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Optimization of cooking for food service: matching quality and nutritional requirements as drivers for the development of innovative tools

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A mamma e papà

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Preface

Food service is a complex system able to create and exchange value. The way value is created and distributed is affected by global megatrends such as rapid urbanisation and demographic increase. Except for Covid-19 year, during which all the services were completely blocked, food service is constantly increasing the catchment area and a continuous growth is expected for the future. From a technological point of view, the increase in the consumption of extra-household food forces the food service to expand the market and boost the production with a consequent need to optimize and enhance food-related machineries. However, consumer remains the main character of food service and satisfaction of consumer needs should be the primary aim to reach in food service. Among consumption habits trends, wellness is driving the shift towards a more sustainable consumption, with a focus on health and nutrition, for instance by means of reduced sugar- and fat-snacks, minor alcohol consumption and attention to food labels and formulations.

In this complex framework food actors are called to fill the gap between consumer and food service, with a particular focus on companies producing cooking devices. The future innovation of cooking lies in providing the best cooking experience thanks to the identification of the optimal cooking time capable of maximizing the sensory and nutritional quality and respecting the safety restrictions. Identify the optimal cooking time capable of achieving these objectives would be beneficial not only to ensure safe and nutritious foods facing the most common challenges of modern society, but also in terms of energy saving and environmental sustainability.

The proper knowledge of food science and technology could be exploited to investigate the chemical and physical modifications affecting foods under cooking. Understanding the events behind the changes in cooking is fundamental to control the cooking process itself.

Based on these considerations, the aim of this Ph.D. thesis was to investigate the possibility of optimizing the oven food cooking, guaranteeing the achievement of a desired sensory and nutritional quality level of the product, by respecting the safety requirements.

Summary

The research activity here described is the result of a joint collaboration between University of Udine and the team of Advanced Development of Electrolux Professional S.p.A. based in Pordenone, which supported this Ph.D.

This research work was aimed at optimization of food cooking by using company oven equipment, in terms of sensory and nutritional quality, by respecting the safety limits. Moreover, the possibility to adopt a suitable and easy to-use indicator instead of temperature to describe the cooking process was investigated. By using a selected quality indicator, less critical than temperature, it was possible to develop a predictive cooking model capable of optimizing the cooking process. Chicken breast meat was considered as study case for its widespread and expected increased consumption worldwide.

A kinetic modelling approach has been proposed to develop a predictive model capable of extrapolating the optimal cooking time of a chicken breast cooking process as function of a quality indicator. The approach was divided in three parts. In the first part a systematic study on the evolution of the main quality indicators during a cooking process was carried out. Three different oven cooking methods were considered, e.g. Grill, Forced Convention, and *Sous Vide*, which are the most common in the food service. Regarding chicken breast meat, emerged that the achievement of the best sensory quality requires less time that the fulfilment of the safety condition of 74 °C at the thermally less favoured point. For this reason, safety time represents the limiting factor in chicken breast cooking.

In the second phase, the effect of cooking method and temperature on the evolution of quality indices was investigated over the cooking process. Rate constants of quality indices linearly increased according to the temperature of the cooking process and they were modelled according to Arrhenius equation.

In the final step of the kinetic modelling approach, a predictive model was elaborated based on Arrhenius parameters and the most sensitive and representative quality indicator of

chicken breast cooking process was identified, i.e. cooking loss. The model was able to predict the optimal cooking time as a function of cooking temperature.

In the second part of the work the nutritional profile in terms of protein digestibility of chicken breast was investigated as affected by different cooking methods and times. Forced convection resulted in the decrease of protein digestibility, while overcooking significantly decreased protein digestibility for all the treatments. Oxidative profile and secondary structure analysis confirmed proteins modifications as affected by prolonged cooking times, due to the modification of the hydrolysis sites for digestive enzymes.

1 Chapter

Introduction

1.1 Food service and its challenges

In the Oxford English Dictionary, Service is described as "the action of helping or doing work for someone; an act of assistance; assistance or advice given to customers during and after the sale of goods; the action of serving food and drinks to customers" (Service, 2022). Service is thus referred to non-physical, intangible parts of economy, as opposed to goods which we can touch or handle. Based on these statements, service creates and exchanges value. The way value is created and distributed across all sectors is changing, underlined by the global societal challenges currently impacting most of the world, such as rapid urbanisation, demographic change, climate change, resource scarcity and food security, shift in political and economic power and increasingly disruptive technological breakthroughs (EY, 2018).

In the food area, service is intended as any activity and business related to meals preparation outside the home. It includes any format connected to outside meal consumption. It implies operations such as clean up, preparation, processing, cooking, storage, service of food intended for consumption in a facility either by facility staff or through a formal agreement that meals will be regularly catered by a third part. In particular, in food service, value is created in a complex socio-technical system that involves a plethora of stakeholders connected by a common interest: the production, procurement, preparation, service, and consumption of food and beverages (Ball et al., 2011). Food service is a highly diversified industry spread across a wide range of operations (Edwards, 2013). This market can be segmented based on the type of restaurant: Full service restaurant, which encompasses all sit-down establishments characterized by table service and a relatively higher quality of food and including fine and casual dining restaurants; Quick service restaurant, which combines

fast food and 100% home delivery/takeaways outlets, usually specialized in one or two main entrees; Cafés and bars, comprising all establishments where the focus is on drinking (either alcoholic or non-alcoholic beverages); Street food, i.e. small foodservice providers, sometimes mobile, characterized by a limited product offering and by low prices (Deloitte, 2022). Other food services can be classified by type of occasion: Freestanding, standalone food service establishments, not operating in a travel, leisure, lodging, or retail location; Retail, establishments located in retail locations including supermarkets, grocery stores, convenience stores, hypermarkets, dept. stores and mass merchandisers; Travel, travel establishments located in travel locations including motorway service or fuel stations, airports, rail stations, coach stations and also establishments located in hotels; Entertainment, establishments located in leisure locations including museums, health clubs, cinemas, theatres, theme parks and sports stadiums (Deloitte, 2022). Finally, yet importantly, we must not forget the segment of the canteen service, i.e. in schools, hospitals, care homes, companies or catering service, for social events, business or celebrations.

According to the report of Food Service Market Monitor published by Deloitte (2022), global food service in Europe has experienced a substantial decline in 2020 due to the Covid-19 pandemic. However, in 2021 it has recorded a significant recovery with a growth of +15.5% compared to 2020 and a market value of 2,221 billion euros, with Asia and Pacific regions (APAC) covering 48% of the market, followed by North America (18%) and Europe (18%), as shown in Figure 1. Full service restaurants account for 48% of total food service, quick service restaurants cover 33%, while cafés and bars, and street food account for 14% and 5%, respectively. In terms of consumption mode, delivery segment boomed in Covid-19 year 2021 (+53.7%) after 5 years of important growth, together with takeaways (+16.88%), but also on site consumption registered an increase (+29.89%), actually a slow growth rate mainly due to Covid-19 on-site restrictions (Deloitte, 2022) (Figure 1).

APAC countries, such as China, India and South Korea, show the highest penetration of full service restaurants, followed by Italy and France. Italy seems to have a leading role in the world. According to the Deloitte report: our country, in fact, ranks first in quality catering in Europe and sixth in the world. For centuries, Italy and France have relied on the catering and gastronomy sector, one of the driving economic factors. Italian cuisine worldwide was worth 205 billion euros in 2021 (19% of global Food service restaurant market), with China and USA accounting for ~60% of the overall market value. 2021 showed a double-digit growth (30%) in terms of Food service restaurant market, but still below pre-pandemic values (2019) when the overall market size was estimated to be 236 billion euros (Deloitte, 2022).

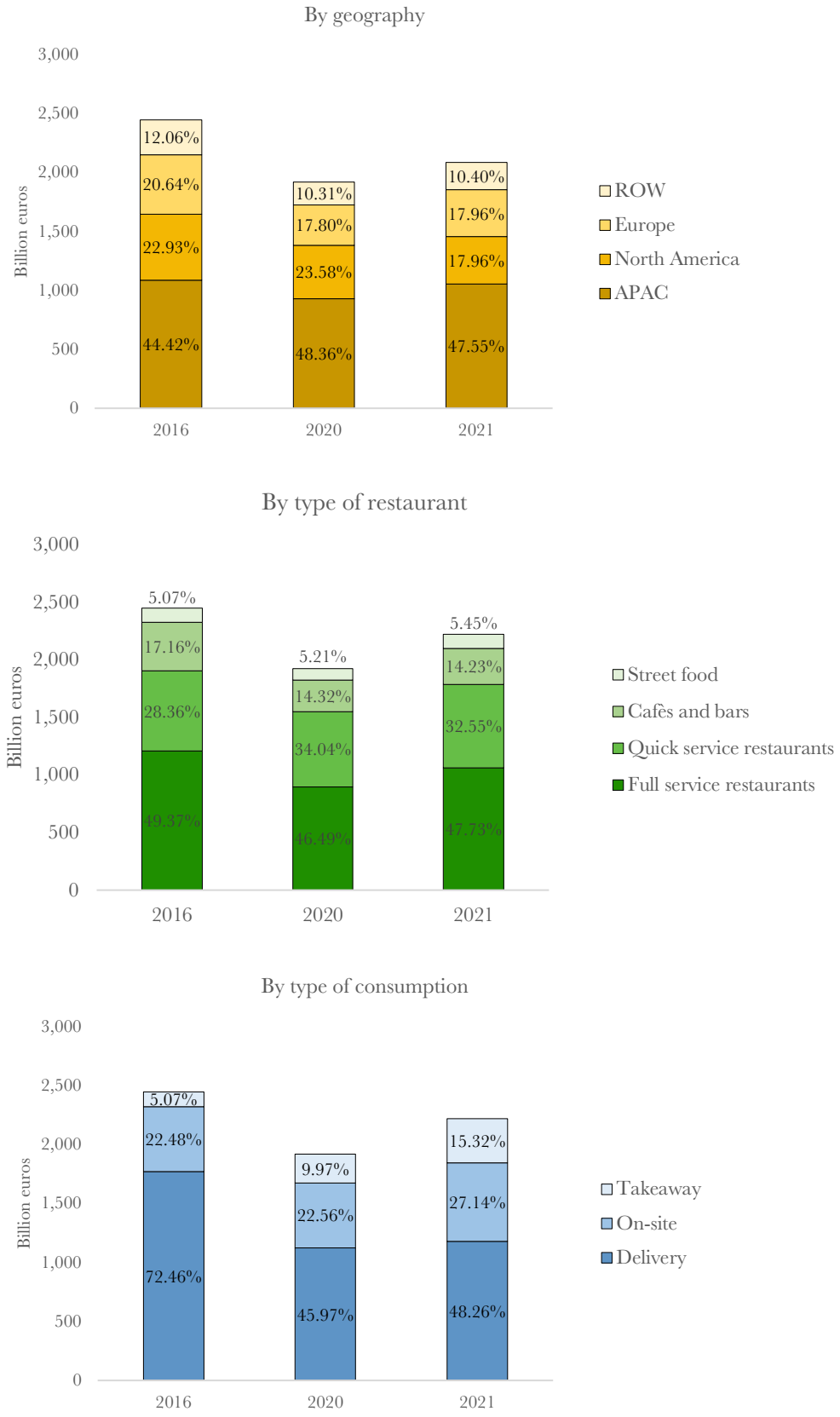


Figure 1 Global food service historical market performance 2016-2020-2021, by geography, by type of restaurant, by type of consumption (% are related to billion euros as % of annual total) (modified from Deloitte, 2022).

However, the recovery is expected to be among the most active, returning to pre-pandemic levels in 2023, with a profit of 2,448 billion euros in 2022, and a forecast of 2,620 billion for 2023. Consumers show an increased spending intent in restaurants/takeout in most countries. Consumers are gradually returning to purchase offline, even though ~23% of them are still heavy online buyers of restaurants/takeout. In the next five years, the sector that will grow more will be that of cafés and bars (8.1%), followed by full service (5.7%), street food (5.6%) and quick service (4%) (Deloitte, 2022). Among consumption habits trends, wellness is driving the shift towards a more sustainable consumption, with a focus on health and nutrition, plant-based food, reduced sugar- and fat-snacks, minor alcohol consumption, clean label and sustainability process (Hassoun et al., 2011; Aschemann-Witzel et al., 2020). The pandemic changed some dynamics in the food service industry, requiring restaurants operators to adopt new strategies to drive their growth path. As technology transforms the broader consumer industry, the capabilities and tools in restaurants must also adopt innovations that serve chefs, managers and staff. To drive growth and profitability, transformation of restaurants should include comprehensive skills, customer and employee engagement combined with next-generation digital technologies (Tuomi & Tussyadiah, 2020). The increase in the consumption of extra-household food forces the food service to increase the production with a consequent need to optimize and enhance food related-machineries, such as professional ovens.

From a cooking quality point of view, modern oven machineries should firstly guarantee the safety of the cooked product by improving the efficiency and effectiveness of food preparation. Nowadays, guarantee food security is one of the most important societal challenge. Optimized ovens can help address societal challenges related to nutrition and health too. By utilizing cooking techniques that preserve nutrients and flavours, professional ovens could produce healthier and more nutritious meals with a positive impact on public health.

From a technological point of view, modern machineries would be able to process larger quantities of products in the shortest possible time, in a controlled and efficient way, in order to optimize process times ensuring minimum energy expenditure. Energy-efficient technologies should be developed thanks to precise temperature control, smart cooking algorithms or improved insulation. By minimizing energy waste and reducing electricity or gas consumption, these ovens could help mitigate the environmental impact of cooking operations. This outline is vital for achieving environmental cleanliness and sustainable economic growth, according to the sustainability goals outlined by 2030 Agenda for

Sustainable Development, endorsed by all United Nations Member States (United Nations General Assembly, 2015), in line in particular with SDG-7 (Affordable and Clean Energy), SDG-9 (Promote sustainable industrialization) and SDG-12 (Responsible Consumption and Production).

Optimization of food devices should progress in parallel to food service and related needs in order to facilitate the work of sector players, optimize profits and ensure the best customer service.

1.2 Global meat consumption

In food service, meat is one of the most requested and expensive items and accounts for a significant percentage of the total cost of production (OECD-FAO, 2021). According to the Organisation for Economic Co-operation and Development (OECD) and FAO Agricultural Outlook 2021-2030, determinants of meat consumption are complex. Demographics, urbanisation, incomes, prices, tradition, religious beliefs, cultural norms and environmental, ethical/animal welfare and health concerns are key factors that affect not only the level but also the type of meat consumption.

Global consumption of meat proteins is projected to increase by 14% by 2030 compared to the base period average of 2018-2020, reaching 374 million tons by 2030. By 2030 protein availability from, beef, pork, and sheep and poultry meat is projected to grow of 5.9%, 13.1%, 15.7%, and 17.8%, respectively (OECD-FAO, 2021).

Population growth is clearly the main driver of increased consumption, with a projected grow by 30% in Africa, 18% in the Asia and Pacific region, and 12% in the Latin American region, 0.4% in Europe and 9% in North America (OECD-FAO, 2021). Economic growth is another important driver of meat consumption. Income growth enables the purchase of meat, which is typically an expensive source of calories and proteins. It is also accompanied by other structural changes such as greater urbanisation, higher labour participation, and food service expenditures that encourage higher meat purchases (Henchion et al., 2014; OECD-FAO, 2021). The term "nutrition transition" describes the major transitions in population-level dietary patterns associated with economic development (Popkin, 2006). The response of *per capita* meat consumption to income increase is higher at lower incomes and less so at higher incomes where consumption is largely saturated and limited by other factors such as environmental, ethical/animal welfare and health concerns (Henchion et al., 2014; OECD-FAO, 2021). In middle-income countries, *per capita* availability of animal protein is

projected to increase by 11% over the coming decade (+2.8% g/person/day), with a different demand of animal products across countries depending on dietary preferences. In high-income countries, *per capita* availability of animal protein is expected to grow slowly over the coming decade (+1.8 g/person/day or 3%) (Figure 2).

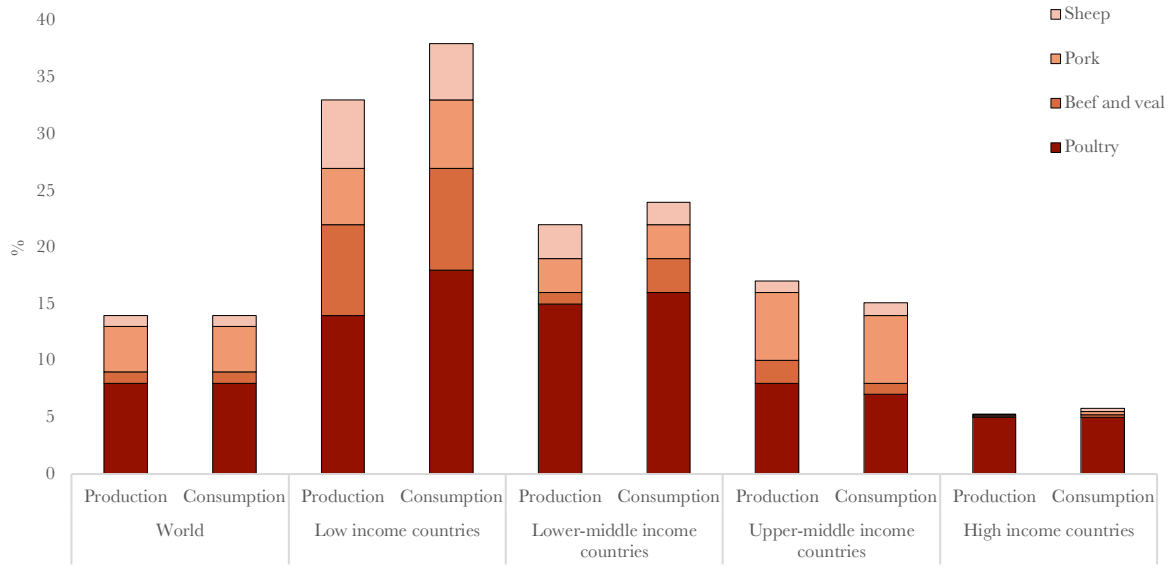


Figure 2 Growth in meat production and consumption on a protein basis, from 2021 to 2030 (modified from OECD-FAO, 2021).

Among all the types of meat, a clear trend is the rise of poultry meat consumption in virtually all countries and regions. At present, poultry meat is the first most-consumed meat in the world and, over the last decades, is having one of the highest growth rates in terms of consumption of animal products (OECD-FAO, 2021). A further growth of the chicken market is expected in the coming years (Windhorst, 2017; Augustyńska-Prejsnar et al., 2018), as shown in Figure 3.

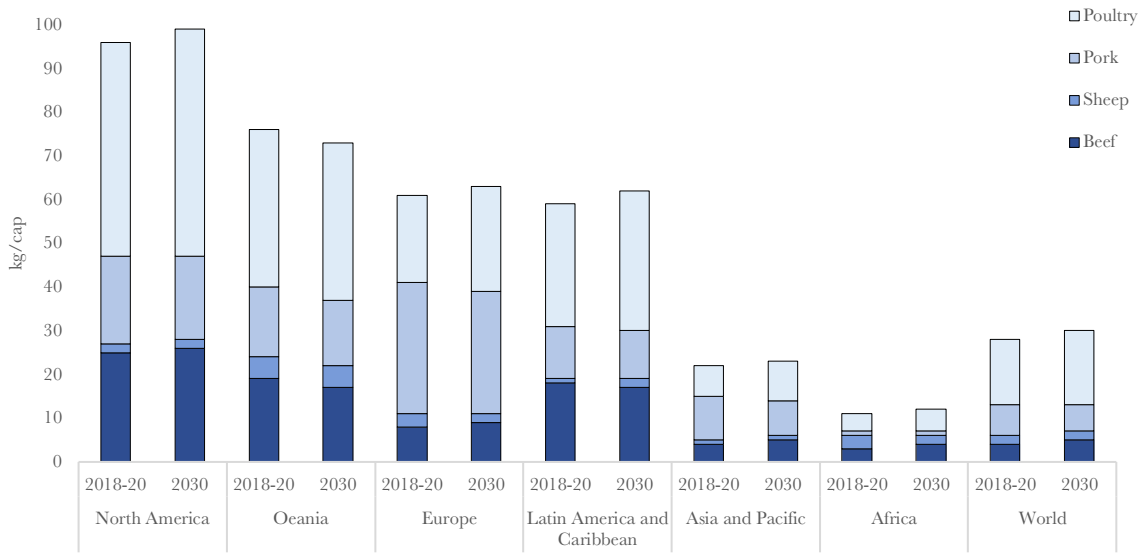


Figure 3 Meat consumption per capita: continued rise of poultry and fall of beef (modified from OECD-FAO, 2021).

Consumption of poultry meat is expected to increase globally to 152 million tons over the projection period of 2030, accounting for 52% of the additional meat consumed. In fact, from the consumer point of view, chicken meat is notoriously a valuable source of nutrients, such as high-quality proteins, micronutrients, and polyunsaturated fatty acids (PUFAs) and is characterised by low fat and cholesterol contents (Sobral et al., 2017). Chicken meat represents a healthier dietary alternative and is subjected to fewer religious barriers in comparison to other meat types, such as beef and pork (Abete et al., 2014; Petracci et al., 2014; Domingo & Nadal, 2017). On a *per capita* basis, the expected robust growth rates in poultry consumption reflect the significant role this meat type plays in the national diets of several populous developing countries, including China and India. From the producers' perspective, chicken is the most cost-effective commercially produced meat in the world and is simpler to handle along the production process with respect to other meat sources (Windhorst, 2017).

The increase in consumer demand for chicken will also lead to a natural increase in demand in food service. The need to implement cooking systems must also take into account this fact. Not only it will be very important to increase the quantity of cooked foods and standardize their quality in the face of a production increase, but also pay even more attention to safety will be essential. In the case of chicken meat, *Salmonella* spp. infections are often associated with the consumption of raw or undercooked poultry (EFSA, 2007). Safety can be achieved by cooking meat until reaching a minimum internal temperature of 74 °C (USDA & FSIS, 2021). Thus, to guarantee safety of this type of meat, the level of safety must be increased

and cooking devices must be equipped with efficient systems capable of monitoring the temperature at the thermally less favoured point of the product.

1.3 Meat and muscle structure

According to European legislation, the term meat refers to the edible portions removed from the carcass of domestic animals or animals farmed as domestic animals, like bovine, porcine, ovine, caprine, and poultry (Reg. No 853/2004). The muscle is a set of different tissues whose aim is to generate strength and movement in the living animal (Purslow, 2023) by converting chemical energy in mechanical energy (Frontera & Ochala, 2015). Several parameters, such as gender, animal species, age, environmental factors and diet, influence muscle composition, but on average, it consists of 75% water, 20% protein, 3% fat and 2% soluble non-protein substances. Out of the latter 2%, metals and vitamins constitute 3%, non-protein nitrogen-containing substances 45%, carbohydrates 34% and inorganic compounds 18% (Tornberg, 2005).

There are three types of muscle tissue: cardiac muscle, smooth muscle, and striated or skeletal muscle. The latter is the one most characterizing the cuts used for human consumption (Purslow, 2023).

The muscular proteins can be divided into three groups: myofibrillar, sarcoplasmic and connective tissue proteins. The myofibrillar proteins constitute between 50 and 55% of the total protein content, while the sarcoplasmic proteins account for approximately 30–34%. The remaining 10–15% of the proteins are the connective tissue proteins (Tornberg, 2005).

The myofibrillar proteins are further divided into three subclasses: the myofilamentous fibrous proteins myosin and actin building up the myofibrillar structure, the regulatory proteins including the tropomyosin-troponin complex, α- and β-actinin, M-protein and C-protein and ultimately the scaffold proteins, such as titin, nebulin, desmin, vimentin and synemin, supporting the whole myofibrillar structure (Tornberg, 2005; Frontera & Ochala, 2015).

The sarcoplasmic proteins are the soluble proteins of the sarcoplasma, to which belong most of the enzymes of the glycolytic pathway, creatine kinase and myoglobin. About 100 different proteins are known to be present in the sarcoplasmic fraction and they are globular proteins of relatively low molecular weight (Tornberg, 2005; Frontera & Ochala, 2015).

The connective tissue proteins are the third class of proteins and they comprehend fibrous proteins such as collagen, reticulin and elastin. Collagen, a glycoprotein, is the main

structural component of the connective tissues (55–95% of the dry matter content) and is composed of tropocollagen monomers (Tornberg, 2005; Frontera & Ochala, 2015).

An individual muscle is surrounded by a layer of connective tissue, known as the epimysium, protecting it (Figure 4). The muscle is built by groups of muscle fibers arranged in bundles and each bundle is enveloped by another layer of connective tissue known as the perimysium. The muscle fiber represents the basic structure of the muscle and consists of a single cell polynucleate. Muscle fiber, also called myofiber, is surrounded by a cell membrane or sarcolemma, also surrounded by another type of connective tissue called endomysium. Myofibers in turn make up contractile structures called myofibrils. Myofibrils present repeated longitudinal structures, the sarcomeres, which are considered the smallest contractile units. The myofibrils are composed of actin and myosin filaments called myofilaments, repeated in units called sarcomeres, which are the smallest contractile units of the muscle fiber necessary for muscle contraction (Ashgar & Pearson, 1980; Frontera & Ochala, 2015).

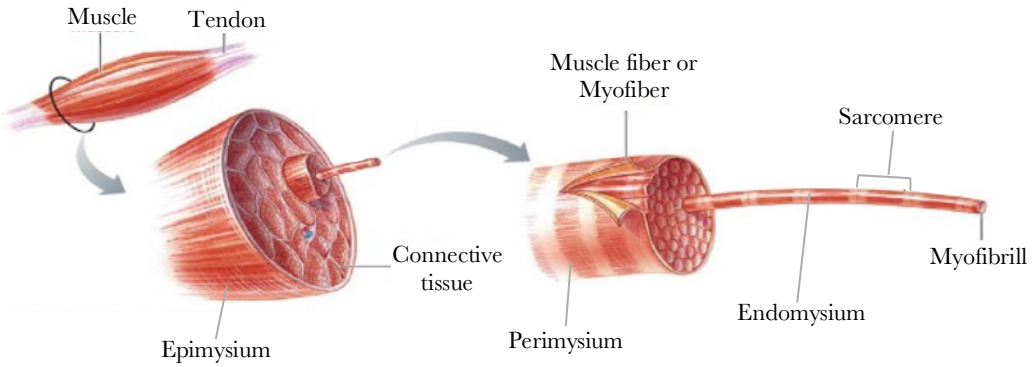


Figure 4 Illustration of internal muscle structure organization (modified from Frontera & Ochala, 2015).

Fibers (or muscles) can be classified as white fibers or red fibers according to the level of organization. Enormous differences in composition and morphology exist between red and white muscle. First of all, red muscle has a greater concentration of the pigment myoglobin and is generally lower in soluble protein content, lower in glycogen content and storage, and higher in lipid than white muscle. Red fibers are smaller in size than white fibers, are better supplied with capillaries, and contain more and larger mitochondria. White fibers are equipped better for glycolytic metabolism than are red fibers, which are designed for oxidative metabolism. Red fibers are high in oxidative enzymes, such as succinate dehydrogenase (SDH), but low in glycolytic enzymes, such as phosphorylase, and also low in ATPase, taking energy by aerobic respiration for their contraction; the opposite situation

exists in white fibers, getting energy for contraction by anaerobic respiration. These features lead red muscle to undergo slow sustained contraction for long periods, while white meat to fast contraction for short periods (Cassens & Cooper, 1971; Klont et al., 1998; Listrat et al., 2015).

1.4 Meat cooking

Cooking of meat is essential to achieve a palatable product. Cooking is the most common heat treatment applied to meat and with the exception of specially dried and fermented products, meat is generally cooked before consumption (Bejerholm, et al., 2014). The primary aim of cooking is to cause structural and chemical changes in the food matrix that will make the meat more palatable and tasty from a sensory point of view. Because of high temperature application, microbiological quality is also affected with a significant reduction of microbial load and an increase in safety. Indeed, for some kind of meat cooking is necessary to reach a safety condition, e.g. poultry meat genetically affected by Salmonellosi, cooking is necessary to reach a safety condition, but for other types of meat not suffering the same type of contamination (e.g. beef) heat treatment is not mandatory before consumption. Furthermore, the heating of meat results in better digestibility and, to some extent, in a change of the nutritive value (Santé-Lhoutellier et al., 2008; Kaur et al., 2014). The sensory changes that are caused by heat treatment, such as doneness, flavour, firmness, consistency, and cured-meat colour development, are time-temperature-dependent processes (Bejerholm, et al., 2014) and are mainly related to Maillard Reaction (Trevisan et al., 2006).

