

Evaluation of DNA extraction methods from green and roasted coffee beans

Stelios Spaniolas ^{a,*}, Maroussa Tsachaki ^b, Malcolm J. Bennett ^{c,d}, Gregory A. Tucker ^{a,d}

^a Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leics LE12 5RD, UK

^b Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leics LE12 5RD, UK

^c Division of Plant Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leics LE12 5RD, UK

^d Authentigene Ltd, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leics LE12 5RD, UK

Abstract

This paper describes a generic approach to the evaluation of DNA extraction methodology using green and roasted coffee beans as a model commodity. The use of *Design-Expert*[®] software was used in the design of experimental work to compare and optimize yields using a variety of commercial DNA extraction kits. The quality of the extracted DNA in terms of PCR inhibitor content is assessed and a judgment made that GeneSpin represents perhaps the best methodology in this instance. Coffee is a major commercial crop and there is the potential for fraudulent adulteration of the more expensive Arabica with Robusta beans. It is demonstrated that the DNA extracted from both green and roasted beans could be used in a PCR–RFLP based analysis to differentiate between Arabica and Robusta types of coffee.

Keywords: Coffee authentication; DNA extraction; DNA quantification

1. Introduction

There is a major requirement nowadays for robust quantitative and qualitative detection methods for the authenticity of foods, particularly at the level of species/variety, and DNA-based techniques often represent the most appropriate approach. Various DNA techniques have been described for food authentication (Lockley & Bardsley, 2000; Popping, 2002). Most, if not all, of these techniques rely at some stage on the polymerase chain reaction (PCR) and as such are reliant on the extraction of high quality, intact DNA from the food material. This can often represent the crucial step in an authentication process, especially if the food commodity under study has been processed in any way. There are usually two problems that have to be overcome during the extraction step. The

first is to maximize the DNA yield. This is particularly important when the authentication process is designed to monitor low levels of possible contamination, such as the legal level of genetically modified material. The second is to ensure that the extracted DNA is amenable to several enzymatic treatments such as PCR amplification and/or restriction fragment length polymorphism digest (RFLP). Consequently, the food analyst requires a robust DNA extraction protocol which provides the highest DNA yield with the lowest amount of PCR/restriction digest inhibitors. This requirement has been well established for the quantitative detection of genetically modified foods (Zimmermann, Luthy, & Pauli, 1998). Therefore, for any food authentication processes it is recommended that a screening and comparison test among different DNA extraction protocols has to be carried out (Csai et al., 1998).

The food commodity studied in this work was coffee and extraction procedures were examined for both green and roasted beans. Coffee is one of the most popular hot beverages worldwide and is of immense socio- economic

importance. It constitutes a significant agricultural export commodity in more than 50 developing countries in Africa, Asia and Latin America. The economically important genus – *Coffea* L. – consists of approximately 100 species so far identified – the most commercially important ones being *C. arabica* L. and *C. canephora* Pierre (*C. robusta*). The Arabica coffees represent over 70% of the world's coffee production and are considered superior because they have a finer flavour and better quality. Therefore, they are sold at approximately 2–3 times the price of the Robustas and some Arabicas from certain geographical origins can attract prices at even 10 times higher (Rubayiza & Meurens, 2005). As a result, fraudulence in coffee products can be considered as an issue of substantial economic importance and therefore consumers should be protected from the likelihood of mis-representation. Towards that direction, an analytical approach based on PCR–RFLP with a lab-on-a-chip capillary electrophoresis has been developed, which is able to detect contamination of Arabica green beans with Robusta (Spaniolas, May, Bennett, & Tucker, 2006).

Recently, the extractability of DNA from roasted and instant coffee and its amenability for PCR amplification was demonstrated. This study tested several commercial extraction kits using a range of starting material dependant on the kit used. The results demonstrated variations in both yields and amenability to PCR (Martelossi, Taylor, Lee, Graziosi, & Donini, 2005). In this present study we have used *Design-Expert*® software to optimize DNA extraction techniques, in terms of both method and amount of starting material, using minimal sample numbers. This approach may represent a generic technique for the optimization of DNA extraction for any commodity.

