Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the conservation conditions of halved fruits in retail establishments

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Working group

Antonio Valero Díaz (Coordinator), Carlos Alonso Calleja, Pablo Fernández Escámez, Carlos Manuel Franco Abuín, Sonia Marín Sillué and Gloria Sánchez Moragas

Technical management of the report AESAN: Paula Arrabal Durán

Abstract

Regulation (EC) No. 852/2004 on the hygiene of foodstuffs, provides that food business operators are primarily responsible for food safety. In addition, it stipulates that food that cannot be stored safely at room temperatures shall maintain a cold chain and temperature requirements should be established based on a scientific risk assessment. However, limited periods outside temperature control are permitted, to accommodate the practicalities of handling, provided that it does not result in a risk to health. Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs lays down certain safety and hygiene criteria for pre-cut fruit and vegetables. However, European legislation does

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not lay down specific storage temperature conditions for these pre-cut fruit and vegetables. In this regard, the obligation to refrigerate may present practical difficulties in the case of bulky fruit such as melon, watermelon, pineapple, and papaya. The Scientific Committee has assessed whether it is possible to keep these bulky fruits halved in retail establishments at ambient temperature for a limited period, ensuring consumer safety. To this end, factors associated with the presence and growth of biological hazards, as well as published studies on pathogen prevalence, alerts and outbreaks of foodborne diseases, challenge studies and studies based on predictive microbiology models have been reviewed for each of the four fruits. Based on the available information, it is concluded that halved melon, watermelon, pineapple, and papaya stored at room temperature may pose a health risk because the physico-chemical conditions are compatible with the growth of foodborne pathogens, such as *Salmonella* spp., verotoxigenic *Escherichia coli* or *Listeria monocytogenes*. Melon, watermelon and papaya tolerate the growth of pathogens, while pineapple does not allow such growth, mainly due to lower pH levels. Despite the above, the storage of halved fruit at ambient temperature for short periods does not seem to have a significant influence on the development of foodborne pathogens, provided that it is accompanied by immediate subsequent cooling and that the product is consumed in a sufficiently short time. Therefore, temperatures <25 °C can be accepted for a time of <3 hours in a sufficiently ventilated and preserved of sunlight place, followed by continuous refrigeration storage at temperatures <5 °C (in the case of pineapple, such conditions could be re-evaluated). It is also recommended that fruits with an excessive degree of maturity, or with wounds or clefts on their surface, should be excluded for cutting. Finally, it is recommended that establishments in the sector follow scrupulous hygienic practices, monitor exposure and storage conditions and put in place the necessary measures to prevent possible cross-contamination.

Key words

Halved fruit, conservation, melon, watermelon, pineapple, papaya, predictive microbiology, *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*.

Suggested citation

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1. Introduction

As regards European legislation on food safety and hygiene, Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food legislation, establishing the European Food Safety Authority and laying down procedures in matters of food safety (EU, 2002) states, in its Article 17 on responsibilities, that food and feed business operators shall ensure, at all stages of production, processing and distribution in the enterprises under their control, that the food or feed comply with the requirements of food law relevant for the purposes of their activities and shall verify that those requirements are met.

For its part, Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs (EU, 2004) states in its Article 1, inter alia, several issues:

- Food business operators have the prime responsibility for food safety. Operators shall ensure food security throughout the food chain, starting with primary production.
- Food that cannot be safely stored at room temperature, in particular frozen food, shall maintain a cold chain.
- Microbiological criteria and temperature requirements need to be set on the basis of a scientific risk assessment.

In addition, Chapter IX of Annex II to that Regulation states that raw materials, ingredients, intermediate products and finished products which may contribute to the multiplication of pathogenic microorganisms or to the formation of toxins must not be stored at temperatures which may give rise to health risks, and, in addition, the cold chain must not be interrupted. However, limited periods outside temperature control due to practical handling needs during the preparation, transport, storage, display and delivery of foodstuffs shall be allowed, provided that this does not pose a health risk (EU, 2004).

For its part, Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for food products (EU, 2005) establishes the following criteria:

- Safety criteria for pre-cut (ready to eat) fruit and vegetables throughout their shelf life:
  - *Listeria monocytogenes* ≤100 CFU/g.
  - *Salmonella* not detected in 25 g.
- Hygiene criterion for pre-cut (ready to eat) fruit and vegetables during manufacturing process: *Escherichia coli* n= 5, c= 2, m= 100 CFU/g, M= 1000 CFU/g, where n is the number of units making up the sample and c is the number of sample units giving values between m and M.

However, European legislation does not lay down specific storage temperature conditions for pre-cut fruit and vegetables.

The *Codex Alimentarius* includes various Codes of Hygiene Practice and Standards regarding fresh fruits, the most prominent being the Code of Hygiene Practice for Fresh Fruit and Vegetables (CAC/RCP 53-2003) (Codex Alimentarius, 2003) and the International Code of Recommended Practice for the Packaging and Transport of Fresh Fruit and Vegetables (CAC/RCP 44-1995) (Codex Alimentarius, 1995). It also includes Standards for pineapple (Codex Alimentarius, 1993a) and papaya (Codex Alimentarius, 1993b). Since pineapple and papaya are tropical or subtropical fruits, these Codes and Standards are of obvious interest (particularly for the international market).

Currently, the Spanish Agency for Food Safety and Nutrition (AESAN) is preparing a Draft Royal Decree for the regulation of certain hygiene requirements for the production and placing on the market of food products in retail establishments, which aims, among other things, to regulate the conditions for the conservation of pre-cut vegetables and fruits in these establishments and which are sold directly to the final consumer.

The obligation to refrigerate pre-cut or peeled vegetables or fruits or ready to eat unpasteurised juices prepared in the retail trade at a temperature below or equal to 4 °C may present practical difficulties for the preservation of bulky fruits such as melons, watermelons or other fruits cut in half. Therefore, the Scientific Committee of AESAN has been asked for a report that determines whether it is possible to keep bulky fruits (in particular melon, watermelon, pineapple and papaya) cut in half in retail establishments at room temperature for a limited time, as well as to store them at the end of the day in refrigeration chambers, ensuring safety for consumers, and also indicating any other necessary considerations.