1.4.1 Maillard Reaction

Maillard reaction is a non-enzymatic chemical set of parallel, complex and consecutive reactions starting from condensation of carbonyls and amines. The overall reaction can be subdivided in three main steps. In the first phase, the reaction begins with the condensation of reducing sugars (such as pentose or hexose) and compounds having a free amino group, such as amino acids or proteins. The resultant condensation product is a glycosylamine (N-glycoside) which rearranges in the Amadori compound. Subsequently Amadori compound degraded into various compounds in the second phase depending on the pH of the system. At medium to low pH (4-7) compounds such as hydroxymethylfurfural (HMF) or furfural are formed by an enolization process, when hexose or pentose sugars are involved, respectively. These molecules are very reactive compounds and are involved in further

reaction of condensation and polymerization, leading to the formation of high molecular weight compounds (Martins et al., 2001). At higher pH (>7) sugars dehydrate and fragment due to β-elimination, compounds break down in Strecker fragmentation resulting in liberation of a wide range of low molecular weight products, including a variety of heterocyclic, carbocyclic and aliphatic compounds such as pyrazines, furans, pyrroli and aldoli. Because of the low molecular weight, these molecules, in particular aldehydes, ketones, thiols and furans, contribute to the sensory profile of the food product. Strecker reaction is one of the most important interactions relating to meat flavour generation (Khan et al., 2015). In the final last step, all the generated compounds polymerize together to form high molecular weight compounds, known as melanoidins (Hodge, 1953). Melanoidins are brown-coloured compounds responsible for brown colour. Their amount is proportional to heating time and temperature. This final step of Maillard reaction has been attributed to colour development (King & Whyte, 2006; Trevisan et al., 2006; Starowicz & Zieliński, 2019). Besides the pH, the specific pathways of the reaction can be affected by several factors such as water activity, nature and concentration of the reagents, presence of additional substances and physical/environmental factors (time, temperature, storage conditions and oxidation state) (Perez-Locas & Yaylayan, 2010).

1.5 Effects of Cooking on the Eating Quality of Meat

Eating quality of meat can change as a result of muscle type, cooking method used, and the temperature in the thermally less favoured point achieved. Three main factors differ depending on the cooking method: temperature at the meat surface, temperature profile through meat and method of heat transfer. The temperature at the surface is important for the colour, odour and flavour of meat. Temperature gradient influences the rate and extent of the changes in protein structure in meat, whereas the mechanism of heat transfer influences the odour, flavour, and colour development (Bejerholm, et al., 2014; James & James, 2014)

The main sensory and safety changes occurring during meat cooking are reported in Table 1 and they will be explained briefly below.

Table 1 Effect of chemical and physical events on meat quality and safety attributes.

Chemical/physical event	Attribute
Proteins denaturation Water loss	Texture
Non enzymatic browning Eme proteins denaturation	Colour
Non enzymatic browning Lipid oxidation	Aroma/Flavour
Protein denaturation Protein oxidation Lipid oxidation Vitamins oxidation Minerals leaching	Nutritional profile
Microorganisms destruction Toxic compounds formation	Hygienic and safety profile

1.5.1 Effect of cooking on texture

Changes in the texture of meat during cooking are due to heat-induced structural changes combined with enzymatic breakdown of proteins catalysed by thermoresistant enzymes (Bejerholm, et al., 2014). The basic effect of the heat treatment is the denaturation of meat proteins, i.e. myofibrillar, sarcoplasmatic and connective tissue proteins. Regarding myofibrillar proteins, the maximum denaturation is reached around 54–58 °C and 80–83 °C for myosin and actin respectively (Tornberg, 2005) (Figure 5).

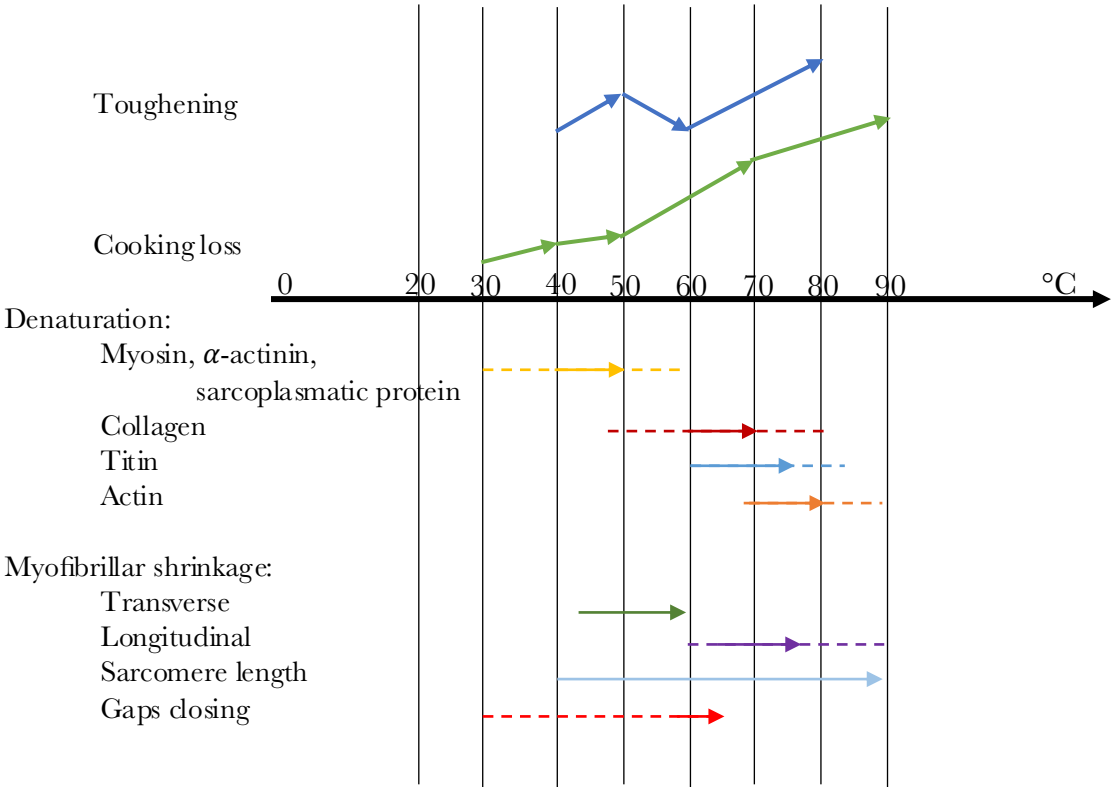


Figure 5 Changes in toughening and cooking loss, and denaturation temperature of different classes of proteins in meat during heating (modified from Bejerholm et al., 2014).

These proteins hold most of the water retained within the muscle and, when they are denatured, the water is released, causing shrinkage and reduction in weight or "cooking loss" (Tornberg, 2005).

Figure 5 shows existing relation between denaturation temperature of different classes of proteins and physical events such as cooking loss and toughening. Due to protein unfolding, hydrophobicity increases and these hydrophobic residues take part in protein-protein interactions leading to a network formation of aggregates (Tornberg, 2005; Yu et al., 2017). Considering connective tissue proteins, collagen denaturation occurs between 53–63 °C (Martens et al., 1982), due to the breakage of hydrogen bonds with a consequent loss of the fibrillar structure and contraction of the collagen molecule. If the collagen fibres are not stabilized by heat-resistant intermolecular bonds, they dissolve completely and form gelatine by further heating. The amount of these bonds is related to the animal age. In older animals, due to the presence of a higher amount of heat-resistant intermolecular bonds, only part of the fiber matrix dissolves (Light et al., 1985). The consequence is a different tenderness in the final cooked meat. Finally, sarcoplasmatic proteins denature and aggregate between 40 and 60 °C. Collagenase enzyme remains active until 60 °C continuing to hydrolyse proteins

until 70–80 °C, causing changes in texture that result in meat softening (Laakkonen et al., 1970).

All these chemical modifications can cause hardening or softening of the meat during cooking, depending on the specific type of meat considered, animal species and consequent protein composition, together with the cooking method, the cooking time and relative temperature (Rao & Lund, 1986; Van Laak & Lane, 2000; Murphy & Marks, 2000; Lawrence et al., 2001; King et al, 2003; Wattanachant et al., 2005; Chiavaro et al., 2009; Roldán et al., 2013; Rabeler & Feyissa, 2018).

1.5.2 Effect of cooking on colour

The colour of meat is a combination of non-enzymatic browning and myoglobin modification. A cascade of events related to meat colour changes are triggered during cooking.

Maillard reaction is the main responsible of colour change and it occurs, due to both temperature increase and meat surface dehydration (Shahidi et al., 2014). As already said in Chapter 1.4.1, in the last phase of the reaction melanoidines compounds are formed. They are high molecular brown compounds which impart the typical brown colour to meat. Their amount is proportional to heating time and temperature (King & Whyte, 2006; Trevisan et al., 2006; Starowicz & Zieliński, 2019).

Simultaneously to denaturation of Maillard reaction, myoglobin modification occurs. Myoglobin is the most responsible pigment of the meat colour. This protein is composed by a globin protein and a heme group containing a central iron atom able to link water or oxygen. Myoglobin can exists in 3 main forms, each of them producing a distinctive colour (Figure 6): the physiologically active oxygen linking oxymyoglobin (oxyMb) and deoxymyoglobin (deoxyMb) forms containing an oxidated iron molecule Fe(II), and the reduced form of metmyoglobin (MetMb) containing a reduced iron molecule Fe(III) (King & Whyte, 2006; Faustman et al., 2023).

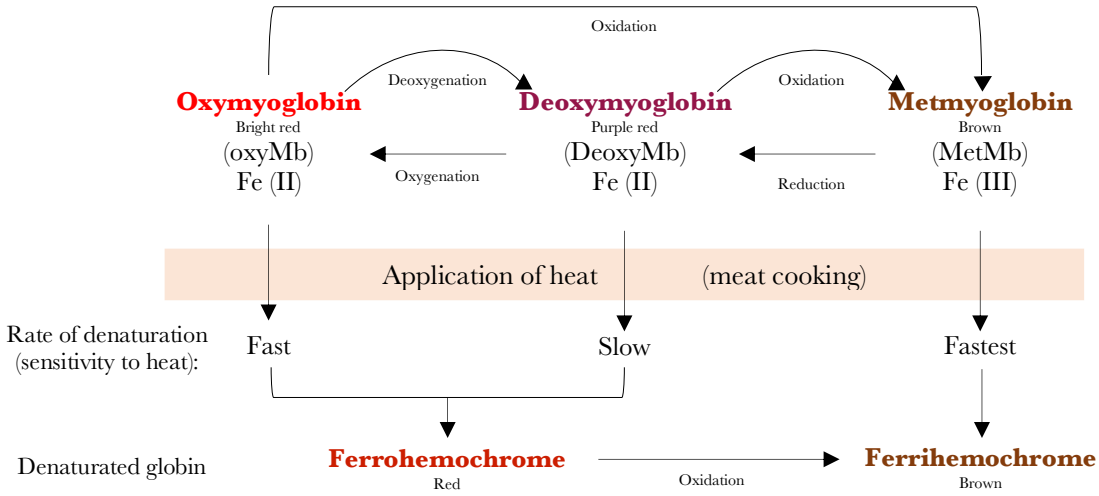


Figure 6 Characteristics of the myoglobin pigments in meat, their dynamic relationships, and the denatured products formed during cooking (modified from King & Whyte, 2006).

The heating process starts denaturing oxyMb and DeoxyMb around 55–65 °C until 75–85 °C in a red compound called ferrohemochrome, which can be oxidised in ferrihemochrome, a brown compound, which can also result from MetMb denaturation. Therefore, the ultimate colour depends on the extent of ferrihemochrome formation (King & Whyte, 2006; Faustman et al., 2023). The rate and the extent of denaturation increase with temperature and cause first whitening of the muscle, then grey or brown hues, depending on the type of muscle (Martens et al., 1982; King & Whyte, 2006).

1.5.3 Effect of cooking on aroma and flavour

Flavour is the result of taste and aroma mixture. Taste is a sensation related to the tongue, whereas aroma is a sensation of volatile compounds related to the epithelia of the nose. Flavour comprises a combination of non volatile and volatile compounds (Pegg & Shahidi, 2014).

The highest amounts of aroma compounds are formed during high thermally induced methods of meat preparation. The main reaction responsible of aroma and flavour development is the Maillard reaction, to confirm the above information. As already said, the intermediate phase of the reaction is responsible for aromatic compound release. Amadori compounds coming from the first phase of the reaction are degraded in numerous low molecular weight volatile products (Khan et al., 2015). It is a matter of fact that, the profile of volatile compounds strictly depends on the content of sugars and amino acids in a raw material (Van Ba et al., 2012). Some derivatives of pyrazines were found to be responsible

for the pleasant aroma of meat, subjected to roasting and grilling, in particular 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine (García-Lomillo, et a., 2016).

Another reaction contributing to the aromatic profile of meat is lipid oxidation. Lipid oxidation is a complex process triggered by high temperature, metals and light whereby unsaturated fatty acids react with molecular oxygen via a free radical mechanism (Asghar & Pearson, 1980). However, the defect may appear over time, in freshly cooked not consumed products stored over time. The major primary products of this lipid oxidation are hydroperoxides, relatively unstable and essentially odourless, which decompose into a wide range of secondary compounds, including alkanes, alkenes, aldehydes, ketones, alcohols, esters, acids and hydrocarbons. Among these compounds, aldehydes are considered the most important breakdown products because they possess low threshold values and are the major contributors to the development of rancidity odours. Some of these give unpleasant odours to food, defined off-flavours, making it unacceptable (Ladikos & Lougovois, 1990).

The higher the degree of unsaturation of fatty acids, the more susceptible they will be to lipid oxidation. From composition point of view, chicken meat has the highest levels of polyunsaturated fatty acids (PUFA) compared to lamb or beef, especially oleic acids and linoleic. For this reason, chicken oxidizes faster compared to beef (Calkins & Hodgen, 2007).

1.5.4 Effect of cooking on nutritional profile

Cooking can improve or reduce nutritional quality of meat due to chemical changes of meat components. First, digestibility and bioavailability of nutrients can increase with cooking (Davey & Gilbert, 1974; Meade et al., 2005). High temperatures lead to denaturation of proteins, unwinding them and causing loss of connections among muscle fibres. Cooked meat is thus easy to chew and digest in the small intestine because proteolytic enzymes have an easier access to hydrolytic sites (Santé-Lhoutellier et al., 2008).

Cooking can affect the overall nutritional composition of meat, including fat profile, amino acids, and bioactive compounds such as vitamins (Lombardi-Boccia et al., 2005; Gerber et al., 2009; Domínguez et al., 2015; Lopes et al., 2015). Cooking is linked with the formation of reactive oxygen species (ROS) which contribute to oxidate nutrients (Heshmati et al., 2013). Lipidic fraction is one of them, responsible for ROS formation, and in particular polyunsaturated fatty acids (PUFA Ω3, Ω6, Ω9) are the most susceptible ones (Calkins & Hodgen, 2007).

Protein can be also oxidated by ROS. Oxidation of proteins can derive from various mechanisms involving protein modifications. It can determine oxidative modification of the amino acids side chains, conversion of one amino acid into a different one, fragmentation of the peptide backbone and the formation of intra- and inter- molecular cross-link (Estevez, 2011).

The main events than can happen inside proteins due to oxidation are an increase in surface hydrophobicity caused by unfolding of proteins with hydrophobic amino acids exposure (Santé-Lhoutellier et al., 2008; Traore et al., 2012); aggregation of meat proteins due to covalent modifications caused by formation of disulfide and dityrosine bridges resulting in oxidation of cysteine and tyrosine (Santé-Lhoutellier et al., 2008; Cui et al., 2009; Gatellier et al., 2010); increase in protein carbonylation (Gatellier et al., 2010; Traore et al., 2012; Roldán et al., 2014). Carbonyls (aldehydes and ketones) can be formed in proteins through four different pathways, namely, i) direct oxidation of the side chains from lysine, threonine, arginine and proline (Requena et al., 2001), ii) non-enzymatic glycation in the presence of reducing sugars (Akagawa et al., 2005); iii) oxidative cleavage of the peptide backbone via the α-amidation pathway or via oxidation of glutamyl side chains (Garrison, 1987; Berlett & Stadtman, 1997) and iv) covalent binding to non-protein carbonyl compounds such as 4-hydroxy-2-nonenal (HNE) or malondialdehyde (MDA) (Feeney et al., 1975). Protein oxidation is responsible for a decrease in their digestibility and so their reduction in the nutritional value (Estévez, 2011).

During food cooking, water content can decrease in the food matrix and consequently the concentration of the remaining substances, such as proteins, fats, but also minerals and vitamins, increase (Hosseini et al., 2014; Karimian-Khosroshahi et al., 2015). On the other hand, with heating, minerals and vitamin can be lost, together with the most heat-labile compounds inside meat. The loss or retention of these nutrients highly depend on the specific cooking method used and the relative temperature adopted (Lund, 1988; Kumar & Aalbersberg, 2006; Lešková et al., 2006).

1.5.5 Effect of cooking on safety

In addition to desirable or undesirable quality modification, heating has an advantageous effect on microbiological spoilage. As already pointed out, in some cases, the cooking of meat is compulsory in order to reduce certain pathogens before consumption, i.e. *Salmonella* spp. in poultry meat (Aiyegoro, 2014). However, sometimes destruction of contaminating microorganisms of meat is not the primary purpose of cooking.

Meat commences as sterile muscle in a living animal. During transport, lairage, stunning, slaughter, and dressing, the exposed muscle is showered by bacteria and other contaminants while it undergoes anaerobic glycolysis and becomes meat. The ever-changing surface of the meat presents a range of suitable environments for growth of various microorganisms. Microorganisms on fresh meat may be pathogens, i.e., microorganisms causing food poisoning that are a high risk to human health. They include *Salmonella* spp.*, Escherichia coli, Campylobacter jejuni-coli, Yersinia enterocolitica (pork), Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens, Clostridium botulinum, Bacillus cereus,* etc. Other microorganisms on fresh meat could be spoilage bacteria that may cause off-odours and slime on meat surfaces. This group include Gram-negative rods (*Acinetobacter–Moraxella, Aeromonas, Alcaligenes, Flavobacterium, Pseudomonas, and Enterobacteriaceae*), Gram-positive rods (*Corynebacterium, Lactic acid bacteria, Brochothrix thermosphacta, Bacillus*), Gram-positive cocci (*Micrococci, Staphylococci, fecal Streptococci*), yeasts, and mold (Aiyegoro, 2014). Each type of microorganism is characterized by specific parameters of thermoresistance (i.e. D_T*, z*) and depending on the intensity and time of the heat treatment, the microbial load is more or less reduced. As already said, in some cases, the cooking of meat is compulsory in order to reduce certain pathogens before consumption, i.e. *Salmonella* spp. in poultry meat (Aiyegoro, 2014).

Cooking meat at high temperatures may lead to the formation of other undesirable compounds, such as heterocyclic aromatic amines (HAAs), formed during Maillard Reaction in presence of creatine (Gibis, 2016) and polycyclic aromatic hydrocarbons (PAHs), deriving from reaction among lipidic free radical (Singh et al., 2016). Epidemiological research suggests that consuming large amounts of well-cooked meat, especially red meat, is associated with a higher likelihood of developing various types of cancers, such as colon, pancreatic, gastrointestinal, lung, liver, prostate, skin, and breast cancers. These risks are connected to exposure to elevated levels of HAAs and PAHs (John et al., 2011).

1.6 Meat cooking methods

The cooking method is one of the major factors that affect the eating quality of meat (James & James, 2014). Cooking is a unit operation involving heat transfer (from heating medium to product) and sometimes mass (product to environment or vice versa). Conduction, convection and radiation are the three heating transfer modalities. According to heat transfer medium and its temperature, cooking methods can be classified in:

- moist heat methods, using hot water as heating medium (boiling, braising, simmering) or steam (steam cooking);
- dry heat methods, transferring heat through air (roasting, oven cooking), direct fire (broiling, barbecuing) or hot oil or fat (frying, confiting);
- alternatives, involving the use of microwave, radio frequency, ohmic heating.

As regards meat cooking in food service, oven is the most used equipment, because it facilitates a high volume of foodstuffs to be cooked at the same time in a controlled and uniform way. In particular in food service, an optimal meat cooking process should firstly ensure safety of meat at point of consumption through pathogen inactivation and also facilitate the development of desirable sensory attributes, while maintaining technological performance e.g. in terms of adequate cooking yield.

Table 2 reports the most commonly used cooking method in food service, where it is possible to see the important use of oven. Conventional oven cooking methods use conduction, convection, and radiation as mechanism for heat transfer. Oven roasting is a common cooking method used to enhance the flavour of meat via caramelization and Maillard reaction occurring on the surface of the food. This is accomplished using dry heat, at temperatures above between 170–180 °C, over variable cooking times (Mottram, 2007; Roldán et al., 2013). Grilling implies the use of a direct heat source or the oven. The heat source, such as thermal radiation or direct conduction, may differ according to the type of grill. Direct grilling can expose food to a temperature up to 260 °C, resulting in grilled meat with aroma and flavour characteristics similar to those achieved by roasting, due to the Maillard reaction. Grilling in the oven involves the achievement of around 200–260 °C and promotes the development of the advanced phases of the Maillard reaction. This means that great flavour and taste will be developed, but also several undesired effects, such as toxic compounds formations. Moreover, high temperature can also promote lipid oxidation, significant shrinkage of meat with related of dryness (Mottram, 2007). Sous-vide is a French expression for "under vacuum". It consists of vacuum-sealing meat in a heat-stable airtight

plastic bag, which is further placed in a water bath, or in a temperature-controlled steam environment, for an extended cooking time at low temperature (<100 °C). Sous-vide prevents the loss of aromatic volatiles and preserves moisture content, resulting in especially flavourful and nutritious food (Baldwin, 2012; Roldán et al., 2015). Boiling is a simple cooking method widely used to cook meat, involving water at the boiling point of 100 °C. This technique is able to retain the taste and aroma due to retention of aromatic volatiles, and nutrients, as well as limiting lipidic oxidation. However, loss of water-soluble vitamins and minerals may occur together with discoloration of the meat (Cupisti et al., 2006; Yu et al., 2017). Frying is a cooking method in which food is submerged in hot fat, most commonly in cooking oil. Typically, it is a rapid preparation technique that promotes physical and chemical changes in the products and leads to unique colour, flavour, texture, and palatability development (Ismail-Fitry et al., 2008; Shabbir et al., 2015).

Table 2 Most common cooking methods adopted in food service, relative cooking settings, equipment, benefits and disadvantages.

Method	Cooking settings	Equipment	Benefits	Disadvantages	References
Roasting	Temperature: 170–180 °C	Oven Pan	Taste and aroma development due to Maillard reaction Fat retention Attractive cooking appearance	Possible formation of burns and high amount of toxic products (HAAs and PAHs) Possible lipid oxidation for T <150 °C	Modzelewska-Kapituła et al., 2012; Roldán et al., 2013
Grilling	Temperature: 200–260 °C	Oven Grid	Intense taste and aroma development due to intense Maillard reaction Attractive cooking appearance	Formation of burns and high amount of toxic products (HAAs and PAHs) Lipid oxidation products formation Significant shrinkage of meat Possible of dryness	Mottram, 2007
Sous-vide	Temperature: 60–90 °C Oven steam: 100 %	Oven Water bath	Prevention of microbial re-contamination Taste and aroma development and retention of aromatic volatiles Retention of the nutrients Texture softness due to preservation of moisture content	No Maillard reaction development Greater time required to reach the final cooking temperature and time Appearance not always attractive due to discoloration of meat	Baldwin, 2012; Roldan et al., 2015
Boiling	Temperature: ~100 °C	Pot	Taste and aroma development due to retention of aromatic volatiles Limited lipid oxidation products formation Retention of the nutrients	Loss of water-soluble vitamins and some minerals (iron, sodium etc.) Discoloration of the meat	Cupisti et al., 2006; Yu et al., 2017
Deep frying	Temperature range: 175–190 °C	Pan Deep fryer	Bacteria destruction Taste and aroma development Crispy texture Brownish crust	Glycotoxins, HAAs, PAHs, acrylamides and trans fatty acids development Fat absorption	Ismail-Fitry et al., 2008; Shabbir et al., 2015

***** HAAs Heterocyclic Aromatic Amines
PAHs Polycyclic Aromatic Hydrocarbon

1.7 Optimal cooking process in food service

When a foodstuff, such as a meat dish, is proposed and served in the food service, firstly safety at the point of consumption must be guaranteed. Microbiological limits must be respected according to the hygiene directives for the specific food considered. For example, in case of chicken meat, The United States Department of Agriculture has proposed a 7-log reduction in the population of *Salmonella* spp. for poultry products (USDA & FSIS, 2021). In fact, *Salmonella* infections are often associated with the consumption of raw or undercooked poultry (EFSA, 2007). Safety can be achieved by cooking meat until reaching a minimum internal temperature of 74 °C (USDA & FSIS, 2021). Moreover, adequate cooking should be performed in order to develop the desired food quality too.

As already pointed out, food quality includes sensory attributes (texture, taste, flavour, colour, etc.), nutritional value (digestibility, bioaccessibility, etc.) and technological performance (cooking yield, hardness etc.) (Figure 7).

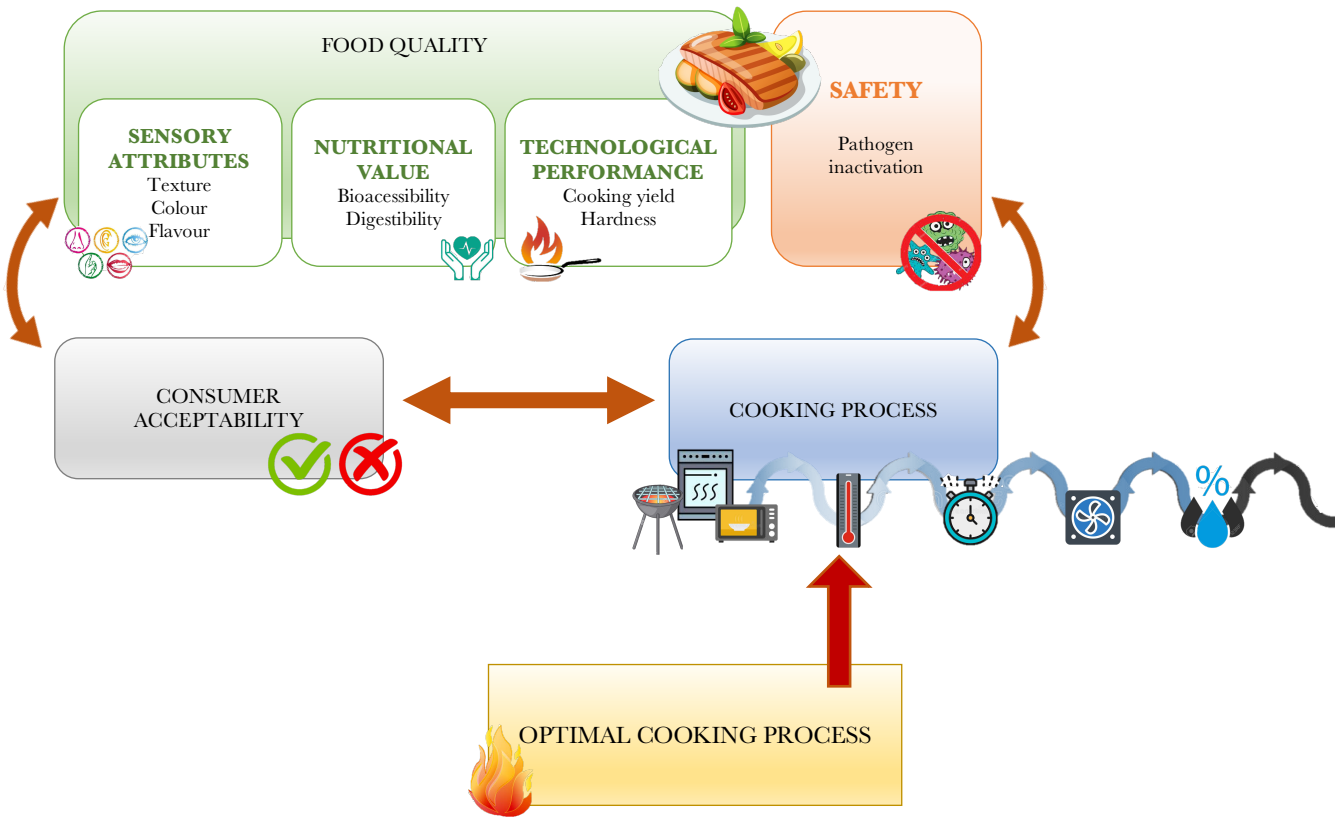


Figure 7 Schematic comprehensive overview of food cooking in the food service.

