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# Anion-exchange chromatography using CIM® DEAE disks as a method of choice for DNA isolation from lecithin

## Ionsko-izmenjevalna kromatografija z uporabo CIM® DEAE diskov kot metoda za osamitev DNA iz sojinega lecitina

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**Abstract.** The most important prerequisite for the application of PCR-based methods, among them the detection and quantification of genetically modified organisms (GMOs) is the ability to extract significant amounts of DNA of adequate quality from the sample under investigation. The sample of interest in our work was soybean lecithin with expected low DNA content. The aim of this study was to set up a fast and effective HPLC (High Performance Liquid Chromatography) method using CIM® (Convective Interaction Media, BIA Separations d.o.o., Ljubljana, Slovenia) DEAE (DiEthylAminoEthyl) anion-exchange disk monolithic columns (disks) for the isolation of DNA from soybean lecithin samples. As the reference isolation procedure we used CTAB (CethylTrimethylAmmonium Bromide) method, which is widely used in GMO detection. It was demonstrated, that CIM® DEAE disks allow efficient isolation of DNA from soybean lecithin. Furthermore, in comparison with the CTAB method, the method was less time-consuming and reduced the use of some aggressive chemicals. The quality of isolated DNA was tested with spectrophotometric analysis, agarose gel electrophoresis and by amplification of soybean specific *lectin* gene with qualitative and real-time PCR. The isolated soybean DNA was of adequate quantity and quality for PCR analysis, even though mostly degraded, present in small amounts and contaminated with some impurities, among them potential PCR inhibitors. The study expanded the applicability of monolithic columns in the isolation of biomolecules from highly processed food materials and their potential use for nucleic acids detection.

**Keywords:** DNA isolation, detection of GMO, soybean lecithin, chromatography, monoliths, CIM, CTAB, PCR, real-time PCR

## Introduction

Rapid development in DNA technology, such as PCR, DNA sequencing, DNA cloning techniques, DNA restriction analysis and *in situ* hybridization have created the need for rapid analytical and preparative separation methods for nucleic acids (Huber 1998). Taking into consideration the difference in type, composition and degree of processing of products analyzed, DNA extraction protocols must be developed on a case-by-case basis and are of the highest importance (Gryson & al. 2004).

Among possible applications, extraction of significant amounts of recombinant DNA of adequate quality, for the use in PCR-based detection methods, represents a crucial step in GMO detection (Wurz & al. 1999). Different extraction methods can influence the efficiency of DNA multiplication (Cankar & al. 2006). According to the European legislation, a labelling treshold value of 0,9 % has been introduced for food and feed, which is a maximum limit for the accidental and unavoidable presence of GMO material per ingredient (Regulation (EC) No. 1829/2003 2003). Labelling is even mandatory for derivatives of GMOs, which do not contain DNA or protein resulting from genetic modification (Gryson & al. 2004).

In 2006, the principle biotech crop, occupying 57 % of global biotech area, continued to be biotech soybean (James 2006). Therefore, our main research object was soybean lecithin, which is the most important byproduct of the edible soybean oil processing industry because of its functionality and wide application in food systems and industrial utility. Lecithin production is an aggressive procedure, that yields lecithin as a highly processed hydrophobic matrice (Szuhaj 1983).

The most widely and frequently used approaches for the isolation of DNA from plant material and plant-derived products are: the CTAB method (Lipp & al. 1999); DNA-binding silica columns in form of commercial kits and the combination of both (Terry & al. 2002). The existing methods for the isolation of DNA from foods are not useful for all matrices and cannot always fulfil the expectations regarding the quantity and quality of DNA. More recently, chromatography gained importance in the isolation of biomolecules, such as recombinant proteins, peptides, polysaccharides and nucleic acids, with the introduction of a novel type of chromatographic supports called monoliths (Levison & al. 1998). CIM® disks allow fast and flow-unaffected separation of several biomolecules, including nucleic acids (Štrancar & al. 2002). With the use of anion-exchange chromatography with CIM® disks, the separation of oligonucleotides (Podgornik & al. 1999); isolation of plasmid DNA from complex matrix, such as bacterial cell lysate (Branovič & al. 2004); separation of bacterial genomic DNA of size up to 200 kbp (Benčina & al. 2004) and even isolation of DNA from processed foods wih decreased DNA content, such as maize and its derivatives (Jerman & al. 2005) is possible.