2. Materials and methods

2.1. Coffee material and methods

Green coffee beans used in this study were kindly provided by Mercanta Ltd “The Coffee Hunters”. Roasted coffee beans were purchased from local shops. Green and roasted beans from *C. arabica* (Café de Cuba) were used in the DNA extraction comparison. Beans from Robusta *C. canephora* (India Mysore Robusta A) were also used in the restriction enzyme assays. All chemicals were purchased from Fisher Scientific Ltd (Loughborough, UK) unless stated otherwise.

2.2. DNA extraction

Coffee beans (20 g) were ground in a milling machine (Glen Creston Ltd, Stanmore UK) using a mesh of 2.0 mm. The commercial DNA extraction kits used were the *DNeasy*® *Plant Mini kit* (Qiagen Ltd, Crawley UK), *GenElute*™ *Plant Genomic DNA Miniprep kit* (Sigma-Aldrich, Dorset UK), *GeneSpin* (GeneScan Analytics GmbH, Germany), *Nucleon PhytoPure Plant DNA extraction kit* (Amersham Biosciences Ltd, Buckinghamshire

UK) and *Wizard*® *Magnetic DNA Purification System for Food* (Promega Corporation, Southampton UK). In addition, a cetyl-trimethyl-ammonium bromide (CTAB) based protocol was also used (Woolley, James, & Manning, 2001). The manufacturers' instructions and published protocols were followed for each of the extraction methods, accordingly. Following extraction, all samples were run on a 1% agarose (Melford Laboratories Ltd, Ipswich UK) electrophoresis gel run in TAE buffer according to standard electrophoresis procedures (Sambrook, Fritsch, & Maniatis, 1989), along with either a 100 bp or 1 kb DNA ladder (Promega Corporation, Southampton UK) as appropriate, stained with ethidium bromide (Invitrogen Corporation, Paisley UK) and visualized.

2.3. DNA quantification

All extracted DNA samples were quantified in triplicate by a fluorescence-based spot array using SYBR® Green I and a gel imaging system (Vitzthum, Geiger, Bisswanger, Brunner, & Bernhagen, 1999). A 10 µL DNA sample containing SYBR® Green I was spotted onto a plastic surface and the whole array was visualized in the Fluoro-S Multi-Imager (Bio-Rad Laboratories, Hertfordshire UK). Lambda DNA (Promega Corporation, Southampton UK) was used for the standard curve and quantification of the DNA samples was carried out using the *Quantity One*™ software (Bio-Rad Laboratories, Hertfordshire UK). In the case of the PCR inhibitory effect, PCR amplicons were pre-stained with SYBR® Green I, electrophorized on a 1.5% agarose gel, run in TAE buffer, and visualized on the Multi-Imager as above. Fluorescence intensities were then measured by using the *Quantity One*™ software (Bio-Rad Laboratories, Hertfordshire UK) and analyzed statistically as described below.

2.4. PCR amplification

PCR was performed in 5.0 µL of 10× *AmpliTaqGold*® buffer, 5.0 µL of 25 mM *MgCl*₂, 1.0 µL dNTPs mixture (10 mM each), 1.0 µL of *AmpliTaqGold*® Polymerase (Applied Biosystems, Warrington UK), 1.5 µL (10 pmol) of each primer (MWG-Biotech GmbH Germany) (Table 1), 2.0 µL (diluted with nuclease free water in a ratio of 1–5 in the case of roasted coffee) of total genomic DNA and was made up to 50 µL final volume with nuclease free water (Sigma-Aldrich, Dorset UK). PCR conditions were 95 °C for 10 min, 40 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min and 1 cycle at 72 °C for 10 min. All PCR amplifications were carried out in an *AB9700* thermocycler (Applied Biosystems, Warrington UK).

2.5. PCR inhibitory effect

Yeast DNA was extracted with the *Easy-DNA*™ kit (Invitrogen Corporation, Paisley UK) according to manufacturer's instructions. PCR inhibitory effects were moni-

