

Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the determination of the date of minimum durability for frozen meat in retail establishments

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Abstract

Delegated Regulation (EU) 2021/1374, which amends Regulation (EC) No. 853/2004 laying down specific hygiene rules for food of animal origin, introduces the possibility for food business operators carrying out a retail activity to freeze meat for food donation purposes. Furthermore, Royal Decree 1376/2003 currently prohibits thawing, refreezing and freezing of meat and meat derivatives in retail establishments in Spain. However, a draft Royal Decree is currently being processed to regulate certain hygiene requirements for the production and marketing of food products in retail establishments, which will repeal this regulation and establish the conditions under which meat can be frozen.

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In order to allow meat to be frozen for purposes other than donation (immediately upon receipt or immediately after a maturation period), operators of such establishments must establish its shelf-life. Carrying out shelf-life studies can be complex and costly, so, in order to facilitate this task for operators, as well as to guide the competent authorities in charge of official control, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) has provided guidelines on the shelf-life period for meat, with the aim of being able to establish a date of minimum durability.

Meat freezing and thawing processes produce changes in microbiological, physicochemical and sensory parameters, directly affecting its shelf-life. Following the review of existing scientific literature and recommendations of different institutions, a series of guidelines are proposed on possible date of minimum durability for frozen meat depending on the species:

- Beef: 12 months at -18 °C, freezing immediately upon receipt. Recommended maturation treatments are 14 days at <2 °C, followed by a 9-month freeze at -18 °C.
- Sheep: 12 months at -18 °C, freezing immediately upon receipt. The recommended maturation treatments are 14 days at -1.5 °C, followed by a 9-month freeze at -18 °C.
- Goat: the recommended refrigeration storage can be between 3-5 days at 4.44 °C, or frozen for 12 months at -18 °C.
- Porcine: 6 months at -18 °C, freezing immediately upon receipt. No conclusive studies on the maturation period prior to freezing have been found.
- Poultry: 12 and 9 months at -18 °C for whole and cut products, respectively, freezing immediately upon receipt. The recommended prior maturation period is 9 days at < -1 °C.
- Rabbit: given the limited information available, it is not possible to establish a global recommendation applicable to this type of meat.

As for thawing, it is recommended to do it under refrigeration, after which the meat must be kept refrigerated and consumed in the shortest possible time. If this is not possible, it can be defrosted in a microwave or by passing it through cold water, in which case it must be cooked immediately afterwards.

Finally, it should be kept in mind that, in the supply and sale of frozen meat by retail establishments, it is essential to comply with good hygiene and handling practices.

Key words

Frozen meat, freezing, shelf-life, date of minimum durability, retail establishment, maturation.

Suggested citation

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1. Introduction

Commission Delegated Regulation (EU) 2021/1374 of 12 April 2021 amending Annex III to Regulation (EC) No. 853/2004 of the European Parliament and of the Council on specific hygiene rules for food of animal origin (EU, 2021), introduces the possibility for food business operators carrying out a retail activity to freeze meat for food donation purposes. To do so, they must meet the following conditions:

- a) in the case of meat to which a “use by” date applies in accordance with Article 24 of Regulation (EU) No. 1169/2011 of the European Parliament and of the Council (EU, 2011), before the expiry of that date,
- b) without undue delay, to a temperature of -18 °C or lower,
- c) ensuring that the date of freezing is documented and stated on the label or by other means,
- d) with the exception of meat which has been previously frozen (defrosted meat), and
- e) in accordance with any conditions laid down by the competent authorities for freezing and subsequent use as food.

Moreover in Spain, Royal Decree 1376/2003, of 7 November, which establishes the sanitary conditions for the production, storage and marketing of fresh meat and its derivatives in retail establishments, prohibits thawing, refreezing and freezing of meat and derivatives in retail establishments (BOE, 2003). However, a Royal Decree regulating certain hygiene requirements for the production and marketing of foodstuffs in retail establishments is currently in process, which will repeal this rule and establish the conditions under which retail establishments will be allowed to freeze meat. In particular, it is foreseen to provide that fresh meat should be frozen immediately upon receipt or immediately after completion of the maturation period, unless it is to be used for donation, in which case it will be governed by the provisions of point 4 of Chapter VII of Section I and point 5 of Chapter V of Section II of Annex III of Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 (EU, 2004). It may not be sold defrosted. It should be noted that, once the meat is frozen, the operator of the retail establishment will have to establish the shelf-life of the meat.

Conducting shelf-life studies can be complex and costly for retail establishments, which are often very small in size and unable to afford it. In order to facilitate this task for operators, in order to provide them with guidance on the shelf-life of frozen products, favour the donation of frozen meat and reduce waste, this report is requested. In parallel, it will provide guidance to the competent authorities responsible for the official control of these establishments, in order to assess whether the established shelf-life for frozen meat is adequate.

Therefore, in order to establish the date of minimum durability, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) is requested to provide a report providing guidance on the shelf-life for frozen beef, sheep, goat, pork, poultry and lagomorphs in retail establishments, taking into account, if deemed relevant, that the time of freezing can be immediately upon receipt, immediately after the end of the maturation period or later in the case of donations.

2. Food Safety Management Systems in retail establishments

Currently, European food safety legislation requires food business operators to develop and implement the well-known Food Safety Management Systems (FSMS), which include the Prerequisite Plans and Programmes (PRP) and the Hazard Analysis and Critical Control Points (HACCP). In the case of retail establishments, supermarkets, restaurants or food donation centres, the difficulty of monitoring this type of system has been reported. The European Commission therefore initially requested the European Food Safety Authority (EFSA) to make recommendations on hazard analysis approaches for retail establishments. EFSA adopted two opinions, on the one hand, focusing on butchers, grocery stores, bakeries, fishmongers and ice cream parlours (EFSA, 2017a), and on the other, on distribution centres, supermarkets and restaurants and on food donation centres (EFSA, 2018). Through the Opinions published by EFSA, a simplified management approach was proposed based on the description of the flow charts of the manufacturing processes and on the evolution of potential hazards of a physical, chemical or biological nature throughout the useful life.

