International Journal of Environment, Agriculture and Biotechnology
Vol-6, Issue-2; Mar-Apr, 2021

Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Assessment of Food Safety and Physicochemical Properties of Sous-vide Cooked Steak in Lebanon

Yasmine Helal[1,*], Antoine Abou Fayad[2], Ali Al Khatib[1]

[1]Department of Nutrition and Food Science, Lebanese International University, Beirut, Lebanon
*Email: 11730670@students.liu.edu.lb
[2]Department of Experimental Pathology, Immunology and Microbiology, Center of Infectious Disease Research, American University of Beirut- Beirut, Lebanon
Samples Provider:
Sofil Catering, Beirut Waterfront, Beirut, Lebanon

Received: 07 Dec 2020; Received in revised form: 21 Feb 2021; Accepted: 14 Mar 2021; Available online: 03 Apr 2021

Abstract**—***Sous-vide cooking was recently introduced to the meat catering and hospitality in Lebanon. It is a heat cooking process that includes either precooked, packed products that need little or no additional heat treatment prior to consumption, doesn't have low pH or low water activity, has an extended chilled shelf life, or ismarketed in sealed packages or containers. The aim of this study is to assess the microbial load and food safety of sous-vide meat produced in Lebanon while maintaining quality and palatable characteristics.*

Sous-vide process used tenderloin meat as raw material. Tenderloin meat was trimmed, cleaned, vacuum packed in plastic pouches and cooked atdifferent low heat, followed by chilled storage. After 6 hours, 24 hours, 5 days and 10 days of chilled storage samples were unpacked, portioned into steakandreheated. Samples were evaluated physicochemically by analysing pH, moister content and extract release volume and microbiologically by preparing a culture media followed by API experimental procedure. Results showed that ERV and moisture content decreased significantly but within acceptable limits and that the cooking method had no effect onpH.A significant decrease to zero microorganisms was observed after sous-vide cooking which remained constant throughout the storage period and after reheating, while pathogenic organisms were not detected. Thus, the present study indicates that sous-vide cooking is a safe technique for mass production of steak with an improved shelf life.

Keywords**—Food Safety, HACCP, Microbiology, Physicochemical assessment, Sous-vide.**

I. INTRODUCTION

The culinary world is rapidly shifting to accommodate the present lifestyle demands. New food processing technologies are applied to produce ready to eat products with improved characteristics and marginal discrepancies. Sous-vide is a fairly novel cooking method that extends shelf life and palatable qualities of food in a way that cannot be achieved through conventional cooking. It involves packing food in heat stable vacuum sealed bags followed by controlled cooking at low temperatures [1]. In meat products, low cooking temperatures help break down of collagen in the connective tissues, preserving tenderness and moisture content whilst improving sensory and nutritional quality of cooked meat [2]. More importantly, sous-vide facilitates safe reproducibility and mass production in a short and controlled timely manner, which is especially valuable for the catering industry. However, there are some limitations to sous-vide cooking. A substantial erraticism in knowledge and training of food handlers applying sous-vide escalates the potential for improper use of sous-vide cooking [3]. The survival of vegetative pathogens is another concern in low temperature long time sous-vide cooking [3]. Furthermore,

protein degradation and lipid oxidation, during storage, may produce off odours and flavours [4].

Sous-vide cooking has been recently introduced to the catering and hospitality industry in Lebanon. In the last three to four years, many catering companies and restaurants started adopting sous-vide as a primary production procedure. Appropriate local food safety standards that regulate the safe production, handling and trade of sous-vide have beenlimited as the Lebanese food safety law has been governed by legislative decrees that date back to the 1960's and 1970's [5]. A centralized and integrated approach that food businesses can consult andconform to have not been available[5]. The accessibility of systematic preventive system that provides basic advice and guidancesuch as Hazard Analysis and Critical Control Points planhas been scarce. The food industry had to rely on self-imposed internal frequent testings to ensure safe food production and avoid food borne illness. Proving that sous-vide produces food that is safe for human consumption demanded research on the safety and quality of this cooking process. The significance of this study is based on the lack of national food safety standards and the rise in public concerns about food safety [5]. The context of work focuses on microbiological assessment in conjunction with physicochemical analysis as safety is not sufficient to indicate palatable quality of food. A suggested HACCP plan offers food businesses a model for formulating a proper food safety management system.

The aim of this work is to evaluate the effect of different time, temperature and storage combinations on microbiological and physicochemical properties of sous-vide cooked steak.

Research Hypothesis

Sous-vide cooking method produces steak that is safe for human consumption while maintaining its quality.This research gave rise to the following questions to be inspected:

1. Does sous-vide produce safe to eat steak?
2. Does sous-vide preserve the physicochemical properties of steak?

1.1 History

What started as a concept to overcome recipe development issues in 1970's by George Pralus developed into a cooking method that became prominent in today's cooking industry[1]. Exceptional outcomes inspired Pralus to adopt this technique and set some rules for achieving perfection. His specifications comprise the acquisition of the highest quality raw material, coordinated with strict hygiene standards and controlled cooking procedure[1]. While others claimed that they are the pioneers of this cooking technique, Pralus' ability to apply this approach in practice and tackle challenges as they arise earned him his title as the founder. In 1971 W.R. Grace, a leading packaging company is USA, took out a patent on the basic concepts of the process [1]. Sous-videhas become widely applied in restaurants and catering industries in 2000's [6]. In Lebanon, utilization of sous-vide in the hospitality industry is fairly new and the first scientific report about it was in 2017 [7].

1.2 The Process

Sous-vide is French term for "under vacuum". It is defined by the Sous-vide Advisory Committee as "interrupted catering system in which raw or par-cooked food is sealed into a vacuumised laminated plastic pouch or container, heat treated by controlled cooking, rapidly cooled and then reheated for service after a period of chilled storage" [1, 8].

The technology involves packaging raw or precooked food under vacuum in hermetically sealed bags then cooking at low temperature. Convection steam ovens and water baths, set at specific precise temperatures, are generally used as a cooking medium. Steam ovens accommodate large quantities of food and distribute heat uniformly as long as they aren't over loaded, while circulating water baths also heat very uniformly when food pouches are completely submerged in water and loading capacity is not exceeded. Probe thermometers, inserted through a closed cell foam tape mounted on vacuumed pouches, are used to monitor and control core temperature of food [6].

Sous-vide differs from conventional cooking as it stipulates several advantages [6]. Vacuum packing inhibits production of off flavours caused by oxidation and prevents evaporative losses of moisture and flavour [10]. It eliminates the risk of contamination during storage and reduces aerobic bacterial growth, thus extending food's shelf life [1,10,11]. Moreover, it promotes efficient heat transfer from the water bath or oven steam to the product [6]. Accurate temperature control permits holding food at low temperatures long enough for fast and slow changes to take place, such as protein denaturation. Also, it allows control over doneness and perfect reproducibility [6]. Sous-vide is known to reduce material costs and enhances superior retention of texture, aroma, flavour and nutrients [12]. Sous-vide can be applied to meat, seafood, fruits and vegetables and supermarket retailed products [13].

1.3 Effect of Heat on Muscle Meat A distinctive feature of sous-vide technique is its ability to cook food at low temperature for a long time. It is particularly favourable for cooking tough meats that require application of low heat for a long time to weaken the connective tissues and

decrease myofibrillar tensile strength [6]. Upon heating, proteins denature and change. The extent at which these changes occur depends mostly on time followed by temperature. Both myofibrillar protein and connective tissues contract and shrink quickly when heated. Sarcoplasmic proteins expand, aggregate and gel also quickly. Collagen dissolves into gelatine, reducing inter-fibre adhesion. However, changes in collagen to allow tenderness of muscle meat demand more time. Moreover, sarcoplasmic protein enzyme, collagenase, remains active at temperature below 60 °C, which can considerably tenderise meat when held for more than 6 hours. Sous-vide grants the flexibility of holding food at desired temperature for enough time to achieve pleasant tenderness of different meats cuts [6].

1.4 Other Physicochemical Changes

Meat undergoes physicochemical changes upon cookingwhich affect quality parameters including colour, flavour, texture, pH, and water holding capacity. Water holding capacity is an imperative property of meat quality. It is influenced by the pH of the tissue and the amount of myofibril in which water resides. Upon heatingmuscle proteinscoagulate and shrink releasing water out of the meat. More water is released as cooking duration increases and juices are lost through drip and evaporation, resulting in dryand tough end product[14].

Previous studies that examined the effect of sous-vide cooking on physicochemical changes have shown that as cooking time and temperature increase weight loss increases [15]. Extract Release Volume (ERV) decreases upon prolonged storage and pH increases [17, 21]. Some studies have shown that sous-vide cooking enhances texture, tenderness and chewiness of meat products [16, 20] while others reported no change [1,18].

1.5 Microbiology and Food Safety

Raw untreated foods are expected to contain varying counts of bacteria, yeasts and moulds.

However, plants and animals which serve as food, have developed defence mechanisms to combat the proliferation and invasion of microorganisms. These mechanisms have become an inherent part of their tissues known as intrinsic parameters. Intrinsic parameters such as pH, moisture contents, oxidation-reduction (Eh), nutrient content, antimicrobial constituents and biological structures govern the initial microbial load of a food product [22].

Bacterial microbiota, in meat, are mostly Gram-negative with some Gram-positive such as enterococci and lactobacilli. A large number of moulds (*Penicillium* and *Mucor*) and some yeasts (*Candida* and *Rhodotorula*) may also be present [22]. *Escherichia coli* (biotype 1) is commonly found in meat products with high rates of incidence. It is regarded as an indicator organism in assessing the sanitary state of fresh food and safety of beef. *Salmonella* is another pathogen common to commercially prepared and packaged food[22]. *Listeria* is also a prevalent microbiota in red meats. Twenty-two percent of tested beef carcases in Belgium reported positive results for Listeria [23].

Meats contain a high amount of water (about 75% when raw) and abundance of nutrients encouraging the growth of microbiota. However, only a few types of spoilage microorganisms can be found in spoiled meat due to intrinsic factors of the product. The surface of meat products tends to create adequate environment for growth of potential aerobes, facultative and strict anaerobes, which may be amplified when extrinsic factors such as suitable growth temperatures are reached [22].

The microbial load in fresh meat may range from a minimum of 10^3 cfu/g to a maximum of 10^{10} cfu/g. Microbial spoilage is generally not recognised in the range of 10^3 cfu/g to 10^6 cfu/g except for milk which might develop a sour taste. Vacuum-packed meats might acquire odours and might be spoiled within a range of 10^6 cfu/g to 10^7cfu/g. Off odours associated with aerobically stored meats occur at microbial count of 10^7cfu/g to 10^8cfu/g. Obvious signs of spoilage are displayed at 10^8cfu/g to 10^9cfu/g in all most all foods. And, a definite change in structure occurs at 10^9cfu/g to 10^{10}cfu/g [2].

Bringing too much technology into sous-vide processing and cooking at lower temperatures raises the risk of growth of pathogenic bacteria [1]. Accordingly, a great deal of research on sous-vide food has focused on the safety of the procedure and on investigating its effect on shelf life extension. Most studies have shown that pathogens and spoilage microorganisms were reduced to an acceptable level [16, 25, 26] and their presence in the final sous-vide product probably results from microbes being in raw ingredients and surviving during processing [24].

1.6 Food Safety Standards

Some countries, such as Australia, Canada and the United States, have set out food safety standards and requirements for sous-vide processing method at a national level. Others have developed guidelines including legal requirements and control measures for food processing industries. Food safety authorities place requirements on food businesses to produce food that is safe and suitable for consumption. They all focus on microbial hazards of concern such as *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria* and *Salmonella* and recommend cooking times and temperatures. Lebanon like many other countries doesn't

have a current standard for this particular procedure [27, 28, 29].

II. MATERIALS& METHODS

2.1 Raw and Cooked Material

Raw and sous-vide cooked steak samples were provided by Sofil Catering, Beirut Water-Front. Three batches of samples were prepared on three successive weeks starting 15th of April 2019 till 8th May 2019. They were divided into 3 groups or blocks (weeks) where each group was considered a replication. Samples were collected as soon as they were prepared and ready for testing. Three replicated identical lots of each batch were preserved in an appropriate freezer where one lot was physiochemically analysed at the Food Science Research Laboratory - Lebanese International University and two lots were microbiologically analysed at the Centre for Infectious Disease Research Laboratory - American University of Beirut.

2.2 Sous-vide Cooking Process, Sampling and Sample Preparation

Nine samples were collected from 9 different stages of sous-vide cooking. Raw vacuum-packed tenderloins were unpacked, drained, trimmed and cleaned then covered with stockinets. They were then processed in two phases. In phase one, tenderloins were cooked, where they were seared, vacuum packed then sous-vide cooked in a steam oven (temperature and time) followed by chilling then refrigeration. In phase two, after being held at chilled storage for varying durations, steak was unpacked, portioned then reheated (time and temperature).

Triplicate batches were collected for three successive weeks from Sofil's kitchen, at various stages of the cooking procedure as described in Table.1 to ensure the method's efficiency and absence of cross contamination.

Fig.1exhibits a step by step chart of sous-vide cooking procedures as well as the stages at which each sample was taken. It is based on an interview with the executive head chef followed by an on-site verification done a week later.

2.3Physicochemical Analysis

Duplicate batches of nine samples each were cooked, collected and tested on differentdates for ERV and moisture content. This was replicated over three weeks.

2.3.1 Determination of Extract Release Volume (ERV)

Sous-vide samples weighing 25g each were blended with 90ml distilled water in an electric blender for 2 minutes. The mix was then poured into a funnel fitted with Whatman filter paper No. 1 folded thrice to make 8 sections. The homogenate was left to seep between the fold into a graduate cylinder for 15 minutes [30]. The released volume was then measured by graduated cylinder to the nearest millilitre.

2.3.2 Moisture Content in Meat

The moisture content of the sous-vide cooked steak samples was measured following the *Official Methods of Analysis of AOAC International* Method 950.46[31].Two grams of sous-vide cooked samples were weighed to the nearest mg and placed in a hot oven at 100°C ±2°C for 16 ±0.5 hours. Final weight of the samples was measured and the moisture content was calculated according to the following formula:

Moisture content = (Initial weight – dry weight)/initial weight *100

2.3.3 pH Test

A calibrated pH meter (Thermo Electron Corporation) adjusted to the temperature of tissues was used to measure the pH.A sample of 15 grams of sous-vide was blended with 30ml of distilled water using a stomacher(BLSmart) at 27-30°Cfor 2 minutes. The pH was then measured by inserting a glass electrode in the prepared sample [30].

2.4Microbiological Assessment

Duplicate batches of 9 samples each were also cooked, collected at the same time as those prepared for physicochemical analysis and tested for spoilage and pathogenic bacteria. This was however replicated over two weeks period.

2.4.1 Sample preparation and culture

Steak samples were plated using standard techniques. Homogenized 1 g of steak in 20 mL of PBS buffer and the supernatant were inoculated on specific agar for bacterial growth.

Serial dilutions of the supernatant plated to MacConkey agar for detection of Gram-negative bacterial species; SS (*Salmonella- Shigella*) agar was used for easier selection of*Salmonella* spp., LB agar (Luria Bertani) used for overall count detection, and shedding samples were directly collected on Brain Heart Infusion Agar for a total viable count.

Plates were incubated at 37 °C in a humidified incubator with ambient air; all other media. For anaerobic bacteria, an anaerobic chamber wasused for incubation. Plates were read at 24 hour and 48 hours of incubation.

Each different isolate was later inoculated in 2 mL of LB broth cells were harvested after 4 hours at 37 °C[32].

2.5 Bacterial Molecular Testing

2.5.1 Analytic Profile Index (API) experimental procedure:

All isolates were grown and streaked on the same type of agar media that they were isolated from and incubated at 37 °C overnight. Fungal growth was eliminated from the study based on colony morphology, and oxidase test was performed on all remaining isolates.

API 20E test was performed on all oxidase negative isolates according to the manufacturer's instructions as follows; isolated bacterial colonies were suspended in 5ml of sterile distilled water and inoculated into the API 20E test strip microtubes. Water was added to the incubation tray to provide a humid setting and the strips were incubated at 37 37 °C for 18 hours after capping them with the provided plastic lid. TDA (Tryptophan deaminase), JAMES, and VP (Voges-Proskauer test for detection of acetoin) (1 and 2) reagents were added to the TDA, IND (Indole Test), and VP microtubes respectively after incubation, and the results were recorded as 7-digit number (excluding the oxidase test). Bacterial samples were characterized and identified with APi LAB Plus V.3.3.3 [32].

API 20NE was performed on all oxidase positive isolates according to the manufacturer's instructions as follows; isolated bacterial colonies were dispersed into 5ml of sterile saline solution (0.85% NaCl) with a turbidity of 0.5 McFarland. API 20NE microtubes NO_3 to PNPG (4-nitrophenyl-βD-galactopyranoside) were inoculated with the saline suspension. 200 µL of the remaining saline suspension was introduced to the API AUX medium and dispensed into GLU (fermentation of glucose test) to PAC (phenylacetic acid) microtubes. Water was added to the incubation tray to provide a humid setting and the strips were incubated at 37 °Cfor 18 hours after capping them with the provided plastic lid. NIT (1 and 2), JAMES reagents were added to the NO_3 (Potassium nitrate)and TRP (L-tryptophane) microtubes respectively after incubation, 2-3 mg of zinc were then added to the negative NO_3 microtubes for confirmation. The results were recorded as 7-digit number (excluding the oxidase test). Bacterial samples were characterized and identified with APiLAB plus V.3.3.3[32].

