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Fish quality

**EFFECT OF COLD STORAGE TIME OF ROACH (*RUTILUS RUTILUS* L.)  
ON MICROBIOLOGICAL, CHEMICAL AND SENSORIC INDICES OF  
FISH QUALITY**

**WPLYW CZASU SKŁADOWANIA CHŁODNICZEGO PŁOCI  
(*RUTILUS RUTILUS* L.) NA MIKROBIOLOGICZNE, CHEMICZNE  
I SENSORYCZNE WSKAŹNIKI JAKOŚCI**

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Changes in roach quality under cold storage in ice were judged with various microbiological, chemical and sensory indices. A visible drop of fish quality was noted between 3<sup>rd</sup> and 6<sup>th</sup> day of cold storage at 2°C. Among the indices applied the total count of H<sub>2</sub>S producers and volatile ammonium bases content were assumed to be best correlated with the sensory assessment and, as such, most useful for the quality assessment of the roach under cold storage.

#### INTRODUCTION

Quality and stability of fish raw material depends on many factors among which the most essential are fish species and its condition, fishing season, fishing area and method, methods of preservation and finally qualitative and quantitative composition of fish microflora.

Microbial activity plays crucial role in spoilage process of fish raw materials under cold storage (Conell 1975; Hudson-Arnold and Brown 1978; Kosak and Toledo 1981; Liston 1982; Okuzumi et al. 1982; Venugopal et al. 1984; Huss 1988; Burt and Hardy 1992). Growth of microorganisms and accumulation of their metabolites in the environment worsen its appearance and result in undesirable changes of taste and flavor. It was generally accepted, that only some representatives of fish microflora play active role in production of metabolites essential for the quality of raw material (Alur et al. 1988; Stenström and Molin 1990).

Changes in quality of fish under cold storage can be followed by various microbiological, chemical and sensoric indices of various usefulness in quality evaluation of such raw material. Such surveys were conducted on several marine and freshwater fish species (Cantoni et al. 1976; Sikorski et al. 1990; Kołakowska et al. 1992; Daczowska-Kozon 1993).

The objective of this work was to follow changes of chosen microbiological, chemical and sensoric quality indices during the cold storage of roach, in ice.

### MATERIAL AND METHODS

Surveys were conducted on the roach (*Rutilus rutilus* L.) caught in the Szczecin Lagoon by the Fishing and Fish Processing Cooperative "Certa" (Tab. 1).

**Table 1**

Characteristics of the roach tested

Period of catch	Gonad maturity Meier's scale	Fish lot
May 1993	III-IV	L 1
April 1994	III-VI	L 2
May 1994	IV-VI	L 3

Fresh, iced fish, delivered in wooden boxes D-40 to the laboratory was repacked into plastic containers, supplemented with ice, when necessary, and stored at 2°C.

Analysis were carried out immediately after fish delivery to the laboratory—on 0 day (up to 8 h from the catch) and after 3, 6, 9, 12, 17, and 22 days of cold storage in ice, at 2°C.

Subject of the analysis was minced, skinned fillets from 4–5 roach individuals. Fish, prior to filleting, were carefully washed under running tap water, be, then headed, gutted and rinsed carefully. Fillets were minced with sterile mincer, with 5 mm sieve diameter, into sterile containers, under sterile conditions.

Minced fillets were subjected to microbiological, chemical and sensoric analysis.

Microbiological analysis included: estimation of total viable count (TVC) of psychrophilic and psychrotrophic bacteria, proteolytic (P) (PN-85/A-82051) and bioluminescent bacteria (Fl) (Barak and Ulitzur 1980) on the Frazier's medium (F); estimation of total count of lipolytic bacteria (L) and H<sub>2</sub>S producers on Nutrient agar + Tween 80 (NAT) and on LAA medium (LAA), respectively (Levin 1968).

At each time interval, three 20-g samples of minced fillets were collected, aseptically, into sterile plastic bags. Initial decimal dilutions of the samples were prepared by homogenising minced meat with 0.1% buffered peptone water, for 2 min, at Stomacher. Then further decimal dilutions of the initial material were prepared and 0.1 ml of chosen decimal dilutions spreaded, in two repetitions, over the surfaces of the above mentioned media. Plates were then incubated for 5–7 days at 20°C and the grown up colonies counted.

Numbers of bioluminescent bacteria (Fl) were estimated by counting luminescent colonies on the Frazier's medium exposed, directly, to the UV radiation. As to give numbers of proteolytic bacteria, Frazier's medium was overlaid with the Frazier's reagent and all colonies with the transparent zone around counted. Lipolytic strains were identified by the precipitation zone around the colonies at NAT medium, while gray to black colonies on the LAA medium were typical for the H<sub>2</sub>S producers and, as such, counted as well.

Chemical assays included: volatile amonium bases (VAB), trimethylamine (TMA) and amonium (NH<sub>3</sub>) determined by the Conway method (Conway 1947); TBA value estimated according to the Vyncke technique (Vyncke 1970), histamine level estimated according to the Polish General Standard PN-87/A-86784, pH value measured with the pH-meter N-5123.

Presented data are the average of three (six—in case of histamine estimation) repetitions.

The sensory assessment of the cold stored material as performed at the whole fishes before and after thermal processing of the fish. It was conducted by the trained sensory panel of 5–8 judges and included assessment of flavour and texture according to the graphic scale (Baryłko-Pikielna 1975) and assessment of freshness according to the graphic scale and EU standards (Baryłko-Pikielna 1975; Howgate et al. 1992).

## RESULTS AND DISCUSSION

Results of the analysis proved there were visible differences in the initial quality of minces produced from fresh roach in ice. The TVC of cold-tolerant bacteria, being one of the indicators chosen for microbiological quality assessment of minced roach fillets, differed by two orders of magnitude, initially, and ranged from  $1.5 \times 10^4$  to  $1.6 \times 10^6$  JTK/g. With permitted level of TVC  $< 1.0 \times 10^5$  JTK/g, the fish lot L1—did not meet microbiological quality standards from the very beginning (Zaleski 1985). Minces from the roach caught in april (L2) were of the best initial microbiological quality.

During cold storage of the tested fish, in minced fillets produced out of it, both the logarithmic growth in numbers of all determined microbiological quality indices (Fig. 1) and linear drop in quality parameters assessed either by chemical methods or sensorically were noted (Tab. 2, Fig. 2). These changes were accompanied by visible, between 3<sup>rd</sup> and 6<sup>th</sup> day of cold storage already, and increasing with the prolonged time of cold storage, drop in total fish freshness and texture (Fig. 2).

Some authors consider total viable count (TVC) of bacteria to be a better indicator of processing hygiene than of microbiological quality of fish raw material (Huss 1988; Gram 1993). They base their judgement on better correlation, in their opinion, between particular chemical or sensoric indices and specific bacteria species rather, than the TVC of bacteria. Others, however, proved there to be a direct relation between the cold-tolerant microbes

presence and activity, and undesired changes in odour and flavour accompanying fish spoilage under cold storage (Zaleski 1985; Makarios-Laham and Lee 1993).

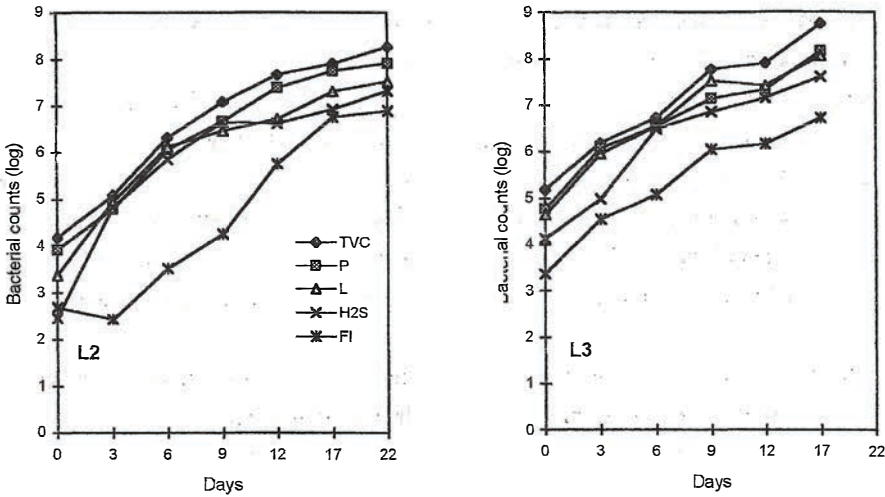


Fig. 1. Effect of cold storage in ice on some microbiological quality indices of the roach

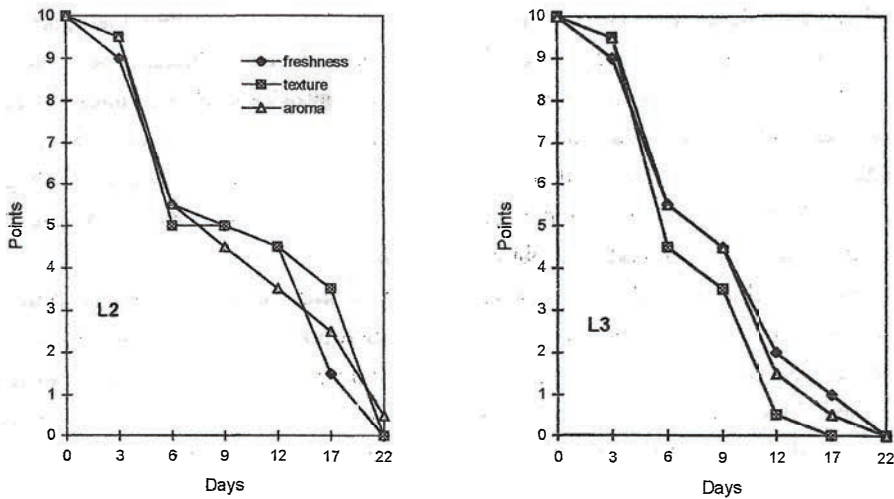


Fig. 2. Effect of cold storage in ice on freshness, aroma and texture of the roach

Table 2

Changes in basic chemical quality indices of minced fillets after various time of cold storage of the roach, in ice (mg/100 g)

Fish lot	Indicator	Day						
		0	3	6	9	12	17	22
L 1	VAB	10	9.38	8.96	—*	—	—	—
	TMA	1.92	1.52	2.04	—	—	—	—
	Histamine	0.24	0.31	0.37	—	—	—	—
	NH <sub>3</sub>	8.08	7.86	6.92	—	—	—	—
	pH	6.52	6.8	7.0	—	—	—	—
L 2	VAB	4.57	3.86	8.53	9.36	12.12	12.36	17.56
	TMA	1.79	1.55	1.96	5.82	8.34	9.24	12.34
	Histamine	0.34	0.4	0.48	0.54	0.6	0.7	1.08
	NH <sub>3</sub>	2.78	2.31	7.06	3.54	3.78	3.12	5.22
	pH	6.65	6.71	7.1	6.6	7.0	7.0	7.3
L 3	VAB	2.25	2.67	6.68	5.25	10.38	15.05	19.31
	TMA	1.22	1.32	3.5	2.57	5.7	9.72	14.44
	Histamine	0.23	0.32	0.48	0.65	0.75	0.97	1.08
	NH <sub>3</sub>	1.03	1.35	3.18	2.68	4.68	5.33	4.87
	pH	6.8	6.84	7.06	7.1	7.05	7.05	7.4

\* not determined.

Microflora typical for fish of the northern hemisphere is dominated by psychrophilic and psychrotrophic bacteria capable of growing under cold storage conditions this is why we found changes in the TVC of cold tolerant bacteria to be useful indicator for the quality assessment of the roach under cold storage.

There is also no agreement on bioluminescent bacteria as a good indicator of fish spoilage. According to Barak and Ulitzur (1980) growth and luminescence of the luminous bacteria correlated well with the total bacterial count at 25°C while under refrigerated temperatures the bacterial proliferation was not accompanied by a parallel increase in luminescence.

Our results confirmed the bioluminescent bacteria to be insignificant criterion of early indication of drop in microbiological quality of the roach under cold storage in ice. Their numbers, at the time of the visible drop in fish quality, were by two orders of magnitude lower than of the other groups tested (Fig. 1) and percentage of bioluminescent bacteria in TVC the lowest one, with no tendency for increasing with the prolonged cold storage (Tab. 3).

Changes in numbers of proteolytic bacteria resembled those noted for the total viable count of cold tolerant microbes (Fig. 1). Percentage of the proteolytic bacteria in the TVC of cold tolerant microbes was the highest one, no matter the day of cold storage, and ranged from 42 to 75 (Tab. 3), except for the fish lot L1—unacceptable from the very beginning. There are various opinions on the TVC of proteolytic bacteria as good indicator of the spoilage process in progress (Chai et al. 1968; Levin 1968; Gram 1992; Makarios-Laham and Lee 1993). Methods of proteolytic activity assessment is one of the reasons for these controversies. Media used for expressing this type of activity include usually gelatin or de-



naturated fish proteins as substrate, which were considered to be substrates attacked more easily by proteolytic bacteria than undenatured fish proteins. This is why total count of bacteria able to split native fish proteins can be, in fact, much lower.

Table 3

Changes in in percentage of the chosen groups of activities in the total viable count of cold tolerant microbes during cold storage at 2°C

Fish lot	Indicator	Day						
		0	3	6	9	12	17	22
L 1	P	3.44	29.1	44.1	—	—	—	—
	L	0.94	9.1	17.6	—	—	—	—
	H <sub>2</sub> S	1.73	8.7	9.7	—	—	—	—
	F1	—*	—	—	—	—	—	—
L 2	P	60	57.7	57.1	50	55.3	62	47.4
	L	16	70	66.7	24.2	11.1	22.8	17.9
	H <sub>2</sub> S	4.1	50.8	36.5	37.5	11.3	7.3	11.1
	F1	3.33	0.38	0.16	0.17	2.13	4.89	3.74
L 3	P	42.1	75	65.5	69.4	71.3	63.8	—
	L	31.4	58.1	65.5	50	32.5	20.7	—
	H <sub>2</sub> S	0.93	6.1	56.4	11.8	17.5	11.1	—
	F1	4.5	2.19	3.33	1.94	2	2.93	—

\* not determined.

Chandrasekaran et. al., cited by Gram (1992) did not find an unambiguous correlation between formation of clearing zones and production of off odours by bacteria. Studies have concluded the proteolysis to be of minor importance in fish spoilage than the breakdown of easily digestible peptides and amino acids.

Makarios-Laham and Lee (1993) found the cold tolerant *Vibrio* spp. strains isolated from seafoods to be capable of hydrolyzing proteins and deteriorating quality of fish and shellfish under refrigeration at 4°C and under frozen storage. Protein hydrolysis of fresh shrimp, haddock fillets and scallops inoculated with *Vibrio* spp. strains, increased by 19.2% after 2 weeks of cold storage at 4°C over the control samples.

A visible decrease in the sensory quality of the roach tested, expressed by increased total defectiveness of whole fish, off odours and by worsened texture, was noted between the 3<sup>rd</sup> and 6<sup>th</sup> day of cold storage, in ice. On the 3<sup>rd</sup> day of storage, numbers of cold tolerant proteolytic bacteria have reached, due to the fish lot, 10<sup>5</sup> to 10<sup>6</sup> CFU/g and percentage of lipolytic bacteria and H<sub>2</sub>S producers in TVC of cold tolerant bacteria were the highest ones (Fig. 1, Tab. 3). It is worth mentioning, that some strains with strongly expressed proteolytic activity were H<sub>2</sub>S producers, as well. This might explain the direct relation, seen by some authors, between deteriorating process and increased proteolytic activity.

Due to the results of this work, increase in total count of H<sub>2</sub>S producers was highest up to the 3<sup>rd</sup>–6<sup>th</sup> day of cold storage, due to the fish lot, and percentage of H<sub>2</sub>S producers in TVC of cold tolerant microbes exceeded 50, at that time (Tab. 3). At the same time the highest (by ~2-times) increase in amount of volatile ammonium bases was noted (Tab. 2). Contrary to the total count of proteolytic bacteria we found the H<sub>2</sub>S producing bacteria to be a valuable criterion in microbiological quality assessment of the roach under cold storage. H<sub>2</sub>S producers number  $1.0 \times 10^5$  CFU/g can be equalized with unacceptable quality of roach under cold storage.

According to Lee (1979) and Gram (1992) only certain strains of bacteria are responsible directly for the spoilage process of the fish under cold storage. Among those responsible ones the most negative role play H<sub>2</sub>S producers. Most of the H<sub>2</sub>S producers are capable of the TMAO reduction, which leads to drop in redox potential and in pH increase of the environment. *Shewanella putrefaciens* is the typical representative of this group of bacteria (Chai et al. 1968; Levin 1968; Lee 1979). According to Shewan cited by Lee (1979) strains of *Shewanella putrefaciens* capable of producing H<sub>2</sub>S and reducing TMAO constitute over 1/3 of the total viable count of bacteria present on fish raw material, with signs of spoilage, under cold storage.

In our experiment, a steady increase in H<sub>2</sub>S producers numbers was noted and their percentage in TVC/g of roach mince, with signs of spoilage, ranged from 7.3 to 17.5. One can assume this group of bacteria to be one of the main producers of VAB in the roach tissue.

Presence of certain microorganisms in fish raw material may result also in biogenic amine formation in this environment if there are certain free aminoacids present. Various strains of bacteria may have different histidine-decarboxylase activity. Fuji et al. (1994) proved, that activity of histidine-decarboxylase of halophilic, histamine-forming marine bacteria of *Photobacterium* spp., estimated at 25°C, 4°C, and 20°C—was highest under cold storage conditions and over 70% of the initial activity value remained after 8 days of storage at 4°C.

During the cold storage of roach histamine content increased only slightly reaching on the 22<sup>nd</sup> day of storage 1.1 mg/100g. Such results are caused probably by low histidine contents in freshwater fish species and/or by low initial number of histamine producing bacteria on fresh fish (Okuzumi et al. 1982).

Level of the VAB was within the permitted value estimated for marine fish species (Sikorski et al. 1990). The TMA content, however, due to the fish lot (Tab. 2), exceeded the permitted values for consumable fish after 6 or 12 days of cold storage (Hebard 1988). Such results seem surprising for a freshwater fish species and need to be confirmed in further surveys.

## CONCLUSIONS

1. A visible drop of the iced roach quality, assessed by microbiological, chemical and sensory indices was noted between 3<sup>rd</sup> and 6<sup>th</sup> day of cold storage.
2. The best correlated with the sensory assessment among the microbiological quality indices was the total count of H<sub>2</sub>S producers, while among the chemical indices the highest agreement was noted for volatile ammonium bases.
3. Regardless of a fishing season, histamine content in the roach tissue poses no threat to the consumer's health.

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#### WPLYW CZASU SKŁADOWANIA CHŁODNICZEGO PŁOCI (*RUTILUS RUTILUS* L.) NA MIKROBIOLOGICZNE, CHEMICZNE I SENSORYCZNE WSKAŹNIKI JAKOŚCI

#### STRESZCZENIE

Przedmiotem analizy była płoć pochodząca z różnych okresów połowu, składowana w warunkach chłodniczych, w lodzie (2°C). Przygotowany, z zachowaniem warunków jałowości, z nieodskórzonych filetów farsz poddawano analizie mającej na celu ocenę jakości surowca. Jakość farszu z płoci oceniano przy pomocy wybranych wskaźników jakości mikrobiologicznej [ogólnej liczby bakterii psychrofilnych i psychrotrofowych (TVC), ogólnej liczby bakterii proteolitycznych (P), lipolitycznych (L), charakteryzujących się bioluminescencją (Fl) i wytwarzających H<sub>2</sub>S (H<sub>2</sub>S)], chemicznej [lotnych zasad amonowych (LZA), trójmetyloaminy (TMA), NH<sub>3</sub>, histaminy, pH] oraz sensorycznej [zapach, tekstura tkanki, świeżość].

W wyniku przeprowadzonych analiz mikrobiologicznych i chemicznych spośród zastosowanych wskaźników jakości za najbardziej przydatne przy ocenie jakości składowanej chłodniczo płoci uznano bakterie zimnolubne, zdolne do wytwarzania  $H_2S$  oraz poziom LZA. Zmiany dotyczące obu wymienionych wyróżników jakości uznano za najlepiej skorelowane z wynikami oceny sensorycznej. Wyraźnemu spadkowi jakości płoci między 3 a 6 dniem (zależnie od partii ryby) przechowywania ryby w lodzie, towarzyszył przyrost liczby bakterii wytwarzających  $H_2S$  w 1 g o 2–3 cykle.log oraz ponad dwukrotny wzrost poziomu LZA w tkance.

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