Short Communication

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A Rapid Molecular Diagnostic Assay for Identification of *Rosa Roxburghii* Juice Products

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ABSTRACT

We devised a DNA-based real time PCR (qPCR) assay for the identification of *Rosa roxburghii*. qPCR technology allows the development of relatively reliable and easy-to-use analytical methods for species identification. The possible use of the method for qualitative measurements was also tested on fruit juice products. The results revealed that this method could be effectively applied to the sample test of commercially available *Rosa roxburghii* products, providing technical support for governmental supervision of markets and it will be of great significance for the quality control of *Rosa roxburghii* related products.

Keywords: *Rosa Roxburghii*; Juice Products; Real Time PCR

Introduction

Rosa roxburghii is widely distributed in the southwest provinces of China. The fruit contains a wide range of nutritional and medicinal components, such as ascorbic acid (AsA), amino acids, dietary fiber, and polysaccharides, believed to have valuable senescence-retarding and cancer-preventative effects [1-3]. Most parts of the R. roxburghii plant are used as traditional herbal products or as food and a series of food and health products have been developed [4].For profit making of the manufacturer, plant-derived foods are often prone to illegal addition and adulteration. The plant-derived food market has become increasingly disordered, due to the appearance of deliberate substitutes and adulteration, especially in processed food products [5,6]. A wide variety of DNA-based polymerase chain reaction (PCR) methods for the species authentication in foods and food products have also been reported [7-9]. However, the adulteration problem of *Rosa roxburghii* products has not been resolved due to lack of detection methods. Therefore, we here develop a simple, rapid, and practical identification method of *Rosa roxburghii* products, using real-time polymerase chain reaction (PCR) method. We further verified the utility of this method for detecting *Rosa roxburghii* in retailed products. Tests proved that this method could be effectively applied to the sample test of commercially available *Rosa roxburghii* products, providing technical support for governmental supervision of markets and it will be of great significance for the quality control of *Rosa roxburghii* relateded products.

Materials and Methods

Rosa roxburghii fruits were collected from Guizhou qiannan. Fruit of 10 other species were purchased from Beijing supermarkets including banana, orange, lemon, strawberry, hawthorn, apple, pear, peach, cherry, kiwifruit. Ten commercial juice products were purchased at local markets.

Fresh fruits were crushed and centrifuged at 15000 rpm for 10 min to obtain a pomace. Fruit juice was centrifuged at 15000 rpm for 30 min to deposit and enrich the sediment. Approximately 1 g of sediment was dissolved in lysis buffer. DN easy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) was used according to the manufacturer protocol with minor modifications. 800 µL AP1 and 10 µL RNase A at 65°C for 2 h, which was then cooled on ice for 20 min.

Table 1: Primers and probes for *Rosa roxburghii.*

Amplified ITS2 gene	Primers and Probe (5′-3′)
Forward	TCGGGAGTTGGATGGGACG
Reverse	GCATCGACGGATCGACAGCT
Probe	FAM-AGAAAGCACTCGATCAACACGAG-CG-BHQ1

Primers and probes were designed with Primer Premier 5.0 [10] using the ITS2 gene sequence. Primer and probe information can be found in Table 1. Real-time PCR reactions were performed in a 20 µL volume with 10 µL of Premix Ex Taq (Takara), 0.4 µL of each primer (10 µM), 0.8 µL of probe (10 µM), and 1 µL template DNA. 7.4 µL ddH2O. Reactions were performed in a 7500fast cycler (Applied Biosystems) at 95°C for 30 s, 95°C for 5 s, 40 cycles of 55°C for 45 s, and 40°C for 10 s.

Results and Discussion

To design species-specific primers and probe, we chose ITS2 as target gene sequences. DNA of *Rosa roxburghii* and 10 other fruits were amplified using ITS2 primers reported by Chen, et al. [11]. All the 11 samples were amplified successfully showing the desired 500bp band (Figure 1). All the sequences were aligned, and species-specific primers and probes were developed (Table 1). The specificity of the primers and probe were evaluated by real time PCR using 11 samples DNA as template. The results showed that only *Rosa roxburghii* fruit sample DNA was tested positive with Ct value of 20 (Figures 2 & 3). To determine the absolute sensitivity of the methods, genomic DNA was extracted from *Rosa roxburghii* fruit. Genomic DNA was diluted with distilled water to 100 ng/µL,10 ng/µL, 1 ng/µL, 0.1 ng/µL, 0.01 ng/µL, 0.1 ng/µL. The Ct values were 21.1, 23.9, 26.7, 30.2, 34.1, 36.2 respectively and Ct values lower than 30 were considered to be positive. Thus, the absolute LOD (limit of detection) was 1 ng/µL for *Rosa roxburghii*. The linear coefficients of the standard curves were all generated by the 7500fast software operation system. R2 values were all greater than 0.99, and the amplification efficiency was greater than 85% (data not shown).

