Food Chemistry xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Adulterations in Basmati rice detected quantitatively by combined use of microsatellite and fragrance typing with High Resolution Melting (HRM) analysis

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ARTICLE INFO

Article history:
Received 29 November 2010
Received in revised form 14 March 2011
Accepted 29 April 2011
Available online xxxx

Keywords:
Adulteration
Basmati rice
Fragrance
High Resolution Melting
Microsatellites

ABSTRACT

The aim of this work was to setup a DNA based method coupled with High Resolution Melting (HRM) analysis for rice products traceability using five different microsatellite markers to genotyping Basmati and non-Basmati varieties. We also exploit the obtained information to detect the presence of Basmati varieties in commercial rice products. Additionally we used the 8 bp deletion in *badh2* gene in combination with HRM to both DNA-typing of the Basmati and non-Basmati varieties and to quantitate accurately adulteration of Basmati rice products with non-Basmati rice products. HRM proved to be a very sensitive tool to genotype rice varieties and detect admixtures as well as able to detect a ratio of 1:100 of non-fragrance in fragrance rice. In conclusion HRM analysis can be a higher resolution, cost effective, alternative method compared to other techniques that could be extended to quantify adulterations in rice varieties and commercial rice food products.

1. Introduction

It is of utmost importance to ensure that raw materials used in food processing and production meet the appropriate standards. This can only be achieved by instituting legislative measures to whom food ingredient suppliers, manufacturers, and retailers must comply to.

Basmati, an aromatic variety of rice of high quality, found in roots of mountain Himalayas and especially all over Pakistan and India. Most typical traits of Basmati are its distinct scent as well as an elongation of a scale of 1–1.5 when cooking (Bligh, 2000). In addition to the genotype the aforementioned scent is owed to specific cultivation practices and environmental conditions of the place (Lopez, 2008). None but few skilled technicians are capable of differentiating rice varieties since they are closely correlated. The UK Food Standards Agency (FSA; www.food.gov.uk/science/surveillance/fsis2004branch/fsis4704basmati) employed to inspect the authenticity of Basmati rice that is being promoted within its borders. European Union has classified certain Indian and Pakistan varieties as "Basmati" which is sold at a very high price in the market at a ratio of 4:1 to common rice (Lopez, 2008). Huge amounts of the rice are exported to USA and Europe alike. However, due to a potential gain, traders tend to heavily adulterate the crop with non-fragrant varieties (Lopez, 2008).

Detection of adulteration in Basmati rice is crucial to guarantee the authenticity of product to abide by the quality regulations of import countries. The occasional incorporation of non-Basmati rice substitutes in Basmati can occur during the production of Basmati rice and hence up to 7% non-Basmati rice may be present legally in 'Basmati rice' (agreed level of admixture in the UK Code of Practice. The presence of more than 20% non-Basmati in some Basmati rice samples reveals deliberate substitution, which unless stated, would be illegal under food labelling rules. Thus, precise quantification is a key point in combating such foul practices (www.food.gov.uk/science/surveillance/fsis2004branch/fsis4704Basmati).

Several methods are available to detect Basmati adulteration such as smelling of grains after boiling in water or treating with potassium hydroxide solution (Sood & Siddiq, 1978); chromatography analysis of aromatic compounds (Lorieux, Petrov, Huang, Guiderdoni, & Ghesquiere, 1996; Widjaja, Craske, & Wootton, 1996). But it is found that these methods are not reliable to detect Basmati adulteration properly. DNA based molecular markers such as microsatellites are, also, routinely used for germplasm identification including rice varieties due to their easiness and reproducibility and for their co-dominant inheritance and high polymorphism (Archak, Lakshminarayanareddy, & Nagaraju, 2007; Vemireddy, Archak, & Nagaraju, 2007). Recently, they have been successfully applied for traceability of sweet cherry, tomato, wheat and olive products (Ganopoulos, Argiriou, & Tsaftaris, 2011; Montealegre, Alrgre, & Garcia-Ruiz, 2010; Pasqualone, Alba, Mangini, Blanco, &

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doi:10.1016/j.foodchem.2011.04.109

Montemurro, 2010; Turci et al., 2010). Furthermore, microsatellite marker based molecular detection of Basmati adulteration is developed and found to be effective in detection of Basmati adulteration (Archak et al., 2007; Vemireddy et al., 2007). Usually, microsatellite analysis requires laborious polyacrylamide gels followed by silver staining or, for better resolution, fluorescently labelled PCR products and automated sequencers. In addition, the method requires post-PCR handling and dilution steps as well as a fluorescently labelled primer for each microsatellite, having as a consequence increases in time and cost of the analysis. Real-time PCR is used more and more frequently to analyse amplified DNA and identify viruses and pathogens and also as an extremely quick analysis for reactions that require no subsequent use of the amplified DNA (Ganopoulos et al., 2011).

In addition to microsatellite markers, a single allele, badh2.1, is the predominant allele in virtually all fragrant rice varieties today. The badh2.1 allele of the *fgr* gene encoding for the BAD2 or badh2 (betaine aldehyde dehydrogenase homologue 2) enzyme, reported an eight base-pairs (8-bp) deletion in exon 7 as the cause of fragrance in many of the fragrant rice varieties including Basmati and Jasmine rice. This deletion was suggested to be used for the distinction of Basmati from non-Basmati rice from several authors applying different genotyping methods (Bradbury, Fitzgerald, Henry, Jin, & Waters, 2005; Bradbury, Henry, Jin, Reinke, & Waters, 2005; Kovach, Calingacion, Fitzgerald, & McCouch, 2009; Sakthivel et al., 2009).

