Mangoes Made in Australia

Agri-Science Queensland Innovation Opportunity

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Queensland Government

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Summary

Australian food products have a reputation for exceptional quality and are valued for by consumers for high safety standards. Recently, there have been several high profile incidents where Australian products “made from local and imported ingredients” have resulted in negative health outcomes which were traced back to produce that was not locally sourced. Local processing company Tropico Fruits produces an extensive range of fruit based products and list an Australian Kensington Pride mango puree among their flagship products. In light of the recent health issues arising from composite products using imported ingredients, the ability to test a processed product and identify the country of origin of it constituents is of great value to commercial enterprises that promote their products as locally sourced. Processed mango products are great candidates for assessing the feasibility of applying DNA-based identification methodologies for a number of reasons. DNA profiles have been determined for over 300 mango accession sourced from around the globe, including all the major domestic and overseas commercial varieties. Within all the commercial varieties, unique combinations of DNA markers exist such that a specific DNA profile can be traced back to the source variety. This means that even in composite purees, the major constituent varieties would be detected. To this end, four commercially produced mango purees were provided by Tropico Fruits and samples were subjected to a number of DNA extraction methodologies. While several of the methods produced workable quantities of highly degraded DNA, none of the extracted samples produced any results in the DNA fingerprinting steps. The objective of this project was to produce a set of reliable protocols that could determine the constituents of a processed product on the basis of a DNA profile. Unfortunately, while DNA was isolated using several techniques, the level of DNA degradation arising from the pureeing and sterilisation processes meant that DNA profiles could not be generated.

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Background

The Department of Agriculture and Fisheries maintains a global collection of mango germplasm at the Walkamin Research Station on the Atherton Tablelands. This collection, which contains over 300 accessions, has been DNA fingerprinted using simple sequence repeat (SSR) markers. An analysis of the DNA profiles revealed that all major varieties could be distinguished using a very small number of molecular markers (see Table 1 for examples). While initially generated to better understand germplasm diversity, these profiles have much broader applications. For example, DNA fingerprints can be used to determine the parentage of seedlings from populations arising from open pollination. This allows any off-types to be identified and discarded and similar technology can also be used to verify that particular varieties are what they're supposed to be.

	SSR Markers					
Variety	LMMA1	LMMA10	LMMA12	LMMA15	MiSHRS18	mMICRO20
Irwin	**203 / 205**	157 / 175	207 /	212 / 220	102 / 104	161 / 171
Keitt	**201 / 203**	**171 / 175**	203 / 207	212 / 220	102 / 104	161 / 171
Kensington Pride	197 / 203	151 / 157	203 / 207	**218 / 220**	**96 / 102**	161 / 167
R2E2	197 / 203	151 / 157	203 / 207	220 /	102 /	**167 / 173**
Tommy Atkins	**199 /**	**173 / 177**	**203 / 205**	220 /	102 / 104	**153 /**

Table 1 DNA profiles for five mango varieties using six microsatellite (SSR) markers. Discriminating markers are underlined and the boxes are coloured to aid in identification. All alleles are described as the size in base pairs of the PCR fragments following capillary electrophoresis with the alleles separated by '/'. Where only a single allele size was detected, typically in homozygotes, the allele following the / is omitted.

Recently, several high profile incidents have been reported by the media where Australian products "made from local and imported ingredients" have raised health concerns. The root cause of these health issues was traced back to produce that was not locally sourced.

Local processing company Tropico Fruits produces an extensive range of fruit based products and list an Australian Kensington Pride mango puree among their flagship products. In light of the recent health issues arising from composite products using imported ingredients, the ability to test a processed product and identify the country of origin of it constituents is of great value to commercial enterprises that promote their products as locally sourced.

Mango products are great candidates for assessing the feasibility of applying DNA-based identification methodologies as mango production is highly regionalised with local varieties grown almost exclusively in Australia. Furthermore, within all local and overseas commercial varieties, unique combinations of DNA markers exist such that a specific DNA profile can be traced back to the source variety, weather sourced domestically or imported.

Project Objectives

The objective of this project was to produce a set of reliable protocols that could determine the varietal constituents of a processed product on the basis of a DNA profile. Specifically, DNA would be extracted from the commercial puree and SSR marker profiles determined for the sample. This profile would then be interrogated against the genetic fingerprints for known mango varieties and the varietal composition determined or verified.

Methodology

Four aseptic mango puree samples were provided by Tropico Fruits. One sample was prepared from Kensington Pride mangos while another was from a known blend of mango varieties. The composition of the remaining two were blind samples, referenced only by lot numbers which related back to production information retained by Tropico Fruits and used to validate the sensitivity of the assays. Each of these samples came from a standard production run which, using high temperature and pressure, produces sterile puree.

