The effects of consumer freezing of food on its use-by date

Strategic review

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1. Summary

The current Food Standards Agency consumer guidance states that consumers can freeze pre-packed food right up to the “use-by” date and, once food has been defrosted, it should be consumed within 24 hours. This strategic review has collated relevant data to determine whether there is an increased risk in relation to freezing ready-to-eat and non-ready-to-eat foods on the use-by date compared to the day before the use-by date. The review has focused on how the shelf-life of a food is determined and the effects of freezing, thawing and refrigeration on foodborne pathogens, including *Bacillus* spp., *Campylobacter* spp., *Clostridium botulinum*, *Clostridium perfringens, Listeria monocytogenes, Salmonella*, pathogenic *Escherichia coli* and *Shigella* spp.

In the UK, food business operators are responsible for setting the safe shelf-life of a food which, in practice, should take into consideration the consumer habits, as well as the factors affecting shelf-life, such as food product characteristics, food processing techniques, transport, retail and domestic food storage temperatures, and type of packaging.

Some countries, such as Ireland, New Zealand and Canada specifically recommend including safety margins within shelf lives. This is used to maintain brand integrity because it ensures that the food is consumed in its optimum condition. The FSA has collaborated with other organisations in the production of several guidance documents; however, there is no explicit requirement for the consideration of a margin of safety when setting shelf-life. There is also no legal requirement in the UK to consider a safety margin when setting shelf-life.

According to regulations, pathogens should not be present in sufficient levels to cause foodborne illness on the use-by date, as food should still be safe to eat on that day. Given that these requirements are met, the risk assessed in this report arises from the processes of freezing, thawing and subsequent refrigerated storage for a further 24 hours, and the potential for these to increase pathogen levels. In this review, it was found that there is a risk of additional growth of certain pathogens

during the refrigerated storage period although the impact of freezing and thawing on the extent of this growth was not readily evident. This risk would relate specifically to ready-to-eat foods as cooking of non-ready-to-eat foods after defrosting would eliminate pathogens.

This report explores the potential issues related to consumer freezing on the use-by date and identifies additional information or research required to understand the risks involved. Overall, there is little evidence to suggest a significant change in risk between consumers freezing ready-to-eat food on the use-by date compared to freezing the food on the day before the use-by date. Specific areas that merit further research include the risks due to low temperature survival and growth of *L. monocytogenes.* There is also a lack of research on the effects of freezing, defrosting and refrigeration on the growth and toxin production of non-proteolytic *C. botulinum*, and the growth of *Salmonella* during domestic freezing and thawing. Finally, more information on how food business operators set shelf-life would enable a better understanding of the process and the extent of the safety margin when determining shelf-life of ready-to-eat and non-ready-to-eat foods.

2. List of acronyms

- a_w – Water activity
- EC – European Commission
- Eh – Redox potential
- EU – European Union
- FBO – Food Business Operator
- FSA – Food Standards Agency
- FSS – Food Standards Scotland
- FSAI – Food Safety Authority of Ireland
- HACCP – Hazard Analysis and Critical Control Point
- IFST – Institute of Food Science and Technology
- MAP – Modified Atmosphere Packaging
- PHE – Public Health England
- RTE – Ready-to-eat
- Spp. – Species
- STEC – Shiga toxin-producing *E. coli*
- VP – Vacuum Packaging

3. Introduction

3.1 Context

The current Food Standards Agency (FSA) consumer guidance states that consumers can freeze pre-packed food right up to the “use-by” date. Once food has been defrosted, it should be consumed within 24 hours (FSA, 2020a). The COVID-19 guidance for consumers is also based on this advice (FSA, 2020b).

There are uncertainties around freezing foods on the use-by date. Advising consumers to consume defrosted food within 24 hours potentially contradicts FSA guidance which states that food should be consumed within its use-by date, if the food was frozen on the use-by date itself (FSA, 2020c).

3.2 Scope and factors considered

A strategic review has been commissioned with the following question:

What is the relative risk to consumers of consuming food frozen at home on the use-by date compared with food frozen at home before the use-by date, assuming defrosting is carried out as recommended by the FSA?

With consideration given to the impact of:

1. Subsequent cook step where applicable, or absence of in the case of ready-to-eat (RTE) foods (i.e. does the risk differ between RTE and non-RTE food)
2. Vulnerable groups (where possible)
3. The temperature of domestic refrigerators/freezers

The review considers the following factors:

- Shelf-life of different food products, including factors affecting shelf-life and how shelf-life is determined in the UK and in EU and non-EU countries;
- Growth of bacteria at refrigeration, freezing and defrosting temperatures;

- Impact of refrigeration, freezing and defrosting on pathogen survival and growth.

The review specifically considers bacterial pathogens such as *Bacillus* spp., *Campylobacter* spp., *Clostridium botulinum*, *Clostridium perfringens, Listeria monocytogenes, Salmonella* spp., Shiga toxin-producing *Escherichia coli* and *Shigella* spp.

This review does not consider:

- Viruses, such as norovirus and hepatitis A virus, because they do not replicate in food;
- Yeasts and moulds which are usually hygiene indicators and responsible for food spoilage rather than food safety
- Toxins such as histamine and mycotoxins
- *Giardia duodenalis*, *Toxoplasma gondii* and *Cryptosporidium* spp. because they do not multiply in foods, are mostly waterborne diseases or because few cases are reported annually by Public Health England (PHE), or they are mostly associated with waterborne outbreaks.
- Cross contamination risks to other foods as a consequence of drip, handling etc.

4. Shelf-life of a food

4.1 Food expiration dates

There are two types of food expiration dates widely applied in the UK:

- “best before” – indicates the period of time for which a food can reasonably be expected to maintain its optimal quality condition (FSA, 2018). A food which is past its “best before” date should be safe to eat, but the quality may have deteriorated (WRAP, 2019).

- “use-by”- should only be applied to foods which, from a microbiological point of view, are highly perishable and are therefore likely, after a relatively short period, to constitute a risk to human health (FSA, 2018).

In addition to the legally required “best before” and “use-by” dates, manufacturers can use other dates such as “Display Until”, “Sell By” and “Open Date”, which help retailers with stock control. These have no legal basis and are not aimed at consumers. These date marks are used for commercial purposes only (Best Food Facts, 2018) (WRAP, 2019).

4.2 Factors affecting shelf-life

Many factors must be considered in determining the appropriate shelf-life of each specific food. These can be divided into intrinsic factors, which are characteristics of the food itself, and extrinsic factors, which refer to the characteristics of the environment surrounding the food. Shelf-life of a food is primarily determined by the potential for contamination with pathogenic microorganisms, and the potential risks for subsequent microbial growth and/or production of toxins (FDA, 2001).

4.2.1 Intrinsic factors

The intrinsic factors influencing microbial growth in/on food include pH, redox potential and water activity (a_w).

pH

pH is a function of the hydrogen ion concentration in the food. Increasing the acidity of foods, also known as lowering the pH, either through fermentation or the addition of acids, has been used as a preservation method since ancient times. The pH can interact with factors such as a_w, salt, temperature, redox potential, and preservatives to inhibit the growth of microorganisms. The pH of the food also has an impact on the effectiveness of heat treatment of a food. Less heat is necessary to inactivate microbes when the pH is low (Mossel et al. 1995). In general, pathogens do not grow, or grow very slowly, at low levels of pH (below 4.6); but many pathogenic microorganisms, and spores in particular, are able to survive in foods at pH levels below their growth minima (FSAI, 2019).

Water activity

Water activity (a_w) is a measure of the amount of free or available water within a food. The a_w of most food products ranges from 0.2 for very dry foods to 0.99 for moist fresh foods. Foods with a low a_w cannot support microbial growth because microorganisms need water for growth. In fact, pathogenic and most spoilage bacteria do not grow in food with an $a_w < 0.85$, but some yeasts and moulds can at a_w values down to around 0.60. The a_w of a food can be altered by processing such as dehydration, concentration or freezing or by the addition of ingredients such as salt and sugar (FSAI, 2019). Different microorganisms have different a_w requirements. Gram-negative organisms require a minimum a_w requirement of 0.96 to 0.93 to grow, whereas Gram-positive, non-spore forming organisms can grow at lower a_w values of 0.85 to 0.94 (Farkas, 2007; FDA, 2012).

Redox potential

Redox potential (Eh) is a measure of the ease by which a substance gains or loses electrons through the reactions of oxidation and/or reduction. The redox potential is measured in terms of millivolts. Fresh fruits and vegetables and raw meat are in a reduced state because of the presence of reducing substances such as ascorbic acid or sugars. The redox potential of a food is influenced by its chemical composition, specific processing treatments and storage conditions in relation to oxygen

concentrations, such as vacuum packaging and modified atmosphere packaging. (Martin et al. 2013).

Each microbial species has a favourable Eh range for growth. In terms of the ability to grow in different Eh environments, there are three major groups of microorganisms, aerobes (+500 to +300 mV), facultative aerobes and microaerophiles (+300 to -100 mV) and anaerobes (+100 to less than -250 mV) (FDA, 2001).

Natural barriers

Some foods have natural barriers which provide protection from external contamination. These barriers can include shells (e.g. nuts and eggs), skins (e.g. vegetable and fruits) and membranes (e.g. meat and fish). The effectiveness of these barriers at preventing contamination of foods will vary considerably, and in some cases, may actually facilitate microbial growth, particularly if the natural covering is damaged.

Antimicrobials

Antimicrobials are naturally present in many plant- and animal-based products, including essential oils and glycosides in plant-based products and lysozyme and lactoferrin in animal-based products. Food processing can also produce antimicrobial compounds, such as smoke condensates (FDA, 2001).

4.2.2 Extrinsic factors

Food processing

Processing methods can improve the shelf-life of a food product. Common technology such as heat treatment will improve food safety and extend shelf-life by destroying pathogens and reducing numbers of other microorganisms. The standard FSA advice is that cooking food at a core temperature of 70 °C for two minutes is sufficient to kill vegetative pathogens (FSA, 2018).

The cooling methods applied to heat-treated products are also important. Some spoilage and pathogenic bacteria produce spores that may survive and be activated during the heating process. If the food is not cooled rapidly after the heat treatment, spores may geminate and bacteria increase rapidly in the warm food, causing spoilage and in some cases food poisoning (FSAI, 2019).

Other processing methods such as high-pressure processing, smoking, fermentation, curing, drying, chilling and freezing alter the properties of the food to kill bacteria or slow the growth of specific microorganisms. Some forms of food processing such as natural smoking may also result in the formation of antimicrobial substances in foods which can result in a delay of microbial growth (FSAI, 2019).

Chemical preservatives and additives can be added during processing in order to increase the shelf-life of a food by inhibiting microbial growth, for example the use of nisin as a preservative in processed cheese, meat and beverages (FDA, 2001).

Type of packaging

Vacuum packaging (VP) is a method of packaging characterised by evacuating the air within the package prior to sealing it. Significant reduction of oxygen concentrations in VP foods will limit growth of aerobic bacteria but in the absence of other controlling factors can provide conditions suitable for growth and toxin production by anaerobic pathogens such as *Clostridium botulinum*. Additionally, the suppression of aerobic organisms may create conditions favourable for the growth of pathogenic facultative anaerobic bacteria such as *Listeria monocytogenes* (Mills et al., 2014).

The gaseous environment within modified atmosphere packaging (MAP) is altered in order to delay the respiration rate of foods as well as microbial growth, and to reduce enzymic degradation and therefore extending the shelf-life of the food. For instance, the presence of carbon dioxide in MAP products inhibits the growth of Gram-negative spoilage organisms such as *Pseudomonas* spp., some moulds and yeasts (Cutter, 2010). MAP is used extensively to extend the shelf-life of meats, fruits, and vegetables, although it can increase the potential for outgrowth of microorganisms

such as *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium botulinum* in comparison with a non-modified gaseous environment (Cutter, 2010).

Active packaging interacts with the internal atmosphere and the food to extend the shelf-life of foods (Labuza and Breene, 1989). For instance, ferrous oxide removes oxygen from the atmosphere inside the package. This technique has been used in several food industries alone or in combination with MAP in the EU and UK market (FSA, 2006; Suneetha et al., 2018).

