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M.Sc. in Food Engineering

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REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

APPLICATION OF SOUS VIDE TECHNOLOGY ON A SEVERAL MEAT PRODUCT; TRIPE

M.Sc. THESIS

IN

FOOD ENGINEERING

BY

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Supervisor

Assoc. Prof. Dr. Kadir Bülent BELİBAĞLI

by

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Mehdiye BULĞAK

ABSTRACT

APPLICATION OF SOUS VIDE TECHNOLOGY ON A SEVERAL MEAT PRODUCT; TRIPE

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Sous-vide technology is a method of cooking under vacuum, so it is aimed to minimize the aroma and taste loss in the food product. By using Sous vide technology; to make the chemical, microbiological and sensorial analysis of the tripe by cooking at a certain temperature and cooking at the specified temperature and time. The most suitable cooking temperature and duration were determined as 86°C and 90 minutes. Samples of tripe taken from the slaughterhouse brought to the laboratory immediately on the day of the experiment; weighing, cleaning, vacuum packaging processes were done. It was placed in salt and hot water solution, and cleaned from dirt and cut in appropriate sizes, weighed and then packed in vacuum bags and ready for cookery. Food samples were cooked in a hot water bath at 86°C for 90 minutes, after the cooking process they were immersed into a cold water bath. In the cooking and cooling processes, instantaneous temperature changes were recorded with food thermometers using the Scan link program. After the initial day analysis, it was kept at + 4°C for weekly analysis. The aim of this study was to investigate acidity, color measurement, TBARs and texture profile analysis (TPA), and total aerobic mesophilic bacteria count, *Salmonella*, and *Enterobacteriaceae* as microbiological analyzes. The panelists selected for the sensorial analysis carried out weekly scoring. At the end of the eight-week storage period, it is observed that the tripe sample preserved as a fresh on the side of chemical, microbiological and physical aspects.

Key Words: Sous-vide, Offal, Tripe, TBARs, Vacuum

ÖZET

SOUS VIDE TEKNOLOJİSİNİN BAZI ET ÜRÜNÜNDE UYGULANMASI; İŞKEMBE

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Sous-vide teknolojisi temel olarak vakum altında pişirme yöntemidir, bu nedenle gıda ürünündeki aroma ve tat kaybını en aza indirmeyi amaçlar. Sous vide teknolojisini kullanarak; işkembenin belirli bir sıcaklıkta pişirerek, belirtilen sıcaklık ve zamanda pişirerek kimyasal, mikrobiyolojik ve duyusal analizlerinin yapılması amaçlanmaktadır. En uygun pişirme sıcaklığı ve süresi 86°C ve 90 dakika olarak belirlenmiştir. Kesimhaneden alınan işkembe örnekleri, deney gününde hemen laboratuvara getirildi; tartım, temizleme, vakum paketleme işlemleri yapıldı. Tuz ve sıcak su çözeltisine yerleştirildi ve kirden temizlendi ve uygun ebatlarda kesildi, tartıldı ve sonra vakumlu torbalara kondu ve pişirmeye hazır hale getirildi. Yiyecek numuneleri, bir sıcak su banyosunda 86°C'de 90 dakika boyunca pişirildi, pişirme işleminden sonra soğuk bir su banyosuna daldırıldı. Pişirme ve soğutma işlemlerinde, Scan link programı kullanılarak gıda termometreleriyle anlık sıcaklık değişiklikleri kaydedildi. İlk gün analizinden sonra, haftalık analiz için +4°C'de tutuldu. Bu çalışmanın amacı, asidite, renk ölçümü, TBAR'lar ve doku profili analizi (TPA) ve toplam aerobik mezofilik bakteri sayısı, *Salmonella* ve *Enterobacteriaceae*'yi mikrobiyolojik analizler olarak incelemektir. Duyusal analiz için seçilen panelistler haftalık puanlama yaptılar. Sekiz haftalık depolama süresinin sonunda, işkembe numunesinin kimyasal, mikrobiyolojik ve fiziksel yönler tarafında taze olarak korunduğu gözlemlenmiştir.

Anahtar Kelimeler: Sous-vide, Sakatat, İşkembe, TBARs, Vakum

TO MY FAMILY

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LIST OF SYMBOLS

SV	Sous Vide
CV	Cook Vide
TC	Traditional Cooking
RSM	Response Surface Methodology
PVC	Polyvinyl Chloride
TMA	Trimethylaluminium
TVBN	Total volatile basic nitrogen
TBARS	Thiobarbituric Reactive
TBA	Thiobarbituric Acid
CCP	Critical Control Point
FSC	Food Supply Chain
PCA	Plate Count Agar
VRBG	Violet Red Bile Glucose
BSA	Bismuth Sulfite Agar
TEP	1,1,3,3-Tetramethoxy Propane
NaOH	Sodium hydroxide
ANOVA	Analysis of Variance
LAB	Lactic Acid Bacteria
MDA	Malondialdehyde
TPA	Texture Profile Analysis

TAMP Total Aerobic Mesophilic Plate

CHAPTER 1

INTRODUCTION

1.1Introduction

Sous Vide is a technique which cooking the food materials under vacuum conditions. It is not a subtitle of vacuum process, only used vacuuming in a step of the process (FSIS 2012). Sous Vide discovered from Sir Benjamin Thompson in 1799, and in the mid-1969 Sous Vide method used to preserve and keep the food products fresh. Bruno Goussault study on the time and the temperature relationship of Sous Vide and provided to reach today (Druitt 2012). These days, the interest in Sous Vide increases due to the number of working people who need to reach the meal fresh and healthy. Sous Vide (SV) is the term used for cooking food samples under vacuum condition with switched temperature and constant time intervals. This cooking style is done by using vacuum bags and cooked under controlled time and temperature. Vacuum bags supply many advantages for the consumers. Food material is become fresh and safe until using in the catering, industry.

Basically, for vacuum bags, polyethylene, and polyvinyl material is used and the features prevent the oxygen entrance into the bags. Food is prevented from external factors; contamination, temperature changes, moisture and etc. Anaerobic conditions prevent the growth of anaerobic and psychrophilic microorganisms. Pasteurization processes inhibit or destroy the growth of microorganisms. This makes the food safer and in high quality. Besides this, the cold chain is an important factor for reaching the meals in safe to an industry, or to the serving places.

The food material is important for processing; each sample has different cooking time and temperature conditions. Traditional cooking styles have limited offers for the consumers and contain some risk of safety. In the sous vide cooking style food placed with special plastic bags and vacuum applied. After that, bags placed into a

water bath adjusted temperature generally lower than 100°C and cooked longtime. Water bath provides the cooking homogeneous of the food sample. Tripe is a by-product of the meat obtained from the stomach of cattle, sheep. A portion of the stomach is known as reticulum or honeycomb is removed and the remaining part is processing. The remainder is cleaned and rinsed with water and treatment applied with soda and hot water. After the cleaning process, there are different types of cooking style in the industry or in catering. Although the cleaning is applied, the cooking style has important consideration. Tripe contains lots of microorganisms especially, *Enterobacteriaceae, Salmonella* and other significant types and these sometimes risk for human health. The necessary heat and time treatments can destroy and inhibited these microorganisms. In Turkey the usage of the tripe is high, especially 'Kokorec' sellers, in restaurants 'tripe soup' and at home, tripe used widely. Beside the microbial consideration, the quality should be taken into account. Unnecessary storage conditions decrease the quality of food and have a short shelf life.

In this thesis study, at the beginning cleaning treatment were applied to tripe samples and debris removed carefully. After that tripe samples cut into small sizes and 100 gram of tripe placed into plastic pouch bags and vacuum applied.

Secondly, heat treatment was applied with adjusted temperature and controlled time conditions. After heating, the tripe samples were cooled rapidly and stored and +4°C to determine the shelf life.

The final part was containing the chemical, microbiological and sensorial analysis. Each week analysis was done duplicated.

Consequently, the aims of this thesis were to;

1. Determine the optimum temperature and time condition for cooking,

2. Provide the safety of the product in order to chemical, microbiological, and sensorial sides.

3. Lead the use of the Sous Vide cooking style in industrial and catering places.

CHAPTER 2

LITERATURE REVIEW

2.1 Sous Vide History

From the ancient, people have to eat in order to survive their life. This began to people lifestyle and now there are a lot of food and different food cultures. In every culture, there are nearly the same food materials but with a little difference, they made totally different meals. Although, this difference made by differing in cooking styles. In traditional cooking, the food sample like a plant, meat or dairy product had to be cooked in like boiling temperature, but when the food sample was reached at the boiling point it results from some nutritional, vitamin and texture loss. According to the traditional cooking method, there are some alternative cooking methods like microwave, radio-waves, ohmic, vacuum treatments, and high-pressure. Recently, the Sous vide cooking method become most popular nearly every cuisine, the principle that under controlled vacuum and short time and low temperature could be done more tasteful and healthy food. (Iborra-Bernad, Tárrega et al. 2014, Candogan and Olum 2015). There is limited studies about Sous vide cooking, but if examined clearly it could be more understood and applicable in every kitchen, restaurant or catering areas. When compare sous vide cooking method with traditional cooking method, the structural, nutritional and sensory properties of food sample show a lot of difference, in one study red cabbage cooked with these two cooking methods and compared by Response Surface Methodology, the food sample which cooked with sous vide method under 87°C/50 min and/or 91°C/30 min conditions. According to color, aromatic properties, and nutritional values, the sous vide foods show more acceptable than traditional cooking method(Iborra-Bernad, Tárrega et al. 2014) Besides this study, some searchers compare sous vide (SV), cook vide (CV) and traditional cooking (TC) in carrots and green peas according to physicochemical and sensorial qualities under selective time and temperature by response surface method cooked these food samples. In the comparison of cooking methods; sous vide

cooking more tasteful and save energy than the other methods. (Koç, Baysan et al. 2017) Sous vide packed product under vacuum condition sometimes used modified atmosphere packaging and cooked definite temperature and time however cook vide unpacked food material cooked in boiling water under vacuum condition. There are some differences between these two methods and there were some limited searches in these methods, but in cook vide method there are limited and generally applicable for vegetables. Although in sous vide cooking method it could be used nearly for all vegetables, meats and it could be enrichment with salt and other spices. In cook vide (vacuum boiling) and Sous vide (cooking with vacuum) had applied to cook some potatoes food samples. Response surface methodology adjusted temperature and time. In that experiment was observing some sensorial and anthocyanin properties. Nearly all results indicated that the sous vide method to supply more advance than the cook vide method. (Iborra-Bernad, García-Segovia et al. 2014). Ready-to-cook Pangasius steaks were cooked by using sous vide. In order to determine the optimum design experiment condition using Response Surface Methodology (RSM) that easily extends the shelf life. For determining the optimum condition, specify the dependent and independent variables. Here in that experiment, they select concentration, cooking time and cooking temperature as a dependent variable on the other hand TBARS value taken as an independent variable. From the designing of experiment determined the number of trials and the optimum concentration, cooking temperature, cooking time and as an independent variable found the TBARS value. (Kumari, Singh et al. 2016). Sous vide technique is the technique of cooking foods based on the temperature-time relationship in vacuumed plastic bags (E Baldwin 2012). Since the cooking time is reduced and the quality of the product increases, it is frequently preferred in restaurants and fast food consumption places. In the term of Sous vide is considered to be a sub-category of the low-oxygen packaging technique, separate from the vacuum packaging technique. Because the vacuum packaging process is only one of the production steps of the sous vide technique. Therefore, the sous vide technique is defined as the technique of cooking under vacuum, unlike vacuum packaging (FDA, 2013). By removing the oxygen in the package, the aerobic microorganism development and the oxidation problem are minimized in vacuum packaging. In addition to this, to reducing the amount of oxygen around the product in the modified atmosphere packaging, carbon dioxide is added to the package and microorganism development is further limited. Through these

packaging systems, it is possible to ensure that the food stored in the cold, especially fresh chicken, fish, and red meat, has a longer shelf life (R. Mccurdy 2006). After pre-preparation, the food product is divided into portions of hermetic pouches in plastic vacuum pouches and hot water baths in a certain temperature and time tracking is made by cooking (Çetinkaya 2015). This process is also called pasteurization. In general, the Sous vide cooking technique is carried out under vacuum and the food product is processed immediately after being cooled, it is carried to fast coolers or in the bathtubs with ice. Sous vide technique is now applied to many different product groups such as vegetables, meat, soup and sauce, and the process steps can vary according to the type of food (Iborra-Bernad, Tárrega et al. 2013). In the study to be done in general, the offal products will be studied, especially the food such as liver and kidney sous vide technique will be looked at. Because the liver has a high amount of fat component, it loses 40% of its mass in the traditional cooking method. It has been observed that the same liver food is only 5% loss and is more delicious when cooked at certain temperature and time controlled by sous vide technique (Hoeche 2016). The red meat contains 75% water, 20% protein and 5% fat and other molecules. When meat is cooked, the proteins are denaturized and the ratio of these proteins to denature is related to the selected temperature and time (McGee 2004). Sous vide technology is used to cook the cooked meat product in a shorter time and at a lower temperature because these losses are less.

2.2 Sous Vide Technology

2.2.1 Sous Vide Methods and Strategies

Sous vide technique is applied with different strategies in industrial and kitchen areas. It is argued that cooking-serving meat and cooking-serving-meat processes are the simplest and safest methods (E Baldwin 2012, Mora 2012). In general, there are four different sous vide strategies which are shown in figure 2.1.

1. Preparation stage: pre-baking processes; fishbone separation, peeling, washing, etc.
2. The cooking stage: heating of food in plastic containers. Food is not directly exposed to heat, but the effect of darkening is minimized
3. Vacuum packaging phase: prepared food, airtight, heat resistant plastic packages are placed. These packages are made of multi-laminated plastic materials. As soon as

the vacuum is created in the package, all of the air is removed. The package is immediately heat-sealed to avoid any loss of vacuum. Vacuum serves to prevent oxidation reactions which cause food spoilage before or after cooking. The vacuum helps tighten the plastic film firmly to the surface of the food. Thus, maximum heat transfer from the plastic to food is possible during cooking.

4. Pasteurization phase: the time and temperature of the packed food according to the characteristics of the packaged food. The cooking is carried out by immersing the vacuum packets in the temperature-controlled water bath. Pasteurization is the most critical stage of the Sous vide technique. This process requires strict control of the temperature to produce fresh and tasty products and ensure that the practitioner is fully pasteurized for all products.

5. Quick cooling stage: after the pasteurization process, the product should be cooled as soon as possible to 1-3°C (within 90 min.). As the food is pre-packed, it is possible to cool directly in the iced water bath. This method is inexpensive and effective but is not possible for standard cooking-cooling menu items.

6. Cold storage stage: the product must be stored at 0-3°C prior to reheating and service. Storage conditions should be strictly applied, such as the rules in the standard cooking-cooling menu items. Vacuum packaging slows oxidation reactions during storage of nutrients. And it prevents the development of aerobic microorganisms.

7. Re-heating stage: the vacuum packaged product is served by reheating in a hot water bath or oven. Another method is; a small hole in the package is opened by heating the package in the microwave. In all methods of reheating, standard cooking-cooling processes are recommended and the central point of the food must be heated to at least 70°C (Ghazala 1994).

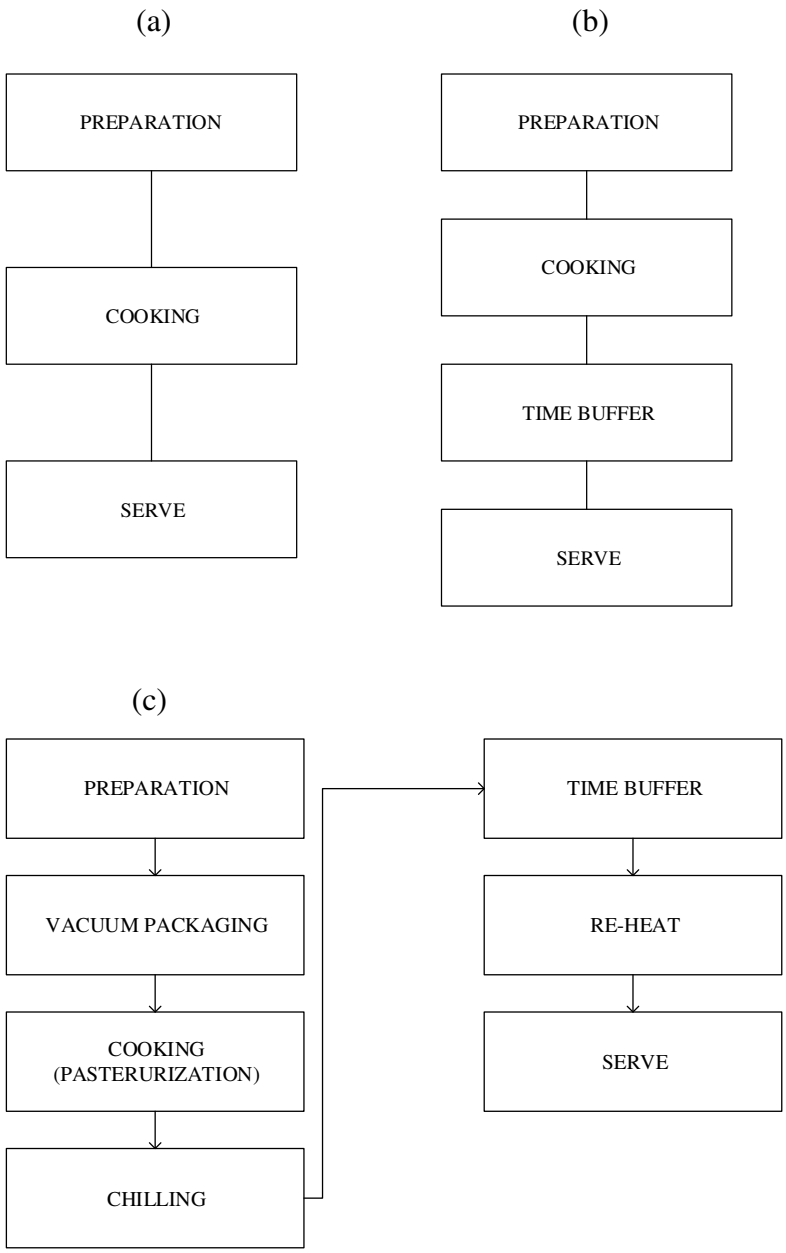

Figure 2 1 (a) Classical cooking style, (b) modern cooking style and (c) Sous-Vide cooking style flow diagram

Meat product is placed in vacuum plastic bags are more crispy and delicious than traditional cooking methods and they extend their shelf life (E Baldwin 2012). By using sous vide technique with carp fillet, quality parameters and microbiological parameters in different temperatures and durations were examined and it was determined that it was safe considering microbiological characteristics. Sous vide technology in the carp fish prepared using heat treatment and storage temperature

shelf life and taste significantly affect. Vacuum packaging with PVC (polyvinyl chloride) coated with packets of bovine liver and kidney cooked by observing microbiological and chemical changes, vacuum-packed lungs and kidney pH value decreased with time; an increase in pH value was observed in the kidney packed with PVC. The microbiological properties were examined, lactic acid bacteria (homo- and heterofermentative *Lactobacilli*, *Streptococci, Leuconostoc spp*.) were observed in vacuum packaging; Gram-negative bacteria were more dominant in products packed with PVC (M.O. Hanna 1982). Meat products cooked and stored using the Sous vide technique were compared with the traditional cooking method and the shelf life was evaluated considering the microbiological and chemical parameters. According to the results, meat products 20ºC observed that the shelf life can be extended up to 28 days when stored (Cobos and Diaz 2007).

When fish were cooked at various temperatures and subjected to sensory analysis with rainbow trout, sous vide technique, 85ºC. The rainbow trout, which was cooked, was the most appreciated (Çetinkaya 2015). In general, meat products are examined and cooked at different temperatures and at different temperatures storage conditions, chemical, microbiological and sensory parameters are compared (Hansen, Knöchel et al. 2007). At the same time, the shelf life-enhancing effects against Listeria monocytogenes microorganisms were investigated by using Sous Vide cooking frost process using thyme and rosemary oil. Sous vide cook-freeze process; after the pasteurization under vacuum by applying the raw meat or pre-preparation phase, it is a fast-freezing technique and storage technology below 3°C. As a result, it was observed that rosemary oil reduced the Listeria monocytogenes microorganisms on to an acceptable level (Rita Gouveia, Alves et al. 2016).

The effects of different salt concentration, temperature and cooking times on the shelf life of fish steaks were investigated with Sous vide technique and it was observed that TMA, TVBN and TBARS values increased during the storage period. As a result, it was found that this fish species cooked using Sous vide method can be stored at 3°C for up to 65 days (Singh 2016). Low temperature and a long time sous vide technology by the effects of the lion meat cooked to the volatile compounds by examining the volatile compounds due to low-temperature oil oxidation occurred in small amounts, high temperature and a long time was observed to be more aromatic in the meat (Roldan, Ruiz et al. 2015). Shelf life is determined as a result of storing

the fish species in the Arizona region under the atmosphere packaging as a result of cooking with sous vide. During the storage period, various microbiological and chemical parameters were investigated and after this fish species was cooked with sous vide, shelf life with modified atmosphere packaging was found to be 49 days (Pino Hernández, Nunes de Carvalho et al. 2017).

2.2.2 Sous Vide Quality Parameters

Recommendations for the determination of microbiological criteria and norms in foods were grouped in 9 sub-categories according to processing techniques, while analytical commission products were classified as category sous vide type meat products. In this category, it is emphasized the necessity to control the application of safety control procedures in time/temperature relationship, especially in the inhibition of *C.botulinum* spores (Anon. 1989a). In the source where processing, distribution and consumption conditions are defined for such products prepared from meat and poultry meat; applied heat treatment with *Salmonella sp. Staphylococcus sp*. *Campylobacter sp*. *Listeria monocytogenes* and *Escherichia coli 0157*. It has been reported that the destruction of bacterial pathogens such as H7 is based on the conditions and the selection of the packaging materials, and the necessity of consumption of a product in the shelf life (FSIS 2012). In the studies; Some physical, chemical and microbiological changes occur such as the formation of aroma, color, size, brittleness, fat content, and protein fractions, increase in pH, mineral losses and decrease of microbiological load during the cooking of meat and meat products.

The loss of cooking and meat is directly proportional to the temperature and time of the meat being cooked. For example, higher water loss and protein denaturation are observed at high temperature (Kolsarıcı 2013). One of the factors leading to the deterioration of products is fat oxidation. Oxidative process, autoxidation, oxygen, and unsaturated oils are formed as a result of the reaction. The resulting hydroperoxides cause a color change in the tissue of the food product. By reducing these compounds, aldehydes and ketones are formed in the bitter taste. One of these compounds is malonaldehyde and is used to measure TBA value. The number of TBARs should be less than 3 and not more than 5 in a good product (Huss 1995). In different sensory analysis studies; the meat cooked in a water bath under vacuum at

60°C has argued that it is more brittle than meat cooked at 94°C in traditional furnaces (Buck, Hickey et al. 1979).

Scientists try to find different cooking styles of sous vide and also one consideration is after cooking in the storage stage. Different food product was cooked by smoked cured pork and sauced. It was cooked with sous vide in the switched temperature between 65°C and 95°C. After pasteurization gamma radiation was applied in order to predict the specific microorganisms and the extending shelf life. To examine the shelf life Total aerobic and anaerobic cell counts and selective microorganisms determination is enough. In addition to that was observed color, lipid oxidation, and sensorial analysis during eight weeks. After all, there is no significant change was observed. The value of pH is nearly 6.0 and was observed for eight weeks. Also, lipid oxidation analyzed by TBARS method and there was no significant alteration. In the sensorial analysis during eight weeks and final week, the food product was being cooked with sous vide was remain in the same nutritional values and physical property. Also, gamma irradiations destroy nearly all inoculated microorganisms (Farkas, Polyák-Fehér et al. 2002). In that study need to compare sous vide with traditional cooking style, and in works of literature, there are some searches that compare sous vide cooking with the traditional cooking method. One search illustrates the cooking pork with different time and temperature combination effect on physical, chemical and sensorial changes and also compare with the traditional cooking method. The pork samples cooked between selective temperature combination and with vacuum and without vacuumed packages. The cooking procedure kept under refrigeration during analysis. Cooking samples were being analyzed with TBARS, color, texture profile analysis and other properties. There are no significant changes in TBARs value in vacuumed packages but in the traditional method there were significant changes during storage due to the lipid oxidation, presence of oxygen and, water loss occurred in traditional method this may result of the shrinkage of the myofibril proteins and their capability of water. Also, color changes were occurring in the traditional cooking method as a result of the degree of myoglobin and rapid protein denaturation. As a result, one may understand that the Sous vide cooking method supply more fresh and intense in taste and also less lipid oxidation was occurring (Sánchez del Pulgar, Gázquez et al. 2012).

In the study of cooking, seabream applied high pressures in addition to sous vide cooking. The fish samples were brought to the laboratory and cutting in the pieces, then cooked and high pressure were applied. In the microbiological analysis total viable count, *Enterobacteriaceae*, *Salmonella* and some other microbiological analysis were done, as chemical analysis and, TBARs and finally, sensory analysis were completed. After all, *Salmonella* and *Enterobacteriaceae* were not detected. Although in some analysis days total viable count was detected, that was indicated may cause from the additives. In chemical analysis results, value nearly remains at the same intervals and TBARs there was no significant change. TBARs shows the oxidative rancidity in the food. And, heat application could cause more rancidity on the food. Also, TBARs value indicates the significant criteria of whether the food product is acceptable or not (Espinosa, Díaz et al. 2015). Besides the effect of heat treatment, cooking of food with different time and temperature combinations, it shows different textural, color, microbiological properties. In the cooking of lamp loins at a different time and temperature combinations were studied and their textural, chemical and microbiological properties were analyzed. 5 different lamp lions were studied at the temperature between 60°C and 80°C and the cooking time were determined between 6 h to 24 h. This study designed using Response Surface Methodology (RSM). The runs were obtained and after cooking procedure samples were kept -80°C until experiment days. Moisture content and water loss were analyzed increasing the temperature increases the water loss and decrease the moisture content. The increasing temperature was led to protein denaturation and decrease water holding capacity. Color measurement was done by using the instrumental device and after observation color parameters lightness (L*), redness (a*) and yellowness (b*) take into account for examination. For lightness, normally L* value increase with temperature but in that study L* value gave higher value at 60°C this is because denaturation of protein and that gave light scattering. The value of a* showed the intensity of the food product. There was an inverse relationship between a* value and denaturation of myoglobin. When the temperature of cooking increased the redness was decrease. The value of b* is related to both cooking temperature and the cooking time. Formation of metmyoglobin lead to denaturation and brownish color were obtained at high temperature. Finally, on the side of microbiology, there was not detected and Total Aerobic Mesophilic,

Enterobacteriaceae, *Salmonella* or *Bacillus* types. For inhibition of microorganisms 70°C 12 h is enough (Roldán, Antequera et al. 2013).

2.2.2.1 Shelf Life Consideration

Sous vide technology is implemented in many areas. Sous vide technique was first used in history in the 1970s (Joan Roca 2005). In the 2000s, studies on increasing the shelf life and quality parameters increased the popularity as the studies on prepared food consumption started (E Baldwin 2012). The most important reason for sous vide is that; it can be applied to fast and easily perishable foods such as meat, poultry, and fish.

It is an important preference for consumers to prepare foods together with spices and in a healthy way. It also offers consumers the opportunity to prepare food and food for consumption in a short time. Extending the shelf life of foods is very important for producers and consumers. Shelf life is an important criterion for food samples. In recent years, people find a way to preserve their food products. Before the refrigeration, the food products could be preserved with a different technique. These ancient methods could be either Drying, Freezing, Smoking, Salting and Pickling. The process should be considered that the side of microbiological, sensorial ways. These oldest methods were even, for now, is used but there are some criteria should be considered. Newest preservation method is vacuum packaging which extends the shelf life of food product without any contamination or growth of microorganisms, besides these protect the nutritional values as a fresh.

Moreover, food waste is an important problem in the world. The food products which expiration date was done become a waste. To avoid this situation, shelf life has to be considered on all sides. Temperature conditions affect directly shelf life. In lack of poor temperature or in unsuitable conditions preventing food waste is become inevitable.

Shelf life consideration become very popular for consumers, people want to be sure that their food products;

- Safety,

- With time has the same sensorial, chemical, microbiological properties until the expired time,
- Nutritional values of food product have to show its safe,
- Acceptable by the consumer. (Mary and Earle 2008). Shelf life described with time, for true shelf-life determination, producer have to consider and have to be accomplished food processing; preparing of food, cooking, packaging, and store of the food product. In the whole process should be certain the sensorial, chemical and microbiological safety. Besides, the shelf-life test should be applied. There are some shelf life tests which are needed to be applied;
- Should know the food product,
- Should be expert in factor affect the product quality,
- Should know the HACCP and the analyze the critical control point(CCP),
- When the processing should predict the microbiological spoilage risk, biochemical possibilities. (Phimolsiripol and Suppakul 2016).

To predict the shelf life basically kinetic data used, based on time which is an important factor that affects the shelf-life;

$$\text{Rate of reactant [A]} = -\frac{d[A]}{dt} = k[A]^n \qquad (2.1)$$

'k' is the kinetic constant, 't' is time, and 'n' in order of the reaction, [A] described as a concentration of a reactant which examined. (Labuza 1982). Shelf life and uncontrolled temperature could cause food waste for the food product. From the food processing to market products need to be under controlled according to be sure that product quality. This quality accuracy could be supplied by temperature control. By new technology scientist suggest that; temperature is controlled food supply chains (FSC). Food supply chains can be combined with different shelf life predictions and decrease the food waste and increase the food quality and consumers are sure that the food product safety (Göransson, Nilsson et al. 2018).

Shelf life can be observed by chemical, physical, microbiological changes during storage conditions. In optimum temperature, could because of some taste, smell, odor and flavor changes. These physical changes could be the result of some chemical or microbiological reactions. In the consideration of microbiological changes, shelf life can be expressed by this equation.

$$\text{Shelf life} = \frac{(\log N2 - \log N1)}{(GrowthRate)} \tag{2.2}$$

In that equation N_1 is the initial number of microorganisms, N_2 is the final number of microorganisms and divided by growth rate. Growth rate can be calculated the exponential growth phase from the curve (Hadawey, Tassou et al. 2017).

2.2.2.2 Consideration of Sous Vide in Thermal Ways

Cooking is a heat treatment; it can be done by application of selected time and temperature combination, which affect the food product. There are different cooking styles; traditional, radiation, conduction, convection cooking. The most important issue in the food industry to extend the shelf life of the food is thermal processing. If meat or pieces of meat cook, after a while protein denaturation will be occurred. In radiation or conduction or convection cooking, they show different denaturation or nutritional loss with time (Adepoju, 2016). The only changes are time and temperature. When the heating process applied, physical and chemical changes are expected. Microbiological preservation should be considered. Each product has different thermal death time. This preservation explained by thermal processes. Thermal process calculation describes the shelf life-extending of food therewithal destroying unwanted microorganisms present in the food product. These affect the quality of the product. Generally, in thermal process calculation F value considered. F value expresses the thermal processing calculation. F value a process is defined as the equivalent time of an assumption produces thermal treatment, T reference, at a constant temperature the same result, until the disappearance of microorganisms. F value considered first-order kinetics model for the destruction of microorganisms. It can be expressed by the below formula.

$$F_{Tref}^{z} = D_{Tref}(\log(C_a) - \log(C_b)) = \int_{t_a}^{t_b} 10^{\frac{T(t)-Tref}{z}} dt \tag{2.3}$$

Here z letter describes the increase or decrease in temperature that reduces or increase the decimal reduction time reducing by one decimal. By using this formula the required temperature can be calculated. Thermal process calculation used in sterilization, pasteurization heating processes to estimate the reduction of unwanted

microorganisms in product. In the canned process, shelf life can be estimated by the thermal process. In our project, we use thermal process calculation in order to determine the cooked value of the product. The safety of the product is important in tripe and other offal's microorganisms can be destroyed at 60°C for 30 minutes. For estimated shelf life by selected weeks the microbial experiments will be done.

Cook value is important and the main thought in our experiment. The selected food products will be cooked in a water bath and under vacuum condition. For estimation, there will need a lot of experiments in order to determine in which time and temperature are optimum for cooking. These procedures take a lot of time, temperature and economic cost. Therefore, the Response Surface Methodology can be estimated which time and temperature interval is better. Here the point which should be considered that which time and temperature interval selected. In some literature suggest the 80°C and 90°C temperature intervals better for cooking meat and meat pieces.

Thermal process important in the cooking process especially pasteurization, microwave in any heating applied the device. Sous vide is a kind of pasteurization process which needs to control the heating and cooling stages. Generally, thermocouple or special devices used in order to determine the changes in the product during the heating process. To control this change of heating is significant in some terms such as control the microbial growth or contamination. The changing of temperature with time is recorded by thermocouple software (Scan Link) and the cooked value is calculated by the formula (*) that given below.

$$C = \int 10^{\frac{[T(t)-T(ref)]}{z}} dt \qquad (2.4)$$

Actually, C-value calculated to estimate the degree of reduction. Each component has different z-value and reference temperature for calculation, in one search, pickled were pasteurized and cook value of product were calculated and for pickled asparagus, reference temperature was taken as a 100°C and z- value 33 min. (Lau and Tang 2002). The point should be considered that when the calculations of cook value based on the chemical properties, not microbial properties. If the microbial side is considered, the F value is calculated. So in the calculation of cook value based on the chemical properties changes and how will be the effect on shelf life. Therefore the z value and T reference value were taken 33 min and 100°C, respectively in the

calculation part. Here in Table 2.1, there are some microorganisms and their properties of heat resistance. Processing of Sous vide cooking style have to consider in all side especially, in some critical parameters. To determine the shelf life heat resistance should be taken into account. That's way after calculation of cook value by the aid of D and z value for any food sample, the processing applied easily. In the experiment, the food sample is Tripe and the Salmonella important microbial criteria, for determining the shelf life and safe production.

Table 2 1 Some critical microorganisms properties and their resistance to heat treatment on Sous vide foods (Brown 2008)

Critical Hazard	Microorganism	T (°C)	pH	a_W	D (T°C)	z (°C)
Infection pathogens	*Salmonella species*	5.1	3.8-4.0	0.92-0.95	1.7 min (60)	5.6
	Listeria monocytogenes	-0.1	4.4	0.90-0.92	2.85 min (60)	5.8-6.3
	Vibrio parahaemolyticus	5	4.8	0.94-0.96	0.8-48 min (47)	
	Aeromonas hydrophilic	0	4.0	0.94	0.19 min (55)	
	Camplobacter jejuni and co	25-30	5.3	0.985	12-21 s (58.3)	6.0-6.4
	Escherichia coli O157	4-7.1	4.4	0.935-0.95		
	Yersinia enterocoliticia	0	4.6	0.95	0.24-0.96 min (62.8)	5.1-5.8
Toxinogenic pathogens	*Staphylococcus aureus*	5.0-7.7	4.0	0.86	5.2-7.8 min (60)	5.4-5.8
Toxinogenic sporeforming pathogens	*Clostridium botulinum*	3.3	5.0	0.96-0.97	0.49-0.74 min (60)	5.6-10.7
	Bacillus cereus	<4	4.4-4.9	0.90-0.93		6.9
	Costridium perfringens	15	5.5	0.93-0.95	1 min (60)	3.8
					7.2 min (59)	

In the study of cooking red cabbage, considered two cooking methods one is the traditional cooking method with boiling water and atmospheric pressure, the other one is Sous vide cooking with modified temperature and time combination. To optimize time and temperature taken as an independent value and for a dependent value color and other textural properties taken using Response Surface Methodology. In the cooking of red cabbage as a traditional one, the cooking time determined 11 minutes, on the other hand, the sous vide conditions optimized 87°C/50min or 91°C/30 min. After cooking procedures, observed the color, and other textural and sensorial properties by panelists. Finally, the red cabbage which is cooked by sous vide found more tasteful and safety when considered from the microbiological way. (Iborra-Bernad, Tárrega et al. 2014). Traditional cooking method boiling in atmospheric pressure the food material like vegetable, meat or fish product have limited shelf life because of the presence of aerobic bacteria and this result of losing nutritional value and off-textural values. The sous vide cooking method improves good taste and more confidence when the consumer used. In the experiment of determining the optimum conditions of sous vide cooking seer fish steak, considered salt concentration, temperature and cooking time as a dependent value and TBARS value considered as an independent value, for the optimization value Response Surface Methodology (RSM) used and evaluated experiment design. From the result of concentration, temperature and cooking time found; 3.5%, 89°C, 13.5 min respectively. By these results, TBARS value increased and shelf-life increase 65 days. In the traditional cooking method usual shelf life is less than the sous vide cooking (Singh, Kumari et al. 2016). Ready-to-cook Pangasius steaks were cooked by using sous vide. In order to determine the optimum design experiment condition using Response Surface Methodology (RSM) that easily extends the shelf life. For determining the optimum condition, specify the dependent and independent variables. Here in that experiment, they select concentration, cooking time and cooking temperature as a dependent variable on the other hand TBARS value taken as an independent variable. From the designing of experiment determined the number of trials and the optimum concentration, cooking temperature, cooking time and as an independent variable found the TBARS value (Kumari, Baru Singh et al. 2016).

Sous vide based on the material cooked under vacuum condition, and with or without the addition of salt or species, or by modification of time, temperature, and modified atmosphere. Cook vide is a different method that like sous vide cooking method but the difference is the food product cooked with vacuum below 100°C temperature condition and time factor changes by temperature and vacuum conditions. The traditional cooking method is totally different from both sous vide and cook vide. The food product cooked in boiling temperature and atmospheric pressure. These changes in cooking styles affect the final food product by means of every property. Bernad and his friends compare the cooking styles and analyze the final product in sensorial and instrumental sides. They have used the carrot as a sample and first determine the cooking time and temperature condition by using Response Surface Methodology (RSM) determine the cooking time and temperature 22-78 minutes and 78-92°C as a temperature. In the traditional cooking method, boiling pan used and cooked for 2-15 minutes under atmospheric pressure. Finally, the carrots were cooked in traditional method found some nutritional loss, less tasteful and firmness, in the comparison of cook vide and sous vide; firmness was found nearly same but in taste and flavor sous vide cooking method was preferred by panelist (Iborra-Bernad, Tárrega et al. 2014). The cooking method is generally considered as a modified time and temperature and concentration combination, the shelf life influence directly. Sous vide cooking method used in several reasons, but the important usage that extending shelf life with prevents the loss of nutritional value and the formation of microbial spoilage. In order to achieve optimum shelf life condition there were a lot of studies about the effect of different products with different time, temperature and concentration combinations, sometimes in Sous vide method modified atmosphere packaging has an alternative effect. Fandos and his friends examine the fish product shelf life which was cooked by Sous vide cooking method. Shelf life could be examined by the microbial side or by quality factors. Generally, for product safety, all properties should be investigated. In the study of cooking rainbow fish, the fish sample vacuumed and stored below 10°C finally the shelf life examined both microbial and sensorial sides. In the microbial search *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens,* and *Listeria monocytogenes* were not found and after storage at 90C for 3.3 minutes products were found more safety and high in nutritional levels (González-Fandos, García-Linares et al. 2004).

2.2.3 Sous Vide Applications

Sous Vide (SV) is in catering places in the last 30 years. The most common ready to eat meals method is producing chilled foods. Chilled foods are similar to homemade meals due to that reason especially working people prefer to buy them. Although chilled food producers have to control shelf life, transportation safety, and consumers have to consider the cold chain. This gives a problem and raises the price of the meals (James 1990). Moreover, have to apply melting and reheating process on chilled food products before to consume the meal. That could cause time buffering, also microbiological, sensorial and chemical threats may occur. Those preservation methods do not become very useful. Manufacturers try to find a new method for preserving the food as fresh and consume safely. Safety is the main consideration issue for manufacturers and consumers when the meals reach to consumers, they want to be sure about there is no pathogenic spoilage, and no color, off-odor and flavor changes, no rancid taste. Sous Vide (SV) cooking style present totally safe food products. It reaches to the consumer without the breaking of the cold chain (Creed 1998). Now, Sous Vide used widely in every area. Sous vide application styles divided into two; home based Sous Vide and Industrial based food products. At home, small portions are prepared and at industrial areas, large portions are prepared.

Sous Vide (SV) cooking procedure includes vacuum packaging components, low-temperature cooking, rapid cooling, refrigerated storage, transport, and reheating before use. These all steps were monitored by the devices and, producer and consumers can be sure about management and hygiene (Anon. 1989a).

In industry, there are different sous vide types of equipment. The designs of the equipment are different but they serve for the same purpose. Generally, for designing Sous vide need polyvinyl chloride (PVC) vacuum sealer shown in figure 2.6 and 2.7(17ninety 2019),(Tools 2019), (Chef 2019) (a), after placing the food sample for vacuuming needs vacuum machine (b) and adjustable temperature device (c) for cooking, after cooking process rapid cooling device and storage rooms.

Figure 2.6 Vacuum sealer bag

Figure 2.7 Vacuuming device

Figure 2.8 Commercial adjustable temperature water bath.

Figures 2.8 show the devices and equipment's of processing Sous vide, in the industry, there are a different type and models of application of Sous vide however the first aim is that create the appropriate conditions. Besides that, vacuuming procedure it could be different, the addition of different gases and obtained Modified Atmospheric Packaging that extends the shelf life in some cases but in the basic principle of Sous vide based on the vacuuming without the addition of any gas.

2.2.4 Advantages and Disadvantages of Sous Vide

- Advantages

Vacuum packaging is done by minimizing the amount of oxygen so that the oxidation risk is reduced. Sealed plastic bags form a biological barrier and prevent product deterioration in processes such as; storage, cooking, cooling and reheating. When compared the Sous vide with the conventional method, the food sample which is cooked with Sous vide, does not moisture and vitamin loss, taste, and odor loss due to the low-temperature cooking process and use of the vacuum bags. The product has become more crispy and delicious. It is suitable for low-sodium diets because of the less salt and spices used in sous vide-cooked meals, according to the grand method. In particular, the traditional marinating process in meat products takes a long time. But with Sous vide, this time is shortened; crispier and delicious meat products are obtained and time loss is minimized (Sarkar and Almeida Costa 2008).

It is preferred in hospitals, schools, factories, restaurants, and hotels because of the high nutritional composition and easy to service of the foods. When pasteurization is applied to the meal, some microorganisms can survive; *C. botulinum*, *B.cereus*, *C. perfringens*, *S. aureus*, *Salmonella sp*., *V. parahaemolyticus*. These are pathogens and make the people sick. Food samples which are cooked by Sous vide technology are become safer according to microbial growth and contamination, protect meals against microorganisms. (Leistner and Gorris 1995).

Another advantage of sous vide technology is to provide a wide range of food alternatives in areas were ready to use food consumption is required (aircraft and train travel, catering, etc.). In addition to all of these, healthy food production is always an important issue for schools, hospitals, factories, kindergartens, hotels, government agencies, and military barracks sous vide method offers a wide range of alternatives and healthy eating facilities is a technology that should be given importance (Mol, Özturan et al. 2009). The Sous vide advisory committee states that the microbiological quality of the products produced by sous vide technology depends on the applied heat, the internal temperature reached in cooking and the temperature at which it is maintained, the cooling rate and the cooling end temperature. Thus, the applied temperature can reduce the microbial load and also the product can be protected with rapid and effective cooling. Studies in Belgium and France show that sous vide technology is widely used in the food industry (Ghazala 1994). It has been reported to be widely used in many countries, especially in restaurants, hotels, schools in the world.

- Disadvantages

Vacuum packaging and pasteurization of the tools used in the application, the high cost of vacuum bags. Need for high-tech equipment in every stage of the food industry due to the need for strict follow-up of each process, the necessity of cold chain tracking at each stage. As a result of poor manufacturing conditions, the product is contaminated during processing, the cold chain cannot be protected and quality loss is observed and the shelf life cannot be expected. The need for trained staff is to determine the conditions of pasteurization and to apply it. The need for high temperature for caramelization is the lack of equipment needed to determine the

pasteurization conditions to be applied in restaurants, especially because of the need for separate temperature and time for each product (Creed 1998).

2.3 Food Sample Used In Sous Vide

Meat and meat product is an enormous amount in daily intake for the human body. There are different cooking styles and procedures until serving. In the beginning, people used fire for cooking meat, and this was changed during time. Finally, Sous vide used for advanced cooking style. There are a lot of species for a cooking meal in Sous Vide, also meats are used widely. Meats do not obtain from one type of animal, but it can be obtained for different animals such as; cattle, sheep, goat. After slaughtering it can be used different parts of meat as a meal. Meats approximately occurred 75 % water, 20 % protein, 2.5 % lipids, 1.2 % carbohydrates, and 1.3 % minerals (Candogan and Olum 2015). Water content is an important chemical parameter for juiciness and showed the freshness of meat and meat products. When the heat is applied to meat and meat products, their properties lead to change and protein denaturation has occurred. Sous vide cooking serve the water loss has become minimum as a result of vacuum packaging and after cooking, meat protects its juiciness.

Table 2.2 The Chemical composition of the fresh cattle.

Composition	% Wet base
Water	75.0
Protein	20.0
Lipid	2.5
Carbohydrate	1.2
Minerals	1.3

2.3.1 Tripe

There are many types of cooking meat and meat species like boiling in water, grilling, frying and these types of cooking styles carried in the presence of oxygen, sous vide cooking technique occur absence of oxygen and carried on the vacuum pads and low temperature and less cooking time. In a study of cooking of lamb lions

by using sous vide method, lamp loins were taken and selective time and temperature conditions determined the TBARS value and other sensorial properties. When the heating process was applied, TBARS (Thiobarbituric reactive substances) value decreased because of the protein could react with malonaldehyde, protein molecules turned into insoluble components. Designing of the experiment considered independent value as a TBARS and across to time, temperature and other sensorial values. From the results found the optimum values for the experiment (Roldan, Antequera et al. 2014).

Meat is the most popular food which is used all over the world. Besides this, offal-by-product of meat- is consumed underestimated amount. By-products of meat are liver, brain, kidney, (white) lung, tripe and small intestinal. Some parts are edible and some parts are known inedible but it varies from region to region. In the comparison of meat and by-product of meat, which edible part decreases year after year. The reason that the offal the high cholesterol content on the other side tripe contains a larger amount of proline, hydroxyproline, and glycine, and a lower level of tryptophan and tyrosine than other meat organs. Tripe is a good source of dietary which include some important vitamins, minerals, fats. Here in Table, 2.3 explained the amount of these sources.

Table 2.3 Amount of percent of nutrients in sheep tripe (a) Vitamins (b) Sterols (c)Minerals (d) fats and fatty acids)

(a)

Per 100 gram of Tripe		**% Daily Value (DV)**
Vitamin A	0.0 IU	0%
Vitamin C	0.0 mg	0%
Vitamin D	-	-
VitaminE(alpha tocopherol)	0.1 mg	0%
Vitamin K	0.0 mcg	0%

Per 100 gram of Tripe		% Daily Value (DV)
Folate	5.0 mcg	1%
Thiamin	0.0 mg	0%
Riboflavin	0.1 mg	4%
Niacin	0.9 mg	4 %
Pantotheniz Acid	0.2 mg	2%
Choline	195 mg	
Betaine	-	-

(b)

Per 100 gram of Tripe		%Daily Value (DV)
Cholesterol	122 mg	1%
Phytosterol	0.0 mg	0%

(c)

Per 100 gram of Tripe		% Daily Value (DV)
Calcium	69.0 mg	7%
Iron	0.6 mg	3%
Magnesium	13.0 mg	3%
Phosphorus	64.0 mg	2%
Potassium	67.0 mg	2%
Sodium	97.0 mg	4%
Zinc	1.4 mg	9%
Copper	0.1 mg	4%
Manganese	0.1 mg	4%
Selenium	125 mcg	18%

(d)

Per 100 gram serving Tripe		%Daily Value (DV)
Total Fat	3.7 g	6%
Saturated Fat	1.3 g	6%
Monounsaturated Fat	1.5 g	-
Polyunsaturated Fat	0.2 g	-
Total Trans Fatty Acids	0.2 g	
Total Trans-monoenoic fatty acids	-	-
Total Trans-polyenoic fatty acids	-	-
Total Omega-3 fatty acids	7.0 mg	-
Total Omega-6 Fatty acids	125 mg	

One of the important by-products of meat is tripe. Stomachs of cattle or lamb composed four-part; the rumen, the reticulum, abomasum, and omnivore. Rumens are most usable part of the stomach known as tripe. First and second parts of stomachs are used. Cattle, sheep or pork stomachs are used for tripe, too. In the processing of tripe; washing, scalding, and bleaching are applied before the processing. Tripe can be used in different areas like poaching, sausages, processing of meat. In Turkey, tripe is widely used in the kitchen; Shkembe soup, Mumbar, and Kokorec are well-known meal dish that consumed in Turkey. Especially Kokorec sold in public places based on the wrapping of intestines around offal. Kokorec is roasted on a horizontal skewer. And after roasting cutting into small pieces and serve it. (Nollet and Toldrá 2011). The food component of tripe is a good source of increasing the nutritional value of the body. Meat and meat products include a high

amount of protein, minerals, fat as a consideration of human dietary. In some countries, offal is banned due to the microbial risk and potentially risky for bodies. In that point, offal such as lung is banned in some areas like the USA. Although the consideration is wrong, the regulation should be considered. The scientist should take microbial consideration and HACCP regulation. Offal includes a high amount of protein and good vitamins that require the take from bodies. In the selected optimum time and temperature condition the tripe is cooked. Sous vide method is useful for cooking tripe. It is cooked well under vacuum pressure. In the search of optimum time combination, tripe samples were boiled in different time intervals and determined in the selective parasites which are inhibited at 30 minutes. In table 2.4 gives the time and percent amount of microorganisms when the tripe is boiled (Li, Wu et al. 2014)

Table 2.4 Inhibition of parasites in selective time

Boiling Time (min)	**Survival of Specific Microorganisms(%)**
0	100
5	13.44
10	3
20	0
30	0

Meat has a short shelf life during storage, with or without cooking procedure, due to the water content (55-65%) decrease the shelf life. This is the same for tripe, after slaughtering of the carcass, the offals should be stored in one or two hours and then cooking procedure is applied, however, the shelf life is nearly about seven days depending on the cooking style. In order to extend shelf life, sous vide cooking method is applied. This method supplies more nutritional value and taste and prevents the microbiological growth because of the absence of oxygen, after weeks the offal could be used as a fresh. Although facultative microorganisms can grow in that condition, in order to prevent microbial spoilage HACCP should be applied and critical control points and all analysis should be done

.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Tripe samples were bought from local butcher in the day of the experiment, chemical reagents were; buffered peptone water (Merck), Plate count agar (PCA, Merck), Violet Red Bile Glucose Agar (VRBG, Merck), Bismuth sulfite agar (BSA, Merck), NaOH solution, 1,1,3,3- tetra methoxy propane (TEP, Sigma Aldrich), %1 thiobarbituric acid (TBA, Sigma Aldrich), 10% trichloroacetic acid (TCA, Sigma Aldrich).

3.2 Packaging of Samples

Approximately 5 kilograms of tripe bought from the local butcher in the day of the experiment, immediately cleaning process was applied. After removing the physical debris from tripe sample, it was disinfected with hot water and salt composition in the solution given in figure 3.1. Tripe was cut into small pieces (20cmx20cmx1cm). The samples were packed (polyethylene/polyamide) with vacuum processed (figure3.2) under a given pressure. Each vacuum package was labeled to show the processing time and temperature condition. The food packages were placed into a water bath that put up the temperature of 86±2°C (figure 3.3). When the pasteurization procedure was completed, the vacuum packages were immersed ice bath and were cooled until the internal temperature reached to +10°C. After the cooling process was completed, all vacuum packaged kept at +2°C into the storage room. Samples were analyzed immediately named as week 0; the other samples were stored and weekly analyzed up to week 8. Analyzes were performed as a microbiological, chemical and sensorial analysis.

Figure 3.1 Disinfection process of tripe

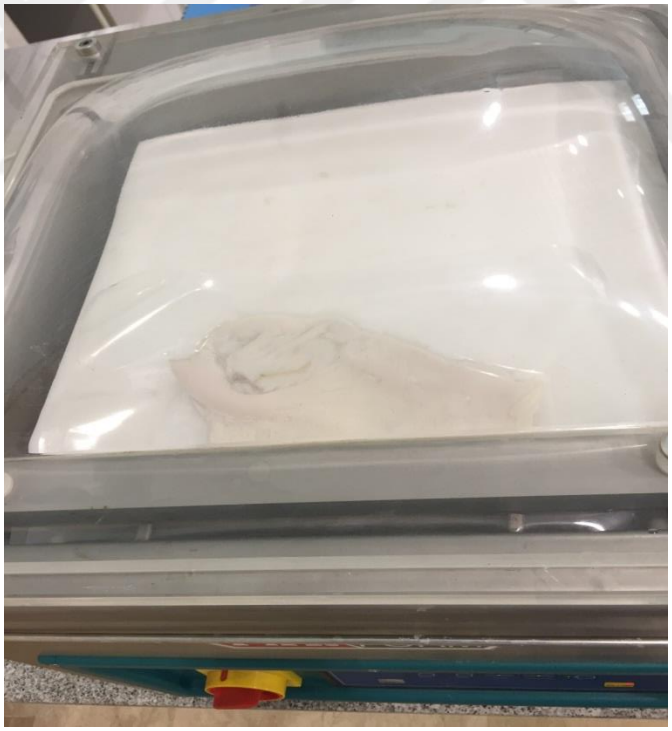

Figure 3.2 Vacuum applied to package tripe sample

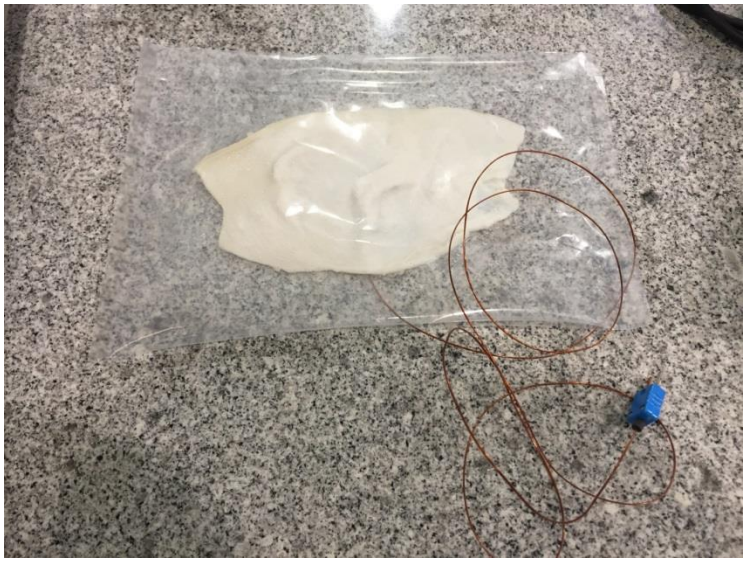

Figure 3.3 After vacuuming control group for cooking procedure

3.3 Chemical Analysis

3.3.1 Determination of Total Acidity in Tripe

3.3.1.1 Preparation of NaOH Solution

In the determination of the total acidity NaOH solution was used. For titration procedure, solution of NaOH was necessary. 4 gram of NaOH weighed in the precision balance and put into a volumetric flask and complete 1 L and 0.1 N NaOH standard solution were ready for titration

3.3.1.2 Titration of Tripe Sample

10 gram of tripe was weighed into precision balance and was put into stomacher flask, and 200 ml of distilled water was added and homogenized around one or two minutes. Then the solution was filtered by Whatman No 54 filter paper into the volumetric flask. 25 ml of tripe water solution were taken by graduated cylinder pour into the flask, and 75 milliliters distilled water poured into the flask. Three drops of phenolphthalein indicator were added and then titrated with the standard solution until were reached the equilibrium point. The amount of NaOH was recorded in order to the calculation of total acidity in tripe sample.

3.3.2 Determination of Color

Color determinations of the tripe samples were carried out using a Hunter Lab Color flex (A-60-1010-615 Model Colorimeter, Hunter Lab, and Reston, VA). In order to determine the effect of pasteurization (cooking) and storage time and condition on tripe sample, color properties were analyzed. The samples were cut (5cm*5cm*1cm) and placed into special glasses then were measured with Hunter LAB Color flex. The values (L* = lightness, a* = redness, and b* = yellowness) were recorded and the analysis was duplicated.

3.3.3 Texture Profile Analysis

In texture profile analysis TAXT plus Stable Microsystem device were used. After the pasteurization process, the samples were taken (5cm*5cm*1cm) and then placed into plate and the with the 3mm/s and 15mm compressed, between two compresses there was 15 seconds. The parameters of hardness, gumminess, chewiness, cohesiveness, springiness, fracturability, and resilience were recorded with the software of TAXT Plus Stable Microsystems (Texture Exponent 32, Stable Microsystems, Godalming, Surrey, UK) The graph and results were recorded.

3.3.4 TBARS (Thiobarbituric Acid Reactive Substance) Analysis

The detection of lipid oxidation has relied largely upon the quantification of compounds such as malondialdehyde (MDA), which are formed by denaturation of samples of radicals. The reaction of MDA with 2-thiobarbituric acid (TBA) one of the most widely used for estimation of lipid oxidation(Janero 1990). TBARS analysis was adapted from the method of Ohkawa and his friends (Ohkawa, Ohishi et al. 1979). In order to determination of lipid oxidation in meat or meat species amount of MDA formation was determined by using spectrophotometry. For these spectrophotometric analyses calibration was necessary.

3.3.4.1 Standard Calibration Curve

A standard calibration was prepared by 1,1,3,3-tetramethoxypropane. For stock solution 170 µl 1,1,3,3-tetramethoxypropane (TEP) solution were taken into a volumetric flask and then completed with 1 liter distilled water. 7 test tubes placed into the stand and 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.7 ml, and 1.0 ml were taken from stock solution with the pipette and completed to 10 ml of distilled water. From each dilute solutions, 0.5 ml of solution were taken and placed into another tube and completed with 5 ml of 10% TCA solution. All test tubes were centrifuged for 20 minutes. After centrifugation 2 ml of supernatant was taken and mixed with 2 ml of 1% thiobarbituric acid (TBA). Then they were placed into 95°C water bath for 30 minutes. After that, shock cold was applied to standard samples. At 532 nm absorbance value against blank and standard curve were obtained (figure 3.4)

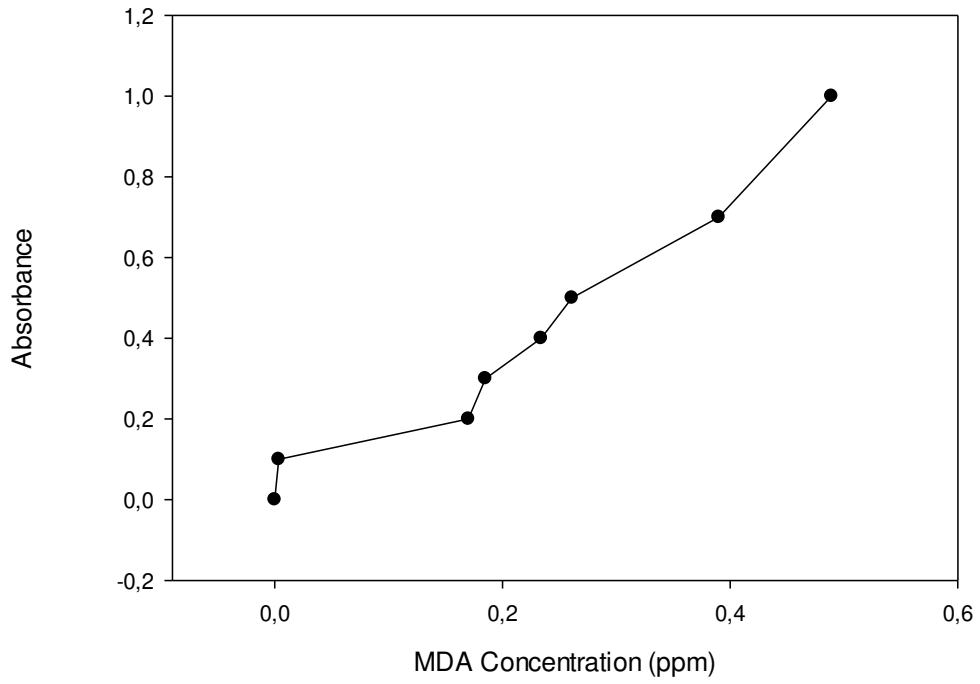

Figure 3.4 Standard calibration curve of MDA (ppm)

3.3.4.2 TBARS Determination

Approximately 1 gram of tripe samples were taken and homogenized with 5 ml of 10 % TCA solution. After homogenization test tubes were placed into a centrifuge for 20 minutes. Nearly 2 ml of supernatant was taken and mixed with 2 ml of 1%TBA. The mix was placed into the water bath at 95°C for 30 minutes. After that, shock cold was applied to samples. At 532 nm absorbance value against blank and TBARS value were determined with the aid of the calibration curve.

3.4 Microbiological Analysis

3.4.1 Preparation of Peptone water

Peptone water was used for dilution and for homogenization of tripe sample.1 gram of Buffered peptone powder (Merck) was weighed and poured into a clean balloon then 1L of distilled water mixed with buffered peptone water. The percent of peptone setting 0.1% point. After the homogenization process, the balloon was sterilized in an autoclave at 121°C for 15 minutes.

3.4.2 Homogenization of Sample

Homogenization process carried under aseptic conditions, 225 milliliters of peptone water poured into a volumetric flask and magnetic stirrer put in it. The mouth of the flask was closed by cotton and aluminum foil. Sterilization was applied by autoclave at 121°C for 15 minutes. After sterilization was completed, 25 gram of meat sample was taken with a sterile knife, at put into the volumetric flask. The sample homogenized approximately 40 minutes by stirrer.

3.4.3 Preparation of Dilutions

For the preparation of dilution test tubes filled with 9 ml of 0.1 % peptone water under aseptic conditions. The mouth of test tubes closed flabbily and covered with aluminum foil. For sterilization, the test tubes were autoclaved at 121°C for 15 minutes. When sterilization was complete, 1 milliliter of the sample which was

homogenized taken by sterile pipette and was put into the first tube. Sterile dilution tube was shaken and tube flamed before and after used. For second dilution 1 milliliter of dilution was taken from the first dilution on pour into the second tube. Serial dilutions were prepared until the sixth dilution was ready.

3.4.4 Total Aerobic Mesophilic Plate Count Experiment

In order to analyze total aerobic mesophilic plate count, Plate Count Agar (PCA, Merck) was used. For each experiment period, the medium prepared in the day of the experiment. 250 ml of agar was prepared. 5.725 gram of plate count agar powder was weighed and was poured into the balloon, 250 milliliters of distilled water poured into the balloon. The mouth of the balloon closed with cotton and aluminum foil and tied. For sterilization agar was autoclave at 121°C for 15 minutes. After sterilization complete agar was allowed to cool until 65°C. For preventing freezing it was kept in heater set to 65°C. Empty Petri dishes prepared under aseptic conditions, each petri dish was labeled with name, date and dilution factor. 1 milliliter of dilution was inoculated to petri dish the approximately 15 milliliters of agar decanted petri dish. Petri dishes were applied 8 movements for three times, made by mixing the dilutions and the medium. Airborne and to minimize the risk of contamination from the environment procedure was completed within 15 minutes. After solidification of the spilled media, the Petri dishes are inverted and incubated at 37±1°C for 48±2 hours. After incubation counted Petri dishes were recorded.

3.4.5 Determination of *Enterobacteriaceae*

In the determination of *Enterobacteriaceae*, Violet Red Bile Glucose (VRBG, Merck) agar was used. Twice pouring plate method was applied. It is used as a selective solid medium for the identification and counting of *Enterobacteriaceae* members in standard microbiological analyzes performed in vitro (except live cells). This medium does not autoclave it was prepared with hot water. Violet is also known as Red Even Glucose (VRBG). It is mixture of Peptone 7.0 g/L; Yeast extract 3.0 g/L; D(+) Glucose 10.0 g/L; NaCl 5 g/L; Ox bile (Bile salt mixture) 1.5 g/L; Neutral red 0.03 g/L; Crystal violet 0.002 g/L; Agar-agar 13.0 g/L (Merck).In the preparation of medium, the balloon poured with 250 milliliters of distilled water and the balloon

was sterilized with an autoclave at 121°C for 15 minutes. After cooling, 9.875 gram of Violet Red Bile Glucose powder was weighed and put into the balloon; it was heated about two minutes. Sterile Petri dished were placed and then 1 milliliter of dilution inoculated to first petri dish then 12.5 ml of agar was poured into sterile Petri dishes and 8 movements were applied three times. After cooling the agar and dilutions 5 or 6 milliliters of agar poured again. Same procedures applied for all dilutions. To prevent the risk of contamination procedure was completed within 15 minutes. After solidification of Petri dishes inverted and incubated at 37±1°C for 24±2 hours. After incubation counted Petri dishes were recorded.

3.4.6 Determination of *Salmonella*

Bismuth Sulfide Agar was used as a selective solid medium for the determination of *Salmonella* in standard microbiological analyzes performed in vitro (except live cells). It was a mixture of Meat extract 5.0 g/L; Peptone from meat 10.0 g/L; D(+) Glucose 5.0 g/L;Na_2PO_4 4.0 g/L; Iron(III) sulfate 0.3 g/L; Brilliant green 0.025 g/L; Bismuth sulfite indicator 8.0 g/L; Agar-agar 15.0 g/L. This medium is not autoclaved. Sterilization was performed while dissolving the medium in a boiling water bath. Agar was used as fresh the medium loses its selectivity when kept for further use. For sampling, 1 milliliter of dilution inoculated to the petri dish and approximately 15 milliliters of Bismuth Sulfite Agar was poured into the dish. Then, 8 movements were applied three times. After solidification of Petri dishes, inverted and incubated at 37±1°C for 48±2 hours. After incubation counted Petri dishes were recorded.

3.5 Sensorial Analysis

Sensory analysis was done in a laboratory conditions. Ten panelists were selected and all of them are University members which had previously received theoretical and practical training in sensory analysis. They had experience in tasting different products such as; wine and, sausage. Panelists were carried the sensory analysis, weekly. Ranking the cooked tripe sample according to its flavor, color, ease of cutting and gives the score from 0(lowest) to 10 (highest) points (Table 3.1). For

evaluation stage, panel performance was evaluated by the addition of scores to each other and overall sensory quality was calculated.

Table 3.1 Sensory analysis evaluation form of Tripe

Score Card for Sensory Analysis

Judging and Grading For Tripe Samples

A. Flavor

(lowest) (highest)

0 10

B. Color

(lowest) (highest)

0 10

C. Ease of Cutting

(lowest) (highest)

0 10

3.6 Statistical Analysis

Sous Vide application on the tripe sample analysis experiment results was compared by one-way analysis of variance (ANOVA) to test for significant differences. Means of the groups were compared using Tukey‘s multiple range test using an SPSS statistical packet (Version 23). In the differences between the groups were to be significant when $p<0.05$. Also graphs of the results created by the Sigma Plot 12.0 (Systat Software Inc. 2011).

CHAPTER 4

RESULTS AND DISCUSSIONS

During this research, Sous vide technology was studied on the tripe sample. After the cooking process, the tripe sample was stored with 8 weeks and the chemical, microbiological and sensorial properties were analyzed.

4.1 Chemical Changes during Storage of the Tripe

4.1.1 Changes in Acidity of the Tripe during Storage

Acidity is an important effect on storage which demonstrates the microbial quality of the meat sample. Generally, acidity means, lactic acid bacteria are growth on the tripe sample. This is cause the decrease in the acidity. The raw tripe sample's acidity was calculated 0.26 as a lactic acid percent, and cooked tripe sample acidity calculated 0.67. First, second and until sixth week the acidity were remain nearly same values ($P>0.05$), but week by week results show that the acidity were decrease.(Figure 4.1) In the storage period there is no significant changes on the means of the acidity until sixth week but after sixth week the changing of the acidity has an importance. Hydrolysis of the collagens caused the protein degradation of the protein molecules and decreases the acidity, by mean the bacterial activity could start. A significant changes obtained on the overall means on changes in acidity ($P<0.05$)

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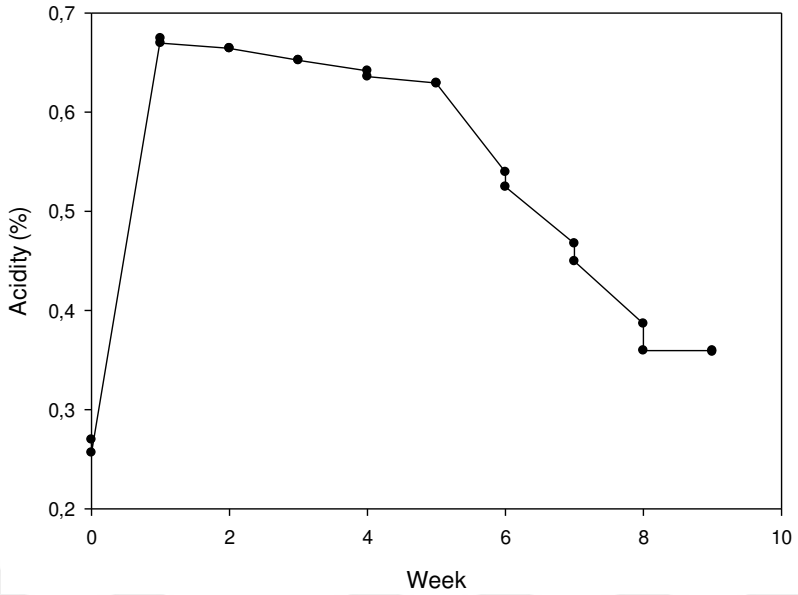

Figure 4.1 Changing in the Acidity during eight weeks

4.1.2 Changes in Color of the Tripe during Storage

Color is an important parameter for determining the quality of food and directly related to the cooking temperature. The color was measured with Hunter Lab device and each week L*, a*, b* values were recorded. Each letter has a different mean and shows different properties of the food. Color values directly affected by the temperature and as a result of cooking temperature and cooking time which affect the protein properties and their response on both physical and chemical appearance. L* value index of the lightness (L=100 white, L=0 black). L* value changed by increasing the cooking temperature. Increasing the cooking temperature change the protein structure in the tripe. Protein especially myofibrillar proteins denatured when the heat was applied (Claus 2019); this changes the value of lightness. In the result of color measurement in raw tripe found; L* was 59.18, after the cooking process and during storage L* values increased to 65.64 to 60.50. In the study of Akoglu et.al, color changes on turkey with Sous vide cooking method had been analyzed and changing in the L* values have support our results (Akoğlu, Bıyıklı et al. 2018).

The value of a* (redness) is related to intensity. Hunter a* value change between negative and positive meaning, negative a* value indicates the greenness and

positive a value mean redness. When the denaturation of myoglobin occurs, the value of intensity is decreased. Results in the Tbale 4.1 shows that the raw tripe of Hunter a* value is 2.49 and after cooking and storage period denaturation occurred and the Hunter a* value were decreased. a* value intervals changes between -0.88 and 0.07.

The Hunter b* value indicates the yellowness and blueness. In the first day of analysis, raw tripe sample measured 15.88 and after storage, the interval changed from 12.85 to 12.06. There was a significant difference between raw tripe sample and the others b* value ($p<0.05$).

Table 4.1 Average hunter color values on tripe during storage

Weeks	**0 (Raw)**	**0 (Cooked**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
L	59.18	60.5	61.1	64.16	65.64	64.15	61.1	63.98	63.25	64.53
a*	2.49	0.10	0.07	-0.68	-0.88	-0.78	-0.66	0.25	-0.56	-0.51
b*	15.88	12.59	12.68	12.85	12.06	12.41	12.37	12.41	12.52	12.55

4.1.3 Changes in Thiobarbituric Acid Reaction (TBARS) on Tripe Sample during Storage

Presence of lipid oxidation is determined by the TBARS method. The standard calibration curve is obtained by measuring standard samples at 532 absorbance value. Lipid oxidation expressed in ppm means milligram of malondialdehyde (MDA) per kilogram tripe sample (Palo, Maggiolino et al. 2013). Lipid oxidation can be a quality factor on the fatty food product. The occurrence of the lipid oxidation causes the off-odor and off-flavor in the meat and by-products of the meat. Moreover, the lipids of the secondary products can react with the protein fragments and cause protein oxidation, too (Kanner 1994).

In the day of analyses, raw tripe sample of calculated MDA (mg/kg tripe) value was found 1.71 ppm and at the same day cooked tripe sample of the MDA (ppm) value was calculated 1.81 ppm. The difference between cooked and uncooked tripe samples nearly was 0.1 ppm. This shows that when heats apply to the sample lipid oxidation will start. In the consideration of all results of the tripe, MDA value increases weekly. In the first five-week increase gradually but in the fifth

week, the value of MDA increase 2.13 ppm suddenly. And final week, this MDA value reaches to 2.21 ppm. In the statistical analysis, the last two week has a significant effect on the other weeks ($P<0.05$).

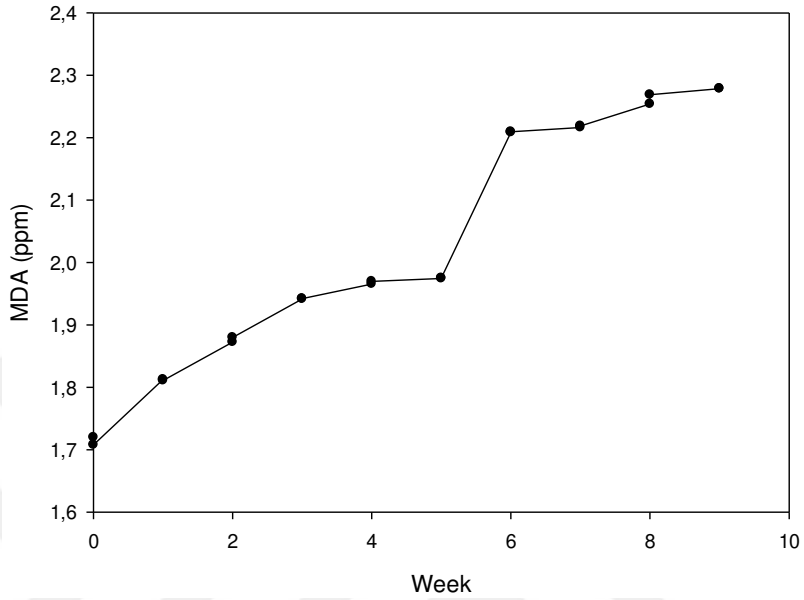

Figure 4.3 Changes in the MDA concentration on the Tripe during Storage

4.1.4 Textural Profile Analysis (TPA) on the Tripe during Storage

The textural analysis on the tripe is providing information about the physical properties on the acceptance level. Consumers take into account the appearance, palatability, and some other physical features. Texture Profile Analysis (TPA) was done with the device known as TAXT plus Stable Microsystem. All samples were analyzed weekly and work in two repeats. The parameters of the TPA were Hardness, Springiness, Cohesiveness, Adhesiveness, Chewiness, and Gumminess. Hardness means the force required for the first compression, Springiness means the distance between the after first compressions with the probe. Cohesiveness means the ratio of the total area under compression curves (A1/A2) shown in the figure. Chewiness means the hardness, cohesiveness and springiness and Gumminess mean the hardness and cohesiveness.

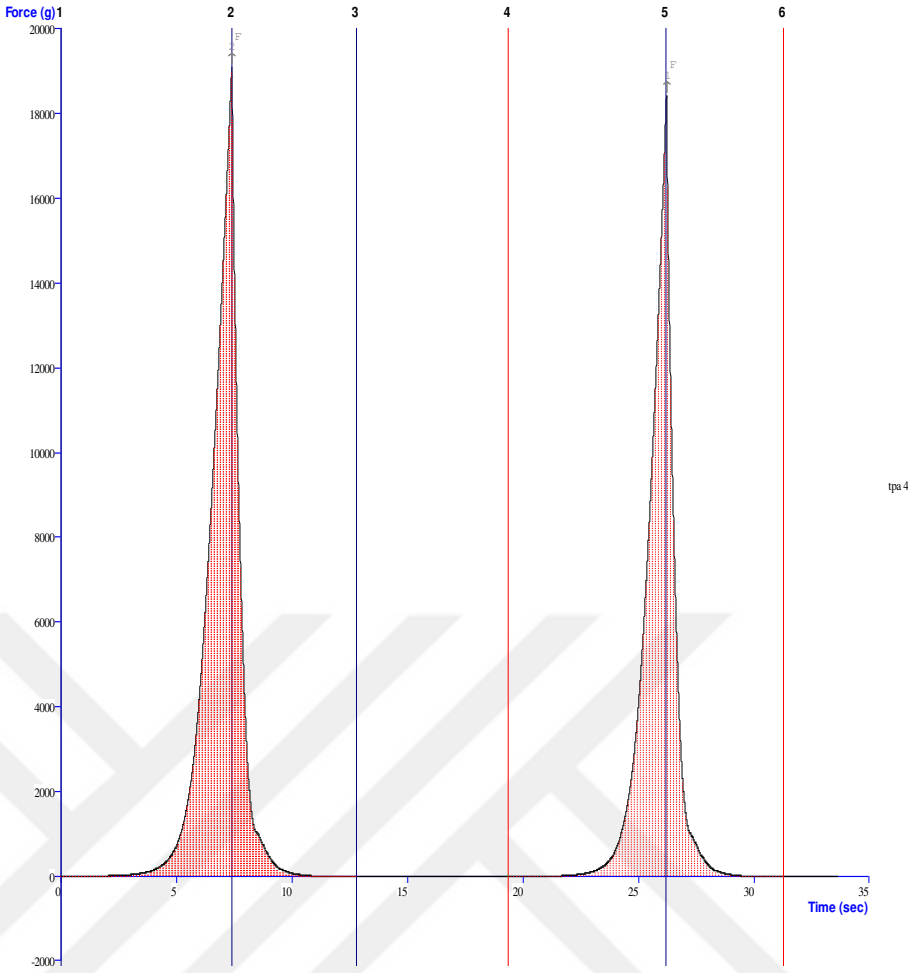

Figure 4.4 Cohesiveness the ratio of the total area under the curve

In the result of the analysis, raw tripe sample hardness average value was found 9959.72 and reach the maximum point on the fourth week. Although there is a difference between the hardness value, the week has no significant effect on hardness ($P>0.05$).

