THESIS OF DISSERTATION (PhD)

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QUALITY ASSURANCE OF FATTEND GOOSE LOVER PRODUCTION SPECIAL REGARDS TO ITS INDUSTRIAL PROCESSING

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1. INTRODUCTION

In spite of introducing quality assurance system in European food industries number of food poisoning rises year by year. Demand of costumers on fresh, natural products which are free from preservers and drastical heat-treatment compels producers to pay bigger attention on microbiological status of the producing circumstances.

Improving microbial quality of raw material contaminated with spore-forming heat-resistant bacteria in a high CFU number can be achieved only by extreme heat treatment or chemical handling (salting).

Spoilage of foodstuffs preserved by heat-treatment caused by mainly termotolerant, termophyl spore forming anaerobic bacteria. The most frequent fault of goose-liver paste (paté de foie dras) is the extreme heat treatment (high F_0-value) in one hand, and the deterioration in flavor of this product caused by this heat on the other hand.

During our work of several years HACCP system of fattened goose-liver production was worked out on processes of goose-breeding to paste production.

2. MATERIALS AND METHODS

2.1. Working out the HACCP system

Hazard analysis of critical control points of fattened goose-liver production was worked out in the slaughterhouse of Merian Finom Szárnyas Különlegességek Részvénytársaság in Orosháza. Management of this factory was committed to introduce and respect the HACCP system on the highest level. The HACCP system of fattened goose and fattened goose-liver production was worked out in 2000.

Development of quality assurance system on fattened goose-liver production was according to the seven principles and the twelve step process. The Seven HACCP Principles are as follows:

1. Analyze hazards
2. Identify Critical Control Points (CCPs)
3. Establish preventive measures with critical limits for each control point
4. Establish procedures to monitor the CCPs
5. Establish corrective actions to be taken when monitoring shows that a critical limit has not been met
6. Establish procedures to verify that the system is working properly
7. Establish effective record keeping

Members of HACCP team were: leader of quality control sector, responsible leader for hygiene, production engineer. Learning the process and method of fattened goose-liver production steps of proceeding were analyzed.

At labor processing on the marked microbiologically critical control point samples for microbiological examination were taken.

2.2. Isolation and identification of *Clostridium* species.

2.2.1. Sampling

Culturing *Clostrium* spp. from meat was according to ÉTI-ML-SOP-VU-FM-C-2.1 and -2.2 (35§ LMBG, L-06.00-20, L-06.00-39). All of glass equipments were cleared and sterilized before use (autoclave – Webeco H-type; LMIM ST 133 121±1°C, t=30s; hot air sterilizer – LMIM ST 222/2 min. 180°C, t=180s).

Droppings were collected with sterile Bactopick® plastic sticks from ten different points of planking of truck carrying living birds and taken into sterile stomacher bags (*Seward, England*). Dirt taken from plucking machine was handled in the same way.

Sampling fattened goose-liver after evisceration and veining was performed with sterile scalpel and samples were taken into sterile stomacher bags. To examine the microbiological status of empty cans samples were taken by swabbing. Number of samples was five in all cases.

2.2.2. Culturing Clostridia

For preparing decimal dilutions 1% peptone water (MERCK, Germany) was used. Temperature of water was between 10-20°C.

1-1 gram of samples of drops and dirt from plucking machine was measured into 9 cm^3 peptone water; while to 10 gram of each goose liver sample 90 cm3 sterile peptone water was added. Solutions prepared this way were declared as starting decimal dilutions (10^{-1}).

To know the number of anaerobic bacterial spores of these samples 10^{-1} dilutions were first heat treated on 75°C for 15 minutes in bath than diluted up to 10^{-5} grade. From each dilution 1-1ml solution was inoculated into 15 ml Reinforced Clostridia (RC) Agar, and Triptose-Soya-Cycloserine (TSC) Agar. Independent decimal dilutions were applied that meant five parallel lines.

Incubation time was 20-24°C on temperature 37°C under anaerobic circumstances: in anaerobic boxes, using anaerocult and anaerobic indicator (*OXOID, England*).

