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ABSTRACT

The technology of processing salmon roe is discussed. The effect of sodium nitrite levels, ascorbate, nicotinamide on the color development of sujiko is examined. A laboratory scale simulated commercial process was devised to investigate the effect of these additives on the quality of sujiko. The penetration of ascorbate in roe and its value in reducing residual nitrite in sujiko prepared from fresh and frozen roe are described. The quality of sujiko prepared from fresh roe stored for up to 15 days on ice and from roe frozen after storage for up to 10 days on ice is examined in relation to color, texture, firmness, residual nitrite levels, rate of sodium chloride penetration. Sujiko prepared from roe extracted and stored in sealed barrier bags and roe kept in the fish for varying periods on ice are compared for quality.

RÉSUMÉ

La technologie de la préparation des oeufs de saumon est traitée. Les effets des teneurs en nitrite de sodium, de l'ascorbate et de la nicotinamide sur le développement de la couleur du sujiko sont examinés. Un procédé commercial simulé a été élaboré afin d'étudier à l'échelle du laboratoire les effets de ces additifs sur la qualité du sujiko. La pénétration de l'ascorbate dans les oeufs et son efficacité sur la réduction du nitrite résiduel dans le sujiko préparé à partir d'oeufs frais et congelés sont décrites. La qualité du sujiko préparé à partir d'oeufs frais conservés dans la glace jusqu'à 15 jours et à partir d'oeufs congelés après conservation dans la glace jusqu'à 10 jours est étudiée du point de vue de la couleur, de la texture, de la tendreté, des teneurs en nitrite résiduel et de la vitesse de pénétration du chlorure de sodium. On compare la qualité du sujiko préparé à partir d'oeufs extraits et conservés dans des sacs fermés de matériaux barrière à celle du sujiko préparé à partir d'oeufs gardés dans le poisson conservé dans la glace pendant diverses périodes de temps.

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INTRODUCTION

Nearly all the Pacific salmon roe (sujiko) production from the west coast of North America are consumed in Japan. Including domestic production from their chum salmon fishery, which has yielded in excess of 1000 metric tons (M/T), the annual consumption in that country for the past 6 years has been around 6000 - 7000 M/T. Of this total, the United States has, in the more productive years, exported in excess of 4000 M/T (Table 1) annually while the Canadian exports over the same years has reached a high of over 2000 M/T in 1973.

While salmon roe has been utilized for a long time as sujiko, caviar, and bait, only 207,000 dollars was realized from 790 M/T of roe produced from the large salmon harvest of 1953. This amounted to less than 0.5% of the total market value of salmon. In contrast, the market value of Canadian salmon roe reached a high of over 18 x 10^6 dollars in 1973 (Table 2) from 2,143 M/T produced from approximately the same landing of salmon as in 1953. This represents more than 8% of the total market value of salmon for that year. Following the liberalization of imports by Japan around the mid 1960's, the production of salmon roe generally increased despite wide fluctuations (Figure 1). However, if production is calculated on the basis of % (w/w) roe harvested from total salmon landings, utilization has increased steadily till 1972 and 1973 when in excess of 2.5% of total was achieved (Figure 2). It would appear that 2.5% of total landings may be approaching maximum utilization as in 1974 and 1975 the values declined somewhat. The % of the market value from the salmon industry shared by roe likewise increased steadily from the mid 1960's from about 1% to a high of over 8% in 1972 and 1973 (Figure 2) and declined subsequently. The value added through recovery of roe has clearly played an important role in the salmon fisheries of British Columbia. A need for research and development in certain aspects of post-mortem storage and processing technology to maintain acceptable quality standards of sujiko has also become evident as increasing amounts of poorer quality roe are being utilized to satisfy the needs of the expanded industry.

The post-mortem history of salmon roe is one of the most important criteria in the preparation of high quality sujiko. Good fish handling practices, minimization of holding salmon at ambient temperatures prior to icing, methods of storing roe, all play important roles in the preservation of roe in good condition. Slight variations in processing techniques may influence the quality of roe products within certain limits but the processors are not expected to perform miracles with poor quality salmon roe. Largely, it is up to the fishermen and subsequent fish handlers to employ such handling practices that would place high quality salmon roe into the hands of the processors. Handling practices adequate for maintaining acceptable quality flesh are often not adequate for maintaining high quality roe. Interests of people involved in the different phases of the industry differ. Roe processors have a more sophisticated feeling for the quality of roe necessary to meet the requirements of the consumers, while those whose primary interests lie in the value of the flesh tend to view roe as an incidental source of income and are less likely to pay attention to quality.

Other than the condition of the starting material, many factors influence the quality of sujiko. Color, salt content, organoleptic characteristics, elasticity, shape and appearance are some of the major criteria in determining the quality of the product. Color is particularly important as it is the first factor that draws the attention of the consumer's sensory perception. Bright, deep orange-red color has become associated with consumer preference. Nitrite is commonly used by the processors to develop part of this color.

The use of nitrate as one of the ingredients in the curing of meat has a long history. It added a characteristic flavor and imparted a desirable pink color to meat. It was not till just before the turn of the century that it was noted that nitrate was reduced to nitrite by bacterial action. Shortly thereafter,it was established that the nitrite so formed was responsible for the pink color. Since then, nitrite was more commonly used directly for developing the pink color in cured meat on a more controlled basis. As much as 625 ppm of sodium or potassium nitrite is permitted for use in dry cured meat with the stipulation that residual sodium nitrite in the finished cured meat product does not exceed 200 ppm (United States Food Additive Regulation 121.1230). For marine products the United States permit

residual nitrite levels not exceeding 10 ppm in smoked tuna fish products and 200 ppm for smoked cured sablefish, salmon and shad (Food Additive Regulation 121.1064). The use of nitrite in fish and fish products for domestic consumption is not permitted in Canada. Japan permits the use of sodium nitrite in fish ham, fish sausage, ikura and sujiko provided the residual level, calculated as the ion, does not exceed 5 ppm.

Astaxanthin is the main carotenoid responsible for the natural orange-red color of salmon eggs. Indeed, this carotenoid is the chief source of color in 'ikura', which is prepared from roe by removing the ovarian membrane, or in 'barako', which are loose eggs recovered in the sujiko process. In roe, however, the mass of eggs are encased in a heavily vascularized ovarian membrane and much of the blood is retained within the vessels and capillaries, even after processing. Blood from extraction of roe from the fish, or hemorrhaging from normal handling practices, may adhere to the egg surface as well as to the membranes holding the mass of eggs together. In the preparation of sujiko, only some of this blood is removed by the washing process. In fact, the wash water as well as the brining solution become colored with blood after the first batch of roe is processed and a film of blood is expected to adhere to the roe after removal from the brine tank. The color transformations which hemoglobin, the red heme protein component of blood, undergoes during the oxidative process profoundly masks the natural color of the carotenoid pigments in roe. The darkening of roe is caused mainly by the oxidative transformation of the pigment hemoglobin to the brown colored methemoglobin which once formed is difficult to reverse by the endogenous reducing capacity of post-mortem roe alone without the aid of external sources of reducing power. Poor storage conditions such as elevated temperatures resulting from inadequate icing, prolonged exposure to light and atmospheric oxygen, hemorrhaging caused by damage to the belly of the fish and other factors tend to accelerate the discoloration of roe. It is also this same hemoglobin which is largely responsible for discoloration which also is primarily chemically involved in developing color in the nitrite process for roe. For those interested, the chemical basis of this transformation is described in Appendix 1.

Ascorbic acid and its sodium salt, or its isomer erythorbate, have been in use in the meat processing industry for some time (reviewed, Counsell, 1971). It helps maintain the reducing conditions on exposed surfaces as well

as within and enhances color development through increasing the rate of formation of nitric oxide from nitrite and by preventing the transformation of the red colored hemoglobin and nitrosylhemoglobin to their respective brown colored oxidation products, methemoglobin and nitrosylmethemoglobin. It has been reported that in meat processing the use of ascorbate can reduce the amounts of nitrite added for good color development by as much as one-half to two-thirds the usual quantities. The residual nitrite levels would also be reduced considerably. Additional benefits of ascorbate in food processing is related to the food hazard problem resulting from the use of nitrite (Anonymous, 1972; Wolff and Wasserman, 1972). Ascorbate inhibits the formation of carcinogenic nitrosamines formed by reaction of nitrite with secondary amines (Kawabata et al., 1973; 1974; Tosawa and Sato, 1974; Fiddler et al., 1973; Mirvish and Shrubik, 1974). This property would be of particular interest in the processing of products from those species like the gadoids with high levels of trimethylamine oxide. Values of around 100 mg% trimethylamine oxide nitrogen have been reported for the Atlantic cod (Dyer, 1952) while about twice this amount has been found in Pacific grey cod and Alaska pollock (Amano et al., 1963). Pacific salmon, on the other hand, have only about one-tenth the amount of this amine oxide compared to the gadoid species (Dyer, 1952).