Table 1
General information about the PCR primers used in this study

Name	Sequence 5'–3'	Target and accession number	Approximate length in base pairs (bp)	Application	Source
Tab-e	GGTTCAAGTCCCTCTATCCC	trnL-trnF intragenic region of chloroplast DNA (U93387 and U93393)	435	PCR prior to restriction digest	Taberlet et al. (1991)
Tab-f	ATTTGAACTGGTGACACGAG				
Coffea1-F	AATCGATCTGGACGGAAAAGC	trnL-trnF intragenic region of chloroplast DNA (U93387 and U93393)	251	Ability of extracted DNA for PCR amplification	This study
Coffea1-R	AGCATCCTCATTTTATGAGAAAAGG				
Spo7a-F	TTGCTGAGGGAATTCGGAAA	Protein of unknown function required for sporulation in yeasts (M36073)	401	PCR inhibition effect on yeast DNA	This study
Spo7a-R	GTAGCCCGCCTGCGAGGC				

tored using a PCR containing 1.5 µL 10× Taq buffer (Promega Corporation, Southampton UK), 1.5 µL of 25 mM MgCl₂, 0.3 µL dNTPs mixture (10 mM each), 0.5 units of Taq Polymerase (Promega Corporation, Southampton UK), 0.75 µL (10 pmol) of each primer (Table 1), 1.5 µL of coffee DNA extract, 0.3 µL of yeast (*Saccharomyces cerevisiae*) DNA template, and made up to 15 µL with nuclease free water. PCR conditions were 94 °C for 5 min, 22 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min and 1 cycle at 72 °C for 7 min.

2.6. Restriction fragment length polymorphism

PCR amplicons were purified using the QIAquick PCR purification kit (Qiagen Ltd, Crawley UK) according to manufacturer's instructions. Amplicons (17.5 µL) were mixed with 2.0 µL of enzyme buffer and 0.5 µL *Pvu*I restriction enzyme (Fermentas GmbH Germany) and digested at 37 °C for 60 min. The resultant fragments were visualized after electrophoresis in a 1.5% agarose gel, run in TAE buffer as above, and stained with ethidium bromide (Invitrogen Corporation, Paisley UK).

2.7. Experimental design and data analysis

The DNA yield experiments were designed, and analysed, using *Design-Expert*® software package version 6.0.6 (Stat-Ease Inc, Minneapolis, Minnesota). This employed a one factor response surface design and used ANOVA to generate a response surface cubic model. Five levels of a single factor (20, 77.5, 135, 192.5, 250 mg of coffee powder) plus replicates were performed for every categorical factor (extraction kit) to allow lack of fit and pure error determination for the model. Analysis of the data was also carried out with the SPSS statistical package by employing two-way univariate ANOVA. Both Bonferroni and Tukey's tests were applied for Post Hoc multiple comparisons, but only *p*-values from the latter are stated. For the PCR inhibitory effect generated by roasted beans a *t*-test for the equality of means was used.

3. Results and discussion

3.1. DNA extraction

Green and roasted *C. arabica* beans (Café de Cuba) were ground to a powder and optimal DNA extraction methodologies investigated. A range of kits were employed—*DNeasy*® Plant Mini kit, *GenElute*™, *GeneSpin*, *Nucleon PhytoPure Plant DNA extraction kit* and *Wizard*® Magnetic DNA Purification System for Food— in each case. In addition, a CTAB based protocol was also used. *Design-Expert*® software was employed to design an experiment in order to investigate the interaction between the “DNA extraction method” and “amount of coffee powder”. The program suggested duplicate extractions for 20, 135 and 250 mg samples and single extractions from 77.5 to 192.5 in order to determine extraction efficiency by the various methods for powders between 20 and 250 mg of powder. This range was based on that which the kit manufacturers' suggest in their instructions. All of the methods, especially those which were not column/filter-based, gave DNA solutions that were both coloured (greenish for green coffee, yellow to dark brown for roasted coffee) and viscous. The extracted DNA was quantified (%CV = 2.5) and expressed as total DNA in µg (green coffee) or ng (roasted coffee) (Fig. 1). The coefficient of variation (%CV) of the model, as given from the analysis with the *Design-Expert*® software, was 9.04 and 7.6 for green and roasted coffee, respectively. It was evident that the amount of DNA extractable from green beans was generally much higher than that from roasted, presumably reflecting the degradation of DNA in the latter during the roasting process. It was also interesting to note that for green beans the methods, especially *GeneSpin*, yielded the highest amounts of DNA and CTAB the lowest, whilst for roasted powder CTAB gave the greatest yield and *GeneSpin* was among a group along with *Nucleon PhytoPure* and *Wizard*® with lower yields and in the case of the *GenElute*™ kit no detectable DNA was extracted from roasted coffee powder. *DNeasy*® gave reasonable yields, relative to the other