Both safety and food quality contribute together to the proprieties of the food served to the final consumer. As shown in Figure 7, food quality also depends on the consumer, who defines it through his/her preferences. Thanks to previous sensory analysis or consumer taste surveys, professional chefs of food service already know on average consumers' tastes and cook a dish according to their suggestions.

Overall, food quality and safety, and consumer acceptability depend on and at the same time outline the specific cooking process used to cook the meal (Figure 7). Each cooking process imparts particular characteristics to the food and is related to the cooking technology adopted and the associated variables, such as temperature, time, ventilation, and relative humidity.

The condition able to harmonize all these different factors is the application of an optimal cooking process (Figure 7). A cooking process can be considered optimal when it is able to maximize the positive and desired effects that cooking can impart, such as safety, sensory attributes, nutritional value and technological performance, and minimize as much as possible the undesired ones, such as burns or dangerous compounds development.

Identify the optimal cooking conditions, in particular in terms of cooking time, could be considered the key for the improvement of food quality and consumer satisfaction. No less identify an optimal cooking time, for example, can represent an economic advantage because it prevents waste energy by avoiding possible cooking longer than necessary.

1.8 Define the optimal end point of cooking

Independently of the cooking technique, generally a cooking process can be defined by cooking temperature and time. The identification of the most appropriate cooking time for the cooking of a specific food can be a hard challenge.

In food service professional chefs cook foodstuffs driven by consumer surveys or sometimes according to their personal experiences or previous cooking trials. This enables them to identify the suitable cooking settings such as temperature and times. However, most of the times, cooking is a subjective process devoid of a methodical approach because driven by chefs' personal tastes. Useful tools can help chefs in monitoring the cooking process and fixing a final cooking time. This is the case of temperature probes, widely used as aid in food service. Professional ovens, which are the most used device in the food service sector, are usually equipped with a temperature probe. At present, temperature probes monitoring the internal temperature of food products during cooking can be considered the only support

able to drive the cooking until reaching the desired internal temperature. They are used mainly to ensure the safety of the product. However, it is not easy for probes to accurately measure the temperature in the centre of the food and deviation of ±1 mm in the temperature sensor position can cause temperature changes of 3–5 °C (Kondjoyan et al., 2013; Moya et al. 2021).

The problem with this type of equipment is that preferences from the point of view of consumer's sensory perception may not coincide with safe temperatures (López Osornio et al., 2008). To our knowledge, no other sensors able to monitor food attributes, such as quality modifications in addition to temperature exist or are used at present in professional kitchens or food service.

Finding an effective way able to establish the end of cooking by combining both the mandatory respect of safety limits and the maximization of sensory quality could be a hard task. The addition of an assistance system to chefs inside cooking machinery able to improve quality and safety and accelerate operations could be of huge help. Integration of on-line sensors capable of securing the firing and monitoring it until the optimal time is achieved is what is needed to meet the need. Especially in food service, where public safety and consumer satisfaction are of main concern, this outcome would be very useful. Even more, in the period of outdoor consumption increase and related safety issues rise (i.e. increase of poultry products consumption), optimization in terms of quality and safety of cooking machinery is mandatory.

1.9 Optimal cooking process identification: the kinetic modelling approach

In order to identify the optimal cooking time as a result of matching both safety and sensory quality, the application of a kinetic modelling approach would be very useful. Kinetic modelling of the evolution of quality indices of the product during cooking would represent a powerful tool for understanding the changes of quality attributes, predicting the outcomes and identifying the optimal cooking process (Ling et al., 2015). As shown in Figure 8, the approach of modelling can be divided in 3 phases: data collection, critical quality index identification and model extrapolation. Then a final step of model application can be considered. The different phases are detailed below.

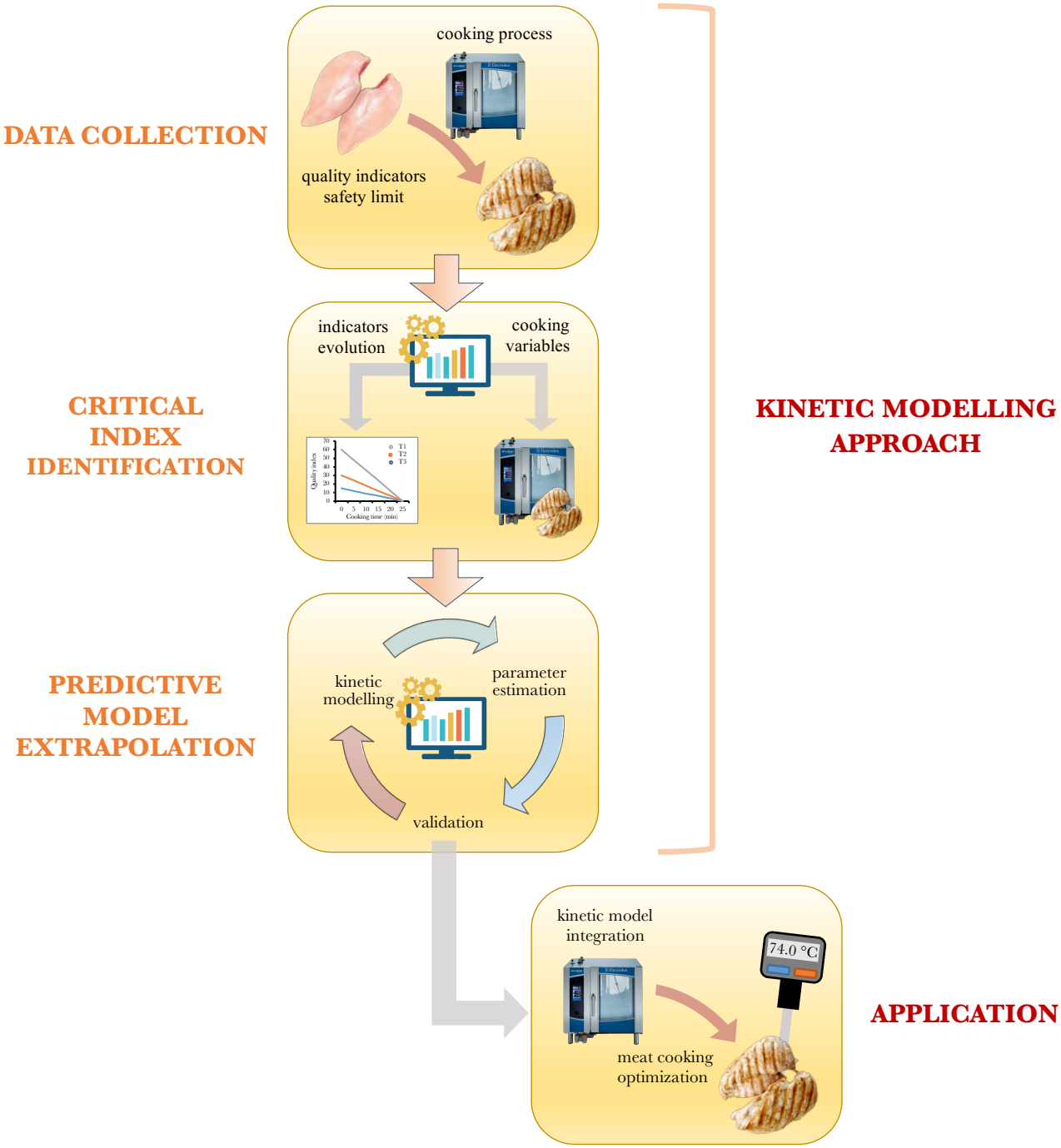


Figure 8 A schematic summary of the kinetic modelling approach and its application.

1.9.1 Data collection and critical index identification

In order to develop a model able to predict a cooking process, the kinetic approach firstly requires identifying the main quality indicators associated to the food being studied able to best describe the effect of cooking process evolution on it. An indicator is a parameter able to vary as affected by a process or time and it can be analysed through a scientific method. The indicators might be determined through fast and cheap instrumental analysis, or sensory analysis, which is slower and more expensive. Quality indicators can be related to physical, chemical, nutritional, sensory or toxicological modifications of the food and could differ in accordance with the food under review. Considering meat cooking in an oven as study case, quality indices to be considered could be cooking loss, texture, colour, moisture, or protein digestibility. Once the indicators have been identified, their evolution should be monitored during the cooking process under examination, by recording their specific amount or value for increasing cooking times.

Among all the different indices, a so-called critical indicator should be then recognised. A critical indicator is the one best describing the evolution of the cooking process on the food under study. Most of the times, the critical indicator corresponds to the indicator which changes faster over time. This can be considered as the most sensitive indicator to temperature changes, but it is not obvious. Sometimes cooking can be limited by regulations and safety directives and for this reason the critical indicator must undergo to this boundary. This is the case of chicken poultry, which must achieve at least 74 °C at the core of the product to be considered safe for the consumption (USDA & FSIS, 2021). The critical indicator will then correspond to the indicator of the alteration event whose trend, between all, better correlate consumers rejection (Kilcast & Subramaniam, 2000; Calligaris & Manzocco, 2012).

1.9.2 Predictive model extrapolation

A mathematical approach to identify the indicator most sensitive to temperature changes should be firstly applied by modelling the data obtained during cooking trials. The most widely used approach to analyse experimental data is the application of the principles of kinetic theory.

Although the general law of a reaction speed has been developed theoretically for chemical reactions, its significance has been demonstrated also for numerous complex chemical, biochemical and physical phenomena that occur in the foods (Calligaris et al., 2012).

Chemical reaction kinetics can be applied to quantify individual attribute of an ideal food system in form of the general rate law (van Boekel 1996; Steinfeld et al. 1998; FDA 2000):

$$\frac{dI}{dt} = \pm kI^n \qquad \textit{Eq. 1}$$

where k is the rate constant, t the reaction time, and n the reaction order. In general, I represents a quantitative value for a quality attribute, enzyme activity, or population of microorganisms. The core of kinetic studies on food quality changes in thermal processing is to quantify a quality attribute value as a function of heating time at a certain temperature (van Boekel, 2008; Ling et al., 2015). The order of kinetics is determined based on the goodness of fit of the observations to a preselected reaction order model. Kinetics of food quality changes generally follows zero-, first-, or second-order reactions as follows:

$$I = I_0 - kt \qquad \text{for zero−order reactions (n=0)} \qquad \textit{Eq. 2}$$

$$I = I_0 e^{-kt} \qquad \text{for first−order reactions (n=1)} \qquad \textit{Eq. 3}$$

$$\frac{1}{I} = kt + \frac{1}{I_0} \qquad \text{for second−order reactions (n=2)} \qquad \textit{Eq. 4}$$

where I_0 is the initial value of the food quality attribute at $t = 0$.

Several studies about muscle foods and vegetables show that a general rate law, describing a zero-, first-, or second-order kinetic models can depict quality degradation during thermal treatments (van Boekel, 2008; Ling et al., 2015).

Extrapolation of rate constant from quality indicators kinetics is fundamental and the comparison among them allows the identification of the most sensitive index to temperature change. The most sensitive indicator has the highest value of rate constant. This means that the quality index changes more markedly than other ones as affected by the same temperature modification.

The kinetic data elaboration is useful not only to identify the critical indicator but also because it represents the basis for further elaborations aimed at the development of a predictive cooking model.

To explore the temperature-dependent quality, the Arrhenius equation is the most common method to describe the temperature (T) effect on the reaction rate constant (k) as follows:

$$k = k_0 e^{-\frac{E_a}{RT}} \quad \textit{Eq. 5}$$

Or in linearized form

$$lnk = lnk_0 - \frac{E_a}{RT} \quad \textit{Eq. 6}$$

where k_0 is the rate constant, R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature (K). E_a is the activation energy (J mol^{-1}) and is defined as the minimum energy needed to start a chemical reaction (sometimes called the energy barrier). A chemical reaction at a reasonable rate happens when an appreciable number of molecules with energy equal to or greater than the activation energy is formed. When temperature increases, the number of molecules with energy greater than the activation energy increases, thus improving the rate of reaction. Therefore, the activation energy is a parameter that indicates the sensitivity of the reaction rate to temperature. For greater activation energy, the rate of reaction is more sensitive to temperature changes and *vice versa* (Ling et al., 2015).

In thermal processing, decimal reduction time (D_T) is defined as the heating interval time required to reduce by 10 times (1 *log*) or 90% the initial value of the indicator at a constant temperature. The D_T value is directly related to the first-order reaction rate constant k by the following equation (Anthon & Barrett, 2002; Awuah et al., 2007; van Boekel, 2008).

$$D_T = \frac{2.303}{k} \quad \textit{Eq. 7}$$

By considering this concept, the variation of the chosen indicator can be easily determined by calculating the D_T value. During the design of a process, this parameter allows to know how fast the indicator changes over time.

These mathematical models can be combined together in order to relate the temperature of the cooking process with the rate of the indicators variation over the cooking process. A model able to predict the quality evolution of a foodstuff during a cooking process can be obtained by considering the critical quality indicator of that food as affected by temperature. The final aim is to extrapolate a predictive model able to establish the optimal cooking time as a function of process temperature, the value of the critical indicator at the beginning and at the end of the cooking:

$$t_{cooking} = f(T, I, I_0) \qquad \text{Eq. 8}$$

where $t_{cooking}$ is optimal cooking time, T is the process temperature, I_0 is the amount of the critical indicator ad the beginning and I is the amount of the critical indicator at the chosen end cooking point.

The end of cooking should identify the optimal cooking time or the value able to maximize the quality of the cooked product. It should be identified by matching safety issue and consumer preferences with the critical quality index.

1.9.3 Application

The predictive model could be integrated inside a cooking device in order to optimize the food cooking. An automatic program could calculate the optimal cooking time starting from the insertion of the chosen variables (T, I, I_0, or other ones).

A further exploitation of the previous findings could be the insertion of a sensor able to analyse the critical quality indicator. By continuously monitoring the real-time evolution of the critical quality indicator, a suitable mathematical expression converted into a program would be capable of controlling the cooking process and stopping it when the indicator reaches a specific end-of-cooking value. Therefore, it is essential to identify a straightforward critical quality index and integrate a practical sensor into the cooking technology for continuous monitoring.

Aim and outline of the Ph.D. thesis

This Ph.D. thesis aimed at investigating a suitable way to optimize the cooking of chicken breast meat by using professional oven equipment. Identification of a valid approach capable of taking into account sensory and nutritional quality with respect of safety requirement was necessary in order to identify an optimal cooking time. Moreover, the possibility of selecting a suitable and easy-to-measure indicator to be adopted instead of temperature, sometimes a critical one, was also examined with the objective of developing a model predicting the cooking time. Nutritional profile modifications as affected by cooking treatment was also examined in terms of protein digestibility.

PART 1: Optimal cooking process identification: the kinetic modelling approach

Part 1 was addressed to establish the kinetic modelling approach. It was divided into 3 phases, as follows.

Chapter 2: Monitoring safety and quality indicators upon cooking of chicken breast meat undergone different oven cooking methods

In chapter 2, quality indicators (i.e. cooking loss, colour, and texture) were monitored for increasing cooking times. Three different cooking methods were identified as the most used in the food service, Grill, Forced Convection and Sous-Vide. Time temperature profiles were collected to investigate the temperature evolution in the thermally less favoured point and establish safety limits. In the end, the above-mentioned indicators were examined in order to identify the best one describing quality evolution of meat during cooking. Safety and critical quality indicators times were finally compared to recognise the limiting factor of the cooking process.

Chapter 3: Kinetic study on quality changes of chicken breast meat undergone different oven cooking methods

In chapter 3, kinetics of quality indicators evolution of chicken breast during cooking were investigated, according to the cooking methods previously mentioned (Grill, Forced Convection and Sous-Vide). Three different cooking temperatures for each cooking method were applied in order to explore the effect of temperature on quality indices evolution. Then, Arrhenius model was applied to study the temperature dependence of quality indicator rates and related Arrhenius parameters were computed.

Chapter 4: Identification of relevant and easy-to-measure cooking indicator for on-line management of chicken breast meat cooking process

In chapter 4, an evaluation was conducted to understand whether a quality indicator other than temperature could be used to predict the cooking process. Then, predictive cooking models were developed by matching kinetic models with Arrhenius model for each quality index. A cross-validation was conducted in order to assess the goodness of the predictions of the model and to understand which model associated to a specific quality indicator best predicted the cooking process.

PART 2

Part 2 was focused on nutritional profile evaluation as follows.

Chapter 5: Evaluation of protein digestibility of chicken breast meat undergone different cooking methods and times

In Chapter 5, the effect of cooking method and time on protein digestibility of chicken breast meat was evaluated. Three different oven cooking methods previously mentioned (Grill, Forced Convection and Sous-Vide) were considered and two different cooking times were assessed, an optimal time coinciding with the safety requirement, and an overcooked time. Static *in vitro* protein digestibility was carried out according to INFOGEST protocol. The method was adapted to chicken breast sample, focusing on oral processing and samples preparation. Then, the effect of cooking method and time on modification of chicken meat was examined according to proteins oxidation analysis (carbonyl groups) and structural analysis (FT-IR analysis).

Part	Chapter	Description	Aim
	Monitoring safety and quality indicators upon cooking of chicken breast meat undergone different oven cooking methods	Monitoring of cooking loss, colour, and texture of chicken breast meat for increasing cooking times according to different oven cooking methods and study of temperature profile for safety assessment	Comparison of safety time and quality indicators evolution to identify the limiting factor of the cooking process among them
Optimal cooking process identification: the kinetic modelling approach	Kinetic study on quality changes of chicken breast meat undergone different oven cooking methods	Investigation of the temperature dependence of quality indices rates by testing three different cooking temperatures for each cooking method	Extrapolation of *Ea* of quality events thanks to the application of Arrhenius equation
	Identification of relevant and easy-to-measure cooking indicator for on-line management of chicken breast meat cooking process	Development of predictive cooking models and relative cross-validation	Identification of the best cooking predictive model for chicken breast meat as a function of temperature and one quality indicator
Nutritional profile evaluation	Evaluation of protein digestibility of chicken breast meat undergone different cooking methods and times	Exploration of the effect of cooking method and time on protein digestibility and protein chemical and structural modification of chicken breast meat	Assessment of the impact of cooking on chicken breast meat nutritional value

PART 1

Optimal cooking process identification: the kinetic modelling approach

In the complex framework of evolving food service over the next years, meat consumption is expected to increase. At the moment, poultry meat consumption is the most requested meat and in the future its demand is projected to grow even more. The high nutritional value, the low religious barriers, the ease of handling and the commercial cost-effectiveness are some of the reasons why an increase in poultry meat consumption is expected in the future.

During cooking, poultry meat undergoes to several chemical-physical modifications able to affect technological performances and final quality of the foodstuff. For example, chicken undercooking may not meet safety requirements due to the failure to *Salmonella* spp. or overcooking may lead to water loss and consecutive dryness (Kondjoyan et al., 2013).

Identify the best cooking conditions of chicken meat in terms of time and temperature is essential to satisfy the continuous and growing requests of an increasingly attentive and demanding food service consumer. Optimizing and improving cooking machinery in the food service is necessary to reach this aim. In particular, professional ovens are widely used to cook meat in the food service because they allow a high volume of foodstuffs to be cooked at the same time in a controlled way. The development of a useful tool such as a predictive model or an innovative on-line sensor able to continuously monitor the evolution of the cooking process could be the right solution. For this purpose, identify the optimal cooking time able to guarantee the safety and maximize the positive effects of cooking while limiting the undesired ones is mandatory.

In the following sections the different steps of the kinetic modelling approach will be explored. In particular, the following chapters deal with:

- Monitoring safety and quality indicators upon cooking of chicken breast meat undergone different oven cooking methods
- Kinetic study on quality changes of chicken breast meat undergone different oven cooking methods

- Identification of relevant and easy-to-measure cooking indicator for on-line management of chicken breast meat cooking process

2 Chapter

Monitoring safety and quality indicators upon cooking of chicken breast meat undergone different oven cooking methods

2.1 Introduction

In order to optimize food quality during cooking, the development of a predictive model based on safety and quality can be considered. In order to develop it, at the beginning of the study an in-depth analysis related to the foodstuff under examination is necessary. In particular, safety limits must be identified together with possible chemical and physical modifications related to quality to which the food is subjected over the cooking time. At first, studying the evolution and the acceptable limits of safety issues is fundamental for ensuring a safe product to the consumers at the point of consumption. For example, regarding poultry meat, cooking until reaching 74 °C at the thermally less favoured point is required for *Salmonella* spp. destruction (USDA & FSIS, 2021). Once establish the safety limitation, quality can be taken in consideration. During cooking, several modifications can occur to the components of the food. Associate each event to a reference indicator measurable through a scientific analysis, give the possibility of monitoring it during the heating process. The comparison among quality evolution trends and safety limits allows to identify the limiting factor for the evolution of the quality during the process.

2.2 Aim of the Study

Based on these considerations, the aim of the study was to monitor safety and quality indicators during chicken breast cooking using different oven cooking methods. Three different oven cooking methods were identified as the most commonly used in the food service and named Grill (G, T=240 °C), Forced Convection (FC, T=170 °C) and Sous-Vide (SV, T=95 °C, RH%=100). Time temperature profiles were analysed to investigate the temperature evolution in the thermally less favoured point and link it with safety. Then quality indicators, i.e. cooking loss, colour, and texture were monitored for increasing cooking times. In the end, the above-mentioned indicators were investigated in order to identify which of them better described quality evolution of meat during cooking. Safety and critical quality indicator were finally compared regarding time in order to identify the limiting factor of the cooking process.

2.3 Materials and Methods

2.3.1 Materials

A batch of individually quick-frozen chicken breast meat (without skin and bones) was purchased from an Italian food service supplier (MARR S.p.A., Rimini, Italy), and kept at -18 °C until use. Chicken breasts with average weight of 240 ± 10 g were selected.
Chicken breasts were removed from the freezer the night before the trials and thawed in a refrigerator overnight.

2.3.2 Cooking treatments

Thawed chicken breasts were cooked individually in the centre of a vessel in an electric professional oven (AOS101ETA1, 17.5 KW, Electrolux Professional, Pordenone, Italy), according to three different cooking methods, Grill (G), Forced Convection (FC) and Sous Vide (SV). The tested cooking processes are reported in Table 3, and for each one temperature was tested, which was the most used in the food service.

Table 3 Tray shape, cavity temperature, cooking time, and cavity humidity of each cooking method considered for chicken breast cooking by using a professional oven.

Cooking method	Tray Shape	Cavity temperature (°C)	Increasing times up to (min)	Relative humidity (%)
Grill (G)	Grid	240	19	---
Forced Convection (FC)	Flat	170	35	---
Sous Vide (SV)	Flat	95	35	100

Before SV cooking, samples were vacuum-packed in polyamide-polypropylene pouches (16x32 cm, 80 μm thickness, water vapour permeability <1 g m^{-2} 24 h^{-1}, Niederwieser Group S.p.A., Campogalliano, MO, Italy) by means of a vacuum machine (Orved, VM-16, Musile di Piave, Italy). Within each cooking process, experiments were replicated three times.
Cooking settings were established after preliminary cooking trials and carried out according to food service cooking guidelines (USDA & FSIS, 2021).

After cooking, samples were cooled in a blast chiller on an open aluminium tray for 30 min until reaching 4 °C in order to ensure temperature equilibration and standardization. Then, samples were immediately analysed for their chemical and physical chemical properties.

2.3.3 Temperature monitoring

Temperature changes of the oven cavity were monitored by a thermocouple (K-type; Ni/Al-Ni/Cr), while sample temperatures modifications were measured by a probe equipped with six internal thermocouples (K-type; Ni/Al-Ni/Cr) connected to a multimeter acquisition system (Multiplexer 34901A, Agilent, United States). In particular, the tip of thermocouple probes was placed both in the thermally less favoured point (i.e. the geometric centre) and adherent to the surface of the sample. Time-temperature profile graphs are the results of at least three replications for each cooking method.

2.3.4 Proximate composition

Grounded samples were poured into previously dehydrated aluminium weigh boat and dried overnight at 105 °C. Moisture content for each sample was calculated according to the official method (method 930.15; AOAC International, 2005) through a gravimetric evaluation as follows:

$$Moisture\ (\%) = \frac{Wet\ weight - dry\ weight}{wet\ weight} * 100 \qquad Eq.\ 9$$

Total protein was calculated from the nitrogen content of the samples by Kjeldahl analysis. The protein factor applied to the nitrogen result was 6.25 (method 968.06; AOAC International, 2005). Total fat was determined by using a standard Soxhlet extraction. This extraction process included submerging the sample into boiling ethyl ether and then lowering the solvent below the sample for a continuous flow of condensed solvent. The solvent was evaporated and recovered by condensation, and the resulting fat residue was gravimetrically determined after drying (method 991.36; AOAC International, 2005). Ash was measured based on the gravimetric loss by heating to 550 °C overnight (method 942.05; AOAC International, 2005). Carbohydrates were finally calculated by difference (total mass of moisture, total fat, ash and protein substrate from the mass of food). Almost five replications were conducted for each analysis.

2.3.5 Cooking loss

Cooking loss was calculated as the percentage weight difference between fresh and cooked samples relative to the weight of fresh meat samples as follows:

$$Cooking\ loss = \frac{Raw\ meat\ weight - cooked\ meat\ weight}{Raw\ meat\ weight} * 100 \qquad Eq.\ 10$$

Reported data are the mean of at least three replications.

2.3.6 Colour analysis

Colour determination of cooked chicken breast surface was carried out using a Minolta colorimeter (Chomameter-2 Reflectance, Minolta, Osaka, Japan) equipped with measuring head CR-200, and standard illuminant C. L* (lightness), a* (redness), b* (yellowness) using 2° position of the standard observer were obtained. Data were expressed as C* (chroma) values calculated as follows:

$$C^* = \sqrt{a^2 + b^2} \qquad Eq.\ 11$$

Reported data are the mean of ten repetitions acquired on different points of the meat surface. Al least three replications were conducted for each analysis.

2.3.7 Texture analysis

Texture analysis was performed by means of a shear test using Instron® (mod. 4301, Instron LTD, High Wycombe, United Kingdom) equipped with a 1 kN load cell.

Samples were cut into two pieces of 3x3x6 cm geometry from the internal part with muscle fibres running parallel to the major dimension. Shear force analysis was performed on raw and cooked samples using a Warner-Bratzler blade (3 mm thick), which sheared the specimen perpendicularly to the muscle fibres at a constant speed of 60 mm min^{-1} and then pushed through the slot (4 mm wide). The maximum force (N) required to shear the sample was measured. For each piece, three determinations were performed in order to obtain six determinations for each cooking treatment. Almost three replications were conducted for each analysis.

2.3.8 Differential Scanning Calorimetry

DSC analysis was carried out using the DSC 3 Star^e System differential scanning calorimeter (Mettler-Toledo, Greifensee, Swiss). Heat flow calibration was carried out using hexane, water, and indium (having melting points of -93.5 °C, 0.0 °C and 156.0 °C, respectively). Samples were prepared by carefully weighing around 10 mg of fresh meat in 40 μL aluminium DSC pans, closed with hermetic sealing. Samples were heated under nitrogen flow (20 mL min^{-1}) during analysis. Each sample was heated from 20 °C until 100 °C at a heating rate of 10 °C min^{-1} under a nitrogen flow. An empty pan was used as a reference in the DSC cell. The start and the end of melting transition were taken as on-set (T_{on}) and off-set (T_{off}) points of transition, that are points at which the extrapolated baseline intersects the extrapolated tangent of the calorimetric peak in the transition state. The machine equipment program STAR ever 16.10 (Mettler-Toledo, Greifensee, Switzerland) was used to plot and analyse the thermal data. Several repetitions were carried out and the most representative one was reported.