For each sample, 1 mL and 5 mL of puree were centrifuged at >16,000g in an Eppendorf 5415D microcentrifuge. The clear supernatants were decanted into sterile tubes. For the 5 mL samples, the gelatinous solids were washed with an equal volume of 10 mM Tris-HCl buffer, pH 7.5. Following centrifugation, the supernatants were again decanted and like samples pooled before the wash, centrifugation and pooling steps were repeated. Final sample volumes of 300 uL and 1.5 mL were obtained from 1 mL and 5 mL of starting material respectively. Frozen leaf tissue samples were included as control reactions.

DNA isolation was performed using the Promega Wizard Magnetic DNA Purification System for Food. This kit was chosen it has protocols for extracting DNA from processed food products including corn chips and soymilk. This approach uses a suspension of paramagnetic beads to bind DNA, which remains coupled to the beads during the purification steps before being chemically eluted into a mild neutral buffer. Purifications were performed as per the manufacturer's protocol except for the initial binding step for the larger sample volumes (1.5 mL of washed sample). For these samples, the DNA binding step was repeated 3 times at which point the total sample was bound to the beads. Following purification, samples were eluted in 100 uL of elution buffer and stored at -20°C. Aliquots (10 uL) of each sample were visualised following agarose gel electrophoresis (0.8%) using Gel Red.

PCR amplifications were performed using primers for the LMMA1 SSR (F:5'-ATG GAG ACT AGA ATG TAC AGA G, R:5'-ATT AAA TCT CGT CCA CAA GT) in a 50 uL as described by Viruel et al (2005)[1]. PCR products were visualised following agarose gel electrophoresis (2%) using Gel Red.

Subsequent DNA concentration and clean-up was performed using QIAGEN min-elute columns as per the manufacturer's recommendations. Cleaned and concentrated samples were eluted in 20 uL of QIAGEN buffer EB.

[1] Viruel, M.A.. P. Escribano, M. Barbieri, M. Fern. and J.I. Horinaza. 2005. Fingerprinting, embryo type and geographic differentiation in mango *(Mangifera indica* L.. Anacardiaceae) with microsatellites. Mol. Breeding 15:383-393.

Results

DNA purification using the Wizard Magnetic DNA Purification System for Food produced a small amount of highly degraded DNA. This is not entirely unexpected given the harsh mechanical disruption and high temperature conditions that the samples were exposed to during processing. DNA extractions from frozen leaf samples produced high quality, high molecular weight DNA which indicated that the extraction methods were performing as expected (see Figure 1).

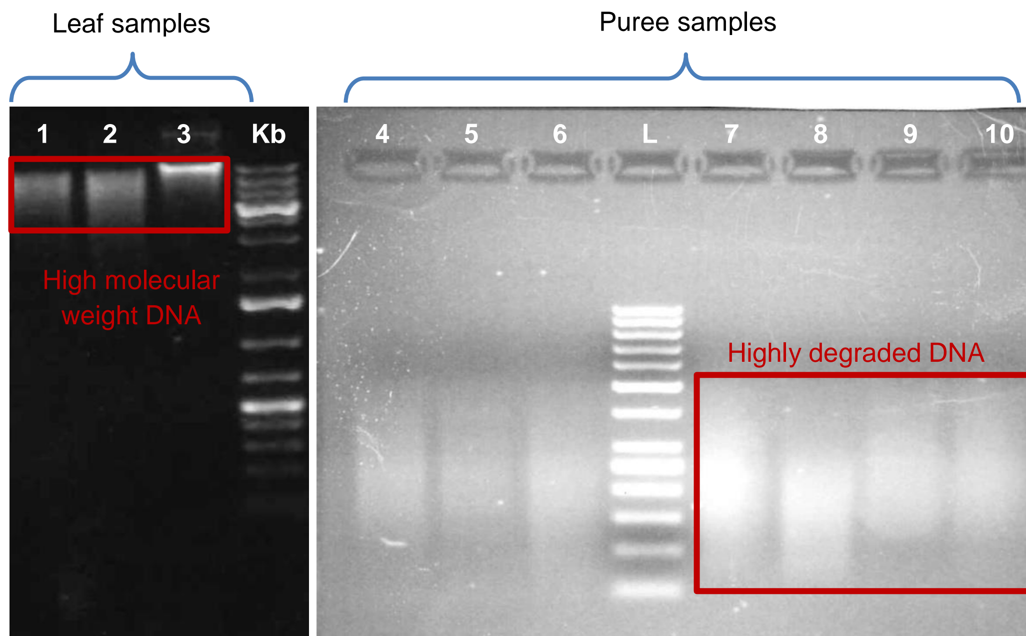

Figure 1 Agarose gel electrophoresis of purified DNA samples. 1. Tommy Atkins leaf, 2. Irwin leaf, 3. Kensington Pride leaf, 4. Kensington Pride puree (1 mL sample), 5. Mixed puree (1 mL sample), 6. unknown puree (1 mL sample, 7. Kensington Pride puree (5 mL sample), 8. Mixed puree (5 mL sample, 9-10. Unknown purees (5 mL sample), Kb 1Kb ladder, L. 50 bp ladder

Despite being highly degraded, the DNA produced from the puree samples should have been of sufficient quality and quality for DNA profiling using PCR. The products generated by the PCR reactions are very short and, generally, have a low DNA template quality requirement. The results of LMMA1 PCR reactions using purified DNA from mango leaf and puree samples are shown in Figure 2.

