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The effect of *sous vide* cooking technique on physicochemical properties of meat

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Considerate la vostra semenza:
fatti non foste a vivere come bruti
ma per seguir virtute e canoscenza.

Consider well the seed that gave you birth:
you were not made to live as brutes,
but to follow virtue and knowledge.

Dante Alighieri (Inferno, Canto XXVI, vv 118-120)

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Riassunto

Il consumatore d'oggi è sempre più attento alla qualità degli alimenti che introduce con la dieta ed è sempre più interessato a quali siano le migliori tecniche di cottura da applicare.

La carne è un alimento fondamentale nella dieta dell'uomo, essa infatti apporta numerosi elementi nutritivi tra cui: amminoacidi essenziali, vitamine, sali minerali e ferro. Sebbene la carne svolga un ruolo importante nell'alimentazione, tecniche di cottura troppo aggressive, caratterizzate da alte temperature possono portare alla formazione di molecole dannose per l'uomo. Tra le cotture più innovative, si menziona la tecnica "*sous vide*", caratterizzata dall'utilizzo di basse temperature per tempi lunghi, applicata ad alimenti confezionati sottovuoto cotti all'interno di un bagno termostatato. Se in passato questa tecnica era utilizzata per lo più nella ristorazione collettiva oggi, invece, trova sempre più utilizzo anche tra le mura domestiche. Tra i vantaggi di questa tecnica vi è una maggiore succosità del prodotto finale, una maggiore concentrazione dell'aroma ed una maggiore conservabilità dell'alimento post cottura.

Lo scopo di questa ricerca è stato quello di valutare l'effetto della cottura "*sous vide*" su alcuni parametri chimico-fisici della carne ritenuti importanti per il consumatore; in particolare sono stati condotti tre differenti studi.

Nel primo studio sono stati sottoposti a due diverse combinazioni di tempo e temperatura di cottura "*sous vide*" (80°C per 1 ora e 60°C per 15 ore) lombi di maiale provenienti da suini alimentati con diete sperimentali (addizionate di semi di lino ed antiossidanti sintetici e naturali). Sono stati valutati parametri della carne quali: perdite di cottura, ossidazione lipidica, colore, tenerezza e profilo degli acidi grassi. Le due combinazioni di cottura hanno avuto effetti significativi su tutti i parametri del colore; i campioni cotti a 60°C per 15 h sono risultati avere livelli di L* inferiori e valori di a* e b* più elevati rispetto ai campioni cotti ad 80°C per 1 h.

Nel secondo studio sono stati utilizzati lombi di suino, acquistati nella grande distribuzione, sottoposti a 12 diverse combinazioni di tempo e temperatura (60°C, 70°C, 80°C, per 60, 90, 120, 150 min) e con o senza un pretrattamento ad ultrasuoni. I parametri valutati in questa sperimentazione sono stati: colore, umidità, ossidazione lipidica, e tenerezza. L'applicazione degli ultrasuoni non ha influenzato alcun parametro valutato, mentre la temperatura di cottura utilizzata ha determinato variazioni importanti sia per i parametri del colore (a* e b*), sia per le perdite di cottura, l'ossidazione lipidica ed infine per la tenerezza.

L'ultimo studio ha preso in considerazione 12 combinazioni di tempo e temperatura "*sous vide*" sul petto di pollo, acquistato presso un centro di grande distribuzione. Oltre ai parametri chimico-fisici valutati negli studi precedenti, è stato effettuato anche uno studio sulla "shelf life", condotto per 21 giorni a 4°C. Anche in questo studio la temperatura di cottura è risultata essere più importante nel determinare variazioni significative sia del colore, sia della stabilità ossidativa e della tenerezza. Per quanto riguarda lo studio di "shelf life", si è riscontrato come già la combinazione 60°C per 60 minuti sia più che sufficiente ad abbattere la carica microbica ed a garantire la stabilità del prodotto per tutto il periodo di conservazione.

Concludendo la cottura "*sous vide*" può rappresentare una valida alternativa alle cotture tradizionali applicate alla carne, incontrando sempre più i gusti del consumatore. Inoltre questa cottura permette di conservare a lungo gli alimenti che devono solo essere riscaldati prima di essere serviti, contrastando lo spreco alimentare e limitando la formazione di composti tossici che si originano dal processo di cottura.

Abstract

The consumer today is increasingly attentive to the quality of food that he introduces with the diet and increasingly pays interest to what are the best cooking techniques to apply.

Meat is important in the human diet it brings fundamental nutritional elements including essential amino acids, vitamins, minerals, and iron heme. However, aggressive cooking techniques for meat, characterized by high temperature can lead to the formation of dangerous molecules for human health. *Sous vide* technique is considered one of the most innovative and recent cooking techniques, it is characterized by the application of low temperatures for long times, applied to under vacuum food cooked in a thermostatic bath. In the past this technique was mostly used at the catering level, nowadays, it is increasingly used also at home. Among the different advantages of this technique, there is greater juiciness of the final product, a greater concentration of the aroma, and the possibility of increasing the shelf life of the cooked product.

The research aimed to assess the effect of *sous vide* cooking on physicochemical parameters of meat that are considered important for the consumer; in this regard, three different studies were carried out.

In the first study, two different combinations of cooking time and temperature were subjected to (80 °C for 1 hour and 60 °C for 15 hours) pork loins from pigs fed experimental diets (linseed, synthetic,

or natural antioxidants). The parameters evaluated were: cooking loss, lipid oxidation, color, shear force, and fatty acids profile. The two cooking combinations had significant effects on all the color parameters evaluated, the samples cooked at 60 °C for 15h had lower L* value and higher values of a* and b* than the samples cooked at 80 °C for 1h.

In the second study, 12 different combinations of temperature and time of *sous vide* were studied (60 °C, 70 °C, 80 °C; 60, 90, 120, 150 min) on pork loins bought in the large-scale distribution, and without or with an ultrasonic pretreatment. The parameters evaluated in this experiment were color, moisture, lipid oxidation, and shear force. The application of ultrasound did not affect any parameter evaluated, while the temperature used determined important variations both for the parameters of color (a* and b*), cooking loss, lipid oxidation, and shear force.

The latest study considered 12 combinations of time and temperature, applying *sous vide* cooking to the chicken breast, purchased in the large-scale distribution. In this study, in addition to physicochemical parameters evaluated in previous studies, a shelf life study was carried out, lasting for 21 days at 4 °C. Also, in this study, the cooking temperature was more important in determining significant variations in the parameters of color (a* and b*), oxidative stability, and tenderness. Overall, the profile of fatty acids was not significantly affected by time or even by the cooking temperature. As far as the shelf life study is concerned, it was found that the lowest temperature combination of cooking, for the shortest time is more than sufficient to break down the microbial load and to ensure the stability of the product throughout the shelf life period.

By concluding *sous vide* cooking can be a valid alternative to traditional cooking applied to meat, meeting the tastes of the consumer who requires more and more tender final products. The use of this cooking also allows keeping for a long time the food that must only be heated before being served, countering food waste and limiting the formation of toxic compounds that originate from the cooking process.

CHAPTER 1.

General Introduction

1.1 The role of meat in human nutrition and evolution

The role and the importance of meat for human evolution are unquestionable. During the time, scientists have studied meat consumption during evolution with two different approaches: indirect and direct (Baltic & Boskovic, 2015). With the indirect approach, the main objects of the studies are the fossils and the remains of plants and animals discovered in archeological sites. Regarding instead of the direct approach, it is based on the study and the quantification of isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) evaluating their quantity on the human rest of bones and teeth (Larsen, 2013). The study of the isotopes quantification is based on two principles:

- the isotopic composition of bone collagen (in humans) depends directly on the isotopic composition of foods that were eaten since the last 15-20 years before the death;
- different foods have different isotopic compositions, for this reason, knowing the composition allows scientists to know which kinds of food were presented in the diet (Tykot, 2004; Hedges 2006).

In his study DeNiro (1985) explained the difference between the quantification of N or C in the human rest of bone. Nitrogen derives only from the consumption of meat or fish; on the contrary, C derives from different sources of food especially from vegetal and cereals but also lipids or protein from meat. A high level of N isotopes means that the diet was rich in animal food or seafood, whilst a high level of C means that the diet was mostly vegetarian.

In human evolution, meat consumption is divided into four periods (Larsen, 2003; Pereira & Vicente, 2013):

- in the beginning the meat consumption was related only to opportunistic hunting; for this reason, the meat dietary intake was not constant but was related to the external conditions;
- communities of hominids started to be more organized and moved from opportunistic hunting to full-scale one. The access to the protein and meat products were more regular and predictable;

- shift from hunting and gathering to domesticated food sources both for animals and plants. This period coincided with the transition from nomadic life to the formation of the first fixed appropriations;
- until our days, where other aspects of meat composition are important for human health, such as the fatty acid profile or the consumption of meat, that is related to the high incidence of cardiovascular disease and cancers.

The most visible and physical effects on *Homo* due to the meat consumption were: an important increase in body mass (44% for males and 55% for females) and an increase of height (33% for males and 37% for females) (McHenry & Coffing, 2000), without forgetting the fundamental evolution step from quadrupedal to bipedalism, necessary to improve the research of food (Baltic & Boskovic, 2015). In his study, Mann (2007) deepened further physiological changes related to a dietary variation such as craniodental change, modification of the morphology of the gut, higher energetic requirements to develop a large ratio of brain to body size. As regards the modification of the craniodental, the teeth, in particular the front ones, became harder and better suited for biting and tearing than grinding. On the contrary, the number of molar teeth decreased and the structure of jaws/skull became frailer. Regarding the study of the morphology of the human gut in comparison with ruminants or carnivores, but altogether it is smaller with a more pronounced small intestine. As regards the development of the brain, what was very important was the right balance of polyunsaturated fatty acids belonging to n-3 and n-6 groups fatty acids longer than C18 (Crawford, 1992).

If in the prehistoric period the consumption of meat was necessary to reach a high level of evolution, from Roman's history until today the consumption of meat or meat products has had also a social role (Murcott, 1995). However, in the years following the economic boom since our days, the attention of experts and consumers has been more focused on the possible negative effects of meat consumption on human health. In this respect, in 2015 red meat and red processed meat were classified as *probably carcinogenic to humans* (Group 2A) and *carcinogenic to humans* (group 1) respectively, by the International Agency for Research on Cancer (IARC).In particular, by the experts, was defined how the consumption of 50 gr per day of processed meat, is responsible for increasing the risk of colorectal cancer by 18% (IARC, 2018)

1.2 The consumption of meat in the World, Europe, and Italy

In the period from 1961 to 2011, the consumption of animal protein in the World increased from 61 g/per day to 80 g/per day. In only 50 years the global consumption increased by 32%; Sans & Combris (2015) found the reason for that in two triggers:

- the economic development;
- the urbanization.

As regards economic development, with the increase in incomes, the budget for the purchase of food also increases, leading, therefore, to choose products with a higher economic value than in the past, such as meat. The effect of urbanization was more effective in emerging countries, where in the last 20 years we have assisted to a "livestock revolution" characterized by an increase in meat consumption, from pork and poultry.

Figure 1. Meat supply quantity (kg/capita/year) in 2017 in different continents (FAO, 2020)

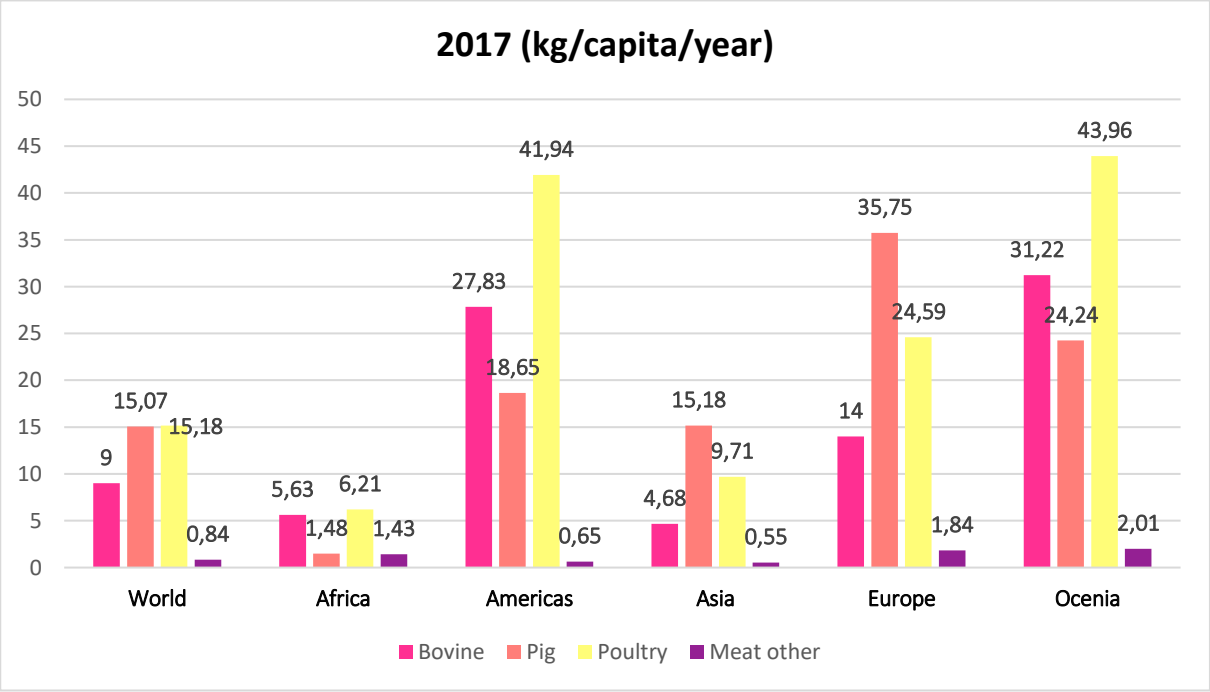
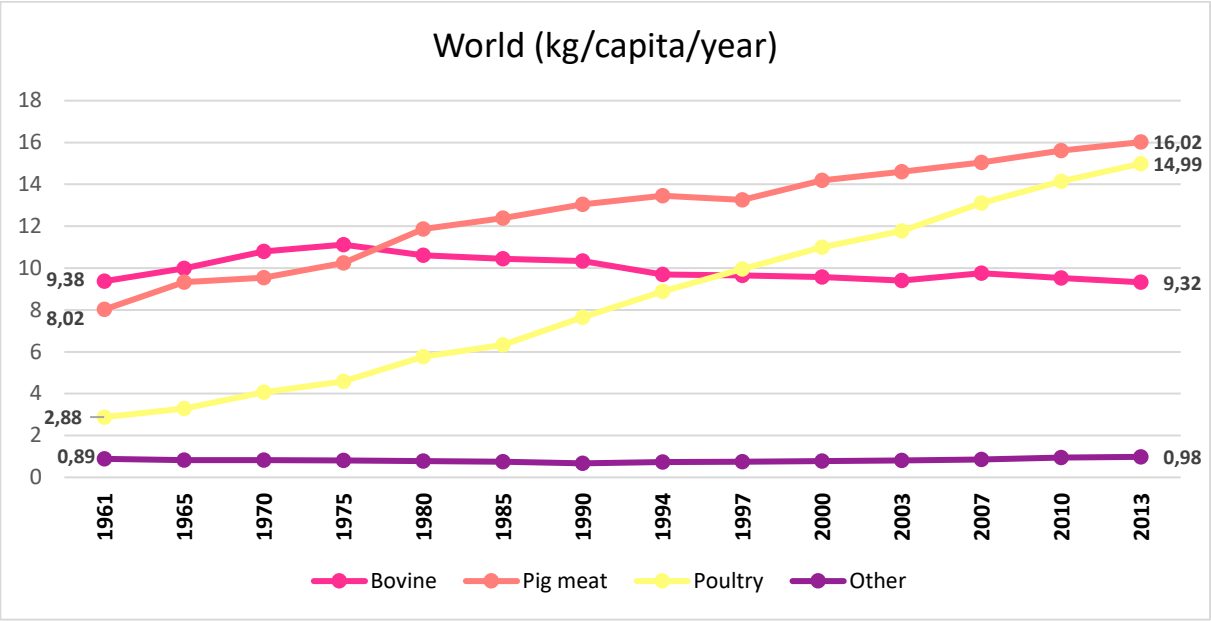


Figure 1 shows the data collected by the Food and Agriculture Organization of the United Nations (FAO) for the year 2017 as regards meat consumption in the World and different continents (www.fao.org/faostat/en, 2020).

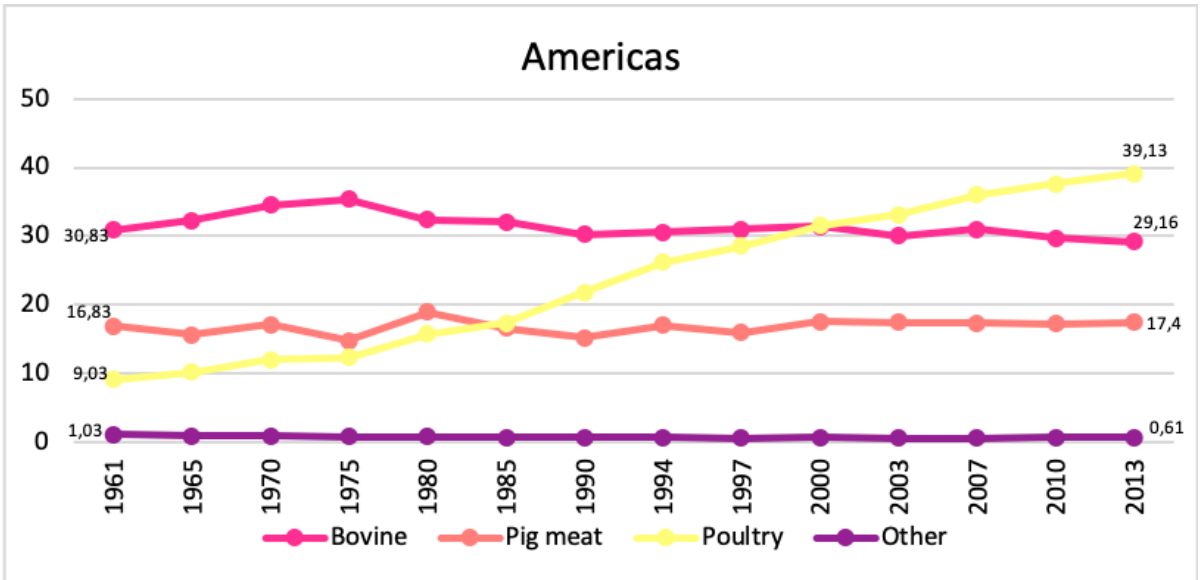
The most eaten meat in the world are pork and poultry, this is due to different reasons, such as the economic cost of the raw meat (for poultry), the easiness of farming, and a large number of product-derived products from pork.

Figure 2. World meat consumption (kg/capita/year) from 1961 to 2013 (FAO,2020)



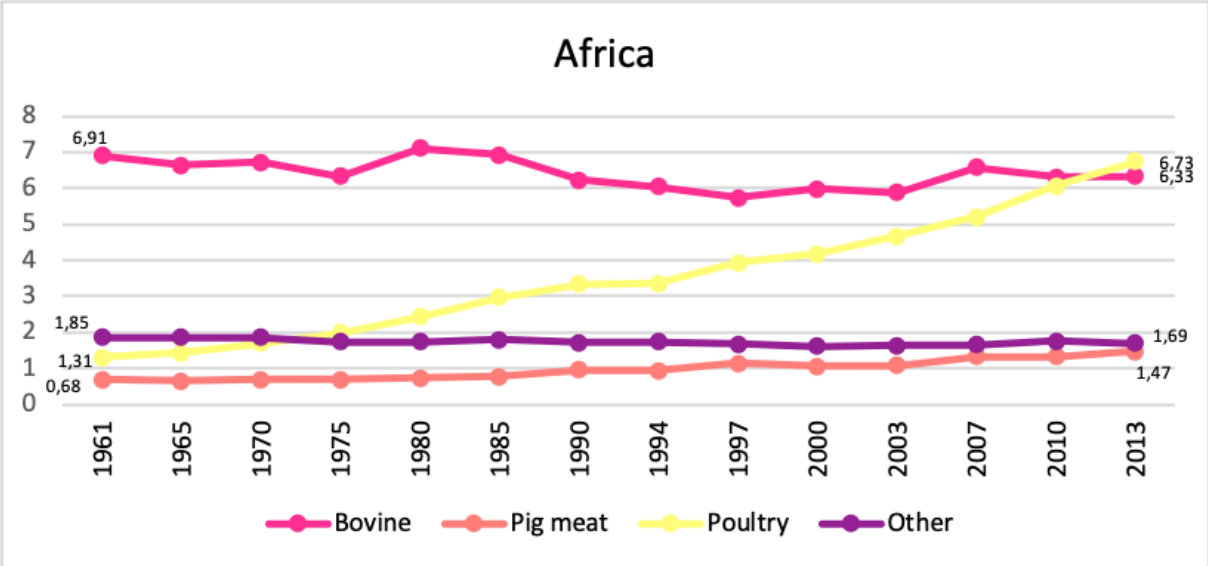
As regards the evolution of meat consumption during the time, Figure 2 shows the World trend of meat consumption from 1961 to 2013 (FAO, 2020). Poultry is the second most eaten meat in the World, but what is surprising is the increase in time, from 2.88 to 14.99 kg/capita/year (+420%) in 52 years. In the same period, the increase for pork is around 100%. About beef, the consumption has remained stable over time (-0.63%), with a slight deflection at the turn of the Bovine Spongiform Encephalopathy (BSE) scandal.

Figure 3. Americas meat consumption (kg/capita/year) from 1961 to 2013 (FAO, 2020)



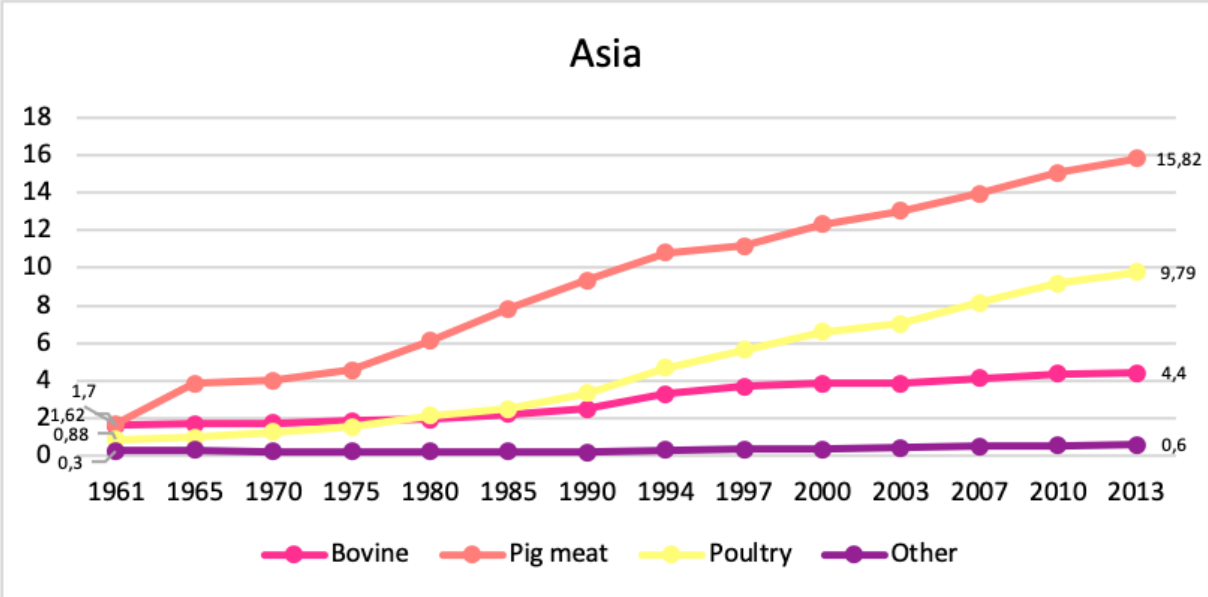
As far as Americas is concerned (Figure 3), there has been an increase over time in the proportion of poultry consumed, while beef and pork have remained stable over time. What is more interesting, however, is the greater quantity of meat consumed in America in comparison with the values recorded globally (86.3 vs 41.31 kg/capita/year).

Figure 4. Africa meat consumption (kg/capita/year) from 1961 to 2013 (FAO, 2020)



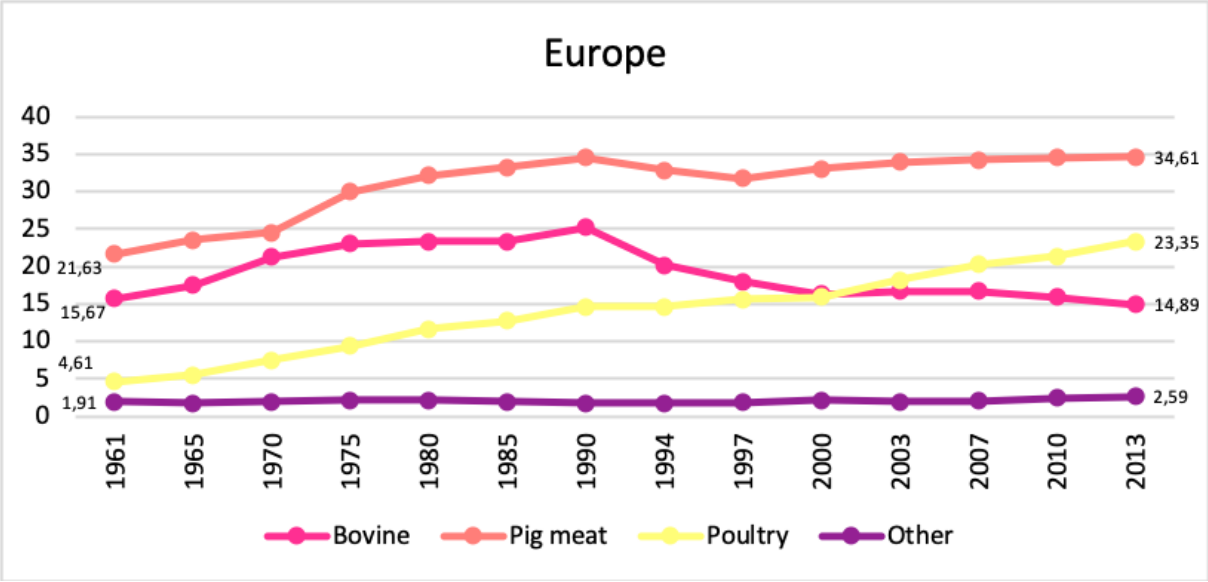
Africa shows an opposed trend to World one (Figure 4). It is easy to understand the reason for this. As regards the low quantity of pork consumed, this is due to religious rules more than 45% of the population is Muslim, in this religion, the consumption of pork is avoided. On the other hand, as regards the increase in poultry consumption, especially from 1980 up today (+413%), this coincided with the increase in the rate of population growth of the continent.

Figure 5. Asia meat consumption (kg/capita/year) from 1961 to 2013 (FAO, 2020)



In Asia, all typologies of meat, except for the voice "Other", have recorded a significant increase in the volume consumed during all the periods considered (Figure 5). The Asiatic continent is the most changed one during the XXI century, recording an extraordinary economic and demographic growth, without forgetting that most of the emerging countries belong to this continent. It is precisely the great demographic growth of Asian countries that is the main reason for the general increase in meat consumption as explained in the study conducted by Sans & Combris (2015).

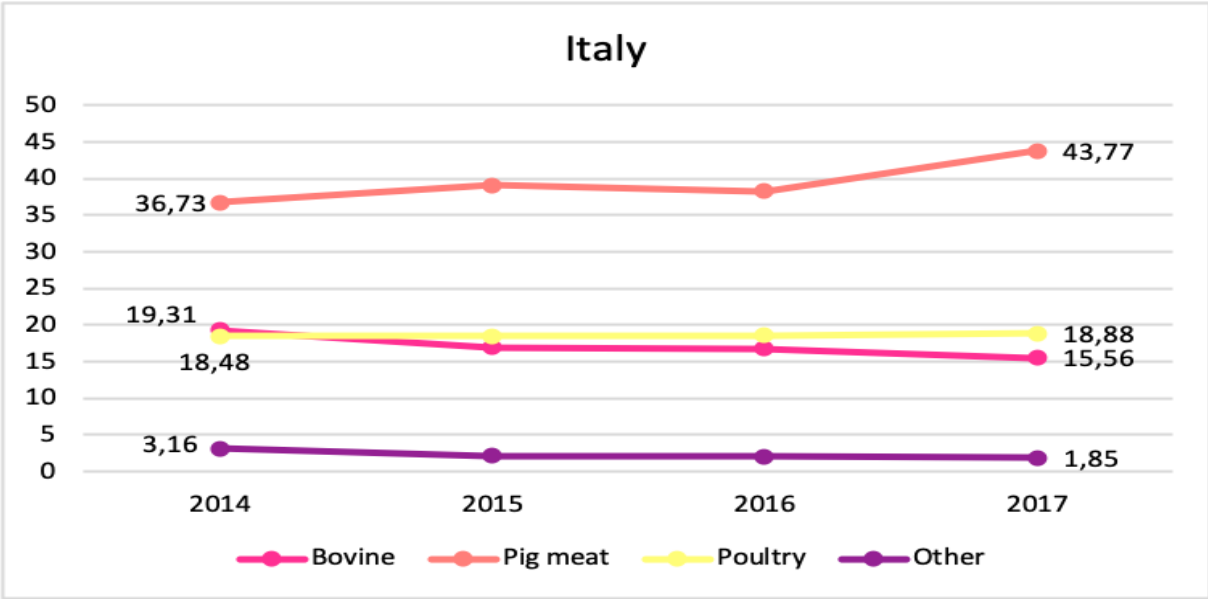
Figure 6. Europe meat consumption (kg/capita/year) from 1961 to 2013 (FAO, 2020)



In Europe, the most eaten meat is pork, with almost 35% of the total (Figure 6). During the last fifty years, the total amount of consumed meat has increased by about 72%, and with the Americas, Europe continent is one of the most meat-eaters in the World.

