

Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the time-temperature combinations necessary for the safe cooking of foods and adequate temperatures for the hot-holding and reheating of cooked foods

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Abstract

Thermal treatment plays an important role in destroying pathogenic microorganisms in food. For this reason, both the temperature at which foods are cooked and the duration (time) thereof, have a special impact from a food safety perspective.

Another important aspect of food safety is the temperature for keeping cooked foods hot. Given that cooking does not inactivate spore-forming pathogenic bacteria, an inadequate temperature could lead to the microbial multiplication and, consequently, may constitute a risk factor. Most pathogenic microorganisms can grow in foods at temperature of between 5 and 60 °C, the range of temperatures that is considered a potential risk.

Refrigeration and subsequent reheating of cooked food before consumption are also factors to be taken into account, being necessary to refrigerate as soon as possible, maintaining refrigeration at an adequate temperature and reheating at a sufficient temperature to inactivate pathogenic

bacteria. Adequate refrigeration is essential to prevent the growth of spore-forming bacteria that have survived the initial thermal treatment.

During preparation, cooking and storage of cooked foods it is essential to maintain good hygiene practices, paying special attention to the cleaning and disinfection of utensil and equipment, and to handlers.

The recommended time-temperature combinations for cooking foods differ between different countries and institutions, much like the scientific publications. The Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) has reviewed the existing recommendations and analysed the effect of the temperature and time combination on the growth and destruction of the principal pathogenic microorganisms and parasites in different foods (meat, fishery products, eggs and egg products and vegetables). There is also reference, from a food safety perspective, to the cooking technique known as "slow cooking".

After this review, the Scientific Committee has proposed a series of time-temperature combinations for the cooking of meat, fish products, eggs and vegetables, considering the temperature to be reached in the centre of the product (coldest point). For the cooking of meat, it is recommended that a temperature of 70 °C is reached in the centre of the food for at least 1 second (or equivalent treatment); for poultry it is recommended that temperature be 74 °C for at least 1 second (or equivalent treatment). For the cooking of fish, it is recommended that a temperature of 68 °C is reached for at least 15 seconds in the centre of the product (or equivalent treatment); in the case of stuffed fish, the temperature to be reached in the centre of the product is 74 °C for at least 15 seconds (or equivalent treatment). Raw molluscs should be cooked at 90 °C for at least 90 seconds in boiling water (or equivalent treatment). The adequate internal temperature for the cooking of dishes containing eggs is 70 °C for at least 2 seconds (or equivalent treatment), which is sufficient treatment to not require the use of pasteurised egg products, and they should be maintained at 8 °C for a maximum of 24 hours. In the case of cooking eggs for immediate consumption, it is recommended that they are cooked so that the centre of the product reaches a temperature of 63 °C for at least 20 seconds (or equivalent treatment). This recommendation applies to different egg-based preparations such as fried eggs and omelettes which, on daily basis, may not set completely (provided they are served for immediate consumption). For the cooking of vegetables, a combination of 70 °C for at least 2 minutes in the centre of the product (or equivalent treatment) is considered adequate.

A minimum temperature of 63 °C is recommended for keeping cooked foods hot. If cooked foods are not to be kept hot, they should be refrigerated immediately, reaching a temperature of 4 °C in the centre in 2.5 hours and should subsequently be maintained at 4 °C or lower. For reheating cooked foods, a temperature of at least 74 °C should be reached in the centre of the product for at least 15 seconds. From a food safety perspective, the use of leftovers is not recommended. However, where they have been cooled and refrigerated in adequate conditions, they may be reheated at a temperature of at least 74 °C for at least 15 seconds in the centre of the product.

If cooking or reheating using a microwave, the time necessary is longer than indicated in the above sections.

All the above recommendations are applicable at all times provided that strict hygiene measures have been correctly applied and the previous stages have been carried out correctly (cooking, cooling, refrigeration).

Key words

Cooking, food safety, pathogens, hot holding, cooling, refrigerated storage, reheating, food service, slow cooking.

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1. Introduction

An important tool that we possess for destroying pathogenic microorganisms in food is thermal treatment. Numerous scientific works have discussed the effects of temperature on the inactivation of pathogenic microorganisms such as *Listeria monocytogenes*, *Salmonella* spp., or *Clostridium botulinum*, among others (Juneja et al., 2011). Therefore, the temperature at which foods are cooked, as well as the duration (time) are especially relevant from the point of view of food safety (Deak, 2014).

Improper cooking can lead to the survival of pathogenic flora and consequently, to the possibility of an outbreak of foodborne diseases. As a matter of fact, improper cooking has been highlighted as one of the possible causes behind outbreaks of foodborne diseases. Additionally, it has been demonstrated that incorrect hygiene practices as well as improper holding temperatures are factors that contribute to outbreaks of foodborne diseases (Lund and O'Brien, 2009) (Gormley et al., 2012). The epidemiological data on outbreaks of foodborne diseases have helped to identify five risk factors linked to the behaviours and practices of food preparation in preparation and sale establishments (FDA, 2017):

- Contaminated foods.
- Contaminated utensils and equipment.
- Lack of personal hygiene.
- Insufficient cooking.
- Improper holding temperatures.

Some foods such as eggs and egg products have been involved frequently in outbreaks of foodborne diseases (EFSA, 2017, 2019). For this reason, specific recommendations have been developed for these cases, such as substituting eggs for pasteurised egg products for when thermal treatment is not sufficient.

In the cooking recommendations on time and temperature, the cooking method must be considered, as for example, in the case of microwave cooking, the temperature distribution may not be homogenous, which implies the need to cook at higher temperatures or for longer periods of time (Szymczak and Dabrowski, 2015) (FDA, 2017). Special mention must be made of the "slow cooking" method where foods may be cooked for several hours (Burnham et al., 2006), under conditions for which there may not be sufficient scientific data on microbial inactivation.

Another highly important aspect of food safety is the hot holding temperature of prepared foods. Given that cooking does not inactivate sporulating pathogenic bacteria, an improper storage temperature may lead to microbial multiplication and consequently become a risk factor. Most pathogenic microorganisms may grow in foods at temperatures between 5 and 60 °C, a temperature range that is deemed potentially risky (Kim et al., 2013).

Special attention must be paid to the storage of prepared foods and their reheating before consumption, as it is necessary to refrigerate them as soon as possible, to hold them in proper refrigeration temperatures, and to reheat them to a sufficient temperature in order to inactivate pathogenic bacteria (Dudeja and Singh, 2017) (FSA, 2020). Proper refrigeration is essential to

preventing the growth of sporulating bacteria that have survived the initial thermal treatment (Poumeyrol et al., 2014).

Cooked foods must either be consumed immediately or held for a short period of time at proper temperatures, or chilled rapidly to prevent the growth of *Clostridium* spp. and other bacteria and reheated properly before consumption (EFSA, 2005) (FDA, 2017) (FSA, 2020).

This report is in response to the request for the opinion of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the following aspects related to biological risks:

- Proper time-temperature combinations for safely cooking foods, as well as the minimum temperature so that eggs do not have to be replaced with pasteurised egg products in the preparation of foods that contain them.
- Whether it is safe to serve eggs whose centres have not reached a temperature of 75 °C, in different preparations such as fried eggs or semi-solid omelettes, provided they are served for consumption after cooking within a time period to be determined by the Scientific Committee.
- The proper temperature for holding hot prepared foods.
- Proper time-temperature combinations for reheating food leftovers.

2. Recommendations regarding existing time-temperature combinations for cooking

The time-temperature recommendations for cooking foods vary according to countries and institutions, as well as scientific publications. In some cases, the recommendations vary depending on the food type. On the other hand, various studies have revealed the failure to comply with recommendations on cooking temperatures, making it necessary to establish training programmes for food safety which focus on correct food cooking practices (Brown et al., 2013).

Spanish legislation does not establish specific requirements regarding cooking temperatures, although it does so for hot holding and refrigeration. In this regard, Royal Decree 3484/2000 establishes that the hot holding temperature of prepared foods must be equal to or higher than 65 °C (BOE, 2001). This Royal Decree also refers to storage temperatures, establishing a low temperature of 8 °C for foods refrigerated for less than 24 hours and 4 °C if the duration is higher.

Different measures have been adopted to reduce salmonellosis outbreaks in food preparation and storage. With regard to the preparation and storage of home-made mayonnaise (prepared on site) and other foods to be consumed immediately where egg is an ingredient, Royal Decree 1254/1991 establishes that when preparing foods that do not follow a thermal treatment higher than 75 °C in their centres, eggs shall be replaced with pasteurised egg products, and in both cases, the foods shall be stored at a temperature of 8 °C until consumption within a maximum of 24 hours. Additionally, for mayonnaise, a pH lower than 4.2 is established for the final product (BOE, 1991).

