Acta Alimentaria, Vol. 30 (1), pp. 113–118 (2001)

Preliminary communication

COMPARISON OF TWO METHODS IN PURIFICATION OF MEAT-DNA FOR PCR

A. JÁNOSI* and J. SZAMOS

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

(Received: 24 May 2000; accepted: 18 September 2000)

The Wizard Clean up System and a three-phase partitioning (TPP) method were used to purify genomic pork-DNA of various food samples for amplification. Quality of DNA purified by Wizard resin and partitioning was controlled by spectrophotometer and electrophoresis, respectively. A 108 bp fragment from the porcine growth hormone gene was applied, according to MEYER and co-workers (1994). Of all the samples prepared, amplicons were obtained by the pork-DNA specific PCR. Partitioning was found to be an efficient DNA purification step in preparation of PCR-grade DNA.

Keywords: DNA purification, three-phase partitioning, PCR

To meet requirements in the field of food analysis, application of specific, reliable, quick and sensitive methodologies are of increasing importance. One of the high sensitivity methods is PCR, the use of which for detection of food components has been successful even in the investigation of heat-treated foodstuff. Microorganisms, genetically modified organisms, raw and processed foods can be detected by PCR (MEYER & CANDRIAN, 1996). However, PCR is inhibited by a number of components originating from the foodstuff or chemicals used for purification of DNA (ROSSEN et al., 1992).

Three-phase partitioning is a protein purification method used primarily for crudes. It was found by LOVRIEN and co-workers (1987) that systems containing water, tert-butanol, dissolved proteins and ammonium-sulphate form a third phase or middle layer, on shaking and successive centrifugation. In case of enzymes, if the third phase is soluble, purification of small apolar molecules is achieved even though the specific activity of the given enzyme remains unchanged. It was found earlier that following proteinase K digestion

* To whom correspondence should be addressed.
E-mail: a.janosi@cfri.hu

0139-3006/2001/$ 5.00

of foods, wheat-DNA could be separated from other constituents by three phase partitioning and applied successfully to PCR (SZAMOS et al., 1998). The objective of the present work was to compare the Wizard DNA Clean up System and the method of interfacial partitioning in the purification of pork meat genomic DNA.

1. Materials and methods

Raw meat samples were obtained from slaughterhouse, foodstuff containing pork meat, Zala meat, pork sausage (red meat products) and winter salami (ripened, fermented product), were purchased in supermarket. All the reagents used were of analytical grade.

1.1. Extraction, purification and characterisation of DNA

DNA isolation was carried out by the method of MEYER and co-workers (1994). Minced meat (0.6 g) was transferred to a sterile 1.5 ml Eppendorf tube; 860 µl extraction buffer (10 mmol TRIS-HCl, 150 mmol NaCl, 2 mmol EDTA, 1% SDS, pH 8.0), 100 µl 5 mol guanidine hydrochloride and 40 µl proteinase K (20 mg ml^{-1} solution) was added. The mixture was incubated at 55 °C for 3 h. After digestion, the samples were centrifuged at 14.500×g for 10 min.

1.1.1. Wizard DNA clean-up procedure. Four hundred and fifty µl aqueous phase was purified with 1 ml of DNA Wizard resin following the instruction of the manufacturer.

1.1.2. Three-phase partitioning method. To prepare DNA for PCR, 450 µl of aqueous phase was pipetted into a 2 ml screw capped tube, then 312 µl of saturated ammonium-sulphate solution (sAS) was added. This solution was thoroughly mixed, then 236 µl of tert-butylalcohol was added. The system was vigorously shaken then let to stand for 15 min. Following low speed centrifugation, 500 µl of the lower aqueous phase was pipetted into a clean Eppendorf tube and 680 µl of sAS and 365 µl of tert-butylalcohol were added.

The system was treated as above, but the disc formed at the interface was separated and dissolved in 450 µl of distilled water.

In the case of Pastanaria sauce /Maggi/ and sausage 145 µl of proteinase-K reaction mixture was diluted to 600 µl then 450 µl of sAS and 310 µl of tert-butylalcohol were added. When partitioning was completed, 1 ml of aqueous phase was pipetted into a bio-vial Beckman (4.5 ml) and 1.36 ml of sAS and 730 µl of tert-butylalcohol were added. Following phase separation, DNA-disc (third phase) was dissolved in 700 µl of TRIS-EDTA buffer, pH 8.0.

1.2. Polymerase chain reaction

The primers of MEYER and co-workers (1994) were used. 250 ng of template DNA was added to 50 µl of reaction mix in a 0.5 ml MARSH tube containing 1× reaction buffer, 2 U Taq polymerase, 0.2 mmol of dATP, dCTP, dGTP and dTTP, 0.5 mmol of each primer and 2 µg ml^{-1} bovine serum albumin. The samples were denatured at 94 °C for 3 min (hot start), then 35 cycles of denaturation at 94 °C for 5 s, was performed, followed by annealing at 60 °C for 30 s and extension at 72 °C 40 s, with a final extension at 72 °C for 3 min (PDR-91, BLS thermocycler). The reaction was stopped by cooling to 4 °C. Ten µl of PCR mixture was separated using a 8% polyacrylamide gel-electrophoresis (AUSUBEL et al., 1989) with TRIS-boric acid-EDTA (0.89 mmol TRIS, 0.89 mmol boric acid, 2 mmol EDTA, pH 8) running buffer, for 3 h at 54 V. The bands were visualized by staining with ethidium bromide and photographed under UV transillumination.

2. Results

UV spectra of DNA-solutions illustrating product related differences are shown in Fig. 1 (JASCO 7850 uv/vis spectrophotometer). The highest DNA yield was obtained for winter-salami with high meat and low water content, while about half of the amount of DNA (compared to salami) could be prepared for heat-treated meat products and Maggi sauce. As it can be seen, purification with a good yield was achieved by the first stage TPP compared to spectrum of the crude DNA solution (Fig. 1). The amount of the DNA prepared by the second TPP amounts roughly one third of the first stage, while its quality proved to be suitable for amplification. Results of DNA purification are summarised in Table 1. In several cases a single three-phase partitioning was sufficient

Table 1

Comparison of Wizard and three-phase partitioning method of DNA purification

Meat products	Wizard DNA preparation technique		Three-phase partitioning (first)		Three-phase partitioning (second)	
	Σ DNS µg	A_{260}/A_{280}	Σ DNS µg	A_{260}/A_{280}	Σ DNS µg	A_{260}/A_{280}
Zala meat	13.4	2.0	57.9	1.6	25.4	1.9
Pork sausage	7.4	1.8	30.8	1.5	–	–
Winter salami	30.4	1.9	47.4	1.2	19.3	1.6
Luncheon meat	31.1	1.9	58.08	1.5	20.9	2.0
Pastanaria Maggi sauce	7.7	1.8	17.4	1.7	–	–