2. Brief description of the characteristics of each type of fruit and main varieties. Production and consumption data

The continuous promotion of healthy diets together with changes in the eating habits of the population has increased the consumption of fruits and vegetables in recent years. Spain remains the leading supplier of fruit and vegetables in the European Union, with approximately 30 % of the total marketed in 2019. Melon and watermelon, belonging to the Cucurbitaceae family, together with strawberries, are the most widespread fruits in Spain, accounting for around 40 % of total sweet fruit production (Mercasa, 2020).

The melon or *Cucumis melo* L. is the fruit of the melon, and has a rounded or oval shape, with a smooth or netted rind. Its pulp can be white, yellow, creamy, orange, salmon or greenish. There are hundreds of varieties of melon, which can be recognized for their shape, colour, taste, place of origin and genetic improvements. The best known and consumed in Spain are *Futuro, Categoría*, *Piel de sapo*, *Tendral*, Honey Dew, *Galia*, *Charentais* and *Cantaloupe* (MAPA, 2021a).

On the other hand, the watermelon or *Citrullus lanatus* L. is the fruit of the watermelon plant that produces a large fruit, a pepo, fleshy and juicy (>90 % of water content) with numerous seeds, almost spherical, greenish, with pink or red pulp, and usually sweet taste (more rarely yellow and bitter). Although there are more than 50 varieties of watermelons, two main genetic groups that can be distinguished: the traditional cultivated diploid watermelons, which produce black or brown seeds of woody consistency, and the triploid or seedless watermelons, which have a rind with green and yellow stripes, and white seeds of non-woody consistency (MAPA, 2021b).

Both types of fruit are widely consumed in Spain (7.8 and 8.1 kg of per capita consumption at home in 2019) due to their high-water content and low sugar content (6 %), as well as their valued organoleptic characteristics. In Spain, watermelon production is 1.2 million tons, while melon production is approximately 50 % lower (641 500 tons) (Mercasa, 2020).

At the same time, other subtropical fruit crops like papaya and pineapple are gaining ground in Spain due to their extended demand in the market.

Pineapple (*Ananas sativus*) is a plant in the Bromeliaceae family. It is a compound fruit (formed by the union of the fruits of several flowers around a fleshy axis) of great size, with a thick and hard rind, with brown scales and a very showy set of green leaves at one of its ends. It has a yellowish, aromatic, and sweet pulp with an acidic touch. Three botanical varieties of tropical pineapple are known: *sativus* (without seeds), c*omosus* (forms seeds that have germination capability) and *lucidus* (allows easier harvesting because the leaves have no thorns). If the ripening in the plant has been carried out correctly, the average carbohydrate content ranges around 11 % (MAPA, 2021c). In 2019, pineapple production gave way to exports exceeding 36 522 metric tons, with a per capita consumption in Spain of 1.9 kg (Mercasa, 2020).

Finally, papaya (*Carica papaya* L.) is a tropical fruit of high nutritional value (mainly rich in vitamins A and C) that belongs to the family Caricacaceae and is the third most produced tropical fruit in Spain, after mango and pineapple. The papaya has a pear shape, and its colour varies depending on the degree of maturity, from green to orange-yellow tones. Different varieties are cultivated in Spain, the predominant being one hybrid variety coming from Mexico, known as *Intenzza*. Other existing varieties are *Siluet, Sweet Sense*, *Tainung*, *BH-65* and *Caballero* (Hueso et al., 2019). The Canary Islands stand out as producing areas in Spain, and increasingly important are the peninsular coasts of southern Spain (Málaga, Granada, Almería, and Murcia), where the greenhouse cultivation of papaya is being developed in a novel way, to which about 575 hectares are allocated (Mercasa, 2020). The growth of the surface of papaya in Spain is due, on the one hand, to the need to look for alternatives that contribute to diversify our agricultural production and increase the supply of products to the European Union and, on the other hand, to the increasing consumption that tropical fruits are experiencing and the excellent results obtained with the introduction of new hybrid varieties of papaya more productive in the climatic conditions of Spain (Almodóvar et al., 2014) (Galán, 2014).

The products studied in this report correspond to watermelons, melons, pineapples, and papayas usually arranged for sale to the public cut in half.

3. Factors associated with the presence and growth of biological hazards in melon, watermelon, pineapple and papaya

Due to its physicochemical characteristics, the inner part of melon, watermelon, pineapple and papaya fruits may present microbial contamination if the cultivation, processing and storage conditions are not properly controlled.

In 2014, the Panel on Biological Hazards (BIOHAZ) of the European Food Safety Authority (EFSA) issued an opinion on the risk associated with pathogens in edible fruits of the cucurbits family, which are sweet and generally large, with multiple seeds, and include melons and watermelons (EFSA, 2014). The report identified some of the main factors associated with food safety for these products. Environmental factors influence primary production, particularly the proximity to animal husbandry operations and climatic conditions (e.g. heavy rainfall) that increase the transfer of pathogens from their reservoirs to melon and watermelon plants or the access of animals to growing areas. On the other hand, the use of untreated or insufficiently treated organic fertilizer or the use of contaminated water, whether for irrigation or agricultural chemicals application such as pesticides, and contamination or cross-contamination by collectors, food handlers and equipment in harvesting or post-harvesting activities, are also sources of contamination. Finally, mechanical damage during harvesting and subsequent handling also poses a risk, with washing water hygiene and washing water temperature being the most important factors favouring cross-contamination.

The proliferation of bacterial pathogens can occur because of delays in cooling melon and watermelon from room temperature (20-35 °C) to the recommended temperatures between 10 and 14 °C. When the rinds are wetted by cooling operations or by dew, they can allow the multiplication of foodborne pathogens on the surface of the rind. Edible portions of melon and watermelon pulp may become contaminated in the process of cutting or removing the rind because the knife blade may spread microbial contamination from the outer rind to the inner edible portions of the fruit. Finally, adequate cooling at the final sale points is crucial to slow down bacterial multiplication since the favourable pH for this to occur is from 5.10 to 6.70.

