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Solid-phase extraction of staphylococcal enterotoxin A in dairy products using an ion exchange resin

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ABSTRACT

In this study, we developed a solid-phase extraction method for staphylococcal enterotoxin (SE), especially SE type A (SEA), in milk and dairy products. Ion-exchange resin, a cation-exchanger, was applied for the extraction of SEA, which was then measured using a sensitive fluorescence immunoassay. First, the optimal pH for the binding of SEA to the cation exchanger was determined to be pH 4.0. Second, the optimal volume of elution buffer, consisting of phosphate buffer with 0.5 M sodium chloride at pH 8.0, was estimated to be 6 ml for a column containing 4 ml wet volume of the cation-exchanger. Food samples (10 ml each) were then studied for the application of the extraction method. High recovery of SEA was obtained from raw milk (approximately 90%), sterilized milk product (approximately 60%), and skimmed milk powder (approximately 60%), whereas the recovery from cheese (approximately 40%) was lower than from other samples. These recoveries were almost constant at different concentrations of spiked SEA. The minimum measurable level with this extraction method was very low, being 0.25 ng/g in skimmed milk powder for example. With a larger volume (60 ml) of sample, this level was as low as 0.025 ng/g SEA in skimmed milk powder. The present extraction method was very simple and time saving in comparison with the conventional method with chloroform and trichloroacetic acid. The results of this study indicate that this extraction method could be applicable for the measurement of SEA in milk and dairy products.

1. Introduction

Staphylococcus aureus food poisoning outbreaks are still a serious health problem worldwide (CDC; Schelin et al., 2011). Over 14,000 individuals who ingested low-fat milk products from a dairy company suffered from staphylococcal food poisoning in Osaka, Japan in 2000 (Anonymous; Asao et al., 2003). Skimmed milk powder, an ingredient for low-fat milk products, was responsible for the outbreak. It had been produced from raw milk at a plant in Hokkaido, Japan, and then sent to the company in Osaka. Exposure of the raw milk to high temperatures because of a loss of power supply during the production in the Hokkaido plant may have been the underlying contributing factor that permitted *S. aureus* growth and subsequent staphylococcal enterotoxin type A (SEA) production in the milk. It was found that the temperature of the in-line milk in the condensing process in the plant remained at about 40 °C for over 9 h (Anonymous).

After the outbreak in Osaka, Fujikawa and Morozumi (2006) quantitatively studied *S. aureus* growth and SEA production in pasteurized milk products at a variety of temperatures and then successfully predicted the growth and SEA production of the microbe in the product. Recently, Sabike, Fujikawa, Sakha, and Edris (2014) further studied *S. aureus* growth and SEA production in raw milk contaminated with natural microflora at various temperatures. They found that the optimal temperature for SEA production in two raw milk samples were as high as 40 °C and 44 °C in the range of 36 °C and 48 °C, which were close to the temperature in the outbreak in Osaka (Sabike et al., 2014).

Since the outbreak in Osaka, SEA in dairy products has been measured using a trichloroacetic acid (TCA) precipitation method in Japan (Anonymous; Asao et al., 2003). Briefly, samples are initially adjusted with hydrochloric acid to pH 3.8 and then centrifuged. The supernatant of the sample is adjusted to pH 6.6–6.8 and then chloroform is added for defatting. After centrifugation, the obtained supernatant is treated with TCA to precipitate proteins in the supernatant and then centrifuged again. The

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obtained pellet is dissolved in Tris buffer and then the pH is adjusted to 7.5. This final sample is subjected to SEA measurement. As is clearly evident, this method consists of many processes, making it time consuming. In the TCA method, moreover, a rotary evaporator is needed for concentration of the samples, but this was not successful for some dairy products, such as ice cream and cheese (Asao et al., 2003).

Solid-phase extraction is a general preparation method for the detection of pesticides and other chemical substances in food in routine examinations (Hernández & Ibáñez, 2013). With this extraction method, the substance of concern dissolved in a liquid sample is easily separated from other compounds according to its physical and/or chemical properties. On the other hand, few investigators have studied this preparation method for the detection of large biomolecules such as proteins.

Ion-exchange chromatography is one of the most-used separation techniques in protein purification, and because elution normally takes place in mild conditions, the protein will not be denatured during the chromatographic process (Giacometti & Josić, 2013). Balaban and Rasooly (2001) recovered SE type B (SEB) from mushroom and infant soy formula using a cation-exchanger, carboxymethylcellulose, and then detected SEB by western blotting. For SEA detection from raw milk and dairy products, Asao et al. (2003) discussed the use of solid-phase extraction with an ion-exchange resin as one possible method. They examined the extraction of SEA from dairy products using a batch absorption method with a cation exchanger (CM-Sepharose™, GE) and then detected SEA using an immunoassay kit (data not shown). Recently, Hamada, Okuno, Ino, and Hosono (2013) detected SEA and SEC_1 using a cation exchanger from a rice cake containing sugared beans, which was incriminated in a staphylococcal food poisoning outbreak. They absorbed SEs in a homogenate of the food sample using a batch method, and then the eluent from the cation exchanger, sulfopropyl-Sephadex™ (GE), was tested for the toxin measurement with mini VIDAS®. However, the optimal extraction conditions were not examined in their study.