The use of external sources of reducing agents in the processing of sujiko appears in the difficult to obtain Japanese scientific literature of a decade ago. Ascorbate and its isomer, erythorbate, other antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), have been added to the brine solution at concentrations of around 300 ppm. Ascorbate may be introduced as a 50 : 50 mixture with nicotinamide in a commercial preparation available by the trade name 'Pinkumin'. A liquid with a trade name 'Sasten', consisting of a 10% emulsion of BHA containing edible oil and surface active agent, and sold on the market for the reddening of meat, sausage or ham, is also used in sujiko processing. Nicotinamide is one of a group of compounds which are known to combine with hemoglobin or myoglobin to form compounds which are red in color. These nitrogenous compounds have been examined by a number of laboratories as possible substitutes for nitrite in developing color. The color formed, however, is not as bright nor as stable as that produced with nitrite. Today the search continues for an effective substitute for nitrite in the color developing process. Much of the rationale,

effectiveness, and the technology of using external sources of reducing agents in the processing of sujiko either do not exist or are available as classified information by the Japanese processors. While well documented for the meat processing industry, it is poorly known for processing fishery products such as roe which is so grossly different in composition and in the processing techniques used.

MATERIALS AND METHODS

Source of salmon roe

Fresh roe from the different species was purchased from commercial sources (Matsuo and Son Ltd., and Fukuyama and Sugiyama Co. Ltd.). Although the post-mortem age of the roe was not available, the freshness was comparable to those used by the processors as the samples were obtained the day the experiments were carried out.

Salmon of known post-mortem age (from the test fishing operation of the IPSFC on the lower Fraser river during the latter part of September and early October) was purchased from the Queen Charlotte Fisheries. The fish were brought to the laboratory 3 to 4 hours following capture in gillnets and both the fish in the round as well as the extracted roe was stored under different conditions. The fish in the round were stored under ice in the refrigerator for 0, 2, 10 and 15 days and the roe was extracted after these times and processed. Roe from one species, the chum, was extracted at day 0, vacuum sealed in barrier bags* with limited permeability to atmospheric oxygen, and stored on ice for 2 and 5 days before processing. In every case the 0 day samples of roe were from fish no more than 3 to 4 hours post-mortem and processed immediately.

To determine the effect of post-mortem age and storage methods on the quality of frozen roe for processing, roe was stored on ice both in the fish and in the extracted form for different periods of time prior to freezing. After 0, 2, 5, 10 and 15 days the roe was extracted from the fish, washed once, vacuum sealed in barrier bags, quick frozen, and stored at -26^{o} C for 3 to 4 months prior to processing. Extracted roe was washed once,

* Purchased from Cryovac Division, Grace Chemicals Ltd., Mississauga, Ontario.

vacuum sealed in barrier bags, held for 0, 2, and 5 days on ice before freezing and stored at -26°C as before. *

More mature (15% body weight, about two weeks before spawning) coho roe was obtained from the Capilano hatchery. The roe from the sockeye, pink, coho, and chinook salmon obtained from the test fishing in the lower Fraser river and used in this study represented about 10% the body weight of the fish while the figure for chum salmon was 15%.

Salmon Roe Processing

The processing method adopted was essentially similar to that practiced at the commercial processors but scaled down to suit experimental laboratory conditions. Briefly, 1 kg lots of roe was washed for about 30 seconds in 8 liters of 3% NaCl solution, drained for a few minutes, and gently stirred in a bucket containing 4 liters of saturated brine solution with the requisite additive. Sodium nitrite at up to 100 ppm or in combination with other chemicals such as sodium ascorbate at up to 2,500 ppm were used as additives. The brining time at ambient temperatures varied from 25 minutes for sockeye, pink and coho roe, 35 minutes for chum roe and 40 minutes for the larger chinook roe. Roe was then drained in a perforated plastic tray for about 25 minutes and packed in polyethylene lined wooden boxes after sprinkling with an amount of fine dry salt equivalent to about 5% the weight of roe. The boxes are filled higher than the sides and the polyethylene liners are folded over the roe. The lid is placed over the box which is then turned upside down and weighted. The roe was cured for 5 days or longer at 15°C (or 20°C) in a temperature controlled incubator.

Color Evaluation

Color was recorded by visual observation under incandescent light, by color photography and occasionally instrumentally with the Gardner Automatic Color Difference Meter. For instrumental measurement, the sample was packed evenly and level in a plastic disposable petri dish 8.8 cm in diameter and 1.3 cm deep. The light source assembly of the Gardner Meter was placed upside down over a wooden stand in which a circular hole, the size of the outside diameter of the petri dish, was cut directly under the opening for the light source. The petri dish was placed in the wooden hole

* Freezing was for convenience only, to permit experimentation later; it is not recommended for commercial practise (see page 15).

under the light source and held in place with a flat board covered with black matte paper to minimize stray light from penetrating the sample area. The instrument was adjusted to a raw salmon flesh color standard in use in this laboratory with the following values; Rd (45^{o} luminous reflectance) = 8.73, 'a' (redness) = 33.8, 'b' (yellowness) = 20.7. Visually, the color of commercial preparations of roe compares more favorably with raw flesh standard than with the canned salmon color standard. For measuring deviations in the red - yellow range, the absolute values of 'a' and 'b' are less meaningful than the a/b ratios.

Because of the large size of the individual eggs in salmon roe, Gardner Meter readings are not easy to reproduce as different reflecting surfaces are exposed by turning the sample container, even though the technique of introducing it into the instrument has been standardized as much as possible. Furthermore, only gross color differences are discriminated by the Gardner Meter and in our experience, smaller and more subtle differences still obvious to the eye is not reflected instrumentally. There is much to be desired in the instrumental measurement of the color of salmon roe.

Determination of Nitrite

The modified method of Mori (1972) was employed.

Reagents: - 1% sulfanilamide solution - 1.0 g of sulfanilamide is dissolved in 100 ml of 10% HCl.

- 0.1% NEDA solution - 0.1 g N-(1-naphthyl)-ethylene diamine dihydrochloride (NEDA) is dissolved in 100 ml nitrite free water.
- zinc-uranyl acetate solution - 15 g zinc acetate and 5 g of uranyl acetate are dissolved in 200 ml water. Shake occasionally and allow to stand overnight and filter.
- nitrite standard solution - 5 µg of sodium nitrite per ml of water.

Procedure: An appropriate amount of roe is sampled and homogenized in a Servall Omni-Mixer. Twenty-five gram aliquots of the homogenized sample are weighed into 500 ml erlenmeyer flasks. Add about 200 ml of nitrite free water and heat to 80^{o} - 85^{o}C in a water bath for 30 minutes with occasional shaking. Cool to room temperature and bring volume to 250 ml. To a 20 ml

aliquot of the extract, add 5 ml of zinc-uranyl acetate solution to precipitate soluble proteins and mix well and filter through Whatman No. 42 (or 44) paper. To 10 ml of the clear filtrate, add 1.0 ml of 1% sulfanilamide solution, let stand for 15 minutes, add 1.0 ml of 0.1% NEDA solution, mix well, and let stand for 15 minutes. Absorbance is read against a blank at 540 nm in a suitable spectrophotometer. A sodium nitrite standard is carried through the procedure for comparison.

Determination of Salt Content

The sodium chloride in the roe was measured with a Quantab Chloride Titrator, available commercially from the Ames Co., Division of Miles Laboratory Inc., Elkhart, Indiana 46514, U.S.A. Sujiko used to determine the effect of post-mortem age on penetration of salt was not dry salted during packing as the uptake during the brining process only was being measured.

Measurement of Drip and Total Weight Difference of Roe Before and After Processing

Drip was measured in frozen roe by weight difference after thawing and draining overnight in the refrigerator. The weight difference of roe was also measured before and after processing, and on the 5th day of curing at $20^{o}C$.

Determination of Ascorbic Acid

The 2, 6-dichloroindophenol method outline in the Official Methods of Analysis of the Association of Official Analytical Chemists, 12th edition, page 829, 1975 was used.

RESULTS AND DISCUSSION

Color

The bright orange to red color of salmon eggs is due to the carotenoids, astaxanthin and its derivatives. Until just before spawning, the eggs are held together in well vascularized ovarian sacs. In fresh roe, blood hemoglobins are mainly in the bright red oxygenated form which is eventually transformed to the reddish purple reduced form as the oxygen is displaced. Further oxidative processes result in the oxidation of the ferrous iron to the ferric state, forming the dark brown colored methemoglobin. Roe is generally processed before this darkening takes place. Processing with brine alone results in a product which tend to be dark with some bright colored ones. It has been the practice of salmon roe processors to add sodium nitrite alone or in combination with nitrates and other additives to produce a product which is bright in color and which appeals to the consumers. Nitrosylhemoglobin, formed during the curing process from nitrite and hemoglobin under endogenous reducing conditions of the roe, is responsible for this color.

The amount of sodium nitrite used in the process should be just sufficient for developing uniform bright red color and low enough for maintaining the residual nitrite levels below 5 ppm after curing as required by the food and drug regulations of the consumer nation. The levels of nitrite required to develop good color varies with species (table 3). The red roes of sockeye and coho form bright red color at 40 ppm while chum roe requires 60 ppm. Gardner Color Meter values indicate that in each species examined, the higher a/b ratios generally correspond to the desirable red color. Generally, coho roes have high ratios while the chum is low. The Gardner values of the experimentally cured coho and chum roes correspond to those of commercial samples.

