FROZEN STORAGE AND CRYOPROTECTION OF EGGS FROM CHUM SALMON (*ONCORHYNCHUS KETA*)

by

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B.Sc. Honours, University of Saskatchewan

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

Department of Food Science

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April 1984

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Date March 19, 1984

ABSTRACT

This study was initiated to assess the influence of frozen storage of eggs from sexually mature female chum salmon (*Oncorhynchus keta*) on the physical properties of the membrane and the yolk. The effectiveness of several salts, sucrose, glycerol and ethylene glycol as cryoprotective agents for preventing any observed cryodamage to the eggs was determined.

Electron microscopy was employed to gain an insight into the micro-structure of the chum egg envelope. In addition, whole eggs were subjected to flat plate compressive loading in order to determine the strength of the membrane. The membrane appeared to be a complex structure which enabled intact eggs to withstand compression forces of 0.47 to 1.22 Newtons. The membrane rupture energy ranged from 2.36 to 7.99 ($\times 10^{-4}$) Joules, and was independent of egg size. Upon freezing fresh eggs at -10°C for 7 days, then thawing in air at 25°C, a drop of 46% in membrane rupture energy was experienced.

The flow behaviour of yolk expressed from fresh chum eggs, as examined with the aid of a Brabender rotational viscometer, displayed a non-Newtonian pseudoplastic nature. Analyses by differential scanning calorimetry and polyacrylamide gel electrophoresis indicated the presence of several major proteins. When the yolk material had been frozen at -10°C for 7 days, then thawed in air at 25°C, little variation was observed to occur by any of the techniques used.

Immersion in a 2.5 M solution of sodium chloride for 30 min at 25°C was the most effective and consistent cryoprotective agent out of all of the potential agents tested. Caviar produced from previously frozen, then thawed eggs that had been so treated before freezing differed from that produced from both

fresh and untreated frozen and thawed eggs in the rate of sodium chloride uptake and yolk apparent viscosity for the same chloride content. Membrane strength of such caviar was similar to that produced from fresh eggs, being greater than that produced from untreated previously frozen and thawed eggs.

Sensory evaluation studies indicated that caviar from eggs that had been cryoprotected were ranked similar to caviar from untreated frozen and thawed eggs in the parameters of yolk viscosity and membrane strength. Both were significantly different from high quality caviar produced from fresh eggs. However, the hedonic ratings indicated a preference for caviar from cryoprotected eggs over that processed from untreated frozen and thawed eggs.

Results of this study indicate that cryoprotection of chum eggs to reduce the defects caused by frozen storage is feasible. With further research, this technique holds potential advantages for the caviar industry.

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ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to Dr. W. D. Powrie for his invaluable support of this study and his review of the manuscript. Special thanks to Dr. M. A. Tung for his many helpful suggestions and also to Dr. S. Nakai and Dr. T. Howard for all their interest in, patience and review of this thesis.

The author is also very grateful for the support and aid extended by the Fisheries Technology Division, B. C. Research. The invaluable technical advice from S. Yee, R. Yada and L. Robinson is also gratefully acknowledged. Appreciation is extended as well for the financial support of the Science Council of British Columbia.

This thesis is dedicated to my mother and father, without whom I would never have been able to begin, and to Mr. Tom Tautorus, without whom I would never have been able to finish.

INTRODUCTION

The term "caviar" is used to describe separated and salted fish eggs. The roe of many teleost species are employed for this purpose; most prized are those of sturgeon and salmon, but carp, whitefish, pike-perch, pike, grey mullet, cod and herring are also utilized (Zaitsev et al., 1969). The USSR presently leads in world salmon caviar production. Sternin and Hori (1982) report that Soviet production of caviar recently reached 600 tonnes per year, while Canada produces only a small fraction of this amount. Most of the roe resulting from the salmon harvest in Canada is considered as offal, however, the amount that was used for human consumption in recent years commanded prices that ranged from seven to eight dollars per kilogram (Fisheries Production Statistics of British Columbia, 1981), second in value only to mild, cured and smoked salmon. Although presently the majority of roe intended for processing is used for "sujiko", a Japanese delicacy involving highly salted intact ovaries, there is increasing interest in salting the separate eggs to produce a European style caviar.

The reddish orange roe of the species *Oncorhynchus keta* is most commonly utilized for salmon caviar production (Sternin and Hori, 1982). Commonly called "chum", this salmon possesses the largest eggs of any of the other fish species used, averaging 200 mg in weight per egg (Smirnov et al., 1968), and occupying 10-11% of the total body weight of the fish (Zaitsev et al.,1969). Chum salmon are among the last of the Pacific salmon to enter the fresh water tributaries to spawn and roe of maturity suitable for caviar production is found only in the months of August to November (Sternin and Hori, 1982).

Fish processors working with roe are faced with the seasonal problem of large influxes of fresh, highly perishable fish roe. Although frozen storage for later processing is one solution to even out the fluctuations in

raw product supply, it has been found that such handling results in the alteration of several aspects of end product quality. One of the most important of these is the texture of the separate caviar grains (Sternin and Hori, 1982; Zaitsev et al., 1969).

The purpose of this thesis was twofold: to determine the location and extent of damage resulting from a freeze/thaw cycle in individual eggs and to investigate the potential use of cryoprotectants in alleviating any such defects. The ultimate goal was to produce a cryoprotected egg that could yield a caviar more nearly resembling caviar prepared with fresh eggs than that produced from frozen eggs.

In the course of the investigation, the composition and physical properties of the chum egg were determined through proximate analysis, scanning electron microscopy, electrophoresis, differential scanning calorimetry, viscometry and force deformation analysis. In addition, since the success of any modified processing technique depends upon acceptance of the final product, sensory evaluation was employed to determine the quality of the caviar ultimately produced from cryoprotected eggs.

LITERATURE REVIEW

A. Yolk Composition

Centrifugation of the interior fluid of coho salmon (*O. kisutch*) eggs by Markert and Vanstone (1968) resulted in the formation of three distinct layers: a clear high density pigmented solution (98%), a waxy material of lower density (1.2%) and a floating pigmented oil (0.8%). Composition of the latter two layers has not been the subject of many studies; however, a considerable amount of attention has been focussed on the proteins that form the principal layer and are the predominant constituents of all egg yolk (Nakagawa and Tsuchiya, 1972). Previous research has revealed that high density proteins located in the egg yolk of many vertebrate oviparous species are comparable (Cook and Martin, 1969). Interspecies characteristics of these proteins are similar, to the point that Wallace et al. (1967) suggested the same names be used, as long as the species is identified, in order to prevent unnecessary complications in nomenclature.

In teleosts, as in other egg laying vertebrates, lipoproteins are considered to be the primary energy source for developing embryos (Nakagawa and Tsuchiya, 1969). Several other proteins, one of which is a phosphoprotein, have also been identified in unfertilized teleost egg yolk. One of the simple proteins, containing no lipid or phosphorus components has been labelled β' component by Markert and Vanstone (1968), while the lipoprotein, phosphoprotein and remaining simple proteins have been identified on the basis of electrophoretic mobility, staining characteristics and precipitation with ammonium sulfate as analogous to the lipovitellin, phosvitin and livetins, respectively, found in avian egg yolk (Markert and Vanstone, 1968).

1. Lipovitellin

Studies by Markert and Vanstone (1971), revealed the lipovitellin in coho *(O. kisutch)* as possessing 12.5% nitrogen, 24.3% lipid and 0.56% phosphorus (all present in the lipid portion), on a dry weight basis. They suggested a molecular weight of 250,000, which agrees well with the value obtained by Ando (1965), of 240,000 to 260,000 for the lipoprotein found in rainbow trout *(Salmo irideus)*. Nakagawa and Tsuchiya (1969) isolated a "component I", that was the main fraction in trout *(S. gairdnerii irideus)* egg yolk, and was similar to the lipovitellin Markert and Vanstone (1971) found in coho egg yolk, in containing 11.9% nitrogen, 0.5% phosphorus and 22.8% lipid, of which some 50% was phospholipid. The lipovitellin found in these teleost egg yolks is most probably present in the form of a molecule and not that of a micelle, according to the theory expressed by Cook and Martin (1962). This theory postulates that a lipoprotein with a lipid content of less that 40% and a neutral lipid:phospholipid ratio of about 1:1 or less would possess a structure consisting of a protein matrix whose molecular integrity is not disturbed by the relatively small amount of lipid present. Such an organization would then be expected to repond in a different manner than a micelle to variations in environmental conditions.

2. Phosvitin

The composition of teleost phosvitin has been studied by several groups of researchers. A phosphorus content of 8.5% was obtained for coho salmon phosvitin by Markert and Vanstone (1971), and a value of 8.33% was obtained for brown trout phosvitin *(S. trutta)* by Schmidt et al.,(1965). Markert and Vanstone (1971) also reported phosvitin nitrogen content to be 12.5% for coho phosvitin and noted the absence of any lipid component. A suggested value of 27,000 for the molecular weight was somewhat higher than other values of

24,000 and 19,350 obtained for rainbow trout phosvitin by Wallace et al., (1966) and Mano and Yoshida (1969), respectively, as well as 19,000 for chum salmon and 18,500 for brown trout phosvitin obtained by Schmidt et al., (1965). An N/P ratio of 3.31 was obtained by Markert and Vanstone (1971) for coho phosvitin, which agrees well with that of 2.79-3.44 obtained for rainbow trout by Wallace et al., (1966).

A study performed by Mano and Lipmann (1966), wherein phosvitin obtained from the eggs of several teleost species was eluted from a DEAE cellulose column by a series of salt gradient solutions, revealed the phosvitin found in salmon (species unidentified) yolk to vary somewhat in organization from that found in other teleosts. All teleost phosvitins, with the exception of that obtained from salmon, yielded a series of distinct phosphoproteins varying in the amount of phosphorus present. These were postulated to have been formed by a stepwise, discontinuous phosphorylation to distinct levels, (but the possibility of a similar stepwise degradation due to sample preparation technique was not ruled out.) However, DEAE chromatography of salmon phosvitin preparation yielded only a single phosphoprotein of 9.9% phosphorus.

Phosvitin and lipovitellin in virtually all vertebrate eggs occur in the form of complexes (Wallace et al., 1966). It has been suggested that these two proteins are laid down in the teleost egg by the same serum protein complex, "vitellogenin", that has been observed in other egg laying vertebrates (Hara et al., 1980). Hara and Hirai (1978), working with rainbow trout eggs, performed an immunological precipitation study that suggested that this serum protein was actually a complex of the two egg yolk proteins, lipovitellin and phosvitin.

Phosvitin, two types of lipovitellin and a small amount of low density lipoprotein are condensed as insoluble "granules" in avian yolk, and in frog egg yolk, phosvitin and a single type of lipovitellin are condensed into "platelets" (Wallace, 1965). In teleosts, however, this is not the case (Wallace

et al., 1966), where a non-granular fluid yolk is the rule, with phosvitin and lipovitellin present in soluble forms. The yolk proteins of teleosts appear to be less phosphorylated than the corresponding proteins of other vertebrates, and this difference may be a cause of the higher degree of water solubility (Jared and Wallace, 1968). Less protein kinase activity has been found in acetone powders of trout and killifish ovaries than those of frog, suggesting a less active phosphorylating system existing in teleosts, which would result in incompletely phosphorylated yolk proteins (Jared and Wallace, 1968).

3. Livetins

An immunodiffusion study by Markert and Vanstone (1971) revealed that only trace amounts of livetins are present in coho egg yolk. This had been previously suggested by the authors', electrophoretic work (1968) on the high density layer of five species of Pacific salmon which failed to yield visible proof of livetins on the resultant electropherograms. Electrophoresis of a concentrated water soluble extract of livetins from egg yolk had produced five to eight zones, depending upon the species. Chum salmon possessed one very major and several minor zones, and the mobility of the major livetin zone corresponded to that of the β' component.

4. β' component

There is some doubt as to the nature of the third major protein of teleost egg yolk, variously labelled β' component (Jared and Wallace, 1968; Markert and Vanstone, 1968; 1971) or "x protein" (Nakagawa and Tsuchiya, 1969). A molecular weight for this simple protein of 30,000, with 14.7% nitrogen was reported by Markert and Vanstone (1971). Jared and Wallace (1968) found that extensive dilution of a saline extract of rainbow trout eggs removed phosvitin, but failed to precipitate this component, and so suggested its

possible identity as that of a serum protein or livetin. Conversely, Markert and Vanstone (1971), working with coho salmon, were unable to repeat such a separation by dilution. They performed an immunodiffusion study in which no precipitation lines occurred between plasma of male coho salmon and those of anti-β' component serum, indicating that β' component is not a livetin, but an unique egg protein.

Fewer studies have been centered on the remaining components of teleost egg fluid. Nakagawa and Tsuchiya (1971) investigated the composition of the oil globule of rainbow trout and found it to be very rich in carotenoids, lacking in phosphorus and comprised mostly of triglycerides, with a small amount of chloesterol, cholesterol esters and higher fatty acids. It is thought that these lipids do provide considerable energy for the developing organism (Nakagawa and Tsuchiya, 1969), but the oil globule is mainly utilized only after hatching, in a later phase of the yolk sac period (Nakagawa and Tsuchiya, 1972; Ando, 1965).

Czeczuga (1979) investigated the source of the intense red color of chum eggs and found the total carotenoid content to range from 2.505 to 2.798 μg/g fresh weight. Of this, astaxanthin ester is the major constituent (67.8%). Apparently, roe possessing higher carotenoid contents are more resistant to environmental factors, particularly low oxygen contents, and tend to hatch fry that show a lower mortality rate. Resistance to bacteria may be conferred by an increase in membrane strength caused by certain carotenoids and low environmental oxygen may possibly be combatted by the potential of carotenoid double bonds to be reversibly saturated with oxygen.

B. Membrane Structure

All oviparous species must develop structures to protect and maintain forming embryos and the teleosts are no exception. During the course of development, embryos of the bony fishes are surrounded with protective membranes and cushioned by the fluid-filled perivitelline space that forms after fertilization (Laale, 1980). Observation of the eggs of many species of fish have revealed species specific differences in the egg membrane, both in the internal structure of the membrane, and in the external surface features (Riehl et al., 1980). Lonning (1972) has suggested that the structure of the egg membrane may be determined more by external environmental influences than by taxonomic affinities.

Salmon species possess demersal eggs that are heavy and tend to sink, with a specific gravity greater than that of water (Lagler et al., 1962). This is a distinct advantage to species who lay their eggs in streams of fast running water. On the other hand, eggs which are deposited in masses in a marine environment are pelagic, tending to float (Stehr and Hawkes, 1979). Differences in time required for development prior to hatching may also be reflected in such characteristics as size of egg and thickness of membrane. Pink salmon (*Oncorhynchus gorbuscha*) eggs that require five to six months to develop are 7.0 mm in average diameter, while egg of the starry flounder (*Platichthys stellatus* (Pallas)) are only 0.9 mm in diameter and require only 14 days to develop (Stehr and Hawkes, 1979). Pelagic eggs have also been found to possess membranes that are simpler and thinner in structure than the demersal eggs of species such as the salmon, whose egg membrane must provide protection from mechanical stresses during a long development within a gravel nest (Stehr and Hawkes, 1979; Lonning, 1972).

Hamor and Garside (1977) published the first detailed composition of

the egg membrane of a teleost, using the species *Salmo salar* L. Made up of 92% water, the majority of the remainder was divided between ash, carbohydrate and protein in a 1:3:1 ratio, with lipid present in a fractional amount. Previously, the membrane had been thought to be composed essentially of a protein called "icthulokeratin" (Bell et al., 1969). This protein was shown to be resistant to the action of most proteolytic enzymes, but readily digested by the hatching enzyme released from the mature embryo, labelled "chorionase" by Iuchi and Yamagami (1976). A more complex membrane composition than previously thought was suggested by Hamor and Garside (1973), who demonstrated the presence of phospholipids, cholesterol, DNA and RNA, as well as enzyme bearing microbodies, in addition to the structural proteins.

Bell et al. (1969) observed that both the hatching enzyme and the proteolytic enzyme "pronase" acted only on the inner surface of the membrane of *O. keta* eggs; those bathed externally were unaffected. A very delicate film shaped like the original whole structure always remained after digestion of membranes immersed in enzyme solution and could not be further digested with the addition of fresh enzyme. They suggested a possible function of a protective coating against the action of proteolytic bacteria and premature emergence by the hatching enzyme of surrounding eggs.

Microstructural studies performed by numerous researchers have established that salmon egg membranes consist of three main layers: The outermost layer is a mucous coat, polysaccharide in nature (Kobayashi, 1982). Below this layer lies the intermediate layer diversely termed cortex radiatus externus (Riehl et al., 1980), also known as the primary oocyte membrane, primary chorion (Grierson and Neville, 1982) or zona pellucida externus (Kobayashi, 1982). This layer has also been shown to be moderately polysaccharide in nature (Kobayashi, 1982), and neither the mucous coat nor the cortex radiatus externus (CRE) are dissolvable in acid (Kobayashi, 1982).

In contrast, the innermost layer, numerously labelled the cortex radiatus internus (CRI) (Riehl et al., 1980), cortical membrane, secondary chorion (Grierson and Neville, 1981) or zona pellucida internus has been shown to partially swell and dissolve in 5 x 10^{-3}N hydrochloric acid (Kobayashi, 1982).

The CRE and CRI have been reported by several researchers to be radially perforated with a series of canals (Laale, 1980). These canals possess microvilli that are actually extensions of both the oocyte membrane and the follicular cells, which shrink as the egg matures (Riehl et al., 1980).

Generally, the CRE appears homogeneous and electron dense in transmission electron microscopy (Grierson and Neville, 1981; Kobayashi, 1982; Hurley and Fisher, 1966), while the CRI reveals a highly complex structure, a herringbone pattern being most prevalent among the teleost species studied. Grierson and Neville (1981) have interpreted this pattern as representing sections of a laminate composed of successive layers of protein microfibrils which change in direction through a small angle in successive layers, resulting in a helicoidal arrangement. The number of layers or lamellae in this arrangement varies between species, from 17-18 observed in *Centrolabrus exoletus* to 5 in *Gadus morrhua* (Lonning, 1972). It has been observed that the eggs of ovoviviparous fish have fewer lamellae than oviparous species (Grierson and Neville, 1981), which again may reflect different requirements for structural durability. Accordingly, studies on the egg membranes of Pacific salmon have revealed a complex laminar structure (Stehr and Hawkes, 1979; Kobayashi, 1982) that is in keeping with the relatively harsh conditions under which embryo development must take place.

C. Mechanism of Ice Formation

The deceptively simple phase transition of liquid water to solid ice

lies well within the realm of the most important biochemical processes. Its effect on the viability and/or physical characteristics of biological tissues has been well documented in the literature, however, the causes of alterations brought about by freezing still remain incompletely understood.

The mechanism of freezing has been the object of much study and is presently thought to occur in two discrete stages: nucleation and crystal growth. Nucleation is essential for any further continuation of the process and describes the condensation of a group of water molecules into a configuration enabling successive water molecules to attach themselves with a resulting reduction in their chemical potential (Franks, 1981). In order for this growth to occur, these "ice embryos" must be of a certain critical size, defined as the point at which there is an equal probability of the structure growing or vanishing (Franks, 1981; Meryman, 1960), and of a sufficient lifetime to allow crystal growth.

At the melting point, water and ice are in equilibrium (Fennema, 1973; Franks, 1981) and any reduction in temperature results in further formation of ice. If there is no ice already present in the system, crystals will not begin to form until the temperature drops to several degrees below 0°C (Fennema, 1973). This phenomenon, known as "supercooling", is thought to be due to the presence of an energy barrier based on the changes in free energy involved in the aggregation of sufficient water molecules to form nuclei of critical size. The fact that tissue systems may be cooled to temperatures well below 0°C and then rewarmed without any alteration in their structure indicates that any damage caused by the freezing process is not due solely to lowered temperatures (Fennema, 1973),

The temperature of the system has a direct effect on both the critical size and lifetime of nuclei and it follows then that the degree of supercooling

in any system is dependent upon the temperature of that system. Lower temperatures result in a decrease in critical size and a lengthening in the lifetime of water molecule clusters (Franks, 1981). The rate of nucleation increases by many orders of magnitude over a narrow range of temperatures. At -39°C, the critical size becomes comparable to the size of random aggregations of water molecules and this temperature is thought to be the limit for supercooling (Meryman, 1960).

Two types of nucleation have been described (Fennema, 1973). Homogeneous, three dimensional nucleation involves the chance formation of water molecules into a critically sized nucleus through random density and energy fluctuations. During heterogeneous, two dimensional nucleation, the aggregation of water molecules occurs on the surface of non-aqueous substances, such as suspended foreign bodies, surface films or even the walls of containers (Fennema, 1973; Franks, 1981). The former process requires very low temperatures and occurs in only pure water (Fennema, 1973). On the other hand, heterogeneous nucleation, though unlikely at 0°C, is much more probable at higher freezing temperatures, and is thought to lower the activation energy of nucleation by either decreasing the number of water molecules required for a critically sized nucleus, or by stabilizing ordered clusters of water molecules in the immediate vicinity (Fennema, 1973). Due to the conditions and higher temperatures at which this process occurs, it is thought to form the predominant mode of nucleation in food systems (Fennema, 1973).

Crystallization is the second stage of the phase transition of water into ice and involves the subsequent build-up of successive water molecules onto the ice nucleus. This process, dependent on the rate of diffusion of water molecules to the active nucleus, is temperature dependent as well, with temperatures close to the melting point favouring crystallization (Franks, 1981).

Due to the contrasting dependence of crystallization and nucleation on temperature, low freezing temperatures result in many small ice crystals, while temperatures closer to the melting point create fewer, larger crystals.

D. Mechanisms of Ice Damage

Alterations in frozen material are a result of one or more processes involved in the transition of liquid water to solid ice crystals. The first of these lies in the physical form of ice crystals acting through mechanical disruption. Dependent upon the structure of individual water molecules, ice crystals must form in the shape of a hexagonal prism (Luyet, 1960). In ideal conditions, the six corners of the forming crystal grow faster than the faces, producing stars and hexagonal rosettes that, with continued growth and added branching yields a "skeleton" - a many branched form, still retaining the basic hexagonal shape (Luyet, 1960).

The transition of water to ice at 0°C results in a volume expansion of approximately 9% (Fennema, 1966). In an aqueous system, depending on composition, the amount of unfreezable water and the temperature range employed, there will be restricted areas of expansion and contraction leading to local stresses. The physical nature of the transformation of water to ice thus may lead to mechanical damage.

Directly related to this mechanical damage, is the process that occurs during frozen storage. Unless frozen very slowly, ice crystals are metastable (Franks, 1981), resulting from a surface:volume ratio too large for the smaller crystals formed (Luyet, 1969), and so recrystallization may occur. This process may take place in one or more of several different ways, but the driving force is towards fewer, larger crystals with an accompanying minimization of free energy (Fennema, 1973).

The second cause of damage to a system results from the effect of ice crystal growth on the surrounding aqueous system and is the direct result of concentration changes. Because this process removes mainly water from the system, the non-aqueous solutes become more concentrated in the remaining unfrozen water (Franks, 1981), and this can have an important effect if compounds sensitive to high levels of electrolytes are present. The extent of this concentration depends upon the freezing rate, the ultimate temperature achieved and the nature of the system (Fennema, 1966).

The rate of freezing has been shown to have a direct effect on the number, size and location of ice crystals with slow freezing resulting in fewer, larger crystals in extracellular spaces, while very rapid freezing creates numerous small intracellular crystals (Luyet, 1969; Fennema, 1966). Slow freezing then, by concentrating extracellular fluid with the formation of large ice crystals leads to the second type of concentration damage, resulting in the creation of an osmotic gradient across the cell membrane (Meryman et al., 1977). Subsequent movement of water out of the cell in an attempt to achieve osmotic equilibrium can result in dehydration of the cell, leaving it shrunken and distorted. Both of these effects are a result of removal of water from the system and a parallel has been drawn between the symptoms of freeze damage and those caused by drought and high salt concentrations (Franks, 1981).

E. Cryoprotectants

It is clear that prevention of damage due to freezing must decrease either the dehydration or the effects of dehydration that occur with the removal of water caused by ice crystal growth (Doebbler, 1966; Meryman, 1960), and to a great extent as possible, prevent the formation of large disruptive ice crystals. Ever since the breakthrough work of Polge et al., (1949), pre-

vention of these effects has been attempted with the use of various compounds, collectively known as cryoprotectants, that appear to work by altering the physical condition of the ice and solutions in the system (Farrant, 1980). In a study by Lusena (1955), effective solutes were observed to alter only slightly the ability of water to nucleate heterogeneously, but were able to markedly retard the rate of ice crystal growth.

General characteristics of all cryoprotectants include a high solubility in water that enables the compound to remain in solution even after the first ice is formed, and a low toxicity to the system being protected (Farrant, 1980). The effectiveness of any particular cryoprotectant has been linked to its capacity to form hydrogen bonds and thus bind water molecules, interfering with their incorporation into ice crystals (Parker, 1972; Farrant, 1980). This is of particular importance for systems in which water exists in narrow capillaries or pores (Franks, 1981).

The correlation of cryoprotectant behaviour with molecular structure has been proposed (Doebbler, 1966; Ling, 1967), based on the effect on water structure of a particular solute's molecules. The presence in a molecule of ionic groups, which act as water structure breakers (Ling, 1967), or of non-polar groups that orient water molecules and interfere with adjacent hydrogen bonding (Doebbler, 1966), decrease any compound's ability to act as a cryoprotectant. However, the more numerous the hydrogen bonding sites on a compound, the more magnified the protective capacity (Doebbler, 1966). In the same way, it has been noted that excessive hydrogen bonding sites on a nucleating agent's surface reduce crystal formation. Agents that possess more than one type of functional group would apparently then behave according to the overall additive effect (Ling, 1967).

There are two basic categories of cryoprotectants, based on their capacity to penetrate into the interior of a cell and the type of freezing for which

protection is offered (McGann, 1978). The fact that agents effective in slow freezing conditions are not necessarily effective during rapid freezing, when added to the variation in penetrability expressed by several successful cryoprotectants, suggests two different modes of action (Meryman, 1960).

Penetrating agents such as glycerol, ethylene glycol, and dimethylsulfoxide act in multimolar concentrations (3 to 4 molar) (Meryman, 1971) that usually result in large extracellular crystals and cell dehydration. Penetration of these agents into the cell is of utmost importance or freezing injuries would be simulated by high extracellular concentrations, resulting in dehydration of the cell.

The rate of penetration of different cryoprotectants varies, with dimethylsulfoxide being particularly successful. Such effectiveness becomes of increasing importance when cryoprotection of whole tissues and organs are considered in which a cryoprotective agent must penetrate into the interior of the system.

Non-toxicity is also very important, considering the large molar concentrations required for effectiveness.

The mechanism of action that has been proposed for penetrating cryoprotectants involves a reduction in the amount of extracellular ice formed. Such an effect can be brought about by a colligative binding of water molecules (Meryman, 1971), or through an increase in the viscosity of the system, resulting in a decreased diffusion rate of water molecules to the ice crystal surfaces (Meryman et al., 1977). Both mechanisms serve to interfere with the formation and growth of ice crystals.

The other category of cryoprotectants includes high molecular weight non-penetrating compounds such as polyvinylpyridone, sugars and sugar alcohols that, unlike penetrating agents, appear to be able to protect against freezing damage in quite low molar concentrations under more rapid freezing conditions

(Meryman, 1971). The exact source of cryoprotection for these compounds is still uncertain, however, a theory has been proposed involving a mechanism acting through the temporary alteration of the cell membrane permeability to solutes. This prevents excessive cellular dehydration beyond a maximum tolerated volume (Meryman, 1971), and rapid cooling rates are then necessary to prevent overly excessive leakage of cell contents before freezing occurs.

F. Processing

The processing of roe for caviar involves several steps that have been described in detail by Zaitsev et al. (1969). The eggs are contained in two ovaries formed by two flattened sacs extending along either side of the spinal column in the abdominal cavity of the female fish. Initially, the ovaries are removed from the fish during dressing of the latter, then washed in water at a temperature under 5°C to remove blood and other extraneous matter. During this washing step, grading takes place, with firm and elastic ovaries of a light orange to orange colour receiving top grade status (Sternin and Hori, 1982).

"Screening" follows grading of the roe, wherein ovaries are manually split open lengthwise, then rotated gently by hand on a stainless steel or cord screen with 1 cm openings. The ovaries are covered by a thin transparent film to which immature eggs adhere firmly. However, as maturity increases, the eggs are less securely attached and separate easily. Eggs fall through the first screen onto a second (also with 1 cm mesh openings), then finally onto a slanting screen of 4 mm mesh, labelled the "strainer". This final screen strains out any water and retains fragments of membranes and burst grains, allowing intact eggs to roll down its surface. Roe that are mature and of grade 1 quality separate very easily, with little breakage

of the grains, and the screening losses are small (Sternin and Hori, 1982).

Salting of the loose eggs takes place through immersion in a preheated saturated salt (sodium chloride) solution (1.2 g/cm^3) at a temperature less than 15°C, and a roe:brine ratio of approximately 1:3.

Salting is performed in large vats, with continual gentle agitation over an 8-18 minute period, depending upon the quality of the roe and the temperature of the brine. Scum, burst membranes and blood clots float on the surface of the brine and can be skimmed off with a mesh net. When the eggs have achieved the desired salt content (measured either organoleptically or with the use of a chloride ion electrode (Sternin and Hori, 1982)), they are removed and placed in baskets or strainers to allow the brine to drain off. This procedure may take from 2-12 hours, again depending upon the quality of the original raw product. Time is an important factor, as eggs left too long become sticky, without the proper loose consistency.

Preservatives of 0.1% hexamethylene tetramine (Zaitsev et al., 1969) or 0.1% benzoic acid (Sternin and Hori, 1982) may then be added to ensure longer shelf-life. Vegetable oil (0.6%) and/or glycerine (0.015%) are added to prevent stickiness of the grains and to lend a luster to the final product.

