EFFECT OF CRYOPROTECTANTS DURING FROZEN STORAGE ON QUALITY OF LEACHED MINCED MEAT FROM TILAPIA *OREOCHROMIS MOSSAMBICUS* (PETERS)

By

RITU AGRAWAL, B. F. Sc.

THESIS

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2003

Dedicated To

My Loving Family

&

Husband

DECLARATION

I hereby declare that this thesis entitled "EFFECT OF CRYOPROTECTANTS DURING FROZEN STORAGE ON QUALITY OF LEACHED MINCED MEAT FROM TILAPIA *OREOCHROMIS MOSSAMBICUS* (PETERS) " is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or society.

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(Chairperson, Advisory Board)
Associate professor,
Department of Processing Technology
College of Fisheries,
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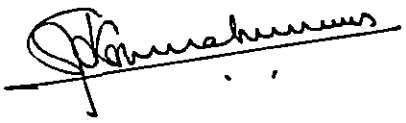

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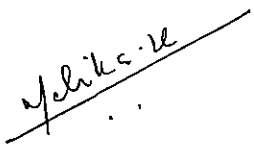

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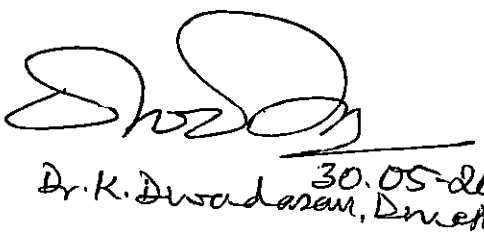

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Introduction

I. INTRODUCTION

In many developing countries the most important source of animal protein is marine fish. However, there is the problem of increasing price of marine fish, in addition to problems such as depletion due to overfishing, pollution and seasonal fluctuation. Freshwater fish is the next best alternative as a protein food supplier. Tilapia *(Oreochromis mossambicus,* Peters*)* is one of the freshwater species, which is abundantly available but has not much commercial value as fresh fish. Several high protein foods such as, salted-dried tilapia, tilapia canned in tomato sauce, curry sauce or chilly sauce prepared from tilapia were found to be well accepted by the taste panelists.

In the seafood industry low value fishes such as tilapia are used for production of various products such as spice-minced fish, fish sausage, fish ball, fish crackers etc. Steinberg *et al.* (1976) had successfully used minced fish as partial replacement for lean beef in sausages. Production of finished products from fish mince often takes place in two phases. The edible fish flesh is first recovered by the use of meat- bone separators, and frozen in the form of blocks. These blocks may contain additives such as cryoprotectants and the separated fish meat may be washed before it is packed and frozen. The second stage of production often takes place in the off-season. It is also common to send the frozen blocks to processors specializing in production of finished product such as fish fingers and fish cakes. As the minced meat has to be stored for some time before it is reprocessed, the changes, which can occur due to protein denaturation, play a significant role in the acceptability of frozen blocks.

The storage stability of minced meat varies considerably for different species, depending upon chemical composition and biological factors. Each species exhibit certain shelf life and storage capabilities, which are different from other species, often within the same biological family.

The products that have good texture, taste and appearance must overcome the reluctance of the consumers to accept minced meat fish products. As would be

expected, protein efficiency ratios of minced flesh are equal to that of whole fillet, indicating the high quality protein found in fish flesh.

In the late fifties, contemporary techniques for utilization of frozen fish flesh were developed. Japanese commercialized a washed minced flesh product, called surimi, in early sixties. It was developed to utilize fish such as Alaska Pollock that did not have sufficient quality or shelf life for the fresh or frozen market under the processing conditions. This may be because at that time freezing techniques were not well developed.

Surimi is a highly functional, pure, fish protein-water-cryoprotectant combination prepared from washed fish flesh. When properly combined with ingredients, it will form a stable gel. The introduction of surimi to the blended seafood market is one of the most explosive new ideas to hit the food industry of the United States.

During the early stages of Japanese surimi industry, Alaska Pollock was the main species, used for surimi production, but many other species such as blue whiting, thread fin bream, hoki, hake, arrow tooth flounder and several small pelagic species were also used.

There are certain advantages of using frozen surimi in place of whole fish. It provides a steady supply of raw material for fish jelly products processors. Processors using surimi need not prepare fish meat daily, thus saving time and money, storing meat as frozen surimi rather than the whole fish is more cost effective in terms of storage space, distribution and transportation. Separating of processing for surimi from that of final product, can result in better hygiene and sanitary control.

There is a common misconception that freezing fish completely prevents deterioration. The truth is that freezing only slows down deterioration. However, chemical and physical changes do take place in minced meat during frozen

storage causing the loss of quality and eventually making it unpalatable. Such changes are more rapid in minced meat compared to whole fish.

Cryoprotectants are compounds that protect or stabilize the product during freezing and thawing. The addition of cryoprotectant is important to ensure the maximum functionality of frozen surimi. Polysaccharides and other saccharides such as sorbitol, sucrose, glucose, galactose, fructose and xylytol are known to be effective in preventing freeze denaturation. Sucrose and sorbitol are being used for frozen surimi in Japan. Sucrose, sorbitol, polyphosphate and salt are most commonly used materials. The amount of each added is considerably different among the processors.

The outstanding functional properties of surimi make it an ideal base for many foods that have immediate market in many western countries. Japan is the largest consumer of surimi-based products. The studies conducted in United Sates and many other countries indicate that health conscious consumers are creating a rapidly growing demand for new high quality food products. Surimi meets these requirements and is an excellent high protein base for a new generation of foods.

The world Tilapia production was 4,73,000 tonnes in 1992 (Cohen, 2001). Many Asian countries including India, found *Oreochromis mossambicus* spreading uncontrollably in their water systems. In the past few years, there has been an explosion of interest in tilapia mainly among farm owners, food corporations, and agriculture companies. Tilapia, on the live weight basis, is currently the third largest imported aquaculture product, entering the USA market.

In 1995, a new trend was apparent, stressing on the quality of fillets. Tilapia found its niche in the market and entered into the restaurants. There is the advantage of consistency in quality and supply and the relatively low cost of tilapia compared to the well known, but rapidly disappearing traditional marine fish fillets.

It was reported that tilapia *(Tilapia nilotica)* was a good material for a minced meat based product called kamaboko (Yamamoto,1977). Taste, flavour and gel strength of tilapia kamaboko were excellent. However, the yield of minced meat was only 25% of total body weight.

Although it is reported that tilapia is good for minced meat production, little work has been reported on the frozen stability of its meat. Hence this study has been proposed to compare the cryoprotectant effects of sugar, sorbitol and polyphosphate against freeze-induced denaturation of the minced meat of tilapia *(Oreochromis mossambicus*, Peters).

Review of Literature

II. REVIEW OF LITERATURE

Chang Lee *et al.* (1990) stated that based on the round fish weight, yields from processing pacific whiting in surimi include 43.3% planks (i.e. after removal of head,backbone, tail and viscera),36.2% minced meat, 23.8% washed and pressed flesh, 19.5% refined flesh and 21.5% surimi(91.5% refined flesh plus 8.3 % cryoprotectants). After washing (water: minced flesh ratio 3:1) and pressing twice, 40.6% of the original lipid and 77.4% of the original ash were removed from the minced flesh. Surimi contained 54% extractable myofibrillar protein.

Blenford, (1992) discussed the changes which occur in products during freeze-thawing cycles (development of large crystals, protein denaturation, starch retro gradation and emulsion breaking) and the resultant effects on the end products. He also described the protection of foods against this damage using cryoprotectants i.e. materials that stabilize the system during freeze-thaw cycles.

Han Ching and Leinot (1993) studied the composition and nutritional properties of surimi made from fish flesh and found that surimi contains 75-78% water, 20% protein, 0-2% lipids, and sugars as sorbitol 4 %, sucrose 4% and polyphosphates 0.2-0.3%. The latter 3 are used as cryoprotectants to prevent protein denaturation during cold storage.

Water binding property of fish during frozen storage was studied by Gormley *et al.* (1993) who analysed the samples of silver melt (*Argentinus silus*), as fillet and as frozen fish, as block frozen fillets, and as block frozen mince. The samples were thawed and tested for toughness (by shearing) and water holding capacity, after storage at -28^{0}C for 150, 235 and 374 days. Cryoprotectants were not used. Water binding capacities were lowest for block-frozen mince and it decreased steadily over the 3 test dates for the 3 types of (frozen fish) materials.

The problems associated with texture changes on freezing of minced fish products, such as surimi was outlined by MacDonald (1995). He also highlighted the need for addition of cryoprotectants to ensure the maintenance of heat setting properties. Minced hoki was used as an example of development of technology to improve frozen shelf life. Use of gelation was considered as the primary indicator of quality .

Anese and Gormley (1995) reported that the use of cryoprotectants can maintain quality in fish mince during freeze thaw cycles and frozen storage. Effect of 9 dairy ingredients, used as potential cryoprotectants, on quality of frozen mince from cod, haddock and salmon were investigated. At an 8% inclusion level, all dairy ingredients exerted a cryoprotectant effect on salmon frozen mince. In frozen cod and haddock mince, only sodium and calcium caseinates and milk protein isolates exerted a cryoprotectants effect, whilst whey protein concentrate gave a reduced water holding capacity in thawed fish. There was generally inverse relationship between water holding capacity of thawed fish and compression strength of fish gels. Depending on the dairy ingredients, there was a variable effect on whiteness of cod and haddock mince and color of salmon. Paired comparison taste panel tests indicated a preference for control samples of cod fish fingers to those containing 4% sodium caseinate or whey protein concentrate; however, all samples were accepted.

Kolsarisi and Ensoy (1996) discussed the manufacture of surimi with reference to the basic principles of the process, washing comminuted fish flesh to eliminate undesirable constituents, setting the purified muscle proteins, incorporation of cryoprotectants, functional properties of surimi and use of surimi in simulated foods.

Types of protein interactions effected by addition of 0% or 8% sucrose during induced frozen aggregation of tilapia *(Tilapia nilotica)* myosin solutions at -20^0 C were examined by Ramirez and Polo (1996), as a function

of time (0-60 days). Protein hydrolysis was not observed. Solubility, and total and exposed SH-group levels decreased during storage. A total loss of Ca^{2+} ATPase activity was observed from day 15 in muscle myosin solutions without sucrose. Partial recovery of sucrose solubility after urea (8M, pH 10) treatments for samples with and without sucrose was directly related to S-S bond formation. Muscle myosin samples with no sucrose showed the highest S-S bond formation and the lowest solubility after 60 days frozen storage. In added muscle myosin samples, they found that addition of sucrose had a cryoprotective effect, associated with inhibition of disulphide bond formation.

Anese and Gormley (1996) investigated the suitability of dairy products as cryoprotectants and to improve the quality of frozen fish mince of cod (*Gadus morhua*), haddock (*Gadus aglefinus*),salmon and spent salmon (*Salmo salar*). Dairy ingredients such as lactose, skim milk, de-mineralized whey, milk protein isolate, whey, whey protein concentrate and spray dried calcium caesinate were added to fish mince at the rate of 80g/Kg. It was concluded that dairy products are suitable for addition to fish based products as improvers of quality of frozen fish.

Tomaniak *et al.* (1998) examined various carbohydrate cryoprotectants, which could be added to frozen ground raw meat. These are sucrose, D-sorbitol, maltodextrin DE 24-38 and synthetic polydextrose. Sensory evaluation of resulting products were evaluated as was sweetness of solutions of cryoprotectants at various concentrations. Overall data indicated that poydextroses should be chosen for use in red meat, as it was least sweet in solutions, its taste was suppressed by inclusion in meat, its duration of sweetness was lowest and its total flavour impact was smallest.

The effectiveness of various cryoprotectants, polyol blends (composed of sucrose, sorbitol and lacitol) in maintaining quality of frozen ling cod surimi stored at -18^0C was investigated by Sultanbava and Li Chan (1998)

(1998) and compared with the commercial cryoprotectants mix (4% sucrose, 4% sorbitol). The blends were added to surimi sample which were frozen and analysed at two week intervals over a four month period. Results showed no significant changes in the gel strength, colour, pH or myosin to actin ratio of surimi with any of cryoprotectant blend used.

A method for improving the flavour and shelf life of frozen fish and shellfish, particularly for lobster, was given by Bayer *et al.* (1999) which comprises of preparation of solution containing antioxidant, cryoprotectants and flavouring agents. This solution was injected into flesh or circulatory system of live fish or shellfish prior to cooking and /or freezing. Injection into live animals ensures that the solution is circulated throughout the tissues.

Krala and Dziomdziora (2000) studied the effect of polydextrose and sorbitol on the functional properties of frozen minced pork during 120 days of storage at -25^0C. Solubility of proteins, emulsifying capacity, water holding capacity and amount of frozen out water were analysed. Polydextrose, sorbitol decreased the amount of frozen out water by 7%. Results showed that none of the tested cryoprotectants protected simultaneously all properties of frozen pork. However, sorbitol had the broadest range of protective action.