The above studies suggested that solid-phase extraction using an ion-exchange resin could be capable of SE extraction from food samples, but the optimal conditions and the performance, such as the detection limit and recovery, with this extraction method have not been not studied. Examination for SE, especially SEA in milk and dairy products, is very important to prevent from staphylococcal food poisoning (Asao et al., 2003). Therefore, in the present report, an extraction method for SE, primarily SEA in milk and dairy products, using ion-exchange resin was studied. Herein, we report the optimal conditions for the extraction method, and then the extraction was applied to detect SEA in milk and dairy products as an evaluation. Furthermore, a larger volume of a sample was also studied to detect lower SEA levels. The toxin measurement was performed with a sensitive, fluorescence immunoassay using mini VIDAS®.

2. Materials and methods

2.1. Toxin

SEA, which was purified by the method of Oda (1978), was kindly provided by Denka Seiken Co. (Tokyo, Japan). SEA produced in the culture supernatant of an SEA-producing strain, 13,008 (Sabike et al., 2014), was also used in this study. Strain 13,008 was cultured in brain heart infusion broth (Becton, Dickinson and Company, Sparks, USA) with shaking at 110 rpm at 37 °C for 24 h, and then the culture was centrifuged at 15,000 *g* for 15 min to obtain the supernatant.

2.2. Toxin measurement

The SEA concentrations in samples were measured using an enzyme-linked fluorescence assay, VIDAS® Staph enterotoxin II (bioMerieux, Marcy-l'Etoile, France) using a mini-VIDAS® automated system. The pH values of samples were adjusted to pH 7.5–8.0 for measurement with a pH meter, in accordance with the manufacturer's instructions. Test values (TVs) of samples measured using the mini-VIDAS® were used for the estimation of toxin concentrations. The estimation was done with a standard curve with purified SEA (Denka Seiken) dissolved in VIDAS ® extraction buffer (Fujikawa & Morozumi, 2006; Sabike et al., 2014). TVs lower than 0.02 were regarded as being potentially unreliable and disregarded.

2.3. Milk and dairy products

Raw milk sample was taken from a Holstein cow, no. 11 at our university farm (Sabike et al., 2014). Ultra-high temperature (UHT) milk product (Snow brand-Megmilk, Tokyo) was obtained from a retailer. Skimmed milk powder (Morinaga Milk, Tokyo) and Parmesan cheese (Union cheese, Atsugi, Japan) were also obtained from a retailer.

2.4. Sample preparation

Raw milk and UHT milk were spiked with SEA at a given concentration and then mixed thoroughly. Skimmed milk powder and Parmesan cheese were spiked with SEA at a given concentration and then mixed thoroughly with warm water at approximately 40 °C at a concentration of 12 g/100 ml for skimmed milk and 20 g/60 ml of Parmesan cheese (VIDAS® technical pamphlet). The pH values of these samples were then adjusted to a specified value with 1 M hydrochloric acid. The volume of the hydrochloric acid needed was taken into consideration for the calculation of recovery described below. Samples were clarified with centrifugation at 15,000 *g* for 20 min. The supernatants of samples were passed through a paper filter or a syringe filter unit with 1.2 μm pore size (Sartorius, Goettingen, Germany).

2.5. Ion exchanger

Four milliliters (wet volume) of a strong cation exchanger, SP Sephadex™ C-25 (GE Healthcare Bio-Sciences AB, Bjorkgatan, Sweden) was placed into a plastic extraction column (15 mm in inner diameter, 80 mm height) (PD-10, GE Healthcare UK, Amersham Place, UK). The outlet of the column was equipped with a two-way stop cock, which can change the flow rate. The flow rates of binding and elution were all set to about 1 ml/min.

For a large volume sample, the cation exchanger (4 ml) was placed into a large column (27 mm in inner diameter, 130 mm height) (GL Science, Tokyo). Some of the eluted samples were further concentrated using a centrifugal filter unit (Amicon Ultra-15, Merck Millipore, Tullagreen, Ireland).

2.6. Buffers

Binding buffers adjusted to various pH values from 3.0 to 6.0 were prepared with 0.05 M sodium phosphate (Wako Pure Chemicals, Tokyo) and 0.02 M citric acid (Wako). Elution buffer was made with 0.1 M sodium phosphate, 0.025 M citric acid, and 0.5 M sodium chloride (Wako) at pH 8.0.

2.7. Determination of pH for extraction

Culture supernatant of strain 13,008 described above was