

Improvement of a concentration protocol based on trichloroacetic acid for extracting staphylococcal enterotoxins in dairy products

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SUMMARY

Different immunological methods have been proposed for detecting staphylococcal enterotoxins. However, the detection methods are not sensitive enough, so purification and concentrations techniques are needed to increase their sensitivity and specificity. The dialysis concentration method against 30% polyethylene glycol (PM 20 000) has to be used prior to detection of enterotoxin according to the official method of concentration recognised by the French Food Safety Agency (AFSSA). However, as this is time consuming and tedious, a quicker and simpler enterotoxin extraction procedure is needed.

The purpose of this study was to evaluate a new extraction protocol based on trichloroacetic acid (TCA) precipitation in dairy products.

Different quantities of purified staphylococcal enterotoxins (A, B, C₂, D and E) were added to different dairy products. Detection of enterotoxins in the food samples was performed using two commercial immunoassays: an automated detection system, VIDASTM SET2 (bioMérieux) and an ELISA method, TRANSIA PLATE *Staphylococcal Enterotoxins* (Diffchamb).

TCA extraction prior to the VIDASTM SET2 improved the detection of staphylococcal enterotoxins from dairy products compared to the dialysis concentration followed by TRANSIA PLATE *Staphylococcal Enterotoxins*.

KEY WORDS : *Staphylococcus aureus*, enterotoxin, milk, cheese, detection, extraction.

RÉSUMÉ

Amélioration d'un protocole de concentration par l'acide trichloracétique des entérotoxines staphylococciques dans les produits laitiers. Par C. VERNOZY-ROZAND, C. MAZUY-CRUCHAUDET, C. BAVAI et Y. RICHARD.

Différentes méthodes immunologiques ont été proposées pour détecter les entérotoxines staphylococciques. Cependant, les méthodes de détection ne sont pas suffisamment sensibles ; c'est la raison pour laquelle des techniques de purification et de concentrations des entérotoxines sont nécessaires. Selon la méthode officielle recommandée par l'Agence Française de Sécurité Sanitaire des aliments (AFSSA), la méthode de dialyse/concentration avec du polyéthylène glycol à 30% (P.M. 20 000) doit être utilisée avant la détection des entérotoxines. Cependant, cette technique est longue et fastidieuse ; un procédé plus rapide et plus simple semble nécessaire. Le but de cette étude a donc été d'évaluer un nouveau protocole d'extraction basé sur la précipitation avec l'acide trichloracétique (TCA) pour les produits laitiers. Différentes quantités d'entérotoxines staphylococciques purifiées (A, B, C₂, D et E) ont été ajoutées à différents produits laitiers. La détection des entérotoxines dans les échantillons alimentaires a été effectuée en utilisant deux tests immunologiques commercialisés : un système automatisé de détection, VIDASTM SET2 (bioMérieux) et une méthode ELISA, le test TRANSIA PLATE *Staphylococcal Enterotoxins* (Diffchamb). La concentration par l'acide trichloracétique suivie d'une détection avec le test VIDASTMSET2 a amélioré la détection des entérotoxines staphylococciques des produits laitiers par comparaison à l'utilisation de la dialyse/concentration suivie du test TRANSIA PLATE *Staphylococcal Enterotoxins*.

MOTS CLÉS : *Staphylococcus aureus*, entérotoxines, lait, fromage, détection, extraction.

Introduction

Methods for fast and reliable detection and identification of food borne pathogens and toxins are highly desired by food industrials to provide early information about the sample being tested.

Staphylococcus aureus is a major cause of food-borne disease in many European countries. This food poisoning is caused by consuming foods containing enterotoxins produced by strains of *S. aureus*. Staphylococci can multiply readily in many foods but in France, dairy products are probably the most frequently implicated [16]. Detection of staphylococcal enterotoxins in implicated foods is essential for confirming staphylococcal food-poisoning. However, this detection in food requires much more sensitive methods than determining enterotoxigenicity by culturing strains under

optimal conditions [7]. The quantity of enterotoxin present in the foods involved in food-poisoning outbreaks may vary considerably from less than 1 ng.g⁻¹ to more than 50 ng.g⁻¹ [3]. Different immunological methods have been proposed for detecting staphylococcal enterotoxins, including immunodiffusion assays [4], radioimmunoassays [19,13] and enzyme linked immunosorbent assays [6, 11, 20, 9, 26].