In 1989, the World Health Organisation (WHO) published guidelines for handling foods in mass catering establishments (WHO, 1989). These guidelines recommended cooking meat, chicken, molluscs and crustaceans at a temperature of 70 °C in the centre of the product. Regarding the storage temperature, the guidelines recommended holding meat-based ready to eat meals at temperatures higher than 60 °C or lower than 10 °C. For reheating, it is recommended to reach an

internal temperature of 70 °C and to maintain this temperature for at least 2 minutes.

The *Codex Alimentarius* establishes that the cooking time and temperature must be sufficient to ensure the destruction of non-sporulating pathogenic microorganisms. For beef, a minimum temperature of 63 °C is established, which is increased to 74 °C for poultry. These thermal treatments are insufficient to inactivate sporulating pathogens such as *Bacillus cereus* or *Clostridium perfringens*, therefore the necessary measures must be taken during storage to limit the growth of said pathogens. The minimum hot holding temperature is set at 60 °C. For reheating, a temperature of 75 °C in the centre of the food or equivalent is established (Codex Alimentarius, 1993).

In the United States, the Food and Drug Administration (FDA) has made recommendations on time-temperature combinations for cooking foods in the Food Code (FDA, 2017). The FDA's Food Code is the basis of local legislation that regulates the requirements to be fulfilled by food preparing establishments. It includes recommendations to ensure that foods are cooked at combinations of temperature and time that are sufficient to inactivate pathogenic microorganisms. Depending on the food, the recommended temperatures vary in general between 60 and 74 °C. In the case of roasted meats, temperatures lower than 60 °C (55 °C) for prolonged time periods (89 minutes) are also included. It must be pointed out that this temperature should be reached in all parts of the food, therefore, it is indicated as internal temperature. In the case of chicken, the Food Code establishes that it must be cooked at a temperature of at least 74 °C for 1 second and that the final cooking temperature must be measured with a thermometer to ensure that this temperature has been reached. Table 1 summarises the recommendations included in the Food Code for different foods. The Food Code also establishes time-temperature combinations for reheating ready-to-eat meals, which are included in Table 2.

Table 1. Cooking temperature-times for different foods as recommended by the Food and Drug Administration

Food	Minimum temperature	Minimum time at specified temperature
Meat	70 °C	1 second
	68 °C	17 seconds
	66 °C	1 minute
	63 °C	3 minutes
Chicken	74 °C	1 second
Eggs prepared to be served immediately	63 °C	15 seconds
Eggs not prepared to be served immediately	70 °C	1 second
	68 °C	17 seconds
	66 °C	1 minute
	63 °C	3 minutes
Fish	68 °C	17 seconds
Meat, fish, chicken, pasta stuffing	74 °C	1 second

Roasted meat	55 °C	89 minutes
	60 °C	12 minutes
	65 °C	85 seconds
	69 °C	14 seconds
Foods cooked in microwave oven	74 °C	2 minutes

Source: (FDA, 2017).

Table 2. Temperature-times for reheating cooked foods according to the Food and Drug Administration

Food	Minimum temperature	Minimum time at specified temperature
Cooked, refrigerated and reheated foods	74 °C	15 seconds
Foods reheated in microwave	74 °C	2 minutes

Source: (FDA, 2017).

In the United Kingdom, the Food Standards Agency (FSA) establishes a cooking temperature of 70 °C for at least 2 minutes or an equivalent thermal treatment (FSA, 2020). The time-temperature combinations established by the FSA are the following:

- 60 °C for 45 minutes.
- 65 °C for 10 minutes.
- 70 °C for 2 minutes.
- 75 °C for 30 seconds.
- 80 °C for 6 seconds.

With regard to the recommendations on holding temperatures for ready-to-eat meals, the FSA establishes a temperature of 63 °C (FSA, 2020).

In Ireland, the Food Safety Authority of Ireland (FSAI) establishes a cooking temperature of 75 °C in the interior of the food for 1 second, or 70 °C for 2 minutes, although it makes exceptions for certain cases (FSAI, 2018). For recommendations on holding temperatures for ready-to-eat meals, the FSAI establishes a temperature of 63 °C (FSAI, 2020). For reheating, it establishes a temperature equal to or higher than 70 °C in the centre of the product (FSAI, 2018, 2020).

3. Calculations regarding the time-temperature combinations for inactivating pathogenic microorganisms of interest in food safety

Generally, the time-temperature combinations are established on the basis of research data which link equivalent D-values to different temperatures, based on z values for the indicated conditions (Equation 1), according to the recommendations of the competent authorities. These data are used to determine how long the foods should be maintained at each of the indicated temperatures in order to ensure their safety. These recommendations assume that the foods shall be constantly maintained at this temperature or they shall remain for the stipulated times at temperatures that are equal to or higher than the recommended ones. If they are held at a higher temperature, the excess

heating is deemed an additional safety margin that would ensure the inactivation of the pathogenic agent of reference in question (Stumbo, 1973).

$$\text{Equation 1: } \log D(T) = \log D_{ref} - \frac{T - T_{ref}}{z}$$

where $D(T)$ is the D-value (decimal reduction time) at the studied temperature; D_{ref} is the D-value at the reference temperature; T is the studied temperature; T_{ref} is the reference temperature, and z is the temperature increase (°C) required to cause a decimal reduction in the $D(T)$ value.

Nevertheless, there are recommendations that are not based on said connections and may be established from empirical values. It is worth substituting these recommendations with different time-temperature combinations that may be deemed equivalent, which would give businesses greater flexibility in food production, based on available facilities or quality criteria. One example is the requirement to reach 90 °C for at least 90 seconds in boiling water to eliminate pathogenic microorganisms in unpurified live bivalve molluscs from B and C production zones, and which have greater levels of contamination (EU, 2004). The most important microbiological hazards associated with these products that have been identified are the Norovirus and the Hepatitis A virus (Messens et al., 2017). The European Food Safety Authority (EFSA) has published a predictive model based on isothermic inactivation data of the Hepatitis A virus in mollusc matrices, which lets us establish alternative processes equivalent to 90 °C/90 seconds. If an Appropriate Level of Protection (ALOP) is established by the risk managers, it may translate into a Performance Criterion (PC) and a Process Criterion (PrC). This would help to establish process criteria based on an F-value (equivalent processing time of a hypothetical isothermic treatment at a reference temperature) which would be more suitable than the time-temperature combination currently in place (EFSA, 2015).

Currently, there are mathematical tools that enable integrating the lethality achieved with dynamic (non-isothermic) temperature profiles, which may lead to replacing the rigidity inherent in pre-established combinations of time and temperature, such as ComBase (Baranyi and Tamplin, 2004) or Bioinactivation FE (Garre et al., 2018). Nevertheless, although there are easy-to-use applications, their use requires a technical training that may limit their application.

Establishing time-temperature pairings based on scientific criteria and ones that permit a wide selection of combinations by the operators may provide greater flexibility and enable compliance with recommendations for a large variety of cases (different equipment, different processing conditions for each food type, different cultural habits, etc.).

4. Effect of the time-temperature combinations in cooking food

Given that there are important differences in microbial composition and flora in different foods, as well as different cooking instructions, it seems appropriate to first explore the pathogenic microorganisms that are most frequently associated with said foods, the heat inactivation of the most relevant pathogens and toxins, as well as calculating the thermal treatment required.

4.1 Meat

The Food and Agriculture Organisation (FAO) defines meat as the flesh of animals intended for human consumption, mainly that which is derived from a series of animal species (for example, beef, small ruminants such as sheep, or goats, birds, pigs, and other species such as camels, deer, buffaloes or horses). Regulation (EC) No. 853/2004 defines meat as the edible parts of domestic animals of the bovine, porcine, ovine and caprine species, as well as domestic solipeds, poultry birds, lagomorphs, wild game and farmed game (EU, 2004).

Meat has the potential to transport pathogenic microorganisms to consumers, classically associated with zoonotic agents. Healthy animals act as reservoirs for these pathogenic microorganisms, without displaying symptoms or pathological changes. Nevertheless, they may contaminate the food chain in the production of meat, for example, during slaughter. In this regard, maintaining good hygiene practices is of vital importance during slaughter, as microbiological hazards are not eliminated during this process.

Bacterial microorganisms that may cause foodborne diseases and may constitute a risk in some meat products are *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., enterohaemorrhagic *Escherichia coli* (for example, serogroup O157), some serovars of *Yersinia enterocolitica*, *Clostridium perfringens*, *Staphylococcus aureus*, *Clostridium botulinum* and *Bacillus cereus*. Likewise, some enteric viruses have demonstrated zoonotic capacity, as is the case of the Hepatitis E virus and its transmission in pork or products thereof, or the rotavirus (type A) and its transmission in beef or products thereof. Besides, different types of parasites associated with the consumption of meat have been frequently described, such as different species of *Echinococcus* (*granulosus* or *multilocularis*), different species of the *Taenia* genus (mainly in their cysticercus form: *Taenia saginata* in beef or *Taenia solium* in pork), nematodes such as *Trichinella spiralis* and more recently, the emerging *Toxoplasma gondii*.