The effect of the additives, ascorbate and nicotinamide, alone or in combination with each other, on color formation at levels of nitrite minimal to the species being examined are shown in table 4. Chum roe was compared both at 40 ppm nitrite, a slightly subminimal amount, and at 60 ppm. The addition of 160 ppm of ascorbate in the brine solution resulted in a

higher a/b ratios in sockeye, coho and chum roe although the differences were not as apparent visually. The addition of nicotinamide to the brine at a concentration of 240 ppm definitely resulted in a less attractive darker product with black borders around the individual eggs in all cases. However, the a/b ratios remained about the same as with ascorbate alone in sockeye and coho but was lower for chum roe. Ascorbate tend to enhance color development with nitrite while nicotinamide darkens the product. In the presence of both, the effect of ascorbate appears to prevail.

Nicotinamide and other nitrogenous ligands are known to form the red colored ferrohemochromes with heme at alkaline pH and the brown colored ferrihemochrome at neutral pH (Akoyunoglou et al., 1963). At pH 5-6, pyridine derivatives (nicotinamide etc.) form ferrohemochromes with myoglobin in the presence of a strong reducing agent, dithionite (Howard et al., 1973). We found that rainbow trout hemoglobin forms with ethylnicotinamide a red color rapidly in the presence of dithionite and slowly in the presence of ascorbate at pH 5.62 but not at pH 7.01. Nicotinamide and ascorbate, used singly or in combination for developing color of pollock roe in the absence of nitrite have resulted only in limited success (Fukumi et al., 1971; Nakamura et al., 1972). Practical application was not realized because the color formed was unstable and gradually faded when exposed to air.

Residual nitrite levels in salmon roe processed with and without sodium ascorbate

The rate of disappearance of residual nitrite was determined in both fresh and frozen salmon roe processed and cured in the usual way with 100 ppm nitrite with and without 2500 ppm of ascorbate. The results are shown in figures 3-6. Ascorbate markedly increased the rate at which residual nitrite was lowered with time in both fresh and frozen salmon roe. Species differences were also observed. In the absence of ascorbate, fresh sockeye roe (figure 3) attained the 7.5 ppm sodium nitrite (equivalent to the permissible level of 5 ppm nitrite) level after 2 days of curing, coho in 5 days (figure 4), pink in 8 days (figure 5), and chum in one day (figure 6). In the presence of ascorbate, little difference was observed in the rate of disappearance of nitrite in the "red roes", sockeye and

coho. In pink roe, the same level of residual nitrite was reached in 5 days and in chum roe, the level was less than 2 ppm after only one day. For frozen roe, all species required a longer time in the absence of ascorbate to reduce the nitrite levels to below 5 ppm: sokeye, 4 days; coho, 12 days; pink, 12 days; and chum, 3 days. Ascorbate reduced these times to 2 - 3 days in all species. Ascorbate is particularly effective in lowering the residual nitrate levels in roe from species such as pink which tend to have high values, and in frozen roe from all species.

The uptake of ascorbate during processing.

To determine uptake of ascorbate during processing, eggs were prepared from freshly caught chum salmon. The eggs were washed twice in 3% salt solution, drained and brined for 30 minutes by the usual method in the presence of 0 to 2,500 ppm of sodium ascorbate (table 5). The eggs were drained thoroughly and stored overnight in sealed glass jars at $0^{o}C$ before ascorbate analysis.

In the absence of added ascorbate (table 5), the value of 70 ppm represents endogenous reducing power of the eggs. The uptake is virtually linear with increasing concentration of ascorbate in brine. The uptake with 2500 ppm ascorbate in the brine reached a value high enough to provide effective reducing power without exceeding the 550 ppm level permitted by the food and drug regulations for this additive. The potential uptake of ascorbate by the ovarian membrane and by tissues other than egg in roe was not determined.

The uptake of ascorbate, at a concentration of 2,500 ppm, by fresh roe was all well under 550 ppm with variations by species and by storage time on ice (table 6). In 5 day old roe from sockeye and pink, the uptake of ascorbate increased and decreased respectively, while those from coho and chum remained the same. The uptake in coho roe treated at 1000 ppm level increased with the post-mortem age of roe. At this lower level, however, the uptake was much lower than at 2,500 ppm. The higher uptake compared to eggs (table 5) reflects uptake by tissues in roe other than egg itself.

The ascorbate uptake by all frozen roe was higher than that for fresh roe except for chum roe stored within the fish. Sockeye roe stored for 5 days in the fish exceeded the 550 ppm value as did most of the frozen roe which were separated from the fish and held on ice for 2 and 5 days except

the 2 day samples of chum and chinook. In all species, increased permeability to ascorbate is indicated in roe separated from the fish prior to freezing compared to those held in the fish before freezing.

Effect of Post-Mortem Age of Fresh Salmon Roe on Quality of Sujiko

Salmon were held on ice in the round for 0 to 15 days and the roe was extracted on days 0, 2, 5, 8, 10, and 15 and processed immediately (table 7). In one species, chum, the roe was removed from the fish, and stored on ice for 2, 5 and 8 days in sealed barrier bags. After 5 days of curing, the sujiko was examined for colour development, texture, firmness, residual sodium nitrite levels and salt content.

The colour development of roe in all species was generally quite good although some fading was noticed in some species at the longer holding times. However, no obvious dark brown discolouration was noticed. Air oxidation, resulting in the formation of brown methemoglobin, is minimized if the roe is left in the fish. The results from chum roe also indicated that if roe is extracted from the fish and held on ice in sealed barrier bags, the colour of the sujiko is good. For the obvious reason that exposure to atmospheric oxygen is known to darken unprotected roe, studies of roe stored under these conditions were not carried out.

The texture of sujiko from all species is good at 5 days but some broken eggs were observed in samples stored for the longer times, although the quality was still good. Some drip was experienced with 10 and 15 day chum roe. Chum roe removed from the fish and stored in sealed barrier bags displayed good texture.

Sujiko from all species were exceptionally firm at day 0 and 2 and still as firm as commercial preparations at day 5. With the exception of coho, all were soft by day 10 or longer. Extracted chum roe was considered soft at day 8. In these and the subsequent studies with frozen salmon roe, it is emphasized that firmness can be controlled to a considerable degree by varying the pressure applied during the curing stage. The days elapsed before the onset of softness in this investigation is, therefore, considered much shorter than that which can be attained. Further studies are planned to assess the effect of pressure on firmness of the product. It was noted that 0 day sujiko was dull in appearance compared to the older roe. We have noted that in Japan where the nature of the salmon fishing industry enables the processors to obtain roe only several hours post-mortem, salad oil is brushed on

the uppermost layers of sujiko to add sheen.

Residual sodium nitrite after 5 days of curing were well below the permissible level of 7.5 ppm (or 5 ppm as the nitrite) in all samples. It is significant that coho roe processed with 1000 ppm ascorbate have much higher residual nitrite levels. The use of 2,500 ppm during the brining step appear, therefore, to be more suitable.

The sodium chloride content in all samples generally increased greatly with age, attaining a 4 fold increase in sockeye roe and about double in the others at day 15. Some variation is expected with the maturity of the roe in all species. The values reported here are minimum figures as the added salt from dry salting during packing was not included. Dry salting will be expected to cause considerable unevenness in the distribution of salt over the length of the roe. Without dry salting, the salt content of the 10 day post-mortem roe from brining alone is within the limiting level for growth of C. botulinum Type A (Southcott et al, 1976). With added dry salt, the level in sujiko prepared from roe less than 10 days post-mortem is expected to be higher and present no bacterial hazard as indicated by Southcott (1976). The freshness of roe prior to processing has, therefore, an important bearing on the penetration of salt.

Effect of Post-Mortem Age and Prefreeze Holding Method on Sujiko Prepared from Frozen Salmon Roe.

Round fish were held on ice in the refrigerator for up to 10 days, except for chinook which were held for only 5 days.

The roe was then extracted at 0, 2, 5, and 10 day intervals, sealed in barrier bags, laid out flat in shallow aluminium trays of the appropriate dimensions, and quick frozen by blast freezing. In this discussion, these samples will be referred to as round roe. Storage temperature was maintained at -28^{o} for 3 months.

In a parallel experiment, roe was extracted immediately from freshly caught fish, sealed in barrier bags, stored in a flat position in the refrigerator for 2 and 5 days before quick freezing as before. Again, for ease of reference, these samples will be termed extracted roe. In both cases, at the end of the 3 months frozen storage, the roe was thawed and processed. Drip after thawing, overall weight change, and the colour, texture, firmness, residual sodium nitrite, and the sodium chloride content were determined (tables 8 - 12).

In all species, both the round roe and the extracted roe developed good colour with some lightening of the colour in sockeye, chum and chinook held for the longer times prior to freezing. As with unfrozen roe, the presence of ascorbate appears to enhance the development of good colour.

The texture of sujiko from pink salmon was considered good for all samples while that of the other 4 species was only fair at 5 days or longer except the round roe of coho and chum which were deemed good at 5 but only fair at 10 days. All samples were shiny in appearance, unlike the product processed from 0 day fresh roe.

Sujiko from sockeye and pink were all considered soft while the day 0 samples from chinook, chum and coho were of commercial grade firmness. Coho sujiko was still assessed to be firm at 2 days. Compared to unfrozen roe, however, the firmness is the quality most critically affected by freezing.