As for viral hazards, melons, watermelons, and pineapples may pose a potential risk of contamination by enteric viruses, especially norovirus, since these fruits are grown in the soil and their surface could potentially be contaminated with viruses through contaminated irrigation waters (FSA, 2019). Norovirus contamination on the surface of these products may be introduced into the fruit pulp during handling and cutting. In the case of papayas, the risk is lower as they grow in trees and are therefore less likely to be contaminated with norovirus, unless it is during handling or processing.

Finally, in relation to the possible presence of mycotoxins, there are no studies that refer to their natural presence in fruits such as melon, watermelon, or papaya. In the case of pineapple, one study noted the presence of fumonisins in pineapple juice and skin (Stepień et al., 2013). However, there is evidence of the presence of aflatoxins in melon and watermelon seeds consumed as such (Iqbal et al., 2018), and said presence generated a total of 10 notifications in the RASFF (Rapid Alert System for Food and Feed) during 2021 (RASFF, 2021).

Based on the scientific literature, the main intrinsic factors that traditionally influence the possible growth of pathogens during the storage of these products are pH, water activity, total sugars or Brix (ºBrix) degrees, and the acidity percentage, among others. The variety in the composition at the physicochemical level will be marked by the degree of maturity of the product, this being a major factor that influences changes under storage conditions (Singh et al., 2021). Likewise, extrinsic factors to this are the packaging conditions in the case of cut products and, above all, the temperature and storage time.

As regards intrinsic factors, the products under study generally have a water content >90 % and internal pH varying according to the type of fruit and variety. Overall, acid pH values tend to affect the growth and survival of foodborne pathogens. In melons, pH can range between 5.78 and 6.67 for the *Cantaloupe*, Honey Dew, *Cassava*, or Persian melon varieties; and in the case of watermelon, the pH can range between 5.18-5.60 (EFSA, 2014). More acidic pH levels (3.2-4.0) can be found in pineapple (Ma et al., 2016), while papaya is considered a product of low acidity, with pH values above 4.60 (Penteado and Leitao, 2004).

Other factors associated with the possible growth of pathogens are the phenomena of build-up of biofilms on work surfaces and their transfer to the product. Abeysundara et al. (2017) studied the growth and formation of *L. monocytogenes* biofilms on *Cantaloupe* melon variety pulp on various work surfaces. The conclusions obtained were that high levels of contamination and high temperatures favour the formation of biofilms and transfer from rubber, polypropylene, or polyurethane surfaces. During handling at the selling points, the possible transfer of pathogenic microorganisms that may be present in the fruit rinds to their interior during the cutting process constitutes a source of contamination in these products. Especially, cutting of surface contaminated fruits, along with the use of contaminated equipment and/or utensils, poor handling conditions, presence of wounds in the rinds or cross-contamination due to mixing with other fruits, are the most frequent factors associated with the occurrence of outbreaks of foodborne infections (Bowen et al., 2006) (Hanning et al., 2009). In this sense, Ukuku and Fett (2002) studied the behaviour of *L. monocytogenes* inoculated on the surface of *Cantaloupe* variety melon rinds as well as the efficiency of washing and disinfection treatments that aim to reduce microbial transfer from the exterior to the interior of the piece. Although at 4 °C there was no growth of the microorganism, *L. monocytogenes* was able to survive during extended storage times (15 days), and presented an obvious growth at temperatures of 8 and 20 °C. However, the competition with the microbiota present in these fruits has an important influence on the development of pathogens (Carlin et al., 1996) related to the availability of nutrients. For instance, it can be said that a high concentration of naturally occurring microorganisms in the product can delay the exponential phase and decrease the growth of *L. monocytogenes* (Koseki, 2015).

4. Main pathogens associated with the contamination of melons, watermelons, pineapples and papayas. Published Prevalence Studies

The most prevalent pathogens in melon, watermelon, pineapple, and papaya fruits are bacteria that contaminate the product during primary production or by cross-contamination, and that have the potential to grow during post-harvest. Among them, *L. monocytogenes*, *Salmonella*, *Bacillus cereus* and *E. coli* are the most prevalent, as described below.

The most recent bibliography on the occurrence of pathogenic bacteria in fresh fruits such as the ones covered in this report shows relatively variable results depending on the country or region in which they are conducted. Thus, we can mention some works such as the one of John et al. (2017), who reported the presence of 75 % of samples contaminated with *Salmonella* in a small number of watermelon and papaya samples purchased in Nigeria, which shows the high possibility of contamination by this pathogen, although none was contaminated with *Listeria*. High values of presence of *Salmonella* similar to those of John et al. (2017) are reported in other countries such as the Philippines, with up to 33 % incidence (Piano and Castillo-Israel, 2019). Jang et al. (2021) studied in South Korea, in fresh fruit cuts (including pineapple, watermelon and melon), *E. coli* (also including *E. coli* O157:H7), *Staphylococcus aureus*, *L. monocytogenes*, *Salmonella* spp., and *B. cereus*, among other dangers.

In 2018, a study in *Cantaloupe* melon marketed in the United States, in which 1075 samples were analysed, showed a prevalence of *Salmonella* of 0.19 % (0.02-0.67 %) and a prevalence of *L. monocytogenes* of 0 % (0-0.34 %) (Zhang et al., 2018). A recent study in Canada did not detect the presence of *Salmonella*, *E. coli* O157:H7, *Shigella* or *Campylobacter* in any of the 1788 samples analysed of melons, watermelons or papayas cut and presented refrigerated at the selling points. However, *L. monocytogenes* was detected with a prevalence of 0.62 % (0.34-1:10 %). Specific data for *Cantaloupe* melon, watermelon, green melon, and papaya were: 0.72 % (0.31-1.66 %), 15 % (0.03-0.86 %), 1 % (0.39-2.54 %) and 3.03 % (0.54-15.32 %) (Zhang et al., 2020).