Focusing more on meat consumption in Italy, the available data of FAO (2020), ranging from 2014 to 2017 are reported in Figure 7.

Figure 7. Italy meat consumption (kg/capita/year) from 2014 to 2017 (FAO, 2020)



The most eaten meat is pork, followed by poultry and beef. During the four years considered, what is interesting to note is that the consumption of pork and beef had respectively an increase (+19,17%)

and a decrease (-19,42%) of the same amount. This situation might indicate that there has been a complete replacement of the proportion of beef consumed in favor of pork.

1.3 The composition of meat

In the European Regulation (2004) we can find the definition of meat "all the edible parts and blood removed from the carcass of domestic ungulates (bovine, porcine, ovine, and caprine), poultry, lagomorphs, wild and farmed game, the large and small wild game".

For most of the scientific community, red meat means meat from the following animals: beef, lamb, pork, and veal; instead, white meat means meat from poultry and lagomorphs. The category of processed meat is the one most implicated in the risk of cardiovascular disease and colon-rectal cancer: this includes all the types of meat that have been stored by different methods such as air-drying, salting, marinating, or smoking (Linseisen et al., 2002).

Meat is an important food source, as it can provide different fundamental nutrients essential for both the development and the maintenance of human health.

Regardless of the type of animal considered, meat is one of the principal sources of protein, characterized by a high digestibility score of 0.92, where the maximum score of 1 is attributed to egg white and casein protein (Boye et al., 2012). In addition, meat can provide different micronutrients such as iron, selenium, zinc, vitamin $B_{12,}$ and vitamin D, and of course different types of long-chain fatty acids.

1.3.1 Protein

Proteins are formed by chains of amino acids, able to confer different structures, which determine the different biological activities of the protein. Proteins are necessary for the growth, maintenance, and repair of the body and some of the amino acids play an essential role such as regulators of gene expression or as a precursor of the synthesis of hormones (Williamson et al., 2005). In nature are present 300 different types of amino acids, but only 20 of them make up the proteins (Wu, 2009). Table 1 shows the list of the 20 amino acids needed for man and used by him for the construction of proteins.

Table 1 Classification of amino acids as nutritionally essential and non-essential for humans (Wu, 2009)

Essential amino acids	Non-essential amino acids
Arginine*	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Taurine
	Tyrosine

*Arginine is essential only for young mammals, a dietary deficit could cause reproductive, neurological, and metabolic dysfunction.

Amino acids are defined as essential when their carbon skeleton cannot be synthesized from human organisms; for this reason, they must be supplied through the diet. However, it is essential to have a good supply of all 20 amino acids, to avoid protein malnutrition, is sufficient that only one of the amino acids is deficient to block the entire line of protein synthesis.

Table 2 Water, protein, fat, and energy content of different types of raw meats (modified from Chan et al., 1995)

	Water	Protein	Fat	Kcal
Beef	71.9	22.5	5.1	136
• Braising steak	72.1	21.8	5.7	139
• Brisket	71.0	21.1	6.1	139
• Fillet steak	72.3	21.2	6.1	140
Veal	75.1	22.7	1.7	106
Lamb	70.6	20.2	8.3	156
Pork	74.0	21.8	4	123
• Chump chops	65.0	20.1	12.6	194
• Chump steaks	69.1	21.3	7.3	151
• Fillet	73.6	22.0	6.5	147
• Loin	59.8	18.6	21.7	270
Chicken (skinless)	74.2	24.0	1.1	106

• Leg	68.1	18.3	13.3	193
• Wing	67.0	20.3	12.4	193
• Breast	67.7	28.4	5.2	114
Rabbit	71.5	21.9	5.5	137
Venison	74.4	22.2	1.6	103

Table 2 shows the water, fat, and protein content of different cuts of raw meat and their energetic density: meat represents a good source of proteins, where the highest value belongs to chicken breast.

At the same time, the biological value of protein is determined by the composition in amino acids; which determines the biological value. Proteins from meat are characterized to have high digestibility and provide all essential amino acids without any limiting amino acids in comparison to vegetal proteins (Williams, 2007).

The protein of the meat can be divided into 3 different classes:

- myofibrillar proteins: they represent 50-55% of the total protein content (Tornberg, 2005). The proteins in this class are divided into other 3 subclasses. 1) *Myofilamentous fibrous protein*: myosin and actin are the main constituents of this class, and they are the main ones responsible for muscle contraction. 2) *Regulatory protein*: they are responsible for the regulation of muscle contraction, of the striated muscle. The most abundant is the tropomyosin-troponin complex. 3) *Scaffold proteins*: these play the role of structural and mechanical support to the structure of myofibrils. Titin is the biggest one of this subclass, others are nebulin, desmin, vimentin, and synemin;
- sarcoplasmic proteins: they represent 30-34% of the total protein content. More than 100 different globular and soluble proteins belong to this class. The most representative are enzymes of the glycolytic pathway, heme pigments (myoglobin, the main responsible of the meat color), and oxidative enzymes (Cobos & Dìaz, 2015);
- connective tissue proteins: they represent 10-15% of the total protein content. The main proteins that belong to this class are collagen and elastin. The role of these proteins is to connect, protect and support different tissues.

1.3.2 Fat content

Fat is the richest source of energy in meat, and is important for the supply of fat-soluble vitamins and essential fatty acids; furthermore, the presence of fat is necessary to guarantee the flavor and palatability of meat (Williamson et al., 2005).

The fat content of meat differs considerably according to the cut of meat considered and especially according to animal species, as can be assessed in Table 2. Other factors that can influence the amount of fat content are the age of the animal, sex, and diet.

However, several epidemiological studies assumed that fat from the meat is implicated in the incidence of severe diseases in the Western World, such as cardiovascular disease (CVD) and colon cancer (Giovannucci et al., 1994; Cross et al., 2007; Kontogianni et al., 2008; McAfee et al., 2010).

To date, the conviction of many authors is that more than the quantity of fat, the quality of the ingested fatty acids influences the incidence of these diseases, together with other factors such as lifestyle and genetics (Stanner, 2005; Wyness et al., 2011). Different fatty acids show different effects on blood pressure, cholesterol, metabolic syndromes, all parameters that are important for the incidence of heart diseases.

In meat, fat is classified as subcutaneous fat (as deposit fat), or intermuscular fat (among the different muscles), and intramuscular fat (among the muscle fascicles). The last category is the most important in the determination of tenderness and flavor of the meat. The main components of lipids are triglycerides (with a share of about >90%) (Wood et al., 2003) and muscle contains more or less 5% of lipids. The most representative fatty acids in meat consist of a carbon skeleton composed of 14-20 atoms of carbon. When there is no unsaturation on the carbon skeleton, the fatty acids are recognized as saturated fatty acids (SFA), the most abundant in meat are myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) (Fink-Gemmels, 1993). Of these three, myristic acid seems to play an important role in the increase of blood cholesterol, with negative effects on human health (Higgs, 2000). Although palmitic acid and stearic acid were believed to have played a neutral role in raising blood cholesterol levels, Shramko et al. (2020) analyzed how instead a diet rich in these two has harmful effects on human health. In particular, it would appear that a diet rich in palmitic acid causes an increase in blood levels of LDL and plays an important role as an inflammatory, increasing the production of cytokines causing an increase in the risk of cardiac damages.

There are two other important categories of fatty acids, based on the number of unsaturation: monounsaturated fatty acid (MUFA) when through the carbon skeleton is present only one unsaturation and polyunsaturated fatty acid (PUFA) when the number of unsaturation is equal to two or more. The MUFA content in meat is around 35-40% of the total, and the most representative of

the category is the oleic acid (C18:1n-9) (Fink-Gemmels, 1993). As regards the PUFA category, the predominant fatty acids in meat belong to the essential n-6 and n-3 fatty acids, such as linoleic acid (LA, C18:2n-6) and alpha-linolenic acid (ALA, C18:3n-3), as the most representative. These types of fatty acids are called essential because the human body is not able to produce them, and it is necessary to introduce them by diet. To have the best health effect such as the prevention of cardiovascular disease, autoimmune disease, or cancer, the ratio of n-6/n-3 consumed into the diet should be around 2-3/1, but the Western diet is characterized by a ratio of 15/1 (Simopoulos, 2002). Several studies (Riley et al., 2000; Bhalerao et al., 2014; Corino et al., 2014; De Tonnac et al., 2017; Kalakuntla et al., 2017; De Tonnac & Mourot, 2018; Minelli et al., 2020) have shown that through the genetic selection of particular breeds and/or the use of feed naturally rich in n3 fatty acids, it is possible to obtain meat enriched in ALA and its precursors. Meats that are richer in n-3 are therefore more beneficial to human health.

1.3.3 Micronutrients

Meat in general, as well as being an essential source of protein and fat as seen above, is also a fundamental resource of other micronutrients such as vitamins or minerals essential for energy production, brain development, growth, bone health, or other biological activities. For the European Union regulation, meat is considered a "*rich source*" of vitamin B_{12}, and a "*source*" of niacin, vitamin B_6, zinc, selenium, and phosphorus, without forgetting the important contribution as iron content (BNF, 2002).

In particular, the complex of vitamins B plays an important role in the production of energy, brain function, carbohydrates, and lipid metabolism, and formation of red blood cells. Animal products, such as meat, are the unique source of vitamin B_{12}, and a quarter of the total vegan and vegetarian population shows plasma levels of B_{12} under the threshold that determines the onset of neurological problems (Biesalski, 2005).

As regards zinc, lean red meat, poultry, seafood, and vegetables are considered as the primary source of the mineral but it is important to underline that its bioavailability is superior in meat than in vegetables (Groff & Grooper, 2000; Williamson et al., 2005).

About selenium, lean cuts provide from 17% to 23% of the daily requirement of this mineral, and an epidemiological study from Clark et al. (1996) showed a correlation between low levels of selenium in plasma and higher incidence of skin, colon, and other types of cancer.

And finally, as regards the contribution of iron content, red meat, but more in general meat and meat products represent the best source of it, and contribute up to 18% of the daily requirement of iron (Geissler & Singh, 2011). Iron, for the human body, is essential for numerous biological activities, such as oxidative metabolism, transport of oxygen around the body, and it is important as a co-enzyme involved in host defense response (Biesalski, 2005; Geissler & Singh, 2011).

There are two different forms of iron present in food, heme-iron, and non-heme iron; the first one is the most bioavailable form concerning the second, and it is largely present in animal foods. The second one, non-heme iron form, is present in a minor part in animal food, and it can be found mainly in vegetables such as legumes or Brassicaceae. Typical molecules of the vegetable world such as phytates or oxalates are the main ones responsible for the lower bioavailability of non-heme iron because it forms a complex with the zinc and stops the process of intestinal absorption of both (Biesalsky, 2005). Different studies (Pereira & Vincente, 2013; Sasso & Latella, 2018) have associated the high intake of iron with damages of intestinal mucosa or increased risk of colorectal cancer, cardiovascular disease, and inflammation in general, for these reasons the maximum intake per day (shown in Table 3) is established because the human body cannot excrete the excess of iron (Geissler & Singh, 2011).

Table 3. International dietary reference values for iron (mg/day) modified from Geissler & Singh (2011).

	EU (recommended nutrient intake based on 15% of absorption)	FAO/WHO (recommended nutrient intake based on 10% of absorption)
Male age <18 year	12.5	18.8
>18 year	9.1	13.7
Female age <18 year	20.7	31.0
>18 year	19.6	29.4

Although an excess of iron in the diet may be harmful to the body, up to 30% of the population suffers from the opposite problem, iron-deficiency anemia, and the most affected part of the population is young women aged 15-49. In particular, one-third of all women of reproductive age are anemic, and this condition represents a serious risk of premature birth and low birth weight (WHO, 2021).

1.4 The conversion from muscle to meat

Before becoming something that can be eaten, muscles from slaughtered animals must switch to meat. This change needs: time, chemical and physical modifications, and biochemical reactions (Lawrie & Leward, 2006, Bekhit et al., 2014).

After the slaughtering of the animal, the carcass will undergo three different phases, the duration of each depends on the type of animal considered, but in general bigger is the animal, the longer will be the duration of the phase:

- *pre rigor*: during this period, the muscle keeps the contraction ability, thanks to the presence of adenosine triphosphate (ATP) that allows the formation and breaking of actin and myosin bonds. The cells show an anaerobic metabolism and in particular, lactic acid production is favored. During this step, because of the lactic acid production, the pH value of the meat drops down;
- *rigor mortis*: the level of ATP is very low, actin and myosin create an irreversible bond, resulting in the shortening of muscle fibers and overall stiffness of the muscle;
- *post rigor*: proteolytic enzymes start their activities and cut the actin and myosin bond. The result is softer meat, suitable to be consumed (Warner, 2016).

1.5 Meat quality

The concept of quality in the food world can have different definitions:

- quality is defined as the totality of characteristics of the product that bear on its ability to satisfy the declared or implied needs (ISO 8402);
- quality is a set of features of the product that are able to satisfy the need of the consumer (ISO 9000).

In general, quality for raw and cooked meat is defined by the compositional quality, in terms of lean cuts, fat content, and their ratio; and by the sensory factors such as visual appearance, smell, juiciness, tenderness, and flavor. The compositional quality and therefore its nutritional aspect is the objective aspect of quality, whereas sensory quality, which depends directly on the sensation of the consumer, is the subjective component of the quality (Heinz & Hautzinger, 2007).

Several factors can influence the meat quality such as the pre-slaughter period, environment, processing, storage conditions, and so on (Heinz & Hautzinger, 2007; Joo et al., 2013).

In addition, the meat quality is determined by:

- technological quality traits;
- sensory quality traits.

The water holding capacity (WHC), texture, pH, color, and fat content determine the technological traits of meat, on the other hand the sensory quality traits are determined by tenderness, juiciness, and flavor.

1.5.1 pH

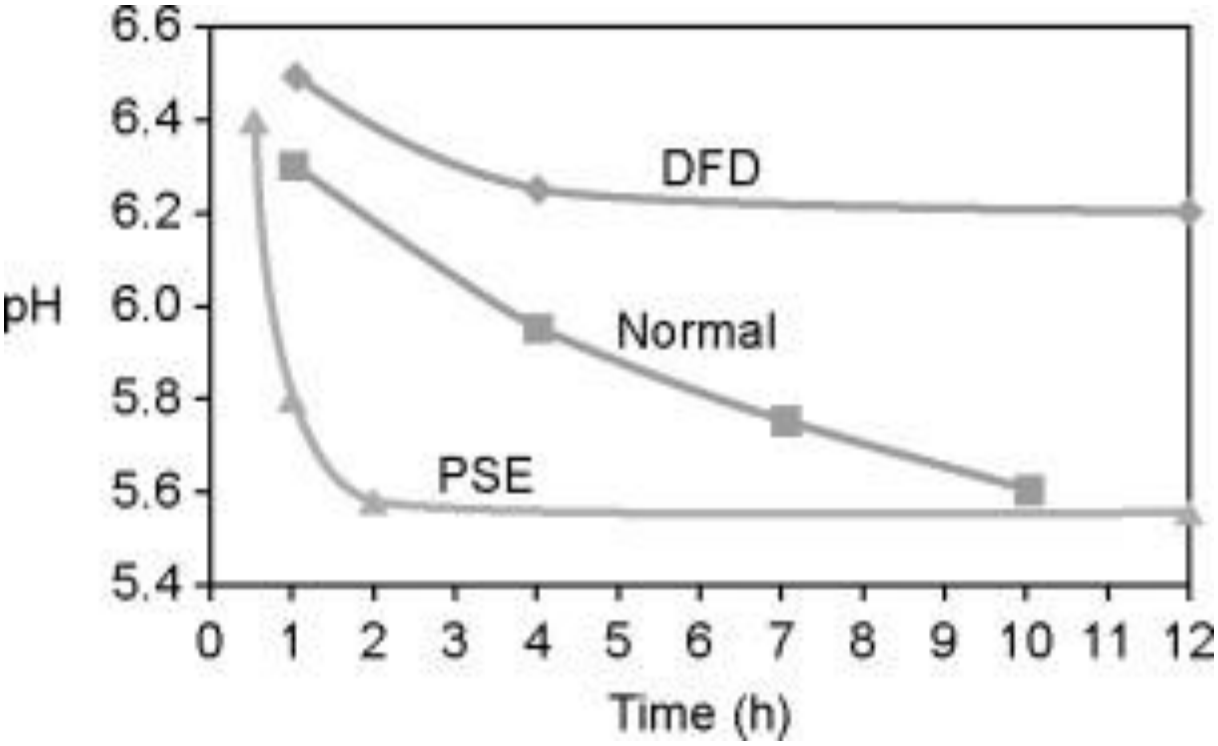
It is correct to start from the pH as the main technological characteristic because it influences the others. The pH is a measurement of acidity, and it can be influenced by several factors. The pH value of a living muscle at rest is 7.2 to 7.4. During the phase of rigor mortis which takes place shortly after slaughter, the glycogen present in the muscle is converted into lactic acid, which causes a drop-down of pH value. The best value of pH after 24 h from slaughter ranges from 5.50 to 6.00 (depending on the type of meat considered), as shown in Table 4.

Table 4. The pH values of some meat products (Anonymous, 1962)

	Approximate pH	Lower range	Upper range
Beef	5.55	5.40	5.70
Chicken	5.90	5.30	6.50
Pork	5.90	5.60	6.20
Turkey	5.70	--	--

Two pH measurements can be obtained on the carcass, one 45 minutes after slaughter and the other at 24 h *post mortem*, the latter is defined as the ultimate pH, and it provides information about the quality of the product. Abnormal pH values are related to meat defects for pork, known as PSE (Pale Soft and Exudative) in particular for the pH_{45min}, characterized by lower values and DFD (Dark Firm and Dry) phenomenon, with higher ultimate pH values (Seideman at al., 1984; Adzitey & Nural, 2011). In particular, the PSE is more common in pork and rarely in double-muscle cattle breeds, and is characterized by a fast glycolysis process with a consequent rapid *post mortem* pH decline. In fact, meat can reach pH value below 5.8 in few minutes after death, as it shows in Figure 8.

Figure 8. Changes in pH value in meat post mortem (Honikel, 2004)



The combined effect of the large amounts of lactic acid produced by the anaerobic glycolysis *post mortem*, and the high temperature of muscle a few minutes later the slaughtered process, is the main cause of the denaturation of the muscle proteins and their reduced ability to hold water. The meat drips and looks soft and mushy. In comparison to normal meat, the PSE has a higher loss of liquids, the color of raw meat is paler, the risk of developing rancidity is higher, and under the technological point of view, the yield is reduced (Fernandez et al., 1994).

In pork, when PSE occurs, all the muscles are interested and show the characteristics of pale and wet surface; in beef, the PSE defect is detected only in the innermost of the thigh.

As regards the DFD defect, beef is the most affected but also pork is afflicted. The DFD meat is characterized by dark color of meat and firmness of muscle fiber.

In DFD meat the glycolysis process does not occur in the normal measure, and due this the pH value recorded at 24 h *post mortem* is over 6.2 (Figure 8), in particular cases up to 7.

As for PSE, for DFD the onset of the phenomenon is associated with the presence of stress situation; but with different mechanism.

What seems to be the trigger element is a prolonged stress condition at the pre-slaughtering. Due to the stress, the animal begins to use glycogen reserves which will no longer be available in the

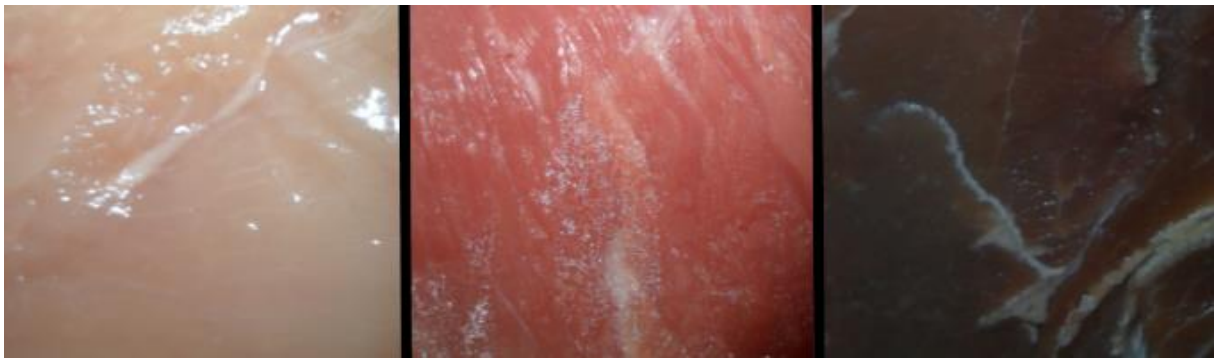
acidification phase *post mortem*. The result is meat with a pH value higher than normal, and this condition promotes microbiological growth (Livingston et al., 2004). Values of pH between 6 and 6.2 are generally considered carefully because, they suggest a tendency of meat to the DFD features (Adzitey & Nurul, 2011).

The color of DFD meat is darker than normal, the volume of the myofibrils is greater, the light can penetrate in deep without being dispersed. The penetration of oxygen results limited while the activity of enzymes with oxygen consumption is still high, this causes a faster formation of metmyoglobin, and the surface appears dry as a result of high water-holding capacity of the meat.

The DFD meat cannot be suitable for seasoned products due to the too high water-holding capacity, but is suitable for the production of cooked or emulsified products (Guàrdia et al., 2005; Holmer et al., 2009).

Figure 9 compares the PSE, normal, and DFD meat.

Figure 9. Example of PSE, normal, and DFD meat (adapted from Adzitey & Nurul, 2011)



PSE	NORMAL	DFD

1.5.2 Water holding capacity

Water holding capacity (WHC) means the ability of meat to retain water, and it is one of the more important quality characteristics for the consumer being also related to the concept of tenderness and juiciness of the product (Huff-Lonergan & Lonergan, 2005).

The WHC is influenced by several factors, like pre-slaughtering condition (stress), genetics, age of the animal, type of muscle, or the oscillation of pH. The variation of pH seems to have the main role in determining different effects such as the modifications of the tertiary structure of proteins that cause their degradation with consequent less retention of water from myofibrils, and the steric effect that

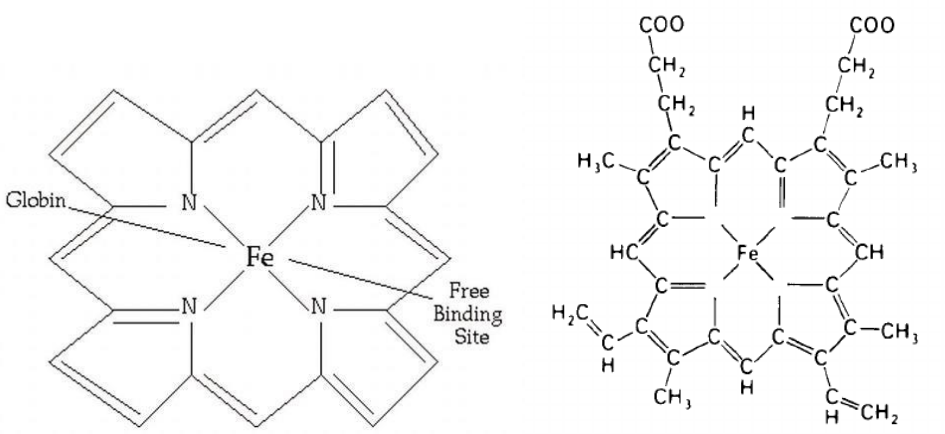
causes a shortening of the distance between the thick filaments, resulting in losing water that is not retained anymore.

The WHC is considered not only an important quality parameter for consumers but also a technological one because depending on it, the meat may be devoted to the fresh market or for transformation industries (Lawrie & Ledward, 2006).

1.5.3 Color

The color aspect of fresh meat is important for consumers and the meat industry (Seideman et al., 1984, Suman & Joseph, 2013). The color of meat is due to two contributions as the chromatic and the achromatic contribution. Chromatic contribution is due to the concentration of the color pigment in meat. Hemoglobin is the predominant pigment in muscle in live animals, and its biological function is to bind oxygen and transfer it to different tissues, but when the animal is stunned and bled, myoglobin is the only pigment still present in muscle tissues.

Figure 10. Chemical structure of Myoglobin (a) and Hemoglobin (b); (Seideman et al., 1984)



a b

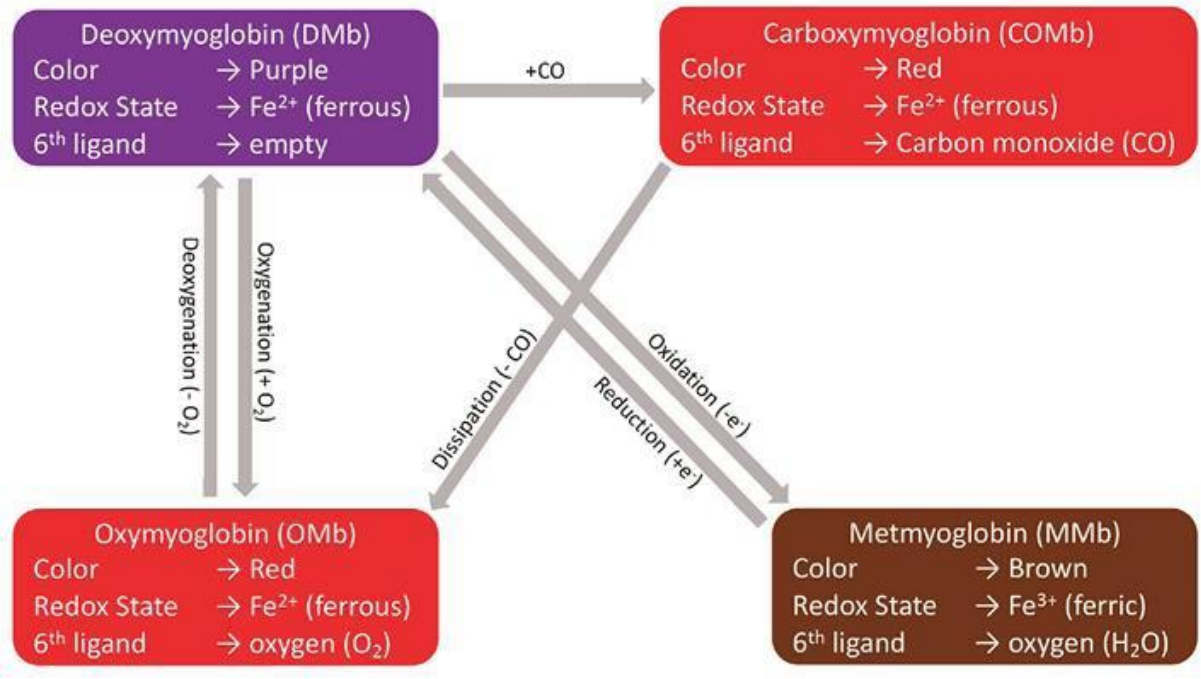
The myoglobin structure is shown in Figure 10 (a). It is a globular protein consisting of a heme group surrounded by globin moiety with a centrally located atom of iron (Fe), it is similar to one of the four subunits that form the structure of hemoglobin, and it represents 95% of the iron reserve in the muscle tissues (Lawrie & Ledward, 2006). What most affects the final color is the state of the valence of the iron atom, instead, the intensity of the color depends on other factors *antemortem* such as species, sex, age of animal, stress condition, muscle source, or *post mortem* pH evolution.

In fresh meat is possible to find myoglobin in four different chemical forms, and each one is characterized by a different color, redox state of the iron atom, and different ligand (Richards, 2013):

- oxymyoglobin (MbO_2): this is the typical condition when myoglobin stores oxygen in muscles, the color of meat is bright red and the atom of iron is in a ferrous oxidation state (Fe^{2+});
- carboxymyoglobin (MbCO): it is similar in color and oxidation state of iron to MbO_2, the only difference is the kind of ligand, in this case, is carbon monoxide. The two red colors, from Mbo2 and MbCo, are indistinguishable by human eyes;
- deoxymyoglobin (DMb): the molecule does not bind oxygen anymore, the oxidation state for iron is Fe^{2+}, and the final color is purplish red;
- metmyoglobin (Met-Mb): now the iron atom is in a ferric oxidation state (Fe^{3+}), the color is brown. The ligand in this form is represented by a water molecule. The passage from DMb to Met-Mb and *vice versa* occurs with a transfer of electrons.

Figure 11 summarizes the characteristics and chemical reactions of Myoglobin in meat.

Figure 11. The forms of Myoglobin in fresh meat, adapted from Mancini & Hunt (2005)



As regards other effects that could influence the color of meat, related to the different amount of myoglobin present, it is possible to summarize (Seideman et al., 1984; Richards, 2013):

- difference between species: the color is strongly correlated to different concentrations of myoglobin beef is darker than lamb because it has the highest value of myoglobin recorded. Pork has a low value;
- sex: in general male has a higher content of myoglobin than female, for this reason, the color of meat from the male is darker than meat from female;
- age: the younger animal has less concentration of myoglobin, so meat from this animal is less dark;
- muscle source: muscle intended for locomotion has a higher content of myoglobin than support muscle, this is due to the physics activity and the need for a large quantity of oxygen essential for aerobic metabolism of red muscle fibers.

As regards the achromatic contribution in determining the color of meat, this is represented by two main factors that are each other interconnected:

- the structural elements of the muscle and their shrinkage;
- the decline of pH *post mortem.*

The transverse shrinkage of the muscle fibers, myofilaments, and myofibrils is responsible to increase the light scattering. In turn, the transverse shrinkages are linked to the value of $pH_{ultimate}$ recorded high pH is related to less shrinkage, smaller extracellular volume, and a reduced value of scattering of light, and darker color of meat as a result. On the contrary, elevated shrinkage of protein structure is related to lower pH and lighter color of meat (Hughes et al., 2020).

