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Degradation of Endogenous and Exogenous Genes of Roundup-Ready Soybean during Food Processing

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Roundup-Ready soybeans have been genetically modified to resist the effects of the herbicidal glyphosate and have become the most prevalent transgenic crop in the world. In this work, Roundup-Ready soybeans were used as raw material to study the effects of critical processing procedures such as grinding, cooking, blending, homogenization, sterilization, and spray-drying on the length of DNA fragments of an endogenous gene (*lectin*) and an exogenous gene (*epsps*) examined in material from three soybean foods of bean curd, soy milk, and soy powder and from samples taken during their processing. The results showed that various processing procedures caused degradations of both the endogenous and exogenous genes to different degrees. In the grinding procedure, endogenous gene DNA was degraded from 1883 to ~836 bp, and exogenous gene DNA was degraded from 1512 to ~408 bp. In the blending and squeeze-molding procedures, exogenous gene DNA was also degraded from about 408 to 190 bp, but there was no obvious action on the endogenous gene. After the endogenous and exogenous genes had been degraded to some degree, such as 836 and 408 bp, respectively, they were not evidently affected by cooking procedure at 100 °C for 15 min. However, the endogenous gene was further considerably degraded from around 836 to 162 bp in the sterilization procedure at 121 °C for 30 s. The effect of the homogenization step on endogenous and exogenous genes was similar to that of the cooking procedure. The coagulation procedure, principally a biochemical reaction, did not greatly affect the exogenous gene but did affect endogenous gene, reducing DNA size from about 836 to 407 bp. Furthermore, the spray-drying procedure, a process of physical shearing, high temperature, and sudden high pressure, distinctly caused degradation of both the *lectin* and *epsps* genes, rapidly decreasing the sizes from about 836 to 162 bp for the endogenous gene and from about 408 to 190 bp for the exogenous gene.

KEYWORDS: Roundup-Ready soybean; processing procedures; endogenous gene; exogenous gene; degradation

INTRODUCTION

In recent years, the utilization of transgenic crops has spread rapidly worldwide. According to statistics released by the International Service for the Acquisition of Agri-Biotech Applications (ISAAA), the area of planted transgenic crops was 81 million hectares in 2004, which is 47 times that in 1996 (*1*). The ongoing development of new characteristics (e.g., disease resistance, insect resistance, herbicide resistance) has made studies on the impact of transgenic crops and their processed food products on human health and the environment a top priority and the focus of worldwide attention (*2*−*5*). More and more countries have established or are establishing related laws and regulations.

Today, efforts to address a series of questions pertaining to the safety of transgenic foods have pointed out the need for studies and discussions on the changes sustained by genomic DNA and transferred exogenous genes in these processed products. There are numerous reported methods to detect transgenic components worldwide (*6*−*11*); however, very little work has been done on DNA degradation (especially transferred exogenous genes) during the processing of transgenic crops into food products. Klien et al. (*12*) explored changes in the DNA and protein of transgenic and nontransgenic sugar beets during processing. Gawienowski et al. (*13*) used a PCR assay to study nucleic acid changes of the specific gene *Rubisco* in corn during processing that included soaking, grinding, and heating. In addition, Chiter et al. (*14*) studied the DNA stability of some vegetable raw materials of feed staples, such as rapes, linseeds, wheat powder, fresh corn powder, corn granules, sugar

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beets, and sugar beet juice. Comprehensive knowledge of the degradation of endogenous and exogenous genes during the various processing procedures of different kinds of foods is required in order to identify the limits of detection method used for authenticity testing and safety assessments of transgenic crops.

In this study, the degradation of the endogenous *lectin* gene and the exogenous *epsps* gene (5-enolpyruvyl-shikimate-3-phosphate synthase gene) from Roundup-Ready soybeans (RRS) during the production of bean curd, soy milk, and soy powder was investigated to understand the effects of different processing methods on genomic and exogenous DNA. It is our purpose to provide a theoretical basis for the reliability of detection methods used for the identification of transgenic products (raw materials, processed products, and especially advanced processed foods) and to make identification systems and security evaluations more practical and reliable. At the same time, we hope to enrich the theoretical studies of this new food resource—transgenic foods—and to provide guidance in controlling their processing.