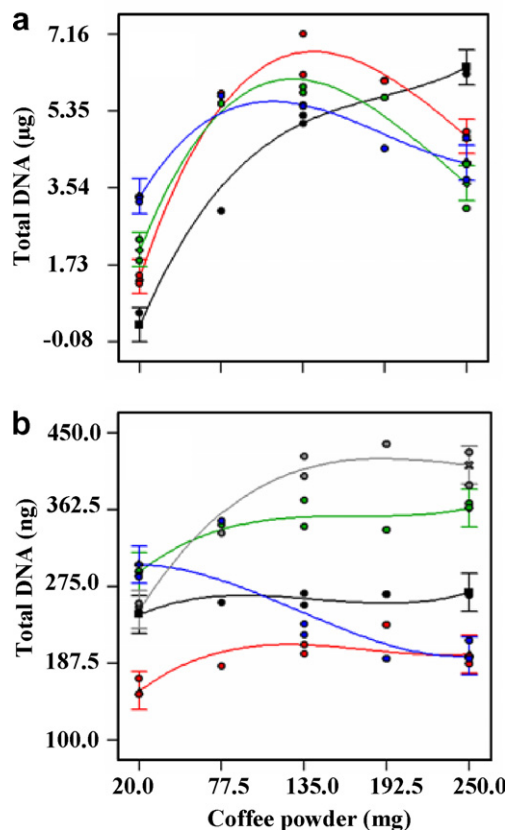


Fig. 1. An interaction graph illustrating the relationship between total DNA and mg of green (a) and roasted (b) coffee powder for each of the kits used in this analysis, as revealed by *Design-Expert*[®]; CTAB (gray), *Wizard*[®] (black), *GeneSpin* (red), *DNeasy*[®] (green) and *Nucleon PhytoPure* (blue). The bars at the end points of each line represent the least significant difference at the 95% confidence interval for that particular average value. If these confidence intervals are not overlapping, then the means are considered significantly different. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

methods, in both cases. Concerning the size of the extracted DNA, that did not seem to differ among the extraction kits and when analysed by agarose gel, its range was always above 10 kb (data not shown).

Design-Expert[®] was used to generate a surface response analysis in order to generate an interaction graph plot (Fig. 1). Since the DNA yields for both CTAB and *GeneElute*[™] used on green coffee powder were at such relatively low values these two methodologies were not included in any further studies. Statistical analysis using a two-way univariate ANOVA demonstrated that both the kit and the amount of starting material had a significant effect ($p < 0.001$) on the yield of DNA. There was also a significant interaction ($p < 0.001$) between the different kits and the mg of coffee powder used.

In the case of green coffee, optimum yields from all methods, except wizard, were obtained using 135 mg of starting material. The DNA yields obtained using *DNeasy*[®], *Wizard*[®] and *GeneSpin* were statistically the same ($0.21 < p < 0.99$). In the case of roasted coffee,

DNeasy[®] and the CTAB method revealed no statistical difference in yield ($p = 0.74$). It is worthwhile mentioning that in a previous study (Martellosi et al., 2005) it was shown that *DNeasy*[®] kit was unable to extract DNA from roasted beans although in the present study the opposite was demonstrated. These contradictory results may possibly suggest the significant effect of the material and especially of the roasting conditions on the DNA extraction step.

3.2. Quality of the extracted DNA

Extracted DNA was analyzed by gel electrophoresis in order to determine size distribution and potential “contamination” with RNA (data not shown). All of the extraction methodologies followed gave detectable DNA from the green beans, whereas in the case of roasted beans only the CTAB and *Nucleon PhytoPure* methods yielded sufficient DNA to be detected from the gel. In no case was there any observable “contamination” with RNA as evidenced by a lack of any detectable characteristic rRNA bands.

The presence of potential PCR inhibitors was tested for by examining the effect of adding DNA extracts from green or roasted coffee beans to a standard PCR targeted at a yeast amplicon (401 bp). This was carried out by setting up a range of PCR reactions containing yeast DNA, primers (specially designed for that species) and 10% (v/v) of coffee DNA extract. A similar approach has been previously used elsewhere for coffee extracts (Martellosi et al., 2005). The number of PCR cycles used was such that the end point of the reaction was in the middle of the exponential phase of the PCR, thus the amplicon could be quantified. Extracts from 135 and 250 mg of powder were tested for their PCR inhibitory effects. This was because 135 mg seems to represent the optimum and 250 mg the maximum extraction amounts, respectively. The results for green bean extracts are shown in Fig. 2. Extracts made from green coffee using the *GeneElute*[™], *DNeasy*[®] and *GeneSpin* kit had no significant effect on the PCR amplification ($0.08 < p < 0.15$). Those using *Nucleon PhytoPure* or the *Wizard*[®] for 250 mg of powder showed some inhibitory effects. The DNA extracted using the CTAB method was

