

DISCUSSION PAPER

**ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD
(ACMSF)**

**EVALUATION OF THE RISK OF GROWTH AND TOXIN PRODUCTION BY
CLOSTRIDIUM BOTULINUM IN SELECTED NEW PRODUCTS OF CONCERN**

1. The attached report was commissioned by the Department of Health following concerns about several food products where there might be potential for growth and toxin production by *Clostridium botulinum*.
2. Members are asked for their views on the findings of the report, any other products of concern, and, in particular, whether the Food Standards Agency should consider commissioning research and/or surveillance in this area. The paper will be introduced by Dr Cook.

**Secretariat
June 2000**

**EVALUATION OF THE RISK OF GROWTH AND TOXIN PRODUCTION BY *CLOSTRIDIUM*
BOTULINUM IN SELECTED NEW PRODUCTS OF CONCERN**

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CONTENTS

1. Introduction

2. Contamination of foods discussed in this report, with *Clostridium botulinum* from soil
3. Vacuum-packed beetroot
4. Vacuum-packed paneer
5. Part-baked bread, stored at ambient temperature
6. Part-baked bread, stored at refrigerated temperature
7. Vegetables/spices/herbs in oil, stored at ambient or refrigerated temperature
8. Canned fruits with a high pH
9. Concluding remarks.
10. References

Tables

1. Properties of bacteria that form botulinum toxin (Lund and Peck 2000b)
2. Reported incidence of *C.botulinum* in soil samples (modified from Notermans 1993)
3. Reports of botulism associated with home-canned beetroot
4. Properties of samples of vacuum-packed beetroot
5. The incidence of *C.botulinum* spores in commercial samples of mascarpone cheese (Franciosa et al. 1999)
6. Outbreaks of botulism associated with dairy products

Tables (contd)

7. Properties of samples of paneer

8. Summary of growth and toxin formation in bakery products inoculated with *C.botulinum* type A and proteolytic type B after baking, packed and stored at 25°C for 42 days. Inoculum 5×10^4 spores/g . (Modified from Daifas et al. 1999a)
9. Properties of part-baked bread stored at ambient temperature
10. The combined effect of water activity, adjusted with NaCl or glycerol, and pH, adjusted with HCl, on growth of *C.botulinum* type A (ZK3), proteolytic type B (ATCC438) and E (Beluga) from spore inocula at 20°C (Modified from Baird-Parker and Freame, 1967).
11. Properties of part-baked bread stored at refrigerated temperature
12. Reported incidence of *C.botulinum* in raw vegetables and fruits (Lund and Peck, 2000a)
13. Reported outbreaks of botulism caused by consumption of vegetables covered by oil

Figures.

1. Possible growth of proteolytic *C.botulinum* in vacuum-packed beetroot at 25°C, based on the measured pH and water activity of samples and on Food MicroModel
2. Possible growth of non-proteolytic *C.botulinum* in vacuum-packed beetroot at 25°C, based on measured pH and water activity of samples and on Food MicroModel
3. Possible growth of non-proteolytic *C.botulinum* in paneer at 8°C, based on measured pH and water activity of samples and on Food MicroModel.
4. Possible growth of proteolytic *C.botulinum* in paneer at 20°C, based on measured pH and water activity of samples and on Food MicroModel.

Appendices

Appendix 1. Methods of sampling foods and measurement of pH and water activity

Appendix 2. Lund, Baird-Parker, Corry et al. (1986) An evaluation of Mester vacuum-packed potatoes for

storage at ambient temperature. Report to the Department of Health and Social Security.

1. INTRODUCTION

This report concerns the following types of food, on which an assessment was requested:

1. Vacuum-packed beetroot
2. Vacuum-packed paneer
3. Part-baked bread stored at ambient temperature
4. Part-baked bread stored at refrigerated temperature
5. Vegetables/spices/herbs in oil, stored at ambient or refrigerated temperature
6. Canned fruits with a high pH

Measurements were made of samples of the first four foods; methods of sampling and measurement of pH and water activity are stated in Appendix 1. The samples tested were selected from those available in supermarkets; there is no implication that they represent more or less of a risk than other similar products that are available.

The properties of bacteria that form botulinum toxin are summarized in Table 1. Strains of *C. butyricum* and of *C. baratii* that form botulinum toxin have only been reported extremely rarely; this report will focus on Group I, proteolytic *C. botulinum* and Group II, non-proteolytic *C. botulinum*, which are the predominant cause of botulism in humans.

The risk of formation, in a food product, of botulinum toxin in sufficient quantities to cause botulism after consumption is a function of:

the probability (p) of contamination of the product; p of survival; p of germination of spores and growth; and the amount of the product consumed.

The major source of contamination of foods discussed in this report with *C. botulinum* is soil, this will be

discussed in section 2; the contamination of each type of product and the probability of survival of spores, germination and formation of toxin will be considered separately for each type of food.

The preservation of foods considered in this report relies mainly on a combination of heat treatment, pH and water activity, and the advice and regulations for the production of heat-preserved foods in hermetically sealed containers, including canned foods, are relevant to these products. Guidelines and regulations for the production of such foods divide them into the following categories.

The definitions of categories used in the UK guidelines are (Department of Health 1994):

Low acid food: Any food product in which any one or more components has a pH greater than 4.5 at the end of the thermal process

High acid food: Any food in which all components have a pH value of 4.5 or below.

Acidified food: A food, any component of which has a neutral pH, to which acidic ingredient(s) are added to bring the pH of all components to 4.5 or below.

The definitions of categories used in the U.S.A regulations are (FDA 2000)

Low-acid foods: Any foods, other than alcoholic beverages, with a finished equilibrium pH greater than 4.6 and a water activity greater than 0.85.

Tomatoes and tomato products having a finished equilibrium less than 4.7 are not classed as low-acid foods, but are classed as acid foods.

Acid foods: foods that have a natural pH of 4.6 or below.

Acidified foods: low-acid foods to which acid(s) or acid food(s) are added and which have a water activity (a_w) greater than 0.85 and a finished equilibrium pH of 4.6 or below.

In discussing the extent to which a preservation procedure reduces the risk of growth of *C.botulinum*, the term "protection factor" will be used in this report to mean the number of 10-fold reductions in the probability of growth and toxin formation per spore. An F_03 process, the minimum heat treatment that should be applied in the thermal processing of low acid foods in cans or other hermetically sealed containers (Department of Health 1994), is regarded as providing a 10^{12} reduction in viable spores of proteolytic *C.botulinum*, equivalent to a protection factor of 12. A 10^6 reduction in the risk of growth of

non-proteolytic *C.botulinum*, equivalent to a protection factor of 6, has been accepted as a criterion for sous vide foods and other prepared, chilled foods with an extended shelf life (Committee on the Microbiological Safety of Food, UK, 1990; Advisory Committee on the Microbiological Safety of Food (ACMSF), UK, 1992; Peck et al. 1995).

2. CONTAMINATION OF FOODS CONSIDERED IN THIS REPORT WITH *CLOSTRIDIUM BOTULINUM* FROM SOIL

The major potential source from which *C. botulinum* could contaminate the foods considered in this report is the soil. Information on the incidence of *C.botulinum* in soil and in other types of sample is limited by the adequacy of the detection/isolation methods used (Lund and Peck 2000a). Reports of the incidence of *C.botulinum* in soil samples are summarized in Table 2. The main extensive studies of soil samples in the UK are those of Smith and co-workers who, unlike some previous workers, used isolation techniques that were designed to detect both Group I, proteolytic and Group II, non-proteolytic strains. In the study of 174 soil samples from various parts of Britain *C.botulinum* was detected in only 10 samples (5.7%), in each case only toxin type B was detected (Smith and Young 1980); the report does not indicate whether these were Group I, proteolytic, or Group II, non-proteolytic *C.botulinum*. These researchers considered that "factors that may conceivably affect the prevalence of *C.botulinum* are type of soil, disturbance of soil by human agencies, climate and the size and rate of change of animal populations responsible for faecal contamination". In a survey of the redeveloped site of the former Metropolitan Cattle market, London, of 60 soil samples 15 (25%) contained *C.botulinum*; of these 15 samples, nine contained strains producing toxin-type B, two type C, two type D, one both types C and D and one type E (Smith and Milligan 1979). In contrast, three further sites in England that had been associated with animals for many years showed an incidence of *C.botulinum* of 3.6%, 2.6% and <3.6% (Smith and Young 1980).

Landfill sites are liable to be a source of *C.botulinum*; of 19 landfill sites sampled by Ortiz and Smith (1994) *C.botulinum* toxin-types B, C and D were each found in 63% and toxin-type E in 5%. The bacteria may be spread by gulls and other scavenging birds that are attracted to the sites, and measures to prevent access of birds to such sites have been advised.

Freshly drained land may also contain large numbers of *C.botulinum* and in clay soil from such land in the Netherlands approximately 450 *C.botulinum* type E per 100g soil were detected (Notermans 1993).

Botulism occurs in animals (both wild and those used in agriculture) in Northern Europe as well as in other parts of the world, this may be due to the ingestion by animals of rotting organic matter. Much of the botulism in animals is caused by toxin types C and D, but other toxin types are also involved;

proteolytic *C.botulinum* toxin type B has caused outbreaks of botulism in cattle and of toxicoinfectious botulism in horses, which are reported to be particularly susceptible to type B toxin (Swerczek 1980). Outbreaks of type B botulism in cattle and horses are reported occasionally, and are due to factors such as the consumption of poorly prepared silage, or contamination of feed with the carcass of a small animal (Chiers et al. 1998; Divers et al. 1986; Anon. 1999; Haagsma and Ter Laak, 1978; Haesebrouck et al. 1990; Notermans et al. 1981; Ricketts et al. 1984). Manure from cattle or horses associated with such outbreaks could be a source of the organism (Notermans et al. 1981).

These reports indicate that the incidence and numbers of *C.botulinum* in soil may be highly variable and influenced by soil type, climate and agricultural practices; it is difficult, therefore, to assess the likely level of contamination of raw food from soil. It is clear, however, that it must be assumed that raw foods may be contaminated with *C.botulinum*, although very often the incidence and numbers present will probably be low.

3. VACUUM-PACKED BEETROOT

3.1 Properties of this type of product.

Beetroot is reported to have a pH between 4.9 and 5.8 (Banwart 1989). An example of this type of product is Long-life vacuum packed beet produced in retail and catering packs and stored at room temperature. Vacuum-packed cooked beetroot was purchased from supermarkets but was not included in the samples tested for this report. The shelf life marked on these products was at least four months at ambient temperature. The beet is described as “prepared, fully cooked and prepacked for convenience”. Some of the beetroot samples tested in the present work were grown in Holland.