2.1. Washing

Kijowski and Richardson (1996) mechanically recovered meat (MRM) from chickens(broilers) and after washing was subjected to either slow or rapid freezing or freeze drying with or without the presence of cryoprotectants. Both freezing and freeze drying reduced the functionality of MRM when no cryoprotectants were used. Sorbitol/sucrose gave some protection to gel forming ability of frozen samples; sucrose /sorbitol with tripolyphosphate gave stronger gels after freezing or freeze drying than fresh samples. Most of the loss of functionality during freezing or freeze drying was caused by loss of solubility of myosin and, to a lesser extent, actin. The

combined presence of the sorbitol/sucrose/tripolyphosphate restored most of the functional properties of the frozen or freeze dried material to that of the fresh material.

Effects of eight months of frozen storage on the protein conformation and functionality of whole, light and dark sardine (*Sardina pilchardus*) muscles, and washed mince were studied by Montero *et al.* (1999). The variation in protein surface hydrophobicity and Ca-ATPase activity was different in dark muscle from that in whole muscle over the storage period. The dark muscle lacked gel forming capacity because of its higher fat content and a greater presence of low molecular weight proteins, and it also became insoluble faster than the light muscle. The Ca^{2+} ATPase activity of the actomyosin extracted from the dark muscle was higher than in light or whole muscle during the first three months. Washing the minced muscle induced conformational changes in the myosin molecule which, together with the added cryoprotectants, contributed to greater functional stability of the washed mince at least during the first 3 months storage, at which stage gels were somewhat softer and more elastic than with unwashed mince.

2.2. Cryoprotectants

Park *et al.* (1988) found that sucrose and/or sorbitol, typically alone or mixed in 1:1 proportion and added to leached fish muscle at 8% (w/w) serve as the primary cryoprotectant in, manufacture of surimi from Alaska Pollock. Polyphosphate at 0.2-0.3% is also commonly added. Polyphosphate works as a synergyst to the cryoprotective effect of the carbohydrate additives.

Yoon and Lee (1990) studied the relative cryoprotective effect of liquid sorbitol(L) alone and in combination with sucrose(L-S) in surimi, extruded cooked and uncooked products, and also compared with the cryoprotective effects of crystalline sorbitol(C), liquid polyol (P) and modified starch (MS).Variables evaluated included gel-forming ability, cooking loss, drip loss and ice crystal formation. A better cryoprotective effect was shown in

uncooked than in cooked products. Results showed no difference in effectiveness of C, C-S, L and L-S. Optimum sweetness was obtained with either C and L at 3 : 3%. Both C-S and L-S at 4 : 4 % were judged to be slightly too sweet.

Sych *et al.* (1990) studied the cryoprotective effect of low or non sweet additives viz. palatinit registered, polydextrose registered, casein hydrolysate and fish protein hydrolysate (at 8%w/w) as well as lactitol (at 4% and 8% w/w) and also compared to an industrial control containing sucrose/sorbilol (8% w/w) and control without additive, in cod surimi stored at – 20^{0}C for four months. Freeze induced denaturation was evaluated monthly by texture and expressible moisture analysis of surimi cooked gels. Results revealed that protein functionality was similarly maintained during frozen storage by lactitol, Palatinit registered and Polydextrose registered when incorporated in cod surimi at 8% level. Surimi gels could be produced with textural attributes compared to 8% sucrose/sorbitol surimi gels, and the level of lactitol in cod surimi could be reduced to 4% w/w without significant alteration of cryoprotection. Some benefits in gel forming properties were found by adding 8% casein hydrolysate to cod surimi.

Jasmine *et al.* (1994) processed fresh threadfin bream (*Nemipterus beekeri*) in minced meat and divided into four lots. Cryoprotectants such as sorbitol (4% w/w), ascorbic acid (0.1% w/w) and mixture of sorbitol and ascorbic acid were added to each lot and one lot was taken as control (without any cryoprotectant). All samples were packed in polyethylene film of 150 gauge in waxed cartons and was frozen stored at –40^{0}C for an hour and a half and further stored at –20^{0}C ± 2^{0}C. Physical, biochemical and textural characteristics were assessed fortnightly. Minced meat mixed with mixture of sorbitol and ascorbic acid represented better quality during frozen storage.

The cryoprotective effect of whey was studied by Dondero *et al.* (1994) in jack mackerel surimi during frozen storage at -18^0C for 5 months. Whey was added @ 4,6 and 8 % (w/w), with and without addition of 0.2% polyphosphate. The results were compared with commercial sucrose/sorbitol (1:1mixture), and a control without cryoprotectants. Surimi quality was assessed by biochemical, physical and organoleptic tests. Decrease in water holding capacity, protein solubility, ATPase activity and gel formation confirmed that protein denaturation occurred during frozen storage. These changes were also detected during sensory evaluation. The decrease in water holding capacity and ATPase activity was minimized by a combination of 0.25% polyphosphate and 8% whey. The best cryoprotective effect was achieved with either 0.2% polyphosphate plus 8% whey, or the commercial mixture. Addition of 8% whey improved sensory quality of the surimi gel after storage for 155 days at -18^0C.

Tomaniak (1995) highlighted the use of cryoprotectants for protection of frozen meat from quality deterioration. The negative quality changes of frozen meat caused by ice crystal growth, ultra-structure injuries and cryodiffusion were studied.

The control of undesirable changes in functional properties of meat proteins by cryoprotectants were studied by Dziomdziora *et al.* (1998). Composition of various cryoprotectants such as sorbitol, sucrose, starch and starch hydrolysates was cited.

Parkington *et al.* (2000) studied functionality changes in oxidatively/ antioxidatively washed beef heart surimi during frozen storage. Effects of antioxidative washing treatments and functional properties were studied by storing at -20^0C and was analysed periodically upto 12 months. All samples showed increased gelling capacity during storage.

2.2.1. Oligosaccharides

Joong *et al.*, (1999) evaluated and compared cryoprotectants of surimi using oligosaccharides and cryoprotectants such as sucrose with or without sorbitol. Surimi samples incorporating the requisite compounds were stored at -18^{0}C for 3 months Physical properties of the resulting gels (texture, water holding capacity, color, microstructure) were studied. Water holding capacities and micro-structural profiles were similar in gels with oligosaccharides and those with sucrose with or without sorbitol, as were colour lightness and whiteness values. Texture profile analysis showed that gels with fructooligosaccharides had significantly higher fracturability, hardness and chewness values than the other gels. Frozen sample was compared with control (no additives). The various treatment had little impact on sensory properties.

Highly concentrated branched oligosacchrides mixture (HBOS) were evaluated as cryoprotactants in a fish protein model system by Auh *et al.* (1999). Effects of HBOS on gelatin properties of surimi were studied, and the effectiveness of sucrose or sucrose + sorbitol as cryoprotanctants were compared. Freeze-thawing studies at different concentration, using an actomyosin solution (extracted from Alaska pollock, *Theragra chalcogramma*), revealed that an 8% (w/v) solution of oligosaccahride mixture (HBOS) was most effective in cryoprotection. During frozen storage at -18^{0}C, HBOS showed cryoprotective effects similar to sucrose and a sucrose + sorbitol mixture (1:1). Surimi (Alaska pollack) gel prepared with HBOS showed higher hardness and more dense microstructure than others, although water holding capacity was slightly lower than the gel with sucrose + sorbitol and HBOS- sucrose + sorbitol. HBOS appeared to have good potential as a non-sweet cryoprotectant of fish protein.

2.2.2. Tripolyphosphates

The cryoprotective effects of various additives (sucrose, sorbitol and phosphates) on frozen surimi made from *Nemipterous tolu* (threadfin bream) were investigated by Yu *et al.* (1994). Samples of surimi containing 5% sorbitol, 0.3% sodium triphosphate(STP), 0.3% sodium pyrophosphate(SPP), 5% sucrose were stored at -30^0C for 16 weeks. Surimi samples containing phosphates increased salt soluble protein, water holding capacity and inhibited the protein denaturation. Overall acceptability was highest in surimi containing 5% sucrose whereas STP was more effective than SPP.

Chang and Regenstein (1995) treated cod mince with various cryoprotectant mixtures @ 0.8% each and frozen stored at –14 or -4^0 C. Expressible moisture, water uptake, cook loss, toughness, dimethylamine levels etc., were determined in treated and untreated fish minces. A combination of sucrose/sorbitol (4% / 4% of mince wt.) and sodium hexametaphosphate (0.5% of mince wt.) improved protein functionality and textural properties of the mince during frozen storage.

Simpson *et al.* (1995) studied the changes in stabilized mince (SM) made from fresh pacific whiting *(Merluccius productus)* mixed with varying concentrations of sucrose (6-12% w/w) including 0.2% w/w polyphosphate. SM samples were frozen stored and used different time intervals for surimi production. Comparison with control surimi made from fresh mince showed that acceptable surimi could be produced from SM stored at -20^0C with cryoprotectant levels at 6% w/w sucrose and 0.2% w/w polyphosphates. There was a slight decrease in whiteness of surimi made from SM samples when compared to control.

2.3. Quality changes

Gomes *et al.* (1995) studied properties of surimi made from freshwater tambacu fish, a hybrid of *Piaractus mesopotamicus* and *Colossoma macropomum.* White fish muscle were ground and washed with phosphoric acid solution

(0.03%) and cold water (approx. $+10^0$C). After rinsing the ground muscle was manually pressed to partially remove the water, cryoprotectants (sorbitol and sucrose) were added and the product was frozen stored. Surimi obtained through this process was graded AA, group 5, indicating excellent elasticity resistance.

Nowsad *et al.* (2000) studied washing and cryoprotectant(CP) effects on frozen storage of spent hen surimi. Chicken mince from 98 week old spent hen was washed with 0.1% NaCl and mixed with cryoprotectants composed of 4% sorbitol, 4% sucrose and 0.2% sodium tripolyphosphate and were immediately frozen stored at -20^0C. Mince without cryoprotectant was run as control. Textural quality parameters of the stored mince and surimi such as gel strength, breaking strength, deformation, protein solubility, expressible moisture, cooking yield, folding test, drip loss, and sensory score were evaluated at 6 months intervals. Results showed that washing protected gel quality of hen mince from degradation in frozen storage. CP could not protect the gel strength or breaking strength, but deformation was slightly improved. Water retention properties were well-protected and folding test and sensory scores were well preserved in meat with cryoprotectants.

Suvanich *et al.* (2000) studied changes in chemical quality characteristics of channel cat fish (*Ictalurus punctatus*) frame mince during chill and frozen storage. Changes in total volatile base nitrogen(TVBN), pH, salt soluble nitrogen(SSN), moisture content and expressible moisture were studied at -20^0C, 0^0C and $+5^0$C. Results showed no change during refrigeration storage. Frozen mince with cryoprotectant would remain acceptable for greater than or equal to three months at -20^0C.

2.3.1. Sensory Quality Changes

Henry *et al.* (1995) compared sensory properties of cryogenically frozen crab meat, treated with polydextrose, a blend of sucrose/sorbitol /phosphate water with those of pasteurised crab meat using both trained and consumer panels. Samples were vacuum-sealed, cryogenically frozen, and stored for 32 weeks at -

29^{0}C. A trained panel evaluated changes in appearance, aroma, flavour and texture after various periods of storage 0, 4, 8, 16, 20, 24, 28 and 32 weeks while a consumer panel evaluated sensory changes after 0.16 and 32 weeks of storage. An untreated cryogenically frozen reference sample was stored at -65^{0}C. Polydextrose and sucrose/sorbitol/phosphate treatments were closest in sensory attributes to fresh crab meat in that they had fewer adverse changes in quality than the water, reference or pasteurized treatments. Panelists detected more sour, rancid and ammonia and less fresh crab flavour in the pasteurised crab meat. Consumer panel evaluations were in agreement with trained panel assessments showing that cryoprotectants were effective in delaying the deterioration of sensory attributes during frozen storage. It was concluded that addition of cryoprotectants to cryogenically frozen crab meat enhanced the quality of the frozen meat compared to effects of pasteurization and/or freezing without preliminary treatment.

Storage stability of cryogenically frozen crab meat treated with cryoprotectants was compared with pasteurized and water treated frozen samples by Henry *et al.* (1995) to determine changes in physical and chemical properties. Commercially processed, hand-picked blue crab meat was treated with solutions of polydextrose, sucrose plus sorbitol/sodium tripolyphosphate water, vacuum packaged, and cryogenically frozen prior to storage at -29^{0}C. All treatments were compared with an untreated reference control stored at -65 ^{0}C and a pasteurized sample stored at 1.1^{0}C. Samples were evaluated for physical and chemical changes at 4 weeks intervals over 32 weeks of storage. It was concluded that cryoprotectants include oxidative stability, drip loss, expressible moisture, texture and colour compared to untreated reference and pasteurized samples.

2.3.2. Changes in myofibrilliar protein

Connell (1961) studied that the myofibrillar protein of most fish, these being cold blooded species, are known to be more labile to denaturation than the

contractile proteins of homeotherms commonly converted to meat for food, including beef, pork and poultry.

Sych, *et al.* (1990) studied the cryoprotective effects of lacitol dihydrate, polydextrose and palatinit at 8% w/w in cod surimi and also compared with an industrial control containing a sucrose/sorbitol 1:1 mixture and control without additive. Surimi was stored at -20^0C for 12 weeks and was analyzed for freeze-induced protein changes every two weeks by salt extractable protein analysis. Results showed that salt extractable protein for surimi were maintained at the same level as the industrial control.