Acceptable processed caviar is divided into two grades, with grade 1 caviar possessing a salinity of between 4-6%, while grade 2 can be as high as 8% (Zaitsev et al., 1969). The highest quality, when judged organoleptically, consists of firm, loose grains that do not stick together when pressed in the palm of the hand. If squeezed between finger and thumb, a single grain will not burst easily and the liquid expressed will retain the shape of a droplet. When squeezed, poorer quality eggs will either burst readily, revealing a thin inner fluid, or will have a waxy appearance and form a solid mass. Other quality factors of the final product include colour, shape and size of the loose grains (Sternin and Hori, 1982). Taste is also of prime

importance. Top quality caviar lacks any sour smell, may possess a slight bitter and/or sharp, but no "skin" aftertaste. The latter sensation results from caviar processed from eggs of a more mature stage than desirable, with a very well developed membrane.

The final yield of caviar from high quality ovaries is 60-65% (w/w) or 80-85% (w/w) of the screened roe (Sternin and Hori, 1982).

MATERIALS AND METHODS

A. Sample Preparation

Ovaries from sexually mature female chum salmon *(Oncorhynchus keta)* were obtained from local fish processors immediately after evisceration. Sample identification was facilitated by assigning a code number to each date of collection as shown in Table 1.

Loose eggs were obtained by "screening", a procedure commonly used in caviar production (Zaitsev et al., 1969). In this method, whole ovaries were manually split open lengthwise, then placed on a stainless steel wired screen with 1 cm square openings and gently rotated with light pressure by hand. Inidividual eggs were separated from the ovary in this manner and were collected in a tray below the screen.

The separate eggs were then prepared in one of the following manners, depending upon the analysis intended:

1. Whole egg blend

Samples were macerated by blending at least 20 eggs with a Polytron homogenizer (Model No. PT 10-35, Brinkman Instruments Inc., Westbury, NY) at 3,000 rpm for 2 min at which time the liquid appeared homogeneous, with no whole eggs or visible pieces of membrane present.

2. Egg magma

Preparation involved placing at least 20 eggs in a plastic strainer (1 mm square openings) and crushing them with a rubber spatula. The free yolk was then allowed to drain through the strainer into a container below.

3. Egg yolk

The immiscible lipid portion of the magma was removed by centrifuging

Table 1. Coding system for egg sample identification.

Date collected	Code no.
1981	
October 15	I
1982	
July 14	II
August 11	III
August 21	IV
September 3	V
September 9	VI
September 16	VII
September 27	VIII
October 8	IX
October 12	X
October 14	XI
October 21	XII

the latter for 90 min at 4°C at 23,500 xg in a Sorvall RC2-B rotor. The higher density layer, labelled the "yolk", was then removed from the bottom of the tube, using a 10 mL disposable tuberculin syringe.

4. Membrane

The residue remaining in the plastic strainer after preparation of egg magma was washed with 2% (w/v) sodium chloride, then blended in 2% (w/v) sodium chloride with the Polytron at 3,000 rpm until no intact membrane fragments remained. The suspension was centrifuged (23,500 xg for 15 min on a Sorvall RC2-B rotor) and washed 3 times with an equal volume of 2% (w/v) sodium chloride, and the final residue was dried overnight in a vacuum oven at 80°C.

B. Proximate Analysis

1. Determination of moisture

Samples of whole egg blend or egg magma (5 g) were mixed with 7 g of washed sand in an aluminum sample pan. These were pre-dried on low heat, stirring with a glass rod to prevent surface case hardening. Final drying was carried out in a vacuum oven at 105 kPa vacuum and 80°C for 18 h. The moisture contents were calculated as a percentage (wet basis) and results presented as the average of a triplicate analyses.

2. Determination of protein

Samples of freeze-dried whole egg blend, egg magma or membrane (12 mg) were placed in a 30 mL micro-Kjeldahl flask and digested using the method of Concon and Soltess (1973). Digested samples were analyzed for nitrogen content by means of a Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, NY). Results were converted into crude protein content by

multiplying % N by 6.25 (Pomeranz and Meloan, 1971). Triplicate samples were digested and the average expressed as crude protein (% wet basis).

3. Determination of crude lipid

Freeze-dried samples of whole egg blend or egg magma (10 g) were placed into 500 mL beakers and carried through a modified Bligh-Dyer extraction (Lee et al., 1966). Aliquots (25 mL) of the chloroform layer were dried under nitrogen in a 50°C water bath, then stored overnight in a desiccator over phosphorus pentoxide before weighing. Extractions were performed in triplicate and the average crude lipid content expressed as a percentage (wet basis).

4. Determination of chloride content

One gram samples of whole egg blend were mixed with 30 mL of 0.1 N silver nitrate (Engelhard Ind., Aurora, ON) for the titration method of the Association of Official Analytical Chemists (AOAC) #18.034-18.035. The chloride content was calculated as a percentage (wet basis), and results were reported as an average of duplicate analyses.

5. Determination of water activity

Samples of egg yolk (10 mL) were placed on a Rotronic Hygroskop DT DMS100 water activity measuring station (Rotronic Ag, Zurich, Switzerland), and allowed to remain at room temperature for 45 min, at which time water activity readings remained essentially constant. Readings were obtained on 3-10 mL portions of yolk and the average value was calculated. The reference sample was a saturated barium chloride solution.

C. Scanning Electron Microscopy

Fresh eggs and several that had been stored at -10°C for 7 d, then thawed

at room temperature were fixed with 4.0% (v/v) glutaraldehyde (J. E. EM Services Inc., Point Claire, Dorvall, PQ) in 0.066 M phosphate buffer pH 7.0 (75 mL 0.066 M sodium phosphate + 48.5 mL 0.066 M potassium phosphate) overnight. The samples were rinsed 3 times (5 min per change) in 0.066 M phosphate buffer (pH 7.0) for 1 h. The eggs were then again rinsed 3 times with a 0.066 M phosphate buffer (pH 7.0), and dehydrated through an ascending ethanol series: 5 min each of 50, 70 and 80% (v/v) ethanol, followed by 2 changes of 10 min each, with 100% ethanol. Subsequent infiltration with amyl acetate (Fisher Chemicals Ltd., purified grade) was achieved with 10 min changes of 25, 50 and 75% (v/v) amyl acetate diluted with absolute ethanol, followed by a 1 h soak in 100% amyl acetate. Samples were then critically point dried in a Parr 5770 Critical Point Drying Bomb (Parr Instrument Co., Moline, IL) using carbon dioxide as the transitional fluid (critical temperature and pressure: 30°C, 7468 kPa). After mounting on aluminum stubs with silver paste and gold coating by vacuum evaporation, the samples were observed with a Cambridge Stereoscan 250 scanning electron microscope. Images were recorded with a Polaroid 545 land film camera on positive/negative film (Type 55). An operating voltage of 20 kV was employed.

D. Electrophoresis

Polyacrylamide gel electrophoresis was performed on salmon egg yolk in an upright slab gel electrophoresis system (#S-1060SG, ATTO Corporation, Tokyo). Using the stock solutions for the zone electrophoresis technique as described by Davis (1964), a 7.5% (w/v) acrylamide (Sigma Chemical Corp., St. Louis, MO) separation gel was polymerized as a 10 cm x 13.5 cm x 0.2 cm slab, leaving a space of 3 cm at the top of the gel mold. A concentration gel of 3.125% acrylamide was carefully pipetted around the comb. Following poly-

merization, the spacer comb was carefully removed and any unpolymerized acrylamide was removed with a syringe. Marker dye was prepared by dispersing 10 mg bromophenol blue (Matheson, Coleman and Bell, Norweed, OH) in a solution of 0.2 mL 0.5 M Tris (Fisher certified ACS Akalimetric Standard) in hydrochloric acid buffer (pH 6.8) and 2 mL glycerol with water to 10 mL. This was applied to each slot with a syringe to a depth of 1 mm, followed by the sample solutions in the order and amounts indicated in Table 2. All electrophoresis experiments were performed on the egg yolk fraction. For each sample 0.1 mL was mixed with 1.25 mL glycerol and 5 mL of the Tris (6.0 mL) : glycine (28.8 g) : water (to 100 mL) running buffer from Davis (1964).

Since some yolk proteins were insoluble in the Tris buffer, the detergents sodium dodecylsulfate (SDS) and deoxycholate (DOC) (BDH Chermicals, Ltd., Poole, England) as well as sodium chloride were evaluated for their effectiveness to completely solubilize the sample. Successive runs were performed with the running buffer containing either 0.5% SDS, 6% DOC or 1.0% sodium chloride. These concentrations were previously found to be the lowest concentration for complete solubilization of the yolk. DOC was found to produce the best well-defined separation; thus all subsequent electrophoretic runs were performed utilizing this detergent in the sample and running buffers.

Each slot containing marker dye and sample solution was overlayered with running buffer and the entire slab carefully placed into the lower reservoir tank of the electrophoretic apparatus. Both upper and lower reservoir tanks had previously been filled with the running buffer. Electrophoresis was performed at 20-25°C with an initial application of 120 mV until the marker dye migrated to the front of the separation gel. Thereafter, the gel was subjected to a 100 mV potential until the marker dye was approximately 0.5 cm from the bottom of the gel.

Fixing and staining were accomplished after a 1 h immersion of the gel in

Table 2. Identity and quantity of yolk sample solutions applied to electrophoresis gels.

Code no.	Condition of yolk	Quantity of sample solution applied (μL)	Gel no.	Slot no.
II	frozen/thawed	5	1	1
		10		2
		5		3
		10		4
	fresh(S)[a]	5		5
		10		6
	fresh	5		7
		10		8
	frozen/thawed(S)	5		9
		10		10
	frozen/thawed	5		11
		10		12
VI	frozen/thawed	10	2	1
	frozen/thawed(S)	10		2
	fresh	10		3
	fresh(S)	10		4
	frozen/thawed	10		5
		5		6
	frozen/thawed(S)	10		7
		5		8
	fresh	10		9
		5		10
	fresh(S)	10		11
		5		12

[a]Yolk from caviar, ie. eggs previously subjected to a saturated sodium chloride colution until chloride content reached 4-5% (measured by chloride ion electrode).

a staining solution of 0.025% (w/v) Coomassie Brilliant Blue R250 (Colab Laboratories Inc., Chicago Height, IL) in 50% (v/v) : 10% (v/v) glacial acetic acid. Destaining was accomplished by an overnight immersion in 25% (v/v) methanol : 7% (v/v) glacial acetic acid, and the gels were stored in fresh destaining solution.

Gels were scanned in a densitometer (Kontes Fiber Optic Scanner Model 800, Vineland, NJ) and data recorded by a Model 42B Varicord variable response recorder (Photovolt Corp., New York City, NY).

Photographs of the gels were recorded on Kodachrome ASA-400, using an Asahi-Pentax Spotmatic F camera.

E. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was performed with a Perkin Elmer DSC-2C (Norwalk, CT). Indium samples were used to calibrate for temperature and to obtain the machine constant used in calculation of heat of enthalpy. The initial temperature was adjusted to 340°K, followed by a programmed scan (at 5 degrees/min) up to 420°K. Thermal scans were controlled, recorded and analyzed by a Perkin Elmer Thermal Analysis Data Station and printout of results was obtained on an interfaced Perkin Elmer AS Plotter.[2]

Samples of 70-80 mg of egg yolk were pressure sealed in stainless steel pans. A sample of lead of sufficient weight for the appropriate heat capacity was sealed in a stainless steel pan and used for a reference. All analyses were performed in triplicate and values expressed as an average.

F. Physical Properties

Since the chum salmon eggs were divided into two discrete components, the membrane and the magma, separate investigations were carried out on these

fractions. A viscometric study of the liquid yolk (ie. magma with the immiscible lipid portion removed) was used to characterize its steady shear flow behaviour and a compression test of the intact egg between parallel flat plates was employed to find the characteristics of the egg membrane upon application of force.

1. Flow behaviour studies

A Brabender Rheotron (Serial No. 802102, C. W. Brabender Instruments Inc., South Hackensack, NJ) rotational viscometer was employed in this study of the flow behaviour of the egg yolk samples. Spring A was used with a couette attachment (C-5) at a controlled temperature of 25°C. The fixture was loaded with 10 mL of the sample and placed into the thermostated jacket for temperature equilibration. At that time (approximately 5 min), a shear rate of 5 rpm (4.5 s^{-1}) was applied, followed by 10 s intervals at successively higher shear rates, up to 904 rpm (814 s^{-1}). This rate was maintained for 30 s, then decreased stepwise back to 5 rpm, again at 10 s intervals. After another 10 s at the lowest rotational speed, the same increase/decrease cycle was conducted. The torsion signal was recorded from the meter for each gear setting. Three replicates were used for each sample tested and the corresponding scale readings for the appropriate rotational speed were averaged for the upcurves and downcurves separately.

For each rotational speed, the shear rate ($\dot{\gamma}$, s^{-1}) was calculated by the equation:

$$\dot{\gamma} = N \times B \qquad (1)$$

where N is the rotational speed in rpm and B is the shear rate constant for the C-5 fixture. (The Kreiger-Maron correction for deviation from Newtonian flow was not applied as the ratio of the radius of the inner cylinder over that of the outer cylinder is very close to 1 for the C-5 couette attachment of the viscometer).

Shear stress (σ, mPa) was calculated by the equation:

$$\sigma = SR \times A \quad (2)$$

where SR is the average scale reading and A is calculated from the following equation:

$$A = 596.1 - 1.943SR + 0.004108SR^2 - 0.00000325SR^3 \quad (3)$$

The equation for the A factor was obtained through multiple regression analyses of corresponding values of the scale readings at various shear rates, using standard oils of know viscosity (No. S-60, S-600 and S-2000, Cannon Instruments Co., State College, PA).

Apparent viscosity (η, mPa·s) was then obtained from the equation:

$$\eta = \sigma / \dot{\gamma} \quad (4)$$

A Monroe programmable calculator (Model 1880) was used with linear regression to determine the fit of the Power Law model to the data, using the form:

$$\log\eta = \log m + (n-1)\log\dot{\gamma} \quad (5)$$

where m is the consistency coefficient (mPa·s^n) and n is the flow behaviour index.

2. Membrane strength studies

One intact egg was placed between parallel flat smooth surface fixtures mounted on an Instron Model 1122 Universal Testing Machine (Instron Inc., Canton, MA), equipped with a 50 kg load cell. Movement of the crosshead was controlled such that the egg was compressed at 10 mm/min up to rupture using the 0.20 kg full scale sensitivity for the load cell. The force deformation curve describing the compression behaviour of the egg was obtained on the Instron stripchart recorder (chart speed set at 50:1 proportional speed with respect to the crosshead speed). Twenty-five individual eggs were used for each treatment tested.

Rupture energy was obtained from areas under the force deformation curves from the point of first resistance to membrane deformation, as determined with a planimeter (Numonics Corp., North Wales, PA) and converted into units of energy (joules). Peak height was measured and converted into units of force (Newtons) to obtain the rupture force. Total deformation (cm), firmness (N/m) and compliance (m/N) were calculated from the area and peak height as previously obtained. Total deformation reflects the crosshead movement from point of first contact to rupture of the egg membrane and was estimated by the formula for triangular area, ie., b = 2(area)/height. The value for b was expressed as deformation of the egg up to the point of rupture. Firmness is defined as the resistance to applied force as reflected by the slope of the curve, and was estimated by the value of peak height/deformation, expressed in units of N/m. Finally, the reciprocal of firmness was defined as compliance, a measure of yielding to applied force.

G. Experimental Procedures

1. Effect of egg size on membrane strength

An investigation was conducted into the relationship between the size of egg and membrane strength to determine whether the variation in membrane strength measurements could be related to the size of the egg. If so, this could be a factor to consider in subsequent experiments.

Fifty fresh eggs (Code VI) were tested for membrane strength, utilizing the Instron machine, according to the procedure previously outlined. Just prior to the test, measurements of egg height and width ($\pm$ 0.1 mm) were taken with the aid of dividers and a scale while the egg was resting on a flat surface. The egg surface area was then calculated with the formula for surface area (SA) of an oblate spheroid (Beyer, 1978):

$$SA = 2\pi a^2 + \pi b^2/\varepsilon \log \varepsilon(1+\varepsilon/1-\varepsilon) \qquad (6)$$

where a is the major axis of the spheroid, b is the minor axis and ε is equal to $\sqrt{a^2 - b^2/a}$.

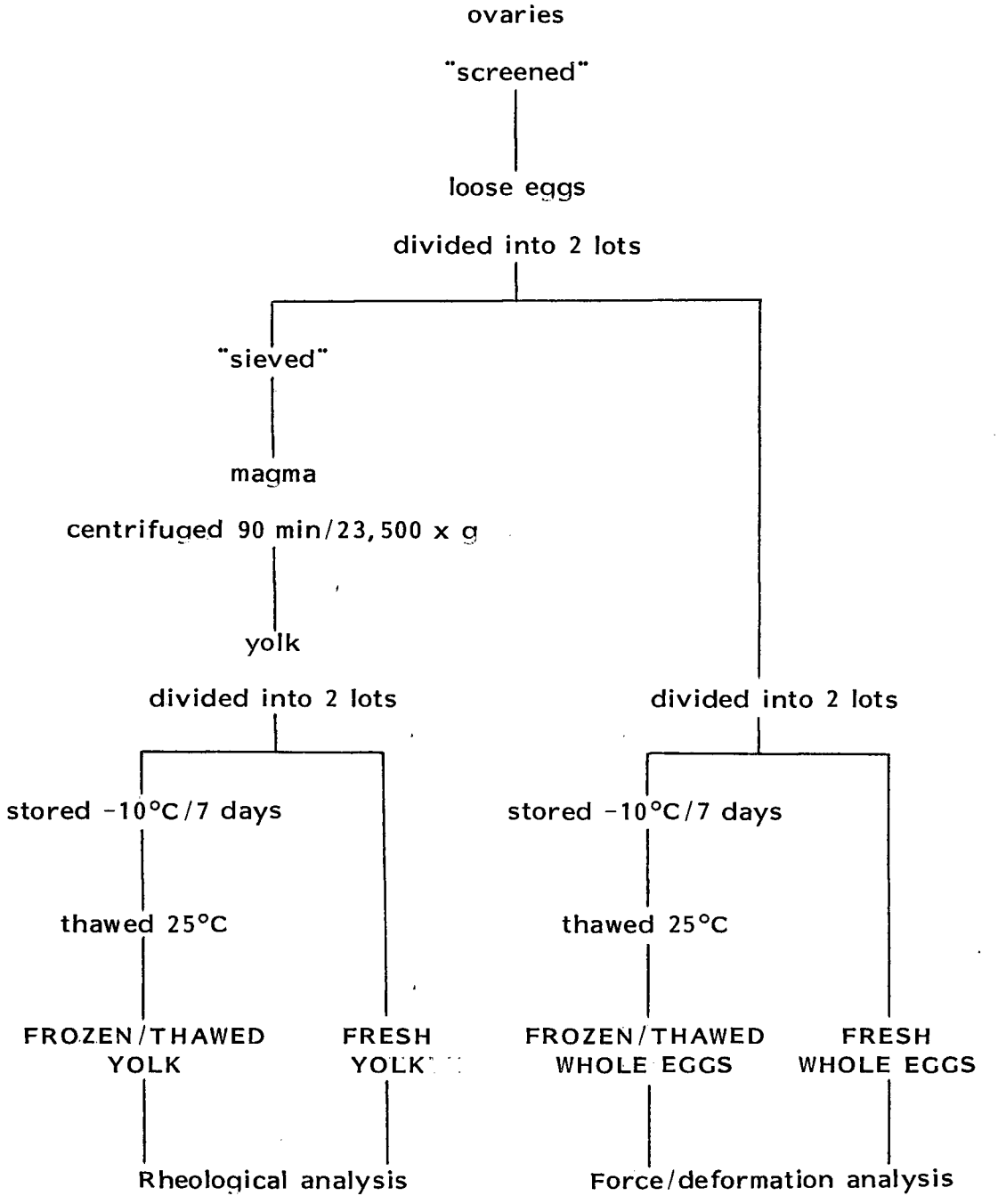
2. Frozen storage effects.

As previously stated, the physical structure of chum salmon eggs were considered as two discrete systems: the inner fluid magma and the surrounding elastic membrane. Evaluation of the effects of frozen storage on physical properties were evaluated in terms of yolk flow behaviour and membrane rupture energy by separate analyses on fresh eggs and those thawed after storage at -10°C for 7 d. The procedure followed is outlined in Figure 1, in which a sample of four ovaries were screened within 2 h of evisceration to produce separate eggs, half of which were crushed to obtain egg magma, which was then centrifuged to isolate the egg yolk. This was collected from the centrifuge tubes, pooled and again divided. In each of six 50 mL polypropylene screw-top disposable centrifuge tubes, 15 mL amounts were placed, and three of these were stored at -10°C for 7 d. The other three tubes were kept unfrozen and used directly for viscometry.

Two lots of approximately 50 eggs were picked from the remaining whole eggs and placed in screw-top polypropylene centrifuge tubes. One of the tubes was stored for 7 d at -10°C, while 25 eggs from the other tube were immediately analyzed by the Instron compression test. After 7 d, the frozen samples were placed at room temperature to thaw, then treated in the same manner as the fresh samples. This experiment was performed with Code II samples, and later with Code VI in order to test whether variation may be attributed to samples from different seasons of the year.

Thermal analysis was conducted on yolk from Codes II and VI in the manner previously described, however, the Perkin-Elmer Data Station was not available for analysis of Code II yolk samples. Instead, data from the calorimeter were recorded on a Perkin-Elmer Single Channel, Multirange Thermal Analysis

Figure 1. Flow diagram of procedure for investigation into frozen storage effects.



recorder with temperature marker (#0056-1003). The areas under curves were obtained with an electronic planimeter and the following equation used for calculation of transition energy:

$$\Delta \text{Heat} = (K \times R \times A)/(W \times S) \quad (7)$$

where ΔHeat is the transition energy (cal/g), K is the calibrated instrument constant (1.0), R is the range sensitivity (mcal·in/s), A is the peak area (in^2), W is the sample weight (mg) and S is the recorder chart speed (in/s).

Electrophoretic analyses were carried out on yolk from Codes II and VI, following the procedure formerly outlined. In addition, to determine whether salting of the eggs (an integral part of caviar production; Zaitsev et al., 1969) produced an effect on yolk proteins, caviar was processed from fresh eggs (Codes II and VI) by immersion in a saturated salt solution until the chloride content reached 4-5%, as determined by a chloride ion electrode (Model 96-17-00, Orion Research, Inc., Cambridge, MA). Yolk was then obtained from these salted eggs and subjected to electrophoresis, both as fresh material and that thawed after 7 d storage at -10°C/

3. Cryoprotectant agent effects

In order to evaluate the effectiveness of various cryoprotectants as a means of maintaining the membrane properties of fresh eggs through frozen storage, selected cryoprotectants were applied at different levels to fresh eggs and the membrane energy determined before and after frozen storage. The procedure involved obtaining 4 ovaries from 4 female chum salmon and screening these within 2 h of evisceration. Thirty mL of the resultant separate eggs were immersed in 50 mL of the cryoprotectant solution, for a period of 30 min, with gentle agitation after 15 min. The samples were drained for 1 min, then divided into two portions. One half was placed in a 50 mL disposable screw-top centrifuge tube, placed at -10°C and stored for 7 d. From the remaining half, 25

eggs were immediately analyzed with the Instron compression test as previously described. After 7 d, the frozen samples were thawed in air at room temperature, then analyzed as for the fresh. The cryoprotectant solutions used were: 0.2, 1.0, 2.0 and 3.0 M concentrations of sodium chloride, 1.0 and 3.0 M concentrations of sodium phosphate, glycerol, ethylene glycol and calcium chloride, and 0.5 and 1.0 M concentrations of sucrose. In addition, sodium phosphate and sodium chloride were combined in concentrations of 0.3 M : 0.2 M, respectively, and 0.9 M : 0.5 M, respectively.

4. Effectiveness of sodium chloride as a cryoprotectant

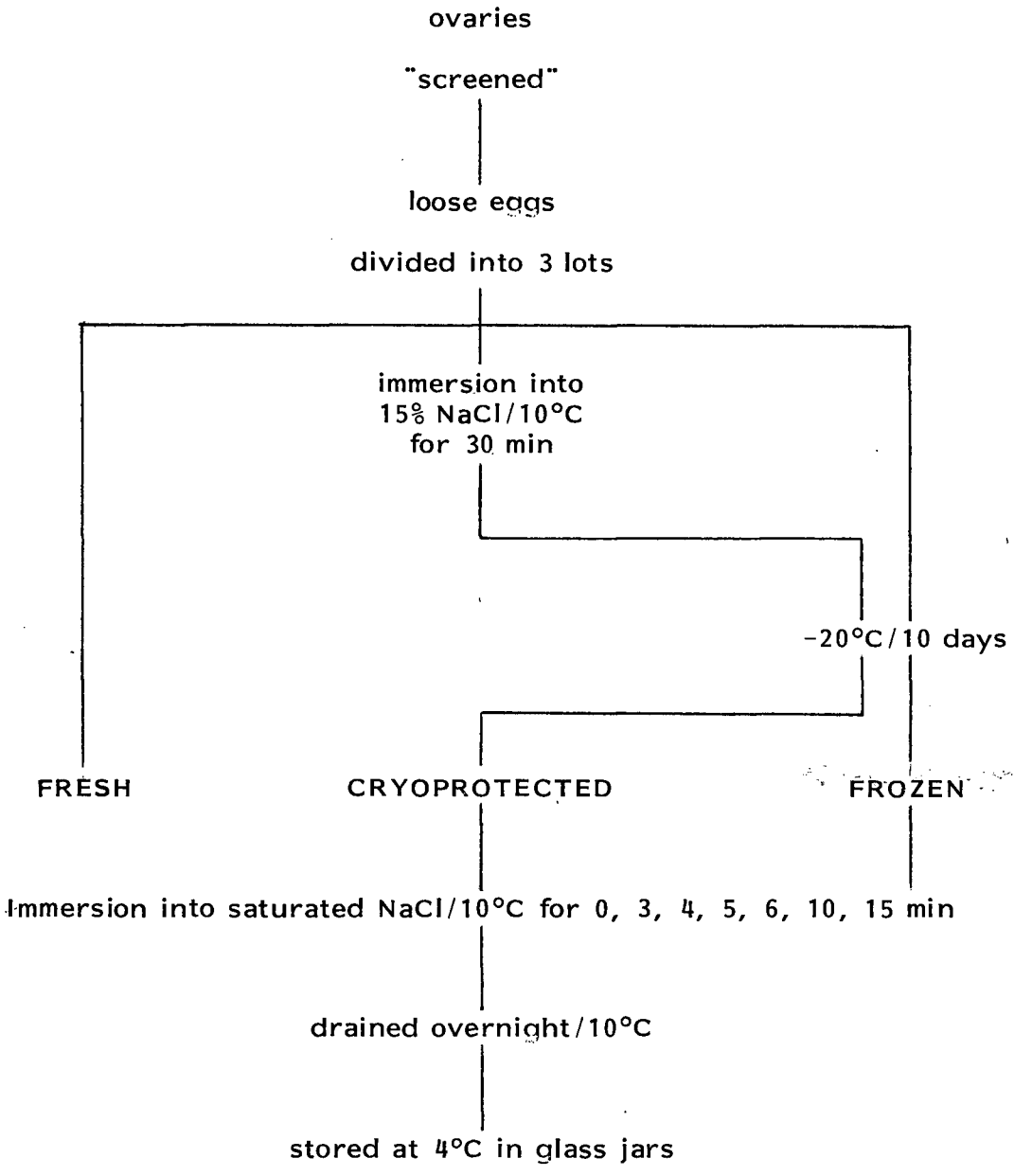
The efficacy of a cryoprotectant found to be effective in the previous section is ultimately determined by the quality of the caviar produced from eggs resulting from such treatment. In order for the cryoprotective treatment to be judged satisfactory, the resulting caviar must be similar in characteristics such as taste, appearance and texture to that produced from fresh eggs. Moreover, the product must be superior to caviar produced from frozen eggs.

Evaluation of textural properties were again carried out in two parts: the analysis of yolk flow behaviour and that of membrane strength. Evaluation was performed both objectively by Instron analyses and the Rheotron, and subjectively, utilizing sensory panel analysis. Caviar from fresh, frozen/thawed and cryoprotected eggs was analyzed and the results compared.

a) Objective analysis - sample preparation

The procedure followed is outlined in Figure 2 and involved screening 12 ovaries within 2 h of evisceration, and dividing the eggs into three portions. One portion was labelled "Frozen/thawed", placed into a polypropylene freezer bag, and sealed so that the mass retained a flat shape of width approximately 2 cm. Another portion which was labelled "Cryoprotected", was immersed in a 10°C solution of 15% (w/v) sodium chloride (ratio of eggs to salt solution, 3 to 5)

Figure 2. Flow diagram of procedure for investigation into effectiveness of sodium chloride as a cryoprotectant.



for 30 min, allowed to drain in a mesh basket for 4 min in a layer 2 eggs deep, then packed in a polypropylene freezer bag and sealed in the same manner as the "Frozen/thawed" sample. It is important here to stress that the term "Cryoprotected" refers to eggs treated with a potential cryoprotectant, then frozen and thawed in the same manner as the "Frozen/thawed" eggs. Both of these were then placed in a still air freezer at -20°C. The final portion was labelled "Fresh" and processed immediately into caviar. Since the immersion time in saturated sodium chloride needed to produce eggs with the appropriate chloride content for caviar (ie., 4-5%, Zaitsev et al., 1969), varies substantially from sample to sample (Hori, 1982), a range of salting times were employed: 0 min (unsalted eggs), 3, 4, 5, 6, 10 and 15 min. Approximately one seventh of the fresh eggs was reserved and the remainder was immersed in a saturated salt solution at 10°C. At intervals of 3, 4, 5, 6, 10 and 15 min, 250 mL of eggs were removed and set to drain in a layer 2 cm deep in a net-lined basket. The unsalted portion was stored in a glass jar at 5°C during the draining period (18 h). Thereafter, the salted eggs were also placed in glass jars and stored at 5°C until analysis.

