THESES OF DOCTORAL (PhD) DISSERTATION

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ENHANCEMENT OF THE MICROBIOLOGICAL QUALITY OF
***SOUS-VIDE* COOK-CHILL FOOD PRESERVATION SYSTEM**

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1. INTRODUCTION

Over the past decades, dietary habits of people have changed considerably. Consumers now prefer fresher, more natural, non-seasonal, „more comfortable", „safer" foods. The production of these types of products presents a major challenge to producers, manufacturers and distributors alike. Mild food production technologies represent a solution to this problem. The word „mild" means that „the food is preserved, while its nutritional and organoleptic properties remain the same, thus reducing the side effects of heat treatment – the main preservation method". Well-regulated, standardized cook-chill systems, including one of the technologies of molecular gastronomy, the sous-vide system, are among new (mild and novel) technologies that produce minimally processed products. The sous-vide („under vacuum") heat treatment technology is a professional cooking method that includes not only cooking, but also preservation in an oxygen-free environment, under accurate temperature control. It produces pasteurized products that can be used later if stored cold. During the procedure, foodstuffs are vaccum-packed, and then subjected to very mild heat treatment. The main point of the technology is to apply heat treatment parameters that take into consideration the biochemical properties of the raw material, especially the thermal denaturation points of proteins. Thus, the structure of the food matrix is preserved, and fragrances, flavors and nutrients are fully retained. Meat is the most expensive and most commonly used raw material for sous-vide processes, therefore, testing its microflora is of high priority when aiming to improve the microbiological quality of the sous-vide technology. Meat tissues are sterile in healthy animals, they can be contaminated during primary processing by saprophytic and pathogenic microorganisms, that can settle on and colonize surfaces. *Clostridium perfringens* can be found in soil, water, dust, spices and in the intestinal tracts of humans and animals, and it is one of the five bacteria that cause food poisoning most often. It is present in 10-80% of raw poultry meat. To cause food poisoning, the pathogen has to reach a level of 10^6 -10^7 cells/g food. It causes food poisoning, if the food is stored inadequately after heat treatment. The temperature range between 30 °C and 50 °C is especially favorable for the rapid growth of the bacterium. Oxygen level is reduced to such an extent during the application of vacuum packaging, that it favors the proliferation of inevitable anaerobic clostridia. According to the 2009 summary of the European Union, 108614 human incidents were caused by salmonellosis, the second most commonly reported infection. *Salmonella* remained the most common pathogen of food-related outbreaks, the bacterium was most often detedted in chicken, turkey and pork. In Hungary, when analyzing the distribution of events of microbiological origin by pathogen, it can be stated that salmonellae are in first place as pathogenic factors, with an absolute dominance of of *Salmonella*

Enteritidis. In 2006, salmonellosis accounted for 93.0% of the total bacterial diseases, and 93.8% of these were caused by Salmonella Enteritidis. Based on the infectious diseases reported in the December 15-21, 2014 period, the epidemiological situation of the country can be summarized as follows: of enteric bacterial infectious diseases, the number of salmonella illnesses reported since the beginning of the year did not differ significantly from the median characterizing weeks 1-51 of years 2008-2012, and it exceeded the corresponding value of 2012 only slightly. Ensuring microbiological quality and the prevention of the proiferation of pathogenic and saprophytic microorganisms are continuous tasks facing technology professionals. A minimum temperature value of 65 °C is prescribed by heat treatment regulations regarding the microbiological safety of foods pasteurized in the conventional way. Sensory properties of foods are also taken into consideration by the sous-vide technology, therefore, a core temperature of 56 °C is recommended for certai meats and fish. Because of the low pasteurization temperature and the long storage time the product is microbiologically sensitive. Therefore, the critical point of applying the sous-vide technology is the sizing of the heat treatment.

OBJECTIVES

The goal of our experiments was to determine optimal heat treatment parameters and thermal death parameters for both pathogens when using the technology, in the case of chicken breast, and determine how thermal deaths of the bacteria are affected by the mode of packaging.