Stabilization of chicken myofibrillar protein isolates ((MPI) during frozen storage was studied by Uijttenboogaart *et al.* (1991). The MPI was treated with high molecular weight carbohydrates with mixtures of 2.8% sorbitol and 4% sucrose, 2% sorbitol, 4% starch, and was exposed to different freezing and thawing treatments. The results showed best in terms of colour, weight loss of gels during cooking and texture with the addition of 2.8% sorbitol and 4% sucrose mixture.

Blanchard and Xiong (1996) studied effect of cryoprotectants (4% sucrose, 4% sorbitol) on the isolated chicken breast myofibrillar proteins (MP) during frozen storage at -20^0 C and evaluated samples every 3 week. MP were mixed with salt, with and without cryoprotectants. Samples were evaluated for protein gelation, solubility and thermal stability. Myofibrillar proteins without cryoprotectant showed 79% reduction in solubility after 3 week and with cryoprotectant solubility decreased by only 16% over 3 week. Storage modulus of gels decreased with time of frozen storage, although addition of cryoportectants helped to maintain the value at a constant level. Total enthalpy of protein denaturation decreased in all salted samples during storage, whereas in non-salted samples with cryoprotectants, total enthalpy increased.

2.3.3. Protein Denaturation

Jae *et al.* (1988) investigated the freeze- induced denaturation of Pollok surimi as affected by addition of sugar and/or polyol, including a starch hydrolysate product, and/or phosphate during 8 months frozen storage. Polydextrose appeared to substitute for the sucrose/sorbitol now used in surimi manufacture without changes in cryoprotective effect. The cryoprotective effect of phosphate addition seemed to depend upon the pH and/or specific phosphate ion used.

Sych *et al.* (1990) studied freeze- induced protein denaturation of cod surimi treated with carbohydrates (sucrose and sorbitol 1:1 mixture at 8% w/w). The surimi was stored for 16 week at -20^0C. Sucrose and sorbitol mixture (at 8% w/w, 1:1) showed best effects.

Reckasi *et al.* (1992) studied the effects of cryoprotectants against proteins denaturation during frozen storage. The ground pork ham meat was treated by 5% 1: 1 sucrose/sorbitol; 5% 89:10:1 glucose/sorbitol/citric acid; or 5% glycerol and was stored at -18^0C for 8 months. Protein denaturation was assessed by microcalorimetry, other parameters such as cooking loss, pH, soluble protein and texture of meat products were also assessed. Results showed inhibition of protein denaturation in frozen meat treated with sucrose /sorbitol.

Denaturation of jack mackerel *(Tracurus murphyi)* actomyosin during frozen storage at – 18^0 C for 16 week was studied by Dondero *et al.* (1996). Effects of cryoprotectants such as sucrose/sorbitol(1:1), maltodextrin (DE25), Whey or sodium lactate (8% by wt.) were investigated and were compared with a control without cryoprotectants. Decrease in the solubility and Ca^{2+} ATPase activity indicated that actomyosin denaturation occurred during both freezing and frozen storage at -18^0 C. The changes in actomyosin properties were greatest during the first week of frozen storage. The most effective cryoprotective agents were sucrose/sorbitol and maltodextrin.

Warangkana *et al.* (1996) studied stability of actomyosin from carp (*Cyprinus carpio*) during storage in ice, with special attention to effects of disulfide bond formation on conformational change of proteins. Effects of cryoprotectants (sorbitol, sucrose and sodium glutamate) and a reducing agent (dithiothreitol) on protein behaviour of actomyosin during storage in ice were assessed by determination of SH content, Ca2+ ATPase activity and surface hydrophobicity, and by SDS-PAGE. When neither cryoprotectants nor reducing agents were added, SH content, Ca2+ ATPase activity decreased, formation of myosin heavy chain dimmers occurred, and surface hydrophobicity increased during frozen storage of carp actomyosin. Myosin heavy chain dimmer formation occurred even in the presence of the cryoprotectants; addition of cryoprotectants stabilized Ca2+ ATPase activity and slightly decreased surface hydrophobicity during storage in ice.

Materials and Methods

III. MATERIAL AND METHODS

3.1. Collection of fish meat

Market fresh tilapia (*Oreochromis mossambicus*) was collected from the wholesale market and transported in an insulated box in iced condition, to the processing laboratory. Only fish that was iced immediately after catch was purchased. Fish was washed thoroughly with chilled water and was dressed in butterfly style. Then meat was collected mechanically with the help of mincing machine.

3.2. Washing of the collected meat

Washing of the collected mince meat was done according to the method of Gopakumar *et al.* (1992).

The collected minced meat was washed and leached with cold water (approx $+10^0$C) in 1:2 ratio (w/v). This was repeated a second time.

3.3. Mixing with cryoprotectants and storage

After washing the minced meat was divided into five lots and was mixed with cryoprotectants @ sorbitol 8%, sucrose 8%, sorbitol : sucrose mixture(1:1) @ 8%w/w, sorbitol : sucrose: polyphosphate mixture(3:4:1) @ 8% w/w, and one lot was taken as control. All the lots were packed in 150 gauge polyethylene sheet and then packed in waxed cartons. All the samples were frozen in deep freezer at a temperature of -40^0C followed by cold storage at -20^0C for 4 months. Samples were drawn fortnightly and assessed for physical quality, biochemical quality, sensory evaluation and folding test.

3.4. Preparation of Kamaboko

The composition of the ingredients used for preparation of kamaboko is given in the Table 1.

The fish meat mixed with the cryoprotectants was first comminuted in food processor for 15 min. at about 2800 cuts per min. Salt was added and ground for 3 min., followed by addition of monosodium glutamate (MSG) and grinding was continued for another 5 minutes. Chilled water was then added followed by refined wheat flour (maida). The blending was continued for another 10 min until a homogeneous mixture was obtained. The mince paste was then filled in wooden slab mold of size 25 ×9×1 cm. Then square pieces of mince paste were cut with knife and steamed for a period of 45 min. The cooked slices were then cooled to room temperature, then packed in polyethylene sheets and were chill stored at $+2^0$C overnight.

Table: 1 Ingredients for Kamaboko

Ingredients	Weight (g)
Minced meat	1000
Refined wheat flour (maida)	100
Salt	20
Monosodium glutamate	2
Iced water	20

3.5 Tests

3.5.1. Moisture Content

Moisture content of minced meat was determined by the method of AOAC(1975). A sample of about 5 gms was accurately weighed in a clean dry petridish using an electronic balance and was dried to a constant weight at a temperature of 105^0C in a hot air oven. The dried material was cooled in a dessicator. The moisture content was calculated as the percentage loss of weight of the minced meat upon drying.

3.5.2. Protein Content

Protein content of minced meat was estimated by the Microkjeldahl's method (AOAC, 1984).

One gram of minced meat was accurately weighed and transferred to a Kjeldahl's flask. A pinch of digestion mixture ($CuSO_4$: K_2SO_4 = 1 : 8) and 10 ml of concentrated H_2SO_4 were added and digested by heating at a temperature of 100^0C for 12h over a heating mantle. About 25 ml of distilled water was then carefully poured into the flask along the side. The flask was swirled to dissipate off the heat evolved. When the solution attained room temperature, it was quantitatively transferred to a 50 ml standard flask with distilled water washings. The solution was then made upto 50 ml using distilled water and mixed thoroughly. Five ml of this solution was subjected to distillation using a distillation unit ('Kjelplus' make). 10 ml 10N NaOH solution was added to the sample solution for distillation. The vapours were collected in 5ml 2% boric acid that was previously mixed with two drops of Tachirho's indicator. The boric acid was titrated against standard N/70 H_2SO_4 to a pink end point.

$$\text{Protein } \% = V \times \frac{14 \times 100 \times 100}{1000 \times 70 \times 5 \times W} \times 6.25$$

where,

V = volume of N/70 H_2SO_4 used
W = weight of sample taken

3.5.3. Fat Content

The Soxhlet Method of fat content estimation was followed (AOAC, 1990). A sample of about 2g of moisture- free minced meat was taken into an extraction thimble. The electrical heating unit was adjusted so that the solvent, petroleum ether (60^0-80^0C), siphons over 5 to 6 times per h. The extraction was carried out for 16 to 20 h. The solvent was then transferred to a pre-weighed beaker and evaporated off on a boiling water bath, then cooled to room temperature in a desiccator, and weighed. The difference in weight was expressed as the percentage of sample weight.

3.5.4. Ash Content

Ash content of minced meat was determined by the method of AOAC (1984). About 3g of the dried sample was weighed accurately in a silica crucible. It was then ignited in a muffle furnace at a temperature of 550^0C until the sample was free of carbon. It was then allowed to cool in a dessicator and weighed. The difference in weight was expressed as the percentage of sample weight.

3.5.5. Carbohydrate Content.

Carbohydrate content of minced meat was indirectly calculated by using Knauer's Procedure (Knauer *et al.*, 1994) using the following formula.

% Carbohydrate content = 100 –(%moisture + % protein + % lipid + % ash)

3.5.6. Cooking Yield

The cooking yield was calculated according to the method given by (Mathews and Garrison, 1975).

A sample of 50g of minced meat was divided into small square pieces and the initial weight of individual piece was taken. The pieces were steam cooked at 100^0C for 30 min. The weight of pieces after cooking was taken and the percentage cooking yield was calculated.

$$\text{Cooking Yield} = \frac{\text{Weight of sample after cooking}}{\text{Weight of sample before cooking}} \times 100$$

3.5.7. Free Drip

It is quantity of aqueous liquid which is freely exuded from the frozen meat when the meat is thawed under natural temperature and pressure. The free drip was calculated according to the method given by Hiremath and Dhananjaya (1980).

A cork borer (20mm diameter) was taken and a piece of meat was kept to a depth of 1 cm at the representative site in the body. Weight of the piece was taken immediately in an analytical balance. The piece was kept in between two rectangular pieces of glass with intervening filter paper in between the material and glass. Sample was allowed to thaw. The filter paper was removed which would have absorbed the exudate. The weight of the thawed sample was taken and weight loss was expressed as free drip in percentage.

$$\% \text{ Drip Loss} = \frac{\text{Free Drip}}{\text{Weight of the sample}} \times 100$$

3.5.8. Centrifugal Drip

The centrifugal drip was estimated according to the method given by Hiremath and Dhananjaya (1980).

About 5g of sample was taken and was placed in the centrifuge tube. Then the sample was centrifuged at 3000 r.p.m. for 10 min. Volume of drip formed was determined. The drip was transferred to 10 ml measuring cylinder and the volume of drip was found out.

3.5.9. Myofibrillar Protein

About 5g of minced meat was weighed and was then blended with 50ml distilled water. Then the blend was centrifuged at 2000 r.p.m. at 4^0C for 20 min. The residue was collected and extracted, with 0.1 M $NaHCO_3$ containing 5% NaCl, with 5 times the volume of the residue and was again centrifuged at 2000 r.p.m. at 4^0C for 20 min. The supernatant was collected and was made upto 100 ml. using 0.1 M $NaHCO_3$ solution containing 5% NaCl. Out of this solution 10 ml was taken. Analysis of the myofibrillar protein was done using AOAC analysis method (1984) for estimation of protein.

$$\% \text{ myofibrillar protein} = V \times \frac{14 \times 100 \times 100 \times 100}{1000 \times 70 \times 5 \times 10 \times W} \times 6.25$$

where,

V = volume of N/70 H_2SO_4 used

W = weight of sample taken

3.5.10. Sarcoplasmic protein

A sample of about 5g minced meat was weighed accurately and was blended with 50 ml distilled water. Then the blend was centrifuged at 2000 r.p.m. at 4^0C for 20 min. The supernatant was collected and was made upto 100ml using distilled water. Out of this solution 10 ml was taken out and analysis of the sarcoplasmic protein was done using AOAC analysis method (1984) for the estimation of protein.

$$\text{Sarcoplasmic Protein} = V \times \frac{14 \times 100 \times 100 \times 100}{1000 \times 70 \times 5 \times 10 \times W} \times 6.25$$

where,

V = volume of N/70 H_2SO_4 used

W = weight of sample taken

3.5.11. Testing the Kamaboko forming ability by Folding Test

The folding test for kamaboko was done according to the method given by Suzuki (1981). **The sample was prepared as per 3.4 Preparation of Kamaboko.**

The folding test was done on a piece of 3 mm thickness cut out from the sample. The test piece was held between thumb and forefinger and folded to observe the way it breaks. Five test pieces from each sample were tested.

The quality was classified according to the following five grades.

Grade AA	no crack seen after folding twice.
Grade A	no cracks seen after folding in half.
Grade B	cracks gradually when folded in half.
Grade C	cracks immediately when folded.
Grade D	breaks by finger pressure.

3.5.12. Sensory Evaluation

A taste panel consisting of seven judges carried out sensory evaluation of the raw and cooked samples. The quality characteristics assessed were whiteness and muddy flavour/muddy odour for raw and cooked meat and sweetness and texture for cooked meat on the basis of a 5-point scale as suggested by Jellinek (1985). Format of the sensory evaluation is given in Appendix I.