The aim of this study was to set up a fast and effective HPLC method using CIM® DEAE disks for the isolation of soybean DNA from soybean lecithin samples with expected low DNA content. As the reference isolation procedure we used CTAB method, which has been widely used in GMO detection (Zimmermann & al. 1998, Lipp & al. 1999, Lipp & al. 2001, Gryson & al. 2004). The quality and quantity of the isolated DNA was tested with spectrophotometric analysis, agarose gel electrophoresis and by amplification of soybean specific *lectin* gene with qualitative and real-time PCR. Gene l*ectin* is normally used for the calculation of the GMO (Roundup Ready® soybean) content, by dividing the amount of transgene with the amount of species specific gene (*lectin* gene) (Holst-Jensen & al. 2006).

## Materials and methods

### Samples

Six different soybean lecithin samples produced from soybean of known or unknown origin and frequently used in manufacture of foods, were used. The samples were: different liquid soybean lecithin (Samples 1, 4, 5, 6 and 7) and granulated soybean lecithin (Sample 8). As control samples with expected absence of soybean DNA, powdered wheat lecithin (Sample 2) and spelt lecithin (Sample 3) were used, even though in the experiment, very small amounts of soybean DNA were unexpectedly detected. The DNA was extracted using two different extraction procedures. Using modified CTAB method, Samples 2, 3, 5, 6, 7 and 8 were used with 10 g starting material. Isolation of DNA from Sample 1 and 4, using CTAB method was not performed, due to the insufficient amounts of these two samples. Using anion-exchange chromatography with CIM® DEAE columns, 10 g starting material was used for Samples 2, 3, 5, 6 and 7 ; and 6,72 g, 4,3 g and 20 g for Samples 1, 4 and 8 respectively.

**DNA extraction using modified CTAB method**

As a reference procedure for extraction of DNA from lecithin samples, we used CTAB method, as described by Lipp & al. 1999, modified with the addition of hexane and double distilled water and greater amounts of starting material (see section: Samples). We used the following starting amounts of chemicals: 10 ml of CTAB-1 buffer (20 g/l CTAB (Acros organics, Germany), 1,4 M NaCl (Merck, Germany), 0,1 M Tris HCl (Sigma, Germany), 20 mM EDTA (ethylenediaminetetraacetic acid, Kemika, Croatia), pH 8), 20 ml of hexane (Merck) and 10 ml of double distilled sterile water. The isolations were done in 50 ml-tubes. After the initial incubation at 65 ºC for 30 min, the aqueous phase was removed and the treatment with addition of 20 mL of hexane was repeated, followed by centrifugation and two successive steps of chloroform addition (20 mL) to the aqueous phase. All the following steps were performed as proposed by Lipp & al. 1999. The final DNA was redissolved in 100 µl of double distilled sterile water, instead of TE buffer, and was stored at –20 ºC.

**Initial lecithin extract preparation for chromatographic isolations**

The extracts from lecithin samples were prepared using the first few steps of the formerly described modified CTAB method. After the second hexane treatment and centrifugation, the aqueous phase was removed and filtered through a filter of regenerated cellulose with pores of 0,45 µm. The extracts were then diluted with double distilled sterile water at the ratio of 3:1 (3 volume units of the extract and 1 volume unit of double distilled sterile water), before the separation, as proposed by Jerman & al. (2005).

**Separation unit, equipment and mobile phase for chromatography**

Separations of DNA from impurities were performed on commercially available anion exchange (DEAE) methacrylate-based CIM® disk monolithic column (BIA Separations d.o.o, Ljubljana, Slovenia), also used by Jerman & al. (2005). The disk in the appropriate housing was integrated in HPLC system. A gradient HPLC system (Knauer, Germany) was built of two pumps, a dynamic mixing chamber, an injection valve with 100 or 500 µl injection loop, a variable-wavelength detector and an interface box, connected to computer with Windows operation system and EuroChrom 2000 software. In all the experiments, the monitor wavelength of 260 nm was used.