Analysis of the samples so obtained included chloride content, flow behaviour and membrane strength, as described (with the exception that for viscosity analysis, only every second shear rate available on the Brabender Rheotron was used and these were applied only once, in increasing progression). Both the viscosity analysis and membrane strength analysis were completed within 48 h post-salting.

After a storage period of 10 d, the frozen/thawed and cryoprotected portions were thawed overnight at 5°C, then treated and analyzed in the same manner as the fresh portion.

The procedure was repeated 3 times using sample Codes IX, X and XI, with the exception that a salting time of 15 min was not included for the first

replicate (ie. Code IX), due to uncertainty of the extent of salting time needed to produce detectable differences in egg characteristics.

b) Subjective analysis - sample preparation

A sample of 4 ovaries were screened within 2 h evisceration and the eggs divided into 3 portions. Frozen/thawed and cryoprotected portions were produced in the same manner as outlined in the previous section on sample preparation for objective analyses, including frozen storage at -20°C in a still air freezer. The final third, labelled "Fresh", was immersed in a saturated sodium chloride solution at 10°C for 8 min, which produced eggs possessing a chloride content of about 4%, as indicated by the chloride contact electrode. This portion was then packed in a glass jar and held at 5°C.

At the end of 7 weeks, the frozen/thawed and cryoprotected portions were each thawed overnight at 5°C, then divided into three. The 3 portions of the frozen/thawed samples were salted at 10°C for 4.0, 4.5 and 5.0 min, and the 3 portions of the cryoprotected sample were salted for 0.0, 1.0, and 2.0 min. Salting times were determined by subjective determination of salinity through sample tasting. All brined eggs were allowed to drain for 18 h in a layer 2 eggs deep in a net-lined basket. On the following day, they were packed into glass jars and held at 5°C.

After a storage period of 6 weeks, mold was discovered in the jar holding the fresh sample and it was discarded for sensory panel analysis. Replacement of this reference was furnished by caviar obtained from the Fisheries Technology Division at B. C. Research that had been determined as being of high quality (Hori, 1982).

c) Subjective analysis - sensory evaluation

A paired comparison test (Larmond, 1977) was chosen for sensory evaluation of membrane strength and viscosity. A nine point scale was used ranging from

"extremely thicker (or tougher)" to "extremely thinner (or weaker)", with a scoring of 5 indicating a sample no different than the reference. The 4.5 min salting time of the frozen/thawed samples and the 1.0 min salting time of the cryoprotected samples were chosen for presentation to the panel on the basis of possessing optimum taste in terms of salinity and low rancidity. Three samples each of the 4.5 min salting time of frozen/thawed eggs and of the 1.0 min salting time of the cryoprotected eggs, and one sample of the high quality fresh caviar made up the seven randomly presented samples (labelled with 3 digit numbers) that were presented to each of the panel members for comparison with the reference (fresh high quality caviar). Eight panel members were chosen, who had been present at caviar taste panels at least once before and so were familiar with the product.

In addition, a nine point hedonic scale, ranging from "like extremely" to "dislike extremely" (Larmond, 1977) was included as a measure of the overall acceptability of the samples.

H. Statistical Analyses

1. Analysis of variance

Using the analysis of variance (ANOVA) program package (*MFAV) (Le, 1980), available through the UBC computer (Amdahl 470 V/8) system, data from several experiments were analyzed. A level of $p<0.05$ was employed in all analyses to determine significance, and if significance was found, a Newman-Keul's multiple range test, available with the *MFAV program was applied.

a) Effect of cryoprotectant

The purpose of the analysis was to determine which, if any, cryoprotective treatments resulted in thawed eggs with membrane strength not significantly different from that of the fresh. A one-way analysis of variance with Treatment

as the single factor was performed separately for each coded lot. Pre and post freezing results of any cryoprotectant treatment were considered as separate treatments (resulting in a number of levels in the analysis twice the number of actual cryoprotective treatments). Newman-Keul's multiple range test then enabled grouping of means not significantly different from each other in codes wherein the factor of Treatment had been evaluated as a significant source of variation.

b) Effect of pre-freezing treatment with sodium chloride:

i. Membrane strength data

A Model III multifactorial ANOVA was applied to membrane rupture energy data with Code (3 levels) as a random factor, and Condition of egg before salting (3 levels) and Minutes in saturated sodium chloride solution (5 levels) as fixed factors. (Only 5 levels of the Minutes factor, representing the salting times of 3-10 min, were used for statistical analysis, due to missing values for the 0 min salting time of Code XI frozen/thawed and cryoprotected eggs, and the 15 min salting time of Code IX eggs.)

Due to the random nature of one or more factors inherent in a Model III ANOVA, the method of calculation differs from an ANOVA wherein all factors are fixed (ie., Model I ANOVA) (Zar, 1974). In the present study, Code represents a random factor, as the three dates of sampling were arbitrarily chosen. A null hypothesis ($H_o=\mu_1=\mu_2=\mu_3$) as applied to this factor would be more aptly expressed as "H_o=there is no difference in membrane strength between days". Because of this difference, the residual error mean square is not used as the denominator of the F statistic for testing every source of variation, rather, testing the main effects of Condition and Minutes is more appropriately done by using the respective interaction mean squares of Condition x Code and Minutes x Code as denominators of F-values. Likewise,

the interaction effect of Condition x Minutes is most aptly assessed by testing the corresponding mean square against the Condition x Minutes x Code mean square (Table C2; p342; Zar, 1974).

ii. Sensory evaluation

Two-way analyses of variance, with Samples (7 levels representing the 7 samples presented in the sensory panel) and Judges (8 levels representing the 8 judges) as sources of variation, were performed for data from the viscosity and the membrane strength assessments. The Sample factor was further divided into three sources of variation expressed as comparisons between: a) the control sample and the remainder of the samples presented, b) caviar from the cryoprotected and the frozen/thawed samples, and c) replicates within each treatment (ie. triplicates each of caviar from cryoprotected and frozen/thawed eggs).

A two-way ANOVA was used to analyze data from the assessment of preference yielded by the hedonic scale, with Samples and Judges as the main effects. Because the interest in this analysis lay only with the comparative preferences of the judges for each separate sample, no further division of the Sample term was carried out.

2. Analysis of regression lines

Comparisons between rheograms were accomplished by utilizing the previously described Power law flow model as a regression equation for data points included within the range 36 to 300 s^{-1}. A pre-written program, titled "NLIN", available on the UBC Food Science Department computing ID (Tung, 1983), was then used to determine the presence of a significant difference between two regression lines. This covariance program compares two sets of data comprising the two regression lines on the bases of homogeneity of residual variances, slopes and levels. The significance of these comparisons is based on an F-value generated in each case. In this comparison of Power law

rheograms, a difference in slopes would indicate that n values differ and a difference in levels would indicate that m values differ.

RESULTS AND DISCUSSION

A. Proximate Analysis

Results of a proximate analysis of Codes V, IX and XII are presented in Table 3. Values for protein and moisture agree well with figures reported in the literature for salmon species (Hamor and Garside, 1977; Smirnov et al., 1968), as well as other species of fish (Linko et al., 1980; Kaitaranta, 1981). The lipid content, however, as yielded by the method employed in this study, exceeds that previously reported by Smirnov et al., (1968) of 4.54% (wet basis) for chum salmon, instead lying closer to those values obtained by Hamor and Garside (1977) of 13.00% (whole egg, wet basis) and 16.00% (yolk, wet basis) for Atlantic salmon *(Salmo salar)*. Variation from Smirnov's values may be due to methods used to analyze lipid content. The ability of the Soxhlet extraction method, as used by Smirnov et al., to extract lipoproteins is known to be inefficient (Lee et al., 1966). The method used by Hamor and Garside involved lipid extraction according to the technique of Bligh and Dyer (1959), and this procedure may then have produced higher yields of lipid material. The method used in this study was a modification of the Bligh-Dyer procedure (Lee et al., 1966) and could then be expected to give similar high yields. In addition, it has been shown that the lipid content of teleost eggs may increase with advancing maturity (Vuorela et al., 1979), so that eggs of Codes V, IX and XII collected late in the season, may actually have possessed a higher lipid content.

The large amounts of protein and lipid found in the salmon egg reflect the need of a growing embryo for an adequate supply of energy to enable development (Hollett and Hayes, 1946).

A value of 0.97 was determined for the water activity of chum egg yolk of Code I, a value which indicates a susceptibility to bacteria, yeasts and

Table 3. Proximate analysis of chum salmon eggs (n=3).

Fraction	Code no.	Moisture (%wb)	Crude lipid (%wb)	Crude protein (%wb)
Whole egg	V	56.9 (0.2)[a]	14.3 (0.3)	27.8 (0.6)
	IX	56.4 (0.1)	12.9 (1.8)	28.2 (0.3)
	XII	57.3 (0.2)	15.0 (3.8)	26.8 (1.3)
Magma	V	56.6 (1.0)	14.1 (0.2)	24.4 (2.3)
	IX	56.0 (0.4)	15.9 (1.8)	26.5 (2.9)
	XII	56.4 (1.0)	15.3 (1.0)	25.8 (3.3)

[a]Standard deviation

molds (Karel, 1975), and necessitates the use of low temperature, sterile conditions and the addition of preservatives in industrial application to ensure adequate preservation.

B. Differential Scanning Calorimetry

Figure 3 represents a typical differential scanning calorimetry (DSC) thermogram of chum egg yolk, revealing three obvious peaks. Previous researchers studying thermal analysis of proteins (Donovan et al., 1975; Wright et al., 1977) have postulated that each major peak in a DSC thermogram represents one thermal process (ie. the denaturation of one protein). Support for this theory has come from comparisons of thermograms of the entire protein system with those of individual purified proteins (eg. the thermogram of whole avian egg white was compared with thermograms of separate proteins including ovalbumin, conalbumen and lysozyme in the study by Donovan et al., 1975). It would appear then, that there are at least three thermal processes taking place in the thermograms obtained in this study, representing the denaturation of three proteins. This agrees well with the presence of three major proteins (lipovitellin, phosvitin and β' component) in chum egg yolk.

Positive identification of each peak in terms of the protein it represents is dubious without comparisons with thermograms of the corresponding isolated protein. However, the size of the peak obtained is proportional to the amount of protein present (Donovan et al., 1975) so that it could at least be suggested that the largest peak is representative of lipovitellin, which has been shown to be the dominant protein in teleost egg yolk (Markert and Vanstone, 1968; Nakagawa and Tsuchiya, 1969). The other two peaks could then represent the β' component and phosvitin.

The temperatures of denaturation, as defined by the peak maxima, are

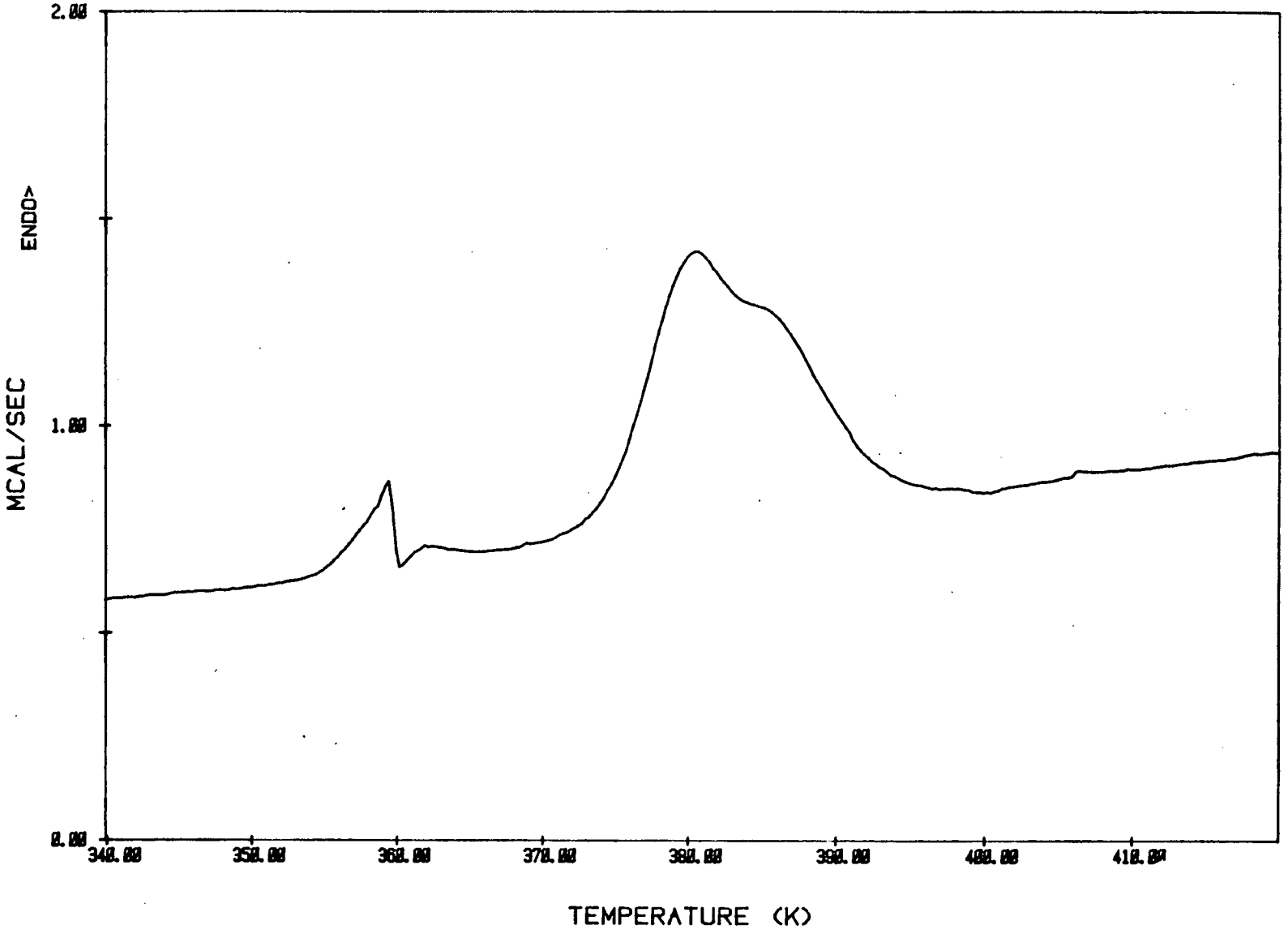


Figure 3. Typical DSC thermogram of fresh yolk from eggs of Code VI.

represented in Table 4. (The maximum for Peak 3 of Code II was estimated from the curves shown by the Perkin Elmer Thermal Analysis recorder.) These values reveal high resistances to heat. Previous research yielded denaturation temperatures of 84.0°C for ovalbumin, 69.5°C for conalbumin (Donovan et al., 1975) and 60-80°C as a range of denaturation temperatures for rabbit muscle proteins (Wright et al., 1977). In comparison, current values of approximately 87°C and 107°C for chum yolk proteins appear to be significantly higher. Likewise, the values for the enthalpies of the various peaks (Table 4) appear diminished in comparison with values obtained for egg white proteins (3.64 cal/g for ovalbumin; 3.63 cal/g for conalbumin (Donovan et al., 1975)) and rabbit muscle proteins (3.46 cal/g for actin; 3.33 cal/g for myosin (Wright et al., 1977)). However, the latter effect may well be due to values for enthalpy being given as calories per gram of yolk dry matter in the present study, and not gram of proteins, since the exact proportion of each protein present is unknown. Any real differences that exist reflect variations in the structure of the proteins. An example of the influence of protein structure on thermal analysis results is found in the case of ovomucin, a mixture of egg proteins having similar properties, and possessing a very simple random coil conformation which yields no peaks in a DSC thermogram (Donovan et al., 1975).

C. Polyacrylamide Gel Electrophoresis

In the present study, an anionic detergent, deoxycholate (DOC) was added to the sample and running buffer to enhance solubilization of the yolk proteins. The densitometer trace resulting from polyacrylamide gel electrophoresis (PAGE) of chum egg yolk is depicted in Figure 4 and reveals the separation of four major discrete bands, labelled I, II, III and IV. Previous electrophoretic studies of teleost egg yolk by other researchers have also indicated the pre-

Table 4. Enthalpy of denaturation and denaturation temperature of proteins of fresh chum egg yolk of two codes.

Code no.	Peak no.	Δ Heat (cal/g yolk, db)	Peak no.	Denaturation temperature (°C)
II	1	0.29 (0.01)[a]	1	86.0 (1.0)
VI	1	0.27 (0.02)	1	86.4 (0.2)
II	2 & 3	2.60 (0.03)	2	106.7 (1.1)
VI	2 & 3	2.95 (0.04)	2	107.5 (0.4)
II			3	110.8 (1.1)
VI			3	-----[b]

[a]Standard deviation (n=3).

[b]value unavailable from Perkin Elmer Thermal Analysis Station.

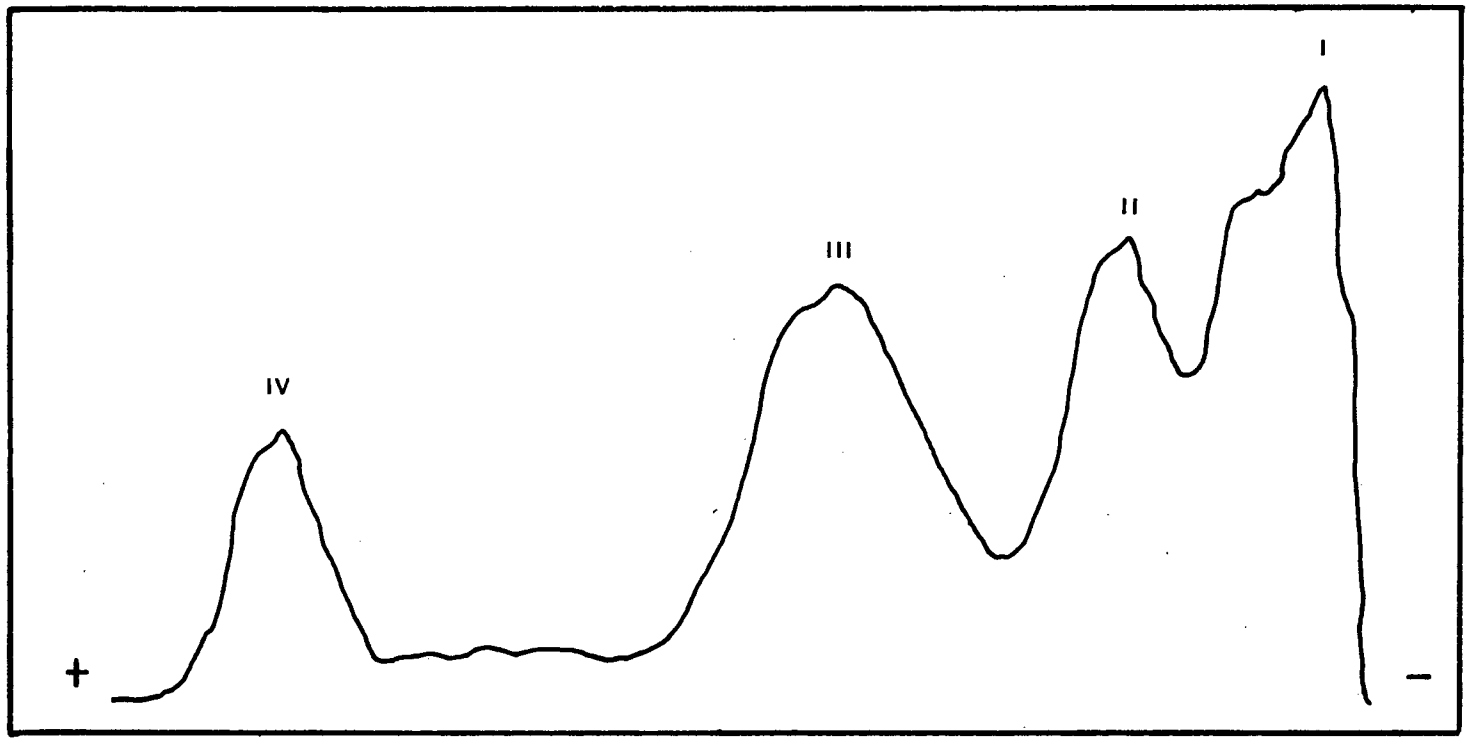


Figure 4. Densitometer tracing of polyacrylamide gels from electrophoresis of yolk from fresh chum eggs.

sence of several proteins. Tiselius and starch gel electrophoretic patterns of rainbow trout egg yolk by Nakagawa and Tsuchiya (1969) revealed three proteins: a lipoprotein, a phosphoprotein and a simple protein which were presumably analogous to the avian egg yolk lipovitellin, phosvitin and livetin, respectively. Working with five species of Pacific salmon, Markert and Vanstone (1968) separated three yolk proteins on cellulose acetate and identified them on the basis of dye-binding capacity, retention on hydroxyapatite and the degree of precipitation with ammonium sulfate. In addition, they were able to isolate a fourth simple protein that migrated the same distance as livetins but could be precipitated out of yolk solution by dilution with water. This component was subsequently named "β' component".

With both starch gel and cellulose acetate electrophoresis, the lipovitellin fraction remained at the origin, followed by one or more bands of livetins and the β' component, and finally the phosvitin. The same pattern occurred in polyacrylamide gel electrophoresis applied to the separation of avian yolk high density proteins (Chang et al., 1970; McBee and Cotterill, 1979; Dixon and Cotterill, 1981), ie. large lipoprotein molecules lipovitellin (α and ββ) remained close to the origin, with the livetins next present in a series of bands, and finally, the phosvitin as a single band migrating the furthest.

Polyacrylamide and starch gel electrophoresis of proteins bring about the formation of separate zones on the basis of electric charge and molecular size of the proteins (Deyl et al., 1979). The addition of DOC in the present study causes the effect of varying molecular charges on protein movement to be cancelled out, making molecular size the major factor in separations (Hjelmeland and Chrambach, 1981). No such detergents were employed in the previously referred to studies on avian yolk, using polyacrylamide gel, and so charge would be expected to play a role in the separation obtained. However, the results remain what may be expected if only the molecular size, based on

molecular weight is considered. Avian lipovitellins (α and β) have been estimated as being approximately 400,000 daltons (dimer forms) (Bernardi and Cook, 1960), livetins (α, β and γ) as ranging between weights of 45,000 to 150,000 (Martin et al., 1957), and phosvitin as 40,000 daltons (Windholz et al., 1976). If it is assumed that molecular size follows molecular weight, then the order of the protein bands achieved with avian yolk high density protein follows that which could be expected. Likewise, the molecular weight of coho lipovitellin, phosvitin and β' component have been estimated as 390,000, 27,000 and 30,000, respectively (Markert and Vanstone, 1968) and the comparative magnitudes thus displayed are reflected in the movement of chum yolk proteins on cellulose acetate, as observed by Markert and Vanstone (1968).

Therefore, based on the postion of the bands, tentative identification of the proteins in the present study may be made. The large peak near the origin (Peak I) is most probably lipovitellin, the β' component and livetin molecules are within Peaks II and III and finally, the smallest, furthest migrating peak (Peak IV) probably represents phosvitin.

D. Scanning Electron Microscopy

A scanning electron micrograph of the cross section of a chum salmon egg membrane is depicted in Figure 5. This and all subsequent views representing the membrane cross section, are in fact actual splits in the egg membrane, most likely the result of stress applied to the egg during removal from the ovary by the technique of screening.

Observation of Figure 5 clearly reveals two layers, the inner of which appears to be fibrous in nature. Such a structure also appears in the micrographs of Kobayashi (1982) who described this inner portion of the chum membrane as a distinct discrete zone of intertwisting fibers, gradually giving

way to a more continuous homogeneous region in the outer portion.

Stehr and Hawkes (1979) produced scanning micrographs of the eggs of pink salmon *O. gorbuscha* (Walbaum) and interpreted the egg membrane to be composed of short, discontinuous lamellae, with pore canals penetrating the zona radiata, producing a columnar like appearance. Such an appearance is indicated in the cross section of the frozen/thawed egg membrane (Figure 6) but is not as apparent in the fresh egg membrane (Figure 7). It is not known whether this difference is due to the frozen storage of the egg, or merely an artifact of preparative technique.

The discreteness of the fibers observed by both Kobayashi (1982) and Stehr and Hawkes (1979) is missing in the micrographs of the present study, however, this may be explained by the fact that if the cross sectional cracks are indeed produced in the egg membrane by the screening process, it is quite likely that inner yolk may have leaked out onto the membrane cross section before fixation occurred, thus obscuring fine detail. Discrete fibers covered by a coat of yolk material, are then represented by inter-twisting ridges.

The width of this part of the membrane appears to be fairly uniform (Figure 8), and measures about 40 µm, in close agreement with values previously obtained for chum salmon of 50 µm (Kobayashi, 1982), 55 to 70 µm for pink salmon (Stehr and Hawkes, 1979) and 18 to 55 µm for other teleost species (Grierson and Neville, 1981; Hurley and Fisher, 1966).

The surface of the chum egg, as revealed in Figure 9, appears as a fibrillar layer similar to that reported by Kobayashi (1982). This layer was found to be polysaccaride in nature by Kobayashi (1982) and may act both to absorb impacts and protect the egg, as well as a possible adhesive apparatus, an adaptation favourable to the demersal eggs characteristic of those species that deposit their eggs in fast running streams (Lagler et al., 1962; Grierson and Neville, 1981).

Figures 5 to 9. Scanning electron micrographs of salmon chum eggs.

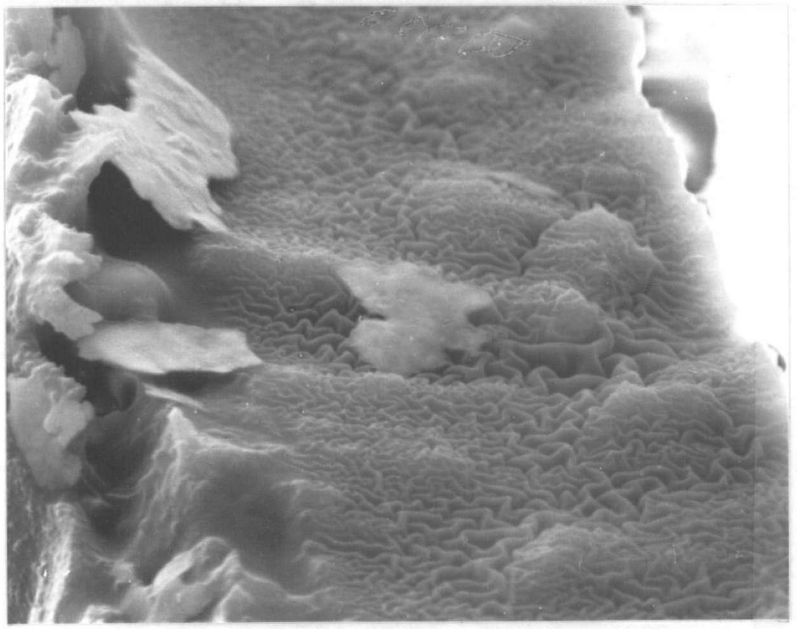


Figure 5. Cross-section of fresh egg membrane as revealed by a rupture in the egg surface (pw = 50 μm).

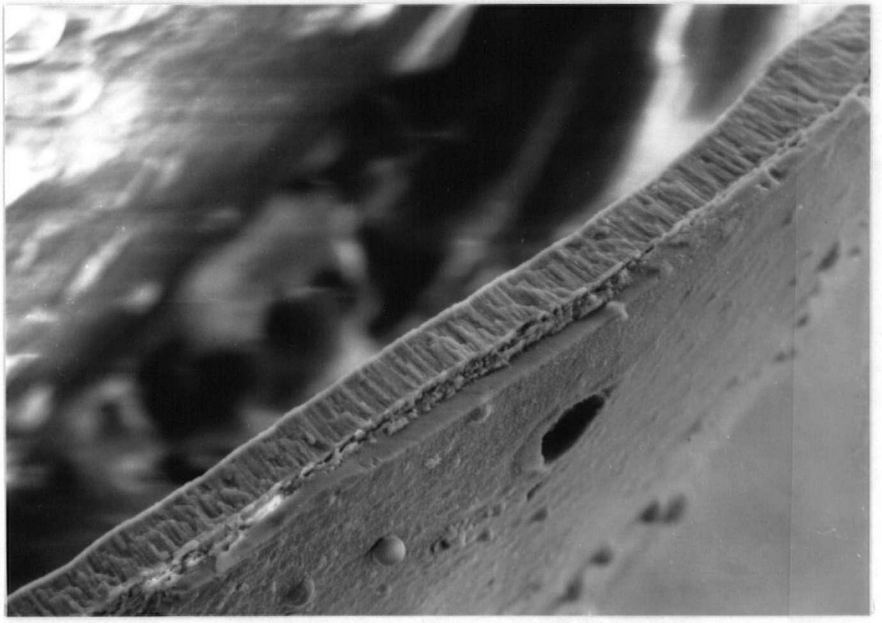


Figure 6. Cross-section of frozen/thawed egg membrane as revealed by a rupture in the egg surface (pw = 490 μm).

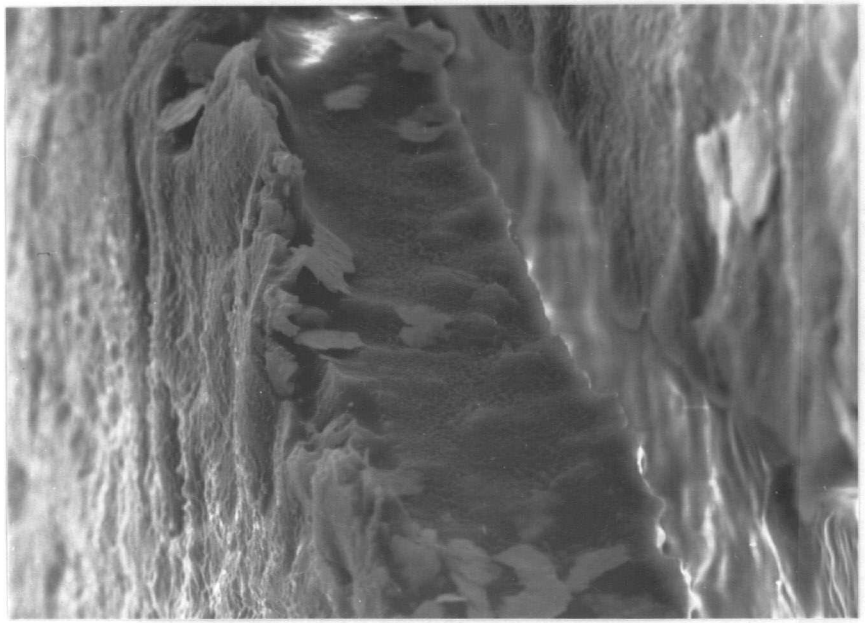


Figure 7. Similar view to Figure 5 (pw = 140 μm).