1.5.4 Texture, tenderness, and juiciness

The definition of texture for meat is difficult to explain, but in general, it is possible to say that it is determined by the size of the bundles of fibers viewed traversal concerning the direction of the muscle fibers (Lawrie & Ledward, 2006). The dimension of the bundles is determined by the numbers and the dimension of the fibers; in general muscles with a high rate of growth are characterized by bigger dimensions of bundles. The texture is recognized as technological quality but more important is the sensory quality as the tenderness.

Tenderness plays a fundamental role in the determination of consumer's acceptability, and it is considered the most important in determining eating satisfaction (Bekhit et al., 2014).

Different factors define the tenderness of the meat, for example, animal age, sex, muscle location, amount of the connective tissue. In particular, the meat of younger animals is more tender because with the age also increases the portion of connective tissue, which tends to be more insoluble making the meat more tenacious. Warner et al. (2021) analyzed how castration, genetic selections, feeding strategies, or the supplementation of vitamin D can have a specific role in the determination of tenderness in meat, for pork and beef.

The degree of tenderness depends on the three different categories of protein: myofibril, connective tissue, and sarcoplasm; and the tenderness is calculated in three different moments:

- ease penetration of the meat by the teeth;
- ease the way with which the meat breaks into fragments;
- the amount of residue remaining after chewing.

More in general, the changes in myofibril reflect the value of shear, compression, and tensile force, on the other hand, the shear force reflects the status of the connective tissue.

The concept and the significance of juiciness in meat are strictly correlated to its water holding capacity since it is related to the degree of shrinkage due to the cooking process (Lawrie & Ledward, 2006). Juiciness, in general, is related to two different organoleptic impressions that occur one after the other:

- the impression of humidity during the first few bites. This phenomenon is due to the fast release of liquids;
- the sustained juiciness (the one that is perceived until the ends of the bites) is largely due to the stimulating effect of intramuscular lipids on the production of saliva.

There is therefore a close correlation between the fat content and the juiciness perceived by the consumers (Hocquette et al., 2010); in fact, meat from young animals, although it gives as a first impression that it is very juicy, eventually leaves the consumer with a feeling of dryness of the product (Jeremiah et al., 2003).

Other studies (Jeremiah et al, 2003; Lawrie & Ledward, 2006; Bekhit et al., 2014) demonstrated that also pH value has a relation with the juiciness; meats with pH equal or superior to 6 are less juicy than meats with lower pH; this is a consequence of the higher retention of water by the protein structure.

1.5.5 Flavor

The flavor is included in the sensory aspects of meat, and it is particularly important in cooked products fresh meat is a source very rich in aroma precursors such as amino acids, peptides, lipids, reducing sugar, vitamins, and nucleotides, and only with the heating process, the flavor of meat can develop. Four different pathways are possible to induce the aroma flavor formation due to heating reaction:

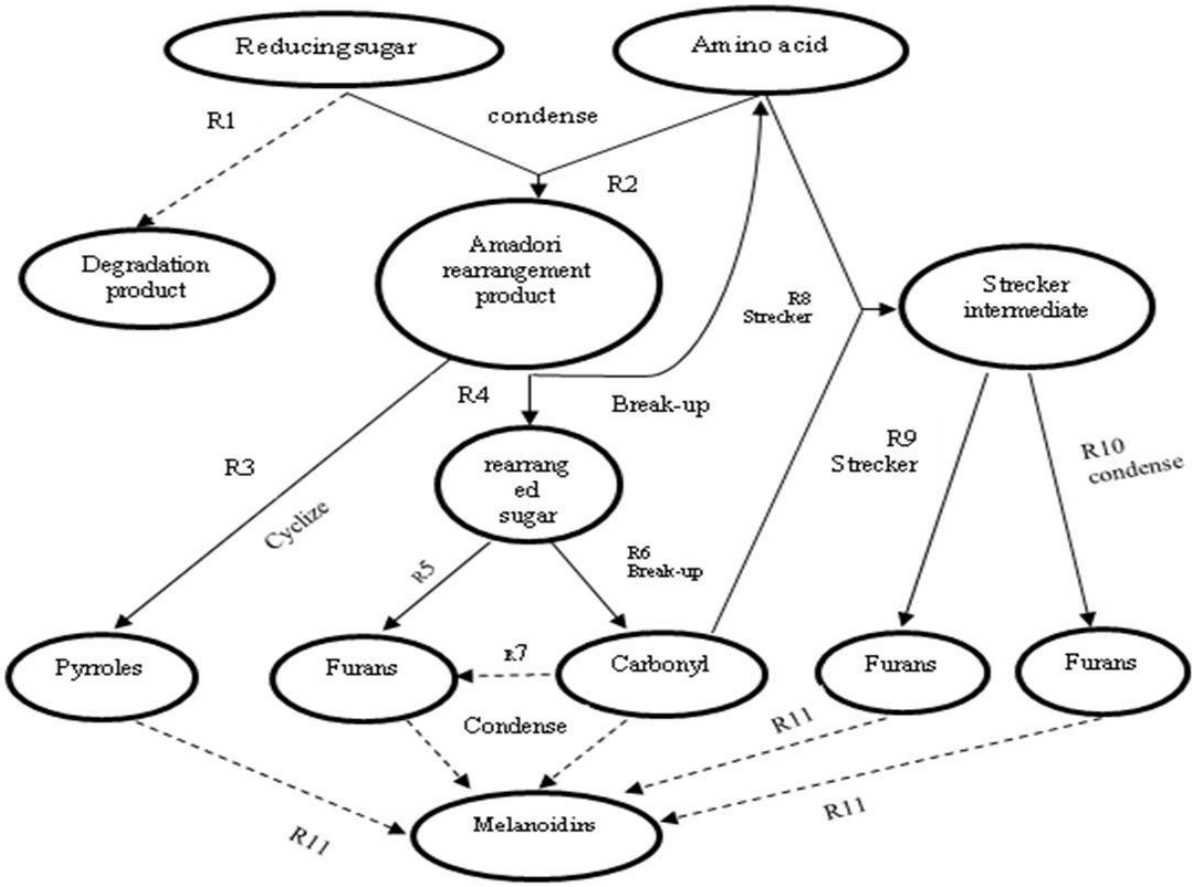
- maillard reaction: the actors of the reaction are reducing sugars and free amino compounds. The reaction is composed of three different stages: the formation of the Amadori product by the condensation between reducing sugar and an amino group, in the second stage the Amadori product is decomposed in an amino group and a sugar fragmentation. The last stage is the most complicated and includes dehydration, fragmentation, polymerization, and cyclization reactions (Figure 12, adapted from Jousse et al., 2002);
- lipid oxidation: the reaction occurs in raw and cooked meat, heating conditions can catalyze the reaction. The odor compound that is originated from lipid oxidation is more detectable than volatile compounds originated by the Maillard reaction. The most detectable are aldehydes included saturated and unsaturated containing from 6 to 10 carbons (Mottram, 1998);
- interaction between lipid oxidized products and Maillard reaction products: typical of this reaction are sulfuring-containing components such as thiols and thiophenes;
- vitamin degradation.

In general, over 1000 different volatile compounds were identified in meat products (pork, beef, poultry, and lamb), but the most representative volatile compounds belong to sulfurous and carbonyl-containing compounds (Ba et al., 2010).

Overall, each animal is characterized by its flavor due to a different lipid composition, not under the qualitative point of view but quantitative of every single fatty acid (Mottram, 1998; Jousse et al., 2002).

And finally, the application of some technological processes such as brining, salting, or smoking, can play an important role in exalting the meat flavor.

Figure 12. Scheme of chemical reaction of flavor formation in Maillard reaction (Jousse et al., 2002)



1.5.6 Safety

Meat, characterized by its values of water activity (0.995) and pH (between 5.3 and 6.2), is an excellent substrate for microbial growth.

The meat contamination may have an endogenous or exogenous origin; the first one is related to aspects of the animal life such as inflammatory state or prolonged fasting, the second type depends on post-slaughtering handling (Dave & Ghaly, 2011).

In general, the typical microbial population of raw meat is composed of a wide variety of species such as *Pseudomonas*, *Staphylococcus*, *Micrococcus*, *Moraxella*, *Psychrobacter*, *Corynebacterium*, *Enterobacteriaceae*, lactic bacteria, *Bacillus*, yeasts, and molds. The % of presence is shown in Table 5 (Parisi & Giaccone, 1993).

Table 5. Summary of Bacteria in fresh meat (Parisi & Giaccone, 1993)

% of the frequency of presence	Microorganism	Animal species
80%	*Pseudomonas*	Poultry, beef, and fish
61%	*Acinetobacter* *Micrococcus* *Enterobacteriaceae*	Ovine, poultry, and beef
40%	*Flavobacterium* *Bacillus* *Microbacterium* *Lactobacillus* *Streptococcus* *Aeromonas*	Lamb, pork, and poultry
10%	*Clostridium*	Poultry
<10%	*Chromobacterium* *Xanthomonas* *Pediococcus*	

The greatest microbial growth occurs from the end of the slaughter process to the storage of the carcasses, in fact, at this stage, a series of manipulations necessary for the commerce of the meat occurs and this promotes contamination. In general, the more numerous manipulations are, the higher degree of final contaminations occurs; as well as the greater number of cuts made, or the surface available for oxygen exchange, induce a shorter shelf life of the product (Cerveny et al. 2009; Dave & Ghaly, 2011). From the past and arriving at these days, several techniques have been used to improve the safety of the meat, for example, refrigeration, fermentation, brining, smoking, or adding preservatives, vacuum packaging, or modified atmosphere.

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Sitography

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www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-anaemia-in-women-of-reproductive-age-(-) (August, 2021)

CHAPTER 2.

The cooking process

2.1 The effect of cooking on meat

During the cooking, several changes and biochemical reactions occur and positive things happen to the food; it becomes safer under the point of view of microbiology, nutrients become more digestible and the taste is developing (Santé-Lhoutellier et al., 2008, Qi et al., 2018). As regards meat, different factors can affect the characteristic of cooked meat, such as contraction state of muscle, *post mortem* aging, fat content, enzymes, rate of temperature rise, and others; instead, the combination of time and temperature used for cooking influences more the biochemical reactions (Laakkonen, 1973).
As previously mentioned, meat represents a good food source of various nutritive elements but also it is an excellent substrate for microbiological contamination, including some pathogens for humans such as *Brucella*, *Clostridium botulinum*, *Salmonella*, *Staphylococcus aureus*, and so on (Leclerc et al., 2002). Salmonella represents one of the most common pathogens in the European Union and the symptoms that can be developed are different, with different intensity due to the level of contamination: headache, fever, diarrhea, and abdominal cramps. In a report, EFSA (2018) reported that turkey, poultry, and pig meat were considered the most *Salmonella* contaminated as well as for not well-cooked meat. For this reason, it is important to avoid eating raw or not well-cooked meat and give more attention to the important step of cooking, which can be considered the first step of the prevention against the food toxic infection (Mari et al., 2012). On the other hand, the heating process has many effects on protein denaturation which will be more easily attacked by digestive enzymes, with an improvement of the digestible rate; moreover, the food allergens present in food are inactivated by the heating process.

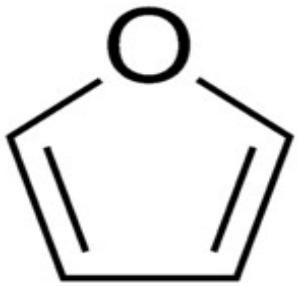
The other side of the medal of the cooking process is represented by the loss of nutrients (such as vitamins) and by the production of harmful substances and toxic compounds, such as furan, heterocyclic aromatic amines (HCAs), acrylamide, acrolein, and polycyclic aromatic hydrocarbons (PAHs).

2.1.1 Furan

The chemical structure of furan is shown in Figure 13. Furan represents the major class of compounds that are formed during the Maillard reaction, and in this category are attributed carcinogenesis

processes and genotoxic mechanisms. The formation of furan happens due to the Maillard reaction, food rich in carbohydrates is characterized by a higher level of furan. Maga (1979), and Santonicola & Mercogliano (2016) reported significant levels also in coffee, canned meat, cooked chicken, and fish protein concentrate, in fact there are multiple and different pathways compared to Maillard reaction that lead the formation of furan, such as the thermal degradation of some amino acids, oxidation of polyunsaturated fatty acids or carotenoids due in particular at high temperature of cooking, or the oxidation of ascorbic acid (Seok et al., 2015).

Figure 13 The chemical structure of furan



2.1.2 Heterocyclic aromatic amines (HCAs)

The International Agency Research on Cancer (IARC), in 1993, has classified HCAs as possible human carcinogens(2B) and probable human carcinogens (2A). More than 25 different HCAs have been identified and the major source of them for humans are cooked meat and fish; the amount of HCAs production depends on different parameters such as the condition of cooking (time and temperature), methods of cooking, moisture, and fat content, creatine content and lipid oxidation (Knize et al., 1994; Szterk et al., 2012).

The family of HCA is composed of two major groups (Mehta, 2015):

- the aminoimidazoazoarenes (AIA): are relevant in cooked foods and formed during the Maillard reaction where the actors of the reaction are free amino acids, in particular creatine, creatinine, amino acids, and hexoses, and the temperature of cooking is between 150°C and 300°C;
- the aminocarbonyl: the formation is due to the reaction of pyrolysis of amino acids and protein at a temperature higher than 300°C.

2.1.3 Acrylamide

Food cooked at high temperatures are rich in acrylamide, the higher is the temperature and the longer time of cooking, the more acrylamide is present in the matrix (Tareke et al., 2002).

Acrylamide is generated from Maillard reaction between a carbonyl group of reducing sugar and the amino group of free amino acid asparagine during deep-frying, baking, grilling, or toasting; in any case, the reaction starts with a temperature above 120°C. The most affected food is potatoes fries followed by baked products and instant coffee; concerning meat, this has not great impact on the acrylamide content in the human diet (Mehta, 2005). Ghasemian et al. (2014) conducted a study with the purpose of evaluate different parameters that can affect the acrylamide content in beef burger. Meat is not a natural source of acrylamide, but the temperature and the time of cooking applied on it, in particular during the frying, promote the formation of the dangerous compound.
Acrylamide is absorbed in the gastrointestinal tract, distributed to all organs, and its by-products with the epoxidation with glycidamide are recognized by EFSA (2015) to have genotoxic, neurotoxic, and cancerogenic effects.

2.1.4 Acrolein

Acrolein derives from the thermal degradation of glycerin that happens when oil is used at a temperature over its smoke point, but it could be originated also from carbohydrates, animal fats, or free amino acids during the cooking process. The deep-fat frying technique is the main responsible for the formation and enrichment of acrolein in food and in the air of the cooking place, although the real quantification of acrolein in food is extremely complicated due to the lack of reliable measurements, as the acrolein molecule is highly volatile and binds to food matrix components. (Abraham et al., 2011; Rietjens, 2018).
More than a toxic molecule, acrolein shows an irritant power beyond a certain threshold of 0.09 mg/kg (for eyes irritation) and 0.30 mg/kg (for respiration problems), but it is also known as an aromatic compound widely diffused in many foods.

2.1.5 Polycyclic aromatic hydrocarbons (PAHs)

The PAHs are originated when meat is cooked using high temperatures, when fat or juices from meat drop-down directly over the flame which in turn in new flame and smoke. The smoke originated is rich in different PAHs (over 16 different PAHs were identified) that adhere to the meat surface (Cross & Sinha, 2004). Grilling is not the only method responsible for the PAHs production: the smoking

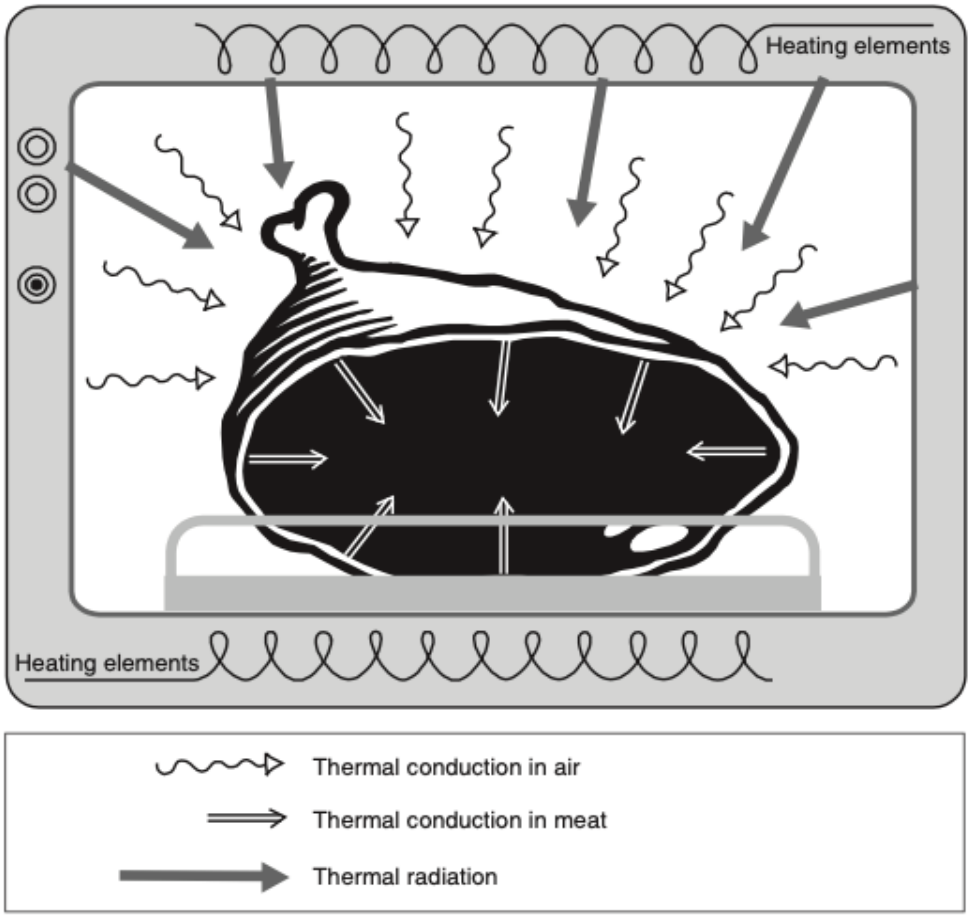
technique, as a preservation method for food, increases the PAHs concentration. In a report, EFSA (2008) affirmed that PAHs have great genotoxic power and carcinogenetic effects.

In general, the cooking technique plays a fundamental role both in formations of volatile compounds fundamental for the taste, and molecules dangerous for health.

2.2 Different types of "home" cooking techniques

From the dictionary, the definition of cooking is "to prepare food by the use of the heat". The cooking process is considered a unitary operation that allows to extend the shelf-life and modify the texture and taste of food. In "traditional" cooking process, the heat can be transferred by conduction, with direct contact from food to heat source, convection, the heat is transferred through a fluid, and radiation, the heat is transferred by waves (Figure 14).

Figure 14. An example of heat transfer by Bejerholm et al. (2014)



All these techniques can be divided according to the type of heat transfer:

- dry heat: it works without the presence of any moisture or broth of water. The heat is transferred to food by the air or fat contact, for example in deep fat frying. It can reach a temperature over 300°C. Products cooked by these methods are characterized by browning;
- moist heat: the food is cooked in water or with steam. This technique allows making healthy dishes because the addition of oils or salt is limited. The brown crust is not produced;
- combination of both: both methods, dry and moisture, are used. Foods are cooked in liquids at low heat for an extended period. This works well with the toughest cuts.

Table 6 illustrates the most common cooking techniques divided by the heat method transfer.

Table 6. Cooking techniques divided by the heat transfer method

Dry heat	Moisture heat	Combination of both
Broiling	Poaching	Braising
Grilling	Simmering	Stewing
Roasting	Boiling	
Baking	Steaming	
Sautéing		

2.2.1 Techniques using dry heat transfer

Broiling: in this method, generally, the heat source is either over or below the food, so the meat must be turned during cooking. The process of cooking is very quick and the use of a thermometer can be helpful to monitor the doneness of the product. Thinner cuts, poultry, or fish represent the best foods where apply this technique. The temperature used in this technique are usually between 230-260°C

Grilling: the method is similar to broiling, the heat source is below the food. Wood or charcoal can be used for heat production. The presence of the fire as source of heat, is fundamental in this technique, the typical temperatures reached are around 350°C. In general, by grilling is possible to obtain the best flavor for cooked meat, the "browning" process is very accentuated. However, the production of PAH is high, due to the direct contact between the fat of food, which drips due to the cooking process on the heat surface, and the flame used for cooking, resulting in the production of smoke rich in toxic molecules.

Roasting: in general, the process happens inside an oven with indirect heat, and all the sides of food are cooked simultaneously. The cooking process is slow and different temperatures can be used depending on the type of meat, from 100°C to 180°C for stiff meat and higher than 230°C for tender meat.

Baking: it is synonymous with roasting, the technique is the same. The term is used when bread, biscuits, or cake, in general, are cooked. The temperatures used are lower than roasting.

Sauteing: the word "*sauté*" has a French origin, and it means "jump"; since this technique involves the use of a low amount of oil and a pan, it is characterized by a very fast cooking process, and the food must be handled (jumped) very often to limit the risk of burns.

2.2.2 Techniques using moisture heat transfer

Poaching: the temperatures used are between 60°C and 80°C, it is a very gentle cooking method, where food is submerged in hot liquid. The use of oil is very limited and the flavor and taste are preserved.

Simmering: is similar to poaching, the temperatures used are higher, a range between 80°C to 95°C (but lower than the boiling point of water).

Boiling: the temperature of this technique is around the boiling point of water. The food is immerged, and the large bubbles of boiling water keep the food in motion. It talks about "slow boil" when water is going to reach the boiling point and the large bubbles of boiling water have slow-moving, and "full boil" when the boiling point is reached, and the large bubbles of boiling water are characterized by fast and rolling moving.

Steaming: the water is used to produce steam, which wraps the foot and allows to obtain uniform cooking of the product while maintaining the internal humidity. At home, steaming is carried out using a pot and a steam basket, while at the industrial level there are combined ovens that allow cooking with steam.

2.2.3 Techniques using a combination of heat transfer

Braising: it is composed of two steps. During the first step, the food is seared in a hot oiled pan to seal it, and avoid the loss of juices. Subsequently, in the second step, the food is transferred to a larger pot, that contains hot liquid (such as water or broth). Food is half-immerged and the application of low heat for a long time is a good combination to obtain softer food and broth concentrated of flavor and taste.

Stewing: the passages of this cooking are the same as braising, but with few differences; the size of food is smaller than in braising, and the liquid completely covers the product.

2.3 Cooking of meat, the chemical and physics changes

Meat is a complex matrix composed of muscle fibers, lipids, water, vessels, nerves and extracellular matrix. During cooking several changes occur, and the choice of the best technique to apply and the right combination of time and temperature are fundamental (Pathare & Roskilly, 2016).

Figure 15. Changes in meat due to cooking process (Bejerholm et al., 2014)

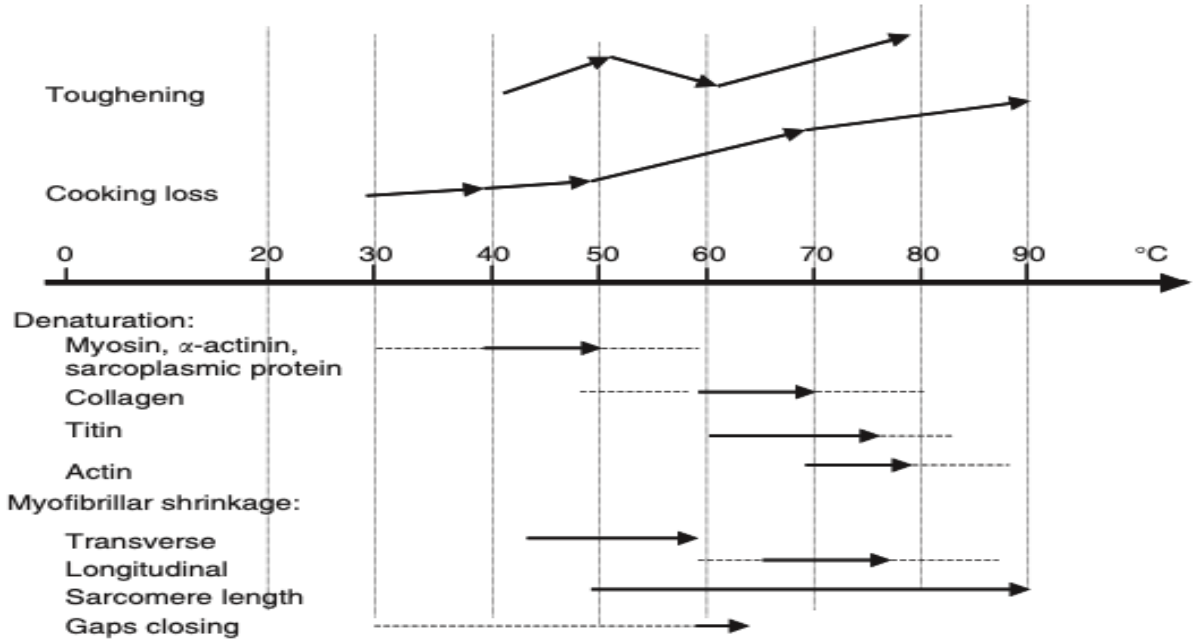


Figure 15 summarizes the main changes in meat during the cooking process, in terms of proteins denaturation, amount of cooking loss, and global value of tenderness.

2.3.1 The change in protein

In general, meat is composed of 75% of water, 20% of protein, 3% of fat, and 2% of soluble non-protein substances such as vitamins, inorganic compounds, or carbohydrates (Tornberg, 2005). The proteins of meat are divided into three main categories; myofibrillar, sarcoplasmic and connective tissue proteins. When cooking meat, heat is used to modify (or denature) these proteins. Each of them shows a different attitude when heating is applied. Due to heating, there are two major changes in proteins:

- conformational changes occurs at a specific temperature for each protein category, named also denaturation temperature;
- structural changes, due to protein-protein interactions that lead to a decrease in solubility and an increased rate of aggregation. For denaturing process of protein means the loss of the thermodynamic stability of the structure, and the rearrangement in a new irreversible structure (Dominguez-Hernandez et al., 2018). Also, the mechanism of proteolysis can promote, in presence of heat, the process of protein degradation, in particular for the collagen. The structural change in the proteolysis is due to the activity of particular enzymes in particular the cathepsins, which are able to degrade both myofibrillar proteins and native collagen (Burleigh et al., 1974).

Sarcoplasmic proteins: they represent 30-34% of the total meat protein, it is a big group of globular and soluble proteins with low molecular weight, consisting of over 100 different proteins including enzymes of the glycolytic pathway and myoglobin (Tornberg, 2005). These proteins do not have a structural function but are important for the determination of color (Dominguez-Hernandez et al., 2018). The denaturation temperature of sarcoplasmic proteins is between 40 and 60 °C, while the aggregation temperature extends over 90°C. The gel that is originated due to the aggregation process can link other structural elements of meat, going to play a role in determining tenderness. In addition, some enzymes, that have a tenderizing effect on meat, work better when the heating process uses low temperature (<60°C) and lasts for 6 h (Laakkonen et al., 1970; Tornberg et al., 1977, Tornberg et al., 2005).

Myofibrillar proteins: There are about 20 different myofibrillar proteins, 65-70% are myosins and actin. The chemical and structural changes of this category are associated with the toughness of meat

(Ayub & Ahmad, 2019). Depending on their activity the myofibrillar proteins can be divided into three sub-groups:

- contractile proteins: the most representatives are myosin and actin, and they build the myofilaments. They are responsible for muscle contraction;
- regulatory proteins: they are involved in the contraction process. The most important is the complex between tropomyosin and troponin, and ð-actinin and ß-actinin;
- scaffold proteins: their main task is to maintain the structure and integrity of myofibril. Titin, nebulin, desmin, and vimentin belong to this group.

In general, the denaturation temperature is different for each protein; in particular, the myosins are rather thermolabile and they star to denature at 40°C, but only when the temperature is above 53°C the denaturation is complete (Brüggemann et al., 2010). Instead, the actomyosin starts to denature at temperatures between 54°C and 58°C, while actinin starts at 80°C. Different studies that use the Differential Scanning Colorimetry (DSC) technique were conducted and affirmed that also the time of cooking plays a role in the denaturation of actin, in fact even at a temperature below 60°C the relative amount of native actin both in beef and pork, decrease with the extension of holding time (Bertola et al., 1994; Christensen et al., 2011).
The denaturation process leads to the formation of a gel made by the organization of the heads and the tails of the myofibrillar proteins. Sharp & Offer (1992), studied and explained how a very small amount of purified myosin can generate a gel in two different steps: the first reaction occurs at a temperature between 30°C and 50°C, where aggregation of the globular heads of myosin occurs; the second step takes place at temperature >50°C, it is characterized by the interaction of hydrophobic groups in a more complex structure with larger aggregates.

Connective tissue (or insoluble proteins): they represent 5% of the total meat proteins. The most representative are elastin and collagen. Elastin is the protein that is largely present in the ligaments, and unlike collagen, it does not degrade due the heating process. Instead, for collagen, the denaturation process occurs in two passages. The first one happens at a temperature between 53°C and 65°C, where connective tissue proteins break the hydrogen bonds with the fibrillar structure, it results in a shrinkage equal to a quarter of the length that the muscle had during the rest phase. At 70°C - 80°C occurs the second step, where the connective tissue proteins break intermolecular bonds and the molecules of collagens that are no longer tied dissolve and form gelatin (Weston et al., 2002;

Tornberg, 2005). The main changes that occur to collagen and other connective tissue proteins influence the tenderness of meat (Ayub & Ahmad, 2019).

2.3.2 The texture and tenderness of cooked meat

When talking about meat, texture and tenderness are strongly interconnected which each other and related to the perception of quality by consumers (Baldwin, 2012).
At the laboratory level, it is possible to evaluate the tenderness of the meat using the Warner-Bratzler shear force test, which can provide objective information on the mechanical forces of meat such as shear, compressive and tensile.
In general, the variation of shear force for meat follows a particular trend during the cooking process: during heating from 50°C to 65°C the value of shear force tends to decrease, this is due to the weakening of the connective tissue and the aggregation of sarcoplasmic proteins, which form a gel that facilitates the chewing process. By increasing the temperature up to 80°C, an increase of shear force is recorded, due to the increase of the elastic modulus linked to the structure of connective proteins (Tornberg, 2005). The last change occurs when the temperature increases from 80°C to 90°C, at this stage there is a decrease in the shear force value since the changes that now take place in the connective tissue have a greater tendering effect on the meat.

2.3.3 The water holding capacity (WHC) and cooking loss

Water holding capacity (WHC) of meat means the ability of meat to retain its water during the application of any type of force; instead, the cooking loss refers to the loss of liquids from meat that occurs because of the cooking process (Honikel & Hamm, 1994).
The water content of raw meat is around 70-75%. In particular, the largest proportion of water is retained within the muscular structures. The water present in meat can be divided into three categories based on its availability (Huff-Lonergan & Lonergan, 2005):

- bound water: due to the dipolar characteristics of the molecule of water, this portion of water has reduced mobility because it is bound to the proteins, and it is resistant to freezing and heating;

- entrapped water: this amount of water is clutched within the structure of muscle, it is not bound to the proteins, but these water molecules are held either by the steric effects or by the attraction to the bound water;
- free water: is the amount of water that has no bonds with protein or structures of the muscles and is easily lost.

The heating process causes structural changes, such as protein denaturation, that in turn cause changes in water retention (Juàrez et al., 2010). The cooking loss process takes place during cooking when myosin is denaturized. The cooking loss is more influenced by the cooking temperature than by the cooking time. In general, the cooking loss begins to develop around 40°C, for pork (with pH value <5.4) the loss of liquids begins earlier, at 30°C. The maximum rate of cooking loss is recorded at temperature between 50°C and 70°C (Aaslying et al., 2003; Bejerholm & Aaslying, 2004).
Other factors can however influence the amount of cooking loss, the quantity of connective tissue the type and size of muscle (if it is whole or portioned), and the cooking method used. In particular, the greatest cooking losses are generally recorded with microwave cooking, follow by the grill and fry cooking (Juàrez et al., 2010; Dominguez et al., 2014)

2.3.4 The formation of flavor

The flavor is an important sensory characteristic able to determine the acceptance or not by the consumer. If the raw meat has no flavor in general, the cooked meat, thanks to the healing process that allows the degradation of precursors present in the raw meat and chemical reactions, is characterized by having a desirable aroma.
The aroma of cooked meat is made up of the composition of sensations between molecules from Maillard reaction and the thermal melting and oxidation of lipids; furthermore, it is influenced not only by the quality of fresh meat but also by the type of cooking, the final core temperature reached, that contribute to the formation of volatile and nonvolatile compounds.
Mottram (1998), in his review, explained the theory about the composition of meat flavor based on two different components. The first one is due to the volatile compounds that originated during the Maillard reaction and are similar in all types of meat. The second component of flavor in meat is species-specific, it allows the consumer to differentiate pork from poultry, and so on, and it is due to

the melting and/or oxidation of lipids; for this reason, lipid composition in terms of qualitative and quantitative fatty acids plays a fundamental role as meaty flavor determinant.

The Maillard reaction is also known as the non-enzymatic browning process of food; it is a very complex reaction, which provides many compounds either for aroma or for color, and it is considered to be one of the most important routes for the generation of flavors in food. In general, it takes place when free amino acids, amine, or part of protein react with carbonyl compounds, in particular reducing sugar. The flavor compounds formed by the Maillard reaction depend directly on the types of sugar and amino acids involved, the temperature and time of the reaction (cooking process) and finally on pH and water content (Jousse et al., 2002).

More specifically, during the reaction of Maillard, occurs the formation of:

- volatile compounds, with potent aroma power and flavor, nevertheless during storage time these compounds can originate undesirable off-flavor;
- brown pigments, such as melanoidins, that contain nitrogen and are soluble in water (more important for bread baking than meat cooking);
- mutagenic compounds;
- loss of essential amino acids such as lysine and so on.

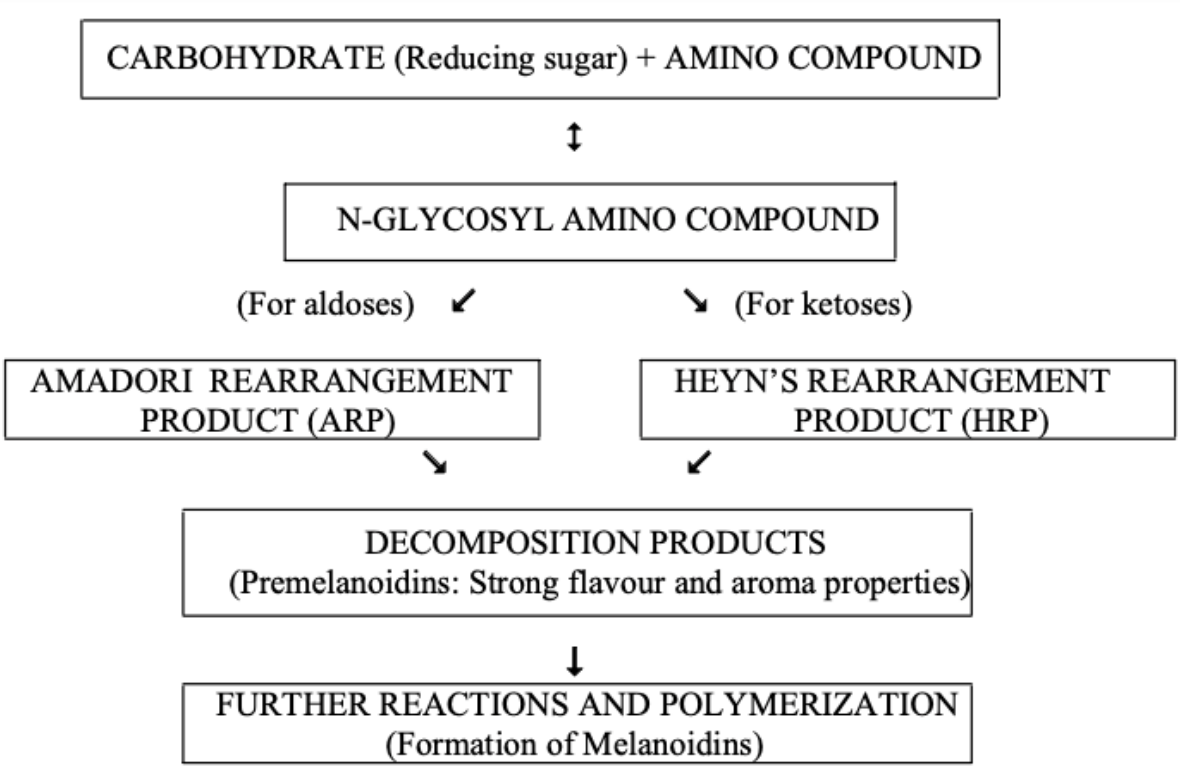
The general mechanism of the Maillard reaction was explained for the first time by Hodge (1953) and later the pathway of the radical formation between amino compound and sugar was explained by Namiki and Hayashi (1983). The three main stages of the Maillard reaction are:

1. The initial stage: starts with a nucleophilic attack from $-NH_2$ of an amino acid group to the carbonyl group of reducing sugar, with the formation of an N-substituted glycosylamine. Subsequently, a re-arrangement of bonds in the molecule takes place, resulting in the formation of an Amadori compound (if the original reducing sugar belongs to aldose) or Heyns compounds (if the original reducing sugar belongs to ketose). From a sensory point of view, the formation of odorous and color compounds has not yet taken place, but the lysine content is reduced.
2. The intermediate stage: the Amadori or Heyns compound is degraded and re-arrangement by a first enolization reaction and by subsequent secondary reactions. The pH value of the product can influence the reaction of enolization: at acid pH, it occurs 1,2-enolization while basic pH favors 2,3-enolization. The molecules that are originated at this stage have high aroma and flavor power, and at the same time represent the precursors of melanoidins.

3. The final stage: at this level occurs the formation of the melanoidins, the main responsible for color.

A summary of the principal steps of the Maillard reaction is shown in Figure 16.

Figure 16. General mechanism for Maillard reaction (Shipar, 2009)



The kinds and the number of compounds of the Maillard reaction are influenced by several factors. For example, in alkaline pH conditions, the reaction is favored, on the other hand, temperature and time of heating are fundamental for the rate of the reaction and the production of molecules for color and aroma. Higher temperature determines a higher rate of reaction, whilst a longer time of heating is responsible for the production of dangerous molecules. Also, the presence of air or oxygen may influence the Maillard reaction: in presence of air melanoidins are degraded at uncolored molecules, the product results in less brown. The presence of metals and ions can promote the rate of the reaction: in particular, the presence of Cu^{2+} and Fe^{3+} accelerates the browning process (Namiki, 1988). Finally, the Maillard reaction tends to occur only on the surface of the product (for meat) because it contains less water that has an inhibiting effect on the reaction.

As previously mentioned, the lipid component, and the products resulting from oxidation (aldehydes and ketones), play an important role in the formation of flavor. Intramuscular fat plays both the role of producer of volatile compounds as well as storage for fat-soluble compounds. The higher the

proportion of unsaturated fatty acids present, the more aroma will be developed (Pegg & Shahidi, 2004).

Figure 17. Compounds derived from lipid precursors (Mottram, 1998)

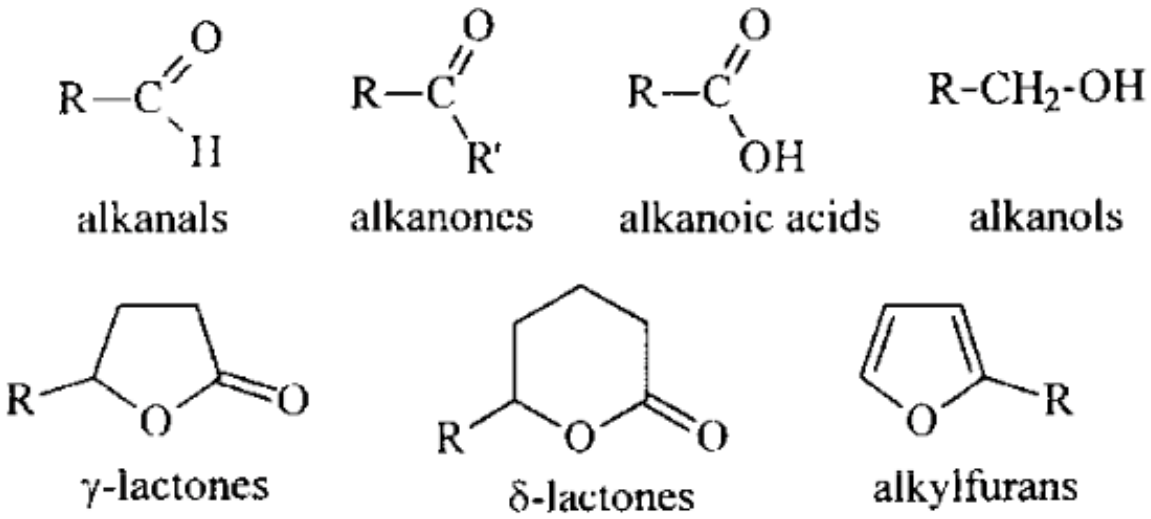


Figure 17 shows the main components that are originated from lipid oxidation, and that are the main precursors of flavor in cooked meat (Mottram, 1998).

From the lipid oxidation reaction are originated reactive carbonyls that can react with the nucleophilic amino group and give the start to Maillard reaction; for this reason, other flavor compounds are originated from the interaction between the lipid oxidation and the Maillard reaction (Mottram, 1998; Zamora & Hidalgo, 2011). The compounds that originated in this way (thiazoles with alkyl substituents in different positions) were found both in beef and poultry, roasted and fried. The precise chemical pathway that regulates the production of these compounds is not yet clear, due to the complexity of the individual reactions and their interactions.

2.3.5 The change in color

The meat color is widely recognized to be a critical and important parameter determining the acceptance of the consumer for raw meat, as an indicator of freshness, and for cooked meat, as a level of doneness (Mancini & Hunt, 2005). In particular, the dull-brown internal color of meat is often related to a well-done cooking process, on the contrary, the pink color is related to uncooked meats

(King & Whyte, 2006). The cooking temperature has an important influence on meat color (Pathare & Roskilly, 2016).

As reported before, the color of meat is made by two components, the amount of myoglobin and the reflectance of proteins. The heat treatments influence the color because they cause the denaturation of myoglobin which precipitates with other proteins (King & Whyte, 2006). The denaturation starts at 55°C and continues during heating, where the maximum level is recorded at 80°C.

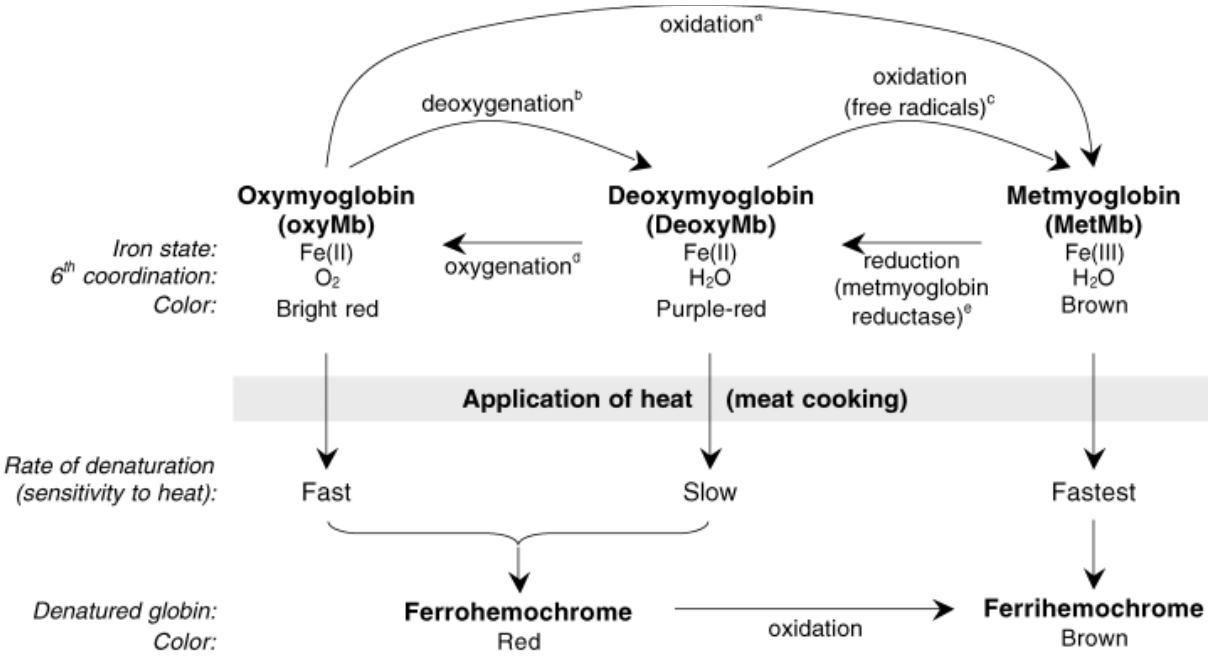
As regards the contribution of myoglobin in determining the color of cooked meat, the oxymyoglobin and metmyoglobin have similar sensitivity to heat and are more susceptible than deoxymyoglobin (Hunt et al., 1999).

The color of the final cooked meat is determined by the final concentration of the ferrihemochrome. The ferrihemochrome originates from the denaturation of metmyoglobin due to the heat process and from the oxidation of ferrohemochrome which in turn results from the denaturation of oxymyoglobin and deoxymyoglobin (Figure 18) (Warren et al., 1996).

Although actin and myosin are not directly related to meat color, their denaturation when occurring at temperature between 45°C and 67°C, affects directly the color opacity of the internal surface, determining a rise of it (Martens et al., 1982).

As regards the change in color due to the reflectance of proteins, during heating the structure of proteins denatures and reconnects in a different configuration. The new configuration, being more compact, prevents the light from passing through, for that the cooked meat looks opaque and no longer translucent (Liu et al., 2011).

Figure 18. Changes of the myoglobin pigments and the denatured products formed during cooking (King & Whyte, 2006)



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CHAPTER 3.

Sous vide

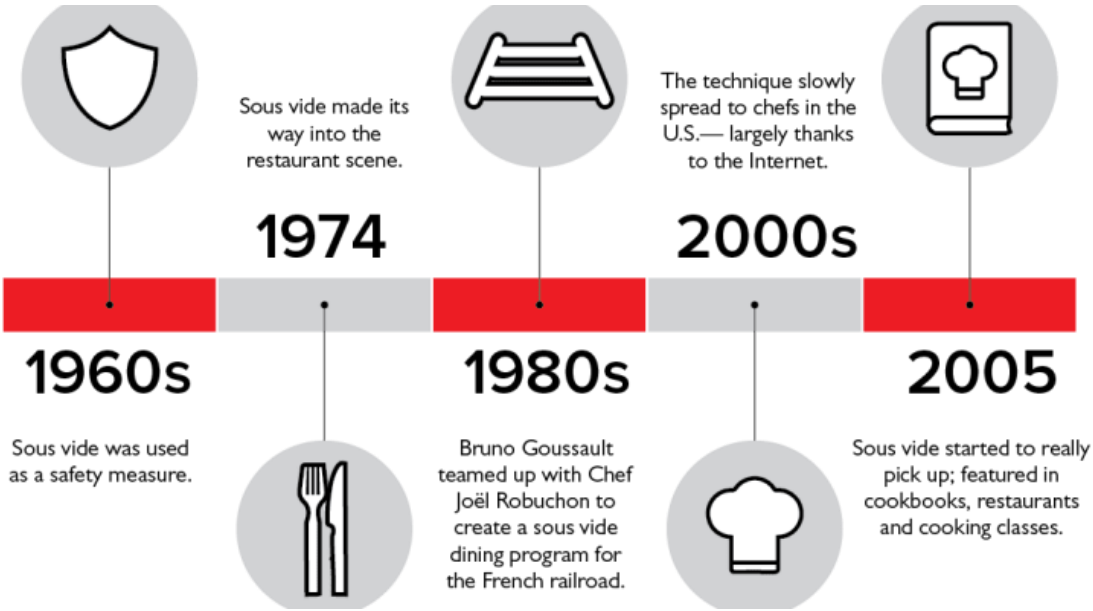
3.1 *Sous vide*

Nowadays, the modern consumer pays attention to different aspects about what he is eating, such as nutritional, organoleptic, and health aspects, but also what is the best technique of cooking to apply to obtain a final product that results tasty and safe. In addition, the changing time has determined a change in consumer's habits and the request for ready-to-eat food is increasing during the last decade.

The *sous vide* is one of the most modern cooking techniques that had French origins, it is considered the evolution of the "*cuisson en papillote*" (or baking in foil) and started to be studied in the 1990s (Baldwin, 2012) by scientists, but the application of technique started in 1980 by Chef Goussalt, who applied it during the cooking of the *foie gras*.

This technique was first used at the level of catering (Majewski, 1990), at the end of 2000s it started to be widely adopted in restaurants and lately at home (Myhrvold et al., 2011; Baldwin, 2012). Figure 19 shows the timeline of the *sous vide* evolution.

Figure 19. Timeline of Sous Vide (www.cookillustrated.com)



Sous vide cooking means "raw materials or raw materials with intermediate foods that are cooked under controlled conditions of temperature and time inside heat-stable vacuumized pounces" (Schellekens, 1996). In fact, this type of cooking tends to apply low temperature (65°C-95°C) for long time (usually from 1h to 7h) (Zavadlav et al., 2020). The control of time and temperature of cooking applied in *sous vide* generally allows to obtain final product which results better in terms of sensory quality and hygiene in comparison to traditional cookings (Baird, 1990; Jang & S Lee, 2005)

In general, the base equipment for the *sous vide* cooking technique is made by: water bath/steam oven, vacuum packaging machine, plastic pouches, and thermometer.
The *sous vide* cooking process is based on three principles, which represent the main differences from traditional cooking processes (Creed, 1995; Baldwin, 2012):

- the raw food, before cooking, is packed in a particular plastic bag, that is heat resistant and food-grade;
- the air inside the bag is removed, creating the vacuum and the plastic bag is sealed to preserve vacuum;
- a constant temperature for a long time is applicated.

In particular, the packaging of the food in a plastic bag during the cooking process allows preventing the loss of food constituents, such as vitamins, water, odors, and flavor compounds, and it avoids the recontamination of the food (Schellekens, 1996).
The absence of oxygen inside the bag plays an important role in preventing the oxidation process, and in addition, the heat transfer from water or steam to the food is more efficient with respect to the traditional cooking process. In addition, with the vacuum-packaging machine is possible to use different degree of vacuum depending on the characteristics of the food. For example, fish needs vacuum degree "more gentle" (residual pressure around 100-120 mbar) respect to which can be used for vegetable of meats, that are characterized by the use of hard vacuum degree to achieve the most efficient thermal treatment (residual pressure 10-15 mbar).
Regards the use of a controlled combination of time and temperature, this is fundamental in the reducing process of the breakdown of food components, and it allows to have control on the fast and slow change that occurs due to the cooking process (Church & Parsons, 2000; Baldwin, 2012).

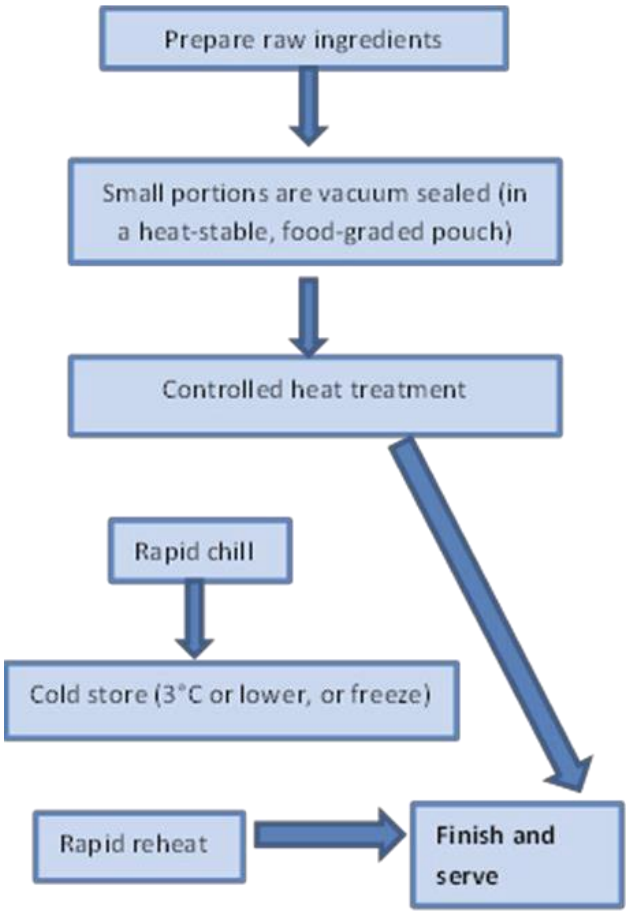
3.2 Overview of the *sous vide* process

Depending on temperature and duration of storage before the consumption, *sous vide* products can be classified in 2 main categories (Baldwin, 2012; Stringer & Metris, 2018):

- *sous vide* cook-serve: preparing the food, vacuum packaging, heating, finishing and service;
- *sous vide* cook-chill: preparing the food, vacuum packaging, heating, rapid chilling, refrigerating, storing until consumption, re-heating, finishing, and serving

Figure 20 shows a generic flow diagram for the *sous vide* cooking process.

Figure 20. Generic flow diagram for sous vide process



In general, the first method is preferred by consumer that tries the *sous vide* technique at home, or by a chef of restaurant, which in this way can easily serve many consumers simultaneously. The second group, the *sous vide* cook-chill is typical of industrial process, that is the author of the passages until the store period, and the last are made by the consumer at home.

3.2.1 Vacuum packaging

Vacuum packaging represents the first step of *sous vide* technique. As it said before, the vacuum packaging provides different advantages, such as: better heat transfer to the food, and preventing the formation of off-flavor. The absence of oxygen is also useful to preserve from the oxidation of natural pigments, and guarantee final product characterized by brightener color, in particular for vegetables.

Another fundamental advantage in the use of vacuum for packaging the food is the improve the shelf life of the products by eliminating the risk of recontamination during the storage period, and the absence of oxygen eliminates the growth of aerobic microorganisms (Church, 1998; Church & Parsons, 2000).

3.2.2 Cooking

Water baths or steam ovens are the most common equipment used for the *sous vide* technique. The use of water baths has several advantages; in particular, the heating process is uniform and temperature fluctuations are less than 0.1. The steam oven, due to the working temperature condition, below 100°C, is characterized by fluctuation of temperature even very high recorded on samples cooked on the same time (Sheard & Rodger, 1995).

In general, the food is immersed in the water which is preheated to the required temperature that must be reached at the core of food, and the pouches have to be completely immersed to guarantee a proper cooking process.

3.2.3 Setting and core temperature

Temperature of cooking, independently on the technique used, is a fundamental parameter. In *sous vide* cooking, the food is cooked at the temperature we want the food to have at the end of the process.

The careful control of cooking temperature, allows to greater control on slow and fast changes that take place in foods (Baldwin, 2012).

In general, it is recommended to choose a right combination of time and temperature in order to achieve a 6-log reduction of Psychrotrophic *C. botulinum* or *L. monocytogenes* (Carlin, 2014; Betts et

al., 2015), always based on the rule “the more intense thermal process the longer the shelf-life and vice versa” (Genigeorgis, 1993).

In the scientific literature, is possible to find tables with the temperature and time of pasteurization to use on meat cooking process in function of thickness the sample and the temperature of water bath. An example is shown in Table 7

Table 7. Time necessary to pasteurize meat, fish, and poultry in water bath, from 55°C to 65°C (modified by FDA, 2011)

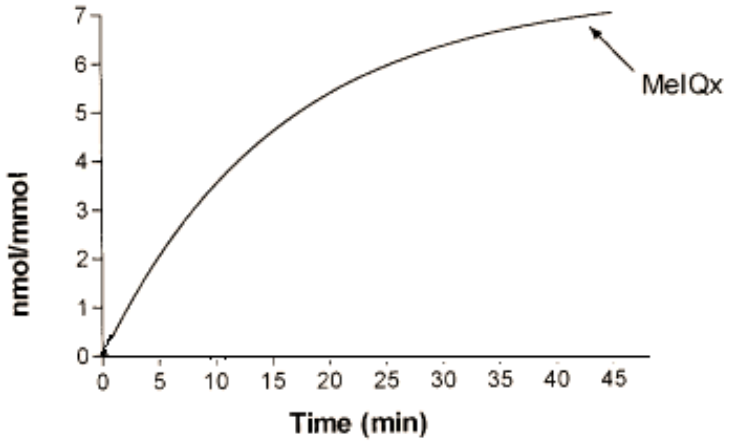
Thickness (mm)	55°C	56°C	57°C	58°C	59°C	60°C	61°C	62°C	63°C	64°C	65°C
5	3:33	2:41	2:00	1:30	1:08	0:51	0:40	0:31	0:25	0:20	0:17
10	3:35	2:43	2:04	1:36	1:15	1:00	0:49	0:41	0:35	0:30	0:27
15	3:46	2:55	2:16	1:48	1:28	1:13	1:02	0:53	0:47	0:42	0:38
20	4:03	3:11	2:32	2:04	1:44	1:28	1:17	1:08	1:01	0:56	0:52
25	4:17	3:25	2:46	2:18	1:57	1:41	1:30	1:21	1:13	1:08	1:03
30	4:29	3:38	3:00	2:32	2:11	1:55	1.43	1:33	1:26	1:19	1:14
35	4:45	3:53	3.15	2:46	2:25	2:09	1:56	1:46	1:38	1.31	1:26
40	4:59	4:07	3.29	3:00	2:39	2:22	2:09	1:59	1:50	1:43	1:37
45	5:21	4:29	3:50	3.22	3:00	2:42	2:29	2:17	2:08	2:00	1:53
50	5:45	4.53	4:14	3:44	3:21	3:03	2:49	2:37	2:27	2:19	2:11
55	6:10	5:18	4:39	4:08	3:45	3:26	3:11	2:58	2:47	2:38	2.30
60	6:38	5:45	5:06	4:35	4:10	3:50	3:34	3:20	3:09	2:58	2:50
65	7:07	6:15	5:34	5:02	4:36	4:15	3:58	3:43	3:31	3:20	3:11
70	7:40	6.45	6:03	5.30	5:04	4:42	4:23	4:08	3:54	3:43	3:32

3.2.4 Finishing and serving

Foods cooked with *sous vide* technique have generally the appearance of being poached (Baldwin, 2012), and generally fish, shellfish, and poultry can be served as they are. As regards other types of meat cooked with this technique such as pork or beef, it can be necessary to sear or sauce before their serving. The searing passage is very common on meat, it can improve the flavor of the products thank to the Maillard reaction, and the oxidative/thermal degradation of lipids (Mottram, 1998; Tamilmani & Pandey, 2016), that occur at high temperature of cooking. The Maillard reaction generally occurs at temperature of 130°C, but the typical roasted aroma is generated when the temperature reaches 150°C, increasing the temperature (> 175°C) and the time of process generally

increases the amount of Maillard compounds as well as increases the production of mutagens such as heterocyclic amines. Jägerstad et al. (1998) conducted a study which demonstrated how the production of HCAs is strongly related on temperature and time of maintaining (Figure 21).

Figure 21. Formation of 2-amino-3,8-dimethylimidazo(4,5-f) quinoxaline (MelQX) at 175°C in a model system simulating beef (adapted from Jägerstad et al. 1998)



3.2.5 Chilling and finishing

For the cook-chill *sous vide* products, inside its vacuum sealed pouch, the food after cooking is rapidly chilled and refrigerated (Baldwin, 2012). After the storage, before the service, the *sous vide* product is generally reheated; for meat, the process occurs in a water bath system.

The main problem related to the consumption of this category of products is that the low temperatures used for the cooking process are not able to guarantee the inactivation or the reduce of pathogenic spore under to safe levels. In addition, a not enough rapidly chilled passage or very long period of refrigerated storage can promote the growth and the multiplication of the spores over than a dangerous level. Table 8 shows a variety of microorganisms (pathogens for human), with their growth temperature, that can survive and grow in *sous vide* products not adequately refrigerated and stored.

Table 8. Pathogen microorganisms of interest for human and their minimum temperature of growing

Organism	Minimum growth temperature (°C)
Clostridium botulinum (mesophilic)	10

Clostridium botulinum (Psychrotrophic)	3.3
Listeria monocytogenes	0
Clostridium perfringens	15
Yersinia enterocolitica	-1
Salmonella	6
Bacillus cereus	4
Escherichia coli	7
Staphylococcus aureus	6

3.3 The nutritional quality of *sous vide* food

The combined effects of the use of mild and precisely cooking temperature and the presence of a low quantity of oxygen in the system plays an important role on preserving the nutritional content of products and on safeguarding the request of the consumer about the tenderness and the juiciness of the product (Iborra-Bernard et al., 2014). It was demonstrated by Rondanelli et al. (2017) on vegetal products and by Da Silva et al (2017) on beef, that the use of plastic pouches in *sous vide* technique is fundamental to limit the loss of liquids, and with them also the loss of minerals and vitamins.

Other researches have demonstrated how vegetables cooked with *sous vide* technique showed amount of polyphenols and anthocyanins comparable to fresh products, and in particular vegetables such as onion, green beans, cauliflower, carrots, or tomato after *sous vide* cooking still showed their antioxidative potential (Iborra-Bernard et al., 2015; Guillèn et al., 2017; Kosewski et al., 2018).

Furthermore, the plastic pouch has a significant role in the improvement the nutritional quality of *sous vide* cooked food, in fact the plastic barrier of the bag limits the diffusion of oxygen into the food, limiting the oxidation of lipids and preserving the quality and the benefit for human health of essential polyunsaturated fatty acids (Schellekens, 1996; Redfern et al., 2021).

3.4 *Sous vide* on meat

The *sous vide* cooking technique is applicated and studied on large amount of different kind of food (Ayub & Ahmed, 2019), this research is focusing on the application of this technique on the cooking of meat.

The cooking process is responsible of the changes that occurs on meat in terms of color, formation of aroma and flavor, and texture. Several studies have analyzed the effects of changes on meat due to the *sous vide* cooking process. Table 9 summarize in brief the main results obtained with the application of *sous vide* on beef, pork, lamb and chicken.

Table 9. Synopsis of studies on *sous vide* meat product

References	Cooking temperature	Cooking Time	Ingredients	Highlights
Laakkonen et al., 1970	60°C	0-6 h	Beef	The WBSF was reduced by the slow heating at 60°C.
Bouton & Harris, 1981	50°C and 60°C	1h and 24h	Beef	The tenderization process is explained by the degradation of collagen
Dinardo et al, 1984	60°C	2h and 4 h	Beef	The use of water bath allowed to obtain more uniform cooking process. The solubilization of the collagen increased with extending the time of cooking at 60°C
Vaudagna et al, 2002	50°C, 55°C, 60°C, and 65°C	1.5 h, 3 h, 4,5 h, and 6,5 h	Beef	With the increasing of temperature from 50°C to 65°C the cooking loss increased to and meat resulted tenderness
Christensen et al., 2011	48°C, 53°C, 58°C, and 63°C	5h and 17 h	Pork	Between 53°C and 58°C the cooking loss increased with the decreasing of shear force value
Sànchez del Pulgar et al., 2012	60°C and 80°C	5 h and 12 h	Pork	Lower temperatures of cooking were related to lower value of cooking loos. At 60°C the collagen fibers were broken but not denatured
Roldàn et al., 2013	60°C, 70°C and 80°C	6 h, 12 h, and 24 h	Lamb	The tenderness increased with the time of cooking, due to gelatinization process of connective tissue surrounding the fibers.
Christensen et al., 2013	53°C, 55°C, 58°C and 63°C	5h and 17 h	Beef	The reduction of shear force value is due to weak of collagen
Roldàn et al., 2015	60°C and 80°C	6h and 24h	Lamb	60°C for 6h decreased the cooking loss and increased the moisture

				content. The shelf life of the product lasted 30 days
Becker et al., 2015	53°C and 58°C	10h, 20h and 30h	Pork	No significant tenderization process or increasing in cooking loss were recorder after 30 h of cooking process
Haghighi et al., 2021	60°C, 70°C and 80°C	1 h, 1,5h, 2h, 2,5h	Chicken	Increasing the temperature cause and increasing of the shear force, cooking loss was affected by both, time and temperature of cooking.

3.4.1 Tenderness

The tenderness is a fundamental parameter evaluated by the consumers on cooked meat. It is directly related to changes in myofibrillar and connective tissues (Tornber, 2005). Different studies on beef (Dinardo et al., 1984; Christensen et al., 2013), lamb (Roldàn et al., 2013), pork (Christensen et al., 2011, Becker et al., 2015) and chicken (Haghighi et al., 2021), confirmed that low temperature (~ 60°C) of cooking for long period is crucial to obtain tender meat.

As it explained in section 2.3.1; the denaturation of protein starts at 35°C-40°C, at 50°C the diameter of fiber is reduced, and at 60°C the shrinkage proceeds in diameter and in longitudinal axis (Warner et al., 2017). Holding for long time temperature of cooking above 60°C does not have any effect of structural changes due to the shrinkage of meat, but results fundamental for the degradation process of connective tissue that is strictly related to the shear force value.

3.4.2 Juiciness and cooking loss

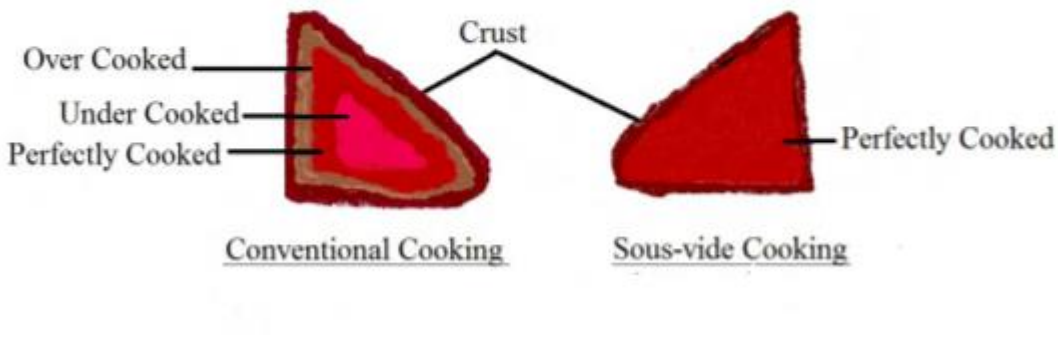
The other sensory aspect largely demanded by the consumer is juiciness, and with the tenderness represents the most important attributes in cooked meat (Dominguez-Hernandez et al., 2018; Ayub & Ahmed, 2019). The *sous vide* cooking technique with the application of long time of heating seems to affect juiciness and tenderness in the opposite way. In fact, longer is the time of cooking, more tender is meat but also it is less juicy. The parameter of cooking loss, which is the evaluation of the amount of liquids and other substances loss during cooking, is correlated to the juiciness. Higher cooking loss are generally related to low value of juiciness. The amount of cooking loss depends not only by the cooking process (temperature and time, cooking technique) but also by the sample size

considered, by the quality of raw meat such as muscle fiber orientation, pre-freezing or ageing time of muscle (Oillic et al., 2011). Compared to other types of cooking such as boiling, deep-frying, oven or pan frying, *sous vide* technique records lower value of cooking loss (Pathare & Roskilly, 2016). The minor cooking loss recorded in *sous vide* is due to the combinations of processing factors: the use of water bath that guarantee a uniform cooking process (Baldwin, 2012), and the vacuum-sealed packed that allows to limit the dehydrate process of meat (Church & Parson, 2000; Ayub & Ahmad, 2019).

3.4.3 Color

By evaluating the color, the consumer determines whether the product is acceptable. The changes in colors for meat are related to the denaturation of myoglobin (King & Whyte, 2006). Respect to high heating cooking technique, the *sous vide* cooked meat is characterized by a less thick brown layer and the appearance is more uniform and appealing (Dinardo et al. 1984, Ayub & Ahmad, 2019) (Figure 22). The external surface of *sous vide* cooked meat does not benefit from the Maillard reaction, and in general results less "browning" aspect with respect to meat cooked at high temperature.

Figure 22. Meat cooked with a conventional method vs sous vide (Ayub & Ahmad, 2019)



With the increase of cooking temperature, on pork, lamb, and beef the redness value is reducing (Vaudagna et al., 2002; Roldàn et al., 2013; Becker et al., 2016), while the yellowness value is higher in very prolonged heating times.

3.4.4 Flavor

Different study demonstrated that the production of volatile compounds in meat occurs when the cooking temperature reaches at least 70°C (Calkins & Hodgen, 2007; Dominguez-Hernandez et al., 2018). In *sous vide* product the meaty flavor is originated by the combined effect of the presence of nonvolatile compounds and lipid degradation (Aaslyng & Meinert, 2017). Dominguez-Hernandez et al. (2018) collected all the studies focused on the formation of flavor in *sous vide* meat products and affirmed that the formation of the desired flavor is strictly related to the duration of cooking when low temperatures are used (60°C-80°C). To improve the aroma, restaurants or catering services usually reheat the meat at 130°C-150°C before serving.

3.5 *Sous vide*, the future of the kitchen?

Different advantages came from the use of the *sous vide* technique. The studies present in literature demonstrated that higher nutritional value of food cooked with this technique. No additional fats are needed for the cooking process, the production of toxic compounds due to the heating process is limited only to the reheating process before service. In addition, the *sous vide* meat products are able to respond to the consumers request, for tender and juicy products. Due to the characteristics of the packaging, the shelf life of cooked product results extended, and this can reduce the food waste. *Sous vide* product can be considered, by the modern consumer which does not have time to cook, the new way of ready-to meat dishes, healthy and tasty, that can be personalized with few and fast passages. For consumers, who love cooking, the use of this technique at home allows to experiment with the application of different times and temperatures on food of different textures and flavors.

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Sitography

https://www.cooksillustrated.com (November 2021)

CHAPTER 4

The aim of the research

Meat and meat products are sources of fundamental nutrients essential for human health and development.

Different cooking techniques are normally used for the cooking of meat, but most of them are responsible of the formation of dangerous compounds due to the application of high temperatures. In addition, modern consumers are requesting increasingly tender and juicy cooked meat, as well as they are looking for cooking techniques that reduce the risk of formation of toxic compounds for human health. One solution can be represented by the use of *sous vide* technique.

This cooking technique is characterized by the application of low temperature for long time of cooking. The food is vacuum-sealed before the cooking process and this reduces the amount of liquids losses while maintaining higher value of moisture, thus providing a juicier product.

The aim of this research was to investigate the potential application of this technique on the cooking of pork and chicken. In particular 3 different experiments were carried out.

The first experiment investigated the effects of two different *sous vide* cooking methods, different for time and temperature combinations on physicochemical characteristics of pork. For the research were used samples coming from a previous study where the aim was to improve the amount of n-3 fatty acids in muscle tissue, supplementing pig diet with a natural source such as extruded linseed, and improve the oxidative stability with supra nutritional dose of synthetic or natural antioxidants.

In order to better assess the effect of cooking time and temperature on the physicochemical parameters of pork, the second experiment investigated the effects of 12 different combinations of time and temperature of cooking, consisting on the application of three temperature levels (60°C, 70°C, and 80°C) and four time levels (60 min, 90 min, 120 min, and 150 min) on *Longissimus thoracis* muscle of pork bought at the local market.

Furthermore, since the application of ultrasound in the food industry is already used for different purpose such as sanitation process, and other research reports positive effects on the improvement of tenderness of beef, in this study was evaluated the effect of an ultrasound treatment before cooking process in order to assesses the difference on the physicochemical parameters of samples

cooked with *sous vide* technique with or without sonication pre-treatment, with a particular attention to the shear force value.

The last study was carried out on chicken breasts bought at the local market, and it investigated the effects of the same twelve combinations of time and temperature of *sous vide* cooking. Since the ultrasound treatment was not effective on the second study, it was not evaluated anymore.

In addition to the physicochemical parameters evaluated in the previous experiments, a shelf life study investigated on the minimum combination of time and temperature of *sous vide* cooking able to guarantee the safety of the final product for a period of 21 days of conservation at 4°C. Finally, the microstructure analysis of the chicken breast cooked with 12 combinations of *sous vide* technique was evaluated.

CHAPTER 5

1ST Experiment

The aim of the study

The aims of the first study were the evaluation of the effects of two combinations of time and temperature conditions of *sous vide* cooking technique on cooking loss, color, oxidative stability, fatty acid composition and shear force (WBSF) of pork loin, and the evaluation of the effect of cooking on physicochemical parameters and fatty acid composition of loins from pig fed with experimental diets. The research is a part of previous study on animals that had undergone an experimental feeding trial aiming to study the effects of dietary linseed supplementation with or without antioxidants of synthetic (vitamin E) or natural origin (grape peels and oregano extracts) on animal performance and meat quality (Scutaru, 2019, Belmonte et al., 2021).

The diet of the animals is reported in Table 10.

Table 10. Composition of the experimental diets (as-fed basis) (modified from Scutaru, 2019)

		C		L		LE		LVE	
Ingredients		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Extruded linseed	%	0.00	0.00	5.00	5.00	5.00	5.00	5.00	5.00
Barley meal	%	85.50	91.00	80.50	86.60	80.30	86.40	80.50	86.60
Soybean meal	%	11.00	5.50	11.00	5.00	11.00	5.00	11.00	5.00
L-Lysine	%	0.31	0.29	0.30	0.29	0.30	0.29	0.30	0.29
DL-Methionine	%	0.06	0.04	0.06	0.03	0.06	0.03	0.06	0.03
L-Threonine	%	0.05	0.04	0.05	0.03	0.05	0.03	0.05	0.03
Calcium carbonate	%	1.18	1.13	1.19	1.15	0.89	0.85	1.19	1.15
Dicalcium phosphate	%	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt (NaCl)	%	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin/mineral pre-mix[1]	%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Vitamin E and Selenium pre-mix^{-1}	%	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00
Vegetal extract (grape skin + oregano)	g per kg of feed	0.00	0.00	0.00	0.00	0.00	0.00	3.00+2.00	3.00+2.00
Fatty acid composition % (of total FAs)									
C14:0	%	0.47	0.39	0.25	0.21	0.25	0.22	0.26	0.22
C16:0	%	29.01	24.25	18.13	15.20	17.78	15.59	18.80	15.31
C16:1	%	0.49	0.34	0.17	0.15	0.17	0.17	0.02	0.15
C18:0	%	2.03	1.51	4.00	3.18	3.88	3.34	4.16	3.23
C18:1 n-9	%	14.92	13.50	20.60	18.12	20.24	18.45	21.29	18.26
C18:2 n-6	%	47.55	53.67	33.50	34.69	33.91	34.09	32.52	34.47
C18:3 n-3	%	4.77	5.70	22.83	28.02	23.25	27.73	22.38	27.95
C20:1	%	0.74	0.64	0.53	0.41	0.52	0.42	0.57	0.41

C, control group; L, group with 5% of extruded linseed; LE, group with 5% of extruded linseed, 200 ppm vitamin E and 0.211 ppm of Selenium; LVE, group with 5% of extruded linseed and vegetal extracts from grape skin (3.00 g per kg of feed) and oregano oil (2.00 g per kg of feed).

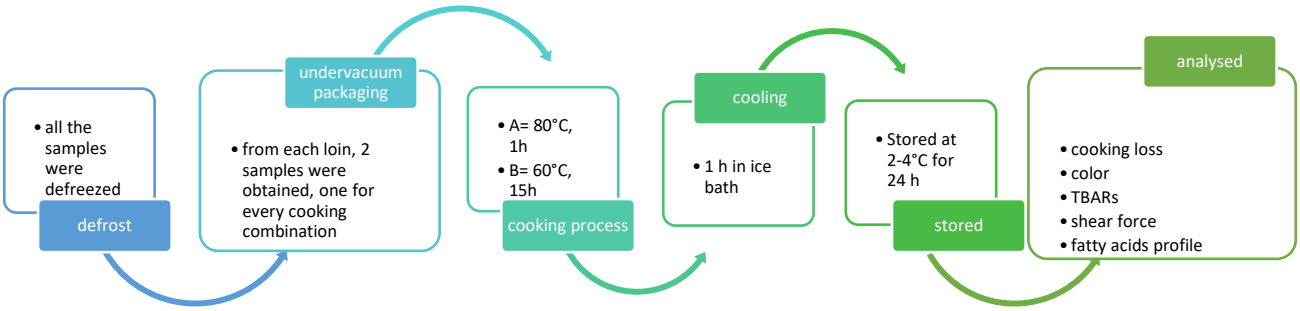
1st= feed administered from an average weight of 80 kg to 120 kg (growing period); 2nd=feed administered from an average weight of 120 kg to slaughter (finishing period).

5.1. Materials and Methods

5.1.1 Preparation of samples

A total of 48 frozen loin, 12 per group coming respectively from pigs subjected to the four dietary groups (Table 10), were involved in the research. Figure 23 shows the main steps of the experiment.

Figure 23. Outline of work, experiment 1



Before vacuum packing the samples, the loins were thawed at 4°C for 24h; subsequently, from each loin, two samples were obtained (364.8 ± 16.3 g), one for each cooking condition, and vacuum-sealed in food-grade nylon-polyethylene plastic pouches (150 x 200 mm^2), with wide thermal stability (-40°C - +120°C) with O_2 permeability of 9 cm^3/day m^2 (4°C/80% relative humidity), and water vapor permeability of 1.2 g/day m^2 (Joeplas SL, Barcelona, Spain). using a vacuum sealer (Elegen, Reggio Emilia, Italy) with a pump flow rate of 30 L per minute to create 98% vacuum degree inside the pouches.

Subsequently, the samples were immersed in the *sous vide* cooker (Elegen, Reggio Emilia, Italy), as shown in Figure 24.

Due to the reduced number of samples available, two combinations of time and temperature of *sous vide* cooking technique were investigated in the study. The two *sous vide* cooking conditions used were:

- condition A: The sample was cooked at 80°C for 1 h;
- condition B: The sample was cooked at 60°C for 15 h.

As regards the temperature used, the reason why 60°C and 80°C were chosen is that these temperatures represent the most used in restaurant and catering service. Regarding instead the choice of time, we decided to apply 1 h for the highest temperature and 15 h for the lowest, to evaluate the maximum possible difference among the samples, both from the point of view of physicochemical changes and about changes in the fatty acids profile. In addition, due to the lack of

a control sample, due to the small numbers of samples available, the choice of the combination of 80°C for 1 h was necessary to guarantee a control sample, as this combination of time and cooking used as control cooking condition.

Figure 24. Sous vide cooking machine

At the end of the cooking process, all the samples were cooled for 1 h in an ice bath and stored overnight at 2-4°C before analysis.

Cooking loss, color measurements, shear force, lipid oxidation, and fatty acid composition were determined after the cooking process.

5.1.2 Cooking loss

The cooking loss was determined according to AOAC 950.46 method (AOAC, 2005), measuring the difference in the weight of sample before and after cooking.

$$[(raw\ weight - cooked\ weight)/raw\ weight] * 100$$

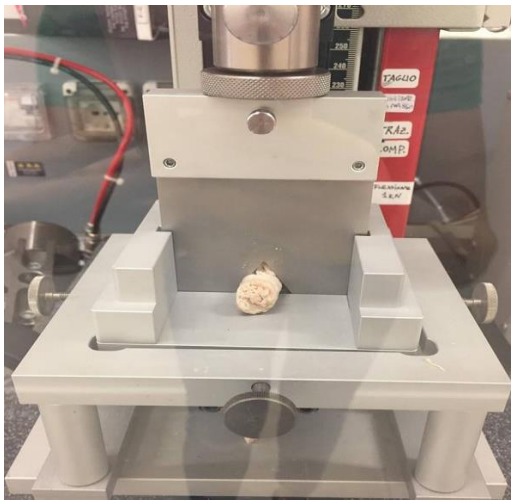
5.1.3 Color measurements

The color of meat samples before and after cooking was measured with spectrophotometer Minolta CM-600d (Konica Minolta Holdings, Inc, Osaka, Japan) equipped with a standard illuminant D65 and an 8 mm diameter aperture. After calibration with a white plate supplied by the manufactured, the results were reported as L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) according to AMSA (2012). The average of five measurements at different positions was calculated.

5.1.4 Warner-Bratzler shear force (WBSF)

On the cooked samples, according to Honikel (1998), the shear force was determined. From each cooked sample, 5 cylindrical cores (diameter of 1.5 cm) were cut and analyzed using the texture analyzer (Z1.0, Zwick/Roell, Ulm, Germany) with a loading cell of 1000 N and crosshead speed 250 mm/min equipped with a V-shaped blade with a triangular hole of 60° (Figure 25). The average of 5 measurements was recorded, and the result was expressed as the maximum peak force recorded (kg) needed to shear the sample.

Figure 25. Dynamometer



5.1.5 Lipid oxidation

Lipid oxidation was evaluated according to Siu and Draper (1978, slightly modified). In duplicate, 2.5g of minced cooked meat and 12.5 ml of distilled water were homogenized at 9500 rpm for 120 seconds using an Ultra-Turrax homogenizer (IKA, Germany), and then vortexed for 1 min at high speed. Subsequently, 12.5 ml of 10% trichloroacetic acid (TCA) (Sigma-Aldrich, Milan, Italy) were added to the homogenate, and centrifuged for 20 min at 2000 rpm at 4°C. The supernatant was filtered by a filter paper (Whatman No.541), 4 ml of clear filtrate were transferred into 15 ml pyrex screw cap test tubes and mixed with 1 ml of 0.06 M 2-thiobarbituric acid (TBA) (Sigma-Aldrich, Milan, Italy), and the solution was heated at 80°C for 90 min. Before reading, the samples were cooled. A distilled water-TCA-TBA reagent was also prepared and heated with the samples, and presented as a blank. The absorbance at 532 nm was measured against the blank, using a Jasco spectrophotometer

(Jasco Corporation, Model V550, UV/VIS, Tokyo, Japan). Using 1,1,3,3 tetraethoxypropane solution (TEP, Sigma-Aldrich, Milan, Italy) as a standard, the results were expressed as mg of malondialdehyde (MDA) /kg of sample.

5.1.6 Fatty acids composition of cooked meat

A methanol-chloroform (Sigma-Aldrich, Milan, Italy) extraction of total lipids from a cooked sample was conducted according to Folch et al. (1957).

Subsequently, according to Ficarra et al. (2010), 25 mg of lipids extract was methylated with 2N methanolic potassium hydroxide solution (KOH from Carlo Erba, Milan Italy, and methanol from Sigma-Aldrich, Milan, Italy). The tridecanoic acid (C13:0) (Larodan Fine Chemicals AB, Malmö, Sweden) was added as internal standard. The fatty acids composition was determined using the TRACE^{TM}GC Ultra (Thermo Electron Corporation, Milan, Italy) equipped with Flame Ionization Detector, a PVT injector, and TR-FAME column (30 m long, 0.25 mm i.d., 0.2 µm thickness) supplied by Thermo Scientific (Rodano, Milan, Italy) were used. In particular, 1 µl of the methylated sample was injected into the gas-chromatograph with a flow rate of splitting of 10 ml/min, and operating at a constant flow of helium as a carrier gas of 1 ml/min. The operating temperature of the detector and injector was the same, 240°C. The initial working temperature, 140°C, was maintained for 2 min, subsequently every 5 min the temperature of working was increased of 4°C until the final temperature of 250°C was reached and maintained for 5 min. To record, identify and integrate the peaks of the fatty acids methyl ester (FAMEs), the Chrom-card software (2.3.3. version, Thermo Electron Corporation, Milan, Italy) was used. A solution with a mix of standard FA (Supelco^{r} 37 Component FAME mix, PUFA standard n.2, Animal Source, Supelco, Bellafonte, PA, USA, and single FAMEs standard, Larodan, Fine Chemicals AB, Malmö, Sweden) was previously used to identify the retention times of the fatty acids. The amount of each FAME was expressed as FAME relative percentage concerning the total amount of FAMEs.

5.1.7 Statistical analysis

The statistical analysis was performed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). The statistical model included the type of cooking and dietary treatments as fixed effects. The

interactions between the cooking conditions and the dietary treatments were considered but they were never statistically significant, therefore they were removed from the statistical model.

5.2 Results and Discussion

5.2.1 The effect of cooking conditions

Table 11 shows the results of the effect of cooking conditions on the physicochemical parameters of meat.

Table 11. The effect of cooking condition on physicochemical parameters of meat

	Condition A	Condition B	RMSE
Cooking loss (%)	16.84	17.35	2.74
WBSF (kg)	4.94[a]	3.28[b]	1.14
MDA (mg/kg of meat)	0.688	0.643	0.329
L*	65.30[a]	61.37[b]	6.06
a*	3.67[b]	4.50[a]	1.51
b*	16.12[b]	18.41[a]	1.87

Condition A: 80°C for 1h; Condition B: 60°C for 15h.
RMSE: Root Mean Square Error
[a,b] P<0.05
WBSF: Warner-Bratzler Shear Force
MDA: malondialdehyde

The parameters of shear force (WBSF) and color were influenced by the cooking condition, while cooking loss and the oxidation of lipids (MDA) did not show any statistical differences between the two cooking conditions (P>0.05).

Cooking loss is in general related to the eating quality of meat, and is intended as the total liquid with other soluble components that are normally lost during the cooking process (Aaslying et al., 2003). In this study the two *sous vide* conditions did not show statistical difference for this parameter, although the cooking loss recorded for condition B was slightly higher than condition A (P>0.05).

Martens et al. (1982) suggested that the loss of liquids in meat is due to the denaturation of proteins structure in particular when the temperature range is between 56°C and 62°C, this produces the contraction of the perimysium, that has the role of wrapping the muscle bonds, and this favors a further loss of liquids. Sànchez del Pulgar et al. (2012), had instead found that with an equal time of

cooking, the higher temperature has a greater influence on liquids loss, in the range of temperature between 45°C and 80°C the loss of liquids is linear to the increase of temperature. Over 80°C this linear relation (temperature and cooking loss) is less evident (Bouton and Harris, 1972).

As regards the value of shear force, samples cooked at 80°C for 1h resulted less tender than the others (4.94 vs 3.28 kg, $P<0.05$). The tenderizing process of cooked meat is mainly due to the changes that occur during cooking in connective tissue, myofibrillar proteins, and sarcoplasmic proteins (Baldwin, 2012; Fabre et al., 2018). The quantity and typologies of collagen play a relevant role on meat tenderness (Maiorano et al., 2000). In general, the gelatinization of sarcoplasmic proteins occurs when the cooking temperature is up to 65°C, in our study probably the long period of cooking at 60°C; 15h, could promote the gelatinization process of sarcoplasmic proteins, and as results, meat cooked for prolonged time resulted more tender.

The combination of time and temperature did not have any influence on the lipid oxidation process. Samples cooked at higher temperatures show only slightly higher numerical values of MDA but not significantly different to the other cooking condition ($P>0.05$); the same result was found also by Sànchez del Pulgar et al. (2012).

The color parameters were affected by the cooking process. L* was higher ($P<0.05$) in samples cooked at 80°C for 1h. The two groups differ from each other also for the a* color parameter, meat cooked at 60°C for 15 h resulted redder than meat cooked at 80°C for 1 h (4.50 vs 3.67, respectively; $P<0.05$). The a* value in meat is determined by the content, the oxidation state and denaturation of myoglobin, the last condition depends directly on the cooking process (King & Whyte, 2006). Based on what Hunt et al. (1999) found in their study, the denaturation process of myoglobin occurs firstly slowly when the temperature is between 55°C and 65°C, while it is faster and easier when the temperature rises to 80°C. It can be inferred that the temperature of cooking in condition B (60°C) was not sufficient to determine the denaturation of a large amount of myoglobin. Our results fit whit what Sànchez del Pulgar et al. (2012) found on pork cheeks and Vaudagna et al. (2008) found on muscle from beef cooked at 50°C-65°C for 1.5 h and 6 h. These authors stated that the redness of cooked meat is more intense on sample cooked at 60°C respect to 80°C.

As regards the b*color parameter, that is providing information on the blue/yellow component of the product, it resulted higher in samples from the B group. Probably it means that a long time of cooking (independently on temperature conditions) favors the formation of metmyoglobin and its denaturation, and the presence of these molecules in high quantities results in browner product, characterized by higher levels of b* value (Suman & Joseph, 2014).

Table 12 shows the fatty acid composition of meat cooked with two different cooking conditions, the values provided by raw meat were also included in the comparison.

Table 12. The effect of cooking condition on fatty acids composition (%) of meat

%	Raw meat	Condition A	Condition B	RMSE
C10:0	0.113[b]	0.125[a]	0.124[a]	0.017
C12:0	0.076[b]	0.106[a]	0.105[a]	0.033
C14:0	1.243[a]	1.477[b]	1.464[b]	0.112
C16:0	23.676[b]	25.905[a]	25.752[a]	1.224
C16:1	3.084[b]	2.485[a]	2.396[a]	0.365
C17:0	0.229	0.227	0.231	0.045
C17:1	0.269[b]	0.219[a]	0.217[a]	0.038
C18:0	12.815[b]	15.799[a]	16,420[a]	1.366
C18:1n-9	38.154	37.800	37.756	2.324
C18:1n-7	4.051[a]	3.219[b]	2.926[b]	0.926
C18:2n-6	9.253[a]	7.591[b]	7.925[b]	1.622
C18:3n-3	1.452[b]	1.823[a]	1.945[a]	0.480
C20:0	0.144[b]	0.197[a]	0.186[a]	0.068
C20:1	0.647	1.141	0.765	1.538
C20:2n-6	0.230[b]	0.320[a]	0.338[a]	0.053
C20:4n-6	2.720[b]	0.481[a]	0.514[a]	0.583
C20:3n-3	0.179[b]	0.289[a]	0.314[a]	0.067
C20:5n-3	0.404[a]	0.021[b]	0.022[b]	0.099
C22:2n-6	0.005[b]	0.089[a]	0.094[a]	0.026
C22:4n-6	0.341[a]	0.096[b]	0.095[b]	0.077
C22:5n-3	0.637[a]	0.131[b]	0.141[b]	0.171
C22:6n-3	0.086[a]	0.021[b]	0.021[b]	0.025
n-6	12.740[a]	8.850[b]	9.197[b]	2.148
n-3	2.757[a]	2.285[b]	2.443[b]	0.711
n-6/n-3	5.721	5.361	5.054	1.679

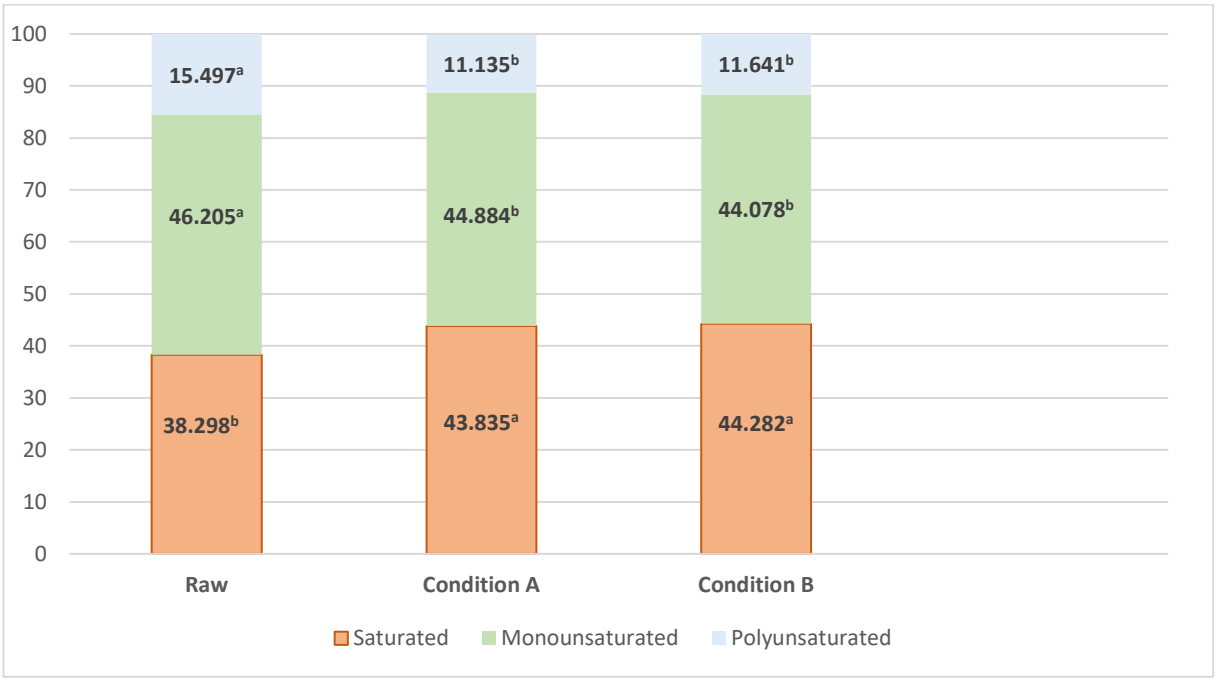
Saturated	38.298[b]	43.835[a]	44.282[a]	2.171
Monounsaturated	46.205[a]	44.884[b]	44.078[b]	2.584
Polyunsaturated	15.497[a]	11.135[b]	11.641[b]	2.732

Condition A: 80°C for 1h; Condition B: 60°C for 15h.
RMSE: Root Mean Square Error
[a,b] P<0.05

In general, independently of the type of cooking A or B, the heat treatment has a significant effect on the fatty acid composition. In fact, except for a few items, the % of almost all fatty acids of cooked samples resulted statistically different from that raw meat, whilst no difference was found between the two cooking conditions.

Notably, the heating process led to an increase in the amount of total saturated fatty acids (38.298% vs 43.835% and 44.282%, respectively raw vs A and B; P<0.05) with a consequent decrease of total monounsaturated (46.205% vs 44.884% and 44.078%, respectively raw vs A and B; P<0.05) and polyunsaturated (15.497% vs 11.135% and 11.641%, respectively raw vs A and B; P<0.05) fatty acids (Figure 26).

Figure 26. Composition (%) of fatty acids of raw and cooked meat with sous vide technique



Condition A: 80°C for 1h; Condition B: 60°C for 15h.

[a,b] P<0.05

As regards the single saturated fatty acids, the most relevant differences were recorded for the palmitic acid (C16:0) (23.676% vs 25.905% and 25.752%, respectively raw vs A and B; P<0.05) and the steric acid (C:18:0) (12.815% vs 15.799% and 16.420%, respectively raw vs A and B; P<0.05).

Among the polyunsaturated fatty acids, the n-6/n-3 ratio is not different between raw and cooked meat, since both the two classes decreased after cooking.

In particular, for the n-6 content the main variations were recorded for the linolenic acid (C18:2n-6), that is the most representative of the category (9.253% vs 7.591% and 7.925%, respectively raw vs A and B; P<0.05),the arachidonic acid (C20:4n-6) (2.720% vs 0.48% and 0.514%, respectively raw vs A and B; P<0.05) and for the docosatetraenoic acid (C22:4n-6) (0.341 vs 0.096; 0.341 vs 0.095, respectively raw vs A and raw vs B; P<0.05). On the other hand, two fatty acids belonging to the n_6 category such as eicosadienoic acid and the docosadienoic acid increased their amount in cooked meat respect to raw meat (Table 12).

Focusing on the total n-3 that has an important role on human health (Simopoulos, 2002), its content resulted lower in cooked samples; in particular, the most affected were the eicosapentaenoic acid (EPA, C20:5n-3) (0.404% vs 0.021% and 0.022%, respectively raw vs A and B; P<0.05) and the docosapentaenoic acid (DPA, C22:5n-3) (0.637% vs 0.131% and 0.141%, respectively raw vs A and B; P<0.05).

The reduction of the total amount of PUFA and of those with longer carbon chain, is probably because of heat that favors the oxidation process. In fact, from a kinetic point of view, the oxidation reaction occurs more easily in the presence of PUFA than MUFA or SFA.

5.2.2 The effect of feeding on cooked meat

Table 13 shows the effect of feeding on different physicochemical parameters of cooked meat.

Table 13. The effect of feeding on the physicochemical parameters of cooked meat

	C	L	LE	LVE	RMSE
Cooking loss (%)	17.78	17.51	16.50	16.58	2.74
WBSF (kg)	4.27	4.05	4.12	4.00	1.14
MDA (mg/kg meat)	0.653	0.752	0.542	0.715	0.329
L*	63.54	64.53	62.39	62.88	6.06
a*	3.96	3.91	4.34	4.13	1.51
b*	16.83^{a}	16.80^{a}	17.13^{ab}	18.29^{b}	1.87

C, control group; L, experimental group with 5% of extruded linseed; LE, experimental group with 5% of extruded linseed, 200 ppm vitamin E and 0.211 ppm of Selenium; LVE, experimental group with 5% of extruded linseed and vegetal extracts from grape skin (3.00 g per kg of feed) and oregano oil (2.00 g per kg of feed).
RMSE: Root Mean Square Error
[a,b] P<0.05

Neither cooking loss nor shear force was affected by dietary treatment, agreeing with Rossi et al. (2013) who found similar results on fresh meat from pig fed with plant extract from Lippia spp. Although cooking loss was lower in the two groups LE and LVE that received the dietary supplementation with the different antioxidants, but the difference was not significant (P>0.05).
Also for the shear force value, no statistical difference was recorded among the groups; the Control group showed a tendentially highest value (P>0.05).

The supplementation with antioxidants (natural or synthetic) seemed not to have detrimental effect on the technological parameters of pork quality.
In LT cooked muscle, no difference was observed among the groups; the L group, where no antioxidants were present showed a tendentially highest value (P>0.05) of malondialdehyde (0.752 mg/kg of meat).
The presence of antioxidants in the diet (natural or synthetic) did not show any effect on preventing the lipid oxidation on cooked samples. This result agrees with Minelli et al. (2020), who did not detect any difference in MDA value of cooked samples of pork fed with linseed and Vitamin E or natural extracts.
It can be noted that all groups had a value of TBARS lower than 1 mg, which is recognized to be the threshold value of the oxidative rancidity in meat products (Akoğlu et al., 2018).

Lightness (L*) and redder (a*) color parameters were not influenced by dietary treatment. The only statistical difference was recorded for the b* parameter among the LVE group against C and L (18.29 vs 16.83 and 16.80, respectively LVE vs C and L; P<0.05).

Data of fatty acid composition of cooked meat was presented in Table 14. Dietary treatment affected the fatty acid composition.

Table 14. The effect of feeding on fatty acid profile of cooked meat

%	C	L	LE	LVE	RMSE
C10:0	0.123	0.122	0.119	0.119	0.017

C12:0	0.088	0.100	0.100	0.094	0.033
C14:0	1.344[a]	1.384[ab]	1.403[ab]	1.488[b]	0.112
C16:0	24.854[a]	24.677[a]	25.208[ab]	25.706[b]	1.224
C16:1	2.881[a]	2.652[b]	2.550[b]	2.535[b]	0.365
C17:0	0.221[ab]	0.217[a]	0.248[b]	0.230[b]	0.045
C17:1	0.245[a]	0.219[b]	0.244[a]	0.233[ab]	0.038
C18:0	14.980	14.933	14.977	15.156	1.366
C18:1n-9	39.994[a]	37.739[b]	37.462[b]	36.417[b]	2.324
C18:1n-7	2.881[a]	2.743[a]	3.937[b]	4.033[b]	0.926
C18:2n-6	8.034	8.678	8.163	8.150	1.622
C18:3n-3	0.620[a]	2.041[b]	2.192[b]	2.108[b]	0.480
C20:0	0.182	0.184	0.155	0.182	0.068
C20:1	0.739	1.243	0.725	0.699	1.538
C20:2n-6	0.300	0.315	0.284	0.286	0.053
C20:4n-6	1.565[a]	1.337[ab]	0.999[b]	1.052[b]	0.583
C20:3n_3	0.142[a]	0.291[b]	0.307[b]	0.302[b]	0.067
C20:5n-3	0.053[a]	0.214[b]	0.156[b]	0.173[b]	0.099
C22:1	0.004[a]	0.018[b]	0.013[b]	0.016[b]	0.013
C22:2n-6	0.031[a]	0.075[b]	0.067[b]	0.077[b]	0.026
C22:4n-6	0.242[a]	0.162[b]	0.146[b]	0.159[b]	0.077
C22:5n-3	0.202[a]	0.397[b]	0.302[ab]	0.311[b]	0.171
C22:6n-3	0.040	0.043	0.042	0.044	0.025
Saturated	41.792	41.615	42.210	42.936	2.171
Monounsaturated	46.745[a]	44.616[b]	44.930[b]	43.932[b]	2.584
Polyunsaturated	11.463[a]	13.769[b]	12.860[ab]	12.938[ab]	2.732

C, control group; L, experimental group with 5% of extruded linseed; LE, experimental group with 5% of extruded linseed, 200 ppm vitamin E and 0.211 ppm of Selenium; LVE, experimental group with 5% of extruded linseed and vegetal extracts from grape skin (3.00 g per kg of feed) and oregano oil (2.00 g per kg of feed).
RMSE: Root Mean Square Error
[a,b] $P<0.05$

The total saturated fatty acid was not different among the 4 diets group, the stearic acid (C18:0) and the eicosanoic acid (C20:0) were not affected by the dietary treatment ($P>0.05$), but some SFAs showed a statistical difference.

The myristic acid (C14:0) recorded the highest value in the LVE group respect to the C group (1.448% vs 1.344%, respectively; $P<0.05$) and the same happen for the palmitic acid (C16:0) (25.706% vs 24.854%, respectively; $P<0.05$). For the heptadecanoic acid the highest value was recorded by LE group respect to L group (0.248% vs 0.217%, respectively; $P<0.05$).

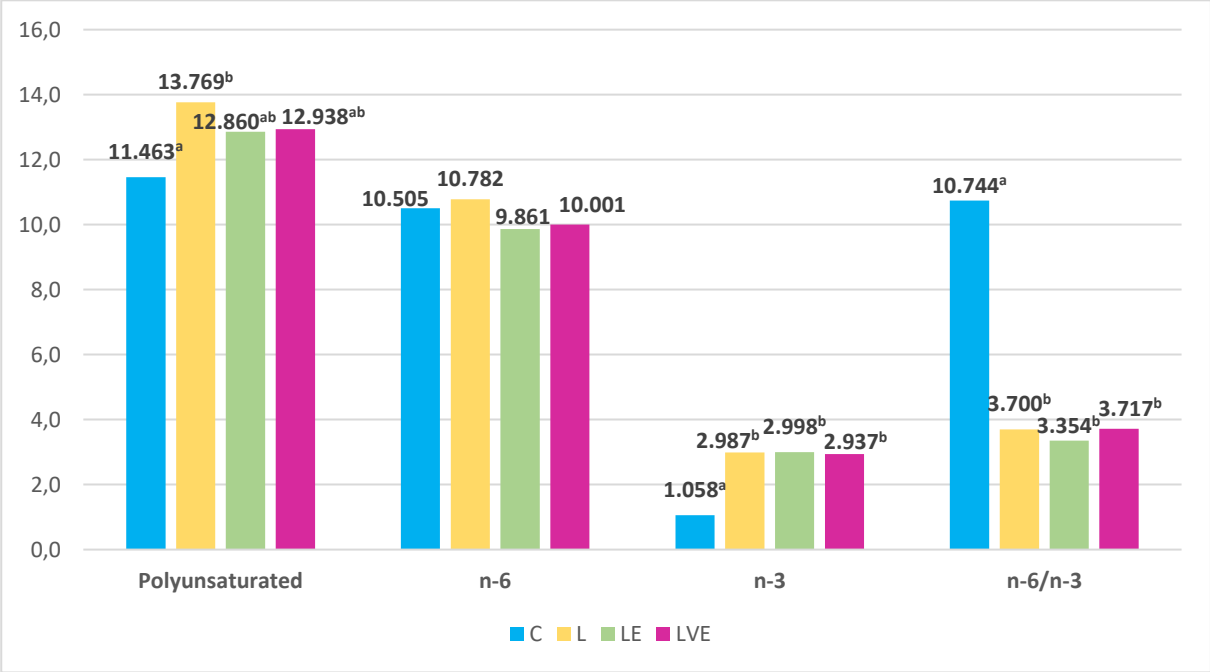
As regards the monounsaturated component, the C group showed the highest value for the total MUFA (46.675% vs 44.616% and 44.930% and 43.932%, respectively; C vs L and LE and LVE; P<0.05). This agrees with the study of Guillevic et al. (2009), who found in cooked chops, smoked belly, and sausages from pig fed with linseed lower value of MUFA whit respect to control.

The most representative for MUFA, the oleic acid (C18:1n-9), was higher in control group respect to all the groups with the linseed (39.994% vs 37.739% and 37.462% and 36.417%, respectively C vs L and LE and LVE; P<0.05), the same trend was recorded for the palmitoleic acid (C16:1, P<0.05).

The eicosenoic acid (C20:1) content was the only one not affected by the dietary treatments, in agreement with the study Enser et al. (2000), on the composition of fatty acid of fresh meat from pig fed with linseed.

Also the PUFA content, and some single PUFAs, were affected by the dietary treatment (P<0.05), as it shown in Table 14.

Figure 27. The polyunsaturated, n-6, and n-3 fatty acids composition (%), and the n-6/n-3 ratio of cooked Longissimus thoracis of pigs fed with 4 experimental diets



C, control group; L, experimental group with 5% of extruded linseed; LE, experimental group with 5% of extruded linseed, 200 ppm vitamin E and 0.211 ppm of Selenium; LVE, experimental group with 5% of extruded linseed and vegetal extracts from grape skin (3.00 g per kg of feed) and oregano oil (2.00 g per kg of feed).
[a,b] P<0.05

Primarily, the total polyunsaturated component is statistically different between the control group (C) and linseed group (L) (11.463 vs 13.769, C vs L, P<0.05) in agree with Guillevic et al. (2009).

In general, the animal diet, for monogastric animals, is a powerful tool that can be used to modify and improve the quality of the meat (Nieto & Ros, 2012). The right choice of a rich source of n-3 is crucial to achieving the goal of improving the n-3 ratio without compromising the n-6 portion.
Different studies on fatty acids composition on fresh meat of pig, confirmed how different levels of linseed in animal fed, were able to increase the n-3 ratio without impairing the n-6 content (Matthews et al., 2000; Kouba et al., 2003; Corino et al., 2008; Haak et., 2008; Minelli et al., 2020).
Our study confirmed the power of linseed in animal feeding also on the n-3 content of cooked meat, higher in groups with the presence of linseed compared to the control group (Figure 27).

In particular, the C group showed higher value of arachidonic acid (20:4n-6) and docosatetraenoic acid (C22:4n-6) than groups with linseed ($P<0.05$), in agreement with the study of Enser et al. (2000). The docosadienoic acid (C22:2n_6) was the only one belonging to n-6 group, which recorded higher values in linseed groups than in control.

The total n-3 resulted significantly higher ($P<0.05$) in all groups with the presence of linseed into the diet in comparison with the control one (Figure 27).
In particular, the fatty acids most affected by the diet were the α-linolenic acid (C18:3n-3), that was three times more abundant in the L, LE, and LVE groups than in the C group (2.041%, 2.192%, 2.108% vs 0.620%, respectively; $P>0.05$), the eicosatrienoic (C20:3n-3) was twice times more abundant in linseed groups than in control group ($P<0.005$) and the eicosapentaenoic acid (C20:5n-3) in L group resulted four times more abundant than in the C group (0.053% vs 0.214%, respectively; $P<0.05$).
The docosahexaenoic acid (C22:6n-3, DHA) was the only, belonging to the n-3 group, not affected by the dietary treatment. Other studies found the lack of effect of the implementation of linseed in the animal diet on increasing the C22:6n-3 content (Enser et al., 2000; Hoz et al., 2007; Guillevic et al., 2009). The α-linolenic acid (ALA) and the DHA compete for the same desaturase enzymes group; consequently, high concentration of ALA in the tissue is the cause of inhibition/low activity of the desaturase enzyme complex, which results in a lower incorporation of DHA (Raes et al. 2004; Juàrez et al., 2010; Karolyi et al., 2012)

As a result of the modification in the two classes of fatty acids, the n-6/n-3 ratio of the samples was influenced by the feeding strategies ($P<0.05$): control group had a significantly higher ratio (10.7)

whilst all groups with the presence of linseed in the diets showed values under 4. The n-6/n-3 ratio is generally used as an indicator to evaluate the nutritional value of dietary fat that has relevance for human health, in particular its value less than 4 is related to a positive effect on human health (Weill et al., 2002; Simopoulos, 2008; Valencak et al., 2015). Valencak et al. (2015) studied how the fatty acids composition changes due to the heating process on different types of game meat, which are normally characterized to have a favorable n-6/n-3 ratio. Their study confirmed that the heating process caused a change in the globally fatty composition but the favorable ratio n-6/n-3 did not result influenced by it.

5.3 Conclusion

Our study gives information about the effect of *sous vide* cooking on the physicochemical characteristics and fatty acids profile of pork, and information about the effect of cooking on sample of meat enriched in PUFA n-3, with particular attention of fatty acid profile.

The study confirms that the combination of a long time and low temperature of cooking for pork allowed to obtain final product tender without worsening the lipid oxidation and other physicochemical parameters. In general the cooking process is the main causes in the variations in fatty acids profile. The SFA component resulted higher respect to other.

In general, the use of linseed in animal feeding is effective in improve the meat quality, in terms of more balance fatty acid profile, with a significant increase of n-3 rate. The ratio n-6/n-3 in samples meat from animal feeding with linseed has value that is considerate adequate for human nutrition and health.

Although the cooking process causing the modification on lipids composition, does not deteriorate the meat enriched in n-3.

Highlights however, that due to the lack of samples, it was not possible to select many time and temperature combinations to be evaluated, causing a lack of direct comparisons, in order to obtain more information on the effects of cooking time and temperature on meat characteristics.

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CHAPTER 6.

2ND Experiment

The aim of the study

The aim of this research was to evaluate the effect of twelve combinations of four cooking time (60 min, 90 min, 120 min, and 150 min) and three temperature (60°C; 70°C, and 80°C) of *sous vide* pork on color, cooking loss, shear force, and lipid oxidation of cooked meat.
In addition, the effect of ultrasonication as a pre-treatment before the cooking process, was studied to evaluate the possible effects on physicochemical parameters of cooked pork.

6.1 Ultrasound

The ultrasounds are defined as acoustic waves that have a specific frequency, in general, greater than 20 kHz, and the ultrasound technique is largely used in food industries (Butz & Tauscher, 2002).
In food manufacturing the ultrasound is mainly used for: peeling, preservation (with the microbial inactivation), or mixing, but the application of ultrasound can also be used in couple with other classic technologies of preservation like sterilization or pasteurization to reduce the time of the working process and improve the efficiency of treatment (Mason et al., 1996; Demirdörven & Baysal, 2008).

Depending on the frequency, two categories of ultrasound are recognized:

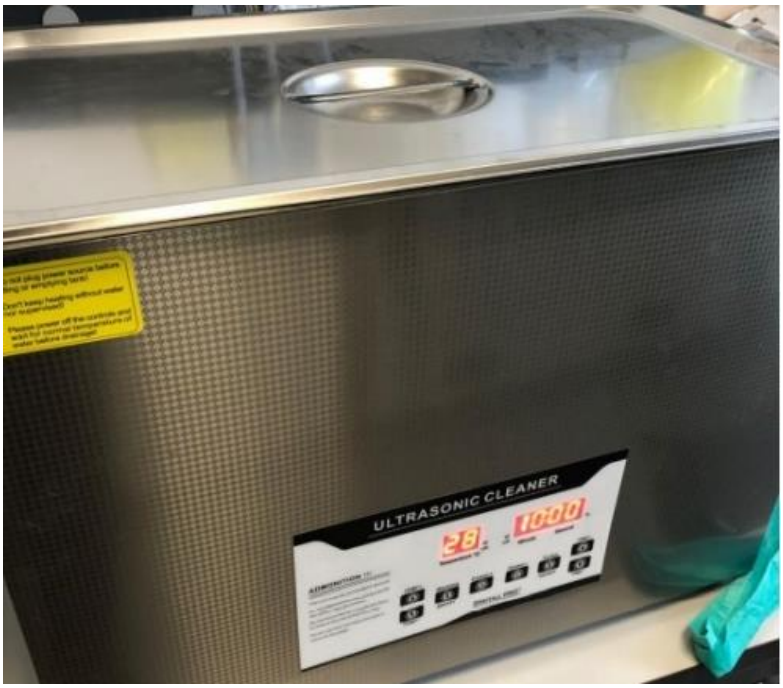
- low-intensity ultrasound at higher frequency (>100 kHz), this type of ultrasound provides information about physical and chemical characteristics of foods, without altering the product;
- higher-power ultrasound at a lower frequency (<100 kHz), is typically used to inactivate microorganisms, thanks to the cavitation process (Piyasena et al., 2003), create emulsions, inhibit enzymes, and tenderize meat (McClements, 1995; Butz &Tauscher, 2002).

6.1.1 Mechanism of functioning

The ultrasound mechanism, used in the food industry with the application of higher-power ultrasound, is based on the cavitation process, whose primary effect is the inactivation of microbes.

Figure 28 shows the ultrasonic cleaner, used in our experimentations. The ultrasound machine consists of a tank containing water inside, where are placed the samples that have to be treated. Through the external screen, there is the possibility to select the power, the temperature, and the duration of ultrasound treatment. More details are provided in section 6.2.1

Figure 28. Ultrasonic cleaner used in the 2nd experiment



In general, the ultrasonic waves, in liquid media, are responsible to generate gas bubbles, which represent the base of the cavitation. The cycle of the cavitation is formed by two different parts (negative and positive cycles) that are repeated for the entire treatment. Because of the negative cycle, the liquid media is subjected to a tensile force, on the contrary during the positive one, the liquid undergoes compression. This alternance causes the formation of microbubbles, which increase their size thousands of times during the process. Subsequently, the gas bubbles, which have reached the critical size, collide with each other and release the energy that they had accumulated, and where this happens, we assist to an instantaneous local increase of temperature which is dissipated without raising the temperature of the treated food (Zinoviadou et al., 2015). The combined effect of the releasing of energy and mechanical shock due to the outbreak of bubble are the main reasons of the structure modifications and inactivation of microorganism.

Remembering that tenderness is one of the most important qualities for consumers, different studies confirmed the effectiveness of the application of ultrasound treatment to improve the tenderness of final products, for beef (Jayasooriya et al., 2007; Stadnik & Dolatowski, 2011; Peña-Gonzalez, et al., 2019).

In this experiment the main idea was to evaluate the possibility to add a pre-higher-power ultrasound treatment before cooking the pork with *sous vide* technique, in order to improve the final tenderness

of meat and evaluate if the ultrasound treatment was responsible to modify other important characteristics of meat such as color or cooking loss.

6.2 Materials and Methods

6.2.1 Preparation of samples

Longissimus thoracis muscle of pig was bought at the local market (Reggio Emilia) supplied by the same producer within 24 h *post mortem* and transported to the Department of Life Sciences, University of Modena and Reggio Emilia, Italy, using a thermocol box filled with ice and used immediately. The combinations of time and temperature of cooking evaluated were twelve (Table 15).

Twenty-four samples, with a weight of 152.3 ± 2.8 g and a thickness of 2.3 ± 0.2 cm, were obtained and randomly designated to the different combination of time and temperature of cooking. Before the cooking process, the samples were vacuum-sealed in a food-grade nylon-polyethylene plastic pouches (150 x 200 mm^2) with a wide thermal stability (-40°C - +120°C) and O_2 permeability of 9 cm^3/day m^2 (4°C/80% relative humidity), and water vapor permeability of 1.2 g/day m^2 (Joeplas SL, Barcelona, Spain) using a vacuum sealer (Elegen, Reggio Emilia, Italy) before cooking and ultrasound process. The vacuum degree of sample was 98%. The samples were randomly assigned into the 12 groups.

Table 15. Combination of time and temperature of *sous vide* cooking, experiment 2

Name of sample	Time of cooking (min)	Temperature of cooking (°C)	Vacuum degree (98%)
LT1	60	60	98%
LT2	90	60	98%
LT3	120	60	98%
LT4	150	60	98%
MT1	60	70	98%
MT2	90	70	98%
MT3	120	70	98%
MT4	150	70	98%
HT1	60	80	98%
HT2	90	80	98%

HT3	120	80	98%
HT4	150	80	98%

Subsequently, 12 of the total 24 samples, were treated before cooking with ultrasound using the Ultrasonic Cleaner, supplied by Elegen (Reggio Emilia, Italy) (Table 16). The working condition of the ultrasound machine was: 10 minutes at 40 kHz at 25 °C of the water bath.

Table 16. Combination of time and temperature of *sous vide* cooking with the ultrasound application as pretreatment, experiment 2

	Name of sample	Time of cooking (min)	Temperature of cooking (°C)	Vacuum degree (%)
Pretreatment with Higher-power ultrasound (10 minutes at 40 kHz)	LT1U	60	60	98%
	LT2U	90	60	98%
	LT3U	120	60	98%
	LT4U	150	60	98%
	MT1U	60	70	98%
	MT2U	90	70	98%
	MT3U	120	70	98%
	MT4U	150	70	98%
	HT1U	60	80	98%
	HT2U	90	80	98%
	HT3U	120	80	98%
	HT4U	150	80	98%

At the end of the ultrasound treatment, all the samples were cooked using the Sous Cooker Professional (Elegen, Reggio Emilia, Italy). Overall, a total of 144 samples were analyzed (24 samples x 2 repeats x 3 independent replicate).

After cooking, the samples were cooled for 1 hour in ice bath and stored at 4°C for 24 hours before the analysis.

Cooking loss, color, shear force and lipid oxidation were evaluated following the same methodologies detailed in section 5.1

6.2.2 Statistical analysis

The statistical analysis was performed using the analysis of variance with SAS (SAS Institute Inc., Cary, NC, USA). The statistical model included the ultrasound treatment, temperature and time of cooking as fixed effects. The interactions among the fixed effects were considered but they were never statistically significant, therefore they were removed from the statistical model.

6.3 Results and Discussions

Table 17 and Table 18 show the effect of temperature and time of *sous vide* cooking on physicochemical parameters of *Longissimus thoracis* muscle.