Springiness for the raw tripe was measured 0.96 and it shows non-linear properties. This non-linear means of values does not affect by the weeks ($P>0.05$).

Cohesiveness for the raw tripe was measured 0.66 and when considered all over the results there are no significant changes with weeks ($P>0.05$)

Table 4.2 Average measured values of Texture Profile Analysis (TPA) during eight week

Week	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
0 (raw)	9959.72	0.93	0.66	6580.7	6091.49	0.41
0 (cooked)	16223.4	0.93	0.93	1455356	14265.65	0.56
1	16456.8	0.96	0.9	14764.23	14217.83	0.51
2	21283.42	0.9	0.89	19026.47	17198.6	0.49
3	22152.94	0.97	0.88	19424.03	18884.86	0.48
4	21450.56	0.92	0.76	7975.49	7307.24	0.48
5	19118.86	0.93	0.86	16400.22	15194.81	0.42
6	10594.74	0.97	0.92	9710.61	9377.24	0.55
7	11569.07	0.97	0.95	10946.79	10618.08	0.57
8	11225.73	0.97	1.14	11589.56	12458.36	0.56

4.2 Microbial Changes during Storage of the Tripe

In the detection of microbiological analysis Total Aerobic Mesophilic Plate (TAMP) count, detection of *Salmonella* and detection of *Enterobacteriaceae* were carried on. For TAMP, Plate Count Agar (PCA), for *Salmonella* Bismuth Sulfite Agar (BSA) and for *Enterobacteriaceae* Violet Red Bile Glucose (VRBG) agar were used. All microbiological analysis were studied two repeated and each week carried on. After detection is applicable calculation was done. Finally, all calculated values were performed on statistical analysis.

4.2.1 Total Aerobic Mesophilic Plate count

Total Aerobic Mesophilic microorganisms show the total viable microorganism in the food sample. Before the heat treatment on the tripe sample, viable microorganisms were found 4.13 log CFU/g in the sample. After heat treatment was applied the number of total viable microorganisms was inhibited. The pasteurization time and temperature affect the inhibition. In the cooking process, pasteurization temperature was found 86°C and time was 90 minutes. This time

and temperature combination was inhibiting the total viable cells during eight weeks.

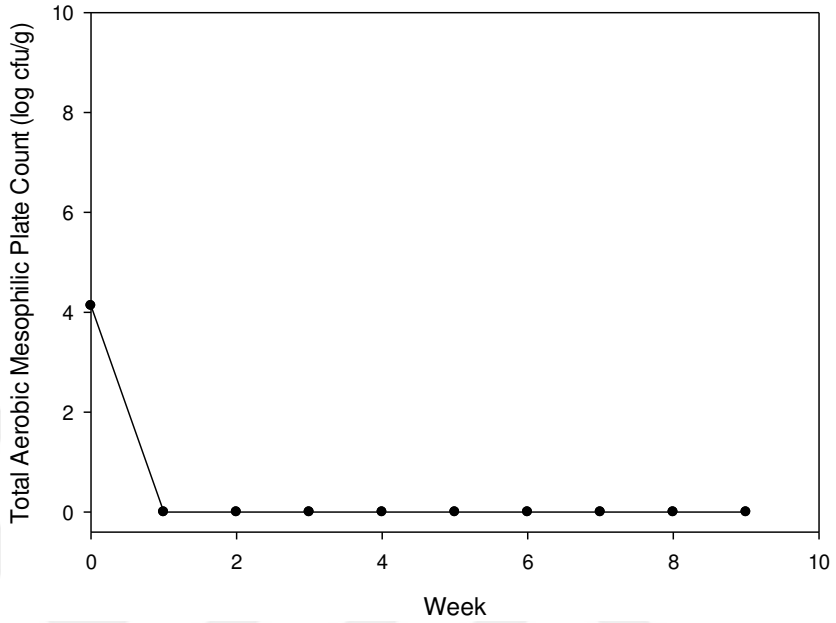

Figure 4.5 Number of viable cells during the storage period

4.2.2 Detection of *Salmonella*

Salmonella is a significant criterion for determining the quality of the food in the consideration of the microbiological side. In the study of Nyati, evaluate the extending shelf life and detect the *Salmonella* presence/absence and they did not found any *Salmonella* species during five-week storage (Nyati 2000). Mol and his friend studied on some species of fish and cooked with Sous Vide method and storage 35 days. During this storage period there was not detect and Salmonella species on the fish sample (Mol, Özturan et al. 2009).

In the analysis of *Salmonella* presence/absence were determined by BSA and after incubation *Salmonella* species were not detected from the first to last week.

4.2.3 Detection of *Enterobacteriaceae*

Enterobacteriaceae family belongs to Gram-negative, non-spore forming and facultative anaerobic bacteria. *Enterobacteriaceae* cause human pathogens when infected to food (Erkmen and Bozkurt 2004). Sous vide vacuum applied process and process carried on the absence of the oxygen, under necessary heat treatment *Enterobacteriaceae* members could be destroyed. On the previous study of the Espinosa, seabream cooked by the sous vide and some microbiological analysis have been done, as a result of their research, they did not detect any *Enterobacteriaceae* during storage (Anandh, Venkatachalapathy et al. 2014).

For inhibition or destroying pH is an important indicative factor, around 6.0 to 5.06 values *Enterobacteriaceae* does not grow. In the result of the analysis, there was not detected any member of the *Enterobacteriaceae* family. And ph ranged from 6.3 to 5.3 all storage period. Previous studies and the indirect results support that the *Enterobacteriaceae* members did not found in the tripe sample.

4.3 Sensorial Analysis Results

Sensorial attributes of raw and cooked and stored tripe samples were analyzed and listed in Table 3.1 since sensory properties of the meat product samples were mainly flavor, color ease of cutting (Callejo, 2015). Their changes for tripe sample during cooking and storage were examined. In the study of buffalo tripe rolls, sensorial scores were decreased weekly. They argue for the increasing the shelf life cause the surface drying and also increasing the TBA value and may due to the microbial growth (Anandh, Venkatachalapathy et al. 2014).

In the day of analysis raw tripe was found undesirable level was 4 points out of 10. Scores were increasing until the fifth week and found better when compared with all weeks was calculated 15 points. From the sixth week to the eighth week the level decreased until 11 points. Overall sensory scores compared with the weeks, and there was a significant effect. ($P<0,05$)

Table 4.3 Sensory scores and average overall sensorial quality during storage

Week	Flavor	Color	Ease of Cutting	Average Overall Sensorial Quality
0 (raw)	2	1	1	4
0 (cooked)	3	1	3	7
1	3	3	3	9
2	4	4	5	13
3	4	4	5	13
4	4	5	5	14
5	5	5	5	15
6	4	4	5	13
7	4	3	5	12
8	3	3	5	11

CHAPTER 5

CONCLUSION

5.1 Conclusion

In this thesis research and the studies in the literature support the Sous Vide (SV) is not a new technology but with the aid of this kind of research; this technology has been developed and used easily in daily life.

Optimum process time and temperature were found to be 86°C and 90 minutes for pasteurization of tripe via Sous vide (SV). At this condition, tripe was cooked well and safe. After cooking, the best temperature for storage determined +4°C and eight-week storage of tripe samples were evaluated.

In the chemical result, acidity, color, TBARs analyses were done and for eight weeks the samples were stored as an acceptable level. In textural profile analysis, the tripe samples were response nearly same results during eight weeks. Some chemical differences take places this was an acceptable level.

In microbiological results, before the cooking process, raw tripe sample includes total aerobic mesophilic bacteria. There is no *Salmonella* or *Enterobacteriaceae* samples were detected at the beginning. After the cooking process, TAMP numbers were decreased and become zero until the eighth week any TAMP was detected. In addition, any *Salmonella* and *Enterobacteriaceae* species were detected. The vacuum cooking and storing could provide the sample, a safe in the microbiological side.

As a result of this search, Sous Vide (SV), make life easier. In the consideration of the quality and safety, Sous Vide (SV) protects the food material either pre-cooked or cooked as a fresh, at the high nutritional level and safety from the microbial contamination or growth. The food material which cooked in Sous Vide (SV) can easily use in every area.

5.2 Future Works

In this search results showed that the Sous Vide (SV) technology can be used in all areas especially in catering and industrial areas. Sous Vide (SV) provides with the high nutritional quality foods, the new trend of healthy foods and beside this safety in microbiological ways.

It will be better to examine the effect of Sous vide techniques on tripe samples contaminated with some microorganisms. In addition, cold chain is an important factor for Sous Vide (SV), in the future works transportation and shelf life needs to be improved.

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APPENDICES

Appendix A

Table A.1 ANOVA for Acidity versus Week

Acidity

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.409	9	0.045	604.642	0.00
Within Groups	0.001	10	0.000		
Total	0.410	19			

Acidity

Tukey HSD[a]

Week	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
week 0 raw	2	.263					
week 8	2		.358				
week 7	2		.373				
week 6	2			.458			
week 5	2				.320		
week 4	2					.629	
week 3	2					.638	.638
week 2	2					.652	.652
week 1	2						.664
week 0 cooked	2						.671
Sig.		1.000	.808	1.000	1.000	.292	.060

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table A.2 ANOVA for MDA (ppm) versus Week

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.737	9	0.082	3683	,000
Within Groups	0.000	10	0.000		
Total	0.737	19			

Tukey HSD[a]

Week	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
week 0 raw	2	1.713						
week 0 cooked	2		1.811					
week 1	2			1.876				
week 2	2				1.941			
week 3	2					1.967		
week 4	2					1.974		
week 5	2						2.208	
week 6	2						2.217	
week 7	2							2.261
week 8	2							2.278
Sig.		1.000	1.000	1.000	1.000	0.854	0.712	0.078

Means for groups in homogeneous subsets are displayed.