On the basis of the work done by Jerman & al. 2005, the following conditions were chosen for the separation of DNA from the samples: loading buffer was 50 mM Tris (Merck), 0,25 M NaCl (Merck) and elution buffer 50 mM Tris, 2 M NaCl. The pH of the buffers was adjusted to pH 8, using HCl (Merck). Loading time was 6 min, followed by 2 min linear gradient of increasing (0,25 – 2 M) NaCl concentration, flow rate ($\Phi_v$) was 1 ml/min. Because of larger amount of impurities in the sample, washing with loading buffer was prolonged in comparison to work proposed by Jerman & al. 2005.

In experiments, where volume of the sample exceeded injection loop volume, multiple injections were performed to avoid loading sample with pumps.

**DNA recovery from the HPLC fractions**

The fractions with purified DNA were manually collected and DNA was precipitated with absolute ethanol, using the procedure proposed by Jerman & al. (2005), modified by the suspension of DNA in 100 µL of double distilled sterile water in the last phase.

**Spectrophotometric analysis**

The measurements of the UV absorbance of the isolated samples of DNA at 230, 260 and 280 nm, for DNA purity determination, were performed on Pharmacia Biotech Ultraspec 2000 UV-VIS spectrophotometer in 0,5 ml quartz cuvette. DNA samples (5 µl) were 100 X diluted with double distilled sterile water (495 µl) as proposed by Karcher (1995). The purity was determined on the basis of $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratios (Somma 2004).

**Qualitative PCR analysis**

PCR primer pair GMO3 and GMO4 (Invitrogen, California, USA) were used for amplification (Querci & al. 2004). This primer pair is specific for the sequence of the single-copy *lectin* gene and yields a PCR product of 118 base pairs. The primers were prepared in a double distilled sterile water. The amplification was performed on Bio-Rad PCR i-cycler 3.021 instrument, with the reaction mixture and amplification programme proposed by Querci & al. (2004). The PCR reaction mixture negative control contained 2 µl of double distilled sterile PCR water instead of the sample. The following control samples for the presence of *lectin* gene were used: positive control – Sample A (DNA from conventional soybean, lectin positive) and negative control – Sample B (corn DNA, *lectin* negative).

**Agarose gel electrophoresis**

Agarose gel electrophoresis was performed for determination of the degree of degradation of the isolated DNA and for visualization of the PCR products. The size-separation was performed with 3 µl of the PCR product solution on a Bio-Rad Power Pac 3000 electrophoresis unit, using 1,5 % (for determination of the degree of degradation) or 2,5 % agarose gel (for visualization of PCR products) in 1 X TAE buffer. 100 bp marker (Fermentas) was used as molecular weight size marker. The electrophoresis was running for 180 – 210 min at 60 V. The gel was subsequently evaluated by 10 min ethidium bromide staining (0,5 µg/ml EtBr) and visualized using Bio-Rad Gel Doc 2000 unit and Quantity One software.

**Real-time PCR analysis**

Real-time PCR was performed for amplification of the sequence of soybean specific *lectin* gene and to determine the eventual inhibition effect of PCR reaction in the sample extracts. Reaction was performed on an ABI PRISM 7900 HT instrument (Applied Biosystems) as proposed by Cankar & al. 2006, with exception of 10 µl reaction mixture used, containing 1 X Taq Man® Universal PCR Master Mix (Applied Biosystems). Taqman® RRS probe (Applied Biosystems, Foster City, CA) was labelled with 5`-FAM (6-carboxyfluoresceine) and 3`-TAMRA (5-carboxytetramethylrhodamine) and TM-lectin-F and TM-lectin-R were used as primers (Bundesamt für Gesundheit, (Eds): Schweizerisches Lebensmittelbuch. CD-ROM, 311.510, BBL-EDMZ 3003, Bern, Switzerland 2001). Reactions were performed in parallels under standard conditions (AbiPrism 7900 HT Sequence detection system and SDS enterprise database, User guide, Applied Biosystems 2002). For determination of PCR inhibition, 10-fold dillutions of the samples were used. Treshold cycle (Ct) values were determined using SDS 2.1 software (Applied Biosystems) after manual adjustment of the baseline and fluorescence threshold. For determination of PCR inhibition, from the difference in Ct values beetween undiluted and 10-fold diluted DNA, slopes of curves and corresponding efficiencies of PCR reactions were calculated using equation $E = (10^{(-1/slope)}) - 1$(Ginzinger 2002).