However, these detection methods are not sensitive enough, so purification and concentrations techniques are needed to increase their sensitivity. Several authors have reported various combinations of ion-exchange chromatography and gel filtration [2, 23, 5, 12, 21]. These procedures are time-consuming and may result in a low recovery of enterotoxins. Different modifications of enterotoxin purification have included chromatofocusing [8, 10, 15], development of dye ligand chromatography [22] and the use of

monoclonal antibody immunoaffinity chromatography [17, 14, 24]. The dialysis-concentration method against 30 polyethylene glycol (PM 20000) has been used prior to detection of enterotoxin with the French reference method [1]. However, as this is time-consuming and tedious, a quicker and simpler enterotoxin extraction procedure is needed.

In a previous work, we have evaluated an alternative extraction procedure for enterotoxin determination in dairy products [18]. More precisely, a concentration protocol based on trichloroacetic acid precipitation was evaluated and compared with the reference method using dialysis concentration.

Different quantities of purified staphylococcal enterotoxins were added to pasteurized camembert-type cheeses. Both enterotoxin extraction methods allowed detection of the lowest enterotoxin concentration level used in this previous study (0.5 ng.g⁻¹).

The purpose of this work is to evaluate a modified protocol including a higher quantity of TCA for extracting the enterotoxin than those used in the previous study and to detect the toxin is the new commercialized test with the VIDAS™ SET2, and an other test the TRANSIA PLATE *Staphylococcal Enterotoxins*. Moreover, the new protocol of extraction and detection of staphylococcal enterotoxin was evaluated in different types of dairy product and not only in pasteurized camembert type cheeses as we did in a previous study [18].

Materials and methods

Five different dairy products were obtained from retail outlets (yoghurts, pasteurized milk and 3 raw milk cheeses : Roquefort, Camembert, Munster). The samples were transported to the laboratory and maintained at 4°C.

Purified *Staphylococcus aureus* enterotoxin (1 ml) was added to the cheeses to give final enterotoxins concentration of:

0.5, 0.25 and 0.1 ng.g⁻¹ for toxin A and B

1.0, 0.5 and 0.25 ng.g⁻¹ for toxin C₂, D and E

The concentration of enterotoxins A, B, C₂, D and E (toxin technology Inc, Sarasota FL, USA) were tested in triplicate.

PROCEDURES FOR EXTRACTION OF STAPHYLOCOCCAL ENTEROTOXINS

Two extraction procedures were tested in parallel.

Method 1 (dialysis concentration).

A 20 g sample of cheese was combined with 40 ml of distilled water and homogenized. The pH was adjusted to 4 using 5 mol.l⁻¹ HCl and the solution was centrifuged at 2000 g for 15 min at 4°C. The supernatant fluid was removed, adjusted to pH6-8, centrifuged and filtered. The extracted samples were concentrated by dialysis against 30 Polyethylene Glycol (Molwt 20000, Merck) with a cellulose dialysis bag retaining molecular weights > 6000-8000 Da (24006; Visking R dialysis bag, Poly Labo, Strasbourg, France) at 4°C ± 2°C over night. After washing in cold water, the bag was placed in phosphate-buffered saline (3.8 g

Na₃P0₄.12H₂O, 8-4 g NaCl l⁻¹, pH7-2) until the concentrated extract was reconstituted to about 1 ml. The samples were tested with the VIDAS™ SET2 test and TRANSIA PLATE *Staphylococcal Enterotoxins*.

Method 2 (TCA precipitation).

