THE EFFECT OF BRINE- AND PLATE-FREEZING AT SEA ON CHEMICAL, PHYSICAL, AND ORGANOLEPTIC PROPERTIES OF THREE SPECIES OF FISH

by

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ABSTRACT

The effect of brine- and plate-freezing and length of subsequent frozen storage upon flesh pH, thaw drip, color, flavor, TBA (2-thiobarbituric acid) values, and long chain free fatty acids of Pacific halibut, Chinook and Coho slamon was determined. The effect of freezing method upon sodium, potassium, and chloride concentration was also determined.

Flesh pH of all three species generally declined significantly ($P \leq 0.05$) with length of storage.

The thaw drip of Pacific halibut and Chinook salmon was less for the brine- than the plate-frozen samples after storage for 9 to 31 weeks whereas subsequently the brine-frozen samples had approximately equal or greater thaw drip than the plate-frozen. The thaw drip of all samples, except those from plate-frozen halibut, tended to increase with length of storage.

The Hunter 'a' and a/b values of Chinook and Coho salmon generally increased during storage.

The difference in flavor between brine- and plate-frozen outside muscle of halibut and Chinook salmon reached a maximum at 31 and 26 weeks of storage respectively, and then steadily decreased. In contrast, the difference in flavor between brine- and plate-frozen Coho salmon outside muscle steadily increased during storage.

The difference in flavor between brine- and plate-frozen inside muscle of all species, except for the Coho

salmon at 10 weeks and halibut at 31, 62 and 81 weeks of storage, was not significant.

The difference in TBA values (an index of oxidative rancidity) between brine- and plate-frozen outside muscle samples rapidly increased and reached a maximum at 45, 26, or 27 weeks (the brine-frozen samples having the higher values) then decreased until there was approximately no difference at 81, 77 and 78 weeks of storage for halibut, Chinook and Coho salmon, respectively.

Method of freezing or length of storage had little effect on the TBA values of inside muscle for all species.

Method of freezing had little effect on the concentration of individual free fatty acids (percentage of total free fatty acids analyzed). The concentrations of several free fatty acids was affected by length of storage but the pattern of change during storage was erratic.

Freezing method had an effect on the concentration of some individual free fatty acids (μg per gram of neutral lipid) of halibut and Chinook salmon but not of Coho salmon. In general, with all species, the concentration of the individual free fatty acids was greatest in the inside muscle. Also for halibut and Chinook salmon, particularly where there was a significant difference among storage times, the concentration of the free fatty acids rapidly increased during the first 26 to 31 weeks of storage.

Method of freezing and length of frozen storage

had a significant effect on total free fatty acids analyzed for only Chinook salmon. Total free fatty acids significantly ($P \leq 0.05$) differed between inside and outside muscle of halibut and Chinook salmon but not of Coho salmon.

The effect of method of freezing upon potassium concentration was small and varied with species.

The effect of brine-freezing upon most variables measured was either small and/or complex. For all three species the sodium and chloride concentration was two to three times greater in the brine-frozen outside muscle than in all other samples. The taste panel results and the TBA values indicate that brine-freezing does impair the quality of the outside muscle of halibut and Chinook salmon during the early stages of frozen storage.

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INTRODUCTION

The nutritional importance of fish has long been recognized (Geiger and Borgstrom, 1962). As a protein source Borgstrom (1962) claims fish is superior to all other major food products whether the protein content is calculated on the basis of grams of protein per 100 calories or as percent protein of the dry matter in food.

Many people incorrectly believe that food supply from the sea is "unlimited". Food production from the sea probably can be increased to an eventual total of only 150 - 160 million metric tons of fish annually (about 2.5 times that produced in 1968). Although world production of fish has increased from 19.6 million metric tons in 1948 to 57.3 million metric tons in 1966 (Bligh, 1969) there have been major declines in annual yields of certain commercially important species. Production of Northwest Pacific Salmon began declining about 1950 and there are as yet no clear signs of recovery (Ricker, 1969).

One possible way of compensating for this decline in catches is to provide better means of preservation. Freezing fish at sea instead of preserving them with flake ice or refrigerated sea water (RSW) would allow the boats (particularly the salmon trollers) to remain out at sea fishing longer and thus increase the number of productive days in a year in addition to improving the quality of the catch (Eddie, 1962).

Freezing fish at sea would also reduce the quantity of spoiled fish sent to reduction plants. However, the method used to freeze fish at sea may affect the quality of the frozen stored product (Harrison and Roach, 1953; Kuprianoff, 1956).

The flesh of chinook salmon (Oncorhynchus tschawytscha), coho salmon (Oncorhynchus kisutch), and Pacific halibut (Hippoglossus stenolepis) is used primarily for the fresh and frozen market. Depending upon the species, the flesh may be kept in frozen storage for up to two years before marketing.

The purpose of the present study was to determine the effects of brine- and plate-freezing at sea and the length of subsequent frozen storage on flesh pH, thaw drip, color, TBA value, flesh content of various long chain free fatty acids, mineral concentration (sodium, potassium, and chloride) and finally flavor of Pacific halibut, chinook salmon, and coho salmon. Evaluations were conducted on inside and outside muscle where appropriate.

LITERATURE REVIEW

A. Freezing Fish At Sea

1) General Considerations

Fish are either killed in catching or shortly thereafter, and as dead tissue, are subject to enzymatic breakdown the rate of which is approximately doubled for each 10°F increase in temperature. In order that a frozen pack of the highest quality can be obtained, it is necessary to freeze fish immediately after they are taken from the water. (Lemon and Carleson, 1948).

Advantages of freezing fish at sea, as compared to preserving them in flake ice are: (a) extension of fishing to more distant grounds, (b) landing fish of high and uniform quality, (c) landing capacity loads, and (d) leveling out supplies of raw materials for the processing plant through frozen storage (Oldershaw, 1955; Eddie, 1959).

Dassow (1963) stated that an obvious solution to the limited storage life of chilled (iced) Pacific halibut is to freeze it at sea. Also Merritt (1969) stated that, in the British fishery, it is conceivable that eventually freezing at sea will be employed on all vessels in which the bulk of the catch must be stored on board for more than seven days before landing.

The different methods of freezing fish at sea as practised by several countries fishing for different or similar fish were reviewed by Heen and Karsti, 1965; Banks and

Waterman, 1968; and Slavin, 1968. The three principal methods used to freeze fish at sea are air-blast, contact plate and brine immersion.

Using a consumer taste panel Lantz and Carter (1951) found that halibut air-blast frozen at sea at -17.8°C (0°F) and stored at -23.3°C (-10°F) were preferred to halibut iced for periods of 12 to 18 days. However, Lantz (1952) reported that the consumer taste panel preferred halibut which were frozen after being stored in ice for three to five days over halibut air-blast frozen at sea.

Depending upon the rate of catching and the rate of freezing or if freezing is purposely delayed, freezing fish at sea can involve freezing fish in various stages of rigor mortis. Freezing of pre-rigor and to a much lesser extent, in-rigor fish, may present certain problems. Jones (1965) stated that changes in appearance associated with textual changes resulting from pre-rigor freezing of fish (Atlantic cod) "on the bone" are uncommon. This is because the musculature is attached to the skeleton and the "thaw rigor" effects (severe contracture and excessive drip) can occur only if the connective structure fails. Partman and Gutschmidt (1963) reported that the concentration of ATP remaining in fish muscle after commercial freezing procedures are unlikely to support "thaw" contracture. These results tend to support those of Torry research scientists (Anon, 1961). However, Tomlinson et al. (1969) observed severe thaw contracture and high free drip in pre-rigor frozen fillets of lingcod and

Pacific cod.

Jones (1969) stated that the flesh of eviscerated fish in rigor can, upon subsequent thawing, present a 'broken appearance' if handled roughly. However, this appearance is commonly the result of passage into and through rigor at high temperatures or the result of undue delay in freezing post rigor.

With fish it is important to eviscerate the fish immediately and wash the cut surfaces well. If this is not done the flesh will be discolored by blood that has not escaped the muscle. Also "belly-burn", proteolysis of the flesh adjacent to the visceral cavity, may develop (Jones, 1965; 1969).

2) Plate-Freezing

Most British vessels that freeze fish at sea use plate freezers, either the horizontal or more commonly the vertical type. Plate freezers are preferred over air-blast freezers for several reasons. The air-blast freezer occupies 2 - 3 times the space and is nearly twice the weight. Air-blast freezers are considerably more complicated in construction than plate freezers. The refrigeration demand is considerably greater than for plate freezers because of the forced-draught fans, and lower refrigerant evaporating temperatures that must be used. Desiccation and oxidation of the surface of the fish may occur with fish frozen in an air-blast freezer. Heavy mechanically operated doors are required in air-blast freezers.

All the above disadvantages are obviated with plate freezers (Ranken, 1958).

It has been reported (Anon, 1952) that Atlantic cod which were plate-frozen immediately after death and kept in frozen storage for one month possessed abnormal qualities which adversely affected acceptability. The texture of the cooked fish was rather soft and 'short', and the smoke cure had a rather poor 'gloss' and 'cut'. Also abnormally large amounts of expressible fluid were obtained. However, after a further two months storage these defects had largely disappeared. It was also reported that cod which had been iced one to three days, and then stored at -30°C (-22°F) for three months, were of very good quality. They had an attractive appearance, filleted well, and yielded excellent smoke cures.

Investigations using a consumer taste panel at the Torry Research Station, (Anon, 1954), showed that cod which had been iced for four days then plate-frozen to -30°C (-22°F) was comparable with good quality fresh fish. Also cod which had been iced for one day then plate-frozen to -30°C (-22°F) was judged superior to good quality fresh fish. It was concluded that the duration of storage in ice before freezing should not exceed three days.

It has also been reported (White Fish Authority, 1957) that Atlantic cod plate-frozen in-rigor or immediately post-rigor yielded a satisfactory product. The thawed fish were firm enough to produce smoothly cut fillets. However, the fillets lacked the sheen of those obtained from iced fish.

Apart from the dullness, the sea-frozen fish possessed a 'sea fresh' flavor, good texture, and made satisfactory smoke cures. The fish could be stored without appreciable deterioration for a period of eight to nine months at -20°F.

Dyer et al. (1962) observed that when Newfoundland trap-caught cod were plate-frozen at sea, thawed and refrozen at -18°C or -23°C there was a rapid decrease in taste panel scores and protein extractability as well as an increase in free fatty acid formation during the first two months after refreezing.

MacCallum et al. (1964) showed that once-frozen Newfoundland trap-caught cod frozen in-rigor in a horizontal plate freezer yielded an acceptable product. Treating fillets with sodium tripolyphosphate significantly improved the texture of the frozen - thawed product but had no effect upon the taste of the fish.

It has also been shown that the quality of cod frozen at sea then thawed, filleted, and refrozen ashore, varies with time and place of catching. However, in all cases an acceptable or better twice-frozen product was obtained (MacCallum et al., 1966).

Tomlinson et al. (1969) studied the effect of the stage of rigor at freezing on the keeping quality of lingcod, Pacific cod, ocean perch, red snapper, orange spotted rockfish, rock sole, sablefish, and Pacific halibut, plate-frozen at sea in the northeastern Pacific Ocean. In general, the thaw contracture of the white muscle of pre-rigor halibut steaks

was very slight (not measurable) while that of the red muscle was quite severe. However, the thaw contracture of the red muscle decreased with time of frozen storage. After nine days of frozen storage the thaw contracture was 35%; after 455 days, 9%. The thaw contracture of the red muscle of the post-rigor steaks was slight in comparison to that of the pre-rigor samples. The in-rigor steaks had a thaw contracture between those frozen pre- and post-rigor. The thaw contracture of the in-rigor steaks decreased from 10% after eight days of frozen storage to 3% after 454 days of frozen storage. The free drip of the post-rigor samples increased more than that of the in-rigor or pre-rigor samples. During frozen storage the pH of the pre-rigor samples decreased the most while the pH of the post-rigor samples decreased the least. At the beginning of the first sampling period the flavor was rated as 'very good' and the texture rated as 'good', while at the last sampling period the flavor of all samples was still good, with no rancidity. However, the pre-rigor samples were preferred over the in-rigor and post-rigor samples, the latter two samples being drier.

The magnitude and changes in pH, free drip and thaw contracture of lingcod, Pacific cod, ocean perch, red snapper, orange spotted rockfish, rock sole, and sablefish varied with the species.

3) Brine-Freezing

The majority of tuna harvested in the U.S. are brine-

frozen aboard the tuna clippers. The hold of a tuna clipper is divided into steel wells or tanks on both sides of the shaft alley. The wells are filled with sea water, the water cooled to +29°F, and the warm tuna are loaded into the well. It requires 24 to 72 hours to bring the temperature of the tuna down to +29°F. After the tuna are precooled, salt is added gradually and mixed by means of the brine circulation pumps.

The brine and tuna are then cooled to about +15°F at which time the chilled brine is pumped to another well or overboard. The tuna are then held at from +10°F to +20°F in the dry refrigerated well. The fish may be unloaded frozen, and thawed at the cannery; or if they are to be processed promptly at the cannery, the refrigeration in the well is shut off and the fish thawed by means of circulating sea water during the last few days of the trip. Salt must be added to the sea water during initial thawing to avoid freezing a solid mass of tuna and ice in the well (Hendrickson, 1959).

There are several advantages of brine immersion freezing in tuna clippers (Slavin, 1956); it requires minimal product handling and also has a low maintenance cost; at rated capacity, it produces a good quality frozen fish in large volume and requires a minimum amount of space because fish are frozen, stored, and thawed in the same tank. There are also several disadvantages. It freezes the product very slowly and requires careful control of temperature because, if proper temperatures are not maintained within very close limits, spoilage of the product may result. This control requires careful

loading to avoid overloading the well and thus exceeding its freezing capacity. It is not versatile, as this freezer is not suitable for freezing ground fish, mackerel, or shell fish. The system requires large amounts of salt for both freezing and thawing and requires a high capacity refrigeration system and considerable auxiliary power for large volume brine pumps. With small tuna, like skipjack, a slow rate of freezing in brine often leads to excessive absorption of salt into the flesh (Slavin, 1956).

Some of the above mentioned advantages and disadvantages apply only to the way brine-freezing is used on tuna clippers. Research conducted by the U. S. Fish and Wildlife Service in the New England area, using the experimental trawler Delaware, has shown that groundfish can be satisfactorily frozen in a 23% sodium chloride brine, thawed in fresh water, filleted and refrozen and marketed as packaged fillets (Slavin, 1968).

The procedure used in brine-freezing ground fish is somewhat different than that used to brine-freeze tuna aboard tuna clippers. Uneviscerated groundfish are put into cylindrical baskets located in the brine tank (+10°F), the freezer-tank doors are closed, and the basket-drive motor is started, causing the baskets to rotate through the brine. The movement of the baskets through the brine provides adequate brine circulation around each fish, thereby insuring uniform, quick and efficient freezing. Fish of approximately the same weight are put together in the same basket. After the proper freezing

time elapses, the fish are removed from their baskets, glazed and conveyed by aluminum chutes to the cold-storage hold. The fish are held in cold storage until the arrival of the trawler at port, when they are discharged, thawed, filleted, and refrozen (Slavin, 1956).

This type of brine-freezing has several advantages. The fish are frozen quickly and efficiently. The system is versatile, as it can also freeze tuna or shrimp, quickly and efficiently. It requires a minimum of handling, uses a sodium chloride brine, which is relatively inexpensive, maintenance cost is low and it produces a high-quality frozen fish. However, in the Delaware experiments the brine-frozen fish were only compared with fish preserved with flake ice and then frozen after landing at port and not with other methods of freezing fish at sea.

There are also several disadvantages. Careful temperature regulation is required in the brine cooler to eliminate the possibility of the brine "freezing out" at -6°F, which might result in bursting tubes within the brine cooler. Also the penetration of salt into the flesh will be excessive if fish are left in the brine considerably longer than the required freezing time (Slavin, 1956).

When a large run of Sockeye salmon enter Bristol Bay, Alaska, the catch often exceeds the local cannery capacity. Consequently part of the Bristol Bay catch is delivered to brine-freezer packers. This brine-frozen salmon is then

delivered to canneries in Alaska, Washington, or Oregon for processing. Salmon contains highly unsaturated oils, and thus the brine-freezing operation may present serious problems from the salt-catalyzed oxidation of these oils during frozen storage prior to canning (Yonker, 1963).

Dassow (1956) stated that salmon, brine-frozen for later canning, should be handled, frozen, and stored with even greater care than that practiced with tuna, because of differences in the subsequent canning process. Oxidative rancidity may occur in the surface fatty flesh of both tuna and salmon. However, with tuna, but not with salmon, the skin and dark flesh are scraped off and not packed with the light meat. Thus oxidative rancidity may be more of a problem with salmon, particularly if it is kept in frozen storage for a prolonged period before being marketed or canned.

Tomlinson and Geiger (1963) reported that with brine-spray frozen tuna, penetration of sodium into the flesh was quite high in the outer layers (outer 3/8 inch) of muscle. However, sodium penetration into the inner layers of muscle was negligible. It was also found that water-thawing reduced the sodium concentration in the outer 1/8 inch of muscle by approximately 50%. Thus the sodium content of the fish after water-thawing is acceptable for canning when a suitable reduction in the salt added is made to compensate for that present in the muscle.

Butler *et al.* (1952) reported that round brine-frozen scrod haddock, when compared to iced haddock, offered no

complications for scaling and filleting. The yield of fillets obtained from the round brine-frozen fish was as high as that from control lots of iced, dressed fish. The appearance of the fillets from brine-frozen haddock was in all instances comparable with that of good quality fillets from iced fish. The appearance, flavor, odor, and texture of the fillets from round brine-frozen fish, thawed in fresh water at +53°F or +72°F, were quite acceptable.

Pottinger (1952) reported that the free drip from fillets of fish brine-frozen at sea was about the same as that from iced fish (3 to 4%). Also palatability tests revealed no objections to the slightly more salty flavor of fillets prepared from fish frozen in circulating brine at +5°F to +10°F and then air-thawed in comparison with fillets prepared from fish frozen on cold plates ashore and then air-thawed. However, when the brine-frozen fish were water-thawed the salt content of the flesh was reduced to pre-freezing levels.

Studies on salt content of haddock which were brine-frozen and water-thawed showed that salt penetration into the meat of fish during immersion freezing varied directly with the temperature of the brine. The increased penetration reached serious proportions from the standpoint of palatability when the brine temperature was +15°F or above. It was also shown that an increase in brine concentration caused a proportionate increase in the penetration of salt into fish during brine-freezing. However, the addition of small quantities

of calcium (1%) and of potassium (0.6%) salts to a sodium chloride brine retarded the penetration of salt. It was also reported that the salt content of commercial fillets prepared from fish that were eviscerated prior to brine-freezing was below the range of 'optimum palatability' for salt (0.9% to 1.2%). Also, water-thawing of the fish prior to filleting reduced the salt content to a level below the taste threshold for salt (0.5% to 0.6%) in fish. Excessive salt penetration occurred only in the nape of the fish, a portion which is not normally incorporated into the commercial fillet (Holston and Pottinger, 1954).

The results of Peters (1959), who investigated the salt content of large eviscerated haddock frozen in brine at +5°F, +10°F, or +15°F, were similar to those of Holston and Pottinger (1954).

Miyauchi and Heerdt (1954) investigated the salt content of sockeye salmon frozen by immersion for 12 hours in brine, cooled to about +5°F, then held in dry storage at +5°F. After thawing in running water, in still water, or in still air the fish were canned. The amount of salt added to each can varied according to the thawing method used. It was concluded that the amount of salt retained by sockeye salmon was not excessive, and that the salt retained from brine-freezing can be compensated for by the reduction of the salt usually added in canning by 20 to 50%. The salt content of canned chum salmon stored in brine at 5°F for approximately

two weeks prior to canning was also determined. When the fish were thawed in running water less than 0.5% salt was present in the canned product. This retained salt could easily be compensated for by decreasing the amount of salt added during the canning process.

Harrison and Roach (1953) froze chinook and chum salmon, and grey cod in an eutectic solution of sodium chloride then rinsed them in fresh water immediately after freezing. The flesh, even the first layer under the skin, had salt concentrations well below the generally acceptable level for palatability.

Miyauchi (1953) observed that the salt absorbed by brine-frozen sockeye salmon interferes with ice glazing of fish at storage temperatures of 0°F to +10°F. The glaze taken by brine-frozen sockeye salmon in this temperature range was not considered satisfactory. However, the glaze taken at -20°F was considered 'good'.

Peters et al. (1968) while investigating the effects of stage of rigor, method of freezing (brine-freezing vs plate-freezing), and the method of thawing (microwave vs water) on refrozen cod showed that neither the average taste panel scores nor the chemical tests for moisture, total lipid, titratable free fatty acids, and extractable protein nitrogen showed any difference attributable to state of rigor, freezing method, or thawing method.

B. Frozen Storage

1) Changes Occurring During Frozen Storage

a) Lipid Hydrolysis

In contrast to oxidative changes in fish lipids, hydrolysis by itself has no obvious nutritional significance (Lovern, 1962). In fish tissue, as such, any effects of lipid hydrolysis on product quality are likely to be due to secondary changes, e.g., possible increased susceptibility to oxidation and development of off-flavors.

Brocklesby (1933) observed a gradual increase in free fatty acids during the frozen storage of chinook and coho salmon.

Dyer et al. (1958) reported that there was almost no hydrolysis in rosefish stored at either +10°F or -10°F but in Atlantic halibut while there was no hydrolysis at -10°F, some free fatty acid formation did occur at 0°F (up to about 10% in 6 months) and when the halibut were stored at +10°F hydrolysis was more rapid (about 20% free fatty acids in 6 months). With plaice there was an increase in free fatty acids to about 32% in 6 months at +10°F. Fresh Atlantic cod had free fatty acid values of about 15%, however, when stored at -10°F the values increased to about 50% in 16 months and when stored at +10°F hydrolysis was very rapid, the free fatty acids reaching values of about 60% in one month and eventually reaching values of 80 to 90% in 16 months.

Wood and Haqq (1962) observed lipid hydrolysis and free fatty acid formation in lingcod and Pacific gray cod stored at +10°F. Fresh lingcod and Pacific gray cod contained 3.4 and 5.2% free fatty acid (%of total lipid), respectively. After 15 weeks of storage at +10°F these values increased to 28 and 42%, respectively. Gray cod resembled Atlantic cod in that there was a period of rapid hydrolysis followed by a slower more uniform rate of hydrolysis. This period of rapid hydrolysis was not observed with lingcod.

Olley et al. (1962) showed that with Atlantic cod, lemon sole, Atlantic halibut, dogfish and eleven other species there was a considerable increase in titratable free fatty acids after 16 weeks at -14°C. The formation of free fatty acids, expressed as a percentage of the total lipid, was very similar in cod, lemon sole, and halibut but was much less in dogfish. Phospholipase activity appeared to be negligible in the Elasmobranchs studied, but in all other species, phospholipase was at least as important as lipase in producing free fatty acids, and in the Gadoids and related species almost all the free fatty acids came from hydrolysis of phospholipids. It was also observed that the average lipid content (%of wet muscle) of halibut varied from 0.75% in June to 1.05% in December, lemon sole varied from 0.78% in July to 1.04% in January while dogfish varied from 4.27% in July to 14.0% in February.

The course of free fatty acid formation in rainbow trout stored at -4°C has been found to be similar to that

in cod (Jonas and Bilinski, 1967).

Free fatty acids of fresh-water whitefish muscle, stored at -10°C for sixteen weeks, have been reported to increase from 4.6% of the total lipid to 21.4% (Awad et al. 1969). About 61% of the total free fatty acid increase was derived from phospholipid and the remainder was probably derived from triglycerides.

Olley et al. (1969) showed that the extent of the initial rapid, first order hydrolysis reaction, appeared to be limited by the amount of free water available in the frozen state. Other results of Olley et al.(1969) showed that with haddock there was a preferential hydrolysis of $C_{16:0}$, $C_{18:0}$ and $C_{20:5}$ phospholipids, and that the rates of hydrolysis of phosphatidylcholine and phosphatidylethanolamine were similar. Previously, Bligh (1961) had shown that phosphatidylethanolamine and phosphatidylcholine hydrolysis were mainly responsible for free fatty acid increase in frozen stored Atlantic cod. Also Bligh and Scott (1966) showed that with cod stored at -12°C the rate of breakdown of phosphatidylcholine was faster than that of phosphatidylethanolamine. Bosund and Ganrot (1969), while studying lipid hydrolysis in frozen Baltic herring, also observed that phosphatidylcholine was hydrolyzed faster than cephalin (phosphatidylethanolamine + phosphatidylserine). They also observed considerably more phospholipid breakdown and free fatty acid formation in the red muscle than in the white muscle. They estimated that only 45% of the free fatty acids in the red muscle and 75% of those

in the white muscle are formed by hydrolysis of phospholipids, the remainder being formed by hydrolysis of triglycerides.

Until recently fish muscle was known only to contain a lipase able to catalyze the hydrolysis of short chain triglycerides (Bilinski, 1969). However, the results of Bilinski and Lau (1969) indicate that rainbow trout muscle also possesses lipolytic activity capable of hydrolyzing depot fat which, in fish, is composed of triglycerides containing predominantly fatty acids with 12 - 24 carbons. Thus, it is now known that hydrolysis of long-chain triglycerides can occur in herring (Bosund and Ganrot, 1969) and in rainbow trout (Bilinski and Lau, 1969). Whether other species of fish, other than Atlantic cod, also possess this ability is not yet known.

The work of Yurkowski and Brockerhoff (1965) indicated that the phospholipids of frozen stored cod are broken down by two enzymes, phospholipase and lysophospholipase. Also their studies on lysolecithinase showed that oleic acid had a strong inhibitory effect.

Although many researchers have investigated the increase in free fatty acid formation during frozen storage of various species of fish, little work has been done to determine if this free fatty acid increase relates to organoleptic changes during frozen storage. Fraser and Dyer (1959) stated that with Atlantic cod the fact that taste panel scores were still high after a year's storage, although the percent free fatty acids had increased to approximately 50% and 80%

when the fish were stored at -10°F and +10°F, respectively, showed that free fatty acids probably do not affect taste.

Peters et al. (1968), while investigating the effect of stage of rigor, method of freezing and method of thawing on the storage of refrozen cod stored at -18°C, observed that the correlation coefficient of free fatty acid production with the average taste panel scores (average of odor, flavor, texture and overall quality scores) of the frozen stored samples was -0.974 ($P \leq 0.01$). However, Olley et al. (1969) stated that if Peters had attempted the correlation with samples stored at a different temperature the significance might not have been so high. Olley's results indicated that free fatty acid production does not go to completion at all temperatures or, if it does so, it is at two distinct rates, an initial rapid reaction followed by a much slower one. Thus she is of the opinion that free fatty acid production and actomyosin insolubilisation cannot both equate to a taste panel for texture at all temperatures of frozen storage.

b) Changes in Protein Extractability

Some workers have observed that, depending upon the species, protein extractability (in 5% NaCl) tends to decrease during frozen storage. This phenomenon has been observed in frozen plaice fillets (Dyer and Morton, 1956); in rosefish (Dyer et al., 1956); in Atlantic cod (Dyer and Fraser, 1959; Olley and Lovern, 1960); in Atlantic halibut, lemon sole, and dogfish (Olley et al., 1962); in saithe, haddock, whiting, and

mackerel (Olley et al., 1967); in Pacific cod, and Pacific halibut (Tomlinson et al., 1969); and in fresh water whitefish (Awad et al., 1969). No detectable change in the extractable protein nitrogen of lingcod, ocean perch, red snapper, orange spotted rockfish, and rock sole stored for 8 1/2 months at -30°C was noticed by Tomlinson et al., (1969). The decrease in protein extractability was also quite slow for lemon sole and dogfish (Olley et al., 1962).

The relationship between the increase in free fatty acid formation and the decrease in protein extractability that occurs during frozen storage appears to vary among species. Olley et al. (1962) while studying frozen stored Atlantic cod, lemon sole, Atlantic halibut, and dogfish observed that the rate of increase of free fatty acids was twice as high for dogfish as for the other species, but the rate of decrease in protein extractability was less for dogfish than it was for cod. Also lemon sole, which was similar to cod in free fatty acid production, showed much less protein denaturation than cod. However, Hanson and Olley (1965) hypothesized that neutral lipids protect protein from free fatty acid denaturation in situ and not only at the homogenization stage of the soluble protein determination. This hypothesis was supported by the work of Olley et al. (1967) who showed that small quantities of neutral lipid may have a protective effect on fish muscle proteins.

Using model systems King et al. (1962) showed that the addition of small amounts of linoleic or linolenic acid

caused cod actomyosin to precipitate from a solution of isolated cod actomyosin. Also using model systems, Anderson et al. (1965) concluded that the interaction of protein with fatty acid resulting in insolubilization of the protein is optimal at an ionic strength of 0.5 at pH 7.2 and that 5u is the ionic strength that should exist in the cellular fluid of cod muscle as a result of freezing to -1.5°C.

In his recent review Connell (1968) stated that the protein extractability of rosefish stored at +10°F and of skate and nursehound stored at -7°C, declined considerably without the formation of free fatty acid. He refers to unpublished data and concludes that free fatty acid production is merely one change which coincides in some species with the decline in protein extractability.

Dyer and Morton (1956) observed that the texture ratings of plaice fillets stored at -12°C, showed an increase in toughness parallel to the decrease in protein extractability. Also Dyer et al. (1956) found that with rosefish stored at -12°C taste panel results correlated reasonably well with protein extractability. However, with rosefish stored at -23°C (Dyer et al., 1956) and Atlantic cod stored at -30°C (Love, 1956) marked increases in toughness occurred before any appreciable decrease in protein extractability had taken place. Luippen (1957) observed a consistent close relationship between the increase in toughness after boiling and the decrease in the ratio of soluble nitrogen to total nitrogen in cod samples that had been stored at -10°C. However, no correlation was observed

with the samples that had been stored at -20°C and at -30°C. Moorjani et al. (1960) reported that with filleted morwong packed in evacuated cans or sealed in cellophane bags and stored at -18°C, the differences in protein extractability were associated with texture differences after storage times of 2 to 6 months. Cowie and Little (1966) studied toughness and protein solubility of cod fillets during storage at -29°C for 82 months. There was a steady decrease in protein solubility from 72% to 45% but the development of toughness during frozen storage was extremely variable. In fact cod which had been stored for 82 months were more tender than some of the control fillets which had been freshly frozen but not stored. Also Cowie and Little (1967) investigated the relationship between toughness and protein extractability with cod stored at -7°C and at -14°C. There was a poor correlation between toughness and protein extractability. They concluded that protein extractability alone cannot accurately describe toughness and suggested that pH must also be considered. Tomlinson et al. (1965a) observed that protein extractability can change markedly during the thawing of frozen flesh. The alteration appeared to be related to flesh pH as extractability decreased to a greater extent at lower pH. Thus one possible explanation of the lack of correlation between protein extractability and toughness of cooked flesh observed by Cowie and Little (1967) and others is that the protein extractability was measured by homogenization of frozen rather than thawed flesh.

In contrast, Peters et al. (1968) found that taste panel scores of cod stored at -18°C for 12 months correlated (r = +0.92) significantly ($P \leq 0.05$) with extractable protein. Connell, (1969) while investigating changes in the eating quality of frozen stored cod, observed that protein extractability correlated much better with flavor (r = -0.730) or firmness (r = -0.664) of cold stored fish than did either color ratio or cell fragility methods.

c) Changes in Thaw Drip

The amount of thaw drip or liquid that exudes when frozen fish tissue thaws is affected by several factors. The amount of drip formed is directly related to the ratio of cut surface area to the weight of flesh. With chinook, coho, and chum salmon and Pacific halibut the rate of freezing influences the amount of drip; rapid freezing results in the least drip. Drip increases with length of frozen storage and is greater at higher temperatures. Brining of fish flesh appears to reduce the amount of drip formed. Increases in drip are probably related to decreased protein extractability (Miyauchi, 1963). Young (1941) observed that with Pacific halibut, over 40 pounds in weight, the amount of drip increased from head to tail but with smaller fish the drip was less and did not always increase in the same sequence.

Tomlinson et al. (1969) reported that the free (thaw) drip of Pacific halibut, frozen pre-rigor, increased during

frozen storage from 5% after nine days to 7.6% after 455 days, while that of the in-rigor samples increased from 5% to 7.8% and the free drip of the post-rigor samples increased from 6.6% to 10.8%.

d) Oxidative Rancidity

One reason rancidity in foods is undesirable is that off-flavors and off-odors develop making the food unsuitable for consumption. It is known that oxidized fats cause the destruction of several fat-soluble vitamins and carotene. It is also claimed that oxidized fats are carcinogenic or in other ways seriously harmful, as very highly oxidized and oxidatively polymerized fats have been shown to produce toxic effects in animals (Lundberg, 1961).

The muscle of most fish is not uniform in color. That part located just beneath the skin, the so called lateral line muscle, is often brown or reddish in color. This red muscle has a high lipid concentration and the lipids are highly unsaturated. Atlantic halibut (Hippoglossus hippoglossus) contains 23.7% lipid (wet weight basis) in the red muscle while the white or ordinary muscle (Fig.1) contains 7.0% lipid (Love, 1970).

