Effects of thermal-acid treatment on degradation and amplification of wheat and maize DNA

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Summary
Food processing causes DNA degradation and may hinder polymerase chain reaction (PCR) based detection of food components. Effects of heating (100 °C) and low pH (2 and 4) on degradation and amplification of maize and wheat DNA were investigated. DNA degradation was assessed by agarose gel electrophoresis and PCR amplification. PCR amplicons of different length ranging from 67 bp to 550 bp specific to plant chloroplast, maize or wheat genomes were used to monitor extent of DNA degradation. Combined thermal-acid treatment intensified time-dependent DNA degradation observed at thermal treatment and more significantly affected amplification than heating. Increase of acidity from pH 4 to pH 2 had negative influence on the integrity and amplifiability of genomic DNA. PCR amplicons were degraded in a time- and size-dependent manner. Small DNA fragments ranging from 67 bp to 140 bp were amplified in all samples while 226 bp, 259 bp and 550 bp amplicons were not detected in certain highly processed samples. Our findings demonstrate that processing parameters such as temperature, pH and exposure time as well as amplicon size have crucial importance for DNA integrity and accurate detection of food components.

Keywords
thermally processed foods; acid treatment; *Triticum aestivum; Zea mays*; plant genomic DNA; polymerase chain reaction

Thermal and acid treatments are widely used in food processing and preservation. They are applied to many types of food products in order to eliminate pathogenic microorganisms, to prolong shelf life, to provide desirable characteristics, taste and flavour. However, technological processing, in particular under strict conditions, may disrupt molecules of food components, which has an impact on molecular diagnostics for food authenticity and traceability. DNA-based polymerase chain reaction (PCR) represents an effective tool for food analysis since DNA is comparatively stable during food processing [1–3]. Nevertheless, food processing may elicit DNA degradation to such an extent that food analysis based on DNA may be affected [4, 5]. This demands comprehensive studies on DNA degradation within processed food matrices. Special attention is paid to investigation of plant DNA due to the following reasons. Firstly, plant-derived food represents an important part of human nutrition and animal feed. Secondly, genetically modified foods derived from transgenic

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plants have been increasingly distributed worldwide. Thirdly, plant materials are often used as ingredients or additives in various products.

In recent years, impact of different processing parameters such as temperature, pressure, pH, ultraviolet (UV) radiation and particle size on plant-derived food DNA was extensively studied [6–9]. It was shown that matrix particle size and thermal treatment have a significant effect on the amount of DNA extracted from food matrix and the size of amplicons obtained. The particle size of flour, degree and duration of thermal treatment, may negatively influence the detection or quantification of food components after technological processing of foods [10]. UCHINO et al. [11] assessed thermostability of wheat DNA in heated food products and reported that degradation ratio of DNA was affected by both heating temperature and time. Studies of HUPFER et al. [12], BAUER et al. [6], BERGEROVA et al. [8] and GODALOVA et.al. [13] revealed that pH and temperature are important factors at DNA degradation in food matrices. BAUER at al. [6] showed a rapid decrease in the maximal detectable length of plant DNA under acidic conditions (pH 4.75). The authors stated that DNA can hardly be detected in acidic foodstuffs, in particular when these are heat-treated. The major impact on DNA integrity was exerted by the combination of high pressure, high temperature and low pH [8, 13]. As each food and each processing step contribute to a unique environment [6], new data on the kinetics of DNA degradation in various food matrices under different processing conditions are of particular interest for reliable food analysis.

In this study, DNA degradation was investigated in raw and experimentally treated important food crops, namely, wheat and maize. The effects of heating at 100 °C and low pH (2 and 4) on stability and amplifiability of plant DNA were studied. Degradation of DNA was monitored by evaluation of PCR amplification efficacy in the range 67–550 bp. Different amplicons of chloroplast, wheat and maize genomes were analysed in specific processing conditions. New DNA markers for wheat identification were proposed.

MATERIALS AND METHODS

Heat and acid treatment, sample preparation

The seeds of wheat (*Triticum aestivum* var. *aestivum)* and maize (*Zea mays* var. *indentata*) were purchased at local markets in Tbilisi (Georgia). Amounts of 150 g each of maize and wheat grains were put in 400 ml distilled water at 22 °C, pH 6.5 separately in different glass containers. The samples were boiled and subjected to thermal treatment at 100 °C till 300 min. Prior to boiling, the temperature increase from 22 °C to 100 °C took approximately 25 min. Samples were taken before putting in water, at the beginning of boiling and then afterwards in 60 min intervals. Subsequently, the samples were dried at room temperature and milled by electric grinder (Siemens, Munich, Germany) to obtain flour. To investigate the combined effect of temperature and pH, 150 g wheat and maize seeds were put separately in three different glass containers filled with 400 ml 0.4% NaCl solution of different pH (6.5, 4.0 and 2.0). Acid pH was adjusted by addition of 0.1 $mol \cdot l^{-1}$ acetic acid (Sigma-Aldrich, St. Louis, Missouri, USA). The samples from each container were treated at 100 °C during 120 min. Samples were taken at the beginning of boiling and then afterwards in 30 min intervals, dried at room temperature and ground to obtain powder.

