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DNA extraction and fingerprinting of commercial rice cereal products

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Abstract

DNA was extracted from commercial rice cereal products using modified conventional methods (CTAB, SDS and a commercial kit) in large fragments (>3 kb) and with relatively high yields (1.4–10.7 μg DNA per g of sample) and was used as template for the amplification of a single copy rice gene (i.e. MIPS) fragment (ca. 850 bp) and microsatellite DNAs (ca. 120–400 bp). The cereal products were further discriminated by using six microsatellite markers. The usefulness of DNA analysis was discussed for quality control and authenticity testing of raw rice materials in rice-based food production, and to monitor genetically modified (GM) rice ingredients in commercial food products.

Keywords: DNA extraction; DNA marker; Fingerprinting; Rice cereal; Quality

1. Introduction

The use of high quality raw materials is a key factor for making genuine products of adequate nutritional value and/or accustomed taste in the food industry; products made from certain raw materials of particular geographic origin are not only preferred in the market but also sometimes privileged by trade arrangements (Council Regulation, 1992), e.g. Basmati rice enjoys zero percent import duty in the European Union (http://inhome.rediff.com/money/2004/dec/06rice.htm). On the other hand, detection of ingredients made from genetically modified (GM) organisms in food products has become an important issue in food production and marketing. Therefore, efficient analytical methods for fingerprinting raw materials and particular ingredients are not only a powerful means of quality control for the food industry, but are also indispensable for the surveillance of the origin, quality, and authenticity of food products to avoid frauds.

Rice is the staple food for more than half of the world population (Khush, 1997) and is rich in diversity regarding structure, functions and properties (Juliano, 1993; Vandeputte & Delcour, 2004; Zhou, Robards, Helliwell, & Blanchard, 2002). Although rice is mainly consumed as milled rice, more and more processed rice products such as infant cereals, cereal drinks, congee, and bars, have become available in food markets in both developing and developed countries. With the quality of both cooked rice and other rice-based products being largely determined by the genotype of the rice variety (Juliano, 1993; Shu & Xia, 1999; Zhou et al., 2002), and the increasingly possible introduction of GM rice in some countries in the near future (Brookes & Barfoot, 2003; High, Cohen, Shu, & Altosaar, 2004), the development of methods for detecting and quantifying GM ingredients and distinguishing varietal differences of rice in processed products becomes increasingly important.

DNA analysis has recently emerged as a novel and efficient technology in food tests, initially developed for the detection of GM food ingredients (Hemmer, 1997; Hurst, Knight, & Bruce, 1999; Peano, Samson, Palmieri, Gulli,

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& Marmiroli, 2004), and now expanding to species and cultivar discrimination of raw material in food and feed (Busconi et al., 2003; Hunt, Parkes, & Davies, 1997; Krcmar & Rencova, 2003; Pasqualone, Montemurro, Caponio, & Blanco, 2004; Siret, Gigaud, Rosec, & This, 2002).The prerequisite for DNA analysis of processed food is the availability of high quality genomic DNA. Studies on DNA extraction from wheat flour and baked products (Tilley, 2004) and soybean and maize products (Peano et al., 2004) demonstrated that there is significant DNA degradation during food processing, particularly when chemical and enzymatic treatment is applied, and that sometimes the extracted DNA is only useful for amplification of DNA fragment of less than 300 bp. There is no report yet on DNA extraction from any processed rice products.

In this study, DNA was extracted from commercially available rice cereal products in China, using three methods modified from the protocol previously used for DNA extraction of milled rice (Pal, Jain, Saini, & Jain, 2001). The yield and quality of extracted DNA was compared among three methods, and their usefulness as DNA template for amplification of target gene fragment and microsatellite DNA markers were investigated.

2. Materials and methods

2.1. Rice materials

The rice cereal products RC1-7 were purchased from a supermarket in Shanghai, with the main ingredients being described in Table 1. Another known rice cereal product (RC8) and the milled rice sample (RC9) from which RC8 was made were used as control.