From the point of view of control by a culinary heating process, the species that may be of greatest interest are *Salmonella* and *L. monocytogenes*. *L. monocytogenes* is the non-sporulating bacteria that is most resistant to thermal treatment. The thermal treatments that inactivate *L. monocytogenes* also inactivate other pathogenic bacteria such as *Salmonella*. In this regard, *L. monocytogenes* has been proposed as a model microorganism for assessing thermal inactivation (ILSI, 2012). *L. monocytogenes* may exist as a saprophyte microorganism in the environment and traditionally, outbreaks of listeriosis in cattle and sheep have been associated with feeding them low-quality silage. Likewise, the presence of *L. monocytogenes* in apparently healthy livestock faeces has been described in many countries, both in farms and at the time of slaughter. *L. monocytogenes* may infect carcasses through the skin, fur or contaminated faeces, and from slaughter and skinning surfaces. Another bacterium to be monitored in cooked meat is *C. perfringens*. This sporulating bacterium usually isolates itself frequently in the surface of beef, ovine and porcine carcasses at the moment of sacrifice, although generally in low numbers (<200 cfu/100 cm²) and mainly as vegetative cells (ICMSF, 2005). Meat contamination arises from faecal material and soil and the dust from animal skin. Food intoxication is due to the survival of the spores in cooked meat and their considerable growth (higher than 10⁹ cfu/g) during improper chilling of the cooked product in anaerobic conditions

(maintaining for some hours between 15 and 50 °C) and subsequent consumption without culinary heat treatment.

Salmonella spp. is slightly resistant to high temperatures. Some serovars are more resistant than others. For example, *S. Senftenberg* is unusually heat-resistant, 10 to 20 times more resistant (Doyle and Cliver, 1990). Other factors that influence heat resistance include composition, water activity and the pH of the food that contains *Salmonella*. This microorganism is more resistant to dry heat than to moist heat and shows greater susceptibility to heat at extreme pH levels (Schuman and Sheldon, 1997). In general, the D-values of *Salmonella* at 60 °C range between 5-6 minutes for chicken, 5-13 minutes for turkey and 3-5 minutes for beef. In most studies, the z-value of *Salmonella* ranges between 5.0 and 6.5 °C. Likewise, the D-values for minced beef at 51.6, 57.2 and 62.7 °C are 61-62, 3.8-4.2 and 0.6-0.7 minutes, respectively (Goodfellow and Brown, 1978), and with a z-value of 6.2 °C (Orta-Ramirez et al., 1997) (Murphy et al., 2000). Juneja et al. (2001) conducted studies on thermal inactivation times in chicken, turkey and beef meat using a *Salmonella* cocktail that included eight representative and appropriate strains in accordance with the recommendations of the Food Safety and Inspection Service of the Department of Agriculture of the United States (Juneja et al., 2001); the D-values calculated for minced meat were lower and the z-values higher than those mentioned previously, and the D-values for chicken were lower.

The thermal inactivation of *L. monocytogenes* has been extensively studied, resulting in a wide range of D-values. The dynamics of inactivation may be influenced by different factors including the type of strain, the physiological status of the microbial cells, and the heating and recovery conditions (Smelt and Brul, 2014). The average D-values for *L. monocytogenes* at 60 °C in broths or buffer solutions (pH 7-7.5; water activity, a_w , 0.99-1.00), are around 1.32 minutes (Wang et al., 2015). Nevertheless, the required thermal treatment times are higher for meat products. D-values for *L. monocytogenes* Scott A at 60, 65 and 70 °C in a mixture of meat (20 % minced meat, 80 % water) are 2.54, 0.75 and 0.23 minutes, respectively (Boyle et al., 1990). Similarly, the D-values for *L. monocytogenes* Scott A at 51.7, 57.2 and 62.8 °C in minced lean beef meat (2.0 % fat) and fatty meat (30.5 %) were 56.1, 34.5 and 2.4 minutes for lean meat and 4.6, 0.5 and 1.1 minutes in meat with a high fat content (Fain et al., 1991), while z-values were 5.4 and 7.3 °C in lean meat and fatty meat, respectively. Likewise, Doherty et al. (1998) reported D-values of 3.14 and 0.33 minutes at 55 and 60 °C respectively, for *L. monocytogenes* in minced meat heated in vacuum packaging. It has been assumed in the food industry that the thermal inactivation survival of microorganisms follows first-order kinetics. Nevertheless, there is growing evidence to suggest it does not always follow a traditional first-order kinetics, especially during mild thermal treatment (Augustin et al., 1998) (Valdramidis et al., 2006). There is general agreement that D-values should be used with caution, as isothermal survival curves are not really log-linear (Peleg, 2006). Therefore, another interesting alternative is to use txD-values that describe the t time required for x reductions in logarithmic units (Valdramidis et al., 2005). Using this parameter, the deviations in the first-order kinetics are considered when estimating the effectiveness of a thermal treatment instead of excluding shoulders and tails (Heldman and Newsome, 2003) (Valdramidis et al., 2005). In this regard, the time required to achieve 6 reductions in logarithmic units (txD) for *L. monocytogenes* at 60 °C in a growth medium is

5.5 minutes (Valdramidis et al., 2005).

Although most meats and meat products undergo full thermal processing before consumption, some types of meat or meat products are often lightly cooked, leaving the meat raw in the centre, as for example in the case of burgers and other similar products. It is important to take special precautions for these products.

The thermal processing of foods at the industrial level can effectively destroy all vegetative forms of bacterial, viral and parasitic pathogens. Traditionally, the recommendations for the food industry have been the treatment of poultry meat and products thereof at an internal temperature of 68.3 and 71.1 °C, respectively, whereas the temperature is up to 63 °C for beef and products thereof (Orta-Ramirez and Smith, 2002). Nevertheless, an outbreak of *E. coli* O157:H7 in several states in the Pacific region of the United States led to modifying the recommendations for beef to time/temperature protocols of: 66.1 °C/41 seconds; 66.7 °C/32 seconds; 67.2 °C/26 seconds; 67.8 °C/20 seconds; 68.3 °C/16 seconds; 68.9 °C/13 seconds and >69.4 °C/10 seconds, and subsequently chilling to a maximum internal temperature of 4 °C in 2 hours (USDA-FSIS, 1993). It must be pointed out that this type of treatment is ineffective for the elimination of bacterial spores, therefore thermal treatment must be followed by specific chilling requirements to be fulfilled. As a result, roasted meats must be rapidly and continuously chilled so that the time at which they are kept at temperatures between 48.9 and 12.8 °C does not exceed a total of 6 hours, with continuous chilling until they reach a temperature of 4.4 °C.

With regard to consumers, the traditional recommendation has been to cook burgers until the internal colour becomes brown (USDA-FSIS, 1985). Colour changes in meat when heated occur at temperatures close to 60 °C, depending on the duration of the heating. Nevertheless, the centre of a fillet 15 mm thick, grill-roasted, barely reaches 40 °C. A temperature within the range of 40 to 60 °C, especially if applied for short intervals of time, will not eliminate even vegetative bacterial forms that are relatively sensitive to heat. Therefore, many microorganisms are able to survive, some of which have pathogenic capacity such as *Salmonella* spp., *Campylobacter* spp., pathogenic strains of *Escherichia coli*, *Yersinia enterocolitica* and parasites. For this reason, government agencies such as USDA-FSIS (*US Department of Agriculture-Food Safety Inspection Service*) have advised against colour-based measurements, recommending instead the use of a thermometer when cooking burgers (USDA-FSIS, 1997), and increasing the cooking recommendations for burgers to a minimum temperature of 71 °C (Taylor, 1992). In 2020, the USDA-FSIS established a series of minimum recommendations for different types of meat products, making some important changes to the recommended cooking temperatures for meats and meat products (USDA-FSIS, 2020). For example, it has recommended reducing the heating temperature for entire cuts of pork (pork fillets and chops or roasts) from 71.1 to 62.8 °C, measured with a food thermometer before withdrawing the meat from the heat source with the addition of a resting time of 3 minutes before cutting or consuming it. In this regard, "resting time" is defined as the amount of time that the product remains at the final temperature after being withdrawn from a grill, oven or other heat source. The USDA-FSIS has established that it is safe to cook pork cuts at 62.8 °C with a resting time of 3 minutes, or to cook them at 71.1 °C which is the previously recommended temperature, without any resting time.

For beef and lamb, the safe temperature remains unchanged at 62.8 °C, with the addition of a resting time of 3 minutes as part of their heating recommendations. In this aspect, it has been deemed worth having a single time-temperature combination for all meats, as it will help consumers to remember the cooking temperature needed to render meat safe for consumption. Table 3 lists the cooking recommendations for meat and meat products established by USDA-FSIS (USDA-FSIS, 2020).