The rate at which oxidative rancidification occurs is affected by (a) the amount of oxygen present, (b) the degree of unsaturation of the lipid components, (c) antioxidants, (d) metals such as copper, (e) organic catalysts such

as hematin compounds and lipoxidases, (f) certain salts, (g) processing treatments, (h) packaging, (i) exposure to light, and (j) storage temperatures. In most types of lipid oxidation it is essential that some oxygen be present. The more highly unsaturated the fatty acids in the lipids, the faster the rate of oxidation. Contamination of foods with inorganic oxidative catalysts such as copper from equipment or other sources, often leads to rapid development of rancidity (Mitchell and Henick, 1961). It has also been shown that certain products, such as frozen meats, become rancid at a slower rate if stored without added salt. Autoxidation takes place at increasing rates as the storage temperature of a food is increased, provided oxygen is present. Thus storage at low temperatures is advantageous in those instances where rancidity may be a problem. Lipids in foods that have been exposed to conditions promoting oxidation may have already passed through the induction period of oxidative rancidity at the initial time of storage and naturally will not have a shelf life as long as that optimally possible (Mitchell and Henick, 1961).

The mechanism of autoxidation of lipids, stored in the presence of oxygen, is the general chain mechanism outlined below (Lundberg, 1962; Ingold, 1968).

Initiation

$$RH + O_2 \longrightarrow \text{free radicals}$$

$$\left.\begin{matrix} ROOH \\ (ROOH)_2 \end{matrix}\right\} \longrightarrow \text{free radicals (e.g., } R^{\cdot}, RO^{\cdot}, ROO^{\cdot}, HO^{\cdot}, \text{ etc.)}$$

Propagation

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

Branching

$$ROO^{\bullet} \longrightarrow RO^{\bullet} + HO^{\bullet}$$

$$ROOH + M \longrightarrow \text{free radicals}$$

Termination

$$\left.\begin{array}{l} R^{\bullet} + R^{\bullet} \\ R^{\bullet} + ROO^{\bullet} \\ ROO^{\bullet} + ROO^{\bullet} \end{array}\right\} \longrightarrow \text{stable (non-radical inactive end products)}$$

ROO• and ROOH represent a peroxy radical and a hydroperoxide, respectively. M represents a metal catalyst which increases the rate of oxidation of the substrate by increasing the rate of decomposition of the hydroperoxide to free radicals. Heavy metals, particularly those possessing two or more valency states with a suitable oxidation-reduction potential between them, e.g., cobalt, copper, iron, manganese, nickel, are the most powerful catalysts (Ingold, 1968).

Castell and MacLean (1964, a) in a study of copper-catalyzed rancidity of cod fillets, observed that muscle from the tail section becomes rancid more rapidly than muscle from the head or centre sections. They also observed that cod caught in the winter and early spring develops rancidity at a greater rate than cod caught in the summer and fall. The seasonal differences were primarily concerned with the induction period in the development of rancidity. Consequently

they thought the seasonal differences could be the result of differences in the natural antioxidants in the muscle, particularly tocopherol, which are known to fluctuate with the feeding cycle of cod. In contrast, Bailey et al. (1952) reported that with chinook salmon the oil from the white muscle is generally more unsaturated than oil from the red muscle and that oil from the flesh near the head is more unsaturated than oil from the flesh near the tail. Thus, with chinook salmon one would expect that muscle near the head would become rancid faster than muscle near the tail although no direct evidence to support this suggestion has been reported in the literature.

Castell and MacLean (1964,b) showed that actively growing bacteria exert an antioxidant effect and suppress the development of copper-catalyzed rancidity in cod muscle.

Castell et al. (1966,a) reported that the addition of free aromatic, heterocyclic, and sulphur containing amino acids retarded copper-catalyzed rancidity as measured by the TBA test. In the absence of added metallic ions, however, the aliphatic amino acids and cysteine showed strong pro-oxidant activity. Also those amino acids which inhibited metal-induced rancidities did not retard rancidity induced by the addition of sodium chloride.

Castell and Spears (1968) added from 1 to 50 ppm of ten different heavy metal ions to blended muscle taken from freshly killed cod, haddock, flounder, redfish, herring, mackeral, scallops, and lobster stored for 24 hours at 0°C.

The resulting rancidities were determined by TBA values and by odours. With some exceptions Fe^{2+}, V^{2+}, and Cu^{2+} were the most active catalysts. Fe^{2+} was always more effective than Fe^{3+} while Cd^{2+}, Co^{2+}, and Zn^{2+} produced rancidity with the fatty species but not with any of the other species while Ni^{2+}, Cr^{2+}, and Mn^{2+} did not accelerate rancidity in any of the muscles. There was considerable difference in the relative susceptibility to rancidity induced by specific metals in muscle from different species.

Banks (1937) found that salt accelerated rancidity in raw herring but not after it was cooked. Tarr (1944,1947) reported that immersion of chinook salmon, pink salmon, and chum salmon fillets in NaCl solutions increased the rancidity (peroxide values) during frozen storage. Also Castell et al. (1965) reported that sodium chloride accelerated rancidity (TBA values) in blended cod muscle at 0°C and that the active agent appeared to be Na^+ ions rather than whole salt or Cl^- ions. In addition, Ellis et al. (1970) observed that sodium chloride had a direct pro-oxidant action on the lard of freezer-stored and dehydrated gels while hydrated gels containing sodium chloride, when stored at 20°C, had an "inhibiting autoxidation pattern" somewhat similar to the quantitative influence of NaCl on pH. Also sodium chloride accelerated heme catalysis regardless of the presence of antioxidants or chelators.

The oxidative rancidity of frozen red salmon (chinook

and coho) has been found to be accompanied by a fading of the red and yellow pigments of the flesh (Tarr, 1947; 1955; Boyd et al. 1957).

METHODS AND MATERIALS

A) Catching and Freezing the Fish

Pacific halibut (Hippoglossus stenolepis) were caught by long line in Queen Charlotte Sound, Northeast of Cape Scott, on June 7, 1969. The fish were eviscerated and placed on ice immediately after being caught. Approximately six hours later three of the fish were frozen to -30°C in a vertical plate freezer during a period of about 3.5 hours. Another three fish were frozen to -5.6°C (22°F) in a 13% NaCl solution. In order to simulate the anticipated worst possible brine-freezing times aboard commercial halibut long liners, the halibut were brine-frozen over approximately a 47 hour period.

Chinook salmon (Oncorhynchus tsawytscha) and coho salmon (Oncorhynchus kisutch) were caught by seining in Goletas Channel and Queen Charlotte Strait near Duval Point on the Northeast coast of Vancouver Island on July 21, 1969. The fish were obtained from commercial salmon seiners on the day of the catch. Three fish of each species were frozen to approximately -30°C (-22°F) in a vertical plate freezer during a period of about 3.5 hours. Another three fish of each species were brine-frozen to -5.6°C (22°F) in a 13% NaCl solution over a 9.5 hour period.

After freezing (whether by brine or plate) the halibut were stored in a home-type chest freezer and the salmon

(chinook and coho) were stored in a plate freezer until arrival at Vancouver. The fish were then glazed twice, using a water and ice mixture, put in plastic bags, and stored at -30°C (-22°F) until required.

B) Sampling and Analysis

Depending on species, the fish were sampled and analyzed 3 or 5 different times during the maximum frozen storage time for that species. The maximum frozen storage times for the three different species were: Pacific halibut 81 weeks, chinook salmon 77 weeks, and coho salmon 78 weeks.

Sampling consisted of sawing one-half inch thick steaks from the anterior end of the fish, immediately reglazing the remainder and replacing it in a plastic bag for further frozen storage. Prior to the first analysis of each species, the six fish of that species were randomly divided into three pairs, each pair consisting of one brine-frozen and one plate-frozen fish. The pairs remained constant throughout the experiment, and provided triplicate determinations per species per time period. Analysis of one species (six fish) required one week (three days for organoleptic analysis at one pair per day, three days for chemical analysis at one pair per day and one day to prepare the samples and reagents for the next six days). Consequently the steaks were sawn from each of the six fish at the beginning of the week, placed in plastic bags and stored over dry ice until needed for analysis. The

2-thiobarbituric acid (TBA) test, long chain free fatty acid analysis, and taste panel evaluation were conducted on both inside and outside muscle (Fig. 1). Color and pH determinations were made only on white muscle (inside muscle plus the white muscle part of the outside muscle). The whole steak(s) or cross-section(s) of a fish was used to determine free drip. Immediately prior to all analyses, the glaze was removed and the skin torn from the frozen steak leaving the red muscle intact on the steak.

a) Determination of Thaw Drip

Thaw drip from the frozen muscle was determined by a slight modification of the U. S. standard method for frozen cod fillets (Anon., 1960). The time of thawing at 20°C (68°F) was fixed at 2 hours for all samples. The samples were drained on paper toweling instead of using a U. S. Standard No. 8 circular sieve. The drip was expressed as percent by weight of the frozen fish.

b) Determination of pH

Measurements of pH were made using the method of Tomlinson, et al. (1966). A combination glass electrode was inserted directly into the thawed flesh. A Metrohm Model E 280 A portable pH meter was used.

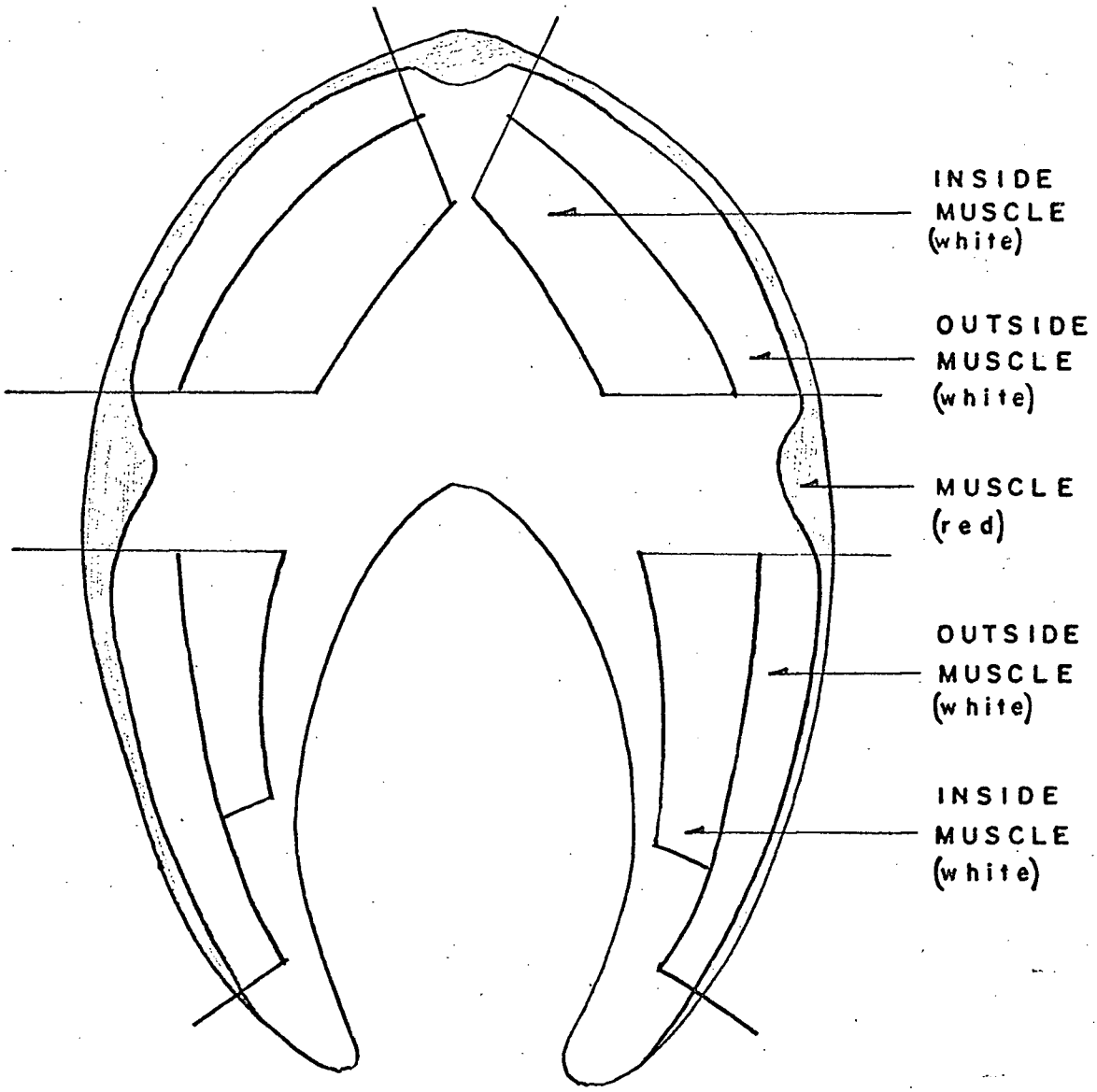

Figure 1. A diagramatic representation of a cross-section of a fish showing the areas sampled as inside and outside muscle.

c) Color Determination

Hunter Rd, a, and b values were determined on the thawed flesh of chinook and coho salmon using the method of Schmidt and Idler (1958). The thawed flesh was placed in a 3.2 cm diameter plastic petri dish and the average of triplicate measurements of Rd, a, and b were taken using a Model C Gardner Color - Difference Meter. The sample in the petri dish was turned twice after the initial measurement. The reference standard was identical to that used by Schmidt and Idler (1958). The reference tile had Rd, a, and b readings of 8.73, 33.8, and 20.7 respectively.

d) Determination of Flavor Differences

Differences in flavor between fish brine-frozen and plate-frozen at sea were determined using a triangle test (Fig. 2) as outlined by Larmond (1967). The samples were prepared for organoleptic evaluation by wrapping the steaks in aluminum foil and steaming them for 12 minutes. The steaks were then unwrapped and divided into samples of inside and outside muscle (Fig. 1). The flavor of the samples was evaluated by a taste panel consisting of 5 experienced panel members. Each panel member was instructed to taste the red muscle on all of the outside muscle samples. Taste panels were conducted daily for three days per week. Each session was devoted to the evaluation of one pair of fish. Thus

TRIANGLE TEST

DIFFERENCE ANALYSIS

DATE_____ TASTER_____

PRODUCT_____

Instructions: Here are three samples for evaluation. Two of these samples are duplicates. Separate the odd sample for flavour difference only.

(1) Sample	(2) Check odd sample
_____	_____
_____	_____
_____	_____

(3) Indicate the degree of difference between the duplicate samples and the odd sample.

Slight_____ Much_____

Moderate_____ Extreme_____

(4) Acceptability:

Odd sample more acceptable_____

Duplicate samples more acceptable_____

(5) Comments including texture and odor abnormalities:

_____

_____

Figure 2. Taste panel questionaire used to determine flavor differences between frozen stored plate-frozen and brine-frozen Pacific halibut, chinook salmon, and coho salmon.

after 3 sessions (days) the organoleptic evaluation of one species was completed for that time period giving 30 observations on the outside muscle (10 observations per pair of fish) and 30 observations on the inside muscle.

e) Determination of 2-Thiobarbituric Acid Reactive Substances

The thiobarbituric acid (TBA) test was conducted using the procedure of Castell et al. (1966) with some modifications. Ten grams of frozen tissue and 1.10 grams of the disodium salt of ethylenediaminetetraacetic acid (EDTA) were homogenized with 50 ml. distilled water for 90 seconds in a Serval Onmi-Mixer. The homogenate was transferred to a 250 ml. beaker and the mixer rinsed with two 20 ml. portions of distilled water giving a total volume of approximately 100 ml. The homogenate was stirred well after the addition of the water and was then acidified to pH 1.5 - 1.6 by the addition of 4N HCl. The homogenate was then transferred to a 500 ml. round bottom flask and the beaker rinsed with 10 ml. of distilled water. Dow Corning Antifoam A was sprayed into the flask and the mixture was then distilled at a rate to give a total of 50 ml. distillate in 18 - 20 min. Duplicate 5 ml. aliquots of the distillate were combined with 5ml. of 0.02 M TBA solution in 90% acetic acid, and heated in a stoppered test tube in a boiling water bath for 35 min. The solution was then cooled for 10 min. in running tap water and the absorbance of the solution was read

in a Bausch and Lomb double beam Precision Spectrophotometer at 538 nm. Blank determinations, using distilled water, were run at the same time as the samples.

f) Lipid Extraction

The lipids were extracted from 20 g. of frozen flesh by a procedure similar to that of Bligh and Dyer (1959). Twenty grams of flesh, 40 ml. methanol and 20 ml. chloroform were homogenized for 2 min. in a Serval Omni-Mixer, to give a chloroform: methanol: water ratio of 1:2:0.8. Twenty ml. of chloroform was then added and homogenization continued for another 30 sec. when 20 ml. of water was added and the mixture homogenized for a further 30 sec. This gave a homogenate with a chloroform: methanol: water ratio of 2:2:1.8. The homogenate was then suction filtered through Whatman No. 1 filter paper on a No. 2 Buchner funnel into a 250 ml. side-arm flask. The tissue residue, filter paper and 40 ml. of chloroform were homogenized for 1 min. and suction filtered as before. The mixer cup and the extracted tissue residue were rinsed with 20 ml. of a (1:1) chloroform: methanol mixture. The combined filtrates were then transferred to a 250 ml. stoppered graduated cylinder and the side-arm flask was rinsed with 20 ml. of a (1:1) chloroform: methanol mixture.

After the chloroform and alcoholic layers completely separated the volume of the chloroform layer was adjusted to

105 ml. and the alcoholic layer was removed by aspiration. Anhydrous sodium sulphate was then added to dry the chloroform layer. The water free lipid chloroform solution was then gravity filtered through Whatman No. 1 filter paper into a 250 ml. round bottom flask. The sodium sulphate was rinsed with 15 ml. of reagent grade chloroform. The lipid-chloroform solution was then evaporated under vacuum to approximately 10 ml. using a Buchler Flash-Evaporator, the water bath temperature being 30°C. The concentrated lipid-chloroform solution was then transferred to a 75 ml. culture tube; the 250 ml. round bottom flask was rinsed with a total of 25 ml. chloroform.

g) Removal of the Phospholipids

The phospholipids were separated from the lipid-chloroform solution using a procedure similar to that of Hornstein et al. (1967). Mallinckrodt 100 mesh silicic acid was activated by heating it overnight at 120°C. Four grams of activated silicic acid was added to the chloroform-lipid solution. The tube was then stoppered and the contents mixed on a Vortex mixer for 1 minute, allowed to stand for 5 minutes and then suction filtered through Whatman No. 1 filter paper on a No. 0 Buchner funnel into a 125 ml. side-arm flask. The silicic acid residue was washed three times with successive 8 ml. portions of chloroform. The filtrate was then transferred to a 100 ml. round bottom flask. The 125 ml. side-arm flask

was rinsed with 15 ml. chloroform. The phospholipid free-lipid chloroform fraction was then evaporated under vacuum to approximately 2 - 5 ml. using a Buchler Flash-Evaporator with a water bath temperature of 30°C.

h) Isolation of Neutral Lipids

An isopropanol-KOH solution was prepared by adding 6.25 gm. KOH to 100 ml. isopropanol then heating and shaking the mixture until the KOH was dissolved. The solution was allowed to cool and then the supernatant was decanted leaving any H_2O present in the flask.

The neutral lipids were separated from the phospholipid-free fraction by a procedure similar to that of McCarthy and Duthie (1962). Four grams of activated 100 mesh silicic acid were weighted into a 100 ml. beaker. Eight ml. of the isopropanol-KOH solution and 24 ml. anhydrous diethyl ether were then added to the silicic acid. The contents were mixed and then allowed to stand for 5 minutes. The mixture was then slurried into a 2 cm. by 26 cm. glass column and washed with 100 ml. of anhydrous diethyl ether. The 2 - 5 ml. of phospholipid-free lipid was dissolved in a small quantity of anhydrous diethyl ether placed on the column and thoroughly washed into the packing by several small portions of anhydrous diethyl ether. The phospholipid-free lipid fraction was placed on the column under a nitrogen atmosphere. Cholesterol, cholesterol esters, mono-, di-, and triglycerides were then eluted in one fraction

from the column with 200 ml. of anhydrous diethyl ether. The neutral lipids eluted from the column were collected in a 250 ml. Erlenmyer flask. When the fraction was collected, the flask was flushed with nitrogen, stoppered, and placed in a refrigerator for subsequent weight determination of the neutral lipids.

i) Removal of Free Fatty Acids

The free fatty acids (FFA) retained on the column were removed by two 10 ml. aliquots of boron trifluoride solution, containing 125 gm. BF_3 per 1000 ml. methanol (Metcalfe and Smith, 1961).

j) Esterification of Free Fatty Acids

The eluted FFA were trans-esterified by mildly refluxing the eluted solution for 15 minutes. The solution was then transferred to a separatory funnel and the round bottom flask rinsed with 5 ml. of n-pentane. Twenty ml. of distilled water was added to the separatory funnel and the layers allowed to separate. The aqueous layer was then transferred into the original round bottom flask and the organic layer was transferred to a 50 ml. Erlenmyer flask containing anhydrous sodium sulphate. Five millileters of spectrascopically analyzed n-pentane was added to the aqueous layer and the solution re-extracted as before. The two organic fractions were combined and well dried with anhydrous sodium

sulphate. The water-free organic fraction was then transferred to a 15 ml. graduated test tube and the sodium sulphate rinsed with two portions of 3 ml. n-pentane. Using nitrogen gas, the volume in the test tube was adjusted to 2 ml. and transferred to a 1 dram vial. The test tube was rinsed well with n-pentane. The air space in the vial was flushed with nitrogen gas and the vial sealed, labelled, and stored at -30°C for subsequent gas chromatographic analysis.

k) Determination of the Weight of the Neutral Lipids

The neutral lipid content was determined by flash evaporating the 200 ml. neutral lipid fraction almost to dryness. Ten millileters of chloroform was then added to the round bottom flask and the contents transferred to a pre-weighed aluminum weighing disk. The round bottom flask was rinsed well with chloroform. The weighing dishes were then placed on a hot plate, (the temperature set at low) in a fume hood, in order to evaporate most of the chloroform. The samples were dried to constant weight by placing them in a vacuum oven at 70°C for 3 hours.

l) Gas Chromatographic Analysis of the Free Fatty Acids

The isolated free fatty acid methyl esters were analyzed using a Micro-Tek Model 220 gas chromatograph equipped with a flame ionization detector. The column used was of

stainless steel, 10 ft. in length and 3/16 in diameter, packed with 3% EGSP-Z (an ethylene glycol-succinic acid diphenyl-diethoxysilane polyester) on 100 - 120 mesh Gas-Chrom Q, Lot 4303, purchased from Applied Science Laboratories. Nitrogen at 5.7 ml/min was employed as the carrier gas. The inlet temperature was 250°C and the detector temperature was 240°C. All analyses were conducted isothermally with a column temperature of 185°C. Methyl tricosanoate (23:0) purchased from Applied Science Laboratories was used as an internal standard. The fatty acid methyl esters were identified by comparison of their retention times with those of herring oil and cod liver oil. The recorder was equipped with a Disc Chart Integrator and quantitation was based on the assumption that detector response was proportional to carbon content. The results were expressed both as a percentage of the total FFA analyzed and as mg. FFA per gm. neutral fat.

m) Determination of the Mineral Concentration in the Flesh

Sodium and potassium concentration in the first half inch of muscle beneath the skin (outside muscle) and in the next adjacent half inch of muscle (inside muscle) was determined on the frozen flesh using the method of Thompson (1969). The dry-ash method was used and the sodium and potassium concentrations in the solutions were determined using an E.E.L. Flame Photometer. The concentrations were expressed as mg/gm flesh.

Chloride concentration in the first half inch of muscle beneath the skin (outside muscle) and in the next adjacent half inch of muscle (inside muscle) was determined using Quantab Chloride Titrators S031 No. 1175. The procedure used was similar to that outlined in the instructions for Quantab Chloride Titrators S041 No. 1176 except a total volume of 50 ml. rather than 100 ml. was used.

C) Statistical Analysis

The sodium, potassium, and chloride concentration data as well as the color, thaw drip, and pH values were statistically analyzed using a randomized complete block split-plot design. The TBA values and the long chain free fatty acid data were statistically analyzed using a randomized complete block split-split plot design.

Pairs of fish were the replicates or blocks, methods of freezing were the whole plots, locations (inside or outside muscle) were the split-plots, and times of frozen storage were the split-split plots for the analyses of the TBA test and long chain free fatty acid data. Pairs of fish were the blocks, methods of freezing were the whole plots, and locations were the split-plots for the analyses of the sodium, potassium, and chloride concentration data. For the analyses of the color, thaw drip, and pH data pairs of fish were the blocks, methods of freezing were the whole plots and times of frozen storage were the split-plots.

Pairs were considered random whereas all other effects were considered fixed in all analyses. The thaw drip values and the free fatty acid data expressed as percentages were transformed, using the arcsine transformation, before any analyses of variance or correlations were conducted. The absolute differences in the pH, thaw drip, color, TBA values, and the free fatty acids[1] between the brine-frozen samples and the plate-frozen samples were correlated with the taste panel scores (number of correct identifications).

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[1] All fatty acids in the text and in all tables are described using the shorthand notation; chain length: number of double bonds, e.g. 18:2 where chain length = 18 carbons, number of double bonds = 2.

RESULTS

A. Analysis of Variance

a) Flesh pH

Analysis of variance of the pH data showed that there were no significant differences in pH between brine-frozen and plate-frozen samples of Pacific halibut, chinook salmon, and coho salmon (Table I).

The analysis (Table I) also revealed significant differences in pH among different frozen storage times for Pacific halibut ($P \leq 0.05$) chinook salmon ($P = 0.01$), and coho salmon ($P \leq 0.001$). Also with chinook salmon there was a significant difference ($P \leq 0.05$) among pairs.

i) Pacific Halibut

The pH of Pacific halibut decreased steadily during frozen storage (Fig. 3). Duncan's new multiple range test showed that the pH at 81 weeks of frozen storage was not significantly different than the pH at 62 weeks but was significantly lower ($P \leq 0.05$) than at 45, 31 and 14 weeks (Table II).

ii) Chinook Salmon

The pH of chinook salmon did not change steadily during frozen storage. The pH increased from 9 weeks until 27 weeks, decreased until 58 weeks and finally increased until 77 weeks of frozen storage (Fig. 4). The pH of chinook salmon at 58 weeks was significantly lower ($P \leq 0.05$) than the pH

TABLE I Analysis of variance of pH values of Pacific Halibut, Chinook Salmon, and Coho Salmon.

	Pacific Halibut			Chinook Salmon			Coho Salmon		
Source	d.f.	M.S.	Prob[1]	d.f.	M.S.	Prob	d.f.	M.S.	Prob
Pairs	2	0.0126	0.1542	2	0.0337	0.0299*	2	0.0009	0.8154
Methods	1	0.0059	0.5168	1	0.0034	0.3236	1	0.0089	0.3128
Error a	2	0.0096		2	0.0020		2	0.0049	
Time	4	0.0265	0.0139*	4	0.0574	0.0014**	2	0.1561	0.0001***
M x T	4	0.0010	0.9525	4	0.0057	0.5784	2	0.0145	0.0812
Error b	16	0.0660		16	0.0077		8	0.0042	

1 Probability of type 1 error

2 Error a was used to test Methods
Error b was used to test all terms except Methods

* Significant at the 5% level

** Significant at the 1% level

*** Significant at the 0.1% level

TABLE II Duncan's new multiple range test on the significant time effects of the analyses of variance on the pH values of Pacific Halibut, Chinook Salmon, and Coho Salmon.(1)

Pacific Halibut

14 weeks(2)	31 weeks	46 weeks	62 weeks	81 weeks
6.203(3)	6.156	6.138	6.118	6.023

Chinook Salmon

26 weeks(2)	9 weeks	40 weeks	77 weeks	58 weeks
6.082(3)	6.067	6.018	5.972	5.838

Coho Salmon

27 weeks(2)	10 weeks	78 weeks
6.310(3)	6.298	6.025

(1) Means not underlined by the same line were significantly different ($P \leq 0.05$) from each other.
(2) Weeks of frozen storage at -30°C.
(3) pH values.

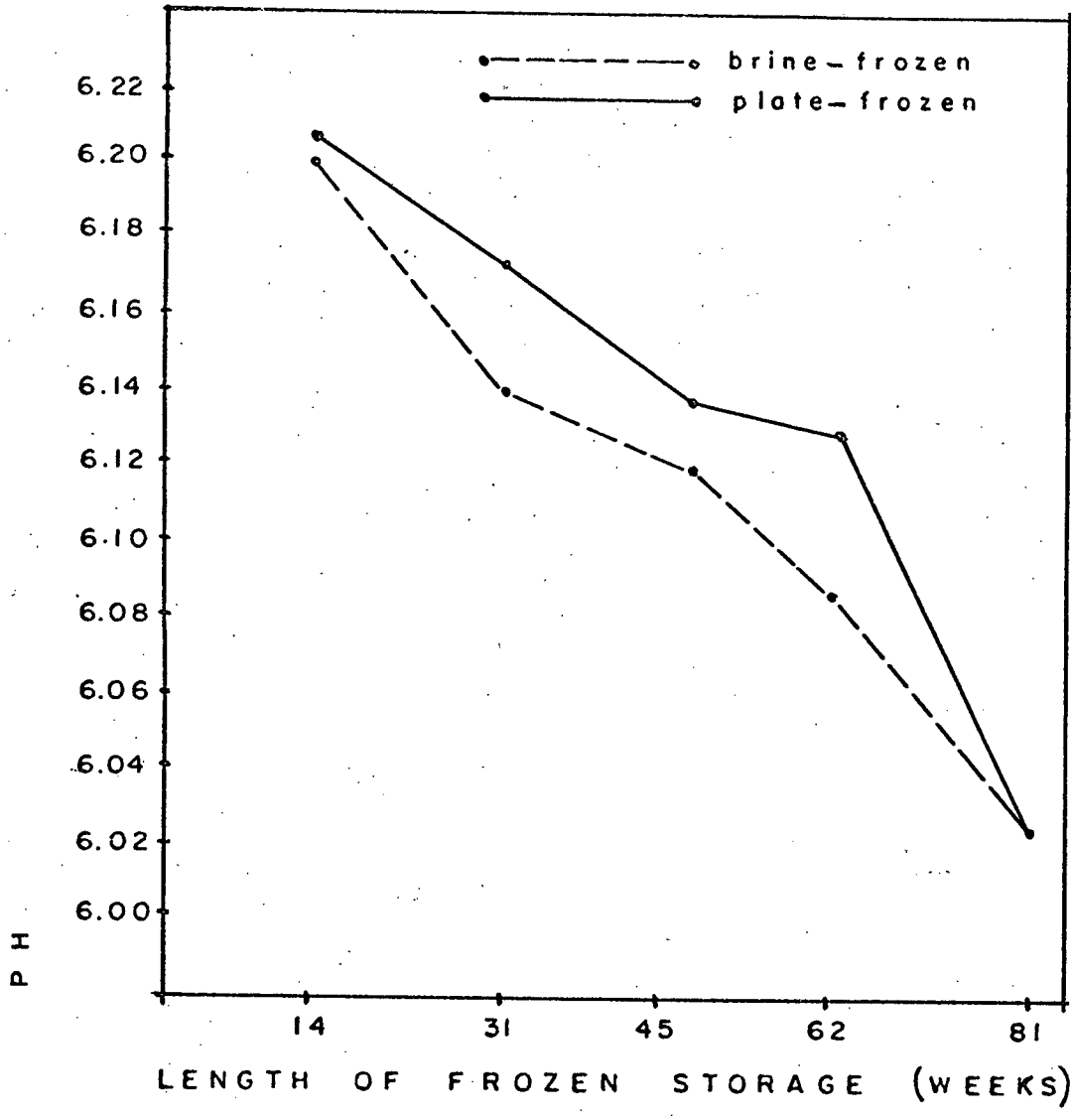


Figure 3 Average flesh pH of Pacific halibut stored at -30°C.