At the national level, for the meat sector, one of the main legislative provisions is Royal Decree 1376/2003 (BOE, 2003). Chapter II on the conditions for handling, storing, preservation, transportation and selling of meat and meat products prohibits the thawing, refreezing and freezing of meat in establishments authorized for that purpose. In this regulation, only those processed meat derivatives that require freezing are allowed to be frozen at a temperature below -18°C .

The latest published provision, Delegated Regulation (EU) 2021/1374 (EU, 2021), recognises that food freezing can be an important means of reducing food waste and ensuring safe redistribution by food banks and other charities. Therefore, the freezing of meat for a donation purpose is allowed, under the requirements specified in the introduction.

When allowing meat to be frozen by retail establishments for purposes other than donation (immediately upon receipt or immediately after the maturation period), operators of such establishments should establish a shelf-life based on the determination of a number of quality and food safety parameters, and reasonably foreseeable conditions of distribution and consumption. The new Prerequisite Programs (PRP) established by EFSA (2018) in retail establishments included, among others, the control of shelf-life, defined as the period of time during which a food maintains its acceptable or desirable characteristics within the framework of specific storage and handling conditions.

Specifically, point 6 of Annex III to Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers (EU, 2011) provides that frozen meat or meat preparations must have a date of freezing and a date of minimum durability. For fresh food, if frozen before "use by" date for subsequent redistribution, the guidelines of some Member States recommend re-labelling the frozen food with a date of minimum durability, in accordance with the procedures relating to the self-monitoring system.

The intensity of application of freezing treatments, and the type of meat matrix appear to be the most important factors in determining the fitness of the final product prior to consumption. In the case of food donations, the influence of these factors is even more critical, since it applies to products close to the end of the shelf-life, which can compromise their safety. The purpose of this re-

port is to provide a response to establishing dates of minimum durability for meat subject to freezing in retail establishments under foreseeable conditions of storage, distribution, sale and consumption.

3. Relevance of the cold chain for meat preservation

Meat and meat products are an essential part of the human diet due to their rich and varied nutritional composition both in macro (proteins, lipids) and in micronutrients (vitamins and minerals such as iron, zinc, selenium, etc.). However, the physicochemical characteristics of unprocessed meat (pH, moisture content, nutrients), as well as the enzymatic activity during maturation, make it a susceptible medium for the growth of spoilage and pathogenic microorganisms during storage. On the other hand, the lipid content of some types of meat makes them equally susceptible to oxidation resulting in a deterioration of their sensory characteristics. Therefore, the preservation of meat by means of refrigeration and freezing is one of the traditional methods for quality assurance and extension of shelf-life (Hammad et al., 2019).

The maintenance of the cold chain is one of the essential principles of hygiene and food safety in the European Union, as set out in Regulation (EC) No. 853/2004 (EU, 2004). These requirements must be met for all foods or intermediate products that tolerate microbial growth, in order to preserve the health and safety of consumers. It should also be noted that, according to EFSA, the cold chain should not be interrupted throughout the meat distribution chain (EFSA, 2014). Currently, European legislation establishes that the maximum conservation temperatures of chilled meat are 4 °C for poultry, 3 °C for offal and 7 °C for the rest of the meat.

In spite of this, in the different steps of the distribution chain, temperature deviations can occur that can significantly impact the quality and safety of the meat. Likewise, other factors such as hygienic conditions in slaughterhouse processing operations, or transport and distribution time and temperature regimes up to the point of sale influence the degree of microbial contamination and meat quality (Wang et al., 2020). This is why maintaining a constant temperature is essential to preserve the quality and safety of the meat through good hygienic practices or the use of cold storage technologies that minimize the deterioration of the food.

The freezing of meat in order to increase shelf-life has been one of the most common practices at industrial and domestic level, although the major advances in new freezing technologies have been made in recent years. The preservation of meat through freezing involves a number of modifications in the water fraction of the meat. Since water is contained within muscle fibres as well as in the interstitial space, as water freezes, differences in osmotic pressure are created due to the increase in solute concentration, thus disrupting the homeostatic balance of the meat system (Lawrie, 1998). The subsequent defrosting of the meat involves a series of changes that affect the shelf-life and that vary depending on the meat piece, the type of system used, or the combinations of time and temperature. The packaging atmosphere, especially vacuum packaging, helps to minimise the deterioration of the meat produced by oxidation, dehydration and burns on the surface of the meat (Muela et al., 2010). Most of the studies have focused on the freezing and thawing effects of meat on water loss that impacts on quality parameters, although other changes such as protein denaturation, texture, colour or modifications in the lipid composition may be affected, as will be described in the next section.

4. Effect of freezing and thawing treatments on the shelf-life of the meat

Shelf-life of meat is typically determined by a number of parameters such as microbial contamination, pH, moisture content, colour, taste/smell, texture, or nutritional value (McMillin, 2008) (Hammad et al., 2017). According to current literature, it seems difficult to establish a useful shelf-life equivalent for all species due to the large number of factors involved, therefore, the main changes in the microbiological, physicochemical and sensory parameters that take place during the freezing and thawing processes of meat will be described.

4.1 Changes in microbiological parameters

Microbial contamination in fresh meat occurs in most cases in surface areas or adjacent tissues such as skin or parts of the slaughtered animal that have been contaminated and come into contact with the meat during processing operations. The sources of transmission of microorganisms to fresh meat are diverse, including the production environment, manipulators, surfaces and/or utensils, etc. Depending on the storage and distribution conditions, on certain occasions the microorganisms can migrate from the surface to internal areas, being able to survive for relatively long periods of time (Tozzo et al., 2018). One of the main factors for microbial contamination control lies in the knowledge of the rate or speed of freezing since, if the temperature drop occurs in a sufficiently short time, microbial proliferation can be largely avoided, as some studies point out (Lu et al., 2022).

Freezing produces a series of mechanical damage due to the formation of ice crystals, as well as a drying of the meat surface caused by a reduction in water activity (a_w), and an oxidative deterioration that largely inhibits microbial growth. Despite this, as is well known, the traditional methods of freezing and thawing do not allow the total inactivation of viable forms of microorganisms. Freezing is usually less efficient in the case of spores, as the damage caused by the temperature reduction is less. However, freezing process makes it possible to keep the microorganisms in a dormant state, since their metabolic activity is reduced. This implies that meat deterioration occurs later, so the shelf-life of frozen meat can be longer than in the case of chilled meat. However, during the thawing process microorganisms can reactivate and proliferate in food, especially if this process takes place slowly and unevenly, which makes certain parts more exposed to microbial growth (Löndahl and Nilaaon, 1993). Additionally, during thawing there is a greater availability of moisture and nutrients produced by the formation of exudate and that favour microbial growth. For this reason, it is essential to maintain good hygiene and handling practices of meat to be frozen in order to avoid microbial contamination (Pham, 2004). From the above, it can be concluded that shelf-life of frozen meat may be limited by the action of spoilage microorganisms, and by the presence or growth of foodborne pathogens which may compromise the safety of consumers.

4.1.1 Spoilage microorganisms

Although, as discussed above, freezing is understood as a bacteriostatic process, some studies suggest that 60 % of the spoilage microbial load present in fresh meat is inactivated at temperatures below -18 °C (Rahman and Velez-Ruiz, 2007). Tolerance of microorganisms to low temperatures varies, depending on the type, physiological status and population level. The alteration of frozen meat

is mainly attributed to psychrotrophic and psychrophilic microorganisms, capable of surviving and growing at temperatures below 0 °C. In the case of bacteria, the outer surface of the cell provides different levels of protection against the penetration of ice crystals. Gram-positive bacteria, with a cell wall composed of 80 % peptidoglycan, are more resistant to freezing than gram-negative microorganisms.

However, surviving microorganisms can proliferate more easily during thawing and may reach meat-like levels before freezing, although available studies indicate conflicting results. One of the most important factors is the stage of maturation of meat under refrigeration conditions prior to freezing. In this regard, Vieira et al. (2009) found that beef frozen for 90 days and previously subjected to a maturation of between 3-10 days, did not present any type of microbiological alteration. However, they did detect a growth of psychrotrophs, attributed to the fact that they were the predominant group in the meat during thawing (48 hours at 4 °C). Similarly, Greer and Murray (1991) reported that the adaptation phase for microbial growth in frozen and thawed pork was lower than that of fresh meat, although no sensory alterations were observed in either case.

In other cases, the temperature and freezing time do not significantly influence the microbial contamination of meat, as demonstrated by Teuteberg et al. (2021) in frozen pork samples at -18 °C and -80 °C, testing times of 12 and 24 weeks. In this case, the concentration of *Enterobacteriaceae* and mesophilic aerobes was similar, although lower than that found in fresh meat. The same authors did obtain lower *Enterobacteriaceae* counts of about 1 log CFU/g in frozen poultry meat compared to unfrozen meat (Kluth et al., 2021). It seems that other factors unrelated to the combinations of freezing time and temperature affect the viability of certain groups of spoilage microorganisms in meat, although if carried out correctly, it does give stability to the product for a more or less prolonged period.

4.1.2 Foodborne pathogens

It is well known that meat is a favourable medium for the possible presence and growth of foodborne pathogens. Depending on the type of matrix, although pathogenic microorganisms such as *Salmonella* spp., *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Campylobacter* spp., *Listeria monocytogenes* and hepatitis E virus (HEV), among others, can survive freezing treatments, in most cases, the altering microbiota inhibits its growth. Therefore, during freezing and thawing treatments, temperature favours the growth of psychrophilic microorganisms, being most of these species, spoilage microorganisms. This is why microbial contamination caused by foodborne pathogens occurs as a result of inadequate hygiene practices during meat processing.

In the case of viral contamination, an increase in the incidence of hepatitis E cases associated with the consumption of raw or undercooked pork and wild boar meat from infected animals has recently been demonstrated (Kupferschmidt, 2016). However, if technological treatments are inefficient, especially meat thawing, it can lead to microbial growth or virus viability that may compromise public health. For hepatitis E virus, the effectiveness of inactivation methods during meat processing is largely unknown. The lack of efficient and easy-to-use methods for the determination of the infectivity of this virus has prevented extensive inactivation studies. Despite this, in recent

years there have been advances that have managed to improve the spread of the virus in cell cultures, although there are still few inactivation studies carried out directly on meat products. Like other enteric viruses, hepatitis E virus is relatively resistant to freezing (EFSA, 2017b).

Freezing treatments appear to be effective in reducing contamination by *Campylobacter* spp. in poultry meat and, in fact, is one of the measures considered by some countries such as Iceland, and also by EFSA (2011) to reduce the incidence of this pathogen. However, the impact of the implementation of this measure is not entirely clear. It is known that a treatment at -20 °C can abruptly reduce the initial concentration of *Campylobacter* spp., followed by a progressive decrease of the population during storage (Maziero and Oliveira, 2010).

In the case of *L. monocytogenes*, an important factor in the incidence of foodborne listeriosis is that it can grow significantly at refrigeration temperatures compared to other pathogens. Growth has been recorded at temperatures as low as -1.5 °C, albeit at a very slow pace (BFFF, 2015). In the refrigeration period prior to freezing, Pradhan et al. (2012) tested the growth of *Listeria innocua* in chicken breasts maintained at 4 and 8 °C for 21 days, where an increase in the population of 2.1 log CFU/g was observed in the first week at 4 °C, and then decreased. The trend was similar at 8 °C, where the population increased 3.7 log CFU/g in 7 days, and then decreased. Consequently, for the management of the presence of *L. monocytogenes* in frozen meat, it will be important to reduce the refrigeration period before freezing, to perform a defrost under conditions of maximum hygiene and to control any shelf-life after defrosting, as well as the correct cooking. As for the effect of freezing, *L. monocytogenes* is not able to grow in frozen meat, but it can survive (Palumbo and Williams, 1991) (Chan and Wiedmann, 2009), unlike that observed in microbial species such as *E. coli* in which the population is reduced along a prolonged storage in freezing (Foschino, 2002). Once the meat is thawed, *L. monocytogenes* can restart growth. It has been observed that increasing the freeze storage time leads to a delay of a few hours in the onset of subsequent growth, possibly due to cell damage, but the growth rate of *L. monocytogenes* does not appear to be affected (Humblot et al., 2015). However, Kataoka et al. (2017) found that growth occurred without a significant lag phase after thawing, so the effect of the freeze on the subsequent growth of the pathogen appears to be influenced by other factors inherent to the meat matrix.

In *Salmonella* spp., growth at temperatures from 3.5 °C has been described, so it does not proliferate at freezing temperatures (Matches and Liston, 2006). There are numerous studies that indicate that it survives prolonged freezing in meat substrates, such as in frozen chicken (Dominguez and Schaffner, 2009). Indeed, numerous outbreaks of foodborne toxi-infection in Canada associated with frozen poultry products (including chicken breaded products) have been described between 2015 and 2019 (Morton et al., 2019). Therefore, freezing cannot be considered to contribute to the control of *Salmonella*, it only prevents its proliferation and prolongs the shelf-life of the product. The temperature prior to freezing and during thawing should be monitored to prevent the proliferation of *Salmonella* and appropriate hygienic practices should be applied to minimise its presence in the meat to be frozen.

Other biological hazards are constituted by certain groups of parasites such as *Toxoplasma*, *Trichinella*, etc., whose presence in fresh meat has been previously reported (Johne et al., 2021)

(Marín-García et al., 2022). In this case, most of the studies on frozen meat show that its viability is significantly reduced at temperatures below $-12\text{ }^{\circ}\text{C}$ (Kotula et al., 1991), although on many occasions it must be accompanied by a subsequent heat treatment to achieve a complete inactivation of these microorganisms.

4.2 Changes in physicochemical parameters

Advances in new freezing technologies have greatly reduced meat quality losses, although freezing and thawing treatments continue to produce a number of changes in physicochemical parameters that can affect the shelf-life of the product. Meat has water as the main constituent, so the greatest modifications occur in the moisture content and water retention capacity of meat after thawing. Water is found in various structures and compartments of muscle fibres. When water is frozen, there is an increase in the concentration of solutes (proteins, carbohydrates, lipids, vitamins and minerals) that cause a disruption in the biochemical balance of the meat system. This series of changes produces damage at the metabolic and cellular levels that ultimately affect the quality of the meat. Changes in the main physicochemical parameters of meat as a result of freezing and thawing treatments are described below.

4.2.1 Moisture

Freezing and thawing of meat affects both content and distribution of moisture in meat tissue. Moisture is assessed based on various measures such as drip loss, weight loss of thawed product, cooking losses, or water retention capacity (Leygonie et al., 2012a). During freezing, since the crystallisation of water makes it unavailable for reactions, it is unnecessary to determine a_w , since it does not depend on dissolved substances but on temperature alone (Schnewberger et al., 1978).

The decrease in moisture content of meat occurs irreparably after slaughter, due to a decrease in pH, depletion of glycogen and ATP reserves, as well as protein denaturation. Freezing and thawing times influence the amount of exudate by size and distribution of the ice crystals (Añón and Cavelo, 1980). It seems that there is no direct correlation between thawing rate and amount of exudate formed, although some recommendations are found in the scientific literature to minimise exudate losses and on the a_w of the thawed product. In fact, Leygonie et al. (2012b) and Vieira et al. (2009) found no significant differences between fresh and frozen meat samples subjected to different thawing treatments. González-Sanguinetti et al. (1985) concluded that a defrost treatment of $-5\text{ }^{\circ}\text{C}$ to $-1\text{ }^{\circ}\text{C}$ in less than 50 minutes could result in water reabsorption by muscle fibres, which results in less moisture loss. No variation in a_w has been observed during freeze storage (Sayas-Barberá et al., 2021). Therefore, a higher defrost rate helps minimise drip losses (Ambrosiadis et al., 1994). In general, a_w is very high at the end of thawing, so it must be done correctly to avoid microbiological proliferation.

4.2.2 Lipids and proteins oxidation

The final temperature at which the meat is frozen and stored determines the amount of thawed water available to participate in chemical reactions. This fraction is important in terms of oxidation, as chemical reactions can occur during frozen storage that initiate primary lipid oxidation (peroxi-

ation) in meat. This can lead to secondary oxidation of lipids after thawing, that are related to modifications in the sensory attributes of the meat. This phenomenon has been demonstrated by Akamittath et al. (1990) and Hansen et al. (2004), who reported accelerated lipid oxidation in frozen and thawed meat subjected to a refrigerated shelf-life study.

The degree of lipid oxidation is usually measured through the presence of thiobarbituric acid reactive substances (TBARS). These secondary metabolites cause defects in meat sensory profile, such as rancidity, fatty flavour, spicy taste and other unpleasant flavours. Vieira et al. (2009), stated that concentration of TBARS in fresh meat was significantly lower than in meat stored for 90 days at -20 °C. Benjakul and Bauer (2001) also found that freezing and thawing of muscle tissue resulted in accelerated accumulation of TBARS, and attributed this finding to damage to cell membranes produced by ice crystals and the subsequent release of prooxidants, specially haem iron.

On the other hand, oxidation of proteins can be linked to any of the prooxidant factors, such as free radicals, haem pigments or malonaldehyde, which is one of the substrates that reacts with proteins to form carbonyl (ketone and aldehyde) (Xiong, 2000). Thus, the oxidation of proteins and lipids is undoubtedly interrelated.

