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Original scientific paper

Effect of vacuum packaging on microbial and sensory quality indicators of cold-smoked freshwater fish

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ABSTRACT

The aim of this research was to monitor the effect of vacuum packaging on selected microbial and sensory parameters of cold-smoked common carp (*Cyprinus carpio*) and cold-smoked bighead carp (*Hypophthalmichthys nobilis*) fillets stored at 3±0.5 °C, and to determine the shelf-life of the products. Fillets were analysed on days 1, 7, 10,12, 14, 15 and 16. The APCs were significantly higher ($p < 0.05$) in common carp than in bighead carp from storage day 12. At the end of study, the APC in both species of fish had not reached 7 logcfu/g. No significant differences ($p > 0.05$) were determined between the PBCs in common carp and bighead carp during the whole period of storage. In cold-smoked bighead carp, the lactobacilli group was dominant at the end of the storage period. According to sensory assessment, it was concluded that vacuum-packaged cold-smoked common carp remained acceptable for up to 15 days of storage, while vacuum-packaged cold-smoked bighead carp remained unchanged until the end of the study (16 days).

1. Introduction

Various preservation techniques, like freezing, drying, salting and smoking, are primarily used to minimize post-harvest fish losses (*Sakyi et al.*, 2019). Among these techniques, fish smoking is one of the oldest and most popular methods, appreciated for the distinct smoky flavour and color it imparts. Additionally, smoked fish is often considered "ready-to-eat," as it can be added directly to meals either whole or in powdered form (*Steiner-Asiedu et al.*, 1991).

Smoking is a traditional preservation method primarily valued for its sensory benefits, such as enhanced taste and color, particularly in minimally processed products with reduced salt content to appeal to consumer preferences. The main preservation mechanisms of smoking include lowering water activity levels through drying, and the antimicrobial and antioxidant properties of smoke components (*Gomez-Guillen et al.*, 2009). Smoke consists of various compounds, including aldehydes, ketones, alcohols, acids, hydrocarbons, esters, phenols and ethers (*Guillen & Errecalde*, 2002). These compounds are deposited on the fish's surface and gradually penetrate into the muscle. Research has shown that phenols in smoke can slow the growth of spoilage microorganisms and inhibit *Listeria monocytogenes* in smoked fish (*Montero et al*., 2007). The main advantages of cold smoking fish are enhanced flavour, extended shelf-life, and the preserved nutritional and textural qualities of fish, making it a popular method in both traditional and modern fish processing.

The quality of smoked fish is influenced by several factors, including: (i) *Fish freshness*. The quality of the raw fish is one of the most critical factors (*Sikorski and Kolodziejska*, 2002). Fish that are fresh and free from spoilage will produce a superior

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smoked product. Degradation of proteins and fats in old or poorly handled fish can lead to off-flavours and undesirable textures in the final product. (ii) *Smoking method*. The choice between hot and cold smoking significantly affects the texture, flavour and microbial stability of the fish. Hot smoking involves cooking the fish while imparting a smoky flavour, while cold smoking mainly adds flavour without fully cooking the product. Both methods require precise control of time and temperature to ensure safety and quality (*Sikorski and Kolodziejska*, 2002). (iii) *Type of wood used*. The type of wood used for smoking greatly influences the flavour profile of the fish. Woods like oak, hickory, alder, or fruitwoods, such as apple and cherry, impart distinct smoky notes. The choice of wood should match the desired flavour characteristics of the final product (*Puke and Galoburda*, 2020). (iv) *Smoking duration and temperature*. Proper control of smoking time and temperature is essential. For hot smoking, maintaining temperatures above 70 °C ensures complete coagulation of proteins and elimination of harmful microorganisms. Cold smoking typically occurs at much lower temperatures, and the duration is longer to allow adequate flavour absorption without cooking the fish (*Puke and Galoburda*, 2020). (v) *Brining process*. Before smoking, fish is often brined to enhance flavour, improve texture and extend shelf-life. The salt concentration and duration of the brining process can affect both the taste and texture of the smoked fish. Over-brining can lead to an overly salty product, while under-brining could result in poor preservation. (vi) *Moisture content*. Proper drying before and after smoking is crucial to achieving the desired texture and microbial stability. Excessive moisture can lead to a soggy product with reduced shelf-life, while overly dry fish can be tough and unappetizing. (vii) *Packaging and storage*. The packaging method, such as vacuum sealing, and storage conditions, particularly temperature control, play a key role in maintaining the quality and extending the shelf-life of smoked fish. Inadequate packaging can result in oxidation, loss of flavour and microbial growth (*Sikorski and Kolodziejska*, 2002). (viii) *Microbial number*. The microbial safety of smoked fish is critical, especially for cold-smoked products, which do not reach high enough temperatures to reduce all potential pathogens. Proper handling, hygiene and storage practices are necessary to minimize contamination and ensure the product's safety. By carefully managing these factors, producers can create a high-quality smoked fish product with excellent flavour, texture and safety characteristics.

Modern consumers looking for high-quality foods that preserve the sensory characteristics and nutritional value of the raw materials used in their production, while also meeting strict safety standards. This demand is largely fulfilled by vacuum or modified atmosphere packaging. In Serbia, most wild-caught and farmed fish are sold for human consumption either fresh or frozen. However, there has been a significant rise in the popularity of smoked fish products.

Cyprinid species, such as common carp, bighead carp and grass carp, are the most commonly farmed in Serbia. This study aimed to monitor changes in selected microbial and sensory parameters in vacuum-packaged cold-smoked fillets of common carp (*Cyprinus carpio*) and bighead carp (*Hypophthalmichthys nobilis*) during storage at 3±0.5 °C, with the goal of determining the products' shelf-life.

2. Materials and Methods

2.1 Fish preparation

Eight common carp and eight bighead carp of 2.50±0.30 kg and 2.70±0.50 mean live weight, respectively, were obtained from a fishpond where semi-intensive rearing system was used. The fish were processed at a freshwater fish processing plant using a standard processing procedure (killing by electrocution, descaling, evisceration and filleting). Two fillets from each carp were prepared, and each fillet was divided into four portions, i.e., a total of eight portions were obtained from one fish. After primary treatment, fish portions were washed and soaked in brine for 24 h, then pressed and laid on the grid in chambers for an hour at 20 ºC. Smoking was performed in an automated smokehouse at the temperature of 28 ºC for 8 h.

The sixty-three portions of cold-smoked common carp and the sixty-three portions of cold-smoked bighead carp were vacuum packaged using a Variovac machine (Variovac Primus, Zarrentin, Germany), and a polyethylene-polyamide film (Suomen Union Verpackungs, Helsinki, Finland) with an oxygen permeability of 29–45 ml O_2 /m^2/24 h/atm (23 ºC, 50% relative humidi ty, RH) and a water vapour permeability of 10–15 g/m^2/24Xh (38 ºC, 90% RH) (1atm=101 325 Pa). All fish portions were stored at the temperature of 3±0.5 °C, and on days 1, 7, 10, 12, 14, 15 and 16 of storage, microbial and sensory testing was performed.

2.2. Microbial analyses

Fish fillets (25g) were homogenized in 225 ml of MRD (Oxoid, Great Britain) in a stomacher (AES; Comburg, France) for 90 sec. Serial dilutions (10-fold) of fish homogenate were spread onto the surface of the appropriate dried media in Petri dishes for enumeration of: aerobic plate count (APC) and psychrotrophic bacteria count (PBC) in plate count agar (PCA, Merck, Germany) incubated at 30 °C for 3 days and at 4 °C for 5 days, respectively; lactic acid bacteria (LAB) on de Man Rogosa Sharpe (MRS) agar (Oxoid, Great Britain) incubated at 30 °C for 2 days under microaerophilic conditions; total yeast and mould count (TYMC) on Dichloran Rose-Bengal chloramphenicol agar (DRBC) (Merck, Germany) incubated at 25 °C for 5 days. All plates were prepared in duplicate and examined visually for typical colony types and morphological characteristics associated with each growth medium. Microbial counts were expressed as logarithms of the number of colony-forming units per gram (logcfu/g).

2.3. Sensory evaluation

The sensory evaluation was performed by six trained panellists. The fish portions were considered for overall acceptability, with regard to odour, flesh color and texture using 1-5 intensity scale, with 5 corresponding to the most liked portion and 1 corresponding to the least liked portion. The product was defined as unacceptable if it achieved a score of less than 2 points recorded by at least of 50% of the judges. Fish from each test group was evaluated throughout the storage period on each sampling day.

2.4. Statistics

The mean values and standard deviations were calculated by using column statistics with the processing of six values for each analyzed group. Significant differences between groups were calculated by using one-way ANOVA. When a significant F was found, additional post-hoc tests with Tukey's adjustment were performed. Differences were considered as significant when p-value was ≤ 0.05. All analyses were performed using the program Microsoft Office Excel (2016).

3. Results and Discussion

The initial low numbers of micro-organisms in common carp and bighead carp (Table 1) suggest that brining and washing as well as smoking process reduced the number of bacteria. At the beginning of our study, the APCs in both groups of fish were the highest compared to other examined micro-organisms. During the storage period, an increase in the APC was observed in both groups of fish. From storage day 12, a significantly higher ($p < 0.05$) APC was detected in common carp than in bighead carp.

Table 1. Aerobic plate count (APC), psychrotrophic bacteria count (PBC), lactic acid bacteria (LAB) and total yeast and mould count (TYMC) expressed as log cfu/g (mean ± SD) of cold-smoked common carp fillets and cold-smoked bighead carp fillets during the storage period

		Days of storage						
Parameter	**Samples**	**1**	**7**	**10**	**12**	**14**	**15**	**16**
APC	Common carp	3.28±0.27^{a}	3.65±0.45^{a}	3.80±0.30^{a}	4.50±0.14^{a}	4.55±0.44^{a}	4.83±0.42^{a}	5.06±0.56^{a}
	Bighead carp	3.06±0.43^{a}	3.40±0.47^{a}	3.43±0.77^{a}	3.82±0.52^{b}	3.85±0.22^{b}	3.93±0.37^{b}	4.09±0.64^{b}
PBC	Common carp	0.56±0.50^{a}	2.10±0.27^{a}	2.31±0.33^{a}	2.53±0.22^{a}	2.56±0.26^{a}	2.76±0.31^{a}	2.82±0.28^{a}
	Bighead carp	0.50±0.07^{a}	2.00±0.16^{a}	2.34±0.27^{a}	2.59±0.23^{a}	2.60±0.22^{a}	2.72±0.19^{a}	2.94±0.32^{a}
LAB	Common carp	0.85±0.25^{a}	2.14±0.40^{a}	2.85±0.58^{a}	3.16±0.20^{a}	3.57±0.30^{a}	4.30±0.24^{a}	4.52±0.32^{a}
	Bighead carp	0.69±0.23^{a}	2.27±0.23^{a}	3.41±0.34^{b}	3.88±0.32^{b}	4.25±0.26^{b}	4.99±0.55^{b}	5.45±0.37^{b}
TYMC	Common carp	0.64±0.50^{a}	1.91±0.62^{a}	2.60±0.23^{a}	3.34±0.27^{a}	3.32±0.23^{a}	3.40±0.47^{a}	2.68±0.12^{a}
	Bighead carp	0.82±0.13^{a}	2.32±0.54^{b}	2.52±0.20^{a}	3.35±0.24^{a}	3.40±0.35^{a}	3.70±0.11^{a}	3.67±0.53^{b}

Legend: Same lowercase letters in a column indicate no significant differences (p>0.05)

During the storage, vacuum packaging influenced microbial growth, and the APCs in both groups of fish did not reach 7 logcfu/g, which likely was reflected in sensory changes of products. Our research showed there was not a good correlation between the APCs of cold-smoked fish and overall sensory acceptability (see below). The low number of aerobic Gram-negative bacteria can be explained by increase of carbon dioxide levels in the gaseous phase inside the packaging due to bacterial metabolism and the gas' bacteriostatic effect (*Radetić et al*., 2007). On the other hand, *Olafsdottir et al.* (2005) reported that APC numbers were from 7 to 8 logcfu/g at the end of the shelf-life of vacuum packaged cold-smoked salmon. They suggested there was good corelation between APC numbers and sensory evaluation of overall acceptability.

Between the PBCs of common carp and bighead carp, no significant differences ($p > 0.05$) were determined during the whole period of storage. During the first seven days of storage, and among the examined microorganisms, the PBC showed the most intensive growth in both fish groups. The reason for that could be the storage temperature, which was very close to the optimal temperature for growth of these micro-organisms. After that period of time, PBC in both fish groups remained quite stable until the end of the study.

In our study, the LAB numbers increased during the storage in both groups of fish. During the storage, LAB numbers determined in bighead carp were significantly ($p < 0.05$) higher compared to LAB numbers determined in common carp. At the end of the storage period, LAB was the dominant microbiota in cold-smoked bighead carp. The dominance of these bacteria has been previously reported in vacuum packaged lightly preserved fish products stored at chilled temperature. In three species of vacuum packaged cold-smoked fish, *Gomez-Guillen et al.* (2009) reported that LAB was the dominant group of micro-organisms. *Truelstrup Hansen et al.* (1998) found the same in vacuum packaged cold-smoked salmon. The ability of these bacteria to grow rapidly under anaerobic conditions at low temperatures, as well as their tolerance to CO_2, could be the reason for dominance of these facultative anaerobic micro-organisms. Although LAB can easily cause spoilage of cold-smoked products (*Lyhs et al.* 1998), it is considered that these micro-organisms in vacuum-packed food contribute to prolonged shelf-life by inhibiting the growth of other bacteria through creating lactic acid and bacteriocins (*Gram and Dalgaard*, 2002). This characteristic of LAB can be a reason for the lower APC and better shelf-life of cold-smoked bighead carp than common carp in our study.

Fungal growth (mainly yeasts) in vacuum-packed cold-smoked fish is quite common, which was confirmed by our study. Intensive growth of TYMC was recorded in our cold-smoked bighead carp compared to in the cold-smoked common carp. According to *Gonzales-Rodriguez et al.* (2002), fungus counts were higher in smoked trout fillets than in smoked salmon, although differences were not significant ($p>0.05$). In *Leroi et al.* (1998), the TYMC in cold-smoked salmon remained low during whole storage period. *Cakli*

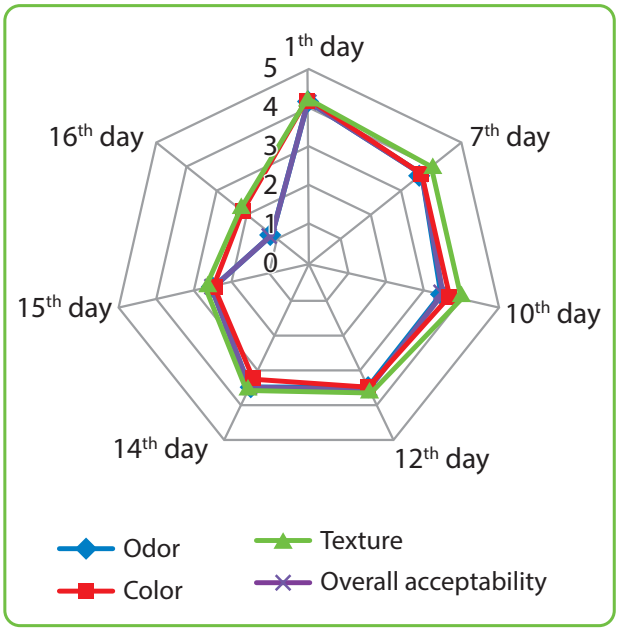

Figure 1. Sensory evaluation of cold-smoked common carp fillets during the storagecategories

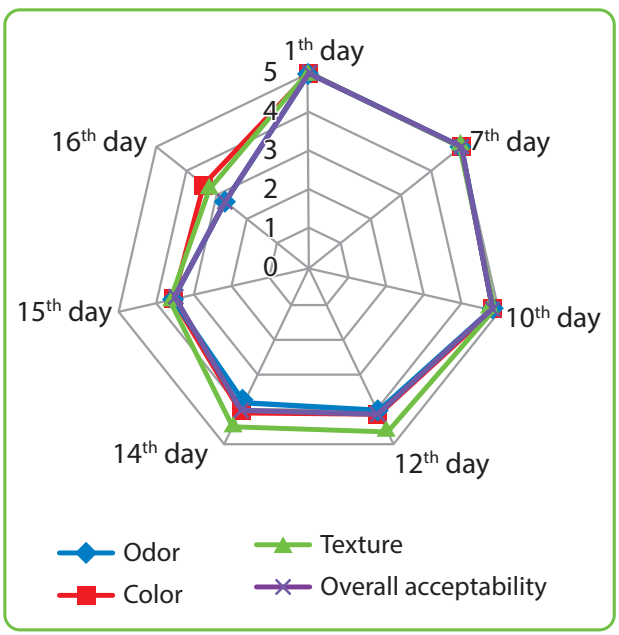

Figure 2. Sensory evaluation of cold-smoked bighead carp fillets during the storage

et al. (2006) did not detect the presence of TYMC in hot-smoked trout packaged in vacuum or in modified atmosphere. They concluded that the growth of yeasts and moulds is influenced by the strong effect of smoke and the temperature during hot-smoke fish processing. The spoilage caused by the growth of fungi is sometimes manifested by an unpleasant smell and taste, but more often, is evident in a change in the appearance of the product which is characterized by surface pigmentation and slime. Since in our research, relatively low numbers of TYMC were recorded, we concluded that TYMC did not cause spoilage of common carp fillets.

The results of the sensory evaluation of cold-smoked common carp fillets and cold-smoked bighead carp fillets are presented in Figs 1 and 2. At the beginning of the storage period, color, flesh texture, odour and overall acceptability were evaluated with very high scores in both groups of fish. During the storage, the average grades decreased for sensory parameters of both fish species. As the results show, all estimated sensory characteristics of common carp received significantly lower ($p < 0.05$) scores on day 15. The odour of fermentation in common carp, detected on day 16, caused the odour score to be lower than the acceptability limit of 2. On the last day of storage, reduced intensity of the pink-cream colouring of common carp muscle was observed, as was softened texture and surface slime. Metabolic activities of microorganisms at the end of storage period could be a reason for the unpleasant fermentation odour, while the changes in texture could be a consequence of the activity of autolytic enzymes, given that the temperature during the cold smoking never exceeded 18 °C , so their inactivation did not occur (*Truelstrup Hansen et al*., 1995). In contrast with common carp, all examined sensory characteristics of bighead carp fillets were within the acceptability level during the study.

4. Conclusion

Cold-smoked freshwater fish packaged in vacuum longer retains its desirable sensory characteristics during cold storage, and the growth of examined micro-organisms are slowed down under these conditions. Based primarily on sensory results, it was concluded that vacuum-packaged cold-smoked common carp remained acceptable up to 15 days of storage, while vacuum-packaged cold-smoked bighead carp remained unchanged until the end of the study (16 days). These results provide useful information about the storage of cold-smoked freshwater fish under vacuum conditions, which could be useful for processor and retailer level.

Uticaj vakuum pakovanja na mikrobiološke i senzorne parametre kvaliteta hladno dimljene slatkovodne ribe

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INFORMACIJE O RADU

Ključne reči:
Šaran (*Cyprinus carpio*)
Tolstolobik (*Hypophthalmichthys nobilis*)
Održivost
Ukupan broj bakterija
Bakterije mlečne kiseline

APSTRAKT

Cilj ovog istraživanja bio je da se ispitaju promene odabranih mikrobioloških i senzornih svojstava hladno dimljenih fileta šarana (*Cyprinus carpio*) i hladno dimljenih fileta tolstolobika (*Hypophthalmichthys nobilis*) pakovanih u vakuum koji su čuvani na temperaturi od 3 ± 0.5 °C, kao i da se odredi održivost proizvoda. Ispitivanja su rađena 1, 7, 10, 12, 14, 15 i 16 dana. Od dvanaestog dana eksperimenta, ukupan broj bakterija hladno dimljenih fileta šarana bio je statistički značajno veći ($P < 0.05$). Ukupan broj bakterija kod obe vrste dimljene ribe nije dostigao vrednost 7 logcfu/g. Tokom celog perioda ispitivanja nije utvrđena statistički značajna razlika ($p > 0.05$) između ukupnog broja psihrotrofnih bakterija. Bakterije mlečne kiseline kod hladno dimljenih fileta tolstolobika bile su dominantna microflora na kraju ispitivanja. Na osnovu promene senzorskih svojstava može se zaključiti da su hladno dimljeni fileti šarana pakovani u vakuumu bili prihvatljivi 15 dana, dok su hladno dimljeni fileti tolstolobika pakovani u vakuumu bili prihvatljivi 16 dana.

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