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Effect of hydrocolloids and pretreatments on the keeping quality of minced fillets of whiting and cod during frozen storage

Proefschrift

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Abstract

The present study examines the effects of additions of hydrocolloids and pretreatments (precooking and irradiation) on the stability of minced fillets of whiting and cod during frozen storage (-18°C).

Some hydrocolloids (Xanthan gum, alginates, carboxymethyl celluloses and Iota and Lambda carrageenan) increase the water holding capacity and have a cryoprotective effect on the texture of the fish minces during frozen storage.

Irradiation (300 krad, -20°C) can be applied to the fish minces for decreasing the number of viable microorganisms, without affecting the keeping quality of the fish minces in the post-irradiation frozen storage. Precooking the fish minces up to an internal temperature of 80°C or higher, for short periods of time (about 1 minute), prevents the production of dimethylamine and formaldehyde in the fish minces during subsequent frozen storage. Both precooking and irradiation can be used in combination with additions of hydrocolloids in the fish minces with beneficial effects.

Hydrocolloids can be used as additives in the manufacture of minced fish products, decreasing the weight loss on cooking, stabilizing the texture and in certain cases increasing significantly the acceptability of the fish products during frozen storage. Iota carrageenan and under certain conditions carboxymethyl cellulose decrease the fat uptake of battered and breaded minced fish products during frying.

Stellingen (Theorems)

1. The conclusion of Matsumoto (1980) that only relative small molecular size saccharides have a cryoprotective effect on fish, is outdated.

This thesis, chapters 3-9.
Matsumoto, J.J. 1980. In: Chemical deterioration of proteins (edited by J. Whitaker and M. Fujimaki) ACS Symp. Nº 123, Pp 95-124. American Chemical Society, Washington, D.C.
Lanier, T.C. 1986. Food Technol. 3, 107-114.
Regenstein, J.M. 1986. Food Technol. 3, 101-106.

2. In comparison with most of the low molecular weight cryoprotectants used in fish, the advantages of the hydrocolloids tested are: effective at very low concentrations and no increase in calorie intake.

This thesis, chapter 9.

3. The characteristics of the TMAO-ase enzymatic system described in the fish muscle of the gadoid family, suggest the involvement of a redox reaction.

Shenouda, S.Y.K. 1980. Adv. Food Res. 26, 275-311.
Sikorski, Z. and Kostuch, S. 1982. Food Chem. 9, 213-222.

4. The healthy image of fish advertised by the fast food chains is contradicted by the high fat content of their products.

5. Dried mechanical deboned lean fish can be an ideal aid food product for the malnourished populations of the world.

Bligh, E.J. and Duclos-Rendell, R. 1986. J. Food Sci. 51(1), 76-78.

6. A widespread usage of fish minces seems to depend on the control and manipulation of their texture.

This thesis, chapter 8.
Connell, J.J. and Hardy, R. 1980. In: Trends in fish utilization (edited by J.J. Connell and R. Hardy) Pp 67. Fishing News Books Ltd. Farnham, Surrey, England.

7. There is a need for inexpensive methods determining quickly and accurately parameters of raw muscle related to the texture of cooked fish.

Stanley, D.W. 1983. In: Physical properties of foods (edited by M. Peleg and E.B. Bagley) Pp 157-206. AVI Publishing Company Inc. Westport, Connecticut.

8. The conclusions of Kim and Heldman (1985) about quantitative analysis of texture change in cod muscle during frozen storage are restricted to their own results.

Kim, Y.J. and Heldman, D.R. 1985. J. Food Process Eng. 7, 265-272.

9. Precooking of tuna by heating in a water system instead of steaming may reduce the occurrence of green discoloration in canned tuna.

10. It is worthwhile to study the possibility of fish farming as a complementary activity to the traditional fisheries in the Azores.

11. Better scientific and technological cooperation among Universities, Research Institutes and Industry could be achieved by a regular and enforced interchange of scientific staff.

12. Intensive gathering of public data may violate the privacy of the individual but can also contribute to decisive advances in science, very difficult to achieve otherwise.

13. Robots will leave to human beings the tasks that are intrinsically human, such as sports, entertainment, and scientific research.

Time magazine, December 8th, 1980.

D.J.B. da Ponte
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Wageningen, 2 september 1986

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To
my wife Orlanda,
and my daughter Cristina

List of abbreviations

C.D.L.	= cook drip loss
CMC	= carboxymethyl cellulose
C.S.	= compressive strength (apparent stress at failure)
DE	= degree of esterification
DMA	= *dimethylamine*
E.M.	= extractable myosin
FA	= formaldehyde
FFA	= free fatty acids
H.P.	= high methoxyl pectin
L.P.	= low methoxyl pectin
M.E.	= modulus of elasticity (calculated as a ratio of the C.S. to the corresponding Cauchy's strain at failure)
M.E. (l)	= modulus of elasticity (calculated as the slope of the initial straight line of the true stress - Cauchy's strain curves)
PVC	= polyvinyl chloride
R.	= resilience (calculate as ½ x C.S. x Cauchy's strain at failure)
SDS	= sodium dodecyl sulfate
S.S.	= shear stress (measured in the Kramer Shear Press and expressed as units of area of the texturegrams obtained)
TBARS	= thiobarbituric acid reactive substances
TC	= total carbonyls
TMA	= *trimethylamine*
TMAO	= trimethylamine oxide
W.H.C.C.	= water holding capacity of the cooked material
W.H.C.R.	= water holding capacity of the raw material

Contents

1. Introduction

In the last three decades, three major developments in world fisheries have affected fish industry and consumption of fish (Spinelli and Dassow, 1982). First, since the late 1940s total fishery landings have increased regularly (Fig. 1). The amount of fish avaiable as food also increased but as percentage of total landings, decreased from about 90% in 1948 to 70% in 1975 (Steinberg, 1980). Second, the expansion of world fisheries showed that we are increasingly dependent on nontraditional and formerly underutilized marine resources to maintain the upward curve of fishery landings. Stocks of some commercial species which had previously supported large catches collapsed to a level at which little can be caught (Lucas, 1980). Third, the establishment by a large and growing number of coastal states of exclusive economic zones (EEZ) has meant that nations with very large fishing fleets had to look for alternative fish resources (Sikorski and Naczk, 1981).

All these facts increased the need for searching for better ways of using the avaiable fish resources for human consumption. Many traditional methods of processing fish are innefficient. Lean fish species, such as cod, are commonly sold as fillets or fish sticks. The yield of edible flesh obtained by the best filleting methods is around 45% of the whole fish while the potentially edible flesh can be as high as 75 to 80% (Keay and Hardy, 1978). The unrecovered edible flesh (30-35%) is normally consigned to the fish meal factories as filleting waste. Small fishes are generally not fully used for human consumption because the fillet size from them is too small for commercial purposes. All these considerations have led, about 15 years ago, to extensive programs of research concerned with recovering as much as possible of the flesh from the skeleton by means of deboning machines. The minced fish obtained by these machines is a starting material for many fine fish and meat products. In occidental countries minced fish is formed into fish sticks, fish burgers, fish portions, seafood patties and products made by extrusion. In oriental countries, specially in Japan, minced fish is mainly used for production of surimi. This product is minced fish which has been washed with water and mixed with cryoprotective agents for increasing the stability during frozen storage. This intermediate product, surimi, is used for a variety of fabricated seafoods, such as Kamaboko, fish sausages and imitation crab legs. The current production of surimi in Japan is about one million tons per year (Grant, 1985). The process of washing the minces removes a good part of the water soluble proteins and enzymes, inorganic salts, blood, pigments and odorous constituents of the flesh. The

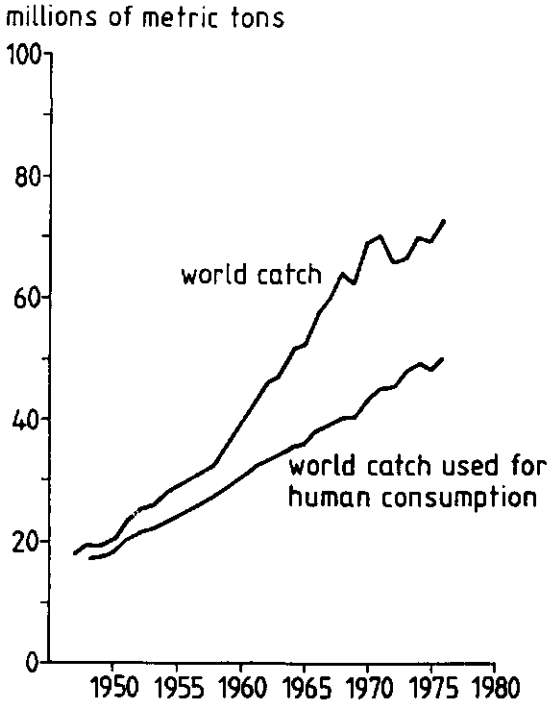

FIG. 1. The world catch (Steinberg, 1980).

addition of stabilizers such as sucrose, sorbitol at levels of about 8%, decreases the protein denaturation and confers a shelf life of up to 12 months at temperatures below -20°C (Lee, 1984; Grant, 1985). On the other hand, minced fish does not have a long frozen shelf life, and must be retailed and consumed within three months of production (Grant, 1985).

Although the production of surimi and its derivative products are well established, in occidental countries its production is very limited. The washing procedures cause losses of proteins and desirable fish flavours and the additions of cryoprotective agents, like sucrose and sorbitol at 8% level brings about changes in taste which lowers the acceptance by occidental consumers. The search for different cryoprotective agents which could decrease the rate of adverse changes of texture, water holding capacity and flavour of the fish minces during frozen storage, is therefore of primordial importance.

The purpose of the present work was to study the effect of different hydrocolloids on the stability of fish minces during frozen storage (chapters 3, 4, 5 and 8). We have also looked for different pretreatments (irradiation

and heating) which could stabilize the fish minces and their products (chapters 6, 7 and 8). The possible mechanisms of cryoprotection of the hydrocolloids were investigated and are discussed in chapters 9 and 10.

2. Review of literature

2.1. MINCED FISH: MECHANICAL DEBONING

Mechanically deboned fish flesh has been the subject of several international seminars and various excellent reviews (Martin, 1974; Martin, 1976a; Martin, 1976b; Keay, 1976; Martin, 1982; Froning, 1981; Expert Panel on Food Safety and Nutrition, 1979; Codex Alimentarius Commission, 1979, 1980 and 1983; Howgate, 1983).

Mechanically deboned fish flesh, or simply minced fish is the edible muscular flesh of fish which has previously been mechanically separated from the bones, skin and scales. There are two basic types of deboners. The first type is illustrated on Fig. 2. Gutted fish, frames, or trimmings enter the deboner at the crusher roll (point A, Fig.2). They are conveyed from B to C between

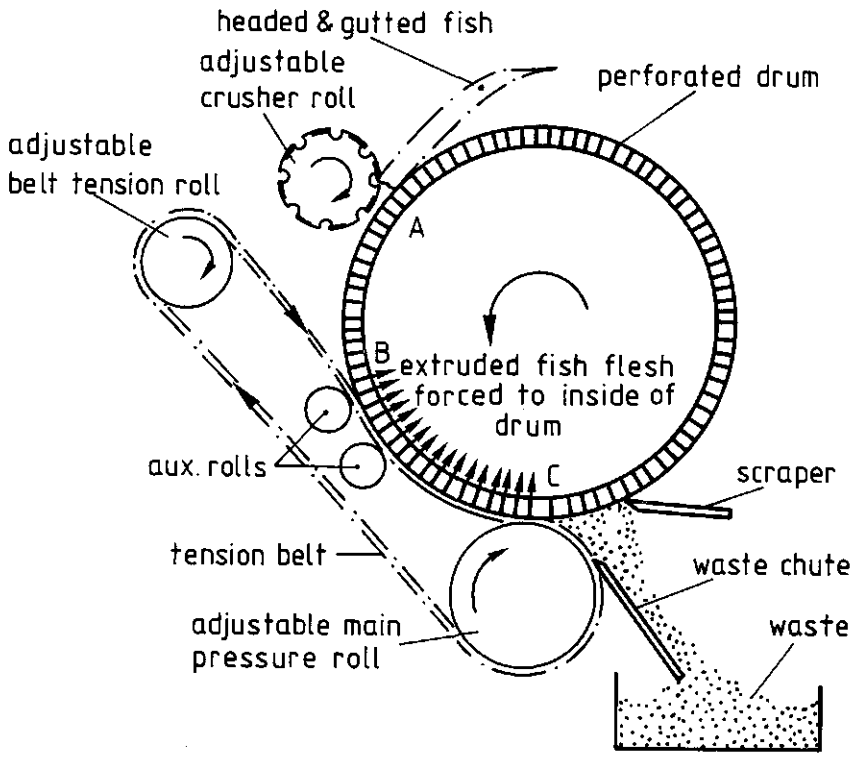

FIG. 2. Operation of a fish-flesh separator (schematic sectional view, Patashnik et al., 1973).

a rotating stainless steel perforated drum and a continuous rubber belt under tension. The belt pressure squeezes the flesh through the perforations of the drum while the skin and bones are scraped off into a waste chute. A slight difference in speed between the belt and the drum helps to shear the skin off of the muscle. The second type uses an auger to convey and push the incoming material against a perforated cylinder. The flesh passes through the holes of this cylinder and the bones, scales and skin which are too large or unable to find the right alignment to pass through the holes, leave the deboner through a separate opening (King, 1972).

The size of the perforations influences the quality of the minced fish. Larger perforations improve the flaky structure of the minces, but let more bone and scales through, and leave more flesh behind. With smaller diameters of perforations the separated flesh has a more uniform appearance, less bones, but a more pasty consistency. For most applications, round perforations between 3 mm and 5 mm in diameter have proven to give the best results.

The advantages of using the deboning machines are three-fold:

-- They process fish that are too small to fillet. FAO (1971) has estimated that an annual potential of 40 million tons of underutilized fish could be used for human consumption by means of mechanical deboners.
-- They permit the recovery of edible flesh from what would otherwise be waste from filleting operations.
-- They produce high yields of edible flesh. Yields of flesh ranging from 65 to 92% were reported on 19 different species of fish processed with flesh--separating machines (King and Carver, 1970).

Minced fish is however a product with entirely different characteristics than the fillet or the whole fish. The shearing action of the mechanical deboning process causes considerable cellular disruption of the fish tissues and an intimate mixing of the skin, blood and residual visceras with the fish flesh. During mincing, the flesh enters in contact with iron surfaces of the equipment which aggravates lipid oxidation during subsequent frozen storage (Lee and Toledo, 1977). Cellular disruption in fish during mechanical deboning accelerates the breakdown of trimethylamine oxide (TMAO) to trimethylamine (TMA) and to dimethylamine (DMA) and formaldehyde (FA) (Babbitt et al., 1972) and increases the concentration of hemoproteins liberated mainly from dark muscles and viscera tissues. Results of microbiological studies on mechanical deboning of either frames or headed and gutted un-filleted fish showed that this operation can increase the bacterial load by a factor of ten (Raccach

and Baker, 1978; Liston, 1980). The shelf life of fish minces at unfrozen temperatures is therefore very short and does not allow supportable commercial distribution, without any other sort of preservation of the fish minces. The frozen shelf life of the fish minces is also shorter than that of the whole fish or of the fillets. Changes in colour, flavour, texture and water holding capacity have been reported to be faster in mechanically deboned minced fish than in the whole fish or fillets, during frozen storage (Froning, 1981; Rodger et al., 1980; Keay and Hardy, 1978).

Mechanical deboning should not be considered an operation for rescue raw material of doubtful quality which was not acceptable for commercial distribution. Special attention should be given to the initial quality of the fish or trimmings for mechanical deboning. Traditional filleting of lean fishes leaves behind the skin, head, frame and trimmings. All this material can be passed through the deboning machines but in terms of yield and product quality only the frames and trimmings are worth processing (Connell and Hardy, 1980). If possible, dark muscles, blood, air bladder, and kidney tissue should be removed from the ordinary muscle, and the gutted fish or trimmings should be well washed before mincing on the bone separator (Fig. 3). A significant retardation of undesirable changes can be obtained by extensive washing of the fish minces. The loss of sarcoplasmic proteins and desirable fish flavours are serious disadvantages of this procedure (Connell, 1982). Babbitt (1986) reports that washing fish minces of pollock causes losses of about 25% of proteins, 46% of lipids and 70% of ash. It should also be pointed out that the operation of washing and the subsequent rinsing or dewatering increases substantially the costs of the final product (Regenstein, 1986).

The addition of substances which depress the freezing point of the tissue liquid, increase the viscosity and or react selectively with some functional groups of the proteins is recommended (Sikorski et al., 1976). Sodium chloride (1.0%), sucrose (4.0-10.0%), sorbitol (4.0%), soya protein (4.0%), milk protein (5.0%), monosodium glutamate (0.3%), polyphosphates (0.2%), sodium or potassium ascorbate (0.6%) and hydrocolloids (0.5%) such as pectins, alginates, carrageenans, carboxymethyl cellulose, Xanthan, Guar gum and Locust bean gum, have been reported to have a cryoprotective effect on the fish minces during frozen storage (Sikorski et al., 1976; Codex Alimentarius Commission, 1981 and 1983).

2.2. STRUCTURE OF FISH MUSCLE

The skeletal musculature of fish is divided into segments called myotomes

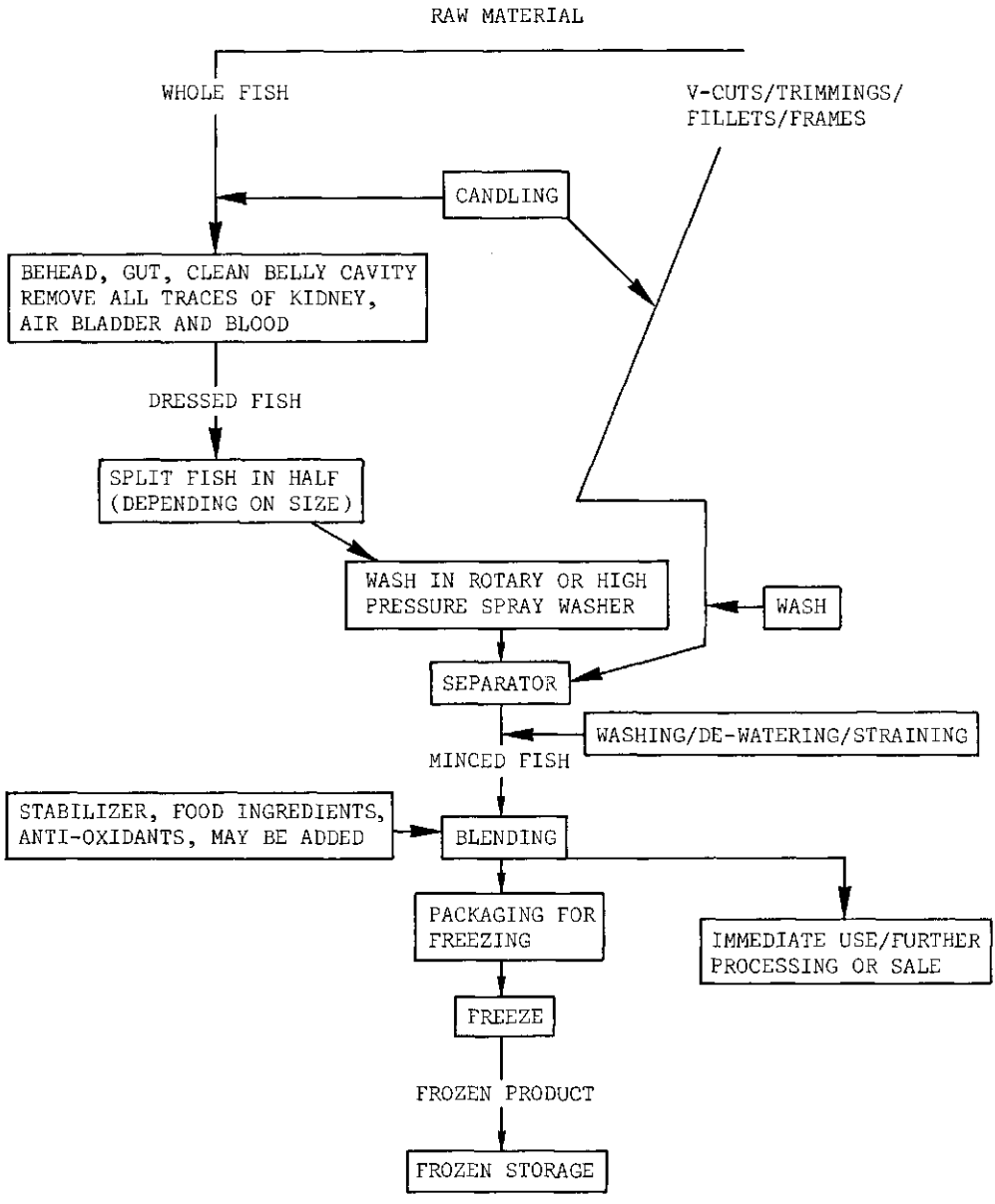

FIG. 3. Flow diagram for processing of minced fish (Codex Alimentarius Commission, 1980).

which are separated from one another by thin sheets of connective tissue, called myocommata (Fig. 4). Each myotome is composed of a large number of parallel muscle fibers, terminating at the boundary myocommata, and generally lying parallel to the main axis of the muscle systems (Dyer and Dingle, 1961). Muscle fibers are bound together by connective tissue (endomysium) and are constituted by many myofibrils which lie parallel and sarcoplasm which fills the space between. The structure of the myofibrils are presented in Fig. 5.

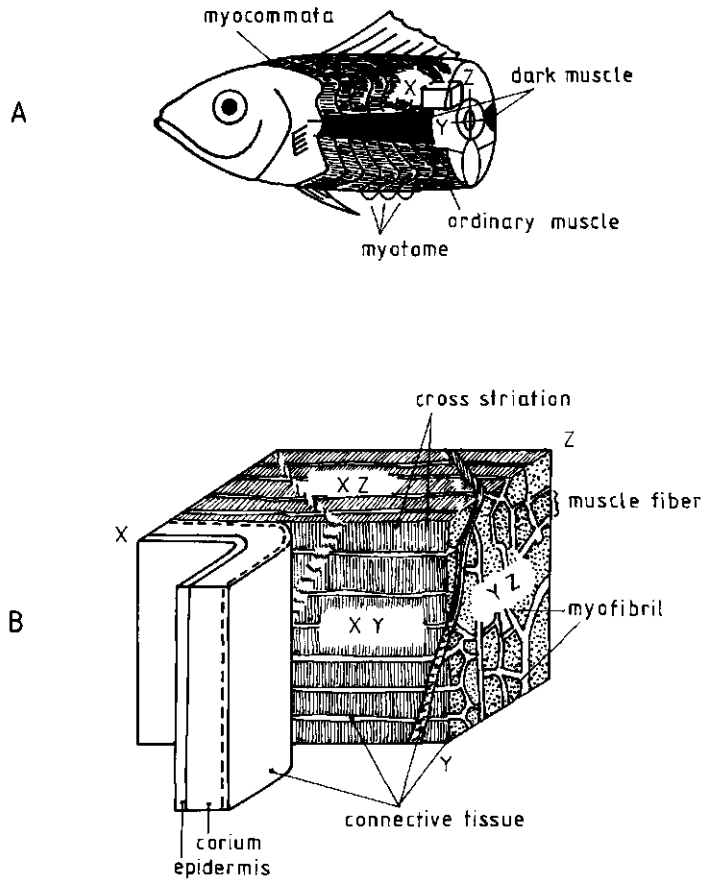

FIG. 4. Macroscopic and microscopic views of horse mackerel (Trachurus japonicus) muscle (Tanaka, 1958).
A: muscle fibers of skinned surface, B: microscopic view of the part of XYZ in Fig. A.

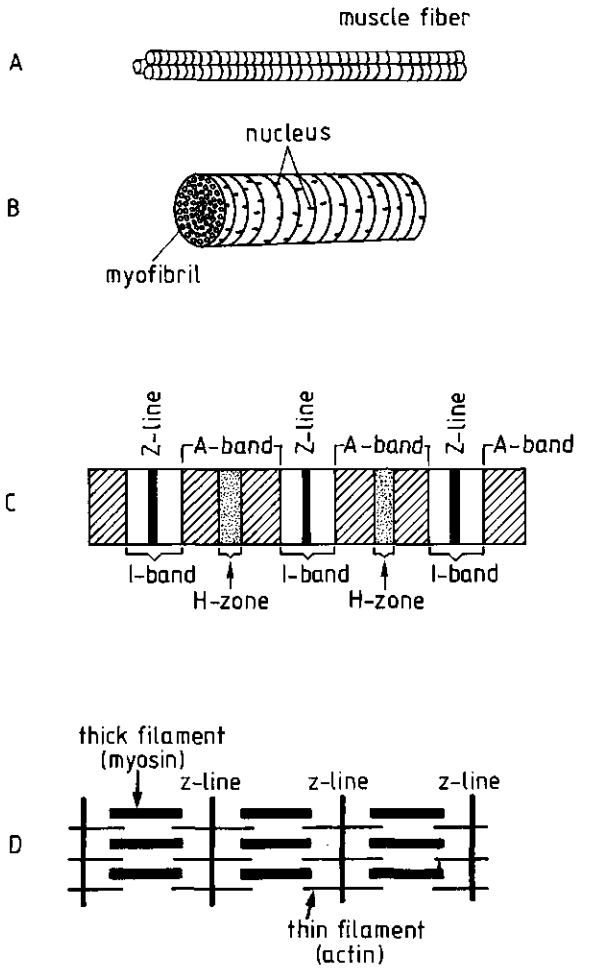

FIG. 5. Structure of striated muscle (Suzuki, 1981).
A: muscle fibers, B: many myofibrils are bundled to make a muscle fiber,
C and D: structure of myofibril.

2.3. COMPOSITION OF FISH

In crude terms, fish is about 66-84% water, 15-24% proteins, 0.1-22% lipids, 1-3% carbohydrates and 0.8 to 2% inorganic substances. Composition of fish is dependent upon species, age, size, maturity, season, feeding conditions, localization of catch and water temperatures. In the same species and during the various seasons of the year, there is normally a negative correlation

between fat and water content (Suzuki, 1981). During the season in which fat content increases, the water content becomes lower; and during the season the fat content lowers the water content rises (Fig.6).

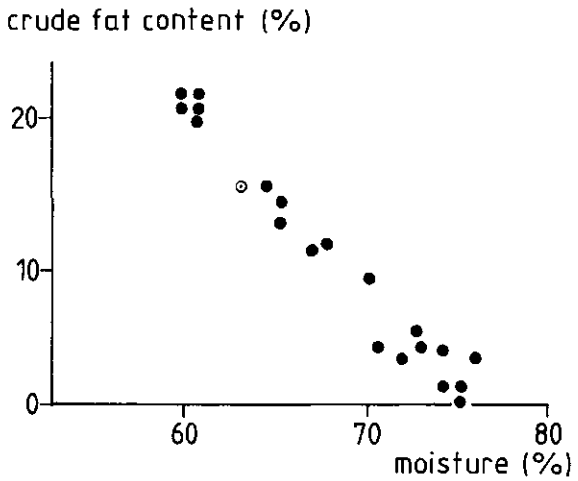

FIG. 6. Relation of crude fat content and water content in sardine (sardinops melanosticta) whole bodies (Sato et al., 1978).

Fish muscle can be divided into white muscle and dark muscle. Dark muscle lies along the side of the body under skin (Fig. 7). In lean fishes (fat content inferior to 4%), such as cod and whiting, the dark fraction can be as low as 1 to 2% of the body weight while in fatty fishes (fat content higher than 4%) it can go over 10%. Dark muscle is very important to the physiology and biochemistry of the fish. A high concentration of lipids, connective tissue, myoglobin, TMAO and enzymes such as succinic dehydrogenase, lecithinase, and respiratory enzymes are found in the dark muscle of the fish (Buttkus and Tomlinson, 1966; Love, 1970). These compounds represent important processing problems because they give rise to postmorten spoilage, such as production of free fatty acids, TMA, DMA, FA and oxidation reactions which severely affect the quality of fish.

The proteins of fish can be divided into 3 major categories based on solubility. Structural proteins or myofibrillar proteins, which are soluble in cold neutral salt solutions of fairly high ionic strength (0.5 μ) and amount to 70-80% of total fish proteins. Sarcoplasmic proteins, which are soluble in water

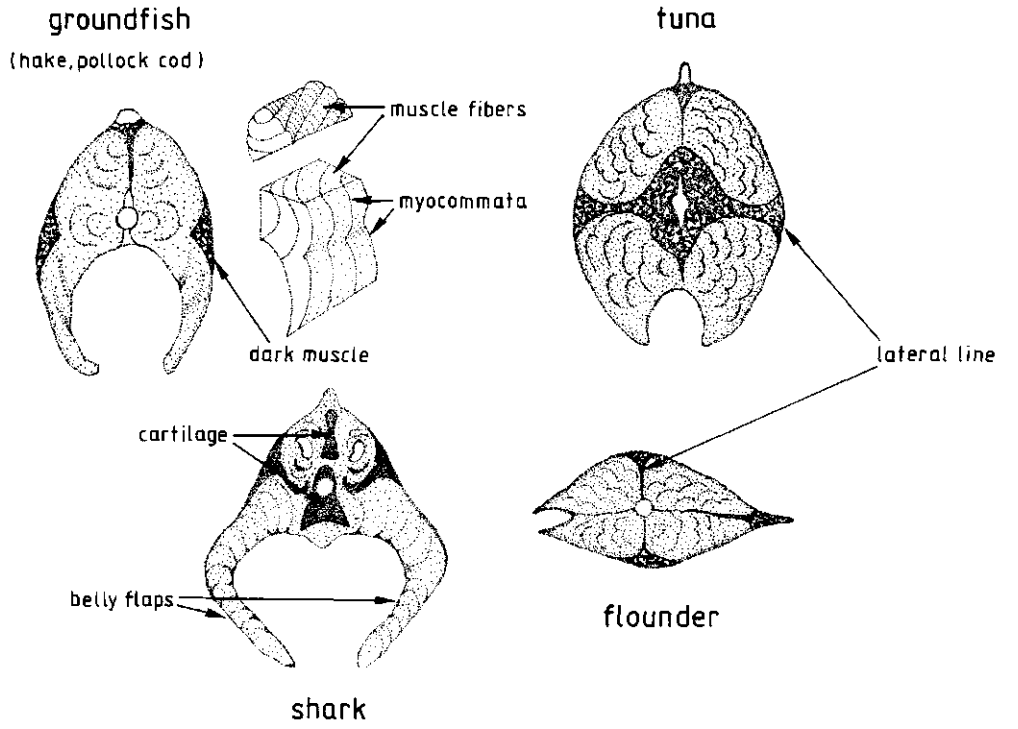

FIG. 7. Transverse sections of various fish species (Spinelli and Dassow, 1982).

and dilute buffers and comprise 20-30% of the total fish proteins. Finally 2-5% of connective tissue, which can not be extracted in acid or alkaline solutions and neutral salt solutions of 0.01-0.1 M concentration (Suzuki, 1981).

Myofibrillar proteins are basically composed of myosin, actin and regulating proteins such as tropomyosin, troponin an actinin. Fish myosin is less resistant to denaturing agents such as urea or guanidine.HCl but is more susceptible to heat denaturation and tripsin digestion than rabbit myosin (Connell, 1960, 1961 and 1968). Actin, tropomyosin and troponin of the fishes seem to have similar structure and properties to the mammalian counterparts.

Sarcoplasmic proteins contain many kinds of water soluble proteins called myogen. They are largely enzymes in solution and although they are heat sensitive and precipitated on cooking they do not appear to have much involvement with textural characteristics of the fish. They seem to be only slightly affected on frozen storage (Mackie, 1984).

The stroma proteins include collagen, elastin and connectin (Maruyama et al., 1975). The approximate protein compositions of various animal muscles are reported in Table 1 (Suzuki, 1981). In comparison with mammalian meat,

TABLE 1 Comparison of protein composition of different animal meats (Suzuki, 1981).

	Sarcoplasmic proteins	Myofibrillar proteins	Stroma
	(% of total proteins)		
Cod	21	76	3
Carp	23-25	70-72	5
Flat fish	18-24	73-79	3
Rabbit	16-28	39-68	16-28
Beef	16-28	39-68	16-28

fish has much less connective tissue.

Fish lipids present a very high degree of unsaturation and a relatively low cholestrol content (Krzynowek, 1985). Fish lipids are an unique source of n-3 polyunsaturated fatty acids (Kinsella, 1986).

Non-protein nitrogeneous constituents (components in the fish tissue which are not precipitable by 5% trichloroacetic acid) comprise 0.5-1.0% of the total weight of the fish muscle. The major components are amino acids, carnosine, anserine, guanidine compounds, TMAO, urea, betaines and nucleotides (Konosu et al., 1974). Many of these compounds have been related to the flavour of fish (Jones, 1963). TMAO can be degraded to TMA, DMA, and FA and the content of these products has been used as spoilage index.

2.4. DETERIORATION OF FISH DURING FROZEN STORAGE

Freezing is one of the best methods of fish preservation and has been applied to an increasing extent both on shore and on board of fishing vessels. As percentage of total fish catch used for human consumption, frozen fish represented about 6% in 1948 and was already about 26% in 1976 (Steinberg, 1980). Although the enormous sucess of freezing as a mean of preservation of fish, deterioration in the quality of fish after frozen storage was already recognized. The shelf life of fatty fishes is generally limited by oxidation

of lipids and pigments which causes rancid odours and discolouration of the flesh. In lean fishes, deterioration of texture, water holding capacity and losses of desirable fish flavours represent serious problems of great commercial importance (Sikorski et al., 1976).

The effect of various freezing conditions on the quality of fish has received considerable attention and can be summarized as follows (Shenouda, 1980):

1. Rapid freezing of the catch without unnecessary delay - that is, freezing at sea is best.
2. Superior quality is produced by fast freezing rate.
3. The advantages and disadvantages of different freezing methods (shelf freezer, blast freezer, refrigerated seawater, fluidized bed and direct contact with freezing media).
4. The deterioration in quality due to fluctuations in frozen storage temperature and relative humidity.
5. The effectiveness of prefreezing treatments for enzyme inactivation or the addition of antioxidants or cryoprotective agents.
6. The importance of packaging as related to moisture vapor and oxygen permeability during frozen storage.
7. Low frozen storage temperature.

Unfortunately, fish deterioration during frozen storage is inevitable, specially after long periods. Changes in fish texture (extra firmness, toughness, springiness, sponginess, stringiness, dryness, rubbery texture, lack of succulence, loss of water holding capacity or loss of juiciness) are considered to be due to protein denaturation during frozen storage, particularly the myofibrillar proteins (Dyer, 1951). Deterioration in flavour (such as off-odor, rancidity, bitterness, or an undesirable fishy taste) is believed to be due to the formation of low molecular weight compounds from lipid oxidation or protein degradation (Shenouda, 1980). Undesirable changes in color and appearance (such as loss of brightness of the coloured tissues, loss of surface glossiness, development of freezer burns and surface dehydration, drip, or muscle opacity or chalky appearance) are thought to be due to irreversible changes that occur in muscle proteins or protein-bound pigments, or to changes in certain pigmented proteins - for example, myoglobin and oxymyoglobin into metmyoglobin, such as is found in frozen tuna meat (Matsumoto and Matsuda, 1967; Shenouda, 1980).

Fish deterioration during frozen storage has been the subject of several

detailed reviews (Dyer, 1951; Dyer and Dingle, 1961; Connell, 1964; Connell, 1968; Fennema and Powrie, 1964; Love, 1966; Powrie, 1973; Sikorski et al., 1976; Banks et al., 1977; Matsumoto, 1979; Matsumoto, 1980; Shenouda, 1980; Suzuki, 1981; Mackie, 1984). Many factors have been reported to cause protein denaturation which has been associated with the fish deterioration during frozen storage. These factors can be divided into three major categories (Shenouda, 1980):

--Factors related to change in fish moisture.

--Factors related to changes in fish lipids.

--Factors related to the conversion of TMAO to DMA and FA.

Factors related to change in fish moisture

For a long time it has been recognized that formation of large ice crystals during freezing and frozen storage has a detrimental effect on the fish tissues. The size and distribution of the ice crystals depends upon the condition of the fish muscle and the rate of freezing (Love, 1968). In pre-rigor fish, the cell fluid is tightly bound to the intracellular proteins, which limit its diffusibility from inside to outside the cell. When pre-rigor fish is frozen, crystallization of water takes place mainly intracellularly regardless the rate of freezing. In post-rigor fish, some of the cellular fluids are free to diffuse into the extracellular spacing, and when the fish is frozen inter and intracellular ice crystals are formed, the proportion depending on the rate of freezing. At a slow freezing rate, the exterior of the cells are believed to cool more rapidly than the interior parts. With the formation of ice crystals in the outside of the cell a high salt concentration in the extracellular fluid will draw more water from inside of the cells by osmosis. Slow rates of freezing induce formation of large extracellular ice crystals (Fennema, 1973; Love, 1968). On the other hand, rapid rates of freezing probably do not allow migration of water into the extracellular spaces and consequently smaller ice crystals, usually spear-like and separated by proteins, are formed in the cells (Love, 1968).

Temperature fluctuations in frozen storage have also a detrimental effect on the quality of fish. Even if a constant temperature is maintained during all period of frozen storage, there is a tendency of the large ice crystals to growth in size at the expenses of the smaller ones (Kent, 1975). Fluctuations of temperature accelerate this process of recrystallization and accretion of the ice crystals.

When water is converted to ice, there is an increase of about 9% in volume.

Therefore freezing causes a continuous pressure of ice crystals in the ultrastructure of cells which may induce disrupture of membranes and reorientation of microorganelles (Shenouda, 1980; Fennema, 1985). During freezing, water is converted into ice crystals increasing the concentration of all the other nonaqueous constituents in the unfrozen water. The net effect is similar to conventional dehydration, except that the temperature is lower and the separated water is deposited locally in the form of ice.

The conformation of most water-soluble proteins follows a general pattern in which hydrophobic amino acids tend to locate toward the inside the molecule, and polar amino acids are mainly at the surface with a rather uniform distribution. However some hydrophobic groups still remain exposed at the molecular surface or in crevices. It has been postulated (Nemethy, 1968; Lewin, 1974) that water molecules arrange themselves around those exposed hydrophobic group side chains so as to form a highly organized water barrier. The stability of the three dimensional structure of protein molecules is also highly dependent on a network of hydrogen bonds, many of which are mediated through water molecules. Thus freezing will disrupt the hydrogen bonding system as well as expose hydrophobic and hydrophilic regions of protein molecules to new enviroments. New hydrophobic-hydrophobic and hydrophilic-hydrophilic interactions could then take place, either within the same protein molecule, causing deconformation of the three-dimensional structure; or between adjacent protein molecules, inducing protein protein interaction and consequently aggregation.

Another important aspect of freezing is the increase of the salt concentration in the remaining unfrozen water. At commercial temperatures of frozen storage, more than 90% of the water is frozen which would give roughly an increase of tenfold of the salt concentration in the remaining liquid water, if the euthetic point is not reached and no precipitation occurres. Salt concentration is known to affect cell permeability and protein properties. At moderate ionic strength (0.5 to 1.0 μ) many salts exhibit a solubilizing effect by associating with ionic linkages of the proteins. At higher concentration however, the competition of inorganic salts for water may result in a salting out effect by reducing the number of hydrophilic protein groups which can associate with water molecules. The high concentration of salts in the unfrozen water can affect the secondary forces (ionic, van der Waals, hydrogen, hydrophilic and hydrophobic) which contribute to the stabilization of the tertiary and quaternary configuration of the proteins (Shenouda, 1980). The stability of electrostatic binding, as well as all the other secondary

forces, is dependent on the dielectric constant, the pH, and the ionic strength of the medium. Increasing the concentration of the salt ions will presumably cause disturbance and competition with the existing bonds and probably the breakdown of some of them.

The effect of salts on the protein denaturation is dependent not only on the concentration of the salts but also on the type of salts and proteins involved.

The increase in salt concentration may also be involved in protein denaturation by enhancing the oxidation and hydrolysis of fish lipids.

Factors related to changes in fish lipids

At commercial temperatures of frozen storage there is normally an increased concentration of free fatty acids (FFA) due mainly to enzymic hydrolysis of phospholipids and triglycerides (Shewfelt, 1981). FFA have been found to reduce the extractability of myofibrillar proteins, specially actomyosin and myosin in model studies and in muscle homogenates (King et al., 1962; Anderson et al., 1963; Anderson and Ravesi, 1968; Poulter and Lawrie, 1979; Ohshima et al., 1984). It has also been demonstrated that the rate of interaction between FFA and myofibrillar proteins is depended on the type and degree of unsaturation of the FFA, their concentrations and the storage time. Polyunsaturated fatty acids insolubilize more fish myofibrillar proteins than do the less unsaturated ones, and shorter fatty acids are more reactive than the high molecular weight FFA.

The mechanism of interaction of the myofibrillar proteins with the FFA has not yet been completely understood. FFA, which can also be considered as surfactants, may attach themselves to hydrophobic, polar or ionized fragments of the protein chain, creating consequently more hydrophobic bonds in place of polar or charged groups and surround the protein with a more hydrophobic enviroment (Sikorski et al., 1976). The low sensitivity of FFA - - protein interactions to salt solution (Anderson and Ravesi, 1970) and their sensitivity to sodium dodecylsulfate (Connell, 1965) suggests involvement of hydrophobic forces in the formation of the aggregates. The dependence on pH (Hanson and Olley, 1965) may indicate the participation of ionic and hydrogen bonding.

Neutral lipid droplets may dissolve FFA and in that way they can produce a dilution effect on the reactivity of FFA towards the myofibrillar proteins (Sikorski et al., 1976; Shenouda, 1980). Species of fish having higher lipid con-

tents require more FFA to produce the same level of inextractability (Anderson and Steinberg, 1964). On the other hand, lipids and proteins which have been removed from their natural compartments in the cell, by the pressure exerted by ice crystals, may produce new lipid-protein complexes different from the native ones, bringing about changes in the fish muscle (Shenouda and Pigott, 1974 and 1975).