The residual nitrite after 5 days of curing were all below permissible levels. In both round roe and extracted roe,there is a general increase in the sodium chloride content with increasing storage time before freezing. Compared to fresh roe, frozen roe takes up significantly more sodium chloride at day 0.

The drip from unprocessed frozen roe was surprisingly low and less than 2% except for the 5 day extracted roe from coho and sockeye and the 10 day round roe from coho which reached a value of 6.6%. Roe obtained from commercial sources and held in frozen storage for 1 - 2 years showed much more drip, in general: chum roe, 15 - 20%; pink roe, 20.8%; coho roe, 13.9%; spring roe, 6.7%; and sockeye roe, 4.3%. The conditions under which roe is held prior to freezing influences greatly the amount of drip. Our samples which were held under ideal conditions produced much less drip.

The overall weight change from the unprocessed to processed and cured roe varied from an increase of 1.8% to a negative value of as high as 19.9% although most samples were in the range of 4 - 8% loss. The major contributing factor in the high loss values result from the breakage of roe and the production of single eggs through handling and particularly during the agitation step in processing. Although roe of approximately the same size were selected for the prefreeze holding times, individual differences in the maturity of the roe, influencing the ease of breakage, no doubt existed.

Conclusions

A minimum of 40 ppm sodium nitrite is required to develop a uniform bright red colour with pink, sockeye and coho roes while 60 ppm was found necessary with chum roe.

The use of ascorbate in processing provides several advantages. It furnishes added reducing power to enhance colour development in the nitrite process and becomes critically important at lower concentrations of nitrite. Enhancement of red colour of sujiko can be measured at a level as low as 160 ppm. At higher levels of 2,500 ppm, residual nitrite levels of sujiko is lowered considerably and is of particular value for certain fresh and most frozen roes in which residual nitrite disappears much more slowly. At a level of 1000 ppm, ascorbate has only a slight influence on residual nitrite.

The penetration of ascorbate into eggs is approximately linear and reaches a value close to the allowable 550 ppm level when used at a concentration of 2,500 ppm in brine for 30 minutes. Penetration into fresh roe varies with species and is generally a little higher in once frozen roe.

Nicotinamide tends to darken the colour of sujiko and its use in processing does not offer much advantage.

Extracted roe held on ice in sealed barrier bags with low permeability to atmospheric oxygen retains the original colour as good as roe retained within the fish and is a desired practice for handling roe.

The degree of salt uptake by salmon roe held on ice increases sharply with post-mortem age but the rate varies with species. After 10 days the salt level is beyond 10% reaching as high as 15 - 16% in sockeye and coho. In chum, the level reached 10% after 8 days of storage. Post-mortem age is therefore an important factor in salt penetration and the effect the salt content has on firmness and taste.

Firmness of sujiko is affected by the post-mortem age of the roe. Roe stored under good icing conditions result in firmer sujiko. After 10 days storage the product tends to be softer.

Sujiko prepared from frozen roe of all species were generally poorer in firmness, the single most important criteria of quality affected by freezing.

The colour does not deteriorate significantly while salt uptake is generally higher compared to unfrozen roe. The post-mortem age prior to freezing also influences firmness. If quick frozen immediately, roe from chinook, chum and coho resulted in reasonably firm sujiko while that from sockeye and pink were softer. It is, however, softer than the product prepared from fresh roe. Coho roe frozen after 2 days on ice resulted in sujiko of reasonable firmness. Drip loss was surprisingly low even from samples stored on ice for as long as 10 days prior to freezing and was generally less than 2% with some exceptions. Commerical sources of roe resulted in drip loss of up to 20% after frozen storage. Drip loss is minimized by good storage practice prior to freezing the roe.

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The figures used for Table 1 were compiled from "Fisheries Market Developments", published by the Fisheries and Fish Products Division. Agriculture, Fisheries and Food Products Branch. Department of Industry, Trade and Commerce, Ottawa, Canada.

Statistical figures used in Table 2 and the data used for Figures 1 and 2 were obtained from "Fisheries Statistics of British Columbia, 1952 - 1974". Department of the Environment. Fisheries and Marine Service. Pacific Region.

Table 1 Domestic Production and Import of Salmon Roe by Japan. Qty. in metric tons

Year	Domestic Production	Imports					
		Canada	United States	China	Sweden	Taiwan	Total Imports
1970	1,100	1,202	4,091				5,293
1971	1,484	1,266	4,182				5,448
1972		1,830	3,022				4,852
1973		2,034	2,826				4,860
1974		1,449	2,882		4.45		4,335
1975		634	2,846			6.5	3,487
1976		1,298	4,473	2			5,773

Table 2. Market Value of Salmon Roe

Year	Market Value $(1000's) Roe	Total Salmon	% of Total
1964	654	63,103	1.04
1965	682	52,071	1.31
1966	1,328	86,572	1.53
1967	1,948	79,747	2.44
1968	2,889	99,956	2.89
1969	1,894	57,982	3.27
1970	3,903	99,597	3.92
1971	4,064	96,926	4.19
1972	9,528	114,349	8.33
1973	18,435	221,245	8.33
1974	11,890	165,841	7.17
1975	6,410	99,748	6.40

Table 3 The Effect of Nitrite on Color Development

Exp. No.	Species	Sod. Nitrite (ppm)	Rd	a	b	a/b	Visual Observation
51	sockeye	20	5.4	19.9	12.8	1.55	slightly dark
52	"	40	5.9	18.1	11.0	1.65	red and bright
53	"	60	5.3	21.1	13.0	1.62	red and bright
54	"	80	5.0	15.7	8.8	1.78	red and bright
31	coho	20	2.9	14.7	8.4	1.75	slightly dark
32	"	40	2.3	11.1	5.3	2.09	red and bright
33	"	60	3.4	10.9	5.6	1.95	red and bright
34	"	80	2.5	11.2	6.0	1.86	slightly dark
23	chum	20	4.8	15.7	10.7	1.47	slightly dark
24	"	40	4.5	12.7	10.3	1.23	dark pale
25	"	60	5.4	18.8	10.5	1.79	good
26	"	80	5.6	17.6	12.1	1.45	slightly pale
*	chum		3.25	11.3	7.3	1.55	
*	chum		3.50	13.1	9.1	1.44	
*	chinook		3.7	10.8	10.1	1.07	
*	chinook		4.3	10.8	8.7	1.24	
*	coho		3.2	12.7	6.6	1.92	

* commercial preparations of sujiko

Table 4 Effect of Ascorbate and Nicotinamide, Alone or in Combination, on Color Development with Sodium Nitrite

Exp.No.	Species	Sod. Nitrite (ppm)	Ascorbate (160 ppm)	Nicotinamide (240 ppm)	Rd	a	b	a/b	Visual Observation
52	sockeye	40	-	-	5.9	18.1	11.0	1.65	bright red
56	"	"	+	-	5.0	17.4	9.9	1.76	bright red
57	"	"	-	+	4.0	13.7	7.7	1.78	slightly darker
58	"	"	+	+	5.7	20.4	11.5	1.77	bright red
59	coho	40	-	-	5.3	18.3	11.3	1.62	bright red
61	"	"	+	-	3.0	14.3	7.4	1.93	bright red
62	"	"	-	+	3.0	12.8	6.7	1.91	slightly darker
63	"	"	+	+	3.8	17.4	8.4	2.07	red
47	chum	40	-	-	4.5	14.5	9.6	1.51	orange red
48	"	"	+	-	4.1	15.0	9.7	1.55	orange red
49	"	"	-	+	4.6	13.9	10.4	1.34	darker
50	"	"	+	+	4.4	15.5	12.8	1.21	darker
64	"	60	-	-	3.3	10.5	8.3	1.27	orange red
66	"	"	+	-	5.3	16.2	10.4	1.56	orange red
67	"	"	-	+	4.5	8.8	7.5	1.17	dark patches
68	"	"	+	+	4.0	11.8	9.0	1.22	orange red

Table 5 The Uptake of Ascorbate by Single Eggs Prepared from Freshly Caught Chum

ppm ascorbate in brine solution	ppm ascorbate found in the eggs
0	70
250	105
500	140
750	181
1000	223
1250	223
1500	258
1750	279
2000	328
2250	335
2500	370

Table 6

Ascorbate Uptake by Fresh Roe and by Roe Frozen after Different Post-Mortem Holding Conditions

	Fresh Roe		Frozen Roe			
			roe extracted from iced fish		roe extracted from fish at day 0 and stored in sealed bags on ice	
	days storage on ice	ascorbate (ppm)	days prior to freezing	ascorbate (ppm)	days prior to freezing	ascorbate (ppm)
sockeye	0	91	0	356	2	655
	5	272	5	712	5	755
pink	0	272	0	420	2	647
	5	153	5	377	5	641
coho	0	391	0	472	2	641
	5	391	5	527	5	805
coho*	0	21				
	2	63				
	5	84				
chum	0	412	0	370	2	434
	5	412	5	370	5	560
chinook			0	342	2	449
			5	370	5	605

* Capilano coho roe (two weeks to spawning) brined in the presence of 1000 ppm sodium ascorbate

