

RAINBOW TROUT (*Salmo irideus*) PRODUCED IN FINLAND

VI. Free amino acid composition of refrigerated and frozen rainbow trout

RITVA-LIISA SIHVOLA, JORMA J. LAINE & FRITZ P. NIINIVAARA

University of Helsinki, Institute of Meat Technology

Received November 21, 1967

Fish is considered a good source of protein and the amino acid composition is the determining factor when evaluating the nutritive value of protein (KONOSU et al. 1956).

During the second year of cultivation the Finnish rainbow trout has a protein content of above 17 per cent and the seasonal variations in the protein content are around 2 per cent (LAINE et al. 1967). When stored in ice at $+4 \dots +6^{\circ}\text{C}$ the changes in the total amino acid content during storage seem to be influenced more by the differences between individual fish than by time (NIINIVAARA et al. 1966).

The purpose of this work was to follow the changes of free amino acids in rainbow trout (*Salmo irideus*) produced in Finland during storage in ice at $+4 \dots +6^{\circ}\text{C}$ and at -18°C .

Material and methods

The trout weighed approximately 250 grams each and were transported to the laboratory alive. After killing and bleeding they were gutted. For refrigerated storage the fish were kept in ice at $+4 \dots +6^{\circ}\text{C}$ on perforated trays and new ice was substituted daily. The fish were packed in polyethylene bags and frozen at -30°C and stored at -18°C .

Free amino acids were determined with an amino acid analyzer (Technicon Auto-Analyzer). Samples of the refrigerated fish were taken after 24 and 72 hours and 10 days. Samples of the frozen fish were taken after 3, 8 and 13 months. The control sample was prepared from fresh gutted rainbow trout.

Samples for the determination of free amino acids were prepared as follows: The fish were homogenized in a blender. An amount of 10 grams of the homogenate was weighed and 90 ml of absolute alcohol was added. The sample was then homogenized with an Ultra-Turrax homogenizer. After homogenization the sample was filtrated and washed with 80 per cent alcohol (WELCHER 1963).



The sample was then evaporated in a vertical vacuum evaporator and double distilled water was added until all the alcohol was evaporated. The pH of the sample was then adjusted to 2.87.

Results

The results obtained from the fish stored in ice at $+4 \text{ -- } +6^\circ\text{C}$ are presented in Table 1. They indicate that the total amount of free amino acids decreased during storage. The development in the individual amino acids followed the same pattern but correlation with the control could not be shown in many instances.

Freezing did not affect the amounts of free amino acids as much as refrigeration (Table 2). During storage at -18°C , the total amount of free amino acids decreased, however, and the results regarding individual acids followed the same pattern as the results of refrigerated fish compared with the control.

Discussion

The studies performed indicated a decrease in the amounts of free amino acids during refrigerated and frozen storage of rainbow trout (Tables 1 and 2). Changes were more distinct in the refrigerated than in the frozen fish.

Quantitative results indicated that the amounts of threonine, serine, alanine, iso-leucine, leucine, valine, ornithine, and lysine at the beginning of refrigerated and frozen storage increased at first slightly compared with the control, decreasing later during the storage. The same phenomenon was more distinct with glutamic acid and proline. The amounts of aspartic acid, methionine, tyrosine, phenylalanine, γ -aminobutyric acid, and arginine remained almost stable throughout the experiments.

A steady decrease occurred in the amounts of glycine, histidine, and, in connection with refrigeration, more distinctly also in tryptophane. These three components formed 76.2 per cent of the total amount of free amino acids in the control sample and constituted the main reason for the decrease of total amounts of free amino acids in rainbow trout during refrigerated and frozen storage.

With the technique used, the taurine, urea, 3-hydroxyproline, glucosamine, norvaline, β -alanine, ethanolamine, hydroxylysine, and α -amino-n-caproic acid could not be quantitatively determined. The results of these substances were presented proportionally according to the analytical values.

Summary

Changes in the amounts of free amino acids in rainbow trout during refrigerated storage in ice at $+4 \text{ -- } +6^\circ\text{C}$ and in frozen storage at -18°C were investigated. The control sample was prepared directly from the killed, bled, and gutted fish.

The results indicated that the total amounts of free amino acids decreased in the refrigerated and in the frozen fish. These decreases depended mostly upon the steady decrease of the three quantitatively largest components, namely histidine, tryptophane, and glycine. Histidine formed 36.1 per cent, tryptophane 25.0 per cent, and glycine 15.1 per cent of the

Table 1. Free amino acid content of gutted trout stored in ice.