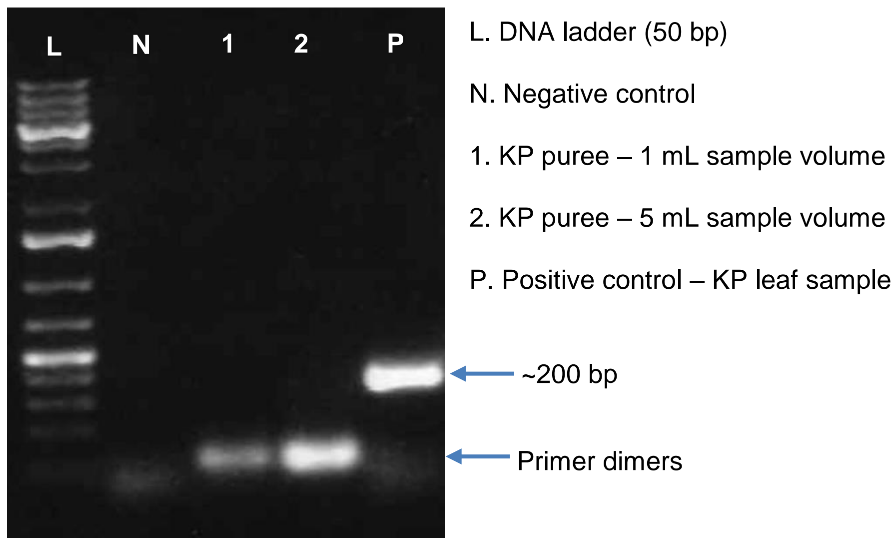

Figure 2 LMMA1 PCR reactions on DNA prepared from mango puree and frozen leaf material. The 200 base pair (bp) arrow indicates the expected location of the LMMA1 PCR product. The primer dimers seen at the bottom of the gel are the result of interactions between the PCR primers during amplification.

Figure 2 shows the unsuccessful amplification of PCR products from the degraded DNA prepared from the puree samples. The bright bands towards the bottom of the image in lanes 1 and 2 are result of interactions between PCR reagents. These bands can also faintly be seen in positive (P) and negative (N) controls. The positive control (P) used DNA extracted from frozen leaf tissue prepared in parallel with the puree samples. The expected PCR product sizes for a KP sample using LMMA1 primers are 197 and 203 base pairs (bp; see Table 1). The positive control reaction generated products of the expected size though the resolving power of the gel was unable to separate the two 197 and 203 bp products; this was expected.

Mango fruit extracts contain many compounds that inhibit *in vitro* enzymatic reactions such as PCR. To this end, puree DNA samples were cleaned and concentrated using QIAGEN Min-elute columns and the PCR reactions were repeated with essentially the same results as those depicted in Figure 2.

In order to generate the DNA profiles, PCR amplification is essential. As this could not be achieved using sensitive DNA extraction methodologies designed for processed food, the project did not proceed past this point.

Conclusions/Significance/Recommendations

The objective of this project was to produce a set of reliable protocols that could determine the constituents of a processed product on the basis of a DNA profile. Unfortunately, while DNA was isolated using the described techniques, the level of DNA degradation arising from the pureeing and sterilisation processes meant that DNA profiles could not be generated.

Key Messages

A need still exists for being able to trace or verify the components of processed fruit products, as was evident during last season Queen Garnet plum season. Processed product and composite product testing have been used in other food industries for many years however it is the nature of the processing the ultimately determines the success of these applications.

Where to next

Colleagues at the USDA in Florida are endeavouring to achieve the same outcomes as those of this project but for an Indian collaborator. At the last update, they were experiencing similar issues and were turning to some older chemistries to treat the puree with prior to DNA extraction. Monitoring the progress in their project may help in progressing this one at a later date either for mango or other processed fruit products.

Budget Summary

The budget for this project was $12,500. As only the DNA extraction component was completed, the total spent was $1,873. Most of the budget was for DNA fingerprinting, which remains a significant cost for microsatellite marker. A smaller amount of the budget was also for use in validating typing using single nucleotide polymorphism markers with the USDA. No genotyping was done.