Report: Establishment of FSO for *Staphylococcus aureus* in pre-cooked frozen shrimps

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Contents:

1. Introduction

Dr. Natalia Gomez Tome visited the Division of Biomedical Food Research at the National Institute of Health Science (Ministry of Health, Labour and Welfare) in Tokyo, Japan, for 3 weeks in November 2009. The visit was funded by the Japan Food Hygiene Association and the host researcher was Dr Fumiko Kasuga, chief of section Food Microbiology. The main purpose of the visit was to incorporate a microbiological risk assessment in the development of food safety objective (FSO) for *S. aureus* in pre-cooked frozen shrimps. Due to the lack of time, it was not possible to do a quantitative risk assessment, but the risk was assessed qualitatively and the assessment was used to suggest processes to establish an FSO. For this study we initially considered the hazards associated with frozen shrimps, but further analysis showed that those hazards only represent a minimal risk for the public health. On the other hand, we found that *S. aureus* has been responsible for a considerable number of outbreaks linked to cooked shrimp and it is described as a significant hazard in cooked crustaceans by the ICMSF (ICMSF 6).

2. Hazard identification - *S. aureus* and frozen shrimps.

S. aureus is responsible for one of the most common types of food poisoning, staphylococcal food poisoning, which is an intoxication caused by the ingestion of foods containing the enterotoxins that are produced by certain strains of *S. aureus*. Following toxin ingestion the onset of symptoms is rapid (from 30min to 8h) but spontaneous remission is usually observed within 24h. The appearance of the symptoms of staphylococcal food poisoning depends on the quantity, type and toxicity of the toxin and the symptoms include abdominal cramps, nausea, vomiting, sometimes followed by diarrhoea (never diarrhoea alone), fever and dehydration. There is a high morbidity rate, but a low mortality rate. The severity of the symptoms and the frequency of occurrence means that it is more costly in terms of medical expenses and loss of working days than all the other food-associated pathogens combined (Halpin-Dohnalek and Marth, 1989; Jablonski and Bohach 1997).

S. aureus is a cause of food borne disease in most countries (Bergdoll, 1989; Zahoor and Bhatia, 2007) because the bacterial agents are ubiquitous and they can easily contaminate food products during preparation and processing. Some strains are able to produce staphylococcal enterotoxins (SEs), a group of low molecular weight proteins which are the causative agents of staphylococcal food poisoning, in conditions that can be found during food manufacture, storage and preparation. When foods containing enterotoxigenic *S. aureus* are stored for long periods at temperatures which permit bacterial growth the

enterotoxin may be produced and this constitutes a hazard for human health. As a guideline the US Food and Drug Administration (2000) indicated that ~10^5 CFU/g *S. aureus* is required to produce sufficient enterotoxins to cause illness in humans. Whether or not *S. aureus* can grow and/or produce detectable amounts of enterotoxins in a given food product depends on the nature of the food product (composition, processing etc.) and on the influence of the environmental conditions during various phases of handling, processing, packaging and storage. Contamination of food with *S. aureus* can be avoided by moderate heat treatment but pre-formed enterotoxins are highly heat resistant (Le Loir et al., 2003) and therefore always represent a hazard.

The number of cases of staphylococcal food poisoning in Japan during the period 1994-1998 comprised 3.1-11.9% of the total cases of bacterial food poisoning (Miwa et al., 2001) and seafood was involved in several reported outbreaks during the period of 1999-2001. In particular, molluscan and shellfish were causing disease being responsible for two third of the diseases caused by seafood (ICMSF 6). *S. aureus* is one of the pathogenic microorganisms reported by the ICMSF as being associated with seafood. Its present in seafood is the result of contamination/pollution from the human/animal reservoir (ICMSF 6). Only a few food-poisoning outbreaks have been ascribed to staphylococcal enterotoxins in cooked peeled shrimp but *S. aureus* can be expected in foods that have been exposed to or handled by food workers. Along the production of pre-cooked frozen shrimps there are several operations that can be carried by hand, therefore there is a potential risk associated with these products. Large numbers of the microorganism usually result from growth and it is frequent that occurs in foods that have been heat processed to eliminate competing microorganisms, as in the pre-cooked frozen shrimps after the cooking process, or in those ones where the normal microflora is very low.