DNA extraction

The genomic DNA was extracted from 100 mg of each sample using the cetyltrimethyl ammonium bromide (CTAB)-based method as was previously described [14]. The quantity and purity of the DNA was evaluated by UV spectrophotometry using a Jenway Genova Life Science Analyzer (Cole-Parmer, Stone, United Kingdom).

PCR analysis

Tab. 1 presents a list of oligonucleotide primers used in the study. Two new wheat-specific primer pairs, namely ACACf/ACACr targeting acetyl-coenzyme A carboxylase gene and GLU1/GLU2 targeting low-molecular weight glutenin subunit, were designed using PrimerQuest tool (Integrated DNA Technologies, Coralville, Iowa, USA), sequence alignment tool Align_MTX [15] and FastPCR [16]. The other PCR primers were taken from previous studies [17–21]. Oligonucleotide primers were synthesized and provided by Integrated DNA Technologies.

PCR amplification was performed in a volume 25 μl, the reaction mixture containing standard Taq Buffer with 1.5 $mmol \cdot l^{-1}$ $MgCl_2$, 1.25 units of Taq DNA polymerase (New England BioLabs, Ipswich, Massachusetts, USA) and 200 $\mu mol \cdot l^{-1}$ of each dNTP (Deoxynucleotide solution mix; New England BioLabs, Ipswich, Massachusetts, USA), 0.5 $\mu mol \cdot l^{-1}$ of each primer and 60–70 ng of genomic DNA. The reactions were carried out in a PCR thermal cycler Techne TC-412 (Techne, Minneapolis, Minnesota, USA).

Amplification conditions for primers

Tab. 1. Oligonucleotide primers used in PCR.

Target	Designation	Sequence	Amplicon size [bp]	Reference
Plant chloroplast genome	Plant1 Plant2	CGAAATCGGTAGACGCTACG GGGGATAGAGGGACTTGAAC	550	[17]
Wheat low-molecular-weight glutenin	TEN2 TEN3	GATATGTCGATGCAGCGGTG ACTAGTTTGGGCGGGTCACA	67	[18]
Wheat acetyl-coenzyme A carboxylase	ACACf ACACr	CCGGTGCTGCATTCCAACCA GCCGGCAAGACCTTGGCGAAT	134	This study
Wheat low-molecular-weight glutenin	GLU1 GLU2	CAAGGTATTCCTCCAGCAGTGCAGC GGGTTGGGAAACACATTGGCCCA	259	This study
Maize zein gene	ZEINf ZEINr	ACACCACCGACCATGGCAGC TGGTGGCAAGTGCGCTGGAA	102	[19]
Maize invertase gene	IVRf IVRr	TCTCCCGTGATCCTGCCCCG GCTTGGACCGTGCGCTTCCT	140	[20]
Maize invertase gene	IVTAS1 IVTAS2	CCGCTGTATCACAAGGGCTGGTACC GGAGCCCGTGTAGAGCATGACGATC	226	[21]

Plant1/Plant2 were as follows: initial denaturation of 4 min at 95 °C; 35 cycles of 30 s at 95 °C, 30 s at 62 °C, 2 min at 72 °C; final extension of 5 min at 72 °C.

Amplification conditions for maize-specific primers IVTAS1/IVTAS2, IVRf/IVRr and ZEINf/ZEINr were as follows: initial denaturation of 3 min at 95 °C; 40 cycles of 30 s at 95 °C, 30 s at 63 °C, 35 s at 72 °C; final extension of 5 min at 72 °C.

The wheat-specific PCR with primers TEN2/TEN3 was conducted in the following conditions: initial denaturation of 2 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C; final extension of 3 min at 72 °C.

In this study, the amplification conditions were optimized for wheat genome-specific new primer pairs, namely, ACACf/ACACr and GLU1/GLU2. The optimized parameters included primer concentration (0.1–1.0 μmol·l^{-1}), number of cycles (30–50), annealing temperature (55–67 °C) and elongation time (20–50 s). The same optimum conditions were determined for both primer pairs, such as 95 °C initial denaturing for 3 min, followed by 40 cycles of 95 °C denaturation for 30 s, 65 °C annealing for 30 s, 72 °C extension for 35 s; 72 °C final extension for 3 min.

