Safety Evaluation of Sous Vide-Processed Products with Respect to Nonproteolytic Clostridium botulinum by Use of Challenge Studies and Predictive Microbiological Models

Eija Hyytti-Trees,1* Elja Skyttä,2 Mirja Mokkila,2 Arvo Kinnunen,2 Mika Lindström,1 Liisa Lähteenmäki,2 Raija Ahvenainen,2 and Hannu Korkeala1

Faculty of Veterinary Medicine, Department of Food and Environmental Hygiene, University of Helsinki, Helsinki,1 and VTT Biotechnology and Food Research, Espoo,2 Finland

Received 21 June 1999/Accepted 2 November 1999

Sixteen different types of sous vide-processed products were evaluated for safety with respect to nonproteolytic group II Clostridium botulinum by using challenge tests with low (2.0-log-CFU/kg) and high (5.3-log-CFU/kg) inocula and two currently available predictive microbiological models, Food MicroModel (FMM) and Pathogen Modeling Program (PMP). After thermal processing, the products were stored at 4 and 8°C and examined for the presence of botulinum spores and neurotoxin on the sell-by date and 7 days after the sell-by date. Most of the thermal processes were found to be inadequate for eliminating spores, even in low-inoculum samples. Only 2 of the 16 products were found to be negative for botulinum spores and neurotoxin at both sampling times. Two products at the high inoculum level showed toxigenesis during storage at 8°C, one of them at the sell-by date. The predictions generated by both the FMM thermal death model and the FMM and PMP growth models were found to be inconsistent with the observed results in a majority of the challenges. The inaccurate predictions were caused by the limited number and range of the controlling factors in the models. Based on this study, it was concluded that the safety of sous vide products needs to be carefully evaluated product by product. Time-temperature combinations used in thermal treatments should be reevaluated to increase the efficiency of processing, and the use of additional antibotulinic hurdles, such as biopreservatives, should be assessed.

The term “sous vide” means “under vacuum” and describes a processing technique whereby freshly prepared foods are vacuum sealed in individual packages and then pasteurized at time-temperature combinations sufficient to destroy vegetative pathogens but mild enough to maximize the sensory characteristics of the product (39, 40). After cooking, the products are chilled, stored refrigerated, and reheated before consumption. Sous vide foods are mainly used in mass catering and restaurants (30). Compared with traditional cooking methods, sous vide has many advantages (40, 42). Economic benefits include better use of labor and equipment through centralized production and extended shelf life due to vacuum packaging, which by excluding oxygen inhibits oxidative processes and growth of spoilage organisms. The shelf life of a sous vide product can be as long as 42 days (42). In addition, the reduced need for preservatives and flavor enhancers, better preservation of vitamins, and retention of most of the original food juices all contribute to higher quality of sous vide foods over conventional meals.

Concerns associated with sous vide processing involve the microbiological safety of the products (40). The psychrotrophic food-borne pathogens and particularly nonproteolytic group II Clostridium botulinum bacteria are of concern due to the methods of preparing, distributing, and storing these products. Mild heat treatments in combination with vacuum packaging may actually select for C. botulinum and increase the potential for botulism. Sous vide products are generally formulated with little or no preservatives and frequently do not possess any intrinsic inhibitory barriers (pH, aw, or NaCl) that either alone or in combination would inhibit growth. Therefore, strict adherence to refrigerated storage below 3.3°C must be maintained to ensure the safety of sous vide products with respect to nonproteolytic C. botulinum (1). However, the temperature control in chill chains is often inadequate, and temperature abuse is common throughout distribution and retail markets and by consumers (8, 16, 27).

Recent research has identified combinations of mild heat treatment and subsequent refrigerated storage that, when combined with a specified shelf life, provide a defined safety margin with respect to nonproteolytic C. botulinum (10, 15, 46). Based on these research results, the Advisory Committee on the Microbiological Safety of Food (1) recommended certain procedures to ensure the safety of refrigerated processed foods of extended durability. According to these recommendations, heat treatments or combination processes should reduce the number of nonproteolytic C. botulinum bacteria by a factor of 106 (a 6-decimal [6-D] process). However, the capability of a combination process to consistently prevent growth and toxin production by C. botulinum in a particular product must be reliably demonstrated.