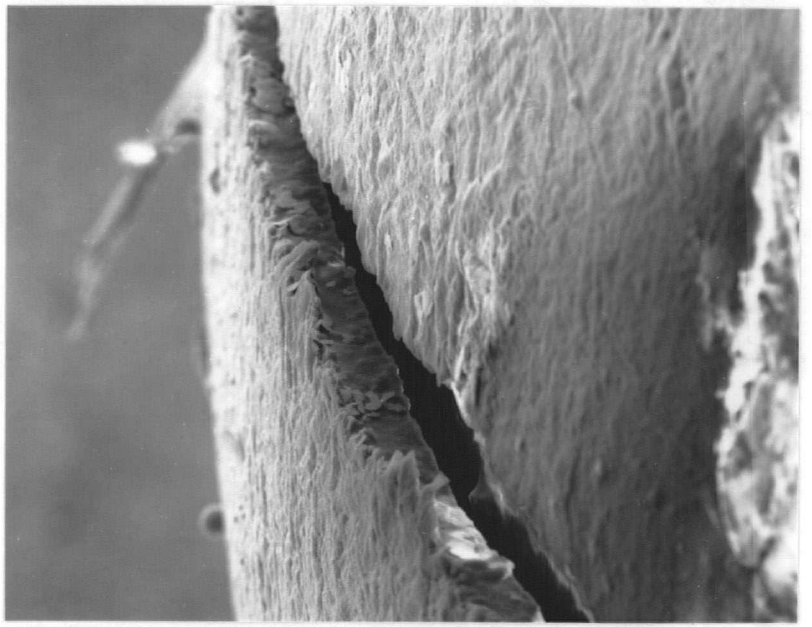


Figure 8. Similar view to Figure 5 (pw = 620 μm).

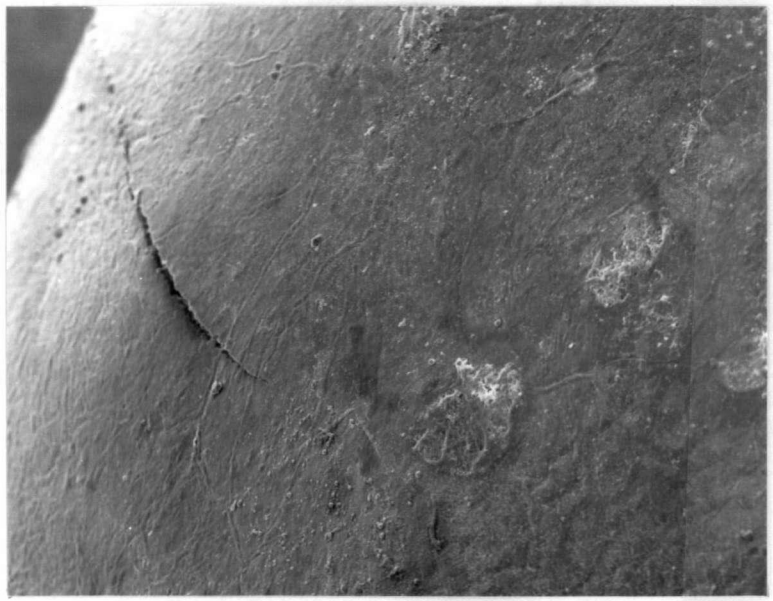


Figure 9. Similar view to Figure 5 (pw = 1350 μm).

A third layer has been reported to exist in the membrane of the eggs of teleost species and labelled the cortex radiatus externus by Riehl et al. (1980). These researchers found this layer to be not readily apparent through scanning electron microscopy, but required transmission EM to allow differentiation from the cortex radiatus internus. The scanning electron micrographs obtained in the present study likewise did not reveal the presence of this layer.

E. Flow Behaviour Studies

The flow behaviour of chum yolk from eggs of four coded samples is demonstrated by the rheograms depicted in Figure 10. The change in apparent viscosity with shear rate is typical of a non-Newtonian fluid, and it appears that both shear thinning at lower rates of shear and, in yolks of greater apparent viscosities (VI and III), shear thickening at higher rates of shear are occurring. Accordingly, discussion of the rheograms will deal with these two phenomena separately: first with the shear thinning portion from 36.0 to 300 s^{-1}.

A decrease in shear rate after achieving a maximum did not result in the hysteresis loop indicative of time dependent behaviour; therefore, the flow behaviour of chum yolk in the shear thinning region was seen to be pseudoplastic in nature. Results of application of the Power-law flow model to the behaviour of yolks from these four different coded samples can be seen from Table 5, which lists the consistency coefficients (m), flow behaviour indices (n), and coefficients of determination (r^2). Values for r^2 indicate the Power-law model to be a good description of the flow characteristics of chum egg yolk. Pseudoplastic behaviour is confirmed by values of less than 1.0 for flow behaviour indices, and it can be seen that both the degree of pseudoplasticity (as indicated by the magnitudes of $\dot{n}$) and the "thickness" (as represented by the consistency coefficients) vary between yolk samples, with the thinnest yolk exhibiting behaviour closest to Newtonian.

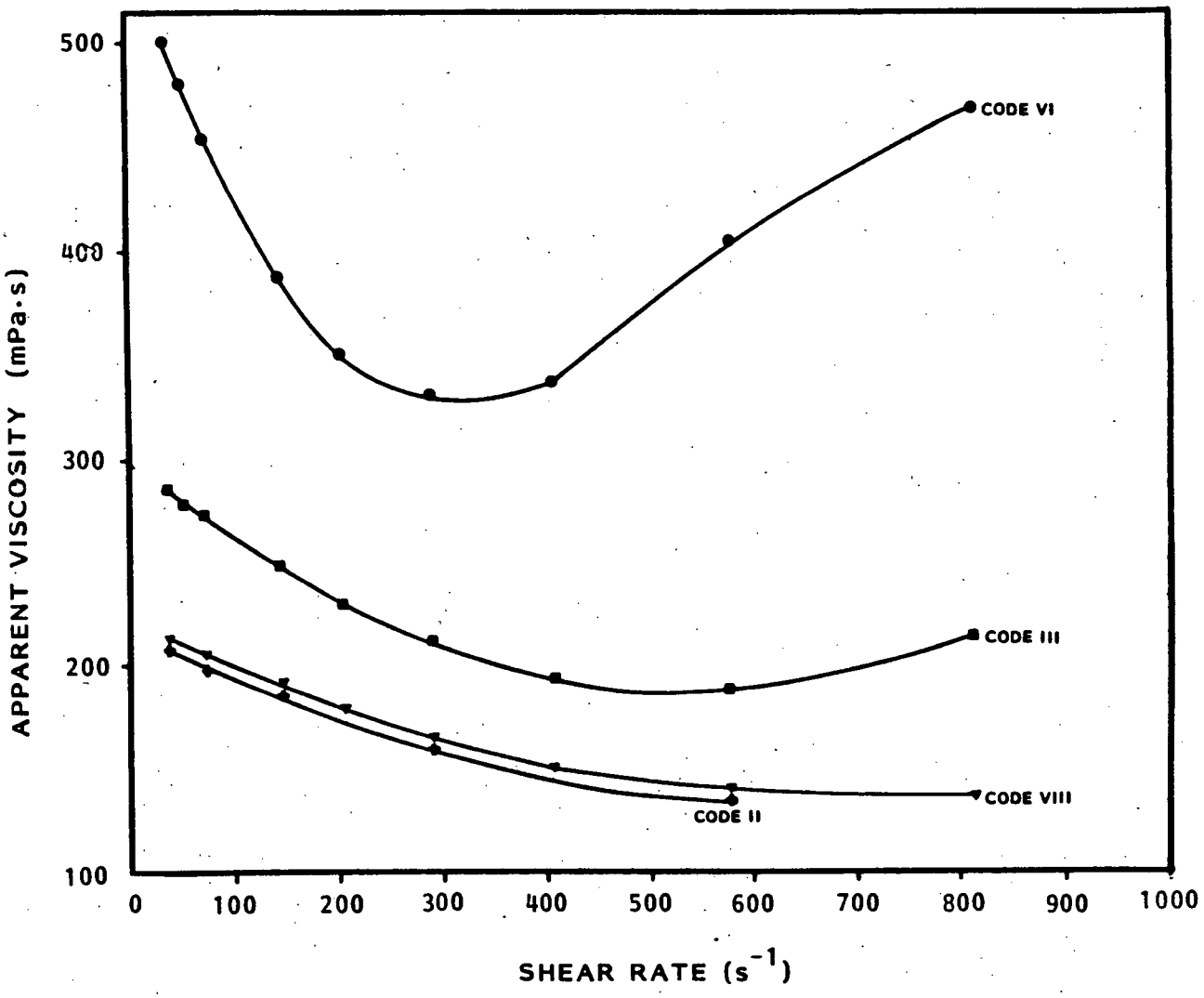


Figure 10. Rheograms of yolk from fresh chum eggs from four codes.

Table 5. Mean values of Power-law flow parameters from rheograms of liquid yolk prepared from fresh chum eggs (n=3).

Code no.	Consistency coefficient m, (mPa s^n)	Flow behaviour index n	r^2
II	330	0.88	0.95
III	525	0.84	0.96
VI	1000	0.81	0.96
VIII	363	0.87	0.92

Such time independent shear thinning is typical of protein solutions and can be postulated as resulting from increasing alignment of the large protein molecules into lamellae with increasing shear rates. Following the tendency to maximize entropy, at low rates of shear, the molecules are oriented at random, causing a greater resistance to flow. However, with higher rates of shear, the molecules become increasingly more aligned along the planes of shear and this progressively more ordered arrangement results in less resistance to flow, by definition a decrease in viscosity (Reiner and Blair, 1967; Tung, 1978; Pradipasena and Rha, 1977).

The second portion of the rheogram in two of the four samples (Codes III and VI) exhibits an apparent increase in viscosity with higher rates of shear. Time dependency is again lacking, as indicated by the absence of a hysteresis loop. Such behaviour has been known to result from turbulent flow, a condition that occurs at high rates of shear when the orderly movement of adjoining lamella are disrupted, whorls and vortices are introduced into the flow, and resistance to shear increases (vanWazer et al., 1963). A value can be calculated using an equation based on fluid density, viscosity, radius of measuring body and mean velocity which, when exceeding a critical value of 2100, indicates the presence of turbulence in a Newtonian fluid. This value, known as the Reynold's Number has been modified for use with non-Newtonian fluids (Whorlow, 1980) and re-labelled the Generalized Reynold's Number. The equation when applied to the coaxial cylinder system as used in this study, gave a rough estimation, sufficient for assessing the presence or absence of turbulent flow at high shear rates.

Generalized Reynold's Numbers for yolks of Code III and VI were calculated, using the following equation:

$$G_{Re} = (8D^{n}V^{2-n}\rho)/(1/n+3)^{n}2^{n}m \qquad (8)$$

where D is the width of the gap (cm), V is the mass average velocity of fluid

(cm/s) and ρ is the density of the fluid (1.6 g/mL, obtained from the average of weight/volume measurements for 4 samples of yolk (Code VIII)). Values calculated were 11.8 and 9.1 for Code III and VI, respectively, so that it can be stated that an increase in viscosity noted at higher shear rates for these codes is probably not due to turbulent flow.

A possible explanation for such behaviour may instead lie in the momentum transfer theory for dilatant fluids discussed by Bauer and Collins (1967). This concept involves the sideways movement of particles from one adjacent lamella into the next, with all particles in the disrupted layer experiencing a change in velocity. Since a shear rate increase results in more particles becoming aligned into lamellae, progressively more particles are affected with the movement of a particle from one lamella into the next, and increased shear rates would then result in a corresponding viscosity increase. Such a theory would suggest that shear thickening should occur at lower shear rates in fluids of overall higher apparent viscosity. This behaviour is noted in yolks from Codes III and VI with the initial occurrence of shear thickening at a shear rate of 400 s^{-1} for yolk from Code VI whose overall apparent viscosity is higher than yolk from Code III, in which shear thickening does not begin until 804 s^{-1}. It is possible that yolks from Codes II and VIII, each of lower overall apparent viscosity than Code III, may yet show shear thickening if higher rates of shear were employed.

Because this shear thickening phenomenon does not appear in all samples, but only in those above a certain value of apparent viscosity, and in order to simplify further analyses, all subsequent discussion of flow behaviour deals with the shear rate interval of 36 to 300 s^{-1}, representing the Power-law pseudoplastic portion of the rheogram. This region is also more comparable to shear rates involved in sensory analysis (Rao, 1977).

F. Force Deformation Analysis

A typical force deformation curve resulting from compression of one egg on an Instron universal testing machine is depicted in Figure 11. The curve appears to be of a form typically obtained from compression of food products, reflecting an ever increasing resistance to force applied, followed by a sudden decrease at what has been referred to as the bioyield point (Mohsenin and Mittal., 1977). The portion of the curve lying between the point of first contact (C) and the point of first resistance (R) is a measure of seating accommodation, or the extent to which the egg could be deformed prior to any resistance by the membrane, and tended to vary depending upon the flaccidity of the egg. Such behaviour was also noted by Pomeranz (1974) in his study on plaice eggs wherein it was found that an egg could be deformed 25% before any appreciable resistance was recorded.

The subsequent gradual increase in resistance resulted from a stretching of the egg membrane around the incompressible yolk, to a point where the deformation limit was reached and rupture occurred. Immediate cessation in resistance followed; (any deviation from the instantaneous nature of this event, as depicted by the curve in Figure 11, resulted from recorder pen response time.)

The two parameters of rupture energy (joules) and rupture force (Newtons) were obtained directly from the force deformation curves and these are presented in Table 6 for fresh eggs of several codes. Although attempts have been made in the past to determine the bursting strength of teleost eggs, results reported in the literature are not in good agreement. Hayes and Armstrong (1942) utilized a system involving an ordinary kitchen spring balance to obtain values for the force in kilograms required to rupture eggs of the species *Salmo salar*. Their experiments yielded an average value of 0.14 kg (1.37 N) which lies close to higher values within the range 1.22 to 0.47 N obtained for eggs in

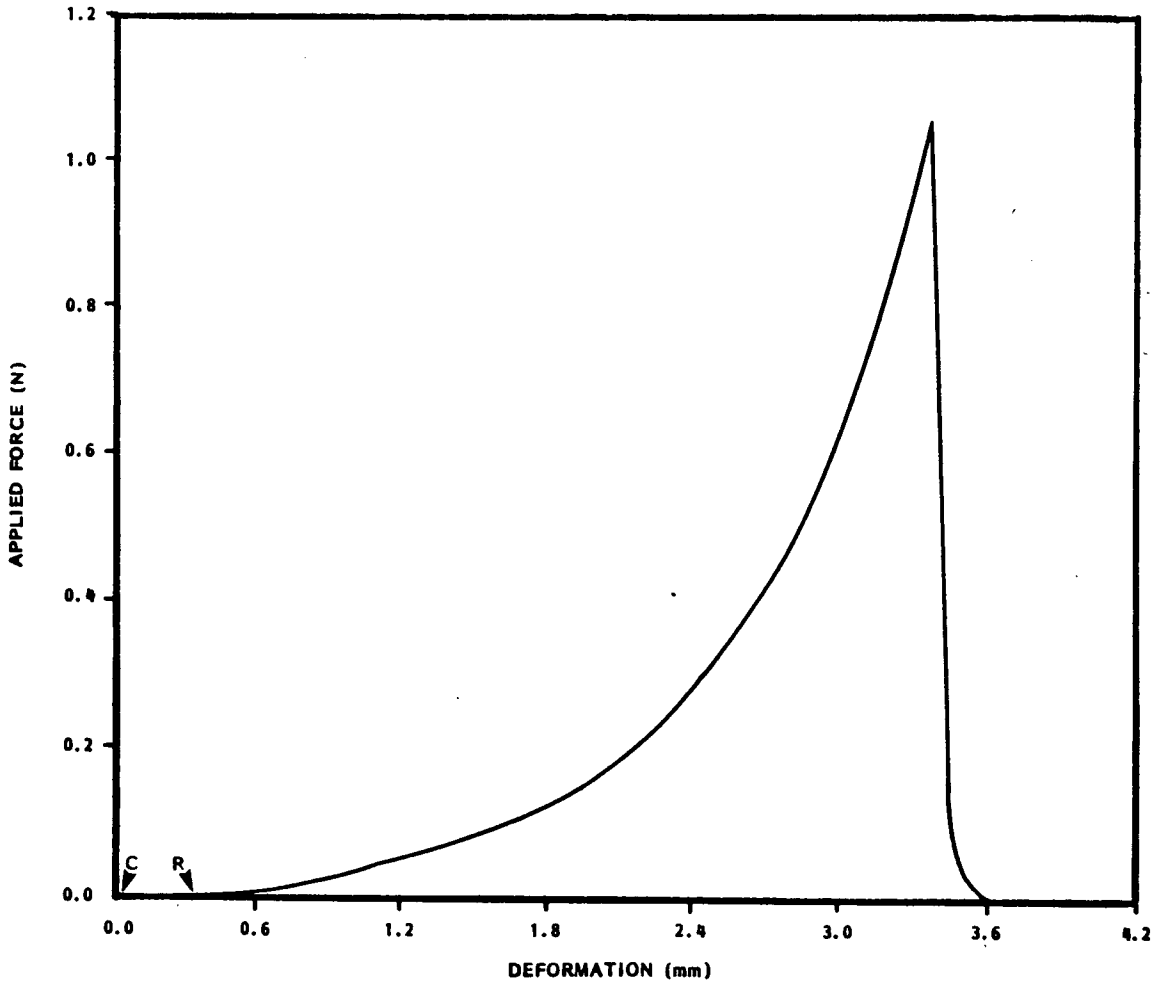


Figure 11. Typical force deformation curve resulting from compression of a single chum egg.

Table 6. Mean membrane rupture energy and rupture force for fresh chum eggs of several codes (n=25).

Code no.	Mean membrane rupture energy ($J \times 10^{-4}$)	Mean membrane rupture force (N)
II	3.14 (0.96)[a]	0,56 (0.19)
III	2.36 (0.94)	0.47 (0.16)
IV	3.34 (1.94)	0.64 (0.19)
IX	7.99 (3.34)	1.22 (0.38)
X	5.82 (3.63)	0.92 (0.43)
XI	4.17 (2.00)	0.74 (0.30)

[a]Standard deviation

the present study. However, eggs of *Salmo gairdneri* were reported by Grierson and Neville (1981) to be capable of withstanding forces of 1 kg (9.8 N) before bursting. This value was obtained by squashing an egg between two glass slides placed under a number of balance weights. The authors do state that other fish species were observed to be far less strong, so the apparent difference between this result and those obtained for chum salmon in the present study may be due to species variation. The disparity between the results of Hayes and Armstrong and Grierson and Neville, working with the same species, could well be a result of the diverse methods employed.

According to Grierson and Neville (1981), the ability to resist large forces of compression, as exhibited by the chum eggs studied in this investigation, is due to the helicoid arrangement of the fibrous laminate of the fish egg membrane. Such strength would be of obvious advantage in the protection of the developing organism; eggs of the Pacific salmon must withstand both the pressure of the surrounding egg mass, as well as the gravel that is piled over top of all in the form of a nest.

Three other parameters were obtained from force deformation analysis of the eggs and these are presented in Table 7. These parameters were all calculated from values for maximum force and the area under the force deformation curve, and represent fresh eggs from codes IX, X and XI. As can be seen, a deformation of approximately 20% resulted in egg rupture (using an average egg height of 5.7 mm, as measured in the study on the effect of egg size on membrane rupture energy), although this value was underestimated somewhat by the method of calculation employed to achieve it. The ability to withstand substantial deformation of structure before rupture is also reflected in values for egg firmness ranging from 670 to 960 N/m, and compliance from 0.0011 to 0.0018 m/N. These values, like that of the rupture force, are indicative of the essential requirements for a structure whose purpose is to protect interior

Table 7. Means of parameters calculated from values of rupture force and energy for membranes of fresh eggs of three codes (n=25).

Code	Deformation (mm)	Firmness (N/m)	Modified modulus of compliance ($m/N \times 10^{-2}$)
IX	1.3 (0.2)[a]	960 (260)	0.11 (0.03)
X	1.2 (0.4)	770 (320)	0.15 (0.06)
XI	1.1 (0.2)	670 (250)	0.18 (0.09)

[a]Standard deviation

contents against physical stress.

The term "firmness" is used in keeping with Mohsenin and Mittal (1977) who differentiate between the "modulus of deformability", a value calculated as the slope of a force-deformation curve in the area of initial resistance when the material is under small strain, and the "modulus of elasticity" representing the region closer to the bioyield point where a higher amount of strain is occurring. In this investigation, assuming the force deformation curve to be a right angle triangle, a value lying intermediate to the two moduli as expressed by Mohsenin and Mittal was obtained from the slope of the hypotenuse, and termed the "firmness" of the egg.

Correlation coefficients of 0.95, 0.87 and 0.96 were calculated for the parameters of membrane rupture energy and force for Codes IX, X and XI, respectively.

Accordingly to Mohsenin and Mittal (1977), mechanical properties reflecting yielding and fracture of the material should correlate well with each other, but not necessarily with those indicating the behaviour of a material under a small strain. The significant correlation found between rupture force and rupture energy is then not unexpected as both are intimately involved with the material under high stress, although the rupture energy is more an indication of a history of resistance forces over the entire period of deformation.

Correlation coefficients for the other parameters were not computed, as these values were actually calculated from measurements used to obtain rupture force and energy. As they were not obtained directly from force deformation curves, the validity of any conclusions drawn concerning interrelationships is dubious.

G. Effect of Egg Size of Membrane Rupture Energy

Of all of the parameters obtained from the force deformation curve, that of membrane rupture energy was considered to be most nearly representative of the overall response of the egg membrane throughout the entire period of compression, and so was used in all successive experiments to evaluate treatment effects.

Preliminary testing was necessary, however, to evaluate the relationship between the rupture energy and egg size, as according to Voisey (1971), samples analyzed in compression studies measuring only force and deformation must be of identical dimensions to make meaningful comparisons. If this is not the case, results must be calculated based on dimensions of the samples analyzed.

In the analysis of fish eggs, biological variation makes the first condition unfeasible. Measurement of every egg tested would provide information of sample dimensions, however, this procedure is also very tedious and time consuming when the numbers of samples analyzed are considered. Therefore, this present investigation was undertaken in order to ascertain whether the effect of egg size on measurement of rupture energy would be of sufficient magnitude to merit consideration in further studies.

A scatter diagram of the results obtained is shown in Figure 12 wherein rupture energy is plotted against egg surface area. From this it can be seen that no apparent correlation existed between these two characteristics. Such a finding agrees with that of Hayes and Armstrong (1942) who reported that no change in the weight of an egg from the species *Salmo salar* L. could be associated with a change in the force required for membrane rupture.

H. Effects of Frozen Storage

Subjective assessment of caviar made from eggs that had been previously

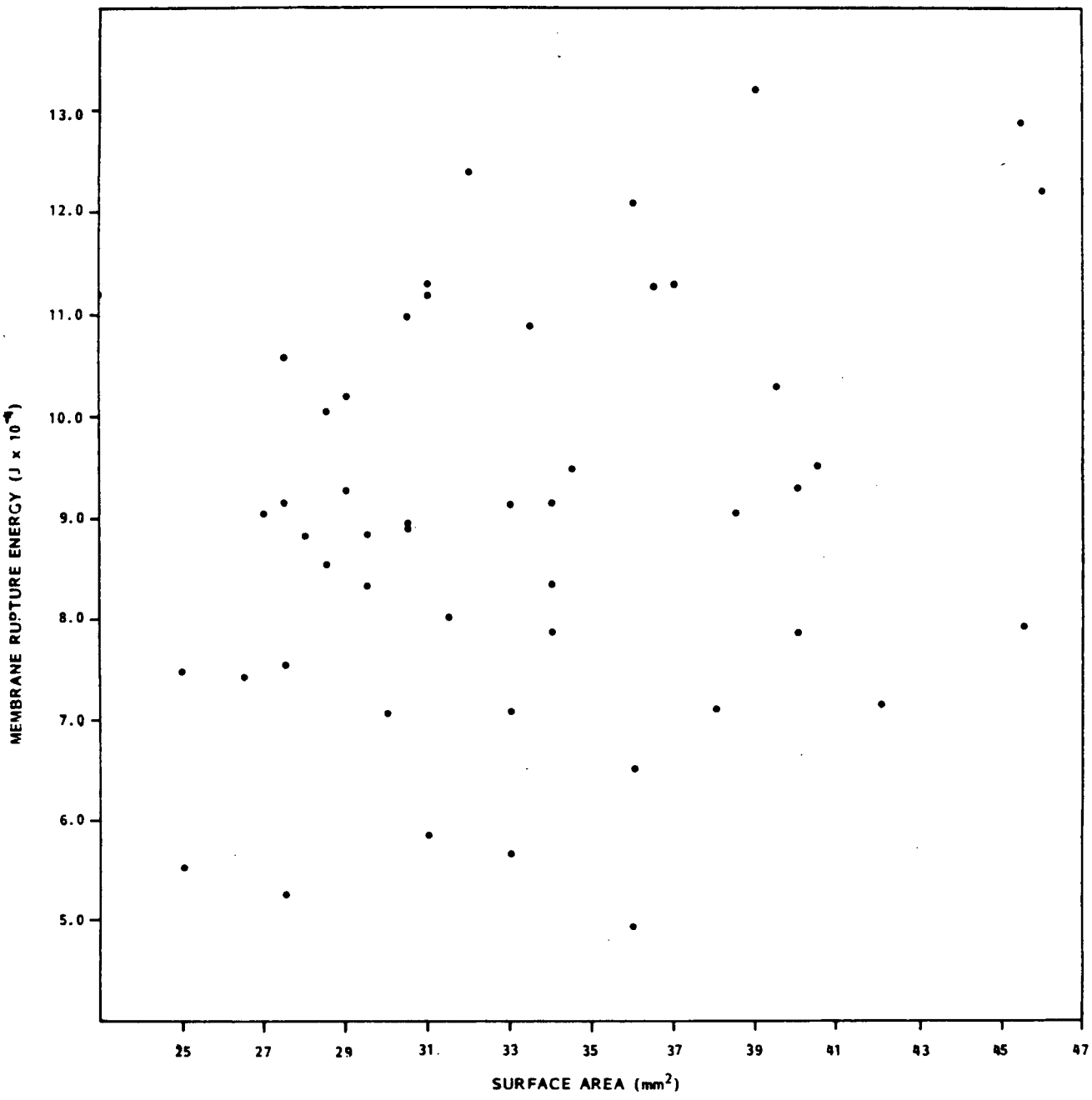


Figure 12. Scatter diagram of membrane rupture energy as function of egg surface area.

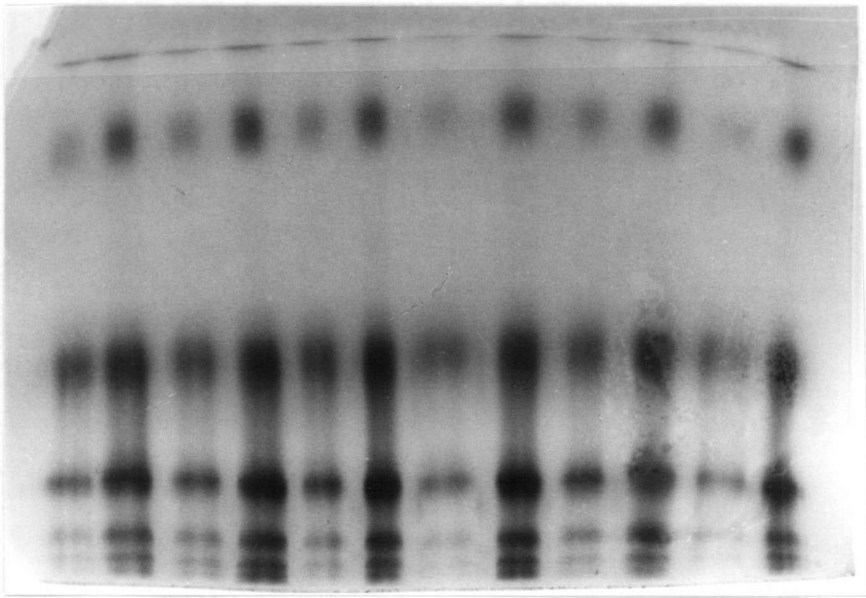
frozen indicated that such caviar was of a different quality than that made from fresh eggs (Sternin and Hori, 1982; Zaitsev, 1969). Accordingly, an investigation was conducted on both fresh and frozen/thawed unsalted eggs to discover where the source of this difference may lie. Since fish eggs are readily divided into the two physical systems of the interior fluid and the surrounding membrane, evaluation of any effects frozen storage may have had was applied to each system separately.