High Resolution Melting (HRM) analysis is an automated analytical molecular technique that measures the rate of double stranded DNA dissociation to single stranded DNA with increasing temperature. This dissociation is monitored by including a fluorescent dye in the PCR reaction that intercalates homogenously into DNA, and only fluoresces, when bound to dsDNA. The change in fluorescence measures the thermally-induced DNA dissociation by HRM and the observed melting behaviour is characteristic of the particular DNA product as determined based on sequence length, GC content, complementarity, and nearest neighbour thermodynamics (Reed & Wittwer, 2004). Increased resolution and precision of the instruments and the development of saturating DNA dyes facilitated the use of HRM for barcoding and genotyping (SNP, SSR markers) (Ganopoulos et al., 2011; Jaakola, Suokas, & Häggman, 2010; Mader, Lukas, & Novak, 2008; Wu, Wirthensohn, Hunt, Gibson, & Sedgley, 2008), for methylation analysis, as an alternative to gel electrophoresis, and for quantification of adulterants (Mader, Ruzicka, Schmiderer, & Novak, 2010). HRM could be used as an alternative technique to investigate microsatellites, especially for those laboratories that do not have immediate access to capillary sequencers (Ganopoulos et al., 2011; Mackay, Wright, & Bonfiglioli, 2008). The sensitivity of the method has already been widely demonstrated (Martino, Mancuso, & Rossi, 2010; Reed & Wittwer, 2004). Results are obtained without additional post-PCR processing in less than 2 h approximately. Herein, we describe a new application of HRM (A) for the rapid detection, quantification and adulteration of Basmati rice cultivars and their products and (B) for the *badh2* typing to detect the quantity of non-Basmati rice present in Basmati rice. The combined used of these two test provide a very fast and accurate quantitative detection of adulterations of Basmati rice with non-Basmati varieties.

2. Materials and methods

2.1. Plant and food material

Isolation of DNA was performed with 0.1 g starting material of fine powder of rice with Qiagen DNeasy plant mini kit according to the manufacturer's instructions. Different percentage mixes of Basmati and non-Basmati rice varieties were obtained by mixing, in the appropriate quantities, rice fine powder from Basmati 6131 and Axios cultivars. The DNA concentration was estimated by standard spectrophotometric methods at 260 and 280 nm UV length by an Eppendorf BioPhotometer and the integrity by gel electrophoresis in a 0.8% agarose gel. Samples were then diluted to 20 ng/mL work concentration.

2.2. PCR amplification

PCR amplification, DNA melting and end point fluorescence level acquiring PCR amplifications were performed in a total volume of 20 μL on a Rotor-Gene 6500 real-time 5P HRM PCR Thermocycler (Corbett Research, Sydney, Australia). The reaction mixture contained 20 ng rice genomic DNA, 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 300 nM forward and reverse primers (Table S2), 1.5 mM Syto® 9 green fluorescent nucleic acid stain, and 0.5 U Platinum Taq DNA polymerase (Invitrogen, Paisley, UK). A third generation DNA intercalating dye, Syto® 9, that at high concentrations can saturate all available sites within double stranded DNA was used. Syto® 9s' fluorescence provides a more accurate assessment of DNA melt status compared to SYBR Green I (Monis, Giglio, & Saint, 2005) and can be used to both monitor the accumulation of the amplified product during PCR and the subsequent product melting on the RotorGene 6000 (software version 1.7.87 Corbett Life Science, Cambridge, UK).

A rapid PCR protocol was conducted in a 36-well carousel using an initial denaturing step of 94 °C for 3 min followed by 45 cycles of 94 °C for 20 s, 55 °C for 20 s and 72 °C for 20 s, then a final extension step of 72 °C for 2 min. The fluorescent data were acquired at the end of each extension step during PCR cycles. Before HRM, the products were denatured at 94 °C for 5 s, and then annealed at 50 °C for 30 s to randomly form DNA duplexes.

HRM was performed as follows: premelt at the first appropriate temperature for 90 s, and melt at a ramp of 10 °C in an appropriate temperature range at 0.1 °C increments every 2 s. The fluorescent data were acquired at the end of each incremental step. End point fluorescence level was acquired following the melting process by holding at 60 °C for 5 min. In order to further increase the reproducibility and reliability of the HRM curve analysis (by obtaining similar amplified quantities of final PCR products before melting), finer adjustments, by diluting, were made to the genomic DNA templates obtained from the eight varieties, were no dilution was made to the DNA extracted from the food products. A Ct parameter of 33 ± 4 cycles at a threshold of 0.01 of the normalised fluorescence was established. All samples were examined in duplicate.

For verification of the validity of the method all PCR products were selected for fragment size analysis. Electrophoresis was performed with the Li-COR 4300 DNA analyzer. Results were analysed using Li-COR SAGA^{GT} software for all SSR loci.