There are two main approaches that can be used to evaluate the stability and the safety of a product with respect to foodborne pathogens. Traditionally, the effect of thermal processing on pathogenic microorganisms, as well as the risk of their growth and possible toxin production in foods, has been determined through the use of inoculated pack studies. Now, however, there are too many products, alternate ingredients, and process variations to conduct a complete laboratory evaluation of each possible contingency and potential food-borne pathogen for each product. Therefore, predictive food microbiology, the modeling of microbial populations, particularly those of food-borne pathogens, has become an active field of research.

* Corresponding author. Mailing address: 1409 Millstream Trail, Lawrenceville, GA 30044. Phone: (678) 380-9923. Fax: (404) 639-3333. E-mail: eih9@cdc.gov.
vacuum pouches (250 by 500 mm) were prepared from nylon-polyethene multi-products or by thoroughly mixing the spore mixture into liquid products. The vacuum pouches by spraying the spore mixture evenly on the surfaces of solid CFU/kg) levels of inoculum were used. The inoculation was performed in vacuum packages at 2°C.

The present study was performed to evaluate the safety of 16 different types of sous vide-processed products with respect to nonproteolytic C. botulinum. The efficiency of thermal processes to inactivate botulinal spores and the subsequent effect of mildly abusive storage temperatures on C. botulinum outgrowth and toxigenesis were studied by using inoculated pack studies and two currently available predictive microbiological programs.

MATERIALS AND METHODS

Products. Sixteen sous vide-processed products of various types were evaluated for safety with respect to nonproteolytic C. botulinum. The details of the products are described in Table 1. The ingredients of each product were obtained from local processors and were transported to the laboratory in refrigerated vacuum packages at 2°C.

Product inoculation and vacuum packaging. A mixture of five nonproteolytic C. botulinum strains was used in the inoculum: three type E strains (31-2570 E, 4062 E, and C-60 E), one type B strain (706 B), and one type F strain (FT 10 F). The strains were of North American and European origin and were isolated from seafood and meat products between the 1960s and the 1980s. The spore suspensions of individual strains were prepared according to the method recommended by the Food and Agricultural Organization (12), and the concentration of each strain was adjusted to 10^9 spores per ml.

Storage conditions and sampling procedures. After processing, samples of each product were stored at 4 and 8°C. The samples were analyzed for the presence of C. botulinum type B, E, and F cells and botulinum neurotoxin after the shelf life typically recommended for a corresponding commercially available product and 7 days after that. The analyses were performed with three parallel samples for each storage temperature and inoculum level. pH was analyzed for four parallel inoculated samples immediately after processing and at both sampling times after storage.

Microbiological quality (aerobic plate count [APC], number of sulfite-reducing clostridia, lactic acid bacteria, and yeasts and molds) of selected products (no. 1, 3, 4, 5, 6, 7, 8, 10, and 11) was determined by using uninoculated samples in parallel with sensory evaluation. All analyses were performed on single samples in duplicate. The samples stored at 8°C were examined immediately after processing, in the middle of the recommended shelf life, after the recommended shelf life, and after the safe-storage time predicted by the Pathogen Modeling Program (PMP).

Detecion of C. botulinum. Twenty grams of each sample was examined for the presence of C. botulinum type B, E, and F cells by PCR analysis as described by Helim et al. (21), with some modifications. The quantification was based on a 1-dilution-level most-probable-number (MPN) series (11). Briefly, 20 tubes containing 10 ml of tryptone-peptone-glucose-yeast extract (Difco, Detroit, Mich.) broth with 625 IU of lysozyme (Sigma Chemical Co., St. Louis, Mo.) per ml were each inoculated with 1 g of thoroughly homogenized sample. Enrichment cultures were incubated at 26°C in an anaerobic cabinet with an internal atmosphere of 85% N2-10% CO2-5% H2 (MK III; Don Whitley Scientific Ltd., Shipley, United Kingdom) for 3 days. Washed and boiled cells from overnight (18-h) cultures were used as a template for PCR. DynaZyme DNA polymerase (Finnzymes, Espoo, Finland) and a 96-well PTC-100 thermal cycler (MJ Research, Watertown, Mass.) were used. The sizes of the amplified PCR products were determined by agarose gel electrophoresis with comparison to standard DNA fragments (DNA molecular weight marker VI; Boehringer Mannheim GmbH, Mannheim, Germany).