The highly unsaturated nature of fish lipids renders them peculiarly susceptible to oxidation (Olcott, 1961). Oxidized lipids in lipid-protein systems are known to induce polymerization and aggregation of the proteins, resulting in decrease of solubility and formation of colored complexes (Karel, 1973; Karel, 1975; Khayat and Schwall, 1983). The mechanism of the reaction between oxidized lipids and proteins is believed to be through unstable free radical intermediates of lipid peroxidation or through stable oxidation products such as carbonyl compounds (Shenouda, 1980). The production of damaging free radicals by lipid oxidation is accelerated by hemoglobin and by certain metal ions (Castell and Spears, 1968; Castell and Bishop, 1969). Lipid oxidation in fish muscle has been generally ascribed to a nonenzymic process. Recent reports however have claimed that an enzyme-catalysed lipid oxidation system exist on the membrane fraction of fish skeletal muscle (Hultin et al., 1982; Mc Donald et al., 1979; German and Kinsella, 1985).

In fatty fishes, formation of rancid odours is an important problem which greatly affects the shelf life on frozen storage. Glazing and addition of antioxidants are the more common methods for preventing lipid oxidation during frozen storage.

Factors related to the conversion of TMAO to DMA and FA

TMAO is a characteristic compound of many marine animals, including fish. In certain fishes, specially in the gadoid ones, formation of FA and DMA, during frozen storage, has been ascribed to an enzymatic reduction of TMAO (Amano and Yamada, 1964a and 1964b and 1965; Yamada and Amano, 1965a and 1965b; Castell et al., 1970; Castell et al., 1971). This enzyme or enzyme system has been only partly isolated (Lundstrom et al., 1982; Parkin and Hultin, 1981; Gill and Paulson, 1982). Its activity depends upon species, type of tissue, and storage temperatures. The activity of TMAO-ase has been found to be higher in viscera tissues (kidney, liver, bile bladder and piloric caeca) and in dark muscles. Processing operations which cause disruption of these tissues, are bound to increase the TMAO-ase activity. Production of FA and DMA in minced

fish is normally higher than in the corresponding intact fillets or the whole fish, during frozen storage (Mackie and Thomson, 1974; Babbitt et al., 1972). In fish muscle, TMAO-ase has a maximum of activity at temperatures around -10°C and is still active at temperatures down to about -29°C (Tokunaga, 1974; Castell et al., 1973). This enzyme appears to be relatively heat resistant, however conflicting results about its thermal inactivation are found in the literature (Tokunaga, 1964; Castell et al., 1971; Lall et al., 1975; Svensson, 1980). Several compounds such as methylene blue, ascorbic acid, cysteine, ferrous chloride, and blood increase the activity of TMAO-ase. On the other hand, oxidizing agents such as hydrogen peroxide, potassium bromate and cyanide seem to inhibit the activity of this enzyme (Racicot et al., 1984; Banda and Hultin, 1983). Packaging in oxygen permeable materials decreases the production of FA and DMA while anaerobic conditions seem to enhance this production (Reece, 1983; Lundstrom et al., 1982).

Harada (1975) proposed the participation of both carboxylic and amino groups in the protein moiety of the enzyme for the rearrangement of TMAO to form the intermediate compound dimethylaminomethylol, which then yields DMA and FA.

While most of the literature speaks about an enzymic reaction for the formation of DMA and FA, there is some evidence that non-enzymic mechanisms can also take place (Tarr, 1958; Spinelli and Koury, 1979 and 1981). Spinelli and Koury (1979) have shown that DMA is produced in very significant quantities in both drum- and freeze-dried fish, and that its formation was neither species related nor enzymatically induced. They proposed a mechanism based on the ability of TMAO to act as an electron receptor when a change in the redox potential of the system occurred. Freezing the fish was cited as a way of enhancing the reaction by concentrating the reactants.

Although the pathways of TMAO reduction to DMA and FA in frozen fish muscle are still not completely understood, there is clear evidence indicating that DMA and FA formation during frozen storage is mainly the result of an enzymic decomposition of TMAO.

FA is a very reactive compound capable of interacting with many functional groups of proteins inducing intra and inter molecular cross links of the molecules. Various reaction sites in protein molecules, sensitive to FA attack were identified (Walker, 1964; French and Edsall, 1945); these included amino, amido, guanido, thiol, phenolic, imidazole and indolyl residues. A typical reaction leading to a methylene bridge is as follows:

$$R\text{-}H + CH_2O \rightarrow R\text{-}CH_2(OH)$$

$$R\text{-}CH_2(OH) + H\text{-}R' \rightarrow R\text{-}CH_2\text{-}R' + H_2O$$

Formation of intermolecular covalent cross-linking of proteins with the methylene group derived from FA has been challenged by Connell (1965 and 1975). He observed that cod muscle stored under conditions that result in considering toughening will dissolve completely in solutions capable of breaking hydrogen bonds and other secondary forces of interaction. The amounts of inextractable proteins in 1% sodium dodecyl sulfate (SDS) were about 0.1 to 0.3% and the average of molecular weight of the dissolved protein was not experimentally different from that of the proteins of unstored muscle similarly dissolved. Connell (1965 and 1975) concluded that no or at most few intermolecular covalent crosslinks might be formed by the action of FA. He pointed out that it was conceivable that during frozen storage some of the smaller polypeptide subunits of the constituent proteins may become covalently crosslinked. The formation of such small quantities of polymeric material which still remains soluble in SDS could go undetected in the bulk of the noncovalently crosslinked polypeptides. The fact that less than the theoretical quantity of FA is recovered from frozen stored fish muscle (Tokunaga, 1964; Castell and Smith, 1973) shows that some irreversible binding to protein (or free amino acids and small peptides) occurs.

Whatever is the nature of the aggregation or denaturation of the fish proteins during frozen storage, there is considerable evidence that production of FA increases fish deterioration. A close correlation between the stability of gadoid species and the rate of production of FA and DMA, during frozen storage, has been reported (Castell et al., 1973; Tokunaga, 1964; Sikorski and Kostuch, 1982). When extracts or parts of visceral organs, such as kidney, liver and piloric caeca, are added to minced fish there is a marked increase in toughening accompanied by a corresponding increase of FA and DMA. Similarly, when minced fish of unstable gadoid species is added to a stable non-gadoid fish such as plaice in a ratio of 1:4, the rate of deterioration of the mixture is equal to that of the gadoid on its own (Dingle et al., 1977).

Concurrent action of the various factors

All the factors, mentioned before, associated with deterioration of fish during frozen storage, were treated separately. However they work as a whole

and interactions among them, causing synergistic or inhibition effects can be expected. An integrated scheme of the various factors and their interactions in the fish muscle during frozen storage is presented on Fig. 8 (Shenouda, 1980).

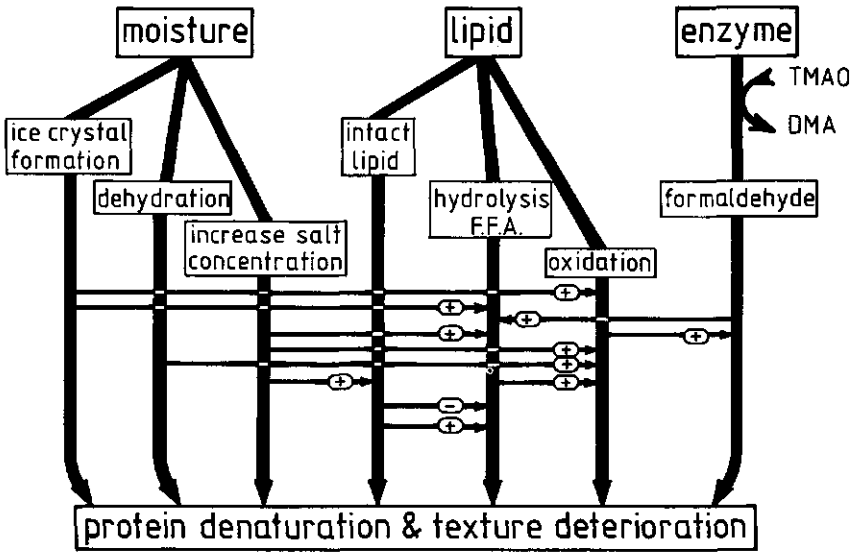

FIG. 8. Various factors that affect directly (vertical pathways) or indirectly (horizontal arrows indicating positive or negative effect on each other) fish protein denaturation during frozen storage (Shenouda, 1980).

The nature of aggregation and cross-linking of fish proteins

While the nature of the changes which take place on frozen storage remain unresolved, it is generally felt that protein aggregation is due mainly to secondary forces (hydrogen bonds, hydrophobic bonds and bonds resulting from van der Waals dispersion forces) (Connell, 1968; Mackie, 1984). It seems obvious that conformational changes as understood to occur in denaturation, play an important part in the overall reaction but the reasons why they occur, their exact nature and their consequences still remain to be elucidated (Connell, 1982).

The arguments against the formation of intermolecular covalent bonds, such as methylene or disulfide bridges are coming from the fact that deteriorated myofibrillar proteins of cod could be solubilized in 1% SDS and the average of molecular weight of the dissolved proteins was not experimentally different from that of the proteins of unstored muscle similarly dissolved

(Connell, 1965). Moreover, during frozen storage of cod muscle, no significant changes were found in the number of free SH-groups of actomyosin (Connell, 1960) and in the number of the titrable acidic and basic groups of myofibrils (Connell and Howgate, 1964).

In contrast with this, Buttkus (1970, 1971 and 1974) was able to observe the side-to-side aggregates of myosin of trout and rabbit, denatured during frozen storage, under electron microscope. He was also unable to detect any significant change in the number of free SH groups before and after frozen storage but he observed that the aggregated myosin formed on frozen storage could be resolubilized by using a solution containing 6M guanidine.HCl and either 0.5M mercaptoethanol, 0.3M sodium sulfite or 0.3M sodium cyanide. From these results, he has attributed the cross-linkages involved in aggregation of myosin during frozen storage to disulfide bonds, hydrophobic bonds and possibly hydrogen bonds. Buttkus (1970 and 1971) postulated a rearrangement of disulfide bonds from intramolecular to intermolecular ones through a sulfhydryl/disulfide interchange reaction, leaving free SH groups basically unchanged.

Recent work of Matsumoto (1980) has demonstrated that carp actomyosin in 0.6M potassium chloride solution decreased as expected with increasing storage time at -20°C, but that in an extractant medium of 0.6M KCl with 8M urea (for testing hydrogen bonds and hydrophobic bonds) and with 0.5M mercaptoethanol (for testing disulfide bonds) it remained almost unchanged. These results led to the conclusion that denaturation and/or insolubilization of actomyosin and myosin during frozen storage is a result of aggregation caused by progressive increase in intermolecular cross-linkages due to the formation of hydrogen bonds, ionic bonds, hydrophobic bonds and disulfide bonds.

Tests used for detection of deterioration in fish during frozen storage

There is a wide variety of methods that have been applied to measure quality changes of fish during frozen storage. Basically we can divide them in subjective tests (sensory tests) and objective tests (instrumental tests).

Changes in appearance, flavour, odour and texture can be assessed by panelists in various ways. A review of sensory methods applicable to fish and fishery products is given by Larmond (1969). Sensory tests still remain today the more preeminent method for detecting relevant changes of fish during frozen storage. Many of the instrumental tests are judged by their performance against sensory analysis. However, sensory methods do not always

differentiate clearly between the changes occurring in iced fish and those occurring in frozen fish. The sensory perception of deterioration during the course of frozen storage can be greatly influenced by the manner of preparation and cooking (Baines et al., 1967; Love, 1966). Moreover although an individual's preferences may be trained to a specific standard, that standard reflects the preference of only one country, or even one part of a country. On the other hand, instrumental tests measure mainly changes of parameters which can or can not be correlated with acceptability of the fish.

In Table 2, from Shenouda (1980), are shown various instrumental and chemical tests used in research and in control of fish during frozen storage. Some comments about the more important instrumental tests are presented next:

-- Protein extractability: Although this method has proven useful, there are several drawbacks to its application. It has not been standardized, it is laborious, the results show a high degree of scatter, and the amount of extractable protein present in fish varies with season, ratio of muscle to extractant, type of homogeniser, type of salts, ionic strength and time of extraction (Mills, 1975; Mackie, 1984; Ironside and Love, 1958; Cowie and Mackie, 1968). Cowie and Little (1966) investigated the relationship between toughness of cod stored at -29°C and its muscle protein extractability and found no correlation between toughness and extractability. Protein denaturation, as measured by decreased extractability into dilute salt solutions, and the complex textural changes brought about during freezing are considered to be separate phenomena which, although they have some interrelation do not always proceed at the same rate (Gould and Peters, 1971).

-- Texture and water holding capacity: In addition to sensory evaluation, which is still the major determination of quality, instrumental textural measurements are now recognized as powerful tools in studying the changes in rheological properties of tissues during frozen storage. The relationship between various instrumental parameters of texture, such as compressibility, deformation, tensile rupture and shear values, and the sensory attributes of texture has been established. As a result, textural changes in tissues during frozen storage can be quantitatively monitored with great accuracy by objective methods. It appears however that because texture includes so many parameters, the majority of which change during frozen storage, it is unlikely that any single instrument will be suitable for measuring deterioration of fish during frozen storage (Mills, 1975).

Measurements of "free drip" (liquid that exudes from frozen fish tissue on thawing without application of force), "press drip" or "centrifuge drip"

TABLE 2 Parameters used to detect or monitor changes or deterioration in fish proteins during frozen storage (Shenouda, 1980).

1. Extractability of fish proteins
   - Total extractable proteins
   - Protein groups: myofibrillar, sarcoplasmic, actomyosin
   - Protein species: myosin, actin, tropomyosin, etc.
2. Protein solubility in
   - Aqueous buffers
   - Detergents
   - Proteolytic enzymes
3. Tissues and texture
   - Drip-thaw
   - Water-holding properties
   - Objective textural measurements: shear, deformation, tensile, and compressibility forces
4. Ultrastructure features
   - Light microscopy , scanning electron microscopy, transmission electron microscopy
5. Extracted proteins
   - Viscosity, molecular weight, specific volume
   - Functional groups: available lysine, reactable SH
   - Spectrometric analysis: UV,ORD, NMR, IR, X-ray patterns
   - Mobility and fractionation under external forces:
     - Ultracentrifuge sedimentation pattern
     - Electrophoretic pattern
     - Isoelectric focusing pattern
     - Chromatographic separation (ion exchange, molecular sieves, adsorption systems)
6. Enzymatic activity
   - ATPase, aldolase, TMAOase, malic enzyme, glycerophosphate dehydrogenase
7. Formation of low-molecular-weight degradation products
   - Lipid hydrolysis: FFA
   - Lipid oxidation: ketones, aldehydes, peroxides, free radicals, TBA
   - TMAO hydrolysis: FA, DMA

(liquid obtained when an external force is applied to the tissue of fish) and "cook drip" (liquid released when the fish tissue is heated or cooked) have been used for measuring deterioration of fish during frozen storage.

These tests reflect the decrease in the capacity of fish muscle to reabsorb the water of melted ice crystals during thawing.

-- Increase in DMA, FA, FFA and products from lipid oxidation: As was appointed out before, FA, FFA and products of lipid oxidation have been correlated with deterioration of fish during frozen storage.

In gadoid fishes, certainly DMA or FA is an important index of spoilage (Castell et al., 1970 and 1971).

In fatty fishes, lipid oxidation can be monitored by determination of thiobarbituric acid reactive substances, peroxide value or carbonyl compounds. The problem with these tests for lipid oxidation is that when rancidity becomes significantly measurable, the product can no longer be considered to have an acceptable degree of freshness (Gould and Peters, 1971). In lean fishes, lipid oxidation is extremely slow and occurs primarily in the phospholipid fraction. Nevertheless, sufficient oxidation takes place during frozen storage which causes a reduction on the acceptability of the fish due to the production of off-flavours, mainly unsaturated carbonyls (Hardy et al., 1979).

Formation of FFA in fish during frozen storage have been extensively examined and can also be used for monitoring deterioration during frozen storage (Jonas, 1969; Wierzchowski, 1969; Hanson and Olley, 1964; Olley et al., 1962).

2.5. CRYOPROTECTIVE AGENTS

The search for compounds (cryoprotective agents) which can increase the rate of survival of living cells and tissues or can minimize deterioration of food during frozen storage, is an important common aim both for cryobiology and food technology. Preservation in frozen state of whole blood, blood components, spermatozoa, bone narrow cells, human skin, human cornea are just a few examples of the huge amount of research and accumulated knowledge, developed in this area, with vital importance to medecine and biology in general. The mechanisms of freeze injury and cryoprotection of living cells are not exactly the same as the ones which cause deterioration and cryoprotection of food during frozen storage. However similarities can be found and it is worthwhile to briefly review the different theories of cryoprotection in cryobiology and then compare with the current knowledge of preservation of food by freezing.

For a long time, cryoprotective agents such as glycerol, dimethyl sulphoxide, sucrose, polyvinylpyrrolidone, hydroxyethyl starch and dextrans have been used in the freezing preservation of living cells (e.g. human erytrocytes, human bone narrow, human leukocytes, human renal cells and human cornea; Karow, 1969; Meryman, 1971). Theories regarding the mechanism of action of the cryoprotective compounds emphasize their role as modifiers of water structure and their interference with growth of ice crystals. The importance of hydrogen binding groups in cryoprotective compounds has been pointed out by several researchers (Doebbler, 1966; Karow, 1969; Meryman, 1971 and 1974). Nash (1962) has observed that cryoprotection seemed to be related to hydrogen bond acceptor capacity (i.e. the presence of lone-pair electrons on the cryoprotectants rather than electron deficient sites). Cryoprotectants by virtue of their ability to create hydrogen bonds with water, may stabilize the hydration lattice surrounding proteins and thereby reduce the probability of protein denaturation during freezing and frozen storage. Another possible way of protection would be the attachement by hydrogen bonds of the cryoprotective compounds to the hydrophilic sites of proteins, and presumably serve to maintain protein conformation and prevent denaturation by forming a protective coat around protein molecules (Santarius, 1968; Tsutsayeva et al., 1978).

In cryobiology, the cryoprotective agents are traditionally divided into two groups: those which penetrate cells and are used at multimolar concentrations and those which do not penetrate cells and are used in quite low molar concentrations (Meryman, 1971). Low molecular weight compounds, such as glycerol or dimethyl sulphoxide, penetrate cells and due to their colligative properties prevent the excessive concentrations of intra and extra cellular solutes. On the other hand, the cryoprotective mechanisms of macromolecules such as dextrans, polyvinylpyrrolidone, hydroxyethyl starch and polypeptides which can not penetrate cellular membranes and which produce their effect at low concentrations, are not yet clear (Santarius, 1982). It has been suggested that high molecular weight compounds have cryoprotective properties which are beyond those that can be attributed solely to colligative factors (Ashwood-Smith et al., 1972; Connor and Ashwood-Smith, 1973; Pribor, 1974; Williams and Harris, 1977; Körber and Scheiwe, 1980). Various possible mechanisms of cryoprotection for the nonpenetrating compounds have been proposed: formation of extracellular ice of fine-grain size and subsequent avoidance of excessive solute concentration gradients (Persidsky and Luyet, 1975), reducing the number of ice nuclei, so that crystallization occurs at lower temperatures (super-

cooling) (Klotz, 1970; Rapatz and Luyet, 1968), formation of lattices of structure water (Doebbler, 1966) and protection of membranes (Connor and Ashwood-Smith, 1973; Lionetti et al., 1976; Allen et al., 1978). Santarius (1982) reported that cryoprotection of chloroplast by equimolar concentrations of dextrans increases with increased molecular weight of dextrans. Ashwood-Smith and Warby (1972) have compared the protective effect of low and high molecular weight compounds on the stability of catalase subjected to freezing and thawing. The stability of catalase after thawing was evaluated by measuring its activity in a standard solution of hydrogen peroxide. They concluded that polyvinylpyrrolidone (molecular weight 20,000-80,000) was 100,000 to 1,000,000 times more effective in protecting catalase from freezing injury than glycerol and dimethyl sulphoxide. Even on a weight basis the factor was more than 1,000, suggesting that the mechanisms of action of the two classes of cryoprotective agents were different. Concentrations as low as 0.005% (W/V) of polyvinylpyrrolidone were effective on protecting catalase from freezing damage while dextran seemed to be effective only at concentrations higher than 0.1% (W/V). They also observed that in equimolar concentrations the protection given by dextrans increased with the increasing of molecular weight of dextrans. Recent studies with hydroxyethyl starch seem to indicate that the cryoprotection action of this compound may be related to its ability to absorb water and to keep it unfrozen at cryogenic temperatures (Körber et al., 1982; Körber and Scheiwe, 1980). Biswas et al. (1975), using differential scanning calorimetry have studied the effect of different carbohydrates on the freezing point of aqueous solutions and on the amount of nonfrozen water. They concluded that the high molecular weight polymers (Locust bean gum, Guar gum, carboxymethyl cellulose, carrageenan and polyvinylpyrrolidone) did not present any observable freezing-point depression of the solutions but did increase significantly the amount of nonfrozen water much more than the low molecular weight compounds (glucose, sucrose and raffinose), on a weight basis. They also observed that anionic polysaccharides were more effective in increasing the amount of nonfrozen water than the neutral ones.

The mechanisms of freezing damage of living cells are still poorly understood, so it is not surprising that accurate and detailed explanations for cryoprotection are not yet available (Fennema, 1973). Similar conclusion can also be expressed for the deterioration of food during frozen storage and for the mechanisms of action of most of the cryoprotective agents used in food. In Table 3, some of the more common compounds added to food in order to minimize deterioration during frozen storage are listed (Fennema, 1973). As can

TABLE 3 Cryoprotectants[a] used in food (Fennema, 1973).

Chemical	Function
Mono- and disaccharides:	
Glucose	Minimize gelation of egg yolk
	Minimize precipitation of milk proteins
Fructose	Minimize gelation of egg yolk
Sucrose	Minimize gelation of egg yolk
	Minimize precipitation of milk proteins
Sucrose syrups and other sugar syrups	Retard oxidative browning of fruit by excluding O_2
Various mono- and disaccharides	Minimize insolubilization of protein in fish
Polyhydric alcohols:	
Glycerol	Minimize gelation of egg yolk
	Minimize insolubilization of protein in fish
	Minimize precipitation of milk proteins
Sorbitol	Minimize precipitation of milk proteins
Salts:	
Sodium chloride	Minimize gelation of egg yolk
	Inhibit oxidative enzymes in fruits
Sodium citrate	Minimize precipitation of milk proteins
Polyphosphates	Minimize thaw-exudate in fish
	Minimize thaw-exudate in poultry
	Minimize precipitation of milk proteins
Other:	
Ascorbic acid	Minimize oxidative browning in fruits by serving as a reducing agent (if present in sufficient concentration it can also inhibit oxidative enzymes)
Citric acid	Minimize oxidative browning in fruits by altering pH to a value less suitable for oxidative enzymes
Carboxymethyl cellulose, alginates, and other gums	Improves texture of ice cream by promoting the formation and stability of small ice crystals
Ice glaze[b]	Minimize desiccation and oxidation of fish
Lactase	Minimize precipitation of proteins in milk by prevention of lactose crystallization
Sulfites (SO_2, $NaHSO_3$)	Inhibit oxidative enzymes in fruits

[a]Chemicals which minimize loss in quality during a freeze-thaw treatment.

[b]A continuous thin film of ice formed in direct contact with the outer surface of the product.

be seen in Table 3, some of the cryoprotective agents, such as sugars and polyalcohols are common both to food and to living cells. This may suggest that mechanisms of freezing injury and cryoprotection might be similar at least in some foods and living specimens (Fennema, 1973). Next, mechanisms of action of some cryoprotectants used in food are examined briefly.

Polyphosphates belong to the earliest compounds added to food in order to minimize deterioration during frozen storage. They are specially used in milk to stabilize casein micelles during frozen storage and in poultry and fish to reduce the thaw drip. In any of these cases the mechanisms of action of the polyphosphates are not completely understood but are probably related to their functional properties such as buffering, sequestering and emulsion stabilization capacity, reaction with proteins and hydration (Krigsman, 1985a). The addition of polyphosphates to milk decreases the avaiable calcium by sequestration which may have an indirect effect on protein stabilization during frozen storage (Fennema, 1973; Muir, 1984). The role of the polyphosphates on increasing the water holding capacity of meat has been attributed to various factors such as pH (Hamm, 1971), ionic strength changes (Swift and Ellis, 1956) and specific ion-induced effects (Hellendoorn, 1962). Treating round fish or fillets with polyphosphate solutions prior to freezing has proven to reduce thaw-drip and to have a protective effect on fish proteins (Linko and Nikkilä, 1961; Tanikawa et al., 1963; Love and Abel, 1966; Matsumoto and Noguchi, 1973). Additional studies with frozen minces of Alaska pollack have shown a marked synergistic effect between polyphosphates and sucrose and sorbitol. This effect was however decreased if 2.5% of NaCl was present in the fish minces which may suggest that the synergistic cryoprotective effect of these substances is dependent on the mode of association of actin and myosin and the ionic state of these proteins (Matsumoto, 1979).

The addition of nonlactose carbohydrates to milk or concentrated milk, has been found to stabilize casein during frozen storage (Babcock et al., 1952; Rose, 1956; Desai et al., 1961; Wells and Leeder, 1963; Lonergan et al., 1981). Rose (1956) using a model system of casein suspended in artificial serum containing various carbohydrates, concluded that different carbohydrates, added

at the same molar concentration, had different cryoprotection effects. He suggested that the differences in protection observed between carbohydrates reflected their different contribution to the viscosity of the unfrozen serum in frozen milk. Wells and Leeder (1963) reported that glycerol delayed lactose crystallization in frozen milk concentrate but did not prevent aggregation of caseinate micelles. On the other hand, glucose, sucrose, fructose and sorbitol not only retarded lactose crystallization but also prevented aggregation of caseinate after lactose crystallization. Kadan (1967) observed that polyhydroxy compounds improved the stability of frozen milk concentrate by increasing its viscosity rather than influencing the salt distribution in the product. Minson et al. (1981) explained the differences in cryoprotection of the various carbohydrates in frozen milk by their differences in hydrogen bonding capacity. At present, it seems not possible to decide which hypothesis, if any, is correct (Muir, 1984).

The addition of neutral and anionic hydrocolloids (stabilizers) to ice cream and other frozen desserts is a well known practice in the dairy industry. The primary reason for using a stabilizer, such as carboxymethyl cellulose, Locust bean gum, Guar gum, alginate and carrageenan, in ice cream is to aid in the maintenance of a smooth texture by preventing the formation of large crystals of ice and lactose, during frozen storage. Compared to ice cream which does not contain stabilizer, properly stabilized ice cream will have a heavier body, will not taste as cold, and will melt more slowly and to a creamier consistency (Keeney, 1982; Sharma, 1981; Arbuckle, 1979). Several theories have been put forward in order to explain the inhibition of lactose and ice crystal growth caused by the addition of hydrocolloids: they may be compatible with the crystal growth and attach themselves to the growing crystal surface thereby altering the normal growth pattern of the crystal; they may form complexes with crystallizing substances (hydrates) enhancing the solubility of the crystalline solute; they may combine with impurities that would enhance crystal growth (e.g. hydrocolloids that combine with calcium would eliminate the effect of calcium on sugar crystallization); they greatly increase the viscosity of the unfrozen liquid, decreasing the molecular diffusion coefficient of water in the liquid state; they bind or hold relatively large amounts of water (Keeney, 1982; Arbuckle, 1979; Shuman, 1960; Shipe et al., 1963). Love and Haraldsson (1961) have observed that smaller but more numerous ice crystals were formed in fish tissue, when a greater amount of water was "bound". The retardation of the migration of free water encouraged nucleation of new ice crystals rather than the continued growth of the existing ice crystals.

It has been shown by several Japanese investigators that the addition of a wide range of compounds, such as monosaccharides, oligosaccharides, polysaccharides of relative small molecular size, di- and tricarboxylic acids, acidic, basic and some other amino acids and their derivatives, have a cryoprotective effect on carp actomyosin, in frozen storage (Arai et al., 1970; Noguchi and Matsumoto, 1970; Matsumoto and Noguchi, 1973; Oguni et al., 1975; Noguchi and Matsumoto, 1975a; Noguchi and Matsumoto, 1975b; Noguchi et al., 1975; Noguchi et al., 1976; Ohnishi et al., 1978). For testing the cryoprotective effect of the various compounds they have used a model system composed of carp actomyosin either in solution (in 0.6M KCl) or in suspension (in 0.05M KCl). Denaturation of carp actomyosin, during frozen storage, was evaluated by measuring solubility, viscosity, ATPase activity and sedimentation pattern of the actomyosin at different intervals of frozen storage. They came to the conclusion that a compound to be an effective cryoprotective agent should have the following attributes: the molecule has to possess one essential group, either -COOH, -OH or $-OPO_3H$ and more than one suplementary group -COOH, -OH, $-NH_2$, -SH, $-SO_3H$ and/or $-OPO_3H_2$; the functional groups must be suitably spaced and properly oriented with each other and the molecule must be comparatively small. In their evaluation of carbohydrates as cryoprotective agents in fish, they have tested monosaccharides, disaccharides, trisaccharides, tetrasaccharides and polysaccharides (inulin and potato starch). Inulin (molecular weight of 5,200) and potato starch were first preheated to be solubilized, and the solution with carp actomyosin added afterwards. They have observed that monosaccharides, disaccharides, trisaccharides and tetrasaccharides showed a marked cryoprotective effect, while inulin showed some and starch had no effect. They concluded that the cryoprotective effect of the saccharides did not vary with molecular size, provided that the molecule was not very large. They did not mention however that starch can undergo retrogradation during frozen storage and if so how it contributes to the results observed. Recently, Regenstein (1986) and Lanier (1986) claim that starch (not mentioned the type of starch) and polydextrose (average molecular weight 5,000) have also a cryoprotective effect on fish minces during frozen storage.

Matsumoto (1979 and 1980) suggested that the different cryoprotective compounds, mentioned before, appear to stabilize fish proteins by association with them through ionic and hydrogen bonding. He proposed that ionic binding to proteins may occur with acidic and basic amino acids and dicarboxylic amino acids while carbohydrates and polyalcohols may interact with fish proteins

through hydrogen bonding. A schematic model of denaturation of α-helical proteins and of globular proteins during frozen storage and its prevention by dianionic cryoprotectants is presented on Fig. 9 (Matsumoto, 1980). Matsumoto (1980) proposed that the cryoprotective compounds would form a protective coat around proteins increasing their hydration and the resistance against displacement of water during freezing. He also admitted the hypothesis of the cryoprotectants, specially carbohydrates and polyalcohols, to interfere with water structure and with formation of and growth of ice crystals. Mackie (1984) suggested that their effects could be due simply to a change in the euthetic point of the fish tissue.

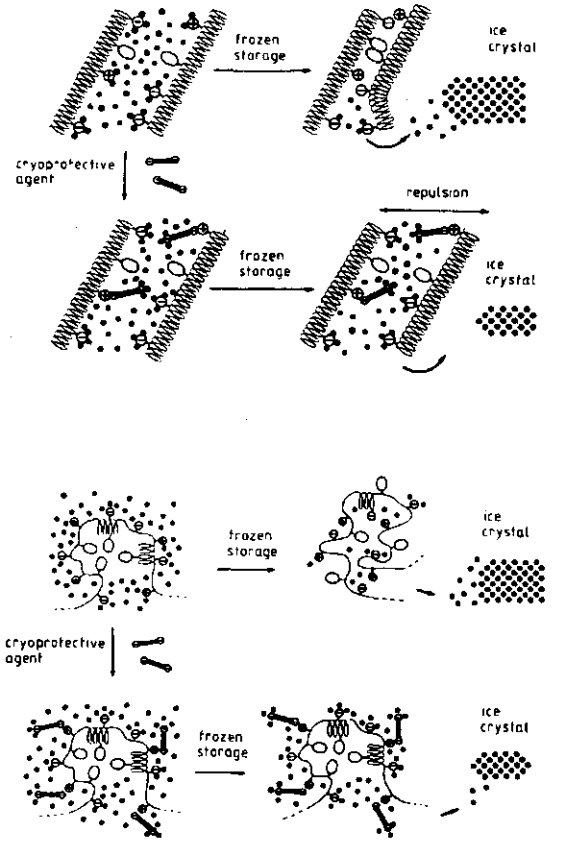

FIG. 9. A schematic model of denaturation of α-helical proteins and globular proteins during frozen storage and its prevention by a dianionic cryoprotectants (Matsumoto, 1980).

2.6. HYDROCOLLOIDS

Hydrophilic colloids or hydrocolloids, also known as "gums" are high--molecular weight polymers that dissolve or disperse in water to give a thickening and sometimes a gelling effect. They also exhibit other functional properties, which are listed in Table 4 (Glicksman, 1982). Edible hydrocolloids are generally classified in various categories depending on their origin and derivation (Table 5; Glicksman, 1982).

Important criteria for selecting a hydrocolloid are its pH stability, its compatibility with other compounds in solution and its solubility, dispersion or hydration at the conditions which it is added to food. Gelatine and most

TABLE 4 Functional properties of hydrocolloids (Glicksman, 1982)

Function:	Example:
Adhesive	Glazes, icings, frostings
Binding agent	Pet foods
Bodying agent	Dietetic beverages
Crystallization inhibitor	Ice cream, sugar syrups, frozen foods
Clarifying agent (fining)	Beer, wine
Cloud agent	Fruit drinks, beverages
Coating agent	Confectionery, fabricated onion rings
Dietary fiber	Cereal, bread
Emulsifier	Salad dressings
Encapsulating agent	Powdered flavors
Film former	Sausage casings, protective coatings
Flocculating agent	Wine
Foam stabilizer	Whipped toppings, beer
Gelling agent	Puddings, desserts, confectionery
Molding	Gum drops, jelly candies
Protective colloid	Flavor emulsions
Stabilizer	Salad dressings, ice cream
Suspending agent	Chocolate milk
Swelling agent	Processed meat products
Syneresis inhibitor	Cheese, frozen foods
Thickening agent	Jams, pie fillings, sauces
Whipping agent	Toppings, marshmallows

TABLE 5 Classification of edible hydrocolloids (Glicksman, 1982).

Starch and derivatives:	Raw starches, pregelatinized starches and modified starches.
Cellulose derivatives:	Microcrystalline cellulose, carboxymethyl cellulose, methyl cellulose.
Seaweed extracts:	Alginates, carrageenans, agar, furcellaran.
Plant exudates:	Gum arabic, gum karaya, gum tragacanth.
Seed gums:	Locust bean gum, guar gum.
Plant extracts:	Pectins.
Fermentation gums:	Xanthan, dextrans, curdlan.
Animal-derived:	Gelatin.
Synthetic:	Polyvinylpyrrolidone (PVP), carboxyvinyl polymers(Carbopol), polyethylene oxide polymers(Polyox).

of the starches are not cold soluble. In addition to that, most of the starches undergo retrogradation during frozen storage. These two hydrocolloids were therefore not considered as additives to fish.

In the following the main properties of the hydrocolloids which were proposed as additives to minced fish (5g/kg) by the Codex Alimentarius Commission (1981 and 1983), are briefly reviewed.

Pectins

Pectins are a group of complex polymers with high molecular weight (50,000--300,000) and whose predominant structural subunit is D-galacturonic acid. A significant proportion of galacturonic acid subunits are methyl esterified, forming regions in the molecule composed of either galacturonic acid or galacturonic acid methyl ester (Fig. 10). Attached to the backbone are neutral sugar side chains of varying length made up of galactose, arabinose and in some cases of xylose and fucose.

For practical purpose, pectins are usually classified according to their degree of methyl esterification. Low methoxyl pectins have a degree of esterification (DE) of less than 50%, and are characterized by their ability to form gels in the presence of calcium or other polyvalent cations (Fig. 11; Pilnik and Rombouts, 1985). On the other hand, gelation of high methoxyl pectins, having DE of more than 50%, is brought about by boiling in the presence of acid and sugar and then allowing the solution to set on cooling (Fig. 12; Pilnik

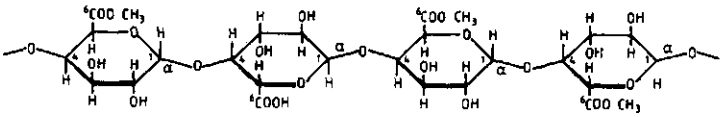

FIG. 10. Segment of high methoxyl pectin (degree of methyl esterification= 75%; Keller, 1984a).

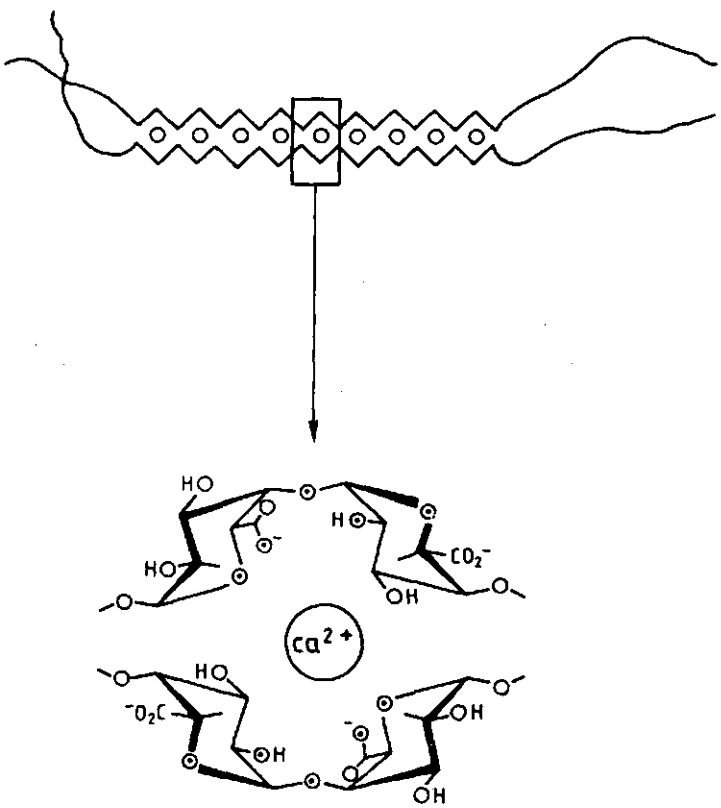

FIG. 11. Gelling mechanism of low methoxyl pectin by calcium ion cross-linking of polymer chains (Pilnik and Rombouts, 1985).
⊙ Denotes sites for calcium binding.

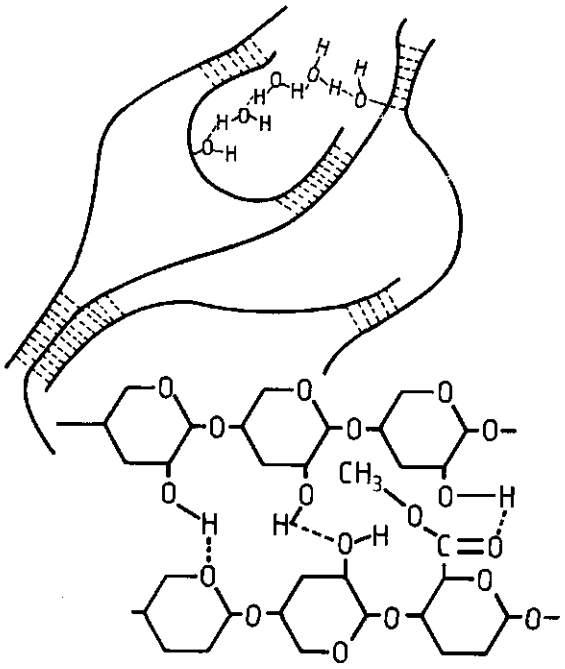

FIG. 12. Gelling mechanism of high methoxyl pectin by hydrogen bonding of polymer chains (Pilnik and Voragen, 1980).

and Voragen, 1980). The acid supresses intermolecular charge repulsions while the sugar competes with pectin for available water; the combined effects induce interchain association and gel formation.

Pectins are mainly used in the jam industry and other applications include confectionery products, fruit drinks, sour milk products and frozen desserts.

Pectins are in general soluble in cold water but the ease of solubilization is depending on several parameters such as number of methoxyl groups and their distribution, molecular weight, pH and type and concentration of salts and organic compounds in solution.

Alginates

Alginates are linear co-polymers (molecular weight of 30,000-200,000) of D-mannuronic and L-guluronic acid monomers and contain regions composed solely of one acid or the other, referred as M-blocks and G-blocks, and regions where the two monomers alternate (Fig. 13). The solution properties of a par-

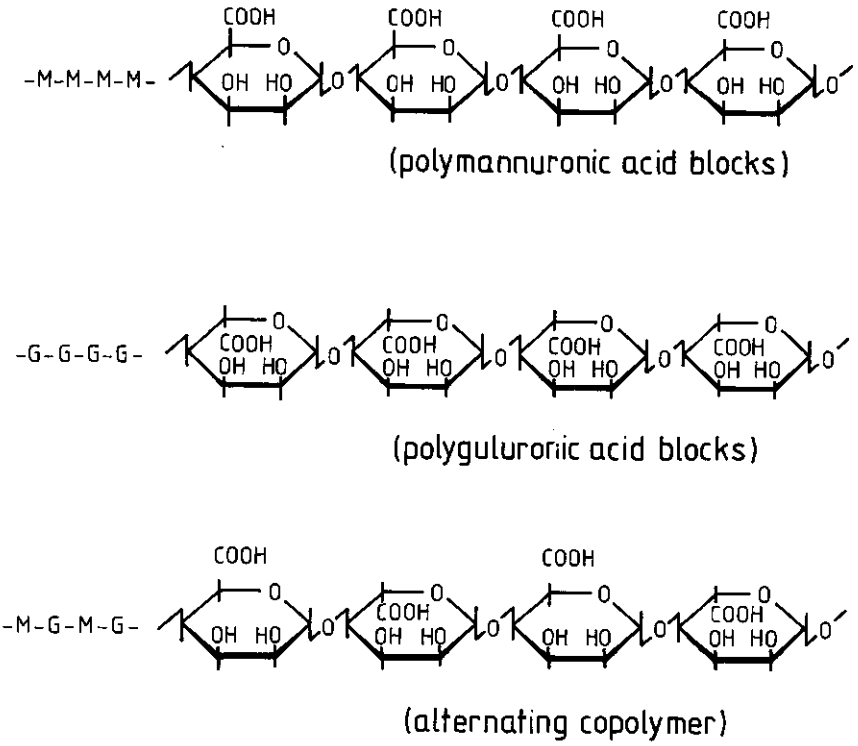

FIG. 13. Structure of the polymer segments contained in alginic acid (Glicksman, 1979).

ticular alginate depend on the ratio of manuronic to guluronic acid (M/G ratio) and the block structure.