		Control	24 hours	72 hours	10 days
taurine	mg/g	**	*	—	*
	%	—	—	—	—
urea	mg/g	***	*	**	**
	%	—	—	—	—
3-hydroxyproline	mg/g	*	*	*	*
	%	—	—	—	—
aspartic acid	mg/g	*	*	0.008	0.004
	%	—	—	0.565	0.312
threonine	mg/g	0.045	0.079	0.052	0.035
	%	2.436	5.223	3.675	2.726
serine	mg/g	0.023	0.070	0.050	0.028
	%	1.245	4.630	3.534	2.181
glutamic acid	mg/g	0.031	0.154	0.182	0.106
	%	1.678	10.185	12.862	8.255
proline	mg/g	0.058	0.115	0.058	0.046
	%	3.140	7.606	4.099	3.583
glycine	mg/g	0.279	0.255	0.135	0.233
	%	15.106	16.865	9.541	18.146
alanine	mg/g	0.087	0.101	0.080	0.104
	%	4.710	6.680	5.654	8.100
glucosamine	mg/g	*	—	—	*
	%	—	—	—	—
valine	mg/g	0.029	0.055	0.037	0.033
	%	1.570	3.638	2.615	2.570
methionine	mg/g	0.015	*	0.015	0.013
	%	0.812	—	1.060	1.012
norvaline	mg/g	*	*	—	*
	%	—	—	—	—
iso-leucine	mg/g	0.016	0.026	0.021	0.018
	%	0.866	1.720	1.484	1.402
leucine	mg/g	0.030	0.052	0.039	0.030
	%	1.624	3.439	2.756	2.336
tyrosine	mg/g	0.013	*	0.022	0.016
	%	0.704	—	1.555	1.246
phenylalanine	mg/g	0.023	0.033	0.030	0.074
	%	1.245	2.183	2.120	5.763
β -alanine	mg/g	*	—	*	*
	%	—	—	—	—
ethanolamine	mg/g	*	—	*	*
	%	—	—	—	—
γ -amino-butyric acid	mg/g	0.016	—	*	*
	%	0.866	—	—	—
hydroxy-lysine	mg/g	*	—	*	—
	%	—	—	—	—
ornithine	mg/g	0.010	0.035	0.013	0.009
	%	0.541	2.315	0.919	0.701
lycine	mg/g	0.037	0.061	0.150	0.275
	%	2.003	4.034	10.601	21.417
tryptophan	mg/g	0.461	*	0.023	*
	%	24.959	—	1.625	—
histidine	mg/g	0.666	0.476	0.487	0.252
	%	36.058	31.481	34.417	19.626
α -amino-n-caproic acid	mg/g	*	—	—	—
	%	—	—	—	—
arginine	mg/g	0.008	*	0.013	0.008
	%	0.433	—	0.919	0.623
	mg/g	1.847	1.512	1.415	1.284
	%	99.996	99.999	100.001	99.999

*analytical value < 5; **analytical value 5—10; ***analytical value > 10.

Table 2. Free amino acid content of gutted trout stored at -18°C .