In order to fragrance typing with HRM we selected a marker (BADEX7-5;(Sakthivel et al., 2009) which can discriminated by the 8 bp InDel polymorphism between fragrant (95 bp) and non-fragrant (103 bp) genotypes. The PCR products were separated by electrophoresis in 3.5% agarose gel. Real time PCR and *BAD2*-typing with HRM, were carried out on standard samples prepared by mixing fine powder from grains of 'Basmati-6131' with 'Axios' in different proportions of 1%, 5%, 7%, 10%, 15%, 30%, and 50%. PCR amplification was performed as described earlier. The difference plot curves of rice genotypes were generated in 3 steps: (1) normalisation, (2) temperature shift, and (3) difference plot. A base curve was chosen arbitrarily to create a more distinguishable plot, especially for those with similar melting curves (Li et al., 2010).

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2.3. Genotyping of PCR products by high-resolution melting analysis

The Rotor-Gene 6000 proprietary software (version 1.7.87) was used to genotype the different varieties and the food products. The negative derivative of fluorescence (F) over temperature (T) (dF/dT) curve primarily displaying the T_m, the normalised raw curve depicting the decreasing fluorescence vs increasing temperature, and difference curves (Wittwer, Reed, Gundry, Vandersteen, & Pryor, 2003) were mainly used. Furthermore, a 2-step procedure was followed to assess similarity of unknown HRM curves with a known one. First, the normalised HRM curve for the unknown type was compared to known normalised HRM profiles (genotypes Basmati T3 and Basmati 6122). These profiles are either generated together with the unknown samples, or more practically, have been previously produced. Second, the closest known HRM profile was selected as the difference graph control, and comparison of the difference curves was used to determine whether the unknown isolate was the same as or different from the known. For each data analysis, the digital filter was set to "heavy" and the replicate grouping option was selected.

Each cultivar was set as a 'genotype' (reference variety) and the average HRM genotype confidence percentages (GCPs) (value attributed to each variety being compared to the genotype, with a value of 100 indicating an exact match) for the replicates (disregarding the most outlying replicate) were tabulated (Hewson, Noormohammadi, Devlin, Mardani, & Ignjatovic, 2009). GCPs were re-coded from a 1–100 to a 1–20 range of values to decrease the number of different genotypes cause of/causing small differences in the shape of the melting curves giving slightly different GCPs (Ganopoulos et al., 2011). The means of the confidence percentage of the varieties replicates assigned to a representative genotype, together with the standard deviation were generated using Microsoft Office Excel.

3. Results

3.1. Microsatellite typing of rice varieties using HRM analysis

The use of HRM to differentiate varieties and by this rice product was examined using five SSR molecular markers (Table S2) to amplify polymorphic products, in eight rice varieties and seven commercial rice products (Table S1). The potential resolving power of this approach is much greater than conventional melting curve analysis because in HRM, melting curves from different amplicons can be differentiated on the basis of shape even when they define the same T_m values because of the composite melting curves of heterozygotes. Heterozygotes include 2 homoduplexes and 2 heteroduplexes contributing to the T_m and thus T_m is ambiguous and less useful as a metric than is the curves' shape. In this study we used the shape of the melting curves that is more informative assessing differences in the varieties under investigation (Fig. 1) and to apply this information in food traceability.

Analysis of the normalised HRM curves with SSR marker RM241 (Fig. 1) revealed that most of the varieties could easily be distinguished, for example 'Basmati T3' and 'Dimitra', the curve profiles of some varieties were similar and could therefore not be visually differentiated. Furthermore, closer examination of the Basmati 6129 HRM difference curve, with the mean Basmati T3 curve as the baseline, revealed part of the curve sitting outside the 95% CI curve, suggesting that the Basmati 6129 and Basmati T3 HRM curves are indeed different (Fig. 1A). Assigning Basmati rice variety 'Basmati T3' as a genotype we were able by subtracting the area (difference graph) from the rest of the produced melting curves by the other varieties, to estimate the confidence value of similarity between Basmati T3 (Fig. 1B). GCPs were calculated and a cut off value of 95% was used to assign a genotype for each locus (Table 1). HRM is proven more sensitive compared to capillary electrophoresis (CE) to discriminate PCR products of the same size but differing in base composition as indicated in Fig. S1 where two rice Basmati varieties (Basmati 6131 and Basmati T3) present the same allele size (112 bp) for RM1 locus but different melting profile.

3.2. Adulterations of rice food products detected by HRM analysis

After the confirmation that single varieties can be identified by HRM analysis we applied the same approach in the identification of the varieties used in rice food products. Five Basmati varieties were used as controls (Table S1). The normalised HRM curves for the amplicons, from the eight rice varieties and seven commercial rice food products, based on HRM analysis with SSR marker RM206 are shown in Fig. 2. Each genotype produced a unique melting plot that was easily distinguishable from other varieties and consistent with the observed nucleotide differences among them, except for Basmati T3 that shared a plot similar to Basmati 6131. Results obtained from the seven different commercial rice products indicate that HRM can be used effectively to assess if a single known variety or more than one was used during the process of the product. In Table 1 we present the average genotype confidence percentages (±3.22) resulting from HRM analysis of the microsatellite locus RM206 of eight rice varieties and seven commercial Basmati rice products at a ramp of 0.1 °C. To further improve our analysis we elaborated a statistical analysis where the GCPs values were re-coded in a range of 1–20 from 1 to 100 to decrease the number of different genotypes (data not shown).