3.6. Statistical analysis

The experiments were carried out using Completely Ramdomised Design(CRD). Data obtained were analysed using analysis of variance (ANOVA) technique(Snedecor and Cochran 1968). Pair-wise comparison of treatment means were done wherever necessary using least significant difference. Sensory evaluation results were analysed using Friedmen test (Sprent, 1989).

Results

V. RESULTS

4.1 Proximate Composition

The results of analysis of proximate composition of minced meat, before water leaching, after water leaching and after 4 months frozen storage are given in Table 2. Minced meat of tilapia had a protein content 16.02%, moisture content 82.41% and fat content 1.05%. Table 2 shows a lower fat content in tilapia *(Oreochromis mossambica)*. On water leaching these values for protein, moisture and fat changed 12.24%, 85.42% and 0.75% respectively

For control, meat moisture content decreased from 85.42% to 53.77% and total protein decreased from 12.24% to 5.27 % after 4 months storage. Moisture content and protein content seems to be maintained during 4 months of storage in treatment of minced meat with sorbitol: sucrose: polyphosphate. In other treatments significant decrease was observed in all the values.

4.2 Storage studies

The minced meat collected from Tilapia (*Oreochromis mossambicus*) was wrapped in 150 gauge polyethylene sheets and then packed in waxed cartons; after treatment with different types of cryoprotectants viz. sorbitol(8% w/w), sucrose(8% w/w), sucrose:sorbitol(1:1,8%w/w) and sorbitol : sucrose : polyphosphate(3:4:1,8% w/w). A control sample without any cryoprotectant was also kept during storage study as reference sample. The packed waxed cartons after freezing at -40^0C were stored in cold storage at -20^0C for 4 months.

4.2.1 Cooking yield

Changes in cooking yield was observed for five different treatments of cryoprotectants during 4 months of storage period. Changes in cooking yield of minced meat treated with different cryoprotectants are shown in Fig. 1. It shows that control, sorbitol, sucrose and sucrose:sorbitol treated meat has not

Table 2 : Proximate composition of minced meat before water leaching, after water leaching and after four months frozen storage

	Before leaching	After leaching	After four months frozen storage				
			T_1	T_2	T_3	T_4	T_5
Moisture	82.41%	85.42%	53.77%	60.94%	83.38%	58.64%	84.38%
Protein	16.02%	12.24%	5.27%	5.25%	5.27%	8.7%	9.25%
Fat	1.05%	0.75%	0.27%	0.34%	0.28%	0.43%	0.45%
Ash	0.4%	0.05%	0.01%	0.02%	0.03%	0.02%	0.03%
Carbohydrate	0.12%	1.54%	42.08%	34.42%	11.04%	32.21%	5.89%

T_1 : minced meat without cryoprotectants (control)

T_2 : minced meat treated with sorbitol

T_3 : minced meat treated with sucrose

T_4 : minced meat treated with sucrose:sorbitol

T_5 : minced meat treated with sucrose:sorbitorl:polyphosphate

Table 3. ANOVA for cooking yield of minced meat treated with different types and levels of cryoprotectants.

Source of variation	Sum of square	Degree of freedom	Mean sum of square	F value
Between Treatment	1435.36	4	371.34	*89.26
Error	41.69	10	4.16	
Total	**152.05**	**14**	**375.50**	

* at 5% level of significance

Treatments	:	T_5	T_4	T_3	T_2	T_1
Means (percentage cooking yield)	:	79	56.07	55.19	53.2	52.7

T_1 : minced meat without cryoprotectants (control)

T_2 : minced meat treated with sorbitol

T_3 : minced meat treated with sucrose

T_4 : minced meat treated with sucrose:sorbitol

T_5 : minced meat treated with sorbitol:sucrose:polyphosphate

T_5 was significantly different from all other four treatments. No significant difference between T_1, T_2, T_3 and T_4

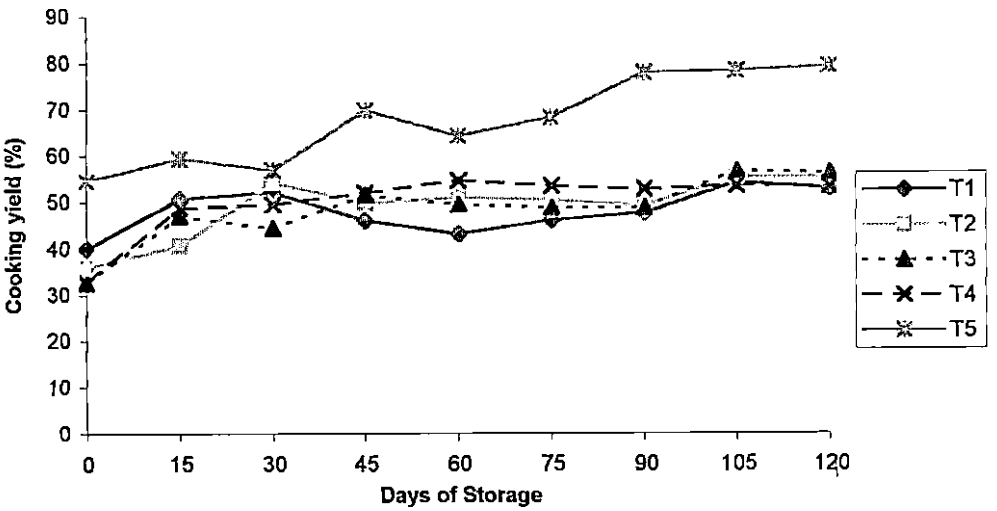

Fig. 1 Changes in cooking yield for five different treatments of cryoprotectants in minced meat during four months storage.

T1 = Control T2 = Sorbitol(8%,Wt./wt.) T3 = Sucrose(8%,Wt./wt.) T4 = Sucrose:Sorbitol(8%,Wt./wt.,1:1)
T5 =Sorbitol : Sucrose : Polyphosphate(8%, Wt./wt.,3:4:1)

Table 4: ANOVA for cooking yield of control minced meat during four months storage

Source of variation	Sum of square	Degree of freedom	Mean sum of square	F value
Between days	535.8	8	66.975	*37.2
Error	32.48	18	1.8	
Total	**568.26**	**26**	**68.775**	

* at 5% level of significance

days	: 0	15	30	45	60	75	90	105	120
means (percentage cooking yield)	: 40.1	50.8	52.1	46.04	43.16	46.04	47.66	54.1	52.77

No significant difference during 75 – 90 days and 105 – 120 days.

Table 5 : ANOVA for cooking yield of sorbitol:sucrose:polyphosphate treated minced meat during four months storage.

Source of variation	Sum of square	Degree of freedom	Mean sum of square	F value
Between days	2136.66	8	267.08	*13.15
Error	366.42	18	20.3	
Total	**2503.08**	**26**	**287.38**	

* at 5% level of significance

days	: 0	15	30	45	60	75	90	105	120
means (percentage cooking yield)	: 54.7	59.42	56.84	69.82	64.2	68.25	77.8	78.16	79

Significant difference in cooking yield only during 30-45 day and 75-90 days.

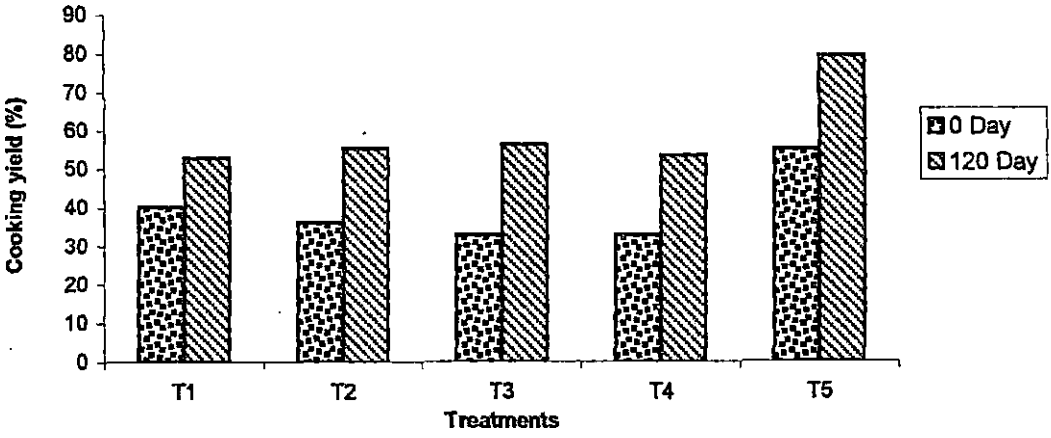

Fig. 2 Changes in cooking yield for five different treatments of cryoprotectants in minced meat on 0 day and 120th day

T_1 = Control T_2 = Sorbitol(8%) T_3 = Sucrose(8%) T_4 = Sucrose:Sorbitol(8%,1:1 Wt./wt.)
T_5 =Sorbitol : Sucrose : Polyphosphate(8%, 3:4:1, wt./wt.)

much difference in cooking yield whereas cooking yield for sorbitol:sucrose:polyphosphate treated meat represents a increasing order during 4 months storage. In Fig. 2 bar diagram shows changes in cooking yield for five different treatments of cryoprotectants in minced meat on 0 day and 120th day. Higher values of cooking yield on 120th day as compared to 0 day can be observed in the Fig. 2.

The statistical analysis showed a significant difference between treatment with sorbitol : sucrose: polyphosphate and all other 4 treatments. The cooking yield of sorbitol: sucrose: polyphosphate treated meat increased from 54.7% to 79% at the end of storage period of 120 days.

There was a significant difference in cooking yield of control on 75 days storage where as no significant difference was observed in 75-90 days storage and 105–120 days storage. In case of treatment with sorbitol:sucrose:polyphosphate, significant difference was observed only during storage period between 30-45 days and 75-90 days.

ANOVA for the cooking yield of minced meat treated with different types and level of cryoprotectants are given in Table 3 and ANOVA for cooking yield for control and sorbitol:sucrose:polyphosphate treated minced meat during 4 months storage are given in Table 4 and Table 5 respectively.

4.2.2. Free drip

The minced meat not treated with cryoprotectants (control) was having 21.4% of free drip whereas sample treated with sorbitol:sucrose:polyphosphate was having 14.96% free drip after 4 months storage. The percentage free drip of other treatments, sorbitol, sucrose and sucrose:sorbitol lie inbetween these values. The results are shown in Fig.3.

The bar diagram showing changes in free drip of minced meat treated with five different levels and types of cryoprotectants for 0 day and 120th day is represented in Fig.4.

ANOVA for free drip of minced meat treated with different type and level of cryoprotectants at the end of 4 months storage has been given in Table 6.

The statistical analysis showed a significant difference in free drip between treatments at the end of 4 months storage. Free drip values for minced meat treated with sorbitol: sucrose: poly-phosphate was significantly different from all other treatments after 4 months of storage.

ANOVA for free drip of control and sorbitol:sucrose:polyphosphate treated minced meat during 4 months storage are given in Table 7 and Table 8 respectively.

Table 7 represents no significant difference in underscore means of free drip of the control minced meat and Table 8 represent no significant difference in free drip was noticed of sorbitol: sucrose: polyphosphate treated minced meat during 4 months storage.

4.2.3 Centrifugal drip

The results of studies on changes in centrifugal drip during 4 months storage of minced meat are shown in Fig.5. Results showed that the values for centrifugal drip in all the treatments were less than that of control at the end of four months storage.

The values of centrifugal drip on 120^{th} day for minced meat treated with sorbitol(8% w/w), sucrose (8% w/w), sucrose:sorbitol (1:1,8% w/w) and sorbitol:sucrose:polyphosphate(3:4:1,8% w/w) are 0.9 ml, 0.5 ml, 0.4 ml, 0.1 ml respectively. The amount of centrifugal drip in sorbitol: sucrose: polyphosphate treated minced meat was less than all other treatments at the end of 4 months storage.

Table 6. ANOVA for free drip of minced meat treated with different types and levels of cryoprotectants after four months storage.

Source of variation	Sum of square	Degree of freedom	Mean sum of square	F value
Between days	86.08	4	21.52	*37.75
Error	5.74	10	0.57	
Total	**91.82**	**14**	**22.09**	

* at 5% level of significance

Treatments	:	T_1	T_3	T_2	T_4	T_5
Means (percentage free drip)	:	21.86	18.9	18	16.06	14.96

T_1 : minced meat without cryoprotectants (control)

T_2 : minced meat treated with sorbitol

T_3 : minced meat treated with sucrose

T_4 : minced meat treated with sucrose:sorbitol

T_5 : minced meat treated with sorbitol:sucrose:polyphosphate

T_5 is significantly different from T_1, T_2, T_3 .

T_1 is significantly difference from all other.

No significant difference between T_2, T_3 and T_4, T_5.