Table 3. Temperature recommendations established by the USDA-FSIS (2020)	
Product	Minimum internal temperature and resting time
Beef, pork and lamb meat	
Fillets, chops, roasts	62.8 °C and allow to rest for at least 3 minutes
Minced meat	71.1 °C
Ham, fresh or smoked (uncooked)	62.8 °C and allow to rest for at least 3 minutes
Fully cooked ham (to reheat)	Reheat packaged cooked hams to 73.9 °C
Poultry meat	
Breasts, whole bird, legs, thighs, wings, giblets, and stuffing	73.9 °C
Minced poultry meat	73.9 °C

To conclude, it is recommended to monitor the heating of the meat at home not by its appearance and colour change but by monitoring its temperature. In this regard, it is especially worth monitoring the temperature in products where the muscular structure has broken down and meat-based masses are produced, as in the case of minced meat and meat products such as burgers.

One of the most important aspects to be taken into consideration regarding safe food processing, especially in their heating and even more so at home, is that the results and parameters obtained in laboratory experiments may vary from real culinary processing, it often being necessary to adopt processing with higher temperatures in real conditions (Kenney and Beuchat, 2004). Wang et al. (2015) showed that the thermal inactivation data based on laboratory experiments with broths reveal a clear overestimation of the degree of inactivation compared to what may be expected in foods cooked in real conditions, which may entail a high-risk scenario. Therefore, it is important to carefully validate the models and to consider the differences that may arise due to the composition, texture and physicochemical characteristics of the food matrix and the competing autochthonous microbiota described (Pin et al., 1999) (Miconnet et al., 2005). Likewise, there are often considerable differences between the results submitted by different research groups. These differences in D-values are mainly due to differences in meat composition and the use of different recovery methods used in *Salmonella* counts. Additionally, the differences in the size and volume of the samples of inoculated meat may affect the calculation of the thermal inactivation parameters.

Following the recommendations of different governmental agencies, when cooking meat, it is recommended that the centre of the product reaches a temperature of 70 °C for 1 second (or equivalent treatment). In poultry meat, the recommended temperature is 74 °C for at least 1 second (or equivalent treatment). Alternatively, if a resting time is considered, then in the case of beef and

pork (fillets, chops, etc.) it is recommended to reach a temperature of 63 °C in the centre of the product during 1 second, with a resting time of 3 minutes, while for poultry meat this temperature must be increased to 74 °C. These recommendations are formulated on the basis of the temperature reached in the centre of the product or its internal part, which is the coldest point of the product, as it must be ensured that the entire product reaches the recommended temperature.

4.2 Fishery products

Fishery products may be involved in foodborne diseases arising from bacteria, viruses and parasites (Safaeian and Khanzadi, 2018).

Pathogenic bacteria transmitted by fishery products are divided into two general groups: autochthonous bacteria, that is to say, those that are naturally present in water (*C. Botulinum*, pathogenic species of the *Vibrio* genus, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*, *Aeromonas*, *Plesiomonas* and *L. monocytogenes*), and non-autochthonous bacteria, generally present as a result of contamination due to waste waters exogenous to fish and fishery products, or due to incorrect handling in later stages, that is to say, by handlers or even end-consumers (*Salmonella*, *Shigella*, *E. coli* or *S. aureus*) (Nilsson et al., 2002).

In general, pathogenic bacteria present in fish, molluscs and crustaceans do not pose an important risk to health because they are not present at high levels and cooking reduces them to acceptable levels (the exception being when there is a greater accumulation of microorganisms such as, for example, *Vibrio* spp. in bivalve molluscs such as clams, oysters or mussels, which are often eaten raw). Refrigeration prevents or slows down the multiplication of the pathogens, while cooking inactivates and eliminates them. If the fish processing is mild, pathogenic agents may survive and be present in the final product. The current mode of consumption of raw fish or less-cooked products may enable these organisms to become commonplace in the list of foodborne pathogens (Rosnes et al., 2011).

In the studies conducted on *L. monocytogenes* in fresh fish, prevalences of 10 % have been detected in Spain (Herrera et al., 2006), 21.6 % in Poland (Wieczorek and Osek, 2017), 2.5 % in China (Li et al., 2019a). With regard to *L. monocytogenes* in crustaceans and molluscs, prevalences of 2.0 % and 2.3 %, respectively, have been highlighted (Li et al., 2019a). In general, the studies that have analysed the levels of *L. monocytogenes* in fresh fish have observed that they are below 2 log cfu/g (Jemmi et al., 2002) (Wieczorek and Osek, 2017), although some authors have found levels higher than 2 log cfu/g in some samples (McLauchlin and Nichols, 1994).

Various studies have been performed on *L. monocytogenes* in fish. The heat resistance of *L. monocytogenes* depends on the fat content and water activity. In this regard, Ben and Huss (1993) point to a higher D_{60} value for *L. monocytogenes* in salmon (4.5 minutes) than in cod (1.8 minutes). These values indicate that fat content may protect this bacterium against heat. The thickness of the fish pieces may also affect the cooking time required.

Non-proteolytic *C. botulinum* is important in the case of fish. Although the incidence of *C. botulinum* in fresh fish is generally low, in some areas, the incidence may be high, as *C. botulinum* type E is the most frequently isolated type (ICMSF, 1998) (Gram and Huss, 2000). The highlighted

$D_{82.2}$ values for this bacteria range between 0.4-2.4 up to 231 minutes (Lund and Peck, 2000). Given that non-proteolytic *C. botulinum* spores are not inactivated by pasteurisation, it is essential that the holding conditions of cooked fish are appropriate. Non-proteolytic *C. botulinum* may grow and produce toxins at temperatures of 3 °C (ICMSF, 1996). Different studies have pointed out that this bacterium grows slowly at 4 °C, but at 8 °C, the growth rate increases by almost five times (Graham and Lund, 1993). A temperature increase of 2-4 °C may result in the growth and production of toxins. Table 4 displays data on the production of toxins at different temperatures in fish inoculated with *C. botulinum* type E.

Table 4. Temperature-time for toxin production in fish inoculated with *Clostridium botulinum* type E spores

Fish	Inoculate/g	Temperature (°C)	Time for toxin production (days)	Reference
Cod	10 ²	10	6	Taylor et al. (1990)
Salmon	10 ²	12	6	García et al. (1987)
Salmon	10 ²	8	9	García et al. (1987)
Salmon	10 ²	4	21	García et al. (1987)

The transmission of viral diseases to human beings from the consumption of fishery products is especially related to the consumption of raw molluscs. Hepatitis A virus, Norwalk type virus, the Norovirus and other enteroviruses are especially significant. Thermal treatment is the only way to eliminate them. It is advisable to heat these higher-risk foods at a temperature of 90 °C for 90 seconds in boiling water (EU, 2004). Some authors even propose extending this time to 3 minutes before consumption (Flannery et al., 2014).

Fish parasites that can generate health problems in people are helminths belonging to the trematode, cestode and nematode classes, such as *Anisakis*. Thermal treatment for inactivation requires fish to reach a minimum internal temperature of 60 °C for 1 minute although it depends on the type of cooking and the size of the pieces. Thus, it is estimated that a 3 cm thick fillet must be cooked at this temperature for at least 10 minutes (EFSA, 2010).

It is well-known that one of the most important benefits of cooking fishery products is improved hygiene quality and safety by inactivating pathogenic microorganisms (Talab, 2014). Proper cleaning, refrigeration (temperatures below 4 °C) and cooking prevents problems related to food toxoinfection. Properly cooking fish reduces the risk of the presence of possible pathogens. Reaching the so-called "safety temperature" (70 °C), increases the possibility of eliminating microorganisms in the interior of the food, but it must be considered that this is not the case for all fishes, molluscs and crustaceans, as for example, mussels (Flannery et al., 2014). It is important to remember that the mode of cooking will affect their destruction. When fried, baked, steamed, or cooked in a water bath, high temperatures are reached in all these cases and therefore there is a high elimination rate of pathogens. If the fish is boiled, it must be fully submerged in water, and if it is cooked in the microwave, it is important to check that the entire piece has been homogeneously cooked.

Various studies have been performed on the effect of different cooking methods (frying, grilling and boiling) on the total count of microorganisms present in different fishes (El-Sheriff et al., 2011)

(Talab, 2014) (El-Lahamy et al., 2019). The results of these studies demonstrate that all cooking methods result in a significant reduction of the microbial load, and the reduction rate is highest, as expected, in the fried samples in comparison to grilled and boiled samples.

Some studies indicate that the minimum internal temperature for cooking fishery products must be 63 °C for 15 seconds. In the case of stuffed fishes, these must be cooked until they reach a minimum temperature of 74 °C for 15 seconds, and if the fish is ground, cut or minced, it must be cooked at 68 °C for 15 seconds. The FDA recommends cooking fish at temperatures of 68 °C for 17 seconds (FDA, 2017), this treatment is sufficient to inactivate *Anisakis* (USDA-FDA, 2020). When fish is microwaved, it must be at a minimum internal temperature of 74 °C (Rabiela, 2015).

In the case of molluscs, it is recommended to apply temperatures higher than 70 °C, as this temperature is insufficient to completely inactivate the virus and therefore it may pose a risk to consumers. In these cases, it is advisable to cook molluscs in boiling water (>90 °C) for a minimum of 90 seconds in order to inactivate possible infectious viruses.

