Review Article

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Factors Determining Meat Quality and Cold Preservation Methods to Extend Shelf Life

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ABSTRACT

Meat refers to animal tissues that can be used as food, its main component being skeletal muscle with some degree of fat and connective tissue. Since the early 1980s, the production, consumption and world trade of meat has increased considerably and these changes are related to world population growth, urbanization and rising domestic incomes in developing countries, as well as changes in consumption patterns, which trigger a global increase in demand for food of animal origin. In general terms, "quality" can be defined as the extent to which a product or service meets consumer expectations over time. When referred to meat quality, it focuses on hygienic aspects during its production, to its nutritional value or to the organoleptic or technological characteristics. The conservation of food implies the action to keep it with the properties or desired nature for as long as possible. The application of cold in meat, as the most used method for its conservation, is mainly due to two purposes: to preserve the initial food quality, with a view to its consumption, and to keep it at an adequate temperature for its maturation and the chemical and biochemical reactions that determine its quality and shelf life. The shelf life of food can be defined as the maximum time in which food maintains its nutritional, sensory, microbiological and safety qualities at levels accepted by consumers. Refrigeration and freezing are the most used traditional methods to preserve meat, based on the application of cold to the carcasses. The main objective is to avoid decomposition due to bacterial alteration.

HIGHLIGHTS

a. The use of antifreeze proteins reduces the size of ice crystals.

b. Chilling causes weight losses in carcasses due to evaporation of water on the surface.

c. Meat quality is due to pH, color, texture, WRC and chemical composition.

KEYWORDS: Temperature; Duration; Organoleptic; Maturation; Carcass; Decomposition

INTRODUCTION

Food has been an important selective force in human evolution. Early hominids obtained energy and protein from fruits, vegetables, and roots. Later, more efficient feeding practices such as hunting large mammals were encouraged, allowing meat to be included in the diet [1]. Since the early 1980s, world meat production, consumption and trade have increased considerably.

These changes are related to world population growth, urbanization and rising domestic incomes in developing countries,

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as well as changes in consumption patterns, which are driving a global increase in demand for food of animal origin [2].

Refrigeration is one of the most widely used techniques in meat preservation, reducing the growth of microorganisms and the chemical reactions that lead to spoilage, with the advantage of maintaining the integrity of the product without altering it [3]. Another technique is freezing, which is mainly applied to further extend the shelf life of the product by largely paralyzing chemical and microbial reactions, using temperatures of -18 °C or lower, which creates a safe medium for freezing meat to the center of the muscle mass [4,5]. As global trade increases and the distance between producer and consumer increases, the need to refrigerate or freeze meat to facilitate transport and marketing increases [6].

Meat refers to animal tissues that can be used as food, the main component being skeletal muscle with some degree of fat and connective tissue [7]. The process of converting muscle into meat is composed of three phases [8]; the pre rigor phase, during which the muscle remains excitable, corresponding to the survival phase of the nervous system [9]; the rigor mortis phase, in which the energy components (ATP, glucose) are exhausted; and finally, the post rigor phase of maturation or tenderization of the meat in which the destructuring of the muscular architecture takes place [10].

When the animals are rendered, because of bleeding, the supply of oxygen and nutrients to the muscle ceases, which will produce a gradual and progressive decrease in available energy. In these circumstances, glycogen stores are used in the muscle to synthesize ATP from glucose to maintain temperature and structural integrity, gradually leading to a shift from aerobic to anaerobic metabolism [10]. This pathway in the absence of oxygen continues until lactic acid is formed and accumulates in the muscle, as the cell is unable to metabolize or transform it. This increase causes a gradual decrease in muscle pH, which will continue until glycogen stores are depleted. The accumulation of this acid in the muscle will cause the pH to drop from 7, which is the pH found in living animals, to acidic values around 5.3 to 5.8 at 24 to 36 hours after the benefit [11,12].

When muscle stores are depleted, the depletion of ATP that maintains the structural integrity of the muscle causes a slow depolarization of the membranes, producing an increase in ionic strength, which causes Ca^{+2} to escape from the sarcoplasmic reticulum into the myofibrillar space. These Ca^{+2} ions react with troponin which, in response, changes its configuration by unblocking the active sites of actin to which it was bound [13].

When these are free, the myosin heads bind to actin, leading to an irreversible union between the two [13], causing muscle contraction or rigor mortis. After rigor, the maturation stage begins, which produces an improvement in the texture of the meat because of the breakdown of the myofibrillar structure by endogenous enzymes present in the post-mortem musculature, producing the degradation of proteins and, therefore, the softening of the meat [14,15]. Through this process the meat becomes more palatable to consumers. For this process to take place, the meat must be chilled and kept at a temperature above freezing point, for a variable number of days and without bacterial contamination [11]. This whole process of transformation from muscle to meat has a variable duration depending on the animal species, the age, the individual and the measures adopted during the processing of the animals [16].

General objective: To describe and analyze the factors that affect the quality and shelf life of meat and the cold preservation methods that extend its shelf life.