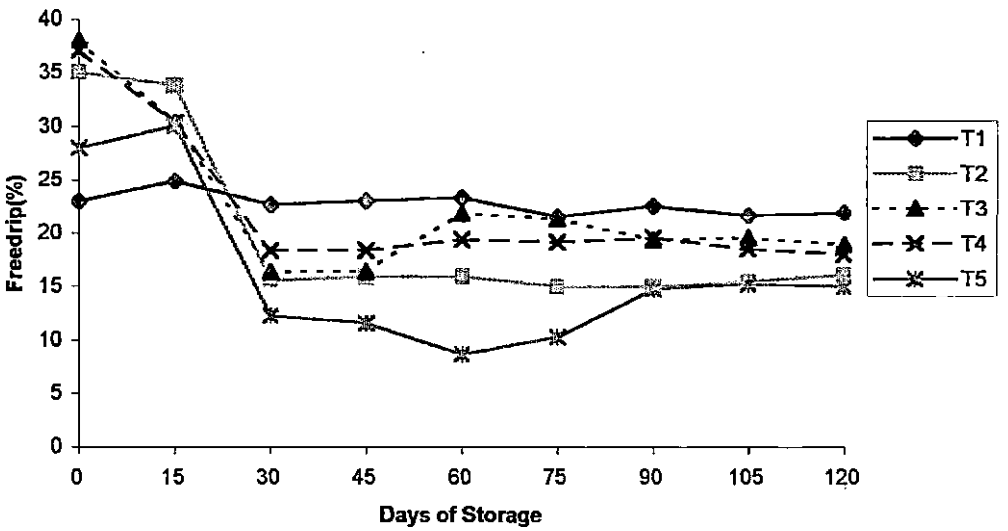

Fig. 3 Changes in freedirp for five different treatments of cryoprotectants in minced meat during four months storage.

T_1 = Control T_2 = Sorbitol(8%) T_3 = Sucrose(8%) T_4 = Sucrose:Sorbitol(8%,1:1 Wt./wt.)
T_5 =Sorbitol : Sucrose : Polyphosphate(8%, 3:4:1, wt./wt.)

Table 7: ANOVA for free drip of control minced meat during four months storage.

Source of variation	Sum of square	Degree of freedom	Mean sum of square	F value
Between days	151.9	8	18.92	*13.15
Error	22.38	18	1.24	
Total	**173.78**	**26**	**20.16**	

* at 5% level of significance

days	: 0	15	30	45	60	75	90	105	120
means (percentage free drip)	: 23	24.9	22.69	28	23.3	21.5	22.53	21.62	21.86

Underscored means are not significantly different.

Table 8: ANOVA for free drip of sorbitol:sucrose:polyphosphate treated minced meat during four months storage.

Source of variation	Sum of square	Degree of freedom	Mean sum of square	F value
Between days	722.54	8	90.31	2.5
Error	648.64	18	36.03	
Total	**1371.18**	**26**	**126.34**	

There is no significant difference.

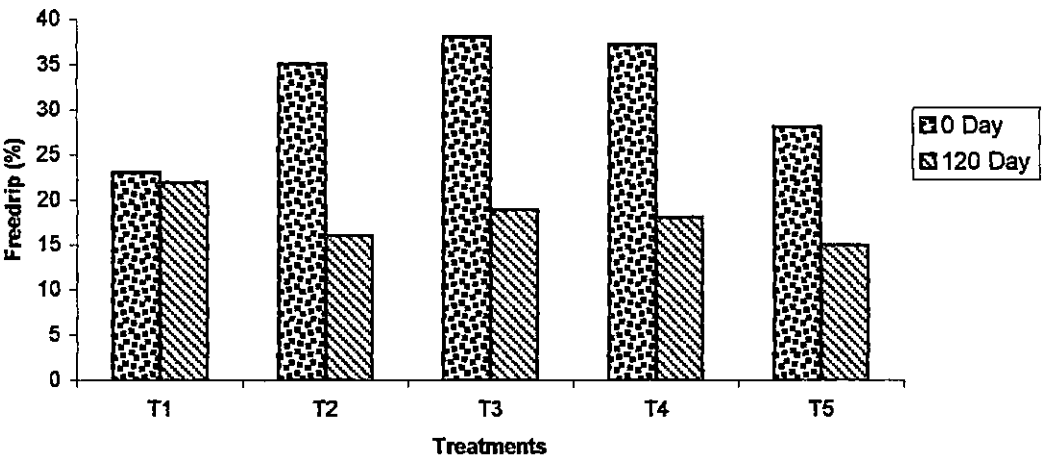

Fig. 4 Changes in freedrip for five different treatments of cryoprotectants in minced meat on 0 day and 120th day

T_1 = Control T_2 = Sorbitol(8%) T_3 = Sucrose(8%) T_4 = Sucrose:Sorbitol(8%,1:1 Wt./wt.)
T_5 =Sorbitol : Sucrose : Polyphosphate(8%, 3:4:1, wt./wt.)

4.2.4. Myofibrillar protein

The observations of changes in myofibrillar protein during 4 months storage are shown in Fig.6. The values of myofibrillar protein on 120th day for minced meat treated with sorbitol(8% w/w), sucrose (8% w/w), sucrose:sorbitol (1:1,8% w/w) and sorbitol:sucrose:polyphosphate(3:4:1,8% w/w) are 1.76%, 3.53%, 3.43%, 5.26% and 8.9% respectively.

ANOVA for the myofibrillar protein after 4 months frozen storage has been given in Table 9. Statistical analysis showed a significant difference between treatments after 4 months storage

Significant difference was found between control and treatments such as sorbitol: sucrose: polyphosphate and sucrose: sorbitol but no significant difference was found between sorbitol and sucrose treated minced meat.

4.2.5. Sarcoplasmic protein

The results of estimation of sarcoplasmic protein during 4 months storage are shown in Fig.7. The values of sarcoplasmic protein on 120^{th} day for minced meat treated with sorbitol (8% w/w), sucrose (8% w/w), sucrose: sorbitol (1:1,8% w/w) and sorbitol: sucrose: polyphosphate (3:4:1,8% w/w) are 0.25%, 0.4%, 0.6%, 1.0% and 1.5% respectively.

Statistical analysis showed no significant difference among the treatments during 4 months storage. Figure shows a very low level of sarcoplasmic protein in control on 120^{th} day.

4.2.6 Sensory evaluation

Average scores of sensory evaluation on 0 day and 120^{th} day of frozen storage for minced meat treated with different level and type of cryoprotectants are given in Table 10. The score sheet has been shown as Appendix I. The quality characteristics tested were whiteness and muddy odour for raw meat and whiteness, muddy taste, sweetness and texture for cooked meat.

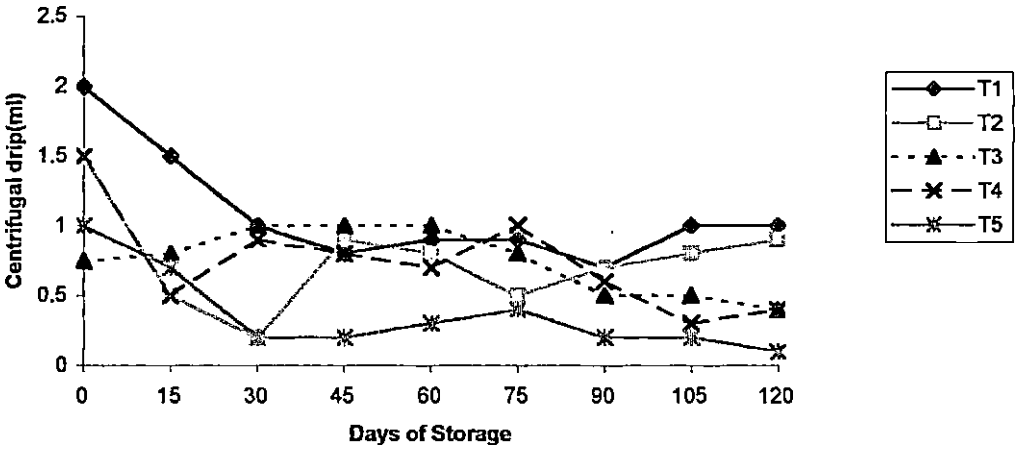

Fig. 5 Changes in Centrifugal drip for five different treatments of cryoprotectants in minced meat during four months storage.

T1 = Control T2 = Sorbitol(8%,Wt./wt.) T3 = Sucrose(8%,Wt./wt.) T4 = Sucrose:Sorbitol(8%,Wt./wt.,1:1)
T5 =Sorbitol : Sucrose : Polyphosphate(8%, Wt./wt.,3:4:1)

Table 9: ANOVA for myofibrillar protein after four months storage.

Source of Variation	Sum of square	Degree of freedom	Mean sum of square	F value
Between days	89.71	4	22.42	*168.57
Error	1.33	10	0.133	
Total	**91.04**	**14**		

* at 5% level of significance

Treatments	:	T_5	T_4	T_2	T_3	T_1
Means (percentage myofibrillar protein)	:	8.9	5.26	3.55	3.43	1.76

T_1 : minced meat without cryoprotectants (control)

T_2 : minced meat treated with sorbitol

T_3 : minced meat treated with sucrose

T_4 : minced meat treated with sucrose:sorbitol

T_5 : minced meat treated with sorbitol:sucrose:polyphosphate

Significant difference between T_1, T_4, T_5

No significant difference between T_2 and T_3.

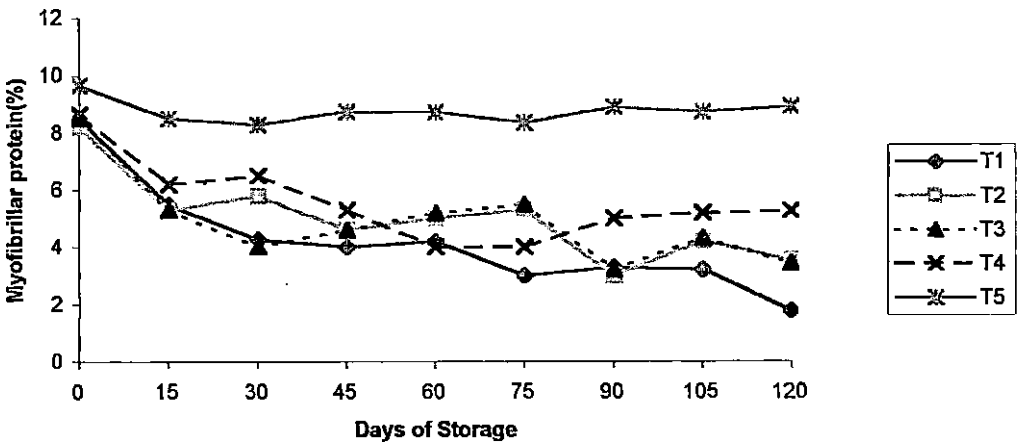

Fig. 6 Changes in Myofibrillar protein for five different treatments of cryoprotectants in minced meat during four months storage.

T1 = Control T2 = Sorbitol(8%,Wt./wt.) T3 = Sucrose(8%,Wt./wt.) T4 = Sucrose:Sorbitol(8%,Wt./wt.,1:1)
T5 =Sorbitol : Sucrose : Polyphosphate(8%, Wt./wt.,3:4:1)

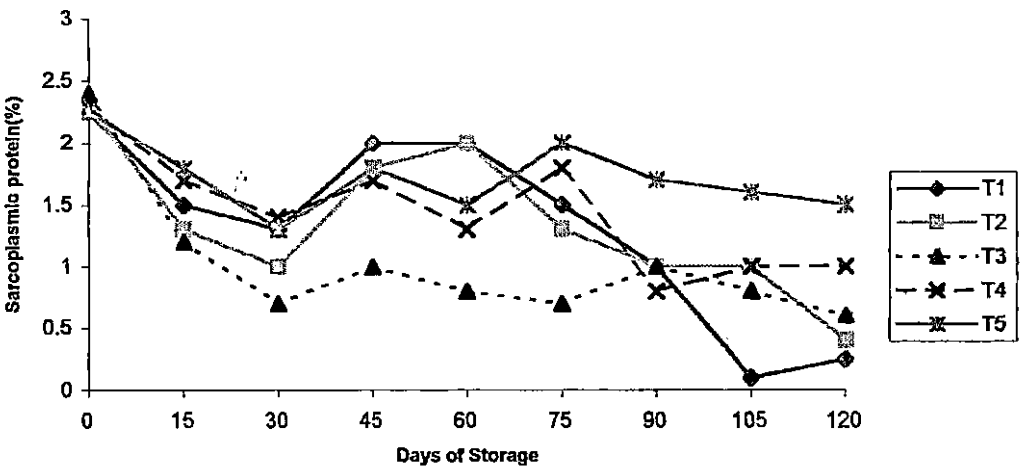

Fig. 7 Changes in Sarcoplasmic protein for five different treatments of cryoprotectants in minced meat during four months storage.

T1 = Control T2 = Sorbitol(8%,Wt./wt.) T3 = Sucrose(8%,Wt./wt.) T4 = Sucrose:Sorbitol(8%,Wt./wt.,1:1)
T5 =Sorbitol : Sucrose : Polyphosphate(8%, Wt./wt.,3:4:1)

Table 10: Average score of sensory evaluation on 0 day and 120 day for raw and cooked minced meat treated with different type and level of cryoprotectants.

