Veterinary Research Communications, 31(Suppl. 1) (2007) 385–388
DOI: 10.1007/s11259-007-0043-2

Detection of Genetically Modified Organisms in Food: Comparison Among Three Different DNA Extraction Methods

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Vodret, B., Milia, M., Orani, M.G., Serratrice, G. and Mancuso, M.R., 2007. Detection of genetically modified organisms in food: Comparison among three different DNA extraction methods. *Veterinary Research Communications*, **31**(**Suppl. 1**), 385–388

Keywords: foods, GMO, real time PCR

Abbreviations: GMO, genetically modified organism; PCR, polymerase chain reaction

INTRODUCTION

European Union consumers are mostly against the use of genetic engineering in the agro-food sector. According to the new European Community regulations on genetically modified food and feed, it is necessary to label food products when the concentration of the genetically modified material is higher than 0.9% (Regulation L268/1, L268/24). In Europe a lot of new transgenic events have recently been authorized (Commission Decision 772/2005, 47/2006, 68/2006, 69/2006). Compulsory labelling and the introduction of new transgenic events highlight the need to develop new analytical methods to quantify the different genetic modified organisms in food. The quantitative real time Polymerase Chain Reaction is currently the main technique used for this purpose and the extraction of DNA from food samples represents the first step for its application. A large number of protocols are currently available: in addition to the standard laboratory techniques, other procedures based on specific kits with faster extraction times can be applied.

In this work, the real time PCR results of the DNA extracted from 45 food samples with three different methods are reported.

MATERIALS AND METHODS

DNA extraction and purification: 45 food samples of soybean from the Italian market were investigated. Samples were homogenized with a mixer; then the DNA was extracted using three different extraction techniques: CTAB extraction (Lipp *et al*., 1999); extraction with a PrepMan™ Ultra commercial kit purchased from Applied Biosystems, whose protocol (Applied Biosystems, 2001) was integrated using a purification step with a Wizard mini column (Promega) (Bononi *et al*., 2002); using the AbiPrism 6100 Nucleic Acid instrument (Applied Biosystems, 2002). After measurement by spec-

trophotometer (Perkin Elmer) and 0.8% agarose gel electrophoresis, extracted DNA was analyzed using real time PCR.

Qualitative analysis: The endogenous lectin gene of soya was detected using a fluorogenic probe and specific primers (Vaitilingom *et al*., 1999). To evaluate transgenic presence in the samples, 35S screening (CaMV) was applied.

Quantitative analysis: to identify and quantify the transgenic species, real time PCR, using a fluorogenic probe and specific primers for Roundup Ready soybean quantification (Vaitilingom *et al*., 1999) in comparison to the lectin gene, was applied. All analyses were performed by application of the real time PCR technique, using the TAQMAN chemistry and ABIPRISM 7700 Sequence Detection System instrument and a fluorogenic probe and specific primers purchased from Applied Biosystems. PCR reactions were run using the following parameters:

$$50^\circ\ \mathrm{C}/120''; 95^\circ\ \mathrm{C}/10'; 50\ \text{cycles}\ \left(95^\circ\ \mathrm{C}/15''; 60^\circ\ \mathrm{C}/60''\right).$$

RESULTS

45 food samples, purchased from different Italian supermarkets and consisting of soybean-derived processed food for human consumption, were analyzed at the "Istituto Zooprofilattico Sperimentale della Sardegna". Results obtained from spectrophotometric measurements and gel agarose electrophoresis showed that all the three extraction meth-

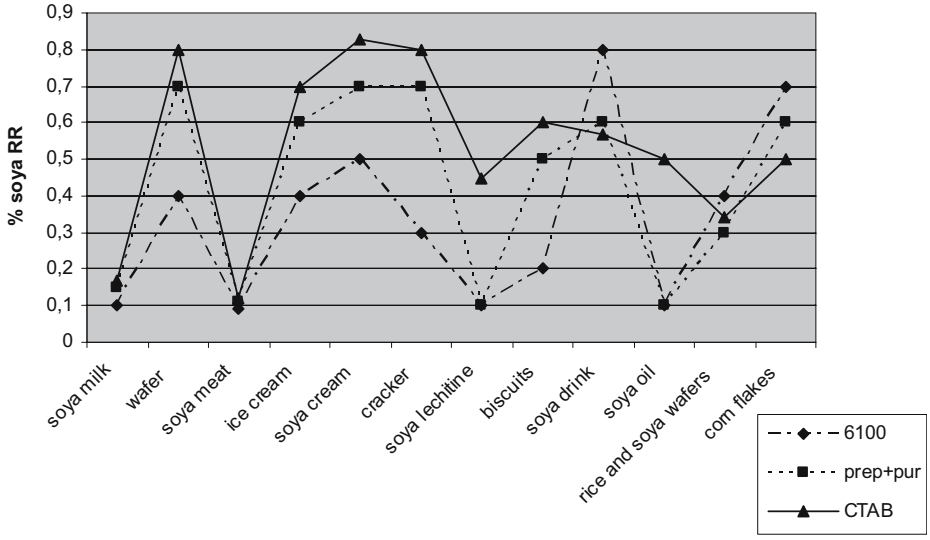

Figure 1. Comparison among results obtained with the three methods

ods are suitable to extract DNA from food. However, DNA extraction with the commercial kit on oil, lecithin and processed food obtained by degradative processes (such as bakery products), was difficult. Real time PCR analysis showed the presence of lectin gene in each sample extracted with different methods. The 35S promotor was found in twelve samples and Roundup Ready soybean was quantified only in these samples. Results are shown in Figure 1.

DISCUSSION

Quantification is important in the analysis of genetically modified organisms in food. The real time PCR tecnhique is widely known as the most sensitive and highly specific method for gmo quantification in food. The critical point for application of this technique is DNA extraction (low degradation, 260/280 ratio, inhibitor free). Looking at our results we can conclude that all the three methods are appropriate for extraction of DNA from food. The CTAB method used in a lot of laboratories, despite needing a long time for its execution, was suitable for the quantification of different processed foods, even complex ones such as oil and lechitine. The PrepMan™ Ultra method followed by purification using the Wizard column has the advantage of being fast, and the quantitative results obtained are comparable to those from the CTAB technique, with some problems for more complex matrixes. We believe that further studies and modifications should be made for use of the 6100 extractor, because results obtained for DNA extraction from oil and lecithin and bakery products were different with respect to other methods.

Our preliminary data show that the commercial kit and the automatic extractor have the advantage of being fast and reliable for simple food. These methods can be used to obtain accurate answers in a short time in GMO monitoring, with the aim of performing screening and then quantification analysis of different transgenic events with real time PCR.

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