In other countries outside the European Union there are some publications concerning *Salmonella*, showing a median prevalence of 0.5-0.8 % of the different studies, with the maximum percentage of 55 % of contaminated samples in a study in Mexico. Most of the existing data correspond to melons (particularly from North and Central America). Some of the studies show the result of sampling the surface of melons, which, unlike other food of non-animal origin (FoNAO) (for example, berries and tomatoes), is not consumed, although it may cause a risk of cross-contamination of the internal edible parts during cutting or damage to the fruit (EFSA, 2014).

On the other hand, there is no routine monitoring for the presence of *Salmonella* in the Member States of the European Union, and there is little data in terms of scientific publications. Available studies on the presence and levels of enteric bacteria such as *E. coli* in melons and watermelons are equally limited (EFSA, 2014).

In 2018, a study that evaluated the microbial contamination of the rind and pulp of 147 melons from international trade was published in Germany (Esteban-Cuesta et al., 2018). *Salmonella* spp., *E. coli* and *B. cereus* group isolates were found in the rind. (1.4 %, 0.7 % and 42.9 %, respectively) and in the pulp (0.7 %, 1.4 % and 4.7 %, respectively). *Clostridium perfringens* was isolated from the rind of 7 melons, while *L. monocytogenes* was not detected in any case. It was concluded that melons that have already been cut, either in halves or in smaller pieces, should always be stored refrigerated at temperatures of 4 °C or lower to prevent the growth of pathogenic microorganisms at the retail stage (Esteban-Cuesta et al., 2018).

Graça et al. (2017) studied the microbiological quality of 160 samples of fruits (including pineapple, melon, watermelon, and papaya) minimally processed in southern Portugal, including, in addition to the study of the counts of several microbial indicators, the investigation of *L. monocytogenes*, *E. coli* and *Salmonella*, without having found these pathogens in any of the samples.

In Spain, the 2019 *Informe de Análisis de Datos de Zoonosis de la AESAN* (AESAN, 2019), which presents the analytical results of the samples taken during 2019 by the autonomous communities, reported a total of 2 positives in 385 samples analysed (i.e. 0.52 %) for *L. monocytogenes*. In the case of *Salmonella*, 10 positives were detected on 387 samples (2.58 %). For *E. coli* VTEC or *Campylobacter*, although the number of samples analysed was clearly lower, no positive fruit samples were found.

The previous studies show that the contamination of the pulp of these fruits with opportunistic microorganisms and pathogens transmitted by food is possible, not only the rind is at risk of infection by numerous micro-organisms, but also the pulp may be contaminated, highlighting the need to control further growth.

5. Alerts and outbreaks of food poisoning associated with melon, watermelon, papaya and pineapple

5.1 Rapid Alert System for Food and Feed (RASFF) and Centers for Disease Control and Prevention (CDC) reports

There have been no alerts in RASFF associated with melon, watermelon, pineapple, or papaya in recent years. Only one alert associated with *Salmonella* contaminated melon seeds has been identified (RASFF, 2022).

The ECDC (European Centre for Disease Prevention and Control) and EFSA published in 2021 a rapid assessment of 1 outbreak of foodborne disease caused by *S.* Braenderup ST22. It was allegedly linked to *Gaul* melons imported from outside the European Union. A total of 348 confirmed cases were reported, with 68 hospitalizations and no deaths (ECDC/EFSA, 2021).

For its part, the CDC (Centers for Disease Control and Prevention) reported in 2018 and 2019 2 outbreaks of foodborne infections caused by *S.* Adelaide and *S.* Carrau, respectively, associated with the consumption of pre-cut melon (CDC, 2018, 2019). A total of 77 cases with 36 hospitalizations were reported in 2018 (CDC, 2018) and 137 cases, with 38 hospitalizations were reported in 2019 (CDC, 2019). They were investigated through epidemiological and traceability evidence.

In 2017, the CDC also reported 2 outbreaks of *Salmonella* in the United States associated with the consumption of papaya (whole fruits) (CDC, 2017a, 2017b). In one of the outbreaks, 220 cases occurred, with 68 hospitalizations and 1 death, due to the strains associated with the outbreak in 23 different States, which were: *S.* Thompson (144 cases), *S.* Kiambu (54), *S.* Agona (12), *S.* Gaminara (7), or *S.* Senftenberg (3) (CDC, 2017a). In a second outbreak, associated with *S.* Anatum, 20 cases, 5 hospitalizations and 1 death were reported (CDC, 2017b). Strains present in papaya fruits were identified and matched those of diseased individuals by whole genome sequencing (Whole Genome Sequencing, WGS).

Another outbreak caused by *L. monocytogenes* in 2011 was also identified, associated with the consumption of *Cantaloupe* melons, marketed whole, resulting in 147 cases, with 143 hospitalizations and 33 deaths (CDC, 2011).

5.2 Scientific literature

In Europe, there was one outbreak caused by *S.* Newport affecting the United Kingdom (UK), Ireland, and Germany in 2011. Sixty-three cases caused by this strain were confirmed. In a study carried out in the UK, the same strain of *S.* Newport was identified in ready-to-eat watermelon coming from Brazil, which was confirmed by WGS. A total of 27 out of 47 reported cases confirmed having consumed watermelon (Byrne et al., 2014).

A study from the United States was published in 2014, using CDC data on foodborne outbreaks from 1973 to 2011. During this period, 34 outbreaks were described due to melon consumption, with *Salmonella* being the main associated pathogen (56 %) followed by norovirus, responsible for 15 % of the outbreaks (Walsh et al., 2014).

In Brazil, foodborne outbreaks associated with fruits and plant products were published in 2014, and one outbreak associated with watermelon/cabbage was described, although the aetiological agent was not established (Elias et al., 2018).

In 2019, a paper was published that analysed in detail the foodborne outbreaks of *Salmonella* in the United States attributed to papayas imported in 2017, listed in the previous section with updated information once the investigation was completed. It was revealed that 4 outbreaks affected several states, and tests were carried out on papayas from different producers in Mexico. It was determined that a total of 4 outbreaks occurred, and different *Salmonella* strains were identified in 4 producing farms. The study made it possible to establish the source of the outbreaks and prevent the emergence of new cases (Hassan et al., 2019).

6. Effect of storage conditions on microbiological safety of pre-cut melon, watermelon, papaya and pineapple

As described in the previous sections, it is evident that the alteration of the fruits under study is influenced by a series of intrinsic, extrinsic, and processing environmental factors, which are relevant for the determination of the shelf life of these products.

One of the tools for the calculation of what is called safe shelf life lies in the determination of the growth potential (δ) of the main foodborne pathogens, which is defined as the difference in logarithmic terms between the levels of contamination at the end and at the beginning of storage. The determination of this growth potential is based on the performance of challenge tests (Behrsing et al., 2003) (Abadías et al., 2012) (Fajar Falah et al., 2015). These studies combine information from possible microbial growth with the evolution of certain physicochemical factors, for the calculation of shelf life, also considering the appearance of alterations in the quality of the product. Because the conditions of pH and water activity (a_w) can support the growth of pathogens, the food safety of this type of fruits cut in half depends especially on the storage conditions at the retail point. Most challenge tests conducted with enteric pathogens have focused on the influence of temperature, along with other factors such as type of product, gaseous composition, or combinations of pH and a_w. The conclusions reached in these studies relate to temperature as the limiting factor to allow microbial growth, although the growth rate of certain pathogens depends on the physicochemical characteristics of the product, which in turn may differ according to their degree of maturity, as well as the site of microbial contamination (rind, skin, or pulp).

Most of the studies found in literature refer to pre-cut melon, especially belonging to *Cantaloupe* and Honey Dew varieties, mainly due to their association with outbreaks of foodborne infections. Behrsing et al. (2003) tested the behaviour of *S.* Salford, *E. coli* and *Listeria innocua* after storage at various temperature/cooling time conditions (8-12 ºC/1-7 days). Among other results, they highlighted the importance of the level of inoculation in the growth capacity of *L. innocua* during storage, since even the lowest levels of contamination (10^3 CFU/g) were related to a growth of over 2 log CFU/g after a 7-day storage, while *S.* Salford and *E. coli* showed no growth. This fact indicates that the usually low counts found in this type of products would not allow the development of the microbial population of *Salmonella* spp. and *E. coli* in refrigeration. However, it should be noted that refrigerated storage does not prevent the growth of *Listeria* in pre-cut melon, given its psychrotrophic nature. Luciano et al. (2022) found that melon had the fastest growth of *L. monocytogenes* compared to other types of pre-cut fruits (mango and papaya) at both 4 and 8 °C, increasing the population between 0.78 and 1.19 log CFU/g after 7 days of storage. At temperatures of 16 °C, the increase in *L. monocytogenes* levels was higher than 5 log CFU/g after 10 days of storage.

In the case of watermelons, the physicochemical composition is similar to that of melon, given the same degree of maturity of both products (pH: 5.50-5.87; ºBrix: 10.25-11.25; acidity (%): 1.55-1.99; total sugars (%): 7.76-8.20), so initially, it could be thought that the microbial behaviour would be similar in the aforementioned storage conditions. However, Penteado and Leitao (2004) found that the growth of *L. monocytogenes* in a range of storage temperatures between 10 and 30 ºC was higher in melon, with increments between 0.04-0.35 log CFU/hour, while, for watermelon, these increases were between 0.02-0.30 log CFU/hour. The explanation for this may arise from other factors not considered in these studies or from small variations in some physicochemical parameters that may result in higher microbial growth in melon.

There are some studies where the growth of enteric pathogens in cut papaya, also considered as a low acidity fruit (pH> 4.60), is evaluated. Some challenge tests prepared on this cut fruit, despite being scarcer than those carried out on melon, show a growth of *L. monocytogenes* at refrigeration temperatures, although it is not as evident as in the other two types of fruit, due to its lower pH value. Penteado and Leitao (2004) found that this microorganism grows approximately 1 log CFU/g in 4 hours at 30 ºC, whereas a reduction of the temperature to 20 °C results in 5 times less growth. However, Luciano et al. (2022) obtained increases in cut papaya of between 0.78 and 1.19 log CFU/g during storage at 4 and 8 °C, although in this last study the pH value of the fruit was higher than 5.40, creating a favourable environment for the growth of *L. monocytogenes* (Colás-Medà et al., 2017). In fact, Zhang et al. (2020) report a higher prevalence of foodborne pathogens in fruits with pH> 5 (melon, watermelon, papaya) compared to other more acidic fruits such as pineapple.

As regards cut pineapple, the studies point to a higher acidity (6.18 g of citric acid/l) and lower pH (3.59) than the rest of the fruits, which prevents microbial development at both refrigeration and room temperatures of pathogens such as *E. coli* O157:H7 (Abadías et al., 2012). Likewise, under the tested conditions, there was greater inactivation in fruits packaged in modified atmosphere at refrigeration temperature, while at room temperature the microbial population remained constant. This fact is confirmed by other studies, such as the one by Huang et al. (2019) where a decrease in the populations of *L. monocytogenes* and *S. enterica* was obtained in cut pineapple kept in storage for 7 days, at static temperatures of 8 and 12 °C.

Other published studies evaluate the behaviour under non-isothermal conditions by simulating a scenario of storage at abuse temperatures followed by subsequent cooling of the cut fruit. The conclusions drawn from these studies suggest that the impact of these abusive conditions on the growth of *Salmonella* spp. or *L. monocytogenes* is influenced by storage at refrigeration temperatures over an extended period after exposure to abusive time and temperature conditions. In this sense, Huang et al. (2019) tested a storage at 35 °C for 2 hours, followed by a refrigeration at 4 °C for 7 days in cut melon of *Cantaloupe* and Honey Dew variety, as well as in cut watermelon and pineapple. The results showed a similar behaviour for both pathogens to the one obtained after an isothermal storage at 4 °C for 7 days, with a slight growth of *L. monocytogenes* at the end of storage (<1 log CFU/g) and a survival of *Salmonella* spp. throughout this period. In a similar study, Ukuku and Sapers (2007) studied the behaviour of *Salmonella* spp. in cut melon (*Cantaloupe* and Honey Dew variety) and watermelon during storage in refrigeration after exposure to 22 °C for 5 hours. Although refrigeration produced a slight decrease of microbial population (1 log CFU/g), storage for more than 3 hours at room temperature can increase the proliferation of *Salmonella* spp. in this type of product, thus compromising its safety. Finally, temperature fluctuations over a short period of time produce a substantial deterioration of the quality of the product, resulting in loss of colour and softening of texture (Huang et al., 2015).

In any case, it is assumed, therefore, that the physicochemical characteristics associated with melon, watermelon, and papaya (pH, acidity, sugar content, etc.) favour the growth of foodborne pathogens to a greater extent, so storage time at abusive temperatures should be limited in order to ensure their safety. For cut pineapple, it has been demonstrated that there is not enough information in the scientific literature showing growth of foodborne pathogens during retail or domestic storage. Studies carried out under non-isothermal conditions under different abuse scenarios show that exposure of these products for a period of more than 3 hours at room temperature can significantly affect their safety. It is therefore recommended that this period be shorter and always followed by refrigeration at temperatures below 5 °C, a limit also established by the FDA (Food and Drug Administration) for foods of plant origin (FDA, 2008).

7. Predictive microbiology models

Several studies have been published in recent years based on the use of predictive models or predictive microbiology, allowing a more accurate estimation of microbial behaviour based on various environmental factors under controlled conditions (Pérez-Rodríguez and Valero, 2013). Predictive models are therefore useful tools for food industry operators, as well as for the health authority to predict the shelf life under reasonably foreseeable storage and distribution conditions.

Several guidelines and technical documents are currently available for conducting challenge tests and predictive modelling in ready to eat foods, primarily focused on *L. monocytogenes* (Fang et al., 2013). Regarding fruits and vegetables, there are publications referring to studies of predictive microbiology in this type of products, mainly in melon and watermelon (Danyluk et al., 2014) (Huang et al., 2019), and being scarcer in pineapple and papaya (Ma et al., 2016). Table 1 summarizes the main studies of foodborne pathogens behaviour in melon, watermelon, pineapple, and papaya based on predictive models.

Table 1. Predictive microbiology models available for cut melon, watermelon, papaya, and pineapple

Product	Part of the product	Factors	Pathogen(s)	Model used	Reference
Cantaloupe and Honey Dew melon; watermelon	Rind, pulp	T °C (4-25)*	*L. monocytogenes*	Secondary: square root	Danyluk et al. (2014)

Table 1. Predictive microbiology models available for cut melon, watermelon, papaya, and pineapple

Product	Part of the product	Factors	Pathogen(s)	Model used	Reference
Cantaloupe melon	Pulp	T °C (4-25)	*Salmonella* spp.	Secondary: square root	Li et al. (2013)
Papaya	Skin and pulp	T °C (4-21), HR % (55-90)**, maturity rate % (0-75)	*Salmonella* spp.	Primary: Baranyi and Roberts/linear	Singh et al. (2021)
Pineapple	Pulp	T °C (4-28)	*Salmonella* spp.	Primary: Baranyi and Roberts Secondary: square root	Ma et al. (2016)
Canary yellow melon	Rind, pulp	T °C (5-35)	*S. enterica*	Primary: Baranyi and Roberts Secondary: square root	Scolforo et al. (2017)
Melon, watermelon and papaya	Pulp	T °C (10-30)	*L. monocytogenes*	Primary: linear	Penteado and Leitao (2004)
Cantaloupe melon	Pulp	T °C (4-43)	*L. monocytogenes*	Primary: logistics Secondary: cardinal model, Arrhenius	Fang et al. (2013)
Cantaloupe melon	Pulp	T °C (5-25)	*L. monocytogenes*	Primary: Baranyi and Roberts	Salazar et al. (2017)
Cantaloupe and Honey Dew melon; watermelon; pineapple	Pulp and extract	T °C (4-12) + 35 °C/2 hours	*S. enterica*, *L. monocytogenes*	Primary: not determined	Huang et al. (2019)

*T °C= temperature in degrees Celsius, **RH %= percentage of relative humidity.

In most cases, these predictive models consider storage temperature as a key factor for the study of microbial behaviour. Other factors such as pH or a_w are not generally included in these models since both factors have values that are compatible with microbial growth (except for pineapple), not observing a significant influence of them under conditions of commercial maturity.

The typology of models more widely collected in the bibliography are those of square root type, in a range that goes from cooling temperatures to ambient temperature conditions. These types of models assume a linear relationship between the root of the maximum microbial growth rate and temperature, under suboptimal growth conditions. In addition, they include a parameter, called T_{min}, which is defined as the theoretical minimum temperature below which there would be no growth of the microorganism (Ratkowsky and Ross, 1995). Therefore, these models allow predictions of microbial behaviour under different time and temperature conditions. In the case of pre-cut fruits, predictive models have been developed on rind, skin and pulp of melons, watermelons, pineapples, and papayas. To estimate the possible growth throughout the storage period, the models developed for pulp have been taken into account, as they represent a more unfavourable scenario in terms of food safety.

One of the most complete studies is from Danyluk et al. (2014), where they evaluated the behaviour of *L. monocytogenes* in cut melons of the *Cantaloupe* and Honey Dew varieties, together with watermelon, at different storage temperatures (4-25 ºC). The predictions generated by this model for *L. monocytogenes* in these pre-cut fruits, based on the increments in logarithmic units versus time, are depicted in Figure 1. It can be seen that the microorganism is able to grow 0.5 log in less than 2 hours, regardless of the storage temperature. As the temperature increases, growth is higher, as the microbial population increases, during 3 hours of storage, in 1 log at 5.5 °C, and in 2 log at 23.5 °C. Given their ability to grow at low temperatures, the authors observed an increase of 4 logs after 15 days of storage at 5 °C. Similarly, Fang et al. (2013) studied the behaviour of different strains of *L. monocytogenes* inoculated on *Cantaloupe* melon, and noticed growth at temperatures below 4 °C. It therefore follows that refrigeration storage does not prevent the growth of *L. monocytogenes* in this type of products (Salazar et al., 2017).

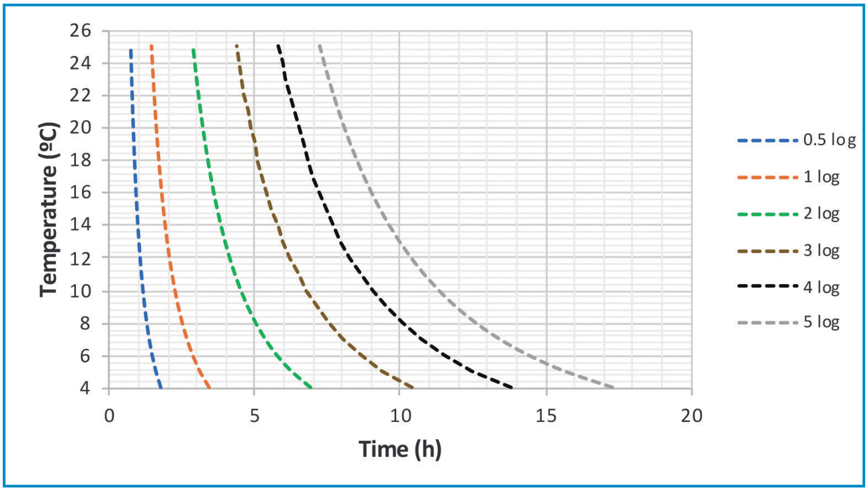

Figure 1. Effect of temperature and storage time on increase in logarithmic units of *Listeria monocytogenes* in cut melon and watermelon pulp. Based on the predictions of the model by Danyluk et al. (2014).

However, as mentioned before, most of the alerts notified for cut fruits under study are related to *Salmonella* spp. This pathogen cannot grow at refrigeration temperatures, although it proliferates rapidly in foods preserved at higher temperatures, provided that physicochemical conditions allow its growth. Li et al. (2013) published a square root model for *Salmonella* and *E. coli* O157:H7 considering the same temperature range and type of fruits as the study by Danyluk et al. (2014). Model predictions for *Salmonella* spp., are shown in Figure 2. *Salmonella* spp. does not grow in this type of products at temperatures <6 °C, while a storage period of 3 hours at 6.68 °C; 9.90 °C and 22.70 °C produces an increase in the population of 0.5; 1 and 2 logs, respectively. Scolforo et al. (2017) obtained similar results in a study on canary yellow melon, where at temperatures above 20 °C, the growth of *S. enterica* and *L. monocytogenes* was between 2-2.5 times higher than at 15 °C, both in the pulp and in the rind of the fruit.

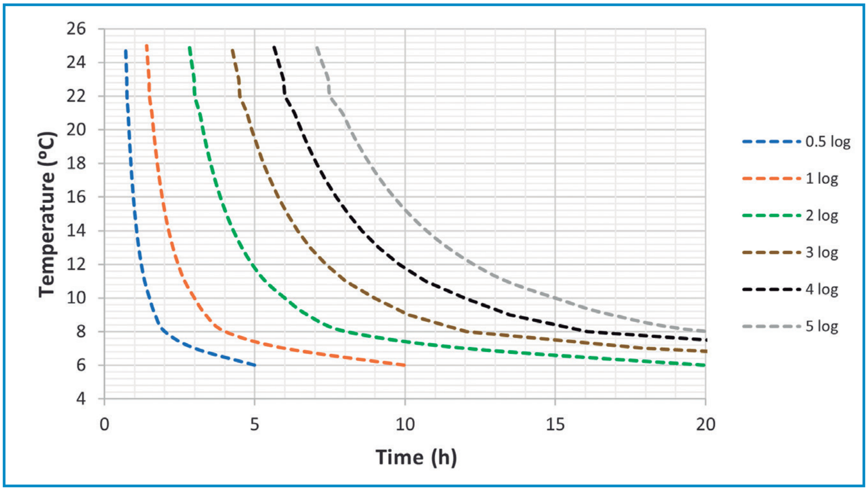

Figure 2. Effect of temperature and storage time on increase in logarithmic units of *Salmonella* spp. in cut melon and watermelon pulp. Based on the predictions of the model by Li et al. (2013).

A comparison of the growth rate of *Salmonella* spp. and *L. monocytogenes* shows that, under the usual storage conditions for this type of fruit, *L. monocytogenes* grows faster, as shown in Figure 3. However, at room temperature (>20 °C), there is similar growth, with both populations reaching high levels in a relatively short time, as mentioned above.

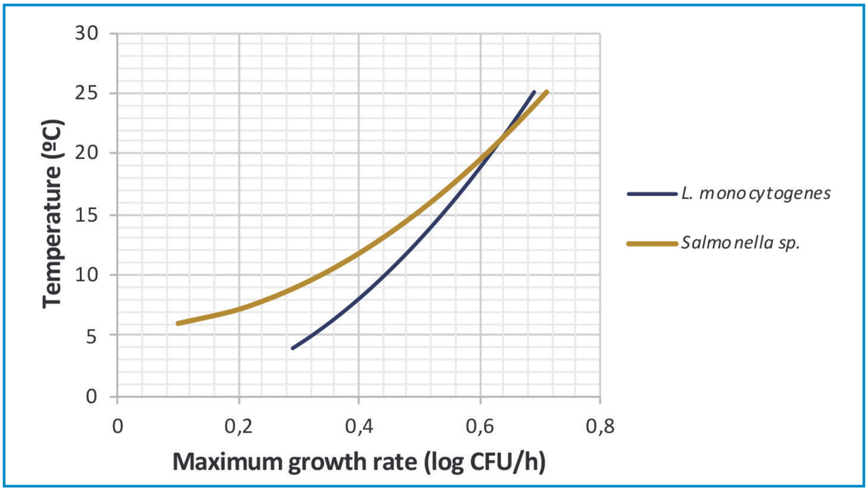

Figure 3. Relationship between maximum growth rate (log CFU/g) and storage temperature for *Salmonella* spp. and *Listeria monocytogenes* in cut melon and watermelon pulp. Based on predictions from models by Danyluk et al. (2014) and Li et al. (2013).

