Strawberry and Raspberry Fruit Differentiation Using the Agilent CE 2100 Bioanalyzer

Application Note

Food

Author

Malcolm Burns, Rebecca Sanders, and Amy Burrell
Bio-Molecular Innovation Team, LGC, Queens Road, Teddington
Middlesex, TW11 0LY
UK

Abstract

Food authenticity is an important and rapidly expanding area that requires development of molecular approaches to help avert fraudulent replacement of expensive food ingredients, and to ensure correct ingredient levels are present in prepacked foods. We report here on the development of a method that uses polymerase chain reaction (PCR) and the DNA 1000 chip to distinguish between DNA derived from the fruits of strawberry and raspberry. Using this approach, characteristic profiles from strawberry and raspberry DNA are generated on the Agilent 2100 capillary electrophoresis system, which may prove useful in food authentication studies.

Agilent Technologies

Introduction

Food authentication is an important and rapidly expanding analytical area that is needed to ensure that food conforms to current international legislation and that policies on food labeling and ingredients are enforced.

Raspberry (genus: *Rubus*) is used in many foods, including purées, jellies, jams, pies, cakes, pastries, dessert toppings, juices, wines, and dairy products such as ice cream and yogurt, as well as being eaten fresh or stored frozen for consumption later. The leaves of raspberry are often used in herbal teas, and the fruit is also used for potential health benefits. Strawberry (genus: *Fragaria*) is also a common type of fruit that is cultivated worldwide and is of global economic significance.

However, recent studies [1, 2] report that some food and drinks (fruit juices in particular) labeled as containing a particular fruit, contain little or no fruit of that particular species, or may have substituted or mixed that fruit with other edible fruits. This may occur through either deliberate adulteration or unintentional processing errors (via contamination through inefficient washing procedures or coprocessing of fruits). Such instances are in contravention of the law, and stakeholders (food retailers, enforcement agencies, etc.) all require access to methods that allow the accurate identification of food ingredients to ensure regulatory compliance and protect consumers. Additionally, correct identification of ingredients in food products is needed to support the authentic composition of food, especially in relation to the declared presence of allergens in a food product, and also in the fraudulent replacement of more expensive food ingredients.

A novel application of the Agilent 2100 capillary electrophoresis (CE) system to differentiate between DNA derived from strawberry and raspberry fruits, is reported here. This approach makes use of microsatellite markers that allow differentiation of strawberry and raspberry DNA based on presence/absence or size differentiation of PCR products, easily measured using the DNA 1000 chip.

Experimental

PCR Primers

Microsatellite markers that have been previously described [3] were used to differentiate between strawberry and raspberry DNA:

Fvi11 Forward: GCATCATCGTCATAATGAGTGC
Fvi11 Reverse: GGCTTCATCTCTGCAATTCAA

Fvi20 Forward: GAGTTTGTCATCCTCAGACACC
Fvi20 Reverse: AGTGACCCAGAACCCAGAA

Samples

Authenticated DNA for Samples A and B were kindly provided by the SCRI (Dundee, UK) and consisted of strawberry samples derived from a numbered selection from a commercial breeding program, and Glen Moy (raspberry), respectively.

Raspberries (Sample D) were bought from a UK supermarket chain as prepacked fruit (225 g) labeled as raspberries (produce of Spain). Strawberries (Sample C) were purchased from the same UK supermarket store at the same time, as prepacked fruit labeled as strawberries. Two individual DNA extractions were taken from each fruit batch and labeled as Samples C1 and C2, and Samples D1 and D2.

DNA Extraction

For Samples C and D, DNA was extracted from strawberry and raspberry fruits using a cetyl trimethylammonium bromide (CTAB) buffer (50 mM tris HCl; 4 M NaCl, 1.8% CTAB; 25 mM EDTA). Approximately 100 mg fruit samples were weighed and homogenized, DNA was extracted using the above CTAB buffer, resuspended in 100 µL of 1x TE buffer, and quantified using a spectrophotometer.

Thermal Cycle Conditions

25 µL PCR reaction mixes were made based on the following components: 12.5 µL of 2x Fast Start PCR Master mix (Product number 04710436001, Roche); 300 nM of appropriate forward primer; 300 nM of appropriate reverse primer; 15 ng of extracted genomic DNA; and sterile distilled water to make a final volume of 25 µL.

