遺伝子組換え（GM）ダイズ系統A2704-12の系統特異的定量検知法の開発および性能指標の評価

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Development and Evaluation of Event-Specific Quantitative PCR Method for Genetically Modified Soybean A2704-12

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A novel real-time PCR-based analytical method was developed for the event-specific quantification of a genetically modified (GM) soybean event; A2704-12. During the plant transformation, DNA fragments derived from pUC19 plasmid were integrated in A2704-12, and the region was found to be A2704-12 specific. The pUC19-derived DNA sequences were used as primers for the specific detection of A2704-12. We first tried to construct a standard plasmid for A2704-12 quantification using pUC19. However, non-specific signals appeared with both qualitative and quantitative PCR analyses using the specific primers with pUC19 as a template, and we then constructed a plasmid using pBR322. The conversion factor (Cf), which is required to calculate the amount of the genetically modified organism (GMO), was experimentally determined with two real-time PCR instruments, the Applied Biosystems 7900HT and the Applied Biosystems 7500. The determined Cf values were both 0.98. The quantitative method was evaluated by means of blind tests in multi-laboratory trials using the two real-time PCR instruments. The limit of quantitation for the method was estimated to be 0.1%. The trueness and precision were evaluated as the bias and reproducibility of relative standard deviation (RSDR), and the determined bias and RSDR values for the method were each less than 20%. These results suggest that the developed method would be suitable for practical analyses for the detection and quantification of A2704-12.

Key words: A2704-12; event-specific; genetically modified (GM); real-time PCR; soybean

Introduction

The use of genetically modified (GM) crops has been increasing since their commercialization in 1996. After more than a decade, the global area of GM crops has increased approximately 80-fold, from 1.7 million hectares in six countries in 1996, to 134 million hectares in 25 countries in 2009.1) The utilization of GM crops has generated substantial economical benefits, but, nevertheless, has been subjected to rigid control. The presence of GM products in crops or foods is obliged to be labeled in the European Union (EU), Korea, Japan, Australia, and many other countries. In Japan, the genetically modified organism (GMO) labeling system has been defined by the “JAS law”1) and the “Food Sanitation law”2), and the thresholds for the unintentional commingling level for approved GM soy and maize were both set at 5%3), 4).

The Japanese food self-sufficiency ratio on a calorie supply basis has been hovering around 40% in recent years, but the ratios among grains, except for rice, are extremely low5). Among them, soybeans are one of the most important crops in Japan. The domestic consump-

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1) Notification No. 1173 (Oct. 1, 2007); Ministry of Agriculture, Forestry and Fisheries of Japan (2007)
tion of soybeans as oil and food is over 4 million tons/year, but the self-sufficiency ratio for this crop is only approximately 5%**, i.e., more than 90% of soybeans are imported into Japan. Most of them are imported from the United States, where GM soybeans account for more than 90% of the soybean cultivation areas**. There are several approved GM soybean events in Japan**. The first commercial GM soybean was a glyphosate-tolerant soybean (GTS), event 40-3-2 [Roundup Ready® soybean (RRS)]. RRS has received regulatory approval in many countries, including Japan. Next, glufosinate-tolerant soybeans such as A2704-12 and A5547-127 were approved, and then the second generation of GTS, MON89788, was recently approved.

A2704-12 and A5547-127 contain the same transgene cassette consisting of cauliflower mosaic virus 35S promoter (P35S), the synthetic pat gene which codes phosphinothricin N-acetyltransferase derived from Streptomyces viridochromogenes**; and cauliflower mosaic virus 35S terminator (T35S)**. Many methods for analyzing RRS and MON89788 have been published**; but there is no report on quantification of A2704-12, although A2704-12 is now being commercially cultivated.

In this study, we developed a new quantitative method for A2704-12, using event-specific PCR, and then evaluated the method with two multi-laboratory trials. The development of the quantitative method was mainly carried out in the National Food Research Institute, and the interlaboratory collaborative study was independently conducted with the National Institute of Health Sciences (NIHS).