These products are analagous to the vacuum-packed potatoes for storage at ambient temperature that were the subject of a report to the Department of Health and Social Security in 1986 (Lund et al. 1986) (Appendix 2).The vacuum-packed, cooked beetroot would be expected to have a pH higher than 4.5 at the end of the thermal process, and thus be subject to the Guidelines for the Safe Production of Heat Preserved Foods (Department of Health, 1994) and to the US Regulations for Low-Acid Canned Foods (FDA 2000).

3.2 The risk of occurrence of spores of *C.botulinum* on beetroot

The risk of contamination of raw beetroot with spores of *C.botulinum* depends largely on the incidence of the bacterium in soil. Removal of the outer tissue of the beet during preparation should remove residues of soil and reduce the level of contamination, but cross-contamination may occur in wash water.

3.3 Reports of outbreaks of botulism associated with beetroot

Evidence of the contamination of beetroot with *C.botulinum* is provided by reports of botulism associated with the consumption of home-canned beets. A review published in 1923 reported one outbreak of botulism in the US associated with preserved beets and one associated with preserved beets and turnip tops (Schoenholz Esty and Meyer 1923). Experiments in which canned beets (in No. 2 cans) were inoculated with a high number of spores of proteolytic *C.botulinum* (10^8 - 10^{10}) and incubated at room temperature or at 35°C, showed that toxin formation occurred in the cans, in some cases this occurred in the absence of obvious spoilage. The pH of the canned beets before inoculation for the experiments was between 4.86 and 5.0. Further reports of botulism in the US associated with home-canned beets are shown in Table 3. In the former USSR an outbreak of botulism involving approximately 10 people was attributed to underprocessed, commercially canned beetroot (Podreshetnikova et al. 1970); type B toxin was detected in a blown can of beetroot.

These reports show that some beets can support growth and toxin formation by *C.botulinum*.

3.4 Probability of survival and growth of *C.botulinum* in vacuum-packed beetroot

Removal of the outer layers of the beet would be expected to reduce the incidence of spores of *C.botulinum* but, conversely, re-contamination and spread of the spores in wash water may occur. Cooked beetroots in a vacuum pack are a low-acid food in a hermetically sealed container, and therefore should receive a minimum thermal treatment equivalent to 3 min at 121°C (an F_{03} process). A heat treatment less than this cannot be relied upon to give the required protection factor against spores of proteolytic *C.botulinum*. Vacuum-packaging and storage at ambient temperature would be expected to allow germination and growth from spores of proteolytic *C.botulinum*. If the heat treatment was not sufficient to inactivate spores of non-proteolytic *C.botulinum* these could also germinate and give growth in the packs.

3.5 Study of samples of vacuum-packed beetroot

Samples of shelf-stable beetroot and measurements of pH and water activity (a_w) are shown in Table 4, this confirms that the pH was higher than 4.5 and the water activity was higher than 0.85. On the basis of the measured pH and water activity (a_w), the ability of proteolytic and non-proteolytic *C.botulinum* to multiply on vacuum-packed beetroot at 25°C as predicted by Food MicroModel is shown in Figs 1 and 2. It should be noted that the Micromodel is based on a_w controlled by NaCl; this was not the case in the samples of beetroot, and equivalent values of water activity controlled by factors other than NaCl may be

less inhibitory to *C.botulinum* (Baird-Parker and Freame 1967). Thus, growth of *C.botulinum* in/on beetroot might be more rapid than is predicted by Food MicroModel. The initial concentration of *C.botulinum* in Figs 1 and 2 is relatively high, at 100 spores/g, but it must be borne in mind that the products are intended to have a long shelf-life, and a substantial protection factor is needed in such products.

3.6 Conclusions regarding vacuum-packed, beetroot

This product is a thermally processed, low-acid food with a pH higher than 4.5 and a water activity higher than 0.85, packed in a hermetically sealed container, and is designed for long-life storage at room temperature. There is evidence that beetroot will support growth and toxin formation by *C.botulinum*, and on the basis of the measured pH and water activity of the product growth of both proteolytic and non-proteolytic *C.botulinum* would be expected to occur within a few days at 25°C. The oxygen concentration is probably low and uncontrolled in the vacuum-packages and cannot be relied upon to prevent growth of *C.botulinum*. Evidence is needed, therefore, either that the product has been subjected to an F₀3 heat treatment (botulinum cook) or that it has been produced by a combination of heat treatment and preservative factors that will prevent provide a protection factor equivalent to an F₀3 process to prevent growth and toxin formation by *C.botulinum*.

If this product was designed for refrigerated storage the heat treatment should be sufficient to give a 6D reduction in viable spores of non-proteolytic *C.botulinum*. This would require a minimum heat treatment equivalent to maintenance at 90°C for 10 minutes throughout the product, (ACMSF 1992), but a more severe heat treatment may be needed if the beetroot contains enzymes with sufficient lysozyme activity to allow growth from heat-damaged spores of non-proteolytic *C.botulinum* (Stringer and Peck 1996). Alternatively a combination of heat and preservative factors should be used that would provide a protection factor of 6 against growth of non-proteolytic *C.botulinum* (ACMSF 1992)

4. VACUUM-PACKED PANEER

4.1 Properties and production of Paneer

Paneer is a traditional dairy ingredient used in India, Iran and Pakistan. It is used primarily as a meat substitute in Asian cookery. In the UK many Asian families are reported to make it in their homes. Paneer is produced commercially in the UK and is sold in selected branches of certain supermarkets and in Specialist Indian shops. The label on the cheese samples obtained states the ingredients as whole milk and vinegar and includes the statement "Protective atmosphere".

A general description of the production of this type of paneer indicates that fresh, whole milk is brought to the boil, vinegar (or lemon juice) is added and the mixture is stirred and set aside to curdle. After the milk has curdled it is wrapped in muslin cloth, rinsed with fresh water and drained. It is then formed into a block and placed under a heavy weight for a specified time, after which it is ready for use.

Correspondence from the Department of Health states that the milk used for production is pasteurized, this would not inactivate spores of *C. botulinum*. When acetic acid is added the casein micelles will begin to precipitate at pH 5.2-5.3, somewhat above the isoelectric point of the casein at pH 4.6 (Teuber 2000). Agitation and heating results in further aggregation of the casein and excretion of whey from the coagulum. Loss of the whey and rinsing of the coagulum with water will result in removal of much of the remaining acid. A report to the Department of Health indicates that a bulk of curd weighing between 15 and 20 kg is pressed, then allowed to cool overnight; temperature measurements during cooling of paneer showed that a drop in temperature from 80°C to 15°C can require about 26 hours. Herbs may be included in some products.

4.2 The risk of occurrence of spores of *C.botulinum* in paneer.

Spores of *C.botulinum* may contaminate milk from soil, faeces or from the environment. There are few data on the incidence of *C.botulinum* spores in dairy products. None of 40 samples of Edam and Cheddar cheese or of 10 samples of cheese spreads was found to contain spores of *C.botulinum* (Insalata et al. 1969b). Unpublished studies at the University of Guelph indicated that the number of *C.botulinum* spores in milk was fewer than one per litre (Collins-Thompson and Wood 1993). In considering these reports, however, it should be recognized that the adequacy of the methods used to detect spores of *C.botulinum* has not always been made clear.

Following an outbreak of botulism in Italy, associated with mascarpone cheese, samples of mascarpone cheese were tested for spores and toxin of *C.botulinum* (Table 5) (Franciosa et al. 1999). The samples containing *C.botulinum* spores were distributed randomly among the different production lots with expiration dates between the end of August and the end of November 1996. The results (Table 5) showed repeated contamination of the product from one plant over a period of three months, and contamination of products from other manufacturers. Of 260 samples of other dairy products tested, 7 (2.5%) were contaminated with spores of *C.botulinum* type A (<10 spores/g); one of these samples, a spread processed cheese, was from the plant implicated in the botulism outbreak. The source of contamination with *C.botulinum* was not determined, but evidence from sampling the environment, packaging materials and other ingredients of the product indicated contamination of the milk, possibly from silage, dust or faeces.

The addition of herbs to some types of paneer could be a source of *C.botulinum* spores.

4.3 Reported outbreaks of botulism associated with milk or dairy products

Further evidence of the contamination of cheese and other dairy products with spores of *C.botulinum* is provided by reported outbreaks of botulism (relatively few) attributed to the consumption of contaminated products. Fourteen outbreaks have been reported (Table 6). In several of these outbreaks there is evidence that the dairy product was contaminated from environmental sources (Collins-Thompson and Wood 1993), for example the Brie cheese associated with outbreaks in 1973 may have been contaminated from straw on which the cheeses were allowed to ripen; the cheese spread involved in the outbreak in 1974 may have been contaminated from onions included in the spread; and the hazelnut yoghurt involved in the outbreak in 1989 was contaminated from hazelnut puree added to the yoghurt.

4.4 Probability of survival and growth of *C.botulinum* in samples of paneer

Heating the milk to about 80°C during production of paneer might be sufficient to inactivate some spores of non-proteolytic *C.botulinum*, depending on the time/temperature relations and the conditions. Spores of proteolytic strains are unlikely to be inactivated. It has been reported that cooling from 80°C to 15°C during production can require about 26 hours, during which there is a possibility that surviving spores could germinate and give rise to growth of vegetative bacteria and toxin formation.

4.5 Study of samples of vacuum-packed paneer

Samples of paneer and measurements of pH and water activity are shown in Table 7. The samples were intended for refrigerated storage. On the basis of the measured pH and water activity, growth of non-proteolytic *C.botulinum* in the product at 8°C, as predicted by Food MicroModel, is shown in Fig. 3. This shows that the products would be expected to support considerable growth of non-proteolytic *C.botulinum* in 20-25 days at 8°C; the shelf-life of one of these products (number 15) was intended to be at least 42 days (Table 7). If the paneer was not refrigerated, but was stored at 20°C, growth of proteolytic *C.botulinum*, as predicted by Food MicroModel, is shown in Fig 4.

4.6 Conclusions regarding paneer

The report on Vacuum Packaging and Associated Processes (ACMSF 1992) contained the following recommendations:

- in addition to chill temperatures of less than 10°C (statutory chill temperature controls require a

maximum temperature of 8°C where applicable) prepared chilled foods with an assigned shelf life of more than 10 days should contain one or more controlling factors at levels to prevent growth and toxin formation by strains of psychrotrophic (non-proteolytic) *C.botulinum*.

- in addition to chill temperatures, which should be maintained throughout the chill chain, the following controlling factors should be used singly or in combination to prevent growth and toxin formation by psychrotrophic *C.botulinum* in prepared chilled foods with a shelf life of more than 10 days:-

- a heat treatment of 90°C for 10 minutes or equivalent lethality
- a pH of 5 or less throughout the food and throughout all components of complex foods
- a minimum salt level of 3.5% in the aqueous phase throughout the food and all components of complex foods
- an a_w of 0.97 or less throughout the food and throughout all components of complex foods
- a combination of heat and preservative factors which can be shown consistently to prevent

growth and toxin formation by psychrotrophic *C.botulinum*.