2.6 Statistical Analysis

The experimental design was a randomized complete block design. The effect of time and temperature combination on Extract Release Volume, Moisture Content, and pH were evaluated using one-way Analysis of variance- ANOVA, where ($p< 0.05$) indicated significant difference between the treatments. Duncan's multiple range test was carried out to determine homogeneous groups. And two-way ANOVA was used to determine the effect of treatments on microbiological content. All ANOVA analysis were performed using IBM SPSS V 22.

III. RESULTS AND DISCUSSIONS

3.1 Extract Release Volume

Extract release volume is a procedure used to indicate spoilage of beef based on the amount of aqueous extract released from a slurry of meat, when allowed to pass through filter paper for a given period of time.

In this study means of all tested samples are expressed in Table.2,where different subscripts denote means of significant difference. Extract release volume ranged between a minimum of 41.00ml and a maximum of 70.67 ml. Raw steak reported 70.67 ml which was the highest. Treatment 1 reported a significantly different ERV than that the rest of treatments and so did treatment 9. A decrease in ERV was noted as cooking temperature and storage duration increased and it reached a minimum for reheated, ready to serve final product at 10 days of chilled storage. The effect of the treatments(Table 3) was significant (p value <0.01) in ERV results as samples were subjected to higher temperatures during the reheating stage and as cold storage duration escalated.

Treatments 2, 3, 4 and 5 reported no significantly different ERVs. This implied that cooking temperature of 57°C in combination with different storage durations had no effect on ERV. Also, treatments 6 and 7 reported no significantly different ERVs. This explains that short durations of cold storage had no effect on extraction release volume. Treatment 5, however, reported a significantly different ERV than treatments 6, 7, 8 and 9. This explains as cooking temperature increased, from 57°C to a reheating temperature of 63°C, ERV decreased. Treatments 7,8 and 9 reported significantly different ERVs proving that prolonged chilled storage of 5 days and above resulted in a decreased extract release volume. The sinusoidal pattern displayed in ERV results may be attributed to deteriorating protein capturing water in the hydrated state or to the accumulation of water vapor on the steak sample during refrigeration and reheating or to an experimental error.

In a similar studies Anandh [17] described the gradual significant decrease of ERV in vacuum packed boiled restructured meat rolls with prolonged refrigerated storage might be attributed to the increase in microbial population. Jay *et al*., [21], explained that fresh beef releases high volumes of extract as opposed to spoiled meat. Additionally, in a study on meat processing, scientists explained that as meats undergo microbial spoilage,

proteins hydrolyse completely bringing down the extract release volume [33].

Nevertheless, all samples exhibited a volume above 25ml indicating good quality of meat as per standards of Food Safety of India which are internationally recognised and easily accessible standard[30].

3.2 Moisture Content

Moisture content values decreased gradually from a top of 77.5% for raw beef to a low of 58.8% for reheated steak held at cold storage for 10 days, as shown in Table 2. Statistical analysis indicated a significant difference in moisture content for raw (treatment 1), cooked (treatment 2, 3, 4, 5) and reheated ready to serve meat (treatment 6,7,8, 9). No significant difference was reported between samples that were only cooked and held at cold storage for 6 hour, 48 hours, 5 days and 10 days. Also, the difference between samples that were reheated after the storage time aforementioned was not significant. This indicates that reheating caused a decrease in moisture content for sous-vide cooked steak as opposed to cooking. On the other hand, different cooking temperatures and duration of chilled storage had no effect on the extract release volume. The slight insignificant rise in moisture content in treatment 5 is probably due to an experimental error.

Previous research described results comparable to this study. Moisture content of meat cooked using three different methods (pressure, microwave and atmospheric cooking) decreased regularly. This was attributed to the fact that as meat cooked progressively the water was being forced out [34]. Moisture content decreased gradually but not significantly during 30 days of storage in a study on vacuum packed restructured buffalo meat rolls [17]. Roldan *et al.*, [35] also reported a difference in moisture content in sous-vide lamb cooked at higher temperatures. Samples cooked at lower temperatures exhibited higher moisture content

3.3 pH

Changes in pH at different stages of cooking for this study are given in Table 2.The highest mean pH was 6.3, reported in sous-vide cooked beef steakchill stored for 6 hours. The lowest mean pH was 5.90, reported in sous-vide cooked samples after 5 days of chilled storage. Analysis of variance showed that different treatments had no significant effect (p value= 0.137) on the pH of meat where samples held at cold storage for 6 hours reported very similar pH readings to those held for 10 days. The pH of beef steak in this study didn't exceed 6.03, which is still within the acceptable range. Since pH plays a role in media for bacteria, the insignificant difference in results suggest a low microbiological activity throughout the samples.

Similar results were depicted in previous studies. When beef pH exceeds 6.0 within 24 hours of harvest, meat quality deteriorated, consumers' eating experience became undesirable and economic losses increased[36]. Özcan[34]studied the effect of different cooking times and treatments (atmospheric, pressure, microwave cooking) on the physical and chemical attributes of ready to eat meat. pH differed significantly between different cooking methods. However, meat of highest quality was indicated by a pH range of 5.7 to 6.0.

Other studies explained that in rested animals the conversion of glycogen to lactic acid causes a depression in pH from 7.4 to 5.6. In fatigued animals, glycogen was utilized, and less lactic acid was formed. Consequently, meat from stressed animals had a pH above 6 upon completion of rigor mortis and spoil faster[37]. This made meat more susceptible to bacterial, mould and yeast spoilage, whereas microorganisms grew best at pH value of 6.6- 7.5[22].

3.4 Microbial Assessment

The mean microbiological population for sous-vide steak determined at different stages of cooking and cold storage duration is presented in Table 5.

The highest reported microbial count was seen on HBI (354,900 cfu/g) and LB plates (26,040cfu/g), particularly in raw samples that have not been subjected to a heat treatment or chilled storage. The lowest count (zero cfu/g) was detected in cooked samples subjected to 6 hours of cold holding post sous-vide cooking. This pattern was sustained along the rest of the samples and no bacterial organisms were detected throughout the rest of the process.

Hence aerobic viable count and overall count detection in raw steak that wasn't subjected to heat treatment and chilled storage conditions decreased significantly ($P< 0.01$) with increasing heat treatment and reached a zero-organism showing that sous-vide cooking decreased the microbiological load to zero. While no increase was detected as refrigerated storage period advanced.

However, total coliforms, faecal coliforms, *Staphylococcus aureus*, *Clostridium*, *Salmonella* and *Shigella* were not detected at any stage of sous-vide cooking procedure nor at short and prolonged refrigerated storage conditions.

The aforementioned results signify that the time-temperature combination in the cooking phase of this sous-vide cooking and 6 hours of chilled storage at 1-3 °Cwere suitable enough to eradicate the spoilage bacteria and

pathogens of interest. The zero microorganisms observed at the rest of the stages of sous-vide cooking and chilled storage denotes that no cross contamination has emerged throughout the process and that reheating and prolonged chilled storage did not affect the safety of the final product.

The very low microbial counts recorded in this study were in accordance or the Lebanese Standards for Food Safety [38] except for the total viable count in raw steak samples which was reduced to zero microorganisms by the first treatment. However, API experimental procedures identified other oxidase negative and oxidase positive bacteria that are worth mentioning and they included *Pasteurella pneumotropica* and *Chryseonomasluteola* in raw steak as presented in Table6.

In a similar study on the microbiological safety and quality of foods processed by sous-vide for commercial catering, results showed that non-spore forming pathogenic bacteria had very low survival rates in sous-vide cooked beef products [39]. Accordingly, the scientist justified the cooking process as limiting the risk associated with these microorganisms as long as raw materials of good microbial quality are used, and the final products are restricted to low storage temperatures.

Babur *et al.*,[40] studied the microbiological quality characteristics of sous-vide cooked meat at different time combinations (2 and 4 hours) at 70°C. Microbiological analysis was performed after 0, 3, 7 days cold storage. None of the tested microorganisms were detected in sous-vide cooked meat refrigerated for 7 days. The results were attributed to good hygienic practices accompanied by proper cooking and storing temperatures.

Anandh[17] studied the shelf life of boiled restructured buffalo meat rolls in refrigerated storage under vacuum packaging conditions. Spoilage bacteria tested in vacuum packed buffalo meat reported an increase in microbial counts of psycrotrophs, *E.-coli*, *Staphylococcus*, *lactobacillus*, yeasts and moulds with increasing storage time. However, the increase was well below the standard of cooked products.

Roldan *et al*. [35]found that very short time-temperature combinations were enough to pasteurize sous-vide cooked lamb. While very low count of LAB, psychorotrophs and Enterobacteriaceae at prolonged refrigerated storage was detected in sous-vide cooked pork loin at 70°C for 11 hours and stored for 10 weeks at 2°C[4].

1. SUGGESTED HACCP PLAN

In consideration of the deficiency of national food safety standards, and a centralized integrated approach to control food safety hazards within a food business that implement sous-vide cooking, the suggested HACCP plan presents a helpful tool to ensure safe sous-vide practices for food businesses. HACCP is an internationally recognized food safety system that is recommended by World Health Organization [41].

4.1Product description

Steak is slice of meat cut from the fleshy part of a beef carcase. It is generally cut across the muscle fibre of a large section of beef and may include a bone. Most steaks come from three prime areas of a cow; short loin, tenderloin and the ribs [42]. A detailed product description is exhibited in table 2.

4.2 Preparation of Sous-vide Steak

Raw vacuum-packed tenderloins are unpacked, drained, trimmed and cleaned then covered with stockinets. They are then processed in two phases. In phase one, tenderloins are fully cooked, where they were seared, condiments were added, and they are all vacuum packed then sous-vide cooked in a steam oven followed by chilling and refrigeration. In phase two, after being held at chilled storage for 6 hours, 48 hours,5 days and 10 days, steak was unpacked, portioned then reheated. Table 4 explains the steps at which critical control point occur including the associated hazards, control measures, critical limits, monitoring tests and frequencies as well as corrective actions to be taken. Fig. 2 exhibits a flow diagram including steps at which critical control points appear.

2. FIGURES AND TABLES

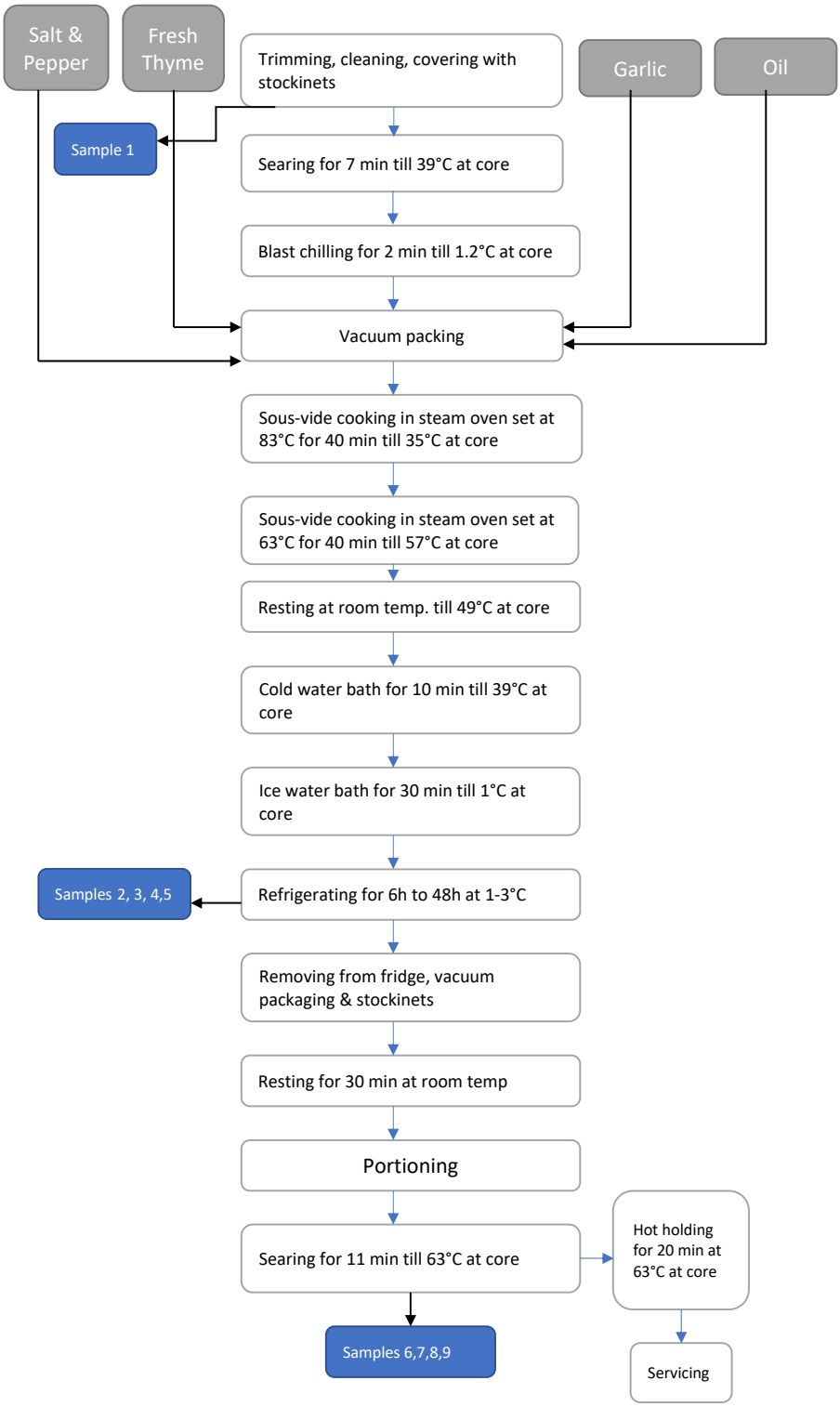

Fig. 1: Process flow diagram for the sous-vide steak showing stages where sampling took place

Fig.2: Process flow diagram for sous-vide steak

Table 1. Sample name and description.

Name	Treatments	Description
Phase 1: Cooked (57 °C at core)		
Sample 1	**1**	collected after cleaning meat and before covering with stockinets. This will be the control sample of the batch tested to identify the original state of the steak prior to handling and heat treatment
Sample 2	**2**	collected after 6 hours of cold holding post sous-vide cooking
Sample 3	**3**	collected after 48 hours of cold holding post sous-vide cooking
Sample 4	**4**	collected after 5 days of cold holding post sous-vide cooking
Sample 5	**5**	collected after 10 days of cold holding post sous-vide cooking
Phase 2: Reheated (63 °C at core)		
Sample 6	**6**	collected after portioning and reheated post 6 hours cold holding
Sample 7	**7**	collected after portioning and reheated post 48 hours cold holding
Sample 8	**8**	collected after portioning and reheated post 5 days cold holding
Sample 9	**8**	collected after portioning and reheated post 10 days cold holding

Table 2. Product Description for a HACCP Plan

Product name(s)	Sous-vide Steak
Important product characteristics	Average composition of Steakper 100 g of edible portion is 66g water, 27g protein and 8g fat per 100g [37] pH is 5.4- 5.8 [41] No preservatives are used
Intended use	*Sous-vide Steak* is prepared for either immediate consumption or long refrigerated storage It is served as a main meal and consumed by general public
Packaging	Served and dispensed on plates
Shelf life	10 days in the refrigerator (below 5 °C*)
Prepared / sold in	Restaurants, hotels, homes
Labelling instructions	Keep refrigerated (below 5 °C*)
Special distribution control	Transport, store, and display refrigerated (below 5 °C) under hygienic conditions

* As recommended by applicable Codex alimentarius standards for refrigerated foods [41, 43].

Table 3. Ingredients

Ingredients	Codex Standard
Beef Tenderloin	As per Codec STAN CXS 88-1981. AMMENDED IN 2019
Salt	As per CODEX STAN 150- 1985 [10]
Black Pepper	Freshly Ground No Codex standard available
Fresh Thyme	No Codex standard available
Garlic cloves	No Codex standard available
Olive oil	*V*egetable oils As per CODEX STAN 210- 2003 [44]

Table 4. HACCP Chart for Sous-vide Steak Production

Step	Hazard	Control Measure	CCP	Critical Limit	Monitoring		Corrective action
					Test	Frequency	
Meat	*Biological* Disease causing microorganisms	Purchase form reputable source During transport & storage temperature constant between 1-4 °C Check upon delivery. For proper shipping conditions-temperature	1	Reputable source and conformance to local specification of meat Transport and storage temperature between 1-4 °C Absence of bones	Check source certificates are consistent with specification Check fridge temperature	Each batch	Reject and change supplier
Trimming	*Physical* Foreign matter from packaging	Use well maintained equipment GMPs	1	Presence of foreign matter Adherence to GMPs	Visual examination for foreign matter	continuous	Remove for matter if possible, discard if not
Salt & Pepper	*Physical* Foreign matter from packaging *Biological* Moulds	Purchase from reputable source Sieve Visual inspection	2	Absence of impurities and foreign matter Mould growth	Ensuring purchase from reputable source Checking sieves Visual inspection	Each batch	Re-sieve salt Discard mouldy pepper
Fresh Thyme	*Biological* Disease causing microorganisms	Decontaminate using sanitizer	3	Dust and soil on produce Free available chlorine (not more or less than 0.05g/l	Measurement of chlorine in water using a certified technique	Each washing step	Re-wash with unchlorinated water in case of high doses

				to 0.1g/l) with a contact time of 30 seconds			
Cooking	*Biological* Growth of Spoilage/ pathogenic microorganisms	Avoid over cooking and charring Discard black crusts	4	Absence of charred crusts	Checking cooked meat during cooking	Each batch	Discard black crusts Readjust oven temperature and or grill
Storage	*Biological* Growth of Spoilage/ pathogenic microorganisms	Preserve meat in refrigerator at temperature between 1-4 °C GMP	5	Storage temperature between 1-4 °C	Temperature measurement	Continuous	Adjust temperature
Hot Holding	*Biological* Growth of Spoilage/ pathogenic microorganisms	Warm holding (60°C / max 1h)	6	Holding at 60°C / max 1h	Temperature and time measuring	Continuous	Adjust holding temperature to the proper level

IV. CONCLUSION

The results obtained in this study pointed out that cooking had a positive impact on quality and safety attributes of sous-vide cooked steak held at different chilled storage durations. Studied parameters were in desirable range and within the Lebanese Safety Standards, verifying the adequacy of the processing method. Further studies can be done on organoleptic and nutritional properties of sous-vide steak.

ACKNOWLEDGEMENT

Thank you to Sofil Catering, Beirut Waterfront, Beirut, Lebanon for providing the samples that were tested for this study.

REFERNCES

[1] Creed, P. G., (1998).Sensory and Nutritional Aspects of Sous-vide Processed Foods.*Sous-vide and Cool Chill Processing for the Food Industry*,57-88.Aspen Publishers Inc. USA.

[2] Florkiewicz, A., Ciska, E., Florkiewicz, A. F., Topolska., K. (2017). Comparison of sous-vide methods and traditional hydrothermal treatments on GLS content in Brassica vegetables. *European Food and Research Technology*, *43*,507-1517.

[3] Briggs, W., Papadopoulos, A., Wilcock, A. (2019). Sous-vide cooking in restaurants: A qualitative study of Ontario public health inspector knowledge, experience, practices and needs. *Food Production Trends*,*39*(1),51-61.

[4] Diaz P, Nieto G, Garrido MD and Ban˜o´n S. (2008). Microbial, physical–chemical and sensory spoilage during the refrigerated storage of cooked pork loin pro- cessed by the sous-vide method. *Meat Science*, *80*(2),287–292.

[5] El Jardali, F., Hammoud, R., Kamleh, R., Jurdi, M. (2014). *Protecting consumers in Lebanon: The Need for Effective Food Safety System.* Faculty of Health Sciences, American University of Beirut, Lebanon. viewed on 23rd February, 2020 from: <https://www.aub.edu.lb/k2p/Documents/K2P%20BN%20Food%20Safety%20English.pdf>

[6] Baldwin, D.E. (2011). Sous-vide cooking: A review. *International Journal of Gastronomy and Food Science*, *1*(1),15-30.

[7] Hilal, Y., Jbeily, C., Othman, H., Rayess, A. &Zaknoun, R. (2017). Sous Vide Food Safety: *A Statistical Approach. International Journal of Innovative Research in Food Sciences, Applied Sciences, and Environmental Studies*. Doi No.: 01.1617/vol4iss9pid1930

[8] SVAC (Sous vide Advisory Committee). (1991). *Codes of Practice for Sous Vide Catering Systems*,12, SVAC, Tetbury Gloucs. UK.

[9] Church, I. Paesons, A. (2001) The Sensory Quality of Chicken and Potato Products Prepared Using Cook-Chill Sous Vide Methods. *International Journal of Food Science & Technology (35)*, 155-162.https://doi.org/10.1046/j.1365-2621.2000.00361.x

[10] Church, I. (1998). The sensory quality, microbiological safety and shelf life of packaged foods.*Sous Vide and Cook–Chill Processing for the Food Industry*, 190–205. Aspen Publishers.

[11] Garcı´a-Linares, M.C., Gonzalez-Fandos, E., Garcı´a-Ferna´ndez, M.C., Garcı´a-Arias, M.T. (2004). Microbiological and nutritional quality of sous vide or traditionally processed fish: influence of fat content. *Journal of Food Quality*,*27*(5),371–387. https://doi.org/10.1111/j.1745-4557.2004.00676.x

[12] Gormley, T. R. Tansey, F. S. (2002). Sous Vide/Freezing Technology for Ready-Meal Components. *American Society of Agriculture and Biological Engineers.*(doi: 10.13031/2013.9792) @2002

[13] Creed, P.G. Reeve, W. (1998). Principles and Applications of Sous Vide Processed Foods.*Sous Vide and Cook–Chill Processing for the Food Industry*, 25-26. Aspen Publishers. ISBN 0-7514-0433-0/ 978-07514-0433-3.

[14] Shabbir, M. A. (2015). Effect of thermal treatment on meat proteins with special reference to heterocyclic aromatic amines (HAAs). *Critical Reviews in Food Science and Nutrition*: *55*(1),82-93.

[15] Gomez I., Ibanez F. C., Beriain, M. J. (2019). Physicochemical and sensory properties of sous-vide meat and meat analog products marinated and cooked at different temperature-time combinations. *International Journal of Food Properties*,*22*(1),1693-1708.

[16] Botinestean, C., Keenan, D., Kerry, J., Hamill, R. (2016). The effect of thermal treatments including sous-vide blast freezing and their combinations on beef tenderness of M.semitendinosus steaks targeted at elderly consumers. *Food Science and Technology*,*47*,154-159.

[17] Anandh M. A. (2015). Shelf life of broiledrestructure buffalo meat rolls in refrigerated storage under vacuum packaging conditions. *Journal of Applied Animal Research*, *43*(3),318–323.

