Analysis of Cereulide in Food Using Triple Quadrupole Mass Spectrometer

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1. Introduction

◆*Bacillus cereus* is a Gram-positive spore-forming rod that commonly contaminates various agricultural products and causes foodborne illness. Its vegetative cells and spores are frequently found in boiled or fried rice, pasta, vegetables, and dairy products[1]. *B. cereus* is known to cause two types of foodborne illnesses: diarrheal and emetic. The emetic type is caused by cereulide (Fig.1), a toxin produced by the bacterium.

◆The measurement of vacuolation degeneration activity using HEp-2 cells and PCR are known methods for the detection of cereulide. However, these methods require several days of cell culture or cannot confirm the production of cereulide itself. Therefore, in recent years, there has been a growing demand for analytical methods that directly detect cereulide using LC-MS/MS[2].

◆We have developed a pretreatment method using acetonitrile as an extraction solvent and an analytical method for cereulide in fried rice using a UHPLC-based LC-MS/MS.

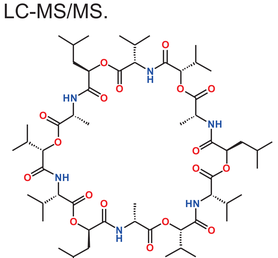

Fig. 1 Structure of Cereulide

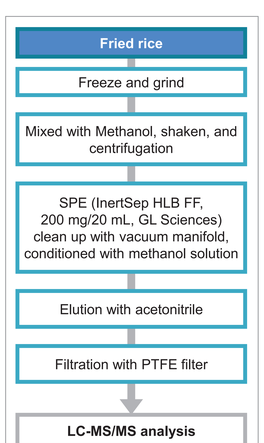

Fig. 2 Workflow of pretreatment

2. Methods

◆The pretreatment method (Fig. 2) was performed with reference to the Annual Report of Kumamoto Prefectural Institute of Public - Health and Environmental Science[3].

◆Fried rice sample preparation : 1 g of frozen pulverized fried rice was mixed with 10 mL of methanol and shaken for 5 min. after centrifugation at 3,000 rpm for 5 min, the supernatant was collected by decantation. The residue was mixed with 5 mL of methanol and subjected to 5 min of shaking treatment. After centrifugation at 3,000 rpm for 5 min, it was added to the extract and adjusted to a final volume of 15 mL with methanol.

◆SPE clean up: The SPE cartridges (InertSep HLB FF, 200 mg/20 mL, GL Sciences), which have large particle sizes in the packing material and improved flowability to reduce clogging, were used to remove lipid components from the extracts of fried rice. Using the vacuum manifold, the SPE cartridges were conditioned with 10 mL of 75% methanol solution. The fried rice extract was then passed through an SPE cartridge and eluted with 10 mL of acetonitrile.

◆LC-MS/MS analysis: The collected eluate was filtered with PTFE filter and the solution was analyzed by LC-MS/MS. In this study, the LCMS-8060RX was used for analysis (Fig. 3). The analytical conditions are shown in Table 1.

High Speed Mass Spectrometer
Ultra Fast Polarity Switching
- 5msec
Ultra Fast MRM
- Max. 555 transition /sec

Fig. 3 Nexera™ and LCMS-8060RX

Table. 1 Analytical condition

[HPLC conditions] (Nexera X3)				
Column	:	Shim-pack Scepter™ C18-120^{*1}(50 mm x 2.1 mm I.D., 1.9 µm)		
Mobile phase A	:	10 mM Ammonium formate-0.1% Formic acid-Water		
Mobile phase B	:	Acetonitrile		
Gradient program	:	B conc. 80% – 95% (6-10 min) – 80% (10.01-15 min)		
Flow rate	:	0.4 mL/min		
Column temp.	:	40 °C		
Injection volume	:	3 µL		
[MS conditions] (LCMS-8060RX)				
Ionization	:	ESI, Positive mode		
Mode	:	MRM (*m/z* 1170.70 > 357.30)		
Interface voltage	:	+1 kV	Focus voltage	: +2 kV
Nebulizing gas flow	:	2.0 L/min	Interface temp.	: 300 °C
Drying gas flow	:	5.0 L/min	DL temp.	: 300 °C
Heating gas flow	:	15.0 L/min	Heat Block temp.	: 500 °C
Probe position	:	+1 mm		

3. Results

◆The calibration curve is shown in Fig. 4. Additionally, Fig. 5 shows the MS chromatograms of cereulide in the standard and recovery test samples. A calibration curve with good linearity (R^2 > 0.999) in the range of 0.005 to 50 µg/L was obtained.

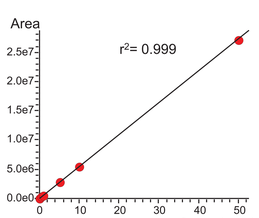

Fig. 4 Calibration curve of Cereulide

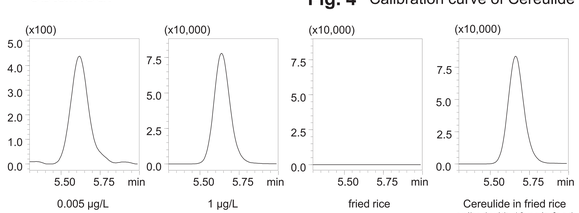

Fig. 5 MS chromatograms of Cereulide

◆Determination of cereulide was performed on frozen fried rice. An additive recovery test was also conducted with fried rice free of cereulide. The amount of cereulide standard solution was added to reach 10 ng/g. A sample of 1 g of frozen pulverized fried rice to which cereulide was added, extracted, and purified in solid phase was analyzed by LCMS-8060RX, and a good additive recovery of 110.1% was obtained. The MS chromatograms are shown in Fig. 5.

4. Conclusion

◆The standard samples and the pretreated samples were analyzed using a triple quadrupole mass spectrometer LCMS-8060RX equipped with a Nexera X3 UHPLC (Shimadzu). Good linearity was maintained over a wide calibration range of 0.005 to 50 µg/L.

◆Determination of cereulide was performed on fried rice. The recovery test was conducted at 10 ng/g, lower than the level reported in a food poisoning incident, using the implicated food as a reference. Using the optimized analytical method, a good recovery rate within the range of 80-120% was achieved in fried rice with simple pretreatment. The conventional cereulide analysis method, the HEp-2 cell vacuolation test, is time-consuming, but this method is a rapid analysis method that can be performed in a short time from sample pretreatment to measurement and analysis.

Reference
1) U.S. Food and Drug Administration (2020). Bacteriological Analytical Manual Chapter 14: Bacillus cereus
2) Julien Masquelier et al. (2023). Validation of a Targeted LC–MS/MS Method for Cereulide and Application in Food and Faeces: Toxins, 16(1), 13
3) Kazuma Yagi et al. (2021). Development of rapid analytical method for cereulide by LC-MS/MS: Annual Report of Kumamoto Prefectural Institute of Public-Health and Environmental Science, No.51

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