Agarose gel electrophoresis

The integrity and quantity of DNA was assessed by agarose gel electrophoresis using an apparatus from VWR International (Radnor, Pennsylvania, USA). Gels of 1.0% and 2.0% SeaKem LE agarose (Cambrex, East Rutherford, New Jersey, USA) were utilized to evaluate the degradation of genomic DNA and formation of PCR products. Electrophoresis was performed in 1× TAE (Tris-acetate) buffer containing 1 μg·ml^{-1} ethidium bromide (Sigma-Aldrich). DNA bands were visualized under UV light and photographed using a gel documentation system PhotoDoc-It imaging system (UVP, Upland, California, USA). In addition, the band intensity of PCR products on agarose gels was determined by image processing program ImageJ version 1.51q (Institut Pasteur, Paris, France). This software allowed semi-quantitative determination of PCR amplicons by digital image analysis of the electrophoretic gel as was previously reported [22, 23]. For monitoring DNA amplifiability, relative intensity of each sample was estimated compared to untreated sample.

RESULTS AND DISCUSSION

Impact of heat on integrity of genomic DNA

Maize and wheat were selected as model plants due to their great significance for food production and nutrition. Processed foodstuffs including bakery, dairy and meat products often contain wheat and maize as ingredients or additives. Moreover, they belong to important allergenic foods. In addition, maize is among two major genetically modified crops distributed worldwide, while transgenic wheat has been already developed. Reliable identification of these species is in high demand for food authentication and traceability. Here we investigated degradation and amplification of maize and wheat DNA under different technological treatments and our results provide valuable information for accurate detection of these crops in processed foods. In this study, edible seeds, namely grains of maize and wheat, were processed and investigated. The whole, refined, canned or pick-

led grains are often used in human diet and animal feeding. Thus seeds are usually considered as raw foods [8, 10].

Firstly, wheat and maize genomic DNA was monitored under heat treatment. Thermal sterilization is a main step in food preservation technology. Boiling grains is generally used in canning production. Canned cereals including maize and wheat are widely distributed in both food and feed industries. Fig. 1 represents influence of heating at 100 °C for 300 min on the genomic DNA of wheat and maize. Comparison of DNA electrophoretic profiles clearly indicates a time dependent disruption of both maize and wheat genomes. The intensive bands of high molecular weight DNA in the control thermally untreated samples were weakened in the samples taken at the beginning of the boil after treatment of grains in water from 22 °C to 100 °C. They completely disappeared after 60 min of boiling (Fig. 1, lanes 1–3), while the intensity of shorter DNA bands increased by the time of exposure (Fig. 1, lanes 4–7). The results obtained demonstrated that the length of the extracted plant genomic DNA shortened with the increasing time of thermal treatment. These results are in agreement with the results of Hrnčírová et al. [10], who showed that higher temperatures (100 °C, 200 °C) considerably reduced the size of the extracted DNA in a time-dependent manner for genomic DNA from maize, soya and pea.

Impact of heat on DNA amplification

In order to investigate amplifiability of the maize and wheat genomic DNA, all DNA samples were subjected to plant-specific PCR. The primers Plant1 and Plant2 targeted conserved sequences of the chloroplast genome and PCR produced one main amplicon of approximately 500–600 bp in size depending on the plant species [14]. In addition, three species-specific PCR amplification systems were selected for each of maize and wheat DNA. The amplicons were chosen in the size range 67–259 bp on the basis of previous results on effectiveness of short amplicons at analysis of processed products [8–10, 20]. The previously published three primer pairs were selected for evaluation of maize DNA amplifiability. Two of them, namely IVTAS1/IVTAS2 and IVRf /VRr, targeted the maize-specific invertase gene. The primers IVTAS1 and IVTAS2 generated an amplicon of 226 bp [21], primers IVRf and IVRr produced an amplicon of 140 bp [20], whereas primer pair ZEINf/ZEINr, which targeted the maize-specific zein gene, produced an amplicon of 102 bp [19].

Three amplification systems were applied to investigate amplifiability of wheat DNA. PCR with primers TEN2/TEN3 targeted the wheat low-molecular-weight glutenin subunit, producing an amplicon of 67 bp, was taken from the publication of Terzi et al. [18]. In this study, two new PCR systems were designed and optimized to amplify PCR fragments of 134 bp and 259 bp with primers targeting wheat-specific genes of acetyl-coenzyme A carboxylase (ACACf/ACACr) and low-molecular-weight glutenin subunit (GLU1/GLU2), respectively. Testing of these primers in amplification of wheat, maize, barley and soya genomes showed their high specificity and sensitivity for reliable detection of wheat (data not shown).

None of the above mentioned amplicons was previously examined under specific processing conditions described in this paper. Fig. 2 shows the impact of boiling at 100 °C on the amplification of wheat and maize DNA. All PCR products of each sample were put together in one well of the gel to eliminate differences between agarose gels and electrophoresis runs at comparison of PCR bands (Fig. 2A, 2B). In order to evaluate kinetics of DNA degradation, intensity of PCR products