MATERIALS AND METHODS

The information was compiled from scientific journals, theses, books, and research found through a search in the databases of the Library of the University of Concepción, using the following search engines: ScienceDirect, Meat Science, PubMed, Taylor & Francis online, Scielo, e-book, among others. We also searched the Internet portals of organizations such as the Food and Agriculture Organization of the United Nations (FAO) and the United States Department of Agriculture (USDA).

The following keywords were used to search for articles: meat, quality, shelf life, preservation methods, refrigeration, freezing. Of all the publications found, those in Spanish and English were selected, preferably from the last 20 years, which were considered relevant to this bibliographic research.

RESULTS

Factors Determining Meat Quality

Quality is a complex term for which there is no single definition valid for all levels of meat production. In general terms, quality can be defined as the extent to which a product or service satisfies the expectations of the user or consumer over time [16]. From another point of view, it can be interpreted in terms of hygienic aspects during production, nutritional value, organoleptic or technological characteristics [17]. Whatever the definition, the main requirement is that meat consumption must not compromise the health of the consumer. There are several factors that could affect the quality of meat [18], and it is necessary to know them to avoid losses at the end of the process. Some of these parameters are the following:

Chemical composition: This is of great relevance as meat is an important component of the human diet providing a wide range of nutrients, such as protein, fat, vitamins, and minerals [19]. These fractions are variable, especially the fat content, depending on the species, the breed, the type of feeding of the animals and even the piece of meat [142]. In general, average values for meat composition can approximate 62% moisture, 17% protein, 20% fat, and 1% ash for fattier meats or 71 to 75% moisture, 20% protein, 1 to 6% fat, and 1% ash in the case of leaner meats [20,21]. From a nutritional point of view, the importance of meat derives from its high-quality protein containing all essential amino acids, as well as a good amount of highly bioavailable minerals and vitamins essential for growth and development [21,22]; it is also an important source of iron, zinc and selenium, and significant amounts of essential fatty acids such as Omega-3 and conjugated linoleic acid [22]. Excluding meat from the human diet and not replacing it with other nutrient-equivalent foods can affect health through nutrient deficiencies [23].

pH: It is a determining attribute of quality, as it affects the biochemical processes that take place during the transformation of muscle into meat, directly influencing the stability and properties of the proteins and the physicochemical characteristics of the meat [24].

In the living animal, the pH value of the muscle is between neutral 6.7 and 7.2. After the death of the animal, the blood circulation and thus the oxygen supply to the muscle is interrupted. However, a certain enzymatic activity continues in the muscle, which causes ATP degradation until its almost complete disappearance.

In this situation, characterized by the absence of oxygen, and in the face of the persistent demand for energy by the muscle, the glycolytic route becomes important, which ultimately degrades the muscle's glucose reserves to obtain energy. The consequence of this situation is an increase in the concentration of lactic acid in the muscle and the result is a decrease in the pH value [16]. In general, 24 hours after the animals have been slaughtered, the pH value in the muscle is around 5.5 [16]. Lactic acid in the muscle has the effect of retarding the development of bacteria that contaminate the carcass during slaughter. These bacteria deteriorate the meat during storage, especially in warm environments, and the meat develops unpleasant odours, colour changes and rancidity [140].

The evolution of pH after rendering can have a profound effect on the sensory and technological properties of meat [24]. There are several factors that are important to consider such as stress. Stress is a hormonal and biochemical adaptation of the animal's internal and intracellular environment to sudden and intense changes in the environment that allow the animal to survive [26]. Gallo [27] points out that muscle glycogen depletion is attributed to situations of physical stress and lack of feed, and this leads to important effects on meat quality. Ante-mortem stress causes an excessive consumption of muscle glycogen, minimizing the amount of lactic acid in post-mortem muscle and thus preventing the natural pH drop in this period. This occurs in animals that are transported to the slaughterhouse and that suffer fears or traumas during loading or unloading and are further stressed by the hierarchy fights between them, or also, during rendering, when it is carried out inadequately [28,29].

Due to these stressful situations, two types of anomalies can occur in the meat that are important to evaluate, as they lead to a loss of quality and, therefore, to its rejection by the consumer. These are:

a) PSE (pale, soft and exudative) meat: this is a condition that occurs in pigs, turkeys and poultry [30,31], is caused by a high sensitivity to stress of some genetic lines of pigs and occurs when they are subjected to stressors immediately before slaughter [32]. PSE meat is a quality defect associated with a rapid rate of post-mortem glycolysis, which is characterized by a high rate of acidification in the first hour after rendering, reaching pH values of 5.4 - 5.5 [25,32]. Troeger & Woltersdorf [33] evaluated the effect of ante-mortem stress and handling during dressing on stress-resistant and stress-sensitive pigs. They showed that muscles of stress-sensitive pigs can achieve pH values at 24 hours post-mortem that are normal and like the pH of muscles of stress-resistant pigs when pre-mortem management and slaughter is appropriate. On the contrary, when handling is inadequate, muscles from stress-resistant pigs can show pH values below normal ranges.

b) DFD (dark, firm, and dry) meat: this condition can occur in cattle or sheep carcasses, and occasionally in pigs and turkeys, soon after processing [25,34]. In the case of cattle, this is referred to as DCB (dark cutting beef) [35]. This develops when muscle glycogen decreases before slaughter resulting in a high muscle pH from reduced post-mortem glycolysis [36]. This type of meat does not reach normal pH at any time, but remains at elevated final pH levels above 6.2, usually higher and even up to 7.0. Muscle glycogen is consumed during transport and handling in the period before slaughter. Consequently, there is little lactic acid generation after rendering, thus producing DFD meat [35,37]. Meat with this condition implies that the carcass came from an animal that was stressed, injured or diseased prior to processing [35].