Protein oxidation in meat can lead to a decrease in the quality of consumption due to reduced tenderness and juiciness, taste deterioration and discolouration (Rowe et al., 2004). Oxidative changes in proteins also have an impact on lower water retention capacity and exudate formation (Liu et al., 2010), as well as on a loss of solubility.

4.2.3 Colour

Meat freezing and thawing treatments produce alterations in colour stability, since myoglobin is part of the exudate. Likewise, it has been shown that myoglobin undergoes a denaturation process during freezing and thawing of meat (Calvelo, 1981), which increases the probability that it will undergo autooxidation which, in turn, manifests itself in a loss of colour of the meat. Indeed, other studies support that in frozen or matured meats there is a greater accumulation of metamyoglobin on the surface (Abdallh et al., 1999).

Meat oxidation phenomena are interrelated, so that when lipid oxidation begins, prooxidant compounds capable of reacting with oxymyoglobin to form metamyoglobin are formed. Therefore, if the oxidation reactions of the lipids are accelerated during meat freezing, the amount of free radicals present that can produce the oxidation of the myoglobin and the deterioration of the colour will increase.

4.2.4 Texture parameters

Meat texture undergoes changes as a result of freezing and thawing treatments. As for tenderness, it has been shown to be positively correlated with meat ripening time prior to freezing (Vieira et al., 2009). The mechanism involved in the tenderness increase resides in a breakdown of muscle fibres by enzymatic action, and a loss of structural integrity of tissues caused by the formation of ice crystals. It should be noted that meat texture may differ depending on whether it is measured instrumentally or evaluated through a sensory panel (Lagersted et al., 2008). It can happen that

the tenderness of a thawed meat perceived by a sensory panel is lower than the measurement instrumentally, due to moisture loss, which causes a lower shear stress (shear force) to break the structure of the meat (Lui et al., 2010).

4.3 Culinary fitness and sensory profile

Meat quality in terms of culinary fitness is usually measured through parameters such as colour, water retention capacity, texture and palatability (Miller, 2002). As described above, the freezing and thawing of the meat may lead to a loss of certain quality attributes that may manifest in lower culinary fitness and sensory profile. Water losses due to meat cooking after thawing are considered in many studies as a parameter that helps define optimal treatments, and usually correlates a greater water loss with a longer freezing and thawing time (Cho et al., 2017). However, other studies indicate that there are no substantial differences in moisture losses during cooking of fresh meat compared to frozen meat (Vieira et al., 2009) (Leygonie et al., 2012b). The factor associated with moisture losses is given by the muscle region present in the meat from which said moisture loss originates. During cooking, in addition to moisture, a fusion of fats takes place, as well as a protein denaturation, which also contribute to a release of juices from bound water (Vieira et al., 2009).

On the other hand, the tenderness of the meat, assessed by the shear force, is also influenced by the freezing and thawing treatments. In this sense, contradictory results are reported in many studies, although most of them postulate that the shear force decreases in those meats subjected to a freezing and thawing treatment. The main associated factors are related to water loss, as well as the rupture of cell membranes produced by ice crystals during freezing (Lui et al., 2010).

In addition to physical properties, the sensory profile of frozen meat can be affected by the interaction of its components through oxide-reduction reactions. There are compounds in meat that act as catalysts for these reactions, such as iron or myoglobin, as well as lipids. The appearance of compounds such as malonaldehyde, ketone, carbonyl groups or free radicals can alter the sensory profile and culinary fitness of the meat.

Although meat freezing may be related to increased lipid and protein oxidation and sensory alteration of meat, some studies indicate that the aromatic profile of meat may improve due to a higher concentration of volatile compounds as a result of short-term freezing storage (Qi et al., 2021). In other cases, the results are not sufficiently conclusive when compared with the cooking of fresh meat because other factors such as the type of culinary treatment, or quality of the raw material, among others, can have greater influence.

Table 1 shows some studies where parameters relating to meat cooking and sensory profile are evaluated, most of which are the loss of moisture, antioxidant capacity, volatile compounds and texture. These studies analyse meat ripening, freezing and thawing treatments and those considered optimal for the improvement of quality and food safety are reported.

Table 1. Studies related to the effect of meat refrigeration, freezing and thawing treatments on the main quality and food safety parameters

Meat type	Treatments			Parameters			Optimal treatment	Reference
	Cooling	Freezing	Thawing	Physicochemicals	Microbiological	Sensory		
Beef (veal)	10 days at 4 °C	90 days at -20 °C and -80 °C	48 hours at 4 °C	Colour, TBARS*, WHC*	Psychrotrophs, <i>Enterobacteriaceae</i> , LAB*	Texture, tenderness, juiciness, smell, flavour	90 days at -20 °C (after storage at 4 °C for a short period of time)	Vieira et al. (2009)
	0-14 days at 2 °C	0-9 months at -18 °C	-	Colour, WHC, TBARS, VBN*, MLcooked*	-	Texture	14 days at 2 °C + <9 months at -18 °C	Cho et al. (2017)
	0-5 weeks <1 °C	0-13 months at -12 °C and -18 °C	-	MLcooling*, MLcooked, colour, MLthawing*, pH, humidity, fat, a _w , glycogen	<i>Brochothrix thermosphacta</i> , <i>Clostridium perfringens</i> , <i>Escherichia coli</i> , <i>Enterobacteriaceae</i> , LAB	Texture	0-5 weeks at <1 °C + 12 months at -12 °C or -18 °C	Holman et al. (2017)
	-	12 months at -18 °C	-	MLcooked, colour, pH, TBARS	Psychrotrophs, <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , coliforms	Aroma, texture, juiciness, flavour	12 months at -18 °C	Fernandes et al. (2013)
Sheep	2-3 weeks at -1.5 °C	7 weeks at -18 °C	12 hours at -1,5 °C	pH, colour, MLcooling, MLcooked	-	Appearance, texture	2 weeks at -1.5 °C + 7 weeks at -18 °C	Kim et al. (2013)
	-	1-21 months at -18 °C	-	pH, colour, TBARS, WHC	-	Texture	21 months at -18 °C	Muela et al. (2015)
	0-60 days at <2 °C	0-13 months at -12 °C and -18 °C	-	Colour, MLthawing, MLcooked, pH, a _w , moisture, glycogen	<i>Brochothrix thermosphacta</i> , <i>Clostridium perfringens</i> , <i>Escherichia coli</i> , <i>Enterobacteriaceae</i> , LAB	Texture	15-30 days at <2 °C + 12 months at -12 °C or -18 °C	Coombs et al. (2017)