An extraction procedure based on TCA precipitation was used to extract enterotoxin from 20 g portions of dairy product. Deionised water (40 ml) was added to 20 g cheese and homogenized time of contact was 30 min. The pH was adjusted with 5 mol l⁻¹ hydrochloric acid to 3.5-4. The suspension was then centrifuged at 3500 g and held at 4°C for 15 min. The supernatant fluid was decanted, measured and treated with 0.05 ml of 5.5 mol.l⁻¹ trichloroacetic acid (TCA, T4885, Sigma Aldrich Chimie) per ml of supernatant fluid. The time of contact was 30 min. The suspension was then centrifuged at 3500 g for 30 min. The pellet was dissolved in Tris buffer 0.3 mol.l⁻¹ at pH 8.0 (37g.l⁻¹ Tris hydroxymethyl amino methane) and adjusted to pH 8 with 5 mol.l⁻¹ sodium hydroxide.

PROCEDURES FOR DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS

VIDAS™ SET2 :

The VIDAS™ Staph Enterotoxin Test 2 (SET2, 30701; bio-Mérieux, Marcy-l'Etoile, France) allows simultaneous detection of the seven enterotoxin serotypes (SEA, SEB, SEC₁, SEC₂, SEC₃, SED and SEE). The method uses an enzyme-linked fluorescent assay (ELFA) with monoclonal anti-enterotoxin antibodies. Filtrate (500µl) was removed and placed in the sample well of the VIDAS™ SET2 reagent Strip. Detection was carried out using the VIDAS automated System and the results given in Relative Fluorescence Value (RFV). This test was qualitative and semi-qualitative. Any result below 450 was considered to be negative. According to the manufacturer, the VIDAS method has a sensitivity of at least 0.5 ng enterotoxin g⁻¹ food. Negative and positive controls were tested.

TRANSIA PLATE *Staphylococcal Enterotoxins* :

TRANSIA PLATE *Staphylococcal Enterotoxins* (ST0796, Diffchamb-LYON-FRANCE) is intended to be used for detection of staphylococcal enterotoxins A, B, C, D and E in food samples and in cultural supernatants. The method is based on a sandwich-type ELISA (Enzyme Linked Immuno Sorbent Assay). The solid support of the reaction is a microtitre plate with divisible strips coated with antibodies specific for staphylococcal enterotoxins. The optical density is the average of negative controls plus 0.20 : $T = (NC1 + NC2) / 2 + 0.20$. The sample is considered positive if its optical density is higher or equal to the threshold. The sample is considered negative if its optical density is lower than T-0.05. Between T-0.05 and T, the sample is considered as doubtful.

Results and discussion

Detection of staphylococcal enterotoxins in food is the only way to be certain that a product has been implicated in

staphylococcal food-poisoning. In order to develop an alternative method to dialysis-concentration, a new enterotoxin extraction procedure based on TCA precipitation was evaluated to compare the sensitivity of the both enterotoxin extraction methods (dialysis concentration and TCA precipitation). Different amounts of purified enterotoxin (SEA, SEB, SEC2, SED and SEE) were added to different dairy products. The results obtained are shown in Table I. For the purpose of underlining the discrepancies between the data obtained, negative and doubtful results are written in bold characters in Tables I and II.

For simplicity and because all yoghurt samples added with enterotoxin gave positive results after TCA and dialysis concentration with VIDAS™ SET2 and TRANSIA PLATE *Staphylococcal Enterotoxins*, the results obtained were not displayed in tables I and II.

Twenty eight of the 300 extractions performed were confirmed after TCA extraction and not confirmed after dialysis concentration. To the hygienist's point of view, these false-negative results are not acceptable. Clear evidence for explaining the false-negative results are not easy to find. The latter might be due at least in part to interferences during the extraction's step. Surprisingly, the pasteurized milk, that is a simple matrices, was the dairy product for which negative results after TCA and positive results after dialysis-concentration were more frequently observed (10 false negative results). Five, 5 and 8 false positive results were respectively noted with Roquefort, Camembert and Munster. When enterotoxins were detected, RFV values given by the VIDAS SET2 after TCA precipitation and those obtained after dialysis concentration were not strikingly different.