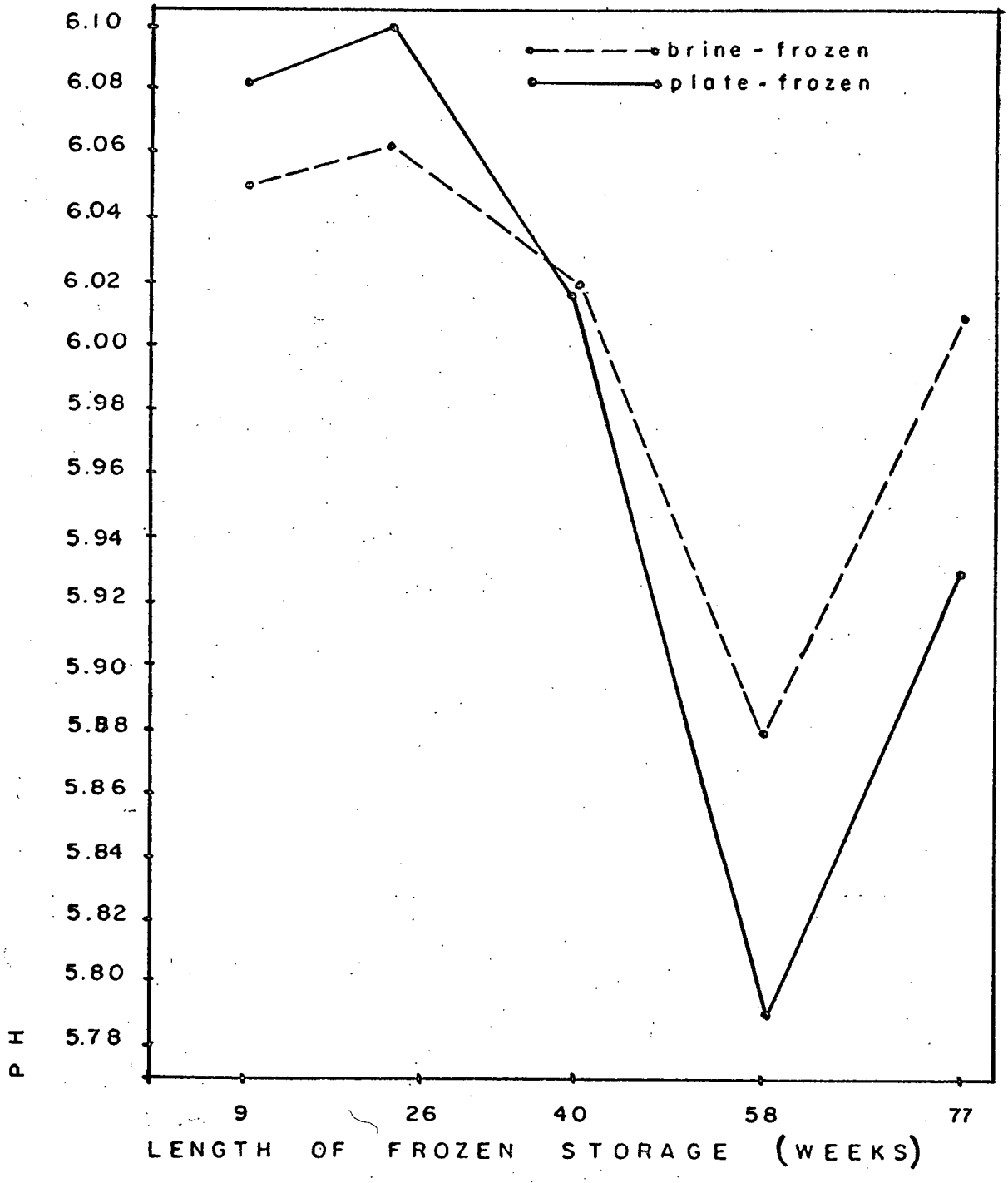


Figure 4 Average flesh pH of chinook salmon stored at -30°C.

at all other frozen storage times (Table II).

iii) Coho Salmon

The pH of the plate-frozen coho salmon decreased steadily during frozen storage while the pH of the brine-frozen samples increased from 10 weeks until 27 weeks then decreased until 78 weeks of frozen storage (Fig. 5). The pH of coho salmon at 78 weeks was significantly lower ($P \leq 0.05$) than the pH at 10 and 27 weeks of frozen storage (Table II).

b) Thaw Drip

i) Pacific Halibut

Analysis of variance on the thaw drip of frozen stored Pacific halibut revealed a highly significant ($P \leq 0.01$) method x time interaction (Table III). At 14 and 31 weeks of frozen storage the thaw drip of the brine-frozen samples was less than that of plate-frozen samples. However, at 45, 62, and 81 weeks of frozen storage the thaw drip of the brine-frozen samples was greater than that of the plate-frozen samples (Fig. 6). The thaw drip of the plate-frozen fish tended to decrease during frozen storage while that of the brine-frozen halibut tended to increase (Fig. 6).

ii) Chinook Salmon

The mean thaw drip of plate-frozen chinook salmon was not significantly different from that of brine-frozen samples (Table III). However, there was a very highly

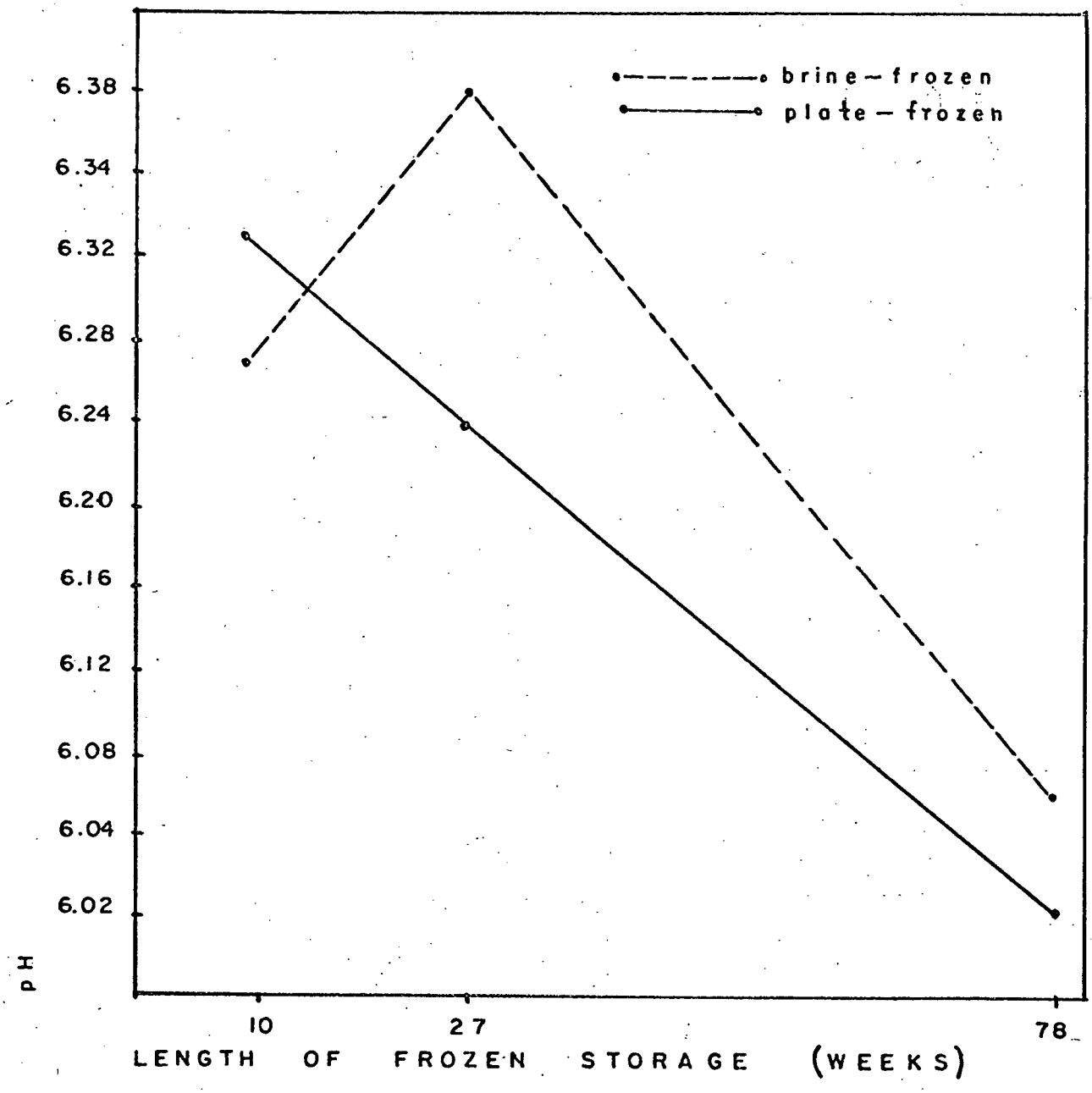


Figure 5 Average flesh pH of coho salmon stored at -30°C.

TABLE III Analysis of Variance of the Thaw Drip Data of Pacific Halibut, Chinook Salmon, and Coho Salmon[1]

	Pacific Halibut			Chinook Salmon			Coho Salmon		
Source	d.f.	M.S.[2]	Prob[3]	d.f.	M.S.	Prob	d.f.	M.S.	Prob
Pairs	2	0.3429	0.0018**	2	0.0051	0.6435	2	0.1509	0.0735
Methods[4]	1	0.0439	0.7788	1	0.0074	0.5441	1	0.6149	0.0341
Error a	2	0.4893		2	0.0141		2	0.0159	
Time	4	0.0485	0.2863	4	0.1138	0.0003	2	0.2201	0.0335*
M x T	4	0.1358	0.0224*	4	0.0323	0.0551	2	0.0937	0.1642
Error b	16	0.0352		16	0.0114		8	0.0412	

1 Analysis was conducted on the arcsin transformed percentages.

2 All mean squares are multiplied by 1.0×10^2.

(3) Probability of type 1 error

(4) Error a was used to test methods
Error b was used to test all terms except methods

* Significant at the 5% level

** Significant at the 1% level

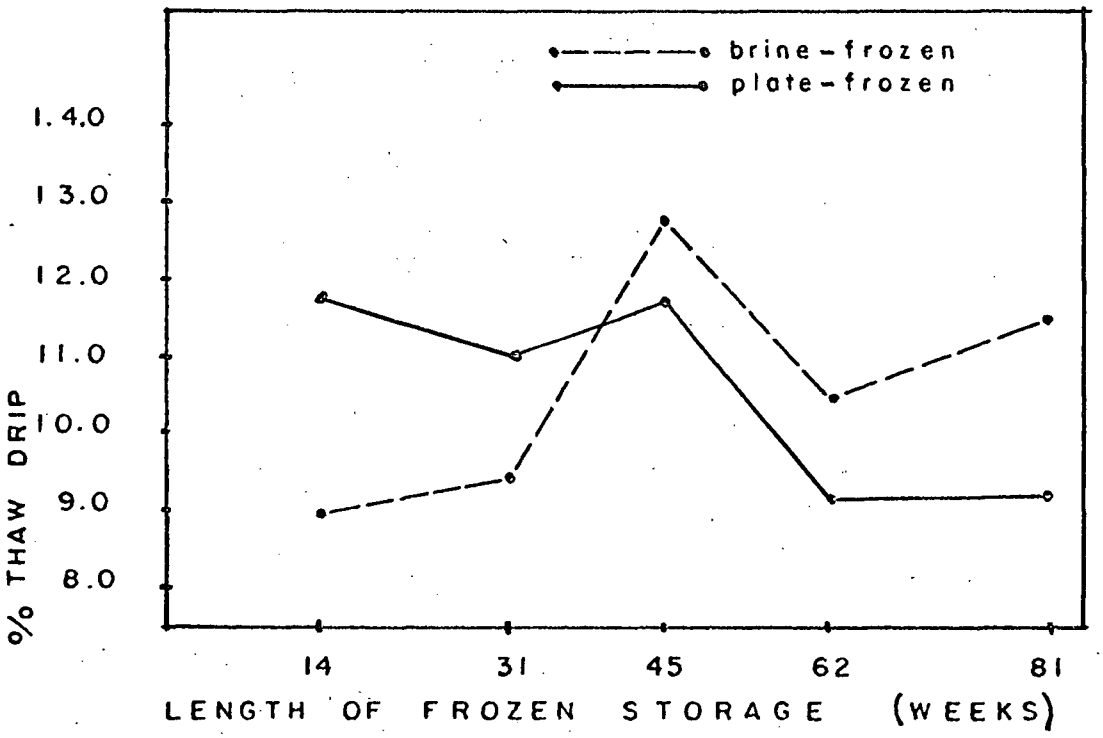


Figure 6 Average thaw drip of Pacific halibut stored at -30°C.

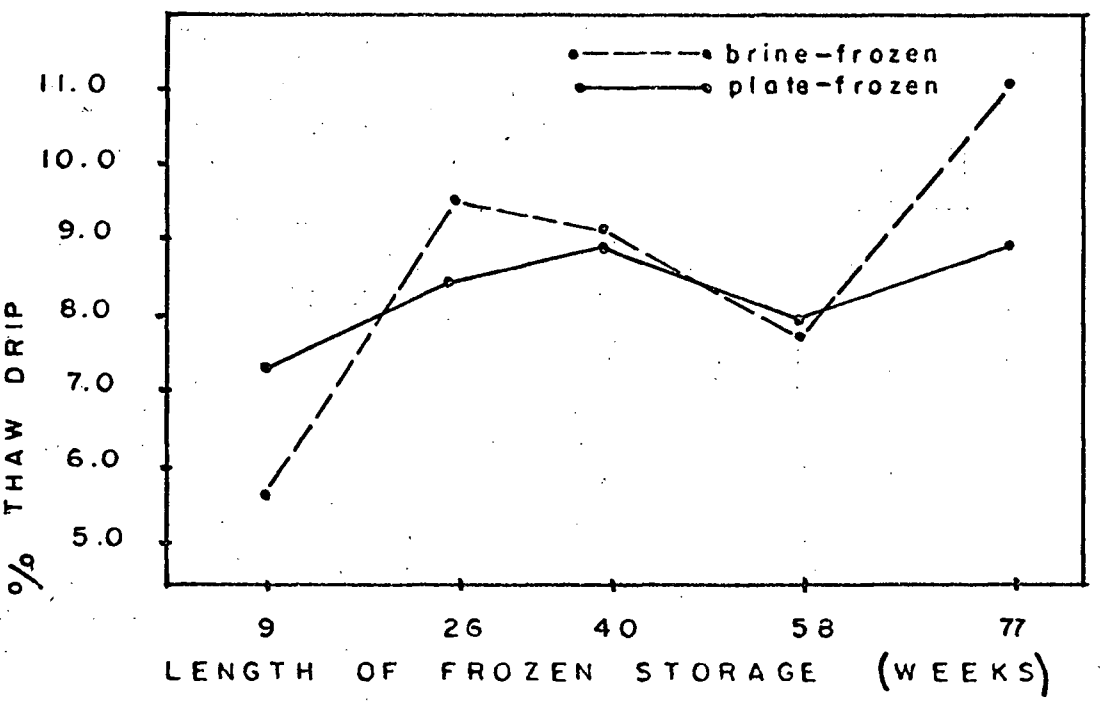


Figure 7 Average thaw drip of chinook salmon stored at -30°C.

significant ($P \leq 0.01$) storage time effect. The thaw drip at 9 weeks was significantly lower ($P \leq 0.05$) at all other frozen storage times (Table IV, Fig. 7). Mean thaw drip of brine-frozen chinook salmon was less than that of the plate-frozen samples at 9 weeks but approximately equal or greater than the plate-frozen samples at other storage times (Fig. 7).

iii) Coho Salmon

Plate-frozen coho salmon had a significantly higher ($P \leq 0.05$) thaw drip than brine-frozen (Table III, Fig. 8). There was also a significant difference ($P \leq 0.05$) in thaw drip among frozen storage times (Table III). The thaw drip at 27 weeks was significantly higher ($P = 0.05$) than at either 10 or 78 weeks (Table IV). This pattern was particularly pronounced in plate-frozen samples (Fig. 8).

c) Color

1) Hunter Rd Values

i) Chinook and Coho Salmon

During frozen storage the Rd values of chinook salmon appeared fairly irregular and variable (Fig. 9). The Rd readings of coho salmon were relatively constant during frozen storage (Fig. 11). Analysis of variance on Hunter Rd readings of both chinook and coho salmon revealed no significant effects due to method or storage time (Table V and VI).

TABLE IV Duncan's new multiple range test on the significant time means from the analyses of variance on thaw drip.(1)

Chinook Salmon(2)

77 weeks(3)	40 weeks	26 weeks	58 weeks	9 weeks
10.0%(4)	9.1%	9.0%	7.8%	6/5%

Coho Salmon

27 weeks	78 weeks	10 weeks
10.7%	8.4%	6.9%

(1) The test was conducted on the arcsine transformed data.

(2) Means not underlined by the same line are significantly different ($P \leq 0.05$) from each other.

(3) Weeks of frozen storage

(4) Original (not transformed) values expressed as % of the weight of the frozen sample.

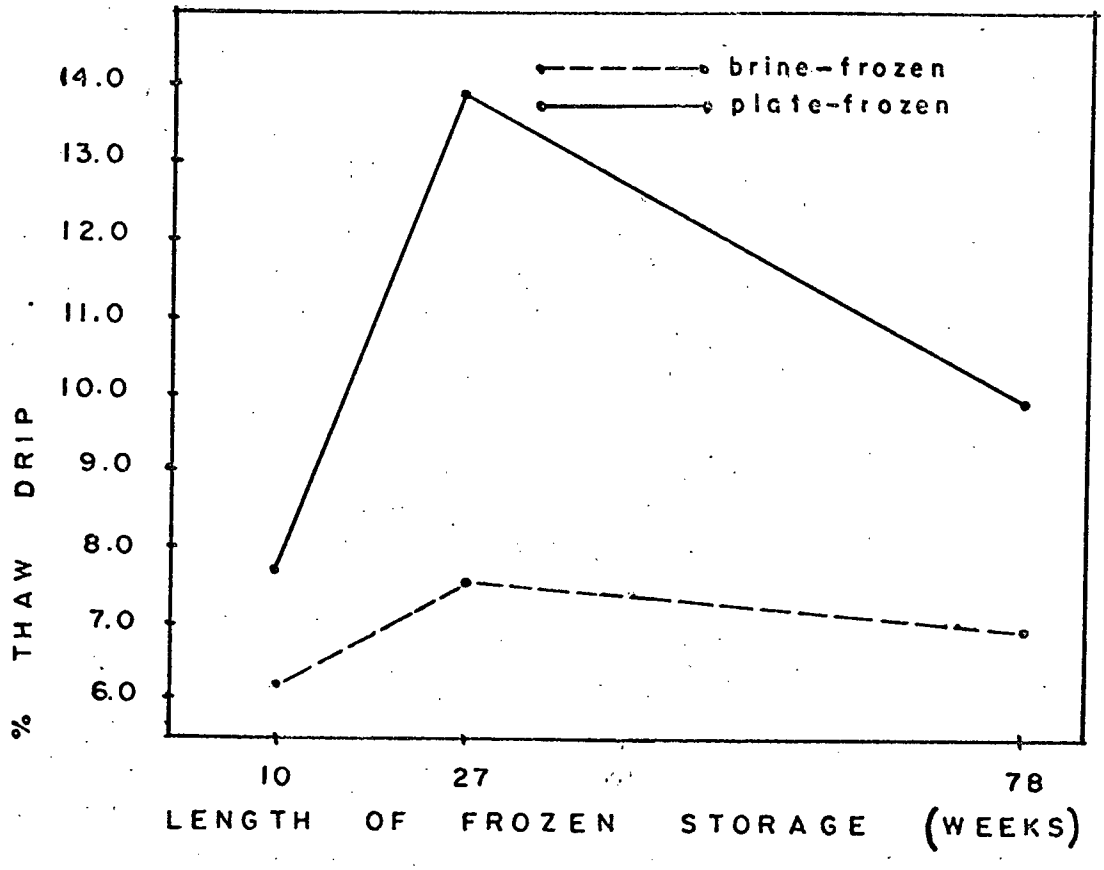


Figure 8 Average thaw drip of coho salmon stored at -30°C.

2) Hunter 'a' Values

i) Chinook Salmon

The mean Hunter 'a' values of Chinook salmon were not significantly affected by method of freezing. However, there was a highly significant difference ($P \leq 0.01$) among frozen storage times. Duncan's new multiple range test showed that the 'a' value at 9 weeks was significantly lower ($P \leq 0.05$) than at any other storage time (Table V). The change in 'a' values during frozen storage was somewhat similar for both the plate-frozen and the brine-frozen chinook salmon (Fig. 10). However, at 9 weeks of frozen storage the brine-frozen samples had slightly higher 'a' reading while at all other frozen storage times the plate-frozen samples had the higher reading (Fig. 10).

ii) Coho Salmon

The mean Hunter 'a' value of plate-frozen coho salmon was not significantly different from that of brine-frozen. There was a significant difference ($P \leq 0.05$) among the storage times means (Table VI). The 'a' reading at 78 weeks was significantly higher than at all other times (Table VII). The 'a' value at 10 weeks was not significantly different from the 'a' value at 27 weeks of frozen storage (Table VII). The general change in 'a' values during frozen storage of coho salmon was similar for both the brine-frozen and the plate-frozen samples (Fig. 12).

TABLE V Analysis of variance of color readings for chinook salmon

		Rd		a		b		a/b	
Source	d.f.	M.S.	Prob[1]	M.S.	Prob	M.S.	Prob	M.S.	Prob
Pairs	2	13.963	0.0005	110.66	0.0000	1.9956	0.3063	0.1422	0.0000
Methods[2]	1	2.945	0.7596	36.81	0.1109	1.9051	0.5811	0.0375	0.4014
Error b	2	26.604		4.63		4.4795		0.0334	
Time	4	1.001	0.4739	28.33	0.0019**	1.4369	0.4783	0.0708	0.0000
M x T	4	1.023	0.4736	5.65	0.2798	1.4199	0.4841	0.0132	0.0128*
Error b	16	1.080		4.05		1.5637		0.0029	

1 Probability of Type 1 error

2 Error a was used to test methods. Error b was used to test all terms except Methods

* Significant at the 5% level ($P \leq 0.05$)
** Significant at the 1% level ($P \leq 0.01$)
*** Significant at the 0.1% level ($P \leq 0.001$)

TABLE VI Analysis of variance of color readings for coho salmon

		Rd		a		b		a/b	
Source	d.f.	M.S.	Prob[1]	M.S.	Prob	M.S.	Prob	M.S.	Prob
Pairs	2	0.1518	0.9185	2.036	0.4963	0.8065	0.7687	0.0078	0.1649
Methods[2]	1	0.3785	0.4181	0.009	0.9214	1.8241	0.2848	0.0029	0.4054
Error b	2	0.3677		4.878		0.8655		0.0027	
Time	2	0.0682	0.9549	12.848	0.0410*	1.2377	0.6740	0.0139	0.0048**
M x T	2	0.0592	0.9591	1.255	0.6413	0.3297	0.8898	0.0045	0.3207
Error b	8	1.9753		2.634		2.9438		0.0034	

[1] Probability of Type 1 error.
[2] Error a was used to test methods
Error b was used to test all terms except Methods.

* Significant at the 5% level ($P \leq 0.05$)
** Significant at the 1% level ($P \leq 0.01$)
*** Significant at the 0.1% level ($P \leq 0.001$)

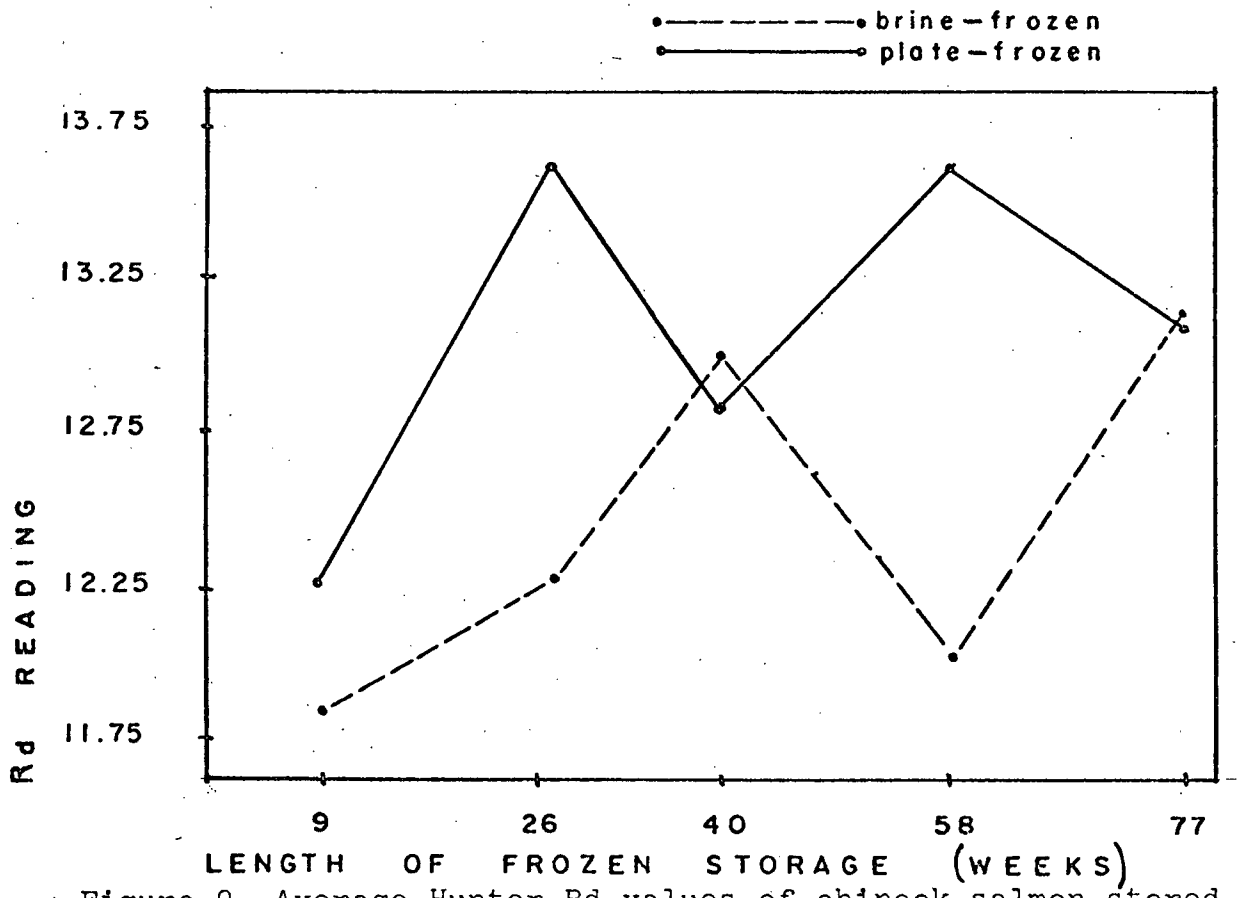


Figure 9 Average Hunter Rd values of chinook salmon stored at -30°C.

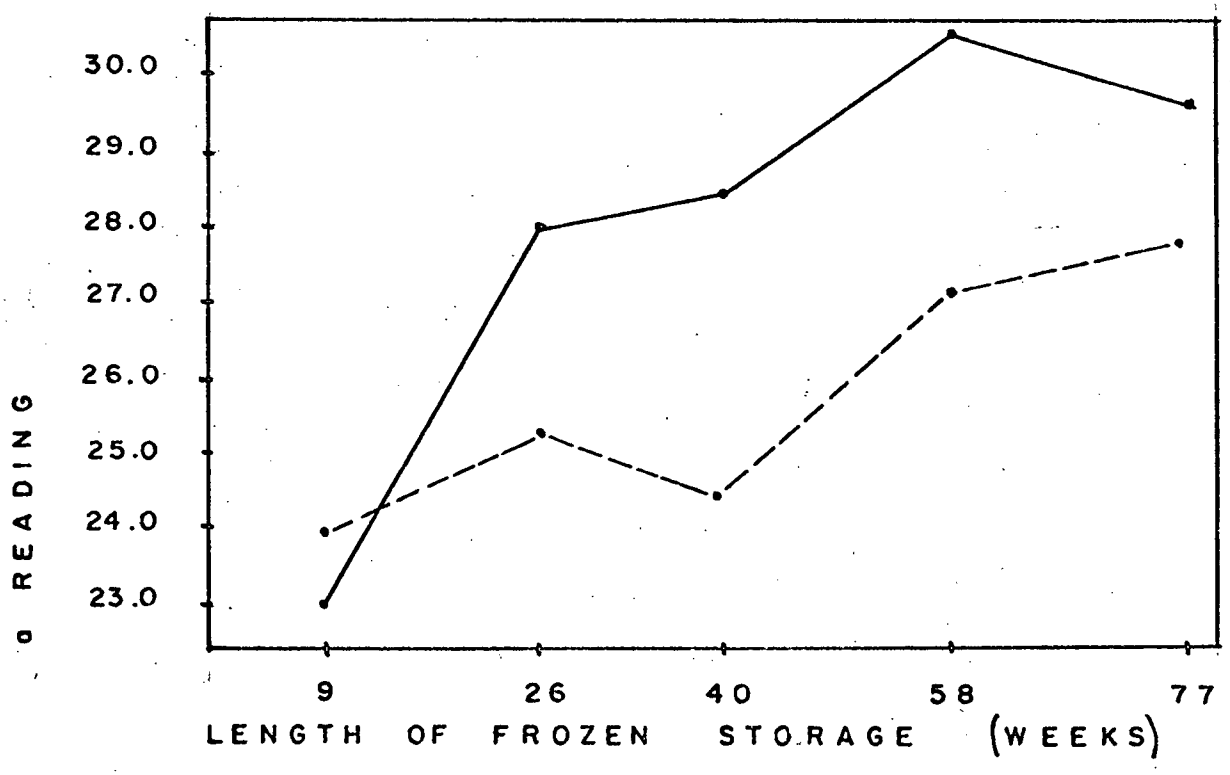


Figure 10 Average Hunter 'a' values of chinook salmon stored at -30°C.

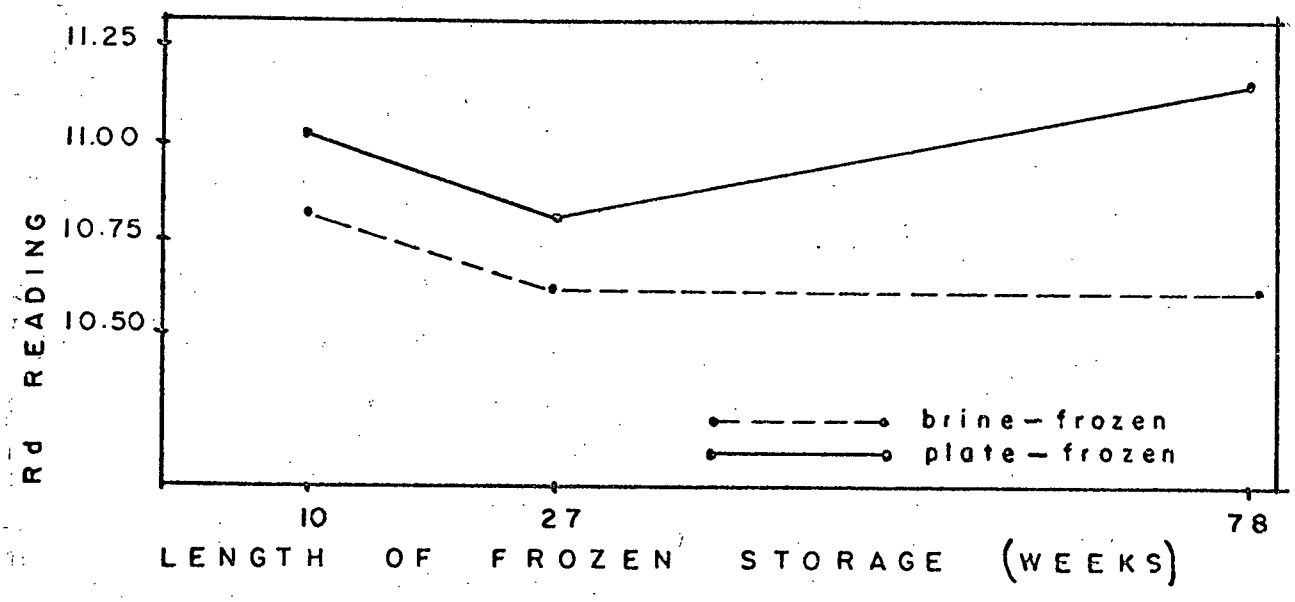


Figure 11 Average Hunter Rd values of coho salmon stored at -30°C.

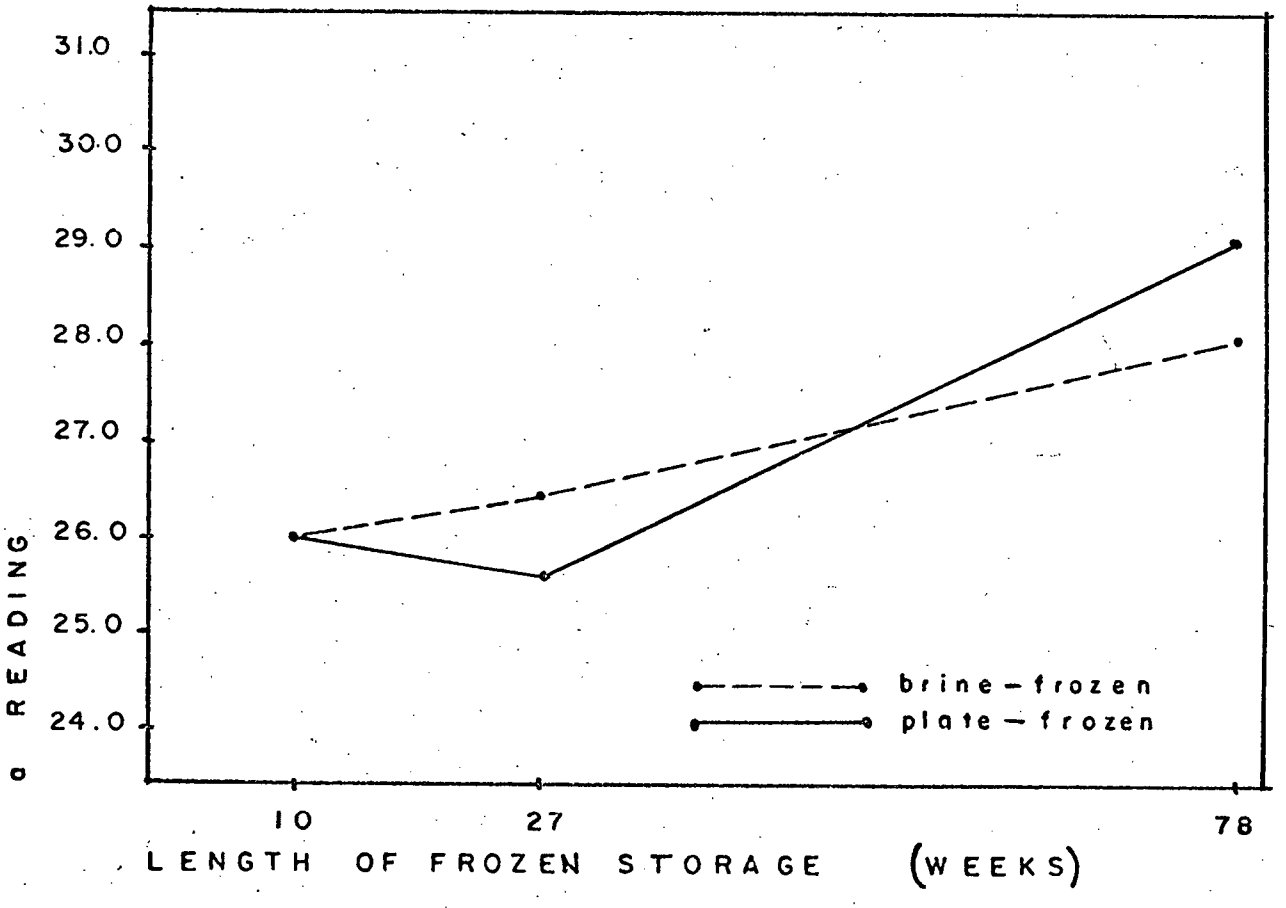


Figure 12 Average Hunter 'a' values of coho salmon stored at -30°C.