Table 7 Effect of Post-Mortem Age of Fresh Salmon Roe on Quality of Sujiko

		days salmon held on ice					
		0	2	5	8	10	15
Colour:	sockeye	deep red (5R5/12)***	deep red (5R5/12)	red (5R6/10)		red (5R6/10)	red (5R6/10)
	pink	red (5R6/10)	red (5R6/10)	pink red (5R7/8)		pink red (5R7/8)	pink red (5R7/8)
	coho	deeper red (5R4/12)	deeper red (5R4/12)	deep red (5R5/12)		deep red (5R5/12)	deep red (5R5/12)
	coho*	deeper red (5R4/12)	deeper red (5R4/12)	deeper red (5R4/12)			
	chum	light red (10R5/12)	light red (10R5/12)	faint red (10R6/10)		faint red (10R6/10)	faint red (10R6/10)
	chum**	light red (10R5/12)	orange red (10R6/12)	orange red (10R6/12)	orange red (10R6/12)		
Texture:	sockeye	good	good	good		some broken eggs observed	
	pink	good	good	good		few broken eggs observed	
	coho	good	good	good		fair	fair
	coho*	good	good	good			
	chum	good	good	good		dripping observed	
	chum**	good	good	good	fair		
Firmness:	sockeye	firm+++	firm++	firm+		soft	softer
	pink	firm+++	firm++	firm+		soft	softer
	coho	firm+++	firm+++	firm++		firm+	soft
	coho*	firm+++	firm+++	firm++			
	chum	firm+++	firm++	firm+		soft	soft
	chum**	firm+++	firm++	firm+	soft		

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Table 7 Contd. Effect of Post-Mortem Age of Fresh Salmon Roe on Quality of Sujiko

		Days salmon held on ice					
		0	2	5	8	10	15
$NaNO_2$ (ppm)	sockeye	N.D.	N.D.	N.D.		0.4	0.4
	pink	0.1	0.3	0.4		2.3	2.0
	coho	0.7	0.3	0.3		0.7	1.5
	coho*	5.8	7.3	7.8			
	chum	0.25	2.0	2.1		2.5	3.4
	chum**	0.25	2.3	3.4	4.6		
NaCl (%)	sockeye	4.1	8.2	8.1		10.4	15.3
	pink	6.5	6.7	10.4		13.6	12.0
	coho	6.7	7.9	8.5		13.0	16.0
	coho*	6.4	7.5	6.4			
	chum	6.2	8.9	9.4		11.6	13.6
	chum**	6.2	7.1	7.8	10.3		

* Coho salmon from Capilano hatchery, 2 weeks prior to spawning. 1000 ppm ascorbate used in brine.
** Chum roe extracted on day 0, sealed in barrier bags, and stored on ice in refrigerator.
*** Munsell colour(Notation)

Table 8 Effect of Post-Mortem Age and Prefreeze Holding Method on Sujiko Prepared from Frozen* Sockeye Salmon

	Prefreeze Holding Conditions					
	days roe held in fish on ice before freezing				days extracted roe held on ice in sealed barrier bags Before freezing	
	0	2	5	10	2	5
Colour	deep red (5R5/12)	deep red (5R5/12)	light red (5R6/10)	light red (5R6/10)	deep red (5R5/12)	light red (5R6/10)
Texture	good	good	fair	fair	good	fair
Firmness	soft	soft	soft	softer	soft	softer
Residual $NaNO_2$ (ppm)	0.4	0.5	0.8	0.8	1.8	1.6
NaCl(%)	9.6	10.4	12.5	13.5	9.1	11.0
Drip (%, w/w) thawing	1.4	0.8	0.4	0.9	1.9	4.1
Overall wt. change** (%)	-6.5	-0.4	-2.6	-0.5	-7.2	-16.1

* all roe described in Tables 6 - 10 have been held in frozen storage for a period of 3 months

** from unprocessed roe to the finished product.

Table 9 Effect of Post-Mortem Age and Prefreeze Holding Method on Sujiko Prepared from Frozen Coho Roe

	Prefreeze Holding Conditions					
	days roe held in fish on ice before freezing				days extracted roe held on ice in sealed barrier bags before freezing	
	0	2	5	10	2	5
Colour	deeper red (5R4/12)	deeper red (5R4/12)	deep red (5R5/12)	deep red (5R5/12)	deeper red (5R4/12)	deep red (5R5/12)
Texture	good	good	good	fair	good	fair
Firmness	firm+	firm	soft	softer	firm	soft
Residual $NaNO_2$ (ppm)	0.5	0.6	0.5	0.5	0.7	0.9
NaCl (%)	7.3	9.1	8.5	10.5	10.0	11.0
Drip (%, w/w) after thawing	0.6	0.7	1.4	6.6	1.4	3.5
Overall wt. change* (%)	-8.7	-4.3	-8.6	-19.9	-6.0	+1.0

*from unprocessed roe to the finished product.

Table 10

Effect of Post-Mortem Age and Prefreeze Holding Method on Sujiko Prepared from Frozen Pink Roe

	Prefreeze Holding Conditions					
	days roe held in fish on ice before freezing				days extracted roe held on ice in sealed barrier bags before freezing	
	0	2	5	10	2	5
Colour	pale red (5R6/10)	pale red (5R6/10)	pale red (5R7/8)	pale red (5R7/8)	pale red (5R6/10)	pale red (5R7/8)
Texture	good	good	good	good	good	good
Firmness	soft	soft	soft	soft	soft	soft
Residual $NaNO_2$ (ppm)	5.1	4.0	3.9	2.0	2.6	2.1
NaCl (%)	8.0	8.3	7.8	10.0	8.4	10.0
Drip (%, w/w) after thawing	0.6	0.4	1.0	1.1	1.4	1.0
Overall wt. change* (%)	-7.6	-6.3	-8.4	-4.2	-4.1	+0.4

* from unprocessed roe to the finished product.

Table 11

Effect of Post-Mortem Age and Prefreeze Holding Method on Sujiko Prepared from Frozen Chum Roe

	Prefreeze Holding Conditions					
	days roe held in fish on ice before freezing				days extracted roe held on ice in sealed barrier bags before freezing	
	0	2	5	10	2	5
Colour	light red (10R5/12)	orange red (10R6/12)	pale orange red (10R6/10)	pale orange red (10R6/10)	orange red (10R6/10)	pale orange red (10R6/10)
Texture	good	good	good	fair, some loose eggs	good	fair
Firmness	firm	soft	soft	soft	soft	soft
Residual $NaNO_2$ (ppm)	1.6	2.5	1.9	2.2	1.8	2.1
NaCl (%)	8.4	8.8	9.6	10.3	9.6	10.5
Drip (%, w/w) after thawing	1.1	1.8	0.8	1.1	1.5	1.3
Overall wt. change* (%)	-2.4	-8.9	-1.2	-2.4	-1.8	+1.8

* from unprocessed roe to the finished product.

Table 12

Effect of Post-Mortem Age and Prefreeze Holding Method on Sujiko Prepared from Frozen Chinook Roe

	Prefreeze Holding Conditions				
	days roe held in fish on ice before freezing			days extracted roe held on ice in sealed barrier bags before freezing	
	0	2	5	2	5
Colour	light orange red (10R 6/10)	light brownish red (10R 6/8)	light brownish red (10R 6/8)	light brownish red (10R 6/8)	light brownish red (10R 6/8)
Texture	good	good	fair, some broken eggs	good	fair, some broken eggs
Firmness	firm	soft	soft	soft	soft
Residual $NaNO_2$ (ppm)	1.6	1.0	2.1	2.3	2.0
NaCl (%)	7.3	8.4	8.6	7.8	9.1
Drip (%, w/w) after thawing	1.0	0.7	0.8	1.1	1.0
Overall wt. change* (%)	-7.7	-4.8	-3.7	-5.0	-1.9

* from unprocessed roe to the finished product.