Toxin analysis. The procedure for the assay of botulinum toxin followed the Nordic Committee on Food Analysis protocol (35), with modifications described by Hyytiä et al. (24).

pH analysis. pHs of homogenates of minced sample and distilled water in a ratio of 1:1 (wt/vol) were determined with a digital Microprocessor pH 537.

TABLE 1. Details of the 16 sous vide-processed products included in the study

| Product no. | Ingredient(s) | Size (g) | \( P \) value (min) | NaCl (% wt/vol) | pH |
|-------------|---------------|---------|---------------------|-----------------|-----|
| 1           | Pork cubes    | 1,500   | 22.9                | 0.7             | 6.0–6.3 |
| 2           | Pork cubes    | 1,500   | 153.5               | 0.7             | 5.8–6.1 |
| 3           | Beef cubes    | 1,500   | 490.7               | 0.2             | 5.8–6.1 |
| 4           | Beef cubes    | 1,500   | 186.7               | 0.7             | 5.6–6.1 |
| 5           | Pork fillet   | 1,300   | 10.6                | 2.0             | 5.8–6.0 |
| 6           | Beef roast    | 1,400   | 19.6                | 1.6             | 5.7–6.1 |
| 7           | Beef roast    | 1,500   | 5.5                 | 1.9             | 5.6–5.8 |
| 8           | Ground beef   | 1,500   | 0.0                 | 0.2             | 5.5–5.9 |
| 9           | Beef liverbeef| 1,500   | 450.8               | 0.3             | 6.1–6.3 |
| 10          | Broiler fillets, marinade | 1,000 | 83.9                | 1.4             | 5.9–6.1 |
| 11          | Rice, vegetables, pork, seafood | 1,500 | 102.0               | 1.9             | 5.8–6.0 |
| 12          | Rice, water, milk | 1,500 | ND<sup>b</sup> (high) | 1.1             | 6.1–6.7 |
| 13          | Beef, pork, water, vegetables | 1,500 | 2.5                 | 1.3             | 4.8–5.1 |
| 14          | Beef, vegetables, water | 1,500 | 349.2               | 1.3             | 4.7–5.3 |
| 15          | Water, potatoes, beef, vegetables | 1,500 | 370.1               | 1.0             | 5.3–5.8 |
| 16          | Pork, vegetables, water | 1,500 | 118.9               | 1.0             | 4.9–5.3 |

<sup>a</sup> Main ingredients in diminishing order.

<sup>b</sup> P value based on the measured temperatures in the product during the processing.

<sup>c</sup> The pH range measured from the samples during the study.

<sup>d</sup> ND, not determined. The measurement did not completely succeed due to the high liquid consistency of the product.

Research. Predictive models are equations which can use the information from a large database to predict inactivation or growth of microorganisms under defined conditions. However, current models cannot be used with confidence until their validation in various foods is tested by comparing the predictions to data obtained from inoculated pack studies (47).
measuring device (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

Determination of microbiological and sensory quality. The APC, lactic acid bacteria, yeasts and molds, and sulfite-reducing clostridia were determined by the methods of the Nordic Committee on Food Analysis (34, 36-38) by using plate count agar (Difco), MRS agar (Oxoid, Basingstoke, United Kingdom), YGC agar (Difco), and SFP agar base (Difco), respectively.

A trained laboratory panel of 10 judges evaluated the appearance and the aroma of the uninoculated samples heated to a service temperature typical of the product by using a five-point structured category scale (32). Each evaluation contained a marked reference sample that was obtained from a fresh production batch. In addition to a marked reference sample, in each session a fresh reference sample was hidden among the stored samples. A score of 2 on the category scale indicated that there was a "just-detectable" deterioration in sensory quality compared to that of the marked reference, a score of 3 indicated a "clearly detectable but not unacceptable" deterioration, and a score of 4 indicated that the judge had considered the sample unacceptable for human consumption. All samples were evaluated twice, and means of scores were calculated over replicates for each sample.

