British Food Journal

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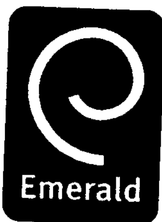

British Food Journal, Vol. 106 No. 9, 2004,
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Isolation and amplification of DNA from turmeric powder

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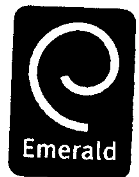
Keywords *Food additives, Food testing*

Abstract *An efficient protocol for the isolation of high molecular weight DNA from dry powdered samples of turmeric including market samples is described which will help in PCR based detection of adulteration in marketed turmeric powders. The method involves a modified CTAB (3 per cent) procedure with 2 M NaCl, 0.3 per cent β-mercaptoethanol coupled with purification of DNA in 30 per cent polyethylene glycol (8000). The yield of the DNA obtained from the samples varied from 2 to 4 μg/g tissue. The DNA obtained from the five different samples were consistently amplifiable (RAPD primers).*

Turmeric (*Curcuma longa* L.) is an important spice as well as a medicinal plant. India is the major producer and exporter of turmeric in the world. Turmeric is exported in dry, fresh, powder, oleoresin and oil forms. Turmeric powder is the major component of curry powder. It has many medicinal properties too. Turmeric powder is known to have antiinflammatory, antidiabetic, antimicrobial and cytotoxic properties. (Govindarajan, 1980; Tonnesn, 1986; Velayudhan *et al.*, 1999) besides anticancerous activity (Kuttan *et al.*, 1985; Rao *et al.*, 1995). Turmeric powder is obtained from the dried rhizomes. Dry turmeric has moisture content of 6-13 per cent, protein 6.3 per cent, fat 1 per cent, carbohydrates 69.4 per cent, ash 3.5 per cent, fiber 2.6 per cent, volatile oils 1.3-5.5 per cent and curcumin 2-6 per cent (Velayudhan *et al.*, 1999).

Though whole dried or fresh turmeric are usually free from adulteration, turmeric powder can be adulterated with powders of certain other species of *Curcuma* besides foreign starch (tapioca, arrowroot, etc.). Adulterated turmeric powder will have low curcumin content (Balasubramanian *et al.*, 1979) and it may also constitute a risk to public health. Powder of *Curcuma zedoaria*, a common adulterant in turmeric powder is known to be toxic (Latif *et al.*, 1979). Techniques like thin layer chromatography are not helpful in identifying cheaper *Curcuma* species in the market samples of turmeric (Govindarajan, 1980). DNA based techniques will be especially relevant in this context. However, turmeric powder being a recalcitrant material, isolation of amplifiable DNA is rather difficult (Bake *et al.*, 1990; Couch and Fritz, 1990; Rogers and Bendich, 1985). The present work reports a protocol for isolation of amplifiable DNA from turmeric powder including market samples.



Materials and methods

Powdered samples of *Curcuma longa*, *Curcuma zedoaria* and three popular market samples are used in the study. The protocol is as below:

This work was supported by an Adhoc scheme of the Indian Council for Agriculture Research (ICAR), Government of India. The authors are thankful to Dr V.A Parthasarathy, Director, Indian Institute of Spices Research, for providing the facilities for the work.

British Food Journal
Vol. 106 No. 9, 2004
pp. 673-678
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0007-070X
DOI 10.1108/00070700410558201

(1) weigh 2 g of each turmeric powder from *Curcuma longa* and *Curcuma zedoaria* as well as three market samples;
(2) homogenize the powder with 10 ml of 3 per cent CTAB buffer containing 0.3 per cent β-mercaptoethanol and transfer to 50 ml Oakridge tubes;
(3) incubate at 60°C for 2 h with intermittent shaking;
(4) add 3 ml of phenol: chloroform: isoamylalcohol (25 : 24 : 1) and 7 ml of chloroform: isoamylalcohol (24 : 1);
(5) mix by inversion for 15 min;
(6) centrifuge at 8,000 g for 15 min at 4°C;
(7) transfer the aqueous phase to a fresh Oakridge tube and add 0.6 volume of ice cold isopropanol;
(8) incubate at −20°C for 2 h to precipitate the DNA;
(9) pellet down the DNA at 4,000 g for 5 min and wash the pellet with 70 per cent ethanol;
(10) dry the pellet and dissolve in minimum volume of sterile double distilled water;
(11) add 10 μg/ml of RNAse and incubate at 37°C for 1 h;
(12) add equal volumes of phenol: chloroform: isoamylalchohol (25 : 24 : 1). Mix for 15 min. Centrifuge at 10,000 g for 15 min at 4°C. Take out the DNA supernatant and repeat the extraction once again;
(13) take the aqueous phase and add equal volume of chloroform: isoamylalcohol (24 : 1);
(14) centrifuge at 10,000 g for 15 min at 4°C using a Superspin R – V/FA from plastocrafts India Ltd;
(15) take out the aqueous phase and add equal volumes of 100 per cent ice-cold ethanol and keep at −20°C for 2 h to precipitate the DNA;
(16) pellet down the DNA at 4,000 g for 5 min and wash with 70 per cent ethanol to remove extra salts dissolved in double distilled water;
(17) add 200 μl 30 per cent of PEG 8000 to 500 μl of DNA sample mix and keep in room temperature for 30 min;
(18) centrifuge at 14,000 g for 15 min;
(19) wash the pellet with 80 per cent ethanol, dry off the ethanol and dissolve the pellet in nuclease free water or Tris-EDTA (10 : 1) buffer;
(20) store the samples frozen at −20°C.

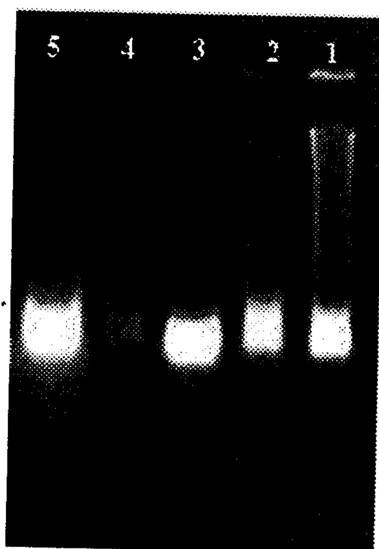
RAPD analysis

PCR amplification of the isolated DNA was done by using random decamer primers obtained from OPERON Technologies, Alameda, USA. The RAPD reaction was performed in 25 μl reaction volume with 20 ng genomic DNA, 0.2 mM dNTPs, 0.3 μM primer, 2 mM $MgCl_2$ and 1 U *Taq* DNA polymerase using a PTC – 100 Programmable Thermal Controller, M J Research, Inc, USA, according to Willams *et al.* (1990). The amplified products were visualized by electrophoresis in a 2 per cent agarose gel containing 0.5 μg/ml of EtBr and documented by a gel documentation system (Alpha Imager 2220, USA).

Results and discussion

Turmeric rhizomes are rich in polysaccharides, proteins, polyphenols, alkaloids and other secondary metabolites that can hamper DNA isolation and amplification. Dry and powdered samples pose further problems in DNA isolation (Do and Adams, 1991; Webb and Knapp, 1990). The problem will be compounded, if the candidate tissue is a dried powdered sample of recalcitrant rhizomatous material like turmeric. The preferred method of DNA extraction for a given recalcitrant tissue can be determined only through trial and error methods. The DNA isolated from recalcitrant tissue by the original CTAB method of Doyle and Doyle (1987) is usually found to be contaminated with high levels of proteins and polysaccharides making it opaque and difficult to dissolve. Nucleic acids form complexes with secondary compounds such as proteins, polysaccharides or polyphenols released by cell disruption leading either to embedding of DNA in a sticky gelatinous matrix or to an off-coloured product (Guillemaut and MarcchaL-Drouard, 1992). Polysaccharides also coprecipitate with DNA after alcohol addition during DNA isolation and lead to high viscosity solutions (Demeke and Adams, 1992; Do and Adams, 1991).

In many plants it is observed that increasing CTAB and NaCl concentrations increase the yield of cellular DNA (Smith *et al.*, 1991). CTAB is generally used as a detergent to separate out polysaccharides. Similarly a NaCl concentration greater than 1.5 M removes the polysaccharides (Fang *et al.*, 1992). Substitution of ethanol for isopropanol in the last step of DNA precipitation is shown to increase the yield and purity of DNA many times (Bult *et al.*, 1992; Rogers and Bendich, 1985). We have successfully isolated DNA from dried powdered samples of turmeric by following a modified CTAB method of Doyle and Doyle (1987). The DNA isolated with the unmodified Doyle and Doyle protocol gave a smear on electrophoresis (lane 1), was not in sufficient quantity and was not amplifiable (Figure 1). In addition to the unmodified



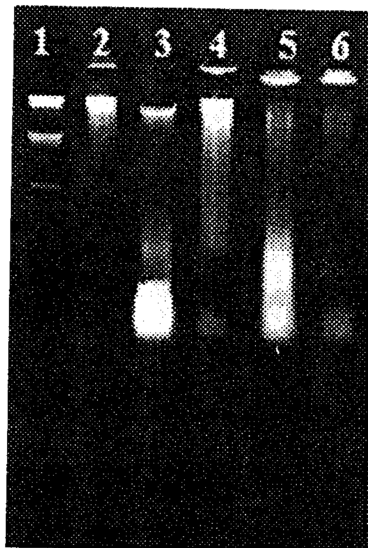
Note: Lane 1 – *Curcuma longa* Lane 2 – *Curcuma zedoaria,* Lane 3 – Market sample 1, Lane 4 – Market sample 2, Lane 5 – Market sample 3

Figure 1. DNA isolated from the dried turmeric powder/market samples using unmodified Doyle and Doyle method

Doyle and Doyle protocol we also tried the modified protocol of Singh *et al.* (1999) for DNA isolation from market samples of dry tea and Cheng *et al.* (1997) for dried rhizomes of *Coptis*. Both these methods failed to yield amplifiable DNA. Hence the original Doyle and Doyle protocol was modified by precipitating the DNA in ethanol instead of isopropanol, increasing the concentrations of CTAB to 3 per cent, NaCl to 2 M, β-mercaptoethanol to 0.3 per cent, adding phenol: chloroform: isoamylalcohol along with chloroform: isoamylalcohol in the isolation protocol (step 4), repeating phenol: chloroform: isoamylalcohol extraction in the purification step (step 12) of the protocol and final precipitation of the DNA with 30 per cent polyethylene glycol (PEG 8000). The results could be exactly reproduced in repeated extractions.

In the polymerase chain reaction, plant DNA contaminated with ubiquitous secondary compounds and carbohydrates is especially difficult to amplify. The precipitation of DNA samples with polyethylene glycol (PEG 8000) yields highly purified homogenous DNA preparations devoid of proteins and polysaccharides. In addition, the coloured pigment present in the samples can also be removed from the DNA preparations by purifying with polyethylene glycol (Agudo *et al.*, 1995; Dixit, 1998).

Spectrophotometric measurements of DNA samples at 260 and 280 nm gave an absorbance ratio (A260/A280) of 1.82-1.91 indicating high purity of the DNA samples. The quality of the total DNA isolated from dried turmeric powder was also checked by agarose gel electrophoresis. Agarose gel electrophoresis of DNA samples showed conspicuous bands of high molecular weight DNA about 22,000 bp with a little shearing (Figure 2). The yield of DNA isolated from the samples ranged from 2 to 4 μg/gm dried powder of turmeric. Figure 3 shows RAPD banding patterns amplified with primer OPA – 02 (5′ TGCCGAGCTG 3′) using DNA of *Curcuma longa*, *Curcuma zedoaria* and the three market samples. The major band of size 750 bp



Note: Lane 1 – Marker Eco RI Hind III double digest, Lane 2 – *Curcuma longa* Lane 3 – *Curcuma zedoaria*, Lane 4 – Market sample 1, Lane 5 – Market sample 2, Lane 6 – Market sample 3

Figure 2.
DNA isolated from the dried turmeric powder/market samples using modified Doyle and Doyle method

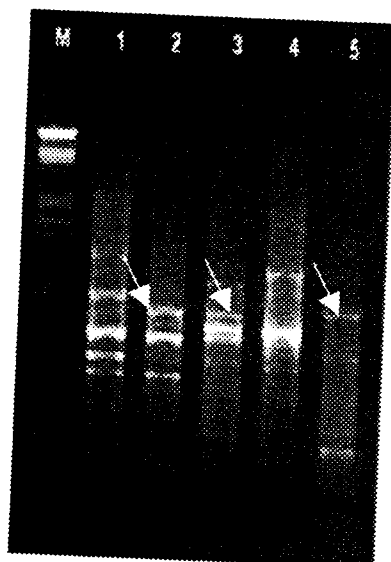


Figure 3.
RAPD profile of the DNA samples isolated from dried turmeric powder/market samples amplified with primer OPA – 02 (5′ TGCCGAGCTG 3′)

Notes: M – Marker Eco R I Hind III double digest, Lane 1 – *Curcuma longa*, Lane 2 – *Curcuma zedoaria*, Lane 3 – Market sample 1, Lane 4 – Market sample 2, Lane 5 – Market sample 3. The arrows indicate the *C. zedoaria* specific bands

produced by the primer which is specific to *C. zedoaria* (lane 2) is observed in the lanes 3 and 5 indicating that these market samples are possibly adulterated with *C. zedoaria*.

This present protocol will be useful in determining the purity/contamination of powdered samples of turmeric which may be very significant from nutritional point of view.

References

Agudo, L.C., Gavidia, P., Perez-Bermudez, P. and Segura, J. (1995), "PEG precipitation, a required step for PCR amplification of DNA from wild plants of *Digitalis obscura* L.", *Bio Techniques*, Vol. 18, pp. 766-8.

Bake, S.S., Rugh, C.L. and Kamalay, J.C. (1990), "DNA and RNA isolation from recalcitrant plant tissues", *Bio Techniques*, Vol. 9, pp. 268-72.

Balasubramanian, N., Kumar, K.R. and Anandswamy, B. (1979), "Packaging and storage studies on ground turmeric (*C. longa* L.) in flexible consumer packages", *Indian Spices*, Vol. 16, pp. 10-13.

Bult, C., Kallersjo, M. and Suh, Y. (1992), "Amplification and sequencing of 16S/18S rDNA from gel purified total plant DNA", *Plant Molecular Biology Reporter*, Vol. 10, pp. 272-84.

Cheng, K.T., Chang, H.C., Su, C.H. and Hsu, F.L. (1997), "Identification of dried rhizomes of *Coptis* species using random amplified polymorphic DNA", *Bot. Bull. Acad. Sin.*, Vol. 38, pp. 241-4.

Couch, J.A. and Fritz, P.J. (1990), "Isolation of DNA from plants high in phenolics", *Plant Molecular Biology Reporter*, Vol. 8, pp. 8-12.

Demeke, T. and Adams, R.P. (1992), "The effects of plant polysaccharides and buffer additives on PCR", *Bio Techniques*, Vol. 12, pp. 332-4.

Dixit (1998), "A simple and rapid procedure for isolation of *Amaranthus* DNA suitable for fingerprint analysis", *Plant Molecular Biology Reporter*, Vol. 16, pp. 1-8.

Do, N. and Adams, R.P. (1991), "A simple technique for removing plant polysaccharide contaminants from plant DNA", *Bio Techniques*, Vol. 10, pp. 162-6.

Doyle, J.J. and Doyle, J.L. (1987), "A rapid DNA isolation procedure from small quantities of fresh leaf tissue", *Phytochemistry Bulletin*, Vol. 19, pp. 11-15.

Fang, G., Hammer, S. and Grumet, R. (1992), "A quick and inexpensive method for removing polysaccharides from plant genomic DNA", *Bio Techniques*, Vol. 13, pp. 52-6.

Govindarajan, V.S. (1980), "Turmeric-chemistry, technology and quality", *CRC Critical Reviews in Food Science and Nutrition*, Vol. 12, pp. 199-301.

Guillemaut, P. and MarcchaL-Drouard, L. (1992), "Isolation of plant DNA: a fast, inexpensive and reliable method", *Plant Molecular Biology Reporter*, Vol. 10, pp. 60-5.

Kuttan, R., Bhanumathy, P., Nirmala, K. and George, M.C. (1985), "Potential anti-cancer activity of turmeric (*C. longa* L.)", *Cancer Letter*, Vol. 29, pp. 197-202.

Latif, M.A., Moris, T.R., Miah, A.M., Hewitt, D. and Ford, J.E. (1979), "Toxicity of shoti (Indian arrwroot: *Curcuma zedoaria*) for rats and chicks", *Br. J. Nutr.*, Vol. 41 No. 1, pp. 57-63.

Rao, C.V., Riverson, A., Simi, B. and Reddy, B.S. (1995), "Chemoprevention of colon carcinogenesis by dietary curcumin, naturally colorin plant phenolic compound", *Cancer Research*, Vol. 55, pp. 259-66.

Rogers, S.O. and Bendich, A.J. (1985), "Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues", *Plant Molecular Biology Reporter*, Vol. 5, pp. 69-76.

Singh, M., Bandana and Ahuja, P.S. (1999), "Isolation and PCR amplification of genomic DNA from market samples of dry tea", *Plant Molecular Biology Reporter*, Vol. 17, pp. 171-8.

Smith, J.F., Sytsma, K.J., Shoemaker, J.S. and Smith, R.L. (1991), "A qualitative comparison of total cellular DNA extraction protocols", *Phytochemistry Bulletin*, Vol. 23, pp. 2-9.

Tonnesn, H.H. (1986), "Chemistry stability and analysis of curcumin: a naturally occuring drug molecule", *Osolo*, p. 91.

Velayudhan, K.C., Muralidharan, V.K., Amalraj, V.A., Gautum, P.L., Mandal, S. and Dineshkumar (1999), *Curcuma* Genetic Resources Scientific Monograph No.4, National Bureau of Plant Genetic Resources, New Delhi.

Webb, D.M. and Knapp, S.J. (1990), "DNA extraction from a previously recalcitrant plant genus", *Plant Molecular Biology Reporter*, Vol. 8, pp. 180-5.

Willams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990), "DNA polymorphism amplified by arbitrary primers are useful as genetic markers", *Nucleic Acid Research*, Vol. 18, pp. 6531-5.