Quality Characteristic/ days / Treatment	Raw meat				Cooked meat							
	Whiteness		Muddy odour		Whiteness		Muddy taste		Sweetness		Texture	
	0	120	0	120	0	120	0	120	0	120	0	120
T_1	1.7	3.28	1.41	2.5	1.28	3.5	2.35	1.78	5	5	3.64	3.35
T_2	2.28	3.57	2.42	3.07	2.57	3.78	2.92	3.21	2.78	3.14	2.42	3.5
T_3	3.35	3.21	2.85	2.92	3.64	4.0	2.71	3.21	1.71	2.42	2	3.28
T_4	3.92	3.50	3.25	3.78	4.07	2.14	4	3.21	2.31	1.71	3	2.85
T_5	3.71	1.42	4.71	2.71	3.5	1.57	2.28	3.57	3	2.71	3.85	2

T_1 : minced meat without cryoprotectants (control)

T_2 : minced meat treated with sorbitol (8% wt/wt)

T_3 : minced meat treated with sucrose (8% wt/wt)

T_4 : minced meat treated with sucrose:sorbitol (8% wt/wt, 1:1)

T_5 : minced meat treated with sorbitol:sucrose:polyphosphate (8% wt/wt, 3:4:1)

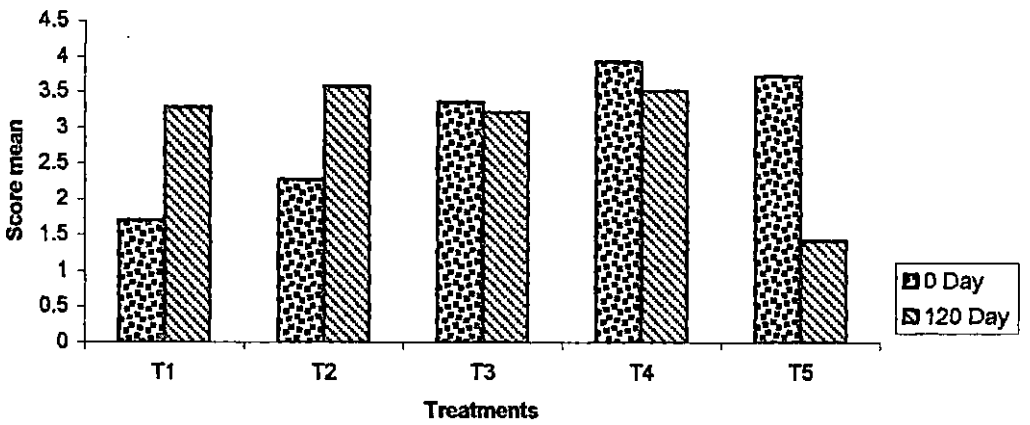

Fig. 8 Changes in Whiteness for five different treatments of cryoprotectants in raw minced meat on 0 day and 120th day

T_1 = Control T_2 = Sorbitol(8%) T_3 = Sucrose(8%) T_4 = Sucrose:Sorbitol(8%,1:1 Wt./wt.)
T_6 =Sorbitol : Sucrose : Polyphosphate(8%, 3:4:1, wt./wt.)

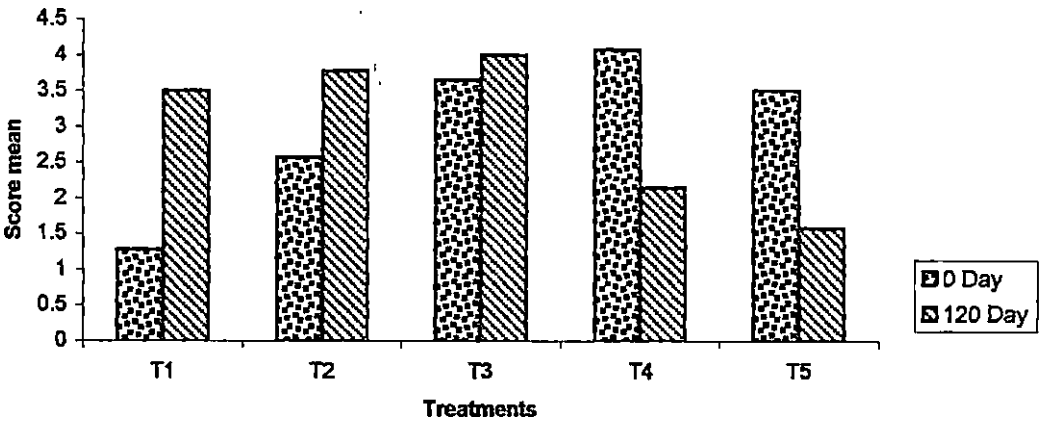

Fig. 9 Changes in Whiteness for five different treatments of cryoprotectants in cooked meat on 0 day and 120th day

T_1 = Control T_2 = Sorbitol(8%) T_3 = Sucrose(8%) T_4 = Sucrose:Sorbitol(8%,1:1 Wt./wt.)
T_5 =Sorbitol : Sucrose : Polyphosphate(8%, 3:4:1, wt./wt.)

4.2.6.1 Whiteness

The changes in whiteness of raw and cooked minced meat on 0 day and 120 day are given in Fig.8 and Fig. 9 respectively.

A mean whiteness score of 1.7 for 0 day increased to 3.28 for 120 day in case of control. For the treatment such as sucrose (8% w/w), sorbitol(8% w/w), sucrose: sorbitol(8% w/w, 1:1), sorbitol: sucrose: polyphosphate(8% w/w, 3: 4: 1) the values for 0 day are 2.28, 3.35, 3.92, 3.71 and for 120 days are 3.57, 3.21, 3.5, 1.42 respectively for 4 months storage. For cooked minced meat, the mean score of 1.28 increased to 3.5 for control and for sorbitol:sucrose:polyphosphate treated minced meat the mean score of 3.5 decreased to 1.57 after 4 months storage.

4.2.6.2 Muddy odour

The observations of changes in muddy odour of raw minced meat during 4 months storage are shown in fig. 10.

The mean score of control increased from 1.41 to 2.5 and for sorbitol: sucrose: polyphosphate treated minced meat the value decreased from 4.71 to 2.71 in 120 days. Friedman test showed no significant difference between treatment after 4 months storage.

4.2.6.3 Muddy taste

The observations recorded for changes in muddy taste of cooked meat during 4 months frozen storage are shown in Fig. 11. For the treatments such as control, sucrose (8% w/w), sorbitol (8% w/w), sucrose: sorbitol (8% w/w, 1:1), sorbitol: sucrose: polyphosphate(8% w/w, 3: 4: 1) the values for 0 day are, 2.35, 2.92, 2.71, 4, 2.28 and for 120^{th} day are 1.78, 3.21, 3.21, 3.21, 3.57 respectively. Friedman test showed no significant difference between treatments after 4 months storage.

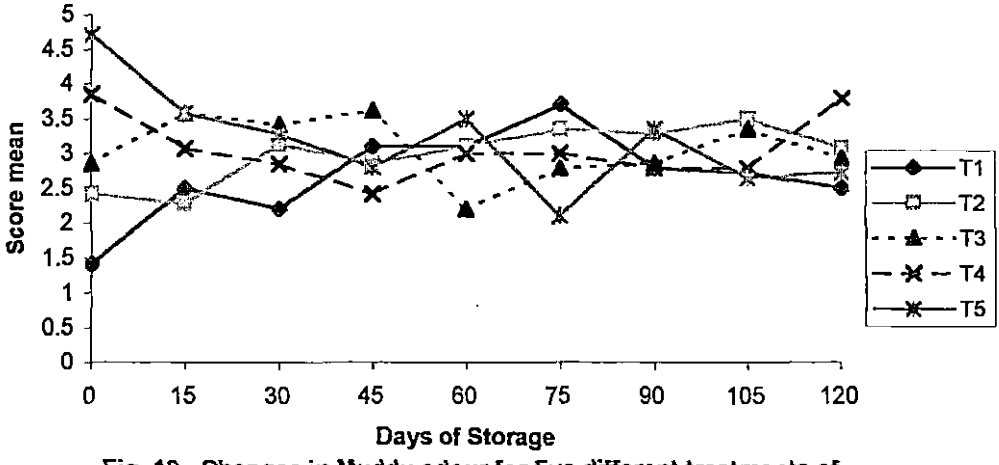

Fig. 10 Changes in Muddy odour for five different treatments of cryoprotectants in raw minced meat during four months storage.

T1 = Control T2 = Sorbitol(8%,Wt./wt.) T3 = Sucrose(8%,Wt./wt.) T4 = Sucrose:Sorbitol(8%,Wt./wt.,1:1)
T5 =Sorbitol : Sucrose : Polyphosphate(8%, Wt./wt.,3:4:1)

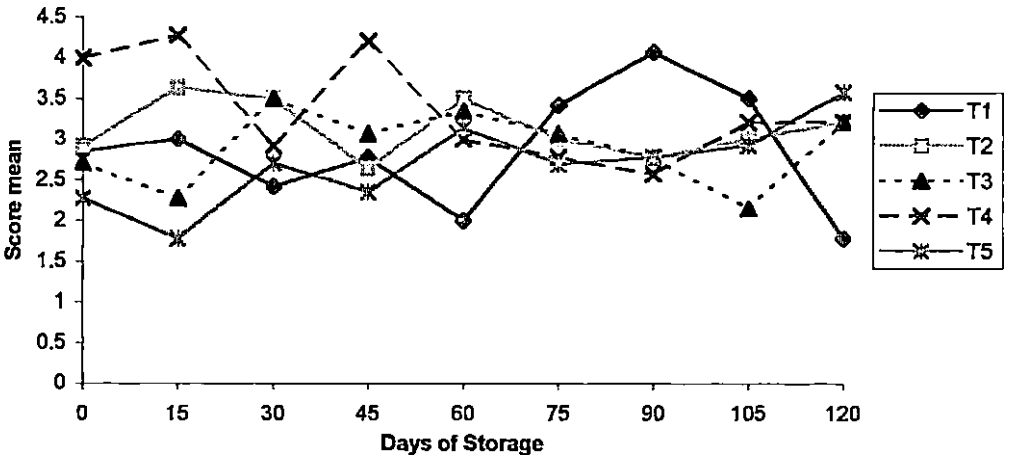

Fig. 11 Changes in Muddy taste for five different treatments of cryoprotectants in cooked meat during four months storage.

T1 = Control T2 = Sorbitol(8%,Wt./wt.) T3 = Sucrose(8%,Wt./wt.) T4 = Sucrose:Sorbitol(8%,Wt./wt.,1:1)
T5 =Sorbitol : Sucrose : Polyphosphate(8%, Wt./wt.,3:4:1)

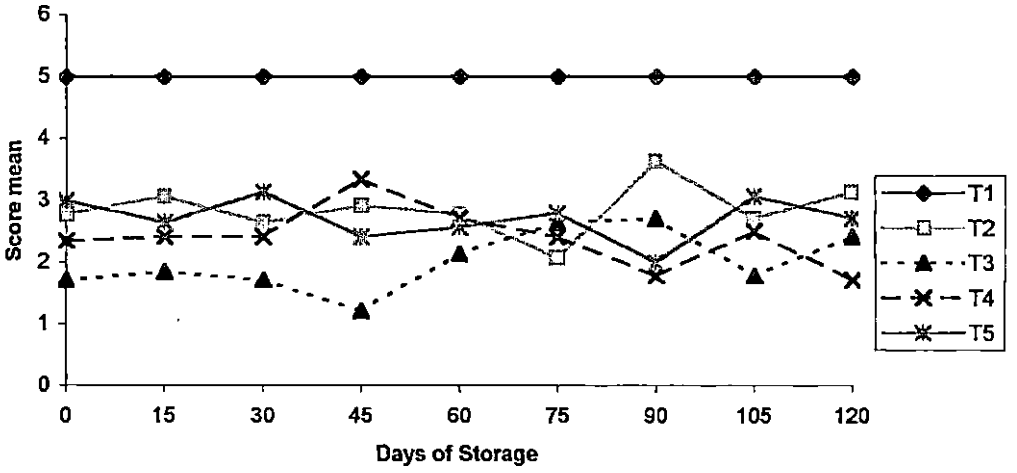

Fig. 12 Changes in Sweet taste for five different treatments of cryoprotectants in cooked meat during four months storage.

T1 = Control T2 = Sorbitol(8%,Wt./wt.) T3 = Sucrose(8%,Wt./wt.) T4 = Sucrose:Sorbitol(8%,Wt./wt.,1:1)
T5 =Sorbitol : Sucrose : Polyphosphate(8%, Wt./wt.,3:4:1)

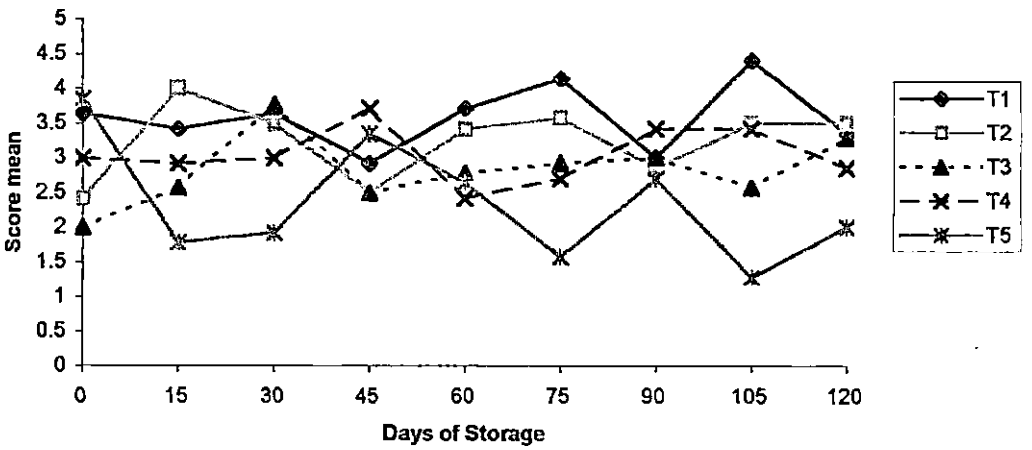

Fig. 13 Changes in texture for five different treatments of cryoprotectants in cooked meat during four months storage.