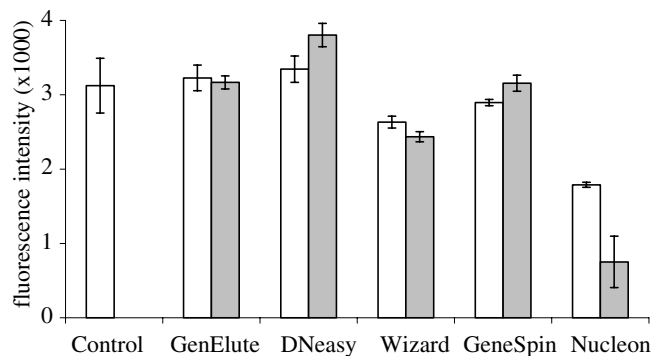


Fig. 2. Inhibition of the yeast PCR system by DNA extracts from 135 mg (white bars) and 250 mg (grey bars) of green beans. All the other extraction methods gave no, or very low, amplification signals.

not used because the amplification signal was very low. The results using extracts from roasted coffee were very different. The addition of undiluted DNA extracts to the yeast PCR resulted in total inhibition, as evidenced by the lack of any detectable amplicon (data not shown). The only exception was with the extract made using the *GeneSpin* kit. In this case 2 of the 6 replicates produced a detectable amplicon. This inhibition test was repeated using extracts that had been diluted with molecular grade water in a 1:1 ratio (Fig. 3). In this case all the extracts (6 at 135 mg and 6 at 250 mg) made using the *GeneSpin*, 2 out of 6 at 135 mg and 3 out of 6 at 250 mg of the extracts using the *DNeasy*[®] and 2 out of 6 at 135 mg and 4 out of 6 at 250 mg of the extracts made using the *Wizard*[®] kit gave detectable amplicons. All extractions made with either *Nucleon PhytoPure* or CTAB failed to give detectable amplicons even after dilution. It seems that, in the case of roasted coffee, the *GeneSpin* kit produced DNA with the highest quality in terms of ability to support PCR. The fact that extracts from roasted coffee exhibit severe PCR inhibition is perhaps not surprising given the nature of the material.

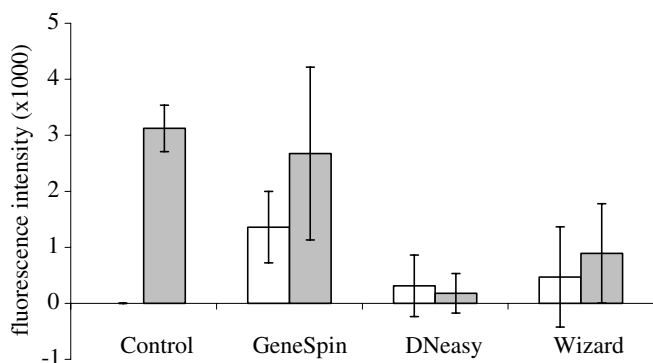


Fig. 3. Inhibition of the yeast PCR system by DNA extracts from 135 mg (white bars) and 250 mg (grey bars) of roasted beans, following a 1:1 dilution with water. All the other extraction methods gave no, or very low, amplification signals.

Given these results the *GeneSpin* kit was selected as potentially the best method for both green and roasted coffee. This does not imply that the other methods are not suitable but that they require modification to the manufacturers' instructions. Although it has been stated elsewhere that amplification of DNA from processed coffee with *Nucleon PhytoPure* was not possible (Martellossi et al., 2005), a treatment of the DNA solution with PVP-40 in a final concentration of 2% (v/v) was proven to be enough for PCR analysis (data not shown). Again, this might be due to different roasting conditions of the beans tested.