An important and unique property of the alginates is their ability to form cold gels instantaneously by reaction with calcium salts. Rees (1969) has suggested that co-operative association of either polymannuronic acid segments or polyguluronic acid segments is involved in the formation of the cross linked network of polymer chains. Grant et al. (1973) proposed that the calcium ions are bound between the associated segments of the polymer chains so that the structure resembles an egg box (Fig. 14).

Alginates are applied in numerous products in food industry due to their thickening properties (sauces, syrups, soups, cake batters and pie fillings), gel formation (milk based desserts, confectionery jellies, backery gels, pet foods, reformed fruits and other food products), stabilization (mayonaise and salad dressings, ice cream and ice confections, fruit pulp and fruit drinks and beer) and flocculation (fining of beer and wine and potable water treatment).

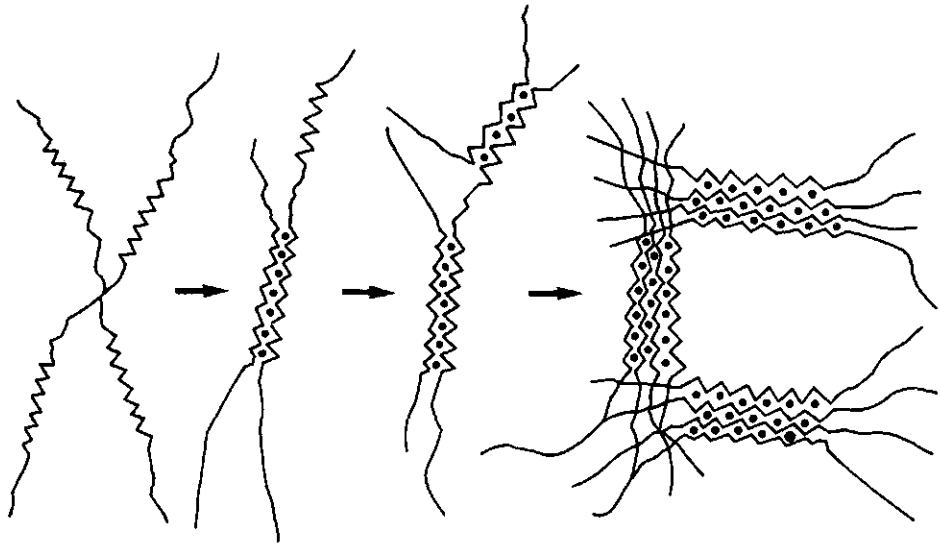

FIG. 14. Various stages of cross-linking of alginate molecules to form a gel (Krigsman, 1985b).

Carrageenans

Carrageenans are high molecular weight (100,000-500,000) linear polysaccharides made up of repeating D-galactose units, and 3,6 anhygalactose (3,6-AG), both sulfated and nonsulfated, joined by alternating α_{1-3} β_{1-4} glycosidic linkages. Various "types" or fractions of carrageenans are defined according to idealized structures and designated by Greek letters (Fig. 15). In practice, pure fractions are not obtained but Kappa-like, Iota-like and Lambda-like depending on the source of red seaweed where they are extracted from. Table 6 summarizes the properties of Kappa, Iota and Lambda carrageenan. Kappa and Iota carrageenan form thermally reversible aqueous gels by a mechanism presented on Fig. 16 (Rees, 1977).

Carrageenans are known for their milk "reactivity" or ability to stabilize milk proteins. Snoeren (1976) has pointed out that Kappa casein contains an extensive and net positively charged region between amino acid residues 20 and 115 at pH 5. It has been suggested that these positive charges may interact with the strongly negative sulphate groups of the Kappa carrageenan chain. This interaction is reduced by raising the pH from 5 to 7 (Stainsby, 1980). Another mechanism proposed, is the formation of a calcium bridge between

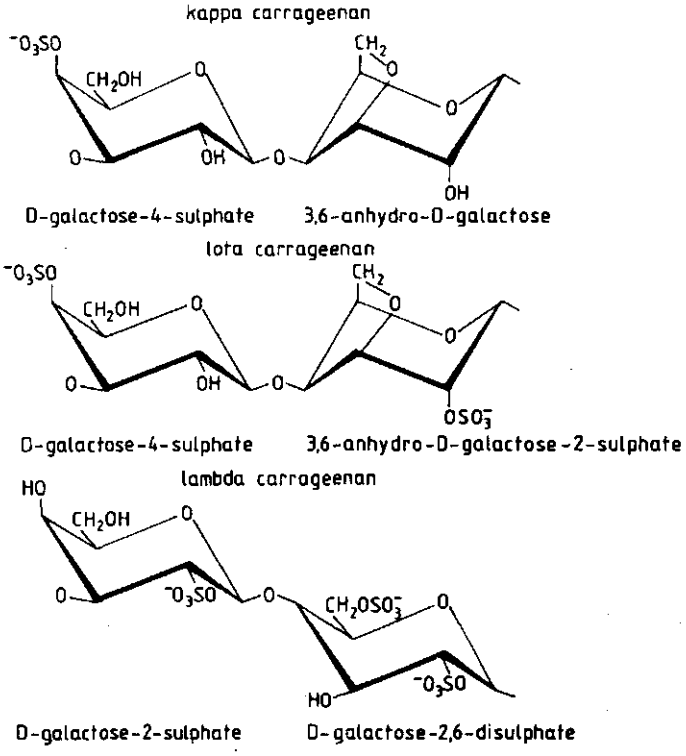

FIG. 15. Carrageenan. Repeating units in 'ideal' fractions (Pedersen, 1980).

the sulphate groups of the carrageenans and the carboxyl groups of the casein at pH above the isoelectric point of the protein (Lin, 1977; Modliszewski, 1984).

Carrageenans are applied in many food products such as ready-to-eat desserts, ice cream and frozen desserts, chocolate milk, aerated toppings, concentrated milk, bread, canned meat, etc.

Carboxymethyl celluloses

Sodium carboxymethyl cellulose (CMC) is a water soluble ether polymer, obtained by treating cellulose with sodium hydroxide and then reacting with sodium monochloroacetate:

$$\underset{\text{cellulose}}{R\text{-}(OH)_3} + NaOH \rightarrow R\text{-}(OH)_2\text{-}O\text{-}Na + H_2O$$

$$R\text{-}(OH)_2\text{-}O\text{-}Na + Cl\text{-}CH_2\text{-}COONa \rightarrow \underbrace{R\text{-}(OH)_2\text{-}O\text{-}CH_2\text{-}COONa}_{\text{carboxymethyl cellulose}} + NaCl$$

An idealized unit structure of CMC with a degree of substitution of 1.0 is presented on Fig. 17. CMC becomes water soluble at a degree of substitution of 0.4. The degree of substitution most used in food ranges from 0.7 to 0.95. The degree of substitution and uniformity of that substitution have an important effect on the hydration rate of CMC and on the flow characteristics and acid stability of its aqueous solutions. Higher degrees of substitution

TABLE 6 General properties of the various commercial fractions of carrageenans (Marine Colloids, 1985).

	Kappa carrageenan	Iota carrageenan	Lambda carrageenan
Solubility:			
80°C water	yes	yes	yes
20°C water	Na^+salt soluble K^+,$Ca^{++}$and NH_4^+ salts swell	Na^+salt soluble Ca^{++}salt swells to form thixotropic dispersion	yes
80°C milk	yes	yes	yes
20°C milk	no	no	thickens
50% sugar solution	hot	no	yes
10% salt solution	no	hot	hot
Gelation:			
strongest gels	with K^+ion	with Ca^{++}ion	no gel
gel texture	brittle	elastic	no gel
regelation after shear	no	yes	no
syneresis	yes	no	no
freeze/thaw stability	no	yes	yes
synergism with locust bean gum	yes	no	no
Acid stability[a]			
gels-pH>3.5	stable	stable	stable
Salt tolerance:	poor	good	good

[a]Hydrolysis in low pH systems accelerated by heat.

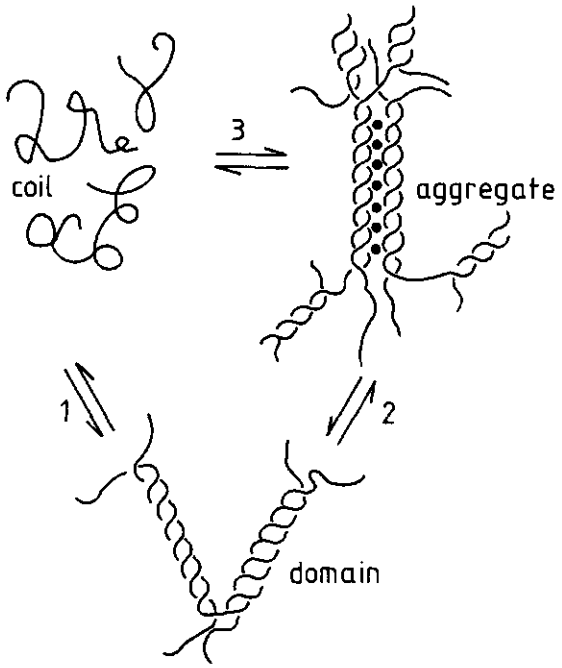

FIG. 16. The domain model of carrageenan gelation. Interchain association of Kappa and Iota carrageenan occurs primarily through double helices (domains) which, with certain cations (•) can aggregate to form a gel (Rees, 1977).

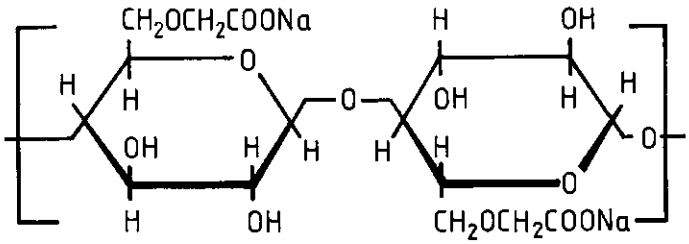

FIG. 17. Idealized unit structure of carboxymethyl cellulose with a degree of substitution of 1.0 (Keller, 1984b).

and better distribution of the substitution improves, hydration, compatibility with other solutes and resistance to acid catalyzed hydrolysis. Another important characteristic of CMC is its degree of polymerization. The longer the chain of CMC the higher is the viscosity and the pseudoplasticity behaviour of its aqueous solutions. Food grade CMC's have molecular weight ranges

between 90,000 and 700,000.

CMC is applied in numerous food products such as ice cream and frozen desserts, confectionery products, cocoa drinks, syrups and fruit drinks, salad dressings and mayonaise, dairy products, bread, frozen snacks, etc.

Xanthan gum

Xanthan gum is a high molecular weight substituted β-glucan polysaccharide, produced by Xanthomonas campestris on a glucose medium. The molecular weight of this polymer is probably in the order of 2 million, but has been reported to be as high as 13-50 millions (Kelco, 1977). The repeating unit structure of Xanthan gum is presented on Fig. 18. As a consequence of this primary struc-

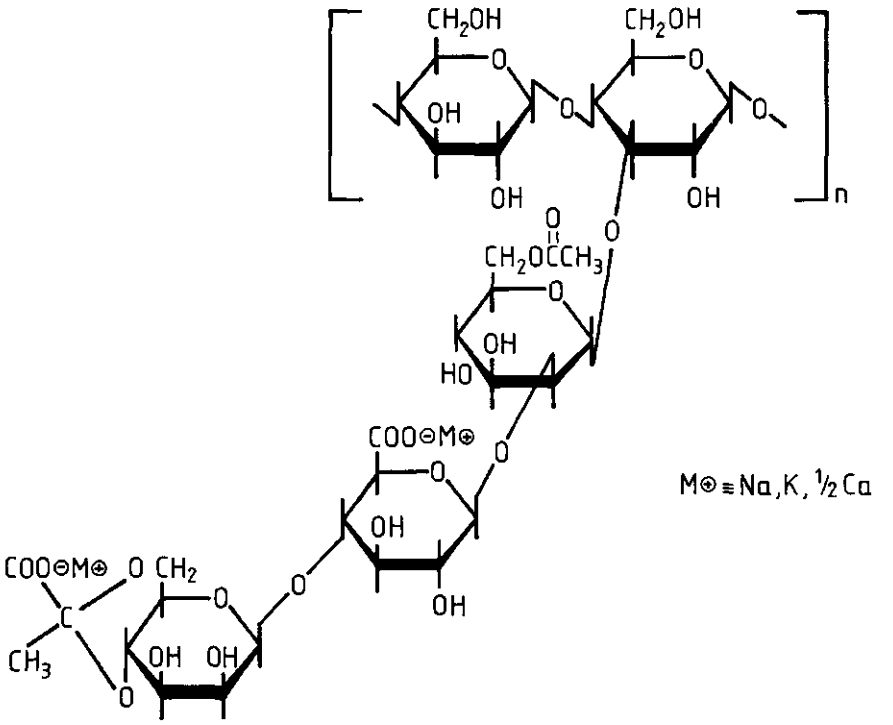

FIG. 18. Xanthan gum. Repeating unit (Kelco, 1977).

ture, the molecule exists in solution as a rigid rod stabilized by noncovalent interactions between the backbone and the side chains (Morris, 1977; Morris et al., 1977; Fig. 19).

Xanthan gum is completely soluble in hot or cold water, displaying unique solution properties (Teague et al., 1982):

-- High at-rest viscosity, even at very low gum concentrations.

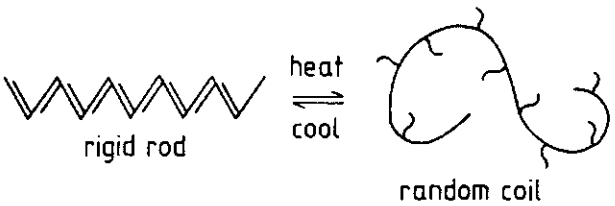

FIG. 19. Molecular structure of Xanthan gum in solution (Morris, 1977).

-- High yield value, and therefore good suspending agent.
-- High pseudoplasticity, i.e., solutions are highly shear thining.
-- Retention of viscosity at elevated temperature, in the presence of low levels of salt.
-- Thermoreversible gelation upon heating and cooling with Locust bean gum.
-- Synergistic viscosity increase with Guar gum.

Xanthan gum is used in many food products such as ice cream, ice milk, milk shakes, salad dressings, bakery products, sauces, gravies, canned food, drinks, etc.

Guar gum and Locust bean gum

Guar gum and Locust bean gum are high molecular weight (220,000-310,000) nonionic hydrocolloids, extracted from seeds, constituted by linear chains of (1 → 4)-β-D-mannopyranosil units with α-D-galactopyranosyl single units side chains attached by (1 → 6) linkages (Goldstein et al., 1973; Rol, 1973; Fig. 20). The ratio of D-galactose to D-mannose in Guar gum is 1:2 (Fig. 20). In Locust bean gum the overall ratio of mannose to galactose is around 3.5:1 and the

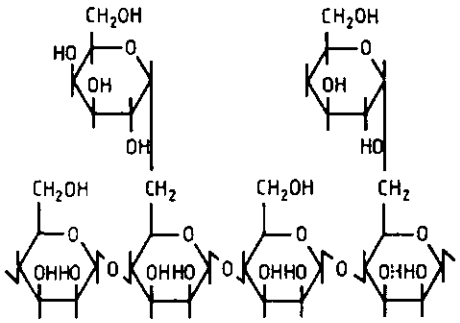

FIG. 20. Structure of Guar gum (Goldstein et al., 1973).

galactose substituents are clustered in long blocks of around 25 residues, called "hairy regions" interspersed by even longer regions of essentially unsubstituted mannan backbone "smooth regions" (Dea, 1979).

Guar gum is soluble in cold water while almost all grades of Locust bean gum require hot water for complete dissolution. Both gums give highly viscous solutions at low concentrations and are therefore used in applications in which thickening, stalilizing, and water binding are required. Typical food uses of these hydrocolloids are: ice cream, fruit drinks, salad dressings, processed cheeses, comminuted meat products, canned meats, pet foods, etc.

Guar gum and Locust bean gum produce a synergistic interaction with carrageenans and Xanthan gum. This interaction is greater with Locust bean gum and is supposed to be caused by intermolecular associations involving unsubstituted or "smooth regions" in the Locust bean gum molecule as presented on Fig. 21 (Glicksman, 1979). In Guar gum, because of the higher degree of

Schematic representation of galactomannan conformation.

Each line represents a sugar unit: the backbone composed of β-D-mannopyranose units and the side chains composed of α-D-galactopyranose units.

Possible model for the interaction between Xanthan gum and Locust bean galactomannan, resulting in gel formation.

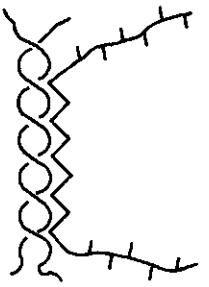

FIG. 21. The proposed mechanism of the interaction between Xanthan and Locust bean gums (Glicksman, 1979).

substitution fewer "smooth regions" exists and interaction is less pronounced, giving rise to a synergistic increase in viscosity rather than gelation.

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3. EFFECTS OF ADDITIONS ON THE STABILITY OF FROZEN STORED MINCED FILLETS OF WHITING: I. VARIOUS ANIONIC HYDROCOLLOIDS.

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ABSTRACT

Pectins, alginates and kappa carrageenan were used as additives (5 g/kg) in ground fillets of whiting. Samples of these treatments were stored at −18°C for 2 months and were evaluated at regular intervals for pH, water-holding capacity, texture, extractable myosin and formation of dimethylamine and formaldehyde.

Remarkable differences in texture and water-holding capacity were observed. The alginates (Kelcogel and Kelcosol) showed the highest values of water-holding capacity in the raw material and the lowest increase in toughness during the 2 months of cold storage.

The hydrocolloids used did not show any protective effect on the extractable myosin and with the exception of Kelcosol, all the treatments presented similar increases of dimethylamine and formaldehyde content.

INTRODUCTION

Mechanical deboners (separators) have been used successfully in beef, poultry and pork industry (Froning 1981; Field 1981; Expert Panel on Food Safety and Nutrition 1979). They have also been employed in the kamaboko and fish sausage industry, with good results (Suzuki 1981). These mechanical deboners seem to be very suitable for fishes for which under conventional conditions, operation of filleting is rather inefficient and expensive, due to the size or boning structure of the fishes.

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The promising prospects of this new technology in the fish industry are contrasted with several problems not yet completely solved. Normally the minced fish is less stable in cold storage than the fish or fillets. Changes in texture, protein extractability and water-holding capacity have been reported to be accelerated in the minced fish (Rodger *et al.* 1980; Webb *et al.* 1976; Grabowska and Sikorski 1973).

Several carbohydrates (glucose, fructose, sucrose and lactose) and certain amino acids and related compounds (glutamate, aspartate, cysteine and proline) have a preventive effect on the denaturation of the fish muscle during frozen storage (Ohnishi *et al.* 1978; Noguchi *et al.* 1976; Noguchi *et al.* 1975). More recently, hydrocolloids such as sodium alginate, locust bean gum, guar gum, pectin, carrageenan, carboxymethyl cellulose and xanthan gum have been proposed as additives to minced fish (Codex Alimentarius Commission, 1981 and 1983).

In our experiments, we added various hydrocolloids to minced fillets of whiting and studied the changes of texture, water-holding capacity, protein extractability and formation of dimethylamine and formaldehyde during 2 months of cold storage. Our idea was to see the effects of these hydrocolloids as cryoprotective agents and as modificators of texture and water-holding capacity.

Control of texture and water-binding capacity could be one of the keys to success in commercialization of new products from minced fish (Keay and Hardy 1978).

EXPERIMENTAL

Whiting (Merlangius merlangus) was caught on the coast of IJmuiden (The Netherlands), in the evening of the 26th of January and was kept on the deck of the boat at temperature just over 0 °C, during the night. In the morning of the next day, in the Institute for Fishery Products TNO in IJmuiden, the fishes were filleted, skinned and deboned manually. Plastic bags with 2 kilos of fillets were blast frozen and were kept frozen at −35 °C in cold storage for 1 week. Thereafter, the bags were transported to Wageningen (1 h by car) in isothermal boxes and were kept frozen in cold storage at −45 °C for 3 weeks. Subsequently, the bags were thawed in cold tap water and different hydrocolloids were spread on both surfaces of the fillets (5 g per kilo of fillets).

Hydrocolloids used were: (1(Low methoxy-esterified pectin (L.P.) and high methoxy-esterified pectin (H.P.) from Obi-Pectin (Bischofszell, Switzerland), (2) Kappa Carrageenan (Carrag.) from Copenhagen Pectin Factory Ltd. (Denmark), (3) Kelcogel HV and Kelcosol (different types of alginates) from Kelco/AIL London (England), and (4) Kelcocolloid HVF (Kelcocol.), a propylene glycol alginate, from Kelco/AIL London (England).

In order to improve the hydration of the hydrocolloids, they were predissolved in water, freeze-dried and ground before adding to the fillets. After addition, the fillets were passed through a Kenwood mincer model A.720, using a mincing screen with a diameter of 4.4 mm. Samples of 150 g were packed in plastic bags, blast frozen and kept at -18 °C for 2 months. Samples of each treatment were withdrawn from the cold storage, thawed in cold tap water and kept for maximum 3 hr in melting ice until analyses were carried out.

pH Measurements

Ten grams of ground fish were blended with 20 ml of distilled water for 5 min. The pH of the resulting mixture was measured with a combined glass calomel electrode.

Water-holding Capacity of the Raw (WHCR) and Cooked (WHCC) Material

Fifteen grams of minced fish were weighed into a 50 ml polycarbonate centrifuge tube and centrifuged in a sorvall RC 5 B at 27750 g for 30 min. Temperature during centrifuging was kept near 0 °C.

Supernatant was discarded and residue reweighed. Water-holding capacity of the raw material was expressed as percentage of residue weight. The same procedure was used to determine water-holding capacity of the cooked material, except that the tubes with the fifteen grams of ground fish were kept in an oven at 100 °C for 1 h, immediately before centrifuging. Four replicates were done for each sample.

Cook Drip Loss

Twenty-two grams of minced fish were laid in a 25 ml beaker and kept in an oven at 100 °C for 1 h. The cake was poured out on 2 layers of filter paper (S&S n 602 h, 12.5 cm of diameter) and kept at room temperature for 1 h. The cake was weighed and the difference to 22 g expressed as percentage of 22 g was taken as a measure of the cook drip loss. Five replicates were done for each sample.

Texture Analysis

Shear stress (S.S.) analyses were carried out in a Kramer Shear Press Model SP-12 Imp.

Seventy-five grams of thawed ground fish were put in a CS-1 standard shear-compression cell, an electronic texturegage of 100 lbf and a speed of 1.2mm/sec were used. Texturegrams were recorded on T-2100 texture-test system and the shear stress was expressed as units of area of the texturegrams. Two replicates were done for each sample. The cakes obtained from the determination of the cook drip loss were used to determine the compressive

strength, modulus elasticity and resilience in an overload dynamic apparatus, model S 100. Cakes were of cylindrical shape with a diameter equal to the height (2.9 cm). A cell of 2000N, a chart speed of 50 mm/min and a plunger speed of 20 mm/min were used.

Curves obtained from the different samples were analysed according to Lee and Toledo (1976). Five replicates were done for each sample.

Extractable Myosin

The procedure used by Lee and Toledo (1976) was followed with minor modifications. Three grams of ground fish muscle were used. The buffer for the extraction of the salt soluble myofibrillar proteins was the same as used by Richard *et al.* (1967) and Chu and Sterling (1970). All the operations were carried out at temperatures between 0 °C and 4 °C. The final supernatant was analysed by the Biuret-Method (Layne 1957) to determine the protein concentration of extractable myosin. Two replicates were done for each sample.

Dimethylamine (DMA) and Formaldehyde (FA)

Twenty grams of ground fish muscle were blended with 80 ml of 5% trichloroacetic acid and the supernatant was used for the determination of DMA and FA. The colorimetric method of Dyer and Mounsey (1945) was used to determine DMA. FA was determined by the colorimetric method of Sawicki *et al.* (1961).

Protein and Total Sugar Content of the Supernatants Released in the WHCR and WHCC Determinations

Proteint nitrogen content and total sugar content of the supernatant obtained from the determination of the water-holding capacity were determined, respectively, by the semi-automatic kjedhal method of Roozen and Ouwehand (1978) and by the colorimetric method of Dubois *et al.* (1956).

Viscosity Determinations

The Rotovisco torque viscosimeter, (model 105/b with a rotary bob NV) was used to measure the shear stress and shear rate of the different supernatants obtained from the determination of the water-holding capacity. A water bath at 20-21 °C was employed to control the temperature of the supernatants in the rotovisco.

Statistical Analysis

Data were analyzed using the statistical analysis systems computer package (Baker and Nelder 1978). Polynomial regression lines were evaluated for each property and for each treatment. The t-test at the 5% level of significance was used to compare different treatments.

RESULTS AND DISCUSSION

pH Measurements

Changes in pH of the different treatments are shown in Fig. 1. In all the treatments, there is small fall (0.2-0.4) of the pH values during the first 2 weeks and then a slight increase of the pH with time of cold storage.

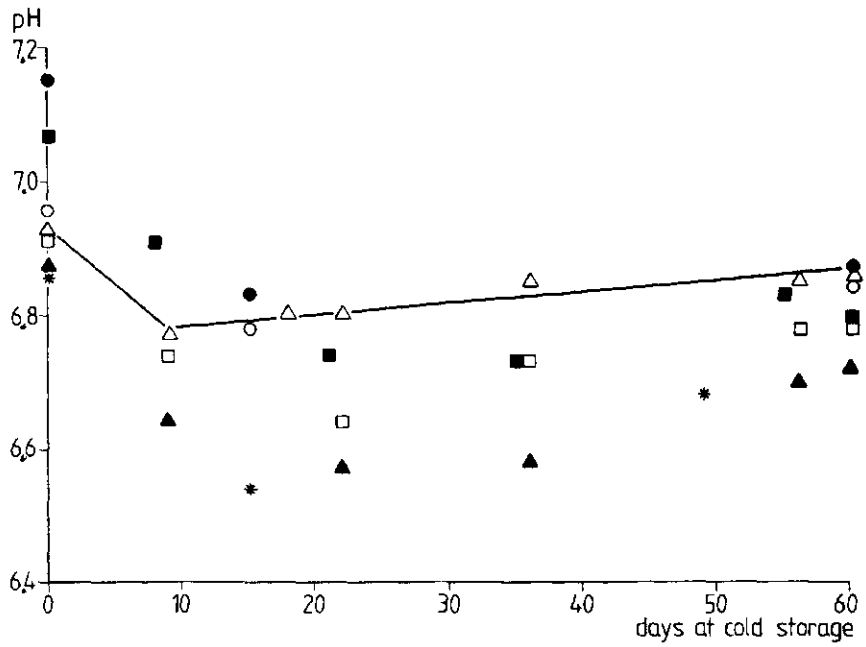

FIG. 1. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON pH OF FROZEN STORED MINCE MUSCLE.
△Blank; ▲L.P.; □H.P.: ■CARRAG.; ○KELCOGEL; ●KELCOSOL and *KELCOCOL.

Many reactions are still possible at temperatures near −18°C. Formation of lactic acid from glycogen, production of DMA, precipitation of alkaline salts (presumably phosphates) and after longer periods of storage precipitation of acid salts are some of the possible explanations for the pH variation presented in Fig. 1 (Licciardello *et al.* 1982).

Water-holding Capacity of the Raw Material (WHCR)

During 2 months of cold storage at −18 °C, WHCR decreased about 12% in all treatments (Fig. 2). Blank and Kelcocol treatments always showed the lowest values and Kelcosol and Kelcogel treatments the highest values. With few exceptions, Carrag. and L.P. treatments showed values significantly

higher than the Blank and Kelcocol treatments. H.P. treatment resulted in higher values than the Blank, but in general they were not significantly different.

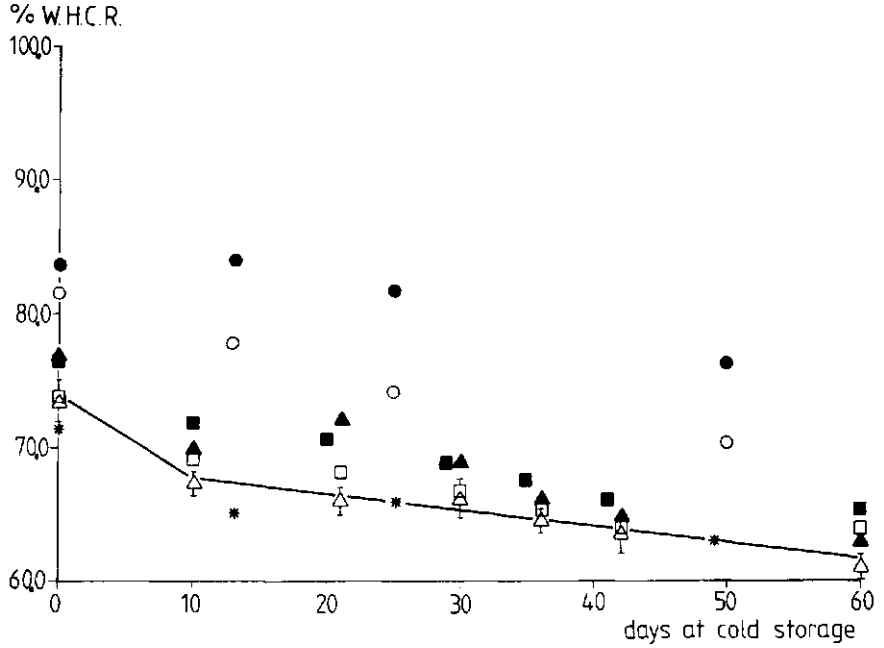

FIG. 2. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON W.H.C.R. OF FROZEN STORED MINCED FISH MUSCLE.

See the note in Fig. 1 for the symbols. The vertical bars represent standard deviation.

This variation of WHCR may be attributed to several features. The hydrocolloids used have different viscosities. The viscosity influences the fluid drainage when fish cakes are submitted to pressure. In some cases, this explanation fits well (Kelcogel, L.P. and Kelcocol. treatments), but in other cases the WHCR behavior does not follow the viscosity. Carrag. treatment gave a supernatant with a viscosity similar to the Blank and much lower than the Kelcocol. and L.P. supernatants (Fig. 9). However, the WHCR of Carrag. was significantly higher than the Kelcocol. and Blank treatments and similar to the L.P. treatments. It seems that the Carrag. was included in the residual fish cake improving the WHCR. At low temperatures, the kappa Carrageenan particles have low solubility but they can be easily dispersed and hydrated. When the ground fish muscle with the Carrag. added was centrifuged for WHCR determination, the Carrag. particles were carried to the bottom of the tubes with the fish cake. This effect is reproduced by dispersions of kappa Carrageenan in cold distilled water in which minced fish was homogenized. When

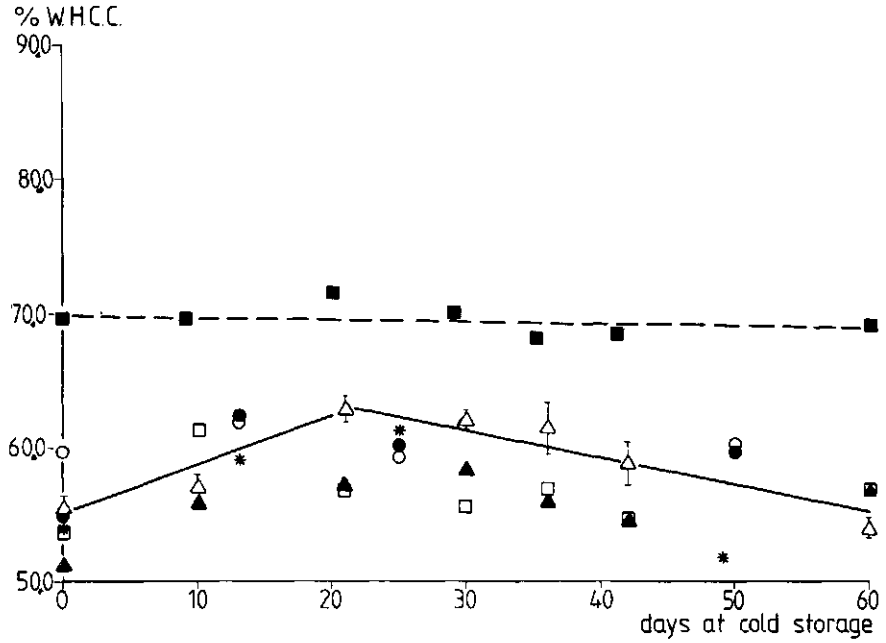

FIG. 3. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON W.H.C.C. OF FROZEN STORED MINCED FISH MUSCLE.

See the note in Fig. 1 for the symbols. The vertical bars represent standard deviation.

these mixtures were centrifuged, the supernatants obtained had very low viscosity and low amounts of Carrag. Mutual interactions among the Carrag. particles or with other compounds in the minced fish may be an additional factor for the fixation of the Carrag. in the fish residue.

The hydrocolloids used have ionic groups which are capable of interacting with other groups of other compounds in the ground fish muscle (Stainsby 1980; Imeson *et al.* 1977).

Differences in pH may be another factor influencing the WHCR, Kelcocol., L.P. and H.P. treatments showed pH values normally lower (0.2-0.3) than other treatments.

Water-holding Capacity of the Cooked Material (WHCC) and Cook Drip Loss (CDL)

WHCC and CDL of different treatments, along the 2 months of cold storage at −18 °C, are presented in the Fig. 3 and 4. With the exception of the Carrag. treatment, there is a decrease of CDL (increase of WHCC) in the first 3 weeks, and then a slight increase of CDL (decrease of WHCC) till the end of cold storage.

It is difficult to find a satisfactory explanation for this. In the literature, conflicting results about CDL have been reported. Moledina *et al.* (1977) found an increase of CDL of 3% in samples of minced fish stored at −25 °C for 8 weeks. For CDL determination, the samples were packed in rigid aluminum dishes and heated in an electric oven at 200 °C for 45 min. Patashnick *et al.* (1976) found a decrease of about 3% in CDL in the first 4 weeks, in samples of minced fish stored at −18 °C. These samples were packed in a covered aluminum container and steamed for 20 min.

Several researchers have reported that prolonged heating of fish gels in the 50-70 °C range causes weakening of gel texture and decrease of WHCC (Lee and Toledo 1976; Cheng *et al.* 1979). This has been suggested to be due to an alkaline protease, particularly active at these temperatures (Lin and Lanier 1981a; Lin and Lanier 1981b; Lanier *et al.* 1981; Cheng *et al.* 1979).

It is possible that the proteolytic activity of this enzyme is the reason for the variations of WHCC and CDL in each treatment.

Carrag. treatment showed always significantly higher WHCC and normally lower values of CDL when compared with the other treatments. The variation of WHCC and CDL in the Carrag. treatment, was very small during the 2 months of cold storage. Formation of a separated gel layer of Carrag. on the cake of the CDL and on the residue of WHCC was observed.

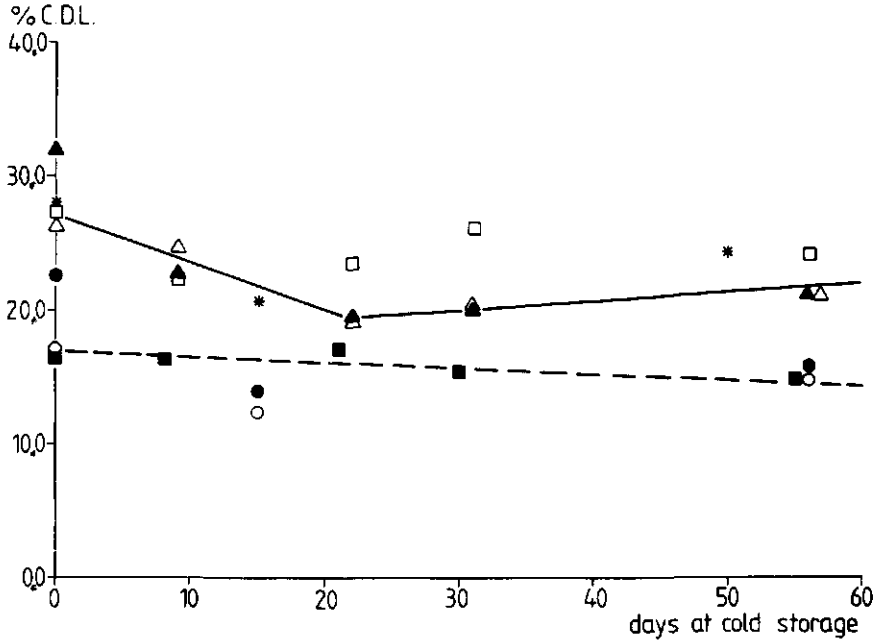

FIG. 4. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON C.D.L. OF FROZEN STORED MINCED FISH MUSCLE.

See the note in Fig. 1 for the symbols.

Texture Analysis

During 2 months of cold storage, it became evident that the samples increase in S.S., C.S., M.E. and R. in all treatments (Fig. 5, 6, 7 and 8). Blank showed the highest values and Kelcogel and Kelcosol treatments the lowest values. At the end of 2 months of cold storage, these latter treatements presented C.S., M.E., R. and S.S. values similar to the initial values of the Blank and Carrag. treatments.

Viscosity of the fish fluid, possible interactions of the hydrocolloids in the ground fish muscle and differences in solubility and pH may explain the variations on shear stress of the different treatments. H.P. and Kelcocol. treatments presented S.S. values in accordance with the viscosity of their supernatants at zero time, but Kelcosol, Kelcogel, L.P. and Carrag. treatments did not show the same correlation. In these latter cases, interaction of the carbohydrates with compounds in ground fish muscle may be expected.

When minced fish muscle was cooked and analysed for C.S., M.E. and R., the more remarkable note was that similar values were obtained from the Carrag. and Blank treatments. The Carrag. was layered on the fish cake as a gel, offering very low resistance to the C.S., M.E. and R. determinations. All the other treatments showed values of C.S., M.E. and R. significantly lower when

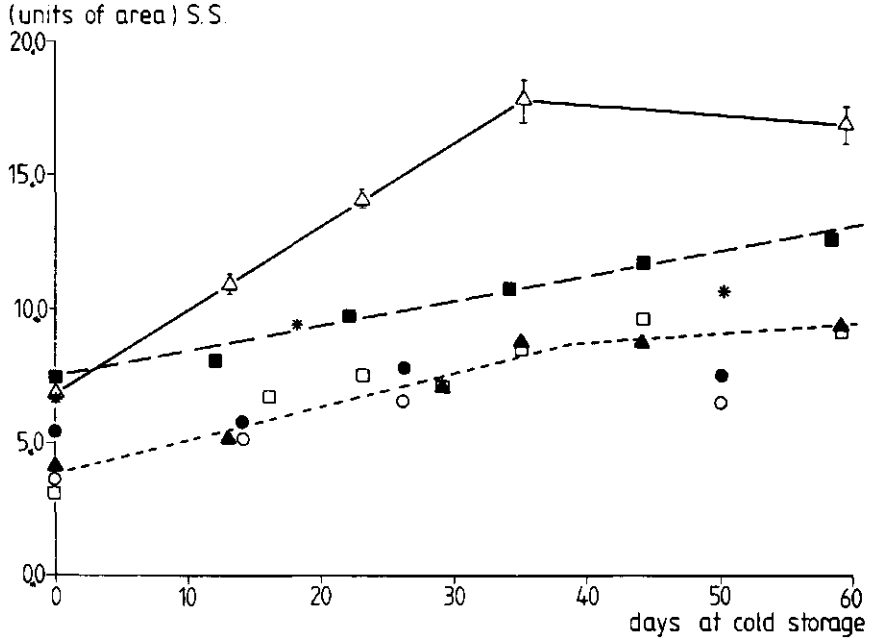

FIG. 5. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON S.S. OF FROZEN STORED MINCED FISH MUSCLE.

See the note in Fig. 1 for the symbols. The vertical bars represent standard deviation.

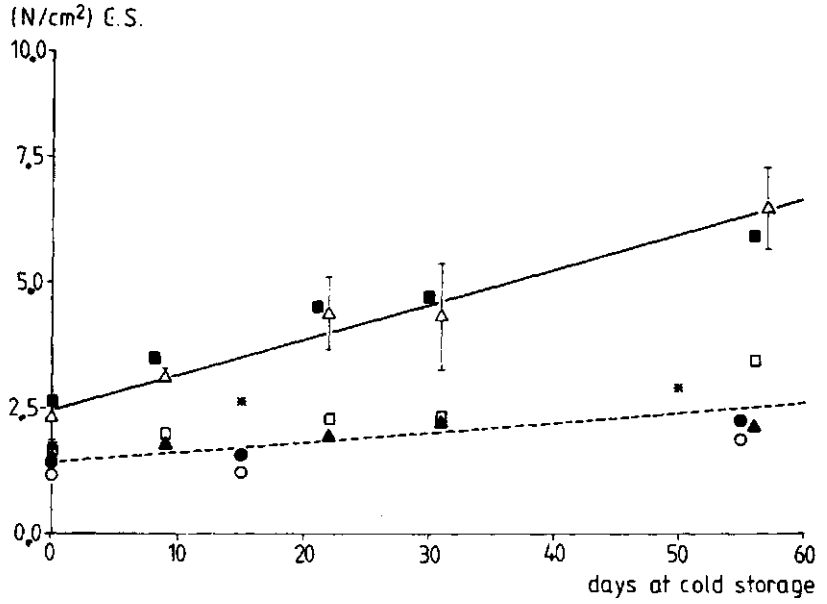

FIG. 6. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON C.S. OF FROZEN STORED MINCED FISH MUSCLE.

See the note in Fig. 1 for the symbols. The vertical bars represent standard deviation.

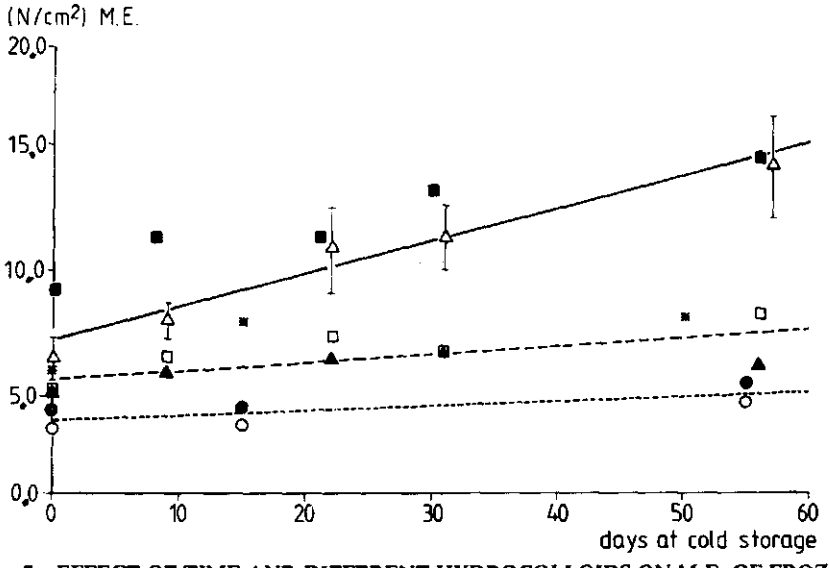

FIG. 7. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON M.E. OF FROZEN STORED MINCED FISH MUSCLE.