MATERIALS AND METHODS

Sample Preparation. Roundup-Ready soybeans were kindly provided by LianYunGang Entry−Exit Inspection and Quarantine Bureau, People's Republic of China. Nontransgenic soybeans were purchased from the Chinese market. Bean curd, soy milk, and soy powder were produced in the processing laboratory at the China Agricultural University according to the standard process chart by using 2.5% (w/w) Roundup-Ready soybean (2.5 g of RRS was mixed with 97.5 g of nontransgenic soybean) as raw material. Samples from the three soybean foods were obtained in the laboratory of the Food Science College of the Chinese Agricultural University according to the processing procedure. For production of soy milk, a mixture of overnight-soaked 2.5% (w/w) RRS and tap water (1:7.5) was ground in a blender; after homogenization, the slurry was sterilized at 121 °C for 30 s to obtain soy milk. For bean curd, after blending and filtration, the resulting filtrate was cooked at 100 °C and mixed with 10% $CaSO_4$ (w/v) first, and then the precipitated protein was squeezed under a pressure of 16 kg/cm^2 at 60 °C for 20−30 min to obtain bean curd. For production of soy powder, the resulting filtrate was directly sprayed for drying (entrance temperature, 160 °C; exit temperature, 80 °C). The samples were collected in eight different critical processing procedures (indicated with asterisks in **Figure 1**) were used for further study.

DNA Extraction. DNA from the soy food samples was extracted with a Promega Wizard Genomic DNA Purification Kit (Shanghai, China) according to the plant tissue DNA extraction procedure with the following modifications. Solid samples were milled and homogenized into fine powders. Six hundred microliters of nuclei lysis solution was added to 30 mg of powdered sample. After homogenization for 30 s using a small homogenizer, all lysates were incubated at 65 °C for 30 min. The subsequent steps were performed according to the manufacturer's instructions.

PCR Amplification and Gel Electrophoresis Analysis. In total, eight pairs of primers were designed using Primer Premier 5 software, on the basis of the published nucleotide sequences of *lectin* and *epsps* genes (GenBank accession numbers *lectin* K00821; *epsps* AY125353), respectively. One primer pair targeted the whole gene sequence, whereas the other three sets were primers that amplified smaller fragments. The sequences of the primers and the locations amplified by each pair are listed in **Table 1**. PCR amplification was carried out in a final volume of 50 μL. Each reaction contained 5 μL of DNA template (100−200 ng) extracted from samples, 5 μL of 10× reaction buffer with $MgCl_2$ (Takara), 0.25 mM dNTPs, 10 μM concentration of each primer, and 0.2 unit *TaKaRa Taq* polymerase (Takara). PCR amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The PCR conditions for the *lectin* gene consisted of an initial denaturation at 94 °C for 5 min, 40 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 7 min. The number of cycles was reduced to 35 for the 836 bp PCR product. Conditions for the amplification of *epsps* gene were an initial denaturation at 94 °C for 5 min, 40 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 7 min. The 807 bp *epsps* PCR product was obtained by decreasing the number of cycles to 35 while increasing the annealing temperature to 60 °C. Ten microliters of each PCR product of the *lectin* gene using the L1/H1863, L928/H1745, L1104/H1488, and L1145/H1290 primer sets were mixed together. Likewise, 10 μL of each PCR product of the *epsps* gene using the L317/H1808, L675/H1462, L1017/H1406, and L1017/H1188 primer sets were mixed together. Mixtures were loaded into a 2% agarose gel containing ethidium bromide (0.5 mg/mL). Following electrophoresis at 80 V for 1 h in 0.5× TBE buffer (90 mM Tris−boric acid, 2 mM EDTA, pH 8.0), fragments were visualized on a long-wavelength UV transilluminator.

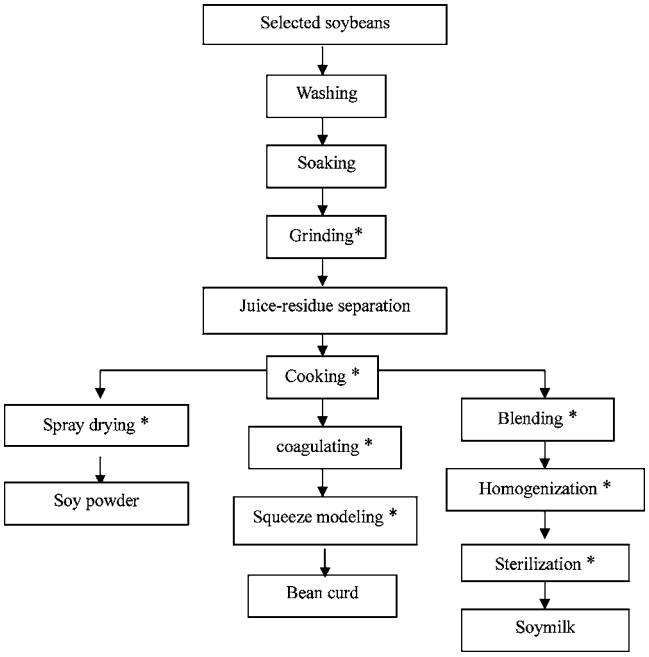

Figure 1. Flowchart of the processes used in the production of bean curd, soy milk, and soy powder. Samples were taken in the eight critical processing procedures marked with asterisks.