1. Effects on yolk

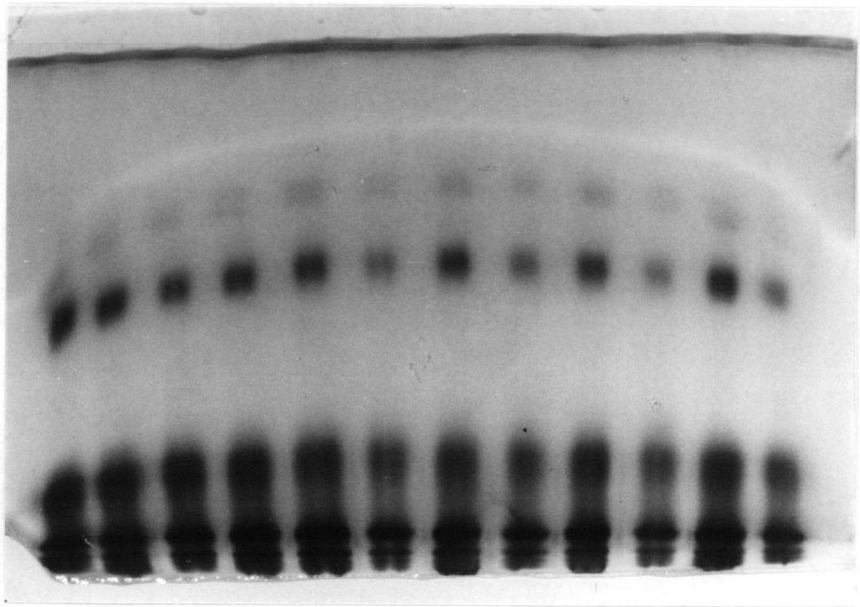
It was considered unlikely that the immiscible lipid droplet forming about 1% (Markert and Vanstone, 1968) of the interior fluid of salmon eggs and consisting of mainly triglycerides (Nakagawa and Tsuchiya, 1971) would have a large effect on yolk consistency. Instead, if any effect were to occur as a result of storage at low temperatures, it would most probably be found in the protein solution forming approximately 98% (v/v) of the egg yolk (Markert and Vanstone,1968). Such an alteration in protein has been noted for avian egg yolk upon storage at temperatures below -6°C (Kamat et al., 1976). On this assumption, analytical methods of polyacrylamide gel electrophoresis, differential scanning calorimetry and viscometric analysis were applied to chum yolk in an attempt to discover if the proteins of the yolk had undergone any modification during the period of frozen storage.

a) Polyacrylamide gel electrophoresis

The results of polyacrylamide gel electrophoresis (PAGE) are represented in gels depicted in Figure 13a and b. From an examination of these gels, it can be seen that no difference in the mobility of the four proteins occurred after seven days storage at -10°C. Therefore, no breakdown or aggregation of any particular protein took place, as was noted for avian egg yolk proteins (Chang et al., 1977). The latter result was found to be due mainly to the



a) 1 2 3 4 5 6 7 8 9 10 11 12



b) 1 2 3 4 5 6 7 8 9 10 11 12

Figure 13. Electrophoretic patterns of fresh and frozen/thawed native and salted chum egg yolk of two codes. (Refer to Table 2 for method of obtaining salted yolk and amounts of sample applied to gel.)

a) Code II - slot 1, 2, 3, 4, 11, 12 : frozen/thawed yolk; slot 5, 6 : fresh, salted yolk; slot 7, 8 : fresh yolk; slot 9, 10 : frozen/thawed, salted yolk.

b) Code VI - slot 1, 5, 6 : frozen/thawed yolk; slot 2, 7, 8 : frozen/thawed salted yolk; slot 3, 9, 10 : fresh yolk; slot 4, 11, 12 : fresh, salted yolk.

breaking up of the micellar structure of low density lipoproteins present in avian yolk as a result of an increasing concentration of salts caused by the incorporation of water into ice crystals through freezing (Powrie, 1968). The difference in behaviour between teleost and avian yolk is most readily explained by the absence of such low density lipoproteins in teleost yolk (Cook and Martin, 1969).

As discussed previously, the addition of deoxycholate to the running buffer in the system involved in this study tended to overcome separation on the basis of charge and so separation would have occurred mainly through differences in molecular size. It is apparent then from results of the electrophoresis performed in this study that frozen storage at -10°C for seven days had no effect on the molecular size of the proteins, nor did it induce any aggregation or breakdown of the molecules.

As mentioned in the Methods and Materials section, samples of yolk from salted eggs were also analyzed in the same manner as fresh and frozen/thawed yolk and it appears from Figure 13 that the addition of salt likewise had no influence on the yolk proteins, as detectable by PAGE.

b) Differential scanning calorimetry

The results of a typical thermal analysis of yolk from fresh and frozen/thawed yolk are presented in Figure 14. Analysis by differential scanning calorimetry (DSC) produces a curve indicating the difference in the amount of heat that must be supplied to a sample in order to maintain its temperature equivalent with that of a reference (Daniels, 1973). Variations in the amount of heat that is required can be caused by endothermic reactions, such as denaturation. In the present study, the denaturation of proteins present in chum yolk are being analyzed and it can be seen from Figure 14 that the curves obtained from fresh and frozen/thawed yolk are very similar. A Student's t

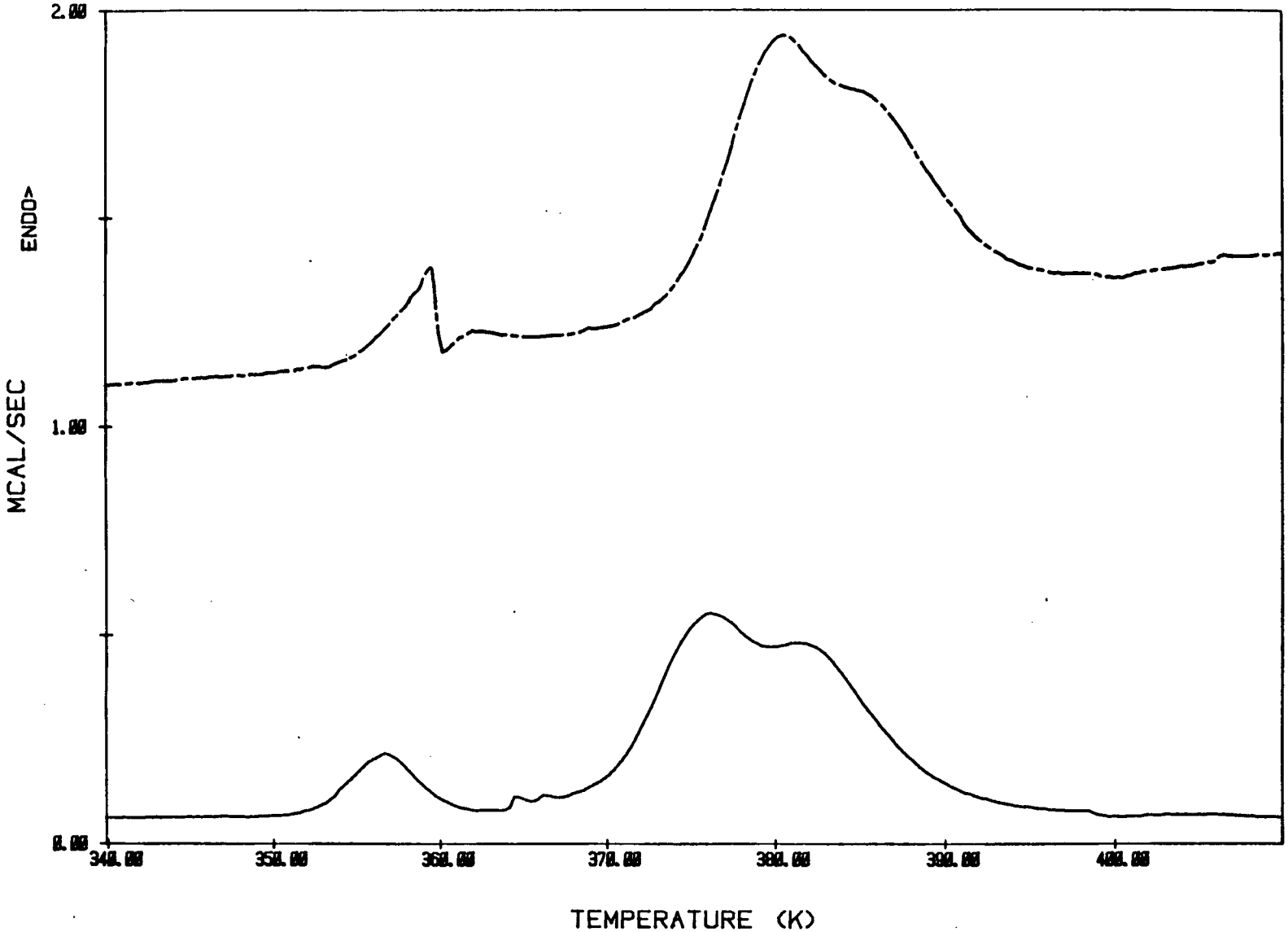


Figure 14. Typical DSC thermograms of fresh and frozen/thawed yolk from eggs of Code VI ((———) = fresh yolk; (– – – –) = frozen/thawed yolk).

test performed on the data reported in Tables 8 and 9 indicated that only the enthalpy of denaturation (Table 9) significantly differed in only one peak of Code II and this significant difference was not repeated in the second code analyzed. Based on these results, it appears that the conformation of the proteins of chum yolk are affected very little, if at all, by frozen storage, since conformational differences should affect the amount of heat required for denaturation to occur.

c) Viscometric analysis

Rheograms of fresh and frozen/thawed yolk for two coding dates are presented in Figure 15. Statistical analysis of the slopes and levels of these curves revealed no significant difference ($p<0.05$), and so it appears that the rheological behaviour of the yolk was unaffected by frozen storage. Since the behaviour of a solution is dependent upon its molecular structure (Pradipasena and Rha, 1977; Hermansson, 1975), this is a further indication that no lasting change has occurred in the proteins.

All of the analyses performed support the conclusion that little or no alteration in proteins as detectable by any of the methods employed, had occurred as a result of storage at -10°C for seven days. Instability in structure of the proteins during the period of frozen storage could be expected since the overall balance of forces that maintain conformation may have been disturbed. Lower temperatures could have resulted in a decrease in the strength of hydrophobic interactions, while increasing the strength of hydrogen bonding (Taborsky 1970). However, it appears that any distortion in structure that may have occurred during storage at low temperatures was reversible and the proteins reverted back to native form when higher temperatures were restored.

Table 8. Enthalpy of denaturation for individual proteins of fresh and frozen/thawed chum egg yolk.

Code no.	Condition of yolk	ΔHeat (cal/g yolk, db)	
		Peak 1	Peak 2 & 3
II	Fresh	0.29	2.60
	Frozen/thawed	0.27	2.41**
VI	Fresh	0.27	2.95
	Frozen/thawed	0.27	3.00

**Significantly different from value for Peak 2 & 3 of fresh yolk from the same code ($p<0.01$).

Table 9. Denaturation temperatures of individual proteins of fresh and frozen/thawed chum egg yolk of two codes.

Code no.	Condition of yolk	Denaturation temperatures (°C)		
		Peak 1	Peak 2	Peak 3
II	Fresh	86.0	106.7	110.8
	Frozen/thawed	85.5	106.6	110.7
VH	Fresh	86.4	107.5	----[a]
	Frozen/thawed	81.3	104.5	----

[a]values unavailable from Perkin Elmer Thermal Analysis Station.

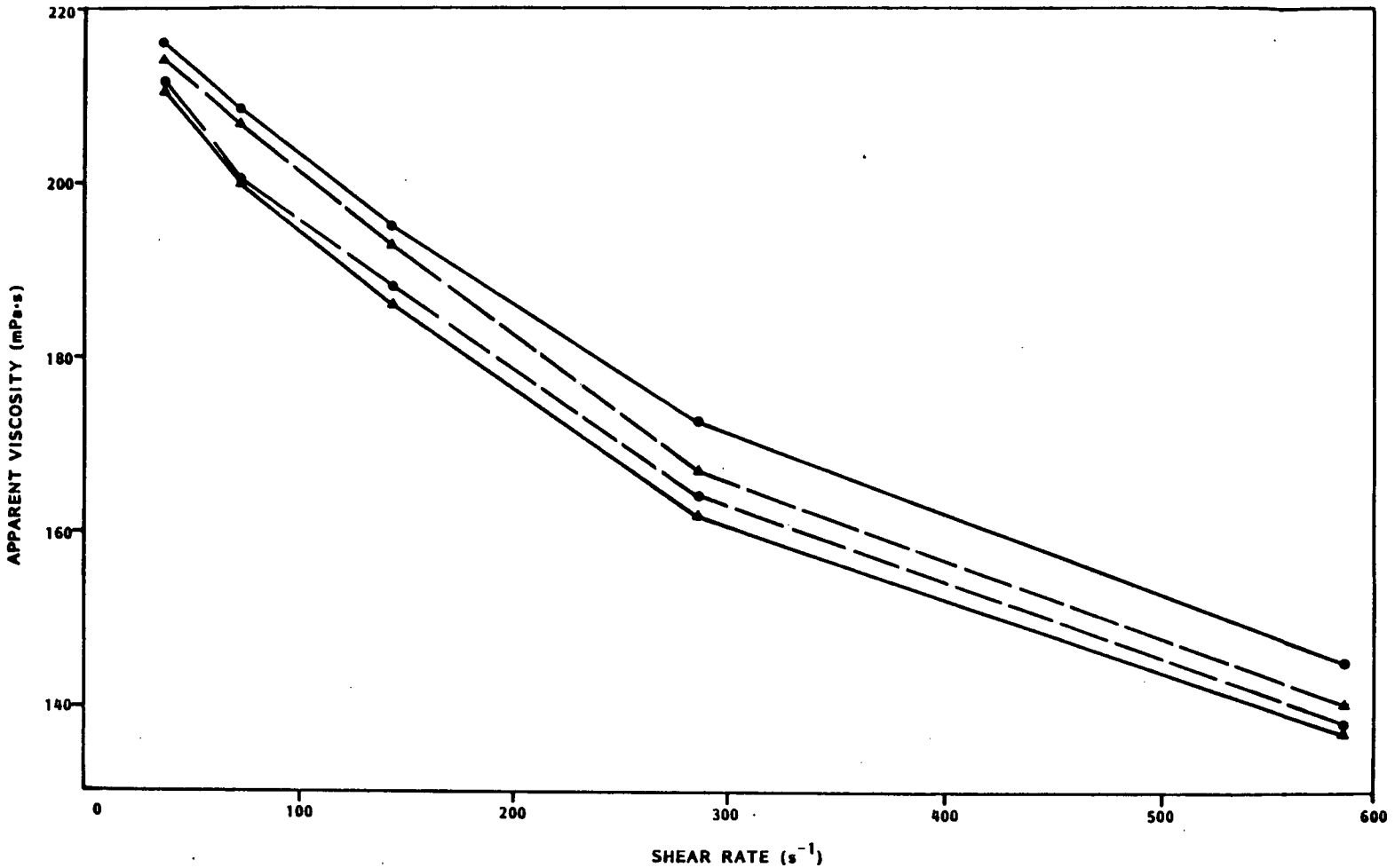


Figure 15. Rheograms of yolk from fresh and frozen/thawed eggs of two codes (● = Code II; ▲ = Code VI; (— — —) = fresh eggs; (————) = frozen/thawed eggs).

2. Effect on membrane rupture energy

The results of force deformation analysis of fresh and frozen/thawed intact eggs are presented in Table 10. A Student's t test indicated a highly significant difference in the rupture energy of egg membranes before and after freeze/thawing with an average 46% decrease in post frozen/thawed egg membrane rupture energy.

It appears then that the alteration that has been observed to occur in chum eggs as a result of frozen storage resides mainly in the membrane. This could be expected, when the composition of the membrane is considered. Chum salmon egg membranes, like those of all teleosts studied, are complex, at least partially laminate structures (Stehr and Hawkes, 1979; Laale, 1980). Hamor and Garside (1977) found the membrane of Atlantic salmon *Salmo salar* eggs to be made up of 92% water, and since the membrane of the related Pacific salmon is in all probability of similar high water content, below freezing point temperatures could be expected to produce ice crystals that would readily disrupt membrane structure and thus weaken it.

I. Effects of cryoprotectants

An important aspect that must be born in mind when considering the effect of cryoprotectants on chum eggs is the difference in structure involved between the cellular suspensions, protein solutions and tissue systems previously reported in the literature dealing with cryoprotectants, and the chum egg membrane.

Individual animal cells are surrounded by an ultra thin plasma membrane and due to their size and membrane permeability, are highly subject to osmotic shock. Vegetable cells are equally fragile, but do possess the added advantage of a rigid cell wall which offers some protection against hypoosmosis. Such

Table 10. Effect of frozen storage on membrane rupture energy.

Code no.	Mean membrane rupture energy ($J \times 10^{-4}$)	
	Fresh	Post freeze/thawing
II	3.14	1.84**
VI	5.87	3.15**

**significant at $p<0.01$

fragility is not reflected to a large degree in the chum egg, which possesses a measurably thicker membrane of greater structural complexity, more likely capable of withstanding greater interior stress than the cellular plasma membrane.

Therefore, the protection against osmotic shock offered by the penetrating class of cryoprotectants is probably not a significant aid in the freezing of chum eggs. More important is the inhibition of ice crystal formation afforded by the water binding capacity of these cryoprotective compounds used.

The results of cryoprotective treatments on the rupture energy of chum eggs from four different codes are depicted graphically in Figures 16, 17, 18 and 19. The individual variability of the eggs can be seen from the large standard deviations. Such variability was also encountered by Hayes (1942) for eggs of *Salmo salar*. When ten recently fertilized eggs were subjected to compression, the sample mean was found to possess a coefficient of variation of 20%. In addition, variation in membrane rupture energy in response to frozen storage between the different codes can be seen from a comparison of values for the control eggs of each lot. The percent decrease from fresh to frozen/thawed control ranged from 9 to 56% in the egg membrane strength of Codes IV and VII, respectively. A similar variation in reaction of the egg membrane to one particular cryoprotectant can also be seen from a comparison of eggs from different codes treated with sodium chloride.

Such variation reveals the influence of factors on results obtained other than the cryoprotective treatment itself, such as the condition and maturity of the egg. Any conclusions drawn regarding the effectiveness of a cryoprotective treatment must then be considered as subject to a certain amount of variability.

Pre and post freeze/thawing results of any cryoprotectant were considered as separate treatments and within each lot, the factor of Treatment was found

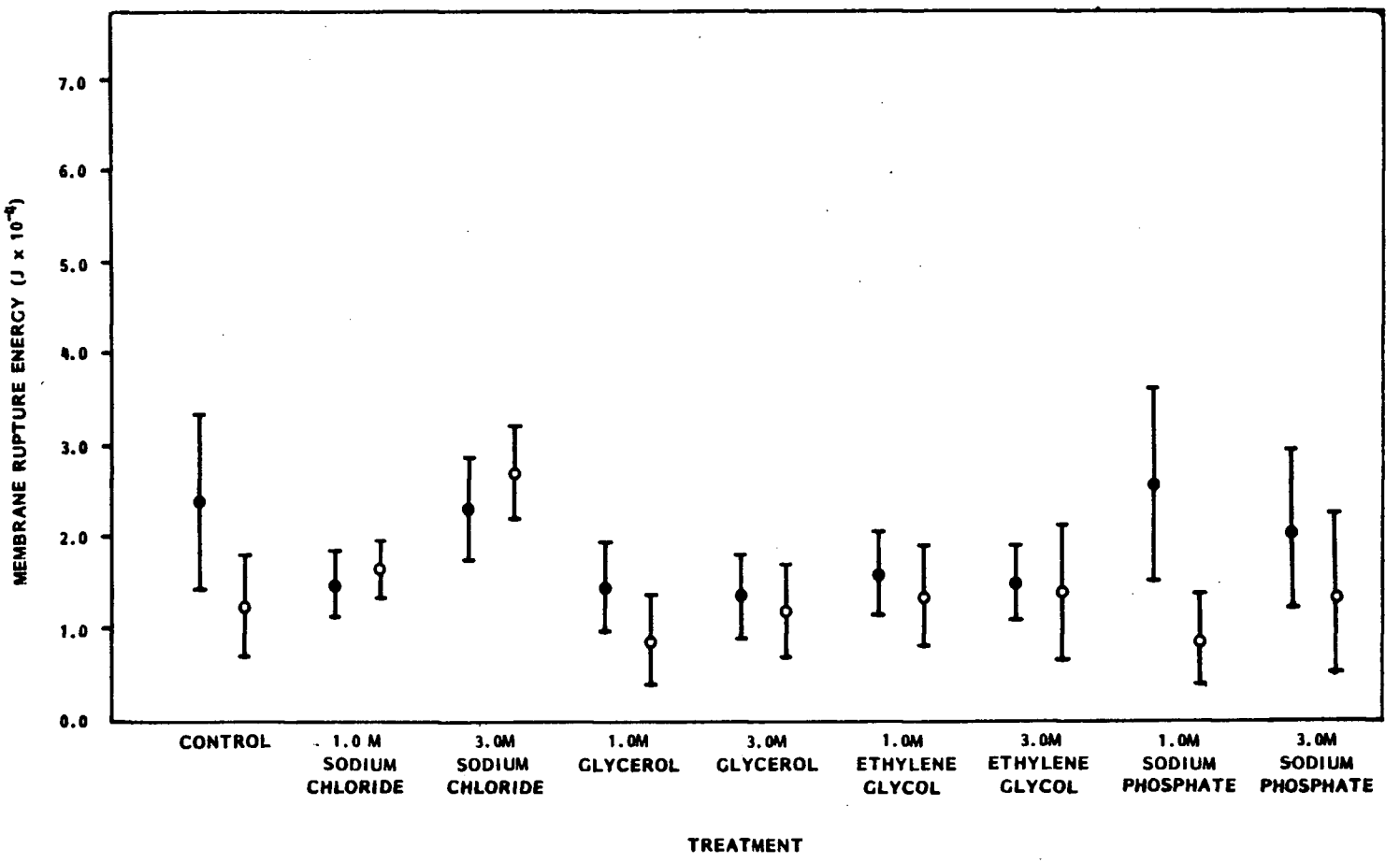


Figure 16. Effect of treatment with various potential cryoprotective agents on membrane rupture energy of eggs from Code III. (● = before freezing; O = post freeze/thawing).

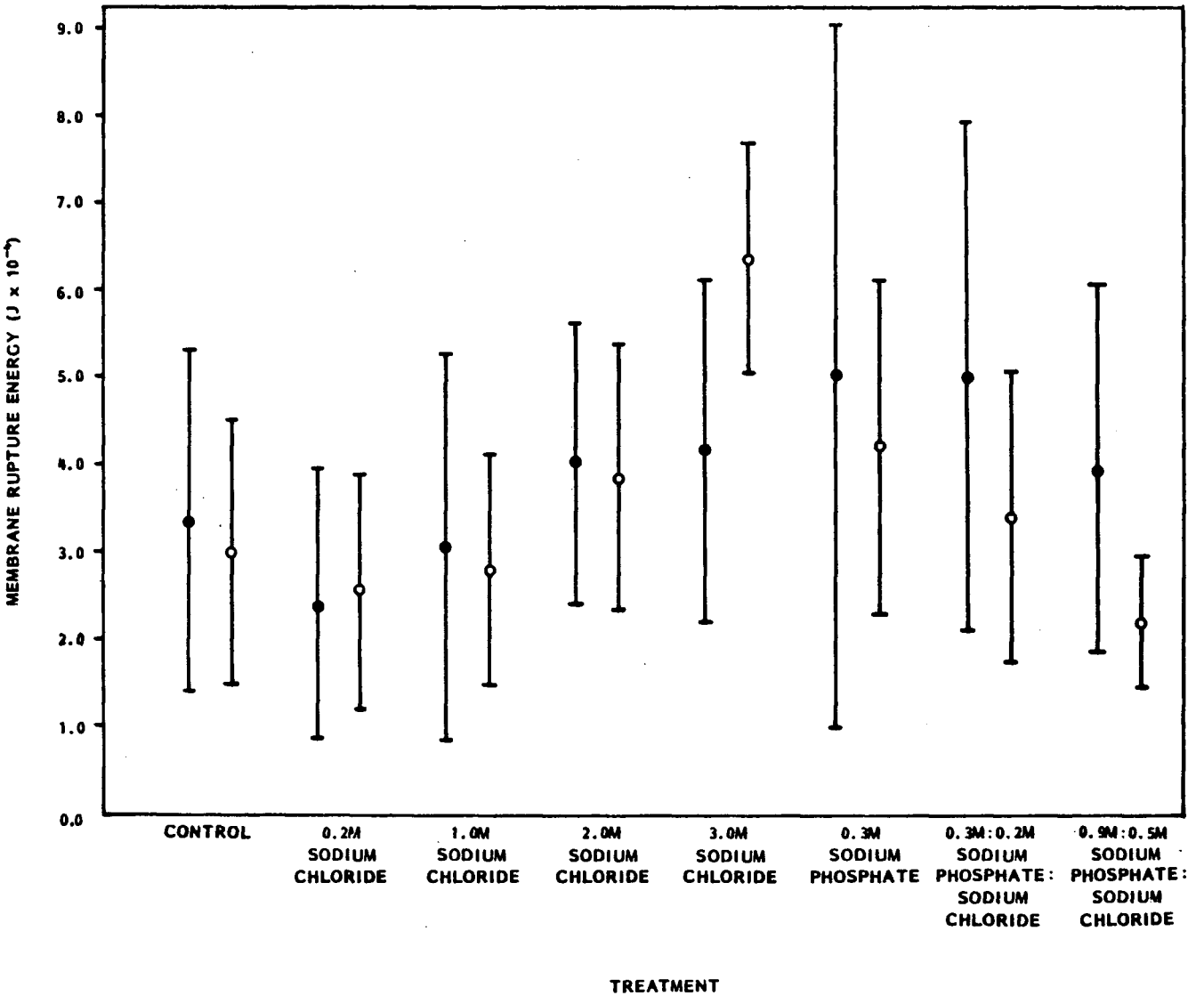


Figure 17. Effect of treatment with various potential cryoprotective agents on membrane rupture energy of eggs from Code IV. (● = before freezing; O = post freeze/thawing).

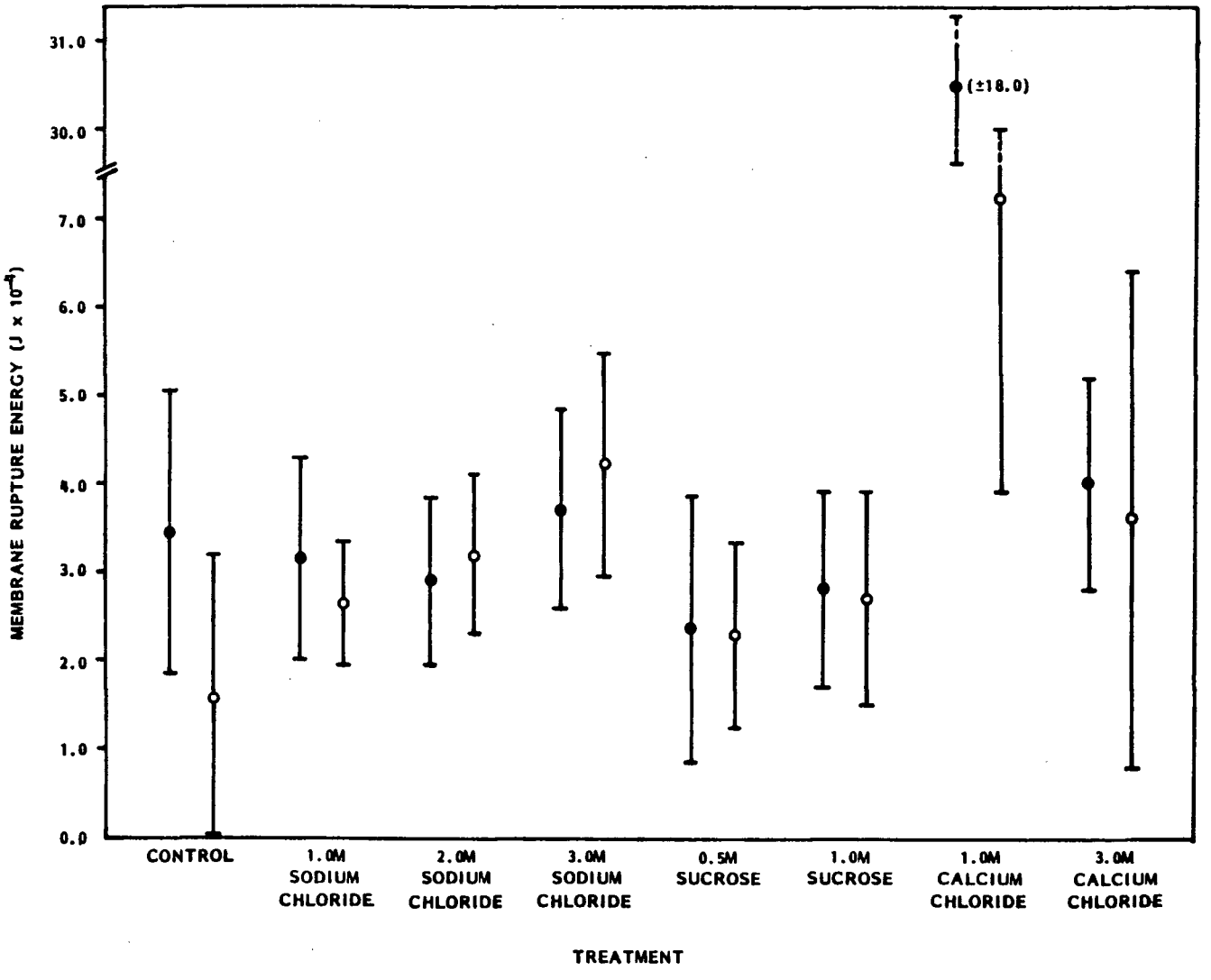


Figure 18. Effect of treatment with various potential cryoprotective agents on membrane rupture energy of eggs from Code VII (● = before freezing; O = post freeze/thawing).

