Hungarian University of Agriculture and Life Sciences

EFFECT OF ONE-STEP AND TWO-STEP SOUS VIDE TREATMENTS ON QUALITY AND STORAGE STABILITY OF CHICKEN BREAST

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LIST OF ABBREVIATIONS

*a** - Redness

ANOVA – Analysis of variance

AOAC – Association of Official Analytical Chemists

*b** - Yellowness

BHI – Brain Heart Infusion

BHT – Butylated hydroxytoluene

BP – Biopackaging

BSE – Bovine Serum Albumin

CATC – Citrate Azide Tween Carbonate

CIE – Commission Internationale de l'Eclairage

COP – Cholesterol oxidation products

DFD – Dark Firm Dry

HHP – High Hydrostatic Pressure

*L** - Lightness

LTLT – Low temperature Long Time

MAP – Modified Atmosphere Packaging

MDA – Malondialdehyde

MRD – Maximum Recovery Diluent

NACMCF – National Advisory Committee on Microbiological Criteria for Foods

NCAIM – National Collection of Agricultural and Industrial Microorganisms

PA/PE – Polyamide / Polyethylene

PET – Polyethylene Terephthalate

PSE – Pale Soft Exudative

PUFA – Polyunsaturated fatty acids

QRA – Qualitative Risk Assessment

RH – Relative Humidity

RTE – Ready To Eat

TBA – Thiobarbituric Acid

TBARS – Thiobarbituric Acid Reactive Substances

TCA – Trichloroacetic acid

TPA – Texture Profile Analysis

TSA – Tryptic-Soy Agar

WBSF- Warner Bratzler Shear Force

WHC – Water Holding Capacity

ΔE – Total color difference

1. INTRODUCTION

According to FAO, roughly half of all meat produced globally is wasted at the household level of the supply chain (FAO, 2011). A significant portion of waste in households is attributed to weight loss during heat processing treatment (Parfitt et. al. 2010). Meat products are perishable by nature and are prone to quality deterioration if not handled and processed properly. For example, conventional heat treatments used for meat processing such as steaming, boiling or grilling have been reported to not only increase the weight loss but also reduce the content of several micronutrients such as vitamins, amino acids and minerals (Silva et. al. 2017). However, consumer habits have shifted away from traditional processed foods and toward high quality and more nutritious food products as a result of technological advancements in the food industry and people's dynamic lifestyle (Juneja et. al. 2006). In this sense, consumers are increasingly interested in mild thermal processed ready-to-eat (RTE) foods due to their excellent sensory qualities, ease of preparation, and high nutritional value. Mild thermal processing refers to the least amount of heat treatment required to achieve necessary preservation of the food product. Sous vide technology has received increased attention from researchers in recent years as one of the mild heat processing methods for the production of ready-to-eat meat products. Optimization of processing parameters in sous vide technology can present a feasible option to obtain higher yields of meat thus reducing the weight loss in meat.

On the other hand, sous vide technology has been shown to improve sensorial characteristics (juiciness, tenderness), oxidative stability, and meat product shelf life. The traditional sous vide method employs only one controlled temperature in the range of 55-70°C. The time required for treatment is determined by the type, shape, and size of the meat (Baldwin, 2012). Selection of proper temperature and time duration in sous vide processing to achieve desired sensory attributes of cooked meat such as tenderness or juiciness is frequently difficult. Most researchers have only looked at the effect of different combinations of a single temperature and time in sous vide processing on the quality attributes of beef (Rinaldi et. al. 2014; Botinestean et. al. 2016; Mortensen et. al. 2012); pork (Christensen et. al. 2011), turkey (Bıyıklı et. al. 2020), and chicken (Głuchowski et. al. 2020). Different meat proteins have different denaturation temperatures and are responsible for different meat quality properties. Therefore, proper selection of temperature and time in sous vide processing to tailor the denaturation of meat proteins can play a major role on the main quality attributes of meat.

Meat contains endogenous proteolytic enzymes, which highest activity has been reported to be at temperatures ranging from 40 to 50 °C (Christensen et. al. 2013). Previous research found that at these temperatures, desmin degradation is higher due to high activity of proteolytic enzymes, indicating an

extension of meat tenderization (Christensen et. al. 2011; Ertbjerg et. al. 2011). The proteolytic activity of the desmin-degrading enzymes calpain-1, calpain-2, and cathepsin B is more intense at temperatures ranging from 40 to 55 °C and negligible at temperatures higher than 60 °C (Ertbjerg et. al. 2012). As a result, using proteolytic enzyme activation temperatures (between 40-55 °C) as the first step temperature in the sous vide processing could potentially improve meat tenderness and other quality attributes. This could be particularly important for elderly people whose meat consumption is limited because of challenges with mastication and swallowing from oral impairments. It was reported that breast muscles in chicken meat tend to be tougher and crumby compared with chicken legs muscles (Zhang et. al. 2020). Thus, this cooking method may be used to produce new poultry-based ready-to-eat foods with higher tenderness. Furthermore, the two-step sous vide method may also be used as a reference for the food industry. Based on our knowledge, there have been no studies that investigate the effect of a two-step temperature in sous vide processing of chicken breast meat. Due to the overlapping temperatures of proteolytic enzyme activation and bacterial growth, careful selection of time and temperature is required to ensure microbial safety (Yang et. al. 2020). Moreover, there is a lack of data in literature to predict the growth of bacteria in the range 42-55 °C (Stringer et al., 2012). Therefore, it is necessary to test the efficiency of two step sous vide treatment using a high thermal resistant microorganism in order to optimize the temperature and time combinations in sous vide for pasteurization of the meat product.

1.1. Hypothesis:

- The two-step sous vide technique containing proteolytic enzyme activation temperature as a first-step temperature combined with the end-step temperature can produce higher quality cooked chicken breast (lower cooking loss, improved texture attributes) than the traditional one-step sous vide technique.
- The two-step sous vide technique can ensure proper thermal inactivation of a high heat resistant microorganism for pasteurization of chicken breast.
- The storage stability of chicken breasts treated with the two-step sous vide technique is similar with those treated with the traditional one-step sous vide technique.

1.2. Objectives

The overall objective of the study was to investigate the application of the two-step sous vide technique in improving the quality of cooked chicken breast. The specific aims of the study were:

- To investigate the effect of two-step temperature sous vide containing a proteolytic enzyme activation temperature (45 °C or 50 °C) as first-step temperature combined with the end step temperature of 60 °C, on different quality attributes of chicken breast.

- To examine the pasteurization efficiency of the studied two-step sous vide treatments by calculation of theoretical pasteurization values based on kinetics data of various target pathogens associated with sous vide treated meat products.

- To investigate the thermal inactivation efficiency of the studied two-step sous vide treatments on *Enterococcus faecalis* which was used as pasteurization indicator microorganism in chicken breast to select the most adequate cooking treatments for further examinations. Are the two-step sous vide treatments enough to pasteurize chicken breast from *Enterococcus faecalis*?

- To compare the storage stability between the two-step temperature sous vide and the traditional one-step sous vide treated chicken breast. Are the two-step sous vide treated chicken breast quality parameters, oxidative and microbiological stability similar or better than the traditional one-step sous vide treated ones?

2. LITERATURE REVIEW

2.1. Composition characteristics and structure of meat

Meat and meat products play an essential role in human nutrition considering their nutritional and biological value. The high level of protein content and essential micronutrients in meat (primarily of vitamins and minerals) provide a healthy diet among different groups of people. Meat is recommended especially for young people, pregnant mothers, elders, and people who have a high physical daily activity. Pork and poultry are the most popular types of meat in Europe, accounting for roughly 80% of total meat consumption. Along with pork meat, global consumption of poultry meat has increased over the last 20 years (EC, 2019). This increase in consumption can be attributed to the beneficial effects of poultry meat on human health as well as its low price in comparison to other meats. Poultry meat has a high protein content, a complete amino acid composition, essential micronutrients, and low-fat levels (Marangoni et. al. 2015). These characteristics reduce the risk of obesity, malnutrition, and cardiovascular diseases in humans (Donma & Donma, 2017). However, poultry meat presents a sensitive and perishable food, owing to its nutrient composition, which promotes microbial growth. Therefore, effective measures must be implemented by both producers and consumers to prevent meat deterioration and the growth of pathogenic microorganisms that may endanger consumer health.

In general, meat contains approximately 75 % water, 20 % protein, 3 % fat, and 2 % non-protein substances. Non-protein substances contain about 3 % vitamins (vitamin A, vitamin B_{12}, folate) and minerals (Zn, Fe, Sn), 45 % nitrogen-containing substances, 34 % carbohydrates and 18% inorganic compounds. Meat composition is influenced by the slaughtered animal breed, age, sex, nutritional status of the animal, and location of the meat part (Tornberg, 2005).

Meat proteins are classified into three categories based on their solubility:

1) myofibrillar proteins (salt soluble) account for approximately 50-55 % (myosin and actin),
2) sarcoplasmic proteins (water-soluble) account for approximately 30-34 % (myoglobin, hemoglobin, creatine kinase, and other enzymes), and
3) stromal proteins (connective tissue proteins) which account for approximately 10-15 % (collagen, elastin).

About 75-92 % of the muscle consists of muscle fibers that hold myofibrils (1μm diameter) inside them, on which sarcomere the contractile unit (2.2 μm length) is lined up (Figure 1). The sarcomere is built by two blocks which contain a thick filament which is extended in the A-band (dark section) and a thin filament which is extended from the Z-line in I-band (light section) towards A-band arranged in a regular manner (Tornberg, 2005). The thick filament contains myosin, M-line protein

and C-protein. The thin filament contains mostly fibrous actin (F-actin), α- and β-actinin, troponin and tropomyosin.

Stromal (connective tissue) membranes namely epimysium and perimysium cover the muscle fibers and form an intra-muscular network which is rich in collagen (Figure 1). This structure is particularly important for the crumbliness of meat which is mostly determined by the heat treatment of the stromal proteins (collagen and elastin) (Lawrie and Ledward, 2006).

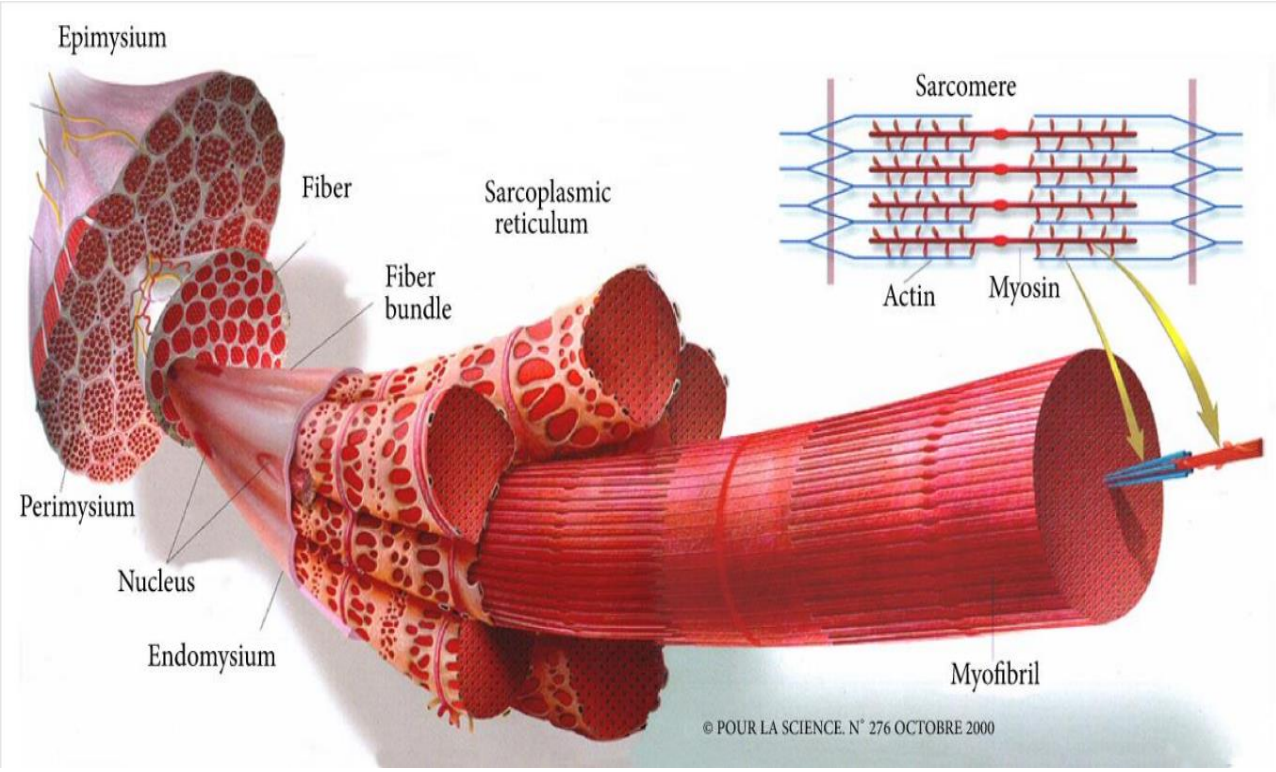


Figure 1: The organizational structure of the muscle (Listrat et. al. 2016)

Two important proteins so called chromoproteins responsible for the color of meat are myoglobin and hemoglobin. Myoglobin consists of a porphyrinic heme group which is a metalloporphyrin group containing an iron atom in ferrous state (Fe II). Myoglobin is the primary contributor to meat color. Hemoglobin, on the other hand, contributes only slightly (around 5%) to the color of the meat because only 0.3% of blood remains in the vascular system during the bleeding process (Swatland, 1995). Color of the meat depends on the oxidation state of the iron in heme group which can interact with several gases and water. The color of the meat can also be influenced by other external factors such as exposure to oxygen, light, microbial growth and rancidity.

2.2. Determinants of poultry meat quality

The quality of meat and meat products can be defined as the overall quality of the meat assessed from the physico-chemical attributes, technological quality, sensorial attributes, microbiological stability and nutritional value of meat. Because meat is typically consumed in processed form or cuts, technological quality, or the ability of meat to be processed, is critical. The main quality attributes of raw meat include technological quality parameters (water-holding capacity, pH, color), texture, oxidative stability, nutritional and microbiological quality. The following sections aim to explain and discuss the main quality attributes of raw meat and different factors that influence them.

2.2.1. Water-holding capacity of poultry meat

WHC is one of the key parameters that affect various quality attributes of raw and cooked meat. WHC, which represents the amount of water retained by meat proteins, has a direct impact on meat sensory characteristics such as tenderness, color and juiciness (Cheng & Sun, 2008). The total water in meat consists of 88-95 % of intracellular water (water held within actin and myosin filaments) and 5-12 % of intercellular water (water between the myofibrils). During the post-mortem process the acid lactic production cause a pH decrease of meat to 5.5 which is close to the isoelectric point of myofibrillar proteins (pH=5.3). At this point there is a high interaction between the positive and negative charges of proteins which results in myofilament shrinkage and less water binding from proteins. The reduced space between filaments allows water to pass into sarcoplasm and then be released in extracellular spaces as free sarcoplasmic water at the surface of meat, resulting in a decrease in meat WHC (Honikel, 2004; Mir et. al. 2017). It is well known that the degree of pH decrease during post-mortem is a critical factor influencing meat's water holding capacity. Thus, a rapid decrease in pH in combination with a high temperature can cause denaturation of protein and myofilament shrinkage, resulting in a decrease in meat WHC. It has been reported that low pH in turkey meat correlates with low water holding capacity, which may result in higher cook-loss and lower tenderness (Barbut, 1993). Therefore, the water holding capacity present one of the crucial parameters for meat industry which has a direct impact in the yield of the product. Aside from the economic value, increased water holding capacity has the greatest impact on meat eating quality, referring to increased tenderness, juiciness, and appearance of meat, thus increasing consumer acceptability of the meat product (Toscas et. al. 1999; Bertram et. al. 2003).

Water holding capacity is associated with juiciness, which can be evaluated using sensorial analysis. However, various feasible and efficient methods for assessing meat's water-holding capacity have

been developed, such as the gravimetric method (drip loss), which uses no force, or the centrifugation method, which uses an external force in meat (expressible moisture) (Huff-Lonergan & Lonergan, 2005).

2.2.2. pH of poultry meat

Normal ranges of pH in poultry meat (24 h post-mortem) are between 6.0 to 6.2. Meat pH is primarily influenced by the post-mortem metabolism processes including lactic acid production and glycolysis process that result in pH decrease to 5.5. The pH changes in meat directly affect other quality characteristics such as water-holding capacity, color, texture and shelf-life of the meat product. It has been known that pH of chicken breast is positively correlated with water binding capacity. Thus, chicken breast with low pH has a lower water holding capacity compared to chicken breast with high pH. According to previous research, the pH of chicken breast correlates with its color (Fletcher, 1995). Thus, high pH meat refers to very dark meat which is characterized with dark color, higher firmness, and dryness (DFD meat). Meanwhile, low pH meat or "acid meat" refers to very light meat which is characterized with pale color, higher softness and higher exudate (PSE meat). Low pH value in meat has been associated with lower water-holding capacity, higher cook loss and lower tenderness (Barbut, 1993). On the other hand, low pH in meat is known to have an important preservation effect against different pathogenic microorganisms that cause meat deterioration. However, frequently low pH or acidification in meat is associated with the growth of lactic acid bacteria, which are responsible for lactic acid production, resulting in meat spoilage (Deumier, 2003; Koutsoumanis et. al., 2006). Thus, monitoring pH changes in meat during storage can provide valuable information on the development of spoilage microorganisms.

2.2.3. Color of poultry meat

Color of poultry meat is the main parameter in terms of appearance and the first attribute consumers notice in meat that affect their purchasing decision (Mancini & Hunt, 2005). The color of meat depends mainly on myoglobin pigment (water-soluble protein) concentration and its biochemical state (oxidation state). Concentration of myoglobin increase with animal age, thus giving a more intense color, but also depends on animal type (beef, pork or poultry). After cutting, meat has a darker color (dark purple red), but when it encounters oxygen in the air (oxygenation reaction), it forms oxymyoglobin, which gives a brighter red color and resembles meat freshness in consumer perception. When myoglobin or oxymyoglobin is oxidized, an electron is removed, resulting in the formation of

metmyoglobin, which gives meat a brown color. Contrary to oxymyoglobin, metmyoglobin is usually associated with deterioration of meat (Boles & Pegg, 2010). Interconversions between the three mentioned pigments (myoglobin, oxymyoglobin and metmyoglobin) in meat are reversible and depend on oxygen availability and enzyme activity (Mancini & Hunt, 2005) (Figure 2).

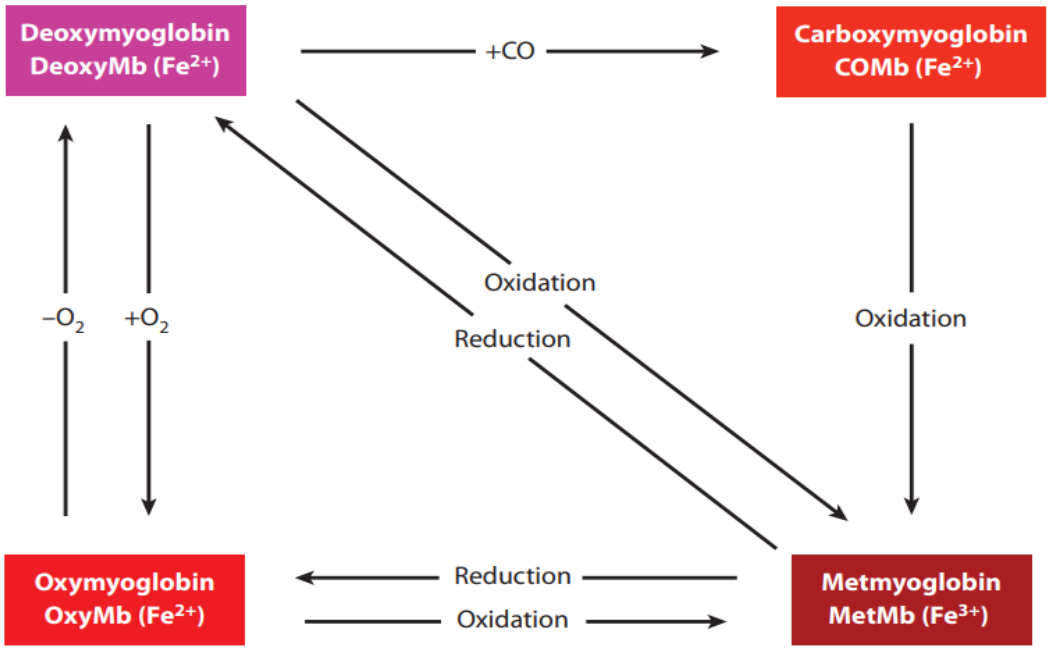


Figure 2: Interconversions between myoglobin redox forms in meat (Mancini & Hunt, 2005)

High pH meat known as dry meat has been associated with reducing the oxygenation process and activity of enzymes, thus making it difficult the reversible conversion of metmyoglobin to oxymyoglobin. Apart from pH, the color of meat is closely related to the length of storage time and the temperature in which it is stored. Long-term storage of meat is associated with metmyoglobin formation, which results in a brown color that reduces consumer color acceptability. In terms of storage temperature, it is recommended that meat be stored at a temperature of -1.5 °C for maximum color retention (Boles & Pegg, 2010).

Various methods have been used to measure color of meat which could be categorized in two types namely, sensory analysis methods which can be conducted by a trained sensory panel or a consumer panel, and instrumental methods such as reflectance spectrophotometry method which use color standards or Video Analysis (VIA), and Near-infrared reflectance (NIR) which is a non-destructive method. Different color systems have been proposed from CIE (Commission Internationale de l'Eclairage) for color assessment such as Munsell system, CIEXYZ System and CIELAB System.

CIELAB System represent the most used system for color measurement which use the coordinates L^*, a^* and b^*, where L^* indicates lightness, with values ranging from 0 to 100 (black to white), a^* belong to wavelength spectrum that correspond to color from green ($-a^*$) to red ($+a^*$), and b* from blue ($-b^*$) to yellow ($+b^*$) (Figure 2). Hue angle and saturation color can be obtained from a^* and b^* coordinates, Hue angle = $\tan^{-1}(b^*/a^*)$ and saturation index = $(a^{*2} + b^{*2})^{1/2}$ (Figure 3) (Konica Minolta, 2007).

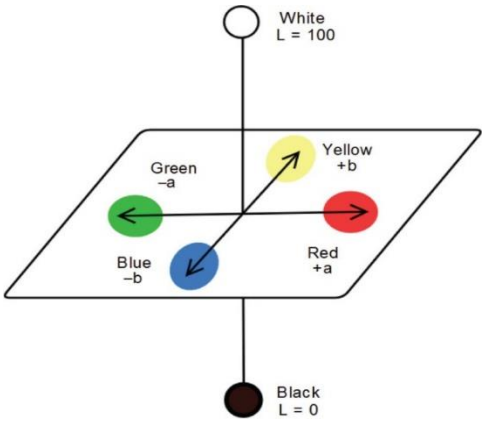


Figure 3: Representation of the CIE L^*, a^* and b^* color space

2.2.4. Texture of poultry meat

Texture of poultry meat is the main quality parameter connected to consumer satisfaction. Texture consists of various parameters such as hardness, chewiness, cohesiveness, springiness, and occasionally juiciness. One of the main factors that affect the texture of poultry meat include connective issue ageing which depends on collagen linkage in the muscle. Thus, higher tenderness of meat is found in younger birds. The hardness of meat (tenderness) is the most important factor for consumers because it determines its commercial value. A sensory panel can assess the hardness of meat based on the sensory tenderness parameter. However, because sensory analysis is considered costly and time-consuming, different objective instrumental methods which are based on measurement of meat tissue resistance to shearing force or compression are being used. Warner-Bratzler shear force (WBSF) is one of the most commonly indicators of meat hardness, with higher shear force indicating greater hardness. It has been reported that Warner-Bratzler shear force has a negative correlation with tenderness of meat, but no correlation with consumer acceptability of meat (Platter et. al. 2003; Safari et. al. 2001). Texture profile analysis (TPA), on the other hand, is a more comprehensive texture analysis that employs a two-step compression cycle test to measure various important texture parameters such as cohesiveness, hardness, springiness, gumminess, chewiness, and so on. Several TPA parameters of raw meat such as hardness, springiness and chewiness were found to be correlated to sensory attributes. Meanwhile, Warner-Bratzler shear force measurement was better to predict the sensory texture attributes of cooked meat (De Huidobro et. al. 2005). Previous research found that TPA analysis was a more accurate method for determining the texture of raw and cooked meat than the Warner-Bratzler shear force test. (De Huidobro et. al. 2005; Onega et. al. 2001).

2.2.5. Oxidative stability of poultry meat

Lipids provide a great importance on nutritional value and sensory qualities of meat. Aside from providing essential fatty acids and fat-soluble vitamins, lipids have a significant impact on the development of sensory properties of meat such as flavor, tenderness, and juiciness, thereby increasing meat consumer acceptability. Poultry meat contains high concentrations of polyunsaturated fatty acids which are susceptible to oxidation thus causing quality deterioration. Oxidation of lipids can reduce the nutritional value (essential fatty acids and vitamins) and cause changes in sensory quality properties of meat particularly color, odour and flavour of meat. Furthermore, lipid oxidation can produce various toxic compounds (hydroperoxides, aldehydes, oxysterols, cholesterol oxidation products) that have been found to be associated with the development of several diseases (Angeli et. al. 2011; Sottero et. al. 2019). However, some products of lipid oxidation are responsible for producing desirable aroma in cured meat products during maturation period.

The first stage of lipid oxidation involves the reaction of unsaturated fatty acids with oxygen, which produces hydroperoxides but causes no discernible change in the aroma of the meat. Meanwhile, in the second stage of oxidation, hydroperoxides start to decompose giving the secondary oxidation products (aldehydes, ketons, esters, hydrocarbons, acids) which can be perceived by consumer as off-flavours and off-oddours. Consumer observations of volatile oxidative rancidity products and color changes in meat can be used as an indicator to determine the shelf-life of meat. The main oxidative rancidity compounds responsible for quality changes in meat are aldehydes, such as malondialdehyde, hexanal and 4-hydroxy-2-trans-nonenal (Estévez, 2005) (Figure 4).

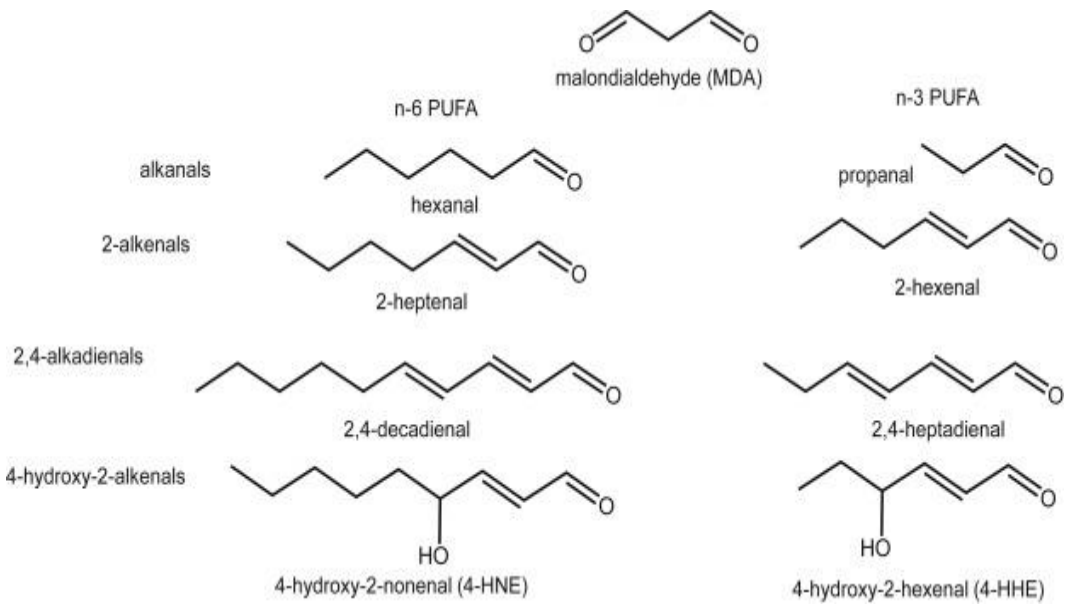


Figure 4. Structures of main aldehydes deriving from lipid oxidation (Guyon et. al. 2016)

The presence of polyunsaturated fats and iron in high concentrations, particularly in poultry meat, increases the likelihood of lipid oxidation (Mercier et. al. 2001). Moreover, cooking increases the susceptibility of meat to lipid oxidation because high temperatures release oxygen and iron, resulting in the production of free radicals. It has been reported that off-odors and off-flavors of cooked meat caused by lipid oxidation products are detected only after two days of storage in refrigerated conditions or after reheating the meat (Byrne et. al. 2002). Thus, lipid oxidation presents a great challenge for ready-to-eat meat products producers, therefore various processing techniques have been employed to prevent this process, namely application of different antioxidants, vacuum packaging and modified atmosphere packaging (MAP). Lipid oxidation can happen because of processing and during the storage of meat products. In this context, sous vide processing is a promising technology for preventing lipid oxidation of meat because it uses low temperatures and longer cooking times while vacuum packaging meat. On other hand, several studies have reported that natural plant extracts and bioactive plant compounds have an important antioxidative and preservative effect on meat storage (Hussein et. al. 2021).

2.2.5.1. Mechanism of lipid oxidation

Lipids present the most unstable components in meat that can be prone to oxidation, thus causing non-desirable quality attributes that influence the acceptability of the meat product by consumer. The main factors that affect lipid oxidation include degree of unsaturated fatty acids, heat and light exposure, molecular oxygen presence and pro-oxidant compounds (Lima et. al. 2013). Muscle components that trigger oxidation of lipids include iron, myoglobin, hydrogen peroxide and ascorbic acid. Meanwhile, other factors such as light, radiation, or enzymes can also initiate lipid oxidation. Main ways of lipid oxidation include auto-oxidation, photo-oxidation and enzymatic oxidation. Autoxidation is a free-radical chain reaction that is dependent on catalysts such as temperature, pH, metal ions, and free radicals. The main difference between autoxidation, photo-oxidation and enzymatic oxidation is the hydroperoxide formation. In photo-oxidation, hydroperoxides are formed from radical reactions in the presence of light and sensitizers such as myoglobin (Wasowisz et. al. 2004). Meanwhile, in enzymatic oxidation lipoxygenase catalyzes the oxidation reaction of fatty acids from which peroxides and hydroperoxides are formed (Schilstra et. al. 1993). Autoxidation presents the most common lipid oxidation process in meat products and has three steps: 1) initiation, 2) propagation, and 3) termination (Chaijan & Panpipat, 2017) (Figure 5).

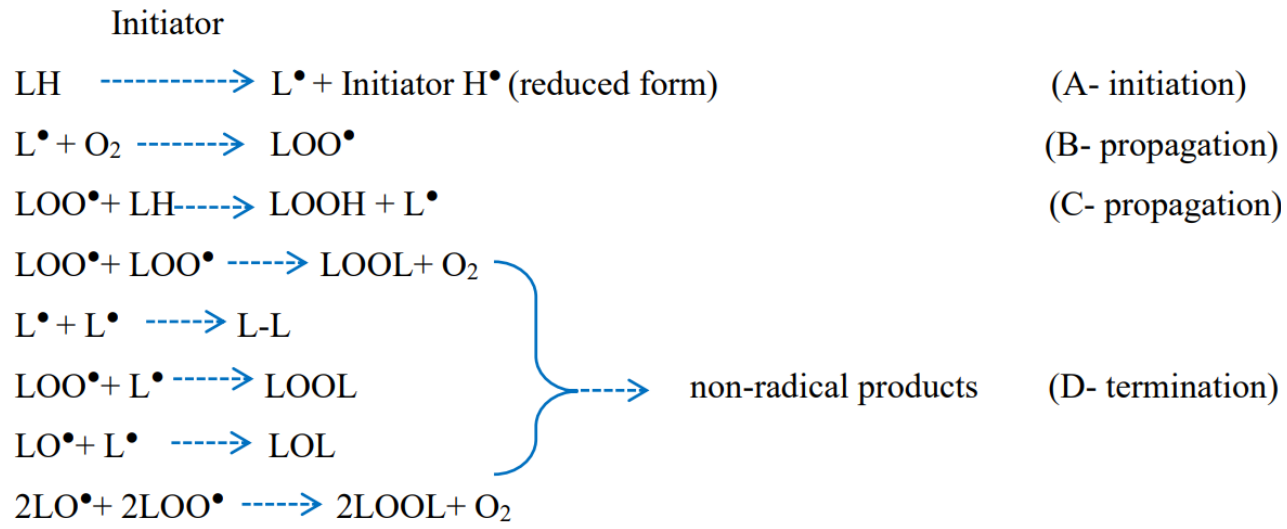


Figure 5: Mechanism steps of lipid oxidation (Erickson, 2008)

*LH indicates the fatty acid, L• – alkyl radical, H – hydrogen atom, LOO• – peroxyl radical, LOOH – hydroperoxide, LOOL – non-radical compound, LO• – alkoxy radical.

1) Prior to initiation, oxygen is activated by a catalyst (transition metals) or an external factor (light, temperature), resulting in the formation of reactive oxygen species with at least one free electron, such as hydroxyl radicals, alkoxyl radicals, hydrogen peroxide, superoxide, or oxygen atoms. The initiation phase (reaction A, Figure 5) begins with the abstraction of a hydrogen atom from a double bond unsaturated fatty acid, resulting in the formation of an alkyl radical (Barriuso et. al. 2013).

2) During propagation phase (reaction B, Figure 5), the alkyl radical reacts with molecular oxygen to form peroxyl radical, which can easily react and abstract hydrogen from the same or another lipid molecule, resulting in the formation of hydroperoxide and the realization of a new alkyl radical (reaction C). The resulting alkyl radical can further react with molecular oxygen, thus repeating propagation process many times (Chaijan & Panpipat, 2017). Moreover, the formed hydroperoxide molecules can get decomposed into other reactive oxygen species such as hydroxyl, alkoxy and peroxyl radicals (secondary initiation) through formation of hydroperoxides bimolecular association or metal-ion mediated pathway (Choe & Min, 2006; Barriuso et. al. 2013)

3) During the termination step (reaction D), radical species such as peroxyl radicals can react with each other and form non-radical compounds, thus terminating the process of propagation. Lipid oxidation termination step can be achieved using antioxidant molecules which provide a hydrogen atom to reactive species of the oxidation, thus creating more stable products (Erickson, 2008). However, different antioxidants have been proved to act as pro-oxidants creating new reactive compounds, thus termination step is not always achieved.

The main way to ensure termination step is the process of degradation of peroxyl and alkoxy radicals into secondary compounds such as hydrocarbons, ketones, alcohols and aldehydes. At this point, off-odours and off-flavours are created which cause sensorial deterioration in meat. The most abundant secondary product responsible for these changes in meat are considered aldehydes, particularly malondialdehyde, hexanal and 4-hydroxy-2-trans-nonenal (Estévez, 2005).

2.2.5.2. Lipid oxidation measurement methods

Various analytical methods have been used to assess lipid oxidation in meat and meat products. Based on the component that is measured, these methods can be categorized in methods that measure the oxygen absorption, the loss of initial substrates, formation of free radicals and the amounts of primary oxidation products (peroxides and hydroperoxides) and secondary oxidation products (aldehydes, ketones, hydrocarbons, alcohols, acids) (Shahidi & Zhong, 2005). Quantification of the amounts of primary oxidation products (hydroperoxides) can be achieved by determining the 'peroxide value' which employs an iodometric titration method, and the result is expressed as milliequivalents iodine per kilogram of fat sample. Peroxide value is a parameter that usually is appropriate to quantify the low levels of lipid oxidation in foods which are stored at very low temperatures. Another method used to monitor primary lipid oxidation is the measurement of the amounts of conjugated dienes and trienes which are an index of the amounts of hydroperoxides formed in meat and meat products (Gray & Monahan, 1992).

On the other hand, when lipid oxidation rates are expected to be high in a food product, it is more suitable to measure the level of formation of secondary oxidation products. The most known method for measurement of secondary lipid oxidation products in meat and meat products is the TBARS value measurement. TBARs measurement is a spectrophotometry method that is based on the measurement of malonaldehyde amounts which are the most important aldehydes in meat products, and the result is reported as milligrams of malonaldehyde equivalents per kilogram of sample. Malonaldehyde is quantified by the measurement of the colored complex which is formed in the reaction with 2-thiobarbituric acid (TBA) which has an absorption maximum at 532 nm wavelength (Grotta et. al. 2017).

Another method used for lipid oxidation quantification in cooked meat products is the measurement of cholesterol oxidation products (COPs) method which includes cold saponification and solid-phase extraction used for COPs purification. Gas chromatography (GC) and High-performance liquid chromatography is usually used to measure COPs in meat products (Shahidi & Wanasundara, 2002). Hexanal is a major component of aldehyde therefore its quantification is a suitable method to monitor

the lipid oxidation and the formation of volatile components in cooked meat products. Hexanal content can be quantified using either Gas chromatography or liquid chromatographic-mass spectrophotometer (LC-MS) (Gray & Monahan, 1992).

2.2.6. Nutritional quality of poultry meat

Poultry meat has a high nutritional and biological value due to its high protein content, complete amino acid composition, high level of micronutrients (minerals and vitamins), and low level of fats and cholesterol. These characteristics make poultry meat an excellent diet component for many people, including pregnant women, young people, and the elderly. The nutritional profile of poultry meat has an important role on prevention of various diseases such as obesity, diabetes, and cardiovascular diseases (Donma & Donma, 2017). The main compounds of poultry meat are proteins (18.4-23.4 %), lipids (1.3-6.0 %) and minerals (0.8-1.2 %). The nutritional composition of poultry meat is mainly determined by the animal feeding, genetics and cuts. It has been reported that chicken thighs have a higher energetic value than chicken breast due to their higher lipid content (Marangoni et. al. 2015). Poultry meat is a good source of unsaturated fatty acids, particularly omega 6 or n-6 linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6). Poultry meat also contains significant amounts of omega 3 fatty acids which are derived from the high content of alpha-linolenic acid in vegetable feed. These components can be increased using various dietary enrichment strategies of poultry products with PUFA (polyunsaturated fatty acids), but the risk of lipid oxidation is high (Rymer & Givens, 2006).

Poultry meat has a high-quality protein due to complete essential amino acids. Because of water loss during cooking process, the protein content and energetic value of cooked poultry meat increases. On the other hand, cooking of poultry meat increase only slightly the fat content and do not significantly reduce the vitamin content. Poultry meat contains substantial amounts of vitamins, particularly B-group vitamins (hydrophilic vitamins) such as vitamin B_{12} and niacin (Vitamin B_3) being the most abundant. Poultry meat, on the other hand, has low levels of lyophilic vitamins such as vitamin E and K, but can be supplemented through different feeding strategies. In this context, vitamin E enrichment improves the oxidative stability and nutritional value of poultry meat products in addition to providing essential nutrients to animals (Bou et. al. 2001). Raw poultry meat is high in selenium, iron, and zinc but low in sodium. However, sodium is high in processed poultry meat because it is commonly used as a preservative in meat industry.

2.2.7. Microbial deterioration of poultry meat

Meat presents an ideal substrate for various microorganism growth including bacteria, moulds and yeasts, because it has a moderate pH and contains high percentage of water and the main micronutrients such as amino acids, vitamins, and minerals which are needed for microorganism growth and metabolism processes. The contamination of meat and meat products with spoilage microorganism can lead to quality deterioration and non-desirable sensory attributes, thus causing food public health issues and food insecurity. Various factors can affect the extent of microorganism growth in meat and meat products including factors inherent to the specific food such as nutrients (water, nitrogen compounds, vitamins, and minerals), water activity (aw), pH, oxidation-reduction potential (Eh), biological structures and antimicrobial substances: enzymes, salt, and other additives). Other factors include storage conditions of the food such as temperature (processing temperatures, storage temperature), relative humidity (% RH), gases presence and their concentration (oxygen or air, anaerobic conditions, carbon dioxide, ozone), and other microorganism presence and their activities (natural microbiota, competition for nutrients and adhesion sites, production of antimicrobials: diacetyl, organic acids, reuterin (3-hydroxypropionaldehyde in several forms), bacteriocins, and killer toxins).

The major challenges in poultry meat microbial safety are considered pathogen microorganisms which can cause a health risk, and spoilage microorganisms which have a key role in quality deterioration leading to various changes such as color, off-flavor and off-odour in poultry meat. The main sources of contamination of poultry meat products include pre-harvest contamination (environmental conditions, employers hygiene, vertical transfer of microorganisms) and processing contamination (cross contamination and fecal matter contamination during processing steps such as scalding, defeathering, evisceration, washing and chilling) (Golden et. al. 2021). Hazard Analysis Critical Control Point (HACCP) system systematic implementation has a major positive effect in controlling the pathogen amounts in poultry industry. Moreover, HACCP present a good tool to decrease the contamination of poultry meat with spoilage microorganisms such as Pseudomonas and other Gram-negative bacteria, but it has little effect on reducing cross-contamination occurrence during the processing steps of poultry industry. Another tool that can be used to control pathogen microorganisms is the Qualitative Risk Assessment (QRA) which consists of hazard identification, exposure assessment and characterization of hazard and risk (CAC, 1999). This tool can be used to determine the effectiveness of an intervention action on a hazard at a particular Critical Control Point (CCP) in the poultry production process.

The main microorganisms associated with poultry meat products include the food-borne pathogen

bacteria such as *Salmonella* and *Campylobacter*. These two pathogens are found at high loads in the gut of birds which after processing can cause contamination of poultry meat. *Salmonella* is classified in two main species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is the most common food-borne pathogen worldwide and consists of Enteritidis which is the most common serotype present in poultry meat products (Jackson et. al. 2013). Meanwhile, *Campylobacter* food related pathogenic species include *Campylobacter jejuni* and *Campylobacter coli*. *Campylobacter* are Gram-negative bacteria, microaerophilic and have an optimal growth temperature range between 37 to 42 °C, which explain the fact of their growth in birds gastrointestinal tract low oxygen conditions and temperature of 42 °C (Park, 2002). *Campylobacter* present one of the leading causes of gastroenteritis in humans likely because its presence in poultry intestinal tract can be as high as 10^9 cfu/g. Moreover, *Campylobacter* counts are much higher in processed poultry (10^9 cfu/carcass) compared to *Salmonella* counts (10^2 cfu/carcass). Other pathogens that may be present in poultry meat include *Clostridium perfringens*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Enterococcus faecalis*. *Listeria monocytogenes* is common in the environment, including food processing plants, water, soil, plants, and animals. Therefore, *Listeria monocytogenes* contamination present a major issue in alternative poultry production (organic poultry) due to bird exposure in natural environments. *Listeria monocytogenes* has been found in more than half of raw chicken samples, usually in low numbers (Gilbert et. al. 1989).

On the other hand, spoilage microorganisms also present a big challenge in meat products being responsible for economic losses and for most of sensory properties deterioration which affect the shelf-life of meat. The most dominant spoilage bacteria during refrigerated storage in aerobic conditions is *Pseudomonas* spp. Other spoilage microorganisms are also present such as *Shewanella putrefaciens* and psychrotrophic strains of *Enterobacteriaceae* (Hinton et. al. 2004). One of the main techniques used for spoilage prevention in meat products is modified atmosphere storage (by adding 10 to 30 % carbon dioxide) which has a key effect in suppressing *Pseudomonas* spp. bacteria but promotes the growth of lactic acid bacteria, which eventually become the dominant bacteria in spoilage microflora of meat products. Various processing technologies has been used to control the spoilage and pathogenic microorganisms in meat and meat products including thermal processing techniques (ohmic heating, infrared heating, sous vide cooking, microwave heating, radiofrequency, reflectance window), irradiation technique, pulsed electric field, ultrasound technique, modified atmosphere packaging (MAP) and high hydrostatic pressure (HHP) (Dang et. al. 2021; Sengun et. al. 2017; Belletti et. al. 2013; Roldán et. al., 2013).

2.2.7.1. Enterococcus faecalis

Enterococci are a microbial group part of gastrointestinal tract microbiota of humans and animals but is also found in vegetables, water and foods of animal and plant origin. After spore-forming microorganisms, *Enterococcus* species have the highest thermal resistance in food processing. They can grow in a wide range of temperatures (10 - 45 °C) and pH values (4.5 - 9.6) and are salt tolerant (6.5 % NaCl) (Moreno et. al. 2006). *Enterococcus* species are responsible for various nosocomial infections including urinary infections, bacteremia, wound infections, and endocarditis (Franz et. al. 2003). Therapy against *Enterococcus* species may be less effective due to their increased resistance to various antibiotics commonly used to treat human infections. Therefore, antibiotics are less preferred for animal growth or disease prevention and treatment due to Enterococcus' ability to acquire resistance genes and become resistant to new antibiotics (Smith et. al. 2002). This poses a risk to consumers who may consume antibiotic-resistant Enterococci from animal food.

The most common *Enterococcus* species present in animal food and clinical samples include *Enterococcus faecalis* and *Enterococcus faecium*. *Enterococcus faecalis* is a Gram-positive, non-spore-forming, facultative-anaerobic, catalase negative and non-motile bacterium, which accounts for roughly 90% of human enterococci infections, followed by *Enterococcus faecium*.

Enterococcus faecalis has been shown to be resistant to extreme conditions such as hydrogen peroxide, bile salts (up to 40%), acidity, and ethanol. *Enterococcus faecalis* starved cells retain viability, making them more resistant to heat, ethanol, acidity, and ultraviolet radiation (Figure 6) (Hartke et. al. 1998). *Enterococcus faecalis* can grow in various agar including chromogenic agar, blood agar and McCongey agar. Burkwall & Hartmann (1964) proposed Citrate Azide Tween Carbonate (CATC) Agar as a selective agar for the isolation of enterococci in meat and meat products. Both citrate and azide have an inhibition effect towards other microbial flora. *Enterococci* appears as red colonies because they reduce the colorless 2,3,5-triphenylterazolium chloride to red formazan.

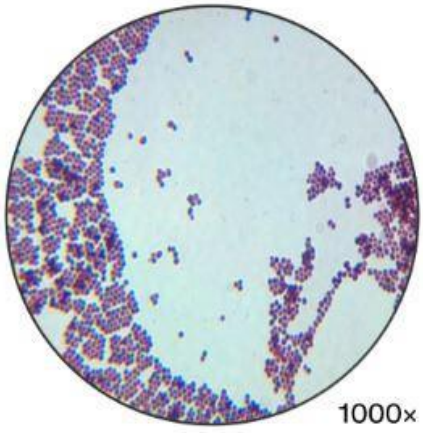


Figure 6: Microscopic view of *Enterococcus faecalis*

Contamination of meat by *Enterococcus* species can occur during the slaughtering process, during processing, or from the environment (Talon et al., 2007). Because of less sanitary slaughtering practices, the prevalence of *Enterococcus* bacteria contamination in poultry meat is up to 96%, which

is higher than in pig or cattle. The Enterococcus counts in poultry carcasses (chicken and turkey) have been shown to be ranging from 1 to 3 log cfu/g (Miranda et. al. 2007). There has been little research on the presence of *Enterococcus* species in ready-to-eat foods because most studies have focused on their presence in raw foods. Enterococcus species, because of their heat resistance, are able to survive the pasteurization stage in the production process of meat products. In a recent published study *Enterococcus faecalis* was found to be the most widespread strain among *Enterococcus* in ready-to-eat meat products with nearly 48.7% followed by *Enterococcus faecium* with 39.7% (Chajecka-Wierzchowska et al., 2016).

Several studies reported that *Enterococcus faecalis* is a high resistant strain to various processing conditions including pressure (Belleti et al., 2013) or heat (Oliviera et al., 2009; McAuley et al., 2012). In case of pathogenic bacteria, a 6-log reduction is required to meet the pasteurization performance criteria for a food product (NACMCF, 2006). To achieve this target level of inactivation, high pressure levels or high temperatures need to be performed in the production process of meat products. High levels of pressure and heat may cause different changes in meat proteins structure that can lead to undesirable changes in the quality and sensory attributes of meat (texture, juiciness, color, etc.). Therefore, current attempts are focused on optimizing the high-pressure parameters (pressure level and time) to achieve a higher quality and safety of meat products. Similarly, future studies need to be done to test mild heat treatments such as sous vide processing to optimize the parameters of temperature and time.

When compared to the other pathogenic bacteria found in meat products, *Enterococcus* species have been found to be the most heat resistant (Kharel et al., 2018). Previous research found that pre-treatment (heat-shock) of cells at 50°C for 15 minutes increased *Enterococcus faecalis* thermal tolerance at temperatures of 55, 60, and 62°C (Ahmad et al., 2002). Refrigeration storage, on the other hand, demonstrated an increased sensitivity of *Enterococcus faecalis* cells to heat (Fernandez et al., 2009). These findings highlight the importance of further research into *Enterococcus faecalis* thermal tolerance during processing and refrigerated storage.

2.3. Thermal processing

Thermal processing is one of the most commonly used methods for extending the shelf life of food products thus increasing their availability to consumers. Various thermal processing methods are used in food treatment for enhancing food palatability such as boiling, baking, frying, and roasting. Meanwhile other thermal processing methods such as pasteurization and sterilization are mainly used

with the aim to inactivate pathogen and spoilage microorganisms and increase the shelf life of food products. Thermal processing methods can be categorized in three main groups: pasteurization (60-80 °C), sterilization (110-120 °C) and ultra-high- temperature (UHT) processing (140-160 °C).

Thermal processing is often associated with in-container sterilization which is used to preserve and extend the storage life of foods. In-container sterilization or canning can be defined also as a heat treatment which use high temperature in sufficient time to achieve the destruction of pathogenic and spoilage bacteria to ensure a long shelf-life of the food product. The reference for safety of low-acid foods (pH above 4.6) in a canning process is 12D-reduction of *Clostridium botulinum* spore-forming bacteria which can produce toxin in anaerobic conditions (Brown, 1993). However, safety and shelf life are no longer the only consumer requirements for food products; consumers are also looking for high sensorial quality, convenience, and value-added products. Traditional canning process, for example, is a conventional thermal processing technique that cause non-desirable sensory and nutritional changes in food products. Therefore, various novel mild thermal processing technologies have been developed which can produce nutritious, high sensorial quality food products and at the ame time ensure proper microbial inactivation by using optimal treatment parameters.

2.4. Mild thermal processing of meat

The sterilization of meat is ensured when all microorganisms including spore-forming bacteria are completely inactivated by the thermal treatment. However, the commercial sterility has been considered excessive and result in high level of degradation of nutritional and sensory properties of meat. On the other hand, mild thermal processing techniques have been shown to inactivate all non-spore-forming bacteria and spoilage microorganisms (Juneja, 2001).

Meat products are perishable by nature and highly susceptible to quality deterioration if not handled and processed properly. Several studies have shown that the most common cause of meat waste is spoilage which can lead to undesirable reactions causing deterioration of sensory properties such as color, flavour, and odour of the meat (Šojić et al., 2017; Díaz et al., 2008). In this context, the application of mild thermal processing techniques is valuable to preserve the microbiological and sensorial quality in order to increase the shelf life of meat products. Among the mild thermal processing techniques with potential application in the meat industry are ohmic heating, radio frequency heating, microwave processing, sous vide technology and infrared heating technology (Hugas et. al. 2002) (Figure 7).

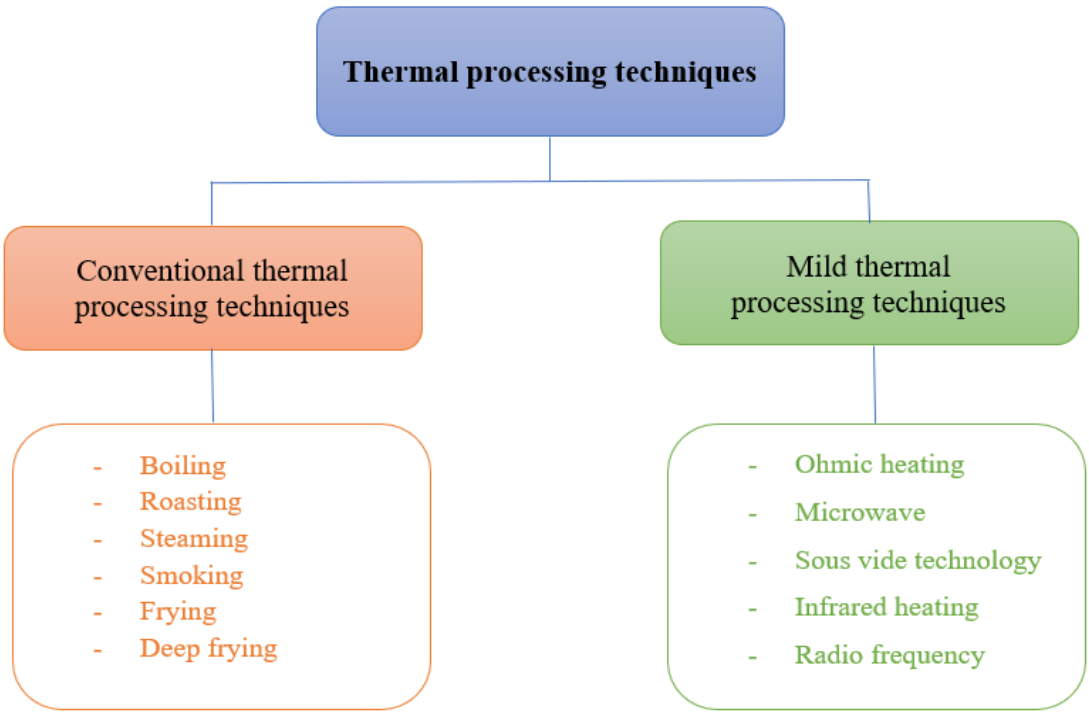


Figure 7: Categorization of thermal processing techniques (Sun, 2005)

2.5. Sous vide technology

In the last decade researchers have had a special focus on sous vide technology as one of the mild-heat processing techniques used in the food industry, households, restaurants and catering industry to produce ready-to-eat foods (Zavadlav et. al. 2020). Sous vide present a low-temperature long-time (LTLT) cooking method and refers to the cooking process of raw or half-processed foods in heat-stable vacuum pouches performed at water bath under carefully monitored temperatures and times (Baldwin, 2012). The optimal cooking temperature and time duration parameters must be chosen to achieve the desired quality attributes of sous vide treated meat, such as juiciness, tenderness, color, and oxidative stability, as well as to meet microbiological safety requirements. It was reported that sous vide technology improves sensory quality properties of meat including tenderness, juiciness, color, taste and flavour (Díaz et. al. 2008). Furthermore, sous vide technology preserves the nutritional composition of meat, including important micronutrients (vitamins, essential amino acids, unsaturated fatty acids) and reduces protein denaturation and lipid oxidation processes (Roascio-Albistur & Gámbaro, 2018; Díaz et. al. 2008). Selection of temperature and time in sous vide cooking has an important impact in denaturation of meat proteins which may cause loss of water and nutrients, myofibrillar shrinkage and texture modification in meat (Zielbauer et. al. 2016). Therefore, sous vide

technology has great potential in improving the yield of the final meat product thus providing an economic advantage but also improve the texture of meat which increase consumer satisfaction. Moreover, sous vide cooking process provides meat products with enhanced shelf life compared to meat which is heat treated by conventional heat processing techniques (boiling, steaming, etc.)

Sous vide cooking of meat is performed at temperatures ranging from 50 to 90 °C for varying time durations depending on the type and size of meat. Sous vide has the potential to improve the quality, sensory and nutritional properties of meat and meat products by tailoring the heat treatment process using different temperature and time parameters. Sous vide technique has some disadvantages such as high cost of installation and total energy consumption compared to the traditional heat treatments techniques (Głuchowski et. al. 2020). Moreover, sous vide cooking requires vacuum packaging using food-grade pouches with specific thickness. Selection of long heating time in sous vide cooking can increase the oxidation of lipids and proteins in meat. Therefore, beside temperature, cooking times must be carefully selected to avoid this drawback but also provide pasteurization of meat. In this context, Baldwin (2012) provided table of time durations for sous vide cooking of meat based on sample thickness from temperature of 55 to 66 °C, based on 6 log reduction of *Listeria monocytogenes* (70 °C for 2 min equivalent heat treatment).

2.5.1. Sous vide technology as a hurdle system

Consumer demand for the food industry is to provide microbiologically safe food products with increased shelf-life and at the same time with less technological impact on their nutritional value and sensory characteristics. As a result, a combination of different technological barriers is required to achieve this goal (Leistner, 2000). Sous vide processing technology present an example of the hurdle principle which includes several technological barriers such as refrigeration, temperature control, heat treatment, rapid cooling, and anaerobic conditions (Baldwin, 2012).

Sous vide technology include several important technological steps that require strict implementation such as:

- vacuum packaging,
- heat treatment,
- rapid chilling,
- refrigerated storage (or frozen storage),
- reheating,
- serving and consumption (Figure 8).

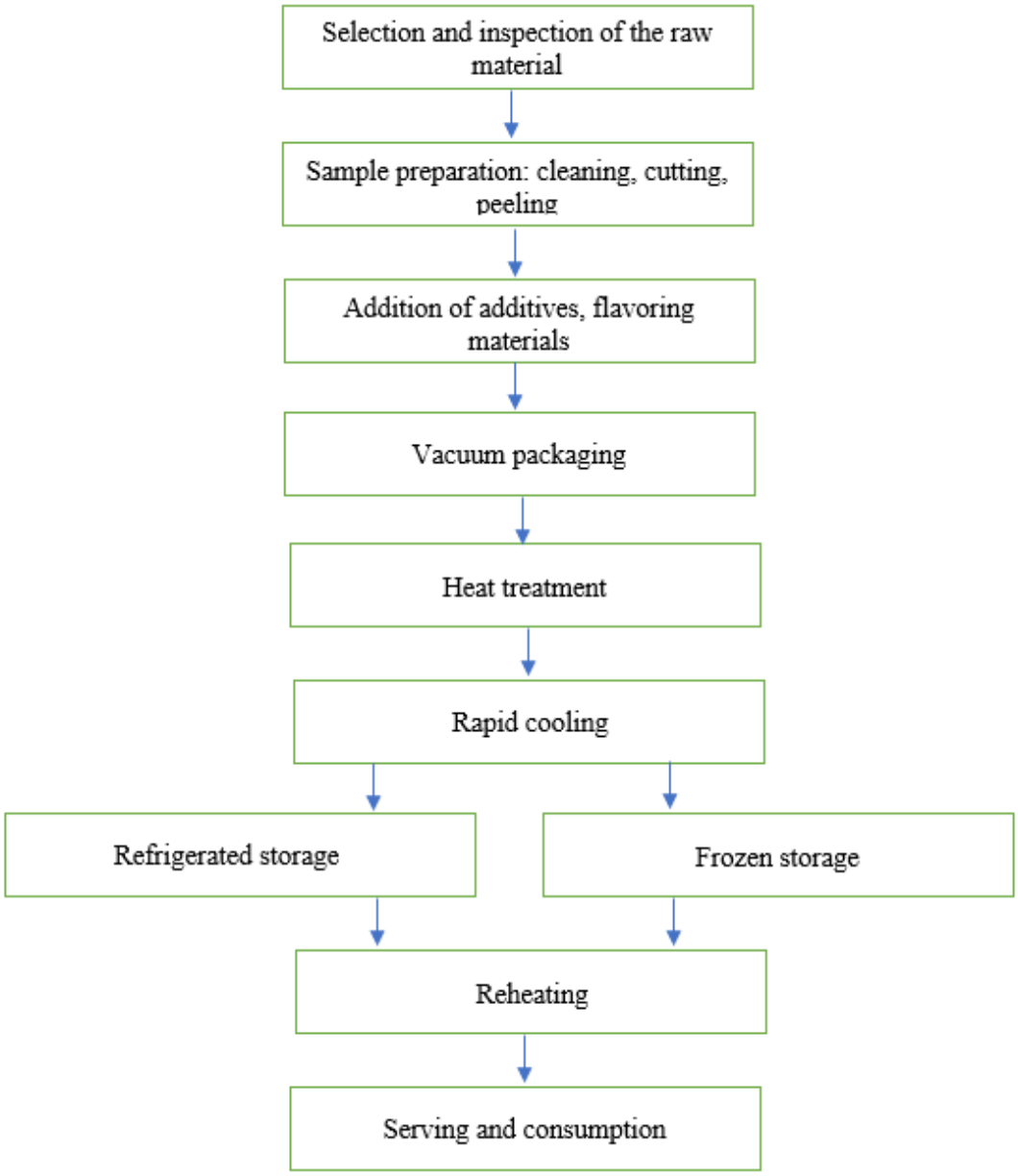


Figure 8: Flow diagram of sous vide cooking (Rozier et. al. 1990)

There are several requirements that must be implemented during each step in sous vide cooking. Before vacuum packaging, meat sample intended for sous vide cooking can be marinated using acidic marinades (e.g., acetic acid or fruit juice) or brined using salt solutions (1-3%) (Myhrvold et. al. 2011; Latoch & Libera, 2019). Vacuum packaging has some major advantages such as efficient heat transfer to meat during cooking in water bath and prevention of water and nutrient loss and oxidation of off-flavors. Furthermore, vacuum packaging reduces the growth of aerobic microorganisms and prevent recontamination during storage (Kehlet et. al. 2017).

Heat treatment in sous vide cooking is performed at precise time and temperature control, resulting in reproducible quality properties of meat. Furthermore, meat in sous vide technique can be cooked for long periods of time at low temperatures to ensure microbiological safety and avoid overcooking. In sous vide, precise time and temperature monitoring can be also used to improve the tenderness of tougher muscle meats (e.g., beef). A needle temperature probe is often used to monitor the internal temperature of meat during sous vide cooking, where the obtained temperature-time profiles can be used to calculate the pasteurization values of the sous vide treatments in meat.

After cooking, an important step that must be implemented without delay is rapid cooling of meat in order to prevent the sporulation of *Clostridium perfringens* that may lead to toxin production during storage (Willardsen et. al. 1978; Jay, 2000). BC Centre for Disease Control (2016) recommends cooling down the meat sample to an ice bath and storing meat at refrigerated storage in order to bring down the temperature of the sample to 4 ℃ within 6 h. If not consumed after treatment, sous vide cooked meat is required to either frozen or refrigerated at temperatures less than 3.3 ℃ in order to prevent the growth of spores from *Clostridium botulinum* and *Bacillus cereus* bacteria that may lead to possible production of toxin (Gould, 1999). If consumed right after treatment, sous vide cooked meat is reheated at temperatures ranging from 53 to 55 ℃ (Charley and Weaver, 1998). The majority of sous vide cooked meat is consumed in its original cooked form. However, some sous vide cooked meats, such as beef and pork chops, are treated with additional techniques such as searing or saucing. Application of searing can enhance the flavor of sous vide cooked meat due to products of Maillard reactions (Cho et. al. 2020).

2.5.2. Effect of sous vide on sensory attributes of meat, mechanism of protein changes

Although sous vide processing has little effect on the nutritional value of meat, it does change and modify proteins during thermal denaturation, thus having a direct effect on the functional and sensorial quality properties of treated meat (Kehlet et. al. 2017). Different proteins get denaturated at different temperatures, resulting in varying quality changes in sous vide treated meat such as weight loss, texture changes, color modification, and muscle shrinkage (Christensen et. al. 2000).

Tenderness and juiciness are the most desired sensorial meat quality properties among consumers (Kerr et. al. 2005). Various meat proteins denaturation state can influence the juiciness of meat products in either a positive or negative way. Actin shrinkage during the denaturation process can cause water to be squeezed out of meat. Meanwhile, collagen and sarcoplasmic protein solubilization during denaturation process can result in gel formation, which causes increased water binding in meat (Shoulders and Raines, 2009). Collagen shrinkage in meat begins between 57 and 58 ℃, resulting in

solubilization and conversion to gelatin after prolonged cooking (Brüggemann et. al. 2009). Meanwhile, actin can be denatured even when cooking at temperatures ranging from 60 to 74 °C if cooking times are long, resulting in water loss in meat (Zielbauer et. al. 2016). Therefore, sous vide technique has the advantage of using a low temperature to maintain actin integrity and a longer cooking time to complete collagen denaturation in order to achieve higher water content in meat products.

In general tenderness of sous vide treated meat has been connected to the solubility of collagen protein, which denatures at temperatures between 55 and 60°C (Purslow, 2018). However, Christensen et. al. (2011) reported that at higher temperatures than 60°C, myofibrillar components have a greater impact on meat texture changes than collagen. Meat tenderization is known to be prolonged because of the desmin degradation process in the myofibrillar component, which is faster between temperatures of 40 and 50 °C but slows down significantly at 60°C and stops completely at higher temperatures (Zhang et. al. 2009). This is due to the desmin degradation specific proteolytic enzymes, which remain active up to 60 °C and become inactive at higher temperatures than 65 °C (Ertbjerg et. al. 2012). Therefore, tailoring the sous vide cooking process through inclusion of cooking steps at proteolytic enzyme activation temperatures (40 - 50 °C) may result in an improve of meat tenderness.

2.5.3. Effect of sous vide on protein solubility and digestibility of meat

Many thermal and non-thermal technologies induce different changes in meat components especially in protein structure which can lead to different quality and sensory attributes. Heat treatment can cause different changes in meat protein such as aggregation, denaturation, crosslinking, degradation, oxidation and protein solubilization (Tornberg, 2005). Protein solubility of cooked meat is an indicator of the degree of protein denaturation that occurred during the cooking process and mainly depends on the myofibril structure, temperature and pH. Hsieh et. al. (2006) reported that the highest protein solubility loss in cooked pork and beef was observed between temperatures of 50 and 70 °C, which can be explained by protein denaturation. Increasing temperatures in cooking of goat meat have been shown to decrease the sarcoplasmic protein solubility, the denaturation process of which begins at 40 °C and ends at 65°C (Liu et. al. 2013). At these temperatures sarcoplasmic proteins aggregate and connect with each other by forming a gel which give consistency to the meat. Protein solubility of meat is connected with other quality attributes of meat such as moisture content, water holding capacity and cooking loss. High protein solubility particularly sarcoplasmic protein solubility in cooked meat has been associated with high water-holding capacity (Joo et. al. 1999). Similarly, Khan et. al. (2016) reported that high protein solubility is correlated to low cooking loss and high water-

holding capacity of meat.

Aside from the desirable quality and sensory attributes caused by sous vide cooking, the effect on meat protein digestion has been rarely studied. Bhat et. al. (2020) reported a positive correlation between protein solubility and protein digestibility in cooked beef meat. Moreover, this study found a positive impact of sous vide cooking on both protein solubility and protein digestibility of beef. This present the potential that sous vide technology has to be used as a tool to induce changes in protein structure and make them more susceptible to enzymatic hydrolysis which is performed by gastrointestinal proteases. Sous vide may also affect the release and bioavailability of various micronutrients such as minerals, amino acids, and peptides from meat during the digestion process, which is still being researched. In this context, the development of new meat products with improved digestibility and higher nutritional content using sous vide would be important for different groups of people especially for young, elderly, and pregnant women. On the other hand, cooking at high temperatures can lead to different structural conformations in proteins which minimize the digestibility of proteins by enzymes, thus also reducing the protein solubility and bioavailability of micronutrients of cooked meat (Kaur et. al. 2014).

2.5.4. Effect of sous vide on color of meat

Color is one of the key factors in determining consumer perception of meat safety and quality. For example, the degree of the pink color in interior of chicken breast is viewed as an indicator by consumers to check the product for proper cooking (King & Whyte, 2006). Changes in the color properties of meat products treated with heat can be attributed to myoglobin denaturation and oxidation, Maillard reactions, packaging, or pH (Del Pulgar et. al. 2012). Compared to conventional cooking, sous vide cooked meat obtains higher redness and lightness which is due to myoglobin changes during denaturation, with deoxymyoglobin being the most resistant to protein denaturation from the three forms of myoglobin (Hunt et. al. 1999). Myoglobin pigment is the main component responsible for color of poultry meat, which when cooking at higher temperatures than 70°C completely gets denaturates and forms a brown color (Suman & Joseph, 2013). When sous vide cooking of poultry meat at low temperatures a pink color can often appear which highly affect consumer perception (Hong et. al. 2016). Aside from cooking parameters, the selection of vacuum packaging material with acceptable degree of oxygen penetration is also important in terms of color stability of meat during storage.

According to Baldwin (2012), cooking temperatures used in sous vide cooking indicate the degree doneness of meat: rare meat at 50 °C, medium rare at 55 °C, medium at 60 °C and well-done at 70

°C. However aside from cooking temperature, consumers frequently use color attributes to determine the degree of doneness of meat. In sous vide cooking of various meats, the main parameter that affect the color changes is temperature (García-Segovia et. al. 2007; Christensen et. al. 2011; Del Pulgar et. al. 2012). Meat processed under sous vide conditions have higher lightness and redness compared to meat processed with conventional thermal processing techniques due to pigment denaturation. Myoglobin is denaturated during sous vide at temperatures ranging from 55 to 65 °C, and the degree of denaturation is negatively correlated with the redness (a*) intensity of meat (King & Whyte, 2006).

2.5.5. Effect of sous vide on oxidative stability of meat

Lipid oxidation is one of the major factors that directly influence the consumer acceptability of poultry meat products. The main factors affecting lipid oxidation reactions in poultry meat include cooking method, cooking temperature and cooking time (Broncano et. al. 2009; Del Pulgar et.al. 2012). Heating parameters temperature and time play a major impact on free radical production which initiate lipid oxidation reactions that directly affect the sensory attributes and nutritional value of cooked meat. In the primary lipid oxidation, hydroperoxides are formed from the oxidation reactions of polyunsaturated fatty acids which are in high amount in poultry meat (Chaijan & Panpipat, 2017). In the second stage of lipid oxidation hydroperoxides are decomposed in secondary compounds such as aldehydes which have a detrimental effect on sensory attributes of cooked meat. One of most used methods to determine lipid oxidation in cooked meat product is assessing TBARS values which is expressed as mg malondialdehyde per kilogram of meat (Dias et al. 2013). During cooking, cholesterol can be also oxidized forming cholesterol oxides products (COPs) in meat which are mainly measured by Gas Chromatography or High-Performance Liquid Chromatography (Broncano et. al. 2009).

Many studies have reported lipid oxidation in meat cooked at high temperatures using conventional thermal processing techniques such as boiling, grilling, steaming, which may be caused from the heating effect on cell membrane disruption and denaturation of hemeprotein (Broncano et. al. 2009; Domínguez et. al. 2014; Conchillo et. al. 2003). On the other hand, there is little information on the effect of mild heat treatments on lipid oxidation of meat. Lipid oxidation can be easily induced by pro-oxidants such as iron present in myoglobin, causing sensory properties to deteriorate and limiting the oxidative shelf life of meat products. The application of sous vide technology in which meat is cooked at mild temperatures and long times under vacuum packaging conditions can be used to prevent excessive lipid oxidation thus increasing the shelf life of meat products (Maqsood et. al. 2015). The main parameters influencing lipid oxidation in sous vide treated meat are cooking temperature, time of cooking, and pre-treatment of raw meat with different additives and flavoring compounds.

Latoch et. al. (2009) reported a decrease in lipid oxidation in pork loin marinated with yogurt before sous vide, which could be attributed to the effect of lactic acid bacteria on reducing reactive oxygen species. Similarly, sous vide treated sausages enriched with rosemary diterpene phenols showed acceptable lipid oxidation levels during 4 months at refrigerated storage (Naveena et. al. 2017).

2.5.6. Shelf life of sous vide meat products and microbial inactivation effect of sous vide

The microbial safety assessment is an important point to determine the efficiency of sous vide treatments on the inactivation of pathogenic microorganisms related to the sous vide products. In case of short-life chilled foods (≤ 10 days), it is recommended a minimum heat treatment that is equivalent to heating at 70°C for 2 min which ensures a 6 log reduction of the heat-resistant pathogen *Listeria monocytogenes* (ECFF, 2006). However, while in sous vide technique, the heat treatment, vacuum packaging, and refrigerated storage limit the growth of most microorganisms, it may create a non-competitive environment that favors anaerobic spore-forming microorganism growth. The highest concern for these types of products is the non-proteolytic *Clostridium botulinum*; thus, the shelf life of these products is limited to 10 days. On the other hand, if foods are treated with a heat treatment equivalent to 90°C for 10 min it ensures a 6 log reduction of non-proteolytic *Clostridium botulinum* spores, thus extending the shelf life to 2-6 weeks (ECFF, 2006).

Recently, several sous vide recipes have been using temperatures below 60°C to cook different types of foods (Stringer et al., 2012). In Figure 9 are presented the sous vide recipes for different food groups found on the internet, books, and Chef's guides. From these data it can be observed that numerous foods are being cooked at temperature and time combinations that do not meet the European guidelines for food safety (ECFF, 2006). In the largest platform for quantitative and predictive microbiology ComBase, there are gaps between temperatures used in growth and thermal inactivation prediction models (intermediate conditions) for food pathogens. Therefore, the extension of these growth and thermal inactivation models for pathogenic bacteria in the temperature range of 40-60 °C would provide valuable information for the improvement of the assessment of sous vide products safety. Previous studies have examined the thermal inactivation of several pathogens such as *Listeria monocytogenes*, *Salmonella*, and *Clostridium perfringens* in sous vide treated meat. These studies showed that microbiologically safe cooking time durations under sous vide condition below 60 °C temperature should be chosen carefully due to the influence of the matrix and the likelihood of pathogenic bacteria being in the internal part of the food product (Abel et al., 2020; El Kadri et al., 2020; Karyotis et al., 2017).

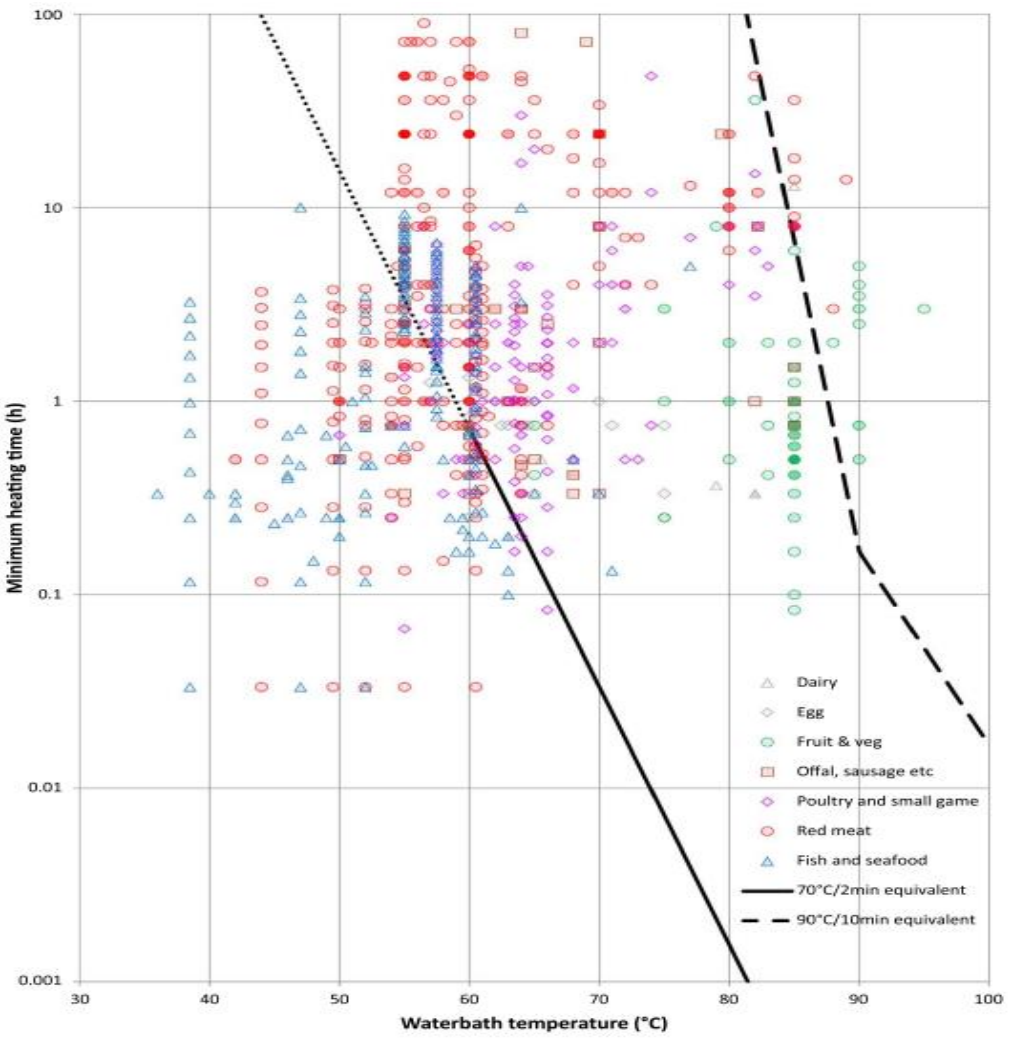


Figure 9: Shortest cooking time against cooking temperature for products in different food groups. The data represents sous vide recipes found in the top 200 internet hits, cook books and guides to sous vide cooking. The solid line represents a heat treatment equivalent to 70 °C for 2 min (i.e. sufficient to reduce contamination by *L. monocytogenes* by 6 log units). The dotted line indicates where this has been extrapolated to below 60 °C for illustrative purposes. The dashed line represents heat treatment equivalent to 90 °C for 10 min i.e. sufficient to reduce contamination by Group II *C. botulinum* by 6 log units (Stringer et. al. 2012).

Additional treatments such as roasting, searing, or frying might be used to improve the appearance and flavour of sous vide products. However, these post-treatments do not pasteurize the sub-pasteurized meat products internally (Ruiz-Carrascal et al., 2019). Therefore, the application of other minimal processing technologies such as high hydrostatic pressure may be effective to achieve full pasteurization of sub-pasteurized meat products.

3. MATERIAL AND METHODS

3.1. Raw meat samples preparation

Fresh chicken breast muscles (24 hours post-mortem) were bought in a local slaughterhouse and transported to the Department of Livestock Products and Food Preservation Technology, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences (Budapest, Hungary) in an ice filled thermos cool box. Chicken breast muscles often referred as *Pectoralis major* muscles represent a suitable raw material for meat research analysis because of its size and homogeneity structure. Chicken breast muscles were skin-off, trimmed of fat and cut in uniform weight of 129.6 ± 2.4 g and thickness of 2.0 ± 0.3 cm (Figure 10). The samples were vacuum packaged in 90 µm PA/PE pouches (200 mm × 250 mm) using a vacuum machine (Multivac C100, MULTIVAC Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany) and were randomly divided in treatment groups.

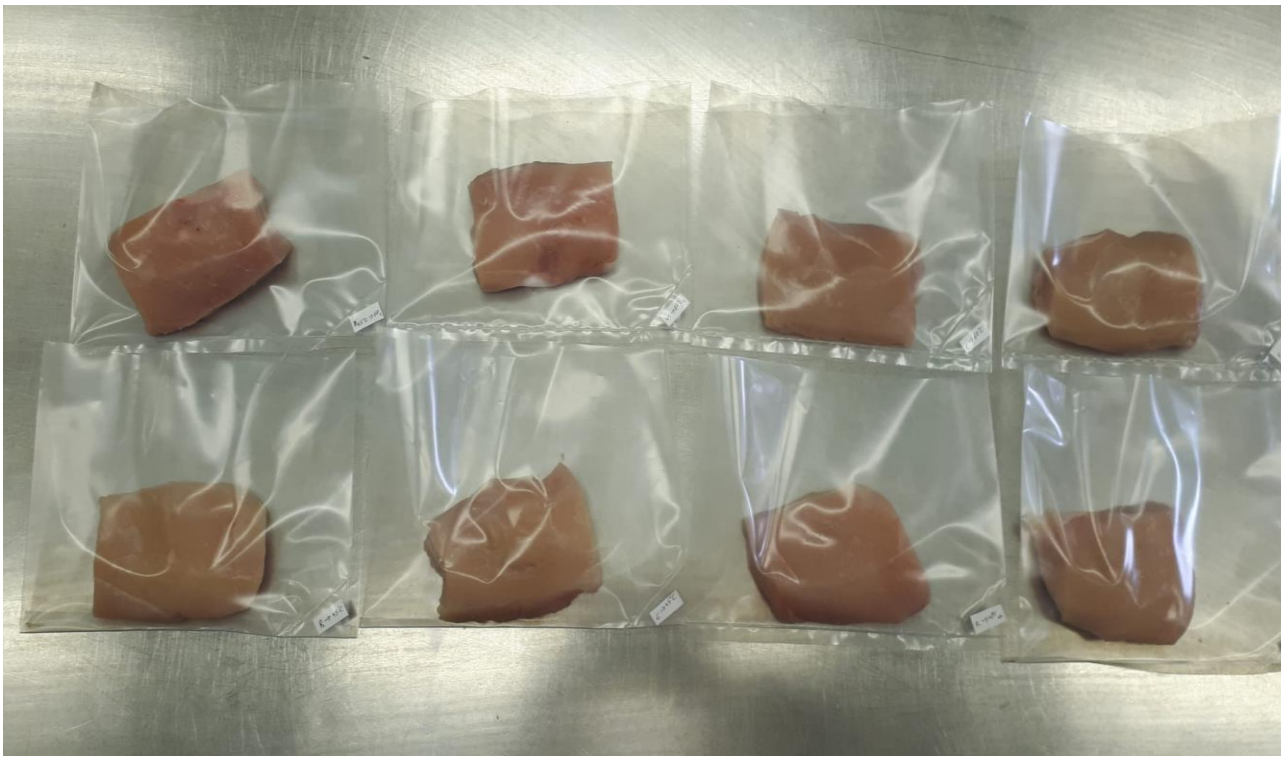


Figure 10: Chicken breast uniform samples in PA/PE pouches (own picture)

The physico-chemical characteristics (weight, thickness, width, length, pH, moisture content and color attributes), oxidative stability of lipids (TBARS) and microbiology properties of the raw chicken breast material used are presented in Table 1.

Table 1: Quality characteristics of the raw chicken breast used in the experiments

Attribute	Unit	Value
Weight	(g)	129.6 ± 2.4
Thickness	(cm)	2.0 ± 0.3
Width	(cm)	7.0 ± 0.15
Length	(cm)	9.5 ± 0.2
pH		5.65 ± 0.03
Moisture content	(%)	74.3 ± 1.24
Color (CIELab)	*L**	50.63 ± 0.93
	*a**	0.6 ± 0.16
	*b**	3.96 ± 0.1
TBARS	mg MDA/kg	0.328 ± 0.62
Enterococcus faecalis	*log CFU/g*	2.69 ± 0.12

The results values are expressed as mean ± standard deviation.

3.2. Experimental design and sous vide treatments

Experiments in the present study were conducted to study the effect of one and two step temperature treatments at different time ratios in sous vide processing of chicken breast muscles. Chicken breasts samples were cooked at one-step temperature of 60 °C and two-step temperatures treatments by combining a first step low temperature of 45 °C or 50 °C and an end step temperature of 60 °C in different treatment time ratios (three levels: 0:1,1:2, 1:1) and total treatment times (two levels: 120 and 180 min) (Table 2). A two-way completely randomized design was applied in the study with three replicates for each sous vide treatment. Sous vide treatments were conducted using two thermostatic water baths (Labor Müszeripari Müvek LP507/01). The internal temperature of the samples during sous vide processing was monitored using a needle probe T-type thermocouple (Figure 11, A) which was placed at the

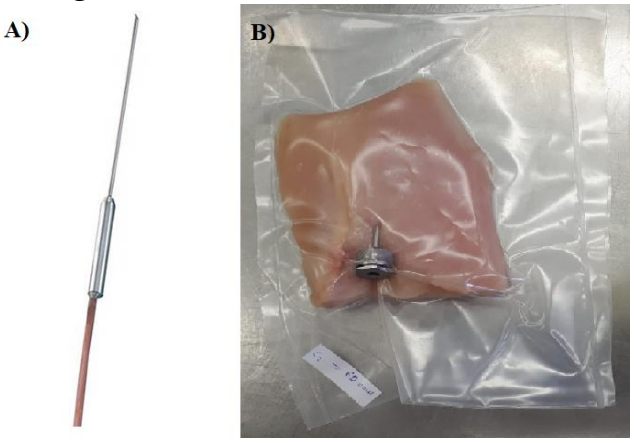


Figure 11: Internal temperature monitoring: A) Needle probe T-type thermocouple, B) nut and bolt in vacuum packaged chicken breast (own picture)

geometric center of vacuum packaged chicken breast sample using a nut and bolt as seen in Figure 11, B, and then were put into water bath. The temperature readings were recorded by a data logger at intervals of 2 s with a ± 0.1 accuracy.

After treatment chicken breast samples were cooled down in ice-cold water (1 °C) and were maintained at refrigerated conditions (2 °C) to achieve a temperature of less than 4 °C for 6 h according to the recommended guidelines (BC Centre for Disease Control, 2016).

Table 2: Sous vide treatments applied in the study.

Group	Time at the temperature of 45 °C (min)	Time at the temperature of 50 °C (min)	Time at the temperature of 60 °C (min)	Treatment time ratio 45 or 50 °C : 60 °C	Total treatment time (min)
T1	-	-	120	0:1	120
T2	-	40	80	1:2	120
T3	-	60	60	1:1	120
T4	-	80	40	2:1	120
T5	40	-	80	1:2	120
T6	60	-	60	1:1	120
T7	80	-	40	2:1	120
T8	-	-	180	0:1	180
T9	-	60	120	1:2	180
T10	-	90	90	1:1	180
T11	-	120	90	2:1	180
T12	60	-	120	1:2	180
T13	90	-	90	1:1	180
T14	120	-	60	2:1	180

Physico-chemical attributes of cooked chicken breast (moisture content, cooking loss, pH, color), lipid oxidation (TBARS), protein solubility and microbiological analysis were conducted on the following days after treatment. In the following experiment, the storage stability of the selected sous vide treated chicken breasts (T1, T2, and T3) was also examined when stored at 4 ± 0.5 °C, at 10 ± 0.5 °C and at -20 ± 0.5 °C for up to 21 days. Samples were taken for physico-chemical analysis, lipid oxidation, protein solubility, odour and microbiological analysis on days 0, 7, 14 and 21 days.

3.3. Determination of pasteurization values

Pasteurization values of each group were calculated by integration of time-temperature profiles provided from the time-temperature readings during sous vide treatments using the following equation:

$$P_{T_{ref}}^{z} = \int_{0}^{t} 10^{(T-T_{ref})/z}\, dt$$

where *t* refers to the heating time (min), T_{ref} is the reference temperature, *T* indicates the measured core temperature (°C) during heating, and *z* indicates the number of degrees of temperature to enhance the thermal death rate of the target microorganism by a factor of 10. Three pathogens of interest were selected to determine the pasteurization values of the studied sous vide treatments, each with specific z-values and reference temperatures (T_{ref}). The selected pathogens were *Clostridium perfringens* (T_{ref} = 60 ◦C, *z* = 6.74 ◦C), *Listeria monocytogenes* (T_{ref} = 70 ◦C, *z* = 10 ◦C), *Clostridium botulinum* spores (T_{ref} = 80 ◦C, *z* = 13 ◦C). Calculations were done based on five independent batches for each group treatment.

3.4. Physico-chemical attributes measurements

3.4.1. Moisture content

The measurement of moisture content of chicken breasts was performed in triplicates following the standard AOAC International 950.46 method (AOAC. 2005). Around 4 g of sample was weighted and dried in an air-forced oven (Labor Müszeripari Müvek, Budapest, Hungary) at 105 ◦C for 16 h. The moisture content of the sample was calculated based on the weight of the sample after drying (M_d) and the initial weight (M_i), following the equation below:

$$\text{Moisture content (\%)} = (M_i - M_d) / (M_i) \times 100$$

3.4.2. Cooking loss

Cooking loss was determined by subtracting the weight of cooked chicken breast (W2) from the weight of initial raw chicken breast (W1), based on the following equation:

$$\text{Cooking loss (\%)} = (W1-W2) / (W1) \times 100$$

3.4.3. pH measurement

The pH value of the chicken breasts was measured before and after sous vide processing using a pH meter (Testo-AG, Germany). The measurement was conducted in triplicate.

3.4.4. Color measurement

The color values of meat samples were determined using the CIELAB scoring system (CIE, 1986). A CR-400-type colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) was used to measure the lightness (L^*), redness (a^*), and yellowness (b^*) values of the samples after white calibration of the instrument. Five parallel readings were performed for each sample. The method described by Knispel (1991) was used to calculate the total color difference (ΔE) using the obtained values of lightness L^*, redness a^*, and yellowness b^*, based on the equation below:

$$\Delta E=\sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

Figure 12: CR-400-type colorimeter (Konica Minolta Sensing Inc.)

However, to understand better the color changes in the sous vide treatments, it is more common to evaluate the total color difference (ΔE) between the studied sous vide treatments. The calculated total color difference (ΔE) between different groups can be classified in five categories according to Mokrzycki and Talol (2011):

- Category I: $0 < \Delta E < 1$, observer perceives no difference
- Category II: $1 < \Delta E < 2$, color difference can be perceived only by experienced observer
- Category III: $2 < \Delta E < 3.5$, color difference can be perceived also by an unexperienced observer
- Category IV: $3.5 < \Delta E < 5$, observer perceives a clear color difference
- Category V: $\Delta E > 5$, observer perceives two distinct colors

Classification is based on the ability of the human eye to perceive the color differences measured with the CR-400-type colorimeter instrument.

3.5. Protein solubility

Protein solubility parameters were assessed using the method applied by Warner et al. (1997). One gram of sample was homogenized in triplicates with 10 mL of ice-cold 25 mM potassium phosphate buffer (pH = 7.2) by a Digital Ultra-Turrax homogenizer (Staufen, Germany) for the extraction of sarcoplasmic proteins. Homogenates were left for 20 h in refrigerated conditions (4 ◦C) and then were centrifuged (1500× g for 20 min) at 4 ◦C. Protein concentrations of the obtained supernatants were

assessed following the Bradford method (Bradford, 1976) using the BSA (bovine serum albumin) as a standard. Similarly, total protein solubility was determined by homogenizing 1 g of sample with 10 mL of 0.55 M potassium iodide and 0.05 M potassium phosphate buffer (pH = 7.2). Myofibrillar protein solubility was calculated as the difference between total protein solubility and sarcoplasmic protein solubility.

3.6. Texture measurement

3.6.1. Warner-Bratzler shear force

For Warner-Bratzler shear force analysis, samples were cut on a slab shape in the size of 15 mm × 15 mm × 50 mm (width, thickness, length) and cut perpendicular to the orientation of fibers with a Warner–Bratzler knife blade with a flat end at 2 mm/s speed using a TA.XT Plus texture analyzer (Stable Micro System, Surrey, United Kingdom) (Figure 13). Texture Exponent 32 software for Windows (Stable Micro System) was used for processing the measured data. The obtained maximum peak force (N) was registered as a shear force value. Six parallel measurements were performed for each sample. The measurements were performed at room temperature (20 ± 1.5 °C).

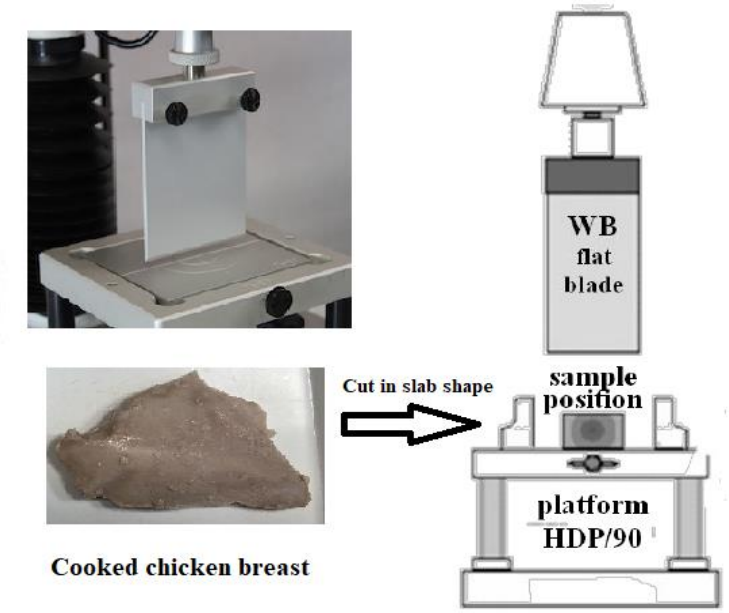


Figure 13: Warner-Bratzler shear force measurement (modified from Belović et.al. 2014)

3.6.2. Texture profile analysis (TPA)

The texture of cooked samples was analyzed through texture profile analysis (TPA) following the method applied by Bourne (1976). Twelve chicken breast cores per treatment in duplicates (diameter

= 12 mm, height = 12 mm), previously tempered at 4 ◦C, were compressed to 70% of their original height in two cycles with a P/35 cylinder probe (35 mm diameter) using a TA.XT Plus texture analyzer (Stable Micro System, Surrey, United Kingdom) (Figure 14, A). A load cell (500 N) was applied at a speed of 2 mm s^{-1} from which maximum force-time deformation curves were obtained. Texture Exponent 32 software for Windows (Stable Micro System) was used for processing data to assess the TPA attributes: hardness (N), springiness (mm), gumminess (N), cohesiveness (-), and chewiness (N * mm).

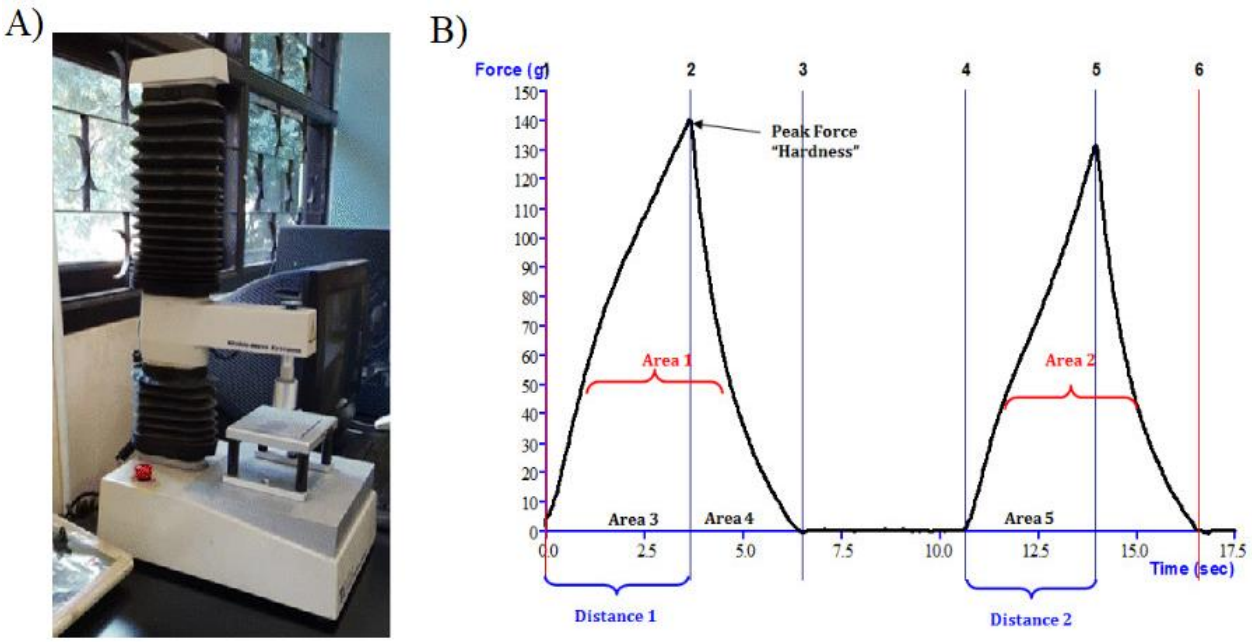


Figure 14: Texture profile analysis (TPA) measurement: A) TA.XT Plus texture analyzer with a P/35 cylinder probe , B) Two-cycle compression graph (Texturetechnologies.com)

Texture profile analysis parameters were calculated based on the obtained data from the two-cycle compression graph (Figure 14: B). The peak force of the first compression (first bite) was registered as hardness and expressed in Newton. Cohesiveness was calculated as the ratio between the area during the second compression to the area during first compression. Gumminess was calculated as the product between hardness and cohesiveness and was expressed in Newton (N). Springiness is described as the sample recovery distance after first compression cycle or the time between the end of the first compression cycle and the start of the second compression cycle. Springiness results are expressed in mm. Chewiness was calculated as the product of gumminess and springiness and was expressed in N * mm.

3.7. Lipid oxidation

Lipid oxidation of chicken breast samples was assessed by measuring the thiobarbituric Acid Reactive Substances (TBARS) values using the method from Dias et al. (2013) with few modifications. The sample (5 g) was homogenized (Digital Ultra-Turrax, Staufen, Germany) for 2 min with 20 mL of 5% trichloroacetic acid (TCA) and 0.5 mL of the artificial antioxidant 0.15% butylated hydroxytoluene (BHT, Sigma-Aldrich, Munich, Germany). After that, centrifugation of the homogenized samples was performed at 5000× g for 10 min. The obtained supernatants were filtered using Whatman No. 1 filter paper into 25-mL volumetric flasks and adjusted to 25 mL with 5% TCA. Two milliliters of the filtrate were mixed with two milliliters of 0.08% thiobarbituric acid (TBA) in glass tubes and were heated in a water bath at 95 ◦C for half an hour. Solutions in glass tubes were cooled down to room temperature, vortexed, and then absorbances were measured against a blank (mixing 2 mL of 5% TCA and 2 mL of 0.08% TBA) at 532 nm by U-2900 UV-visible spectrophotometer (Hitachi Ltd., Tokyo, Japan) (Figure 15). TBARS values represented the mean of triplicate measurements for each sample and were reported as mg malondialdehyde/kg of meat sample.

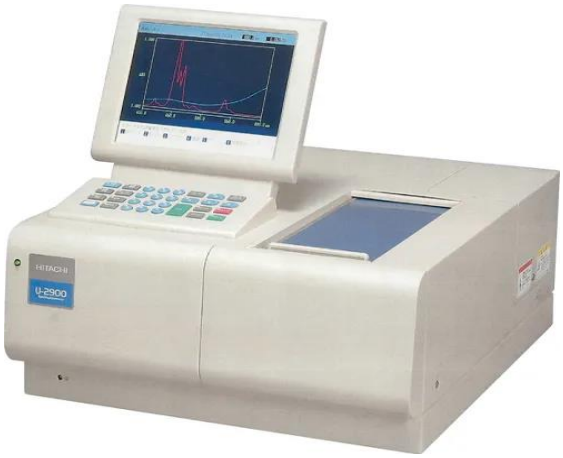


Figure 15: U-2900 UV-visible spectrophotometer (Hitachi Ltd.)

3.8. Odour acceptability

During the storage experiment, at each sampling day the pouches were aseptically opened and the samples were evaluated for odour acceptability. The samples were scored using a five-point scale: 1 = acceptable, 2 = slightly acceptable, 3 = neutral, 4 = slightly unacceptable, 5 = unacceptable. The panel consisted of 5 members of researchers and teachers familiar with sensory evaluation of cooked chicken breast in the Department of Livestock Products and Food Preservation Technology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary.

3.9. Microbiological analysis

Microbiological analysis were conducted to check the thermal inactivation of the studied treatments on chicken breast by using *Enterococcus faecalis* as a reference microorganism. *Enterococcus faecalis*

counts were also tested during the storage of sous vide treated chicken breast at temperature of 4° C, 10 °C and at – 20 °C for up to 21 days, for the selected sous vide treatments.

3.9.1. Determination of Enterococcus faecalis

The microbiological challenge test was used to determine the pasteurization level of the chicken breast samples treated with the studied sous vide treatments. *Enterococcus faecalis* was selected as a target microorganism to conduct the thermal inactivation challenge tests due to its high thermal heat resistance. Three replicates were performed for each treated sample.

3.9.1.1. Preparation of bacterial strain and inoculum

The *Enterococcus faecalis* strain (NCAIM B. 01312) used in the present study was obtained from the National Collection of Agricultural and Industrial Microorganisms (NCAIM, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary). Recovery of the lyophilized culture was performed in Brain Heart Infusion (BHI, Sigma-Aldrich, Munich, Germany) broth and incubated for 24 hours at 37 °C. The culture was streaked on the selective *Enterococcus faecalis* agar and incubated for 24 hours at 37 °C. Pure cultures were maintained on Tryptic-Soy Agar (TSA, Biokar Diagnostic BK046HA, Sigma Aldrich) slands at refrigeration conditions (4° C) until use. The first step of preparation of *Enterococcus faecalis* inoculum was preparation of an overnight culture on the non - selective TSA Caso Agar TSA, Biokar Diagnostic BK046HA, Sigma Aldrich). After that, a cell suspension of the culture was prepared using MRD diluent and the cell concentration was set to approx. 10^8 CFU/ml using a McFarland densitometer.

3.9.1.2. Bacterial inoculation on chicken breast

Approximately 10 grams chicken breast sample was taken in a stomacher bag and inoculated with 0.1 ml cell suspension (10^8 CFU/ml) of *Enterococcus faecalis* B. 01312 in order to have an initial cell count of 6 log CFU/g in the samples. Then the chicken breast samples were vacuum packaged in 90 µm PA/PE pouches (200 mm × 250 mm) using a vacuum machine (Multivac C100, MULTIVAC Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany) and after sous vide treated in a water bath in one of the treatment conditions. In storage experiment, inoculated sous vide cooked chicken breast (those treated in T1, T2, and T3 treatment conditions) were stored at temperature of 4°C, 10°C and at -20°C for up to 21 days.

3.9.1.3. Microbiological enumeration

After treatment or storage sampling day each vacuum packaged sous vide treated chicken breast sample was suspended aseptically with 90 ml Maximum Recovery diluent (MRD, Sigma-Aldrich, Munich, Germany) and homogenized in a stomacher bag for 2 min using a stomacher. After that the samples were 10-fold serially diluted in MRD diluent and were plated on Citrate azide tween carbonate Agar (CATC Agar, Sigma-Aldrich, Munich, Germany) by pour plating of 0.1 ml of sample/dilution and spreading with a sterile glass spreader. The inoculated selective *Enterococcus faecalis* Agar (CATC Agar) plates were then incubated at 37 °C for 24 hours and the red obtained colonies were counted using a colony counter (Figure 16). The results were presented as the logarithms of colony-forming units per gram of sample (log CFU/g).

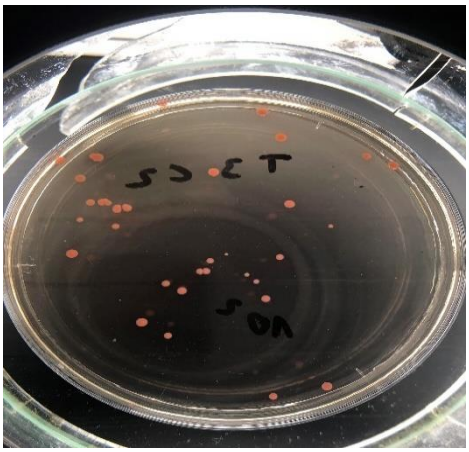


Figure 16: *Enterococcus faecalis* colonies in CATC Agar (own photo)

3.10. Sensory analysis

Sensory analysis of sous vide treated chicken breast samples was performed for different sensory characteristics such as intensity of flavour, tenderness, juiciness, and overall acceptability. The sensory panel consisted of 11 professors and researchers at Hungarian University of Agriculture and Life Sciences who were familiar with sensory analysis of cooked chicken breast. Nine-point hedonic scale was used to perform the sensory assessment: flavour intensity (1 = very weak, 5 = neutral, 9 = very strong), tenderness (1 = extremely dry, 5 = neutral, 9 = extremely tender), juiciness (1 = not juicy, 5 = neutral, 9 = extremely juicy), color and overall acceptability (1 = dislike extremely, 5 = neutral, 9 = like extremely). The samples were labeled with three-digit random codes and the order of treatments given to panelist was randomized.

3.11. Statistical analysis

The experimental data were analysed using IBM SPSS (Version 27.0, Armnouk, NY, 2020). Data were analysed using the analysis of variance (ANOVA) and General Linear Model (GLM). Normality of the residuals was tested by Kolmogorov-Smirnov test ($p>0.05$). Homogeneity of error variances

was accepted by Levene's test ($p>0.05$). Tukey's post hoc tests were run if homogeneity of variances was satisfied, and Games-Howell's method was used when this assumption was violated. The obtained microbiological data were converted to log CFU/g.

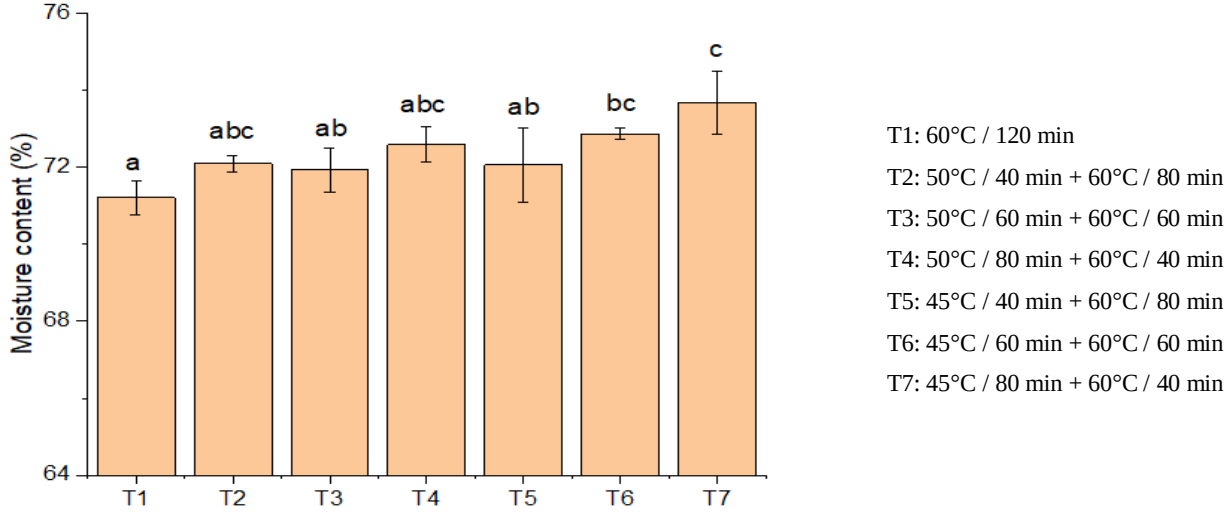
4. RESULTS AND DISCUSSION

4.1. COMPARISON BETWEEN THE EFFECT OF ONE-STEP AND TWO-STEP SOUS VIDE ON QUALITY AND MICROBIOLOGICAL ATTRIBUTES OF CHICKEN BREAST

4.1.1. Physicochemical attributes of chicken breast

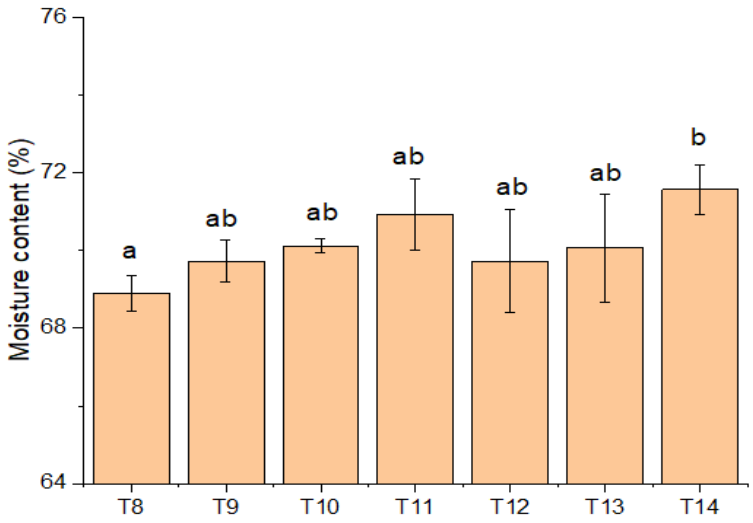
4.1.1.1. Moisture content and cooking loss

A comparison of moisture content between the studied sous vide treatments of same total treatment time are displayed in Figure 17 and Figure 18. In 120 min sous vide treatments, the two-step sous vide treated chicken breast (T6 and T7) showed significantly higher moisture content than the one-step sous vide ones (T1) ($P < 0.05$). Higher moisture content was observed with increasing the cooking time of the low temperature (45 °C) from 40 min (T5 treatment) to 80 min (T7 treatment) in the two-step sous vide treatments (Figure 17). Similarly, higher moisture content was observed with increasing the application time of the low temperature (45 or 50°C) from 0 to 120 min in the 180 min sous vide treatments, having a significant increase only between T8 and T14 ($P < 0.05$) (Figure 18). Our results are in agreement with those reported by other authors (Bıyıklı et.al. 2020; Hwang et. al. 2019). Bıyıklı et.al. (2020) founded that moisture content was significantly higher in turkey cutlet cooked at lower sous vide cooking temperatures. The moisture content of meat depends on the degree of thermal protein denaturation which causes shrinkage of muscle fibers (Offer et. al. 1984) leading to a volume reduction and consequently water loss at high cooking temperatures.



Different letters (a-c) above the bars indicate a significant difference according to Tukey's post hoc test ($P < 0.05$).

Figure 17: Moisture content of chicken breast cooked with 120 min sous vide treatments.

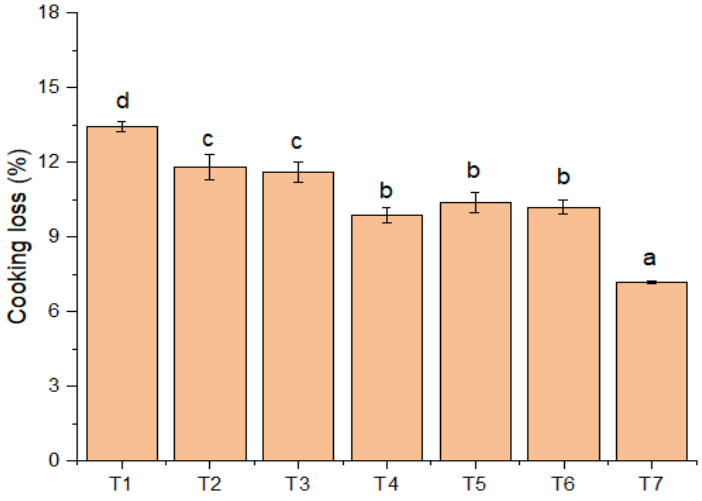


T8: 60°C / 180 min
T9: 50°C / 60 min + 60°C / 120 min
T10: 50°C / 90 min + 60°C / 90 min
T11: 50°C / 120 min + 60°C / 60 min
T12: 45°C / 60 min + 60°C / 120 min
T13: 45°C / 90 min + 60°C / 90 min
T14: 45°C / 120 min + 60°C / 60 min

Different letters (a-b) above the bars indicate a significant difference according to Tukey's post hoc test ($P < 0.05$).

Figure 18: Moisture content of chicken breast cooked with 180 min sous vide treatments.

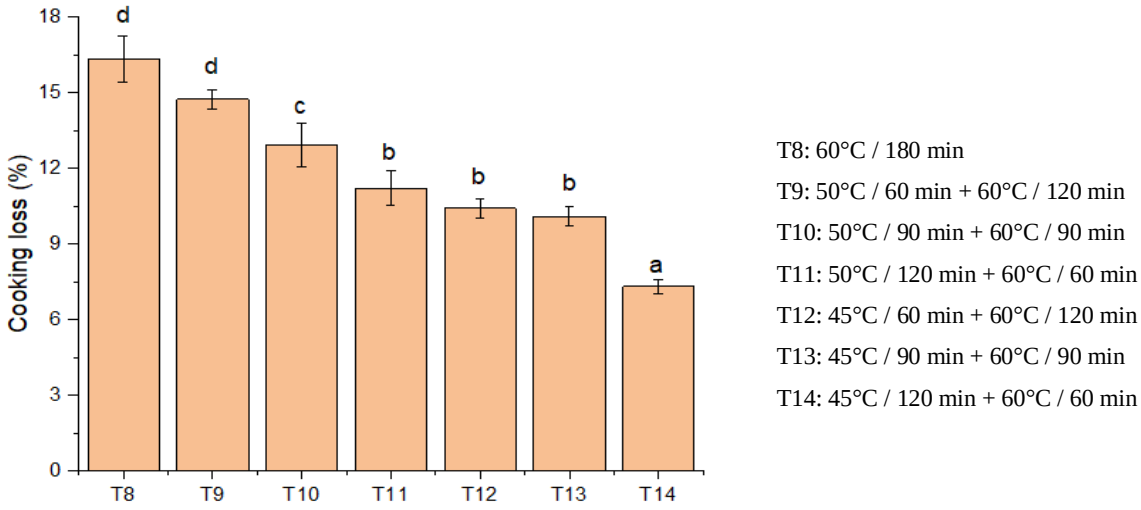
Cooking losses during heat treatment contribute on a considerable share of food wastes in households and catering sector (Sałek et. al. 2020). Sous vide presents a valuable method to reduce food waste, by the combination of optimal temperature and time duration of cooking in heat-stable pouches. In our study, cooking loss of the chicken breasts cooked with different sous vide treatments ranged from 7.20 % to 16.35 % (Figure 19 and Figure 20).



T1: 60°C / 120 min
T2: 50°C / 40 min + 60°C / 80 min
T3: 50°C / 60 min + 60°C / 60 min
T4: 50°C / 80 min + 60°C / 40 min
T5: 45°C / 40 min + 60°C / 80 min
T6: 45°C / 60 min + 60°C / 60 min
T7: 45°C / 80 min + 60°C / 40 min

Different letters (a-d) above the bars indicate a significant difference according to Tukey's post hoc test ($P < 0.05$).

Figure 19: Cooking loss of chicken breast cooked with 120 min sous vide treatments.



Different letters (a-d) above the bars indicate a significant difference according to Tukey's post hoc test ($P < 0.05$).

Figure 20: Cooking loss of chicken breast cooked with 180 min sous vide treatments.

In 120 min treatments, chicken breasts cooked with the two-step sous vide treatments (T2-T7) had significantly lower cooking loss than the chicken breast cooked with the one-step sous vide treatment T1 ($P < 0.05$) (Figure 19). Similar result was observed in 180 min treatments, with the exception of cooking loss of one-step sous vide treated chicken breast (T8) which was not significantly different with the two-step sous vide treatment (T9) (Figure 20). Similarly, Ismail et. al. (2019) reported reduced cooking loss when beef was pre-cooked at 49°C and then cooked at a higher temperature of 60 or 65°C.

The low cooking loss is in correlation with higher juiciness which has a positive impact on consumer preference for chicken meat (Zielbauer et. al. 2016). Apparently, the application of the lower temperatures (45 and 50 °C) in the two-step sous vide had a positive effect on lowering the cooking loss thus it improved the juiciness of the chicken breast. The cooking loss which is released in the vacuum pack at the initial cooking temperature, seems to be reabsorbed by the meat structure before reaching the final temperature of 60 °C. Zielbauer et.al (2016) reported lower values of cooking loss compared with the trend during long time of cooking pork at the temperature of 51 °C. This could be explained by collagen solubilization under denaturation which results in gel formation and higher water content in meat (Del Pulgar et. al. 2012).

4.1.1.2. Color attributes

In Table 3 and Table 4 are presented the instrumental color properties (L^*, a^*, b^*, and ΔE) of the chicken breast cooked in different combinations of temperature and time. Color is one of the main

indicators in consumer perception of meat quality and safety (King & Whyte, 2006). The present study shows that two-step sous vide treated samples had significantly lower lightness (L^* value) than the one-step sous vide treated samples within the same total treatment time ($P < 0.05$), with the exception of T6 and T12 being non-significant (Table 3 and 4). This can be explained by the fact that higher temperatures can increase the protein denaturation process which results in a higher brightness of meat (Christensen et. al. 2011). Cooked chicken breast can be classified into three categories based on degree of lightness: pale ($L^* < 53$), normal (L^*= 46-53) and dark ($L^* < 46$) (Da Silva-Buzanello et. al. 2019). Based on the obtained lightness values, all the studied sous vide treated chicken breasts belonged to the pale appearance classification group ($L^* > 53$).

Table 3: Means ± standard deviations of color attributes (L^*, a^*, b^*, ΔE) of 120 min cooked chicken breast with different combinations of temperatures and time durations.

Time	**120 min**						
Treatments	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
	60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**	**50°C / 80 min + 60°C / 40 min**	**45°C / 40 min + 60°C / 80 min**	**45°C / 60 min + 60°C / 60 min**	**45°C / 80 min + 60°C / 40 min**
L*	80.0±0.24c	78.79±0.30b	78.36±0.23b	77.93±0.43ab	78.79±0.17b	79.01±0.23bc	77.07±0.89a
a*	1.79±0.3a	2.10±0.17ab	2.22±0.17abc	2.28±0.21abc	2.34±0.05bc	2.32±0.14bc	2.72±0.13c
b*	9.55±0.18a	11.55±0.39b	13.1±0.13c	13.15±0.38c	9.6±0.1a	11.68±0.22b	10.12±0.19a
ΔE	29.34±0.26b	28.64±0.28b	28.69±0.18b	28.3±0.44b	28.2±0.19b	28.9±0.16b	26.66±0.9a

ΔE – Total color difference of chicken breast before and after cooking. Mean values with different letters (a-c) in the same row are significantly different according to Tukey's post hoc test ($P < 0.05$).

Redness values (a^* value) were between 1.34 and 2.72. The results of the present study revealed that chicken breast cooked with the 180 min sous vide had significantly higher redness values with increasing application time of the low temperature of 45 or 50°C from 0 to 90 min (Table 4). This might be attributed to myoglobin pigment concentration which is presumed to be higher in two-step sous vide treated samples because its denaturation starts between 55 and 65 °C (Hunt et. al. 1999). The pink color in poultry meat has been shown to be the first indicator that consumers check for a properly cooked product (Langsrud et. al. 2020). The degree of the pink color on chicken breast can be evaluated using a subjective pink threshold of a^*= 3.8 (Holownia et. al. 2003). In the present study, all the sous vide treated samples obtained redness values under this threshold level.

Table 4: Means ± standard deviations of color attributes (*L**, *a**, *b**, ΔE) of 180 min cooked chicken breast with different combinations of temperatures and time durations.

Time	**180 min**						
Treatments	**T8**	**T9**	**T10**	**T11**	**T12**	**T13**	**T14**
	60°C / 180 min	**50°C / 60 min + 60°C / 120 min**	**50°C / 90 min + 60°C / 90 min**	**50°C / 120 min + 60°C / 60 min**	**45°C / 60 min + 60°C / 120 min**	**45°C / 90 min + 60°C / 90 min**	**45°C / 120 min + 60°C / 60 min**
L*	80.83±0.05d	78.96±0.22abc	78.38±0.21abc	77.62±0.46a	79.52±0.96cd	79.23±0.56bc	78.0±0.54ab
a*	1.34±0.18a	1.94±0.19ab	2.49±0.22bc	2.65±0.35c	1.94±0.3ab	2.54±0.17bc	2.58±0.25bc
b*	9.11±0.34a	12.59±0.39b	12.87±0.65b	13.09±0.63b	8.98±0.14a	9.56±0.15a	10.08±0.43a
ΔE	30.06±0.27c	29.08±0.26bc	28.65±0.23abc	28.02±0.41ab	28.77±0.97abc	28.63±0.58abc	27.55±0.63a

ΔE – Total color difference of chicken breast before and after cooking. Mean values with different letters (a-d) in the same row are significantly different according to Tukey's post hoc test ($P < 0.05$).

Significant negative correlation was observed between cooking loss and redness in sous vide cooked chicken breast ($R=-0.691$, $P < 0.01$). Meanwhile significant positive correlation was observed between cooking loss and lightness in cooked chicken breast ($R=0.640$, $P < 0.01$). Ismail et. al. (2019) reported similar Pearson correlation coefficients but not significant between cooking loss and color attributes lightness and redness of cooked beef.

The yellowness values (*b** value) of the chicken breasts cooked with the two-step temperature (50°C + 60°C) were significantly higher than those processed with the one-step temperature within the same total treatment time ($P < 0.05$). This might be due to lower exposure of meat to the temperature of 60°C during cooking in the two-step sous vide treatments (40–120 min) in comparison with the one-step sous vide treatments (120 and 180 min) (Table 3 and 4).

Results of the total color difference (ΔE) between raw and cooked chicken breasts are presented in Table 3 and 4. The highest total color difference (ΔE) was obtained for treatment T8 (30.06) and the lowest for treatment T7 (26.66). According to Mokrzycki and Tatol (2011) observer notices two different colors when $\Delta E > 5$. In this context, authors believe that for a better understanding of changes on the color attributes of the samples it is more common to evaluate the total color difference (ΔE) between the studied sous vide treatments. Total color difference (ΔE) results between sous vide treatments were classified based on five categories as described by Mokrzycki and Tatol (2011) and are presented below in Table 5 and Table 6.

According to the study of Tomasevic et.al. (2019), a clear color difference between meat products is

noticeable if ΔE > 3.5. From the pairwise comparison between the 120 min sous vide treatments, a clear difference in color can be noticed between the chicken breast cooked with one-step temperature treatment (T1) and the two-step temperature treatments T3 and T4 where the total color difference (ΔE) was 3.94 and 4.19, respectively (Table 5). Similar results were obtained for total color difference (ΔE) between the 180 min one-step (T8) and two-step sous vide treated chicken breasts (T9, T10, T11) (ΔE > 3.5) (Table 6). It can be inferred that the first-step temperature of 50°C and the time duration in which it was used in two-step sous vide treatments had a major impact on the color attributes between the studied sous vide treatments. On contrary, the total color difference between chicken breast cooked at one step temperature of 60 °C and at the two step temperature 45 °C + 60 °C can be perceived only by an experienced observer (ΔE > 2) when the low step temperature (45 °C) is applied for one third of the total treatment time both in 120- and 180-min two-step sous vide treatments. However, color difference between one-step and two-step sous vide (45 °C + 60 °C) treated chicken breasts can be perceived also by an unexperienced observer (2<ΔE < 3.5) when the time ratios between the low step temperature (45 °C) and end step temperature (60 °C) is 1:1 and 2:1, both in 120- and 180-min two-step sous vide treatments.

Table 5: Total color difference (ΔE) between chicken breasts cooked with 120 min sous vide treatments

	Total color difference (ΔE)						
Time	**120 min**						
Treatment	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
	60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**	**50°C / 80 min + 60°C / 40 min**	**45°C / 40 min + 60°C / 80 min**	**45°C / 60 min + 60°C / 60 min**	**45°C / 80 min + 60°C / 40 min**
T1	-	Cat. III	Cat. IV	Cat. IV	Cat. II	Cat. III	Cat. III
T2	2.36	-	Cat. II	Cat. II	Cat. II	Cat. I	Cat. III
T3	3.94	1.62	-	Cat. I	Cat. IV	Cat. II	Cat. III
T4	4.19	1.83	0.44	-	Cat. IV	Cat. II	Cat. III
T5	1.33	1.96	3.53	3.66	-	Cat. III	Cat. II
T6	2.41	0.35	1.57	1.83	2.09	-	Cat. III
T7	3.13	2.32	3.29	3.18	1.85	2.52	-

Latin numbers in the table indicate the total color difference (ΔE) categories: Category I: 0<ΔE < 1- observer perceives no difference ; category II: 1<ΔE < 2 - Only the experienced observer perceives the difference; category III: 2<ΔE < 3.5 - the color difference is perceived also by unexperienced observer; category IV: 3.5<ΔE < 5- clear color difference is perceived; category V: 5<ΔE - observer perceives two distinct colors.

Table 6: Total color difference (ΔE) between chicken breasts cooked with 180 min sous vide treatments

	Total color difference (ΔE)						
Time	**180 min**						
Treatments	**T8**	**T9**	**T10**	**T11**	**T12**	**T13**	**T14**
	60°C / 180 min	**50°C / 60 min + 60°C / 120 min**	**50°C / 90 min + 60°C / 90 min**	**50°C / 120 min + 60°C / 60 min**	**45°C / 60 min + 60°C / 120 min**	**45°C / 90 min + 60°C / 90 min**	**45°C / 120 min + 60°C / 60 min**
T8	-	Cat. IV	Cat. IV	Cat. V	Cat. II	Cat. III	Cat. III
T9	4.00	-	Cat. I	Cat. II	Cat. IV	Cat. III	Cat. III
T10	4.63	0.85	-	Cat. I	Cat. IV	Cat. III	Cat. III
T11	5.27	1.59	0.80	-	Cat. IV	Cat. IV	Cat. III
T12	1.45	3.66	4.09	4.58	-	Cat. I	Cat. II
T13	2.05	3.10	3.41	3.87	0.89	-	Cat. II
T14	3.23	2.77	2.82	3.04	1.97	1.32	-

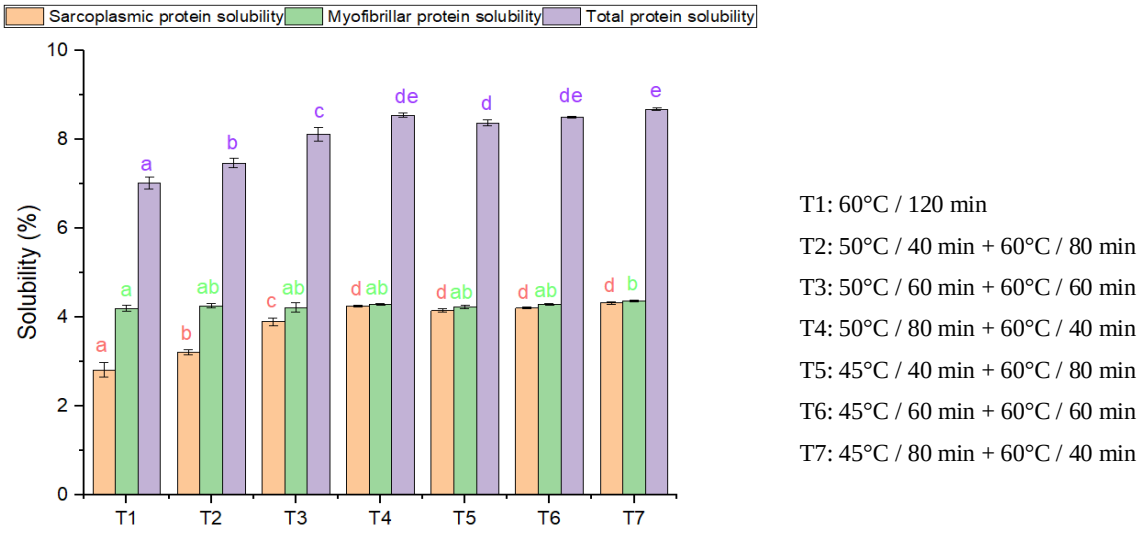
Latin numbers in the table indicate the total color difference (ΔE) categories: Category I: $0<\Delta E < 1$- observer perceives no difference ; category II: $1<\Delta E < 2$ - Only the experienced observer perceives the difference; category III: $2<\Delta E < 3.5$ - the color difference is perceived also by unexperienced observer; category IV: $3.5<\Delta E < 5$- clear color difference is perceived; category V: $5<\Delta E$ - observer perceives two distinct colors.

4.1.2. Protein solubility

The results for sarcoplasmic, myofibrillar, and total protein solubility of chicken breasts cooked at different combinations of temperatures and time durations are shown in Figure 21 and Figure 22. The present study showed a significant decrease ($P < 0.05$) in total protein solubility of chicken breast cooked with the one-step sous vide in comparison with the ones cooked with the two-step sous vide within the same total treatment time (120 min and 180 min). Lower total protein solubility indicates the heat denaturation of sarcoplasmic and myofibrillar proteins. Protein denaturation increased with longer cooking time durations of chicken breast at the high temperature of 60°C, which is in agreement with the results of Cropotova et al. (2019).

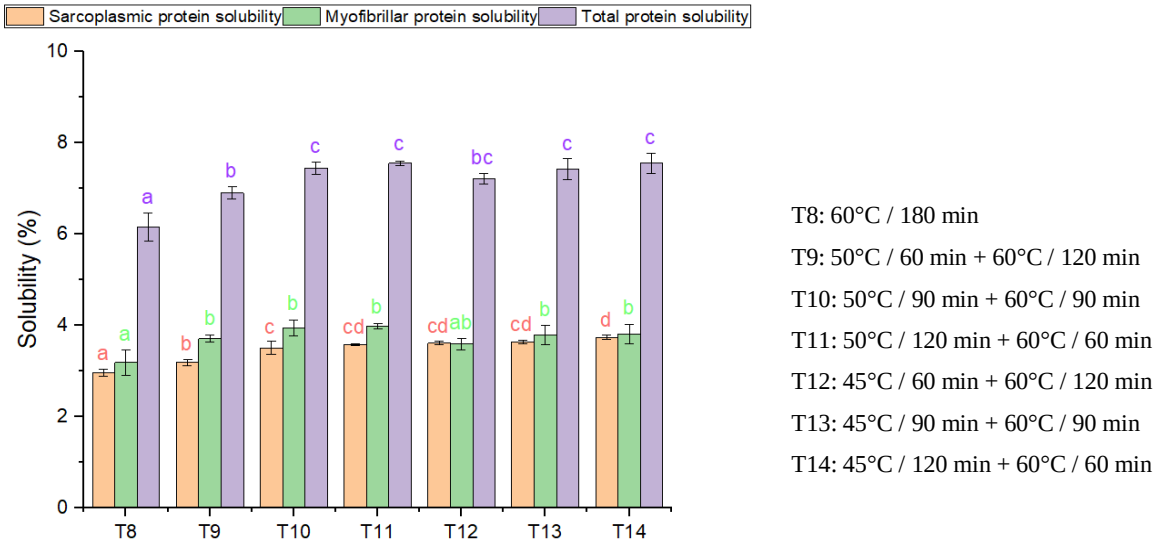
According to our results, sarcoplasmic protein solubility in two-step sous vide treated chicken breast was significantly higher than one-step sous vide ones both in 120- and 180-min treatments ($P < 0.05$). Sarcoplasmic protein solubility of chicken breasts cooked with the two-step sous vide was increased at a higher rate than myofibrillar protein solubility in comparison to chicken breasts cooked with the one-step sous vide in the 120 min total treatment time but not in the 180 min (Figure 21 and Figure 22). Myofibrillar protein solubility was not significantly increased in 120 min two-step sous vide

treatments compared to one-step ones, with the exception of T7 treatment. On contrary, myofibrillar protein solubility was significantly higher in 180 min two-step sous vide treatments compared to one-step ones, with the exception of treatment T12 (Figure 22).



Different letters (a-e) above the bars indicate a significant difference according to Tukey's post hoc test or *Games-Howell's post hoc test ($P < 0.05$).

Figure 21: Means ± standard deviations of protein solubility of 120 min sous vide treated chicken breast.



Different letters (a-d) above the bars indicate a significant difference according to Tukey's post hoc test or *Games-Howell's post hoc test ($P < 0.05$).

Figure 22: Means ± standard deviations of protein solubility of 180 min sous vide treated chicken breast.

There is a relation between protein solubility, moisture content, and cooking loss of meat (Murphy and Marks, 2000; Joo et. al. 1999). Murphy and Marks reported a strong correlation between protein solubility and cooking loss in heat-treated chicken breast patties (Murphy and Marks, 2000). Similarly, our results shows that cooking loss was significantly correlated with sarcoplasmic and total protein solubility of sous vide cooked chicken breast (R=-0.744 and R=-0.715, both with $P < 0.01$). Joo et. al. (1999) has also reported a correlation between sarcoplasmic protein solubility and water holding capacity. On the other hand, moisture content was strongly and significantly correlated with total protein solubility (R=0.806, $P < 0.01$) and medium and significantly correlated with sarcoplasmic and myofibrillar protein solubility (R=0.638 and R=0.792, both with $P < 0.01$).

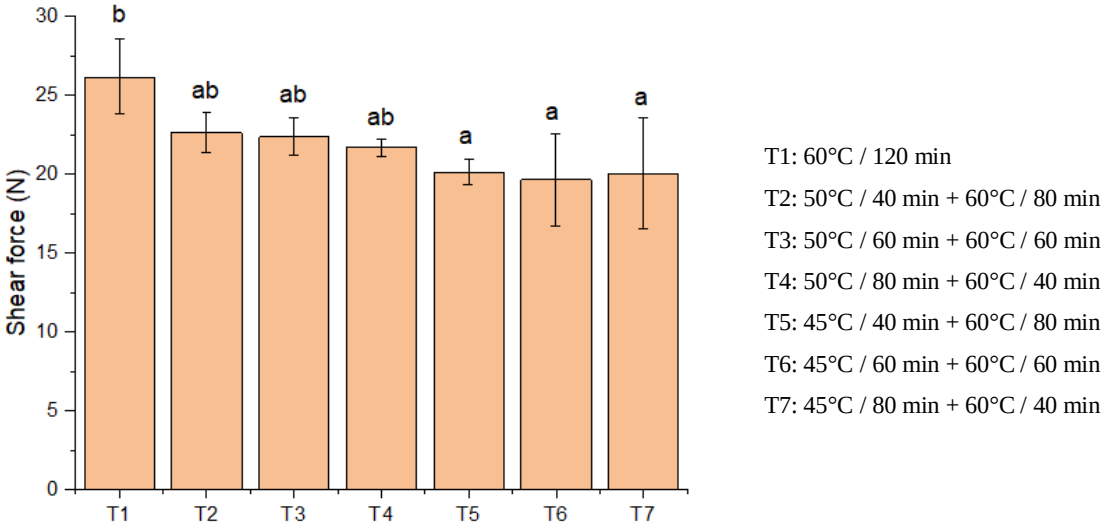
Sarcoplasmic, myofibrillar and total protein solubility were significantly correlated with lightness of sous vide chicken breast (R=-0.620, R=-0.527, R=-0.666, all with $P < 0.01$). Similarly, Ismail et.al. (2019) reported significant Pearson correlation coefficient between lightness and sarcoplasmic protein solubility of cooked beef samples. On the other hand, redness was also significantly correlated with sarcoplasmic, myofibrillar and total protein solubility of sous vide treated chicken breast (R=0.597, R=0.434, R=0.606, all with $P < 0.01$). Meanwhile, Ismail et. al. (2019) observed significant correlation only between myofibrillar protein solubility and redness of cooked beef.

4.1.3. Texture attributes

Shear force is the most common instrumental method to evaluate the tenderness of cooked meat. Shear force values of the studied sous vide treatments on the chicken breast are displayed in Figure 23 and 24. Cooked chicken breast using 120 min one-step temperature 60°C (T1) had significantly higher shear values (lower instrumental tenderness) compared to the cooked chicken breast using 120 min two-step temperature 45°C + 60°C (T5-T7) ($P < 0.05$) but was insignificant compared to the 120 min two-step temperature treatments 50°C + 60°C (T2-T4) ($P > 0.05$) (Figure 23).

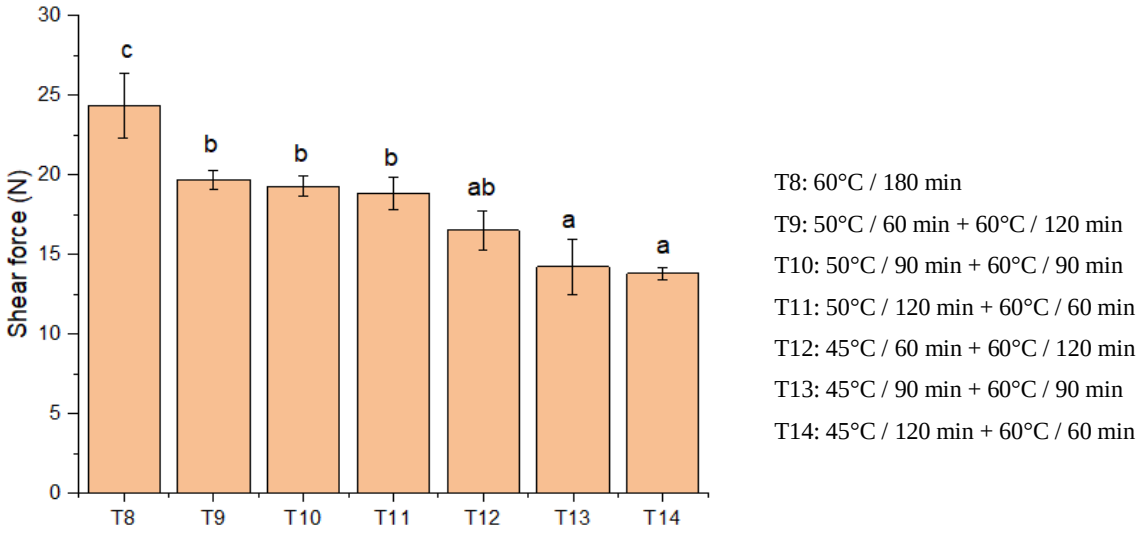
On the other hand, in the case of 180 min treatments chicken breasts cooked with the two-step temperature (T9-T14) had significantly lower shear force values than chicken breasts cooked with the one-step temperature (T8) ($P < 0.05$) (Figure 24). These results showed the effect of the first-step temperature 45°C and 50 °C applied in the two-step temperature treatments on increasing the tenderness of sous vide chicken breast. Ismail et. al. (2019) reported reduced shear force values in beef when cooked at a pre step temperature of 45°C and then at a higher temperature (60 or 75°C) for a total period of 6 hours. Hwang et al. (2019) reported also that shear force values were lower when pork loins were cooked at 50°C compared to those cooked at 55 or 60°C. This tenderization effect might be attributed to the specific proteolytic enzymes for desmin degradation which are largely active

up to 50°C (Ertbjerg et. al. 2012). Chicken breasts cooked with the one-step sous vide obtained higher shear values because at 60°C the main proteolytic enzymes, calpains are inactivated (Dransfield, 1994) while cathepsins operate with only 50% of their proteolytic activity (Spanier et.al. 1990).



Different letters (a-b) above the bars indicate a significant difference according to Tukey's post hoc test ($P < 0.05$).

Figure 23: Shear force values of chicken breast cooked with 120 min sous vide treatments.



Different letters (a-c) above the bars indicate a significant difference according to Tukey's post hoc test ($P < 0.05$).

Figure 24: Shear force values of chicken breast cooked with 180 min sous vide treatments.

The results of TPA parameters are shown in Table 7 and 8. The highest TPA-hardness mean value (49.78 N) was obtained in chicken breasts cooked with the 120 min one-step sous vide (T1), which was significantly higher compared to chicken breast cooked with the 120 min two-step sous vide (T2-T7) ($P < 0.05$) (Table 7). However, hardness was not significantly different in the 180 min two-step sous vide treatments, with the exception of T14 treatment, compared to the 180 min one-step sous vide treatment (T8) (Table 8). No significant differences were observed between one-step and two-step sous vide on cohesiveness and springiness values within the same total treatment time (120 min and 180 min) ($P > 0.05$) (Table 7 and Table 8). Previous studies have also reported no difference in springiness and cohesiveness of the sous vide chicken breast and beef cooked at different combinations of temperature and time durations (Park et. al. 2020; Rinaldi et. al. 2014).

Table 7: Means ± standard deviations of shear force and TPA attributes of chicken breast (pectoralis major) heated with different combinations of temperatures and time durations.

Time	**120 min**						
Treatments	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
	60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**	**50°C / 80 min + 60°C / 40 min**	**45°C / 40 min + 60°C / 80 min**	**45°C / 60 min + 60°C / 60 min**	**45°C / 80 min + 60°C / 40 min**
Hardness (N)	49.78±1.05b	39.84±3.44a	37.79±4.16a	37.02±1.66a	40.02±1.83a	38.77±1.16a	38.04±1.8a
Cohesiveness (-)	0.23±0.01ab	0.186±0.01a	0.25±0.03b	0.23±0.03ab	0.22±0.01ab	0.24±0.02b	0.24±0.01b
Springiness (mm)	1.47±0.11a	1.47±0.09a	1.51±0.04a	1.53±0.05a	1.54±0.04a	1.55±0.04a	1.53±0.04a
Gumminess (N)	11.3±0.3c	7.38±0.64a	9.34±0.1b	9.31±0.05b	8.94±0.65b	9.3±0.6b	9.12±0.21b
Chewiness (N * mm)	16.58±1.45c	10.86±1.65a	14.05±0.33abc	13.16±1.20ab	13.77±0.89abc	14.43±1.31bc	13.92±0.61abc

Mean values with different letters (a-c) in the same row are significantly different according to Tukey's post hoc test or *Games-Howell's post hoc test ($P < 0.05$).

In addition to hardness, two other important texture parameters especially for elderly consumers are chewiness and gumminess. According to the results presented in Table 7 and 8, gumminess was significantly ($P < 0.05$) reduced in the two-step sous vide treated samples in comparison with the one-step sous vide treated ones within the same total treatment time (120 min and 180 min), except for T9. The trends for gumminess values were just slightly different from chewiness values in sous vide chicken breast treated at 180 min total treatment time.

Table 8: Means ± standard deviations of shear force and TPA attributes of chicken breast (pectoralis major) heated with different combinations of temperatures and time durations.

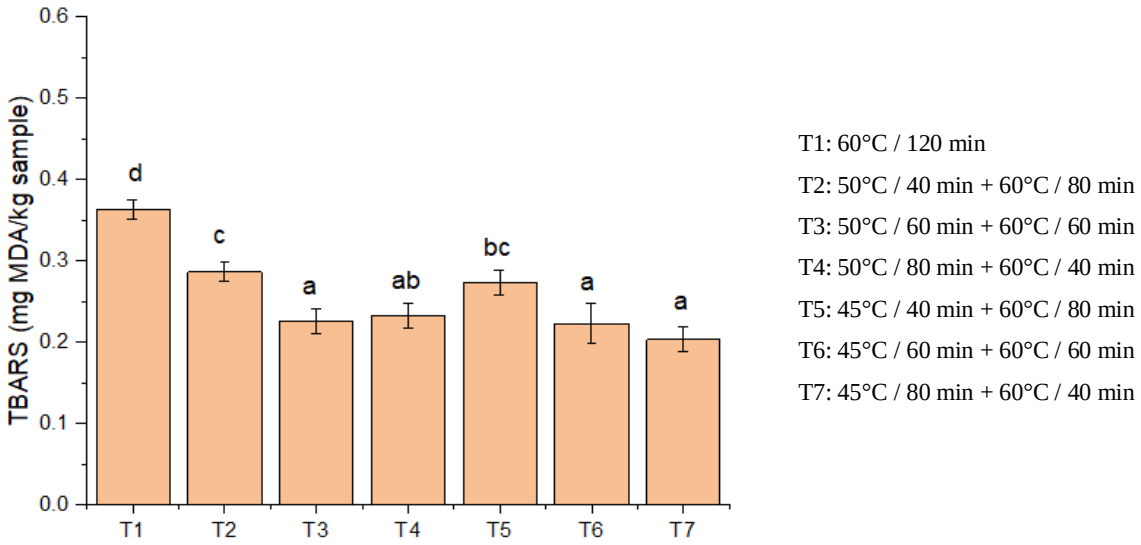
Time	**180 min**						
Treatments	**T8**	**T9**	**T10**	**T11**	**T12**	**T13**	**T14**
	60°C / 180 min	**50°C / 60 min + 60°C / 120 min**	**50°C / 90 min + 60°C / 90 min**	**50°C / 120 min + 60°C / 60 min**	**45°C / 60 min + 60°C / 120 min**	**45°C / 90 min + 60°C / 90 min**	**45°C / 120 min + 60°C / 60 min**
Hardness (N)	46.45±3.39b	44.55±4.7ab	41.08±3.91ab	39.15±1.75ab	41.72±2.67ab	38.76±2.37ab	35.91±2.4a
Cohesiveness (-)	0.26±0.02a	0.27±0.02a	0.24±0.02a	0.24±0.01a	0.25±0.01a	0.24±0.01a	0.23±0.01a
Springiness (mm)	1.44±0.08a	1.52±0.16a	1.50±0.06a	1.44±0.05a	1.48±0.14a	1.5±0.06a	1.53±0.08a
Gumminess (N)	12.11±0.32d	11.89±0.23cd	9.7±0.11ab	9.5±0.07ab	10.38±0.57bc	9.07±0.94ab	8.25±0.95a
Chewiness (N*mm)	17.38±1.37b	18.07±1.88b	14.52±0.64ab	13.68±0.53ab	15.44±2.22ab	13.58±1.7ab	12.66±2.11a

Mean values with different letters (a-d) in the same row are significantly different according to Tukey's post hoc test or *Games-Howell's post hoc test ($P < 0.05$).

The chewiness was also reduced in case of 120 min two-step sous vide cooked chicken breast with T2 and T4 treatments having a significant decrease ($P < 0.05$) (Table 7). On contrary, chewiness values of 180 min two-step sous vide treated chicken breasts were not significantly lower compared to one-step ones, except for T14 treatment (Table 8). The reduced shear force and hardness (initial bite tenderness) and the reduced gumminess and chewiness obtained in two-step sous vide treated chicken breast present important texture attributes for an increase in meat consumption by elderly consumers (Forde et. al. 2013).

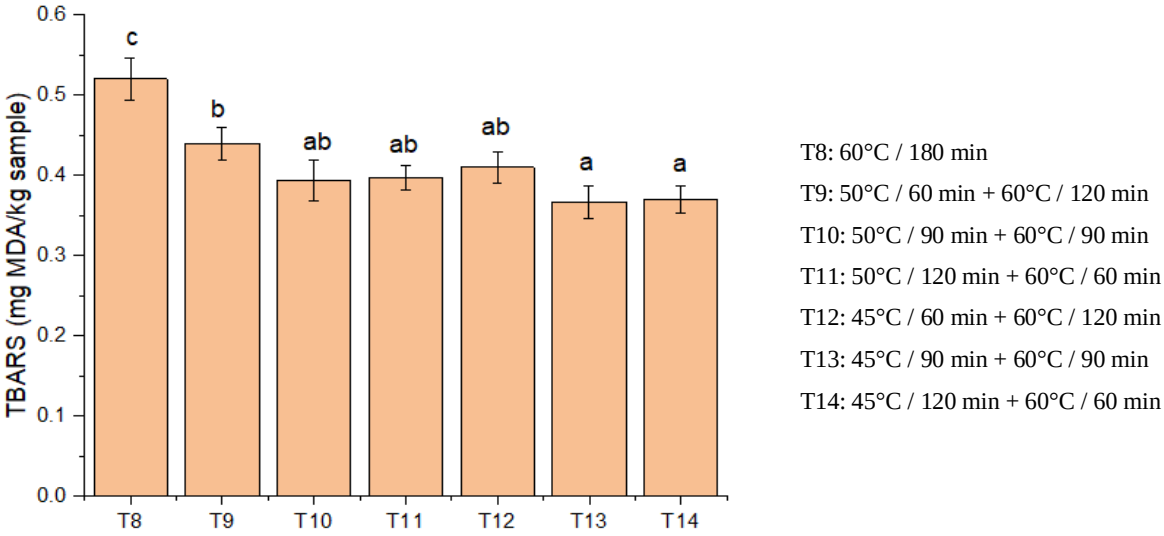
4.1.4. Lipid oxidation

To evaluate the rate of oxidation in sous vide treated chicken breast, the level of the secondary lipid oxidation compounds such as aldehydes (like malonaldehyde) was measured using a TBARS test. This method is used to indirectly detect the level of oxidative rancidity in meat products. The results of TBARS values of sous vide treated chicken breast with different combinations of temperatures and time durations are shown in Figure 25 and Figure 26. Chicken breasts treated with the one-step temperature treatments obtained significantly higher TBA values than those treated with the two-step temperature treatments within the same total treatment time, 120 min (Figure 25) and 180 min (Figure 26) respectively ($P < 0.05$). This might be due to the longer application time of the high temperature (60°C) in one-step sous vide treatments compared to the two-step ones which caused higher protein denaturation thus higher release of iron ions which have a strong prooxidant effect (Estévez et. al. 2011).



Different letters (a-d) above the bars indicate a significant difference according to Tukey's post hoc test ($P < 0.05$).

Figure 25: Means ± standard deviations of TBARS of 120 min sous vide treated chicken breast.



Different letters (a-c) above the bars indicate a significant difference according to Tukey's post hoc test ($P < 0.05$).

Figure 26: Means ± standard deviations of TBARS of 180 min sous vide treated chicken breast.

Increasing the total treatment time from 120 min to 180 min within the same treatment time ratio caused an increase in TBARS values of the cooked chicken breast. In agreement with our result, Karpinska-Tymoszczyk et al. (2020) reported that increasing the cooking time from 90 to 150 minutes at 61°C increased the TBARS values of sous vide chicken breast fillets. The highest mean TBA value (0.520 mg MDA per kilogram of sample) was observed in chicken breasts cooked at 60°C for 180 min (T8 treatment). According to Baker et al. (1972), consumers are not able to detect the oxidative rancidity in sous vide treated chicken breast with TBARS values below 1 mg/kg of sample. None of

the tested sous vide treatments in our study exceeded this threshold level (Figure 25 and Figure 26).

4.1.5. Pasteurization values

The pasteurization process has an important role in the sensorial attributes, nutritional value, and microbial stability of sous vide meat products (Bıyıklı et. al. 2020; Głuchowski et. al. 2020). Pasteurization values (P-values) are defined as the duration of the pasteurization process to achieve a particular reduction of a target pathogen at a specified temperature. To calculate pasteurization values of sous vide treatments, three target pathogens associated with sous vide meat products were considered: *C. perfringens* ($P_{60}^{6.74}$), *L. monocytogenes* (P_{70}^{10}) and *C. botulinum* spores (P_{80}^{13}). Pasteurization values ($P_{60}^{6.74}$, P_{70}^{10}, P_{80}^{13}) of the studied sous vide treatments derived from the recorded time-temperature profiles are presented in Table 9 and Table 10. As expected, chicken breasts treated with the one-step sous vide treatments obtained higher pasteurization values than the chicken breasts treated with the two-step sous vide treatments within the same total treatment time.

Table 9: Pasteurization values ($P_{60}^{6.74}$, P_{70}^{10}, P_{80}^{13}) of chicken breast (*pectoralis major)* cooked in 120 min sous vide treatments.

Treatments	Pasteurization values								
	$P_{60}^{6.74}$ (min)			P_{70}^{10} (min)			P_{80}^{13} (min)		
	Average	St.dev	Min.	Average	St.dev	Min.	Average	St.dev	Min.
T1	130.26	3.92	123.81	12.21	1.06	11.05	4.01	0.33	3.65
T2	86.68	5.09	80.21	9.31	0.37	8.84	2.92	0.19	2.68
T3	67.57	2.73	64.58	7.15	0.68	6.52	2.18	0.12	2.01
T4	44.52	1.88	42.15	4.22	0.48	3.54	1.39	0.05	1.30
T5	84.89	1.62	83.18	8.38	0.44	7.88	2.71	0.07	2.64
T6	65.79	1.27	64.06	6.60	0.46	6.05	1.90	0.22	1.68
T7	43.68	1.23	41.98	3.83	0.62	3.22	1.28	0.07	1.19

T1: 60°C for 120 min; T2: 50°C for 40 min and 60°C for 80 min; T3: 50°C for 60 min and 60°C for 60 min; T4: 50°C for 80 min and 60°C for 40 min; T5: 45°C for 40 min and 60°C for 80 min; T6: 45°C for 60 min and 60°C for 60 min; T7: 45°C for 80 min and 60°C for 40 min. $P_{60}^{6.74}$ - Pasteurization value for *Clostridium perfringens* (T_{ref} = 60 ◦C, z = 6.74 ◦C P_{70}^{10} - Pasteurization value for *Listeria monocytogenes* (T_{ref} = 70 ◦C, z = 10 ◦C), P_{80}^{13} - Pasteurization value *Clostridium botulinum* (T_{ref} = 80 ◦C, z = 13 ◦C).

Table 10: Pasteurization values ($P_{60}^{6.74}$, P_{70}^{10}, P_{80}^{13}) of chicken breast (*pectoralis major)* cooked in 180 min sous vide treatments.

Treatments	Pasteurization values								
	$P_{60}^{6.74}$ (min)			P_{70}^{10} (min)			P_{80}^{13} (min)		
	Average	St.dev	Min.	Average	St.dev	Min.	Average	St.dev	Min.
T8	197.55	7.73	188.86	17.34	1.02	16.24	6.01	0.31	5.66
T9	136.17	4.41	130.24	13.50	0.71	12.62	4.47	0.28	4.17
T10	99.07	3.70	93.51	10.06	0.52	9.59	3.06	0.16	2.89
T11	68.32	1.01	66.87	7.15	0.54	6.64	2.15	0.18	1.96
T12	132.67	2.05	130.02	11.46	0.35	11.05	3.93	0.20	3.72
T13	97.98	1.10	96.54	8.87	0.43	8.39	2.84	0.12	2.68
T14	65.24	0.81	64.18	5.91	0.39	5.55	1.93	0.13	1.79

T8: 60°C for 180 min; T9: 50°C for 60 min and 60°C for 120 min; T10: 50°C for 90 min and 60°C for 90 min; T11: 50°C for 120 min and 60°C for 60 min; T12: 45°C for 60 min and 60°C for 120 min; T13: 45°C for 90 min and 60°C for 90 min; T14: 45°C for 120 min and 60°C for 60 min. $P_{60}^{6.74}$ - Pasteurization value for *Clostridium perfringens* (T_{ref} = 60 ◦C, z = 6.74 ◦C, P_{70}^{10} - Pasteurization value for *Listeria monocytogenes* (T_{ref} = 70 ◦C, z = 10 ◦C), P_{80}^{13} - Pasteurization value for *Clostridium botulinum* (T_{ref} = 80 ◦C, z = 13 ◦C).

For pasteurization of sous vide foods, European Chilled Food Federation (ECFF, 2006) recommends at least a heat treatment equivalent to 70 °C for 2 min to ensure a 6-log reduction of *L. monocytogenes* (z-value = 10 °C). According to our results, all the studied sous vide treatments achieved sufficient inactivation of *L. monocytogenes* from chicken breast. Previous studies have also reported successful inactivation of *L. monocytogenes* and other vegetative microorganisms by sous vide treatments (Karyotis et. al. 2017; Peck et. al. 2006). On the other hand, both one-step and two-step sous vide treatments were insufficient to pasteurize chicken breast for *C. botulinum* spores as the pasteurization values (P_{80}^{13}) were lower than 26 min (SVAC, 1991). This poses a potential safety risk, thus it requires proper storage at temperatures lower than 3 °C or at freezing temperatures to prevent the spore multiplication of *C. botulinum* (Peck et. al. 2006).

Clostridium perfringens is a mesophilic spore-forming bacteria usually used as an indicator of thermal efficiency in sous vide treatments. From the thermal inactivation data of *C. perfringens* (D_{60} = 5.3 min and z-value = 6.74 °C) (ECFF, 2006), meat need to be pasteurized with a heating process equivalent to 60 °C for 32 min to achieve a 6-log vegetative cell reduction. From our results, it can be seen that all the studied sous vide treatments achieved the minimum pasteurization value ($P_{60}^{6.74}$= 32 min).

4.1.6. Microbiological analysis

The thermal inactivation of *Enterococcus faecalis* NCAIM B. 01312 on chicken breast after one-step and two-step sous vide treatments are presented in Figure 27 and Figure 28. According to our results, the thermal resistance of *Enterococcus faecalis* NCAIM B. 01312 on sous vide treated chicken breast was higher in two-step sous vide treatments compared to one-step treatments within the same total treatment time (120 and 180 min). Both one-step sous vide treatments (T1 and T8) were enough to inactivate the initial count of 6.85 ± 0.56 log CFU/ g of *Enterococcus faecalis* NCAIM B. 01312 inoculated in raw chicken breast during challenge test. On the other hand, in two-step sous vide treated chicken breast *Enterococcus faecalis* were countable even after treatments T9 and T12 which had the longest cooking time (120 min) at the end step temperature of 60 °C, resulting in 4.34 ± 0.15 and 3.34 ± 0.11 log reduction, respectively (Figure 28). Previous studies have also reported higher thermal resistance of *Enterococcus* species compared to other pathogens such as *Listeria monocytogenes*, *Salmonella enterica*, *Escherichia coli* O157:H7 (Kharel et. al. 2018; Dhowlaghar et. al. 2021). Due to their higher thermal resistance, Enterococci species have been proposed to serve as surrogate microorganisms for *Salmonella, E. coli* O157:H7 and *L. monocytogenes* to validate the heat treatments used in food industry (Shah et. al. 2017).

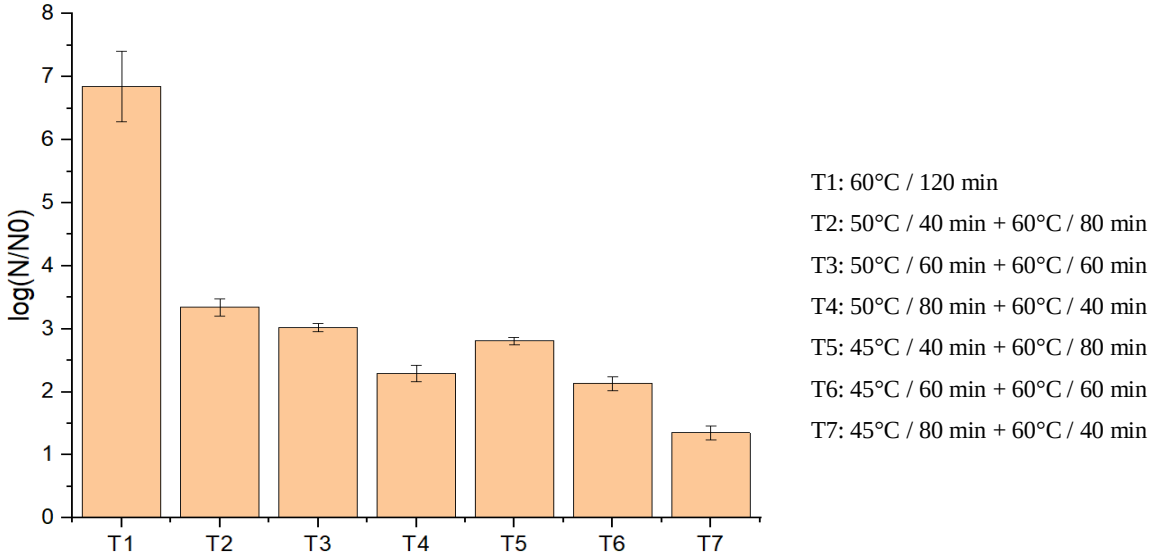


Figure 27: The effect of 120 min sous vide treatments on log inactivation of *Enterococcus faecalis* NCAIM B. 01312 in chicken breast.

The lowest inactivation value of *Enterococcus faecalis* NCAIM B. 01312 was obtained in 120 min two-step sous vide treatment T7, which resulted in 1.35 ± 0.11 log reduction. Meanwhile, the highest log inactivation (4.34 ± 0.15) of *Enterococcus faecalis* NCAIM B. 01312 was obtained in two-step sous vide treatment T9. In 120 min two-step sous vide treated chicken breast the log inactivation was

decreased with higher application time of the first step temperature of 50°C (T2-T4 treatments) and 45°C (T5-T7 treatments) in sous vide cooking (Figure 27). Similar results were observed in 180 min two-step sous vide treated chicken breast when the log inactivation's were decreased from 4.34 to 3.38 and from 3.34 to 1.37 with higher application time of the first step temperature of 50 °C (T9-T11) and 45 °C (T12-T14) respectively, in sous vide cooking (Figure 28).

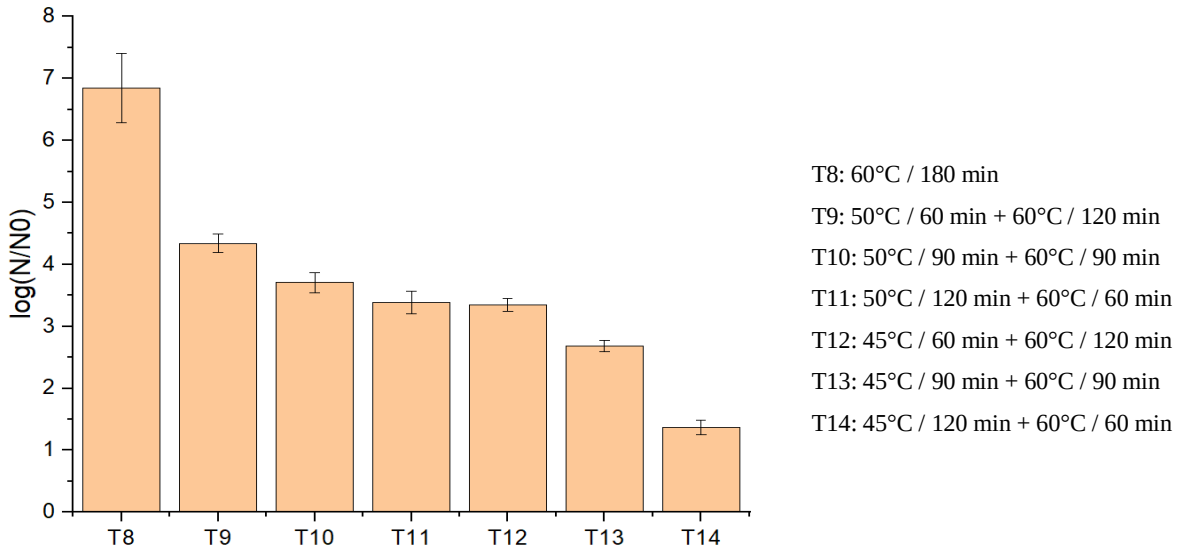


Figure 28: The effect of 180 min sous vide treatments on log inactivation of *Enterococcus faecalis* NCAIM B. 01312 in chicken breast.

For pasteurization of food product, 6 log reduction of the pathogenic bacteria is defined as a pasteurization performance criterion (NACMCF, 2006). Based on our results, only the one-step sous vide treatments (T1 and T8) achieved this criterion by complete inactivation of 6.85 log CFU/ g of *Enterococcus faecalis* NCAIM B. 01312 inoculated in chicken breast before treatments (Figure 27 and Figure 28). However, Enterococcus counts in poultry (chicken and turkey) have been shown to be in the range from 1 to 3 log CFU/g (Miranda et. al. 2007). Therefore, a pasteurization target criterion of sous vide cooking treatment will be 3-log reduction of *Enterococcus faecalis* in chicken breast. From the studied two-step sous vide treatments, this criterion was achieved in 120 min treatments (T2, T3) and 180 min treatments (T9, T10, T11, T12).

4.1.7. Sensory analysis

For consumers 120 min two-step sous vide treatments T2 and T3 would be more convenient for chicken breast cooking due to shorter treatment time compared to 180 min treatments. Therefore 120 min two-step sous vide treated chicken breast T2 and T3 that achieved sufficient inactivation of

Enterococcus faecalis were tested on different sensory attributes including flavour, color, tenderness and juiciness, and compared to one-step sous vide treated ones T1. In general, shorter sous vide treatments would be more important as well for the catering industry and restaurants. However, this would impose increasing temperature in sous vide cooking which may cause different changes in meat proteins structure that can lead to undesirable changes in the quality and sensory attributes of meat (lower tenderness and juiciness, color modification, etc.). Therefore, future studies need to be done to further test mild heat treatments such as two-step sous vide processing to optimize the parameters of temperature and time to achieve high quality and safety of meat products.

The effects of three selected sous vide treatments (T1, T2 and T3) on different sensory quality characteristics of chicken breast are presented in Table 11. Our results showed that sensory attributes of sous vide cooked chicken breast improved with longer application time of low temperature (50 °C) in two-step sous vide treatment, except for flavour. Similarly, Bıyıklı et. al. (2020) reported higher sensory rating scores for sous vide cooked turkey cutlet at low temperatures than those cooked at higher temperatures. On contrary, in flavour results of sous vide cooked chicken breast, it was observed that longer application time of 60°C in sous vide treatments gave higher flavour scores.

Table 11: Effect of sous vide treatments on sensory quality characteristics of chicken breast.

Time	**120 min**		
Treatments	**T1**	**T2**	**T3**
	60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Flavour[1]	7.27 ± 0.79b	6.18 ± 0.75a	6.09 ± 0.94a
Tenderness[2]	6.36 ± 1.29a	7.55 ± 0.93b	7.64 ± 0.81b
Juiciness[3]	5.64 ± 1.03a	6.82 ± 0.98b	7.09 ± 1.14b
Color[4]	5.91 ± 0.75a	6.72 ± 0.9 b	6.64 ± 0.51ab
Overall acceptability[4]	6.09 ± 1.14a	7.18 ± 0.87b	7.46 ± 0.93b

Mean values with different letters (a-c) in the same row are significantly different according to Tukey's post hoc test ($P < 0.05$).

[1]Scale: 1 = very weak, 9 = very strong

[2]Scale: 1 = very tough, 9 = very tender

[3]Scale: 1 = not juicy, 9 = extremely juicy

[4]Scale: 1 = dislike extremely, 9 = like extremely

One-step sous vide treated chicken breast (T1) had significantly higher flavour scores than two-step sous vide ones ($P < 0.05$). Similarly, Park et. al. (2020) reported higher flavour intensity scores for chicken breast cooked at 60°C for 120 min than those cooked for 60 min. On the other hand, tenderness scores were significantly higher in two-step sous vide cooked chicken breast (T2 and T3)

than in one-step sous vide ones (T1) ($P < 0.05$) (Table 11). Apparently, the inclusion of proteolytic enzyme activation temperature of 50°C in two-step sous vide treatment had an impact on increased tenderness scores of cooked chicken breast.

Two-step sous vide treatments (T2 and T3) scored significantly higher for juiciness compared to one-step sous vide one (T1) ($P < 0.05$) (Table 11). The highest score for juiciness was observed in T3 sous vide treated chicken breast (7.09 ± 1.14) which was not significantly different with T2 (6.82 ± 0.98) ($P > 0.05$). Our results shows that the shorter application time of the high temperature (60 °C) in sous vide treatment produce cooked chicken breast with higher juiciness. Similarly, Park et. al. (2020) reported higher juiciness scores for chicken breast cooked at 60°C for 60 min compared to chicken breast cooked for 120 and 180 min.

Two-step sous vide treated chicken breast (T2) had significantly higher color liking scores compared to one-step sous vide ones (T1) (6.72 vs. 5.91, $P < 0.05$). However no significant differences on color scores were observed between treatments T2 and T3, or T1 and T3 ($P > 0.05$). In the end despite lower flavor scores, two-step sous vide cooked chicken breasts (T2 and T3) scored higher in overall acceptability compared to one-step sous vide ones (T1).

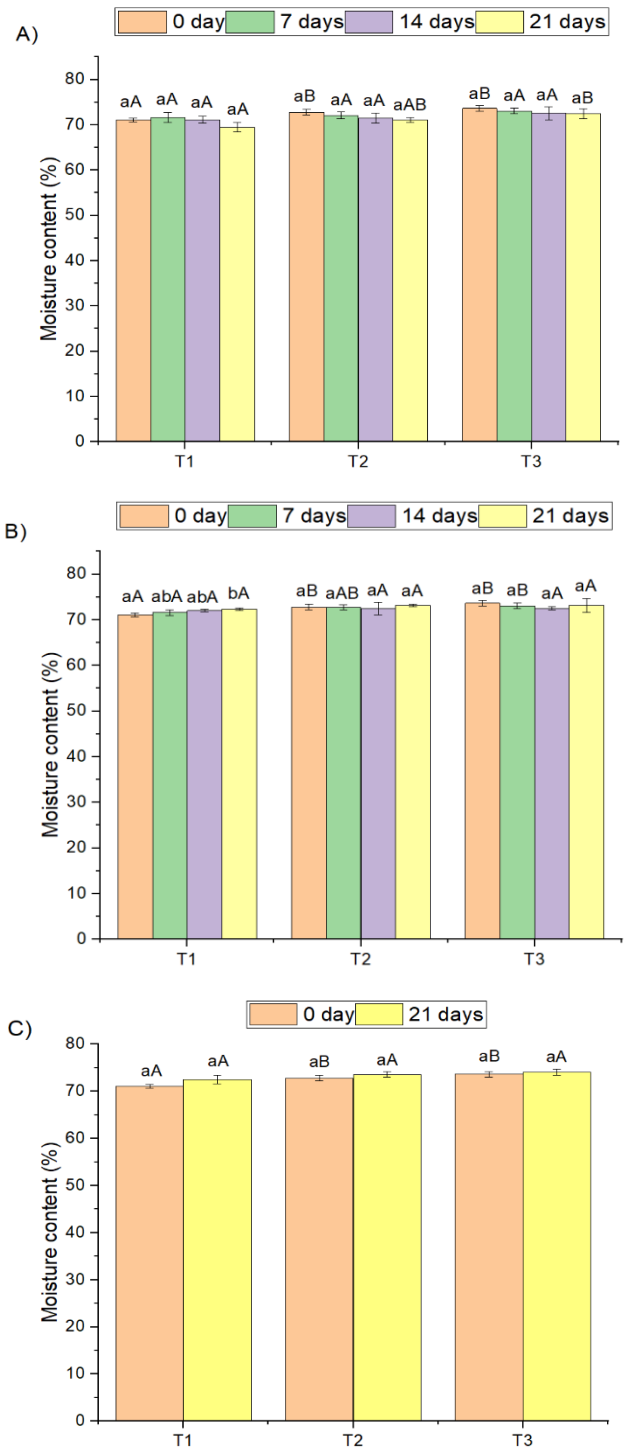
4.2. STORAGE STABILITY OF ONE AND TWO-STEP SOUS VIDE TREATED CHICKEN BREAST

4.2.1. Physicochemical attributes

4.2.1.1. Moisture content and cooking loss

The results of moisture content of sous vide treated chicken breasts stored at different storage temperatures are presented in Figure 29. According to the present study results, moisture content values of sous vide treated chicken breast during storage at 4 °C had a decreasing trend but did not significantly change between sampling days 0 to 21 days ($P > 0.05$). Similar result was observed in samples stored at 10 °C with the exception of T1 sous vide treatment which increased significantly after 21 days (Figure 29: B). Similarly, Hong et. al. (2015) reported no significant difference on moisture content during chilled storage at 4 °C for 14 days. Roldan et. al. (2015) reported that moisture content of sous vide cooked lamb loins did not significantly change during refrigerated conditions (2 °C) for 30 days. In the present study, moisture content values of sous vide treated chicken breast during storage were between 69.47 and 73.66 %, which are higher than moisture content levels of cooked chicken breast reported by other studies (Kim et. al. 2015; Sampaio et. al. 2012). According to our results, moisture content of sous vide treated chicken breast had a non-significant increase after frozen storage at -20 °C for 21 days (Figure 29, C). On contrary, Ji et. al. (2019) reported that 30 days of freezing had a significant effect on increasing the moisture content of sous vide treated pork meat. Moreover, moisture content of two-step sous vide treated chicken breasts (T2 and T3) was not significantly higher than one-step sous vide ones (T1) after frozen storage at -20 °C ($P < 0.05$) (Figure, 29, C).

The results of cooking loss of sous vide treated chicken breasts stored at different storage temperatures are presented in Figure 30. Cooking losses of sous vide treated chicken breast during 21 days of storage were between 11.98 and 17.65 %. Cooking loss of both one-step (T1) and two-step (T2 and T3) sous vide treated chicken breast were significantly increased after 7 days of storage at 4°C or at 10 °C (Figure 30, A and B). However, there were no significant difference between cooking loss values of sous vide treated chicken breast between 7, 14 and 21 days of storage at 4°C or at 10 °C ($P > 0.05$). Similarly, Hong et. al. (2015) reported significant increase in cooking loss after 5 days of chilled storage of sous vide cooked chicken breast. Two-step sous vide treated chicken breast had significant lower cooking loss compared to one-step sous vide treated ones in all storage days of 4°C temperature ($P < 0.05$). Similar result was observed during storage at 10 °C of sous vide treated chicken breast (Figure 30, B).



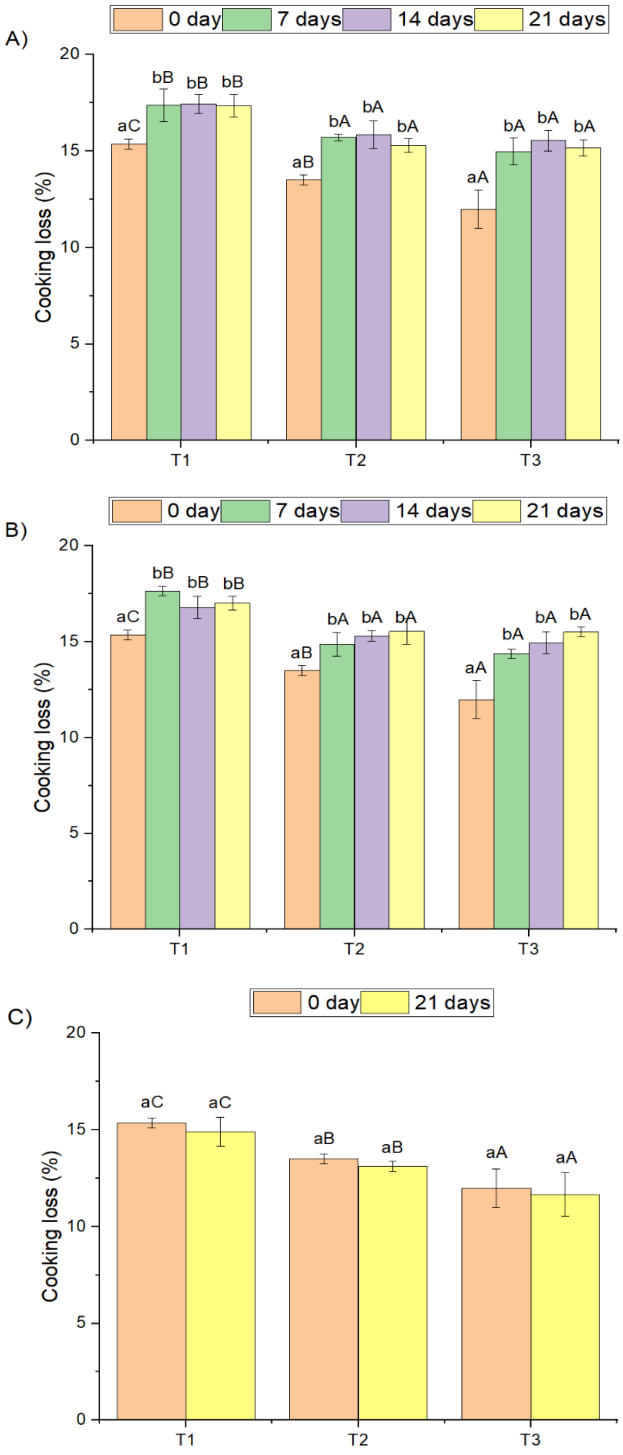
T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

∗a,b,c means with different letters are significantly different for storage days. ∗A, B, C means with different letters are significantly different for treatment type ($P < 0.05$).

Figure 29: Moisture content (%) of sous vide treated chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.



T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

$*^{a,b,c}$ means with different letters are significantly different for storage days. $*^{A, B, C}$ means with different letters are significantly different for treatment type ($P < 0.05$).

Figure 30: Cooking loss (%) of sous vide treated chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.

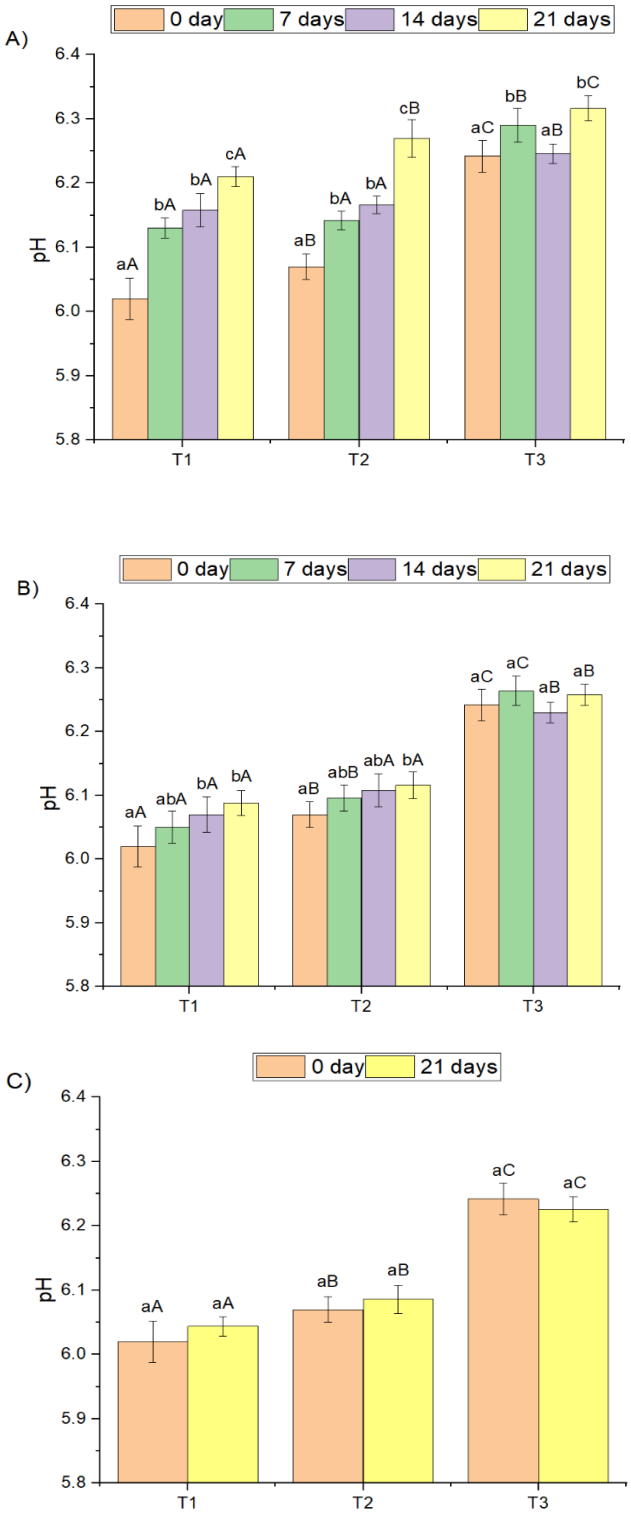
On the other hand, no significant differences were observed in cooking loss of sous vide treated chicken breast between 0 and 21 days of frozen storage at - 20°C (Figure 30, C). This presents a positive effect of freezing on maintaining the cooking loss values of sous vide treated chicken breast similar for 21 days storage which may result in improvement of the eating quality of sous vide treated chicken (increased juiciness). Moreover, one-step sous vide cooked chicken breast (T1) had significantly higher cooking loss compared to two-step treated ones (T2 and T3) after frozen storage at – 20 °C ($P < 0.05$).

4.2.1.2. pH values

The pH values of sous vide treated chicken breasts showed significant changes for 21 days storage period (Figure 31). During storage at 4 °C pH values of sous vide treated chicken breast were increased significantly between 0 and 7 days. Significant increase of pH values was observed also between 7 and 21 days of storage at 4°C with the exception of T3 treatment ($P < 0.05$). Hong et. al. (2015) reported significant pH increase of sous vide cooked chicken breast only after 21 days of chilled storage. Cosansu et. al. (2013) reported steady increase of pH values of sous vide cooked whiting fish during 42 days of chilled storage at 4 °C. One-step sous vide treated chicken breast T1 showed significantly lower pH values compared to two-step sous vide treated ones T3 in all sampling days of storage at 4°C ($P < 0.05$).

The highest pH value was observed in two-step sous vide treatment (T3) at 21 days of storage at 4 °C where pH value was equal to 6.32 ± 0.02 and at 7 and 21 days of storage at 10 °C where pH value was equal to 6.26 ± 0.02. No significant difference on pH values of sous vide cooked chicken breast was observed between 0 and 21 days of storage at 10 °C ($P > 0.05$). Meanwhile significant differences on pH values were observed only in treatment T1 between 0 and 14 days and in treatment T2 between 0 and 21 days of storage at 10 °C ($P < 0.05$) (Figure 31, B). On contrary, Akoğlu et. al. (2018) reported no significant increase between pH values of sous vide cooked chicken during 21 days of storage at 12 °C.

There was no significant effect of freezing at -20 °C on pH values of sous vide cooked chicken breast between 0 and 21 days of storage ($P > 0.05$) (Figure 31, C). On the other hand, two-step sous vide cooked chicken breast (T2 and T3) had significantly higher pH values compared to one-step ones (T1) both before and after frozen storage at -20 °C ($P < 0.05$). This shows that freezing has a positive effect on maintaining pH values of sous vide cooked meat during storage.



T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

*a,b,c means with different letters are significantly different for storage days. *A, B, C means with different letters are significantly different for treatment type ($P < 0.05$).

Figure 31: pH values of sous vide treated chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.

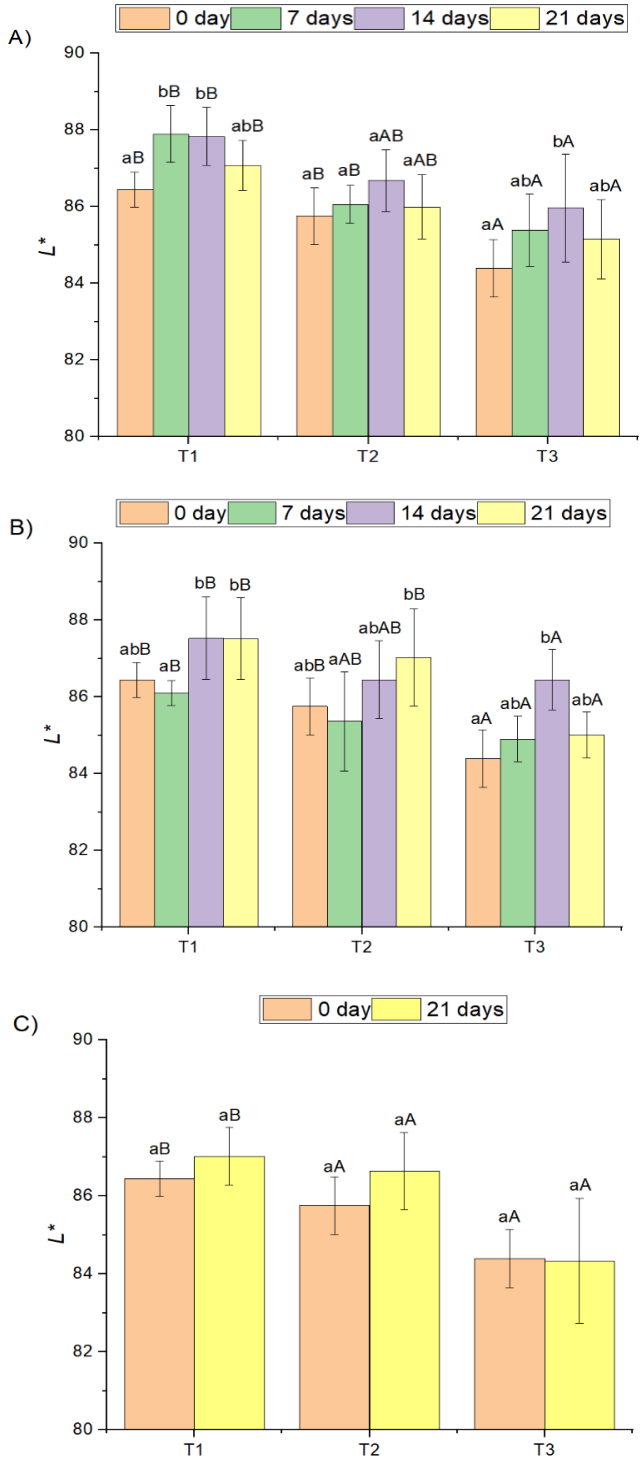
4.2.1.3. Color attributes

Color attributes of sous vide treated chicken breast during storage at different temperatures are presented in Figure 32, 33 and 34. Lightness values (*L**) of sous vide treated chicken breast changed significantly after 7 days (T1 treatment) and 14 days (T3 treatment) of storage at 4°C ($P < 0.05$) (Figure 32). Similarly, Kim et. al. (2015) reported significantly higher lightness values (*L**) of sous vide treated broiler breast during 14 days at 4° C. On contrary, Akoğlu et. al. (2018) reported no significant difference on lightness values of sous vide treated turkey cutlet during storage at 4°C for 35 days and at 12°C for 21 days.

On the other hand, lightness values (*L**) of one-step sous vide treated chicken breast (T1) were significantly higher than those treated with the two-step sous vide treated ones (T3 treatment) during storage at chilled temperature of 4°C ($P < 0.05$). Similar result was observed when sous vide treated chicken breast was stored at 10 °C (Figure 32, B). These results are in agreement with the outcome of our previous study studying the effect of two-step sous vide treatment on color attributes (*L**, *a**, *b**) of chicken breast (Hasani et. al. 2022). According to the results presented in Figure 32: C, frozen storage for 21 days at -20 °C did not have a significant effect on lightness values of sous vide treated chicken breast ($P > 0.05$). Lightness value (*L**) was significantly higher in one-step sous vide treated chicken breast (T1) compared to two-step sous vide ones (T2 and T3) before and after 21 days of frozen storage ($P < 0.05$).

In general, redness values (a* values) of sous vide treated chicken breast did not significantly changed during storage at 4 °C and 10 °C, except for T1 treatment (Figure 33, A and B). Similarly, Roldán et. al. (2015) reported no significant effect of 30 days storage at 2 °C on redness of sous vide cooked lamb loins. Redness of one-step sous vide treated chicken breast (T1 treatment) were significantly increased after 21 days of storage at 4°C and after 7 days of storage at 10 °C. Akoğlu et. al. (2018) reported that redness values of sous vide treated turkey cutlet were significantly increased after 3 days at 4°C and after 9 days at 12 °C.

According to the results in Figure 33: C, frozen storage at -20 °C for 21 days did not have a significant effect on the redness of sous vide cooked chicken breast. Redness values of sous vide cooked chicken breast varied from 1.89 to 3.10. During all the used storage temperatures, none of the studied sous vide treatments did not exceed the pink color subjective threshold of a*=3.8 (Holownia et. al. 2003). From the consumer perspective this is very important as pink color plays a key factor to purchase poultry meat products. Therefore, chicken breasts cooked with the two-step sous vide technique can be considered acceptable for consumers regarding color attributes.



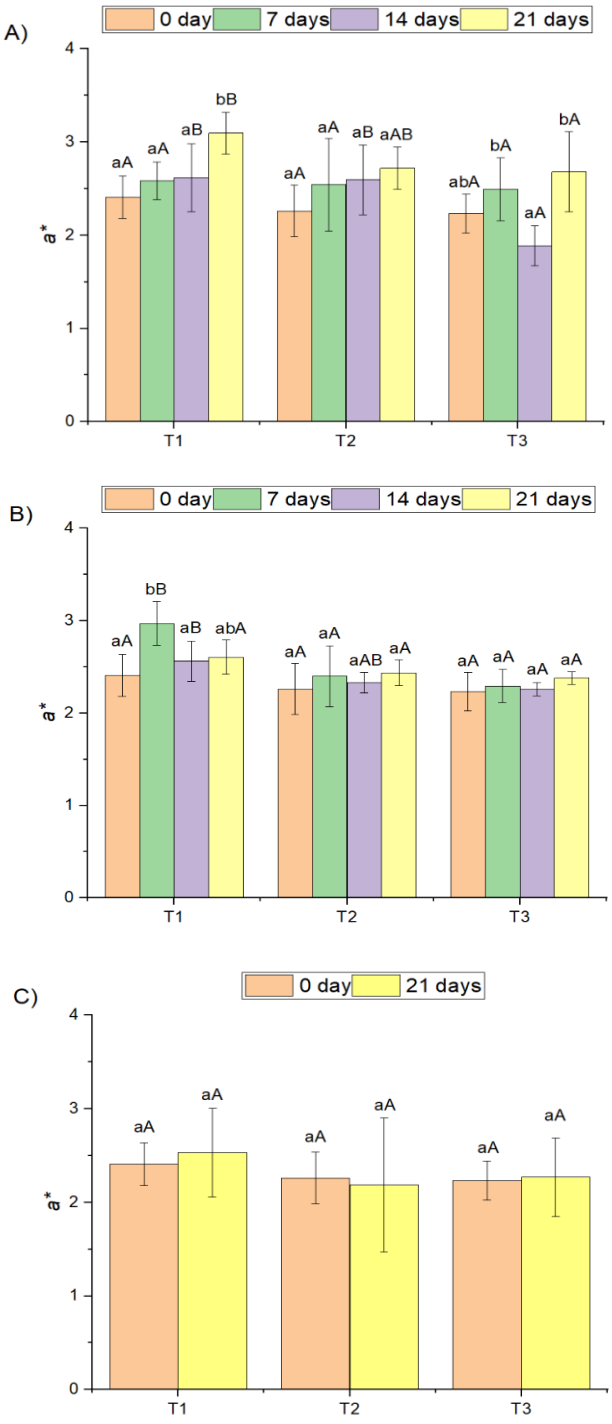
T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

*a,b,c means with different letters are significantly different for storage days. *A, B, C means with different letters are significantly different for treatment type ($P < 0.05$).

Figure 32: Lightness values of sous vide chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.



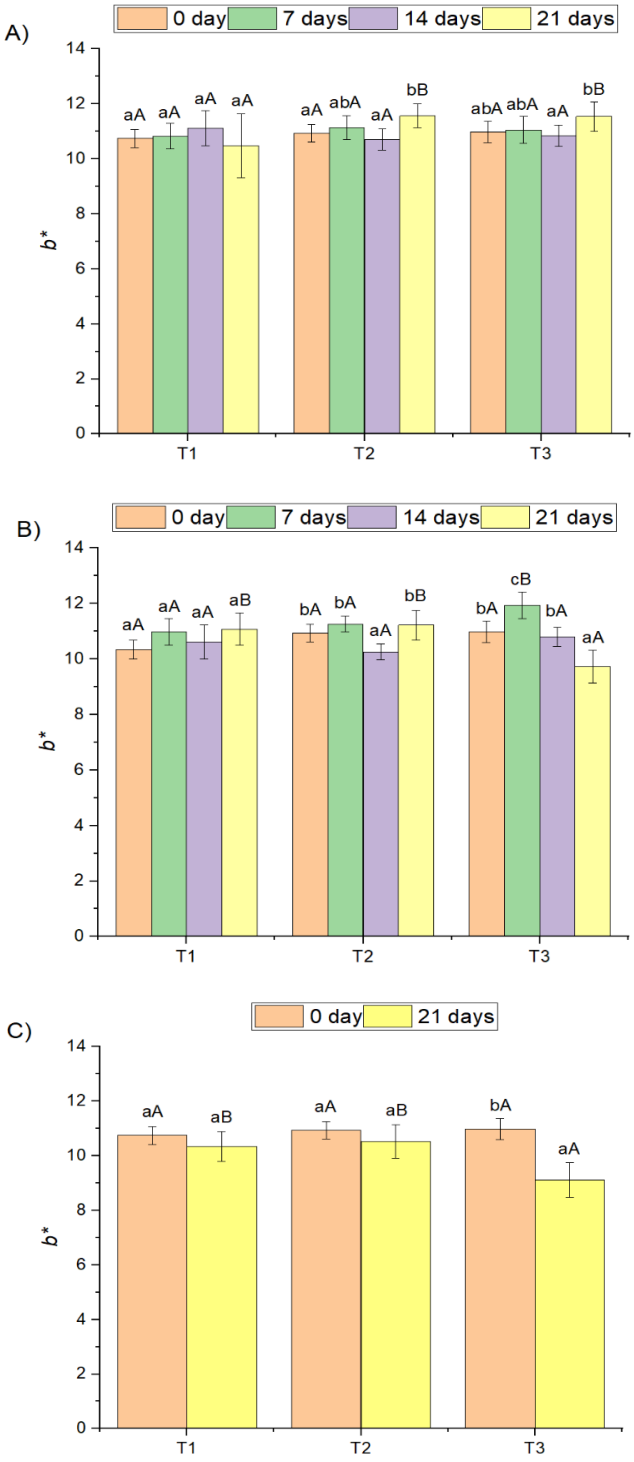
T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

*a,b,c means with different letters are significantly different for storage days. *A, B, C means with different letters are significantly different for treatment type ($P < 0.05$).

Figure 33: Redness values of sous vide treated chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.



T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

∗a,b,c means with different letters are significantly different for storage days. ∗A, B, C means with different letters are significantly different for treatment type ($P < 0.05$).

Figure 34: Yellowness values of sous vide treated chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.

Yellowness values (*b** value) of all sous vide cooked chicken breasts were significantly increased after 21 days of storage at temperature of 4 °C with the exception of T1 treatment ($P < 0.05$) (Figure 34: C). Previous studies have also reported that yellowness values of sous vide cooked turkey meat and chicken breast were significantly increased during storage at 4 °C (Akoğlu et. al. 2018; Roldán et. al. 2013). On the other hand, no significant differences on yellowness values (*b** value) of cooked chicken breast were observed during storage at temperatures of 10 °C and – 20 °C with the exception of T3 treatment (Figure 34, B and C). On contrary, in two-step sous vide treatment T3 yellowness values of chicken breast were significantly reduced after 21 days of storage at 10 °C and frozen storage at – 20 °C.

4.2.2. Protein solubility

Protein solubility properties of sous vide cooked chicken breasts stored at 4 °C, 10 °C and -20 °C are presented in Table 12, 13 and 14. According to the results, sarcoplasmic protein solubility of all the studied sous vide treated chicken breasts was not significantly changed during 21 days of storage at temperature of 4 °C ($P > 0.05$) (Table 12). Similarly, myofibrillar protein solubility of all the studied sous vide treated chicken breast was not significantly changed during 21 days of storage at temperature of 10 °C ($P > 0.05$) with the exception of T3 treatment. Meanwhile, total protein solubility was significantly increased only in the two-step sous vide treated chicken breast (T2 and T3 treatments) after 21 and 14 days of storage at 4°C, respectively ($P < 0.05$). Increased protein solubility property may be explained by the reduced protein denaturation and degradation processes in meat at the end of storage (Van Laack et al., 2000).

On the other hand, sarcoplasmic protein solubility and total protein solubility of two-step sous vide cooked chicken breast (T2 and T3 treatments) were significantly higher than one-step sous vide ones (T1 treatment) during 21 days of storage at 4 °C ($P < 0.05$). This result agrees with the previous study outcomes regarding the effect of heat treatment on protein solubility properties of chicken breast (Hasani et. al. 2022). Similarly, two-step sous vide treated chicken breast (T3 treatment) had significantly higher myofibrillar protein solubility than one-step ones (T1 treatment) only on 14 and 21 days of storage at 4°C. According to the results presented in Table 13, there was no significant effect of storage at 10 °C on sarcoplasmic protein solubility (%) on all the sous vide cooked chicken breast ($P > 0.05$). On contrary, after 21 days of storage at 10 °C myofibrillar protein solubility (%) was significantly increased from 4.09 ± 0.04 to 4.25 ± 0.05 in T1 one-step sous vide treatment and from 4.14 ± 0.12 to 4.5 ± 0.1 in T3 two-step sous vide treatment.

Table 12: Means ± standard deviations of protein solubility of sous vide cooked chicken breast (pectoralis major) during storage at 4 °C.

	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Sarcoplasmic protein solubility (%)	0	2.75 ± 0.09 aA	3.15 ± 0.13 aB	3.34 ± 0.16 aB
	7	2.81 ± 0.15 aA	3.26 ± 0.16 aB	3.43 ± 0.18 aB
	14	2.92 ± 0.1 aA	3.36 ± 0.14 aB	3.61 ± 0.15 aB
	21	2.85 ± 0.11 aA	3.47 ± 0.07 aB	3.71 ± 0.25 aB
Myofibrillar protein solubility (%)	0	4.09 ± 0.04 aA	4.18 ± 0.02 aA	4.14 ± 0.12 aA
	7	4.02 ± 0.07 aA	4.06 ± 0.07 aA	4.2 ± 0.09 aA
	14	3.98 ± 0.07 aA	4.16 ± 0.1 aA	4.4 ± 0.09 abB
	21	4.03 ± 0.05 aA	4.1 ± 0.08 aA	4.51 ± 0.15bB
Total protein solubility (%)	0	6.84 ± 0.1 aA	7.33 ± 0.12abB	7.47 ± 0.05aB
	7	6.82 ± 0.09 aA	7.32 ± 0.1aB	7.63 ± 0.12aC
	14	6.9 ± 0.16 aA	7.52 ± 0.04 abB	8.01 ± 0.09bC
	21	6.88 ± 0.13 aA	7.56 ± 0.1bB	8.21 ± 0.11bC

*a,b means with different letters in the same column are significantly different for storage days.
*A, B, C means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

On the other hand, no significant effect of storage at 10 °C was observed in total protein solubility of sous vide cooked chicken breast with the exception of T3 treatment ($P < 0.05$) (Table 13). Sarcoplasmic and total protein solubility of two-step sous vide cooked chicken breast (T2 and T3) were significantly higher than one-step sous vide ones (T1) during 21 days of storage at 10 °C ($P < 0.05$). Total protein solubility of sous vide treated chicken breast (T3) was significantly increased only after 14 days of storage at 10 °C. The increased total protein solubility in the end of storage can be related to the reduced activity residual proteases on protein degradation and the reduced rate of chemical reactions and protein denaturation in meat (Sun et. al. 2002).

Table 13: Means ± standard deviations of protein solubility of sous vide cooked chicken breast (pectoralis major) during storage at 10 °C.

	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Sarcoplasmic protein solubility (%)	0	2.75 ± 0.09 aA	3.15 ± 0.13 aB	3.34 ± 0.16 aB
	7	2.77 ± 0.07 aA	3.21 ± 0.07 aB	3.41 ± 0.12 aB
	14	2.84 ± 0.05 aA	3.22 ± 0.11 aB	3.61 ± 0.12 aC
	21	2.73 ± 0.11 aA	3.3 ± 0.05 aB	3.62 ± 0.18 aC
Myofibrillar protein solubility (%)	0	4.09 ± 0.04 aA	4.18 ± 0.02 aA	4.14 ± 0.12 aA
	7	4.10 ± 0.03 aA	4.21 ± 0.01 aA	4.14 ± 0.08 aA
	14	4.09 ± 0.04 aA	4.19 ± 0.06 aA	4.32 ± 0.04 abA
	21	4.25 ± 0.05 bA	4.2 ± 0.1 aA	4.5 ± 0.1 bB
Total protein solubility (%)	0	6.84 ± 0.1 aA	7.33 ± 0.12 Ab	7.47 ± 0.05 aB
	7	6.86 ± 0.07 aA	7.42 ± 0.07 aB	7.56 ± 0.06 aB
	14	6.93 ± 0.08 aA	7.41 ± 0.12 aB	7.93 ± 0.09 bC
	21	6.98 ± 0.06 aA	7.5 ± 0.07 aB	8.12 ± 0.09 Cc

*a,b,c means with different letters in the same column are significantly different for storage days.

*A, B, C means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

According to the results presented in Table 14, there was no significant effect of frozen storage at -20 °C on sarcoplasmic and total protein solubility on all the sous vide cooked chicken breasts ($P > 0.05$). Similarly, myofibrillar protein solubility was not significantly changed during 21 days of frozen storage with the exception of T2 treatment. These results show a positive effect of freezing at -20 °C on maintaining the protein solubility properties of sous vide cooked chicken breasts stable during 21 days of storage. The protein solubility properties showed significant correlation with cooking loss of sous vide cooked chicken breast, in accordance with the findings of Murphy and Marks, (2000).

Table 14: Means ± standard deviations of protein solubility of sous vide cooked chicken breast (pectoralis major) during storage at -20 °C.

	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Sarcoplasmic protein solubility (%)	0	2.75 ± 0.09 aA	3.15 ± 0.13 aB	3.34 ± 0.16 aB
	21	2.89 ± 0.12 aA	3.32 ± 0.09 aB	3.57 ± 0.07 aC
Myofibrillar protein solubility (%)	0	4.09 ± 0.04 aA	4.18 ± 0.02 bA	4.14 ± 0.12 aA
	21	3.95 ± 0.19 aA	4.02 ± 0.01 aA	4.0 ± 0.09 aA
Total protein solubility (%)	0	6.84 ± 0.1 aA	7.33 ± 0.12 aB	7.47 ± 0.05 aB
	21	6.83 ± 0.29 aA	7.34 ± 0.1 aB	7.57 ± 0.16 aB

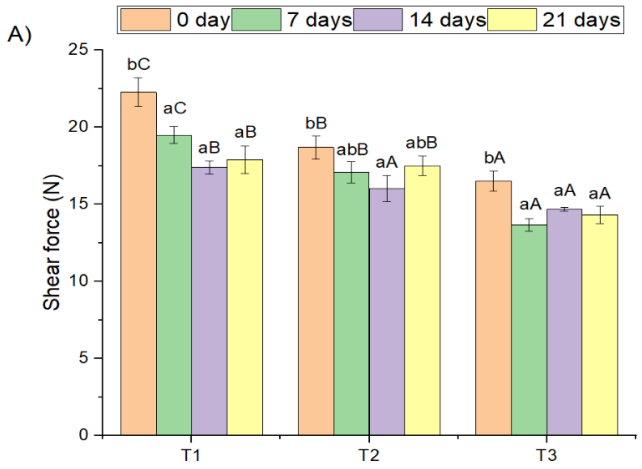
*a,b means with different letters in the same column are significantly different for storage days.

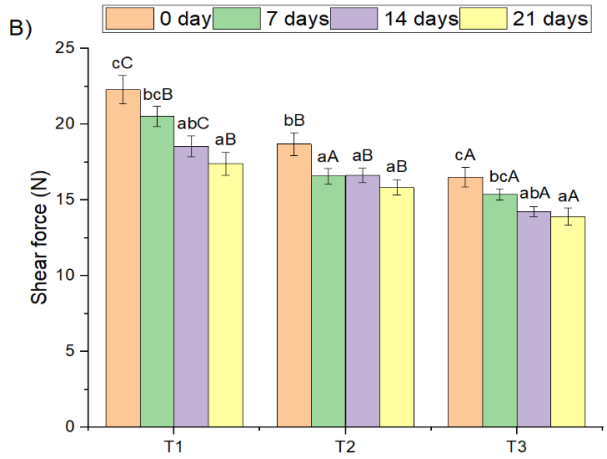
*A, B, C means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

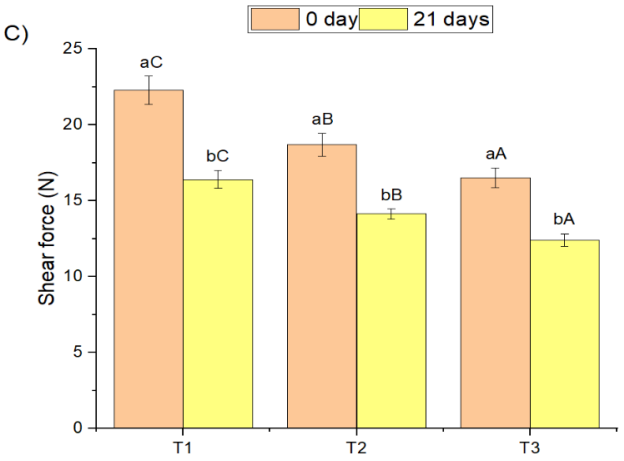
Several authors have analysed the effect of heat treatments on protein solubility properties of sous vide cooked pork and beef meat (Ji et. al. 2019; Bhat et. al. 2020). In our study, sarcoplasmic and total protein solubility of two-step sous vide cooked chicken breasts (T2 and T3 treatments) was significantly higher than one-step sous vide ones (T1 treatment) before and after frozen storage at -20 °C ($P < 0.05$). Similarly, Ismail et. al. (2019) reported higher protein solubility in sous vide cooked beef on which lower temperatures were applied in the treatment. Bhat et. al. (2020) reported that protein solubility in sous vide cooked meat correlates with protein digestibility which is an important attribute particularly for people with sensitive digestive tract system and elderly.

4.2.3. Texture properties

Shear force values (instrumental tenderness) of sous vide treated chicken breast were significantly decreased after 7 days of storage at 4 °C, with the exception of T2 treatment which decreased after 14 days, and then did not significantly change till the end of storage period (Figure 35, A). Similarly, Roldán et. al. (2015) reported a significant decrease of shear force values only in sous vide cooked lamb loins at temperature of 60 °C for 6 h or 24 h after the first week and then steady or slightly higher shear force values till the end of storage period.







T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

*a,b,c means with different letters are significantly different for storage days. *A, B, C means with different letters are significantly different for treatment type ($P < 0.05$).

Figure 35: Shear force of sous vide treated chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.

Hong et. al. (2015) reported significant increase of shear force values of sous vide cooked chicken breast during 14 days of chilled storage at 4 °C. Meanwhile our results showed no significant changes on shear force values between 7-, 14-, and 21-days of storage at 4°C of sous vide cooked chicken breast ($P > 0.05$). On the other hand, shear force values of sous vide cooked sample had a decreasing trend during storage at 10 °C (Figure 35, B). Storage temperature at 10 °C had a significant effect on reducing shear force values of sous vide cooked chicken breast (T1 and T2) after 7 days of storage and after 14 days of storage for T3 treatment respectively ($P < 0.05$). Frozen storage at -20 °C had significantly decreased the shear force values on all the studied sous vide chicken breast ($P < 0.05$) (Figure 35, C). Botinestean et. al. (2016) reported a positive effect of freezing on lowering the shear force values (increasing tenderness) of sous vide cooked beef steaks. Two-step sous vide cooked chicken breast (T2 and T3) remained tender than one-step sous vide ones (T1) having significantly lower shear force values even after 21 days of frozen storage at -20 °C ($P < 0.05$).

From TPA parameters hardness, gumminess and chewiness of sous vide cooked chicken breast were significantly changed during storage at 4 °C ($P < 0.05$), except for T1 for chewiness (Table 15). Hardness values of all the studied sous vide cooked chicken breast were significantly decreased after 7 days of storage at 4°C, followed by a non-significant increasing at day 14 and 21. Similarly, Akoğlu et. al. (2018) reported a significant decrease of hardness of sous vide turkey cutlet after 14 days and then a steady but significant increase to 35 days. Gumminess and chewiness values of sous vide treated chicken breast had similar trends as hardness values during storage at 4 °C, except of T1. Meanwhile no significant changes were observed on cohesiveness and springiness values of all the studied sous vide cooked chicken breasts during 21 days of storage at 4°C ($P > 0.05$). Two-step sous vide cooked chicken breast (T2 and T3) had significantly lower gumminess and chewiness values than one-step sous vide ones (T1) during 21 days of storage at 4°C ($P < 0.05$).

Hardness values of sous vide cooked chicken breast were significantly reduced during storage at 10 °C, except of hardness in T1 treatment ($P < 0.05$) (Table 16). Similarly, gumminess values of sous vide treated chicken breast were significantly decreased during 21 days of storage at 10°C except of T2 treatment. On the other hand, chewiness values were significantly reduced after 14 days of storage at 10 °C in T1 and T2 treatment and after 7 days in T3 treatment. Meanwhile, there were no significant storage day effect at 10 °C on cohesiveness and springiness values of sous vide cooked chicken breast ($P < 0.05$). Apparently, cooked chicken breast become softer during storage at 10 °C because of residual proteases that remain active after heat treatment which activity continue during storage (Diaz et. al. 2008).

Table 15: Means ± standard deviations of texture attributes of sous vide cooked chicken breast (pectoralis major) during storage at 4 °C.

	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Hardness (N)	0	39.28 ± 1.75 bB	35.95 ± 1.22 bA	34.56 ± 1.08 bA
	7	33.68 ± 3.56 aB	28.77 ± 3.82 aAB	27.36 ± 2.36 aA
	14	37.19 ± 2.08 abB	33.64 ± 1.34 abAB	31.74 ± 1.51 abA
	21	38.96 ± 1.89 abA	34.62 ± 3.22 abA	33.83 ± 4.94 abA
Cohesiveness (-)	0	0.28 ± 0.01 aB	0.26 ± 0.01 Aa	0.26 ± 0.01 aAB
	7	0.29 ± 0.03 aB	0.24 ± 0.02 aA	0.23 ± 0.03 aA
	14	0.25 ± 0.03 aA	0.24 ± 0.02 aA	0.24 ± 0.02 aA
	21	0.25 ± 0.01 aB	0.22 ± 0.01 aA	0.22 ± 0.02 aA
Springiness (mm)	0	1.45 ± 0.1 aA	1.45 ± 0.08 aA	1.51 ± 0.04 aA
	7	1.43 ± 0.09 aA	1.42 ± 0.1 aA	1.53 ± 0.05aA
	14	1.49 ± 0.1 aA	1.42 ± 0.03 aA	1.51 ± 0.02 aA
	21	1.47 ± 0.11 aA	1.47 ± 0.09 aA	1.50 ± 0.03 aA
Gumminess (N)	0	11.08 ± 0.76 bB	9.12 ± 0.31 bA	9.02 ± 0.26 bA
	7	9.78 ± 0.93 abB	6.9 ± 1.26 aA	6.18 ± 1.13 aA
	14	9.35 ± 0.68 aB	8.01 ± 0.48 abA	7.52 ± 0.48 abA
	21	9.9 ± 0.39 abB	7.7 ± 0.61 abA	7.37 ± 0.49 abA
Chewiness (N*mm)	0	15.97 ± 1.02 aB	13.22 ± 0.38 bA	13.59 ± 0.7 bA
	7	13.97 ± 0.83 aB	9.7 ± 1.29 aA	9.4 ± 1.5 aA
	14	13.92 ± 0.84 aB	11.41 ± 0.9 abA	11.33 ± 0.63 abA
	21	14.54 ± 1.62 aB	11.3 ± 1.17 abA	11.12 ± 0.93 abA

*a,b means with different letters in the same column are significantly different for storage days.