Temperature

Temperature has a significant impact on food product shelf-life by affecting microbial growth rates. Different microbial groups can grow at different temperature ranges. Thus, psychrophiles tend to grow between 15 and 20 °C, and their fastest (optimal) growth is around 10 °C. Psychrotrophs tend to grow between -5 and 35 °C and their fastest growth is between 20 and 30 °C. Mesophiles tend to grow between 10 and 35 °C and fastest at 30°C. Thermophiles tend to grow between 40 to 90 °C and fastest between 55 and 66°C (FDF, 2017). At temperatures above the optimal growth temperature for each microbial group, growth rates decrease precipitously. At temperatures below the optimum growth temperature for each microbial group, growth rates decrease more gradually (FDF, 2017). In more general terms, most bacterial pathogens are able to grow at temperatures between 8.0 °C and 63.0 °C, and this temperature range is called the “Danger Zone” (FSA, 2018).

The control of temperature during all stages of food manufacture, storage, and distribution should be carefully considered, measured and documented by food business operators (FBOs) as it can significantly affect shelf-life (FSAI, 2019).

4.3 UK legislation

Under Regulation No 1169/2011, shelf-life of a foodstuff should be indicated by a date of minimum durability (“best before”). In the case of foods which, from a microbiological point of view, are highly perishable and are therefore likely after a short period to constitute an immediate danger to human health, the date of minimum

durability is replaced by a “use-by” date. After the “use-by” date, a food shall be deemed to be unsafe in accordance with Article 14(2) to (5) of Regulation No 178/2002.

Under Regulation No 2073/2005, food should “not contain microorganisms, their toxins and metabolites in quantities that present an unacceptable risk for human health”. Furthermore, FBOs may be required to demonstrate that the foods they produce comply with specified microbiological criteria throughout the shelf-life under reasonably foreseeable conditions of distribution, storage and use. Under this regulation, a RTE food or ingredient with a shelf life of less than 5 days is considered to be unable to support the growth of *L. monocytogenes*. CFU

The exception is foods that contain ingredients that support the growth of *L. monocytogenes*, where FBOs must demonstrate the *L. monocytogenes* levels do not exceed 100 CFU/g during the shelf life.

4.4 Setting product shelf-life

4.4.1 In the UK

FBOs are responsible for setting the shelf-life of a food product as part of their Hazard Analysis and Critical Control Point (HACCP) plan and for ensuring that the information provided on the label includes clear advice on the conditions in which the food should be kept. The shelf-life of food must be determined carefully and with full knowledge of the risks involved. Setting shelf-life typically involves a number of steps as outlined in the Campden BRI “Evaluation of microbiological shelf-life of foods” guidance and in the Food and Drink Federation “Industry Guidance on Setting Product Shelf-Life” (Campden BRI, 2019, FDF, 2017). These include shelf-life studies which determine the amount of time the product will maintain certain properties such as acceptable microbiological counts, taste, appearance and aroma. Some consumers keep food in their domestic refrigerator which is operating higher that the recommended temperature of 4-5 °C, therefore FBOs are encouraged to consider a buffer when setting the shelf-life of a food product to allow for this temperature abuse (FDF, 2017).

Predictive microbiology models can also be useful in estimating food safety and shelf-life, once the food's characteristics have been established.

There is some evidence that FBOs use a safety margin when setting shelf life. In 2012 WRAP was commissioned to undertake a study examining how manufacturers and retailers set product life of cheddar cheese and yoghurt in the UK. WRAP reported that the shelf-life of cheddar cheese and yoghurt is 15-25% less than the total life of these foods. This buffer is necessary to maintain brand integrity and trust, because this ensures that the food is consumed in its optimum condition. Moreover, WRAP showed that retailers are consistent in requiring a safety margin of shelf-life: evidence suggests that products can be delivered with a shorter use-by date than 75% of the total life (WRAP, 2012). There is little information on whether manufacturers consider a safety margin when setting shelf-life of other food products.

The FSA has collaborated with other organisations in the production of guidance for FBOs to determine shelf-life of RTE food in relation to *L. monocytogenes* (CFA, 2010). In December 2020, the FSA and FSS published an updated version of vacuum packaging technical guidance ("The safety and shelf-life of vacuum and modified atmosphere packed chilled foods with respect to non-proteolytic *Clostridium botulinum*") which provides advice on food safety for raw and RTE VP or MAP chilled foods (FSA, 2020d). However, these two guidance documents do not mention the consideration of a margin of safety when setting shelf-life.

It is therefore uncertain whether consumers freezing food on the use-by date and consuming it within 24 hours of defrosting is factored in when setting shelf life.

WRAP freezing guide for FBOs suggests that a 'Freeze by date shown' or a 'Freeze as soon as possible' label is included on products, as well guidance for FBOs to include 'defrosting and/or cook from frozen advice, e.g. defrost in fridge and use within 24 hours (which is important to ensure that the original 'Use By' period is not exceeded)' (WRAP, 2019).

4.4.2 In other countries

The Food Safety Authority of Ireland (FSAI) has published a guidance document which outlines good practice for FBOs to estimate, set and verify food shelf-life (FSAI, 2019). This document describes the use of predictive microbiology models and laboratory testing to determine the shelf-life of a food. However, this guidance outlines that while the accuracy and reproducibility of shelf-life will be affected by the characteristics of the food, it is not possible to expect that the shelf-life of foods is consistently accurate and reproducible under all circumstances. Therefore, a margin of safety should be applied when setting shelf-life. The margin should be determined after examining all reasonably foreseeable conditions of processing, storage, distribution and use (FSAI, 2019).

The New Zealand Ministry for Primary Industries has also published a guidance document on how to determine the shelf-life of food (NZG, 2016). This guidance document describes how to perform a shelf-life study based on laboratory testing. Moreover, as in the FSAI's guidance, the shelf-life should be no longer than the number of days before unacceptable deterioration occurs plus a safety margin. A safety margin is needed because the shelf-life is only an approximation and not a fixed value and will vary from time to time. The size of the safety margin needs to take into account the potential for the shelf-life to be easily compromised by less than ideal conditions for storage, distribution and use (NZG, 2016).

The Canadian Food Inspection Agency has guidance providing an overview of the process for conducting a shelf-life study (Government of Canada, 2018). The two most common methods used for setting shelf-life are the direct or real-time study where the food is stored under normal conditions for a period of time greater than the estimated shelf-life. The state of food is then verified at regular intervals to determine the point at which it deteriorates. The second method described is the indirect or accelerated shelf-life. Acceleration factors such as increased temperatures are applied to the food to increase the rate of deterioration. The use of an indirect study should be validated to be appropriate and effective in predicting the shelf-life. The document also outlines that the declared shelf-life should be the actual shelf-life with the inclusion of a safety margin (Government of Canada, 2018).

5. Effects of freezing, defrosting and refrigeration on foodborne pathogens

5.1 Freezing

Freezing slows down chemical reactions within food and pauses growth and toxin production of bacteria. The rate of heat removal is dependent on the surface area of the food, the temperature difference between the food and the air, and the nature of the food matrix. For instance, the mean time required to reduce the temperature of chicken portions from 0 to -5 °C was 372 minutes (McIntyre et al., 2007).

Although some microorganisms are killed during the freezing process, the majority can survive freezing for a long period of time. Cellular damage or death occurs due to dehydration of the cell caused by formation of extracellular ice or injury due to formation of intracellular ice. Pathogens are unable to grow below around -1 °C and spoilage microorganisms are not able to grow below -10 to -12 °C (James and James, 2014). Freezing can also cause sub-lethal damage to bacteria. Some of these damaged cells can slowly recover and multiply after an extended lag phase (Wesche et al., 2009).

Only food that is labelled as suitable for home freezing should be frozen, however, labels may not always be provided for deli counter items, or items purchased from independent butchers, fishmongers and other food stores. The recommended temperature for domestic freezers is -18 °C. A study examining 745 freezers in England found that their average temperature over 7 days was -20.3°C (Biglia et al., 2018). The maximum mean temperature of a single device over the 7 days was −5.6 °C and the minimum mean temperature was −37 °C. These variations in temperature above the recommended -18 °C are an issue in terms of food quality rather than food safety (Biglia et al., 2018).

In a majority of experiments, freezing results in a decrease or no change to the number of bacterial cells on food. See Section 5.4 for results for specific bacterial pathogens.

The effects of freezing are mainly thought to depend on:

I. the kind of bacteria - Different genera (and different species and strains within a single genus) can have different responses to freezing. Example of this include *Campylobacter* spp., which are more susceptible to freezing than most other pathogens and *L. monocytogenes*, different strains of which have been reported to show significantly different freezing tolerances (El-Kest and Marth 1992).

II. duration of the freezing process - Usually the longer the freezing duration, the lower the chances of survival of bacterial cells – for instance, *E. coli* counts continued to decrease during storage at -22 °C, for 1, 2, 4 and 8 weeks (Foschino, 2002).

III. the pH - Very low or high pH values can affect pathogen survival during freezing. For instance, viable numbers of *S. aureus* cells did not decline at pH values of 4.4 to 7.0 in liquid buffer, or at pH values of 4.3 to 6.3 in meat (Demchick et al., 1982). Similarly, viable *L. monocytogenes* cell numbers did not decline in five foods with a pH > 5.7, but did decline in foods at a pH of 4.74 (Palumbo and Williams, 1991).

IV. the food matrix - The food matrix refers to the overall structure and physicochemical properties of food. The matrix can have a protective effect on bacterial cells. For instance, glycerol is a more successful cryoprotectant for *L. monocytogenes* than 2% or 4% milk fat (El-Kest and Marth 1992).

V. the physiological state of the bacterium - *E. coli* cells in the stationary phase of growth are less susceptible to injury and death during freezing than cells in the multiplication phase (Foschino 2002). Bacterial spores are especially resistant to freezing (Archer 2004).

Some of these factors are further addressed in the section 5.4 on foodborne pathogens. There are other factors that can act in combination with the five described above and have an effect on the survival of bacteria during freezing and thawing, such as the rate of freezing and freezing temperature (FSA, 2020a).

5.2 Defrosting

Thawing/defrosting of frozen food and subsequent storage at significantly higher temperatures facilitates the metabolism and growth of surviving microorganisms, increasing the risks of food spoilage and human foodborne illness. It is therefore important that the processes of thawing and post thawing storage are properly carried out. The size and shape of frozen products will greatly influence the rate of thawing. Thawing of a product is non-uniform, as external areas of the product will be exposed to higher temperatures, encouraging microbial growth. There is also an increase in the availability of moisture and nutrients creating a medium rich in proteins, vitamins and minerals which proves excellent for microbial growth (Leygonie et al., 2012). The process of freezing and thawing stresses and damages the bacterial cell, which can lead to an increase in the lag period of bacterial growth, increased sensitivity to chemicals, and decrease in growth rate (El-Kest and Marth, 1992).

Thawing is recommended before cooking of frozen foods as otherwise cooking will take longer and may not be sufficient to ensure that the food has reached the internal temperature necessary to kill pathogens (FSA, 2020a). The cross-contamination risk associated with drip and leakages from the packaging or product onto ready-to-eat food must also be considered when thawing food, especially raw meat and poultry – extra care should be taken to prevent this. To prevent microbial growth, thawing should be undertaken in accordance with current FSA advice (FSA, 2020a).
Once food is thawed, it should be stored in the refrigerator and consumed within 24 hours of thawing.

5.2.1 Defrosting in the refrigerator

It is recommended that food is defrosted in the refrigerator where possible and consumed within 24 hours of it being fully defrosted (FSA, 2020a).
Large items, such as a 6-7 kg Christmas turkey, can take up to 4 days to defrost fully in the refrigerator, therefore some planning ahead is required. While defrosting in the refrigerator takes longer than defrosting in a microwave oven or at room temperature, this approach ensures that the defrosted food remains at microbiologically safe

temperatures throughout the defrosting process and pathogen growth is minimal. Cross-contamination from unpacked/leaking raw meat and poultry onto other foods in the refrigerator is a possible hazard.

