DETERMINATION OF DECOMPOSITION RATES IN SELECTED MID-ATLANTIC FISH SPECIES STORED UNDER ICED AND SUPER-CHILLING TEMPERATURES.

BY

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DETERMINATION OF DECOMPOSITION RATES OF SELECTED MID-ATLANTIC FISH SPECIES STORED UNDER ICED AND SUPER-CHILLING TEMPERATURES

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(ABSTRACT)

Three different species of fish (sea trout, spanish mackerel and catfish) were studied. Samples undergoing normal spoilage were compared with samples which had undergone a sanitizing treatment with alcohol. Differential temperature storage studies were conducted at 29°F (-1.7°C) and 32°F (0°C). Fish quality was assessed by means of microbiological, chemical and sensory analyses. Quality assessment via measurement of proteolytic and lipolytic enzymes was attempted, but these enzyme activities were not detected in any of the samples. It was not possible to differentiate between the contributions of microbial and autolytic spoilage. Alcohol treated samples (reduced numbers of microorganisms) had shelf-lives extended by 6-10 days over untreated samples. The shelf-life of samples stored at 29°F was extended by 6-10 days over the shelf-life of samples stored at 32°F. Treated samples stored at 29°F received highest sensory scores and untreated samples stored at 32°F received the lowest scores.

It was seen that the three fish species studied had

different shelf-lives: sea trout-6 days, spanish mackerel-10 days and catfish-16 days. Decomposition rates differed significantly between species and this factor must be taken into account when marketing strategies are developed by firms engaged in fresh fish sales.

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I. INTRODUCTION

The demand for fresh fish has rapidly increased as consumers have become more aware of the nutritional benefits. Furthermore, potential markets are expanding inland as non-coastal consumers are introduced to fresh fish. In order to supply these markets, seafood processors are required to extend the shelf life of products traditionally confined to coastal areas (1). Decomposition rates differ from one species of fish to another, and if the demand in the inland non-coastal markets is to be met, this must be taken into consideration and marketing strategies accordingly planned for each individual species.

It is generally accepted that the main cause of spoilage of fish and fishery products are tissue enzymes and bacteria (95,94). Recent studies by Virginia Tech and the Virginia Institute of Marine Science (VIMS) on a mid-west fresh fish marketing project revealed that the shelf-life of fresh fish having similar microbiological profiles at different storage temperatures was dependent on fish species (1). The rate and extent of autolytic spoilage in fish is considerably less than bacterial spoilage, but autolysis plays an important role in characteristic flavor development and the onset of bacterial spoilage (73).

The main difficulty in attempting to differentiate between the changes resulting from tissue enzyme activtiy

and those due solely to bacterial action has been the technical one of obtaining sterile raw tissue. Numerous attempts to overcome this difficulty have been made (42) and a variety of techniques have been employed with varying degrees of success.

Seafood is a very perishable food commodity. It has been shown that reducing the storage temperature from 32°F (crushed ice) to 29°F results in substantially lower microbial counts and an extension in the shelf-life of the fish. However, fish marketing practices today are such that the fish are stored and transported by a processor at 29°F and subsequently merchandised by a retailer at 32°F (1).

It is recognized that the quality of fish is dependent on intrinsic (catheptic enzymes, lipases, lipoxygenases) as well as extrinsic (microbiological profiles) factors. Numerous trial fish studies have compared the effect of varying microflora type and quantity on acceptability and shelf-life. However, in none of these studies have fish with reduced microbial populations been evaluated for effects of storage at differential temperatures.

The purpose of the present study was three-fold. One was to differentiate between the contributions of microbial and autolytic spoilage in fish. Secondly, to examine the effect of storage temperature on the quality and shelf-life of three different species of fish, and thirdly to determine the decomposition rates of sea trout, spanish mackerel and

catfish.

II. LITERATURE REVIEW

1. BACTERIAL SPOILAGE

1.1 Introduction

The microbiological changes that occur during the handling and processing of fish and fishery products depend upon many factors. Also, the microflora of live fish is greatly influenced by the environment. The gross chemical composition of fish species can vary widely: non-fatty fish such as cod and haddock commonly have 20 % protein, 1 % fat, 1 % carbohydrate and 80 % water whereas; fatty fish such as herring or mackerel can have 15-20 % protein, 3-25 % fat, 1 % carbohydrate and 70 % water (43). These components also vary markedly, depending on the season and sexual maturity of the individual fish. Once fish is caught subsequent changes in the bacterial flora will depend upon the conditions imposed by the processing procedures and the flora introduced during these processing procedures. Changes in the bacterial flora and the degree to which these bacteria are able to grow will determine the spoilage pattern of a particular fish (43).

1.2 Bacteriology of Fresh Fish

Although the flesh of newly caught, healthy fish is

sterile, the skin, gills and, in fish which have recently been feeding, the intestines may carry considerable bacterial loads (96). Microbial populations of 10^2 to 10^7 per cm^2 (at 20^oC) of skin with adhering slime have been recorded and populations of 10^3 to 10^8 and 10^3 to 10^6 per milliliter of intestinal fluid and per gram of gill tissue respectively have also been observed (94). Both quantitatively and qualitatively the flora appears to be a function of the environment. Higher microbial loads are found on marine fish from tropical and subtropical areas than from colder regions. More important is the fact that psychrophiles are more abundant in fish from the colder areas than in tropical or subtropical areas (96).

Qualitatively the flora, particularly of the skin and gills, differs considerably from the varying areas. Fish from the northern areas are dominated by the large numbers of psychrophilic gram-negative genera (Pseudomonas, Alteromonas, Moraxella, Acinetobacter, Flavobacterium/Cytophaga and Vibrio) whereas in fish from warmer waters (Adriatic, S. Africa, Indian Ocean, Pacific and Australian) the more mesophilic gram-positive genera such as the Micrococcus, and Corynebacterium and Bacillus seem to predominate (96).

The intestinal flora differs quite considerably from that of the slime and gills. Quantitatively in ´feeding´fish the numbers are often much greater than in either gills or slime. Moreover, qualitatively, the flora is conditioned not

so much by the food or external environment as by the special ecological conditions, existing in the stomach and intestines, such as pH, anaerobiosis, and presence of bile salts. Accordingly, as can be expected there is a preponderance of facultative and/or strict anaerobes such as Aeromonas, Vibrio and Clostridium species (95), as compared with the slime.

As far as fresh water fish are concerned, data are too few to generalize. However, the bacterial loads on the skin, gills and intestines are somewhat lower than a marine fish and generally the gram-negative heterotrophs predominate. In the intestines, the Vibrio genus does not seem to be as predominant as in marine species (96).

Both species of fish and the method of catching may also affect the bacterial load. Thus in a study conducted over a continuous 27 month period of observation, it was seen that the population on the surfaces, and in particular on the gills, of sole was somewhat different from that of skate, caught in the same area at the same time. It seems very probable that the microflora environment on the gills and surfaces of these two species of fish are sufficiently different to account for these findings. With regard to method of catching, it has been shown that trawled fish usually carry loads 10 to 100 times heavier than lined fish. The increased infection in trawled fish is probably the result of dragging along the sea floor, where the mud is

known to contain immense numbers of bacteria and to the expression of the gut contents among the fish during the hoisting of the trawl net on board. Seasonal variations in the gut flora seem to be less evident than in either the gills or slime. The fast that normally occurs during spawing with many species should of course be reflected in the bacterial loads in the intestines (94).

1.3 Handling of Fish

Fish is one of the most perishable of all foods and needs proper care from the time it is caught until it is served or processed.

The flora of fish will be conditioned both qualitatively and quantitatively to some extent by the handling and storage conditions aboard the fishing vessels and by the prevalent conditions being discharged from the fishing vessel (94). The fishing industry is an extremely competitive one with limited financial resources and furthermore, traditional methods are difficult to eradicate. However, the improvement in the quality of the product if proper handling techniques are adopted is undeniable and changes are taking place gradually (47).

A. Species and Fishing Methods

Fish that are very active in their normal habitat, e.g., tuna and mackerel, may become excited and lie in a frenzied state when seized. Similarly, certain types of gear e.g., salmon gill nets, may kill the fish after an exhausting struggle. Such activity before death results in rapid development of rigor mortis followed by earlier signs of deterioraton during icing. On the other hand, many salmon are caught by surface hook and line, brought to the boat swiftly and dispatched quickly with a blow on the head. Halibut caught on a bottom hook and line usually come to the surface easily and are quickly dispatched. Such clean kills are significant in extending freshness and quality as is well known in slaughtering livestock. Modern aquaculture operators have found that use of refrigerated brine immersion or an electric shocker to stun or kill the fish immediately after harvest is important in quality control.

B. Physical Damage

The fishing gear and the handling of the fish when the gear is brought aboard often contribute to bruising or tearing the flesh. Transfer of fish in and out of the boat with gaff hooks, pughs or forks takes a toll in terms of unsightly and unsanitary holes in some fish. Quick bacterial

spoilage follows in these pugh marks. Rough weather on the trip back to port and excessive ice pressure in the bins accelerates the deterioration and increases the shrinkage of fish.

C. Dressing

Fish that are feeding actively at the time of catch show greater incidence of autolytic spoilage by digestive enzymes and need prompt dressing and icing. It is usually desirable, where possible, that all fish be dressed, i.e., gills and viscera removed, immediately after catching. The gut cavity should be washed with clean sea water before icing (20). Tests aboard an Atlantic trawler (59) showed that use of 50 ppm chlorine in the sea water was more effective than plain sea water in rinsing the blood and slime from the fish. In some fisheries, e.g., the Pacific trawl fishery, dressing is deemed impractical for the value and size of the fish (20).

D. Chilling Procedures

Lowering the temperature of the fish by a prompt and efficient and chilling procedure is fundamental for preservation of fish freshness. Proper use of crushed fresh water ice is the oldest and simplest method. The most significant rule in use of ice of any chilling procedure is

that eventual spoilage is only retarded, not stopped (9).

Ice, when properly used in adequate amounts, aids in preservation in two ways: 1) the temperature of the fish is lowered to approximately 32 to 36°F, which slows the bacterial and enzymatic changes and 2) the melting of the ice bathes the fish in clean cold water and, with proper storage, washes away considerable slime, blood and bacteria.

To ice the fish correctly in the boat hold, three things should be accomplished. First, the fish should be placed with sufficient ice around them to cool them promptly and to maintain their temperature as close to the melting point of ice (32°F) as is practical for the duration of the trip. Second, the ice and fish should be arranged to allow accumulated water, blood and slime to drain through the mass into the bilge. Third, the fish should not be subjected to great pressure from the weight of fish ice placed above. Otherwise, the physical damage as well as the shrinkage or loss of weight by the fish will be excessive.

E. Sanitation

Fish buyers know from experience that under comparable conditions the cleanest boats bring in the best quality fish. "Good housekeeping" aboard the vessel is associated with careful handling methods because the fisherman who keeps the fish hold, gear, and deck clean is likely to be quality

conscious when he ices his fish. Contamination from any source will affect fish quality. Dirt, slime, fuel oil, rust, grease, blood, scales and bits of viscera and flesh must be removed and washed away constantly in order to keep the deck and the hold clean. The problem of sanitation and housekeeping aboard a boat depends greatly upon its design and construction. The use of concrete mastic, or metal (corrosion-resistant types) to eliminate the hard-to-clean corners in the holds saves many hours of labor and cleaning during the busy season. Bilges should be cleaned frequently during the fishing season, using a good detergent or bilge cleaning compound to remove accumulated dirt, oil and slime. Pen boards should be scrubbed, sanitized and allowed to dry after each trip.

Although the cold storage plants prepare the ice from clean potable water, ice can be contaminated with dirt or bacteria during crushing and delivery at the dock and during handling aboard the vessel. Since the ice comes in intimate contact with the fish, old ice should be discarded at the end of the fishing trip and new ice should be kept clean. Shovels, utensils, gloves and the hold should be cleaned at frequent intervals and rinsed with chlorinated water or other effective sanitizing solutions.

F. Unloading

Unloading is the final operation for the fishermen who must transfer the fish from the iced bins by hand, shovel or pugh to the unloading box or net. The ice is removed and the fish are dumped on a table or a belt for grading. Buyers grade most fish species before washing since the slime provides a quick index to the quality. The fish are generally weighed in tared carts or boxes. During these unloading and transfer operations it is common to find that iced fish may increase several degrees in temperature (20).

G. Boxing at Sea

Boxing is one method used on vessels fishing the inshore and near waters. If done properly, boxing is usually the best way of stowing iced fish. The boxes in use on vessels vary considerably in size, design and material of construction.

Boxing and icing can be the best method of retaining the quality of white fish. This is because there is less handling during discharge, less crushing in stowage, and a lower temperature is maintained after unloading. Bulked fish may also lose weight during storage; cod boxed with plenty of ice and without crushing may gain 1-2 % in weight.

Oily fish such as herring, mackerel and sprats are so perishable that even an hour or two at summer temperatures may cause severe loss of quality; they are so easily damaged

that, at the bottom of a pile 0.5 m deep as is common in bulk white fish stowage, severe damage due to crushing can render them fit only for conversion to fish meal. Boxing reduces pressure damage and, when used with sufficient ice properly applied, is an excellent method of stowage.

Boxed fish are easier to handle on discharge than bulk or shelfed fish and centralized cleaning of standard boxes is easier to organize than that of pound boards. The following procedure should be used for icing fish in boxes. The bottom of the box should be covered with a layer of ice about 5 cm thick. A layer of fish is laid on the ice, and more ice is shovelled over to cover it lightly before the next layer is stowed and so on to a depth of not more than 50 cm. Finally another layer of ice at least 5 cm deep should be added to top off the box.

Although boxing is more labor intensive than chilled seawater stowage, it is also suitable for stowing small fish. Ideal boxing in a temperate climate requires the use of about 1 part of ice to 2 parts of fish by weight and careful distribution of ice throughout the fish. Nevertheless, even with a box specifically designed to hold the required quantity of fish and ice in the correct ratio, it is difficult to achieve this during periods of heavy fishing. Tests have proved, however, that a fish temperature approaching that of melting ice can be reached on short trips by covering the whole of the bottom of the box with a good

layer of ice before the box is filled with fish, and putting another layer on top of the fish.

Boxes should be of light alloy or plastics. They are easier to clean than wooden ones and, once cleaned, will not support growth of moulds. They do not become water-logged and are light and easy to handle. Light alloy and plastic boxes should be so designed that a stack of them is stable. Drainage should preferably be from the corners of the base, rather than from the center; if possible, a box should not drain into the box below. The box should have a smooth finish with no internal corners that are difficult to clean. Some types of open boxes have been made to nest when empty to save space. A box that both nests and drains outside the area of the box is ideal (50).

H. Filleting

A major portion of the fish are sold as fresh fillets. In large operations many of the washing and handling operations are mechanized. In small operations, the fish are washed and filleted by hand on a cutting board placed next to a sink (20). Much filleting is still done by hand and the filleting trough is still seen far too often. Filleting should be carried out as a line process on a properly constructed board either under a spray of ice-cold potable water or using frequent washings with the same quality water.

A trough of stagnant water containing fish heads, gut, slime, and scales and whole fish, waiting to be filleted, is not satisfactory. Immediately after filleting the fish should be placed in clean boxes in plenty of ice; it may be necessary to separate the fillets from the ice because of possible softening effects. If filleting machines are used, great care is necessary to ensure that they are thoroughly cleaned and sterilized at the end of each days work (47).

Mechanization of the fillet plant is becoming increasingly important because of the larger volume of the operation, the relatively low yield of the product (e.g. 25 % in many flat fish), and the stiff economic competition from other protein foods and imported fishery products. A typical filleting plant consists of a mechanical washer, which may also scale the fish, a manual fillet line or a filleting machine, a washer and/or briner, a draining table or belt, an inspection area, and a mechanized packaging line.

Whereas shipment within a few hundred miles provides no problem, the fish must be most carefully re-iced and boxed for refrigerated truck or air shipment when greater distances are involved. Large shipments of fresh dressed fish are made daily from the large ports of both the Atlantic and the Pacific to the large inland markets (20). Transportation of the fish away from the ports should be in properly designed, insulated or refrigerated trucks. The fish should be well iced and loaded in metal or plastic boxes. The trucks should

be enclosed and the interiors lined with hard, easily cleanable material. Sheet metal is probably the best. Once again, maintenance of a sufficiently low temperature is of great importance. At the inland markets the same precautions to prevent contamination, cross-infection and rise in temperature with consequent loss of quality must be taken (47).

I. Handling Effects

Handling procedures have an influence on the bacterial populations in fish. Rough handling of the fish which results in breaks in the skin barrier or crushing of tissue will facilitate primary penetration of the tissues by bacteria and will hasten spoilage. Dirty ice, reused ice, or ice stored for long periods in fish rooms will harbor large numbers of psychrophilic fish spoilage bacteria and, therefore the use of such ices can bring about a shortening of the lag-acceleration phase of bacterial growth again leading to more rapid spoilage. A similar effect will be obtained by exposure of fish to improperly cleaned boxes, pen boards, etc.

Of all the physical and chemical factors which affect bacterial growth, temperature is probably the most important. The psychrophilic bacteria which normally cause fish to spoil, grow many times faster at temperatures close to 70°F

than they do at temperatures close to 32°F (60). The effect on bacterial growth of raising the temperature a few degrees in the region of 32°F is out of all proportion to the effect of raising the temperature through the same number of degrees in the region of 70°F (13). Any laxity in temperature control (e.g. through use of insufficient ice or poor brine cooling) will result in accelerated bacterial growth and more rapid spoilage.

Filleted and steaked fish are particularly susceptible to the deleterious effects of bad handling since the naked flesh provides an excellent medium for bacterial growth. Filleting knives, tables and boards, filleter's gloves, static wash water, etc, rapidly accumulate large populations of fish bacteria if proper sanitary precautions are not observed (60).

1.4 Bacteriological Changes Post Mortem

A. Quantitative Changes

Except in the case of disease during the lifetime of the fish or shellfish there is no effective penetration of the tissues by the bacteria present in the body surfaces and in the intestines, and a balanced situation seems to exist whereby the numbers of these bacteria remain at a rather

stable level. On the death of the fish, the humoral defences against bacterial invasion cease to operate and the mechanical barriers such as skin and membranes gradually lose their impermeability. As a result of these and other changes post mortem, the balance between bacteria and host animal is upset, and qualitative and quantitative changes in these bacterial populations soon become evident.

Very little is known concerning the nature and extent of these changes when the dead fish remains in its natural environment, but considerable information is available on the changes which take place in dead fish stored on ice. Technologically, these changes in the bacterial flora are of enormous importance since, together with associated endogenous biochemical and perhaps physical changes in the fish tissue, they lead to the process of spoilage and ultimate decay and dissolution of the cadaver.

For a short period after death, corresponding rather closely to the onset, duration, and resolution of rigor mortis, there is little change in the numbers of bacteria present. This is succeeded by a period of gradually accelerating growth associated with organoleptic changes in the fish, typified by a loss of the characteristic fresh fish flavor. Next the bacterial population enters a phase of more or less exponential growth corresponding to the initial appearance of such well known spoilage indicator substances as trimethylamine and other related bases. This phase is of

short duration and is succeeded by a more or less stationary terminal growth period, during which there is little change in numbers among the surface bacterial populations. Despite the absence of quantitative bacterial change, this is the period of maximum spoilage activity terminating practically, when the fish is approaching putridity.

It is well established by experiment and observation that this classical growth sequence occurs in all fish and shellfish samples held under chilling conditions (i.e., greater than 32°F, less than 50°F) and applies equally to whole fish and to fillets or pieces.

B. Qualitative Changes

The qualitative alterations in the bacterial populations which accompany these quantitative changes are not well established. However, recent work has provided some information on this point. The predominant gram-negative rod floras of living fish are frequently altered by the handling procedures which precede stowage in ice so that gram-positive organisms such as Micrococcus and Corynebacterium attain a temporarily important quantitative position in the surface bacterial populations. This is also frequently true in the case of filleted products. However, during the apparent lag period and the phase of accelerating growth, qualitative changes in the flora re-establish the predominance of the

gram-negative rod forms so that by the time logarithmic growth is under way usually over 90 % of the total bacteria present are of this type (60).

Organisms of the Pseudomonas-Alteromonas and Moraxella-Acinetobacter groups increase in numbers more quickly than the other organisms, and in the later stages of spoilage comprise 80 % of the total flora. Putrefaction proceeds rapidly even at chilled temperatures once the bacterial load attains or exceeds 10^6 organisms per gram. The rate at which putrefaction proceeds depends upon the rate of growth of these organisms and this is largely governed by two factors: the temperature and the number of organisms present (43). There is also apparently a shift in balance among pseudomonad types themselves, typified by a relative occurence of typical marine forms and an increase in the proportions of types of Pseudomonas (P. fragi, P. putida, P. putrefaciens and similar species) commonly found on all spoiling "animal protein" foods held under refrigeration, i.e., meat, turkeys, etc. (60).

1.5 Penetration of Tissues

The degree of penetration of the surface bacteria into the tissue of the fish is not well established. Three possibilities exist: movement inwards from cut surfaces such as belly flaps and from gills - possibly mainly via the major

blood vessels, movement outwards from the gut or gut cavity, and movement inwards from the skin surface.

The small amount of experimental evidence available indicates that while all three possibilities may be realized to some extent, general penetration from the surface inwards is most significant. Some evidence strongly suggests that significant penetration through the belly wall may also take place. However, movement of bacteria into tissues is apparently a slow process and multiplication within tissues is not very rapid. Even when the fish is clearly spoiling, only small numbers of bacteria can be found in the deep muscle. Consequently, the most widely accepted view is that the most primary activity of the spoilage bacteria occurs in the surface layers of the fish, and the total effect is largely due to secondary diffusion of bacterial enzymes and products into the deep tissues.

Penetration of bacteria into cut fish and fillets is certainly more rapid than in the case of the whole fish but is still sufficiently slow to justify the application of the above theory to these products also. It is possible that the reason for the primary surface action of the spoilage flora is its obligate aerobic nature (60).