Taking into consideration the available scientific information, it is recommended that fish be cooked at a temperature of 68 °C for 15 seconds, the temperature to be measured in the centre of the fish (or equivalent treatment). In the case of stuffed fish, the temperature to be reached in the centre of the product is 74 °C for 15 seconds (or equivalent treatment). In the case of raw molluscs, it is recommended to cook them at 90 °C for 90 seconds in boiling water.

4.3 Eggs and egg products

Eggs and egg products are susceptible to contamination by *B. cereus*, *S. aureus*, *L. monocytogenes* and *Campylobacter* spp., although they are most frequently involved in outbreaks of *S. Enteritidis* (EFSA, 2014). Salmonellosis has traditionally been associated with the consumption of eggs and it constitutes one of the riskier pathogenic agent/food combinations. Within the European Union, the prevalence of *Salmonella* in eggs is 0.1-0.4 %. Although its prevalence is low, there is still an elevated number of egg-related cases of salmonellosis in human beings as 45.6 % of all reported cases of salmonellosis have been due to eggs and egg products. This is especially because eggs are consumed and used in many dishes that are often not properly heated. Salmonellosis is the second-most reported foodborne disease in the European Union, involving 30.7 % of all cases reported. *S. Enteritidis* is responsible for 49.9 % of all cases of salmonellosis, followed by *S. Typhimurium* (13.0 %) and *S. Infantis* (2.3 %) (EFSA, 2017, 2019).

Eggs may be contaminated with *Salmonella* in different ways. The outside of the egg may be contaminated with faeces after laying, or the inside may be contaminated with *Salmonella* during laying if the reproductive tract was infected prior to egg development (Humphrey, 1994). If *Salmonella* is present on the outside of the egg, it may occasionally migrate to the interior through the porous shell, especially when the eggs have been recently laid or are in humid conditions (De Buck et al., 2004), but it is considered unusual in real-life situation, as opposed to laboratory studies. Extending the storage time of eggs may increase this risk, except when they are refrigerated (EFSA, 2014).

Thermal processing continues to be one of the most common and effective methods to inactivate *Salmonella* in eggs. Any bacteria (except bacterial spores) may be inactivated by cooking eggs

at an internal temperature of 70 °C for 2 minutes. Thermal treatments that are usually applied in cooking are temperatures of 65 to 68 °C during 5 to 6 minutes for the whole egg and the egg yolk. The treatments are milder for egg whites (55-57 °C for 2-5 minutes) (Baron and Jan, 2011), owing to the greater heat sensitivity of egg white proteins. These treatments are adequate to reduce vegetative flora by at least 6 log cfu in whole eggs or egg yolks (Baron et al., 2010). On this basis, different regulatory agencies have recommended for the specific control of *Salmonella*, that eggs should be cooked at an internal temperature of at least 71 °C, so that both the yolk and the egg white are solidified before serving (CDC, 2011) (FDA, 2016). When the temperature reaches 70 °C, the egg yolk coagulates and the ovomucoid protein denatures, thus the egg white acquires consistency. Nevertheless, many restaurants, especially gourmet restaurants, cook eggs at relatively low temperatures ranging around 60 °C (Vega and Mercadé-Prieto, 2011), in order to keep the egg white and yolk soft or slightly modified.

Thomas et al. (2006) estimated *Salmonella* reduction to be on average 12 log cfu (standard deviation 1) in full-boiled (10 minutes) or scrambled eggs (reaching 80 °C or fried on both sides for 1.5-2 minutes) while it was only 2 log cfu (standard deviation 0.5) in less-cooked eggs (boiled for 4 minutes, pan-fried with a liquid yolk, or microwaved for 50-90 seconds).

With regard to prolonged thermal treatments (at least 1 hour) at low temperatures (62-65 °C), in order to achieve more fluid textures, Machado et al. (2020) demonstrated that although it does not comply with the minimum recommendation of 70-75 °C, when contaminated eggs were processed at 62 °C for 60 minutes and *Salmonella* survival was checked, the results indicated that the temperature in the centre of the egg reached 61.7 ± 0.4 °C after 30 minutes, inactivating 7.7 log of *Salmonella* spp. After 30 minutes of cooking, the yolk remained liquid and the egg white became slightly opaque, showing that *Salmonella* inactivation was not related to the solidification of the egg white or yolk.

Recently, the FDA (2020b) has recommended the following time-temperature combinations for cooking dishes that contain egg:

- Until 74 °C in the microwave and then leave it covered for 2 minutes.
- At 68 °C for 17 seconds if not serving immediately.
- At 63 °C for 15 seconds if serving immediately.

However, Royal Decree 1254/1991, which establishes the regulations for the preparation and storage of home-made mayonnaise and other foods for immediate consumption that contain egg as an ingredient, stipulates that when preparing foods that are not subjected to thermal treatment of at least 75 °C in their centres, the eggs shall be replaced with pasteurised egg products (BOE, 1991).

Several experimental studies have been conducted on the heat resistance of *S. Enteritidis* (Table 5), owing to health and food safety concerns in the egg and egg product sector, and more recently, on *L. monocytogenes*, as it is known to possess greater heat resistance than *S. Enteritidis*. It continues to be difficult to compare the studies, given that heat resistance depends, among other factors, on the strain, the culture conditions, the size of the inoculation and the equipment used.

As observed in the Table, in the case of *S. Enteritidis*, at 50-55 °C, D-values display wide variability between studies, whereas at 60 °C, the values are between 0.17 and 1.1 minutes, and <6 seconds at

65 °C. The heat resistance of *Salmonella* is lower in egg white, followed by liquid egg and finally, in the yolk (Figure 1). Nevertheless, the addition of salt increases the heat resistance of *S. Enteritidis*, particularly 10 % of salt leads to increased D-values in yolk of 19, 49, 51 and 66 % at 53, 55, 57 and 59 °C, respectively (Kang et al., 2018). Similarly, Michalski et al. (2000) found increases of 66, 255 and 133 %, at 58, 61 and 64 °C respectively, also using 10 % of salt, a value that may be uncommon in cooking, but highlights that it is necessary to leave a safety margin.

Table 5. D-values (minutes) for different *Salmonella* serovars in egg and its components

	Liquid egg	Liquid yolk	Liquid egg white	Reference
<i>Salmonella</i> Enteritidis	D ₅₅ 3.9-6.4 D ₆₀ 0.22-0.44 D ₆₄ 0.22	D ₅₅ 21.0 D ₆₀ 1.1	D ₅₅ 1.5 D ₆₀ 0.2	Humphrey et al. (1990)
	-	D ₆₀ 0.55-0.75 D _{61.1} 0.27-0.35 D _{62.2} 0.21-0.30	-	Palumbo et al. (1995)
	D ₅₆ 7.9 D ₆₀ 0.62 D ₆₄ 0.07	-	-	Gurtler et al. (2013)
	D ₅₃ 3.62 D ₅₅ 1.75 D ₅₇ 0.66 D ₅₉ 0.40	D ₅₃ 5.21 D ₅₅ 2.28 D ₅₇ 1.04 D ₅₉ 0.54	D ₅₁ 2.52 D ₅₃ 1.10 D ₅₅ 0.61 D ₅₇ 0.28	Kang et al. (2018)
	-	D ₅₈ 1.83 D ₆₀ 0.69 D ₆₂ 0.26 D ₆₄ 0.096 D ₆₆ 0.036	-	Jordan et al. (2011)
	D ₅₄ 5.70 D ₅₆ 0.82 D ₅₈ 0.27 D ₆₀ 0.17	-	D ₅₂ 6.12 D ₅₄ 1.51 D ₅₆ 0.42 D ₅₈ 0.19	Jin et al. (2008)
	D ₅₀ 9.3-16.5 D _{52.5} 1.5-3.3 D ₅₅ 1.4-1.6 D _{57.5} 0.5-0.7	-	-	Muriana et al. (1996)
	D ₅₅ 12.39 D ₅₈ 1.5 D ₆₁ 0.20 D ₆₄ 0.04	D ₅₅ 10.36 D ₅₈ 1.49 D ₆₁ 0.27 D ₆₄ 0.09	D ₅₁ 7.66 D ₅₃ 2.76 D ₅₅ 1.23 D ₅₇ 0.47	Michalski et al. (2000)
<i>Salmonella</i> Typhimurium	D ₅₅ 2.3-4.7 D ₆₀ 0.20-0.26 D ₆₄ 0.15	D ₅₅ 8.0 D ₆₀ 0.8	D ₅₅ 1.0 D ₆₀ 0.3	Humphrey et al. (1990)
	-	D ₆₀ 0.67 D _{61.1} 0.20 D _{62.2} 0.14	-	Palumbo et al. (1995)