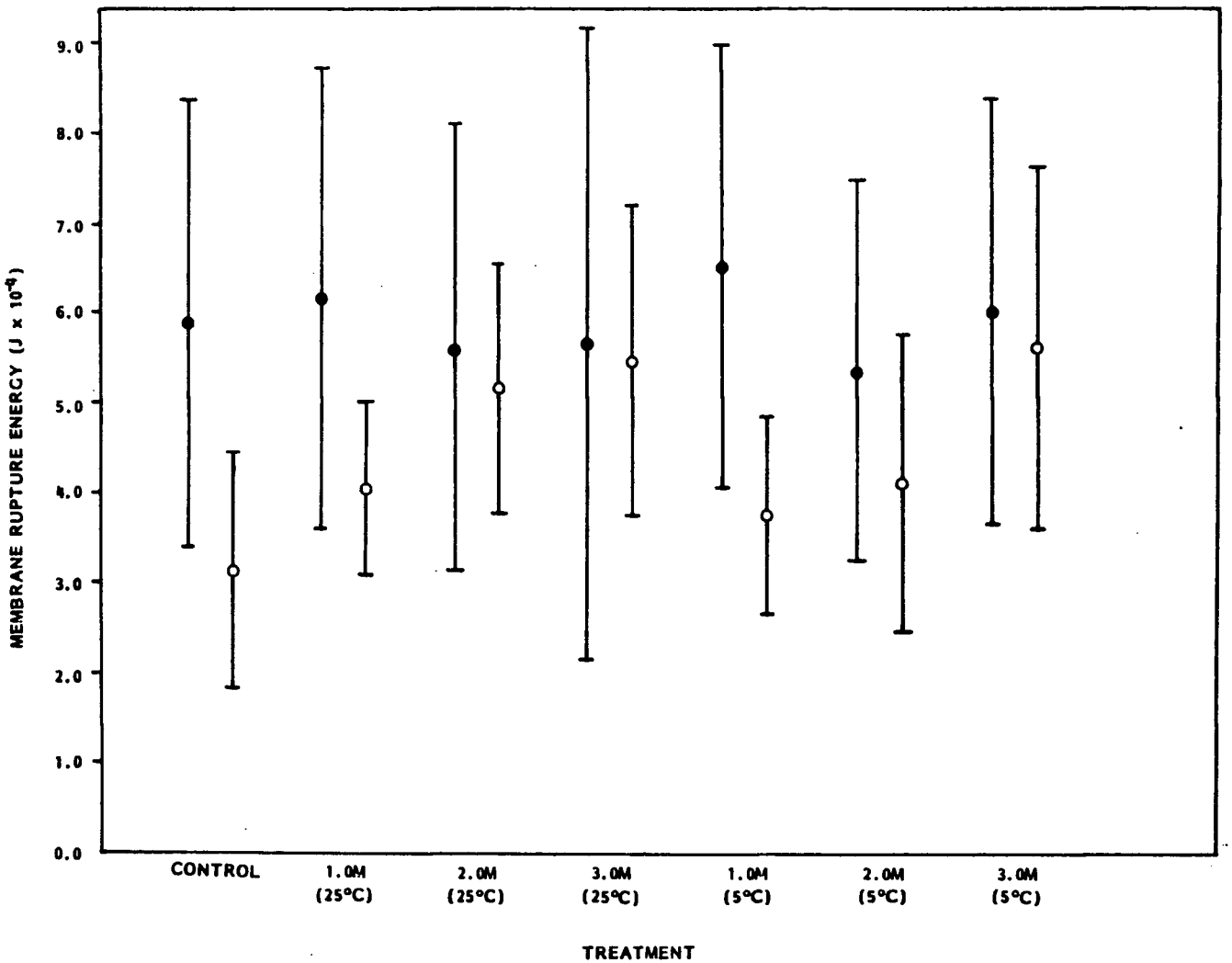


Figure 19. Effect of treatment with various potential cryoprotective agents on membrane rupture energy of eggs from Code VIII (● = before freezing; O = post freeze/thawing).

was found to be a significant source of variation. A Newman-Keul's multiple range test then enabled the grouping of means not significantly different from each other.

To indicate the initial effect of the cryoprotective treatment on the membrane rupture energy, as well as the degree to which membrane rupture energy was maintained through freeze/thawing, Table 11 reports the per cent differences in membrane rupture energy between the fresh and pre-freeze/thaw treated eggs, as well as the percent difference between the pre and post freeze/thawed membrane rupture energies. Non significant differences are indicated by the letters "N.S.". Of particular importance are the treatments which maintained a membrane rupture energy both before and after freeze/thawing that was not significantly different from that of the fresh control. These compounds were effective cryoprotectants in this context.

Both ethylene glycol and glycerol significantly decreased the rupture energy of eggs before frozen storage (Table 11), most probably the result of a reaction between these compounds and the proteins present in the membrane. With the exception of eggs treated with 1.0 M glycerol, no further damage resulted from frozen storage of eggs, which may indicate some degree of cryoprotection, however, the initial loss of membrane strength renders these compounds unsuitable as cryoprotectants. The failure of the 1.0 M glycerol treatment to preserve any degree of membrane rupture energy may be a reflection of the multimolarity required for effectiveness of some agents (Meryman, 1971).

Membrane rupture energies after treatment with sodium phosphate varied somewhat. In eggs of Code III, no initial membrane weakening occurred when treatment was with sodium phosphate alone. However, neither was cryoprotection apparent and this lack may be a result of damage to membrane components caused by the greater concentrations of phosphate occurring in the liquid phase as a result of the freezing process. Code IV eggs, treated with a lower initial con-

Table 11.. Effect of cryoprotective treatments on membrane rupture energy of eggs before and after freeze/thawing.

Code	Treatment	Level	Per cent difference in mean membrane rupture energy	
			Fresh eggs after[b] cryoprotective treatment	Cryoprotected eggs[c] after frozen storage
III	None	----	-------	-46.2
	Sodium chloride	1.0M	-37.0	+11.8 N.S.[a]
		3.0M	- 2.6 N.S.	+17.8 N.S.
	Glycerol	1.0M	-38.2	-45.1
		3.0M	-43.6	- 4.1 N.S.
	Sodium phosphate	1.0M	+ 8.4 N.S.	-65.4
		3.0M	-10.4 N.S.	-34.5
	Ethylene glycol	1.0M	-32.3	-15.8 N.S.
		3.0M	-38.4	- 1.2 N.S.
IV	None	----	-------	- 9.3 N.S.
	Sodium chloride	0.2M	-27.2 N.S.	+ 5.4 N.S.
		1.0M	- 8.9 N.S.	- 8.3 N.S.
		2.0M	+19.4 N.S.	- 3.6 N.S.
		3.0M	+24.5 N.S.	+53.0
	Sodium phosphate	0.3M	+50.2	-16.7 N.S.
	Sodium phosphate:sodium chloride	0.3M:0.2M	+50.3	-31.8 N.S.
		0.9M:0.5M	+18.1 N.S.	-43.5 N.S.

Continued...

Table 11. Continued.

Code	Treatment	Level	Per cent difference in mean membrane rupture energy: Fresh eggs after cryoprotective treatment	Per cent difference in mean membrane rupture energy: Cryoprotected eggs after frozen storage
VII	None	-----	-----	-56.7 N.S.
	Sodium chloride	1.0M	- 8.7 N.S.	-15.8 N.S.
		2.0M	-15.4 N.S.	+ 8.9 N.S.
		3.0M	+ 7.9 N.S.	+12.0 N.S.
	Sucrose	0.5M	-32.0 N.S.	- 3.0 N.S.
		1.0M	-18.8 N.S.	- 2.3 N.S.
	Calcium chloride	1.0M	+788.0	-76.5
		3.0M	+15.2 N.S.	-10.1 N.S.
VIII	None	-----	-----	-46.3
	Sodium chloride (25°C)	1.0M	+4.9 N.S.	-34.0
		2.0M	- 4.5N.S.	- 8.0 N.S.
		3.0M	- 3.9 N.S.	- 3.5 N.S.
	Sodium chloride (5°C)	1.0M	-11.4 N.S.	- 4.3
		2.0M	- 8.9 N.S.	-21.4 N.S.
		3.0M	+ 1.8 N.S.	- 6.4 N.S.

[a] N.S. indicates per cent differences that are not significant ($p < 0.05$).

[b] 100(fresh untreated membrane rupture energy - fresh cryoprotected membrane rupture energy)/fresh untreated membrane rupture energy

[c] 100(fresh cryoprotected membrane rupture energy - frozen/thawed cryoprotected membrane rupture energy)/fresh cryoprotected membrane rupture energy.

centration (ie. 0.3 M as compared to 1.0 M and 3.0 M) had a significant strengthening effect on the membrane, the cause of which is unknown, but probably due to an interaction with the membrane proteins that proves favourable to membrane strength only at low concentrations of sodium phosphate. From Table 11, it can be seen that no significant decrease in this enhanced membrane rupture energy occurred as a result of frozen storage.

The use of sucrose as a cryoprotectant did appear to have favourable results (Code VIII), with membrane rupture energy preserved both before and after frozen storage at the 1.0 M level. Based on its polyhydroxy nature, sucrose could be expected to exhibit cryoprotective properties and has been identified as doing so in other systems (Soliman and VandenBerg, 1971; Meryman, 1971).

The 1.0 M concentration of calcium chloride added had a substantial effect on the membrane rupture energy of eggs of Code VII, causing a drastic increase in membrane toughness before freeze/thawing. The divalent cation of calcium has been shown to interact with both protein and polysaccharides, two of the main components of chum egg membrane (Hamor and Garside, 1977; Kobayashi, 1982). Interaction with a protein has been established in the case of the milk protein casein (Brunner, 1976) and is integral to micelle structure. A gel or precipitate may form on the addition of calcium to some polysaccharides and is a result of association of the divalent ion with acidic groups of the polysaccharide (Hodge and Osman, 1976). Such interactions may have occurred between the calcium added and the protein and/or polysaccharide components of the membrane to produce the initial increase in strength noted.

However, no cryoprotection was subsequently indicated, with a 76% decrease in membrane rupture energy after freeze/thawing, indicating that such interactions must have been disrupted.

The higher concentration of calcium chloride (3.0 M) utilized appeared to

be of value, having both a smaller initial effect on, and preserving more of, the membrane rupture energy during freeze/thawing. However, examination of the eggs produced by this treatment revealed that the inner fluid yolk had increased in consistency to an unacceptable extent. Any apparent cryoprotective effect was probably caused not by a preservation of membrane rupture energy but by the resistance to force offered by a semi-solid yolk.

Generally, the application of sodium chloride not only appeared capable of initially maintaining membrane rupture energy, but actually afforded some degree of cryoprotection (Codes III, IV, V and VI). This result is somewhat unexpected, since this compound is not generally considered to possess useful cryoprotective qualities, rather it is thought to be among the injurious compounds present during the process of freezing (Soliman and VandenBerg, 1971).

However, sodium chloride has been used as a cryoprotectant in the food industry in the preservation of several foods, including egg yolk and fruit tissue. In the former case, it was found to act during freezing by decreasing the concentration of phosphate in the unfrozen water phase (Soliman and VandenBerg, 1971), however the system described in that investigation was a protein solution and so the same mode of action may not apply to preservation of membrane structure. In the present study, results from treatments involving combinations of sodium phosphate and sodium chloride (Code VI) are indecisive, due to the variability in reaction of the chum egg membrane to sodium phosphate, however, it appears that the addition of sodium chloride had little significant influence.

One of the mechanisms thought to be involved in preservation of fruit tissue firmness was dehydration of the cell wall (Sterling, 1968) and this is a plausible explanation for the action of sodium chloride in this study. By removing water molecules from the laminar system of the membrane and interfering with the

production of large ice crystals during the process of freezing, membrane structure could be expected to remain intact to a greater extent.

Of all of the cryoprotective treatments tested, sucrose and sodium chloride were the best choices in terms of relative ineffectiveness on membrane strength before freezing and maintainence of rupture energy during freezing. The use of a sucrose solution would necessitate a post-freezing treatment in order to rid the eggs of the undesirable sweet-taste. Sodium chloride, on the other hand, is actually used in the processing of the eggs into caviar, and so little or no post-freezing treatment should be required. Of all levels tested, a 2.5 M solution of this compound gave the most uniform results, and consequently, this concentration of sodium chloride was chosen as the most consistent and convenient cryoprotectant to be used in successive experiments.

Results reported in Table 11 for Code VIII reveal that no significant difference in maintenance of membrane rupture energy occurred at treatment levels of 2.0 M and 3.0 M sodium chloride, whether the eggs were treated at 25°C, as in all previous tests, or at 5°C. This indicated then that the 2.5 M sodium chloride cryoprotective treatment would be capable of being used in industry with no alteration in the usual packing plant temperature of 10°C.

J. Effectiveness of Cryoprotection by Sodium Chloride

1. Effect of cryoprotection on rate of sodium chloride uptake

The percent chloride contents as a function of salting time for the fresh, frozen and cryoprotected treatments of the Codes IX, X and XI are depicted graphically in Figure 20. From this, it can be seen that in all three codes there was a difference in salt uptake by the cryoprotected eggs, compared to the fresh and frozen/thawed eggs, with cryoprotected eggs having a higher chloride content for the same period of immersion in the saturated sodium chloride

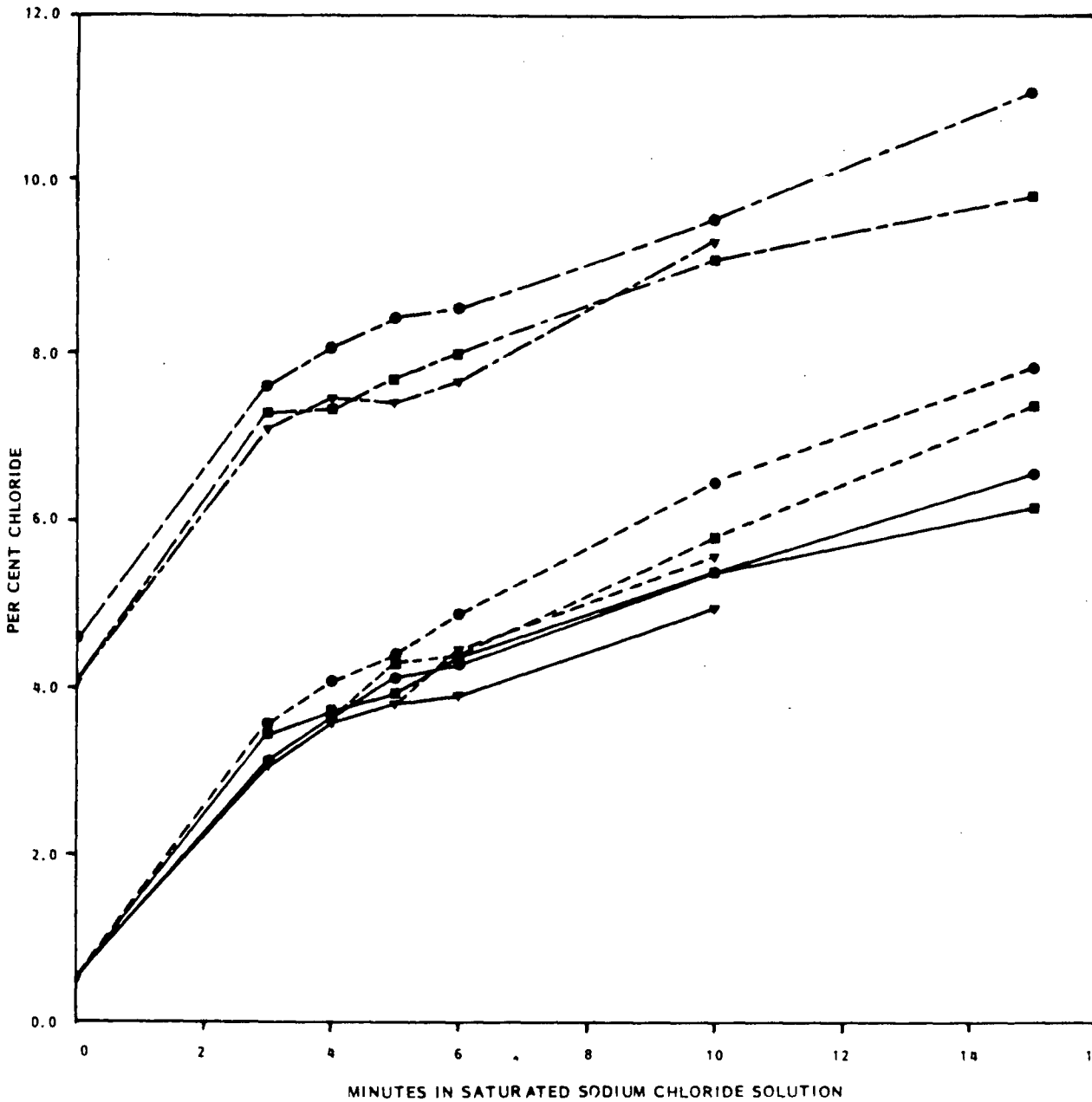


Figure 20. Effect of immersion in a saturated sodium chloride solution for various periods on the chloride content of fresh eggs, eggs previously frozen/thawed, and cryoprotected eggs from three codes (● = Code IX; ▲ = Code X; ■ = Code XI; (———) = fresh eggs; (- - - - -) = frozen/thawed eggs; (— - —-) = cryoprotected eggs).

solution. The fresh and frozen/thawed treatments appeared similar in their rate of salt uptake, although eggs previously frozen consistently possessed a higher chloride content for the same amount of salting time, especially for periods longer than six minutes.

The curves described a logarithmic relationship and Table 12 presents the results of linear regression using the following equation:

$$\%Cl = m(\log \text{time}) + b \qquad (9)$$

The coefficients of determination obtained indicate that this equation is a good description of the relationship between salting time and chloride content for all of the treatments. Comparison of slope and intercepts for fresh, frozen/thawed and cryoprotected eggs support earlier observations on the similarity of the behaviour (ie. salt content and rate of salt uptake) of fresh and frozen/thawed eggs, as compared to cryoprotected. Explanation for the slightly larger value for slope (indicating a more rapid uptake of salt) reported for the previously frozen eggs can be found in the probable damage to the membrane resulting from frozen storage. Such injury could then alter the permeability of the membrane and allow a more rapid penetration of salt.

The large difference in behaviour of the cryoprotected eggs observed in this study can be attributed to the higher salt content possessed at the initiation of salting, due to the pre-freezing treatment with 2.5 M sodium chloride for 30 min. In fact, the chloride content present in the cryoprotected eggs at the start of the salting period is in the normal range for the final product of caviar and so little or no further salting is actually necessary.

With respect to the use of this cryoprotective treatment in industry, these results indicate that there is a difference in the brining process required for fresh eggs and for those previously treated with the cryoprotectant, in order to achieve eggs of the correct salt content for caviar. Further experimentation is needed to determine more exactly the optimum timing of the initial cryopro-

Table 12. Parameters obtained from linear regression of salting curves for chum eggs.

Condition of unsalted eggs	Code no.	Slope	Intercept (% chloride)	r^2
Fresh	IX	0.38	2.1	0.96
	X	0.46	1.9	1.00
Frozen/thawed	XI	0.37	2.2	0.98
Frozen/thawed	IX	0.47	1.9	0.98
	X	0.50	2.0	1.00
	XI	0.53	1.7	1.00
Cryoprotected	IX	0.21	5.5	0.86
	X	0.22	5.9	0.98
	XI	0.20	5.6	0.96

tective treatment to ensure effective protection through frozen storage, yet still produce high quality caviar of the required salt content. It would be desirable to have a cryoprotected egg with a post freeze/thawed salt content lower than that required for caviar, so that a post freeze/thaw salting can be used to adjust the eggs to the desired chloride content.

Further investigation into the logarithmic relationship between salting time and chloride content may reveal this to be equally applicable to eggs of any initial known chloride content. If so, the equation could be used to calculate the time period required to produce any desired final chloride content, thus removing some of the conjecture concerning salting time presently inherent in the procedure.

2. Effect of cryoprotection on development of apparent viscosity

Results of viscometric analysis performed on yolk from fresh, frozen/thawed and cryoprotected eggs of Codes IX, X and XI that were subjected to 3 to 15 minutes immersion in saturated sodium chloride, are presented in the form of rheograms in Figures 21 to 29. There appears to be enough variation in quantitative response to treatments and salting times between the three codes tested to make combining the results undesirable. However, the flow behaviour of all samples appears to be similar in that, all yolks exhibit a pseudoplastic nature that is close to Newtonian and the level of overall apparent viscosity within each treatment and code is affected by the chloride content.

Regression analysis was performed on the rheograms depicted in Figures 21 to 29, using the Power law flow behaviour model, and the parameters of flow behaviour and consistency coefficient are presented in Table 13. It is apparent that all flow behaviour indices (n) were only slightly less than 1.00, indicating a pseudoplastic, near Newtonian behaviour that was supported by the actual observed rheograms. In all codes, both the flow behaviour indices and the

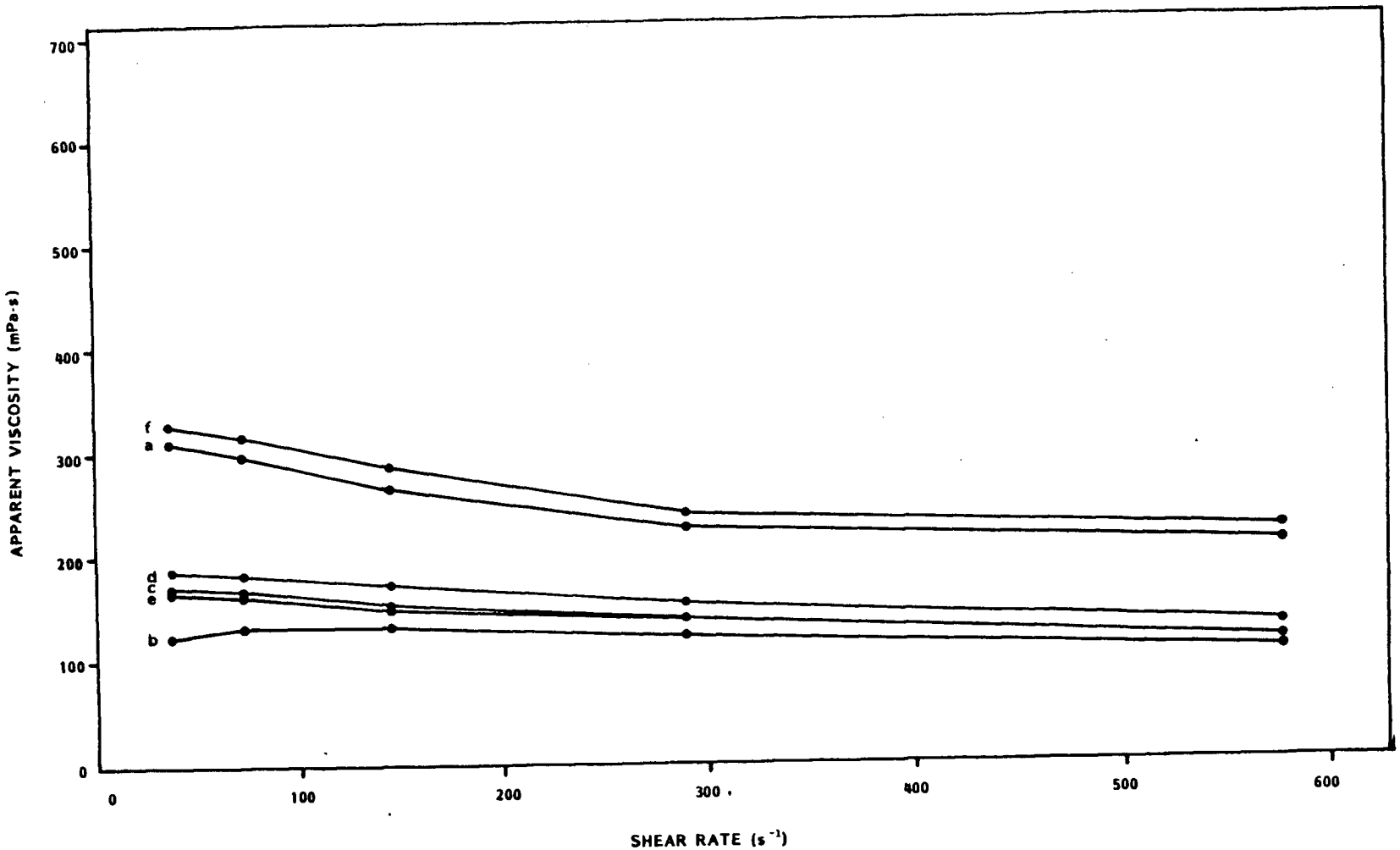


Figure 21. Rheograms of yolk from fresh eggs of Code IX immersed for various periods in a saturated sodium chloride solution (a = 0 min; b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min).

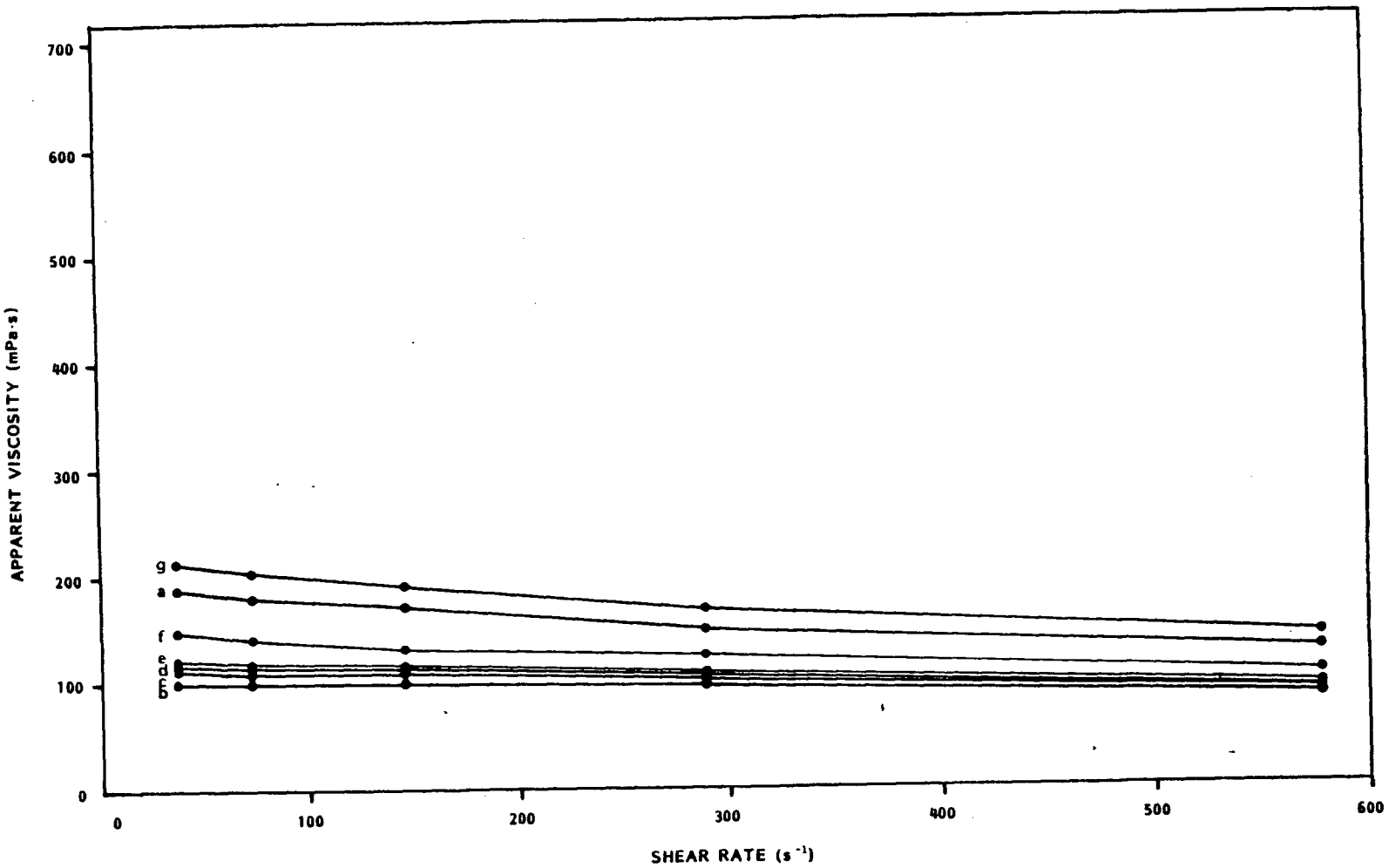


Figure 22. Rheograms of yolk from fresh eggs of Code X immersed for various periods in a saturated sodium chloride solution (a = 0 min; b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min; g = 15 min).