Predictive microbiological models. Food MicroModel (FMM), version 2.5 (Leatherhead Food Research Association, Leatherhead, Surrey, United Kingdom), was used to generate predictions for the lethal effect of each thermal process on nonproteolytic C. botulinum spores. FMM and PMP, version 5.0 (U. S. Department of Agriculture Eastern Regional Research Center, Wyndmoore, Pa.), were used to generate predictions for the safe-storage times after a thermal process on nonproteolytic C. botulinum types B, E, and F by the FMM has temperature (4 to 30°C), pH (5.1 to 7.5), and water-phase NaCl concentration (0 to 4.5%) as controlling factors. In both growth models, 5°C was used as the lower storage temperature to enable the comparison of models.

RESULTS

During storage at 4 and 8°C, a distinct difference between low- and high-inoculum samples was observed, in that substantially fewer samples with a low inoculum were positive for C. botulinum in PCR analysis and no toxigenesis was observed (Tables 2 and 3). Storage temperature did not appear to have a considerable effect on the number of PCR-positive samples or on the number of C. botulinum bacteria in positive samples at either inoculum level. However, toxigenesis was detected only in samples stored at 8°C (products 1 and 8). Eighty-seven percent of the PCR-positive samples contained serotype E. The prevalence of serotypes B and F in positive samples was 15% and 9%, respectively.

The microbiological quality of the products studied remained unchanged during the storage period at 8°C with APCs being mainly <1.0 log CFU/g. The highest counts (3.0 log CFU/g) were detected in products 5 and 11. The numbers of sulfite-reducing clostridia, lactic acid bacteria, and yeasts and molds were below detectable levels (1.0 log CFU/g) in all products studied. The sensory quality remained good with 4 of the 126 evaluated samples having a mean score of 2 or below, indicating that difference from the fresh reference sample was just detectable in most cases, and all scores being below 3, showing no unacceptable deterioration in examined samples.

Two distinct groups could be discerned among the 16 products subjected to challenge testing. A high-risk group consisted of four products (no. 1, 7, 8, and 13) that had high numbers of C. botulinum bacteria in PCR analysis with one or both inoculum levels at both sampling times and/or that showed toxigenesis (Tables 2 and 3). Two products (no. 14 and 16) were designated safe since they were negative for C. botulinum cells and botulinum neurotoxin at both sampling times in all different treatment groups. Differences in P value, NaCl concentration, and pH were observed between high-risk and safe products (Table 4). The remaining 10 products presented increased

### TABLE 2. Detection of nonproteolytic C. botulinum types B, E, and F by quantitative PCR analysis in sous vide-processed products with a low inoculum level (2.0 log CFU/kg) after storage at 4 and 8°C until sell-by date and 7 days after sell-by date

| Product no. | No. of PCR-positive samples/no. of samples analyzed<sup>a</sup> | Sell-by date | 1 wk after sell-by date |
|-------------|---------------------------------------------------------------|--------------|------------------------|
|             | 4°C | 8°C | 4°C | 8°C |
| 1 (14)      | 0/3 | 0/3 | 0/3 | 0/3 |
| 2 (30)      | 3/3 (1.19 ± 0.61; E, B) | 2/3 (0.83; E) | 0/3 | 0/3 |
| 3 (21)      | 0/3 | 0/3 | 0/3 | 0/3 |
| 4 (14)      | 0/3 | 0/3 | 0/3 | 0/3 |
| 5 (10)      | 3/3 (0.41; E, F) | 2/3 (0.60; E) | 0/3 | 0/3 |
| 6 (30)      | 0/3 | 0/3 | 0/3 | 0/3 |
| 7 (9)       | 1/3 (0.41; E) | 1/3 (0.41; E) | 0/3 | 0/3 |
| 8 (21)      | 0/3 | 0/3 | 0/3 | 0/3 |
| 9 (21)      | 2/3 (0.83; E) | 1/3 (0.41; E) | 0/3 | 0/3 |
| 10 (21)     | 0/3 | 0/3 | 0/3 | 0/3 |
| 11 (21)     | 1/3 (0.41; E) | 0/3 | 1/3 (0.90; E, B) | 1/3 (0.90; B) |
| 12 (21)     | 0/3 | 0/3 | 0/3 | 0/3 |
| 13 (21)     | 0/3 | 0/3 | 0/3 | 0/3 |
| 14 (30)     | 0/3 | 0/3 | 0/3 | 0/3 |
| 15 (21)     | 3/3 (0.74; E) | 1/3 (0.74; F) | 0/3 | 0/3 |
| 16 (21)     | 0/3 | 0/3 | 0/3 | 0/3 |