		Control	3 months	8 months	13 months	
taurine	mg/g	**	*	**	*	
	%	—	—	—	—	
urea	mg/g	***	***	***	***	
	%	—	—	—	—	
3-hydroxyproline	mg/g	*	*	—	—	
	%	—	—	—	—	
aspartic acid	mg/g	*	0.004	*	0.005	
	%	—	0.218	—	0.359	
threonine	mg/g	0.045	0.074	0.025	0.081	
	%	2.436	4.037	1.983	5.823	
serine	mg/g	0.023	0.034	0.022	0.060	
	%	1.245	1.855	1.745	4.313	
glutamic acid	mg/g	0.031	0.165	0.086	0.035	
	%	1.678	9.002	6.820	2.516	
proline	mg/g	0.058	0.097	—	0.086	
	%	3.140	5.292	—	6.183	
glycine	mg/g	0.279	0.149	0.124	0.077	
	%	15.106	8.129	9.833	5.536	
alanine	mg/g	0.087	0.109	0.080	0.094	
	%	4.710	5.946	6.344	6.758	
glucosamine	mg/g	*	*	—	—	
	%	—	—	—	—	
valine	mg/g	0.029	0.042	0.028	0.055	
	%	1.570	2.291	2.220	3.954	
methionine	mg/g	0.015	0.012	0.015	0.009	
	%	0.812	0.655	1.190	0.647	
norvaline	mg/g	*	*	—	—	
	%	—	—	—	—	
iso-leucine	mg/g	0.016	0.022	0.012	0.020	
	%	0.866	1.200	0.952	1.438	
leucine	mg/g	0.030	0.035	0.029	0.055	
	%	1.624	1.909	2.300	3.954	
tyrosine	mg/g	0.013	0.013	0.016	0.011	
	%	0.704	0.709	1.269	0.791	
phenylalanine	mg/g	0.023	0.030	0.007	0.023	
	%	1.245	1.637	0.555	1.653	
β -alanine	mg/g	*	*	—	*	
	%	—	—	—	—	
ethanolamine	mg/g	*	*	—	—	
	%	—	—	—	—	
γ -amino-butyric acid	mg/g	0.016	0.009	0.011	—	
	%	0.866	0.491	0.872	—	
hydroxy-lysine	mg/g	*	—	—	—	
	%	—	—	—	—	
ornithine	mg/g	0.010	0.013	*	*	
	%	0.541	0.709	—	—	
lycine	mg/g	0.037	0.038	0.163	0.033	
	%	2.003	2.073	12.926	2.372	
tryptophan	mg/g	0.461	0.468	0.630	0.228	
	%	24.959	25.532	49.960	16.391	
histidine	mg/g	0.666	0.519	0.013	0.519	
	%	36.058	28.314	1.031	37.311	
α -amino-n-caproic acid	mg/g	*	*	—	—	
	%	—	—	—	—	
arginine	mg/g	0.008	*	*	*	
	%	0.433	—	—	—	
		mg/g	1.847	1.833	1.261	1.391
		%	99.996	99.999	100.000	99.999

*analytical value < 5 ; **analytical value 5—10; ***analytical value > 10 .

total amount of free amino acids in the control sample. In connection with many other amino acids a decrease in their amounts was noted during storage. Changes appearing in refrigerated fish were more distinct than in frozen fish.

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

REFERENCES

- 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.
- LAINEN, J. J., VARESMÄÄ, ELINA & NIINIVAARA, F. P. 1967. Rainbow trout (*Salmo irideus*) produced in Finland. III. Seasonal variations in rainbow trout. J. Sci. Agric. Soc. Finland 39: 133
- NIINIVAARA, F. P., SIHVOLA, RITVA-LIISA & LAINE, J. J. 1966. Rainbow trout (*Salmo irideus*) produced in Finland. I. Bacterial spoilage and amino acid composition of fresh rainbow trout during refrigerated storage. Ibid. 38: 210.
- WELCHER, F. J. 1963. Standard methods of chemical analysis. Vol. 2. Industrial and natural products and noninstrumental methods, Part A. D. Van Nostrand Company, Inc., New York.

SELOSTUS

TUTKIMUKSIA SUOMESSA KASVATETUSTA KIRJOLOHESTA (*Salmo irideus*)

IV. Kirjoloihen vapaissa aminohapoissa tapahtuvat muutokset jäädytyksen ja pakastuksen aikana

RITVA-LIISA SIHVOLA, JORMA J. LAINE & FRITZ P. NIINIVAARA

Helsingin Yliopisto, Lihateknologian laitos

Suoritetussa tutkimuksessa seurattiin kirjoloihen vapaissa aminohapoissa tapahtuvia muutoksia säilytyksen aikana jäähileessä +4...+6°C:ssa ja pakastettuna -18°C:ssa. Vertailu suoritettiin kontrollinäytteen, joka valmistettiin tuoreesta, peratusta kirjolohesta.

Tulokset osoittivat, että vapaiden aminohappojen määrät alenivat säilytyksen aikana sekä jäähileeseen pakatuissa että pakastetuissa kaloissa. Aleneminen johtui lähinnä kolmen kvantitatiivisesti suurimman aineen määrissä tapahtuneesta vähenemisestä. Histidiinin osuus oli 36.1 %, tryptofaanin 25.0 % ja glysiinin 15.1 % kontrollinäytteen vapaiden aminohappojen kokonaismäärästä. Muidenkin aminohappojen kohdalla oli havaittavissa laskua varastoinnin aikana. Muutokset pakastetun kalan kohdalla olivat pienemmät kuin jäähileeseen pakatussa kalassa.