**Materials and Methods**

**Plant materials**

Regarding soybean and maize seeds, A2704-12 and a non-GM isoline of A2704-12 used as a non-GM soy sample were kindly provided by the developer, MON 89788, RRS, MON810, MON863, MON88017, and NK 603 by Monsanto Company (St. Louis, MO, USA), Bt11, Event176, GA21, and MIR604 by Syngenta Seeds AG (Basel, Switzerland), TC1507 and DAS59122 by Pioneer Hi-Bred International (Johnston, IA, USA), and T25 was directly imported from the USA. QC9651 maize from Quality Technology International, Inc. (Elgin, IL, USA) was used as a non-GM maize. Seeds of rice (*Oryza sativa*) variety Kinuhikari, wheat (*Triticum aestivum*) variety Haruyutaka, and barley (*Hordeum vulgare*) variety Harrington were harvested in Japan. A5547-127 was purchased from the American Oil Chemists’ Society (AOCS) (Urbana, IL, USA) as DNA extract (AOCS 0707-C; above 999.9 ng/μL of GM DNA).

**Oligonucleotide primers and probes**

For the specific detection of A2704-12, a pair of primers (KVM175; 5'-GCACCCCGGTGTTACCTCT-3' and SM0001; 5'-ATTCAGGCTGCGCAACTGTT-3') and a fluorescent dye-labeled probe (TM031; 5'-CGG-TCTCCTGATCGCCCTTCC-3') were used for real-time PCR. The sequences of these primers and probe were taken from the report of the European Commission’s Joint Research Centre (JRC, IRMM, Retieseweg, Geel, Belgium)**. Le1** was used as a soybean-specific endogenous reference DNA for quantitative analysis. For specific detection of Le1, the primers ([Le1n02-5'; 5'-GCCCTCTACTCCACCCCCCA-3' and Le1n02-3'; 5'-GCCCATCTGCAAGGCTTCTTTT-3']) and fluorescent dye-labeled probe ([Le1-Taq; 5'-AGGCTTTGCCGCTTCCCTTACAATCAC-3']) were used**. The oligonucleotide primers and TaqMan® probes were synthesized by FASMAC Co., Ltd. (Kanagawa, Japan) and Life Technologies (Carlsbad, CA, USA), respectively. The oligonucleotide probes were labeled with 6-carboxy-fluorescein (FAM) at the 5' ends and 6-carboxy-tetramethylrhodamine (TAMRA) at the 3' ends.

**Preparation of calibrant plasmid**

Specific sequence fragments from A2704-12 and the endogenous soybean Le1 gene were synthesized as a single oligonucleotide in tandem and inserted into a pBR322 vector. The constructed plasmid was purified by equilibrium centrifugation in a CsCl gradient to collect the covalently closed circular DNA, which was linearized by cutting at a restriction site located outside the integrated fragment. The concentration of the linearized DNA was calculated from the ultraviolet (UV) absorbance measured with a spectrophotometer, DU 800 (Beckman Coulter, Fullerton, CA, USA) as described previously**; and converted to the molar concentration. The solution was then diluted to theoretical concentrations of 20, 125, 1,500, 20,000, and 250,000 copies per 2.5 μL, with 5 ng/μL of ColE1 DNA (NIPPON GENE, Tokyo, Japan) solution. Finally, the copy numbers of the diluted plasmids were adjusted based on the Le1 segment of pMuSL2, which has been adopted in the Japanese standard analytical method**, using quantitative real-time PCR analyses.