		ROQUEFORT				CAMEMBERT			
		Dialysis/ concentration		TCA		Dialysis/ concentration		TCA	
		Transia	Set2	Transia	Set2	Transia	Set2	Transia	Set2
Negative control	0 ng.g ⁻¹	0.19 (0.08)	38 (9)	0.121 (0.1)	37 (5)	0.109 (0.12)	34 (5)	0.07 (0.02)	15 (3)
	0.5 ng.g ⁻¹	2.591 (0.32)	7866 (1085)	1.837 (1)	5469 (800)	1.062 (0.6)	5337 (952)	2.28 (1)	6151 (1236)
SEA	0.25 ng.g ⁻¹	1.264 (0.98)	5111 (985)	0.896 (0.6)	2656 (952)	1.233 (0.8)	5390 (981)	0.78 (0.2)	3056 (695)
	0.1 ng.g ⁻¹	0.639 (1)	2778 (395)	0.468 (0.06)	1236 (209)	0.329 (0.2)	103 (10)	0.348 (0.1)	1485 (265)
SEB	0.5 ng.g ⁻¹	1.356 (1.3)	8299 (1022)	2.256 (1.02)	8089 (1230)	0.317 (0.1)	2425 (406)	1.81 (1)	7133 (1364)
	0.25 ng.g ⁻¹	0.699 (0.69)	6154 (1122)	1.249 (0.5)	5441 (658)	0.174 (0.05)	1519 (318)	1.08 (0.56)	4747 (965)
	0.1 ng.g ⁻¹	0.399 (0.8)	1422 (241)	0.665 (0.06)	2957 (409)	0.134 (0.2)	429 (174)	0.386 (0.09)	1917 (369)
SEC2	1 ng.g ⁻¹	0.262 (0.5)	2658 (652)	1.32 (0.4)	6811 (1036)	1.57 (0.5)	3578 (678)	1.41 (0.2)	7215 (967)
	0.5 ng.g ⁻¹	0.223 (0.7)	228 (174)	1.324 (0.9)	2558 (953)	0.243 (0.09)	599 (174)	2.9 (1)	4927 (706)
	0.25 ng.g ⁻¹	0.192 (0.09)	85 (10)	0.619 (0.3)	650 (145)	0.136 (0.02)	817 (145)	1.269 (0.5)	1890 (319)
SED	1 ng.g ⁻¹	1.024 (0.1)	5280 (800)	0.719 (0.9)	1195 (224)	0.84 (0.09)	4373 (706)	0.83 (0.05)	2566 (498)
	0.5 ng.g ⁻¹	0.721 (0.3)	3387 (350)	0.439 (0.06)	1125 (264)	0.32 (0.1)	582 (174)	0.34 (0.03)	1120 (208)
	0.25 ng.g ⁻¹	0.525 (0.05)	1283 (223)	0.365 (0.8)	620 (145)	0.232 (0.05)	257 (56)	0.235 (0.09)	245 (69)
SEE	1 ng.g ⁻¹	Do>	7280 (962)	2.674 (1.3)	6193 (1039)	0.83 (0.3)	2652 (406)	2.218 (1.05)	6014 (1198)
	0.5 ng.g ⁻¹	1.608 (1)	4542 (893)	0.91 (0.06)	3644 (489)	1.684 (1.02)	5203 (956)	1.06 (0.1)	2263 (479)
	0.25 ng.g ⁻¹	0.954 (0.9)	2695 (1020)	1.619 (1.3)	2046 (302)	0.94 (0.8)	3018 (479)	0.798 (0.03)	1917 (208)

TABLE I. — Amount of purified staphylococcal enterotoxins detected in «Roquefort» and «Camembert» with different extraction and detection procedures.