3.3. Fragrance typing rice varieties and rice products using HRM analysis

The *BAD2* gene has been a well-accepted marker for fragrance rice genotyping for over 2 decades (Bradbury et al., 2005; Bradbury et al., 2005; Sakthivel et al., 2009). In this study we have developed a genotyping method based on *BAD2* deletion (8 bp) using HRM analysis. The procedure is a single step and a closed tube procedure taking approximately 2 h to perform, and costs approximately €1.00. In our protocol we analysed fifteen samples in total including seven commercial rice products and eight rice varieties. As depicted in Fig. 3A all samples have been successfully fragrance typed and assigned to aromatic or non-aromatic genotypes. In one commercial (Commercial 2) rice product adulteration was revealed by applying our genotype method (Fig. 3A and B). Assigning fragrance rice variety 'Basmati T3' as control genotype we were able by subtracting the area from the rest of the produced melting curves to distinguish the genotypes with better visualisation (Fig. 3B).

3.4. Adulterations of rice food products detected by HRM analysis

The method was validated with non-Basmati rice (Axios) spiked with Basmati rice (Basmati T3) in different proportions and also with commercial market samples (Fig. 4). DNA was extracted in duplicate from all the validation samples and HRM analysis was performed. When our data were analysed by means of conventional derivative plots in the "genotyping" mode, each genotype was represented by a single peak (data none shown). Heterozygous samples had only a shoulder instead of forming double peaks. The del/del samples showed a peak at 76.83 °C (SD = 0.10).

In contrast to the results of the first method described above, mere fluorescence normalisation suffices to distinguish the genotypes with melting curve shapes after applying the Corbett software. Nevertheless, temperature-shifted melting curves (Fig. 4A) or difference curves (Fig. 4B) demonstrated more definite

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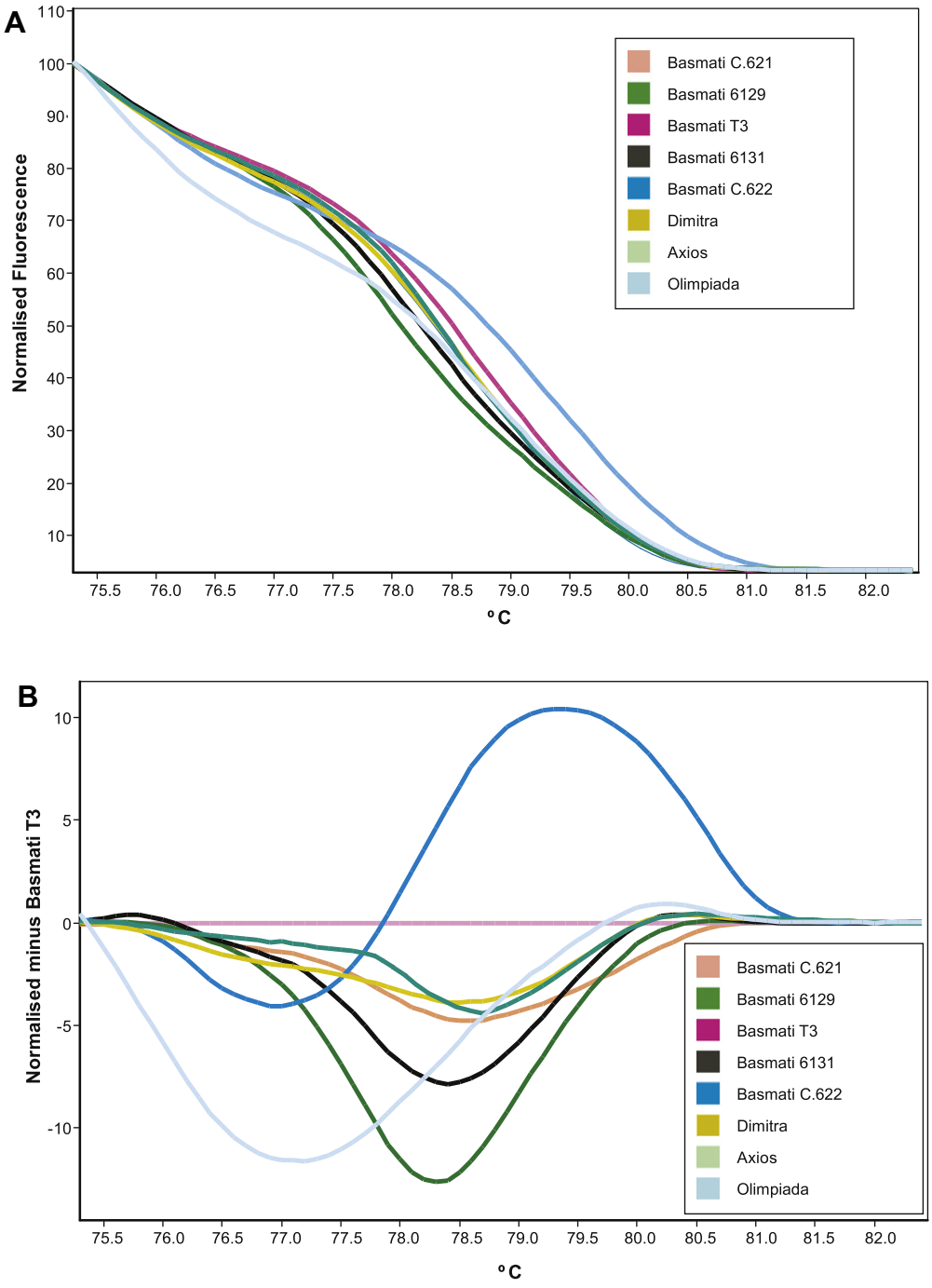