**Footnotes**


** List of products that have undergone safety assessment and been announced in the Official Gazette (May 12, 2010) of the Department of Food Safety, Ministry of Health, Labour, and Welfare; http://www.mhlw.go.jp/english/topics/food/pdf/sec01-2.pdf


Preparation of test samples and DNA extraction

To evaluate the quantitative method, we used six mixing levels of test materials containing 0%, 0.10%, 0.50%, 1.0%, 5.0%, and 10.0% A2704-12. To prepare the mixed samples, we ground A2704-12 seeds and non-GM seeds using MM200 and 2M100 grinders (Retsch, Haan, Germany), respectively, as described previously\(^{15}\), and then mixed the samples on a weight-to-weight basis. DNA was extracted from the ground materials using GM quicker (NIPPON GENE) according to the manufacturer’s manual. The concentration and quality of extracted DNA solutions were evaluated by measuring UV absorbance with a spectrophotometer, ND-100 (NanoDrop Technologies, Wilmington, DE, USA). Soybean genomic DNA solutions were adjusted to a concentration of 20 ng/\(\mu\)L.

Qualitative PCR

Qualitative PCR using a thermal cycler, GeneAmp PCR system 9700 (Life Technologies), and agarose gel electrophoresis were performed as described previously by Kuribara et al. (2002).

Quantitative PCR

TaqMan\(^{\text{®}}\) real-time PCR assays were carried out using the Applied Biosystems 7900HT (AB 7900) or the Applied Biosystems 7500 (AB 7500) (Life Technologies), in 25 \(\mu\)L final volume, containing 50 ng of sample DNA, 12.5 \(\mu\)L Universal Master Mix (Life Technologies), 0.5 \(\mu\)M primer pairs, and 0.2 \(\mu\)M probe. The step-cycle program was as follows: 2 min at 50°C, 10 min at 95°C, 45 cycles, 30 s at 95°C, and 1 min at 59°C. In the reaction plate, each sample was measured in triplicate.

Multi-laboratory trial

Multi-laboratory trials were performed with the AB 7900 and the AB 7500 independently and consisted of 2 separate stages: measurement of the \(C_{\text{t}}\) value and a blind test. All measurements were conducted by 5 laboratories for the AB 7900 and 3 laboratories for the AB 7500. Experimental protocols were provided by the NIHS. Quantitative real-time PCR was performed with primers, probes, Universal Master Mix, and blind DNA solutions supplied by NIHS.

The first stage was the experimental determination of the \(C_{\text{t}}\) value as the ratio of the copy number of recombinant DNA (r-DNA) to the taxon-specific sequence in the GM plant genome. To calculate the \(C_{\text{t}}\) value for A2704-12, we extracted the genomic DNA from genuine GM seeds and determined the copy numbers of r-DNA and taxon-specific sequences. The measurement was conducted twice at each laboratory. The \(C_{\text{t}}\) value for each real-time PCR instrument was separately determined as the mean of the obtained values.

A blind test was carried out as the second stage. Blind samples designed as blind duplicates of the soybean genomic DNAs extracted from 6 different concentrations of A2704-12, 0.0%, 0.10%, 0.50%, 1.0%, 5.0%, and 10.0%, were sent to the participants. All participants were requested to submit the data from the real-time PCR analyses. All submitted data were analyzed by means of Cochran’s test\(^{41}\) and Grubbs’ test\(^{15,16}\).

Results and Discussion

Specificity of the PCR system for A2704-12

A2704-12 contains two copies of the pat gene cassette inserted in a head-to-tail configuration\(^{10}\). The biotic transformation of soybeans was conducted by microparticle bombardment with a pUC19-based plasmid containing the pat gene cassette. The pUC19 plasmid contains an antibiotic resistance gene; beta-lactamase (bla), and a lac operon, and several fragments derived from pUC19 DNA exist in the A2704-12 genome. Partial sequences derived from the bla and lac operon are integrated side-by-side between the two pat gene cassettes\(^{10}\), and this site is unique to A2704-12. For specific detection of A2704-12, the junction site was used (Fig. 1). The specificity of the primer set was confirmed by qualitative PCR. The expected 64-bp product was detected using genomic DNA solutions from A2704-12, but not from non-GM soybeans; GM soybeans RRS, MON89788, and another glufosinate-tolerant soybean: A5547-127; non-GM maize: 11 lines of GM maize; rice, wheat, and barley; and the no template control (Fig. 2).