3) Hunter 'b' Values

i) Chinook and Coho Salmon

There was no significant difference in Hunter 'b' values between plate-frozen and brine-frozen samples of either chinook (Table V) or coho salmon (Table VI). All other main effects and interactions were also non-significant for both species (Table V and VI). Except for the 'b' values at 58 weeks of frozen storage the values of the brine-frozen chinook salmon samples remained fairly constant. With the plate-frozen samples, except for a sharp decrease between 26 weeks and 40 weeks the values were also relatively constant (Fig. 13). From 9 weeks until 78 weeks of frozen storage the 'b' values of plate-frozen coho salmon decreased about 0.90 units while the brine-frozen samples decreased about 0.85 units (Fig. 14).

4) Hunter a/b Ratios

i) Chinook Salmon

The method x time interaction of the a/b ratios for chinook salmon was significant ($P = 0.05$) (Table V). Except for the 9th week of frozen storage the plate-frozen had higher a/b ratios than the brine-frozen samples (Fig. 16). Also except for the period between 26 and 40 weeks of frozen storage the changes in the a/b ratio of chinook salmon were similar for both the plate- and brine-frozen samples (Fig. 16).

ii) Coho Salmon

There was no significant difference in the mean a/b ratio between brine-frozen and plate-frozen samples

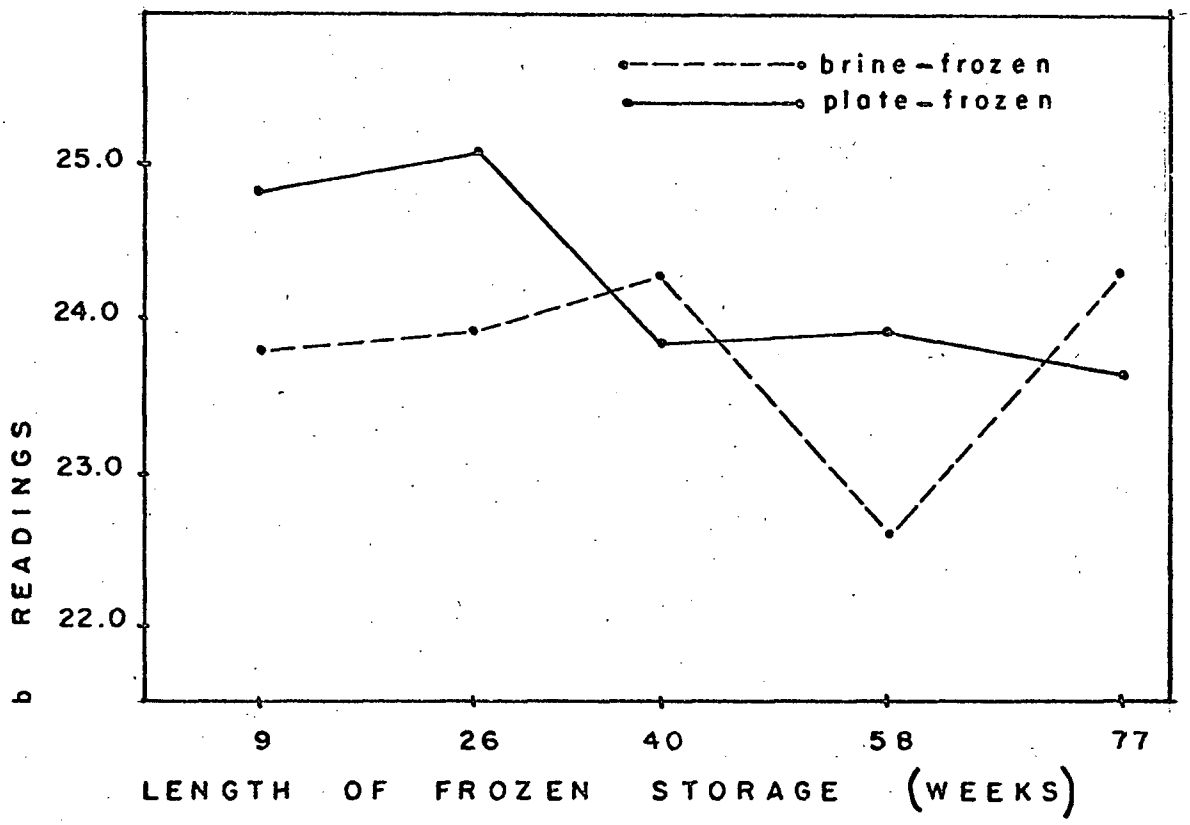


Figure 13 Average Hunter b values of chinook salmon stored at -30°C.

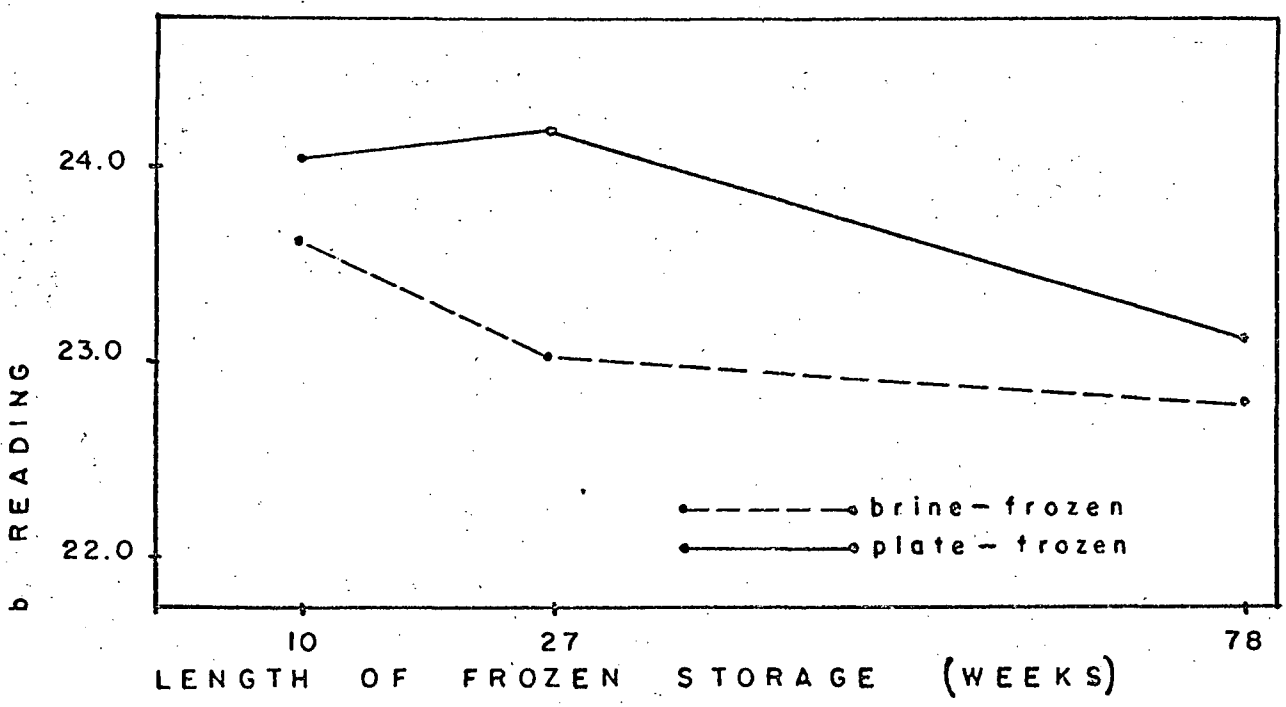


Figure 14 Average Hunter b values of coho salmon stored at -30°C.

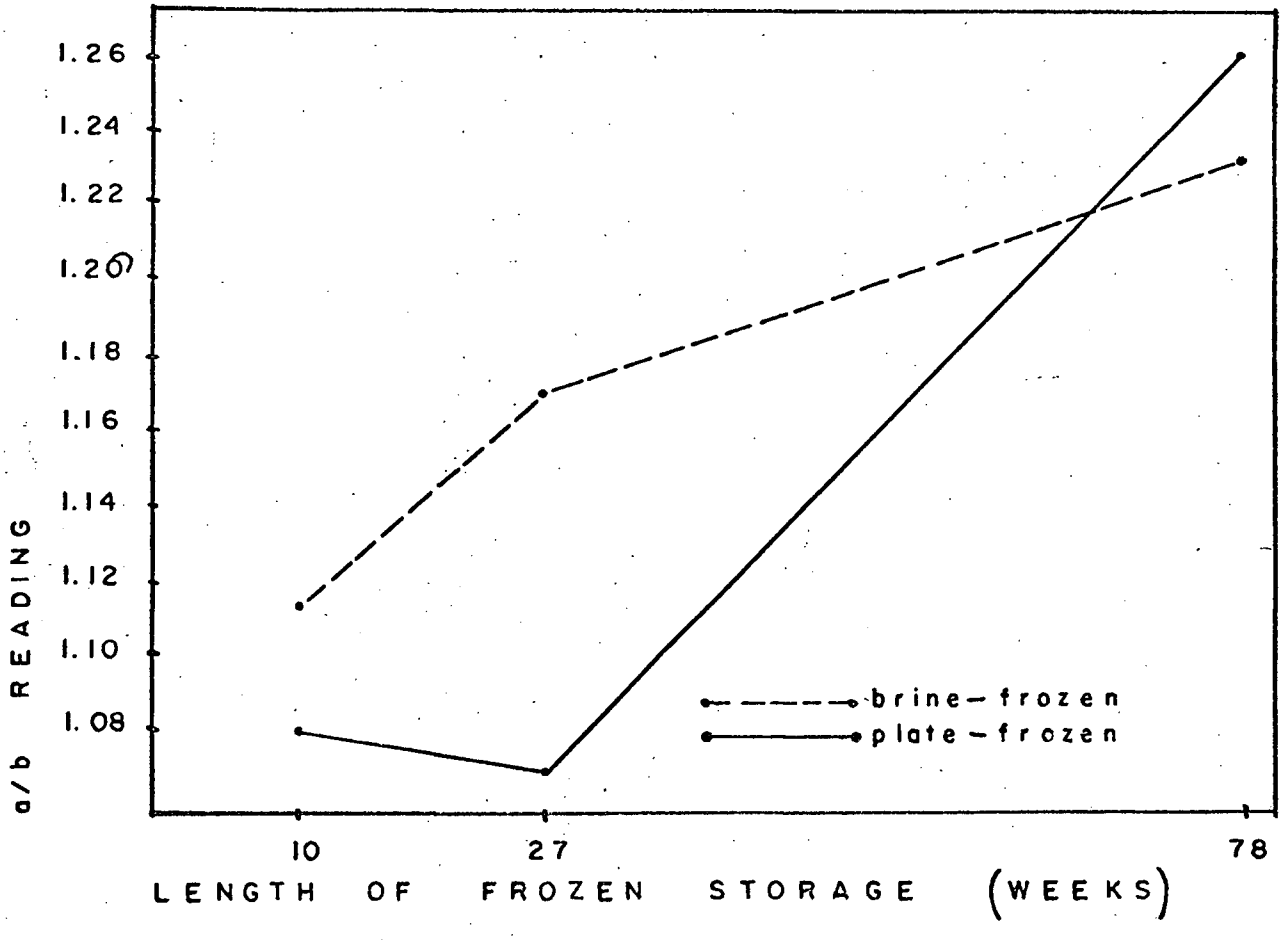


Figure 15 Average Hunter a/b ratios of coho salmon stored at -30°C.

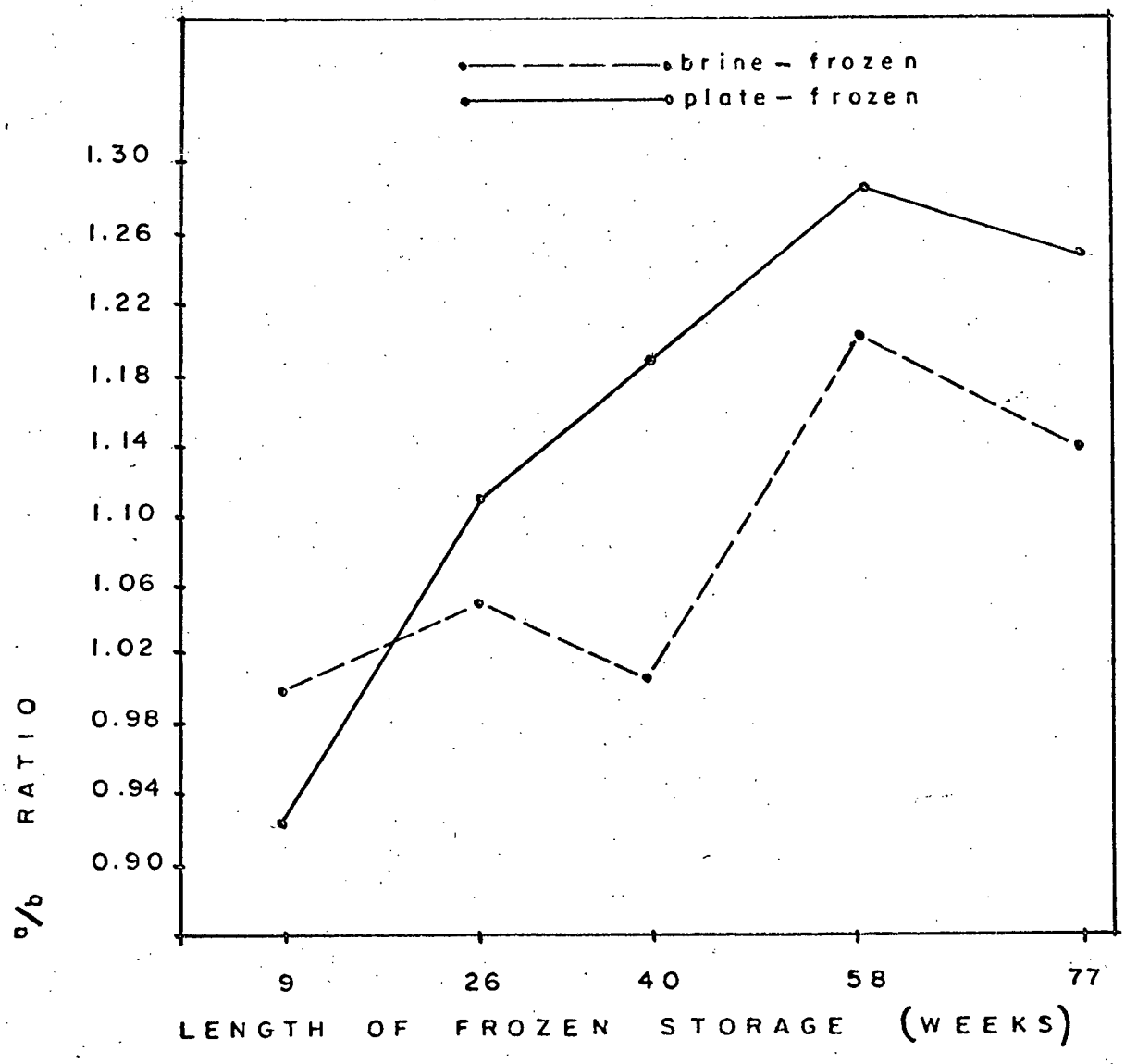


Figure 16 Average Hunter a/b ratios of chinook salmon stored at -30°C.

(Table VI). However, there was a highly significant difference ($P \leq 0.01$) among frozen storage times (Table VI). The a/b ratio at 78 weeks was significantly higher ($P \leq 0.05$) than at 10 and 27 weeks of frozen storage (Table VII). From 10 until 78 weeks of frozen storage the a/b ratio of the plate-frozen samples increased 0.173 units while the brine-frozen samples increased 0.127 units (Fig. 15).

d) Flavor Differences

The results of the triangle tests on inside and outside muscle samples of Pacific halibut, chinook salmon and coho salmon are shown in Figure 17. Levels of significant difference were obtained from tables prepared by Larmond (1967).

i) Pacific halibut

There were no significant differences in flavor between freezing methods for either inside or outside muscle samples at 14 weeks of frozen storage. At 31 weeks, however, there was a very highly significant difference ($P \leq 0.001$) between the outside muscle of the plate-frozen halibut and the outside muscle of the brine-frozen fish (Fig. 17). These differences between freezing methods steadily declined after 31 weeks of frozen storage until they were just significant at the 5% level after 81 weeks (Fig. 17).

The taste panel results for halibut inside muscle appeared a little more erratic in comparison with those for

TABLE VII Duncan's new multiple range test on the significant time effects of the analyses of variance on the Rd, a, b, and a/b values of Chinook Salmon and Coho Salmon. (1)

Chinook Salmon: a values

58 weeks(2)	77 weeks	26 weeks	40 weeks	9 weeks
28.89(3)	28.66	26.63	26.45	23.50

Coho Salmon: a values

78 weeks(2)	10 weeks	27 weeks
28.60(3)	26.11	26.02

Coho Salmon: a/b ratio

78 weeks(2)	10 weeks	27 weeks
1.25(4)	1.11	1.10

(1) Means not underlined by the same line are significantly ($P \leq 0.05$) different from each other.

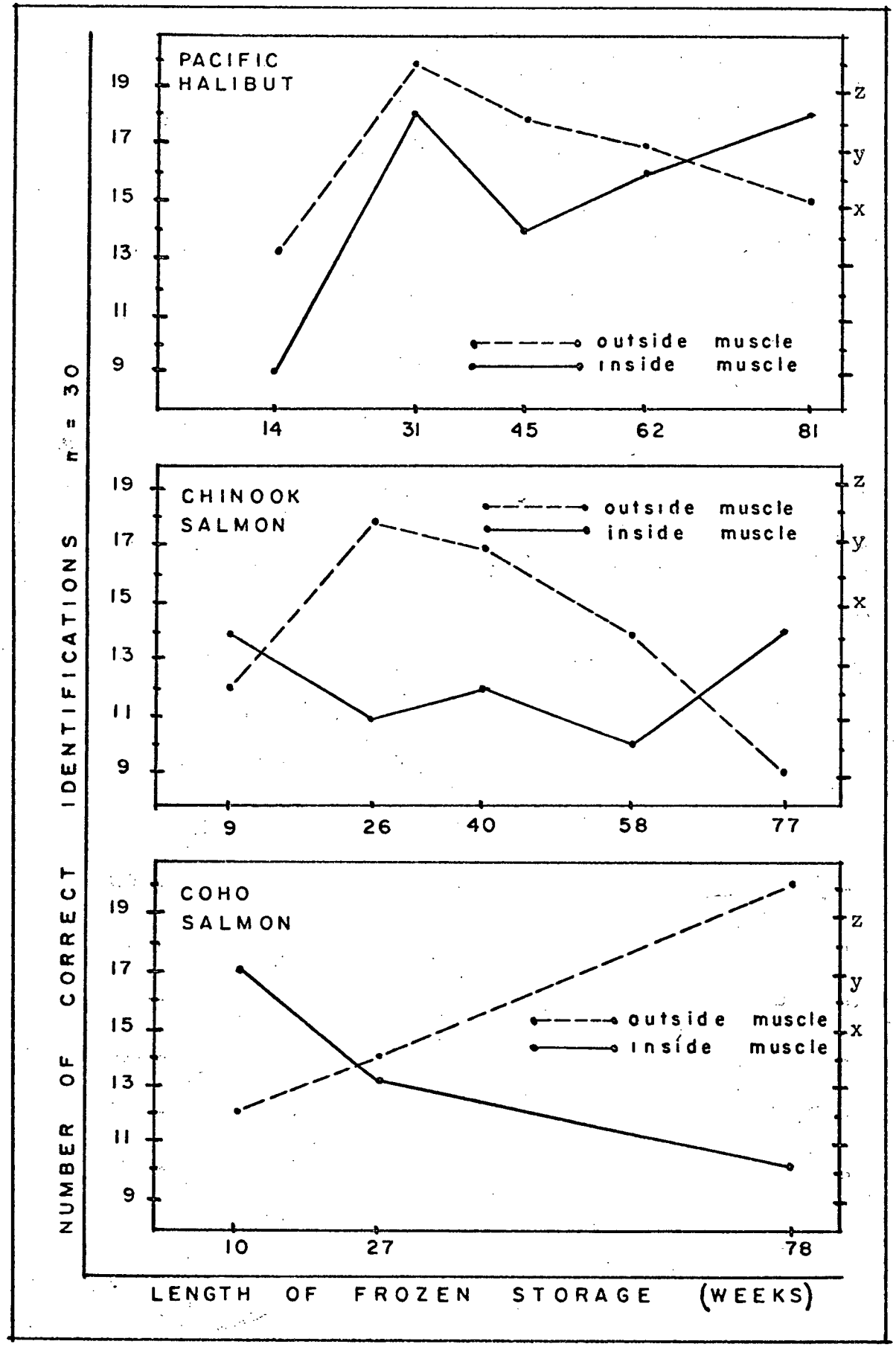
(2) Weeks of frozen storage at -30°C.

(3) Hunter a values.

(4) a/b ratio.

Figure 17 Taste panel (triangle test) results of Pacific halibut, chinook salmon, and coho salmon.

x = significant at the 5% level
y = significant at the 1% level
z = significant at the 0.1% level


PACIFIC HALIBUT
CHINOOK SALMON
COHO SALMON
outside muscle
inside muscle
NUMBER OF CORRECT IDENTIFICATIONS n = 30
LENGTH OF FROZEN STORAGE (WEEKS)
19
17
15
13
11
9
z
y
x
14
31
45
62
81
9
26
40
58
77
10
27
78

outside muscle. There were highly significant differences ($P \leq 0.01$) between freezing methods at 31 and 81 weeks whereas the differences were non-significant at 14 and 45 weeks and significant ($P \leq 0.05$) at 62 weeks of frozen storage (Fig. 17).

ii) Chinook Salmon

The taste panel results for chinook salmon were somewhat similar to those for halibut. There were no significant differences between freezing methods at the first analysis (9th week of frozen storage) for either inside or outside muscle samples and with outside muscle there was a highly significant difference ($P \leq 0.01$) between freezing methods at the second analysis (27th week of frozen storage) (Fig. 17). This difference steadily declined until it became non-significant at 58 and 77 weeks (Fig. 17). The taste panel results for inside muscle samples were different from those for halibut as with chinook salmon the differences between freezing methods were non-significant at each sampling time (Fig. 17).

iii) Coho Salmon

Taste panel results for coho salmon were very different from those for halibut and chinook salmon. At the first analysis (10th week of frozen storage) there was a non-significant difference between freezing methods for the outside muscle and a highly significant difference ($P \leq 0.01$) for the inside muscle (Fig. 17). The number of correct identifications steadily increased with time and culminated with a very highly

TABLE VIII Acceptability Preferences for Pacific Halibut, Chinook Salmon and Coho Salmon

Species	Location	Length of Frozen Storage	Number of Correct Identification[1]	Preferences Plate-Frozen %	Brine-Frozen %	No Pref. %
Pacific halibut	Outside muscle	14 weeks	13	69	31	
		31 "	20	60	40	
		45 "	18	39	50	11
		62 "	17	29	59	12
		81 "	15	47	53	
	Inside muscle	14 weeks	9	22	78	
		31 "	18	50	50	
		45 "	14	43	50	7
		62 "	16	50	38	13
		81 "	18	44	28	28
Chinook Salmon	Outside muscle	9 weeks	12	67	33	
		26 "	18	56	44	
		40 "	17	59	35	6
		58 "	14	50	50	
		77 "	9	33	56	11
	Inside muscle	9 weeks	14	57	43	
		26 "	11	55	45	
		40 "	12	50	50	
		58 "	10	-	70	30
		77 "	14	50	21	29
Coho Salmon	Outside muscle	10 weeks	12	83	17	
		27 "	14	57	43	
		78 "	20	40	55	5
	Inside muscle	10 weeks	17	53	47	
		27 "	13	54	46	
		78 "	10	40	30	30

1 Number of correct identifications out of 30 observations.

2 Percentage of correct identifications.

significant difference ($P \leq 0.001$) between freezing methods at 78 weeks (Fig. 17). With coho salmon inside muscle there was a significant difference between freezing methods only at the 10th week. Differences at all other times were non-significant (Fig. 17).

Examination of the data for outside muscle revealed that for all species plate-frozen samples were preferred to brine-frozen at the first sampling time. The preference declined as storage time increased until at the last sampling period brine-frozen samples were slightly preferred over the corresponding plate-frozen ones. For both the inside and outside muscle samples the percentage of "no preferences" tended to increase with increasing time of frozen storage (Table VIII).

e) Mineral Concentration

i) Pacific Halibut

Insofar as sodium and potassium concentrations in the flesh were concerned, the differences between the two methods of freezing were not statistically significant and no conclusions could be made about differences in chloride concentration as there was a very highly significant ($P \leq 0.01$) method times location interaction (Table IX). However, graphical analysis of the means indicated that the sodium and chloride concentrations of the plate-frozen inside muscle, plate-frozen outside muscle and the brine-frozen inside muscle were quite similar (Fig. 18). However, the brine-frozen outside

TABLE IX Analysis of variance of mineral concentration of Pacific Halibut muscle

		Sodium		Potassium		Chloride	
Source	d.f.	M.S.	Prob(1)	M.S.	Prob	M.S.	Prob
Pairs	2	0.4532	0.4373	0.4760	0.0321	0.007	0.8749
Methods(2)	1	1.9764	0.1795	0.0675	0.7137	10.660	0.0131
Error a	2	0.4710		0.3954		0.047	
LOCATION	1	2.3497	0.0817	2.2188	0.0037**	12.669	0.0004
M x LOC.	1	1.6502	0.1241	0.1633	0.1445	9.031	0.0006***
Error b	4	0.4404		0.0501		0.050	
Total	11						

1 Probability of Type 1 error
2 Error a was used to test methods.
Error b was used to test all terms except methods

* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level

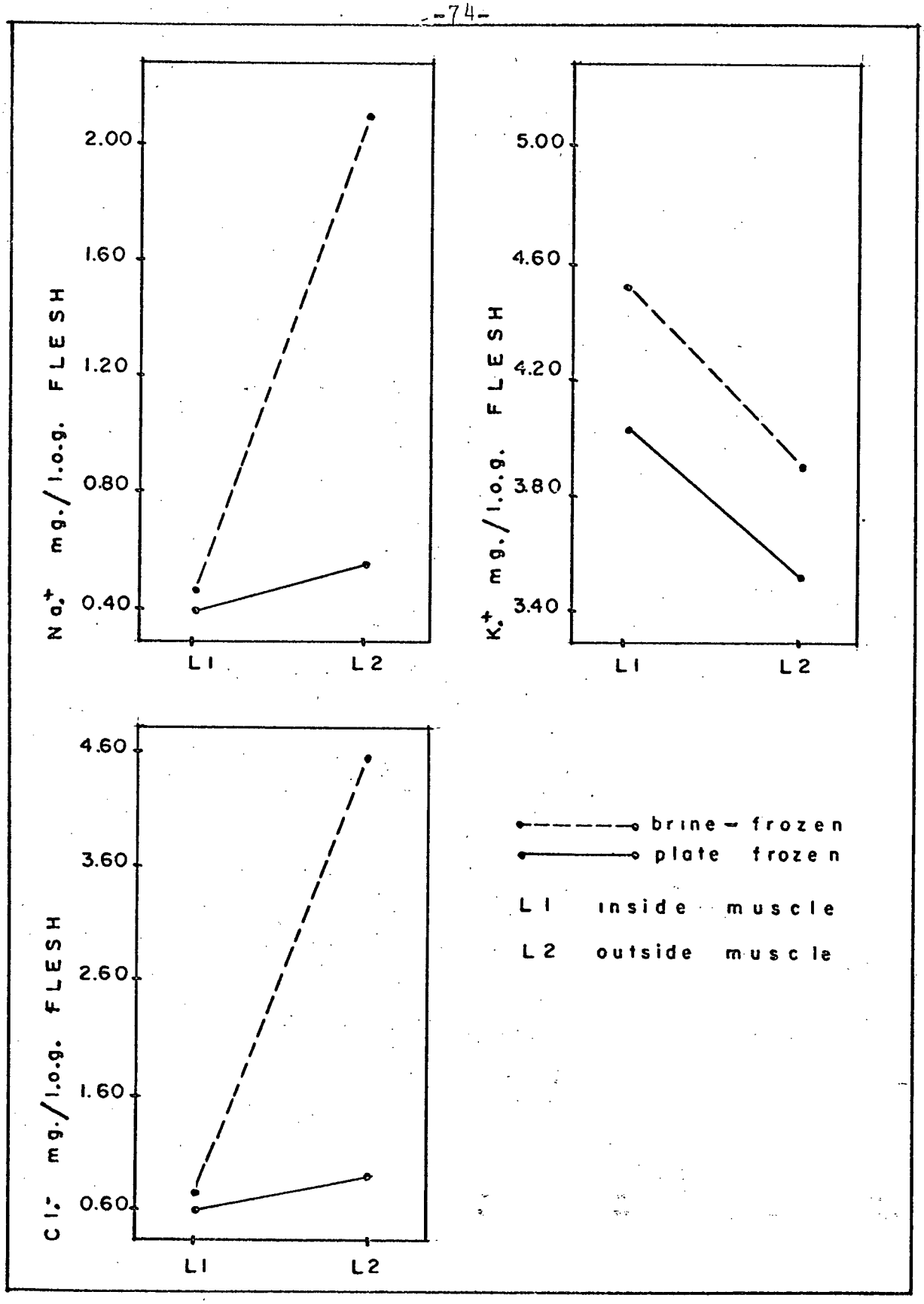


Figure 18 Average sodium, potassium, and chloride concentration in the flesh of Pacific halibut.

muscle had 3.82 times as much sodium and 4.09 times as much chloride as the plate-frozen outside muscle (Fig. 18). The potassium concentration of the brine-frozen samples was about 1.11 times greater than the plate-frozen samples for the outside muscle and about 1.02 times greater for the inside muscle (Fig. 18). The sodium and chloride concentrations did not differ significantly among pairs (Table IX). Sodium concentration did not differ significantly between the inside and outside muscle samples whereas potassium concentration was significantly ($P \leq 0.01$) greater in the inside muscle (Table IX, Fig. 18). No conclusions about differences in chloride concentration between the inside and outside muscle samples could be made as there was a very highly significant ($P \leq 0.001$) method times location interaction (Table IX). Chloride concentration in the outside muscle was 6.03 times greater than that of the inside muscle with the brine-frozen samples and only 1.53 times greater with the plate-frozen samples (Fig. 18).

ii) Chinook Salmon

No simple assessment of the quantitative differences in sodium concentration between methods and between locations could be made as there was a highly significant ($P \leq 0.01$) method x location interaction (Table X). Sodium concentration of inside muscle was essentially the same for the two methods of freezing but the concentration in outside muscle was about 3 times higher in brine-frozen compared to plate-frozen samples (Fig. 19).