Values reported are means of three replicates, Values in parentheses are standard deviations

* VIDAS™ SET2 : RFV Relative Fluorescence Value

* Transia : optical density. Positive results : T = 0.316, Negative results : optical density is lower than 0.266, Doubtful results : Between 0.266 and 0.3

* Negative results and Doubtful results are written in bold characters

TCA : Trichloroacetic acid

Transia : TRANSIA PLATE staphylococcal enterotoxins

Set2 : VIDAS™ SET2

SEA : Staphylococcal enterotoxin serotype A

		MUNSTER				PASTEURIZED MILK			
		Dialysis / Concentration		TCA		Dialysis / Concentration		TCA	
		Transia	Set2	Transia	Set2	Transia	Set2	Transia	Set2
Negative control	0 ng.g ⁻¹	0.059 (0.02)	35 (6)	0.07 (0.03)	18 (2)	0.23 (0.01)	13 (2)	0.122 (0.01)	35 (9)
	0.5 ng.g ⁻¹	1.651 (0.9)	7493 (1298)	1.111 (0.85)	4617 (697)	1.91 (1)	3323 (655)	0.633 (0.03)	7667 (1152)
SEA	0.25 ng.g ⁻¹	0.77 (0.06)	3793 (678)	0.449 (0.05)	1800 (697)	0.148 (0.01)	1163 (209)	2.557 (1.6)	5247 (698)
	0.1 ng.g ⁻¹	0.28 (0.05)	1615 (289)	0.382 (0.03)	1375 (206)	0.105 (0.06)	14 (6)	1.417 (0.98)	2237 (459)
SEB	0.5 ng.g ⁻¹	0.471 (0.06)	5580 (986)	0.382 (0.04)	6710 (1239)	0.324 (0.1)	2966 (406)	0.848 (0.32)	6208 (1039)
	0.25 ng.g ⁻¹	0.236 (0.03)	3034 (649)	0.702 (0.2)	4564 (703)	0.290 (0.03)	1556 (241)	0.566 (0.08)	3103 (678)
	0.1 ng.g ⁻¹	0.142 (0.07)	1439 (241)	0.347 (0.06)	1807 (206)	0.238 (0.1)	276 (159)	0.218 (0.03)	1176 (208)
SEC2	1 ng.g ⁻¹	0.121 (0.03)	206 (12)	1.951 (1.02)	5630 (952)	0.106 (0.02)	230 (98)	0.887 (0.05)	2985 (406)
	0.5 ng.g ⁻¹	0.126 (0.08)	196 (9)	1.101 (0.6)	1762 (269)	0.216 (0.07)	43 (8)	0.477 (0.11)	1362 (209)
	0.25 ng.g ⁻¹	0.097 (0.02)	105 (10)	0.399 (0.09)	720 (149)	0.104 (0.05)	41 (9)	0.278 (0.06)	650 (69)
SED	1 ng.g ⁻¹	1.389 (1)	4676 (713)	0.236 (0.05)	887 (269)	2.588 (1.2)	7163 (1395)	0.572 (0.09)	1708 (319)
	0.5 ng.g ⁻¹	0.696 (0.3)	2391 (406)	0.134 (0.06)	609 (178)	1.109 (0.89)	4583 (623)	0.779 (0.06)	2992 (318)
	0.25 ng.g ⁻¹	0.215 (0.08)	258 (75)	0.141 (0.05)	327 (147)	0.498 (0.20)	1667 (289)	0.423 (0.01)	1376 (208)
SEE	1 ng.g ⁻¹	2.578 (1.3)	7077 (1023)	2.831 (1.2)	6138 (1039)	2.739 (1.02)	7367 (1198)	Do>	8471 (1239)
	0.5 ng.g ⁻¹	1.547 (1)	4701 (796)	1.476 (1.03)	3650 (703)	0.978 (0.3)	3168 (617)	2.096 (1.06)	5639 (952)
	0.25 ng.g ⁻¹	0.837 (0.5)	2810 (409)	0.858 (0.09)	1996 (229)	0.631 (0.08)	2316 (406)	0.919 (0.65)	2890 (442)

TABLE II.—Amount of purified staphylococcal enterotoxins detected in «Munster» and pasteurized milk with different extraction and detection procedures.