3. Hazard characterization

Staphylococcus aureus

Staphylococcus aureus is a facultative anaerobic Gram-positive coccus (spherical bacterium); it is non-motile and catalase and coagulase positive. Cells are spherical single or paired cocci, or form grape-like clusters (*staphylo* means grape in Greek). *S. aureus* is a ubiquitous organism whose major habitats include the skin and mucous membranes of the nose and oropharynx of warm blooded animals (Schleifer 1986). In healthy individuals it can exist in a carrier state (up to 30-50% of the human population are carriers) but it is also the cause of a variety of diseases in humans and animals.

Growth of *S. aureus*

S. aureus is able to grow in a wide range of temperatures, pH and sodium chloride concentrations (Temperature range is 7 to 48.5°C with an optimum temperature of 30 to 37°C (Schmitt et al., 1990), pH range is 4.2 to 9.3 with an optimum of 7 to7.5 (Bergdoll, 1989) and sodium chloride concentrations up to 15% NaCl support growth). These characteristics enable *S. aureus* to grow in a wide variety of foods but, on the other hand, *S. aureus* is quite sensitive to microbial competition. Foods where most of the normal flora has previously been destroyed, like the cooked shrimps, present a good substrate for the growth of *S. aureus*.

Toxins types for *S. aureus*

The SEs are a group of short proteins secreted in the medium and soluble in water and saline solutions. They are highly stable, resist most proteolitic enzymes such as pepsin or trypsin (enzymes secreted along digestion process), and thus keep their activity in the digestive tract after ingestion. Five classical enterotoxin types, i.e. SEA, SEB, SEC, SED and SEE (Betley and Mekalanos 1985; Jones and Khan 1986; Couch et al. 1988; Bayles and Iandolo 1989, Dinges et al., 2000), and many new types of SEs or superantigens (Sags), i.e. SEG through SEU, have been reported (Ren et al. 1994; Su and Wong 1995; Munson et al. 1998; Zhang et al. 1998; Fitzgerald et al., 2001; Jarraud et al., 2001; Novick et al., 2001; Letertre et al., 2003; Orwin et al., 2003). SEA is the enterotoxin most commonly produced by *S. aureus* from human sources (Casman el at., 1967) and the strains from animal sources are predominantly SEC and SED (Olson et al., 1970; Wieneke, 1974; Harvey and Gilmour, 1985; Stephan et al. 2001).

Enterotoxigenic strains of *S. aureus*

Some strains of *S. aureus* are enterotoxigenic. After significant population growth enterotoxigenic strains of *S. aureus* produce toxins that cause human illness. Although *S. aureus* can grow in foods within a broad range of environmental conditions, production of enterotoxins occurs within a much narrower range, i.e. temperatures from 10 to 40-45°C. In situations that permit growth of *S. aureus* oxygen tension and associative growth of other microorganisms affect enterotoxin production more adversely than other factors such as temperature, pH and water activity.

Generally, a high percentage of isolates from healthy humans are enterotoxin producers. Casman et al. reported incidence values of 47% for clinical and 31% for non-clinical strains in 1967. In a more recent study, Al Bustan et al. (1996) observed that 86.6 % of 133 strains isolated from nasal swabs of restaurant workers in Kuwait City were enterotoxigenic.
Among the strains isolated from food the percentage of enterotoxigenic strains is estimated to be around 25% (Bergdoll, 1989). Nevertheless estimations vary considerably from one food to another and from one report to another. Types A and D are the most common SEs involved in food poisoning and these SEs are formed during the exponential growth of the bacteria whereas the other types are predominately formed when the bacteria enter the stationary phase (ICMSF 5). In most food borne outbreaks a single enterotoxigenic strain is isolated; however a number of these strains produce more than one enterotoxin (Bryant et al., 1988; Carmo and Bergdoll, 1990).

Heat sensitivity for *S. aureus* and *S. aureus* enterotoxins

S. aureus toxins are highly heat resistant and they are thought to be more heat resistant in foodstuffs than in a laboratory culture medium (Bergdoll, 1983). Staphylococcal enterotoxins have z values ranging from 25 to 33°C and D-values from 8.3 to 34 min at 121°C. Therefore SEs are not normally inactivated during food processing, storage, distribution or during the preparation of the food at home but they can be inactivated by heat treatment used in the sterilization of canned foods when they are present at low concentrations (Bergdoll, 1983). The impact of heat treatment on SE activity depends on SE type, SE concentration and on the food matrix (Le Loir et al., 2003).

Dose-response relationship for *S. aureus* enterotoxins

The amount of enterotoxin necessary to cause intoxication is very small (Balaban and Rasooly, 2000) but there is not complete agreement about the dose-response relationship. Evenson et al. (1988) estimated that the infective dose required to induce staphylococcal food poisoning in humans is around 0.1µg but it may vary with patient sensitivity. Mossel et al. (1995) concluded that an adult has to ingest approximately 10-20µg of SE to show symptoms and Martin et al. (2001) considered that less than 1µg of SE may cause food-poisoning symptoms in sensitive individuals. The smallest dose of SE reported to cause illness in susceptible individuals has been around 0.1µg of toxin per consumption event (Roberts, 1996). In a recent staphylococcal intoxication incident in Japan the total intake of SEA from low-fat milk was estimated as 0.02-0.1µg per person (Asao et al., 2003).

S. aureus in pre-cooked frozen shrimps

In 1985, Beckers et al. conducted a study examining the phage type and enterotoxin production of strains of *S. aureus* isolated from shrimps and the results of that study indicated that *S. aureus* is able to produce enterotoxin in shrimp. From that study the authors concluded that the presence of *S. aureus* on commercially produced shrimp represents a potential hazard to human health.

Pre-cooked frozen shrimps are classified as ready-to-eat products and they can be consumed without further treatment. Therefore it is very important to prevent contamination after the initial heat treatment. If processed properly, the cooking step is sufficient to inactivate *S. aureus*, but it is a potential hazard if re-contamination occurs afterwards (ICMSF 6). Peeling of cooked shrimp can introduce *S. aureus* in the product and if temperature-abused, it will grow well and can produce enterotoxin. If the product is hand peeled, the risk represented by the introduction of *S. aureus* in the product is higher and the level of risk will be determined by the extend of human handling in the production process. In temperature-abused products, significant growth of staphylococci can occur, particularly if the cooked shrimp, where the spoilage microflora has been suppressed as a result of the thermal processing. This has resulted in occasional staphylococcal food poisoning outbreaks (ICMSF 6).

Since peeling of shrimp is mostly carried out by hand, it may be assumed that it will be contaminated by *S. aureus* of human origin (Beckers et al., 1985). However, only a few food-poisoning outbreaks have been ascribed to staphylococcal enterotoxins in cooked peeled shrimp (Gilbert and Wieneke, 1973; Turnbull and Gilbert, 1982).

But in the ICMSF 6 it is reported that a significant percentage of the outbreaks linked to the cooked shrimp have been attributed to long-established food-borne pathogens including *S. aureus*, Salmonella, Shigella and virus.

We considered the 2 main sources of *S. aureus* in the product after processing as a) insufficient thermal processing or b) post-processing contamination, together with inappropriate maintenance of refrigerated of frozen storage.

4. Production pathway

Sixteen steps were identified to describe the pre-cooked frozen shrimp production chain; reception, ice-frozen storage, de-ice/thaw – wash, size/grade, cook, cool, peel, dip (additives), drain, individual quick frozen, glaze, package, weigh, label, frozen storage and ship (fig 1.).

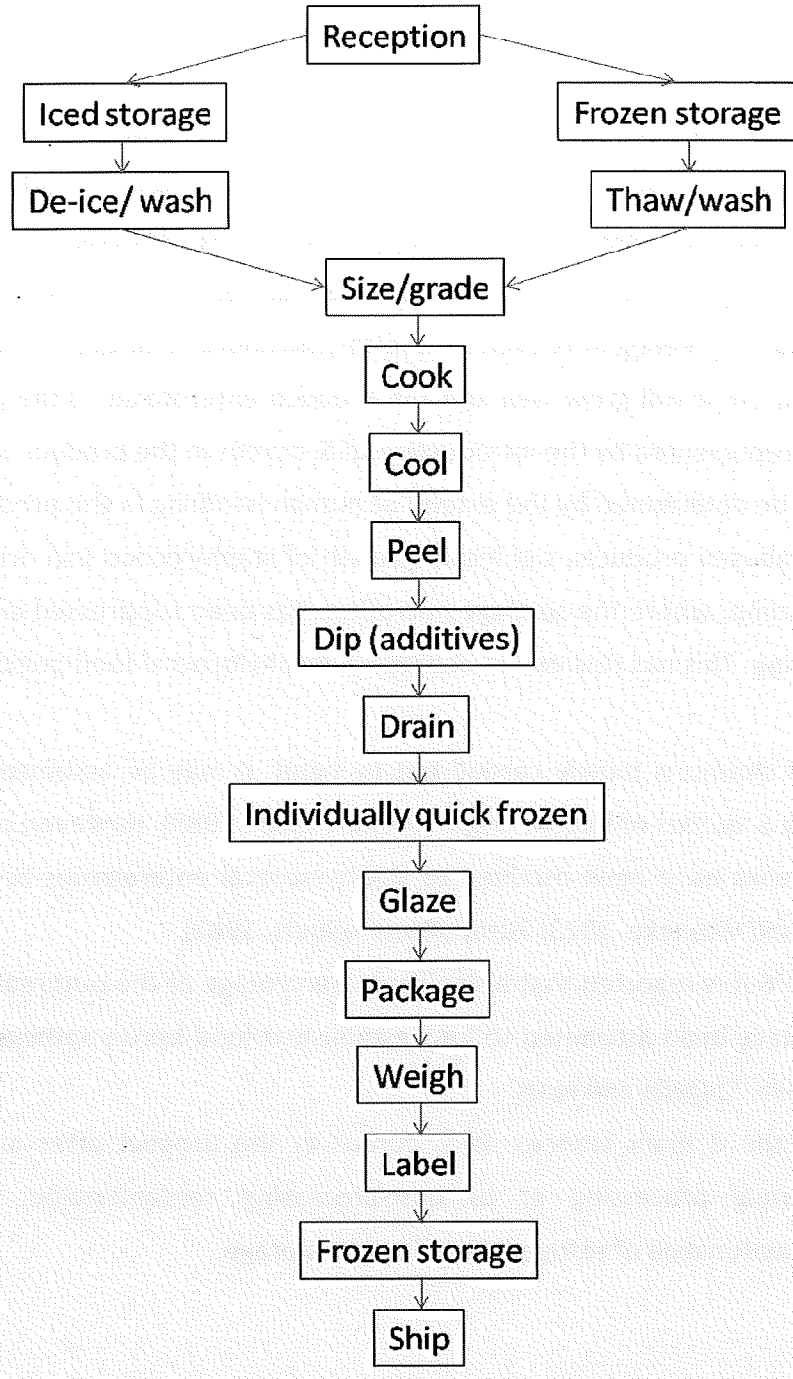

Figure 1. Flow diagram for raw frozen shrimp (from the Code of Practice for fish and fishery products (CA/RCP 52-2003)

1. Reception

Shrimps die immediately upon capture and as a consequence of that the initial bacterial level at the processing plant will be a function of the quality and extent of shipboard storage. Most shrimps have high counts (10^5-10^7 cfu/g) at the time of receipt at the processing plant. They are commonly captured by trawlers and iced or held in refrigerated sea-water from transport to processing plants. In large shrimp fisheries, the animal may be "headed" on board the vessel, the tail is removed from the head, gills, and thorax. This process removes a large external source of bacterial contamination but at the same time it exposes flesh at the broken surfaces and the microbiological benefits derived from this process are considered to be minimal (ICMSF 6).

2. Iced/frozen storage

An increasing proportion of the shrimps supply is produced by aquaculture and these shrimps are either processed directly at the rearing ponds or shipped to distant processing plants. During the transport, they are kept iced or frozen. Storage at chill temperatures (0-5°C) can cause growth of Gram-positive micrococci and lactic acid bacteria.

3. De-ice/thaw – wash

At the processing plants, each shrimp is thawed by enclosing it in a film type bag and immersing in water at room temperature (not greater than 35°C). The complete thawing of the product is determined by gently squeezing the bag occasionally so as not to damage the texture of the shrimp, until no hard core or ice crystal are left (Codex Stan 92-1981).

4. Size/grade

After being washed, shrimps are sorted for size.

5. Cook

Shrimps are cooked whole and the duration of the cooking is normally short to minimize quality lost. Cooking means to boil the shrimp in potable water, clean sea water or brine or heating in steam for a period of time sufficient for the thermal centre of the shrimp to reach a temperature adequate to coagulate the protein. The cooking step will reduce the bacterial counts in shrimps, but cooked shrimps are susceptible for posterior cross-contamination.

6. Cool

Cooked or parboiled shrimp deteriorate rapidly. Therefore shrimp should be cooled down to the temperature of melting ice, as quickly as possible. Any careless treatment or delay in reducing the temperature of shrimp will have a marked detrimental effect on their potential keeping time (Recommended International Code of Practice for Frozen Shrimps or Prawns (CAC/RCP 17-1978 and Supplement November 1989)).

7. Peel

Cooked shrimp products are generally peeled after cooking. Peeling involves the separation of the edible tail meat from the carapace and removal of the lower intestine. Peeling of shrimp is increasingly done by machine, but a large amount of shrimps are still hand peeled, particularly in non-industrialised countries, and often under less than ideal sanitary conditions. Besides most processing plants will have a manual "fine peeling" step following a mechanical peeler. This has been a source of contamination by *S. aureus* and Salmonella and other pathogens.

8. Dip (additives)

9. Drain

10. Individually quick frozen

Each shrimp is frozen separately and apart from other shrimp. Freezing injury is generally more pronounced with Gram-negative bacteria than with Gram-positive species, Staphylococcus usually survives well (ICMSF 6).

11. Glaze

The processing of glazing consist in the application of a layer (coating) of ice to the product's surface to serve as a barrier to air with the purpose to retard dehydration of the product. According to the Codex standard for quick frozen shrimps or prawns the water used for glazing or preparing glazing solutions should be of potable quality or clean sea-water (Codex Stan 92-1981).

12. Package

13. Weigh

14. Label

15. Frozen storage

16. Ship

Due to time constrictions, we decided to simplify the production chain in only two steps: cooking and post-cooking processes (considering cooling, peeling, dipping (additives), draining, individual quick frozen step, glazing, packaging, weighing, labelling, frozen storage and shipping as one single step).

5. Risk management

A microbiological FSO is the maximum frequency and/or concentration of a microbial hazard in a food considered tolerable for consumer protection.

Food safety objectives can be translated into a mathematical form using the following inequality:

$$H_0 - \sum R + \sum I \leq FSO$$

Where: FSO = Food safety objective

H_0 = Initial level of the hazard

$\sum R$ = Total (cumulative) reduction of the hazard

$\sum I$ = Total (cumulative) increase of the hazard

FSO, H_0, R and I are expressed in $\log_{10}$ units.

From the mathematical expression, we can observe that when establishing a FSO, we should consider the initial level of a hazard and changes occurring during production, distribution, storage, preparation and use of the food.

The initial level of the hazard in the product should be estimated, including uncertainty and variability. The way of including prevalence has not been established yet (this could be an interesting topic for further investigation). The way to establish the uncertainty and variability on the establishment of a FSO could also be another interesting topic for further research.

The methods of catching, or harvesting, and processing are varied, and they will affect the initial level of microorganisms in shrimps, but in our study we will consider the reception of shrimps at the factory as the first step of the production chain.

Swartzentruber et al. (1980) reported that, in general, shellfish have low bacterial counts when freshly caught. In this study, we assumed that the initial level of *S. aureus* in shrimps is 1 $\log_{10}$ CFU/g.

Establishing a Food Safety Objective

The US Food and Drug Administration (2000) indicated that ~10^5 CFU/g *S. aureus* is required to produce sufficient enterotoxins to cause illness in humans. Therefore we decided to suggest a FSO of 5 $\log_{10}$ CFU/g in pre-cooked frozen shrimp at the point of consumption.

We simplified the production chain as only 2 steps:

1. Cooking process

The reduction of the hazard is mainly obtained at this process. If the cooking is done correctly will be sufficient to inactivate *S. aureus*.

2. Post-cooking steps

Post-cooking contamination or increase in the initial concentration of *S. aureus* can occurs, especially when peeling is done by hand.

A performance objective (PO) is the maximum frequency and/or concentration of a hazard in a food at a specific step in the food chain before the time of consumption that provides or contributes to an FSO.

In the pre-cooked frozen shrimp process, the cooking process is where the reduction of pathogens will occur. This process will usually destroy *S. aureus*, but not the toxins.

After the heat treatment, the hazard can increase if the shrimps are recontaminated. Excessive handling of shrimps by humans can be a source of *S. aureus* and the primary concern is the handling of the product after the cooking treatment, particularly because of the possible contamination with *S. aureus* at the peeling step and the adequacy of refrigerated transport and storage. The sources associated with the reintroduction of the microorganism include food handlers, raw product/seawater, and the production-distribution-retail environment.

The potential for the presence of *S. aureus* in cooked shrimp is a function of the adequacy of the thermal process, the level of post-processing contamination, and the maintenance of proper refrigerated or frozen storage. Temperature abused shrimp can support the growth of *S. aureus*, particularly if competitive microorganisms have been eliminated as result of the thermal processing.

At this point of the study we will suggest one PO after the cooking process, because we consider that the contamination post-contamination together with temperature abuse is the main contributor to the possible increase of the hazard. In order to calculate the PO, two different scenarios were considered:

a) Normal scenario.

The final product will be kept at appropriate temperatures and therefore growth of *S. aureus* will not be possible. In this case the PO will have the same value as the FSO, 5 $\log_{10}$ CFU/g.

b) Bad scenario.

Temperature abuse may happen and in this case multiplication of *S. aureus* may happen before consumption. We assume that from the time of cooking to the time of consumption, a 2 $\log_{10}$ CFU/g increase in *S. aureus* could occur. In this case the PO should be lower than the FSO. The required PO should be 3 (5-2, the maximum increase of *S. aureus* after the cooking step) $\log_{10}$ CFU/g. To ensure that the PO would be met by 99% of the food in the lot, the maximum permitted level should be 3 SD bellow the calculated PO value of 3. Accordingly, the corresponding log-normally distributed population with an s.d. of 0.8 $\log_{10}$ CFU/g should have a mean concentration of 2.6 $\log_{10}$ CFU/g or less ($5 - 3 \times 0.8$).

6. Discussion

In this study we developed a qualitative microbiological risk assessment to estimate the risks associated with *S. aureus* in pre-cooked frozen shrimps. Due to the lack of time it was not possible to develop a quantitative risk assessment, although we are aware that it would have been more appropriate for the purpose of the study.

The qualitative assessment was used to describe the production process of pre-cooked frozen shrimps and a better understanding of the production, processing and product handling different scenarios was achieved. Knowing the different microbial processes that occur along the processing chain, facilitates the evaluation of the impact of different control measures on the risk to which consumers may be exposed and the impact of different FSOs on the risk estimated.

One of the limitations that we identified while doing the study was the difficulty for including variability and uncertainty in the establishment of the FSO and POs. A few years ago Havelaar et al. (2004) reported this difficulty and one of their statements was the need of modifying the definition of FSOs to account for variability and uncertainty about the contamination of food with pathogenic microorganisms. These authors recommended alternative definitions for the FSO, performance criterion and process criterion. More recently, Rieu et al. (2007) addressed this problem as well. In their study they tried to develop a methodological tool to derive a FSO from an ALOP being expressed as a maximal annual marginal risk and one of their conclusions was that the definition of the FSO should be improved.

But despite these limitations, quantitative risk assessments can be used to establish FSOs and it can be a very powerful tool. Risk assessment can help to indentify how the frequency and/or concentration of a microbiological hazard in a food can influence the incidence of a disease and the relationship between these 2 elements can be represented by a curve that can be used to estimate FSOs.

Appendix : Some definitions of interest (by the Codex Alimentarius)

Shellfish: Those species of aquatic molluscs and crustaceans that are commonly used for food.

Freezing process: A process that is carried out in appropriate equipment in such a way that the range of temperature of maximum crystallization is passed quickly. The quick freezing process shall not be regarded as complete unless and until the product temperature has reached –18C (0F) or lower at the thermal centre after thermal stabilization.

Frozen storage facility: A facility that is capable of maintaining the temperature of fish at –18 °C.

Frozen fish: Fish that have been subjected to a freezing process sufficient to reduce the temperature of the whole product to a level low enough to preserve the inherent quality of the fish and that have been maintained at this low temperature as specified in the Standard for quick frozen finfish, uneviscerated and eviscerated (Codex Stan 36-1981) during transportation, storage and distribution up to and including the time of final sale. For the purposes of this Code, the terms "frozen", "deep frozen", "quick frozen", unless otherwise stated, shall be regarded as synonymous.

Dehead: To remove the head from the whole shrimp or prawn.