TABLE X Analysis of variance of mineral concentration of Chinook Salmon muscle

		Sodium		Potassium		Chloride	
Source	d.f.	M.S.	Prob[2]	M.S.	Prob	M.S.	Prob
Pairs	2	0.0281	0.2453	0.0652	0.2173	0.0893	0.5904
Methods[2]	1	0.4485	0.0371	1.1844	0.2372	1.3267	0.0824
Error a	2	0.1311		0.4218		0.1139	
Location	1	1.0208	0.0018	2.0750	0.0019**	3.7969	0.0083**
Method x LOC.	1	0.5043	0.0049**	0.0014	0.8158	0.9241	0.0661
Error b	4	0.0137		0.0284		0.1469	
Total	11						

[1] Probability of Type 1 error
[2] Error a was used to test methods
Error b was used to test all terms except methods

* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level

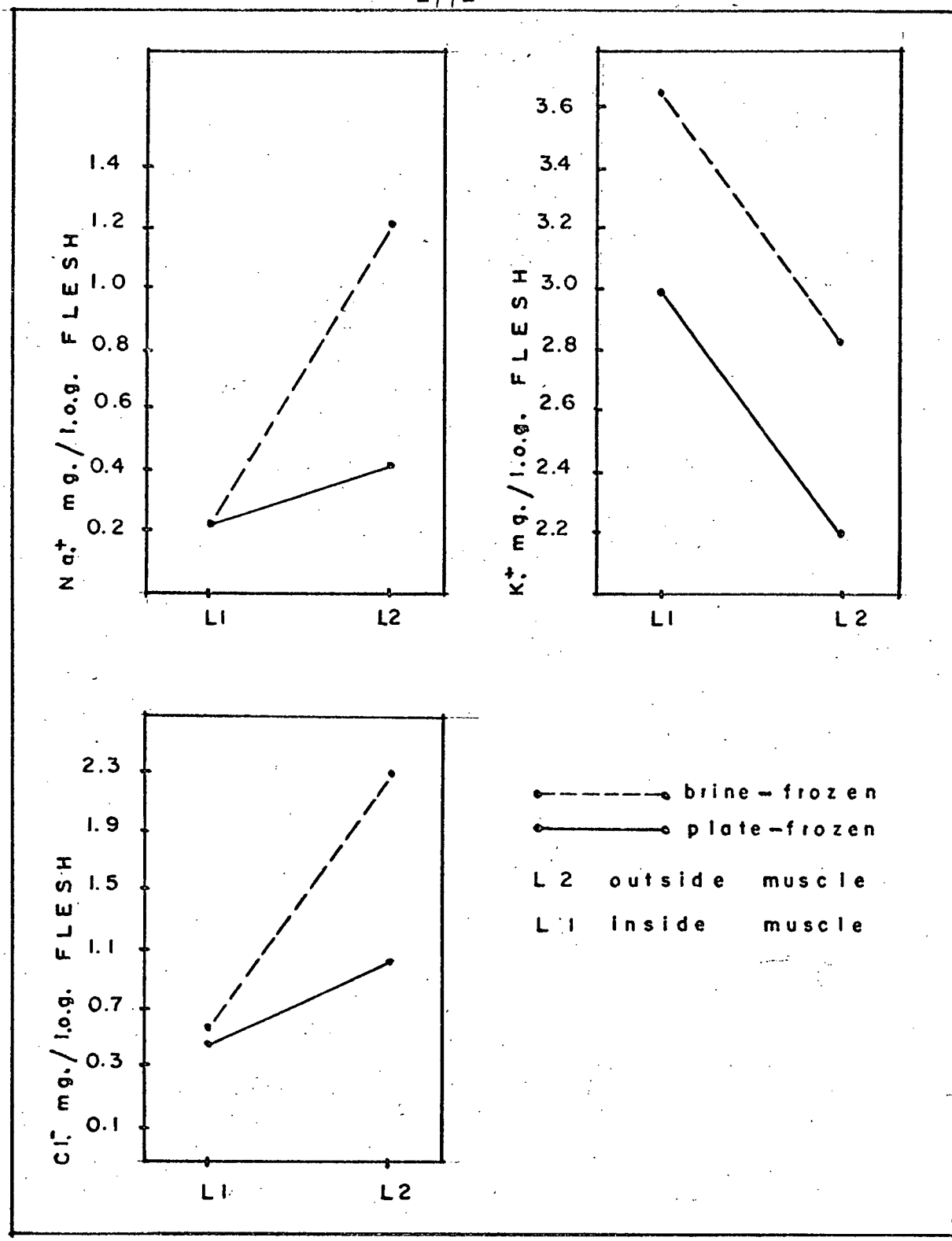


Figure 19 Average sodium, potassium, and chloride concentration in the flesh of chinook salmon.

Potassium and chloride concentrations of chinook salmon did not differ significantly between the plate-frozen and brine-frozen samples (Table X). The interaction between methods and locations for chloride approached significance ($P \leq 0.07$) (Table X). The difference in chloride concentration between brine-and plate-frozen samples was much greater for outside than for inside muscle (Fig. 19).

The potassium concentration was significantly ($P \leq 0.01$) higher in the inside muscle than in the outside whereas chloride concentration was significantly ($P \leq 0.01$) lower in the inside muscle (Table X, Fig. 19).

iii) Coho Salmon

Assessment of differences in sodium, potassium, and chloride concentration between methods and between locations was complicated by the presence of significant ($P \leq 0.05$) method x location interactions (Table XI). Graphical analysis indicated that freezing method had little effect on concentration of sodium or chloride in inside muscle. However, concentration of these ions in outside muscle were 2.65 times greater in brine-frozen than in corresponding plate-frozen samples (Fig. 20). Potassium concentrations of samples of inside muscle was higher in plate-frozen than in brine-frozen fish but the reverse was true for outside muscle (Fig. 20).

TABLE XI Analysis of variance of mineral concentration in Coho Salmon muscle

		Sodium		Potassium		Chloride	
Source	d.f.	M.S.	Prob[1]	M.S.	Prob	M.S.	Prob
Pairs	2	0.0023	0.8084	0.0485	0.1301	0.4096	0.3895
Methods[2]	1	0.5002	0.0577	0.0002	0.9200	3.4027	0.1951
Error a	2	0.0270		0.1022		0.9115	
Locations	1	1.1594	0.0010	3.1930	0.0005	6.4387	0.0133
Method x LOC.	1	0.4524	0.0037**	0.4447	0.0059**	3.4669	0.0337*
Error b	4	0.0101		0.0137		0.3389	

[1] Probability of Type 1 error
[2] Error a was used to test methods
Error b was used to test all terms except methods

* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level

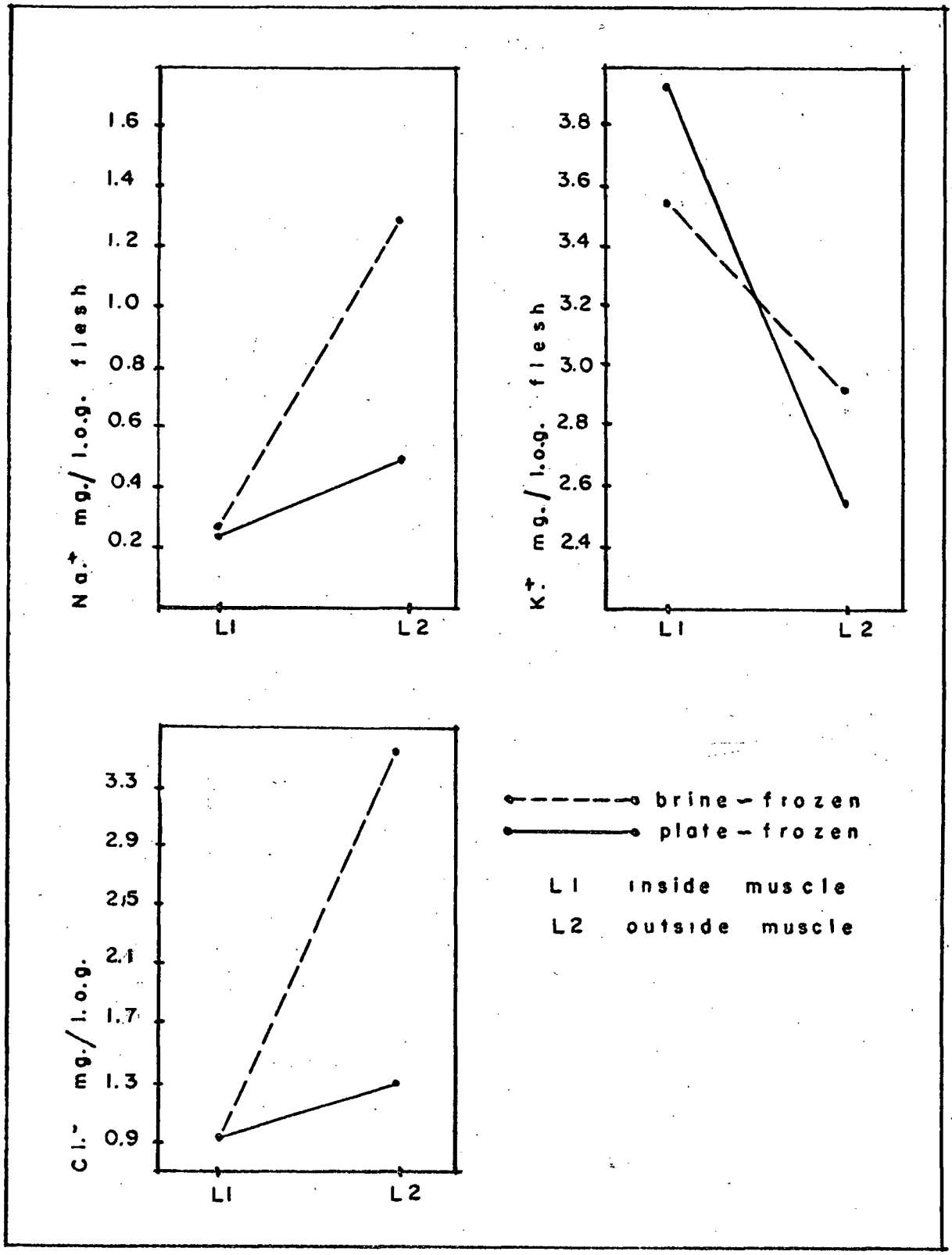


Figure 20 Average sodium, potassium, and chloride concentration in the flesh of coho salmon.

f) TBA Values

i) Pacific Halibut

The TBA values of the brine-frozen samples were significantly greater ($P \leq 0.05$) than those of the plate frozen samples (Table XII). A significant ($P \leq 0.05$) location x time interaction precluded testing of location or time effects (Table XII). TBA values of outside muscle were generally greater than those of inside muscle with the greatest differences at 45 and 62 weeks of storage (Fig. 21).

ii) Chinook Salmon

The significant ($P \leq 0.05$) method x location interaction effect revealed by analysis of variance of TBA values of chinook salmon (Table XII) is graphically represented in Figure 22. It is obvious that the extremely high TBA values for outside muscle of brine-frozen fish at 26 and 40 weeks of storage were largely responsible for the interaction. There were highly significant differences ($P \leq 0.01$) in the TBA values among the different times of frozen storage (Table XII). Mean TBA values at the first and last two sampling periods were similar (Table XIII). The highest mean TBA value occurred at 26 weeks of storage and was significantly higher ($P \leq 0.05$) than those at 9, 77, and 58 weeks but was not significantly different from that at 40 weeks of frozen storage (Table XIII).

TABLE XII Analyses of variance of TBA values of Pacific halibut, chinook salmon and coho salmon

	Pacific Halibut		Chinook Salmon		Coho Salmon	
Source	d.f.	M.S.[1]	d.f.	M.S.	d.f.	M.S.
Pairs [2]	2	11.234	2	0.455	2	0.278
Method	1	20.795*	1	87.554	1	14.161
M x P = Error a	2	0.333	2	11.498	2	19.664*
Location	1	80.887	1	249.360*	1	212.210
M X L	1	14.260	1	74.202*	1	12.469
Error b	4	2.822	4	4.360	4	6.415
Time	4	7.561	4	41.541**	2	64.677
M x T	4	4.510	4	20.500	2	3.826
L x T	4	12.004*	4	22.356	2	47.325**
M x L x T	4	3.969	4	14.553	2	3.535
Error c	32	4.418	32	9.032	16	5.388

1 All mean square values are multiplied by 1×10^{-3}
2 Methods were tested by error a
Location and M X L were tested using error b
All other terms were tested by error c

* Significant at the 5% level
** Significant at the 1% level

TABLE XIII Duncan's new multiple range test on significant time means from the analysis of variance of the TBA values of chinook salmon.

26 weeks[1]	40 weeks	9 weeks	77 weeks	58 weeks
0.2138[2]	0.1695	0.0929	0.0902	0.0820

1 Weeks of frozen storage at -30°C

2 The average TBA value in absorbance units. Each mean is the average of 3 observations. Means not underlined by the same line are significantly (P = 0.05) different from each other.

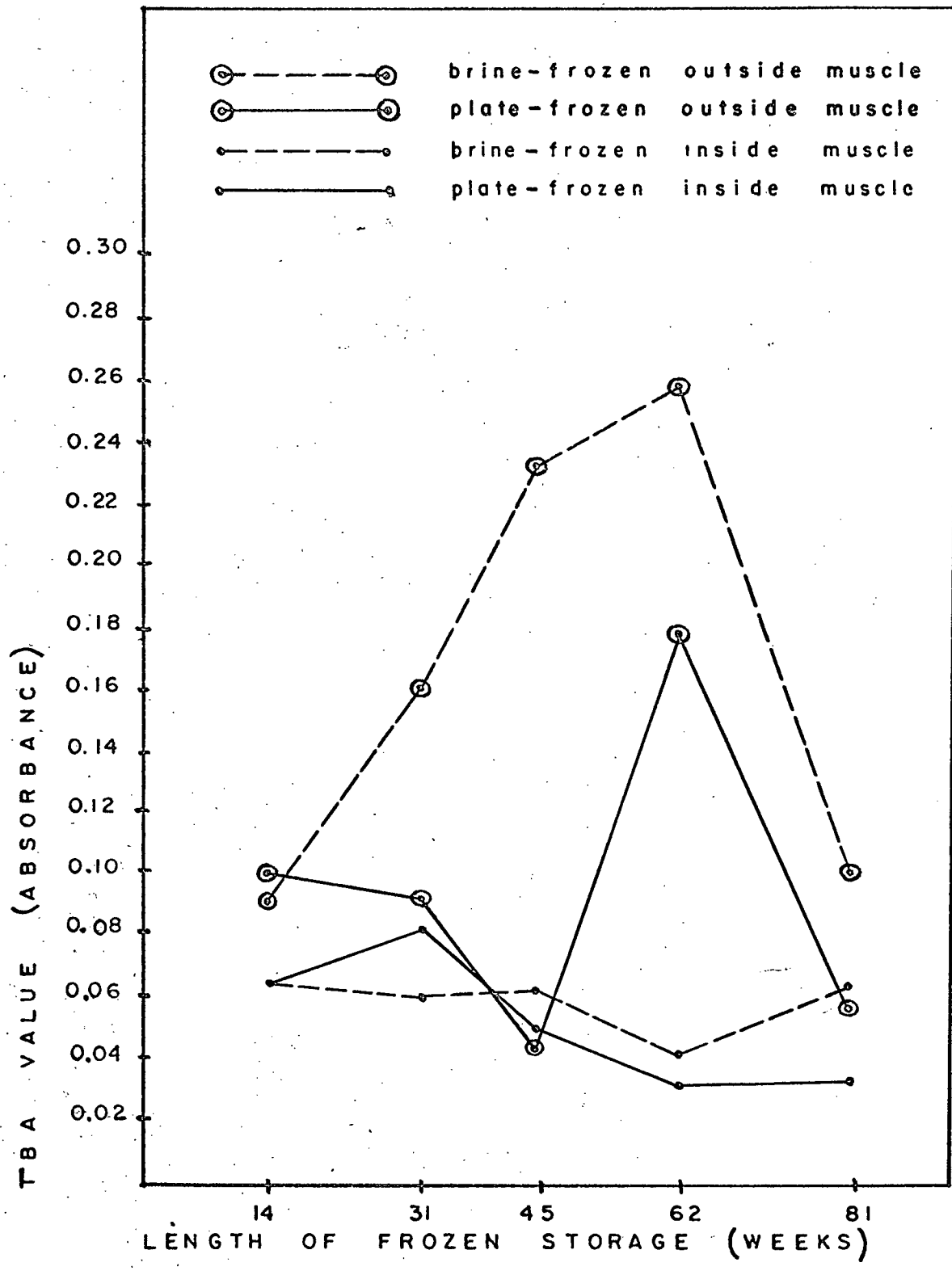


Figure 21 Average TBA values of Pacific halibut stored at -30°C.

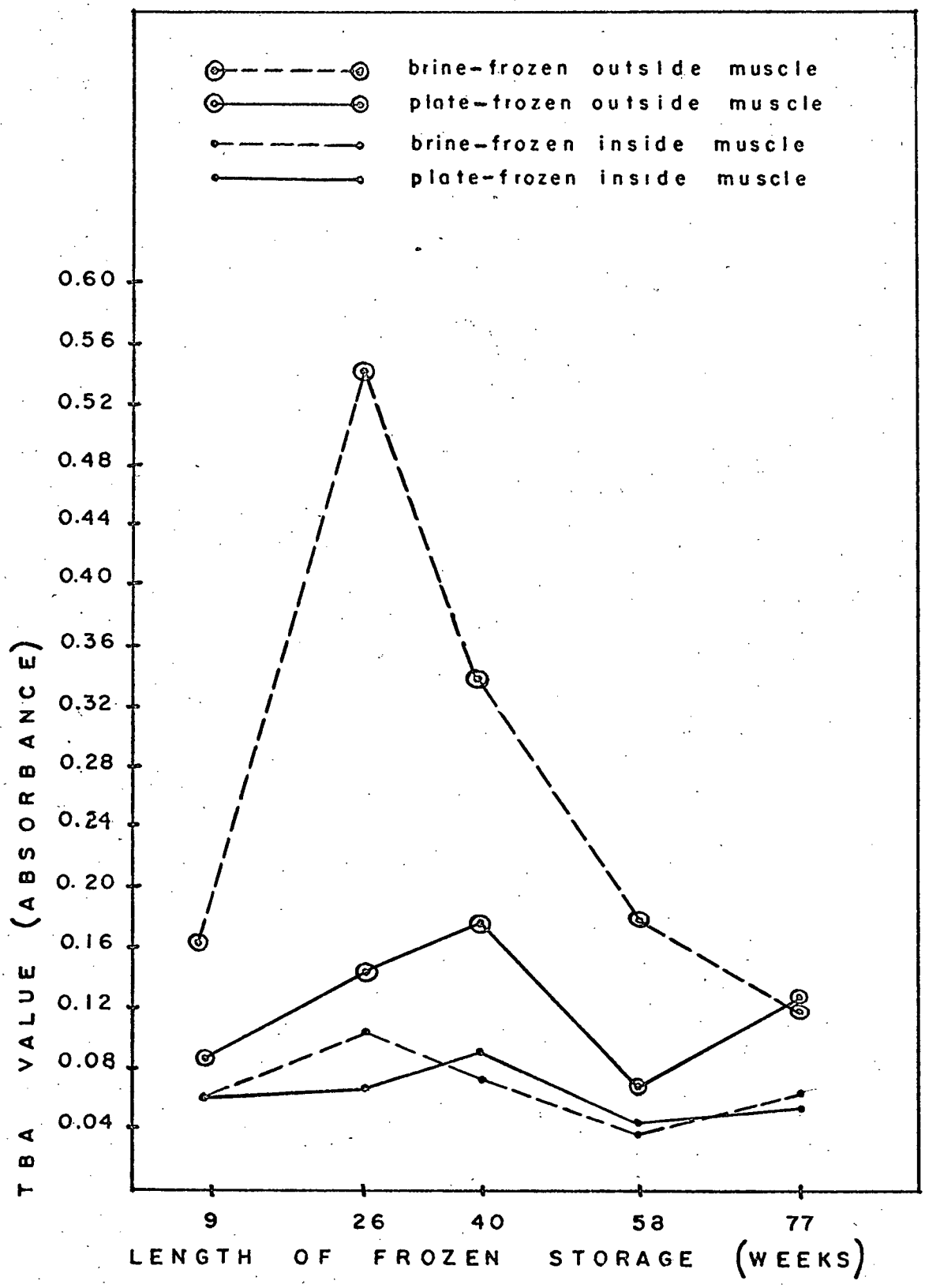


Figure 22 Average TBA values of chinook salmon stored at -30°C.

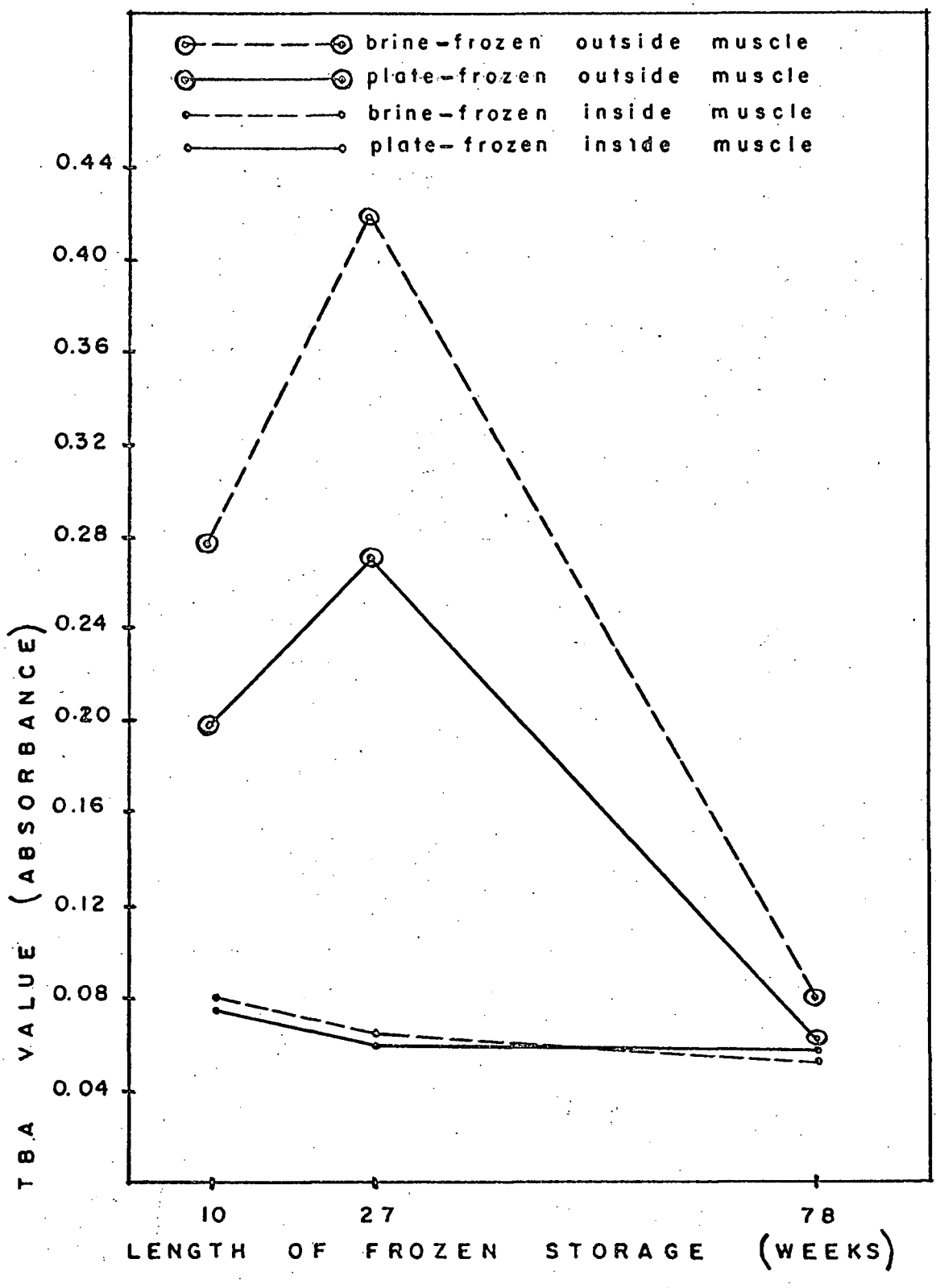


Figure 23 Average TBA values of coho salmon stored at -30°C.

iii) Coho Salmon

No simple evaluation of differences between inside and outside muscle or among different times of frozen storage was possible as there was a highly significant ($P \leq 0.01$) location x time interaction (Table XII). Graphical analysis of the means indicated that exceptionally high values for outside muscle at the first two sampling periods with a peak at 27 weeks were responsible for the interaction effect (Fig. 23) and that by 78 weeks, differences between inside and outside muscle had virtually disappeared.

g) Long Chain Free Fatty Acids

i) Pacific Halibut

1) Free Fatty Acids Expressed as Percent of Total Free Fatty Acids Analyzed

There was a significant difference ($P = 0.05$) between freezing methods only for fatty acid 15:0 (Table I, Appendix) which was greater in the brine-frozen than in the plate-frozen samples. A significant ($P = 0.05$) method x time interaction existed for fatty acid 17:0 (Table I, Appendix). Visual analysis indicated that the changes in fatty acid 17:0 over time were qualitatively different for the two methods and no obvious trends could be observed (Table II, Appendix).

The percentage of fatty acid 18:0 was significantly greater ($P \leq 0.05$) for inside muscle while the percentage of fatty acids 16:1, 17:0, 18:2, 18:4, 20:1 and

and 22:1 was significantly ($P \leq 0.05$) greater for the outside muscle (Tables I and II, Appendix). A significant ($P \leq 0.05$) time x location interaction existed for both 14:0 and 20:5 and significant ($P \leq 0.05$) method x location, and method x location x time interactions for 18:3 (Table 1, Appendix). Visual analysis of the means for these three fatty acids did not reveal any meaningful trends (Table II, Appendix).

There were significant differences ($P \leq 0.05$) between frozen storage times for fatty acids 16:0, and 16:1, 16:2, 18:2, 18:4, 20:1, 20:2, 22:1, 22:5, and 22:6 (Table 1, Appendix). No conclusions could be made about differences between frozen storage times for fatty acids 14:0, 17:0, 18:3, and 20:5 as there were significant ($P \leq 0.05$) location x time interactions for 14:0 and 20:5, a significant ($P \leq 0.05$) method x time interaction for 17:0 and a significant ($P \leq 0.05$) method x location x time interaction for 18:3 (Table I, Appendix).

The changes in the percentages of free fatty acids 16:0, 16:1, and 16:2 during frozen storage, although significant ($P \leq 0.05$), were random in nature and no meaningful trends could be observed (Table XIV). Free fatty acids 18:2, 18:4, 20:1, 20:2, 22:1, and 22:5 were lowest at 14 weeks of frozen storage and increased erratically thereafter (Table XIV). In contrast, the percentage of free fatty acid 22:6 declined steadily from 14 to 81 weeks of frozen storage.

2) Free Fatty Acids Expressed as µg Fatty Acid per Gram of Neutral Lipid.

TABLE XIV Method, location, and time means from the analyses of variance of free fatty acids (expressed as percent of total free fatty acids analyzed) of Pacific halibut[1].

	14:0	15:0	16:0	16:1	16:2	17:0
M1[2]	2.18 ns	0.35*	15.93 ns	5.33 ns	0.29 ns	1.21
M2	2.35	0.29	15.26	6.29	0.38	1.30
L1[3]	1.94[5]	0.32 ns	16.29 ns	5.13*	0.29 ns	1.15*
L2	2.60	0.33	14.88	6.49	0.37	1.36
T1[4]	1.98	0.35[6] a	15.99 ab	5.69 ab	0.27 ac	0.96
T2	2.13	0.35 a	17.62 a	4.91 b	0.39 ab	1.26
T3	2.33	0.37 a	13.69 b	6.40 ab	0.49 b	1.42
T4	1.68	0.30 a	14.67 b	4.95 b	0.21 c	1.13
T5	3.22	0.23 a	15.79 ab	7.08 a	0.31 ac	1.51

	18:0	18:1	18:2	18:3	18:4	20:1
M1	7.23 ns	17.58 ns	1.33 ns	0.41	0.33 ns	5.28 ns
M2	4.90	15.36	1.30	0.46	0.72	6.83
L1	6.84*	16.24 ns	1.19*	0.40	0.34**	5.74**
L2	5.32	16.63	1.43	0.48	0.71	6.36
T1	6.35 a	16.21 a	1.06 a	0.41	0.34 a	4.90 a
T2	6.21 a	15.85 a	1.45 b	0.31	0.39 ab	5.30 a
T3	6.12 a	16.35 a	1.30 ab	0.53	0.62 bc	6.55 bc
T4	6.62 a	16.99 a	1.44 b	0.52	0.53 abc	6.39 b
T5	4.17 a	17.00 a	1.32 ab	0.42	0.74 c	7.65 c

TABLE XIV (Continued)

	20:2	20:4	20:5	22:1	22:5	22:6
M1	0.22 ns	2.61 ns	11.80 ns	1.58 ns	1.85 ns	22.56 ns
M2	0.19	1.38	14.07	3.54	1.61	22.00
L1	0.20 ns	2.19 ns	12.99	1.96**	1.67 ns	23.18 ns
L2	0.20	1.80	12.89	3.16	1.80	21.36
T1	0.10 a	1.61 a	13.48	1.90 a	1.44 a	24.76 a
T2	0.28 b	2.76 a	11.88	2.39 a	1.52 ab	22.93 ab
T3	0.25 bc	1.94 a	12.95	2.64 a	1.82 bc	22.48 ab
T4	0.20 c	2.08 a	14.00	2.27 a	2.02 c	22.13 b
T5	0.18 c	1.59 a	12.38	3.62 b	1.86 bc	18.85 c

1 The analyses were conducted on the arsine transformed percentages but the actual percentages (not transformed) are recorded in TABLE XIV.

2 M1 = brine-frozen and M2 = plate-frozen and each mean is the average of 30 observations.

3 L1 = inside muscle and L2 = outside muscle and each mean is the average of 30 observations.

4 T1 = 14 weeks, T2 = 31 weeks, T3 = 45 weeks, T4 = 62 weeks and T5 = 81 weeks of frozen storage. Each mean is the average of 12 observations.

ns = no significant difference at the 5% level.

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

5 If a column of methods, locations, or storage times is completely blank (i.e. no *, letter or ns) then the methods, locations, or storage times for that fatty acid were not tested for significance.

6 Time means sharing the same letter are not significantly ($P \leq 0.05$) different from each other.

Free fatty acids 14:0, 17:0, 18:1, 18:3, and 20:2 were significantly greater ($P \leq 0.05$) in the brine-frozen than in the plate-frozen halibut. There were no significant differences between freezing methods for fatty acids 15:0, 16:0, 16:1, 16:2, 18:2, 18:4, 20:1, 20:5, 22:1 and 22:6 (Table III, Appendix). Differences between brine-frozen and plate-frozen samples for fatty acids 18:0, 20:4 and 22:5 were dependent upon location as indicated by a significant ($P \leq 0.05$) method x location interaction (Table III, Appendix). Visual analysis of the means indicated that inside muscle contained more 20:4 and 22:5 than outside muscle with both the brine- and plate-frozen samples but that the differences between freezing methods was far greater for the inside muscle than for the outside muscle samples, the brine-frozen samples having the greater content of both free fatty acids (Table IV, Appendix). In contrast the inside muscle contained more 18:0 than the outside muscle with the brine-frozen samples but the reverse was true for plate-frozen samples. Consequently, the magnitude by which the fatty acid 18:0 content of the brine-frozen samples exceeded that of the plate-frozen samples was very much greater with the inside than with the outside muscle (Table IV, Appendix).

The inside muscle samples contained significantly ($P \leq 0.05$) more 14:0, 15:0, 16:0, 16:1, 17:0, 18:1, 18:2, 18:3, 18:4, 20:1, 20:2, 20:5, 22:1, and 22:6 free fatty acids than the outside muscle samples (Table XV and Appendix, Table III).

TABLE XV Method, location, and time means from the analyses of variance of free fatty acids (expressed as µg free fatty acid per gram of neutral lipid) of Pacific halibut.

	14:0	15:0	16:0	16:1	16:2	17:0
M1[1]	2386.8*	423.4 ns	22737.8 ns	6365.9 ns	448.2 ns	1534.7*
M2	1257.9	229.8	9183.0	3272.6	208.7	710.6
L1[2]	2889.9**	551.5*	27299.1*	7871.3*	549.4 ns	1860.4*
L2	754.7	101.6	4621.7	1767.2	107.5	384.9
T1[3]	571.2[4]a	119.9 a	5247.5 a	1385.7 a	93.3 a	286.4 a
T2	2140.3 ab	369.4 a	25863.4 a	6726.5 a	681.9 a	1658.0 a
T3	2662.8 b	636.5 a	18753.4 a	6795.9 a	544.4 a	1636.6 a
T4	1087.6 ab	236.9 a	10780.7 a	2803.7 a	117.4 a	752.1 a
T5	2649.7 b	270.2 a	19157.1 a	6384.4 a	205.1 a	1208.2 a

	18:0	18:1	18:2	18:3	18:4	20:1
M1	9858.1[5]	22245.2*	1513.8 ns	502.5*	260.9 ns	6255.4 ns
M2	3427.7	8636.5	696.7	252.5	264.0	3706.4
L1	11463.7	25833.0**	1783.6**	617.8**	366.4***	8156.0**
L2	1822.1	5048.7	426.9	137.1	158.5	1805.8
T1	2214.6 a	4861.2 a	360.9 a	116.0 a	42.4 a	1206.4 a
T2	8340.7 a	19580.9 a	1331.5 b	403.4 a	234.6 b	5222.8 b
T3	9550.8 a	21004.5 a	1524.8 b	607.2 a	384.7 bc	7665.1 b
T4	5507.5 a	12753.2 a	956.7 ab	359.1 a	222.9 b	4067.2 ab
T5	7601.0 a	19004.4 a	1352.3 b	401.8 a	427.6 c	6742.9 b

TABLE XV (Continued)

	20:2	20:4	20:5	22:1	22:5	22:6
M1	304.4*	3239.7	1626.89 ns	1466.1 ns	2352.8	31428.6 ns
M2	125.1	893.5	8268.2	1441.5	928.2	13889.0
L1	368.3*	3559.5	20743.0*	2101.1**	2747.8	38694.7**
L2	61.2	573.8	3794.1	806.5	533.2	6622.8
T1	38.4 a	546.9 a	3747.3 a	321.7 a	467.6a	7894.4 a
T2	376.8 a	3246.8 a	14607.1 a	1649.1 b	1786.6a	30118.8 a
T3	330.6 a	2708.6 a	16817.5 a	1891.9 b	2530.8a	33630.3 a
T4	144.9 a	1599.7 a	9834.5 a	1285.1 ab	1374.4a	16815.5 a
T5	183.0 a	2231.0 a	16336.4 a	2121.3 b	2039.1a	24834.8 a

1 M1 = brine-frozen and
M2 = plate-frozen and each mean is the average of 30 observations.

2 L1 = inside muscle and
L2 = outside muscle and each mean is the average of 30 observations.

3 T1 = 14 weeks, T2 = 31 weeks, T3 = 45 weeks, T4 = 62 weeks, and T5 = 81 weeks of frozen storage.

ns = no significant difference at the 5% level
* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level.

4 Time means sharing the same letter are not significantly ($P \leq 0.05$) different from each other.

5 If a column of methods, locations, or storage times is completely blank (i.e. no *, letter, or ns) then the methods, locations, or storage times for that fatty acid were not tested for significance.

Fatty acid 16:2 concentration was not significantly different between the inside and outside muscle samples.

There was a significant difference ($P \leq 0.05$) among frozen storage times for free fatty acids 14:0, 18:2, 18:4, 20:1, and 22:1 (Table IV, Appendix). In general, the concentration of free fatty acids 14:0, 18:2, 20:1 and 22:1 was significantly different from the concentration at 62 weeks of frozen storage (Table XV). With free fatty acid 18:4 the concentration at 14 weeks was significantly lower than that at any other frozen storage time while the concentration at 81 weeks was significantly greater than the concentration at 13, 31, and 62 weeks (Table XV).

ii) Chinook Salmon

1) Free Fatty Acids Expressed as Percent of Total Free Fatty Acids Analyzed

The percentage of free fatty acid 17:0 was significantly ($P \leq 0.05$) greater in brine-frozen samples than in plate-frozen samples (Table XVI and Appendix, Table V).

The percentage of fatty acid 18:3 was significantly greater ($P \leq 0.05$) in the outside than in the inside muscle of chinook salmon (Table XVI and Appendix, Table V). The inside and outside muscle samples did not differ significantly in the percentages of fatty acids 14:0, 15:0, 16:1, 17:0, 18:0, 18:1, 18:2, 18:4, 20:1, 20:2, 20:4, 20:5, 22:5, and 22:6 (Table V, Appendix). Conclusions about differences between inside and outside muscle could not be easily drawn

TABLE XVI Method, location and time means from the analyses of variance of free fatty acids (expressed as percent of total free fatty acids analyzed) of Chinook salmon[1]

	14:0	15:0	16:0	16:1	16:2	17:0
M1[2]	5.07 ns	0.26 ns	16.75[6]	9.13 ns	0.45	1.17*
M2	4.58	0.28	16.16	8.23	0.49	1.04
L1[3]	4.89 ns	0.27 ns	16.56	8.98 ns	0.43	1.02 ns
L2	4.75	0.27	16.34	8.39	0.50	1.19
T1[4]	4.43[5]a	0.33 a	20.40	7.89 a	0.32	0.78 a
T2	4.59 a	0.19 b	18.93	8.52 a	0.67	1.15 bc
T3	5.20 a	0.35 b	13.49	9.20 a	0.65	1.37 c
T4	4.82 a	0.27 b	14.87	8.80 a	0.31	0.97 ab
T5	5.07 a	0.18 a	14.40	9.02 a	0.39	1.24 bc

	18:0	18:1	18:2	18:3	18:4	20:1
M1	3.26 ns	24.23 ns	2.19 ns	1.18 ns	1.72 ns	4.32 ns
M2	3.85	23.51	2.10	1.22	1.64	4.57
L1	3.49 ns	23.72 ns	2.03 ns	1.16*	1.61 ns	4.40 ns
L2	3.63	24.02	2.26	1.24	1.75	4.49
T1	3.80 a	23.46 ab	1.35 a	1.01 a	1.07 a	3.69 a
T2	2.99 b	21.83 b	3.12 b	1.23 ab	1.62 b	3.68 a
T3	3.89 b	24.80 a	1.97 c	1.29 b	1.84 b	4.89 bc
T4	3.71 b	25.10 a	1.88 c	1.41 b	1.97 b	5.26 bc
T5	3.39 ab	24.11 a	2.41 d	1.05 a	1.89 b	4.68 b

TABLE XVI (Continued)

	20:2	20:4	20:5	22:1	22:5	22:6
M1	0.28 ns	0.50 ns	13.90 ns	3.05 ns	2.14 ns	8.62 ns
M2	0.25	0.45	13.10	2.86	2.62	11.24
L1	0.30 ns	0.44 ns	13.56 ns	2.86	2.30 ns	10.14 ns
L2	0.24	0.51	13.44	3.04	2.46	9.73
T1	0.20 a	0.64 a	13.70 a	2.66	2.28	9.87 ab
T2	0.64 b	0.53 a	12.19 a	3.44	2.06	10.84 a
T3	0.17 a	0.38 a	13.71 a	2.92	2.42	9.62 ab
T4	0.18 a	0.42 a	13.63 a	3.16	2.54	8.70 b
T5	0.14 a	0.41 a	14.30 a	2.57	2.60	10.62 a

1 The analyses were conducted on the arcsine transformed percentages but the actual percentages (not transformed) are recorded in TABLE XVI.

2 M1 = brine-frozen and M2 = plate-frozen and each mean is the average of 30 observations.

3 L1 = inside muscle and L2 = outside muscle and each mean is the average of 30 observations.

4 T1 = 9 weeks, T2 = 26 weeks, T3 = 40 weeks, T4 = 58 weeks, and T5 = 77 weeks of frozen storage. Each mean is the average of 12 observations.

ns = no significant difference at the 5% level

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

5 Time means sharing the same letter are not significantly ($P \leq 0.05$) different from each other.

6 If a column of methods, locations, or storage times is completely blank (i.e. no *, letter, or ns) then the methods, locations, or storage times for that fatty acid were not tested for significance.

for free fatty acids 16:0, 16:2, and 22:1 as there were significant ($P \leq 0.05$) method x location x time interactions for 16:0 and 16:2 and a significant ($P \leq 0.05$) location x time interaction for fatty acid 22:1 (Table V, Appendix). Visual analysis of the means indicated that although the percentage of 22:1 of both inside and outside muscle increased to a maximum and then rapidly decreased, the outside muscle reached a maximum at 26 weeks while that of the inside muscle did not reach a maximum until 58 weeks of frozen storage (Table VI, Appendix).

The percentages of free fatty acids 15:0, 17:0, 18:0, 18:1, 18:2, 18:3, 18:4, 20:1, 20:2, and 22:6 differed significantly ($P \leq 0.05$) among storage times (Table V, Appendix). There were no significant differences between frozen storage times for fatty acids 14:0, 16:1, 20:4, 20:5, and 22:5. Differences between frozen storage times for fatty acids 16:0, 16:2, and 22:1 could not be simply evaluated as there were significant ($P \leq 0.05$) method x location x time interactions for 16:0 and 16:2 and a significant ($P \leq 0.05$) location x time interaction for 22:1 (Table V, Appendix).

The percentages of fatty acids 15:0, 17:0, and 18:0 reached a maximum at 40 weeks while 18:1, 18:3, 18:4, and 20:1 reached a maximum at 58 weeks and fatty acids 18:2, 20:2 and 22:6 reached a maximum at 26 weeks of frozen storage (Table XVI). In all cases the maximum concentration was significantly ($P \leq 0.05$) greater than the concentration at one or more other frozen storage times (Table XVI).

2) Free Fatty Acids Expressed as µg Fatty Acid per Gram of Neutral Lipid

The brine-frozen chinook salmon contained significantly ($P \leq 0.05$) less 16:0, 18:2, and 22:6 free fatty acids than plate-frozen samples (Table XVII and Appendix, Table VII). There were no significant differences between freezing methods for fatty acids 15:0, 16:2, 18:0, 18:1, 18:3, 20:1, 20:2, 20:4, 20:5, and 22:5 (Table XII, Appendix). No conclusions could be made about differences between freezing methods for fatty acids 14:0, 16:1, 17:0, 18:4, and 22:1 as there were significant ($P \leq 0.05$) method x location x time interactions (Table VII, Appendix).

There were significant differences ($P \leq 0.05$) between inside and outside muscle for free fatty acids 15:0, 18:0, 18:1, 18:2, 18:3, 20:1, 20:4, 20:5, and 22:5 (Table VII, Appendix). The concentration of all the above mentioned fatty acids was greater in the inside muscle than in the outside muscle (Table XVII). No conclusions could be made about differences between inside and outside muscle for free fatty acids 14:0, 16:0, 16:1, 16:2, 17:0, 18:4, 20:2, 22:1, and 22:6 as there were significant ($P \leq 0.05$) method x location x time interactions for fatty acids 14:0, 16:1, 17:0, 18:4, and 22:1 and significant ($P \leq 0.05$) location x time interactions for fatty acids 16:0, 16:2, 20:2, and 22:6 (Table VII, Appendix). Visual analysis of the means of fatty acids 16:0, 16:2, 20:2, and 22:6 indicated that in each case there were no obvious meaningful trends (Table VIII, Appendix).

TABLE XVII Method, location, and time means from the analyses of variance of free fatty acids (expressed µg free fatty acid per gram of neutral lipid) of Chinook salmon.

	14:0	15:0	16:0	16:1	16:2	17:0
M1[1]	622.7[4]	30.5 ns	2005.6*	1143.2	61.1	157.6
M2	695.2	38.8	2350.7	1260.1	71.4	157.8
L1[2]	895.7	47.1**	2987.0	1651.8	88.0	203.4
L2	422.2	22.2	1369.3	751.5	44.5	112.0
T1[3]	272.5	21.1[5] a	1227.0	502.3	19.8	48.7
T2	558.1	24.0 a	2630.5	1073.5	95.4	162.3
T3	873.0	56.6 b	2362.1	1575.0	107.8	218.3
T4	652.8	38.3 a	2036.6	1201.1	42.0	130.8
T5	938.3	33.4 a	2634.6	1656.2	66.3	228.3

	18:0	18:1	18:2	18:3	18:4	20:1
M1	390.0 ns	3061.9 ns	278.4**	150.3 ns	225.7	536.6 ns
M2	546.2	3676.7	312.8	174.0	248.6	696.0
L1	623.4**	4511.9**	395.9	213.2*	312.8	820.1*
L2	313.6	2226.7	196.2	111.1	161.5	412.5
T1	236.7 a	1502.4 a	87.0 a	61.0 a	69.2	241.4 a
T2	371.3 ab	2945.1 b	391.8 b	164.5 b	206.2	441.3 b
T3	654.6 c	4415.0 c	321.5 bc	213.0 b	301.4	832.1 c
T4	497.0 bc	3531.3 bc	251.5 c	185.6 b	255.7	697.9 c
T5	583.0 c	4452.6 c	426.1 b	186.6 b	353.2	868.7 c

TABLE XVII (Continued)

	20:2	20:4	20:5	22:1	22:5	22:6
M1	30.8 ns	55.4 ns	1682.4 ns	368.0	254.6 ns	1051.2*
M2	37.8	63.0	1964.7	420.9	387.2	1715.5
L1	51.7	78.5*	2477.8*	531.5	430.7**	1920.8
L2	16.9	40.0	1169.4	257.4	211.2	845.9
T1	7.0	34.1 a	870.9 a	164.2	145.0 a	681.5
T2	79.2	64.2 a	1535.8 b	392.6	265.8 b	1407.6
T3	33.5	69.0 a	2327.3 cd	483.3	419.7 c	1721.5
T4	24.5	53.9 a	1822.3 bc	432.5	307.2 b	1116.1
T5	27.5	75.0 a	2561.4 d	499.7	466.8 c	1990.0

M1[1] Brine-frozen and
M2 Plate-frozen and each mean is the average of 30 observations.

L1[2] = inside muscle and
L2 = outside muscle and each mean is the average of 30 observations.

T1[3] = 9 weeks, T2 = 26 weeks, T3 = 40 weeks, T4 = 58 weeks, and T5 = 77 weeks of frozen storage.

ns = no significant difference at the 5% level.

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

4 If a column of methods, locations, or storage times is completely blank (i.e. no *, letter, or ns) then the methods, locations, or storage times for that fatty acid were not tested for significance.

5 Time means sharing the same letter are not significantly ($P \leq 0.05$) different from each other.

There were significant differences ($P \leq 0.05$) among frozen storage times for free fatty acids 15:0, 18:0, 18:1, 18:2, 18:3, 20:1, 20:5, and 22:5 (Table VII, Appendix). Free fatty acid 20:4 did not differ significantly between frozen storage times. No conclusions about differences between frozen storage times could be made for free fatty acids 14:0, 16:0, 16:1, 16:2, 17:0, 18:4, 20:2, 22:1, and 22:6 as there were significant ($P \leq 0.05$) method x location x time interactions for 14:0, 16:1, 17:0, 18:4, and 22:1 and significant ($P \leq 0.05$) location x time interactions for 16:0, 16:2, 20:2 and 22:6 (Table VII, Appendix).

The concentration of free fatty acid 15:0 appeared to increase to a maximum at 45 weeks and then decrease until the 77th week of frozen storage (Table XVII). In general, with free fatty acids 18:1, 18:2, 18:3, 20:1, 20:5, and 22:5 there was a significant increase in concentration between 9 and 26 weeks but the differences among the other frozen storage times (i.e. 45, 58, and 77 weeks) were variable (Table XVII).

iii) Coho Salmon

1) Free Fatty Acids Expressed as Percent of Total Free Fatty Acids Analyzed

None of the free fatty acids analyzed differed significantly between freezing methods (Table IX, Appendix).

The percentage of fatty acid 18:4 was significantly ($P \leq 0.05$) greater in outside muscle than in inside muscle

(Table XVIII and Appendix, Table IX). Free fatty acids 14:0, 15:0, 16:0, 16:1, 16:2, 17:0, 18:0, 18:1, 18:2, 18:3, 20:1, 20:2, 20:4, 22:1, 22:5 and 22:6 did not differ significantly with location (Table IX, Appendix). No conclusions about differences between inside and outside muscle or between freezing methods could be made for free fatty acid 20:5 as there was a significant ($P \leq 0.05$) method x location x time interaction (Table IX, Appendix).

There was a significant ($P \leq 0.05$) difference among the different frozen storage times for free fatty acids 15:0, 16:1, 17:0, 18:1, 18:2, 18:4, 20:2, 20:4 and 22:1 (Table IX, Appendix). No conclusions about differences among frozen storage times could be made for free fatty acid 20:5 as there was a significant ($P \leq 0.05$) method x location x time interaction (Table IX, Appendix).

The concentrations of free fatty acids 17:0, 18:0, and 18:4 were significantly greater ($P \leq 0.05$) at 78 weeks than at 27 and/or 10 weeks of frozen storage (Table XVIII). The concentrations of free fatty acids 15:0, 16:1, 18:1, 18:2, 20:2, 20:4 and 22:1 were significantly greater ($P \leq 0.05$) at the 10th or 27th week than at the 78th week (Table XVIII).

2) Free Fatty Acids Expressed as µg Free Fatty Acid per Gram of Neutral Lipid

There were not any significant differences due to freezing method, location of or storage time for any of the 18 free fatty acids analyzed (Table XIX, and Appendix Table XI).

TABLE XVIII Method, location, and time means from the analyses of variance of free fatty acids (expressed as percent of total free fatty acids analyzed) of Coho salmon.

	14:0	15:0	16:0	16:1	16:2	17:0
M1[1]	3.37 ns	0.23 ns	15.58 ns	8.44 ns	0.43 ns	1.05 ns
M2	3.49	0.18	14.49	9.07	0.50	1.19
L1[2]	3.24*	0.20 ns	15.40 ns	8.78 ns	0.35 ns	0.95 ns
L2	3.62	0.21	14.67	8.34	0.57	1.29
T1[3]	3.66[4]a	0.30 a	15.20 a	8.37 a	0.43 a	0.90 a
T2	3.19 a	0.15 b	14.44 a	10.06 b	0.55 a	1.15 ab
T3	3.44 a	0.17 b	15.47 a	7.26 a	0.41 a	1.31 b

	18:0	18:1	18:2	18:3	18:4	20:1
M1	3.26 ns	21.60 ns	2.01 ns	1.10 ns	1.55 ns	4.24 ns
M2	3.09	20.81	2.01	1.00	1.51	4.56
L1	3.12 ns	21.05 ns	1.87 ns	0.99 ns	1.41*	4.30 ns
L2	3.23	21.37	2.15	1.10	1.65	4.49
T1	3.19 a	20.94 a	1.49 a	1.11 a	1.57 a	4.37 a
T2	2.20 b	22.81 b	2.47 b	0.93 a	1.19 b	4.21 a
T3	4.15 c	19.84 a	2.07 c	1.11 a	1.83 c	4.61 a

TABLE XVIII (Continued)

	20:2	20:4	20:5	22:1	22:5	22:6
M1	0.68 ns	0.72 ns	15.11[5]	3.07 ns	2.87 ns	13.04 ns
M2	0.59	0.76	15.16	4.09	2.96	13.02
L1	0.70 ns	0.77 ns	15.16	3.07 ns	2.66 ns	13.71 ns
L2	0.57	0.77	15.02	3.46	3.17	12.34
T1	0.39 a	1.06 a	15.23	3.69 a	2.62 a	13.78 a
T2	1.22 b	0.56 b	13.28	4.17 a	2.80 a	12.54 a
T3	0.29 a	0.60 b	16.88	2.89 b	3.33 a	12.78 a

M1[1] Brine-frozen and
M2 Plate-frozen and each mean is the average of 30 observations.

L1[2] = inside muscle and
L2 = outside muscle and each mean is the average of 30 observations.

T1[3] = 9 weeks, T2 = 26 weeks, T3 = 40 weeks of frozen storage.

ns = no significant difference at the 5% level.

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

4 If a column of methods, locations, or storage times is completely blank (i.e. no *, letter, or ns) then the methods, locations, or storage times for that fatty acid were not tested for significance.

5 Time means sharing the same letter are not significantly ($P \leq 0.05$) different from each other.

TABLE XIX Method, location, and time means from the analyses of variance of free fatty acids (expressed as μg free fatty acid per gram of neutral lipid) of Coho salmon.

	14:0		15:0		16:0		16:1		16:2		17:0	
M1[1]	4817.8	ns	201.7	ns	17661.9	ns	9743.2	ns	632.9	ns	1876.0	ns
M2	804.1		41.0		3692.8		2120.8		100.3		308.4	
L1[2]	5288.5	ns	224.7	ns	20002.2	ns	11064.2	ns	671.9	ns	2053.6	ns
L2	333.4		18.0		1352.5		799.8		61.4		130.8	
T1[3]	372.2[4]	a	31.1	a	1640.7	a	872.0	a	43.4	a	93.8	a
T2	371.4	a	18.6	a	1800.5	a	1270.1	a	59.3	a	122.5	a
T3	7690.3	a	314.3	a	28590.8	a	15653.9	a	997.2	a	3060.3	a

	18:0		18:1		18:2		18:3		18:4		20:1	
M1	3999.3	ns	24819.3	ns	2138.4	ns	1441.0	ns	2799.6	ns	7468.6	ns
M2	904.2		5148.3		520.7		288.5		421.0		1106.1	
L1	4533.1	ns	27932.5	ns	2464.0	ns	1621.4	ns	3056.0	ns	8162.9	ns
L2	370.4		2035.1		195.0		108.2		164.7		411.8	
T1	344.8	a	2233.6	a	151.4	a	114.6	a	166.0	a	442.0	a
T2	275.2	a	2836.4	a	289.4	a	112.7	a	133.9	a	483.7	a
T3	6735.1	a	39881.5	a	3547.8	a	2367.1	a	4531.1	a	11936.3	a

TABLE XIX (Continued)

	20:2	20:4	20;5	22:1	22:5	22:6
M1	804.5 ns	569.3 ns	19905.2 ns	4600.8 ns	2691.0 ns	14666.1 ns
M2	106.9	154.4	3998.9 ns	849.0	779.3	3725.1
L1	873.1 ns	664.6 ns	22377.3 ns	5165.5 ns	3152.8 ns	17163.9 ns
L2	38.3	59.2	1526.8	284.2	317.5	1227.3
T1	37.9 a	104.5 a	1622.2 a	364.4 a	253.3 a	1541.7 a
T2	159.3 a	70.4 a	1636.2 a	507.3 a	348.9 a	1637.5 a
T3	1169.9 a	910.8 a	32597.8 a	7302.9 a	4603.1 a	24407.6 a

M1[1] Brine-frozen and
M2 Plate-frozen and each mean is the average of 30 observations.

L1[2] = inside muscle and
L2 = outside muscle and each mean is the average of 30 observations.

T1[3] = 9 weeks, T2 = 26 weeks, T3 = 40 weeks of frozen storage.

ns - no significant difference at the 5% level.

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

4 Time means sharing the same letter are not significantly ($P \leq 0.05$) different from each other.

h) Total Free Fatty Acids (Expressed as µg Free Fatty Acid per Gram of Neutral Lipid)

i) Pacific Halibut

There was no significant difference between method of freezing or among storage times (Table XIII, Appendix). The concentration of total free fatty acids was significantly ($P \leq 0.01$) greater in the inside than in the outside muscle (Table XX and Appendix, Table XIII).

ii) Chinook Salmon

The concentration of total free fatty acids was significantly greater ($P = 0.05$) in the plate-frozen than in the brine-frozen samples (Table XX and Appendix, Table XIII). Inside muscle contained significantly ($P \leq 0.01$) greater free fatty acids than the outside muscle (Table XX and Appendix Table XIII). There was also a very highly significant difference ($P \leq 0.001$) among storage times (Table XIII, Appendix). The samples at 9 weeks were significantly ($P \leq 0.05$) lower in total free fatty acid concentration than samples at any other storage time (Table XX). The samples at 77 weeks were significantly ($P \leq 0.05$) greater than samples at any other storage time except those at 58 weeks (Table XX).

iii) Coho Salmon

There were no significant differences between method of freezing, location of sampling, or among storage

TABLE XX Method, location, and time means from the analyses of variance of total free fatty acids (expressed as ug free fatty acid per gram of neutral lipid) of Pacific halibut, Chinook salmon and Coho salmon.

	Pacific halibut	Chinook salmon	Coho salmon
M1[1]	12959.3 ns	1210.7*	12083.6 ns
M2	5739.2	1481.7	2506.9
L1[2]	15745.6**	1824.0**	13647.2 ns
L2	2952.8	868.4	943.4

PACIFIC HALIBUT

14 weeks[3]	31 weeks	45 weeks	62 weeks	81 weeks
2952.2[4] a	12433.9 a	12967.6 a	7070.3 a	11322.2 a

CHINOOK SALMON

9 weeks	26 weeks	40 weeks	58 weeks	77 weeks
619.2 a	1280.9 b	1698.5 cd	1327.7 bc	1804.8 d

COHO SALMON

10 weeks	27 weeks	78 weeks
1042.9 a	1213.3 a	19629.6 a

1 M1 = brine-frozen and M2 = plate-frozen.

2 L1 - inside muscle and L2 = outside muscle.

3 Weeks of frozen storage.

ns = no significant difference at the 5% level.

* Significant at the 5% level.
** Significant at the 1% level.

4 Time means sharing the same letter are not significantly (P = 0.05) different from each other.

times (Table XIII, Appendix).

B. Correlations

a) Correlations of pH, Thaw Drip and Color (Hunter Rd, 'a', b, and a/b values) with Each Other

i) Pacific Halibut

There was a significant ($P \leq 0.05$) negative correlation ($r = -0.358$) between thaw drip and pH (Table XV, Appendix).

ii) Chinook Salmon

There were significant ($P \leq 0.05$) correlations among the three color parameters (Table XV, Appendix).

iii) Coho Salmon

The negative correlations between pH and Hunter 'a' values ($r = -0.596$) and between pH and Hunter a/b values ($r = -0.721$) were both highly significant ($P \leq 0.01$). There were also significant ($P \leq 0.05$) correlations among the three color parameters (Table XV, Appendix).

b) Correlations of pH, Thaw Drip, Color, TBA values and Free Fatty Acids with Flavor

i) Pacific Halibut

Flavor was not significantly correlated with any of the other parameters measured (Table XV, Appendix).

ii) Chinook Salmon

Only the Hunter b values ($r = -0.527$), the TBA values ($r = +0.460$), and free fatty acid 20:1 (expressed as percent of total free fatty acids analyzed ($r = -0.410$) correlated significantly ($P \leq 0.05$) with flavor (Table XVI, Appendix).

iii) Coho Salmon

Only the Hunter 'a' values ($r = +0.653$) and free fatty acid 18:0 (expressed as percent of total free fatty acids analyzed ($r = +0.515$) correlated significantly ($P \leq 0.05$) with flavor (Table XVI, Appendix).

DISCUSSION

a) pH

The presence of a small and non-significant difference in pH between brine-frozen and plate-frozen halibut indicates that brine-freezing likely would not increase the incidence of the undesirable chalky condition in halibut as it has been found that chalkiness is related to the pH of the muscle (Tomlinson *et al.*, 1965).

The decline in pH during frozen storage of Pacific halibut agrees with the findings of Tomlinson *et al.* (1969). However, in both cases sampling was confounded with the position along the length of the halibut and Tomlinson *et al.* (1966) observed that the pH of halibut tends to be higher near the head of the fish and lower near the center of the body. Thus the observed decline in pH may be related to the position of sampling, or it may have resulted from a combination of frozen storage time and position of sampling.

No data could be found in the literature regarding flesh pH at different points along the length of chinook and coho salmon. Nevertheless this does not preclude a positional effect on flesh pH, particularly in view of the findings for Pacific halibut (Tomlinson *et al*, 1966). Therefore the results are inconclusive.

b) Thaw Drip

The mean thaw drip of Pacific halibut and chinook salmon, at the first and/or second sampling time, was less for the brine-frozen than for the plate-frozen samples. At all other frozen storage times the brine-frozen samples had approximately equal or greater thaw drip than the plate-frozen samples (Fig. 6 and Fig. 7).

Fish frozen slowly tends to form more drip than those frozen quickly and drip increases with increased frozen storage time (Miyauchi, 1963). Also Tarr (1942) showed that NaCl can markedly reduce free drip from fish muscle. The brine-frozen halibut were frozen over a 47 hour period and the chinook salmon were brine-frozen over a 9.5 hour period whereas both the halibut and chinook salmon were plate-frozen in approximately 3.5 hours.

Thus, the fact that the brine-frozen halibut and chinook salmon had less thaw drip than the plate-frozen samples, during the early part of frozen storage, may be related to the effect NaCl has on reducing drip. Whereas brine-frozen halibut and chinook had approximately the same (particularly the chinook salmon) or more (particularly the halibut) thaw drip than the plate-frozen samples during the later part of frozen storage suggests that freezing rate may have been a determinant. However, this explanation does not apply to the thaw drip from coho salmon as the plate-frozen samples

consistently had greater thaw drip than the brine-frozen samples.

The decline in thaw drip during frozen storage of plate-frozen halibut has been observed in other work with this species (Roach et al., 1966). The thaw drip values of Pacific halibut were higher than those reported by Tomlinson et al. (1969) but this may be due to differences in the ratio of cut surface area to total weight of the fish flesh as the amount of drip formed is related directly to the above mentioned ratio (Miyauchi, 1963). Differences in pH may also be involved as Tomlinson et al. (1966) found that thaw drip of halibut increased continuously with decreasing pH in the range pH 6.8 - 5.7. The flesh pH of the halibut in the present work varied between pH 6.25 and pH 5.85 whereas the flesh pH of the halibut used by Tomlinson et al. (1969) varied between pH 6.66 and pH 6.25.

c) Color

The Hunter Rd, a, and b color readings on raw salmon have, by themselves, little practical meaning. However, Schmidt and Idler (1958) found that the 'a' reading alone was a suitable measure of the color of processed salmon and that it could best be predicted from the a/b ratio of the raw flesh. The higher the a/b ratio of the raw flesh, the higher the 'a' value of the resulting processed product and the higher the visual redness.

Analysis of variance indicated that in comparison

with plate-freezing, brine-freezing did not significantly decrease the a/b ratio of coho salmon. For chinook salmon, between 26 and 77 weeks of frozen storage, the brine-frozen samples always had a slightly lower a/b ratio than the plate-frozen (Fig. 16).

Oxidative rancidity of frozen red salmon (chinook and coho salmon) is accompanied by a fading of the red pigments of the flesh (Tarr, 1947; 1955; Boyd et al., 1957). Thus, if oxidative rancidity was occuring during the frozen storage of chinook and coho salmon one would expect a decrease in the a/b ratio. However, with both species the a/b ratio increased during frozen storage. With the coho salmon the a/b ratio at 78 weeks was significantly higher ($P \leq 0.05$) than the a/b ratio at 10 and 27 weeks of frozen storage (Table VI). With brine-frozen chinook salmon the a/b ratio increased from a low of 1.003 at 9 weeks to a high of 1.203 at 58 weeks of frozen storage (Fig. 16). Thus either oxidative rancidity was not taking place to a sufficient degree to reduce the a/b ratio or oxidative rancidity does not significantly affect the red pigments of salmon.

d) Flavor Differences

For halibut and chinook salmon the taste panel found that the differences in flavor between outside muscle samples from brine-frozen and plate-frozen fish reached a maximum at the second period (i.e., at the end of approximately 29 weeks

of frozen storage at -30°C) and then steadily decreased. This relationship may be related to the higher salt content in the brine-frozen outside muscle samples. The higher salt content may have accelerated the induction period of lipid oxidation as in the chinook salmon the greatest difference in TBA values between the brine-frozen and plate-frozen outside muscle samples occurred at the second sampling period and in halibut at the third sampling period. After approximately 30 weeks of frozen storage the plate-frozen samples may begin to 'catch-up' with the brine-frozen samples in development of rancidity and off-flavors.

The fact that, with all three species, the plate-frozen outside muscle samples were preferred at the first two or three sampling periods while at the last sampling period the brine-frozen outside muscle samples were slightly preferred suggests that salt possibly masked increasing deterioration in flavor.

It is unknown to what degree the higher salt content of the brine-frozen outside muscle samples influenced the panel members ability to identify the odd sample and no explanation is apparent for the difference between coho and the other two species.

The taste panel also detected little difference in flavor between the brine-frozen and plate-frozen inside muscle samples from the two salmon species. One possible reason for

the difference detected between inside halibut muscle samples is that halibut were brine-frozen during a 47 hour period while the salmon were brine-frozen in 9.5 hours. The fact that the present results differed from those of Peters et al. (1968) may be related to the fact that Atlantic cod is a much leaner fish than salmon and to a lesser degree halibut.

e) Mineral Concentration

The sodium concentration in the brine-frozen inside muscle, plate-frozen inside muscle and plate-frozen outside muscle of Pacific halibut, chinook salmon and coho salmon was similar to that reported by McBride and MacLeod (1956) but potassium concentration was slightly lower.

The fact that the percentage change in potassium in the outside muscle was much less than the percentage change in sodium and chloride concentrations may be related to the length of time the fish were in the brine. Tomlinson et al. (1965 a; 1965 b) reported that the decrease in potassium concentration of coho and sockeye salmon and rainbow trout was much slower than the increase in sodium concentration that occurs during storage of fish in refrigerated sea water (RSW) or fortified refrigerated sea water (FRSW).

Even though there was an increase in sodium and chloride content of the brine-frozen outside muscle of all three species, the salt concentration was still below that

generally accepted for palatability, which is usually taken to be 1%. Similar results were found by Harrison and Roach (1953) for chinook salmon, chum salmon and gray cod and by Holston and Pottinger (1954) for haddock.

f) TBA Values

The TBA values for the inside muscle of all three species were relatively similar for both brine- and plate-frozen samples suggesting little difference in degree of oxidation in inside muscle frozen by the two different methods. In outside muscle, by contrast, between 26 and 45 weeks of frozen storage (depending on the species) there occurred a relatively large maximum difference in TBA values between the brine-frozen and plate-frozen samples. The differences then decreased as storage progressed until at approximately 80 weeks the differences in TBA values between the inside and outside muscle samples were relatively small. The fact that, except at the first or last analyses, (depending on the species) average TBA values of the brine-frozen outside muscle were greater than those of the corresponding plate-frozen samples indicated that oxidative rancidity, particularly at approximately 29 weeks of frozen storage, was greater in the brine-frozen outside muscle than in the plate-frozen outside muscle.