Table 1. Primer Sequences and PCR Product Sizes of *lectin* and *epsps* Genes

gene	location of amplified fragment in GenBank sequence		primer sequence	amplicon size
lectin	1–1883	L1	5′caatgccatcgtatcgtgtc3′	1883
		H1863	5′gcgatcgagtagtgagagtgg3′	
	928–1764	L928	5′gactccccatgcatcacagt3′	836
		H1745	5′ggcaaattggaagcaaaaga3′	
	1104–1511	L1104	5′gaagcaaccaaacatgatcctc3′	407
		H1488	5′atggatctgatagaattgacgtta3′	
	1145–1307	L1145	5′cctcctcgggaaagttacaa3′	162
		H1290	5′gggcatagaaggtgaagt3′	
epsps	317–1828	L317	5′ ggcgaggacgtcatcaatac 3′	1512
		H1808	5′ tcgatccccgatctagtaaca 3′	
	675–1481	L675	5′ cctccgcacaggtgaagt 3′	807
		H1462	5′ ccatcaggtccatgaactcc 3′	
	1017–1424	L1017	5′ cgacatcgaagtcatcaacc 3′	408
		H1406	5′ gtgacagggttttccgacac 3′	
	1017–1206	L1017	5′ cgacatcgaagtcatcaacc 3′	190
		H1188	5′ ttcttccagaccgttcatca 3′	

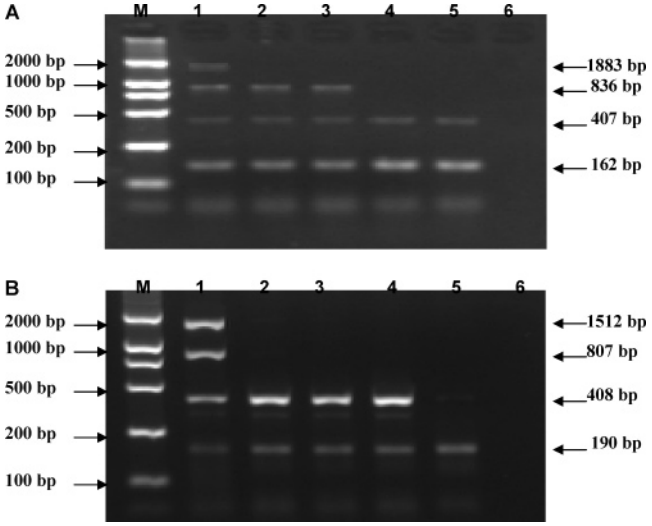

Figure 2. PCR amplification of the *lectin* and *epsps* genes in samples taken after critical procedures of bean curd production: amplicons of the endogenous *lectin* gene (**A**) and the exogenous *epsps* gene (**B**) amplifications. [Lane M, molecular weight marker; lane 1, raw materials; lane 2, grinding; lane 3, cooking; lane 4, coagulation; lane 5, squeeze modeling (bean curd); lane 6, negative control.]

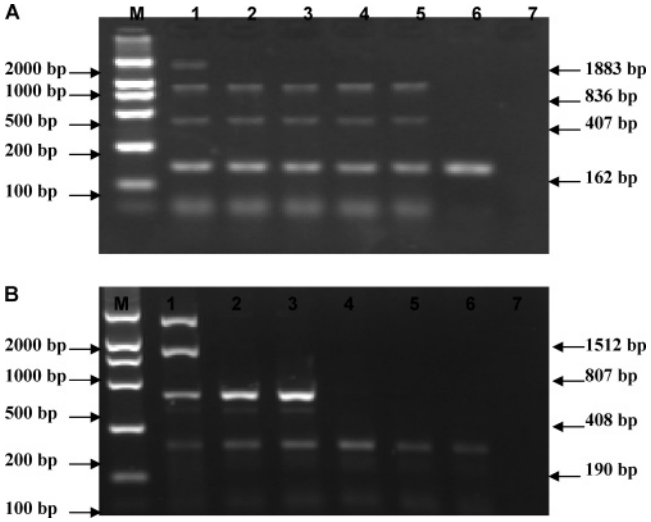

Figure 3. PCR amplification of the *lectin* and *epsps* genes in samples taken after critical procedures of soy milk production: amplicons of the endogenous *lectin* gene (**A**) and the exogenous *epsps* gene (**B**) amplifications. [Lane M, molecular weight marker; lane 1, raw materials; lane 2, grinding; lane 3, cooking; lane 4, blending; lane 5, homogenization modeling; lane 6, sterilization (soy milk); lane 7, negative control.]