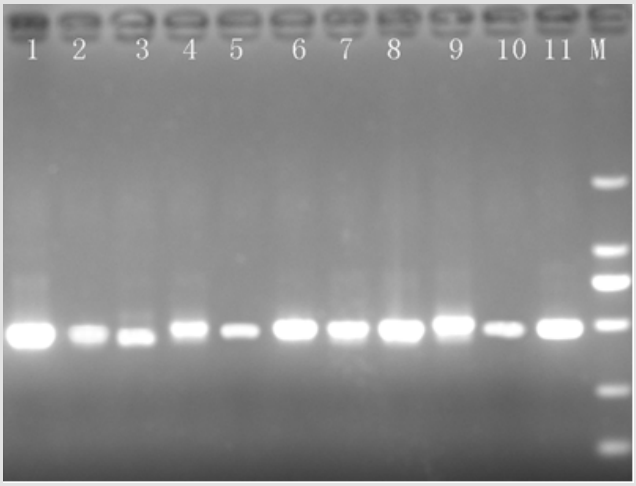

Figure 1: PCR amplification of 11 samples using ITS2.

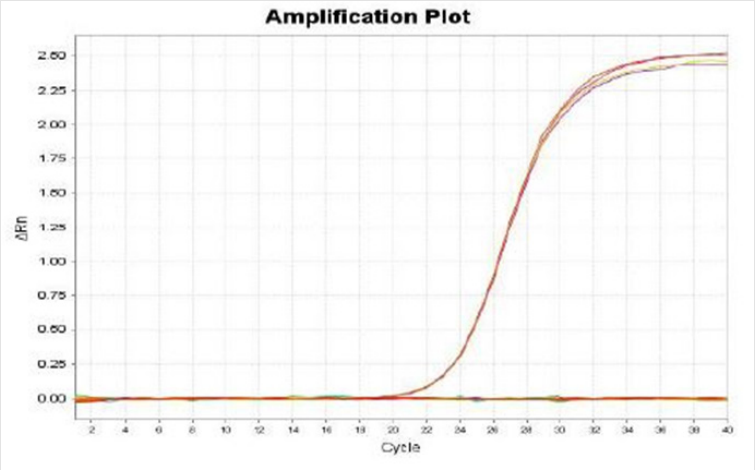

Figure 2: Species specificity detection of *Rosa roxburghii*.

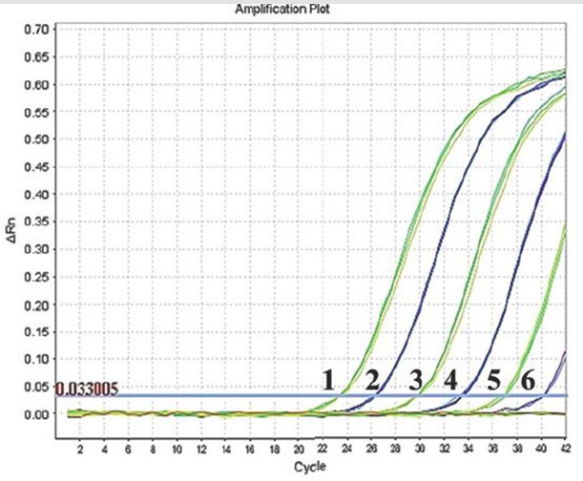

Figure 3: Sensitivity analysis of qPCR. gDNA was diluted across six concentration gradients from 100 ng/μL to 0.1 ng/μL.

Furthermore, we test the ability of the above designed primers and probe to detect *Rosa roxburghii* in commercial juice products. Five *Rosa roxburghii* not-from-concentrate juice samples and five other fruits juice samples were analyzed in the test. First, the quality of extracted DNA was determined through the amplification of the 18S rRNA reference gene. We excluded DNA that could not be extracted which may cause failure of the target species signal. It can be observed that all the ten samples were successfully amplified. Only the five *Rosa roxburghii* not-from-concentrate juice samples showed the positive signals (Ct values of 20–30) and five other fruits juice samples were negative. In summary, a total of ten commercial juice samples were analyzed in the test with a correct assignation of 100%. Mislabeling and adulteration can lead to serious and even fatal health consequences. The assay presented a standard TaqMan-based real-time PCR method with species-specific probe, when the method was applied to commercial product testing, only the pure juice detection results were satisfactory, indicating that this method is sensitive, reliable, simple and can increase the power of detection in future validation. However, the approach was initially developed as a qualitative but not quantitative method, so further experiments may be performed to ascertain the approximate proportion of the target species in the commercial product.

Conclusion

A rapid molecular diagnostic assay was developed for detecting *Rosa roxburghii* in commercial juice products. The developed assay is an accurate and sensitive methodology for the detection of ingredients in food samples. Commercial *Rosa roxburghii* products were

tested to investigate the suitability of the method to authenticate commodities in the market and furthermore the method can be used for the quality control of related products.

Conflicts of Interest

The authors declare no competing interests.

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