<i>Salmonella</i> Senftenberg	D ₅₅ 34.3 D ₆₀ 5.60 D ₆₄ 2.80	D ₅₅ 42.0 D ₆₀ 11.8	D ₅₅ 3.0 D ₆₀ 0.8	Humphrey et al. (1990)
	-	D ₆₀ 0.73 D _{61.1} 0.28 D _{62.2} 0.21	-	Palumbo et al. (1995)
Mixture of <i>Salmonella</i> species	-	-	D _{55.5} 2.74 D _{56.6} 1.44 D _{57.7} 0.09	Palumbo et al. (1996)
	-	D ₆₀ 0.28 D _{61.1} 0.16 D _{62.2} 0.087	D _{55.1} 7.99 D _{56.7} 2.96 D _{58.3} 1	Schuman and Sheldon (1997)

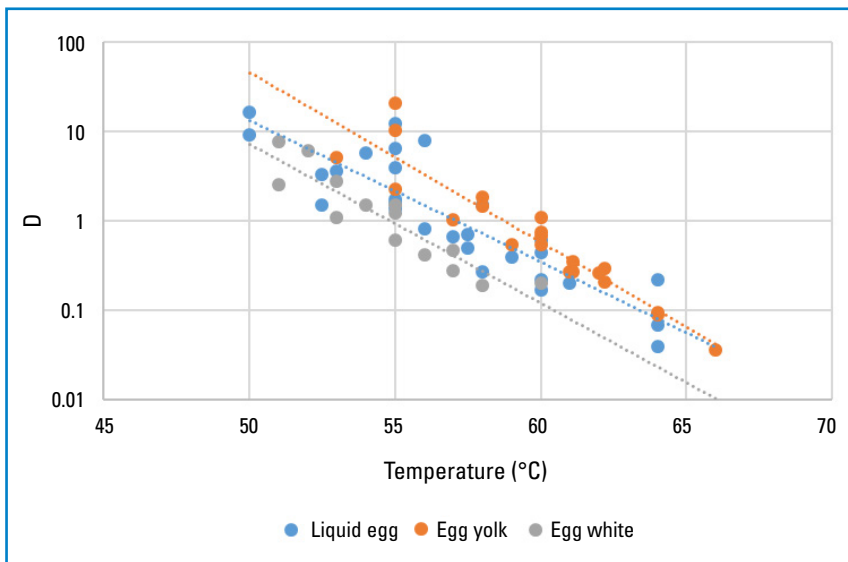


Figure 1. Variation in D-values depending on the thermal treatment temperature.

In general, it is necessary to increase the temperature by at least 4 °C to achieve a decimal reduction of the D-value for *S. Enteritidis* (Table 6).

Liquid egg	Egg yolk	Egg white	Reference
-	6.6	-	Palumbo et al. (1995)
3.7-4.2	-	-	Gurtler et al. (2013)
6.1	6.1	6.4	Kang et al. (2018)
-	4.7	-	Jordan et al. (2011)
4	4	5	Michalski et al. (2000)
4	-	4	Jin et al. (2008)

On the other hand, studies on the heat resistance of *L. monocytogenes* in eggs reveal greater resistance of this bacteria, therefore treatments calculated for *Salmonella* could permit the survival of *L. monocytogenes*, should it be present (Table 7).

Table 7. D-values (minutes) for *Listeria monocytogenes* in egg and its components

Product	55-56 °C	57-58 °C	59-60 °C	61-62 °C	63-64 °C	65-66 °C	Reference
Liquid egg	-	-	1.8-1.95	-	0.49-0.55	-	Muriana et al. (1996)
Egg yolk	-	-	-	0.70-2.30	0.35-1.28	0.19-0.82	Palumbo et al. (1995)
Egg yolk	-	-	1.34	0.89-0.58	-	-	Schuman and Sheldon (1997)
Egg white	13	12-8.3	-	-	-	-	Palumbo et al. (1996)
Egg white	7.58	4.76	3.47	-	-	-	Schuman and Sheldon (1997)

Nevertheless, and given that the presence of *L. monocytogenes* in eggs and egg products is less frequent, the safe cooking values are calculated based on *Salmonella*. A reduction of 5 log cfu is deemed an effective treatment for the expected presence of *Salmonella* in raw egg (CDC, 2011). Based on this safety value, and the values derived from Figure 1 on egg yolk as the most adverse case, it may be concluded that the proper treatments may be, taking into account the internal temperature:

- 121 minutes at 55 °C.
- 7 minutes at 60 °C.
- 37 seconds at 65 °C.
- 2 seconds at 70 °C.

Treatments in line with those proposed by the FDA (2020a, b). With regard to the value established in Royal Decree 1254/1991 (BOE, 1991), the internal temperature needed to avoid using pasteurised egg products may be set at 70 °C for 2 seconds, in which case, it is not necessary to immediately consume the cooked food, but it must be maintained at 8 °C for a maximum of 24 hours, as there is the possibility of cross-contamination after cooking, and *Salmonella* multiplies rapidly at room temperature, as shown in Figure 2.

Nevertheless, these values do not include the short periods that occur on a daily basis when cooking eggs that do not solidify completely. Given that the prevalence of *Salmonella* is low, and the levels in eggs have been quantified at 1-400 cells, normally with values lower than 20 cells (EFSA, 2014), milder thermal treatments that seek to reduce 2-3 log cfu, with immediate consumption

afterwards, may be considered. Based on the values displayed in Figure 1, these treatments may be, according to the internal temperature: 65 °C for 9 seconds, 63 °C for 20 seconds or 61 °C for 48 seconds, and equivalent treatments.

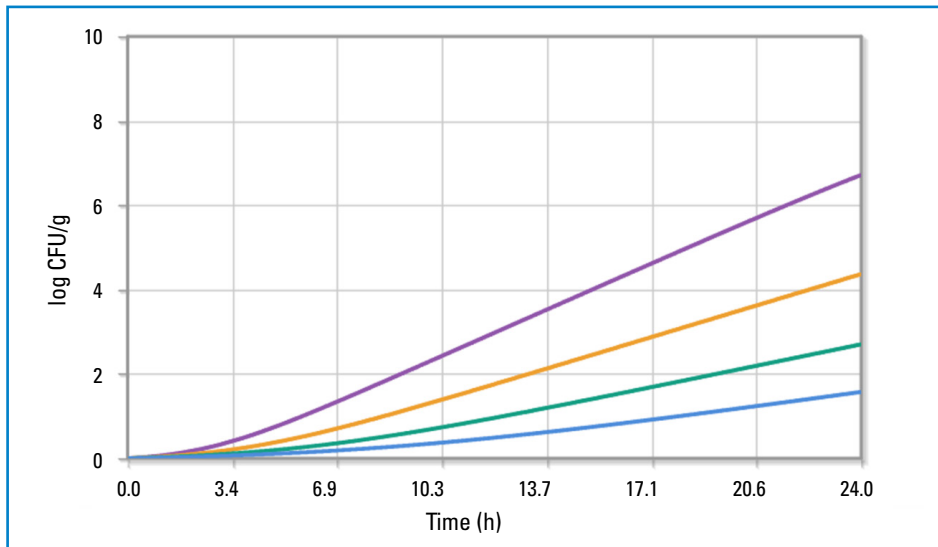


Figure 2. *Salmonella* growth in eggs at 25 °C, 30 °C, 35 °C and 40 °C.

Therefore, it may be considered that the proper internal temperature for cooking dishes that contain egg is 70 °C for 2 seconds (or equivalent treatment). This internal temperature is the temperature needed to avoid the use of pasteurised egg products, with subsequent holding at 8 °C for a maximum of 24 hours. In the event of cooking eggs for immediate consumption, the centre of the product must reach 63 °C for 20 seconds during cooking (or equivalent treatment). This recommendation is applicable to different egg-based preparations such as fried eggs or omelettes that may not be completely solidified on a daily basis, provided they are served immediately for consumption.

4.4 Vegetables

Foods of plant origin include fruits, leaf vegetables and fresh herbs, roots and tubers, dried legumes, grains, edible seeds, flours, seeds for sprouting and sprouts, nuts, spices and dried herbs. Given their diversity, the sources of contamination may vary significantly depending on the crop type and production systems. Possible sources of contamination in the environment, animals' access to the crops, the quality of the water for irrigation, soil decontamination, and hygiene conditions during and after the harvest, are considered determining factors for safety. Vegetables usually contain a greater variety and concentration of sporulating microorganisms, compared to fruits. Additionally, viruses may be transferred by food handlers.

By order of prevalence, *Yersinia* spp. (23.66 %), staphylococcus toxins (6.98 %), *L. monocytogenes* (2.68 %), *S. aureus* (1.71 %), *Campylobacter* spp. (0.73 %), *Salmonella* spp. (0.48 %) and enteropathogenic

E. coli (0.28 %) are the biological hazards in foods of non-animal origin. By number of outbreaks, noroviruses (34 %), *B. cereus* (23 %), *Salmonella* spp. (17 %), *S. aureus* (11 %), enteropathogenic *E. coli* (4 %), *Shigella* spp. (4 %), *C. perfringens* and *C. botulinum* are the main microorganisms involved. However, enteropathogenic *E. coli* has been responsible of the greatest number of cases in human beings in recent years, mainly due to the widespread VTEC O104 outbreak in Germany in 2011, linked to sprouted fenugreek seeds (3793 cases in human beings, 2353 hospitalisations and 53 deaths). Outbreaks linked to foods of non-animal origin are mainly related to the consumption of raw vegetables, whereas the number of outbreaks attributed to plant foods that include a treatment aimed at inactivating vegetative cells make up 24.1 % and those that include one or more cooked ingredients, 11.4 % of all outbreaks attributed to foods of non-animal origin (EFSA, 2013).