See the note in Fig. 1 for the symbols. The vertical bars represent standard deviation.

HYDROCOLLOIDS FROZEN MINCED WHITING

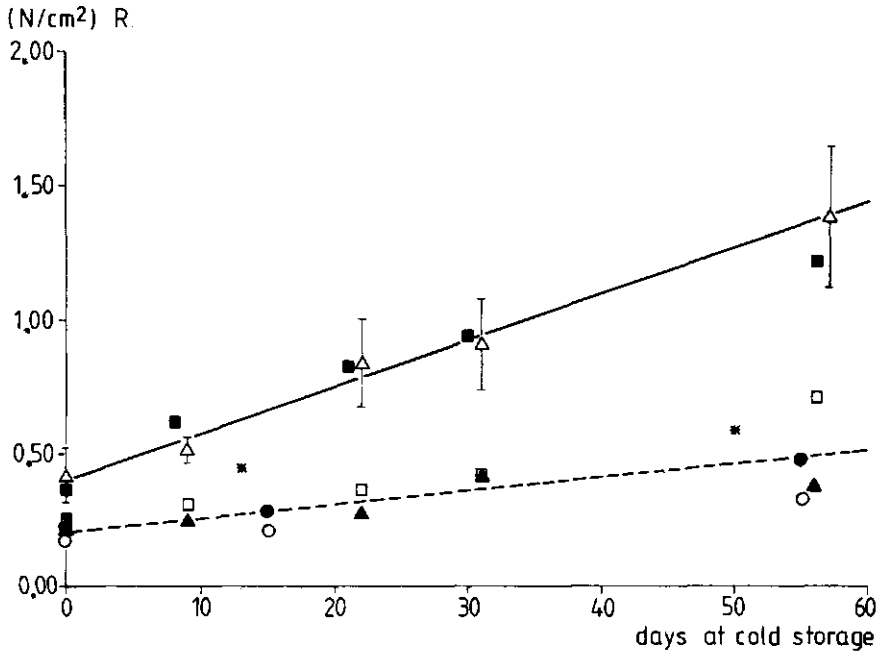

FIG. 8. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON R. OF FROZEN STORED MINCED FISH MUSCLE.
See The Note In Fig. 1 For The Symbols. The Vertical Bars Represent Standard Deviation.

Table 1. Extractable myosin (mg/g of fish) of frozen stored minced fish at the time intervals.

Days at	Blank	L.P.	H.P.	Carrag.	Kelcogel	Kelcosol	Kelcocol
-18°C							
0	9.2±1.3[a]						
6	5.1±0.6	3.2±0.1	4.2±0.2	5.2±1.3			
8					4.7±0.4	4.0±0.1	5.0±0.1
12				4.1±0.2			
13		3.0±0.1					
14					4.1±0.1	3.7±0.4	
16			3.3±0.2				3.5±0.2
18	3.1±0.1						
22				2.8±0.1			
23	2.6±0.1						
29	2.5±0.1	2.6±0.1	2.6±0.1	2.8±0.1			
36		2.6±0.1		2.7±0.1			
37	2.5±0.1	2.5±0.1					
49					2.5±0.1	2.5±0.1	2.5±0.1

[a]Mean of duplicate samples ±standard deviation.

compared with the Blank or Carrag. treatments. In general, the highest values of viscosity (Fig. 10) gave the lowest values of C.S., M.E. and R.

Extractable Myosin (E.M.)

Losses of extractable myosin were very critical in the first week where about 50% of initial E.M. became insoluble (Table 1). These losses continued until the 4/5 weeks, when a plateau of 28% of the initial solubility was reached.

All the treatments showed similar values of E.M. No effect of the different hydrocolloids added were noticed. Similar decreases of E.M. have been reported in the literature (Rodger *et al.* 1980; Bremner 1977; Gill *et al.* 1979).

Table 2. Dimethylamine content[a] (μg DMA-Nitrogen/g of fish) of frozen stored minced fish at time intervals.

Days at -18°C	0	19	20	59	60
Blank	28.1±0.7		46.2±0.5	69.9±1.7	
L.P.		31.9±0.3		61.6±0.4	
H.P.		36.5±1.1		66.1±0.1	
Carrag.		31.8±0.3		60.5±0.2	
Kelcogel			59.5±0.2		79.1±0.9
Kelcosol			41.4±0.6		46.3±0.5
Kelcocol.			48.5±0.1		62.0±0.7

[a]Mean of triplicate samples ± standard deviation.

Table 3. Formaldehyde content[a] (μg/g of fish) of frozen stored minced fish at time intervals.

Days at -18°C	0	19	20	59	60
Blank	8.0±0.1		11.1±0.2	27.8±0.5	
L.P.		12.7±0.3		23.1±0.6	
H.P.		11.8±0.2		25.7±0.9	
Carrag.		10.8±0.3		21.5±0.3	
Kelcogel			15.9±0.1		30.1±0.1
Kelcosol			9.0±0.2		16.4±0.5
Kelcocol.			12.8±0.1		23.2±0.2

[a]Mean of triplicate samples ± *standard deviation.*

Dimethylamine (DMA) and Formaldehyde (FA)

With the exception of the Kelcosol treatment, there was a similar increase on DMA and FA formation in all treatments (Tables 2 and 3). The lower values of DMA and FA in the Kelcosol treatment should be confirmed with more experimental data.

HYDROCOLLOIDS FROZEN MINCED WHITING

Table 4. Protein nitrogen content[a] (mg/ml) of the supernatants obtained from the WHCR and WHCC determinations at zero time.

Treatments	Raw samples	Cooked samples
Blank	11.5±0.2	7.0±0.1
L.P.	9.6±0.2	6.6±0.1
H.P.	9.6±0.3	6.2±0.1
Carrag.	10.9±0.2	7.1±0.3
Kelcogel	10.1±0.1	6.3±0.5
Kelcosol	9.9±0.1	7.5±0.3
Kelcocol.	9.7±0.1	7.3±0.4

[a]Mean of duplicate samples ± standard deviation.

Table 5. Concentrations of carbohydrates in the supernatants obtained from the WHCR and WHCC determinations at zero time.

TREATMENTS	RAW SAMPLES			COOKED SAMPLES		
	[a]mg glucose per ml sup.	[b]mg carb. per ml. sup.	[c]mg carb. in sup. per g of fish	[a]mg glucose per ml sup.	[b]mg carb. per ml-sup.	[c]mg carb. in sup. per g of fish
Blank	1,40			1,6		
L.P.	5,8	(0,46) 9,6	2,2	4,8	(0,52) 6,2	3,0
H.P.	6,5	(0,48) 10,6	2,8	6,1	(0,60) 7,5	3,5
Carrag.	2,2	(0,42) 1,9	0,5	2,2	(0,42) 1,4	0,4
Kelcogel	4,4	(0,34) 8,8	1,6	4,7	(0,40) 7,8	3,1
Kelcosol	2,8	(0,32) 4,4	0,7	4,0	(0,38) 6,3	2,8
Kelcocol	4,8	(0,30) 11,3	3,2	4,0	(0,29) 8,3	3,8

[a]Equivalents of glucose obtained from the colorimetric method of Dubois *et al.* (1956).

[b]Calculated by subtracting the glucose concentration of the blank (a) and multiplying with factors which were determined for each carbohydrate (--).

[c]These concentrations were calculated by multiplying the carbohydrate content of the supernatant (b) by:

$\frac{100\text{-W.H.C.R.}}{100}$ for the raw samples.

$\frac{100\text{-W.H.C.C.}}{100}$ for the cooked samples.

Protein, Total Sugar Content and Viscosity of the Supernatants Released in WHC Determination at Zero Time

The protein nitrogen content varied from 9.6 to 11.5 mg/ml in the raw supernatants and from 6.2 to 7.5 mg/ml in the cooked supernatants (Table 4). The carbohydrate released in the raw supernatants varied from 1.9 mg/ml, in the Carrag. treatment, to 11.3 mg/ml, in the Kelcocol. treatment, and in the cooked supernatant from 1.4 mg/ml, in the Carrag. treatment, to 8.3 mg/ml, in the Kelcocol. treatment (Table 5).

The protein nitrogen differences among the supernatants are small and cannot be responsible for the variation in the flow curves presented in Fig. 9 and 10. These variations should be ascribed to the nature and content of the hydrocolloids in the supernatants and to the effect of the heat treatment on the hydrocolloid conformations.

Carrag. stayed almost completely in the residual cake, only minor fractions came into the supernatants.

With the exception of the Kelcosol treatment, there was a slight decrease of

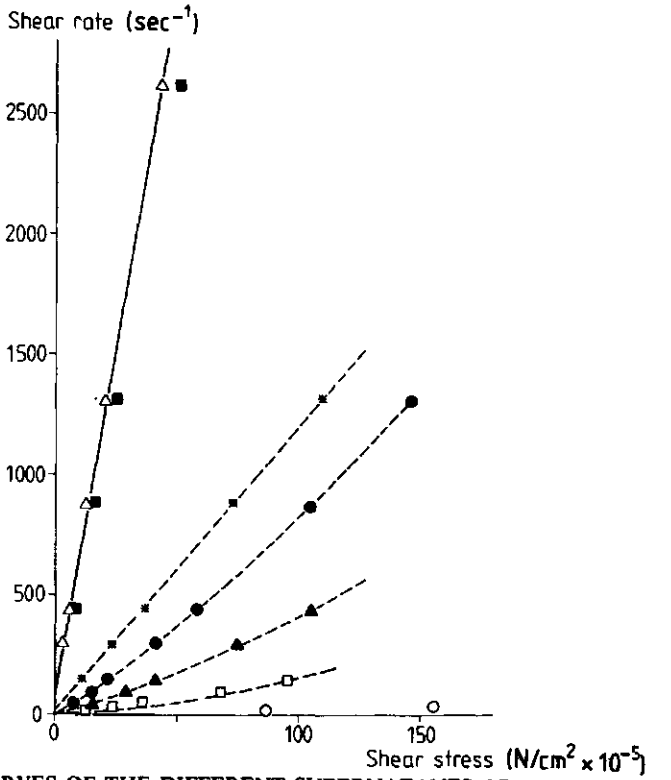

FIG. 9. FLOW CURVES OF THE DIFFERENT SUPERNATANTS OBTAINED FROM W.H.C.R. AT ZERO TIME.
See The Note In Fig. 1 For The Symbols.

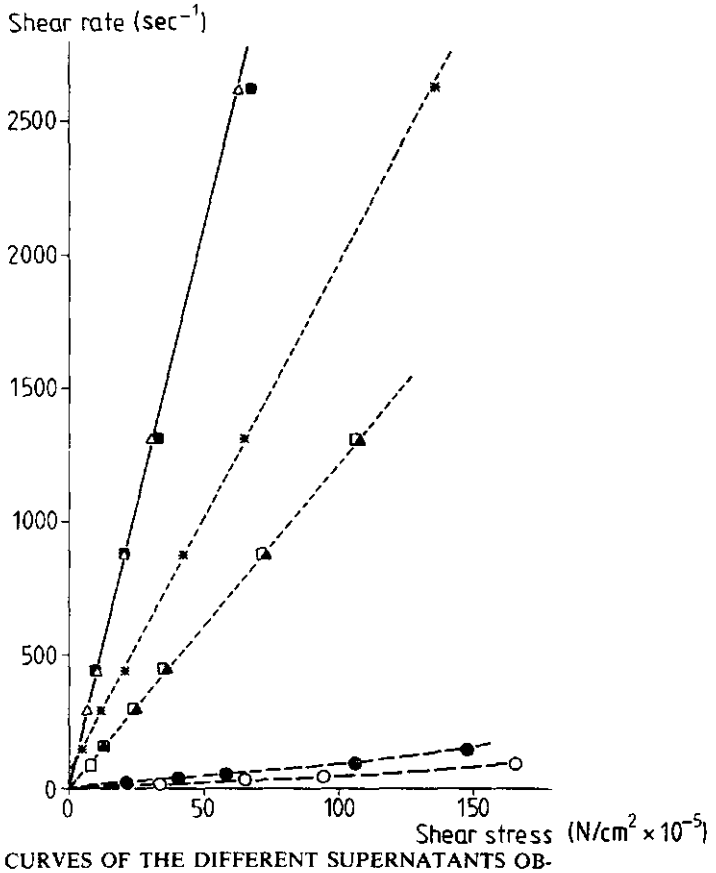

FIG. 10 FLOW CURVES OF THE DIFFERENT SUPERNATANTS OBTAINED FROM W.H.C.C. AT ZERO TIME.
See The Note In Fig. 1 For The Symbols.

the carbohydrate content in the cooked supernatants when compared with the raw supernatants (Table 5). This decrease should be attributed to the increase of fluid release in the WHCC determination. The surprising increase of the carbohydrate content in the supernatant of the cooked sample of the Kelcosol treatment is accompanied by an increase of about 28% of the fluid released. Mutual interactions among the Kelcosol particles or with other compounds in the raw fish muscle may be the reason for the lower concentrations found in the supernatant of the raw sample (Stainsby 1980; Imeson *et al.* 1977). The carbohydrate content and the viscosity of supernatant increased inthe cooked sample. Apparently, the Kelcosol dissolved by the heat treatment of WHCC determination.

CONCLUSIONS

The hydrocolloids used did not show any protective effect on the extractable myosin. Only Kelcosol addition slowed down DMA and FA formation.

Some hydrocolloids exhibited remarkable changes on texture and water-holding capacity. Kelcosol and Kelcogel presented promising results on the WHCR and texture; Carrag. revealed very high values of WHCC which is, however, due to the formation of a separated gel.

At the end of the 2 months of cold storage, the Blank samples had very intensive off-flavors (fishy odours). This fact was not observed in the samples of the other treatments. The increase of off-flavors in the Blank treatment was not correlated with the increase of DMA. Other chemical analysis should be considered to give a better understanding of this fact.

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4. EFFECTS OF ADDITIONS ON THE STABILITY OF FROZEN STORED MINCED FILLETS OF WHITING. II: VARIOUS ANIONIC AND NEUTRAL HYDROCOLLOIDS

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ABSTRACT

Locust bean gum, Xanthan, Iota carrageenan and a combination of Locust bean gum and Xanthan were used as additives (5 g/kg) in minced fillets of whiting. Samples of these treatments were stored at −18°C for 3 months and were evaluated at regular intervals for pH, water-holding capacity, texture, extractable myosin, color change and formation of dimethylamine and formaldehyde.

Xanthan, Iota carrageenan and the combination of Locustbean gum and Xanthan presented the highest values of water holding capacity. The treated samples showed much less increase in compressive strength, modulus of elasticity, resilience and shear stress than the Blank samples during the three months of frozen storage.

Xanthan and the combination of Locust bean gum and Xanthan improved the whiteness of the cooked samples. In the same samples and the Iota carrageenan one, the formation of dimethylamine and formaldehyde was slowed down during frozen storage.

INTRODUCTION

In Japan and some oriental countries, mechanical deboned fish meets the requirements of a raw material for well-established traditional products (Suzuki 1981), but in western countries the interest is still limited. Incorporation of minced fish in products as fish burgers, fish sticks, patties and spreads was achieved and a growing market for these products can be forseen (Martin 1976). At the moment, however, much more minced fish can be produced than marketed (Connell 1982).

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The poor keeping quality of frozen deboned fish flesh is the main factor which hinders the full utilization of this new technology (Arocha and Toledo 1982). Generally, changes in texture, water-holding capacity, flavor and color are accelerated in the minced fish (Tran and Han-Ching 1982; Froning 1981). Additions of sucrose (4 to 10%), sorbitol (4 to 5%) and polyphosphates (0.2 to 0.5%) have been used to improve the quality of frozen minced fish in Japan ("surimi") (Suzuki 1981; Matsumoto 1979). However, these additions caused changes in taste which lowered the acceptance by occidental consumers.

In our previous paper (da Ponte *et al.* 1985), we found that certain alginates (Kelcosol and Kelcogel) have a beneficial effect on the texture and water-holding capacity of minced fillets of whiting kept in frozen storage. We continued this study by examination the additions of Xanthan, Locust bean gum, Iota carrageenan, and a combination of Locust bean gum and Xanthan.

EXPERIMENTAL

Whiting (Merlangius merlangus) was caught in the southern part of the North Sea on the 24th of October and handled as before (da Ponte *et al.* 1985). The bags obtained with 2 kg of fillets were thawed in cold tap water and were passed through a Kenwood mincer model A.720, using a mincing screen with 4.4 mm ϕ holes. The resulting minced fish was divided in portions and different hydrocolloids were spread over the surface (5 g per kg of minced fish, 1:1 ratio for the combination of Xanthan and Locust bean gum). In order to improve the hydration of the hydrocolloids, they were predissolved in water, freeze-dried and ground before adding to the minced fish. The hydrocolloids and the minces were gently mixed with a spoon and kept in plastic bags on melting ice for two hours.

Hydrocolloids used were as followed: (1) Xanthan gum, Keltrol F (KTLF-67203A), from Kelco (New Jersey, U.S.A.), (2) Locust bean gum (LBG), Meyprodin 200, from Meyhall Chemical (Switzerland) and (3) Iota carrageenan (HF33895-96), from Copenhagen Pectin Factory Ltd. (Denmark).

Samples of 150 g were repacked in plastic bags, blast frozen and kept at −18°C for three months. Samples of each treatment were withdrawn from the frozen storage, thawed in cold tap water and kept for 3 h in melting ice until analyses were carried out.

Extractable myosin (EM), dimethylamine (DMA) and formaldehyde (FA) formation, texture analysis, pH measurements, water-holding capacity of the raw minced fish (WHCR) and of the cooked minced fish

(WHCC), cook drip loss (CDL), protein and carbohydrate content of the supernatants released in the WHCR and in the WHCC, and viscosity determinations of these supernatants were determined in the same way as described before (da Ponte *et al.* 1985). The method outlined by Graham (1977) was used to determine the carbohydrate content of the supernatants of the combination of LBG and Xanthan.

A Hunterlab model D25D3P color difference meter was used for color determinations. It was standardized with a white color standard, Hunterlab standard DC-220 (L = 91.40, a = −0.9, b = 0.5). Twenty-five grams of minced fish were cooked in the same way as for the CDL determination. The resulting fish cake was packed in a 5 cm ϕ plastic petri dish and the three color co-ordinates measured. The petri dish was inserted three times to obtain three sets of color co-ordinates. Two samples were measured for each treatment.

Statistical Analysis

Data were analysed using the statistical analysis systems computer package (Baker and Nelder 1978). Polynomial regression lines were evaluated for each property and for each treatment. The t-test at 5% level of significance was used to compare different treatments.

RESULTS AND DISCUSSION

pH Measurements

After preparation, the pH's of the different treatments were between 6.7 and 6.8. There was a slight increase (0.1) during the first 12 days of frozen storage and afterwards the pH was almost constant.

Water-holding Capacity of the Raw Material (WHCR)

Remarkable differences in WHCR were observed in the different treatments (Fig. 1). Xanthan presented the highest values of WHCR: about 20% more than the Iota carrageenan and the combination of LBG and Xanthan, about 25% more than the LBG and about 30% more than the Blank. Xanthan is a well-known thickening agent, it dissolves readily in hot or cold water, producing relatively high viscosity solutions at low concentrations (Kovacs and Kang 1977).

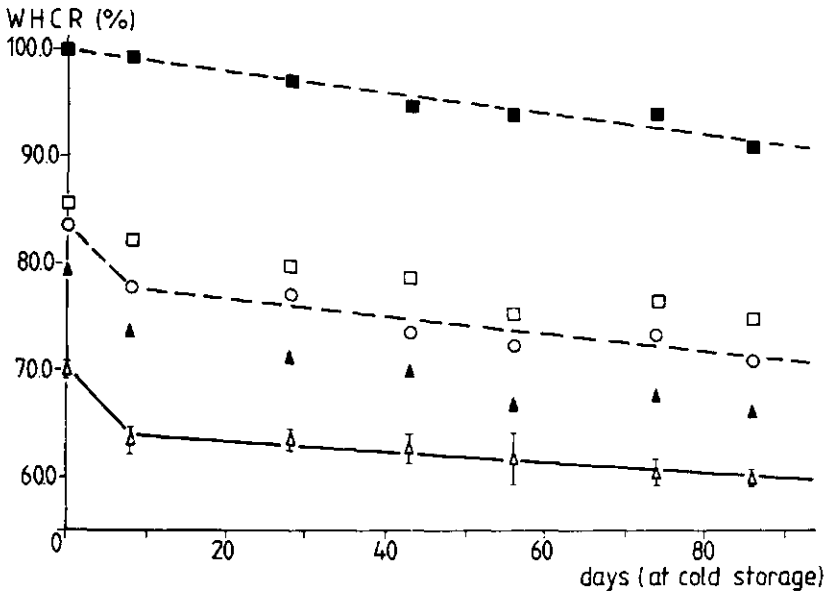

FIG. 1. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON WHCR OF FROZEN STORED MINCED FISH MUSCLE.
△ Blank, ▲ LBG, □ Iota carrageenan, ■ Xanthan and O LBG and Xanthan.
The vertical bars represent standard deviation.

The supernatant obtained from the second highest WHCR (Iota carrageenan) was a heterogeneous system. The fluid obtained had lumps probably consisting of hydrated particles of Iota carrageenan. It is possible that some of these hydrated particles got stuck in the residue and increased the WHCR.

The combination of LBG and Xanthan showed also high values of WHCR. This combination is well-known for its formation of strong cohesive and thermoreversible gels. This property is used to make instant puddings (Cottrell *et al.* 1980; Mc Neely and Kang 1973). In our experiments, it was noticed that only small amounts of carbohydrates, mainly LBG, were found in the supernatants (Table 6). This was confirmed by the low viscosities of these supernatants (Fig. 9).

Among the different additions, LBG presented the lowest WHCR. LBG is only partly soluble in cold water (Meer 1977), demonstrated by some hydrated particles remaining suspended in aqueous solution even after long stiring at room temperature. This insufficient solubilization is shown by the viscosity and the carbohydrate content of the supernatants (Fig. 9 and Table 6). The WHCR of the LBG was, however, significantly higher than the Blank (about 6 to 10%). As with Iota carrageenan, this

may be due to hydrated particles of LBG which were not solubilized and so carried to the bottom of the tubes with the fish residue.

Water-holding Capacity of the Cooked Material (WHCC) and Cook Drip Loss (CDL)

WHCC was almost constant during the 3 months of frozen storage. During this time, six different measurements were carried out and the average values of WHCC are presented in Table 1A. CDL showed a slight decrease during the first 4 weeks of the frozen storage and afterwards the CDL presented a small increase (Fig. 2). Similar results were described in our previous paper (da Ponte *et al.* 1985).

WHCC and CDL are both indicators of the water holding capacity but different in their meaning. The CDL measured the drip which flows through the fish cake by the action of the gravity and by the sucking effect of the filter paper. The WHCC measured not only this "free drip" but also the fluid which is expelled from the possibly destroyed gel by the pressure of the centrifugal force.

WHCC, 100-CDL and the liquid expelled extra to the CDL in the WHCC determination ((100-CDL)-WHCC) are presented in Table 1A for the actual additions and Table 1B for the additions of our previous paper

Table 1. Comparison of the CDL and WHCC determinations (w/w%) among the different additions.

A. ACTUAL ADDITIONS

	BLANK	LBG	IOTA CARRAG.	XANTHAN	LBG+XANTHAN
100-$\overline{CDL}$ [a]	74.3±1.3	79.9±0.9	82.6±1.8	90.5±0.5	85.7±1.1
$\overline{WHCC}$ [b]	57.8±0.5	67.6±0.9	74.4±1.5	75.8±0.9	77.7±2.2
(100-$\overline{CDL}$)-$\overline{WHCC}$ [c]	16.5	12.3	8.2	14.7	8.0

B. DATA FROM THE PREVIOUS PAPER DA PONTE et al. (1985)*

	BLANK	LP	HP	CARRAG.	KELCOGEL	KELCOSOL	KELCOCOL.
100-$\overline{CDL}$ [a]	77.6±3.0	76.8±5.1	75.2±1.9	84.4±1.5	85.2±2.4	83.1±3.7	75.6±3.7
$\overline{WHCC}$[b]	59.0±3.4	55.7±2.4	56.5±2.4	69.5±1.1	60.3±1.1	59.2±3.1	56.5±4.4
(100-$\overline{CDL}$)-$\overline{WHCC}$[c]	18.6	21.1	18.7	14.9	24.9	23.9	19.1

[a]Average and standard deviation of different measurements of CDL and subtracted from 100% (minced tissue).
[b]Average and standard deviation of diferent measurements of WHCC
[c]Fluid expelled from the fish cake of WHCC extra to the CDL.
*LP-Low Methoxy-esterified Pectin; HP-High Methoxy-esterified Pectin; CARRAG.-Kappa carrageenan; and Kelcogel, Kelcosol, and Kelcocol.-different types of alginates.

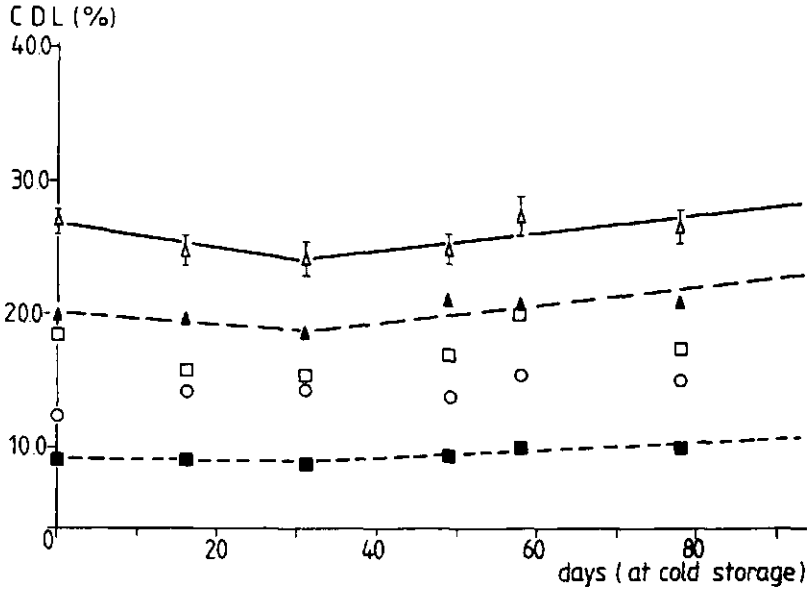

FIG. 2. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON CDL OF FROZEN STORED MINCED FISH MUSCLE.
See the note in Fig. 1 for the symbols.

(da Ponte *et al.* 1985). Xanthan, the combination of LBG and Xanthan, Kelcosol and Kelcogel (different types of alginates), Kappa carrageenan and Iota carrageenan showed average values of 100-CDL significantly higher than the Blank. However, in the WHCC determination only LBG, Kappa carrageenan, Iota carrageenan, Xanthan and the combination of LBG and Xanthan were significantly higher than the Blank (more than 10%).

The high WHCC of the Kappa carrageenan, Iota carrageenan and the combination of LBG and Xanthan may be explained by the formation of a hydrocolloid gel separated from the fish cake. The relative good resistance of the LBG treatment to the centrifugal force (12.3%) may be due to the hydrated particles of LBG which were not solubilized and were carried to the bottom of the tubes with the fish residue. Xanthan treatment had the lowest CDL, while its WHCC was similar to the Iota carrageenan and the combination of LBG and Xanthan. The alginates (Kelcosol and Kelcogel), experimented in our previous paper, showed also good retention of water in the CDL determination but poor resistance to the centrifugal force. Viscosity seems to be more important in holding water in the CDL determination than in the WHCC.

HYDROCOLLOIDS FROZEN MINCED WHITING

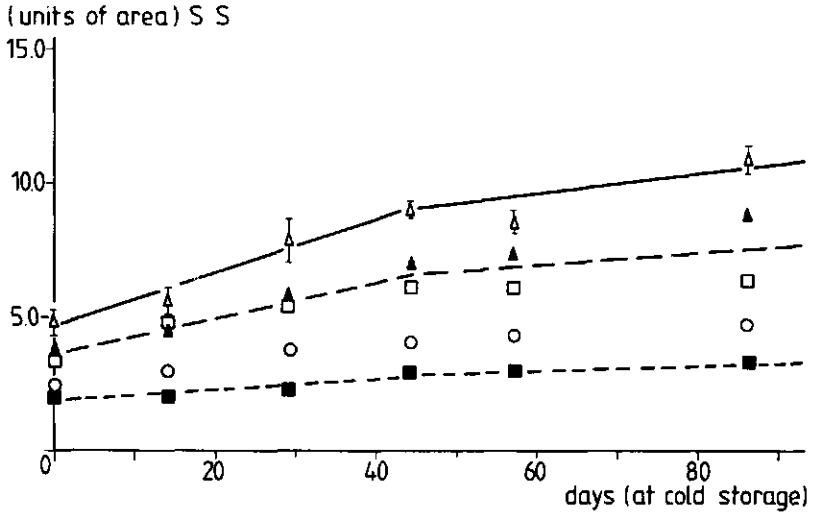

FIG. 3. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON SS OF FROZEN STORED MINCED FISH MUSCLE.
See the note in Fig. 1 for the symbols.

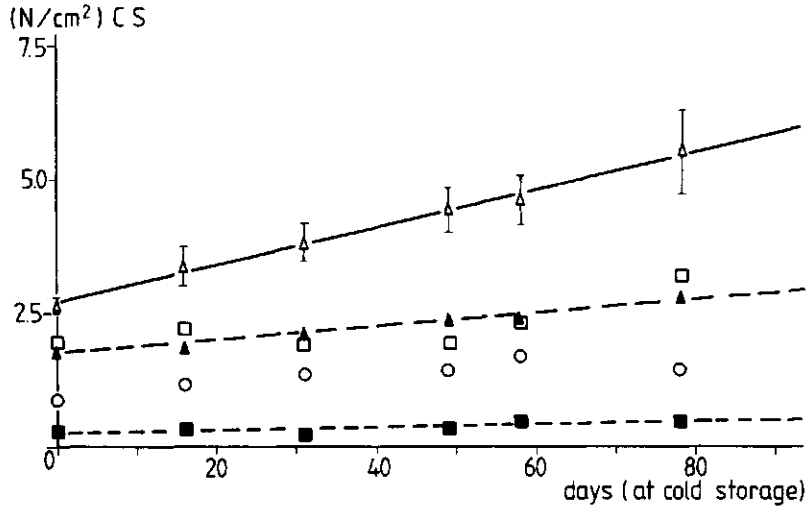

FIG. 4. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON CS OF FROZEN STORED MINCED FISH MUSCLE.
See the note in Fig. 1 for the symbols.

Texture Analysis

The shear stress (SS) measured in the Kramer Shear Press on the raw minced fish samples is shown in Fig. 3. The compressive strength (CS), modulus of elasticity (ME) and resilience (R) measured in an overload dynamic apparatus on the cooked minced fish are reported, respectively, in the Fig. 4, 5 and 6.

The Blank samples became much more tough, elastic and resilient than the treatments, while the Xanthan samples showed the lowest increase after three months of frozen storage. The addition of hydrocolloids has a big impact on texture parameters. The samples with Xanthan were very soft, like a paste or a pudding. The texture of LBG and Iota carrageenan samples was similar to the Blank. These distinct effects may be profited in existing products or in the development of new products from minced fish (Kogyo 1982). Xanthan will give products as pastes with very soft texture, excellent water holding capacity and good stability during frozen storage. Fish minces with Iota carrageenan and LBG can be appropriate for the production of fish sticks or fish burgers.

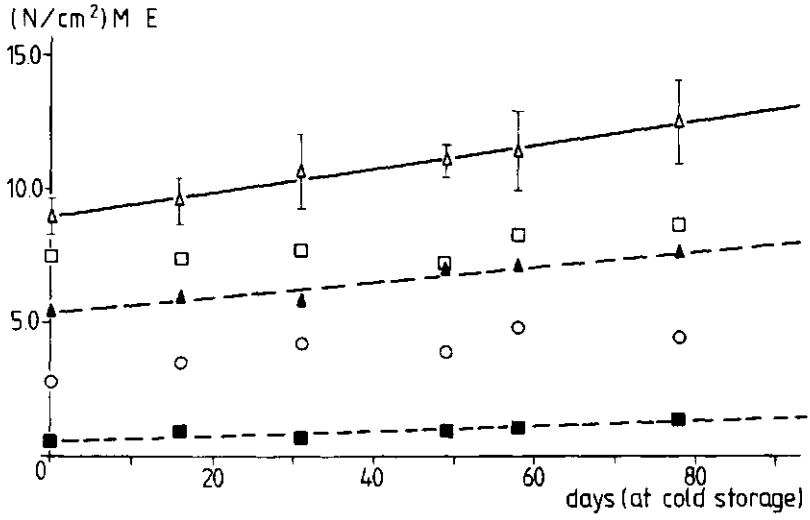

FIG. 5. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON ME OF FROZEN STORED MINCED FISH MUSCLE.
See the note in Fig. 1 for the symbols.

HYDROCOLLOIDS FROZEN MINCED WHITING

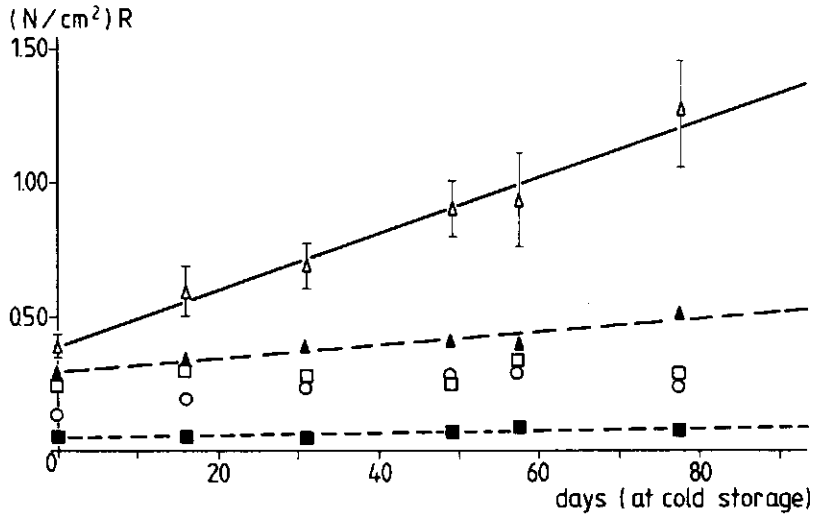

FIG. 6. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON R. OF FROZEN STORED MINCED FISH MUSCLE.
See the note in Fig. 1 for the symbols.

Extractable Myosin (EM)

During the first week the decrease of EM was very sharp in all treatments (Table 2). In agreement with our previous results (da Ponte *et al.* 1985) most of the initial EM disappeared within 1 month.

Table 2. Extractable myosin[a] (mg/g of fish) of frozen stored minced fish at time intervals.

DAYS AT -18°C	BLANK	LBG	IOTA CARRAG.	XANTHAN	LBG+XANTHAN
0	6.6±1.1	5.4±1.0	5.4±0.9	4.0±0.1	4.9±0.5
9	2.8±0.1	2.8±0.1	2.9±0.1	2.8±0.1	2.8±0.1
25	2.6±0.0	2.5±0.2	2.6±0.1	2.7±0.1	2.6±0.1
42	2.5±0.1	2.4±0.0	2.6±0.0	2.5±0.1	2.4±0.1
63	2.4±0.1	2.4±0.0	2.5±0.0	2.4±0.0	2.4±0.0

[a]Mean of duplicate samples± standard deviation.

Table 3. Dimethylamine[a] (μg DMA-Nitrogen/g of fish) of frozen stored minced fish at time intervals.

DAYS AT -18°C	BLANK	LBG	IOTA CARRAG.	XANTHAN	LBG+XANTHAN
0	41.2±2.1	42.3±2.7	40.1±0.8	37.1±1.4	38.3±1.2
24	132.1±1.1	146.0±5.2	124.1±4.5	113.2±4.9	125.8±2.0
39	161.9±3.3	161.4±5.4	154.5±3.1	126.9±3.8	149.5±6.1
53	184.1±1.8	179.6±4.7	167.5±7.5	150.4±7.6	164.5±1.1
65	233.8±0.8	209.0±3.8	192.4±3.4	169.0±1.4	188.5±5.2
79	189.8±2.9	189.6±4.3	169.4±5.4	153.3±2.3	176.3±3.6

[a]Mean of triplicate samples ±standard deviation.

Table 4. Formaldehyde content[a] (μg/g of fish) of frozen stored minced fish at time intervals.

DAYS AT -18°C	BLANK	LBG	IOTA CARRAG.	XANTHAN	LBG+XANTHAN
0	13.4±0.5	11.1±0.9	12.3±0.1	9.7±0.4	12.4±0.3
24	42.9±0.9	45.5±0.5	40.4±0.1	39.4±0.2	41.1±2.4
39	76.4±0.4	73.5±0.5	61.3±1.5	48.1±1.2	55.7±2.0
53	85.4±3.0	89.6±2.5	67.0±4.0	54.2±3.4	65.3±3.2
65	113.8±2.8	94.9±2.4	72.6±2.2	60.4±2.2	69.4±1.0
79	97.0±1.1	103.8±0.2	83.0±0.1	67.4±1.7	84.0±0.5

[a]Mean of duplicate samples±standard deviation.

Dimethylamine (DMA) and Formaldehyde (FA)

The formation of DMA and FA during 3 months of storage at −18°C is reported in Tables 3 and 4. Xanthan showed the lowest increase in DMA and FA formation. Iota carrageenan and the combination of LBG and Xanthan had also significantly lower values of DMA and FA in comparison with the Blank and LBG treatments. These results may suggest an inhibitory effect of Xanthan and Iota carrageenan on the formation of DMA and FA. A special study is in progress for better understanding of these experimental data.

Table 5. Protein nitrogen content[a] (mg/ml) of the supernatants obtained from the WHCR at time intervals.

DAYS AT -18°C	BLANK	LBG	LBG+XANTHAN
0	13.0±0.9	12.6±0.8	13.1±0.1
8	13.4±0.3	13.0±0.7	12.9±0.6
28	10.2±0.1	10.9±0.7	12.9±0.6
43	10.4±0.5	10.2±0.5	12.0±0.8
56	9.7±0.1	10.0±0.4	10.1±0.4
74	10.2±0.5	9.7±0.1	10.0±0.1
86	10.1±0.3	10.1±0.2	10.8±0.8

[a]Mean of duplicate samples ±standard deviation

Table 6. Protein nitrogen content[a] (mg/ml) of the supernatants obtained from the WHCC at time intervals.

DAYS AT -18°C	BLANK	LBG	IOTA CARRAG.	XANTHAN	LBG+XANTHAN
0	7.2±0.1	7.4±0.1	7.2±0.3	9.4±0.6	7.3±0.4
8	7.0±0.2	7.6±0.6	7.2±0.1	9.3±0.2	7.7±0.4
28	7.1±0.6	7.2±0.4	6.8±0.1	8.8±0.3	6.7±0.1
43	6.7±0.6	7.4±0.4	6.4±0.3	8.7±0.2	6.8±0.3
56	6.9±0.3	7.1±0.4	6.9±0.3	8.4±0.1	6.8±0.2
74	7.0±0.2	6.9±0.1	6.7±0.3	8.4±0.2	7.3±0.1

[a]Means of duplicate samples±standard deviation

Protein, Carbohydrate Content and Viscosity of the Supernatants Released in WHC Determination

The protein nitrogen contents of the supernatants of WHCR were similar (Table 5). A decrease of about 3 mg/ml of protein nitrogen was observed in all treatments at the end of the storage. In the WHCC, the protein nitrogen content was about 7.0 mg/ml, except for Xanthan at which it varied from 9.4 to 8.4 mg/ml (Table 6). In this case, the supernatants looked milky which could indicate the aggregation of

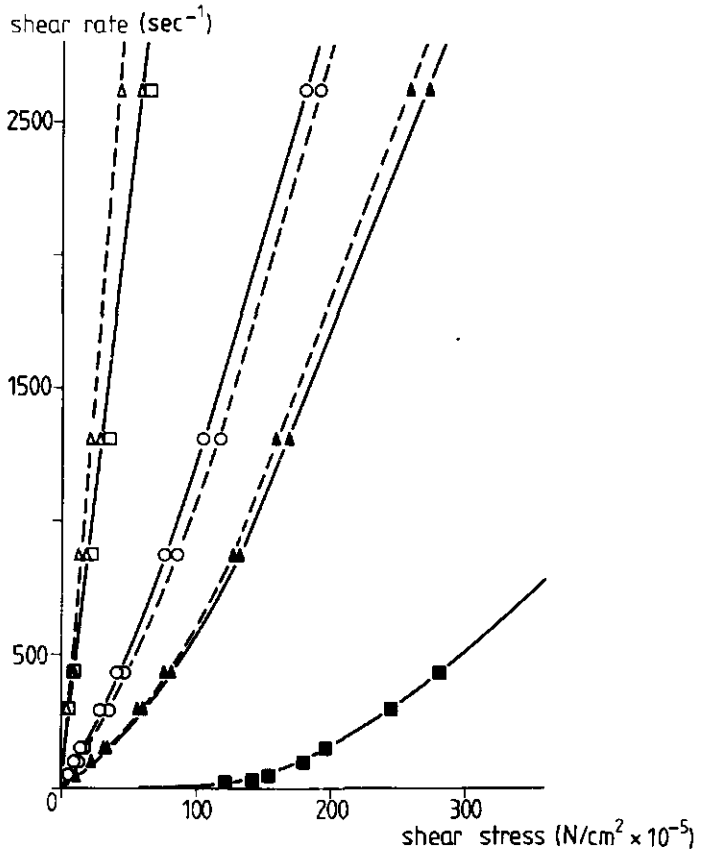

FIG. 7. FLOW CURVES OF THE DIFFERENT SUPERNATANTS OBTAINED FROM THE WHCR AND WHCC AT ZERO TIME. THE BROKEN LINES (--) REPRESENT THE SUPERNATANTS OF THE WHCR AND THE FULL LINES (-) REPRESENT THE SUPERNATANTS OF THE WHCC.
See the note in Fig. 1 for the symbols.

particles with sol-stability. This suspending action of Xanthan gum (Cottrell *et al.* 1980) may be responsible for the high protein nitrogen content of the Xanthan supernatant.