RESULTS AND ANALYSIS

Degradation of Endogenous and Exogenous Genes during Bean Curd Processing. Grinding, cooking, coagulation, and squeeze-molding are four critical procedures of bean curd processing. PCR amplifications of the different length fragments of the endogenous *lectin* gene and the exogenous *epsps* gene were performed after extracting DNA from each processing step material. The results (**Figure 2**) show that the mechanical treatment of grinding has the greatest effect on the degradation of both endogenous *lectin* gene and the exogenous *epsps* DNA; however, the degrees of effect are very different for these two genes. After grinding, only full-length fragment could not be detected for the *lectin* gene, but both full-length and 807 bp fragments could not be detected for the *epsps* gene. The cooking technique, a heating process at 100 °C for 15 min, did not have much of an effect on the degradation of either gene. Amplification yielded the same size fragments as those after the grinding step. Coagulation is a chemical reaction process with a coagulator, $CaSO_4$, and induces the denaturation of soybean protein. After coagulation, no further degradation was detected for the exogenous *epsps*; the DNA fragment that could be detected after this step was of the same length as those after grinding and cooking, whereas further degradation of the endogenous *lectin* gene occurred so that no fragments larger than 407 bp were detected. Squeeze-molding, the last step in the manufacture of bean curd, is a time-consuming, low-intensity mechanical treatment. The *lectin* gene was not very affected by this process, and no further degradation was observed in PCR analysis. However, squeeze-molding had a definite impact on the exogenous *epsps* gene, such that the DNA was degraded from 408 to 190 bp fragments in the final product (**Figure 2**).

Degradation of Endogenous and Exogenous Genes during Soy Milk Processing. The main processes used in the manufacture of soy milk are grinding, cooking, blending, and sterilization. DNA was extracted from the raw material, Roundup-Ready soybeans, and from samples after each of these four critical procedures was performed. Because the processes of grinding and cooking are similar to those used in the production of bean curd, the PCR results (**Figure 3**) show that the patterns of DNA degradation of both *lectin* and *epsps* genes were also similar in these two steps. Blending and homogenization did not significantly affect the endogenous *lectin* gene, as all DNA fragments other than the 1883 bp could still be detected in the samples. However, blending significantly affected degradation of the exogenous gene, *epsps*. The 408 bp fragment that existed after grinding and cooking steps was further degraded, and only 190 bp fragments were detected, a pattern that was maintained even after homogenization. Sterilization using high temperature (121 °C for 30 s) and pressure is the last step in the manufacturing of soy milk and resulted in a further large-scale degradation of the *lectin* gene from 836 to 162 bp DNA fragments. Meanwhile, 190 bp fragments were still present in the *epsps* gene mixture. Therefore, in the final analysis, only DNA fragments of <200 bp could be detected for both the endogenous and exogenous genes (**Figure 3**).

Degradation of Endogenous and Exogenous Genes during Soy Powder Processing. The processing of soy powder is much easier and includes soaking, grinding, cooking, and spray-drying. PCR results for the endogenous gene, *lectin*, and the exogenous gene, *epsps*, from transgenic soybean and from three critical procedures for processing soy powder are shown in **Figure 4**. DNA degradation patterns after the grinding and cooking steps were similar to those in bean curd and soy milk processing. Spray-drying is the last step in the processing of soy powder from soybeans and incorporates violent processes such as high temperature, shearing, and instantaneous high pressure. Before spray-drying, DNA fragments of the *lectin* gene and the *epsps* gene were degraded to about 836 and 408 bp, respectively. After spray-drying and processing to the final product, the endogenous *lectin* gene was degraded from 836 to ~162 bp abruptly, and the exogenous *epsps* gene was also degraded from about 408 to 190 bp. Therefore, the physical and chemical actions during spray-drying processing affected the degradation of both the endogenous and exogenous genes (**Figure 4**).

