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Liver patè: process hygiene, quality parameters and thermal process

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A b s t r a c t: The aim of this paper was to assess the manufacturing process of liver patè (150 g) packed in cans designed for the domestic market. The investigation included hygiene of the manufacturing process, product quality and control of heat treatment. Process hygiene was assessed through determining microbial numbers in canned stuffing before heat treatment, immediately after filling and during the wait time for the canning process (up to 150 minutes). Sulphate reducing Clostridia were not detected, Escherichia coli numbers ranged from 3,08 up to 3,25log CFU/g^{-1}, Enterobacteriaceae from 2,96 up to 3,08 log CFU/g^{-1} and total aerobic colony count from 5,75 up to 6,36 log CFU/g^{-1}. Average chemical and physico-chemical product paramaters were determined: total protein content (9,66±0,61%), fat content (22,32±1,05%), sodium chloride content (1,49±0,04%), a_w (0,96±0,005), pH (6,44±0,11) and nitrite content as $NaNO_2$ (7,44±1,97 mg/kg^{-1}). Determined chemical and physico-chemical parameters were in accordance with the requirements of domestic legislation. Heat tr eatment lasted 1 hour and 30 minutes. Effective sterilization time was 55 minutes, at autoclave medium temperature 114°C and a pressure of 3.2 bar. Lethality of the heat treatment was controlled by thermocouple probes placed in thermal centre of cans in six point checks and determining F_o values which ranged from 7,24–8,58. This thermal regime, supported by can hermeticity, was sufficient to ensure the safety of the canned liver patè.

Keywords: *Liver patè, process hygiene, quality, heat treatment.*

Introduction

Liver patè is a meat product derived from meat, fat, liver (at least 10%), other offal, connective tissue, blood, blood preparations, bouillon, soup, onion and additives, and protein preparations may also be included. Mostly, these patè are placed on the Serbian market as sterilized canned products as well as pasteurized liver patè in casing (*Anonymous*, 2015). Pork liver patè is a very popular and cheap cooked meat product manufactured and consumed all around the world (*Ivanovic et al.*, 2015). Due to high amounts of fat and non-haeme iron as well as the manufacturing process itself, liver patè is highly sucseptibile to lipid oxidation (*Lorenzo and Pateiro*, 2013).

The shelf life of canned meat products, when hermeticity is preseverved, in the first place depends on the degree of microbial destruction during heat treatment. Cans are considered commercially sterile. Comercial sterilization implies successful destruction of vegetative forms of bacteria (*Enterobacteriaceae*, staphylococci, micrococci) and especially spores of *Clostridium botulinum* and *Cl. perfringens,* as well as the absence of toxins and tissue enzymes, but with a tolerance for non-pathogenic microbiota that are not capable of causing failure of cans (*Anonymous*, 2005). The desirable taste of liver patè is significantly influenced by tissue composition (porcine, bovine, poultry meat, fat and liver) and spices and additives used. Also the intensity of heat treatment, which contributes to the appearance of a bitter taste, is correlated with the sterilization temperature. Because of that and especially in order to preserve the biological value of the product, the heat treatment should be carried out at temperatures ≤120°C, but with the imperative that the product must stay safe during the defined shelf life. The type of package (plastic film, cans) causes no difference in the taste of products which are subjected to an equal thermal treatments (*Anomymous*, 2011; *Uhler*, 2016).

According to epidemiological data and risk analysis, the major causes of foodborne diseases are microorganisms and toxins of microorganisms.

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Therefore, with the new approach to food safety it is necessary to set up adequate microbiological criteria for foods (*Buncic and Katic*, 2011). Principles for the development of risk assessment of microbiological hazards have been developed by the Codex Alimentarius (CAC) and EU Scientific Committee for Food (*Anonymous*, 2011a). This takes into account the principles of CAC and opinions, the EU Commission laid down the microbiological criteria for food in 2005 (*Anonymous*, 2005a).

The aim of this study studywas to evaluate the manufacturing process hygiene, safety and quality parameters of commercially sterilized liver patè packed in 150 g amounts in a three-part hard tin can intended for the local market. The process hygiene of liver patè production was determined by systematic monitoring of microorganisms present in patè stuffing at different waiting times before heat treatment. Product safety was monitored by determining the F_o value during the heat treatment.

Materials and Methods

Examinations were carried o ut in a meat processing plant while a total of six test series were performed. The tests consisted of three parts. The first part was related to the determination of process hygiene, the second to the examination of quality parameters, and the third to the heat treatment.

During the first stage, samples were taken after the closure of the stuffing (150 grams) into cans (three parts hard tin, manufacturer Silgan Holdings Inc., Skydra, Greece) before the heat treatment. The filled cans were sampled and investigated four times:

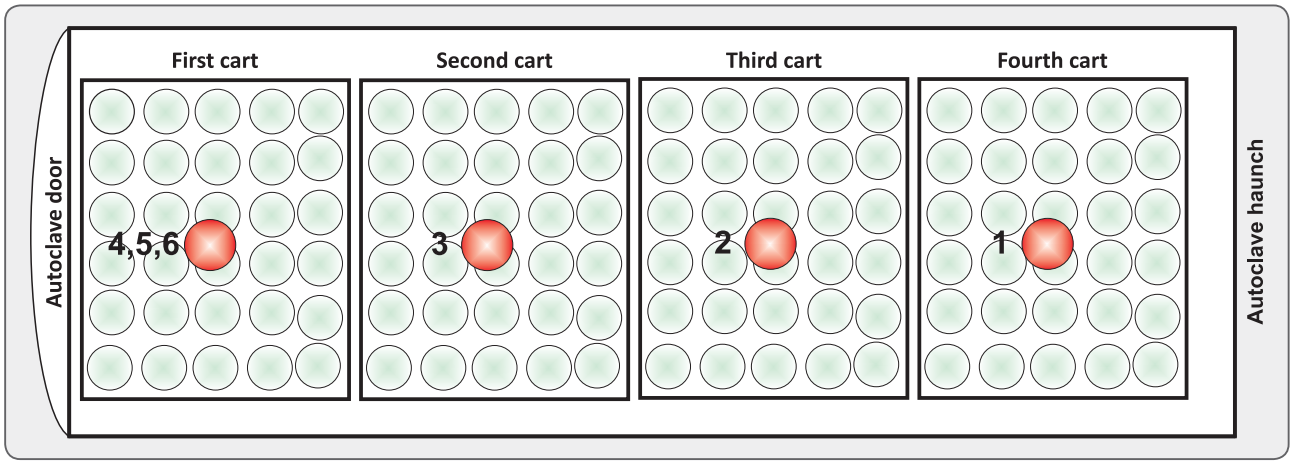
1. Immediately after the filling
2. After 90 minutes of waiting before the heat treatment
3. After 120 minutes of waiting before the heat treatment
4. After 150 minutes of waiting before the heat treatment

Table 1. List of reference methods used for enumeration of microbiota testing in the liver patè

Microbiota	Reference method
Sulphate-reducing clostridia	ISO (2011) 15213:2011 – Horizontal method for the enumeration of sulfitereduction bacteria that grow under anaerobic conditions
Escherichia coli	ISO (2008) 16649–2: 2008 – Horizontal method for the enumeration of β-glucuronidase positive *Escherichia coli* Part 2: Colony count technique at 44°C with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide;
Enterobacteriaceae	ISO (2009) 21528–2: 2009 – Horizontal method for the detection and enumeration of *Enterobacteriaceae*. Part 2: Colony-count method;
Total aerobic colony count	ISO (2008b) 4833: 2008 – Horizontal method for the enumeration of microorganisms-count technique at 30°C

Table 2. List of reference methods used for quality parameters testing in the samples

Quality parameter	Reference method
Total protein content	ISO (2008a) 16634:2008. Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content
Sodium chloride content	ISO (2004). 1738:2004. Meat and meat products – Determination of salt content
a_w value	ISO (2004b). 21807:2004. Microbiology of food and animal feeding stuff-Determination of water activity
Fat content	ISO (1998). 1444:1998. Meat and meat products – Determination of free fat content
pH value	ISO (2004a). 2917:2004. Meat and meat products – Measurement of pH – Reference meth*od*
Nitrite content	ISO. (1999). 2918:1999. Meat and meat products – Determination of nitrite content



Legend: Probe placed into the the thermal centre of the product.

Figure 1. Schematic diagram of probes positions in thermal centre of the liver patè cans in carts, in autoclave during heat treatment, viewed from above

Each sample consisted of five filled and closed units (cans). Five units were labeled and transported under cold chain conditions (+4°C) for up to two hours to the laboratory. Immediately upon arrival at the laboratory, the samples were examined for numbers of of micro-organisms: sulphite reducing *Clostridia*, *E. coli*, *Enterobacteriaceae* and total aerobic colony counts, in order to determine the process hygiene conditions. Micro-organisms were determined using reference methods, as presented in table 1.

The quality parameters of liver patè were determined in parallel by means of physico-chemical (pH value, a_w), and chemical tests (protein, fat, sodium chloride and nitrite contents).

Quality parameters in liver patè were determined using reference methods, as presented in table 2.

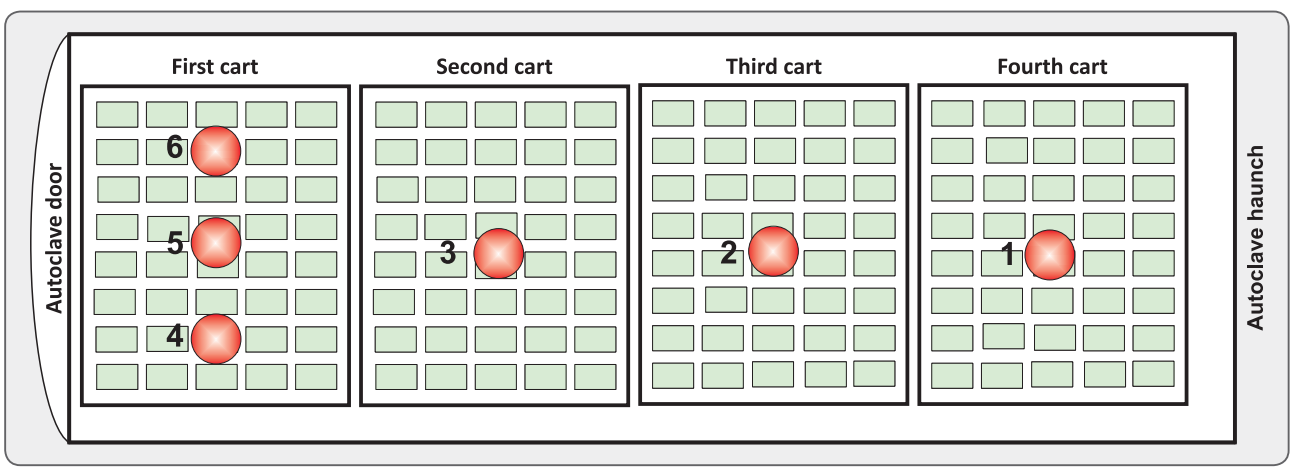
Heat treatment was validated for the same production batch. Validation was performed in a horizontal autoclave with overpressure that can receive four carts ("Sterilflow, type 1341, EA, manufactured 2013" – Roanne France), with thermocouple "ELLAB", model CTF 84with six compensating cables). The temperature and F_o values were recorded and printed every five minutes. A total of six probes were placed in four carts in the middle section, in the thermal centre of filled cans (Figures 1 and 2). Heat treatment was performed in accordance to the following formula:

$$To = 15' + \frac{55'}{114°C/3.2bar} + 20'$$

15' – was the heating time to the required autoclave temperature

55' – was the effective sterilization time under autoclave medium (114°C and pressure 3,2 bar)

20' – was the cooling time



Legend: Probe placed into the the thermal centre of the product.

Figure 2. Schematic diagram of probes positions in thermal centre of the liver patè cans in carts, in autoclave during heat treatment, viewed from viewed from the side

Statistical analysis was performed by using commercial statistical software (MS Office 2010, Excel 2010)

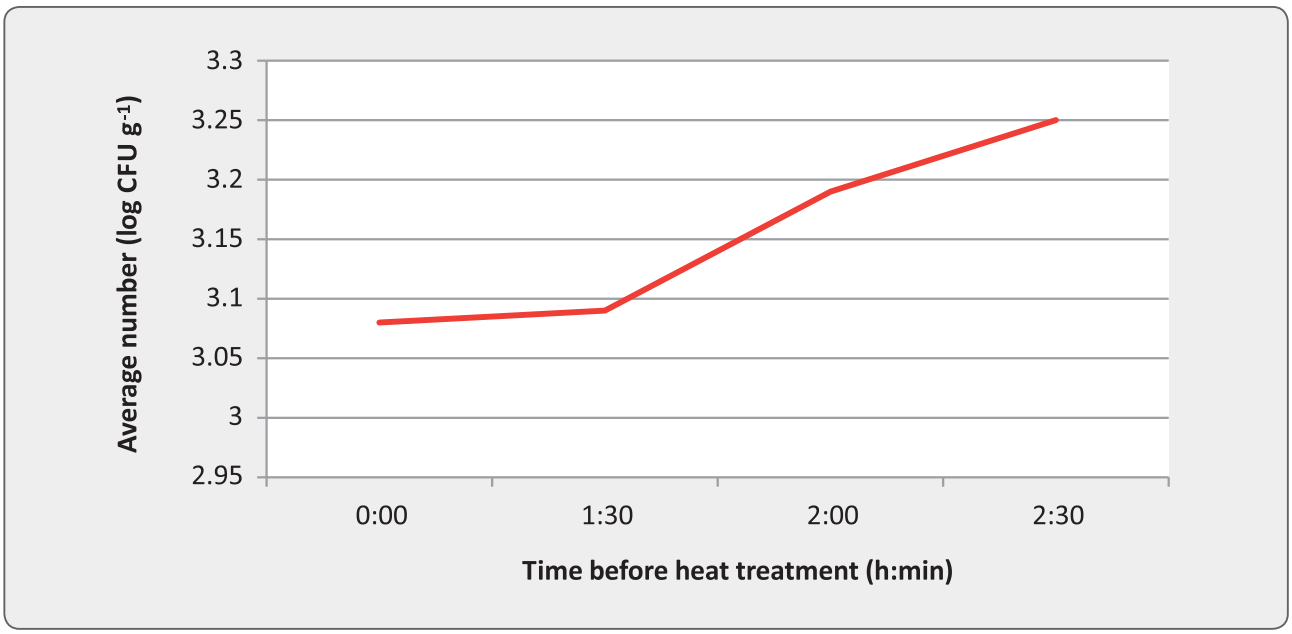
Results

Microbiological investigation showed that sulphite reducing *Clostridia* were not detected in any sample of canned stuffing before heat treatment.

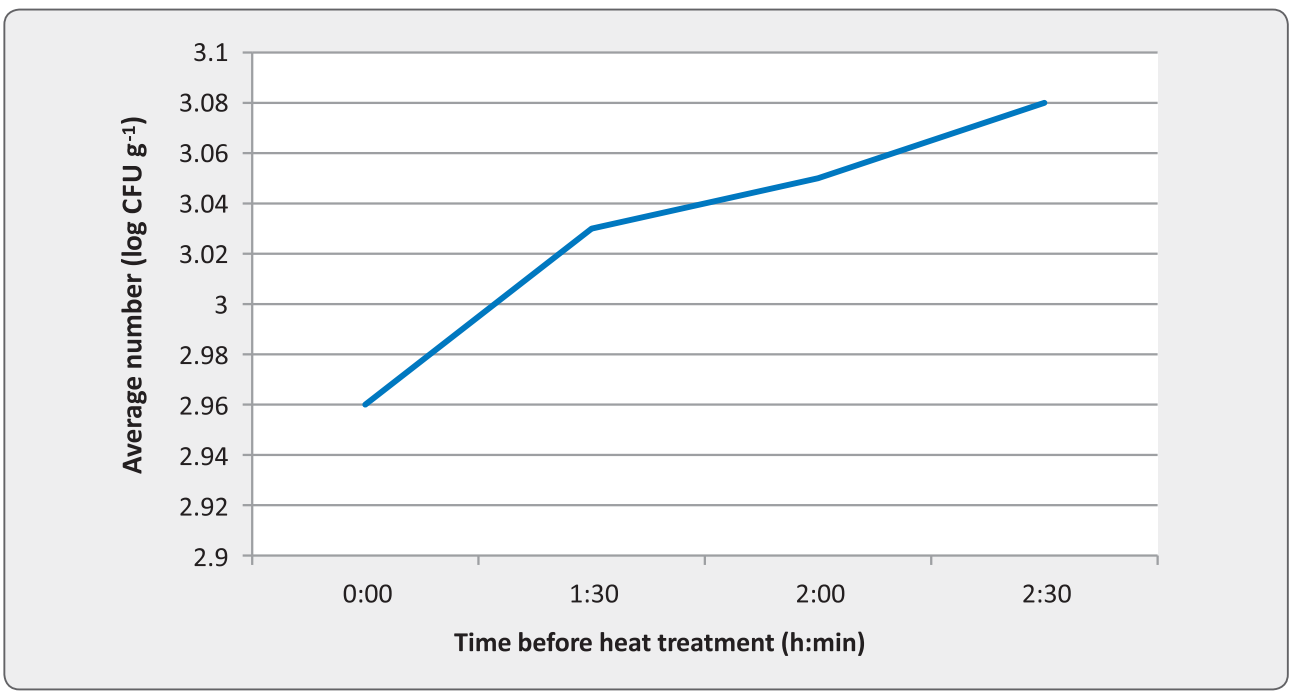
The presence of other tested microbiota is presented in Figures 3–5.

During the waiting time, in hermetically sealed cans with stuffing, an increase of the number of tested microorganism was observed.

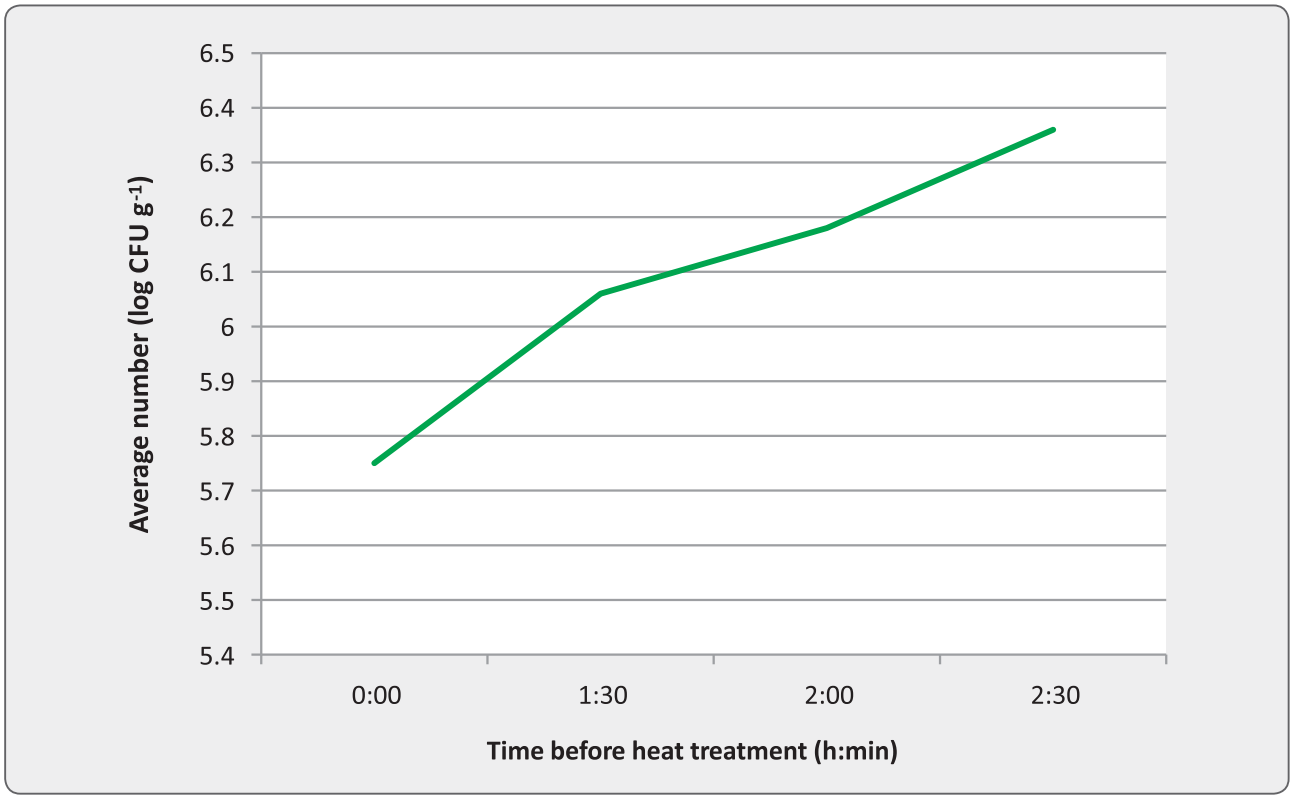
The number of *E. coli* increased from 3,08±0,36 log CFU g^{-1}, (immediately after sealing) to 3,25±0,24 log CFU g^{-1}, (after 2 hours and 30 minutes, before heat treatment).



Graph 3. Numbers of *Escherichia coli* in canned liver patè stuffing before heat treatment



Graph 4. Numbers of *Enterobacteriaceae* in canned liver patè stuffing before heat treatment



Graph 5. Numbers of total aerobic colony count in canned liver patè stuffing before heat treatment

The number of *Enterobacteriaceae* increased from 2,96±0,26 log CFU g^{-1}, (immediately after sealing) to 3,08±0,16 log CFU g^{-1}, (after 2 hours and 30 minutes, before heat treatment)

The total aerobic colony count increased from 5,75±0,39 log CFU g^{-1}, (immediately after sealing) to 6,36±0,33 log CFU g^{-1}, (after 2 hours and 30 minutes, before heat treatment)

Before heat treatment, quality parameters were determined in six repetitions, through determination of chemical and physico-chemical properties. Results are presented in table 3.

The chemical and physico-chemical parameters determined were in accordance with domestic legislation (*Anonymous*, 2015).

Heat treatment was monitored during regular production (Figures 6 and 7).

Table 3. Physical and psysico-chemical parameters of liver patè

Parameters	X±SD	Min	Max	Iv
Total protein content (%)	9,66±0,61	8,57	10,13	1,56
Fat content (%)	22,32±1,05	20,97	23,5	2,53
Sodium chloride content (%)	1,49±0,04	1,45	1,56	0,11
a_w	0,96±0,005	0,954	0,972	0,018
pH	6,44±0,11	6,32	6,56	0,24
Nitrite as $NaNO_2$ content (mg kg^{-1})	7,44±1,97	5,42	9,36	3,96

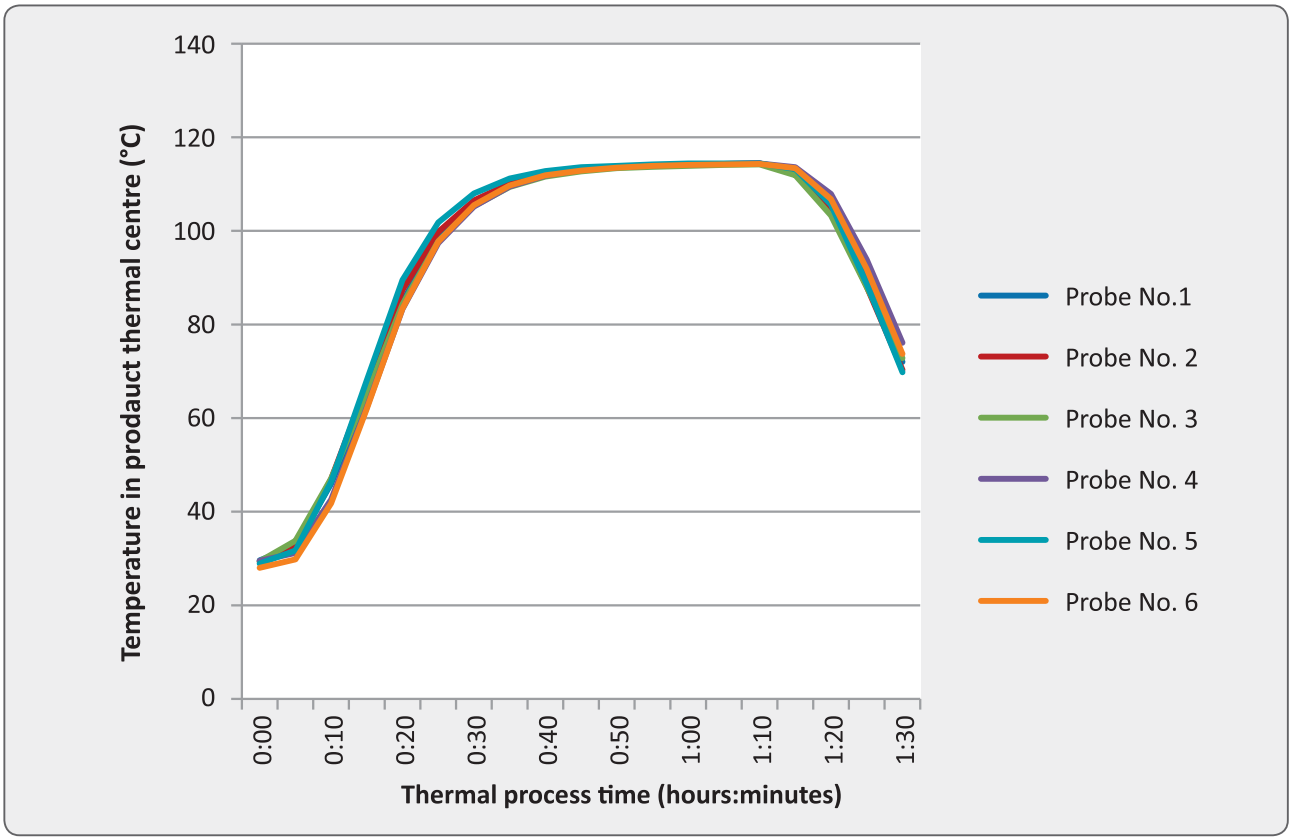


Figure 6. Temperature change measured in thermal centre during heat treatment of liver patè (150 g, in three part tinplate)

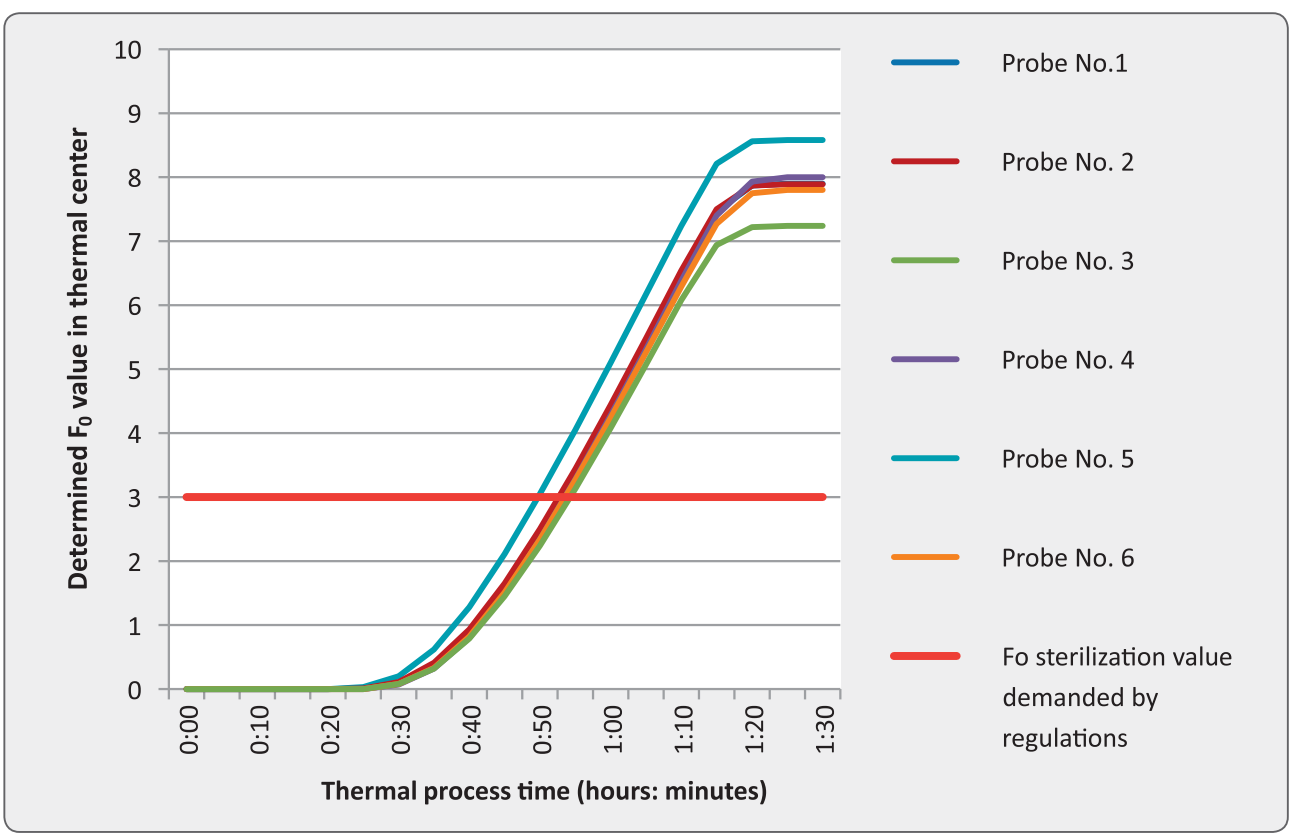


Figure 7. F_o value change during heat treatment of liver patè (150 g, in three part tinplate)

Discussion

E. coli in canned stuffing before heat treatment

E. coli is considered as an indicator of fecal contamination. Its presence in raw material generally indicates improper hygiene and/or improper handling of raw material. *E. coli* O157:H7 (causative agent of hemolytic uraemic colitis and thrombotic thrombocytopaenic purpura syndrome) is especially significant for human health. Since the early 1980s, *E. coli* O157 has emerged as one of the most significant pathogens of public health relevance not because of the incidence of the illness, which is much lower than that of other foodborne pathogens such as *Campylobacter* or *Salmonella*, but becouse of the severity of symptoms, the low infections dose and potential sequelae (*Nastasijevic et al.*, 2014). The EU Scientific Council of Veterinary Public Health issued an opinion on verotoxin-positive *E. coli* (VTEC) in food (*Anonymous*, 2003). The Council concluded that it is unlikely that the application of microbiological standards for VTEC O157 to the end product has led to a significant reduction of the related risks for consumers. However, microbiological guidelines aimed at reducing faecal contamination along the food chain can contribute to reducing public health risks, including VTEC (*Anonymous*, 2005a). Therefore, it is difficult to provide clear recommendations on the admissibility of the presence of *E. coli* in raw material which is being directed to the intensive heat treatment at temperatures of over 100°C. However a statement in Serbian regulation covering general and specific food hygiene requirements in any stage of production, processing and transport pertaining to the processing of meat preparations, (Annex 2, 2.1.8.) relevant and requires counts of 2,7–3,7 log CFU g^{-1}, immediately before heat treatment (*Anonymous*, 2010). Unsatisfactory microbial counts require measures to improve production hygiene and improvements in selection and/or origin of raw materials. Although these statements are present in the scientific literature (*Peran et al.,* 2015*)* there are also different opinions because the infectious dose of E. coli O157 is not known. In some cases of foodborne disease, only a few cells, perhaps lower than 2 log CFU, may have been ingested (*Tilden et al.,* 1996*).* Therefore, the prevention of foodborne *E. coli* O157 infections requires not only growth suppression in foods, but also elimination of the pathogen from foods (*Buncic et al.,* 2004).

In the current study *E. coli* numbers ranged from 3,08 to 3,25 log CFU g^{-1} (depending on the waiting time of canned stuffing before heat treatment), which was acceptable from the aspect of process hygiene, because all production batches were sent to commercial sterilization. Additionally the *E. coli* level was in accordance with the recommended limit (*Anonymous*, 2010). Good hygiene and manufacturing principles must be applied in all production stages, in order to maintain *E. coli* (if present at low levels) (waiting time of canned stuffing intended for heat treatment must be as short as the production process allows). At the same time, the infectious dose for vulnerable groups (elderly, children, pregnant women) is often lower than 2 log CFU g^{-1} (*Tilden et al.*, 1996), particularly in the case of the most pathogenic serovars. Therefore delays between preparation of stuffing, filling and heat treatment process must be avoided or reduced to minimum. Any deviation is unacceptable, and such product must be removed from production and adequately destroyed (*Wiliam and Doyle*, 2009).

Enterobacteriaceae in canned stuffing before heat treatment

The family *Enterobacteriaceae* includes a large group of biochemically and genetically-related bacteria, the presence/absence of which in feed/food generally indicates the level of hygiene. Their presence in heat-treated foods indicates an inadequate thermal regime or contamination after the completion of heat treatment. The European Food Safety Authority (EFSA) issued an opinion on the microbiological risks, which stated that the presence of *Enterobacteriaceae* can be used as an indicator of risk in the finished food (*Anonymous*, 2004). Moreover EFSA recommended monitoring and testing for *Enterobacteriaceae* in the production setting, and in finished product intended for special vulnerable groups (*Anonymous,* 2005). However, besides pathogenic species, the family *Enterobacteriaceae* also includes saprophytic species, which often occur in environments where food is produced without posing any health hazard. Therefore, the family *Enterobacteriaceae* can be used for routine monitoring and, if present, can trigger testing for specific pathogens. The presence of *Enterobacteriaceae* in raw materials intended for heat treatment is taken as an indication of the general hygienic manufacturing process and manufacturing plant hygiene (*Anonymous*, 2011a).

In the current study the average *Enterobacteriaceae* count in canned stuffing intended for heat treatment ranged from 2,96 to 3,08 lof CFU g^{-1}. This may be the result of poor selection of raw materials or errors during manipulation of raw material during the preparation of the product.

Total aerobic colony count of canned stuffing before heat treatment

Aerobic colony count on the surface of fresh meat directly influences its shelf life. Spoilage of fresh meat occurs when the aerobic colony count reaches 6 log CFU cm^{-2} (*Anonymous*, 2016), followed by strange smell, discolorations and texture changes if total aerobic colony counts reach 8 log CFU cm^{-2}. Because of that, the principles of Good Hygiene (GHP) and Good Manufacturing practice (GMP) are of particular importance. In the Serbian legislation on general and specific food hygiene requirements at any stage of production, processing and transport (*Anonymous*, 2010) of minced meat (which has increased surface contact with microorganisms) in Annex 2 (hygiene parameters of the production process), paragraph 2.1.6. cited the limit for aerobic colony count as being 5,7 to 6,7 log CFU g^{-1}, whereby counts ≤ 5,7 log CFU g^{-1} are satisfactory, counts between 5,7–6,7 log CFU g^{-1} are acceptable, but counts > 6,7 log CFU g^{-1} are unacceptable. Unsatisfactory results trigger improvements to production hygiene and improvements in selection and/or origin of raw materials.

In the current study the average total aerobic colony countin canned stuffing intended for heat treatment ranged from 5,75 to 6,36 log CFU g^{-1} and so were satisfactory (*Anonymous*, 2010). Special attention must be devoted to the waiting time of canned stuffing prior to heat treatment. Excessive waiting time, over two hours, led to increases of total aerobic colony count.

Physical and physico-chemical parameters of liver patè

Physical and physico-chemical parameters of liver patè can influence the optimal regime of thermal processing, canning and sustainability at recommended storage temperature.

The a_w of 0,96±0,005 was not an obstacle to microbial growth and development. Generally, common spoilage bacteria are inhibited at approximately a_w 0,97 and some pathogens (e.g. *Clostridia* spp.) at 0,94. In order to ensure human health protection by lowering food a_w, this should be lowered to at least 0,62 (*Karolyi*, 2004) to 0,75 (*Vukovic,* 2012).

Nitrite, measured as $NaNO_2$, 7,44±1,97 mg kg^{-1} was relatively low. Similar levels were observed in Germany – 11 mg kg^{-1} (*Sabine et al*., 2011), while the highest observed nitrite levels in that study were around 50 mg kg^{-1}, which is significantly higher than in our study (highest nitrite level: 9,36 mg kg^{-1}). Nitrite is applied as a preservative through curing salt, a homogenous mixture of table salt and nitrite (0,5–0,6%). In the EU the use of nitrite and nitrate in meat products is regulated (*Anonymous*, 2008). The amount of residual nitrite allowed in this type of product (patè) in Serbia is up to 100 mg kg^{-1} (*Anonymous*, 2013). Nitrites are able to prevent formation of toxins in products containing spores of *Cl. botulinum* types A and B, via an inhibitory effect on spore germination. The amount of nitrites decrease during the thermal treatment of meat products because of reaction with proteins and other components in the meat stuffing. The presence of nitrite residues ensure sustainability of the product during its shelf life. Sodium chloride plays a very important role in the production of meat products and provides a favorable effect on the texture, smell, taste and sustainability. The perception of saltiness of sodium chloride arises from a combination of sodium and chlorine ions (*Miller and Bartosuk*, 1991) and this combination gives a clean salty taste in the mouth receptor corpuscles (*Lilic et al.,* 2014).

The pH of the liver patè was 6,44±0,11 on average. This pH would not on its own effectively prevent the growth of microorganisms in the stuffing. In a product such as liver patè, low pH would negatively affect the sensory properties of the products, so the other factors are needed to achieve microbial stability.

The fat content of the liver patè was 22,32±1,05% on average. Fat content can be of great importance to the dynamics of heat treatment, as fat can act as an insulator and prevent effective heat penetration to the can thermal centre, so the heat treatment of a product containing more fat should last longer than the same product containing less fat.

Total protein content is prescribed as a quality parameter in domestic law (*Anonymous*, 2015), were liver sausage and patè must contain at least 9% meat proteins or total proteins. The average total protein content in the liver patè was 9,66±0,61 so this product met local requirements.

During storage, over longer periods of time , can stuffing is susceptible to oxidative changes. Longer storage leads to increases in peroxide and thiobarbituric acid content, due to the decomposition of fat. To decrease the intensity of fat oxidation processes antioxidants can be justifiably added to stuffing, during production. Natural antioxidant, such as that based on rosemary, would provide added value to commercial liver patè due to both its natural origin and potential bioactive properties (*Ivanovic et al.*, 2015).

Heat treatment

Commercial sterilization is a heat treatment of cans, at temperatures exceeding 100 °C, wherein, a lethality of at least F_0=3 must be achieved in the thermal centre of the product (*Anonymous,* 2015). F_o value describes heat treatment lethality. It is used for reliable control of sterilization and expresses the lethal effect of the reference temperature (121,1°C) for one minute (*Teodorovic et al*., 2015; *Vukovic*, 1996, 2006). According to FAO recommendations (*Anonymous*, 2016a) based on microbiological risk assessment, sterilization of canned meat products should achieve F_o values of 4–5,5, while the temperature should be in the range 117–130°C, depending on the characteristics of the products. If these thermal requirements are met, 1–4 year shelf life is achieved by storage temperature ≤ 25°C.

In the current study, the heat treatment of liver patè (150 g, three piece tinplate can) lasted 1 hour and 30 minutes (15 minutes warming up, 55 minutes effective sterilization and 20 minutes cooling). In the thermal centre of the cans, the achieved Fo values ranged from 7,24–8,58, using a six point check (with six probes) which ensured the safety of the product. During the sterilization process, workflow variations were not observed, which indicated that the heat treatment process occurred uniformly in all areas inside the autoclave.

Sterilization should eliminate vegetative bacteria, spores of clostridia, especially thermoresistant spores of mesophilic *Cl. botulinum* types A and B, while spores of some thermophilic bacteria, such as *Bacillus stearothermophilus*, could survive sterilization, with lethality of F_o 4–5,5. Because of that, proper storage conditions (temperature ≤25°C,dark and dry place) should be provided in order to ensure stability of the product without microbial growth. However, the general commercial intention is to implement mild thermal processing in order to preserve nutritional value and sensory characteristics (*Anonymous*, 2016a).

In order to ensure product safety food business operators must have HACCP-based self-control plans. The EU regulation on hygiene of foodstuffs states that food and staff who come into contact with food must be controlled and must adhere to all food safety instructions (*Anonymous*, 2004). Regular hygiene control must be performed by taking swabs from surfaces that come into contact with food. The elapsed time from filling stuffing cans to the beginning of the thermal treatment should not be longer than two hours as is recommended in FSIS documents.

The ability to produce microbially safe commercially sterilized canned liver patè depends on low initial microbial contamination of raw materials, adequate application of Good Hygiene (GHP) and Good Manufacturing Practice (GMP), adequately conducted technological processes, as well as adequate heat treatment.

Conclusions

The safety of canned liver patè is based on proper hermetic sealing and consecutively applied stages of intensive heat treatment. The commercial sterilization process of liver patè studied in cans with 150 g net weight, which lasted 1 hour and 30 minutes, with an effective sterilization time of 55 minutes, autoclave medium temperature 114°C and a pressure of 3,2 bar bar resulted in F_o values ranging from 7,24–8,58. This values are adequate and suitable for the canned liver patè produced.

However the efficiency of heat treatment of canned liver patè also depends on the microbiological profile of the stuffing, and the waiting time prior to heat treatment.

The microbiological profile of the liver patè stuffing before heat processing (*E. coli* from 3,08 to 3,25 log CFU g^{-1}; *Enterobacteriaceae* from 2,96 to 3,08 log CFU g^{-1}; total aerobic colony count from 5,75 to 6,36 log CFU g^{-1}) indicates a need to improve general hygiene conditions during the liver patè production process.

In general the main recommendations for the treatment of canned meat products are: 1) proper selection of raw materials with suitable of hygienic standards; 2) treatment of raw material in accordance with food safety procedures; 3) a high level of hygiene built in to the product design and manufacture; 4) manufacturing, including adequate heat treatment, in accordance with food safety objectives; 5) the preservation of can hermeticity after closure and 6) proper can storage.

Physico-chemical parameters (a_w 0,96±0,04; pH 6,44±0,11) and chemical composition (sodium chloride 1,49±0,04, nitrite content 7,44±1,97 mg kg^{-1}) of liver patè are not sufficient to prevent microbial growth on their own, so these antimicrobial parameters have to be combined with some other hurdles including the heat treatment.

The quality of the liver patè was ensured by total protein content being on a average 9,66±0,61% which is in accordance with regulations.

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