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Short Communication

Monitoring of Red Pepper Powder and Seasoned Red-Pepper Sauce using Species-Specific PCR in Conjunction with Whole Genome Amplification

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ABSTRACT - Red pepper is one of the most important spices popularly utilized in Korea. Because of the differences in tariff rates between red pepper powder and seasoned red-pepper sauce, seasoned red-pepper sauce is often therefore imported by consumers, then dried, ground, and added to red pepper powder for cost effective purposed to use the product the most effectively. In this study, we combined species-specific polymerase chain reaction (PCR) assays (for red pepper, garlic, onion, spring onion, and ginger) with whole-genome amplification (WGA). Thirty-nine red pepper powders were well in accordance with their labels. However, six red pepper powder and five seasoned red-pepper sauce products failed to meet their compliance requirements. As a consequence, our monitoring results revealed that the overall mislabeling rate detected in this study was identified at 22%. Thus, our findings showed that the species-specific PCR in conjunction with WGA was an ideal method to identify raw materials that are used in the manufacturing of red pepper powder and seasoned red-pepper sauce.

Key words : Species-specific polymerase chain reaction, Red pepper powder, Labeling compliance, Economically motivated adulteration

Abbreviations : WGA, whole genome amplification, PCR, polymerase chain reaction

Red pepper has been used in the form of powder for traditional fermented foods such as kimchi and red pepper paste since approximately 400 years ago in Korea, making red pepper an important spice in Korean food culture. The import of red pepper has increased in recent years, because the domestic production of red pepper in the Republic of Korea cannot satisfy the demand[1]. As of 2012, over 95% of Korea's pepper products are imported from China. Although Koreans cultivate pepper domestically, the production volume is just 68% that of China, and the price of domestically cultivated pepper is 7.1 times higher than that of China[2]. In this sense, higher tariffs (270% as of 2016) have been applied to Chinese dried pepper products in an effort to protect domestic red pepper farms in Korea. Nevertheless, some companies have attempted to take advantage of these tariffs for economic gains. According to a press release from the Korea Customs Service in 2013, some companies have evaded high tariffs applied to red pepper powder (270%) by manufacturing economically motivated adulteration (EMA) of red pepper powder. Specifically, seasoned red-pepper sauce, which is subjected to relatively lower tariffs (45%), was imported, then dried, ground, and added to red pepper powder. However, according to the Food Code, no ingredient other than red pepper should be added when red pepper powder is manufactured[3].

Polymerase chain reaction (PCR) technique using species-specific primer sets has been widely used to identify the ingredients used in processed products[4,5]. Previously, Park et al. (2012) reported that ingredients (red pepper, garlic, and onion) used in seasoned red-pepper sauce could be specifically identified using species-specific PCR[6]. In this study, in order to identify the ingredients used processed red pepper products, five species-specific PCR conditions were optimized and then combined with the whole-genome amplification (WGA) method. With this PCR-based method, we successfully to monitored the labeling compliance of forty-five red pepper powders and five seasoned red-pepper sauces.

Materials and Methods

Samples and pretreatment

All of the samples (forty-five red pepper powders and five seasoned red-pepper sauces) used in this study were

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provided by the Ministry of Food and Drug Safety (MFDS) and the Korea Customs Service (KCS). Among them, ingredients used in the two seasoned red-pepper sauces have been already verified by the KCS, mostly composed of red pepper and garlic. Pretreatment of the samples was conducted according to the method reported by Park et al. (2012)[6]. Briefly, 40 mL of distilled water was added to 10 g of sample, and the mixture was agitated and then centrifuged at 5,000 × *g* for 5 min. After centrifugation, the supernatant was removed. Samples were washed three times. The purified samples were then dried at 50°C for 1 day and used for DNA extraction.

DNA extraction

In order to extract DNA from commercial red pepper powder and seasoned red-pepper sauce, DNeasy Plant Mini Kit (Qiagen Inc., Hilden, Germany) was used, according to the manufacturer's instructions.

Whole-genome amplification (WGA)

In order to randomly amplify the extracted genomic DNA, we used a GenomePlex Whole Genome Amplification Kit (Sigma-Aldrich Inc., St. Louis, MO, USA), according to the manufacturer's instructions. After WGA step, the amplified DNA was purified using an AccuPrep PCR Purification Kit (Bioneer Co., Daejeon, Korea), according to the manufacturer's instructions.

Species-specific PCR and sequencing

Species-specific PCR was performed in 20 μL of reaction volume containing 10 ng of template DNA, 0.5 μM of each primer, 1 × PCR buffer, 0.2 mM of deoxynucleosides (dNTPs), 2.0 mM of $MgCl_2$, and 1 U of rTaq polymerase (Takara Bio Inc., Otsu, Japan). The reactions were carried out in a thermal cycler C1000 Touch (Bio-Rad Laboratories, Hercules, CA, USA). Information regarding the primers used in this study was described in Table 1[7-11]. The amplification conditions for red pepper, garlic, onion, spring onion, and ginger were summarized in Table 2. PCR products were separated from agarose gels for sequencing and cloned into the pGEM-T Easy Vector (Promega Co., Madison, WI, USA). The cloned plasmid DNA was sequenced by Bioneer (Daejeon, South Korea).