*A, B means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

Table 16: Means ± standard deviations of texture attributes of sous vide cooked chicken breast (pectoralis major) during storage at 10 °C.

	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Hardness (N)	0	39.28 ± 1.75 aB	35.95 ± 1.22 bcA	34.56 ± 1.08 bA
	7	38.43 ± 0.98 aB	36.98 ± 0.5 cB	32.23 ± 1.83 abA
	14	36.48 ± 4.25 aA	33.68 ± 1.88 abA	29.87 ± 2.22 aA
	21	35.74 ± 0.95 aC	32.68 ± 0.53 aB	29.54 ± 0.78 aA
Cohesiveness (-)	0	0.28 ± 0.01 aB	0.26 ± 0.01 aA	0.26 ± 0.01 bAB
	7	0.27 ± 0.02 aB	0.23 ± 0.01 aA	0.24 ± 0.01 abA
	14	0.27 ± 0.03 aA	0.25 ± 0.03 aA	0.23 ± 0.02 aA
	21	0.25 ± 0.01 aB	0.24 ± 0.01 aB	0.21 ± 0.01 aA
Springiness (mm)	0	1.45 ± 0.1 aA	1.45 ± 0.08 aA	1.51 ± 0.04 aA
	7	1.47 ± 0.11 aA	1.47 ± 0.09 aA	1.51 ± 0.04 aA
	14	1.43 ± 0.08 aA	1.38 ± 0.06 aA	1.51 ± 0.03 aA
	21	1.48 ± 0.1 aA	1.45 ± 0.07 aA	1.5 ± 0.02 aA
Gumminess (N)	0	11.08 ± 0.76 bB	9.12 ± 0.31 aA	9.02 ± 0.26 cA
	7	10.56 ± 0.72 bB	8.6 ± 0.29 aA	7.76 ± 0.42 bA
	14	9.61 ± 0.56 abB	8.43 ± 1.04 aAB	6.81 ± 0.4 aA
	21	8.81 ± 0.26 aC	7.76 ± 0.34 aB	6.3 ± 0.31 aA
Chewiness (N*mm)	0	15.97 ± 1.02 cB	13.22 ± 0.38 bA	13.59 ± 0.7 cA
	7	15.45 ± 0.58 bcB	12.6 ± 0.71 abA	11.68 ± 0.66 bA
	14	13.72 ± 0.44 abB	11.62 ± 0.92 aA	10.27 ± 0.84 abA
	21	13.03 ± 0.65 aC	11.26 ± 0.34 aB	9.43 ± 0.45 aA

*a,b means with different letters in the same column are significantly different for storage days.

*A, B, C means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

Table 17: Means ± standard deviations of texture attributes of sous vide cooked chicken breast (pectoralis major) during frozen storage at -20 °C.

	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Hardness (N)	0	39.28 ± 1.75 aB	35.95 ± 1.22 aA	34.56 ± 1.08 aA
	21	31.71 ± 0.88 bB	26.91 ± 0.49 bA	25.92 ± 0.33 bA
Cohesiveness (-)	0	0.28 ± 0.01 aB	0.26 ± 0.01 aA	0.26 ± 0.01 aAB
	21	0.26 ± 0.01 bB	0.24 ± 0.01 aA	0.24 ± 0.01 aAB
Springiness (mm)	0	1.45 ± 0.1 aA	1.45 ± 0.08 aA	1.51 ± 0.04 aA
	21	1.42 ± 0.03 aA	1.4 ± 0.01 aA	1.42 ± 0.02 bA
Gumminess (N)	0	11.08 ± 0.76 aB	9.12 ± 0.31 aA	9.02 ± 0.26 aA
	21	8.32 ± 0.42 bB	6.46 ± 0.3 bA	6.28 ± 0.11 bA
Chewiness (N*mm)	0	15.97 ± 1.02 aB	13.22 ± 0.38 aA	13.59 ± 0.7 aA
	21	11.77 ± 0.52 bB	9.02 ± 0.39 bA	8.94 ± 0.17 bA

*a,b means with different letters in the same column are significantly different for storage days.

*A, B means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

Hardness, gumminess and chewiness values of sous vide cooked chicken breast were significantly reduced after frozen storage at -20 °C ($P < 0.05$). Similarly, previous studies have reported that freezing technology can improve the texture attributes of sous vide cooked meat mainly shear force, hardness and chewiness (Botinestean et. al. 2016; Ji et. al. 2019). On the other hand, there was no frozen storage effect on cohesiveness and springiness of sous vide cooked chicken breast, with the exception of T1 treatment ($P > 0.05$). Two-step sous vide treated chicken breast (T2 and T3) had significantly reduced hardness, gumminess, and chewiness than one-step sous vide cooked chicken breast (T1) before and after frozen storage at -20 °C ($P < 0.05$) (Table 17).

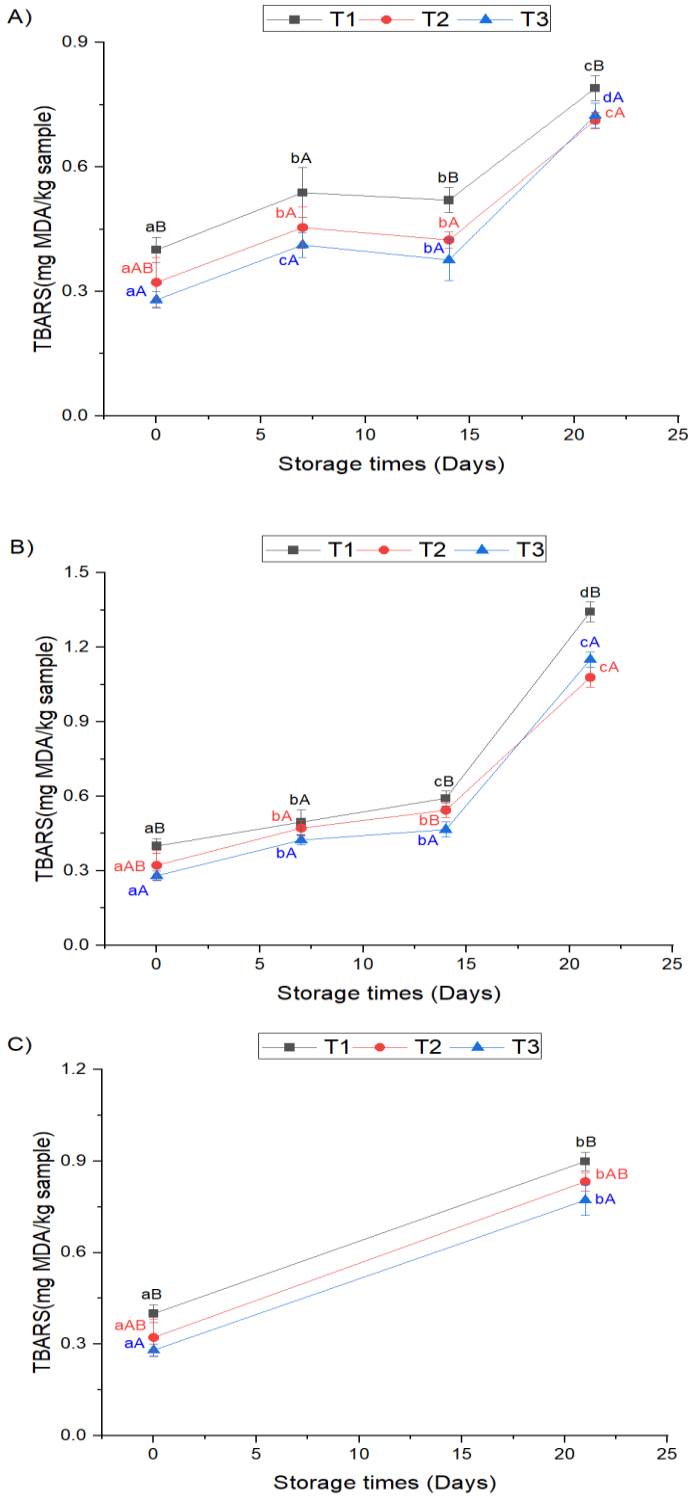
4.2.4. Lipid oxidation

TBARS values measures the amount of secondary lipid oxidation products specifically malonaldehyde which is known to have a major effect on the aroma deterioration of meat products during storage. In the present study, TBARS values of all the studied sous vide treated chicken breast were significantly increased after 7 days of storage at 4 ℃ ($P < 0.05$). Furthermore, significant increase in TBARS values were observed also between 14 and 21 days of storage at 4℃ in all the studied sous vide treatments ($P < 0.05$) (Figure 36: A). According to the study of Hong et.al., (2015), the TBARS values of sous-vide cooked chicken breast were significantly increased after 10 days of chilled storage at 4 ℃. On contrary, Baker et. al. (1972) reported that TBARS values of sous vide treated turkey cutlet were significantly increased after 21 days of storage at 4 ℃.

On the other hand, TBARS values of all the studied sous vide chicken breast were significantly increased after 7 days of storage at 10 ℃ ($P < 0.05$) (Figure 36: B). Akoğlu et. al. (2018) reported a significant increase of lipid oxidation rates (TBARS) of sous vide cooked turkey cutlet after 12 days of storage at 12 ℃.

The highest values of TBARS were noticed in one-step sous vide treated chicken breast (T1 treatment) after 21 days of storage at 10 ℃ with 1.34 ± 0.04 mg MDA/kg of sample. Meanwhile, the lowest TBARS value 0.28 ± 0.02 mg MDA/kg was observed at two-step sous vide treated chicken breast (T3 treatment) at 0 day. It has been reported that the threshold level of TBARS for consumers to detect oxidative rancidity in meat is higher than 1 mg malonaldehyde per kilogram of sample (Baker et al. 1972). Based on our study results, this threshold level was exceeded only in sous vide treated chicken breast after storage at 10 ℃ for 21 days. It can be emphasized that storage at 4 and -20 ℃ were more stable in lipid oxidation compared with storage 10 ℃. Furthermore, low lipid oxidation rates observed in our study can be explained by low level of fat in chicken breast muscles, heat treatment parameters and the effect of vacuum packaging on prevention of lipid oxidation.

TBARS values of one-step sous vide treated chicken breast (T1 treatment) were significantly higher during each sampling days of storage at 10 ℃ ($P < 0.05$). In general, two-step sous vide treated chicken breast (T3 treatment) had significantly lower TBARS values than one-step ones (T1 treatment) at all sampling days ($P < 0.05$) except at 7 days of storage at 4℃ and 10 ℃. At the end of both storage temperatures (4℃ and 10 ℃) TBARS values of two-step sous vide chicken breasts (T2 and T3) were significantly lower than one-step sous vide treated ones (T1) ($P < 0.05$). This can be explained by low oxidation rates of chicken breast after being cooked with the low temperature treatments at 0 day compared to traditional sous vide one.



T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

∗a,b,c means with different letters are significantly different for storage days. ∗A, B, C means with different letters are significantly different for treatment type ($P < 0.05$).

Figure 36: TBARS values (mg MDA/kg sample) of sous vide treated chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.

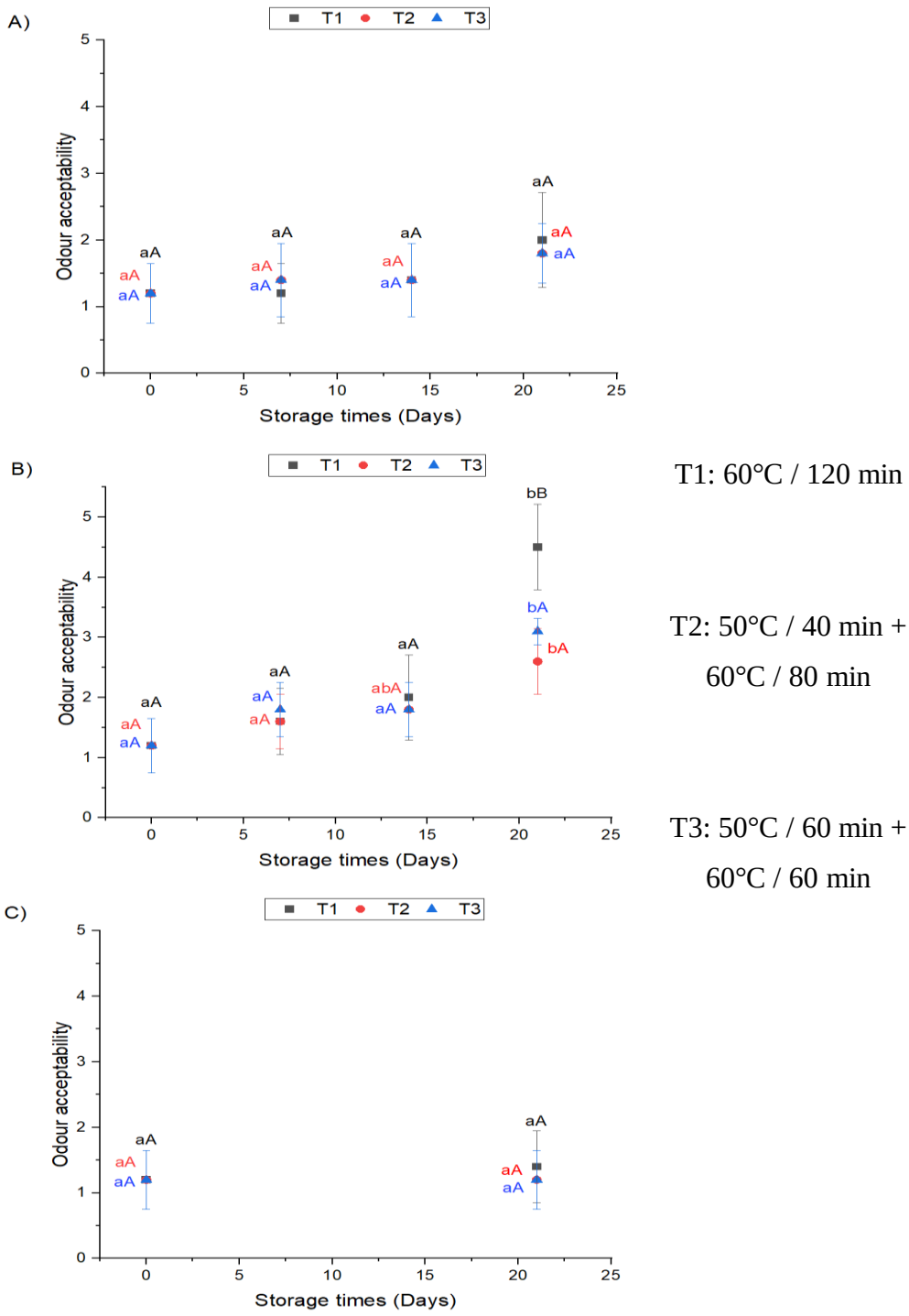
According to the results presented in Figure 36 C, TBARS values of all the studied sous vide treated chicken breast were significantly increased during 21 days of frozen storage at – 20 °C ($P < 0.05$). This finding suggests that freezing is insufficient to prevent lipid oxidation in meat. Water that does not freeze during frozen storage is available for chemical reactions like lipid and protein oxidation. The accumulation of lipid oxidation products in muscle is thought to occur as a result of ice crystal damage to cell membranes and the release of pro-oxidants such as haem iron, which may cause deterioration of color, flavor, and pigment oxidation in meat products (Leygonie et.al., 2012).

On the other hand, significant difference was observed on TBARS values between chicken breasts cooked with T3 treatment and those cooked with T1 treatment before and after frozen storage ($P < 0.05$). Apparently, the increase of oxidation rates was similar during frozen storage for two types of treatments of sous vide chicken breasts. The high oxidative storage stability of two-step sous vide chicken breast compared to one-step sous vide ones is an indicator of a higher quality and shelf life of this product.

4.2.5. Odour acceptability

According to the results presented in Figure 37 A, sous vide cooked chicken breasts had acceptable odor scores during storage at 4 °C except for T1 treatment which resulted slightly acceptable after 21 days with score equal of 2 (Figure 37, A). These results suggests that sensory quality of two-step sous vide cooked chicken breasts might be stable for 21 days of storage at 4 °C.

On the other hand, odor of sous vide treated chicken breast resulted acceptable during 14 days of storage at 10 °C except of one-step sous vide treatment (T1 treatment). After 21 days of storage at 10 °C, two-step sous vide treated chicken breast T2 had slightly acceptable odour with acceptance scores of 2.6. Meanwhile, two-step sous vide treated chicken breast T3 and one-step ones T1 after 21 days of storage at 10 °C had the highest acceptance scores of 3.1 and 4.5, respectively. The unacceptable odor of one-step sous vide treated chicken breast (T1) can be associated with the development of secondary lipid oxidation products during storage at 10 °C which were over the sensorial threshold limit of 1 mg MDA/kg sample (Baker et al. 1972) (Figure 37, B). Furthermore, the sensory quality of cooked meat can be a pre-indicator of microbiological spoilage during storage (Díaz et. al. 2008). Based on the results, odor of all the studied sous vide treated chicken breasts remained acceptable during 21 days of frozen storage at -20 °C with scores lower than 2 (Figure 37, C). There was no significant difference between one-step (T1 treatment) and two-step sous vide cooked chicken breasts (T2 and T3 treatments) during storage at 4 °C, 10 °C and -20 °C, with the exception of 21 sampling day at 10 °C.



T1: 60°C / 120 min

T2: 50°C / 40 min + 60°C / 80 min

T3: 50°C / 60 min + 60°C / 60 min

*a,b,c means with different letters are significantly different for storage days. *A, B, C means with different letters are significantly different for treatment type ($P < 0.05$). The odour acceptability was evaluated using five-point scale: 1 = acceptable, 2 = slightly acceptable, 3 = neither acceptable nor unacceptable, 4 = slightly unacceptable, 5 = unacceptable.

Figure 37: Odour acceptability scores for sous vide treated chicken breasts stored at 4°C (A), 10°C (B) and -20 °C (C) for 21 days.

4.2.6. Microbiological stability

In microbiological storage stability experiment of sous vide treated chicken breasts, around 6.45 ± 0.08 log CFU/g *Enterococcus faecalis* NCAIM B. 01312 were inoculated in raw samples before treatments. Based on our results, one-step sous vide treatment (T1) of chicken breast samples reduced *Enterococcus faecalis* NCAIM B. 01312 counts to non-detectable levels (Table 18). On the other hand, two-step sous vide treated chicken breast (T2 and T3) resulted in higher than 3 log reduction of *Enterococcus faecalis* NCAIM B. 01312, having 3.01 ± 0.07 and 3.37 ± 0.07 log CFU/g respectively at 0 day of storage (Table 18).

The thermal inactivation of T2 and T3 treatments can be considered effective for pasteurization taking into account that initial counts of *Enterococcus faecalis* in raw chicken breast (without inoculation) were around 2.69 ± 0.12 log CFU/g (Table 1). Similarly, Miranda et. al. (2007) reported 1-3 log CFU/g *Enterococcus* counts in raw poultry meat (chicken and turkey). According to the results presented in Table 18, *Enterococcus faecalis* NCAIM B. 01312 counts did not significantly changed during 21 days of storage at 4°C of sous vide treated chicken breast ($P < 0.05$). Similarly, Ingham & Tautorus (1991) reported no significant change in *Enterococcus faecalis* counts in cooked turkey meat for 15 days storage at 3 ± 1 °C.

Table 18: Means ± standard deviations of *Enterococcus faecalis* NCAIM B. 01312 (log CFU/g) of sous vide cooked chicken breast (pectoralis major) during storage at 4 °C.

	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Enterococcus faecalis NCAIM B. 01312 (log CFU/g)	0	ND	3.01 ± 0.07 aA	3.37 ± 0.07 aB
	7	ND	3.07 ± 0.04 aA	3.40 ± 0.02 aB
	14	ND	3.11 ± 0.02 aA	3.39 ± 0.03 aB
	21	ND	3.09 ± 0.02 aA	3.40 ± 0.02 aB

ND – not detected.

*a,b,c means with different letters in the same column are significantly different for storage days.

*A, B means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

The highest *Enterococcus faecalis* NCAIM B. 01312 counts were observed after 21 days of storage at 4°C in T3 sous vide cooked chicken breast with 3.4 ± 0.02 log CFU/g, which if it is subtracted from the inoculated counts before heat treatments gives higher that 3 log reduction of *Enterococcus faecalis* NCAIM B. 01312. This shows that sous vide treated chicken breast were microbiologically stable during 21 days of storage at 4 °C regarding *Enterococcus faecalis* NCAIM B. 01312. Chicken breast cooked with T2 treatment had significantly lower *Enterococcus faecalis* NCAIM B. 01312 compared to chicken breast cooked with T3 treatment in all sampling days of storage at 4°C ($P < 0.05$). This result shows that T2 sous vide treated chicken breast had higher microbiological stability during storage at 4 °C from two-step sous vide treatments.

Enterococcus faecalis NCAIM B. 01312 counts of sous vide cooked chicken breasts had an increasing trend during 21 days of storage at 10 °C (Table 19). A significant increase on *Enterococcus faecalis* NCAIM B. 01312 counts in T2 sous vide treated chicken breast was observed only after 7 days of storage at 10 °C and after 14 days in samples cooked with T3 treatment ($P < 0.05$). At the end of storage at 10 °C, *Enterococcus faecalis* NCAIM B. 01312 in sous vide treated chicken breasts were increased to 6.37 - 6.64 log CFU/g, similar to initial counts inoculated before treatments. This result is expected as *Enterococcus faecalis* has been reported to grow at temperatures as low as 10 °C (Moreno et. al. 2006). From two-step sous vide treatments (T2 and T3), chicken breast treated with treatment T2 had significantly lower *Enterococcus faecalis* NCAIM B. 01312 compared to T3 treatment during 21 days of storage at 10 °C, with the exception of 7 day ($P < 0.05$).

Table 19: Means ± standard deviations of *Enterococcus faecalis* NCAIM B. 01312 counts (log CFU/g) of sous vide cooked chicken breast (pectoralis major) during storage at 10 °C.

Bacteria	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Enterococcus faecalis NCAIM B. 01312 (log CFU/g)	0	ND	3.01 ± 0.07 aA	3.37 ± 0.07 aB
	7	ND	3.24 ± 0.06 bA	3.47 ± 0.03 aB
	14	ND	4.22 ± 0.09 cA	5.08 ± 0.05 bB
	21	ND	6.37 ± 0.03 dA	6.64 ± 0.04 cB

ND – not detected.

*a,b,c,d means with different letters in the same column are significantly different for storage days.

*A, B means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

Table 20: Means ± standard deviations of *Enterococcus faecalis* NCAIM B. 01312 counts (log CFU/g) of sous vide cooked chicken breast (pectoralis major) during storage at -20 °C.

Bacteria	Storage time (d)	Treatments		
		T1	T2	T3
		60°C / 120 min	**50°C / 40 min + 60°C / 80 min**	**50°C / 60 min + 60°C / 60 min**
Enterococcus faecalis NCAIM B. 01312 (log CFU/g)	0	ND	3.01 ± 0.07 bA	3.37 ± 0.07 bB
	21	ND	2.05 ± 0.05 aA	2.91 ± 0.05 aB

ND – not detected.

*a,b means with different letters in the same column are significantly different for storage days.

*A, B means with different letters in the same row are significantly different for treatment type ($P < 0.05$).

Enterococcus faecalis NCAIM B. 01312 counts in sous vide cooked chicken breast were significantly decreased during 21 days of frozen storage at -20 °C ($P < 0.05$) (Table 20). Similarly, Mohammed et. al. (2021) reported a significant decrease on bacteria present in minced chicken meat during frozen storage at -20 °C. From the two-step sous vide treatments, cooked chicken breast (T2) had significantly lower *Enterococcus faecalis* NCAIM B. 01312 counts compared to T3 cooked chicken breast before and after frozen storage at -20 °C ($P < 0.05$). This shows that chicken breasts cooked with the two-step sous vide treatment (T2) had the highest microbiological stability, after the traditional one-step sous vide one (T1 treatment).

4.3. NEW SCIENTIFIC RESULTS

1- I found that 120 min two-step sous vide, which contains either 45°C or 50°C initial temperature for 40 to 80 min (in order to increase proteolytic enzyme activity) and 60°C end step temperature, decreased hardness and gumminess values of chicken breast and had higher yield (lower cooking loss) compared to the 120 min traditional one-step sous vide (only treated at 60°C).

2- I observed that two-step sous vide treatments (which contain either 45°C or 50°C initial temperature and 60°C end step temperature) increases sarcoplasmic and total protein solubility and decrease TBARS values of chicken breast compared to traditional one-step sous vide treatments (only treated at 60°C) within the same total treatment time (120 or 180 min).

3- I found that one-step sous vide treatments 120 and 180 min at 60°C fulfilled the pasteurization performance criteria for a 6-log reduction of pathogenic bacteria *Enterococcus faecalis* NCAIM B. 01312 in chicken breast. Meanwhile, the 120 min two-step sous vide in which the first step temperature of 50°C was performed for 40 to 60 min of the total treatment time achieved the 3-log reduction of the *Enterococcus faecalis* NCAIM B. 01312 defined as target pasteurization performance based on its incidence in chicken meat. Similar outcome was achieved by the 180 min two-step sous vide treatments where the first step temperature 50°C was performed for 60 to 120 min or where 45°C was performed for 60 min of the total treatment time.

4- I observed that lipid oxidation of chicken breasts cooked with 120 min two-step sous vide (treated for 40 or 60 min at 50°C and then at 60°C) and 120 min one-step sous vide (only treated at 60°C) exceeded the sensorial threshold limit of TBARS (1 mg MDA per kilogram of meat) after 21 days of storage at 10 °C.

5- I found that both one-step and two-step sous vide treated chicken breasts were microbiologically stable regarding *Enterococcus faecalis* NCAIM B. 01312 during 21 days of storage at 4 °C and – 20 °C, but not at 10 °C.

5. CONCLUSIONS AND RECOMMENDATIONS

Sous vide mild thermal processing technology have had a growing attention in recent years in the catering sector, households and restaurants as it presents a feasible option to obtain higher yields of meat, improve sensorial characteristics such as juiciness and tenderness, oxidative stability and shelf life of meat products. Different meat proteins have different denaturation temperatures which are responsible for the main quality attributes of meat. Therefore, selection of proper temperature and time in sous vide processing allows to tailor the denaturation of meat proteins in order to achieve desired sensory attributes of cooked meat such as tenderness and juiciness. Endogenous proteolytic enzymes of meat which highest activity is between 40 to 50 °C temperatures have been shown to extent meat tenderization. As a result, application of proteolytic enzyme activation temperatures as first step temperature in the sous vide processing could potentially improve meat tenderness and other quality attributes.

The current study aimed to investigate the effect of sous vide treatments using the one-step temperature of 60 °C and two-step temperatures (45 °C + 60 °C) and (50 °C + 60 °C) applied in different time ratios of the same total treatment times, on physicochemical characteristics, texture attributes, lipid oxidation and protein solubility of chicken breast muscles (pectoralis major). In addition, the pasteurization efficiency of the studied sous vide treatments was examined by calculation of theoretical pasteurization values and microbiological analysis. In the second experiment the storage stability of one-step and two-step sous vide treated chicken breast were investigated during 21 days at 4°C, 10 °C, and -20 °C.

Two-step sous vide technique provides a valuable cooking alternative for elderly consumers as it significantly reduced the main texture parameters (shear force, hardness, chewiness, and gumminess), but at the same time preserved the moisture content, redness, and oxidative stability in chicken breasts. Furthermore, significantly lower cooking loss and increased protein solubility values were observed in chicken breast cooked with the two-step sous vide compared to the ones cooked with the traditional one-step sous vide. Future studies need to be carried out to investigate the possible positive effect of two-step sous vide on protein digestibility of chicken breast which is related to protein solubility.

Two-step sous vide treatments were able to successfully inactivate the vegetative cells of two main pathogens of interest (*C. perfringens* and *L. monocytogenes*) in chicken breast based on the calculated theoretical pasteurization values. However as expected, none of the studied sous vide treatments were enough to inactivate the *C. botulinum* spores, thus proper refrigeration storage of these products is required. On the other hand, microbiological analysis showed that one-step sous vide treatments 120

and 180 min at 60°C successfully inactivated *Enterococcus faecalis* NCAIM B. 01312 in chicken breast. Meanwhile from the studied two-step sous vide treatments only 120 min treatments (T2 and T3) and 180 min treatments (T9, T10, T11, and T12) achieved the target pasteurization performance criterion of 3 log reduction of *Enterococcus faecalis* NCAIM B. 01312 in chicken breast. Regarding sensory attributes, two-step sous vide treated chicken breasts had less flavour but higher tenderness and juiciness than traditional one-step sous vide ones. Future investigations need to be done to examine the effect of different post treatments such as roasting, searing, or frying on sensory attributes of two-step sous vide cooked chicken breast.

In the second experiment, two-step sous vide treated chicken breast exhibited lower cooking loss, gumminess and chewiness, as well as higher sarcoplasmic and total protein solubility compared to one-step sous vide treated ones during storage at 4 °C and 10°C. On the other hand, during frozen storage at –20 °C two-step sous vide cooked chicken breast had lower cooking loss, shear force, gumminess, chewiness and hardness as well as higher lightness, sarcoplasmic and total protein solubility than one-step sous vide treated ones. Our results showed that combination of two-step sous vide treatment and frozen storage provided better quality attributes of cooked chicken breast.

On the other hand, one-step and two-step sous vide treated chicken breast had lipid oxidation rates within the sensorial threshold limit (> 1 mg MDA/kg of sample) during 21 days of storage at 4 °C and -20 °C. On contrary, lipid oxidation rates of all the studied sous vide treated chicken breast exceed the sensorial threshold limit after 21 days of storage at 10 °C, which was supported by higher odor acceptability scores. Regarding microbiological stability, both two-step sous vide cooked chicken breast (T2 and T3) resulted within the criterion limits levels for *Enterococcus faecalis* NCAIM B. 01312 during 21 days of storage at 4 °C and at – 20 °C. On the other hand, only T2 sous vide treated samples were stable for 7 days at 10 °C regarding *Enterococcus faecalis* NCAIM B. 01312. Two-step sous vide treated chicken breast (T2) that remained stable for one week at abusive chilled storage temperature (10 °C) present an example of a proper combination of temperatures and times in the two step sous vide treatment.

6. APPENDIXES

6.1. References

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