C

Colour	Variety	Genotype	Confidence %
	Basmati C.621	Variation	42,78
	Basmati 6129	Variation	90,42
	Basmati T3	Basmati T3	100,00
	Basmati 6131	Variation	78,84
	Basmati C.622	Variation	0,06
	Dimitra	Variation	73,71
	Axios	Variation	16,80
	Olimpiada	Variation	0,15

Fig. 1. Microsatellite typing of rice varieties using HRM analysis with the RM241 marker. (A) Difference graph of eight rice varieties using Basmati T3 as genotype. (B) Assigned genotypes using a cut off confidence value of 95%. Used as Basmati T3 control for HRM assay. The HRM of all other varieties were compared to this control and were resulted as Basmati T3 at ⩾95% confidence or as variation if <95% confidence. (C) Colour code table with the varieties used.

variances. Aromatic varieties with non-deletion specific amplicon melt at a lower temperature than non-aromatic once with the deletion in the amplicon. While on the other hand, heterozygous variants genotypes exhibit a wider alteration in melting

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Table 1
Average genotype confidence percentages (±3.2) resulting from HRM analysis of the microsatellite locus RM206 of eight rice varieties and seven commercial Basmati rice products at a ramp of 0.1 °C.

	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	BAS C.621	Bas 6129	Bas T3	Bas 6131	Bas C.622	Dimitra	Axios	Olimpiada
Com 1	100														
Com 2	0.0	100													
Com 3	0.4	8.0	100												
Com 4	0.2	24.0	48.3	100											
Com 5	7.7	0.5	44.2	19.6	100										
Com 6	0.5	8.8	94.2	45.7	42.1	100									
Com 7	1.2	4.6	93.5	42.1	60.0	92.9	100								
Bas C.621	29.5	0.0	4.4	1.9	42.9	3.8	8.2	100							
Bas 6129	20.9	0.0	0.0	0.0	0.1	0.0	0.0	0.8	100						
Bas T3	14.5	0.0	9.4	4.6	64.2	8.2	15.6	81.5	0.3	100					
Bas 6131	24.5	0.0	7.5	3.3	59.2	7.0	13.4	87.7	0.6	93.9	100				
Bas C.622	60.1	0.00	0.3	0.1	4.0	0.4	0.8	10.1	44.0	5.5	10.6	100			
Dimitra	28.1	0.00	0.00	0.00	0.2	0.0	0.0	1.5	93.3	0.5	1.1	47.8	100		
Axios	75.8	0.00	0.1	0.0	3.8	0.1	0.4	22.5	34.1	10.5	18.0	63.8	47.5	100	
Olimpiada	0.0	61.9	30.8	72.4	5.8	32.3	22.6	0.2	0.00	0.8	0.5	0.0	0.00	0.00	100

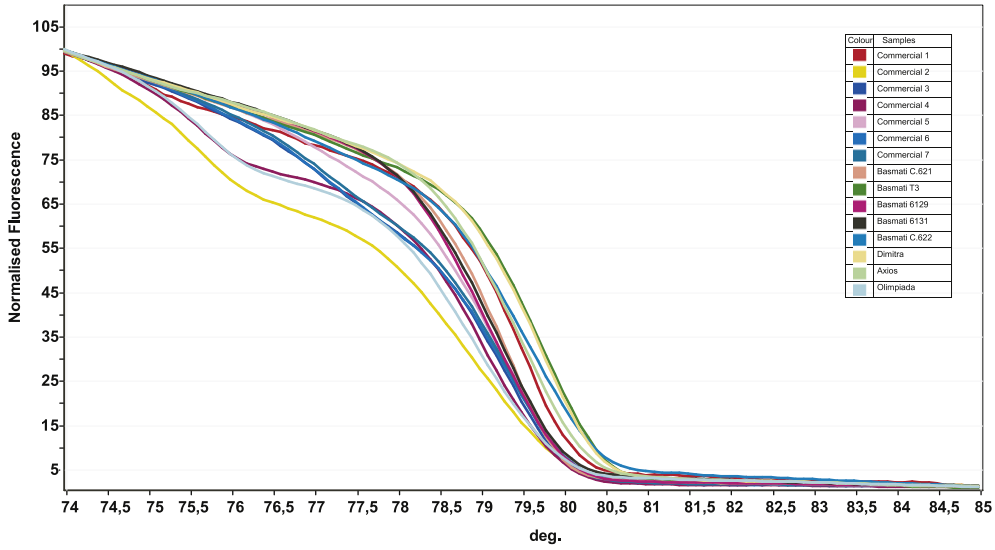

Fig. 2. Microsatellite typing of eight rice varieties and seven commercial rice food products using HRM analysis with microsatellite marker RM206.

temperature degrees which starts to melt with the no-deletion amplicon and ended with the deletion-specific amplicon.