DISCUSSION AND CONCLUSION

Processing techniques vary for different categories of food. They usually involve physical treatment (e.g., mechanical

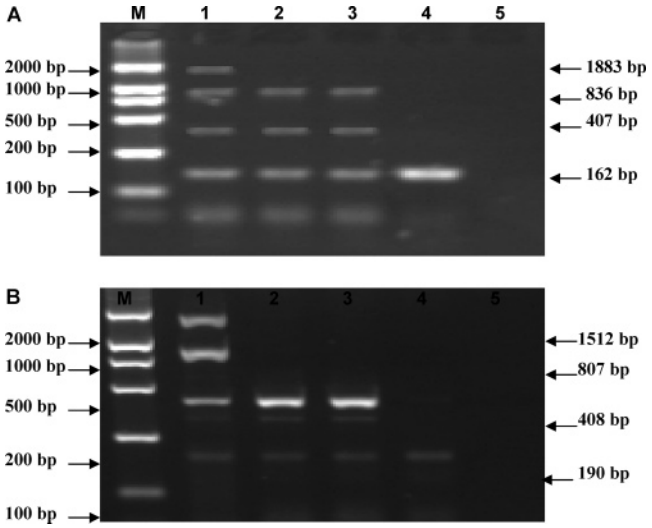

Figure 4. PCR amplification of the *lectin* and *epsps* genes in samples after critical procedures during soy powder productio: amplicons of the endogenous *lectin* gene (**A**) and the exogenous *epsps* gene (**B**) amplifications. [Lane M, molecular weight marker; lane 1, raw materials; lane 2, grinding; lane 3, cooking; lane 4, spray drying (soy powder); lane 5, negative control.]

treatment, high temperature, and high pressure), chemical changes (e.g., acid, alkali, and sulfur treatment), or biological reactions (e.g., enzymolysis and fermentation). These different processing procedures will bring about different degrees of DNA degradation in the resulting food products. For example, sterilization and sealing can cause DNA degradation in vacuum-sealed food, whereas the nucleases may degrade DNA as the storage period of fresh food is prolonged; low pH values adopted in the manufacture of tomato juice induce chemical modifications and DNA degradation (*7*, *15*−*17*).

In this study we analyzed the changes of the endogenous *lectin* gene and exogenous *epsps* gene in transgenic soybean raw materials and in samples from three soybean foods (bean curd, soy milk, and soy powder) during eight critical processing steps such as grinding, cooking, coagulation, squeeze-molding, blending, homogenization, sterilization, and spray-drying. Our results showed that almost all of the processing procedures affected the degradation of both the exogenous target gene *epsps* and the exogenous gene *lectin* in transgenic foods, but that the degree of the effect is evidently different between those two genes in some steps. Mechanical processing such as grinding caused the degradation of both the exogenous gene *lectin* and the endogenous gene *epsps* but caused more extensive degradation on the exogenous gene. After grinding, only the fragment around 800 bp of the *lectin* gene could be amplified, but only the ∼400 bp fragment of the *epsps* gene could be detected. Blending and squeeze-molding caused remarkable degradation of the exogenous gene, but had no evident effect on the endogenous gene. When DNA was degraded within the range of 400−800 bp, short periods of high temperature, such as that used for cooking and homogenizing, at 100 °C for 15 min did not obviously change the size of the DNA fragments of either gene, whereas super-high-temperature and high-pressure sterilization at 121 °C for 30 s caused a further large-scale degradation of the endogenous gene. Coagulation, for the most part a chemical reaction, had an obvious effect on the degradation of the large DNA fragment; the DNA fragment around 800 bp was degraded to ∼400 bp after this processing step. Furthermore, spray-drying, a process that includes shearing, high temperature, and instantaneous high pressure, unquestionably produced a remarkable effect on both the endogenous gene, *lectin*, and the exogenous gene, *epsps*.

In conclusion, during advanced food processing, the endogenous gene *lectin* in transgenic foods is more stable than the exogenous gene *epsps*; the large DNA fragments will be affected more than small ones during processing, and DNA undergoes a slower degradation when degraded to certain small fragments. The fragment sizes of the endogenous and exogenous genes in final products will be different even when produced with the same transgenic raw materials treated with different techniques. Furthermore, more complicated and violent processing conditions affect the DNA of endogenous and exogenous genes much more than simple and more moderate processing. These conclusions undoubtedly provide the most direct contribution for the detection, identification, and evaluation of the safety of transgenic crops and enrich and support the theoretical basis of this new food source. At the same time, these conclusions can also provide guidance for the processing of transgenic foods. Moreover, the results of this study indicate that choosing primers for amplifying suitable fragments of endogenous and exogenous genes can avoid false negative results in the testing and characterization of processed food products.

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