RAINBOW TROUT (*SALMO IRIDEUS*) PRODUCED IN FINLAND

I. Bacterial spoilage and amino acid composition of fresh rainbow trout during refrigerated storage

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The first problem with the decay of foodstuffs under natural conditions is usually microbiological, while enzymatic and chemical deterioration occurs after the spoilage by micro-organisms has started (4). The development of micro-organisms during the storage of fresh fish is accompanied by decomposition of the muscle carbohydrates, proteins, and lipids; and because of variations in composition of the muscle of different species and the complex nature of the bacterial populations involved, no consistent degradative pattern can be expected (8). On the other hand, the chemical and physical composition of an individual fish depends on the season, sex, age, food, and environment (2, 7). Psychrophilic bacteria occurring commonly in water, air and soil form the natural microflora on the external surfaces of fish, while the flesh and internal organs of healthy freshly caught fish are considered to be bacteriologically sterile (8). The microbial invasion into the originally sterile flesh of the fish begins from the surface slime through the skin, but invasion also takes place through the gills and viscera (7). In spoiling fish, defects in color, odor and taste are usually the most striking changes but also questions in connection with public health must be taken seriously into consideration because clostridia, including *Cl. botulinum*, *Cl. tetani* and *Cl. sporogenes* occur in the intestines of fish (7, 9).

The storage life of trout produced on Danish trout farms is about nine days in ice, for whole and for gutted trout (1). Vacuum packing under the same conditions clearly improved the keeping quality (3). Irradiation of vacuum packed gutted trout prolonged the storage life further (5).

Fish is generally considered a rather good source of animal protein. The value of protein does not depend merely upon the total amount of proteins but also on the amino acid composition (9).

In this sense, essential amino acids are the determing factor when the quality of fish is judged on this basis. In some cases the amounts of essential amino acids are almost the same even in different species of fish (6).

In the present study the bacteriological spoilage, organoleptic quality and amino acid composition of rainbow trout (*Salmo irideus*) produced in Finland was investigated.

Material and methods

Experiments were carried out with 2-year old trout cultivated in Sysmä. The mean weight of the fish was 242 grams (range 162—302 gram). The control fish were transported to the laboratory alive, the rest of the fish was first killed and gutted. All the fish was in the laboratory within 4 hours.

In approximate analyses the whole fish contained 70.4 % water, 8.5 % fat, 17.7 % protein, 3.6 % ash, its pH was 6.70. The corresponding figures for the gutted fish were 71.1 %, 8.0 %, 17.6 %, 3.2 % and pH 6.40, respectively.

The samples were kept 1) in air, 2) in ice, 3) in polyethylene bags and 4) in vacuum bags at +4—+6° C throughout the experiment.

Bacteriological experiments. For each sample 11 grams of fish were aseptically weighed in 99 ml of 0.9 % NaCl-solution. The sample was then homogenized and the necessary decimal dilution series was prepared.

Experiments were carried out with living fish, after storage periods of 4 and 30 hours, and 5, 7 and 13 days. All samples were tested for total viable aerobic counts on SPC-agar (Orion), for total coliforms on VRB-agar (Orion), and the vacuum-packed samples also for anaerobic sulphide producers on iron-sulphite agar (Orion). Changes in the pH in all samples were measured during storage. Incubation for total viable aerobes was 72 hours at 20° C, for coliforms 48 hours at 37° C and for anaerobic sulphide producers 120 hours at 37° C.

Organoleptic evaluation. Organoleptic evaluation was made with raw fish and after it had been cooked in fysiological NaCl-solution for 10 minutes at 90° C.

The evaluation panel consisted of four tasters who gave scores as follows:

Appearance	(scores from 0 to 4)
Structure	(» » 0 to 4)
Color	(» » 0 to 4)
Odor	(» » 0 to 2)
Flavor	(» » 0 to 6)

maximum score 20

Besides these scoring numbers, faults were also noted verbally.

Determination of amino acids. Amino acid determinations were carried out with an amino acid analyzer (Technicon Auto-Analyzer). Determinations were made on living fish, after 4 hours, and on fish stored in ice for 3, 6 and 9 days.

Samples were prepared as follows: The fish was homogenized in a blender. An amount of 3 grams of the homogenate was weighed out in 1000 ml of HCl (20 %).

The hydrolysis was carried out in a vertical condenser for 20 hours at 110° C. Then the sample was evaporated in a vertical vacuum evaporator. Thereafter the sample was washed until the pH remained between 2 and 3. After filtration the pH was adjusted to 2.87 and the sample was diluted into 1000 ml of double distilled water and a portion of 1 ml was used for the analyses.

Results

Bacteriological experiments. The total viable aerobic counts of different samples are presented in Fig. 1. The results show clearly that counts are highest in the samples kept in air. After five days of storage when the relative

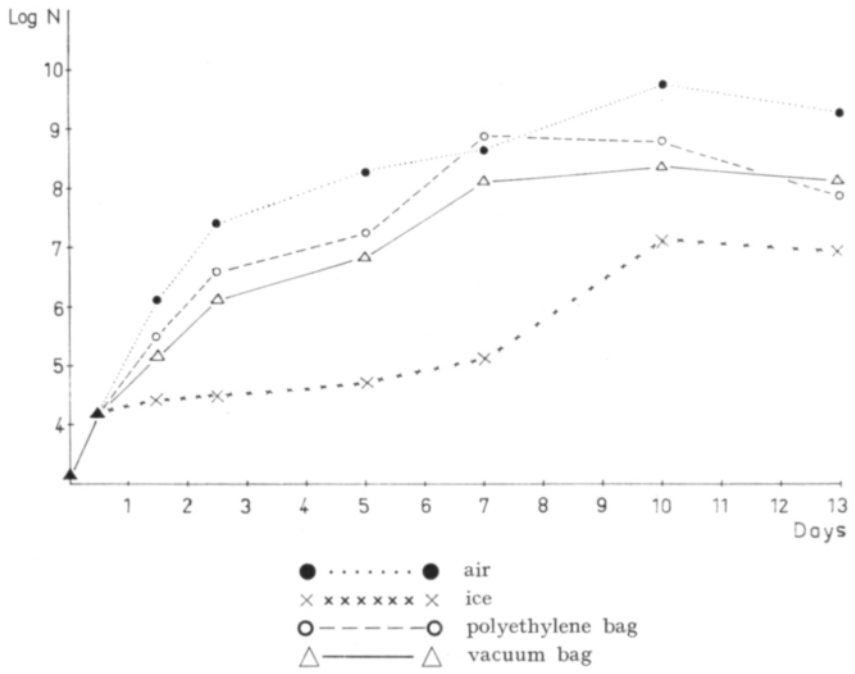

Fig. 1. The total viable aerobic counts in gutted trout on SPC-agar at +4 — +6° C.

differences between the samples were at their greatest, the counts in air were 445 × 10^6/gram, in polyethylene bags 47 × 10^6/gram, in vacuum bags 19.51 × 10^6/gram and in ice 84 × 10^3/gram. In ice the amount of bacteria increased slowly and after 7 days of storage it was 210 × 10^3/gram. The bacterial invasion in the ice-stored fish was first clearly noted after 10 days of storage.

The amount of total coliforms was considered as an indication of hygiene. Fig. 2 reveals that the tendency in the different samples followed the same pattern as that for the total bacterial counts. Counts were highest in the fish kept in air, while storing in ice was the most effective method also against coliforms. The

relative differences between the different types of treatments in this test (Fig. 2), however, were smaller than in the previous test (Fig. 1).

Vacuum-packed trout was also tested for anaerobic sulphide producers in iron-sulphide agar. The amount of this type of organisms was unexpectedly high (Fig. 3).

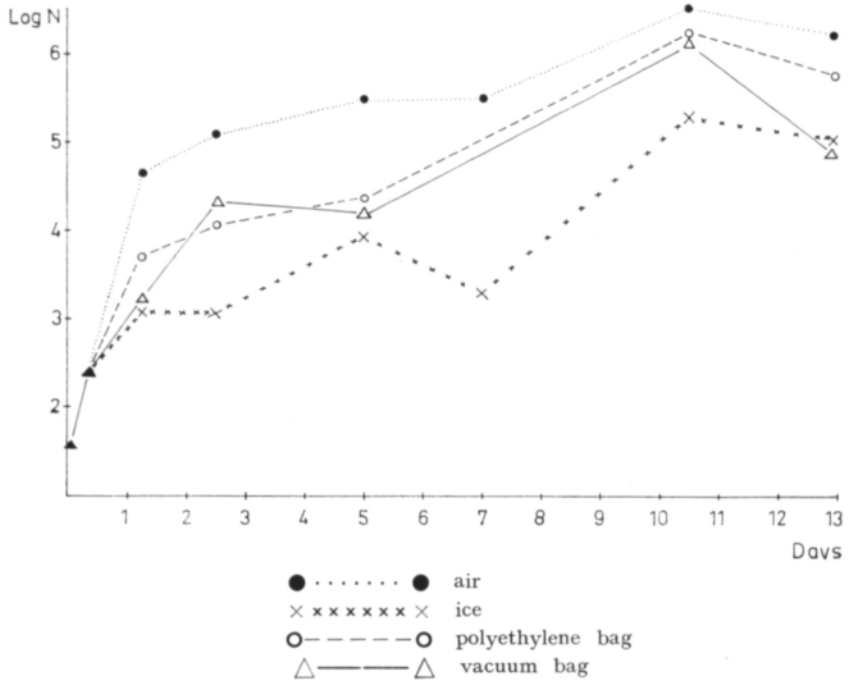

Fig. 2. The total counts of coliforms in gutted trout on VRB-agar at +4 — +6° C.

Fig. 4 shows the changes in the pH during storage. The pH initially decreased naturally in all the samples, but thereafter an increase did not take place until after 12 days. It occurred first in fish kept in air, then in ice, while it was slowest in the fish packed in polyethylene bags and vacuum bags. On the other hand, in the two latter instances the pH reached its lowest level, being after 10—13 days of storage 5.85—5.90.

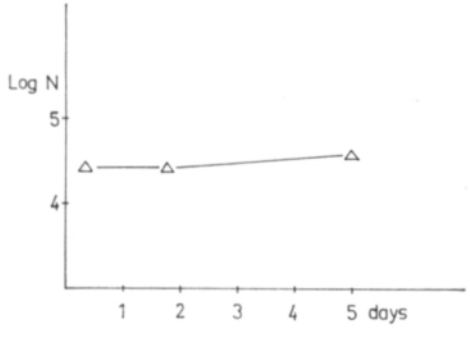

Fig. 3. The anaerobic sulphide-producing bacteria in gutted trout stored in ice on iron-sulphide agar at +4 — +6° C.

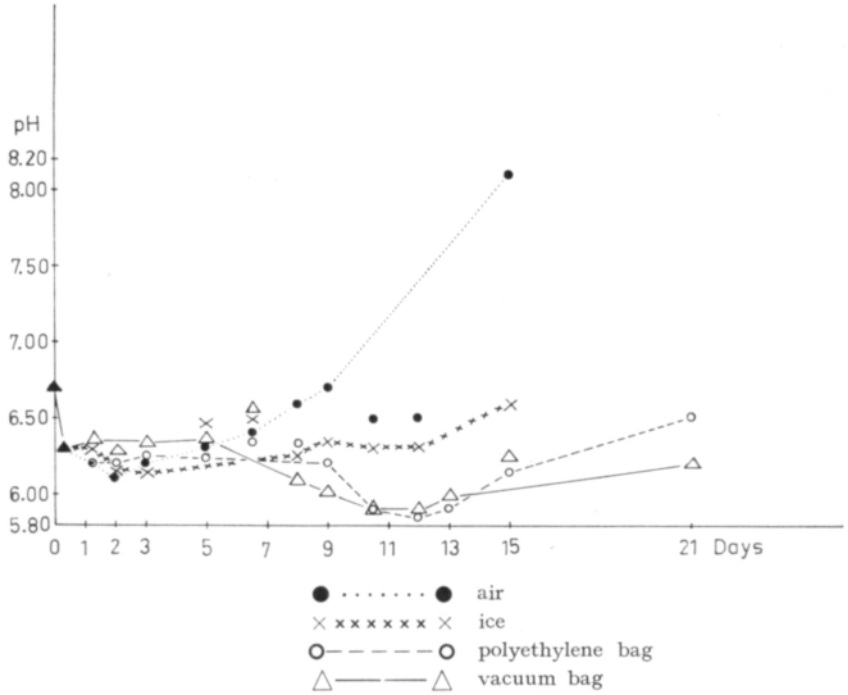

Fig. 4. The pH of gutted trout during storage at +4 — +6° C.

Organoleptic evalution. Table 1 shows the organoleptic quality of living and gutted (4 hours) fish. These results served as controls for the different types of packages (Tables 2, 3, 4 and 5) during storage.

In comparing the organoleptic quality of the different types of packages (Tables 2, 3, 4 and 5), differences were clearly observed. In air the fish was unacceptable after 6 days (Table 2), while in ice this happened after 11 days (Table 3) and in

Table 1. Organoleptic quality of fresh and gutted (4 hours) trout.

		Appear-ance	Struc-ture	Color	Odor	Flavor	Total score
Fresh	Raw	3.5 pale	4	3.5 red	2 neutral		
	Cooked	4— turbid	4	4 salmon red	2	4.5 neutral	18+
4 hours	Raw	3+ pale	4— soft	3+ light	2 neutral		
	Cooked	4— turbid	4— soft	4 salmon red	2	4.5	18

Table 2. Organoleptic quality of gutted trout stored in air.

Time days		Appearance	Structure	Color	Odor	Flavor	Total score
2	Raw	3 dry	4 rigor	3+ light	2		
	Cooked	4	4	4	2	4 rancid	18
3	Raw	3 dry	3.5 post rigor	3+ light	2		
	Cooked	4	3.5 soft	4	2	3 rancid dry	16.5
5	Raw	1 slimy turbid	3 soft	3 colorless	1 rancid		
	Cooked	3 turbid	2.5 soft	4	1 rancid	1.5 spoiled	12
6	Raw	0.5 slimy	1 loose	1 milky	1— rancid		
	Cooked	0.5 slimy	0 loose juicyless	1 milky	1— rancid	0 completely spoiled	2+

Table 3. Organoleptic quality of gutted trout stored in ice.

Time days		Appearance	Structure	Color	Odor	Flavor	Total score
2	Raw	3 turbid	4	3 light	2		
	Cooked	3 turbid	3.5 dry	3 pale	2	3 dry	14.5
5	Raw	2.5 turbid	3 dry	3 pale	2		
	Cooked	2.5 turbid	3.5 dry	3 pale	2— odorless	2.5 dry rancid	13+
8	Raw	1 turbid	3 soft	1 colorless	1 odorless		
	Cooked	3.5 turbid	2 dry	0.5 colorless	0.5 odorless	1 rancid denatured	7.5
11	Raw	0 slimy	1 dry soft	0 colorless	0 spoiled		
	Cooked	2 slimy	2 soft	0 colorless	0 spoiled	0 rancid denatured	4

Table 4. Organoleptic quality of gutted trout stored in polyethylene bags.

Time days		Appearance	Structure	Color	Odor	Flavor	Total score
2	Raw	4	4 rigor	3 pale	2		
	Cooked	4	4	3— pale	2	4,5	17+
5	Raw	4	3 soft	3— pale	2		
	Cooked	4 juicy	3 soft	3.5 pale	2— off-odor	4 rancid	16+
11	Raw	2.5 slimy	1 soft	2— pale	0.5 rancid		
	Cooked	3 turbid	3 soft	2— pale	1.5 rancid	2.5	12—
13	Raw	2 slimy	0 soft	1 colorless	0 spoiled		
	Cooked	2 turbid	0 dis-integrated	0 colorless	0 sour	1 rancid	3

Table 5. Organoleptic quality of gutted trout stored in vacuum bags.

Time days		Appearance	Structure	Color	Odor	Flavor	Total score
2	Raw	4	3 soft	4 red	2		
	Cooked	4	4—	4	2	5 juicy	19—
5	Raw	4	3.5 firm	3 pale	2		
	Cooked	4	4	3 pale	2	4+ off-flavor	17+
11	Raw	2 slimy	3 soft	2 pale	1 off-odor		
	Cooked	3	2 dis-integrated	2.5 pale	1 off-odor	3 off-flavor	11.5
13	Raw	2 slimy	2.5 soft	2 pale	1 off-odor		
	Cooked	2.5 turbid	2 dis-integrated	1.5 color-less	1 off-odor	3 sour	10

polyethylene bags after 13 days. In vacuum bags the fish was considered edible still after 13 days of storage.

Determination of amino acids. Experiments made with the fish stored in ice (Table 6) reveal that no great changes in the total amino acid composition occurred during the experiment. The relative changes between the different amino acids were rather similar in the tests, and the quantitative changes probably depended upon the individual fish investigated. Glutamic acid was the greatest component (15.713—17.217 %) followed by lysine (12.38—14.353 %). With the method used, 17 different amino acids could be detected in amounts from a

Table 6. Amino acid content of gutted trout stored in ice.

		Control	4 hours	3 days	6 days	9 days
aspartic acid	mg/g	8.24	10.108	8.06	7.226	9.310
	%	9.67	9.138	8.72	9.136	9.650
threonine	mg/g	2.93	4.641	4.04	3.372	4.283
	%	3.44	4.196	4.37	4.263	4.440
serine	mg/g	4.65	4.375	3.53	3.185	3.850
	%	5.46	3.955	3.82	4.027	3.990
glutamic acid	mg/g	14.02	17.380	15.34	13.585	16.610
	%	16.46	15.713	16.61	17.175	17.217
proline	mg/g	—	1.265	—	—	0.117
	%	—	1.144	—	—	0.121
glycine	mg/g	5.22	6.475	5.20	3.675	7.327
	%	6.12	5.854	5.63	4.165	7.595
alenine	mg/g	4.92	7.446	5.36	4.628	5.727
	%	5.77	6.732	5.80	5.851	5.936
valine	mg/g	4.09	5.226	5.03	4.251	4.913
	%	4.80	4.725	5.44	5.374	5.093
cystein	mg/g	—	1.408	1.35	1.239	—
	%	—	1.273	1.46	1.566	—
methionine	mg/g	3.37	3.526	2.83	2.781	2.930
	%	3.95	3.188	3.06	3.516	3.037
iso-leucine	mg/g	4.67	4.279	4.19	3·406	4.017
	%	5.48	3.869	4.53	4.306	4.164
leucine	mg/g	6.50	8.340	7.03	6.375	6.943
	%	7.63	7.540	7.61	8.060	7.197
tyrosine	mg/g	3.31	3.017	3.31	3.017	3.380
	%	3.88	2.728	3.58	3.814	3.504
phenylalanine	mg/g	3.57	3.630	4.01	3.630	3.740
	%	4.19	3.282	4.34	4.589	3.877
lysine	mg/g	10.55	15.799	11.71	10.065	13.847
	%	12.38	14.283	12.68	12.725	14.353
histadine	mg/g	3.50	3.920	6.65	1.960	3.290
	%	4.10	3.544	7.20	2.478	3.410
argenine	mg/g	5.62	9.776	4.71	6.611	6.190
	%	6.59	8.838	5.10	8.358	6.416
	mg/g	85.16	110.611	92.35	79.006	96.474
	%	99.92	100.002	99.95	99.403	100.000

few mg/g to about 10 mg/g. However, the amounts of proline and cystein did not exceed these figures in all instances. Proline was present in all samples, but cystein could not be detected in the control nor in the 9-day sample.

Discussion

The keeping quality of rainbow trout varied considerably according to the packing method used in an experiment under laboratory conditions. In many instances bacteriological and organoleptical results were not directly correlated with each other. Fish stored in air spoiled most rapidly both bacteriologically and organoleptically.

Storage in ice was most effective from the bacteriological standpoint (Figs. 1 and 2). When the experiments were carried out at +4—+6° C there may have been a somewhat lower temperature in fish stored in ice compared to the other packing methods used. On the other hand, bacteria could be washed out from the surface of the fish when the ice was changed; not until after 7 days of storage did the total viable count exceed 10^5 bacteria/g. The bacterial counts in fish packed in polyethylene and vacuum bags were similar throughout the experiment, although the total viable count and the coliform count in vacuum were somewhat lower (Figs. 1 and 2).

The high count of anaerobic sulphide producers must, however, be taken seriously under consideration in connection with vacuum-packed fish (Fig. 3). These could be detected immediately after gutting in amounts which exceeded the total viable aerobic count. Because of the relatively high anaerobic counts, vacuum-packing cannot be recommended at present as a method for fresh trout. In this respect, more information is needed about the pathogenity of these bacteria.

The changes in pH (Fig. 4) showed a typical initial decrease. An increase in pH occurred first with the fish kept in air. In polyethylene and vacuum bags the pH reached its lowest level, namely 5.85—5.90, after 10 and 13 days of storage. Only thereafter was there a slow increase in the pH.

Organoleptic studies showed that vacuum-packed trout was still edible after 13 days (Table 5). Slime-formation occurred only after 10 days, when degradation in structure, color and odor began to become more distinct. Fish packed in polyethylene bags held their shelf life for 11 days but after that complete spoilage set in rapidly (Table 4). Fish in ice showed unfavorable features of sensory quality most rapidly. Already after 2 days the flesh was lacking in juice and tasted dry (Table 3). The washing effect of melted ice may account for this.

Comparing these results with those obtained in the Danish experiments (1, 2, 3 and 5) it can be noted that storage in ice showed similar results in the organoleptical tests (2). Vacuum packing had a favourable effect in organoleptic quality in both cases, but the bacterial counts were lower in Denmark (3). However, the Danish experiments were carried out at lower temperatures. The temperature, however, is not the only factor involved, because big differences have been noted depending on handling, season and sex (2). Radiation pasteurization in connection with vacuum packing (5) seems to improve the keeping quality of fresh trout considerably, but

the public health aspects should be examined in detail before vacuum packing can be recommended as a method for storing fresh trout.

Total amino acid analyses showed that 17 different amino acids were detectable with the method and concentration used (Table 6). There were no considerable changes during storage between the different amino acids. Quantitative differences are thought to depend on the variations between individual fish. In the next study an investigation will be made on the volatile amino acid composition of fresh trout during storage.

Summary

Bacteriological spoilage, organoleptical quality and amino acid composition of fresh trout were studied during storage at +4—+6° C.

Experiments were carried out with living fish (control), with fish 4 hours after killing and during storage. The fish were kept in air, in ice and packed in polyethylene and vacuum bags.

It was observed that the type of packing considerably influences both the bacteriological and organoleptical quality. These changes were not, however, directly correlated with each other. In connection with vacuum packing, the amounts of anaerobic sulphide producing bacteria were so high that this aspect needs a detailed investigation before vacuum packing can be recommended for fresh trout.

The amino acid composition of iced trout changed only slightly during storage. Current experiments concerning changes in volatile amino acid contents will provide additional information in this respect.

Recognition and appreciation is extended to the Institute of Dairy Science, University of Helsinki, for cooperation and for making available the amino acid analyzer in this study.

REFERENCES

(1) Hansen, P. 1963. Fat oxidation and storage life of iced trout. I. Influence of cutting. J. Sci. Fd Agric. 14: 781.

(2) Hansen, P. 1964. Fat oxidation and storage life of iced trout. II. The influence of sex and season. Ibid. 15: 344.

(3) Hansen, P. & Jørgensen, B. V. 1965. Storage life of vacuum-packed iced trout. I. Influence of packing material. Ibid. 16: 150.

(4) Ingram, M. 1962. Microbiology, biochemistry and food. Recent Advances in Food Science, Vol. 2: 307. London.

(5) Jørgensen, B. V. & Hansen, P. 1966. Storage life of vacuum-packed iced trout. II. Influence of radiation pasteurization. J. Sci Fd Agric. 17: 140.

(6) Konosu, S., Katori, S., Ota, R. Eguchi, S. & Mori, T. 1956. Amino acid composition of fish muscle protein. Bull. Jap. Soc. Sci. Fish. 21: 1163.

(7) Shewan, J. M. 1961. The microbiology of sea-water fish. In Fish as Food, Vol 1: 487. New York.

(8) Tarr, H. L. A. 1954. Microbiological deterioration of fish *post mortem*, its detection and control. Bact. Reviews 18: 1.

(9) Venkataraman, R. & Chari, S. T. 1957. Amino acid composition of some marine fishes. Ind. Jour. Med. Res. 45: 77.

SELOSTUS:

TUTKIMUKSIA SUOMESSA KASVATETUSTA KIRJOLOHESTA (*SALMO IRIDEUS*)

I. Tuoreen kirjolohen säilyvyys ja aminohappokoostumus

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Tuoreen peratun kirjolohen bakteriologista pilaantumista, organoleptista laatua ja aminohappokoostumusta seurattiin koesarjalla, joka suoritettiin +4 — +6° C:ssa. Kokeita tehtiin elävästä kalasta, 4 tuntia teurastuksen ja perkauksen jälkeen sekä säilytyksen aikana. Kaloja säilytettiin kokeen aikana perkaamattomina sellaisenaan, jäähileessä, muovikalvoon pakattuna ja vakuumipakkauksessa.

Havaittiin, että pakkaustavalla oli selvä vaikutus kalan bakteriologiseen ja organoleptiseen laatuun. Muutokset eivät kuitenkaan olleet suorassa korrelaatiossa keskenään. Suoritetun tutkimuksen valossa ei vakuumipakkausta voida suositella tuoreen kirjolohen pakkaustavaksi ennen kuin klostriidikysymys on perusteellisesti selvitetty. Anaerobisten sulfidinmuodostajien määrä oli odottamattoman korkea vakuumipakatuissa kaloissa.

Aminohappokoostumuksessa esiintyi sangen vähäisiä vaihteluita. Liukoisten aminohappojen tutkiminen tulee selventämään kokonaiskuvaa tuoreen kirjolohen aminohappojen kohdalla tapahtuvista muutoksista säilytyksen aikana.