The observation that the brine-frozen outside and the plate-frozen outside muscle of chinook and coho salmon

as well as the brine-frozen outside muscle of halibut reached a maximum and then steadily decreased are not in agreement with the results of Awad et al. (1969). They observed that the TBA values of fresh-water whitefish, stored at -10°C, steadily increased with time of frozen storage. In contrast, Castell et al. (1966) observed that the lipids of cod muscle stored at -18°C and at -25°C did not undergo oxidation as measured by the TBA method. Castell et al. (1966 b) also found that during frozen storage the lipids of cod became markedly more resistant to metal - (Cu^{2+} or Fe^{2+}) or to hemoglobin-catalyzed oxidation. They suggested that the free fatty acids may have reacted with proteins or some other component of the muscle in a manner that protected their double bonds against oxidation. An analogous reaction may explain the decrease in the TBA values that occurred during the present study.

The TBA test is based on the reaction of malonaldehyde with 2 Thiobarbituric acid to form a pink to red colored product in solution. Consequently, a possible explanation for the observed decrease in TBA values of the outside muscle samples is that malonaldehyde may have reacted with some other components of the muscle and thus would have been unavailable for the TBA reaction. Buttkus (1967) showed that malonaldehyde reacted with the E-amino groups of trout myosin. The rate of reaction at 0°C was less than that at 20°C while the reaction rate at -20°C was almost equal to that at +20°C. Buttkus (1969) also showed that malonaldehyde

reacted with cysteine and methionine. Kwon et al. (1965) reported that thiobarbituric acid reactive substances react with protein and in tuna stored at -18°C became partially unrecoverable for the TBA test.

An additional factor probably contributing to the decrease in TBA values observed in the present study could be the fact that the presence of oxygen is necessary for oxidative rancidity to occur (Lundberg, 1961). Prior to freezing, the fish flesh was exposed to oxygen whereas after the fish were frozen and heavily glazed access of oxygen to the flesh would have been very greatly impeded. During each sampling, the fish were reglazed immediately after a sample was taken. Thus even at this point very little oxygen would penetrate the flesh. Consequently, oxidative rancidity could only proceed while oxygen was still present in the flesh. Once the original oxygen was used up randidity could no longer continue as no 'new' oxygen (or very little) should have been entering the flesh. Awad et al. (1969) observed increasing oxidative rancidity (TBA values) with increasing storage time. Although the fish used in their study were wrapped in saran film prior to freezing, the fish were not frozen until approximately 5 days post-mortem.

g) Free Fatty Acids

The percentage of free fatty acid 17:0 was significantly ($P \leq 0.05$) greater in the brine-frozen than in the

plate-frozen chinook salmon (Table XVI and Appendix, Table V. The concentrations (μ g free fatty acid per gram of neutral lipid) of fatty acids 16:0, 18:2, and 22:6 as well as the concentrations of total free fatty acids analyzed were significantly smaller ($P = 0.05$) in the brine-frozen than in the plate-frozen chinook salmon (Table XVII and Appendix, Table VII). With halibut the percentage of fatty acid 15:0 and the concentrations of free fatty acids 14:0, 17:0, 18:1, 18:3, and 20:2 were all significantly greater ($P \leq 0.05$) in brine-frozen than in plate-frozen fish (Tables XIV and XV and Appendix, Tables I and III). There were no significant differences in the percentages or concentrations of any individual free fatty acids between freezing methods for coho salmon (Tables IX and XI, Appendix). Also there were no significant differences between freezing methods in the concentration of total free fatty acids analyzed for either coho salmon or halibut (Table XIII, Appendix). Thus brine-freezing appeared to affect the formation of some individual free fatty acid in both chinook salmon and halibut but brine-freezing did not significantly affect the total free fatty acid formation in either coho salmon or halibut while it did significantly decrease the concentration of total free fatty acid analyzed in chinook salmon. The fact that there were many more significant ($P \leq 0.05$) differences between methods when free fatty acids were expressed as μ g per gram of neutral lipid rather than as percent of free fatty acids analyzed indicated that brine-freezing

alters the composition of the free fatty acids formed by a very slight degree. The fact that brine-freezing significantly affected the total free fatty acid concentration (total of those analyzed) of chinook salmon but not of coho salmon or of halibut may be related to the higher lipid content in chinook salmon.

In general, where there was a significant difference, the inside muscle contained more free fatty acids per gram of neutral lipid than the outside muscle. This may be the result of the higher neutral lipid content of the outside muscle.

The concentration of the total free fatty acids of chinook salmon and the individual free fatty acids of chinook salmon and halibut that differed significantly ($P \leq 0.05$) among frozen storage times tended to increase rather rapidly during the first 26 or 45 weeks of frozen storage and then increase (if at all) at a much slower rate. Even with the concentration of total free fatty acids of halibut, although they did not differ significantly among frozen storage times, the greatest increase occurred between 14 and 31 weeks. This trend (if present) was not very evident with the coho salmon. This rapid increase in free fatty acids followed by a much slower increase is somewhat similar to the pattern observed in Pacific gray cod and Atlantic cod (Wood and Haqq, 1962) except in the present study the slower rate of increase (if any) was not as uniform as that observed by Wood and Haqq (1962).

The results of the present study differ from those of Varesmea et al. (1969) who observed that with rainbow trout stored for 8 months at -18°C or at -32°C the percentage of fatty acid 18:1 decreased from 23% to approximately 0% while the percentage of fatty acid 18:0 increased from 6% to approximately 24%. Olley et al. (1969) showed that with haddock and lemon sole there was a preferential hydrolysis of 16:0, 18:1, and 20:5 phospholipids. In the present study the concentration of fatty acids 16:0, 18:1, and 20:5 of Pacific halibut, chinook salmon, and coho salmon did not significantly increase as time of frozen storage progressed (Tables XIV, XVI, and XVIII and Appendix, Tables I, V, and IX).

The significant differences in total free fatty acid concentration between brine-frozen and plate-frozen chinook salmon differs from the findings of Peters et al. (1968) who observed no significant difference in total titratable free fatty acids between brine-frozen and plate-frozen Atlantic cod. The discrepancy between the two observations may be related to the much higher lipid content of chinook salmon (Stansby and Olcott, 1963).

The results suggest that lipid hydrolysis may occur at a greater rate in Pacific halibut than in Atlantic halibut. In the present study, although there were no significant differences among storage times and the concentration at 31 weeks was not significantly greater than that at 14 weeks the concentration of the total long chain free fatty acids increased from 2,952.2 u g per gram of neutral lipid at 14

weeks to 12,433.9 μg at 31 weeks of frozen storage at -30°C whereas Dyer et al. (1958) observed no lipid hydrolysis in Atlantic halibut stored at -10°F for approximately 6 months.

It should be emphasized that when the free fatty acids were expressed a u g free fatty acid per gram of neutral lipid it was assumed that the increase in free fatty acids arises from hydrolysis of phospholipids and not from hydrolysis of triglycerides. During the course of this study Bilinski and Lau (1969) showed that rainbow trout possessed lipolytic activity towards long-chain triglycerides. Also Bosund and Ganrot (1969) showed that hydrolysis of long-chain triglycerides occurs during the frozen storage of herring. However, with other species, notably Atlantic cod and related species (ling, saithe and hake) several workers have shown that almost all the free fatty acids arise from hydrolysis of phospholipids (Olley et al., 1962; Bligh, 1961; and Bligh and Scott, 1966).

It should also be emphasized that the free fatty acid called 22:6 probably also contains (depending on the species) 5 to 10% fatty acid 24:1 (Ackman, 1966; and Gruger et al., 1964).

SUMMARY AND CONCLUSIONS

Experiments were conducted to determine the effect of brine- and plate-freezing at sea and the length of subsequent frozen storage upon flesh pH, thaw drip, color, flavor, TBA values, and various long chain free fatty acids of Pacific halibut, chinook salmon, and coho salmon. The effect of the two freezing methods upon mineral (sodium, potassium, and chloride) concentration was also determined. Evaluations were conducted on outside and inside muscle, where appropriate.

Method of freezing had no significant effect upon the flesh pH of any of the 3 species. Flesh pH significantly ($P \leq 0.05$) decreased (the pattern of decrease varied with the species) as length of frozen storage increased but this decrease in pH may have been the result of the method of sampling.

The mean thaw drip from Pacific halibut and chinook salmon was less for the brine-frozen than for the plate-frozen samples after storage for 9 to 31 weeks whereas subsequently the brine-frozen samples had approximately equal or greater thaw drip than the plate-frozen samples. The plate-frozen coho salmon had a significantly ($P \leq 0.05$) higher average thaw drip than the brine-frozen.

During frozen storage the thaw drip of the plate-frozen halibut tended to decrease while that of the brine-frozen halibut tended to increase. In general, with both chinook and coho salmon, the thaw drip of both the brine- and plate-frozen samples tended to slightly increase.

In general, redness ('a'-value) and the a/b ratio tended to increase with length of frozen storage of both brine- and plate-frozen chinook and coho salmon.

The differences in flavor between brine- and plate-frozen samples of outside muscle of both halibut and chinook salmon reached a maximum, which depending upon the species was either highly ($P \leq 0.01$) or very highly ($P \leq 0.001$) significant at 26 or 31 weeks of frozen storage. The differences then steadily decreased. In contrast, the differences in flavor between brine- and plate-frozen coho salmon outside muscle steadily increased. With the exception of the samples of coho salmon stored for 10 weeks there appeared to be little difference in flavor of inside muscle of the salmon species frozen by the two different methods. Conversely, significant ($P \leq 0.05$) differences in flavor between brine- and plate-frozen samples of halibut inside muscle were detected at 31, 62, and 81 weeks of storage.

In general, with all three species, during frozen storage the TBA values of both brine- and plate-frozen outside muscle increased to a maximum then decreased. With all three species, the differences in TBA values between brine- and plate-frozen outside muscle samples rapidly increased and reached a maximum at 45, 26 or 27 weeks then decreased until there was approximately no difference at 81, 77, and 78 weeks of frozen storage for halibut, chinook, and coho salmon, respectively.

The TBA values for the inside muscle of all three species were relatively constant during frozen storage and there appeared to be little difference between samples frozen by the two different methods.

When expressed as percent of total free fatty acids analyzed there was a significant difference between freezing methods for only free fatty acids 15:0 of halibut and 17:0 of chinook salmon. In both cases the percentage was greatest in the brine-frozen samples. Although for each species there were a fair number of free fatty acids that differed significantly ($P \leq 0.05$) among frozen storage times the changes were erratic and no meaningful trends could be discerned.

When expressed a μg per gram of neutral lipid brine-frozen halibut contained significantly ($P \leq 0.05$) more free fatty acids 14:0, 17:0, 18:1, 18:3, and 20:2, and brine frozen chinock salmon contained significantly ($P \leq 0.05$) less 16:0, 18:2, and 22:6 than the corresponding plate-frozen samples. In general, with all three species, although not always significant, the concentration of the individual free fatty acids was greater in the inside than in the outside muscle. Also for halibut and chinook salmon, particularly where there was a significant difference among storage times, the concentration of the free fatty acids appeared to rapidly increase during the first 26 to 31 weeks of frozen storage.

Total free fatty acids analyzed per µg of neutral lipid was significantly ($P \leq 0.05$) lower in the brine-frozen than in the plate-frozen chinook salmon whereas there were no significant differences between freezing methods for halibut and coho salmon.

The concentration of total free fatty acids was highly significantly ($P \leq 0.01$) greater in the inside than in the outside muscle for halibut and chinook salmon. With coho salmon the concentration of total free fatty acids did not differ significantly between locations.

The concentration of total free fatty acids differed significantly ($P \leq 0.05$) among storage times for only chinook salmon. There was a large significant ($P \leq 0.05$) increase between 9 and 26 weeks of storage. Although not significant there was a fairly large increase in the concentration of total free fatty acids between 14 and 31 weeks of storage for halibut.

With all three species the sodium and chloride concentrations of the brine-frozen outside muscle was two to three times greater than that of the brine-frozen inside, plate-frozen inside and plate-frozen outside muscle samples. Method of freezing had a small and variable effect upon potassium concentration of the flesh.

There was a significant ($P \leq 0.05$) negative correlation ($r = -0.358$) between pH and thaw drip of halibut. There

were also highly significant ($P \leq 0.01$) negative correlations between pH and Hunter 'a' values ($r = -0.596$) and pH and Hunter a/b ratios ($r + -0.721$) for coho salmon.

With halibut flavor was not significantly correlated with any of the other parameters measured. Only the Hunter b values ($r = -0.52$), the TBA values ($r = +0.460$) and free fatty acid 20:1 (expressed as percent of total free fatty acids analyzed ($r = -0.410$) correlated significantly ($P = 0.01$) with flavor of chinook salmon. With coho salmon only the Hunter 'a' ($r = +0.653$) values and free fatty acid 18:1 (expressed as percent of total free fatty acids analyzed) ($r = +0.515$) correlated significantly with flavor.

The effect of brine-freezing upon most variables measured was either small and/or complex. For all three species the sodium and chloride concentration was two to three times greater in the brine-frozen outside muscle than in all other samples. When the concentration (µg per gram of neutral lipid) of free fatty acids differed significantly ($P \leq 0.05$) among frozen storage times the free fatty acids increased most rapidly during the first 26 to 31 weeks of storage. The taste panel results and the TBA values indicate that brine-freezing does impair the quality of the outside muscle of halibut and chinook salmon during the early stages of frozen storage in comparison to plate-freezing.

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APPENDIX

TABLE I Analyses of variance of free fatty acids (expressed as percent of the total free fatty acid analyzed) of Pacific halibut[1]

		14:0	15:0	16:0	16:1	17:0	16:2
Source	d.f.	M.S.[3]	M.S.	M.S.	M.S.	M.S.	M.S.
Pairs	2	7.80	0.50	77.07	4.55	2.19	0.38
Method[2]	1	4.09	0.49*	77.41	145.03	1.27	1.27
Error a	2	3.64	0.02	6.21	25.47	2.11	0.53
Location	1	65.32	0.01	335.06	281.93*	6.53*	0.90
M x L	1	1.39	0.05	41.66	0.58	3.97	1.21
Error b	4	0.37	0.20	231.86	33.28	0.57	0.25
Time	4	40.94	0.40	310.04*	108.84*	5.90	1.43**
M x T	4	0.66	0.07	62.89	12.87	3.33*	0.44
L x T	4	9.50*	0.37	63.40	29.24	2.44	0.67
M x L x T	4	2.53	0.14	45.44	24.56	0.90	0.35
Error c	32	2.97	0.23	95.72	28.62	1.22	0.29
		18:0	18:1	18:2	18:3	18:4	20:1
Pairs	2	2.93	184.12	1.40	0.03	1.98	58.55
Method[3]	1	847.16	865.61	0.15	0.40	23.64	372.47
Error a	2	62.13	396.77	0.95	0.48	1.69	54.21
Location	1	358.40*	52.72	8.79*	1.09	20.64**	60.69**
M x L	1	0.68	30.40	0.05	0.54*	4.11	0.50
Error b	4	40.77	13.49	0.86	0.06	0.56	1.14
Time	4	30.51	36.15	3.10*	0.98	3.14**	418.08***
M x T	4	20.41	9.07	0.81	0.18	0.80	1.86
L x T	4	14.52	21.91	1.40	0.17	0.51	16.77
M x L x T	4	6.45	62.51	1.00	0.39*	0.69	3.00
Error c	32	519.89	37.97	1.02	0.13	0.75	6.45

TABLE I (Continued)

		20:2	20:4	20:5	22:1	22:5	22:6
Pairs	2	0.24	36.20	218.05	9.52	4.98	192.54
Method	1	0.19	225.39	843.65	582.37	8.89	61.52
Error a	2	0.32	96.67	127.79	68.65	265.62	865.71
Location	1	0.00027	23.01	1.46	216.54**	2.36	676.40
M x L	1	0.00027	37.25	15.03	28.96	1.23	67.85
Error b	4	0.17	32.95	57.60	5.42	1.21	96.62
Time	4	0.59***	27.22	94.81	50.54**	7.17**	724.28***
T x M	4	0.069	28.99	8.40	18.13	0.80	43.63
T x L	4	0.088	24.02	161.01**	19.16	1.64	213.04
T x M x L	4	0.027	31.37	21.48	0.43	2.24	236.95
Error c	32	0.070	27.74	29.43	11.04	1.80	96.76

1 Analyses of variance was conducted on arcsin transformed percentages.

2 Methods were test by error a.
Location and M x L were tested using error b.
All other terms were tested by error c.

3 Mean square values are all multiplied by $1.0 \times 10^{+5}$

* Significant at the 5% level ($P \leq 0.05$)
** Significant at the 1% level ($P \leq 0.01$)
*** Significant at the 0.1% level ($P \leq 0.001$)

TABLE II. Method x location x time interaction means from the analyses of variance of the free fatty acids (expressed as percent of total free fatty acids analyzed) of Pacific halibut[1].

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outstide Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
14:0	14 weeks	1.98[2]	1.99	1.97	1.99
	31 "	1.54	1.78	2.66	2.52
	45 "	1.91	2.27	2.41	2.73
	62 "	1.54	1.39	1.71	2.06
	81 "	2.06	2.90	4.06	3.85
15:0	14 weeks	0.41	0.34	0.38	0.27
	31 "	0.31	0.21	0.46	0.43
	45 "	0.36	0.50	0.38	0.26
	62 "	0.38	0.23	0.34	0.26
	81 "	0.22	0.21	0.26	0.22
16:0	14 weeks	18.27	15.19	16.09	14.43
	31 "	18.39	20.37	16.19	15.76
	45 "	14.12	14.43	13.37	12.88
	62 "	15.56	15.14	13.92	14.47
	81 "	15.54	15.75	17.82	14.02
16:1	14 weeks	4.67	5.90	5.47	6.70
	31 "	4.73	5.03	3.52	6.37
	45 "	5.47	5.94	7.30	6.91
	62 "	3.61	3.67	5.70	6.81
	81 "	4.88	7.39	7.83	8.22
16:2	14 weeks	0.29	0.28	0.33	0.17
	31 "	0.37	0.53	0.22	0.45
	45 "	0.51	0.34	0.44	0.67
	62 "	0.16	0.13	0.16	0.40
	81 "	0.14	0.20	0.26	0.62
17:0	14 weeks	0.94	0.83	1.05	1.01
	31 "	1.29	1.38	0.99	1.37
	45 "	1.60	1.15	1.57	1.35
	62 "	1.13	0.75	1.05	1.59
	81 "	0.97	1.47	1.50	2.11
18:0	14 weeks	8.91	5.62	6.51	4.25
	31 "	8.01	5.58	6.62	4.58
	45 "	6.64	6.06	6.59	5.19
	62 "	8.65	6.52	6.90	3.92
	81 "	7.90	4.39	5.66	2.94

TABLE II (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
18:1	14 weeks	17.23	14.71	17.75	15.16
	31 "	17.11	14.99	16.31	15.31
	45 "	15.78	16.01	19.14	14.38
	62 "	18.68	15.56	17.49	16.20
	81 "	17.07	15.23	19.54	16.10
18:2	14 weeks	1.19	1.02	1.09	0.92
	31 "	1.46	1.19	1.42	1.75
	45 "	1.03	1.33	1.42	1.40
	62 "	1.26	1.26	1.54	1.70
	81 "	1.14	1.03	1.73	1.30
18:3	14 weeks	0.36	0.44	0.34	0.52
	31 "	0.33	0.22	0.35	0.35
	45 "	0.46	0.55	0.59	0.52
	62 "	0.51	0.32	0.49	0.78
	81 "	0.34	0.43	0.37	0.53
18:4	14 weeks	0.09	0.37	0.23	0.66
	31 "	0.18	0.22	0.26	0.92
	45 "	0.41	0.54	0.62	0.90
	62 "	0.23	0.28	0.43	1.19
	81 "	0.21	0.85	0.60	1.28
20:1	14 weeks	4.17	6.01	3.96	5.45
	31 "	4.23	5.56	4.94	6.47
	45 "	5.91	6.49	6.24	7.55
	62 "	5.04	7.41	5.85	7.27
	81 "	7.41	7.27	7.08	8.82
20:2	14 weeks	0.16	0.11	0.06	0.07
	31 "	0.29	0.31	0.25	0.28
	45 "	0.27	0.20	0.29	0.23
	62 "	0.21	0.19	0.20	0.21
	81 "	0.17	0.12	0.29	0.14
20:4	14 weeks	1.72	1.21	2.01	1.49
	31 "	6.32	1.11	2.11	1.47
	45 "	2.29	1.68	2.23	1.54
	62 "	2.50	1.54	2.73	1.54
	81 "	2.41	1.10	1.73	1.13

TABLE II (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
20:5	14 weeks	11.11	14.06	13.17	15.74
	31 "	9.75	12.37	11.79	13.59
	45 "	12.23	14.03	11.12	14.40
	62 "	12.70	15.11	13.15	15.01
	81 "	14.20	14.17	8.94	12.05
22:1	14 weeks	0.96	2.33	1.00	3.30
	31 "	0.98	2.52	1.75	4.33
	45 "	1.72	2.40	2.34	4.11
	62 "	1.46	2.53	1.59	3.51
	81 "	0.88	3.87	3.12	6.58
22:5	14 weeks	1.33	1.49	1.85	1.07
	31 "	1.36	1.37	1.87	1.50
	45 "	2.03	1.84	1.95	1.47
	62 "	2.14	1.56	2.31	2.08
	81 "	1.88	1.71	1.83	2.02
22:6	14 weeks	23.94	25.84	24.62	24.64
	31 "	21.83	23.09	25.89	20.89
	45 "	25.14	22.48	20.35	21.96
	62 "	22.23	24.25	22.59	19.45
	81 "	22.64	20.23	15.75	16.77

1 The analyses were conducted on the arcsine transformed percentages but the actual percentages (not transformed) are recorded in TABLE II.

2 Each value is the average of 3 observations.

TABLE III Analyses of variance of free fatty acids (expressed as μg free fatty acid per gram of neutral lipid) of Pacific halibut

		14:0	15:0	16:0	16:1	16:2	17:0
Source	d.f.	M.S.[1]	M.S.	M.S.	M.S.	M.S.	M.S.
Pairs	2	12.54	0.98	2203.00*	162.5*	1.37	7.31*
Method[2]	1	19.12*	0.56	2756.00	143.52	0.86	10.19*
Error a	2	0.61	0.04	299.28	17.56	0.13	0.15
Location	1	68.38**	3.04	7714.00*	558.88*	2.92	32.66**
M x L	1	5.65	0.20	1663.20	71.75	0.87	6.52
Error b	4	1.96	0.23	514.28	30.74	0.40	1.06
Time	4	10.79*	0.46	773.06	77.54	0.86	4.23
M x T	4	3.02	0.25	409.26	33.15	0.33	1.05
L x T	4	5.13	0.30	689.17	53.00	0.70	3.11
M x L x T	4	2.33	0.23	404.04	38.90	0.36	1.35
Error c	32	3.85	0.26	628.72	45.13	0.60	2.16

		18:0	18:1	18:2	18:3	18:4	20:1
Pairs	2	203.07*	1247.60*	5.43**	0.88*	0.12949	79.43*
Method	1	620.26	2777.90*	10.02	0.94*	0.00014	97.46
Error a	2	27.83	121.54	0.90	0.04	0.02179	30.06
Location	1	1394.40	6479.80**	27.61**	3.47**	0.64833***	604.90**
M x L	1	356.31*	1319.50	3.51	0.39	0.00006	46.30
Error b	4	37.76	186.11	1.08	0.12	0.00280	21.45
Time	4	99.47	539.84	2.59*	0.37	0.27891***	76.35*
M x T	4	52.66	294.60	1.55	0.21	0.05578	15.34
L x T	4	74.01	384.27	1.76	0.24	0.06924	35.09
M x L x T	4	47.98	306.63	1.33	0.25	0.08484	11.19
Error c	32	62.05	303.20	0.93	0.20	0.04440	20.31

TABLE III (Continued)

		20:2	20:4	20:5	22:1	22:5	22:6
Pairs	2	0.411	16.65	1126.60**	8.964**	11.48	2396.10
Method	1	0.483*	82.57	960.18	0.009	30.44	4614.60
Error a	2	0.007	1.16	160.09	3.601	0.21	481.04
Location	1	1.414*	133.72	4309.00*	25.141**	73.57	15429.00**
M x L	1	0.264	48.28*	581.08	0.048	15.85*	2788.40
Error b	4	0.104	3.09	223.02	0.726	1.11	500.91
Time	4	0.230	13.08	363.73	5.957*	7.25	1298.70
M x T	4	0.113	10.37	162.53	1.561	1.87	397.36
L x T	4	0.163	12.29	288.89	1.734	5.22	1035.00
M x L x T	4	0.116	10.96	138.36	1.778	1.83	394.46
Error c	32	0.178	5.06	206.95	1.530	4.53	817.26

1 All mean square values are multiplied by 1.0×10^{-6}.

2 Methods were tested by error a.
Location and M x L were tested using error b.
All other terms were tested by error c.

* Significant at the 5% level ($P \leq 0.05$)
** Significant at the 1% level ($P \leq 0.01$)
*** Significant at the 0.1% level ($P \leq 0.001$)

TABLE IV Method x location x time interaction means from the analyses of variance of the free fatty acids (expressed as μg free fatty acid per gram of neutral lipid) of Pacific halibut

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
14:0	14 weeks	1128.4[1]	368.2	688.8	99.4
	31 "	5903.7	1474.8	786.9	396.8
	45 "	4215.7	4671.0	874.5	890.1
	62 "	2732.6	760.7	549.9	307.4
	81 "	4826.0	2819.2	2161.4	792.2
15:0	14 weeks	237.4	66.2	162.4	13.6
	31 "	1132.2	166.6	129.9	48.9
	45 "	817.6	1377.8	188.7	161.8
	62 "	666.5	129.4	112.3	39.4
	81 "	677.8	243.8	109.3	50.1
16:0	14 weeks	10515.6	3221.1	6513.8	739.3
	31 "	79619.9	16563.6	4901.5	2368.5
	45 "	33961.1	27829.4	6189.7	7033.2
	62 "	28666.6	8285.5	4018.0	2152.5
	81 "	43944.3	20383.9	9047.5	3252.7
16:1	14 weeks	2661.7	991.2	1560.0	330.1
	31 "	20494.9	4272.3	1058.1	1080.5
	45 "	9872.9	12048.4	2927.6	2344.7
	62 "	6555.7	1945.6	1662.7	1050.8
	81 "	12971.6	6898.1	3903.4	1764.6
16:2	14 weeks	196.3	53.7	114.5	8.7
	31 "	2122.3	437.6	66.8	101.1
	45 "	928.8	822.6	175.1	251.2
	62 "	294.3	66.3	52.8	56.3
	81 "	404.9	167.3	126.1	122.1
17:0	14 weeks	572.4	168.2	353.5	51.6
	31 "	4841.9	1269.5	274.8	245.7
	45 "	3025.8	2361.3	600.9	558.5
	62 "	2026.7	426.1	329.1	226.4
	81 "	2543.7	1368.9	778.6	429.7
18:0	14 weeks	4705.8	1231.0	2705.1	216.6
	31 "	26261.2	4353.4	2001.7	746.6
	45 "	17485.6	14438.8	3193.5	3085.1
	62 "	15887.5	3728.7	1854.4	559.4
	81 "	21239.3	5306.0	3247.3	611.2

TABLE IV (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
18:1	14 weeks	9917.2	2589.4	6181.8	756.2
	31 "	60580.9	10543.3	4913.0	2286.3
	45 "	34699.3	34752.9	8820.8	5744.7
	62 "	34814.0	8471.5	5319.3	2408.0
	81 "	46622.3	15338.8	10582.8	3473.7
18:2	14 weeks	682.9	209.9	504.4	46.3
	31 "	3708.4	923.1	433.4	261.4
	45 "	2233.0	2877.6	567.3	421.3
	62 "	2347.9	685.6	553.9	239.4
	81 "	3152.7	1015.2	954.2	287.1
18:3	14 weeks	200.3	73.1	164.8	25.7
	31 "	1275.9	175.4	100.6	61.6
	45 "	846.5	1162.2	256.1	164.1
	62 "	946.5	189.2	188.4	112.1
	81 "	846.6	462.6	199.0	98.8
18:4	14 weeks	40.6	44.2	52.4	32.6
	31 "	527.3	193.6	77.9	139.6
	45 "	390.4	762.0	213.2	173.3
	62 "	405.2	152.4	162.9	171.1
	81 "	455.9	692.4	283.5	278.6
20:1	14 weeks	2237.5	1079.1	1236.6	272.5
	31 "	14121.2	4320.5	1505.9	943.8
	45 "	12216.5	12826.8	2670.7	2946.2
	62 "	9278.1	4198.2	1757.8	1034.8
	81 "	13691.3	7590.9	3838.4	1851.1
20:2	14 weeks	108.6	23.0	18.9	3.3
	31 "	1191.0	209.0	70.9	36.1
	45 "	549.2	543.6	137.6	92.0
	62 "	375.1	122.5	53.4	28.7
	81 "	397.5	163.0	142.0	29.3
20:4	14 weeks	1089.2	219.5	804.3	74.7
	31 "	11207.2	944.4	612.8	222.9
	45 "	5224.5	3890.8	983.6	735.5
	62 "	4390.5	830.2	961.8	216.7
	81 "	6236.5	1562.2	886.7	238.5

TABLE IV (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
20:5	14 weeks	6735.7	2534.2	4926.6	793.0
	31 "	42409.6	10155.9	3604.7	2258.2
	45 "	26434.3	29085.8	5017.2	6734.7
	62 "	23352.8	8771.1	5028.9	2185.0
	81	40346.8	17606.1	4834.9	2557.9
22:1	14 weeks	477.4	355.3	283.9	170.4
	31 "	3455.8	2000.9	540.3	599.5
	45 "	1829.9	3659.7	943.1	1134.6
	62 "	2680.9	1381.6	553.8	524.0
	81 "	1981.6	3188.3	1914.6	1400.8
22:5	14 weeks	851.7	269.1	694.1	55.4
	31 "	5252.6	1113.6	543.5	236.7
	45 "	4913.2	3832.8	726.4	651.0
	62 "	3718.9	824.1	663.0	307.5
	81 "	5133.8	1567.9	1030.0	423.9
22:6	14 weeks	14342.0	5844.1	10092.0	1299.6
	31 "	88118.4	20767.9	8051.7	3536.9
	45 "	65744.5	48089.6	8737.8	11949.2
	62 "	41765.0	14384.6	8140.7	2971.7
	81 "	61437.8	26452.8	7855.4	3593.1

[1] Each mean is the average of 3 observations.

TABLE V Analyses of variance of free fatty acids (expressed as percent of the total free fatty acids analyzed) of Chinook Salmon[1].

		14:0	15:0	16:0	16:1	16:2	17:0
Source	d.f.	M.S.[2]	M.S.	M.S.	M.S.	M.S.	M.S.
Pairs	2	2.39	0.26865*	73.91	23.58	0.46	2.67
Method[3]	1	36.13	0.04507	64.36	128.30	0.28	2.28*
Error a	2	74.31	0.00522	166.03	14.54	0.13	0.03
Location	1	3.16	0.00007	9.95	54.81	0.72	4.17
M x L	1	10.24	0.00427	9.12	5.86	1.06	0.38
Error b	4	0.74	0.01277	90.32	18.96	0.54	3.00
Time	4	12.31	0.69965***	1332.90	33.30	3.65	6.40**
M x T	4	3.68	0.06573	26.30	16.86	0.17	0.65
L x T	4	4.10	0.07073	143.45	2.21	0.01	0.28
M x L x T	4	0.71	0.03168	179.50*	2.98	0.78*	2.91
Error c	32	6.49	0.07954	58.34	12.92	0.27	1.27

		18:0	18:1	18:2	18:3	18:4	20:1
Pairs	2	11.20	292.34	0.55	0.069	5.24	85.230
Method	1	52.58	70.45	1.18	0.232	0.87	9.463
Error a	2	11.61	543.81	2.92	2.695	18.06	75.094
Location	1	2.87	12.17	8.46	1.110*	3.04	1.147
M x L	1	4.60	9.02	0.12	0.003	1.94	0.006
Error b	4	1.21	47.50	5.25	0.070	1.18	12.523
Time	4	16.55*	275.47**	52.29***	3.352**	15.87***	63.175***
M x T	4	9.64	37.62	5.23	0.623	1.49	3.324
L x T	4	6.15	19.33	1.70	0.904	0.63	2.019
M x L x T	4	3.08	67.47	1.15	0.190	1.43	4.149
Error c	32	5.70	69.60	2.53	0.736	2.38	4.667

TABLE V (Continued)

		20:2	20:4	20:5	22:1	22:5	22:6
Pairs	2	2.33*	4.1924**	109.54	55.92	2.86	123.94*
Method	1	0.11	0.3174	106.20	5.23	25.46	1090.90
Error a	2	0.05	0.5202	192.63	71.55	29.25	128.92
Location	1	0.60	0.6017	2.49	5.08	4.24	25.48
M x L	1	0.04	0.0006	0.09	0.21	3.03	1.38
Error b	4	0.29	0.3079	13.16	3.35	5.94	9.77
Time	4	5.27***	1.3597	80.99	15.51	5.67	90.21*
M x T	4	0.26	0.7080	8.95	8.47	4.27	19.96
L x T	4	0.17	0.4411	24.67	26.97**	4.66	65.52
M x L x T	4	0.48	0.4775	7.42	7.88	2.53	2.82
Error c	32	0.50	0.5207	38.44	5.85	4.04	33.55

1 Analyses of variance was conducted on the arcsin transformed percentages.

2 All mean square values are multiplied by $1.0 \times 10^{+5}$.

3 Methods were tested by error a.
Location and M x L were tested using error b.
All other terms were tested using error c.

* Significant at the 5% level ($P \leq 0.05$).
** Significant at the 1% level ($P \leq 0.01$).
*** Significant at the 0.1% level ($P \leq 0.001$).

TABLE VI Method x location x time interaction means from the analyses of variance of the free fatty acids (expressed as percent of total free fatty acids analyzed) of chinook salmon[1]

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
14:0	9 weeks	4.70[2]	4.58	4.81	3.64
	26 "	4.63	4.18	5.20	4.35
	40 "	5.42	5.06	5.46	4.85
	58 "	5.39	4.83	5.02	4.05
	77 "	4.89	5.26	5.13	4.99
15:0	9 weeks	0.31	0.38	0.27	0.37
	26 "	0.17	0.19	0.23	0.18
	40 "	0.30	0.33	0.38	0.38
	58 "	0.30	0.30	0.28	0.21
	77 "	0.19	0.19	0.14	0.22
16:0	9 weeks	21.39	17.14	19.62	23.35
	26 "	20.47	20.14	18.45	16.28
	40 "	13.93	14.80	13.27	12.87
	58 "	14.66	14.23	15.49	14.16
	77 "	14.09	14.38	15.69	13.64
16:1	9 weeks	8.33	8.33	7.87	7.03
	26 "	9.10	8.26	8.67	8.03
	40 "	9.93	8.78	9.49	8.61
	58 "	9.96	8.43	9.72	7.07
	77 "	9.34	8.70	8.93	8.47
16:2	9 weeks	0.30	0.31	0.38	0.32
	26 "	0.73	0.51	0.46	0.96
	40 "	0.62	0.61	0.60	0.75
	58 "	0.23	0.33	0.32	0.38
	77 "	0.38	0.30	0.43	0.43
17:0	9 weeks	0.59	0.81	1.02	0.71
	26 "	1.45	0.81	0.99	1.35
	40 "	1.49	1.01	1.52	1.45
	58 "	1.04	0.79	1.15	0.90
	77 "	0.97	1.24	1.44	1.33
18:0	9 weeks	3.26	4.21	3.28	4.45
	26 "	2.45	3.23	2.65	3.61
	40 "	3.29	3.89	3.77	4.62
	58 "	3.60	4.44	3.14	3.67
	77 "	3.80	2.70	3.37	3.69

TABLE VI (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
18:1	9 weeks	23.73	23.89	24.25	21.98
	26 "	20.87	22.40	23.09	20.98
	40 "	24.52	24.88	24.66	25.30
	58 "	25.27	23.83	25.35	25.95
	77 "	25.28	22.50	25.28	23.38
18:2	9 weeks	1.17	1.46	1.45	1.33
	26 "	3.38	2.32	3.65	3.10
	40 "	1.76	1.84	2.09	2.18
	58 "	1.90	1.94	1.93	1.76
	77 "	2.22	2.28	2.35	2.80
18:3	9 weeks	0.98	0.99	1.06	1.02
	26 "	1.21	1.45	0.98	1.29
	40 "	1.26	1.08	1.42	1.41
	58 "	1.36	1.32	1.50	1.48
	77 "	0.87	1.06	1.17	1.11
18:4	9 weeks	1.08	1.01	1.22	0.98
	26 "	1.61	1.61	1.70	1.57
	40 "	1.89	1.46	2.11	1.88
	58 "	1.87	1.79	2.19	2.04
	77 "	1.49	2.26	2.01	1.80
20:1	9 weeks	3.42	4.00	3.76	3.56
	26 "	3.42	3.55	3.83	3.92
	40 "	4.69	4.98	5.16	4.74
	58 "	5.48	5.32	4.83	5.42
	77 "	4/32	4.78	4.21	5.37
20:2	9 weeks	0.47	0.07	0.10	0.14
	26 "	0.67	0.73	0.63	0.53
	40 "	0.24	0.18	0.13	0.14
	58 "	0.11	0.23	0.23	0.16
	77 "	0.11	0.18	0.10	0.18
20:4	9 weeks	0.77	0.52	0.78	0.47
	26 "	0.48	0.37	0.53	0.75
	40 "	0.46	0.31	0.37	0.39
	58 "	0.29	0.37	0.61	0.39
	77 "	0.33	0.53	0.36	0.41

TABLE VI (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
20:5	9 weeks	14.14	14.02	1398	12.69
	26 "	12.09	11.37	12.57	12.73
	40 "	14.30	13.07	14.29	13.19
	58 "	14.14	14.40	13.42	12.56
	77 "	15.03	13.21	14.99	13.98
22:1	9 weeks	2.51	2.51	3.34	2.27
	26 "	3.29	2.08	4.16	4.24
	40 "	3.21	2.48	3.17	2.84
	58 "	3.89	3.60	2.39	2.77
	77 "	1.96	3.06	2.53	2.73
22:5	9 weeks	1.83	2.55	2.37	2.36
	26 "	1.99	2.17	1.76	2.32
	40 "	2.56	2.32	2.20	2.60
	58 "	1.75	2.49	2.24	3.69
	77 "	2.49	2.80	2.18	2.92
22:6	9 weeks	8.71	11.42	8.28	11.08
	26 "	9.83	12.65	8.58	12.30
	40 "	9.51	10.97	8.05	9.94
	58 "	5.76	9.60	8.19	11.28
	77 "	10.44	12.47	8.74	10.83

1 The analyses were conducted on the arcsine transformed percentages but the actual percentages (not transformed) are recorded in TABLE VI.

2 Each value is the average of 3 observations.

TABLE VII Analyses of variance of free fatty acids (expressed as μg free fatty acid per gram of neutral lipid) of Chinook salmon.

		14:0	15:0	16:0	16:1	16:2	17:0
Source	d.f.	M.S.[1]	M.S.	M.S.	M.S.	M.S.	M.S.
Pairs	2	33.86*	0.045	408.40**	110.50**	0.83*	3.83*
Method[2]	1	7.87	0.105	178.72	20.49	0.16	0.0008
Error a	2	14.32	0.021	4.00	1.99	0.14	0.32
Location	1	336.19	0.930**	3925.20	1215.80	2.85	12.54
M x L	1	31.83*	0.107	283.44	90.73	0.006	0.33
Error b	4	1.94	0.014	106.86	13.19	0.54	1.44
Time	4	85.01	0.237***	411.48	255.43	1.60	6.38
M x T	4	12.08	0.041	73.31	38.40	0.40	1.08
L x T	4	7.33	0.009	273.12*	32.70	0.52	1.10
M x L x T	4	20.10*	0.011	171.04	61.22*	0.30	2.84*
Error c	32	6.72	0.037	69.00	20.09	0.18	0.98

		18:0	18:1	18:2	18:3	18:4	20:1
Pairs	2	12.92	809.55*	3.21*	1.69	8.61**	66.26***
Method	1	36.21	567.07	1.78**	0.88	0.79	38.09
Error a	2	8.32	126.58	0.01	0.44	1.96	26.37
Location	1	143.97**	7833.20**	59.22**	15.63*	34.32	249.11*
M x L	1	15.56	815.31	1.36	1.26	4.21	22.93
Error b	4	3.48	197.07	2.09	0.76	1.35	3.53
Time	4	33.52***	1787.50***	21.73***	4.19***	14.13	86.43*
M x T	4	3.01	136.20	3.06	0.33	2.45	10.07
L x T	4	2.49	259.96	5.63	1.09	1.62	5.22
M x L x T	4	2.02	237.21	5.45	1.00	6.22**	12.27
Error c	32	4.99	164.01	2.27	0.53	1.48	5.76

TABLE VII (Continued)

		20:2	20:4	20:5	22:1	22:5	22:6
Pairs	2	0.11	0.86**	130.41*	44.80***	11.02***	276.10***
Method	1	0.07	0.09	119.55	4.20	26.38	661.92*
Error a	2	0.12	0.17	41.95	17.81	4.65	25.54
Location	1	1.82	2.22*	2568.00**	112.67	72.26**	1733.20
M x L	1	0.10	0.21	200.37	2.71	7.04	283.04
Error b	4	0.06	0.18	52.69	4.81	1.91	37.94
Time	4	0.87	0.31	536.58***	22.03	19.56***	314.11
M x T	4	0.02	0.15	26.04	8.02	1.12	19.01
L x T	4	0.54**	0.18	44.52	2.33	3.32	91.46*
M x L x T	4	0.02	0.22	96.87	13.19**	2.91	33.64
Error a	32	0.11	0.13	38.00	2.49	1.48	28.76

1 All mean square values are multiplied by 1.0×10^{-4}.

2 Methods were tested by error a.
Location and M x L were tested using error b.
All other terms were tested by error c.

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

TABLE VIII Method x location x time interaction means from the analyses of variance of the free fatty acids (expressed as μg free fatty acid per gram of neutral lipid) of chinook salmon.

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
14:0	9 weeks	275.0[1]	531.6	146.0	137.5
	26 "	920.9	781.7	227.1	302.7
	40 "	1102.4	1270.4	588.8	530.4
	58 "	906.0	758.6	560.9	385.5
	77 "	728.5	1681.3	771.4	571.9
15:0	9 weeks	18.3	44.7	7.9	13.6
	26 "	37.2	37.8	9.1	11.8
	40 "	59.6	87.2	38.7	40.8
	58 "	50.9	48.2	34.7	19.4
	77 "	27.6	59.8	20.9	25.2
16:0	9 weeks	1200.6	2135.0	613.6	958.7
	26 "	4380.9	4071.8	800.2	1269.2
	40 "	2659.7	3881.9	1454.2	1452.6
	58 "	2690.0	2333.6	1779.7	1343.1
	77 "	2053.9	4462.3	2422.8	1599.3
16:1	9 weeks	465.9	1010.2	233.7	299.4
	26 "	1820.7	1561.7	348.2	563.5
	40 "	1977.4	2347.7	1019.7	955.0
	58 "	1713.4	1343.8	1090.2	656.9
	77 "	1374.3	2902.4	1388.1	959.8
16:2	9 weeks	14.3	40.3	11.6	12.9
	26 "	175.2	117.2	20.5	68.5
	40 "	124.9	164.5	63.5	78.3
	58 "	38.6	53.1	41.2	35.3
	77 "	56.5	95.6	65.0	47.9
17:0	9 weeks	36.9	96.7	31.2	30.2
	26 "	324.9	180.2	43.2	100.9
	40 "	300.9	256.9	159.3	156.1
	58 "	176.9	124.3	137.4	84.6
	77 "	139.8	396.6	225.4	151.5
18:0	9 weeks	159.7	505.4	96.7	185.0
	26 "	502.6	629.4	104.6	248.6
	40 "	627.8	1085.3	387.3	517.9
	58 "	608.7	703.1	333.5	342.9
	77 "	575.3	837.1	512.4	407.4

TABLE VIII (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
18:1	9 weeks	1235.6	3081.7	755.0	937.3
	26 "	4445.7	4785.6	986.4	1562.7
	40 "	5012.3	6958.1	2757.7	2932.0
	58 "	4524.0	4012.9	3041.4	2546.9
	77 "	3961.5	7101.5	3898.9	2848.4
18:2	9 weeks	66.3	185.3	43.4	52.9
	26 "	747.6	466.6	139.7	213.1
	40 "	346.0	486.3	216.4	237.4
	58 "	317.5	307.9	211.3	169.1
	77 "	335.7	690.0	359.5	319.3
18:3	9 weeks	46.7	123.2	32.0	42.1
	26 "	272.6	254.4	38.6	92.4
	40 "	253.6	295.4	152.5	150.4
	58 "	229.7	208.9	167.1	136.5
	77 "	131.6	315.4	178.3	120.9
18:4	9 weeks	69.8	129.3	37.3	40.4
	26 "	369.6	257.4	74.1	123.8
	40 "	397.9	378.4	233.3	196.1
	58 "	311.5	286.9	234.2	189.9
	77 "	225.2	701.5	304.0	182.1
20:1	9 weeks	201.9	496.5	114.6	152.8
	26 "	668.1	639.3	156.3	301.4
	40 "	964.3	1281.8	540.4	541.7
	58 "	913.7	857.8	520.1	499.9
	77 "	644.6	1532.0	641.9	656.1
20:2	9 weeks	9.8	9.0	3.2	6.2
	26 "	122.8	142.0	26.8	25.3
	40 "	52.6	54.5	12.9	13.9
	58 "	18.9	36.2	28.6	14.3
	77 "	17.0	54.8	16.0	22.0
20:4	9 weeks	38.5	53.9	23.7	20.4
	26 "	103.8	84.6	25.5	42.7
	40 "	101.3	93.9	37.9	42.8
	58 "	50.7	60.3	68.9	35.6
	77 "	49.7	148.2	54.3	47.8

TABLE VIII (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
20:5	9 weeks	737.4	1778.1	421.5	546.6
	26 "	2409.3	2344.1	530.9	859.1
	40 "	2848.2	3494.8	1505.1	1461.1
	58 "	2503.6	2330.2	1274.4	1181.1
	77 "	2270.9	4061.2	2322.7	1590.9
22:1	9 weeks	145.9	305.1	102.5	103.3
	26 "	680.3	410.4	179.9	299.8
	40 "	674.2	600.6	341.1	317.1
	58 "	626.8	588.8	255.9	258.7
	77 "	291.6	991.2	381.8	334.1
22:5	9 weeks	83.3	325.8	69.2	101.8
	26 "	377.9	460.2	74.6	150.7
	40 "	515.7	640.9	225.2	296.9
	58 "	306.7	402.8	195.0	324.5
	77 "	366.8	826.2	331.5	342.5
22:6	9 weeks	459.2	1534.2	248.6	483.7
	26 "	1803.4	2543.9	365.4	917.6
	40 "	2000.6	2907.3	869.5	1108.4
	58 "	1009.5	1557.7	843.7	1053.3
	77 "	1584.4	3807.5	1327.3	1240.9

[1] Each value is the average of 3 observations.

TABLE IX Analyses of variance of free fatty acids (expressed as percent of the total free fatty acids analyzed) of Coho salmon[1].

		14:0	15:0	16:0	16:1	16:2	17:0
Source	d.f.	M.S.[2]	M.S.	M.S.	M.S.	M.S.	M.S.
Pairs	2	22.00*	0.147**	227.31	115.81*	0.61	3.46
Method[3]	1	1.28	0.187	120.15	36.27	0.39	1.65
Error a	2	38.36	0.206	21.24	5.94	0.09	4.31
Location	1	12.48	0.009	54.69	0.10	4.35	10.47
M x L	1	0.66	0.009	0.18	0.01	0.35	0.43
Error b	4	1.61	0.024	37.34	17.82	0.84	3.29*
Time	2	6.73	0.787***	39.48	335.62***	0.68	5.13*
M x T	2	1.33	0.004	20.50	78.46	0.25	0.44
L x T	2	3.05	0.003	51.28	6.28	0.20	0.65
M x L x T	2	0.40	0.002	248.00	6.96	0.55	0.03
Error c	16	3.05	0.019	102.80	29.33	0.35	0.84

		18:0	18:1	18:2	18:3	18:4	20:1
Pairs	2	4.62	131.72	0.2707	1.93	6.3806	3.50
Method	1	2.60	73.23	0.0001	0.84	0.1691	9.36
Error a	2	57.64	935.48	0.0751	6.17	14.9650	107.93
Location	1	1.14	11.43	7.2296	1.06	5.1376*	3.14
M x L	1	11.09*	35.25	0.4142	0.44	0.0001	2.78
Error b	4	0.88	83.52	0.5400	0.19	0.5353	4.24
Time	2	114.54***	357.48**	29.1910***	1.32	11.3670***	4.79
M x T	2	2.67	12.56	1.4854	0.38	0.9405	0.02
L x T	2	0.40	97.98	2.7911	0.14	0.0568	4.24
M x L x T	2	16.73	173.87	7.0184	0.10	0.5089	5.18
Error c	16	8.00	59.89	2.0885	0.55	0.6689	3.46

TABLE IX (Continued)

		20:2	20:4	20:5	22:1	22:5	22:6
Pairs	2	2.43	2.13*	151.82	4.85	2.29	282.89
Method	1	0.71	0.12	291.67	94.10	0.75	0.05
Error a	2	0.07	0.50	139.56*	207.95***	7.90	95.90
Location	1	1.50	0.37	0.22	5.46	22.72	187.03
M x L	1	0.62	0.01	33.47	0.35	14.57	2.67
Error b	4	2.21	0.42	34.97	1.20	3.82	82.31
Time	2	30.79***	9.11***	449.19	49.75*	16.77	56.91
T x M	2	0.15	0.65	35.16	2.60	8.38	2.97
T x L	2	1.80	0.14	7.59	11.76	8.65	8.16
T x M x L	2	1.10	0.27	115.60*	2.69	6.65	56.39
Error c	32	0.88	0.53	25.76	6.18	4.99	152.23

[1] Analyses of variance was conducted on the arcsin transformed percentages.

[2] All mean square values are multiplied by 1.0×10^5

[3] Methods were tested by error a.
Location and M x L were tested using error b.
All other terms were tested by error c.

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

TABLE X Method x location x time interaction means from the analyses of variance of the free fatty acids (expressed as percent of total free fatty acids analyzed) of coho salmon[1].

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
14:0	10 weeks	3.51[2]	3.63	3.60	3.93
	27 "	3.14	3.05	3.36	3.20
	78 "	3.03	3.10	3.58	4.04
15:0	10 weeks	0.32	0.27	0.33	0.27
	27 "	0.16	0.12	0.18	0.14
	78 "	0.17	0.15	0.20	0.14
16:0	10 weeks	18.48	13.98	13.29	15.02
	27 "	13.71	14.90	15.21	13.77
	78 "	15.63	15.64	16.92	13.65
16:1	10 weeks	8.78	6.90	9.39	7.92
	27 "	9.74	11.77	10.42	11.02
	78 "	6.87	7.53	6.42	8.20
16:2	10 weeks	0.27	0.36	0.58	0.49
	27 "	0.52	0.44	0.55	0.69
	78 "	0.26	0.26	0.40	0.73
17:0	10 weeks	0.73	0.90	1.00	0.97
	27 "	0.79	1.10	1.24	1.48
	78 "	1.03	1.16	1.54	1.53
18:0	10 weeks	3.22	3.06	3.49	2.98
	27 "	2.26	1.90	2.48	2.15
	78 "	4.66	3.63	3.46	4.83
18:1	10 weeks	21.46	19.58	20.57	22.09
	27 "	21.44	22.61	25.39	21.74
	78 "	21.48	20.57	20.00	18.20
18:2	10 weeks	1.45	1.48	1.77	1.26
	27 "	2.37	1.94	2.38	3.18
	78 "	1.88	2.08	2.20	2.11
18:3	10 weeks	1.20	0.95	1.25	1.04
	27 "	0.98	0.80	0.92	1.00
	78 "	1.05	0.97	1.18	1.22

TABLE X (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
18:4	10 weeks	1.49	1.45	1.80	1.52
	27 "	1.06	1.08	1.16	1.46
	78 "	1.74	1.64	2.04	1.89
20:1	10 weeks	4.01	4.76	4.40	4.27
	27 "	3.54	4.24	4.56	4.47
	78 "	4.58	4.63	4.26	4.92
20:2	10 weeks	0.56	0.24	0.26	0.50
	27 "	1.45	1.39	1.15	0.87
	78 "	0.34	0.21	0.31	0.32
20:4	10 weeks	0.99	1.14	1.06	1.04
	27 "	0.53	0.74	0.43	0.55
	78 "	0.72	0.54	0.60	0.57
20:5	10 weeks	14.99	16.53	15.23	15.06
	27 "	15.11	11.85	12.29	13.80
	78 "	17.03	16.26	16.84	17.38
22:1	10 weeks	2.75	4.33	3.27	4.37
	27 "	4.34	4.91	3.05	4.33
	78 "	2.57	3.29	2.43	3.28
22:5	10 weeks	1.30	2.94	3.24	2.99
	27 "	2.91	2.75	2.95	2.58
	78 "	3.05	3.04	3.78	3.47
22:6	10 weeks	13.55	14.93	13.98	12.59
	27 "	14.25	12.72	11.12	12.00
	78 "	13.12	13.67	12.18	12.15

1 The analyses were conducted on the arcsine transformed percentages but the actual percentages (not transformed) are recorded in TABLE X.

2 Each value is the average of 3 observations.

TABLE XI Analyses of variance of free fatty acids (expressed as μg free fatty acid per gram of neutral lipid) of Coho salmon.

		14:0	15:0	16:0	16:1	16:2	17:0
Source	d.f.	M.S.[1]	M.S.	M.S.	M.S.	M.S.	M.S.
Pairs	2	16.11	0.026	215.82	64.44	0.28	24.90
Method[2]	1	14.50	0.023	175.62	52.23	0.26	22.12
Error a	2	18.05	0.026	208.99	66.90	0.30	28.67
Location	1	22.10	0.038	313.03	94.82	0.34	33.28
M x L	1	14.83	0.023	176.00	54.10	0.28	22.59
Error b	4	17.67	0.028	220.22	69.35	0.31	27.84
Time	2	21.42	0.034	288.81	85.11	0.36	34.86
M x T	2	14.83	0.023	178.28	54.96	0.26	22.85
L x T	2	18.90	0.030	251.99	75.38	0.30	30.24
M x L x T	2	15.46	0.024	181.89	58.05	0.29	23.59
Error c	16	17.38	0.027	216.07	68.53	0.31	27.47

		18:0	18:1	18:2	18:3	18:4	20:1
Pairs	2	11.19	422.40	3.09	1.45	5.88	42.82
Method	1	8.62	348.25	2.36	1.20	5.09	36.64
Error a	2	10.27	405.71	3.09	1.59	6.36	44.20
Location	1	15.60	603.61	4.63	2.06	7.52	54.07
M x L	1	9.61	352.09	2.44	1.26	5.21	37.52
Error b	4	11.20	437.03	3.22	1.55	6.25	44.30
Time	2	16.51	558.01	4.43	2.03	7.67	52.65
M x T	2	8.81	257.71	2.41	1.22	5.23	37.78
L x T	2	13.27	489.40	3.80	1.75	6.79	47.74
M x L x T	2	10.16	372.20	2.50	1.30	5.41	39.36
Error c	16	11.00	432.91	3.20	1.53	6.13	43.61

TABLE XI (Continued)

		20:2	20:4	20:5	22:1	22:5	22:6
Pairs	2	0.48	0.18	270.26	16.07	4.61	150.27
Method	1	0.44	0.15	227.71	12.67	3.29	107.74
Error a	2	0.46	0.21	285.96	16.55	4.69	148.06
Location	1	0.63	0.33	391.27	21.45	7.35	228.58
M x L	1	0.45	0.16	235.33	13.46	3.44	111.47
Error b	4	0.46	0.21	276.43	16.55	4.64	154.42
Time	2	0.46	0.27	383.62	18.87	7.41	208.27
T x M	2	0.44	0.17	232.17	13.65	3.64	112.25
T x L	2	0.45	0.23	328.47	17.33	5.74	179.59
T x M x L	2	0.46	0.18	244.04	14.26	3.91	119.98
Error c	32	0.48	0.20	283.41	16.14	4.76	151.29

[1] All mean square values are multiplied by 1.0×10^{-7}

[2] Methods were tested by error a.
Location of M x L were tested using error b.
All other terms were tested by error c.

* Significant at the 5% level ($P \leq 0.05$).
** Significant at the 1% level ($P \leq 0.01$).
*** Significant at the 0.1% level ($P \leq 0.001$).

TABLE XII Method x location x time interaction means from the analyses of variance of the free fatty acids (expressed as ug per gram of neutral lipid) of coho salmon.

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
14:0	10 weeks	466.7[1]	649.2	238.2	154.7
	27 "	540.7	598.2	172.0	174.9
	78 "	26986.9	2509.5	522.5	738.2
15:0	10 weeks	41.3	52.5	20.0	10.7
	27 "	29.7	27.1	9.8	7.9
	78 "	1082.2	115.4	27.3	32.1
16:0	10 weeks	2590.4	2514.0	848.5	610.0
	27 "	2431.5	3180.3	803.1	787.2
	78	96914.8	12382.6	2383.0	2683.0
16:1	10 weeks	1126.3	1424.4	620.9	316.3
	27 "	1661.2	2300.8	493.4	624.9
	78 "	53468.6	6404.1	1089.1	1654.0
16:2	10 weeks	38.1	67.7	48.1	19.6
	27 "	92.1	77.4	27.6	40.2
	78 "	3522.6	233.4	69.0	163.7
17:0	10 weeks	94.7	169.9	72.1	38.7
	27 "	151.3	196.3	63.2	79.1
	78 "	10643.0	1066.7	231.9	299.8
18:0	10 weeks	431.9	606.0	224.3	117.0
	27 "	385.6	462.6	130.6	122.0
	78 "	22326.5	2985.8	496.8	1131.5
18:1	10 weeks	3034.8	3605.5	1377.8	916.4
	27 "	3792.8	4866.1	1404.8	1281.8
	78 "	136144.9	16151.0	3160.7	4069.0
18:2	10 weeks	187.5	262.1	106.1	49.7
	27 "	431.7	430.2	121.1	174.7
	78 "	11669.0	1803.7	314.7	403.7
18:3	10 weeks	150.2	198.5	69.0	40.5
	27 "	164.2	183.0	46.1	57.6
	78 "	8052.3	980.2	164.3	271.4

TABLE XII (Continued)

Free Fatty Acid	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
18:4	10 weeks	188.0	304.3	112.5	59.0
	27 "	175.4	220.6	56.2	83.4
	78 "	15982.1	1465.4	283.7	393.2
20:1	10 weeks	515.2	807.2	274.7	170.8
	27 "	605.6	859.9	222.9	246.2
	78 "	42595.8	3593.4	597.0	958.9
20:2	10 weeks	78.1	43.9	9.7	19.9
	27 "	246.7	287.7	53.2	49.6
	78 "	4407.3	174.9	32.2	65.1
20:4	10 weeks	119.9	195.0	61.5	41.6
	27 "	99.3	127.7	23.4	31.1
	78 "	3021.7	423.9	90.3	107.1
20:5	10 weeks	1822.1	3061.3	997.3	607.9
	27 "	2726.6	2384.0	662.2	772.0
	78 "	110697.7	13572.0	2525.1	3596.3
22:1	10 weeks	364.6	720.0	198.0	175.0
	27 "	746.1	899.9	150.9	232.2
	78 "	25813.9	2448.7	331.1	617.9
22:5	10 weeks	167.0	549.5	179.4	117.4
	27 "	517.3	590.8	143.2	144.5
	78 "	1457.3	2517.8	564.7	755.5
22:6	10 weeks	1821.9	2816.5	1025.5	502.7
	27 "	2526.3	2816.5	530.7	676.4
	78 "	80248.7	12753.5	1843.5	2784.8

[1] Each value is the average of 3 observations.

TABLE XIII Analyses of variance of total free fatty acids (expressed as µg free fatty acid per gram of neutral lipid) of Pacific halibut, chinook salmon and coho salmon.

Source	Pacific Halibut		Chinook Salmon		Coho Salmon	
	df	M.S.[1]	df	M.S.	df	M.S.
Pairs	2	497.51*	2	1.546**	2	1015.60
Method[2]	1	781.96	1	1.102*	1	825.41
Error a	2	54.37	2	0.041	2	1025.00
Location	1	2454.80**	1	13.698**	1	1452.50
M x L	1	433.13	1	1.225	1	848.29
Error b	4	83.88	4	0.257	4	1062.40
Time	4	217.85	4	2.603***	2	1369.30
M x T	4	92.55	4	0.234	2	851.34
L x T	4	162.04	4	0.438	2	1191.40
M x L x T	4	88.36	4	0.566	2	892.84
Error c	32	128.75	32	0.213	16	1044.80

1 All mean square values are multiplied by 1.0×10^{-6}

2 Methods were tested by error a, Location and M x L were tested using error b. All other terms were tested using error c.

* Significant at the 5% level ($P \leq 0.05$)

** Significant at the 1% level ($P \leq 0.01$)

*** Significant at the 0.1% level ($P \leq 0.001$)

TABLE XIV Method x location times time means from the analyses of variance of total free fatty acids (expressed as µg free fatty acid per gram of neutral lipid) of Pacific halibut, chinook salmon, and coho salmon[1].

Species	Length of Frozen Storage	Inside Muscle		Outside Muscle	
		Brine Frozen	Plate Frozen	Brine Frozen	Plate Frozen
Pacific Halibut	14 weeks	5670.1	1934.0	3705.8	498.9
	31 "	37222.4	7983.5	2967.4	1557.1
	45 "	22538.6	20503.3	4321.4	4507.1
	62 "	18090.5	5535.3	3196.3	1459.1
	81 "	26691.0	11282.7	5189.6	2125.5
Chinook Salmon	9 weeks	526.5	1238.6	299.2	412.5
	26 "	2016.4	1976.8	415.1	715.4
	40 "	2002.0	2628.6	1060.3	1102.9
	58 "	1699.7	1601.5	1081.8	927.8
	77 "	1483.5	3066.5	1522.2	1146.8
Coho Salmon	10 weeks	1321.9	1804.7	648.4	396.8
	27 "	1732.4	2050.9	511.4	558.6
	78 "	66814.9	8158.2	1472.7	2072.5

[1] Each mean is the average of 3 observations.

TABLE XV Correlation coefficients (r) for the correlations of pH, thaw drip, color (Hunter Rd, a, b, and a/b values) with each other for Pacific halibut, Chinook salmon, and Coho salmon.

PACIFIC HALIBUT

Variable	pH	Thaw Drip
pH	1.000	
Thaw Drip	-0.358*	1.000

CHINOOK SALMON

Variable	pH	Thaw Drip	Rd	a	b	a/b
pH	1.000					
Thaw Drip	0.074	1.000				
Rd	-0.336	0.163	1.000			
a	-0.017	0.187	-0.232	1.000		
b	0.290	0.021	0.417*	0.263	1.000	
a/b	-0.103	0.193	-0.398*	0.928**	-0.112	1.000

COHO SALMON

Variable	pH	Thaw Drip	Rd	a	b	a/b
pH	1.000					
Thaw Drip	-0.132	1.000				
Rd	-0.033	0.124	1.000			
a	-0.596**	0.041	0.556*	1.000		
b	0.270	0.237	0.774**	0.217	1.000	
a/b	-0.721**	0.201	-0.062	0.725**	-0.511	1.000

* Significant at the 5% level ($P \leq 0.05$).
** Significant at the 1% level ($P \leq 0.01$).

TABLE XVI Correlation coefficients (r) for the correlation of pH, thaw drip, color (Hunter Rd, a, b, and a/b values), TBA values, and free fatty acids with flavor (number of correct identifications) for Pacific halibut, Chinook salmon, and Coho salmon.

Variable	Pacific halibut	Chinook salmon	Coho salmon	Variable	Pacific halibut	Chinook salmon	Coho salmon
pH	-0.258	-0.102	-0.737	%C 22:1	-0.160	-0.338	0.223
Thaw Drip	-0.198	0.116	-0.136	%C 22:5	0.012	0.113	0.075
Hunter Rd		-0.375	0.455	%C 22:6	-0.077	0.009	-0.240
Hunter a		-0.042	0.653*	FFA[2]			
Hunter b		-0.527*	0.059	C 14 F[2]	0.016	0.046	0.038
Hunter a/b		0.024	0.147	C 15 F	-0.051	-0.029	0.039
TBA	-0.039	0.460**	0.152	C 16 F	0.070	-0.059	0.031
FFA[1]				C 16:1 F	0.028	-0.039	0.032
%C[1] 14	0.086	0.038	0.147	C 16:2 F	0.052	-0.127	0.044
%C 15	0.114	-0.271	0.368	C 17 F	0.044	-0.024	0.032
%C 16	0.006	-0.099	0.261	C 18 F	0.046	0.065	0.031
%C 16:1	0.086	-0.057	0.176	C 18:1 F	0.069	-0.119	0.038
%C 16:2	0.109	0.058	-0.115	C 18:2 F	0.030	-0.093	0.020
%C 17	0.215	0.142	-0.040	C 18:3 F	0.017	-0.069	0.028
%C 18	-0.180	0.332	0.216	C 18:4 F	0.261	0.090	0.041
%C 18:1	0.121	-0.046	0.515*	C 20:1 F	0.017	0.023	0.044
%C 18:2	0.114	0.192	0.308	C 20:2 F	0.024	-0.102	0.053
%C 18:3	0.076	0.110	0.117	C 20:4 F	0.091	0.053	0.023
%C 18:4	0.063	0.204	0.193	C 20:5 F	0.056	-0.276	0.032
%C 20:1	-0.315	-0.410*	0.141	C 22:1 F	0.247	-0.112	0.043
%C 20:2	0.064	0.156	0.060	C 22:5 F	-0.0003	-0.0003	0.021
%C 20:4	0.096	-0.131	-0.385	C 22:6 F	0.023	0.036	0.020
%C 20:5	-0.017	-0.256	-0.020	TFFA/GF[3]	0.036	-0.053	0.031

1 Free fatty acids expressed as percent of total free fatty acids analyzed.
2 Free fatty acids expressed as µg free fatty acid per gram of neutral fat.
3 Total free fatty acids expressed as ug total free fatty acid per gram of neutral fat.
* Significant at the 5% level.
** Significant at the 1% level.