Table 17. The effect of temperature of cooking (60°C, 70°C, and 80°C) on physicochemical parameter of *Longissimus thoracis* muscle cooked with *sous vide* technique

Temperature	60 °C (LT)	70°C (MT)	80°C (HT)	RMSE
L*	68.36	67.73	69.04	2.74
a*	4.50^{a}	4.03^{ab}	3.61^{b}	0.87
b*	14.72^{c}	16.77^{b}	17.58^{a}	1.13
WBSF (kg)	3.96^{c}	4.30^{b}	5.07^{a}	0.46
Cooking Loss (%)	14.27^{c}	23.42^{b}	29.83^{a}	2.16
MDA (mg/kg)	1.13^{c}	3.42^{a}	3.06^{b}	0.46

^{a,b} P<0.05
RMSE: root mean square error
WBSF: Warner-Bratzler Shear Force
MDA: malondialdehyde

The temperature of cooking resulted significant for all the physicochemical parameters evaluated, except for L* (lightness, P>0.05) (Table 17), while the time of cooking affected only cooking loss (P<0.05) (Table 18).

Table 18. The effect of time of cooking (60 min, 90 min, 120 min, and 150 min) on physicochemical parameters of *Longissimus thoracis* muscle cooked with *sous vide* technique

Time	60 min (1)	90 min (2)	120 min (3)	150 min (4)	RMSE
L*	69.27	68.53	67.68	68.02	2.74
a*	4.07	4.07	4.13	3.91	0.87
b*	15.97	16.48	16.67	16.30	1.13
WBSF (kg)	4.42	4.54	4.39	4.41	0.46
Cooking Loss (%)	20.37^{b}	22.53^{a}	23.25^{a}	23.88^{a}	2.16

MDA (mg/kg)	2.45	2.51	2.57	2.62	0.46

[a,b] P<0.05
RMSE: Root Mean Square Error
WBSF: Warner-Bratzler Shear Force
MDA: malondialdehyde

Cooking loss (%), resulted influenced by both time and temperature of cooking (Table 17 and Table 18). A significant increase of the loss of liquids occurred with the increase in cooking temperature (14.27 % vs 23.42% vs 29.83%, respectively 60°C vs 70°C vs 80°C; P<0.05). Our results agree with Sànchez del Pulgar et al. (2012) found on pork cheeks, and Vaudagna et al. (2002) and Garcià-Segovia et al. (2007) found on different muscles of beef.

Van der Sman (2007) explained using the Flory-Rehner theory performing the cooking process on a rectangular of piece of beef, how the temperature can affect the cooking loss. In summary, the increase of cooking temperature causes as the primary effect, the denaturation of proteins, with a consequent shrinkage of myofibrillar proteins, which are responsible to hold the main quantity of water in muscle. Primarily, up to 60°C the shrinkage is transverse, and secondly, up to 90°C, the shrinkage is longitudinal, with the main loss of water; thus, the loss of liquids increases with the increasing of temperature

The values of cooking loss were statistically different also depending on the time of cooking (P<0.05) (Table 18). In particular, the shorter cooking time produced the lower value of liquid losses (20.37% vs 22.53%, respectively 60 min vs 90 min; P<0.05). Probably, the longer is the cooking time and greater is the portion of proteins that undergoes the structural changes explained before.

The redness (a*) and the yellowness (b*) were affected by cooking temperature (P<0.05).

Various elements play an important role of determining the color of cooked meat, the content of myoglobin and its redox status are the most important in the determination of a* parameter (King & Whyte, 2006).

In our study, the lower value of the redness parameter (a*) was recorded by the group cooked at the higher temperature (3.61 vs 4.50, respectively 80°C vs 60°C; P<0.05). Probably this result is due to the denaturation process of myoglobin during the cooking, which occurs faster at temperature over 70°C, as confirmed also by several studies on pork, lamb and beef (Vaudagna, et al., 2002; Roldàn et al., 2013; Becker et al., 2016; Dominguez-Hernandez et al., 2018); and the time of cooking resulted not influent on redness.

The change in b* parameter, as it for a*, is affected by the change of oxidation status of the myoglobin structure; the cooking process leads to the denaturation of myoglobin and to the formation of met-myoglobin that is the responsible for the dull brown appearance of cooked meat (Suman & Joseph, 2013).

The yellowness parameter (b*) showed an opposite trend respect to a*, since samples cooked at lower temperature had lower value, and the ones cooked at the highest temperature had the highest value (14.72 vs 17.58, respectively 60°C vs 80°C; $P<0.05$). The same trend for a* and b* was recorded by Garcià-Segovia et al. (2007) on beef samples cooked at 60°C and 80°C for 15 min and 60 min, by Christensen et al. (2011) on pork cooked at 48°C, 53°C, 58°C and 63 °C for 5h and 17h, and by Roldàn et al. (2013) on lamb cooked for at 60°C, 70°C and 80°C for 6h, 12h and 24h.

The tenderness values (WBSF) were significantly affected by the cooking temperature ($P<0.05$), but not by the cooking times. In particular the lowest cooking temperature produced the more tender product, whilst the tenderness dropped with the increase in temperature (3.96 vs 4.30 vs 5.07, respectively 60°C vs 70°C vs 80°C; $P<0.05$). Our results fit with what Baldwin (2012) found in his study; in particular, under 70°C the sarcoplasmic protein tends to aggregate with the formation of a gel, which makes easier the chewing process. On the other hand, when the cooking temperature reach the 80°C, the tensile module of muscle fibers change, it increases the elastic one, and consequently, the meat resulted tougher.

The temperature of cooking affected also the lipid oxidation (Table 17), specifically only the cooking temperature of 60°C recorded value of malondialdehyde slightly higher than 1 mg/kg, recognized as the threshold of perception by the consumers of the lipid oxidation compounds (Akoğlu et al., 2018). The highest value of MDA was recorded by the group cooked at 70°C. Roldan et al. (2014) found higher values of MDA in lamb samples cooked at 70°C and 60°C than at 80°C. In his study the time of cooking affected the lipid oxidation, on the contrary in our study, MDA value shows a not significant tendency to increase with the time of cooking

The ultrasound pre-treatment did not show any effects on the parameters evaluated ($P>0.05$) (Table 19 and Table 20).

Unexpectedly, the ultrasound pre-treatment seemed to have no effect on the determination of tenderness (as shown in Table 19 and Table 20), contrary to what was found by other authors (Yeung

& Huang, 2017; Contreras-Lopez et al., 2020). Yeung & Huang (2017) found that pretreatment with ultrasound for 6 min at 15 kHz on pork loin was able to reduce the shear force value, while Jayasooriya et al. (2007) found that sonication of beef at 24 kHz for 4 min was enough to recorded lower value of shear force on the samples treated without affecting the color parameters and reducing the cooking loss. The absence of the effect of ultrasound in our experiment could be due to choose of not adequate pre-treatment conditions, since is the tenderization process of meat dependently by the time and intensity of sonication process (Alarcon-Rojo et al., 2019).

Hong et al. (2014) found that the addition of brine during the ultrasound treatment (40 kHz) before cooking of pork loin played a positive effect on reduce the shear force value and reduce the cooking loss. On the other hand, Barekat & Soltanizadeh (2017) studied the effect of addition of papain to bull meat treated with ultrasound (20 kHz for 10min, 20min, and 30min), and reported that the lowest shear force was recorded with a treatment of 20 min.

other author found that the addition of brine may play a synergic role in improving the tenderizing process of meat using the ultrasound (Hong et al. 2014; Barekat & Soltanizadeh, 2017).

Table 19. The effect of time of cooking and ultrasound on physicochemical parameters of *Longissimus thoracis* muscle cooked with *sous vide* technique

	Ultrasound				Without ultrasound				RMSE
	60 min (1)	90 min (2)	120 min (3)	150 min (4)	60 min (1)	90 min (2)	120 min (3)	150 min (4)	
L*	69.00	68.85	67.43	68.24	69.54	68.21	67.93	67.80	2.74
a*	4.23	3.96	4.21	3.96	3.91	4.19	4.05	3.87	0.87
b*	16.43	16.56	16.83	16.35	15.52	16.39	16.52	16.25	1.13
WBSF (kg)	4.46	4.58	4.32	4.38	4.37	4.49	4.47	4.45	0.46
Cooking loss (%)	20.18^{d}	21.87^{cd}	23.40^{bc}	24.08^{ab}	20.57^{b}	23.19^{a}	23.09^{a}	23.67^{a}	2.16
MDA (mg/kg)	2.40	2.55	2.64	2.64	2.50	2.47	2.49	2.61	0.46

^{a,b,c,d} P<0.05 within ultrasound treatment
RMSE: root mean square error
WBSF. Warner-Bratzler Shear Force
MDA: malondialdehyde

Table 20. The effect of temperature of cooking and ultrasound on physicochemical parameters of *Longissimus thoracis* cooked with *sous vide* technique

	Ultrasound			Without ultrasound			RMSE
	60°C (LT)	70°C (MT)	80°C (HT)	60°C (LT)	70°C (MT)	80°C (HT)	

L*	68.05	68.03	69.07	68.67	67.43	69.01	2.74
a*	4.63^{a}	4.00^{ab}	3.63^{b}	4.37^{a}	4.06^{ab}	3.59^{b}	0.87
b*	15.12^{c}	16.78^{b}	17.73^{a}	14.32^{b}	16.76^{a}	17.43^{a}	1.13
WBSF (kg)	3.90^{b}	4.24^{b}	5.17^{a}	4.01^{c}	4.35^{bc}	4.97^{a}	0.46
Cooking loss (%)	14.71^{c}	23.03^{b}	29.42^{a}	13.83^{c}	23.81^{b}	30.24^{a}	2.16
MDA (mg/kg)	1.10^{c}	3.55^{a}	3.02^{b}	1.16^{b}	3.29^{a}	3.10^{a}	0.46

^{a,b,c,d} P<0.05 within ultrasound treatment
RMSE: root mean square error
WBSF. Warner-Bratzler Shear Force
MDA: malondialdehyde

6.4 Conclusion

Sous vide cooking is increasingly attracting the attention of consumers, restaurateurs, and scientists. This technique makes it possible to obtain final products with better chemical and physical characteristics than cooked with traditional techniques, as regards the parameter of tenderness.

In this research we carried out the study of 12 different combinations of *sous vide* cooking on samples of *Longissimus thoracis* muscle of pig, and with a pretreatment with low-frequency ultrasound, to which several authors attribute an improvement on the shear force value.

The temperature of cooking played a significant role more than the time of cooking on determining effects on the main psychochemical parameters evaluated. Cooking loss was the only parameter affected by both, time, and temperature

In our study, 60°C for 60 min was the best combination of temperature and time of cooking in terms of tenderness, color, and lipid oxidation values of the final products, and confirmed how important is the choice of the right combination of time and temperature of cooking to obtain final products which better can answer to the request of the consumer. The first and the second experiments confirmed how the low temperature of cooking was able to obtain the final product with the best physicochemical characteristics.

The application of ultrasound is widely used in the food industry for known effect on bacterial inactivation and emulsification.

In this research, the ultrasound did not show any statistical differences on the parameters evaluated. This result was probably due to the non-adequate sonication conditions chosen in terms of power or time applicated.

6.5 References

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CHAPTER 7

3RD Experiment

The aim of the study

The aim of the 3rd study was the evaluation of the effects of 12 combinations of cooking time (60 min, 90 min, 120 min, and 150 min) and temperature (60°C, 70°C, and 80°C) on quality characteristics such as moisture content, cooking loss, shear force, lipid oxidation, pH, color, and microbial safety of *sous vide* on chicken breast.

It was also studied the effect of the *sous vide* temperature and time combination on the shelf life (21 days at 4°C) in terms of contamination from total mesophilic aerobic bacteria, *Enterobacteriaceae* bacteria and *Psychrotrophic* bacteria.

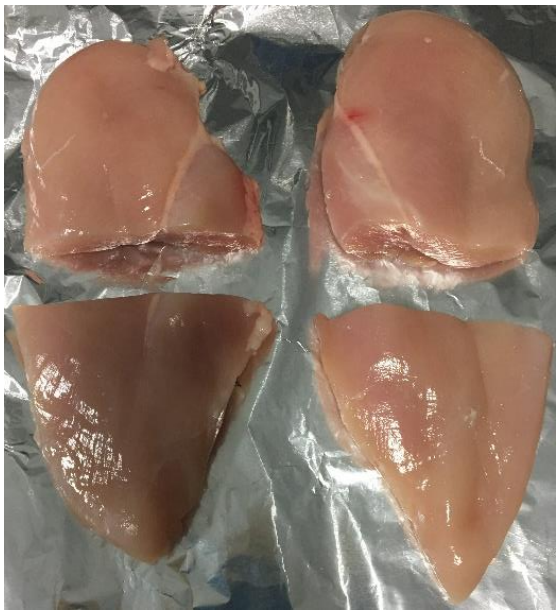
Finally, the microstructure analysis was carried out to evaluate the difference among the protein structures of the cooked samples at different *sous vide* combinations of time and temperature.

7.1 Materials and Methods

7.1.1 Preparation of samples

Samples of boneless raw chicken breast, purchased from the local market (Reggio Emilia, Italy) supplied by the same producer within 24 h *post mortem*, were used for the experiment. The chicken breasts were transported to the Department of Life Sciences, University of Modena and Reggio Emilia, Italy using a thermocol box filled with ice and used immediately. The chicken breasts, without the surface fat, were cut (Figure 29), weighted (125 ± 5 g and thickness 2.5 ± 0.2 cm), and vacuum-sealed in a food-grade nylon-polyethylene plastic pouches (150 x 200 mm^2) with thermal stability (-40°C – +120°C) and O_2 permeability of 9 cm^3/day m^2 (4°C/80% relative humidity), and water vapor permeability of 1.2 g/day m^2 (Joelplas SL, Barcelona, Spain) using a vacuum sealer (Elegen, Reggio Emilia, Italy). The vacuum degree in the sample was 98%.

Figure 29. The division of chicken breast into samples for sous vide cooking process



As it shows in Table 21, twelve different combinations of 3 temperatures (60°C, 70°C; 80°C) and 4 times (60 min, 90 min, 120 min, 150 min) were evaluated. In addition, one sample of chicken breast sealed in plastic pouches without vacuum (0% of vacuum degree), was evaluated as a control group. The condition of cooking for the control group was 100°C for 60 min. Samples were randomly assigned into the 13 groups.

Table 21. Temperature, time, and vacuum conditions applied in the study

Group	Temperature (°C)	Time (min)	Vacuum Degree (%)
Control	100	60	0
1	60	60	98
2	60	90	98
3	60	120	98
4	60	150	98
5	70	60	98
6	70	90	98
7	70	120	98
8	70	150	98
9	80	60	98
10	80	90	98
11	80	120	98
12	80	150	98

After the packaging process, the samples were cooked in a *sous vide* cooker (Elegen, Reggio Emilia, Italy). Three independent replicate trials with two repeats founded on different combinations of temperature (60°C, 70°C, and 80°C) and times (60 min, 90 min, 120 min, and 150 min) were analyzed. A total of 78 samples of chicken breast were examined (13 combination of time and temperature of cooking x 3 independent replicate x 2 repeats).

At the end of cooking process, the samples were cooled in an ice bath for 1 hour and stored overnight at 2°C before analysis. Moisture content, cooking loss, pH, color, lipid oxidation, and shear force were evaluated 24 hours after cooking process.

A different set of samples was used for the shelf life analysis. After cooking and cooling in ice bath, the samples were stored at 4°C for 21 days. At 0, 5, 10, 15, and 21 days the microbiological controls were made.

Table 22 shows the moisture content, lipid oxidation (expressed as mg of malondialdehyde per kg of meat), color parameters (L*, a*, b*) pH values of the raw chicken breast fillet 24 *post mortem*.

Table 22. Physicochemical characteristics of raw chicken breast 24 h *post mortem.*

Physicochemical parameter	Results
Moisture (%)	72.4 ± 1.02
MDA (mg/kg)	0.08 ± 0.011
Weight (g)	125 ± 5
L*	58.4 ± 1.7
a*	0.8 ± 0.1
b*	9.1 ± 0.9
pH	5.8 ± 0.03

Cooking loss, color, shear force and lipid oxidation were evaluated following the same methodologies detailed in section 5.1

7.1.2 Moisture content

The moisture content, for raw meat and cooked samples, was determined according to AOAC 950.46 (AOAC, 2005). Briefly, 5 g of treated sample were drying in an oven at 105°C for 6 hours. The moisture content (%) was calculated using the formula:

$$(Mi - Md)/Mi * 100$$

Where Mi = weight of wet meat

And Md = weight of the dried meat

7.1.3 pH

The pH value was measured before and after cooking process, according to AOAC 981.12 method (AOAC, 2005). A pH meter equipped with a Xerolite electrode (Crison Instrument, Allela, Spain) was used, after calibration. Three different measurements for each sample were recorded, and the result was expressed as a mean.

7.1.4 Microbiological analysis

The microbiological controls were made at 5 different times (0, 5, 10, 15, and 21 days). During storage period of 21 days the samples were kept at 4°C. For each day of analysis, ten grams of cooked chicken breast was added to 90 mL of sterile saline solution (0.9% NaCl) and homogenized for 2 min in a stomacher (Lab blenders Stomacher 400, Instrument Lab Control, Reggio Emilia, Italy).

After appropriate dilutions in saline solution, 1 mL of sample was plated onto the culture media. The evaluation of total mesophilic aerobic bacteria was made using the Plate Count Agar (Biolife, Milan, Italy), incubated in aerobic condition for 48 h at 30°C according to ISO 4833-1:2013; and with the same media, but incubated for 10 days at 4°C, the evaluation of the total *Psychrotrophic* bacteria was made (according to ISO 17410:2019). Using the Violet Red Bile Glucose Agar, (Biolife, Milan, Italy) incubated at 37°C for 24 h, the *Enterobacteriaceae* were counted (agreeing with ISO 21528-1:2017). The average of three measurements was recorded.

7.1.5 Microstructure analysis

After the cooking process a piece of chicken breast (5*5*2 mm^3) for each combination of time and temperature was freeze dried for 24 h before the measure of the microstructure. Before the reading at the microscope, according to Jeong et al. (2018), the samples were mounted over the stubs with a double-side conductive tape and a thin layer of gold for 120 s (25 mA and 20 Pa). The coated over the samples was made using an automated gold sputter coater (Emitech K550, Quorum Technologies

Ltd, UK). Using a scanning electron microscope (Nova NanoSEM^{TM} 450, FEI, USA), working in a low vacuum (80KPa) and at an acceleration voltage of 15 KV, the microstructure of the samples was evaluated.

7.1.6 Statistical analysis

The data were analyzed using a two-way analysis of variance (ANOVA). The differences between means were compared by Tukey's post hoc test ($P<0.05$). In addition, a principal component analysis (PCA) was performed to establish the variations and relationship among physicochemical properties of *sous vide* chicken breast fillets cooked at twelve different combinations of temperature and time. All the analyses were performed by SPSS software (IBM SPSS 20, New York, USA).

7.2 Result and Discussion

7.2.1 Moisture content, cooking loss and pH

Table 23 shows the values of moisture content, cooking loss, WBSF, MDA, color, and pH of the *sous vide* chicken breast fillets cooked at 12 different temperature and time combinations.

The moisture content (%) recorded was between 68.25 % and 71.89 % (100°C *60 min *vs* 60°C*150 min, respectively; $P<0.05$), and both time and temperature, and their interaction were statistically significant. As expected, we assisted to a reduction of moisture content by increasing the temperature of cooking, from 60°C to 80°C, the lowest value was recorded by the control sample (cooked at 100°C*60 min).On the other hand, increasing the cooking time from 60 min to 150 min at higher temperature (70°C and 80°C) caused a reduction in moisture content (70.43 % *vs* 69.02 %, respectively 80°C* 60 min *vs* 80°C *150 min; $P<0.05$). The liquid lost during cooking, is not composed only by water but also by other elements such as fat, soluble vitamins, and soluble proteins. As confirmed by several authors (Murphy & Marks, 2000; Tornberg, 2005; Dominguez-Hernandez et al., 2018) the releasing of the sarcoplasmic fluid from muscle fibers due to the cooking process, reduces the water content of meat; the releasing of liquids is more pronounced with the increasing of cooking temperature. In addition, changes due to the denaturation of myosin and actin at a higher temperature, cause a change in the ability to retain water by protein structure, which directly affects

the moisture content of the final (Murphy et al., 2001). Our results fit whit what Sànchez del Pulgar (2012) found in pork cheeks and Ismail et al., (2019) found in beef.

Table 23. The physicochemical parameters of *sous vide* chicken breast fillets cooked with 12 different time and temperature combinations and the control (100°C *60 min without vacuum)

Temp (°C)	60				70				80				100	SEM	Temp	Time	Temp * Time
Time (min)	60	90	120	150	60	90	120	150	60	90	120	150	60		(°C)	min	
Moisture (%)	71.41^{fg}	71.30^{efg}	71.72^{fg}	71.89^{g}	71.71^{fg}	70.86^{efg}	69.97^{cde}	70.46^{def}	70.43^{def}	69.76^{bcd}	69.47^{abc}	69.02^{ab}	68.25^{a}	0.21	*	*	*
Cooking loss (%)	10.23^{a}	11.02^{a}	12.42^{ab}	12.47^{ab}	14.01^{abc}	16.88^{bcd}	18.38^{cde}	18.69^{cde}	17.86^{cde}	21.77^{def}	22.77^{ef}	24.23^{fg}	28.08^{g}	3.11	*	*	N.S.
WBSF (kg)	0.75^{a}	0.83^{ab}	0.76^{a}	0.62^{a}	0.66^{a}	0.73^{a}	0.62^{a}	0.63^{a}	0.88^{ab}	0.97^{b}	0.79^{a}	0.88^{ab}	1.37^{c}	0.02	*	N.S.	N.S.
MDA (mg/kg)	0.29^{a}	0.77^{ab}	0.92^{ab}	0.94^{ab}	1.50^{b}	1.47^{b}	1.63^{b}	1.71^{b}	2.31^{c}	2.42^{c}	2.54^{c}	2.60^{c}	2.91^{d}	0.12	*	N.S.	N.S.
L*	80.94	81.71	80.11	79.63	80.82	81.72	82.27	82.43	81.39	81.19	80.85	81.15	80.75	2.65	N.S.	N.S.	N.S.
a*	1.95^{a}	1.95^{a}	1.81^{a}	1.71^{a}	1.73^{a}	1.71^{a}	1.74^{a}	1.50^{ab}	1.44^{b}	1.39^{b}	1.33^{b}	1.29^{b}	1.29^{b}	0.05	*	*	N.S.
b*	14.71	14.65	14.95	15.15	14.91	14.83	14.95	15.40	15.64	15.55	15.60	15.36	14.82	0.41	N.S.	N.S.	N.S.
pH	6.17^{abc}	6.14^{a}	6.07^{a}	6.08^{a}	6.11^{a}	6.14^{ab}	6.07^{a}	6.13^{a}	6.15^{ab}	6.25^{bc}	6.30^{d}	6.27^{cd}	6.17^{abc}	0.93	*	N.S.	*

^{a,b,c}: P<0.05

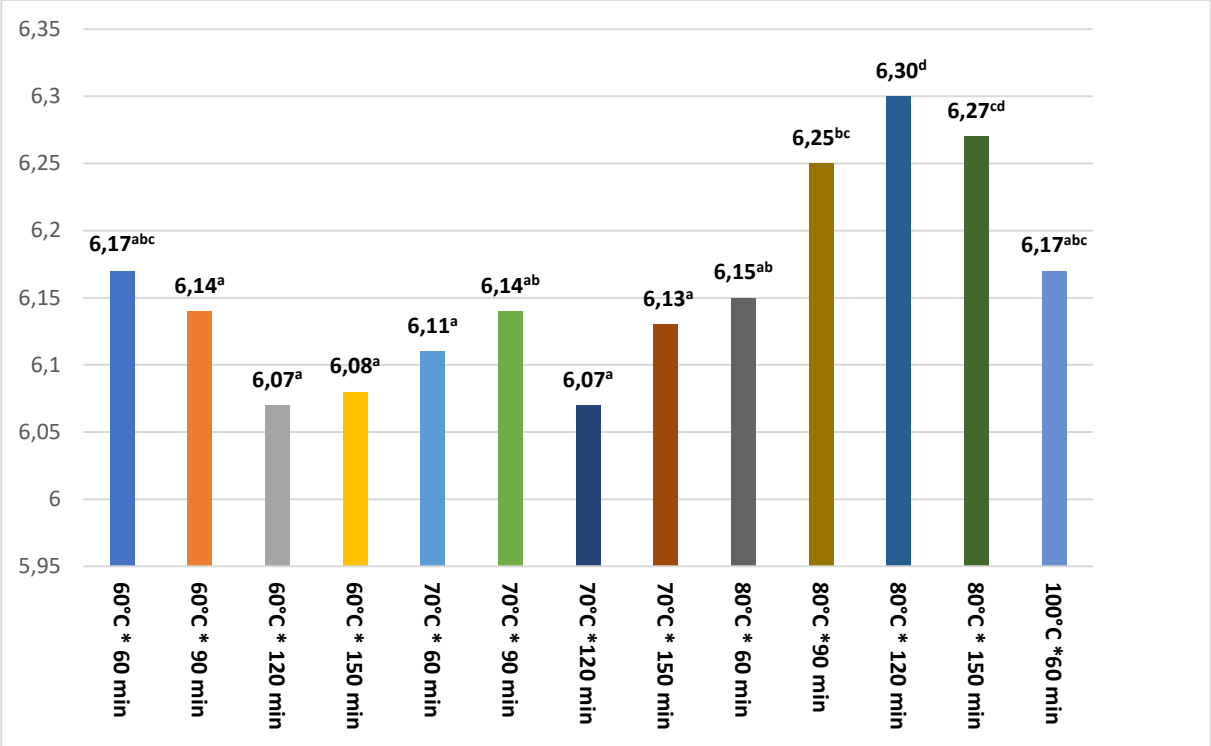
N.S.: not significant, *: P<0.05

SEM: Standard Error of the Mean

Cooking loss is considered as the total liquid and soluble components that are lost from the meat during the cooking (Aaslying et al., 2003). In general, it is considered by the consumer an important technological parameter because it is related to the juiciness of the product (Gòmez et al., 2019), and high value of cooking loss is related to a general less eating quality (Aaslying et al., 2003). In our study the cooking loss is affected by both, time and temperature, and the highest value was recorded by the control sample (28.08 % vs 10.23 % respectively 100°C*60min vs 60°C*60 min; $P<0.05$). We assisted to an increase of the cooking loss value due to increasing temperature and time. The cooking loss is mainly established by the shrinkage of myofibrillar proteins, and collagen which occurs at temperature of 40°C-60°C for the first, and 60°C-70°C for the second, and by the denaturation of actin which occurs between 70°C and 80°C (Tornberg, 2005; Purslow et al., 2016). Thus, increasing the temperature results in increasing the cooking loss ($P<0.05$) Our result confirms what other previous studies found on pork, lamb, beef, and chicken cooked with *sous vide* technique (Sànchez del Pulgar et al., 2012; Roldàn et al., 2013; Naavena et al., 2017; Gòmez et al., 2019; Ismail et al., 2019).

Figure 30 shows the pH values of chicken breast fillets cooked with the *sous vide* technique at different combinations of temperature and time of cooking. In the study the pH value of raw meat was 5.8 (Table 22) and the pH values of cooked samples increased and ranged from 6.07 to 6.3 (60°C*120 min and 70°C*120 min vs 80°C*120 min, respectively; $P<0.05$), and were significantly affected by temperature and the interaction between time and temperature (Table 23).
We assisted to an increase of pH value when the cooking temperature increased from 60°C to 80°C. Becker et al. (2016) reported that increasing temperatures caused an increase in pH value mainly due to the protein denaturation and the change in protein charge.

Figure 30. pH values of sous vide chicken breast fillets cooked at different time and temperature combinations



7.2.2 Color

The lightness (L*) value of raw chicken breast fillet at 24 h *post mortem* was 58.4, the redness (a*) value was 0.8 and the yellowness (b*) was 9.1 (Table 22).

The color of cooked meat has an important impact in determining the degree of attraction and appearance for the consumer, that therefore finds in the evaluation of the final color one of the few instruments that can be used for the determination of the doneness degree of the meat and the final acceptability of the product (Dominguez-Hernandez et al. 2018; Gluchowski et al., 2019).

Different elements play an important role in the determination of meat color, for example, the myoglobin content (which mainly affects the a* parameter) and oxidative state of myoglobin, the orientation of muscle fiber, the space between the muscle fibers (which mainly affects the L* parameter) packaging conditions, Maillard reaction, and pH value (Seideman et al., 1983; Cobos et al., 2014; Wideman et al., 2016).

The values of color parameters of chicken breast fillets *sous vide* cooked with 12 different combinations of temperature and time are showed in Table 23. The L* and b* coordinates were not affected either by cooking temperature, time, and their interactions (P>0.05). This fit with the study

reported by Can & Harun (2015) about chicken meat balls cooked with *sous vide*. On the contrary, Sànchez del Pulgar et al. (2012), reported that *sous vide* pork cheeks cooked at 60°C showed higher value of L* compared to those cooked at 80°C. Based on the table defined by Da Silva-Buzanello et al. (2019), to determine the appearance of the cooked chicken breast based on L* value; where L* <46 dark, normal for 46 < L* < 53 and pale when L* > 53; in our experiments all the samples showed a pale appearance independently on time and temperature (P>0.05).

In this experiment, the a* values ranged from 1.29 to 1.95 (100°C*60 min *vs* 60°C*60 min and 60°C*90 min, respectively; P<0.05), and it was affected by time and temperature of cooking (P<0.05). The lowest value of a* was recorded by the control group than those cooked at 60°C and 70°C (P<0.05) The same trend was recorded by Garcià-Segovia (2007) on beef, and Naavena et al. (2017) on chicken sausages. In general, the a* value in cooked meat is conversely linked to the degree of myoglobin denaturation, that occurs quickly when the cooking temperature is higher than 70°C (Sànchez del Pulgar et al. 2012).

