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Pasteurization radiation offers a new means of extending the refrigerated storage life of foods. This procedure avoids many of the undesirable changes which result when sterilization radiation is used and still eliminates most of the spoilage organisms.

Shrimp was treated with 5 ppm chlortetracycline and subjected to pasteurization levels of gamma radiation and stored at 38° F. The levels of irradiation used were 0.5 and 0.75 megarad. These levels were based on the flavor threshold of irradiation intensity.

The quality of the stored irradiated shrimp was determined by subjective evaluation, chemical analyses and microbiological examinations.

The storage life of the shrimp irradiated at 0.5 megarad was extended to 5 weeks, compared with 1 week for the unirradiated samples held at the same temperature. Throughout the ten week storage period, the samples which received doses of 0.5 megarad

and CTC and those which received 0. 75 megarad with and without CTC remained in good condition.

RADIATION PASTEURIZATION OF RAW AND CHLORTETRACYCLINE-TREATED SHRIMP

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AHMED ALI AWAD

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Associate Professor of Food Science and Technology

In Charge of Major

Head of Department of Food Science and Technology

Dean of Graduate School

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INTRODUCTION

The quality of seafoods during storage is largely dependent on the microbial population and the activity of tissue enzymes. The reactions which take place often result in a loss of desirable flavor and the development of off-odors and flavors, sometimes accompanied by marked changes in texture. To a major extent the deteriorative changes that occur during the storage of fresh seafoods may be attributed to bacteria, yeasts and molds. Elimination of microorganisms would provide means of greatly extending storage life. Chemicals, heat and solar radiation have been used by man to prevent bacterial attack and preserve his food supply. The destruction of microflora by gamma radiation is a new approach to an old problem -- the preservation of food for man.

Ionizing radiation or gamma radiation, which results from the decay of radiation elements is effective in destroying microorganisms. Complete sterilization, the destruction of all life, may be achieved by high dose levels -- those in excess of 4.5 megarad. Pasteurization levels which are below this value destroy almost all spoilage organisms, but do not achieve complete sterility. Sterilization levels of radiation often impart off-odors and flavors to the food. Pasteurization radiation could conceivably avoid many of the undesirable flavor changes and still destroy most of the spoilage organisms and

greatly extend the refrigerated storage life.

With many products such as raw meat and fish the initial microbial number may be high. Radiation pasteurization will kill many bacteria, but still leave some survivors to grow at room temperature. Therefore, refrigeration is required for the control of the surviving microbial population, enzymic and chemical action. Temperatures below 40°F. slow the development of spoilage bacteria and could prevent the production of some of the microbial toxins.

The radiation pasteurization level needed usually depends on the initial bacterial number and the type of product. Generally, it varies in a range between 0.05 to 1.00 megarad (68, p. 423).

The use of chlortetracycline (CTC) for seafood preservation has been used by several workers. Inclusion of CTC in dips and washes for shrimp prior to packaging has resulted in an extension of their storage life (73, p. 585). Tarr (92, p. 363) reported that CTC is the most effective antibiotic for retarding microbial growth in fish.

The pasteurization levels used in this research were determined by flavor evaluation of samples receiving various amounts of gamma irradiation. The flavor threshold level of irradiation intensity was established by a trained panel. The flavor threshold level may be defined as the level at which no significant odor or taste is imparted to the product by irradiation (78, p. 119).

The purpose of this research was to subject raw shrimp to CTC and pasteurization levels of gamma radiation and study their effects on the extension of refrigerated storage life. The quality of the shrimp was ascertained by objective and subjective evaluations during the storage period.

LITERATURE REVIEW

Radiation is the propagation or transmission of energy through space (45, p. 59). This may occur by means of wave motion or by means of atomic or subatomic particles moving at great velocities and set in motion by the action of electric fields or by emission from radioactive (unstable) substances (57, p. 304).

Ionizing radiation, including pasteurization and sterilization doses has been used for food preservation. Coleby (20, p. 877) stated "unfortunately in radiation sterilization of many foods, some undesirable chemical and physical changes take place." Shewan and Liston (81, p. 377) indicated that irradiation produced noticable changes in odor, taste, color and texture of many foods. Clifcorn (18, p. 40) pointed out that these changes were in direct proportion to the radiation dose applied and vary in degree from product to product.

McNamara et al. (64, p. 68) demonstrated through extensive laboratory tests and feeding trials that no toxic substances were produced by the irradiation process. Cook (21, p. 7) reported that the irradiated foods were not toxic, harmful or radioactive and appear as wholesome as or better than foods preserved by other methods. Kraybill (56, p. 14) found no differences in growth rate or food consumption after feeding irradiated ground beef as 50 percent of the

calories in the daily ration of female beagle pups over eight months.

Up until 1957, most studies on radiation preservation were based on the premise that a dose on the order of 2 megarad was sufficient for effective sterilization of foods (72, p. 516). This level of "sterilization" dose may be adequate for a small number of moderately contaminated samples of simple substrates. Food preservation must necessarily consider, in addition to limiting spoilage, elimination of all of the pathogenic species in an extremely large number of complex food samples.

Morgan and Reed (65, p. 359) found that the organisms most resistant to radiation were the spores of the Clostridium botulinum. The rate of survival of this species has become the limiting factor in establishing the sterilizing dose.

Shewan (82, p. 143) said that the sterilization of the common varieties of seafoods by irradiation requires doses which almost invariably produce undesirable and unacceptable organoleptic changes in these products. It seems unlikely therefore, Shewan continued, that this method can at present compete commercially with the more conventional preservation processes. He concluded that recent work, particularly in the U. S. A. suggests that pasteurizing doses either alone or along with a variety of combination treatments could give a satisfactory product with greatly enhanced shelf life particularly at temperatures below 40^{o} F.

Hannan in 1955 (50, p. 67) estimated that sterilizing doses of about 5 megarad would be necessary for food sterilization. The Quartermaster Corp's contractors found a "best estimate" of a 4.5-4.8 megarad (10, p. 109; 46, p. 489; 67, p. 24; 72, p. 516) based on LD_{90} for Cl. botulinum spores of about 400,000 rad. Recent appraisals suggested that each food may have to be evaluated separately (10, p. 110; 23, p. 50; 72, p. 513; 75, p. 56).

Radiation processing promises major economic benefits according to recent observations made by Mosan (70, p. 704). He reported that savings will be made between the farmer and the consumer by reducing food spoilage and costs due to transportation, storage, and in-store marketing, in spite of the necessary capital outlay for installation and maintenance of radiation sources. The U. S. Quartermaster Corp's Food and Container Institute (62, p. 8, 10) indicated that fresh foods processed by radiation sterilization can be stored and transferred without use of expensive and frequently inconvenient refrigeration equipment. This indicates that there can be little doubt that the process will be used. The unit processing cost obtained in this study is only a fraction of a cent per pound. This cost is so small that radiation processing will certainly be used on a large scale in the food industry.

Radiation Pasteurization

Desrosier and Rosenstock (25, p. 331) pointed out that low level pasteurization radiation treatment of foods may be used to prolong the storage and marketing time of perishable commodities such as fruits, vegetables, meats, fish, poultry and prepared foods for dinner meals. The level of irradiation used is related to the degree of microbial and/or parasite destruction.

Morgan (68, p. 423) defines this low level irradiation as radiation pasteurization, that is, the killing of 98 percent of the microorganisms. Ingram (53, p. 106) defined refrigeration, which includes "chilled" storage at 32° F. to 41° F., as a process which confers a storage life of a week or two for perishable foods such as eggs, fish, meat, milk and vegetables by delaying changes in quality caused by bacteria, enzymes and chemical reactions. The value of refrigeration in addition to the slowing down of enzymic, chemical and microbial changes, is that temperatures below 40° F. prevent the development of food poisoning organisms.

Shewan (82, p. 143) reported that the doses in excess of 1.0 megarad for shrimp usually produce undesirable and unacceptable organoleptic changes. The undesirable odors and flavors produced, which have been variously described as "metallic",

"burnt-feather like" and "rubbery" can be of course, detected after irradiation at high levels.

Desrosier and Rosenstock (25, p. 331) reported that the storage and distribution life of fresh foods can be extended by a factor of 5 to 10 using radiation treatments of a million rads or less. Furthermore, most packaging materials suitable for cool storage of perishable foods are unaltered functionally with such low levels of ionizing radiations. Costs of irradiation are at least one fifth less than those for sterilizing treatments. Clifcorn (18, p. 39) pointed out that the use of radiation pasteurization may alter the storage and distribution patterns of the food products thus allowing areas of production greater freedom of marketing. Eukel and Huber (34, p. 198) concluded that low dose radiation processing has been extensively considered as a useful tool for extending shelf-life of fresh foods, particularly when used in conjunction with refrigerated storage and other auxiliary methods of preservation.

The levels of radiation needed to bring about pasteurization of the product depends on the initial bacterial load and the type of product. Pasteurization is usually effected at levels ranging from 0.05 to 1.00×10^6 rads followed by refrigerated storage (19, p. 115).

Uses of Antibiotics in Fish Preservation

Tarr and his co-workers (90, p. 257) in Canada were among

the first to realize the potential of antibiotics as preservatives for flesh foods, and they reported the result of their first efforts in 1944. Since that time many investigations concerning the application of antibiotics to fisheries have been made by Tarr.

In 1952, Tarr et al. (92, p. 363) reported that, of the 14 antibiotics investigated, CTC and oxytetracycline (OTC) most effectively retarded bacterial growth in fish flesh. Tarr (91, p. 199) later reported that CTC was about five times as active as OTC which in turn was more active than tetracycline.

Lerke, Farber and Huber (58, p. 145) reported that early in 1959 the U. S. Food and Drug Administration approved the use of chlortetracycline for treating whole or gutted fish, shucked scallops and unpeeled shrimp and set a tolerance of 5 ppm of CTC in these products. Both the skin and the outer layers of flesh act as quite efficient barriers to the penetration of CTC into the deeper layer of flesh. Similar results in live fish were found by Kline et al. (55, p. 305). They showed that after CTC treated shrimp were boiled, they retained only small amounts of CTC. From 65 to 96 percent of the CTC was inactivated through boiling. They concluded that for average cooking conditions and normal pickling and smoking procedures, practically no CTC was found if antibiotics were employed at the recommended levels (5-20 ppm).

De Silva and Hughes (24, p. 161-168) studied the effect of two antibiotics (CTC and OTC) on the spoilage of whole iced herring. They reported that spring and summer herring were improved (tase panel assessment, determination of total volatile bases) by the antibiotics, but autumn herring were unaffected. This may be attributed to a seasonal variation in the nature of the bacterial flora present on the fish. Peroxide values were higher for oil from treated compared with that from untreated fish, but the production of free fatty acids and volatile fatty acids was not affected. The production of some free amino-acids was higher in the treated compared with the untreated fish.

Castell and Greenough (13, p. 771) in a study on the effect of certain antibiotics on the production of trimethylamine (TMA), reported that chlortetracycline (CTC) did not retard the bacterial reduction of trimethylamine oxide to TMA, but sodium nitrite did retard the production of TMA.

Vance, et al. (94) in studies of the use of CTC in the control of spoilage in ice-stored shrimp, state,

> The extension of storage life of treated shrimp was determined both from organoleptic judgments of odor and by objective tests of bacterial numbers. Fairly close agreement was noted between these two criteria. Taste panel did not always show as marked an extension of acceptable flavor. This may be due in part to enzymatic changes occurring in the shrimp in spite of reduced bacterial numbers, and may reflect also the fact that flavor evaluation seemed more affected by personal preference than did odor evaluation.

Hillig, et al. (52, p. 695) indicated that using CTC ice or dipping fillets in a brine solution containing CTC increases the shelf life of fish by suppressing bacterial decomposition. Most of these conclusions are based on organoleptic and bacteriological observations. However, Farber (36, p. 503) reported that OTC and CTC in concentrations of 2 ppm had no preservative effect upon shrimp; Fieger, Novak and Bailey (39, p. 21-25) also reported that antibiotics were ineffective in shrimp preservation.

A Combination Treatment of Low Level of Radiation and Antibiotics

Liston and Shewan (61) reported that irradiation appears to have three effects on the bacterial population in fish -- a bactericidal one, a bacteriostatic one and a devitalizing one. The first two obviously have a direct effect on the bacterial counts. The latter effect shows up in two ways. First, there appears to be a number of bacteria growing in the agar plates from the fish samples taken immediately after irradiation, which fail to grow on subsequent subcultures. This effect probably accounts for the continued drop in numbers during the first few days of storage in the fish subjected to 10×10^6 rads. Secondly, there appear to be some bacteria which after a period of time, recover from the damage caused by irradiation and then grow in the normal way. The additional treatment with antibiotics seems to

prevent this recovery process so that in these samples the counts are always comparatively lower.

Much attention is now being directed to pasteurization radiation usually along with "combination treatments" such as blanching, addition of antibiotics or chemical protectors with a view to improving both the shelf-life and the organoleptic properties of the irradiated materials.

Shewan (82, p. 143) reported a beneficial effect of combination treatments with gamma radiation and CTC on the spoilage retardation of cod fillets. Lerke, Farber and Huber (58, p. 145) reported similar results with low level radiation in combination with CTC on the spoilage retardation of shell fish.

Radiation Induced Chemical Changes

Willard (97, p. 141) said,

> "In passing through matter all of these radiations bore their energy by transferring it to electrons thus producing electronically excited or ionized molecules. Chemical effects follow as a result of the transfer of energy of electronic excitation or ion neutralization into vibrational energy sufficient to break bonds. Most of these chemical events result in the formation of free radicals which may combine with each other or react with other species of the medium, their fate being determined by their proximity to each other and the activation energies of possible competing reaction."

He added,

> "In radiation chemistry the energy of each incident photon

> or particle is sufficient to produce many ionized and many excited molecules."

Black (9, p. 88) reported that as an ionizing particle travels through matter, its energy is dissipated primarily by collision with electrons. If the contact of interaction is sufficiently energetic, an electron is ejected from its parent atom or molecule. This electron is thereby ionized. A less direct or energetic contact may result in a transfer of energy sufficient to raise the atom or molecule to a high state of electronic excitation. He concluded (9, p. 88) that an ionizing particle passes through a number of atoms before effecting ionization by the ejection of an electron from an atom.

Black (9, p. 88, 89) also said,

> "...when a molecule or ion has been given sufficient excitation energy by collision or irradiation to rupture one or more chemical bonds, the rearrangement of atomic configuration takes place and new chemical bonds are formed. At least 3.6 electron volts of excitation energy are required for breaking the carbon ——carbon bond and 4.2 electron volts for the serving of the carbon-hydrogen bonds. This minimum amount of energy must be concentrated in the particular bond in order that it vibrate with sufficient amplitude to rupture the bond. As the amount of available energy is increased, the rate of decomposition or bond breaking increases and at any energy, the reaction with the lower activation energy will be favored."

Black (9, p. 90) added,

> "...for a transfer of energy to take place it is not essential that the excited molecule come into direct contact with another molecule, it is possible for the transfer to occur at distances that are great relative to the effective molecular diameter."

Morgan (68, p. 424) reported that about 0.003 percent of the CH_3 bonds in food have been estimated to be broken by a sterilizing dose of radiation. In meat, the objectionable odor producing compounds formed by radiation are measured in ppm, which indicates that human discernibility is, at times, more sensitive than many of our chemical methods. Hamill in 1960 (49, p. 95) added that irradiation of water can be described in terms of H_2, H_2O_2 (the "molecular yield") and of H, OH (the "radical yield"). So in the radiolysis of aqueous solutions containing H_2, H_2O_2 and O_2, the "H atom" formed by free radical oxidation of H_2 is shown to react with O_2 much faster than with H_2O_2. The "H atom" formed by radiolysis of water reacts with O_2 and H_2O_2 at comparable rates. Barr and Allen (3, p. 928) concluded that the two kinds of "H atom" may be the basic and acidic forms of H, i.e., the solvated electron and H_2.

Siu and Bailey (85, p. 97) stated that the interaction of ionizing radiation with water gives rise to four principal products, the radicals H and OH and the molecular products H_2 and H_2O_2. These are formed through electronic excitation, ionization and dissociation of irradiated water molecules. The importance of these breakdown products in the preservation of food is based largely on their great chemical activity. Following the formation of the free radicals, a series of other reactions takes place as a result of their activity.

Recombination leads to the formation of H_2O, H_2 and H_2O_2 as follows:

$$H + OH \longrightarrow H_2O$$

$$H + H \longrightarrow H_2$$

$$OH + OH \longrightarrow H_2O_2$$

As the H_2O_2 accumulates it begins to compete for the free radicals through the following reactions:

$$H_2O_2 + H \longrightarrow H_2O + OH$$

$$H_2O_2 + OH \longrightarrow HO_2 + H_2O$$

H_2 also reacts with the free radical OH

$$H_2 + OH \longrightarrow H_2O + H$$

and H radical in the presence of O_2 forms:

$$H + O_2 \longrightarrow HO_2$$

and

$$HO_2 + HO_2 \longrightarrow H_2O_2 + O_2$$

Overall

$$H_2O \longrightarrow H_2 + H_2O_2 + H + OH$$

Ise and Fox (54, p. 1-16) have shown that within the last decade, evidence has been presented to indicate that the direct "hits" may be responsible for some specific biological effects, but that many effects are caused in whole or in part by the solvent. The effect of irradiation on the action of the radiation products of water were formed by the splitting of water into hydrogen atoms and hydroxyl radicals. These two radicals are known to be chemically very

reactive and can act as reducing and oxidizing agents as well as to cleave C-C bonds. However, the secondary products of irradiation may be of more importance, since in the presence of dissolved oxygen the hydrogen atom can combine with molecular oxygen to form the very reactive O_2H radical,

$$H + O_2 \longrightarrow O_2H$$

which by dismutation can form hydrogen peroxide

$$2\ O_2H \longrightarrow H_2O_2 + O_2$$

The hydroxyl radical may also form H_2O_2

$$OH + OH \longrightarrow H_2O_2$$

The theories of direct and indirect action are not incompatible and they describe mechanisms which may apply within the same system. While there is no doubt that both modes of action are valid, the indirect theory offers a wider basis for chemical changes.

Radiation Induced Changes in Protein

Desrosier and Rosenstock in 1960 (25, p. 154) reported that the effect of radiation on proteins can be divided into three classes:

A. Specific chemical changes.

B. Changes in physiochemical properties including denaturation.

C. Less clearly defined changes particularly odor formation,

which are of technological interest. They concluded that the demonstrable chemical changes in irradiated proteins include liberation of ammonia, oxidation of SH groups and formation of peroxide. The ammonia may be liberated from free NH_2 groups which occur at one end of the molecule, from NH_2 groups which are not integral parts of the protein backbone (as in a diabasic amino acid), and from ruptured peptide linkages. The oxidation of SH groups probably occurs by attack of an OH radical formed in water. Another effect on sulfur in proteins is the liberation of H_2S. They added (25, p. 154) that radiation produces changes in viscosity, ultraviolet absorption spectra, electrophoretic behavior, altered sensitivity towards enzymes, increases in exposed SH groups and coagulation or precipitation. These changes are characteristic of denaturation.

Ise and Fox (54, p. 2), claim that although the radiation effect is possibly caused by radiation products of water, it has been proposed that when the protein concentration or the radiation dose is increased, there may be in addition the direct collision of the ionizing particles with the protein itself. This behavior results in denaturation and precipitation. They concluded that the denaturation of the protein by radiation is similar to that produced by heat, acid and alkaline denaturation; and also reported that it occurred in two steps--the first, a preliminary temperature-independent process, in which

the protein molecule became a new chemical entity. The second, a physical process, caused the formation of visible coagulation and had a large temperature coefficient. Later, Clark (17, p. 199) suggested that the denaturation occurred in three steps: (1) an alteration of the protein molecule by the radiation, (2) a reaction between the radiation altered molecule and water, and (3) the formation of a visible coagulation.

Dale, Davis and Gilbert (22, p. 93) suggested two possible mechanisms of deamination by radiation: one involving the formation of several pairs of radicals per ion pair and the other a chain reaction. The non-chain mechanism is based on the indirect theory which postulated that the action of ionizing radiation is due to the hydrogen atom and hydroxyl radical formed by the splitting of the water molecule. The chain reaction is as follows:

$$OH + H_3^+NCH_2COO^- \longrightarrow OH.CH_2COO^- + NH_3$$

$$NH_3^+ + H_2O \longrightarrow NH_4^+ + OH$$

The reformed hydroxyl radical could contact with another glycine and carry on the chain (26, p. 134).

Morgan (68, p. 425, 426) reported that the sulfur-containing amino acids (methionine, cysteine and cystine) and the ring-containing amino acids (proline, tyrosine, phenylalanine, histidine and tryptophan) are the most radiosensitive. Morgan (68, p. 426) found that

in irradiated beef some compounds increased with total dose; these include the lower boiling alcohols, mercaptans, sulfides, disulfides, unsaturated lower hydrocarbons and aldehydes as well as pyrrole, pyridine, methylethylketone and other aromatics. Drake and Giffee (26, p. 149) concluded that it is evident that fragmentation as well as aggregation of proteins occur when they are subjected to ionizing radiation.

Ise and Fox (54, p. 10) further suggested that the principal products of deamination of amino acids are ammonia and the corresponding aldehyde. Morgan (68, p. 426) suggested that volatile bases such as methylamine and ethylamine could react with CO_2 to form low molecular weight volatile compounds. This could explain the loss of CO_2 during irradiation.

Ise and Fox (54, p. 2) concluded that the cause of these changes was shown to be in part the indirect action of ionizing radiation on serum albumin in the presence and absence of oxygen. If the direct action was responsible for the alterations, there should be no difference in the absence of oxygen, but if there was a difference, then the irradiation products of water (O_2H and H_2O_2) would be contributing to the observed results in as much as the lack of oxygen should have no effect on the direct collisions.

Radiation Induced Changes in Quality

Ionizing radiation side effects often produce changes in the flavor, color and odor of the food. The taste changes are usually described by taste panel members as being oxidative in character (77, p. 192). Objectionable radiation side effects, such as flavor change and alteration of texture and color, are generally small and often undetectable in the range of 100,000 to 300,000 rads (34, p. 198).

It was shown that off-flavors produced by cathode ray sterilized materials were probably due to free radicals produced by the effect of ionizing radiation on water. These free ions which may be strong oxidizing or reducing agents, react with any number of the components of the food being irradiated. This may result in an oxidized flavor (77, p. 192).

Batzer, et al. (4, p. 702) reported that the carbonyl compounds produced by irradiation of meat and meat fats probably do not directly contribute to the off-odors produced in irradiated beef; they may possibly have a role in decreasing the apparent off-odors by reacting with the compounds that do contribute, i. e., with sulfhydryl compounds and amines. These reaction products would then be of more importance than the residual unreacted carbonyls. An undesirable "irradiation" odor can develop due to the formation of a complex mixture of volatile compounds including sulphur-containing carbonyl

compounds, and corresponding changes in flavor can be observed (51, p. 287).

Fats and oils are responsible for many of the pleasant odors and flavors in foods. Irradiation could destroy, change or create unpleasant odors and flavors from them so that irradiation of fatty or oily foods should be approached with caution (50, p. 150). Protein components might also be responsible for the characteristic "irradiation" odor but this is less certain (50, p. 150).

Drake and Giffee (26, p. 148) reported that the major odor-forming reactions occurring during irradiation of meat have been found to involve the water-soluble proteins. They concluded (26, p. 150) that the extent of undesirable flavor and odor type compounds formed from protein as a result of irradiation appears to be extremely small, but, nevertheless, is large enough to be perceived by the taster. A research on flavor and odor of gamma-irradiated meat by Witting and Batzer (98, p. 237) has led to the detection of mercaptans, β-unsaturated aldehydes and peroxides in the meat. They said that the crude reaction mixture containing methional had an odor typical of ground beef which had received 2-4 megarad of gamma radiation while the mixture containing 3-methylthiobutyraldehyde had an odor typical of meat at higher radiation doses (8-10 megarad). Morgan (69, p. 45) concluded that irradiated odors in meat appear, at least in beef, to arise from sulfur-containing compounds such as

glutathione.

Schweigert (79, p. 137) reported that meats treated with sterilization dosages show an increase in the amounts of methyl mercaptan, hydrogen sulfide and carbonyl compounds produced, and a rise in pH values. A decrease in the amount of sulfur-containing tripeptide, glutathione and of glycogen was also observed. Morgan (68, p. 426) found that it is doubtful whether or not free amino acids are directly associated with changes in odor or taste due to irradiation. He further stated that the odors produced by irradiation are not directly related to the functional groups of the amino acids. In fact, a decrease in odor is caused as the functional groups complex by sulfation of the aliphatic hydroxyls, sulfhydryl and phenol hydroxyls.

In seafoods higher doses of radiation generally result in increasing off-flavor and odor and these characteristics vary quantitatively with the pre-treatment and the species of fish. McGill, et al. (63, p. 75) concluded that organoleptic acceptability of the food is probably not reduced when low levels of radiation are applied.

With increased radiation dosage, the red color of beef was changed to a dull red and at 30 megarep it was tan in color. The destruction of carotenoid pigments mainly astaxanthin, was a function of radiation dose in salmon (48, p. 557). If the pigment is solubilized in a lipid, the pigment will be much more susceptible to oxidative degradation during radiation than when it is bound to the protein fraction (68, p. 424).

The carotenoids associated with the normal pink color of salmon or shrimp may turn brown or be destroyed by sterilization dosages which make the products undesirable from the standpoint of appearance (79, p. 137).

Morgan (68, p. 427) suggested that radiation treatment, like heat treatment, tends to decrease the level of vitamins below that of fresh foods. Vitamins which are quite stable to radiation are, D, K, riboflavin, niacin, folic acid and B_{12}. Vitamins A, E, C, and thiamine, however, are quite unstable when subjected to radiation. About 20 percent more thiamine is lost by sterilization irradiation than by heat treatment.

Proctor and Goldblith (76, p. 379) and Hannan and Shepherd (51, p. 286) indicated that simple freezing and irradiation of the food in the frozen state minimize the off-flavor in milk and orange juice probably by lowering the diffusion rate of the free radicals produced by the ionizing radiation. The addition of chemical compounds as free radical acceptors may be another means of obtaining the same result, thus avoiding the necessity of freezing. Eukel and Huber (34, p. 198) concluded that the alteration of texture and color is generally small and often undetectable in the low doses (100,000-300,000 rad.). On an over all basis, therefore, irradiation pasteurization of meats, poultry and fish appears promising (79, p. 137).

Fish Spoilage and Its Detection

Fieger, Bailey and Novak (41, p. 297) examined fresh and frozen shrimp and considered spoilage of this product to be due largely to biochemical changes induced by the microbial population and to a lesser degree to enzymes and chemical compounds inherent in shrimp. Spoilage of shell fish has been attributed to enzymic degradation caused by contaminating microorganisms (73, p. 585). Fieger, Bailey and Novak (41, p. 297) stated that spoilage of the fish is believed to be mainly the result of bacterial action, and due to the consequent formation of compounds which impart off odors, colors and flavors. They concluded that during the critical period between the time the fish are killed and are actually processed, the problem is to minimize degradation in quality caused by the action of bacterial and autolytic enzymes.

Stansby (88, p. 260) reported that two general types of changes occurred when fish deteriorated. The first may be termed a fundamental change and tests used to measure such changes can be termed fundamental tests. The fundamental tests are those which measure factors that contribute to the quality of fish. These may be based upon changes that result directly in alteration of texture, flavor, odor or appearance or may be changes that alter the keeping quality. Examples of the latter include development of lactic acid in the flesh,

at low concentrations not to affect the flavor, yet in sufficient quantity to lower the pH to retard bacterial action, which is not apprarent organoleptically and would increase the spoilage rate. The second type of change is an accessory change, and accessory tests are used to measure them. Accessory changes include those that occur in deteriorating fish without directly affecting quality. Changes in the appearance of the eyes of fish is an example of an accessory change. The formation of chemical substances in the flesh of the fish that do not in any way alter flavor, odor, texture or appearance like the formation of CO_2 is considered an accessory change.

Beatty and Collins (6, p. 412-423) reported that spoilage in sea fish always occurred in two stages irrespective of the availability of air; first the oxidation of lactic acid and sugar, and second that of oxidation of amino acids and the hydrolysis of proteins. The second stage represents advanced spoilage. After the death of fish and warm blooded animals the onset of rigor mortis is generally the first obvious sign of change in the muscle (83, p. 892). While the flesh is still in rigor, the deteriorative changes do not generally proceed to a measurable extent, but after a resolution of rigor the effect of autolytic and bacterial changes becomes apparent (83, p. 892).

Faulkner and Watts (38, p. 632) reported that the red color of

cooked shrimp is due to a carotenoid pigment, astaxanthin (or astacin). There are two types of color changes which occur in stored shrimp. The change which occurs first is the conversion of the original pinkish-red pigment to one of a more orange color. The other color change occurring in stored shrimp is the gradual fading of the red pigment to an almost colorless state. It is possible that the bleaching of astaxanthin, the carotenoid pigment of shrimp, is related to the oxidation of the fat present. However, the amount of fat is so small and so intimately associated with the protein that attempts to extract it in an unchanged condition for rancidity determinations were not successful (38, p. 632).

It is well known that indole may be formed through bacterial action from protein containing tryptophan as Duggan and Strasburger (28, p. 177) mentioned. They concluded that indole could be used as an index of decomposition. There is a relationship existing between the degree of decomposition and the indole content of shrimp. The indole content of individual shrimp from the same class may vary widely. No indole is formed by cooking. Duggan and Strasburger (28, p. 177-188) found that the indole content increases as the decomposition advances, therefore the indole content of shrimp is a measure of the extent of decomposition. Small amounts of indole are normally found in commercially packed

shrimp (0-4. 3 mcg/100 g).

Farber (35, p. 319) found that the measurement of indole is of no practical significance for such a fish as tuna. But Sigurdsson (83, p. 892) said, "although the determination of indole cannot supplant odor and physical appearance in the examination of canned salmon, it affords considerable information as to the previous history of the sample." Duggan concluded (29, p. 507) that different types of decomposition produced varying amounts of indole. The decomposition characterized as ammonical produced smaller amounts of indole than was found in the putrefactive type of decomposition.

Beatty and Gibbons in 1936 (5, p. 77) reported that the separation of a fraction of the volatile nitrogenous bases from the decomposition of cod muscle, was found to develop only as a result of bacterial action. This fraction was believed to consist mainly or wholly of trimethylamine derived from the reduction of trimethylamine oxide.

The increase of trimethylamine in the muscle of marine fishes runs closely parallel to the degree of decomposition. The trimethylamine value of fish muscle fulfills all the conditions required of an effective test for freshness (5, p. 77-91). Later Beatty demonstrated that at least 94 per cent of the TMA is derived from TMAO naturally present in the muscle which is quantitatively reduced to TMA in the advanced stage of decomposition.

Beatty (7, p. 63-68) reported that the most probable sources of TMA in spoilage are those compounds such as creatine, betaine and TMAO which have groupings similar to TMA. Of these, TMAO is the most readily converted to TMA. Wood and Baird (99, p. 194) had shown that TMAO could be considered as a hydrogen acceptor in the respiration of muscle. It has been found that the family Enterobacteriaceae reduces TMAO to TMA. The only exceptions have occurred within the genus Shigella and the genus Erwinia. Shigella dysenteriae and S. paradysenteriae are unable to reduce TMAO.

Beatty and Collins (6, p. 412-423) reported that TMA production occurred mainly during the first stage of spoilage. It is a good criterion as to the probable production of toxic compounds resulting from protein and amino acid breakdown. TMA is produced rapidly during the period of rapid multiplication of bacteria in cod muscle press juice, and very small amounts are produced by autolysis. It is apparent that when TMAO is reduced, it provides a source of oxygen for the oxidation of the substrate and that at least to a considerable degree, the spoilage is anaerobic. Watson (95, p. 252-255) has shown that two types of spoilage, aerobic and anaerobic do exist, and that anaerobic spoilage predominates.

A general equation involving the reduction of TMAO by achromobacter is derived (96, p. 267-280):

$$AH_2 + (CH_3)_3\,NO \longrightarrow A + (CH_3)_3\,N + H_2O$$

Where AH_2 is the hydrogen donator and A the oxidized substrate. The reduction of TMAO as hydrogen acceptor with the evolution of TMA is a linear function of time in the presence of cell suspensions and single hydrogen donators including glucose, glycogen, lactate, and pyruvate. If there is a suitable hydrogen donator present such as lactic acid, it becomes activated by the dehydrogenases, the hydrogen from the donator is transferred to, and accepted by, the oxygen from the oxide. Thus the steps of the reaction are as follows:

$$CH_3CHOHCOOH + (CH_3)_3\,NO \longrightarrow CH_3COCOOH + (CH_3)_3\,N + H_2O$$

$$CH_3COCOOH + (CH_3)_3\,NO + H_2O \longrightarrow CH_3COOH + (CH_3)_3\,N + CO_2 + H_2O$$

Overall:

$$CH_3CHOHCOOH + 2(CH_3)_3\,NO + H_2O \longrightarrow CH_3COOH + 2(CH_3)_3\,N + CO_2 + H_2O$$

It has been seen that two molecules of TMAO were reduced for each molecule of lactic acid oxidized. It is possible therefore, that TMA produced in cod muscle press juice is a result of energy yielding reactions between TMAO and appropriate hydrogen donators, especially carbohydrates and their derivatives, during the anaerobic respiration of reducing achromobacter.

The enzymic reduction of TMAO is affected by pH. As the pH change of the medium becomes more acid in reaction the enzyme

activity is less, and below 6.0 it is almost insignificant (14, p. 561).

Beatty and Gibbons (5, p. 77-91) found that odors always appear at approximately the same level of "TMA", the increase resulting from autolysis is negligible. The increase during the development of spoilage is 15-20 times the original value. Farber and Lerke (37, p. 191) concluded that TMA content enabled a good estimate of shrimp freshness.

The concentration of volatile bases cannot be used to detect the earlier stages of fish spoilage unless the original value for the fish under examination is known. While there is a rise in volatile bases due to autolysis, it is negligible in comparison to that due to bacteria (5, p. 77-91). Beatty and Gibbons (5, p. 77-91) found that the increase in volatile nitrogenous bases in cod fish muscle between the pre-rigor period and the first appearance of odor is approximately 6 mg./100 g. of tissue, and due almost entirely to the action of bacteria. The volatile bases used by Beatty (7, p. 63-68) as a measure of fish spoilage was shown to be at least 95 percent TMA.

Bacteria were found mainly responsible for protein decomposition of fish and only slight autolytic changes occurred in haddock muscle held at 30 and 36° F. (44, p. 73). Fieger et al. (42, p. 85) found that amino nitrogen values in shrimp became smaller as the storage

period progressed. Since deamination occurred only after the fish had become definitely spoiled, decarboxylation and higher amine formation would likewise occur after the fish was no longer acceptable as food (6, p. 412-423).

Fieger and Friloux (40, p. 35) postulated that two types of changes account for loss of quality and spoilage of shrimp. One of these causes loss of sweet flavor and some softening of the tissue. These changes predominate during the first seven days of ice storage and probably are catalyzed by tissue enzymes and can be designated as tissue autolysis while loss of quality progresses during this period, spoilage has not occurred. The other type of change predominates during the later storage period and results from the multiplication of bacteria. Some of the products of bacterial action give to the shrimp the odors and flavors associated with spoilage. Loss of quality during the early period of storage is mainly caused by autolysis while with longer storage spoilage is mainly the result of microbial action.

Stansby and Lemon (86, p. 208) discussed deteriorative changes in fish flesh and concluded that protein is the only substance whose decomposition is of any importance during spoilage. At the higher temperatures (about 25° C.), the development of volatile acids and TMA is followed very closely by the hydrolysis of protein while at

10° C. the proteolysis lags behind the other changes. At 0° C. on the other hand, the fermentation of carbohydrates and lactic acid leading to the formation of the acids and the amine is inhibited to such an extent that it does not occur until proteolysis also becomes appreciable and then these two kinds of changes occur at similar rates (83, p. 900).

Beatty and Collins (6, p. 412-423 followed the protein decomposition by determining the amino nitrogen in the Van Slyke apparatus and never found more than a very slight increase in amino groups over the initial level. On the other hand the amino nitrogen decreased as spoilage proceeded. They assumed that this was due to bacterial deamination and judged the extent of protein decomposition by the decrease in amino nitrogen.

The spoilage pattern varies widely from one fish to another but is usually a combination of loss of flavor, development of off-odors and off-flavors, alteration of texture, and reduction of keeping qualities. The resulting pattern is dependent upon many factors, especially those related to the original handling conditions (88, p. 260).

Microbial methods may give a fairly good picture of the sanitary history and handling of the product, but a high bacterial count does not always mean that the product is spoiled (83, p. 892). Microbial action on fish gives rise to volatile breakdown products which

are responsible for marked odors that develop during spoilage (35, p. 319). These changes in the natural odors led to the use of organoleptic tests for measuring the quality of the fish. Farber (35, p. 319) found that this method proved unsatisfactory because it was limited by an individuals sense acuity and other environmental conditions.

Products of the fermentation of the carbohydrates in fish are volatile acids such as propionic acid, acetic acid, formic acid, and other products such as ethanol, carbon dioxide, acetyl methyl carbonyl, lactic acid, and diacetyl, according to Sigurdsson (83, p. 899). He also observed that the production of volatile acids in herring closely followed the TMA production.

Wood, Sigurdsson and Dyer (100, p. 53-62) used surface pH as an index of spoilage in cod and haddock. They found that these fish had a pH of 6.4 when fresh, and a pH of 8.4 when badly spoiled. The pH was measured by placing the glass electrodes in the moist surface of the tissue (30, p. 183-184). Charnley and Goard (15, p. 32) also studied pH as a measure of spoilage.

Irradiation of Shrimp

It has been shown that irradiation produces changes in the natural constituents of fish. These changes occur largely in the protein

constituents. Shrimp contains about 25 percent protein which is composed primarily of the amino acids arginine, lysine, histidine, tryptophan and cystine (Tressler and Lemon, 93, p. 289, 292). Shrimp is also rich in thiamine, riboflavin, and niacin. It also contains significant amounts of vitamin A. Riboflavin and niacin are quite stable to irradiation but thiamine and vitamin A are measurably affected by radiation treatment (59, p. 195-196). Ring-containing amino acids such as histidine and tryptophan are especially susceptible to radiation damage (68, p. 426).

The carotenoid pigment in shrimp is astaxanthin which is bound to protein and is more resistant to radiation than it is in salmon where it is not bound (68, p. 425).

The color and taste of shrimp were normal at radiation doses of 0.50×10^6 reps according to work done by Tappel, Knapp and Brack (89, p. 274). After a flavor panel of 176 members evaluated samples of freshly thawed shrimp irradiated at 0.50, 1.00 and 1.5×10^6 rads, Sinnhuber (84) reported that no significant flavor due to irradiation was present in the samples irradiated at 0.5×10^6 rads. In all samples of shrimp tested, the radiation odor and taste gradually diminished and in some cases disappeared after storage at temperatures of 45° F. or higher. Samples of shrimp irradiated at 0.50 and 0.75×10^6 rads were not spoiled during a storage period of 180

days and samples irradiated at 0.25×10^6 rads remained unspoiled for 60 days. No bacterial spoilage was observed in samples receiving a minimum of 1.0×10^6 rads.

Scholz et al. (78, p. 118-120) reported that the loss of irradiated taste and odor in the irradiated, stored shrimp was quite definite, and no adverse change in quality was observed.

METHODS

Source of Raw Materials

The shrimp (Penaeus setiferus) used in this investigation came from Mexico and were packed by Congeladora Union S. A. Mazatlan Sin., Mexico. They were obtained from a commercial firm located in Portland, Oregon. The shrimp were frozen in five pound packages.

Handling Operation

Beam trawl nets were used for the commercial catching of shrimp. The shrimp were subjected to a cleaning operation, followed by a deheading and shelling process performed mechanically by a machine peeler. The frozen samples were sent under refrigeration from Portland to the Oregon State University laboratories.

Pre-irradiation Treatment

The five pound packages of shrimp were thawed, washed, and packed in 1/2 pound C enamel cans. The cans were divided into six treatments as follows:

1-0.00 Megarad
2-0.00 Megarad + 5 ppm CTC
3-0.50 Megarad
4-0.50 Megarad + 5 ppm CTC
5-0.75 Megarad
6-0.75 Megarad + 5 ppm CTC

A 5 ml. water solution of CTC was added to the shrimp to give a concentration of 5 ppm. For the other samples 5 ml. of distilled water were added and sealed in the tin. The shrimp were immediately frozen and shipped under dry ice by railway express to Material Testing Reactor at Idaho Falls, Idaho, for radiation service.

Irradiation

The shrimp were subjected to gamma radiation while in the frozen state. The average dose rate was 0.88×10^6 rad per hour for 0.5 megarad and 0.83×10^6 rad per hour for 0.75 megarad samples.

The radiation levels selected (0.5 and 0.75) megarad were previously determined by flavor evaluation of samples receiving various amounts of gamma radiation ranging from 0.25 to 2.0 megarad. The flavor threshold level is the point at which a definite taste and aroma due to the irradiation is significantly present (78, p. 118-120). The flavor threshold level of irradiation intensity was established by a trained panel of 19 members. The irradiation intensity ballot used for this experiment is shown in the appendix. The irradiated samples were served with a non-irradiated control for comparison. Significance was determined statistically. The flavor threshold levels varied between 0.50 and 0.75 megarad. These levels of irradiation were used for all subsequent studies.

Storage

Upon receipt of the shrimp from MTR at the laboratory, the irradiated shrimp samples were held at 38° F. for the duration of the storage period. A maximum-minimum thermometer was used for accurate measurement of the temperature. The shrimp samples were stored for a total of 10 weeks. Subsamples were taken at 0, 1, 2, 3, 4, 5, 8, and 10 weeks for the bacteriological, analytical and flavor evaluations.

Flavor Preference

The shrimp were subjected to a flavor preference test involving a trained panel of 15 members. (The ballot used is the flavor preference ballot shown in the appendix.) The flavor evaluations were made at 0, 1, 2, 3, 4, 5, 8 and 10 weeks of storage at 38° F.

Objective Evaluations

Chemical analysis, bacterial counts, and toxicity tests were conducted on the stored and frozen samples.

The chemical tests included:

1. Determination of indole
2. Determination of trimethylamine

3. Determination of volatile base

4. Determination of pH

5. Determination of free amino nitrogen

6. Determination of volume of drip.

All bacteriological analyses and toxicity tests were performed in the laboratories of the Microbiology Department at Oregon State University under the supervision of Dr. A. W. Anderson.

Toxicity Test

Prior to the taste panel evaluations of the stored samples, toxicity tests using mice were conducted to insure the safety of the shrimp for human consumption. For each sample three mice were injected subcutaneously with an aqueous supernatant of the blended fish. The mice were observed during a 72-hour period for appearance of toxic effects such as sluggishness, abnormal behavior, or death.

Determination of the Total Plate Count

Analyses for the total plate counts were made on various types of media. The media used were tryptone glucose yeast extract agar and the following selective medium: Desoxycholate agar and eosin methylene blue agar. Plates poured with the tryptone glucose yeast

extract agar were incubated at 30° C.; all other plates were incubated at 37° C. After an incubation period of about 40 hours, the plates were examined and the colonies were counted.

Chemical Tests

A. Indole

The method used for this determination depends on the reaction between indole and *p*-dimethylaminobenzaldehyde in the presence of hydrochloric or sulfuric acid. A reddish-pink color is produced (16, p. 273). The method used is approved by the Association of Official Agriculture Chemists (1, p. 241-242) briefly as follows:

1. Apply enough heat to the steam distillation apparatus to maintain a volume of 80-90 ml. Collect 350 ml. distillate in about 45 minutes. (If alcohol were used in preparation of sample collect 450 ml.)

2. Weigh 25 g. of the ground sample. Transfer weighed portion to high speed blender; add 100 ml. alcohol and mix 3-5 minutes. Transfer mixture to distillation flask quantitatively.

3. Transfer distillate to 500 ml. separator and add 5 ml. of dilute HCl and 5 ml. saturated Na_2SO_4 solution.

4. Extract successively with 25, 20, and 15 ml. portions $CHCl_3$, shaking vigorously at least one minute each time.

5. Combine the 25 and 20 ml. extracts in a 500 ml. separator and wash with 400 ml. H_2O, 5 ml. saturated Na_2SO_4 solution and 5 ml. of dilute HCl. Save wash H_2O.

6. Filter combined extracts through cotton plug into dry 125 ml. separator.

7. Wash the 15 ml. portion using same wash H_2O and combine with other portions in same 125 ml. separator.

8. Add 10 ml. of the color reagent to combined extracts, shake vigorously exactly 2 minutes and let acid layer separate as completely as possible.

9. Transfer 9.0 ml. acid layer to 50 ml. vol. flask. Make up the volume to 50 ml. with HOAc. Mix well.

10. Transfer to suitable photometer cell, and measure color photometrically at 560 mμ.

11. Prepare standard curve as above by steam distillation of freshly prepared dilutions of standard indole solution.

12. The indole content was calculated as mcg. N/100 g. shrimp.

B. Trimethylamine

Determination for TMA was carried out according to the method of Dyer (32, p. 292-294). The procedure was slightly modified as follows:

1. Weigh 50 g. of the ground sample. Add 100 ml. 7.5% TCA. Shake occasionally over a period of several hours.

2. Centrifuge 5 minutes at 1800 rpm.

3. Pipette off into a plastic bottle.

4. Add 2 ml. H_2O, 1 ml. HCHO, 10 ml. toluene and 3 ml. K_2CO_3.

5. Shake for 2 minutes, pipette off 5 ml. (toluene layer) into a test tube containing approximately 0.3 g. sodium sulfate.

6. Shake, decant to another test tube, add 5 ml. picric acid.

7. Read in a Beckman DU spectrophotometer at 410 mμ against a blank carried through the procedure.

8. TMA is expressed as mg. N/100 g. sample.

C. Volatile bases

The procedure for "volatile bases in fish" described by Stansby, et al. (87, p. 593) was used with some modifications and is briefly as follows:

1. Forty grams of fish are placed in a blender with 100 ml. of 60% ethanol and mixed for 5 minutes.

2. The contents of the blender are transferred quantitatively to 250 ml. centrifuge bottle, using 60% ethanol as wash solution, centrifuged for 10 minutes, and decanted into a 250 ml. volumetric flask.

3. The solids in the centrifuge bottles are stirred with 25 ml. of 60% ethanol, centrifuged, and decanted into the volumetric flask.

The washing and centrifuging is repeated with a second 25 ml. 60% ethanol. The volume is made up with 60% ethanol. The contents of the volumetric flask are transferred to a 500 ml. Kjeldahl flask and 4 glass beads and 5 grams of powdered borax are added. The flask is quickly connected to the distillation equipment and 100 ml. of distillate are collected in 20 ml. of 4% boric acid. The distillate is titrated with 0.05 HCl using methyl red and bromo cresol green as indicators. The volatile bases contents were calculated as mg..N/100 g. of the sample.

D. pH Tests

The samples were thawed to warm to room temperature. The pH of the shrimp was measured with a glass electrode Beckman Zeromatic pH meter which had been standardized with a buffer at pH 7.0. The pH of the drip from the frozen stored samples was taken with the same apparatus.

E. Free Amino Nitrogen

The amino nitrogen method used in this investigation was described by Pope and Stevens (74, p. 1070). The procedure was followed with some slight modifications as follows:

(1) Forty grams of the sample and 80 ml. of distilled water were blended in a high speed blender for two minutes and then centrifuged at 1800 rpm for 5 minutes.

2. 15 ml. were decanted to a 50 ml. volumetric flask, 4 drops of thymolphthalein were added, followed by normal NaOH to a faint blue color.

3. 30 ml. of the copper phosphate suspension were added and the volume made to 50 ml. with distilled water, well mixed and filtered through a No. 5 Whatman paper.

4. 10 ml. of the filtrate were acidified with 0.5 ml. acetic acid and approximately 1.0 g. of Kl was added.

5. The solution was titrated with standardized thiosulphate, 4 drops of starch solution being added towards the end of the titration. The amino nitrogen values were expressed as mg. N/100 g. sample.

F. Determination of drip.

The cans were thawed and the drip was weighed separately. The drip calculated as a percentage of the shrimp before free liquor separation.

RESULTS AND DISCUSSION

The samples of shrimp prepared for this storage study were analyzed objectively and subjectively at designated weekly intervals.

1. Indole in Stored Shrimp

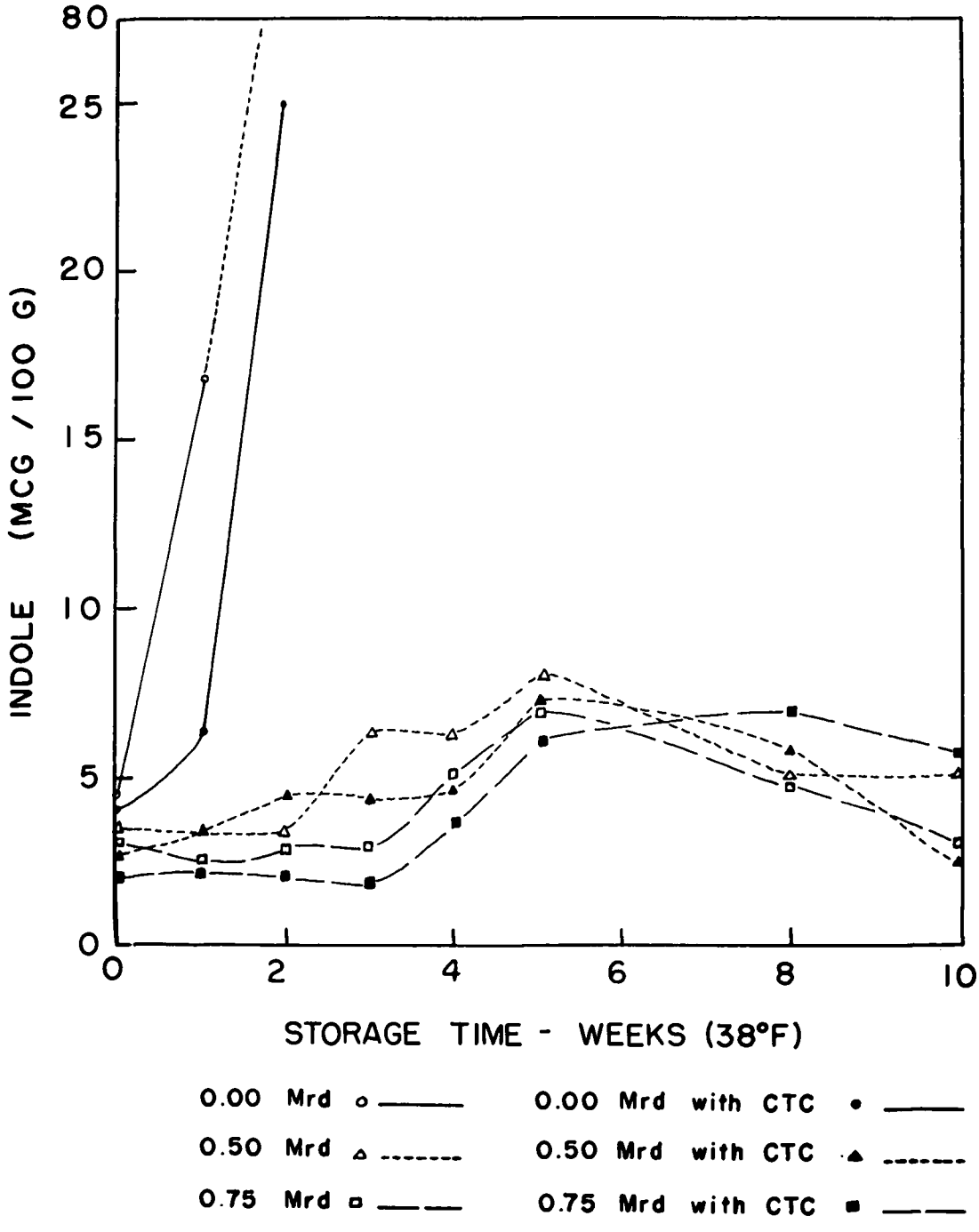
Data for the indole content are presented in Table 1.

Table 1. INDOLE CONTENT OF STORED, IRRADIATED AND CTC TREATED SHRIMP

Storage (weeks)	Indole Content					
	0.00 Mrd		0.50 Mrd		0.75 Mrd	
	==	5 ppm CTC	==	5 ppm CTC	==	5 ppm CTC
	Mcg./100 g.					
0	4.0	4.0	3.5	2.8	3.2	2.1
1	16.8	6.4	3.5	3.5	2.5	2.5
2	79.0	25.0	3.5	4.5	3.1	2.2
3	==	==	6.4	4.4	3.1	2.0
4	==	==	6.4	4.8	5.0	3.9
5	==	==	8.0	7.8	7.0	6.0
8	==	==	5.0	5.8	4.9	6.7
10	==	==	5.0	2.6	2.9	5.8

The indole content is a measure of the extent of decomposition. Duggan and Strasburger (28, p. 188) found that the indole content increased as the decomposition advanced. They found that small

FIGURE 1. INDOLE CONTENT OF STORED IRRADIATED AND CTC TREATED SHRIMP



amounts of indole were normally found in commercially packed shrimp and ranged between 0 to 4.3 mcg/100 g. sample. They also (28, p. 180) found that shrimp having a "strong", "old", or "fishy" odor contained 9-11.2 mcg. indole/100 g. sample. It can be seen that the production of indole increased rapidly and continued at a rapid rate in the stored, non-irradiated samples. There was less indole in the non-irradiated sample treated with CTC, and no appreciable change in indole in the irradiated samples. A graphical representation for the indole content is presented in Figure 1.

2. Trimethylamine in Stored Shrimp

Data on the TMA content of the stored shrimp are presented in Table 2.

It can be seen that the production of TMA increased rapidly and continued at a rapid rate in the stored non-irradiated samples. A very slight increase in TMA was noted in the irradiated samples. This change was much less significant except for the samples of 0.5 megarad without CTC, where there was an increase in the third week and remained insignificant thereafter. At the end of 10 weeks the TMA content was less than 6 mg. per 100 g. for the irradiated samples. Beatty and Gibbons (5, p. 90) stated that at concentrations of 4-6 mg. of trimethylamine nitrogen per 100 g. of fish, off odors began to appear and at 10 mg. they were definite. A graphical presentation of the trimethylamine content of the stored shrimp appears in Figure 2.

Table 2. TRIMETHYLAMINE CONTENT OF STORED, IRRADIATED AND CTC TREATED SHRIMP

Storage (weeks)	TMA Nitrogen					
	0.00 Mrd.		0.50 Mrd.		0.75 Mrd.	
	--	5 ppm CTC	--	5 ppm CTC	--	5 ppm CTC
	Mg. N/100 g.					
0	1.5	1.5	1.3	1.2	1.2	2.6
1	3.5	3.8	2.2	1.6	1.7	2.6
2	8.5	6.4	3.8	1.3	1.5	1.9
3	10.3	7.8	4.0	1.5	1.3	2.2
4	14.6	12.0	4.0	1.5	1.9	2.7
5	--	--	4.0	2.8	2.9	2.7
8	--	--	5.9	2.1	3.0	2.4
10	--	--	4.7	2.2	2.6	2.4

3. Volatile bases in Stored Shrimp

As decomposition proceeds, it has been shown by Beatty and Gibbons (5, p. 90) that ammonia accumulates in the fish. Stansby, et al. (87, p. 593) reported that total volatile bases is widely used as an index of freshness of fish. The actions of enzyme systems in many bacterial species cause hydrolysis of the amino acids and proteins yielding low molecular weight basic volatiles. Values representing the volatile bases in stored shrimp appear in Table 3.

FIGURE 2. TRIMETHYLAMINE CONTENT OF STORED IRRADIATED AND CTC TREATED SHRIMP

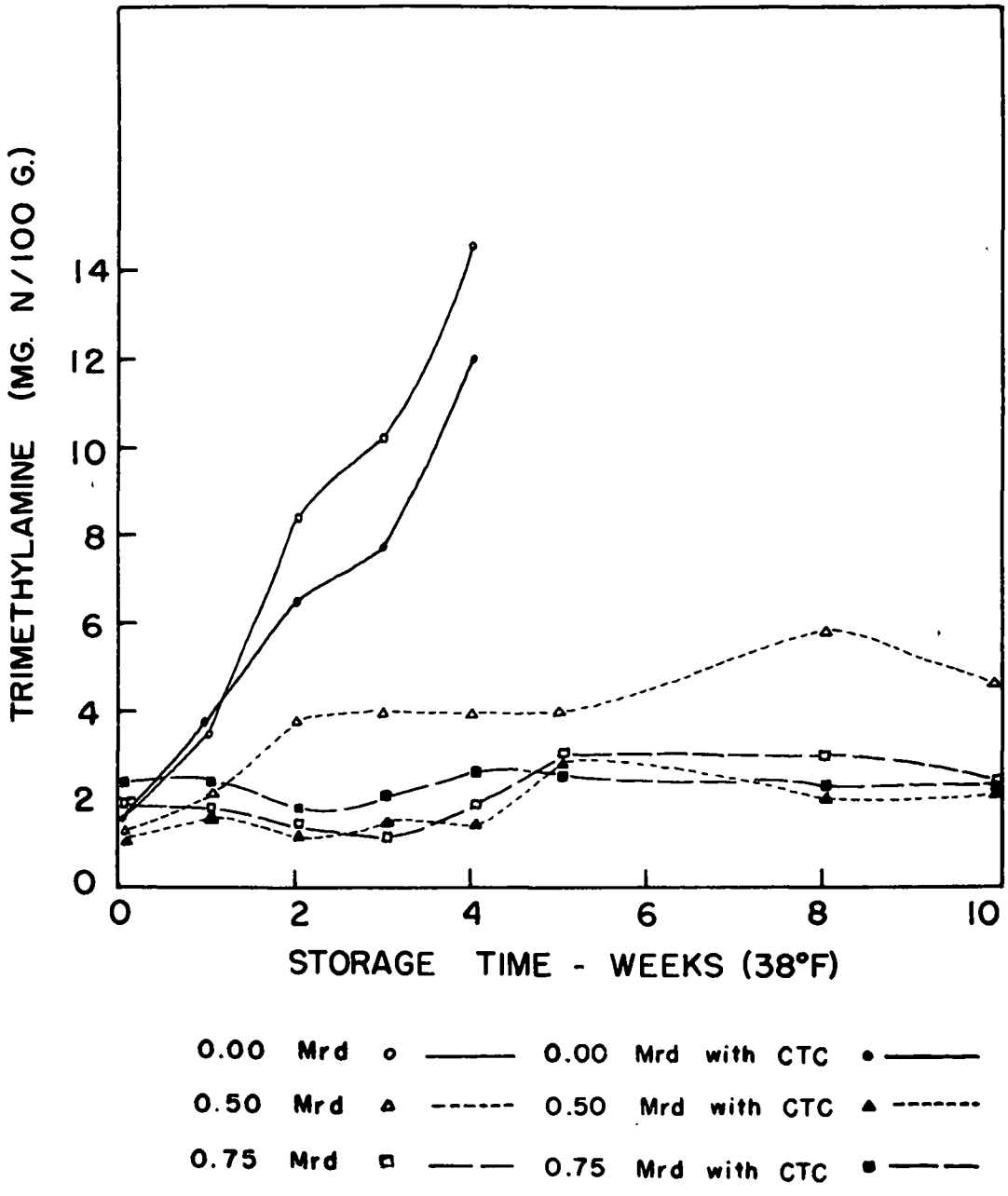


Table 3. VOLATILE BASES IN STORED, IRRADIATED AND CTC TREATED SHRIMP

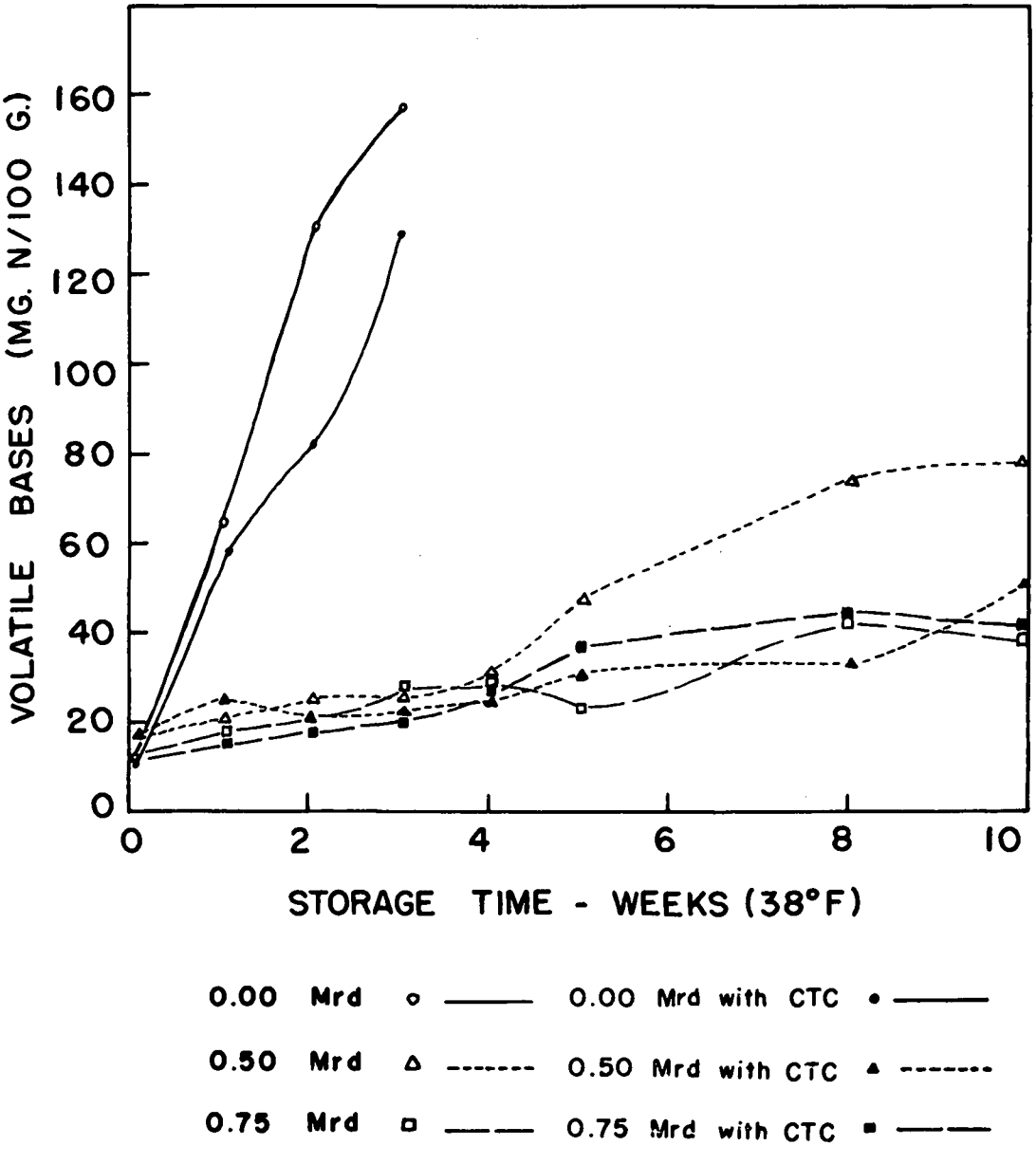
Storage (weeks)	Volatile Bases					
	0.00 Mrd.		0.50 Mrd.		0.75 Mrd.	
	--	5ppm CTC	--	5 ppm CTC	--	5 ppm CTC
	Mg. N/100 g.					
0	11.8	11.7	14.6	14.2	12.9	12.5
1	64.5	59.0	20.3	25.1	18.7	15.7
2	130.3	82.6	24.3	22.1	21.6	19.9
3	---	---	24.4	24.7	26.9	21.6
4	---	---	31.0	25.9	28.4	27.5
5	---	---	47.9	31.6	23.9	37.0
8	---	---	73.2	32.4	45.7	45.1
10	---	---	76.8	50.3	38.8	43.2

The data are presented graphically in Figure 3. There was a rapid increase of the volatile bases in the non-irradiated samples by the first week. Samples irradiated at 0.5 megarad and 0.75 megarad showed a slight increase in the volatile bases content.

4. pH of Stored Shrimp

Bethea and Ambrose in 1962 (8, p. 9) reported that the pH of peeled brown shrimp appeared to be useful as an indication of quality. Data indicating the pH of the stored irradiated and CTC treated

FIGURE 3. VOLATILE BASES IN STORED IRRADIATED AND CTC TREATED SHRIMP



shrimp are represented in Table 4.

Table 4. pH OF STORED, IRRADIATED AND CTC TREATED SHRIMP

Storage (weeks)	pH					
	0.00 Mrd.		0.50 Mrd.		0.75 Mrd	
	--	5 ppm CTC	--	5 ppm CTC	--	5 ppm CTC
0	7.05	7.05	7.05	7.06	7.10	7.18
1	7.36	7.28	7.15	7.12	7.10	7.12
2	7.72	7.58	7.00	7.16	7.0	7.10
3	8.16	7.95	7.10	7.10	7.10	7.08
4	---	---	7.12	7.20	7.12	7.05
5	---	---	7.15	7.20	7.11	7.05
8	---	---	7.10	7.00	7.06	7.04
10	---	---	7.20	7.19	7.15	7.12

There was an increase of pH in the non-irradiated samples at one week, continuing rapidly after that. Bailey, et al. (2, p. 611) reported that the pH of shrimp increased with spoilage to a level of about 7.95 when the samples are considered unfit. Scholz, Sinnhuber, East and Anderson (78, p. 118-120) found that pH did not appear to be indicative of the quality of irradiated crab, but the pH of the irradiated cooked shrimp seemed to parallel the rate of deterioration.

The pH of the drip was also measured from stored, irradiated and CTC treated shrimp. It is presented in Table 5.

Table 5. pH OF THE DRIP FROM STORED, IRRADIATED AND CTC TREATED SHRIMP

	pH					
	0.00 Mrd.		0.50 Mrd.		0.75 Mrd.	
Storage (weeks)	---	5 ppm CTC	---	5 ppm CTC	---	5 ppm CTC
0	7.12	7.05	7.05	7.10	7.0	7.02
1	7.32	7.25	7.12	7.08	7.10	7.00
2	7.92	7.48	7.07	7.02	7.0	7.00
3	---	7.90	7.00	6.90	6.9	7.00
4	---	---	7.00	6.98	6.98	7.10
5	---	---	7.00	7.22	7.25	7.05
8	---	---	7.30	7.20	7.18	7.08
10	---	---	7.48	7.15	7.20	7.00

The non-irradiated samples show a rapid pH increase after two weeks. The values indicate that there is no appreciable increase of the pH in the irradiated samples.

A graphical representation for pH of the shrimp meat and of the drip are shown in Figures 4 and 5 respectively. There is a

FIGURE 4. pH OF STORED IRRADIATED AND CTC TREATED SHRIMP

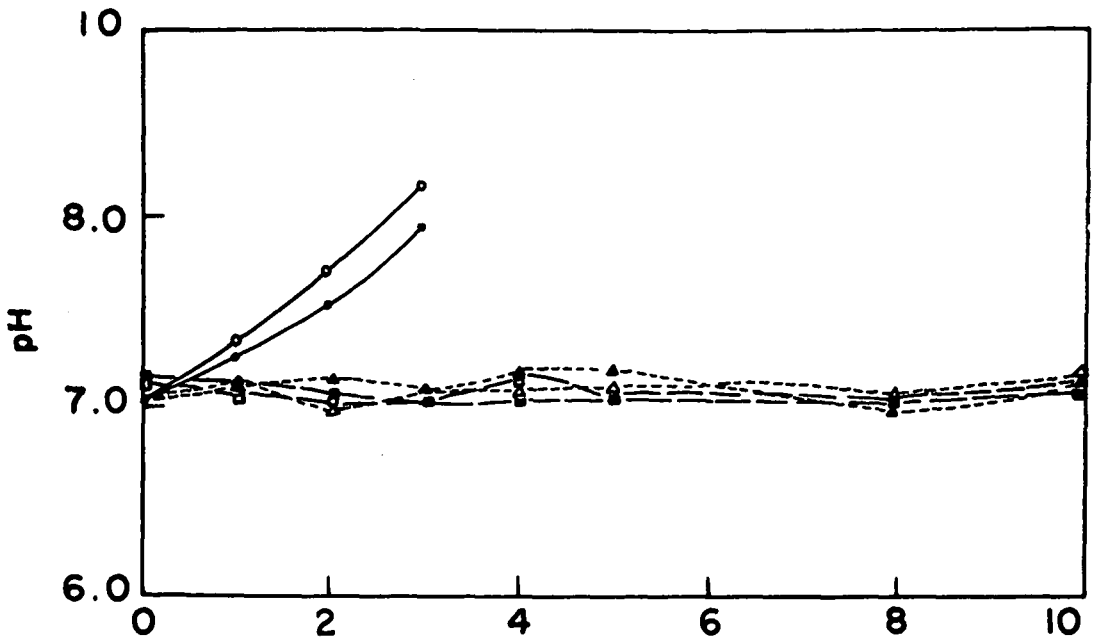
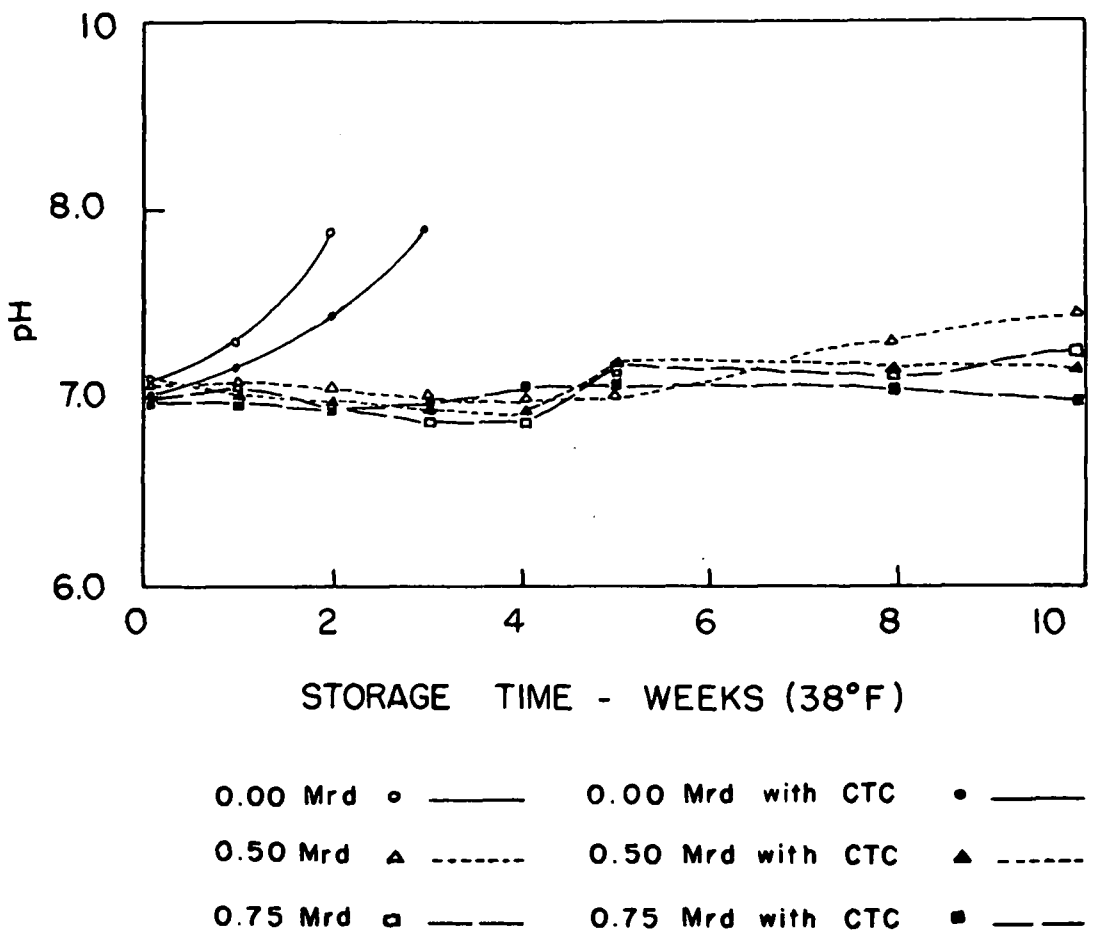


FIGURE 5. pH OF THE DRIP FROM STORED IRRADIATED AND CTC TREATED SHRIMP



good agreement between the pH values of both meat and drip.

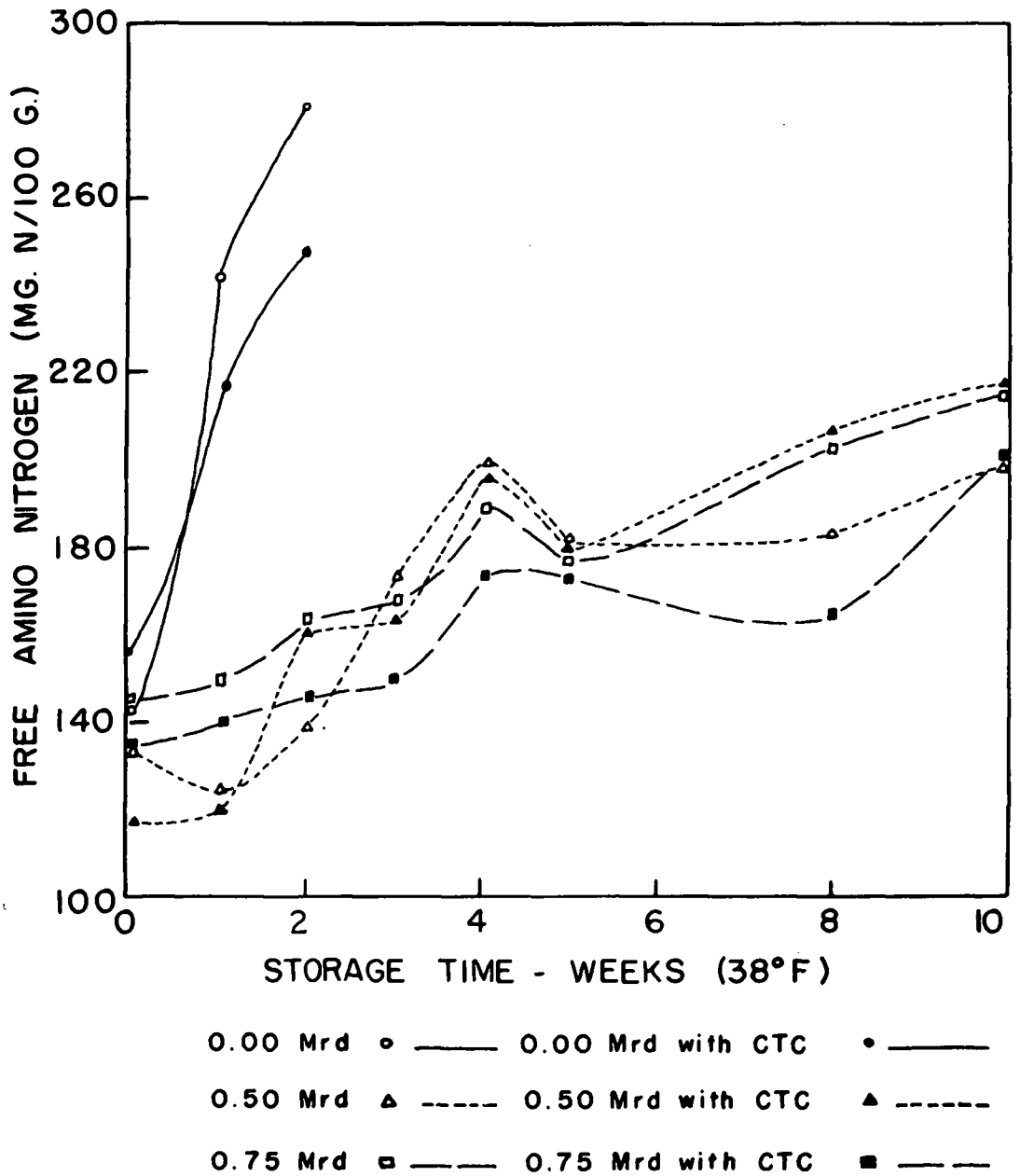
5. Free Amino Nitrogen in Stored Shrimp

This procedure is used to measure the degree of hydrolysis of proteins by enzymes. Data presenting the change in free amino nitrogen content during storage are presented in Table 6 and a graphical representation is given in Figure 6.

Table 6. FREE AMINO NITROGEN CONTENT OF STORED, IRRADIATED AND CTC TREATED SHRIMP

	Free Amino Nitrogen					
	0.00 Mrd.		0.50 Mrd.		0.75 Mrd.	
Storage (weeks)	---	5 ppm CTC	---	5 ppm CTC	---	5 ppm CTC
	Mg. N/100 g.					
0	148	156	134	117	145	134
1	252	218	123	118	151	140
2	280	245	149	160	164	145
3	---	---	162	162	169	151
4	---	---	200	196	189	173
5	---	---	180	180	178	172
8	---	---	184	207	204	163
10	---	---	210	218	216	201

FIGURE 6. FREE AMINO NITROGEN CONTENT OF STORED IRRADIATED AND CTC TREATED SHRIMP



In this determination one can notice the increase of the free amino nitrogen content in both the irradiated and the non-irradiated samples. An increase in free amino nitrogen indicates that protein degradation has occurred and is due to the activity of either bacterial enzymes or autolytic enzymes present in the tissue, or both. Beatty and Collins (6, p. 412-423) found that the amino nitrogen content of press cod fish juice decreased during storage and that the decrease approximately equaled bacterial deamination. Opposite results for amino nitrogen content were obtained by Campbell and Williams (12, p. 125) and by Sigurdsson (83, p. 892) for herring. Both experiments indicated an increase in amino nitrogen during ice storage. These different results can possibly be explained on the basis of different bacterial flora in various seafoods resulting in different degradative products, although it has been shown that amino nitrogen content of fish depends on the autolytic changes in fish flesh, as well as the chemical composition of the flesh (71, p. 84). Another possibility is the rapid association of amino groups with denatured protein molecules masking them from detection by the procedure used (2, p. 613).

6. Drip in Stored Shrimp

The data are shown in Table 7.

The value of the weight of the drip was of little or no value as quality index. This statement agrees with that of Bethea and

Ambrose in 1962 (8, p. 11).

Table 7. THE AMOUNT OF THE DRIP IN STORED, IRRADIATED AND CTC TREATED SHRIMP

Storage (weeks)	Free Liquor (Drip) Percent					
	0.00 Mrd.		0.50 Mrd.		0.75 Mrd.	
	---	5 ppm CTC	----	5 ppm CTC	----	5 ppm CTC
0	5.0	3.4	3.5	5.0	5.4	5.3
1	5.3	7.6	4.6	6.0	6.3	9.1
2	3.4	7.9	7.5	6.5	15.3	11.8
3	10.7	8.8	9.5	6.6	4.7	10.8
4	12.9	9.4	7.3	4.9	7.9	6.3
5	36.0	7.4	5.2	6.2	6.2	5.3
8	---	---	---	5.1	5.5	7.0
10	---	---	6.1	6.5	9.4	7.2

Flavor preference of Stored Shrimp

The shrimp was subjected to a flavor preference test involving a trained panel of 15 members. The flavor evaluations were made at 0, 1, 2, 3, 4, 5, 8, and 10 weeks of storage at 38° F. Samples were heated for 3-4 minutes in boiling water and served in small paper cups coded with three-digit random numbers. In all flavor

tests a non-irradiated sample which had been held at 0° F. was presented with the irradiated samples. The non-irradiated samples were served only at "0" weeks as they were obviously spoiled by the end of the first week.

It was seen that in most cases the non-irradiated samples at 0° F. were preferred. The samples receiving 0.5 megarad were acceptable until five weeks and at eight weeks were considered spoiled. The samples receiving 0.5 megarad + CTC and all the samples of 0.75 megarad remained in good condition by the end of ten week storage. The results were determined statistically; no significant differences were obtained.

A score of four indicated a neutral response to the samples, that is, the samples were neither better nor poorer, than the reference. A score below four indicated a poorer flavor than the reference and a higher score indicated a better flavor than the reference (see appendix). Data for the flavor preference are presented in Table 8. A graphical representation for the data is presented in Figure 7.

8. Total Plate Count of Stored Shrimp

The total plate count was made in the Microbiology Department at Oregon State University under the supervision of Dr. A. W. Anderson. The medium used was the tryptone glucose yeast extract agar. The total plate count data are presented in Table 9.

FIGURE 7. FLAVOR PREFERENCE OF STORED IRRADIATED AND CTC TREATED SHRIMP

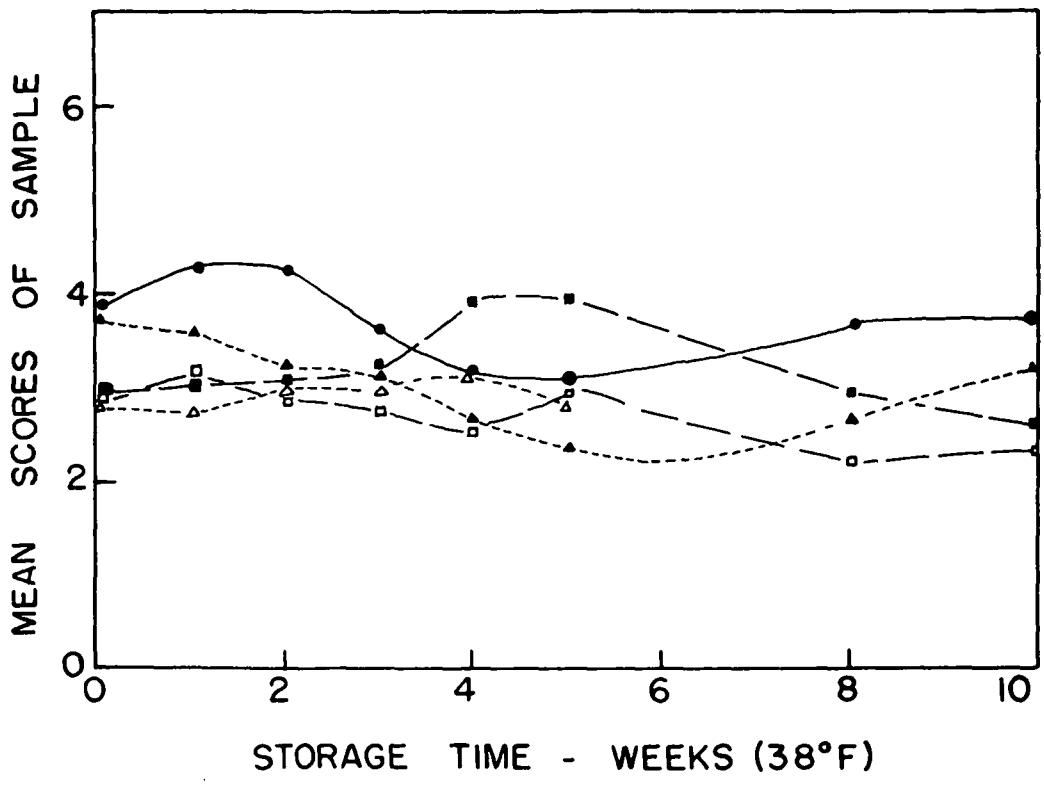


Table 8. FLAVOR PREFERENCE IN STORED, IRRADIATED AND CTC TREATED SHRIMP

Storage (weeks)	Mean Scores of Samples					
	0.00 Mrd.		0.50 Mrd.		0.75 Mrd.	
	0° F.	38° F.	---	5 ppm CTC	---	5 ppm CTC
0	3.9	3.9	2.8	3.7	2.9	3.1
1	4.4	---	2.7	3.6	3.2	3.3
2	4.3	---	3.0	3.3	2.9	3.1
3	3.7	---	3.0	3.1	2.8	3.3
4	3.2	---	3.2	2.7	2.6	4.1
5	3.2	---	2.8	2.5	3.1	4.1
8	3.7	---	---	2.7	2.3	3.0
10	3.7	---	---	3.1	2.3	2.5

Table 9. TOTAL PLATE COUNT IN STORED, IRRADIATED AND CTC TREATED SHRIMP

Storage (weeks)	Total Count in thousands per gram					
	0.00 Mrd.		0.50 Mrd.		0.75 Mrd.	
	---	5 ppm CTC	---	5 ppm CTC	---	5 ppm CTC
0	7300	5000	10	7.5	1.6	1
1	12000	21000	1	3	1	1
2	20000	29000	3	1	0	1
3	---	---	3	1	1	0
4	---	---	1	1	0	0
5	---	---	1	0	0	0
8	---	---	0	0	0	0
10	---	---	---	0	0	0

From the table it is obvious that the total counts of the non-irradiated samples are much higher than the irradiated ones. Increasing the level from 0.5 to 0.75 megarad reduced the total plate count. Using 5 ppm CTC reduced the total plate count for each level. In the irradiated samples at 0.5 megarad the count seemed to show a limited increase in number and then a decrease. At 0.75 megarad the total plate count was insignificant and less than 1000 per gram.

The increase and subsequent decline may have been due to the survival of organisms which were obligately aerobic and which eventually exhausted the supply of oxygen or possibly to the survival of microorganisms which required a growth factor which was in limited supply (23).

SUMMARY AND CONCLUSIONS

The feasibility of extending the storage life of shrimp by the use of pasteurization levels of ionizing radiations in combination with refrigerated storage at 38° F. and chlorotetracycline was investigated. Levels of irradiation which would not impart a significant irradiation odor or taste (pasteurization radiation threshold) to raw shrimp were determined. These levels were 0.50 and 0.75 megarad.

Irradiated and non-irradiated samples were subjected to a storage period of ten weeks. These samples were compared at specific intervals for changes in quality. Methods of analysis included organoleptic, microbiological and chemical evaluations.

Conclusions

1. Significant increases in degradative products in the non-irradiated samples were noted by the end of the first-week of storage according to chemical and microbial tests.

2. In almost all cases the irradiated samples after ten weeks storage at 38° F. gave chemical results equal to or less than the values of the non-irradiated samples after one week.

3. Pasteurization radiation effected about 4 to 5 fold extension of storage life based on the flavor preference test.

4. CTC treatments have a complimentary effect to radiation and yield a more desirable product. This is more noticeable at the lower pasteurization level.

5. The TMA, volatile bases and the indole content agreed closely and appeared to be good indices of the quality of the irradiated raw shrimp.

6. pH of the irradiated raw shrimp remained unchanged during the storage period.

7. The volume of the drip changed little, if any, during the storage period in all samples.

8. Organoleptic evaluations of the shrimp receiving 0.5 megarad of irradiation without CTC showed a development of off-flavors at eight weeks. In the samples which received 0.5 megarad + CTC and in all the samples treated with 0.75 megarad, no off-flavors had developed by 10 weeks.

9. The free amino nitrogen content increased after the first week in both irradiated and non-irradiated samples and continued to increase during the storage period. This was due to the autolytic enzymes which are still active after irradiation of the raw shrimp. This may be used as a measurement of storage time of irradiated, raw shrimp.

10. According to the total plate count results, there was an increase and then a subsequent decline of microorganisms in the

irradiated raw shrimp at 0.5 megarad. Samples which received 0.5 megarad + CTC showed a decrease in the total plate count. In all other samples treated with 0.75 megarad, the total plate count was insignificant and less than 1000 per gram.

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APPENDIX

FLAVOR PREFERENCE BALLOT

Date: _____ Product _____ Name _____

INSTRUCTIONS:

The side dish contains a reference sample. Compare the flavor of each sample directly with the flavor of the reference sample.

(a) Taste the reference and the sample as many times as necessary;

(b) Write the number which is on sample container in a space after the statement which indicates your judgments.

Much better flavor than reference				
Moderately better flavor				
Slightly better flavor				
Neither better nor poorer flavor				
Slightly poorer flavor				
Moderately poorer flavor				
Much poorer flavor than reference				

Comments:

Sample of Irradiation Intensity Ballot

Irradiated Seafood Project

Name _____ Product _____ Date _____

The reference sample is a normal non-irradiated sample. Please score the intensity of irradiated odor and flavor in the coded samples. Mark sample numbers opposite horizontal lines where you think the flavor intensity lies. Do not mark between the lines.

IRRADIATED ODOR		IRRADIATED FLAVOR	
None		None	
Slight Amount or intensity		Slight Amount or intensity	
Moderate Amount or intensity		Moderate Amount or intensity	
Large Amount or intensity		Large Amount or intensity	
Extreme Amount or intensity		Extreme Amount or intensity	
Very Extreme intensity		Very Extreme intensity	

Comments:

Comments: