EFFECT OF ICE STORAGE ON THE CHEMICAL AND NUTRITIVE PROPERTIES OF SOLVENT-EXTRACTED WHOLE FISH—RED HAKE, *Urophycis chuss*

DAVID L. DUBROW, NORMAN L. BROWN, E. R. PARISER, HARRY MILLER, JR., V. D. SIDWELL, AND MARY E. AMBROSE[1]

ABSTRACT

Because red hake that are to be used in the future production of fish protein concentrate will be caught in quantity, the preservation of the hake during periods of glut will present a problem that possibly can be solved by storage of the hake in ice.

In our study of this problem, whole red hake were held in ice for 2, 6, 8, and 11 days. Organoleptic tests on the fresh fish showed that they were edible on the 8th day but were not edible on the 11th day.

Samples of fish were removed during each period of storage and were processed (1) by freeze-drying to produce a reference sample (2) by solvent extraction with isopropyl alcohol to produce a fish protein concentrate. Proximate composition, amino acid composition, and nutritive quality were determined comparatively on both of these two kinds of processed samples.

From the data obtained, we concluded that red hake stored in ice for 8 days are suitable for use in the production of fish protein concentrate and that they would be suitable for this use up to the point of spoilage of the fish, which occurs sometime between 8 and 11 days.

In the period between the capture and processing of fish that are to be used in products for human consumption, they must be preserved in a manner that maintains their food-grade quality. This requirement applies to the production of fish protein concentrate (FPC) as well as to that of more common fish products.

The preservation of fish is a problem not only aboard the harvesting vessel but at the shore processing plant as well. The problem ashore becomes especially important during periods of glut when the fresh fish must be held several days before being processed.

In the manufacture of FPC by the method we use, oil and moisture are removed from the fish with isopropyl alcohol. We therefore investigated the possibility of holding fish in this solvent (Dubrow and Hammerle, 1969). We found the method to be entirely suitable for periods of holding up to 11 days.

Although storage in isopropyl alcohol was satisfactory, more conventional means of holding the fish, such as storing them in ice, are likely to be used in commercial operations. During the time fish are held in ice, however, considerable change may occur in the components of the fish tissue. Endogenous and bacterial enzymes may break down protein into water-soluble and volatile components, causing off-flavors and odors in the fish. In addition, the highly unsaturated lipids of the fish may oxidize rapidly, causing the fish to become rancid.

While these changes are taking place in iced fish, the water from the melting ice is leaching out some of the compounds that are forming. Furthermore, the subsequent extraction with alcohol during the production of FPC, if adequate, removes most of the undesirable compounds that were not leached out by the melt water.

Just what effect the enzymatic and oxidative changes have on the various components of the tissues as well as on the nutritive quality of the protein in the finally processed FPC is not known. Accordingly, solubilization of the components of the fish tissues could alter the composition of the finally processed FPC. We should

[1] National Marine Fisheries Service National Center for Fish Protein Concentrate, College Park, Md. 20740.

Manuscript received August 1970.

know, of course, what occurs, because FPC is of value solely as a protein supplement of high quality.

The aim of this study therefore was to determine the effect that storage of food-grade fish in ice has on the chemical composition of the components of the tissue and on the nutritive quality of the protein. We accomplished this aim by comparing FPC made from samples of the ice-stored fish with reference samples made by freeze-drying samples of the fish. We used freeze-drying because we believe that this method of production results in minimum alteration in the samples during drying.

CHEMICAL COMPOSITION

Both the proximate composition and the amino acid composition of the samples were determined.

PROXIMATE COMPOSITION

As indicated earlier, we used standard reference samples produced under ideal conditions, as a basis on which to evaluate our samples of FPC.

Standard Reference Sample

About 600 lb of red hake were caught on January 6, 1965, in 25 to 26 fathoms of water off the coast of Rhode Island. The fish were divided randomly into lots of 100 lb each, were iced immediately, and then were taken to the Bureau of Commercial Fisheries (BCF) (now National Marine Fisheries Service) Technological Laboratory at Gloucester, Mass., where they were held in ice.

During the next 11 days, each lot of fish was inspected periodically for freshness by experienced BCF fish inspectors at Gloucester. The factors they considered were (1) damage to the fish, (2) conditions of the skin, eyes, and gills, and (3) texture, odor, and flavor of cooked samples. A numerical score ranging from one to four was used to rate fish of varying quality for each of the factors. Fish of perfect or nearly perfect quality were assigned a value of 1, whereas those at the limit of acceptability or beyond the limit were assigned a value of 4.