Table 1. Studies related to the effect of meat refrigeration, freezing and thawing treatments on the main quality and food safety parameters

Meat type	Treatments			Parameters			Optimal treatment	Reference
	Cooling	Freezing	Thawing	Physicochemicals	Microbiological	Sensory		
Caprine	24 hours at 4 °C	16 weeks at -10 °C	-	pH, WHC, TBARS, protein	MA*	-	24 hours at 4 °C + 8 weeks at -10 °C	Ahmad et al. (2017)
Pork	16 days at 5 °C	6 months at -18 °C	-	pH, VBN, TBARS, biogenic amines	MA, coliforms, psychrotrophs	-	8 days at 5 °C or 90 days at -18 °C	Custódio et al. (2018)
Pork	-	12-24 weeks at -18 °C and -80 °C	24 hours at 4 °C	Colour, MLthawing, pH, TBARS, moisture, MLcooked	<i>Enterobacteriaceae</i> , <i>Pseudomonas</i> spp., MA	Texture	24 weeks at -18 °C (after rapid freezing at -80 °C)	Teuteberg et al. (2021)
Poultry (chicken)	9 days at -1.3 °C or 28 days at 2-4 °C	30 days at -20 °C	-	pH, MLfreezing*, colour, humidity, TBARS	MA, LAB	Texture	30 days at -20 °C (prior storage at -1.3 °C extends shelf-life)	Kaewthong et al. (2019)
Poultry (turkey)	-	12-24 weeks at -18 °C and -80 °C	24 hours at 4 °C	MLthawing, MLcooked, colour, pH, a _w , TBARS	<i>Enterobacteriaceae</i> , <i>Pseudomonas</i> spp., MA	Texture	24 weeks at -18 °C or -80 °C	Kluth et al. (2021)
Poultry (chicken)	-	2-8 weeks at -18 °C	12 hours at 10-15 °C	MLcooked, free amino acids, nucleotides	-	Volatile compounds, flavour generating compounds	6 weeks at -18 °C	Qi et al. (2021)
Rabbit	36 days at -2.5 °C or 36 days at -4 °C or 10 days at 4 °C	36 days at -18 °C	12 hours at 4 °C	pH, VBN, soluble proteins, MLthawing, MLcooked	MA	Texture, colour	20 days at -2.5 °C or 36 days at -4 °C or 6 days at 4 °C + 36 days at -18 °C	Lan et al. (2016)

a_w: water activity; LAB: Lactic Acid Bacteria; MA: mesophilic aerobes; MLcooked: Moisture Loss after cooking; MLcooled: Moisture Loss after cooling; MLrefreezing: Moisture Loss after refreezing; MLrefrigeration: Moisture Loss after refrigeration; MLthawing: Moisture Loss after defrosting; TBARS: reactive substances of thiobarbituric acid; VBN: Volatile Basic Nitrogen; WHC: Water Holding Capacity.

5. Guidance on the establishment of dates of minimum durability for frozen meat intended for consumption

The main factors attributed to meat ripening, freezing and thawing treatments and their impact on the alteration and safety of the final product have been described in the previous sections.

The directly applicable legal provision to take into account for retail trade is Royal Decree 1376/2003 (BOE, 2003). This Royal Decree refers to some hygienic requirements, as well as of temperature ranges of storage, conservation, transport and sale of meat, which must be ≤ -12 °C. Also, the marking of the date of minimum durability is a mandatory indication under Regulation (EU) No. 1169/2011 (EU, 2011). The food operator responsible for this information must ensure the accuracy of the information.

In order to be able to give guidance on the estimation of the freezing life of meat in retail establishments it is necessary to provide for the possibility of freezing immediately upon receipt or after prior ripening treatment.

A great deal of work can be found in the scientific literature related to the evaluation of the impact of meat ripening, freezing and thawing treatments on food quality and safety. In other cases, studies are more directed towards determining shelf-life in terms of fitness of frozen meat for consumption (Table 1). Maturation of the meat is known to produce an improvement in tenderness and water retention capacity, and its combination with a subsequent freezing treatment can help extend the shelf-life. Based on the information available, it can be concluded that there is a variability in the times and temperatures of the treatments reported in the different studies depending on the objective of the same, type of meat and parameters evaluated, as well as criteria used to determine the suitability of the meat. That is why the global shelf-life estimate should consider all these limitations.

Some recommendations for meat freezing treatments provided by different institutions at the national and global level are summarised in Table 2. In order to be able to respond adequately to the purpose of this report, all available information will be compared in order to provide guidance on the dates of minimum durability of frozen meat for sale in retail establishments.

Table 2. Recommendations on meat frozen storage conditions provided by different worldwide organisms

Meat type	Description	Shelf-life	Organism
Beef, sheep and pork	Steaks	6-12 months at -18 °C	HHS-FDA (2018)
	Chops	4-6 months at -18 °C	
	Other	3-4 months at -18 °C	
Poultry	Chicken, turkey (whole)	12 months at -18 °C	HHS-FDA (2018)
	Chicken, turkey (parts)	9 months at -18 °C	
	Giblets	3-4 months at -18 °C	
Beef	Minced meat	2-3 months at -12 °C or -18 °C	PrimeSafe (2022)
Sheep		2-3 months at -12 °C or -18 °C	
Pork		2-3 months at -12 °C or -18 °C	
Poultry		2-3 months at -12 °C or -18 °C	
Beef	Meat pieces	8 months at -12 °C or 18 months at -18 °C	PrimeSafe (2022)
Sheep		12 months at -12 °C or 18 months at -18 °C	
Pork		6 months at -12 °C or 10 months at -18 °C	
Poultry		9 months at -12 °C or 18 months at -18 °C	
Beef	Meat pieces	12 months at -18 °C or 18 months at -25 °C or 24 months at -30 °C	FAO (1991)
Sheep		9 months at -18 °C or 12 months at -25 °C or 24 months at -30 °C	
Pork		6 months at -18 °C or 12 months at -25 °C or 15 months at -30 °C	
Poultry		12 months at -18 °C or 24 months at -25 °C or 24 months at -30 °C	

In the case of beef, Cho et al. (2017) considered that a maturation time of 14 days at 2 °C implies an improvement in meat quality, and recommend a freezing time of less than 9 months at -18 °C. Instead, Holman et al. (2017) reported longer times, up to 12 months at -12 °C or -18 °C after a period of maturation at <1 °C for 5 weeks. The type and size of the meat pieces evaluated may be the cause of these differences. Regarding the shelf-life recommendations presented in Table 2, it should be noted that the HHS-FDA (United States Department of Health and Human Services-Food and Drug Administration) divides beef, sheep and pork into different categories of meat pieces, while PrimeSafe considers a distinction between minced meat and meat pieces.

Taking storage at $-18\text{ }^{\circ}\text{C}$ as a reference, freezing storage times range from 6 to 18 months for meat pieces. On the basis of the information available, a freezing storage time of 12 months at $-18\text{ }^{\circ}\text{C}$ can be considered, provided the meat is frozen immediately upon receipt. The recommended maturation treatments are 14 days at $<2\text{ }^{\circ}\text{C}$ followed by a 9-month freeze at $-18\text{ }^{\circ}\text{C}$, according to the study by Cho et al. (2017).

For sheep meat there is a greater discrepancy in the freeze storage times reported in the studies. Fernandes et al. (2013) recommended a time of 12 months at $-18\text{ }^{\circ}\text{C}$, while Muela et al. (2015) argued that sheep meat is acceptable for consumption after a storage time of 21 months at $-18\text{ }^{\circ}\text{C}$. The recommendations provided in Table 2 range from 4 to 18 months of storage at $-18\text{ }^{\circ}\text{C}$ for sheep meat pieces. Taking these collected values, a time of 12 months at $-18\text{ }^{\circ}\text{C}$ can be recommended, provided the meat is frozen immediately upon receipt. In case a previous maturation period is carried out, Kim et al. (2013) reported values of 14 days at $-1.5\text{ }^{\circ}\text{C}$, so it can be considered as a reference followed by a freezing treatment of 9 months at $-18\text{ }^{\circ}\text{C}$, based on the recommendations of the Food and Agriculture Organization of the United Nations (FAO, 1991) (Table 2).

Regarding goat meat, there is little information in the scientific literature. Ahmad et al. (2017) studied the effect of pre-cooling at $4\text{ }^{\circ}\text{C}$ for 24 hours and subsequent freezing at $-10\text{ }^{\circ}\text{C}$ for 4 months of various pieces of goat meat under polypropylene packaging conditions. Their study concluded that the organoleptic quality of the meat was adequately maintained for 8 weeks. Despite this, there are recommendations provided by the USDA-FSIS (United States Department of Agriculture-Food Safety Inspection Service) where they stipulate storage times in refrigeration and freezing, as well as the way in which the thawing of goat meat should be carried out (USDA-FSIS, 2013). Recommended refrigeration storage can be from 3-5 days at $4.44\text{ }^{\circ}\text{C}$, or frozen for 12 months at $-18\text{ }^{\circ}\text{C}$.

In the case of pork, Custódio et al. (2018) studied the effect of refrigeration versus meat freezing and recommend a shelf-life of 8 days at $5\text{ }^{\circ}\text{C}$ or 3 months at $-18\text{ }^{\circ}\text{C}$. These values are lower than those reported by Teuteberg et al. (2021) who estimated a freezing shelf-life of 6 months at $-18\text{ }^{\circ}\text{C}$, although meat quality improved after previous rapid freezing at $-80\text{ }^{\circ}\text{C}$. The recommendations provided in Table 2 range from 4 to 10 months of storage at $-18\text{ }^{\circ}\text{C}$ for the pork pieces. Thus, a 6-month freeze storage period at $-18\text{ }^{\circ}\text{C}$ can be recommended immediately upon receipt of the meat at the retail establishment. No conclusive studies on the maturation period of pork meat prior to freezing have been found in the literature.

Regarding poultry meat, Kluth et al. (2021) evaluated a series of physicochemical and microbiological parameters on the quality and safety of turkey meat, recommending a storage period of 6 months at $-18\text{ }^{\circ}\text{C}$. Qi et al. (2021) demonstrated that storage at $-18\text{ }^{\circ}\text{C}$ for 6 weeks achieved an improvement in chicken meat quality, while Kaewthong et al. (2019) suggested a storage of 30 days at $-20\text{ }^{\circ}\text{C}$ for chicken meat after a maturation period of 9 days at $-1.3\text{ }^{\circ}\text{C}$. Based on the HHS-FDA recommendations, a distinction is made between whole and cut product, with shelf-life being 12 and 9 months at $-18\text{ }^{\circ}\text{C}$, respectively. This 12-month recommendation coincides with that provided by FAO and is lower than that reported by PrimeSafe (18 months). Based on the available information, a freeze storage time of 12 and 9 months at $-18\text{ }^{\circ}\text{C}$ can be established for whole and cut products,

respectively (in the event that a freeze is carried out immediately upon receipt), as well as a prior maturation period of 9 days at < -1 °C.

In the case of rabbit meat there are fewer studies available, although some studies have focused on the evaluation of super-cooling treatments and their comparison with traditional meat ripening and freezing treatments. In this regard, Lan et al. (2016) investigated the effect of two ripening treatments at -2.5 °C and -4 °C for 36 days on the quality of rabbit meat. In this study, ripening periods of 20 days at -2.5 °C and up to 36 days at -4 °C were recommended, followed in both cases by freezing storage of 36 days at -18 °C. However, given the limited information available, it is not possible to establish a recommendation at global level that could be applicable to this type of meat.

6. Hygiene requirements of retail establishments for the freezing of meat

The Joint FAO/WHO *Codex Alimentarius* Commission, among its various Codes of Hygiene Practice, presents one relating to the meat sector. This is the Code of Hygienic Practice for Meat (CXC 58-2005-CAC/RCP 58-2005), developed by the *Codex* Committee on Meat Hygiene (*Codex Alimentarius*, 2005). This is a vertical code, ranging from sacrifice and slaughter. In Chapter 9 "Process Control", Article 151, some interesting and fundamental guidelines in relation to packaging and freezing of meat are detailed.

In the supply and sale of frozen meat by retail establishments, it is essential to comply with good hygiene and handling practices. Undoubtedly, some of the points indicated in Royal Decree 1376/2003 (BOE, 2003), can continue to be applied to those retail establishments that supply frozen meat, as reflected below:

- In relation to counters, display cases and other items where frozen meat is displayed for sale to the public, it is recommended that they be designed to avoid any contamination.
- On the other hand, refrigeration facilities for the preservation, chilling or freezing of meat should be equipped with temperature reading and checking devices so as to meet the shelf-life verification requirements reflected in this report.
- In order to avoid cross-contamination, chilled and frozen meat should not be kept in the same refrigeration compartment and non-simultaneous preservation or storage with other raw materials or products determined by sanitary regulations should be observed at all times.

In addition to the above, different entities and organisations of the meat sector present very useful sectoral guides as a basis for the implementation of a Food Safety Management System (FSMS, Prerequisite Plans and Programs, PRP, and Safety Plan or Hazard Analysis and Critical Control Points, HACCP) in meat retail establishments, such as the guide prepared by the Agri-Food Business Federation of the Valencian Community (FEDACOVA, 2012).

7. General recommendations for the thawing of meat

In addition to the above, it is recommended that retail establishments provide sufficient, clear and accurate information on the conditions of storage, handling and cooking of meat. The guidelines

and recommendations of international organisations indicate that, after the freezing period, food must be defrosted before cooking, since, otherwise, cooking will take more time and may not be sufficient to ensure that the food has reached the internal temperature necessary to eliminate the pathogens (FSA, 2020). Once thawed, food should be kept refrigerated and consumed within 24 hours of thawing. While thawing in refrigeration takes longer than thawing in a microwave oven or at room temperature, it ensures that thawed food remains at microbiologically safe temperatures throughout the thawing process and that pathogen growth is minimal. If not possible, it can be defrosted in a microwave in a defrost setting. This method is faster and can be performed shortly before cooking the food. However, it should be noted that food thaws unevenly in the microwave and can reach temperatures above 8 °C, which favours microbial growth. Therefore, once thawed in the microwave, food should be cooked immediately afterwards. Certain foods can be thawed under cold water, which is faster than thawing under refrigeration and will not allow the foods to get too hot, although it should not be the most common practice.

According to USDA-FSIS recommendations (2005), the shelf-life of thawed product under refrigeration is set at 3-5 days, while products thawed in cold water or thawed by microwave must be cooked immediately after thawing and before consumption.

Conclusions of the Scientific Committee

In this report, the main factors that affect the quality and safety of meat have been studied, taking into account the different stages of pre-cooling or maturation, freezing, and thawing, in order to establish an indicative useful life period for its marketing in retail establishments.

The main conclusions drawn from the report are the variability between the different animal species, treatments applied and factors involved, which makes it difficult to establish a date of minimum durability that is valid for all types of meat.

As the subject of the report is the assessment of unprocessed fresh meat subject to freezing, it has been demonstrated when establishing the shelf-life that short chilling periods, followed by an immediate freezing afterwards, can help to maintain or even improve the organoleptic profile of the meat, although it is important that the operator of the establishment takes into account the type of meat piece, and the intended use thereof.

Although pathogenic microorganisms can survive under freezing conditions in meat, provided that good hygienic practices based on the principles of Hazard Analysis and Critical Control Points (HACCP), are carried out in the establishment, it can be concluded that microbiological safety is not compromised, as indicated by the recommendations provided by the USDA, so the evaluation of the shelf-life should take into account mainly quality parameters. However, as the freezing *per se* does not achieve a total inactivation of microorganisms, it is important that hygiene rules based on the application of good hygiene practices and HACCP are followed in the retail establishment in order to avoid contamination of the meat during the storage and handling period.

The physicochemical and sensory properties of meat during the freezing period can undergo significant variations due to lipid and protein oxidation, as well as the generation of volatile compounds that can affect its sensory and culinary fitness. It is therefore important for the establishment to

make a number of recommendations to consumers concerning the defrosting of meat as well as cooking treatments in order to preserve its organoleptic characteristics.

Finally, the dates of preferential consumption provided in the report are merely indicative, being the responsibility of the operator of the retail establishment to set a useful life period for each type of meat piece depending on the conditions of conservation and intended use by the consumer.

These dates of minimum durability are summarised in Table 3.

Animal species	Freezing after receipt	Maturation	Freezing after maturation
Beef	12 months at -18 °C	14 days at <2 °C	9 months at -18 °C
Sheep	12 months at -18 °C	14 days at -1.5 °C	9 months at -18 °C
Caprine	12 months at -18 °C	3-5 days at 4.44 °C	UD*
Pork	6 months at -18 °C	UD	UD
Poultry	12 and 9 months at -18 °C for whole and cut products	9 days at < -1 °C	UD
Rabbit	UD	UD	UD

*UD: not possible to establish general recommendations.

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