One of the two samples of paneer was intended to have a shelf-life of at least 42 days, as both samples were produced by the same dairy this was probably true for all their samples. There was no evidence that any of the above controlling factors were used in the products. On the basis of the measured pH and water activity, predictions from Food MicroModel indicate that non-proteolytic *C.botulinum* would be capable of growth and formation of toxin in the products.

The following possible measures could be used to control the safety of this paneer and similar products, either

- (a) the shelf-life could be limited to less than 10 days
- or (b) the pH could be reduced to less than 5.0
- or (c) it should be demonstrated experimentally that the combination of the method of production of paneer and its composition provide a protection factor of 6 against growth and toxin formation from spores of non-proteolytic *C.botulinum*.

5. PART-BAKED BREAD STORED AT AMBIENT TEMPERATURE

5.1 Properties and production

All of the shelf-stable, part-cooked breads sampled contained salt; the low water activity of these products was due, probably, to a combination of factors, with a small effect due to the salt.

5.2 The risk of occurrence of spores of *C.botulinum* in part-baked, bread products

Flour has been reported to contain between between 10^2 and about 10^5 bacteria/g and moulds may reach a similar number (ICMSF 1998). The bacteria include spore-formers such as *Bacillus subtilis* and *B.licheniformis*, which can survive the baking process and are the main cause of “ropy” bread. *Clostridium botulinum* has been reported “to occur frequently” in flour in the USA (Ingram and Robinson 1951). During baking of home-style, canned quick bread the internal temperature of the batter was reported to reach $106^\circ\text{--}108^\circ\text{C}$ (Aramouni et al. 1994); another report states that during a full baking process, the internal temperature of a loaf reaches almost 100°C (ICMSF 1998), this is probably sufficient to be lethal to a high proportion of spores of non-proteolytic *C.botulinum* but is not sufficient to inactivate spores of proteolytic *C.botulinum*.

5.3 Reports on survival and growth of *C.botulinum* in bread products

There appear to be no reports of botulism associated with bread products, but it has been shown that *C.botulinum* can multiply and form toxin in some types of product. Several groups of workers have reported that inoculation of *C.botulinum* types A and proteolytic B into canned bread resulted in toxin formation (refs cited in Daifas et al. 1999a). In the most recent of these reports over 240 cans of two different types of bread (with a pH between 6.1 and 6.7) were each inoculated with 3×10^5 spores of a mixture of *C.botulinum* type A and proteolytic type B, processed commercially (F_0 minimum 0.14, i.e. much less than a “botulinum cook”) and stored at 29.5°C for a minimum of two years, after which toxin was not detected in any of the cans (Denny et al. 1969). Further experiments indicated that water activity was the main factor preventing growth and toxin formation from the spores in these products, and that toxin formation occurred in canned bread with an a_w of 0.955 but not in canned bread with an a_w at or below 0.95. It was concluded that the water activity of these breads was only influenced to a small extent by the moisture content; the addition of invert sugar or of sorbitol lowered the water activity and breads with raisins or other dried fruits had a lower a_w than those without.

A recent study showed that following inoculation of a mixture of 5×10^4 spores of type A and proteolytic type B *C.botulinum* per g. into bakery products, growth and toxin formation occurred in crumpets (a_w 0.990, pH 6.00) and pizza crust (a_w 0.960, pH 5.62) that were packaged in air, in air plus oxygen absorbent, or CO_2/N_2 (60%:40%) and stored at 25°C for 42 days, but not in bagels (a_w 0.944, pH 5.63) stored in similar conditions (Daifas et al. 1999a) (Table 8). During storage in packs containing air, or air plus oxygen absorbent, production of CO_2 occurred due, probably, to growth of heterofermentative lactic acid bacteria. The crumpets and pizza crust were sensorily unacceptable after 42 days but the bagels were marginally acceptable. Crumpets with a pH of 6.5 or 8.5 and a water activity of 0.990 inoculated

with 500 spores of proteolytic *C.botulinum*/g, packaged under CO₂/N₂ (60%:40%) or under CO₂ (100%) and stored at 25° C were toxic in between four and seven days, and prior to spoilage (Daifas et al. 1999b), showing a potential hazard in such products; it was concluded that additional barriers were needed to inhibit growth of *C.botulinum* in this type of product.

5.4 Study of samples of part-baked bread stored at ambient temperature

Samples of products and measurements of water activity are shown in Table 9. A further product, on which measurements were not made, was a ready-to-bake garlic baguette purchased from a supermarket and stored at ambient temperature.

The water activity of the products was below the minimum a_w , 0.97, that can be used for predictions of growth from Food MicroModel. It should be noted, however, that in Food MicroModel the water activity is controlled by NaCl. In the samples of part-baked bread examined the low water activity was due, probably, to a combination of factors and only in part to NaCl. A comparison of the combined effect of pH and water activity controlled by NaCl or by glycerol is shown in Table 10. If the water activity of the products was due mainly to NaCl, Table 10 indicates that the conditions in the bread samples would prevent the growth of *C.botulinum*. Table 10 shows that, in conditions where the water activity was controlled by a factor other than NaCl, in this case by glycerol, growth of *C.botulinum* could occur at a combination of pH 5.5 and a water activity in the region of 0.95-0.96, as was found in some of the samples tested by Daifas et al. (1999a) and shown in Table 8. The combination of water activity and pH in samples 1, 9 and 12 of part-baked bread (Table 9) indicates that there may be a risk of growth of *C.botulinum* in these products.

The composition of the wrappers used has not been determined and these may be expected to be permeable to oxygen and allow the maintenance of aerobic conditions in the breads. The possibility that growth of other bacteria in the breads, e.g. lactic acid bacteria, might cause depletion of oxygen in the products and facilitate growth of *C.botulinum* (Daifas et al. 1999a) should be considered.

5.5 Conclusions regarding part-baked bread stored at ambient temperature

There is a low risk that *C.botulinum* will occur in products of this type. Spores of proteolytic strains would be expected to survive the baking process. The combinations of pH and water activity in some products of this type are such that growth of *C.botulinum* might occur, particularly at high ambient temperatures. The "Use-by" date on some of the products in Table 9 showed a long intended shelf life.

The risk that *C.botulinum* would be able to grow and form toxin in these products is probably low, but it is advisable to confirm experimentally that a suitable protection factor is provided in these foods. The addition of further ingredients, to control the water activity and to reduce it slightly to ensure that it is below a_w 0.95, and to give a small reduction in pH, may be a method of increasing the protection factor against *C.botulinum*.

6. PART-BAKED BREAD, STORED AT REFRIGERATED TEMPERATURE

6.1 Properties and production

The list of ingredients in these products is more complex than that of the shelf-stable breads on which measurements were made. All contained NaCl, but the water activity may be controlled mainly by a combination of factors rather than mainly by NaCl.

6.2 The risk of occurrence of spores of *C.botulinum* in part-baked bread stored under refrigeration

The risk of occurrence of spores of *C.botulinum* in these products may be greater than that in the shelf-stable breads examined because of the presence, in the refrigerated products tested, of ingredients such as garlic puree and parsley, which can be a source of contamination with bacterial spores. Most of the relevant information in the literature appears to refer to proteolytic strains of *C.botulinum*, but non-proteolytic strains may also be present.

6.3 The risk of survival and growth of *C.botulinum* in part-baked bread stored under refrigeration

Provided that part-cooked bread is maintained below 10°C growth of proteolytic *C.botulinum* will be inhibited. There do not appear to be reports in the literature concerning growth of non-proteolytic *C.botulinum* in bread stored under refrigerated conditions, and the extent to which they may survive the part-cooking is not known.

6.4 Study of samples of part-baked bread stored under refrigeration

Information on samples examined is shown in Table 11. All the products had a water activity lower than 0.970. If this water activity was due to the content of NaCl, growth of non-proteolytic *C.botulinum* would be inhibited (Baird-Parker and Freame 1967; Graham, Mason, Maxwell and Peck, 1997). If, as seems likely, the water activity is controlled by a combination of factors, the a_w of 0.95 or lower in products 7, 13

and 14 combined with a pH lower than 6.0 and a temperature below 10°C will probably prevent growth of non-proteolytic *C.botulinum*. In the case of the fresh dough chocolate croissants, with a pH 6.83 and a_w 0.953 and a long shelf life, confirmation is needed that these conditions will prevent growth of non-proteolytic *C.botulinum*. If the products are not stored at a temperature below 10°C, but are maintained at 20°C, and water activity is not controlled by NaCl, the conditions in samples 7 and 24 may not be sufficient to prevent growth of *C.botulinum*.

6.5 Conclusions regarding part-baked bread stored at refrigerated temperature

There is a low risk that *C.botulinum* will occur in products of this type. Spores of proteolytic *C.botulinum* would be expected to survive the part-baking process, it is not known whether spores of non-proteolytic *C.botulinum* would do so. It is advisable to confirm experimentally that suitable protection is provided against growth of non-proteolytic *C.botulinum* in the case of products such as sample 24; further protection probably could be provided, if necessary, by a small reduction in the water activity.

7. VEGETABLES/SPICES/HERBS IN OIL, STORED AT AMBIENT OR REFRIGERATED TEMPERATURE.

7.1 Properties of this type of product.

Samples of these products were not tested, but we note that jars of vegetables in oil (including those labelled as antipasto) are on sale at ambient temperature in the UK. A range of related products has been noted, these include antipasti which contain grilled aubergines, or artichokes, or sun-dried tomatoes, or mushrooms in olive oil and pasta sauces in olive oil.

The following examples of jars of vegetables in oil were purchased from a supermarket :

(i) Italian mixed mushrooms. Mixed mushrooms in a blend of sunflower and extra virgin olive oil.

Ingredients: mushrooms, sunflower oil, extra virgin olive oil, porcini extract, salt, white wine vinegar, garlic powder, antioxidant L-ascorbic acid.

Purchased 2 April 2000, Best before date September 2001.

(ii) Italian mixed peppers. Mixed peppers in a blend of sunflower and extra virgin olive oil. Ingredients: red, yellow and green peppers, sunflower oil, sugar, extra virgin olive oil, parsley, salt, white wine vinegar, flavouring, garlic powder.

Purchased 2 April 2000, best before date December 2001.

According to one particular recipe, a mixture of fresh and canned vegetables may be used. The recipe includes a small volume of vinegar and directs heating at a temperature lower than 100°C for 15 minutes.

Immersion of the vegetables in oil is liable to create anaerobic conditions. When these products are prepared with fresh vegetables, spices or herbs, they are subject to the same risk associated with *C.botulinum* as are canned foods. If the final pH throughout the food is higher than 4.5 the product will be classed as a low-acid food and should be processed using at least an F₀3 heat treatment; alternatively the food should be acidified to a pH of 4.5 or lower throughout the product and be processed using sufficient heat treatment to prevent microbial spoilage. If the natural pH of the product is 4.5 or lower it should receive sufficient heat treatment to prevent microbial spoilage.