Results

Verification of DNA extracts

In this study, 18S ribosomal RNA (18S rRNA) gene was

Table 1. Species-specific primer sets used in this study

Targets	Name	Sequences (5'→3')	Size (bp)	References
Red pepper	CCS-F	CTAATGGAAACCCTTCTAAAGC	102	Song et al. (2007)
	CCS-R	GGTTGGATTTGGAAAAGTGG		
Garlic	GM-ASM-080-F	AATCTCCCTCCAAAGTCCC	180	Zhao et al. (2011)
	GM-ASM-080-R	CTGTATTTTGTGTAAAGCATCA		
Onion	API73-F	GTTTCTTGGATGCGATTTTG	280	McCallum et al. (2006)
	API73-R	GCAACTGTATAATCAGCATATGC		
Spring onion	SFI12-Spronin-F	ACCCACCTACGGTAAACTTACAC	136	NIFDSE (2014)
	SFI12-Spronin-R	TGTGTTAACTCGATACACCATT		
Ginger	SFI12-Ginger-F	CTCGGAATCAATCAATCAACC	113	NIFDSE (2014)
	SFI12-Ginger-R	TCACGAGATTCTGCAATTCACAC		
18S ribosomal RNA	18S rRNA-F	TCTGCCCTATCAACTTTCGATGGTA	137	Allmann et al. (1993)
	18S rRNA-R	AATTTGCGCGCCTGCTGCCTTCCTT		

Table 2. Species-specific PCR conditions for red pepper, garlic, onion, spring onion, and ginger

Step	Red pepper			Garlic and Onion			Spring onion			Ginger		
	Temp.	Time	Cycles	Temp.	Time	Cycles	Temp.	Time	Cycles	Temp.	Time	Cycles
Pre-denaturation	95°C	5 min	1	94°C	10 min	1	94°C	5 min	1	94°C	5 min	1
Denaturation	95°C	30 s		94°C	30 s		94°C	30 s		94°C	30 s	
Annealing	62°C	30 s	40	50°C	10 s	35	68°C	30 s	35	66°C	30 s	35
Extension	72°C	30 s		72°C	30 s		72°C	30 s		72°C	30 s	
Final extension	72°C	5 min	1	72°C	5 min	1	72°C	5 min	1	72°C	5 min	1

Table 3. Amplification results of red pepper products using species-specific primer sets after whole genome amplification

Sample	Primer set						Sample	Primer set					
	18S rRNA	Pepper	Garlic	Onion	Spring onion	Ginger		18S rRNA	Pepper	Garlic	Onion	Spring onion	Ginger
S1	+	+	+	-	-	-	P21	+	+	-	-	-	-
S2	+	+	+	-	-	-	P22	+	+	-	-	-	-
S3	+	+	-	-	-	-	P23	+	+	-	-	-	-
S4	+	+	-	-	-	-	P24	+	+	-	-	-	-
S5	+	+	-	-	+	-	P25	+	+	-	-	-	-
P1	+	+	-	-	-	-	P26	+	+	-	-	-	-
P2	+	+	-	-	-	-	P27	+	+	-	-	-	-
P3	+	+	-	-	-	-	P28	+	+	-	-	-	-
P4	+	+	-	-	-	-	P29	+	+	-	-	-	-
P5	+	+	-	-	-	-	P30	+	+	-	-	-	-
P6	+	+	-	-	-	-	P31	+	+	-	-	-	-
P7	+	+	-	-	-	-	P32	+	+	-	-	-	-
P8	+	+	-	-	-	-	P33	+	+	-	-	-	-
P9	+	+	-	-	-	-	P34	+	+	-	-	-	-
P10	+	+	-	-	-	-	P35	+	+	-	-	-	-
P11	+	+	-	-	-	-	P36	+	+	-	-	-	-
P12	+	+	-	-	-	-	P37	+	+	-	-	-	-
P13	+	+	-	-	-	-	P38	+	+	-	-	-	-
P14	+	+	-	-	-	-	P39	+	+	-	-	-	-
P15	+	+	-	-	-	-	P40	+	+	-	-	+	-
P16	+	+	-	-	-	-	P41	+	+	-	-	+	-
P17	+	+	-	-	-	-	P42	+	+	-	-	+	-
P18	+	+	-	-	-	-	P43	+	+	-	-	+	-
P19	+	+	-	-	-	-	P44	+	+	-	-	+	-
P20	+	+	-	-	-	-	P45	+	+	-	-	+	-

+; detected, -; not detect.
S1-5; seasoned red-pepper sauce, P1-45; red pepper powder.

used as a positive amplification control (PAC) to verify the integrity of DNA extracted from processed red pepper products[11]. Additionally, Park et al. (2012) reported that garlic- and onion-specific PCR failed to amplify DNA extracted directly from seasoned red-pepper sauce[6]. Thus, DNA extracts from red pepper products, including seasoned red-pepper sauce, were subjected to WGA before identifying ingredients (red pepper, garlic, onion, spring onion, and ginger) used. As shown in Table 3, PAC was amplified in all of the 50 samples, suggesting that the extracted DNA was appropriate for use in species-specific PCR assays.

