ORIGINAL SCIENTIFIC PAPER

EFFECTIVENESS OF HACCP SYSTEM IN CONTROL OF MICROBIOLOGICAL HAZARDS IN THE PRODUCTION OF SMOKED TROUT (ONCORHYNCHUS MYKISS) 1*

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Abstract: The paper describes the application of the HACCP system in the production of cold smoked trout. The production of cold smoked trout fillets (Oncorhynchus mykiss) was analyzed from the standpoint of the safety of ready-to-eat products. The processing was observed and adapted in accordance with HACCP principles. In the presented model, three critical control points (CCP) for biological hazards were identified for which critical limits, monitoring, corrective measures and verification methods were defined. During the 12 months, the microbiological safety of 65 fresh trout samples and 195 samples of finished product, smoked trout was analyzed. The results of microbiological analyzes of fresh and smoked trout, during the examined period, were in accordance with the prescribed values, which confirms the efficiency of the application of the HACCP system in the production process of the smoked trout.

Key words: HACCP system, smoked trout, critical control point (CCP)

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INTRODUCTION

The fish prepared by roasting or frying, preserved by marinating, salting, brining or drying, as well as smoking, has highly appreciated nutritive and sensory properties, which is an additional, determining factor for both consumers and fish producers. Fish preserved by salting, brining and smoking is a highly regarded gastronomic specialty. Smoking fish is usually carried out in two ways: cold smoking and hot smoking. Cold smoking is most often performed at a temperature of 30 °C to 40 °C, and hot smoking at 80 °C to 90 °C (Alam, 2007). Smoking affects the flavor and color of products and contributes to the preservation of the product due to the bactericidal and bacteriostatic effects of the smoke components as well as the antioxidant properties of phenols present in the smoke (Toldra, 2002, Bortolomeazzi et al. 2013). Therefore smoking is increasingly important in modern fish processing, and is a technological way of producing a distinctive flavor, color, aroma and texture of the final product. When it comes to food safety standards, cold-smoked fish is categorized as ready for consumption ( RTE-as ready-to-eat.) Due to the fact that the product is not subjected to heat treatment and that no additives are usually added during production process, the basic, qualitative characteristics of the product are retained and, as such, it is defined as a product of exceptional quality.

Consumption of fishery products, as well as other food products, is associated with certain biological and chemical risks. The most common chemical risks in the consumption of fish, crustaceans and shellfish are histamine and heavy metals (Alic et al., 2004; Bergman et al., 2015). Biological hazards include: parasites, microorganisms (bacteria, viruses) and toxins (Baltić et al., 2009). The degree of contamination of the fish depends on the environment and the bacteriological correctness of the water in which the fish is caught (Kozačinski et al., 2009).

Food business operators at the level of primary production are obliged to establish and implement regular controls of hygienic, health and technical conditions of production in each facility, by conducting self-control preventive procedures developed in accordance with good manufacturing practice. An integrated control system, HACCP system is used in the food production chain in order to avoid any potential chemical, physical and biological / microbiological hazards that can endanger the health of consumers. (Grujić i sar., 2003, Gramza-Michalowska and Korczak 2008, Kozačinski i sar., 2009; Savanović et al., 2017).

In food production HACCP proved to be a control system that gives good results (Lu & sar., 2014; Hung and sar, 2015; Novaković i Savanović, 2017). The introduction of the HACCP system enables enterprises to develop their own system of controlling the production and management of hygienically proper
and quality food, as well as to determine allowed permits and undertake corrective actions before the emergence of serious problems (Stanley et al., 2011; Hung and sar., 2015).

In fishponds with good manufacturing practice and all prerequisite programs, HACCP system implementation is recommended in order to produce quality and healthy fish (Kozačinski i sar., 2009). The HACCP system, which is science based and systematic, identifies specific hazards and measures for their control which ensures the safety of food. (Jeličić et al., 2009; Singh, 2015; Savanović et al., 2017). The aim of this paper was to define critical control points (CCPs) and examine the effectiveness of the HACCP system in controlling microbiological hazards in the production of smoked trout (Oncorhynchus Mykiss).

**MATERIAL AND METHODS OF WORK**

The food safety management system in the process of smoked trout production was prepared according to the HACCP Codex Alimentarius Instruction (Annex CAC / RCP 1-1969. Rev.4 (2003)), while respecting certain recommendations (the Guidance document on the implementation of the HACCP principles in certain food businesses, 2012). After the application of prerequisite programs (ISO, 2009), a company engaged in the production of smoked trout should develop a HACCP plan. The HACCP food safety system is based on seven basic principles, and the HACCP plan is implemented in twelve steps (Scheme 1). The basic postulates of the HACCP system were applied in all phases of the smoked trout production, in such a way that the risk analysis system at critical control points of production was gradually incorporated into the production process.

![Scheme 1. The course of application of the HACCP system in the production of smoked trout](image-url)
The microbiological quality of fresh and smoked trout is continuously monitored over a period of 12 months. 65 samples of fresh trout and 195 samples of smoked trout were analyzed. In the analyzed samples of fresh trout the presence of the following microorganisms was examined: *Salmonella* (BAS EN ISO 6579 / Cor2: 2010); *Listeria monocytogenes* (BAS EN ISO 11290-1 / A1: 2005); Sulfate-reducing bacteria (BAS ISO 15213: 2008); Coagulase positive staphylococci (BAS EN ISO 6888-1 / A1: 2005); *Enterobacteriaceae* (BAS ISO 21528-2: 2013). In the samples of smoked trout, the presence of the following microorganisms was examined: *Salmonella* (BAS EN ISO 6579 / Cor2: 2010); *Listeria monocytogenes* (BAS EN ISO 11290-1 / A1: 2005); Sulfate-reducing bacteria (BAS ISO 15213: 2008); Coagulase positive staphylococci (BAS EN ISO 6888-1 / A1: 2005); Aerobic mesophilic bacteria (BAS EN ISO 4833: 2006).

**RESULTS AND DISCUSSION**

After the formation of the HACCP team, a product description is created. The HACCP team prepared the Product Description (Table 1) and the Flowchart for smoked trout production (Scheme 2), according to the principles of the HACCP system. The product description contains information regarding product safety (CAC / RCP 1-1969, Rev. 4-2003).

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>SMOKED TROUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRODUCT GROUP NAME</td>
<td>Vacuum-packed cold-smoked fish</td>
</tr>
<tr>
<td>INTENDED USE</td>
<td>For all consumer categories (except those with fish allergies); Product is ready to eat</td>
</tr>
<tr>
<td>INGREDIENTS</td>
<td>Rainbow trout fillet (<em>Oncorhynchus mykiss</em>), salt, spices.</td>
</tr>
<tr>
<td>SENSORY PARAMETERS OF THE PRODUCT</td>
<td>Skin undamaged and of metallic glow. The meat does not show signs of drying (dehydration), has a characteristic color, smell and taste of smoked fish, it is dry and firm in consistency. Fillets do not contain residues of inedible parts (bones).</td>
</tr>
<tr>
<td>TECHNOLOGICAL PROCESS OF PRODUCTION</td>
<td>Trout is taken from out of the pool, the weight is measured, and then it is put into the processing plant. There the fish is slaughtered, eviscerated and washed and then filleted, immersed into brine and cold smoked. After smoking, the fillets are sliced, vacuumed and stored in the chamber until dispatch</td>
</tr>
</tbody>
</table>
**PACKAGING**
Primary packaging: vacuum bags, boxes. Transport packaging: cardboard box.

**STORAGE CONDITIONS**
Chambers with a temperature range of 0-4 °C or -18 °C.

**TRANSPORT CONDITIONS**
A vehicle equipped with a cooling system to achieve a transport temperature of 0-4 °C or -18 °C.

**SHELF LIFE**
30 days from the date of production for the product or 12 months for the frozen product.

**LABELLING INSTRUCTIONS**
No thermal treatment is required. Keep the product in the refrigerator and use it for up to 3 days after opening the package.

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**CHEMICAL AND MICROBIOLOGICAL PARAMETERS**

- **Allowed harmful substance content:**
  - Pb max 0.3 mg/kg
  - Cd max 0.05 mg/kg
  - Hg max 0.5 mg/kg
  - As max 2.0 mg/kg
  - Sum of dioxin: max. 3.5 pg/g wet weight
  - Sum of dioxin and PCB: max 6.5 pg/g wet weight
  - Sum of PCB 28, PCB 52, PCB 101, PCB 138, PCB 153: max 75 ng/g wet weight
  - Benzo[a]pyrene max 2.0 µg/kg.
  - Benzo[a]pyrene, benzo(a)anthracene, benzo[b]fluoranthene and chrysene max 12.0 µg/kg
  - Polycyclic aromatic hydrocarbons (PAH) max 2.0 µg/kg

- **Microbiological criteria:**
  - *Salmonella* spp 0 u 25 g
  - *L. monocytogenes* 0 u 25 g
  - sulphate-reducing Clostridium M=10 cfu/g
  - coagulase positive staphylococci M=10 cfu/g
  - aerobic mesophilic bacteria m=100 cfu/g do M=1000 cfu/g

* Guidance on Microbiological Criteria for Food (BiH Food Safety Agency, 2013)
** Rulebook on microbiological criteria for foodstuffs ("Official Gazette BiH", No. 11/13)
Scheme 2. Flowchart for cold smoked trout fillet
Proper application of the HACCP system requires scientifically documented procedures and preventive measures that exist, effectively applied to the established critical control points (CCPs). Determination of critical control points for the implementation of the HACCP system is possible for chemical, physical and biological hazards. According to the Guidance document on the implementation of the HACCP principles in certain food businesses from the receipt of the raw material to the final product on the market, including preparation, processing, packaging, storage and distribution should be analyzed in the given order. In order to define hazards, it is necessary to list all those biological, chemical and physical hazards associated with the production process, which are the result of direct or indirect contamination. During the HACCP study for the production of smoked trout, a total of 47 hazards were analyzed, of which 30 were biological, 10 chemical and 7 physical hazards. By applying prerequisite programs (ISO, 2009) it is possible to eliminate 20 biological, 10 chemical and 7 physical hazards, ie a total of 37 hazards. At control points (CP) it is possible to eliminate 8 biological hazards, while 2 biological hazards are eliminated at critical control points (CCP) (Table 2).

Table 2. Overview of the hazards analyzed during the development of the HACCP study

<table>
<thead>
<tr>
<th>Types of hazards</th>
<th>Number of analyzed hazards</th>
<th>Number of hazards eliminated by the application of pre-requisite programs</th>
<th>Number of hazards eliminated at CP</th>
<th>Number of hazards eliminated at CCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological hazards</td>
<td>30</td>
<td>20</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Chemical hazards</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Physical hazards</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>37</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

During the implementation of the hazard analysis phase, the HACCP team identified 8 control points (CP) and 2 critical control points (CCPs). The identified control points (CPs) are:
- preparation of brine;
- brining;
- cold smoke;
- storage of semi-finished product at +4 °C;
- storage of finished product at +4 °C;
- freezing;
- storage of frozen product at -18 °C;
- delivery and transport

Identified critical control points (CCPs) are:
- Storage of eviscerated and fresh chilled fish (keeping fish at +4 °C);
- Smoked fillet cooling.

With regular and continuous monitoring of all control points in production, identified hazards and risks are eliminated before the emergence of a defective product, which is the key to successful implementation of the HACCP system. Critical control points are included in the HACCP plan and critical limits, monitoring methods, corrective measures and verification methods are defined. HACCP plans are extensive documents, which is why in this paper an excerpt of the most important elements for the identified CCPs (Tables 3 and 4) is given.

Table 3. Excerpt from the HACCP plan, related to CCP1

<table>
<thead>
<tr>
<th>CP/CCP Process step</th>
<th>Hazard</th>
<th>Critical limit</th>
<th>Monitoring</th>
<th>Corrective measures</th>
<th>Verification</th>
</tr>
</thead>
</table>
| CCP 2 | Storage of eviscerated and frozen fish | Biological: pathogenic microorganism | Temperature in refrigeration units must be 0-4 °C | **What:** Temperature control in refrigeration units  
**How:** By reading from the thermometer display  
**When:** twice a day (at the beginning and at the end of the working day)  
**Who:** A member of the HACCP team responsible for controlling the temperature of refrigeration units  
**Record:** A report on room temperature | - In the event of a deviation, the HACCP team responsible for temperature control adjusts the temperature in the chamber (if possible). If the desired temperature can not be achieved, the product is transferred to another chamber or refrigerated car until the cause of the deviation is eliminated.  
- The leader of the HACCP team identifies the cause of the deviation and establishes measures to prevent repetition of deviations.  
- If the cause of the deviation is the failure of the equipment, the maintenance service is contacted. The preventive maintenance plan will be reviewed and, if necessary, changed  
- The head of the HACCP team makes an assessment of the product's usefulness based on changes in product temperature and time spent at elevated temperature, and makes a decision about further treatment  
- The implemented corrective measure is recorded in the prepared form. | - The temperature of the cooling chambers is automatically read and recorded on the computer.  
- The HACCP team's head is checking the temperature records.  
- The absence of the identified hazard is confirmed by laboratory analysis, according to the sampling plan. |
Microbiological contamination of food products is a major obstacle to food security (Dutta et al., 2017). Some of the advantages of the HACCP system over traditional food control systems are that the control parameters are easily monitored, the work is controlled on the spot, which can identify and eliminate the existing and predicted hazards, thus ensuring health in the product stage, and corrective measures are implemented proactively ie before the emergence of more difficult problems (Jeličić et al., 2009). In order to analyze the efficiency of HACCP implementation in the process of production of smoked trout, certain microbiological analyzes of starting material and finished product were performed during 12 months of production. The obtained results are shown in Table 5 and Table 6.
Effectiveness of HACCP system in control of microbiological hazards in the production of smoked trout (Oncorhynchus mykiss)

Table 5. Results of microbiological analysis of fresh trout

<table>
<thead>
<tr>
<th>Sample</th>
<th>Microorganisms</th>
<th>Number of analyzed samples</th>
<th>UM</th>
<th>Prescribed value</th>
<th>Established value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh trout</td>
<td><em>Salmonella</em></td>
<td>20</td>
<td>u 25g</td>
<td>Not allowed</td>
<td>Not isolated</td>
</tr>
<tr>
<td></td>
<td><em>Listeria monocytogenes</em></td>
<td>5</td>
<td>u 25g</td>
<td>Not allowed</td>
<td>Not isolated</td>
</tr>
<tr>
<td></td>
<td><em>Sulfate-reducing anaerobic bacteria</em></td>
<td>15</td>
<td>cfu/g</td>
<td>m=100 M=1000</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>Coagulase positive staphylococci</td>
<td>5</td>
<td>cfu/g</td>
<td>m=10 M=100</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacteriaceae</em></td>
<td>20</td>
<td>cfu/g</td>
<td>m=100 M=1000</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

m - limit value - the results are considered satisfactory if all the values obtained are less than or equal to “m”

M- the maximum allowable value beyond which the results are considered unsatisfactory; JM- unit of measure

Table 6. Results of microbiological analysis of smoked trout

<table>
<thead>
<tr>
<th>Sample</th>
<th>Microorganisms</th>
<th>Number of analyzed samples</th>
<th>UM</th>
<th>Prescribed value</th>
<th>Established value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoked trout</td>
<td><em>Salmonella</em></td>
<td>60</td>
<td>u 25g</td>
<td>Not allowed</td>
<td>Not isolated</td>
</tr>
<tr>
<td></td>
<td><em>Listeria monocytogenes</em></td>
<td>60</td>
<td>u 25g</td>
<td>Not allowed</td>
<td>Not isolated</td>
</tr>
<tr>
<td></td>
<td>Sulfate-reducing anaerobic bacteria</td>
<td>50</td>
<td>cfu/g</td>
<td>M=10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Coagulase positive staphylococci</td>
<td>15</td>
<td>cfu/g</td>
<td>M=10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Aerobic mesophilic bacteria</td>
<td>10</td>
<td>cfu/g</td>
<td>m=100 M=1000</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>
The results of microbiological analysis of fresh and smoked trout samples indicate the absence of Salmonella spp. and Listeria monocytogenes in all examined samples during the examined period. The number of sulfate-reducing bacteria, coagulase positive staphylococci, enterobacteriaceae and the number of aerobic mesophilic bacteria was in accordance with the values prescribed by the Rulebook on Microbiological Criteria for Food (Official Gazette of BiH, No. 11/13) and the Guidance on Microbiological Criteria for Food (BiH Food Safety Agency, 2013). Microbiological contamination of smoked fish can be caused by several factors such as faults during the smoking process or inadequately conducted smoking phase, poor hygiene conditions, the use of inadequate packaging, and the use of inadequate processing plants, etc. (Adegunwa et al., 2013). In order to provide a microbiologically correct and safe product that meets the quality requirements, the smoking process must be carried out under strictly controlled conditions and in production facilities with a high level of hygiene. Numerous authors in their research (Kök, 2009; Kafetzopoulos, 2013; Novakovic and Savanovic, 2017) unambiguously confirm that the application of the HACCP Food Safety System is scientifically and practically justified and legally binding for most business entities in food business. Based on the results of the microbiological examinations presented in this paper it is evident that there was no deviation from the prescribed values during the observed period and that this aspect of the HACCP plan was successfully tested.

CONCLUSION

This paper presents the HACCP concept in order to ensure safety at all stages of the production of smoked trout and to obtain a microbiologically correct product. By conducting the Hazard Analysis phase, 8 control points (CPs) and 2 critical control points (CCPs) were identified. Identified control points (CP) are: - preparation of brine, brining, cold smoking, storage of semi-finished product at +4 °C, storage of finished product at +4 °C, freezing, storage the frozen product at -18 °C, delivery and transport. The identified critical control points (CCPs) are: storage of eviscerated and fresh chilled fish (keeping at + 4 °C) and cooling of smoked fillet. The results of this paper confirm that the HACCP plan is part of a food safety management system in the production
of smoked trout. The results of the microbiological analysis of basic raw materials and finished products have not shown any deviations from the defined criteria, which confirms the efficiency of the application of the HACCP system in smoked trout production.

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