Values reported are means of three replicates, Values in parentheses are standard deviations

* VIDAS™ SET2 : RFV Relative Fluorescence Value

* Transia : optical density. Positive results : T = 0.316, Negative results : optical density is lower than 0.266, Doubtful results : Between 0.266 and 0.3

* Negative results and Doubtful results are written in bold characters

TCA : Trichloroacetic acid

Transia : TRANSIA PLATE staphylococcal enterotoxins

Set2 : VIDAS™ SET2

SEA : Staphylococcal enterotoxin serotype A

TCA was quicker, taking only 1 h compared with the 18-24 h required for the dialysis concentration procedure. With respect to purification procedures, REYNOLDS et al. (1988) found ion-exchange chromatography, gel filtration and chromatofocusing to be expensive and not easily adaptable for large-scale enterotoxin purification. Although immunoaffinity chromatography resulted in a significant improvement in time taken, ease of use, yield and purity of enterotoxin from culture supernatant fluids [24], enterotoxin extraction was difficult, especially from dairy products. The extraction of enterotoxins by dialysis concentration in these complex matrices with high protein content was difficult, often incomplete and labour-intensive. Adding 0.05 ml of trichloroacetic acid at 5.5 mol.l⁻¹ instead of 0.05 ml of trichloroacetic acid at 3 mol.l⁻¹ significantly improved the extraction recovery (data not shown). That is the reason why this modified protocol was used in this study.

The VIDAS™ SET2 detection test needed only 80 min to perform. This automated detection System is simple to use, 'user-friendly' and quick [25]. Except for SED, performed after TCA extraction, the test consistently detected the lowest inoculum of enterotoxins used in this study : e.g. 0.1 ng.g⁻¹ (SEA, SEB); 0.25 ng.g⁻¹ (SEC₂, SEE).

These results suggest that TCA precipitation appears to be more sensitive and reproducible than dialysis concentration. Furthermore, as TCA precipitation is a user-friendly, inexpensive, simple, reliable extraction method, it could replace dialysis concentration for assessing the safety of dairy products. The technique can be easily adapted for large-scale enterotoxin extraction.

TCA extraction used prior to the VIDAS™ SET2 improved the detection of staphylococcal enterotoxins from dairy products.

