THESIS OF THE DOCTORAL DISSERTATION

Endrit Hasani

Budapest,

2023

Hungarian University of Agriculture and Life Sciences

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

Endrit Hasani

Budapest

2023

PhD school

Name: PhD School of Food Science

Field: Food Science

Head: Dr. Livia Simonné Sarkadi
Professor, DSc
Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Department of Nutrition Science

Supervisors: Dr. István Dalmadi
Associate professor, PhD.
Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Department of Livestock Products and Food Preservation Technology

Dr. György Kenesei
Senior lecturer, PhD.
Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Department of Livestock Products and Food Preservation Technology

Approval signature of Head of the doctoral school and supervisors:

The candidate has fulfilled all the conditions prescribed by the Doctoral School of Hungarian University of Agriculture and Life Sciences, the comments and suggestion at the thesis workshop were taken into consideration when revising the thesis, so the dissertation can be submitted to a public debate.

…… ……..……..

Signature of Head of PhD School Signature of Supervisors

INTRODUCTION AND OBJECTIVES

According to FAO, roughly half of all meat produced globally is wasted at the household level of the supply chain (FAO, 2011). A significant portion of waste in households is attributed to weight loss during heat processing treatment (Parfitt et. al. 2010). Conventional heat treatments used for meat processing such as steaming, boiling or grilling have been reported to not only increase the weight loss but also reduce the content of several micronutrients such as vitamins, amino acids and minerals (Silva et. al. 2017). Consumers are increasingly interested in mild thermal processed ready-to-eat (RTE) foods due to their excellent sensory qualities, ease of preparation, and high nutritional value. Among mild thermal processing techniques sous vide has received increased attention from researchers in recent years as one of the mild heat processing methods for the production of high quality ready-to-eat meat products. The traditional sous vide method employs only one controlled temperature in the range of 55-70°C (Baldwin, 2012). Different meat proteins have different denaturation temperatures and are responsible for different meat quality properties. Therefore, proper selection of temperature and time in sous vide processing to tailor the denaturation of meat proteins can play a major role on the main quality attributes of meat.

Meat contains endogenous proteolytic enzymes, which highest activity has been reported to be at temperatures ranging from 40 to 50 °C (Christensen et. al. 2013). Previous research found that at these temperatures, desmin degradation is higher due to high activity of proteolytic enzymes, indicating an extension of meat tenderization (Christensen et. al. 2011; Ertbjerg et. al. 2011). Therefore, using proteolytic enzyme activation temperatures (between 40-55 °C) as the first step temperature in the sous vide processing could potentially improve meat tenderness and other quality attributes. It was reported that breast muscles in chicken meat tend to be tougher and crumby

compared with chicken legs muscles (Zhang et. al. 2020). Thus, this cooking method may be used to produce new poultry-based ready-to-eat foods with higher tenderness. Based on our knowledge, there have been no studies that investigate the effect of a two-step temperature in sous vide processing of chicken breast meat.

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

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

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

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

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

MATERIAL AND METHODS

Fresh chicken breast muscles (24 hours post-mortem) were skin-off, trimmed of fat and cut in uniform weight of 129.6 ± 2.4 g and thickness of 2.0 ± 0.3 cm. The samples were vacuum packaged in 90 μm PA/PE pouches (200 mm × 250 mm) using a vacuum machine (Multivac C100, MULTIVAC Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany) and were randomly divided in treatment groups.

Samples were cooked at one-step temperature of 60 °C and two-step temperatures treatments by combining a first step low temperature of 45 °C or 50 °C and an end step temperature of 60 °C in different treatment time ratios (three levels: 0:1,1:2, 1:1) and total treatment times (two levels: 120 and 180 min) (Table 1).

Table 1: Sous vide treatments applied in the study.

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

A two-way completely randomized design was applied in the study with three replicates for each sous vide treatment. Sous vide treatments were conducted using two thermostatic water baths (Labor Müszeripari Müvek LP507/01). The internal temperature of the samples during sous vide processing was

monitored using a needle probe T-type thermocouple which was placed at the geometric center of vacuum packaged chicken breast sample. After treatment chicken breast samples were cooled down in ice-cold water (1 °C) and were maintained at refrigerated conditions (2 °C) to achieve a temperature of less than 4 °C for 6 h according to the recommended guidelines (BC Centre for Disease Control, 2016).

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

Determination of pasteurization values

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

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

where t refers to the heating time (min), T_{ref} is the reference temperature, T indicates the measured core temperature (°C) during heating, and z indicates the number of degrees of temperature to enhance the thermal death rate of the target microorganism by a factor of 10. Calculations were done based on five independent batches for each group treatment.

Physico-chemical attributes measurements

The measurement of moisture content of chicken breasts was performed in triplicates following the standard AOAC International 950.46 method (AOAC. 2005). Cooking loss was determined by subtracting the weight of cooked chicken breast from the weight of initial raw chicken breast. The pH value of the chicken breasts was measured before and after sous vide processing using a pH meter (Testo-AG, Germany). The measurements were conducted in triplicate. The color attributes of meat samples were determined using the CIELAB scoring system (CIE, 1986). A CR-400-type colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) was used to measure the lightness (L^*), redness (a^*), and yellowness (b^*) values of the samples after white calibration of the instrument. Five parallel readings were performed for each sample.

Protein solubility

Protein solubility parameters were assessed using the method applied by Warner et al. (1997). Protein concentrations of the obtained supernatants were assessed following the Bradford method (Bradford, 1976) using the BSA (bovine serum albumin) as a standard.

Texture measurement

Warner-Bratzler shear force

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

Texture profile analysis (TPA)

The texture of cooked samples was analyzed through texture profile analysis (TPA) following the method applied by Bourne (1976). Texture Exponent 32 software for Windows (Stable Micro System) was used for processing data to assess the TPA attributes: hardness (N), springiness (mm), gumminess (N), cohesiveness (-), and chewiness (N * mm).

Lipid oxidation

Lipid oxidation of chicken breast samples was assessed by measuring the thiobarbituric Acid Reactive Substances (TBARS) values using the method from Dias et al. (2013) with few modifications. TBARS values represented the mean of triplicate measurements for each sample and were reported as mg malondialdehyde/kg of meat sample.

Odour acceptability

During the storage experiment the pouches were aseptically opened and the samples were evaluated for odour acceptability. The samples were scored using a five-point scale: 1 = acceptable, 2 = slightly acceptable, 3 = neutral, 4 = slightly unacceptable, 5 = unacceptable. The panel consisted of 5 members of researchers and teachers familiar with sensory evaluation of cooked chicken breast.

Microbiological analysis

The microbiological challenge test was used to determine the pasteurization level of the chicken breast samples treated with the studied sous vide treatments. *Enterococcus faecalis* was selected as a target microorganism to conduct the thermal inactivation challenge tests due to its high thermal heat resistance. Enterococcus faecalis counts were also tested during the storage of sous vide treated chicken breast at temperature of 4° C, 10 °C and at – 20 °C for up to 21 days, for the selected sous vide treatments. Three replicates were performed for each treated sample.

Approximately 10 grams chicken breast sample was taken in a stomacher bag and inoculated with 0.1 ml cell suspension (10^8 CFU/ml) of *Enterococcus faecalis* B. 01312 in order to have an initial cell count of 6 log CFU/g in the samples. Then the chicken breast samples were vacuum packaged in 90 µm PA/PE pouches (200 mm × 250 mm) using a vacuum machine (Multivac C100, MULTIVAC Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany) and after sous vide treated in one of the treatment conditions.

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

Sensory analysis

Sensory analysis of sous vide treated chicken breast samples was performed for different sensory characteristics such as intensity of flavour, tenderness, juiciness, and overall acceptability. The sensory panel consisted of 11 professors and researchers familiar with sensory evaluation of cooked chicken breast. Nine-point hedonic scale was used to perform the sensory assessment.

Statistical analysis

The experimental data were analysed using IBM SPSS (Version 27.0, Armnouk, NY, 2020). Data were analysed using the analysis of variance (ANOVA) and

General Linear Model (GLM). Normality of the residuals was tested by Kolmogorov-Smirnov test ($p>0.05$). Homogeneity of error variances was accepted by Levene's test ($p>0.05$). Tukey's post hoc tests were run if homogeneity of variances was satisfied, and Games-Howell's method was used when this assumption was violated.

RESULTS AND DISCUSSION

Comparison between the effect of one-step and two-step sous vide on quality and microbiological attributes of chicken breast.

Chicken breast cooked with two-step sous vide treatments which contains a low first step temperature of 45°C or 50°C and an end step temperature of 60° C exhibited lower cooking loss compared to one-step sous vide treated ones within the same total treatment time. On the other hand, moisture content of two-step sous vide chicken breast was significantly higher than one-step sous vide ones, only when the first step temperature 45°C was applied for half or two third of total treatment time (120 or 180 min). Cooking loss was significantly correlated with sarcoplasmic and total protein solubility of sous vide cooked chicken breast ($R=-0.744$ and $R=-0.715$, both with $P < 0.01$). Therefore, both sarcoplasmic and total protein solubility were significantly higher in two-step sous vide chicken breast compared to one-step sous vide ones.

Two-step sous vide treated samples exhibited lower lightness values (L^* value) than one-step sous vide treated chicken breast due to lower protein denaturation. On the other hand, higher redness (a* value) and yellowness (b* value) were observed in two-step sous vide cooked chicken breast compared to one-step sous vide ones. However, redness values of either one or two-step sous vide cooked chicken breasts did not exceed the pink color threshold limit ($a^*= 3.8$). From the pairwise comparison on color attributes between chicken breast cooked with one-step sous vide and the two-step sous vide within the same total treatment time, in most of cases color difference can be perceived even by an unexperienced observer.

Chicken breast cooked with 120 min two-step sous vide had lower hardness, gumminess and chewiness values compared to one-step sous vide ones. However, chicken breast cooked with 180 min two-step sous vide had lower shear force and chewiness values compared to one-step sous vide. Lipid oxidation rates of

chicken breasts were lower when cooked with two-step sous vide compared when cooked one-step sous vide. However, lipid oxidation rates (TBARS) all the studied treatments were under the sensorial threshold limit of 1 mg MDA/kg sample.

Regarding microbiological safety, both one-step and two-step sous vide treatments had higher pasteurization values than required to be able to reduce by a 6 log the vegetative cells of two main pathogens of interest *C. perfringens* and *L. monocytogenes* in chicken breast. Meanwhile as expected, none of the studied sous vide treatments resulted in required pasteurization values to reduce 6 log of *C. botulinum* spores. On the other hand, based on microbiological analysis results, only the one-step sous vide treatments (T1 and T8) achieved the pasteurization performance criteria by inactivation of more than 6 log CFU/ g of *Enterococcus faecalis* NCAIM B. 01312. Meanwhile the pasteurization target criterion of sous vide treatment to pasteurize chicken breast (defined by the incidence of the tested microorganism in poultry meat) was achieved in 120 min treatments (T2, T3) and 180 min treatments (T9, T10, T11, T12) which resulted in more than 3-log reduction of *Enterococcus faecalis* NCAIM B. 01312.

For consumers 120 min two-step sous vide treatments T2 and T3 would be more convenient for chicken breast cooking due to shorter treatment time compared to 180 min treatments. Therefore 120 min two-step sous vide treated chicken breast T2 and T3 which achieved sufficient inactivation of *Enterococcus faecalis* and had higher quality attributes (texture, lipid oxidation, cooking loss) were tested on different sensory attributes including flavour, color, tenderness and juiciness, and compared to one-step sous vide treated ones T1. Based on sensory results, one-step sous vide cooked chicken breast had higher flavour scores but lower tenderness, juiciness and overall acceptability scores compared to two-step sous vide ones. Sensory attributes of two-step sous vide cooked chicken breast

improved with longer application time of low temperature (50 °C), except for flavour.

Storage stability of one-step and two-step sous vide treated chicken breast

In the following experiment, the storage stability of the selected sous vide treated chicken breasts (T1, T2, and T3) was examined when stored at 4 ± 0.5 °C, at 10 ± 0.5 °C and at -20 ± 0.5 °C for up to 21 days. Cooking loss was significantly increased after 7 days of storage at 4 °C and at 10 °C in both one-step and two-step sous vide cooked chicken breast and did not change after 14 and 21 days ($P > 0.05$). This might be due to lower protein denaturation process in cooked chicken breast at the end of storage. On the other hand, no significant difference was observed in cooking loss between one and two-step sous vide treated chicken breast during frozen storage. Moisture content, redness and yellowness of either one-step or two-step sous vide cooked chicken breast did not change during storage at refrigerated or frozen conditions. On the other hand, lightness and pH values of chicken breast cooked with either one-step or two-step sous vide were increased during refrigerated storage but did not change during frozen storage.

There were no significant changes in protein solubility properties during refrigerated or frozen storage with the exception of total protein solubility of two-step sous vide chicken breast which increased only after 14 and 21 days of storage in both refrigerated conditions (4 and 10 °C) which can be explained by low protein denaturation and degradation processes at the end of storage.

Shear force values of both one-step and two-step sous vide cooked chicken breast were decreased after 7 days of storage at 4 °C and then remained steady till the end of 21 days of storage. Meanwhile the decrease of shear force values of both one and two-step sous vide cooked chicken breast stored at 10 °C continued till the end of storage and is attributed to residual proteases activity which remained active after heat treatment. Frozen storage at -20 °C had significantly decreased the shear force values on all the studied sous vide chicken breast ($P < 0.05$) (Figure

35, C). Similarly, Botinestean et. al. (2016) reported a positive effect of freezing on lowering the shear force values (increasing tenderness) of sous vide cooked beef steaks. From TPA parameters hardness, gumminess and chewiness of sous vide cooked chicken breast were reduced during storage at 4 °C and 10 °C ($P < 0.05$). Meanwhile no significant changes were observed on cohesiveness and springiness values of both one and two-step sous vide cooked chicken breasts during 21 days of storage at 4°C and 10 °C ($P > 0.05$). Hardness, gumminess and chewiness values of sous vide cooked chicken breast were significantly reduced after frozen storage at -20 °C as well ($P < 0.05$). Similarly, previous studies have reported that freezing technology can improve the texture attributes of sous vide cooked meat mainly hardness and chewiness (Botinestean et. al. 2016; Ji et. al. 2019). On the other hand, there was no frozen storage effect on cohesiveness and springiness of sous vide cooked chicken breast, with the exception of T1 treatment ($P > 0.05$).

TBARS values of both one and two-step sous vide treated chicken breast were significantly increased during 21 days of storage at 4 °C, 10 °C and -20 °C. It has been reported that the threshold level of TBARS for consumers to detect oxidative rancidity in meat is higher than 1 mg malonaldehyde per kilogram of sample (Baker et al. 1972). Based on our study results, this threshold level was exceeded in both one and two-step sous vide treated chicken breast after storage at 10 °C for 21 days. It can be emphasized that at storage at 4 and -20 °C both one and two-step sous vide cooked chicken breast were more stable regarding lipid oxidation compared with storage 10 °C, not exceeding the sensorial threshold of 1 mg MDA/kg of sample. Based on the results, odor of both one and two-step studied sous vide treated chicken breasts remained acceptable during 21 days of refrigerated storage at 4 °C and frozen storage at -20 °C with scores lower than 2. However, after 21 days of storage at 10 °C, two-step sous vide treated chicken breast T2 had slightly acceptable odour with acceptance scores of 2.6. Meanwhile,

two-step sous vide treated chicken breast T3 and one-step ones T1 after 21 days of storage at 10 °C had the highest acceptance scores of 3.1 and 4.5, respectively.

According to the results, *Enterococcus faecalis* NCAIM B. 01312 counts did not significantly changed during 21 days of storage at 4°C of sous vide treated chicken breast. This shows that sous vide treated chicken breast were microbiologically stable during 21 days of storage at 4 °C regarding *Enterococcus faecalis* NCAIM B. 01312. *Enterococcus faecalis* NCAIM B. 01312 counts of sous vide cooked chicken breasts had an increasing trend during 21 days of storage at 10 °C. A significant increase on *Enterococcus faecalis* NCAIM B. 01312 counts in T2 two-step sous vide treated chicken breast was observed only after 7 days of storage at 10 °C and after 14 days in samples cooked with T3 treatment ($P < 0.05$). *Enterococcus faecalis* NCAIM B. 01312 counts in sous vide cooked chicken breast were significantly decreased during 21 days of frozen storage at -20 °C ($P < 0.05$). From the two-step sous vide treatments, cooked chicken breast (T2) had significantly lower *Enterococcus faecalis* NCAIM B. 01312 counts compared to T3 cooked chicken breast before and after frozen storage at -20 °C ($P < 0.05$). This shows that chicken breasts cooked with the two-step sous vide treatment (T2) had the highest microbiological stability, after the traditional one-step sous vide one (T1 treatment).

NEW SCIENTIFIC RESULTS

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

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

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

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

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

CONCLUSIONS AND RECOMMENDATIONS

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

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

Two-step sous vide technique provides a valuable cooking alternative for elderly consumers as it significantly reduced the main texture parameters (shear force, hardness, chewiness, and gumminess), but at the same time preserved the moisture content, redness, and oxidative stability in chicken breasts. Furthermore, significantly lower cooking loss and increased protein solubility values were

observed in chicken breast cooked with the two-step sous vide compared to the ones cooked with the traditional one-step sous vide. Future studies need to be carried out to investigate the possible positive effect of two-step sous vide on protein digestibility of chicken breast which is related to protein solubility.

Two-step sous vide treatments were able to successfully inactivate the vegetative cells of two main pathogens of interest (*C. perfringens* and *L. monocytogenes*) in chicken breast based on the calculated theoretical pasteurization values. However as expected, none of the studied sous vide treatments were enough to inactivate the *C. botulinum* spores, thus proper refrigeration storage of these products is required. On the other hand, microbiological analysis showed that one-step sous vide treatments 120 and 180 min at 60°C successfully inactivated *Enterococcus faecalis* NCAIM B. 01312 in chicken breast. Meanwhile from the studied two-step sous vide treatments only 120 min treatments (T2 and T3) and 180 min treatments (T9, T10, T11, and T12) achieved the target pasteurization performance criterion of 3 log reduction of *Enterococcus faecalis* NCAIM B. 01312 in chicken breast. Regarding sensory attributes, two-step sous vide treated chicken breasts had less flavour but higher tenderness and juiciness than traditional one-step sous vide ones. Future investigations need to be done to examine the effect of different post treatments such as roasting, searing, or frying on sensory attributes of two-step sous vide cooked chicken breast.

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

that combination of two-step sous vide treatment and frozen storage provided better quality attributes of cooked chicken breast.

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

LIST OF JOURNAL PUBLICATIONS IN THE FIELD OF STUDIES

IF articles or Q1-Q4 articles in foreign language:

Hasani, E., Kiskó, G., Dalmadi, I., Hitka, G., Friedrich, L. F., & Kenesei, G. (2023). Effect of Two-Step Sous Vide Cooking and Storage on Microbiological and Oxidative Stability of Chicken Breast. *Foods*, 12(6), 1213. **IF: 5.561 (Q1)**

Hasani, E., Csehi, B., Tóth, A., Dalmadi, I., & Kenesei, G. (2023) Development of innovative hurdle systems using minimal processing techniques for meat preservation. *Journal of Hygienic Engineering and Design*, 41, 103-111. **IF: 0.16 (Q4)**

Hasani, E., Csehi, B., Darnay, L., Ladányi, M., Dalmadi, I., & Kenesei, G. (2022). Effect of Combination of Time and Temperature on Quality Characteristics of Sous Vide Chicken Breast. *Foods*, 11(4), 521. **IF: 5.561 (Q1)**

Hasani, E., Kenesei, G., & Dalmadi, I. (2021). Comparison of the single-step and double-step sous-vide treatment effect on the quality attributes of chicken breast•: A novel approach to sous-vide. *Progress in Agricultural Engineering Sciences*, 17(S1), 61-68. **(Q4)**

Hasani, E., Labidi, S., Mohácsi-Farkas, C. and Kiskó, G., (2021). Comparison of biofilm formation between non-pathogenic Listeria strains under different stress conditions. *Progress in Agricultural Engineering Sciences*, 16(S2), pp.73-80. **(Q4)**

REFERENCES

AOAC. (2005) Official Methods of Analysis, 18th ed.; AOAC International: Rockville, MD, USA; ISBN 0935584870

Baker, R.C., Darfler, J. and Vadehra, D.V., (1972). Effect of storage on the quality of chicken frankfurters. *Poultry Science*, 51(5), 1620-1624.

Baldwin, D.E., (2012). Sous vide cooking: A review. *International Journal of Gastronomy and Food Science*, 1(1), 15-30.

BC Centre for Disease Control (2016). Guidelines for Restaurant Sous Vide Cooking Safety in British Columbia. Available online: http://www.bccdc.ca/search?k=svguidelines_finalforweb.pdf (accessed on 10 March 2021)

Botinestean, C., Keenan, D.F., Kerry, J.P. and Hamill, R.M., (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. *LWT*, 74, 154-159.

Bourne, M.C. and MC, B., (1978). Texture profile analysis.

Bradford, M.M., (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254.

Christensen, L., Ertbjerg, P., Aaslyng, M.D. and Christensen, M., (2011). Effect of prolonged heat treatment from 48 C to 63 C on toughness, cooking loss and color of pork. *Meat Science*, 88(2), 280-285.

Christensen, L., Ertbjerg, P., Løje, H., Risbo, J., van den Berg, F.W. and Christensen, M., (2013). Relationship between meat toughness and properties of connective tissue from cows and young bulls heat treated at low temperatures for prolonged times. *Meat Science*, 93(4), 787-795.

Dias, M.V., Nilda de Fátima, F.S., Borges, S.V., de Sousa, M.M., Nunes, C.A., de Oliveira, I.R.N. and Medeiros, E.A.A., (2013). Use of allyl isothiocyanate and carbon

nanotubes in an antimicrobial film to package shredded, cooked chicken meat. *Food Chemistry*, 141(3), 3160-3166.

Ertbjerg, P., Christiansen, L.S., Pedersen, A.B. and Kristensen, L., (2012). The effect of temperature and time on activity of calpain and lysosomal enzymes and degradation of desmin in porcine longissimus muscle. In Proceedings of the 58th international congress of meat science and technology, Montreal, QC, Canada, 12-17.

FAO. (2011). FAO-World Livestock —Livestock in food security. Www.Fao.Org/Docrep

Ji, D.S., Kim, J.H., Yoon, D.K., Kim, J.H., Lee, H.J., Cho, W.Y. and Lee, C.H., (2019). Effect of different storage-temperature combinations on Longissimus dorsi quality upon sous-vide processing of frozen/thawed pork. *Food Science of Animal Resources*, 39(2), 240.

Parfitt, J., Barthel, M. and Macnaughton, S., (2010). Food waste within food supply chains: quantification and potential for change to 2050. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1554), 3065-3081.

Silva, F.L.; de Lima, J.P.; Melo, L.S.; da Silva, Y.S.; Gouveia, S.T.; Lopes, G.S.; Matos, W.O., (2017). Comparison between boiling and vacuum cooking (sous-vide) in the bioaccessibility of minerals in bovine liver samples. *Food Res. Int*., 100, 566–571.

Warner, R.D., Kauffman, R.G. and Greaser, M.L., (1997). Muscle protein changes post mortem in relation to pork quality traits. *Meat Science*, 45(3), 339-352.

Zhang, J., Bowker, B., Yang, Y., Pang, B. and Zhuang, H., (2020). Effects of deboning time and thawing method interaction on sensory descriptive profiles of cooked chicken breast and thigh meat. *LWT*, 120, 108939.