5.2.2 Defrosting in a microwave oven

If defrosting in the refrigerator is not possible, food can be defrosted in a microwave on a defrost setting. This method is quicker than the other two methods and can be carried out shortly before cooking the food. Food will defrost unevenly in the microwave and may reach temperatures above 8 °C where microbial growth is favoured. Therefore once this has been carried out, food should be cooked immediately after or placed in the refrigerator.

5.2.3 Defrosting at room temperature

Defrosting high risk foods, such as RTE products that support pathogen growth, at room temperature is not recommended, as it can be difficult to control the temperature of the food during the defrosting process. The uneven thawing rates can lead to favourable growth conditions for pathogens on the surface of the food for a long time while the centre of the product fully defrosts.

5.2.4 Cold water thawing

Certain foods can be defrosted by running it under cold water, which is faster than defrosting in the refrigerator and will not allow the food to get too warm. Raw meat and poultry should not be defrosted using this method unless they are in a sealed watertight container, to prevent cross-contamination from splashing water onto surfaces/RTE foods. Larger food items may need to be submerged in cold water, which should be changed periodically to ensure it stays cold, for a longer time to thaw. Ideally, food should be cooked immediately after thawing using this method or placed in the refrigerator.

5.3 Refrigeration

The low temperature of a refrigerator is used to control the safety of food. Domestic refrigerators are recommended to function in between 1 and 5 °C.

A number of studies have been published on domestic refrigerators, which have generally concluded that many refrigerators run at higher temperatures than recommended. Mean temperatures exceeding 5 °C were recorded in the majority (91%) of refrigerators of UK consumers (Evans and Redmond, 2016). A similar study of domestic cold appliances in England found that the average temperature over 7 days of 671 refrigerators was 5.3°C (Biglia et al. 2018). The largest overall mean temperature in a single refrigerator was 14.3 °C and the overall smallest mean temperature was −4.1 °C (Biglia et al. 2018).

The mean operating temperature of the refrigerator central storage area was recorded to be below 8 °C in 41 out of 43 kitchens (Evans and Redmond, 2016). In a 2017 review paper, around 80% of recorded refrigerator appliances in northern European countries had a mean temperature below 8 °C (Roccato et al., 2017). As a result, in this report when discussing microbial growth, refrigeration temperatures are considered to be between 4 and 8 °C, to more accurately represent the situation in consumers' homes.

5.4 Foodborne pathogens

Common foodborne pathogens are discussed in the following sections, including typical growth characteristics, symptoms, vulnerable groups, common food pathways for transmission and the effects of freezing, thawing and refrigeration.

5.4.1 Bacillus spp. (diarrhoeal type)

Introduction

Bacillus is a genus of Gram-positive and Gram-variable rod-shaped bacteria that can form spores typically in aerobic conditions, and are found in a large range of habitats, from soils, rice and vegetables to hot springs. Bacterial spores are able to survive environmental stresses such as heat, dehydration, extremes in pH and nutrient limitations (Soni et al., 2016).

Bacillus cereus is the *Bacillus* species most commonly associated with food poisoning, causing either emetic or diarrheal illness. *Bacillus anthracis*, *Bacillus thuringiensis* and *Bacillus cytotoxicus* have also been associated with foodborne illness. The toxin responsible for diarrheal illness is produced during *B. cereus* growth in the small intestine. The toxin responsible for emetic illness is produced when food, particularly rice, is cooked and then held at room temperature. Emetic illness caused by *B. cereus* is not considered in this review, as correct refrigeration will not significantly increase spore numbers over 24 hours and *B. cereus* spores are activated at temperatures of 65 – 75 °C (Schoeni and Lee Wong, 2005). Therefore, there is no difference in risk between freezing of products on the use-by date and the day before the use-by date with respect to emetic illness.

Diarrheal illness symptoms typically include diarrhoea, abdominal pain, and occasionally nausea, fever and vomiting. Onset varies from around 8 – 16 hours after consumption of contaminated food, and the symptoms usually resolve after 12 – 14 hours. The illness is usually mild and therefore it is difficult to accurately estimate case numbers. More severe disease, including systemic infection, meningitis,

gangrene, can be seen in the immunosuppressed population, those with indwelling catheters or surgical wounds and neonates (McDowell et al., 2020)

The infectious dose is estimated to be from 5 x 10^4 to 10^{11} *B. cereus* cells (Schoeni and Lee Wong, 2005). Although infants are particularly susceptible to illness, there are not many documented foodborne outbreaks of *B. cereus*. PHE reported either 0 or 1 *B. cereus* outbreak a year from 2004 to 2013 ("Reported Outbreaks of *Bacillus* spp. from 1992 to 2013" 2013).

Food pathways

Rice and meat dishes especially are associated with *B. cereus* diarrheal illness, as well as vegetables, soups, sauces, spices and dairy products (Schoeni and Lee Wong, 2005).

Effects of temperature

Bacillus spp. grow optimally at 25 to 37 °C, although psychrotrophic strains of *B. cereus* (strains capable of growth and reproduction at low temperatures) have been recorded growing at 5 °C (Schoeni and Lee Wong, 2005) (Dufrenne et al., 1995). *B. cereus* can survive freezing.

The spores of *B. cereus* are heat resistant so may not be inactivated by some cooking treatments.

Following pasteurisation treatment, mesophilic *B. cereus* counts increased by approximately 1 $\log_{10}$ every week for the first two weeks, and by approximately 4 $\log_{10}$ within the third week at refrigeration temperatures of 4 ± 2 °C (Aires et al., 2009). The doubling time of 12 mesophilic *B. cereus* strains in milk at 7 °C was found to vary considerably - between 9.4 hours to 75.2 hours in milk and brain heart infusion broth (Dufrenne et al., 1995). However, a lag phase of 0.7 days to 6.4 days was observed in half the experiments. All 12 strains were capable of producing diarrheal toxin (in broth at 30 °C). In zucchini broth, at pH 6.5, no growth was observed for 5 *B. cereus* strains held at 8 °C, and slow growth was observed for

1 mesophilic strain, after a lag time of 88 hours. At a lower pH of 5, none of the 5 strains grew even at 12 °C (Valero et al., 2003). Given these observations, it is unlikely that 24 hours storage in consumer refrigerators would have a large impact on *B. cereus* numbers.

There was no significant difference in *B. cereus* levels between fresh hams and hams that were frozen for 2 months and then either thawed at 2 °C or at room temperature (16 °C) (Kemp et al., 1982).

5.4.2 Campylobacter spp.

Introduction

Campylobacter species are Gram-negative spiral, rod-shaped, or curved bacteria which do not form spores. There are more than 20 species of *Campylobacter*, with the most common pathogenic species being *C. jejuni* and *C. coli*.

The incubation period for *Campylobacter* is usually 2 to 5 days but can be 1 to 11 days. The most common clinical symptoms of *Campylobacter* infections are diarrhoea (frequently bloody), abdominal pain, fever, headache, nausea and/or vomiting. These symptoms typically last 3 to 6 days. Gastroenteritis induced by *C. coli* is clinically indistinguishable to that of *C. jejuni.* Whilst the diarrhoea is self-limiting, excretion of *Campylobacter* can continue for two to three weeks.

Campylobacter infection can lead to long term complications such as reactive arthritis (9 in every 1,000 cases), Guillain-Barré syndrome (1 in every 1,000 cases) and other rare late consequences, such as Miller Fisher syndrome, haemolytic uremic syndrome, inflammatory bowel disease and functional gastrointestinal disorders (ACMSF, 2019).
Campylobacter infections are equally common in males and females, with babies and children in the 0–4 age group more likely to be affected. It has been found to be more prevalent during the summer months.

The infectious dose of *Campylobacter* is low, and thought to be around a few hundred cells (ACMSF, 2019).

The IID2 study showed that *Campylobacter* was the most common bacterial pathogen isolated from the stools of patients reporting infectious intestinal disease (Tam et al., 2012). The IID2 study extension model found that *Campylobacter* was the most common foodborne pathogen in the UK with an estimated 280,000 cases per year (O'Brien et al. 2016). Reported yearly cases of campylobacteriosis are shown in Figure 1. Most *Campylobacter* cases are unrelated to outbreaks, but due to sporadic cases.

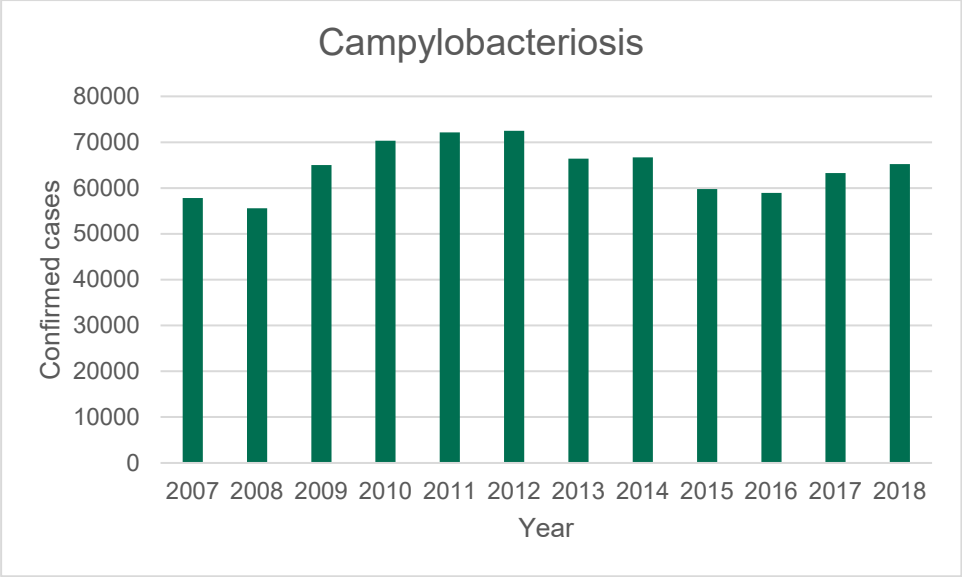

Figure 1: Laboratory-confirmed cases of campylobacteriosis in the UK from 2007 to 2018. Data from the Surveillance Atlas of Infectious Diseases, ECDC.

Food pathways

The main reservoir of *Campylobacter* is poultry; it can also live in the gastrointestinal tract of mammals including livestock and pets, such as cats and dogs (Kaakoush et al., 2015). Common food pathways for *Campylobacter* infection therefore include raw and undercooked meat, especially poultry, unpasteurised milk and untreated water. Of the 143 outbreaks of campylobacteriosis in England and Wales between 1992 and 2009, 80% were due to consumption of contaminated food or water, while the remainder were due to person-to-person transmission (3%), contact with animals (1%) or an unknown mode of transmission (16%) (Little et al., 2010). A predominant

dish associated with *Campylobacter* outbreaks is poultry liver pâté (Little et al., 2010) (Edwards et al., 2014)

Sampling carried out by the 9 major UK retailers from April to June 2019 of fresh UK-produced chicken found contamination levels as depicted in Table 1. This meets the FSA/FSS/industry risk reduction strategy of reducing the percentage of birds with contamination levels of > 1000 CFU/g to less than 7%.

Table 1: Contamination levels of fresh chicken following sampling at the major 9 UK retailers.

Contamination levels	April - June 2019
< 10 CFU/g	59%
10 – 99 CFU/g	25.3%
100 – 1000 CFU/g	12.1%
> 1000 CFU/g	3.6%

Packaging of fresh chicken at retail was also found to have between 100 to 4,500 CFU *Campylobacter* spp. per swab which had been rubbed on the entire outer packaging in 1.6% of cases (Jorgensen et al., 2015). Cross contamination is still a potential issue but can be mitigated by good kitchen hygiene. Washing raw chicken is considered a key contributor to cross-contamination in domestic settings (ACMSF, 2005).

Effects of temperature

Campylobacter spp. are readily destroyed by heat at recommended time-temperature combinations of 70 °C for 2 minutes or equivalent.