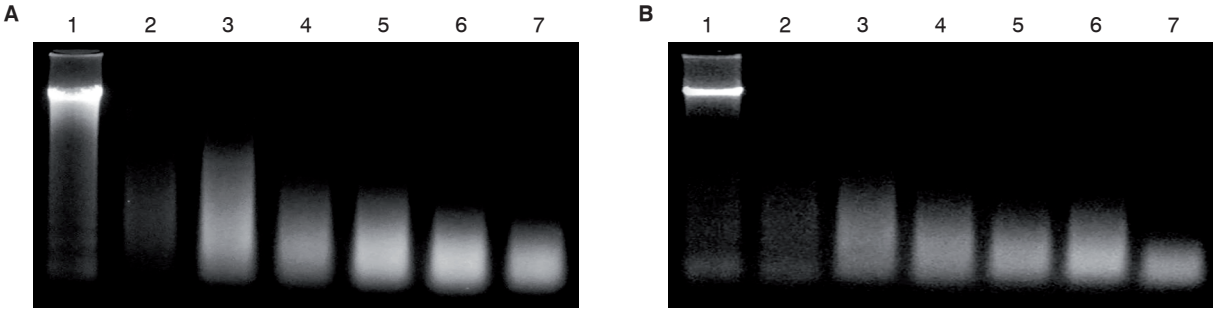

Fig. 1. Influence of heating (100 °C) on integrity of genomic DNA.

A – wheat samples, B – maize samples.
Lanes: 1 – without treatment, 2 – 0 min, 3 – 60 min, 4 – 120 min, 5 – 180 min, 6 – 240 min, 7 – 300 min.

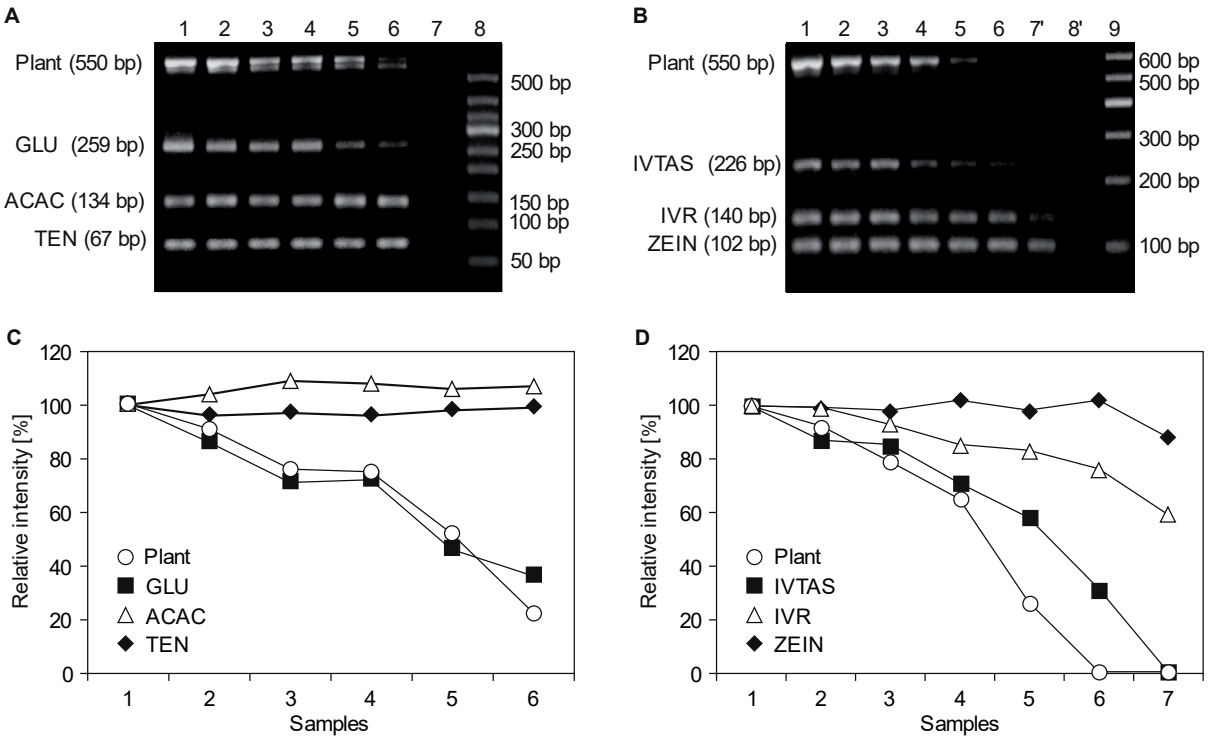

Fig. 2. Influence of heating (100 °C) on DNA amplification.

A – agarose gel electrophoresis of wheat samples, B – agarose gel electrophoresis of maize samples, C – relative intensities of wheat samples, D – relative intensities of maize samples.
Lanes: 1 – without treatment, 2 – 0 min, 3 – 60 min, 4 – 120 min, 5 – 180 min, 6 – 240 min, 7 – water, 8 – GelPilot 50 bp molecular size standard, 7' – 300 min, 8' – water, 9 – GelPilot 100 bp molecular size standard.

was determined and compared. Relative intensity of each sample was calculated as percentage of its intensity in comparison with the intensity of the control untreated sample (Fig. 2C, 2D). The semi-quantitative analysis of electrophoresis gels facilitated a more reliable interpretation of conventional PCR results than previously reported by other authors [22, 23]. Gel electrophoresis demonstrated that the expected PCR product was amplified using each primer pair for both maize and wheat DNA templates (Fig. 2A, 2B). However, PCR with primers Plant1/Plant2 gave a minor additional band for wheat samples, as was previously reported [14]. These primers amplify the non-coding regions of chloroplast DNA over a wide taxonomic range of plants. They were found to produce one main amplicon and additional minor PCR products for several species including wheat [14, 17] suggesting that primers Plant1 and Plant2 may have some homology with other regions of plant genome.

The long amplicons such as the 550 bp fragment of chloroplast genome, the 259 bp fragment of wheat low-molecular-weight glutenin gene and the 226 bp fragment of maize invertase gene exhibited significant time-dependent degradation. Moreover, the 550 bp and 226 bp fragments of maize genome disappeared after boiling for 240 min or 300 min (Fig. 2B, 2D, samples 6, 7). The 140 bp fragment of maize invertase gene revealed a slight decrease of amplification efficacy by time of exposure, the DNA band intensity being reduced by 40 % within 300 min of boiling (Fig. 2D). The short PCR products, such as the 67 bp fragment of wheat glutenin subunit gene, the 134 bp fragment carboxylase gene and the 102 bp fragment of maize zein gene, maintained almost invariable amplifiability within the total exposure time (Fig. 2).

Our results coincide with those obtained by Hrnčírová et al. [10] regarding the decreased amplification of long PCR products. Those authors showed that amplification of the 1339 bp DNA fragment of maize invertase gene failed when maize seeds were boiled at 100 °C for 15 min. Uchino et al. [11] reported about the presence of the 1 794 bp PCR fragment of 18S rDNA in wheat samples treated at 100 °C for 60 min. This result

may be explained by using different techniques of heat treatment. The findings obtained in the present work are in accordance with the earlier published data [9, 12]. HUPFER et al. [12] showed that the 211 bp PCR fragment of *cry1Ab* gene of transgenic maize was still present even after 105 min of boiling at 100 °C. BALLARI and MARTIN [9] demonstrated a reduced band intensity of the 356 bp fragment of transgenic maize from the model plasmid after conduction heating at 100 °C for 30 min and 60 min, while this treatment did not affect amplification of the 84 bp fragment of maize-specific zein gene. BERGEROVÁ et al. [4] reported that small DNA fragments ranging from 100 bp to 200 bp of maize zein gene were amplified after dry heating of maize flour at 100 °C or 220 °C for 60 min. Overall outcomes suggested that thermal degradation of DNA is dependent on the duration, temperature and approaches of thermal treatment.

Combined thermal-acid influence on genomic DNA

The combination of high temperature and low pH is often applied in food processing and preservation steps. To investigate the combined thermal-acid influence, the seeds of wheat and maize were treated in 0.4% NaCl solution of different pH (6.5, 4.0 and 2.0) together with high temperature (boiling at 100 °C). Treatment of seeds at 100 °C in 0.4% NaCl (pH 6.5) solution gave results (data not shown) similar to those observed after heating at 100 °C in distilled water (Fig. 2). As shown in Fig. 3, agarose gel electrophoresis revealed time-dependent degradation of the both wheat and maize genomic DNA and an intensive genomic DNA band in control untreated samples (Fig. 3, lane 1) disappeared in samples taken at the start of boil, after treatment of grains in 0.4% NaCl solution pH (4 and 2) from 22 °C to 100 °C (Fig. 3, lane 2). In addition, an increase in acidity from pH 4 (Fig. 3A, 3C) to pH 2 (Fig. 3B, 3D) intensified DNA degradation.

Combined thermal-acid influence on DNA amplification

Combined effect of thermal and acid treatment on the amplification efficacy of wheat DNA was assessed using four PCR systems targeting the chloroplast genome, wheat-specific genes of acetyl-coenzyme A carboxylase and low-molecular-weight glutenin subunit as already mentioned above (Fig. 2A). As shown in Fig. 4, the smallest 67 bp amplicon at both pH 4 and pH 2, and the 134 bp amplicon at pH 4, revealed almost invariable amplifiability during the total time of exposure (Fig. 4A) as was previously observed for thermal treatment (Fig. 2A). However, the band intensity of the 134 bp fragment was decreased

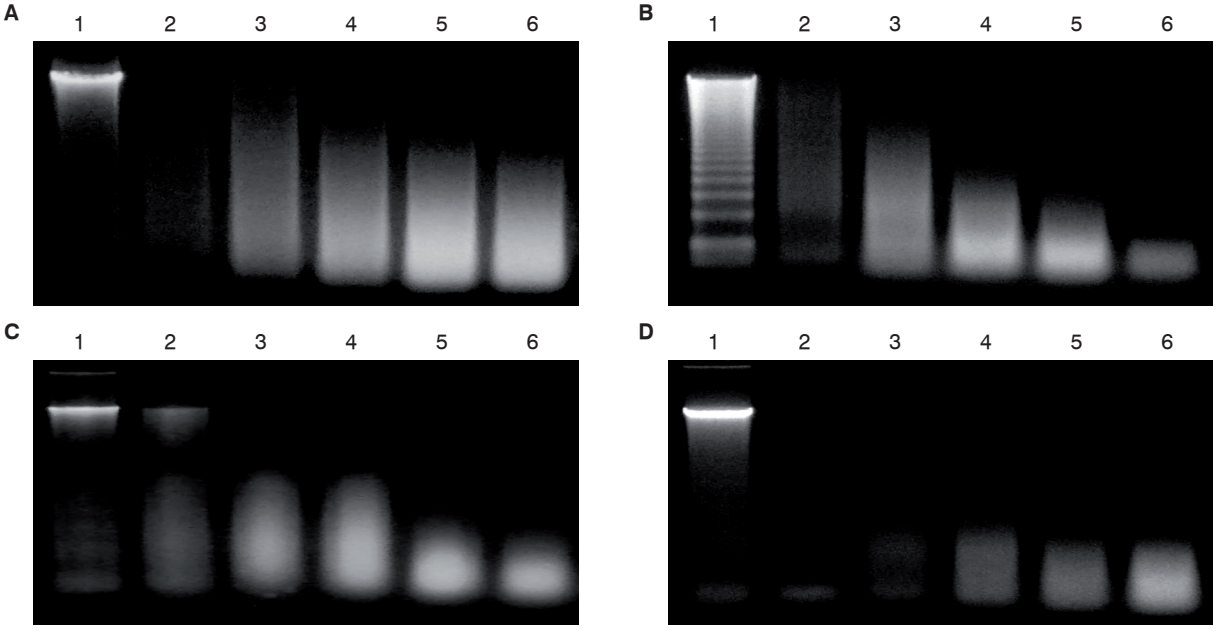

Fig. 3. Combined thermal-acid treatment effects on integrity of genomic DNA.

A – wheat samples at 100 °C, pH 4; B – wheat samples at 100 °C, pH 2; C – maize samples at 100 °C, pH 4; D – maize samples at 100 °C, pH 2.
Lanes: 1 – without treatment, 2 – 0 min, 3 – 30 min, 4 – 60 min, 5 – 90 min, 6 – 120 min.

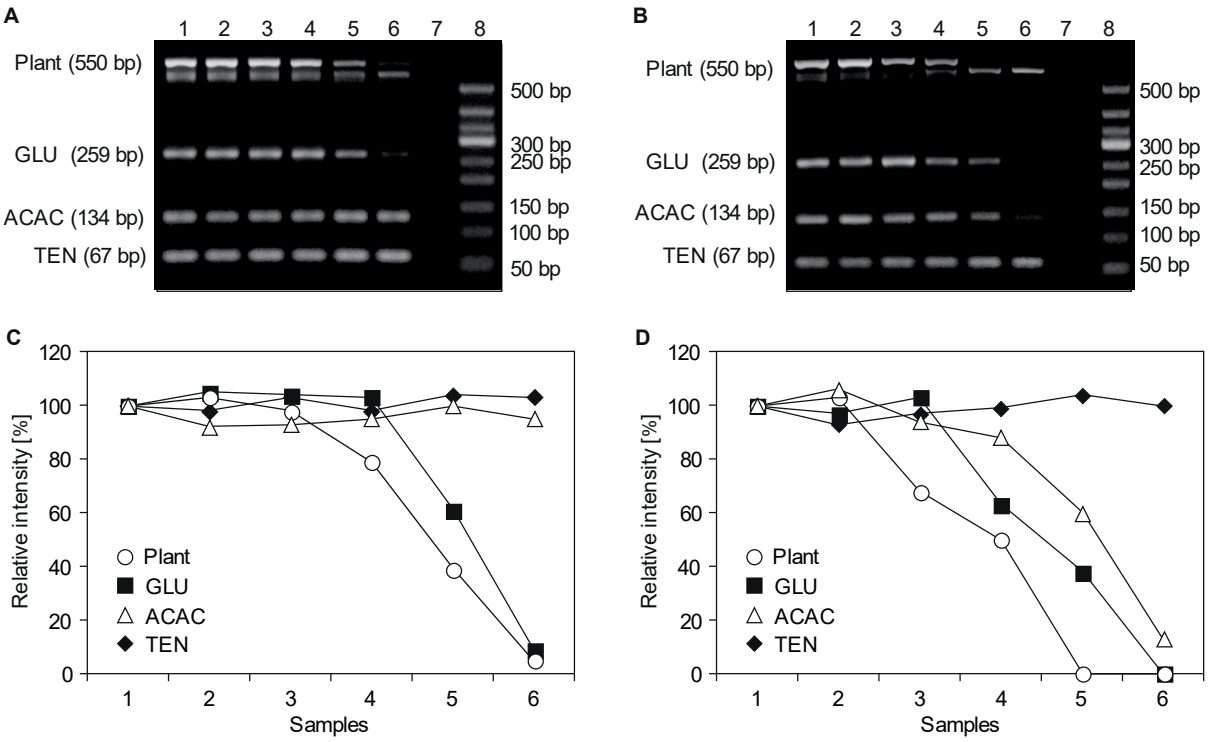

Fig. 4. Combined thermal-acid effects on wheat DNA amplification.

A – agarose gel electrophoresis of samples treated at 100 °C, pH 4; B – agarose gel electrophoresis of samples treated at 100 °C, pH 2; C – relative intensities at 100 °C, pH 4; D – relative intensities at 100 °C, pH 2.
Lanes: 1 – without treatment, 2 – 0 min, 3 – 30 min, 4 – 60 min, 5 – 90 min, 6 – 120 min, 7 – water, 8 – GelPilot 50 bp molecular size standard.

by the time of processing at pH 2, while the PCR product remained detectable (Fig. 4B). In addition, long DNA fragments, such as those of 550 bp and 259 bp, were degraded in a time-dependent manner in both acid conditions, while degradation was faster at pH 2 than at pH 4 (Fig. 4). Comparison of relative intensities of amplicons in different processing conditions demonstrated that DNA amplifiability significantly decreased at low pH (Fig. 4C, 4D). In particular, during 120 min of processing, intensity of the 550 bp and 259 bp fragments decreased by 25 % and 28 % at 100 °C, pH 6.5, respectively, while it decreased by 95 % and 91 % at 100 °C, pH 4. Moreover, intensity of the 550 bp and 259 bp fragments was reduced by 100 % in 90 min or 120 min at 100 °C, pH 2 (Fig. 4C, 4D). The results obtained suggested that the two PCR methods producing 67 bp and 134 bp amplicons are useful to precisely detect wheat in highly processed foods. Up to now, very limited data are available about thermostability of wheat DNA. Uchino et al. [11] investigated amplification of a PCR fragment of 1794 bp in thermally treated wheat samples. Our study filled the gap with wheat DNA and presented data regarding amplifiability of smaller products in the range of 67–259 bp. Moreover, two new wheat DNA markers, namely the 134 bp fragment of acetyl-coenzyme A carboxylase gene and the 259 bp fragment of low-molecular-weight glutenin subunit, were identified.

To monitor the degradation of maize DNA at thermal –acid combined treatment, the above mentioned four PCR systems (Fig. 2B) targeting the chloroplast genome, maize invertase and zein genes were used. Agarose gel electrophoresis demonstrated almost invariable amplifiability of small 102 bp and 140 bp amplicons at both 100 °C, pH 4 and 100 °C, pH 2 conditions within the entire time of exposure (Fig. 5A, 5B). Moreover, intensity of these PCR products in several samples increased by 10–17 % compared to the intensity of the control untreated sample (Fig. 5C, 5D). This suggested that the amount of short fragments increased by degradation of high molecular weight genomic DNA (Fig. 3C, 3D). The results obtained here for the experimentally processed samples are in agreement with our findings about suitability of these PCR systems to identify maize DNA in commercially available processed products [20, 21].

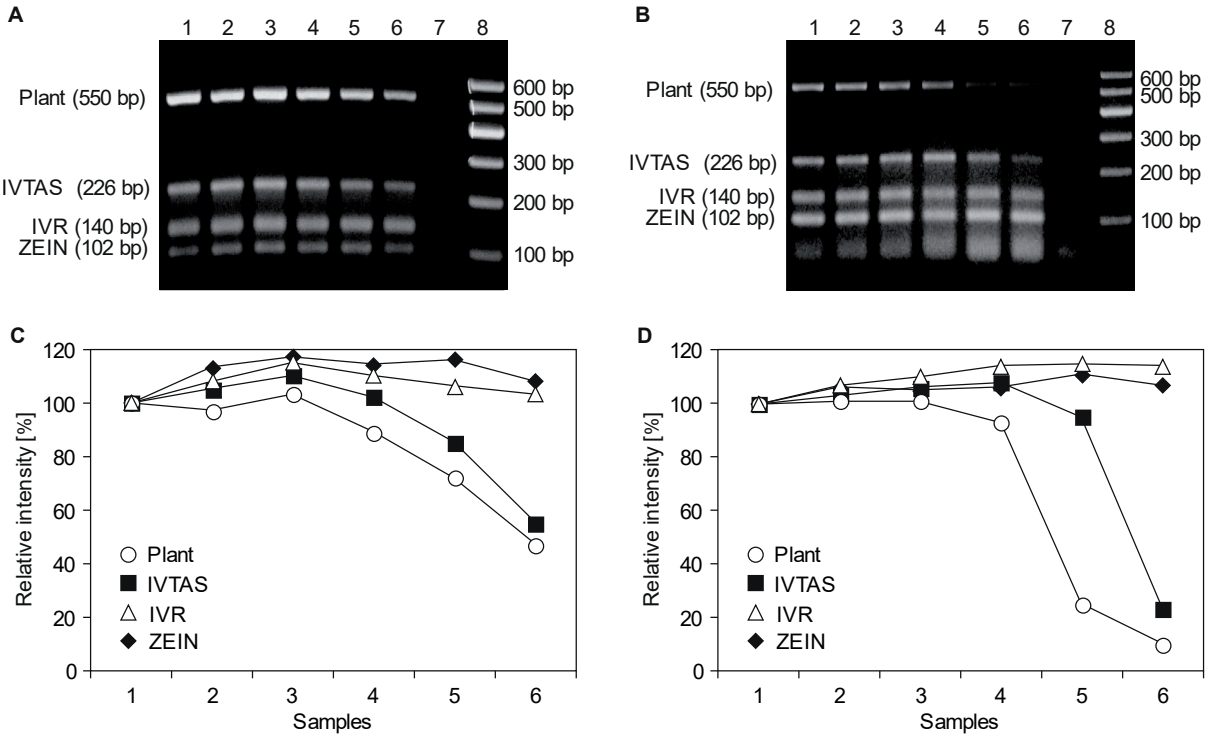

Fig. 5. Combined thermal – acid effects on maize DNA amplification.

A – agarose gel electrophoresis of samples treated at 100 °C, pH 4; B – agarose gel electrophoresis of samples treated at 100 °C, pH 2; C – relative intensities at 100 °C, pH 4; D – relative intensities at 100 °C, pH 2.
Lanes: 1 – without treatment, 2 – 0 min, 3 – 30 min, 4 – 60 min, 5 – 90 min, 6 – 120 min, 7 – water, 8 – GelPilot 100 bp molecular size standard.

The amplification efficacy of both 226 bp and 550 bp fragments was almost constant for 30 min and 60 min (Fig. 5A, 5B). Afterwards, time-dependent degradation of these amplicons was revealed (Fig. 5A, 5B). Analysis of relative intensities of maize PCR fragments in different treatment conditions clearly exhibited a crucial effect of low pH, which decreased the integrity of maize genomic DNA. In particular, relative intensity of the 550 bp and 226 bp PCR fragments decreased to 65 % and 71 % at 100 °C, pH 6.5 compared with 47 % and 55 % at 100 °C, pH 4 as well as 10 % and 23 % at 100 °C, pH 2, respectively, during a 120 min treatment. Our results coincide with previous findings that the 224 bp amplicon of the maize MON 810 invertase gene was detected after sterilization at 100 °C, pH 2.25 for 30 min [8] and fragments of up to 957 bp were still amplifiable after 90 min of incubation at 85 °C, pH 8.4 or 65 °C, pH 4.0 [6]. However, in contrast to these data, the 211 bp transgenic fragment of Bt maize polenta was not detected after boiling at pH 2–3 for 15 min [12]. In addition, BERGEROVÁ et al. [8] reported that the 469 bp fragment of bean genome was not amplifiable after sterilization at 100 °C, pH 7.6 for 10 min. These discrepancies may be due to differences in the processing technologies or differences in amplification efficiency of PCR systems. Our findings on the effects of the combined treatment by high temperature and acidic pH on DNA integrity are in agreement with those obtained previously by several authors [6, 8, 12, 13].

Taken together, results obtained in the present study confirm the importance of the length of the PCR amplicon to the detection of food components and suggest that amplicons of up to 140 bp in size are suitable for analysis of high-processed food products. Our outcomes support the previously published data [7, 8, 10]. PCR amplicon size of less than 200 bp facilitated successful detection of genetically modified organisms (GMOs) in processed foods by VIJAYAKUMAR et al. [7]. HRNČÍROVÁ et al. [10] concluded that the amplicon size should be around 200 bp for analysis of processed foods by PCR. BERGEROVÁ et al. [8] reported that the maximum amplicon length for highly processed matrices should be 300 bp. In addition, our study demonstrated temperature-, pH-, and time-dependent degradation of plant genomic

DNA. The reduced length of amplifiable DNA correlated with fragmentation of the genomic DNA during the time of processing.

CONCLUSIONS

The outcomes of the present study clearly demonstrate that heat and acid treatment cause degradation of plant DNA and may affect PCR amplification. Extent of DNA degradation was found dependent on temperature, pH and duration of food processing. Disruption of genomic DNA observed at heating was intensified by combined heat-acid treatment. The size of PCR amplicon was found to have a crucial importance for efficiency of amplification. Decreased amplification of long amplicons was in correlation with DNA degradation. However, short amplicons with a size less than 140 bp maintained invariable amplifiability even in highly processed samples. Therefore, they may be used for reliable detection of wheat and maize in food products.

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