[18] Thorsell, U. (1996). Quality of meat prepared by the sous-vide method.*Proceedings of Second European Symposium on Sous Vide*, 10-12, Alma University Restaurants/FAIR, University of Leuven, pp. 387-388.

[19] Hansen, T.B., Knochel, S., Juncher, D. and Bertelsen, G. (1995). Storage characteristics of sous-vide cooked roast beef. *International Journal of Food Science and Technology*, *30*,365-378.

[20] Choain, F. and Noel, P. (1989).Le Sous-Vide en Cuisine - Des fondementsscientifiques aux applications pratiques, *Editions Jacques Lanore*, 207.

[21] Jay J. M., Kontou K. S. (1964). Evaluation of extract release volume phenomenon as a rapid test for detecting spoilage in beef. *Applied Microbiology*, *12*(4),378-383.

[22] Jay. J. M. Loessner, M.J. Golden, D. A. (2005). *Modern Food Microbiology* (7th ed.). Springer Science + Business Media Inc.

[23] Korsak N. Daube, G. Chahed, A, Jolly, S. Vindevogel, H. (1998). An Efficient Sampling Technique Used to Detect Four Foodborne Pathogens on Pork and Beef Carcasses in Nine Belgion Abattoirs. *Journal of Food Protection*. *61*(5), 535-541. https://doi.org/10.4315/0362-028X-61.5.535

[24] Kilibarada, N., Brdar, I., Baltic, B., Markovic, V., Mahmutvic, H., Karabasil, N., Stanisic, S. (2018). The Safety and Quality of Sous Vide Food. *Meat Technology*. 9(1), 38-45. https://doi.org/10.18485/meattech.2018.59.1.5

[25] Sebastia C., Sorian, J. (2010). Microbiological quality of sous-vide cook-chil preserved food at different shelf life. *Journal of Food Processing and Preservation*, *34*(6), 964-974.

[26] Florkiewicz, A., Ciska, E., Florkiewicz, A. F., Topolska., K. (2017). Comparison of sous-vide methods and traditional hydrothermal treatments on GLS content in Brassica vegetables. *European Food and Research Technology*, *243*,1507-1517.

[27] Food and Drug Administration (2017).*Supplement to the 2017 Food Code*, U.S. Public Health Service, College Park, USA. Viewed on 25th February 2020 from: <https://www.fda.gov/media/133749/download>

[28] NSW Food Authority. (2012). Sous Vide. Food Safety Precautions for Restaurants, pp 1-31.

[29] BC Centre for Disease Control Environmental Health Services and the BC Sous-vide Working Group. (2014). Guidelines for restaurant sous-vide cooking safety in British Columbia.

[30] FSSAI (2012). Manual of Methods of Analysis of Foods. *Meat & Meat Products Fish and Fish Products*, Ministry of Health and Family Welfare. New Delhi, India.

[31] AOAC (2000). *Official methods of analysis of AOAC International. 1, agricultural chemicals, contaminants, drugs / edited by William Horwitz.*, CEU Repositorio Instituciona. Viewed on 3rd February 2020 from: http://www.temoa.info/node/111911

[32] Isenberg, H, D., Garcia, L.S. (2007). *Clinical Microbiology Procedures Handbook*, ASM Press, Washington D.C., USA.

[33] NPCS Board of Consultants & Engineers. (2013).*The Complete Technology Book on Meat, Poultry and Fish Processing*, Second Edition, India.

[34] Özcan, A. U., Bozkurt, H. (2015). Physical and chemical attributes of a ready-to-eat meat product during the processing: effects of different cooking methods, *International Journal of Food Properties*,*18*(11), 2422-2432.

[35] Roldan M., Antequera T., Hernandez A., Ruiz J. (2014). Physicochemical and microbiological changes during the refrigerated storage of lamb loins sous-vide cooked at different combinations of time and temperature, *Food Science and Technology International*, *21*(7), 512-522.

[36] Tapp, W. N., Christjohn, C. T., Grigging, D. A., Bratcher, C. L. (2017). Evaluation of meat quality on high pH strip loins injected with buffered acetic acid, *Meat and Muscle*

Biology,1, 218-226.

[37] FAO. Meat quality, *Agriculture and Consumer Protection Department, Food* And Agriculture Organization of The United Nations, viewed on 22nd February, 2020, from: <http://www.fao.org/3/t0562e/T0562E02.htm>

[38] Sahili T. (2006). Decision of the Minsiter of Agriculture no.1/57; Sampling methods of food products of animal origin, *Lebanese Official Journal*, (12), 1097-1108 (in Arabic)

[39] Bolton, D. J. (1998). The microbiological safety and quality of foods processed by the sous-vide system as a method of commercial catering, *The National Food Centre*, 1.

[40] Babur, T. E., Tekinsen, K. K., Gurbuz, U. (2019). Some quality qualifications of cooked meat sous-vide in the storage process, *Manas Journal of Engineering*, *7*(1), 34-41.

[41] World Health Organisation. (2008). Regional Office for the Eastern Mediterranean & Regional Center for Environmental Activities, Hazard analysis and critical control point generic model for some traditional foods; A Manual for the Eastern Mediterranean Region.

[42] Gallary, C. (2019). Kitchen, A complete guide to steak, viewed on 3rd of February, 2020, from: <https://www.thekitchn.com/shopping-for-steak-here-are-the-4-cuts-you-should-know-207368>

[43] FAO. (1997). Hazard analysis and critical control point (HACCP) system and guidelines for its application, Annex to CAC/RCP 1-1969, Rev. 3, viewed on 2nd March 2020 from < http://www.fao.org/3/y1579e/y1579e03.htm>

[44] FAO and WHO. Codex alimentarius official standards, *International Food Standards*, viewed on 22nd February 2020 from <http://www.codexalimentarius.net/web/standard_list.do?lang=en>