## Results and discussion

Isolation of DNA from lecithin with anion-exchange chromatography using CIM® DEAE disks was tested and effective separation with clear peaks was achieved. As an example, chromatogram of Sample 5 is shown in Fig. 1. The peak area depended on the volume of the sample extracts used,

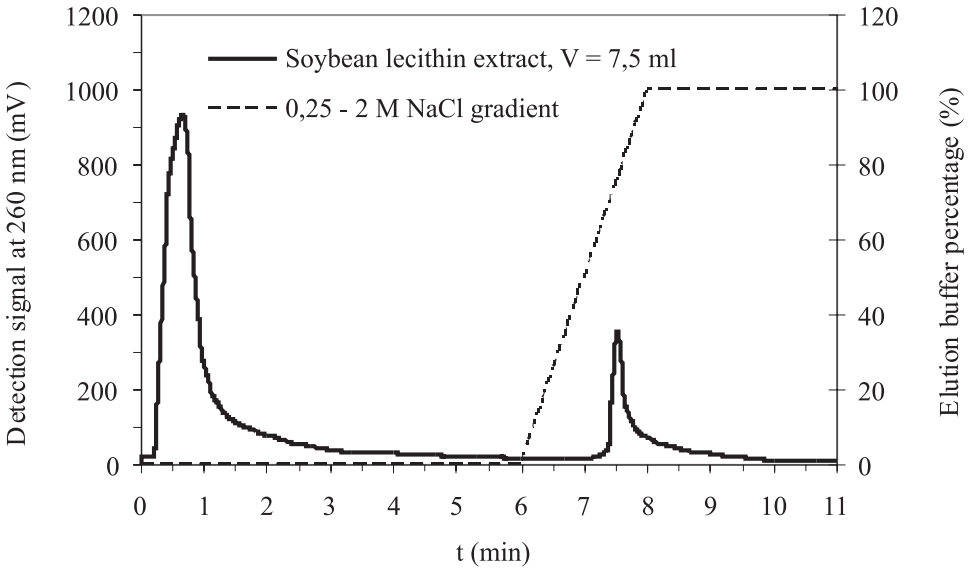


Figure 1: Chromatogram of DNA isolation from extract of soybean lecithin (Sample 5), with anion-exchange chromatography using CIM® DEAE monolithic columns (7,5 mL is correspondent to 10 g of soybean lecithin sample; 10 mL load volume after dilution with double distilled sterile water at the ratio of 3:1, mobile phase pH 8, flow rate $\Phi_v$ = 1 ml/min; using 500 µl inj. loop; 1st peak – elution of impurities, 2nd peak – elution of DNA).

Slika 1: Kromatogram osamitve DNA iz ekstrakta sojinega lecitina (vzorec 5) z ionsko-izmenjevalno kromatografijo z uporabo CIM® DEAE diskov (7,5 mL ekstrakta odgovarja 10 g vzorca sojinega lecitina; naložen volumen 10 mL po redčenju z dvakrat destilirano vodo v ramerju 3:1, pH = 8, $\Phi_v$ = 1 ml/min; uporaba 500 µl inj. zanke; 1. vrh – elucija nečistoč, 2. vrh – elucija DNA).

which was 0,075 – 7,5 ml, and on the amount of the DNA, which was sample dependent (Tab. 1). In chromatography, using CIM® DEAE disks, retention of dsDNA is influenced by electrostatic interactions between the surface potentials created by the positively-charged DEAE groups of an anion-exchange stationary phase and the negatively-charged phosphodiester groups of dsDNA (Huber 1998). In comparison with the CTAB method, the steps after the initial extraction, consisting of purification with chloroform, few centrifugations and a 60 min precipitation (Lipp & al. 1999), were substituted with a single-step chromatographic isolation. The method was less time-consuming and reduced the use of some aggressive chemicals. CIM® DEAE disks were previously successfully used by Jerman & al. (2005) for isolation of DNA from corn meal and thermally pre-treated corn meal for the preparation of »polenta«.