\[^{10}\] Agbios database: http://www.cera-gmc.org/?action=gm_crop_database&

Fig. 1. Schematic diagrams of the target positions in A2704-12

The event-specific target sequence of A2704-12 is the junction region of two partial sequences derived from the bla gene and lac operon, corresponding to positions 248-267 and 2036-2056 in pUC19, between the two pat gene cassettes. The nucleotide sequence of pUC19 is available under accession number L09137.
Fig. 2. Specificity test of the designed primer pair for genomic DNAs from several GM events or crops
Agarose gel (3.0%) electrophoretogram of the amplified PCR products corresponding to the 64 bp of A2704-12 DNA. Arrowhead indicates the expected amplified product. Lanes 1-11, eleven GM maize events, namely NK603, Event176, T25, GA21, MON810, TC1507, Bt11, MIR604, MON88017, DAS59122, and MON863, respectively; 12 and 13, non-GM maize and non-GM soy, respectively; 14-17, two GM soybean events, namely, RRS, MON89788, A2704-12, and A5547-127, respectively; 18-21, rice, wheat, barley, and no template, respectively. Lane M shows 100 bp ladder size markers.

Fig. 3. Specificity test of the designed primer pair and probe for plasmid DNAs
A: Qualitative PCR analysis. Lanes 1-4, A2704-12 genomic DNA, CoIE1 DNA, pUC19, and pBR322, respectively. The arrow indicates a non-specific amplification product. Lane M shows 100 bp ladder size markers.
B: Quantitative PCR analysis. pUC19, pBR322, or CoIE1-derived signals in the amplification profile using the AB 7900 are shown. Concentrations of DNA solutions of A2704-12 genomic DNA, CoIE1 DNA, pUC19, and pBR322 were 20, 5, 10, and 10 ng/µL, respectively.

Construction of calibrant plasmid for A2704-12
In Japan, one of the features of standard quantitative methods is the utilization of standard plasmid DNA as reference molecules and calibrators. We have developed several standard plasmids utilizing pUC19 or pBR322. Although pUC19 is a useful plasmid containing a high-copy-number replicon, when the pUC19 plasmid was used as a template, a non-specific amplified product of around 1.0 kb was observed (Fig. 3A). Furthermore, a non-specific signal was detected by quantitative PCR analysis (Fig. 3B). As mentioned above, for the specific detection of A2704-12, the pUC19-derived sequences, KVM175 and SMO001, which are a part of the sequences of bla gene and lac operon, respectively, were used as primers. The unexpected band may have been caused by the sequences of these primers, indicating the possibility that inaccurate quantification could occur if pUC19-based plasmids were used as a calibrator for quantification. Thus, we used pBR322 plasmid for the construction of the standard plasmid, designated as pLLS. To prepare the pLLS plasmid, we removed a bla gene partial sequence that included KVM175 from pBR322 and then inserted the A2704-12 and Leu segments (Fig. 4). Figure 5 shows the amplification plots of the pLLS plasmids diluted to a concentration ranging from 20 to 250,000 copies per reaction (Fig. 5A and B).
2 values from both A2704-12 and Le1 were above 0.999 (Figs. 5C and D), which is an acceptable level (R² should be above 0.990)⁸. The slopes, representing the amplification efficiencies, were -3.44 and -3.32 for A2704-12 and Le1 standard curves, respectively. PCR efficiency was calculated by use of the following equation: PCR efficiency = 10^{(1/slope)}¹⁹. The theoretical value with an efficiency of 100% in each cycle is 2.00, and, in this study, PCR efficiencies of 1.95 and 1.99 were obtained for A2704-12 and Le1, respectively.

**Determination of the Cᵢ value for A2704-12**

To determine the experimental Cᵢ value for A2704-12, we measured the copy numbers of Le1 and A2704-12 in the extracted DNA from A2704-12 seeds. The Cᵢ value was determined with two real-time PCR instruments (the AB 7900 and the AB 7500) independently, from the results of 5 laboratories for the AB 7900 and 3 laboratories for the AB 7500. The measurement was repeated twice, and the Cᵢ value was determined as the mean of values measured by these laboratories. The determined Cᵢ values with the AB 7900 and the AB 7500 were similar; in fact, they took the same value of

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SD: Standard deviation  
RSD: Relative standard deviation

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Fig. 4. Schematic diagram of the construction of pLLS plasmid

The region corresponding to positions 3613-3843 in pBR322 was removed, and the specific sequences of A2704-12 and the Le1 were inserted in tandem. The nucleotide sequence of pBR322 is available under accession number J01749.

Fig. 5. Amplification plots for dilution series of pLLS plasmid and corresponding curves

Typical amplifications of A2704-12 and Le1 segment are shown in A and B, respectively. The respective standard curves are shown in the panel below, and the R² and slope values of each standard curve are indicated at the upper-right corners. The dilutions contained 250,000, 20,000, 1,500, 125, and 20 initial template copies per reaction, respectively. These analyses were performed with the AB 7900.
Evaluation of the solution-based blind samples as described trials analytical methods which were developed to quantify. The obtained precisions were significantly smaller than those of the extraction step. However, the obtained precisions were significantly smaller than those of the extraction step. After that, the established methods were evaluated with another interlaboratory study which was performed using the same materials without the DNA extraction step. From these results, we used 0.98 in the following quantifications as the common C<sub>t</sub> value for the two instruments.

**Evaluation of the PCR quantification by multi-laboratory trials**

We performed multi-laboratory trials to evaluate the developed quantitative method for A2704-12 as a blind test using the AB 7900 and the AB 7500 instruments in 5 and 3 laboratories, respectively. We used DNA solution-based blind samples as described previously. For PCR quantification, analytical procedures would be divided into two main steps, that is, DNA extraction from samples and real-time PCR measurements. In our previous study, the Japanese standard analytical methods which were developed to quantify RRS and several GM maize events, were validated with an interlaboratory study which consisted of 2 steps, namely, DNA extraction and PCR quantification. After that, the established methods were evaluated with another interlaboratory study which was performed using the same materials without the DNA extraction step. However, the obtained precisions from the two studies were almost the same, suggesting that, at least in our system, the repeatability of relative standard deviation (RSD<sub>r</sub>) and reproducibility of relative standard deviation (RSD<sub>b</sub>) of the DNA extraction step were significantly smaller than those of the PCR quantification step. It was also suggested that the relatively large RSD<sub>r</sub> and RSD<sub>b</sub> values which were obtained from GMO quantification, may be attributed to the principle of real-time PCR, which is based on relative quantification between target and taxon-specific sequences, rather than absolute quantification.

All the participants received primers, probes, and test samples consisting of six different concentrations of A2704-12, and the measurement was performed twice. All the submitted data were examined for outlier laboratories with extreme variation using Cochran’s test (p < 0.025) and with an extreme average level using Grubbs’ test (p < 0.025) for the AB 7900 and the 7500 independently, as previously described, and no outlier was found. The blank sample, 0% A2704-12, was used to estimate invalid laboratories, and no laboratory was eliminated. We then used all of the submitted data obtained from mixed samples with five different GM contents (0.1%, 0.5%, 1.0%, 5.0%, and 10.0% concentrations) for further statistical analyses. The trueness and precision were determined for the AB 7900 and the 7500 as previously described. The mean, bias (mean-value, %), RSD<sub>r</sub>, and RSD<sub>b</sub> of blind samples were measured (Table 2). The determined bias, RSD<sub>r</sub>, and RSD<sub>b</sub> for the AB 7900 ranged from −14.6% to 1.0%, from 6.4% to 9.9%, and from 7.5% to 10.7%, respectively. The determined bias, RSD<sub>r</sub>, and RSD<sub>b</sub> for the AB 7500 ranged from −7.1% to 11.7%, from 3.0% to 13.2%, and from 4.0% to 13.2%, respectively. The obtained bias, RSD<sub>r</sub>, and RSD<sub>b</sub> here were similar to or within a narrower range than those in previously reported GMO events. The data below 20 copies were extrapolated from the standard curve in our method because there was no calibrant below 20 copies.

We developed a specific quantification method for

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<sup>a</sup> RSD<sub>r</sub>: Repeatability relative standard deviation
<sup>b</sup> RSD<sub>b</sub>: Reproducibility relative standard deviation
<sup>c</sup> Number of values less than 20 copies per the total number of retained data.
<sup>d</sup> When RSD<sub>r</sub> was above RSD<sub>b</sub>, RSD<sub>b</sub> was considered to be the same as RSD<sub>b</sub>.
GM soybean A2704-12. The experimentally determined C_r value was 0.98. The LOQ, trueness, and precision of this method were similar to or better than those of previous methods^{20-23}. ISO 24276 specifies the LOQ in GMO analysis, and the values are generally observed to have a RSD of 25% or less at the lowest level^{24}; all the RSDs obtained in this study met this criterion. Therefore, we concluded that the developed method would be applicable for the detection and quantification of A2704-12 to monitor the validity of the food labeling system in Japan.

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FASMAC Co., Ltd., Kanagawa, Japan
Food and Agricultural Materials Inspection Center, Saitama, Japan
Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan
Hiroshima Prefectural Technology Research Institute, Public Health and Environment Center, Hiroshima, Japan
Japan Food Research Laboratories, Tokyo
Kanagawa Prefectural Institute of Public Health
National Food Research Institute, Ibaraki, Japan
Tokyo Metropolitan Institute of Public Health

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References

6) De Beuckeleer, M. Elite event A5547-127 and methods and kits for identifying such event in biological samples.

*11 International Standard 24276, Foodstuffs—Nucleic acid based of analysis for the detection of genetically modified organisms and derived products—General requirements and definitions.

14) Cochran, W. G. The distribution of the largest of a set of estimated variances as a fraction of their total, Annals of Eugenics, 11, 47-52 (1941).


遺伝子組換え(GM)ダイズ系統A2704-12の系統特異的定量検知法の開発および性能指標の評価（報文・英文）
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小村雄宏 布野 聡 島崎孝明 手塚玲子
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GMダイズ系統A2704-12の系統特異的定量検知法の開発は、ゲノムに複数のpUC19由来のDNA断片が存在し、特異的PCR增幅領域としてこれらを配列を用いた。しかしながら、pUC19を用いたPCRを行ったところ非特異的な増幅が観察されたため、定量用プロック標準プルシックの構築にはpUC19ではなくpBR322を用いた。また、本研究によって、A2704-12混合比率率の際に必要な数値である内部補正を決定した。さらに、さまざまな濃度のA2704-12を含む疑似混合試料を調製し、複数の試験室において性能指標を評価したところ、本分析法の定量下限値は0.1％と見込まれ、偏差、室間再現性とも20％を下回る結果が得られた。
*（独）農研機構 食品総合研究所

液中食品用容器PID(Pouch in Dispenser)の製造に関する試験検査（報文）
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二階俊規 根尾大介 一色啓司*
食衛誌52(2)、108－111(2011)