2.3.9 Statistical analysis

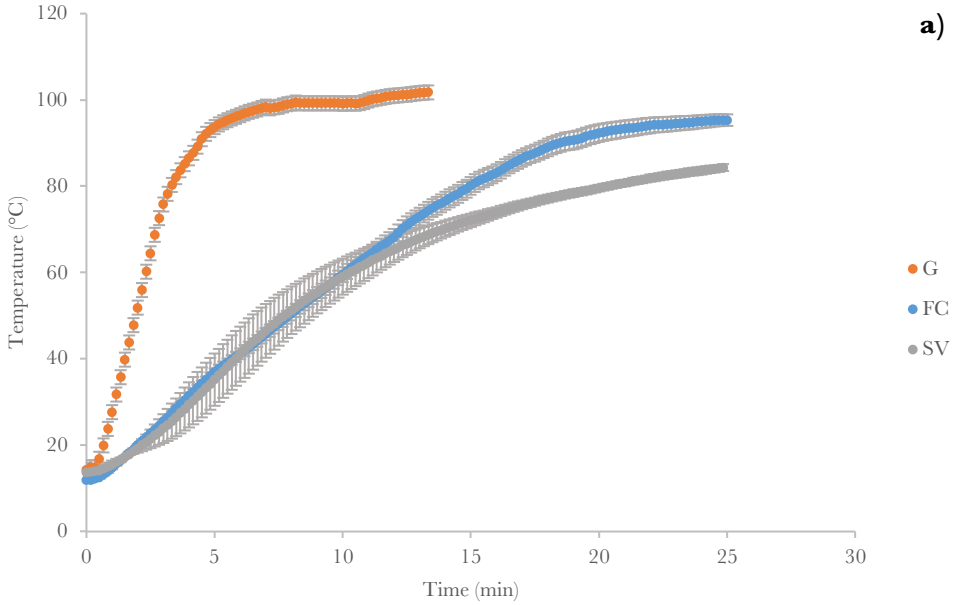
Data are expressed as means ± standard deviation. Data elaboration, representation and regression analysis was performed using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). Statistical analysis was performed using RStudio (version 4.2.2, The R Foundation for Statistical Computing, Vienna, Austria). Bartlett's test was used to check the homogeneity of variance. Analysis of variance (ANOVA) was conducted in case of homogeneity or Welch test in case the variances were not homogeneous. In both cases Tukey-HSD test was used to assess differences between means ($p < 0.05$). The goodness of models fitting was evaluated by using the coefficient of determination (R^2).

2.4 Results and Discussion

Average composition of raw chicken breast was as follows: moisture, 76.0 ± 1.3%; protein, 20.8 ± 2.2%; lipids, 0.62 ± 0.05%; ash, 1.14 ± 0.08%; carbohydrates, 1.47%. These data were in agreement with those reported by Lonergan et al. (2003) and Taşkıran et al. (2020). In particular, chicken breast has a high protein and a low fat content as compared to the other anatomical parts of the chicken itself as well as to other animal muscles (Soriano-Santos, 2010; Petracci et al., 2014; López-Bote, C., 2017).

Chicken breast was cooked by using three different cooking methods, namely Forced Convection (FC), Grill (G) and Sous-Vide (SV), which were chosen because they are the most commonly applied in the food service. The temperature values of 170 °C for FC, 240 °C for G and 95 °C for SV represent the reference cooking temperatures previously identified by professional chefs as the optimal conditions for development of chicken breast sensory properties meeting consumers' requirements.

As an example, Figure 9 shows the time-temperature profiles of the surface (a) and of the thermally less favoured point until reaching 74 °C (b) of chicken breast cooked according to FC, G and SV, with oven temperature set at 170 °C, 240 °C and 95 °C, respectively.



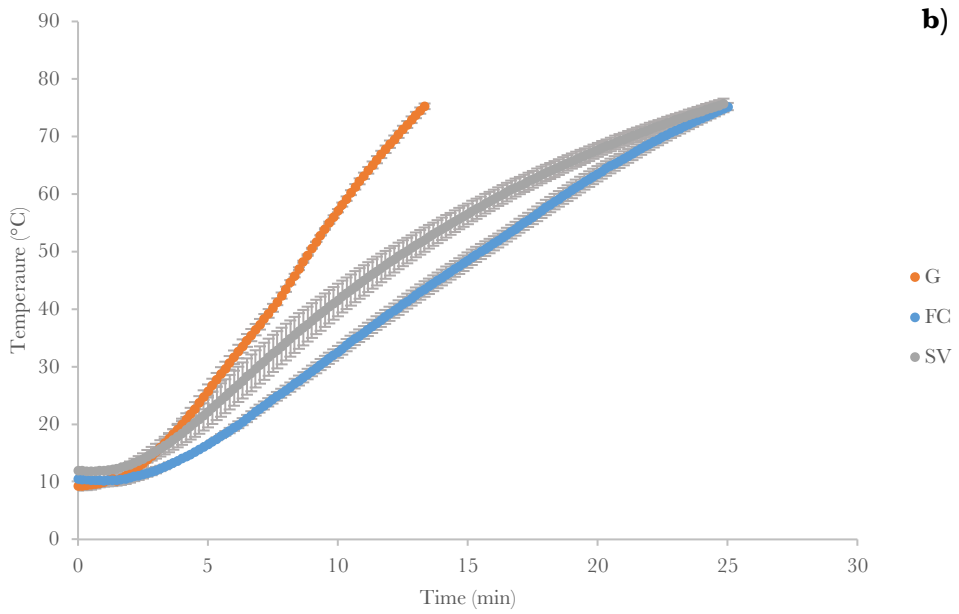


Figure 9 Temperature profile of chicken breast at the surface (a) and at the thermally less favoured point (b) as a function of cooking time under Forced Convection (FC), Grill (G) and Sous Vide (SV) cooking methods. Cavity temperature was set at 170 °C for FC; 240 °C for G; 95 °C for SV.

As regards the chicken breast surface (Figure 9a), it is possible to notice that temperature surface increased differently according to the cooking method considered until reaching a constant value of plateau. Temperature increases lead to water evaporation from the surface and migration within the product. Free water inside the muscle migrates by diffusion and, during cooking, also water trapped inside proteins is released due to denaturation and unfolding of proteins and diffuses. An evaporative front is maintained at the surface as long as the cavity humidity and conditions allow it. When sample internal moisture significantly decreases, evaporative front starts moving towards the centre of the sample becoming responsible of crust formation (Goñi & Salvadori, 2010; Kondjoyan et al., 2013). In particular, G temperature increase is the fastest among the three cooking methods because the highest cavity temperature is applied. Sample surface reached a value around 95 °C and it remained constant due to an important crust formation able to limit heat exchange. For FC and SV instead, temperature surface increased with the same speed until 12 minutes, regardless the different cavity temperature. This behaviour could be related to a constant water evaporation rate for both FC and SV samples, in which the surface remained wet, therefore limiting the temperature increase. The evaporative front is maintained near the surface, avoiding the formation of a crust (Goñi e Salvadori, 2010; Kondjoyan et al., 2013). Initially, FC samples acted similarly to SV samples cooked in a 100% moisture environment. However, after 12 min, the evaporative front in FC samples started to move towards the

centre of the sample and the temperature surface increased very quickly, greatly differentiating from SV treatment. Towards the end of the cooking, a slight temperature plateau was seen for FC due to crust formation but not for SV.

Considering the temperature profiles at the meat thermally less favoured point (Fig. 9b), samples subjected to G cooking showed the fastest increase in temperature. As regards SV cooking procedure, the initial rise of internal temperature seemed faster than FC, despite the lower cavity temperature than FC. It is likely that the vacuum package in SV favours the water evaporation from meat generating a high moisture environment surrounding the meat able to speed the heat transfer.

In Table 4, cooking processes settings are reported together with the final temperature at the centre and surface of the meat samples.

Table 4 Heating times needed to reach the safety standards corresponding to the achievement of 74 °C in the thermally less favoured point of the chicken breasts and corresponding surface temperature for different cooking methods under study: Forced Convection (FC) at 170 °C, Grill (G) at 240 °C and Sous Vide (SV) at 95 °C.

Cooking method	**Cavity temperature (°C)**	**Heating time (min)**	**Heating phase final temperature (°C)**	
			Centre	**Surface**
Grill (G)	240	13.33 ± 0.19^{b}	74.26 ± 0.48^{a}	101.74 ± 1.63^{a}
Forced Convection (FC)	170	25.00 ± 0.63^{b}	74.21 ± 0.63^{a}	95.32 ± 1.34^{b}
Sous Vide (SV)	95	24.83 ± 0.87^{b}	74.72 ± 0.87^{a}	84.33 ± 0.80^{c}

**Means in column with the same letters are not significantly different with ANOVA, Tukey-high significant different test ($p<0.05$).*

Table 4 shows the results about the heating time needed to reach the safety standard corresponding to the achievement of 74 °C in the thermally less favoured point of the chicken breasts. Such a reference temperature is in accordance with guidelines (USDA & FSIS, 2021). The corresponding temperature achieved at the surface is also reported.

Quality indicators such as cooking loss, colour and texture changes of chicken breasts during the cooking process were also monitored. Figure 10 shows the evolution of cooking loss (a) and chroma (b) of meat cooked according to FC at 170 °C, G at 240 °C and SV at 95 °C.

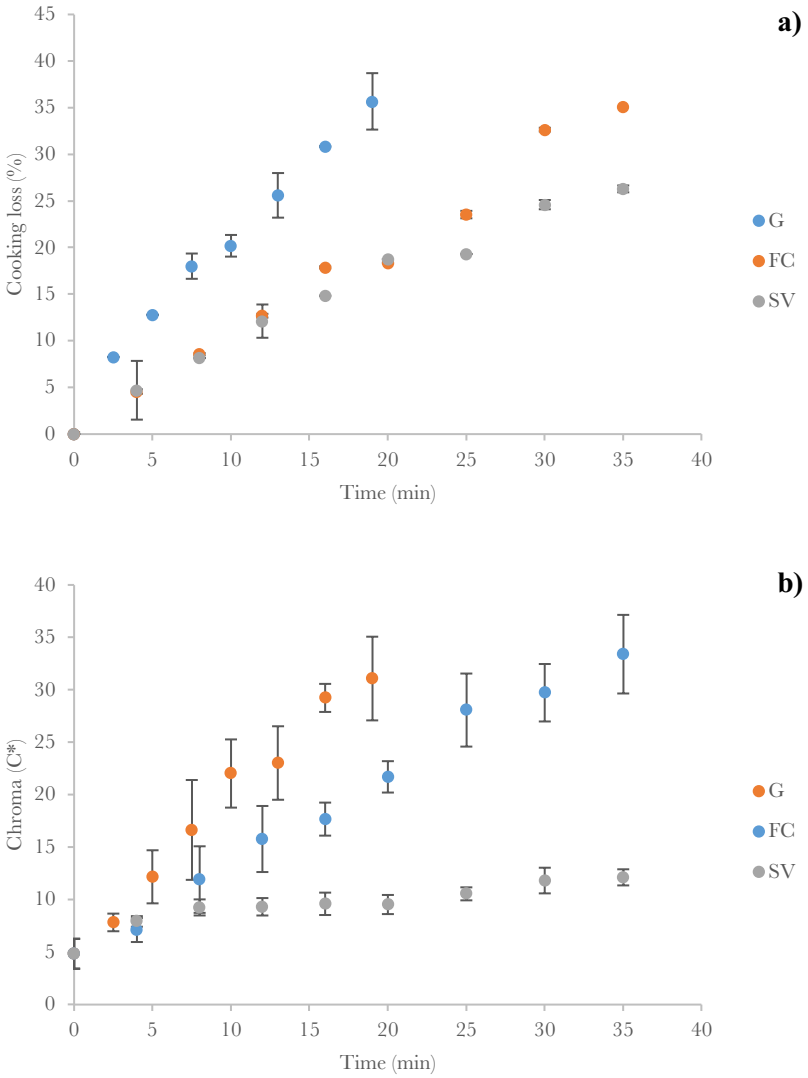


Figure 10 Changes in cooking loss and chroma of chicken breast as a function of time under different cooking methods: Forced Convection (FC) at 170 °C, Grill (G) at 240 °C and Sous Vide (SV) at 95 °C.

Cooking loss and colour changes resulted to be significantly affected by both the cooking method and heating time. Such differences can be mainly attributed to the fact that the cooking procedures under investigation were characterised by different time-temperature profiles as previously discussed (Figure 9). As expected, despite being cooked for a long time, SV showed the least loss of fluids (Figure 10a), because of low temperature, uniform heating and the vacuum being used, which limited meat dehydration (James & Yang, 2012; Roldán et al., 2013; Silva et al., 2016). Results were consistent with those reported in the literature regarding oven cooking of meat considering different cooking settings (Davey & Gilbert, 1974; Bendall & Restall, 1983; Wattanacht et al. 2005; Roldàn et al., 2015; Vaskoska et al., 2020).

Cooking loss is a time-dependent event mainly attributable to myofibrillar protein denaturation. These proteins hold most of the water retained within the muscle and, when they are denatured, the water is released, causing shrinkage and reduction in weight (Tornberg, 2005). Denaturation and shrinkage of myofibrillar proteins take place in the range of 40–90 °C, while collagen denatures in the range of 56–62 °C (Larick & Turner, 1992; Vaudagna et al., 2002; Tornberg, 2005). In particular, up until 60 °C the muscle fibres shrink transversely and widen the gap between fibres. However, above this temperature the muscle fibres shrink longitudinally and cause substantial water loss. The extent of this contraction increases with temperature (Roldàn et al., 2013). King et al. (2003) demonstrated that in beef muscle fast cooking caused greater water losses than slow cooking. This is consistent with the results of Cross et al. (1976), who reported that faster heating rates caused greater evaporative and total cooking losses.

Cooking method and heating time also affected colour changes (Figure 10b). Results were in accordance to data by Rabeler and Feyissa (2018) and Wattanacht et al. (2005). A cascade of events related to meat colour changes are triggered during cooking. As known, with heating, myoglobin, which is responsible for the typical red colour of raw meat, starts denaturing at 55–65 °C until 75–85 °C (King & Whyte, 2006). The rate and the extent of denaturation increase with temperature and cause first whitening of the muscle and then formation of brown precipitates due to oxidation of myoglobin into metmyoglobin, and to denaturation of all the different forms of heme proteins in browner substances (Martens et al., 1982; Shahidi et al., 2014). Thus, the cooked samples of the present study may have undergone a different pattern of thermal denaturation of red heme proteins as a result of different final temperatures reached at the surface (Figure 9b) (Vittadini et al., 2005). Simultaneously to denaturation of heme proteins, also Maillard reaction would occur, due to both temperature increase and meat surface dehydration (MacLeod & Seyyedainardebili, 1981). As a result, high molecular weight melanoidins are formed which confer the typical brownish colour of chicken breast. For this reason, due to higher temperature applied and the dry air, browning was more intense for G than FC. Regarding SV, chroma remained almost constant during the entire thermal treatment: the moist environment and the limited oxygen availability of vacuum packaging would have prevented surface dehydration, myoglobin oxidation, and protein denaturation. The samples whitened and no intense browning was detected in accordance with the literature (Roldàn et al., 2013; Silva et al., 2016; Ayub & Ahmad, 2019; Ortuño et al., 2021).

Figure 11 shows the effect of the different cooking methods on chicken breasts shear force, which represents a hardness indicator mainly related to protein denaturation (Warner et al., 2021).

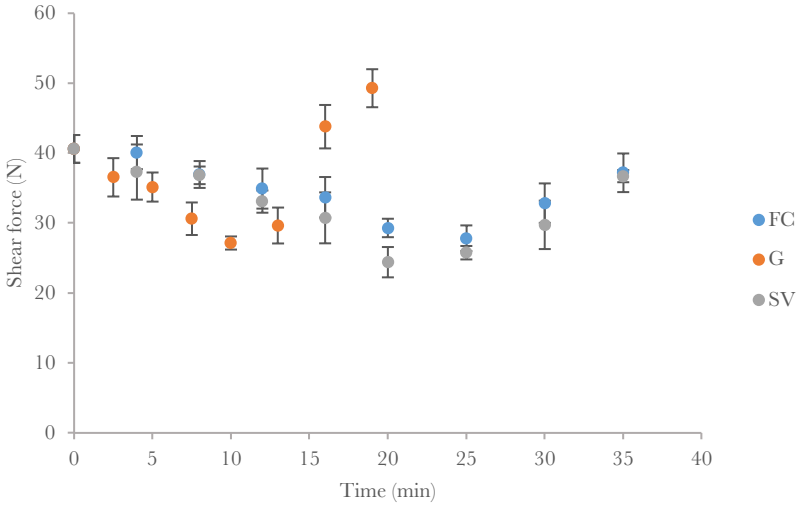


Figure 11 Changes in texture of chicken breast as a function of time under different cooking methods: Forced Convection (FC) at 170 °C, Grill (G) at 240 °C and Sous Vide (SV) at 95 °C.

Shear force significantly decreased during the first minutes of cooking for all the cooking treatments, meaning that an increase in softness occurred for all the samples. In particular, the fastest shear force decrease was associated to the G treatment, which employed the highest process temperature. SV and FC showed a similar decrease in shear force versus cooking time. However, for longer cooking times, a significant increase in texture values was observed. In the case of G, meat became softer until reaching the minimum value of 27.15 ± 0.94 N after 10 min cooking. Beyond this heating time, hardness increased, reaching a value of 49.28 ± 2.72 N in correspondence of 19 min of cooking. Similar trends were observed for both FC and SV.

Results from the literature report either texture decrease or increase, depending on the cooking conditions applied. For instance, studies involving dry cooking found out that slow cooking rates improved tenderness (Lawrence et al., 2001) due to slower collagen solubilisation (Møller, 1981) and a lesser reduction in sarcomere length than fast cooking (Bayne et al., 1969; Cross et al., 1976; Lewis et al., 1977; Cheng & Parrish, 1979; King et al, 2003). Roldán et al. (2013) demonstrated that shear force of sous-vide cooked lamb loins decreased during cooking time. They postulated that raw meat is tougher than the cooked one due to the presence of a viscous flow in the fluid-filled channels between the fibres and fibre bundles, which provides viscoelastic characteristics to the raw meat. Roldán et al. (2013)

stated that tenderness increases with heating because the sarcoplasmic proteins aggregate and form a gel, facilitating the fracture of meat with the teeth. By contrast, several authors showed that texture increased during cooking mostly due to shortening of myofibrillar proteins (Bouton & Harris, 1972; Davey & Gilbert, 1974; Rao & Lund, 1986; Zayas & Naewbanij, 1986) together with a concomitant shrinkage and water loss (Chiavaro et al., 2009; Murphy & Marks, 2000; Van Laak & Lane, 2000). Rabeler & Feyissa (2018), Murphy & Marks (2000) and Wattanachant et al (2005) found that hardness, gumminess, and chewiness of chicken breast samples heated in thermostatic water bath up to 100 °C increased according to the cooking time. They assumed that this increase might be due to the combination effect of the denaturation and shrinkage of myofibrillar proteins, in particular dehydration and shrinkage of actomyosin, as well as the shrinkage of intramuscular collagen.

Heat-induced meat softening is generally attributed to the increase of collagen solubility, which begins to shrink at 60–70 °C and is converted to gelatine at 80 °C, which weakens the connective tissue by forming a gel (Rao & Lund, 1986; Wattanachant et al 2005). To investigate protein denaturation behaviour and support meat tenderness trend, DSC analysis was performed on chicken meat and the relative thermogram is shown in Figure 12.

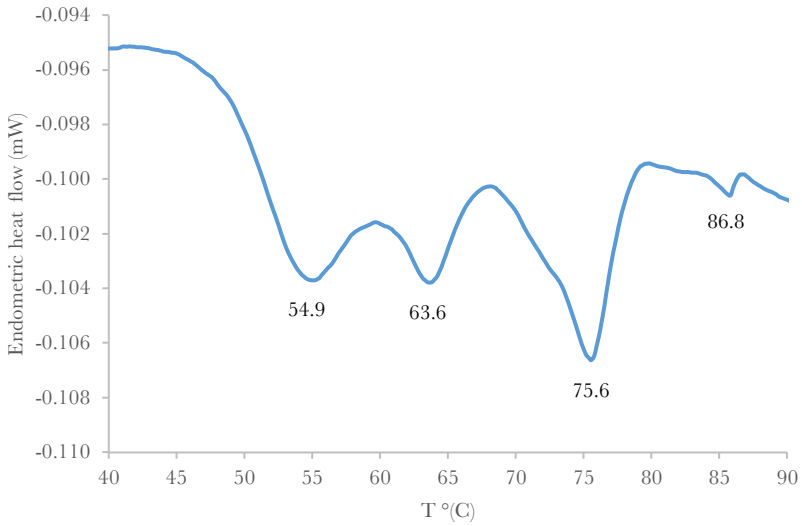


Figure 12 DSC Thermogram of raw chicken meat.

The thermogram of chicken meat (Figure 12) showed three major peaks of thermal transitions. The first transition displayed its maximum at 54.9 °C with a transition range between 48.67 and 83.41 °C. This peak was attributed to the myosin denaturation (Martens & Vold, 1976; Wright et al., 1977). The second transition, which occurred between 56.6 and

68.2 °C, with a maximum temperature at 63.6 °C was assigned to a denaturation of collagen (Martens & Vold, 1976; Stabursvik & Martens, 1980) and to sarcoplasmic proteins (Wright et al., 1977). The third and last transition was assigned to actin denaturation and was found between 70.1 and 78.2 °C (Wright et al., 1977), with a maximum at 75.6 °C. It is clear that until 74 °C denaturation of myosin and collagen drives tenderness modification of meat during cooking, in particular collagen, which is able to increase the tenderness of meat during its denaturation. A further peak was found at 86.6 °C and might be related to the titin denaturation (Pospiech et al., 2002).

Among the analysed quality indices, it is noteworthy that texture assumes a minimum value able to affect the food quality. In consideration of the biphasic evolution of meat shear force under cooking (Figure 11), changes in texture were modelled by using a second order polynomial equation. Equation parameters are reported in Table 5.

Table 5 Second order polynomial equation of texture trend of chicken breast as a function of time under different cooking methods: Forced Convection (FC) at 170 °C, Grill (G) at 240 °C and Sous Vide (SV) at 95 °C.

Cooking method	**Cavity temperature (°C)**	**Second order equation**	**R^2**
Grill (G)	240	$y = 0.1824x^2 - 3.0725x + 42.442$	0.87
Forced Convection (FC)	170	$y = 0.0252x^2 - 1.0924x + 42.857$	0.80
Sous Vide (SV)	95	$y = 0.0335x^2 - 1.4121x + 42.836$	0.80

It can be observed that the second order polynomial equation allows a good description of the texture evolution of chicken breast under cooking. On the basis of the equations reported in Table 5 the minimum values of shear stress (SF_{min}) were estimated. Results are shown in Table 6. The corresponding cooking times necessary to achieve such shear stress values, corresponding to the maximum level of meat tenderness, ($t_{maxtenderness}$) are also reported.

Table 6 Minimum values of shear stress (SF_{min}) and cooking time corresponding to the maximum level of meat tenderness ($t_{maxtenderness}$) extrapolated by second order polynomial equation of texture trend of chicken breast as a function of time under different cooking methods: Forced Convection (FC) at 170 °C, Grill (G) at 240 °C and Sous Vide (SV) at 95 °C.

Cooking method	**Cavity temperature (°C)**	**SF_{min} (N)**	**$t_{maxtenderness}$ (min)**
Grill (G)	240	29.50	8.42
Forced Convection (FC)	170	31.02	21.67
Sous Vide (SV)	95	27.96	21.07

From Table 6 is possible to see that times necessary to reach the maximal softness ($t_{maxtenderness}$) are shorter than safety cooking times (Table 4) and this means that the achievement of the safety condition needs more time than the achievement of tenderness quality. Thus, the achievement of 74 °C at the thermally less favoured point of the sample is the limit to take into account for these types of cooking. This agrees with literature since the final internal temperature was reported to have a large effect on the textural properties of cooked meat (Parrish et al., 1973; Wood et al., 1995).

2.5 Conclusions

After investigating the evolution of quality indices over a cooking process, it has been established that texture represented the most critical one due to its biphasic behaviour, while water loss and chroma seemed to change linearly versus cooking time. Shear force resulted to achieve a minimum value representing the maximum level of meat tenderness mainly attributable to connective tissue proteins aggregation and gelling. However, longer cooking times caused a progressive increase in shear force. The latter is attributable to meat shrinkage mainly due to shortening of myofibrillar proteins, together with water loss.

For each cooking method, times required to achieve the safety issues of 74 °C resulted longer than those necessary to achieve the maximum meat tenderness level. It can be inferred that, independently by the cooking method, time necessary to achieve 74 °C at the thermally less favoured point of the chicken breast during cooking drives the cooking process.

On the basis of these observations, for the considered cooking procedures, temperature measured at the thermally less favoured point of the meat represents the most important indicator to set up a cooking process.

3 Chapter

Kinetic study on quality changes of chicken breast meat undergone different oven cooking methods

3.1 Introduction

In the second phase of the kinetic modelling approach, quality indicators have to be monitored over the cooking processes according to different temperatures. In this way, quality attributes are quantified as function of heating time for a defined temperature. Kinetic evolution of indicators over cooking time can be elaborated and relative kinetic order established based on how well the observations match a preselected reaction order model. Extrapolation of rate constant from quality indicators kinetics is fundamental for further elaborations. Different mathematical models can be combined together in order to relate the temperature of the cooking process with the rate of the indicators variation over the cooking process, such as Arrhenius model. Thanks to these elaborations, the most sensitive quality indicator to temperature can be determined and considered as the critical indicator, best describing the evolution of the cooking process.

3.2 Aim of the Study

The aim of this work was to study the kinetics of quality indicators of chicken breast during cooking by using different oven cooking techniques, in particular Grill (G), Forced Convection (FC) and Sous Vide (SV), the most common in the food service. The effect of temperature on quality indices evolution was investigated by testing three different cooking temperatures for each cooking method (G, T=240, 260, 280 °C; FC, T=150, 170, 190 °C; SV, T=80, 95, 120 °C). Cooking treatments were performed until the achievement of the safety time, the limiting factor for chicken breast cooking. Then the temperature dependence of the rate of quality indicator evolution was investigated by applying the Arrhenius model and computing the related Arrhenius parameters.

3.3 Materials and Methods

3.3.1 Materials

Chicken breasts samples were prepared as reported in paragraph 2.3.1.

3.3.2 Cooking treatments

Thawed chicken breasts were prepared and cooked individually as reported in 2.3.2. Samples were cooked according to the three different cooking processes Grill (G), Forced Convection (FC) and Sous Vide (SV), and three different temperatures were tested for each cooking treatment. Samples were cooked for increasing times until reaching 74 °C in the thermally less favoured point of the chicken breast, as reported in Table 7.

Table 7 Tray shape, cavity temperature, cooking time required to achieve 74 °C at the thermally less favoured point, and cavity humidity of each cooking method considered for chicken breast cooking by using a professional oven.

Cooking method	Tray Shape	Cavity temperature (°C)	Time (min)	Relative humidity (%)
Grill (G)	Grid	240	13	---
		260	15	---
		280	11	---
Forced Convection (FC)	Flat	150	31	---
		170	25	---
		190	29	---
Sous Vide (SV)	Flat	80	35	100
		95	25	100
		120	21	100

3.3.3 Cooking loss

Cooking loss was calculated as reported in paragraph 2.3.5.

3.3.4 Colour analysis

Colour determination of cooked chicken breast surface was carried out as shown in 2.3.6.

3.3.5 Texture analysis

Texture analysis was performed as indicated in paragraph 2.3.7.

3.3.6 Kinetic data analysis

Kinetic data of cooking loss, colour and texture changes during oven cooking process were elaborated to identify the reaction order best describing their evolution (Karel & Lund, 2003). Experimental values were expressed as follow:

Zero order, n=0

$$I = kt + I_0 \qquad \textit{Eq. 12}$$

First order, n=1

$$\ln I = kt + lnI_0 \qquad \textit{Eq. 13}$$

Second order, n=2

$$\frac{1}{I} = kt + \frac{1}{I_0} \qquad \textit{Eq. 14}$$

where I is the value of the selected indicator, I_0 is the value of the indicator at time zero and k is the rate constant. Results were plotted against time and interpolated by linear regression analysis. The reaction order of quality indices evolution was associated to the best linear trend and apparent constant rates were calculated.

The effect of temperature on the rate of quality indicators changes was evaluated by the Arrhenius equation. To make a better estimate of the apparent activation energy, a one-step nonlinear regression was applied to all data by using the reparametrized Arrhenius equation, in which a reference temperature was inserted, chosen in the middle of the temperature range considered (G, T_{ref} = 240 °C; FC, T_{ref} = 170 °C; SV, T_{ref} = 100 °C) in the experimental plan:

$$lnk = lnk_{ref} - \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right) \qquad \textit{Eq. 15}$$

where k is the apparent reaction rate, R is the molar gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature (K), and k_{ref} is the apparent reaction rate at T_{ref} (170 °C for FC, 260 °C for G, 100 °C for SV). Ea and k_{ref} were determined by linear regression analysis and used to calculate the frequency factor k_0:

$$k_0 = e^{\left(lnk_{ref} + \frac{E_a}{RT_{ref}}\right)} \qquad \textit{Eq. 16}$$

3.3.1 Statistical analysis

Data are reported as means ± standard deviation. Data elaboration, representation and regression analysis were performed using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). The goodness of models fitting was evaluated by using the coefficient of determination (R^2), the corresponding *p* values and standard errors (SE) thanks to RStudio (version 4.2.2, The R Foundation for Statistical Computing, Vienna, Austria).

3.4 Results and Discussion

Water loss and colour changes of chicken breast subjected to different cooking temperatures for increasing times by means of G, FC, and SV cooking methods were monitored until reaching 74 °C at the thermally less favoured internal point, which is the safety time or the limiting factor for chicken breast cooking, as assessed in chapter 2 (Appendix 7.1 and 7.2). As already reported in chapter 2, cooking loss and chroma linearly increased over the cooking time proportionally to the temperature of the cooking process. Only for SV no chroma variation was seen due to process settings and no browning development.

Data of cooking loss and chroma kinetic evolution over time were elaborated according to a zero order kinetic. Texture evolution was not considered due to its polynomial trend. Table 8 shows the apparent zero-order rate constants of cooking loss and colour changes of chicken breasts subjected to different cooking procedures at different oven temperature settings. Chroma of SV samples was not considered because no colour changes were detected.

Table 8 Apparent zero order rate constants and the corresponding regression parameters (Standard Error (SE), p-value and coefficient of determination (R^2)) of cooking loss and chroma of chicken breast cooked according to different cooking methods at different oven temperature settings.

Cooking method	Quality index	Temperature (°C)	*k* (index min^{-1})	SE	*p*-value	R^2
Grill (G)	Cooking loss	240	1.978	2.20	<0.001	0.99
		260	2.520	1.19	0.0173	0.99
		280	3.073	2.50	0.0453	0.99
	Chroma	240	1.526	1.37	<0.001	0.97
		260	1.893	1.32	<0.001	0.99
		280	2.549	1.14	0.0557	0.99
Forced Convection (FC)	Cooking loss	150	0.768	1.40	0.0015	0.99
		170	1.008	1.26	<0.001	0.99
		190	1.271	1.52	0.0014	0.99
	Chroma	150	0.642	0.16	0.0103	0.99
		170	0.892	1.17	<0.001	0.99
		190	1.044	1.46	0.0069	0.99
Sous Vide (SV)	Cooking loss	80	0.539	2.18	0.0582	0.98
		95	0.888	1.83	<0.001	0.99
		120	1.190	1.55	0.0072	0.97

Dimensions of k were as follows: Cooking loss: % min^{-1}; Colour: C min^{-1}*

As expected, in all cases, the rate constants *k* of quality indicators increased with the increasing of temperature set in the oven. For G and FC, cooking loss showed higher values of *k* than colour, indicating a faster evolution of the index. In overall, the treatment characterized by the highest process temperature (G) showed the fastest quality indices evolution, followed by FC and SV. So independently from the cooking process applied, higher was the process temperature, faster were the reactions, in accordance with literature (Martens et al., 1982; Rabeler & Feyissa, 2018; Murphy & Marks, 2000).

Few kinetic studies relevant to meat quality changes during cooking are available in the literature. They are mostly focused on describing the changes in colour, texture and cooking loss of meat during cooking. For instance, Rabeler & Feissa (2018a) investigated the changes of texture and colour of chicken breast meat during a thermal process in thermostatic water

bath, aiming at the development of kinetic models capable of describing these changes. They studied modifications of texture (TPA) and colour (CIE L*a*b* colour parameters) as a function of temperature and time. The two quality indicators showed a rise with heating time until reaching an equilibrium value, while the rate of change increased with temperature. The authors computed reaction rate constants by fitting the differential form of a modified general rate law which took into account a condition of non-zero equilibrium, i.e. where quality value changes over time compared to the initial time. Data were best fit by the first order kinetic model. Rabeler et al. (2019) instead studied the evolution of colour of chicken breast meat during a convective roasting. They started from the consideration that consumers assess firstly the quality of roasted chicken meat by its appearance and so a study related to this topic was necessary, since related knowledge missed. The authors studied the evolution of Lightness (L*) at the top and bottom surface of the meat during a roasting process, following the whitening of meat at the beginning of the cooking treatment and then the browning. The whitening of chicken breast meat during heating was studied and the order 1.1 kinetic model best suited the trends. Thanks to this finding a model predicting the whitening of meat during cooking was elaborated as function of heating time and sample temperature. Instead, browning was elaborated according to a first order kinetic model which was further related to heating time, sample temperature and water activity, since browning relies also in the water content. Moya et al. (2021) investigated the kinetics of heat-induced colour changes in beef meat under three different storage conditions (fresh, refrigerated and frozen) during pan cooking over time and for different temperatures. Turn-over of meat during cooking was also taken into account and standardized. The CIELab colour parameters lightness (L*) and the reddish tone (a*) were considered and related to cooking time and temperature. The parameters were first modelled according to two successive first-order reaction models, but the observed reactions showed a non-Arrhenius behaviour. So, to describe the evolution of the parameters, a response surface of each colour coordinate against time and temperature was fitted. The equation representing the response surface of L* corresponded to a five-degree polynomial function for temperature and linear for time, while for a*, the surface was represented by a polynomial function of degree five for both temperature and time. Also Goñi & Salvadori (2011) investigated the colour changes (a* in particular) of thin slices of beef semitendinosus muscle subjected to different time-temperature treatments (between 2.5 to 30 minutes, and between 40 to 100 ^{o}C) using a thermostatic water bath. To describe the variation of a* values a simple kinetic model was proposed. A first order fractional kinetic model was used, and the reaction rates were

correlated with temperature according to an Arrhenius relationship. Portanguen et al. (2009) and Pakula & Stamminger (2012) also investigated colour changes of red meat during cooking in an electric oven and employed successive first order kinetics to predict the whitening initial phase (lightness L* increases) followed by the browning stage. These authors indicated that lightness recording was sufficient to characterize colour kinetics. Also Kondjoyan et al. (2014) analysed colour evolution relating it to protein denaturation. They described both consecutive stages (whitening followed by browning) and computed the rates with first order kinetics.

Regarding cooking loss, Oillic et al. (2011) investigated the cooking loss kinetics on meat by heating samples in a water bath at three different temperatures for five different times. The authors investigated the effect of different sample shapes, pre-freezing, muscle type, ageing time, animal species and age (beef, veal, horse, lamb) on cooking losses. Results showed that cooking losses were dependent on both the raw-product water content and process temperatures for longer treatment times, while pre-freezing of the sample, sample size, difference in ageing time, and in muscle fiber orientation had little influence on cooking losses. At a given temperature, kinetics of water content over cooking time showed similar first-order kinetics patterns regardless of species or muscle.

As known, to describe the relationship between the temperature and the reaction rate constant, the common Arrhenius model is mostly used (Goñi & Salvadori, 2011; Kong et al., 2007; Rabeler & Feissa, 2018). Therefore, to further demonstrate the dependency of quality indices from temperature, the Arrhenius model was applied. The values of the zero order rate constants expressed in logarithmic terms were plotted as a function of the reciprocal of the temperature minus the reciprocal of the reference temperature of the specific cooking method considered, in accordance with the reparametrized Arrhenius model, as shown in Figure 13 and Figure 14.

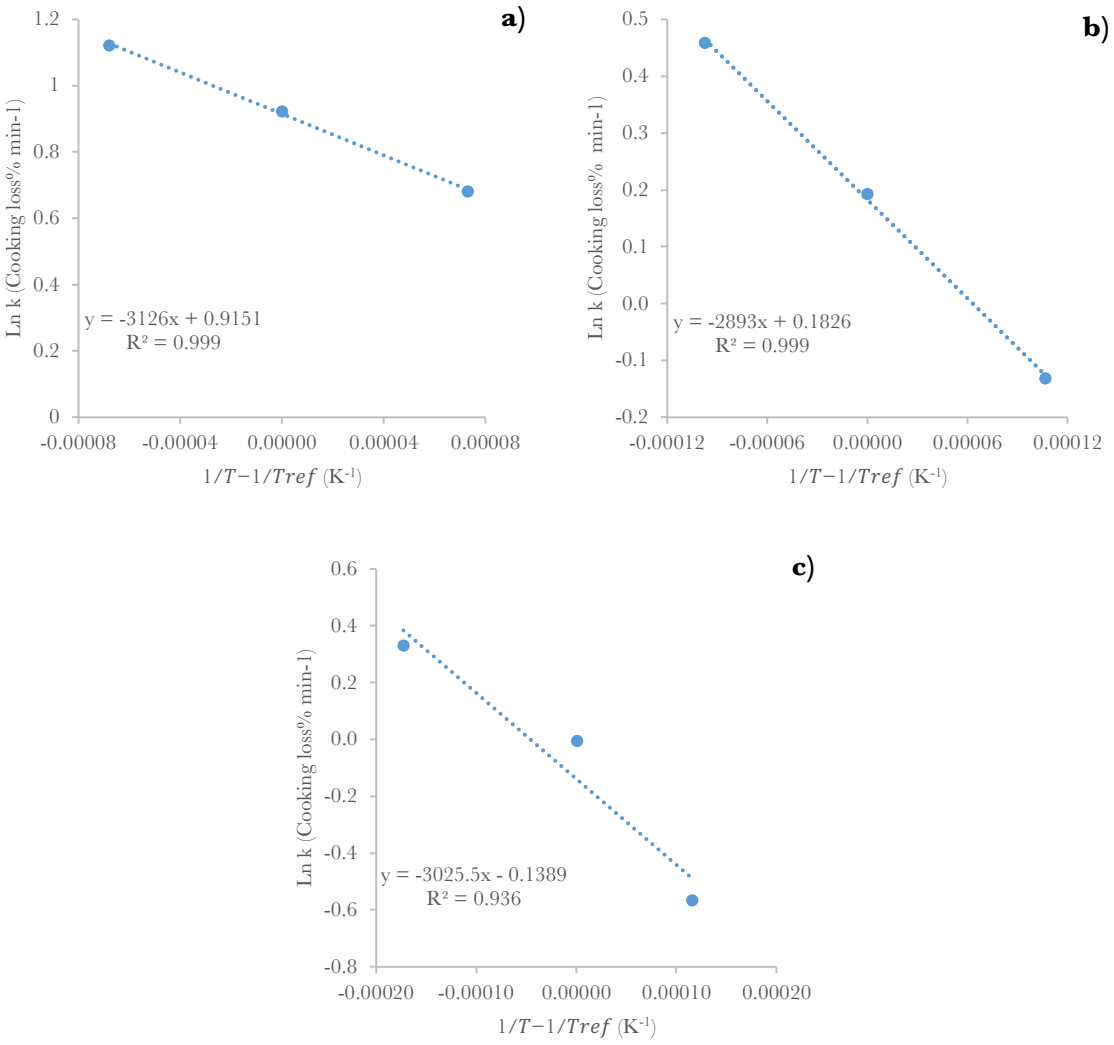


Figure 13 Logarithm of zero order rate constant of cooking loss and chroma evolution as a function of the temperature reciprocal minus the reciprocal of the reference temperature of the specific cooking method considered, in particular (a) G, (b) FC and (c) SV.

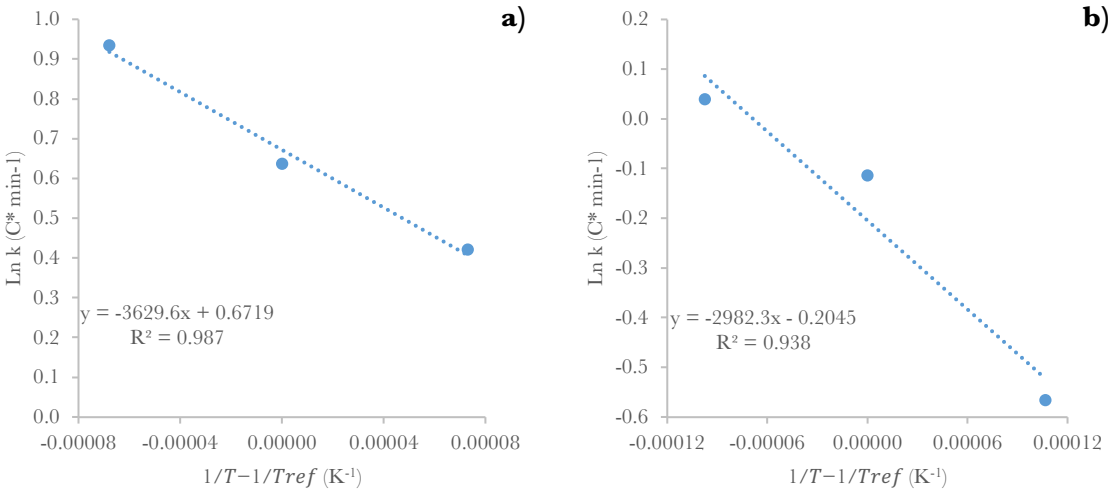


Figure 14 Logarithm of zero order rate constant of cooking loss and chroma evolution as a function of the temperature reciprocal minus the reciprocal of the reference temperature of the specific cooking method considered. In particular (a) G and (b) FC.

As shown in Figure 14, in all the cases the Arrhenius equation well described the temperature dependence of the rate constants of the considered quality indices. Based on this evidence, activation energies (*Ea*) were calculated and results are reported in Table 9.

Table 9 Frequency factor (k_0), activation energy (Ea) and corresponding regression parameters (Standard Error (SE), p-value and coefficient of determination (R^2)) of cooking loss and chroma evolution of chicken breast cooked according to different cooking methods at different oven temperature settings.

Cooking method	Quality index	k_0	*Ea* (kJ mol^{-1})	SE	*p* value	R^2
Grill (G)	Cooking loss	880.08	25.99	0.01	0.02	0.99
	Chroma	1775.18	30.19	0.076	0.16	0.98
Forced Convection (FC)	Cooking loss	262.01	20.50	0.0072	0.01	0.99
	Chroma	1029.22	24.79	0.22	0.01	0.94
Sous Vide (SV)	Cooking loss	4274.26	25.15	0.14	0.17	0.94

Ea values were consistent with data reported in the literature (Ling et al., 2015). It must be pointed out that estimated *Ea* for both quality indices was of the same magnitude. The phenomena of cooking loss could be associated to water migration inside the sample and evaporation of exudate from meat to oven air. Therefore, cooking loss *Ea* could be associated to moisture transfer or water evaporation.

Regarding colour parameters, the *Ea* values of chroma obtained in the present study resulted significantly lower (reported in Table 9) than the ones reported in literature for colour changes: 101.59 kJ mol^{-1} for lightness changing in chicken (Rabeler & Feyissa, 2018) and 80.74 kJ mol^{-1} for a* modifications during beef roasting (Goñi & Salvadori, 2011).

From these results it can be observed that cooking loss showed the lowest activation energy between the two indicators. This means that the energy necessary to trigger the reaction associated to water loss is lower than the one to colour and so cooking loss needs a smaller temperature variation than chroma to be modify. Therefore, for this reason cooking loss was considered the most sensitive quality index to temperature modification or the critical indicator.

3.5 Conclusions

The results acquired in this study confirmed that cooking generates a series of complex physical and chemical modifications, which are significantly affected by cooking procedure and temperature. In fact, evolution of quality indices, i.e. cooking loss and colour, over different cooking methods and temperatures showed a linear increase during cooking in a time- and temperature-dependent way. Grill cooking method was able to accelerate the chemical physical reactions more than forced convection and sous vide.

Extrapolation of *Ea* could be very useful since this parameter is able to link the temperature to the rate of quality indicators changes. Thanks to it, the most sensitive quality indicator was identified in cooking loss.

4 Chapter

Identification of relevant and easy-to-measure cooking indicator for on-line management of chicken breast meat cooking process

4.1 Introduction

Based on conclusions of previous parts, the limiting factor of chicken breast cooking is safety because the achievement of 74 °C in the thermally less favoured point of the meat requires a longer time than the achievement of the maximal quality. However, replacement of the heart temperature indicator with another qualitative one could be a beneficial resource, in terms of safety, accuracy and ease of use.

Since respect of safety time is mandatory, identify the value related to the most representative quality indicator of the cooking process at the safety time is the starting point. This limiting value establish the end of the optimal cooking process. The achievement of the optimal condition would guarantee the respect of safety condition despite being based on a quality index. With this information, a predictive model related to the indicator can be developed in order to predict the cooking time as a function of the quality indicator. This model could be useful in the prospective of the development of an alternative on-line cooking sensor based on the monitoring of the quality index.

4.2 Aim of the Study

The aim of the study was at first to evaluate whether a different indicator rather than temperature measured at the meat thermally less favoured point could be used to describe the cooking process. Comparison of quality indicator values of cooking loss and chroma were conducted in order to assess differences among them at the final safety time. Then, predictive cooking models were developed for each quality index by coupling their respective kinetic model with Arrhenius model. A cross-validation with a new data set of chicken breasts samples was conducted in order to assess the goodness of the predictions of the model and to understand which quality indicator best predicted the cooking process.

4.3 Materials and Methods

4.3.1 Materials

Chicken breasts samples were purchase by an Irish food service supplier (O'Mahony Meats Ltd., Dublin, Ireland). Chicken breasts with average weight of 240 ± 10 g were selected and prepared as reported in paragraph 2.3.1.

4.3.2 Cooking treatments

Thawed chicken breasts were cooked individually in an electric professional oven (Rational AG, SCC WE 61, Landsberg am Lech, Germany). Samples were cooked according to three different cooking processes Grill (G), Forced Convection (FC) and Sous Vide (SV). Three different temperatures were tested for FC and one for G and SV and samples were cooked for increasing times until reaching 74 °C in the thermally less favoured point of the chicken breast, as reported in Table 10.

Table 10 Tray shape, cavity temperature cooking time required to achieve 74 °C at the thermally less favoured point, and cavity humidity of each cooking method considered for chicken breast cooking by using a professional oven.

Cooking method	Tray Shape	Cavity temperature (°C)	Time (min)	Relative humidity (%)
Grill (G)	Grid	240	20	---
Forced Convection (FC)	Flat	150	30	---
	Flat	170	26	---
	Flat	190	26	---
Sous Vide (SV)	Flat	95	21	100

Samples were prepared as reported in 2.3.2.

4.3.3 Cooking loss

Cooking loss was calculated as reported in paragraph 2.3.5.

4.3.4 Colour analysis

Colour determination of cooked chicken breast surface was carried out as shown in 2.3.6.

4.3.5 Texture analysis

Texture analysis was performed as indicated in paragraph 2.3.7.

4.3.6 Optimal cooking time computation

Optimal cooking time of a specific cooking process was identified thanks to a predictive model as follows

$$t_{cooking} = \frac{I_{lim} - I_0}{k_T} \qquad \textit{Eq. 17}$$

where I_{lim} is the limiting value of quality indicator, I_0 is the value of the indicator at time zero and k_T is the quality index modification rate constant at a specific process temperature.

4.3.7 Predictive model validation

Validation of the predicted model of cooking time was carried out with an external validation by using new data. New data were collected by purchasing new chicken breast samples (as reported in paragraph 2.3.1) and cooking them as reported in 3.3.2. Three different cooking times were analysed for each cooking method and temperature. Samples were then analysed for cooking loss and chroma quality indicators as reported in paragraph 2.3.5 and 2.2.6. Three replications were conducted at least. Data were used to assess the quality of the predictions of the model.

4.3.1 Statistical analysis

Data were expressed as the mean of at least three analytical determinations on three replicated samples and are reported as means ± standard deviation. Data elaboration, representation and regression analysis was performed using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). Statistical analysis was performed using RStudio (version 4.2.2, The R Foundation for Statistical Computing, Vienna, Austria). Bartlett's test was used to check the homogeneity of variance. Analysis of variance (ANOVA) was conducted in case of homogeneity or Welch test in case the variances were not homogeneous. In both cases Tukey-HSD test was used to assess differences between means ($p < 0.05$). The goodness of regression models fitting was evaluated by using the coefficient of determination (R^2), the corresponding p values and standard errors (SE).

The adequacy of the model to predict data was assessed on the basis of statistical indices (Te Giffel & Zwietering, 1999), in particular:

Mean Square Error (MSE) was calculated as follows:

$$MSE = \frac{RSS}{n} = \frac{\sum_{i=0}^{n}(y_{i,observed} - y_{predicted})^2}{n} \quad \textit{Eq. 18}$$

where RSS is the residual sum of squares, $y_{i,observed}$ is the observed variable value, $y_{predicted}$ is the related predicted value and *n* is the number of degrees of freedom;

Accuracy factor:

$$Accuracy\ factor = 10^{\left(\frac{\sum_{i=0}^{n}\left|\frac{\log y_{predicted}}{y_{i,observed}}\right|}{n}\right)} \quad \textit{Eq. 19}$$

where $y_{i,observed}$ is the observed variable value, $y_{predicted}$ is the related predicted value and *n* is the number of degrees of freedom;

Bias factor:

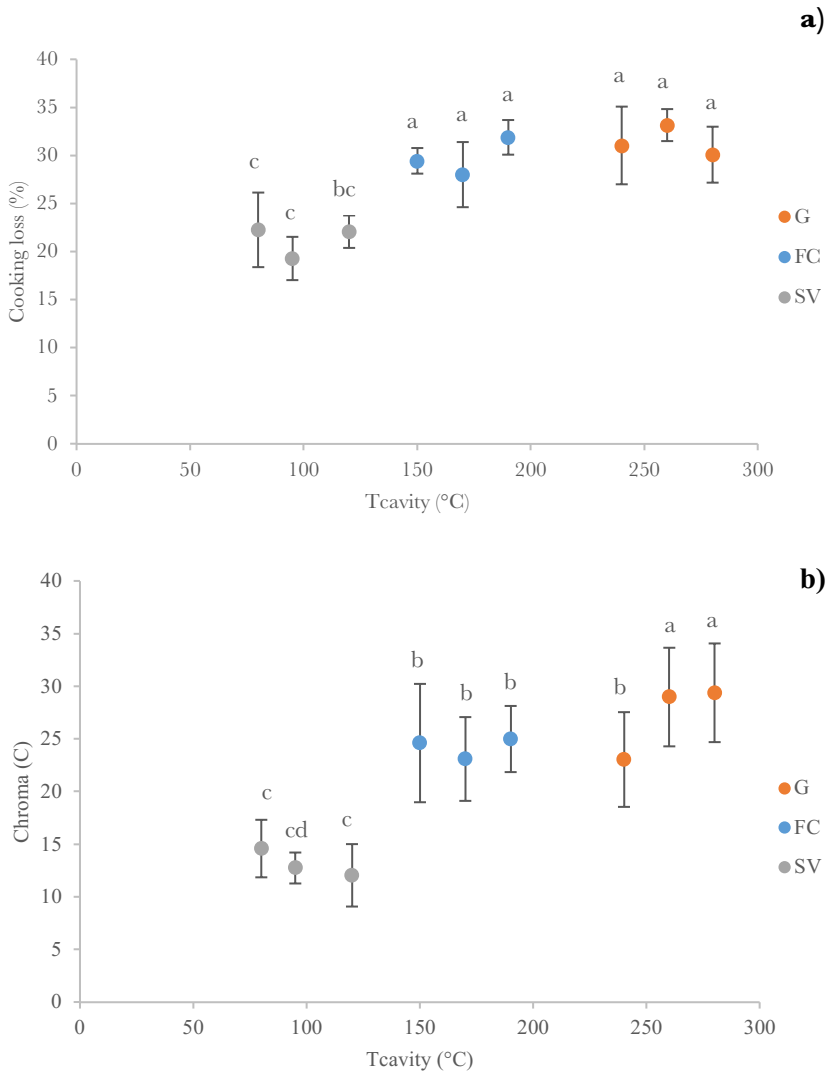
$$Bias\ factor = 10^{\left(\frac{\sum_{i=0}^{n} log\left(\frac{y_{i,observed}}{y_{predicted}}\right)}{n}\right)} \quad \textit{Eq. 20}$$

where $y_{i,observed}$ is the observed variable value, $y_{predicted}$ is the related predicted value and *n* is the number of degrees of freedom.

4.4 Results and Discussion

In order to increase system variability and obtaining a more reliable predictive model, other kinetic studies were conducted considering a different chicken sample supplier and a different professional oven. Evolution of cooking loss, shear force and chroma was monitored until the achievement of 74 °C in the thermally less favoured point of the sample over the different cooking methods previously analysed, i.e. Grill (G, 240 °C), Forced convection (FC, 150, 170 and 190 °C) and SV (95 °C), as shown reported in Appendix 7.3. The figures showed the same trends as reported in chapter 2. Cooking loss linearly increased over the cooking time proportionally to the temperature of the cooking process and chroma too, again with the exception of SV where no colour variation was seen. Regarding texture, trends were unclear and almost constant and no clear differences were found according to the cooking method and temperature. So for these reason texture trends were not even considered (data not showed).

Values of cooking loss and chroma at safety time here discussed (Appendix 7.3) and obtained from kinetic evolution study of chapter 3 (Appendix 7.1 and 7.2) are reported all together in Figure 15.



[a,b] Means related to the specific cooking method with the same letters are not significantly different with ANOVA, Tukey-HSD test ($p<0.05$).

Figure 15 Average values of cooking loss (a) and chroma (b) corresponding to the safety time of achievement of 74 °C at the less thermally favoured point and according to the cooking method and the tested temperatures: Grill (G) at 240, 260, 280 °C, Forced Convection (FC) at 150, 170, 190 °C, Sous Vide (SV) at 80, 95, 120 °C.

Figure 15 shows the average values of cooking loss and chroma corresponding to the safety time of achievement of 74 °C at the less thermally favoured point according to the cooking method and the tested temperature. Statistical analysis showed that values at safety time were always the same according to the specific cooking method used and independently by cooking temperature.

Thanks to this result, it was possible to calculate an average value of quality indicator at safety time for each treatment, as shows in Table 11.

Table 11 Cooking loss and chroma corresponding to the safety time of achievement of 74 °C at the less thermally favoured point and according to the cooking method and the tested temperatures: Grill (G) at 240, 260, 280 °C, Forced Convection (FC) at 150, 170, 190 °C, Sous Vide (SV) at 80, 95, 120 °C.

Cooking method	T (°C)	Cooking loss (%) at t_{safety}	Average cooking loss (%) at t_{safety}	Chroma (C*) at t_{safety}	Average chroma (C*) at t_{safety}
Grill (G)	240	31.04 ± 4.04 $^{a, A}$	31.22 ± 3.90	23.04 ± 4.50 $^{b, B}$	26.40 ± 5.51
	260	33.16 ± 1.66 $^{a, A}$		28.97 ± 4.68 $^{a, A}$	
	280	30.08 ± 2.91 $^{a, A}$		29.38 ± 4.69 $^{a, A}$	
Forced Convection (FC)	150	29.44 ± 1.33 $^{ab, A}$	29.71 ± 2.83	24.60 ± 5.62 $^{a, B}$	24.22 ± 6.09
	170	28.01 ± 3.39 $^{b, AB}$		23.09 ± 3.98 $^{a, B}$	
	190	31.88 ± 1.80 $^{a, A}$		24.99 ± 3.14 $^{a, B}$	
Sous Vide (SV)	80	22.26 ± 3.88 $^{a, C}$	21.49 ± 2.56	14.59 ± 2.73 $^{a, CD}$	12.99 ± 2.80
	95	19.29 ± 2.25 $^{a, C}$		12.74 ± 1.47 $^{b, D}$	
	120	22.07 ± 1.69 $^{a, BC}$		12.04 ± 2.97 $^{b, C}$	

^{a,b} Means in column related to the specific cooking method with the same letters are not significantly different with ANOVA and Tukey-HSD test ($p<0.05$).

$^{A-C}$ Means in column with the same letters are not significantly different with ANOVA and Tukey-HSD test ($p<0.05$).

Considering individually each cooking method, statistical analysis assessed that average values of cooking loss and chroma corresponding to the achievement of safety time were mostly independent from temperature, apart from chroma values in G and SV (Table 11). By comparing the different cooking processes instead, the final values of cooking loss and colour statistically differed according to the cooking method applied. The higher were the temperatures of the cooking process, the higher were the final values of the two quality indices. Only cooking loss in G and FC methods showed statistically equal values.

At this point, a model capable of predicting the optimal cooking time was elaborated from the following equation

$$I = I_0 + kt_{cooking} \qquad \text{Eq. 21}$$

where I is the value of quality indicator at the chosen $t_{cooking}$, I_0 is the initial value of the quality indicator and k is the specific constant rate of that cooking method. The equation was elaborated as a function of cooking time as showed in Eq. 17.

According to Arrhenius equation (Eq. 5), *k* could be re-written as follows

$$k = e^{\left(lnk_0 - \frac{E_a}{RT}\right)} \quad \textit{Eq. 22}$$

where k_0 is the rate constant, R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature (K), and E_a is the activation energy (J mol^{-1}). In the end *k* was substituted to the Eq. 17 resulting in the following equation

$$t_{cooking} = \frac{I_{lim} - I_0}{e^{\left(lnk_0 - \frac{E_a}{RT}\right)}} \quad \textit{Eq. 23}$$

Eq. 23 can be considered the model able to predict the cooking time of a cooking process as a function of the limiting value of the quality indicator, the initial value of the indicator, and the process temperature of oven cavity.

A predictive model specific for each cooking process and related to cooking loss or chroma was elaborated by substituting k_0 and E_a with the respective previously calculated values (Table 9). Each predictive model can be written as reported in Table 12.

Table 12 Predictive model of optimal cooking time as a function of limiting value of the quality indicator (I_{lim}), the initial value of the indicator (I_0), and the temperature (T) for cooking loss and chroma quality indicators for Grill (G), Forced Convection (FC) and Sous Vide (SV) cooking methods.

Cooking method	Cooking loss	Chroma
Grill (G)	$t_{cooking} = \frac{31.22}{e^{\left(ln880.08 - \frac{25{,}990}{8.314*T}\right)}}$	$t_{cooking} = \frac{26.40 - 4.84}{e^{\left(ln1775.18 - \frac{30{,}190}{8.314*T}\right)}}$
Forced Convection (FC)	$t_{cooking} = \frac{29.71}{e^{\left(ln832.10 - \frac{24{,}050}{8.314*T}\right)}}$	$t_{cooking} = \frac{24.22 - 4.84}{e^{\left(ln1029.22 - \frac{24{,}790}{8.314*T}\right)}}$
Sous Vide (SV)	$t_{cooking} = \frac{21.49}{e^{\left(ln4274.26 - \frac{25{,}150}{8.314*T}\right)}}$	---

Where I_0=0 for cooking loss and I_0=4.84 for chroma. Chroma predictive model for SV was not elaborated due to the absence of colour changes during the cooking process as a consequent of no browning development.

In literature only few kinetic studies related to the development of cooking predictive models are available. In particular, the authors elaborated models capable of describing the evolution of cooking processes over the cooking time as a function of different quality indicators.

For instance, Rabeler & Feissa (2018a) investigated the temperature dependence of texture of chicken breast meat during a thermal process in thermostatic water bath and were able to elaborate kinetic models describing the evolution of the quality change. Thanks to these findings, the authors coupled the kinetic models of heat-induced textural changes with models for heat and mass transfer. They developed a mechanistic model able to predict the texture evolution during the roasting process as function of process parameters, i.e. temperature and ventilation (Rabeler & Feissa, 2018b). In the same way, Rabeler et al. (2019) developed also another combined mathematical model able to predict the lightness (L*) changes as a function of temperature and water activity of chicken breast meat during convective roasting. At the beginning they developed non-isothermal kinetic models, one described the browning (decrease of the lightness) of the surface of the meat as function of temperature, water activity and roasting time and the other described the whitening as function of temperature and roasting time. The two kinetic models were then coupled to validated mechanistic model for chicken meat roasting. This enabled the prediction of internal as well as surface lightness development from temperature and water activity changes. Actually, several authors developed kinetic models to predict colour variations during meat processing, in particular for red meat. Colour measurement in fact appears to be a suitable quality indicator to monitor in real time during a cooking process as well as an applicable method for industrial food processes monitoring (Goñi & Salvadori, 2017). Moya et al. (2021) developed a model capable of describing the evolution of beef colour during domestic pan cooking, including the turnover process. In particular, the model aimed to predict the turning over time for the meat in order to achieve a similar colour profile throughout the thickness of the meat. The authors developed a 3D mathematical model by coupling the kinetics of L* and a* colour parameters change to the heat and mass transfer model during the two-sided pan cooking. The model was able to adequately predict the colour changes of the meat by the good agreement between the numerical and experimental results for the three degrees of doneness tested, very rare, medium, rare and done. Also Goñi & Salvadori (2011) investigated the colour changes (a* in particular) of beef muscle subjected to different time-temperature treatments in a thermostatic water bath. The kinetic model of a* colour parameter evolution was coupled to a previously developed and validated beef

roasting model, which considered simultaneously heat and mass transfer during the process. Their predictions were in good agreement with experimental tests. Predictive models able to monitor colour variation to determine the degree of cooking of beef meat were also developed by Portanguen et al. (2009) and Pakula & Stamminger (2012). They both modelled the evolution of L* parameter during cooking and they develop a model able to predict the remaining cooking time by measuring lightness of beef while roasting until reaching different end temperatures and by using different oven temperatures. They also suggested that real-time measurement of meat colour opacity might therefore be used in automatic cooking programs to determine the degree of doneness.

Kondjoyan et al. (2013) instead develop a combined heat transfer and kinetic model to predict weight losses during cooking of *Semimebranosus* muscle of beef meat in an oven. The parameters of the kinetic models were identified using a set of experiments performed on meat cubes of different sizes heated in a water bath (Oillic et al., 2011). Then the model was validated in a fan-assisted oven under different air/steam conditions and was able to predict cooking losses of meat cuts of very different dimensions with an average absolute difference between simulations and the measurements of 6.8% of the cooking loss value during water bath experiments, and of 8.7% during oven wet cooking treatments.

A cross-validation with external data was conducted in order to assess the quality of the predictions of the model by comparing experimentally obtained values of cooking loss and chroma recorded at a specific cooking time to the predicted ones obtained by the model. In order to statistically and mathematically evaluate the models, firstly a graphical comparison was conducted, as shown in Figure 16 and Figure 17.

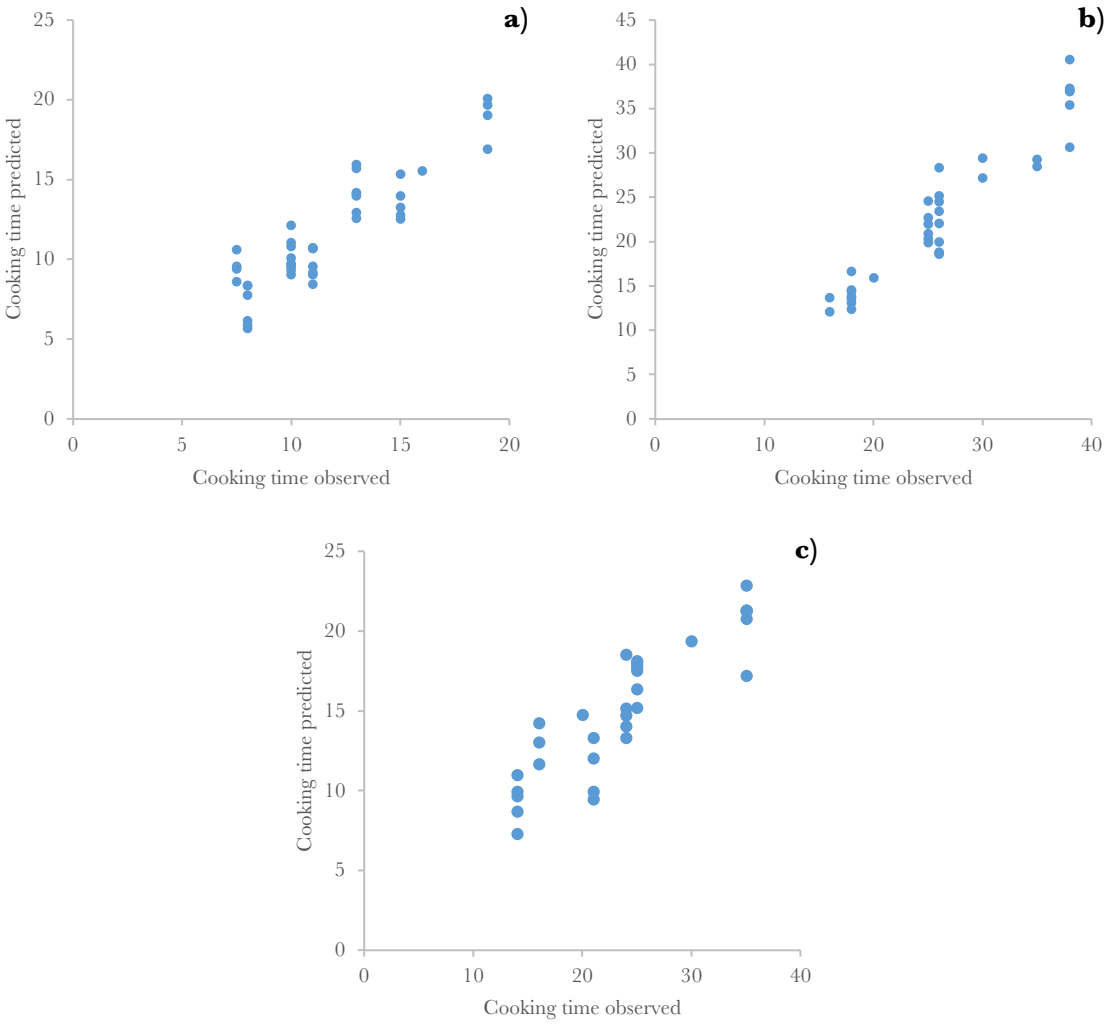


Figure 16 Comparison of observed and predicted cooking time values calculated using the cooking loss predictive models for (a) Grill (G) (b), Forced Convection (FC) (c) Sous Vide and evaluation of coefficient of determination (R^2).

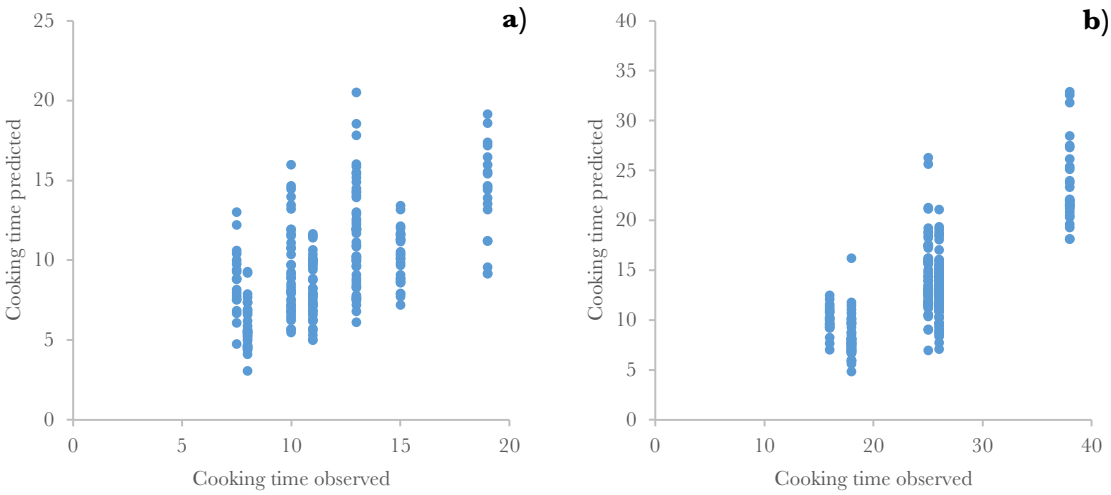


Figure 17 Comparison of observed and predicted cooking time values calculated using the chroma predictive models for (a) Grill (G) and (b) Forced Convection (FC) and evaluation of coefficient of determination (R^2).

From Figure 16 and Figure 17, coefficients of determination (R^2) were calculated in order to study the overall measure of the prediction attained and are shown in Table 13. Statistical parameters coefficient of determination (R^2), Mean Square Error (*MSE*), accuracy factor and bias factor were then calculated to evaluate the quality of the fitting and results are reported in Table 13.

Table 13 Mean Square Error (MSE), coefficient of determination (R^2), accuracy factor, and bias factor statistical evaluation of cooking loss and chroma predicted models according to Grill (G), Forced Convection (FC), and Sous Vide (SV) cooking method.

		R^2	**MSE**	**Accuracy factor**	**Bias factor**
Grill (G)	Cooking loss	0.817	2.288	1.240	1.015
	Chroma	0.331	1.734	1.323	0.884
Forced Convection (FC)	Cooking loss	0.868	3.377	1.116	1.143
	Chroma	0.631	19.086	1.111	1.913
Sous Vide (SV)	Cooking loss	0.787	27.024	1.128	1.444

As can be seen by Table 13, the highest R^2 values were related to cooking loss predictive models rather than chroma; in particular cooking loss model fitted very well for G and FC cooking method. The worst values were related to chroma models, in particular to chroma under G conditions.

MSE is a measure of the remaining variability. The lower the *MSE* the better the adequacy of the model to describe the data. *MSE* of cooking loss values were better estimated by cooking loss and chroma models for G, and by cooking loss for FC cooking method. The worst prediction was related to cooking loss for SV, showing a value of about 27, followed by chroma for FC, about 19.

The accuracy factor averages the distance between each point and the line of equivalence as a measure of how close predictions are to observations. The larger the value, the less accurate is the average estimate. Similar low accuracy values were assigned to models related to FC and SV cooking method. Models related to G cooking methods showed instead the highest accuracy factors, chroma in particular.

The bias factor answers the question whether, on average, the observed values lie above or below the line of equivalence and, if so, by how much. It gives the structural deviations of a model. According to bias factor, the models best fitting the data were again related to cooking

loss and chroma for G and cooking loss for FC, while the worst prediction was given by chroma predictive model for FC, with a very high value of 1.91, followed by cooking loss for SV (1.44).

In overall according to the four statistical parameters (Table 13), the best prediction models were those related to cooking loss for G and FC cooking methods, because they produced the closest prediction to the observed one. Therefore, cooking loss could be considered as the alternative indicator to temperature probe for chicken meat cooking inside a professional oven.

4.5 Conclusions

It was demonstrated that cooking loss can be considered the alternative indicator to temperature probe for chicken meat while cooking inside a professional oven. It is an easy-to-measure indicator and a balance could be the useful instrument for an on-line monitoring during a cooking process, by placing the meat on top of it. By monitoring the weight and so the water loss evolution in real time over a cooking process, the predictive model could calculate the final cooking time and automatically set it as end of the process. Another possibility could be to set the final cooking loss value, as associated to a qualitative noun or as entering a desirable specifically value beyond this point. Thanks to this input the model could extrapolate the cooking time and stop the cooking process when the meat cooking loss reaches that value.

The kinetic modelling approach can drive the optimization of cooking. Identification and control of the most sensitive quality index can drive the optimization of cooking through predicted models aimed at the development of cooking programs in cooking equipment. Another option is the use of innovative sensors monitoring the index during cooking and allowing a better management of the industrial cooking process.

PART 2

5 Chapter

Evaluation of protein digestibility of chicken breast meat undergone different cooking methods and times

5.1 Introduction

Chicken meat is considered healthy by consumers because is characterized by high-quality proteins, micronutrients, and polyunsaturated fatty acids (PUFAs) and is characterised by low fat and cholesterol contents (Sobral et al., 2017). Nowadays consumers are always more interested in well-being and their eating choices are always more driven by health aims.

Cooking generates a series of complex chemical and physical modification which can influence and compromise the final quality of meat if they are not properly controlled. A minimal loss of regulation of the cooking process can affect important aspects of foodstuffs, in particular the nutritional value. Understanding what happens during a cooking process and which consequences a cooking process can represent towards the nutritional profile allow the management of the cooking process itself.

Literature affirms that meat cooking can affect the physicochemical state of proteins and thus the bioavailability of amino acids. Indeed, during these processes, proteins are the target of free radical attack and consequent oxidation (Santé-Lhoutellier et al., 2008). Many authors have demonstrated that oxidation of meat proteins can negatively affect their degradation by enzymes of the digestive tract (Kamin-Belsky et al., 1996; Liu & Xiong, 2000; Santé-Lhoutellier et al., 2007; Santé-Lhoutellier et al., 2008). Oxidation not only leads to a decrease in protein hydrolysis, resulting in a lower absorption of amino acids in the intestine but also can lead to the accumulation of undigested protein in the intestinal tract, causing

fermentation and elevating the risk of colon cancer. (Pereira et al., 2013; De Smet & Vossen, 2016).

Understanding the impact of cooking on protein digestibility can be a useful task in order to enhance the nutritional value and limiting eventually undesired effects.

5.2 Aim of the Study

The aim of this study was to investigate the effect of cooking method and time on protein digestibility of chicken breast meat. Three different oven cooking methods were considered, in particular Grill (G, T=240 °C), Forced Convection (FC, T=170 °C) and Sous-Vide (SV, T=95 °C, RH%=100). Two different cooking times were assessed, an optimal time coinciding with the safety time, and an overcooked one. Static *in vitro* protein digestibility was carried out according to INFOGEST protocol (Brodkorb et al., 2019). The method was adapted to chicken breast sample, focusing on oral processing and samples preparation. Then, the effect of cooking method and time on chicken meat modification was examined according to protein oxidation analysis (carbonyl groups) and structural analysis (FT-IR analysis). Finally, a correlation analysis was conducted among all the different indices.

5.3 Materials and Methods

5.3.1 Materials

Chicken breasts samples were prepared as reported in paragraph 2.3.1.

5.3.2 Cooking treatments

Thawed chicken breasts were prepared and cooked individually as reported in 2.3.2. Samples were cooked as reported in Table 14. Two different cooking times were considered in this part: an optimal cooking time, i.e. safety time or time necessary to the achievement of 74 °C in the thermally less favoured point, and an overcooked time, obtained by increasing the optimal one by around 33%.

Table 14 Tray shape, cavity temperature, cooking time, and cavity humidity of each cooking method considered for chicken breast cooking by using a professional oven.

Cooking method	Tray Shape	Cavity temperature (°C)	Cooking times (min)	Relative humidity (%)
Grill (G)	Grid	240	13 – 19	---
Forced Convection (FC)	Flat	170	25 – 35	---
Sous Vide (SV)	Flat	95	25 – 35	100

5.3.3 Moisture

The moisture of the samples was determined using the gravimetric official method AOAC (method 930.15; AOAC International 2005), as reported in Chapter 2.3.4.

5.3.4 Image acquisition

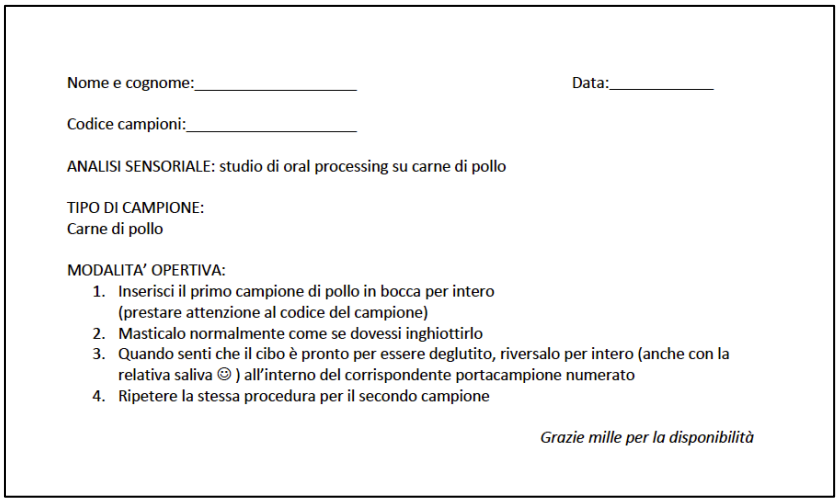
Images were acquired using image acquisition cabinet (Images & Computers, Bareggio, Italy), equipped with a digital camera (EOS 550D, Canon, Milan, Italy). Samples were placed on a black background and the digital camera was located on an adjustable stand positioned 45 cm above of the samples. The light was provided by 4100 W frosted photographic floodlight. Images were saved in .jpeg format, resulting 5184x3456 pixels.

5.3.5 Oral processing set up

5.3.5.1 Boluses collection

The collection of the boluses has been set up to evaluate the oral processing of cooked meat. 15 subjects recruited from Ph.D. students and thesis students within the Department of Agricultural, Food, Environmental and Animal Sciences of University of Udine, aged between 20 and 35, were chosen as subjects of study. No participants had adverse medical conditions for the trial, such as dental problems or chewing disorders.

Each judge was provided with 2 samples of cooked chicken, each weighing 5.0 ± 0.1 g (Pematilleke et al., 2020) and cooked according to the method of forced convection 170 °C for 25 min. Subjects were required to chew the samples individually and, when the stimulus to swallow was perceived, to pour the bolus into specimen holders. It was not required to carry out a fixed number of chewing cycles nor instructions were given on how to conduct chewing. The instructions were provided by means of a tab, which was supplied to each individual (Figure 18).



Nome e cognome:_____ Data:_____

Codice campioni:_____

ANALISI SENSORIALE: studio di oral processing su carne di pollo

TIPO DI CAMPIONE:
Carne di pollo

MODALITA' OPERTIVA:

1. Inserisci il primo campione di pollo in bocca per intero (prestare attenzione al codice del campione)
2. Masticalo normalmente come se dovessi inghiottirlo
3. Quando senti che il cibo è pronto per essere deglutito, riversalo per intero (anche con la relativa saliva ☺) all'interno del corrispondente portacampione numerato
4. Ripetere la stessa procedura per il secondo campione

Grazie mille per la disponibilità

Figure 18 Form for the oral processing study presented to the participants.

The analysis was carried out in a single day between 12:00 and 13:30, lunch time.

Collected boluses were subsequently photographed inside an image acquisition booth (Images & Computers, Bareggio, Italy), using a digital camera (EOS 550D, Canon, Milan, Italy) and the moisture was evaluated.

5.3.5.2 Boluses simulation

Cooked chicken samples were ground using a meat grinder (TritaExpress, R.G.V., Cermenate, Italy) fitted with a 7 mm-hole plate and the two possible speeds were tested. Different quantities of water were then added to the minced meat, in particular a quantity of water equal to 18.90% (w/w) of the total (value related to the average quantity of saliva added in the real boluses) and one equal to 21.48% (w/w) of the total (value equivalent to the dry matter of the meat, as required by the INFOGEST method for simulating food boluses) were then added to the minced meat.

5.3.5.3 Protein *in vitro* digestibility analysis

The static *in vitro* digestion method was carried out according to INFOGEST protocol (Brodkorb et al., 2019). Simulated salivary (SSF), gastric (SGF) and intestinal (SIF) fluids were prepared and stored at 4 °C until analyses and preheated to 37 °C just before use. Stock solutions of pepsin (60 U/mL) (Sigma-Aldrich, St. Louis, USA) in water, and pancreatin (800 U/mL) (Sigma-Aldrich, St. Louis, USA) and bile (134 mM) (Sigma-Aldrich, St. Louis, USA) in SIF were freshly prepared just before the experiments. Meat samples were ground in a meat grinder (Trita express, R.G.V, Cermenate, Italy to simulate the oral processing. Aliquots (1 g) of ground meat were added with a volume of SSF, $CaCl_2(H_2O)_2$, and water to guarantee the osmolarity and fluid to meal ratio (1:1, dry weight of food:volume). Due to the absence of starch in the matrix used during this phase, salivary amylase was omitted. (Brodkorb et al., 2019). The oral phase was simulated by maintaining the sample at 37 °C in a thermostat (Thermocenter TC- 40T, SalvisLab, Rotkreuz, Switzerland) under stirring with a rotatory shaker (F205, Falc Instruments s.r.l., Treviglio, Italy) for 2 min at 15 rpm.

The gastric phase was started by mixing the oral bolus with a volume of pepsin solution providing the required enzymatic activity of 2000 U/mL in the final gastric mixture. SGF and $CaCl_2(H_2O)_2$ were added, the pH was adjusted with HCl (6 M) (Carlo Erba, Milano, Italia) to 3 and water was added to maintain a 1:1 (v/v) fluid to bolus ratio. The sample was stirred at 37 °C during 2 h.

The intestinal phase was started by mixing the chyme with a volume of pancreatin solution providing the required enzymatic activity of 100 U/mL in the final intestinal mixture. 10 mM of bile salts were also added. SIF and $CaCl_2(H_2O)_2$ were added, the pH was adjusted with NaOH (1 M) (Carlo Erba, Milano, Italia) to 7 and water was added to maintain a 1:1 (v/v) fluid to chyme ratio. The sample was stirred at 37 °C during 2 h at 15 rpm.

At the end of the gastric and intestinal phases, samples were collected to analyse the degree of protein digestion. Samples collected at the end of the gastric phase were blocked by the addition of 2 M sodium hydroxide until pH 8 was reached to interrupt the enzyme activity. Samples obtained from the gastric and intestinal phases were centrifuged at 7000 rpm, 4 °C for 10 minutes (Avanti J-25, Beckman, Fullerton, California). The resulting supernatant was removed and 5 mL of ethyl alcohol (Merck KGaA, Darmstadt, Germany) was added and samples were centrifuged again. The supernatant was removed again and the solid residue was dried overnight in a vacuum oven at 70 °C. All the samples obtained have been stored at -18 °C in the freezer and thawed before the analysis.

5.3.5.4 BCA assay

The quantification of digested proteins was performed by bicinconinic acid (BCA) assay according to the method of Smith et al. (1985) with slight modifications.

About 0.02 g of the dried insoluble fraction of the gastric and intestinal phases were weighed in an Eppendorf by adding 1 mL of buffer. This solution was obtained by mixing 0.9456 g of Tris-HCl (Sigma-Aldrich, St. Louis, USA) and 2 g of SDS (Sigma-Aldrich, St. Louis, USA), reaching the volume of 100 mL with milli-Q water and adjusting the pH to 7.5. The samples were treated with ultrasound for about 130 minutes at room temperature (USC900D, VWR Avantor, Radnor, Pennsylvania) to help the breakage of the sample. Subsequently the samples were treated at 90 °C for 10 minutes to denature the proteins and centrifuged at 7000 rpm, 4 °C for 10 minutes. The samples were diluted 1:20 (v/v) with Milli-Q water and mixed with a vortex for a few seconds to promote homogenization. 25 µL of diluted sample were deposited in 3 different wells of a microplate. Immediately before inserting the microplate into the absorbance reader (Sunrise-basic tecan, Tecan Austria, Grödig, Austria), 200 µL of BCA working reagent was added to each well containing the sample to be analysed. The BCA working reagent was prepared at the time of analysis and obtained by mixing bicinconinic acid (Sigma-Aldrich, St. Louis, USA) and 4% copper sulphate (J.T. Baker, Deventer, Holland) in a 50:1 ratio and keeping it away from light. The samples were incubated at 37 °C for 30 minutes, after which the absorbance reading at 562 nm was carried out using a microplate reader (Sunrise-Basic Tecan, Tecan GmbH, Grodig, Austria).

The blank was prepared by mixing 200 µL BCA working reagent at 25 µL of water Milli-Q. The protein concentration of the samples was obtained by comparison with a calibration line constructed by producing different concentrations of standard protein of BSA bovine

serum albumin (Sigma-Aldrich, Milan, Italy). The results obtained from the analysis of the insoluble fraction were analysed in accordance with Bhat et al., (2019) and expressed as shown in the following equation:

$$Insoluble\ fraction\ (\%) = \frac{insoluble\ BSA\ equivalent}{raw\ meat\ BSA\ equivalent} * 100 \qquad Eq.\ 24$$

Where $insoluble\ BSA\ equivalent$ were BSA equivalent obtained from the insoluble part derived from protein *in vitro* digestion, while $raw\ meat\ BSA\ equivalent$ were BSA equivalent of raw meat. Then protein digestibility was obtained as:

$$Protein\ digestibility\ (\%) = 100 - Insoluble\ fraction\ (\%) \qquad Eq.\ 25$$

5.3.6 Protein carbonyl groups analysis

Total carbonyl content was evaluated by derivatisation with dinitrophenilidrazine (DNPH) as described by Oliver et al. (1987) with slight modifications.

Chicken meat samples (1 g) were minced and then homogenized 1:10 (w/v) in 20 mM sodium phosphate buffer containing 6 M NaCl (pH 6.5) (Sigma-Aldrich, St. Louis, USA) using an ultraturrax homogenizer for 30 s. Two equal aliquots of 0.2 mL were taken from the homogenates and dispensed in 2 mL Eppendorf tubes. Proteins were precipitated by cold 10% Trichloroacetic Acid TCA (1 mL) (Sigma-Aldrich, St. Louis, USA) and subsequent centrifugation for 5 min at 4200g. One pellet was treated with 1 mL 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH (Sigma-Aldrich, St. Louis, USA) in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10% TCA (1 mL) and washed three times with 1 mL ethanol:ethyl acetate (1:1, v/v) (Sigma-Aldrich, St. Louis, USA) to remove excess DNPH. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5) (VWR, Radnor, USA), stirred and centrifuged for 2 min at 4200g to remove insoluble fragments. Protein concentration was calculated from the absorption at 280 nm using BSA as standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of 21.0 $nM^{-1}\ cm^{-1}$ at 370 nm for protein hydrazones.

5.3.7 FT-IR

FT-IR spectra were recorded on freeze-dried chicken meat samples at 25 ± 1 °C by using a FT-IR instrument, equipped with an ATR accessory and a Zn-Se crystal that allow collection of FT-IR spectra directly on freeze-dried sample without any special preparation (Alpha-P, Bruker Optics, Milan, Italy). The "pressure arm" of the instrument was used to apply constant pressure to the sample, positioned onto the Zn–Se crystal, to ensure a good contact between the sample and the incident IR beam. All FT-IR spectra were collected in the range from 4000 to 400 cm^{-1}, at a spectrum resolution of 4 cm^{-1} and with 32 coadded scans. Background scan of the clean Zn–Se crystal was acquired prior to sample scanning. The collected FT-IR spectra were pre-processed (baseline corrected, smoothened, and normalized) using the OPUS software (version 7.0 for Microsoft Windows, Bruker Optics, Milan, Italy) and Gaussian curve fitting of deconvoluted amide I (1600–1700 cm^{-1}) was performed using Origin Pro 9 (OriginLab, Northampton, MA, USA) accordingly with Sow & Yang (2015) and Sow et al. (2017). The fitting quality of the Gaussian curves was confirmed by having $R^2 > 0.997$.

5.3.8 Statistical analysis

Data were expressed as the mean of at least three analytical determinations on three replicated samples and reported as ± standard deviations.

Data elaboration and representation was performed using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). Statistical analysis was performed using RStudio (version 4.2.2, The R Foundation for Statistical Computing, Vienna, Austria). Bartlett's test was used to check the homogeneity of variance. Analysis of variance (ANOVA) was conducted in case of homogeneity or Welch test in case the variances were not homogeneous. In both cases Tukey-HSD test was used to assess differences between means ($p<0.05$). A Pearson correlation analysis was also conducted.

5.4 Results and Discussion

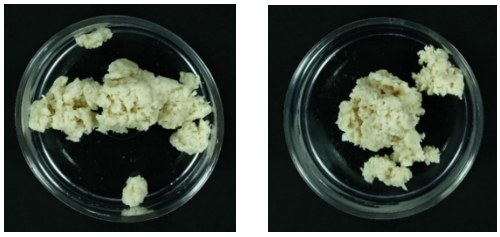
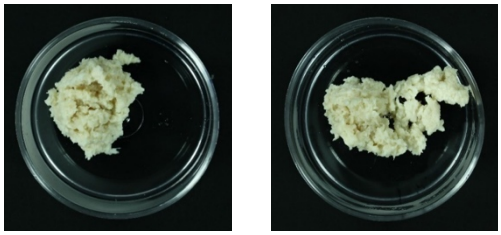
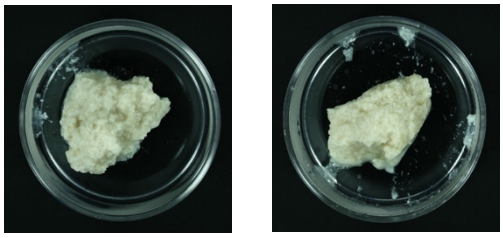
5.4.1 Collection and characterization of boluses obtained from oral processing

In order to develop a method of *in vitro* preparation of boluses that corresponds to what actually happens in human oral processing, some preliminary experiments were carried out on a sample of tasters aimed at evaluating the characteristics of the boluses obtained.

For this purpose, samples (approximately 5 g) of chicken subjected to forced convection cooking treatment were administered. Meat samples after cooking had an initial moisture content of 72.65 ± 1.01% (w/w) (dry matter 27.35 ± 1.51% (w/w)).

Food boluses obtained from samples of chicken breast chewed by the participants of the trial were collected, photographed and the images analysed, as can be seen from Table 15. At the same time, moisture analysis was carried out on the samples.

Table 15 Classification of collected food boluses of chicken breast meat according to the amount of saliva, related images and average amount of saliva.

Boluses classification	Collected boluses	Average saliva amount (%)
Low Moisture (saliva <12 % w/w)		9.13 ± 3.75% [c]
Intermediate Moisture (12< saliva<24 % w/w)		17.26 ± 3.18% [b]
High Moisture (saliva >24 % w/w)		32.744 ± 3.39% [a]

**Means in column with the same letters are not significantly different with ANOVA, Tukey-HSD test (p<0.05)*

As is clearly shown from Table 15, the obtained boluses were grouped into three distinct classes by saliva content. The saliva content was calculated thanks to a mass balance, by knowing the moisture amount and dry matter of the initial cooked sample and of the final boluses. The mass balance considers that the content of total solids does not change during chewing while increases the moisture content due to the addition of saliva. Images reflected the results obtained from the analysis of saliva. Boluses characterized by low saliva content appeared slightly cohesive, fragmented and dry and no drops of saliva were found at the bottom of the specimen. Their average saliva content calculated was about about 9% (w/w). Boluses characterize by intermediate saliva were more cohesive than the previous ones with the presence of sporadic droplets of saliva on the specimen, with an average saliva of about 17% (w/w). Then for high saliva content boluses appeared evident that the amount of embedded saliva was very high, because boluses were very cohesive with the presence of evident droplets of saliva in the sample holder. The average saliva amount of this class of boluses was around 33% (w/w). The average amount of saliva among all the boluses resulted to be 18.90 ± 8.93% (w/w), while the average humidity resulted to be 77.82 ± 2.44% (w/w) (dry matter average 22.18 ± 1.99% (w/w)).

Table 15 showed considerable variability in the results. Great variability of these data could also be due to the great suggestion aroused in participants by this kind of tests where it is required not to swallow the sample. The request could affect the chewing phase, prolonging or decreasing it according to the panellist state of mind and this influence the saliva addition, increasing or decreasing it respectively. A partial swallowing of the bolus caused by involuntary reflexes may also happen during the experiment (Pematilleke et al., 2020). This loss stands at less than 12% (Yven et al., 2005). Moreover, saliva addition is characterized by a very high inter-individual variability due to the different amount of saliva produced, number of chewing cycles and force exerted in the single bite (Yven et al., 2005; Pematilleke et al., 2020) related to factors such as period of day, age, gender, and presence of disease (Chen, 2009; Wang & Chen, 2017).

The strength exerted by the muscles of the mouth and the number of cycles of chewing lead to a different degree of rupture of the muscle fibers of the meat, resulting in a more or less pushed release of water still present in them. This can then be incorporated back into the bolus or partially swallowed and this affects the final bolus moisture (Yven et al., 2005; Pematilleke et al., 2020).

For the development of the oral phase of the meat digestion method, the *in vitro* digestion method INFOGEST (Brodkorb et al., 2019) was considered as the starting point for the evaluation of food digestibility. This method involves the use of a quantity of salivary fluids in the first phase or salivary phase equal to the dry matter amount of the sample under analysis, then this ratio 1:1 is maintained also for the following phases, i.e. between bolus and gastric fluids first, and between chyme and intestinal fluids later. Considering 1 g of cooked meat characterized by a moisture of 72.65%, it contained 0.2735 g dry matter and 0.7265 g of water as shown in Table 16. The INFOGEST method affirms that for each gram of dry matter there must be an equal amount of saliva or salivary fluid, that is 0.2735 g dry matter and 0.2735 g of saliva. As the oral phase involved 1 g of sample and 0.2735 g of saliva, the corresponding total weight of the sample was 1.2735 g, as shown in Table 16.

Table 16 Average composition by weight (g) of a sample of cooked chicken breast and corresponding chicken breast bolus.

Chicken meat sample	**Sample (g)**	**Water (g)**	**Dry matter (g)**	**Saliva (g)**
Cooked	1	0.7265	0.2735	0
Simulated INFOGEST bolus	1.2735	0.7265	0.2735	0.2735

In order to verify whether the quantity of saliva suggested by the INFOGEST method reflected the real quantity incorporated in the bolus, the average percentages of saliva in collected food boluses were calculated as shown in Table 17.

Table 17 Amount of saliva in chicken meat boluses obtained by the INFOGEST method and real amounts reported as percentage and saliva on dry matter (w/w).

Chicken meat bolus	Saliva in the bolus	
	(saliva/bolus %)	**(g saliva/g dm)**
Real bolus	18.90 ± 8.93[a]	0.91 ± 0.55[b]
INFOGEST bolus	21.48[a]	1.00[b]

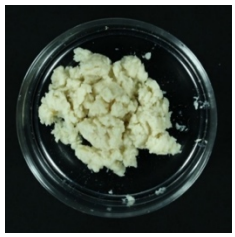
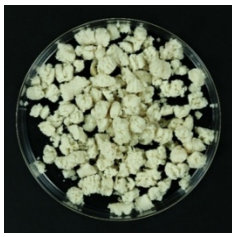
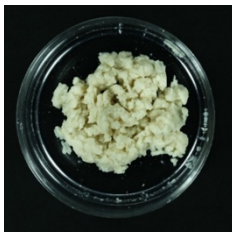
**Means in column with the same letters are not significantly different with ANOVA, Tukey-high significant different test ($p<0.05$)*

As Table 17 shows, the amount of saliva obtained from real boluses is not significantly different from the INFOGEST reference value (p-value = 0.06) and is in line with the results obtained from other meat studies (Pematilleke et al. 2020). Therefore, the ratio 1:1 between saliva and dry matter indicated by the INFOGEST method is consistent with the amount of saliva that would actually be incorporated into the bolus by a person.

5.4.2 Set up of food bolus on a laboratory scale for *in vitro* digestion

With the amount of saliva indicated by the INFOGEST method, boluses were prepared *in vitro*. To simulate chewing, a meat grinder was used as often employed in literature (Bax et al., 2012, Xiao et al., 2020). Two different machine speeds were tested (speed I slower and speed II faster) to see which one simulated the real boluses better (Table 18). In order to simulate an oral phase, after meat grinding water was added to mimic the contribution of saliva. The decision to use only water was made because it represents 99% of saliva and therefore the proportion of dry matter theoretically present would not affect the final result (Pematilleke et al., 2020).

Table 18 Samples of chicken breast meat minced by a meat grinder according to a low speed (I) and a high speed (II) and containing different amount of water: 0% (w/w) and 21.48% (INFOGEST quantity).

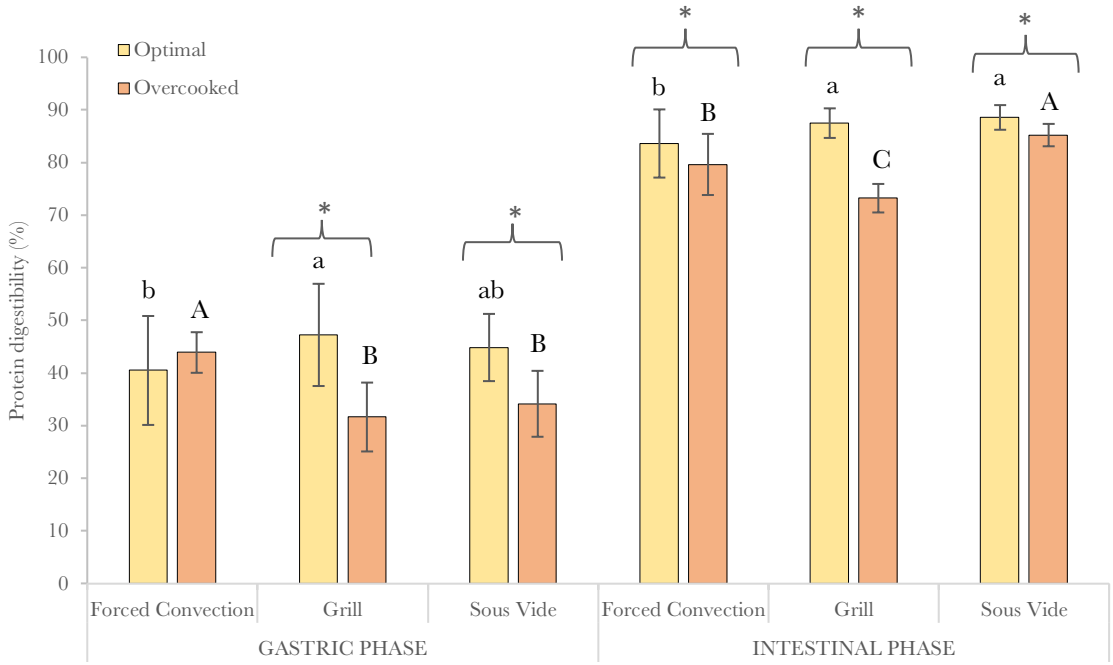
Grinder speed	**Water amount (%)**	
	0	21.48 (INFOGEST)
Low (Speed I)		
High (Speed II)		

As shown in Table 18, samples ground at higher speeds (Speed II) were smaller and packaged, because they were overly compressed against the perforated cutting plate. The added water could not properly rehydrate the whole mass, but only the surface of the meat fragments. This led to the creation of a more cohesive bolus but less homogeneous in terms of hydration. The lower speed (Speed I) produced less compact minced meat that resulted in less cohesive bolus but with a more homogeneous hydration level and therefore more similar to reality. For these reasons speed I of the meat grinder was used for the preparation of the simulated boluses for the *in vitro* digestion.

Literature shows that the amount of saliva incorporated into the meat is extremely variable and can be affected by cooking conditions. Pematilleke et al. (2020) assessed that different cooking times and temperatures led to variable incorporation of saliva between 15.93% and 24.58%. In fact, more intense treatments in terms of temperature and time cause a greater loss of water and hardening of meat. The resulting bolus will therefore need a greater amount of saliva, as well as longer chewing times (Mioche et al., 2003) to be suitable for swallowing (Pematilleke et al., 2020). Other studies have also reported how different moisture amounts of meat after cooking can be compensated by the intake of different amounts of saliva, obtaining boluses with the same final moisture (Rizo et al., 2019).

5.4.3 Effect of cooking method and time on protein *in vitro* digestibility of chicken breast

After optimization of the oral phase, the digestibility of proteins in chicken breast cooked according to different cooking methods for optimal cooking times was evaluated during the gastric and intestinal phase of digestion, as shown by the blue bars in Figure 19.



a-b means related to optimal cooking time indicated by different letters are significantly different at the same digestion phase ($p<0.05$)
A-B means related to overcooking time indicated by different letters are significantly different at the same digestion phase ($p<0.05$)
** means related to optimal and overcooking time are significant different for the same cooking method and digestion phase ($p<0.05$)*

Figure 19 Protein digestibility of gastric phase and intestinal phase of chicken breast meat cooked according to different cooking method Forced Convection (FC), Grill (G) and Sous-Vide (SV) cooking methods until the achievement of the optimal cooking time or exceeding it by 30% (overcooked samples).

Figure 19 shows the influence of the cooking method adopted on protein digestibility. Considering the optimal cooking time, protein digestibility in the gastric phase varied between 40 and 50%. The highest percentage of digested proteins was related to chicken cooked according to G (47.29 ± 9.71%) cooking method, while the lowest to FC (40.50 ± 10.35%). FC showed significantly lower protein digestibility than G. FC value resulted very close to the one obtained by Lian et al. (2023), estimating a value of 38.28% of gastric protein digestibility when they cooked chicken wings samples in an electric oven at 180 °C for 20 min. SV protein digestibility (44.87 ± 6.39%) did not result significantly different from both G and SV. Wen et al. (2015) found a similar value of SV protein digestibility (44.67%) when they cooked chicken breast samples in plastic pouches in a 72 °C water until the centre

temperature of meat reached 70 °C (approximately 30 min). Moreover, they also assessed that protein digestibility of chicken breast meat was not significantly different from pork, beef and fish meat, both in gastric and in intestinal phase. Concerning the cooking methods, Yin et al. (2020) found out that cooking beef *Semimembranosus* muscles according to a SV cooking process at 55 °C for 4 h significantly increased protein digestibility in the gastric phase as compared to boiling and roasting treatment (respectively by immersion in 100 °C water and cooking at 180 °C in an electric oven until both interior temperature of the samples reached 90 °C).

Regarding the intestinal phase, a higher protein digestibility was observed as compared to those in the gastric digestion. Several studies have shown that protein hydrolysis for meat is higher during the intestinal phase as compared to the gastric one (Lian et al., 2023; Wen et al., 2015; Yin et al., 2020; Bax et al., 2012; Sayd et al., 2015): in fact in the gastric phase proteins are hydrolysed into big peptides by pepsin, while in the intestinal phase these peptides are hydrolysed into polypeptides, tri/dipeptides and amino acids by trypsin and chymotripsin, present in pancreatin. Small peptides and amino acids can be so absorbed into the bloodstream (Dupont, 2017). The bioavailability of amino acids increases protein digestibility (Deb-Choudhury et al., 2014). Protein digestibility in the intestinal phase did not result significantly different between G (87.50 ± 2.80%) and SV (88.58 ± 2.34%), while FC cooking method showed significantly lower protein digestibility values (83.62 ± 6.48%) than both G and SV. The high standard deviation related to FC might suggest that the digestibility result could be affected by experimental variability. Lian et al. (2023) found a value of 84.47% related to roasting of chicken wings, comparable to FC value. In the literature, very few studies are available that compare the *in vitro* protein digestibility of meat, particularly chicken meat, using different cooking methods, as applied in the present work. Yin et al. (2020) investigated the effect of SV, roasting and boiling cooking method on beef *Semimembranosus* muscle and established that SV is the cooking method able to guarantee the highest protein digestibility.

Then the effect of meat overcooking on protein digestibility was also investigated. Overcooking can causing on one hand a lower nutritional quality, and on the other an increase in the amount of undigested proteins in the large intestine, which can be fermented into mutagenic products by intestinal microbiota, raising the risk of bowel diseases (Pereira et al., 2013). Chicken meat was cooked according to the 3 different cooking methods of G, FC and SV until the achievement of an overcooking time, obtained by increasing the optimal safety time by 33%. The overcooking time and the safety cooking time were compared as

shown in Figure 19. Considering G cooking method, protein digestibility significantly decreased in overcooking conditions, both in the gastric (-33%) and in intestinal phase (-16%). The cooking time influenced protein digestibility also in SV, which significantly decreased both in gastric (-24%) and in intestinal phase (-4%). Also FC cooking method, protein digestibility was affected by cooking time, but only in the intestinal phase (-5 %). Also in this case, only few studies were found in literature where the effect of cooking time on protein digestibility was assessed. A decrease in protein digestibility of chicken meat as effect of cooking time was discovered by Sangsawad et al. (2016). A cooking treatment of 60 min at 121 °C in an autoclave was able to reduce the protein digestibility of about 10% respect to a treatment of 15 min, and a treatment of 24 h at 70 °C in a water bath decreased it of about 5% respect a treatment of 0.5 h, both in the gastric and in the intestinal phase. Han et al. (2023) investigated protein *in vitro* digestibility of *Longissimus thoracis et lumborum* (LTL) of sheep meat cooked in an electric oven at 220 °C for increasing cooking times (0, 5, 10, 15, 20, 25, 30 min). It was interestingly observed that sheep meat protein digestibility increased according with the cooking time until reaching a peak at 15 min (about 50% of protein digestibility in the intestinal phase) and then dramatically decreased, reaching a minimum at 30 min (about 20% of protein digestibility).

Several reason could have affected these results of the protein digestibility, so further analysis about protein oxidation level and secondary structure were conducted to investigate.

5.4.4 Effect of cooking method and time on protein oxidation and secondary structure of chicken breast meat

In order to explore protein modifications caused by heating and influencing protein digestibility, oxidation level of proteins was analysed.

As first step, carbonyl groups quantification was conducted as shown in Table 19.

Table 19 Carbonylic groups in chicken breast cooked according to different cooking methods (FC, G, SV) until the achievement of an optimal time and an overcooked time. Data referred to raw meat are also shown.

Cooking method	**Cooking time**	**Carbonylic groups** (nmol/mg prot)
Raw	Raw	4.31 ± 1.18^{d}
Grill (G)	Optimal	14.69 ± 1.24^{bc}
	Overcooking	21.01 ± 4.06^{a}

Forced Convection (FC)	Optimal	11.62 ± 1.30[c]
	Overcooking	17.31 ± 3.28[ab]
Sous Vide (SV)	Optimal	13.12 ± 2.66[bc]
	Overcooking	13.97 ± 2.66[bc]

[a-d] In the same column, means indicated with different letters are significantly different according to ANOVA and Tukey-HSD test ($p<0.05$).

The increase in carbonyl groups content is considered an important protein oxidation marker that is linked to the reaction between proteins and the aldehydes, as well as oxidation of side chains of amino acids (Zhang et al., 2013). Carbonyl groups are usually produced on the side chains of amino acids such as Pro, Arg, Lys and Thr when they are oxidized with reactive oxygen species mediated by iron (Dalle-Donne et al., 2003). Results about carbonyl groups content are showed in Table 19. It can be observed that carbonyl groups content significantly increased in cooked samples respect the raw ones. The effect of time on oxidation was seen in G overcooking treatment, with the highest amount of carbonylic group in overcooking conditions, followed by FC overcooking. No differences were seen in terms of cooking method. Sobral et al. (2020) found out that oven and microwave cooking significantly increased the amount of carbonyl groups in chicken meat cooking. Also Silva et al. (2016) and Yin et al. (2020) affirmed that carbonyl content significantly increased in chicken meat after cooking treatment and in particular increased differently according to the cooking method applied, i.e. grilling, roasting, boiling frying or sous vide. For the authors, sous vide always showed the lowest carbonyl groups amount. This outcome coincided with the results of the present study. In fact, SV overcooking resulted in the same oxidation level of optimal cooking treatment. This can be explained by the vacuum packaging and low cooking temperatures, which are able to prevent intramuscular fat oxidation in comparison with meat cooking without vacuum (Yin et al., 2020).

Protein secondary structure modifications was also investigated. Figure 20 shows FT-IR spectra in the range 4000 – 400 cm^{-1} of freeze-dried meat samples cooked according to different cooking methods and times. During spectra elaboration, the bands between 1650 and 1670 cm^{-1} were associated to *α*-helix structure and bands between 1640 and 1650 cm^{-1} are due to random/non-ordered structures. *β*-sheet were detected in the range of 1618–1640 cm^{-1} and 1670–1680 cm^{-1} respectively. Additionally, *β*-turns appeared between 1675 and 1695 cm^{-1} (Kong & Yu, 2007).

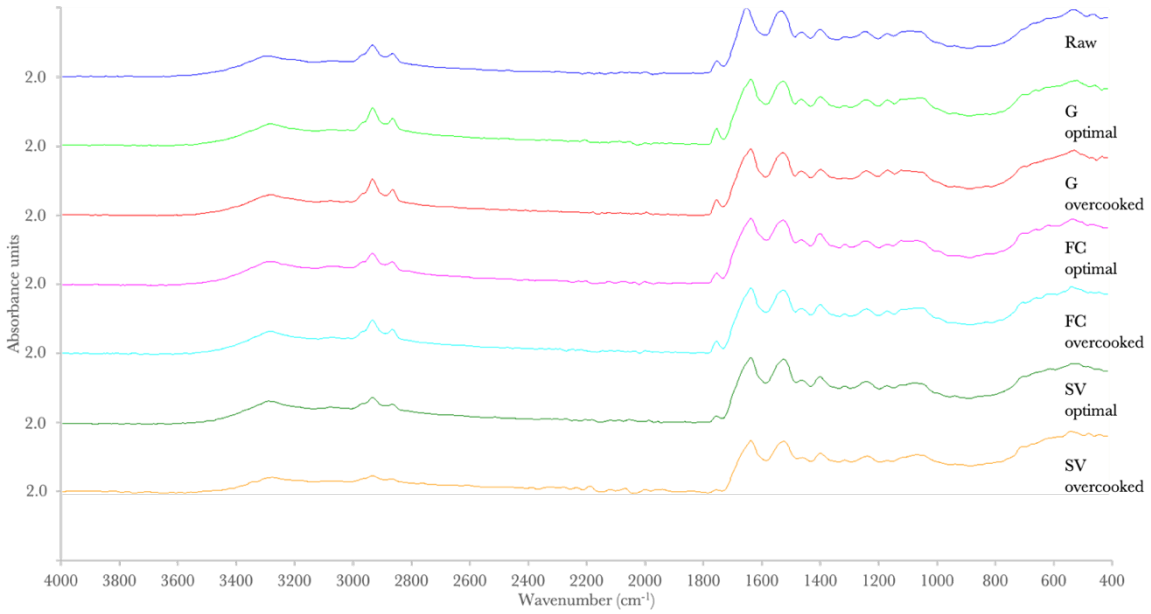


Figure 20 FT-IR spectra of chicken breast meat cooked according to different cooking methods (G, FC, SV) and times (optimal, overcooked). Data referred to raw meat are also shown.

Table 20 reports the relative percentage of *β*-sheet, random coil, *a*-helix, β-turn identified in samples spectra.

Table 20 Percentage content of secondary structures (β-sheet, random coil, a-helix, β-turn) in chicken breast cooked according to different cooking methods (FC, G, SV) until the achievement of an optimal time and an overcooked time. Data referred to raw meat are also shown.

Cooking method	**Cooking time**	**Protein secondary structure (%)**			
		***β*-sheet**	**Random coil**	***a*-helix**	***β*-turn**
Raw	Raw	22.65 ± 2.39^{c}	30.64 ± 1.62^{a}	29.45 ± 5.1^{a}	17.26 ± 1.87^{a}
Grill (G)	Optimal	30.18 ± 5.64^{ac}	30.26 ± 5.52^{a}	10.54 ± 0.39^{b}	29.03 ± 9.49^{a}
	Overcooking	37.83 ± 4.99^{a}	34.33 ± 6.82^{a}	8.51 ± 0.71^{b}	19.33 ± 2.34^{a}
Forced Convection (FC)	Optimal	30.98 ± 5.54^{ac}	30.88 ± 4.64^{a}	20.58 ± 3.27^{ab}	17.56 ± 3.97^{a}
	Overcooking	34.03 ± 5.65^{ab}	34.12 ± 2.16^{a}	12.04 ± 0.1^{ab}	19.81 ± 2.41^{a}
Sous Vide (SV)	Optimal	27.07 ± 3.83^{bc}	32.11 ± 1.38^{a}	22.57 ± 3.66^{ab}	18.25 ± 4.28^{b}
	Overcooking	28.75 ± 4.79^{ac}	36.85 ± 2.16^{a}	13.00 ± 0.1^{ab}	21.40 ± 2.41^{b}

$^{a-c}$ In the same column, means indicated with different letters are significantly different according to ANOVA and Tukey-HSD test ($p<0.05$).

Results of Table 20 shows that *β*-sheet structure amount increased in cooked meat with respect to the raw one and in particular it significantly increased in G cooking method and FC under overcooking conditions. No differences were seen between raw meat and SV. Regarding *a*-helix, the proportion seems to be lower for cooked samples than the raw one. In particular, G cooking treatment presented the lowest amount of α-helix. The analysis seems to indicate that during cooking processing α-helix gradually decreased and β-sheet gradually increased. This could mean that α-helix fractions turned into β-sheet and β-turns. These results are consistent with previous studies where modification of secondary structure was reported to be affected by heating temperature and cooking method. In particular, authors agree that a gradual increase of *β*-sheet in the detriment of α-helix in proteins is expected during a cooking process, both in whole meat and extracted myofibrillar protein (Xu et al., 2011; Berhe et al., 2014; Zhou et al., 2019; Yin et al., 2020).

The reason is that cooking process can break the hydrogen bonds between the amino hydrogen and carbonyl oxygen, which is critical to the maintenance of α-helix structure (Liu et al., 2008). The increase in β-sheet could be attributed to the rebuilding of unfolded proteins and the aggregation of proteins by hydrophobic interaction between nonpolar amino acids, which was caused by the effects of heating and time (Bouraoui et al., 1997; Okuno et al., 2007). The intensity of heating rules the conversion of α-helix in other forms (Chen et al., 2018).

Results indicated that heat treatments can easily cause denaturation and changes of conformation and local microenvironment of proteins. In order to understand whether the changes of structure and local microenvironment of proteins are related with the digestibility, a final correlation study was performed to investigate links among parameters of oxidative modifications (carbonylic groups), secondary structure modifications (*a*-helix, *β*-sheet) and protein digestibility in both gastric and intestinal phase. Correlation matrix showing Pearson correlation indices is presented in Table 21.

Table 21 Correlation matrix among parameters of secondary structure modifications (a-helix, β-sheet), oxidative modifications (Carbonylic groups) and protein digestibility in gastric phase and intestinal phase.

	a-helix	β-sheet	Carbonylic groups	Protein digestibility in gastric phase	Protein digestibility in intestinal phase
a-helix	1				
β-sheet	-0.67 *	1			
Carbonylic groups	-0.78 *	0.87 *	1		
Protein digestibility in gastric phase	0.35 NS	-0.48 NS	-0.46 NS	1	
Protein digestibility in intestinal phase	0.58 NS	-0.97 ***	-0.86 *	0.65 *	1

*Significance is noted as follows: p>0.05, NS; p<0.05, *; p<0.01, **; and p<0.001, ****

Thanks to Pearson correlation analysis, a negative correlation was found between carbonylic groups and protein digestibility in intestinal phase (r=-0.86, p-value=0.02). The reason is linked to the fact that proteins oxidation, crosslinks, aggregation, and polymerization as well as complexation with aldehydes may alter the protease-active sites, hindering the enzymatic proteolysis and consequently reducing the amino acid release during digestion (Santé-Lhoutellier et al., 2008). Moreover, a different susceptibility to digestion has been associated to protein secondary structure. A very high significant negative correlation was found between *β*-sheet and protein digestibility in intestinal phase (r=-0.97, p-value=0.001). According to protein secondary structures, proteins seemed to have a different susceptibility to digestion. A higher proportion of *β*-sheet may partly cause a low access to gastrointestinal digestive enzymes, which may lead to lower protein nutritional value (Calabrò & Magazù, 2012; Yu, 2005). Thanks to a more flexible structure α-helix has also been associated to an easier proteolysis (Zhou et al., 2014).

Results showed that the functionality of the total protein pool might be adversely affected because of stronger cooking conditions. Results are in good agreement with literature. Several studies indicate that protein digestibility is compromised as a result of the oxidative modifications suffered by proteins during processing (Santé-Lhoutellier et al., 2008; Chen et al., 2013; Oueslati et al., 2016).

5.5 Conclusions

The obtained results showed that INFOGEST method for *in vitro* digestion was successfully optimized for the digestion of chicken meat. In particular, according to the indications of the INFOGEST protocol, boluses developed *in vitro* were comparable with real boluses in terms of saliva amount.

Regarding cooking methods, FC cooking method seemed to significantly decrease protein digestibility both in gastric and intestinal phase. Significant differences were also identified according to the cooking time. In fact, overcooking condition significantly decreased the protein digestibility for G and SV in the gastric phase and for all the three cooking methods examined in the intestinal phase.

Protein digestibility in intestinal phase was negatively correlated with carbonyl groups, meaning that the more proteins were oxidated and the less proteolytic enzymes access to protein hydrolysis sites. Protein digestibility in intestinal phase was also negatively correlated to β-sheet secondary structure, which caused limited access to gastrointestinal digestive enzymes leading to a decrease of protein nutritional value.

Results showed that the achievement of safety time, for G, FC and SV guarantees the best protein digestibility and so the highest protein quality. Instead, longer cooking times decrease the protein digestibility.

A cooking machinery such as a professional oven could be implemented with this information to create an added value. So, for example, the creation of a program certified to reach the optimal cooking time related to the maximum value of protein digestibility or protein quality could increase satisfaction in consumers.

6 Chapter

General discussion

6.1 Context

Food service is expected to grow in the future and, to drive growth and profitability, restaurants should be transformed by including innovative digital technologies. Cooking equipment should possess the capability to efficiently process larger product quantities within minimal timeframes, all while maintaining strict control, ensuring product standardization, upholding food safety, and delivering the highest quality. This addresses the evolving preferences of consumers who prioritize wellness, health, and nutrition, and simultaneously addresses societal and sustainability challenges.

Cooking machineries, such as professional ovens, at the moment are able to monitor only food temperature during cooking thanks to integrated temperature probes. Monitoring the temperature in the thermally less favoured point in foods guarantees the respect of safety issues if present. At the moment, several automatic programs in professional ovens aimed at the reduction of microbiological load exists and can be applied to guarantee food safety. For this purpose, instruments such as temperature probes, sometimes very inaccurate, are employed.

An appealing cooked dish is not only characterized by the respect of the mandatory safety limits, but also by a precise quality. Food quality is defined by sensory attribute, nutritional value and technological performance. Food quality is sometimes taken for granted or underestimated and subjected to personal tastes of chefs, providing their experience. At present, cooking machineries are not aimed at optimization of a cooking process.

In the context of this Ph.D. thesis, aligning the mandatory safety standards with the perspective on food quality has yielded novel insights that contribute to the knowledge necessary for the enhancement of food cooking processes. These processes are designed to

achieve both safety and sensory food quality simultaneously. In this sense, optimization of a cooking process by matching the two issues can be accomplished thanks to the application of the kinetic modelling approach. The modelling approach aimed at evaluation of quality or nutritional indices modification of a food subjected to a cooking process and developing a predictive model able to combine quality and nutritional indices, as long as safety limits are ensured. The result represents the premise for the development of an innovative on-line sensor based on quality modification.

The pursued approach has introduced several innovative elements that may serve as a foundation for future research avenues in the realm of cooking for high-quality food and sensory experiences.

6.2 Main findings

Chapter 2 concerned the first step of the kinetic modelling approach based on data collection about quality indicators. Cooking loss and colour evolution during chicken breast cooking linearly increased as a function of cooking time while a biphasic behaviour was observed regarding texture evolution: shear force decrease until reaching a minimum point of tenderness and then increase. Similar trends were observed as regards of all tested cooking process (Grill, Forced Convection and Sous Vide). However, times required for the achievement of the best quality associated to the tenderest point was shorter than the achievement of the safety limits. Safety was recognised as the limiting factor for chicken breast cooking.

Chapter 3 was related to the second phase of the kinetic modelling and in particular to kinetic data elaboration. The effect of cooking method and temperature was investigated on the evolution of quality indices over the cooking process. Rate constants of quality indices linearly increased according to the temperature of the cooking process and they were modelled according to Arrhenius equation. Grill cooking method was able to accelerate the chemical physical reactions more than forced convection and sous vide. Arrhenius model allowed to evaluate the existing relation between process temperature and quality indicators changes.

Chapter 4 involved the final step of the kinetic modelling approach aimed at the development of a predicting model for cooking control. Models as function of quality indices

were developed by combining Arrhenius equation with quality indices kinetics. Cross-validation showed that cooking loss-based model was the one best predicting cooking time as a function of temperature. The value of cooking loss attributable to the end of the optimal cooking process was the one associated to safety time.

Chapter 5 examined the nutritional aspect of proteins digestibility of chicken breast as affected by cooking process. With regards of oral processing studies, meat sample preparation was optimized for static *in vitro* protein digestibility analysis. Thank to this, protein digestibility of meat emerged to be not influenced by the cooking method, while overcooking conditions were able to significantly decrease the protein digestibility, regardless of the cooking method applied. Oxidative profile and secondary structure analysis confirmed proteins modifications as affected by prolonged cooking times, capable of hiding the hydrolysis sites for digestive enzymes.

Based on the results acquired during the research activity, the following can be considered as the major findings of this Ph.D. thesis:

- Evolution of chicken breast shear force during cooking is characterized by a biphasic behaviour, decreasing until reaching a minimum value of tenderness and then increasing. This behaviour was attributable to connective tissue proteins aggregation and gelling.
- During cooking of chicken breast, safety time of achievement of 74 °C in the thermally less favoured point represents the limiting factor of an optimal cooking process. Times required for the achievement of the tenderest point considered as the most representing index of the cooking process are shorter than safety time.
- During chicken breast cooking, quality indices of cooking loss and chroma linearly increase as a function of the temperature of the cooking process. Arrhenius model allowed to evaluate the existing relation between process temperature and quality indicators changes.
- Kinetic modelling approach can be considered as a valid approach to optimize a cooking process through the analysis of quality indicators and maximization of sensory quality by respecting the safety issues.
- Cooking time significantly affects chicken breast protein digestibility and in particular overcooking conditions are able to decrease it.

6.3 Innovative aspects

The approach followed during the progress of this Ph.D. thesis presented some innovative aspects pointing out key features relevant to the optimization of the cooking process. In particular:

- The kinetic modelling approach represents a valid and objective method to identify the optimal cooking time of a cooking process by matching quality and safety aspects. The model can maximize the sensory and nutritional quality while minimizing any adverse effects of the cooking process within the bounds of safety constraints.
- This approach was developed by taking into consideration chicken breast meat cooking, but its applicability extends to various other food items for the purpose of optimizing their cooking. It represents an easy and objective way of determining the optimal cooking time. An in-depth analysis of quality indices indicators related to the specific food under examination would be necessary at the beginning.
- The predictive model output can be considered a starting point for cooking optimization, by integration in cooking machineries.

6.4 Future perspective

The predicted model extrapolated thanks to the kinetic modelling approach would be suitable to be integrated inside a cooking machinery. The development of an automatic program able to predict the optimal cooking time would be possible by integrating a sensor such as an on-line sensor capable of monitoring in real time the evolution of the critical quality indicator. Selection of an easy-to-measure critical quality indicator would be fundamental.

In case of meat, a balance would be considered as a suitable on-line sensor since the critical quality index is cooking loss. So in this way, by monitoring the weight loss evolution in real time over a cooking process, the predictive model would be able to calculate the final cooking time and automatically setting it as end of the process. An alternative application of the program would involve establishing a desired final cooking loss value. Users could input this value manually or select a predefined qualitative label associated with a specific value (e.g. optimal cooking label = achievement of 30% of cooking loss). Thanks to this input the model could extrapolate the cooking time and stop the cooking process when the meat cooking loss reaches that value. In this way, cooking process would be optimized: no undercooking risks

linked to safety for consumer health, no overcooking time decreasing the nutritional value, only the certainty that cooking reached the maximum sensory quality.

Other foodstuffs could be investigated and specific automatic cooking programs could be extrapolated for each one. Efficient sensors could be considered or developed for the food matrix under examination, opening the way to many possibilities. In the coming years, several innovative sensors could be integrated into the machines. A good example can be provided by Computer Vision Systems (CVS) which are relatively new research tools. They are commonly employed for computer-based image acquisition and analysis, enabling the extraction of information or the control of processes (Brosnan & Sun, 2004). They can be applied to macro (Balage et al., 2018) and microscopic subjects (Monteschio et al., 2019). Images might be taken using digital cameras, even those from smartphones (Meunier et al., 2021), ultrasound (US) methods (Fiore et al., 2020), dual energy X-ray absorptiometry (DEXA) (López-Campos et al., 2018), near infrared (NIR) spectroscopy (Rust et al., 2008; Wyrwisz et al., 2019) and hyperspectral (HS) imaging (Balage et al., 2018; Ma & Sun, 2020). These are very promising analytical techniques, with a high acquisition speed, no sample preparation, rapid predictions, some of them are very cheap and versatility. These devices, once trained, are able to collect input from outside, recognizing the item and transmit the signal to the processor. Some of these instruments can correlate a specific physical property, such as colour, with its chemical components in specific real-time food processes (Nguyen et al., 2022: Pedreschi et al., 2006). It cannot be difficult to imagine a CVS sensor inside an oven able to monitor in real-time the status of a food during a cooking process (Lin et al., 2021).

Improving the technology of cooking machineries is necessary in the next years due to the fact that food service is getting bigger. Optimize the cooking means guarantee the best service for the consumer, enhancing the food and nutritional quality, ensuring food safety, but also rendering the process more sustainable from energy and environmental point of views.

6.5 Research advancements for company profit

These main findings obtained by this academic research can be considered a significant starting point for a technological advancement of the cooking machineries, contributing to the economic growth of the company and its promotion in the market. Cooking devices integrated with advanced predictive models and algorithms are able to optimize cooking from a quality and technological point of view.

From a quality point of view, such cooking programs assure the achievement of food safety by improving the efficiency of food preparation, guaranteeing consumer protection and public health. Wellness can also be promoted by proposing cooked foodstuffs characterized by high sensory and nutritional standards. Foods cooked by optimized ovens can also assure appetizing foods and the highest preservation of nutrients and flavours. Food waste (e.g. due to overcooking) can be limited or avoided thanks to the control of cooking programmes. Both safety and nutritional control can have a great impact on society, particularly in terms of delivering well-balanced and nourishing meals in institutional settings like schools, hospitals, and care facilities. Moreover, typical of this kind of institutions is the high demand for meals to be cooked in large quantities in a short period, problem that can be faced by the optimized ovens able to faster cooking times and to a precise temperature control.

From a technological point of view, optimized cooking times can ensure minimum energy expenditure, avoiding energy waste and promoting environmental sustainability. Nowadays this evolution is fundamental for the industry and their products in order to ensure a transition towards a low-carbon economy and a sustainable economic growth. In fact to combat climate change, the European Parliament approved the European Climate Law (Reg. No 2021/1119), which raises the target of reducing net greenhouse gas emissions by at least 55% by 2030 (from the current 40%) and makes climate neutrality mandatory by 2050. European Climate Law is part of the European Green Deal, the EU roadmap to climate neutrality, and all industries of Member States have to face with it in the next years.

6.6 Main conclusions

Innovation and technology of cooking machineries should keep up with global trends and in particular consumers needs and new habits. Food service is expected to grow in the future and so consumer worries about well-being and health, driving them towards a greater consumption of chicken meat. Optimizing the food cooking process, and in particular of chicken breast cooking, answer to the request of improving sensory and nutritional quality of a food.

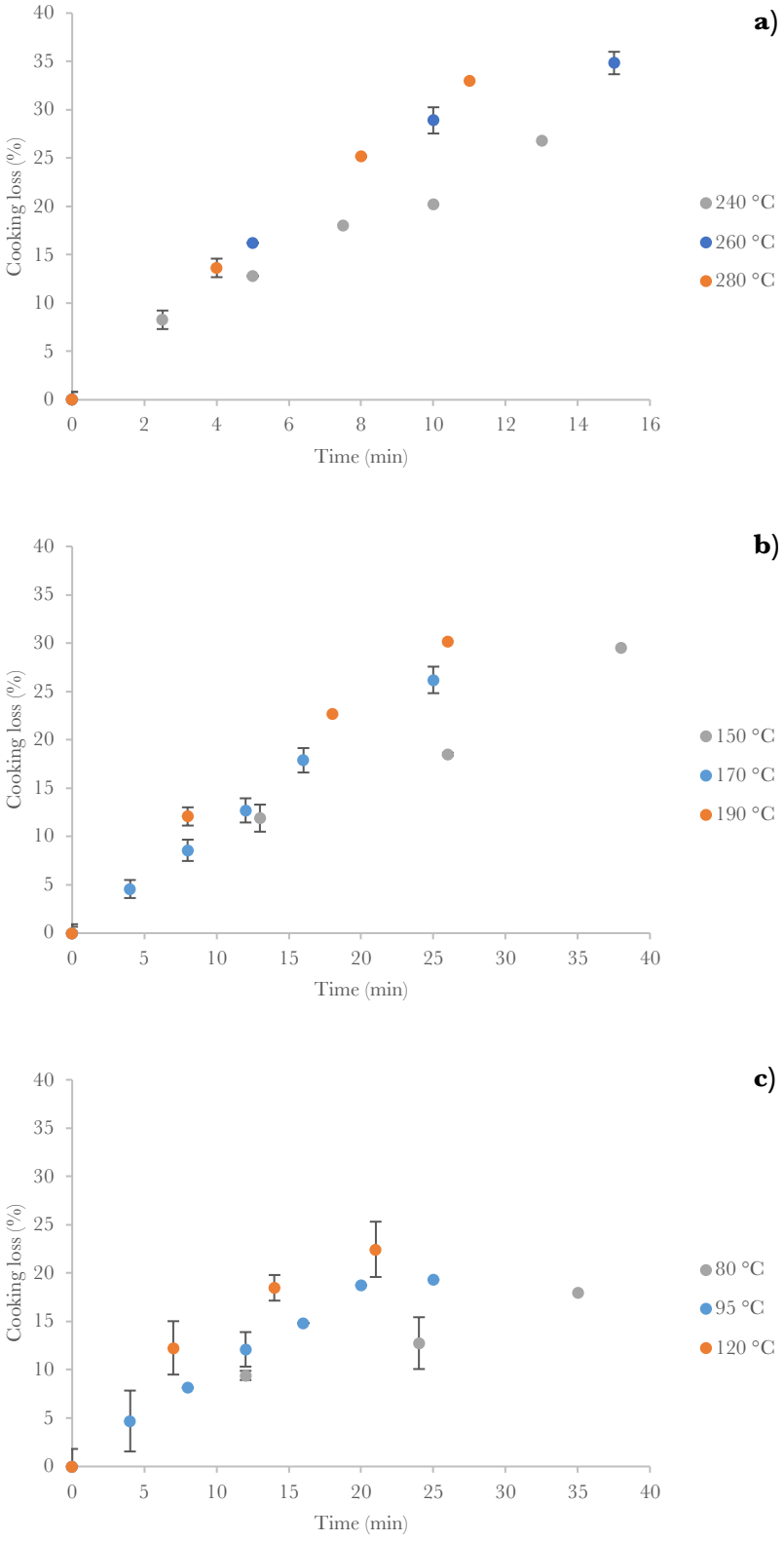
Since only inaccurate temperature probes for safety control exist and are used as cooking monitoring sensors in professional kitchen, the necessity of an objective way able to establish the achievement of the maximum sensory and nutritional quality is essential.

The application of the kinetic modelling approach to a food can be a useful tool to investigate the complex chemical and physical modifications subjected to the food during a cooking

process. The approach elaborates a predictive model providing an optimal cooking time capable of matching and maximizing both sensory and nutritional quality and safety requirements. Identification of a suitable quality index representative of the cooking process for the food under examination can drive the development of alternative on-line sensors capable of monitoring the cooking process.

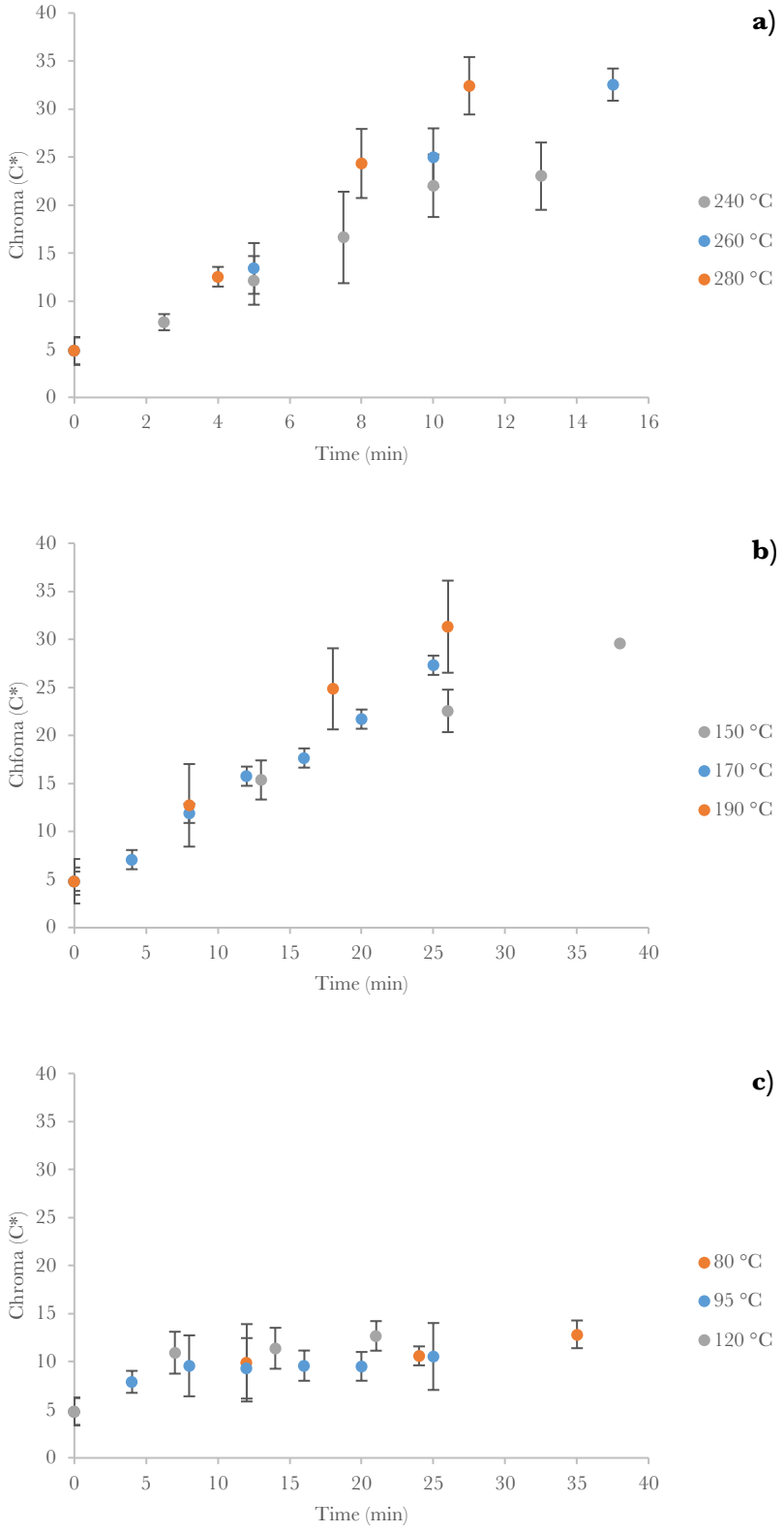
7 Appendix

7.1 Cooking loss evolution of chicken breasts undergone different cooking treatments at different cooking temperatures



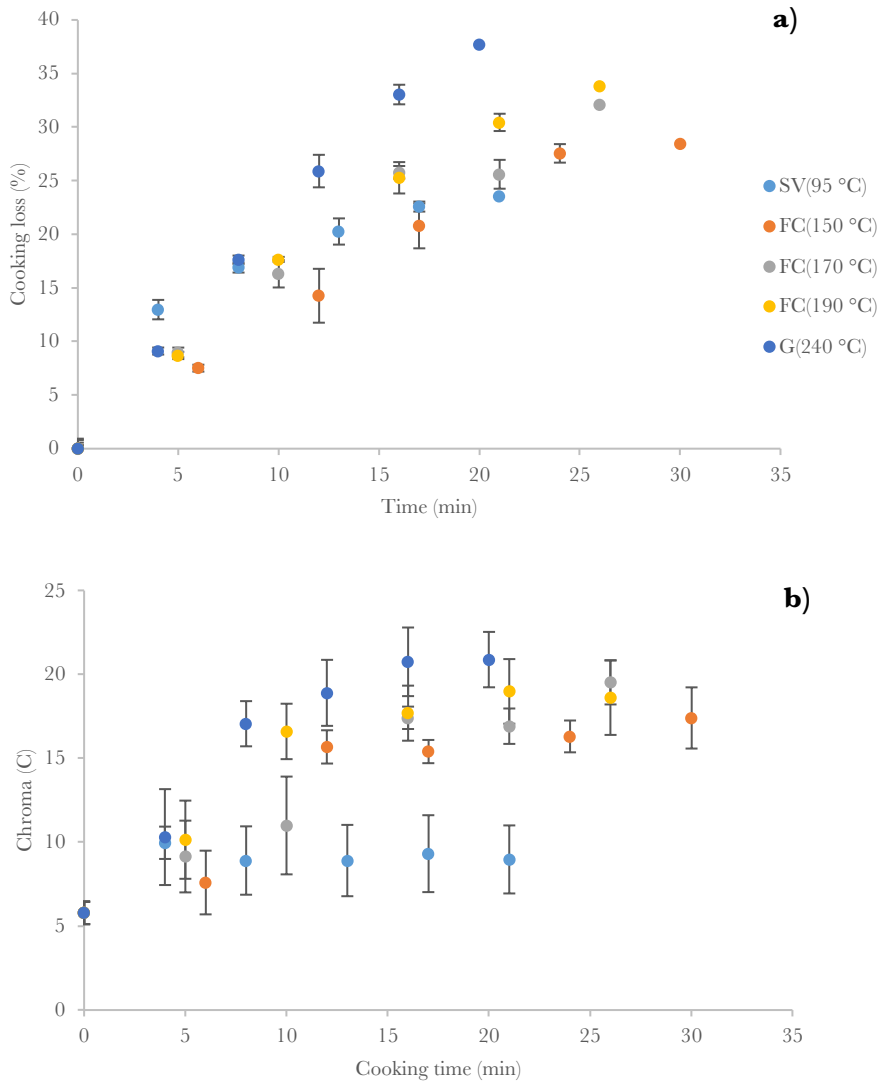
Appendix 7.1 Cooking loss evolution as a function of cooking time of chicken breast undergone different cooking methods and temperatures:(a) Grill (G) at 240, 260, 280 °C (b) Forced Convection (FC) at 150, 170, 190 °C (c) Sous Vide (SV) at 80, 95, 120 °C.

7.2 Chroma evolution of chicken breasts undergone different cooking treatments at different cooking temperatures



Appendix 7.2 Chroma evolution as a function of cooking time of chicken breast undergone different cooking methods and temperatures:(a) Grill (G) at 240, 260, 280 °C (b) Forced Convection (FC) at 150, 170, 190 °C (c) Sous Vide (SV) at 80, 95, 120 °C.

7.3 Cooking loss and chroma evolution of chicken breasts samples provided by a new supplier undergone different cooking treatments at different cooking temperatures in a distinct oven



Appendix 7.3 Evolution of (a) cooking loss and (b) chroma of chicken breast batch for cross-validation as a function of time under different cooking methods and temperatures: Grill (G) at 240 °C, Forced Convection (FC) at 150, 170, 190 °C, Sous Vide (SV) at 95 °C.

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Giulia Romano was born in Treviso (Italy) on 31st December 1991. After attending the scientific high school, she enrolled at the University of Udine in the Food Science and Technology course. She earned the master's degree in 2017 with the highest marks, presenting a dissertation on modelling the effect of the oxidation status of the ingredient oil on stability and shelf life of low-moisture bakery products, supervised by Professor Lara Manzocco and Professor Sonia Calligaris. She won a position as a research fellow in 2017 working with the Food Technology Research Group of the University of Udine in collaboration with Advance Development Group of Electrolux Professional S.p.A. The project was aimed to the design and development of a high-pressure device for storing food and to study its functionality and potential. In 2019 she was awarded a research doctorate scholarship in "Food and Human Health" by University of Udine and funded by Electrolux Professional S.p.A., under the scientific supervisor of Professor Maria Cristina Nicoli and Professor Monica Anese and the company supervisor of Mr. Daniele Turrin and Dr. Arianna Bozzato. From April to August she was a visiting student at Teagasc Food Research Centre in Dublin (Ireland), where she started to acquire knowledge about spectrophotometric techniques and relative modelling. During her Ph.D. she held seminars relevant to her research activity within the bachelor's and master's degree study courses in Food Science and Technology at the University of Udine. She assisted professors in examinations for invigilation and marking, and supervised bachelor and master students during their dissertation work. She also attended at several national and international conferences, presenting oral and poster contributions on her research activity.

Publications relevant to the Ph.D. activity

Publications on international peer reviewed journals

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Romano, G., Nicoli, M. C., Bozzato, A. & Anese, M. A kinetic study on quality changes of chicken breast using different oven cooking procedures. *Food Research International*. Under review.

Romano, G., Nicoli, M. C., & Anese, M. Effect of different oven cooking procedures on in vitro protein digestibility of chicken meat by using INFOGEST digestion protocol. Under drafting.

Romano, G., Ferragina, A., Hamill, R., Nicoli, M. C.., & Anese, M. Exploratory study on the effect of time and temperature in different cooking methods on colour and texture of chicken meat and effect on spectral profile of different spectrophotometric instruments. Under drafting.

Contributions to national and international conferences

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