2.2. DNA extraction and quantification

The modified SDS mini-prep method developed by Pal et al. (2001) for DNA extraction from milled rice was adopted as the basic protocol. A couple of modifications were also formulated to make it suitable for DNA extraction from cereal products, a brief account of the methods used in this study is given below: 140 mg samples were ground into fine powder in liquid N_2. Pre-warmed (at 65 °C) 600 μL of DNA extraction buffer [*CTAB method* (Murray & Thompson, 1980): 100 mM Tris–HCl pH 8.0, 20 mM Na_2EDTA pH 8.0, 1.4 M NaCl, 2% CTAB, 0.4% (v/v) β-mercaptoethanol; *SDS method* (Doyle & Doyle, 1990): 100 mM Tris–HCl pH 8.0, 50 mM Na_2EDTA pH 8.0, 500 mM NaCl, 1.5% SDS, 0.38% sodium bisulfite] was added to ground samples, mixed well and incubated for 60 min at 65 °C with intermittent mixing every 5–10 min. Chloroform extraction was done using equal volume of ice-cold chloroform. Phase-separation was accom plished by centrifugation at 5000 rpm for 5 min. Upper aqueous phase was transferred to another Eppendorf tube and re-extracted with ice-cold chloroform for 3–4 times. DNA was precipitated with 2/3 volume of ice-cold isopropanol and kept at 4 °C overnight. DNA was pelleted down by centrifugation at 4 °C for 10 min at 10,000 rpm and washed with 75% ethanol and air dried for 1 h. Air-dried DNA was re-suspended in 200 μL TE buffer [10 mM Tris–HCl (pH 8.0), 1 mM EDTA (pH 8.0)], and treated with RNase A (10 μg/μL) at 37 °C for 30 min. RNase reaction was terminated by adding 1 volume of phenol: chloroform (1:1 v/v). DNA was again precipitated with 2 volumes of absolute ethanol mixed with 1/10 volume of 3 M NaAc at −20 °C for 3 h and centrifuged at 13,000 rpm for 10 min. DNA pellet was rinsed twice with 75% and once with 95% ethanol, air-dried, and dissolved into 100 μL sterile double distilled water (ddH_2O).

A commercial DNA extraction kit (SAAS-SCIQ-CBSR kit, Shanghai Academy of Agricultural Science, Shanghai, China), developed for DNA extraction from soybean seeds and their product (Liu et al., 2002), was also used. Since the amount of extract buffer provided in the kit (30 ml) was not sufficient for 50 extractions as marked, therefore the same procedure of chloroform extraction (4–5 times) as SDS method stated above, was applied to the samples. DNA precipitation and purification was accomplished using the buffers and columns provided in the SAAS-SCIQ-CBSR kit. The DNA was also dissolved in ddH_2O to a final volume of 100 μL.

For simplicity, the above-modified methods are hereafter still referred to as CTAB method, SDS method and SAAS method.

The purity of extracted DNA was checked on the basis of UV absorbance at 260/280 nm using a spectrophotometer (DU Series 500 model, Beckman Instrument, Inc. USA) according to manufacturer's instruction. The concentration of DNA was determined by using 5 μL of diluted DNA (1:10) on the basis of UV absorption at 260 nm (OD_{260}) with 1 OD equaling 50 μg/ml DNA (Sambrook, Fritsch, & Maniatis, 1989). The extraction yield was then calculated according to the dilution factor and initial mass of sample and presented as μg DNA per gram of sample. The quality of extracted DNA was also analyzed

Table 1
Rice materials used for DNA extraction and genotype fingerprinting

Code	Manufacturer[a]	Description
RC1	A	Infant rice cereal – plain
RC2	B	Infant rice cereal – with egg yolk
RC3	C	Infant multi cereal – with oat, wheat, beef and vegetables
RC4	D	Infant rice cereal – plain
RC5	E	Infant rice cereal – plain
RC6	F (2001 production)	Infant rice cereal – with fish and vegetables
RC7	F (2003 production)	Infant rice cereal – with fish and vegetables
RC8	G	Infant rice cereal – plain
RC9	G	Raw white rice, polished

[a] The letters are used for avoiding any conflict with manufacturers' interest.