Campylobacter spp. are able to persist both in the environment and in contaminated foods, despite being highly sensitive to atmospheric oxygen concentrations. Studies investigating the survival of *Campylobacter* spp. show that this increases with decreasing temperature, with survival lasting a few hours at 37 °C and several days or longer at 4 °C. Optimal temperatures for growth occur at 37 - 43 °C in the microaerophilic environment of poultry guts. The organisms are not able to grow

below 30 °C. *Campylobacter* spp. exhibit a relatively high susceptibility to the effects of freezing (ACMSF, 2005). Effects of temperature on *Campylobacter* spp. in different food matrices is shown in Table 2. Freezing, even if carried out on the use-by date, will decrease the levels of *Campylobacter* spp. in food, therefore decreasing the risk under consideration in this review.

Table 2: Effects of temperature on Campylobacter *spp.* in different food matrices.

Type of meat	Pathogen	Temperature, ºC	Time	Change reported	Magnitude of change (log_{10} CFU/g if not specified)	Reference
Chicken	*C. jejuni*	-70	56 days	Decrease	2.5 log_{10} CFU/ml/cm^2	Lee et al., 1998
Chicken	*Campylobacter* spp.	-20	9 days	Decrease	5 log_{10} CFU/cm^2	El-Shibiny et al., 2009
Chicken	*Campylobacter* spp.	-20	2 weeks	Chicken skin: Decrease Skinned muscle: Decrease	2.96 log_{10} CFU/cm^2 2.51 log_{10} CFU/cm^2	Ritz et al., 2007
Chicken	*Campylobacter* spp.	-20	55 days	Decrease	2.87-3.16	Huang et al., 2012
Chicken	*C. jejuni*	-20	182 days	Decrease	2.99 log_{10} CFU/cm^2	Yogasundram and Shane, 1986
Chicken	*C. jejuni*	-20	28 days	Decrease	2.33	Maziero and de Oliveira, 2010
Beef	*C. jejuni*	-18	112 days	Decrease	1.5 - 2.5	Moorhead and Dykes, 2002
Chicken	*C. jejuni*	-18	20 days	Decrease	0.76	Maziero and de Oliveira, 2010

Chicken	*C. jejuni*	-18	20 days	Ground chicken: Decrease Chicken skin: Decrease	1.48 – 1.52 1.65 – 2.26	Maziero and de Oliveira, 2010
Chicken	*C. jejuni*	-18	32 days	Decrease	2.2 log_{10} CFU/ml	Maziero and de Oliveira, 2010
Chicken	*Campylobacter* spp.	Freezing storage	56 days	Decrease	3.5 log_{10} CFU/ml/cm^2	Ivić-Kolevska et al., 2012
Chicken	*Campylobacter* spp.	4	48 hours	No change	-	Ingham et al., 2005
Chicken	*C. jejuni*	4	7 days	Decrease	0.9 log_{10} CFU/cm^2	Yogasundram and Shane, 1986
Chicken	*C. jejuni*	4	7 days	Decrease	1.89	Maziero and de Oliveira, 2010
Chicken	*C. jejuni*	4	7 days	Decrease	0.63 – 0.81	Bhaduri and Cottrell, 2004
Chicken	*C. jejuni*	4	7 days	Decrease	0.58	Eideh and Al-Qadiri, 2011
Chicken	*C. jejuni*	4	9 days	Decrease	3.3-4.3 log_{10} CFU/cm^2	El-Shibiny et al., 2009
Chicken	*C.coli*	4	9 days	Decrease	2.6-4.0 log_{10} CFU/cm^2	El-Shibiny et al., 2009
Chicken	*C. jejuni*	4	10 days	Decrease	3.35-3.51	Huang et al., 2012
Chicken	*C. jejuni*	4	24 days	No change	-	Zhao et al., 2003

5.4.3 Clostridium botulinum

Introduction

Clostridium botulinum is a Gram-positive, obligate anaerobic, spore-forming, rod-shaped bacterium. Strains of *C. botulinum* fall into two categories, non-proteolytic and proteolytic strains. Neurotoxin production in strains relevant to public health is shown in Table 3.

Table 3: Neurotoxin production in C. botulinum strains.

	Non-proteolytic (Psychrotrophic)	**Proteolytic (Mesophilic)**
Neurotoxin	B, E or F	A, B and/or F
Lower limit for Growth / Neurotoxin production	Cold storage – minimum temperature 3 °C	Higher temperatures (10-12°C)

The risk from proteolytic *C. botulinum* is not considered in this review as its toxin is produced at higher temperatures (10 °C and above).

Failure to include controlling factors or temperature abuse allow *C. botulinum* spore germination, multiplication and neurotoxin production. Non proteolytic *C. botulinum* will not grow in acidic conditions, therefore one of the controlling factors is a pH of 5.0 or below. It can also be controlled by reducing the water activity to 0.97 or below in a food. Combinations of factors can also control the organism such as pH and water activity. Sodium, nisin and potassium nitrite are also used to control the growth of *C. botulinum* in meat, poultry, cheese and fish products. Synergism between *C. botulinum* and other microorganisms is possible – for instance the growth of mould on acidic foods can raise the pH; the growth of *C. perfringens* at warm temperatures on meat can lower the redox potential of tissues, allowing the growth of *C. botulinum* (Hamad, 2012).

Botulinum toxin is the most potent biological toxin known, with an estimated median lethal dose of 1 ng per kg of body weight. Foodborne botulism is an intoxication caused by consumption of botulinum toxin formed by *C. botulinum* in food. With

treatment most people will make a full recovery, but paralysis can spread to the muscles that control breathing if it is not treated rapidly and can prove fatal. Initial neurologic symptoms can include double or blurred vision, drooping eyelids, slurred speech, dry mouth, difficulty swallowing and breathing. Vomiting, diarrhoea, constipation, and abdominal swelling may also occur. Symptoms usually appear within 12 to 36 hours (with minimum and maximum range of 4 hours to 8 days) and generally last for 2 to 8 weeks.

Infant botulism occurs when infants under 1 ingest bacterial spores, particularly from honey. Spores can germinate within their gut and outgrow to form new vegetative cells that produce botulinum neurotoxin and/or multiply. These spores are harmless to older children and adults because the body develops defences against them from about the age of 1.

Botulism cases are rare in the UK – see Figure 2.

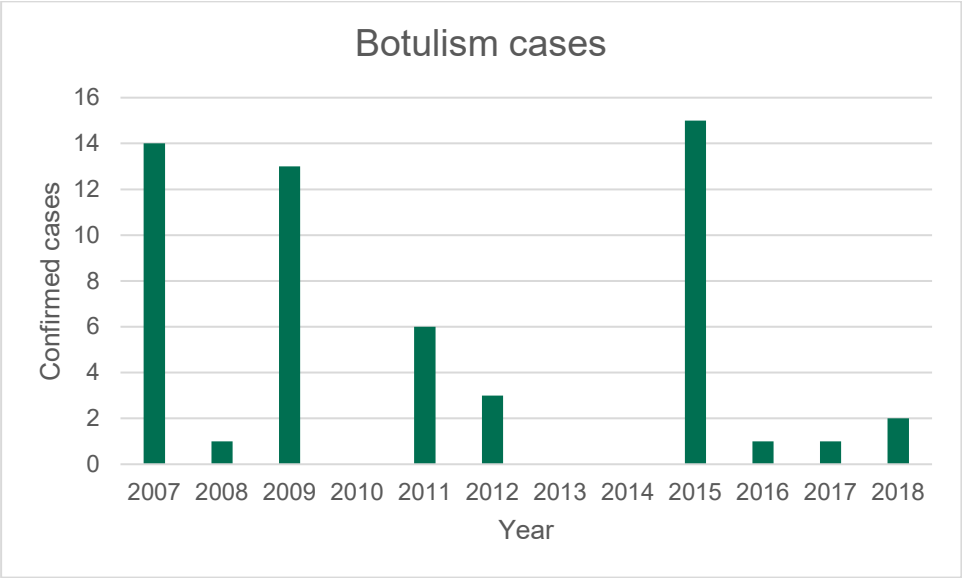

Figure 2: Confirmed cases of botulism in the UK from 2007 to 2018. Data from the Surveillance Atlas of Infectious Diseases, ECDC.

Food pathways

Spores of *C. botulinum* are widely distributed in the environment and may be present in a variety of foods. Spores germinate, leading to growth and toxin formation, at low oxygen concentrations and in foods with a low redox potential. Outbreaks of

foodborne botulism have been associated with foods sealed in airtight containers including VP and MAP foods and cans. A common source of foodborne botulism is home-canned or preserved foods that are low in acid, such as vegetables, meat products and fish. It is important to note that the presence of air, or a similar oxygen-containing atmosphere, cannot be relied upon to prevent growth and toxin formation by non-proteolytic *C. botulinum*. Such foods can contain areas of low oxygen and low redox potential that will allow *C. botulinum* to grow and form toxin.

Effects of temperature

C. botulinum spores are heat resistant – the recommendation is to heat treat refrigerated processed foods at 90 °C for 10 minutes in order to achieve a 6 log kill of non-proteolytic *C. botulinum*.

Placing the VP/MAP foods in refrigeration may not inhibit the growth of non-proteolytic *C. botulinum* given that some strains can grow and produce fatal toxin as low as 3 °C in the absence of oxygen, however the rate at which this will occur at low temperatures is slow (FSA, 2020). The optimum temperature of growth of these strains is 30 °C.

If the storage temperature may reach or exceed 10 °C, then the production of botulinum neurotoxin from proteolytic *C. botulinum* is a risk.

C. botulinum spores are resistant to freezing (James, 1933) as is botulinum toxin (Archer, 2004) (Wallace and Park, 1933), although there is little research available on the effects of freezing on non-proteolytic *C. botulinum*.

Toxin production at different temperatures was measured in pureed cooked mushroom (pH 6.29), cauliflower (pH 5.56) and potatoes (pH 5.71) (Carlin and Peck, 1996). All foods had an a_w of 0.99 and were inoculated with 3 $\log_{10}$ (10^3) of *C. botulinum* type B spores. The lag time, the doubling time, the time to visible growth (production of gas) and to toxin production are recorded in Table *4* below. The higher the temperature, the shorter the lag time and the doubling time (Carlin and Peck, 1996). Peck et al., 2020 stored fresh lamb, beef and pork at < 3 °C for 1 day, then at

5 °C for 1 day, 22 °C for 2 hours (to simulate potential abuse during consumer purchase and transportation) and then at 8 °C for the remaining incubation period to reflect domestic storage. Toxin production was detected in a pork product at 35 days, but not in lamb stored for 35 days or beef stored for 50 days. The typical shelf lives for different red meat species were 8-13 days for beef, 8-11 days for pork and 8-11 days for lamb (Peck et al., 2020).

Table 4*:* Toxin production and growth of food inoculated with *C. botulinum* spores. There was no growth in pureed potato at 5 and 10 °C.

Food/matrix	Temperature, °C	Lag time, hours	Doubling time, hours	Time to visible growth, days	Time to toxin production, days	Reference
Pureed mushroom	16	38	2.1	2.9	2.9	(Carlin and Peck, 1996)
Pureed mushroom	10	161	7.0	10.5	10.5	(Carlin and Peck, 1996)
Pureed mushroom	8	146	8.9	7.1	10.2	(Carlin and Peck, 1996)
Pureed mushroom	5	304	12.4	20.0	20.0	(Carlin and Peck, 1996)
Pureed cauliflower	16	52	3.0	3.9	3.9	(Carlin and Peck, 1996)
Pureed cauliflower	10	235	12.6	13.1	13.1	(Carlin and Peck, 1996)
Pureed cauliflower	8	288	11.3	14.9	17.1	(Carlin and Peck, 1996)
Pureed cauliflower	5	383	8.5	21.0	19.0	(Carlin and Peck, 1996)
Pureed potato	16	83	2.6	4.5	5.3	(Carlin and Peck, 1996)
Pureed potato	10	-	-	-	-	(Carlin and Peck, 1996)
Pureed potato	8	628	10.3	31.0	33.9	(Carlin and Peck, 1996)
Pureed potato	5	-	-	-	-	(Carlin and Peck, 1996)
Beef	0.6	26	-	-	-	Ali and Vanduyne, 1981

A review of botulism outbreaks, where the implicated product was commercially-prepared food meant to be refrigerated, found that illness occurred only as a result of time and temperature abuse rather than correctly stored products (Peck et al., 2020).

5.4.4 Clostridium perfringens

Introduction

Clostridium perfringens is a Gram-positive, anaerobic, spore-forming, rod-shaped bacterium. It is found ubiquitously in soil and in the intestines of warm-blooded animals, including humans. Spore formation enables *C. perfringens* to resist extremes in temperatures. Illness occurs due to toxin production in the intestines by vegetative cells.

C. perfringens enterotoxin (CPE) producing, or type A, strains are a very common cause of foodborne illness (Kiu and Hall, 2018). Common clinical symptoms include abdominal cramps, nausea, and diarrhoea which persists for 12 to 24 hours. The onset of symptoms occurs 8 to 18 hours after ingestion of the contaminated food.

Linking the cause of an outbreak to *C. perfringens* is difficult as healthy individuals can have high numbers of spores in their faeces and not all strains are able to produce enterotoxin (Brynestad and Granum, 2002). Given the mild illness caused by *C. perfringens*, there is significant underreporting associated with it. In 2018, *C. perfringens* caused an estimated 85,000 cases and 13,000 GP presentations in the UK (Holland and Mahmoudzadeh, 2020). It has a high under-ascertainment ratio, as testing is only done for the enterotoxin during outbreaks (Holland and Mahmoudzadeh, 2020).

Food pathways

C. perfringens food poisoning commonly occurs when meat, poultry products or other cooked foods are undercooked or kept warm for prolonged periods, at temperatures of 12°C – 60 °C, allowing the spores to germinate. Food products with a pH of 5.5 or below were shown to inhibit *C. perfringens* spore germination during extended cooling from 54 to 7 °C of up to 15 hours (Juneja et al., 2013).

Effects of temperature

Rapid *C. perfringens* growth is observed from 43 to 46 °C (Brynestad and Granum, 2002). As described in Table 5, some experiments found no growth of *C. perfringens* in ground beef held at 0.6 °C and 4 °C for 26 and 28 days respectively (Ali and Vanduyne, 1981, Cosansu and Juneja, 2018). The minimum growth temperature for 13 *C. perfringens* isolates was found to be 12 °C, with a maximum of 53.3 °C.

Table 5: Effects of temperature on C. perfringens in different food matrices

Type of meat	Temperature, ºC	Time (hours if not specified)	Change reported	Magnitude of change (log_{10} CFU/g if not specified)	Reference
Beef tongues	-29	4 weeks	No change	-	Rothenberg et al., 1982
Beef livers	-29	4 weeks	Decrease	1.37 log_{10}/cm^2	Rothenberg et al., 1982
Beef	0.6	26	No change	-	Ali and Vanduyne, 1981
Beef	4	28	No change	-	Cosansu and Juneja, 2018

5.4.5 Listeria monocytogenes

Introduction

Listeria monocytogenes is a zoonotic, Gram-positive, facultatively anaerobic rod-shaped bacterium that does not sporulate. *Listeria* spp. are widely dispersed in the environment and can enter food-processing settings via incoming raw materials or the movement of personnel and equipment.

L. monocytogenes can cause listeriosis, the symptoms of which range from mild flu-like illness to septicaemia and bacteraemia which can be fatal, particularly to vulnerable groups. *Listeria* spp. other than *L. monocytogenes* are rarely pathogenic and as such are not considered a risk to human health but can be used as hygiene indicators.

Clinical manifestations associated with listeriosis can be grouped into two categories: invasive listeriosis and non-invasive listeriosis. Symptoms vary in infected people from mild flu-like or gastroenteritis symptoms, such as nausea, vomiting, fever, headache, myalgia and diarrhoea (non-invasive listeriosis), to more serious infections such as meningitis and other life-threatening complications (invasive listeriosis). Non-invasive listeriosis outbreaks generally involve the ingestion of high doses of *L. monocytogenes* by otherwise healthy individuals.

Invasive listeriosis is relatively rare in comparison to foodborne illnesses caused by other pathogens (see Figure 3) but is a serious disease with high fatality rates (20-30%) compared with other foodborne pathogens. This illness mainly affects vulnerable groups such as those over 60, those with underlying medical conditions (e.g. immunosuppression, HIV/AIDS and chronic conditions such as cirrhosis and diabetes that impair the immune system), infants and pregnant women (and their unborn child). Levels of miscarriage are around 30% and an *L. monocytogenes* infection can be asymptomatic in the mother. While the infectious dose is not known, and is reported to vary by 5 orders of magnitude for the vulnerable and non-vulnerable populations (EFSA Panel on Biological Hazards, 2018), an observation from an outbreak connected to contaminated ice cream estimated that hospital patients had 10,000 CFU per serving (Buchanan et al., 2017). Lower levels (circa. 100 CFU per g) are considered potentially hazardous in foods (Buchanan et al., 2017).

L. monocytogenes infection has a long incubation time, and it can take up to 90 days for the onset of listeriosis or development of symptoms, but, in some cases, symptoms can appear after a few days only (Goulet et al., 2013). The confirmed cases of listeriosis in the UK shows a slightly downward trend over the years (Figure 3).

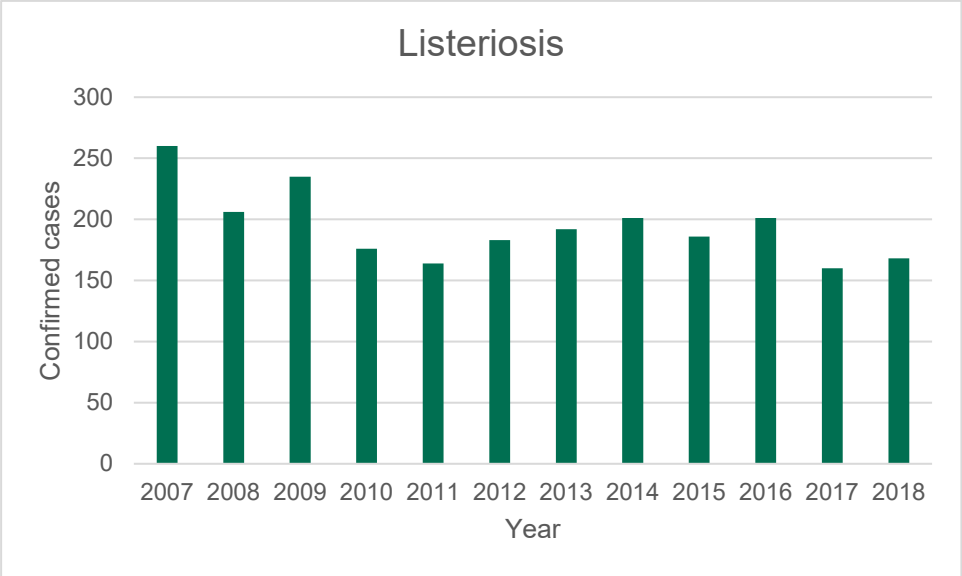

Figure 3: Confirmed cases of listeriosis in the UK from 2007 to 2018. Data from the Surveillance Atlas of Infectious Diseases, ECDC.

Food pathways

L. monocytogenes can form biofilms on food-processing equipment and food-contact surfaces, therefore persisting for prolonged periods in food-processing environments. *Listeria* spp. can also develop resistance to some biocides, increasing their persistence in biofilms and as environmental contaminants. Hence, a wide range of foodstuffs can become contaminated throughout the various stages of food production and distribution, particularly during the food-processing stage.

Although a wide variety of foods may be contaminated with *L. monocytogenes*, outbreaks and sporadic cases are predominantly associated with "ready-to-eat" foods as *L. monocytogenes* can grow at low temperatures (Walker et al., 1990). A recent UK study found that domestic refrigerators run at a range of operating temperatures varying from 1.1 to 11.4 °C (Evans and Redmond, 2016), with higher temperatures potentially allowing *L. monocytogenes* to grow significantly. Listeriosis is therefore usually associated with ingestion of refrigerated products such as contaminated milk products, meat or vegetable products that are RTE or eaten without being cooked properly.

Listeria spp. have been found in a range of chilled RTE foods, including: pre-packed sandwiches, pâté, butter and other milk products, mould-ripened soft cheeses – such

as Brie, Camembert, or others with a similar rind, soft blue-veined cheese, cooked sliced meats, crab meat, and cooked and cured smoked fish, including smoked salmon. Other foods implicated in foodborne *L. monocytogenes* outbreaks include salad, vegetables, and frozen vegetables, especially frozen sweetcorn.

Absence of *L. monocytogenes* in 25g is required by UK law in some foods, e.g. ready-to-eat foods intended for infants and those for special medical purposes; while for other ready-to-eat foods (including those able to support growth of the pathogen), *L. monocytogenes* should not exceed 100 CFU/g throughout the shelf-life. For the latter group of foods, growth under poor temperature and time control during this period should be taken into account.

Effects of temperature

L. monocytogenes is able to grow at temperatures ranging from <0 - 45 °C and pH values of between pH 4.2 and pH 9.5 (although optimal growth occurs around pH 7.0) and at a minimum water activity of 0.92 (Walker et al., 1990).
L. monocytogenes are readily destroyed by heat at recommended time-temperature combinations of 70 °C for 2 minutes or equivalent (FSA, 2018).

Effects of refrigeration

An important factor in the incidence of foodborne listeriosis is that *L. monocytogenes* can grow significantly at refrigeration temperatures compared to other pathogens (Chan and Wiedmann, 2009). Growth has been recorded at temperatures as low as -1.5 °C, although this is at a very slow rate (BFF, 2015) (Walker et al., 1990).

Several studies showed an increase of levels of *L. monocytogenes* under refrigeration temperatures in raw milk (Leclair et al., 2019), avocado pulp (Iturriaga et al., 2002), egg salad (Hwang and Marmer, 2007), pasta salad (Hwang and Marmer, 2007), roasted turkey (Jiang et al., 2011) and cucumbers (Bardsley et al., 2019) – see Table 6 for more detail. These increases were measured over 3 days to 8 weeks. For instance, Hwang and Marmer (2007) observed an increase of 3 log CFU/g in pasta salad and egg salad held at 8 °C over 9 days and 5 days,

respectively. However, after 24 hours at 8 °C, there did not seem to be significant growth.

Other studies at refrigeration temperatures showed no significant change in levels of *L. monocytogenes* in tomato juice, pasteurised milk, chocolate milk, processed guacamole, avocado pulp, beef frankfurters and camel milk.

A reduction was reported in tomato juice at 5 °C after 2 days, though no significant change in levels of *L. monocytogenes* was reported for the next 10 days (Diakogiannis et al., 2017). Reduction of *L. monocytogenes* was also observed in cut strawberry, peeled oranges and whole tomatoes after 4 days at 4 °C (Flessa et al., 2005). The authors suggested that the reduction of *L. monocytogenes* levels is due to the acidic characteristics of these food products.

Effects of freezing

L. monocytogenes can survive freezing – see Table 6 for more detail. Studies showed no change in levels of *L. monocytogenes* in broccoli and cauliflower frozen up to 168 days (Pinton et al., 2020), cheese frozen up to 30 days (Metzger et al. 2015), mango pulp frozen for 4 weeks (Penteado et al., 2014), feta cheese frozen for 4 weeks (Papageorgiou et al., 1997), cooked MAP shrimps frozen for 120 days (Mejlholm et al., 2005), and ground beef for 14 weeks (Palumbo et al., 1991).

Other authors reported a decrease of levels of *L. monocytogenes* under freezing conditions in avocado pulp (Iturriaga et al., 2002), whole and sliced cucumbers (Bardsley et al., 2019), processed guacamole (Iturriaga et al., 2002) tomato soup (Palumbo et al., 1991) and milk (El-Kest and Marth, 1992). However, some of the reductions seen (such as in the processed guacamole) may be caused by unfavourable characteristics such as low pH, or bacteriostatic agents added during processing.

Increased storage time in the freezer has been seen to lead to a lag time of a few hours, potentially due to injury of the cells, but growth rate did not appear to be affected (Humblot et al., 2015). However, Kataoka et al. (2017) found that growth

occurred without a significant lag phase once food had thawed. pH, water activity and the presence of bacteriostatic agents also have an effect on the lag time (Chan and Wiedmann, 2009).

Table 6*:* Effects of temperature on L. monocytogenes in different food matrices.

Food	Temperature, °C	Time	Change reported	Magnitude of change *	Statistically significant change?	Reference
Tomato juice	5	2 days	Decrease	1.3 log_{10} CFU/ml	Yes	Diakogiannis et al., 2017
Strawberry, peeled oranges and whole tomatoes	4	7 days	Decrease	3	Yes	Flessa et al., 2005
Pork	4	24 hours	Decrease	0.08 – 0.14 log_{10} CFU/cm^2	No	Chang et al., 2003
Tomato juice	5	10 days	No change	-	No	Diakogiannis et al., 2017
Pasteurised milk	4	24 hours	No change	-	No	Pricope-Ciolacu et al., 2013
Chocolate milk	4	24 hours	No change	-	No	Pricope-Ciolacu et al., 2013
Processed guacamole	4 to 7	15 days	No change	-	No	Iturriaga et al., 2002
Avocado pulp	4 to 7	2 days	No change	-	No	Iturriaga et al., 2002
Camel milk	4	14 days	No change	-	No	Al-Nabulsi et al., 2016
Beef frankfurters	0.5	1 day	No change	-	No	Čaklovica et al., 2011
Beef frankfurters	0.5	15 days	Increase	3	N/A	Čaklovica et al., 2011
Raw milk	4	5 days	Increase	1	Yes	Leclair et al. 2019
Roasted turkey	4	1 week	Increase	2	Yes	Jiang et al., 2011
Pasteurised milk	4	3 weeks	Increase	3.1	Yes	Pricope-Ciolacu et al., 2013

Whole and sliced cucumbers	4	21 days	Increase	2.8-2.9	Yes	Bardsley et al., 2019
Pasta salad	4	21 days	Increase	3	Yes	Hwang and Marmer, 2007
Pasta salad	8	9 days	Increase	3	Yes	Hwang and Marmer, 2007
Pasta salad	12	5 days	Increase	3	Yes	Hwang and Marmer, 2007
Egg salad	4	10 days	Increase	3	Yes	Hwang and Marmer, 2007
Egg salad	8	5 days	Increase	3	Yes	Hwang and Marmer, 2007
Egg salad	12	3 days	Increase	3	Yes	Hwang and Marmer, 2007
Milk	-18	4 weeks	Decrease	0.21-0.42 log_{10} CFU/ml	N/A	El-Kest and Marth 1992
Avocado pulp	-18	58 weeks	Decrease	2	N/A	Iturriaga et al., 2002
Processed guacamole	-18	58 weeks	Decrease	3	N/A	Iturriaga et al., 2002
Tomato soup	-18	14 weeks	Decrease	-	Yes	Palumbo et al., 1991
Pork	-20	24 hours	No change	-	No	Chang et al., 2003
Broccoli and cauliflower	-18	Up to 168 days	No change	-	No	Pinton et al., 2020
Cheese	-20	2,7, 30 days	No change	-	No	Metzger et al. 2015
Mango pulp	-18	4 weeks	No change	-	No	Penteado et al. 2014
Feta cheese	-18	4 weeks	No change	-	No	Papageorgiou et al. 1997
Cooked MAP shrimps	-22	120 days	No change	-	No	Mejlholm et al., 2005
Ground beef	-18	14 weeks	No change	-	No	Palumbo et al., 1991

*** The units are log CFU/g unless otherwise specified**

Effects of thawing

While freezing has been shown to stop the growth of *L. monocytogenes* in various foods, regrowth of the pathogen can occur once the foods are thawed. Beauchamp

et al. (2010) investigated the effect of different methods of thawing on *L. monocytogenes* in frankfurters. Thawing for 24 hours at 7°C, at 22 °C for 8 hours or in the microwave for approximately 4 minutes did not have an effect on the level of *L. monocytogenes* (Beauchamp et al., 2010).

Similar results were reported in another study on cheese. Metzger et al. (2015) showed that the thawing treatments at 4 °C for 14 hours or 20 °C for 4 hours did not result in a significant difference in *L. monocytogenes* levels. Leclair et al. 2019, reported that raw drinking milk thawed overnight at 4 °C or held at 22 °C until thawed did not significantly change the levels of *L. monocytogenes* in the product. Similarly, turkey disks inoculated with *L. monocytogenes* and thawed at 4 °C overnight showed no significant difference in levels straightaway (Jiang et al., 2011).

Kataoka et al. (2017) also showed that a defrosting treatment of 24 hours at 4 °C or 8 °C in 25 grams of crabmeat, corn, green peas and shrimp did not significantly increase *L. monocytogenes* levels. Levels of *L. monocytogenes* did increase after storage at 8 °C for a prolonged period of time – however it is unclear from the study at what point the food was fully thawed, as time zero was the point when the food was taken from the freezer and placed at 4 °C or 8 °C. Therefore, it is not possible to say whether there was a significant increase in the 24 hours following thawing. The pH of the crabmeat, corn, green peas and shrimp was 7.2, 7.2, 6.8 and 7.5, respectively, which is close to the optimal pH for growth of the pathogen.

5.4.6 Salmonella spp.

Introduction

Salmonella are Gram-negative, non-spore forming rods that are facultatively anaerobic, and motile. The genus *Salmonella* is divided into two species: *Salmonella enterica* and *Salmonella bongori*. *S. bongori* has mainly been isolated from cold-blooded animals and is only usually a human pathogen in vulnerable groups such as immunocompromised individuals or infants. *S. enterica* has numerous serovars which account for over 99% of human *Salmonella* isolates. Henceforth, *Salmonella enterica* is simply referred to as *Salmonella*.

Salmonella serovars are commonly sub-divided into two subgroups based on disease symptoms: typhoidal and non-typhoidal. Typhoidal serovars, such as *Salmonella* Typhi and Paratyphi, cause typhoid fever, an endemic problem in the developing world due to poor sanitation. It is uncommon in the UK, with the majority of cases linked to foreign travel (PHE, 2018b). Non-typhoidal serovars, however, are commonly associated with foodborne infection and, based on the most recent data from Public Health England (PHE) published in May 2018, the major serovars in the UK are *S.* Enteritidis and *S.* Typhimurium, which together accounted for nearly 50% of lab-confirmed isolates in 2016 (PHE, 2018a). The number of confirmed cases of salmonellosis is summarised in Figure 4.

The symptoms of *Salmonella* infection can range from asymptomatic carriage to severe diarrhoea. The incubation period is typically between 6 and 48 hours. The principal symptoms of mild fever, nausea and vomiting, abdominal pain and diarrhoea last for a few days but can persist for a week or more. Whilst the illness is usually self-limiting, it can be more severe in vulnerable groups, including the elderly, young and immunocompromised, potentially leading to systemic infection and death.

The infectious dose is generally high at around 10^6 infectious cells; however, this varies between serovars and food vehicles. High fat foods consumed by vulnerable groups could have an infectious dose as low as 10-100 cells.

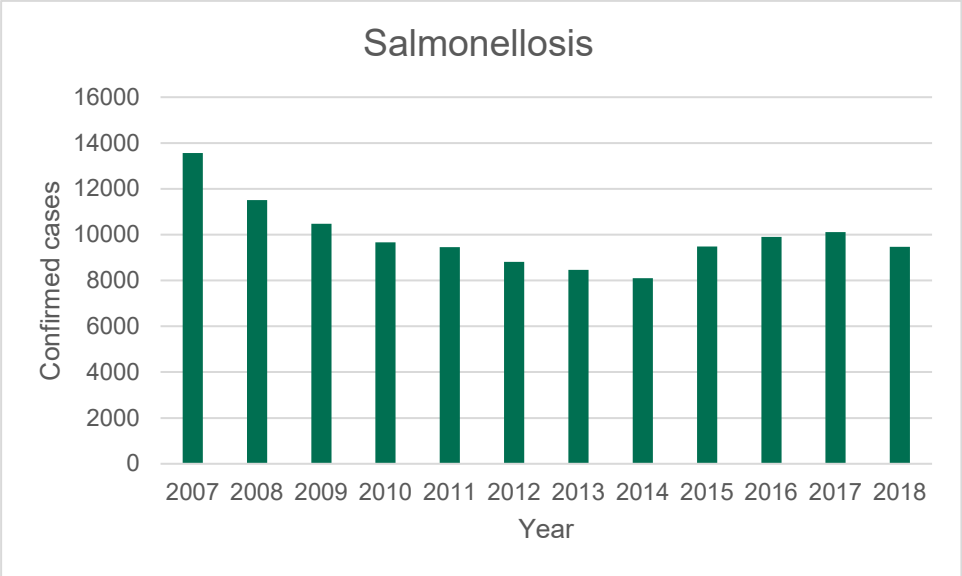

Figure 4: Confirmed cases of non-typhoidal salmonellosis in the UK from 2007 to 2018. Data from the Surveillance Atlas of Infectious Diseases, ECDC.

Food pathways

Transmission of *Salmonella* occurs via the faecal-oral route. The primary vehicles for *Salmonella* infection are animal products such as meat and dairy products due to under-processing or cross-contamination. Processing failures commonly associated with *Salmonella* contamination include temperature abuse, inadequate heat treatment and unhygienic handling. Many kinds of food can become contaminated from eggs to fruits and vegetables, and even dry foods, such as spices and raw tree nuts, though meat in general and poultry in particular are the most common sources of foodborne illness by *Salmonella* spp. (Smadi et al., 2012).

Effects of temperature

Salmonella growth has been observed between 5 and 47 °C with an optimum growth temperature of 37 °C. *Salmonella* are readily destroyed by pasteurisation temperatures and the standard 70 °C for two minutes cooking advice is normally sufficient (FSA, 2018). This is affected by the food matrix, however, for example in low water activity foods, such as peanut butter, the survival of *Salmonella* at 70 °C is increased (Beuchat et al., 2013).

The minimum water activity that permits growth of *Salmonella* is 0.94, however, cells are able to survive in dried foods for extended periods of time (Beuchat et al., 2013).

Cells exposed to desiccation are also more tolerant to heat, UV and chemical treatments. It has been reported that *Salmonella* can grow at pH 3.8-9.5 although the optimal pH for growth is 7. Chlorine and ozone-based treatments have been shown to reduce *Salmonella* counts in a variety of foods. UV treatment, curing and fermentation are also generally effective at reducing bacterial loads (Mandal and Kwon, 2017).

The effects of cold temperatures on *Salmonella* spp. in different food matrices are summarised below in Table 7. Most studies report either a decrease or no change in *Salmonella* spp. levels when stored at freezing or refrigerator temperatures. Pradhan et al., 2012 does report a significant change in *Salmonella* spp. levels in chicken products held at 8 °C, however this is over a longer period of time (7 days).

Table 7: Effects of temperature on Salmonella spp. in different food matrices.

Food	**Temperature, °C**	**Time**	**Change reported**	**Magnitude of change ***	**Statistically significant change?**	**Reference**
Beef	-22	75 days	Decrease	0.5 – 0.7	Yes	Manios and Skandamis, 2015
Cut pineapple	-20	7 days	Decrease	2	Yes	Strawn and Danyluk, 2010
Cut pineapple	-20	180 days	No change from day 7 to day 180	-	No	Strawn and Danyluk, 2010
Cut papayas	-20	21 days	Decrease	1.7	Yes	Strawn and Danyluk, 2009
Cut mangoes	-20	14 days	Decrease	2	Yes	Strawn and Danyluk, 2009
Cheese	-20	2 days	Decrease	>2		Metzger et al. 2015

Orange juice	-20	14 days	Decrease	1.2 log_{10} CFU/ml		Niemira et al. 2003
Raw tuna	-18	42 days	No change	-	No	Liu et al. 2016
Raw tuna	5-7	12 days	Decrease	1-2		Liu et al. 2016
Cut pineapples	4	28 days	Decrease	>4	Yes	Strawn and Danyluk, 2010
Pork	4	24 hours	Decrease	-	N/A	Chang et al., 2003
Cut mangoes	4 ± 2 °C	24 hours	No change	-	No	Strawn and Danyluk, 2009
Cut papayas	4 ± 2 °C	24 hours	No change	-	No	Strawn and Danyluk, 2009
Cut pineapples	4	24 hours	No change	-	No	Strawn and Danyluk, 2010
Chicken	4	20 hours	No change	-	No	Chaves et al., 2011
Chicken	0	7 days	No change	-	No	Pradhan et al., 2012
Chicken	4	7 days	No change	-	No	Pradhan et al., 2012
Chicken	0 and 4	2 weeks	No change	-	No	Bailey et al., 2000
Burgers	4 and 8	11 days	No change		No	Roccato et al., 2015
Pork	4	7 days	No change	-	No	Silva et al., 2016
Chicken	8	7 days	Increase	1.2	Yes	Pradhan et al., 2012
Beef	Thawing at 20 °C	16 hours	Increase	0.4 and 0.7	Yes	Manios and Skandamis, 2015
Beef	Thawing at 4 °C	12 hours	No change	-	No	Manios and Skandamis, 2015
Beef	Thawing in a microwave	24 minutes	No change	-	No	Manios and

						Skandamis , 2015
Kebabs	Thawing at 23 °C	overnig ht	Increas e	-	Yes	Roccato et al., 2015
Kebabs	Thawing at 4 °C	overnig ht	No change	-	No	Roccato et al., 2015
Beef	Thawing at 22 °C	9 hours	Increas e	N/A	N/A	Ingham et al., 2005
Beef	Thawing at 30 °C	9 hours	Increas e	N/A	N/A	Ingham et al., 2005
Whole chicken	Thawing at 30 °C	9 hours	No change	-	No	Ingham et al., 2005

*** The units are log_{10} CFU/g unless otherwise specified**

5.4.7 Shiga toxin-producing Escherichia coli

Introduction

Shiga toxin-producing *E. coli* (STEC) are a group of *E. coli* characterised by their ability to produce Shiga toxins. The two main types of toxin are Stx1 and Stx2, and these are split into three Stx1 (Stx1a, Stx1c and Stx1d) and seven Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g) subtypes. Shiga toxins are also known as verocytotoxins and the terms STEC and verocytotoxin-producing *E. coli* (VTEC) are synonymous. *E. coli* attaching and effacing (*eae*), Shiga toxin (*stx*) and cytolethal distending toxin (*cdt*) genes encode important virulence factors in diarrheagenic *E. coli* such as STEC (Hassan et al., 2018).

The symptoms of STEC infection can be variable, from asymptomatic to diarrhoea, abdominal pain, bloody diarrhoea, and haemolytic uremic syndrome (HUS), a serious condition that can lead to kidney failure and can be fatal. HUS develops in approximately 10% of patients infected with STEC O157 and is the leading cause of acute renal failure in young children. The incubation period is generally between 1 and 6 days, with an average onset of illness of 3 to 4 days.

All STEC strains should be regarded as potentially pathogenic and the serotype should not be considered a virulence criterion (ACMSF, 2018). The infective dose of STEC O157:H7 is estimated to be low (10 to 100 cells), and can result in serious illness, particularly in children and other vulnerable populations (Leclair et al., 2019).

The yearly number of STEC cases in the UK is given in Figure 5.

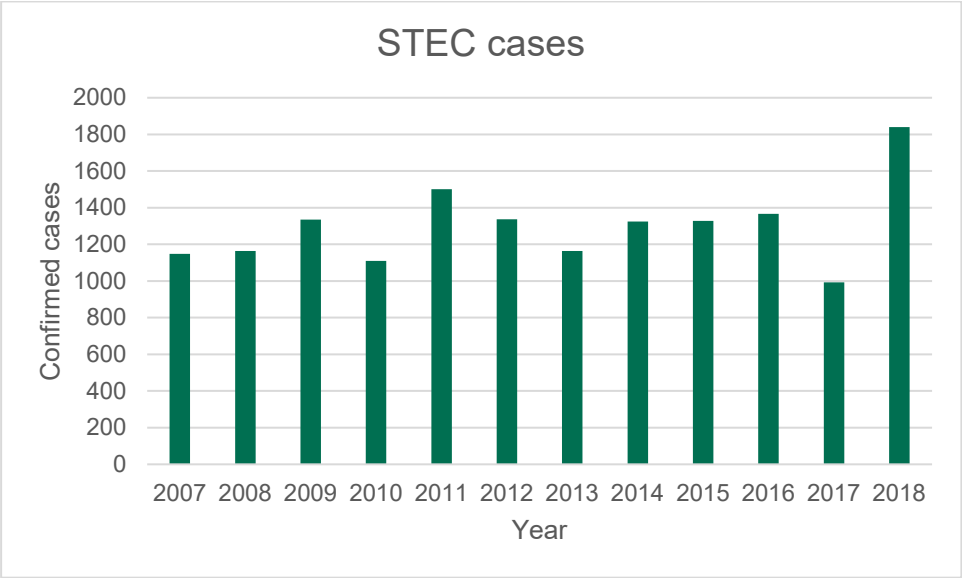

Figure 5: Laboratory-confirmed cases of STEC in the UK from 2007 to 2018. Data from the Surveillance Atlas of Infectious Diseases, ECDC.

Food pathways

STEC illness has been generally related to meat (beef, lamb and pork), but it can also occur due to consumption of other food types such as raw drinking milk, fruit, vegetables, raw milk cheese and fish. Beef cattle are the main reservoir of STEC.

Effects of temperature

STEC growth has been observed in the range of 7 - 50 °C, although growth has been also reported at 6 °C in minced beef (Tamplin et al., 2005) and at 6.5 °C in milk (Kauppi et al., 1996). Effects of temperatures on STEC in different food matrices are summarised below in Table 8. Storage at freezer or refrigerator temperatures generally leads to a decrease or no change in the level of STEC. Manios and Skandamis, 2015 also observed a small but significant increase in STEC levels after thawing beef at 20 °C for 16 hours.

Table 8: Effects of temperature on STEC in different food matrices

Food	Temperature, °C	Time	Change reported	Magnitude of change *	Statistically significant change?	Reference
Beef	-22	5 days	Decrease	0.7	Yes	Manios and Skandamis, 2015
Cheese	-20	2 days	Decrease	1.6	Yes	Metzger et al. 2015
Cut pineapple	-20	21 days	Decrease	1.7	Yes	Strawn and Danyluk, 2010
Cut mangoes	-20	14 days	Decrease	0.8	Yes	Strawn and Danyluk, 2009
Cut papayas	-20	7 days	Decrease	1.1	Yes	Strawn and Danyluk, 2009
Beef	Freezing storage	30 days	Decrease	1	N/A	Black et al., 2010
Beef	-20	90 days	Decrease	2	Yes	Keeling et al., 2009
Beef	-18	90 days	Decrease	0.8 – 1.7	N/A	Luchansky et al., 2013
Beef	-23	40 and 44 hours	No change	-	No	Dykes, 2006
RDM	-23	40 and 44 hours	No change	-	No	Leclair et al. 2019
Beef	-22	75 days	No change from day 5 to day 75	-	No	Manios and Skandamis, 2015
Cut pineapple	-20	180 days	No change from day 21 to day 180	-	No	Strawn and Danyluk, 2010

Beef	Freezing storage	28 days	No change	-	No	Bollman et al., 2001
Cut pineapple	4 ± 2 °C	10 days	Decrease	1.2	Yes	Strawn and Danyluk, 2010
Cut papayas	4 ± 2 °C	28 days	No change	-	No	Strawn and Danyluk, 2009
Cut mangoes	4 ± 2 °C	28 days	No change	-	No	Strawn and Danyluk, 2009
Beef	4	2 weeks	No change	-	No	Keeling et al., 2009
Beef	4	5 days	No change	-	No	Black et al., 2010
Beef	Thawing at 4 °C	12 hours	No change	-	No	Manios and Skandamis, 2015
Beef	Thawing at 20 °C	16 hours	Increase	0.7 – 0.9	Yes	Manios and Skandamis, 2015
Beef	Thawing in a microwave	24 minutes	No change	-	No	Manios and Skandamis, 2015
Beef	Thawing at 4 °C		No change	-	No	Luchansky et al., 2013
Beef	Thawing at 21 °C		No change	-	No	Luchansky et al., 2013
Beef	Thawing at 22 °C	9 hours	Increase	N/A	N/A	Ingham et al., 2005
Beef	Thawing at 30 °C	9 hours	Increase	N/A	N/A	Ingham et al., 2005
Whole chicken	Thawing at 30 °C	9 hours	No change	-	No	Ingham et al., 2005

*** The units are log_{10} CFU/g unless otherwise specified**

5.4.8 Shigella spp.

Introduction

Shigellae are Gram-negative, non-motile, non-spore forming, rod-shaped bacteria. *Shigella* species, which include *S. sonnei, S. boydii, S. flexneri* and *S. dysenteriae*, are highly infectious agents. Some strains produce enterotoxins and Shiga toxins, which are also produced by STEC O157:H7.

The illness caused by *Shigella* is shigellosis (also called bacillary dysentery). In healthy individuals, the disease usually consists of self-limiting diarrhoea, fever, and stomach cramps. Severe cases, which tend to occur primarily in immunocompromised or elderly people and young children, are associated with mucosal ulceration, rectal bleeding, and potentially drastic dehydration. Uncomplicated cases usually resolve in 5 to 7 days.

S. dysenteriae type 1 causes the most severe disease and is the only serotype that produces the Shiga toxin, which is responsible for cases in which haemolytic uremic syndrome (HUS) develops. *S. sonnei* produces the mildest form of shigellosis, usually involving water diarrhoea. *S. flexneri* and *S. boydii* infections can be either mild or severe.

The potential for illness following consumption of contaminated foods is relatively high, since the infective dose of *Shigella* may be as low as 10 to 500 organisms. In particular, *S. flexneri* has a low infectious dose of 10 to 100 organisms (Ranganathan et al., 2019). The number of confirmed shigellosis cases in the UK is shown in Figure 6.

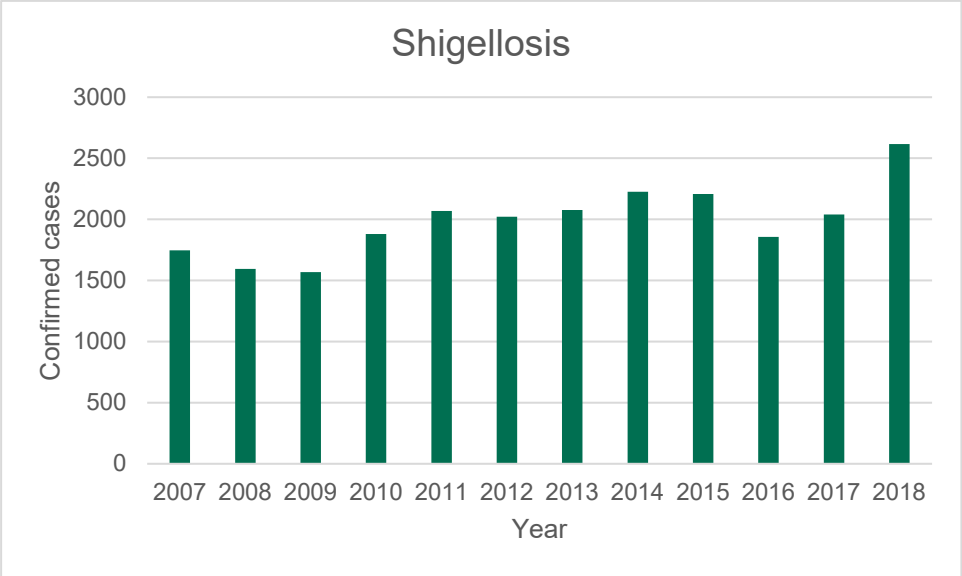

Figure 6: Laboratory-confirmed cases of Shigellosis in the UK from 2007 to 2018. Data from the Surveillance Atlas of Infectious Diseases, ECDC.