<sup>a</sup> Data in parentheses are mean MPN log CFU per kilogram in PCR-positive samples (standard deviation of the cell counts is reported if more than two parallel samples were positive) and the serotype(s) detected, in diminishing order.
surviving spores were increased. According to the PMP, all products were safe at 5°C with their recommended shelf life regardless of the storage temperature. Several occasions. The thermal death predictions agreed well with the measured P values but not with the observed PCR results. The safe-storage times predicted by the FMM were in good agreement with the maximum incubation times observed in inoculated pack studies. The results of the inoculation studies question the current recommendations for safe processing set out by the Advisory Committee on the Microbiological Safety of Food. Based on the calculated P values, the thermal processes of five products (no. 3, 9, 12, 14, and 16) appeared to be adequate to achieve the 6-D reduction in botulinal spores (criterion: the ratio of the maximum incubation time required for 6-D reduction being ≥1) which is recommended to ensure the safety of refrigerated processed foods of extended durability with respect to nonproteolytic C. botulinum (1, 3). However, only one of these products (no. 14) was determined to be safe in challenge tests. The results of the inoculated pack studies revealed that the majority of thermal processes were inadequate to eliminate the spores even with the low inoculum level. The presence of botulinal spores in nonsterile low-acid vacuum-packaged foods must be considered a serious risk due to the high probability of temperature abuse and mishandling of these types of products (8, 16, 27). The botulism risk of sous vide products is additionally increased by the absence of spoilage flora and by the long sensory shelf life which allows toxigenesis before sensory spoilage occurs. However, to our knowledge, sous vide products have not been implicated as a cause of a botulism outbreak so far. The comparison of high-risk products with safe products pointed out the factors contributing to the increased botulism risk. A low P value seemed to be the most significant single factor increasing the botulism risk in the products. A considerable overlap was observed in NaCl content and pH values between different risk groups, though high-risk products

**TABLE 3. Detection of nonproteolytic C. botulinum types B, E, and F by quantitative PCR analysis in sous vide-processed products with a high inoculum level (5.3 log CFU/kg) after storage at 4 and 8°C until sell-by date and 7 days after sell-by date**

| Product no. (recommended shelf life in days) | High risk (products 1, 7, 8, and 13) | Safe (products 14 and 16) | No. of PCR-positive samples/no. of samples analyzed |
|---------------------------------------------|------------------------------------|--------------------------|----------------------------------------------------|
| Slight increase | No increase | Both samples positive | Serotype(s) detected, in diminishing order |
| 4°C | 8°C | 1 wk after sell-by date |
| 4°C | 8°C |
| 1 (14) | 1/3 (0.74; F) | 3/3 (1.91 ± 2.01; E) | 1/3 (2.18; E) | 3/3 (1.93 ± 1.75; E) |
| 2 (30) | 3/3 (0.80 ± 0.16; E, B) | 3/3 (0.55 ± 0.23; E) | 0/3 | 0/3 |
| 3 (21) | 0/3 | 2/3 (1.01; E) | 1/3 (0.41; B) | 2/3 (0.60; E) |
| 4 (14) | 0/3 | 0/3 | 0/3 | 0/3 |
| 5 (10) | 2/3 (0.41; E, F) | 2/3 (0.41; E) | 0/3 | 0/3 |
| 6 (30) | 0/3 | 0/3 | 0/3 | 0/3 |
| 7 (9) | 2/3 (0.41; E) | 1/3 (0.41; E) | 0/3 | 0/3 |
| 8 (21) | 3/3 (1.36 ± 0.79; E) | 3/3 (0.97 ± 0.79; E) | 0/3 | 0/3 |
| 9 (21) | 1/3 (0.90; E, B) | 0/3 | 0/3 | 0/3 |
| 10 (21) | 0/3 | 0/3 | 0/3 | 0/3 |
| 11 (21) | 0/3 | 0/3 | 0/3 | 0/3 |
| 12 (21) | 0/3 | 0/3 | 0/3 | 0/3 |
| 13 (21) | 2/3 (1.54; E) | 1/3 (0.41; E) | 0/3 | 0/3 |
| 14 (30) | 0/3 | 0/3 | 0/3 | 0/3 |
| 15 (21) | 0/2 | 0/3 | 0/3 | 0/3 |
| 16 (21) | 0/3 | 0/3 | 0/3 | 0/3 |

- a Data in parentheses are mean MPN log CFU per kilogram in PCR-positive samples (standard deviation of the cell counts is reported if more than two parallel samples were positive) and the serotype(s) detected, in diminishing order.
- b One sample contained botulinum neurotoxin.