PETボトルおよびガラス瓶のリユースに関する検証（ノート）
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PETボトルおよびガラス瓶のリユースに際し、汚染物質に対するリユースシステムの安全性を検証するため、汚染物質10種類を用いて検証試験を行った。污染物質として1,1,1,2－トリフルオロエタン、クロホルム、トルエン、ベンゼン、コハク酸エチル、フルオロホルムを用い、これらをPETボトルに充てんし50℃で7日間保存後、水洗浄後にアルカリ洗浄したものを材質試験および溶出試験を実施した。その結果、各物質はPETボトル材質中に水洗浄後430～1,400μg/g。3.5％アルカリ洗浄後225～925μg/g。残留したが、それらのボトルに95℃の水をかいて10℃以下で7日間保存したところ、内包液に0.096～7.35μg/mLに減少した。清浄飲料水の含有成分であるミネラルがアルカリ洗浄後48μg/g。10μl/mLに減少した。一方、アルカリ洗浄後のガラス瓶ではいずれの物質もほとんど溶出しなかった。これらの結果から、PETボトルおよびガラス瓶に比べ、汚染物質の質保証や衛生安全性の観点からリユースには適さないと結論された。
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LC-MS/MSによる味付け海藻中のチアアメンゾールの分析法の検討（ノート）
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香油中の味付け海藻中のチアメンゾール(TBZ)を検出した事例を経験した。原因調査の結果、味付け工程で調味液を製造する機械に使用されているスプーンに汚染された。抗酸剤で使用されていたTBZが海藻に移行したことが判明した。今回、LC-MS/MSを用いて味付け海藻中のTBZの定量方法を検討した。味付け海藻の製造工程でこのスプーンが広く普及していたことから、改良した定量方法を用いて同時期と翌年の市販品についてTBZの調査を実施した。分析例のあった平成20年3月3日まで6検体中5検体で0.014～1.736μg/g。翌年の平成21年7月では6検体中1検体で微量のTBZが検出された。以上の結果から味付け海藻の製造工程が見直され、現在では改善されていると考えられた。
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輸入冷凍野菜・果実中の残留農薬実態（1986年4月～2003年5月）（調査・資料）小林雅統* 大倉健治 田村寛也 高山明夫 田村重雄
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1989年4月から2003年3月にかけて東京都内で市販されていた輸入冷凍野菜・果実595検体について農薬の残留調査を行った。182検体から45種類の有機剤やリン系農薬、有機リン系農薬、ビペラチル系農薬、カルバメート系農薬、ペラメート系農薬、その他農薬の検出数（0.01ppm未満）4.6ppm)の範囲で検出された。農薬類（みそ酵素およびかぼす類）は、スクロビオール、メルトロンおよびオトニオエート、豆科野菜（えだもおよび新鮮ないんげん）では、ギリシャトウおよびカルガレル（NAC）の検出数が高かっただった。また、トウモロコシ、豆、菜豆などの蔬菜に農薬の検出が高かった。その結果、農薬の検出が高かった野菜の結果が、食前調査を用いて推定された高水分の効果、食品の摂取条件の悪化、それに伴う食事の不適度な状態、公衆衛生の重要性が示された。特に、農薬の摂取が健康に影響を及ぼす可能性を考える必要がある。
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鮮魚中の一酸化炭素分析法の改良（調査・資料）
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通知で規定されている鮮魚中の一酸化炭素(CO)分析法のうちA法（通称A法）は、試料を多重に必要とし、また試料調剤調製時に鮮魚中のCOの一部が散逸するなどの問題が指摘されている。そこで、本研究では、これらの問題点の解決ならびに適正なコントロールのため、分析方法を一部変更した分析法（改良A法）の適用性を検討した。また、改良方法を用いて通知されているマグロ、ブリ、ハマチおよびヒラスアヒのCO濃度のパーセントルート値を調査した。その結果、改良A法は、試料調剤調製時のCOの散逸抑制、試料量の減少、操作の簡便性の点で通称A法よりも優れており、鮮魚中のCO分析に適用可能であることが確認された。さらに、4種類共同実施した鮮魚中のCO濃度のパーセントルート値においては、改良A法が通称A法と比較してCOの回収率を向上させること、特にCO未処理のヒラスアヒ中のCO濃度が現在の推定値と同程度に測定することができた。このことから、改良A法を今後新たに急性アシモフ中毒の場合のCO分析法として適用する場合には、プリアシモフの測定値の変化が必要であると考えられた。
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