By subtracting 7 curves from the base curve Basmati T3, all of the difference plot curves of the 8 genotypes are distinguishable from each other, although genotypes with dilutions 99:1, 97:3 and 95:5 are similarly shaped and run in parallel to each other (Fig. 4B). To avoid mistyping these 3 genotypes, we generated a second subset using curves 99:1, 97:3 and 95:5 (Fig. 4C). The difference plots are more distinguishable in the second subset analysis because there are fewer curves. Although genotypes with dilutions 97:3 and 95:5 have more similar melting profiles during melt curve and genotyping analysis, it can still be identified by the construction of heteroduplex formation using 99:1 as a reference genotype (Fig. 4C). In addition, the presence of nucleotide variants can be resolved by detecting altered high-resolution melting (HRM) profiles using heteroduplex formation.

The process of the Basmati T3 amplicon dissociation reveals the actual degree of contamination resulting from adulteration on a more precise level, as it is suggested by Mader et al. (2010). Based on these approaches we were able to produce a standard curve (Fig. 5) that shows significant correlation between the level of fluorescence and the percentage of contamination (R^2 = 0.957). An alternative approach would be the usage of genotype confidence percentage (GCP) in lieu of the level of fluorescence with a similar outcome (R^2 = 0.922) (Ganopoulos et al., 2011).

4. Discussion

Rice varieties are highly homozygous due to its autogamy. Furthermore, the greatest genetic diversity was found among rice populations and the least within population variation. Even if non-Basmati varieties are cultivated as standard crops, Basmati rice deemed as more commercial was intentionally better preserved within populations. Besides, analysis conducted with SSR and F-ISSR (Fluorescence-Inter Simple Sequence-Repeat) markers depicted trivial divergences. This seems to support the theory that Basmati varieties stem from a unique landrace that survive alterations due to ongoing interest of farmers all over (Nagaraju, Kathirvel, Kumar, Siddiq, & Hasnain, 2002).

Due to its commercial value Basmati rice is frequently subject to adulteration. In order to setup a DNA based method for rice products traceability we evaluated five different SSR markers to genotype five Basmati and three non-Basmati varieties and exploit the obtained information to verify the presence of Basmati varieties in commercial rice products. Furthermore, we established an HRM

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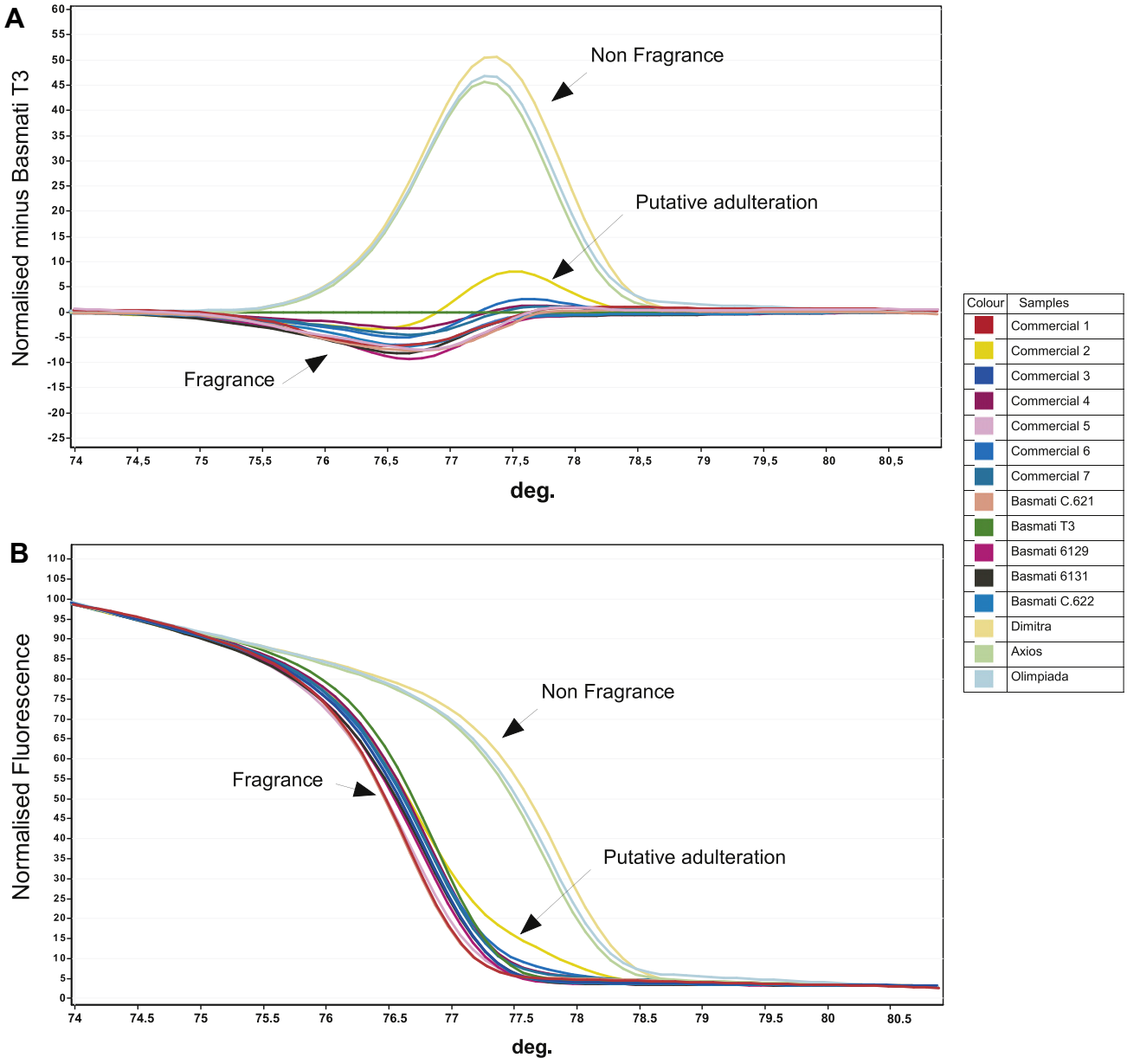