In our experiment, the quality of the isolated DNA was tested using: agarose gel electrophoresis, spectrophotometric analysis, qualitative PCR and real-time PCR. To test the degradation of DNA, agarose gel electrophoresis was applied. The isolated DNA from soybean lecithin samples showed very

Table 1: Total gained extract volumes for different lecithin samples, loading extract volumes for separation and corresponding chromatogram peak areas for DNA from lecithin extracts, isolated with anion-exchange chromatography using CIM® DEAE disks (detection signal at 260 nm; Samples 1, 4, 5, 6 and 7 – liquid soybean lecithin; Sample 8 – granulated soybean lecithin; negative control Samples 2 and 3 – powdered wheat and spelt lecithin).

Preglednica 1: Dobljeni volumni ekstraktov vzorcev lecitina, na diske nanešeni volumni ekstraktov in pripadajoče površine kromatogramov za DNA, osamljeno iz vzorcev lecitina z ionsko-izmenjevalno kromatografijno z uporabo CIM® DEAE diskov (merjenje absorbance pri 260 nm; vzorci 1, 4, 5, 6 in 7 – tekoči sojin lecitin; vzorec 8 – granuliran sojin lecitin; vzorca 2 in 3 za negativno kontrolo – lecitin pšenice in pire v prahu).

| Sample number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Total extract volume (ml) | 4,6 | 10 | 6,5 | 8 | 7,6 | 17 | 7,5 | 7,1 |
| Loading extract volume for separation (ml) | 4,5 | 0,075 | 0,075 | 7,5 | 7,5 | 7,5 | 7,5 | 7 |
| Peak area (mVmin) | 20,8 | 122,6 | 513,0 | 278,8 | 128,2 | 88,8 | 80,2 | 88,4 |

weak signal or no signal on 1,5 % agarose gel and was highly degraded, for both extraction methods used (data not shown). The term »DNA quality« is defined as the degree of degradation of DNA and by the presence of potential inhibitors of the PCR (Meyer 1999).

Spectrophotometric analysis was performed to determine DNA purity ($A_{260}/A_{280}$, $A_{260}/A_{230}$) . As shown in Tab. 2, values of the $A_{260}/A_{280}$ ratios showed very variable and sample dependent results. For soybean lecithin samples (Samples 1, 4 and 5-8), ratios were 1,9 or 2, which suggested, that isolation

Table 2: Comparison of the calculated $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratio values for purity determination of DNA from extracts, isolated with anion-exchange chromatography using CIM® DEAE disks or with modified CTAB procedure (spectrophotometric analysis of absorption at 230, 260 and 280 nm; Samples 1, 4, 5, 6 and 7 – liquid soybean lecithin; Sample 8 – granulated soybean lecithin; negative control Samples 2 and 3 – powdered wheat and spelt lecithin). Remarks: / – analysis for Samples 1 and 4, isolated with CTAB method, were not performed.

Preglednica 2: Primerjava izračunanih vrednosti razmerij $A_{260}/A_{280}$ ter $A_{260}/A_{230}$ za čistosti DNA, osamljene z ionsko-izmenjevalno kromatografijo z uporabo CIM® DEAE diskov ali z modificirano CTAB metodo (spektrofotometrične meritve absorbance pri valovnih dolžinah 230, 260 in 280 nm; vzorci 1, 4, 5, 6 in 7 – tekoči sojin lecitin; vzorec 8 – granuliran sojin lecitin; vzorca 2 in 3 za negativno kontrolo – lecitin pšenice in pire v prahu). Opombe: / – analize za vzorce 1 in 4, osamljene po metodi CTAB, niso bile opravljene.