Deveined shrimps: All the shrimps that have been peeled, the back of the peeled segments of the shrimps have been opened out and the gut ("vein") removed.

Fresh shrimps: Freshly caught shrimps that have received no preserving treatment or that have been preserved only by chilling. It does not include freshly cooked shrimps.

Peeled shrimps: Shrimps with heads and all shell removed.

Raw headless shrimps: Raw shrimps with heads removed and the shell on.

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Report of visit to the National Institute of Health Sciences.

Dr. Natalia Gomez Tome visited the Division of Biomedical Food Research at the National Institute of Health Science (Ministry of Health, Labour and Welfare) in Tokyo, Japan, for 3 weeks (from the 18th of November to the 8th of December 2009). The visit was funded by the Japan Food Hygiene Association and the host researcher was Dr Fumiko Kasuga, chief of section Food Microbiology. The main purpose of the visit was to incorporate a microbiological risk assessment in the development of food safety objective (FSO) for *S. aureus* in pre-cooked frozen shrimps.

At the beginning of the stay Dr Gomez assisted to one of the meetings of the Division of Biomedical Food Research where she met the sections chiefs and the director of the division, Dr Shigeki Yamamoto.

A meeting with the director of the Institute, Dr Masahiro Nishijima was held on the 26th of November.

An initial discussion with Dr Kasuga about specifications and standards for frozen foods in Japan was held on the 19th of November 2009. The Minister of Health, Labour and Welfare (MHLW) classifies frozen foods in 3 categories and establishes microbiological standards for each of them:

1. Frozen food to be consumed without heating.

APC < 100,000/g

Coliform negative.

2. Frozen food to be consumed after heating.

2.1. Those pre-heated before freezing process

APC < 100,000/g

Coliform negative.

2.2. Those not heated before freezing process

APC < 3,000,000/g

E. coli negative

3. Seafood consumed raw.

APC < 100,000/g

Coliform negative.

Vibrio parahaemolyticus < 100/g (MPN)

The Minister of Health, Labour and Welfare is interested in re-formulate the specifications and standards for frozen foods and Dr Kasuga is working in that project.

During the first week a literature review was done. Operational information of the food production chain was gathered and a flowchart of the production chain of pre-cooked frozen shrimps was drawn, including a description of each of the steps of the chain. Microbiological implications were described; microbiological risks associated with the production chain and the final product, prevalence and levels of *S. aureus* at different steps of the production chain, information of the growth and enterotoxin production of *S. aureus* in shrimp and outbreaks of food poisoning due to *S. aureus* in shrimps. Information of guidelines and recommendations about microbiological criteria for frozen foods was also obtained.

Along the three weeks several meetings were held between Dr. Kasuga and Dr Gomez to discuss about different tools used to link food safety with public health: food safety objective (FSO), performance objective (PO), appropriate level of protection (ALOP), distribution of pathogens in food, operating characteristic (OC) curve and probabilities of accepting or rejecting lots. There were further discussions about the role of these concepts in microbiological food safety management and the mathematical relationship between FSOs, POs and microbiological criteria. Dr Kasuga explained the role of different organizations such as the Codex Alimentarius Commission (CAC), the Codex Committee on Food Hygiene (CCFH), the International Commission on Microbiological Specifications for Foods (ICMSF), the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and the World Trade Organization (WTO) and its Sanitary and Phytosanitary Agreement (SPS).

On the 30th of November Dr Gomez and Dr Kasuga visited the National Food Research Institute and the National Institute of Infectious Diseases in Tsukuba. At the National Food Research Institute, Dr Gomez attended to a presentation offered by Dr Shigenobu Koseki about Microbial Responses Viewer (MRV). After the presentation, a discussion was held between Dr Koseki, Dr Kasuga and Dr Gomez. Afterwards Dr Gomez presented the work that she is doing at the Institute of Food Research, Norwich, as part of the European Integrated project BIOTRACER (www.biotracer.org) to Dr. Koseki, Dr Kasuga and members of the National Institute of Animal Health. A group discussion was held after the presentation about the use of Bayesian networks in food risk assessment.

With information obtained from the literature review, processes to establish an FSO and two different PO were suggested; one for a normal scenario where no growth of *S. aureus* would happen and another PO to describe the possibility of growth in the product in case of temperature abuse.

Finally, a scientific report was written.