The flow curves presented in Fig. 7 are in agreement with the carbohydrate concentrations (Table 7 and 8). LBG is limited soluble in cold water and even at 100°C this gum is not fully soluble. The carbohydrate content of the Xanthan supernatant was almost the double of the LBG (Table 8).

In the determination of the WHCR, the volume of Xanthan supernatant was insufficient and the Iota carrageenan supernatant was too heterogeneous for carrying out any of the analyses.

HYDROCOLLOIDS FROZEN MINCED WHITING

Table 7. Carbohydrate content (mg/ml) of the supernatants obtained from the WHCR at time intervals.

DAYS AT -18°C	BLANK[1]	LBG[2]	LBG+XANTHAN[3]
0	2.1	3.5	2.3
8	2.2	3.5	2.1
28	2.0	3.2	2.4
43	2.0	3.6	2.0
56	2.1	3.4	1.9
74	2.0	3.0	2.3
86	2.1	3.3	2.1

[1]Equivalents of glucose obtained from the colorimetric method of Dubois et al. (1956)
[2]Calculated by subtracting the glucose content of the Blank(1)
[3]Calculated in the same way as (2). No Xanthan was detected by the method of Graham (1977)

Table 8. Carbohydrate content (mg/ml) of the supernatants obtained from the WHCC at time intervals.

DAYS AT -18°C	BLANK[1]	LBG[2]	IOTA CARRAG.[2]	XANTHAN[2]	LBG+XANTHAN[3]
0	2.3	3.6	0.6	7.2	2.2
8	2.2	3.5	0.7	6.5	1.9
28	2.1	2.9	0.4	6.3	2.3
43	2.3	3.4	1.1	6.4	1.7
56	2.1	3.2	0.7	6.5	2.2
74	2.1	3.7	1.1	6.3	2.4

[1]Equivalents of glucose obtained from the colorimetric method of Dubois et al. (1956)
[2]Calculated by subtracting the glucose content of the Blank (1) and multiplying with factors which were determined for each carbohydrate (LBG=1.00; IOTA CARRAGEENAN=0.54; XANTHAN=0.84)
[3]Calculated in the same way as (2). No Xanthan was detected by the method of Graham (1977)

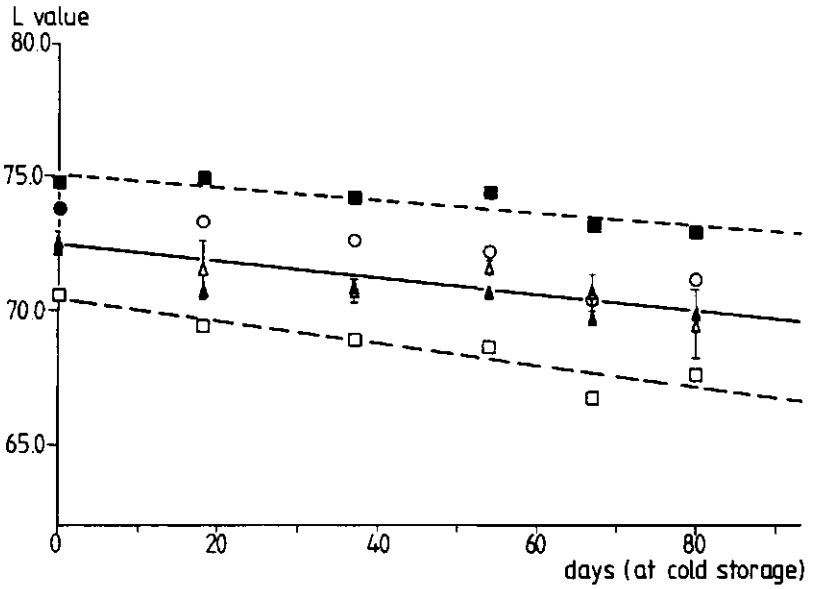

FIG. 8. EFFECT OF TIME AND DIFFERENT HYDROCOLLOIDS ON THE WHITENESS (HUNTER L COLOR) OF THE FROZEN MINCED FISH AFTER COOKING.
See the note in Fig. 1 for the symbols.

Color Changes

A small decrease of the whiteness (Hunter L-color) was observed in all treatments during the frozen storage (Fig. 8). This was accompanied by an increase in greeness (Hunter a-value) and increase in yellowness (Hunter b-value). The Xanthan addition showed a significant improvement in whiteness of the fish cakes after cooking. The combination of LBG and Xanthan improved slightly the whiteness of the fish cakes while Iota carrageenan showed a small reduction.

CONCLUSIONS

The hydrocolloids used change the texture and water-holding capacity of minced fillets of whiting, during frozen storage. To a certain extent, it is possible to control and stabilize the different parameters of texture. This may be useful for the development of products from minced fish.

Xanthan and Iota carrageenan slowed down the formation of DMA and FA. Xanthan improved the whiteness of the minced fish cakes.

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5. Effects of different types of carrageenans and carboxymethyl celluloses on the stability of frozen stored minced fillets of cod

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Summary

Different types of carrageenans and carboxymethyl celluloses were used as additives (5 g/kg) for minced fillets of cod. Samples of these treatments were stored at −18°C for 10 weeks and were evaluated at regular intervals for pH, water holding capacity, texture, extractable myosin and formation of dimethylamine and formaldehyde.

Except Kappa carrageenan the additions improved the water holding capacity of the raw and cooked minced fish and decreased toughening during frozen storage. The addition of Iota carrageenan resulted in samples with remarkable texture stability and so after 10 weeks of frozen storage the texture of this treatment was similar to fresh minced cod. The higher the viscosity of carboxymethyl cellulose, the more it increased the softness and water holding capacity.

Introduction

Several attempts have been made to preserve the functional properties of minced fish during frozen storage. Additions of carbohydrates, amino acids and related compounds, polyphosphates, sodium chloride and anti-oxidants have been tried (Tran & Hang-Ching, 1982; Suzuki, 1981; Codex Alimentarius Commission, 1981; Matsumoto, 1979; Ohnishi, Tsuchiya & Matsumoto, 1978). More recently, hydrocolloids such as sodium alginate, pectin, carboxymethyl cellulose, locust bean gum, guar gum, carrageenan and xanthan gum were listed as additives by the Codex Alimentarius Commission (1981, 1983).

In previous papers we described the effect of additions of different types of alginates, carrageenans, pectins, xanthan andlocustbean gum on the stability of frozen stored minced fillets of whiting (da Ponte, Roozen & Pilnik, 1985a; 1985b). Remarkable changes in texture and water holding capacity were observed in the minced whiting depending on the type of hydrocolloid used. These important effects may find use in products already marketed, such as fish sticks and fish burgers and in the development of new products from minced fish. In these experiments, we pre-dissolved the hydrocolloids in water, freeze dried and ground them before adding to the minced fish. However, this is impractical because of the costs involved in the freeze drying operation. In the present study, we omitted this pre-treatment of the hydrocolloids. Three types of carrageenans were compared of which Kappa and Iota are not completely soluble and Lambda is soluble in cold water. Four types of carboxymethyl

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cellulose with similar degrees of substitution (0.80–0.95) and viscosities from 20–30 to 2000–4500 centipoises (at 1% concentration in water at 25°C) were studied.

Materials and methods

Skinned fillets of cod were bought in the local market of Wageningen on 25 January 1984. They were packed in 2 kg plastic bags, blast frozen and kept frozen at −45°C for 2 weeks. Subsequently they were thawed in cold tap water and were passed through a Kenwood mincer model A.720, using a mincing screen with a 4.4 mm mesh. The resulting minced fish was divided in portions and different hydrocolloids were spread over the surface (5 g per kg of minced fish). The hydrocolloids and the minces were gently mixed with a spoon and kept in plastic bags on melting ice for 2 hr. Hydrocolloids used were:

(i) Potassium Kappa carrageenan (HF 55758-63) and Iota carrageenan (HF 33895-96), from Copenhagen Pectin Factory Ltd (Denmark);

(ii) Lambda carrageenan, Viscarin 402, (Batch 321403), from Marine Colloids Division, FMC Corporation (Springfield, New Jersey, U.S.A.);

(iii) Carboxymethyl cellulose (CMC), Akucell AF 705, AF 1505, AF 2205 and AF 2805 from Enka bv, Industrial Colloids (Holland).

Samples of 150 g were repacked in plastic bags, blast frozen and kept at −18°C for 10 weeks. Samples of each treatment were withdrawn from the frozen storage, thawed in cold tap water and kept for 3 hr in melting ice until analyses were carried out.

Extractable myosin (EM), dimethylamine (DMA) and formaldehyde (FA) formation, texture analysis, pH measurements, water holding capacity of the raw and cooked minced fish (WHCR and WHCC), cook drip loss (CDL), protein and carbohydrate content of the supernatants in the WHCR and in the WHCC, and viscosity determinations of these supernatants were determined in the same way as described before (da Ponte *et al.*, 1985a).

Statistical analysis

Data were analysed using the statistical analysis systems computer package (Baker & Nelder, 1978). Polynomial regression lines were evaluated for each property and for each treatment. The *t*-test at the 5% level of significance was used to compare different treatments.

Results and discussion

pH measurements

In all cases (different treatments, time of storage) the pH's were between 6.5 and 6.7. The pH of the blank and the carrageenan additions showed a slight fall of 0.1 during the first 2 weeks of frozen storage and afterwards a small increase of 0.2. The pH of the CMC additions increased steadily from 6.5 to 6.7 during frozen storage.

Water holding capacity of the raw material (WHCR)

During the 10 weeks of frozen storage at −18°C, WHCR decreased in all cases, and, with the exception of the Kappa carrageenan, the WHCR of the treatments were significantly higher than the ones of the blank (Fig. 1). In the case of the CMC additions, the WHCR's correlate positively with their viscosities (Fig. 7).

The Kappa carrageenan treatment had a similar or lower WHCR than the blank.

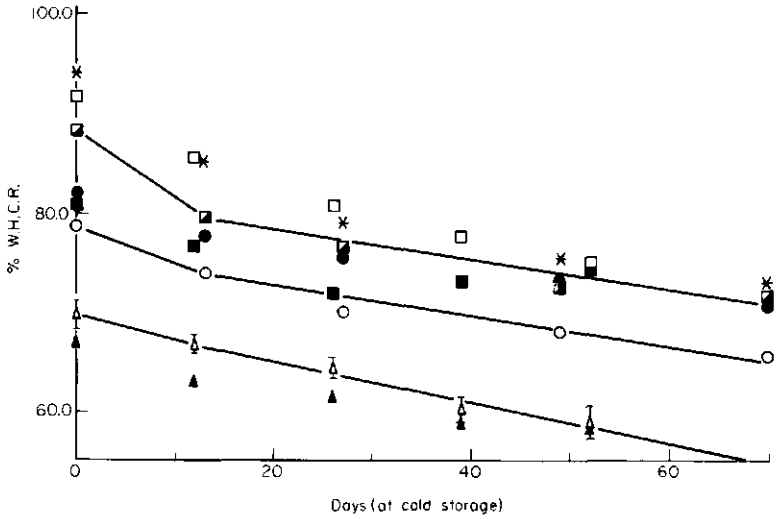

Figure 1. Effect of time and different hydrocolloids on WHCR of frozen stored minced fish muscle. △ Blank; ▲ Kappa carrageenan; □ Lambda carrageenan; ■ Iota carrageenan; O Carboxymethyl cellulose AF 705; ● Carboxymethyl cellulose AF 1505; ◪ Carboxymethyl cellulose AF 2205; and ★ Carboxymethyl cellulose AF 2805.

The mineral content of cod is about 0.320% for potassium, 0.077% for sodium, 0.016% for calcium and 0.023% for magnesium (Paul & Southgate, 1978). At this potassium content and at room temperature Kappa carrageenan is difficult to hydrate and form a solution in the raw minced fish. In a previous experiment, freeze drying helped Kappa carrageenan to hydrate and so an improvement of the WHCR of whiting was observed (da Ponte *et al.*, 1985a).

The Iota and Lambda carrageenan additions improved the WHCR. This should be ascribed to hydrated particles of Iota and Lambda carrageenan which remained in the fish residue during the WHCR determination. Similar phenomena were observed in our previous experiment (da Ponte *et al.*, 1985b) in which the use of freeze dried Iota carrageenan was shown to enhance the value of the WHCR. The supernatants obtained in that case were heterogeneous with visible lumps of hydrated particles of Iota carrageenan.

Water holding capacity of the cooked material (WHCC) and cook drip loss (CDL)

WHCC and CDL were almost constant during the ten weeks of frozen storage (Table 1a and Fig. 2). The increase of WHCC and decrease of CDL correlates with ranking of the viscosities of the CMC additions. For these hydrocolloids the differences of $(100-\overline{CDL})-\overline{WHCC}$ were similar or higher than the blank (Table 1a). The CMC additions did not seem to influence the resistance of the fish cake to the impact of the centrifugal force.

In the case of the carrageenan treatment, the difference $(100-\overline{CDL})-\overline{WHCC}$ were lower than the blank. Comparing the present results of the carrageenan additions, with previous results (Table 1b), it is clear that freeze drying improved the water holding capacity of the Kappa and Iota carrageenan significantly. Formation of separated gels of Kappa, Iota and perhaps Lambda carrageenan may explain the good resistance of the

Table 1. Comparison of the CDL and WHCC determinations (w/w %) among the different additions

(a) Actual additions to minced fillets of cod

	Blank	Kappa carrag.	Lambda carrag.	Iota carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
$100-\overline{CDL}$*	72.6±0.8	77.1±1.9	78.0±0.9	77.6±1.9	73.4±1.6	77.1±1.8	79.6±1.4	85.7±1.5
$\overline{WHCC}$†	55.3±2.4	61.1±2.4	62.3±1.9	64.0±2.1	56.6±1.0	59.4±1.5	61.9±3.9	67.0±1.5
$(100-\overline{CDL})-\overline{WHCC}$‡	17.3	16.0	15.7	13.6	16.8	17.7	17.7	18.7

(b) Kappa and Iota carrageenan were freeze dried before adding to minced fillets of whiting (da Ponte *et al.*, 1985a; 1985b)

	Kappa carrageenan		Iota carrageenan	
	Blank	Addition	Blank	Addition
$100-\overline{CDL}$*	77.6±3.0	84.4±1.5	74.3±1.3	82.6±1.8
$\overline{WHCC}$†	59.0±3.4	69.5±1.1	57.8±0.5	74.4±1.5
$(100-\overline{CDL})-\overline{WHCC}$‡	18.6	14.9	16.5	8.2

*Average and standard deviation of different measurements of CDL and subtracted from 100% (minced tissue).

†Average and standard deviation of different measurements of WHCC.

‡Fluid expelled extra from the fish cake of the WHCC compared to the CDL.

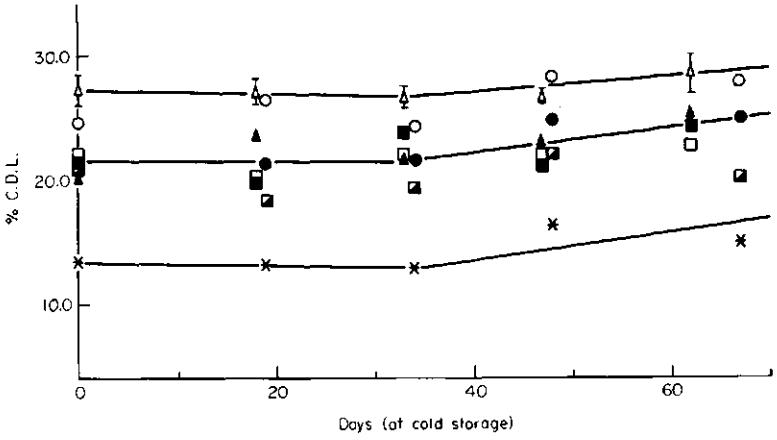

Figure 2. Effect of time and different hydrocolloids on CDL of frozen stored minced fish muscle. (See Fig. 1 for symbols.)

fish fluid against being expelled by the centrifugal force of the WHCC test. The commercial Lambda carrageenan might be a mixture of different types of carrageenan relatively rich in the Lambda type.

Texture analysis

The blank and Kappa carrageenan containing samples became much tougher (compressive strength, CS and shear stress, SS), elastic (modulus of elasticity, ME) and resilient (R) than all the other treated samples during frozen storage (Figs 3, 4, 5 and 6). In the SS determination of the raw minced fish samples, the Kappa carrageenan gave values similar or higher than the blank. It seems that the Kappa carrageenan had no effect on the texture of the raw minced fish and the initial high values of SS of this

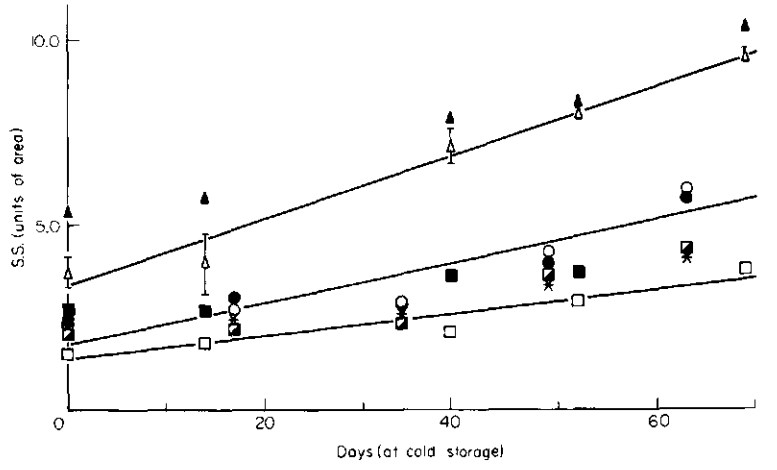

Figure 3. Effect of time and different hydrocolloids on SS of frozen stored minced fish muscle. (See Fig. 1. for symbols.)

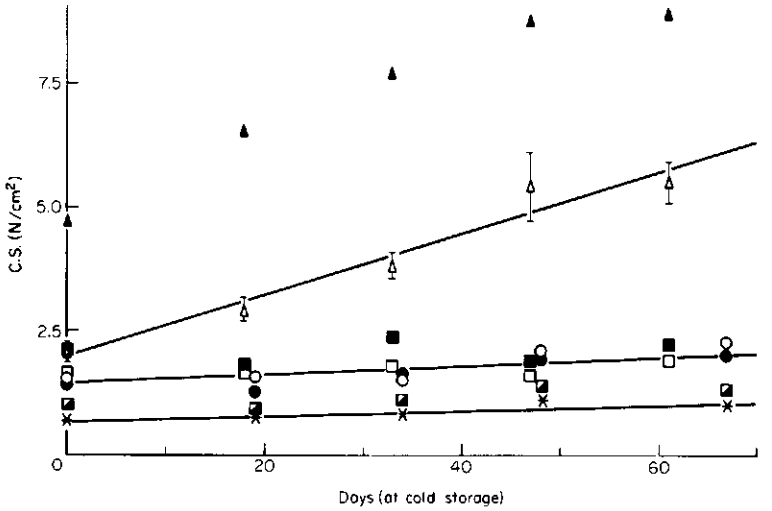

Figure 4. Effect of time and different hydrocolloids on CS of frozen stored minced fish muscle. (See Fig. 1 for symbols.)

treatment were probably due to the heterogenity of the mixture of the hydrocolloid with the minced fish. After heating, the Kappa carrageenan addition showed significantly higher values of CS, ME and R than all the other treatments. This should be attributed to the formation of a rigid gel of Kappa carrageenan separated from the fish cake and additional to the toughening of the blank.

The Iota and Lambda carrageenan additions showed remarkable stability of SS, CS, ME and R during storage. After 10 weeks the values of CS, ME and R of the Iota carrageenan treatment were (on average) similar to the initial values of the blank. The

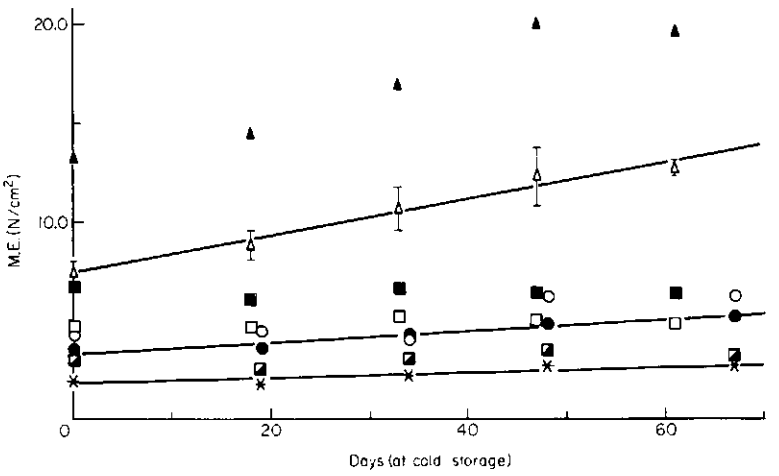

Figure 5. Effect of time and different hydrocolloids on ME of frozen stored minced fish muscle. (See Fig. 1 for symbols.)

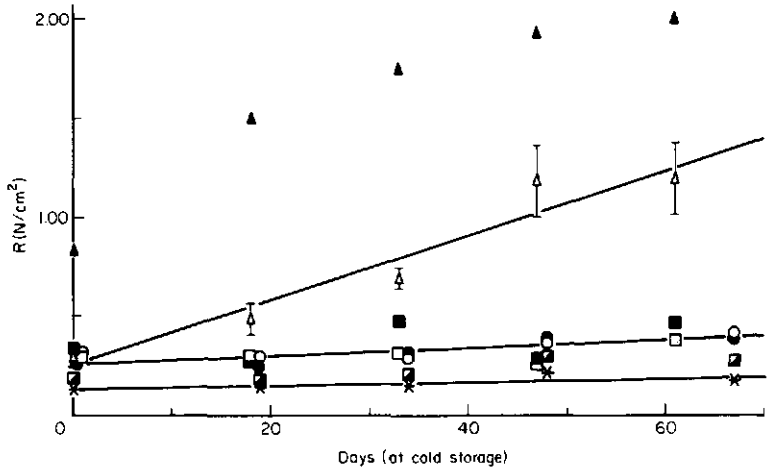

Figure 6. Effect of time and different hydrocolloids on R of frozen stored minced fish muscle. (See Fig. 1 for symbols.)

addition of Lambda carrageenan resulted in somewhat lower values than Iota carrageenan and they were in the range of the CMC treatments.

The effect of the viscosity of the hydrocolloid on the texture of the minced fish can clearly be seen in the CMC additions. With the increase of the viscosity, the minced fish became softer and less elastic. Similar results were obtained in our previous experiments with the additions of alginates and xanthan gum (da Ponte *et al.*, 1985a; 1985b).

Extractable myosin (EM)

With the exception of the Lambda carrageenan addition, all treatments exhibited similar losses of EM during the 10 weeks of frozen storage (Table 2). More experimental data are necessary to get a better understanding of the Lambda carrageenan results.

Table 2. Extractable myosin* (mg/g of fish) of frozen stored minced fish at time intervals

Days at −18°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
0	5.0±0.3	4.3±0.2	4.2±0.2	3.4±0.3	5.4±1.4	6.4±0.1	5.2±0.8	6.9±0.4
10	3.2±0.2	2.9±0.7	3.6±0.2	2.9±0.2				
11					2.9±0.1	2.8±0.1	2.6±0.1	2.6±0.1
24	3.2±0.2	3.5±0.2	4.1±0.1	3.3±0.2				
25					3.0±0.2	3.1±0.2	2.9±0.0	2.8±0.1
45	2.9±0.3	2.8±0.3	3.2±0.2	2.6±0.3				
46					2.5±0.1	2.6±0.3	2.5±0.3	2.1±0.3
54	2.1±0.2	1.8±0.2	2.6±0.3	1.8±0.4				
60					2.0±0.4	1.9±0.4	1.7±0.1	1.7±0.2

*Mean of duplicate samples ± standard deviation.

Dimethylamine (DMA) and Formaldehyde (FA)

During frozen storage there was an increase of DMA and FA content in all treatments (Tables 3 and 4). Kappa carrageenan addition showed apparently the lowest increase while CMC 2805 presented the highest increase.

Table 3. Dimethylamine content* (μg DMA nitrogen/g of fish) of frozen stored minced fish at time intervals

Days at −18°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
0	12.3±0.6	8.1±0.2	10.6±1.1	8.7±0.4	10.3±0.8	9.3±0.8	10.6±0.3	10.7±0.3
20	28.9±4.0	15.8±0.5	24.2±1.3	22.6±1.7	22.0±0.9	16.5±1.1	19.8±1.3	27.6±0.9
40	25.2±1.9	15.1±1.9	28.8±2.0	26.0±2.9	24.9±1.6	18.5±1.5	23.3±6.2	26.5±3.9
53	41.7±1.1	25.3±1.4	45.6±2.0	50.2±1.0	45.3±4.8	31.3±0.3	42.7±6.1	59.6±2.0
68	41.5±1.0	30.5±1.1	64.4±0.1	50.2±2.6	53.2±2.1	36.3±2.0	42.2±3.3	67.9±2.4

*Mean of triplicate samples ± standard deviation.

Table 4. Formaldehyde content* (μg/g of fish) of frozen stored minced fish at time intervals

Days at −18°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
0	6.2±0.3	5.7±0.2	6.5±0.1	5.5±0.1	6.0±0.2	6.2±0.0	9.4±0.0	8.8±0.2
21	13.8±0.6	7.9±0.1	11.1±0.3	12.0±0.3	12.5±0.0	13.9±0.1	21.1±0.4	11.9±0.1
31	19.7±0.8	11.7±0.1	19.3±0.3	18.6±0.3	19.7±0.1	17.0±0.2	24.6±0.4	18.2±0.5
41	22.6±0.8	10.4±0.2	24.4±0.4	20.6±0.8	21.9±1.5	14.5±0.8	20.8±0.9	36.0±1.7
67	32.1±0.4	16.7±2.0	39.3±0.9	24.3±2.0	39.0±0.6	34.6±2.2	52.0±1.1	68.8±0.7

*Mean of duplicate samples ± standard deviation.

Protein, carbohydrate content and viscosity of the supernatants released in WHC determination

The supernatants of all treatments of the WHCR presented a slight decrease of protein nitrogen content in the course of the frozen storage while the protein nitrogen content of the supernatants of the WHCC were almost constant during the same time (Tables 5 and 6).

Table 5. Protein nitrogen content* (mg/ml) of the supernatants obtained from the WHCR at time intervals

Days at −18°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205
0	10.9±0.5	11.0±0.0	10.6±0.1	10.8±0.2	10.8±0.2	11.5±0.2	n.d.
12	10.6±0.1	10.2±0.3	10.4±0.2	9.8±0.4			
13					10.4±0.5	11.1±0.1	10.9±0.1
26	10.3±0.0	10.5±0.6	9.8±0.2	9.8±0.6			
27					10.5±0.4	11.2±0.5	10.5±0.0
39	9.3±0.1	9.5±0.0	9.7±0.1	9.6±0.5			
49					10.7±0.2	10.6±0.1	10.9±0.3
52	9.3±0.1	9.2±0.0	8.8±0.1	9.2±0.0			
70					10.3±0.1	10.4±0.2	10.7±0.2

*Mean of duplicate samples ± standard deviation.
n.d. = Not determined.

Table 6. Protein nitrogen content* (mg/ml) of the supernatants obtained from the WHCC at time intervals

Days at −18°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
0	6.4±0.1	6.1±0.1	6.5±0.1	6.4±0.1	6.7±0.2	6.9±0.2	7.0±0.3	7.2±0.1
12	6.5±0.1	6.2±0.3	5.7±0.3	6.3±0.1				
13					7.0±0.1	6.8±0.2	6.9±0.0	7.0±0.3
26	6.0±0.3	6.1±0.0	6.5±0.3	6.1±0.0				
27					6.7±0.1	7.0±0.3	7.1±0.5	7.3±0.5
39	6.8±0.2	6.4±0.1	7.0±0.1	6.7±0.1				
49					6.9±0.2	6.9±0.2	7.2±0.4	7.5±0.4
52	6.6±0.4	6.0±0.2	6.6±0.2	6.3±0.2				
70					7.0±0.2	7.2±0.4	7.3±0.3	7.4±0.2

*Mean of duplicate samples ± standard deviation.

The carbohydrate content of the supernatants of the WHCR and WHCC are reported in Tables 7 and 8 respectively. The supernatants of the CMC additions of the WHCR showed a decrease of carbohydrate content with the increase of fluid released in the WHCR (Table 7). The heat treatment of WHCC determination caused a further increase in fluid released which was accompanied by a decrease in the carbohydrate content of the supernatants of the CMC treatments (Table 8). It seemed that competition existed for water between the fish tissue and the hydrocolloid. Whenever the capacity of the fish tissue to hold water decreased, more water became available for the CMC additions.

Table 7. Carbohydrate content (mg/ml) of the supernatants obtained from the WHCR at time intervals

Days at −18°C	Blank*	Kappa-carrag.†	Lambda-carrag.†	Iota-carrag.†	CMC 705	CMC 1505†	CMC 2205†
0	1.4	0.5	4.4	0.3	10.2	10.3	n.d.
12	1.5	0.6	4.0	0.2			
13					9.4	10.1	9.5
26	1.3	0.2	3.1	0.5			
27					8.3	9.7	9.3
39	1.5	0.8	2.8	0.1			
49					7.6	9.2	8.2
52	1.4	0.3	2.6	0.1			
70					7.8	8.6	7.9

*Equivalents of glucose obtained from the colorimetric method of Dubois *et al.* (1956).

†Calculated by subtracting the glucose content of the blank and multiplying with factors which were determined for each carbohydrate (Kappa-carrageenan = 0.67, Lambda-carrageenan = 0.59, Iota carrageenan = 0.58, CMC 705 = 0.58, CMC 1505 = 0.58, CMC 2205 = 0.62).

n.d. = Not determined.

The flow curves of the supernatants of the WHCR and WHCC determinations at zero time are presented in the Figs 7 and 8. The Iota and Kappa carrageenan treatment exhibited supernatants with flow curves similar to the blank and with very low carbohydrate content. The supernatants of the Lambda carrageenan used, Viscarin 402, had intermediate viscosities and carbohydrate

Table 8. Carbohydrate content (mg/ml) of the supernatants obtained from the WHCC at time intervals.

Days at −18°C	Blank*	Kappa-carrag.†	Lambda-carrag.†	Iota carrag.†	CMC 705†	CMC1505†	CMC 2205†	CMC 2805†
0	1.7	0.3	2.7	0.5	6.8	7.4	7.5	6.8
12	1.8	0.3	2.4	0.2				
13					7.0	7.5	6.3	6.2
26	1.9	0.1	2.8	0.2				
27					6.7	5.8	5.9	6.0
39	2.1	0.6	3.3	0.3				
49					6.7	6.3	6.2	6.9
52	2.0	0.6	2.9	0.1				
70					6.4	6.9	6.7	6.3

*Equivalents of glucose obtained from the colorimetric method of Dubois *et al.* (1956).

†Calculated by subtracting the glucose content of the blank and multiplying with factors which were determined for each carbohydrate (Kappa-carrageenan = 0.67, Lambda-carrageenan = 0.59, Iota-carrageenan = 0.58, CMC 705 = 0.58, CMC 1505 = 0.58, CMC 2205 = 0.62 and CMC 2805 = 0.56).

contents. Probably only the Lambda carrageenan fraction was in solution and the other types of carrageenans present in Viscarin 402 remained in the fish cake as hydrated particles for the raw sample and as separated gel for the cooked sample.

The volumes of supernatants of the WHCR of the CMC 2805 addition are too small

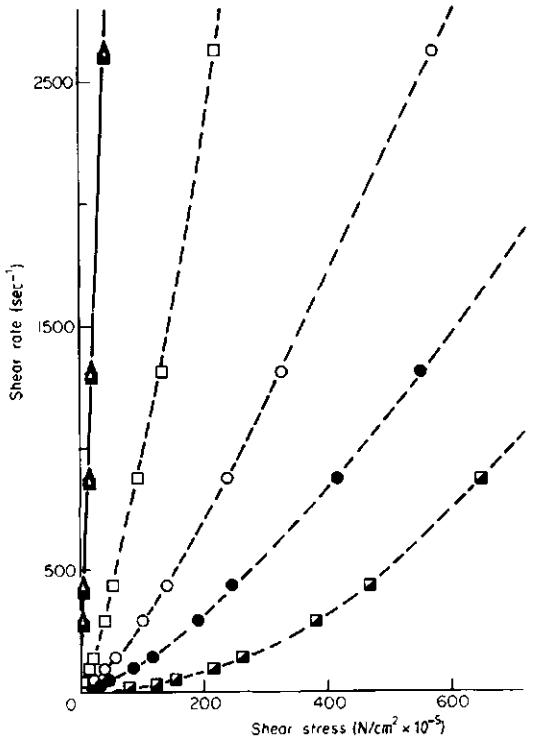

Figure 7. Flow curves of the different supernatants obtained from the WHCR at zero time. (See Fig. 1 for symbols.)

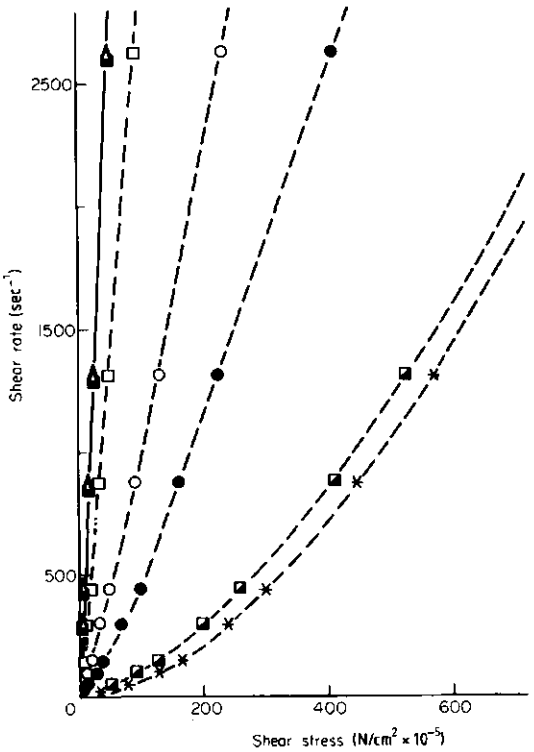

Figure 8. Flow curves of the different supernatants obtained from the WHCC at zero time. (See Fig. 1 for symbols.)

to determine the protein nitrogen and carbohydrate content and the flow curves. The same is true for the supernatant of the WHCR at zero time of CMC 2205 addition, which was only enough for the determination of the flow curve.

Conclusions

Viscosity of the fish fluid influenced the texture and water holding capacity of the minced fillets of cod. Carboxymethyl celluloses with high viscosity made samples softer in texture and higher in water holding capacity.

Iota carrageenan caused remarkable texture stability during frozen storage. Kappa, Iota and Lambda carrageenan significantly improved the WHCC due to the formation of a separated gel, while only Iota and Lambda carrageenan significantly improved the WHCR.

The carboxymethyl cellulose can be blended directly with the minced fish while freeze drying increased the effects of Kappa and Iota carrageenan.

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6. Effects of additions, irradiation and heating on the activity of Trimethylamine oxide-ase in frozen stored minced fillets of whiting

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Summary

Fish fluid was obtained by centrifuging minced fillets of whiting and used to study the effects of several treatments on TMAO-ase activity during storage at −18°C. Additional experiments were carried out with the whole mince. When the fish fluid or mince was heated for 1 min at 80°C or more, the production of dimethylamine and formaldehyde ceased. Xanthan was the only one of the hydrocolloids studied (pectins, carrageenans, alginates, locust bean gum and carboxymethyl celluloses), which showed a significant reduction of TMAO-ase activity. A low dose of irradiation (300 Krad) did not change TMAO degradation of the fish fluid during subsequent frozen storage.

Introduction

Many gadoid fishes produce large amounts of formaldehyde (FA; about 50 ppm) and dimethylamine (DMA; about 500 ppm) in muscle and intestine tissues during frozen storage (Tokunaga, 1964; Amano & Yamada, 1964; Castell, Neal & Smith, 1970). This production has been attributed to an endogenous enzyme (more concentrated in kidney, pyloric caeca and liver) capable of reducing trimethylamine oxide (TMAO) to the intermediate dimethylaminomethylol which then yields DMA and FA (Harada, 1975). Probably FA contributes to the textural toughening and to the loss of water holding capacity of fish muscle during frozen storage (Connell, 1975; Poulter & Lawrie, 1978). DMA could be a potential precursor of the carcinogenic compound N-nitrosodimethylamine (Matsui, Ishibashi & Kawabata, 1984; Mackie & Thomsom, 1974). The continuing production of DMA in frozen storage may also impair the flavour of fish products.

During the past several years, increasing numbers of new frozen fish products have been introduced which have been pre-cooked before freezing. Among them, pre-fried, battered and bread coated fish and seafood products with a wide range of ingredients, have become well accepted by consumers (Sikorski, Olley & Kostuch, 1976; Hansen, 1980; Lopez-Gravito & Pigott, 1983). As the gadoid fishes play an important role in this industry there is a need for studying practical pretreatments which could prevent the production of DMA and FA in these products during frozen storage. Several attempts have already been made: removal of TMAO and soluble proteins by washing the minces, addition of oxidizing agents and packaging in oxygen permeable materials (Landolt & Hultin, 1981; Reece, 1983; Racicot *et al.*, 1984). Heat treatments have also

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been used to arrest the TMAO-ase activity of frozen stored fish, but the results obtained are not clear in respect to time and temperatures of inactivation (Tokunaga, 1964; Castell, Neal & Smith, 1971; Lall, Manzer & Hiltz, 1975).

In order to avoid fluctuations in DMA and FA determinations fish fluid was studied, which was obtained by centrifuging minced fillets of whiting. The effects of different times of heating at various temperatures, irradiation and additions of several hydrocolloids were examined. The effects of heat treatments on the whole minced fish were also investigated.

Materials and methods

Mince of skinned fillets of whiting (*Merlangius merlangus*) with a high TMAO-ase activity (da Ponte, Roozen and Pilnik, 1985b) was kept in frozen storage at −45°C during 12 months. It was then thawed, centrifuged at 25000 **g** for 30 min and the supernatant liquid (fish fluid) stored for 4 weeks at −80°C.

Skinned fillets of whiting were bought in the local market of Wageningen on 12 December 1984. They were kept on ice and passed twice through a Kenwood mincer model A. 720, using a mincing screen with 4.4 mm holes. The resulting minced fish was divided in portions of 25.0±0.1 g and packed in polyvinyl chloride (PVC) plastic bags (180×300×0.065 mm) in such a way that the minced fish formed a rectangular slab of 178.5×93.5 mm and 1.5 mm thick, on average. The slabs were stored at −80°C for 1 week.

Heat treatments

Fifty-two ml of fish fluid were put in a 500 ml Erlenmeyer flask, in an ice water bath to 0°C, then heated in a microwave oven (Philips, model 2010C) to the desired temperature and immediately afterwards transferred to a water bath at the same temperature as the fish fluid, and held there for different intervals of time. Subsequently the fish fluid was cooled by intensive swirling of the Erlenmeyer flask in an ice water bath. The heat treated fish fluid was then mixed well, divided into 8 ml portions in screw cap tubes and stored at −18°C. Two replicates of each treatment were used to determine the DMA and FA content at various storage intervals. The different heat treatments of the fish fluid are reported in Table 1. The heating and cooling curves of the various treatments were determined with a thermocouple placed in the centre of the Erlenmeyer (Fig. 1).

Table 1. Heat treatments used for the inactivation of TMAO-ase in fish fluid from minced fillets of whiting

Heating time in the microwave oven (sec)	Temperature reached (°C)	Different holding times in the water bath (sec)
8	55	10, 50, 200, 400 and 800
9	60	30, 60, 120, 240, and 480
10	65	10, 30, 90, 180 and 270
11	70	20, 40, 80, 160, and 320
13	80	15, 30, 60, 120 and 240
14.5	85	10, 20, 40, 80 and 160

TMAO-ase activity frozen minced whiting

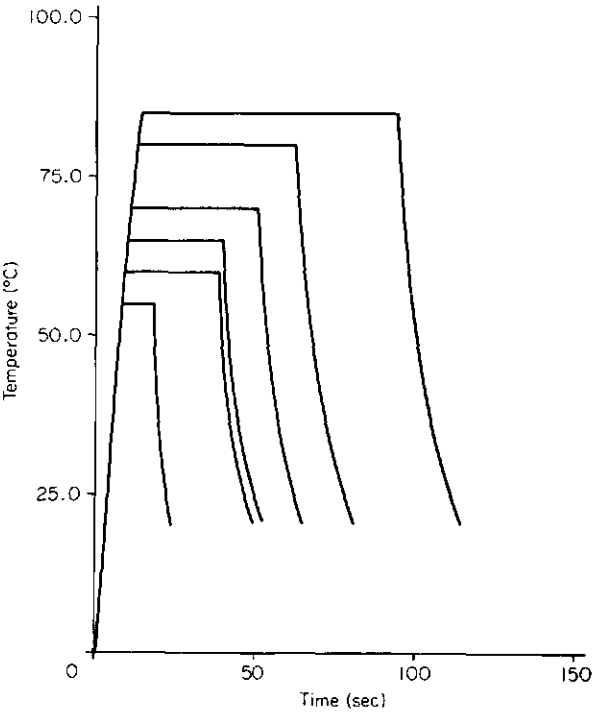

Figure 1. Heating and cooling curves of the different heat treatments as determined for fish fluid from minced fillets of whiting.

The bags with 25.0 g of minced whiting were thawed in an ice water bath and then dipped in a water bath at a desired temperature for 1 min. Immediately afterwards, the bags were cooled by intensive agitation in an ice water bath. Subsequently the bags were blast frozen and stored at −18°C. The various temperatures used in the water bath are shown in Table 2. The heating and cooling curves of these heat treatments were determined theoretically (Fig. 2) using the thermal diffusity of PVC (9.55×10^{-8} m^2/sec), the thermal diffusity of minced fish (1.47×10^{-7} m^2/sec; Aitken & Connell, 1979) and assuming a distance of 0.9 mm for reaching the coolest point of the fish slab. Two replicates of each treatment were used for determining the DMA and FA contents at various intervals of storage.

