Andrade, P. B., Mendes, G. C., Valentão, P., Seabra, R. M. and Ferreira M. A. 2000. Analysis of Phenolic Compounds in the Evaluation of Commercial Quince Jam Authenticity. Journal of Agricultural and Food Chemistry 48 (7): 2853–2857.

Taberlet, P. , Gielly, L. and Bouvet, J. 1992. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology, 17: 1105–1109.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 1994: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research. 22: 4673-4680.

Turci, M., Savo Sardaro, M. L., Visioli, G., Maestri, E., Marmiroli, M. and Marmiroli, N. 2010. Evaluation of DNA extraction procedures for traceability of various tomato products. Food Control 21: 143–149.

Weising, K, Nybon, H., Wolff, K. and Kahl, G. 2005. DNA fingerprinting in plants: Principles, methods, and applications. Second Edition, p. 81-107. Boca Raton: Taylor and Francis group.

Yamamoto, T., Kimur, T., Soejima, J., Sanada, T., Ban, Y. and Hayashi, T. 2004. Identification of Quince varieties using SSR markers developed from Pear and Apple. Breeding Science 54: 239-244.