Good management of livestock, in an efficient, expert, and calm manner, using appropriate techniques and facilities and taking measures to avoid pain and accidental injuries, will reduce stress in the animals and thus avoid deficiencies in the final quality of the meat [38].

Colour: If it is considered that the consumer judges the quality of meat at the time of purchase by means of the perception of colour, then this is a parameter of high economic importance [39]. Sepúlveda et al. [40] mention that colour is the main aspect that consumers evaluate as an indicator of quality above others, such as price, in the same way, if the consumer considers colour to be unacceptable, the rest of the attributes lose all importance, as this characteristic is what makes the consumer decide whether or not to buy the product [41].

The factors that determine the colour of meat are the redox reactions of myoglobin and the amount of myoglobin present in the muscle. This protein is composed of a prosthetic group that is globin and a center that has a heme group, which is responsible for the colour, as it can bind with other molecules giving it oxidative and reductive properties [42]. The myoglobin content accounts for 95% of the total pigments and the function of this protein is to facilitate the supply of oxygen to the muscle fiber, as it binds reversibly to oxygen, thus improving the availability of oxygen particularly when the partial pressure of oxygen is low [43]. In fresh meat, colour is influenced by the amount and chemical state of myoglobin, which can be found in three different states depending on the oxidation phase of the iron and the ligands that bind to its free bonds [44,45]. Inside the meat, where the partial pressure of oxygen is low, myoglobin is in a reduced state, called deoxymyoglobin (Mb or DeoxyMb), giving the meat a purple or purplish red colour. This state can only be maintained under anoxic conditions, such as in vacuum packaging and in the muscle immediately after cutting [46]. When DeoxyMb is exposed to oxygen, it picks up an oxygen molecule and oxymyoglobin (MbO2 or OxyMb) is formed, which manifests itself in the bright red colouring that is characteristic of the surface of fresh meat [47]. As oxygen exposure increases, OxyMb penetrates deeper beneath the meat surface. The depth of oxygen penetration and the thickness of the OxyMb layer depend on meat temperature, oxygen partial pressure, pH and competition for oxygen by other respiratory processes [48]. With the increase in the thickness of this layer, there is a simultaneous increase in the saturation of the bright red colour that consumers find more aesthetically desirable [45]. The third chemical state of myoglobin is metamyoglobin (MMb or MetMb), which is produced by the oxidation of the iron atom and the binding of a water molecule, giving a dull brown or brownish colour that consumers associate with a loss of quality or deterioration of the product [48], and is therefore rejected at the time of purchase. MetMb is formed by prolonged exposure of OxyMb to oxygen when meat has been stored for a long period in these conditions or directly from DeoxyMb when oxygen pressures are low. However, the deeper the OxyMb layer, the longer it takes for MetMb to appear on the surface and affect the tone, leading to discoloration of the meat [42]. It is well established that oxygen is required to preserve the typical bright red colour of meat, however, oxygen also accelerates biochemical changes, chemical oxidation, and bacterial growth, and all these factors contribute to the loss of colour [44]. In vacuum-packed meat, the penetration of a small amount of oxygen leads to the formation of a MetMb layer on the surface of the meat due to the fact of actual oxidation and not oxygenation [44].

As meat comes from live animals, skeletal muscle adapts to the environment in which the animal resides and its need to respond to various stimuli [49,50], so there are numerous extrinsic and intrinsic factors that can alter the colour of fresh meat.

Extrinsic factors, i.e., not dependent on the animal, include:

a) **Feeding management**: Priolo et al. [51] compiled a review on the effects of pasture and concentrate feeding on meat colour in ruminants. Meat from animals finished on grass diets was darker than meat from animals finished on concentrate diets. pH was one of the main reasons for the reported differences between pasture and cereal based systems. Vestergaard et al. [52] reported that forage-based diets fed in restricted amounts could promote oxidative metabolism, rather than anaerobic muscle metabolism and glycogen storage. Bulls fed restricted forage-based diets were observed to have lower glycogen, higher pH and darker muscle colour than bulls fed ad libitum concentrates. The higher pH in pasture-raised animals could also be attributed to differences associated with production systems, as cattle raised in extensive pasture systems have minimal human contact and handling compared to feedlot animals. Therefore, pasture-raised animals may be more susceptible to pre-mortem stress, which in turn may lead to a decrease in muscle glycogen content prior to slaughter and a high final pH in the meat [53].

b) **Ante-mortem stress**: Acute and short-term ante-mortem stress can lead to the formation of PSE meat. These undesirable conditions increase protein denaturation, which decreases water holding capacity, resulting in a shift of intracellular water and myoglobin into interfibrillar spaces [54]. Thus, the muscle structure becomes softer and "open", which increases light scattering and has a paler visual appearance [54,55]. In contrast, animals subjected to prolonged ante-mortem stress result in meat with a higher pH (>6) and dark cut, described as DFD [54]. This high pH is the result of low energy storage in the muscle caused by prolonged pre-mortem stress. This condition results in the meat having a higher water holding capacity and creates a more compact tissue structure that reflects less light [55].

c) **Storage temperature**: myoglobin is sensitive to temperature fluctuations, the rate at which it oxidises accelerates with increasing temperature [56], and meat discolouration is delayed at lower temperatures [57]. This is because higher storage temperatures result in increased reaction rates of prooxidants (chemicals that induce oxidative stress) present within the muscle [58]. High storage temperatures also increase the activity of oxygen-consuming enzymes in meat [138], enhance microbial growth [55] and accelerate lipid oxidation [59], all of which increase meat discolouration. Martin et al. [60] observed that minced meat samples stored at 2.3 °C discoloured more rapidly than those stored at -1.7 °C. The accelerated discolouration was attributed to increased microbial growth and lipid oxidation, as both processes produce undesirable by-products, which negatively affect meat colouration.

Intrinsic, i.e., animal-dependent factors include:

a) **Species**: The colour of fresh meat depends on the species [48]. Pork is lighter, greyish pink in colour and is accepted by consumers, whereas meat from ruminant livestock (cattle, goat and lamb) is darker than pork, and a bright cherry red colour is considered acceptable in these species. Differences in meat colour between species are largely due to differences in myoglobin content [28,61], as shown in Table 1. Also, to the proportion of muscle fiber type [52].

Table 1: Differences in myoglobin content between different species [146].

Species	mg Myoglobin/g Fresh Meat
Cattle	15
Sheep	10
Pigs	5
Poultry	<5

Poultry meat is lighter in colour than mammalian meat and the reason for this difference is due to the type of muscle fiber they are composed of. There are basically two types of muscle fibers, those belonging to the muscles that carry out intense work (white fibers) and those that carry out slow, repetitive work (red fibers). White fibers are mostly found in birds, as they require fast movements, while large mammals have red fiber muscles that are necessary to withstand great efforts [44].

b) **Sex**: another factor that influences the colour of the meat is the sex of the animals, however, the conclusions of the authors have not coincided. Renerre [62] point out that females tend to have a higher concentration of myoglobin than males at the same age, because they are more precocious. Seideman et al. [63] report that the meat of intact males is darker than that of females and castrated males, which is attributed to higher myoglobin concentrations, probably due to higher levels of physical activity. In another study by Ronda et al. [64], where the colour of beef of both sexes was assessed, no significant sex differences in colour were found. This indicates that sex differences were minimal, or at least in the animals studied, which were of the same breed, reared under the same farming regime and slaughtered at the same age. Therefore, studies on the effects of sex on meat colour are inconclusive.

c) **Age**: Myoglobin concentration in meat increases with increasing age of the animals [65], as shown in Table 2. Myoglobin concentration affects the perceived colour of meat [28] such that older animals have redder and darker meat. Dhanda et al. [66] compared kid and goat meat, finding that as the age of the animal increased, the myoglobin concentration also increased, and the meat appeared darker.

Table 2: Myoglobin concentration in relation to age in cattle [41].

Age	Myoglobin Content
Calf	2mg/g
Steer	4mg/g
Young cattle	8mg/g
Older cattle	18mg/g

Water Retention Capacity (WRC): Water holding capacity was described by Hamm [67] as the ability of meat to retain its constituent water during the application of external forces, such as gravity, cutting, heating, chopping or pressure [68]. Meanwhile, Pearce et al. [18] describe it as the ability of meat proteins to retain water, both their own and added water due to the processes they undergo. WRC determines the weight loss, mainly through fluid release, that occurs throughout the meat distribution and processing chain and can also affect meat quality, especially in terms of juiciness, texture, and palatability [68].

WRC is of sensory importance because of its association with so-called juiciness [69], which is a unique subjective property of meat [70]. Juiciness is defined as the impression of moisture and lubrication when meat is chewed in the mouth [71], and this is expelled in the form of exudate. The exudate depends on the amount of fluid released from the muscle protein structure and the ease with which that fluid is released from that structure [72]. Approximately 50-55% of the constituent water in fresh meat is found in the muscle myofibrils, 30-34% in the sarcoplasm and 10-15% in the connective tissue [73]. Of the total water in muscle, 7-8% is strongly associated with the polar groups of the protein and is known as "bound water" [74]. Bound water remains tightly bound to proteins even when intense external forces are applied to the muscle. Next, water molecules bound by less intense forces become available, this water is called "retained" varying from 74 to 75% [74] and the amount that is released depends on the intensity of the external force applied to the muscle. Water that remains bound to the muscle structure only by surface forces is called "free water" and corresponds to 16.6 to 17.6%, which is easily expelled from the muscle when an external force is applied [74]. The water content of meat products is one of the most essential quality parameters for the meat industry, as it is related to the final yield of the product [75]. Any loss of water will obviously reduce the weight of the product, which implies an important economic loss [76], if the loss is excessive, it will reduce the sensory perception of the product and consequently, it will reduce the acceptability by consumers, which will result in a decrease in the sale value [77].