Food intoxication due to *B. cereus* has been frequently linked to thermally treated foods that favour the growth of this bacteria, especially in the case of storage at improper temperatures. Dishes of cooked pasta and rice, in addition to vegetarian meat substitutes, vegetable purees, potato salads, concentrated orange juice and onion powder, are foods that have been involved in the transmission of emetic *B. cereus* (EFSA, 2005).

Vegetables that are more mildly treated make up cooked or pasteurised foods. Cooking techniques include baking, boiling, grilling, steam cooking and frying. Thermal treatments vary according to the characteristics of each product. For example, in order to obtain the proper texture, many vegetables require at least a few minutes above 90 °C, and for some products, several minutes at 100 °C. The inactivation of the enzymes present in fruits and vegetables, a frequent prerequisite to obtaining a product with sensory stability for the duration of its shelf life, requires several minutes of treatment at temperatures beyond 80 °C. Vegetable products are pasteurised at many different temperatures, but they generally take several minutes at 70 °C or higher. These thermal treatments generally reduce non-sporulating bacterial pathogens by several logarithmic cycles (above 5), including *Salmonella*, *L. monocytogenes* and enteropathogenic *E. coli*, and the product must be refrigerated to limit the growth of bacterial spores of *Bacillus* spp. and *Clostridium* spp. that have survived the treatment (Nguyen-The and Carlin, 2000). In this case, it is not the cooking, rather the storage conditions that determine the risk, it being necessary to correctly hold hot foods or to quickly and correctly lower the temperature. Low heat treatment may even activate inactive spores that may sprout, grow and multiply if the products are chilled incorrectly (Juneja et al., 2018). In any case, *S. aureus* and *B. cereus* toxins are not destroyed by these treatments (EFSA, 2013).

Generally, foods with low water activity increase the heat resistance of foodborne bacterial and viral pathogens, a situation that generally does not occur in the case of vegetable products used in cooking. Additionally, the different pH values of foods have different D-values, and have different effects depending on the pathogenic microorganisms under study. Consequently, there is great diversity within the published data on the inactivation kinetics of foodborne pathogens, depending on their determining medium or matrix.

Table 8 displays the estimated D-values according to the predictive models included in Combase (2021) at different pH, assuming an a_w of 0.98 for the food to be cooked (except for *Salmonella*, where the a_w value is 0.997).

Table 8. D-values (minutes) for the most frequent bacterial pathogens in vegetables (values obtained in culture medium)

pH	Pathogenic bacteria	D ₆₀ (minutes)	D ₇₀ (minutes)	D ₈₀ (minutes)	D ₉₀ (minutes)	z (°C)
4.2	Non-proteolytic <i>Clostridium botulinum</i>	2795.8*	246.9*	10.0-38.5	1.0-4.1	9.5
	<i>Listeria monocytogenes</i>	0.32-1.24	0.04*	-	-	8.9
	<i>Escherichia coli</i>	0.80-3.24	0.01*	-	-	4.9
	<i>Salmonella spp.</i>	0.19-0.80	0.01*	-	-	6.1
4.6	Non-proteolytic <i>Clostridium botulinum</i>	5011.9*	423.6*	16.3-63.8	1.6-6.3	9.3
	<i>Listeria monocytogenes</i>	0.49-1.90	0.06*	-	-	8.4
	<i>Escherichia coli</i>	0.93-3.73	0.02*	-	-	4.9
	<i>Salmonella spp.</i>	0.24-1.00	0.01*	-	-	5.5
6.0	Non-proteolytic <i>Clostridium botulinum</i>	25 061.1*	1774.2*	57.7-230.8	4.7-18.6	8.7
	<i>Listeria monocytogenes</i>	0.97-3.85	0.07*	-	-	7.0
	<i>Escherichia coli</i>	1.02-4.11	0.02*	-	-	5.2
	<i>Salmonella spp.</i>	0.50-2.11	-	-	-	4.3

*Extrapolated values.

Since *L. monocytogenes* is the most heat-resistant vegetative pathogen, whose inactivation, as may be observed in the Table, guarantees the inactivation of *E. coli* and *Salmonella*, and considering a target of 6D for this microorganism, the recommended treatment would be a minimum of 23 minutes at 60 °C, 5 minutes at 65 °C, 30 seconds at 70 °C, 5 seconds at 75 °C, or 1 second at 80 °C. Keeping in mind the variability linked to the process of inactivation, as well as intraspecific differences, the time-temperature combinations for cooking vegetables provided by bodies such as the FSA (2020) are confirmed as suitable (Table 9).

Temperature (°C)	Required time
60	45 minutes
65	10 minutes
70	2 minutes
75	30 seconds
80	6 seconds

All equivalent and calculated for z= 7.6 °C.

With regard to the virus, Peng et al. (2017) showed a D₆₀ of 1-3 minutes for murine norovirus in foods such as spinach and strawberries. For the Hepatitis A virus, most authors showed a D of

several minutes at 60 °C, whereas Harlow et al. (2011) showed a D_{60} of 109 minutes or Gibson and Schwab (2011) of 74.6 minutes, in this case, a thermal treatment developed for the reduction of 6 log of *L. monocytogenes* is unable to reduce 6 log of the Hepatitis A virus. The z values for this virus would be in the interval 10-20 °C, in comparison to ~10 °C for bacterial spores (*C. botulinum*) and ~7 °C for vegetative cells of bacterial pathogens (*L. monocytogenes*) (Peng et al., 2017).

Finally, it is worth highlighting that the application of heat by microwaving has shown that longer treatments are required to achieve the same effect of *L. monocytogenes* inactivation as in conventional thermal treatments (Szymczak and Dabrowski, 2015).

Therefore, it may be considered that the proper temperature for cooking vegetables is 70 °C for 2 minutes in the centre of the product (or equivalent treatment).

5. Hot holding

There are certain differences regarding the recommendations on holding hot foods. Thus, the FDA recommends that hot foods should be held at a temperature higher than 60 °C. Even the FDA's Food Regulation (2017) mentions that this temperature should be above 57 °C.

In Spain, pursuant to Spanish legislation, and more specifically to Article 7 of Royal Decree 3484/2000 (BOE, 2001), the storage, preservation, transportation, sale temperatures and when applicable, temperatures in ready-to-eat catering that hold foods at a regulated temperature, must be ≥ 65 °C in the case of hot foods. However, the persons in charge of the establishments may set different temperatures, provided they are supported by scientific or technical evidence and have been verified by the competent authority.

B. cereus is the only aerobic sporulating pathogenic microorganism that can survive culinary treatment. The maximum growth temperature of *B. cereus sensu lato* described in the literature has been, for strains of the phylogenetic group VII, 58.1 °C (having estimated an interval of 57.1-59.2 °C as maximum temperature) (Carlin et al., 2013). Therefore, it would be unable to grow at temperatures exceeding 60 °C.

C. perfringens is an anaerobic sporulating pathogen that can also survive culinary treatment. The maximum growth temperature described in the literature has been 51 °C (Li and McClane, 2006) or 52.3 °C in strict conditions of anaerobiosis (Juneja et al., 2010), therefore it is unable to grow at temperatures higher than 53 °C.

In a recent study, Ricci et al. (2020) have shown that holding prepared foods at 62 °C for various days not only prevented the growth of different pathogenic microorganisms, but also reduced the concentration of *L. innocua* and *E. coli* by at least, 5 logarithmic cycles. With regard to *B. cereus*, there was no growth beyond 100 cfu/g in any of the cases.

Although based on a single study, this information does indicate that temperatures equal to or greater than 62 °C enable the hot holding of different foods without microbiological risks for several days. Additionally, *B. cereus* is unable to grow or produce toxins at temperatures equal to or higher than 60 °C (Carlin et al., 2013). Therefore, a temperature of 62 °C is the lowest temperature that guarantees the prevention of foodborne pathogenic microorganisms in holding, given that there is no comparable scientific data for lower temperatures.

In order to have a sufficient safety margin and given that we only have data from one study at 62 °C, it is recommended that hot holding temperature be at least 63 °C.

6. Reheating

There is little scientific information currently available on the reheating of prepared foods. We may especially consider the study by Ricci et al. (2020), the one highlighted by the ICMSF (1998) and the European Union Project EU-RAIN (Bolton and Maunsell, 2004).

In accordance with Bolton and Maunsell (2004), reheating is included within the list of Critical Control Points in mass catering (Table 10).