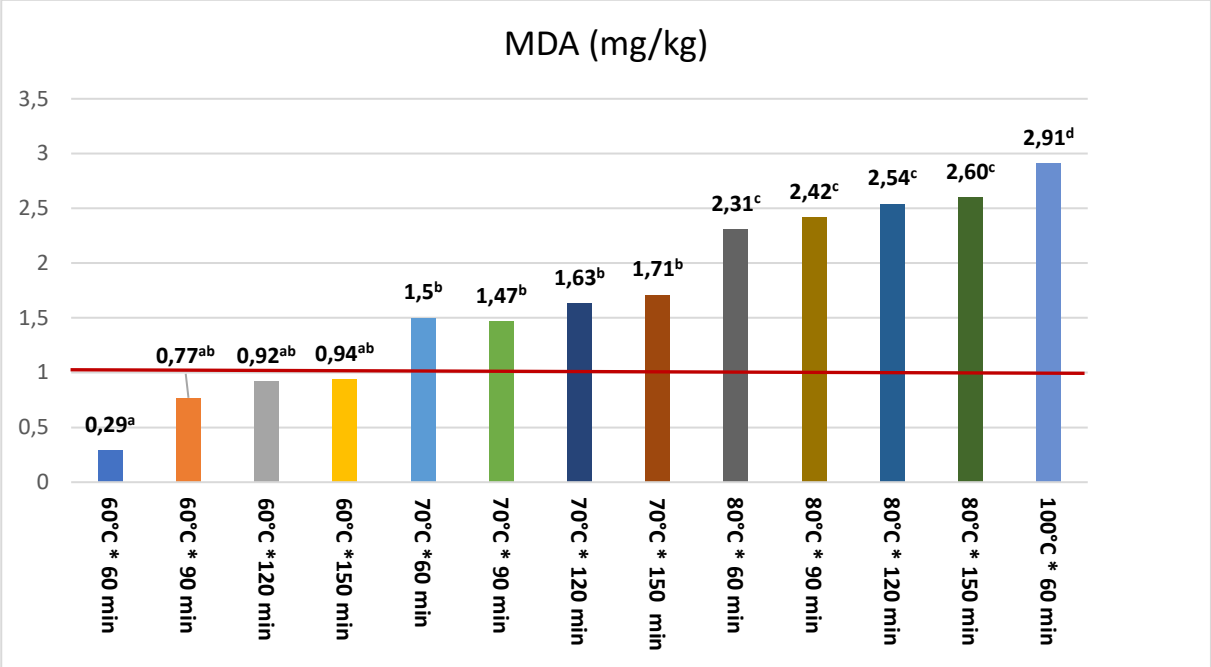
However, for all the temperature and time combinations considered in this study, the a* value was always lower than 3.8, which is considered as a threshold value below which poultry products are considered not well-cooked (Halownia et al., 2003).

In the experiment, the b* value ranged from 14.65 to 15.64, no statistical differences among the samples were recorded (P>0.05). This result was in contrast with Park et al. (2020), who reported that b* value was affected by cooking temperature in *sous vide* chicken samples cooked at different combinations of temperature (60°C and 70°C) and time (60 min, 120 min, and 180 min).

7.2.3 Lipid oxidation

The lipid oxidation products were expressed as mg/kg of malondialdehyde (MDA), the values of cooked samples are shown in Figure 31, and for raw meat was in Table 22. The parameter is affected by cooking temperature (P<0.05, Table 23).

Figure 31. Content of lipid oxidation products, expressed as malondialdehyde mg/kg, of chicken breast fillets cooked at different time and temperature combination of sous vide



In general, the presence of lipid oxidation products (e.g. aldehydes) in meat is the main cause of off-flavors. It exists a threshold value for MDA, equal to 1mg/kg of meat below which is impossible for the consumer to perceive the oxidative rancidity (Akoğlu et al., 2018). In this study, raw chicken breast fillets showed a MDA value of 0.08 mg/kg (Table 22). In the samples cooked at different temperature-time combinations, MDA value ranged from 0.29 to 2.91 ($P<0.05$, Table 23). This parameter was affected by the cooking temperature ($P<0.05$) but not by time. Only samples cooked at 60°C were under the threshold individuated by Akoğlu et al. (2018). We found an increase in the value of MDA according to the increase of cooking temperature ($P<0.05$), and, the control sample showed the highest value recorded 2.91 mg/kg of meat, followed by the values recorded by the group of samples cooked at 80°C and then by those cooked at 70°C. Our result was in contrast with Sànchez del Pulgar et al. (2012), who found on pork cheeks, samples cooked at 80°C for 12 h showed lower value of lipid oxidation products than those cooked at 60° for the same time. In his study, time, temperature, and their interaction affected the MDA content. The reason for this difference could probably be due to the reactive characteristics of the compounds measured by the TBARs test, and the interaction with other elements present in meat such as proteins and amino acids, which give a rise to a variety of different compounds (Draper et al., 1986). On the contrary, Roldan et al. (2014)

found on lamb loins for lipids oxidation the same trend of our study, but in their research not only temperature affected the MDA content, but also the time of cooking and the interaction of both.

7.2.4 Warner-Bratzler Shear Force (WBSF)

The shear force parameter is an important eating quality due to the link with texture and consumer acceptance, and WBSF test is commonly used for the objective evaluating of tenderness (Destefanis et al., 2008).

In our study the WBSF (kg) ranged from 0.62 to 1.37 (Table 23), and was only affected by cooking temperature; it was increase by increasing temperature ($P<0.05$). As expected and confirmed by other studies (Murphy & Marks, 2000; Park et al., 2020), due to higher moisture content and lower cooking loss, the lowest values of shear force were recorded by samples cooked *sous vide* at lower temperatures (60°C and 70°C). On the contrary, control sample, cooked at 100°C, and characterized by the highest value of cooking loss, recorded the highest value of shear force ($P<0.05$).

In general, the tenderness process in cooked meat is caused by the solubilization of connective tissue (Barbanti & Pasquini, 2005; Tornberg, 2005) and on the contrary, the denaturation of myofibrillar proteins is the main cause of the toughening process. According to several authors, the tenderness of meat is mainly due to the interaction of three factors: the solubilization of connective tissue, the degree of the aggregation of sarcoplasmic proteins, and the water retention inside the muscles (Tornberg, 2005; Baldwin, 2012; Garcià-Segovia et al., 2007; Warner et al, 2017; Fabre, et al 2018; Gòmez et al, 2019.

7.2.5 Principal Component Analysis (PCA)

Figure 32 shows the loading plot of the physicochemical parameters evaluated in the study. PC1 and PC2 explained the 74.42% of the total variance. The PC1, which explained more than 59% of variance, is positively correlated with cooking loss, lipid oxidation, shear force, and pH, while it had negative correlation with moisture and a* color parameter. The negative correlation between moisture from one side, and cooking loss and shear force on the other, confirms the results that we found. The PC2, which explained the 14.58 % of total variance, is only positively related to L* color value. This tendency agrees with Fabre et al. (2018).

Figure 32. PC1 vs PC2. The loading plot of physicochemical variables evaluated on sous vide chicken breast cooked at different combinations of temperature (60°C, 70°C, 80°C, and 100°C) and time (60 min, 90 min, 120 min, and 150 min)

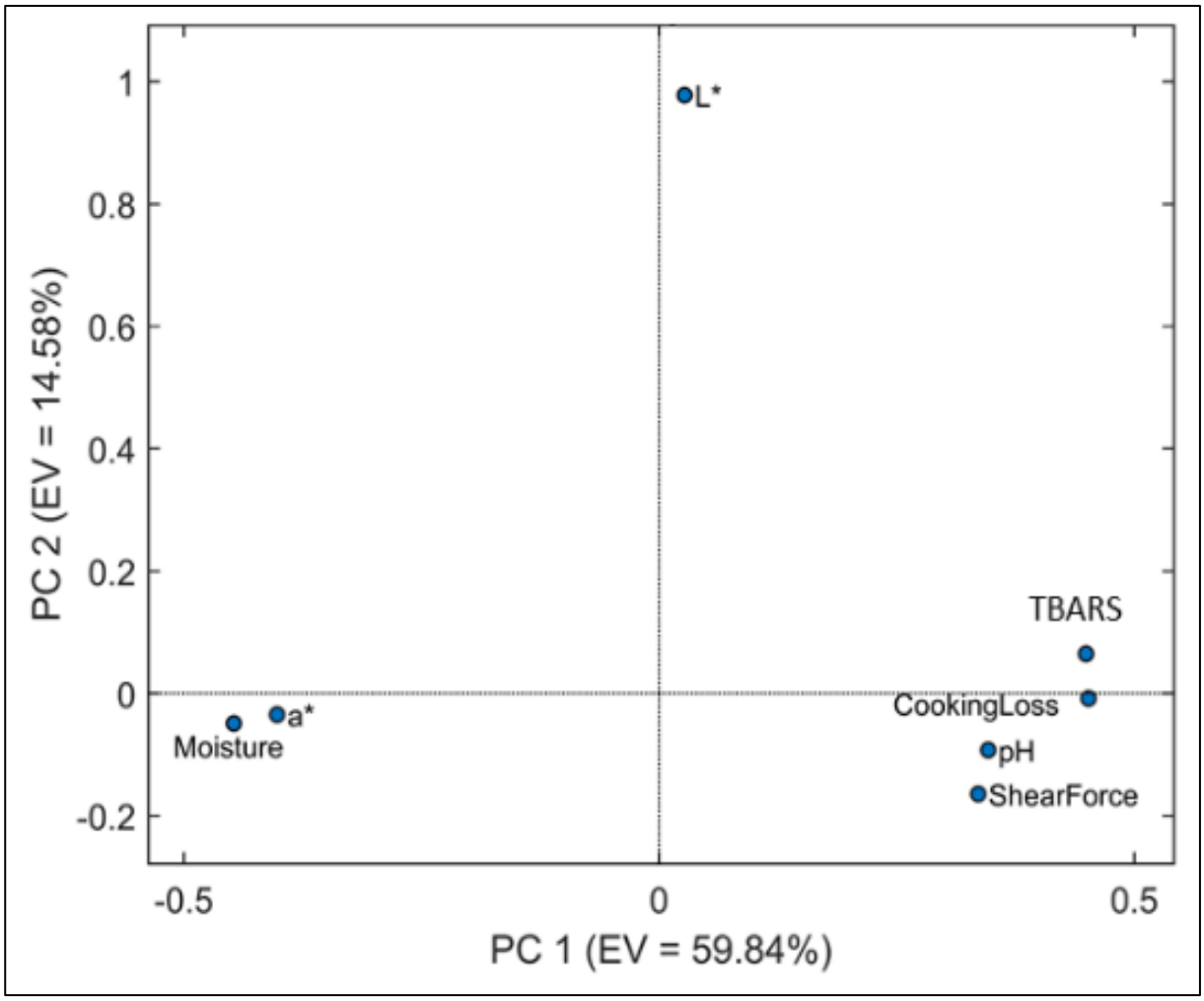
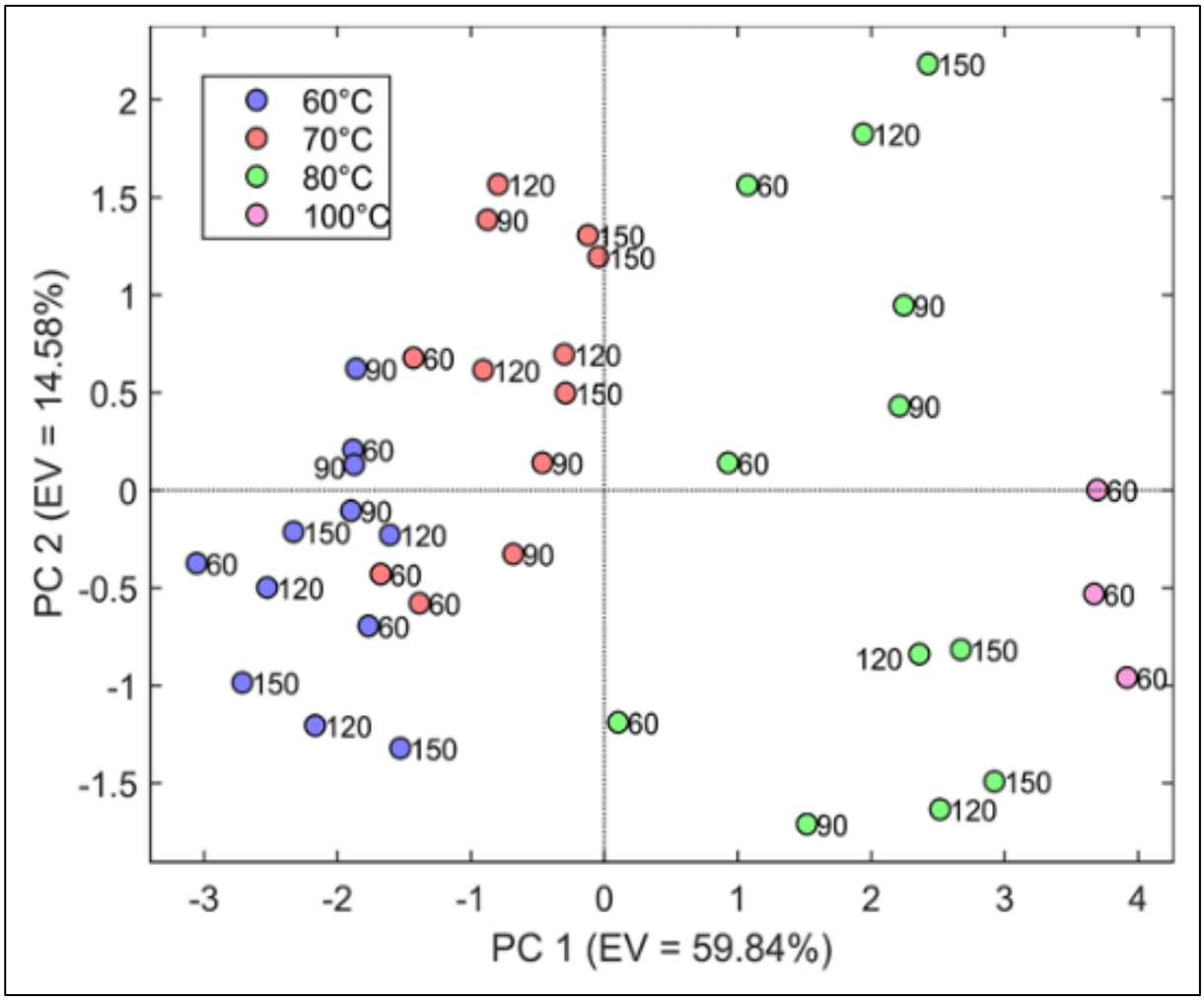


Figure 33 shows the score plot of the present study. Different colors indicate different temperatures of cooking, and for each point is indicated the cooking time. Different clusters could be detected in the plot.

The first cluster was represented by the samples cooked at 100°C (pink dots), the second cluster was represented by the samples cooked at 80°C (green dots) and the last two cluster were represented by the samples cooked at 70°C (red dots) and samples cooked at 60°C (blue dots). The numbers near the dots indicate the cooking time. The samples cooked at lower temperatures, 60°C and 70°C, were at the negative value of PC1 while samples cooked at 80°C and 100°C were at the positive value.

Figure 33. The score plot of physicochemical variables evaluated on sous vide chicken breast cooked at different combinations of temperature (60°C, 70°C, 80°C, and 100°C) and time (60 min, 90 min, 120

min, and 150 min). Different color corresponds to different temperature of cooking, the number beside the dot corresponds to cooking time. PC1 vs PC2



Thanks to the comparison of the loading plot with the score plot (Figure 32 and Figure 33) is possible to determine the relationship between variables and samples. The samples cooked at higher temperature are characterized by higher value of cooking loss, lipid oxidation, pH, and shear force, and on the opposite hand, lower temperature of cooking were characterized by higher value of moisture and a* color value. From the analysis of PCAs and the values shown in Table 22, is possible to affirm that the temperature of cooking was more effective in determining the difference among the samples cooked at different *sous vide* temperature and time combinations, while the cooking times did not show the same effect.

7.2.6 Microbiological analysis

The microbiological analysis was conducted to evaluate whether the different combinations of time and temperature of cooking were able to guarantee the microbiological safety of chicken breast fillets. The values of the microbiological analysis of raw meat and cooked samples are shown in Table 24.

Table 24. Microbiological counts of raw chicken breast fillets 24 h post mortem (day 0) and *sous vide* chicken breast fillets (cooked at all different combinations of temperature and time) during 21 days of storage at 4°C.

Days	Treatment	Total mesophilic aerobic Log (CFU/g)	*Enterobacteriaceae* Log (CFU/g)	*Psychrotrophic* aerobic Log (CFU/g)
0	Raw meat	2.8 ± 0.6	2.3 ± 0.4	< 1
0	*Sous vide*	n.d.	n.d.	n.d.
5	*Sous vide*	n.d.	n.d.	n.d.
10	*Sous vide*	n.d.	n.d.	n.d.
15	*Sous vide*	n.d.	n.d.	n.d.
21	*Sous vide*	n.d.	n.d.	n.d.

Values are presented as means ± standard deviations
n.d.: not detected

At day 0, the microbiological analysis was conducted on the raw chicken breast fillets, before the cooking process, and immediately after the cooking process. The raw meat of our study, showed value of 2.8 and 2.3 log CFU/g respectively for the total mesophilic aerobic bacteria and *Enterobacteriaceae*; the *Psychrotrophic* bacteria were < 1 log CFU/g. The selection of these 3 groups of microorganisms was due to their importance in food quality and safety. The level of contamination of the raw meat was below the reference value of $5*10^6$ CFU/g of total bacterial contamination, recommended by quality standards for fresh poultry meat (EC No. 2073/2005).

For all combinations of time (60 min, 90 min, 120 min, and 150 min) and temperature (60°C, 70°C, and 80°C) used in this study, none of microbial groups were detected during the storage time at 4°C. Our result confirms that even the combination of the lowest temperature (60°C) for the shortest time (60 min) can pasteurize the final product, which could be stored for more than 21 days at 4°C.

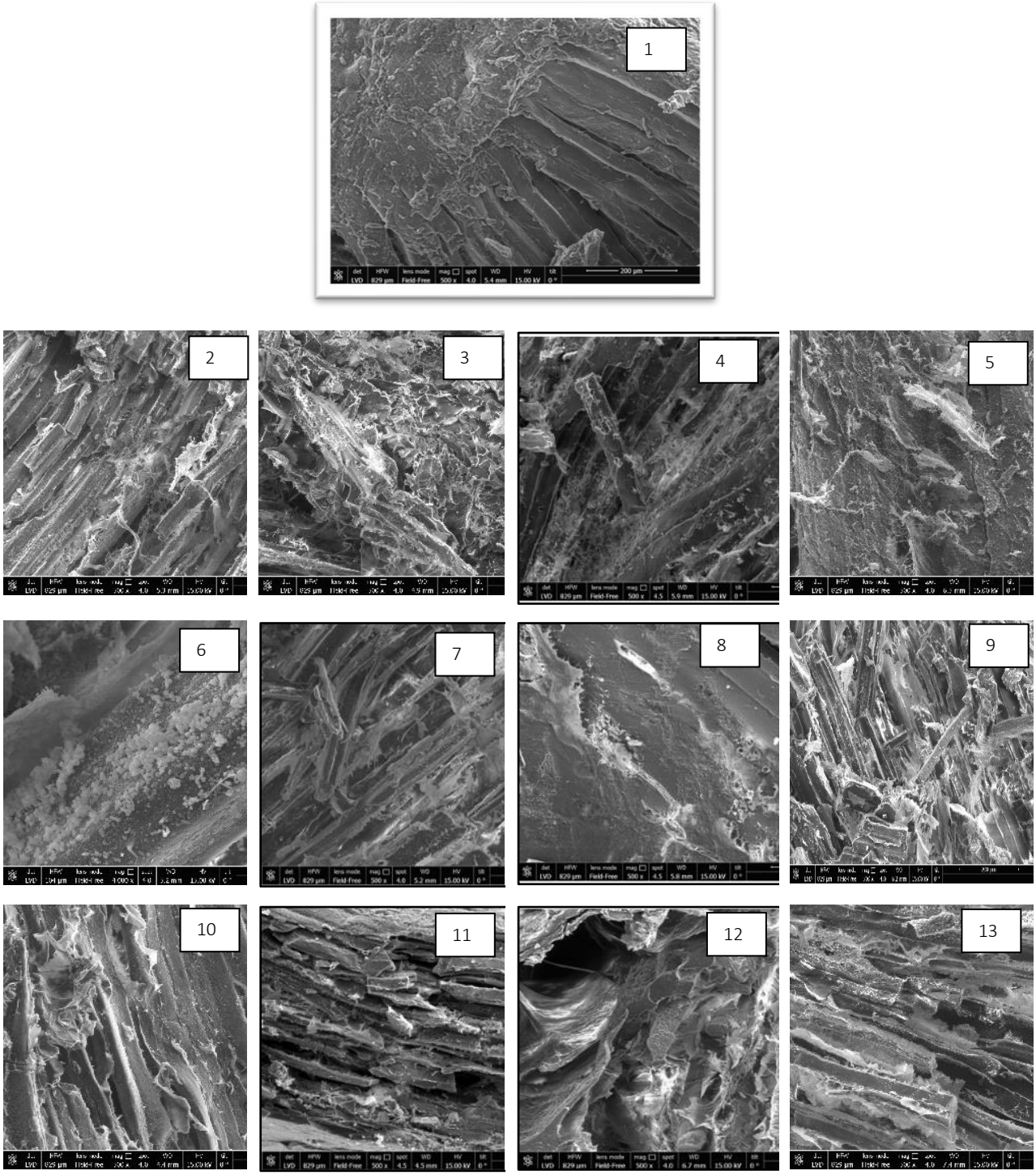
Probably the inhibition of microbial growing was not only due to the cooking process, but also the vacuum packed played a role in the inhibition of the growth of the aerobic microorganisms that normally contaminate the surface of the fresh meat, such as the low temperature of storage (4°C) (Hong et al., 2015; Akoğlu et al.,2018).

Similar results were reported in *sous vide* chicken wings stored for 7 weeks (Wang et al., 2004) or chicken meat balls stored for 10 weeks (Can & Harun, 2015), lamb (Roldàn et al., 2013), pork loin stored for 10 weeks (Dìaz et al., 2008), and beef stored for 5 weeks (Hansen et al., 1995).

7.2.7 Microstructure analysis

The cooking process is responsible for the physical-chemical changes that occur in meat, and determines important changes in the microstructure of muscle fiber (Palka, 2003).
Using scanning electron microscopy, the microstructure of the chicken breast fillets cooked with the *sous vide* technique was evaluated.
Figure 34.1 represents the control group, cooked without vacuum to simulate a normal boiling cooking. It is possible to see how the structure of the control sample was denser, compacter and with the absence of irregularities in the fiber structures in comparison with all the *sous vide* samples. Another study on chicken (Wattanachant et al., 2005) and one on duck (Li et al., 2013), reported the same compact structure which we found for the control group. As explained by other authors (Barbut et al., 2005; Garcìa-Segovia et al., 2007), the reason for compact microstructure of samples cooked at 100°C is due to different factors, such as: higher value of cooking loss, shrinkage of fibers, gelatinization process of sarcoplasmic proteins and narrowing of connective tissue, which all have as their main consequence the determination of significantly higher shear force values respect to the *sous vide* samples.
In Figure 34 samples 2,3,4,5 are the samples cooked at 60°C, arranged in increasing order of cooking time. Respect to control group, these samples show abundant areas of broken fibers and is possible to identify the free space among the fibers. The free space among fibers is reduced with the increase of coking temperature, while the cooking time did not show the same effect. To better understand this concept of reduction in free space among the fibers, it can be useful to look samples 3,7, and 11 in sequence (Figure 34). Similar studies and results were conducted and obtained on beef and pork microstructure (Garcìa-Segovia, 2007; Sànchez del Pulgar, 2012).

Figure 34. Scanning electron microscopy images of chicken breast fillets cooked with sous vide



1: control sample, 100°C *60 min
2,3,4,5: samples cooked at 60°C, respectively for 60 min, 90 min, 120 min, and 150 min
6,7,8,9: samples cooked at 70°C respectively for 60 min, 90 min, 120 min, and 150 min
10,11,12,13: samples cooked at 80°C respectively for 60 min, 90 min, 120 min, and 150 min.

7.3 Conclusion

The *sous vide* cooking method can be a good instrument that can be used by catering or by simple consumer to obtain meat products juicy and tender. Moreover, since the cooking process happens inside a specific plastic bag, this allows extending the shelf life of the product, reducing the risk of recontamination until the moment of consumption.

The choosing of the right combination of time and temperature of *sous vide* cooking is central to obtaining final meat dishes which can satisfy the consumer palate. In addition, the use of low temperature of cooking for meat reduce the risk of the formation of dangerous compounds for human health.

The findings in our study showed that cooking temperature is more effective in the determination of moisture content, cooking loss, shear force, lipids oxidation, a*color value, and pH value.

While due to the increase of cooking time from 60 min to 150 min, the values of moisture content and a* reduced and the cooking loss increased.

The lowest value of cooking loss was recorded by chicken samples cooked at 60°C, and also other parameters such as lipid oxidation, shear force were lower at this temperature, compared with samples cooked at 70°C and 80°C.

From the safety point of view, the lowest combination of time and temperature of cooking (60°C*60 min) resulted enough in terms of pasteurizing effect on chicken meat, in fact in all samples cooked at all different time and temperature combinations, total mesophilic aerobic bacteria, *Psychrotrophic* bacteria and *Enterobacteriaceae* were never detected during the storage period of 21 days at 4°C.

The cooking process generates structural changes in the proteins, which not only change in conformation but also reduce their power to retain water. The analysis of the microstructure confirmed that samples cooked at higher temperature showed a more compact structure due to lower value of moisture and higher cooking loss value recorded.

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CHAPTER 8

General Conclusions

Nowadays, the consumer is aware and attentive to the quality of the food he consumes and what is the better way to prepare it. Several studies have confirmed that meat is an essential source of nutrients such as proteins, vitamins, and microelements in particular heme iron. All these elements proved to be fundamental during human development.

The cooking process of meat is fundamental for improving the digestibility and bioavailability of nutrients, and also leads to the formation of odorous molecules and flavors which are the main responsible for the increase in general palatability and acceptability by the consumers of cooked meat.

However, the heating process of meat is also responsible to produce a large number of molecules, which have shown to have detrimental effects on human health. In particular, the heterocyclic aromatic amines (HCAs) are formed quickly when the cooking temperature exceeds 150°C, or polycyclic aromatic hydrocarbons (PAHs) are formed when there is a direct contact with the fat that is dripping on the heating sources during the cooking process. The most common cooking techniques applied to meat, are: grilling, roasting or pan-broiling. All of them are characterized using high temperature, that in turn cause the formation of smoke rich in dangerous compounds.

Another sensorial parameter important for cooked meat is the tenderness, in fact, modern consumers increasingly prefer more juicy and tender meats. For this reason, the attention has been increasingly focused on innovative cooking techniques, which allow reconciling the needs of the consumer both from the sensory and from the health point of view.

The *sous vide* cooking technique, although it has been already used in the past by some great chefs, has only recently begun to have widespread use both in catering, in ready-to-eat dishes, and in the possibility of being used in the home walls.

This technique is characterized using long cooking times and the application of low temperatures (<85 °C), which therefore reduce the risk of formation of the carcinogenic compounds mentioned above. The raw food is vacuum packed and immersed in a water bath, this allows to have careful control of the cooking temperature and limits the excessive loss of juices, thus ensuring greater juiciness of the final product. In addition, the long cooking times play an important role in increasing

the degree of tenderness of the final product, ensuring coagulation of muscle proteins and a softening of collagen.

The research conducted during Ph.D. studies led to have important results on the application of this technique to the cooking of meat. In all studies conducted, it emerged that the choice of the right combination of time and temperature of cooking is essential to obtain final products with adequate values of physicochemical parameters, but the temperature is the variable that proved to have major influence.
The temperatures range evaluated in the different studies was between 60°C and 80°C, except for 100°C used as a control cooking.
In general, a temperature of 60 °C allowed to obtain proper cooking for samples of chicken and pork of about 150 g, which resulted better than those cooked at higher temperatures, both regarding the cooking loss values, humidity, color, and tenderness.
Cooking time had little influence on determining changes on physicochemical changes, however it is possible to affirm that longer cooking time causes an increase in cooking loss.

One of the studies carried out had the objective to fortify pig meat with PUFA-n-3 by a particular feeding strategy. Although the overall fatty acid profile of cooked meat was different from that of raw meat, the *sous vide* cooking process nevertheless maintained the favorable n-6/n-3 ratio, which resulted below 4, in accordance with what is required by the most important health organizations to maintain a good state of health.

It was also evaluated the possibility to add an ultrasound treatment before *sous vide* cooking process to further improve the tenderness of meat. However, in our study the effectiveness of ultrasound on improving shear force or any physical and chemical parameter evaluated, was not recorded, this was probably due to the not right choice of power/frequency of ultrasound applicated due to the limited capacity of the ultrasound bath used. More research in terms of evaluation of different ultrasound frequencies, times of application need to be carried out.

The greatest risk related to the consumption of *sous vide* cooked products may be related bacterial contamination, since the low temperatures used may not be sufficient to ensure the safety of the final product.

In the study on the chicken breast, starting from a raw product with a normal level of bacterial load, we observed how even the lowest combination of time (60 min) and temperature (60°C) was enough to guarantee a shelf life period of 21 days stored at 4°C.

Future studies using inoculums of pathogens for human, on food cooked with *sous vide* technique can be carry out to evaluate the real effectiveness of this techniques to guarantee the safety of food. In additions, what has been achieved in the three studies may be a basis for future research to assess whether the final consumer is satisfied with these products, consuming them immediately after cooking and verifying also if their acceptability lasts throughout a long storage period.

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E quindi uscimmo a riveder le stelle

And then we came out to see again the stars

Dante Alighieri (Inferno, Canto XXXIV, vv 139)

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