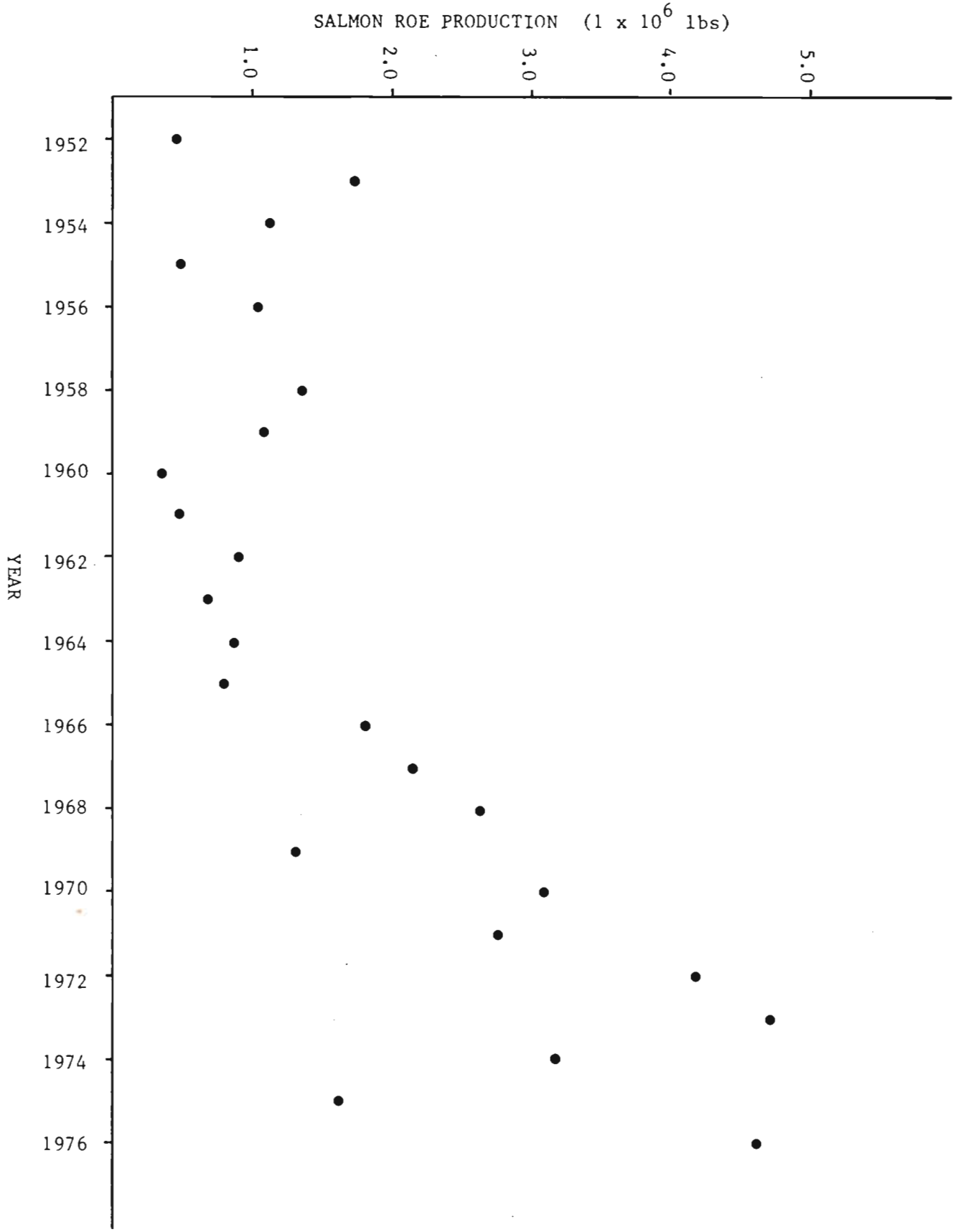


Figure 1

Salmon Roe

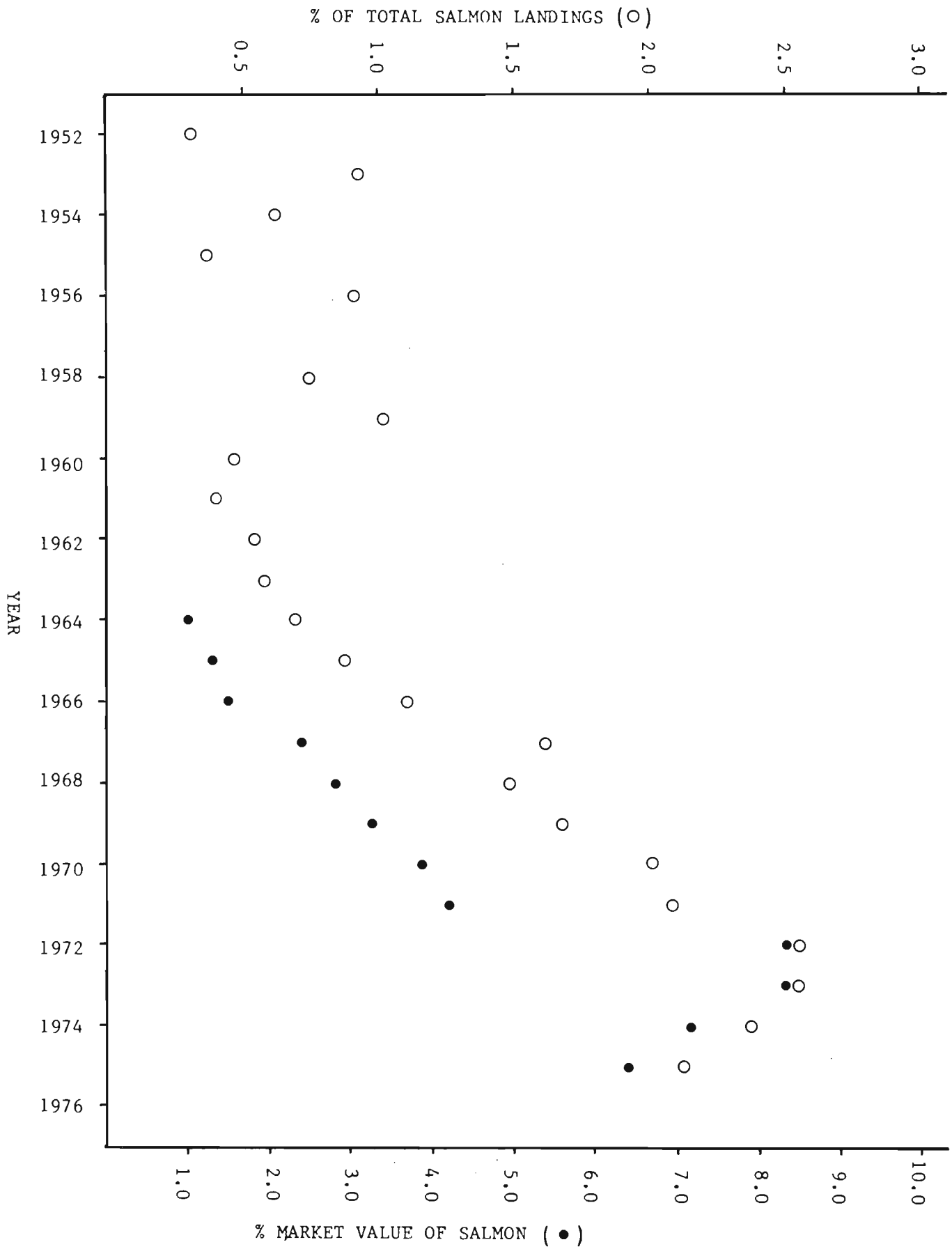


Figure 2

Salmon Roe

Figure 3

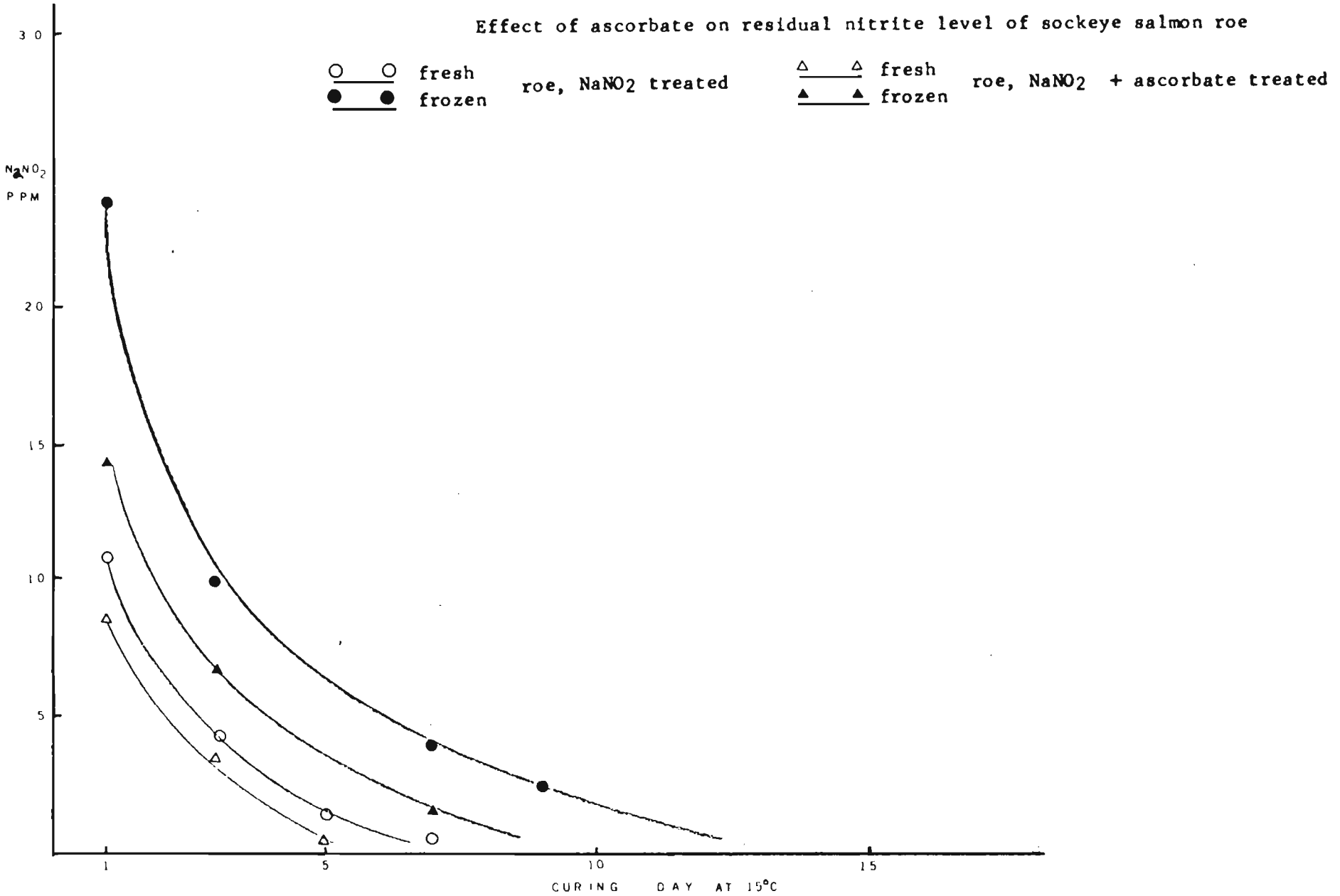

Effect of ascorbate on residual nitrite level of sockeye salmon roe
fresh
frozen
roe, NaNO2 treated
fresh
frozen
roe, NaNO2 + ascorbate treated
NaNO2 PPM
30
20
15
10
5
1
5
10
15
CURING DAY AT 15°C