**TABLE 4. Physical and chemical features of high-risk and safe sous vide products**

| Physical or chemical feature | High risk (products 1, 7, 8, and 13) | Safe (products 14 and 16) |
|-----------------------------|------------------------------------|--------------------------|
| P value*                   | 7.7 (0.0–22.9)                     | 234.1 (118.9–349.2)      |
| NaCl (% [wt/vol])          | 1.0 (0.2–1.9)                      | 1.2 (1.0–1.3)            |
| pH#                        | 5.8 (5.1–6.3)                      | 5.3                      |

- a Samples strongly positive for C. botulinum and/or botulinum neurotoxin at both sampling times.
- b Samples negative for C. botulinum and botulinum neurotoxin at both sampling times.
- c Means of the lowest values measured for each product and the range.
- d Means of the highest values measured for each product and the range.

**DISCUSSION**

Due to the ubiquitous spread of C. botulinum in nature (17, 19, 20), contamination of raw ingredients of food products by botulinal spores is possible and even probable. However, the number of spores in different foods has been generally reported to be low (17, 18, 23, 26). The challenge tests of this study were designed to simulate as closely as possible the natural contamination level in foods (low-level inoculum) and to present a worst-case scenario to obtain an adequate margin of safety (high-level inoculum). The results gained from the inoculation studies question the current recommendations for safe processing set out by the Advisory Committee on the Microbiological Safety of Food. Based on the calculated P values, the thermal processes of five products (no. 3, 9, 12, 14, and 15) appeared to be adequate to achieve the 6-D reduction in botulinal spores (criterion: the ratio of P value to processing time required for 6-D reduction being ≥1) which is recommended to ensure the safety of refrigerated processed foods of extended durability with respect to nonproteolytic C. botulinum (1, 3). However, only one of these products (no. 14) was determined to be safe in challenge tests. The results of the inoculated pack studies revealed that the majority of thermal processes were inadequate to eliminate the spores even with the low inoculum level. The presence of botulinal spores in nonsterile low-acid vacuum-packaged foods must be considered a serious risk due to the high probability of temperature abuse and mishandling of these types of products (8, 16, 27). The botulism risk of sous vide products is additionally increased by the absence of spoilage flora and by the long sensory shelf life which allows toxigenesis before sensory spoilage occurs. However, to our knowledge, sous vide products have not been implicated as a cause of a botulism outbreak so far.
TABLE 5. Thermal inactivation of nonproteolytic *C. botulinum* spores based on the measured temperatures during the processing of 16 sous vide products as predicted by FMM and safe-storage times as predicted by FMM and PMP

| Product no. (recommended shelf life in days) | Predicted reduction in amt of spores (log units) | Lag time for growth by FMM (days)* | Time to turbidity by PMP (days) for initial no. of nonproteolytic *C. botulinum* bacteria before thermal processing (log CFU/kg) |
|--------------------------------------------|-----------------------------------------------|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
|                                             |                                               | 5°C  | 8°C  | 2.0  | 5.3  | 8°C  | 2.0  | 5.3  |
| 1 (14)                                     | NE                                            | 7    | 3    | 32   | <4.4*| 18   | <2.4*|
| 2 (30)                                     | 1.4                                           | 9    | 3    | >42* | 13   | 23   | 7    |
| 3 (21)                                     | >12.0                                         | 9    | 3    | >90  | >90  | >90  | >90  |
| 4 (14)                                     | 1.6                                           | 9    | 3    | >42* | 15   | 22   | 9    |
| 5 (10)                                     | NE                                            | 13   | 5    | >36* | <4.0*| 9    | <2.5*|
| 6 (30)                                     | NE                                            | 10   | 4    | >36* | <4.0*| 21   | <2.5*|
| 7 (9)                                      | NE                                            | 19   | 8    | >42* | >4.4*| 25   | >2.5*|
| 8 (21)                                     | NE                                            | 12   | 5    | 42   | <6.2*| 24   | <3.5*|
| 9 (21)                                     | >12.0                                         | 7    | 3    | >90  | >90  | >90  | >90  |
| 10 (21)                                    | 0.5                                           | 9    | 4    | >41* | 48   | 24   | 3    |
| 11 (21)                                    | 2.2                                           | 13   | 5    | >90  | 22   | >90  | 13   |
| 12 (21)                                    | >12.0                                         | 7    | 3    | >90  | >90  | >90  | >90  |
| 13 (21)                                    | NE                                            | >90  | >69  | >90  | >90  | >90  | >90  |
| 14 (30)                                    | 6.1                                           | >90  | >90  | >90  | >90  | >90  | >90  |
| 15 (21)                                    | >12.0                                         | 21   | 15   | >90  | >90  | >90  | >90  |
| 16 (21)                                    | 3.8                                           | >90  | >65  | >90  | >90  | >90  | >90  |

* The initial number of nonproteolytic *C. botulinum* bacteria before thermal processing was 5.3 log CFU/kg.

b NE, no effect; processing temperature was below the limits of the model.

* The initial number of organisms was outside the limits of the model.

d The result was extrapolated from the probability curve generated by the model.

To our knowledge, the predictive models used in the present study have not been previously validated in sous vide products with respect to nonproteolytic *C. botulinum*. The predictions by the FMM thermal death model were found to be unreliable in the 16 sous vide products studied. The model appeared to give high values for the logarithmic reduction of spores, since spores were observed even in those products with a low inoculum level which were predicted to have 6-D reduction in spore numbers. The FMM growth model predictions cannot be directly compared with the data obtained from the present challenge tests, since the model predicts lag time for growth and the observed results do not give evidence as to when growth began. The PMP time-to-turbidity model appeared to generate long safe-storage times for low-inoculum samples, since spores and/or slight growth was detected in most products. However, with the high inoculum level the model predicted considerably shorter safe-storage times for most products, including those that were considered to exhibit high risk. The failure of both growth models to predict safe-storage times for different types of vacuum-packaged fishery products with respect to *C. botulinum* type E and *Listeria monocytogenes* has been recently reported (7, 25). The poor agreement of predicted and observed results in the above-mentioned studies and in this study was partly due to the limited number of controlling factors in the models. For example, the level and nature of the natural bacterial flora in the products and the product formulation have an effect on growth by food-borne pathogens. The models have been developed in broths under constant conditions and do not account for different changing variables in food products and characteristics of different bacterial strains that affect microbial behavior (41, 47). Additionally, in many cases the levels of the controlling factors in the products studied were simply out of range or operated near the outer limits of those set by the models, which contributed to inaccurate predictions. With these types of products, models should not be used. Instead, safety evaluation should be done by inoculation studies.
The results of the present study indicate that the safety of sous vide products with respect to nonproteolytic C. botulinum has to be carefully evaluated product by product. An increase in processing time and temperature would seem a logical solution in view of the difference in the $P$ values observed between high-risk and safe products. However, the degree of benefit gained from increased thermal processing is obviously greatly dependent on the type of product. Additionally, adverse effects on sensory and nutritional qualities by increased thermal treatment are the opposite of the original idea of sous vide processing. Another alternative to improve safety would be to add additional hurdles to products. Biopreservatives, such as nisin and organic acids, are known to have an anti-botulinum effect (33, 43, 45). However, even a slight change in formulation or processing conditions warrants a safety evaluation by challenge tests since the predictive models available to date appear to frequently provide misleading predictions. Furthermore, use of time-temperature indicators in individual product packages would record the storage history of a product (40, 44) and might lead to enhanced temperature control in chill chains. Additionally, for evaluators to be able to make confident risk assessments and to avoid being unnecessarily overcautious, additional data on the prevalence and numbers of spores of psychrotrophic C. botulinum in different categories of sous vide-processed foods is needed (15).

ACKNOWLEDGMENTS

This work was supported by grants from the Technology Development Centre, the Academy of Finland, the Walter Ehrstro¨m Foundation, and the Finnish Veterinary Foundation.

We are grateful to Kärsi Risktari and Maria Stark for their invaluable technical assistance.

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