T1 = Control T2 = Sorbitol(8%,Wt./wt.) T3 = Sucrose(8%,Wt./wt.) T4 = Sucrose:Sorbitol(8%,Wt./wt.,1:1)
T5 =Sorbitol : Sucrose : Polyphosphate(8%, Wt./wt.,3:4:1)

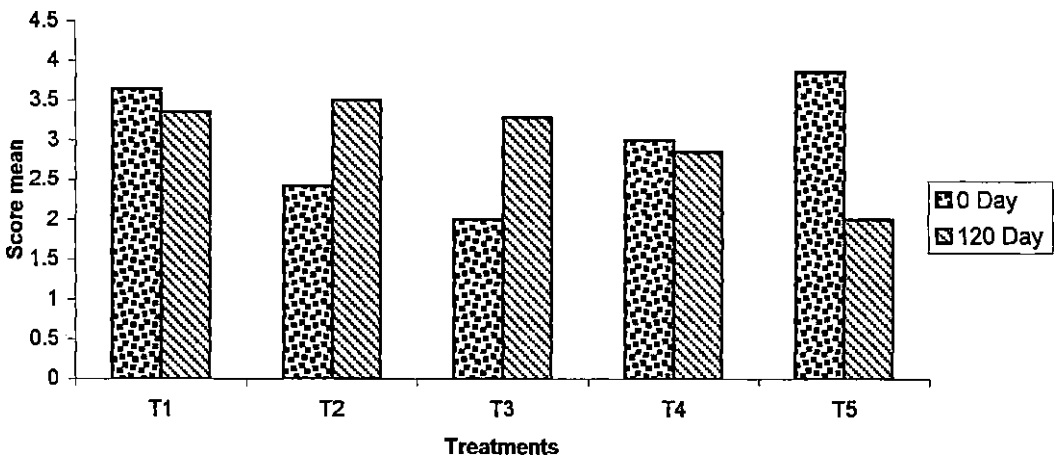

Fig. 14 Changes in Texture for five different treatments of cryoprotectants in cooked meat on 0 day and 120th day

T_1 = Control T_2 = Sorbitol(8%) T_3 = Sucrose(8%) T_4 = Sucrose:Sorbitol(8%,1:1 Wt./wt.)
T_5 =Sorbitol : Sucrose : Polyphosphate(8%, 3:4:1, wt./wt.)

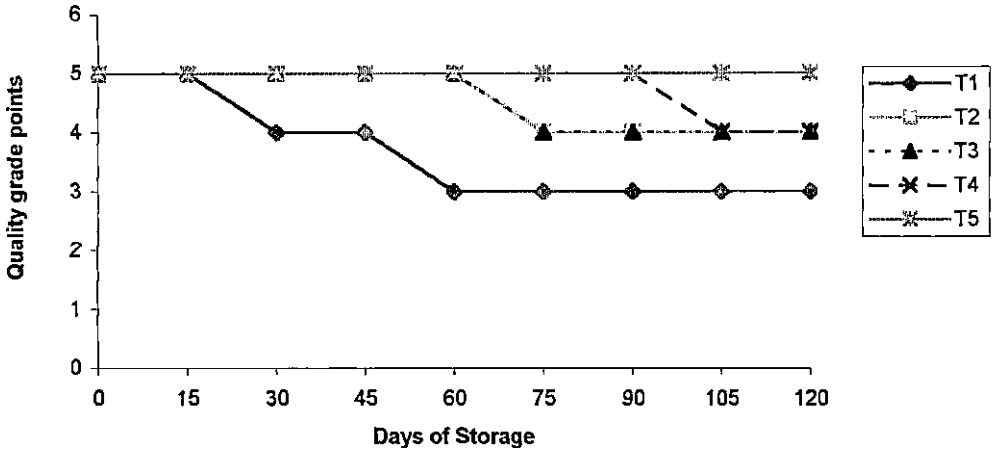

Fig. 15 Changes in Quality grade of Kamaboko during four months storage.

Treatments : T1 = Control T5 = Sorbitol : Sucrose : polyphosphate (8%, wt./wt., 3:4:1)

Grade Points : 5 = AA 4 = A 3 = B

4.2.6.4 Sweet taste

The results of sensory analysis for sweet taste of cooked meat during 4 months storage are shown in fig. 12. For the treatments such as control, sucrose (8% w/w), sorbitol (8% w/w), sucrose: sorbitol (8% w/w, 1:1), sorbitol: sucrose: polyphosphate(8% w/w, 3: 4: 1) the values for 0 day are 5, 2.78, 1.71, 2.31, 3 and for 120th day storage are 5, 3.14, 2.42, 1.71, 2.71 respectively.

Friedman test have shown a significant difference between control and sucrose (8% w/w) treated minced meat up to 60 days storage. For sorbitol: sucrose: polyphosphate treated minced meat, the mean score of 3 on 0 day decreased to 2.71 on 120 days frozen storage.

4.2.6.5 Texture

The observations for texture score of cooked meat during 4 months frozen storage are shown in fig. 13. Texture score for the treatments such as control, sucrose (8% w/w), sorbitol (8% w/w), sucrose: sorbitol (8% w/w, 1:1), sorbitol: sucrose: polyphosphate (8% w/w, 3: 4: 1) for 0 day are 3.64, 2.42, 2, 3, 3.85 and for 120 day are 3.35, 3.5, 3.28, 2.85, 2. Changes in texture of cooked meat on 0 day and 120 days are shown in fig. 14.

Friedman test was applied for the scores obtained from the control meat during 4 months storage. Significant difference was found in 60 – 75 days.

4.2.7 Folding test

The observations for the folding test of Kamaboko during 4 months storage are shown in fig. 15.

Kamaboko prepared from sorbitol: sucrose: polyphosphate treated minced meat showed quality grade AA throughout 4 months storage period. Kamaboko prepared from control minced meat showed quality grade B after 60 days.

Discussion

V. DISCUSSION

5.1 Proximate composition:

It is evident from the literature that tilapia is a good material for Kamaboko preparation (Yamamoto, 1977). Proximate composition of tilapia has been estimated under the present study. Results show a lower fat content in tilapia. Yamamoto, (1977) found similar results and concluded that lower fat content is important in product development because the problem of rancidity can be reduced.

Gomes *et al.* (1995) also found similar composition for surimi made from fresh water tabacu fish (a hybrid of *Piaractus mesopotamicus* and *Colossoma macropomum*).

The process of washing increases the moisture content of minced meat whereas the protein content is reduced after washing. Purpose of washing process is not only to obtain a white colour product but also to remove sarcoplasmic protein. The halibut meat had no kamaboko forming ability unless the water washing process was applied. Removal of sarcoplasmic protein is necessary for preventing inhibition of ashi forming ability. The water soluble protein inhibit the formation of ashi because when actomyosin is denatured by heat, the heat coagulative sarcoplasmic protein binds with actomyosin and coagulates.

Results from Table 2 shows that even after 4 months storage minced meat treated with sorbitol : sucrose : polyphosphate has higher moisture content The hydration is always an important economic consideration and one that affects the eating quality of finished product. The use of polyphosphate can stabilize suitable water content and thus aid processing and improve quality. Montero *et al.* (1999) found greater functional stability of washed meat when added with cryoprotectants. They also reported that during first three months of storage, gels were somewhat softer and more elastic than with unwashed mince.

In the present study minced meat treated with cryoprotectants has more or less stable amount of protein even after four months of storage. Han Ching and Leinot (1993) also found similar results for surimi composition containing sugars (4%

sucrose, 4% sorbitol) and polyphosphate (0.2 to 0.3 %). Reckasi (1992) reported inhibition of protein denaturation in frozen pork meat treated with sorbitol /sucrose. Sych *et al.* (1990) also found inhibition of freeze-induced denaturation of cod surimi with combination of sucrose and sorbitol 1:1 mixture at 8% w/w. It has been explained by Buttkus (1970) and Arakawa and Timashaff (1982) that sugars protect protein from freeze denaturation by increasing surface tension of water as well as amount of bound water. This prevents withdrawal of water molecules from protein thus stabilizing protein.

5.2 Storage Studies

Changes during storage of minced meat were studied based on various physical, biochemical and organoleptic tests. All the tests used are dependent on quality of the minced meat and hence, are useful for determining its shelf life. In addition, sensory evaluation was used as a means of determining consumer acceptability of minced meat, used for preparation of final product. It is well known that quality of fish products are most satisfactorily judged by organoleptic methods rather than by objective tests. It may be noted that most of the parameters used in present investigation are inter related and all these in turn can determine quality of minced meat.

Samples of fish meat mixed with different combination of cryoprotectants or without cryoprotectants were frozen stored at –20°C for 4 months. Minced meat mixed with different cryoprotectants were reported to have a longer shelf life than minced meat having no cryoprotectants.

5.2.1 Cooking yield

In the present study minced meat mixed with the cryoprotectants sorbitol 8%, sucrose 8%, and sucrose : sorbitol 1:1, 8% w/w and without cryoprotectants has no significant difference in cooking yield after four months of frozen storage. However treatment with sorbitol : sucrose : polyphosphate (8% w/w, 3:4:1) has resulted in higher value of cooking yield on 4 months frozen storage (Fig 1 and Fig 2). Similar results were found by Nowsad *et al.* (2000) for chicken mince. Water retention properties were reportedly well protected and thus having higher cooking

yield in minced meat treated with sorbitol : sucrose : polyphosphate (8% w/w, 3:4:1). Yoon and Lee (1990) found greater cooking loss in surimi at 4% level of sorbitol and sucrose while at 8% level, no cooking loss was observed. Baowu and Youling (1998) found that cooking yield for beef heart surimi (without cryoprotectants) decreased during storage at -15^0C and -29^0C. However it was well preserved by addition of cryoprotectants. Chang and Regenstein (1995) found similar results for cooking loss in cod mince subjected to treatment of sucrose/sorbitol (4% /4% mince weight).

5.2.2 Free drip

In the present study changes in free drip shown in Fig 3 and 4 indicated that amount of free drip is significantly different for mince meat treated with different type and level of cryoprotectants. Fig 3 shows that control mince (without cryoprotectants) formed higher level of free drip throughout the frozen storage period. Also mince treated with sucrose and combination of sucrose : sorbitol has similar amount of free drip during storage. Mince treated with sorbitol was having lower amount of free drip and treated with sorbitol : sucrose : polyphosphate was having least amount of free drip after storage period. Sudden fall in amount of free drip was observed at the end of 30 days frozen storage in both the samples. After 90 days storage there was slight increase in amount of free drip. In all the treatments after 90 days amount of free drip form is almost stable (Fig 4). Similar results were reported by Yoon and Lee (1990). They studied drip loss in both cooked and uncooked mince extruded products and concluded that gel with sorbitol and sucrose mixture showed least drip loss in either cooked or uncooked products. The largest drip loss was found in gel without cryoprotectants. The addition of cryoprotectants did not prevent thaw drip from whether cooked or uncooked products when incorporated into extruded products. Cooked products had considerably more drip loss than uncooked, indicating that cryoprotectants worked better in uncooked products. Sorbitol : sucrose : polyphosphate treated mince was having less amount of free drip because water retention properties were well preserved in mince with added cryoprotectants (Nowsad *et al.* 2000). Increasing moisture retention is another function of polyphosphates when added to surimi.

The protein's ability to reabsorb liquids during thawing is due to provision of more polar sites on protein surface. Polyphosphates when used in conjunction with sugar or sorbitol as cryoprotectants, slows denaturation of actomyosin during storage. Sugars act as an anti freeze agent, preventing formation of large crystals, while phosphates are thought to prevent denaturation of actomyosin by binding to active sites of proteins, preventing them from either irreversibly binding to each other or denaturing (unwinding). Upon thawing, water may again be held by charged sites on the still soluble proteins. Krala and Dziomdziora (2000) have found that sorbitol alone decreased amount of frozen out water by 70% and showed broadest range of protective actions. In frozen stored beef heart surimi containing cryoprotectants (4% sorbitol, 4% sucrose) found increased gel water holding capacity.

5.2.3 Centrifugal drip

In the present study, same as free drip, amount of centrifugal drip / expressible moisture were more or less same for sucrose and sucrose : sorbitol combination whereas mince treated with sorbitol : sucrose : polyphosphate has least centrifugal drip during 4 months storage (Fig 5). The reasons are same as free drip. Chang and Regestein (1995) have also found similar results and concluded that cryoprotectants treated mince (sorbitol : sucrose 4% /4% w/w with sodium hexametaphophate 0.5% mince wt.) has better capacity to hold expressible moisture and water uptake than any other combination of cryoprotectants in frozen cod mince. Nousad *et al.* (2000) also reported decreased amount of expressible moisture in cryoprotectants treated frozen spent hen surimi.