| | CIM® DEAE | | CTAB | |
|---|---|---|---|---|
| Sample number | $A_{260}/A_{280}$ | $A_{260}/A_{230}$ | $A_{260}/A_{280}$ | $A_{260}/A_{230}$ |
| 1 | 2 | 0,7 | / | / |
| 2 | 1,5 | 1,3 | 1,8 | 2,4 |
| 3 | 1,4 | 0,2 | 1,7 | 0,8 |
| 4 | 2 | 0,5 | / | / |
| 5 | 1,9 | 1,7 | 1,7 | 1 |
| 6 | 1,9 | 1,7 | 1,3 | 1,3 |
| 7 | 2 | 1,4 | 2 | 1 |
| 8 | 2 | 1,4 | 2 | 1 |

using CIM® DEAE disks satisfactory purified the DNA of proteins (Somma 2004), with exception of wheat and spelt lecithin (Samples 2 and 3). The modified CTAB method showed acceptable results for Samples 2, 7 and 8 and worse results for Samples 3, 5 and 6. Values of the $A_{260}/A_{230}$ ratios showed a high degree of contamination of DNA by substances, such as carbohydrates, peptides, phenols or aromatic compounds (Somma 2004), for almost all lecithin samples analysed, using either CIM® DEAE disks or modified CTAB method as isolation methods. The values, with exeption of Sample 2, isolated using CTAB method, were all under 2,2 (Tab. 2).

Amplification potential of the isolated DNA was tested by amplification of the sequence of *lectin* gene with qualitative PCR. DNA, isolated from all 8 lecithin samples using CIM® DEAE disks, contained amplifiable soybean DNA (Fig. 2). Additionally, *lectin* gene DNA was also amplified in the samples of wheat and spelt lecithin, which suggested that traces of soybean were also present in these two samples. The amplification of *lectin* gene was also performed for the samples, isolated using modified CTAB method. The PCR products were visible for 4 out of 6 lecithin samples (data not shown). Gryson & al. (2002) showed that degumming in the refining process is the most important step in transferring DNA from crude soybean oil to the water fraction (lecithin).

To test the possibility of PCR inhibition, real-time PCR was performed. DNA was assayed for the *lectin* gene at two 10-fold dilutions (undiluted and 10-fold diluted sample). In Tab. 3, the numerical results (Ct and E values) for real-time PCR of samples, isolated with modified CTAB method and using

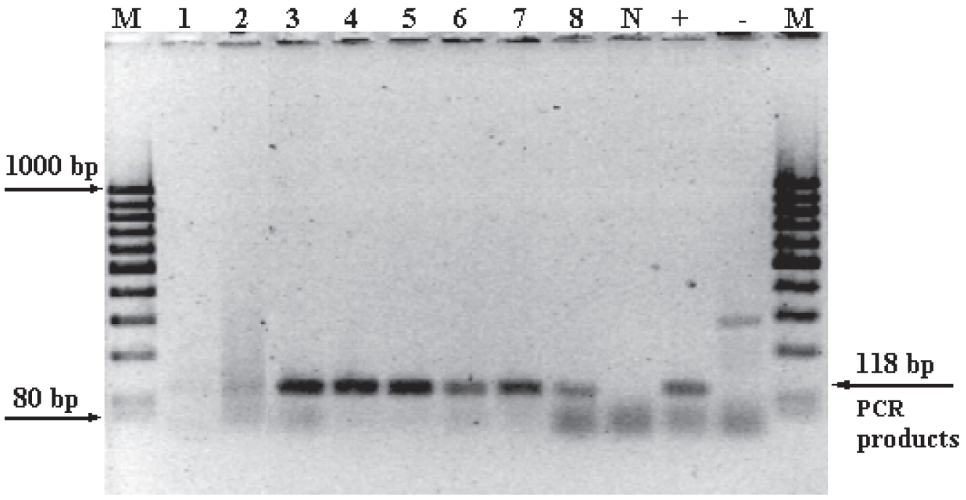


Figure 2: Agarose gel electrophoresis for visualization of PCR products (118 bp size) of amplification of soybean DNA (GMO3 and GMO4 primers used, specific for soybean *lectin* gene) isolated with anion-exchange chromatography using CIM® DEAE disks (lane 1, 4, 5, 6, 7 in 8: Samples 1, 4, 5, 6 and 7 (liquid soybean lecithin) and 8 (granulated soybean lecithin); lane 2: Sample 2 for negative control (wheat lecithin); lane 3: Sample 3 for negative control (spelt lecithin); lane N: PCR reaction mixture negative control; lane +: reference Sample A for pozitive control (*lectin* poz.); lane –: reference Sample B for negative control (*lectin* neg.); M: 100 bp molecular weight size marker).

Slika 2: Agarozna gelska elektroforeza pomnožkov PCR (velikost 118 bp), za odkrivanje sojine DNA v vzorcih sojinega lecitina (uporaba začetnih oligonukleotidov GMO3 in GMO4, specifičnih za sojin *lectin* gen), osamljene z ionsko-izmenjevalno kromatografijo z uporabo CIM® DEAE diskov (proge 1, 4, 5, 6, 7 in 8: vzorci 1, 4, 5, 6, 7 (tekoči sojin lecitin) in 8 (granuliran sojin lecitin); proga 2: vzorec 2 za negativno kontrolo (lecitin pšenice); proga 3: vzorec 3 za negativno kontrolo (lecitin pire); proga N: neg. kontrola PCR reakcijske mešanice; proga + : ref. vzorec A za poz. kontrolo (*lectin* poz.); proga –: ref. vzorec B za neg. kontrolo (*lectin* neg.); M: molekulski označevalec dolžin pomnožkov 100 bp).

Table 3: Ct and E values for determination of inhibition of *lectin* gene amplification with real-time PCR.
Preglednica 3: Ct in E vrednosti za določanje inhibicije pomnoževanja *lectin* gena s PCR v realnem času.

| Isolation method | Sample number | | Ct | E (%) | Inhibition |
|---|---|---|---|---|---|
| CIM® DEAE | 1 | | 34,35127 | 9900# | YES# |
| | | | 33,43676 | | |
| | | 10 X R | 33,82257 | | |
| | | | 34,87834 | | |
| | 2 | | 32,795334 | 364# | YES# |
| | | | 33,864666 | | |
| | | 10 X R | 35,62797 | | |
| | | | 34,094425 | | |
| | 3 | | 23,401003 | 142 | YES |
| | | | 23,426117 | | |
| | | 10 X R | 26,106941 | | |
| | | | 25,897501 | | |
| | 4 | | 26,325968 | 142 | YES |
| | | | 26,243254 | | |
| | | 10 X R | 28,75998 | | |
| | | | 28,975714 | | |
| | 5 | | 26,04777 | 110 | NO |
| | | | 26,083706 | | |
| | | 10 X R | 29,08203 | | |
| | | | 29,172789 | | |
| | 6 | | 30,621397 | 184 | YES |
| | | | 30,521042 | | |
| | | 10 X R | 32,649963 | | |
| | | | 32,955513 | | |
| | 7 | | 26,466047 | 89 | NO |
| | | | 26,284533 | | |
| | | 10 X R | 29,997795 | | |
| | | | 29,856054 | | |
| | 8 | | 30,247734 | 128# | YES# |
| | | | 29,934256 | | |
| | | 10 X R | 33,386494 | | |
| | | | 32,515114 | | |
| CTAB | 2 | | / | / | / |
| | | | / | | |
| | | 10 X R | / | | |
| | | | / | | |
| | 3 | | ~ | ~ | YES |
| | | | ~ | | |
| | | 10 X R | 25,738462 | | |
| | | | 25,745096 | | |
| | 5 | | 31,464403 | 105# | NO# |
| | | | 31,637342 | | |
| | | 10 X R | 33,677097 | | |
| | | | 35,842133 | | |
| | 6 | | 32,044857 | 80# | NO# |
| | | | 32,421013 | | |
| | | 10 X R | 36,970497 | | |
| | | | 35,285854 | | |
| | 7 | | 32,17105 | 142# | YES# |
| | | | 32,099506 | | |
| | | 10 X R | 33,97479 | | |
| | | | 35,506104 | | |
| | 8 | | ~ | ~ | YES |
| | | | ~ | | |
| | | 10 X R | 35,271873 | | |
| | | | 34,3671 | | |

Legende – Legenda:
Ct; E – treshold cycle; efficiency
/: *lectin* gene (soybean DNA) not present
~: results lower than the limit of detection
10 X R: 10-fold diluted parallel samples
[#] – variability of the Ct values between parallel samples, because of low amounts of the isolated soybean DNA.