2. MATERIALS AND METHODS

These experiments were performed in the accredited (NAT-1-1674/2012) Food and Water Test Laboratory of the Institute of Food Sciences at the University of West Hungary, Faculty of Agricultural and Food Sciences. NCAIM B 01417T strain of *Clostridium perfringens* and ATCC-13076 of *Salmonella* Enteritidis were used to determine the heat resistance in normal (atmospheric conditions) and sous-vide type vacuum packing.

2.1. Study of *Salmonella* Enteritridis heat resistance

2.1.1. Study of *Salmonella* Enteritridis heat resistance in model media

After opening the double plastic vial, the liophilized *Salmonella* Enteritridis ATCC (American Type Culture Collection)-13076 strain culture was transferred

into the diluting solution of the kit following the protocol, and spread on XLD (Xilóz-Lizin-Dezoxikolát) plates. After 24-48 hours of incubation at 37 °C, 0.5 McFarland (1.5×10^8, determined by Densimat®, BioMerieux) suspension was prepared from the pure culture, and in sterile buffered pepton water the final concentration (10^8/ml) was adjusted.

From the suspension 50 ml units were filled in sterile heat stable plastic bags suitable for the number of samples. Half of them were vacuum-packed modelling sous-vide conditions (99% vacuum). The samples were heat treated in circulating water bath. During sampling bags were removed from the water bath and the content was used for the inoculation. This assured continuous and quick sampling, which is necessary for the immediate processing and reduces inaccuracy derived from the time factor in heat resistance investigations. Investigations were conducted parallel at 55 °C, 60 °C, 65 °C, with same treatment time and inoculation frequency under atmospheric pressure and vacuum-packed samples. Sampling frequency was 5 minutes at 55·°C, 1·minutes at 60·°C, 0.5 minutes at 65·°C for atmospheric pressure and vacuum packed samples

2.1.2 Study of Salmonella Enteritridis heat resistance in chicken meat model media

A suspension adjusted to 0.5 to 1.0 McFarland units (10^8 CFU/ml) was prepared from a pure culture of Salmonella Enteritidis prepared on XLD (Xylose-Lysine-Deoxycholate) agar and incubated under aerobic conditions for 24 hours. According to the sous-vide technology, the surface of the chicken breast was baked in a pan for a few seconds, then it was ground. To 10 g of the ground chicken breast, 80 ml BPW and 10 ml of the suspension were added. From the mixture thus obtained, a number of samples, corresponding to the sampling frequency, was prepared, and half of them were vacuum packed. The sampling frequency was 5 minutes at 55 °C, 5 minutes at 60 °C, and 0.5 minute at 65 °C both for samples packed under atmospheric conditions and under vacuum.

2.2. Study of *Clostridium perfringens* heat resistance

2.2.1. Study of *Clostridium perfringens* heat resistance in model media

NCAIM B 01417[T] *Clostridium perfringens* strain was obtained as liophilized preparate in vacuum packed double vial from the National Collection of Agricultural and Industrial Microorganisms NCAIM), Hungary. Based on protocol, sterile physiologic saline solution was pipetted after opening, and after a 20 minutes rehydration time it was inoculated into the advised RCM (Reinforced Clostridial Medium) broth followed by an anaerobic incubation at

37 °C for 24-72 h. For the reproducibility of the experiment, the strain was preserved in Microbank® system as well. From the inoculated and translucent broth TSA (Tripton-Soya Agar) plates were streaked and incubated at 37 °C for·72 h. The pure culture was processed to a 0.5 McFarland ($1.5x10^8$/ml) suspension, adjusted for inoculating the study suspension.

To prepare the experimental samples, 50 ml RCM broth was pipetted to vacuumable poliethylene foil under sterile conditions. The same way samples were prepared under 99% vacuum in a sous-vide vacuum equipment. The unit number of the samples (64) for preparation was determined upon the heat treatment temperature values, thus the sampling frequency. For both sample series heat treatment was performed in sous-vide circulation termostated water-bath with core thermometer, at 55, 60, 65°C, respectively (accuracy: 0.1°C). Sampling frequency was 10 minutes at 55·°C, 5 minutes at 60·°C, 0.5 minutes at 65·°C for atmospheric pressure and vacuum packed samples

2.2.2. Study of *Clostridium perfringens* heat resistance in in chicken meat model media

A suspension adjusted to 0.5 to 1.0 McFarland units (10^8 CFU/ml) was prepared from a pure culture of Clostridium perfringens prepared on RCM agar and incubated under anaerobic conditions for 48 hours. Samples were inoculated with 10 ml of this suspension. According to the sous-vide technology, the surface of the chicken breast was baked in a pan for a few seconds, then it was ground. To 10 g of the ground chicken breast, 80 ml physiological saline and 10·ml of the suspension were added. From the mixture thus obtained, a number of samples, corresponding to the sampling frequency, was prepared, and half of them were vacuum packed.. Sampling frequency was temperature dependent, 10 minutes at 55 °C, 5 minutes at 60 °C, 1 minute at 65 °C

2.3. Cultivation microbiological methods

Decimal dilution series were prepared from the heat treated samples up to the 10^8 member, 1ml quantities of each of these were pipetted into sterile Petri dishes, then agar plates were poured using TSA (Tripton-Soya-Agar) culture medium. After solidification, they were incubated at 37 °C for 72 hours under anaerobic conditions in the case of *Clostridium perfringens*, and at 37 °C for 24 hours in the case of *Salmonella* Enteritidis. Two parallel inoculations were performed for each dilution. Our tests were performed as 3 independent experiments. Those dilution levels were included in the evaluation, where the number of developed colonies on the plate was between 10 and 300. The number of cells surviving the heat treatment was given as the weighted average

of the colony counts of the assessable plates, taking into consideration the degree of dilution, based on a certain formula.

3. RESULTS AND DISCUSSION

3.1. Result of study of *Salmonella* Enteritidis heat resistance

3.1.1. Result of study of *Salmonella* Enteritidis heat resistance in model media

A survival curves is obtained by plotting the change in the common logarithm of the living cell count (lgN CFU/ml) of the *Salmonella* Enteritidis strain samples packed under atmospheric conditions and under vaccum as a function of time, the slope of which can be used for the calculation of the decimal reduction time. Survival curves based on data obtained during the heat treatment of *Salmonella* Enteritidis ATCC-13076 strain samples at 55 °C, 60 °C and 65·°C are shown in Figures 1-3.
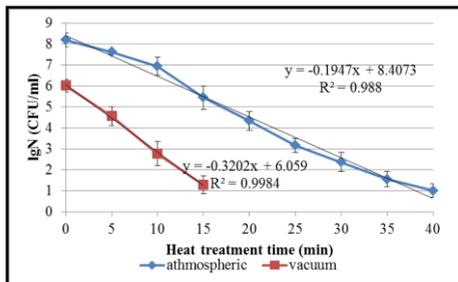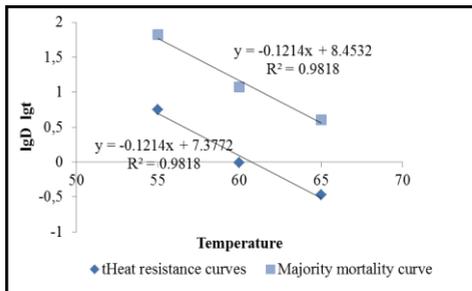
**1. Figure 55 °C**
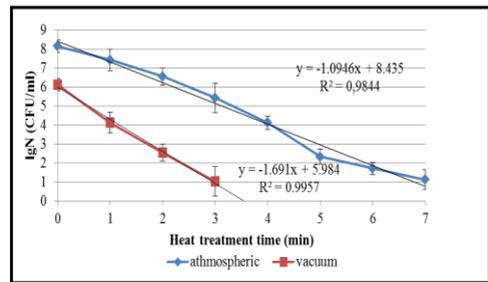
2. Figure60 °C
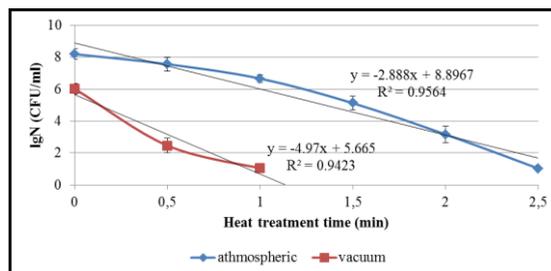
3. Figure 65 °C

By plotting the logarithms of the decimal reduction times against treatment temperatures, the heat resistance curve is obtained, shown in Figures 4-5. The change in the resistance of the microorganism as a function of the deadly heat intensity is indicated by the slope of the
curves, and from it the z value can be determined.

4. Figure atmospheric
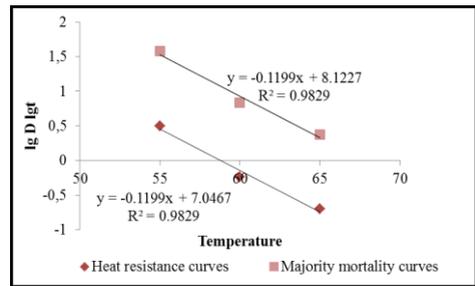
5. Figure vacuum-packed

Based on Figures 4-5, heat destruction parameters, the z value, the value of the Q_{10} temperature coefficient, the relative thermal death rate (RTDR) and the relative thermal death time (RTDT) were determined. Parameters are summarized in Tables 1-2.

1. Table athmospheric sample

Temperature	D (min)	log D	log t	z °C	Q_{10}	RTDR (1/ min)	RTDT (min)
55°C	5,57	0,74	1,82			0,015	66,22
60°C	0,99	-0,004	1,07	8,23	16,36	0,061	16,37
65°C	0,34	-0,468	0,60			0,24	4,05

2. Table vacuum-packed sample

Temperature	D (min	log D	log t	z °C	Q_{10}	RTDR (1/ min)	RTDT (min)
55°C	3,16	0,49	1,58			0,0159	62,88
60°C	0,58	-0,23	0,84	8,34	15,81	0,0632	15,81
65°C	0,2	-0,69	0,38			0,2514	3,98

3.1.2. Result of study of *Salmonella* Enteritidis heat resistance in chiken meat model media
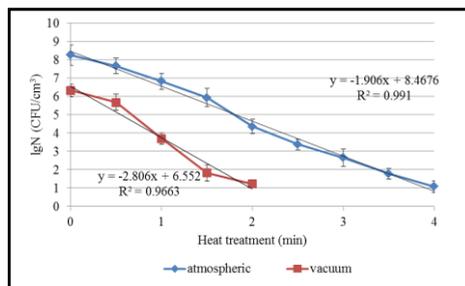
Survival curves based on data obtained during the heat treatment of *Salmonella* Enteritidis strain samples at 55 °C, 60 °C and 65 °C are shown in Figures 6-8.
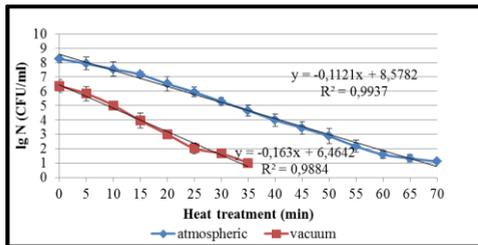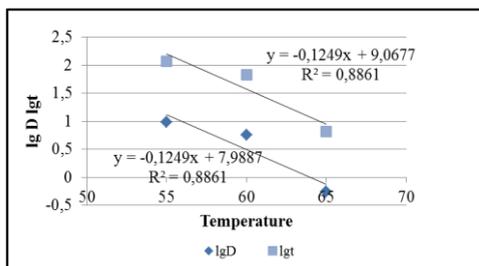

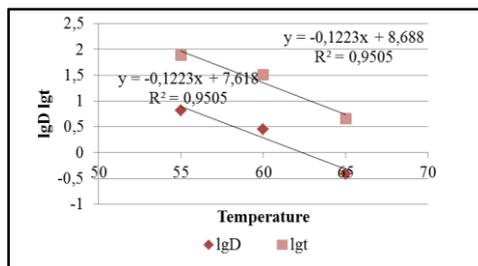
6. Figure 55 °C
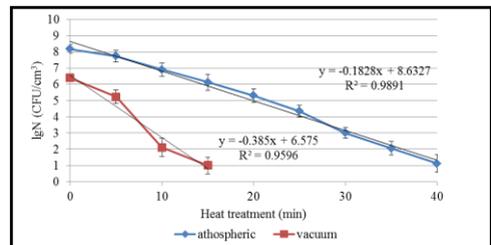
7. Figure 60 °C

8. Figure 65 °C

By plotting the logarithms of the decimal reduction times against treatment temperatures, the heat resistance curve is obtained, shown in Figures 9-10.

9. Figure atmospheric

10. Figure vacuum-packed

Based on Figures 4-5, heat destruction parameters, the z value, the value of the Q_{10} temperature coefficient, the relative thermal death rate (RTDR) and the relative thermal death time (RTDT) were determined. Parameters are summarized in Tables 3-4.

3. Table athmospheric sample

Temperature	D (min)	log D	log t	z (°C)	Q_{10}	RTDR (1/min)	RTDT (min)
55°C	9,76	0,96	2,04			0,0145	74,73
60°C	5,66	0,75	1,83	8,1	16,74	0,0597	17,74
65°C	0,55	-0,25	0,81			0,2443	4,21

4. Table vacuum-packed sample

| Temperature	D (perc)	log D	log t	z °C	Q_{10}	RTDR (1/min)	RTDT (min)
55°C	6,51	0,81	1,88			0,015	67,61
60°C	2,78	0,44	1,51	8,6	16,5	0,060	16,60
65°C	0,39	-0,40	0,66			0,245	4,07

3.2. Result of study of *Clostridium perfringens* heat resistance

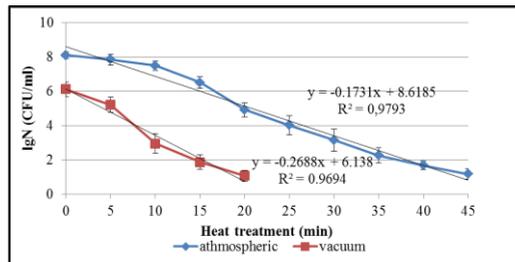3.2.1. Result of study of *Clostridium perfringens* heat resistance in model media

Survival curves based on data obtained during the heat treatment of *Clostridium perfringens* strain samples at 55 °C, 60 °C and 65 °C are shown in Figures 11-13.
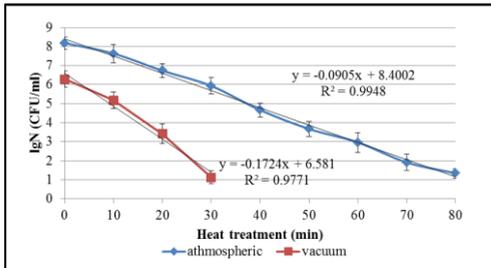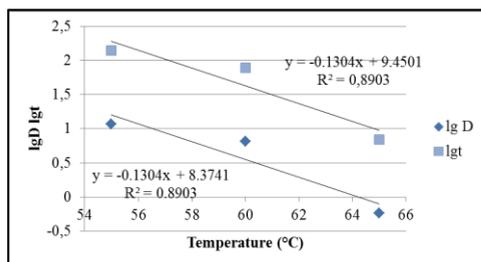

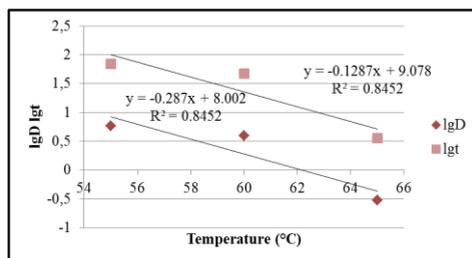**11. Figure55 °C**
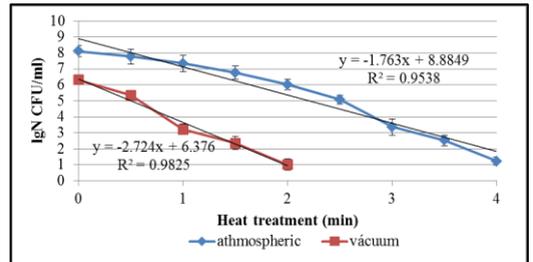
12. Figure 65 °C

13. Figure 60 °C

By plotting the logarithms of the decimal reduction times against treatment temperatures, the heat resistance curve is obtained, shown in Figures 14-15.

14. Figure atmospheric

15. Figure vacuum-packed

Based on Figures 4-5, heat destruction parameters, the z value, the value of the Q_{10} temperature coefficient, the relative thermal death rate (RTDR) and the relative thermal death time (RTDT) were determined. Parameters are summarized in Tables 5-6.

5. Table athmospheric sample

Temperature	D (min)	log D (min)	log t (min)	z (°C)	Q_{10}	RTDR (1/ min)	RTDT (min)
55°C	11,69	1,06	2,14			0,0110	90,36
60°C	6,49	0,81	1,88	7,6	20,1	0,0496	20,13
65°C	0,58	-0,23	0,83			0,2228	4,48

6. Table vacuum-packed sample

Temperature	D (min)	log D (min)	log t (min)	z (°C)	Q_{10}	RTDR (1/ min)	RTDT (min)
55°C	5,81	0,76	1,84			0,0117	85,21
60°C	3,96	0,59	1,67	7,5	19,36	0,0516	19,36
65°C	0,3	-0,52	0,55			0,2272	4,04

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3.2.2. Result of study of *Clostridium perfringens* heat resistance in chiken meat model media

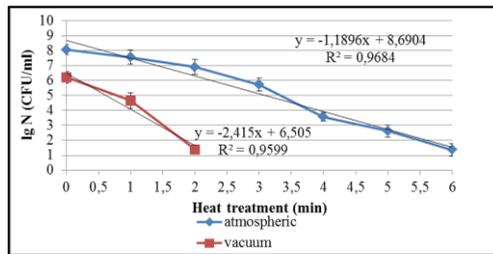Survival curves based on data obtained during the heat treatment of *Clostridium perfringens* strain samples at 55 °C, 60 °C and 65 °C are shown in Figures 16-18.
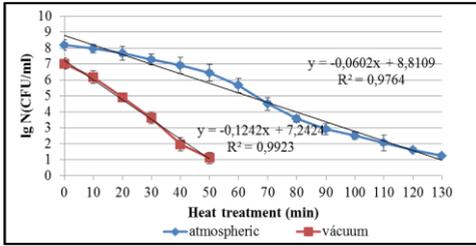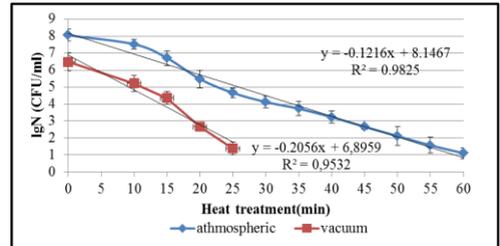
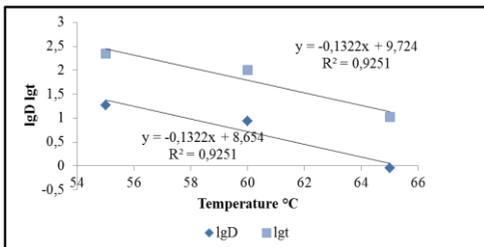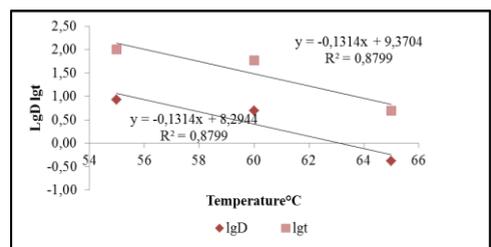**16. Figure 55 °C**   **17. Figure 60 °C**

18. Figure65 °C

By plotting the logarithms of the decimal reduction times against treatment temperatures, the heat resistance curves is obtained, shown in Figures 19-20

19. Figure atmospheric   **20. Figure vacuum-packed**

Based on Figures 4-5, heat destruction parameters, the z value, the value of the Q_{10} temperature coefficient, the relative thermal death rate (RTDR) and the relative thermal death time (RTDT) were determined. Parameters are summarized in Tables 7-8.

7. Table athmospheric sample

Temperature	D (min)	log D (min)	log t (min)	z (°C)	Q_{10}	RTDR (1/ min)	RTDT (min)
55°C	18,7	1,27	2,34			0,010	96,16
60°C	8,64	0,93	2,01	7,5	20	0,048	20,99
65°C	0,89	-0,05	1,02			0,218	4,58

8. Table vacuum-packed sample

Temperature	D (min)	log D (min)	log t (min)	z (°C)	Q_{10}	RTDR (1/ min)	RTDT (min)
55°C	8,45	0,93	2,00			0,010	93,54
60°C	4,9	0,69	1,77	7,6	20	0,048	20,6
65°C	0,41	-0,39	0,69			0,22	4,05

4. CONCLUSIONS

<u>Effect of vacuum packaging</u>

Meats prepared by sous-vide technology are vacuum packed before the start of the heat treatment, thus, part of the experimental samples were packed like this, and control samples were traditionally packed and were called atmospheric samples.

Based on the experimental results, the applied vacuum packaging alone has an important antimicrobial effect for *Salmonella* Enteritidis ATCC-13076 and *Clostridium perfringens* NCAIM B 01417[T] strains, because even in the 0 min. sample the survived vegetative cell number is 2 order of magnitude lower in vacuum-packed, than in atmospheric samples.

Vacuum packaging has effect during the heat treatment as well, for both strains at the investigated temperatures (55 °C, 60 °C and 65 °C) the slope of the survival curves were higher for vacuum packed samples, indicating a higher heat destruction.

Based on the slopes the decrease of vegetative cell number under the detection limit is faster in vacuum-packed samples, thus, for sous-vide products the time required for the destruction of pathogens is shorter, than for atmospheric samples.

Statistical probes demonstrated a significant difference between the two sample groups in heat destruction of bacterial cells, which is the result of vacuum packaging.

Based on these it can be stated, that vacuum packaging of sous-vide technology enhances microbiological quality of sous-vide products, and during the heat treatment the microbial destruction is higher.

For optimal heat treatment parameters the following is suggested:

For sous-vide vacuum packed chicken meat, to reach cell numbers under detection limit for *Salmonella* Enteritidis ATCC-13076 at 55·°C 40 min, at 60·°C 20 min, at 65 °C 2.5 min heat treatment is necessary. For *Clostridium perfringens* NCAIM B 01417[T] strain at 55 °C 60 min, at 60°C 30 min, at 65·°C 3 min is necessary.

5. NOVEL SCIENTIFIC RESULTS

1. Applying sous-vide technology for *Salmonella* Enteritidis ATCC-13076 strain at 55 °C 40 min, at 60 °C 20 min, at 65·°C 2.5·min heat treatment in vacuum packaging, significantly higher heat destruction can be reached than in control atmospheric pressure samples.
    Applying sous-vide technology *Clostridium perfringens* NCAIM B 01417[T] strain at 55 °C 60 min, at 60 °C 30 min, at 65 °C 3 min heat treatment in vacuum packaging, significantly higher heat destruction can be reached than in control atmospheric pressure samples.

2. Heat treatment parameters were determined for *Salmonella* Enteritidis ATCC-13076 strain in vacuum packed chicken meat model media, which are the following: $D_{(55)}$=6.51 min, $D_{(60)}$=2.78 min and $D_{(65)}$=0.39·min. z-values for 55-65 °C range is 8·°C. RTDR at 55·°C is 0.015, at 60 °C 0.069, at 65 °C 0,245. RTDT value at 55·°C is 67.61 min, at 60 °C 16.6 min, at 65 °C 4.07 min.

3. Heat treatment parameters were determined for *Clostridium perfringens* NCAIM B 01417[T] strain in vacuum packed chicken meat model media, which are the following: $D_{(55)}$=8.45 min, $D_{(60)}$=4.9 min and $D_{(65)}$=0.41·min. z-values for 55-65 °C range is 7.6·°C. RTDR at 55·°C is 0.01, at 60 °C 0.048, at 65 °C 0,22. RTDT value at 55·°C is 93.5 min, at 60 °C 20.6 min, at 65 °C 4.05 min.

6. SCIENTIFIC PAPERS, LECTURES WRITTEN IN THE TOPIC OF THE DISSERTATION

Reviewed publications in scientific journals:

Vajda K., Szigeti J., Ásványi B.,& Szűcs P. (2015): Sous-vide húsokban előforduló humán patogén baktériumok hőrezisztenciájának vizsgálata, Élelmiszervizsgálati közlemények 2015/3 szeptember 30. (In Hungarian: Heat resistance of human pathogenic bacteria in sous-vide meat)

Szücs P., **Vajda K.,** Szigeti J., Molnár J., Ásványi B.: A Sous-vide, mint kíméletes hőkezelési technológia élelmiszer-higiéniai vonatkozásai
Acta Agraria Kaposváriensis, In press (in Hungarian: Food hygieny of sous-vide, as a mild heat treatment technology)

Reviewed publications in scientific journals in English:

Vajda K, Halbritter A., Szűcs P., Szigeti J. & Ásványi B. (2015): Heat resistance of human pathogens in in sous-vide products studied in model nutrition media, Acta Alimentaria, In press 2016 2.(jún)

Journals, conference proceedings

Régaiszné **Vajda Katalin**: A XXI. század konyhája, a molekuláris gasztronómia Proceedings of the 14th APÁCZAI DAYS Scientific Conference (2010), Apáczai Csere János Faculty, University of West Hungary, Győr. (In Hungarian: Kitchen of the 21st century: molecular gastronomy)

Régaiszné **Vajda Katalin**: A „sous- vide" cook&chill élelmiszer tartósító rendszer mikrobiológiai minőségének kérdései Proceedings of the 15th APÁCZAI DAYS Scientific Conference (2011), Apáczai Csere János Faculty, University of West Hungary, Győr. (In Hungarian: Microbial quality questions of „sous- vide" cook&chill food preserving system)

Lectures in Hungarian:

1.   A sous - vide cook&chill élelmiszertartósító rendszer mikrobiológiája, Magyar Tudomány Ünnepe 2010, BGF – Budapest Conference lecture (In Hungarian: Microbial quality questions of „sous- vide" cook&chill food preserving system)

2.   A XXI század turizmusának gasztronómiája és mikrobiológiai minőségének biztosítása, 17th APÁCZAI DAYS Scientific Conference (2013), Apáczai Csere János Faculty, University of West Hungary, Győr. (In Hungarian: Gastronomy and its microbial quality assurance in the tourism of the 21st century)

3.   Kíméletes tartósító eljárások, V. International Tourism Conference (2013) NYME-AK-Győr (In Hungarian: Mild food preserving methods)

4.   Turizmus és gasztronómia, VI. International Tourism Conference (2014) NYME-AK-Győr (In Hungarian: Tourism and gastronomy)