Some other factors not usually considered in predictive models are fruit maturity rate, sugar content or total soluble solids. In some studies, on cut papaya, it has been found that the maturity rate is directly related to the growth of *Salmonella* spp. (Singh et al., 2021), as shown in Figure 4. Such growth is in turn favoured by storage at higher temperatures and high levels of relative humidity.

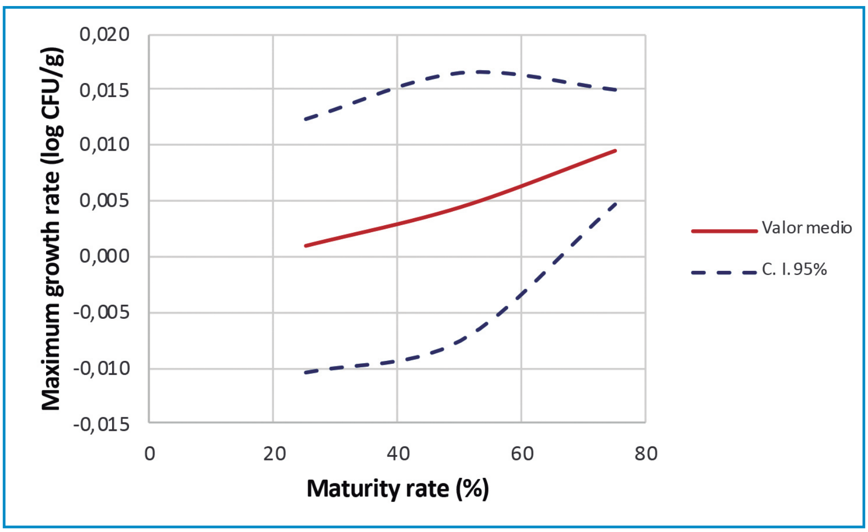

Figure 4. Relationship between the maximum growth rate of *Salmonella* spp. and the maturity rate of cut and stored papaya at 21 °C/90 % relative humidity. Based on the parameters of maximum growth rate from Singh et al. (2021).

From all the studies presented in this report, it is concluded that predictive models estimate a significant growth of *L. monocytogenes* and *Salmonella* spp. in melon, watermelon and cut papaya, while no growth would be observed in cut pineapple throughout storage. However, predictive models present some limitations to describing microbial behaviour in these products, which must be considered.

In the first place, most of the studies consider high inoculation levels that rarely correlate with the actual contamination that may occur in these products (Salazar et al., 2017). Luciano et al. (2022) considered low levels of inoculation of *L. monocytogenes* (1-4 cells/sample) in studies where low levels are inoculated in melon, mango, and papaya pulp, although storage conditions were at constant temperature for 10 days, which could not be extrapolated to the purpose of this report, where different temperature scenarios are evaluated. There are publications that report no significant difference in microbial behaviour if the cut fruit is kept at room temperature for a short time, followed by immediate refrigeration and storage at constant refrigeration temperature (Huang et al., 2019).

Finally, it is important to consider the existence of an adaptation phase of the microorganisms, which is especially relevant at refrigeration temperatures. While some studies claim that microbial growth is immediate and, therefore, there would be no adaptation phase (Fang et al., 2013), others do take this phase into account and consider it for the development of predictive models (Scolforo et al., 2017).

Therefore, predictions based on the use of predictive models should be taken with due caution, as the conditions under which these studies have been carried out cannot always be extrapolated to real cases, prior knowledge being necessary for the correct interpretation of the results.

Conclusions of the Scientific Committee

Based on the available information and the results displayed in this report, the following conclusions can be drawn:

- The storage at room temperature of melon, watermelon, papaya, and pineapple cut in half can pose a health risk since the physicochemical conditions (pH, water activity, total soluble solids, availability of nutrients, etc.) are compatible with the growth of foodborne pathogens such as *Salmonella* spp., verotoxin-producing *E. coli* or *L. monocytogenes*.
- Microbial behaviour differs according to the type of product, where melon, watermelon and papaya tolerate pathogen growth, while pineapple does not allow pathogen growth, mainly due to lower pH levels.
- In addition to temperature, some studies indicate that the maturity rate and post-harvest storage conditions (temperature and relative humidity) substantially influence microbial growth during retail storage.
- Notwithstanding the above, the storage of fruit cut in half at room temperature for short periods of time does not appear to have a significant influence on the development of foodborne pathogens, provided that it is followed by immediate refrigeration and that the product is consumed soon.
- It is concluded that, based on the information gathered so far, in order to make more flexible the conditions of storage at the point of sale of melon, watermelon, papaya and pineapple cut in half,

temperatures <25 °C can be admitted, since they do not pose a significant microbiological risk, provided is for <3 hours in a sufficiently ventilated place and preserved from sunlight, followed by continuous storage in refrigeration at temperatures <5 °C. In the case of pineapple, these conditions could be revised, if sufficiently representative complementary studies are available.

- To minimize the health risk that these practices may pose, it is recommended to exclude for cutting fruits with an excessive degree of maturity, or that present wounds or clefts in their surface, since they can be a source of contamination.
- It is recommended that establishments in the sector, regardless of their volume of sales, follow strict hygienic practices, especially with regard to cutting tools and, in general, to all the utensils used (for example, by carrying out adequate cleaning and disinfection). In addition, exhibition and storage conditions should be monitored (e.g. by keeping food away from sources of sunlight and heat, and by properly recording storage temperature), and the necessary measures should be established to prevent possible cross-contamination. The minimally processed fruits and vegetables sector has very useful Guidelines and Codes in this context (FEPEX, 2010).

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