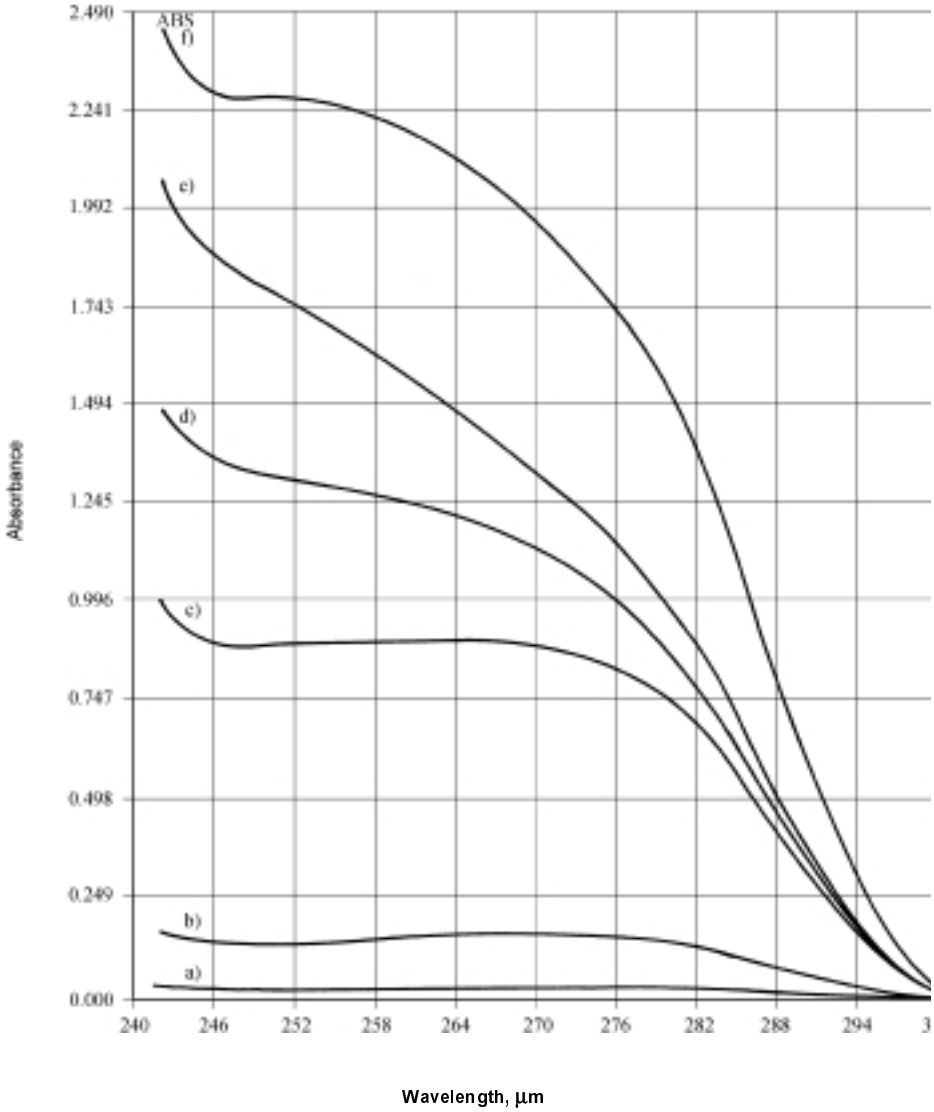

Fig. 1. The UV spectra of proteinase-K digested foodstuff samples. a: fat; b: lard; c: pork sausage; d: luncheon meat conserve; e: goliat salami; f: winter salami

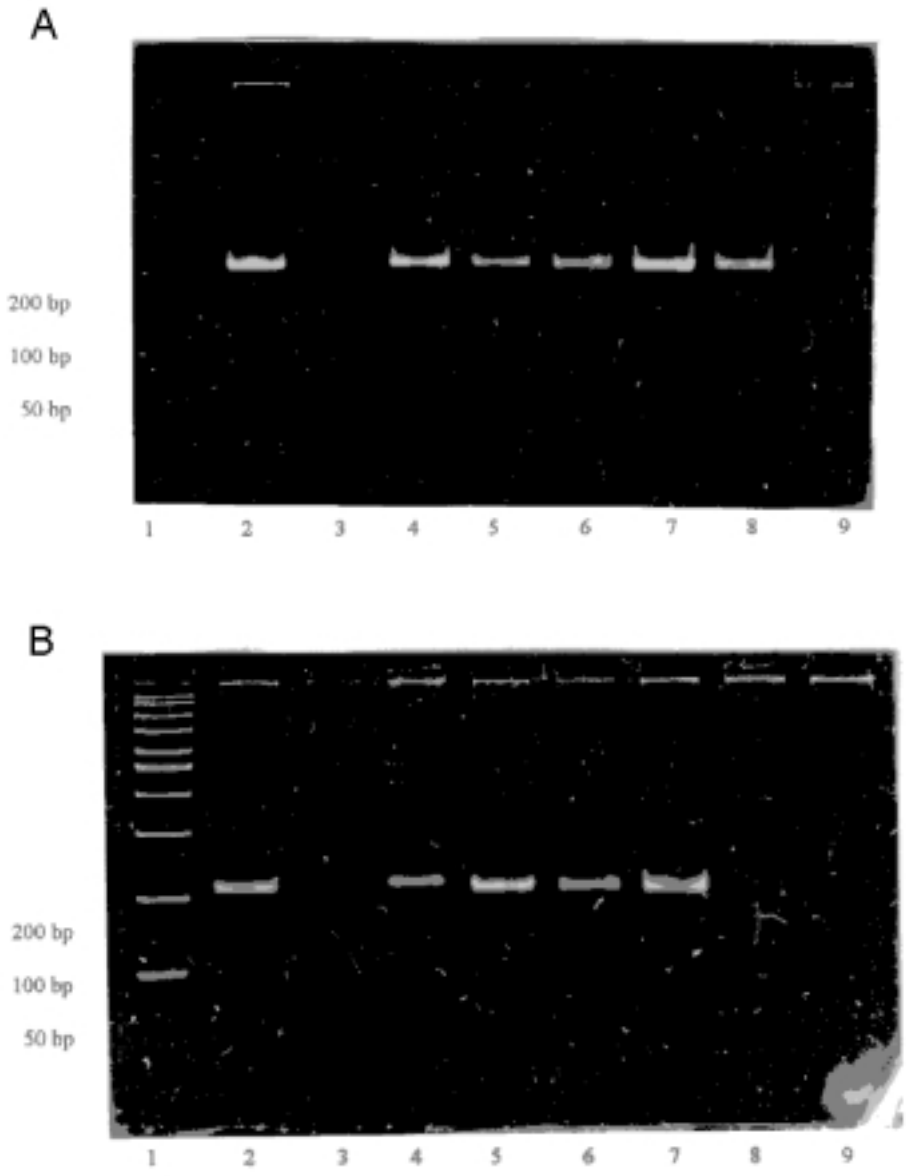

Fig. 2. PCR product of different meat and meat products DNA (DNA was purified with A-Wizard technique, B-TTP method). Lanes: 1: DNA ladder (50–2000 bp); 2: positive control (pork meat); 3: negative control; 4: pork sausage; 5: Zala meat; 6: luncheon meat conserve; 7:winter salami; 8: Pastanaria Maggi sauce; 9: beef meat

to prepare higher amounts of DNA, though accompanied by small amount of protein compared to that obtained by the Wizard system. Higher purity DNA could be obtained by a second partitioning, more over in case of salami, ratios of A_{260} and A_{280} for DNA partitioned are close to those of prepared by the Wizard system. DNAs prepared by both methods could be amplified by PCR at 250 ng 50 μl^{-1}. In Fig. 2 the electrophoretogram of PCR products of different food samples are shown. Signals (108 bp) were obtained for pork meat and all food samples investigated, while negative controls did not result in amplicons.

3. Conclusion

From different foods containing pork meat PCR-grade DNA could be purified by three-phase partitioning. Compared to purification method by Wizard resin, TPP is rapid and of lower cost, moreover it can easily be scaled up. This fact is of significance when inhomogenic materials are investigated. Compared to common DNA isolation methods (ZIMMERMANN et al., 1998) TPP can be primarily characterised by its low cost and short time of manipulations.

*

This work was supported by Ministry of Agriculture and Regional Development. The authors would like to thank Mrs. C. MOLNÁR for technical assistance and Mr. I. MAKK for photographs.

References

AUSUBEL, F. M., BRENT, R., KINGSTON, R. E., MOORE, D., SEIDMAN, J., SMITH, J. & STRUHL, K. (1989): *Short protocols in molecular biology*. Harvard Medical School, Boston, pp. 72–75.

LOVRIEN, R. E., GOLDENSOPH, C., ANDERSON, P. C. & ODEGARD, B. M. (1987): Three phase partitioning (TTP) via t-butanol: Enzymes separation from crudes. -in: BURGES, R. (Ed.) *Protein purification micro to macro*. Alan R.Liss.Inc. New York, pp. 131–148.

MEYER, R. & CANDRIAN, L. (1996): PCR-based DNA analysis for the identification and characterisation of food components. *Lebensm. Wiss. Technol., 29,* 1–9.

MEYER, R., CANDRIAN, L. & LÜTHY, J. (1994): Detection of pork in heated meat products by polymerase chain reaction. *J. A.O.A.C., 77,* 617–622.

ROSSEN, J., NORSKOV, P., HOLMSTROM, K. & RASMUSSEN, O. F. (1992): Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA extraction solutions. *Int. J. Fd Microbiol., 17,* 37–45.

SZAMOS, J., AUBRECHT, E. & GELENCSÉR, É. (1998): Detection of wheat by adapted polymerase chain reaction (PCR) methodology. *Acta Alimentaria, 27,* 87–95.

ZIMMERMANN, A., LÜTHY, J. & PAULI, U. (1998): Quantitative and qualitative evaluation of nine different extraction methods for nucleic acids on soya bean food samples. *Z. Lebensm. Unters. Forsch, 207,* 81–90.