Critical Control Points	Critical limit
1. Chilling	The food must be placed in refrigerated storage within 90 minutes after cooking. That is, <10 °C in <150 minutes
2. Chilled storage	-1 to 5 °C
7. Reheating the food	≥70 °C (temperature in the centre) which must be reached immediately and served within ≤30 minutes

Source: (Bolton and Maunsell, 2004).

Regarding the control of cooked foods, the ICMSF (1998) also considers reheating to be a Critical Control Point for refrigerated and cooked foods, if there is any doubt about the storage time after cooking, or if it was chilled slowly. Temperatures of 70 °C or higher, with exposure times of at least 1 minute, should inactivate the levels of vegetative bacterial cells that are most likely present in foods with sufficient moisture, when handled correctly. However, these temperatures are insufficient for inactivating thermostable toxins.

We must point out that it is necessary to pay attention to the possible presence of thermostable toxins, given that enterotoxins developed by *S. aureus* (Bergdoll, 1979, 1989) and the emetic toxin produced by *B. cereus* are thermostable and not inactivated when foods are heated again. Attention must also be paid to other possible thermostable toxins produced by *E. coli* and other enteric pathogens (ICMSF, 1998).

We may emphasise that the *B. cereus* emetic toxin is a low molecular-weight peptide (molecular weight <5000 daltons) that is not an antigen but is extraordinarily resistant to heat (126 °C for 90 minutes), at extreme pH values (stable in the pH range between 2 and 11) and at enzymatic digestion, that is to say, resistant to trypsin and pepsin (Melling and Capel, 1978) (ICMSF, 1996).

A foodborne process that is greatly related to hot holding (already mentioned in earlier paragraphs) and should never be neglected is possible food intoxication from *C. perfringens* in some products that are heat-treated, insufficiently chilled and held at temperatures within the critical or risk range.

Another important point to be considered is microwave reheating and food safety, as it is necessary to extend reheating times in order to achieve the same effect as with other conventional heating methods (FDA, 2017).

Evidently, for the purposes of food safety, the ideal approach would be to not use leftovers (cooked foods that have not been consumed and have not been held at proper temperatures), given that various and sometimes complex factors may come into play, such as cross contamination, possible temperature abuses in storage, handler training, etc. As mentioned previously, compliance with Good Hygiene Practices or Guides to Correct Hygiene Practices must be rigorously implemented and enforced.

Special attention must be paid in connection with reheating to a culinary technique widely used today in mass catering, the so-called “cold line” or “complete cold line”, which entered the market a few years ago (Bouétard and Santos, 2009), and in many cases, reduces costs. Reheating is key to the organoleptic quality of the product, however the presence of “danger zones” or “temperature abuse” that could lead to the growth of foodborne pathogenic microorganisms must also be prevented.

The FDA recommends reheating cooked foods until the centre of the product reaches a temperature of 74 °C for 15 seconds (FDA, 2017). For fish, the Official Mexican Regulation (or NOM) sets an internal temperature requirement of 74 °C for 15 seconds (NOM, 2019).

A study conducted in Turkey (Däg, 2020) pointed out that when reheating foods, the centre of the product should reach a temperature between 75-80 °C and it should be maintained for 2 minutes, similar to the conditions recommended for cooking in the same study. Additionally, it stated that when chilling foods, the temperature should be quickly reduced to 21 °C in 2 hours and <4 °C in 4 hours.

Studies conducted on meat have noted that to prevent the growth of *C. perfringens* that may have survived the cooking process, it is necessary to quickly chill to 27 °C in 30 minutes and to 4 °C in 2.5 hours (Li et al., 2019b).

Taking into consideration the existing recommendations and the literature consulted, it is recommended that cooked foods be rapidly chilled, with the centre reaching temperatures of 4 °C in 2.5 hours and subsequent storage at temperatures of 4 °C or lower. It is also recommended to reheat ready-to-eat and conveniently refrigerated meals at temperatures of at least 74 °C in the centre of the product for 15 seconds, for food safety or innocuousness purposes. From the perspective of food safety, using leftovers is, in principle, not recommended. Nevertheless, if they have been chilled and refrigerated under suitable conditions, they may be used after reheating to temperatures of at least 74 °C for a minimum of 15 seconds in the centre of the product. The need for strict temperature control (time and temperature) as well as following good hygiene practices must be stressed.

7. Slow cooking

The system of slow cooking comes from the United States. Its origins go back to the 1940's, when women began working outside their homes and using this system ensured the food would be cooked and ready when they returned.

The basic design of a slow cooker (or crock-pot) consists of a ceramic or porcelain interior set on an electric heating base which permits cooking at low temperatures for long periods of time. Thus, cooking temperatures are, by way of indication, between 71-74 °C; although there are slow cookers that can reach 79-93 °C and always maintain a constant temperature. These cookers are hermetically sealed; therefore, the steam becomes liquid once again. It is necessary to ensure that

the crock pot ceramic is safe and easy to clean, for proper and safe cooking.

Crock-pots only have two cooking temperature settings (HIGH and LOW); they also have a heating function, which keeps the food hot after cooking. There are different brands available in the market, which makes it difficult to establish the temperatures in Celsius that correspond to the high and low settings. In theory, for some brands, the LOW setting can reach 90 °C, therefore temperature/time graphs in Celsius must be made available to consumers in order to control cooking safety, as many graphs are displayed in Fahrenheit units or even in the imperial system, given the American origin of this cooking system.

The USDA-FSIS (2021) has a webpage dedicated to slow cookers and their safety. Slow cooking is safe as the temperature range lies between 170 and 280 degrees Fahrenheit, or 76.6 to 137 °C. Given that the ceramic container is above the electric base and is fully covered, these two factors combine to lower the incidence of bacteria in cooking. In this regard, it would be worth having a clear idea of the temperatures used, as normally commercial establishments provide certain margins within which slow cookers work, as there is no correlation between the HIGH and/or LOW settings and different temperatures.

Additionally, there is a certain leaching of water-soluble vitamins, although they are concentrated in the liquid in question and therefore the loss is lower than in other traditional treatments.

The safety of slow cooking has been demonstrated in the study by Burnham et al. (2006), through a predictive model on *Salmonella* serovars, *E. coli* O157:H7 and *S. aureus*.

Conclusions of the Scientific Committee

- When cooking and handling foods, it is essential to maintain correct hygiene practices to avoid contamination and possible outbreaks of foodborne diseases. Special attention must be paid to personal, equipment and utensil hygiene.
- Contaminated foods and ingredients should not be used, nor ones whose provenance is unknown.
- To ensure safe cooking of the foods, it is worth establishing proper time-temperature combinations based on scientific criteria.
- Generally, meat should be cooked at a temperature of 70 °C in the centre of the product, for at least 1 second (or equivalent treatment); for poultry, the recommended temperature is 74 °C for at least 1 second (or equivalent treatment). In the case of beef and pork (fillets, chops, etc.) it is recommended to reach a temperature of 63 °C in the centre of the product for at least 1 second, with a resting time of 3 minutes, whereas for poultry meats, this temperature must be increased to 74 °C.
- Fish must be cooked at a temperature of 68 °C for at least 15 seconds (or equivalent treatment), which is the temperature to be reached in the centre of the product, although it depends on the cooking method. In the case of stuffed fish, the temperature to be reached in the centre of the product is 74 °C for at least 15 seconds (or equivalent treatment). Molluscs must be cooked at 90 °C for at least 90 seconds in boiling water (or equivalent treatment).
- The proper internal temperature for cooking dishes that contain egg is 70 °C for at least 2

seconds (or equivalent treatment). This internal temperature is needed to avoid the use of pasteurised egg products, with subsequent holding at 8 °C for a maximum of 24 hours.

- In the event of cooking eggs for immediate consumption, the centre of the product must reach 63 °C for at least 20 seconds during cooking (or equivalent treatment). This recommendation is applicable to different egg-based preparations such as fried eggs or omelettes that may not be completely solidified on a daily basis, provided they are served immediately for consumption.
- When cooking vegetables, the proper combination is considered to be 70 °C for at least 2 minutes in the centre of the product (or equivalent treatment).
- For holding hot prepared foods, a temperature of at least 63 °C is recommended.
- Ready-to-eat meals must be immediately chilled reaching a temperature of 4 °C in 2.5 hours.
- Ready-to-eat meals must be kept in chilled storage at temperatures of 4 °C or lower.
- Ready-to-eat meals must be reheated at temperatures of at least 74 °C for a minimum of 15 seconds in the centre of the product.
- From the perspective of food safety, using leftovers is not recommended. If they have been chilled and refrigerated under suitable conditions, they may be used by reheating to temperatures of at least 74 °C for a minimum of 15 seconds in the centre of the product.
- In the event of microwave cooking or reheating, the cooking time required is longer than that mentioned in the previous sections.
- All the previous recommendations are applicable provided the hygiene measures are strictly complied with and the previous stages are conducted correctly (cooking, chilling, chilled storage).
- Strict temperature and time control is required at all stages.

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