Figure 4

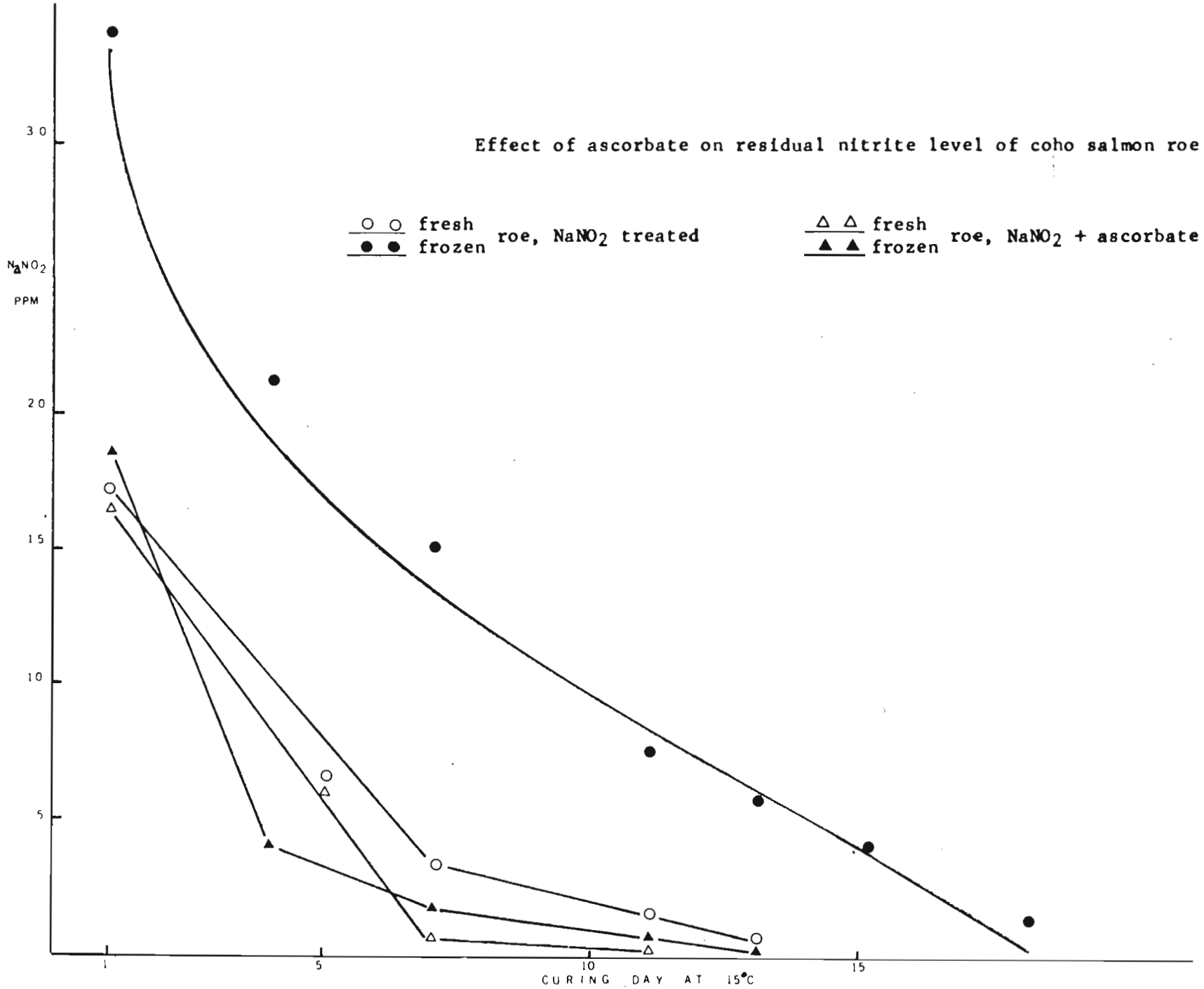

Effect of ascorbate on residual nitrite level of coho salmon roe
○ ○ fresh
● ● frozen roe, NaNO2 treated
△ △ fresh
▲ ▲ frozen roe, NaNO2 + ascorbate treated
NaNO2 PPM
30
20
15
10
5
1
5
10
15
CURING DAY AT 15°C

Figure 5

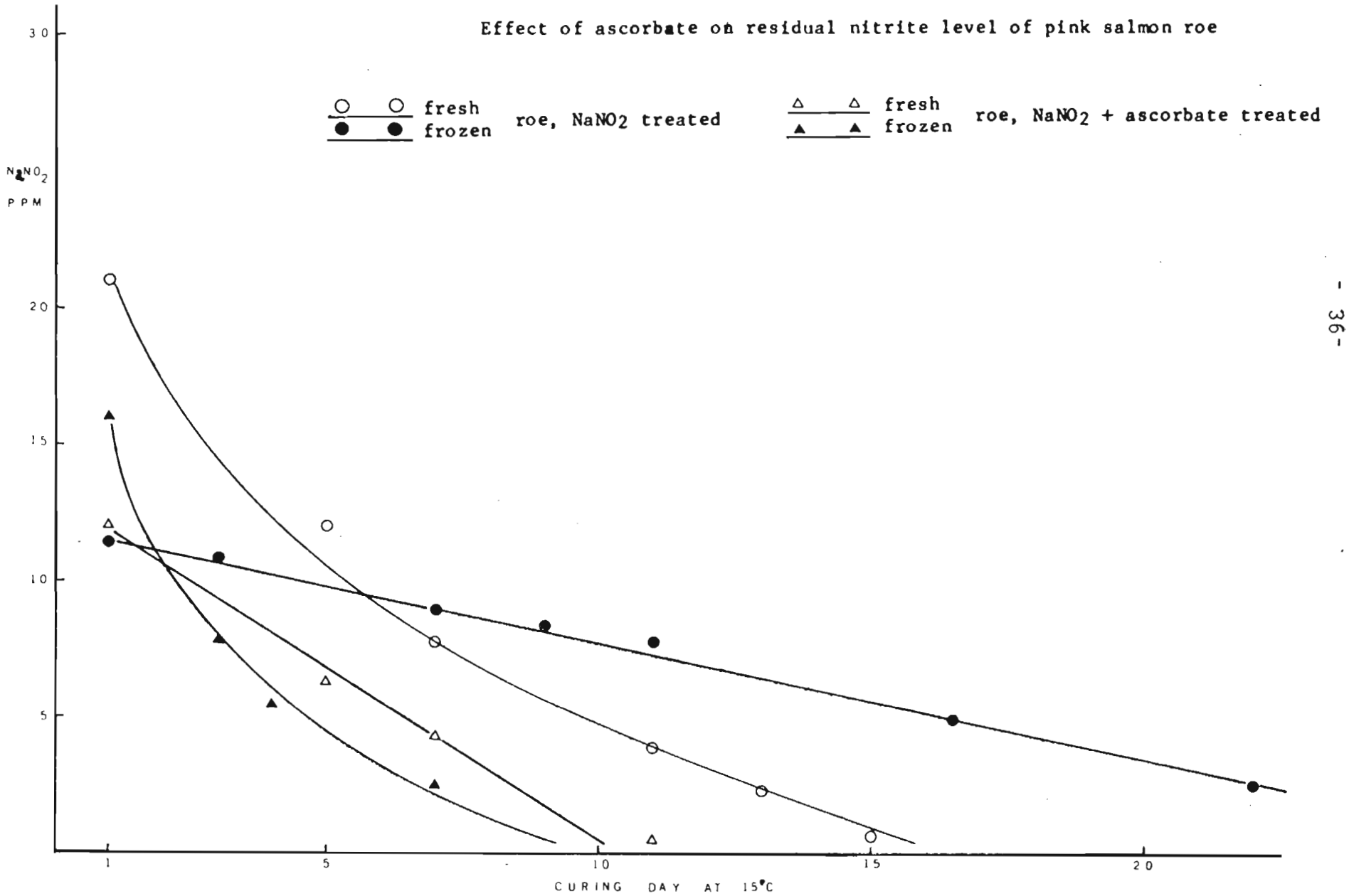
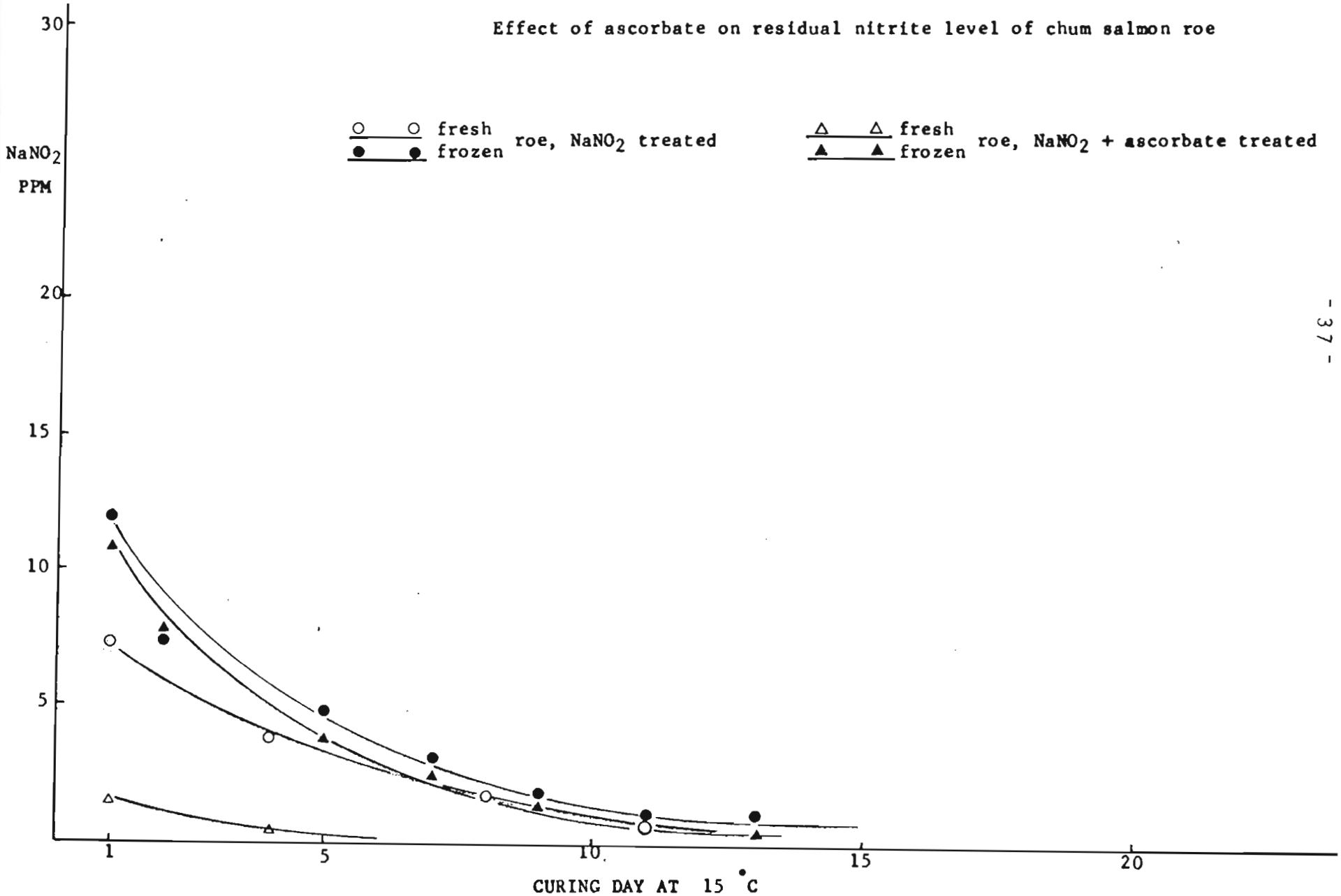