1.6 Bacterial Metabolites

On a quantitative basis, the major chemical changes in

fish resulting from microbial spoilage are the production of nitrogenous bases, particularly trimethylamine (TMA) and ammonia, and to a lesser extent of volatile fatty acids. The TMA is principally derived from the reduction of trimethylamine oxide, which is present in nearly all marine species and absent in fresh water species. Experimental evidence suggests that the ammonia and volatile fatty acids result from the oxidative deamination of non-protein nitrogenous compounds (NPN) of fish muscle tissues. However, these substances alone do not account for the total taste and odor characteristics of spoiling fish, and in recent years there have been extended analysis by Gas Liquid Chromatography (GLC) and other methods, of volatile products from spoiling fish muscle, which might be implicated as a source of spoilage odors, even though they are present in small amounts (109). Much attention is currently being focussed on the sulfur-containing compounds, since these are reported to be present in amounts well above odor threshold levels (8).

1.7 Effect of Storage Temperature

The most important single factor controlling spoilage of fresh fish is storage temperature. Temperature regulates the onset of rigor mortis and also the lag period and growth rate of spoilage microorgansisms. The types of bacteria that

eventually induce spoilage in iced mid-Atlantic (temperate) fish are termed psychrophilic or psychrotrophic which signifies a preference or tolerance for low temperatures. These organisms constitute the natural microflora of newly caught fish and may also be picked up during subsequent handling. These bacteria are capable of growing at temperatures slightly below freezing but grow most rapidly in the temperature range of 68° to 77°F (20 to 25°C). As the temperature is lowered and approaches 32°F (0°C), the growth rate of fish spoilage bacteria is drastically retarded. A reduction in storage temperature of 3° at just above freezing adds proportionately to the keeping time of fish than a similar reduction at a higher temperature (36). In previously conducted shelf-life studies with bluefish, it has been found that storage at 29°F resulted in an increase of approximately 5 days over the 33°F stored fish (1).

1.8 Differing Rates of Spoilage of Various Fish Species

If it be accepted that the spoilage of fish stored at chill temperatures or in ice occurs at or in the surface slime, and the organisms therein are dependent on environment, then it would be logical to conclude that the spoilage of any fish caught in the same environment and stored under identical conditions would spoil at the same rates. However, various species of fish, even those

belonging to the same family such as the gadoids (whiting, cod, coalfish), spoil at different rates. It has, of course, been well known to the practical fisherman for decades that whiting spoil very rapidly, much more so than codling of the same size, and considerably quicker than plaice and other flatfish. These different spoilage rates can be expressed by organoleptic assessment and by chemical or physical measurements; both show that the spoilage rates of different species of fish differ remarkably (Table 1). When such studies are extended to tropical species stored in ice, even more remarkable periods of keeping fresh have been claimed. Table 1, for example, shows that Indian bream is considered still edible after storage for up to 40 days in ice and Nile perch and Mrigal carp (both freshwater species) for up to 29 days, whereas U. S. freshwater perch (northern temperate waters) remain edible for about 17 days and trout 9-11 days. The most obvious explanation is that in such species the psychrophilic bacteria are absent as members of the initial floras of the fish and are only gradually introduced with the ice.

It is probable that the defense mechanism, more powerful in some species of fish than in others, might still operate for some time after death. Certain histological examination of the integuments of various species of fish show that there are considerable differences in their structures and their physical properties. Plaice, which as already mentioned

keeps well, besides having a copious covering of mucus, has a robust well-defined epidermis and dermis, whereas whiting, a quick spoiler, has an extremely poor dermis with a very fragile and easily removable epidermis. Mechanical damage to whiting during handling on board ship or on shore is therefore very great resulting in rapid spoilage. Blue whiting, a recently exploited species, has similarly a poor integument and also keeps poorly.

There may well be other considerations to explain the differing keeping qualities of the various fish species. Plaice has been shown to possess a powerful lysozyme whereas cod has none. Lysozyme, effective against some gram-positive bacteria, requires the help of chelating agents and surfactants to lyse gram-negative organisms in vitro. It is possible that cellular components in plaice slime may provide the necessary cofactors to the enzyme to broaden its activity against microbial invasion. Certain antibacterial properties have also been ascribed to some fish slimes (97).

Recent studies by Virginia Tech and the Virginia Institute of Marine Science (VIMS) on a mid-west fresh fish marketing project revealed an interesting fact. The shelf-life of fresh fish having similar microbiological profiles at different storage temperatures was dependent on fish species. Bluefish and Atlantic mackerel caught in the same net and handled under identical conditions on board a fishing vessel by Virginia Tech and VIMS personnel had a shelf-life of 9 and

13 days respectively when stored at 33°F. Cod, flounder, and ocean perch caught at the same place and at the same time and bulk packed together had different spoilage rates among the three species when stored at both 29 and 33°F. Cod had a 16 day shelf-life while ocean perch lasted only 10 days at 33°F. A similar relationship was observed at 29°F storage except the shelf-life was extended. The difference in shelf-life has been observed from other published reports even when the same fish genus was utilized. It has also been discovered that bluefish appears to have a 9 day maximum shelf-life irrespective of handling conditions while other fish species can achieve a maximum 17 day shelf-life. The presence of dark muscle in bluefish is not the problem since other red muscle containing fish can have a significantly longer shelf-life if handled under identical conditions. This data indicates that varying autolytic spoilage between fish species may be present even though the microbial flora is similar (1).

2. AUTOLYTIC SPOILAGE

2.1 Introduction

Autolysis is defined as the degradation of muscle and skin constituents by endogenous enzymes. The rate and extent of autolytic spoilage in fish is considerably less than

bacterial spoilage, but autolysis plays an important role in flavor development and the onset of bacterial spoilage. Absolutely fresh and healthy fish is impermeable to bacteria due to the intact skin. Further, the absence of simple and easily available nutrients in absolutely fresh fish makes it difficult for bacteria to grow and multiply. However, after the death of the fish, autolysis sets in, making the fish skin permeable to bacteria and the same time releasing simple sugars, free amino acids, free fatty acids, etc., which provide a nutrient rich medium for bacteria to thrive.

Live fish contain numerous enzyme systems required for the complex metabolic reactions taking place. These enzymes are distributed both in the intracellular and extracellular compartments throughout the fish muscle, but their individual concentrations vary with the nature and functions of the tissue. In live fish, all these enzyme systems are put to use only as and when they are actully required. Consequently, most of the enzymes occur as some sort of inactive precursors, while in certain other cases the enzymes are kept isolated from their substrates. Some enzymes are present in the active form in tissues, but their concentration will be very low and their distribution will be limited to specialized tissues.

Once the fish is dead, the ability of its body to regulate the enzyme will be lost. The absence of blood circulation, and hence the absence of oxygen and nutrient

supply to the tissue, depletion of energy sources such as ATP, and the breakdown of the body's scavenging system will bring an end to all anabolic or biosynthetic processes. In effect, in post mortem fish muscle, only the catabolic or the degenerative and degrading reactions are active, leading to the accumulation of catabolic products (73).

2.2 Methods Used in the Study of Autolysis

1) Utilization of the lag phase of the spoilage microflora at the skin surface:

The flesh of healthy live fish is sterile. After death and evisceration, some time elapses before the invasion of the muscle by the spoilage microflora, this lag depending on the experimental conditions. Advantages in the technique are that the muscle is not stimulated by dissection and that there is no intimate mixing of enzymes and substrates not in contact under normal commercial conditions of practice. The flesh is, however, subject to losses of substrate and ionic environment by the leaching action of ice-melt water.

2) Preservation of muscle minces, homogenates or extracts by toluene:

This was at one time, a popular technique. Preparations were incubated frequently at a pH far removed from the post-rigor level. As protein breakdown was the usual criterion of autolysis, this led to serious inaccuracies. Substrates and

enzymes which are physically separate in intact muscle are brought together in these procedures.

3) Preparation of minces under aseptic conditions:

In this procedure the skin of the fish is flamed before putting the flesh through a sterile meat chopper. Such procedures are much preferred to the previously described ones, but they are still open to the objections inherent in experiments with minces.

4) Supression of bacteria in intact muscles by ionizing radiation:

This procedure has been used for various studies on autolysis. Evidence has been accumulating in other fields of radiation sterilization, that some enzymes are less stable under sterilizing conditions than was generally accepted in the past.

5) The dissection of sterile blocks of muscle and their maintenance under aseptic conditions:

Probably, this is the best of the procedures available at the present time. The development of a satisfactory technique, though difficult, is essential. Provided that the temperature of the operation is kept sufficiently low to avoid denaturation of the enzymes, the only objection to the technique is the possible stimulation of the muscle during dissection (46).

6) Suppression of bacteria by using ethylene oxide:

Ethylene oxide appears to be the most promising for

widespread use in the sterilization of many materials that are so sensitive to dry or moist heat as to preclude the use of these agents in their sterilization. Moreover, for many applications, sterilization with ethylene oxide has been found more convenient and economical even though heat or some other agent might well be employed (69).

7) The examination of enzyme systems isolated from muscle:

While bearing in mind the biochemical commonplace that the reactions of an isolated enzyme do not necessarily reflect its role in the multi-enzyme system of intact muscle, it is this approach which will lead eventually to a full understanding of autolysis in fish muscle (46).

2.3 Products of Autolysis

It is accepted generally that the flavor of foods is derived from the presence of compounds which are usually of low molecular weight and soluble in either the lipoprotein complexes of the nasal epithelium or in the water. Autolytic changes produce a breakdown of complex structures to simpler, usually water-soluble compounds, which form part of the so-called `extractive´ fraction of the fish. Imidazoles, which are much affected by autolytic action, are important flavoring agents (2,44). Flavor has been related also to the free amino acids which are the final products of proteolysis (76).

Since the earliest studies on fish muscle autolysis, there have been suggestions that the products of autolytic action would provide a readily assimilable source of nutrients for the growth of spoilage microorganisms. Since the introduction of chromatographic techniques, a closer investigation of these ideas has been made possible (46). Comparison of the free amino acid compositions of iced herring and haddock, treated with antibiotics or untreated (21), supports the view that bacteria control the concentration of some amino acids in the flesh by the assimilation of the products of autolysis rather than their production (46). There are indications in the literature that certain autolytic actions within the flesh modify bacterial growth or metabolism. The post-rigor pH, which depends primarily on the course of glycolysis, appears to be particularly important (101,51). The concentration of sugars, which is dependent on the activities of muscle enzymes initially, may affect the course of spoilage in so far as their presence suppresses the formation of volatile basic nitrogen and amino nitrogen in tissues.

Most evidence suggests that the white muscle of fish does not normally suffer significant spoilage through the actions of its enzymes - although these do affect subsequent bacterial attack. In the spoilage of red muscle in fish the common attributes of spoilage - proteolysis and the production of volatile bases and other amines - can and do

take place independently of bacterial attack on the flesh. Under commercial conditions, bacteria would, of course make an eventual contribution to putrefaction (46).

2.4 Enzymatic (Proteolysis and Lipolysis) Analyses

It is well known that the softening rate of fish muscle after death is different among fish species. It is reported that the variation rate of physical properties of fish meat during processing is different among species. At least two factors may concern such a variation rate of fish meat protein: the stability of meat tissue or protein and the activity and/or the quantity of muscle proteinase. In fish muscle there exist various proteases. Among these, proteinase, is responsible for the variation of the physical properties of meat protein (66). The term protease is understood to apply to an enzyme having endo-peptidolytic activity (i.e. one which cleaves peptide bonds internal to a polypeptide protein chain). A peptidase is an enzyme with exo-peptidolytic activity, cleaving peptide bonds either at the N-terminal end of the polypeptide (aminopeptidase) or at the C-terminal end (carboxypeptidase). Both endo- and exopeptidases are involved in the total proteolysis of fish muscle (105). In post mortem fish muscle the lowering of pH due to glycolysis/glycogenolysis and lipolysis will upset the regulation of proteolytic enzymes. The increased acidic

environment of lysosomes causes rupture of the membrane, resulting in the release of cathepsins, proteases and other hydrolases. Drastic changes in tissue temperature, such as incorrect chilling and freezing make the condition worse as such treatments break open almost all the lysosomal packets releasing the entire stock of cathepsins and other hydrolases into the tissue (73).

Another set of enzymes that are active post mortem are lipases including phospholipases. These enzymes are widely distributed in almost all fishes, especially the fatty fishes and the ones with red meat. The primary products of lipolysis are free fatty acids and glycerol. Further degradation of fatty acids via beta-oxidation does not occur in sterile tissue due to the absence of respiratory oxygen. Hence, in dead fish the products of lipolysis also accumulate at the site of formation. The lower free fatty acids formed as a result of lipolysis will also impart off-flavor to fish muscle and is known as hydrolytic rancidity. The higher unsaturated fatty acids released will easily become vulnerable to oxidation by atmospheric oxygen, resulting in oxidative rancidity (73).

In reviewing the literature on proteolytic and lipolytic activity it was seen that most of the research in which enzyme activity was reported was carried out on frozen fish stored over long periods of time. Lipid hydrolysis in the flesh of fish stored on ice has been reported to exhibit a

lag period at the initial stages, characterized by very low rates of hydrolysis (37). This lag period has been shown to extend for 10 days to 2 weeks depending on the species (77, 64,78). In a study conducted by Lovern et al., (65) it was seen that in the flesh of certain species of fish, autolytic hydrolysis of some lipids may be totally inhibited. Examples are the hydrolysis of triglycerides in herring and of phospholipids in dogfish. In other cases an initially slow rate of hydrolysis of phospholipids may be followed by a faster rate. Such initial partial inhibition is exhibited, for example, in cod flesh maintained at 0°C. This initial "lag" may be abolished by freezing at either a very fast or a very slow rate, followed by thawing. No comparable lag has been observed in fish maintained in the frozen state at any temperature, regardless of freezing rate. One hypothesis set forth to explain the delay in the start of lipolysis in fish stored on ice required cell damage before the enzyme was able to act since, according the the authors, the enzyme was in some way kept apart from the substrate in the intact cell (65). Results obtained by other workers were in line with this hypothesis (37). Proteolytic activity in fish during frozen storage has been reported (105,98). Other workers have reported proteolytic activity in fish during iced storage and frozen storage (66), in surf clam viscera (16) and in surimi from Pacific whiting (15). In the present study, however, neither proteolytic nor lipolytic activity

was detected in any of the fish samples. One can only conclude that the enzymes were not present at detectable levels during the 16 day storage period used in this study.

3. TECHNIQUES TO OBTAIN STERILE FISH TISSUE

3.1 Introduction

It is generally accepted that the main cause of spoilage of fish and fishery products are tissue enzymes and certain groups of gram-negative bacteria, in particular members of the genera Pseudomonas and Achromobacter (95,94).

The main difficulty in attempting to differentiate between the changes resulting from tissue enzyme activity and those due solely to bacterial action has been the technical one of obtaining sterile raw tissue. Numerous attempts to overcome this difficulty have been made (42). One of the earliest techniques employed muscle minces and homogenates with or without added toluene to prevent bacterial growth (90,38,4). The method however is open to severe criticism - substrates and enzymes, normally separate in the intact cell, are brought together; protein denaturation occurs at the interface between the aqueous and organic phase; toluene does not completely supress the bacterial spoilage flora (81).

The use of ionizing radiation (75,86) and antibiotics (21) to supress or eliminate the bacterial flora have had

only a moderate degree of success. Seafoods have been sterilized by irradiation but doses required to sterilize fish are so high that significant chemical changes have resulted. Hobbs and Hodkiss (43) reported that the flesh had sufficient unpleasant odors and flavors to mask any changes resulting from storage. Pasteurization by irradiation results in some shelf-life extension, but the products undergo a progressive loss in quality even in the absence of significant bacterial spoilage. Others have used sterile muscle press juice which in many respects is further removed from the natural environment than muscle homogenates (34).

3.2 Utilization of Alcohol as a Sterilizing Agent

The only satisfactory technique is to dissect sterile muscle sections from newly killed fish under aseptic conditions and to maintain them under such conditions for the duration of the experiment. Such a technique ensures the cellular integrity of the tissue, the minimum degree of protein denaturation, and pH values and buffering capacities similar to those in natural fish muscle. It is a difficult technique and if conditions are not carefully controlled, can yield a low percentage of sterile blocks. As a result, Castell and his co-workers (13,10,11,12) finally had to use cooked mince fish muscle for large-scale work and they maintained it gave results comparable to those with sterile

raw muscle tissue. Lobben and Lee (62) obtained sterile fish flesh by washing the whole fish in running water and thoroughly swabbing with 95 % ethyl alcohol. After removal of the skin, the sterile flesh was excised under a UV lamp. The flesh samples from several fish were then pooled and then ground in a sterile meat grinder. Partmann (82) excised sterile blocks of muscle from newly killed fresh water fish after descaling, immersion for 5 min in 70 % alcohol and irradiation under UV for 15 min (42).

At Torry a technique has been perfected which gives a high proportion (> 80 %) of sterile muscle blocks which can then be used for storage experiments at various temperatures with or without inoculation with bacteria. In this technique aquarium kept cod were killed and immediately washed in a 5 % sodium carbonate solution to remove the outer slime which contains most of the surface and adhering bacteria. The skin surface was then washed in 2 % formalin solution. The skin was removed under aseptic conditions and the underlying tissue (42), which is sterile in newly killed, normal, healthy fish (94), was excised in blocks about 80 x 80 x 15 mm and placed in sterile petri dishes. The blocks were then stored at chill temperatures (usually 2°C) until required, generally within a week or so, by which time any non-sterile blocks would be evident. The sterile blocks could then be used for further storage experiments either as sterile blocks or inoculated with pure cultures of bacteria (42).

In other studies conducted by Fletcher and Statham (34,35), and Fletcher and Hodgson (33), sterile fish samples were prepared in the following way. The fish were held in tanks in the laboratory and then killed by a blow on the head. They were then immediately immersed in a sodium carbonate solution (5 % w/w) and gently scrubbed to remove most of the surface slime (42). After rinsing in tap water, the surface was sterilized by immersing for at least 5 min in 70 % ethanol containing crystal violet (28). The fish surface was then dried with sterile air in a laminar flow cabinet. Sterile flesh samples were obtained as follows: on one side of each fish a cut around the edge of the skin was made with sterile forceps. Two to four sections of flesh (1-6 g/section) were then excised from each fish, avoiding the gut cavity. The flesh excised from each fish was placed in a sterile glass jar (100 ml) and sealed. The jars were chilled in ice then placed at 4 +/- 0.5^{o}C (34). In this experiment with mullet, of 43 jars of flesh, only 19 proved to be sterile prior to sensory assessment, a far lower proportion than that obtained by the Torry laboratories or in other experiments using the same technique on white fish (34). In a study on snapper by Fletcher and Hodgson (33) in which this same technique was used to obtain sterile fish, 93 % of the nominally sterile flesh packages were found to be sterile in practice. This may be due either to mullet being a smaller fish, and hence more difficult to sample successfully or may

indicate that the flesh of some species is not as uniformly sterile as that of others (34).

3.3 Utilization of Ethylene Oxide as a Sterilizing Agent

The use of vapor phase sterilants dates back to the late 1800's when such gases as formaldehyde and sulfur dioxide were used to fumigate sick rooms. This practice was discontinued due to an apparent lack of effectiveness and not until recently has renewed interest occurred in the use of vapor phase sterilization. This interest has risen primarily from the needs of the food, agricultural, medical and space industries for methods of sterilizing items that cannot be subjected to excessive heat, liquid chemical sterilization or radiation. Since sterilization with chemicals in the vapor phase may be carried out at relatively low temperatures and moisture levels and because most sterilants can diffuse through plastic, paper or fabric, and are easily removed, the use of this method offers advantages not afforded by other means of sterilization (5).

Therefore, for many years bactericidal gases have been used by both the food and medical industry to achieve sterilization without a comitant loss in another biological activity. It has been well established that certain chemicals in the vapor phase are effective microbicides. Of the chemicals thus far tested, ethylene oxide appears to be

the most promising for widespread use in the sterilization of many materials that are so sensitive to dry or moist heat as to preclude the use of these agents in their sterilization (69). The chief disadvantage of flammability is overcome by dilution with inert gases such as carbon dioxide, nitrogen and chlorofluorohydrocarbons (5). Moreover for many applications, sterilization with ethylene oxide has been found more convenient and economical, even though heat or some other agent might well be employed (69). During the past 50 years, ethylene oxide vapor phase sterilization has been studied by many investigators (5,40,48,49,83,84,85,79, 29,39,22,61,54,93,106,52).

It has been established that death kinetics of microorganisms subjected to ethylene oxide are influenced by a number of factors, principally by temperature, ethylene oxide concentration in the sterilant atmosphere, relative humidity of the sterilant atmosphere, water activity of the microorganisms to be killed, level of contamination, microbial species to be eliminated, and physical and chemical nature of the material to be sterilized (69). The death of *Clostridium botulinum* when exposed to gaseous ethylene oxide followed first order kinetics (108). Vegetative cells of *Salmonella senftenberg* and *Escherichia coli* also followed first order kinetics when exposed to ethylene oxide (69).

The toxicity and mutagenic potential of ethylene oxide are well recognized (32); however, because of its volatile

nature, it has generally been assumed that ethylene oxide was safe, not leaving a residue (92). It has been found, however, that under conditions for effective fumigation, ethylene oxide reacted with moisture and choride ions to form ethylene chlorohydrin, a non-volatile (bp 129°C) toxic substance found in foods exposed to ethylene oxide (107,87). Another study was initiated to determine the potential hazard of ethylene chlorohydrin (ECH) as an indirect food additive and was investigated in 90 days studies in rats, dogs and monkeys. No adverse effects were noted in rats fed dose levels of 30 and 45 mg ethylene chlorohydrin/kg body weight/day (80). A long term study with rats fed on ECH, and on the growth of three generations, revealed no difference in body weights or histological changes in sections of liver and kidney. Also fertility did not appear to be affected by the diets (67). Other workers examined four ethylene oxide produced esters for metabolism of mammalia enzyme systems. Based on expected levels, it is unlikely that significant levels would remain intact long after digestion (100).