5.2.4 Myofibrillar Protein

Surimi (washed minced meat with cryoprotectants) produces an elastic and chewy texture that resembles texture of shell fish due to its high concentration of myofibrillar protein. During frozen storage, alteration in myofibrillar protein have been largely accepted as principal cause of loss of protein functional properties (Shenouda, 1980). Cryoprotectants prevent muscle protein, particularly actomyosin, from denaturation during frozen storage (Matsumoto 1980).

Lower level of myofibrillar protein and sarcoplasmic protein in untreated minced meat and minced with sucrose, sorbitol alone can be seen in present study in Fig 6 and Fig 7. Whereas combination of sucrose : sorbitol and sorbitol : sucrose : polyphosphate show higher values of myofibrillar protein and sarcoplasmic protein on 4 months of frozen storage. Loss of sarcoplasmic protein is due to washing operation. Fig 6 shows remarkable decrease in amount of myofibrillar protein during initial 30 days of storage in untreated mince and in mince treated with sucrose and sorbitol alone whereas changes are not remarkable in the case of sorbitol : sucrose treated mince meat. Sych *et al.* (1990) found similar results in cod surimi salt extractable protein (SEP) frozen at –20°C over 12 weeks. Results showed that surimi treated with 8% sorbitol / sucrose SEP remained relatively stable throughout frozen storage. The cryoprotective effects of sugar have been explained by several researchers (Buttkus 1970, Arakawa, 1982).

Denaturation of actomyosin during frozen storage is a result of aggregation which can be caused by the progressive increase in intermolecular cross linking of myosin (Connell, 1959, 1961; Buttkus, 1970; Jiang and Lee,1985). Hydrogen bonds, ionic bonds, hydrophobic bonds and disulphide bonds are believed to be involved in formation of intermolecular cross linkages (Matsumoto, 1980; Jiang *et al.*, 1988 a,b). Denaturation of actomyocin during frozen storage can be prevented by cryoprotectants which increase surface tension of water (Arakawa and Timasheff, 1982) as well as the amount of bound water (Matsumoto, 1980). This prevents ice crystal growth and migration of water molecules from protein, thus stabilizing the protein in its native form during frozen storage (Buttkus, 1970; Matsumoto, 1980) and by polyphosphates which also have been shown to induce stabilization of myosin (Park and Lanier, 1987; Naguchi and Matsumoto, 1971). Jae *et al.* (1998) also obtained similar results for freeze induced denaturation of Pollock surimi affected by addition of sugar and/or polyol, including a starch hydrolysate product and /or phosphate during eight months storage .

Reckasi *et al.* (1992) also found similar results for inhibition of protein denaturation in frozen pork ham meat treated with sucrose/sorbitol.

Sych *et al.* (1990) reported best effects of sucrose and sorbitol 1:1 mixture @ 8% w/w against induced denaturation of cod surimi.

5.2.5 Sensory evaluation

The parameters used for orgenoleptic tests were whiteness and muddy odour of the raw mince meat and for cooked meat whiteness, muddy taste, sweet taste and texture. A brief score sheet was given to the panelists before the test regarding the parameters. The panelists were asked to judge each parameter according to their liking as would be done by consumers for mince based extruded products .

5.2.5.1 Whiteness

In the present study results of changes in whiteness on 0 and 120th day for raw meat and cooked meat as depicted in the Fig. 8 and Fig 9 shows decrease in whiteness of the raw mince meat treated with sucrose and also the minced meat samples treated with other cryoprotectant combinations with sucrose. Sucrose causes a brown colour change due to maillard reaction during frozen storage whereas sorbitol did not cause any discoloration. Thus in all the samples treated with sucrose whiteness was decreased on 4 months frozen storage. Grant *et al.* (1991) have also reported similar results for bright white Kamaboko product treated with sorbitol.

Simpson *et al.* (1995) found that there was slight decrease in whiteness of surimi made from stabilized mince of pacific whiting *(Merlussius productus)* mixed with the varying level of sucrose (6-12%) including 0.2 % w/w polyphosphate.

5.2.5.2 Muddy odour

In the present study remarkable change in muddy odour of raw meat was seen during initial 30 days frozen storage in mince treated with combination of sucrose:sorbitol and sorbitol:sucrose : polyphosphate (Fig. 10). Mince frozen stored without cryoprotectants or treated with sucrose showed an increase in muddy odour during initial 15 days. After 120 days of storage mince treated with sucrose:sorbitol was having maximum muddy odour. According to Friedman Test there was no significant difference between the other 4 treatments of cryoprotectants.

5.2.5.3. Muddy taste

Regarding changes in muddy taste of cooked meat variations are found among all the treatments of cryoprotectants during 4 months storage. Fig . 11 shows that changes in muddy taste of mince treated with Sucrose: sorbitol and sorbitol:sucrose:polyphosphate are similar during 4 months study whereas mince without cryoprotectants showed fluctuations during study period of 4 months .

5.2.5.4 Sweet taste

In present study sweetness scores of cooked mince meat shows changes during 4 months frozen storage. As control does not contain any carbohydrate as cryoprotectant it does not show any sweet taste during study. Friedman test shows significant difference between the control and treatments of sucrose:sorbitol:polyphosphate and sucrose:sorbitol. Mince treated with sucrose or sorbitol alone was having moderate sweetness whereas combination of sorbitol: sucrose and sorbitol:sucrose:polyphosphate showed lower level of sweetness which decreased after 120 days of storage.

Higher level of sucrose (8% w/w) made surimi very sweet. Hence to reduce level of sucrose, sorbitol was used as it was not as sweet as sucrose. Grant *et al.* (1991) reported that these cryoprotectants impart considerable sweet taste to surimi which many of western consumers find objectionable for certain product application. There has been some effort in United States to replace sucrose with sorbitol or polydextrose.

5.2.5.5 Texture

Changes in texture of cooked mince meat have been shown in Fig. 13 and Fig 14. Friedman test presents significant difference in texture of mince without cryoprotectants and in treated with different types of cryoprotectants. Fig 14 shows significant change in texture of mince meat treated with sorbitol:sucrose:polyphosphate which was having sticky texture after 4 months of storage. This may be due to use of 1 % level of polyphosphate whereas recommended dose in literature is only 0.2-0.5 %. Mince without cryoprotectants has shown tough texture on 4 months of storage. Gormley *et al.* (1993) found

similar results in silver melt (*Argentinus silus*) fillets frozen without cryoprotectants at –28^{0}C for 150 days.

5.2.6 Folding test

Grant *et al.* (1991) reported that bright white Kamaboko are typically enjoyed by Japanese. Results of folding test shown in Fig 15 represents that Kamaboko prepared with minced meat treated with sorbitol:sucrose:polyphosphate has quality grade AA throughout study of 4 months whereas in case of Kamaboko prepared from mince meat as control quality grade changes from AA to A after 15 days and to B grade after 60 days frozen storage. Kamaboko prepared from mince treated with sucrose:sorbitol have shown quality grade AA up to 90 days and afterwards it changes to quality grade A. Kamaboko treated with different type and level of cryoprotectants has either AA or A after 4 months storage. Gomes *et al.* (1995) found that surimi having different cryoprotectants was graded AA, group 5, indicating excellent elasticity resistance.

According to present study cryoprotectant combination of sorbitol:sucrose:polyphosphate (8%w/w,3:4:1) have shown greater functional stability of the minced meat stored at -20^{0}C for 4 months. Combination of sucrose: sorbitol have also shown good results for many of parameters used for study.

Henry *et al.* (1995) concluded that cryoprotectants improve drip loss, expressible moisture, texture and color compared to untreated reference sample . Suvanich *et al.* (2000) reported that frozen mince with cryoprotectants would remain acceptable for greater than or equal to 3 months at –20° C.

Montero *et al.* (1999) concluded that washed mince meat with added cryoprotectants, contributed to greater functional stability at least for first 3 months of storage, at which stage gels were some what softer and more elastic than with unwashed mince. Sultanbawa and Li Chan Ecy (1998) reported that blend giving the best economy and lowest calorie content in surimi was that containing 1 % of each cryoprotectants. Kijowski and Richardson (1996) concluded that combined presence of sorbitol:sucrose :polyphosphate restored most of the functional properties of the frozen or freeze-dried material to that of the fresh material.

Summary

VI. SUMMARY

1. The main objective of the study was to develop a suitable combination of cryoprotectants for preventing quality changes in minced meat of tilapia, during frozen storage.

2. Tilapia *(Oreochromis mossambicus* Peters*)* in fresh condition was transported from fish market to the laboratory in insulated box under iced condition.

3. Minced meat collected with the help of meat- bone separator, after washing, was divided into five lots and afterwards it was mixed with different types and levels of cryoprotectants viz., sucrose (8% w/w), sorbitol (8%w/w), sucrose: sorbitol (8%w/w, 1:1 mixture), and sorbitol: sucrose: polyphosphate (8%w/w, 3:4:1). A reference sample as control (without cryoprotectants) was also maintained.

4. Minced meat treated with different cryoprotectants was wrapped in 150 gauge polythene sheets and then was packed in waxed cartons. These waxed cartons were then frozen in deep freezer at -40^0C followed by storage in cold storage at -20^0 C for 4 months.

5. A Japanese paste product called Kamaboko was also prepared to study the effect of cryoprotectants on quality of minced meat based product.

6. For evaluating quality changes, physical and biochemical tests, folding test and sensory evaluation were carried out on samples drawn each fortnight.

7. Quality changes during storage period were monitored based on various tests, viz., cooking yield, free drip, centrifugal drip, myofibrillar protein content, sarcoplasmic protein content, folding test and sensory evaluation based on whiteness, muddy taste, sweet taste and texture of cooked meat.

8. The values of all the quality parameters generally showed a progressive change during storage in case of all the treatments of the cryoprotectants.

Minced meat treated with sorbitol: sucrose: polyphosphate and sucrose: sorbitol showed comparatively lowest rate of deteriorative changes while control (without cryoprotectants) sample showed highest rate.

9. Minced meat treated with sorbitol: sucrose: polyphosphate and sucrose: sorbitol remained acceptable for the entire storage period of four months and no significant difference in the sensory evaluation was obtained between the two treatments. However minced meat treated with sucrose and sorbitol alone and control was found to be acceptable only for 45 days storage.

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VII. REFERENCES

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EFFECT OF CRYOPROTECTANTS DURING FROZEN STORAGE ON QUALITY OF LEACHED MINCED MEAT FROM TILAPIA *OREOCHROMIS MOSSAMBICUS* (PETERS)

By

RITU AGRAWAL, B. F. Sc.

ABSTRACT OF THE THESIS

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ABSTRACT

A study was conducted to develop the combination of cryoprotectants for preventing quality changes in minced meat of tilapia *(Oreochromis mossambicus* Peters*)*, during frozen storage. A Japanese paste product called Kamaboko was also prepared to study the effect of cryoprotectants on quality of minced meat based product.

The cryoprotectants used for study were sucrose (8% w/w), sorbitol (8%w/w), sucrose: sorbitol (8%w/w, 1:1 mixture) and sorbitol: sucrose: polyphosphate (8%w/w, 3:4:1). A reference sample as control (without cryoprotectants) was also maintained. Minced meat treated with different cryoprotectants was wrapped in 150 gauge polythene sheets and then was packed in waxed cartons. These waxed cartons were then frozen in deep freezer at -40^0 C followed by storage in cold storage at -20^0 C for 4 months.

Quality changes during storage were monitored at fortnightly interval based on various tests viz., cooking yield, free drip, centrifugal drip, myofibrillar protein content, Sarcoplasmic protein content, folding test and sensory evaluation based on whiteness, muddy taste, sweet taste and texture of cooked meat.

Minced meat treated with sorbitol: sucrose: polyphosphate and sucrose: sorbitol remained acceptable with no significant difference in sensory quality, for all four months of study. However minced meat treated with sucrose and sorbitol separately and control (without cryoprotectant) showed significant lowering in quality parameters during storage and were acceptable only for 45 days.

APPENDIX I.

Key To Score

Date: Name:

Samples of washed fish meat are given. Kindly evaluate them for the various quality characteristics using the appropriate scales given.

Scale:

Whiteness	Score	Muddy odour/ Muddy taste/ Sweet taste	Score	Texture	Score
Very White	5	Absent	5	Excellent	5
White	4	Slight	4	Good	4
Slightly off-white	3	Moderate	3	Average	3
Fairly off-white	2	High	2	Poor	2
Slightly dark	1	Very high	1	Bad	1

Score Sheet

	Quality Characteristics	Sample No	Score
Raw meat	Whiteness	a b c d e	
	Muddy odour	a b c d e	
Cooked meat	Whiteness	a b c d e	
	Muddy taste	a b c d e	
	Sweet taste	a b c d e	
	Texture	a b c d e	

Valued as Recommended by the Board of M.F.Sc degree

26/3/20

Dr K. Divakaran
Director
Cochin