Effect of ascorbate on residual nitrite level of pink salmon roe
fresh
frozen
roe, NaNO2 treated
fresh
frozen
roe, NaNO2 + ascorbate treated
NaNO2
PPM
30
20
15
10
5
1
5
10
15
20
CURING DAY AT 15°C

Figure 6


Effect of ascorbate on residual nitrite level of chum salmon roe
○ ○ fresh
● ● frozen
roe, NaNO2 treated
△ △ fresh
▲ ▲ frozen
roe, NaNO2 + ascorbate treated
NaNO2 PPM
30
20
15
10
5
1
5
10
15
20
CURING DAY AT 15 °C

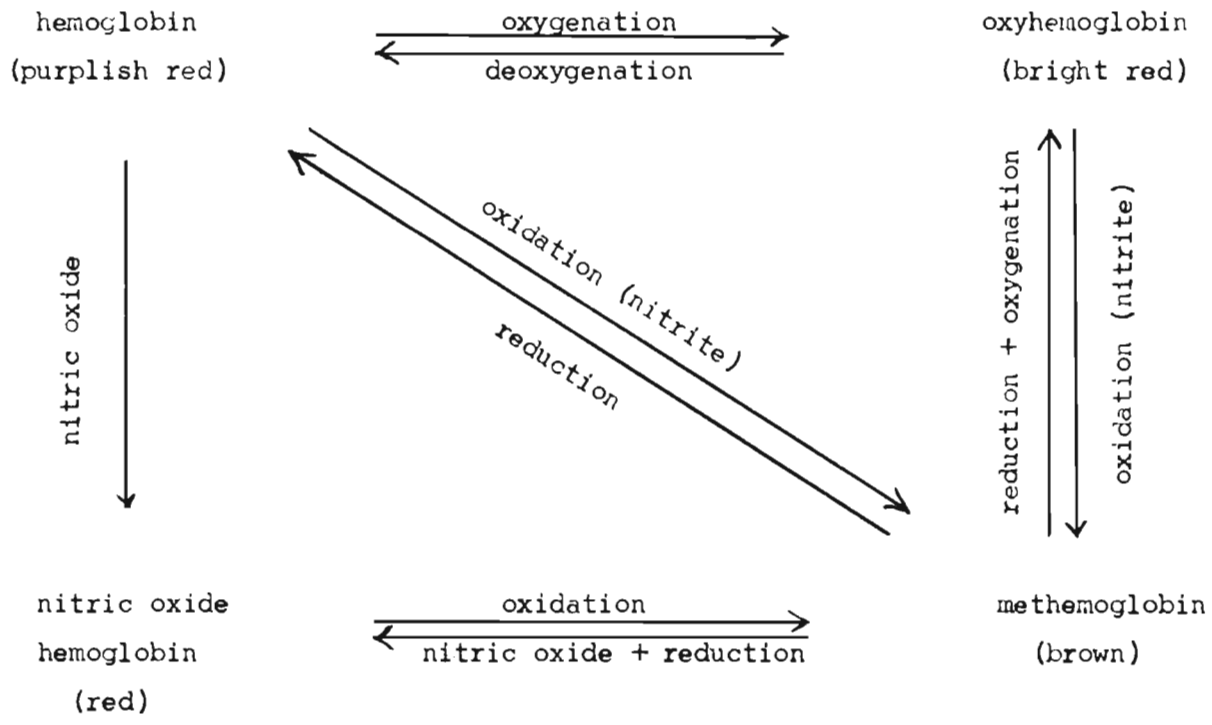
APPENDIX 1

Upon death of the salmon, the bright red oxygenated oxyhemoglobin gradually deoxygenates to the dull red to purplish reduced hemoglobin. Initially, endogenous reducing agents such as tocopherol (E vitamin), K vitamines, coenzymes, and enzymatic processes may help maintain hemoglobin in this reduced deoxygenated state. There is, however, a slow and continuous oxidation of the ferrous iron in hemoglobin to the ferric form of methemoglobin which is brownish in color.

(see diagram)

In the nitrite process, hemoglobin reacts with the nitric oxide, which is formed from nitrite by reduction by endogenous reducing agents, to form the bright red colored nitric oxide hemoglobin (nitrosylhemoglobin) (Fox, 1966, Kemp; 1974) so desired in salmon roe products. The formation of nitric oxide is rate limiting and influenced both by the concentration and the kind of reductant. In the presence of methemoglobin the process yields the brown colored nitrosylmethemoglobin which must be reduced to form the red colored nitrosylhemoglobin. The met- form of this compound is reduced only with difficulty by endogenous reducing agents which tend to become less effective with the age of roe and external sources of reducing agents enhance the transformation. At around pH 6.0, a strong reducing agent such as dithionite can reduce nitrosylmethemoglobin to nitrosylhemoglobin about 50 times faster than ascorbate which in turn is about 4 times more effective than cysteine. The choice of reducing agents that can be used, however, is limited by Food and Drug regulations and by the organoleptic characteristics introduced to the product.

Ascorbate can also react with oxyhemoglobin to form an unstable hydrogen peroxide derivative of hemoglobin (Chang and Watts, 1949). During the subsequent oxidation of hemoglobin, methemoglobin and small amounts of a green decomposition product, choleglobin, are formed. Temperature and concentration of ascorbic acid affect the oxidation of hemoglobin. At low concentrations and temperatures, ascorbic acid protects the color. In the presence of nitrite, however, ascorbate forms a relatively stable red color with heme-proteins.


hemoglobin
(purplish red)
oxygenation
deoxygenation
oxyhemoglobin
(bright red)
nitric oxide
oxidation (nitrite)
reduction
reduction + oxygenation
oxidation (nitrite)
nitric oxide
hemoglobin
(red)
oxidation
nitric oxide + reduction
methemoglobin
(brown)