In the case of green coffee beans, 135 mg of extraction material seemed to give higher DNA yield, whereas in the case of roasted coffee it seemed that there was no statistical difference between 135 and 250 mg. As far as concerns the PCR inhibition effect, this seemed statistically higher when using 135 mg of green coffee powder, although each value from 135 to 250 mg did not seem to statistically differ from the control. Concerning the inhibition from roasted coffee, it is believed that the standard deviation is relatively big and therefore, apart from a subjective point of view, no concrete conclusions can be reached. However, to elucidate whether the use of 135 or 250 mg of starting material would be more appropriate, one should conduct an authentication experiment using two sets of coffee beans mixtures of Arabica spiked with Robusta in several ratios (w/w). Each set of mixtures should correspond to different weight of coffee powder and hence, the set that leads to better analytical performances should presumably indicate the optimum weight of starting material.

3.3. Coffee discriminatory test

Given the nature of coffee as a commodity it would be useful to be able to detect contamination of Arabica coffees with Robusta. A potential DNA target was identified based on a phylogenetic study, where a number of single nucleotide polymorphisms (SNPs) were found among several *Coffea* species, indicating the existence of different

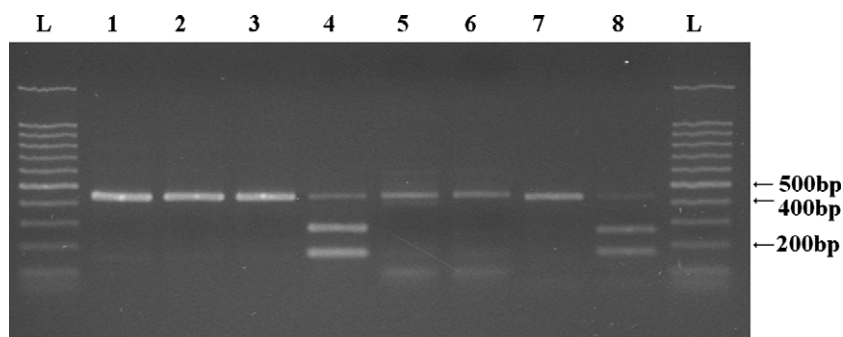


Fig. 4. Restriction digests of the PCR amplicons from the chloroplastic DNA target *trnL(UAA) - trnF(GAA)* from Arabica and Robusta coffee beans with the *PstI* restriction enzyme. (L) 100 kb DNA ladder, (1) Arabica green; undigested, (2) Arabica green; digested, (3) Robusta green; undigested, (4) Robusta green; digested, (5) Arabica roasted; undigested, (6) Arabica roasted; digested, (7) Robusta roasted; undigested, and (8) Robusta roasted; digested.

chlorotypes between Arabicas and Robustas (Cros et al., 1998). The selected DNA target was the chloroplast *trnL*(UAA) – *trnF*(GAA) intraspacer region which exhibits a single nucleotide polymorphism (SNP) that resides in a *PvuI* restriction site resulting in the site being present in Robusta but absent in Arabica. An experiment was thus carried out to test whether DNA extracted using the *GeneSpin kit* could be used directly for a PCR–RFLP analysis based on this SNP. DNA was extracted, using the *GeneSpin kit*, from green and roasted Arabica or Robusta coffee beans. This was then used as the template in a PCR targeted at the chloroplast *trnL*(UAA) – *trnF*(GAA) intraspacer region. The tab PCR primers used (Table 1) amplify approximately a 435 bp section of the above region, containing the *PvuI* restriction endonuclease site. The resultant amplicon was then incubated with the restriction endonuclease *PvuI* prior to analysis by gel electrophoresis. The results are shown in Fig. 4. The *PvuI* restriction site was located asymmetrically within the amplicon such that, in the presence of an intact restriction site, digestion with *PvuI* would result in two fragments of 170 and 265 bp, respectively. The Arabica coffee gave a single product of around 435 bp, indicating that, as expected, the *PvuI* restriction site has been disrupted by the SNP. In contrast the Robusta sample showed two smaller fragments at around 170 and 265 bp as expected. Although the roasted Robusta profile seems partially undigested, that was likely due to the participation of two chlorotypes in the coffee sample, as confirmed with SNaPshotTM assay (Spaniolas, Tsachaki, Bennett, Kalaitzis, & Tucker, 2007) and not due to partial inhibition of restriction digest.

4. Conclusions

In this work, an experimental design was successfully applied to compare and evaluate both quantitatively and qualitatively DNA extraction methods on green and roasted coffee. By employing a quantitative fluorescence-based spot array using SYBR[®] Green I and a gel imaging system (Vitzthum et al., 1999), it was demonstrated that DNA could be extracted from both green and roasted coffee beans using all the extraction procedures tested. Whilst DNA yield is an important factor, DNA quality is likely to be a more decisive factor, and it was found that of all the methods tested only the *GeneSpin kit* provided an extract from roasted coffee beans that did not show a 100% inhibitory effect on yeast PCR. As stated elsewhere (Martelossi et al., 2005), additional purification steps could be employed to enhance the quality of the DNA extracted by the other kits such that the PCR inhibitors are removed. Generally, the column-based methods were less laborious and provided DNA of higher quality. Among these, *GenElute*TM and *DNeasy*[®] involve the use of two kinds of columns, whereas *GeneSpin* had only one making it even less laborious. As a result, it seemed from the overall data

that *GeneSpin* might be the kit of choice particularly for roasted coffee. The DNA extracted with that kit could be employed in a PCR–RFLP based assay to differentiate between Arabica and Robusta coffee types (Spaniolas et al., 2006). However, the effectiveness of such an assay for the qualitative and quantitative analysis of coffees remains to be elucidated.

Acknowledgement

Mercanta Ltd “The Coffee Hunters” is acknowledged for providing all coffee material used in this work. This work was funded by the Greek State Scholarships Foundation (IKY).

References

- Cros, J., Combes, M. C., Trouslot, P., Anthony, F., Hamon, S., Charrier, A., et al. (1998). Phylogenetic analysis of chloroplast DNA variation in *Coffea* L. *Molecular Phylogenetics and Evolution*, 9, 109–117.
- Csaikl, U. M., Bastian, H., Brettschneider, R., Gauch, S., Meir, A., Schauerte, M., et al. (1998). Comparative analysis of different DNA extraction protocols: A fast, universal maxi-preparation of high quality plant DNA for genetic evaluation and phylogenetic studies. *Plant Molecular Biology Reporter*, 16, 69–86.
- Lockley, A. K., & Bardsley, R. G. (2000). DNA-based methods for food authentication. *Trends in Food Science & Technology*, 11, 67–77.
- Martelossi, C., Taylor, E. J., Lee, D., Graziosi, G., & Donini, P. (2005). DNA extraction and analysis from processed coffee beans. *Journal of Agricultural and Food Chemistry*, 53, 8432–8436.
- Popping, B. (2002). The application of biotechnological methods in authenticity testing. *Journal of Biotechnology*, 98, 107–112.
- Rubayiza, A. B., & Meurens, M. (2005). Chemical discrimination of arabica and robusta coffees by Fourier transform Raman spectroscopy. *Journal of Agricultural and Food Chemistry*, 53, 4654–4659.
- Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning: A laboratory manual*. New York: Cold Spring Harbour Laboratory Press.
- Spaniolas, S., May, S. T., Bennett, M. J., & Tucker, G. A. (2006). Authentication of coffee by means of PCR–RFLP analysis and lab-on-a-chip capillary electrophoresis. *Journal of Agricultural and Food Chemistry*, 54, 7466–7470.
- Spaniolas, S., Tsachaki, M., Bennett, M. J., Kalaitzis, P., & Tucker, G. A. (2007). PCR–RFLP and lab-on-a-chip technology as a means to authenticate roasted coffee beans. *Proceedings of the fifth international congress on food technology* (Vol. 2, pp. 365–370). Thessaloniki, Greece: Congress Center of HELEXPO.
- Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of 3 non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17, 1105–1109.
- Vitzthum, F., Geiger, G., Bisswanger, H., Brunner, H., & Bernhagen, J. (1999). A quantitative fluorescence-based microplate assay for the determination of double-stranded DNA using SYBR Green I and a standard ultraviolet transilluminator gel imaging system. *Analytical Biochemistry*, 276, 59–64.
- Woolley, L. C., James, D. J., & Manning, K. (2001). Purification and properties of an endo-beta-1,4-glucanase from strawberry and down-regulation of the corresponding gene, cell. *Planta*, 214, 11–21.
- Zimmermann, A., Luthy, J., & Pauli, U. (1998). Quantitative and qualitative evaluation of nine different extraction methods for nucleic acids on soya bean food samples. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung a-Food Research and Technology*, 207, 81–90.