Additions

Different hydrocolloids were mixed with the fish fluid (5 mg/ml) for 1 hr at refrigerated temperatures (4°C). Afterwards the fish fluids with the different additions were divided into 8 ml portions and stored in screw cap tubes at −18°C. Two replicates of each addition were used to determine the DMA and FA content before and after 46 days in storage.

Hydrocolloids used were: (i) Low esterified pectin (L.P.) and High esterified pectin (H.P.) from Obi-Pectin (Bischofszell, Switzerland); (ii) Potassium Kappa carrageenan (HF 55758-63) and Iota carrageenan (HF 33895-96) from Copenhagen Pectin Factory

Table 2. Effect of various heat treatments on TMAO-ase activity in mince of whiting during frozen storage

Heat treatments (1 min at T°C)	Days at (−18°C)	Production of DMA-Nitrogen		Production of FA	
		μg DMA-N/g mince	(%)*	μg FA/g mince	(%)†
	0	38.0±1.5	100	9.2±0.5	100
Blank	21	93.3±3.0	100	28.6±2.0	100
	45	147.7±7.5	100	43.3±3.5	100
	0	37.3±0.2	98	5.9±0.4	64
50	21	87.7±4.8	91	22.0±1.5	83
	45	139.7±5.9	93	34.3±0.3	83
	0	38.4±2.0	101	5.1±0.3	55
55	21	82.8±0.3	80	19.0±1.4	72
	45	119.4±1.8	74	27.6±1.2	66
	0	39.7±0.5	104	5.0±0.2	54
60	21	80.2±2.6	73	16.6±1.3	60
	45	109.3±4.5	63	25.2±0.3	59
	0	39.0±2.1	103	3.6±0.3	39
65	21	72.7±2.7	61	13.3±0.8	50
	45	103.1±0.6	58	23.4±1.8	58
	0	36.7±1.7	97	3.0±0.2	33
70	21	54.7±3.5	33	9.4±1.0	33
	45	77.0±1.9	37	13.6±0.6	31
	0	38.5±0.9	101	2.6±0.1	28
75	21	43.8±0.1	10	5.3±0.1	14
	45	54.8±1.7	15	7.1±0.4	13
	0	36.9±1.4	97	2.5±0.2	27
80	21	37.9±1.8	2	3.2±0.3	4
	45	41.7±1.6	4	3.4±0.2	3
	0	37.8±0.5	99	2.6±0.2	28
85	21	37.2±0.6	−1	3.4±0.3	4
	45	40.3±0.5	2	3.5±0.2	3
	0	36.2±1.5	95	2.6±0.2	28
90	21	36.8±1.7	1	2.8±0.2	1
	45	39.3±2.1	3	2.9±0.3	1

*$\frac{\text{DMA-Nitrogen of the treat. at } t \text{ time} - \text{DMA-Nitrogen of the treat. at 0 time}}{\text{DMA-Nitrogen of the Blank at } t \text{ time} - \text{DMA-Nitrogen of the Blank at 0 time}} \times 100\%$.

†$\frac{\text{FA of the treat. at } t \text{ time} - \text{FA of the treat. at 0 time}}{\text{FA of the Blank at } t \text{ time} - \text{FA of the Blank at 0 time}} \times 100\%$.

Ltd. (Denmark); (iii) Lambda carrageenan, Viscarin 402, (Batch 321403), from Marine colloids Division, FMC Corporation (Springfield, New Jersey, U.S.A.): (iv) Kelcogel HV, Kelcosol and Kelcocolloid HVF (different types of alginates) from Kelco/AIL London (U.K.); (v) Xanthan gum, Keltrol F (KTLF-67203A), from Kelco (New Jersey, U.S.A.); (vi) Locust bean gum (L.B.G.), Meyprodin 200, from Meyhall Chemical AG (Switzerland); and (vii) Carboxymethyl cellulose (CMC), Akucell AF 705, AF 1505, AF 2205 and AF 2805 from Enka bv, Industrial Colloids (The Netherlands).

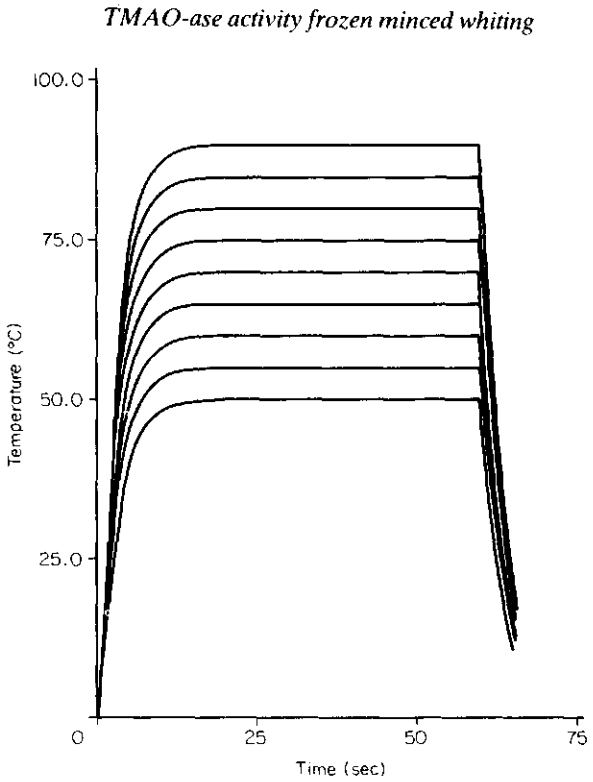

Figure 2. Heating and cooling curves of the different heat treatments as determined for the coolest point of slabs of minced fillets of whiting packed in PVC bags.

Irradiation

Screw cap tubes with 8 ml of fish fluid were gamma irradiated (cobalt 60, 300 Krad, −20°C) in the Pilot Plant for Food Irradiation, Wageningen, and then stored at −18°C. Two replicates were used for determination of DMA and FA during storage.

DMA and FA determination

Five percent trichloroacetic acid extracts of mince or fish fluid were used for the determination of DMA and FA. DMA was determined by the colorimetric method of Dyer & Mounsey (1945). The formaldehyde (FA) content was determined by the procedure outlined by Castell & Smith (1973) using the Nash reagent (Nash, 1953).

Statistical analysis

Differences were analysed by Duncan's multiple range test at a probability level of 1% (Steil & Torrie, 1982).

Results and discussion

Heat treatments

During heating and cooling of both the fish fluid and mince the heat transfer proceeded rapidly (Figs 1 and 2). The fish fluid was heated from 50 to 85°C in about 7 sec and the mince in 12 sec.

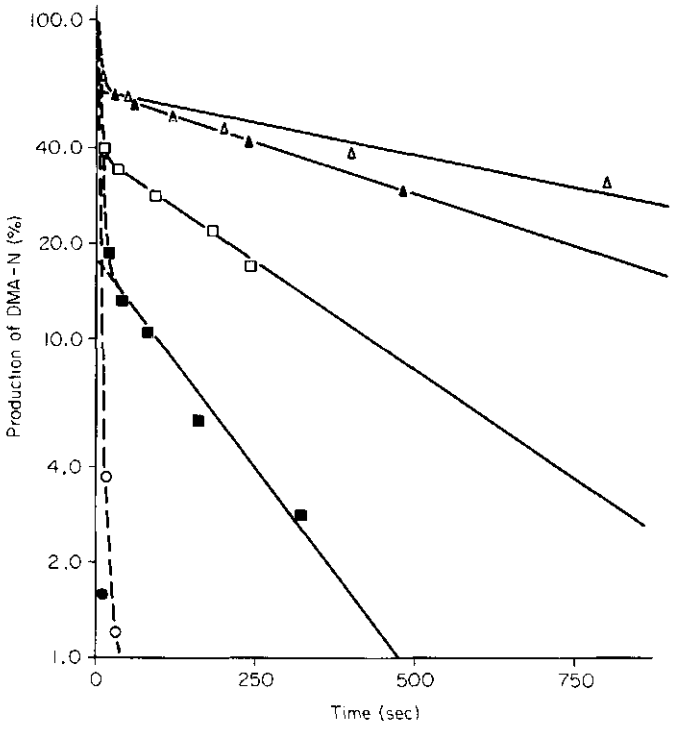

Figure 3. Thermal inactivation of TMAO-ase in fish fluid from minced fillets of whiting. Production of DMA as a function of time and temperature: △ 55°C, ▲ 60°C, □ 65°C, ■ 70°C, O 80°C and ● 85°C.

The thermal inactivation curves of TMAO-ase in respect to the production of DMA and FA in the fish fluid are presented respectively in Figs 3 and 4. In both cases the thermal inactivation curves consist of initial steep straight lines, which seem to be the result of very quick (almost instantaneous) inactivation rates. Then the curves show small transition parts and final straight lines with flat slopes, which indicate relatively moderate rates of inactivation.

At present, little is known about the exact structure of the TMAO-ase molecule and its localization in the fish muscle (Parkin & Hultin, 1981; Lundstrom, Correia & Wilhelm, 1982). The fish fluid is a complex mixture of soluble proteins, suspended particles and minor organic and inorganic compounds. It is possible that differences in heat stability of TMAO-ase are due to differences in thermostability of isoenzymes or of complexes of proteins and TMAO-ase. Also TMAO-ase aggregation caused by the heat treatment may protect the enzyme (Schleusener, 1982).

The effect of various heat treatments on the TMAO-ase activity of the mince of whiting during frozen storage is reported in Table 2. Comparing these values with the curves in Figs 3 and 4 it seems that TMAO-ase is somewhat more thermostable in the mince than in the fish fluid. Perhaps a higher proportion of TMAO-ase is complexed in the fish muscle than in the fish fluid thus protecting it from heat denaturation.

Heating reduced the free FA content of the samples determined by the Nash reagent

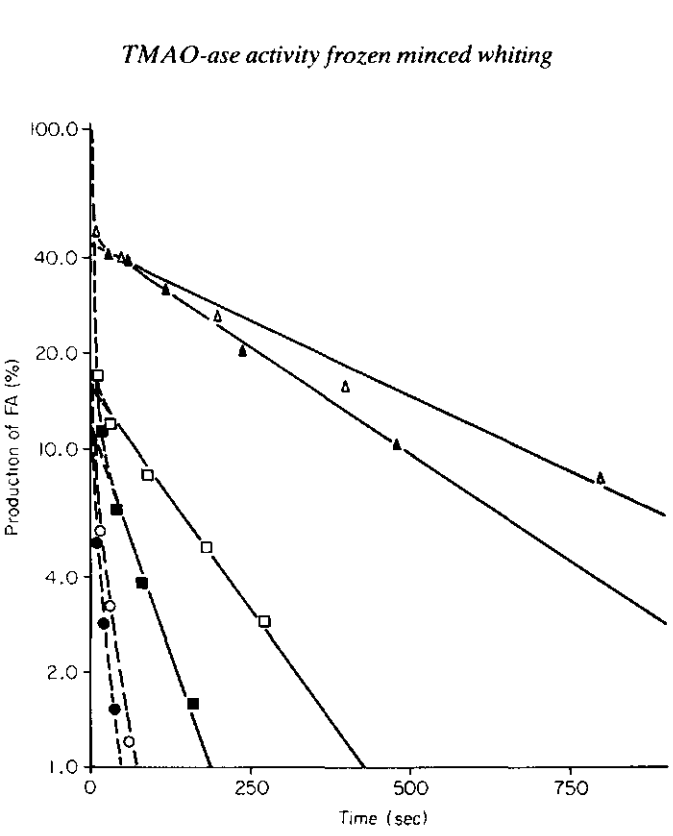

Figure 4. Thermal inactivation of TMAO-ase in fish fluid from minced fillets of whiting. Production of FA as a function of time and temperature. See Fig. 3 for symbols.

(Nash, 1953). This may be due to heat accelerating the FA reactions. It seems also that heated mince and heated fish fluid increased their capacity to bind FA during storage at −18°C and therefore the percentages of FA found were somewhat lower than the ones of DMA (Table 2 and Figs 3 and 4). Similar results were reported by Castell & Smith (1973).

Reports about thermal inactivation of TMAO-ase are difficult to interpret. Tokunaga (1964) found that heating of muscle tissue of Alaska pollock for 30 min at 40°C caused 20% decrease in FA production after 4 weeks at −17 – −19°C. This production disappeared almost totally when heating was carried out at 50°C. Heating of fillets and minced flesh of silver hake up to 60°C had little or no effect on the rate of DMA formation during subsequent storage for 1 month at −10°C (Lall *et al.*, 1975). Heating of silver hake mince to 80°C was found to stop the DMA formation totally. Sevensson (1980) concluded that thermal inactivation of partially purified TMAO-ase from kidney tissue homogenates of cod and blue whiting followed first order reaction kinetics. In our experiments, the thermal inactivation curves seem to be the resultants of two first order reactions. It can be concluded that holding at 80°C or higher for 1 min is sufficient to arresting TMAO-ase activity in minced fillets of whiting. At lower temperatures a quick partial inactivation can be obtained but eliminating the remaining TMAO-ase activity will require much longer holding times.

Additions

In previous reports (da Ponte, Roozen & Pilnik, 1985a and b: da Ponte *et al.*, 1985c) we described the effect of various hydrocolloids on the stability of frozen minced fillets of whiting and cod. Some of the hydrocolloids used (Kelcosol, Xanthan, Iota and Kappa carrageenan) diminished the production of DMA and FA during storage at −18°C. In the present experiments only xanthan reduced the DMA and FA contents of the fish fluid stored at −18°C (about 86% of the blank, Table 3).

Table 3. Effect of additions of different hydrocolloids on the TMAO-ase activity of fish fluid of whiting during frozen storage

Additions	Days in frozen storage (−18°C)	μg DMA-Nitrogen per g of fish fluid	μg FA per g of fish fluid
Blank	0	75.9±1.6	24.2±0.1
	46	158.0±3.4*	81.7±1.6*
K-carrageenan	0	74.9±2.0	23.9±0.3
	46	154.5±4.0*†	79.2±1.1*
I-carrageenan	0	74.7±2.7	23.5±0.4
	46	157.6±3.6*	82.4±0.6*
λ-carrageenan	0	74.6±1.6	23.6±0.2
	46	155.9±4.7*	80.4±1.0*
Xanthan	0	74.5±2.3	23.3±0.1
	46	145.0±4.1†	70.1±0.1†
CMC 705	0	75.5±0.1	23.2±1.1
	46	158.1±2.1*	79.4±1.4*
CMC 1505	0	75.1±0.4	24.0±0.1
	46	154.1±2.5*†	79.3±0.1*
CMC 2205	0	74.4±0.6	24.6±0.8
	46	152.7±4.4*†	79.3±0.8*
CMC 2805	0	75.3±2.0	23.6±0.4
	46	156.5±2.4*	79.6±0.8*
L.B.G.	0	73.8±1.3	24.8±0.5
	46	157.5±0.3*	80.2±1.4*
L.P.	0	75.0±2.0	24.9±0.3
	46	154.7±4.0*†	78.4±0.7*
H.P.	0	72.4±1.3	24.4±0.1
	46	157.3±2.2*	78.4±0.7*
Kelcogel	0	72.6±0.0	24.3±0.3
	46	160.0±0.1*	80.9±3.0*
Kelcosol	0	73.9±1.1	24.7±0.6
	46	158.5±3.0*	79.8±2.8*
Kelcocolloid	0	74.3±0.5	24.9±0.1
	46	157.0±1.3*	80.1±1.7*
L.B.G.+Xanthan	0	74.2±0.6	23.4±0.8
	46	156.0±4.0*	78.2±2.7*

*†Values in the same column with the same superscripts were not significantly different ($n = 2$; $P < 0.01$).

TMAO-ase activity frozen minced whiting

Irradiation

The bacterial load of mechanical deboned fish minces can increase rapidly unless strict hygienic procedures are used. Raccach & Baker (1978) showed that mechanical deboning of either frames or headed and gutted fishes increased the bacterial load by a factor of ten. Minced fish, even of good initial quality, has very short shelf life at chill temperatures.

Radiation could be employed for decreasing the number of viable microorganisms present in the fish minces and consequently it could increase the shelf life of this raw material for the manufacture of fish products. Studies on the cleavage of TMAO in fish flesh showed that irradiation enhanced the production of FA and DMA in gadoid fishes (Tozawa & Amano, 1969a and b). In these studies, doses of 600 Krad and more were used.

In our experiments we determined the effect of a lower irradiation dose (300 Krad) for the production of DMA and FA in the fish fluid stored at −18°C. Irradiation did not show any noticeable effect, also not during subsequent frozen storage (Table 4).

Table 4. Effect of irradiation on the production of DMA and FA in fish fluid of whiting during frozen storage

	Not irradiated		Irradiated	
Days in frozen storage (−18°C)	μg FA/g fish fluid	μg DMA-Nitrogen/g fish fluid	μg FA/g fish fluid	μg DMA-Nitrogen/g fish fluid
0	28.9±1.0	69.4±2.4	28.9±0.5	72.1±1.1
21	53.7±0.1	108.1±0.9	49.6±0.3	103.8±0.5
35	62.8±0.0	121.5±0.1	60.0±0.5	118.3±1.3
53	69.3±0.2	135.9±2.1	67.7±0.9	133.1±2.3

Conclusions

Heat treatment of frozen gadoid mince could overcome some of the undesirable changes in texture and water holding capacity attributed to FA production by TMAO-ase. In our experiments, it was found that heating of mince of whiting up to 80°C or more for short periods of time reduced drastically the production of DMA and FA during subsequent frozen storage. At lower heating temperatures it took much more time. This information may be useful for the pre-frying of ready-to-serve fish products which are becoming increasingly popular. The addition of xanthan caused about 14% reduction of DMA and FA production during frozen storage. A low dose of irradiation (300 Krad) did not enhance the formation of DMA and FA in the fish fluid.

Acknowledgments

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7. Effect of Irradiation on the Stability of Minced Cod, with and without Hydrocolloids during Frozen Storage

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Minced fillets of cod were used to determine the effects of a low dose of irradiation (300 krad, −20°C) on the stability of the minces during frozen storage.

Texture, water holding capacity, lipid oxidation, production of dimethylamine and formaldehyde and lipid hydrolysis were evaluated at regular intervals during 3 months of post-irradiation frozen storage (−18°C). Combinations of irradiation with 0.5% cryoprotective additives (Xanthan, Carboxymethyl cellulose and Iota carrageenan) were also studied.

During three months of frozen storage the irradiated samples did not present any major change in comparison with the non-irradiated ones. As before, Xanthan, Carboxymethyl cellulose and Iota carrageenan showed their stabilizing effect on texture and cook drip loss.

Introduction

Mechanical deboning machines are more and more used for obtaining higher yields of flesh from traditionally exploited fish (recovery of flesh from trimmings and fillet frames) and for utilising species of fish which were formerly considered uneconomic for commercial preparations. The deboning operation causes disrupture of fish tissues and an intimate mixing of the flesh and bacteria normally resident on the skin of the fish. The results of studies on mechanical deboning of either frames or headed and gutted fishes showed that this operation can increase the bacterial load of the minces by a factor ten (1, 2).

Irradiation could be employed for decreasing the number of viable microorganisms present in the fish minces and consequently increasing the shelf life of this raw material at chill temperatures. It could also be combined with other treatments for making stable fish products. Most of the work in fish irradiation has dealt with the extension of the shelf life in chilled storage. Normally only low doses of irradiation are applied due to the formation of undesirable off-flavours and off-odours in the irradiated fish. The maximum dose level for acceptable cod has been reported to range from 150–300 krad (3). The parameters used for evaluation of irradiated fish at chill temperatures are generally correlated with changes in the bacterial load. Irradiation, however, can promote autoxidation of fat in meat and fish tissues (4–6), decrease the water holding capactiy in meat (7, 8), and enhance the production of dimethylamine (DMA) and formaldehyde (FA) in gadoid fishes (9, 10). Recent reports claim that irradiation could increase the release of enzymes in fish tissues even when low doses (100 krad) are applied (11–13).

In our experiments we used minced fillets of cod for testing the effects of a low dose of irradiation (300 krad) on the stability of the minces during frozen storage. The purpose was to find out if irradiation and post-irradiation frozen storage could produce changes in texture, water holding capacity, lipid oxidation, and in the formation of DMA and free fatty acids (FFA). The combinations of irradiation and additives (Xanthan, Carboxymethyl cellulose and Iota carrageenan), which showed a stabilizing effect on fish minces during frozen storage (14), were also studied.

Materials and Methods

Skinned fillets of cod were bought in the local market of Wageningen on December 5, 1984. They were packed in plastic bags of 1 kg, blast frozen and kept frozen at −45°C for 2 weeks. Subsequently they were thawed in cold tap water and were passed through a mincer (Zellweger s.a. Uster, Switzerland), using a mincing screen with 4 mm holes. The resulting minced fish was divided in portions and different hydrocolloids were spread over the surface (5g kg^{-1} of minced fish). The hydrocolloids and the minces were gently mixed with a spoon and kept in plastic bags on melting ice for two hours.

Hydrocolloids used were:

– Iota carrageenan (HF–33895–96), from Copenhagen Pectin Factory Ltd. (Denmark).

– Carboxymethyl cellulose (CMC), AF 2805 from Enka bv, Industrial Colloids (Holland).

– Xanthan gum, Keltrol F (KTLF–67203A), from Kelco (New Jersey, U.S.A.).

Samples of 150 g mince were repacked in polyvinylchloride plastic bags and blast frozen. After one day of frozen storage at −20°C, they were gamma irradiated (cobalt 60, 300 krad, −20°C) in the Pilot Plant for Food Irradiation, Wageningen, and then stored at −18°C.

Samples of each treatment were withdrawn from the frozen storage, thawed in cold tap water and kept for 3 hours in melting ice until analyses were carried out.

Texture analysis, pH measurements, water holding capacity of the raw minced fish (W.H.C.R.) and cook drip loss (C.D.L.) were determined in the same way as described before (15).

DMA and FA determination

Five percent trichloroacetic acid extracts of mince were used

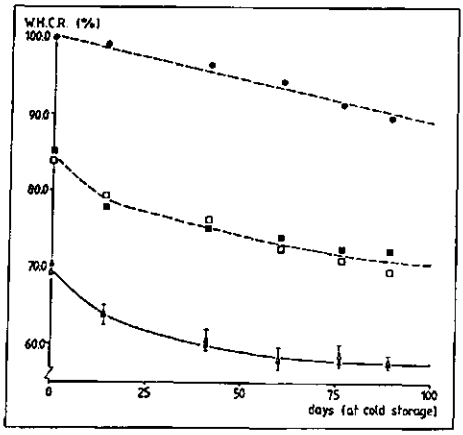

Fig. 1 Effect of time, irradiation and different additives on water holding capacity of the raw material (W.H.C.R.) of frozen stored minced cod
△ Blank not irradiated, ▲ Blank irradiated, □ Iota carrageenan irradiated, ■ Carboxymethyl cellulose irradiated and ● Xanthan irradiated
The vertical bars represent standard deviations

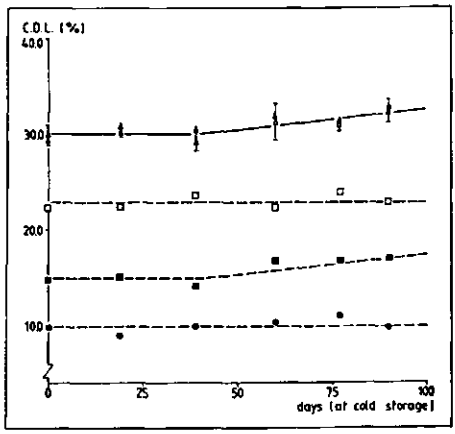

Fig. 2 Effect of time, irradiation and different additives on cook drip loss (C.D.L.) of frozen stored minced cod
See Fig. 1 for symbols

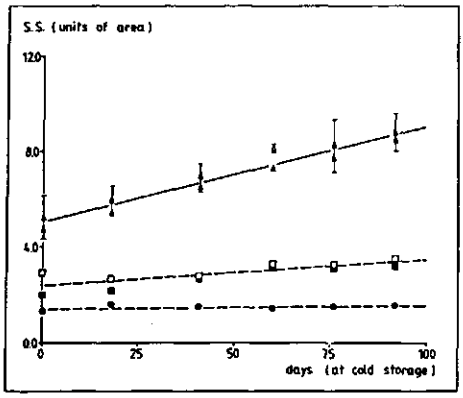

Fig. 3 Effect of time, irradiation and different additives on shear stress (S.S.) of frozen stored minced cod
See Fig. 1 for symbols

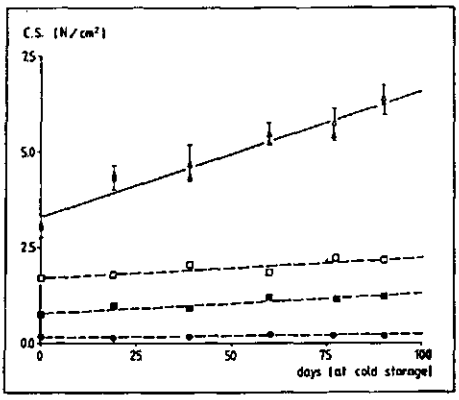

Fig. 4 Effect of time, irradiation and different additives on compressive strength (C.S.) of frozen stored minced cod
See Fig. 1 for symbols

for determination of DMA and FA. DMA was determined by the colorimetric method of DYER and MOUNSEY (16). Three replicates were done for each sample.
The free FA was determined by the procedure outlined by CASTELL and SMITH (17) using the Nash reagent (18). Two replicates were done for each sample.

Lipid oxidation
Thiobarbituric acid reactive substances (TBARS) were determined by the distillation method of TARLADGIS *et al.* (19) as modified by KE *et al.* (20).

Total carbonyls (TC) were measured by the procedure of LAWRENCE (21) using EM=22500 M^{-1} cm^{-1} at 340 nm reported by SCHWARTZ *et al.* (22).

Lipid extraction, separation and determination
Lipids were extracted from the minced fish by the BLIGH and DYER method (23) with a mixture of chloroform, methanol and water.
Total free fatty acids measured in the chloroform extracts by the colorimetric end point titration method of DRIESSEN (24) using petroleum ether (60–80°C): propanol-2 (4:1) as

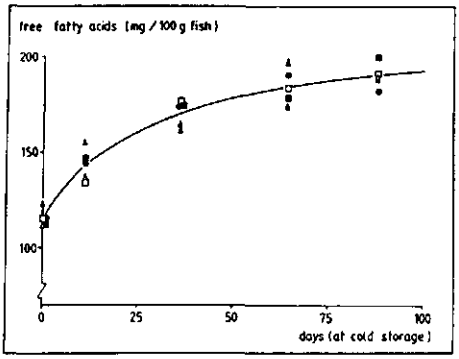

Fig. 5 Effect of time, irradiation and different additives on free fatty acid formation of frozen stored minced cod
See Fig. 1 for symbols

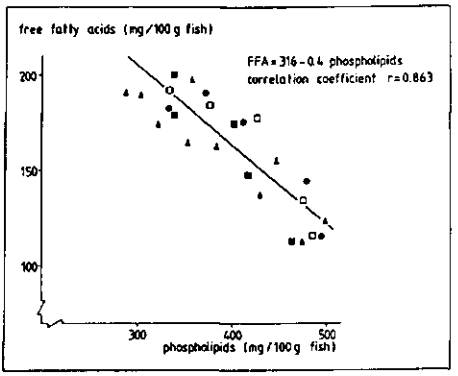

Fig. 6 Decrease in phospholipids content compared with development of free fatty acids, both measured in the different treatments of frozen stored minced cod
See Fig. 1 for symbols

Tab. 1 Dimethylamine content (µg DMA-Nitrogen/g of fish) of frozen stored minced cod at time intervals

Days at		Irradiated			
−18°C	Blank	Blank	ι-carrag.	CMC	Xanthan
0	12.7 ± 0.3	12.6 ± 0.2	12.1 ± 0.5	12.4 ± 0.3	12.9 ± 0.5
20	13.3 ± 0.1	13.2 ± 0.4	12.4 ± 0.3	13.3 ± 0.5	13.1 ± 0.3
42	17.4 ± 0.6	16.7 ± 0.9	18.2 ± 0.1	18.4 ± 0.6	15.3 ± 0.6
60	18.8 ± 0.7	18.7 ± 0.5	19.8 ± 0.5	18.8 ± 1.3	16.3 ± 0.2
77	20.9 ± 0.3	20.1 ± 0.5	20.4 ± 1.0	20.7 ± 0.5	18.7 ± 0.6
91	24.0 ± 0.9	23.6 ± 0.8	23.1 ± 1.0	23.1 ± 0.8	22.5 ± 1.4

fat solvent. Total free fatty acids were expressed in mg per 100 g of sample, assuming an average molecular weight of 300.
Total phospholipids were determined in the chloroform extracts by the colorimetric method of NEN 1046 (25) using an acid digestion for the conversion of organic into inorganic phosphorous. Total phospholipids were expressed in mg per 100 g of sample, assuming a conversion factor of 25.

Results and Discussion

pH measurements
During the first two weeks of frozen storage there was a slight fall of about 0.1 in the pH of all samples. Afterwards a small increase of about 0.2 was observed.
In all treatments (blank, blank irradiated and the irradiated treatments with additives) the pH's were between 6.7 and 6.9.

W.H.C.R. and C.D.L.
The low dose of irradiation (300 krad) did not affect the W.H.C.R. and C.D.L. of the fish minces with and without additives (**Figs. 1 and 2**). The results obtained for W.H.C.R. and C.D.L. were as before (14).

Texture analysis
Comparing the raw and cooked samples of the blanks, the irradiated blank seemed to have a more tender texture than the non-irradiated ones (**Figs. 3 and 4**). SEGARS *et al.* (26) reported similar results e.g. that the shear stress of cooked beef samples measured in the Instron decreased with the increase of the dose level of irradiation.
Irradiation did not affect the remarkable stabilizing effect of the additions in the texture, which was observed before.

DMA and FA production
The low dose of irradiation used did not show any noticeable effect on DMA and FA production (**Tab. 1 and 2**). All treatments had similar increases during frozen storage at −18°C. This is in accordance with our previous work (27) in which the same dose of irradiation was applied to the fish fluid of minced fillets of whiting. A recent report of LICCIARDELLO *et al.* (3) showed also that a low dose of irradiation (100 krad) did not affect significantly the formation of DMA in cod. It seems to be that only high doses of irradiation (600 krad and more) are able to increase the production of DMA and FA (9,10).

Lipid oxidation
During frozen storage, the oxidation in lean fishes is usually extremely slow and occurs primarily in the phospholipids fraction (28, 29). Normally the TBARS test doesn't show any substantial change during long periods of frozen storage (28), but off-flavours can still be developed from unsaturated carbonyls (29).
In our experiments, it seems to be that neither the TBARS nor TC were affected by the dose of irradiation used

Tab.2 Formaldehyde content (µg/g of fish) of frozen stored minced cod at time intervals

Days at		Irradiated			
-18°C	Blank	Blank	ι-carrag.	CMC	Xanthan
0	2.4 ± 0.0	2.8 ± 0.1	2.3 ± 0.0	2.5 ± 0.1	2.4 ± 0.1
20	3.7 ± 0.1	4.4 ± 0.0	3.4 ± 0.1	4.4 ± 0.2	3.6 ± 0.1
42	4.7 ± 0.2	5.8 ± 0.3	5.5 ± 0.2	4.9 ± 0.2	4.8 ± 0.1
60	5.7 ± 0.1	6.3 ± 0.3	5.4 ± 0.3	6.6 ± 0.3	6.0 ± 0.3
77	7.7 ± 0.3	7.6 ± 0.0	7.1 ± 0.2	7.8 ± 0.3	7.0 ± 0.2
91	8.6 ± 0.2	10.6 ± 0.4	9.3 ± 0.3	9.2 ± 0.2	8.5 ± 0.3

Tab.3 Thiobarbituric acid reactive substances content (µmol/kg of fish) of frozen stored minced cod at time intervals

Days at		Irradiated			
-18°C	Blank	Blank	ι-carrag.	CMC	Xanthan
0	5.7	5.2	5.7	5.7	5.7
13	4.2	5.1	4.1	4.8	4.6
40	3.9	3.7	4.0	3.5	5.1
55	3.9	3.8	4.8	4.9	3.2
68	4.9	4.7	5.0	4.5	4.8
90	3.5	3.1	3.8	4.6	3.7

Tab.4 Total carbonyls content (µmol/kg of fish) of frozen stored minced cod at time intervals

Days at		Irradiated			
-18°C	Blank	Blank	ι-carrag.	CMC	Xanthan
0	66	56	49	40	52
36	94	96	84	93	95
54	129	123	131	141	120
69	140	156	161	122	132
90	173	173	173	158	161

(**Tab.3 and 4**). In all treatments the TBARS values were low and almost constant during the 3 months of frozen storage. Values of TBARS lower than 8 µmol kg^{-1} fish are an indication of no rancidity while values between 9 and 20 µmol kg^{-1} fish indicate slight rancidity (20). During the same time of frozen storage TC showed a steady increase. TARLADGIS *et al.* (19) noted already that there is no need for a relationship between TBARS andTC. The increase observed in TC in all treatments (**Tab.4**) may be ascribed partly to small oxidative changes in the phospholipid fraction and mainly to other reactions capable of producing carbonyl compounds at -18°C (30).

Lipid hydrolysis

During frozen storage lipid hydrolysis proceeded in all cases without significant differences between treatments (**Figs.5 and 6**). In lean fishes, like cod, lipid hydrolysis is mainly due to phospholipase activity. The increase in acidity measured in the chloroform extracts was proportional to the decrease of phospholipids (**Fig.6**).

Conclusions

The low dose of irradiation used (300 krad, -20°C) had no significant effect on the water holding capacity, texture and DMA production of the minced fillets of cod during the 3 months of frozen storage (-18°C). Irradiation did not disturb the stabilizing effect of the additions used (Iota carrageenan, Carboxymethyl cellulose and Xanthan).

The rates of lipid hydrolysis and lipid oxidation were similar for all our experiments.

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8. Effects of Iota carrageenan, carboxymethyl cellulose and Xanthan gum on the stability of formulated minced fish products during frozen storage

8.1. INTRODUCTION

Minced fish is becoming an important raw material. Several products using minced fish are already marketed, like fish sticks and fish burgers. However the minces obtained by mechanical deboners from the whole fish or from the fillet rests have a texture and water holding capacity which deteriorate more rapidly than whole fish or fillet during frozen storage (Froning, 1981; Rodger et al., 1980; Keay and Hardy, 1978).

In previous work we have shown that several hydrocolloids have a cryoprotective effect on texture and water holding capacity of minced fillets of cod and whiting during frozen storage (chapters 3, 4 and 5). In these experiments the evaluation of texture and water holding capacity was done by instrumental methods. In the present study we used some of these hydrocolloids (Iota carrageenan, carboxymethyl cellulose and Xanthan) as additives in formulated minced fish products (fish chips and fish sticks) and determined their effects on weight loss, texture and formation of dimethylamine and formaldehyde during frozen storage. The purpose was to find out if in formulated minced fish products, the hydrocolloids had similar effects on texture and water holding capacity as observed before (chapters 4 and 5) and if the instrumental measurements of texture and water holding capacity could be correlated with the sensory evaluation of these parameters by an analytical panel. It was also important to know if the hydrocolloids would impart any off-taste which could lower the acceptance of the fish products by consumers. As carboxymethyl cellulose and carrageenan have been reported to reduce fat absorption of several products during frying, we also looked at this aspect in the different treatments of the various products during frozen storage (Akzo Zout Chemie, 1985; Whistler and Daniel, 1985).

8.2. MATERIALS AND METHODS

A schematic presentation of the preparation of fish sticks and fish chips is shown on Fig. 1.

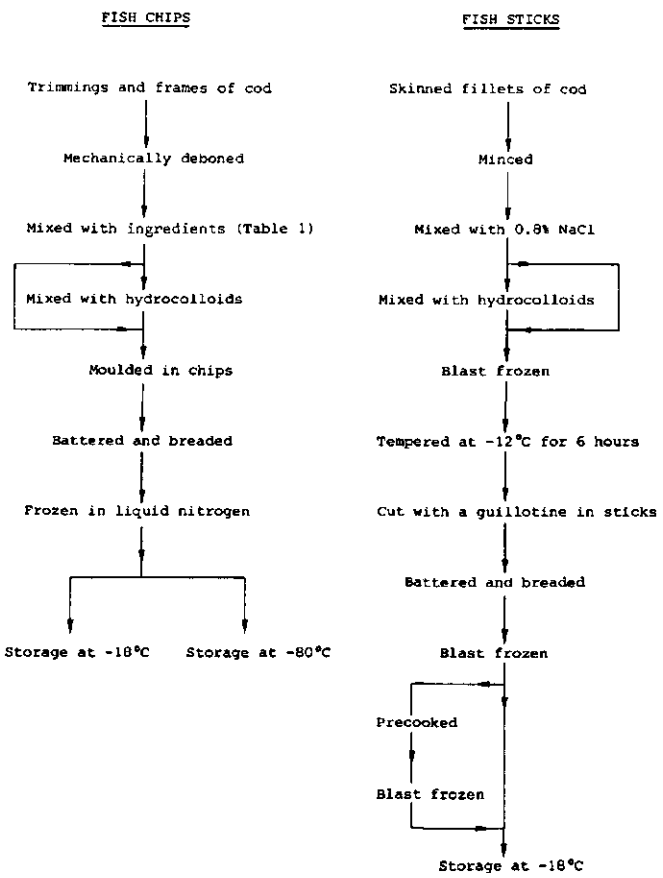

FIG. 1. Schematic illustration of the manufacture of fish sticks (precooked and unprecooked) and fish chips.

Cod bought in the trade market was manually filleted and the frames and trimmings were mechanically deboned at the "Rog's Visspecialiteiten" Factory, Deventer, on May 31, 1985. The minces were kept in a cold storage (2°C) until the next day and were mixed with several ingredients (Table 1) in a Hobart Mixer for the normal production of fish chips. The resulting minced fish was divided in portions and different hydrocolloids were spread over the surface (5g per kg of minced fish). The hydrocolloids and the minces were gently mixed with a spoon and kept in a cold storage (2°C) for two hours.

Hydrocolloids used were: (i) Iota carrageenan (HF-33895-96), from Copenhagen Pectin Factory Ltd (Denmark); (ii) Carboxymethyl cellulose (CMC), AF 2805

TABLE 1. Composition of fish chips (Blank).

Fish minces	90.07%
Wheat flour	6.76%
Fish broth	1.91%
Monosodium glutamate	0.34%
Salt (NaCl)	0.56%
Bread Crumbs	0.36%

from Enka bv, Industrial Colloids (Holland); (iii) Xanthan gum, Keltrol F (KTLF--67203A), from Kelco (New Jersey, U.S.A.).

The different treatments were passed through a moulding machine to produce chips of 7cm x 1cm x 1cm. These chips were immediately battered, breaded and frozen in liquid nitrogen. Chips were transported to Wageningen (1 hour by car) in isothermal boxes and were kept at -18°C for about 8 months. A portion of the treatment without hydrocolloids (Blank) was kept at -80°C for the same time.

Skinned fillets of cod were bought in the local market of Wageningen on July 3, 1985. They were passed through a mincer (Zellweger s.a. Uster, Switzerland) using a mincing screen with 4mm holes. The resulting minced fish was divided in portions and 0.8% sodium chloride and 0.5% of different hydrocolloids (the same as used for the fish chips) were spread over the surface and gently mixed with a spoon. The fish minces were then kept in plastic bags on melting ice for two hours. Samples of 1015g of minces were repacked in plastic bags (300mm x 500mm x 0.065mm) in a way that the minced fish formed a rectangular slab of 290mm x 350mm and 10mm thick on average. The slabs were blast frozen, tempered for 6 hours at -12°C and then cut with a guillotine in the form of fish sticks (60mm x 25mm x 10mm). These portions were battered, breaded and blast frozen. All treatments were divided in two parts: one was kept immediately at -18°C for about 5 months and the other was first precooked in a microwave oven (Philips, model 2010C) till an internal temperature of 80°C (20 seconds) and then blast frozen and kept at -18°C.

Skinned fillets of cod bought in the local market of Wageningen, on October 16, 1985, were minced as before. The resulting minced fish was divided in portions and 0.8% of sodium chloride and different concentrations (0.5% and 2%) of hydrocolloids (the same as for the fish chips) were added. The different treatments were treated as before for the production of fish sticks.

One part was kept at -45°C for the weight loss and fat uptake determination and another part (0.5% addition) was kept at -18°C for sensory analysis.

Weight loss and fat uptake determination

Batches of 30 preweighed frozen fish chips were fried in a semi-solid, partially hydrogenated, palm oil (Romi, Holland) at 180°C in an electric frying pan (Giorik, model 500, 3000 watt, Bribano, Italy) for 3 minutes and 10 seconds. Immediately after, the frying pan basket was suspended and the fish chips were allowed to drip for 30 seconds, then they were put on two layers of filter paper (S&S 602 h, 18.5 cm of diameter) and after 10 minutes were transfered to a preweighed stainless steel dish. After 3 hours of adjustment to room temperature the dish was reweighed and the weight loss calculated as follows:

$$\text{Weight loss} = \frac{\text{initial weight - weight after frying}}{\text{initial weight}} \times 100\%$$

The fish chips were then freeze-dried and the fat was extracted by the Soxhlet method using hexane as solvent. Fat uptake was expressed as fat weight (g) and as percentage of the fish chips after frying.

The weight loss and fat uptake of the fish sticks were determined in the same way as for the fish chips, except that size of the batches were of 4 fish sticks each. The initial weight of the precooked fish sticks was considered to be the weight before precooking in the microwave oven.

Two replicates were done for each sample of the fish chips and three replicates were done for each sample of the fish sticks.

Texture analysis

Texture analyses were carried out in a Kramer Shear Press Model SP--12 Imp. Four fish chips, fried in the same way as for the weight loss determination, were put in a CS-1 standard shear-compression cell (flat on grid at 90° angle to the slits), an electronic textureguage of 5000 lbs (22241 N) and a speed of 2.1 mm/second were used. Texturegrams were recorded on T-2100 texture test system and the height of the peak (Fig. 2) used for comparison the different treatments.

The same procedure was applied to the fish stick samples with the exception that two fish sticks were used in the shear compression cell.

Three replicates were done for each sample.

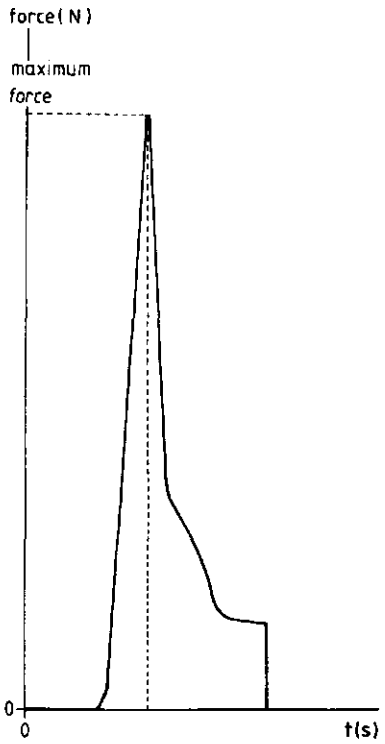

FIG. 2. Typical recorder curve obtained with the CS-1 standard shear-compression cell of the Kramer Shear Press on the different fried formulated minced fish products.

Dimethylamine (DMA) and Formaldehyde (FA) determination

DMA and FA determination was carried out as before (chapter 6). Two replicates were done for each sample.

Sensory analysis

At zero time and at 8 months of frozen storage, fish chips were evaluated for preference using the forced-choice, paired comparison test. At zero time, four treatments (Blank, Iota carrageenan, CMC and Xanthan) were evaluated by visitors (relatives of the students) during the Open Day of the Food Chemistry Department and at 8 months of frozen storage, five treatments (Blank kept at -80°C and Blank, Iota carrageenan, CMC and Xanthan kept at -18°C) were evaluated by students of the course of sensory analysis. In both cases,

the assessors were divided in small groups. The fish chips were fried in the same way as for the weight loss determination and were hot served to the assessors in individual booths, in coded trays.

At 14 weeks of frozen storage, fish sticks were evaluated for assessment of sensory attributes, using 18 members of the analytical panel of the Spelderholt Centre for Poultry Research and Extension, Beekbergen. The fish sticks were also evaluated for preference by 80 co-workers of Spelderholt Centre and of the Sprenger Institute. Preliminary sessions with the analytical panel were carried out in order to define the sensory attributes which could describe this type of product and which could be relevant for differentiating the various treatments. The following sensory attributes were chosen:

-- Fish taste: Resemblances to fish. Any taste detected during biting, chewing or swallowing which was judged not fish like had a negative contribution for the evaluation of the sample.
-- Fibrosity: Impression of fibers in the sample on chewing.
-- Softness: Impression of the force required to break the structure of the fish stick by biting or chewing. Soft is the opposite of tough.
-- Juiciness: Impression of moistness and succulence during chewing prior to swallowing. Dry is the opposite of succulent or juicy.
-- Crustiness: Impression of the force required to break the structure of the crust of the fish stick on biting.

Forced-choice, paired comparison test was used for the preference and sensory attributes of the fish sticks. Fish sticks were fried and served in the same way as the fish chips.

Statistical analysis

Differences in weight loss, fat uptake and percentage of fat were analysed by Duncan's multiple range test at a probability level of 1% (Steel & Torrie, 1982). Differences in the total scores of the paired comparison test were analysed by the least significant difference method at a probability level of 5% (David, 1963).

8.3. RESULTS AND DISCUSSION

Weight loss and Fat uptake

Xanthan and CMC were the most effective additions to reduce weight

loss during frying after frozen storage (Tables 2 and 3). The effect of the additions on the weight loss of the fish chips were masked to some extent by the overwhelming amount of flour (6.8%) in this product (Table 2A). Fish chips without additives which were kept at -80°C for 8 months showed a weight loss (24.8 ± 0.6%) very similar to the initial value of the Blank. Fish sticks which were precooked in the microwave oven had a drastic increase in weight loss (Table 2C). Increasing the concentration of the additives resulted in a corresponding significant reduction in weight loss (Table 3).

TABLE 2. Effect of time and different hydrocolloids (0.5% w/w) on the weight loss and fat uptake of different types of formulated minced fish products at -18°C.

A - Fish chips.[1]

Days at -18°C		Blank	Iota carrag.	CMC	Xanthan
	Weight loss (%)	24.6 ± 1.3^{a}	24.5 ± 1.4^{a}	22.4 ± 0.5^{a}	23.3 ± 2.7^{a}
0	Fat uptake (g)	25.8 ± 0.1^{a}	21.8 ± 0.7^{a}	24.3 ± 0.1^{a}	25.4 ± 1.7^{a}
	Fat (%)	14.8 ± 0.4^{a}	12.4 ± 0.1^{b}	13.6 ± 0.1^{ab}	14.4 ± 0.3^{a}
	Weight loss (%)	27.2 ± 1.1^{a}	26.3 ± 0.7^{a}	24.7 ± 1.3^{a}	25.3 ± 0.3^{a}
92	Fat uptake (g)	25.2 ± 1.0^{a}	21.1 ± 0.6^{b}	22.3 ± 0.4^{ab}	23.2 ± 0.5^{ab}
	Fat (%)	15.1 ± 0.9^{a}	12.4 ± 0.2^{b}	12.9 ± 0.1^{ab}	13.6 ± 0.1^{ab}
	Weight loss (%)	26.1 ± 0.5^{a}	25.1 ± 0.6^{ab}	23.1 ± 0.6^{b}	22.3 ± 0.7^{b}
167	Fat uptake (g)	25.5 ± 0.7^{a}	22.4 ± 0.4^{b}	22.6 ± 0.5^{b}	24.6 ± 0.5^{ab}
	Fat (%)	15.0 ± 0.3^{a}	12.8 ± 0.2^{b}	12.8 ± 0.2^{b}	13.9 ± 0.4^{ab}

B - Fish sticks.

Days at -18°C		Blank	Iota carrag.	CMC	Xanthan
	Weight loss (%)	25.9 ± 1.6^{a}	23.8 ± 3.3^{a}	20.0 ± 3.4^{ab}	16.0 ± 0.2^{b}
0	Fat uptake (g)	7.9 ± 0.1^{a}	7.0 ± 0.3^{b}	7.6 ± 0.4^{ab}	8.3 ± 0.4^{a}
	Fat (%)	13.1 ± 0.4^{a}	11.4 ± 0.3^{b}	12.1 ± 0.4^{ab}	12.5 ± 0.7^{ab}
	Weight loss (%)	29.4 ± 1.2^{a}	25.0 ± 1.7^{b}	20.8 ± 1.4^{c}	16.7 ± 1.6^{d}
56	Fat uptake (g)	7.8 ± 0.2^{a}	7.2 ± 0.2^{b}	7.1 ± 0.1^{b}	7.7 ± 0.4^{ab}
	Fat (%)	12.9 ± 0.6^{a}	11.5 ± 0.3^{b}	11.2 ± 0.2^{b}	11.1 ± 0.4^{b}
	Weight loss (%)	30.3 ± 1.2^{a}	26.2 ± 0.6^{b}	19.7 ± 1.7^{c}	16.9 ± 1.6^{c}
131	Fat uptake (g)	7.9 ± 0.4^{a}	7.0 ± 0.3^{b}	7.8 ± 0.3^{ab}	7.4 ± 0.2^{ab}
	Fat (%)	13.2 ± 0.7^{a}	11.3 ± 0.4^{b}	11.6 ± 0.4^{b}	10.8 ± 0.5^{b}

C - Precooked fish sticks.

Days at -18°C		Blank	Iota carrag.	CMC	Xanthan
0	Weight loss (%)	39.8±1.6[a]	39.5±1.0[a]	32.8±0.1[b]	29.3±0.5[c]
	Fat uptake (g)	8.1±0.3[b]	6.3±0.4[c]	8.5±0.9[ab]	9.9±0.4[a]
	Fat (%)	17.2±0.6[a]	13.6±0.3[b]	16.5±1.8[a]	17.0±0.5[a]
56	Weight loss (%)	39.2±0.7[a]	39.5±2.0[a]	32.0±2.6[b]	28.3±1.4[b]
	Fat uptake (g)	9.3±0.7[a]	7.2±0.2[b]	10.±0.4[a]	8.7±0.7[a]
	Fat (%)	18.8±1.6[a]	15.6±0.5[b]	18.7±0.6[a]	14.1±1.1[b]
131	Weight loss (%)	38.6±1.9[a]	37.9±0.5[a]	31.5±1.4[b]	27.5±0.3[c]
	Fat uptake (g)	9.8±0.4[a]	7.1±0.3[b]	9.4±0.3[a]	9.1±0.3[a]
	Fat (%)	19.0±0.4[a]	14.3±0.7[c]	16.6±0.6[b]	14.3±0.2[c]

[1]Blank of the fish chips kept at -80°C for 8 months presented a weight loss of 24.8±0.6%.

[a,b,c,d]Values in the same horizontal line with the same superscripts were not significantly different ($P < 0.01$).

TABLE 3. Effect of different concentrations of hydrocolloids on the weight loss and fat uptake, during frying of frozen fish sticks.

%(w/w) of hydrocolloids		0.0	0.5	2.0
Iota carrag.	Weight loss (%)	24.1±0.6[a]	19.8±1.2[b]	15.7±0.9[c]
	Fat uptake (g)	8.4±0.2[a]	7.5±0.1[b]	7.3±0.2[b]
	Fat (%)	13.2±0.4[a]	11.5±0.0[b]	10.7±0.2[b]
CMC	Weight loss (%)	24.1±0.6[a]	15.7±1.3[b]	10.1±0.1[c]
	Fat uptake (g)	8.4±0.2[a]	8.1±0.4[a]	7.1±0.3[b]
	Fat (%)	13.2±0.4[a]	11.1±0.8[b]	9.4±0.5[b]
Xanthan	Weight loss (%)	24.1±0.6[a]	10.6±0.5[b]	6.2±0.3[c]
	Fat uptake (g)	8.4±0.2[b]	9.3±0.2[a]	9.3±0.2[a]
	Fat (%)	13.2±0.4[a]	12.7±0.3[a]	12.1±0.5[a]

[a,b,c]Values in the same horizontal line with the same superscripts were not significantly different ($P < 0.01$).

Fat uptake remained almost constant in all treatments of the various formulated minced fish products, after different times of frozen storage (Table 2). All additions reduced the percentage of fat uptake in the fried product (Table 2). The same fact is reported on Table 3. In Xanthan this was due to the decrease of weight loss, in the CMC and Iota carrageenan

this was also due to the reduction of the fat absorbed during frying. Increasing the concentration of the additives resulted in the reduction of the fat uptake of the CMC treatment but did not have any significant effect on the fat absorbed by the Iota carrageenan and Xanthan treatments. Precooking seemed to destroy the capacity of the CMC to reduce the fat uptake during frying.

Very few studies have dealt with the mechanisms of fat absorption during frying. This phenomenon seems to be dependant upon several parameters like type of food, surface conditions, formation of crust, moisture content, type of fat used, frying temperature and time of frying (Thorner, 1973). The ability of the CMC to reduce fat uptake may be related to the formation of a film at the surface of the product which would prevent in part the penetration of too much oil or fat (Akzo, 1985; Ludwig, 1968; Ludwig, 1971). Film forming agents, like gelatins and certain starches have also been reported to reduce oil absorption during frying (Olson and Zoss, 1985). The capacity of Iota carrageenan to form a gel may account for the reduction of fat uptake.

Texture analysis

The Blank of the fish chips and of the fish sticks showed the highest increase of maximum force during frozen storage (Figs. 3 and 4). On the other hand, the fish chips without additions, which were kept at -80°C for about 8 months showed values of maximum force similar to the initial values of the Blank or even lower (Fig. 3). Xanthan and CMC presented a remarkable stabilizing effect in all products during frozen storage. Precooking greatly increased the maximum force necessary to extrude the fish sticks through the Kramer Shear Press cell; only Xanthan and CMC showed values of maximum force similar to the Blank of the fish sticks not precooked.

Dimethylamine (DMA) and Formaldehyde (FA)

With the exception of the precooked fish sticks all treatments showed increase in DMA and FA during frozen storage (Tables 4 and 5). The fish chips, which were made from mechanical deboned frames and trimmings of cod, presented the highest rates of DMA and FA formation. Precooking the fish sticks to an internal temperature of 80°C almost completely arrested the formation of DMA and FA, in agreement with previous work (chapter 6).

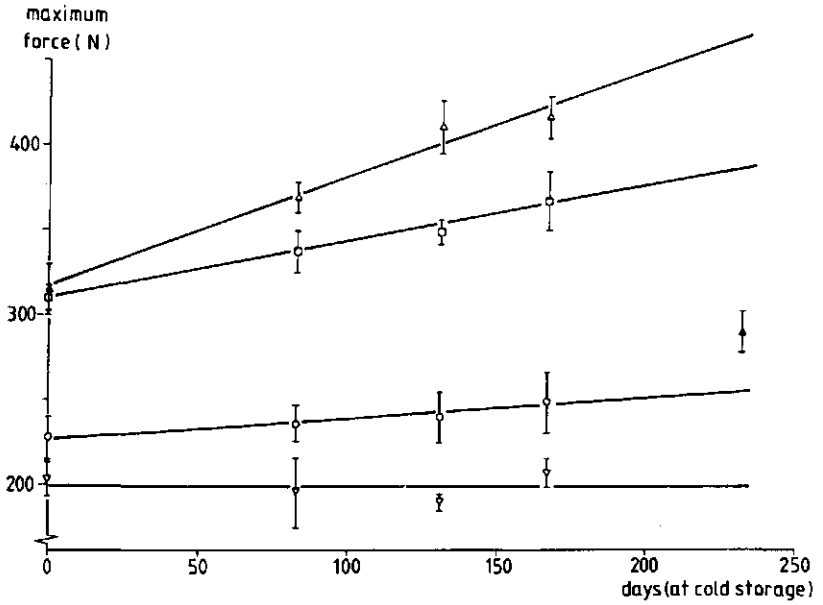

FIG. 3. Effect of time and different hydrocolloids on the maximum force, measured in the Kramer Shear Press, of the frozen stored fish chips. △ Blank at -18°C, ▲ Blank at -80°C, □ Iota carrageenan at -18°C, ○ Carboxymethyl cellulose at -18°C and ▽ Xanthan at -18°C.
The vertical bars represent standard deviation.

Moreover, the precooked fish sticks appeared to be relatively stable in texture (Fig. 4), which could be an indication of the involvement of FA on toughening during frozen storage.

Sensory analysis

The response of the analytical panel to the various sensory attributes of the unprecooked fish sticks at 14 weeks of frozen storage is reported on Table 6. Blank, Iota carrageenan and CMC did not show any significant difference on fish taste and even Xanthan, which had significantly lower score than Iota carrageenan, was not significantly different from the Blank (Table 6I). Blank and Iota carrageenan were significantly less juicy than CMC and Xanthan (Table 6II). High correlations were obtained between the scores of juiciness and the different instrumental measurements of weight loss (interpolated from Table 2B), cook drip loss and water holding capacity

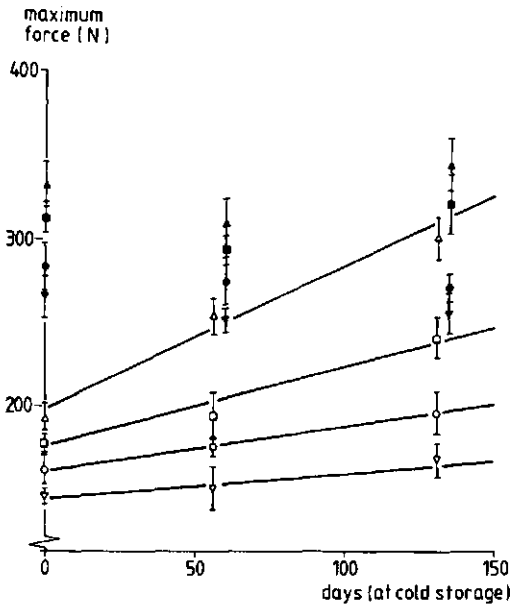

FIG. 4. Effect of time and different hydrocolloids on the maximum force, measured in the Kramer Shear Press, of the fish sticks stored at -18°C. △ Blank, □ Iota carrageenan, ○ Carboxymethyl cellulose, ▽ Xanthan, ▲ Blank precooked, ■ Iota carrageenan precooked, ● Carboxymethyl cellulose precooked and ▼ Xanthan precooked.
The vertical bars represent standard deviation.

TABLE 4. Dimethylamine content (μg DMA-Nitrogen/g of fish) of different types of frozen formulated minced fish products at time intervals.

	Days at -18°C	0	77	128	150	180
Fish chips	Blank	37.7±0.9		85.6±3.3		108.4±1.6
	Iota carrag.	36.5±1.1		85.7±3.0		110.7±3.4
	CMC	37.1±1.5		102.2±3.3		112.1±5.9
	Xanthan	38.0±0.8		90.1±1.3		104.1±4.7
Fish sticks	Blank	10.0±0.3	52.7±2.1		65.4±2.0	
	Iota carrag.	9.2±0.4	59.3±1.3		62.0±1.7	
	CMC	10.3±0.3	52.8±2.2		67.8±2.9	
	Xanthan	10.5±0.4	48.6±1.1		59.0±1.4	
Precooked fish sticks	Blank	13.3±0.2	14.1±0.4		13.5±0.5	
	Iota carrag.	13.0±0.1	14.5±0.4		14.6±0.4	
	CMC	13.1±0.4	14.7±0.5		13.7±0.7	
	Xanthan	14.2±0.5	14.9±0.3		14.3±0.4	

TABLE 5. Formaldehyde content (μg/g of fish) of different types of frozen formulated minced fish products at time intervals.

Days at -18°C		0	77	128	150	180
Fish chips	Blank	8.6 ± 0.2		23.3 ± 0.6		34.1 ± 1.2
	Iota carrag.	8.4 ± 0.4		21.2 ± 0.4		32.2 ± 1.5
	CMC	8.5 ± 0.1		25.0 ± 0.8		35.2 ± 1.1
	Xanthan	8.8 ± 0.3		22.3 ± 0.1		32.0 ± 0.7
Fish sticks	Blank	2.7 ± 0.0	6.0 ± 0.1		12.9 ± 0.6	
	Iota carrag.	2.7 ± 0.1	5.7 ± 0.3		12.5 ± 0.5	
	CMC	2.8 ± 0.1	5.8 ± 0.4		13.5 ± 0.7	
	Xanthan	2.9 ± 0.2	5.3 ± 0.4		11.9 ± 0.9	
Precooked fish sticks	Blank	2.6 ± 0.0	2.7 ± 0.1		2.7 ± 0.1	
	Iota carrag.	2.5 ± 0.2	2.3 ± 0.1		2.8 ± 0.1	
	CMC	2.4 ± 0.1	2.7 ± 0.2		2.6 ± 0.1	
	Xanthan	2.6 ± 0.1	2.7 ± 0.1		2.6 ± 0.0	

TABLE 6. Response of the analytical panel, in paired comparison test, to various sensory attributes of the different treatments of fish sticks at 14 weeks of frozen storage. A = Blank, B = Iota carrageenan, C = Carboxymethyl cellulose and D = Xanthan.

I - Fish taste.

Pairs	A	B	C	D	Significance[1]
A,B	7	11			n.s.
A,C	9		9		n.s.
A,D	12			6	n.s.
B,C		10	8		n.s.
B,D		12		6	n.s.
C,D			10	8	n.s.
Total scores	28^{ab}	33^{a}	27^{ab}	20^{b}	

II- Juiciness.

Pairs	A	B	C	D	Significance[1]
A,B	6	12			n.s.
A,C	2		16		(1.0%)
A,D	2			16	(1.0%)
B,C		2	16		(1.0%)
B,D		1		17	(0.1%)
C,D			2	16	(1.0%)
Total scores	10^{a}	15^{a}	34^{b}	49^{c}	

III - Fibrosity.

Pairs	A	B	C	D	Significance[1]
A,B	17	1			(0.1%)
A,C	15		3		(1.0%)
A,D	17			1	(0.1%)
B,C		16	2		(1.0%)
B,D		18		0	(0.1%)
C,D			16	2	(1.0%)
Total scores	49^{a}	35^{b}	21^{c}	3^{d}	

IV - Softness.

Pairs	A	B	C	D	Significance[1]
A,B	3	15			(1.0%)
A,C	2		16		(1.0%)
A,D	1			17	(0.1%)
B,C		4	14		(5.0%)
B,D		0		18	(0.1%)
C,D			3	15	(1.0%)
Total scores	6^{a}	19^{b}	33^{c}	50^{d}	

V - Crustiness.

Pairs	A	B	C	D	Significance[1]
A,B	15	3			(1.0%)
A,C	10		8		n.s.
A,D	15			3	(1.0%)
B,C		9	9		n.s.
B,D		17		1	(0.1%)
C,D			9	9	n.s.
Total scores	40^{a}	29^{ab}	26^{b}	13^{c}	

[1] Level of significance of difference in each pair (American Society for Testing and Materials, 1968); n.s.= not significant.

^{a,b,c,d} values with the same superscripts were not significantly different ($P < 0.05$).

(obtained by extrapolation from chapters 4 and 5; Fig. 5 and Table 7).

All treatments presented significantly different scores in respect of fibrosity and softness (Table 6III and IV). Xanthan was judged the least fibrous and softest and the Blank the most fibrous and the toughest. Blank gave also the highest crustness and Xanthan the lowest crustness (Table 6V). The scores for fibrosity and softness were in good agreement with the instrumental measurements of maximum force (interpolated from Fig. 4 at 14 weeks), compressive strength, modulus of elasticity and resilience (obtained by extrapolation from chapters 4 and 5; Fig. 6 and Table 8).

The instrumental measurements of texture and water holding capacity and the scores of the respective sensory attributes are dependant upon the type of fish and the batch of fish used (see differences on Table 2B and Table 3 for weight loss). It was not possible to do all the measurements on the same batch of fish. The differences however would be proportional and certainly similar correlations would be obtained.

The results of the preference test on the different treatments of the fish chips and fish sticks, not precooked, are reported on Table 9. For most of the assessors these fish products were entirely new and their references for judgement were probably potato chips for the case of fish chips and fish sticks made from fillets for the fish sticks. We asked the assessors to write

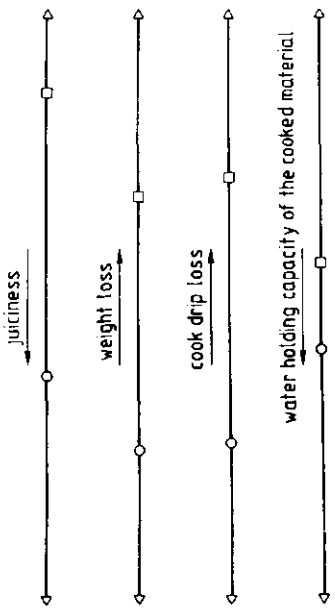

FIG. 5. Comparison of the different instrumental measurements of water holding capacity with the scores of the sensory attribute of juiciness. Weight loss was obtained by interpolation from Table 2B. Cook drip loss and water holding capacity of the cooked material were obtained by extrapolation from chapters 4 and 5. See Fig. 3 for symbols.

the reasons for their preferences. Most of the panelists indicated structure and taste as the main reasons for their preferences. Xanthan was not considered appropriate for the manufacture of fish sticks. It gave a very soft product perhaps suitable for a croquette. The fish chips with Xanthan were however acceptable and even after 8 months of frozen storage at -18°C were

TABLE 7. Correlation coefficients (r) between the scores of the sensory attribute of juiciness and the instrumental measurements of weight loss, cook drip loss and water holding capacity of the cooked material (Fig. 5).

	Cook drip loss	Weight loss	Water holding capacity of the cooked material
Juiciness	-0.985	-0.979	0.941

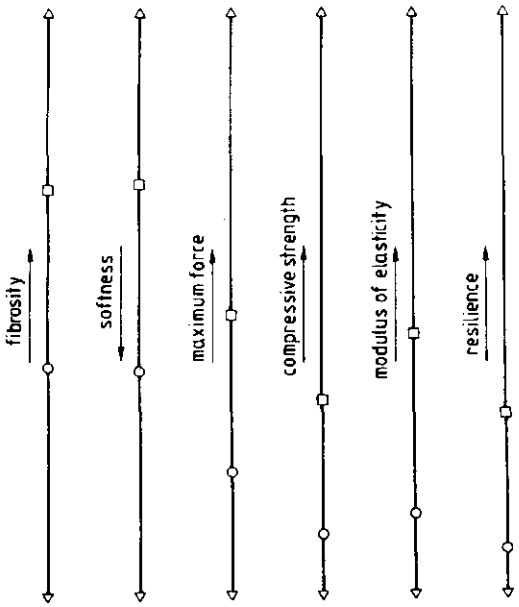

FIG. 6. Comparison of different instrumental measurements of texture with the scores of the sensory attributes of fibrosity and softness. Maximum force values were obtained from Fig 4. Compressive strength, modulus of elasticity and resilience were obtained by extrapolation from chapters 4 and 5. See Fig. 3 for symbols.

significantly preferred to the Blank (Table 9II). In this product, CMC addition was significantly preferred to all the other treatments, including the Blank kept at -80°C for 8 months. Iota carrageenan seemed to be highly recomended for the production of fish sticks (Table 9III). After longer periods of storage,

TABLE 8. Correlation coefficients (r) among the scores of the sensory attributes of fibrosity and softness and the instrumental measurements of maximum force, compressive strength, modulus of elasticity and resilience (Fig. 6).

	Maximum force	Compressive strength	Modulus of elasticity	Resilience
Fibrosity	0.964	0.909	0.946	0.897
Softness	-0.963	-0.907	-0.945	-0.895

Table 9. Response of assessors, in paired comparison test, to the preference of the different treatments of the various formulated minced fish products. Ao = Blank at -80°C, A = Blank at -18°C, B = Iota carrageenan at -18°C, C = Carboxymethyl cellulose at -18°C and D = Xanthan at -18°C.

I - Fish chips at zero time of frozen storage.

Pairs	A	B	C	D	Significance[1]
A,B	22	25			n.s.
A,C	26		21		n.s.
A,D	28			19	n.s.
B,C		23	24		n.s.
B,D		24		23	n.s.
C,D			23	24	n.s.
Total scores	76[a]	72[a]	68[a]	66[a]	

II- Fish chips at 8 months of frozen storage.

Pairs	Ao	A	B	C	D	Significance[1]
Ao,A	18	12				n.s.
Ao,B	15		15			n.s.
Ao,C	14			16		n.s.
Ao,D	15				15	n.s.
A,B		10	20			n.s.
A,C		6		24		(1%)
A,D		13			17	n.s.
B,C			8	22		(5%)
B,D			8		22	(5%)
C,D				20	10	n.s.
Total scores	62[b]	41[c]	51[cb]	82[a]	64[b]	

III - Fish sticks at 14 weeks of frozen storage.

Pairs	A	B	C	D	Significance[1]
A,B	31	49			n.s.
A,C	48		32		n.s.
A,D	63			17	(0.1%)
B,C		47	33		n.s.
B,D		66		14	(0.1%)
C,D			68	12	(0.1%)
Total scores	142[ab]	162[a]	133[b]	43[c]	

[1]Level of significance of difference in each pair (American Society for Testing and Materials, 1968); n.s. = not significant.

[a,b,c]Values with the same superscripts were not significantly different ($P < 0.05$).

it is possible that Iota carrageenan and perhaps CMC would be significantly preferred to the Blank. Each fish product has an ideal texture which should be maintained during frozen storage. For each product the right addition or combination of additions has to be found.

8.4. CONCLUSIONS

All additions had a remarkable stabilizing effect on texture and weight loss in all products, during frozen storage. Precooking to an internal temperature of 80°C arrested the production of DMA and FA but increased the weight loss and maximum force. Results of sensory analysis showed good correlation between sensory attributes and the instrumental measurements of texture and water holding capacity. After 8 months of frozen storage, the fish chips with carboxymethyl cellulose and Xanthan additions were significantly preferred to the Blank. After 14 weeks of frozen storage, the fish sticks samples with Iota carrageenan presented the highest score of preference although not significantly different from the Blank. Iota carrageenan and to some extent carboxymethyl cellulose reduced the fat absorption during frying.

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9. Effects of additives made under specific conditions of viscosity on the stability of frozen stored minced fillets of cod

9.1. INTRODUCTION

The experiments described in previous chapters (3, 4, 5, 7 and 8) strongly indicated that the capacity of the hydrocolloids to decrease toughening of the fish minces during frozen storage was related to their ability to hold water in the raw minced fish (increase in W.H.C.R.). This phenomenon might be explained by increase of viscosity of the fluid phase or, in case of incomplete dissolution, by the formation of hydrated particles of hydrocolloids. In order to obtain more insight in these hypothetical factors of the mechanism of action of the hydrocolloids we investigated these aspects further. We compared carboxymethyl celluloses with similar degrees of substitution but with different degrees of polymerization and at different concentrations in the fish fluid, in order to give similar flow curves at low shear rates. We also compared different hydrocolloids which gave, at the same concentration in the fish fluid, similar flow curves at low shear rates. We investigated further the effects of the addition of Iota carrageenan by comparing it with a mechanically degraded Iota carrageenan which had a smaller average molecular weight and had lost its gel formation capacity.

We have used empirical parameters of the structural failure of the cooked fish minces to determine changes of texture during frozen storage. Sensory texture profile evaluation of fish gels tends to correlate much better with destructive mechanical tests than with small deformation tests (Hamann, 1983; Lanier, 1986). However as we were interested in having a better understanding of the viscoelastic system of the fish minces and of the possible influences of the hydrocolloids on fish texture, we also determined the modulus of elasticity of the Blank, Iota carrageenan and of a highly viscous carboxymethyl cellulose, in the linear region of the true stress-Cauchy's strain curves, at various speeds of the plunger.

9.2. MATERIALS AND METHODS

Skinned fillets of cod were bought in the local market of Wageningen, on December 4, 1985. They were passed through a mincer (Zellweger s.a. Uster,

Switzerland) using a mincing screen with 4mm holes. The resulting minced fish was divided in portions and different hydrocolloids and sucrose and sorbitol were spread over the surface and gently mixed with a spoon. The fish minces were then kept in plastic bags on melting ice for two hours. The various additions were:

-- Blank (no addition).
-- 0.5% Iota carrageenan, coded Iota (0 hour), (HF 33895-96) from Copenhagen Pectin Factory Ltd. (Denmark).
-- 0.5% Iota carrageenan, coded Iota (8 hours), (HF 33895-96) mechanically degraded for 8 hours in a vibrating mill (Vibratom SM, Siebtechnik GmbH, Mühlheim, FRG). The volume of the cylindrical jars of the vibrating mill is 300 cm^3 and was filled to 90% with porcelain cylinders 13mm x 13mm and with 18g of Iota carrageenan. The vibration had a frequency of 24 sec^{-1} and an amplitude of 3.5mm.
-- 0.5% Carboxymethyl cellulose, Akucell AF 2805, coded CMC 2805, (average molecular weight =500,000) from Enka bv, Industrial Colloids (Holland).
-- 1.0% Carboxymethyl cellulose, Akucell AF 1505, coded CMC 1505, (average molecular weight =250,000).
-- 1.5% Carboxymethyl cellulose, Akucell AF 0705, coded CMC 705, (average molecular weight = 150,000).
-- 0.5% Carboxymethyl cellulose, Akucell MF 971, coded CMC 971, (average molecular weight =300,000).
-- 0.5% Alginate, Manucol DH, (average molecular weight =100,000) from Kelco/AIL, London, England.
-- 4% Sucrose and 4% sorbitol.

Samples of 150g were repacked in plastic bags, blast frozen and kept at -18°C for about 2 months. Samples of each treatment were withdrawn from the frozen storage, thawed in cold tap water and kept for 3 hours in melting ice until analyses were carried out.

Texture analysis (S.S., C.S., M.E. and R.) W.H.C.R., W.H.C.C. and C.D.L. were determined in the same way as previously described (chapter 3).

Blank, Iota (0 hour) and CMC 2805 were selected for further analysis of texture. The cooked cylindrical fish cakes of C.D.L. determination were compressed in an overload dynamic apparatus, model S100, equiped with a cell of 100N. The various speeds of the plunger used were 4mm/min., 20mm/min. and 200mm/min. while the speed of the chart was adjusted to a speed 2.5 times that of the plunger. Photographs of the compression behaviour of the fish cakes, in which parallel vertical lines were printed with India ink, were taken

at each 1.6mm of compression. The force-compression curves were recalculated as true stress (force/true area) - Cauchy's strain curves (Sherman, 1983). The slope of the initial straight line of these latter curves was considered to be the modulus of elasticity, M.E. (1) (Fig.4).

Five replicates were done for each sample.

Iota (0 hour) and Iota (8 hours) were characterized by their intrinsic viscosity, gel permeation elution pattern and ability to form a gel.

Intrinsic viscosities were obtained by extrapolation of the curves showing the relation between reduced viscosity versus concentration to zero concentration. Reduced viscosity was obtained from $(t_s-t_o)/(t_o.c)$ where t_s and t_o are the flow times of the solution and the solvent (>70 sec) respectively and c is the concentration in g of Iota carrageenan per 100ml. Preparations were dissolved in 0.1M pH 6 tris-succinate buffer and the flow times were measured at 20°C in an Ubbelohed glass viscosimeter. For complete dissolution of preparations with Iota carrageenan it was necessary to heat first to 65°C.

The gel permeation elution patterns of Iota (0 hour) and Iota (8 hours) were determined on a column (Pharmacia K26/100) packed with Sephacryl S400 Superfine (Pharmacia) eluted with 0.2M LiCl (pH=7.0) at 65°C. Samples of 5 mg of Iota carrageenan in 2 ml of eluent were applied to the column and fractions of 6 ml were collected and analysed for carbohydrate content by the colorimetric method of Dubois et al. (1956).

The gel formation ability of Iota (8 hours) was evaluated by heating dispersions of this hydrocolloid, at various concentrations, in distilled water, to 95°C followed by the addition of calcium chloride. The hot solution of Iota (8 hours) with calcium chloride was poured into molds and left to cool to room temperature.

The various additives used in the present work, were mixed with fish fluid (for 1 hour at 4°C, 5 mg/ml) obtained by centrifuging (at 25000g for 30 minutes) a portion of the minced fillets of cod. The flow curves of these solutions were determined as described in chapter 3.

9.3. RESULTS AND DISCUSSION

The flow curves of the various additives mixed with fish fluid, obtained by centrifuging minced fillets of cod, are presented in Fig. 1. At low shear rates the 0.5% CMC 2805, 1.0% CMC 1505 and 1.5% CMC 705 showed similar flow curves. The same is true for 0.5% Manucol DH and 0.5% CMC 971 additions. The addition of 4% sucrose and 4% sorbitol showed the lowest viscosity (next to

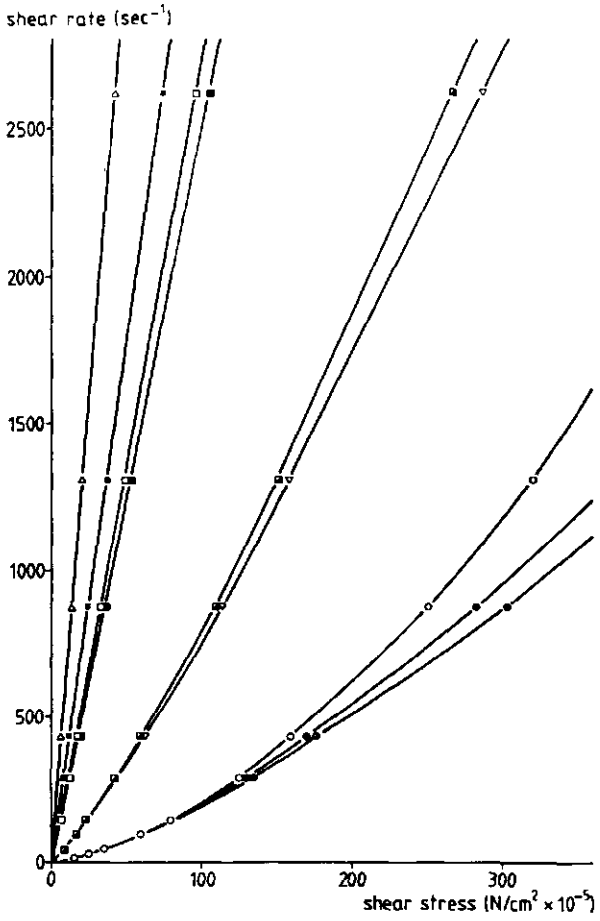


FIG. 1. Flow curves of the various additions mixed with fish fluid, obtained by centrifuging minced fillets of cod. △ Blank; □ 0.5% Iota (0 hour); ■ 0.5% Iota (8 hours); ○ 0.5% CMC 2805; ● 1.0% CMC 1505; ◕ 1.5% CMC 705; ◪ 0.5% CMC 971; ▽ 0.5% Manucol DH; ✱ 4% Sucrose and 4% Sorbitol.

the Blank), immediately followed by the Iota carrageenan additive (Fig. 1). The elution patterns of Iota (0 hour) and of Iota (8 hours) are presented in Fig. 2. The intrinsic viscosities of these hydrocolloids were 38.1 dl.g^{-1} for Iota (0 hour) and 3.1 dl.g^{-1} for Iota (8 hours) respectivelly. The degraded Iota (8 hours) was unable to form any kind of gel at the various concentrations of the hydrocolloids (1-8%) and at the various concentrations of the calcium chloride (0.2-2%) tried.

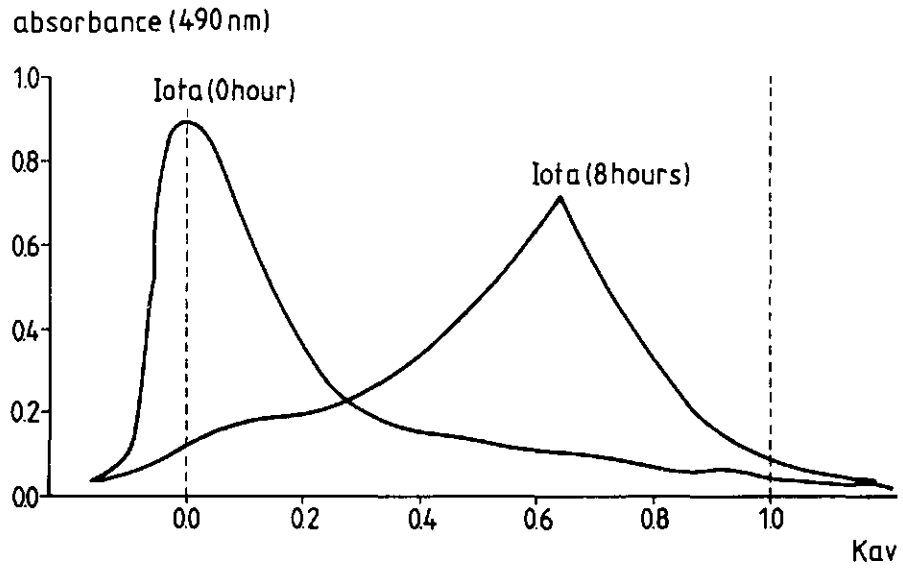


FIG. 2. Elution profiles on Sephacryl S400 of Iota (0 hour) and Iota (8 hours). Carbohydrate content was determined by the colorimetric method of Dubois et al. (1956). Eluent 0.2M lithium chloride.

During 2 months of frozen storage all treatments showed similar losses of W.H.C.R. (Table 1). It should be noticed however that W.H.C.R. of the various

TABLE 1 Effect of time and different additions on W.H.C.R. of minced fillets of cod stored at -18°C.

Additions	W.H.C.R.		W.H.C.R.(0) - W.H.C.R.(57)
	At 0 day	At 57 days	
Blank	70.3±0.8	60.1±1.2	10.2
0.5% Iota(0 hour)	84.4±1.1	74.4±0.3	10.0
0.5% Iota(8 hours)	85.9±0.3	74.9±1.9	11.0
0.5% CMC 2805	84.6±0.7	76.8±2.9	7.8
1.0% CMC 1505	83.2±1.3	73.4±0.3	9.8
1.5% CMC 705	81.9±0.3	73.8±0.6	8.1
0.5% CMC 971	80.6±0.6	69.6±0.3	11.0
0.5% Manucol DH	79.6±0.7	67.9±0.5	11.7
4% Sucrose, 4% Sorbitol	73.4±1.5	65.8±1.8	7.6

CMC's at different concentrations and of the Iota carrageenan additions at 57 days of frozen storage were in general higher than the W.H.C.R. of the Blank even at zero time of frozen storage. Not much difference was observed between Iota (0 hour) and Iota (8 hours) probably because the values of W.H.C.R. of these treatments were not due the viscosity of the hydrocolloids in dissolved state but to hydration of undissolved particles.

W.H.C.C. and C.D.L. of the various additions are presented in Tables 2 and 3 respectively. Manucol DH and the various CMC's at various concentrations

TABLE 2 Effect of time and different additions on W.H.C.C. of minced fillets of cod stored at -18°C.

Additions	W.H.C.C.		$\overline{\text{W.H.C.C.}}$[1]
	At 0 day	At 57 days	
Blank	53.5±0.5	51.2±0.5	52.4±1.6
0.5% Iota(0 hour)	63.1±3.5	65.1±3.1	64.1±1.4
0.5% Iota(8 hours)	56.0±1.2	55.6±1.1	55.8±0.3
0.5% CMC 2805	64.6±1.3	64.0±1.1	64.3±0.4
1.0% CMC 1505	62.4±0.6	62.2±1.9	62.3±0.1
1.5% CMC 705	64.5±2.0	61.5±1.1	63.0±2.1
0.5% CMC 971	56.4±0.9	55.9±0.2	56.2±0.4
0.5% Manucol DH	54.0±0.1	54.1±0.6	54.1±0.1
4% Sucrose, 4% Sorbitol	52.6±0.6	53.4±0.8	53.0±0.6

[1]Mean and standard deviation of W.H.C.C. at zero day and at 57 days of storage at -18°C.

showed values of C.D.L. and W.H.C.C. which are in proportion to the viscosity of their flow curves of the fish fluid (Fig. 1). Iota (8 hours) which is unable to form a gel exhibited values of C.D.L. and W.H.C.C. close to the Blank. CMC 971 and Manucol DH were not very effective on improving W.H.C.C..

All additions increased the resistance of the fish minces to toughening during frozen storage (Tables 4, 5, 6 and 7). They also exerted a softening effect in the fish minces which was approximately proportional to the increase in viscosity of the fish fluid brought about by the additions (Fig. 1). Blank samples showed the highest increases in S.S., C.S., M.E. and R. during the two months of frozen storage while the Iota carrageenan additions and the CMC additions with high viscosity showed the lowest increases (Tables 4, 5, 6 and 7). These results in respect to Iota (8 hours) rule out the hypothesis that

TABLE . Effect of time and different additions on C.D.L. of minced fillets of cod stored at -18°C.

Additions	C.D.L.		$\overline{C.D.L.}$[1]
	At 0 day	At 61 days	
Blank	33.1±0.5	34.1±0.3	33.6±0.7
0.5% Iota(0 hour)	22.0±2.5	22.2±0.8	22.1±0.1
0.5% Iota(8 hours)	30.3±1.1	31.2±1.4	30.8±0.6
0.5% CMC 2805	10.6±1.7	14.6±1.3	12.6±2.8
1.0% CMC 1505	11.6±1.0	12.8±1.7	12.2±0.8
1.5% CMC 705	12.3±0.7	12.6±1.1	12.5±0.2
0.5% CMC 971	17.8±1.8	18.7±1.0	18.3±0.6
0.5% Manucol DH	18.4±1.7	16.8±2.0	17.6±1.1
4% Sucrose, 4% Sorbitol	29.5±1.1	32.7±2.3	31.1±2.3

[1]Mean and standard deviation of C.D.L. at zero day and at 61 days of storage at -18°C.

TABLE 4 Effect of time and different additions on S.S.[1] of minced fillets of cod stored at -18°C.

Additions	S.S.		Increase in S.S./day[2]
	At 0 day	At 58 days	
Blank	4.47±0.50	11.06±0.74	0.1136
0.5% Iota(0 hour)	2.63±0.24	3.76±0.04	0.0195
0.5% Iota(8 hours)	1.98±0.11	3.10±0.11	0.0193
0.5% CMC 2805	1.66±0.21	2.88±0.29	0.0210
1.0% CMC 1505	1.39±0.21	2.44±0.15	0.0181
1.5% CMC 705	1.46±0.12	2.54±0.17	0.0186
0.5% CMC 971	1.93±0.24	3.62±0.33	0.0291
0.5% Manucol DH	1.85±0.09	3.48±0.24	0.0281
4% Sucrose, 4% Sorbitol	3.75±0.01	5.47±0.07	0.0297

[1]S.S. was measured in the raw minced fish, in the Kramer Shear Press (chapter 3). S.S. is expressed as units of area.

[2]Obtained by dividing the increase in S.S. by the 58 days of frozen storage.

TABLE 5 Effect of time and different additions on C.S. of minced fillets of cod stored at -18°C.

Additions	C.S.(N/cm^2)		Increase in C.S./day[a]
	At 0 day	At 63 days	
Blank	1.84 ± 0.11	5.38 ± 0.09	0.0562
0.5% Iota(0 hour)	1.63 ± 0.16	1.83 ± 0.22	0.0032
0.5% Iota(8 hours)	1.71 ± 0.08	1.92 ± 0.17	0.0033
0.5% CMC 2805	0.39 ± 0.04	0.63 ± 0.08	0.0038
1.0% CMC 1505	0.31 ± 0.01	0.57 ± 0.03	0.0041
1.5% CMC 705	0.30 ± 0.03	0.49 ± 0.05	0.0030
0.5% CMC 971	0.56 ± 0.07	1.14 ± 0.14	0.0092
0.5% Manucol DH	0.64 ± 0.08	1.18 ± 0.18	0.0086
4% Sucrose, 4% Sorbitol	1.90 ± 0.21	3.48 ± 0.24	0.0251

[a]Obtained by dividing the increase of C.S. by the 63 days of frozen storage.

TABLE 6 Effect of time and different additions on M.E. of minced fillets of cod stored at -18°C.

Additions	M.E.(N/cm^2)		Increase in M.E./day[a]
	At 0 day	At 63 days	
Blank	6.50 ± 0.51	14.68 ± 0.68	0.1298
0.5% Iota(0 hour)	6.00 ± 0.43	7.85 ± 0.83	0.0294
0.5% Iota(8 hours)	5.60 ± 0.40	6.70 ± 0.72	0.0175
0.5% CMC 2805	1.09 ± 0.12	1.92 ± 0.29	0.0132
1.0% CMC 1505	0.91 ± 0.05	1.58 ± 0.09	0.0106
1.5% CMC 705	0.86 ± 0.14	1.42 ± 0.13	0.0089
0.5% CMC 971	1.83 ± 0.22	3.07 ± 0.24	0.0197
0.5% Manucol DH	1.70 ± 0.18	2.98 ± 0.26	0.0203
4% Sucrose, 4% Sorbitol	5.81 ± 0.68	10.26 ± 0.86	0.0706

[a]Obtained by dividing the increase in M.E. by the 63 days of frozen storage.

TABLE 7 Effect of time and different additions on $R_.$ of minced fillets of cod stored at $-18^{\circ}C$.

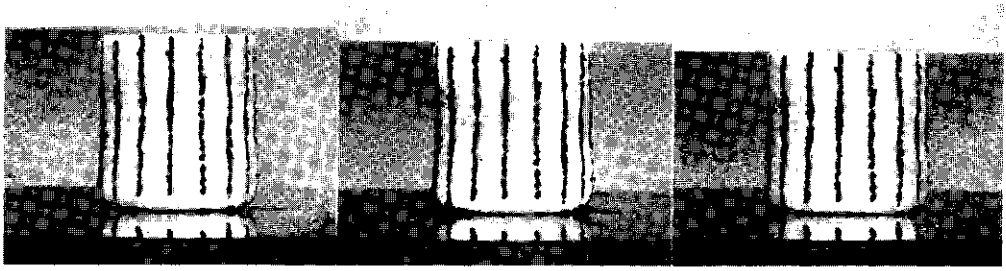
Additions	$R.(N/cm^2)$		Increase in R./day[a]
	At 0 day	At 63 days	
Blank	0.262 ± 0.024	0.882 ± 0.072	0.00984
0.5% Iota(0 hour)	0.239 ± 0.030	0.403 ± 0.055	0.00260
0.5% Iota(8 hours)	0.221 ± 0.026	0.325 ± 0.029	0.00165
0.5% CMC 2805	0.071 ± 0.014	0.132 ± 0.011	0.00097
1.0% CMC 1505	0.051 ± 0.009	0.104 ± 0.008	0.00084
1.5% CMC 705	0.053 ± 0.004	0.093 ± 0.009	0.00063
0.5% CMC 971	0.127 ± 0.019	0.214 ± 0.037	0.00138
0.5% Manucol DH	0.121 ± 0.014	0.232 ± 0.024	0.00176
4% Sucrose, 4% Sorbitol	0.298 ± 0.024	0.658 ± 0.070	0.00571

[a]Obtained by dividing the increase in R. by the 63 days of frozen storage.

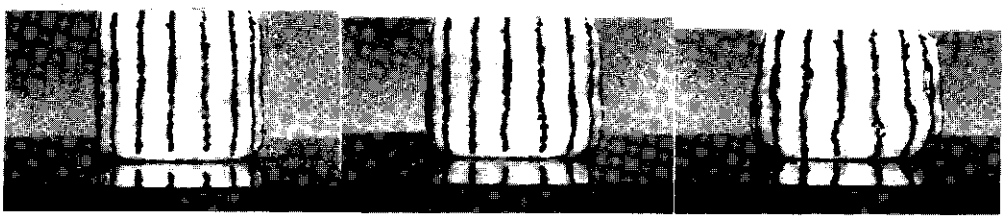
the gel formation was necessary for the cryoprotective effect of this hydrocolloid on texture. The cryoprotective effect of the CMC's and of the alginate (Manucol DH) on the various parameters of texture of the cooked fish minces, was to some extent in agreement with the viscosity of these additions exhibited in the fish fluid (Fig. 1).

Failure strength of materials depends on many factors. Photographs of the fish cakes, obtained from the C.D.L. determination of the Blank, at zero day of frozen storage, at various stages of compression and at a speed of the plunger of 20mm/min., are presented in Fig. 3. Up to 18% compression there was a small but continuous increase in the surface area in contact with the plunger. The failure of the fish cake structure caused a rapid increase of the overall area in contact with the plunger. The fish cake cylinder developed a barrel shape during compression which was associated with a greater distortion of the ink parallel lines in the central region than in either end of the sample. This was initiated between 16.5 and 22% of compression and is connected with the friction between the fish cake sample and the compression surfaces (Sherman, 1983).

The various parameters of texture used for evaluating the cooked fish cakes of the various additions, during frozen storage, are schematically illustrated in Fig. 4. M.E. (1) was calculated as the slope of the initial straight



0.0% Compression 0.0% Compression 5.5% Compression



11.0% Compression 16.5% Compression 22.0% Compression

27.5% Compression 33.0% Compression 38.5% Compression

FIG. 3. Photographs of the fish cakes of the C.D.L. determination of the Blank at zero day of frozen storage, at various stages of compression and at a speed of the plunger of 20mm/min., in an overload dynamic apparatus.

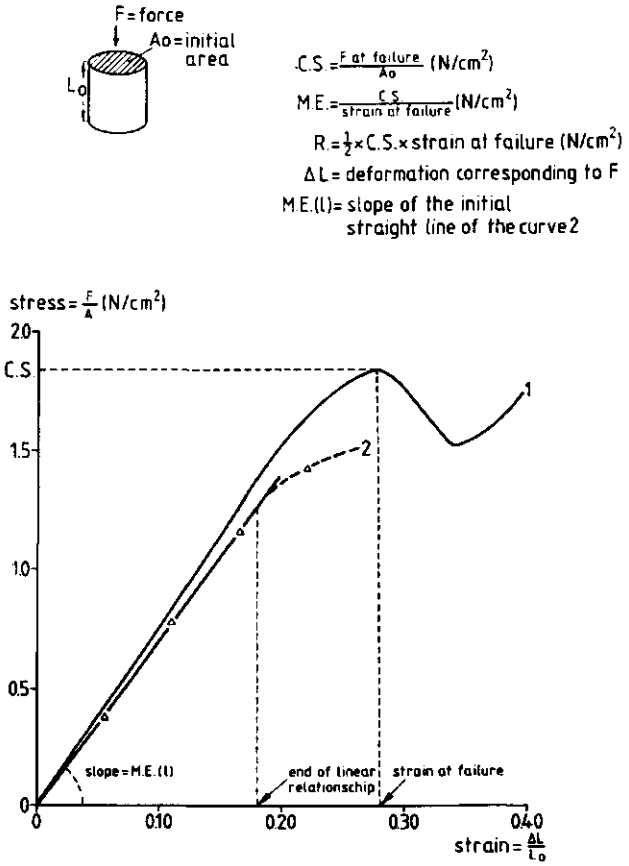


FIG. 4. Schematic diagram of the various parameters of texture obtained by compression the fish cakes of the C.D.L. determination in an overload dynamic apparatus.

1- Stress calculated from the initial surface area in contact with the plunger of the fish cake.

2- Stress calculated from the actual surface area in contact with the plunger during compression of the fish cake.

line of the true stress-Cauchy's strain curve (curve 2 of Fig. 4). In this range of compression (0-18%) the differences between the apparent strain or Cauchy's strain $\left(\frac{\Delta L}{L_0}\right)$ and the true strain or Henky's strain $\left(\ln \frac{L_0}{L_0 - \Delta L}\right)$ are small (maximum difference 1.8%) so the values of M.E. (l) will not be significantly altered (Peleg, 1977; Peleg, 1984). The speed of the plunger was limited to 200mm/min.

to minimize errors due to the recorder pen response. The effect of the various speeds of compression in the C.S., M.E., R. and M.E. (1) of the Blank, Iota (0 hour) and CMC 2805 during frozen storage are presented in Figs. 5, 6, 7 and 8 respectively. There was an increase of all these parameters of texture with the increase of the speed of the plunger. M.E. (1) of the Blank, Iota (0 hour) and CMC 2805 increased in similar way as the M.E. of these additions during frozen storage (Figs. 6 and 8). These results show that the behaviour of the additions in relation to texture of the fish minces were not due to the use of a low speed of compression. From the present data it is reasonable to conclude that at higher speed of compression the relative effects of the additions will be observed. Good agreement was found between the values of C.S., M.E. and R. of the Blank, Iota carrageenan, CMC 2805 and Xanthan, obtained with a speed of compression of 20mm/min., and the scores of the texture attributes of fibrosity and softness obtained with an analytical panel (chapter 8).

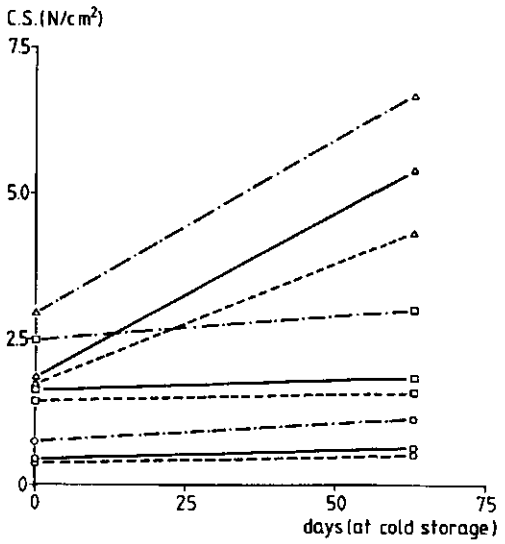


FIG. 5. Effect of time, different hydrocolloids and various speeds of the plunger of the overload on C.S. of frozen stored minced fish muscle. (----) Speed of the plunger of 4mm/min.; (—) Speed of the plunger of 20mm/min.; (—·—·—) Speed of the plunger of 200mm/min.. See Fig. 1 for the other symbols.

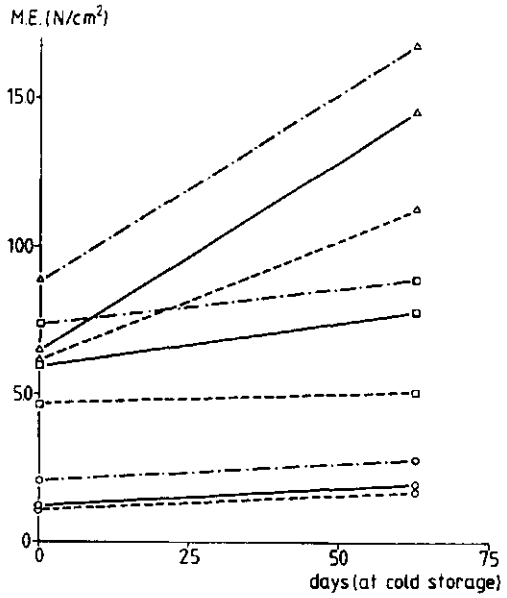


FIG. 6. Effect of time, different hydrocolloids and various speeds of the plunger of the overload on M.E. of frozen stored minced fish muscle. See Fig. 5 for the symbols.

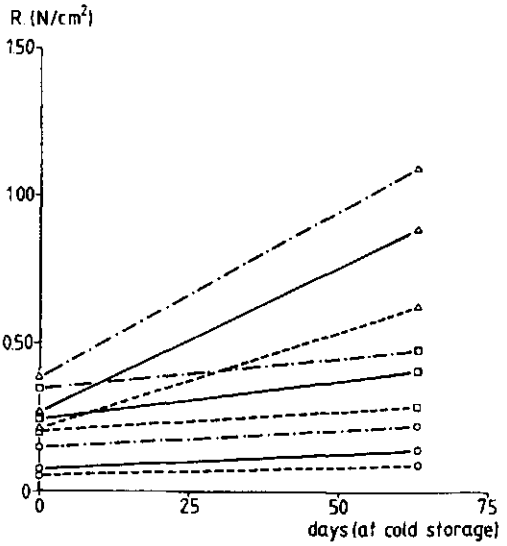


FIG. 7. Effect of time, different hydrocolloids and various speeds of the plunger of the overload on R. of frozen stored minced fish muscle. See Fig. 5 for the symbols.

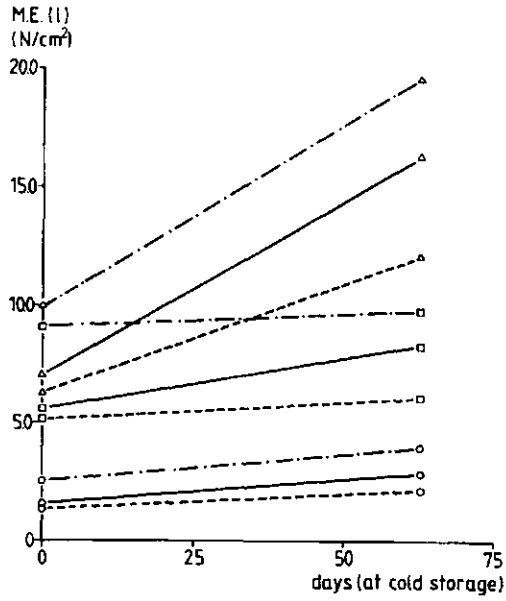


FIG. 8. Effect of time, different hydrocolloids and various speeds of the plunger of the overload on M.E. (I) of frozen stored minced fish muscle. See Fig. 5 for the symbols.

Finally it is worthwhile to examine the effects of frequently used cryoprotective agents, sucrose and sorbitol, on the stability of minced fillets of cod during frozen storage. The combination of 4% sucrose and 4% sorbitol showed, as did the hydrocolloids used, a significant reduction of toughening of the fish minces during frozen storage but failed to show any protective effect on the water holding capacity or C.D.L. of the fish minces during frozen storage.

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10. Possible mechanisms of action of the hydrocolloids as cryoprotective agents

Deterioration of fish during frozen storage is a complex phenomenon not yet completely understood (Connell, 1982; Mackie, 1984). As pointed out in chapter 2.4., there are several factors contributing to the deterioration of fish during frozen storage. Among them, products of lipid oxidation, FFA and FA are especially important. With the exception of certain anti--oxidants which can retard lipid oxidation, most of the other cryoprotectants used in fish do not have any influence on lipid oxidation or on the formation of FFA and FA. Theories regarding the mechanisms of cryoprotection of these compounds do not differ from the general ideas of how most of the cryoprotectants act in food or in living cells or tissues during frozen storage (chapter 2.5.).

In previous chapters (3, 4, 5, 7, 8 and 9) we have shown that several hydrocolloids can exert a cryoprotective effect in fish minces of whiting and cod during frozen storage. In these experiments, there was a common pattern of effects of the hydrocolloids which can be summarized as follows:

-- Hydrocolloids did not influence the decrease of E.M. during frozen storage.
-- With the exception of Xanthan gum, the hydrocolloids used did not have any influence on the formation of DMA and FA during frozen storage.
-- Iota carrageenan, carboxymethyl cellulose and Xanthan gum did not retard lipid hydrolysis during frozen storage.
-- Some of the hydrocolloids increased W.H.C.R. of the fish minces but during frozen storage the Blank and the fish minces with the additions showed a similar decrease of W.H.C.R..
-- Some of the hydrocolloids improved W.H.C.C. and C.D.L. of the fish minces during frozen storage.
-- Some of the hydrocolloids reduced toughening of the fish minces during frozen storage.

Some authors (Matsumoto, 1979 and 1980; Grabowska and Sikorski, 1973) have shown cryoprotective effects of sucrose, lactose, monosodium glutamate and polyphosphates on myofibrillar proteins, during frozen storage, in model studies. Indeed, some of these compounds are used in the surimi industry (fish minces which are throughly washed and intimately blended with cryoprotective agents). However when these compounds are added to fish minces which are not extensively washed the results obtained in respect to E.M. or extractable

myofibrillar proteins are contradictory. The addition of 2% monosodium glutamate, 0.2% or 0.5% polyphosphates or 5% sucrose to mechanically deboned gutted cod, did not prevent significantly the decline of solubility of the myofibrillar proteins during storage at -18°C (Grabowska and Sikorski, 1973). Paired cod fillets dipped in tripolyphosphate solution did not show any significant difference from the control in respect to protein solubility and lipid hydrolysis during storage at -12°C (Dyer et al., 1964). Rodger et al. (1980) reported that the addition of 3% of either lactose or monosodium glutamate or sodium citrate to fish minces, obtained from trimmings and frames of cod, showed beneficial effects on the solubility of myofibrillar proteins, when the fish minces were stored at -29°C, but had little or no effect when the fish minces were stored at -14°C or -7°C. They also showed that the major part of the solubility losses of the myofibrillar proteins occurred in the first week of frozen storage while deterioration of texture continued steadily till the end of frozen storage. Love (1958) already pointed out that "denaturation" as measured by the extractable myofibrillar proteins and toughening were two separate phenomena and that previous parallelism reported earlier was coincindental, although parallelism can undoubtedly occur. It is therefore not surprising that in unwashed minces and at -18°C no effect of hydrocolloids on E.M. was noticed by us.

The effect of hydrocolloids on W.H.C.R. of fish minces during frozen storage is attributed by us to the increase in viscosity of the fluid of the fish minces (pectins, alginates and carboxymethyl celluloses) to the formation of a gel (combination of Locust bean gum and Xanthan) or to undissolved hydrated hydrocolloid particles which retain large amounts of water (carrageenans and Locust bean gum) (chapters 3, 4, 5, 8 and 9).

In our experiments, E.M. and W.H.C.R. seemed to decrease in a similar way during frozen storage. The correlation coefficients between E.M. and W.H.C.R. of the Blank, in the various experiments with whiting and cod, were respectively 0.986 (chapter 3), 0.971 (chapter 4) and 0.882 (chapter 5). In our opinion the effects in the W.H.C.R. were merely additive to the Blank value, they seem not to influence the nature of the changes occurring during frozen storage which affect W.H.C.R.. The addition of 4% sucrose and 4% sorbitol to minced fillets of cod did not show either any significant effect on the decrease of W.H.C.R. during frozen storage (chapter 10). It should be emphasized however that some additions (Xanthan gum, high viscous alginates and carboxymethyl celluloses and Iota and Lambda carrageenan) are able to increase the W.H.C.R.

to such a degree that thaw drip is prevented.

In the cooked fish minces the increase in W.H.C.C. and decrease of C.D.L., caused by addition of hydrocolloids are ascribed by us to the increase in viscosity brought about by the hydrocolloids (high viscous alginates and carboxymethyl celluloses and Xanthan gum) or to the formation of separated hydrocolloid gels (combination of Locust bean gum and Xanthan gum and carrageenans).

The cryoprotective effect of the hydrocolloids on the texture of the fish minces during frozen storage, can be summarized as follows:

-- The hydrocolloids which did not hydrate or enter in solution in the raw fish minces did not show any stabilizing effect on texture (e.g. Kappa carrageenan).
-- The hydrocolloids which entered in solution in the raw fish minces increasing markedly the viscosity of the fish fluid showed a cryoprotective effect on texture which was approximately proportional to the increase in viscosity brought about by the hydrocolloids (e.g. Xanthan gum, carboxymethyl celluloses, alginates and pectins).
-- The hydrocolloids which did not enter in solution or were solubilized to a very small extent only but were able to hydrate as essentially undissolved particles in the raw fish minces can have a cryoprotective effect on texture. This was found to be approximately proportional to the capacity of the hydrocolloids to hold water in the raw fish minces (e.g. Iota and Lambda carrageenan).
-- The capacity of the hydrocolloids to form a gel in the cooked minced fish was not relevant for the cryoprotection effect of the hydrocolloids on texture (e.g. Kappa carrageenan formed a strong gel in the cooked minced fish without showing any protection on the texture of the fish minces during frozen storage; the mechanically degraded Iota carrageenan, on the other hand had lost its ability to form a gel in the cooked fish minces but did show a remarkable stabilizing effect on texture).

Current theories regarding the mechanisms of cryoprotectants appear to emphasize their role as modifiers of water structure which then interfere with formation of and growth of ice crystals (chapter 2.5.). It is generally accepted that inevitable formation of large ice crystals during frozen storage has deleterious effects on fish quality. The addition of hydrocolloids to the fish minces may decrease the size and the number of the large ice crystals formed during freezing and frozen storage. This property of the hydrocolloids

has been recognized and applied in the ice cream and frozen desserts industry since a long time. It should be noticed that the concentrations of hydrocolloids (stabilizers) used in the ice cream industry are in the same range as of the additions used in the fish minces. Discussion of the various theories regarding the effect of the hydrocolloids on ice crystal growth are presented in chapter 2.5..

Many of the functional properties of the hydrocolloids are related to their ability to imbibe and retain large amounts of water and interact with water (Wallingford and Labuza, 1983). Several methods have been used to measure the tendency of water to associate, with various degrees of tenacity, to the hydrocolloids (water binding capacity; Fennema, 1985). Recently, Wallingford and Labuza (1983) have used the Baumann capillary suction apparatus to evaluate water binding of several food grade hydrocolloids. They have found that this method gave the best prediction of the hydrocolloid behaviour in a low fat meat emulsion food system, when compared to other methods of measuring water binding capacity such as cryoscopy osmometer or the moisture sorption isotherm. The values of water binding capacity obtained in the Baumann capillary suction apparatus were in good agreement with the viscosity and molecular structural characteristics of the hydrocolloids. They have rated Xanthan gum as the hydrocolloid which binds most water followed next by carrageenan which was followed by Locust bean gum and then by high methoxyl pectin and low methoxyl pectin. Biswas et al. (1975) using differential scanning calorimetry have concluded that in aqueous solutions, carboxymethyl celluloses with higher molecular weight bind more water than the low molecular weight carboxymethyl celluloses. In our experiments similar ranking of the hydrocolloids in respect to water holding capacity and cryoprotective effect on texture of the fish minces was observed.

Another possible mechanism of cryoprotection would be the attachement of the hydrocolloids to the fish proteins creating lattices of structured water surrounding the proteins. This would result in a model of cryoprotection similar to the one proposed by Matsumoto (1980) (Fig. 9, chapter 2.5.). The pH of fish is around 6.5 - 7.0 and even assuming a small fall on freezing, strong interactions between the fish proteins and hydrocolloids at these pH's are not favoured. Another point to be considered is the high ionic strength of the unfrozen water phase of the fish minces which reduces the electrostatic interactions between fish proteins and hydrocolloids (Stainsby, 1980). However, freezing causes an increase on the concentration of both proteins and hydro-

colloids in the unfrozen water bringing closer these possible reactants. Experiments in our laboratory showed that the addition of salt extracts of fish proteins to solutions of various hydrocolloids in 5% sodium chloride, at room temperature and at pH 6.0 and 6.5, did not have any substantial effect on the viscosity (unpublished data). On heating the salt extracts of fish proteins precipitated at higher temperatures in the presence of the various hydrocolloids. In this respect Iota carrageenan was more effective than Manucol DH (alginate) and Guar gum. Similar results were reported by Imeson et al. (1977) and Ledward (1979). It is difficult to draw any conclusion from model studies to the real situation in the frozen fish minces, specially when important parameters are not known. Strong interactions between the fish proteins and hydrocolloids in the frozen fish minces are unlikely to occur but weak interactions can not be ruled out.

Hydrocolloids could also exert their cryoprotective effects on fish proteins by scavenging compounds such as FA, FFA or products of lipid oxidation. Miller and Cornwell (1978) have reported that classic cryoprotective agents such as dimethyl sulphoxide and glycerol are hydroxyl radical scavengers. However if this is the case with the hydrocolloids an important question would be to know to what extent the hydrocolloids can compete with the fish proteins.

Hydrocolloids also increase the amount of nonfrozen water reducing therefore the amount of ice formed and the concentration of solutes in the unfrozen water.

Whatever are the mechanisms of action of the hydrocolloids on protecting fish minces from toughening during frozen storage, we think that our experiments show that these mechanisms are closely related to the ability of the hydrocolloids to retain or hold large amounts of water in fish minces during frozen storage.

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11. Summary and conclusions

1. Pectins, alginates, carrageenans, carboxymethyl celluloses, Xanthan gum, Locust bean gum and a combination of Xanthan and Locust bean gum were used as additives (5g/kg) in minced fillets of whiting and cod. Samples of these treatments were stored at -18°C for about 3 months and were evaluated at regular intervals for water holding capacity, texture, extractable myosin and formation of dimethylamine and formaldehyde.

   The hydrocolloids did not show any protective effect on the extractable myosin. The hydrocolloids changed the texture and water holding capacity of minced fillets of whiting and cod during frozen storage. Some hydrocolloids showed remarkable stabilizing effect on texture, reducing toughening of the fish minces during frozen storage. Some hydrocolloids seemed to decrease the formation of formaldehyde and dimethylamine but this effect might be due to heterogeneity of fish minces.
2. Fish fluid, obtained by centrifuging minced fillets of whiting, was used to study the effect of several pretreatments (additions of hydrocolloids, irradiation and precooking) on trimethylamine oxide-ase (TMAO-ase) activity during frozen storage at -18°C. Xanthan gum was the only one of the hydrocolloids studied which showed a significant reduction of TMAO-ase activity. Irradiation (cobalt 60, 300 krad, -20°C) did not enhance the formation of dimethylamine and formaldehyde in the fish fluid. When the fish fluid or minced fillets of whiting were heated up to 80°C or higher for short periods of time (about 1 minute), the production of dimethylamine and formaldehyde was reduced drastically during subsequent frozen storage. At lower heating temperatures a quick partial inactivation of TMAO-ase could be obtained but eliminating the remaining activity required much longer holding times.
3. The effect of irradiation with and without the additions of hydrocolloids in fish minces were further examined. Minced fillets of cod were used to determine the effects of a pasteurization dose of irradiation (300 krad, -20°C) on the stability of the fish minces during frozen storage. Texture, water holding capacity, lipid oxidation, production of dimethylamine and formaldehyde and lipid hydrolysis were evaluated at regular intervals during 3 months of post-irradiation frozen storage (-18°C). Combination of irradiation with 0.5% cryoprotective additives (Xanthan gum, carboxymethyl cellulose and Iota carrageenan) were also studied.

   Irradiation had no significant effect on the water holding capacity,

texture and dimethylamine production of the minced fillets of cod during 3 months of frozen storage (-18°C). Irradiation did not disturb the stabilizing effect of the additions used. Lipid hydrolysis and lipid oxidation of the irradiated samples were not significantly different from the non-irradiated ones.

4. The effect of additions of hydrocolloids and the combination of precooking and additions of hydrocolloids on the stability of formulated minced fish products were studied. Iota carrageenan, carboxymethyl cellulose and Xanthan gum were used as additives (5g/kg) in the manufacture of different kinds of minced fish products (fish chips, fish sticks and precooked fish sticks). Samples of these products were stored at -18°C and were evaluated at regular intervals for weight loss, fat uptake, texture, formation of dimethylamine and formaldehyde and for sensory properties.

   All additions had a remarkable stabilizing effect on weight loss and texture in all products, during frozen storage. Precooking up to an internal temperature of 80°C prevented the production of dimethylamine and formaldehyde but increased the weight loss and the maximum force necessary to extrude the fish sticks through the Kramer Shear Press cell. From the hydrocolloids studied only Xanthan gum and carboxymethyl cellulose were able to reduce these latter deleterious effects. Results of sensory analysis showed good correlation between sensory attributes and the instrumental measurements of texture and water holdind capacity. At 8 months of frozen storage the fish chips with carboxymethyl cellulose and Xanthan gum additions were significantly preferred to the Blank. At 14 weeks of frozen storage, the fish sticks with Iota carrageenan presented the highest score of preference although not significantly different from the Blank. Xanthan gum impaired a very soft texture in the fish sticks which was considered undesirable for this type of product. It was concluded that each fish product has an ideal texture which should be maintained during frozen storage. To achieve this, the right addition or combination of additions has to be found for each product. Iota carrageenan and under certain conditions carboxymethyl cellulose decreased the fat uptake of battered and breaded minced fish products during frying.

5. The possible mechanisms of action of the hydrocolloids as cryoprotective agents in fish minces during frozen storage were further investigated. We examined the effects of additions of carboxymethyl celluloses with similar degrees of substitution but with different degrees of polymerization and

at different concentrations in order to bring about similar viscosities in the fish fluid of the fish minces. We also studied the effects of different hydrocolloids which gave at the same concentration similar viscosities in the fish fluid of the fish minces. We also examined the effects of the Iota carrageenan addition by comparing it with a mechanically degraded Iota carrageenan which had a smaller average molecular weight and had lost its gel formation ability. We came to the following conclusions:

-- The hydrocolloids which did not hydrate or enter in solution in the raw fish minces did not show any stabilizing effect on texture of the fish minces during frozen storage (e.g. Kappa carrageenan).
-- The hydrocolloids which entered in solution in the raw fish minces increasing markedly the viscosity of the fish fluid showed a cryoprotection effect on the texture of the fish minces during frozen storage which was approximately proportional to the increase in viscosity brought about by the hydrocolloids (e.g. Xanthan gum, carboxymethyl celluloses, alginates and pectins).
-- The hydrocolloids which did not enter in solution or were solubilized to a very small extent only but were able to hydrate as essentially undissolved particles in the raw fish minces had a cryoprotective effect on the texture of the fish minces during frozen storage. This was found to be approximately proportional to the capacity of the hydrocolloid to hold water in the raw fish minces (e.g. Iota and Lambda carrageenan).
-- The capacity of the hydrocolloids to form a gel in the cooked minced was not relevant for the cryoprotection effect of the hydrocolloids on the texture of the fish minces during frozen storage (e.g. Kappa carrageenan formed a strong gel in the cooked minced fish without showing any protection on the texture of the fish minces during frozen storage; the mechanically degraded Iota carrageenan, on the other hand had lost its ability to form a gel in the cooked fish minces but did show a remarkable stabilizing effect on the texture of the fish minces during frozen storage).

It is postulated that the mechanisms of cryoprotection of the hydrocolloids on the texture of the fish minces during frozen storage are closely related to the ability of the hydrocolloids to retain large amounts of water in the fish minces during frozen storage.

12. Samenvatting en conclusies

1. Diverse hydrocolloiden zoals pektines, carragenanen, carboxymethylcelluloses en een combinatie van xanthaan en Johannesbroodpitmeel werden gebruikt bij de bereiding van gemalen visweefsel van filets van wijting en kabeljouw in hoeveelheden van 5 g per kg. De verkregen produkten werden gedurende ongeveer 3 maanden opgeslagen bij -18°C en op bepaalde tijdstippen werden monsters beoordeeld op waterhoudend vermogen, textuur, extraheerbaar myosine en vorming van formaldehyde en dimethylamine. De hydrocolloiden toonden geen enkel beschermend effect op het extraheerbare myosine, ze beïnvloedden wel de textuur en het waterhoudend vermogen tijdens bevroren opslag. Sommige hydrocolloiden hadden een aanzienlijk stabilizerend effect op de textuur en verminderden het taai worden van de gemalen visweefsels tijdens bevroren opslag, sommige leken de vorming van formaldehyde en dimethylamine te verminderen. Dit laatste effect moet mogelijk echter toegeschreven worden aan de heterogeniteit van de monsters.
2. De vloeistof, die verkregen werd door centrifugeren van gemalen visweefsel gemaakt van wijting, werd gebruikt om het effect van diverse voorbehandelingen (toevoeging van hydrocolloiden, bestraling en voorkoken) op de trimethylamine oxide-ase (TMAO-ase) activiteit tijdens opslag bij -18°C na te gaan. Alleen xanthaan gaf een aanzienlijke reductie in TMAO-ase activiteit te zien. Bestraling (Cobalt 60, 300 Krad, -20°C) bevorderde de vorming van dimethylamine en formaldehyde in het visvocht niet. In het geval visvocht of gemalen filets van wijting gedurende korte tijd (ca. 1 minuut) verhit werden tot 80°C of hoger nam de vorming van dimethylamine en formaldehyde tijdens de daarop volgende bevroren opslag drastisch af. Bij lagere behandelingstemperatuur werd een snelle partiële inactivering van TMAO-ase verkregen. De eliminatie van de overblijvende activiteit vereiste veel langere behandelingstijden.
3. Het effect van bestraling in aan- en afwezigheid van toevoegingen van hydrocolloiden werd nader onderzocht. Gemalen visweefsel van kabeljouw werd gebruikt om het effect van een pasteurisatie bestralingsdosis (300 Krad, -20°C) op de stabiliteit van visweefsel gedurende

bevroren opslag te bepalen. Textuur, waterhoudend vermogen, vetoxydatie, vorming van dimethylamine en formaldehyde en de verzeping van het vet werden na bepaalde tijdsintervallen over een bewaarperiode van 3 maanden (-18°C) na de bestraling bepaald. De combinatie van bestraling met de toevoeging van 0,5% cryoprotectanten (xanthaangom, carboxymethylcellulose en iota-carragenaan) worden eveneens bestudeerd.

De toegepaste bestralingsdosis had geen significant effect op het waterhoudend vermogen, textuur en dimethylamine vorming gedurende 3 maanden bevroren opslag (-18°C). De bestraling had ook geen effect op de stabiliserende werking van de onderzochte hydrocolloiden. De mate van vetverzeping en vetoxidatie in de bestraalde monsters verschilden niet significant van de niet-bestraalde monsters.

4. Het effect van de toevoeging van hydrocolloiden, wel of niet gecombineerd met voorkoken, op de stabiliteit van produkten van gemalen visweefsel werd eveneens onderzocht. Toevoegingen van iota-carragenanen, carboxymethylcellulose en xanthaangom (5 g/kg) werden gebruikt voor de bereiding van verschillende soorten produkten bereid van gemalen vis (visfrites, vissticks en voorgekookte vissticks). Monsters van deze produkten werden bewaard bij -18°C en na bepaalde tijdsintervallen onderzocht op gewichtsverlies, vetopname, textuur, vorming van dimethylamine en formaldehyde en sensorische eigenschappen.

Alle onderzochte hydrocolloiden hadden een opmerkelijk stabiliserend effect op gewichtsverlies en textuur in alle produkten tijdens bevroren opslag. Voorkoken tot een inwendige temperatuur van 80°C verhinderde de vorming van dimethylamine en formaldehyde maar verhoogde het gewichtsverlies en de maximale kracht nodig om de vissticks door de perscel van de Kramer Shear pers te extruderen. Van de voorgekookte vissticks toonden alleen de monsters met xanthaan en carboxymethylcellulose toevoegingen hetzelfde gewichtsverlies en dezelfde maximum kracht als de niet-voorgekookte monsters zonder toevoegingen. De resultaten van sensorisch onderzoek toonden een goede correlatie met instrumentele metingen van textuur en waterhoudend vermogen. Vischips met carboxymethylcellulose of

xanthaantoevoegingen werden na 8 maanden bewaren in bevroren toestand duidelijk geprefereerd boven de contrôle. Visweefsels met iota-carragenaan kregen na 14 dagen bewaren in bevroren toestand de voorkeur, ofschoon ze niet significant beter waren dan de contrôle. Xanthaangom gaf een zeer zachte structuur aan vissticks en werd daarom niet geschikt bevonden voor dit type produkt. Geconcludeerd werd dat ieder visprodukt zijn eigen typische textuur heeft welke gehandhaafd moet blijven tijdens bevroren opslag. Om dit te bereiken moet voor elk produkt vastgesteld worden welke toevoegingen of combinaties van toevoegingen hiervoor nodig zijn. Iota-carragenaan en onder bepaalde omstandigheden ook carboxymethylcellulose verlaagden de vetopname van gepaneerde produkten bereid van gemalen vis tijdens het braden.

5. Er werd ook onderzoek verricht naar de mogelijke mechanismen voor de vriesbeschermende werking van de hydrocolloiden in gemalen vis tijdens bevroren opslag. Zo werd het effect van toevoegingen van carboxymethylcellulose preparaten met dezelfde substitutiegraad maar verschillende polymerisatiegraad gemeten in de concentraties die dezelfde viscositeit aan het visweefselvocht gaven. Ook werden de effecten van verschillende hydrocolloiden bestudeerd die bij een gegeven concentratie ongeveer gelijke viscositeiten aan het visweefselvocht gaven. Het effect van iota-carragenaan werd onderzocht door zijn werking te vergelijken met de werking van een mechanisch afgebroken iota-carragenaan preparaat dat een lager gemiddeld molecuulgewicht had en zijn vermogen tot gelvorming verloren had. De volgende conclusies werden getrokken:

   - De hydrocolloiden die moeilijk te hydrateren zijn of moeilijk oplossen in het vocht van het rauwe, gemalen visweefsel hebben geen enkel stabiliserend effect op de textuur van het visweefsel tijdens bevroren opslag (bijv. kappa-carragenaan).
   - De hydrocolloiden die wel oplossen in het vocht van het rauwe, gemalen visweefsel verhogen de viscositeit van het weefselvocht aanzienlijk en beschermen het gemalen visweefsel tegen textuurverandering tijdens bevroren opslag. Deze beschermende werking

is vrijwel evenredig aan de toename in viscositeit veroorzaakt door de hydrocolloiden (bijv. xanthaangom, carboxymethylcellulose, alginaten en pektines).

- De hydrocolloiden die niet of zeer slecht oplossen maar wel water opnemen en als onoplosbare deeltjes in het gemalen visweefsel aanwezig zijn kunnen een vriesbeschermend effect op de textuur van het visweefsel hebben tijdens de bevroren opslag. De bescherming bleek ongeveer evenredig aan het vermogen van de hydrocolloiden om water te binden in de rauwe, gemalen visweefsels (bijv. iota- en lambda-carragenaan).
- Het vermogen van de hydrocolloiden om een gel te vormen in de gekookte, gemalen visweefsels is niet relevant voor het vriesbeschermend effect van de hydrocolloiden op de textuur van het visweefsel tijdens bevroren opslag (bijv. kappa-carragenaan vormt een sterk gel in gekookte, gemalen visweefsel zonder dat het echter enige bescherming geeft aan de textuur van het weefsel tijdens de bevroren opslag). Het mechanisch afgebroken iota-carragenaan daarentegen had wel een aanzienlijke stabiliserende werking op de textuur van visweefsels tijdens bevroren opslag ondanks het feit dat het zijn vermogen om een gel te vormen verloren had. Er wordt aangenomen dat de vriesbeschermende werking van de hydrocolloiden op de textuur van de gemalen visweefsels tijdens bevroren opslag nauw samenhangt met het vermogen van de hydrocolloiden om grote hoeveelheden water in de visweefsels vast te houden tijdens de bevroren opslag.

Curriculum vitae

Duarte José Botelho da Ponte was born in Pico da Pedra, S. Miguel, Azores, on 3th of February, 1954. From 1971 to 1976 he studied Chemical Engineering at the University of Porto, Portugal. In 1977 he joined the Department of Food Technology of the University of Azores. Since then he undertook several trainings in Food Technology and Food Chemistry: University of Rhode Island, U.S.A. (May-July, 1978), National Institute for Food Technology, Lisbon, Portugal (March-July, 1979) and Laboratory of Food Chemistry, Department of Food Science, Agricultural University of Wageningen (November-July, 1981/1982).

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