Originally in this study the fish samples were to be sterilized using a mixture of ethylene oxide (12 %) - dichlorodifluoromethane (88 %) at 33 % relative humidity at a pressure of 5 psig at 40°C, so as to obtain an ethylene oxide concentration of 700 mg per litre of sterilant atmosphere. The exposure system essentially consisted of a battery of three stainless steel anerobic jars with clamp on lids. The

jars were to be equipped for either vacuumizing or pressurizing. The first two jars were to be used as preheating chambers to bring the incoming gas to any desired temperature. These would be immersed near the lids in a water bath, the temperature of which would be thermostatically controlled. The third jar was to be used as the exposure chamber in which the fish samples were to be placed and exposed to the sterilizing action of the gas. The jar would be immersed upto near its lid in a mineral oil bath, the temperature of which would be thermostatically controlled. This jar would be equipped with a humidity sensing element attached to an electric hygrometer, a rubber diaphragm protected water injection port and a standardized thermometer (61). In order to provide protection to project and other personnel, the gas was to have been evacuated out of the building with an exhaust or vacuum system. In addition to this a 13X - molecular sieve composed of crystalline aluminum silicate would be employed at the vacuum or exhaust outlet. The silicate pores are capable of absorbing 20 % (w/w) ethylene oxide.

However, it was not possible to use the above described system and procedure to sterilize the fish samples, due to health and safety considerations and equipment availability.

4. CHEMICAL TESTS TO ASSESS THE QUALITY OF FISH SAMPLES

In this study, fish sample quality was determined by microbiological, chemical and enzymatic analyses in addition to a sensory evaluation.

CHEMICAL ANALYSES

4.1 Total Volatile Bases

Microbiological activity and biochemical changes during iced or chilled storage of fish are responsible for the progressive decline of organoleptic quality. Specifically, enzymatic (bacterial and natural) breakdown of both proteins and the osmoregulatory agent trimethylamine oxide (TMAO) present in marine species (63) result in a build-up of odoriferous compounds, namely: ammonia, monomethylamine, dimethylamine, trimethylamine and other volatile amines (91,55,41).

Total volatile base (TVB) analyses, measuring low molecular weight volatile bases and amine compounds produced by microbial decarboxylation of amino acids (45,30), have been commonly used for assessment of fresh fish quality (31,19). In fact, more data have probably been accumulated on this test than for any other spoilage indicator (31). Of 65 papers attempting to correlate TVB with organoleptic quality reviewed by Borgstorm (31), 39 were positive, 17 were negative and 9 found variable results. However, excellent

linear correlation between TVB and TMA values have been reported for cod fillets (111). Recently researchers (6,7) applied six different published methods to Atlantic cod stored on ice for up to 18 days and found that although TVB nitrogen increased with time for all procedures, values differed from each other substantially.

The TVB test involves distillation of amine compounds into boric acid solution and titration with a standardized acid (hydrochloric or sulfuric acid). Samples have been in the form of press juice (99,3), comminuted tissue (18) and 60 % ethanol (99), 5 - 20 % TCA (74), perchloric acid (102) or magnesium sulfate extracts (19). Techniques for amine to boric acid transfer have included microdiffusion (99,71,74, 17,72), atmospheric distillation (99,18,110), vacuum distillation (57) and aeration (99,103). With this choice in methodology, literature values for organoleptic acceptability limits have ranged from 20 to 30 mg TVB nitrogen per 100 gram fish. Additional discrepancies in data have also arisen among laboratories due to the varied nature of heating apparatus in use. These factors coupled with species differences are responsible for the range of reported values and the risk involved in making direct comparisons (31).

4.2 Gas Chromatograph Total Volatiles Profile

Seafood has long been recognized as a highly perishable

product which must be refrigerated immediately upon harvest from the water to retard deterioration. Even with reasonable handling and storage, bacterial growth in time tends to impart odors and flavors to the product that reflect on freshness and quality. Thus, sensory evaluation has long been recognized as a most important factor for assessing the wholesomeness of seafood (88). Unfortunately, establishing effective taste panels for such purposes is time consuming, costly, and ultimately is limited by the tasters' subjectivity (68). Many objective chemical tests have been proposed to measure decomposition of seafood (31), but such tests are not always applicable to detect the various types of spoilage which occur in different products (88). With the advent of direct gas chromatography, it has become feasible to detect many of the volatile components that contribute to the flavor quality of certain food products (23). Further, when direct gas chromatography (24) is coupled with mass spectrometry (25,56) it becomes possible to identify the specific volatile components that characterize flavor and indicate the sensory qualities of food products (88). Such techniques are readily adaptable to seafoods (58).

In recent years, considerable effort has been expended to examine the volatiles and trace components that characterize and contribute to food flavors. Some early attempts to measure food volatile components by gas chromatographic methods consisted of analyzing headspace

vapors to detect vegetable and fruit aromas and volatiles of various food products. These methods require special preparation of the sample and subsequent transfer of a vapor aliquot to the gas chromatograph. Extraction and distillation techniques have been proposed to provide quantities of volatiles sufficient for instrumental detection and analysis. These methods are complex, tedious, time consuming operations that may also produce artifacts (26). More recently, a direct gas chromatographic procedure was reported for the examination of volatiles in salad oils and peanut butters (24). The method does not require prior enrichment of volatiles and is rapid and efficient (26). This method has been used in this study and is described in 'Materials and Methods'.

Examination of spoiling muscle (cod and haddock) has shown that, organoleptically, the most important constituents are the volatile sulfur compounds such as hydrogen sulfide, dimethyl sulfide and methyl mercaptan (14,42) accompanied, when fruity odors are present, by the esters of lower fatty acids such as acetic, butyric, propionic and hexanoic (89,70). The volatile sulfur compounds are produced from the sulfur amino acids, methionine and cysteine, and the esters from the amino acids such as glycine, serine and leucine. With marine fish there is also the production of volatile amines such as TMA and DMA from TMAO which contributes to the ammoniacal odors of spoiling fish (96).

III. MATERIALS AND METHODS

1. Introduction

In this project three species of fish were studied:

A. Sea trout (*Cynoscion regalis*)

B. Spanish mackerel (*Scromberomorus maculatus*)

C. Catfish (*Ictalurus punctatus*)

The sea trout and spanish mackerel were obtained from Hampton, VA and the catfish from Martinsville, VA and were filleted between 24 and 48 hours after harvest. In each study, some of the fish were treated to obtain fish samples with reduced microbial counts. To obtain fish tissue with reduced microbial counts, two methods were considered:

A. Utilization of ethylene oxide

B. Utilization of alcohol

The alcohol treatment seemed more feasible under the existing laboratory conditions. The treatment involved the immersion of the whole fish in alcohol after which pieces of flesh were excised aseptically.

The rest of the fish samples were not treated with alcohol, i.e. underwent normal spoilage. Small pieces of both the treated and untreated samples were individually packaged in sterile whirl-pak bags and stored at 29°F (-1.7°C) and 32°F (0°C-crushed ice). A few fillets were

placed in the freezer and these served as frozen references for the sensory evaluation.

2. Utilization of Alcohol as a Sterilizing Agent

Reagents

i)	Sodium carbonate solution	5 % w/w
ii)	Ethyl alcohol	70 %
iii)	Formalin	2 %

Procedure

The procedure to obtain treated fish was implemented as follows:

i) The dead fish were immersed in a sodium carbonate solution and scrubbed to remove most of the surface slime (42).

ii) The fish surface was sterilized by immersing for 5 min in 70 % ethyl alcohol (28). Two test tubes were placed at the bottom of the container of 70 % alcohol. The fish was placed on top of these two test tubes, so as to ensure that the alcohol sterilized both sides of the fish uniformly. After 2 1/2 min the fish was turned over and allowed to remain in that position for another 2 1/2 min. The 5 min exposure time was selected as a higher exposure time resulted in alcohol odor being detected in the fish by the sensory panelists.

iii) The fish was then removed from the container of alcoholand placed on a clean tray. The surface was then scrubbed with a swab of sterile cheese cloth dipped in 70 % ethyl alcohol twice, after which the fish surface was swabbed twice with formalin to further sanitize the fish surface.

iv) Treated flesh samples were obtained as follows: On one side of each fish a cut around the edge of the skin wasmade with a sterile scalpel; and the skin was removed using sterile forceps and knives. Small pieces of flesh were then excised from each fish, avoiding the gut cavity. The fish was then turned over and the same procedure was applied to the other side.

v) Each fish piece was then individually packed in a sterile whirl-pak and stored at 29 or 32°F.

3. Tests to Assess the Quality of Fish Samples

The fish samples were evaluated by:

3.1 Microbiological analysis

3.2 Chemical analyses

3.3 Enzymatic analyses

3.4 Sensory evaluation

Sampling was done on days 1, 5, 9, 12, 14 and 16. On each sampling day the following tests were carried out:

3.1 Microbiological Analysis

Total aerobic plate count was carried out as follows:

Media

i) Standard Methods Agar

ii) Dilution blanks

salt-peptone water (1 % NaCl, 0.1 % peptone)

or

peptone water (0.1 % peptone)

Equipment

i) Stomacher Lab-Blender 400

Procedure

i) 11 grams of fish were weighed out and were diluted with 99 ml dilution blanks of salt-peptone water (or peptone water in the case of catfish).

ii) Homogenize in the stomacher for 2 min.

iii) Appropriate dilutions were prepared and plated out using the pour plate method.

iv) Plates were incubated for 6 days at 20°C before reading.

v) Duplicate samples were plated out for each treatment. Duplicate plates were prepared for each sample.

3.2 Chemical Analyses

A. Total Volatile Bases

Reagents

i) Boric acid solution 2 %

ii) Sodium hydroxide 0.1 N

iii) Sulfuric acid 0.050 N

iv) Screened methyl red indicator

v) Magnesium oxide

vi) Boileezers (anti-bumping granules)

vii) Anti-Foam A concentrate and Anti-Foam A emulsion (Sigma)

viii) Potassium acid phthalate

ix) Phenolphthalein indicator

Equipment

i) Kjeldahl steam distillation apparatus

ii) Drying oven

ii) Waring blender

Procedure

The original procedure had to be modified so that it could be carried out with existing equipment and supplies. The following modifications were made:

- The distillation equipment used was the Kjeldahl

steam distillation unit.

- A 500 ml round bottom distillation flask was used instead of a 1000 ml round bottom flask.
- 5 gm sample and 150 ml distilled water were used instead of 10 gm sample and 300 ml distilled water.
- 1 gm magnesium oxide was used instead of 2 gm magnesium oxide.

The procedure was implemented as follows:

i) 0.1 N NaOH and 0.05 N H_2SO_4 were standardized and their normalities calculated.

ii) 5 gm sample and 150 ml distilled water were blended for 1 min in a Waring blender and transferred to a 500 ml round bottom distillation flask.

iii) Anti-bumping granules, 1 gm magnesium oxide, 25 drops Anti-Foam A emulsion and 15 drops Anti-Foam A concentrate were added to the sample and mixed thoroughly.

iv) The flask was connected to the still.

v) 25 ml of 2 % boric acid and 5 drops of indicator were added to a 250 ml Erlenmeyer receiving flask.

vi) The receiving flask was installed so that the receiver tube dipped below the boric acid solution.

vii) The distilling flask was heated so that the mixture within boiled in approxiamately 10 mins.

viii) Using the same rate of heating the sample was distilled

for exactly 25 mins.

ix) After distillation the solution in the receiver flask was titrated with standard 0.05 N H_2SO_4 solution, back to the original color.

x) A blank was titrated which had all the reagents except the sample. If the blank requires more than 0.1 ml titrant, new boric acid should be used.

xi) Total volatile bases expressed as milligrams nitrogen per 100 gm sample were calculated as

$$TVB = (V_4 - V_5) \times N_2 \times 100 \times 14 / W_2$$

where

V_4 = Volume (ml) H_2SO_4 used for sample

V_5 = Volume (ml) H_2SO_4 used for blank

N_2 = Normality of H_2SO_4

W_2 = Weight of sample in grams

xii) The TVB readings were obtained for 3 different samples of each treatment. The treated and untreated samples stored at 29°F were analyzed on each sampling day. The treated and untreated samples stored at 32°F were analyzed on the day following the sampling day.

B. Gas Chromatograph Total Volatiles Profile

Reagents

i) Tenax GC 60-80 mesh

ii) Helium

iii) Acetone

iv) Dry ice

v) Hyrdrogen

vi) Air

Equipment

i) Hewlett Packard (HP) 5890 Gas Chromatograph (GC). A HP 5890 GC series equipped with a flame ionization detector (FID) was used to measure tissue volatiles. Flow rates for hydrogen and air were 30 and 240 ml/min respectively. Helium flow rates were 1.2 ml/min through the column and 30 ml/min for auxiliary make-up gas. A HP ultra performance capillary column was used (50 mm X 0.31 mm ID column coated with 0.52 micron film of cross linked 5 % phenyl methyl silicone). An external closed inlet device (Scientific Instrument Service, River Ridge, Louisiana) was interfaced at the carrier gas arm of the inert Weldment assembly of the GC to facilitate direct gas chromatography (27).

ii) HP GC Chemstation.

Used for data acquisition and analysis.

iii) HP Inkjet Printer Model.

Used to plot GC curves.

Procedure

Moisture-free volatiles from the fish samples were collected on a Tenax-GC trap, as follows:

i) About 200 mg of Tenax was secured in a Pyrex glass liner (0.84 x 8.50 cm) between two plugs of glass wool and conditioned at 200^{C} ^{o}overnight.

ii) 20 g of fish sample and 100 ml of distilled water was placed in a Ziploc bag, in a bowl, and microwaved until the fish sample attained an internal temperature of 175°F. The fish and water were homogenized by hand through the plastic bag.

iii) The sample was then transferred to a 500 ml Erlenmeyer flask equipped with a 20 x 1-cm tap-water cooled condenser. A standard taper Teflon plug was positioned on top of the condenser to hold the Tenax trap in place. A nut with a minute perforation was placed on top of the glass wool plug of the Tenax trap to prevent migration of the Tenax material under vacuum.

iv) The Erlenmeyer flask was placed in a water bath on a hotplate.

v) Volatiles were then stripped from the fish samples for 2 hours at approximately 60°C, under vacuum. The vacuum pressure was adjusted to maintain a sufficiently low

level of boiling.

vi) Adsorbed moisture-free volatiles were then desorbed from the Tenax trap by the flow of the carrier gas (helium) in the heated ECID of the modified GC system. Inlet temperature of the ECID was set at 180°C, and the valve temperature was also set at 180°C. The GC detector was heated to 275°C (104).

vii) A portion of the column was immersed for 2 min in an acetone-dry ice bath with the six-port valve positioned in the "inject" mode to direct the carrier gas through the sample and purge the volatiles onto the head of the cold column. The oven temperature was programmed immediately from 30 to 150°C at 2.5°C/min, and from 150 to 250°C at 5°C/min. Final hold was 250°C for 30 min (27).

viii) A GC profile was obtained for one sample of each treatment on sampling days 1, 5, 12 and 16.

3.3 Enzymatic Analyses

A. Proteolytic Activity

Two methods were used to measure proteolytic activity:

Method I: Utilizing change in absorbance readings.

Method II: Utilizing casein substrate plates.

(see Appendix 1 for detailed methodology)

Method I: The experiment was carried out using the procedure described by Chen et al. (16).

The following modifications were made to the original procedure:

- 1.0 ml of fish extract was used instead of 0.50 - 0.25 ml fish extract.
- 1.0 ml buffer was used instead of 1.95 - 1.75 ml buffer.
- 0.5 ml hemoglobin substrate was used instead of 1.0 ml substrate.

In this procedure, the hemoglobin substrate and the enzyme extract were incubated together to allow digestion of protein in the substrate by the protease present in the enzyme extract. The undigested protein was precipated by the addition of trichloroacetic acid (TCA). The amount of TCA soluble peptides produced by enzymic digestion was measured spectrophotometrically at 280 nm., thus giving a measure of protease activity present in sample.

Method II: The experiment was carried out using Bio-Rad Protease Substrate Gel Tablets (Bio-Rad Bulletin).

In this procedure, agar diffusion plates were used for the determination of proteolytic activity. Bio-Rad Protease Substrate Gel Tablets produced a 1% agar gel containing a bovine casein preparation in a Tris buffered physiological saline solution at pH 7.2. Protease diffusion into the

substrate gel was accompanied by digestion of the casein. This forms a transparent ring around the sample wells in a turbid gel. The size of the ring was a measure of the proteolytic activity of the sample (Bio-Rad Bulletin).

B. Lipolytic Activity

Two methods were used to measure lipolytic activity:

Method I: Utilizing change in absorbance readings.

Method II: Utilizing a titrimetric method.

(see Appendix 1 for detailed methodology)

Method I: This experiment was carried out using the procedure described by Geromel et al. (37).

This test involves the hydrolysis of the triglycerides in the substrate to fatty acids by lipase present in the enzyme extract. The free fatty acids (FFA) are transferred from the aqeous phase into chloroform, in which the copper complex of FFA is formed on the addition of a copper reagent. Addition of a copper complexing agent results in the formation of a color complex which is measured colorimetrically at 540 nm.

Method II: This procedure was carried out using the Sigma Lipase Kit.

This test involves the hydrolysis of triglycerides in olive oil into fatty acids, diglycerides and to some small

extent into monoglycerides and glycerol, by lipolytic activity. The amount of fatty acids formed, under the specific conditions of the test, is a measure of lipase activity in the sample. The fatty acids formed are determined by titration with sodium hydroxide.

3.4 Sensory Evaluation

In this study the modified nine-point hedonic method of the National Marine Fisheries Service (NMFS) was used. The samples were rated for taste and odor on a nine-point scale ranging from ´excellent´ (rating of 9) to ´inedible´ (rating of 1). The panel consisted of six people. The size of the panel was small due to the limited quantity of treated sample available. For each panel, six panelists were chosen from a group of eight selected people, so that the panel was relatively trained and variation due to subjectivity was thus minimized.

The difference between the modified nine-point method and the original NMFS method is that a high quality frozen fish was provided as a reference for the panelist. This reference sample served as an objective measure in place of extensive panel training to determine relative sample quality. Panelists compared treatment samples to the reference sample in order to rate their relative quality (53).

Each individual sample was wrapped in aluminum foil, placed in a conventional oven pre-heated to 350°F (177°C), baked for 15 min, and distributed to sensory panelists. To sample the products, panelists were directed to open the foil and place samples close to their nose to capture the odor of escaping volatiles and evaluate for odor. They were then instructed to evaluate the samples for taste. They could refuse to taste the product if the odor and appearance were not acceptable.

IV. RESULTS AND DISCUSSION

1. Introduction

Fish quality during storage for up to 16 days was assessed by means of microbiological, chemical and enzymatic tests and sensory evaluations. Two replications were carried out for each of the three fish species studied (sea trout, spanish mackerel and catfish). In each study, samples with reduced numbers of microorganisms were obtained by treating the fish with alcohol and excising the flesh aseptically. These samples were compared with samples undergoing normal spoilage. Differential temperature storage studies were conducted by storing samples at 29 and 32°F. The super-chilling temperature (29°F) has been suggested for the storage and transportation of fish since the spoilage rate is significantly reduced over a 32°F or greater temperature environment. The 32°F temperature was selected to approximate that of crushed ice storage which is the most utilized commercial storage environment. Storage temperatures used in this study have been reported in degrees Farenheit as this study has been done mainly to benefit the seafood industry which utilizes the Farenheit scale and instruments are calibrated in this scale.

Data obtained were statistically analyzed by means of ANOVA using the GLM procedure. A factorial design was used;

the three factors being treatment, storage time and storage temperature. All statistically significant data have been represented graphically. In some instances data were not statistically significant but have been graphed to explain specific conclusions.

Though an attempt was made to assess proteolytic and lipolytic activity in fish samples, no enzymatic activity was detected. It was concluded that enzymatic activity was not present in the fish samples at detectable levels.

A correlation was attempted between total area of the peaks of the GC profile and fish quality, as proposed by other researchers (104). However, no distinct correlation was obtained; nor could specific individual peaks correlating with fish quality be identified. Various areas under the curve were examined and correlations attempted with fish quality. However, no distinct patterns could be identified. Lack of correlation might be attributed to the variation present between samples and also due to the variability of the technique utilized. Also, volatiles present in the fish samples could have been affected by the alcohol treatment used in the present study (see Appendix 3 for gas chromatograms showing variability present and the complexity of the profile).

Despite having undergone an alcohol sanitizing treatment, significantly reduced microbial populations were observed in some of the treated samples. Therefore treated

samples have been reported as samples with reduced microorganisms instead of being reported as sterile samples.

Variation was seen to be present in the data and standard deviations have been reported in Appendix 2. Due to variations in the data, power regression curves have been plotted for the sensory evaluation data and for the TPC data. TVB data has been represented by best-fit curve.

2. Sea Trout

Total Plate Count (TPC): Due to the presence of two factor interactions, independent effects of the factors can not be discussed.

As seen in Figure 1, storage temperature influenced the TPC, as samples stored at 32°F had a higher TPC than samples stored at 29°F. At 29°F (super-chilling) microbial growth was inhibited in the fish sample. There was an increase in TPC for samples stored at both 29 and 32°F over the 16 day storage period. This increase was more gradual in samples stored at 29°F than in samples stored at 32°F. Samples stored at 32°F showed a sharp increase of 1.5 log in the TPC until day 5. The TPC increased only 2 logs during the next 11 days. Samples stored at 29°F showed a 0.5 log increase in TPC by day 5. However, the TPC increased only 0.5 logs for the remainder of the storage period. From Figure 2, it can be seen that for both the treated and untreated samples

(normal spoilage), there was a steady increase in TPC over the 16 day storage period. During the study, the TPC for the treated samples was 4 to 6 log cycles lower than the TPC for the untreated samples. Both storage temperature and treatment had a significant ($p < 0.05$) effect on TPC (Figure 3). For the treated samples, the TPC remained practically the same at both 29 and 32°F, indicating that the treatment had a greater effect on TPC than the storage temperature. In the untreated samples the storage temperature had an effect on TPC, as the TPC of samples stored at 32°F was higher than the TPC of samples stored at 29°F.

Total Volatile Bases (TVB): In the treated samples, the alcohol treatment had a significant ($p < 0.05$) effect on TVB as there was only a marginal increase of 6 mg-N % in the TVB values of treated samples stored at 29 and 32°F (Figure 4). At the end of the storage period, the treated samples stored at 32°F had levels of TVB only 3 mg-N % higher than samples stored at 29°F. This is probably due to the reduced microbial populations present in the treated samples. Storage temperature had an effect ($p < 0.05$) on TVB in the untreated samples as the samples stored at 29°F did not show a significant ($p < 0.05$) increase in TVB levels during storage, and had only 20 mg-N % of TVB present at the end of the storage period whereas samples stored at 32°F achieved a value of 67 mg-N %. This was probably also due to the

inhibition of microbial growth due to the super-chilling conditions. The untreated samples stored at 32°F showed a gradual increase from 20 to 40 mg-N % in the level of TVB up until day 14, at which point the TVB level showed a sharp increase, reaching a value of 69 mg - N %. Microbial populations counts need to attain a certain level before rapid production of TVB occurs. On day 14, the TPC for sea trout was 9.1 log/gm which was probably sufficient for the sudden increase in the production of TVB due to the breakdown of TMAO and non-protein nitrogen into compounds such as TMA, ammonia and other volatile bases.

Overall, the treated samples had significantly ($p < 0.05$) lower levels of TVB present than the untreated samples, which is attributed to the reduced number of microorganisms present in the treated samples due to the sanitizing effect of the alcohol treatment.

Sensory Evaluation: Similar data were obtained (Figures 5 and 6) from the sensory evaluation of odor and taste. The fish was considered to have high quality only until day 3 (sensory score of 7 or higher). There was a rapid decline in quality during the first three days, after which the deterioration in quality was more gradual. At the end of the 16 day storage period, however, the samples were still acceptable (sensory score of 5 or higher) to the panelists for both odor and taste sensory parameters. For this fish,

storage temperature and treatment were not significant ($p < 0.1$) in the sensory evaluation. Though not statistically significant, (Figures 7 and 8) the odor and taste of all treated samples were preferred to that of the untreated samples stored at 32°F. Also, within treatments and between treatments, samples stored at 29°F received higher scores for odor and taste as compared to samples stored at 32°F. Untreated samples stored at 32°F received a score of 5 or lower (borderline) on day 6, which was decided to be the cut-off point for consumer acceptability. Untreated samples stored at 29°F and treated samples stored at both temperatures were still acceptable to the sensory panelists at the end of the 16 day storage period. Thus there was an extension in the shelf-life of treated samples and untreated samples stored at 29°F of at least 10 days over the shelf-life of untreated samples stored at 32°F. Treated samples stored at both temperatures and the untreated samples stored at 29°F would probably have been acceptable to the sensory panel for at least 5 days more, thereby having an effective shelf-life of 21 days.

A Pearson Correlation Coefficient of -0.74 with a significance probability of 0.0001 was obtained for the correlation of taste with TVB and for the correlation of odor with TVB, showing that taste and odor scores decreased as TVB levels increased. A Pearson Correlation Coefficient of 0.95

with a significance probability of 0.0001 was obtained for the correlation of taste with odor (Table 2).

3. Spanish Mackerel

Total Plate Count (TPC): Treatment and storage temperature had a significant ($p < 0.05$) effect on the TPC (Figure 9). TPC for the treated samples at both storage temperatures remained similar. Treated samples stored at 29°F had a TPC of 0.5 log/gm whereas treated samples stored at 32°F were only 0.5 log higher with a TPC of 1.0 log/gm. This shows the effectiveness of the alcohol treatment. The storage temperature was important as the TPC of untreated samples stored at 29°F was significantly ($p < 0.05$) lower by 2.5 log than the TPC of untreated samples stored at 32°F. The effectiveness of the alcohol treatment can be seen in Figure 10 ($p < 0.05$) as the TPC of treated samples over the 16 day storage period was 4 to 6 log cycles lower than the TPC of untreated samples. The TPC of the treated samples remained practically the same (0.5-1.0 log/gm) over time whereas the TPC of untreated samples showed a rapid increase of 1.5 log up to day 5 after which there was a gradual increase of only 2 log over the remaining 11 days of the storage period.

Total Volatile Bases (TVB): None of the factors could be

evaluated independently due to three factor interactions between storage time, storage temperature, and treatment. In Figure 11 it can be seen that in the treated samples, the TVB level at the end of the 16 day storage period was higher by only 1 mg-N % in samples stored at 32°F than in samples stored at 29°F. The data for the untreated samples (Figure 11) followed similar patterns at both storage temperatures until day 8, after which TVB levels in samples stored at 29°F remained unchanged at about 26 mg-N %, whereas samples stored at 32°F showed a gradual increase in TVB levels from 25 to 27.5 mg-N % until day 14, with a sharp increase in TVB production only at the end of the storage period on day 16 achieving a value of 41 mg-N %. As discussed earlier, microbial numbers have to attain a certain level before sharp increases in TVB levels can be observed. On day 16, the TPC for untreated samples stored at 32°F was 7.2 log/gm, at which time rapid production of TVB was observed.

Sensory Evaluation: Storage time and treatment were both significant ($p < 0.05$) for odor. Mackerel were rated as high quality fish (sensory score of 7 or higher) during the first three days of storage after which there was a gradual decline in odor scores (Figure 12). At the end of the 16 day storage period, odor ratings were still acceptable as judged by the sensory panel. Treated samples had a higher score for odor as compared to untreated samples, as the alcohol treatment

inhibited microbial growth and spoilage. However, certain off-odors were still present due to autolytic spoilage reactions. Treatment, storage temperature and storage time were all significant ($p < 0.1$) for the taste evaluation (Figure 13). However, interactions were present between these three factors and therefore independent factor effects cannot be discussed. Treated samples at both storage temperatures produced similar results, with fish having high quality until day 3 after which there was a gradual decline in taste. The untreated samples stored at 29°F had a longer shelf-life than untreated samples stored at 32°F, due to reduced microbial growth at the lower temperature. At the end of the 16 day storage period, untreated samples stored at 29°F were still acceptable for taste and judged to have a fair quality by the panelists, whereas untreated samples stored at 32°F were rated as being ´borderline´ (score = 5) by day 10 and thus effectively having a shelf-life of approximately 10 days after processing. Thus the shelf-life of untreated samples stored at 29°F was extended by at least 6 days over the shelf-life of untreated samples stored at 32°F at the end of the 16 day storage period. Treated samples stored at 29 and 32°F were still acceptable to the sensory panelists at the end of the 16 day storage period being rated as ´fair´. Thus there was an extension of at least 6 days in the shelf-life of treated samples stored at both storage temperatures over the shelf-life of untreated

samples stored at 32°F. Extrapolating, the treated samples would probably be acceptable to the sensory panelists for at least another 5-6 days, resulting in a further extension of shelf-life over the untreated samples stored at 32°F and effectively having a shelf-life of 21-22 days.

A Pearson Correlation Coefficient of 0.95 with a significance probability of 0.0001 was obtained for the correlation of taste with odor (Table 3).

4. Catfish

Total Plate Count (TPC): The only significant ($p < 0.05$) interaction was that of treatment and storage temperature (Figure 14). The TPC for treated samples was much lower than the TPC for untreated samples due to the effectiveness of the alcohol treatment which resulted in low numbers of microorganisms in the treated samples. The TPC for treated samples stored at 29°F and 32°F were almost identical, with the TPC of samples stored at 32°F being less than 1 log cycle higher. The microbial counts for untreated samples stored at 32°F were significantly ($p < 0.05$) higher by at least 2 log cycles than the microbial counts for samples stored at 29°F.

Total Volatile Bases (TVB): Storage time, storage temperature, and the alcohol treatment were not significant

($p < 0.1$), and this was attributed to the catfish used in this study being fresh water fish. These fish do not contain TMAO which is a major osmoregulatory agent present in marine fish. In marine species, TMAO is broken down by enzyme activity into TMA, ammonia and other volatile bases. This test was performed for catfish to see if any other volatiles were being produced which could be detected and measured by the TVB test. There was no further increase in TVB from initial levels of about 20 mg-N % over the 16 day storage period.

Sensory Evaluation: Two factor interactions between treatment and storage temperature and between storage temperature and storage time were significant ($p < 0.05$) in odor evaluations (Figure 15). Untreated samples stored at both storage temperatures obtained practically the same odor scores (Figure 16). Treated samples stored at 29°F obtained lower scores for odor than samples stored at 32°F. This can possibly be explained by the fact that catfish being cultured fish, have a greater concentration of fat between the skin and the flesh layer, and since alcohol is slightly lipophilic this might have resulted in greater absorption of the alcohol by the catfish tissue. Super-chilling at 29°F resulted in the alcohol odor not evaporating over time, whereas it dissipated gradually over time in samples stored at 32°F due to the volatility of the alcohol at this temperature.

However, treated samples stored at 32°F were judged to have a better odor than untreated samples stored at 32°F. Over the 16 day storage period, samples stored at 29°F showed a steady decline in odor and were rated by the sensory panelists as fair at the end of 16 days. Samples stored at 32°F showed a slight increase in odor ratings over the storage period and obtained a rating of between ´fair´ and ´good´ at the end of 16 days. This can again be attributed to the fact that treated samples stored at 29°F had a lower odor rating than samples stored at 32°F due to the strong alcohol odor present. Significant interactions ($p < 0.1$) were present between treatment and storage time. Treated samples had higher taste scores than untreated samples (Figure 17) due to higher numbers of microorganisms being present in untreated samples thus leading to faster spoilage. Initial sensory scores for taste obtained for catfish were lower than those obtained for sea trout and spanish mackerel. Treated samples had initial high quality (score of 6.5 and above) for the first 5 days. There was a decline in taste evaluation scores from day 1 to day 16 for both treated and untreated samples. Treated samples however obtained higher ratings for taste than untreated samples showing the effectiveness of the alcohol treatment. Treated samples stored at 32°F were preferred to treated samples stored at 29°F. This was probably due to the strong alcohol odor in the fish tissue at 29°F (Figure 18) as explained earlier. At the end of 16 days

both treated and untreated samples were still acceptable to the panelists. Catfish undergoing normal spoilage had a shelf-life of at least 16 days after processing. Treated samples could be expected to have an extension in shelf-life of at least 5-6 days over untreated samples.

A Pearson Correlation Coefficient of 0.65 with a significance probability of 0.0006 was obtained for the correlation of taste with odor (Table 4).

V. SUMMARY AND CONCLUSIONS

In order to be able to differentiate between microbial spoilage and enzymic spoilage it is necessary to obtain sterile raw fish tissue. Numerous experimental sterilization techniques have been employed with varying degrees of success. In this study, two such methods were examined; one method, utilizing alcohol as a sterilizing agent, was selected due to health and safety conditions and equipment availability. Samples were treated with alcohol to obtain fish tissue with greatly reduced microbial populations. It proved to be extremely difficult to maintain absolute sterility in treated samples, especially towards the end of the 16 day storage period used in this study.

Overall, it was seen that treated samples had a longer shelf-life than untreated samples. Sensory panelists generally preferred treated samples to untreated samples, especially during the latter part of the storage period. The shelf-life of the fish was extended. However it was only the period of moderate to low quality which was extended. This is in accordance with previous studies (34). The reason offered for the inability to preserve the initial high quality is that non-microbial factors such as autolysis or oxidation (free radicals) have contributed to product quality loss (34).

Conflicting results have been reported from studies on

proteolysis and lipolysis in fish. Most of the research in this area has been conducted on storage of frozen fish, usually over long periods of times. In this study, enzymatic activity was not detected in any of the samples. It could be possible that more sensitive assays are necessary to detect enzyme activity in fish samples. As autolytic enzymes play a major role in the development of unpleasant odors and flavors in fresh fish, any method to extend the shelf life of fish must inhibit autolysis as well as be able to limit oxidation and bacterial spoilage. Further work should be carried out to determine the relative effects of oxidation and autolysis on the deterioration of chilled fish in the absence of bacteria (33).

Crushed ice storage (32°F) is the most utilized storage environment for fresh fish. In previous studies it has been observed that fish samples stored at 29°F have significantly reduced microbial populations as compared to samples stored at 32°F (1). The results obtained in this study were in accordance with the previously reported results in that it was seen that all samples stored at 29°F had lower microbial counts than samples stored at 32°F. In most cases, samples stored at 29°F were preferred to those stored at 32°F by the sensory panelists, for both taste and odor. Storage at 29°F results in super-chilling conditions, thus resulting in the drastic inhibition of bacterial growth. Consequently, TVB levels were also observed to remain practically unchanged

over time in the samples stored at 29°F.

A correlation was attempted between total area of the peaks of the GC profile and fish quality, as proposed by other researchers (104). However, no distinct correlation was obtained; nor could specific individual peaks correlating with fish quality be identified. Various areas under the curve (eg. the area under the curve between 20 and 40 min.) were examined and correlations attempted with fish quality. However, no distinct patterns could be identified. Lack of correlation might be attributed to the variation present between samples and also due to the variability of the technique utilized. Also, volatiles present in the fish samples could have been affected by the alcohol treatment used in the present study (see Appendix 3 for gas chromatograms showing variability present and the complexity of the profile).

The method used to obtain GC profiles samples is an extremely time-saving and simple procedure. However, a lot of variability was observed using this technique, and further research needs to be conducted so as to be able to obtain more consistent and reproducible results. Also, with the aid of a mass spectrometer it would be possible to identify the various volatile compounds present in the fish and correlate the various off-flavors and off-odors that develop in fish during storage with the particular volatile compounds.

Comparing the three fish, the initial TPC was highest for sea trout at 4.2 log/gm and lowest for catfish at 3.4 log/gm; spanish mackerel had an initial TPC of 3.8 log/gm. These initial counts are lower than the results obtained in commercial plants, as lesser quantities of fish were filleted in this study, and filleting was carried out under more sanitary conditions. At the end of the 16 day storage period, the TPCs for all three fish were very similar ranging from 8.9 log/gm for sea trout, 8.8 log/gm for spanish mackerel and 8.6 log/gm for catfish. TVB was detected in both sea trout and spanish mackerel towards the end of the storage period, after the bacterial load had attained the level of 10^6/gm, and putrefaction due to microbial spoilage had begun. Sea trout had higher levels of TVB resulting in a value of 69 mg-N % on day 16, whereas spanish mackerel reached a value of 41 mg-N %. Catfish, being a freshwater fish, lacks the major osmoregulatory agent TMAO present in most marine fish species, and therefore did not show an increase from initial TVB levels present.

Alcohol treated samples had a longer shelf-life than untreated samples. Samples stored at 29°F were preferred by sensory panelists to samples stored at 32°F in most cases. Both sea trout and spanish mackerel maintained the initial high quality for 3 days, whereas the catfish had high quality for 5 days. However, for all three fish there was a rapid decline in the high quality itself during the initial 3 or 5

days, after which the deterioration in quality was more gradual.

Sea trout had a shelf-life of 6 days after processing when stored at 32°F. Sea trout treated with alcohol and stored at 29 and 32°F, and untreated samples stored at 29°F had shelf-lives extended by 10 days over the sea trout samples undergoing normal spoilage, and were still acceptable to the sensory panelists on day 16. Speculating, these samples would probably have been acceptable to the sensory panel for at least another 5 days, effectively having a shelf-life of 21 days.

Spanish mackerel had a shelf-life of 10 days after processing when stored at 32°F. Alcohol treated samples stored at 29 and 32°F, and untreated samples stored at 29°F had an extension of at least 6 days in shelf-life. Since the mackerel were still acceptable to the sensory panel at the end of 16 days, on conjecture, these samples would probably have had a shelf-life of 21-22 days.

Catfish, being cultured fish, have a high concentration of fat between the skin and the tissue, and since alcohol is slightly lipophilic, this resulted in greater absorption of the alcohol in the catfish tissue. Super-chilling at 29°F resulted in the alcohol odor not evaporating over time and this resulted in lower sensory scores for odor for treated samples stored at 29°F than treated samples stored at 32°F. At the end of the 16 day storage period, both treated and

untreated samples were still acceptable to the sensory panelists. Treated samples were preferred for taste to untreated samples. Catfish undergoing normal spoilage had a shelf-life of at least 16 days. Treated samples could be expected to have an extension in shelf-life of at least 5-6 days over untreated samples. This long shelf-life could be attributed to initially lower TPCs and to lack of TMAO in catfish.

The decomposition rates of the three fish were determined: sea trout had a shelf-life of 6 days, spanish mackerel 10 days, and catfish atleast 16 days.

From this study it is seen that the storage temperature plays a very important role in determining the shelf-life of fish. Samples stored at 29°F had longer shelf-lives than samples stored at 32°F (crushed ice). It would be extremely beneficial if fish were stored at 29°F throughout the journey from the processor to the retailer. The 29°F must be maintained by the retailer, or else microbial counts will increase when fish are stored at 32°F as reported in previous studies (1).

The shelf-life of a fish is largely determined by microbial spoilage reactions and care should be taken to minimize microbial contamination by ensuring good handling, processing, and transportation procedures to be carried out at a low temperature of 29°F. Though microbial spoilage plays an important role in the deterioration of fish quality,

autolytic spoilage reactions also seem to result in the production of off-flavors and off-odors in fish during storage. Treated samples were also seen to lose the initial high quality rapidly, showing that autolytic reactions do occur in fish. However, neither proteolytic nor lipolytic enzyme was detected in any of the fish samples. It can be concluded that certain other enzymatic or chemical reactions must be occurring in the fish tissue. Some of the products of autolysis could be present in trace amounts adequate to affect the organoleptic quality of the fish. However it is possible that these substances are not present at levels at which they can be detected by various chemical tests. At present we have no reliable method to differentiate between the contributions of autolytic and microbial spoilage in the deterioration of fish quality during storage. Thus, in the present study we were unable to distinguish between the roles played by autolytic and microbial spoilage in deterioration of fish quality.

As can be seen from the present study, the TPC and TVB tests and sensory evaluation are good indices for determining fish quality. Decomposition rates differ from one species of fish to another, and if the demand for fresh fish in inland non-coastal markets is to be met, this factor must be taken into account and marketing strategies accordingly planned for each individual species. It is clear that certain fish species, irrespective of microbial profiles, have very short

shelf-lives and these fish cannot be marketed fresh to distant markets as they would be of unacceptable quality on arrival. It would be economically beneficial to either market these fish in the frozen state or develop markets closer to the place where the fish are harvested.

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VII. LIST OF TABLES

Table 1: The shelf-life of various species of fish stored in ice.

Area	Species	Shelf-life (Days)
Northern temperate waters	Marine	
	Fatty Scotch summer herring	2-4
	Lean Norwegian herring	12
	Mackerel	6
	Whiting	9
	Cod, haddock	12
	Sole, plaice	7-18
	Halibut	21
	Freshwater	
	Perch	15-17
	Trout	9-11
India	Marine	
	Pomfret	7-9
	Horse mackerel	10-45
	Bonga	27
	Perch	30-45
	Freshwater	
	Nile perch, Mrigal carp	29
West Africa	Marine	
	Sea bream	26
East Africa	Freshwater	
	Kariba bream	15
	Tilapia sp.	28

Table 2: Pearson Correlation Coefficients for sea trout data.

	MTVB	MTPC	MTASTE	MODOR
MTVB	1.00	0.66	-0.74	-0.74
	0.00000	0.0005	0.0001	0.0001
MTPC	0.66	1.00	-0.58	-0.50
	0.0005	0.0000	0.0030	0.0124
MTAS	-0.74	-0.58	1.00	0.95
	0.0001	0.0030	0.0000	0.0001
MODOR	-0.74	-0.50	0.95	1.00
	0.0001	0.0124	0.0001	0.0000

Table 3: Pearson Correlation Coefficients for spanish mackerel data.

	MTVB	MTPC	MTASTE	MODOR
MTVB	1.00 0.0000	0.17 0.4229	-0.42 0.0405	-0.44 0.0316
MTPC	0.17 0.4229	1.00 0.0000	-0.61 0.0014	-0.58 0.0029
MTAST	-0.42 0.0405	-0.61 0.0014	1.00 0.0000	0.95 0.0001
MODOR	-0.44 0.0316	-0.58 0.0029	0.95 0.0001	1.00 0.0000

Table 4: Pearson Correlation Coefficients for catfish data.

	MTVB	MTPC	MTASTE	MODOR
MTVB	1.00 0.0000	0.0 0.7678	0.24 0.2595	0.34 0.1048
MTPC	0.06 0.76	1.00 0.00	-0.43 0.0358	-0.12 0.5826
MTASTE	0.24 0.2595	-0.43 0.0358	1.00 0.0000	0.65 0.0006
MODO	0.34 0.1048	-0.12 0.5826	0.65 0.0006	1.00 0.0000

VIII. LIST OF FIGURES

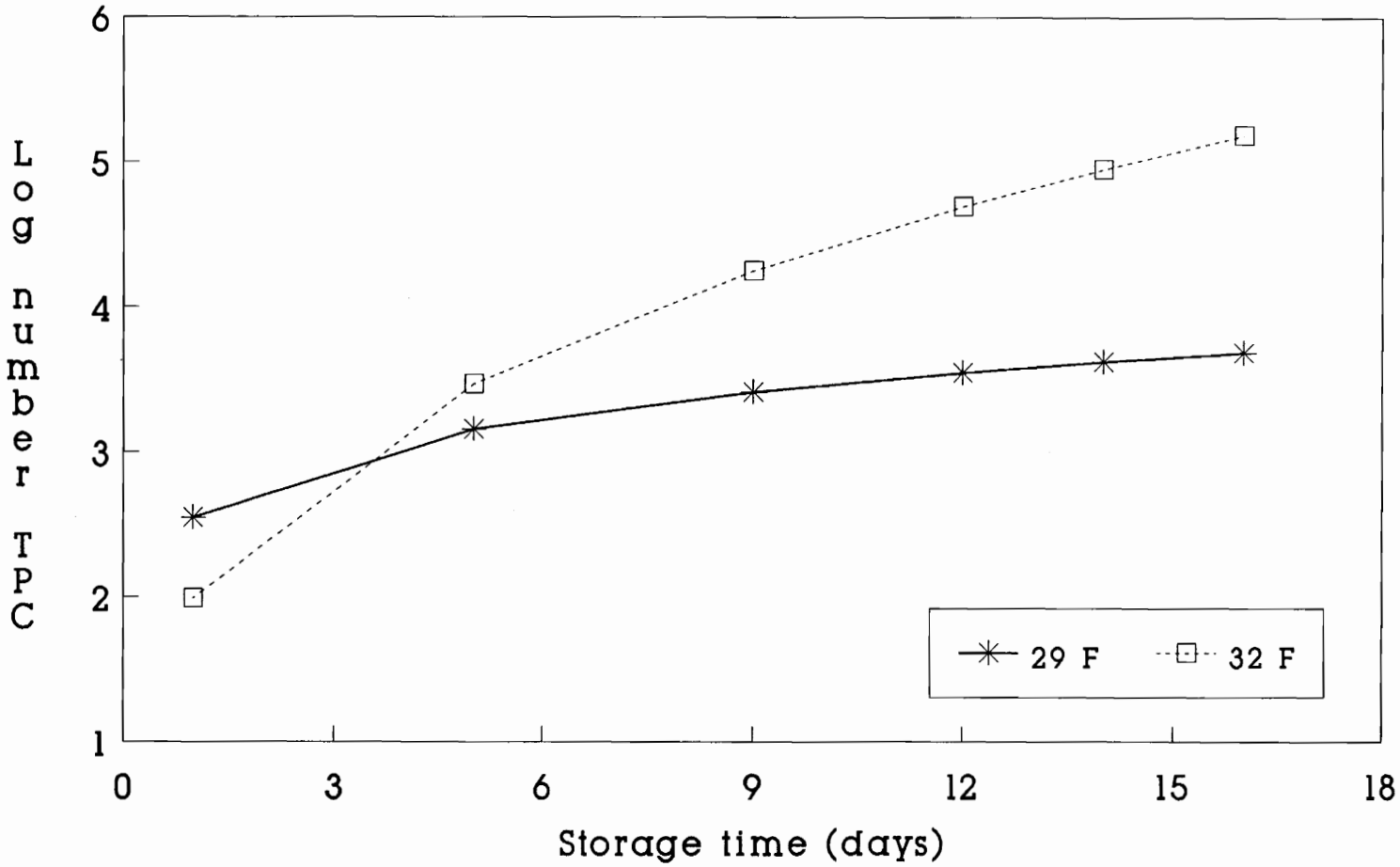

Figure 1: Effect of storage temperature and storage time on the TPC of sea trout.

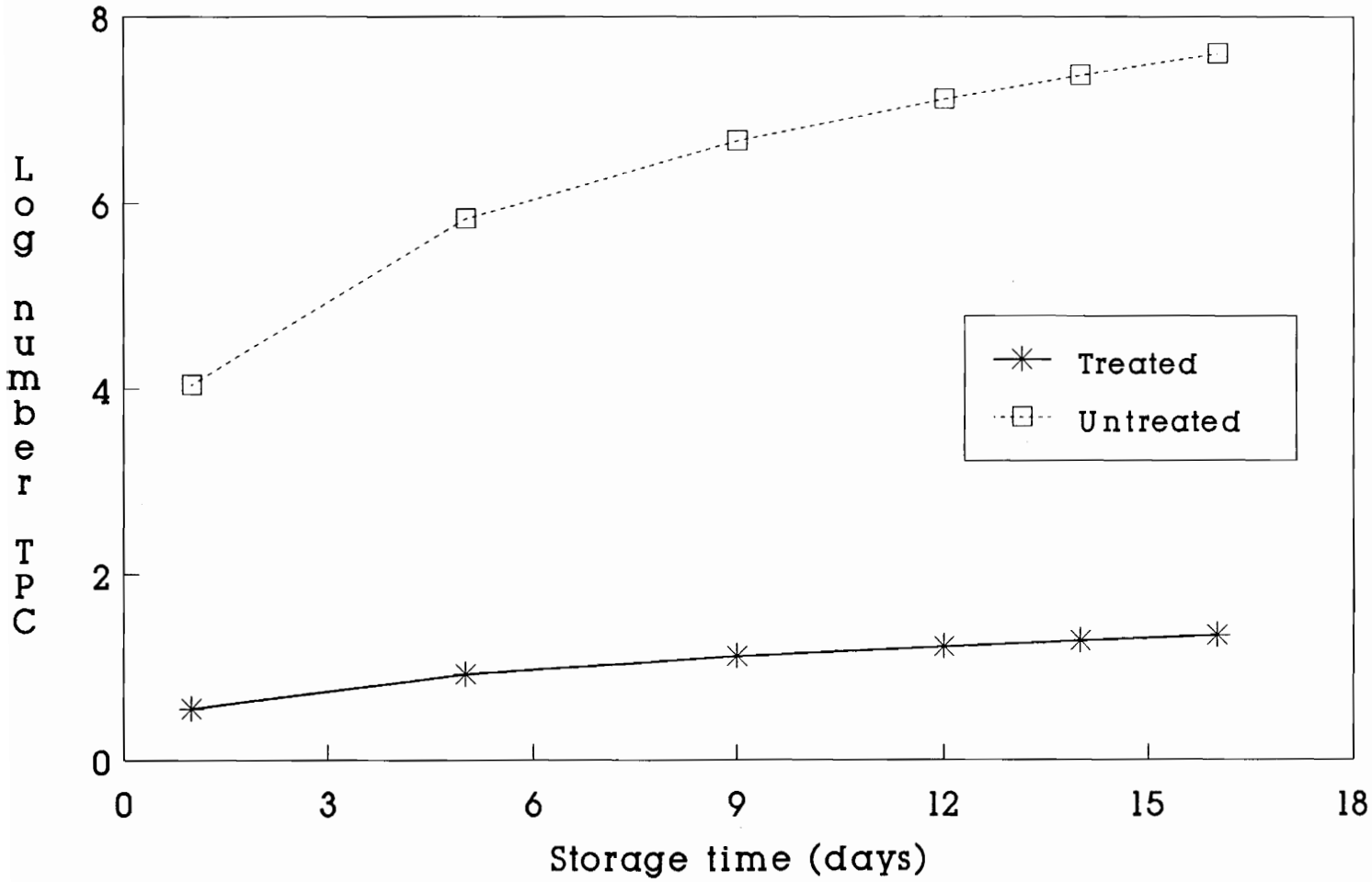

Figure 2: Effect of treatment and storage time on the TPC of sea trout.

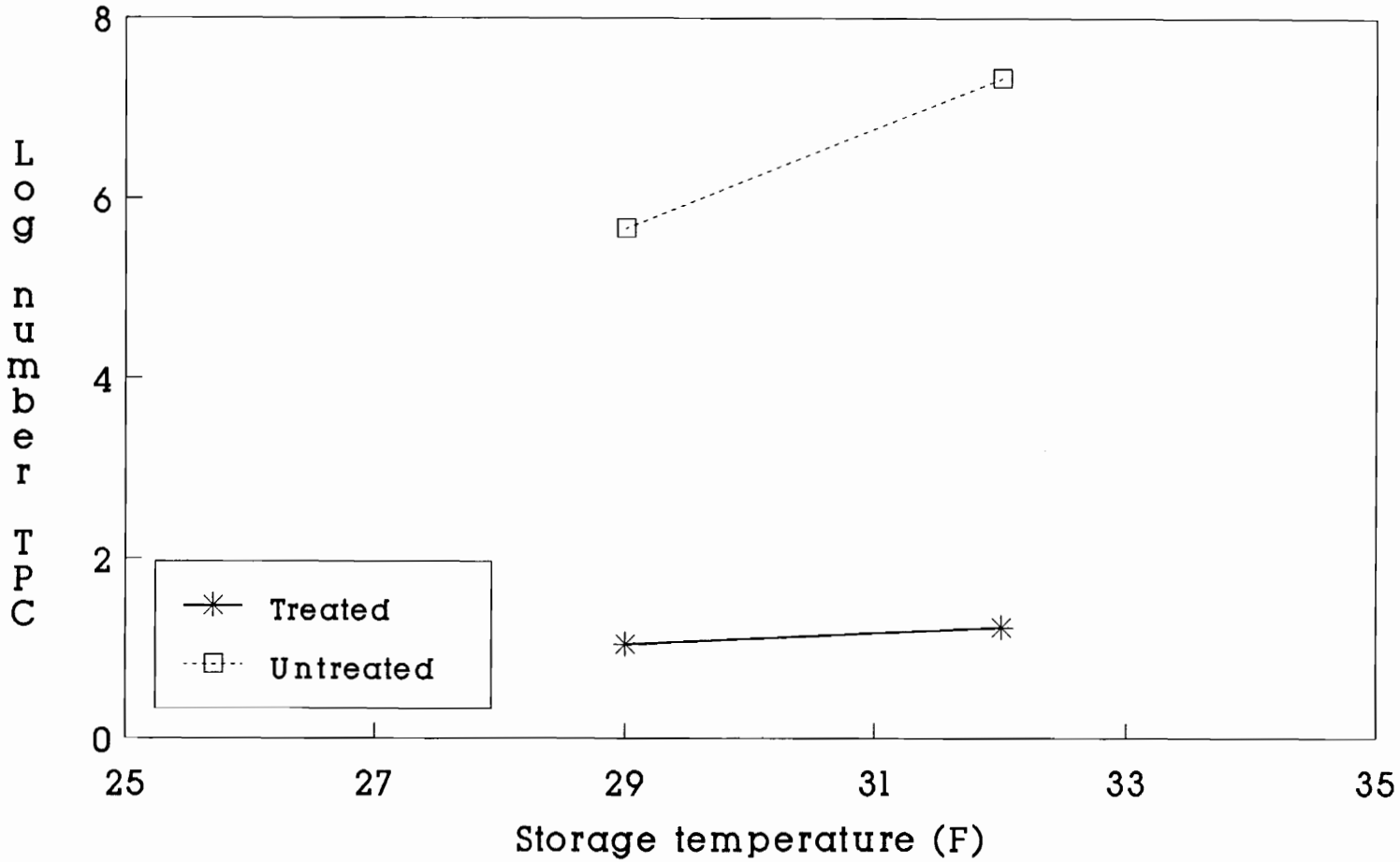

Figure 3: Effect of treatment and storage temperature on the TPC of sea trout.

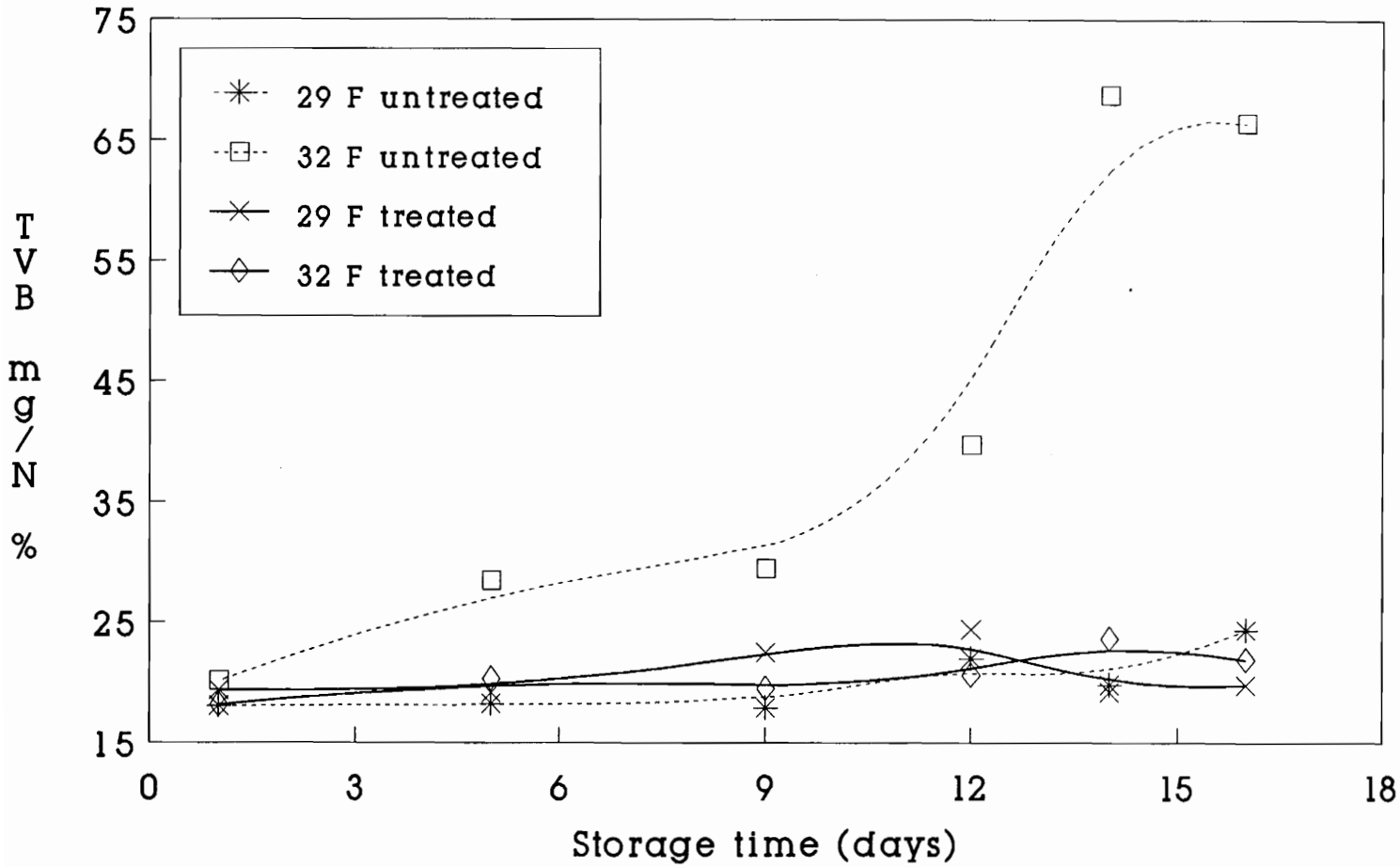

Figure 4: Effect of treatment, storage temperature and storage time on TVB present in sea trout.

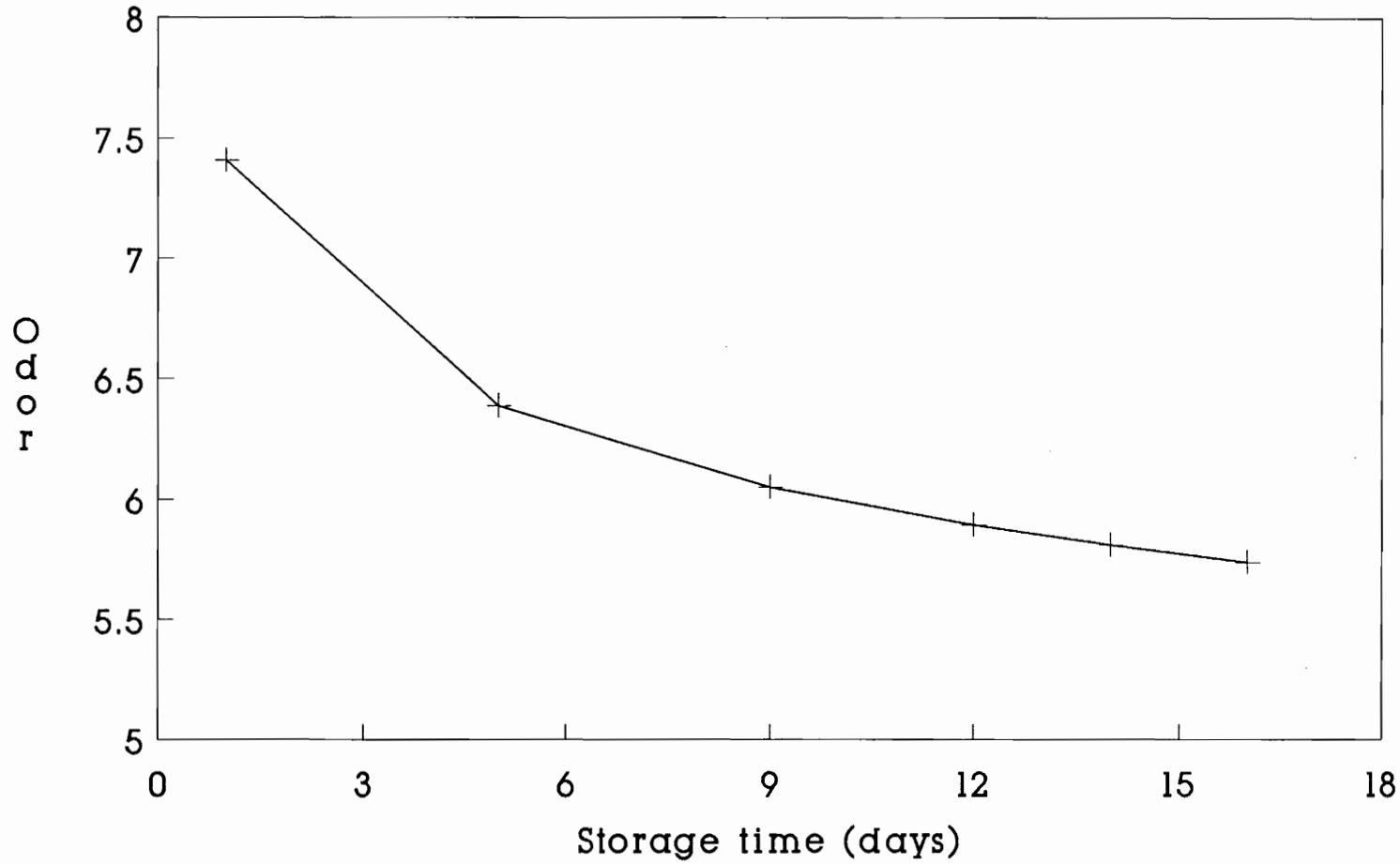

Figure 5: Effect of storage time on the odor of sea trout as determined by sensory evaluation.

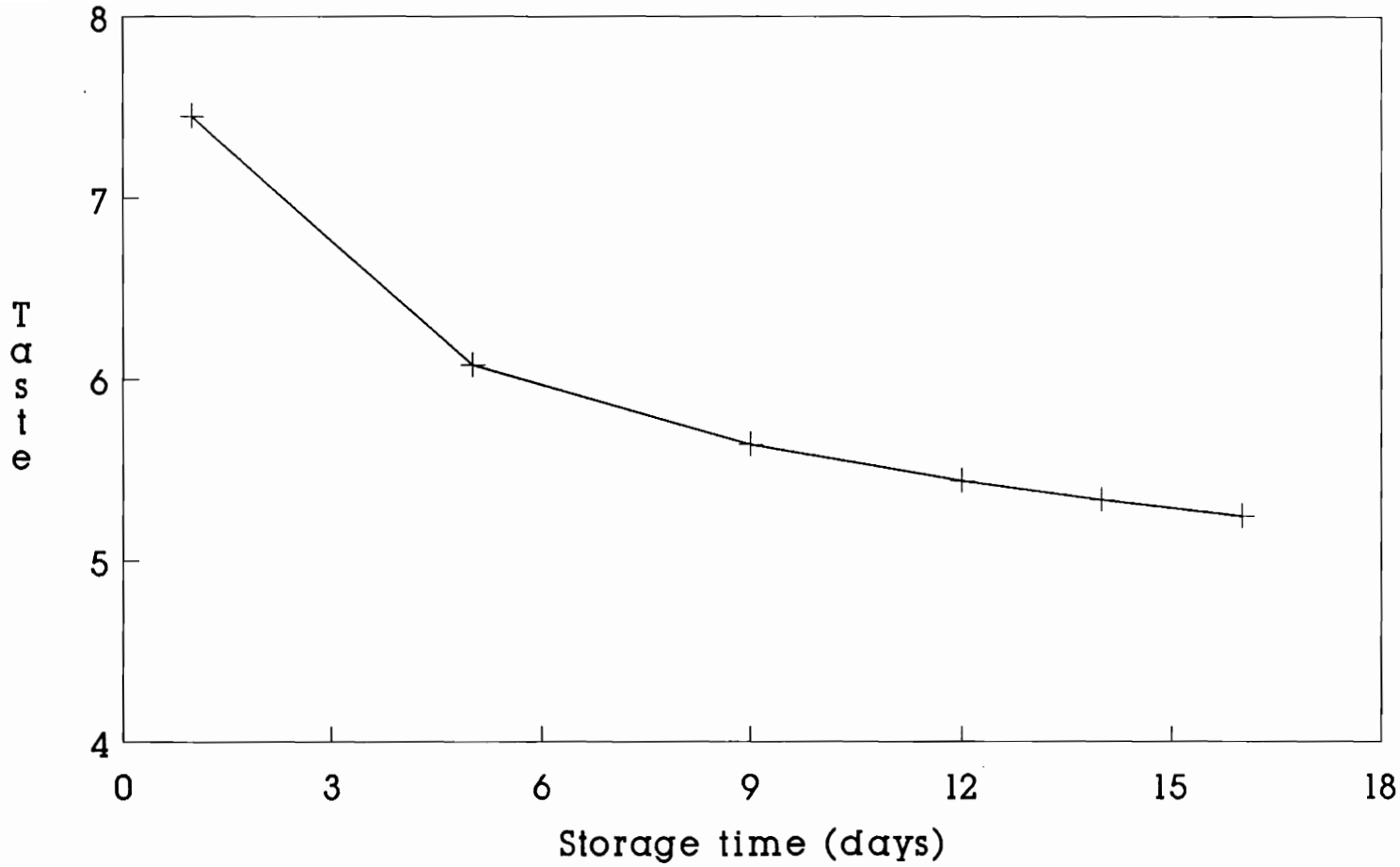

Figure 6: Effect of storage time on the taste of sea trout as determined by sensory evaluation.

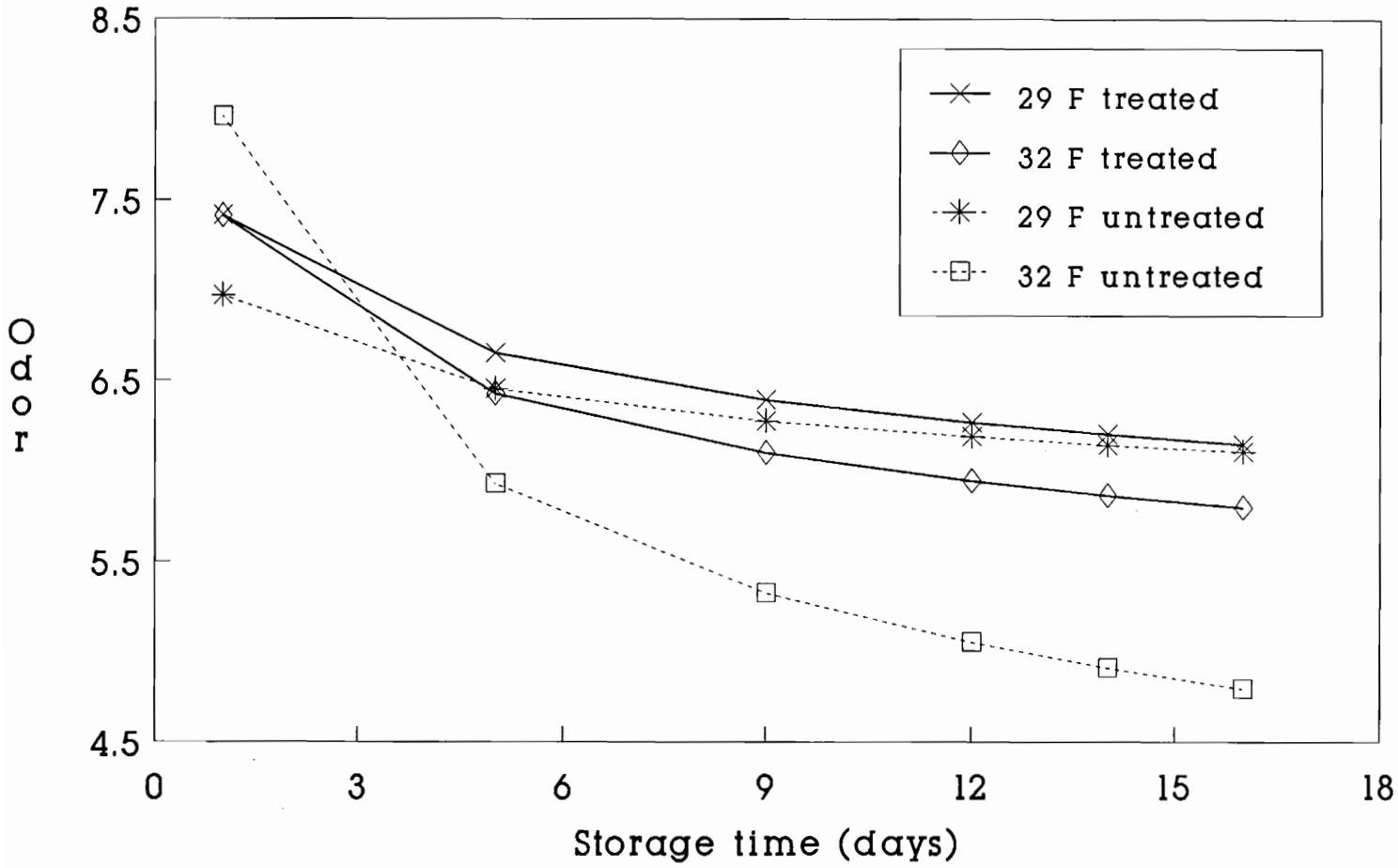

Figure 7: Effect of treatment, storage temperature and storage time on the odor of sea trout.

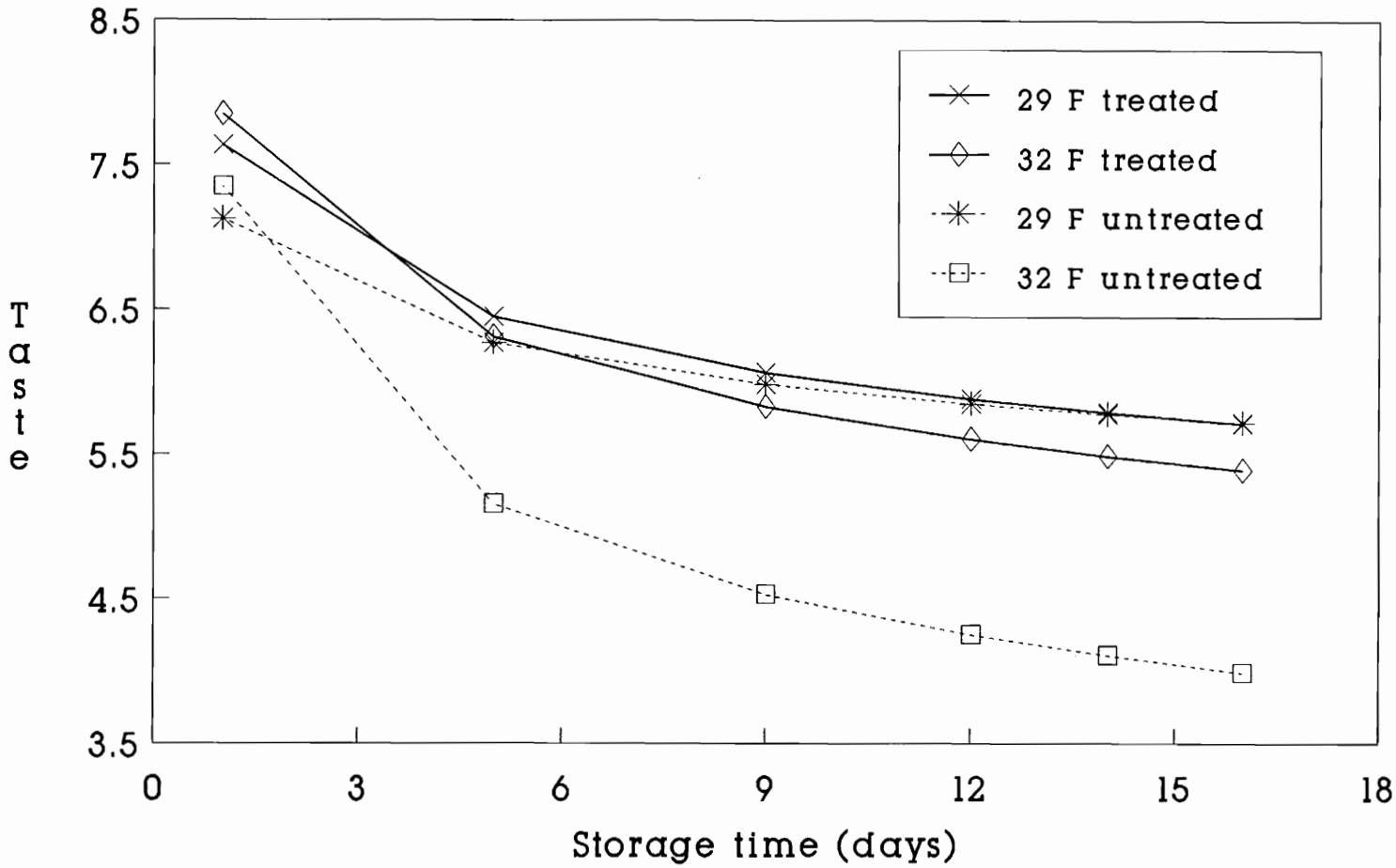

Figure 8: Effect of treatment, storage temperature and storage time on the taste of sea trout.

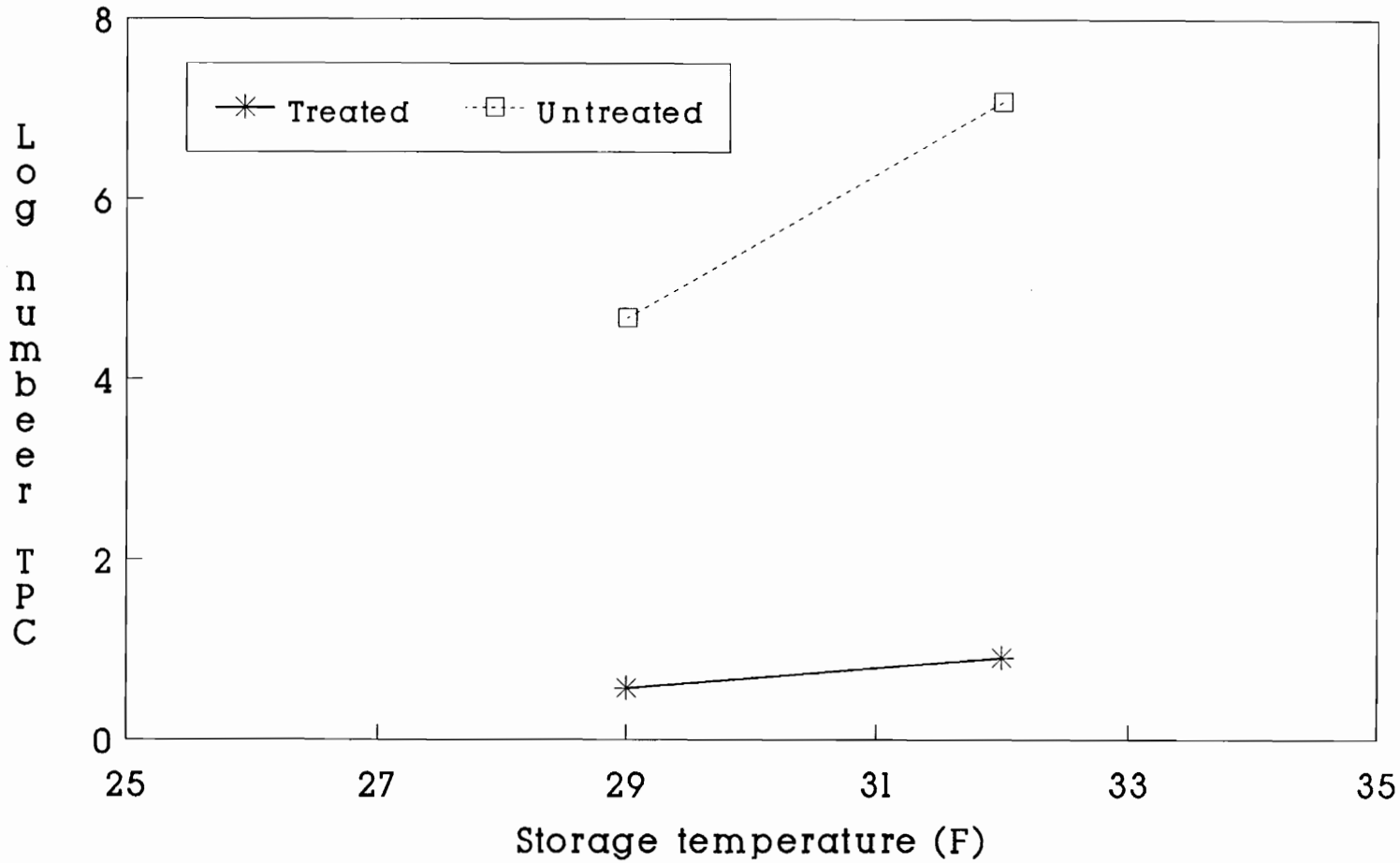

Figure 9: Effect of treatment and storage temperature on the TPC of spanish mackerel.

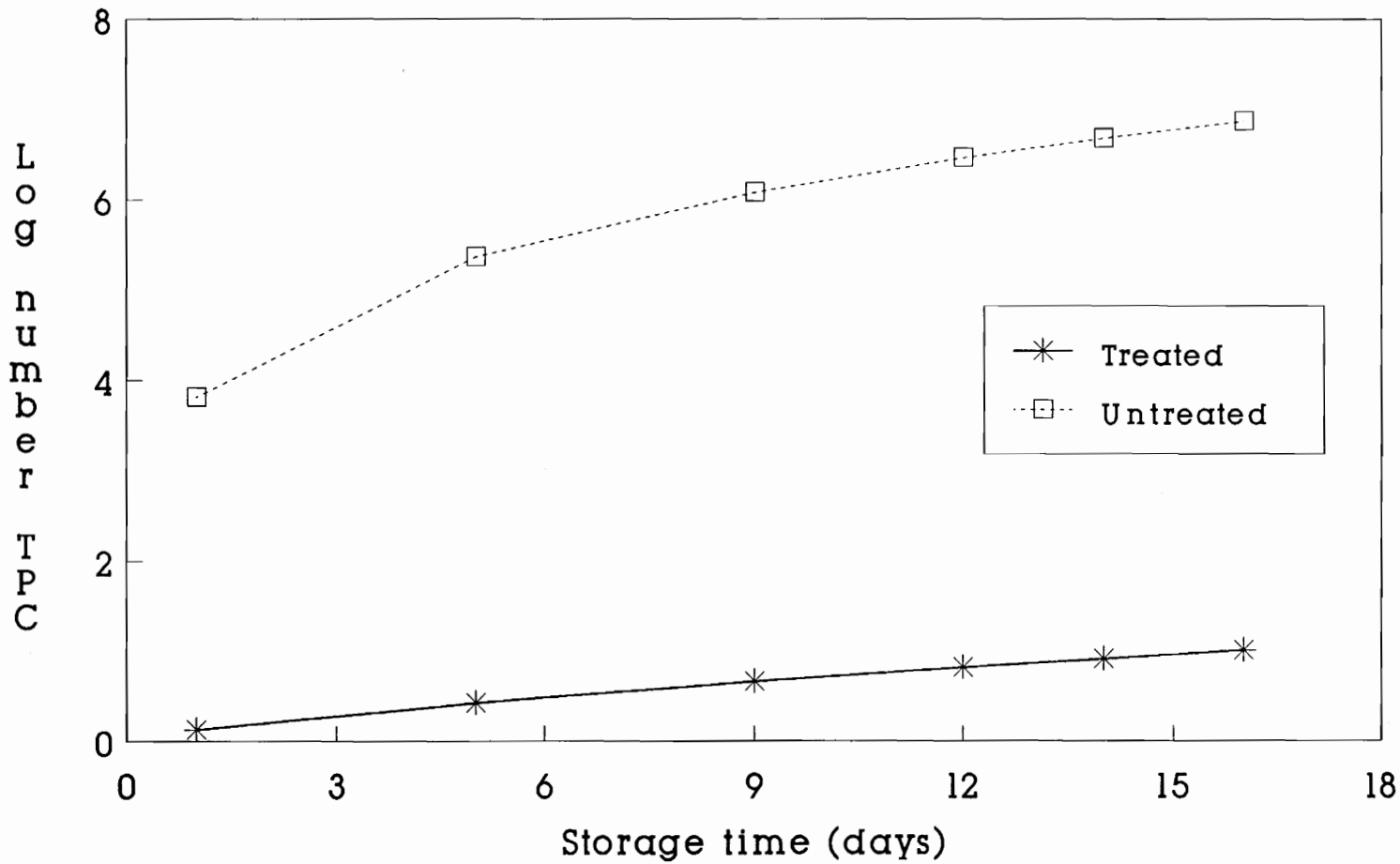

Figure 10: Effect of treatment and storage time on the TPC of spanish mackerel.

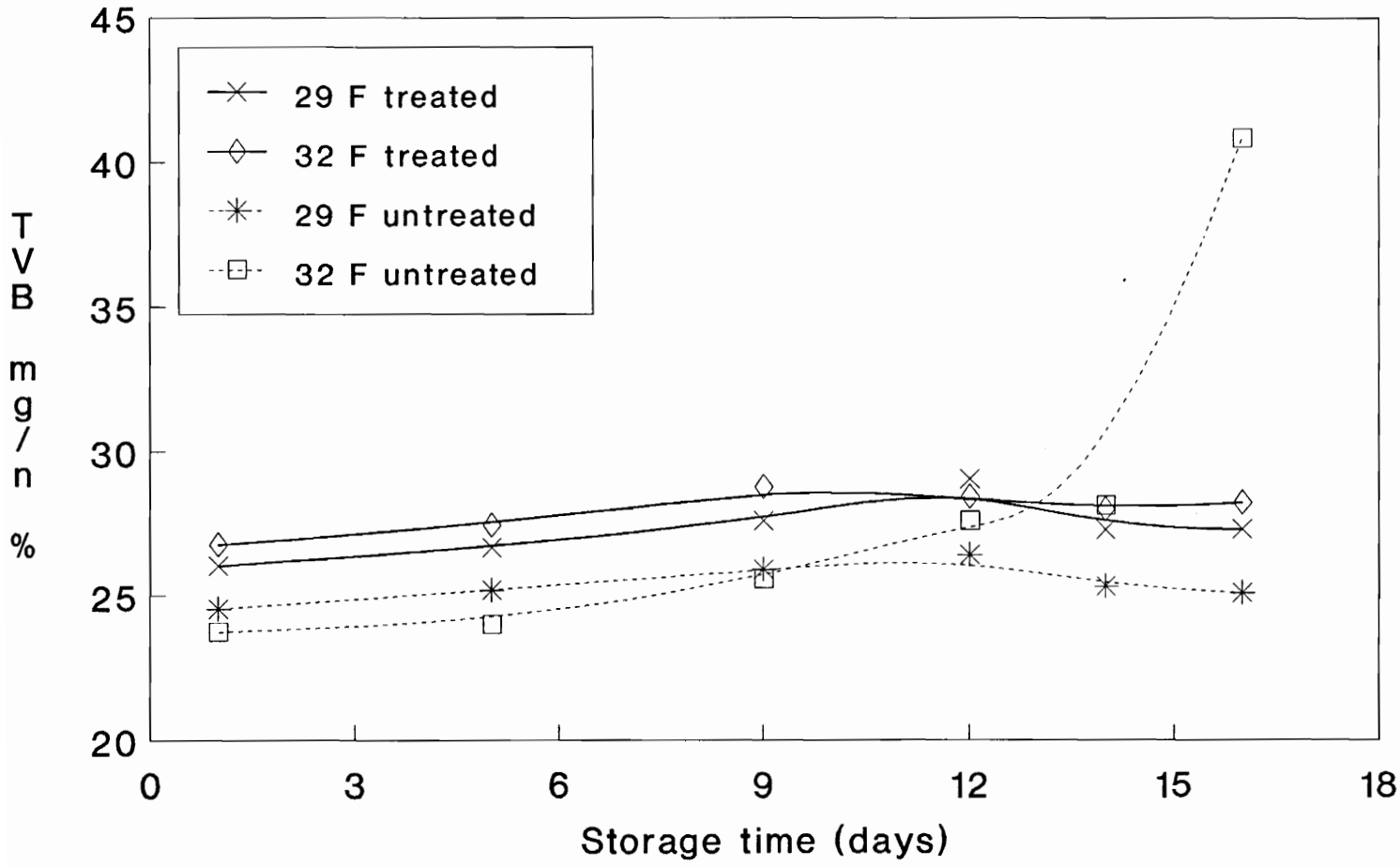

Figure 11: Effect of treatment, storage temperature and storage time on the TVB in spanish mackerel.

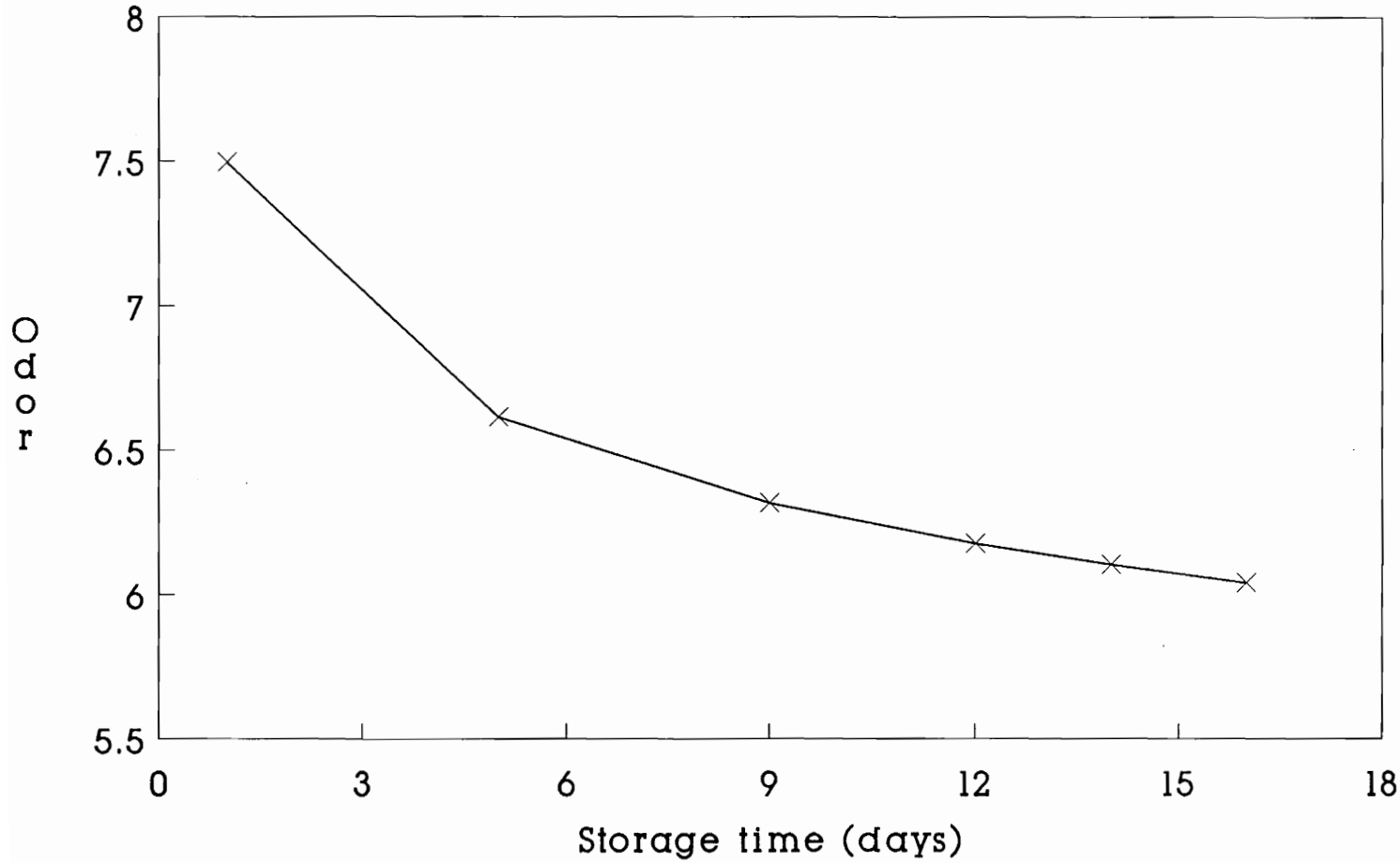

Figure 12: Effect of storage time on the odor of spanish mackerel as determined by sensory evaluation

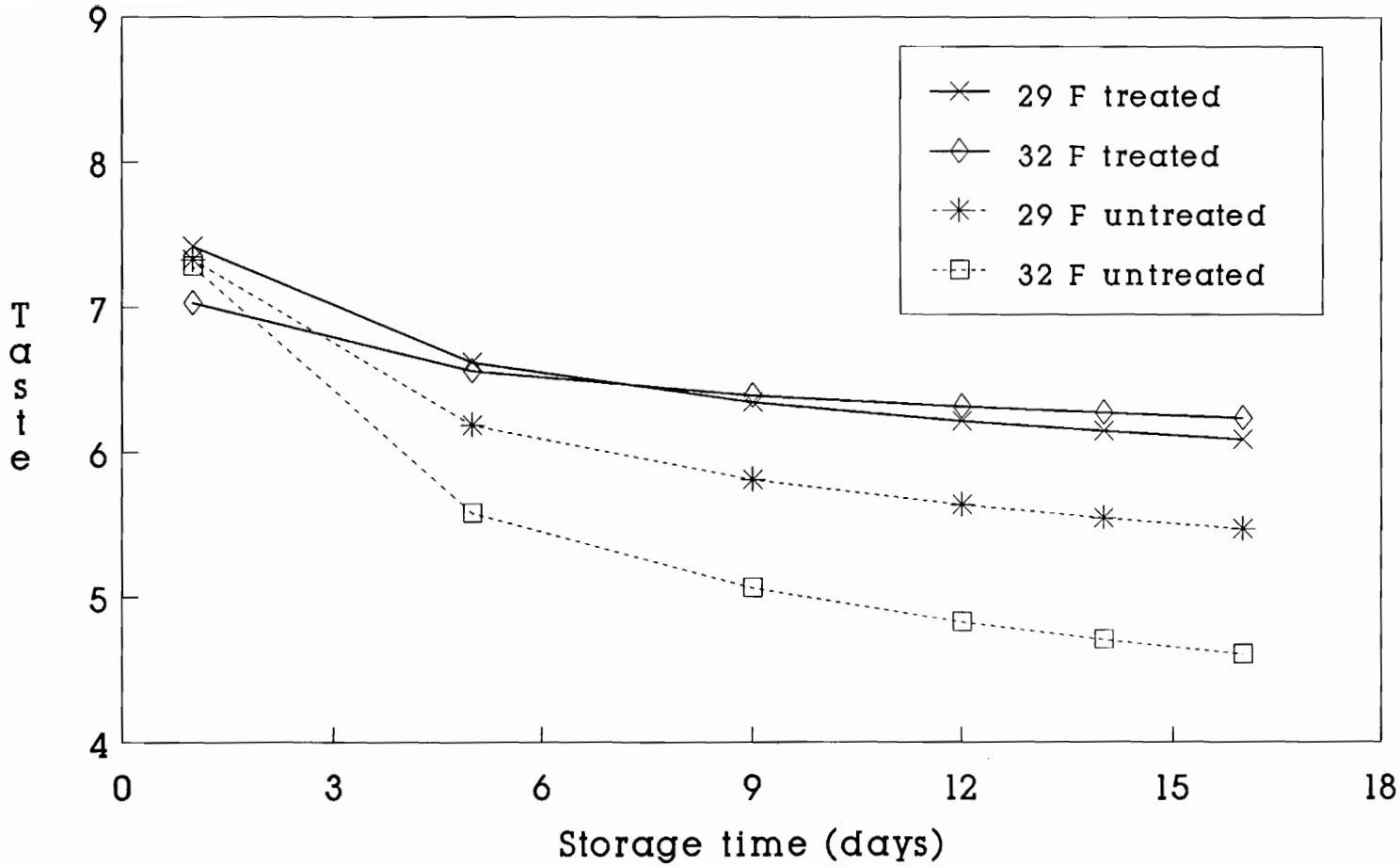

Figure 13: Effect of treatment, storage temperature and storage time on the taste of spanish mackerel.

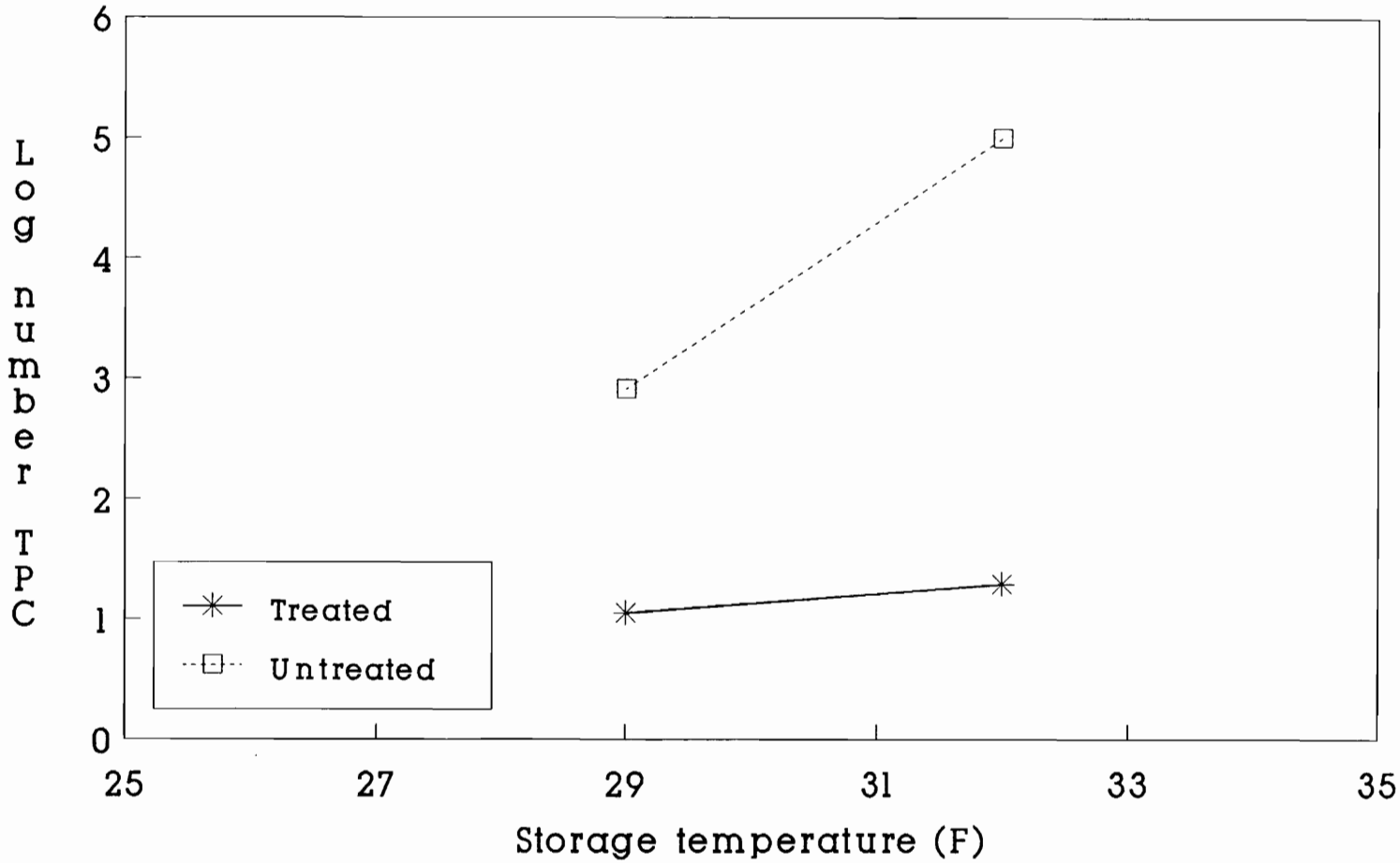

Figure 14: Effect of treatment and storage temperature on the TPC of catfish.

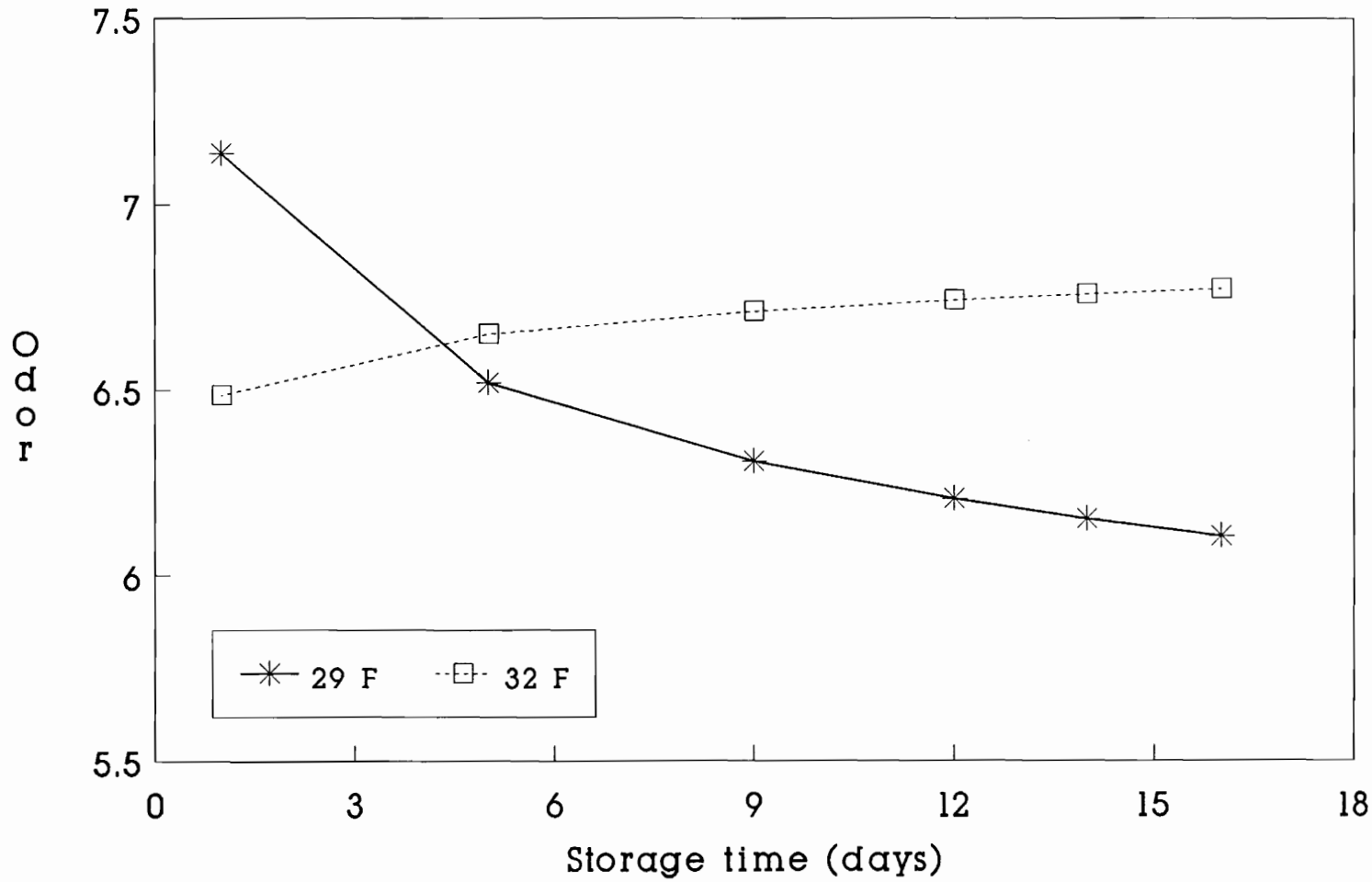

Figure 15: Effect of storage temperature and storage time on the odor of catfish

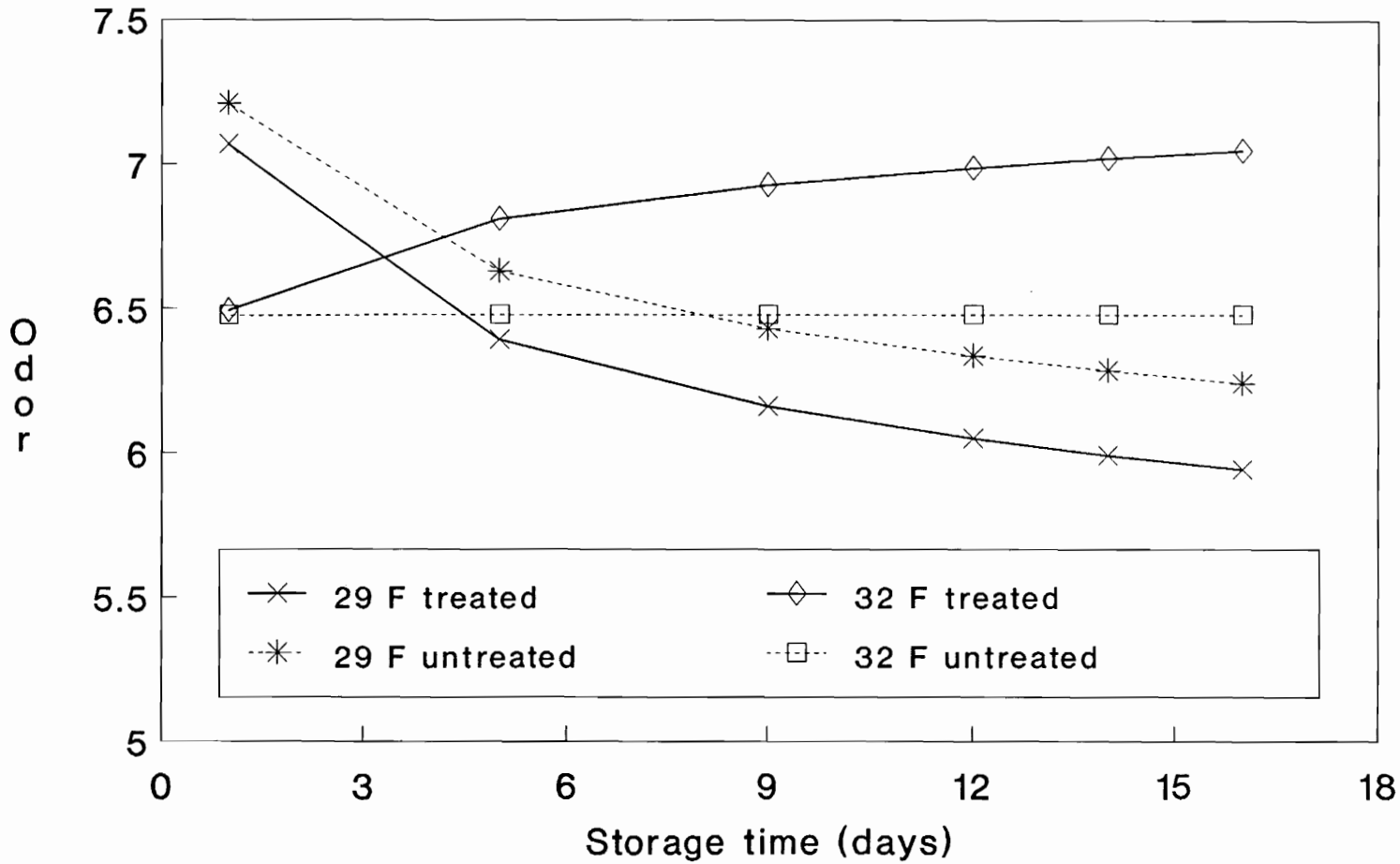

Figure 16: Effect of treatment, storage temperature and storage time on the odor of catfish.

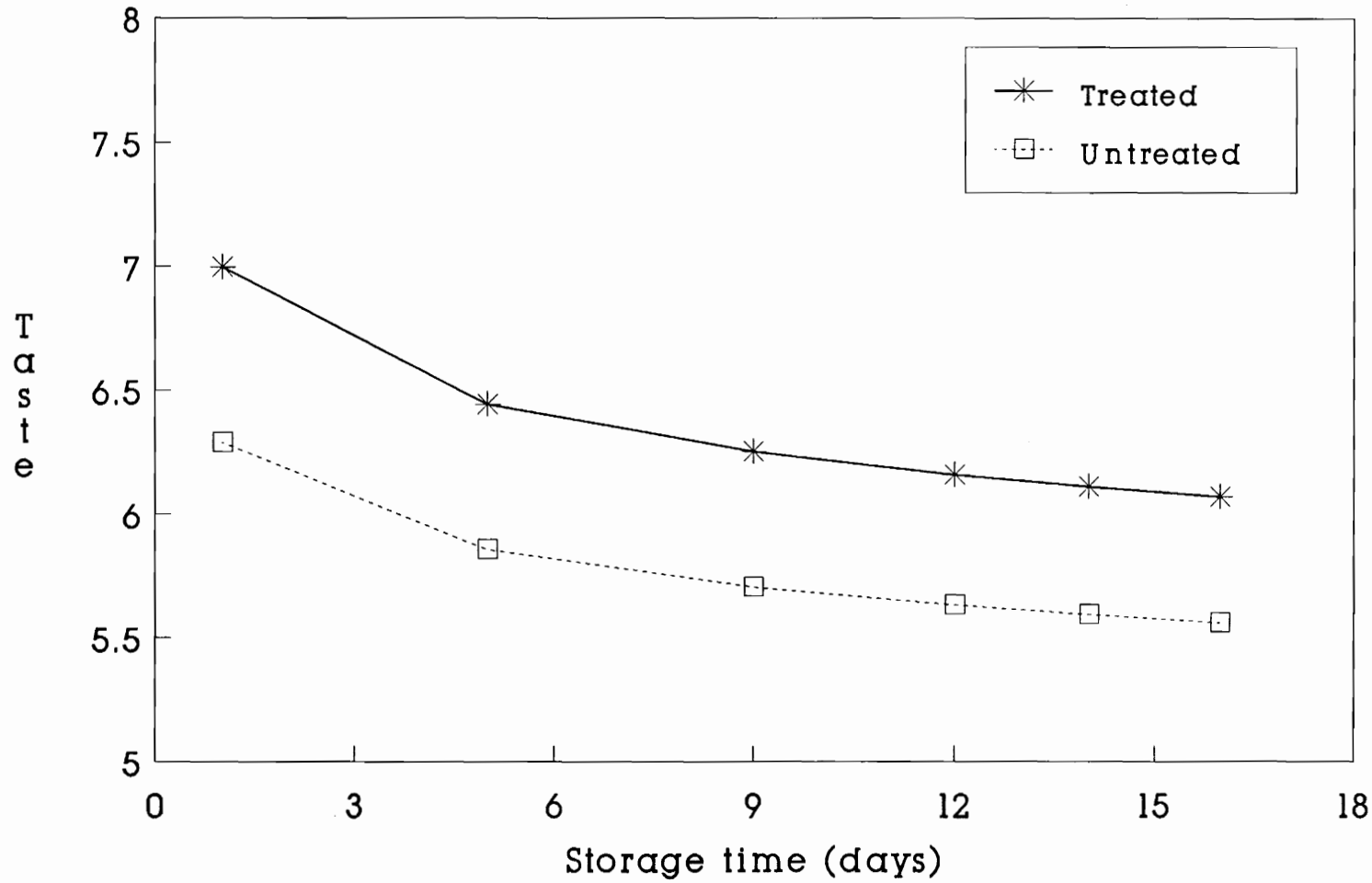

Figure 17: Effect of treatment and storage time on the taste of catfish.

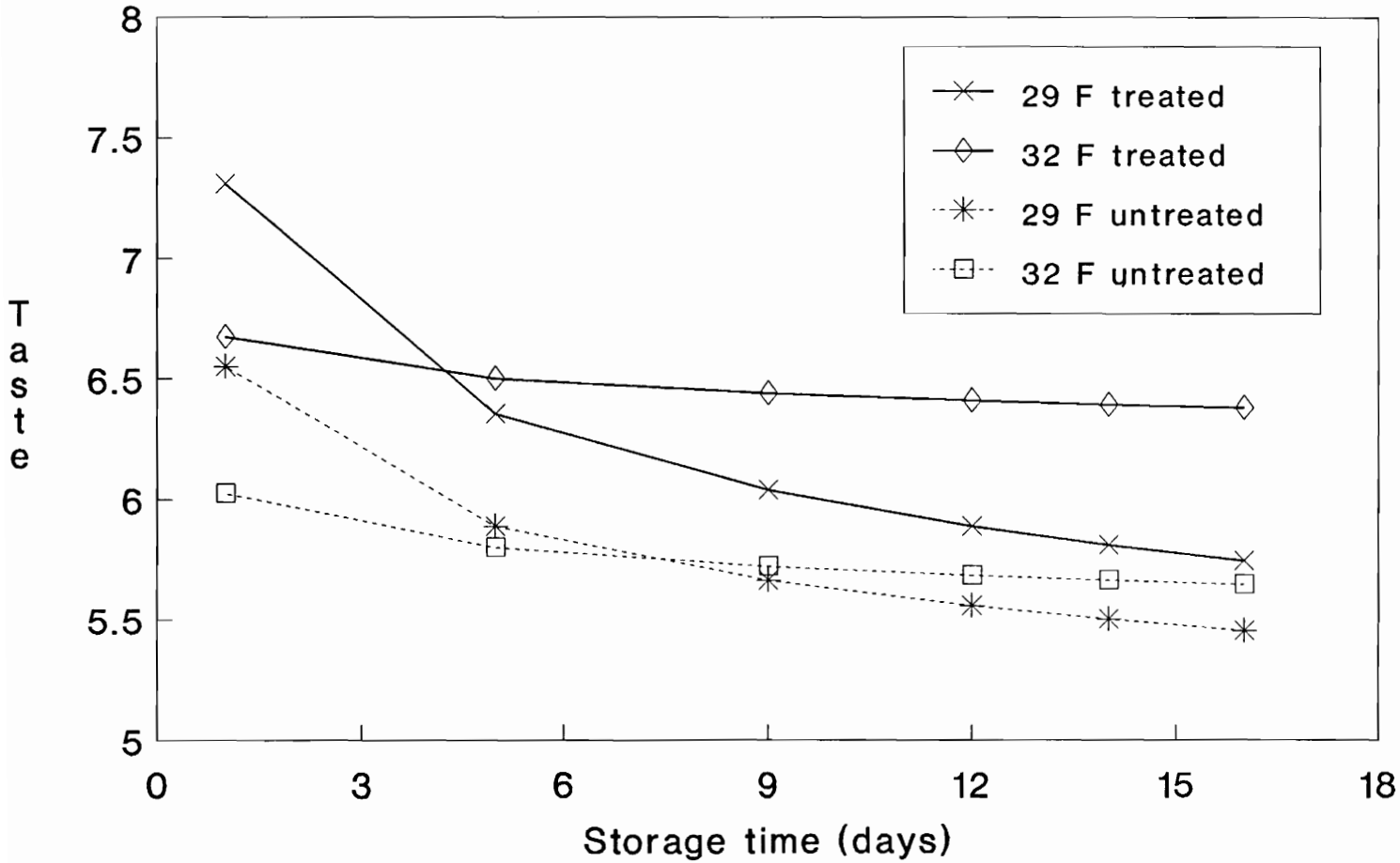

Figure 18: Effect of treatment, storage temperature and storage time on the taste of catfish.

IX. APPENDICES

Appendix 1: Enzymatic Analyses Methodology

B. Proteolytic Activity

Method I

Reagents

i)Buffer - citrate phosphate buffer and Tris-HCL buffer.

ii)Hemoglobin solution 2 %.

iii)Trichloroacetic acid (TCA)0.2 M

Equipment

i)Waring blender.

ii)Sorvall RC-5B Refrigerated Superspeed Centrifuge.

iii)Perkin-Elmer Lambda 3 UV/VIS Spectrophotometer.

Procedure

In this experiment various concentrations of the fish extract, various pH values of the buffer and different buffers were tried out to obtain a combination resulting in optimal enzyme activity.

Crude enzyme extract

The fish extract was prepared by the addition of an adequate amount of buffer to the fish sample, so as to obtain the desired concentration of the fish extract. This was homogenized for 2 min in a commercial Waring blender at high

speed. The homogenate was then centrifuged at 4°C and 13000 x g for 20 min. The supernatant was used as a crude enzyme extract for further study.

Assay

i)To 1.0 ml fish extract (enzyme solution) was added 1.0 ml buffer solution, followed by 0.5 ml substrate.

ii)The mixture was incubated at 37°C for 15 min before the addition of 5.0 ml 0.2 M TCA to precipitate undigested protein. The 15 min incubation time was selected by running a time course of the assay. Within this time interval the reaction is zero order.

iii)After another 10 min, the mixture was filtered through Whatman paper # 1.

iv)The amount of TCA soluble peptides produced by enzymic digestion was measured spectrophotometrically at 280 nm.

v)A blank was run simultaneously with each determination by reversing the addition order of substrate and TCA.

vi)The activity of proteolytic enzymes was expressed in hemoglobin units (HU). One HU was arbitrarily defined as 0.001/min increase in A_{280} under assay conditions (6).

Method II

Reagents

i)Protease Substrate Gel Tablets (Bio-Rad).

ii)Sodium phosphate buffer pH 5.70, 0.1 M.

iii)Calbiochem 53910 Protease.

iv)Acetic acid 3 % (v/v).

Equipment

i)Diffusion plates (Bio-Rad).

ii)Polytron homogenizer.

Procedure

Sample preparation

To 1.0 gm fish tissue was added 10.0 ml buffer. Homogenize for 2 min using a Polytron homogenizer.

Assay

i)Two substrate gel tablets were placed in an 18 x 150 mm test tube and 10.0 ml distilled water was added. The tablets were allowed to hydrate for 15 min at room temperature, and then the mixture was thoroughly vortexed to obtain complete suspension of the tablets. Failure to adequately solubilize the substrate (casein) and buffer components before heating will result in a heterogenous plate.

ii)The suspension was heated in a boiling water bath for 4 min (until the agar dissolved completely). The solution was vortexed and checked visually to ascertain that all solids

had dissolved.

iii)The hot agar melt was pipeted onto a plastic diffusion plate and the solution was allowed to cool undisturbed to room temperature.

iv)After the gel had set and cooled to room temperature, 5.0 mm diameter sample wells were punched in the gel using a cork borer.

v)Using a micropipete the sample wells were filled with 25 microlitres of sample.

vi)The plates were covered and incubated at 20°C for 24 hr.

vii)Termination of the protease digestion and enhancement of the rings is accomplished by overlaying the plate with a solution of 3 % (v/v) acetic acid. The plates can be rinsed after 10 min with water and the diameter of the rings measured.

viii)Units of activity can be read off the standard curve.

B. Lipolytic Activity

Method I

Reagents

i)Extracting solution

0.25 M sucrose/0.175 M KCl/1 mM EDTA solution

pH adjusted to 7.2 - 7.3

ii)Citrate - phosphate buffer0.1 M, pH = 4.2 - 4.3

iii)Triton X-100 15 % (w/v).

iv)Substrate dispersion

Tridecanoylglycerol

Gum Arabic solution (10 % in citrate-phosphate buffer)

v)Chloroform

vi)Copper reagent:

1 N acetic acid

1 M 2,2′,2"-nitrilotriethanol

Cupric nitrate solution 6.45 %

vii)DPCH solution - 1,5-diphenylcarbohydrazide

0.5 % (w/v) solution in methanol.

Equipment

i)Sorvall RC - 5B Refrigerated SuperspeedCentrifuge.

ii)Perkin-Elmer Lambda 3 UV/VIS Spectrophotometer.

iii)Shaking water bath.

iv)Clinical centrifuge.

Procedure

Sample preparation

i)To 4.0 gm muscle was added 16.0 ml cold extracting solution and this was blended for 1 min in the blender.

ii)The homogenate was centrifuged at 1000 x g for 10 min and the supernatant was kept aside.

iii)The pellet was resuspended in 10.0 ml of extracting solution and centrifuged at 1000 x g for 10 min. The

supernatant was kept aside.

iv)To 0.4 g of pellet was added 4.0 ml of cold extracting solution. This is called the ´sediment preparation´.

v)The supernatants obtained from steps ii) and iii) were combined and centrifuged at 27000 x g for 20 min.

vi)The pellet obtained was resuspended in 3.0 ml cold extracting solution and mixed together by a short burst of blender. This is called the ´lysosomal preparation´.

vii)The supernatant from step v) is called the ´supernatant preparation´.

Assay

i)0.80 ml citrate phosphate buffer, 0.20 ml 15 % Triton X-100, 0.25 ml substrate dispersion and 0.25 ml enzyme preparation (pre-incubated for 60 min in a water bath) at 37°C) were added together in a test tube. This is the test sample. For the blank, all the reagents as above, were placed in a test tube, except for the substrate dispersion.

ii)The blank and sample were incubated at 37°C for three hours in a shaking water bath. Test tubes were tilted at 60° angle and 200 oscillations/min.

iii)At the end of three hours the reaction was stopped by the addition of 2.5 ml copper reagent (at this time 0.25 ml substrate dispersion was added to the blank).

iv)The mixture was vortexed for 30 seconds.

v)10.0 ml chloroform was added and the tubes were shaken

manually 100 times.

vi)4.0 ml of the chloroform was transferred with a Pasteur pipet to a 15 x 125 ml tube. 2.0 ml of water was added on top of chloroform. This mixture was centrifuged at 510 x g for 10 min in a clinical centrifuge.

vii)The aqueous layer was removed completely.

viii)Aliquots of 0.5 ml of chloroform solution of copper soap were diluted to 2.0 ml with chloroform and 1.0 ml DPCH solution.

ix)The absorbance was read at 540 nn.

x)Reagent blank was prepared by the addition of 2.0 ml chloroform solution of copper soap and 1.0 ml complexing agent (DPCH).

Method II

Reagents

i)Sigma lipase substrate

Olive oil 50 % (v/v) and sodium azide 0.1 %

ii)Trizma buffer.

Tris(hydroxymethyl)amino methane 0.2mol/litre pH 8.0 and sodium azide 0.1 %

iii)Thymolphthalein indicator solution.

iv)Sodium hydroxide 0.05 N.

v)Ethyl alcohol 95%

Equipment

i)Water bath at 37°C.

ii)Polytron homogenizer.

Procedure

Sample preparation

To 1.0 g fish sample was added 10.0 ml distilled water. This was homogenized for 2 min with a Polytron homogenizer. Various concentrations of fish extract were tested.

Assay

i)Into each of two test tubes were pipeted the following:

Reagent (ml)	Test (ml)	Blank (ml)
Water	2.5	2.5
Sigma lipase substrate	3.0	3.0
Trizma buffer	1.0	1.0
Enzyme extract	1.0	-

ii)The tubes were capped with clean rubber stoppers and shaken vigorously for 5 seconds.

iii) Both tubes were placed in a constant temperature water bath at 37°C, and incubated for 6 hours.

iv)Immediately after starting the incubation, 1.0 ml enzyme extract was pipeted into a 50.0 ml Erlenmeyer flask. This flask was marked ´Blank´ and stored in the

refrigerator.

v)After the 6 hour incubation period, the contents of the ´Blank´ tube were poured into the ´Blank´ flask and the contents of the ´Test´ tube were poured into a clean 50.0 ml Erlenmeyer flask, and this was marked ´Test´.

vi)Into each of the two tubes (´Blank´ and ´Test´) were pipeted 3.0 ml 95 % ethyl alcohol. Shake to rinse and pour into their respective flasks.

vii)To each flask was added Thymolphthalein indicator.

viii)A 25.0 ml buret was set up, filled with 0.05 N NaOH solution.

ix)Each flask was titrated to a slight but definite blue(not dark blue) color.

Note:

- the ´Blank´ and ´Test´ must be titrated to the same color intensity.

-the colored end point is stable for about 30 seconds and then gradually fades.

x)Sigma-Tietz Units of lipase are exactly equal to the ml of 0.05 N NaOH required to neutralize the fatty acids liberated during incubation period of the test. The ml of 0.05 N NaOH required is obtained by subtracting the ml of NaOH used for ´Blank´ from the ml of NaOH used for the ´Test´. Lipase activity in Sigma-Tietz Units/ml may be converted to International Units/litre (U/L) by multiplying by 280. Sigma Enzyme Control 2E Elevated Range can be used for the

preparation of the standard curve and controls.

Appendix 2: Standard Deviation Data

Table A: Standard deviation data for the effect of storage temperature and storage time on the microflora (Total Plate Count) of sea trout.

Storage temp. (°F)	Storage time (days)	Standard deviation
29	1	1.71
29	5	2.16
29	9	2.47
29	12	2.99
29	14	3.05
29	16	2.76
32	1	2.24
32	5	2.75
32	9	3.92
32	12	3.68
32	14	4.40
32	16	3.15

Table B: Standard deviation data for the effect of treatment and storage time on the microflora (Total Plate (Count) of sea trout.

Treatment	Storage time (days)	Standard deviation
Treated	1	0.70
Treated	5	0.84
Treated	9	0.69
Treated	12	0.34
Treated	14	0.66
Treated	16	0.74
Untreated	1	0.24
Untreated	5	0.74
Untreated	9	1.35
Untreated	12	1.12
Untreated	14	1.64
Untreated	16	1.34

Table C: Standard deviation data for the effect of treatment, storage temperature and storage time on the Total Volatile Bases in sea trout.

Treatment	Storage temp. (°F)	Storage time (days)	Standard deviation
1	29	1	1.29
1	29	5	1.56
1	29	9	5.48
1	29	12	10.54
1	29	14	1.40
1	29	16	1.27
1	32	1	1.39
1	32	5	3.25
1	32	9	2.02
1	32	12	3.18
1	32	14	4.19
1	32	16	1.92
2	29	1	1.70
2	29	5	1.26
2	29	9	2.17
2	29	12	4.83
2	29	14	1.23
2	29	16	9.11
2	32	1	0.88
2	32	5	13.88
2	32	9	15.08
2	32	12	17.45
2	32	14	22.11
2	32	16	20.82

Treatment: 1=treated; 2=untreated

Table D: Standard deviation data for the effect of treatment and storage time on the microflora (Total Plate Count) of spanish mackerel.

Treatment	Storage time (days)	Standard deviation
Treated	1	0.25
Treated	5	0.59
Treated	9	0.48
Treated	12	0.59
Treated	14	0.72
Treated	16	0.49
Untreated	1	0.47
Untreated	5	1.98
Untreated	9	1.95
Untreated	12	1.58
Untreated	14	2.06
Untreated	16	1.68

Table E: Standard deviation data for the effect of treatment, storage temperature and storage time on Total Volatile Bases in spanish mackerel.

Treatment	Storage temp. (°F)	Storage time (days)	Standard deviation
1	29	1	1.60
1	29	5	2.21
1	29	9	1.12
1	29	12	1.97
1	29	14	1.19
1	29	16	1.17
1	32	1	1.45
1	32	5	1.79
1	32	9	1.42
1	32	12	0.78
1	32	14	0.71
1	32	16	1.16
2	29	1	1.33
2	29	5	1.43
2	29	9	1.59
2	29	12	1.68
2	29	14	1.48
2	29	16	2.08
2	32	1	0.81
2	32	5	2.13
2	32	9	1.47
2	32	12	2.01
2	32	14	1.17
2	32	16	4.25

Treatment: 1=treated; 2=untreated

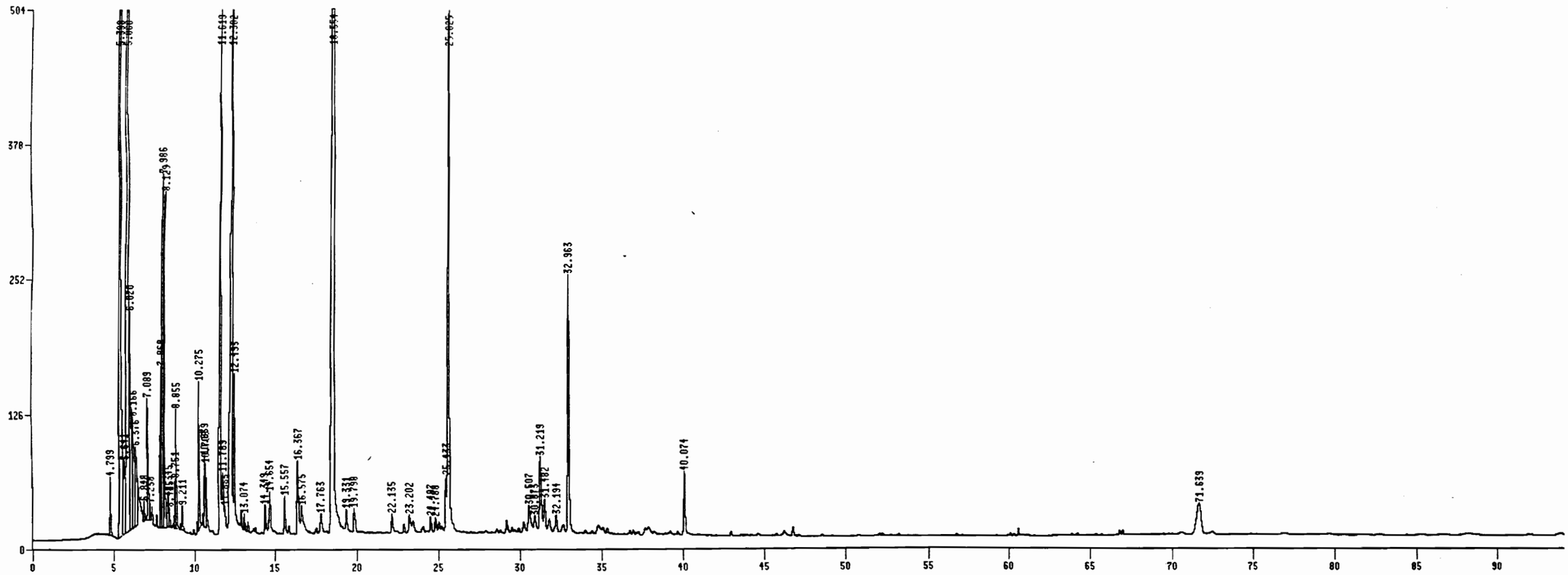

Figure 1: Gas chromatogram of treated spanish mackerel stored at 32^{0}F for 16 days.

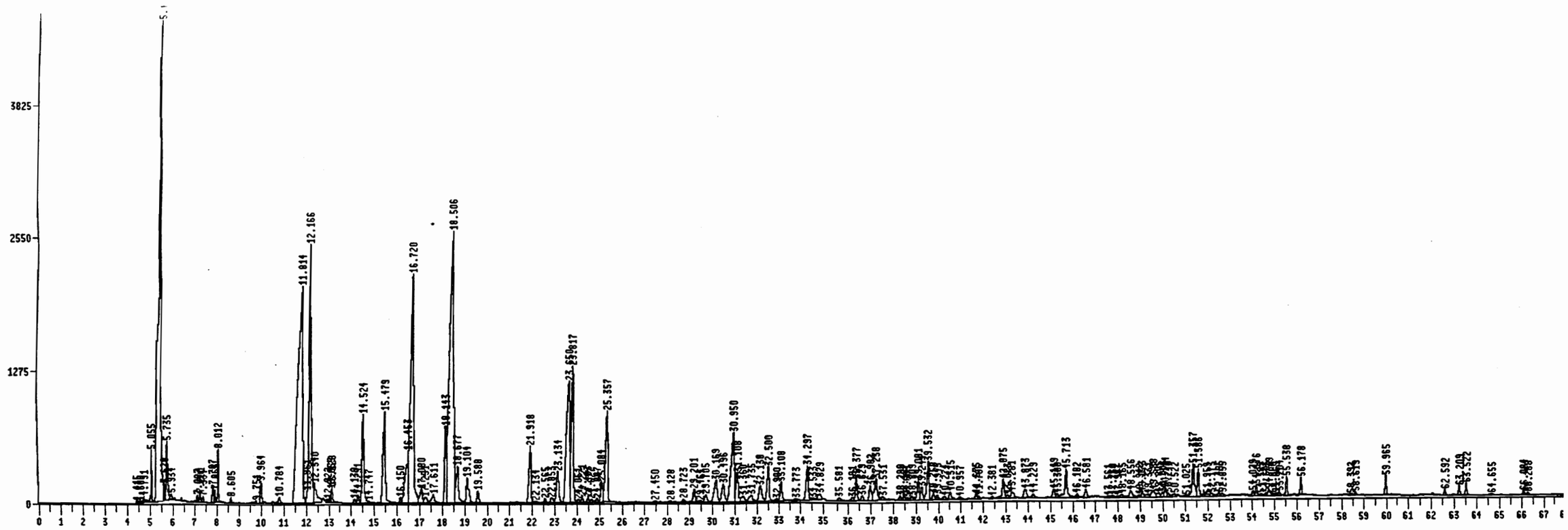

Figure 2: Gas chromatogram of untreated sea trout stored at $32^{0}F$ for 5 days.

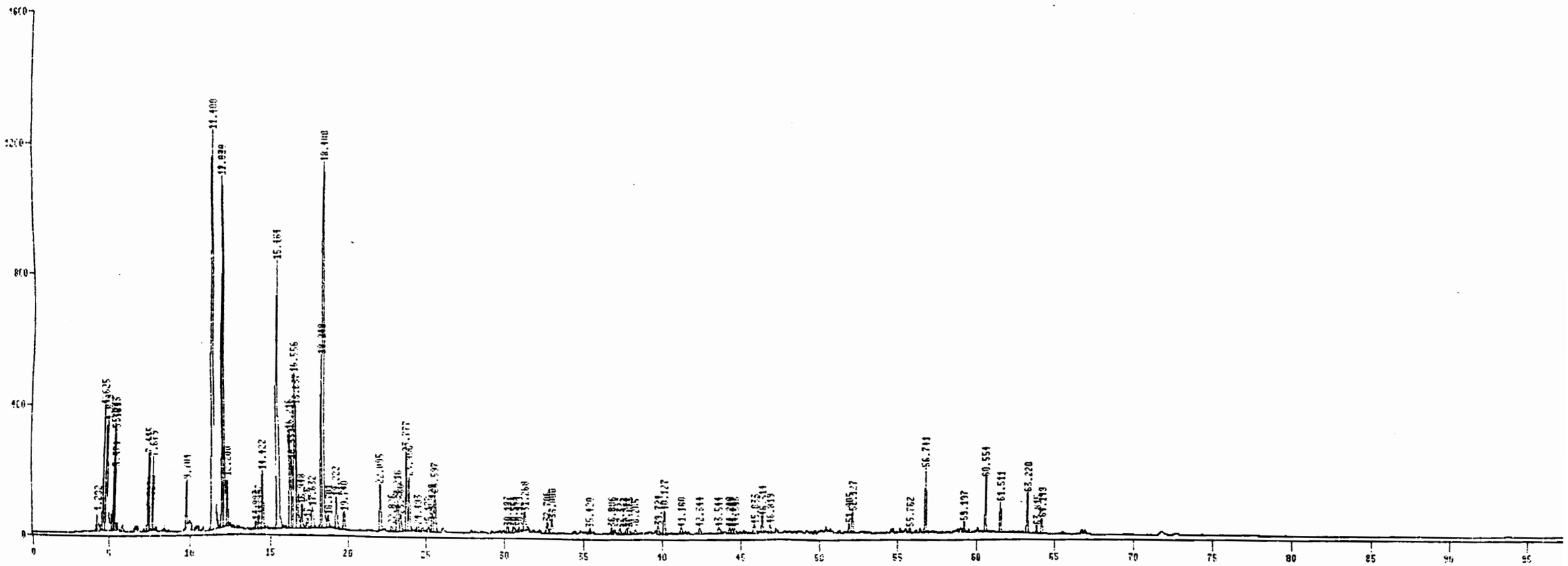

Figure 3: Gas chromatogram of untreated sea trout stored at $29^{0}F$ for 12 days.

VITAE

Mala Barua, the author of this thesis, was born on February 3, 1966 in Bombay, India. In 1982 she graduated from Bombay International School and entered St. Xavier´s College and was awarded a Bachelor of Science degree in Life Sciences in 1987.

In Fall of 1987, Ms. Barua joined the Graduate School of Virginia Polytechnic Institute and State University to pursue a Master of Science degree in Food Science and Technology.

Ms. Barua is a member of the Institute of Food Technologists.