Table 1 shows the data on the subjective evaluation of the raw fish. The samples of fish tested after storage for 11 days in ice were judged to be at the limit of acceptability. The fish that had been stored in ice for 8 days were of acceptable quality and were considered to be of food grade.

TABLE 1.—Freshness evaluations of raw red hake stored in ice for periods up to 11 days.
[Each sample had 50 fish.]

Storage time	Average subjective evaluations of:						
	Damage	Skin	Eyes	Gills	Texture	Odor	Flavor
Days							
2	1.00	1.00	1.02	1.40	1.50	1.08	1.0
6	2.26	2.22	2.04	2.32	2.60	2.38	2.0
8	2.44	2.50	3.00	2.92	3.18	3.10	2.5
11	3.10	4.00	3.86	3.92	3.98	4.00	--

Fish of perfect or nearly perfect quality were assigned a value of 1; those of unacceptable quality were assigned a value of 4.

After the iced fish had been inspected for quality, they were shipped in ice to College Park, Md. Each box of fish, upon receipt at College Park, was divided into two groups and were processed immediately—one into a standard reference sample and the other into FPC.

One portion of 20 lb was selected at random from the group of fish to be used as a standard reference sample. The standard reference sample was prepared by freezing the fish in liquid nitrogen and grinding the whole fish through a Rietz Disintegrator[2] under a stream of liquid nitrogen, and then freeze-drying the liquid-nitrogen slurry of ground fish. The freeze-drying step was carried out under a pressure of 500μ of mercury and at a platen temperature of 40° C. The dried samples were then removed from the freeze dryer in an atmosphere of nitrogen and were sealed in containers. The containers were maintained at —40° C until the samples were needed.

The freeze-dried samples were analyzed for crude protein, ash, and volatiles in accordance with standard procedures (Horwitz, 1965). Total lipids were determined by the method of Smith, Ambrose, and Knobl (1964).

Table 2 shows the proximate composition of

[2] The use of trade names is merely to facilitate description; no endorsement of products is implied.

TABLE 2.—Proximate composition of freeze-dried, ground whole hake (standard reference samples) stored in ice for periods up to 11 days.

Storage time	Volatiles	Lipids[1]	Ash[1]	Crude protein[2]
Days	*Wt. %*	*Wt. %*	*Wt. %*	*Wt. %*
2	3.80	15.30	13.44	74.47
6	2.49	14.06	12.84	77.34
8	2.46	14.36	12.43	77.38
11	4.70	15.07	12.49	77.01

[1] The data on lipids, ash, and protein were based on the dry weight of sample.
[2] Crude protein was calculated as N X 6.25.

the various samples of freeze-dried whole fish. Data are presented on a dry-weight basis to reveal possible losses during storage.

The concentration of lipid varied between 14 and 15%; that of ash, between 12 and 13%. The data indicate that the nitrogen fraction did not change greatly. The crude protein remained relatively constant at about 77% (on a dry-weight basis) except on the second day of sampling. This deviation on the second day was probably the result of a sampling error. Analyses for nonprotein nitrogen would have been helpful for interpretive purposes. Unfortunately, they were not made. Dassow[3] has reported that the nonprotein nitrogen fraction of whole Pacific hake stored in ice did not change significantly over a period of 11 days.

Fish Protein Concentrate

From the remaining portion of each lot of fish, 20 lb were selected at random and were extracted with isopropyl alcohol according to established procedures (Brown and Miller, 1969). In brief, the fish were ground through a Hobart meat grinder, were slurried with 15 liters of 91% (v/v) isopropyl alcohol for 30 min, and were centrifuged. The centrifuged solids were then extracted continuously with hot isopropyl alcohol at 60° to 70° C and at a rate of flow of 0.2 gal per minute. After 2 hr the solids were removed by centrifugation and were desolventized under vacuum at 60° C.

[3] Dassow, John A. 1966. Statement of project accomplishment, Utilization of fishery resources program. *In* Quarterly progress report of the BCF Technological Laboratory, Seattle, Wash., July 1 - September 30, 1966. Unpublished report, 6 p.

This method of processing was not intended to be representative of commercial methods. It was used in our laboratory at that time solely as an experimental technique to evaluate selected variables in the preparation of FPC by solvent extraction. It has since been replaced by a several-stage countercurrent extraction system, which is both much more economical in the volume of solvent needed and is more representative of commercial processing methods. A comparison of FPC made by each system has shown no significant differences, however, either in chemical composition or in nutritive value.

The proximate composition was determined by the same method used with the freeze-dried fish.

Table 3 lists the proximate compositions of the FPC's prepared from the fish stored in ice for various periods. The concentrations of lipids and volatiles remained essentially unaffected by storage. The concentration of ash increased, however, and that of protein (that is, of nitrogen) decreased. The major change occurred after the 8th day of storage. Because the concentration of protein in the standard reference samples did not drop in the same manner as the concentration of protein did in the FPC's, the loss of protein could not have occurred during storage but must have occurred during processing. This conclusion could be accounted for by the formation, during storage, of soluble nitrogenous products resulting from enzymatic breakdown or bacterial breakdown, or from both, that were not leached out of the fish during storage but that were subsequently leached out during the extraction process used in making the FPC. This conclusion was further support-

TABLE 3.—Proximate composition of FPC prepared from raw fish stored in ice for periods up to 11 days.

Storage time	Volatiles	Lipids[1]	Ash[1]	Crude protein[2]
Days	*Wt. %*	*Wt. %*	*Wt. %*	*Wt. %*
2	4.25	0.18	12.30	89.70
6	5.10	0.13	12.44	89.65
8	5.12	0.10	13.19	89.30
11	4.10	0.21	16.04	86.94

[1] The data on lipids, ash, and protein were based on the dry weight of sample.
[2] Crude protein was calculated as N X 6.25.

ed by the observed decrease in yield after processing—namely, 12.0 percent of 2-day-old fish to 10.0 percent of 8-day-old fish.

Storage of whole red hake in ice up to 11 days did not influence the extractability of the lipids. A slight loss of nitrogen occurred, however, during the processing of whole fish stored for 11 days as compared with fish stored for shorter periods.

AMINO ACID COMPOSITION

Standard Reference Sample

Amino acids were determined by the method of Spackman, Stein, and Moore (1958).

Table 4 shows that the recovery of amino acids was relatively constant at about 92% of the protein. The essential amino acids for which analyses were made ranged between 45.5 and 46.3% of the total. No major change in the pattern of any one particular amino acid resulted from storage.

In general, this finding agrees with those by Cohen and Peters (1963) on whiting, *Merluccius bilinearis,* that were stored in ice. These authors reported, however, that methionine decreased after the 13th day with a subsequent increase in methionine sulfoxide. We do not know whether this compound was present in the hake that we studied.

Fish Protein Concentrate

The same methods were used as with the standard reference sample. That is, the amino acids were determined by the method of Spackman, Stein, and Moore (1958).

Table 5 shows the concentration of amino acids in the FPC's processed from the fish held in ice. The data indicate that about 100% of the amino acids were recovered.

The essential amino acids constituted 47% of the total amino acids in the FPC made from fish stored 2 days, but the concentration of these amino acids dropped to 43% after the fish had been stored 11 days. Individual amino acids decreased in concentration. Of these amino acids, leucine and isoleucine decreased slightly, whereas lysine and histidine decreased markedly after the 8th day of storage. The total concentration of lysine was about 11% less in the FPC made after the fish had been stored for 11 days than in the FPC produced after they had been stored for 2 days. The concentration of histidine

TABLE 4.—Amino acid composition of raw freeze-dried whole ground fish. The samples were prepared after fish were held in ice for periods up to 11 days.

Amino acid	Concentration of the given amino acid in the Standard Reference Samples after they were held:			
	2 days	6 days	8 days	11 days
	— — — *Percent of the protein (N X 6.25)* — — —			
Lysine	7.63	7.44	7.72	7.56
Histidine	1.92	1.77	1.92	1.76
Ammonia	1.68	1.57	1.58	1.59
Arginine	5.96	5.82	6.05	5.76
Aspartic acid	9.50	9.41	9.45	9.53
Threonine	4.07	4.16	4.08	4.14
Serine	4.08	4.22	4.11	4.11
Glutamic acid	14.05	14.32	14.27	14.23
Proline	4.57	4.79	4.69	4.76
Glycine	7.70	8.23	7.68	7.52
Alanine	6.50	6.56	6.36	6.41
Valine	4.88	4.78	4.63	4.98
Methionine	3.02	3.00	2.91	3.05
Isoleucine	4.21	4.17	4.13	4.32
Leucine	7.07	7.03	7.06	7.21
Tyrosine	3.01	2.90	2.88	2.99
Phenylalanine	3.82	3.98	3.98	3.94
Total amino acid recovery	91.99	92.58	91.92	92.27
Percent essential amino acids	46.29	45.51	46.21	46.30

TABLE 5.—Amino acid composition of FPC prepared from raw fish held in ice for periods up to 11 days.

Amino acid	Concentration of the given amino acid in the samples extracted after they were held for:			
	2 days	6 days	8 days	11 days
	— — — *Percent of the protein (N X 6.25)* — — —			
Lysine	8.67	8.14	8.38	7.72
Histidine	2.06	1.88	2.00	1.74
Ammonia	1.47	1.57	1.39	1.44
Arginine	7.12	6.98	7.24	6.92
Aspartic acid	10.36	10.36	10.17	10.00
Threonine	4.45	4.48	4.51	4.44
Serine	4.60	4.59	4.70	4.75
Glutamic acid	15.47	15.57	15.34	15.27
Proline	5.01	5.64	5.59	6.47
Glycine	8.04	9.20	9.12	10.23
Alanine	6.78	7.10	6.96	7.23
Valine	5.14	5.24	4.95	4.90
Methionine	3.32	3.32	3.46	3.29
Isoleucine	4.52	4.46	4.37	4.26
Leucine	7.70	7.54	7.44	7.17
Tyrosine	3.39	3.31	3.28	3.16
Phenylalanine	4.12	4.05	4.07	3.91
Total amino acid recovery	100.75	101.86	101.58	101.46
Percent essential amino acids	47.00	45.25	45.70	43.71

decreased about 15.5% within the same period of time. Both glycine and proline increased in percentage of the total amino acid concentration. This increase could possibly be due to the lack of enzymatic breakdown of the fish collagens, thereby increasing the percentage of these amino acids as compared with that of the amino acids of the myofibrillar proteins.

In retrospect, an analysis of the raw, unprocessed fish for free amino acids or total nonprotein nitrogen would have made the interpretation of these results more certain.

No marked differences in the amino acid pattern of the standard reference sample could be detected after storing the whole fish in ice for periods up to 11 days. The amino acid pattern of the FPC's produced from the same batch of fish as was the standard reference sample, did, however, show changes, which were more pronounced in the FPC processed from 11-day-old fish. These changes appeared to be the result of alcohol extraction of solubles that were apparently formed during ice storage and not leached out by the melt water from the ice.

PROTEIN QUALITY

STANDARD REFERENCE SAMPLE

Protein efficiency ratios were determined by the method of Campbell (1960). Diets of the standard reference samples and of FPC prepared from raw fish stored in ice were fed *ad libitum* to male albino rats (Charles River strain), which were randomly allotted to groups of 10 animals. The samples were added to a basal diet at a 10% level of crude protein. Gain in weight and consumption of food were recorded each week for 4 weeks, and the protein efficiency ratio was calculated as (weight gain)/(weight of protein consumed). A diet in which casein was the source of protein was used as a reference.

Table 6 shows the data obtained from the animal-feeding studies comparing the quality of the protein of the various samples. Except for the sample prepared from fish held 11 days, the protein quality of the standard reference samples was better than that of casein. The protein quality of the standard reference sample taken on the 11th day was similar to that of casein and therefore was lower than that of the three samples taken earlier.

TABLE 6.—Mean weight gained, food consumed, and adjusted protein efficiency ratio of groups of eight rats fed freeze-dried whole hake prepared from fish stored in ice, compared with casein.

Storage time	Mean weight gained	Mean weight of food consumed	Adjusted protein efficiency ratio[1]
Days	*Grams*	*Grams*	
2	158.6 ± 3.16	390 ± 5.7	3.46 ± .05
6	150.8 ± 3.08	385 ± 5.9	3.35 ± .08
8	155.6 ± 5.28	381 ± 7.7	3.49 ± .07
11	148.6 ± 3.35	400 ± 3.9	3.18 ± .07
Casein	113.5 ± 5.65	323 ± 3.9	3.00 ± .00

[1] The protein efficiency ratios were adjusted to a protein efficiency ratio of 3.00 for casein.

Proximate composition and concentrations of amino acid do not account for the difference obtained in the quality of the protein in the sample of fish held in ice for 11 days. Because the fish were from the same lot and were chosen randomly, we can only speculate either that the utilization (digestibility) of the protein (amino acids) was decreased or that compounds depressing growth were formed during storage.

FISH PROTEIN CONCENTRATE

The same methods were used to determine protein quality as were used with the freeze-dried fish.

Table 7 shows the data obtained from the feeding tests made on FPC's produced from the fish held in iced storage. All the FPC's gave a greater gain in weight and a higher pro-

TABLE 7.—Mean weight gained, food consumed, and protein efficiency ratio of groups of eight rats fed diets of FPC prepared from raw fish stored in ice for periods up to 11 days compared with casein.

Storage time	Mean weight gained	Mean weight of food consumed	Adjusted protein efficiency ratio[1]
Days	*Grams*	*Grams*	
2	154.0 ± 8.63	363 ± 12.0	3.62 ± .05
6	155.1 ± 8.12	362 ± 12.3	3.65 ± .09
8	154.4 ± 4.95	368 ± 7.6	3.59 ± .10
11	145.4 ± 4.80	358 ± 10.3	3.47 ± .10
Casein	113.5 ± 5.65	323 ± 3.9	3.00 ± .00

[1] The protein efficiency ratios were adjusted to a protein efficiency ratio of 3.00 for casein.

tein efficiency ratio than did the casein. Diets containing FPC made from fish stored for 2, 6, and 8 days in ice resulted in protein efficiency ratios ranging between 3.59 and 3.65. The diet containing FPC from the 11-day-old fish yielded a slightly lower gain in weight and a protein efficiency ratio of 3.47. These results agree with those obtained with the standard reference samples made from the same fish. The nutritive quality of the 11-day standard reference samples, however, was poorer than that of the FPC sample. This anomalous result suggests either an improved utilization of protein as a result of extraction with isopropyl alcohol or the removal of some factor that may have depressed growth.

Freeze-dried fish produced from whole red hake stored in ice 2 to 8 days did not differ in protein quality. Freeze-dried fish produced from whole red hake stored for 11 days, however, was lower in protein quality but still had a protein efficiency ratio equal to that of casein. FPC produced from whole fish stored for 2 to 11 days showed no differences in protein quality. All the FPC's had protein efficiency ratios higher than that of casein.

SUMMARY AND CONCLUSIONS

Whole red hake were stored in ice for 2, 6, 8, and 11 days. The fish were organoleptically evaluated for freshness at each storage period and were then processed by freeze-drying to form a reference sample or by solvent extraction with isopropyl alcohol to form a fish protein concentrate. These products were then analyzed for proximate composition and amino acid concentration and for protein quality.

The results of the subjective evaluation for freshness indicated that the fish stored up to 8 days were still acceptable for food but that those stored for 11 days were not acceptable.

The proximate composition and the amino acid concentration of the freeze-dried whole samples of fish showed very little change as a result of the storage of the raw fish in ice. Rat-feeding tests indicated a loss in protein quality of the freeze-dried sample prepared from 11-day-old raw fish. Protein efficiency ratio values ranged from 3.35 to 3.49 for fish stored up to 8 days, whereas the 11th-day sample resulted in a protein efficiency ratio of 3.18. All protein efficiency ratio values, however, were equal to the value for casein or were higher.

The proximate composition of FPC's produced from fish stored up to 8 days in ice remained relatively constant. The crude protein in the concentrate produced from fish stored for 11 days decreased about 2.5%. The concentration of amino acids also followed this pattern with a resultant lowering in the concentration of lysine and a slight increase in that of proline and glycine. The protein quality of the FPC processed from the 11-day-old fish was also slightly lower than that of FPC processed from fresher fish. All FPC's, however, had protein efficiency ratios higher than that of casein.

We conclude that storage of whole hake in ice up to 8 days is a satisfactory means of holding them prior to extracting the ground hake with isopropyl alcohol to produce FPC.

LITERATURE CITED

BROWN, NORMAN L., AND HARRY MILLER, JR.
1969. Experimental production of fish protein concentrate (FPC) from Mediterranean sardines. Commer. Fish. Rev. 31(10): 30-33.

CAMPBELL, J. A.
1960. Evaluation of protein in foods for regulatory purposes. J. Agr. Food Chem. 8(4): 323-327.

COHEN, EDWARD H., AND JOHN A. PETERS.
1963. Effect of storage in refrigerated sea water on amino acids and other components of whiting (*Merluccius bilinearis*). Fish. Ind. Res. 2(2): 5-11.

DUBROW, DAVID, AND OLIVIA HAMMERLE.
1969. Holding raw fish (red hake) in isopropyl alcohol for FPC production. Food Technol. 23(2): 254-256.

HORWITZ, WILLIAM (chairman and editor).
1965. Official methods of analysis of the Association of Official Agricultural Chemists. 10th ed. Association of Official Agricultural Chemists, Washington, D.C., xx + 957 pp.

SMITH, PRESTON, JR., MARY E. AMBROSE, AND GEORGE N. KNOBL, JR.
1964. Improved rapid method for determining total lipids in fish meal. Commer. Fish. Rev. 26(7): 1-5.

SPACKMAN, DARREL H., WILLIAM H. STEIN, AND STANFORD MOORE.
1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30(7): 1190-1206.