There are several factors that affect WRC, as explained above. Stress before profit is known to be particularly critical [78]. The water binding capacity of the musculature is directly influenced by pH. When meat reaches a low pH quickly, it will lose more liquid, because it will have a lower WRC (this is the meat described as PSE). Conversely, if the meat has only a small drop in pH, i.e., it does not drop much after the death of the animal, it will lose less liquid, as it will have a high WRC [70] (this is the meat described as DFD). Around the isoelectric point of the meat protein (pH 5.0 - 5.3), the protein repels water intensely, this is sought after so that the meat is better preserved [37]. However, if the meat has a high pH, the isoelectric point is not reached, thus increasing its water retention capacity and the preservation is diminished due to bacterial growth [28]. As the pH of the meat moves away from its isoelectric point, the proteins tend to increase their binding strength, which means that the WRC remaining inside the myofibrillar structures increases, so that water is tightly bound and little or no exudate is formed. Thus, the muscle appears "closed" and the structure does not reflect light, but absorbs it, making the flesh appear darker [54]. Muscle prone to the PSE condition, high temperatures and low pH, caused by rapid glycolysis, lead to myosin denaturation along with early destruction of the membrane. When the myosin head is denatured, it contracts causing contraction of the myofibrillar network [79]. These phenomena cause excessive contraction in the myofibril, sufficient to explain the excessive water loss in this type of meat [70]. The final tenderness of meat depends largely on the degree of alteration and weakening of myofibrillar structures, which would explain the increase of this characteristic in matured meat compared to unripened meat [80,149,154]. The structural integrity of myofibrils changes during meat maturation, which contributes to the tenderness of matured meat [81]. Tenderness increases if chilling time is extended [82]. Thus, post-mortem storage has a positive effect on the final texture [137,152].

The process known as "maturation" comprises a series of biochemical and structural changes that transform the characteristics of the meat. These changes are mainly due to the action of proteolytic enzymes, which contribute to the dissociation of contractile proteins and thus produce a softening of the meat known as "tenderization" [84,144]. The most important impact of maturation on meat quality is the significant tenderization of the meat. The tenderization of meat under maturation is considered to reach full tenderness after a period at refrigeration temperature of two to three weeks [84,139,148,151].

Meat tenderness generally decreases with the age of the animal [153]. A study by Jeremiah et al. [85], in which rectus femoris, vastus lateralis, biceps femoris, semitendinosus and semimembranosus muscles of the hind leg of sheep aged 74 to 665 days were evaluated for shear strength and tenderness, showed a positive correlation between age and decreased tenderness. Hopkins et al. [86] also reported much higher shear force values for the semimembranosus muscle of 14- to 20-month-old ewes compared to 8- to 14-month-old ewes. Young et al. [87] report that as the age of the animals increases, the solubility of collagen decreases, i.e., the cross-links become non-reducible, while the concentration of collagen remains relatively constant.

Collagen is the major protein component of endomysia and epimysia connective tissue and contributes to the tenderness of meat through the total collagen content and the degree of development of thermostable cross-links [88], the greater the amount of collagen or the number of cross-links, the tougher the meat [89]. The cross-links become thermostable as the animal matures, increasing the toughness of the meat of older animals [90], this is referred to as intrinsic toughness.

Cattle go through a series of phases or stages from farm to slaughter. Animals facing stressful situations will have an altered metabolism depending on the type of stress suffered [25], and this can lead to a significant depletion of muscle glycogen [28], if this happens, a normal final pH will not be reached when the animal is slaughtered, as demonstrated by PSE and DFD meats, the texture can vary considerably. In extreme cases, PSE meat appears extremely soft and moist, with muscle separation and rough texture. In contrast, DFD meat has a firm and rigid structure that retains its shape, and its high-water holding capacity gives the cut surface a sticky texture [91].

As can be seen, all the factors described above are interrelated, which is why proper animal management, both before and after processing, is of vital importance for obtaining high-quality meat that is accepted by the consumer (Figure 1). The level of demand on meat quality is increasing, especially in the organoleptic qualities of colour, juiciness, texture, and flavor [72,150].

Cold Preservation Methods

Food preservation involves the action of maintaining food with the desired properties or nature for as long as possible [92]. In 2011, the Food and Agriculture Organization of the United Nations (FAO) published a study in which they estimated that one third of food produced for human consumption is lost or wasted globally [93]. Food spoilage is characterized by any change in food that makes it sensory unacceptable to consumers. This may be due to physical damage, chemical changes or the appearance of off-flavors and odours resulting from microbial growth and metabolism in the food [94]. Fresh meat is a food that, due to its nature, decomposes easily, generally due to environmental factors such as temperature, oxygen, humidity, light, meat enzymes and bacterial flora, the latter being the most relevant factor in its deterioration [5] and affecting its shelf life [142].

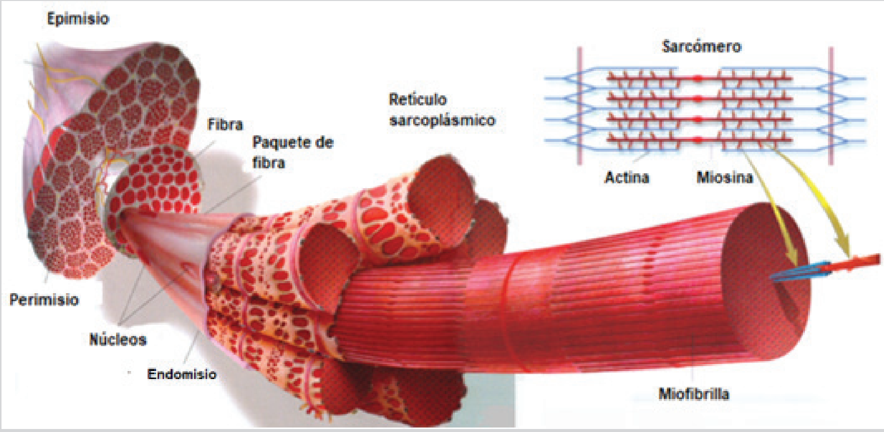

Figure 1: General structure of skeletal muscle [143,145].

Borch et al. [95] defined shelf-life as the storage time until spoilage. It can also be defined as the maximum time for which foods maintain their nutritional, sensory, microbiological and food safety qualities above a level considered acceptable by consumers [96]. However, the biological and chemical characteristics of meat make it an excellent medium for the growth of microorganisms that produce undesirable sensory changes, which is why preservation methods are aimed at delaying or inhibiting microbial growth to increase the shelf life of fresh meat [97].

The preservation of perishable foods at warm temperatures is the main cause of spoilage [98]. As the temperature approaches 0 °C, bacterial growth is much slower and fewer and fewer types of microorganisms can grow [99]. Micro-organisms have the ability to multiply at high rates when favourable conditions exist [92], however, each micro-organism has an optimum temperature for best growth, a minimum temperature below which they no longer grow and a maximum temperature above which all growth is suppressed. Bacteria that grow well at low temperatures, 15 °C or below, are called psychrophilic, those that grow at temperatures of 20-45 °C are mesophilic and those that grow above 45 °C are thermophilic [100].

Microbial growth in food results in the development of undesirable sensory characteristics and, in certain cases, food may become unsafe for human consumption. The application of cold to meat as the most widely used method for its preservation is mainly due to two purposes: to preserve the initial quality of the food, with a view to its consumption, and to maintain it at a suitable temperature so that it matures and the chemical and biochemical processes that determine the final colour, flavor, odour and texture develop [44].

It should be borne in mind that refrigeration does not improve the quality of the meat, it only maintains it, therefore, it should be applied when the meat has an adequate hygienic-sanitary quality [44]. The shelf life of meat can be extended by several days by chilling and several weeks or months by freezing [98].

Refrigeration: The preservation of meat by refrigeration is a widely used method that reduces the physical, chemical, and microbiological changes that occur as a result of high temperature, largely preserving the nutritional value and the original organoleptic characteristics of the food [44].

Refrigeration is the action of cooling, i.e., removing heat from a product [101]. The vapour compression refrigeration cycle is the most used in refrigerators or air conditioning systems and is composed of four processes: Refrigerant compression in the compressor; Heat removal in the condenser; Refrigerant inlet to the expansion valve; Heat absorption in the evaporator [102].

A temperature of 4 °C or less is considered a safe refrigeration temperature [98], as cold inhibits the growth of microorganisms, but does not destroy them. These bacteria generally thrive in the temperature range of 4.4 °C and 60 °C, which is why, to maintain product safety and quality, refrigeration systems should be kept below that range [103]. Lawrie [104], states that the refrigeration temperature that is considered critical for the handling and storage of meat and that cannot be exceeded for any reason without substantial loss of quality and appearance is 5 °C. Prevention of food spoilage and premature loss of quality, due to microorganisms, is the major area of application of refrigeration [98].

Refrigeration is the most widespread and widely applied food preservation treatment in both domestic and industrial settings [105]. The improvement, in days, of the shelf life of refrigerated food will depend on the initial contamination of the product. If the initial contamination is low and the food is of good quality, optimal results will be achieved. This is not the case if the raw material is of poor quality, with high contamination, as the delay caused by chilling will only have a minor effect [105]. Although most carcass chilling processes are primarily aimed at ensuring food safety and security by inhibiting bacterial growth, it is known that temperature management also has a great influence on the final characteristics of the meat [11,106].

There are two basic forms of refrigeration: the natural way, in which freezing derivatives such as ice, dry ice, or simply that the food is introduced in water that has depth, are used to maintain a stable temperature and not allow its deterioration to accelerate [107], and the mechanical way, which is based on a process of evaporation of the liquid refrigerant that is inside a machinery, this allows the cold to constantly run through the refrigerator and the temperature to be artificially regulated, and well below the temperature of the environment [107]. The biggest problem with refrigeration lies in the weight loss suffered by the carcasses, due to the evaporation of water on the surface of the meat, which depends on the speed of the temperature drop, generating a greater weight loss when the cooling speed is slower [108].

Freezing: This method of preservation provides a longer shelf life to meat by paralysing the chemical and bacterial reactions present in it [5]. Freezing consists in the extraction of sensible and latent heat from the product, which causes the crystallization of the water it contains. In other words, it is the lowering of the temperature of the product below its freezing point [11]. The formation of ice crystals in meat is mainly determined by the freezing speed of the products, and large extracellular crystals can form when freezing is slow, causing great alteration in the quality of the meat [109].

It has been suggested that large crystals with sharp edges can rupture cell walls and contribute to texture deterioration due to drip water loss [110], whereas rapid freezing generates small, fine intracellular crystals in the muscle, which are evenly distributed and do not cause significant losses [109]. Therefore, the speed of freezing is the parameter used to control the size and distribution of ice crystals in the system [111]. A further complication is recrystallisation, which occurs due to temperature fluctuations during freezing, causing the formation of larger crystals from the smaller crystals formed at the beginning [112]. It is the process in which, over time, the average size of ice crystals increases, and their number decreases due to the redistribution of water from smaller ice crystals to larger ones [111]. One way to prevent recrystallisation is to keep the temperature constant throughout the storage process of frozen meat [3]. Freezing food involves three steps: (1) cooling to the freezing point (removing sensible heat), (2) freezing (removing latent heat) and (3) further cooling to the desired temperature at which the product will be maintained during storage [98].

Although the average freezing point of lean meat is -2 °C, it should be noted that freezing occurs over a temperature range, which goes from -1 to -4 °C. However, meat must be frozen and stored at much lower temperatures for long-term storage [98]. As shown in Table 3, the lower the storage temperature, the longer the shelf life of meat products.

Table 3: Storage life of frozen meat products at different storage temperatures [136,141].

Storage Life in Months			
	Temperature		
Product	-12 °C	-18 °C	-23 °C
Beef	4-12	6-18	12-24
Lamb	3-8	6-16	12-18
Veal	3-4	4-14	8
Pork	2-6	4-12	8-15

aW is one of the determining factors in the prediction of food stability. The rate of many spoilage changes has been related to this parameter, as it determines the water that is available for microbial growth [123]. Most microorganisms in food proliferate at high aW values. In general, the lower limit of aW for microbial growth is 0.90 for most bacteria [113]. However, growth of most bacteria ceases below aW of 0.90 [114]. Thus, if the aW decreases, few genera of micro-organisms will be able to multiply on the food [114].

To obtain good freezing results, several strategies have been applied to increase the heat extraction rate, however, for this, the product should ideally be small and individually frozen. Freezing large products only results in the formation of large crystals that reduce the quality of the food [111]. New preservation technologies have been explored to improve the quality of meat reaching the consumer. To solve this problem, research focuses on the nucleation phase to produce numerous and small-sized crystals [115]. However, these technologies are generally more expensive than their conventional counterparts and are therefore not yet applied on a large scale. Some of the processes that stand out are the following:

Ultrasound-assisted freezing: Power ultrasound can be defined as a type of acoustic wave of low frequency (between approximately 20 and 100,000kHz) and high intensity (generally greater than 1W/cm2). If power ultrasound is applied to a product that is freezing, it produces cavitation on the liquid phase that has not yet frozen [116]. Cavitation, as shown in Figure 2, consists of the formation of small bubbles that grow as the acoustic pressure decreases and compress as the acoustic pressure increases until they collapse violently. These bubbles act as nucleating agents favouring the formation of ice nuclei throughout the sample volume [117]. The oscillatory motion of the bubbles also induces strong microcurrents that facilitate heat and mass transfer, thus accelerating the freezing rate [118]. On the other hand, the stresses exerted by ultrasound cause fractures in the ice crystals, resulting in smaller ice crystals in the final frozen product [119]. On the other hand, the higher the power and the longer the exposure time, the faster the temperature of the sample drops. However, it must be considered that the higher the power of the ultrasound passing through the medium and the longer the application time, the more acoustic energy is converted into heat.

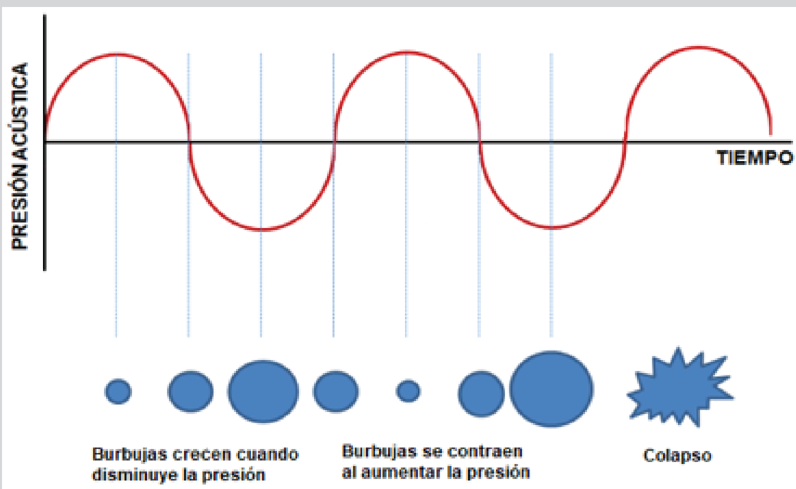

Figure 2: Descriptive diagram of cavitation caused in liquids by ultrasound [118].

For this reason, the treatment is carried out intermittently, to avoid a temperature, increase of the coolant. Therefore, it is necessary to find a balance between these parameters to optimize the process [120].

Dehydrofreezing: A technique in which a food is dehydrated to a desirable moisture content and then frozen [121]. Much of the water in the product is removed prior to freezing using an osmotic solution or air drying, and results in a freezing process that is faster [122]. The water content of food is one of the single most important factors influencing its preservation [113]. The higher the aW and the closer it is to 1.0, the higher the probability of food spoilage. Fresh meat has an aW of about 0.97. Reducing the amount of water in dehydration processes results in intermediate or low moisture foods, which corresponds to an aW of 0.65 to 0.86 or less. This decrease in water often increases the shelf life of meat due to the reduction of water available for the growth of microorganisms [123].

High pressure freezing: When water freezes at atmospheric pressure, its volume increases. This increase in volume is attributed to the ice that forms, which has a lower density than liquid water, resulting in a volume increase of approximately 9% when freezing at 0 °C and approximately 13% at -20 °C [124]. This causes tissue damage upon freezing. During the phase transition, high-pressure ice does not expand in volume, which may reduce tissue damage. Changing the physical state of food can be achieved by external manipulation of temperature or pressure [125]. Food can be frozen without any form of cooling, only by controlling the pressure. However, few experiments have been carried out in this area, due to the high pressure required [124]. It is important to note that freezing of the feed does not occur at high pressure but at atmospheric pressure after expansion [116]. The main advantage of high-pressure freezing is that the initial ice formation is instantaneous and homogeneous throughout the product volume, due to the high supercooling achieved on pressure release. Therefore, this technology can be especially useful for freezing foods with large dimensions where a uniform distribution of ice crystals is required [77,126].

Antifreeze protein: Initial interest in antifreeze protein was sparked by observation of fish inhabiting polar and northern coastal waters. It was discovered that antifreeze proteins found in the blood and tissues of fish prevent them from freezing [127]. The function of antifreeze proteins is to reduce the freezing temperature and inhibit the growth of ice crystals [122]. In addition, they can inhibit recrystallisation during storage and transport, thus preserving food texture by reducing cell damage and minimizing nutrient loss by reducing drip [128]. There are many types of antifreeze proteins synthesized within each organism, Hew & Yang [129] classified antifreeze proteins into two main groups: antifreeze glycoproteins and antifreeze (non-glycosylated) proteins. Antifreeze proteins are present in a wide range of cold-adapted organisms in nature such as: fish, plants, insects, fungi and bacteria [130]. Antifreeze (non-glycosylated) proteins can be either thermal hysteresis proteins (THPs) or ice structuring proteins (ISPs). THPs are known to prevent ice crystal formation by lowering the freezing point of water [131]. Whereas ISPs control the size, shape, and aggregation of ice crystals rather than preventing water from freezing [132].

Ice crystal growth is inhibited because the antifreeze proteins adhere to the ice surface, thus restricting ice crystal growth to the exposed surface between adjacent antifreeze molecules [133]. A study in which beef cuts were soaked in a solution containing 1mg/mL of antifreeze proteins before freezing at -20 °C showed that the crystals were significantly reduced in size [135]. Also, a study investigated the effect of administration of Antarctic cod antifreeze protein prior to processing of lambs on meat quality after slaughter and freezing. The lambs were injected intravenously at various times prior to processing, the meat samples were vacuum packed and stored at -20 °C for 2 to 16 weeks [134]. It was observed that injection of antifreeze glycoprotein 1 or 24 hours prior to dressing reduced drip loss and ice crystal size in meat samples. The smallest ice crystals were found in lambs injected with 0.01mg/kg antifreeze glycoprotein 24 hours before rendering [136].

CONCLUSION

Meat quality will be optimal if the animals are handled properly, both before and after slaughter. In addition, cold preservation methods are very useful and necessary to maintain meat quality over a long period of time.

The preservation of meat, regardless of the method used, seeks primarily to avoid its decomposition due to bacterial alteration, reducing to a minimum, a possible drop in the quality of the product. The temperature of the musculature in the pre-rigor state is around 38 to 40°C, because metabolic activity continues to develop in the muscles, even though the animal is dead. This temperature provides an optimal environment for the development of bacterial growth, a fact that supports the application of chilling of the carcasses as quickly as possible.

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