References

1. — ANON : Note de service DGAL/SDHA/N.97/N°8097. Techniques d'identification et de détection des entérotoxines staphylococques dans les produits laitiers. 1997.
2. — BERGDOLL M.S. : Staphylococcal intoxications. In *Fond-Borne Infections and Intoxications* 2nd edn, ed. Riemann, H. and Bryan, F.L. pp. 443-494. London: Academic Press, 1983.
3. — BERGDOLL M.S. : *Staphylococcus aureus*. *J. Assoc. Off Anal. Chem.*, 1991, **74**, 706-710.
4. — CASMAN E.P., BENNETT R.W., DORSEY A.E., STONE J.E. : The microslide gel double diffusion tests for the detection and assay of staphylococcal enterotoxins. *Health Lab. Sci.*, 1979, **6**, 185-189.
5. — CHANG H.C., BERGDOLL M.S. : Purification and some physicochemical properties of staphylococcal enterotoxin D. *Biochemistry* 1979, **18**, 1937-1942.
6. — CHANG P.C., YANO Y., DIGHTON M., DICKIE N. : Fractionation of staphylococcal enterotoxin C₂ by isoelectric focusing. *Can. J. Microbiol.*, 1971, **17**, 1367-1372.
7. — DONNELLY C.B., LESLIE J.E., BLACK L.A., LEWIS K.H. : Serological identification of enterotoxinogenic staphylococci from cheese. *Appl. Microbiol.*, 1967, **15**, 1382-1387.
8. — ENDE LA., TERPLAN G., KICKHOFEN B., HAMMER D.K. : Chromatofocusing: a new method for purification of staphylococcal enterotoxins B and C₁. *Appl. Environ. Microbiol.*, 1983, **46**, 1323-1330.
9. — FEY H., PFISTER H., MULLER C. : Simple purification of staphylococcal enterotoxins with chromatofocusing and isoelectric focusing in flat bed gels. *Zentralblatt Veterinarmedizin Reihe*, 1984a, **531**, 508-517.
10. — FEY H., PFISTER H., RUEEGG O. : Comparative evaluation of different enzyme-linked immunosorbent assay Systems for the detection of staphylococcal enterotoxins A, B, C and D. *J. Clin. Microbiol.*, 1984b, **19**, 34-38.
11. — FREED R.C., EVENSON M.L., REISER R.F., BERGDOLL M.S. : Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. *Appl. Environ. Microbiol.*, 1982, **44**, 1349-1355.
12. — IGARASHI H. : Staphylococcal enterotoxins. In : *Protein Toxins* (Ed.) Takeda, Y. and Kato, I. Tokyo: Ishiyaku Publisher Co. 1983, 91-116.
13. — JANIN F., DE BUYSER M.L., LAPEYRE C., FEINBERG M. : Radioimmunological quantitative determination of *Staphylococcus aureus* enterotoxin A in various foods. *Sci. Aliments*, 1985, 3397-412.
14. — LAPEYRE C., KAVERI S.V., JANIN F., STROSBERG A.D. : Production and characterization of monoclonal antibodies to staphylococcal enterotoxins: use in immunodetection and immunopurification. *Mol. Immunol.*, 1987, **24**, 1243-1254.
15. — LEI Z., REISER R.F., BERGDOLL M.S. : Chromatofocusing in the purification of staphylococcal enterotoxin D. *J. Clin. Microbiol.*, 1988, **26**, 1236-1237.
16. — LEPOUTRE A., SALOMON J., CHARLEY C., LE QUERREC F. : Les toxiinfections alimentaires en 1993. Bulletin Epidémiologique Hebdomadaire, 1994, **52**, 245-247.
17. — MEYER R.F., MILLER L., BENNETT R.W., MACMILLAN J.D. : Development of a monoclonal antibody capable of interacting with five serotypes of *Staphylococcus aureus* enterotoxin. *Appl. Environ. Microbiol.*, 1984, **47**, 283-287.
18. — MEYRAND A., ATRACHE V., BAVAI C., MONTET M.P., VERNOZY-ROZAND C. : Evaluation of an alternative extraction procedure for enterotoxin determination in dairy products. *Lett. Appl. Microbiol* 1999, **28**, 411-415.
19. — MILLER B.A., REISER R.F., BERGDOLL M.S. : Detection of staphylococcal enterotoxins A, B, C, D and E in foods by radioimmunoassay, using staphylococcal cells containing protein A as immunosorbent. *Appl. Environ. Microbiol.*, 1978, **36**, 421-426.
20. — NEYERMANS S., BOOT R., TIPS P.D., DE NOOY M.P. : Extraction of staphylococcal enterotoxins (SE) from minced meat and subsequent detection of SE with enzyme-linked immunosorbent assay (ELISA). *J. Food Prot.*, 1983, **46**, 238-241.
21. — REISER R.F., ROBBINS R.N., NOIETO A.L., KHOE G.P., BERGDOLL M.S. : Identification, purification, and some physicochemical properties of staphylococcal enterotoxin Q. *Infection and Immunology* 1984, **45**, 625-630.
22. — REYNOLDS D., TRANTER H.S., SAGE R., HAMBLETON P. : Novel method for purification of staphylococcal enterotoxin A. *Appl. Environ. Microbiol.*, 1988, **54**, 1761-1765.
23. — SCHANTZ E.J., ROESSLER W.G., WOODBURN M.J., LYNCH J.M., JACOBY H.M., SILVERMAN S.J., GORMAN J.C., SPERO L. : Purification and some chemical and physical properties of staphylococcal enterotoxin A. *Biochemistry*, 1972, **11**, 360-366.
24. — SHINAGAWA K., MITSUMORI M., MATSUSAKA N., SUGII S. : Purification of staphylococcal enterotoxins A and E by immunoaffinity chromatography using a murine monoclonal antibody with dual specificity for both of these toxins. *J. Immunol. Methods*, 1991, **139**, 49-53.
25. — SU Y.I., WONG C.L. : Current perspectives on detection of staphylococcal enterotoxins. *J. Food Prot*, 1997, **60**, 195-202.
26. — WIENEKE A.A., GILBERT R.G. : The use of a sandwich ELISA for the detection of staphylococcal enterotoxin A in foods from outbreaks of food poisoning. *J Hyg (Lond)*, 1985, **95**, 131-138.