Fig. 3. (A) Fragrance typing with HRM analysis in aromatic and non-aromatic rice varieties and products. (B) Difference graph of eight rice varieties and seven commercial rice products using Basmati T3 as genotype.

protocol in order to distinguish and quantify the presence of non-fragrance rice varieties in fragrance rice products, combining microsatellites and fragrance typing, for adulteration purposes. HRM is able to detect and screen single locus markers without the need of labelled primers, product fractionation, DNA restriction or individual sequence analyses (Ganopoulos et al., 2011). This makes the technique ideal for variety identification studies where large populations are to be scored with numerous marker loci. Moreover, HRM is more sensitive compared to CE to discriminate PCR products of the same size but differing in base composition (Ganopoulos et al., 2011) as indicated in Fig. 4 where two varieties present the same allele size for RM1 but different melting profile. In terms of cost, although there is an initial capital cost for real-time thermocycler acquisition, the described method has advantages over sequencing and capillary electrophoresis, as SYTO-9 dye is the only added cost to a standard PCR. Unlike nucleotide sequencing and CE, the HRM analysis is proven to be rapid and expedient, while other relevant techniques i.d. PCR and melt-curve analysis are capable of being performed in a single close tube and one machine. An extra gain of HRM analysis is that it can be executed automatically, rendering unneeded a further clarification of findings. Furthermore, with each unknown variety, a library of prototype profiles can be used to facilitate determination of the identity of the profile and possible inter-variety diversity or to facilitate the discovery of new varieties. In order to increase the affidability of the assigned genotype we preferred to use the confidence percentage values. It is believed that confidence percentage and sequence identity are correlated but confidence percentage is more sensitive to sequence variation, as shown in previous publications (Hewson et al., 2009; Robertson et al., 2010; Steer, Kirkpatrick, O'Rourke, & Noormohammadi, 2009).

Here we present, to our knowledge, the first adaptation of novel HRM assays demonstrating the potential of microsatellite and fragrance typing with HRM analysis as a sensitive and reliable diagnostic tool for simultaneous detection and quantification of rice food products adulterants.

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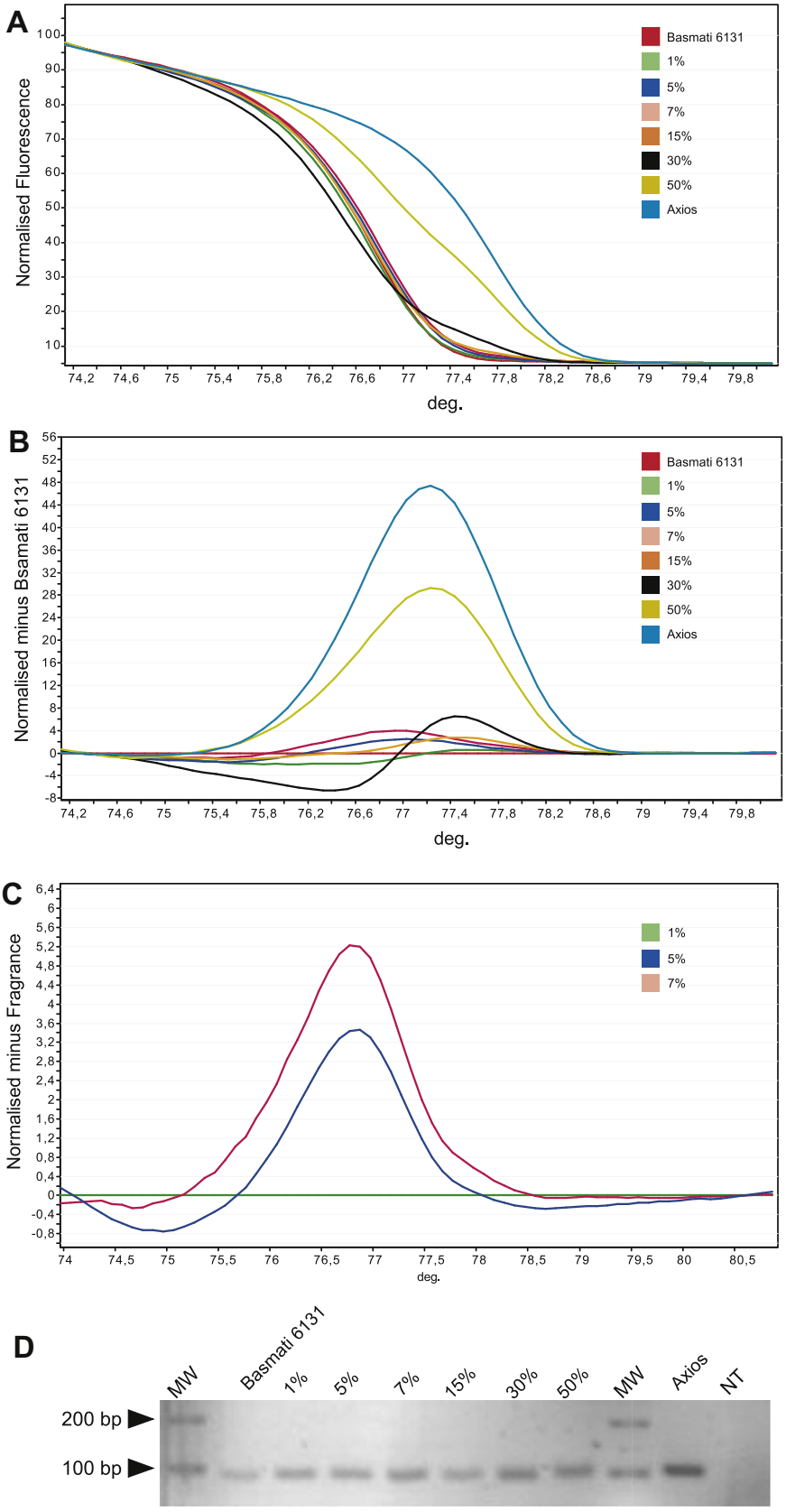

Fig. 4. Adulterations of rice varieties detected by HRM analysis. (A) High-resolution melting curves of the BAD2 gene primer assay. The HRM data are presented as graphs displaying the difference in fluorescence (ΔF) of the two different amplicons and Basmati amplicon mixtures in relation to the Basmati. Each variant amplicon was mixed with a Basmati amplicon at a ratio of 1:99, 5:95, 7:93, 15:85, 30:70 or 50:50, respectively. (B) Diagrams of normalised fluorescence of mixed Basmati/no Basmati DNA dilutions normalised against Basmati control fluorescence. (C) A second subset with curves 99:1, 95:5, and 93:3 resulted in clear and legible differentiation between the 3 mixes. (D) Electrophoresis on 3.5% agarose gel of the PCR products of the marker BADEX7-5 in aromatic and non-aromatic rice varieties. All the aromatic rice varieties amplified a 95 bp fragment, while the non-aromatic varieties amplified a 103 bp fragment. MW, size marker, NT, No template PCR mix.

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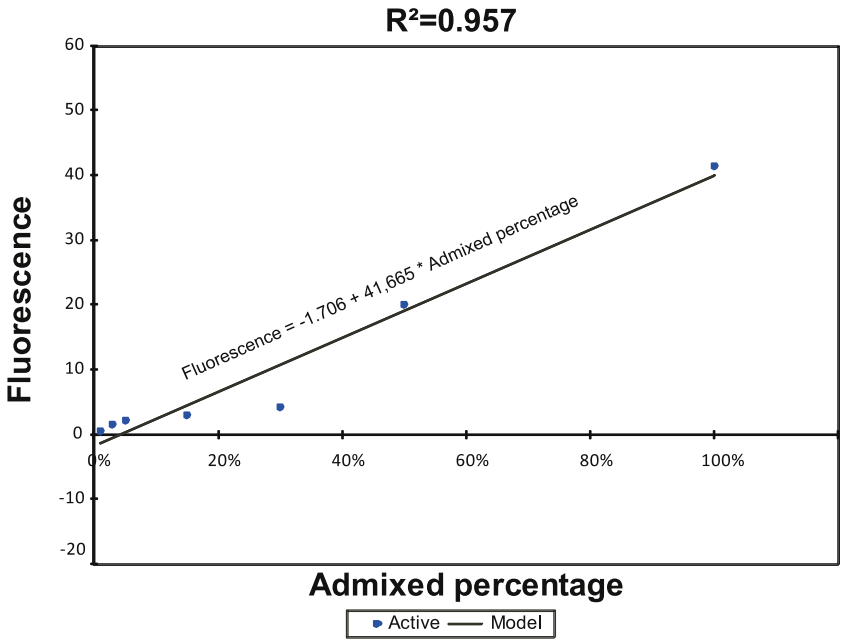

Fig. 5. Pure Basmati T3 was mixed with Axios in progressive proportions (1%, 3%, 7%, 15%, 30%, and 50%). Values of fluorescence were plotted against the percentage of admixture of each dilution to generate a typical standard curve. All the experiments were performed in duplicate.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2011.04.109.

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Please cite this article in press as: Ganopoulos, I., et al. Adulterations in Basmati rice detected quantitatively by combined use of microsatellite and fragrance typing with High Resolution Melting (HRM) analysis. *Food Chemistry* (2011), doi:10.1016/j.foodchem.2011.04.109