CIM® DEAE disks, are presented. Inhibitors were present in some of the lecithin samples, isolated with modified CTAB method and also in some samples, isolated using CIM® DEAE disks. Because of the very low amounts of soybean DNA, the variability of the Ct values between some parallel sample dilutions is present. With 10-fold linear dilutions, a 100 % efficiency of the real-time PCR corresponds to a -3,32 slope of a curve (Ginzinger 2002). In our work, we used a slope interval from -4,1 to -2,9, corresponding to efficiencies from approximatelly 75 to 121 % respectively, which suggested an absence of PCR inhibitors. Complex plant-derived matrices, usually contain a number of PCR inhibitors, such as polysaccharydes, proteines and phenolic compounds. Additionally, some extraction chemicals can also act as PCR inhibitors, such as ethanol, EDTA, NaCl, chloroform, isopropanol, CTAB and other. Their presence can influence the amplification of DNA (Rossen & al. 1992).

## Conclusions

The aim of this study was to investigate the applicability of CIM® DEAE disks for the isolation of DNA from highly processed hydrophobic matrice, with decreased DNA content – soybean lecithin. It was demonstrated, that:

– the anion-exchange chromatography using CIM® DEAE disks, allows efficient isolation of DNA from soybean lecithin. In comparison with the CTAB method, the method was less time-consuming and reduced the use of some aggressive chemicals;
– the isolated DNA was of adequate quality for the real-time PCR analysis;
– the soybean DNA was present in small amounts and mostly contaminated with some impurities, including potential PCR inhibitors;

The study expanded the applicability of monolithic columns in the isolation of biomolecules from highly processed food materials and their potential use for nucleic acids detection.

## Povzetek

Prvi in zelo pomemben korak v procesu detekcije nukleinskih kislin, je njena osamitev in čiščenje, ki mora zagotoviti DNA primerne količine in kakovosti za nadaljnjo uporabo. Eden od aktualnih primerov uporabe osamljene DNA je pomnoževanje genskih elementov v reakcijah PCR, za zagotavljanje natančnega določanja prisotnosti GSO v živilih. Sojin lecitin je eden izmed najbolj pomembnih stranskih produktov v industriji procesiranja soje, ki je danes najbolj razširjena gensko spremenjena poljščina. Zagotovo je njegova uporaba najpomembnejša in najbolj razširjena v živilski/prehranski industriji. Da bi potrošnikom zagotovili sledljivost proizvodov, je odkrivanje morebitno prisotne gensko spremenjene soje v tem proizvodu nujno. Cilj našega dela je bil postaviti učinkovito metodo za osamitev DNA soje iz sojinega lecitina z ionsko-izmenjevalno kromatografijo z uporabo CIM® DEAE diskov. V ta namen smo skušali osamiti DNA iz osmih vzorcev lecitina z uporabo ionsko-izmenjevalne kromatografije s CIM® DEAE diski, ter ločeno iz šestih vzorcev lecitina z referenčno metodo CTAB. Za negativno kontrolo smo uporabili pšenični in pirin lecitin, kjer smo pričakovali odsotnost sojine DNA. Za uspešno sta se izkazali tako ionsko-izmenjevalna kromatografija z uporabo CIM® DEAE diskov, kot referenčna metoda CTAB za izolacijo DNA iz sojinega lecitina. S pomnoževanjem za sojo specifičnega *lectin*

gena s kvalitativno reakcijo PCR in reakcijo PCR v realnem času smo preverili kakovost osamljene DNA. Prisotnost sojine DNA smo dokazali v vseh vzorcih sojinega lecitina. Osamljena DNA je bila torej prisotna v dovoljšnji količini, njena kakovost pa je bila primerna za pomnoževanje v reakciji PCR. Z reakcijo PCR v realnem času smo ugotovili, da so bili v nekaterih ekstraktih analiziranih vzorcev lecitina prisotni inhibitorji reakcije PCR. Agarozna gelska elektroforeza je pokazala močno razgrajenost osamljene DNA. Spektrofotometične analize za določanje čistosti nukleinskih kislin so pokazale, da so v večini analiziranih ekstraktov vzorcev lecitina poleg DNA prisotne tudi številne nečistoče. Z ugotovitvami smo razširili področje možne uporabe CIM® DEAE diskov pri osamitvi biomolekul iz visoko procesiranih živil in njihovo potencialno uporabo v molekularnih metodah določanja nukleinskih kislin.

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