To determine *Clostridium* number only those Petri dishes were evaluated in which the number of colonies were 15-150. Dishes containing small but well-countable colonies were also counted if the number of colonies did not exceed 200. >From the colonies of the smallest and the following countable dilution level mean was established as follows: **1st. Equatation**

$$č = \frac{\Sigma c}{n_1 * 1 + n_2 * 0{,}1}$$

where:
č maen of colonies,
Σc number of evaluated dishes,
n_1 number of dishes belonging to the lowest evaluated dilution level,
n_2 number of dishes belonging to the next dilution level.

CFU/g or CFU/cm^3 of examined samples was calculated by multiplication of č with the dilution factor. Results were given in standard form.

After 24 hour incubation time black colored colonies were counted. From two dishes of each sample 1-1 colony was streaked onto Columbia Blood Agar (MERCK, Germany) and incubated on 37^{o}C for 20-24 hours under both anaerobic and aerobic circumstances.

2.2.3. Identification of *Clostridium* species

Colonies formed only anaerobic circumstances were further examined as follows:

- ✓ Absence or existence of bacterial flagella was examined with semi-liquid agar (ffNMOT) (t=24h, T=37^{o}C).
- ✓ Reduction of lactose and gelatin with inoculation colonies into Lactose-gelatin Agar (LG) (t=24h, T=37^{o}C)
- ✓ To study nitrate-nitrite reduction of these bacteria ffNMOT and nitrite reagent were used.
- ✓ Sulfide reduction was observed by Differential Reinforced Clostridia Medium (DRCM, *OXOID, England*).
- ✓ Localization of bacterial endospores Malachit-green processing was applied.
- ✓ Further biochemical reactions were examined by rapid ID 32 A test kit (*bioMérieux, France*) that contains 29 biochemical reagents in dehydrated from. After 24 hour incubation time (T=37oC)

biochemical reactions were evaluated both manually and with ATB computer system.

2.2.4. Examination of temperature needed for growth

Solitary colonies grew on Columbia agar were inoculated into Reinforced Clostrtidial Media (RCM, *OXOID, England*) and incubated under anaerobic circumstances on the following temperatures: 25, 30, 42 and 50^{o}C (t=24h). Growth of bacteria was declared if turbidity in glasses was observed.

2.2.5. Applying ATB automatic identification system

This ATB automatic identification system contains a measuring unit, a computer that analyses results, and a printer connected to the computer.

Measuring unit notices test kit and measuring is performed with colorimeter and/or nephelometer. With the help of ATB Identification Software the computer analyses the biochemical profile given from measuring unit and compares it to taxonomic categories. After calculating identification index (id%) and taxonomy index (T) it identifies the bacterium. If id% value is above 80% and T-value higher than 0,5, the result can be accepted.

Preparing the sample to ATB identification system, from solitary colony formed on Columbia Blood Agar under anaerobic circumstances suspension with 4 McF density and volume 2 ml was made. From the suspension 55 µl was taken with automatic pipette and inoculated into

each whole of test kit. Finally, the test whole testing urease activity was covered with paraffin oil. Test kit was incubated under aerobic circumstances for 4 hours on 37^{o}C temperature. To study the nitrate reduction into the whole number "0.0" nitrate reagent (NIT1-NIT2) was dropped. To observe exist of alkalic-phosphatase enzyme and indol formation fast blue (FB) and JAMES reagents were given into whole "0,2" and "0,1".

2.2.6. Maintenance of identified species

Isolated *Clostridial* species were maintained in test-tubes on 10cm^3 RCM, under anaerobic circumstances. To obtain anaerobic circumstances sterile paraffin oil was disposed onto the agar. Bacteria were monthly inoculated into fresh RCM.

2.3. Examination of heat resistance of isolated *Clostridia*l species

2.3.1. Heat resistance of bacterial spores isolated *Clostridial* species

Heat treatment of bacterial spores was performed on 80^{o}C for 10 minutes. Decimal dilutions were made with buffered 1% peptone water to 10^{-7} grade. After learning starting spore number with Bürker chamber 0,1cm^3 of dilution 10^{-1} was inoculated into TSC Agar.

Heat resistance of *Clostridial* spores was examined on 85, 95, 105 and 115^{o}C (t=1, 3, 5, 10, 20, 30 min.). Heat treatment on 85 and 95^{o}C was prepared in bath while on 105 and 115^{o}C was in oil-bath.

To imitate the physical characteristics of fattened goose liver *Clostridial* spores were inoculated into solution of glycerin and water with 0,982 water activity and 5,5±0,2 pH.

3 parallel dilution lines were prepared after heat treatment from which each dilution 1 cm^3 was inoculated into RCM. Colonies formed in dishes after 24 hour incubation (T=37^oC, anaerobic circumstances) were counted.

2.3.2. Verifying the degree of heat resistance of bacterial spores

Destruction rate of a microbial population can be described with the rate coefficient. Time of decreasing CFU tenfold (D=2,303/k), is that time whilst number of bacteria decreases one grade under a given destructive effect. Calculating this value is possible as follows:

2nd. Equatation

$$\mathbf{D_t=t/(lgN_0-lgN_t),}$$

wherel:

- N_t means the given cell concentration in a given time,
- N_0 cell concentration at start,
- t time of heat treatment

Destruction rate's dependence on temperature is given by "z" value that is that temperature rising which decreases time of heat-destruction tenfold.

3rd. Equatation

$$z = (T'' - T') / (\log D_{T'} - \log D_{T''}),$$

where:

- T' lower applied temperature (°C),
- T'' higher applied temperature (°C),
- $\log D_{T'}$ logarithm of D on lower applied temperature,
- $\log D_{T''}$ logarithm of D on lower applied temperature.

To calculate heat destruction the following equitation was used:

4th. Equatation

$$N_t = N_o \times e^{-kt},$$

where

- N_t means the given cell concentration in given time
- N_o cell concentration at start
- t time of heat treatment
- k destructional rate coefficient

Changing in CFU number in the function of time of temperature effect is given by the survival curve.

5th. Equatation

$$\lg N_t = \lg N_o - (k \times t) / 2{,}303$$

6th. Equatation

$$F_o = (t/60) \times 10^{(T-121^oC)/z},$$

Where?

- F_0 equivalent of heat treatment (minute),
- t time of heat treatment (second),
- T temperature of heat treatment (°C),
- z temperature dependence of destructional rate (°C).

3. RESULTS AND DISCUSSION

3.1. Isolation of *Clostridia*l species from samples given during fattened goose-liver production

3.1.1. Examination with TS Agar

Numbers of anaerobic bacteria cultured from goose faeces, plucking machine and fattened goose-liver are showed in table 1.

1st. table

Number of isolated anaerobic bacterial spores grew on TSC Agar

Sample	Number of Clostridia CFU/cm^3
Goose faeces (drops)	$3{,}65 \times 10^1$
Dirt from plucking machine	$6{,}50 \times 10^1$
Fattened goose-liver after evisceration	10^2
Fattened goose-liver after veining	10^2
Empty cans	0

3.1.2. Isolation of *Clostridia* with RCA

>From the samples taken marked critical control points of fattened goose-liver production *Clostridium sordellii* could be isolated.

2nd Table

Numbers of *Clostridium spp* and *Cl. sordellii* spores

Sample	Clostridial spore number (CFU/g)	*Cl. sordellii* spores (CFU/g)
Goose faeces (Drops)	$1,77*10^3$	10^2
Feather	$1,53*10^3$	$1,2*10^1$
Fattened goose-liver after evisceration	$8,13*10^1$	10^1
Fattened goose-liver after veining	$8,67*10^2$	$2,1*10^2$
Empty cans	0	0

Number of *Clostridium spp*. and *Cl. sordellii* spores was one fold higher in fattened goose-liver after veining than that of after evisceration. The cause of it might be either the insufficient transporting circumstances of livers into canning factory or the equipments and workers inadequate hygiene.

Noticeable higher number *Clostridial* colonies formed in RC agar than in TSCA. This can be because of the high selectivity of TSCA on *Cl. perfringens.*

4.1.3. Biochemical examination of *Cl. sordellii*

Mucous colonies with white-opal color covered the RC agar were streaked onto Columbia agar. After incubation greyish, flat diffuse and mucous colonies were observed. Hence clearing up the agar around the colony was observed ß-hemolytic activity was declared.

Although exist of bacterial flagella was justified by the diffuse characteristic of the colonies, it was verified also by hanging-drop

equipment. Isolated bacteria were short, bacilli with rounded-end moving vigorously. Many of these bacteria also had sub-centrically localized endospores.

In ffNMOT bacteria spread diffuse that also justified the existing of flagella. Since in the color of ffNMOT was not changed after adding nitrate reagent this bacteria did not reduced nitrates.

Isolated bacteria reduced neither lactose nor gelatin.

Verifying Cl. sordellii

Evaluatiung our results given from rapid Id 32 a test kits, it can be said that identification was "good". Identification index was 97,8%, T-value was 0,54. Although this latest value is low a bit, but the next choice of the computer was also *Cl. sordelli*. Thus the result was accepted. According to data given to ATB automatic identification system our newly isolated *Cl. sordellii* species differed in 3 biochemical characteristics than that of the others. Our species showed ß-galactosidase, piroglutaminacid-arilamidase and α-fuconodase activity.

Verifying Cl. perfringens

75% of *Cl. perfringens* species was positive in α-galactosidase activity and 95% had raffinose-reduction ability. *Cl. perfringens* species isolated from goose-liver belonged to those which are negative to these tests. Identification index was 99,9%, that enforced us in our results (T=0,65).

3.2. Examination of heat-resistance

3.2.1. Spore-staining

Staining spores of *Cl. sordellii* green colored endospores were seen localized sub-central in the cell. Some spores of *Cl. sordellii* were released during cell-lysis. Spores of *Cl. perfringens* localized central in the bacteria.

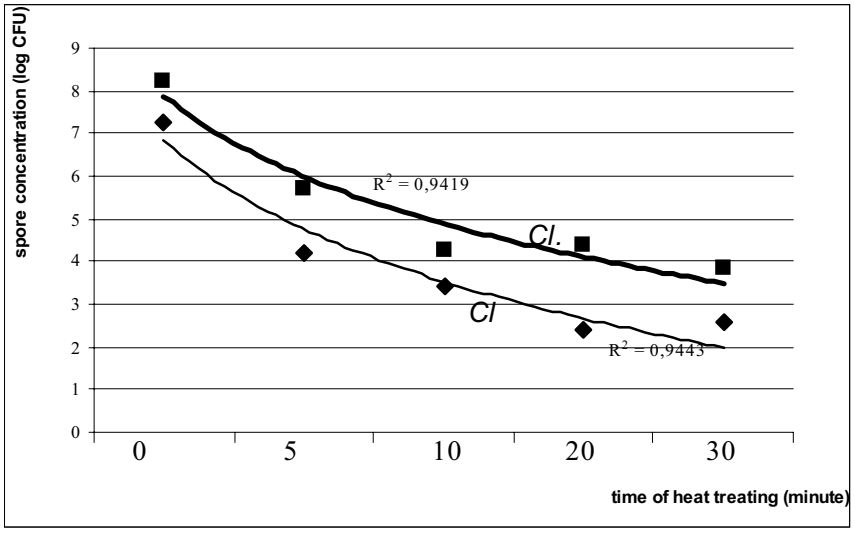
Examination of heat-resistance of these two bacteria was performed with high spore concentration.

3.2.2. Results of heat-resistance on 95^{o}C

Starting number of *Cl. sordellii* spores was 1,571*10^{8}CFU/ml that is decreased three fold (5,125*10^{5} CFU/ml) after the fifth minute of heat treatment. The next 5 minutes caused only one fold decrease in CFU (2,275*10^{4} CFU/ml) and this tendency did not change in the twentieth minute of treatment. (1,857*10^{4} CFU/ml). At the and of heat treatment on 95^{o}C number of *Cl. sordellii* was 6,727*10^{3} CFU/ml.

When heat treating *Cl. perfringens* spores the same tendency was noticed than that of *Cl. sordellii*. Starting spore concentration (1,76*10^{7} CFU/ml) decreased three fold (1,62*10^{4} CFU/ml) in the first five minute of treatment, but in the next five minute only one fold (2,60*10^{3} CFU/ml) decreasing was counted. In the following examination times

(20^{th} and 30^{th} minutes) the spore concentration did not vary significantly ($2{,}37*10^2$ CFU/ml, $3{,}66*10^2$ CFU/ml).



1.st figure: Curve calculated according to heat-resistance of *Cl. Perfringens* spores and *Cl. Sordellii* spores

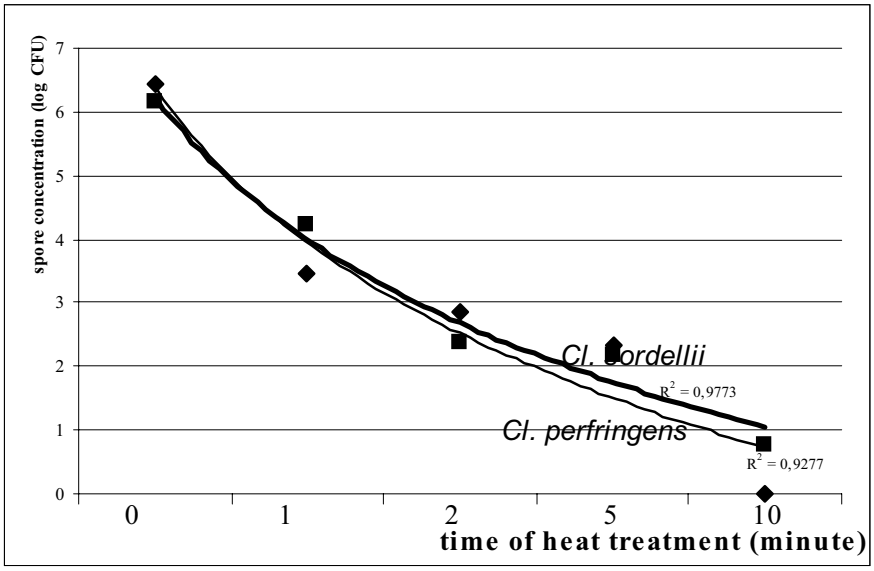
First figure shows that during heat treatment on 95°C *Cl. sordellii* and *Cl. perfringens* spore number did not decrease significantly but in the first 5 minutes CFU was reduced noteworthy. This phenomenon can be explained by the sudden heat-shock of bacterial cells and the heat-adaptation of spores.

High CFU ($6{,}727*10^3$ CFU/ml) of *Cl. sordellii* after the thirtieth minute of treatment reflects on further examinations of these spores in the future.

3.2.3. Results of heat-resistance on 105°C

In the case of *Cl. sordellii* spores results of heat-treatment on 105°C maintained in oil-bath showed the same tendency as was on 95°C.

In the starting high spore numbers of both bacteria three fold reduction occured in the first minute. In the second minute reduction of *Cl. sordellii* spores was two fold while that of *Cl. perfringens* spores was only one fold. Although in the fifth minute of heat treatment number of both bacterial spores showed 10^2 CFU/ml grade, in the following five minutes *Cl. perfringens* spore was not cultured.



2nd figure: Curve calculated according to heat-resistance of *Cl. Perfringens* spores and *Cl. Sordellii* spores

3.3. Calculated D- and z-values of *Cl. sordellii* and *Cl. perfringens*

D-value on 95°C of Cl. sordellii isolated from raw fattened goose-liver was 6,86 minute while on 105°C was 1,86 minute. The calculated z-value was 17,63°C that can be declared as a high z-value.

According to the equitations described above D (95^{o}C) of *Cl. perfringens* was 6,407 minute and on 105^{o}C was 1,255 minute. z-value calculated by D_{95oC} and D_{105oC} was 14,12^{o}C.

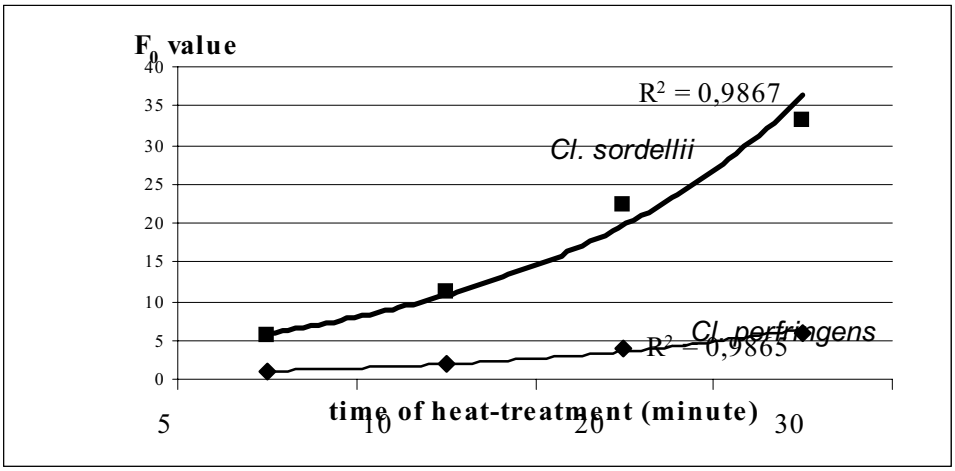
F_0 values calculated with help of D- and z-values are sowed in table 3.

3rd Table

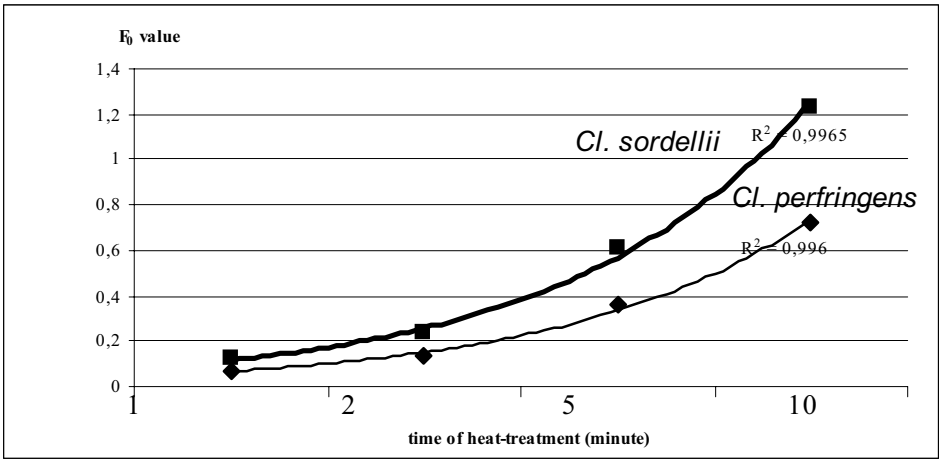
Summary of F_0 values of *Cl. sordelli* and *Cl. perfringens* spores

Name	Temperature (°C)	Time of heat-treatment (minute)	F_0 value
Cl. perfringens	95	5	0,99
		10	1,99
		20	3,99
		30	5,99
Cl. sordellii	95	5	5,53
		10	11,07
		20	22,15
		30	33,23
Cl. perfringens	105	1	0,07
		2	0,14
		5	0,36
		10	0,72
Cl. sordellii	105	1	0,12
		2	0,24
		5	0,61
		10	1,23

For the adequate heat-treatment of fattened goose-liver paste applying the lowest F_0-value examining heat-conductivity of cans and warming up of heating equipment further examination are necessary.



3rd figure: F_0 values of *Cl. perfringens* and *Cl. sordellii* on 95°C.



4th figure: F_0 values of *Cl. perfringens* and *Cl. sordellii* on 105°C

The high F_0-value of *Cl. sordellii* examined on 105°C was due to the high starting spore concentration ($1{,}571*10^8$ CFU/ml).

Number of *Cl. sordellii* isolated from fattened goose-liver was 10^1 and $2{,}1*10^2$ CFU/ml that may be decreased on 95°C in the first 5 minute of heat treatment.

4. NEW SCIENTIFIC RESULTS

1. From our results presented here it an be said that number of *Clostridium* CFU isolated from veined fattened goose-liver was higher than that of livers gained after evisceration. Microbiological contamination of these products might result from either inadequate parting of carcasses or insufficient personal hygiene.

2. Numbers of critical control points in production of fattened goose-liver and reprocessing of livers might be reduced and the microbiological quality of these products might be improved by elimination of pre-cooling of carcasses. Although this method is not introduced in Hungarian poultry slaughter house with this technique F_0-value applied to heat-treat cans could be decreased.

3. Considering our results on heat-resistance of *Cl. sordellii* spores, it can be established that to reduce the CFU of these spores more drastical heat-treatment is needed. Presence of these heat-resistant anaerobic bacterial spores in fattened goose-liver jeopardizes both the microbiological and physical-chemical quality of canned products.

4. To get end products with higher grade HACCP system also on goose-breeding farms was introduced. Improving the hygiene of breeding circumstances and feeding quality of fattened goose-liver can be increased.

5. SUMMARY

To determine the microbiologically critical control points during fattened goose-liver microbiological status of carcasses – especially focused on anaerobic bacteria - on recent fabricating steps was examined. Higher *Clostridium* CFU numbers both of eviscerated and veined fattened goose-liver suggest insufficient personal and tool hygiene in the slaughterhouse. Eleven critical control points were pointed on the line of fattened goose-liver production to reveal and avoid factors that might cause contamination of carcasses. By omitting air pre-cooling improving microbiological status of fattened goose liver might be produced. Avoiding cross-contamination of liver lower F_0-value (F_0=2-3) might be sufficient to degerminate preserved goose-liver products that can improve the market of these hence canned foods treated on low F_0 value are preferred in West-European countries (e.g. France, Spain), to where most of the canned fattened goose liver products of Merian Rt. are exported.

6. PUBLICATIONS IN THE THEME OF THESIS

4.1. Publications in foreign language

Turcsán J., Varga, L., Turcsán Zs., Szigeti J. – Farkas L., 2001: *Occurence of Anaerobic Bacterial Spores, Clostridial and Clostridium perfringens Spores in Raw Goose Livers from a Poultry-Processing Plant in Hungary*. Journal of Food Protection 64 (8), 1252-1254

Turcsán Zs., Szigeti J., Varga L., Farkas L., Birkás E. – **Turcsán J.,** 2001: *The effects of electrical and controlled atmosphere stunning methods on meat and liver quality of geese.* Poultry Science, 80 (11), 1647-1651

Turcsán Zs., Varga L., Szigeti J., **Turcsán J.,** Csurák I. – Szalai M., 2003: *Effect of electrical stunning frequency-voltage combinations on the presence of engorged blood vessels in goose liver.* Poultry Science, 82 (6), 1816-1819. (2002)

4.2. Publications in Hungarian language

Turcsán J. (2000): *Nyers libamájból izolált Clostridum perfringens hőtűrésének vizsgálata*. Thesis, Mosonmagyaróvár

Turcsán Zs., Szigeti J., Tenk A., Birkás E. - **Turcsán J**., 2002: *A magyar hízott libamáj ágazat helyzete és fejlesztésének lehetőségei a legújabb*

hazai és nemzetközi kutatási eredmények tükrében. Állattenyésztés és Takarmányozás, 51. (2.) 157-464.

cumulative impact factor: 4.29