The need to apply an F₀3 process to low acid foods of this type could be relaxed if the foods were shown to contain preservative factors that, together with a mild heat treatment, give a protection factor equivalent to that provided by an F₀3 process.

7.2 The risk of occurrence of spores of *C.botulinum* on a wide range of vegetables.

Reports of the incidence of *C.botulinum* on a range of vegetables are summarized in Table 12.

Mushrooms have caused some botulism concerns in the past. In 1973, during an investigation of a spoilage problem, a can company in the US found a toxic can of mushrooms (Lynt et al. 1975). The presence of botulinum toxin in the can was confirmed by the FDA and 19 cans in 7 codes of canned mushrooms from this producer were found to contain viable spores of *C.botulinum* type B. About one month later, upon inspection of another plant following a customer complaint, canned mushrooms from the plant were found to contain viable spores of *C.botulinum* type B; no toxin was found in the product. Following these episodes the FDA ordered a survey of domestic and imported canned mushrooms. In this survey 30 cans of mushrooms were found to contain botulinum toxin and an additional 11 contained viable spores of *C.botulinum* (Lynt et al. 1975). In the majority of the cans contamination was with *C.botulinum* type B, although one can of US origin and one of foreign origin were also contaminated with type A. As far as is known, no cases of clinical botulism were traced to any of these products.

In 1973 in Canada a jar of imported, marinated mushrooms in oil was implicated as the cause of type B botulism in Montreal (Hauschild et al. 1975). As a result, over 8,000 jars of the product were recalled, of these 10 contained *C.botulinum* type B toxin. All the toxic jars had a butyric odour and a pH above 4.8. Following these findings, in a survey of 12 samples of fresh mushrooms from retail stores in several parts

of Canada, spores of *C.botulinum* type B were found in all the samples at estimated most probable numbers of between 15 and 41 spores per 100g (Hauschild et al. 1975).

In a survey in the Netherlands, *C.botulinum* was not detected in fresh mushrooms, indicating that numbers were fewer than one spore per 100g (Notermans 1989).

7.3 Reports of botulism associated with vegetables/spices/herbs in oil

Examples of outbreaks of botulism caused by the consumption of vegetables stored in oil are shown in Table 13. A large outbreak in Canada in 1985 was associated with home-bottled, chopped garlic in soybean oil, that was used to prepare garlic butter included in sandwiches sold in a restaurant (St. Louis et al. 1988). The chopped garlic was rehydrated, sun-dried garlic without added chemicals or acid. The bottles of chopped garlic in soybean oil were labelled with instructions to refrigerate, but unopened bottles were reported to have been stored in the restaurant unrefrigerated for eight months.

A further outbreak of botulism associated with garlic bread made from garlic in oil occurred in the US in 1989. The garlic in oil had been prepared by mixing chopped garlic, ice-water and olive oil, without further additives. The bottles were labelled "Keep refrigerated" in small print. Despite this label, a bottle had been kept at room temperature for about three months. Morse et al (1990) stated "because of the inherent danger associated with this type of product if left unrefrigerated, the FDA and the New York State Department of Agriculture and Markets ordered companies to stop making any garlic-in-oil mixes which are only protected by refrigeration. For safety, such products must now contain specific levels of microbial inhibitors or acidifying agents such as phosphoric acid or citric acid".

Home-prepared mushrooms in olive oil were responsible for reported outbreaks due to type B botulinum toxin in 1982 and 1998 and commercially produced, sliced, roasted eggplant in oil was responsible for an outbreak due to type B toxin in 1993 (Table 13). A large outbreak of botulism in the US was caused when sauteed onions covered by a thick layer of margarine were maintained at about 41°C throughout one day and possibly held overnight.

Vegetables in oil have contributed to 54% of the cases of botulism in Italy between 1992 and 1996, where approximately 40 cases per annum have been reported (Aureli et al. 1999; Squarcione et al. 1999). Other vegetable preserves have been implicated in 10% of cases. While many of the products implicated were home-prepared, a proportion was produced commercially.

From these reports it is clear than some vegetables in oil can support growth and toxin formation by *C.botulinum*. In a study by Solomon et al. (1991), however, several unacidified products in oil, inoculated

with a mixture of spores of five strains of *C.botulinum* type A failed to show toxin formation. The products were: oil extract of garlic; black beans in oil, chili-garlic in oil; chopped shallots in oil; walnuts in oil; sun-dried tomatoes in oil; dried tomatoes in olive oil; dried tomatoes in sunflower oil; and two sauces. The pH of the tomatoes in oil was 4.0-4.5, thus the failure to support growth of *C.botulinum* was not surprising. The pH of the chopped shallots in oil was 4.7 and the pH of the remaining products was 6.0. It is rather surprising that some of these products did not show formation of botulinum toxin, possibly the packaging and the repeated sampling of the products prevented anaerobiosis from developing. The authors stated "However, new and unusually potent strains of *C.botulinum*, or a combination of optimum conditions, might induce their growth in these products and could be a potential hazard. Therefore caution in the manufacture and distribution of these items is still urged"

7.4 Conclusions

Vegetables, spices and herbs are obtained from many countries and are liable to be contaminated with both proteolytic and non-proteolytic *C.botulinum*. There are reported outbreaks of botulism due to some products of this type kept at ambient temperature. The ingredients used in this type of product appear to include raw, precooked and canned vegetable components; cooking of ingredients would be likely to inactivate spores of non-proteolytic *C.botulinum* but not necessarily to inactivate spores of proteolytic strains. Evidence from outbreaks of botulism indicates that *C.botulinum* can grow and form toxin in some products of this type.

Antipasto products have been produced commercially in Italy for many years. Further information is needed regarding the composition and processing of the products that are sold in the UK. The possible variations that may be introduced in composition and processing of such foods mean that it is important that the protection factor against *C.botulinum* should be determined and its basis should be understood. If foods of this type are for sale at ambient temperature, either (1) it needs to be demonstrated that the combination of processing and formulation provides a high protection factor against growth of *C.botulinum*, or (2) if the pH is higher than 4.5 the product should receive at least an F₀3 heat treatment, or (3) the product should be acidified to a pH of 4.5 or lower and a heat treatment should be used similar to that given to acid canned foods, in order to inactivate spoilage microorganisms, or (4) products with a pH of 4.5 or lower should be treated as high-acid foods.

If low-acid products are sold that are designed to be maintained at chilled temperatures, they should not rely solely on refrigeration for safety and should be produced using a method that can be relied upon to give a protection factor of at least 6 against the growth of non-proteolytic *C.botulinum*.

8. CANNED FRUITS WITH A HIGH pH

8.1 Properties and production of products.

No products of this type were obtained for testing. Most fruits have a pH lower than 4.5. Those with the highest pH include banana, papaya, watermelon and other melons (Lund and Snowdon, 2000), other fruits that may have a pH higher than 4.5 include cherries, pears, figs, tomatoes and peppers.

There are several reports of botulism associated with heat-processed **acid** fruits with a low pH (Odling and Pflug 1978); in each of these cases there was evidence that growth of yeasts or moulds in the product had raised the pH sufficiently to allow growth of *C.botulinum*. Two deaths from botulism were attributed to home-canned pears, which were stated usually to have a pH lower than 4.0. Yeasts were found to have grown in the pears and the pH of the toxic samples was ≥ 6.0 (Meyer and Gunnison, 1929 cited by Sperber 1982). In the 76 years prior to 1978, 34 outbreaks of botulism were reported in the US that were associated with home-canned, high-acid foods and in every case there was evidence of concomitant growth of yeast or mould that would raise the pH (Odling and Pflug, 1978). Thirteen of these outbreaks were associated with fruit or fruit juice, 18 with tomatoes or tomato preparations and three with pickles.

In a review of human botulism in the US and Canada, Meyer and Eddie (1965) (cited by Ito et al. 1978) referred to 12 outbreaks associated with home-canned figs, but no outbreaks are known to have been reported due to commercially canned figs. Because of the high pH of figs, it has been commercial practice in the US to add lemon juice, concentrated lemon juice or organic acid, when necessary, to reduce the pH to 4.9 or below (Ito et al. 1978). Work by Townsend et al (1955) and Ito et al. (1978) showed that maintenance of a pH of 4.9-5.1 or lower (adjusted with lemon juice in the work by Ito et al ,1978) prevented the outgrowth of spores of proteolytic strains of *C.botulinum* in figs packed in water, light or heavy syrup. The protection factor at pH 5.1 in the experiments by Ito et al. (1978) was approximately 6.

Acidified canned foods were covered briefly in US Regulations published in 1973. In the same year seven cases of botulism were reported in West Virginia, US, after consumption of peppers that had been acidified improperly (FDA 2000). A case of botulism occurred in Canada after consumption of marinated mushrooms packed in the US, that were acidified improperly and in 1974 FDA found inadequately acidified pimentos and hearts of palm processed by 29 firms in other countries, but no illnesses were documented. In 1976 eight people were diagnosed with botulism that was linked epidemiologically to the consumption of sweet cherry peppers. Home-canned jalapeno peppers underprocessed and used in food served in a restaurant caused an outbreak of type B botulism in the US in 1977 that affected 59 people (Terranova et al. 1978).

In 1976 the FDA concluded that certain manufacturers of acidified foods did not realize the importance of adequate control of pH, and that a separate, more detailed regulation was needed. The final regulations covering acidified foods were published on March 16, 1979 and are now included in the Code of Federal Regulations Title 21, Parts 108, 113, and 114 revised in April 1999.

8.2 Conclusions

It is important that producers of processed fruits with a high pH understand the implications of the pH for processing. If these products have a pH higher than 4.5 they will be classified in the UK as low-acid foods and should receive at least an F₀3 heat treatment. If these products are acidified and are processed by a heat treatment less than an F₀3 process the importance of pH control as a critical control point should be recognised.

9. CONCLUDING REMARKS

The samples tested for this report were selected from those available in supermarkets; there is no implication that they represent more or less of a risk than other similar products that are available.

Based on the considerations in this report, the following measures are suggested, in approximate order of priority:

1. **Vacuum-packed beetroot for extended shelf-life at ambient temperature.** Information is needed to show that this product provides the same protection factor against survival and growth of *C.botulinum* as do other low-acid, heat-processed foods in hermetically sealed containers (Department of Health 1994).

2. **Vegetables/spices/herbs preserved in oil.** Further information is needed about the composition and method of processing of products that are on sale in the UK, and an understanding is needed of the basis for their safety in relation to *C.botulinum*. In the case of low-acid products for storage at ambient temperature, information is needed to show that these products provide the same protection factor against survival and growth of *C.botulinum* as do other low-acid, heat-processed foods in hermetically sealed containers that are given an F₀3 thermal process. If these products in oil are acidified and given a heat treatment less than an F₀3 process there is a need to ensure that the pH is measured and controlled. In the case of products classified as high-acid foods there is a need to ensure that the pH of products remains at 4.5 or lower.

3. **Paneer** is a refrigerated food with an intended shelf-life of at least 42 days. Although it is used largely in cooking, it is not acceptable to rely on cooking by the consumer to provide protection against *C.botulinum* toxin. It is appropriate, therefore, to consider this product in relation to the report on Vacuum Packing and Associated Processes (ACMSF 1992). Measures are needed to ensure that the production and/or formulation provide a suitable protection factor against growth of *C.botulinum*.

4. **Canned fruits with a high pH**. It is important that manufacturers of this type of product understand the significance of the pH of such products. There is a need to ensure that these canned fruits are classified correctly as low-acid, or high-acid, or are acidified, that the pH is measured and controlled where necessary, and that the thermal process relevant to the product is applied .

5. **Part-baked bread stored at ambient temperature** . Some of these foods have a long intended shelf-life, the processing would probably not inactivate spores of proteolytic *C.botulinum* and the combination of pH and water activity in some of the products may allow growth of *C.botulinum*. It is advisable to ensure that protection against *C.botulinum* is provided. This could be done by adjusting the process and/or altering the ingredients to ensure control of water activity and pH. The presence of garlic or herbs in these breads is liable to increase the risk of contamination with *C.botulinum*.

6. **Part-baked bread stored at refrigerated temperature** . Some of the breads have a long intended shelf-life, the baking would probably not inactivate spores of proteolytic *C.botulinum*, the extent to which spores of non-proteolytic *C.botulinum* would be inactivated is not known. Storage below 10°C would prevent growth of proteolytic *C.botulinum*. A product such as the fresh dough chocolate croissants with a neutral pH might allow growth from surviving spores of non-proteolytic *C.botulinum*; it may be possible to increase the protection factor for *C.botulinum* by adjustment of the ingredients to reduce and control the water activity.

The extent to which oxygen is depleted in packs will differ in the different types of food. The remaining concentration of oxygen and the oxidation-reduction potential in the food will affect the risk of growth of *C.botulinum*. This factor is often an uncontrolled variable in foods and is influenced by variations in method of packing, in the permeability of packs, and in growth of spoilage bacteria. Unless it is possible to ensure that depletion of oxygen does not occur in packs, tests of the ability of *C.botulinum* to multiply and form toxin in foods should be made under strictly anaerobic conditions, to show the maximum risk of growth of these bacteria in the foods and to provide the “worst case” situation.

Emphasis is placed generally on the use by the food industry of the Hazard Analysis and Critical Control Point (HACCP) approach to identify and analyse microbiological hazards associated with food production and to design in appropriate control measures (Jouve 2000). This approach includes consideration of hazards due to pathogenic microorganisms, in particular *C.botulinum*.

The present report points to the need for control measures to be designed into the foods considered in a deliberate way; evidence of analysis of the risk due to *C.botulinum* and of the design and implementation of adequate control measures should be provided in the HACCP plan drawn up by the food producer.

The fact that an outbreak of botulism can be due to a food not previously associated with, or implicated in, the disease is illustrated well by the outbreak associated with hazelnut yoghurt in 1989 (O'Mahony et al. 1990). This outbreak was caused by the failure to apply a sufficient thermal process in the production of canned hazelnut puree, a low-acid canned food, and presumably an inadequate HACCP analysis by the company involved.

A thoroughly researched and understood HACCP approach by food companies is needed in order to prevent incidents of this kind.

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Table 1. Properties of bacteria that form botulinum toxin (Lund and Peck, 2000b)

| | <i>C.botulinum</i> group | | | | <i>C. butyricum</i> | <i>C. baratii</i> |
|-------------------------------------|--------------------------|--------------------------|----------------|---------------------------------|--------------------------------|------------------------------|
| | I | II | III | IV | | |
| Toxins formed | A, B, F | B, E, F | C, D | G (<i>C. argentinense</i>) | (E) ^a | (F) ^a |
| Proteolysis ^b | + | - | - | + | - | - |
| Liquefaction of gelatin | + | + | + | + | - | - |
| Lipase | + | + | + | - | - | - |
| Optimum growth temperature (°C) | 30- 40 | 25 - 37 | 40 | 37 | 30 - 37 | 30 - 45 |
| Minimum temperature for growth (°C) | 10-12 | 3 | 15 | NR | ~10 | NR |
| Spore heat-resistance | high | low | moderate | moderate | NR | NR |
| Similar non-toxic organisms | <i>C. sporogenes</i> | no species name assigned | <i>C.novyi</i> | <i>C. subterminale</i> | typical <i>C. butyricum</i> | typical <i>C. baratii</i> |

^a, strains that form botulinum toxin have been reported very rarely.

^b, proteolysis denotes the ability to degrade native proteins such as coagulated egg yolk, cooked meat particles and casein; Groups I - IV of *C.botulinum* can degrade the derived protein gelatin.

NR, not reported.

Table 2. Reported incidence of *Clostridium botulinum* in soil samples^a

| Location | No. of samples | % of positive samples | Toxin type, % | | | | Ref. ^b |
|--------------|----------------|-----------------------|---------------|-----------------|-----|----|-------------------|
| | | | A | B | C/D | E | |
| USA | 260 | 24 | 42 | 35 | 13 | 10 | 1 |
| Argentina | 722 | 34 | 67 | 20 | 0 | 0 | 2 |
| Netherlands | 135 | 29 | 0 | 24 | 2 | 74 | 3 |
| Italy | 144 | 0.7 | 0 | 100 | 0 | 0 | 4 |
| Italy (Rome) | 520 | 1.3 | 86 | 14 ^c | 0 | 0 | 5 |
| Britain | 174 | 5.7 | 0 | 100 | 0 | 0 | 6 |
| Denmark | 215 | 13.5 | 0 | 93 | 7 | 0 | 7 |
| former USSR | 4242 | 10.5 | 8 | 28 | 2 | 62 | 8 |

^a Modified from Notermans 1993

^b Refs. 1, Smith 1978; 2, Ciccarelli and Gimenez, 1981; 3, Haagsma, 1973; 4 Barbuti et al. 1980; 5, Creti et al. 1990; 6, Smith and Young, 1980; 7 Huss, 1980; 8 Kravchenko and Shishulina, 1967.

^c Type B Group I

Table 3 Reports of botulism associated with home-canned beetroot in the US

| Year | Toxin type | Location | Cases | Deaths | Ref. |
|------|-----------------|------------|-------|--------|------|
| 1952 | A | Oregon | 2 | 2 | 1 |
| 1953 | NK ^a | Colorado | 3 | 3 | 1 |
| 1954 | NK | Nevada | 3 | 3 | 1 |
| 1954 | A | Oregon | 1 | 0 | 1 |
| 1959 | NK | Idaho | 6 | 3 | 1 |
| 1959 | NK | Michigan | 4 | 0 | 1 |
| 1960 | NK | Kentucky | 2 | 0 | 1 |
| 1960 | NK | Michigan | 3 | 0 | 1 |
| 1966 | A | Indiana | 2 | 0 | 1 |
| 1974 | B | California | 1 | 0 | 1 |
| 1974 | A | Nevada | 4 | 0 | 1 |
| 1975 | A | Montana | 1 | 0 | 1 |
| 1993 | A | Washington | 3 | 0 | 2 |

Refs. 1, Center for Disease Control, 1979. 2. Friedman and Hatheway, 1996.

^a, not known

Table 4. Properties of samples of vacuum-packed beetroot

| Sample number | 18 | 19 | 21 | 22 |
|-----------------------|-------------------|----------------------|-----------------|------------------|
| Description | Cooked beetroot | Pure cooked beetroot | Cooked beetroot | Organic beetroot |
| Purchase date | 02-March-00 | 02-March-00 | 02-March00 | 03-March-00 |
| Use-by date | 04-June-00 | 13-June-00 | 18-August-00 | 15-June-00 |
| Storage conditions | Keep refrigerated | Longlife | Longlife | Keep cool |
| Measurement date | 08-March-00 | 08-March-00 | 08-March-00 | 08-March-00 |
| Measured pH (mean) | 5.22 | 5.25 | 5.26 | 5.33 |
| Measured a_w (Mean) | 0.984 | 0.987 | 0.982 | 0.988 |
| Additional comments | Vacuum packed | Vacuum packed | Vacuum packed | Vacuum packed |

Ingredients of foods sampled: no information on the labels

Table 5. The incidence of *C.botulinum* spores and toxin in commercial samples of mascarpone cheese (Franciosa et al. 1999)

| | Production plant A ^a | Other production plants |
|--|---------------------------------|-------------------------|
| Total samples | 878 | 139 |
| Samples positive for spores of <i>C.botulinum</i> type A | 325 ^b (37%) | 4 ^b (3%) |
| Samples positive for spores of <i>C.botulinum</i> type B (proteolytic) | 2 ^b | 0 |
| Samples containing pre-formed type A toxin | 7 | 0 |
| Samples containing pre-formed type B toxin | 0 | 0 |

^a Production plant that produced the mascarpone cheese implicated in the outbreak of botulism in Italy in 1996.

^b Numbers of *C.botulinum* spores were < 10/g, except in samples that contained *C.botulinum* toxin.

Table 6. Reported outbreaks of botulism associated with dairy products. (Modified from Collins-Thompson and Wood 1993)

| Year | Toxin type | Product | Location | Cases | Deaths | Ref |
|------|------------|--|-------------------|-------|--------|-----|
| 1912 | - | cottage cheese | California | 7 | 2 | 1 |
| 1914 | - | Neufchatel cheese | California | 2 | 2 | 1 |
| 1914 | B | cottage cheese | New York | 3 | 3 | 1 |
| 1931 | - | milk | California | 1 | 0 | 1 |
| 1935 | - | curd cheese | California | 3 | 0 | 1 |
| 1939 | A | cottage cheese | New York | 3 | 0 | 1 |
| 1951 | B | cheese spread, Liederkranz | California | 1 | 1 | 1 |
| 1973 | B | Brie | France | 22 | - | 2 |
| 1973 | B | Brie | Switzerland | 43 | - | 2 |
| 1974 | A | cheese spread | Argentina | 6 | 3 | 3 |
| 1978 | B | soft cheese | France | - | - | 4 |
| 1989 | B | hazelnut yoghurt | England and Wales | 27 | 1 | 5 |
| 1996 | A | mascarpone | Italy | 8 | 1 | 6 |
| 1997 | A | locally made cheese preserved with oil | Iran | 27 | 1 | 7 |

Refs. 1. Meyer and Eddie, 1965. 2. Sebald et al. 1974. 3. De Lagarde, 1974. 4. Billon et al. 1980. 5. O'Mahony et al. 1990. 6. Aureli, Franciosa and Poushaban, 1996. 7. Poushafie et al. 1998.

Table 7. Properties of samples of Paneer.

| | | |
|-----------------------|--------------------------------------|--------------------------------------|
| Sample number | 15 | 16 |
| Description | Paneer | Paneer |
| Purchase date | 02-March-00 | 02-March-00 |
| Use-by-date | 20-April-00 | 03-March-00 |
| Storage conditions | Keep refrigerated | Keep refrigerated |
| Measurement date | 08-March-00 | 08-March-00 |
| Measured pH (mean) | 5.69 | 5.64 |
| Measured a_w (mean) | 0.985 | 0.990 |
| Additional comments | Protective atmosphere Acetic acid | Protective atmosphere Acetic acid |

Ingredients of foods sampled: Full cream milk; Acetic acid.

Table 8. Summary of growth and toxin formation in bakery products inoculated with *C.botulinum* type A and proteolytic type B, 5×10^4 /g, after baking, packed and stored at 25°C for 42 days (Modified

from Daifas et al. 1999a)

| | Packaging under: | | |
|--|------------------|-----------------------------------|---|
| | Air | Air + O ₂ absorbent | CO ₂ /N ₂ (60:40) |
| Crumpets, a _w 0.990, pH 6.00 | + ^a | + ^a | + ^a |
| Pizza crust, a _w 0.960 pH 5.62 | + ^a | + ^a | + ^b |
| Bagels, a _w 0.944 pH 5.63 | - ^b | - ^b | - ^b |

^a, sensorily unacceptable; ^b, sensorily marginally acceptable

Table 9. Properties of part-baked bread stored at ambient temperature.

| Sample number | 1 | 2 | 8 | 9 | 12 | - |
|--------------------------------|-------------------------------------|-------------------------------------|---------------------------------|----------------------------|---------------------------------|--|
| Description | Part baked French stick | Part baked Baguettes | 2 Baguettes | Ready to bake Baguettes | 2 half Baguettes | Ready to bake Garlic Baguette ^a |
| Purchase date | 02-March-00 | 02-March-00 | 02-March-00 | 03-March-00 | 02-March-00 | 22-March-00 |
| Use by date | 16-May-00 | 10-May-00 | 07-April-00 | 04-March-00 | 08-April-00 | 24-March-00 |
| Storage conditions | Room temperature, can freeze | Room temperature | Cool dry place, can freeze | Cool dry place, can freeze | Cool dry place, can freeze | Not specified |
| Measurement date | 07-March-00 | 07-March-00 | 09-March-00 | 08-March-00 | 09-March-00 | |
| Measured pH (Mean) | 5.86, 5.86, 5.89 5.87 | 5.62, 5.65, 5.63 5.63 | 5.31, 5.33, 5.33 5.32 | 5.67 | 5.89, 5.95, 6.02 5.95 | |
| Measured a _w (Mean) | 0.952, 0.950, 0.952 0.951 | 0.940, 0.940, 0.934 0.938 | 0.953 | 0.948 | 0.946 | |
| Additional comments | Protective atmosphere | Protective atmosphere | Protective atmosphere | Protective atmosphere | Protective atmosphere | Sealed, possibly in waxed paper |

^a, A large white crusty baguette filled with garlic and herb butter (300g)

Table 9 (contd) Ingredients of samples of part-baked bread stored at ambient temperature (where packaging was retained)

| Sample 1 | Sample 2 | Sample 8 | Sample 12 | Garlic Baguette |
|-------------|-------------|-------------|-------------|-----------------|
| Wheat flour | Wheat flour | Wheat flour | Wheat flour | Wheat flour |
| Water | Water | Water | Water | Butter |
| | | | | |

| | | | | |
|------------------------------|------------------------------|----------------------------|--|------------------------------|
| Yeast | Yeast | Yeast | Yeast | Water |
| Salt | Salt | Salt | Salt | Garlic puree |
| Emulsifier (E472(e)) | Emulsifier (E472(e)) | May contain traces of nuts | Emulsifiers (Mono- and Di-Glycerides of Fatty Acids, Mono- and Di-Acetyltartaric Esters of Mono- and Di-Glycerides of Fatty Acids) | Yeast |
| Dried Autolysed Yeast | Dried Autolysed Yeast | | Antioxidant (Ascorbic Acid) | Salt |
| Flavouring | Flavouring | | | Sugar |
| Flour treatment Agent (E300) | Flour Treatment Agent (E300) | | | Soya flour |
| Yeast extract | Yeast Extract | | | Emulsifier (E472(e)) |
| | | | | Parsley |
| | | | | Concentrated lemon juice |
| | | | | Dextrose |
| | | | | Vegetable oil |
| | | | | Flour Treatment Agent (E300) |

Table 10. The effect of water activity, adjusted with NaCl or glycerol, and pH, adjusted with HCl, on growth of *C.botulinum* type A (ZK3), proteolytic type B (ATCC 438) and E (Beluga) from spore inocula at 20°C. (Modified from Baird-Parker and Freame 1967)

| water activity | pH | | | | | | | | |
|----------------|-----|---|---|-----|----|----|-----|---|---|
| | 6.0 | | | 5.5 | | | 5.3 | | |
| | A | B | E | A | B | E | A | B | E |
| 0.98, NaCl | + | + | + | - | + | - | - | + | - |
| 0.98, glycerol | + | + | + | nt | nt | nt | + | + | + |
| 0.97, NaCl | - | + | - | - | - | - | - | - | - |
| 0.97, glycerol | nt | + | + | nt | nt | nt | + | + | - |
| 0.96, NaCl | - | - | - | - | - | - | - | - | - |
| 0.96, glycerol | + | + | + | + | + | nt | - | ± | - |
| 0.95, NaCl | - | - | - | - | - | - | - | - | - |
| 0.95, glycerol | + | + | ± | - | ± | nt | - | - | - |
| 0.94, NaCl | - | - | - | - | - | nt | - | - | - |
| 0.94, glycerol | - | + | - | - | - | nt | - | - | - |
| 0.93, glycerol | - | - | - | - | - | nt | - | - | - |

nt, not tested.

Table 11. Properties of part-baked bread stored at refrigerated temperature

| Sample number | 7 | 13 | 14 | 24 |
|-----------------------------------|-------------------|---|-------------------------------------|--------------------------------------|
| Description | Garlic Baguette | Garlic Bread | Italian style garlic Ciabatta | Fresh dough Chocolate Croissants |
| Purchase date | 02-March-00 | 03-March-00 | 02-March-00 | 07-March-00 |
| Use by date | 08-March-00 | 11-March-00 | 08-March-00 | 19-April-00 |
| Storage conditions | Keep refrigerated | Keep refrigerated below 5°C, can freeze | Keep refrigerated, can freeze | Keep refrigerated, less than 5°C |
| Measurement date | 09-March-00 | 07-March-00 | 07-March-00 | 10-March-00 |
| Measured pH (Mean) | 5.87 | 5.66, 5.68, 5.61 5.65 | 5.42, 5.45, 5.47 5.45 | Dough 6.83 Chocolate 5.47 |
| Measured a _w (Mean) | 0.950 | 0.922, 0.946, 0.950 0.94 | 0.925, 0.954, 0.952 0.944 | Dough 0.953 Chocolate 0.269-0.276 |
| Additional comments | Wrapper | "Flow wrap PP5" | Wrapper | - |

Table 11 (contd) Ingredients of part-baked bread stored at refrigerated temperature

| Sample 7 | Sample 13 | Sample 14 | Sample 24 |
|----------|-----------|-----------|-----------|
|----------|-----------|-----------|-----------|

| | | | |
|------------------------------|---|---------------------------------------|--|
| Wheat flour | Wheat flour | Wheat flour | Wheat flour |
| Water | Water | Water | Water |
| Butter (16%) | Garlic butter (19%) ^a | Butter | Vegetable Fat |
| Garlic puree (1%) | Salt | Pomace Olive Oil | Wheat Gluten |
| Yeast | Yeast | Yeast | Raising Agents (Sodium Acid Pyrophosphate E450, Sodium Bicarbonate E500ii) |
| Salt | Emulsifier (Mono- and Di-Acetyltartaric Acid Esters of Mono- and Di- Glycerides of Fatty Acids) | Garlic puree | Dextrose |
| Parsley | Sugar | Salt | Sugar |
| Full fat Soya flour | Flour treatment agent L- Ascorbic acid | Vegetable oil | Flavour |
| Flour treatment agent (E300) | | Malt flour | L-Ascorbic Acid |
| Emulsifier (E472(e)) | | Parsley | 12 Vegetarian Chocolate Pieces |
| | | Flour treatment Agent (Ascorbic Acid) | |

^a, Garlic Butter contains butter, garlic puree and dried parsley

Table 12. Reported incidence of *C.botulinum* in raw vegetables and fruits. (Modified from Lund and Peck, 2000a)

| Country of origin and year of report | Product | No. of samples | % samples positive | Toxin types detected ^a | Ref. |
|--------------------------------------|---------|----------------|--------------------|-----------------------------------|------|
|--------------------------------------|---------|----------------|--------------------|-----------------------------------|------|

| | | | | | |
|---|---|-------|-------------------------|-------------|-------------------------|
| | | | | | |
| US (California), 1922 | Fruit and vegetables | 189 | 19 | A(15);B(21) | Meyer & Dubovsky, 1922a |
| US, 1922 | Fruit and vegetables | 431 | 13 | A;B | Meyer & Dubovsky, 1922b |
| Sweden, 1963 | Potatoes | 40 | 67 | E(27) | Johannsen, 1963 |
| US, 1969 | Chopped spinach | 50 | 12 | A,B | Insalata et al. 1969 |
| US, 1969 | Cut green beans | 50 | 0 | | Insalata et al. 1969 |
| Former USSR, 1972 | Vegetables and herbs | 30 | 43 | A,B | Rosanova et al. 1972 |
| Canada, 1975 | Mushrooms | 12 | 100 (MPN 150-410/kg) | B | Hauschild et al. 1975 |
| Hungary, 1979 | Potatoes | 26 | 0 | | Vegieva & Incze, 1979 |
| Hungary, 1979 | Carrots | 18 | 0 | | " |
| Italy, 1983 | Vegetables | 296 | 4 | B | Quarto, 1983 |
| US, 1986 | Onions | 75 | 6.7 | A | Solomon & Kautter, 1986 |
| Germany, 1987 | Potatoes, peeled, washed. | 72 | 0 | | Baumgart, 1987 |
| US (California), 1988 | Garlic | 115 | 4.4 | A | Solomon et al. 1988 |
| The Netherlands,1989 | Mushrooms | 50 | 0 | | Notermans et al. 1989 |
| US (Washington DC),1990 | Cabbages | 88 | 13.6 | A | Solomon et al. 1990 |
| US, source companies in Florida, New York, California, 1996 | Range of MAP ^b vegetables | 1,118 | 0.36 | A(4) B(1) | Lilly et al. 1996 |

^a Figure in brackets is the number of samples in which the toxin type was detected

^b Modified atmosphere-packed (MAP)

Table 13. Reported outbreaks of botulism associated with vegetables preserved or cooked in oil

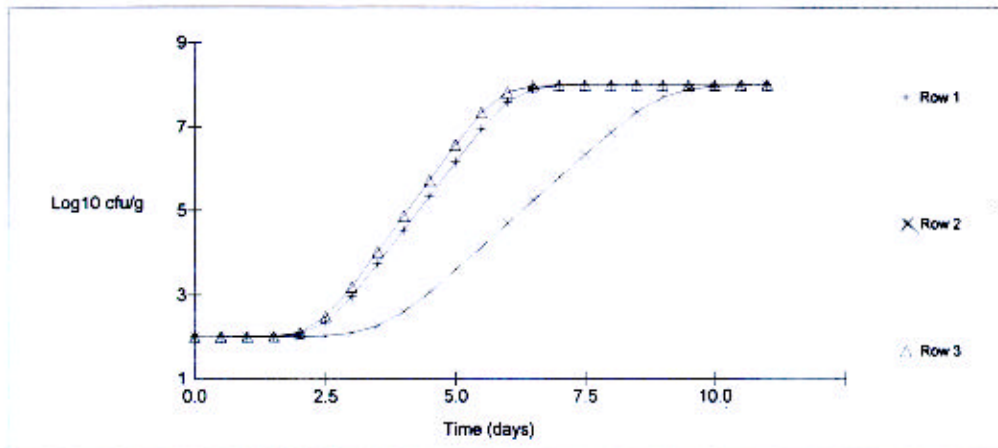
| Year | Toxin type | Product | Location | Cases | Deaths | Ref |
|------|------------|--|----------|-------|--------|------|
| 1982 | B* | home-prepared mushrooms under olive oil | Italy | 2 | | 1 |
| 1983 | A | sauteed onions, covered by a thick layer of margarine | US | 28 | 1 | 2, 3 |
| 1985 | Bp** | home-bottled, chopped garlic in soybean oil | Canada | 36 | 0 | 4 |
| 1989 | A | home-bottled, chopped garlic in olive oil | US | 3 | 0 | 5 |
| 1993 | Bp | commercially produced, sliced, roasted eggplant in oil | Italy | 7 | 0 | 6 |
| 1998 | B | home-preserved mushrooms bottled in oil, imported | UK | 2 | 1 | 7 |

* not reported whether proteolytic or non-proteolytic

** proteolytic

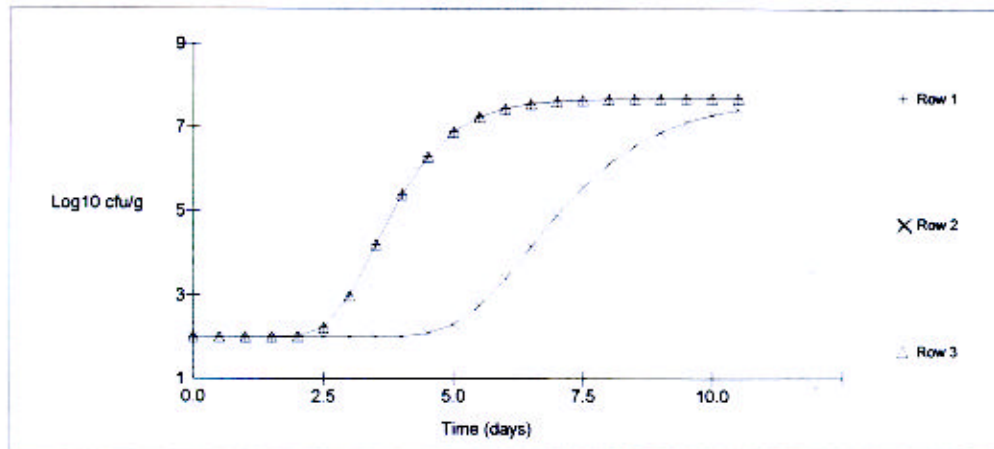
Refs. 1, Dessi et al. 1983; 2, Center for Disease Control, 1984; 3, MacDonald et al. 1985; 4, St. Louis, M.E. et al. (1988) 5. Morse, D.L. et al. 1990. 6. Center for Disease Control 1995; 7. Communicable Disease Surveillance Centre, 1998.

Fig. 1 Possible growth of proteolytic *C.botulinum* in vacuum packed beetroot at 25°C, based on measured pH and water activity and on Food MicroModel



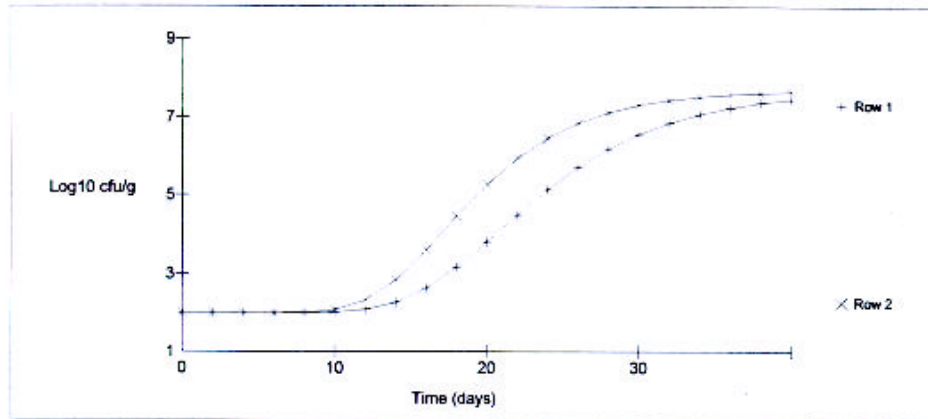
| Growth & Survival Model Name (Initial Count=1E2 cfu/g) | Temp °C | NaCl %w/w aq | aW | pH | Lag days | Double days | Growth Rate /h | days to 1E5 | log cfu/g 15 days |
|--|---------|--------------|---------|-----|----------|-------------|----------------|-------------|-------------------|
| 1 Clostridium botulinum (P) | 25.0 | 1.9 | (0.989) | 5.3 | 2.4 | 0.19 | 0.068 | 4.29 | 8.0 |
| 2 Clostridium botulinum (P) | 25.0 | 2.6 | (0.985) | 5.3 | 3.6 | 0.27 | 0.046 | 6.29 | 8.0 |
| 3 Clostridium botulinum (P) | 25.0 | 1.8 | (0.990) | 5.3 | 2.3 | 0.18 | 0.071 | 4.08 | 8.0 |

Fig. 2 Possible growth of non-proteolytic *C.botulinum* in vacuum packed beetroot at 25°C, based on measured pH and water activity and on Food MicroModel



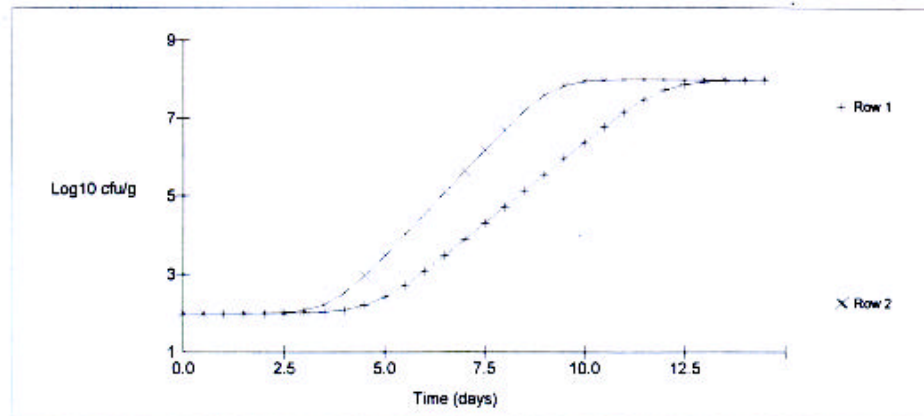
| Growth & Survival Model Name (Initial Count=1E2 cfu/g) | Temp °C | NaCl %w/w eq | aW | pH | Lag days | Double days | Growth Rate /h | days to 1E5 | log cfu/g 15 days |
|--|---------|--------------|---------|-----|----------|-------------|----------------|-------------|-------------------|
| 1 Clostridium botulinum (N-P) | 25.0 | 1.8 | (0.990) | 5.3 | 2.6 | 0.12 | 0.106 | 3.83 | 7.7 |
| 2 Clostridium botulinum (N-P) | 25.0 | 2.6 | (0.985) | 5.3 | 5.1 | 0.20 | 0.064 | 7.07 | 7.7 |
| 3 Clostridium botulinum (N-P) | 25.0 | 1.8 | (0.990) | 5.3 | 2.6 | 0.12 | 0.106 | 3.83 | 7.7 |

Fig. 3 Possible growth of non-proteolytic *C.botulinum* in paneer at 8°C, based on measured pH and water activity and on Food MicroModel



| | Growth & Survival Model Name (Initial Count=1E2 cfu/g) | Temp °C | NaCl %w/w aq | aW | pH | Lag days | Double days | Growth Rate /h | days to 1E5 | log cfu/g 15 days |
|---|--|---------|--------------|---------|-----|----------|-------------|----------------|-------------|-------------------|
| 1 | Clostridium botulinum (N-P) | 8.0 | 2.6 | (0.985) | 5.7 | 14.7 | 0.88 | 0.014 | 23.6 | 2.4 |
| 2 | Clostridium botulinum (N-P) | 8.0 | 1.8 | (0.990) | 5.6 | 12.3 | 0.70 | 0.018 | 19.3 | 3.2 |

Fig. 4 Possible growth of proteolytic *C.botulinum* in paneer at 20°C, based on measured pH and water activity and on Food MicroModel



| | Growth & Survival Model Name (Initial Count=1E2 cfu/g) | Temp °C | NaCl %w/w aq | aW | pH | Lag days | Double days | Growth Rate /h | days to 1E5 | log cfu/g 15 days |
|---|--|---------|--------------|---------|-----|----------|-------------|----------------|-------------|-------------------|
| 1 | Clostridium botulinum (P) | 20.0 | 2.6 | (0.985) | 5.7 | 4.8 | 0.36 | 0.035 | 8.35 | 8.0 |
| 2 | Clostridium botulinum (P) | 20.0 | 1.8 | (0.990) | 5.6 | 3.7 | 0.28 | 0.045 | 6.42 | 8.0 |

An evaluation of Mester vacuum-packed
Potatoes for storage at ambient temperatures

Report to Department of Health and
Social Security, February 1986

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For storage at ambient temperatures

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Contents

1. Summary
2. Background
3. Review of Mester vacuum-packed potatoes
4. References

Summary

Mester vacuum-packed potatoes are packs of peeled potatoes that are reported to be produced by a process that involves a light pasteurization, followed by vacuum packing and a storage period up to 27h at a temperature of 25-35°C, after which the packs are again pasteurized. It is claimed that the packs can then be stored for many months at ambient temperature without deterioration in quality or appearance.

This product is not regarded as satisfactory for the following reasons:-

- 1) Potatoes are liable to be contaminated with spores of Clostridium botulinum, the most dangerous of the food-poisoning bacteria.
- 2) It has been shown that C. botulinum spores are capable of germination and growth resulting in toxin formation in vacuum packs of potatoes.
- 3) No data have been supplied that show whether, and to what extent, the process used to produce Mester vacuum-packed potatoes reduces the risk of growth and toxin formation of C. botulinum in the product.

The production of Mester vacuum-packed potatoes has not been shown to provide the standard of safeguards that are used in the production of comparable foods, i.e. commercially sterile, low-acid canned foods and similar foods in hermetically sealed containers, in the U.K. These foods include canned potatoes. Codes of practice agreed internationally and also produced in the UK require that the heat process applied to such products should reduce the risk of survival and growth of C. botulinum in the product by a factor of 10^{12} . The process described for the Mester vacuum-packed potatoes has not been demonstrated to reduce the risk of growth of C. botulinum by a factor of this order and, moreover, we do not know of a way in which the process could be validated and controlled to assure adequate safety.

The same considerations would also apply to any similar packs of other vegetables such as carrots, turnips and asparagus.

Background

The major concern regarding this product is in relation to the risk of growth of, and toxin formation by, Clostridium botulinum in packs.

C. botulinum is the most dangerous of the food-poisoning organisms. It produces a powerful toxin, and ingestion on 0.1ml (or 0.1g) or more of poisoned food (which could contain 1 µg of neurotoxin) might be expected to cause botulism in man. (Lamanna and Carr, 1967). The bacterium forms spores that occur in soil and on raw food. Botulism in man is very rare in the U.K. Since 1955 there have been only 2 outbreaks involving 6 cases, both resulted from imported foods. The fact that food-borne botulism is so rare in this country is probably because (1) high standards of processing and control are used by food industry (2) home canning of all foods except acid fruits is actively discouraged (3) we do not consume foods such as uncooked fish. In France foodborne botulism occurs more frequently; between 1979 and 1981 35 outbreaks were reported involving 77 people (Anon 1983) (these outbreaks were not attributable to vacuum-packed potatoes).

Spores of C. botulinum are likely to be present on raw potatoes; in Sweden they were found on peel of 68% of 40 samples of stored, unprocessed potatoes (Johannsen 1963). C. botulinum was isolated from each of 3 samples of unpeeled potatoes from an American supermarket (Sugiyama et al. 1981) and in the Netherlands each of seven samples of soil from potato fields contained C. Botulinum (1-5 spores per 10g soil) (Notermans et al. 1985).

Growth of C. botulinum on potato can lead to human botulism; potato salad served in restaurants was implicated in two separate outbreaks of botulism in the USA in 1978, and potato salad was one of the possible foods responsible for a third outbreak in 1978 involving 32 reported cases (Sugiyama 1982; Centers for Disease Control 1978). Subsequent experiments suggested that the toxin had been formed during storage of baked potatoes prior to preparation of the salad. In these experiments potatoes were inoculated with C. botulinum spores, wrapped in aluminium foil, baked at 204°C for 50 min. then stored at 30°C or 22°C; toxin was formed within 3-7 days at 22°C (Sugiyama 1982).

C. botulinum can grow in vacuum-packed, cooked potatoes (Notermans et al. 1981). In these experiments spores of C. botulinum were inoculated into packs of peeled potatoes that were then evacuated sealed, heated in a water bath at 95°C for 40 min, cooled and stored at temperatures between 4°C and 20°C. Spores survived this heat treatment and toxin was formed in packs stored at 20°C, 15°C and 10°C within 4, 8 and 11 days, respectively, but not at 4°C in 60 days.

Vacuum-packed, cooked or part-cooked potatoes are an example of a low-acid food (ie. A food with a pH of 4.5 or above) that has been subjected to a heat-

treatment capable of eliminating most vegetative bacteria, and that if stored at ambient temperature will support growth of C. botulinum. In these circumstances vacuum-packed potatoes are in the category of commercially sterile foods in hermetically sealed containers and should receive a processing that ensures the same reduction in risk of growth of C. botulinum as is provided in the canning of potatoes.

In the U.K. manufacturers of canned foods and of other commercially sterile foods in hermetically sealed containers adhere to the code of practice on the canning of low-acid foods issued by the DHSS (Department of Health and Social Security 1980-1983) and to the International Code of Practice (FAO/WHO 1979). According to the codes of practice, these low-acid foods (including canned potatoes) must receive a thermal process at least sufficient to kill 10^{12} spores of C. botulinum (i.e. to reduce the risk of growth of C. botulinum spores by a factor of 10^{12}), a process at least equivalent to heating at a temperature of 121°C in the centre of the product for 3 min. Moreover, the processing should be continuously monitored, and records retained to ensure that the scheduled process is properly applied. In the USA the Food and Drug Administration has promulgated regulations that require the above processing for low-acid canned foods and include further measures to assure the safety of such foods (National Research Council Committee of Food Protection 1975).

The processing applied in production of vacuum-packed potatoes should provide the same reduction in risk of growth of C. botulinum as is provided by the canning process, but no evidence of such a reduction in risk has been provided in relation to the Mester process.

Review of Mester vacuum-packed potatoes

According to a relevant patent (GB 2098 850 A 1982) the peeled potatoes undergo the following process:-

- 1) A light pasteurization at a temperature between 75°C and 85°C for between 5 and 15 min.
- 2) Vacuum packaging in bags of plastics material.
- 3) Storage at a temperature between 25°C and 35°C for between 24h and 14h.
- 4) A pasteurization at a temperature between 85°C and 90°C for time that depends on the temperature to which the potatoes were raised during the storage period, and on the thickness of the layer of potatoes contained in the bags. For a bag with a thickness of 40mm. containing 500g of potatoes the duration of pasteurization would be between 22 and 35 min.
- 5) Cooling.

According to a letter from Professor Beerens to Dr. Charles dated 4th January 1985, the processing includes the following treatments:-

1. Potatoes are passed through water at 90°C for 5-10 min.
2. They are transported in sterile air to a site where they are packed in vacuum-sealed trays.
3. Packed potatoes are stored at 25°C for 27h.
4. Packs are pasteurized at 90°C for 37 min.
5. Packs are stored at room temperature.

Mester claim that the first heat treatment would ensure that all spores of C. botulinum that may be present would germinate and become heat-sensitive during storage of the potatoes at 25°C - 35°C, and that after germination the bacteria would be killed by the second heat process. In relation to this claim, the following points should be recognized.

1. The second heat process applied is recognised to be only a pasteurization and would not be lethal to C. botulinum spores. From known scientific data it can be calculated that in order to provide the same safety factor that is provided by a canning process, potatoes would have to be heated for about 40h at 91°C (the approximate temperature used by Mester).

2. The process relies on germination of spores within 27h after the first pasteurization. No evidence is provided of the proportion of spores that can be relied upon to germinate rapidly in these conditions. Within a sample of spores, the individuals do not necessarily germinate simultaneously and there is evidence that germination times show an approximately log normal distribution (Gould, 1970). Some spores can remain dormant (i.e. fail to germinate in favorable conditions) for long, and very variable periods of time. Dickson et al. (1925) heated spores of C. botulinum in sealed glass tubes of medium and found that occasional tubes only showed growth after incubation for 72 months (Dickson 1927), i.e. spores remained dormant for this time. Moreover, conditions on the potato surface may well be less than optimal for rapid germination and therefore reduce its rate.

In addition, in the process the potatoes are subject to possible re-contamination after the first heat treatment when they are transported to another site before vacuum-packaging. Although the transport is said to be "in sterile air", no evidence has been provided of the degree of safety against re-contamination during transfer on equipment.

No evidence is provided of the degree to which germination of C. botulinum spores can be assured, or of the degree to which re-contamination can be prevented prior to the second heat treatment. If C. botulinum spores are present that do not germinate prior to the second heat treatment, there is a very high probability that they will survive that heat treatment; there is, in fact, a risk that this second heat treatment will help to activate germination. When the packs of potatoes are then stored at ambient temperature for several weeks there is a risk of germination, growth and toxin formation.

In a letter, dated 4th January 1985, to Dr. R.H.G. Charles, Professor Beerens stated that after incubation at 25°C for 24h the pH of the potato tissue was lower than 5.5 and cited work by Montville (1984) reporting that the chance of germination and growth of C. botulinum spores on a culture medium at pH 5.5 and 30°C in 7 days was only 1 in 20,000. The pH of raw potato is usually between 5.6 and 6.2 (Burton, 1966) and the pH of vacuum-packed, cooked potatoes was reported to be 5.9-6.0 (Notermans et al. 1985). If the pH of the potato tissue was 5.5 this could cause a delay in germination compared with a rate at pH 7, but could not be relied upon to prevent germination and growth. In contrast to the results of Montville (1984), in work by Graham, Lund & Wyatt (1986) the probability of germination and growth of spores of a strain of C. botulinum in a culture medium at pH 5.5 and 30°C in 14 days was between 1 in 9 and 1 in 19 while growth of vegetative C. botulinum was not significantly inhibited at this pH.

In a letter dated 6th August 1984, from Mr. P. Montaner, French Agricultural Adviser, to Dr. R.H.G. Charles, DHSS, it was claimed that the presence of C.

botulinum spores in the vacuum packs would, in a few days, produce distension of the bags and therefore ensure that the product was not consumed. In fact it has often been found that C. botulinum can form toxin in a food before obvious spoilage has occurred; for example, Notermans et al. (1981, 1985) reported that C. botulinum formed toxin in vacuum-packed potatoes stored at 10°C, 15°C or 20°C before significant gas production or sensory deterioration.

In his letter of 4th January 1985, Professor Beerens stated “Moreover these potatoes cannot be eaten as they are, they must be cooked. Cooking would destroy the botulinum toxin”. Although the potatoes would normally be heat-treated prior to consumption, this would not necessarily always be the case despite any instructions that might be given on the package; for example the appearance of the potatoes might suggest that they could be used without heating to make a potato salad. Moreover, it is not regarded as an adequate assurance of safety to market a product in which there (?) a significant risk that toxin has been formed, on the assumption that cooking the product will destroy the toxin.

Tests for the presence of C. botulinum in the packs produced by Mester are inadequate to demonstrate the safety of the process. The risk of growth and toxin formation by C. botulinum in packs stored at ambient temperature must be reduced to a very low level. In order to achieve this, the incidence of remaining viable spores of C. botulinum in packs may have to be of the order of less than one viable spore in many millions of packs. Clearly it is not possible, therefore, to assess the safety of this product by testing for C. botulinum in the final packs. The assurance of safety depends on the use of a process that provides an adequate degree of lethality and/or inhibition.

In these circumstances the safety of the Mester product cannot be assured if it is stored at ambient temperatures.

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