Food pathways

The faecal-oral route is the primary means of human-to-human transmission of *Shigella* spp. With regards to foods, numerous outbreaks have been associated with foods that are consumed raw, as well as with multiple-ingredients foods. Salads, milk and dairy products, and poultry are among the foods that have been associated with shigellosis (Zaika, 2001). Contaminated water is another vehicle for transmission of *Shigella* spp. and this can occur because of inadequately treated contaminated water used for drinking and food preparation (Warren et al., 2006).

Effects of temperature

Shigella spp. are tolerant of low pH and are able to transit the harsh environment of the stomach. These pathogens survive and, in some cases, grow in foods with low pH, such as fruits and vegetables (Bagamboula et al., 2002). They are also able to survive on produce packed under vacuum or modified atmosphere and in water, with only a slight decrease in number (Zaika, 2001). *Shigella* spp. grow at a temperature range of 10-40 °C with an optimum temperature of 37 °C (Schneider et al., 2012). Under frozen (-20 °C) or refrigerated (4 °C) conditions *Shigella* spp. can survive for extended periods of time but cannot grow (Warren et al., 2006).

6. Summary and conclusion

Shelf-life of food

The shelf-life of a food is the period of time for which it remains safe and suitable for consumption under specified storage and handling conditions. There are many factors that can affect the shelf-life of a food. These can be food product characteristics, food processing techniques, temperature, and the type of packaging.

In the UK, FBOs are responsible for setting the shelf-life of a food following consideration of the intrinsic and extrinsic factors, as well as consumer habits.

The inclusion of a safety margin when setting shelf-life is recommended in the guidance documents of other countries, but not specifically factored into UK guidance. While there is no legal requirement in the UK to consider a safety margin when setting shelf-life, Regulation No 2073/2005 states FBOs should ensure that "the food safety criteria applicable throughout the shelf-life of the products can be met under reasonably foreseeable conditions of distribution, storage and use", which should include freezing of the product by consumers on the use-by date.

Effects of freezing, defrosting and refrigeration on foodborne pathogens

Pathogens should not be present or exceed infection-causing levels on the use-by date, as food should still be safe to eat on that day. Therefore, the risk arises from the processes of freezing, thawing and subsequent refrigerated storage, and the potential for these to increase microbial levels. This was explored for foodborne pathogens of concern in the UK.

Although *B. cereus* spores are heat resistant, there does not appear to be increased risk from this pathogen as the lag time and growth rate at refrigeration temperatures are slow, making it unlikely that it will grow to levels capable of causing disease over a 24-hour period. However, the information on *B. cereus* growth during freezing and thawing is limited.

Campylobacter spp. should not be present in ready to eat foods. The bacteria do not multiply at refrigeration temperatures and the numbers are greatly reduced by freezing. In addition, the organism should not be present in ready-to-eat foods and would be readily destroyed by any subsequent cooking.

Literature on the effects of freezing and thawing on *C. botulinum* growth is limited. From a 2006 report on botulism cases in the UK, none appear to be related to frozen or thawed food (McLauchlin et al., 2006). A large proportion of these cases were linked to home-preserved or home-canned products, and products subject to temperature abuse. A review of commercially produced foods intended to be stored chilled also concluded that illness occurred due to time or temperature abuse or preformed toxin from an ingredient added to the chilled food (Peck et al., 2020).

C. perfringens does not appear to grow at refrigeration temperatures and has a minimum growth temperature of 12°C, therefore for the purposes of this review, there would not be an increase in risk from this pathogen.

L. monocytogenes can grow significantly at refrigeration temperatures compared to other pathogens, with growth promoted by higher refrigeration storage temperatures (8 -12 °C) compared to 4 °C. Lag time following defrosting also appears to be quite short. The studies summarised in section 5.4.5 show significant increases of 1 to 3 log CFU/g in some products, over a course of 5 days to 3 weeks. This rate of growth makes it unlikely that there will be a large change (1 log CFU/g or more) in levels of *L. monocytogenes* over the course of 24 hours at 8 °C. However, given that the infectious dose for vulnerable groups is unknown, but thought to be quite low, and the uncertainty around the effect of thawing on *L. monocytogenes* growth, it is not clear whether there may be an increased risk posed to vulnerable groups from RTE foods frozen on the use-by date compared to RTE foods frozen the day before the use-by date.

Salmonella spp. should not be present in RTE foods. Most data collected in this review show no change or a decrease in *Salmonella* spp. levels in refrigerated or frozen foods; where growth occurs at refrigeration temperatures, it is fairly slow –

1.2 log CFU/g over 7 days (Pradhan et al., 2012). Thawing according to recommendations (in a microwave or at refrigeration temperatures) does not appear to significantly increase *Salmonella* spp. levels.

STEC should not be present in RTE foods. No significant increase was found in a number of studies looking at STEC growth in frozen and refrigerated foods. Thawing according to recommendations (in a microwave or at refrigeration temperatures) does not appear to significantly increase STEC levels. The standard cooking advice of 70 °C for two minutes is sufficient to destroy STEC in foods such as raw meat which in most cases is intended to be thoroughly cooked before consumption.

Shigella spp. do not grow at temperatures below 10 °C and no studies were found on the effects of thawing. In addition, *Shigella* spp. should not be present in ready-to-eat products and would be eliminated by cooking.

Conclusion

In principle, FSA advice allows the freezing of products at the end of the use-by date and consumption within 24 hours of subsequent defrosting. If an FBO is not required to incorporate a margin of safety, the consumer may be exposed to additional risk presented by the growth of any foodborne pathogens present during the period that it takes to freeze the product, defrost the product and then store it for a further 24 hours under refrigerated conditions (8°C).

In assessing the risk, it is important to distinguish between RTE foods and foods that will be cooked prior to consumption. In the case of RTE foods, the levels present after thawing and prior to consumption will be the levels that the consumer is exposed to and which present the risk of infection. Keeping such food for extended periods after thawing or without appropriate temperature control will increase the risk. In the case of foods cooked prior to consumption, the risk relates to the presence of increased levels of pathogens following freezing and thawing which are not destroyed by the subsequent cooking process. The data indicates that although levels of some pathogens may increase during freezing, thawing and subsequent storage at 8 °C for 24 hours, such increases would be insignificant in comparison

to the reduction achieved by subsequent cooking and therefore the predominant risk is likely to be from RTE foods. For pathogens such as *B. cereus* and *C. botulinum* whose spores are more resistant to heat, there is likely to be limited growth (and toxin production) during freezing, thawing and subsequent storage at 8 °C for 24 hours.

There was little evidence to suggest a significant change in risk between consumers freezing RTE food on the use-by date compared to freezing the food on the day before the use-by date. RTE processed foods may also contain added substances which decrease the pH of the original products, contributing to preservation. Other bacteriostatic substances are also used in the food industry to inhibit the growth of pathogens, extending the shelf-life of the food products. However, a review of the literature on the effects of refrigeration, freezing and defrosting on *L. monocytogenes* showed that there may be potential for concern, particularly for vulnerable groups where the infectious dose is low. A very limited number of studies focused on the effects of thawing on *L. monocytogenes*, and further investigation would be beneficial to fully understand the risks to vulnerable consumers of *L. monocytogenes* growth in ready-to-eat food during defrosting in a domestic environment.

This work is based on the assumption that the food is safe to eat on the use-by date, which is a requirement of UK regulation, and it has not spent significant amounts of time in the danger zone (8 to 60 °C) if it is meant to be refrigerated. It is also important that thawing is carried out as recommended in a microwave, in a refrigerator or using cold potable water, rather than at room temperature, and that food is refrigerated or cooked straight after being defrosted.

In assessing the risk from consumer freezing of food on the use-by date, there are various other uncertainties that need to be considered. It is relevant to note that any pathogen growth will not be uniform as the organism will be subject to significant temperature fluctuations during freezing, thawing and then subsequent storage. The impact of such fluctuation on the metabolism of the organism and in particular whether any additional lag time is conferred is difficult to estimate but would have a significant impact on the estimate of the risk. The type of food under consideration

is another critical factor that will determine pathogen growth. The circumstances around handling, cooking, and storage will also influence the likelihood of illness. Therefore, the risk to consumers from freezing food on its use-by date can only be estimated on a product-by-product basis, and this report simply attempts to summarise the factors that need to be taken into consideration when making this assessment.

7. Future considerations

The work carried out is a strategic review rather than a systematic literature review. Therefore, it must be read as an overview of the potential issues related to freezing on the use-by date and there may be additional scientific papers on the effects of freezing, thawing and refrigeration on foodborne pathogens that have been overlooked in this review.

A more in-depth literature review was carried out in order to understand the risks associated with thawing foods in relation to *L. monocytogenes*. There is a lack of information on the effects of defrosting RTE foods on *L. monocytogenes* growth, therefore further research into the growth of *L. monocytogenes* in various ready-to-eat food items during the thawing process would be beneficial. A few studies reported that during freezing, bacterial cells may be protected from damage by certain solutes called cryoprotectants, a phenomenon known as cryoprotection. Glycerol and milk components are known to act as cryoprotectants in bacterial cell. Further investigation would be necessary to understand the role of these cryoprotectants, especially in dairy products.

Further studies could focus on *C. botulinum* as limited information is available especially on the effects of defrosting and subsequent refrigeration on the pathogen.

Further information on how industries set shelf-life would be beneficial to fully understand the process and the extent of the safety margin when determining shelf-life of RTE and non-RTE foods

Annex I

Literature search

Two databases were searched to retrieve relevant literature. These were PubMed and a database maintained by EBSCO: the Food Science Source. Returns were imported directly into the reference management software (Zotero 5.0.82, https://www.zotero.org/). Searches were conducted looking for keywords in the title and abstract.

The search string used for PubMed is shown below. Searches in other databases used similar strings but had minor syntax differences.

(meat OR raw meat OR sausage OR bacon OR burger OR kebab OR lamb OR pork OR beef OR mutton OR poultry OR chicken) AND (pathogen OR Escherichia coli OR VTEC OR listeri* OR STEC OR salmonell* OR campylobacte* OR bacill* OR clostridi* OR staphylococc* OR Yersinia OR toxoplasm*) AND (freez* OR thaw* OR defrost*)

Screening studies for inclusion or exclusion

To include only relevant returns, an automated sift using keywords was performed, first in the title and then in the abstract of each reference. Titles or abstracts in a language other than English were excluded at this stage. To ensure that results focused on meats, pathogens and processes of interest, literature which did not refer to a relevant combination of meat, process (freezing, thawing and chilled storage) and pathogen (including hygiene indicators) were excluded. Following title screening, a more specific screen of the abstracts was performed. This narrowed the search to screen out less specific papers. After key word screening 126 papers remained, which were then manually screened by abstract to determine suitability for inclusion. This process was performed independently by two FSA researchers in line with good practice guidance for systematic literature reviews. In the case of disagreements, papers were discussed until a consensus was achieved, with the default of continuing to include the paper in the next stage of the process.

After screening was completed, the full text of the 48 remaining papers was examined and assessed. The data were extracted and collated using a standardised system independently by two FSA researchers.

Annex II

Literature search

Two databases were searched to retrieve relevant literature. These were PubMed and Scopus. Resulting publications were exported directly. Literature searches were carried out in October 2020.

For the literature search, which aimed to collate all relevant literature regarding the effects of refrigeration, freezing and thawing on *L. monocytogenes*, the search used was: ((smoked OR meal OR food OR cheese OR dairy OR cooked OR vegetable OR fruit) AND listeri* AND (freez* OR thaw* OR defrost*))

Screening studies for inclusion or exclusion

A screen of the titles and abstracts of the papers was performed manually to determine suitability for inclusion. This process was performed independently by two FSA researchers in line with good practice guidance for systematic literature reviews. Papers considered irrelevant because they did not include information on the effects of freezing and chill temperatures on *L. monocytogenes* were excluded based on reviewer interpretation. In case of disagreement, papers were discussed until a consensus was achieved. At the end of this process, 19 papers covering the period from 1988 to 2020 were identified as suitable to include in this more in-depth literature review.

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