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Technological Aspects of Fish Processing with Determination of Critical Points and Identification of Microbial Contamination (of Fish Material, Devices and Product)

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1. Introduction

The fish market is recently developing and demand for fish products is growing. Total sells value of processed fish products runs at a level of approximately 2 billions (2×10^9) PLN, which represents 2% of production of food industry in Poland [14]. According to the Polish Central Statistical Office, fish consumption per capita reaches 7 kilograms per year. Total catch of sea fishes in the year 2006 reached 125.6 tonnes and total catch of fresh water fishes reached 53.9 tonnes [11]. Fish processing encompasses two types of actions: pre-processing, which means separation of edible parts of fish material and actual processing, which means preparing final products.

A vast majority of fish industry products is represented by frozen fishes (93.8 thousands tonnes in 2006) and smoked fishes (64.3 thousands tonnes in 2006). Canned fishes are also an important part of fish industry together with fish preserves (39.6 thousands tonnes in 2006) [11]. Fishes that are usually canned are herrings, sprats and mackerels. Despite the fact that canned fishes, marinades and frozen fishes require advanced processing and special technological lines, there are nearly 350 companies (of which 95% are private owned ones) that process fish products. Half of them are located in seaside provinces. After joining the EU, most of these companies faced the necessity of their production processes adaptation to the sanitary requirements of the EU.

The consumer expects fishes and fish products to be of high quality considering sensory impressions. To fulfil such an expectation, the fish processing companies should implement the Good Manufacturing Practice

(GMP) procedures, the Good Hygiene Practice (GHP) procedures and Hazard Analysis and Critical Control Point (HACCP) system. The GMP / GHP practices consist of various procedures such as: washing and disinfection, personnel hygiene, water tests, storage, protection against pests etc. The basic documentation of HACCAP system consists of: description of the product, the system is implemented for, block diagram of production process, risk assessment sheet with risk prioritization and containment methods, Critical Control Point determination sheet, monitoring sheet and rectifying actions sheet for each CCP, and finally sheet of quality control loop for each CCP.

The HACCAP system is based on six fundamental principles (actions):

1. Possible hazards analysis, i.e. identification of factors that may result in a creation of product that may be dangerous for the consumer.
2. Critical Control Points identification.
3. Determination of critical limits for each CCP identified.
4. Establishing the parameters monitoring system for each CCP, i.e. introducing the control system with parameters storage capability.
5. Establishing rectifying actions.
6. Establishing verification procedures [9].

One of verification methods of HACCAP systems are microbiological tests. To prove the effectiveness of hazards control, tests of fish material, final products, spices, production lines, devices and personnel are usually conducted [8]. Identification of potential hazards at every stage of production process from the material delivery through the processing to the final consumption is a basic method of CCPs determination.

The aim of this paper are quantitative and qualitative analyses of microbial contamination of fish material, semi-finished and final products to determine control points of microbial contamination.

2. Materials and methods used

The research was conducted during two one-day sessions i.e.: 15th of February (session I) and 17th of May (session II).

The examined material originated from production line of oil steamed herrings. The microbiological purity of material has been examined at every stage of production process:

1. Material delivery and pre-processing: the material examined was a raw one – **frozen Baltic herring** formed into blocks (defrosted over period of 6-8 hours) and **fresh Baltic herring** (inedible parts have been removed by beheading and gutting). Test samples of 1kg weight (raw material, chamber "0") have been collected in accordance with Polish norm PN-85/A-86752 (Fresh and deep frozen fishes and aquatic invertebrates - Sampling). The

microbiological examinations have been conducted in accordance with Polish norm PN-A-86730 referring to fish and fishery products;

2. Material rinsing and brining: **water** both **from internal intake and public water supply** system has been examined as well as **the brine** and **the brined fish**. Water test sample (of 500ml volume) have been collected in accordance with Polish norm PN-74/C-04620/02. Water examinations have been conducted in accordance with Polish norms PN-EN ISO 9308/2004 (Water quality - Detection and enumeration of Escherichia coli and coliform bacteria) and PN-EN ISO 6222/2004 (Water quality – Enumeration of culturable micro-organisms-Colony count by inoculation in a nutrient agar culture medium). In case of a brine (taken in a volume of 500ml for each sample) examinations of overall number of micro-organisms have been conducted with plate method at temperature of 30^0C in accordance with Polish norm PN-EN ISO 4833/2003 (Microbiology – General guidance for the enumeration of micro-organisms – Colony count technigue at 30°C);
3. Canning and oil steaming: **the fish after steaming** process has been examined as well as **the oil marinade** itself. Fish test samples of 1kg weight have been collected in accordance with Polish norm PN- A -86752/1985 (Fresh and deep frozen fishes and aquatic invertebrates – Sampling); oil marinade have been collected in a volume of 500ml. Microbiological test have been conducted in accordance with Polish norm PN-A-86730/1989. Packages have also been examined with rinsings method;
4. Sterilization of cans: **the can after sterilization and after ripening** processes has been examined. Test samples have been collected in accordance with Polish norm PN-A-86731. The quality of cans of herrings in oil marinades has been evaluated in accordance with Polish norm PN-A-86762/1994;
5. Storage: **can from retail market** has been examined. Test samples have been collected in accordance with Polish norm PN-A-86731. Examinations have been conducted in accordance with Polish norm PN-A-86762/1994;
6. Microbiological analyses of air inside production hall have been conducted as well. Microbiological purity of air has been evaluated with sedimentation method in accordance with Polish norm PN-ISO-7218/1998 (Microbiology of food and animal feeding stuffs – Geneal rules for microbiological examinations). The scope of basic tests concerned overall number of mesophilic bacteria and number of moulds and yeasts fungi in m^3 of air. Bacteria were cultured on a nutrient agar, while fungi on a Sabouraud's medium with chloramphenicol and on a potato - dextrose agar (PDA agar). Assessment of a contamination level of atmospheric air with bacteria has been interpreted in accordance with Polish norm PN-89/Z-04111/02, while fungal contamination has been interpreted in accordance with Polish norm PN-89/Z-04111/03.

The assessment of microbiological purity of examined material has been conducted as a deep-seated inoculation of diluted material with the Koch's inoculation method. The media used and culturing parameters has been presented in Table 1. The assessment criteria were numbers of vegetative and spore forms of bacteria. Identification of cultured bacteria has been conducted with use of API mini analyzer made by bio Merieux company with API 50 CHB, ID 32 STAPH, ID 32 GN tests. Identification of fungi genera has been made on a base of their macroscopic and microscopic features taking into account morphological structures such as: structure of hyphae, sporangium (sporangial) and spores (sporangial) as well as conidiophores and (or) conidia spores. During identification of yeasts cultured, test ID 32 C of bioMerieux company was used.

Table 1. The medium used and culturing parameters
Tabela 1. Zastosowane media i parametry kultur

Lp.	Medium	Incubation parameters	Kind of microorganisms
1.	Nutrient agar	37°C / 48 hours	Mesophilic bacteria
2.	Nutrient agar	20°C / 72 hours	Psychrophilic bacteria
3.	Nutrient agar	37°C / 72 hours	The bacterial spore
4.	Endo's medium	44°C / 48 hours	*Escherichia coli*
5.	Chapman's medium	37°C / 48 hours	*Staphylococcus sp.*
6.	Sabourod's agar with chloramphenicol	20°C / 5 days	Moulds and yeast fungi
7.	PDA agar	30°C / 72 hours	Yeast
9.	Brilliant Green Agar -BGA	44°C / 48 hours	*Escherichia coli*
10.	Azide Dextrose broth	37°C / 48 hours	*Enterococcus sp.*

3. Tests results

Results of microbiological tests that characterize microbial contamination have been collected in Tables 2, 3 and 4.

Results of quantitative analysis of microbial air contamination (Table 2) showed, that contamination of particular parts of production line with bacterial and fungal flora was insignificant. The water from internal intake appeared to be microbiologically clean as well. The water from public water supply appeared to be contaminated with bacterial micro flora during both tests sessions, especially with psychrophilic bacteria. Number of psychrophilic bacteria ($1.2x10^3$cfu cm^{-3}) was higher than allowable by appropriate norm (Dz.U. Nr 203 Pos. 1718 dated 19.11.2002; $1.0x10^2$cfu cm^{-3}). Likewise, number of mesophilic bacteria ($5.0x10^1$cfu cm^{-3}) exceeded value allowable for water from public water supply system ($2.0x10^1$cfu cm^{-3}). Water samples (both from

internal intake and from public water supply system) during both test sessions did not reveal *Escherichia coli*.

Bacterial contamination of material originated from the cold room was detected during both test sessions. Mesophilic bacteria have also been isolated (first test session $8.9x10^3$ cfu g^{-1}, second tests session $1.2x10^4$cfu g^{-1}), as well as psychrophilic (respectively: $1.4x10^5$ cfu g^{-1} and $5.1x10^4$ cfu g^{-1}). The brined and steamed fishes have been highly contaminated with mesophilic microorganisms (1st test session $3.4x10^4$ cfu g^{-1}; 2nd test session $1.3x10^4$ cfu g^{-1}). Contamination with psychrophilic bacteria was lower and run at a level of $5.0x10^1$ cfu g^{-1} during 1st test session and at $6.6x10^3$ cfu g^{-1} during a second one. Number of mesophilic bacteria isolated from fishes after steaming process run during 1st test session at a level of $1.0x10^4$ cfu g^{-1} and during the 2nd one at a level of $1.2x10^4$ cfu g^{-1}. Psychrophilic bacteria have been cultured only during 2nd test session. Their number run at a level of $2.8x10^3$ cfu g^{-1}.

Microbial contamination of raw material and pre-processed products with fungi run at a lower level. Number of fungi in the material from „0" chamber during both test sessions run at a similar level of ($1.0x10^1$ g^{-1} nad $1.1x10^1$ g^{-1}). Fungi have been isolated in fishes after brining process only during 2nd test session ($1.1x10^3g^{-1}$). However, during both test session, number of fungi isolated from fishes after steaming process was relatively high ($2.5x10^3g^{-1}$ and $2.3x10^3g^{-1}$).

Additional materials (brine and oil marinade) have been microbiologically pure. Analogically, cans after sterilization and ripening processes have not revealed microbial contamination. An insignificant contamination has been revealed in case of cans from retail market (similar levels during both test sessions: $7.3x10^1$ cfu g^{-1} and $5.2x10^1$ cfu g^{-1}). Likewise, packaging examinations with rinsings method revealed, during both test sessions, insignificant contamination with both mesophilic bacteria ($4.0x10^1$ cfu cm^{-3} i $3.5x10^1$ cfu cm^{-3}), and fungi (**$7.0x10^1$**, 8.1x101cfu cm^{-3}**)**.

The qualitative examinations of bacterial microflora in the air revealed an existence of the *Bacillus licheniformis* (4.3×10^3 cfu m^{-3}) and *Micrococcus lylae* ($1.7x10^3$ cfu m^{-3}) bacteria during the first test session and *Bacillus licheniformis* (2.6×10^4 cfu m^{-3}) and *Micrococcus luteus* (3.5×10^4cfu m^{-3}) during the second session. The *Acinetobacter baumanni* (1.0×10^2cfu cm^{-3}) bacteria dominated in the water from public water supply system during the first session, and *Flavobacterium odoratum* **(**1.3×10^3 cfu cm^{-3}) during the second one.

Table 2. Quantitative analysis of microbial contamination
Tabela 2. Analiza ilościowa zanieczyszczeń mikrobiologicznych

Material examined	**Session I**		**Session II**	
	Microorganisms	Quantity	Microorganisms	Quantity
Air	Bacteria Fungi	**cfu m^{-3}** 39.5 6	Bacteria Fungi	**cfu m^{-3}** 41.2 4.1
Water from internal intake	Mesophilic bacteria Psychrophilic bacteria *Escherichia coli* Fungi	Not observed	Mesophilic bacteria Psychrophilic bacteria *Escherichia coli* Fungi	Not observed
Water from public water supply system	Mesophilic bacteria Psychrophilic bacteria *Escherichia coli* Fungi	**cfu cm^{-3}** 5.0 x 10^1 1.2 x 10^3 - -	Mesophilic bacteria Psychrophilic bacteria *Escherichia coli* Fungi	**cfu cm^{-3}** 5.0 x 10^1 1.0 x 10^4 - -
Material from cold room	Mesophilic bacteria Psychrophilic bacteria Fungi	**cfu g^{-1}** 8.9 x 10^3 1.4 x 10^5 1.0 x 10^1	Mesophilic bacteria Psychrophilic bacteria Fungi	**cfu g^{-1}** 1.2 x 10^4 5.1 x 10^4 1.1 x 10^1
Brined fish	Mesophilic bacteria Psychrophilic bacteria Fungi	**cfu g^{-1}** 3.4 x 10^4 5.0 x 10^1 -	Mesophilic bacteria Psychrophilic bacteria Fungi	**cfu g^{-1}** 1.3 x 10^4 6.6 x 10^3 1.1 x 10^3
Steamed fish	Mesophilic bacteria Psychrophilic bacteria Fungi	**cfu g^{-1}** 1.0 x 10^4 - 2.5 x 10^3	Mesophilic bacteria Psychrophilic bacteria Fungi	**cfu g^{-1}** 1.2 x 10^4 2.8 x 10^3 2.3 x 10^3
Brine	Mesophilic bacteria Psychrophilic bacteria Fungi	Not observed	Mesophilic bacteria Psychrophilic bacteria Fungi	Not observed
Oil marinade	Mesophilic bacteria Psychrophilic bacteria Fungi	Not observed	Mesophilic bacteria Psychrophilic bacteria Fungi	Not observed
Can after sterilization	Mesophilic bacteria Psychrophilic bacteria Fungi	Not observed	Mesophilic bacteria Psychrophilic bacteria Fungi	Not observed
Can after ripening	Mesophilic bacteria Termophilic bacteria Fungi	Not observed	Mesophilic bacteria Termophilic bacteria Fungi	Not observed
Can from retail market	Mesophilic bacteria Termophilic bacteria Fungi	**cfu g^{-1}** 7.3 x 10^1 - -	Mesophilic bacteria Termophilic bacteria Fungi	**cfu g^{-1}** 5.2 x 10^1 - -
Packaging	Mesophilic bacteria Fungi	**cfu cm^{-3}** 4.0 x 10^1 7.0 x 10^1	Mesophilic bacteria Fungi	**cfu cm^{-3}** 3.5 x 10^1 8.1 x 10^1

There were only *Bacillus subtilis* (4.1 x 10^1cfu g^{-1}) detected during both sessions in cans from retail market. The packaging was contaminated during the first session with *Micrococcus luteus* (2.3 x 10^1cfu/g) and during the second one with *Bacillus subtilis* (2.1 x 10^1cfu g^{-1}). Detailed results of qualitative analyses of bacterial microflora have been collected in Table 3.

Table 3. Qualitative analysis of bacterial microflora
Tabela 3. Analiza jakościowa flory bakteryjnej

Material examined	**Session I**		**Session II**	
	Microorganisms	Quantity	Microorganisms	Quantity
Air		**cfu m^{-3}**		**cfu m^{-3}**
	Bacillus licheniformis	4.3 x 10^3	*Bacillus licheniformis*	2.6 x 10^4
	Micrococcus lylae	1.7 x 10^3	*Micrococcus luteus*	3.5 x 10^4
Water prom public water supply system		**cfu cm^{-3}**		**cfu cm^{-3}**
	Acinetobacter baumanni	1.0 x 10^2	*Flavobacterium odoratum*	1.3 x 10^3
Material from cold room		**cfu** g^{-1}		**cfu** g^{-1}
	Staphylococcus aureus	2.1 x 10^3	*Bacillus subtilis*	1.4 x 10^3
	Staphylococcus xylosus	1.3 x 10^3	*Moraxella lacunata*	9.0 x 10^2
			Escherichia coli	3.0 x 10^3
			Staphylococcus cochin	1.5 x 10^2
			Bacillus psychrophilus	3.0 x 10^2
			Bacillus laterosporius	2.0 x 10^4
			Staphylococci	2.5 x 10^4
Brined fish		**cfu g^{-1}**		**cfu g^{-1}**
	Moraxella lacunata	1.8 x 10^3	*Bacillus halophilus*	5.0 x 10^3
	E. coli	4.1 x 10^3	*Enterobacter amnigenus*	1.0 x 10^1
	Staphylococcus aureus	2.9 x 10^3	*Bacillus subtilis*	
			Staphylococcus capitis	1.0 x 10^2
				1.0 x 10^2
Steamed fish		**cfu g^{-1}**		**cfu g^{-1}**
	Escherichia coli	2.0 x 10^3	*Bacillus subtilis*	1.5 x 10^3
	Proteus mirabilis	1.0 x 10^2	*Staphylococcus gallinarum*	1.0 x 10^2
	Staphylococcus aureus	4.2 x 10^2		
Can from retail market		**cfu g^{-1}**		
	Bacillus subtilis	4.1 x 10^1		
Packaging		**cfu cm^{-3}**		**cfu cm^{-3}**
	Micrococcus luteus	2.3 x 10^1	*Micrococcus luteus*	1.3 x 10^1
			Bacillus subtilis	2.1 x 10^1

There were fungi from the genus *Rhizopus sp.* (2.5 x 10^1cfu m^{-3}) type present in the air during the first session and from the genus *Penicillium sp.* (6.1 x 10^1cfu m^{-3}) type during the second one. Material originated from the cold room contained *Geotrichum sp.* (1.0 x 10^1cfu g^{-1}, 1.1 x 10^1cfu g^{-1}) fungi during both sessions. The brined fish revealed during the second session yeasts of

Rhodotorula sp. (1.1 x 10^3cfu g^{-1}) type. The steamed fish contained during the first session *Rhizopus* (2.5 x 10^3cfu g^{-1}) and during the second one *Rhodotorula sp.* (1.3 x 10^3cfu g^{-1}).

The packaging has been contaminated with *Rhizopus sp.* (7.0 x 10^1cfu cm^{-3}; 5.0 x 10^1cfu cm^{-3}) during both sessions. Detailed results of qualitative analyses of fungal microflora have been collected in Table 4.

Table 4. Qualitative analyses of fungal microflora
Tabela 4. Analiza jakościowa flory grzybowej

Material examined	**Session I**		**Session II**	
	Microorganisms	Quantity	Microorganisms	Quantity
Air		**cfu m^{-3}**		**cfu m^{-3}**
	Rhizopus sp.	2.5 x 10^1	*Penicillium sp.*	6.1 x 10^1
	Penicillium sp.	1.3 x 10^1	*Cladosporium sp.*	2.3 x 10^1
	Mucor sp.	1.2 x 10^1	*Mucor sp.*	5.7 x 10^1
Material from cold room		**cfu g^{-1}**		**cfu g^{-1}**
	Geotrichum sp.	1.0 x 10^1	*Geotrichum sp.*	1.1 x 10^1
Brined fish	-	-		**cfu g^{-1}**
			Rhodotorula sp.	1.1 x 10^3
Steamed fish		**cfu g^{-1}**		**cfu g^{-1}**
	Rhizopus sp.	2.5 x 10^3	*Rhodotorula sp.*	1.3 x 10^3
			Rhizopus sp.	1.0 x 10^3
Packaging		**cfu cm^{-3}**		**cfu cm^{-3}**
	Rhizopus sp.	7.0 x 10^1	*Rhizopus sp.*	5.0 x 10^1
			Aspergillus sp.	3.1 x 10^1

4. Discussion and Critical Control Points description

The assessment of sanitary and hygienic condition of the company encompassed microbial contamination tests (of fish material, devices and product) at the production line of oil steamed herrings with critical points designation.

The company that produces food and has already implemented GHP, GMP practices and HACCP system should possess appropriate cloakrooms and locks that minimize possibility of external microflora transmit into the production halls. Biological hazard factors, that exist in the environment, lead to various unfavourable results, first of all they cause hazards of contamination of working place as well as production one. Taking the fact into account, that the most hazardous factor for the product are employees – unaware pathogenic microorganisms carriers, control of air contamination parameters at the

production hall was indispensable during own examinations. Values of isolated bacteria and fungi concentration run at a safe level $7.5 \times 10^2 - 1.0 \times 10^7$ cfu m^{-3}, i.e. they did not exceed norms allowable for air in the production halls [7] .

While determining conditions of production hall, special attention has been paid to contamination of water from public supply water system. The production process specificity imposes large consumption of water, which microbiological condition has a substantial influence over clearness and hygiene at every stage of technological process. Cleaning devices or floors with microbiologically contaminated water results in an ease of bacteria transit to the fish material, semi-finished product, supplementary ingredients and packages. Therefore investments in the sanitary infrastructure facilities of food processing companies are undoubtedly required. It came out, that water contamination revealed in this particular case was probably a result of defective water supply system or faucets contamination at place, where test samples have been acquired [10].

The comparison of results of water tests with requirements defined by regulation of Ministry of Health, dated November 19th 2002 [16], indicates that overall quantity of bacteria cultured during both sessions has significantly exceeded allowable values, whereas microbiological condition of water originated from internal intake was of high sanitary level. It is used directly in a production process i.e.: during fish material pre-processing, for fish steaming, for brine preparation and packages washing.

An optimal conditions of unloading and transport of fish material at the quay are the fundamental factors to assure the highest freshness of products delivered to the consumer. According to the tests conducted, the material delivered has been contaminated, while contamination depended on a material type (fresh or frosted). Frozen material (1st test session) had larger psychrophilic microflora. Pre-processing of frozen material took place in an another company. Discovered contaminations may be a result of natural psychrophilic microflora of raw material, but may also come from unhygienic pre-processing. Leakage of refrigerating chain from the catch moment to the distribution moment or an inappropriate transport conditions are another factors that may have create contamination. Modernization of unloading operations, improvement of storage conditions of the catch and unloading security are the most important aspects that may reduce contamination.

The fresh Baltic herring (test session II) is characterized by much more luxuriant bacterial microflora in respect of both qualitative and quantitative analyses. It was significantly influenced by the fact, that the raw material has been delivered directly to the company and pre-processed there. Available in the

literature [15] descriptions of fishes' surface covered with mucus indicate presence of psychrophilic bacteria of the genus *Pseudomonas, Achromobacter, Flavobacterium* and *Micrococcus* and mesophilic bacteria of the genus *Sarcina* and *Serratia*. These bacteria penetrate gradually into fish muscles. During transport and distribution one may notice the rise of mesophilic microflora, bacteria from coli group, campylobacter, coccus and bacillus. The microflora that have been identified in the material has probably cultured as a result of derivative contamination during storage, pre-processing or transport [2]. Modernization or creation of a new pre-processing halls (e.g. sorting plants, cold rooms for fresh fishes storage, ice plants), improvement of the quality of water used during production processes are just some factors that may improve safety of raw material reception [14].

Devices and tools used during production processes create another source of microorganisms. The only dubious devices was the evaporator. The evaporator had a cover bent at its opening, which may result in a microbial contamination of semi-finished product – fishes after steaming.

Available literature [13] contains data showing, that during machine pre-processing phase, the contamination with microorganisms rises from the level of 3×10^3cfu g^{-1} at the beginning of the process to 5.3×10^4cfu g^{-1} after 10 minutes and to 1.3×10^5cfu g^{-1} after one hour. Average level of initial contamination starts at the level of 1.2×10^5cfu g^{-1}, but rises to 4.9×10^5cfu g^{-1} after one hour. The bacteria culturing during pre-processing phase is mostly influenced by microbiological conditions of devices i.e. frequency and precision of their cleaning. It has been stated, that initial contamination level is much higher (reaching 1.4×10^6cfu bacteria g^{-1}) in case of pre-processing conducted by devices that have not been precisely cleaned the previous day. An improvement of sanitary and hygienic conditions of fish processing companies has to be connected with technological lines modernization, increase of technical standards of production halls, as well as proper washing of a raw material, knifes, hands and production surfaces [15]. Managers of the company are expected to organize seminars, trainings or conferences concerning the possibilities and ways of quality improvements of processed material. The priority is to obey sanitary and hygienic principles at every stage of production process [1].

Eliminating brine as a medium that transferred contaminants onto fish material (examinations revealed neither bacterial nor fungal microflora in the oil marinade and the brine) one should consider other possibilities of such a large contamination of material after brining process. The most widespread hazard in working environment comes from bio-aerosoles transmitted over the air. Taking

this fact into account together with *Staphylococcus aureus* presence in the examined material, one may consent that contaminations could be a result of inappropriate hygiene of personnel [12].

The own examinations revealed large microbial contamination of material after steaming process, that may have appeared during processing. The source of such a contamination at this stage was probably, already indicated, defect of evaporator. As far as there is no comparative literature which would define allowable limits of microorganisms content at this phase of production process, results of own examinations were compared to delicatessen semi-finished products [2]. This showed, that the quantity of identified *Staphylococcus aureus* in fishes after steaming process runs at acceptable level when compared to limits defined for delicatessen semi-finished products, whereas amount of mesophilic bacteria in the fish after steaming process, during 2nd test session was higher than allowable. Examinations results showing contamination with bacteria potentially dangerous for consumers are, in this case, acceptable because examined fishes after steaming process were a semi-finished product ready for next phase: sterilization.

Examinations of cans after sterilization and ripening processes revealed no contamination. The sterilization process run properly and efficiently eliminated previous contaminations with microorganisms. An insignificant contamination may be noticed in case of the can taken "out of the market's shelf". The can contained *Bacillus subtilis*. Detected contamination may be a result of a production process of that particular can or unhygienic opening. Anyway, the quantity of detected bacteria does not disqualify the product.

According to the regulation of Ministry of Health, dated September 6th 2001 (about materials and products designed for food keeping), the use of particular packaging material should be permitted by an appropriate certificate made out by National Institute of Hygiene. The packages examined revealed significant level of fungal contamination, which may be a result of improper conditions of packages storage or improper transport conditions. During both test sessions insignificant contamination with air originated bacteria has been noticed. The micro flora of the packaging may influence microflora of semi-finished products used during further processing [5, 6].

During production process of canned fish products, there is a hazard of resting bacteria existence, especially the hazard of its disclosure in finished product, which has already been sterilized. In case of examined material, existence of resting bacteria has not been detected regardless of test sessions.

The results revealed the most important sources of hazards during technological process of canned fish production, and created the fundament for

critical control points determination. The common available research publications, when referring to critical control points determination, usually point to two phases of canned fish production process: cans' **closing** and **sterilization**. During hazards analyses, three critical control points have been identified: **material delivery**, cans **filling** and **sterilization;** an additional non-critical control points have been also identified: storage of fresh and frozen material, fish canning and pouring out the remains of marinade oil after steaming process. The control points identification conforms with general literature guidelines, as every analysis of hazards is to be conducted for specific technological line of particular product, with respect to the conditions of particular production company [4].

CCP1 – Raw fish material, both fresh and frozen, that is delivered to the company should be reliably certified. Usually the material comes from reliable suppliers. During the delivery, material's organoleptic features are visually tested as well as the cleanness of transport means. The parasite contaminations are observable at once, while physicochemical and microbial contaminations require the conduct of appropriate tests. According to the microbiological tests of the material, one may note, that the material is contaminated and that contamination removal process is very difficult. Initial contamination accompanies the material up to the sterilisation phase. An examination of material should be precisely and carefully planned, conducted and documented. Eliminating the maximum amount of potential material contaminants at the beginning of technological process results in production of product of high sanitary quality [3].

CCP 2 – The cans' closing phase is a critical control point, because it influences sanitary suitability of product for consumption. At this critical point, the **tightness of cans** is tested. Thorough washing allows to detect the leakiness of seams. After washing, the can is immersed for 15 minutes in the water (at temperature of 45-50°C), and than carefully dried with clothes. The can's casing is tightly enveloped with filter paper, which width corresponds with can's height. Subsequently, cans are placed in a vacuum chamber with pressure set at 9.25 atmospheres for the period of 3 minutes. After taking cans out, the filter paper is examined for possible dampness. The tightness tests are conducted daily.

CCP3 – The sterilization process, as a critical control point, has to be carefully controlled. Inappropriate determination of sterilization time or wrong maintenance of autoclaves are the most frequently identified reasons of inappropriate cans' sterilization. Such a mistakes may become even more costly when not detected promptly, as they lead to the spoiling of an entire batch of product. The measurement of production parameters, especially temperature in

this case, has to be credible and reliable. It is indispensable to use an appropriate measurement gauges that are capable of precise measurements of processing values at a given moment. It is recommended that gauges are connected to the computers, which store and archive measurement data and their changes over time [4].

CP1 – Material storage, both fresh and frozen, has been identified as a control point, because proper storage conditions could minimize culturing both initial and derivative microflora of the material.

CP2 – Can's filling is a control point owing to the fact, that the process is conducted manually. The personnel that fills cans with fish material should wear proper working aprons, caps, masks and gloves. Personnel should obey the hygienic rules. Every worker who conforms to such rules does not expose the product to the possibility of derivative contamination. It is also very important, that personnel should control cleanness of their working stations, packaging and the material they have a direct contact with. The obligatory, cyclic trainings on the area of health and safety at work and the workers' awareness are also key issues. Therefore, it is necessary to strive for build-up of self-control habits of personnel at their working stations.

CP3 - Pouring out the remains of marinade oil after steaming process has also been identified as a control point. This phase is initially conducted manually and is associated with sliding cans out of the conveyor belt. Cans are sent to the casting wheels than. Derivative contamination of the material is highly influenced both by peoples and devices. Wheels have a number of curvatures, corners and fastening belts that hamper their cleaning. Conducted research confirmed significant contamination at this phase of production. Observations clearly strengthened the importance of hygienic state of casting wheels during production process and the need of their inspection. The hygienic state of both personnel and devices should be carefully inspected to minimize hazards of microbial contamination. The regularity and precision of devices' cleaning should be obeyed. More frequent inspections for microbial contamination should be planed and appropriate documentation should be kept [4, 5, 6].

5. Conclusions

1. The dominating bacterial microflora of raw material, semi-finished products and finished products from production lines of oil-steamed herrings consisted of the bacteria of the genera *Staphylococcus* and *Bacillus*, while fungal microflora consisted mainly of *Rhizopus* genus.

2. The largest microbial contamination has been identified in raw material. The microbial contamination of final product run at allowable levels and did not pose health hazards for consumers.
3. The critical control points identified in en examined company were: material delivery and cans' closing.

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Technologiczne aspekty przetwórstwa ryb z określeniem punktów krytycznych i identyfikacją zanieczyszczeń mikrobiologicznych (ryb, urządzeń i produktów)

Streszczenie

Ogromna większość produktów przemysłu rybnego to ryby mrożone (93,8 tysięcy ton w 2006) i wędzone (64,3 tysięcy ton w 2006). Rybne w puszkach to również ważna część przemysłu rybnego wraz z konserwami rybnymi (39,6 tysięcy ton w 2006) [11]. Ryby, które zwykle sprzedawane są w puszkach to śledzie, szproty i makrele. Pomimo faktu, że ryby w puszkach, marynaty i ryby mrożone wymagają zaawansowanego przetwarzania i specjalnych linii technologicznych, istnieje 350 zakładów (w tym około 95% prywatnych) które przetwarzają ryby. Połowa z nich położona jest w paśmie nadmorskim. Po wstąpieniu do Unii, większość z tych zakładów stanęła przed koniecznością adaptacji procesu produkcyjnego do wymagań sanitarnych UE.

Konsument oczekuje od ryb i produktów rybnych wysokiej jakości pod względem wrażeń organoleptycznych. Aby spełnić te wymagania zakłady przetwórstwa ryb powinny wprowadzać procedury Dobrej Praktyki Produkcyjnej (GMP), Dobrej Praktyki Higieny (GHP) oraz system Analizy Zagrożenia i Krytycznych Punktów Kontrolnych (HACCP). Praktyki GMP/GHP składają się z różnych procedur, takich jak: mycie i dezynfekcja, higiena personelu, badania wody, przechowywanie, ochrona przeciw szkodnikom itd. Podstawowa dokumentacja systemu HACCAP składa się z: opisu produktu, wprowadzonego systemu, schematu blokowego procesu produkcji, arkusza oceny ryzyka z priorytetyzacją ryzyka i metodami zapobiegania, arkuszem wyznaczającym Krytyczne Punkty Kontrolne, arkuszem monitoring i arkuszem mechanizmów naprawczych dla każdego CCP i w końcu arkusz pętli sterowania jakością dla każdego CCP.

Przeprowadzono analizę ilościową i jakościową zanieczyszczeń mikrobiologicznych surowców, półproduktów oraz gotowych produktów z linii produkcyjnej śledzia parowanego w oleju i na tej podstawie wyznaczono punkty kontroli zanieczyszczeń mikrobiologicznych. Badania przeprowadzono w 2007 roku w dwóch cyklach: tj. 15 lutego i 17 maja. Skontrolowano także czystość mikrobiologiczną materiału na wszystkich etapach tej produkcji. Kryterium oceny była liczba form wegetatywnych i przetrwalnych bakterii oraz grzybów. Badanie taksonomiczne bakterii wykonano przy użyciu testów API firmy bio Merieux (API 50CHB, ID 32STAPH, ID 32GN). Identyfikację grzybów pleśniowych na podstawie cech makro- i mikroskopowych, a drożdży przy użyciu testu ID 32C.

Badania wykazały, że dominującą mikroflorę bakteryjną stanowiły bakterie z rodzajów *Staphylococcus* oraz *Bacillus*, a grzybową – *Rhizopus*. Najwyższe skażenie mikrobiologiczne wykazywał surowiec. Skażenie mikrobiologiczne gotowego produktu kształtowało się poniżej dopuszczalnych norm i nie stanowiło zagrożenia zdrowotnego dla konsumenta. Punktami krytycznymi skażeń mikrobiologicznych w monitorowanym zakładzie były: przyjęcie surowca i zamykanie puszek.