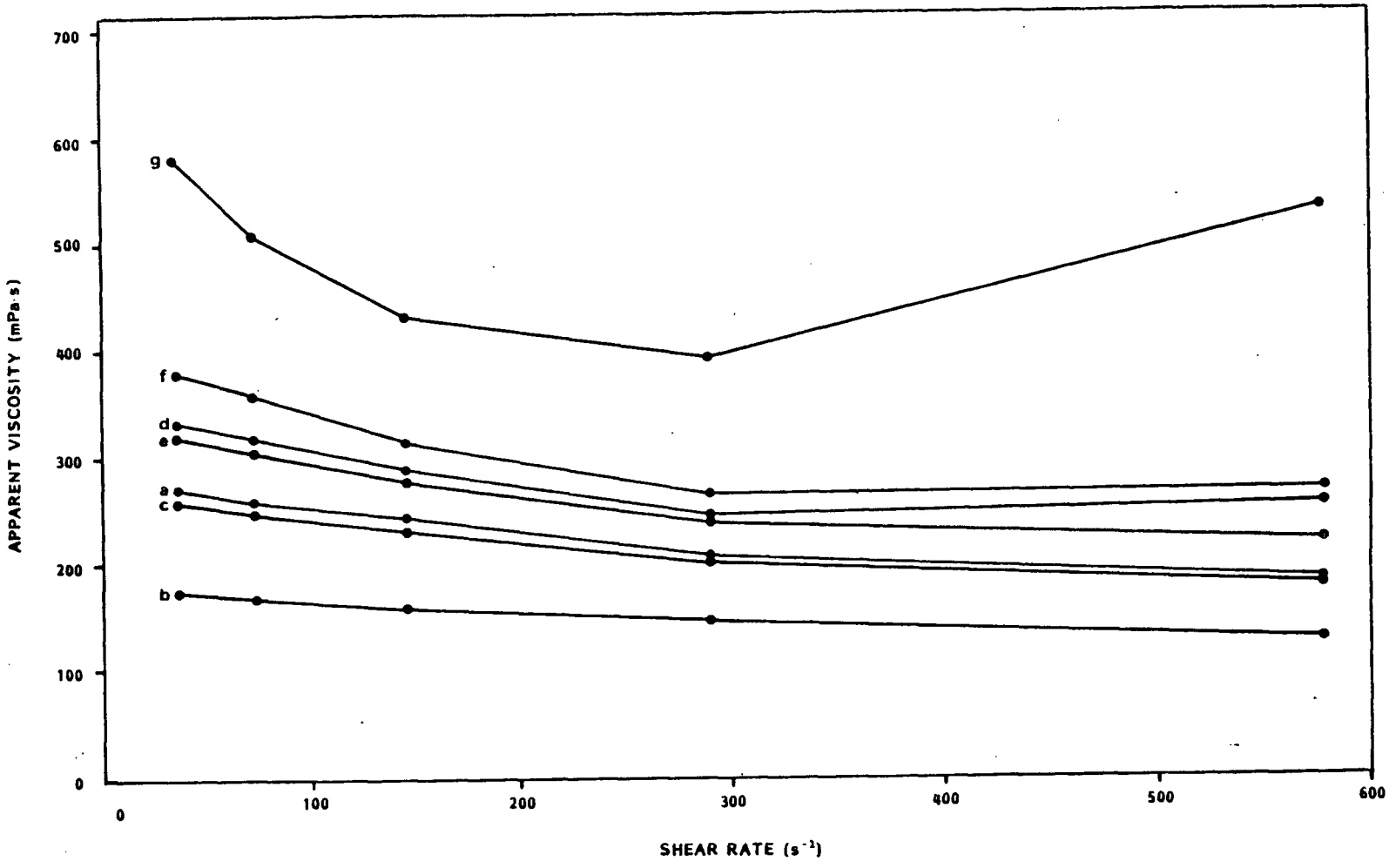


Figure 23. Rheograms of yolk from fresh eggs of Code XI immersed for various periods in a saturated sodium chloride solution (a = 0 min; b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min; g = 15 min).

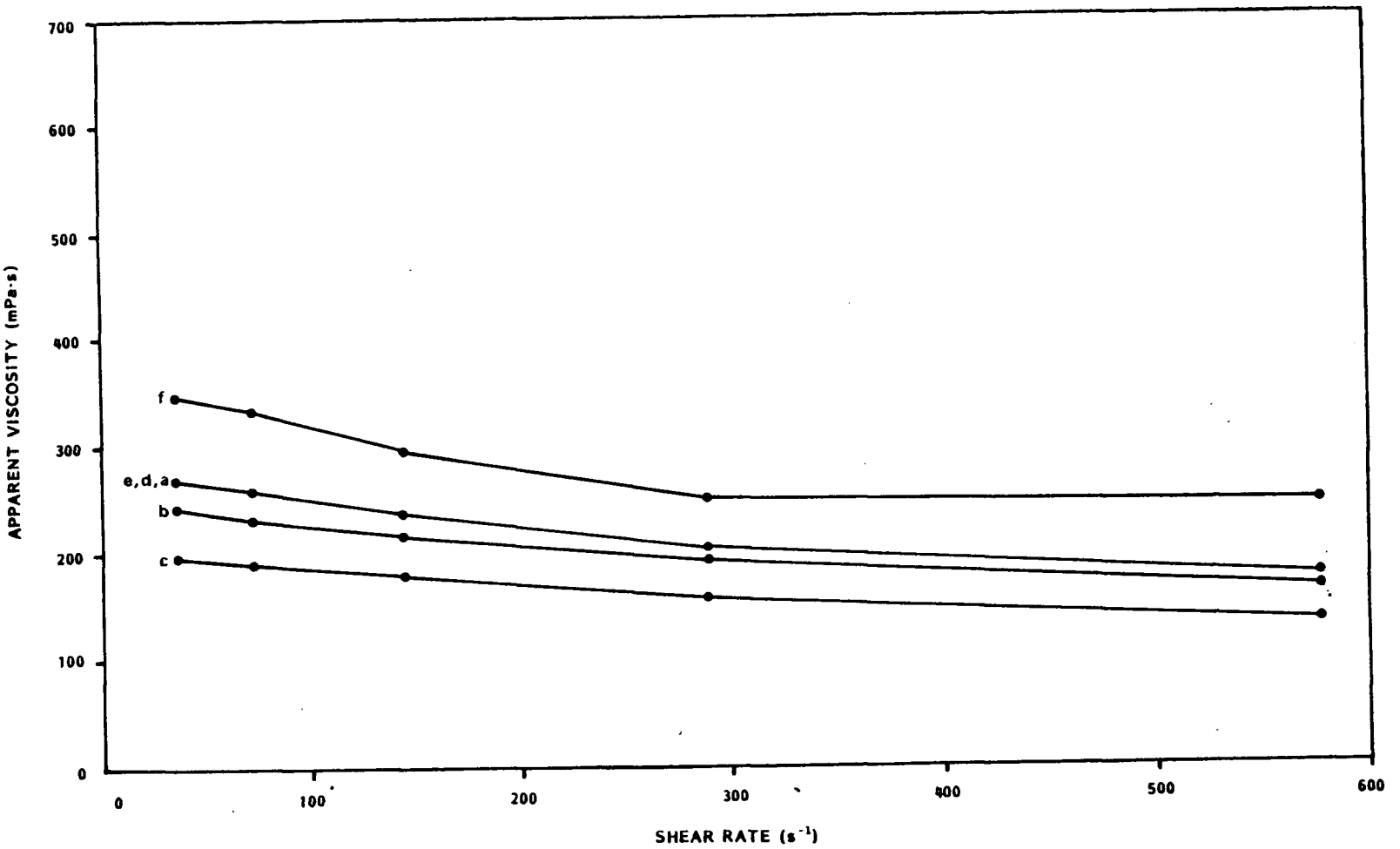


Figure 24. Rheograms of yolk from frozen/thawed eggs of Code IX immersed for various periods in a saturated sodium chloride solution (a = 0 min; b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min).

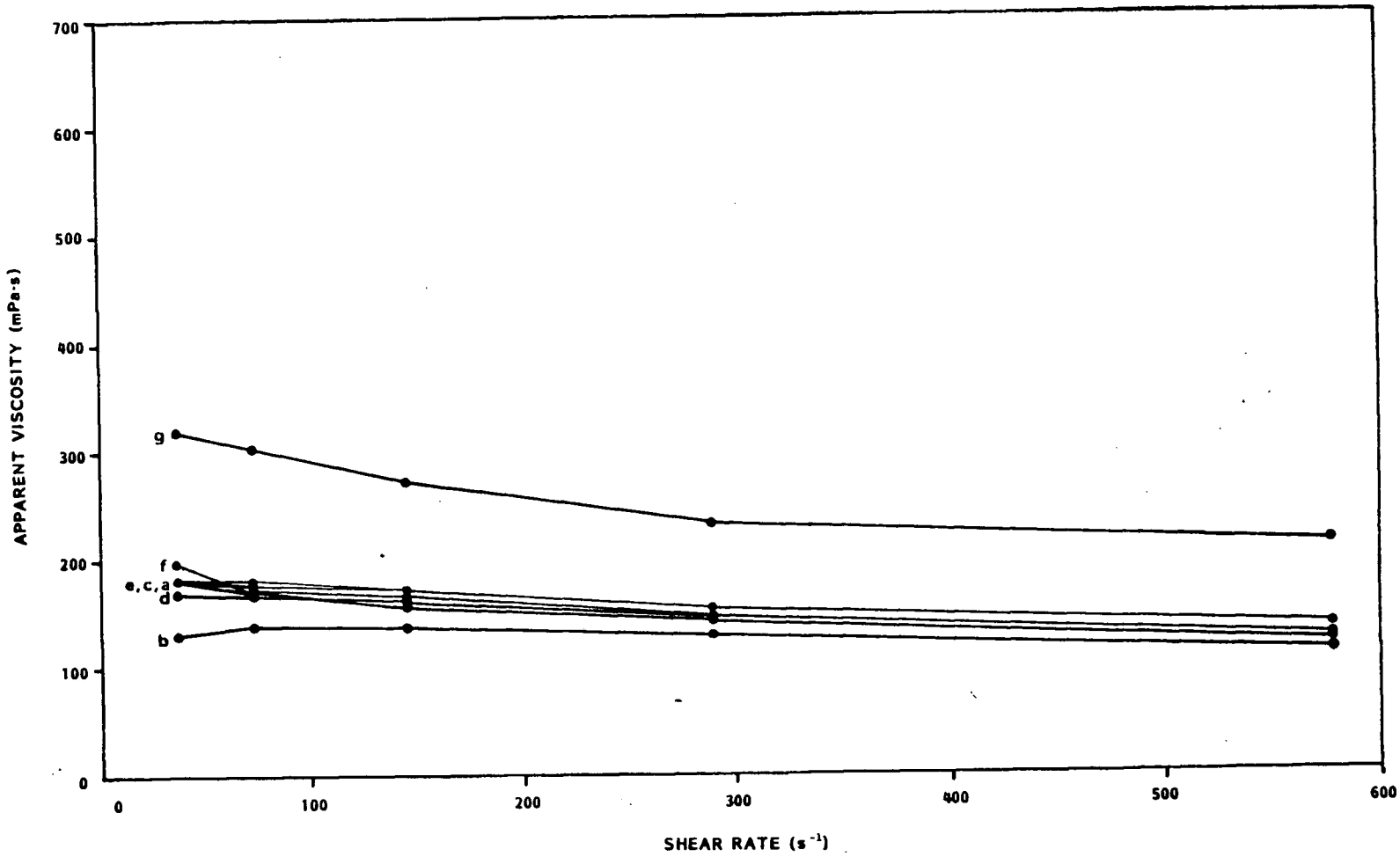


Figure 25. Rheograms of yolk from frozen/thawed eggs of Code X immersed for various periods in a saturated sodium chloride solution (a = 0 min; b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min; g = 15 min).

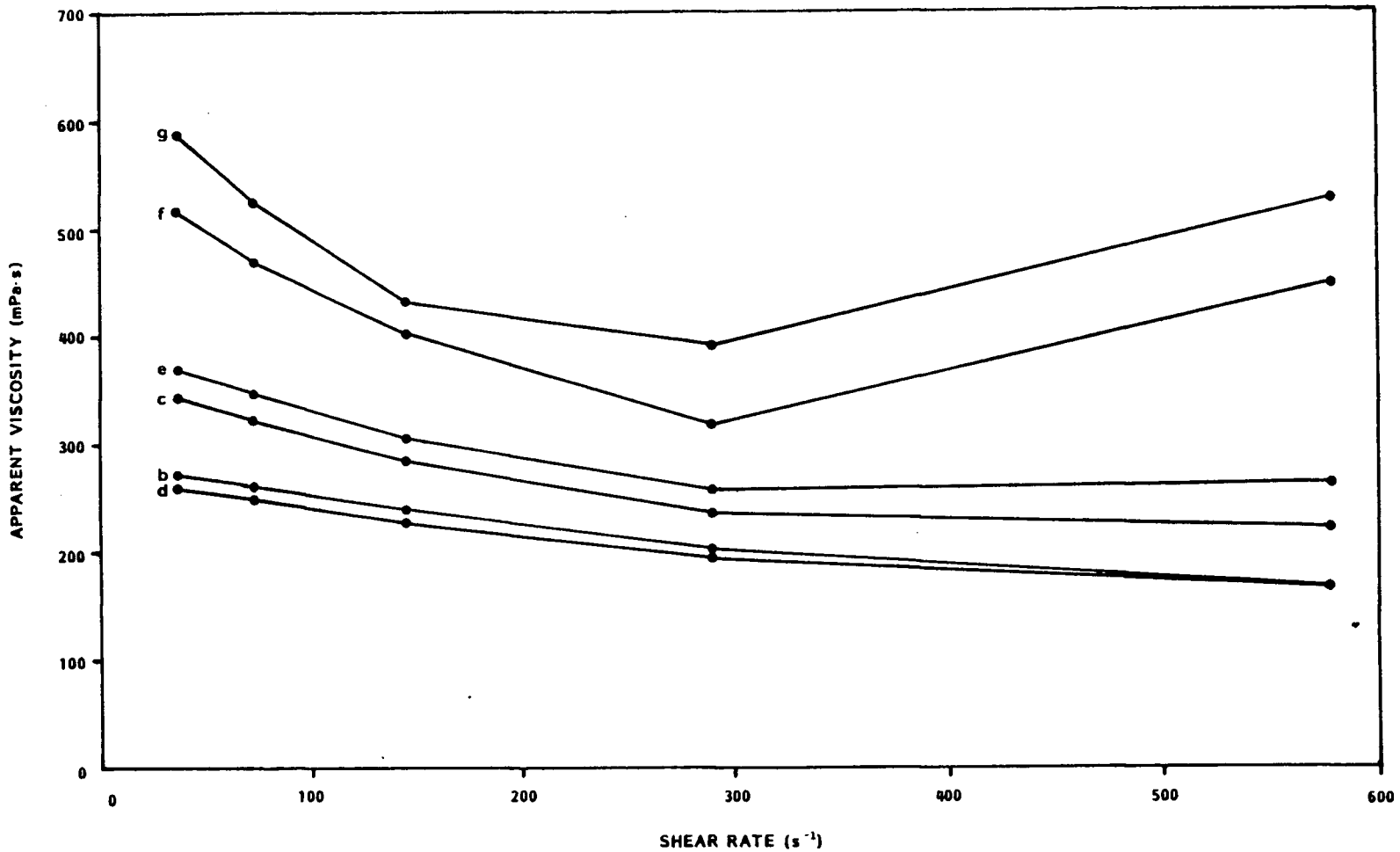


Figure 26. Rheograms of yolk from frozen/thawed eggs of Code XI immersed for various periods in a saturated sodium chloride solution (b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min; g = 15 min).

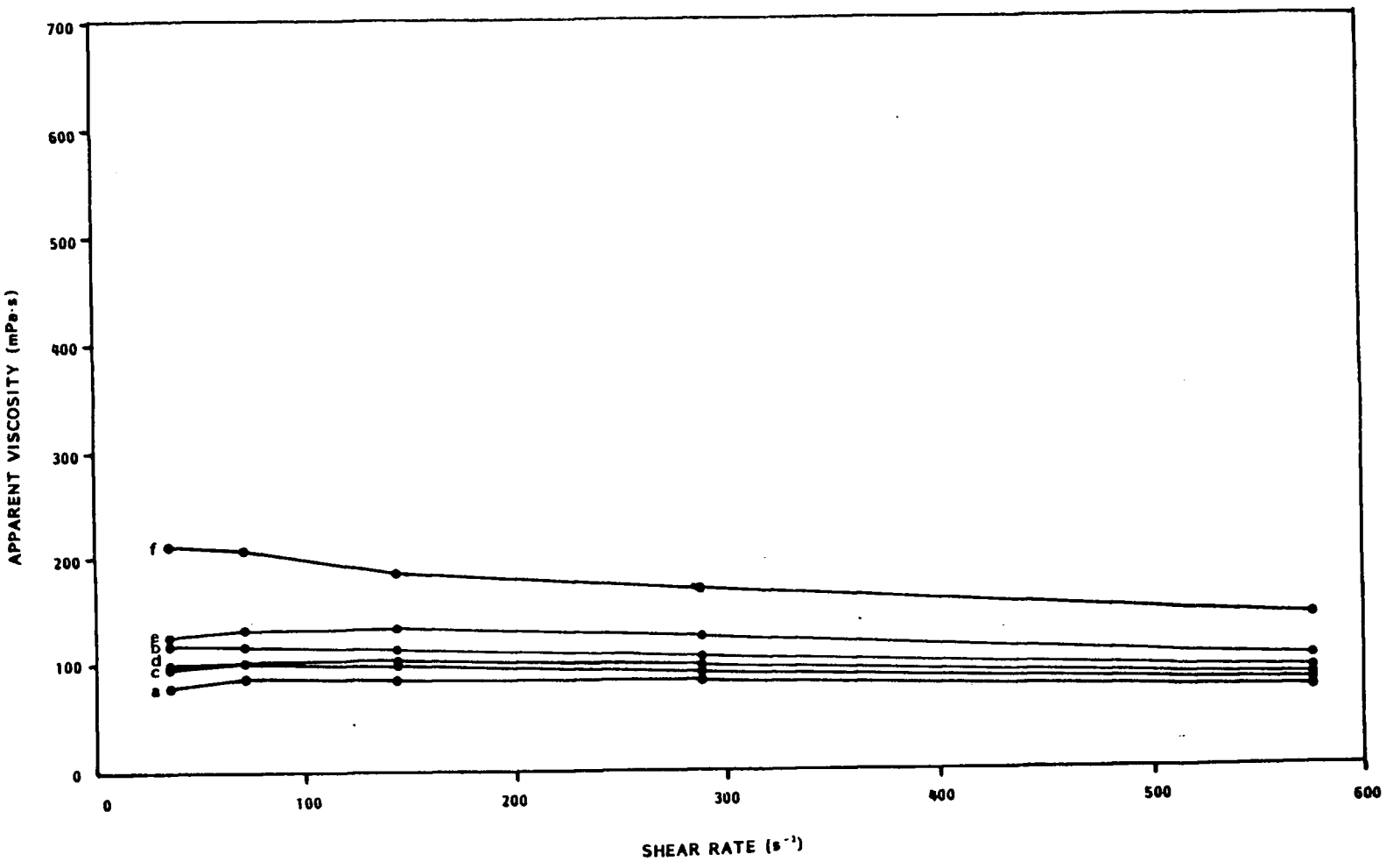


Figure 27. Rheograms of yolk from cryoprotected eggs of Code IX immersed for various periods in a saturated sodium chloride solution (a = 0 min; b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min).

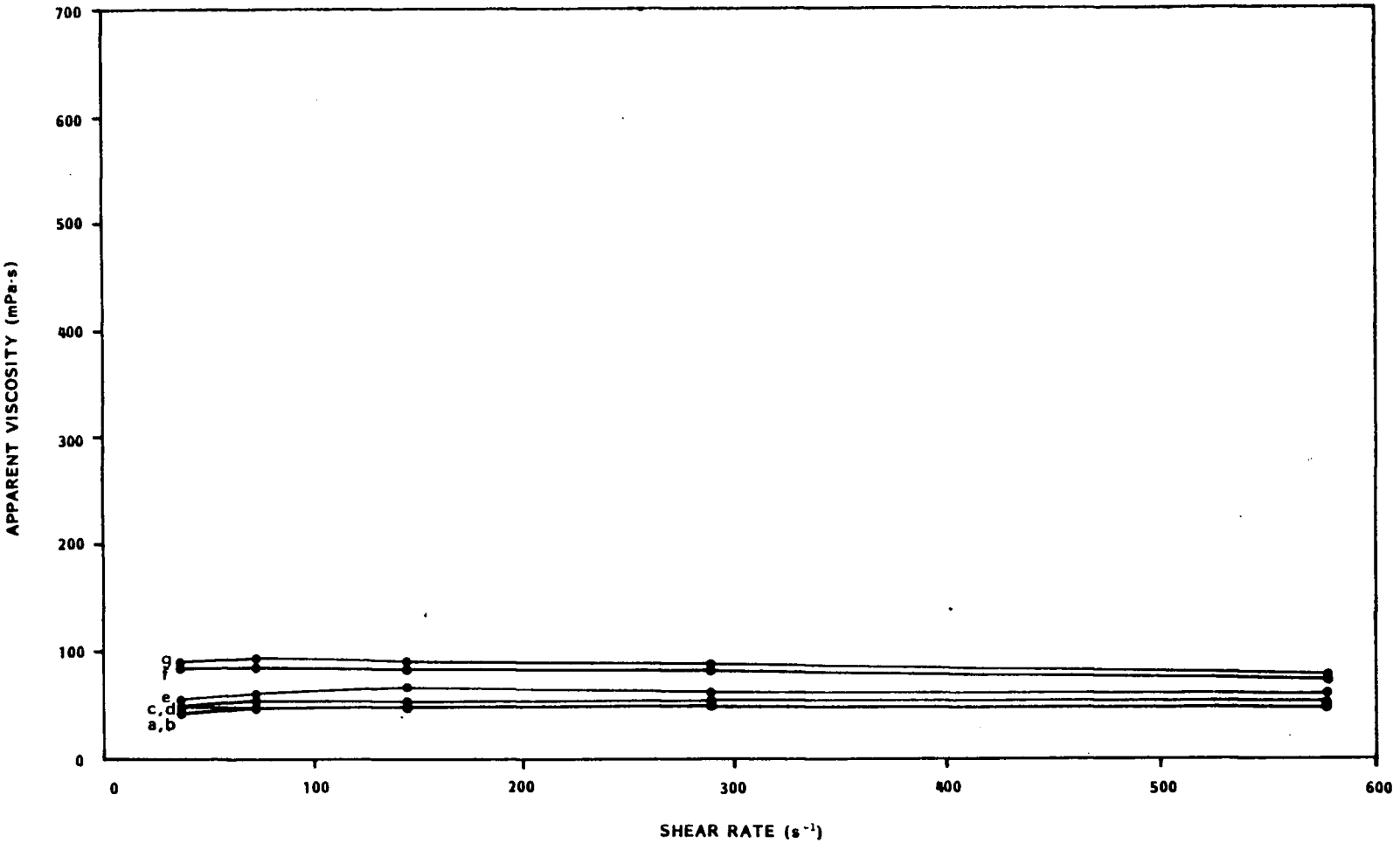


Figure 28. Rheograms of yolk from cryoprotected eggs of Code X immersed for various periods in a saturated sodium chloride solution (a = 0 min; b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min; g = 15 min).

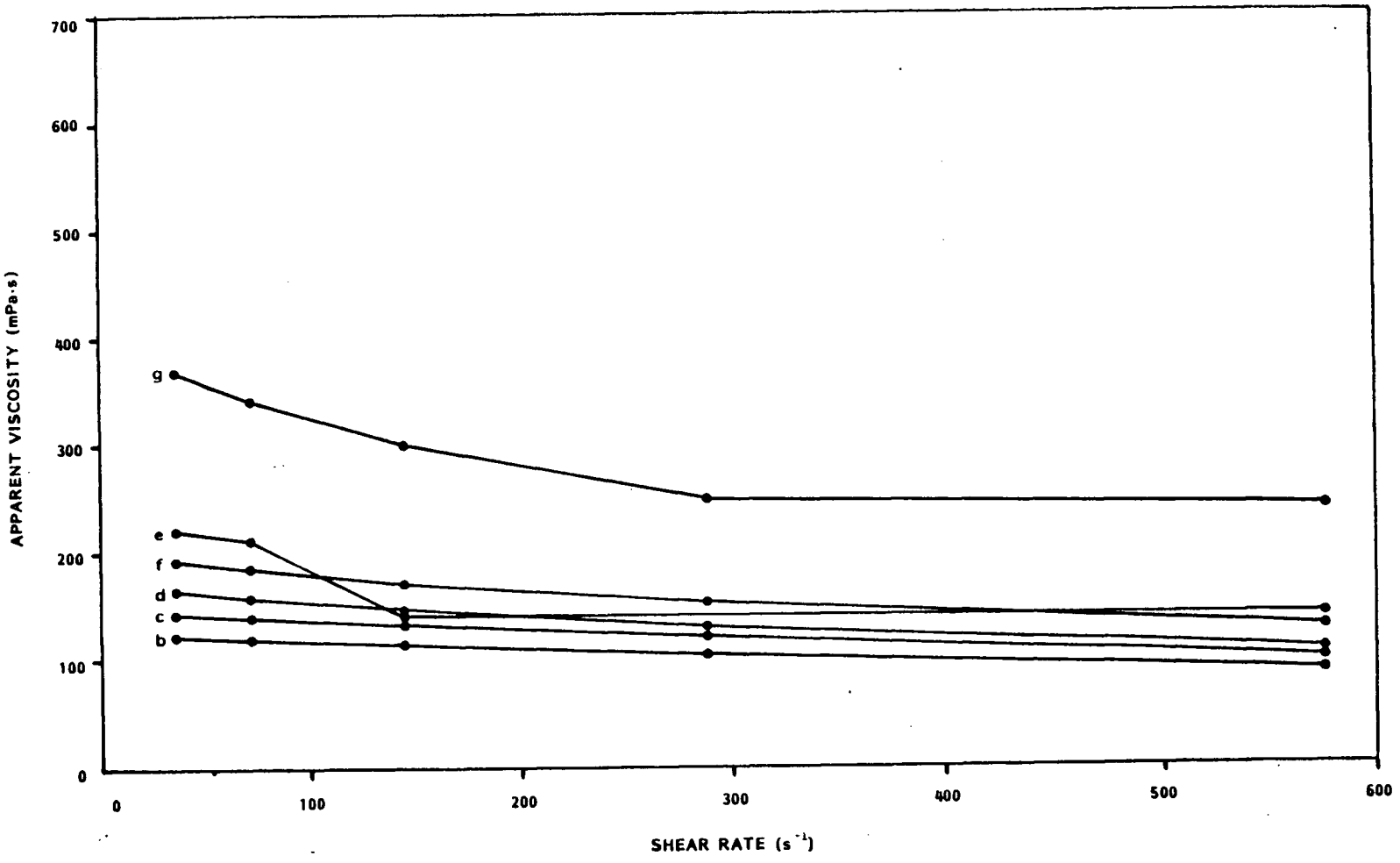


Figure 29. Rheograms of yolk from cryoprotected eggs of Code XI immersed for various periods in a saturated sodium chloride solution (b = 3 min; c = 4 min; d = 5 min; e = 6 min; f = 10 min; g = 15 min).

Table 13. Mean values of Power-law flow parameters of liquid yolk prepared from fresh, frozen/thawed and cryoprotected eggs of various chloride contents from three codes (n=3).

Condition of unsalted eggs	Salting period (min)	Code IX			Code X			Code XI		
		Chloride content (%)	m (mPa s^n)	n	Chloride content (%)	m (mPa s^n)	n	Chloride content (%)	m (mPa s^n)	n
Fresh	0	0.48	5555	0.85	0.49	317	0.87	0.44	---	---
	3	3.06	160	0.95	3.16	137	0.93	3.50	288	0.87
	4	3.65	299	0.86	3.57	163	0.91	3.73	451	0.86
	5	3.83	310	0.88	4.14	163	0.92	3.94	525	0.88
	6	3.91	281	0.87	4.30	175	0.91	4.41	571	0.85
	10	4.90	583	0.85	5.42	236	0.88	5.41	666	0.85
	15	--	-	--	6.63	377	0.85	6.17	684	0.83
Frozen/thawed	0	0.43	498	0.84	0.41	294	0.88	--	-	--
	3	3.18	417	0.86	3.56	183	0.93	3.12	552	0.82
	4	3.63	347	0.86	4.08	315	0.86	3.60	650	0.83
	5	3.80	519	0.84	4.37	275	0.88	4.33	497	0.84
	6	4.47	515	0.84	4.93	299	0.88	4.35	629	0.86

Continued...

Table 13. Continued.

Condition of unsalted eggs	Salting period (min)	Code IX			Code X			Code XI		
		Chloride content (%)	m (mPa s^n)	n	Chloride content (%)	m (mPa s^n)	n	Chloride content (%)	m (mPa s^n)	n
Frozen/thawed	10	5.58	600	0.86	7.15	349	0.84	5.83	683	0.91
	15	--	-	--	7.13	592	0.84	7.43	711	0.93
Cryoprotected	0	4.07	92	0.98	4.63	50	0.96	--	-	--
	3	7.12	165	0.92	7.62	40	1.04	7.32	180	0.90
	4	7.49	124	0.95	8.07	47	1.02	7.40	218	0.89
	5	7.44	125	0.95	8.40	47	1.01	7.70	277	0.86
	6	7.66	167	0.94	8.52	52	1.03	7.96	314	0.89
	10	9.30	367	0.86	9.59	101	0.96	9.10	338	0.86
	15	--	-	--	11.08	109	0.95	9.86	665	0.84

consistency coefficients calculated indicated the yolk of cryoprotected eggs to be dissimilar in comparison with those of the fresh and frozen/thawed treatments. The higher values for n obtained for cryoprotected egg yolks indicated a behaviour closer to Newtonian, and the lower values of m revealed thinner consistencies than the yolk from the other two treatments, for the same salting times and chloride contents.

Both chloride content and viscosity are important to the quality assessment of caviar, and high quality caviar must then possess optimum values for these two parameters at the same time. Eggs that require too high or too low a salt content to achieve the optimum viscosity will never produce caviar that will be judged of high quality. The relationship between chloride content and apparent viscosity in the salted eggs produced from fresh, frozen and cryoprotected treatments for the three codes was investigated and is depicted graphically in Figures 30, 31 and 32.

Two conclusions are apparent from the curves obtained. In the first place, for each code, the apparent viscosities as a function of per cent chloride are similar for the fresh and frozen/thawed eggs, while those of the cryoprotected eggs are considerably less. Secondly, there is a significant drop in apparent viscosity for all curves at values of 3 to 4% chloride, followed by a rise with further increasing chloride contents.

The causes of these effects can only be surmised on the information available. It is known that the chum egg membrane is permeable to both water and inorganic ions, but relatively impermeable to high molecular weight substances (Eddy, 1974). Immersion of chum eggs into a saturated sodium chloride solution appeared to result in not only an immediate intake of salt, but also of water, as is revealed by the puffing up of the eggs that was observed, as the flaccidity commonly observed in unfertilized fresh eggs was lost (Eddy, 1974). Such an intake of water may appear to be an unlikely flow against the osmotic pressure

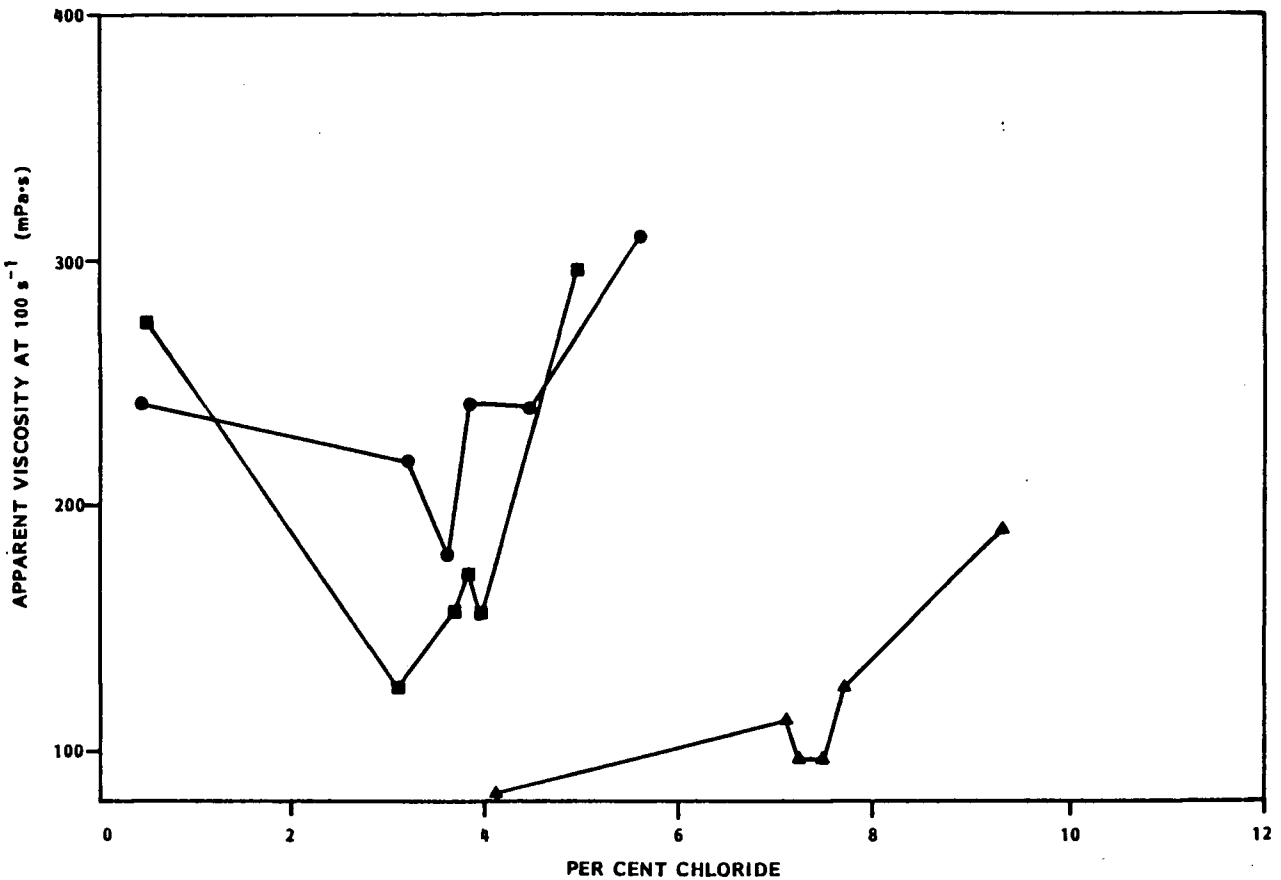


Figure 30. Effect of chloride content of eggs on apparent viscosity of yolk from fresh, frozen/thawed and cryoprotected eggs of Code IX (● = fresh eggs; ■ = frozen/thawed eggs; ▲ = cryoprotected eggs).

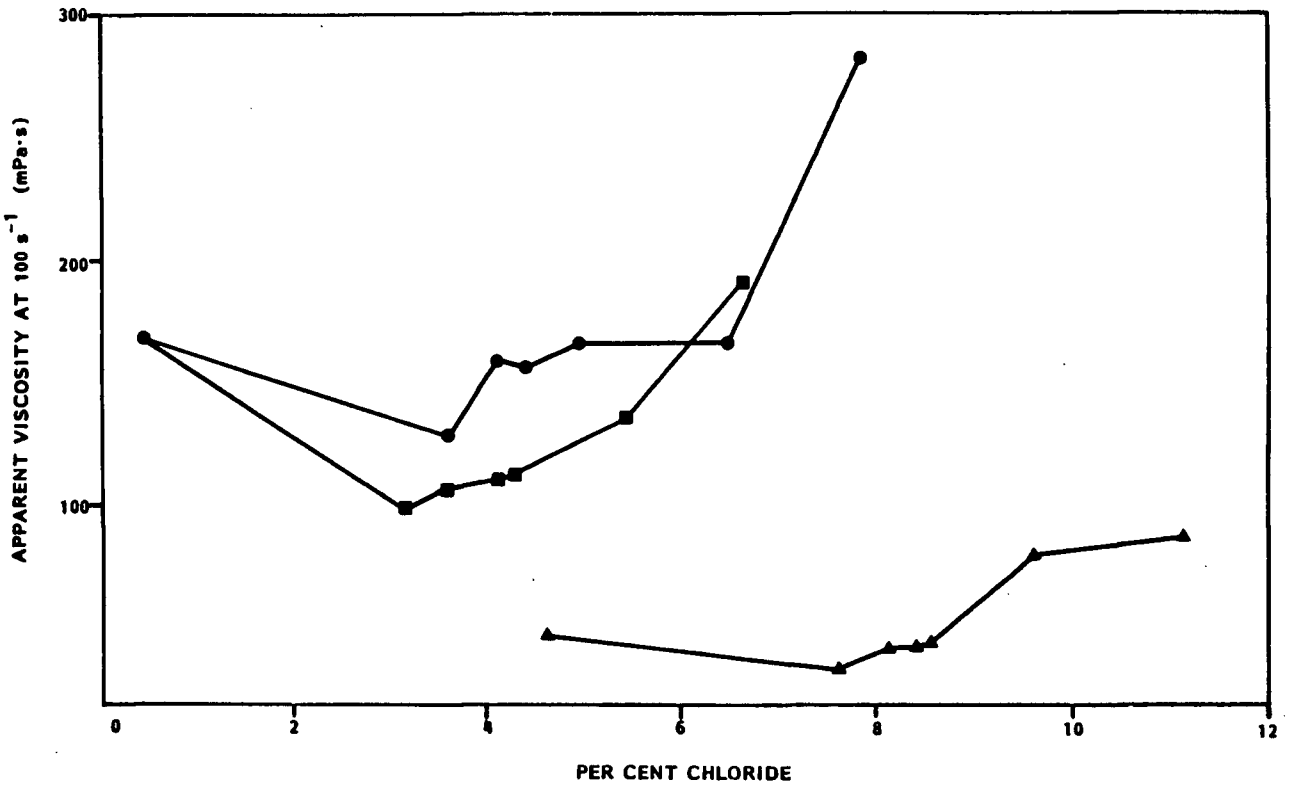


Figure 31. Effect of chloride content of eggs on apparent viscosity of yolk from fresh, frozen/thawed and cryoprotected eggs of Code X (● = fresh eggs; ■ = frozen/thawed eggs; ▲ = cryoprotected eggs).

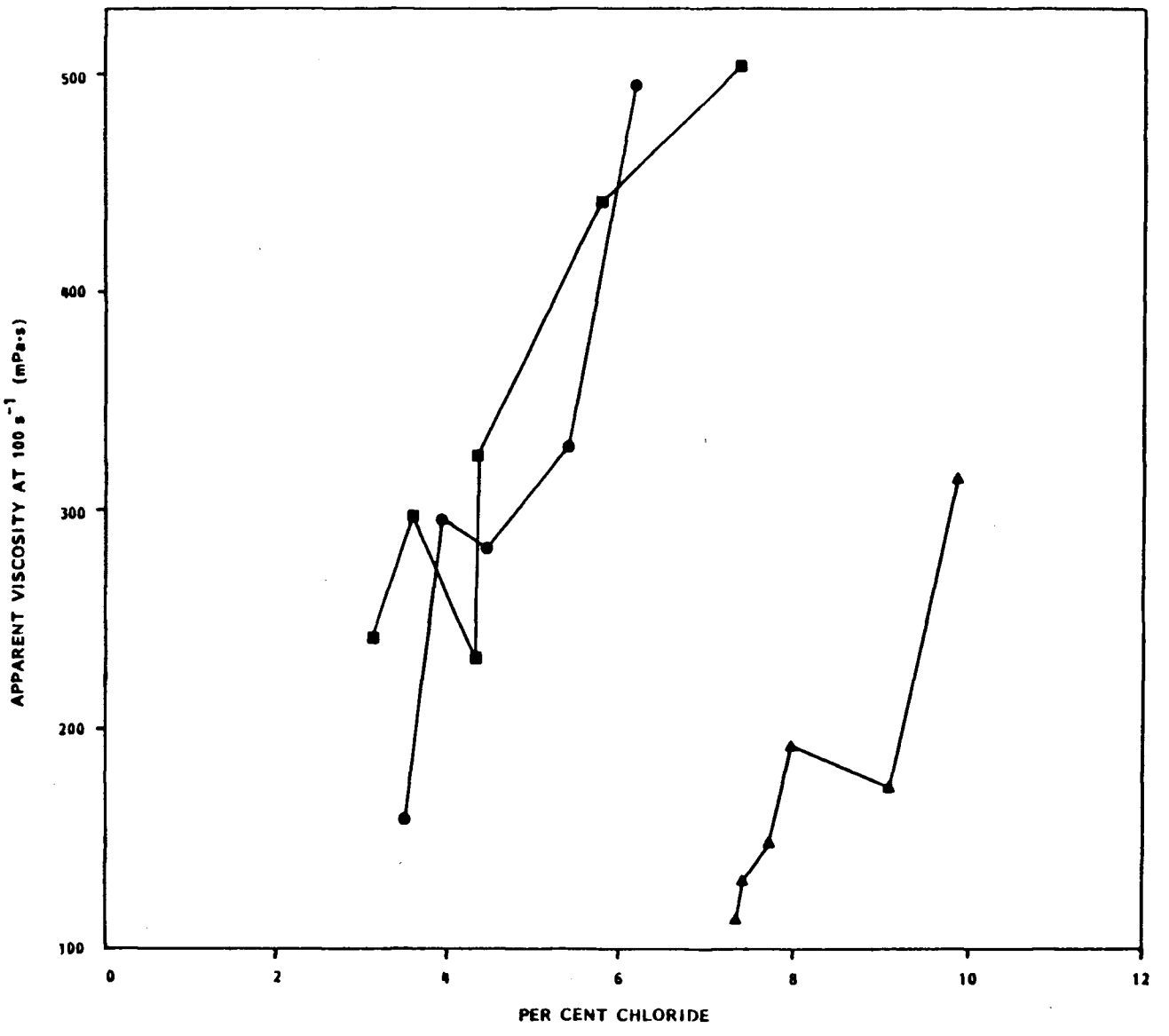


Figure 32. Effect of chloride content of eggs on apparent viscosity of yolk from fresh, frozen/thawed and cryoprotected eggs of Code XI (● = fresh eggs; ■ = frozen/thawed eggs; ▲ = cryoprotected eggs).

caused by the external concentration of sodium chloride, but similar behaviour across a semi-permeable membrane has previously been recorded in the literature. Meschia and Setnikar (1958) observed the flowing of water across a semi-permeable membrane into a dextran solution from a urea solution with 87 times the osmolality. Hargens (1972) found similar results when a 0.0015 M dextran solution drew across a dialyzing membrane a 0.2 M sodium chloride solution approximately 800 times stronger.

Such behaviour may be explained by the theory of Scholander (Scholander, 1967; Hammel and Scholander, 1976) which takes into account hydrostatic pressure in an explanation of solute/solvent movements across semi-permeable membranes. The mechanism postulates that whenever solute molecules are confined in a solvent, they exert pressure on the solvent surface. This stress is transmitted hydraulically throughout the solvent, giving rise to a "partial hydrostatic solvent pressure". If placed on one side of a rigid membrane impermeable to the solute, this pressure hydraulically pulls through all molecules from the other side that are able to penetrate the membrane, until an equilibrium is reached. Therefore, high molecular weight substances such as yolk proteins confined within an expansible semi-permeable membrane, as is the case with the chum egg membrane, could create a pressure that would pull in molecules capable of penetrating the membrane (such as sodium chloride ions and water molecules) until turgor pressure equals the interior solute pressure. At this point, further inward movement of sodium chloride could then still be expected to occur due to diffusion.

In the present study then, when the eggs were first immersed in the saturated sodium chloride solution, there was an initial influx of water as well as salt. This immediate inrush of water served to decrease the apparent viscosity of the yolk of eggs that were removed from the solution at this point. For the eggs immersed for longer periods, while an increasing turgor pressure slowed and finally halted

the influx of water, the concentration of salt continued to increase by diffusion, producing a corresponding increase in yolk viscosity. The increase in apparent viscosity in a protein solution with increasing concentration of sodium chloride has been noted before (Hermansson, 1975). Such events may explain the relationship revealed by Figures 30, 31 and 32.

Explanation for the dissmimilarity of the cryoprotected egg yolk may also be found within Scholander's theory. Prior to freezing, the cryoprotected eggs were immersed for 30 minutes in a 2.5 M solution of sodium chloride and could have thus imbibed more water for a given influx of salt than would be expected to occur in a saturated salt solution. The result of this would be a yolk with a lower apparent viscosity for the same chloride content due to higher water content. The apparent viscosity would then be expected to increase with further exposure to a saturated solution of sodium chloride..

The significance that these results have on the possible use of cryoprotection with sodium chloride in the caviar industry is substantial. Caviar produced in this study from cryoprotected eggs possessed an appreciably lower viscosity than that produced from fresh eggs for the range of 4 to 5% chloride content desired in caviar, and this poses a large problem in achieving a caviar possessing at the same time optimum values of consistency and salinity.

3. Effect of cryoprotection on membrane rupture energy

Values for membrane rupture energy obtained through force deformation analysis of fresh, frozen/thawed and cryoprotected eggs immersed in a saturated sodium chloride solution for periods ranging from 3 to 15 minutes for 3 codes are presented in Table 14. Results of a three way analysis of variance are reported in Table 15. The factors of Code and the Condition of egg before salting were computed to be highly significant sources of variation ($p<0.01$). The results of a Newman-Keul's multiple range test revealed each code to be significantly different from the other. This agrees with the trend noted in previous experi-

Table 14. Mean membrane rupture energies (J x 10^{-4}) for salted fresh, frozen/thawed and cryoprotected eggs (n=25).

Condition of egg before salting	Code	0	3	4	5	6	10	15
Fresh	IX	7.99 (0.48)[a]	6.11 (3.06)	9.03 (3.65)	7.12 (3.83)	6.64 (3.91)	7.76 (4.90)	---- ------
	X	5.82 (0.49)	5.68 (3.16)	4.95 (3.57)	6.47 (4.14)	5.72 (4.30)	6.12 (5.42)	6.70 (6.63)
	XI	4.17 (0.44)	4.31 (3.50)	3.96 (3.73)	4.24 (3.94)	4.04 (4.41)	4.10 (5.41)	4.70 (6.17)
Frozen	IX	3.01 (o.43)	4.04 (3.18)	4.94 (3.63)	5.21 (3.80)	4.91 (4.47)	5.76 (5.58)	---- ------
	X	2.94 (0.41)	4.16 (3.56)	3.86 (4.08)	3.90 4.37)	4.43 (4.93)	4.05 (7.15)	4.65 (7.13)
	XI	---- ------	2.66 (3.12)	2.46 (3.60)	2.01 (4.33)	2.39 (4.35)	2.95 (5.83)	3.26 (7.43)
Cryoprotected	IX	6.00 (4.07)	6.80 (7.12)	7.79 (7.49)	9.25 (7.44)	7.74 (7.66)	7.61 (9.30)	---- ------
	X	4.62 (4.63)	6.32 (7.62)	6.35 (8.07)	6.42 (8.40)	6.58 (8.52)	7.71 (9.59)	7.84 (11.08)
	XI	---- ------	3.85 (7.32)	4.64 (7.40)	4.47 (7.70)	5.17 (7.96)	4.88 (9.10)	4.83 (9.86)

[a]Values expressed in brackets represent mean percent chloride content (n=3).

Table 15. Analysis of variance of membrane rupture energy for eggs of various chloride contents.

Source of variation	D.F.	Mean square	F-ratio
Code	2	80.92×10^7	139.39**
Condition of egg before salting	2	65.52×10^7	74.76**
Minutes in saturated sodium chloride solution	4	1.60×10^7	1.26
Code x condition	4	0.88×10^7	1.51
Code x minutes	8	1.26×10^7	2.18*
Condition x minutes	8	0.36×10^7	0.46
Code x condition x minutes	16	0.78×10^7	1.34
Error	1080	0.58×10^7	
Total	1124		

*significant at $p<0.05$
**significant at $p<0.01$

ments, is. that there may be considerable variation in response to treatments from one shipment of roe to the next. The multiple range test of the factor of Condition revealed no significant difference between results obtained from fresh and cryoprotected treatments, but such a difference did exist between these and results obtained from frozen/thawed eggs. Such an outcome reflects the successful cryoprotective ability of sodium chloride in chum egg membrane in yielding eggs that could be processed into caviar of comparable membrane strength to that produced from fresh.

A last significant source of variation was revealed in the interaction of Code and Minutes in saturated sodium choride solution. It is unknown why this interaction would appear as a significant factor, since there is no apparent reason why an interaction between egg shipment and length of salting time should occur, when a significant interaction between egg shipment and Condition of egg (ie. fresh, frozen or cryoprotected) is missing.

K. Sensory Evaluation

The two characteristics of yolk viscosity and membrane rupture energy have formed the basis of all the objective rheological studies in this investigation. Bourne (1975) states that ultimately, it is the subjective human response to a product that determines its success or failure. Accordingly, a sensory panel analysis was conducted in an attempt to determine if judges could discern any difference in yolk viscosity and membrane strength between caviar produced from frozen eggs and that from cryoprotected eggs. A control sample of high quality caviar was included in the samples presented as both the reference and as one of the trial samples. This was performed to provide a basis for determining the judges' ability to match the two control samples presented, as well as to see how the caviar from frozen and cryoprotected eggs would be assessed in relation to the high quality control.

Results of sensory evaluation of the characteristics of membrane strength and of yolk viscosity are presented in Table 16. In the nine point scale used for the paired comparison test, a value of 5 was used as the center point and any sample so ranked would be considered as equal to the reference. A value of 1 was given to samples ranked "extremely tougher or thicker", while a value of 9 was assigned to samples ranked at the other end of the scale: "extremely weaker or thinner". Intermediate values were then given to the descriptions lying between these two extremes (see sample tests in Appendix A). Values obtained for each test were subjected to an analysis of variance which is presented in Tables 17 and 18. The particular analysis employed permits partitioning of the Samples factor to allow evaluation of any variation introduced by the single control caviar sample in comparison with the rest of the trial samples presented (Control vs. caviar from cryoprotected and frozen/thawed samples), or caviar produced from frozen eggs in comparison with that produced from cryoprotected eggs (Frozen/thawed vs. cryoprotected samples), as well as variation introduced within the three replicates of each of the two experimental treatments (Replicates within cryoprotected and frozen/thawed samples).

Of the sources of variance evaluated, only Judges and Control vs. caviar from cryoprotected and frozen/thawed samples were significant. The significance of the factor Judges would indicate that individual variability was high in the sensory panel. Such a result illustrates the problems in specifying to a taste panel the particular criteria being evaluated. The significance of the factor Control vs. caviar from cryoprotected and frozen/thawed samples shows that the high quality control sample made from fresh eggs was readily discerned from the remaining samples of caviar, based on both membrane strength and yolk viscosity. Some experimental error may have been introduced at this point due to the rancidity of the caviar produced from frozen/thawed and cryoprotected eggs. This flavour was commented on fairly consistently in the questionnaire

Table 16. Mean ratings (nine point scale) for yolk viscosity and membrane strength judged by sensory panel for each caviar sample presented (n=8).

Condition of unsalted eggs	Replicate	Viscosity	Membrane strength
Frozen/thawed	1	5.4 (1.7)[a]	5.6 (0.7)
	2	5.0 (1.1)	5.3 (0.7)
	3	5.9 (1.0)	5.1 (0.6)
Cryoprotected	1	6.1 (1.0)	5.9 (1.3)
	2	5.5 (1.2)	5.8 (1.3)
	3	6.0 (1.3)	5.5 (1.7)
Fresh (control)		4.5 (0.9)	4.6 (0.5)

[a]Standard deviation

Table 17. Analysis of variance of ratings from sensory analysis of membrane strength.

Source of variation	D.F.	Mean square	F-ratio
Samples	6	1.48	1.35
Control vs. cryoprotected and frozen samples	1	5.50	5.02*
Frozen vs. cryoprotected samples	1	1.69	4.05
Replicates within cryoprotected and frozen samples	4	0.42	0.38
Judges	7	3.21	2.93**
Error	42	1.10	
Total	55		

*significant at $p<0.05$
**significant at $p<0.01$

Table 18. Analysis of variance of ratings from sensory analysis of yolk viscosity.

Source of variation	D.F.	Mean square	F-ratio
Samples	6	2.72	2.11
Control vs. cryoprotected and frozen samples	1	9.00	6.97**
Frozen vs. cryoprotected samples	1	2.52	2.09
Replicates within cryoprotected and frozen samples	4	1.21	0.94
Judges	7	2.77	2.15*
Error	42	1.29	
Total	55		

*significant at $p<0.05$
**significant at $p<0.01$

distributed, and could have been caused by inadequate frozen storage (due to freezer breakdown that occurred during the storage period).

A hedonic scale was included in the sensory evaluation, to ascertain whether there was overall preference for any particular caviar sample, and results are reported in Table 19. On the nine point scale used, values of 1 and 9 represented the judgements of "dislike extremely" and "like extremely", respectively. For statistical analysis, a simple two way analysis of variance was performed on these results to determine if there was any significant difference in preference for any particular sample/s. Calculations as reported in Table 20 showed that both Samples and Judges were significant sources of variation. The significance of the Judges term again reflects individual variability in preference of the samples. A Newman-Keul's multiple range test was performed on the significant term Samples and it was found that mean preference scores for the three samples of caviar processed from cryoprotected eggs were not significantly different than that of the high quality control, while the caviar processed from the frozen/thawed eggs ranked significantly lower in preference scores for two out of the three replicates. This indicates that although no difference was discerned in the membrane strength and yolk viscosities between caviar from the frozen/thawed and cryoprotected eggs, general preference was evinced for that produced from cryoprotected eggs.

There are two limitations inherent in a study of this sort. Firstly, as stated by Bourne (1975), during the mastication of a food in order to assess quality, many sequential judgements take place, and there is a continuous change in physical properties with respect to destruction of structure, mixing with saliva and temperature effect. Considering only the "one event" parameters of membrane rupture strength and yolk viscosity is limiting in an assessment of caviar quality and not representative of the entire nature of the final product. However, as these two parameters formed the basis of the development of the

Table 19. Mean ratings from nine point hedonic scale for each caviar sample presented.

Condition of unsalted eggs	Replicate	Preference rating
Frozen/thawed	1	$3.8^{a[z]}$ $(1.8)^{[zz]}$
	2	5.1^{abc} (2.0)
	3	4.9^{ab} (2.2)
Cryoprotected	1	5.9^{bc} (1.6)
	2	5.6^{abc} (1.3)
	3	5.4^{abc} (1.3)
Fresh (control)		7.0^{c} (1.1)

[zz] standard deviation

[z] means sharing the same superscript are not significantly different ($p<0.05$) as determined by Newman-Keul's multiple range test.

Table 20. Analysis of variance of ratings from nine-point hedonic scale

Source of variation	D.F.	Mean square	F-ratio
Samples	6	7.88	4.44**
Judges	7	8.49	4.78**
Error	42	1.77	
Total	55		

**significant at $p<0.01$

cryoprotective technique devised, sensory evaluation of these two characteristics can be said to form the first phase of future, more intense investigations.

In addition, the rancidity of the trial samples was probably an influence in judgement of parameters. Further evaluation is then necessary to more surely state the success of the cryoprotective technique in producing a higher quality caviar than that processed from frozen/thawed eggs.

CONCLUSIONS

Frozen storage for later processing would be a viable solution to even out the large fluctuations in roe supply that processors dealing with this commodity are seasonally presented with. This study was performed to determine the extent and location of damage in frozen/thawed eggs and to investigate the possibility of utilizing a cryoprotectant to alleviate any such alterations as were found to occur.

The first part of the study investigated the physical aspects of the chum egg. Previous reports of the occurence of at least three major proteins in salmon egg yolk (Markert and Vanstone, 1968; 1971) were confirmed. Differential scanning calorimetry revealed three major proteins; electrophoresis indicated the presence of more, however, this difference may have been due to either the differences in sensitivity of the two analyses, or to a masking of the heat denaturation peaks of minor proteins by the large peaks observed in the DSC thermogram. The flow behaviour of chum egg yolk was non-Newtonian, pseudoplastic in nature. The degree of deviation from a Newtonian fluid and the consistency varied from one coded lot of eggs to another.

The previously reported (Kobayashi, 1982; Stehr and Hawkes, 1979) complex nature of the chum membrane was also confirmed, consisting of an inner layer of intertwisting fibers and an outer fibrous layer. A third intermediate layer postulated to be present in teleost egg membranes was not readily apparent in the present study. As in previous investigations with other teleost eggs, the membrane was capable of withstanding large forces of compression, a feature advantageous to species whose eggs lie in a mass on the bottom of stream beds under a nest of gravel.

The second part of the study revealed that frozen storage for 7 d at -10°C followed by thawing in air at room temperature had little or no effect on the

egg yolk proteins as detectable by either heat of denaturation or denaturation temperature, electrophoretic patterns, or flow behaviour. However, an average 46% decrease in membrane rupture energy was observed, indicating that the membrane is the source of the difference detected by subjective observations between the fresh and the frozen/thawed eggs.

A study of the potential cryoprotectants tested in this investigation revealed that cryoprotection of the chum egg membrane involved different principles than that operating in the usual cellular suspensions reported in the literature. In this study, highly effective cellular cryoprotectants such as ethylene glycol and glycerol had an adverse effect on membrane rupture energy, even prior to freezing. The most effective cryoprotectant studied, in terms of consistent ineffectiveness on membrane rupture energy before, and maintenance during, freeze/thawing was a 30 min immersion in a 2.5 M solution of sodium chloride.

Results of analysis of caviar produced from this cryoprotectant treatment indicated that eggs so produced did indeed result in caviar with a membrane rupture energy closer to that produced from fresh eggs, than that produced from frozen eggs. However, a modification in the usual brining procedure is called for to produce caviar of the proper chloride content, as much shorter salting times appeared to be required, than were necessary for either fresh or frozen/thawed eggs. In addition, the consistency of the resulting caviar appeared to be adversely affected, with a lower apparent viscosity for a given chloride content that occurs with caviar from either fresh or frozen/thawed eggs. Further experimentation with the cryoprotective treatment using a more concentrated sodium chloride solution for a shorter period of time may not even be necessary, since the range of acceptable apparent viscosities revealed by previous sensory analyses is on the order of 400 to 1500 mPa·s ($10s^{-1}$) (Sternin and Hori, 1982).

Sensory panel analysis indicated that the fresh high quality control caviar was readily differentiated on the basis of both membrane strength and yolk viscosity from the caviar produced from both the cryoprotected and the frozen/thawed eggs that had been stored for six weeks at -20°C in a still air freezer. However, the rancidity of the caviar from the pre-frozen eggs may well have been a large source of error. More experimentation with the effect of long storage times in a more controlled frozen storage condition on the quality of caviar produced from cryoprotected eggs is needed. Even with the presence of a rancid taste, hedonic studies were promising, revealing a preference for caviar from cryoprotected eggs over that of that produced from frozen/thawed eggs.

Results of this study indicate that it is possible to treat chum eggs prior to freeze/thawing such that the membrane, the major site of freezing damage, is protected. What remains is the need for more extensive studies to co-ordinate the optimum concentration and duration of cryoprotective treatment with that of any modifications of present caviar processing technique to produce a higher quality caviar than that previously attainable with the use of unprotected eggs.

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APPENDIX

MULTIPLE COMPARISONS TEST

-CAVIAR-

Name _____ Date _____

You are receiving samples of caviar to compare for viscosity and membrane strength. You have been given a reference sample, marked R, with which you are to compare each sample. Make judgements on more than one egg and retest as necessary to be sure. Test the samples in the order in which they have been presented (left to right).

1. Viscosity - Bite eggs with front teeth; make judgement on the inner liquid of the eggs for viscosity. Determine whether the yolk is thicker than, comparable to, or thinner than the reference. Then mark the amount of difference that exists.

Sample number							
Thicker than R							
Equal to R							
Thinner than R							
Amount of Difference:							
None							
Slight							
Moderate							
Much							
Extreme							

2. Membrane Strength - Bite eggs with front teeth; make judgement on force required to rupture eggs. Determine whether the membrane is tougher than, comparable to or weaker than the reference. Then mark the amount of difference that exists.

Sample number							
Tougher than R							
Equal to R							
Weaker than R							
Amount of Difference:							
None							
Slight							
Moderate							
Much							
Extreme							

Hedonic Scale
Scoring

Date _____ Taster _____

Product _____

Taste these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us.

Code _____	Code _____	Code _____	Code _____
___ Like extremely	___ Like extremely	___ Like extremely	___ Like extremely
___ Like very much	___ Like very much	___ Like very much	___ Like very much
___ Like moderately	___ Like moderately	___ Like moderately	___ Like moderately
___ Like slightly	___ Like slightly	___ Like slightly	___ Like slightly
___ Neither like nor dislike	___ Neither like nor dislike	___ Neither like nor dislike	___ Neither like nor dislike
___ Dislike slightly	___ Dislike slightly	___ Dislike slightly	___ Dislike slightly
___ Dislike moderately	___ Dislike moderately	___ Dislike moderately	___ Dislike moderately
___ Dislike very much	___ Dislike very much	___ Dislike very much	___ Dislike very much
___ Dislike extremely	___ Dislike extremely	___ Dislike extremely	___ Dislike extremely
Reason:	Reason:	Reason:	Reason: