RADIATION PASTEURIZED SHRIMP AND CRABMEAT

by

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TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

LIST OF TABLES

LIST OF FIGURES

RADIATION PASTEURIZED SHRIMP AND CRABMEAT

INTRODUCTION

Ionizing radiations have been employed effectively in destroying or reducing microbial numbers of food products. Elimination of micro-organisms by this method of food preservation should permit a greatly extended storage life. Unfortunately, the high levels of radiation necessary to completely destroy all life frequently imparts off-flavors or has a deleterious effect on the food. Low level or pasteurization doses of radiation could conceivably avoid many of the undesirable changes and still destroy most of the spoilage organisms and extend the refrigerated shelf life.

Shrimp have become the most valuable product to the fishing industry and recent investigations have indicated that shrimp may be one of the largest untapped fishery food resources in the sea (1, p. 357). Crab is also commercially important and the crabmeat has become a popular food item with the consumer. The spoilage pattern of radiation pasteurized shrimp and crabmeat may be different from the spoilage of the fresh shellfish. Therefore, it was the purpose of this research investigation to subject shrimp and crabmeat to pasteurization levels of gamma radiation and study the spoilage pattern that develops

during refrigerated storage. The pasteurization doses used were determined previously as those levels of irradiation which did not impart a pronounced irradiation flavor to the shrimp and crabmeat. The quality of the shellfish was ascertained by objective and subjective evaluations during the storage period.

The majority of fish and fish products are sold fresh or frozen, resulting in the problem of marketing in inland areas. An extension of the storage life of the shrimp and crabmeat by radiation pasteurization would enable the expansion of existing markets and the development of new markets in inland areas, while eliminating some of the expense involved in frozen storage.

LITERATURE REVIEW

Foods soon after harvest start to deteriorate and lose their original fresh quality. Food science and technology is dedicated to a better understanding of this deteriorative process and the means of preventing or allaying this spoilage action. The principal causes of spoilage in foods result from the growth of microorganisms, the action of naturally occurring enzymes, chemical reactions, and physical degradation and desication. The type of spoilage in a particular food item depends to a great extent on the composition, structure,

microflora, and storage conditions.

The growth of micro-organisms is the main cause of food spoilage according to Hannan (29, p.752). Desrosier and Rosenstock (15, p.290), report that microbiological deterioration of a food can occur within a day or two following the initiation of growth, but is a function of time, temperature and environment as well as the initial population of organisms. Logically then, whatever can be done to reduce the numbers of spoilage micro-organisms will prolong the useful life of the food product, provided there is adequate protection against other types of spoilage.

Food Preservation by Irradiation.

Research in many laboratories has shown that ionizing radiations has effectively destroyed micro-organisms. Morgan and Siu (43, p.277), pointed out, however, that the application of heat remains the most practical method for inactivating enzymes. Results of initial investigations by Shewan and Liston (49, p.377) indicated that irradiation produced noticeable changes in odor, taste, color, and texture of many foods. Subsequent chemical studies of these changes showed that the substances responsible were present only in trace amounts. It is possible, however, that toxic products also might have been

produced in minute but significant amounts. McNamara et al. (39, p.68) demonstrated through extensive laboratory tests and feeding trials that no toxic substances were produced by the irradiation process. Induced radioactivity does not occur at the levels used in the irradiation of food according to existing information compiled by Meinke (40, p.37). Doty et al. (17, p.414) suggested that with the proper application of ionizing radiations, wholesome and nutritious foods could be obtained.

Foods that are sensitive to thermal processing can be preserved by ionizing radiation since it is a cold process (27, p.488).

Radiation processing promises major economic benefits according to recent observations by Mason (38, 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.

Morgan (41, p.423) found that sterilization of food products is effected between 3.0 and 5.0 x 10^6 rads varying with the original bacterial population. By definition, one rad equals 100 ergs of absorbed energy per gram of material (31, p.287). The killing dose to insure preservation against spoilage from micro-organisms

is about 3.0 to 4.0 x 10^6 rads, but a dose of 4.5 x 10^6 rads is required to insure safety from the more resistant spores of Clostridium botulinum in foods above pH 4.5 (42, p.357).

Unfortunately in radiation sterilization of many foods, some undesirable chemical and physical changes take place. These may adversely affect the quality of the food through changes in odor, taste or texture (13, p. 877). Clifcorn (11, p.40) pointed out that these changes are in direct proportion to the radiation dose applied and vary in degree from product to product.

Radiation Pasteurization.

Research investigators recently have used low level doses of radiation to achieve some extension of storage life with only slight changes in quality. Morgan (41, p.423) defines this low level irradiation as radiation pasteurization; that is, the killing of 98 per cent of the micro-organisms.

If a food is to be stored in the frozen state, spoilage organisms are not a great problem. Therefore irradiation is not needed simply to prolong the storage life, according to Ingram (32, p.106). Ingram (32, 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, fruits 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. Therefore, Ingram (32, p.105) stated, chilling seems necessary to supplement the process of radiation pasteurization which might allow the food poisoning organisms to survive. The virtue of pasteurization radiation, he concluded, is its use in reducing those micro-organisms which spoil the food above 40°F., as these are especially sensitive to irradiation. Moderate doses of radiation reduce the microflora without causing appreciable changes in quality. Since pathogenic bacteria do not develop below 40°F., pasteurization radiation in combination with low temperature storage is a very feasible process (32, p.106).

The level 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 x 10^6 rads followed by refrigerated storage (12, p.115). Niven (44, p.518) stated that even though the

predominant organisms are destroyed by this process, a less dominant but more resistant bacterial species survives and ultimately causes spoilage.

Clifcorn (11, 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. The storage and distribution life of fresh foods can be extended by a factor of 5 to 10 and the costs for irradiation at these lower levels can be reduced by one-fifth from the cost of sterilizing doses.

Radiation Induced Chemical Changes.

An understanding of the chemical changes induced by ionizing radiations is essential before the elimination of undesirable side reactions such as radiation flavor, poor texture, and adverse color changes can be brought about. The ultimate goal of the radiation chemist is to trace in detail the energy and charge transfer steps of the ionized particles and the resultant chemical steps which occur from the moment the high energy particles pass through a system until thermal equilibrium is again achieved. Willard (61, p.141) suggested that this is a difficult task due to the many states of excitation and the many secondary physical and chemical reactions theoretically

possible.

Radiation produces a variety of chemical species such as ions, free radicals and excited molecules. "These species undergo further reactions among themselves and with their environment to form radiation products," Willard (61, p.141) reported.

As an ionizing particle travels through matter, its energy is dissipated primarily by collision with electrons. If the contact or interaction is sufficiently energetic, an electron is ejected from its parent atom or molecule. A less direct or energetic contact may result in a transfer of energy sufficient to raise the atom or molecule to a higher state of electronic excitation (8, p.88). 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 severing of 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 (8, p.88-89). Morgan (41, p.424) reported that about 0.003 per cent of the CH_3 bonds in food are broken with sterilization doses of irradiation.

Free radicals which are formed by the decomposition of excited molecules undergo a variety of reactions of which the following types predominate: 1. abstraction, 2. recombination, 3. disproportionation, and 4. polymerization. The types of reactions undergone by ions are similar to the above. These are: 1. decomposition of excited ions, 2. charge transfer, 3. ion-molecule reactions, and 4. ion-electron recombination (15, p. 40-44).

Direct "hits" may be responsible for some specific biological effects, but many effects are caused in whole or in part by the solvent. Siu and Bailey (53, p.97) attributed effects of irradiation to the actions of radiation products of water. They claimed these were formed by the splitting of water into hydrogen atoms and hydroxyl radicals. These two radicals are known to be chemically very active and can act as reducing and oxidizing agents as well as carbon-carbon bond cleaving devices. The secondary products of irradiation may be of greater 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 hydrogen peroxide.

$$OH + OH \longrightarrow H_2O_2$$

Black (8, p.88-89) concludes that there is no doubt that both direct and indirect action take place, but the indirect theory offers a wider basis for chemical change.

Radiation Induced Changes in Proteins.

Considerable denaturation, fragmentation, and aggregation of proteins occur due to the effects of ionizing radiation according to Morgan (41, p.424). Irradiation has three main effects on proteins: 1. specific chemical changes, 2. changes in physiochemical properties including denaturation, 3. less clearly defined changes such as off-odor formation. Since proteins are condensation polymers of a number of amino acids joined by peptide linkages, the effects on proteins depend on the amino acid composition, the sequential arrangement of the amino acids, and the length of the polymer chain. A protein molecule may behave in the presence of radiation both as a single molecular entity, such as when it is denatured, and as a composite

of various amino acids which each display a characteristic radiation sensitivity (18, p.156-158).

Sulfur-containing amino acids (methionine, cysteine, and cystine) and ring-containing amino acids (proline, hydroxyproline, phenylalanine, histidine, and tryptophan) are the most sensitive to irradiation (41, p.426).

Recent investigations by Drake and Giffee (18, p. 157-158) have shown that chemical changes in irradiated proteins include liberation of ammonia, oxidation of S - H groups and formation of peroxides. Morgan (41, p. 426) reported that low molecular weight volatile compounds such as ketones, aldehydes, alcohols, mercaptans, and amines are formed.

The deamination of amino acids appears to be a characteristic action of ionizing irradiations, according to Ise and Fox (33, p.7). Ise and Fox (33, p.10) further suggested that the principal products of deamination of amino acids are ammonia and the corresponding aldehyde. For example glycine, $(NH_2)CH_2COOH$, produce formaldehyde, HCHO, and alanine, $CH_3(NH_2)CHCOOH$, produce acetaldehyde, CH_3CHO. 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 an integral part of the protein backbone (as in dibasic amino acids) and from rupture of the peptide linkages (18, p.156-158). The mechanism is

not yet clear but probably involves direct deamination resulting from attack by H· and ·OH radicals. The deamination process produces a radical formed by removal of the NH_2 group and the subsequent reaction of the radical in irradiated aqueous solution to produce a variety of oxidative products. The mechanisms of the deamination:

$$OH + H_3N^+CH_2COO^- \longrightarrow OH\cdot CH_2COO^- + NH_3^+$$

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

Investigations by Garrison and Weeks (26, p.618) have shown that hydrogen and hydroxyl radicals act on glycine in two ways: removal of amino groups or removal of hydrogen from the amino-bearing carbon.

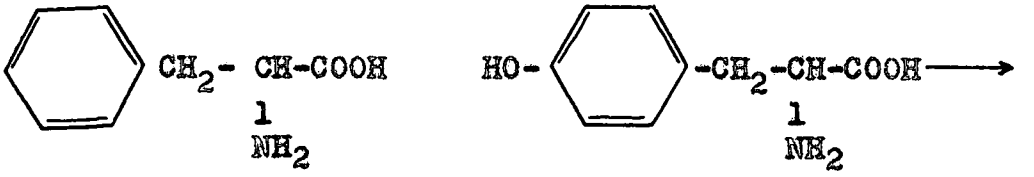
$$(H\cdot \ \cdot OH) + (NH_2)CH_2COOH \longrightarrow \cdot CH_2COOH + NH_3$$

$$(H\cdot \ \cdot OH) + (NH_2)CH_2COOH \longrightarrow (NH_2)\dot{C}HCOOH + H_2 \text{ or } H_2O$$

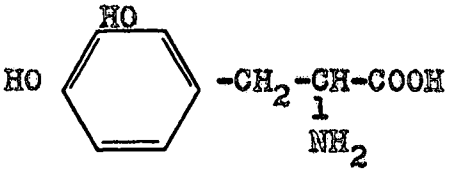
Dale and Davies (14, p.129-134) reported that irradiation of sulfur-containing amino acids, liberates hydrogen sulfide or oxidizes the S-H groups to disulfide and finally sulfinic $-SO_2H$ and sulfonic $-SO_3H$ acid groupings. According to Ise and Fox (33, p.9), H_2S is liberated when cysteine hydrochloride or glutathione is irradiated in the reduced state.

Hydroxylation of the ring in aqueous solutions of cyclic amino acids has occurred in some cases (45, p.535-540). Evidence of this is the hydroxylation of

phenylalanine to tyrosine and a second hydroxylation forming 3,4-dihydroxy phenylalanine.



phenylalanine tyrosine



3,4-dihydroxy phenylalanine

Ise and Fox (33, p.13) reported that rupture of the aromatic ring can also occur due to irradiation.

Morgan (41, 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.

Doty, et. al. (17, p.414), stated that there is an appreciable reduction of soluble protein in ground lean beef after irradiation, but an increase in the non-protein nitrogen compounds. There is also an increase in the formation of hydrogen sulfide and methyl mercaptans. Batzer and Doty (4, p.64) found that the pH and the carbonyl compounds increased in meat with increasing

radiation dosages.

An increase in the total carbonyl content of irradiated beef was reported by Morgan (41, p.425). His studies indicated that low molecular weight extractable carbonyls were obtained from the protein fraction.

Radiation Induced Changes in Quality.

The changes brought about in a food product by the ionizing treatments are minute and in many cases beyond the sensitivity of the usual chemical and physical methods of measurement (23, p.198). Chemical changes are frequently detected by the slightly modified odor, taste, or texture of the product because humans have sense acuity in parts per billion (19, p.1394).

Results of research by Robinson (46, p.192) showed that the changes in flavor and color are probably due to free radicals produced by the effect of the ion radiations on water. These ions are either reducing or oxidizing substances and react with components of food being irradiated.

Hannan and Sheppard (31, p.287) reported that the irradiation odor in foods is due to the formation of complex mixtures of volatile compounds including H_2S and other sulfur containing compounds, soluble proteins and amino acids, and carbonyl compounds.

Morgan (41, p.426) suggested, however, that it is doubtful that 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, sulfhydryls and phenol hydroxyls.

Water soluble proteins, on the other hand, have been implicated in studies by Morgan (41, p.426) as major contributors to odors in meat after irradiation.

The threshold dose at which quality changes can be distinguished depends upon the method of assessment. Coleby (12, p.116) reported that the change in odor of raw meat is one of the most sensitive sensory tests, and it is often possible to detect changes in odor due to irradiation with doses as low as 0.05×10^6 rads. Cooking usually makes it difficult or impossible to detect such low doses. Experiments by Hannan and Sheppard (31, p.286) have shown that after light cooking, a dose in excess of 0.25×10^6 rads was necessary before discrimination of irradiated flavor could be made in minced chicken. Doses of 0.8×10^6 rads given roasted whole chickens produced scarcely detectable flavor changes Coleby (12, p.116) reported. The relative threshold levels for the detection of

irradiation induced flavor varies with the type of product and its eventual destination. For example, a product intended for use in restaurants or hotels will be cooked before reaching the consumer, who will thus be spared any unpleasant odors evolved during cooking (12, p.117).

Morgan (41, 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 irradiation. About 20 per cent more thiamine is lost by irradiation than by heat treatment.

The susceptibility of carotenoids to radiation damage is controlled by the environment in the food in which they are found according to Morgan (41, p.424). If the pigment is solubilized in a lipid, the pigment will be much more susceptible to oxidative degradation during radiation then when it is bound to the protein fraction.

Fish Spoilage and Its Detection.

Spoilage of fish has been described by Reay and Shewan (47, p.384) as the interaction between bacteria and the natural components of the fish. Stansby (54, p.261) attributed fish spoilage to oxidation and enzymatic action.

In addition, autolysis accounts for a small part of fish decomposition, according to Sigurdsson (50, p.892). The spoilage of fish is a complex process that can proceed along many diverse lines, Stansby (54, p.262) concluded.

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 (54, p.261).

Microbiological 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 (50, p.892). Microbial action on fish gives rise to volatile breakdown products which are responsible for marked odors that develop during spoilage (24, p.319). These changes in the natural odors led to the use of organoleptic tests for measuring the quality of the fish. Farber (24, p.319) found that this method proved unsatisfactory because it was limited by an individuals sense acuity and other environmental conditions.

A number of chemical tests were proposed to measure these changes in spoilage; but according to Stansby

(54, p.260), none were consistently accurate in measuring fish freshness for all species. Sigurdsson (50, p.893) reported that available evidence indicated the impossibility of ascertaining with any degree of certainty which of several tests was the best for evaluating the quality of fish products.

In 1937 Beatty and Gibbons (7, p.91) observed that the increase in trimethylamine in the muscle of marine fish closely paralled the degree of decomposition and concluded that "the trimethylamine in fish muscle fulfills all the conditions required of an effective test for freshness". Later, Beatty (5, p.63-68) demonstrated that at least 94 per cent of the trimethylamine is derived from trimethylamine oxide naturally present in the muscle, which is quantivatively reduced to trimethylamine in the advanced stages of decomposition. Watson (59, p.266) showed that trimethylamine oxide functions as a hydrogen acceptor during the decomposition of the flesh and is reduced to trimethylamine during the fermentation of lactic acid by certain facultatively anaerobic bacteria. According to Reay and Shewan (47, p.367), trimethylamine is responsible for the stale fishy odor of fish products. Beatty and Gibbons (7, p.85-90) stated that at concentrations of 4 to 6 milligrams of trimethylamine nitrogen per hundred grams of fish, off-odors began to appear and at 10

milligrams they were definite. Variations were noted among species according to the initial content of trimethylamine. They also reported that the lowest value for trimethylamine found was 0.06 milligrams per hundred grams although 0.17 milligrams per hundred grams was found to be the average initial concentration of the trimethylamine in fish.

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 carbinol, lactic acid, and diacetyl, according to Sigurdsson and Wood (51, p.45-52). Sigurdsson (50, p.899) observed that the production of volatile acids in herring closely followed the trimethylamine production. He also found that off-odors were detected at a concentration of 60 milliliters of 0.01 N acid per hundred grams and at 70 to 90 milliliters per hundred grams, the fish was definitely spoiled. Research by Farber (24, p.323) has shown that the volatile acids content is a variable quantity and depends largely on the types of spoilage micro-organisms predominating during deterioration.

Beatty and Gibbons (7, p.77) found that ammonia accumulates as decomposition proceeds. They pointed out that the volatile base content in the flesh approximated the advent of odors of incipient spoilage. Beatty and

Gibbons (7, p.91) have shown that the development of ammonia is not as good an index of spoilage as trimethylamine.

Research by Beatty and Collins (6, p.422-423) demonstrated that fermentation of carbohydrates and carbohydrate derivatives accompanied by production of trimethylamine, constitutes the first phase of spoilage, the second phase being the degradation of proteins and amino acids, which does not begin until the trimethylamine has reached a fairly high concentration. Sigurdsson (50, p.899) observed in his studies with herring that the temperature of storage was a factor in determining the onset of the two phases of spoilage. At 25°C. the development of volatile acids and trimethylamine was followed very closely by protein decomposition, whereas at 10°C. proteolysis lagged behind the other changes. When the storage temperature was reduced to 0°C., fermentation of carbohydrates and lactic acid leading to the formation of the acids and the amines was inhibited to such an extent that it did not occur until proteolysis also became appreciable. Then the two phases occurred at similar rates. At temperatures below 0 C., the development of volatile acids and trimethylamine was largely inhibited.

Wood, Sigurdsson and Dyer (62, 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 (21, p.183-184). Charnley and Goard (10, p.32) also studied pH as a measure of spoilage. They concluded that although there was considerable fluctuation in pH values, pH could be used for grading salmon.

Sigurdsson (50, p.892) observed that the indole test afforded considerable information as to the previous history of the sample. Volatile reducing substances closely approached specifications of a useful test of spoilage in all kinds of fish and fish products according to Farber and Lerke (25, p.680). Other tests for determining fish quality have been studied but have not been found as useful (24, p.319).

Reay and Sheawan (47, p.383) concluded that of all the objective tests reviewed the most useful and reliable for routine checking and grading of quality appeared at that time to be the measurement of trimethylamine production. Sigurdsson (50, p.899) reported that for fish stored above $0^{o}C.$, determination of the total volatile acids or trimethylamine is found to be the most reliable measurement of the rate of spoilage. Stansby (54, p.261), however, stated that we should determine which chemical or other tests, when run in conjunction with one another, will give us the best overall criteria as to the

deterioration that has taken place and that the development of a single reliable test was not feasible.

Irradiation of Shrimp and Crabmeat.

It has been shown that irradiation produces changes in the natural constituents of fish. These radiation induced chemical changes occur largely in the protein constituents of crabmeat and shrimp. Shrimp contains about 25 per cent protein, which is composed primarily of the amino acids arginine, lysine, histidine, and tryptophan (34, p. 945). It had been pointed out earlier that ring containing amino acids such as histidine and tryptophan are especially susceptible to radiation damage (41, p.426). Crab which contains about 17 per cent protein is rich in the same four amino acids as shrimp and, in addition, contains significant amounts of cystine (34, p.946). Since cystine is a sulfur containing amino acid, it is also quite sensitive to irradiation effects.

Shrimp and crab are both rich in thiamine, riboflavin, and niacin. Shrimp also contain fair amounts of vitamin A. Riboflavin and niacin are quite stable to irradiation but thiamine and vitamin A are measurably affected by radiation treatment. (36, p.195-196).

The carotenoid pigment of crab and shrimp, which is astaxanthin, is quite stable to irradiation due to

its inherent association with the protein. This is in contrast to the same pigment found in salmon which is very susceptible to degradation by radiation due to its association with the lipid fraction (41, p.424).

Studies of the effects of irradiation on crabmeat appearing in a review by Morgan and Siu (43, p.277) showed that the bacterial count of crabmeat was reduced from 3.0 to 0.78 x 10^6 by dosages of 1.2 x 10^5 reps. One rep equals 95 ergs of absorbed energy per gram of material by definition (30, p.44). After one week in the refrigerator, the irradiated crabmeat was still in excellent condition, whereas the control was spoiled.

Sinnhuber (52) used a consumer panel of 163 members to evaluate crabmeat irradiated at 0.50, 0.75, and 1.00 x 10^6 rads. The crabmeat was served freshly thawed and was graded slightly poorer to poorer than a non-irradiated reference sample. Gradual loss of irradiated flavor took place after storage of the crabmeat. Storage studies conducted on the same lot of crab showed that irradiation levels of 0.25 x 10^6 rads kept the crab from spoiling for a period of 20 days at 45°F., whereas 0.50 x 10^6 rads preserved the crab for 60 days at the same temperature. Sinnhuber (52) also found that irradiation of frozen crabmeat suffered no loss of pigment after doses up to 2.0 x 10^6 rads.

Workers at the Engineering Research Institute (9, p. 218) irradiated raw fresh crabmeat of the Atlantic blue crab at 0.04, 0.08, 0.12, and 1.00 x 10^6 reps. The samples were stored on ice for 1 week and then observed for spoilage and off-odors. At 0.12 x 10^6 reps, the crab was of excellent quality and had no off-odors. Levels less than that, however, were slightly spoiled and the samples receiving 1.0 x 10^6 reps had a dry cooked appearance and a musty off-odor.

The color and taste of shrimp were normal at radiation doses of 0.50 x 10^6 reps according to work done by Tappel, Knapp, and Brock (56, p.274). After a flavor panel of 176 members evaluated samples of freshly thawed shrimp irradiated at 0.50, 1.00, and 1.50 x 10^6 rads, it was ascertained by Sinnhuber (52) that no significant flavor due to irradiation was present in the samples irradiated at 0.50 x 10^6 rads. In all samples of the 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 x 10^6 rads were not spoiled during a storage period of 180 days and samples irradiated at 0.25 x 10^6 rads remained unspoiled for 60 days. No bacterial spoilage was observed in samples receiving a minimum of 1.0 x 10^6 rads.

METHOD

Source of Raw Materials.

The crab and shrimp used in this investigation were the common Pacific Coast varieties of *Cancer magister* and *Pandalus jordani*, respectively. They were obtained from commercial fisheries located in Astoria, Oregon, and were purchased frozen in #10 "C" enamel cans. The shrimp was of excellent quality and somewhat better than the crab. Because of the extremely rapid increase in bacterial counts of the crab, there was reason to believe that it had passed its peak quality (22, p.23-45).

Handling Operations.

Commercially, crabs are caught in traps or "crab pots" in inlet and coastal areas. After they are brought to the canneries, they are killed by breaking off the back shell. They are then eviscerated, washed and boiled in a brine solution. After cooling, the meat is picked from the cracked shell. Particles of shell are separated from the meat by suspending it in a saturated brine solution and agitating briefly. This causes the meat to float to the surface but the shell particles sink to the bottom. Equal portions of body and leg meat are packed in "C" enamel cans and vacuum sealed before

freezing (16, p.474-477).

Beam trawl nets are used for the commercial catching of shrimp off the Oregon coast. The shrimp are subjected to a cleaning operation followed by a deheading and shelling process performed mechanically by a machine peeler. They are then blanched in a mild salt brine prior to packing and freezing (35).

The frozen samples were sent under ice by air express from Astoria to the Oregon State University laboratories.

Pre-irradiation Treatment.

At the laboratory, the #10 cans were thawed at room temperature prior to repacking for irradiation services. The crab and shrimp to be used for bacteriological and chemical analyses were ground in a meat grinder and mixed well to obtain homogeneous samples. Equal parts of crab body and leg meat were ground together to insure uniformity. The shrimp and crab to be used for taste panel evaluations were packed whole. Both the ground and whole samples were packed in 211 x 400 "C" enamel tin cans and sealed under 20" of vacuum. Sanitary conditions prevailed, as much as practical, during this entire packing operation. Immediately after sealing the cans were frozen to -18°F. where they were held until shipped for irradiation service. The cans for shipment were packed in insulated Shamrock carriers

with 50 pounds of dry ice and sent via Railway Express to the Materials Testing Reactor, Arco, Idaho. The samples were held frozen preceding and following the exposure to the gamma irradiation source.

Irradiation.

The cans were subjected to gamma radiation while in the frozen state. The average dose rate was 2.79×10^6 rads per hour for the crab and 6.00×10^6 rads per hour for the shrimp. In order to obtain the desired levels of 0.25 megarads and 0.50 megarads, the crab was exposed to the gamma grid for 5.5 minutes and 11 minutes, respectively. Exposure times of 4.8 minutes and 7.5 minutes were given the shrimp to attain the respective irradiation levels of 0.50 megarads and 0.75 megarads. Irradiation was effected in ambient water at 70°F. Shipped controls, which did not receive irradiation, were sent as a check on the handling of the samples or possible thawing enroute. These were removed from the freezer during the irradiation process. The lot was returned to Oregon State University under the same shipping conditions.

Radiation levels used for the crab and shrimp were determined by flavor evaluation of samples receiving various amounts of gamma irradiation ranging from 0.1 to 4.5 megarads during previous investigations at this

laboratory. The flavor threshold level of irradiation intensity was established by a trained staff panel of twelve members. The flavor threshold level is the point at which a definite taste and aroma due to the irradiation is significantly present. Significance was determined on the statistically computed panel results at the 5 per cent significance level (37, p.151-180, 234-238, 520). The irradiated samples were served with a non-irradiated control for comparison. The flavor threshold levels varied between 0.25 and 0.50 megarads for the crab and 0.50 and 0.75 megarads for the shrimp. These levels of irradiation were used for the storage studies.

Storage.

Upon receipt at the laboratory, the cans 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 crab samples were stored for a total of 9 weeks with sub-sampling for microbiological and chemical analysis at 0,1,2,3,4,6, and 9 weeks. An 18 week storage period was given the shrimp because of its apparent high quality and lack of deterioration with storage. Sub-samples were taken out at 0,1,2,3,4,6,7.5,9,12,15, and 18 weeks for bacteriological and analytical evaluations. Flavor panel analyses were made on both the shrimp and

crab at weekly intervals through the fourth week. For all three methods of evaluation, comparisons were made with non-irradiated samples held at 0°F. and 38°F.

Flavor Preference.

In addition to the above storage studies, the shrimp was subjected to a flavor preference test involving a student panel of 150 members. The flavor evaluations were made at 0,1, and 3 week intervals of storage at 38°F. The shrimp for these tests was from a different shipment but was handled similarly and irradiated at the same levels as the shrimp for the storage studies. The shrimp was not repacked as before, but was sent in #10 "C" enamel cans for irradiation.

Objective Evaluations.

Chemical analyses, bacterial counts and toxicity tests were conducted on the stored and frozen samples. The chemical tests included determinations for pH, trimethylamine, volatile bases, and volatile acids. All bacteriological analyses and toxicity tests were performed in the laboratories of the Bacteriology Department at Oregon State University under the supervision of Dr. A. W. Anderson and Dorothy East (22).

Toxicity Tests.

Prior to all taste panel evaluations of the stored samples, toxicity tests using mice were conducted to insure the safety of the shrimp and crab 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 appearances of toxic effects such as sluggishness, abnormal behavior, or death.

Determination of the Microflora.

Analyses for total plate counts were made on various types of media. The media used were tryptone glucose yeast extract agar and the following selective medium: high salt medium agar, 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.

1. pH. After thawing the samples to room temperature, ten grams was weighed out and ground with 10 milliliters

of water in a mortar with a pestle. The pH of the shrimp obtained was measured with a glass electrode Beckman Zeromatic pH meter which had been standardized with a buffer at pH 7.0.

2. Trimethylamine. Determinations for trimethylamine nitrogen were carried out according to the method of Dyer (20, p.292-294). The procedure was followed exactly with the exception that 50 grams of ground sample was mixed with 100 milliliters 7.5 per cent trichloracetic acid instead of 100 grams and 200 milliliters, respectively. The developed color was read against a blank carried through the same procedure at 410 mu. in a Beckman DU Spectrophotometer.

3. Volatile Bases. The procedure for "Volatile Bases in Fish" by Stansby (55, p.593) was used in these determinations. However, there were some modifications in the procedure. The distillate was collected in 20 milliliters of 4 per cent boric acid instead of 50 milliliters of 0.05 N hydrochloric acid. In addition, 0.05 hydrochloric acid was used in lieu of a standard alkali for titrating the distillate. Methyl red-bromo cresol green was used as the indicator.

4. Volatile Acids. Distillation with steam according to the Association of Official Agriculture Chemists (2, p.236-237) was used for the determination

of volatile fatty acids in the shrimp and crabmeat. The distillate was titrated with 0.01 N sodium hydroxide in a carbon dioxide free atmosphere provided by a stream of nitrogen bubbling into the solution.

Subjective Evaluation.

Flavor panels were also used for the evaluation of the samples. These tests included a small trained staff panel and a large student panel. All flavor evaluations were conducted in the Flavorium of the Food Technology Department with the help of Professor (Mrs.) Lois Sather and her staff.

Samples were served at room temperature in small paper cups coded with three-digit random numbers. Each group of samples to be analyzed was served on metal trays to the judges seated in individual flavor testing booths. The booths were uniformly lighted with white incandescant light and equipped with individual porcelain sinks, hot and cold water, and paper drinking cups so that the panel members could freshen their mouths between samples (48).

Trained Panel.

Fifteen staff members of the Food Technology Department were selected for the panel because of their familiarity with irradiated flavor. There were usually at

least twelve trained members available for all tests. The training process consisted of presenting to the panel labeled, irradiated samples of shrimp or crabmeat which had been allowed to come to room temperature. The levels of irradiation ranged from 0.1 megarads to 4.5 megarads. The taste and aroma at the various levels of irradiation was discussed and compared with non-irradiated samples. On two consecutive days following this discussion period, the labeled samples were served in the flavor testing booths and the members were asked to rate them according to intensity of irradiation odor and taste on an 11 point intensity scale. Following this, the panel was used for the actual testing of the irradiation intensity of the samples in the coded cups. A non-irradiated sample was always presented in a cup marked "R" to be used as a reference. In addition, another non-irradiated sample was served in a coded cup as a blind reference.

Student Panel.

A large student panel of 150 members was used in evaluation of the shrimp for flavor preference. Irradiated samples at 0.50 and 0.75 megarads stored at 38°F. for one and three weeks were served with irradiated samples held at 0°F. In all flavor tests a non-irradiated sample which had been held at 0°F. was presented with the irradiated

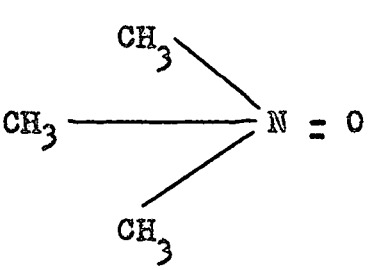
samples. The code numbers on the sample cups were marked for preference on a 9 point Hedonic scale ballot. The scale went progressively from dislike very much to like very much, with the lowest score being 1 and the highest score being 9. Results were computed statistically for significant differences among the samples tested.

RESULTS AND DISCUSSION

The samples of shrimp and crabmeat prepared for this storage study were analyzed objectively and subjectively at designated weekly intervals. The shrimp and crab will be discussed separately in this section since their spoilage patterns differed considerably.

Trimethylamine Nitrogen in the Stored Crab.

Trimethylamine oxide is a naturally occurring constituent of many marine animals and is found quite abundantly in crab, according to Groninger (28, p.6). Groninger points out that the trimethylamine oxide



is reduced to trimethylamine by the enzyme triamine-oxidase which is present in a number of different species

of bacteria. Beatty and Gibbons (7, p.90) observed a parallel between the increase in trimethylamine of the muscle in marine animals and the degree of decomposition. Trimethylamine is a suitable test for quality of fish stored at temperatures above 0°C. according to Sigurdsson (19, p.900). Data on the trimethylamine content of the stored crab are presented in Table 1.

TABLE 1

Trimethylamine Content of Stored Irradiated Crab

(mg. N/100 g. sample)

Storage Time-weeks (38°F.)	TMA of Samples		
	0.00 megarads	0.25 megarads	0.50 megarads
0	0.21	0.31	0.38
1	1.89	0.98	0.39
2	10.44	1.35	0.43
3	16.33	-	0.49
4	65.50	2.84	-
6	95.30	7.54	3.55
9	101.52	35.91	10.62

TMA of Frozen Control held at 0°F. 0.21 ± 0.02

It can be seen that the production of trimethylamine from the oxide increased rapidly and continued at a rapid rate in the stored non-irradiated samples. By comparison, the rate of trimethylamine production in the irradiated samples is considerably less. At the end of two weeks, the trimethylamine nitrogen content of the non-irradiated samples was approximately 10 milligrams per 100 grams of sample. It was also obvious by the odor that the non-irradiated crabmeat was spoiled at this point. Using the trimethylamine content as a criteria of spoilage, the samples receiving 0.25 megarads of radiation were spoiled after six weeks of storage and the samples receiving 0.50 megarads of radiation were spoiled after nine weeks of storage. No change occurred in the trimethylamine content of the frozen non-irradiated samples during the nine weeks of storage. A graphical presentation of the trimethylamine content of the stored crab appears in Figure 1.

Volatile Bases in Stored Crab.

As decomposition proceeds, it has been shown by Beatty and Gibbons (7, p.90) that ammonia accumulates in the fish. Stansby (55, p.593) reported that total volatile bases have been 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 volatiles. The volatile bases include ammonia and various low molecular weight amine constituents. Values representing the volatile bases in the crab appear in Table 2.

TABLE 2

Volatile Bases in Stored Irradiated Crab

(mg. N/100 g. sample)

Storage Time-Weeks (38°F.)	Volatile Bases of Samples		
	0.00 megarads	0.25 megarads	0.50 megarads
0	5.29	5.27	5.36
1	7.60	6.55	5.40
2	44.51	7.30	5.29
3	90.79	21.15	5.51
4	100.26	47.95	12.16
6	102.81	56.91	49.14
9	172.81	98.18	-

Frozen Control held at 0°F. 5.17 ± 0.37

Data indicating the volatile base production in the non-irradiated samples closely paralled the trimethylamine values as indices of spoilage. Volatile bases in the irradiated samples, however, indicated a more rapid deterioration than did the trimethylamine. The volatile bases at four and six weeks, respectively, of the samples

irradiated at 0.25 megarads and 0.50 megarads, were approximately equal to the volatile bases in the non-irradiated samples at two weeks. The data are presented graphically in Figure 2.

Volatile Acids in Stored Crab.

Volatile acids are the products of the fermentation of the carbohydrates in fish. They may also be formed in part by the hydrolysis of the proteins and amino acids which yield equal quantities of amino and carboxylic groups thus indicating that equivalent amounts of acidic and basic groups are simultaneously liberated (60, p.128). Sigurdsson (50, p.899) showed that the production of volatile acids was directly related to the production of trimethylamine in fish. Volatile acids include the low molecular weight fatty acids: formic acid, acetic acid, propionic acid, and butyric acid. The volatile acids in the stored crab samples are recorded in Table 3 and presented graphically in Figure 3.

TABLE 3

Volatile Acids in Stored Irradiated Crab

(meq. V.A./100 g. sample)

Storage Time-Weeks (38 F.)	Volatile Acids of Samples		
	0.00 megarads	0.25 megarads	0.50 megarads
0	2.00	3.40	3.00
1	3.80	3.20	-
2	30.40	-	3.80
4	98.20	54.40	4.80
6	192.60	106.80	49.40
9	529.00	126.00	74.20

Frozen Control held at 0°F. 2.20 ± 0.50

The onset of spoilage in the samples receiving 0.25 and 0.50 megarads of irradiation was four and six weeks, respectively, according to the volatile acids produced. These results are in agreement with the production of volatile bases. During the first four weeks of storage, the development of volatile acids and bases in the irradiated samples was closely related, but by the end of the storage period the volatile acid contents was much higher than the volatile base content.

pH Values of Stored Crab.

Typical spoilage patterns of crab reported by Tobin (57, p.165) show an increase in pH from pH 7.2 in fresh crab to pH 8.0 or higher in the spoiled crab. He found that this increase in pH was directly related to the bacterial count.

In Table 4 and Figure 4 are presented the data and graphs representing the pH values of the stored crab.

TABLE 4

pH Values of Stored Irradiated Crab

Storage Time-Weeks (38°F.)	pH Values of Samples		
	0.00 megarads	0.25 megarads	0.50 megarads
0	7.33	7.28	7.34
1	7.36	7.43	7.55
2	7.53	-	7.45
4	8.30	7.53	7.43
6	8.05	7.51	7.05
9	7.75	7.65	7.05

pH of Frozen Control held at 0°F. 7.33 ± 0.01

The non-irradiated samples showed a rapid increase from pH 7.3, which was the initial pH of the crab, to pH 8.3 at 4 weeks. The crab was quite putrid and spoiled at

this point. After 4 weeks, the pH decreased steadily to a value of 7.75. This decrease in pH can be explained in part by the relative amounts of volatile acids and bases present. At four weeks, the production of volatile bases nearly ceased, whereas the formation of volatile acids continued to increase. After this point the concentration of volatile acids was always higher than the concentration of volatile bases. It is expected, then, that the pH should be somewhat reduced.

A gradual increase in the pH over the nine week period occurred in the samples receiving 0.25 megarads. At four weeks pH values were the same as for the non-irradiated samples at two weeks.

The pH values of the samples which received irradiation doses of 0.50 megarads were quite different from the normal. The sudden decline in pH to the acid side is probably due to a higher concentration of carboxyl groups than basic groups within the crab tissue.

Flavor Evaluation of Stored Irradiated Crab.

The irradiation intensity of the odor and taste of the crab was determined by a trained flavor panel. Results are presented in Tables 5a and 5b and are illustrated graphically in Figures 5a and 5b.

TABLE 5a

Irradiated Taste Intensity of Stored Crab

Storage Time-weeks (38°F.)	Mean Scores of Samples		
	0.00 megarads	0.25 megarads	0.50 megarads
0	0.50	4.17	4.92
1	0.50	2.08	4.17
2	-	1.75*	3.42
3	-	1.08**	3.08
4	-	2.67	2.92

TABLE 5b

Irradiated Odor Intensity of Stored Crab

Storage Time-weeks (38°F.)	Mean Scores of Samples		
	0.00 megarads	0.25 megarads	0.50 megarads
0	0.17	3.50	4.50
1	0.50	2.00*	4.25
2	-	1.33**	3.12
3	-	1.25**	2.67
4	-	3.25	3.17

* Barely significant from non-irradiated control
** No significant difference from non-irradiated control

Samples were scored on a 10 point scale for irradiation intensity. 0 signifies no irradiation
10 signifies very extreme irradiated intensity

The non-irradiated stored samples were served only after the first week of storage as they were obviously spoiled by the second week. The irradiated samples were served weekly through four weeks of storage.

It can be seen from the data and curves that the loss of irradiation odor and taste occurred on storage. This agrees with the work of Sinnhuber (52) who found that irradiated flavor in crab was lost on storage. Samples receiving 0.25 megarads of irradiation were not rated significantly different in odor from the non-irradiated samples at 2 and 3 weeks or in taste at 3 weeks. However, by the fourth week, the flavor scores of the irradiated samples were again significantly different. The reason for this rise was probably due to the development of spoilage flavors which gave the samples a different flavor than the non-irradiated reference.

According to the panel, irradiated flavor was significantly present in all of the samples receiving 0.50 megarads although the intensity of irradiation flavor decreased measurably with storage.

Overall Quality Changes of Stored Crab.

Results of the chemical tests indicated slight increases of decompotition products at the end of one week of storage in the non-irradiated samples. A slight rise

in pH was also noted. By two weeks more than a five-fold increase of the degradation products had occurred according to all of the chemical tests. Sensory observations indicated that the crab was definitely spoiled at this time. The appearance of off-odors in samples irradiated at 0.25 megarads was noted at four weeks, although it was not considered definitely spoiled by sensory observations until after six weeks of storage. Results of the determination for trimethylamine nitrogen agrees closely with the organoleptic evaluations. The production of volatile bases and volatile acids, however, indicated the onset of spoilage at about four weeks compared with equivalent values for the non-irradiated samples. Organoleptic evaluations of the crab receiving 0.50 megarads of irradiation indicated the development of off-odors such as mustiness at six weeks with spoilage at nine weeks. No changes in composition of the frozen non-irradiated samples resulted during the nine weeks of storage according to all of the chemical tests. The levels of irradiation used caused little if any change in the original composition of the crabmeat.

Results seemed to indicate that the trimethylamine production in crab is closely related to sensory evaluations. The production of volatile acids and bases, however, predicted the development of spoilage in the

irradiated samples before spoilage was ascertained organoleptically. The pH of irradiated crabmeat did not seem to be definitely related to quality.

A three-fold increase in the storage life has been achieved in the crab irradiated at 0.25 megarads and a four-to five-fold increase in the storage life was brought about in the crab irradiated at 0.50 megarads.

Trimethylamine Nitrogen in Stored Shrimp.

Data on the trimethylamine nitrogen content of the stored shrimp are presented in Table 6. In Figure 6, the data are shown in graphical form. A steady increase in trimethylamine content with storage occurred in the non-irradiated samples indicating spoilage at 15 weeks. Very slight increases in trimethylamine nitrogen were noted in the irradiated samples, but these changes were much less significant. The non-irradiated frozen samples remained unchanged during the storage period.

TABLE 6

Trimethylamine Content of Stored Irradiated Shrimp

(mg. N/100 g. sample)

Storage Time-weeks (38°F.)	TMA of Samples		
	0.00 megarads	0.50 megarads	0.75 megarads
0	0.12	0.15	0.19
1	0.10	0.13	0.16
2	0.17	0.15	0.17
3	0.29	0.18	0.18
4	0.41	0.25	0.28
6	1.10	0.26	0.30
7½	2.17	0.26	0.32
9	2.30	0.33	0.30
12	2.88	0.37	0.49
15	7.75	0.44	0.26
18	-	0.40	0.28

TMA of Frozen Control held at 0°F. 0.11 ± 0.02

Volatile Bases in Stored Shrimp.

Values indicating total volatile base content in shrimp during the 18 week storage period are presented in Table 7. The volatile base and the trimethylamine content of the shrimp show similar trends. Both the volatile bases and trimethylamine values started to increase in the

TABLE 7

Volatile Bases in Stored Irradiated Shrimp

(mg. N/100 g. sample)

Storage Time-weeks (38°F.)	Volatile Bases of Samples		
	0.00 megarads	0.50 megarads	0.75 megarads
0	2.94	3.40	3.03
1	3.18	3.18	3.24
2	3.66	3.38	3.32
3	6.39	-	3.15
4	8.09	3.05	3.70
6	18.66	3.76	3.68
7½	19.78	3.34	3.59
9	21.12	3.83	3.33
12	31.24	3.83	3.33
15	31.89	3.99	3.50
18	46.81	-	3.76
Frozen Control held at 0°F.	3.09 ± 0.10		

stored non-irradiated shrimp after two weeks. By three weeks the volatile base content doubled and the trimethylamine nitrogen had almost doubled. Only a slight increase in volatile bases occurred in the irradiated samples. At 15 weeks, the samples receiving 0.50 megarads and at 18 weeks the samples receiving 0.75 megarads had volatile

base contents nearly the same as the non-irradiated samples at two weeks.

Volatile Acids in Stored Shrimp.

Results of the production of volatile acids in the stored shrimp are presented in Table 8. Graphical illustrations of the data appear in Figure 8.

TABLE 8

Volatile Acids in Stored Irradiated Shrimp

(meq. V.A./100 g. sample)

Storage Time-weeks (38°F.)	Volatile Acids of Samples		
	0.00 megarads	0.50 megarads	0.75 megarads
0	1.00	1.60	1.00
1	1.20	1.60	1.20
2	2.40	2.20	1.80
3	9.60	2.40	2.20
4	20.20	1.80	2.00
6	43.60	1.80	2.00
7½	55.60	2.40	2.20
9	108.80	2.20	2.20
12	324.20	3.20	2.20
18	-	3.20	4.40

Frozen Control held at 0°F. 1.00 ± 0.20

The increase in volatile acids after storage of the shrimp was more pronounced than the increase in either the volatile bases or trimethylamine. At two weeks, the volatile acids had doubled in the non-irradiated samples and thereafter the increase in volatile acids was quite constant. The volatile acids in the samples irradiated at 0.50 megarads had doubled after 12 weeks of storage. Samples at 0.75 megarads, however, showed a two-fold increase in volatile acids at three weeks but this concentration remained quite constant until the 18th week. Both of the irradiated samples had values at 12 weeks which were only slightly more than the non-irradiated samples at two weeks.

pH Values of Stored Shrimp.

Appearing in Table 9 are the values for pH in the stored shrimp. A graphical representation appears in Figure 9. After two weeks of storage, the non-irradiated samples increased in pH from the original values of about 7.4 to a pH of nearly 8.0 at 18 weeks. A slight increase in pH was observed in the irradiated samples at 18 weeks but appeared unchanged through the first 12 weeks of storage. Bailey (3, p.611) reported that the pH in shrimp increases with spoilage to a level of about 7.95 when the samples are considered poor. At pH values higher than

7.95, he adds, the shrimp are considered quite poor in quality.

TABLE 9

pH Values of Stored Irradiated Shrimp

Storage Time-weeks (38°F.)	pH Values of Samples		
	0.00 megarads	0.50 megarads	0.75 megarads
0	7.43	7.43	7.42
1	7.42	7.42	7.42
2	7.44	7.42	7.42
3	7.51	7.42	7.42
4	7.52	7.42	7.40
6	7.52	7.43	7.40
7½	7.53	7.43	7.40
9	7.62	7.42	7.40
12	7.65	7.42	7.40
15	7.78	-	-
18	7.98	7.47	7.43

pH of Frozen Control held at 0°F. 7.42 ± 0.01

Flavor Evaluation of Stored Shrimp.

The trained panel rated the samples of stored shrimp against non-irradiated reference samples that had been freshly thawed. Mean scores for irradiated flavor of the

shrimp are presented in Tables 10a and 10b. The stored non-irradiated samples were served weekly through the first two weeks and the stored irradiated samples weekly through a four week period. As in the case of the crab, irradiated odor and taste diminished on storage. This correlates with the work of Sinnhuber (52) who found that irradiated flavor in shrimp held at 45°F., or higher, decreased on storage. Samples irradiated at 0.50 megards had an insignificant amount of irradiated taste present at three and four weeks and an insignificant amount of irradiated odor present at three weeks. The panel found no significant differences between the taste of the non-irradiated samples and those irradiated at 0.75 megarads at three weeks of storage.

TABLE 10a

Irradiated Taste Intensity of Stored Shrimp

Storage Time-weeks (38°F.)	Mean Scores of Samples		
	0.00 megarads	0.50 megarads	0.75 megarads
0	0.33	3.25	4.00
1	0.33	3.75	3.12
2	0.33	3.50	3.42
3	-	1.50**	1.12**
4	-	1.50**	2.83

TABLE 10b

Irradiated Odor Intensity of Stored Shrimp

Storage Time-weeks (38 F.)	Mean Scores of Samples		
	0.00 megarads	0.50 megarads	0.75 megarads
0	0.33	3.42	4.08
1	0.33	4.08	3.67
2	0.50	3.08	3.08
3	-	1.42**	2.00
4	-	2.08	2.58

Samples were scored on a 10 point scale for irradiated intensity
0 signifies no irradiation
10 signifies very extreme irradiated intensity

** No significant difference from non-irradiated control

Flavor Preference of Irradiated Shrimp.

A large student panel of 150 members evaluated the shrimp freshly thawed and after storage of one and three weeks for flavor preference. Table 11 contains the mean scores for the shrimp.

TABLE 11

Flavor Preference of Irradiated Stored Shrimp

Storage Time in Weeks	Samples held at 0°F.			Samples held at 38°F.	
	0.00 megarads	0.50 megarads	0.75 megarads	0.50 megarads	0.75 megarads
0	6.66	6.05	5.58	-	-
1	6.83	6.36	6.02	6.79**	6.26
3	6.91	6.22	6.05	6.53	6.47

Samples scored on a 9 point Hedonic Scale for preference

9 is most preferred - like extremely

1 is least preferred - dislike extremely

** No significant difference from non-irradiated sample

It can be seen that in most cases the non-irradiated samples were preferred. Samples receiving 0.50 megarads of irradiation, however, were equally preferred after one week of storage. Irradiated samples stored at 38°F. were significantly preferred to the irradiated samples held

frozen and served freshly thawed. A score of 5 would indicate a neutral response to the samples; that is, the samples were neither liked nor disliked. All of the samples were rated above this level at - liked slightly, or - liked moderately. The irradiated samples held at 38°F., however, were all rated in the range - liked moderately, as were all of the non-irradiated samples.

Overall Quality Changes of Stored Shrimp.

Significant increases in degradative products of the non-irradiated samples by the third week of storage was noted in results of all the chemical tests. Although decomposition continued quite steadily, off-odors were not noted until 12 weeks and the shrimp were not considered spoiled until after 15 weeks of storage. The composition of the non-irradiated frozen samples remained unchanged during the 18 weeks of storage. No degradative effect due to the radiation process was noted by the methods of analysis employed in this study.

The loss of irradiated taste and odor in the irradiated samples was quite definite on storage, but no adverse changes in quality were observed. The trimethylamine test indicated that small changes occurred in the irradiated samples illustrating that degradation was taking place but on a greatly reduced scale. In

almost all cases the irradiated samples at 18 weeks had values determined by the various chemical analyses approximately equal to or less than the values for the non-irradiated shrimp after three weeks of storage.

The trimethylamine nitrogen and the volatile bases were closely related and both seemed to be good indices of the quality of the shrimp.

The shrimp used in this study was unquestionably of excellent quality and probably contained only small numbers of spoilage organisms. It is obvious that the irradiation had halted or greatly delayed the deterioration but it is difficult to estimate the increase in storage life of the irradiated samples because it is impossible to tell when the irradiated samples will spoil. Unfortunately, the lengthy storage life was not anticipated and consequently there were not enough samples to permit the extension of the storage period.

FIGURE 1. TRIMETHYLAMINE CONTENT OF STORED IRRADIATED CRAB

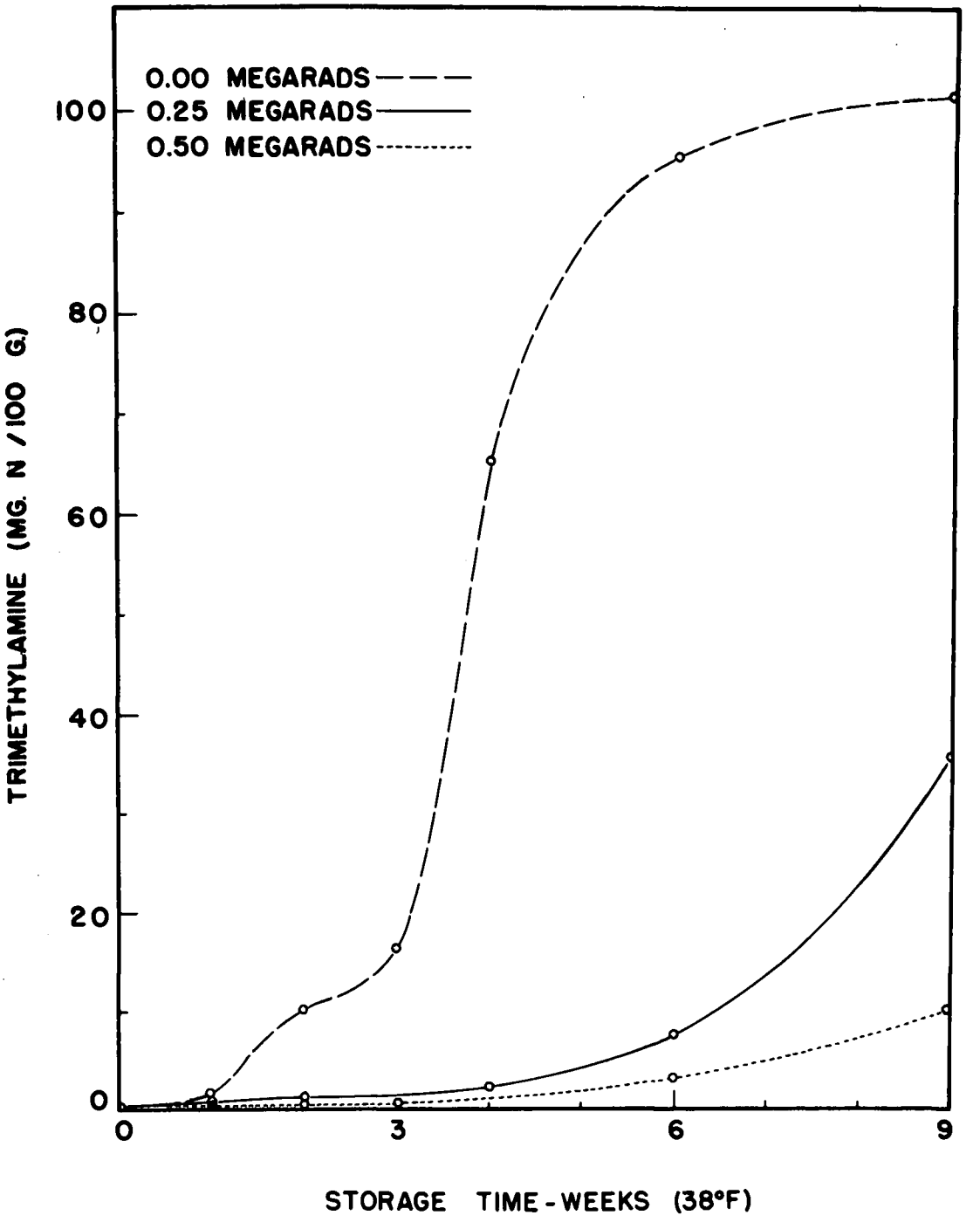


FIGURE 2. VOLATILE BASES IN STORED IRRADIATED CRAB

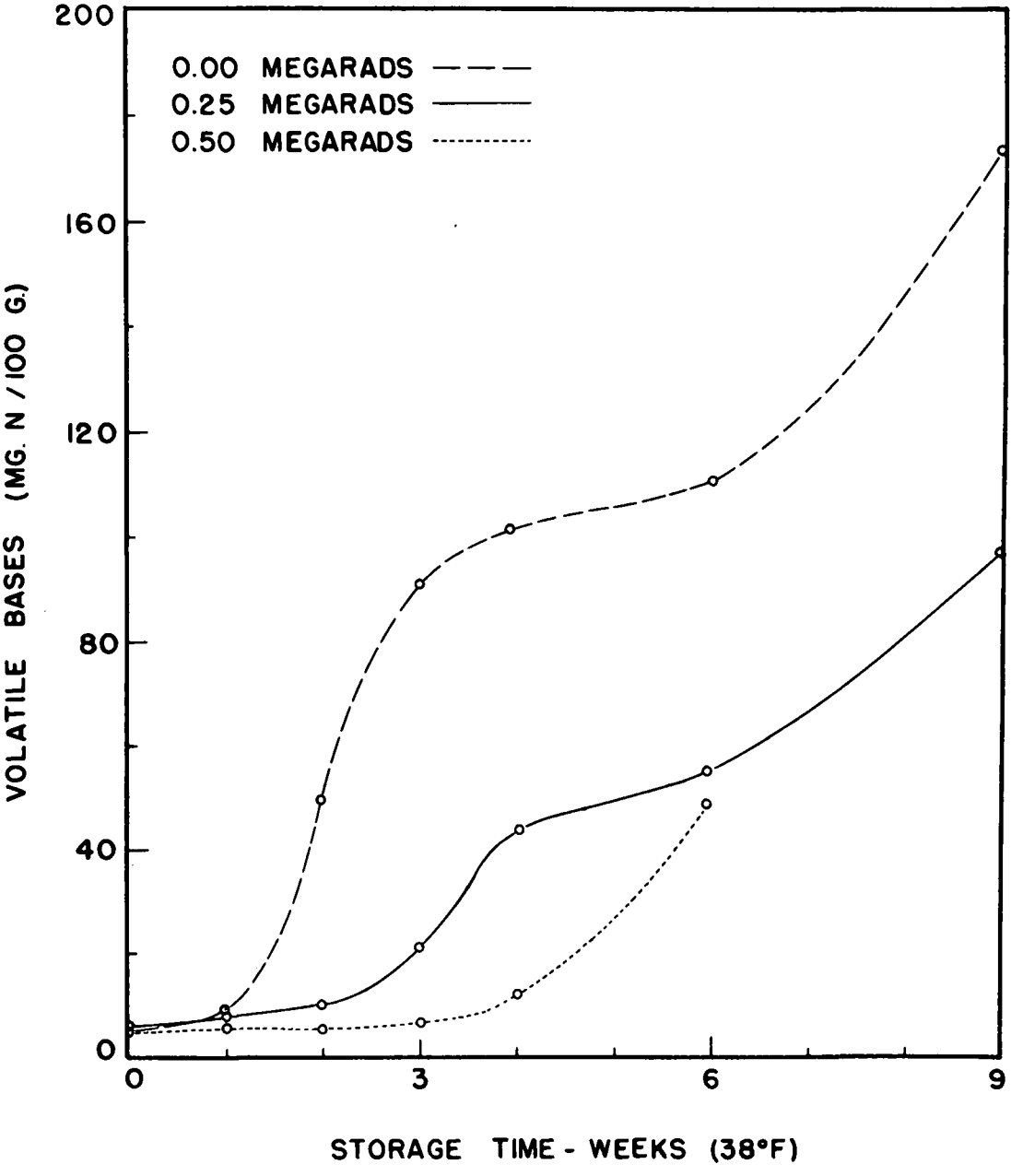


FIGURE 3. VOLATILE ACIDS IN STORED IRRADIATED CRAB

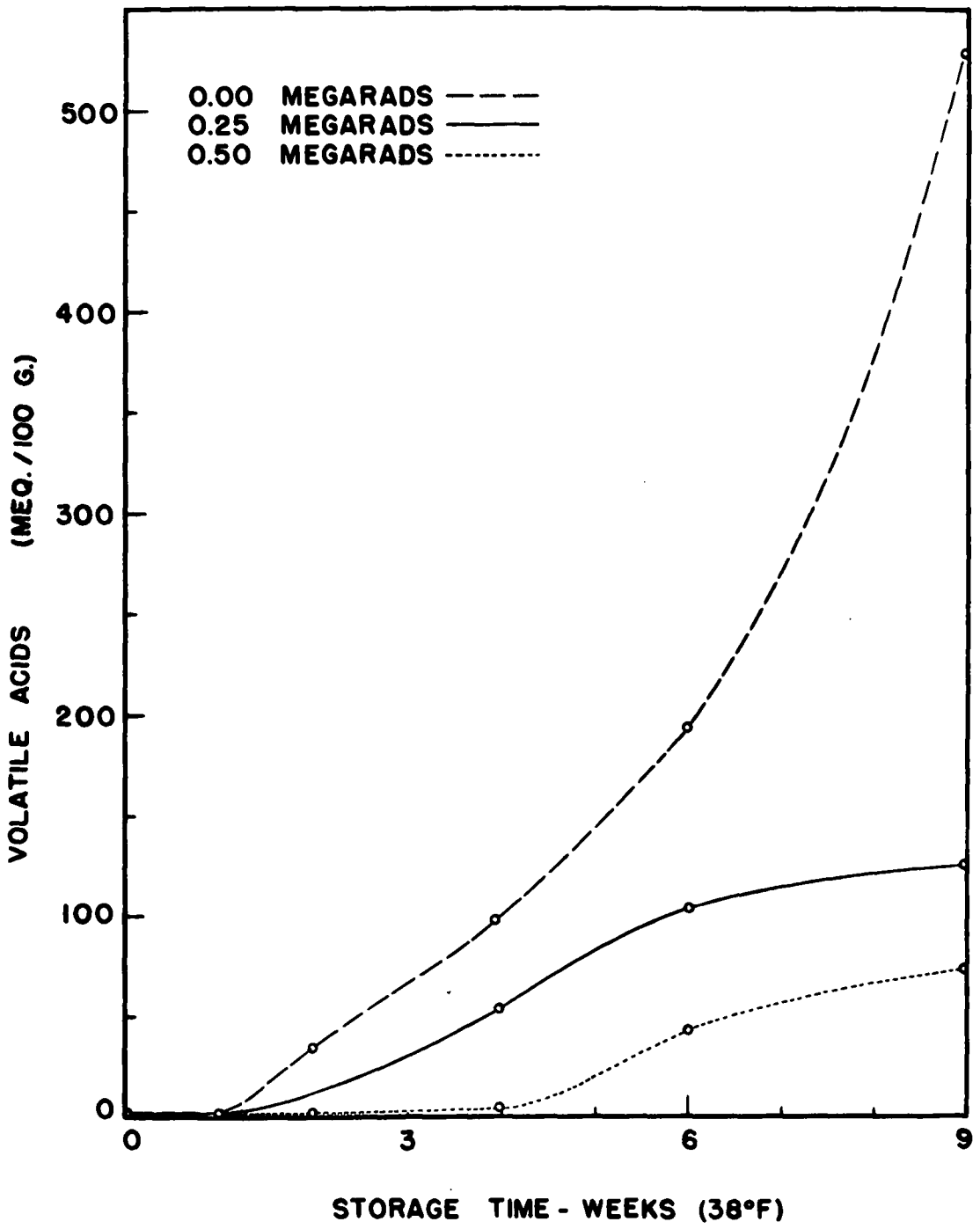


FIGURE 4. pH VALUES OF STORED IRRADIATED CRAB

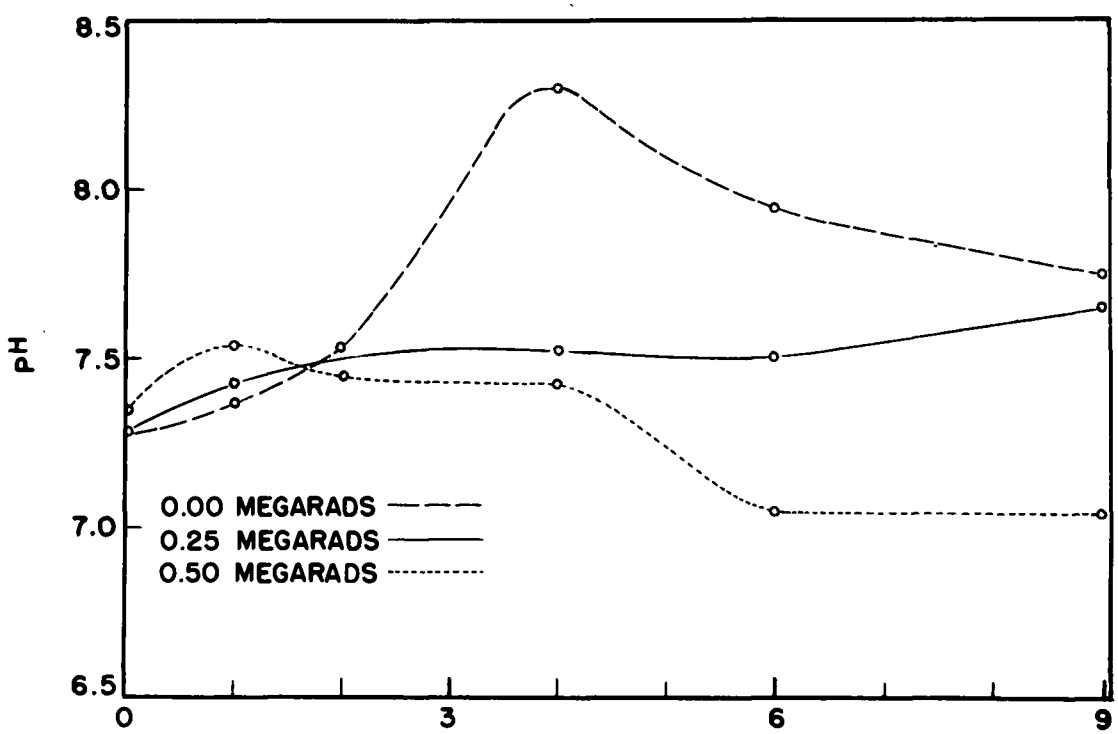


FIGURE 5A. IRRADIATED TASTE INTENSITY OF STORED CRAB

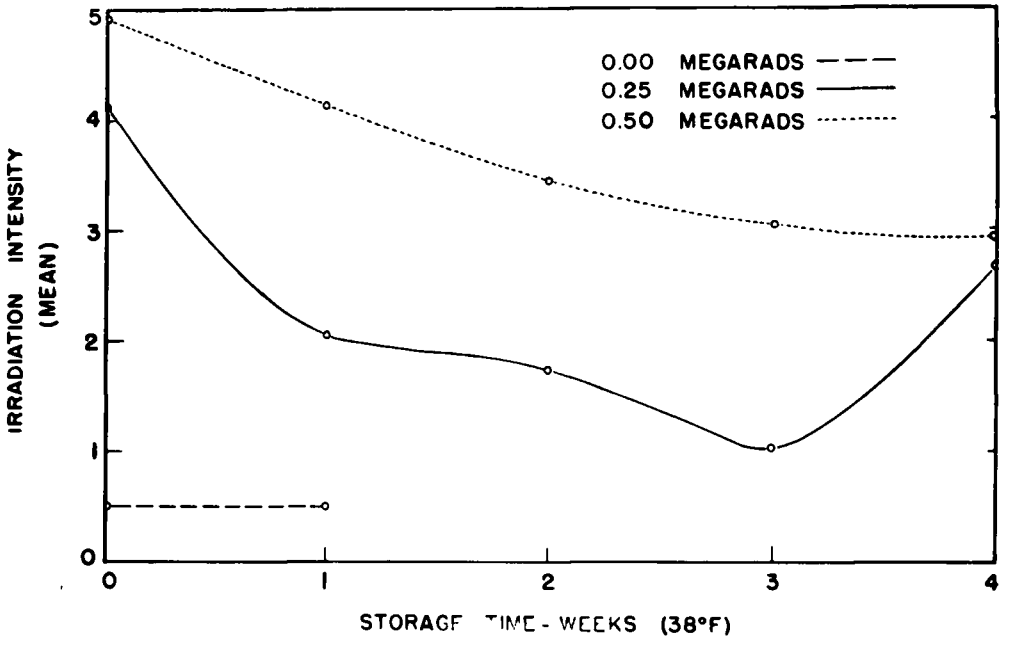


FIGURE 5B. IRRADIATED ODOR INTENSITY OF STORED CRAB

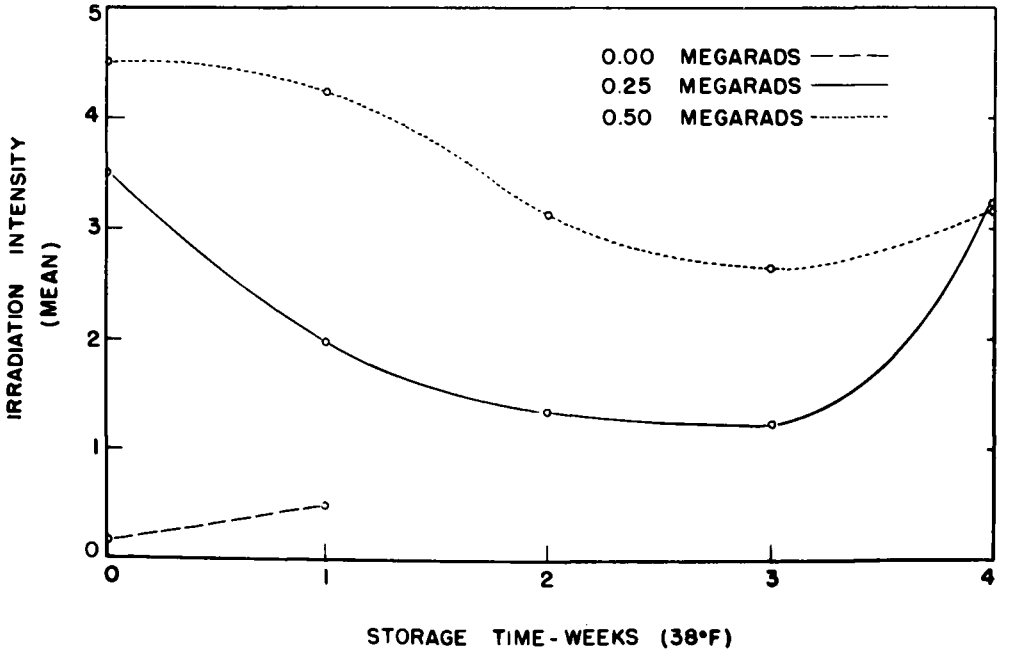


FIGURE 6. TRIMETHYLAMINE CONTENT OF STORED IRRADIATED SHRIMP

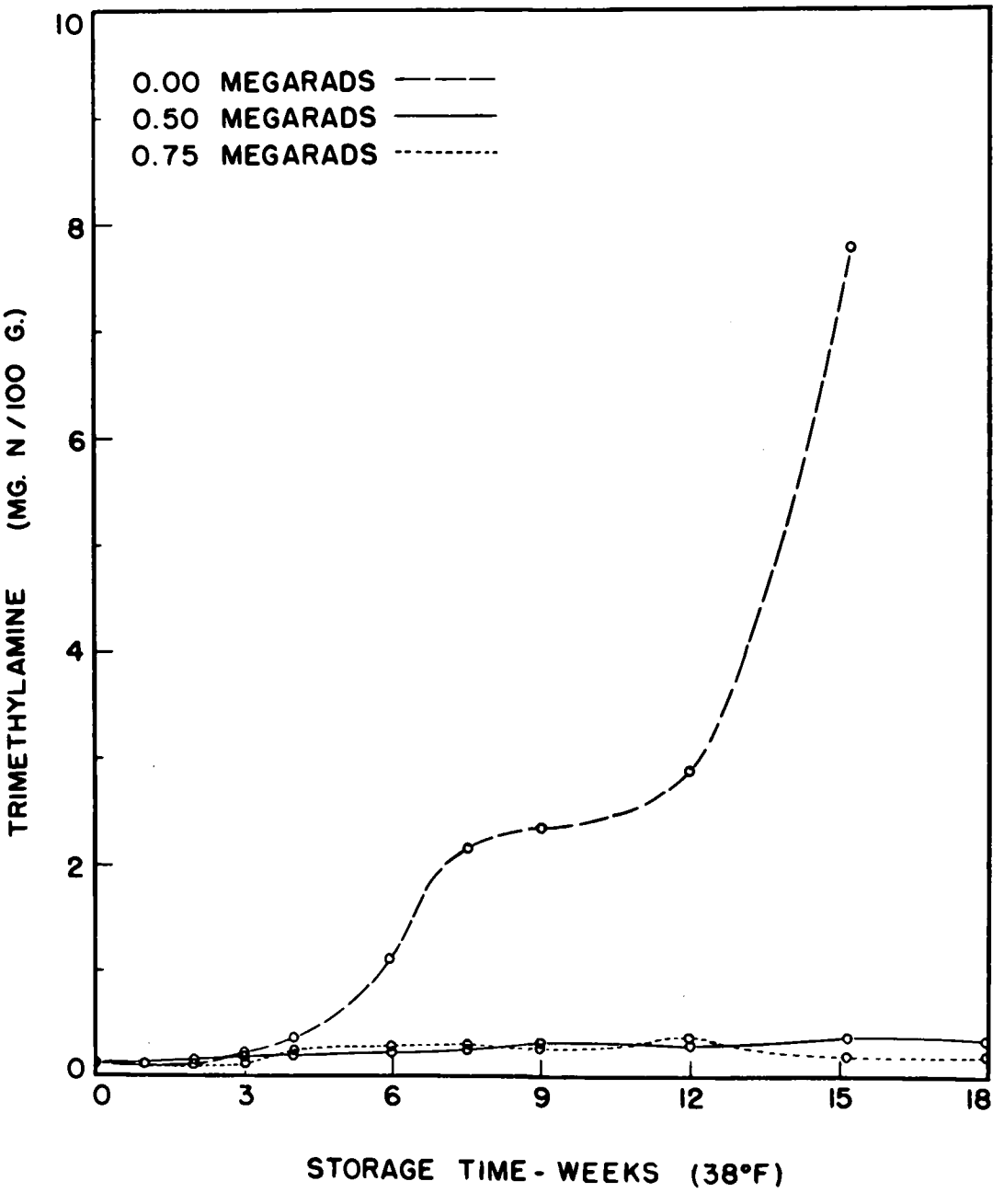


FIGURE 7. VOLATILE BASES IN STORED IRRADIATED SHRIMP

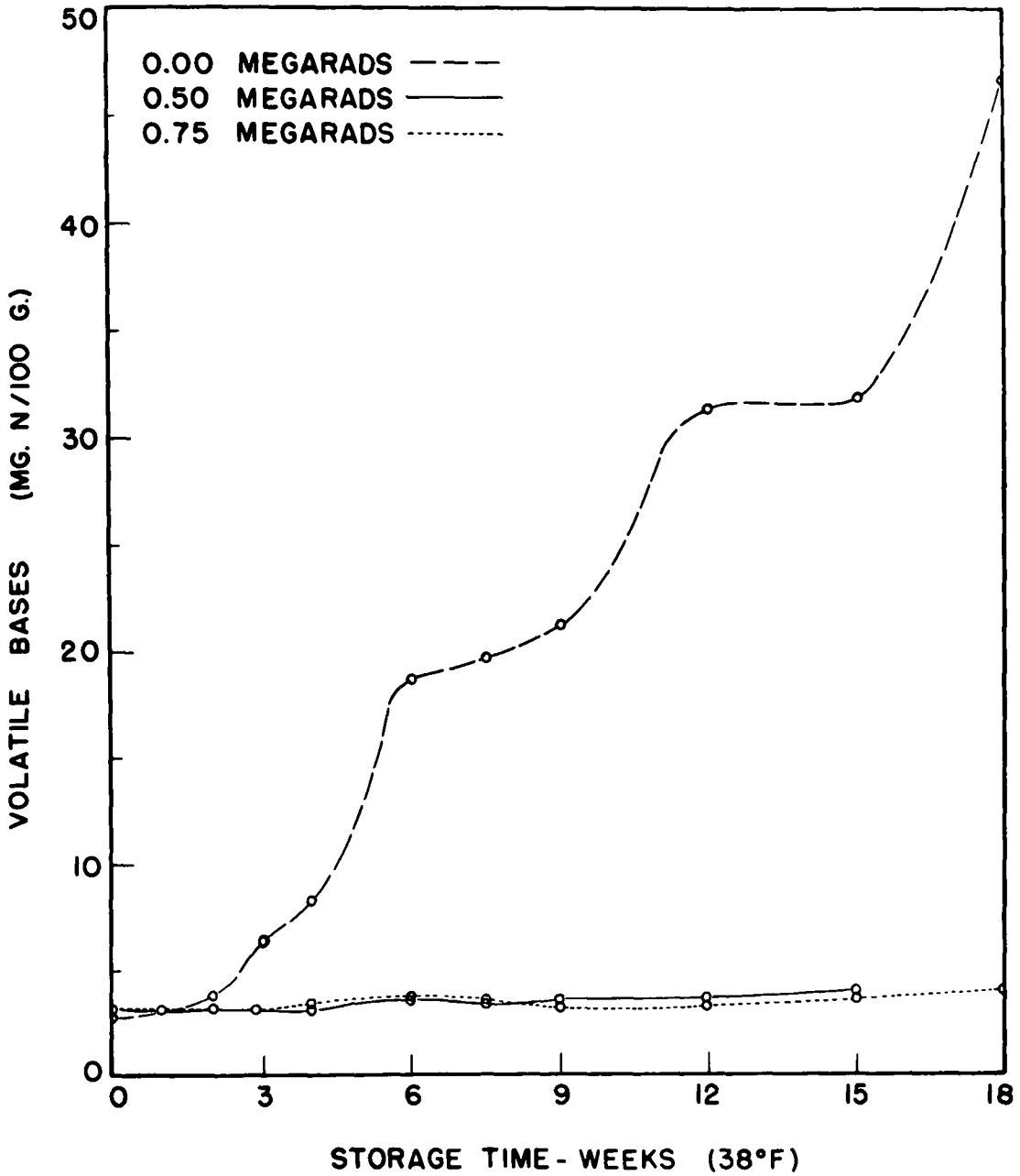


FIGURE 8. VOLATILE ACIDS IN STORED IRRADIATED SHRIMP

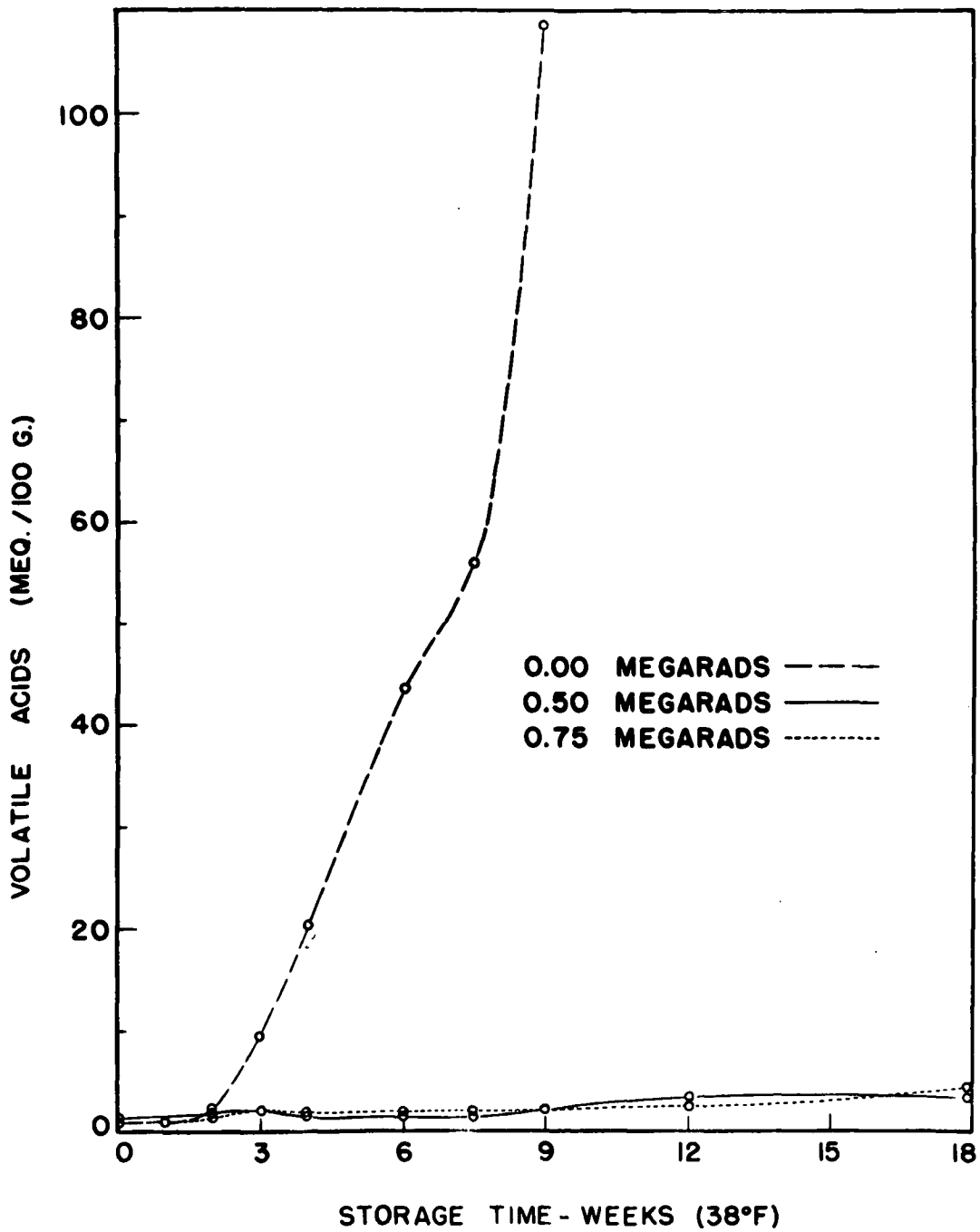


FIGURE 9. pH VALUES OF STORED IRRADIATED SHRIMP

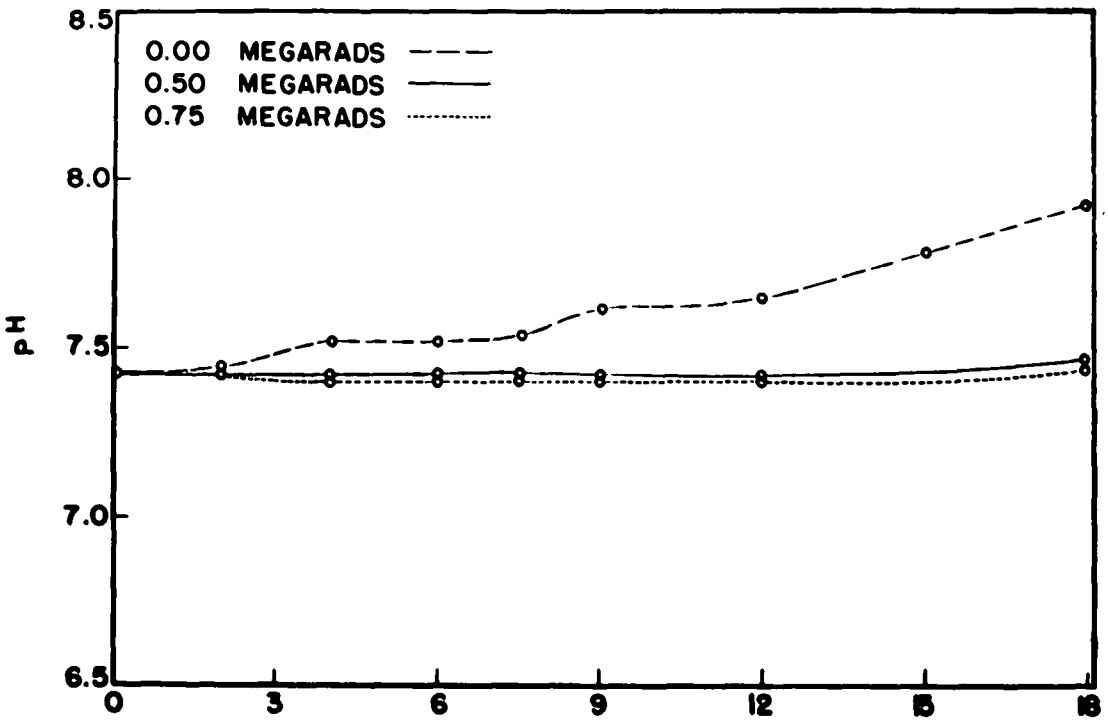


FIGURE 10A. IRRADIATED TASTE INTENSITY OF STORED SHRIMP

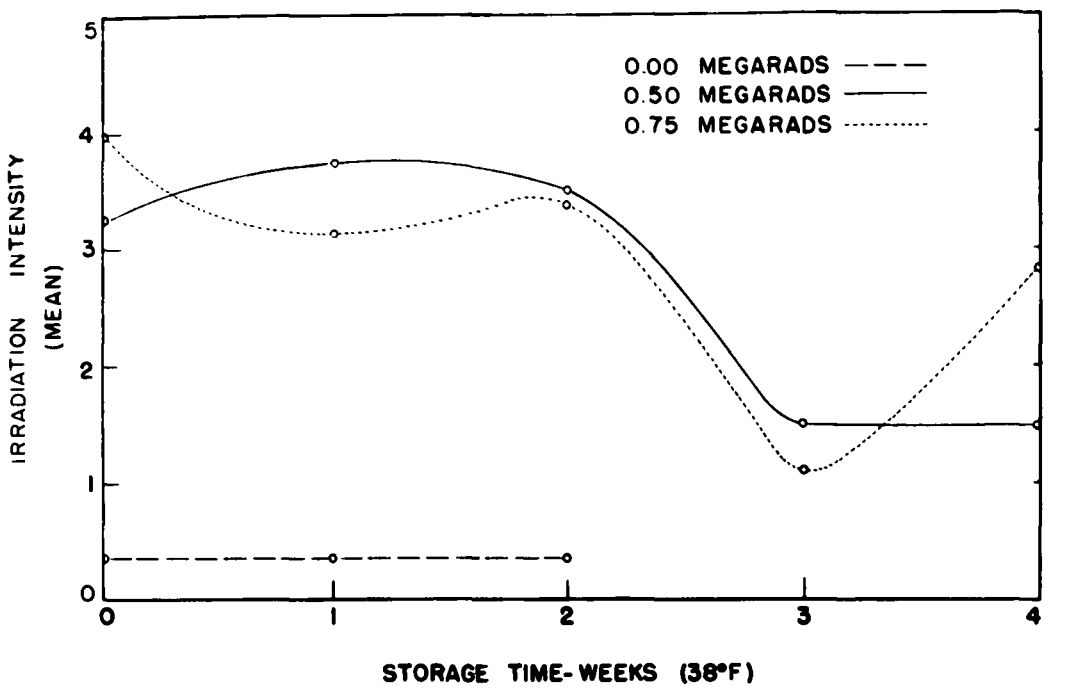
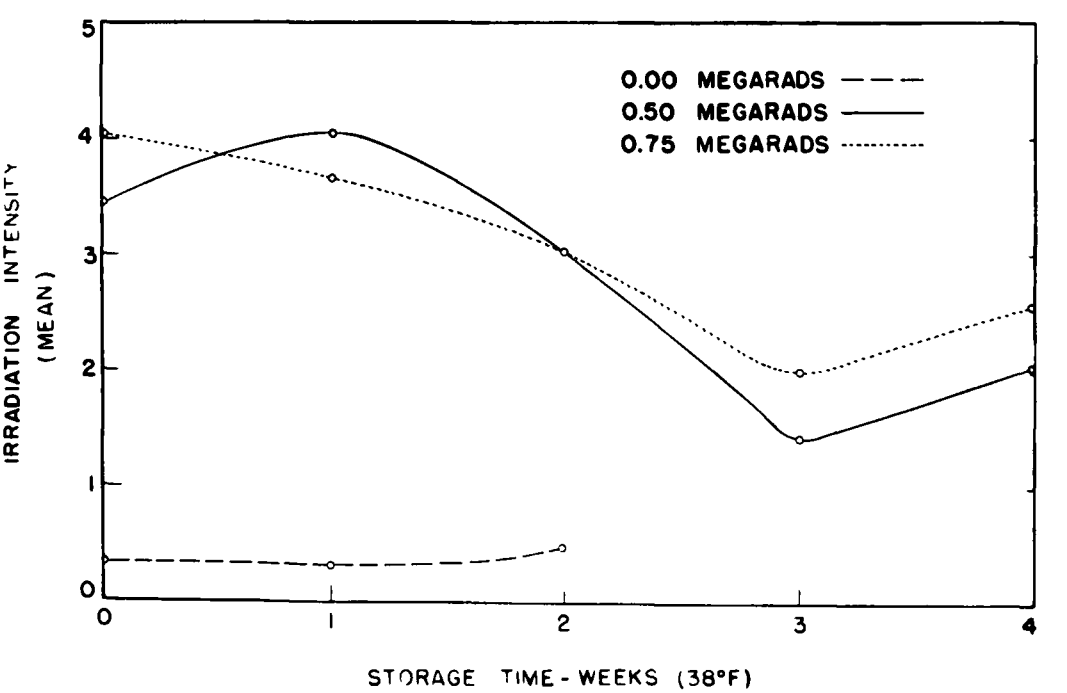


FIGURE 10B. IRRADIATED ODOR INTENSITY OF STORED SHRIMP



SUMMARY AND CONCLUSIONS

The feasibility of extending the storage life of shrimp and crabmeat by the use of low levels of ionizing radiations in combination with refrigerated storage at 38°F. was investigated. Levels of irradiation which would not impart a significant irradiated odor or taste to the shrimp and crabmeat were determined during previous investigations. These levels were 0.25 and 0.50 megarads for the crab and 0.50 and 0.75 megarads for the shrimp.

Irradiated and non-irradiated samples were subjected to a storage period of nine weeks for the crabmeat and eighteen weeks for the shrimp. These samples were compared at specified intervals for changes in quality. Methods of analysis included organoleptic, microbiological and chemical evaluations. Results of microbiological investigations appear in a master's thesis by East (22).

Conclusions.

1. No change in composition of the samples of shrimp and crabmeat was caused by the levels of irradiation used according to results of the chemical analyses. Likewise, no changes occurred in the frozen non-irradiated samples during the storage periods.

2. A three-fold increase in the storage life was achieved in the crab irradiated at 0.25 megarads and a

four- to five-fold increase in the storage life was effected in the crab irradiated at 0.50 megarads. The quality of the irradiated shrimp remained good throughout the eighteen week storage period, thus indicating the effectiveness of the irradiation in extending the shelf-life.

3. In chemical analysis of both the shrimp and crabmeat, the trimethylamine production was closely related to sensory evalutations. The test for trimethylamine was the most sensitive of the chemical analyses for the irradiated shrimp, indicating small changes in quality not detected by the other methods.

4. The pH did not appear to be indicative of the quality of the irradiated crab, but the pH of the shrimp seemed to parallel the rate of deterioration.

5. Measurement of the volatile acids and bases produced in the irradiated crab and the volatile acids produced in the non-irradiated shrimp demonstrated the onset of spoilage before sensory observations could ascertain spoilage. The volatile base production was closely related to the trimethylamine production in the shrimp.

6. Organoleptic evaluations demonstrated that a loss of irradiated taste and odor occurred on storage of the samples at 38°F. At two to three weeks of storage, the crabmeat at 0.25 megarads was not rated significantly different from non-irradiated samples and at three to four

weeks of storage the shrimp irradiated at 0.50 megarads was not significantly different from non-irradiated samples.

7. A large flavor preference panel of 150 members preferred the irradiated stored samples over the irradiated frozen samples. The non-irradiated shrimp was preferred except at one week of storage where both the non-irradiated and irradiated samples were preferred equally.

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APPENDIX

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:

Sample of Preference Ballot

NAME: _____ PRODUCT: SHRIMP DATE: _____

Please write the sample numbers in the spaces provided, then check your opinion of each sample.

	Sample # ___	___	___	___	___
Like Extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					