With the exception of two seasoned red-pepper sauces (S1 and S2) provided by the KCS, ingredient information for the other three seasoned red-pepper sauces (S3-5) was unavailable. Red pepper and garlic DNA were detected in the two samples (S1 and S2). It was in well accordance with the results informed by the KCS. In one sample (S5), red pepper and spring onion genes were detected, and red pepper was only identified in two samples (S3 and S4). Therefore, our findings demonstrated that these five samples failed to meet the requirements of seasoned red-pepper sauce.

In addition, we monitored 45 red pepper powders currently available in the market to examine the possible presence of garlic, onion, spring onion, and ginger. As shown in Table 3, red pepper was detected in all red pepper powders, while spring onion was identified in six red pepper powders (P40-45), despite the statement on the label describing red pepper as the only ingredient. Garlic, onion, and ginger were not identified in the 45 samples (P1-45).

In order to verify the reliability of our results, species-

specific PCR products were cloned into the TA vector and sequenced. The analyzed sequences were subjected to species identification through the Basic Local Alignment Search Tool of the National Center for Biotechnology Information. The sequences of species-specific PCR products were identified as red pepper (accession no. X78030.1, 100% identity), spring onion (accession no. KT781691.1, 100% identity), and garlic (accession no. EU909138.1, 100% identity).

Discussion

In this study, species-specific PCR in conjunction with WGA was used to monitor raw materials used in commercial red pepper products. When red pepper powder and seasoned red-pepper sauce were produced, DNA could be degraded into the short-length fragments during their processing steps, including drying and grinding. Therefore, DNA extracts were subjected to WGA, and their integrity was verified using the PAC system. Our strategy was suitable for the monitoring of labeling compliance of commercial red pepper products.

According to the KCS, red pepper in seasoned red-pepper sauce should account for less than 40% (w/w) of the ingredients. At least two other components (such as garlic, onion, spring onion, and ginger) should be mixed over 10% of the sauce to improve flavor in seasoned red-pepper sauce. However, all seasoned red-pepper sauce samples used in this study did not contain at least two additional ingredients mentioned above. These ingredients could not be detected if the amounts of garlic, onion, spring onion, and ginger used in the seasoned red-pepper sauce were below the limit of detection (LOD). In a previous study, Park et al. (2012) reported that species-specific PCR could detect 3.6-6.3% of onion and 4.0-20.0% of garlic in season red-pepper sauce after WGA[6]. Thus, when considering the LOD of this previous study, the five seasoned red-pepper sauce samples analyzed in this study failed to satisfy the 10% requirement, suggesting a possible example of EMA of seasoned red-pepper sauce. According to their labels, forty-five red pepper powders examined in this study should have only contained red pepper. In six red pepper powders, spring onion-specific PCR products were detected regardless of WGA, and therefore these six products failed to meet the requirement. However, it should be noted that unintentional mislabeling may occur, when multiple ingredients are ground on the same equipment without sufficient cleaning. The qualitative method employed in this study cannot exactly discriminate between this possible cross-contamination and intentional mislabeling.

Because WGA includes a nonspecific pre-amplification step during which all DNA can be amplified, its weakness is that it is susceptible to cross-contamination with other samples[12]. Therefore, the sequences of PCR products were analyzed after WGA, suggesting that the method used in this study was reliable and did not generate errors in DNA sequences or nonspecific reactions arising from cross-contamination during WGA. Therefore, our findings suggested that species-specific PCR in conjunction with WGA method was suitable for the screening of commercial red pepper products.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

국문요약

고추는 한국에서 매우 중요한 양념 중 하나이다. 하지만 수입 고춧가루와 다진 양념(다대기)에 부과되는 관세율(45%/270%)의 차이로 인해, 다진 양념이 수입된 후, 건조 및 분쇄 과정을 거쳐 고춧가루로 제작되고 있는 실정이다. 본 연구에서는 종 특이 PCR 기술과 whole-genome amplification 방법을 접목하여 고춧가루(N=45) 및 다진 양념(N=5) 제품의 사용원료(고추, 마늘, 양파, 파, 생강)를 분석하였다. 모니터링 결과, 39개 고춧가루 제품은 표시사항을 준수하였으며, 6개 고춧가루 및 5개 다진 양념 제품은 제조 기준을 충족시키지 못했다. 따라서 분석 제품의 22%가 표시사항을 준수하지 못한 것으로 밝혀졌으며, 본 연구에 사용한 분석 방법은 고춧가루 제품에 사용된 원료 분석에 적합한 방법임을 입증하였다.

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