Thermal cycle conditions (MJ Research Tetrad #2 PCR machine) consisted of 95 °C for 6 min; 40 cycles of 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; followed by 72 °C for 10 min and hold at 4 °C.

Use of the DNA 1000 Chips on the Agilent 2100 CE System

All chips were prepared according to the instructions provided with the Agilent DNA 1000 LabChip kit. The gel-dye mix was prepared by mixing 400 µL of the gel matrix with 20 µL of the dye concentrate, then filtering the mixture through a spin filter. The separation chip was filled with the gel matrix/dye mixture, and 5 µL of the markers was added to each sample well. After adding samples (1 µL each) to the sample wells and the DNA sizing ladder (1 µL) to the assigned ladder well, the chip was vortexed and run on the Agilent 2100 bioanalyzer.

Results and Discussion

Fvi11 Assay

According to published literature [3, 4], Fvi11 is based on a $(GA)_{16}$ repeat motif and should give an amplicon of around 137 bp in length with strawberry, but also exhibits polymorphism in amplicon size between *Fragaria* varieties. However, Fvi11 should not cross-react with raspberry, and so no amplicon should be present.

The results from this preliminary study shown in Figures 1A and 1B indicate that Fvi11 gives an amplicon of around 122 bp in the samples that contained strawberry (A and C). Additional amplicons were also sometimes observed at 282 and 290 bp, but within the confines of the limited experimental data presented here, not on a repeatable basis. In line with expectations, Figures 1A and 1C show that Fvi11 did not cross-react with samples that contained raspberry (B and D).

Negative controls showed no detectable amplification. Additionally, Fvi11 showed a positive result and amplified the same 122-bp fragment when tested on a commercially available strawberry sauce sample with a listed ingredient of 40 percent whole strawberries (Figure 1D), inferring the assay's applicability to processed food samples containing fruit.

Fvi20 Assay

According to published literature [3, 4], microsatellite marker

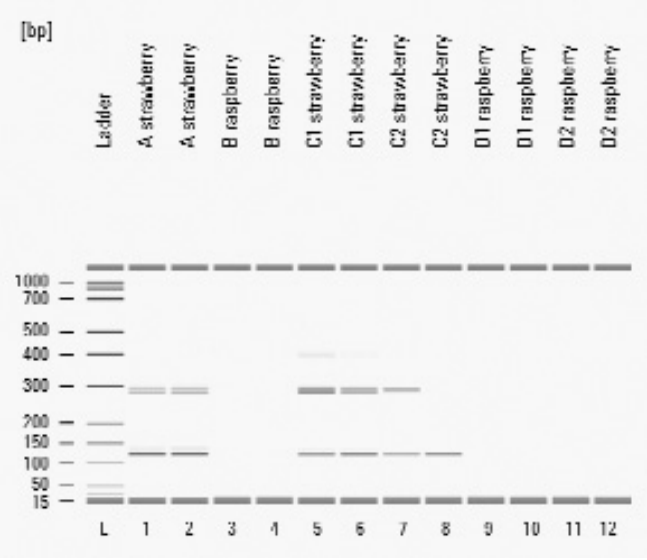


Figure 1A: Gel-like image based on Fvi11 assay on all samples.

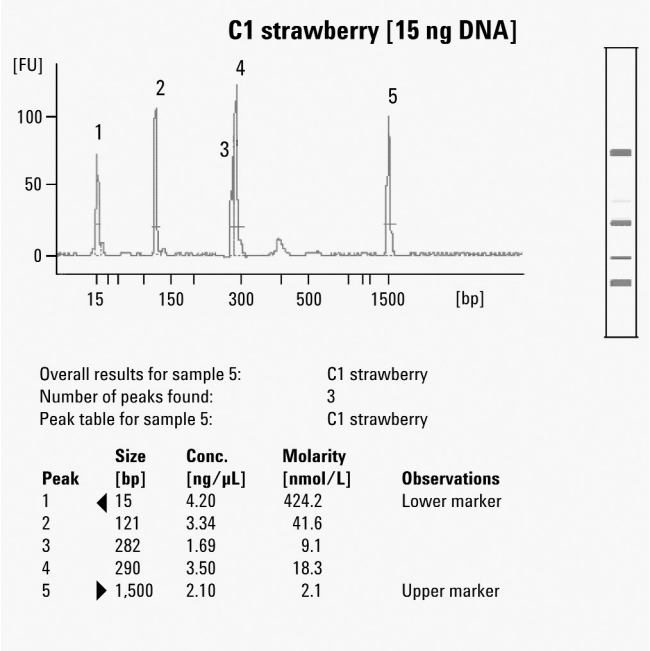


Figure 1B. Electropherogram to show profile generated using Fvi11 with strawberry DNA.

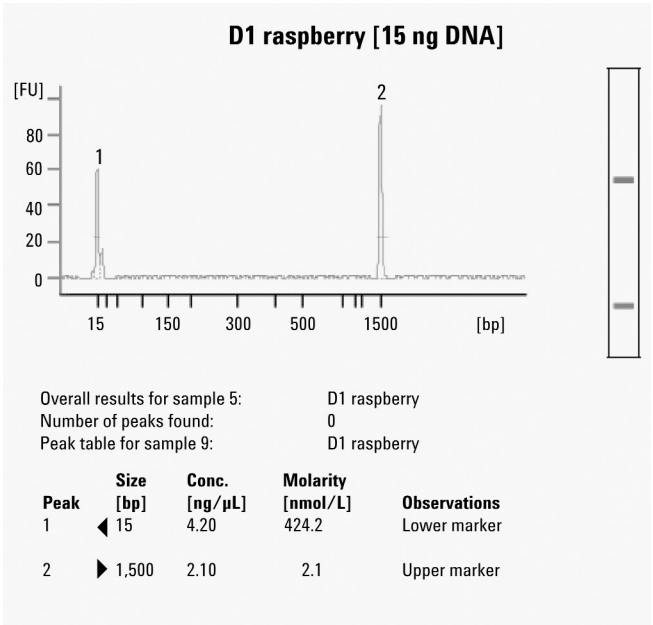
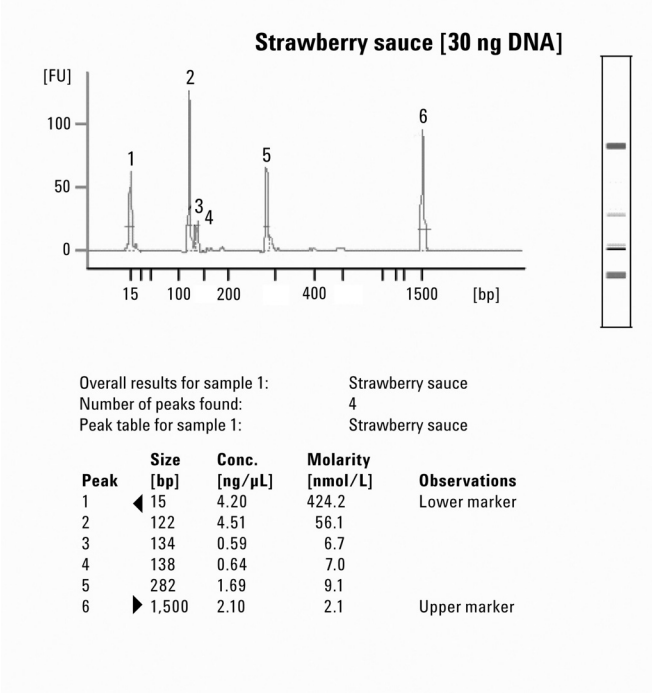


Figure 1C. Electropherogram to show absence of bands when using Fvi11 with raspberry DNA.



Overall results for sample 1: Strawberry sauce
Number of peaks found: 4
Peak table for sample 1: Strawberry sauce

Peak	Size [bp]	Conc. [ng/µL]	Molarity [nmol/L]	Observations
1	◀ 15	4.20	424.2	Lower marker
2	122	4.51	56.1	
3	134	0.59	6.7	
4	138	0.64	7.0	
5	282	1.69	9.1	
6	▶ 1,500	2.10	2.1	Upper marker

Figure 1D. Electropherogram to show characteristic strawberry DNA profile generated following application of Fvi11 to a strawberry sauce sample.

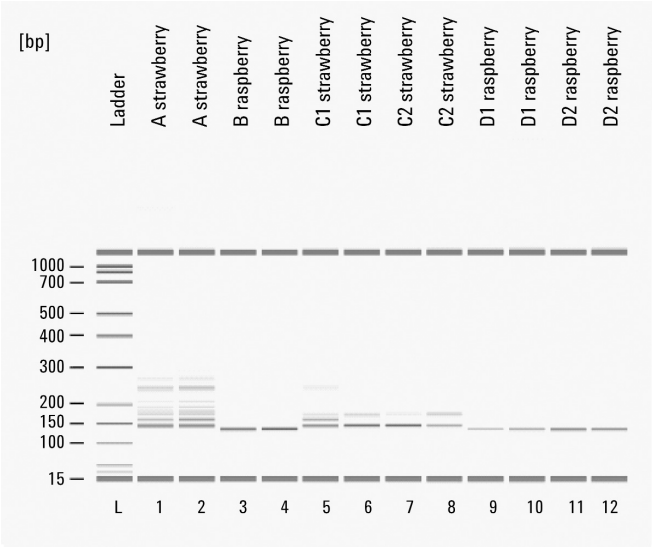


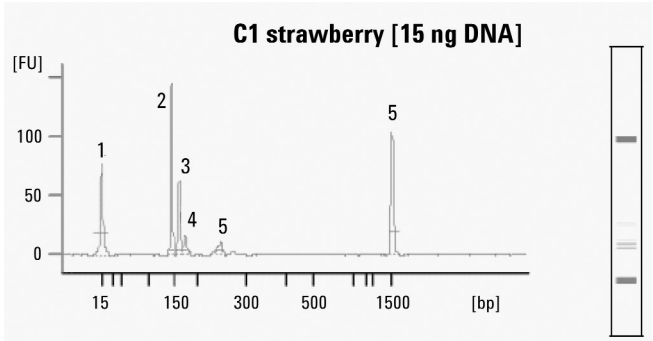
Figure 2A. Gel-like image based on Fvi20 assay on all samples.

Fvi20 is based on a $(GA)_{20}$ simple sequence repeat motif sequence, and has been shown to give a single or multiple bands around 162 bp in length for *Fragaria* varieties, but only a single amplicon with raspberry (*Rubus*) varieties.

Based on the results from this preliminary study, shown in Figures 2A and 2B, the application of the Fvi20 assay to samples that contained strawberry (A and C) gave single or multiple amplicons at around 144, 162, and/or 175 bp. In the limited strawberry samples tested in this study, the 144-bp fragment was present and predominated, while the occurrence of the other bands was less repeatable.

The application of the Fvi20 marker locus to samples that contained raspberry (B and D), shown in Figures 2A and 2C, showed the presence of a single band at 136 bp, which was easily distinguished from the 144-bp amplicon characteristic of strawberry cultivars.

Negative controls and extraction blanks showed no detectable amplification. Furthermore, the application of Fvi20 to the commercially available strawberry sauce sample showed bands around 143 and 161 bp, characteristic of strawberry DNA being present (Figure 2D).



Overall results for sample 5: C1 strawberry
Number of peaks found: 3
Peak table for sample 5: C1 strawberry

Peak	Size [bp]	Conc. [ng/µL]	Molarity [nmol/L]	Observations
1	◀ 15	4.20	424.2	Lower marker
2	144	4.13	43.6	
3	161	2.01	18.9	
4	174	0.76	6.6	
5	248	0.60	3.7	
6	▶ 1,500	2.10	2.1	Upper marker

Figure 2B. Electropherogram to show profile generated using Fvi20 with strawberry DNA.

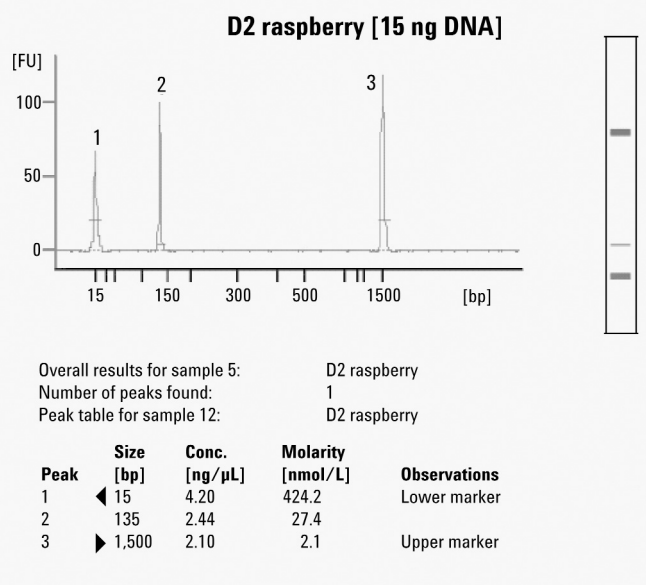


Figure 2C. Electropherogram to show presence of one band when using Fvi20 with raspberry DNA.

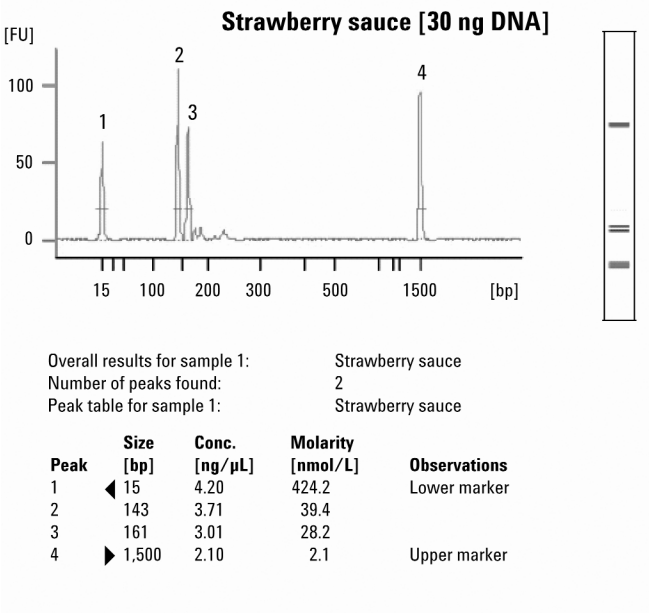


Figure 2D. Electropherogram to show characteristic strawberry DNA profile generated following application of Fvi20 to the strawberry sauce sample.

Conclusions

Originally, the microsatellite markers of Fvi11 and Fvi20 were used to assess genetic variability in strawberry (*Fragaria*) varieties. We have shown the novel application of these primer pairs using PCR and the Agilent CE 2100 system, to differentiate between samples that contain strawberry and raspberry DNA. Based on initial studies and amplicon sizes, these may prove useful in food authentication studies. The results from this small study are only representative of specific varieties of *Fragaria* and *Rubus*, but demonstrated clear differentiation between strawberry and raspberry DNA using traditional PCR followed by resolving the PCR products on the Agilent 2100 CE system.

References

1. Food Commission Survey results, The Food Magazine, 25 February 2008, http://www.foodmagazine.org.uk/press/food_flavourings/
2. A. Stój and Z. Targoński, "Use of amino acid analysis for estimation of berry juice authenticity," *Acta Sci. Pol., Technol. Aliment* (2006) 5(1):61–72
3. M.V. Ashley, J.A. Wilk, S.M.N. Styan, K.J. Craft, K.L. Jones, K.A. Feldheim, K.S. Lewers, T.L. Ashman, "High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill. (Rosaceae)," *Theor. Appl. Genet.* (2003) 107:1201–1207
4. K.S. Lewers, S.M.N. Styan, S.C. Hokanson, "Strawberry GenBank-derived and Genomic Simple Sequence Repeat (SSR) Markers and Their Utility with Strawberry, Blackberry, and Red and Black Raspberry," *J. Amer. Soc. Hort. Sci*. (2005) 130(1):102–115

Acknowledgements

We gratefully acknowledge provision of strawberry Sample A and raspberry Sample B as authenticated samples from Julie Graham, SCRI, for the purposes of this study.

The work presented here was part of the "Government Chemist 2008-2011 Programme" and was funded by the UK Department for Innovation, Universities and Skills.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Published in the USA
January 19, 2009
5990-3327EN

Agilent Technologies