# STUDIES ON THE PRESERVATION OF CRAB PROCESSING WASTE AND EVALUATION OF THE QUALITY OF THE PROTEIN FROM CRAB WASTE

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Key words: Crab waste, Preservation, Crab waste protein supplement, Protein quality

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(ABSTRACT)

Three experiments were conducted to determine the effect of different chemicals on the preservation of crab waste at room temperature. In Exp. 1, .2 and .4% NaOCl and $H_2O_2$ were used and the waste was stored for 17 d. In Exp. 2, NaCl (10%), $NaNO_2$ (1%), NaOCl (.4%), $NaN_3$ (.065%), $KNO_3$ (.1%), Tert butyl hydroperoxide (TBHP) (50 ppm), and $I_2$ (25 ppm) were used. After mixing with the chemicals the waste was stored for 21 d. In Exp. 3, the waste was treated with NaCl (10%), $NaNO_2$ (1%) and $NaN_3$ (.065%) and the mixtures were kept for 20, 30 and 40 d. In the first experiment the waste treated with .4% NaOCl preserved better than for the other treatments, with lower ($P < .05$) $NH_3$ and trimethylamine (TMA). In Exp. 2, treatment with NaCl, $NaNO_2$ and $NaN_3$ did not produce any change in the physical characters of the crab waste. The TMA, indole and $NH_3$ were lower ($P < .05$) and no $H_2S$ was detected in the waste treated with those chemicals. In Exp. 3, treatment with NaCl did not alter the physical characteristics of crab waste. The waste had lower ($P < .05$) $NH_3$, TMA and indole on d 20, 30 and 40 than those treated with $NaNO_2$ and $NaN_3$.

Protein separated from crab waste was evaluated by *in vitro* and *in vivo* with chicks. Treatment with 1% KOH for 1 h gave maximum ($P < .05$) protein recovery. Approximately 25 kg of crab waste protein supplement (CWPS) were recovered, which contained on an average 43.2% CP, 30.5% ether extract and 17.8% total ash. *In vitro* protein digestibility was similar to that for soybean meal (SBM). A feeding experiment was conducted to evaluate the quality of CWPS with 180, 1-d old broiler male chicks. Crab waste protein supplement replaced 0, 25 and 50% SBM protein. The five dietary treatments were: A - 0% CWPS fed *ad libitum*; B - 25% CWPS diet fed *ad libitum*; C - 50% CWPS diet fed *ad libitum*; D - 25% CWPS diet pair-fed to diet C; and E - 0% CWPS diet pair-fed. The birds were fed for 21 d and the excreta were collected during the last 3d. Blood samples and pancreas weights were obtained from three birds from each pen. Linear decreases ($P < .05$) were recorded in body weight gain, and gain / feed with increasing levels of CWPS in the *ad libitum* and pair-fed chicks. The N and energy retention decreased linearly with increased CWPS in the *ad libitum* fed birds. Average weight of pancreas increased linearly with increased levels of CWPS. The uric acid index was higher for chicks fed the control diet and lowest for those fed 25% CWPS diet (quadratic effect, $P < .05$). It is concluded that the poor performance of chicks fed CWPS diets may be due to lower feed intake and digestibility of CWPS, and that CWPS can substitute for SBM protein at 25 % level in the diets of chicks with little effect on performance.

**Key words**: Crab processing waste, Preservation, Crab waste protein supplement, Protein quality.

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# CHAPTER 1

## Introduction

The increased competition for food between humans and animals leads to a great demand for foodstuffs for animals. Efficient use of rangelands, forages, and other materials such as industrial and agricultural wastes must be made if the livestock flocks and herds are to be maintained at their present levels. Widespread use is made of by-products in feeding farm animals. Increased use of underutilized by-products, such as crab processing waste, is necessary to meet increased demand for feedstuffs.

Crabs are classified in the phylum Arthropoda and class crustacea. Crab meat is regarded as a specialty food that adds variety to the diet, but the yield of meat, when hand picked, is only 10% of the total crabs harvested. Crab processing waste is thus a byproduct of crab industry, consisting of the shell, viscera and part of the meat. The global availability of dry crustacean waste is estimated to be 1.46 million tons per year (Knorr, 1991). It presents environmental problems because of its odor, high moisture content, and statutes governing disposal of waste. Thus, utilization of crab waste for feeding of animals could help to solve the problem of waste disposal and provide a high-protein feed source for animals.

Crab waste has been ensiled satisfactorily with wheat straw in the presence of added molasses. The quick deterioration of crab waste is the major limitation for use as animal feed. Trimethylamine oxide is a normal component of marine animals, which forms trimethylamine (TMA), one of the most important factors contributing to the offensive odor of the product upon deterioration. The TMA level is considered as an indirect measure of spoilage of sea foods. Earlier studies on the preservation of crab waste using $NaNO_2$, NaOCl, $H_2O_2$ and mixture of

organic acids have shown that NaOCl and $NaNO_2$ could preserve the waste for short periods of time. Research conducted using bacterial cultures have shown promising results in reducing the production of TMA using $NaNO_2$, $NaNO_3$ and sodium azide ($NaN_3$).

Crab waste, because of its high mineral and chitin contents, cannot be incorporated in the diets of animals in large amounts. Crab waste has been used to extract the protein, pigments and chitin. The protein thus recovered is of high quality. Experiments were conducted to evaluate the value of different chemicals in preserving crab waste for long periods of time at room temperature, to separate the protein from crab waste and to evaluate its quality for nonruminants.

## Objectives

The objectives of the research were to:

1. Study the effects of different chemicals in preserving crab processing waste at room temperature;
2. Standardize the procedure for the recovery of protein and separate protein from crab waste in bulk;
3. Study the effect of replacing soybean meal with crab waste protein supplement in the diets of chicks.

# CHAPTER 2

# Review of Literature

*Crab Industry*

*Classification of Crabs.* Crabs are classified in the phylum Arthropoda, class Crustacea, subclass Malacostraca, order Decapoda, suborder Reptantia (Poppke, 1977). The suborder Reptantia is divided into Macrura (crayfish and lobsters), Anomura (hermit crabs), and Brachyura (true crabs). The true crabs are divided into four groups: (1) Gymnopleura, (2) Dromiacea, (3) Oxystomata, and (4) Brachynatha. The majority of strong shelled crabs including blue crab, dungeness, stone crab, red or rock crab, pea crab, and land crabs, come under the group Brachynatha.

The crab industry of the Pacific Coast involves two species of major importance, King (*Paralithodes camtschatica)* and Dungeness (*Cancer* species) crabs and two species of minor importance, rock crab (*Cancer* species) and tanner crab (*Chinoecetes* species). In Hawaii, Kona crabs (*Ranina ranina*) are found (Firth, 1969). The blue clawed crab (*Callinectes sapides*) is found in the East Coast, around the Florida peninsula and along the Texas coast (Poppke, 1977).

*Life Cycle of Crabs.* A new generation of crabs is produced each year, usually between May and October ( Poppke, 1977). At this time the adult female crabs migrate to more salty waters of the bays and extrude the eggs in large masses known as "sponge". The young, after hatching appear as larvae, are called Zoea or surface plankton. These larvae feed on smaller plankton and after the fifth molt, the young crab becomes a megalops and looks more like a crab. This stage lasts for about a week and then it molts again to become "miniature crab".

The body of crab is enclosed by a hard exoskeleton which prevents the growth within the shell. Thus, crabs increase in size by molting or ecdysis (Spence, 1989). At the time of molting, the crabs seek shelter among the rocks. The shell cracks along a precise line dividing the upper and lower halves, and the soft crab slowly backs out through the gap. It then absorbs water and swells, increasing the size across the back by 20 to 30% in one molt. After the molt, the shell slowly hardens and no further increase in size will occur until the next molt. Molting takes place at frequent intervals during the early life, but after it has reached a size of 12 cm, molting takes place once every 2 yr.

The total amount of crabs harvested in US was estimated to be $1 * 10^6$ t in 1991 (Anonymous, 1993). The meat represents about 29 to 30% body weight with the most meat in the head and shoulders (Firth, 1969). On an average, 24% of the meat was in the claws, 36% in the legs, and 40% in the body.

*Crab Meat.* Crab meat is classified as a specialty food that adds variety to the diet (Firth, 1969). Dungeness crab meat contained 18 to 20% protein, and .7 to 1.1% fat (Firth, 1969) while blue crab contained 16.64% protein, 1.96% ether extract, and 3.13% ash, on fresh basis (Tressler, 1940). Canned crab meat contained 90% moisture, and 434 kcal of energy, 76.5 g protein, 10.2 g fat, 5.1 g carbohydrates, .19 g Ca, 4 mg Fe, .4 mg thiamin, and 8.1 mg of niacin per 100 g DM (Reed, 1980).

Fresh and 40-yr old pasteurized crab meat were analyzed for proximate composition, mineral, heavy metal, and amino acid volatile concentrations (Reddy et al., 1991). Fresh pasteurized crab meat was found to contain 87.6% crude protein, 1.2% crude fat, 8.3% ash, and 2.9% carbohydrates on DM basis. It contained 2.5 mg Ca and 10.7 mg P per g of dry crab meat. The 40-y old crab meat contained high levels of Fe, Mn, Cu, and heavy metals, compared to the

fresh. The major amino acids were aspartic acid, glutamic acid, lysine, and arginine. The 40-yr-old crab meat contained higher concentrations of ethanol and trimethylamine (TMA) than the fresh meat.

Boiled crab meat contained 72.5% water, 20.1% crude protein, 5.2% fat, and 127 kcal of energy / 100 g on fresh basis (Holland et al., 1992). It was also found to contain .7% of saturated, 1.4% of mono-unsaturated, and 1.5% poly-unsaturated fatty acids.

Gates et al. (1993) investigated the efficiency of commercial packaging materials on the quality, shelf life, and safety of fresh and pasteurized crab meat. Meat pasteurized in plastic and aluminum cans had better sensory and microbiological quality and longer shelf life than meat packed in steel cans. They also found that vacuum skin packaging resulted in improved sensory qualities of freshly cooked and packed meat.

Hollingworth et al. (1991) studied the correlation of spoilage of imitation crab meat with sensory analysis after storage at 4, 10, and 22° C using various procedures. Total volatile acids, total volatile bases, cadavarine, putrescine, histamine, aerobic plate count, and proteolytic count were found to be highly correlated with product spoilage at 22° C. Neither the chemical nor microbial indicators were found to be adequate to assess the quality of product stored at 4 and 10° C, concluding that sensory analysis was the only method currently available to assess the product acceptability after prolonged storage at reduced temperature.

*Crab Processing Waste*

The fish processing industry uses many types of fish and shellfish and is widely spread along the coastal lines. The quantity of waste varies among plants and among fish and shellfish types, ranging from 0% for whole rendered fish to

90% for crabs (Brinsfield, 1980). Sea food is a perishable product which must be refrigerated soon after harvest from water to prevent deterioration. Global annual production of dry crustacean waste was estimated to be $1.46 * 10^6$ t (Knorr, 1991), the major components of which are protein, chitin and minerals. In Virginia and Maryland, approximately 21 million metric tons of crab are processed annually and crab processing waste amounts to 19 million metric tons (Brinsfield, 1980). The waste undergoes degradation within 5 h after harvest (Brooks, 1980). Crab waste includes the shell, viscera, and part of the meat (Lubitz, 1943) and it is a very good source of crude protein and minerals (Samuels et al., 1991, 1992; Abazinge et al., 1993, 1994). Chitin, one of the most abundant polysaccharides of the world, comprises 12.3% of fresh water crayfish waste meal, 12.9% of crab meal, and 7.6% of shrimp meal (Patton and Chandler, 1975).

Chitin is a homopolysaccharide of β, 1,4-linked N-acetyl-D-glucosamino-pyranosal residues. This polysaccharide is found in fungi (molds and mushrooms), plantae (green algae), and in animalia (segmented worms, mollusks, and arthropods) (Yu et al., 1991). Chitin, designated as "animal cellulose", is a major component of the exoskeleton of insects and crustacea. Chitin occurs in close association with other substances. In crustaceans, $CaCO_3$ serves as the cementing substance (Lovell et al., 1968).

Chitin turn-over is important for recycling of C and N in marine ecosystems (Yu et al., 1991). The authors reported that the key step in this process is the adhesion of marine bacteria to chitin containing particles. They found that a single Ca requiring lectin is responsible for adhesion of *Vibrio furnissi* to N-acetyglucosamine.

Bassler et al. (1991) reported the steps in the catabolism of the oligosaccharides of which, N-acetyl glucosamine (GlcNAc), N,N'-

diacetylchitobiose $(GlcNAc)_2$, and chitotriose $(GlcNAc)_3$ were consumed very rapidly by the intact cells, while chitotetraose $(GlcNAc)_4$ was utilized rather slowly. The oligosaccharides enter the periplasmic space and are hydrolyzed by a unique membrane bound endoenzyme, Chitodextrinase and an endoenzyme, N-acetyl-β-glucosamidase. The chitodextridase cleaves soluble oligomers, but not chitin to di- and trisaccharides, while the periplasmic N-acetyl-β-glucosaminidase hydrolyzes the GlcNAc termini from the oligomers.

A thermostable exochitinase was purified from the culture of *Bacillus stearothermophilus* CH-4, which was isolated from agricultural compost containing shrimp and crabs (Sakai et al., 1994). This enzyme was found to hydrolyze C-4 β-anomeric bonding of N-acetyl chito oligosaccharides, as well as their p-nitrophenyl (pNP) derivatives.

*Crab Meal.* Crab meal is defined as that product prepared from the undecomposed dried waste of the crab industry and consists of shell, viscera, and part or all of flesh (AAFCO, 1992). It should contain 25% protein and not more than 3% salt.

Stelmock et al. (1985) compared the Van Soest ADF method with the modified Welinder method for chitin analysis of Alaskan crab and shrimp meal and found that Van Soest ADF method was acceptable for determination of shell fish chitin. Similar results were reported by Ayangbile (1989) when he compared different methods of estimating chitin of crab waste.

Lubitz et al. (1943) reported the mean chemical composition of commercial crab meal made by steam drying of blue crab. It contained 6.3% moisture, 32.7 % CP, .8% crude fat, 12.9% crude fiber, 41.6% ash, 16.4% Ca, 1.64% P, .4% K, and 2.9% NaCl. It also contained 180 ppm Mn, 1063 ppm Fe, 2.9 μg of riboflavin and less than 1 I. U. of vitamin A per gram. The average availability of protein was

84.5% in rats with a biological value of 76%. Chitin was found to have no protein supplementing value for chicks.

Lovell et al. (1968) determined the nutritive value of fresh water crayfish waste meal. It contained 40.1% CP, 4.9% ether extract, 29.0% ash, 18.1% Ca 1.2% P, .27% Mg, .14% K, 157 ppm Mn, .13% I, 8.8% Fe, and 14.1% chitin. The digestion coefficients for chitin-free protein was 87.54 in rats.

Rutledge (1971) reported that decalcification of crustacean meals (Blue crab and freshwater crayfish) could be done by drying to a moisture level of 6% or less, grinding through a Wiley mill through a .6 cm screen and sieving through a No.12 U. S. Standard mesh. The protein content was doubled and the Ca level reduced by 68% by the process.

Patton et al. (1975) determined the nutritive value of crab meal for young ruminating calves. Three trials were conducted with 26 male Holstein calves. In trials 1 and 2, crab meal was substituted for the basal ingredients up to 20% of the basal diet and was fed to young rapidly growing calves. They found no significant reduction in body weight gain, feed intake or feed efficiency while the N retention was reduced when crab meal was substituted at 20% level. There was an increased urine output which was attributed to the excessive amounts of Ca, Na, Cl, and Br in the crab meal diets. The digestibility of chitin from crab meal was 66%, but varied from 26 to 87%. In trial 3, crab meal was added to a protein deficient diet and it increased the average daily gain and feed intake over the unsupplemented group.

In another study Patton and Chandler (1975) evaluated the *in vivo* rumen digestibility of crab meal, shrimp meal, cockroaches, grasshoppers and purified chitin using cannulated steers. The DM solubility was 35.7% for crab meal while it was 66.5% for cockroaches and 21.5% for pure chitin.

Brundage et al. (1981) compared the feeding value of King crab meal for lactating cows. Crab meal was incorporated at 11 and 22.5%, replacing soybean meal. Milk production was lowest for the negative control group and the next to the lowest for those receiving smaller amounts of supplemental soybean and crab meal. It was concluded that crab meal can be a potential source of supplemental protein in concentrates for lactating cows.

In another study, Brundage et al. (1984) compared the acceptability of tanner crab meal with soybean meal in 30 lactating Holstein cows. Crab meal was substituted for soybean meal at 0, 25, 50, 75, or 100% on an isonitrogenous basis. Concentrates contained 19 : 0, 14 : 7.5, 9.5 : 15, 5 : 22.5, 0 : 30% soybean meal : crab meal, respectively. Feed intake was lowest for cows fed 30% crab meal concentrate and was highest for those fed the 15% crab meal diet. Persistency of 4% fat-corrected milk production was highest for cows fed the 0% crab meal concentrate. Cows fed the 30% crab meal concentrate lost body weight, while those fed 0 and 15 % crab meal gained the highest amount of weight during the study. The effect of crab meal on palatability and animal performance was found to be of greater consequence than its effect on milk flavor.

Anderson and Van Lunen (1986) fed isocaloric and isonitrogenous diets containing 0, 5, 10, or 15 % crab meal to 88 growing Yorkshire pigs. The body weight gains of the growing pigs fed different diets were not significantly different while the feed consumed per day and feed / gain ratio were higher for 10% crab meal fed group, compared to the 5% crab meal diet. Carcass index was significantly better with less backfat in pigs fed 15% crab meal than those on 5% crab meal diets.

Laflamme (1988) conducted two experiments to assess the nutritive value of crab meal for weaned beef calves. In experiment 1, the nutritive value of crab

meal for replacement heifers was compared with soybean meal. Forty-five heifer calves were fed barley-based concentrate mixtures containing 0, 10, and 20% soybean meal or 15 and 25% crab meal. Incorporation of crab meal reduced the feed consumption and growth rate initially, and these measures improved with time. In experiment 2, 24 steer calves were fed up to 1% of body weight of concentrate mixtures containing 20% soybean or 35% crab meal. Crab meal inclusion in the diet reduced feed intake and performance by the calves, but most of the negative effects were eliminated after a period of adaptation.

Sticker et al. (1989) evaluated ruminal degradation and subsequent absorption of menhaden fish meal and blue crab meal in cannulated sheep and cattle. In trial 1, four Suffolk wethers fitted with ruminal and duodenal cannulae were fed diets containing 52 to 59% corn, 21 to 24% cottonseed hulls and .2% chromic oxide mixed with menhaden fish meal, blue crab meal, or soybean meal. Wethers fed soybean meal had higher fecal N percentage on DM basis than those fed crab meal or fish meal. In trial 2, two rumen fistulated Holstein steers were used to determine the *in situ* N and DM disappearance of fish meal, crab meal and soybean meal. It was found that fish meal and crab meal did not differ significantly in the percent N disappearance at any given time period, suggesting that crab meal protein has similar ruminal escape potential as menhaden fish meal.

Husby (1990) compared tanner crab shell to chopped brome grass hay as a potential roughage source in hulless barley diets for finishing steers and found that feeding diets containing crab shell reduced the growth and feed intake.

Bunting et al. (1994) conducted two experiments to determine the feeding value of crustacean processing waste using either ground dehydrated crustacean meal (mixed crayfish and crab) or fresh, partially commuted crayfish waste. It was found that the daily DM intake and body weight gains were similar for lambs fed

soybean meal or crustacean waste. Blood and ruminal metabolite data suggested that ruminal adaptation may be required to elicit maximum degradation of dehydrated crustacean waste protein. In a second experiment, 11 male lambs from the previous trial were used in a 20 d metabolism trial. Fresh crayfish waste was fed to those lambs that received dehydrated crustacean waste in the previous experiment. Apparent N retention was greater for lambs fed fresh crayfish than those fed soybean meal, suggesting that crustacean meal can be incorporated in the diets of growing ruminants to comprise 35 to 40 % of protein intake with positive growth response. It was further suggested that more than 4 wk may be required for full ruminal adaptation to crustacean waste.

*Ensiling of Crab Waste*. Samuels et al. (1991) ensiled fish waste and crab waste with wheat straw using 5% dry molasses as an additive. Fin fish waste was ensiled with wheat straw in combinations of 70:30 and 51:49, whereas crab waste was ensiled with wheat straw in combinations of 60:40 and 40:60. Glacial acetic acid (16% vol/wt) was added to the crab waste mixtures. In a feeding trial with sheep the DM intake was greater for crab waste-straw at 60:40 and fish waste-straw at 51:49 proportions. The apparent digestibility of CP was greater for diets containing fish waste than those containing crab waste. In another study ensiling fish waste and crab waste with corn stover or peanut hulls with or without 5% dry molasses or 1% formic acid and with wilted Johnson grass with and without 5% dry molasses decreased or eliminated coliforms and fecal coliforms (Samuels et al. 1992). Addition of 5% dry molasses decreased the pH of fish waste silages but had no effect on crab waste silages.

Ensiling characteristics of crab waste and wheat straw treated with different additives was studied by Abazinge et al. (1993). They found that the lactic acid levels increased and pH decreased linearly with increasing levels of molasses.

Trimethylamine level was lower for molasses or the inoculant (.1% microbial silage inoculant) treated silages. Addition of 10 to 12% dry molasses to crab waste-straw mixtures resulted in silages with substantial amounts of lactic acid. Addition of molasses and inoculant resulted in silages with lower pH and higher lactic acid compared to those with molasses alone.

In another study crab waste ensiled with wheat straw with different additives (16% v / wt glacial acetic acid, 20% dry molasses and microbial inoculant) were used to study the digestibility, N utilization and voluntary intake ( Abazinge et al., 1994). The voluntary intake and apparent digestibility of DM and CP were lower for acetic acid treated silages than those containing molasses. The N retention was higher for the molasses-inoculant treated silages than the molasses treated silages. Treatment of crab waste-straw mixtures with molasses produced a palatable silage that was efficiently utilized by wethers.

*Preservation of Crab Waste.*

Spinelli (1971) reported that the loss of freshness of fish resulting from autolytic changes begins immediately after catching. The loss of freshness led to loss of flavor and textural alterations in the tissue. Accumulation of lactic and pyruvic acids and depletion of 5'-inosine monophosphate were attributed for the loss of quality.

Trimethylamine oxide (TMAO) is a constituent of marine animals including both fish and invertebrates ( Strom et al., 1979). After death TMAO is reduced by bacteria, notably by gram negative rods, to TMA which gives rise to the characteristic off-flavor in the early stages of decay. The enzyme responsible for the reduction of TMAO to TMA, originally termed as "triamine oxidase" has been classified as NADH: trimethylamine-N-oxidoreductase (Easter et al., 1982). Physiological significance of TMAO reduction was recognized with the discovery

that TMAO can act as a terminal electron acceptor supporting the growth of some anaerobic bacteria. Reduction of TMAO was also demonstrated by *Halobacterium*, *Haloarcula*, and *Haloferax* bacteria that grow at salt concentrations of 15% and higher (Oren, 1990).

Parkin and Hultin (1982a) found that mincing stimulated the breakdown of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde in red hake muscle at both above and below freezing temperatures. They also stated that presence of TMA, DMA and citrate inhibited the reaction in frozen minced tissue. Kim and Chang (1974) found that TMAO supported the anaerobic growth of *Salmonella* on glycerol and they concluded that TMAO reductase and nitrate reductase systems have common components. Tomioka et al. (1974) found that the enzyme prepared from the pyloric caeca of fish, *Theragra chalcogramma* could convert TMAO to DMA and formaldehyde.

Strom et al. (1979) found that TMAO stimulated the anaerobic growth of *Proteus* NTHC 153. Similar stimulatory effect on the microbiological growth of *Alteromonas* sp. was reported by Easter et al. (1982). They reported that pyruvate, lactate, formate and peptone acted as good electron donors; succinate, fumarate, malate, and acetate served as moderate donors; and oxaloacetate, citrate, isocitrate, glucose and glycerol acted as poor donors to support TMAO reduction. Oren (1990) demonstrated that TMA could be formed from choline and glycine betaine.

A microsomal fraction isolated from the skeletal muscle of red hake (*Urophycic chuss*) was capable of breaking down TMAO to DMA and formaldehyde *in vitro* (Parkin and Hultin, 1982b). They also found that either cysteine or ascorbate could serve as the substrate and iron was required for the reaction. Phenazine methosulfate and methylene blue activated the process, while

certain other chemicals, such as TMA, choline, EDTA, citrate, and Cu inhibited the process.

Joly et al. (1992) reported that TMAO-demethylase (TMAO-ase) was present in large amounts in the kidney of gadoid fishes. During mechanical deboning of fish, contamination from kidney was found to cause the production of dimethylamine (DMA) and formaldehyde from TMAO.

Patterson and Hespell (1979) studied the effect of TMA as growth substrates for ruminal bacteria, *Methanosarcina barkeri*, for methanogenesis. They found that when TMA and methylamine were added as the only added C and N sources, up to 80% of the theoretical methane production was obtained while pure cultures of various ruminal bacterial species including *Methanococcoides ruminantium* were not able to utilize these substrates for energy / N source. They suggested that in the rumen, TMA and methylamines are primarily degraded by *Methanosarcina*, resulting in release of $NH_3$, which then can be utilized by other ruminal bacteria.

Sowers and Ferry (1983) isolated a new genus of methylotropic marine methanogen, *Methanobacterium methylutens*, and the growth on TMA was stimulated by ruminal fluid, yeast extract, trypticase or B vitamins. Maximum growth was obtained between pH 7.0 and 7.5.

King (1984) studied the response of methanogenesis and sulfate reduction to TMA, choline, and glycine betaine in surface sediments from intertidal regions of Maine and found that methanogenesis was stimulated by addition of the above substrates.

Naumann et al. (1984) reported the presence of a TMA:2 mercaptoethanesulfonate methyltransferase in *Methanosarcina barkeri.* This organism was found to be able to utilize methanol, acetate, methanol + $H_2$, carbon

monoxide, methylamine, DMA and TMA, besides $CO_2$ and $H_2$. They prepared cell extracts from *Methanosarcina barkeri* which had been grown on methanol, dimethylamine or trimethylamine and studied the ability of the extracts to catalyze the formation of methane. They found that the use of extract from methanol grown cells resulted in methane formation from methanol but not from any of the methylamines, whereas extract from TMA grown cells resulted in methane from all four substrates tested. This demonstrated that the methanol : HS-coenzyme M methyltransferase did not act with significant activity on TMA, DMA and methylamine. Dimethylamine grown cells showed very little activity with trimethylamine, so that a specific enzyme seemed to be responsible for the conversion of TMA into DMA. They found that HS-coenzyme M was required for TMA degradation to form methyl-S-coenzyme M as an intermediate. Addition of 2-bromoethanesulfonic acid (BrES) inhibited methane production from TMA but TMA conversion rate remained constant. Both TMA:HS-coenzyme M methyltransferase and Methanol:HS-coenzyme M methyltransferase require ATP for activity and are activated by reducing agents such as hydrogen.

Muller et al. (1987) studied the energy conservation in resting cells of *Methanosarcina barkeri* during methanogenesis from methanol + $H_2$ or formaldehyde + $H_2$. Addition of TMA to resting cells of M. barkeri grown on TMA increased methane production (up to .4 micromol. $min^{-1}$. mg $protein^{-1}$), ATP concentration in the cell (4.6 nmol/ mg protein), and the proton motive force ( $\Delta$ p = -130 mv). They found that in the presence of $H_2$, TMA was exclusively reduced to methane according to the equation,

$$(CH_3)_3NH + 3H_2 \rightarrow 3\ CH_4 + NH_4.$$

The addition of an uncoupler, 3,5,4'5'- tetrachlorosalicylanilide (TCS) led to a drastic decrease of the intracellular ATP content and the transmembrane electrical

gradient, but stimulated methanogenesis. The ATPase inhibitor N-N'-dicyclohexyl carbodiimide (DCCD) caused a rapid exhaustion of the ATP pool and inhibited methane formation. The inhibition of methane formation by DCCD could be relieved by the addition of TCS, indicating a chemiosmotic coupling between methane formation and ATP synthesis.

Peterek and Smith (1988) isolated a methanogenic organism, *Methanohalophilus mahii*, from the sediments of the Great Salt Lake in Utah. The microorganisms of this genus require NaCl concentrations of 1.0 to 2.5 M for optimum growth and methanogenesis. These organisms utilize TMA, methylamine and methanol for growth.

Trimethylamine production leads to undesirable flavor in marine products and TMA production by TMAO reduction is one of the best known bacterial decomposition reactions studied in fish (Malle et al., 1986). It represents an indirect expression of the microorganisms present to decompose muscle tissue. Many methods for detection of specific spoilage bacteria in fish and fish products are based on TMA and $H_2S$ production. Gram (1992) described the traditional and rapid methods for estimation of bacterial levels in seafoods which include the reduction of TMAO to TMA. Malle et al. (1986) developed a rapid method for assessing the sanitary quality of deep frozen fish fillets based on the reduction of TMAO to TMA. Singh et al. (1992) studied the effect of TMA in rats and the results revealed that 10 meq of TMA reduced body weight gains and protein efficiency ratio. They also found that there was a reduction in the packed cell volume, neutrophils and monocytes and an increase in the reticulocytes, leukocytes, lymphocytes and eosinophils in the blood of rats fed TMA-containing diets. Since TMA level is an indirect index of spoilage of marine products,

attempts to decrease TMA production will improve the keeping quality of the product (Kim and Chang, 1974).

Indole is a product of decomposition of proteins containing tryptophan. It is formed from proteins under anaerobiasis in the absence of carbohydrates (Clarke et al., 1937). They found high indole in butter made from decomposed cream. Indole content of raw oysters was 1.6 μg / 100 g at 0 h and it increased to 21.3 μg / 100 g in 168 h (King et al., 1945). Duggan and Strasburger (1946) estimated the indole content of different classes of shrimp and found that class 4 shrimps contained 1.053 mg indole / 100 g while class 1 had 8 μg / 100g. Chung and Cadwallader (1993) analyzed the indole content of fresh crab meat and crab byproduct and found that crab meat had higher indole (8.6 μg/ 100 g) than crab byproduct (3.9 μg / 100g).

Frampton et al. (1990) compared a method based on indole production for enumerating *E. coli* in artificially inoculated ground meats to the standard methods. They found that a significantly lower number of *E. coli* cells was recovered from chicken for the method based on indole production, but no difference was obtained in ground beef. Indole was identified from water boiled duck meat and was attributed to its specific aroma (Wu and Liou, 1992).

Different indole alkaloids occur in plants and many of them have pharmaceutical properties. An alkaloid 3α*(S)*-strictocidine has been isolated from the genomic libraries prepared from *Rauvolfia serpentina* and *Rauvolfia mannii* (Bracher and Kutchen, 1992). Pasquali et al. (1992) reported the presence of a wide range of indole alkaloids in *Cantharanthus roseus* and they determined the mRNA sequence for the enzyme, strichnosidine synthase. The enzyme, tryptophan decarboxylase, was found to convert tryptophan to tryptamine and thereby channels primary metabolites into indole alkaloid biosynthesis (Goddijn et al.

1992). Vetter et al. (1992) demonstrated that two p-450 enzymes, geraniol-10-hydroxylase and nerol-10-hydroxylase, are involved in the synthesis of indole alkaloids in *Catharanthus roseus*. Auxins induced tryptophan decarboxylase (TDC) activity in *Catharanthus* seedlings (Aerts et al., 1992). Fernandez and Luca (1994) suggested that ubiquitin proteolytic pathway plays an important role in the development regulation of TDC in *Catharanthus roseus*.

Sidransky et al. (1994) evaluated the effects of indole-related compounds on binding of L-tryptophan to rat hepatic nuclei and found that 3-methyindole reduced tryptophan binding to nuclear envelopes even though it did not alter hepatic protein synthesis.

Gillies (1975) reviewed the effect of different chemicals on preservation of seafood, of which some of them were 5-amino hexapyrimine (.01 to .1%), sorbic acid (.1 to .5%), antioxidants (.2%), 2-haloacetic acid (400 to 800 ppm), EDTA (5 to 50 ppm), and tert-butyl hydroperoxide (5 to 50 ppm). These chemicals were found to inhibit the growth of a wide range of microorganisms, thus extended the storage life of seafoods.

Easter et al. (1982) studied the effects of different chemicals on the production of TMA from TMAO in bacteriological cultures of *Alteromonas* sp. NCMB 400. Sodium azide ($NaN_3$) at 1 and 5 mmol / L inhibited TMA production by 45 and 68% respectively, while 5 mmol / L of $KNO_3$ and $NaNO_2$ inhibited the TMA production by 52 and 69%, respectively.

George and Gopakumar (1988) studied the spoilage changes in crab muscle stored at three different temperatures and found that crab muscle could be preserved at ambient temperature (25 to 28°C) for 8 to 9 h, at refrigerated temperature (6.6 to 7.5°C) up to 6 d and at 0°C up to 11 d. They also reported that the bacterial count, TMA, and lactic acid contents increased with storage time

while the organoleptic score was decreased. They attributed the changes in flavor and texture of the muscle to the postmortem degradation of glycogen and accumulation of lactic acid.

Degnan et al. (1994) evaluated the effect of lactic acid bacteria fermentation products and food grade chemicals to control *Listeria monocytogenes* in blue crab meat. They found that washing of pathogen inoculated crab meat using lactic acid fermentation products decreased (3.16 to 10 units / g) the number of bacterial cells initially, but original numbers were noted in 6 d. Washing with food grade chemicals such as sodium acetate (4M), sodium diacetate (.5 or 1 M), sodium lactate (1 M), or sodium nitrite (1.5 M) caused modest reductions in the number of bacterial counts (2.5 to 6.3 units / g), while sodium diacetate (2M) decreased the counts to 398 units / g within 6 d, compared to the control values of $31.6 \times 10^4$ units / g. Trisodium phosphate at 1 and 2.5M reduced the counts to $> 4 \times 10^4$ and 50 units / g, respectively, within 6 d, indicating that the number of *L. monocytogenes* could be reduced by washing with select antibacterial agents.

*Salt.* Salting is one of the traditional methods of preserving fish. There are two fundamental methods of salting, dry salting which is used for non-fatty fish and pickling for fatty fish ( Hockenhull, 1967 ). Most of psychrophilic proteolytic *Pseudomonas* and *Achramobacter* sp. were found to be killed by the pickling process. Dry salting destroyed most of the organisms, but *Micrococci* were found to survive if the salt concentration was less than 12 %. Duncan and Foster (1968c) determined the effect of sodium nitrite, salt, and sodium nitrate on the germination and growth of anaerobic spores, and found that as much as 4% nitrite failed to prevent germination and swelling of spores, while salt concentrations above 6% prevented germination. In the presence of 3 to 6% NaCl in the medium, most of

the spores germinated and produced vegetative cells, but cell divisions were blocked.

Rhodes and Jarvis (1976) reported no effect of varying the severity of thermal processes on the inhibitory action of sodium nitrite in the presence of NaCl, on growth and toxin production by *Clostridium botulinum*. Roberts et al. (1976) studied the interaction of NaCl / $NaNO_2$ and storage temperature on toxin production and spoilage by *C. botulinum* in meat slurry at pH 6.0. They found the greatest inhibition at 1.8% or 3.5% NaCl at 15°C over 6 mo of storage. A pronounced salt-nitrite-time interaction was observed at lower incubation temperatures and the greatest inhibition was seen at salt concentrations of 1.8% or 3.5% at 15°C. Jarvis et al. (1976) reported that a significant NaCl by $NaNO_2$ interaction occurred only at NaCl levels of 4.5% and above. They reported spores of *C. botulinum* type B heated at 70 or 80°C were sensitized to 4.5% and 5.5% NaCl. Gram (1992) studied the inhibition of mesophilic spoilage *Aeromonas* species on fish by NaCl, potassium sorbate, liquid smoke and chilling and found that a combination of 5% NaCl and 1000 ppm of sorbate inhibited growth at 25 to 37°C.

Coleman et al. (1986) studied the ultrastructural changes of 0° C blast frozen and brine immersed (23% NaCl) blue crab levator muscle by transmission electron microscopy in comparison to the unfrozen. They found a decreased density and change in the shape of myofibrils and 'Z' lines, and disappearance of the sarcoplasmic matrix due to blast freezing (slow process), while the change was less severe due to brine immersion freezing (rapid method). The distance between myofibrils was found to be compacted in brine immersed crab muscle samples, which was related to the firmer texture.

The mode of bacterial inhibition by NaCl was found to be primarily by its plasmolytic effect (Busta and Foegeding, 1983). Other antimicrobial effects, include dehydration, interference with enzymes, $O_2$ removal, or the toxicity of high sodium or chloride ion concentration. The effect of NaCl was attributed to the loss of Mg from bacterial cells (Sato et al., 1972; Ito et al., 1977). Ito et al. (1977) further observed degradation of RNA during incubation of *E. coli* cells in tris buffer containing .15M NaCl, which was suggested to be due to the enzyme action of RNAase 1.

A 10% concentration of NaCl was found to inhibit *Clostridium botulinum* and other microorganisms in fish (Schmidt, 1964). The tolerance of microorganisms to NaCl was found to vary greatly (Banwart, 1989). He reported that the mesophilic gram negative rods and psychrotrophic bacteria were most sensitive at concentrations of 4 to 10%, while the lactic acid and spore forming bacteria could tolerate about 4 to 15% and 5 to 16% NaCl respectively. The *Halophiles*, *Halobacterium* and *Halococcus* organisms need relatively high NaCl concentrations for their growth.

*Sodium Nitrite.* Sodium nitrite has been used as a food preservative to inhibit the development of *Clostridia* and other food spoiling microorganisms in preserved foods (Walters and West, 1964).

Duncan and Foster (1968a) found that $NaNO_2$ stimulated the germination of putrefactive anaerobe 3679 strain h spores in culture media and the process was accelerated by using higher concentrations of nitrite, a low pH, and a high temperature of incubation. It was found that hydroxylamine inhibited the nitrite induced germination, while nitrite inhibited germination induced by L-alanine. In another study, Duncan and Foster (1968b) studied the mechanism by which NaCl, $NaNO_2$, and $NaNO_3$ supplement the action of heat in preserving canned cured

meat products. The results of their study revealed that when the spores of the putrefactive anaerobe 3679 strain h were heated and cultured in the presence of curing agents (1) the nitrate and NaCl increased the apparent heat resistance at low concentrations (.5 to 1%) but decreased it at concentrations of 2 to 4%; (2) nitrite was markedly inhibitory, especially at pH 6.0. At the normal pH of canned meat of approximately 6.0, nitrite was found to be the chief preservative against spoilage by putrefactive bacteria.

Fox (1974) reported that reductants such as ascorbate and cysteine, histidine, and the reduced coenzyme NAD caused important losses of nitrite added to meat slurries by reducing nitrite to nitric oxide. Christiansen et al. (1974) treated pork bellies to 0, 30, 60, 120, 170, or 340 μg of nitrite and the bellies were inoculated with *Clostridium botulinum* via pickle or after processing and slicing. Processed bacon was stored at 7° and 27°C. No toxins were found in samples incubated at 7°C throughout the 84-day period. Increased levels of formulated nitrite decreased the probability of botulinal toxin formation in bacon inoculated by both methods.

Schlyter et al. (1993) studied the effects of diacetate with nitrite, lactate, or pediocin on the viability of *Listeria monocytogenes* in turkey slurries and found that sodium diacetate alone or in combination with sodium lactate or pediocin could delay the growth of *L. monocytogenes* in turkey meat. Using nitrite with diacetate did not affect the growth of *L. monocytogenes*.

Perigo et al. (1967) studied the effect of nitrite on the inhibition of vegetative cells of *Clostridium sporogenes* in a laboratory medium and found that at pH values of 6.0 and above, the inhibitory effect of a given concentration of nitrite was enhanced when it was first autoclaved in the medium. They reported that the nitrite on heating reacts with some components of the medium, producing

an unknown substance which was extremely inhibitory to the vegetative growth of *C. sporogenes*. The formation of a potent antimicrobial substance with a broad spectrum of action was also reported by Ashworth et al. (1974) when nitrite was heated in certain systems. The inhibitory substance was found to be similar to the iron nitrosyl co-ordination complexes, termed as Roussin black salts.

Moran et al. (1975) further studied the inhibitor of *Clostridium perfringens* formed by heating sodium nitrite and found that the inhibitor was formed from cysteine, ferrous sulfate and sodium nitrite. They also found that S-nitrosocysteine, Roussin black salt, and cysteine complex could inhibit *C. perfringens*, but since no single compound was present in levels adequate for inhibition, the net effect could be the combined effects of small amounts of each of these compounds.

Tompkin et al. (1979) compared the combinations of nitrite with isoascorbate, and EDTA for their antibotulinal efficacy in perishable canned meat. They found that EDTA (500 μg /g) was more effective than isoascorbate for enhancing the effect of nitrite.

Nitrite was shown to inhibit active transport, $O_2$ uptake and oxidative phosphorylation of *Pseudomonas aeruginosa* (Rowe et al., 1979). The inhibitory effect of nitrite was by oxidizing ferrous iron of an electron carrier such as cytochrome oxidase, to ferric iron. They also found that glucose transport by *Streptococcus faecalis* was not inhibited by nitrite, presumably because these species lack cytochromes, and glucose was transported by the phosphoenolpyruvate : phosphotransferase system rather than active transport.

Benedict (1980) reported that inhibition of *Clostridium botulinum* in cured meat was due to several interacting mechanisms: (a) reaction and oxidation within the spores and vegetative cells; (b) restriction of use of iron (or other metal ion)

through inhibition of solubilization, transport, or assimilation, thus interfering with metabolism and repair mechanisms and cell surface membrane activity limiting substrate transport by the outgrowing cell.

Nitrite was found to inhibit the active transport of proline in *Escherichia coli* but not the group translocation of sugar via the phosphoenolpyruvate : phosphotranferase system (Yarbrough et al., 1980). Nitrite also inhibited the $O_2$ uptake and oxidative phosphorylation in aerobic bacteria and enzyme aldolase in *E. coli*, *Streptococcus faecalis*, and *Pseudomonas aeruginosa*, indicating that nitrite has more than one site of attack in the bacterial cell.

The addition of nitrite to a suspension of *Clostridium sporogenes* in glucose medium resulted in a rapid decrease in intracellular ATP concentration which was accompanied by an accumulation of pyruvate in the medium (Woods et al., 1981). The accumulation of pyruvate was found to be caused by inhibition of the phosphoroclastic system by the nitrite. Nitrite inhibits the system by the reaction of nitric oxide formed from nitrite with the non-heme iron of pyruvate : ferrodoxin oxidoreductase.

Glidewell and Glidewell (1993) have shown that in the presence of iron salts, nitrite reacts with cysteine and other sources of cysteine to yield antibacterial iron-sulfur nitrosyl salt (Na $[Fe_4S_3(NO)_7]$ which was more potent than nitrite itself under a range of substrates. Ayangbile (1989) studied the preservation of crab waste using different preservatives such as 1.5% propionic / formic acid (1:1), 0.2% sodium hypochlorite, 0.4% hydrogen peroxide, 0.4% NaOCl + 1.5 % acetic acid, 1.5 % NaOH, and 1% $NaNO_2$. The TMA concentration was the lowest for the sodium nitrite treated material during a 10 d preservation period. Hydrogen peroxide treatment did not increase the TMA content during a 7 d period. Waste

treated with NaOCl + acetic acid preserved for 4 d. Sodium nitrite at 1% level was found to be effective in preserving crab waste up to 10 d (Ayangbile, 1989).

Potassium nitrate / nitrite were found to be essential for a stable color typical of meat to become established in the frankfurter product (Mautes, 1995). As nitrites are reduced, nitrogen oxide become attached to myoglobin to produce nitrosomyoglobin, which was found to responsible for the color promoting and color stabilizing effects.

*Organic Acids.* Mountney and O'Malley (1965) studied the effect of different acids as poultry meat preservatives and found that acetic, adipic and succinic acids increased the shelf life by 6 d more than the control. The inhibitory effect of adipic and succinic acids was found to be dependent upon the pH. The meat preserved with adipic and succinic acids had lower microbial growth and more acceptable taste, compared to the other treatments.

Allen et al. (1975) studied the effect of adding formic acid, propionic acid, formic-propionic mixture, and molasses in the preservation of wet brewers grain stored in uncovered piles, and found that the formic-propionic mixture (1:1) at 4% level reduced all aspects of deterioration (both surface and subsurface deterioration), and maintained quality of the material during the 14-d period.

Woolford (1975) studied the effect of some food preservatives, cold sterilants, and specific antimicrobial agents against a range of microorganisms at pH 3, 4, 5 and 6. The author reported that lactic acid was effective only in anaerobiasis and potassium sorbate and the antibiotic, pimaricin, increased the aerobic stability of the silage. Formaldehyde and paraformaldehyde were found to have potential in the production of nonfermented silages, while hexamine and propylene oxide were found to be less attractive as silage additives because of the

need for large quantities. Sodium nitrite was found to restrict or suppress the fermentation in silage.

Wing et al. (1976) determined the effect of different levels of formic acid, formaldehyde and propionic acid in the preservation and digestibility of alfalfa haylage. Formaldehyde protected hemicellulose from degradation, reduced lactic and acetic acid contents, and the apparent digestibility of CP, while propionic acid treatment increased the rumen propionate and reduced ruminal butyrate levels.

Fesyun et al. (1985) suggested that formic acid, acetic acid, propionic acid, benzoic acid, sodium pyrophosphate, sodium bisulfate, and low molecular acid mixtures (mixture of formic, propionic, acetic, butyric acids and water) could conserve green herbage. Mel'nik (1987) also used a mixture of low molecular fatty acids for the preservation of green feeds and obtained an increase in the utilization of nutrients by 15 to 20% compared to untreated silage when fed to cattle.

Selivanov (1985) obtained satisfactory results when ewes were fed on silages preserved by addition of formic acid, benzoic acid, or sulfite liquor. Tagaeu et al. (1985) stated that formic acid is the most suitable chemical preservative for feeds because of its high bactericidal and fungicidal properties. They reported that the most suitable doses of formic acid for ensiling clover, lucerne, and maize were .3 to .5% of ensiled material.

Baron and Greer (1988) compared the effectiveness of six commercial hay preservatives and found that a preservative containing 67% propionic acid partly neutralized with ammonia gave the best results in terms of IVDOM. The preservatives inhibited the growth of molds and aerobic bacteria.

Kalac and Wohlrab (1988) ensiled *Dactylis glomerata* and oats with additives of formic acid, silostan, acrylic acid, and sodium acrylate. The best

conservation was obtained with 2.5 to 3 kg of acrylic acid / t, but its relatively high cost indicated that it might be used in a mixture with other silage additives.

Abazinge et al. (1993) preserved crab waste with 1.5% formaldehyde, .75% propionic acid, .75% formic acid, and 10% liquid molasses for 4 wk. A higher CP and a lower TMA was obtained for the formaldehyde treated crab waste samples even though the material treated with propionic / formic acid mixture showed better fermentation when ensiled.

Ayangbile et al. (1987) found that addition of 1.5% propionic acid and formic acid in 1:1 proportion prevented the degradation up to 14 d. Desirable fermentation was achieved when the preserved waste was ensiled with wheat straw, liquid molasses and water.

Wohlt et al. (1994) studied the composition, preservation and use of sea clam viscera as a protein supplement for growing pigs. They found that fresh clam viscera could be preserved up to 3 wk by adding formic or propionic acid at 3.5 % (wt / wt). Diets containing wet viscera preserved with propionic acid were palatable for pigs and did not affect the growth rate of pigs, compared to that of controls. Incorporation of viscera at 5 or 10% of diet DM imparted a distinct fishy aroma for the cooked loin roast.

Acetic acid was found to inhibit many species of bacteria, yeast, and molds and the inhibitory effect was greater than that of citric or lactic acid at the same pH. Undissociated acetic acid penetrates the microbial cell and exerts a toxic effect (Banwart, 1989). The antimicrobial effect of propionic acid was reported to be due to nutrient transport inhibition (Eklund, 1980), inhibitory enzymes (Lueck, 1980) and partly by competing with necessary growth substances such as alanine and other amino acids (Hasseltine, 1952).

Lactic is a weak lipophilic acid and causes leakage of $H^+$ ions across the cell membrane, resulting in acidification of cell interior and inhibition of nutrient transport (Woolford, 1975). Its antimicrobial effect is directed primarily against anaerobic bacteria such as putrefying anaerobes and butyric acid bacteria. The antimicrobial action of lactic acid was only moderate and concentrations above .5% are needed for a preservative effect (Busta and Foegeding, 1983).

Formic acid was found to exert its antimicrobial action against yeast and some bacteria by inhibiting decarboxylases and heme enzymes, especially catalase (Lueck, 1980).

*Hydrogen Peroxide.* Hydrogen peroxide has been used in the dairy industry for two purposes: (a) as a preservative for raw milk and (b) in combination with heat treatment to reduce the total bacterial count of milk before cheese making (El-Gendy et al., 1980). El-Gendy et al. (1980) studied the effect of $H_2O_2$ on the survival and growth of *Clostridium* species in litmus milk (containing 10% yeast-lactate-liver bouillon) and found that at .02%, peroxide inhibited the growth and gas production of milk containing 50 or 100 spores / ml. The effect of $H_2O_2$ on the activity of lactic acid cultures in milk was studied by Subramanian and Olson (1968). They found that $H_2O_2$ inhibited the acid production by all cultures.

Toledo et al. (1973) studied the sporicidal properties of $H_2O_2$ at concentrations of 10 to 41% at temperatures of 24 to 76°C against food spoilage organisms grown in bacterial culture. They found that heat shocking spores prior to $H_2O_2$ treatment decreased their resistance, and wet spores were more resistant than dry spores to peroxide inactivation. The resistance of dry spores of *Bacillus subtilis* var. *globilii* to solutions of $H_2O_2$ was studied at concentrations of 10 and 30% at temperatures from 20 to 50°C. Smith and Brown (1980) found that there

was a logarithmic relationship between decimal reduction time and temperature at different concentrations of $H_2O_2$. Hydrogen peroxide was found to be a good preservative and a bleaching agent when it was used for marinating herring (Sims et al., 1975).

Racicot et al. (1984) reported that addition of oxidizing agents such as $H_2O_2$, NaOCl, and $KBrO_3$ to minced red hake muscle, reduced the rate of DMA and formaldehyde formation, while addition of reducing agents accelerated their production. They also found that $H_2O_2$ at .05, .1, and .25 % levels was most effective in slowing the reaction rate and imparting a better texture than that of the control.

Lewis et al. (1987) found that presoaking in either NaOH or $NH_3$ before peroxide treatment improved the effectiveness of the treatment. Ayangbile et al. (1987) observed that preserving crab waste with .4% $H_2O_2$ prevented deterioration up to 10 d without any increase in TMA production. Bas et al. (1989) observed an increase *in vitro* digestibility of NDF with alkaline $H_2O_2$ treatment of wheat straw. They reported increased total VFA and bacterial N production with treated wheat straw.

*Sodium Hypochlorite.* Sodium hypochlorite has been used extensively in preservation of edible products (Lechowich, 1981). Narasimhalu et al. (1981) reported that the broiler litter aerobes and coliforms were reduced by sodium hypochlorite. Ayangbile (1989) found that .2% of NaOCl prevented putrefaction of crab waste for 5 d during which the TMA increased from 3.7 to 12.85 mg N / 100 g. The author reported further that the combination of NaOCl and calcium hypochlorite extended the storage period of crab waste to 7 d and the addition of .4% NaOCl and 1.5% acetic acid retarded the degradation of crab waste for 4 d.

The efficiency of buffered NaOCl solution 'Bionox' in controlling bacterial contamination was evaluated on fresh-cut poultry, fish fillets, fruits and vegetables ( Park et al., 1991). The results showed that the sanitizing solution was effective in reducing *Salmonella enteritidis* on all test foodstuffs, but its effect on fish fillets depended on the background bacterial contamination.

The antibacterial action of chlorine was based on its strong oxidizing effect and involves penetration of cell wall (Busta and Foegeding, 1983). Chlorine reacts with cellular protoplasm, enzyme systems, and cell membranes, causing oxidation or denaturation of proteins, inactivation of enzymes, inhibition of respiration or altered membrane permeability.

The effectiveness of chlorine sprays on *E. coli* 0157:H7 was evaluated by Cutter and Siragusa (1995). Beef tissue was inoculated with *E. coli* and was sprayed with NaOCl solutions of 50, 100, 200, 250, 500 or 800 ppm concentrations, and chilled for 24 h. The results showed that the NaOCl solutions were not effective for reducing *E. coli* on beef.

*Sulfur Dioxide*. Gibson et al. (1986) fed sulfur dioxide treated high moisture barley (35% of DM) to lactating dairy cows and found no significant influence on feed intake, milk production, milk composition or flavor, body weight change, or feed efficiency.

Mathison et al. (1985) successfully preserved barley and a barley oats mixture at moisture levels of 20 to 25 % with about 1% added sulfur dioxide. Sulfur dioxide inhibited the microbial growth in the high moisture barley grain stored in plastic bins of 18 t capacity, but a feeding trial revealed poor feed efficiency in cattle fed the treated grain (Mathison et al., 1988).

The antibacterial action of sulfur dioxide depended on the pH. In water, sulfur dioxide forms $H_2SO_3$, which is effective against molds, yeast and certain

species of bacteria (Leuck, 1980). Molecular sulfur dioxide or undissociated $H_2SO_3$ were found to more inhibitory than sulfite ions, and thus the antibacterial effect was greatest at pH values below 4.0.

*Recovery of Protein From Crab Processing Waste*

Peniston et al. (1969) described the process by which protein could be extracted from shellfish waste. The solid waste would be fed continuously to a series of countercurrent vessels in which protein would be solubilized and extracted with dilute (1 to 2%) NaOH solution at a temperature of approximately 60°C. Residence time in the extraction system would be about 4 h and the flow of waste and alkali would be adjusted to accomplish complete protein removal and to yield an effluent liquor in which excess alkali is completely consumed. The sodium proteinate concentration of 8 to 10% would be attained in the extract. The extract solution could be processed in several ways depending upon the type of product desired. Neutralization of alkali to pH 7.0 with HCl and spray drying is one possibility. This yields a sodium proteinate in the form of a light non-hygroscopic powder which is readily redispersable and soluble in water. This was found to contain 90% protein, 6 to 8% ash, and 1 to 3% moisture. Protein can also be removed from the clarified extract by neutralization to the isoelectric point (about pH 4.5). The precipitate can be collected by filtration or centrifugal separation, washed and spray dried to yield a very pure protein product which may be applicable as a human food supplement.

Johnson and Peniston (1971) reported that protein removal from shellfish waste would be an economical procedure at Kodiak, Alaska. They also reported that both shrimp and crab waste show an initial period of very rapid extraction amounting to 30 to 50% of the total protein, followed by a rather sharp break and a slow extraction period. The rate constants obtained for shrimp waste were found

to be considerably higher than for Dungeness crab waste under the same conditions. The solubility of alkali extracted protein at its isoelectric point would be about .5%. Since there is a minimum alkali combining capacity of about 10% for the extracted protein equivalent to about 14.6% of the protein as salt on neutralization, it is not possible to increase the protein concentration without increasing the salt concentration. It is also reported that the added time of treatment to achieve high protein concentration in the effluent would result in more degradation and more isoelectric solubility. The amino acid contents of both crab waste and shrimp waste proteins were found to be similar and their total essential amino acid contents were comparable to that of casein. The percentages of arginine and isoleucine were higher in shell fish proteins, while casein showed higher values for valine and leucine.

Johnson and Peniston (1982) found that the protein recoverable from shrimp and crab waste by mild alkaline extraction and isoelectric precipitation has an amino acid profile similar to casein except for a lower cystine and methionine. Feeding tests with rats and mink indicated it to be of good nutritional value when supplemented with a small amount of methionine. It was recovered in 85 to 90% yield as a dry light tan powder containing up to 90% protein. The extraction of the protein was done with 1 or 2% NaOH at 60 to 70°C in two or more stages to produce sodium proteinate. The extract was clarified to remove extraneous solids, cooled in a heat exchanger, and treated with dilute hydrochloric or sulfuric acid to reduce the pH to the isoelectric point of about 4.5. The protein precipitated in granular curds, which with proper controls, was recovered by decantation or centrifugal means. The washed protein slurry was dewatered to 15 to 20% solids and dried, using either a roll or spray drier.

The optimum conditions for the recovery of protein involved treatment of the decalcified shell with 3.5% (wt / wt) NaOH solution for 2 h at 65°C with constant stirring with a solid to solvent ratio of 1:10 (No et al., 1989). Then the reaction mixture was filtered and the filtrate centrifuged at 16300 *g* for 15 min to remove extraneous solids. The clarified extract was adjusted with 1 N HCl to pH 4.5 with stirring and was allowed to settle for 3 h at ambient temperature. The protein precipitates were separated by centrifugation at 16300*g* for 15 min, washed with deionized water and dried in a vacuum oven at 50°C for 8 h. The amino acid analysis of the dried protein was done after hydrolyzing .1g of sample with 6N HCl for 24 h at 110°C under vacuum.

Shahidi and Synowiecki (1991) isolated nutrients and value-added products from snow crab and shrimp processing wastes. Protein was separated by KOH treatments of the waste under different experimental conditions. They found that a solid to solvent ratio of 1:20 removed almost all of the proteins at 90°C when a 1.0 or 2.0% (wt / vol) KOH solution was used for shrimp and crab waste, respectively. It was also found that a 2 h extraction period was required for the removal of all the proteins present in the offal.

Protein recovered from shrimp waste was higher in quality than that from crab waste (Shahidi and Synowiecki, 1992). The protein efficiency ratios for extracted proteins from shrimp waste and crab waste were 2.72 to 2.88 and 2.3 to 2.42, respectively.

No and Meyers (1989) used crawfish chitosan as ligand-exchange column material for recovery of amino acids from seafood processing waste water.

Jaswal (1990) produced amino acid hydrolysate from crab waste using 5 N HCl. Amino acid yields from a 12 h hydrolysis varied from about 28 to 31%, and increased marginally to 29 to 32 % with 24 h hydrolysis. Approximately 42 to

44% of the total amino acids were found to be essential amino acids, principally leucine, arginine, valine and threonine. The nonessential amino acid fraction was composed mainly of glutamic acid, aspartic acid and glycine.

Manu-Tawiah and Haard (1987) recovered carotenoprotein from the exoskeleton of snow crab, using trypsin. A 30% slurry of crab shell in .1 M sodium phosphate, pH 7.5 buffer was homogenized for 2 min. The slurry temperature was brought to 50°C, and bovine trypsin (.3%) was added and stirred continuously for 30 min. Then it was filtered through four layers of cheese cloth. The filtrate was cooled to 4°C and the pH was adjusted to 7.0 with HCl, made to 65% saturated with solid ammonium sulfate and was stored overnight at 4°C. The carotenoprotein was recovered as a pellet by centrifugation at 2000 *g* for 15 min, suspended in 10 ml 5 mM sodium phosphate, pH 7.0, and dialyzed for about 18 h against 3 L of the same buffer at 4°C. After dialysis, carotenoprotein was lyophilized. The recovery of carotenoprotein complex from crab shell was lower than that from shrimp waste.

Gates and Parker (1992) mechanically extracted blue crab meat from picking room by-products, which yielded 3.18% white, 10.71% mixed, 6.39% claw, and 2.6% leg meat. Extraction within 1.5 h of picking, or icing prior to mechanical extraction stabilized microbial levels. Pasteurization of meat at 80.3°C and addition of citric acid buffer to pasteurized meat reduced the darkening of meat. Lee et al. (1993) determined the quantitative and qualitative characteristics of mechanically separated minced meat from crab processing by-product. Separation produced 50 to 58% food grade mince from picking table by-products and 49% from undersized claws. Minced meat was included in various proportions in crab cake.

Chung and Cadwaller (1993) compared the volatile components in fresh-picked blue crab meat and processing byproduct by simultaneous steam distillation-solvent extraction / gas chromatography / mass spectrometry. They found that TMA, four alkanes (C15-17, C19), and indole contents were highest in crab meat. Of compounds from both samples, 23 were higher in byproduct and seven were higher in the meat, indicating that crab processing byproduct may be a good source for volatile flavor recovery.

Chen and Mayers (1993) reported that acid ensiling of crawfish waste prior to pigment extraction increased concentration of the extaxanthin oil extract by 40 to 50%, and oil recovery by 10%. A two-fold increase in free amino N, and a 70% reduction in exoskeleton $CaCO_3$ were observed in crawfish silage from acid enzymatic hydrolysis, compared to the control.

There are several reports regarding the preparation of fish protein concentrates and fish protein hydrolysates. Cobb and Hyder (1972) reported that an ideal fish protein concentrate should have the following attributes: (1) should have nutritional properties similar to the original fish muscle; (2) should regain water to the original fish muscle content; (3) should be bacteriologically sterile; (4) should be odorless and tasteless or possess a pleasing taste; (5) should be stable to atmospheric conditions; (6) should have no toxic residues. Sen et al. (1962) studied the rate of hydrolysis of fish flesh with papain and found that the recovery of solids and N was greater at pH 7.0 than at 5.0. The hydrolysate constituted of coagulable protein, proteoses, peptones and sub-peptones including amino acids (Sripathy et al., 1962). Hale (1974) compared the relative activities of 23 commercially-available proteolytic enzyme preparations acting on fish protein substrate, and found that pancreatin, pepsin and papain had the highest activity per unit cost of enzyme. Yanez et al. (1976) found that the enzymatic fish protein

hydrolysate contained about 63% protein which was rich in all essential amino acids, with slight deficit of threonine. Lalasidis et al. (1978) produced low molecular weight enzymatic fish protein hydrolysates from deboned cod filleting offal and found that it had a balanced amino acid composition. Nutritional evaluation of the hydrolysate in N balance experiments in rats showed it to have a high nutritive value.

The rates of proteolytic breakdown of fish-protein concentrate were derived from the increase in solubility during enzyme digestion (Hevia et al., 1976). They compared the activities of three concentrations each of bromelain, pronase, ficin, and activated ficin, and found that pronase, at 50°C had twice the activity of ficin (40°C) or bromelain (50°C), and was as active as cysteine activated ficin (40°C). The soluble fraction was bitter in taste. Nair et al. (1976) used 8 M urea and papain for 24 h at 37°C to render food proteins soluble in the preparation of protein hydrolysate. The urea was removed by use of a highly acidic cation exchanger. The food proteins thus obtained were completely water soluble and partially hydrolyzed.

Spenelli et al. (1977) described the preparation of fish protein derivatives. The derivatives were prepared by reacting the myofibrillar protein with the acetic or succinic anhydride, under slightly alkaline condition. The reacted proteins were then precipitated from solution with HCl and extracted with hot (70°C) azeotropic isopropanol to remove residual lipids. The acylated proteins were then neutralized (pH 7.0) with NaOH to solubilize the derivatives, and were dried.

The use of proteolytic enzymes in liquefying fish and fish waste was reported to be suitable for conversion to fish meal which could be readily dispersed in water (Mackie, 1982). The products thus obtained were found to be of high nutritive value.

Yu et al. (1990) studied the acceptability of crackers containing fish protein hydrolysate. The proteins from the fish *Oreochromis mossambicus* were hydrolyzed using alkalase .61 to produce a spray dried hydrolysate. The hydrolysis was carried out at 50°C, using a ratio of one part water and one part fish mince, and an enzyme:substrate ratio of 1:50 at pH 8.0. Reaction was terminated by heating to 90°C for 20 min. After neutralization, the soluble fraction was obtained by centrifugation, then was spray dried at an inlet temperature of 170°C. Incorporation of 10% of hydrolysate in crackers did not affect the overall acceptability. Valisek et al. (1991a) reported the presence of 3-chloro-1,2 propanediol in protein hydrolysates formed by the interaction of HCl with lipids and $NH_3$. This product was found to react with amino acids in model aqueous solutions to form the corresponding glycerylaminoacids, N-(2,3-dihydroxypropyl derivatives (Valisek, 1991b). These amino acid derivatives were detected in stored soybean hydrolysate, the physiological properties of which were unknown..

Shih (1993) reported that a thermophilic anaerobic digester system developed for the conversion of animal manure to methane, solid residues for feed supplements, and liquid nutrients for aquaculture. The digester was found to destroy pathogens. An enzyme, keratinase, secreted by *Bacillus lichenifirmis* was isolated and was used for fermenting feather meal to feather-lysate, which could be used as a digestible protein source.

*Evaluation of Protein Quality*

*In Vitro Methods.* Sheffner et al. (1956) studied the relationship between the pattern of amino acids released by digestive enzymes and the biological values of food proteins. The pattern of amino acids released *in vitro* by pepsin revealed differences between proteins which were not apparent from their total amino acid content nor from the patterns existing when the pepsin digest was further digested

with trypsin and erepsin. They also described the amino acid index which took into account the physiological availability of amino acids during digestion. The amino acids released by *in vitro* pepsin digestion was used with the amino acid pattern of the remainder of the protein to produce the pepsin-digest-residue amino acid index, which was found to correlate well with the net protein utilization value of a variety of proteins

Akeson and Stahmann (1964) devised a pepsin-pancreatin digest index for a rapid, accurate estimation of protein quality. The index was calculated from the amino acids released by an *in vitro* digestion with pepsin followed by pancreatin. They observed good correlation between the pepsin-pancreatin index and their reported biological values. Better correlation was observed with biological values for growing rats than the essential amino acid index or the chemical score.

Maga et al. (1973) studied the initial rate of proteolysis with trypsin of some commonly used protein sources as an *in vitro* means of measuring gastronomic acceptability and found that sodium caseinate was the most easily digestible protein source and the isolated soybean protein was the least easily digestible. It was also reported that steaming increased the hydrolysis rate.

Saunders et al. (1972) compared the *in vitro* protein digestibility by pepsin, pepsin-pancreatin and pepsin-trypsin to that of *in vivo* digestibility in rats and found that the *in vitro* systems involving pepsin showed a high correlation with protein digestibility values obtained from rat feeding trials.

A multi-enzyme technique was developed for estimating protein digestibility (Hsu et al., 1977) using trypsin, chymotrypsin, and peptidase. Fifty milliliters of aqueous protein suspension (6.25 mg / ml) were adjusted to pH 8.0 with .1N NaOH / HCl in a 37°C water bath. The multi enzyme solution (1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase /ml) was maintained in an ice

bath and the pH was adjusted to 8.0 with .1N NaOH / HCl. Five milliliters of the multi enzyme solution were then added to the protein suspension at 37°C and the pH drop was recorded automatically over a 10 min period using a recording pH meter. The results revealed that the drop in pH of a protein suspension immediately after 10 min digestion was highly correlated with the *in vivo* apparent digestibility in rats. This method was found to be quick, sensitive and could detect the effects of trypsin inhibitor, chlorogenic acid, and heat treatment on protein digestibility.

Alder-Nissen (1979) developed a method for determining the degree of hydrolysis of food protein hydrolysate by using trinitrobenzenesulfonic (TNBS) acid. The method was a spectrophotometric assay of the chromophore formed by the reaction of TNBS with the primary amines. Satterlee et al. (1979) discussed two assays (C-PER and T-PER) that were tested with a wide variety of foods and which took less than 72 h to complete. The data on the *in vitro* protein digestibility and essential amino acid composition of the food protein were used to predict its protein quality as the computed protein efficiency ratio (C-PER). The T-PER assay involved the use of *in vitro* protein digestibility along with the growth of the protozoan *Tetrahymena thermophila* $WH_{14}$ on hydrolyzed food sample. The *in vitro* protein digestibilities of various protein samples were measured using a modification of the multi-enzyme technique described by Hsu et al. (1977). The modifications were (a) at exactly 10 min after the time the enzyme trypsin-chymotrypsin-peptidase was added to the protein sample; (b) immediately the solution was transferred to a 55°C water bath; (c) nine min after adding the bacterial protease solution to the sample, the sample was removed from 55°C and returned to the 37°C water bath; (d) at exactly 10 min after the sample had received the bacterial protease, the pH of the enzyme hydrolysate was recorded; (e)

the pH measured in step 4 was recorded as the 20 min pH; (f) the *in vitro* protein digestibility was calculated using the equation,

Digestibility (%) = 234.84 - 22.56 (X), where X = the pH recorded in step 5.

Pedersen and Eggum (1981) estimated the protein digestibility of 61 foods and feeds by the three-enzyme method by Hsu et al. (1977) and a four-enzyme method by Satterlee et al. (1979). The *in vitro* results were compared with true digestibility obtained by rat assays. For plant proteins and combinations of plant-animal proteins significant correlations were obtained between *in vitro* and *in vivo* methods, while no correlation was found in animal proteins. The buffer capacity of test proteins affected the *in vitro* values. Both methods were found to be sensitive to the presence of tannins.

The suitability of the rapid multi-enzyme assay for *in vitro* digestibility estimation was determined by using a group of native and thermally processed vegetable proteins (Wolzak et al., 1981). The *in vitro* digestibility was assessed by measuring the extent to which the pH of the protein suspension dropped when treated with a multi-enzyme system consisting of trypsin, chymotrypsin and peptidase for 10 min and protease isolated from *Streptococcus griseus* for another 10 min. Best correlation was observed between *in vivo* protein digestibility in rats and the pH of the protein suspension after 15 min of enzymatic treatment. It was also reported that there were differences in the responses of different types of proteins to the multi enzyme assay.

Pedersen and Eggum (1983) developed a pH-stat procedure for the *in vitro* estimation of protein digestibility. They used pepsin (Merck 7192, 100mu / mg), porcine pancreatic trypsin (Type ix, 1450), bovine pancreatic chymotrypsin (Type 11, 47 units / mg), porcine intestinal peptidase (Grade 1, 22 units / g) and protease from *Streptomyces griseus* (4.4 units / mg). Ten milliliters of an aqueous protein

suspension (1 mg N / mg) were equilibrated with .1N NaOH or HCl at pH 8.0 at 37°C. One ml of the three enzyme solution (1.61 mg trypsin, 3.96 mg chymotrypsin, and 2.36 mg peptidase per ml) was added to the protein suspension. The pH was kept constant at 7.98 during the incubation using .1N NaOH. At the end of incubation period of 10 min, the amount of alkali added was recorded to estimate the *in vitro* protein digestibility. Sodium caseinate was used as reference protein. The pH of the three-enzyme solution was adjusted to 8.0 at 37°C for 2 min before adding to the protein suspension. The results showed that the pH-stat technique improved the *in vitro* estimation of protein digestibility when compared with pH-drop method of Hsu et al. (1977) and Satterlee et al. (1979). The estimation was found to be better only when the three-enzyme solution was added. For the 30 proteins tested, a correlation coefficient of .96 was obtained with *in vivo* digestion trials with rats. Predigestion of protein with pepsin was found to be beneficial in test proteins containing protease inhibitors.

Kan and Shipe (1984) determined *in vitro* protein digestibility by pepsin hydrolysis in a diafiltration reactor and observed that it was comparable to the reported *in vivo* values of the 16 samples from both plant and animal sources studied. The *in vitro* method was done by placing .5g protein in a 50 ml ultra filtration stirred cell containing 50 ml of .03 N HCl and 250 mg pepsin, and kept in a 37°C water bath on top of a multi-magnetic stirrer. When diafiltration was started, the system was kept at .85 kg / $cm^2$ pressure with compressed N. A blank was prepared by the same procedure using 250 mg pepsin without substrate. The percent of protein hydrolyzed was calculated using the equation,

$$\textit{in vitro}\ \text{digestibility} = \frac{\text{N retention in diafiltrate - N of blank x 100.}}{\text{N content of the sample}}$$

Jecsai et al. (1984) determined the digestibility of protein using an *in vitro* methylene blue method. They incubated plant protein fodder with trypsin, chymotrypsin and pancreatin for 10 to 15 min and observed similar digestibility values to those obtained by *in vivo* experiments. They also reported that preliminary digestion with pepsin had no effect on the hydrolysis of animal proteins.

Porter et al. (1984) used porcine pepsin, trypsin, α-chymotrypsin and intestinal mucosal peptidases, covalently immobilized on porous glass beads to determine the digestibilities of a number of proteins from plant and animal sources. Immobilized pepsin was used in a separate reactor maintained at low pH, whereas trypsin, chymotrypsin and intestinal peptidase were placed in a single reactor maintained at pH 7.5. The digestibility was determined by using the reaction of orthophthaldialdehyde (OPA) and 2-mercaptoethanol with the alpha amino groups freed by hydrolysis of peptide bonds. It was observed that the digestibilities determined with pepsin alone were lowest for those proteins having high structural stability, whereas the combination of pepsin pretreatment with pancreatic and intestinal enzymes indicated more rapid initial digestion rates by the latter enzymes. The complete system gave digestibility values in agreement with *in vivo* studies.

Threshner et al. (1989) determined the digestibility of protein of various foods by the immobilized digestive enzyme assay (IDEA). The assay consisted of two bioreactors, one containing pepsin and the other containing trypsin, chymotrypsin and intestinal mucosal peptidases. The digestibility values obtained by the assay were found to correlate well with the *in vivo* values. The method was also found to be sensitive to structural modification of protein. Chang et al. (1992) further studied the effects of sucrose, starch and oil on the *in vitro* determination of

protein digestibility using the immobilized digestive enzyme assay system. They observed that varying concentrations of sucrose (0, 5, 10 and 15%), starch (0, .5, and 2%), emulsified fat (1% lecithin), and vegetable oil (0, .5, 1, and 2%) did not affect the digestibility of protein, indicating the suitability of this method for complex foods.

Papadopoulos (1987) studied the relationship between *in vitro* tests and protein-amino acid digestibility of feather meal subjected to different periods of processing by autoclave hydrolysis. He used pepsin digestion, N solubility in .02 N NaOH, and chemical score as the *in vitro* tests and observed a poor correlation between *in vitro* and *in vivo* tests of protein quality.

Bodwell et al. (1989) evaluated the different methods of protein quality and confirmed the usefulness of *in vitro* digestibility procedures. They reported that it was difficult to rank one system as more powerful than the others because of the differences in the relative values for net protein utilization (NPU) and net protein ratio (NPR). The problems associated with the *in vitro* evaluation of heat damaged proteins and some types of beans were also emphasized.

The different *in vitro* digestibility procedures were reviewed and the *in vitro* enzymatic pH-stat procedure of Pedersen and Eggum (1983) was recommended as more accurate for predicting protein digestibility of food and feed proteins (FAO/ WHO, 1989). This method used three enzymes, porcine pancreatic trypsin (Type IX, Sigma 7-0143), bovine pancreatic chymotrypsin (Type11, Sigma C-4129), and porcine intestinal peptidase (Grade K, Sigma P-7520) in solution to give 23100 units, 186 units, and .052 units per milliliter, respectively. The pH was adjusted to 8.0 at 37°C and was maintained for exactly 2 min, then transferred to an ice bath and kept at 0°C. The enzyme solution was made fresh daily and the activity was checked with sodium caseinate (1 mg N / ml distilled water) as the standard. The

suspension was allowed to stand at 4°C for at least 1 h but no longer than 24 h. Then, 10 ml of the sodium caseinate suspension was taken in a reaction vessel, warmed to 37°C, and pH was maintained at 8.0 for 5 to 10 min before adding 1.0 ml of the 3-enzyme solution. While stirring, the amount of .1N NaOH required to maintain the pH at 7.98 for exactly 10 min was recorded and the true digestibility was calculated using the equation, TD = 76.14 + 47.77 B, where B = ml of .1N NaOH added. Values for sodium caseinate were 98 to 102%. Digestibilities of the test proteins were estimated by using samples containing exactly 10 mg N dissolved in 10 ml distilled water. Sodium caseinate was used as a control to give a laboratory correction factor for adjusting the final values as, 100 divided by digestibility of sodium caseinate.

Eggum et al. (1989) determined the protein quality and digestible energy of selected foods by balance trials with rats. They found that the multi enzyme test for *in vitro* true protein digestibility showed good agreement with *in vivo* values with the exception of two legumes. The amino acid digestibility showed similar pattern of the corresponding protein digestibility.

Araba and Dale (1990a) compared urease activity (UA), Orange G-binding capacity (OGBC) and protein solubility (PS) in KOH solution of SBM as indicators of over processing to *in vivo* trial using chicks. The results indicated that the PS values more closely reflected the impaired chick performance from over heated SBM than UA or OGBC. The PS values also were found to be useful in detecting under processed SBM (Araba and Dale (1990b). They reported that a PS value in excess of 85% or less than 70% indicated under and over processing of SBM, respectively, while Parsons et al. (1989) found that the growth performance of chicks and pigs fed the autoclaved SBM was depressed when PS was less than 61%.

Parsons et al. (1991) found that the PS decreased as the particle size of SBM increased. Increasing autoclaving time from 0 to 40 min at 120°C resulted in a quadratic decrease in PS. The feed efficiency of chicks and pigs were found to decrease when the PS was less than 66%.

Kim and Barbeau (1991) evaluated the protein digestibility of unheated and autoclaved soy protein concentrate samples *in vitro* by sodium dodecyl sulfate-polyacrylamide gel electrophpresis (SDS-PAGE) method. The results were compared with those determined by the pH drop method and with apparent *in vivo* digestibility in rats. They found that the pH drop method had a better correlation with apparent *in vivo* digestibility than SDS-PAGE. However, the SDS-PAGE technique allowed estimation of the molecular weight of polypeptides remaining in protein hydrolysate and from densitometric analysis, visualization of the process of protein digestion. Spencer et al. (1988) used SDS-PAGE technique to monitor the fate of dietary protein in ruminal fluid *in vitro* and found that the protein components of different seed meals showed a wide range of resistance to degradation.

Madi (1993) reported the proposed changes in the protein quality measurements as outlined by FDA in the new food labeling regulations. It was reported that the protein digestibility corrected amino acid score (PDCAAS) method determined protein quality based on established human amino acid requirements corrected for digestibility, and that the use of PDCAAS was consistent with a consensus of nutritionists represented worldwide by FAO / WHO/ UNO agreement. Henley and Kuster (1994) described the protein quality evaluation by PDCAAS in which the essential amino acid profile of the food, corrected for digestibility was compared to the FAO / WHO essential amino acid requirement pattern for 2 to 5 yr old children.

*In Vivo Methods.* Numerous methods have been proposed for the determination of protein quality. Of these, the most important are the chemical methods based on the amino acid content of the test protein and the biological assays based mainly on the *in vivo* growth response or the N retained by the experimental animals fed the test protein (Vit et al., 1993).

Protein Efficiency Ratio (PER) is defined as gain in body weight (g) divided by the protein consumed (g) (McDonald et al., 1995). This method can be used to compare specific proteins or protein sources. A N-free otherwise adequate diet is used in which the protein sources to be compared are included for different groups of young animals and records are kept of growth and feed consumption (Maynard et al., 1979). A limitation of measuring protein efficiency in terms of body gain is that the protein content may be variable. Inclusion of slaughter data will help to overcome this limitation.

Net protein ratio is calculated as: (weight gain of the group fed the test protein minus weight loss of group fed protein-free diet) divided by weight of protein consumed (McDonald et al., 1995).

For calculating the Gross Protein Value, the live weight gains of chicks receiving a basal diet containing 8% CP are compared with those receiving the basal diet plus 3% of a test protein, and a group receiving the basal diet plus 3% of casein. The extra live weight gain per unit of supplementary test protein as a proportion of the extra live weight gain per unit of supplementary casein is the GPV of the test protein (McDonald et al., 1995).

The term Biological Value (BV) denotes the percentage of the protein intake which is utilized in the body (Maynard et al., 1979). This procedure measures the efficiency of the absorbed protein in supplying the amino acids needed for the synthesis of body protein and is calculated as follows:

$$BV = \frac{\text{N intake - (fecal N + urinary N ) x 100}}{\text{N intake- fecal N}}$$

This formula measures the BV of protein for growth purposes only. It was then modified to take into account the maintenance requirement also. For this the endogenous losses are subtracted separately from the fecal and urinary excretions and BV can be calculated using the formula,

$$BV = \frac{\text{N intake - (fecal N - metabolic N) - (urinary N - endogenous N) x 100}}{\text{N intake - (fecal N - metabolic N)}}$$

The level of protein fed must be high enough so that marked growth will actually result as indicated by a positive N-balance, but it must not be in excess of the amount needed to cause maximum growth since the excess will be catabolized and excreted and give a lower BV than the true value.

The values for metabolic and endogenous N cannot be determined while the protein is under study, but must be calculated from values obtained in separate periods when the animals are receiving a protein-free diet. The low palatability of the protein-free diets poses problems in determining the endogenous losses which could be overcome by incorporating small amounts of milk or egg proteins which are utilized almost completely.

Net Protein Utilization (NPU) is based on a comparison of the body N content resulting from a test protein with that resulting over the same period on a N-free diet (Maynard et al., 1979). It can be calculated as :

$$NPU = \frac{\text{Body N content with test protein - body N content with N-free diet}}{\text{N intake}}$$

Lyman et al. (1953) evaluated the protein quality of cottonseed meals by chick growth and chemical index method. They used 20 chicks for each treatment. They gave a chick growth index of 100 to butanone-extracted cottonseed meal.

The chemical index was based on the percentage N solubility in dilute NaOH and total gossypol content of the sample.

Featherston and Scholz (1968) observed an increased liver xanthine dehydrogenase activity in chicks fed a high-protein diet (75% isolated soybean protein). They also found a direct relationship between the elevated activity of hepatic xanthine dehydrogenase and uric acid excretion.

Miles and Featherston (1976) used uric acid excretion by the chick as an indicator of protein quality since less N is incorporated into the body protein and more is excreted as uric acid by chicks fed a poor-quality protein. They used day-old male broiler type chicks. Uric acid excretion was determined for the last 24 h of the 14 d experimental period. Excreta was collected in .5% lithium carbonate solution placed in stainless steel pans under each pen. They fed 15% protein diets using either whole egg protein, soybean meal, safflower meal or casein, or the latter three proteins supplemented with their most limiting amino acids. They found good agreement between uric acid excretion and protein efficiency ratio (PER) as indicators of protein quality.

The PER in chicks fed diets containing 11, 20, 43, and 83% CP correlated negatively and curvilinearly with the enzyme adenosylosuccinate activity, and negatively and linearly with the purine nucleoside phosphorylase and xanthine dehydrogenase of hepatic tissue (Hevia and Clifford, 1977a). The uric acid excretion and uric acid concentration in the excreta were very useful methods for measuring dietary protein quality in chicks (Hevia and Clifford, 1977b). They also studied the changes in hepatic purine enzyme activities of chicks fed diets containing 11, 20, 43, and 80% CP (Hevia and Clifford, 1978). Nucleoside phosphorylase, xanthine dehydrogenase, adenylosuccinate synthetase and adenosine kinase activity were positively correlated with protein intake, while the

activities of adenosine deaminase 5'-nucleotidase (AMP), adenylate deaminase and adenine phosphoribosyl transferase were negatively correlated with protein intake and uric acid production. A high correlation between the PER, NPR, or N retained and the activities of the enzymes nucleoside phosphorylase, xanthine dehydrogenase or the concentration of uric acid in the excreta was reported by Millan et al. (1984). Similar results were also obtained when chicks were fed graded L-Lysine diets (Vit et al., 1993) and they suggested that the activities of the hepatic enzymes, nucleoside phosphorylase and xanthine dehydrogenase and uric acid excretion can be used as indicators in protein quality evaluation.

Watkins et al. (1982) evaluated three products derived from shrimp processing waste and a protein concentrate extracted from king crab waste as feed supplements for mink , replacing 10 or 20% protein in a standard wet diet. Crab waste protein contained 67.15 CP, .1% Ca, trace chitin and .5% ether extract. Minks fed crustacean waste had lower final weights and weight gains and greater feed consumption than control group fed the standard diet. It was concluded that crustacean waste product could be a satisfactory protein supplement for minks, provided the protein and energy concentrates of diets are maintained at sufficient levels and dietary Ca does not become excessive.

Groninger and Miller (1979) studied the chemical and nutritional properties of acylated fish protein in rats. The PER of highly (75%) and medium (50%) acetylated myofibrillar protein was 85% and 73%, respectively of that of casein. Feeding of $^{14}$C-labeled acetyllysine and $^{14}$C-labeled acetylated protein to rats showed that both free and protein bound acetyllysine were partially utilized.

Sibbald (1979) conducted an experiment to determine whether the methodology of the bioassay for true ME can be applied to the measurement of available amino acids in feedstuffs. They found that feeding graded levels of

soybean meal, alone or in combination with glucose, caused a linear increase in amino acid excretion. They described a bioassay for the determination of available amino acid which could be combined with the measurement of true ME.

Protein quality of rape seed meal (RSM) was evaluated by both chemical and biological methods (Goh et al., 1980). The rape seed meal studied consisted of six pairs, of which each pair represented the meals with the highest and lowest protein solubilities in .2% KOH. The protein solubilities of the RSM used in the chick growth trial ranged from 33.9 to 72.6% and the total protein efficiency (TPE) was not different among the different RSM samples, concluding that solubility in KOH is not a good measure of protein quality of RSM. The TPE values of RSM varied from 2.57 to 2.71 and were not different from SBM. They also found that the dye-binding capacity of protein (DCCP) with Acid Orange 12 correlated well with the TPE values.

Rua et al. (1985) evaluated the protein quality of a meal consisting of a mixture of byproducts from shark filleting and shrimps, using weanling rats. They were given the meal to supply 3, 6, 9, and 12% of protein in their diets. The meal had a PER of 1.6 and apparent digestibility of 88.8%.

Papadopoulos et al. (1985) studied the effect of different processing conditions on amino acid availability of feather meal by chick assay. They measured the apparent and true digestibility of all individual amino acids and observed higher values for the enzymatically treated samples than that of chemically treated ones.

Landry et al. (1986) evaluated the protein quality of six lepipteran species of larvae in chicks. Larvae contained 49.4 to 58.1% CP and were deficient in arginine, methionine, cysteine. The incorporation of larval meal as the sole source

of protein replacing SBM in diets of chicks did not cause any change in average body weight gains compared to that of chicks fed conventional corn / SBM diet.

Escalona et al. (1986) compared different methods of determining the protein quality of poultry by-product meal. A sample of poultry by-product meal was compared with soybean meal fortified with .5% L-methionine. Each was incorporated at 2.5% increments between 0 and 30%. Diets were fed for 7 d starting when the chicks were 8 d old. Slope-ratio and saturation kinetics models were fitted for the two protein sources. The PER was found to be the most discriminating method of estimating protein quality, especially at lower protein levels.

The effect of single essential amino acid deprivation on chick growth and N and energy balances was determined at *ad libitum* and equalized-food intakes (Kino and Okumura, 1986). The effects were found to be different depending upon the amino acid omitted from the diet, and were associated with voluntary food intake. Among the essential amino acids, lysine and histidine had the least effect and methionine plus cystine had the maximum effect. When the food intake was equalized among the amino acid deficient groups, the reduction in body weight was diminished by about 44% and the reduction in N balance was diminished by about 29%, and the difference in energy utilization disappeared.

Nakagaki et al. (1987) conducted two experiments to evaluate the protein quality of house cricket protein in broiler chicks. In the first experiment they used semi- purified diets to identify the limiting amino acids and the results revealed that the crickets were limiting in arginine, methionine, and tryptophan. In the second experiment when dried cricket meal was incorporated into practical diets of broiler chicks as the major source of protein replacing SBM, no difference in

weight gain was observed from that of the control. Feed-gain ratios improved significantly when diets were supplemented with methionine and arginine.

Boushy et al. (1990) reported the processing and utilization of feathermeal as a feedstuff for poultry. Processing by means of pressure was reported to break down the keratinous material, resulting in a hydrolyzed feather meal with a 70% digestible CP. It was found that addition of synthetic amino acids (methionine and lysine), fish meal or dried whey powder to hydrolyzed feather meal during processing improved the nutritive value of the product. Hydrolyzed feather meal could be added at levels up to 6% of the diets of broilers, 7% for layers, and 5% for turkeys in well balanced diets, without any harmful effects on production or health of the birds.

Hahn et al. (1990) evaluated the protein quality of oat meal and oat bran and found that they were similar in quality to that of dehulled SBM in slope ratio assessment and chick growth method. The oat bran and oat meal were incorporated in the chick diets to supply 3, 6, and 9% CP. A protein free diet was also included for the calculation of NPR.

Han et al. (1991) conducted a series of experiments to assess the nutritive value of raw, Kunitz inhibitor-free, low trypsin-inhibitor soybean (LTS) in comparison with raw conventional soybeans (RCS) and heated dehulled soybean meal (HDS). The protein quality of the soybeans was compared in two trials in which young chicks were fed 9% or 16% CP diets containing one of the soybeans as the sole source of dietary protein. They found that LTS was superior to RCS but was inferior to HDS. In another experiment, when HDS protein was replaced with LTS or RCS protein at 25, 50, 75, or 100% of a 22% CP diet, performance of chicks fed LTS was found to be better than that of chicks fed RCS at all replacement levels. The feed efficiencies of chicks fed 25 or 50% dietary soybean

protein as LTS were not different from that of chicks fed the control diet, indicating that the nutritive value of LTS was greater than that of RCS but somewhat lower than that of HDS.

Anderson-Hafermann et al. (1992) conducted five 10-d chick growth experiments and an amino acid digestibility assay to assess the effect of steam heating on *in vivo* protein quality of raw full fat Kunitz trypsin inhibitor-free soybeans (KFSB) compared with raw, conventional full fat soybeans (CSB). The CSB and KFSB were autoclaved for 0 to 21 min at 121°C. The growth performance of chicks fed autoclaved KFSB or CSB increased and pancreas weight as percentage of body weight decreased as autoclaving time increased, indicating that the raw KSSB must be heated to obtain maximum protein quality for chicks.

The nutritive value of canola meal autoclaved for 0 to 90 min at 121C at 105 kilo pascal (kPa) was evaluated by Anderson-Hafermann et al. (1993). It was found that the growth of chicks fed autoclaved canola meal decreased linearly as autoclaving time increased. The lysine content and the digestibilities of indispensable amino acids also decreased as autoclaving time increased.

The protein quality of insect infested cereal grains was measured biologically using rat growth and N balance studies (Jood et al., 1992, 1993; Jood and Kapoor, 1992). Insect infestation above 25% decreased the protein quality significantly.

Furuse et al. (1992) studied the effect of dietary medium chain triglycerides on protein and energy utilization in growing chicks. Diets containing 10 and 20% fat with either maize oil or glyceryl tricaprylate were used as source of long chain or medium chain triglyceride respectively. Results showed that body weight gain, food intake, protein, fat and energy retention were lower in chicks fed medium

chain triglyceride diet. Improved body weight gain and protein utilization were reported in chicks fed medium chain triglyceride diet under equalized feeding conditions (Mabayo et al. 1993). The effect of different dietary supplemental fat and oils (5%) on tissue fatty acid composition and growth of female broilers was determined by Scaife et al (1994). They found that the feed conversion ratio was poor for diets with added tallow. Lipid concentration in the abdominal pad was higher for the soybean-oil fed chicks. They found a significant correlation between the dietary fatty acid and tissue fatty acid composition for all fatty acids except myristic, arachidic and arachidonic acids.

Morris et al. (1992) studied the effect of dietary protein concentration on the response of chicks to methionine and the results of the experiment revealed that the methionine requirement (g / kg diet) for maximum performance increased as a linear function of dietary CP concentration. They suggested that the methionine concentration in chick diet should not be lower than .025 times the dietary CP concentration. Leclercqe et al. (1993) reported that genetically lean chickens require a higher dietary concentration of S containing amino acids than the fat chickens because of their lower food intake and their greater feather synthesis.

Luo et al. (1992) studied the effect of dietary Cl and Mg on the incidence of tibial dyschondroplasia in chickens fed on Chinese practical diets and found that the high chloride induces a high incidence of tibial chondroplasia which could be ameliorated by Mg supplementation. Dietary Mg improved the deposition of Cu and Zn in the bone tissue.

The effect of extrusion and expelling on the nutritive value of conventional (CSB) and Kunitz trypsin inhibitor free (KFSB) soybeans was studied by Zhang and Parson (1993). They found that the growth performance was greater and

pancreas weights were lower for chicks fed KFSB extruded at 104 or 121°C compared, compared to chicks fed CSB extruded at the same temperature.

Fernandez et al. (1994) studied the effect of over heating on the nutritive value of cottonseed meal for chicks and the protein solubility in .2% KOH. The growth performance of chicks was depressed by feeding CSM autoclaved for 40 to 60 min or more. The digestibilities of amino acids in CSM were reduced by 60 and 120 min of autoclaving.

The digestible lysine and valine in SBM and CSM as measured by the precision-fed cecectomized rooster assay were found to be totally bioavailable for protein synthesis (Fernandez and Parsons, 1996a), while the digestible lysine in heat-damaged SBM was not totally bioavailable for protein synthesis (Fernandez and Parsons, 1996b).

# CHAPTER 3.

## Studies on the Effect of Different Chemicals on the Preservation of Crab Processing Waste at Ambient Temperature.

**ABSTRACT:** Three experiments were conducted to determine the effect of different chemicals on the preservation of crab waste at room temperature. In Experiment 1, two levels of NaOCl and $H_2O_2$ (.2 and .4%) were used and the waste was stored in 20 L plastic buckets for 17 d. The gases, $NH_3$ and $H_2S$, were measured on d 5, 11, and 17. In Experiment 2, NaCl (10%), $NaNO_2$ (1%), NaOCl (.4%), $NaN_3$ (.065%), $KNO_3$ (.1%), tert-butyl hydroperoxide (TBHP) (50 ppm), and $I_2$ (25 ppm) were used. After mixing, the mixtures were kept inside a building at 21°C for 21 d. The $NH_3$ and $H_2S$ were measured on d 6, 13 and 21. In Experiment 3, NaCl (10%), $NaNO_2$ (1%), and $NaN_3$ (.065%) were used as preservatives. Crab waste treated with these chemicals was kept at room temperature for 20, 30, and 40 d. The $NH_3$ and $H_2S$ were measured on those days. Physical characteristics such as color, smell, and mold growth were recorded on the final samples in all the three experiments. The initial and final samples were analyzed for DM, CP, ash, chitin, trimethylamine (TMA) and indole. In the first experiment crab waste treated with NaOCl (.4%) preserved better than the other treatments, with lower ($P < .05$) $NH_3$ and TMA contents. However, there were numerous maggots in all the buckets. In Experiment 2, treatment with NaCl, $NaNO_2$ and $NaN_3$ did not cause any change in the physical parameters studied. The TMA, indole, and $NH_3$ were also lower ($P < .05$) and no $H_2S$ was detected in the material treated with those chemicals. In Experiment 3, treatment with NaCl did not alter the physical characteristics of crab waste. The waste had lower ($P <$

.05) $NH_3$, TMA and indole on d 20, 30 and 40, compared to $NaNO_2$ and $NaN_3$ treatment. Treatment with $NaN_3$ gave higher ($P < .05$) indole, compared to waste treated with $NaNO_2$ on d 20, 30 and 40. Hydrogen sulfide could not be measured in any of the treatments during the experiment. From the overall results it could be concluded that .4% NaOCl preserved crab waste for 17 d while, $NaNO_2$ (1%) and $NaN_3$ (.065%) preserved the waste for at least 21 d. Treatment with 10% NaCl could preserve crab waste for at least 40 d without any change in the physical or chemical parameters studied.

**Key words**: Crab waste, preservation, sodium chloride, sodium nitrite, sodium azide.

## Introduction

Global annual production of crustacean wastes is estimated to be 1.44 million metric tons, DM basis ( Knorr, 1991). The major components of crustacean waste are protein, chitin, and minerals. The wastes present environmental problems because of the odor, high moisture content, and statutes governing disposal of wastes. Ensiling has been shown to be effective in preservation of various types of animal waste (Fontenot et al., 1971). Crab waste was successfully ensiled with wheat straw using glacial acetic acid (16% v / wt) (Samuels et al., 1991). Abazinge et al. (1994) ensiled crab waste and wheat straw satisfactorily with glacial acetic acid (16%), dry molasses (20%) or glacial acetic acid (20%) with or without microbial inoculant. They obtained lower apparent digestibility of DM and CP for the acetic-acid treated silage than the silages containing molasses when fed to sheep. Addition of 10 to 20% dry molasses to

crab waste-wheat straw mixtures prior to ensiling, resulted in silages with substantial amounts of lactic acid (Abazinge et al., 1993).

Ensiling must be done within hours to prevent deterioration if the waste is not preserved. Crab waste was preserved with different chemicals such as 1.5% formaldehyde, for 4 wk (Abazinge et al., 1985), 1.5% propionic / formic acid (1:1), for 14 d (Ayangbile et al., 1987), and 1% sodium nitrite, for 10 d (Ayangbile, 1989). Gilles (1975) found that tert-butyl hydroperoxide (TBHP) at 5 to 50 ppm could be used to prevent low-temperature spoilage of fish. Sodium azide ($NaN_3$), $KNO_3$ and $NaNO_2$, each at 5 mmol / L inhibited TMA production in bacteriological cultures of *Alteromonas* species by 68, 52 and 69 %, respectively (Easter et al., 1982). Three experiments were conducted to determine the effect of different chemicals on preservation of crab processing waste at ambient temperature.

## Materials and Methods

Three experiments were conducted and the general procedures were similar for all experiments. Fresh crab waste, obtained from the crab processing plant (Little River Seafoods, Reedville, Virginia), was ground in a food grinder. The crab waste was mixed with chemicals using a horizontal mixer and was stored in 20 L plastic buckets. The buckets were filled to approximately 75% capacity and were closed with lids. Initial samples were taken from each bucket in double-lined polyethylene bags and were frozen immediately for analysis. There were six replicates for each treatment. Two polyvinyl chloride (PVC) pipes 1 m in length and 2 cm internal diameter were introduced into each bucket through holes made through the lids of the buckets for measuring the gases produced in the crab waste

during preservation. Several holes were made on the bottom half of the PVC pipes so that the gases produced in the buckets could get into the pipe for measurement. The pipes were closed with rubber stoppers 24 h before gas measurement. The gases, $NH_3$ and $H_2S$, were measured using a Gastek analyzer (Precision Gas Detector System, Industrial Products Co, Langhorne, PA.). The buckets were kept inside a building maintained at 21°C. After opening the buckets, physical characteristics such as color, smell and appearance were recorded. The smell of the material was evaluated by giving scores from 1 to 4. Number 1 was given if there was no change in smell from that of the original material and No. 4 when it was highly objectionable. The contents of each bucket were emptied on a stainless steel pan, mixed thoroughly and samples were placed in double lined polyethylene bags for analysis.

Both initial and final samples were analyzed for DM, CP, ash and indole by AOAC (1990) procedure. Trimethylamine was analyzed by the colorimetric method of Dyer (1959) and chitin was determined by the Van Soest acid detergent fiber (ADF) method (Van Soest, 1963; Ayangbile, 1989). Wet samples were used for the determination of CP, TMA and indole. Crab waste samples were dried and ground in a Wiley mill to pass a 1 mm screen for ash and chitin analyses.

*Experiment 1*

Crab waste was mixed with NaOCl and $H_2O_2$ at .2 and .4% of the total mixtures (w/w). A negative control was also included without any additives. Materials were kept for 17 d and the gases were measured on d 5, 11 and 17.

The data were analyzed by the General Linear Model procedure of SAS (1989). The mean of the control was compared with the treated means. The model included comparisons of treatments (NaOCl and $H_2O_2$), levels (.2% vs: .4%) and the interaction.

*Experiment 2*

Crab waste was mixed with the following chemicals: NaCl, 10%; $NaNO_2$, 1%; NaOCl, .4%; $NaN_3$, .065%; $KNO_3$, .1%; TBHP, 50 ppm; and $I_2$, 25 ppm. A negative control group was also included without any additives. Commercial "bleach" containing 5.25% NaOCl was used as a source of NaOCl. Chemicals added in small amounts ($NaN_3$, $KNO_3$, TBHP, and $I_2$) were diluted to 1 L with deionized water before mixing with the crab waste.

Buckets were placed inside a building for 21 d and were covered with a thin transparent net to keep the flies from the buckets. Ammonia and $H_2S$ production were measured on d 6, 13, and 21.

The data were analyzed by the Analysis of Variance by the General Linear Model (GLM) Procedure of SAS (1989). The treatment means were compared by Tukeys HSD method.

*Experiment 3.*

Fresh crab waste, after grinding through a food grinder, was mixed with 10% NaCl, 1% $NaNO_2$, or .065% of $NaN_3$. Forty kilograms of crab waste were mixed with the chemical in a horizontal mixer in each batch and six batches were made for each treatment. After mixing, the mixtures for each treatment were stored in 18 plastic buckets of 20L capacity. The buckets were covered using a thin net to protect against the flies.

The $NH_3$ and $H_2S$ were measured from six buckets randomly selected from each treatment on d 20, 30, and 40, the buckets were opened, the physical characteristics such as color, smell, and fungal growth were evaluated and samples were taken for chemical analysis.

Statistical analysis of the data was done by the Analysis of Variance of General Linear Model Procedure of SAS, 1989. The means were compared using Tukey's HSD method.

## Results

*Experiment1*

The lids of the buckets were bulged starting on the second day and there was fluid accumulation on the lids. On d 4, maggots were found on the lids of all the buckets.

*Physical Characteristics.* Upon opening the buckets, the color of the shells was similar to the original color of the shells, but the remainder of the crab waste was light gray. The smell was slightly objectionable. Numerous maggots were found in all buckets up to a depth of approximately of 10 cm except for the .4% NaOCl treated materials where maggots were few and were only up to a depth of 4 to 6 cm.

*Chemical Composition.* The chemical composition of the initial and final samples are given in Table 1. The DM of the control samples was higher ($P < .05$) than for the treated samples for both the initial and final samples. The DM of initial samples treated with NaOCl was lower ($P < .05$) than that of the $H_2O_2$ treated materials. The CP, total ash, true protein, and NPN contents were not different ($P > .05$) among the treatments, in the initial and final crab waste samples.

*Ammonia and Hydrogen Sulfide.* The data for the $NH_3$ and $H_2S$ are presented in Table 2. On d 5, the $NH_3$ in the control samples (4.0 ppm) was higher

**Table 1. Chemical composition of initial and final crab waste samples (Exp. 1)**

| Treatment | Dry matter[ab] | Crude protein[c] | True protein[c] | Non protein nitrogen[c] | Ash[c] |
|---|---|---|---|---|---|
| | % | | | | |
| | | | Initial | | |
| Control | 35.8 | 43.64 | 33.31 | 1.55 | 37.97 |
| .2% NaOCl | 33.75 | 43.78 | 33.53 | 1.53 | 38.15 |
| .4% NaOCl | 33.85 | 43.7 | 32.99 | 1.63 | 37.73 |
| .2% $H_2O_2$ | 35.65 | 44.19 | 33.27 | 1.75 | 37.99 |
| .4% $H_2O_2$ | 34.37 | 43.09 | 33.46 | 1.54 | 37.31 |
| SEM | .31 | .55 | .66 | .12 | .44 |
| | | | Final | | |
| Control | 34.07 | 48.11 | 28.97 | 3.06 | 40.01 |
| .2% NaOCl | 33.38 | 46.07 | 28.21 | 2.86 | 39.29 |
| .4% NaOCl | 32.86 | 47.02 | 31.03 | 2.54 | 39.28 |
| .2% $H_2O_2$ | 34.57 | 46.9 | 31.65 | 2.44 | 39.17 |
| .4% $H_2O_2$ | 31.68 | 47.34 | 30.1 | 2.93 | 39.19 |
| SEM | .22 | .38 | .73 | .10 | .53 |

[a] Control differs from treated ($P < .05$)

[b] NaOCl differs from $H_2O_2$ ($P < .05$) treated materials for the initial samples.

[c] DM basis

**Table 2. Ammonia and hydrogen sulfide in treated crab waste samples after 5, 11 and 17 d (Exp. 1)**

| Item | Day | Treatment | | | | | SEM |
|---|---|---|---|---|---|---|---|
| | | Control | .2% NaOCl | .4% NaOCl | .2% $H_2O_2$ | .4% $H_2O_2$ | |
| | | ---------- ppm ---------- | | | | | |
| Ammonia | 5[a] | 4.00 | 1.75 | 2.00 | 2.67 | 2.83 | .78 |
| | 11 | 26.17 | 25.17 | 19.83 | 26.92 | 55.17 | 13.89 |
| | 17[b] | 17.08 | 38.58 | 10.58 | 79.83 | 158.17 | 45.3 |
| Hydrogen sulfide | 5[ac] | 576.67 | 333.33 | 3.38 | 354.17 | 87.83 | 70.07 |
| | 11[ac] | 1983.33 | 1258.3 | 203 | 725 | 581.67 | 243.8 |
| | 17[a] | 388.33 | 2450 | 1183 | 883.33 | 1253.3 | 462.1 |

[a] Untreated differs from treated ($P < .07$)
[b] NaOCl differs from $H_2O_2$ ($P < .05$)
[c] .2 differs from .4% ($P < .05$)

($P < .07$) than that of the treated samples (2.31 ppm). On d 17, NaOCl treated materials showed lower ($P < .05$) $NH_3$ than those of $H_2O_2$ treated waste. The $H_2S$ of the control was higher ($P < .05$) on d 5 and 11, and lower ($P < .06$) on d 17 than those of the treated materials. The waste treated at .2% levels of NaOCl and $H_2O_2$ had higher ($P < .05$) $H_2S$ than those treated at .4% levels on d 5 and 11, but the difference was not significant ($P > .05$) on d 17.

*Trimethylamie and Indole Concentration.* The TMA and indole contents of the initial samples were 12.15 and .01 mg /100 g, respectively. The TMA and indole concentrations of the final untreated samples were greater than that of the initial samples (Figures 1 and 2). The TMA and indole values were lower for the control than the treated crab waste samples. The TMA values were lower ($P < .05$) for the NaOCl treated materials than those of $H_2O_2$ treated waste. Numerically, the values for .2 and .4% NaOCl were similar to the control values. The indole value for the .4% $H_2O_2$ treated material was higher numerically than for the other treatments.

*Experiment 2*

*Physical Characteristics.* There was dark discoloration of the control and $KNO_3$ and $I_2$ treated materials and slight discoloration for materials treated with TBHP (Table 3). The waste treated with NaCl, $NaNO_2$, NaOCl and $NaN_3$ did not show any change in color. Treatment with NaCl, $NaNO_2$, and $NaN_3$ did not cause any change in the smell of the product but the smell of waste treated with other chemicals was similar to that of untreated waste. There was no mold growth in any of the materials except on top of the NaOCl treated material.

*Chemical Composition.* The chemical composition of the initial and final crab waste samples are given in Table 4. The DM content of the crab waste samples varied depending upon the type and quantity of chemicals used. Material

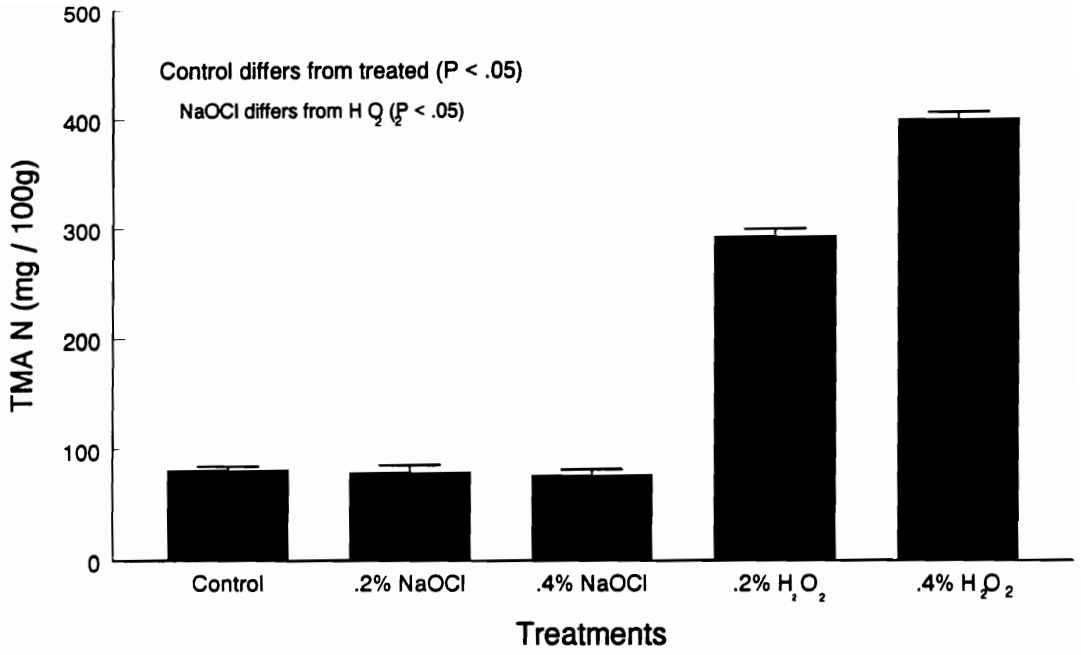


**Figure 1. Trimethylamine (TMA) N content of treated crab waste samples after 17 d (Exp. 1).**

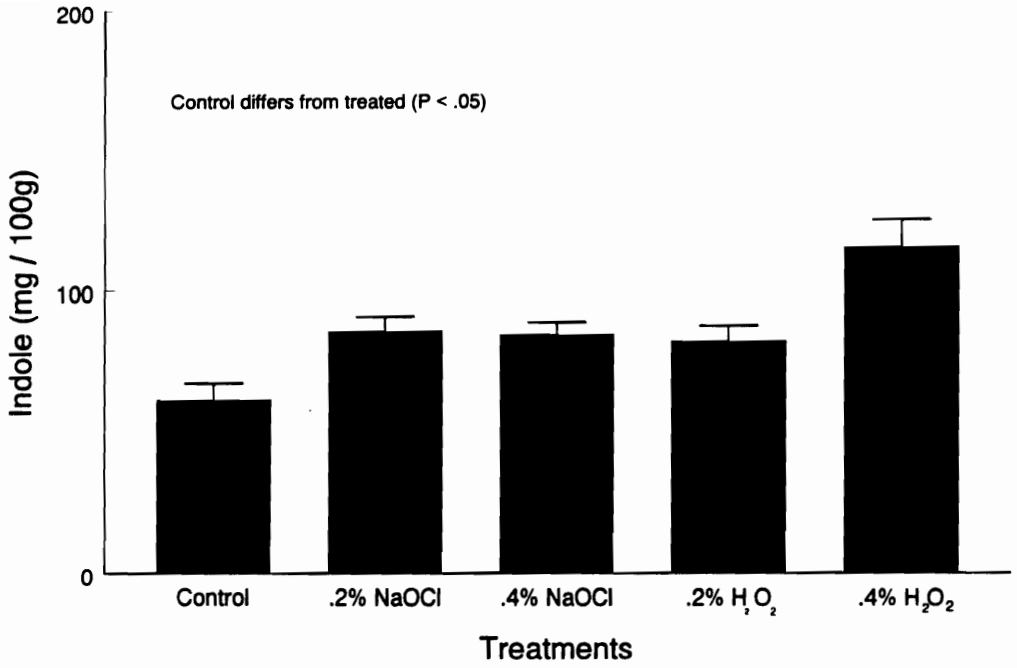


**Figure 2. Indole content of treated crab waste samples after 17 d (Exp. 1).**

**Table 3. Physical characteristics of the final crab waste samples (Exp. 2)**

| Treatment | Color | Smell[a] | Mold growth |
|---|---|---|---|
| Control | Dark discoloration | 3 | No mold |
| NaCl | No discoloration | 1 | No mold |
| $NaNO_2$ | No discoloration | 1 | No mold |
| NaOCl | No discoloration | 2 | Mold on top |
| $NaN_3$ | No discoloration | 1 | No mold |
| $KNO_3$ | Dark discoloration | 3 | No mold |
| TBHP[b] | Slight discoloration | 3 | No mold |
| Iodine | Dark discoloration | 3.5 | No mold |

[a] Code:
1 - No change
2 - Slightly objectionable
3 - Objectionable
4 - Highly objectionable

[b] Tert-butyl hydroperoxide

**Table 4. Chemical composition of initial and final crab waste samples (Exp. 2)**

| Item | Dry matter | Crude protein[a] | Ash[a] | Chitin[a] |
|---|---|---|---|---|
| | % | | | |
| | Initial | | | |
| Control | $45.52^{bc}$ | $40.78^{b}$ | $45.56^{b}$ | 12.56 |
| NaCl | $51.55^{d}$ | $30.45^{c}$ | $54.88^{c}$ | 11.56 |
| $NaNO_2$ | $46.01^{bc}$ | $37.97^{b}$ | $45.29^{b}$ | 12.51 |
| NaOCl | $43.47^{e}$ | $38.47^{b}$ | $45.29^{b}$ | 12.51 |
| $NaN_3$ | $46.58^{b}$ | $37.82^{b}$ | $46.61^{bc}$ | 12.96 |
| $KNO_3$ | $46.26^{b}$ | $38.13^{b}$ | $43.18^{b}$ | 13.36 |
| TBHP[g] | $45.28^{bc}$ | $41.48^{b}$ | $41.4^{b}$ | 14.36 |
| Iodine | $44.87^{c}$ | $39.73^{b}$ | $40.08^{b}$ | 13.5 |
| SEM | .31 | 1.07 | .71 | 1.84 |
| | Final | | | |
| Control | $43.78^{bcd}$ | $40.78^{b}$ | $48.6^{bc}$ | $13.13^{bc}$ |
| NaCl | $51.02^{e}$ | $30.45^{c}$ | $55.57^{b}$ | $10.98^{c}$ |
| $NaNO_2$ | $45.12^{bf}$ | $37.97^{b}$ | $45.85^{c}$ | $12.87^{bc}$ |
| NaOCl | $41.96^{d}$ | $38.47^{b}$ | $45.9^{c}$ | $12.74^{bc}$ |
| $NaN_3$ | $46.37^{f}$ | $37.82^{b}$ | $41.66^{c}$ | $12.72^{bc}$ |
| $KNO_3$ | $44.59^{bcf}$ | $38.13^{b}$ | $46.73^{c}$ | $13.58^{b}$ |
| TBHP[g] | $43.22^{cd}$ | $41.48^{b}$ | $44.67^{c}$ | $12.84^{bc}$ |
| Iodine | $43.31^{bcd}$ | $39.73^{b}$ | $47.76^{c}$ | $13.82^{b}$ |
| SEM | .41 | 1.07 | .556 | 1.72 |

[a] DM basis

[bcdef] Means within columns within initial and final with different superscripts differ ($P < .05$).

[g] Tert-butyl hydroperoxide

treated with NaCl had highest ($P < .05$) DM (51.6% and 51.02%) and the NaOCl treated waste numerically had the lowest (43.47% and 41.96%) DM for the initial and final samples, but the difference was not always significant.

Within initial and final samples, the CP contents of the untreated and the treated wastes were similar, except for the lower ($P < .05$) values for the NaCl treated waste. The ash content of crab waste was similar ($P > .05$) in all treatments except that addition of NaCl increased the ash content, and the difference was usually significant ($P < .05$). The chitin content of the initial crab waste did not differ ($P > .05$) among treatments. For the final samples, NaCl treatment resulted in lower ($P < .05$) values than the $KNO_3$ and $I_2$ treatments.

*Ammonia and Hydrogen Sulfide.* Mean $NH_3$ in the crab waste samples measured on d 6, 13 and 21 are shown in Figures 3, 4 and 5, respectively. On d 6, the $NH_3$ concentration was lower ($P < .05$) in all the treated wastes compared to the control. Wastes treated with $NaNO_2$ did not show any $NH_3$ and the level was not different ($P > .05$) from those treated with NaOCl, $NaN_3$, TBHP and $I_2$. The waste treated with $KNO_3$ had higher levels ($P < .05$) of $NH_3$ than the $NaNO_2$ treated waste. Treatment with NaCl showed lower $NH_3$ than that of control, but the difference was not significant.

On d 13, no $NH_3$ was detected for wastes treated with NaOCl and $NaNO_2$ (Figure 4). Low levels of $NH_3$ were measured in NaCl and $NaN_3$ treated materials. There was no difference ($P > .05$) in the $NH_3$ levels among the different treatments and were below 1.5 ppm in all the treatments. On d 21, the untreated waste had higher ($P < .05$) $NH_3$ than NaCl, $NaNO_2$, NaOCl and $NaN_3$ treated materials (Figure 5). The average value for the control was approximately double those of $KNO_3$, TBHP and $I_2$ treated material, but the difference was not significant due to large variation of individual values.

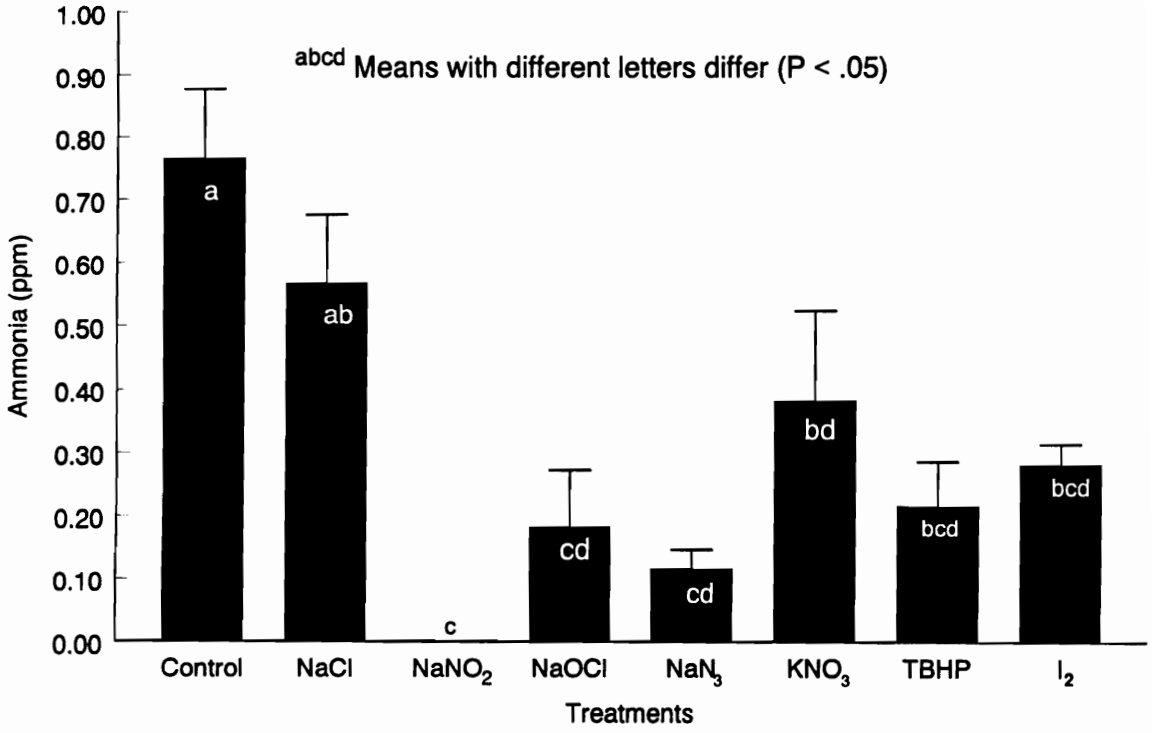


**Figure 3. Ammonia in treated crab waste samples after 6 d (Exp. 2).**

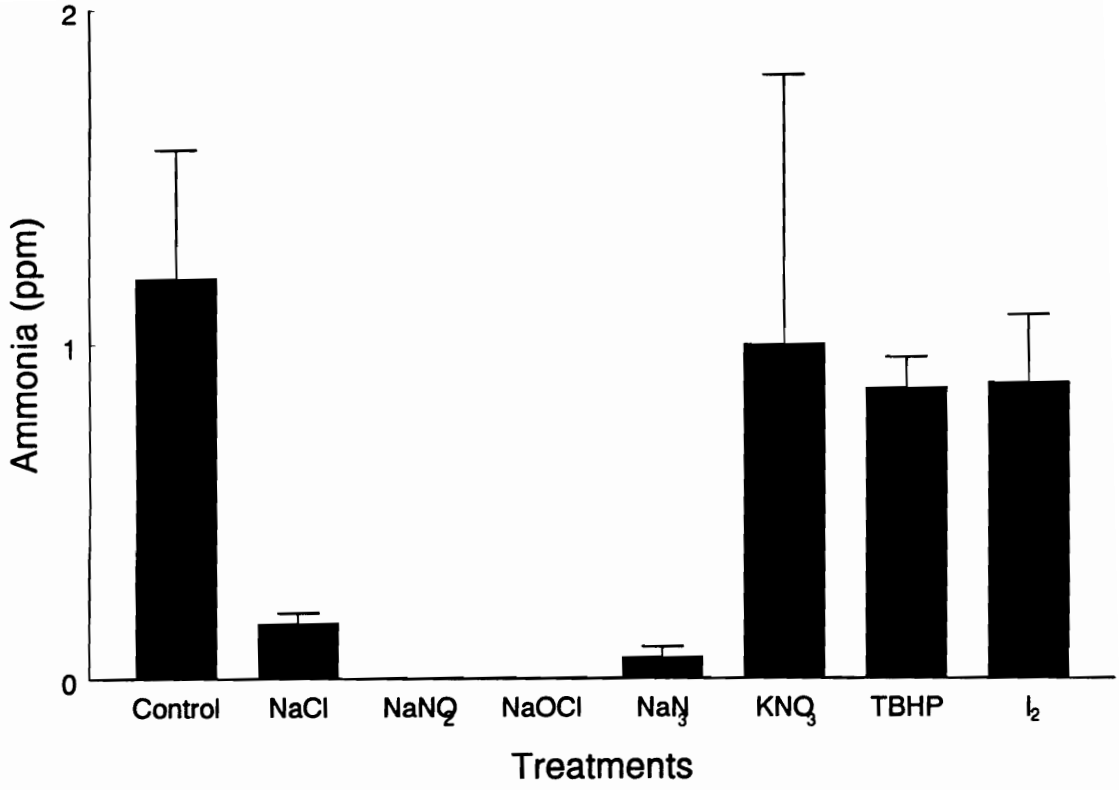


**Figure 4. Ammonia in treated crab waste samples after 13 d (Exp. 2).**

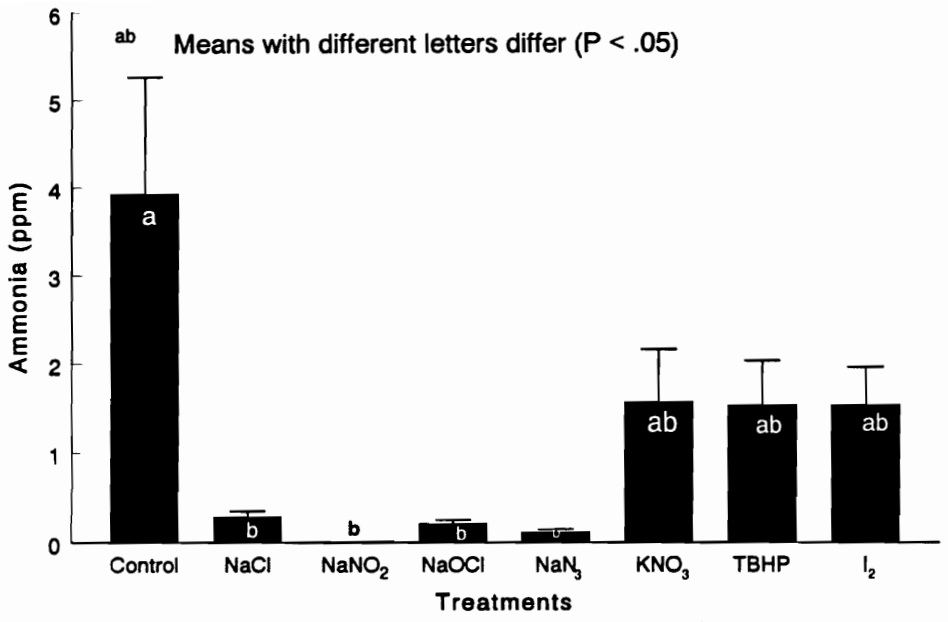


**Figure 5. Ammonia in treated crab waste samples after 21 d (Exp. 2).**

Hydrogen sulfide was not detected on d 6, 13, and 21 in material treated with NaCl, $NaNO_2$, NaOCl and $NaN_3$ (Figures 6, 7 and 8). On d 6, among the treated wastes, the waste treated with $I_2$ gave the highest ($P < .05$) $H_2S$. On d 13 and 21, treatment with $I_2$ and TBHP showed higher ($P < .05$) $H_2S$ than those treated with NaCl, $NaNO_2$, NaOCl and $NaN_3$. The waste treated with $KNO_3$ gave similar $H_2S$ values to that of the untreated on d 13. On day 21, higher ($P < .05$) $H_2S$ values were recorded for waste treated with TBHP and $I_2$ (Figure 8).

*Trimethylamine Content.* There was no difference ($P > .05$) in TMA content of the initial crab waste samples among treatments. The average TMA was 3.98 mg N / 100g. The TMA content of the final samples is presented as Figure 9. The materials treated with NaCl, $NaNO_2$, NaOCl and $NaN_3$ did not show large increase in TMA after 21 d. The levels (5.23, 7.74, 12.42 and 6.57 mg / 100 g) were lower ($P < .05$) than in the untreated waste or waste treated with other chemicals. The values for the $KNO_3$ treated waste were lower ($P < .05$) than for the control, TBHP and $I_2$ treated materials.

*Indole Content.* The indole content of the initial crab waste samples (.11 mg / 100g) was not different ($P > .05$) among treatments (Figure 10). Values were higher ($P < .05$) for the control and the material treated with $KNO_3$, TBHP, and $I_2$ than those of the other treatments of the final samples. Differences were up to eight-fold. There was no difference ($P > .05$) in indole contents among materials treated with NaCl, $NaNO_2$, NaOCl and $NaN_3$.

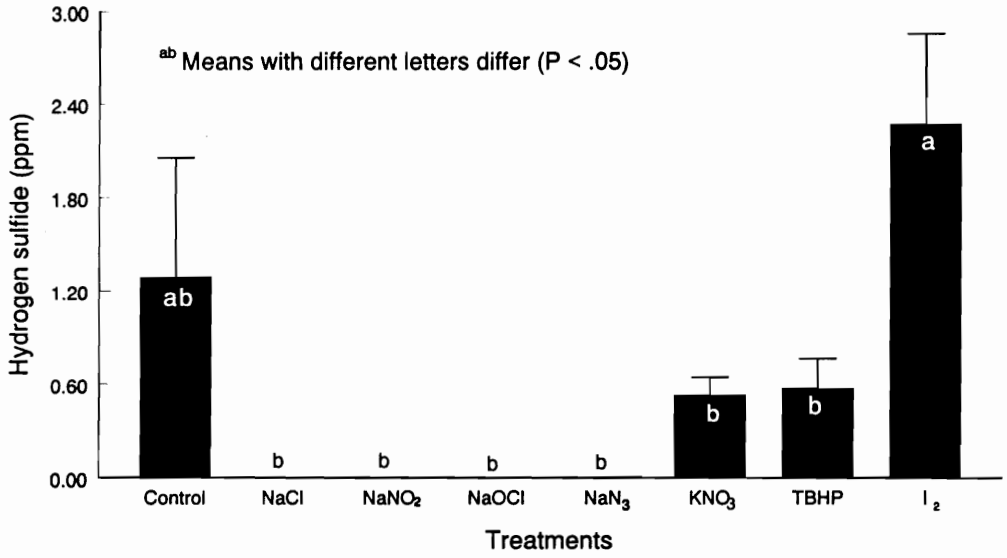


**Figure 6. Hydrogen sulfide in treated crab waste samples after 6 d (Exp. 2).**

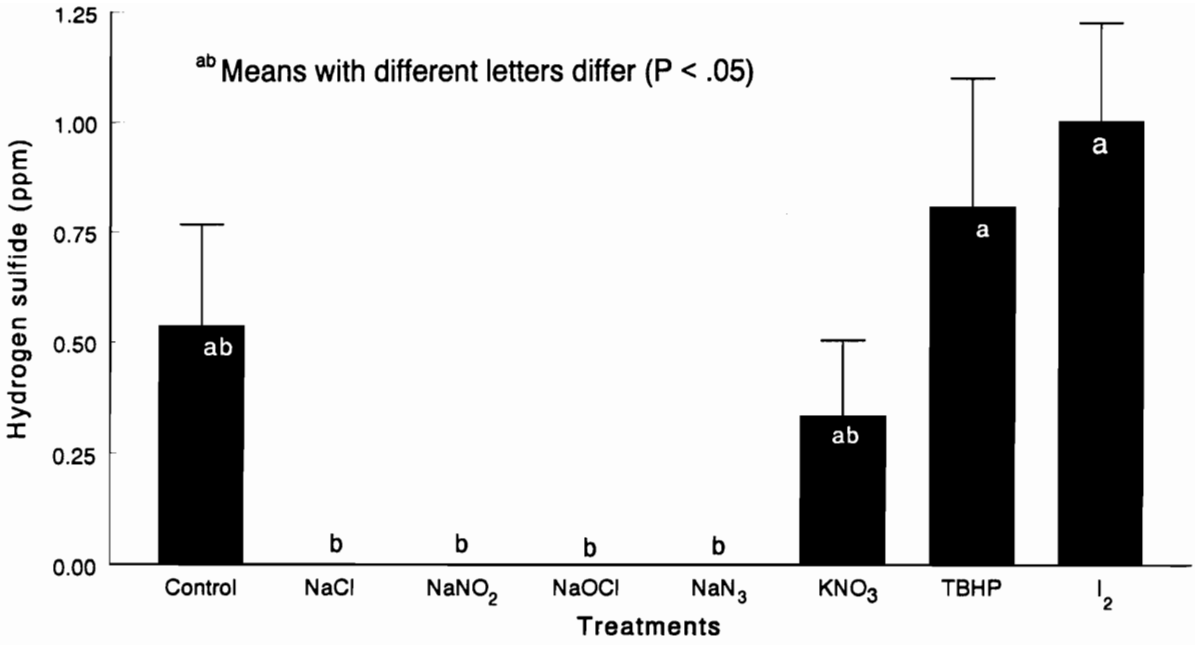


**Figure 7. Hydrogen sulfide in treated crab waste samples after 13 d (Exp. 2).**

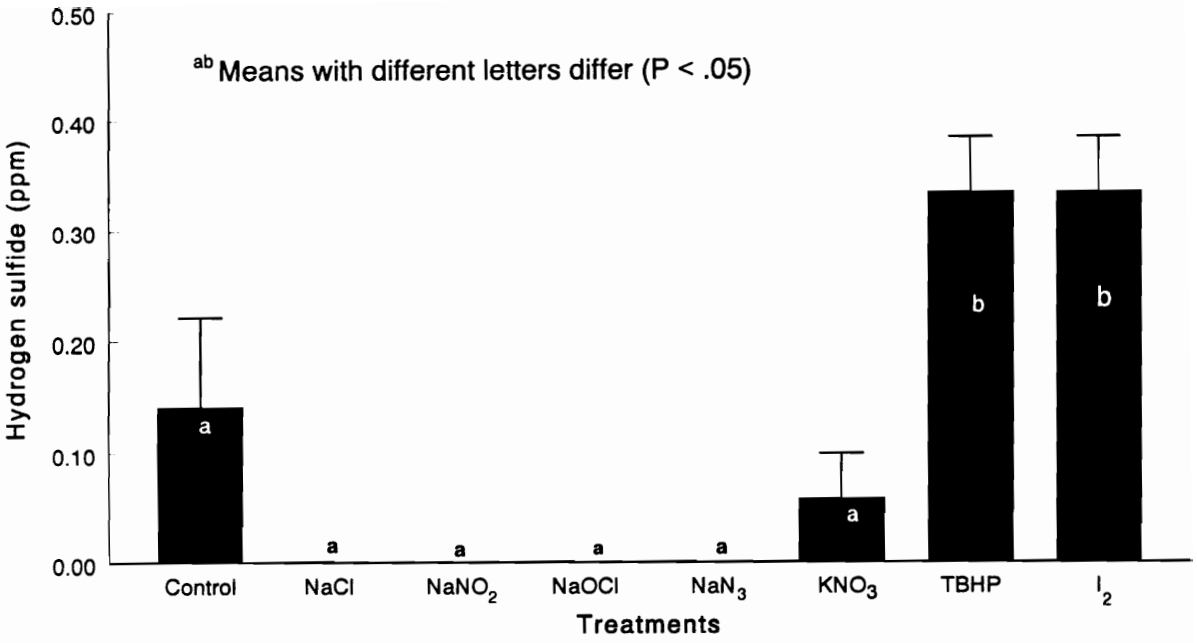


**Figure 8. Hydrogen sulfide in treated crab waste samples after 21 d (Exp. 2)**

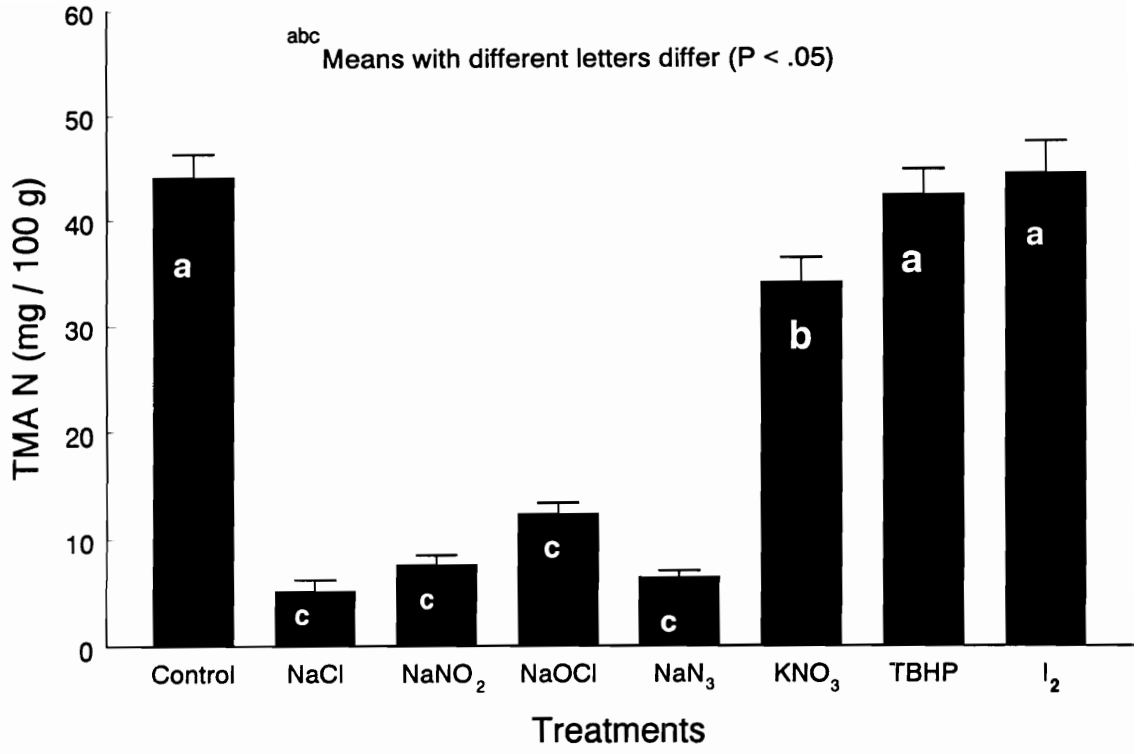


**Figure 9. Trimethylamine (TMA) N content of treated crab waste samples after 21 d (Exp. 2).**

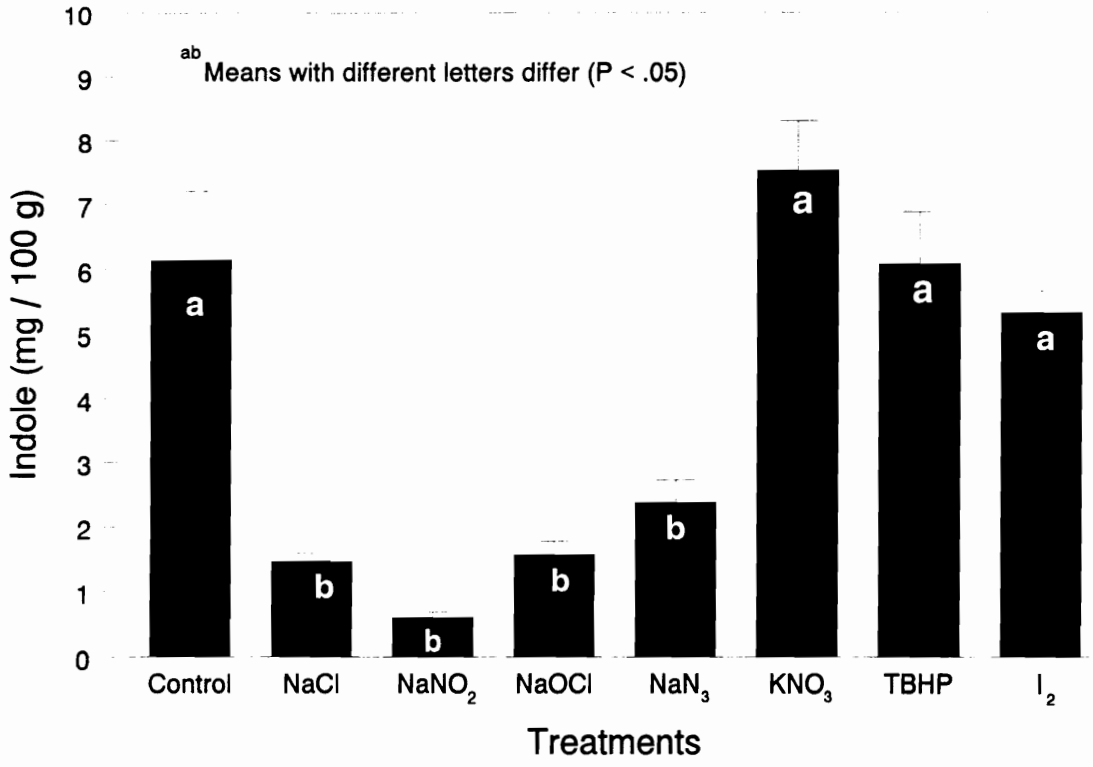


**Figure 10. Indole content of treated crab waste samples after 21 d (Exp. 2).**

*Experiment 3.*

*Physical Characteristics.* The physical characteristics of crab waste preserved for 20, 30 and 40 d are given in Table 5. There was no change in the color or smell of the material treated with NaCl after 40 d of preservation. The $NaNO_2$ treated material showed spotted darkening on the surface of crab waste starting with d 30. On d 40, a greenish and pinkish discoloration was seen. On d 40, there was a dark discoloration at the bottom 5 cm for the $NaN_3$ treated waste. A slight $NH_3$ smell was detected in the $NaNO_2$ treated waste after 20 d. There was no mold growth on the materials treated with NaCl and $NaN_3$ even after 40 d, but white spotted fungi were noted on the surface of crab waste treated with $NaNO_2$, starting with d 20.

*Chemical Composition.* The chemical composition of crab waste preserved for 0, 20, 30, and 40 d are presented in Table 6. Initially, the DM of $NaN_3$ treated material was lower ($P < .05$), compared to the NaCl and $NaNO_2$ treated material. Initially and throughout the preservation period, DM was higher ($P < .05$) for the NaCl treated material. The NaCl treated material had higher ($P < .05$) ash and lower ($P < .05$) CP than the other treatments. There was a trend for chitin to be lower for the NaCl treated waste.

*Ammonia and hydrogen sulfide.* The data on the $NH_3$ measured on d 20, 30 and 40 are shown as Figure 11. There was no difference ($P > .05$) among treatments on d 20 and 30. On d 40, $NaNO_2$ treated material showed the highest ($P < .05$) $NH_3$ compared to the NaCl and the $NaNO_2$ treated samples. Hydrogen sulfide was not detected in any of the treatment groups even after 40 d of preservation.

*Trimethylamine and Indole.* The TMA contents of crab waste preserved for 0, 20, 30, and 40 d are presented in Figure 12. The initial samples contained on an

**Table 5. Physical characteristics of crab waste preserved for 20, 30 and 40 days (Exp. 3)**

| Days | Treatment | Color | Smell[a] | Mold growth |
|---|---|---|---|---|
| 20 | NaCl | No change | 1 | No mold |
| 20 | $NaNO_2$ | No change | 2 ($NH_3$) | Spotted white mold on top |
| 20 | $NaN_3$ | No change | 1 | No mold |
| 30 | NaCl | No change | 1 | No mold |
| 30 | $NaNO_2$ | Spotted darkening | 2 ($NH_3$) | White spots of mold on top |
| 30 | $NaN_3$ | No change | 1 | No mold |
| 40 | NaCl | No change | 1 | No mold |
| 40 | $NaNO_2$ | Greenish and pinkish discoloration on top | 2 ($NH_3$) | White mold on top |
| 40 | $NaN_3$ | No change on top but dark discoloration at the bottom for 5 cm. | 1 | No mold |

[a] Code:
1 = No change
2 = Slightly objectionable

**Table 6. Chemical composition of crab waste samples preserved for 0, 20, 30 and 40 days (Exp. 3)**

| Days of the experiment | Treatment | Dry matter | Crude protein[a] | Ash[a] | Chitin[a] |
|---|---|---|---|---|---|
| | | % | | | |
| 0 | NaCl | 44.62[b] | 31.72[b] | 48.73[b] | 9.55[b] |
| 0 | $NaNO_2$ | 39.42[c] | 40.4[c] | 39.93[c] | 10.3[bc] |
| 0 | $NaN_3$ | 38.51[d] | 40.85[c] | 37.74[c] | 10.96[c] |
| 0 | SEM | .23 | .57 | .92 | .34 |
| 20 | NaCl | 44.85[b] | 31.63[b] | 50.85[b] | 8.89[b] |
| 20 | $NaNO_2$ | 39.29[c] | 42.41[c] | 40.08[c] | 11.25[c] |
| 20 | $NaN_3$ | 38.55[c] | 42.47[c] | 41.02[c] | 10.99[c] |
| 20 | SEM | .28 | .47 | .87 | .40 |
| 30 | NaCl | 45.09[b] | 34.6[b] | 49.91[b] | 9.18[b] |
| 30 | $NaNO_2$ | 38.6[c] | 44.06[c] | 40.19[c] | 10.73[c] |
| 30 | $NaN_3$ | 38.85[c] | 42.77[c] | 40.3[c] | 12.22[d] |
| 30 | SEM | .17 | .59 | .78 | .18 |
| 40 | NaCl | 44.24[b] | 35.03[b] | 49.69[b] | 10.02[b] |
| 40 | $NaNO_2$ | 37.76[c] | 44.95[c] | 40.15[c] | 11.63[c] |
| 40 | $NaN_3$ | 37.48[c] | 44.39[c] | 42.12[c] | 10.53[bc] |
| 40 | SEM | .26 | .37 | .97 | .37 |

[a] DM basis

[bcd] Means within columns with different superscript letters differ ($P < .05$).

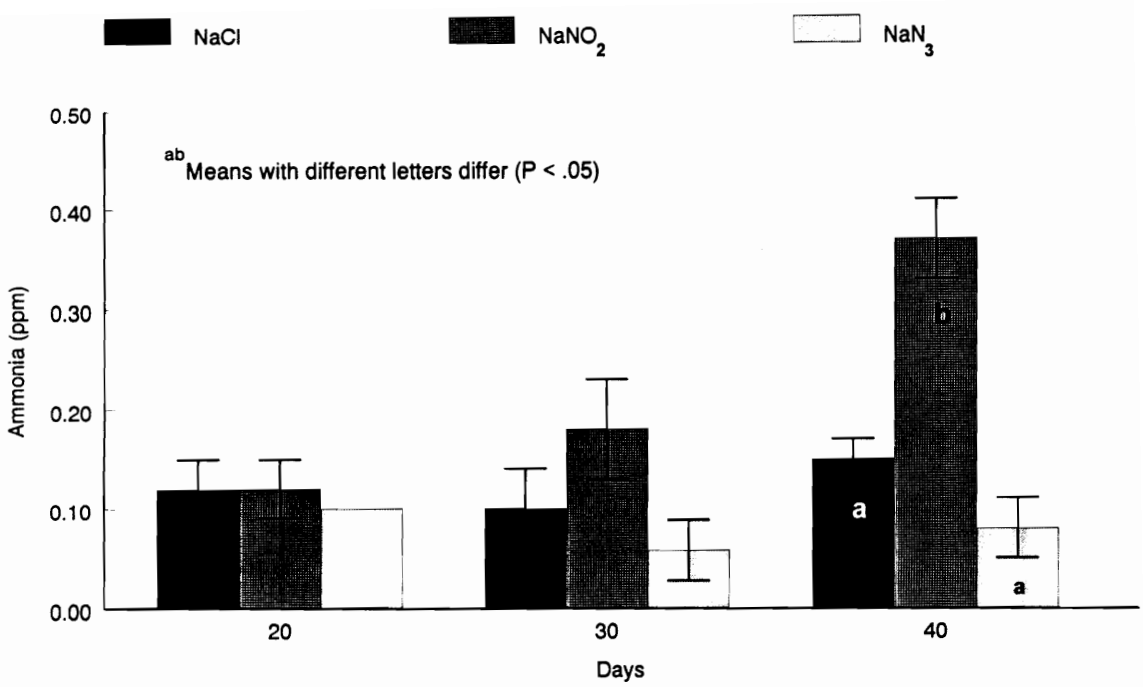


**Figure 11. Ammonia in treated crab waste samples after 20, 30 and 40 d (Exp. 3).**

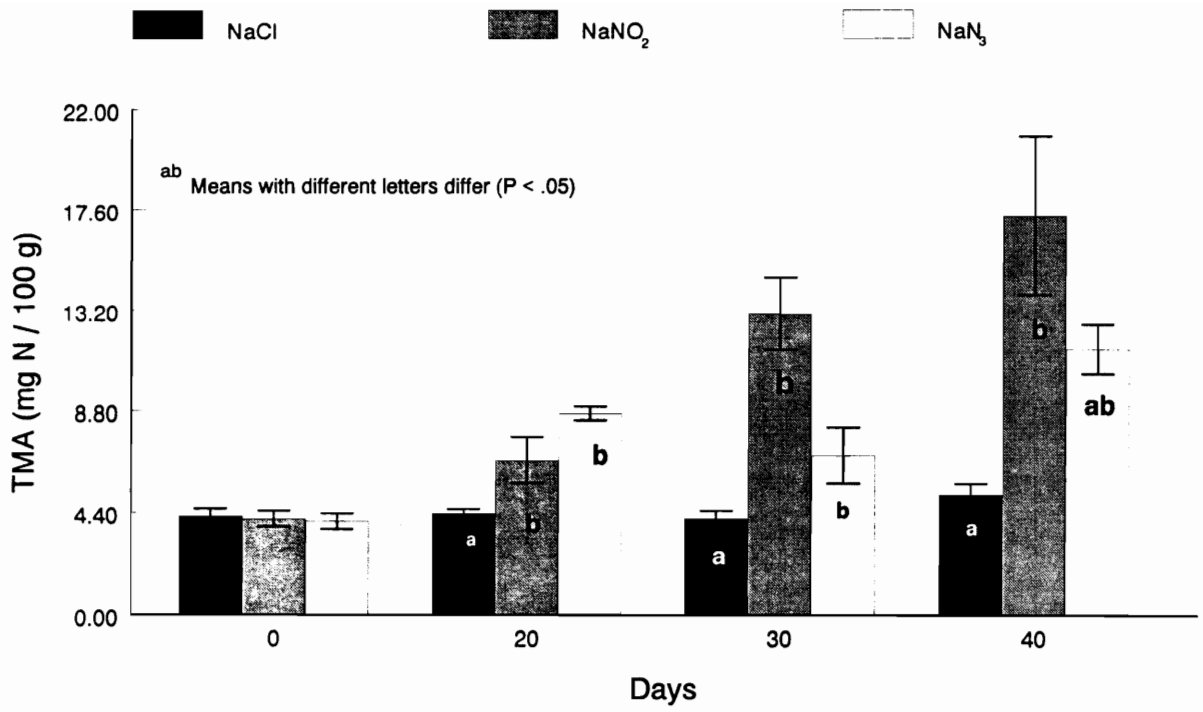


**Figure 12. Trimethylamine (TMA) content of treated crab waste samples after 0, 20, 30 and 40 d (Exp. 3).**

average of 4.15 mg TMA N / 100 g and the level was not different ($P > .05$) among treatments. The TMA content of NaCl treated material was lower than that of the other treatments at 20, 30 and 40 d of preservation. The differences were significant ($P < .05$) between NaCl and $NaNO_2$. At d 40, no significant differences were detected between waste treated with $NaNO_2$ and $NaN_3$.

The data on indole contents of crab waste preserved for 0, 20, 30, and 40 d are shown in Table 7. The average indole content of the initial samples were 15.57, 15.29 and 14.79 μg / 100 g, respectively, for the NaCl, $NaNO_2$ and $NaN_3$ treated materials and were not different ($P > .05$) among treatments. The NaCl treated material showed very low ($P < .05$) indole during the 40-d preservation period. The $NaN_3$ treated material showed a very high value on d 20 (8.32 mg / 100 g), which was higher ($P < .05$) than for the other treatments. The levels for the $NaN_3$ treated material decreased for d 30 and 40 but were higher ($P < .05$) than for the NaCl treated waste.

## Discussion

*Physical Characteristics*

*Color.* Crab waste treated with NaOCl and $H_2O_2$ showed dark discoloration after 17 d which may be due to the growth of numerous maggots. The growth of maggots may have been due to over filling of the buckets and seepage of fluid on the lids. Waste treated with NaCl and $NaN_3$ did not show any change in the color of the material after 40 d, while those treated with $KNO_3$, TBHP and $I_2$ showed discoloration after 21 d which was indicative of the deterioration of the material. Treatment with $NaNO_2$ showed spotted discoloration on the surface after 20d.

*Smell.* The NaOCl and $H_2O_2$ treated materials had slightly objectionable smell after 17 d of preservation, while treatment with NaCl and $NaN_3$ did not

**Table 7. Indole content of treated crab waste after 0, 20, 30 and 40 d (Exp. 3)**

| Day of experiment | Treatment | | | SEM |
|---|---|---|---|---|
| | NaCl | $NaNO_2$ | $NaN_3$ | |
| | ---------- mg / 100g ---------- | | | |
| 0 | .015 | .016 | .016 | 0 |
| 20 | .11[a] | .38[a] | 8.32[b] | .49 |
| 30 | .05[a] | 1.71[b] | .78[ab] | .33 |
| 40 | .04[a] | .52[a] | 1.13[b] | .13 |

[ab] Means within rows with different superscript letters differ ($P < .05$).

cause any change in the smell of the product even after 40 d. The $NaNO_2$ treated waste had slight $NH_3$ smell after 20d of preservation. Absence of any change in the smell of the product also indicates the effectiveness of NaCl, and $NaN_3$ to preserve the waste at room temperature.

*Mold Growth.* White mold growth on the surface was noticed on crab waste treated with NaOCl, after 21 d. The $NaNO_2$ treated materials also showed white spotted fungal growth after 20 d of preservation.

*Chemical Composition*

*Dry matter.* The DM content of the crab waste samples varied depending upon the type and quantity of chemicals used as evidenced by the higher DM (51.6% and 51.02%) for the NaCl treated material and the lower DM (43.47% and 41.96%, respectively) for the NaOCl treated material of both the initial and final samples, compared with the other treated materials. The $NaN_3$ treated material had lower DM than those treated with NaCl and $NaNO_2$ in the initial samples. This may be due to the fact that $NaN_3$ was added at very low level and was dissolved in water before mixing with crab waste. The DM content of crab waste used for the present study was higher than that reported by Samuels et al. (1991, 1992).

The treatment of waste with NaOCl and $H_2O_2$ lowered the DM content compared to that of the untreated materials. This was due to the addition of solutions to the crab waste of the treated groups. Similarly, the NaOCl treated materials had lower DM than the $H_2O_2$ treated of the initial samples. This was because of the difference in the volume of solutions added to obtain the required levels of NaOCl and $H_2O_2$. The commercially available 'Bleach' contained 5.25% NaOCl while the concentration of $H_2O_2$ solution was 30%.

*Crude Protein.* The CP content of the treated materials was similar except for the NaCl treated material, which was lower. This could partly be explained by

the dilution effect as a result of adding salt. The CP content on an ash-free basis was also low for the NaCl treated material (67.5% vs. 74.9% in control) indicating the possibility of sampling error. The CP content of crab waste used for the present study was higher than that reported for crab meal (Patton et al., 1975; Laflamme, 1988; Husby, 1990), similar to that reported by Brundage et al. (1981) and was lower than those reported by Samuels et al. (1992) for crab processing waste. The difference in the CP content may be due to the difference in the processing methods used in different crab processing plants, or loss of some protein as a result of bacterial degradation and solubilization of protein.

*Ash.* The ash content of crab waste was similar in all treatment groups except for the NaCl-treated material. Addition of NaCl increased ($P < .05$) the ash content of crab waste, due to addition of salt. The ash content of crab waste used for this study was higher than that reported by Samuels et al. (1992) but lower than that reported by Husby (1990) and Laflamme (1988) for crab meal. Similar ash values as those obtained in this study were reported by Stelmock et al. (1985) for crab meal.

*Chitin.* The NaCl treatment resulted in lower chitin values than the other treatments of the final samples which may be due to the dilution effect as a result of addition of salt. The chitin values, when expressed on ash free basis, showed less variation among treatments (24.7 in the NaCl treated vs. 25.54 in control samples). The chitin values obtained in this experiment were similar to those given by Stelmock et al. (1985) while higher chitin values were reported by Laflamme (1988) for crab meal.

*Ammonia and Hydrogen Sulfide.* The $NH_3$ measured for the control material was higher on d 5, but was not different from the crab waste samples treated with NaOCl and $H_2O_2$ on d 17. This may be due to the quick deterioration

of the material of the control. The $H_2S$ was lower for the .4% NaOCl and $H_2O_2$ treated material than those treated with .2% level except on d 17, which indicates an effect of level. The $NH_3$ and $H_2S$ values obtained in the second experiment were very low compared to the values obtained for the first experiment which may be due to the growth of maggots in the crab waste samples in Exp. 1. Preserving crab waste with .2 % NaOCl for 7 days produced 14.7 ppm of $NH_3$ and 2.3 ppm of $H_2S$ (Ayangbile et al., 1987) which is higher than the values obtained in the present study for the waste treated with NaCl, NaOCl, $NaNO_2$ and $NaN_3$. The significantly lower levels of $NH_3$ and $H_2S$ production in the crab waste samples treated with NaCl, $NaNO_2$, NaOCl and $NaN_3$ indicate that very little degradation occurred in those samples during preservation. Hydrogen sulfide could not be detected in the waste treated with NaCl, $NaNO_2$ and $NaN_3$ even after 40 d.

*Trimethylamine and Indole.* The TMA and indole contents were higher in the $H_2O_2$ treated material than that of the NaOCl treated material after 17 d. This was in contrast to the finding of Ayangbile et al. (1987). They did not observe any increase in TMA content when crab waste was preserved with .4% $H_2O_2$ for 10 d. Treatment of crab waste with .2% NaOCl for 5 d resulted in an increase in TMA from 3.7 to 12.85 mg / 100g. (Ayangbile, 1989). In the present experiment, materials treated with NaCl, $NaNO_2$, NaOCl and $NaN_3$ had lower TMA levels than those treated with $KNO_3$, TBHP and $I_2$. These values were lower than those reported by Abazinge et al. (1993) when crab waste-wheat straw silage was treated with molasses and Ayangbile and Fontenot (1987) when crab waste was treated with .2% NaOCl . The lower TMA production also indicated low spoilage of crab waste treated with NaCl, $NaNO_2$, NaOCl, and $NaN_3$ after 21 d.

Trimethylamine is produced as a result of reduction of trimethylamine oxide (TMAO) which is a natural constituent of marine animals including both fish and

invertebrates (Strom et al. 1979). Trimethylamine production leads to undesirable flavor in the marine products and TMA producing TMAO reduction is one of the best known bacterial decomposition reactions studied in fish (Malle et al., 1986). It represents an indirect expression of the microorganisms present to decompose muscle tissue. Many methods for detection of specific spoilage bacteria in fish and fish products are based on TMA and $H_2S$ production. Gram (1992) described the traditional and rapid methods for estimation of bacterial levels in seafoods which include the reduction of TMAO to TMA. Malle et al. (1986) developed a rapid method for assessing the sanitary quality of deep frozen fish fillet based on the reduction of TMAO to TMA. Singh et al. (1992) studied the effect of TMA in rats and the results revealed that 10 meq of TMA reduced the body weight gains and protein efficiency ratio. They also found that there was a reduction in the packed cell volume, neutrophils and monocytes and an increase in the reticulocytes, leukocytes, lymphocytes and eosinophils in the blood of rats fed TMA containing diets. Since TMA level is an indirect index of spoilage of marine products, attempts to decrease TMA production will improve the keeping quality of the product (Kim and Chang, 1974). In the present study, the lower TMA levels of crab waste treated with $NaNO_2$, and $NaN_3$ after 21 d and that treated with NaCl after 40 d shows that these chemicals are effective in preserving crab waste.

The indole content of the crab waste treated with $NaN_3$ was higher (8.32 mg / 100 g) on d 20 than the other two treatments. The indole value of the $NaN_3$ treatment was reduced to 1.14 mg /100g on d 40, but it was higher than those of NaCl and $NaNO_2$ treated samples. Indole is a product of decomposition of proteins containing tryptophan. It is formed from proteins under anaerobiasis in the absence of carbohydrates (Clarke et al., 1937). Duggan and Strasburger (1946) estimated the indole content of different classes of shrimp and found that class 4

shrimp contained 1.053 mg indole / 100 g while class 1 had 8 μg / 100g, indicating that a high indole is an indirect index of the freshness of the product. Indole was isolated from water boiled duck meat and it was found to be responsible for its specific aroma (Wu and Liou 1992). Chung and Cadwallader (1993) analyzed the indole content of fresh crab meat and crab byproduct and found that crab meat had higher indole (8.6 μg/ 100 g) than crab byproduct (3.9 μg / 100g). Thus, the lower indole content of crab waste treated with NaCl even after 40 d confirms its effectiveness to prevent deterioration of the waste.

*Salt.* Salting is one of the traditional method of preserving fish. There are two fundamental methods of salting, dry salting which is used for non-fatty fish and pickling for the fatty fish (Hockenhull, 1967). Most of psychrophilic proteolytic *Pseudomonas* and *Achramobacter* sp. were found to be killed by the pickling process. Dry salting destroyed most of the organisms, but *Micrococci* were found to survive if the salt concentration was less than 12%. With concentrations above 6%, NaCl was found to prevent germination of anaerobic spores (Duncan and Foster, 1968), while 10% NaCl inhibited *Clostridium botulinum* and other microorganisms (Schmidt, 1964). The tolerance of microorganisms to salt was found to vary greatly (Banwart, 1979). He reported that the mesophilic gram negative rods and psychrotrophic bacteria were most sensitive at concentrations of 4 to 10%, while the lactic acid and spore forming bacteria could tolerate about 4 to 15% and 5 to 16% salt, respectively. The *Halophiles*, *Halobacterium* and *Halococcus* species need relatively high salt concentrations for growth.

The mode of bacterial inhibition by salt was found to be primarily by its plasmolytic effect (Busta and Foegeding, 1983). Other antimicrobial effects include dehydration, interference with enzymes, $O_2$ removal or the toxicity of high

Na or Cl ion concentration. The effect of NaCl was attributed to the loss of Mg from bacterial cells (Sato et al., 1972; Ito et al., 1977). Ito et al. (1977) further observed degradation of RNA during incubation of *E. coli* in tris buffer containing .15M NaCl which was suggested to be due to the enzyme RNA-ase 1. The results of the present study showed that the crab waste preserved with 10% NaCl for 40d at room temperature, could be by inhibiting the anaerobic and other bacterial organisms.

*Sodium Nitrite.* Sodium nitrite has been used as a food preservative and inhibited the development of *Clostridium* species and other food spoiling microorganisms in preserved foods (Walters and West, 1964). The formation of a potent antimicrobial substance with a broad spectrum of action was also reported by Ashworth et al. (1974) when nitrite was heated in certain systems. The inhibitory substance was found to be similar to the iron nitrosyl co-ordination complexes, termed as black Roussin salts. Moran et al. (1975) further studied the inhibitor of *Clostridium perfringens* formed by heating $NaNO_2$ and found that the inhibitor was formed from cysteine, ferrous sulfate and sodium nitrite. They also found that S-nitrosocysteine, black Roussin salt, and cysteine complex could inhibit *C. perfringens* but since no single compound was present in levels adequate for inhibition, the net effect could be the combined effects of small amounts of each of these compounds.

Benedict (1980) reported that inhibition of *Clostridium botulinum* in cured meat was due to several interacting mechanisms: (a) reaction and oxidation of cellular biochemical compounds within the spores and vegetative cells; (b) restriction of use of iron (or other metal ion) through inhibition of solubilization, transport, or assimilation, thus interfering with metabolism and repair mechanisms

and cell surface membrane activity limiting substrate transport by the outgrowing cell.

Nitrite was found to inhibit the active transport of proline in *Escherichia coli* but not the group translocation of sugar via the phosphoenolpyruvate: phosphotranferase system (Yarbrough et al., 1980). Nitrite also inhibited the $O_2$ uptake and oxidative phosphorylation in aerobic bacteria and enzyme aldolase in *E. coli*, *Streptococcus faecalis*, and *Pseudomonas aeruginosa* indicating that nitrite has more than one site of attack in the bacterial cell. Glidewell and Glidewell (1993) have shown that in the presence of iron salts, nitrite reacts with cysteine and other sources of cysteine to yield antibacterial iron-sulfur nitrosyl salt (Na $[Fe_4S_3(NO)_7]$ which was more potent than nitrite itself under a range of substrates.

Ayangbile (1989) studied the preservation of crab waste using different preservatives such as 1.5% propionic / formic acid (1:1), 0.2% sodium hypochlorite, 0.4% hydrogen peroxide, 0.4% NaOCl + 1.5 % acetic acid, 1.5 % NaOH and 1% $NaNO_2$. The TMA concentration was the lowest for the sodium nitrite treated material during a 10-d preservation period. Hydrogen peroxide treatment did not increase the TMA content during a 7-d period while NaOCl + acetic acid preserved for 4 days. Sodium nitrite at 1% level was found to be effective in preserving crab waste up to 10 d. In the present study, sodium nitrite at 1% level preserved crab waste for 21 d. However, after 20 d there was slight ammonia smell and spotted fungal growth on the surface of the material. The TMA values were also higher after 20 d than that of the NaCl treated material.

## Implications

NaCl at 10% level was found to preserve crab waste for 40 d and was found to be better than the other treatments studied. However, more work needs to be

done to find out the efficacy of NaCl at lower concentrations. Sodium nitrite (1%) and sodium azide (.065%) were found to be effective in preserving crab waste for 20 d. These results indicate that the crab waste could be accumulated for upto 40 d, then ensiled for use as ruminant feed.

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# CHAPTER 4.

## Quality of Protein from Crab Processing Waste.

**ABSTRACT:** Crab waste was treated with 0, 1, 2 and 4% KOH at 90°C for 1, 2, and 3 h in a 4 x 3 factorial design to standardize the procedure for extracting protein from crab waste. Treatment with 1% KOH at 90°C for 1 h gave maximum protein recovery. Approximately 240 kg of crab waste were extracted using the above procedure, processing 10 kg batches in a 250 L steam jacketed kettle. The dissolved protein was separated from the shell by filtering through eight layers of cheese cloth. The protein was precipitated by reducing the pH to 4.5 with 1N HCl. The precipitate was separated by decanting the supernatant liquid and was dried in a forced draft oven at 60°C for 48 h. The crab waste protein supplement (CWPS) contained 43.23% CP, 30.57% ether extract, 17.82% total ash, and 7.1% Cl, DM basis. *In vitro* digestibility of CWPS was determined. True digestibility of protein from CWPS and soybean meal (SBM) was similar. Preliminary feeding trials using 1-d old chicks showed that chicks performed very poorly if CWPS replaced the protein from SBM at 100% level. Based on the results from the preliminary feeding trials, an experiment was conducted with 180, 1-d-old male broiler chicks allotted randomly to 30 pens of six chicks. The chicks were housed in brooder batteries where the temperature was controlled by individual thermostats in each pen. The pens were divided into six blocks of five pens based on location. The five treatments were allotted at random to each block. The treatments were as follows: A - 0% CWPS diet fed *ad libitum*; B - 25% CWPS diet fed *ad libitum*; C -50% CWPS diet fed *ad libitum*; D - 25% CWPS diet pair-fed to the 50% CWPS diet; E - 0% CWPS diet pair-fed to treatment C. The feeding

experiment was conducted for 21 d during which feed intake was recorded daily and body weights at weekly intervals. Excreta were collected during the last 3 d to determine N retention, uric acid excretion and the ME value of the diets. The day before the end of the experiment, blood was collected from three birds per pen for estimation of serum uric acid levels. The birds were sacrificed at the end of the experiment and the pancreas was removed from the same chicks from which blood samples were obtained. Body weight gain, Protein Efficiency Ratio (PER) and gain / feed decreased linearly ($P < .05$) with increasing levels of CWPS in both *ad libitum* and pair-fed chicks. The N and energy retention decreased linearly ($P < .05$) with increased levels of CWPS in *ad libitum* fed chicks, but differences were not significant among the pair-fed birds. Feed intake decreased linearly ($P < .05$) with increased levels of CWPS, although the reduction in feed intake was less severe with 25% CWPS diet fed chicks. The uric acid index increased with decreasing levels of CWPS (linear and quadratic effect, $P < .05$) both for *ad libitum* and pair-fed birds. Serum uric acid concentrations were higher for the pair-fed than those of *ad libitum* fed chicks and showed a linear decrease ($P < .05$) with increasing levels of CWPS in the pair-fed birds. The calculated digestion coefficients of N decreased with increased levels of CWPS (linear and quadratic, $P < .05$). The ME and N-corrected ME values were higher ($P < .05$) for the pair-fed compared to that of the *ad libitum* fed chicks. The pancreas weight increased linearly ($P < .05$) with increased CWPS in the diets. It could be concluded that digestion and utilization were lower for CWPS than SBM. However, difference in performance was less with 25% CWPS diets, indicating that CWPS can be incorporated in the diets of chicks at 25% level.

**Keywords**: Crab waste protein, Protein efficiency ratio, Pancreas weight, Uric acid index, Metabolizable energy.

## Introduction

Crab waste is an excellent source of crude protein, but its high mineral and chitin contents limit its use at high levels in the diets of animals (Chung and Cadwallader, 1992). Recovery of protein and other value added products, such as carotenoid pigments and chitin, from crab waste was shown to be an effective method for utilizing the waste (Johnson and Peniston, 1982; No et al., 1989; Shahidi and Synoweicki, 1992). The protein thus recovered had very low ash and chitin contents, and contained all the essential amino acids with lower levels of methionine, leucine and isoleucine than FAO / WHO standards (Shahidi and Synoweicki, 1992).

The enzymatic digestion of protein *in vitro* has been used as a measure of protein quality. Single enzyme digestion was used to evaluate the relationship between the pattern of amino acids released and its biological value (Sheffner et al., 1956; Maga et al., 1973). A mixture of two proteolytic enzymes was used by Akeson and Stahmann (1964) and Saunders et al. (1972). A multi-enzyme technique was developed for estimating the protein digestibility based on the drop in pH of the protein suspension after the addition of enzyme mixture (Hsu et al., 1977). Pedersen and Eggum (1983) developed a pH-stat procedure for determining true digestibility of protein of both plant and animal origin with a high correlation to that of *in vivo* digestibility in rats.

The chick growth method has been employed for the biological assay of protein quality (Lyman et al., 1953; Feathterston and Scholz, 1968; Papadopoulos et al., 1985). Uric acid excretion and the liver xanthine dehydrogenase activity in chicks were reported to be indicative of protein quality (Featherston and Scholz,

1968; Miles and Featherston, 1976; Hevia and Clifford, 1977; Vit et al., 1993). Organ weights, especially the pancreas weight expressed as percentage of body weight was also found to correlate to the protein quality (Anderson-Hafermann et al., 1992; Zhang and Parsons, 1993). Thus, the objectives of the present experiment were to develop a method for the separation of protein from crab processing waste and to evaluate the quality of CWPS by *in vitro* and *in vivo* methods.

## Materials and Methods.

Crab processing waste was obtained from a hand picked crab processing plant (Little River Seafoods, Reedville, VA) and was ground through a food grinder. It was then packed in double lined polyethylene bags, kept in large plastic cans, layered with ice during transportation for 480 km, then frozen until used.

*Separation of Protein from Crab Processing Waste*

*Standardization of Procedure.* Samples of 25 g of fresh crab waste were treated with 250 ml of 0, 1, 2, and 4% KOH solutions and were kept in a water bath at 90°C for 1, 2 and 3 h in a 4 x 3 factorial design. There were six replicates for each treatment. The samples were then filtered through four layers of cheese cloth and the filtrate was clarified by centrifuging at 1600 *g* for 15 min in a refrigerated centrifuge (20°C) (IEC Centras-7R, International Equipment Company, USA). The clear filtrate was adjusted to a pH of 4.5 with 1 N HCl with constant stirring to precipitate the protein. The precipitate was allowed to settle at room temperature for 3 h, then was transferred to weighed centrifuge tubes and centrifuged at 1600 *g* for 15 min. The supernatant solution was decanted, and the

precipitate was freeze dried and was weighed. The protein recovery was then calculated as percentage of fresh crab waste.

The N and ash contents of the precipitate were determined by AOAC (1990) procedure.

The statistical analysis of the data was conducted by the General Linear Model (GLM) Procedure of SAS (1989) in a 4 x 3 factorial, with levels of KOH and the processing time as the factors.

*Recovery of Protein in Bulk from Crab Processing Waste.* Crab processing waste, obtained from the same processing plant, was treated by the method which gave the maximum recovery of protein in the previous experiment. Modifications in the procedure were made due to the lack of facilities for centrifugation and freeze-drying large quantities of protein.

Samples of 10 kg of crab waste were treated with 100 L of 1% KOH in a steam jacketed kettle of approximately 240 L capacity at 90°C for 1 h. The mixture was allowed to cool to room temperature, then was filtered through eight layers of cheese cloth kept over a strainer of 20 cm in diameter into 20 L buckets. The pH of the solution in each bucket was adjusted to 4.5 by adding 1N HCl slowly, with constant stirring to precipitate the protein. The precipitate was allowed to settle at room temperature for 3 h. The supernatant solution was decanted slowly to prevent spilling of the precipitate or mixing of the precipitate in solution. The precipitate was then transferred quantitatively to stainless steel pans 56 x 46 x 6.4 cm (L x B x D), and was dried for 48 h in a forced-draft oven at 60°C. The dried precipitate was transferred to double-lined polyethylene bags and was kept frozen. A total of 24 batches was done to obtain the quantity of crab waste protein required to conduct the chick feeding trial. The protein recovered from four consecutive batches was pooled, and ground through a 2 mm screen

using a stainless steel hammer mill (W. J. Fitzpatrick Co., Chicago, IL). Samples were taken from all the six pooled materials for analysis.

*In Vitro Digestibility of Crab Waste Protein Supplement*

The *in vitro* digestibility of crab waste protein supplement (CWPS) and soybean meal was determined by the procedure described by Pederson and Eggum (1983). Samples of 1.41 g CWPS, 1.31g of SBM or .70 g of sodium caseinate were dissolved in 100 ml deionized water to obtain 1 mg N / mL of suspension. The protein suspension was allowed to stand at 4°C for 1 h. Aliquots of 10 mL of the protein suspension were placed in 50 mL beakers, warmed to 37°C, the pH was adjusted to 8.0 using .1N NaOH, and was maintained for 5 to 10 min before adding the enzyme solution. The enzyme solution was prepared by mixing 32.81 mg porcine pancreatic trypsin (Type 11, Sigma T 7409), 89.42 mg bovine pancreatic chymotrypsin (Type 11, Sigma C 4751) and 12.75 mg of porcine intestinal peptidase (Sigma P 7500) to obtain 23100 units of trypsin, 186 units of chymotrypsin, and .052 units of peptidase / mL of solution. The pH was adjusted to 8.0 at 37°C and was maintained for 2 min. The enzyme solution was then transferred to an ice bath and kept at 0°C. One milliliter of the enzyme solution was added to the protein suspension, and while stirring, the pH was maintained at 8.0 for exactly 10 min by adding .1N NaOH solution, and the volume of alkali added was recorded. Six samples of each protein were used in the study.

Sodium caseinate was used to calculate the laboratory correction factor (100 divided by sodium caseinate digestibility) for adjusting the final values. True digestibility (TD) of protein was calculated using the equation, TD = 76.14 + 47.77 B, where B = mL of .1 N NaOH added.

*Evaluation of Crab Waste Protein Supplement in Chicks*

*Preliminary Feeding Trials.* Three trials were conducted to determine the effect of incorporating CWPS in the diets of 1-d-old chicks. The diets were formulated to meet the nutrient requirements of growing chicks (NRC, 1994).

In Trial 1, 20 1-d-old White Leghorn chicks were allotted at random to four pens of five chicks each and the pens were allotted to two dietary treatments. The corn-soybean diet was the control diet. The protein of the SBM was replaced by CWPS and the diets were formulated to be isonitrogenous and isocaloric. The ingredient composition and the calculated nutritive value of the diets are given in Table 8. Corn oil was added to the control diet to equalize the ether extract of the two diets. The chicks were wing banded and weighed individually at the start of the experiment and weekly thereafter. Feed intake was recorded during the experimental period of 16 d. Feed and water were available *ad libitum* throughout the experiment. Light was also provided 24 h /d. The chicks were kept in temperature controlled (33°C) pens of brooder batteries.

In Trial 2 a control diet was formulated, incorporating 6% corn oil. It contained 51.94% corn and 37.8% SBM which gave a calculated CP value of 22.74 and 3.15 Mcal of ME per kg of diet. The birds of the control group of the previous experiment were allotted to two pens of five chicks each and were allotted at random to the old and the new control diets and were fed for a period of 7 d. The body weights of the chicks were recorded at the beginning and the end of experiment. Feed, water, and light were provided 24 h and feed consumption was recorded.

In Trial 3, 40 1-d-old male broiler chicks (Peterson / Ross) were obtained from Wampler-Longacre Hatchery, Harrisonburg, VA. They were wing banded, weighed and were allotted at random to four pens of 10 chicks. The pens were

**Table 8. Ingredient composition and calculated composition of diets used in preliminary trial 1**

| Item | Diet | |
|---|---|---|
| | Control | Crab waste protein supplement |
| Ingredient composition, % | | |
| Corn | 45.59 | 61.44 |
| SBM | 37.85 | _ |
| Crab waste protein supplement | _ | 34.00 |
| Corn oil | 12.00 | _ |
| Limestone | 1.86 | 1.86 |
| Defluorinated phosphate | 1.70 | 1.70 |
| Vitamin premix[a] | .20 | .20 |
| Trace mineral premix[b] | .20 | .20 |
| Salt | .40 | .40 |
| DL-methionine | .20 | .20 |
| Calculated composition | | |
| CP, % | 22.23 | 22.22 |
| ME, Mcal / kg | 3.43 | 3.42 |
| Ca, % | 1.5 | 1.5 |
| P, % | .68 | .60 |

[a] Supplied per kilogram of diet: vitamin A, 2640 I. U.; vitamin D3, 2640 I. C. U.; vitamin E, 26.4 I. U.; vitamin K, .75 mg; riboflavin, 7.48 mg; Ca pantothenate, 9.68 mg; niacin, 26.4 mg; vitamin B12, .011 mg; choline chloride, 1012 mg; biotin, .31 mg; folacin, 3.08 mg; thiamin HCl, 3.08 mg; pyridoxine HCl, 3.08 mg; ethoxyquin, 5.28 mg; virginiamycin, 2.9 mg.

[b] Supplied per kilogram of diet: Mn, 88 mg; Zn, 95 mg; Fe, 100 mg; Cu, 12.5 mg; $I_2$, 4 mg; and Se, .6 mg.

allotted to two diets, the ingredient composition of which is given in Table 9. The CWPS was used to replace 50% of SBM protein. The quantity of SBM was adjusted to make the diets isonitrogenous. The diets contained 22% CP and 3.14 Mcal of ME and were equalized for the ether extract and Cl contents. Potassium chloride was added to the control diet to equalize the Cl content to that of CWPS diet. Sodium bicarbonate was added instead of NaCl to both the diets to supply the required amounts of Na and to reduce the level of Cl content of the diets. In the experimental diet, CWPS was substituted for 50% of the SBM protein.

The chicks were kept in battery brooder pens where the temperature was controlled at 33°C with individual thermostats in each pen. Feed, water, and light were provided 24 h /d throughout the experimental period of 15 d. The birds were weighed and the feed consumption was recorded at weekly intervals.

*Feeding Trial with Chicks to Evaluate Crab Waste Protein Supplement.* One hundred and eighty 1-d-old male broiler (Peterson / Ross) chicks were obtained from the Wampler-Longacre Hatchery, Harrisonburg, VA. The birds were wing banded, weighed individually and were allotted at random to 30 pens of six birds per pen. The chicks were kept in pens of the brooder batteries and the temperature was controlled by the individual thermostats (33°C) in each pen. There were 24 pens in one battery, two rows of six pens each, on each side. The remaining six pens were located in another battery, four pens on the bottom of one side and two pens on the bottom of the other side. The pens were divided into blocks of five pens, in an attempt to eliminate the differences between batteries and the position of pens in each battery. Five dietary treatments were allotted at random to the five pens of each block. Corn-SBM based diet containing 22% CP formed the control diet. The ingredient composition and the calculated composition of the diets are given in Table 10. The five treatments were : A - 0%

**Table 9. Ingredient composition and calculated analysis of the diets used in preliminary trial 3**

| Item | Diet | |
|---|---|---|
| | Control | Crab waste protein supplement |
| Ingredients composition, % | | |
| Corn | 52.61 | 59.76 |
| SBM | 34.75 | 18.00 |
| Crab waste protein supplement | – | 17.50 |
| Corn oil | 5.25 | – |
| Limestone | 1.86 | 1.86 |
| Defluorinated phosphate | 1.70 | 1.70 |
| Vitamin premix[a] | .20 | .20 |
| Trace mineral premix[b] | .20 | .20 |
| $NaHCO_3$ | .58 | .58 |
| DL-methionine | .20 | .20 |
| KCl | 2.65 | – |
| Calculated composition | | |
| CP, % | 21.34 | 21.34 |
| ME, Mcal / kg | 3.14 | 3.14 |
| Ca % | 1.54 | 1.54 |
| P, % | .68 | .66 |
| Lysine, % | 1.10 | 1.03 |
| Methionine + cystine, % | .64 | .66 |
| Chloride, % | 1.36 | 1.36 |

[a] Supplied per kilogram of diet: vitamin A, 2640 I. U.; vitamin D3, 2640 I. C. U.; vitamin E, 26.4 I. U.; vitamin K, .75 mg; riboflavin, 7.48 mg; Ca pantothenate, 9.68 mg; niacin, 26.4 mg; vitamin B12, .011 mg; choline chloride, 1012 mg; biotin, .31 mg; folacin, 3.08 mg; thiamin HCl, 3.08 mg; pyridoxine HCl, 3.08 mg; ethoxyquin, 5.28 mg; virginiamycin, 2.9 mg.

[b] Supplied per kilogram of diet: Mn, 88 mg; Zn, 95 mg; Fe, 100 mg; Cu, 12.5 mg; $I_2$, 4 mg; and Se, .6 mg.

**Table 10. Ingredient composition and calculated composition of diets used in trials to evaluate crab waste protein supplement**

| Item | Crab waste protein supplement | | |
|---|---|---|---|
| | 0 (control) | 25 | 50 |
| Ingredient composition, % | | | |
| Corn | 45.377 | 50.967 | 56.507 |
| SBM | 38.85 | 28.26 | 17.74 |
| Crab waste protein supplement | - | 10.50 | 21.00 |
| Corn oil | 7.85 | 3.92 | - |
| Limestone | 1.86 | 1.86 | 1.86 |
| Defluorinated phosphate | 1.70 | 1.70 | 1.70 |
| Vitamin premix[a] | .20 | .20 | .20 |
| Trace mineral premix[b] | .20 | .20 | .20 |
| $NaHCO_3$ | .58 | .58 | .58 |
| DL-methionine | .20 | .20 | .20 |
| KCl | 3.17 | 1.59 | - |
| Santoquin | .013 | .013 | .013 |
| Calculated composition | | | |
| CP, % | 22.02 | 22.00 | 22.01 |
| GE, Mcal / kg | 4.202 | 4.202 | 4.203 |
| Ca, % | 1.54 | 1.53 | 1.50 |
| P, % | .71 | .70 | .69 |
| $Cl^-$, % | 1.61 | 1.61 | 1.61 |
| Lysine, % | 1.20 | 1.13 | 1.08 |
| Methionine + cystine, % | .85 | .87 | .87 |

[a] Supplied per kilogram of diet: vitamin A, 2640 I.U.; vitamin D3, 2640 I. C. U.; vitamin E, 26.4 I.U.; vitamin K, .75 mg; riboflavin, 7.48 mg; Ca pantothenate, 9.68 mg; niacin, 26.4 mg; vitamin B12, .011 mg; choline chloride, 1012 mg; biotin, .31 mg; folacin, 3.08 mg; thiamin HCl, 3.08 mg; pyridoxine HCl, 3.08 mg; ethoxyquin, 5.28 mg; virginiamycin, 2.9 mg.

[b] Supplied per kilogram of diet: Mn, 88 mg; Zn, 95 mg; Fe, 100 mg; Cu, 12.5 mg; $I_2$, 4 mg; and Se, .6 mg.

CWPS diet fed *ad libitum*, B - 25% CWPS diet fed *ad libitum,* C - 50% CWPS diet fed *ad libitum*, D - 25% CWPS diet pair-fed, E - 0% CWPS diet pair-fed. The chicks fed diets D and E were pair-fed with those fed diet C. On d-1, the chicks of all the five treatment groups were fed *ad libitum*, but starting on d-2, the chicks of treatments D and E were fed the same amount of feed consumed by the birds of treatment C on the previous day. Feed intake by pens was recorded daily and individual body weights were recorded weekly. Water and light were provided 24 h /d throughout the experimental period of 21 d. Samples of feed were taken daily and were composited at the end of the experiment into six samples of each diet for analysis.

A nitrogen balance trial was conducted during the last 3 d of the experiment. The feed troughs were removed before starting the collection and the birds were fed after starting the collection. Excreta were collected from all the pens at exactly the same time daily and were weighed, sampled and frozen. Excreta trays were first cleaned, dried and numbered. Wooden bars were used to separate the tray for the different pens. Aluminum foil was cut according to the size of the tray, was formed into a tray, and was weighed and numbered. It was then placed on the tray. A thin polyethylene wrap, cut according to the size of the partitions of the tray, was weighed and spread over the aluminum foil to prevent leakage of any liquid from the excreta since the excreta were very wet.

The excreta were collected once daily. The foil and the polyethylene wrap were kept in position by using small pieces of tape which were removed before weighing.

Approximately 100 g of excreta were taken from each pen after mixing thoroughly and were kept on dry ice until transferred to the freezer. The daily samples of each day were analyzed separately. After thawing, the samples were

mixed in a blender for 2 min before taking samples for DM and N. The N-corrected ME value was calculated by deducting 8.22 kcal / g N retained from the ME value obtained (Hill and Anderson, 1958).

After the balance trial, blood samples were collected by cardiac puncture from three birds from each pen. The chicks were weighed and sacrificed at the end of the experiment by cervical dislocation. The pancreases were removed from the birds from which blood samples were taken, and were weighed.

From the body weight gain and the feed intake data, feed efficiency was calculated. The protein efficiency ratio of the three diets was calculated for birds fed both at *ad libitum* and pair-fed.

*Chemical Analyses and Energy Determination*

The isolated protein samples were analyzed for DM, CP, EE and ash and samples of SBM, corn and diets were analyzed for DM, CP and ash by AOAC (1990) procedures. Chitin was analyzed by the ADF procedure (Van Soest, 1963; Ayangbile, 1989). Acid insoluble ash was determined by the AOAC (1984) procedure. Samples were wet ashed by the procedure of Sandel (1959) for the determination of Ca, Mg, Cu, Zn, and K contents by Atomic Absorption Spectrophotometry. Chloride content of the CWPS, SBM and corn samples were determined at the Forage Laboratory, Northeast Dairy herd Improvement, NY. Gross energy content of CWPS, SBM, corn, the three diets and the excreta samples was determined using the Parr Bomb calorimeter. The P content of the wet ashed samples was determined by colorimetry (Fiske and Subbarow, 1925). Amino acid analysis of crab waste protein and SBM samples was done at the Experiment Station Chemical Laboratories, University of Missouri, Columbia.

The DM and the N contents of the excreta were analyzed by AOAC (1990) procedures. Nitrogen was determined on approximately 1 g samples of wet

excreta. The excreta were then dried in a forced-draft oven maintained at 60°C for 24 h, then were ground using a mortar and pestle for uric acid and energy determinations.

The uric acid was extracted from the excreta by the procedure described by Pudelkiewicz et al. (1968). One gram samples of finely ground excreta were quantitatively transferred to 250 mL volumetric flasks. The necks of the flasks were washed with two 25 mL portions of .5% lithium carbonate solution. After extracting for 30 min with frequent swirling of the samples, the flasks were made up to volume (250 mL) with distilled water and mixed by inversion. A portion was centrifuged at 1600 *g* to remove solids. A volume of 25μL was used for the determination of uric acid. The uric acid was estimated using a Sigma Diagnostic Kit. The uric acid index was calculated by dividing the uric acid excretion per day by N intake as described by Vit et al. (1993). Digestibility of N was calculated assuming that the uric acid N represents 80% of urinary N, since uric acid N was reported to constitute 60 to 80% of the total urinary N excretion (Coulson and Hughes, 1930). Uric acid was determined in the serum using a Sigma Diagnostic Kit.

*Statistical Analysis*

The data were statistically analyzed by the analysis of variance of GLM procedure of SAS (1989) with treatments and blocks as variables. The means were compared using contrast comparisons to determine linear and quadratic effects separately for the *ad libitum* and pair-fed chicks. The F values were compared with Bonferroni F (Games, 1977) to reduce the possible error of treating the 50% CWPS fed group both as *ad libitum* and pair-fed.

## Results and Discussion

*Separation of Protein from Crab Waste*

*Standardization of Procedure.* The CP content (66.6%, DM basis) of the recovered material was highest ($P < .05$) for the 1% and lowest for 4% ($P < .05$) KOH treatments, while material from treatments with 0 and 2% KOH were intermediate (Figure 13). The CP content was lower than that reported by Peniston et al. (1969) and Johnson and Peniston (1982) when they obtained up to 90% CP in the recovered material. Approximately 8.9% of the crab processing waste (DM basis) was recovered as protein, when treated with 1% KOH. Treatment with 4% KOH resulted in lower ($P < .05$) protein recovery (2.7%). Manu-Tawiah and Haard (1987) used trypsin for the recovery of carotenoprotein from the exoskeleton of snow crab and they obtained 9% recovery (DM basis) as lipoprotein fraction.

Processing time had an effect on both the CP content and the percent recovery of the material. The CP contents of the recovered product (59.8%) was highest ($P < .05$) for 1 h KOH treatment and lowest for 3 h treatment (51.1%) (Figure 14). Treatment with KOH for 3 h resulted in lowest ($P < .05$) protein recovery. Approximately 5.8, 4.8 and 3.6 % of crab processing waste (DM basis) were recovered as CP for 1, 2 and 3 h, respectively. Peniston et al. (1969) used 1 to 2% NaOH solution at 60°C for 4 h for extracting protein from shell fish waste. A higher concentration of NaOH (3.5%) was used at a temperature of 65°C for 2 h for extraction of protein from decalcified shell (No et al., 1989), while, Shahidi and Synowiecki (1991) used 1 and 2% KOH solutions for extracting protein from shrimp and crab waste, at a temperature of 90°C for 2 h.

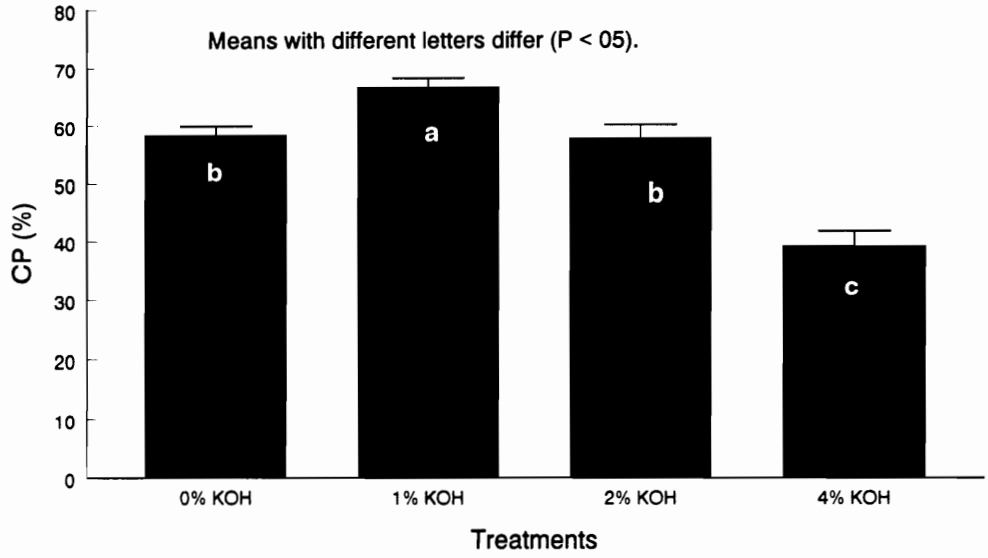


**Figure 13. Crude protein content of recovered material (dry matter basis).**

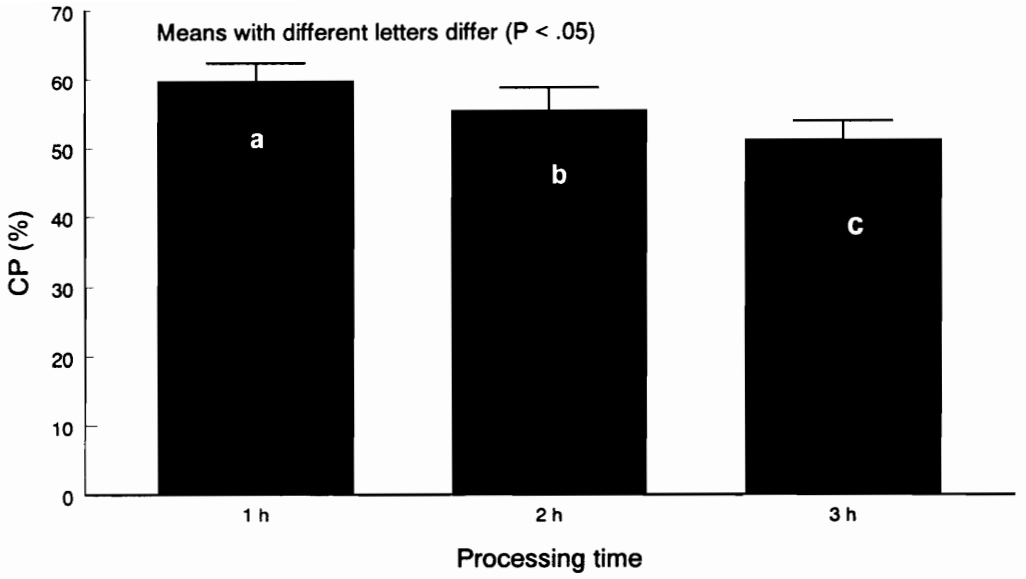


**Figure 14. Effect of processing time on crude protein content of recovered material (dry matter basis).**

Protein has been separated from fish for producing fish protein concentrate. Cobb and Hyder (1972) reported the ideal characteristics of fish protein concentrate which stated that it should be sterile, odorless and tasteless, should have similar nutritional properties as that of original fish muscle, and should be free of any toxic residues. Different enzymes were used for preparing fish protein concentrate. Papain was used by Sen et al. (1962) and Sripathy et al. (1962). The proteolytic enzymes such as pancreatin, pepsin and papain were found to have highest activity for producing fish protein concentrate (Hale, 1974). Fish protein hydrolysate was found to contain 63% CP and was rich in all essential amino acids except threonine (Yanez et al. 1976). Lalasidis et al. (1978) produced low molecular weight enzymatic fish protein hydrolysates from deboned cod filleting offal, which was balanced in amino acid composition. Nair et al. (1976) used 8M urea and papain for the preparation of fish protein hydrolysate. Spinelli et al. (1977) described the preparation of fish protein derivatives by treating the myofibrillar protein with acetic or succinic anhydride, under slightly alkaline condition. Mackie (1982) reported that the enzymes that are used to liquefy fish and fish waste can be used for the conversion of fish meal which could be readily dispersed in water. Yu and Tan (1990) incorporated fish protein hydrolysate at 10% level in crackers without any effect on their acceptability.

*Evaluation of Protein Quality*

*In Vitro Protein Digestibility.* The *in vitro* true digestibility of protein was 87.39 and 86.39% respectively, for the CWPS and SBM samples after applying the laboratory correction factor. The value obtained for the sodium caseinate which was used as the reference protein was lower than that reported by Pedersen and Eggum (1983). The *in vitro* digestibility is based on the rate of release of amino acids as a result of enzyme action. Similar values obtained in the present study for

CWPS and SBM may be because they released amino acids at similar rates. Pedersen and Eggum (1983) reported that there was no under-estimation of digestibility of animal proteins with the exception of egg protein with the pH-stat procedure. Pedersen and Eggum (1981) found no correlation between the *in vitro* and *in vivo* digestibility of animal proteins when the *in vitro* digestibility was estimated by the three-enzyme method of Hsu et al. (1977) and the four-enzyme method of Satterlee et al. (1979). The pH-stat procedure by Pedersen and Eggum (1983) was used to determine the *in vitro* protein digestibility of CWPS and SBM in the present study since it was recommended as more accurate for predicting protein digestibility of food and feed proteins after reviewing the different *in vitro* digestibility methods (FAO / WHO, 1989).

*Preliminary Feeding Trials Using Chicks.* At the end of wk 1, chicks fed control and CWPS diets weighed an average 45.6 and 78.6 g, respectively. After 16 d, chicks fed the CWPS diet lost weight and four chicks had died. The feed per gain ratio was 2.2 and 7.8, respectively for the chicks on control and CWPS diet. In the second trial, chicks gained 112.86 and 121.4 g, respectively when they were fed the old and new control diets. The feed per gain was also improved (1.61 vs. 2.07) when the level of corn oil was reduced from 12 to 6%. In the third trial, the total feed intake per bird was 388.64 and 258.62 g, respectively, for the control and CWPS diets. The overall body weight gains were 288.89 and 166.24 g with a feed per gain ratio of 1.35 and 1.56, respectively, for those fed the control and CWPS protein diets. The data on body weight gain and feed to gain ratio obtained in the preliminary feeding trial showed that CWPS cannot be used to replace SBM at 100% level. The birds on the control diets containing 12% added corn oil performed poorly compared to those with 6% oil diet. The utilization of dietary fat is dependent on the age of the birds (NRC, 1994), and birds after 2 wk of life are

found to utilize it better (Lessire et al., 1982). Diets with 10 and 20% fat with either corn oil or glyceryl tricaprylate were used as a source of long and medium chain triglyceride in chicks. Lower body weight gain, food intake, protein and energy retention were recorded in chicks fed medium chain triglyceride diet (Furuse et al., 1992). Improved body weight gain and protein utilization were reported in chicks fed medium chain triglyceride diet under equalized feeding conditions (Mabayo et al., 1993). A poor feed conversion ratio was reported in female broilers fed 5% added tallow (Scaife et al., 1994).

The results of the preliminary feeding trial using CWPS at levels to replace 50% of protein from SBM resulted in lower feed intake, weight gain and higher feed to gain ratio. The subsequent feeding experiment was planned to mask the effect of reduction in feed intake by equalizing the feed intake of the chicks fed the control and 25% CWPS diets to those fed 50% CWPS diet.

*Feeding Trial in Chicks to Evaluate Crab Waste Protein*

The composition of CWPS, SBM and corn used for mixing the diets are given in Table 11. The composition of corn and SBM was similar to the reported values (NRC, 1994). The CWPS contained on average 43.23% CP, 30.57% ether extract and 17.82% ash. It also contained .08% Mg, 5.9% K, 7.1% Cl, 256 ppm Cu, and 285 ppm Zn, DM basis. The low CP value obtained may be due to the high fat (30.57%) and ash (17.82%) contents. A further step to remove the fat may have been beneficial to concentrate the protein of the product. The higher ash content may be due to the inability to remove all the supernatant liquid which was dried along with the precipitated protein. The major components of total ash were K and Cl. Higher K and Cl levels may be due to KCl formed by the reaction of KOH and HCl during the precipitation of protein. A high Cl content in the diets of chicks induced development of tibial chondroplasia which was found to be

**Table 11. Chemical composition of the ingredients used for mixing the diets[a]**

| Item | Crab waste protein supplement | Soybean meal | Corn |
|---|---|---|---|
| DM, % | 98.95 | 90.78 | 86.79 |
| CP, % | 43.23 | 47.75 | 8.15 |
| Ether extract, % | 30.57 | -[b] | -[b] |
| Total ash, % | 17.82 | 12.34 | 7.45 |
| Acid insoluble ash, % | 1.34 | -[b] | -[b] |
| Chitin, % | .02 | -[b] | -[b] |
| Ca, % | .59 | .74 | .01 |
| P, % | .36 | .64 | .28 |
| K, % | 5.9 | 1.83 | .41 |
| $Cl^-$, % | 7.1 | .13 | .10 |
| GE, kcal / g | 5.60 | 4.26 | 4.02 |

[a] DM basis except DM
[b] Not determined

ameliorated by Mg supplementation (Luo et al 1992). Peniston et al. (1969) reported an ash value of 6 to 8% in the protein separated from crab waste.

The amino acid composition of CWPS and SBM samples are presented in Table 12. The supplements were similar in amino acid composition, although the levels of cysteine, lysine and arginine were lower in the CWPS than that of SBM. The total amino acids were 40%, which was approximately 93% of the CP of CWPS. This value is higher than that reported by Jaswal (1990) when amino acid hydrolysate was prepared from crab waste using 5N HCl. The amino acid yield varied from 28 to 31% after 12 h hydrolysis. This could be because he used crab waste instead of CWPS which was used in the present study. The amino acid contents of protein extracted from shrimp and crab were found to be similar and were reported to be comparable to that of casein (Johnson and Peniston, 1971), with higher percentages of arginine and isoleucine, and lower valine and leucine than that of casein. Shahidi and Synoweicki (1992) found a lower methionine, leucine and isoleucine in crab waste protein than the FAO / WHO standards.

The DM, CP and GE of the diets and the excreta are given in Table 13. The DM content of the excreta was low for chicks on all treatments. The DM was highest for the 50% CWPS diet fed chicks (18.51%). The CP and GE values of the excreta were also higher for the 50% and lowest for the chicks fed the 0% CWPS diet.

The average initial weight of chicks was 38.20 g. A total of seven chicks died during the course of the experiment (four from the 0% CWPS *ad libitum*, two from 50% CWPS diet and 1 from 25% CWPS diet pair-fed), and feed intakes were corrected accordingly.

**Table 12. Amino acid composition of crab waste protein supplement and soybean meal[a]**

| Item | Crab waste protein | Soybean meal |
|---|---|---|
| | % | |
| Taurine | .3 | .05 |
| Hydroxyproline | .05 | .11 |
| Aspartic acid | 4.37 | 4.87 |
| Threonine | 1.24 | 1.68 |
| Serine | .96 | 1.87 |
| Glutamic acid | 5.34 | 7.41 |
| Proline | 2.1 | 2.24 |
| Lanthionine | .15 | 0.00 |
| Glycine | 2.27 | 1.89 |
| Alanine | 2.21 | 1.92 |
| Cysteine | .14 | .667 |
| Valine | 2.27 | 2.15 |
| Methionine | 1.07 | .59 |
| Isoleucine | 2.02 | 1.99 |
| Leucine | 3.59 | 3.36 |
| Tyrosine | 2.29 | 1.57 |
| Phenylalanine | 2.54 | 2.24 |
| Hydroxylysine | .01 | .02 |
| Hystidine | 1.45 | 1.18 |
| Ornithine | .48 | .03 |
| Lysine | 2.11 | 2.77 |
| Arginine | 2.04 | 3.2 |
| Tryptophan | .93 | .57 |
| Total | 39.93 | 42.38 |

[a] DM basis

**Table 13. Chemical composition of the diets used in the diets and chicken excreta**

| Treatment | Dry matter | Crude protein[a] | Gross energy[a] |
|---|---|---|---|
| | % | % | kcal / kg |
| | | Diets | |
| 0% CWPS diet | 90.72 | 22.18 | 4086 |
| 25% CWPS diet | 90.6 | 21.7 | 4114 |
| 50% CWPS diet | 91.85 | 22.25 | 4118 |
| SEM | .25 | .11 | 6.39 |
| | | Excreta[b] | |
| 0% CWPS diet *ad lib* | 12.76$^{cd}$ | 26.75$^{cd}$ | 3444$^{c}$ |
| 25% CWPS diet *ad lib* | 14.67$^{c}$ | 29.38$^{e}$ | 3646$^{e}$ |
| 50% CWPS diet *ad lib* | 18.51$^{e}$ | 33.98$^{f}$ | 3774$^{f}$ |
| 25% CWPS diet pair-fed | 13.32$^{cd}$ | 30.33$^{ce}$ | 3590$^{e}$ |
| 0% CWPS diet pair-fed | 10.78$^{d}$ | 26.41$^{d}$ | 3367$^{c}$ |
| SEM | .62 | 1.8 | 21.5 |

[a] DM basis

[b] Average of 3 d

[cdef] Means within columns with different superscript letters differ ($P < .05$).

By the end of the first week, the body weight had increased three to four fold (Table 14). Daily gain for the first week decreased linearly ($P < .05$) with increased levels of CWPS for the *ad libitum* and pair-fed birds. Daily gains were quite good even for the birds fed 50% CWPS diet (8.37 g / d). Feed intake for the first week followed a similar trend as daily gain in the *ad libitum* (linear effect $P < .05$). Thus the difference in the performance was due at least partly to difference in feed intake. The difference in feed intake between the chicks fed 0 and 25% CWPS diet at *ad libitum* was small. In the pair-fed chicks, the reason for the lower feed intake for the chicks fed the 0 and 25% CWPS diets than those fed the 50% CWPS diet was that the adjustment in intake was made a day later. The gain / feed showed a linear decrease ($P < .05$) with increased levels of CWPS in the diets for the chicks fed *ad libitum* and pair-fed.

At the end of the second week, the body weights increased for all treatments. Weights decreased linearly ($P < .05$) with increased levels of CWPS for both *ad libitum* and pair-fed chicks. Daily gains of chicks increased over two folds in the second week for all treatments compared to wk 1. The daily gains again showed a linear decrease ($P < .05$) with increased levels of CWPS in both *ad libitum* and pair-fed chicks. The feed intakes of chicks fed 0 and 25% CWPS diets at *ad libitum* were similar, (285 and 279 g, respectively) while it was lower for the 50% CWPS diet fed chicks (241 g) and the decrease was linear ($P < .05$). The gain / feed was highest for the chicks fed 0% CWPS diets. There was a linear decrease ($P < .05$) in the gain / feed with increased levels of CWPS in both *ad libitum* and pair-fed chicks. The chicks fed the 0 and 25% CWPS diets had higher gain / feed when pair-fed than when fed *ad libitum*.

**Table 14. Performance of chicks fed differential proportions of crab waste protein supplement and soybean meal**

| | | Feeding method and percent crab waste protein supplement | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Ad libitum* | | | Pair-fed | | |
| Item | Week | 0 | 25 | 50 | 25 | 0 | SEM |
| Body weight, g | 0[a] | 38.28 | 37.28 | 38.48 | 38.83 | 38.14 | .58 |
| | 1[bd] | 140.17 | 124.63 | 97.05 | 103.99 | 114.29 | 3.06 |
| | 2[bd] | 351.22 | 305.89 | 226.53 | 260.11 | 292.53 | 7.55 |
| | 3[cd] | 619.75 | 566.66 | 408.08 | 458.3 | 501.07 | 11.3 |
| Daily gain, g | 1[bd] | 14.56 | 12.46 | 8.37 | 9.31 | 10.88 | .42 |
| | 2[bd] | 30.15 | 25.9 | 18.5 | 22.3 | 28.57 | 1.09 |
| | 3[c] | 38.36 | 37.25 | 25.94 | 28.31 | 29.85 | 1.04 |
| | Cum.[cd] | 27.67 | 25.21 | 17.6 | 19.97 | 22.43 | .61 |
| Feed intake, g | 1[bd] | 131.46 | 128.55 | 116.81 | 99.12 | 97.95 | 3.16 |
| | 2[b] | 284.53 | 278.9 | 240.73 | 213.98 | 215.47 | 8.97 |
| | 3[c] | 393.33 | 399.1 | 337.01 | 340.72 | 332.91 | 8.94 |
| | Cum.[b] | 809.33 | 806.56 | 694.55 | 653.82 | 646.33 | 16.91 |
| Gain / feed | 1[bd] | .78 | .68 | .50 | .66 | .78 | .02 |
| | 2[bd] | .75 | .66 | .55 | .73 | .84 | .03 |
| | 3[bd] | .69 | .65 | .53 | .58 | .63 | .01 |
| | Cum.[bd] | .74 | .66 | .53 | .66 | .75 | .01 |
| PER[e] | 1[bd] | 3.85 | 3.46 | 2.46 | 3.34 | 3.87 | .09 |
| | 2[bd] | 3.72 | 3.33 | 2.7 | 3.71 | 4.17 | .15 |
| | 3[cd] | 3.4 | 3.31 | 2.63 | 2.95 | 3.11 | .08 |
| | Cum.[bd] | 3.66 | 3.37 | 2.6 | 3.34 | 3.72 | .07 |

[a] - Initial weight
[b] - Linear effect among *ad libitum* fed ($P < .05$)
[c] - Linear and quadratic effect among *ad libitum* fed ($P < .05$)
[d] - Linear effect among pair-fed ($P < .05$)
[e] - Protein Efficiency Ratio

The body weights of chicks at the end of the third week were 620, 567 and 408 g, when fed the 0, 25 and 50% CWPS diets *ad libitum*. There was a linear and quadratic decrease ($P < .05$) in the body weight with increased levels of CWPS among the *ad libitum* fed and a linear decrease in the pair-fed chicks. The daily gains of the *ad libitum* fed chicks showed linear and quadratic decreases ($P < .05$) when CWPS was incorporated at increasing levels. However, there were no significant differences among the pair-fed birds. The daily gains of the 0 and 25% CWPS diets fed chicks were similar both in the *ad libitum* (38.4 vs. 37.3g) and pair-fed birds (29.9 vs. 28.3g). Feed intake followed a similar trend as daily gain. A slightly higher feed intake for the chicks pair-fed the 25% CWPS diet than that of 50% CWPS diet fed birds may be due to the error when the feed intake was corrected for the dead bird of the 50% CWPS group. The gain / feed was lower during the third week than the first and second weeks. Chicks fed 0 and 25% CWPS diets at *ad libitum* showed higher feed efficiency than those pair-fed. Gain / feed decreased linearly ($P < .05$) with increased levels of CWPS both among the *ad libitum* and pair-fed chicks.

The cumulative daily gains were 27.7, 25.2 and 17.6 g respectively for the 0, 25 and 50% CWPS diets fed ad libitum. The decrease in gains observed with the incorporation of CWPS showed linear and quadratic effects ($P < .05$). The cumulative gains among the pair-fed birds showed a linear decrease ($P < .05$) with increased levels of CWPS in the diet. However, the cumulative daily gains of 25% CWPS diet fed chicks were not very different from those of 0% CWPS diet fed birds, both for *ad libitum* and pair-fed. The cumulative feed intake was not different among the pair-fed birds but there was a linear decrease ($P < 05$) among the *ad libitum* fed chicks as the level of CWPS was increased from 0 to 50%. The decrease in feed intake was less severe between chicks fed 0 and 25% CWPS diets

*ad libitum*, compared to those fed the 50% CWPS diet. The overall gain / feed showed a linear decrease ($P < .05$) among the *ad libitum* and pair-fed chicks with increased levels of CWPS in the diets. Reduced weight gains and higher food consumption were reported in minks when the protein concentrate from king crab waste was used to supply 10 and 20% of the protein of standard diets (Watkins et al., 1982).

The PER decreased linearly with increased levels of CWPS both in *ad libitum* and pair-fed chicks for wk 1 and 2 (Table 14). The effect was linear and quadratic ($P < .05$) at the end of third week in chicks fed *ad libitum*, an indication of only a small difference between the chicks fed 0 and 25% CWPS diets. The PER showed a linear decrease ($P < .05$) with increased level of CWPS in pair-fed chicks. The cumulative PER of chicks were 3.66, 3.37 and 2.6 when fed *ad libitum* and 3.72, 3.34 and 2.6 when pair-fed with 0, 25 50% CWPS diets, respectively (linear effect, $P < .05$). A low PER value was reported by Rua et al. (1985) in rats when they were fed a mixture of byproducts from shark fillets and shrimps. The PER of protein recovered from shrimp waste and crab waste were reported to be from 2.72 to 2.88 and 2.3 to 2.42, respectively in rats (Shahidi and Synowiecki, 1992) which agrees with the values obtained for the 50% CWPS diet in the present study.

The N intake of chicks fed *ad libitum* showed a linear decrease ($P < .05$) with increased levels of CWPS (Table 15) because of lower feed intake. The N excretion increased linearly ($P < .05$) with increased CWPS levels in the pair-fed chicks, indicating a reduced utilization of N of CWPS diets. The N retention was reduced linearly ($P < .05$) with increased CWPS levels in the *ad libitum* fed chicks, but there was no significant effect in the pair-fed chicks.

**Table 15. Nitrogen and energy balance of chicks fed differential proportions of crab waste protein supplement and soybean meal**

| | Feeding method and percent crab waste protein supplement | | | | | |
|---|---|---|---|---|---|---|
| | *Ad libitum* | | | Pair-fed | | |
| Item | 0 | 25 | 50 | 25 | 0 | SEM |
| N intake, g[a] | 1.92 | 1.92 | 1.62 | 1.51 | 1.50 | .05 |
| N excretion, g[c] | .82 | .90 | .82 | .69 | .59 | .03 |
| N retention, g/d[a] | 1.1 | 1.02 | .79 | .82 | .91 | .03 |
| GE intake, kcal[a] | 249.11 | 250.86 | 205.27 | 196.87 | 189.92 | 7.5 |
| GE excretion, kcal[a] | 66.98 | 69.77 | 56.38 | 51.28 | 47.38 | 2.07 |
| Energy retention, kcal / d[a] | 181.97 | 180. 87 | 148.89 | 145.59 | 142.54 | 6.16 |
| ME, Mcal / kg[c] | 2.97 | 2.97 | 2.97 | 3.04 | 3.07 | .02 |
| N-corrected ME, Mcal / kg[c] | 2.97 | 2.92 | 2.93 | 3.00 | 3.03 | .02 |
| N digestibility, %[bde] | 69.27 | 57.65 | 54.52 | 58.02 | 71.51 | 1.14 |

[a] - Linear effect among *ad libitum* fed ($P < .05$)
[b] - Linear and quadratic effect among *ad libitum* fed ($P < .05$)
[c] - Linear effect among pair-fed ($P < .05$)
[d] - Linear and quadratic effect among pair-fed ($P < .05$)
[e] - Calculated by taking uric acid N as 80% of urinary N excretion.

The GE intakes were similar for the chicks fed 0 and 25% CWPS diets *ad libitum* or pair-fed, but was lower for the 50% CWPS diet fed chicks. This was due to the lower feed intake of chicks fed the 50% CWPS diet. The decrease was linear ($P < .05$) for *ad libitum* fed birds. The GE excretion was reduced linearly with increased levels of CWPS in diets of chicks fed *ad libitum*. The energy excretion tended to be higher for the chicks fed the 50% CWPS diet, compared to those fed the 0 and 25% CWPS diets. Energy retention was higher for chicks fed 0 and 25% CWPS diets at *ad libitum* (linear effect, $P < .05$). Energy retention of the pair-fed chicks was not significantly different ($P > .05$). The ME / kg of diet was not different among the 0, 25 and 50% CWPS diet when fed *ad libitum*, but decreased linearly ($P < .05$) with increased CWPS levels when pair-fed. However, the differences were small. The N-corrected ME values decreased linearly ($P < .05$) with increased levels of CWPS only in the pair-fed chicks. The ME and N-corrected ME values were lower when fed *ad libitum* than when the same diets were pair-fed. This could be due to lower efficiency of utilization of nutrients when the intake is high (Maynard et al., 1977).

The calculated N digestibility showed linear and quadratic effects ($P < .05$) among the *ad libitum* and pair-fed chicks. The values decreased with increased levels of CWPS in the diet. The N digestibility was calculated taking uric acid N as 80% of urinary N (Coulson and Hughes, 1930) and the results showed a lower N digestibility for all diets containing CWPS. This may be due to the heat and alkali treatment of crab waste in the process of extraction of protein which could have made it less available.

There was a linear decrease ($P < .05$) in serum uric acid levels with increased levels of CWPS in the pair-fed chicks (Table 16) but there were no

**Table 16. Serum uric acid, uric acid index and the weight of pancreas of chicks fed differential levels of crab waste protein supplement and soybean meal**

| | Feeding method and percent crab waste protein supplement | | | | | |
|---|---|---|---|---|---|---|
| | *Ad libitum* | | | Pair-fed | | |
| Item | 0 | 25 | 50 | 25 | 0 | SEM |
| Serum uric acid, mg / dL[c] | 4.29 | 3.93 | 3.78 | 5.79 | 8.02 | .40 |
| Uric acid index [bde] | .29 | .11 | .13 | .10 | .27 | .02 |
| Pancreas wt[acf] | .31 | .36 | .42 | .36 | .30 | .02 |

[a] - Linear effect among *ad libitum* fed ($P < .05$)
[b] - Linear and quadratic effect among *ad libitum* fed ($P < .05$)
[c] - Linear effect among pair-fed ($P < .05$)
[d] - Linear and quadratic effect among pair-fed
[e] - Uric acid excretion (g) / N intake (g)
[f] - Expressed as percentage of body weight.

significant differences in *ad libitum* fed chicks. The serum uric acid values were higher for the pair-fed chicks of both control and 25% CWPS diets than the *ad libitum* fed ones. This was in contrast to many of the reports where there was a similar pattern in the uric acid excretion and uric acid levels in blood (Miles and Featherston, 1974; Hevia and Clifford, 1977). This could be due to the fact that most of the research was conducted with amino acid deficient diets or with very high protein levels. Since serum uric acid results from uric acid production from endogenous and dietary sources (Garrel et al., 1991), the higher serum levels of uric acid in the pair-fed ones may be due to some tissue break down to meet their energy requirement for fast growth. However, similar serum uric acid values were reported by Singh and Ray (1980) and Hevia and Clifford, 1977), while Miles and Featherston (1974) observed a higher uric acid levels (12.35 mg /dL) when the diet was supplemented with lysine.

The uric acid index showed linear and quadratic decreases ($P < .05$) in chicks fed CWPS diets both at *ad libitum* and when pair-fed. The uric acid excretion, expressed per unit of N intake, was lower for chicks fed diets containing CWPS. The chicks fed the control diet *ad libitum* and pair-fed showed uric acid index values of .29 and .27 respectively. Similar values were reported by Solsberg (1971), but slightly higher values were reported in chicks fed amino acid supplemented diets(Miles and Featherston, 1976; Millan et al., 1984; Vit et al., 1993). Chicks fed low quality proteins had higher uric acid excretion as reported by Salter et al. (1974) and Miles and Featherston (1974). The total uric acid excretion and uric acid concentration in the excreta were found to be indicative of protein quality of chicks (Hevia and Clifford, 1977). A higher uric acid excretion was reported in children as a result of high protein intake (Sirivech et al., 1978). However, the uric acid index values obtained for the control diet fed chicks in the

present study were within the normal range of reported values. The lower values obtained for the CWPS diets could be due to the lower digestion and absorption of amino acids from the gastro-intestinal tract, since uric acid is produced as result of catabolism of absorbed amino acids. Uric acid is formed from xanthine in bird by a reaction catalyzed by NAD-dependent enzyme xanthine dehydrogenase (Miles and Featherston, 1974). Thus, the poor performance of chicks fed CWPS diets may be due to lower digestibility along with reduced feed intake and may not be due to lower utilization of absorbed amino acids.

The pancreas weight of chicks, when expressed as percentage of body weight, showed a linear increase ($P < .05$) with increased CWPS in diets at both feeding regimens. This is indicative of poor digestibility of CWPS diets since the intake of protein of low digestibility could lead to the hypertrophy of the gland to produce more proteolytic enzymes. Higher pancreas weight was also reported when chicks were fed raw SBM (Miles and Featherston. 1976; Anderson-Hafermann et al., 1992) and conventional soybeans instead of Kunitz trypsin inhibitor free soybeans (Zhang and Parson, 1993).

The lower calculated N digestibility and higher pancreas weight indicate lower digestibility of CWPS. The lower uric acid excretion of chicks fed CWPS diets indicates more efficient utilization of the absorbed amino acids for growth. The amino acid composition of CWPS did not indicate a deficiency of any of the essential amino acids. The poor performance of chicks fed CWPS could be due to the lower feed intake and poor digestibility of the material.

## Implications

Chicks fed diets containing CWPS showed lower growth, N-retention and PER, due at least partly to lower intake and digestibility of CWPS diets. The chicks fed 25% CWPS diets showed satisfactory intakes and gain / feed ratios indicating that CWPS can be incorporated at 25% level in the diets of chicks. The similar essential amino acid contents of CWPS and SBM, along with lower uric acid excretion in chicks fed CWPS indicated the high quality of protein and the better utilization of the absorbed amino acids. A lower temperature treatment for the extraction of protein might have been beneficial in preventing lowering the protein availability in chicks. Purification of protein to remove the fat and minerals may also be beneficial to concentrate the CP content and to improve the feeding value of the product.

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# CHAPTER 5

## General Discussion

Crab processing waste, consisting of the shell, viscera and part of meat, is a rich source of protein, minerals and chitin. It is been used as crab meal for feeding of ruminant animals. It has also been shown from the research conducted in this laboratory that it could be successfully ensiled with forages of low protein content with the addition of molasses (Abazinge et al., 1993; 1994). The fast deterioration of the product is the major constraint in the utilization of the crab waste as a feed ingredient. The loss of freshness of fish was found to start immediately after catching and the accumulation of lactic and pyruvic acids and depletion of 5'-inosine monophosphate were found to be responsible for the loss of quality (Spinelli, 1971).

Trimethylamine is produced as a result of bacterial action on TMAO which is a natural component of marine animals (Strom et al, 1979). The TMAO acts as a terminal electron acceptor under anaerobiasis and thus support the growth of some of anaerobic bacteria including *Halobacterium*, *Haloarcula* and *Haloferax* (Oren, 1990). The TMAO was found to stimulate the growth of *Salmonella* (Kim and Dhang, 1974), *Proteus* NTHC 153 (Strom et al., 1979) and *Alteromonas* sp. (Easter et al., 1982). Various enzymes have been isolated having the property of reducing TMAO to TMA (Kim and Chang, 1974; Parkin and Hultin, 1982; Joly et al., 1992).

Malle et al. (1986) reported that TMA production leads to undesirable flavor in marine products. They developed a rapid method for assessing the sanitary quality of deep frozen fish fillet based on the reduction of TMAO to TMA. Thus, TMA level represents an indirect measure of the microorganisms

present to decompose the muscle tissue. In the present study treatment with .4% NaOCl resulted in lower TMA and indole during a period of 17 d while treatments with $NaNO_2$ and $NaN_3$ preserved crab waste for 21 d. Treatment with NaCl was effective in keeping the waste without deterioration for up to 40 d. The lower TMA values obtained in the present experiment for the crab waste treated with $NaNO_2$, $NaN_3$ and NaCl indicate low deterioration of the product.

The production of gases such as $NH_3$ and $H_2S$ is related to the bacterial decomposition of the product. The $NH_3$ and $H_2S$ levels were lower for materials which were preserved well in the present study. The $NH_3$ increased with time in all the treatments except that of the NaCl- and $NaN_3$-treated materials. The $H_2S$ tended to decrease with storage time in all the treated as well as untreated materials. Lower $NH_3$ and $H_2S$ values were reported in crab waste treated with .2% NaOCl, compared to the untreated waste (Ayangbile et al., 1987). Many of the methods for the detection of specific spoilage bacteria are based on TMA and $H_2S$ production (Gram, 1992). Thus, low levels of $NH_3$ and $H_2S$ indicate less spoilage of the material.

Indole is a product of decomposition of proteins containing tryptophan and is formed under anaerobiasis in the absence of carbohydrates (Clarke et al., 1937). The level of indole in shrimps was used to classify them into different classes (Duggan and Strasburger, 1946). Indole was found to be responsible for the specific aroma of water-boiled duck meat (Wu and Liou, 1992). Indole content was higher in fresh crab meat than fresh crab byproducts (Chung and Cadwallader, 1993). The higher indole values obtained in some of the treatments in the present study indicate their inefficiency in preventing protein degradation and thus to preserve the material.

Sodium chloride has been used to preserve fish by two fundamental methods: dry salting for non-fatty fish and pickling for fatty fish (Hockenhull, 1967). Most of the psychrophilic proteolytic *Pseudomonas* and *Achramobacter* sp were killed by pickling, while dry salting destroyed most of the organisms except *Micrococci*, if the NaCl concentration was less than 12%. A NaCl concentration of 6% prevented the complete germination of anaerobic spores (Duncan and Foster, 1968). These results generally agree with those of the present study in which it was found that 10% NaCl preserved crab processing waste for at least 40 d. Perhaps a lower NaCl level would have been effective.

The mode of bacterial inhibition by salt was found to be primarily by its plasmolytic effect (Busta and Foegeding, 1983). Other antimicrobial effects include dehydration, interference with enzymes, $O_2$ removal or toxicity of high Na or Cl ion concentration. The effect of NaCl was attributed to the loss of Mg from bacterial cells (Sato et al., 1972; Ito et al., 1977). Ito et al. (1977) further observed degradation of RNA during incubation of *E. coli* cells in tris buffer containing .15M NaCl which was suggested to be due the enzyme action of RNAase 1.

Easter et al. (1982) found a reduction in TMA production when $NaN_3$, $NaNO_2$ and $KNO_3$ were added to the culture media of *Alteromonas* sp NCMB 400. Positive results were obtained with $NaN_3$ and $NaNO_2$ in the present experiment, although $KNO_3$ was not found to be effective in preserving crab waste. Sodium nitrite was found to be effective in preserving crab waste for 10 d by Ayangbile et al. (1989).

Sodium nitrite has been used as a food preservative which inhibited the development of *Clostridium* species and other food spoiling microorganisms in preserved foods (Walters and West, 1964). Decreased botulinal toxin formation with increased levels of nitrite was observed in bacon inoculated with *Clostridium*

*botulinum*. The inhibitory effect of nitrite on *Clostridium sporogenes* was found to be due to the reaction of nitrite with some compounds of the medium producing unknown inhibitory substances (Perigo et al., 1967). Moran et al. (1975) found that the inhibitor was formed of cysteine, ferrous sulfate and $NaNO_2$.

Nitrite inhibited the active transport, $O_2$ uptake, and oxidative phosphorylation in *Pseudomonas aeruginosa* (Rowe et al., 1979). Benedict (1980) reported that the inhibition of *Clostridium botulinum* in cured meat was due to several mechanisms which included oxidation of cellular biochemical compounds, restriction of use of iron and inhibition of cell membrane activity. Glidewell and Glidewell (1993) have shown that in the presence of iron salts, nitrite reacts with cysteine and other sources of cysteine to yield iron-sulfur nitrosyl salt ($Na\{Fe_4S_3(NO)_7$) which was more potent than nitrite itself under a wide range of substrates.

Thus, the positive results obtained up to 20 d with the addition of 1% $NaNO_2$ to crab waste samples in the present study may be due to the inhibition of the growth of microorganisms in the material. Fox et al. (1974) reported that reductants such as ascorbate and cysteine, histidine and the reduced coenzyme NAD caused the important loss of nitrite added to meat slurries by reducing nitrite to nitric oxide. Thus the loss of nitrite may be one of the causes of the negative results obtained starting with d 30 in the present experiment.

Sodium hypochlorite (.4%) preserved crab waste for 17 d in the present study. Ayangbile (1989) reported that NaOCl at .2% level preserved crab waste for 5 d. Sodium hypochlorite has been used in the preservation of edible products as reported by Lechowich (1981) and Park et al. (1991). The antibacterial action of Cl was based on its strong oxidation effect and involves penetration of the cell wall (Busta and Foegeding, 1983). It also reacts with cellular protoplasm, enzyme

systems and cell membranes causing oxidation or denaturation of protein, inactivation of enzymes, inhibition of respiration or altered membrane permeability.

Gillies (1975) reviewed the effect of different chemicals on preservation of seafood and found that tert-butyl hydroperoxide (5 to 50 ppm) inhibited the growth of a wide range of microorganisms and extended the storage life at refrigerated temperature. The negative results obtained with TBHP in the present study may be because of the storage temperature: the waste was stored at room temperature instead of the refrigerated temperature used by the above author.

Separation of protein and chitin from crab waste is a possible alternative for the utilization of crab waste. Treatment of waste with 1% KOH at 90°C for a period of 1 h gave the maximum recovery of protein in the present study. Johnson and Peniston (1982) and Shahidi and Synowiecki (1991) treated crab waste with 1 to 2% NaOH or KOH, while No et al (1989) used 3.5% KOH for the protein isolation. Treatment of crab waste with alkali for a period of 2 h was recommended by No et al. (1989) and Shahidi and Synowiecki (1991), while Johnson and Peniston (1982) treated the waste for 4 h. Treatment with alkali for 4 h resulted in lowest recovery of protein in the present experiment.

The CP content of the freeze-dried protein was 67% while it was only 44% when dried in forced-draft oven in bulk in the present study. The CP content of the recovered material was found to be lower than that reported by Peniston et al. (1969). This could be due to the high ether extract and ash contents of the material recovered in the present study. The oven dried material contained 30% ether extract and 17% ash. Johnson and Peniston (1971) reported that both shrimp and crab waste show an initial period of rapid extraction amounting to 30 to 50% of

the total protein, followed by a sharp break and a slow extraction period. They used a period of 4 h for the extraction of protein.

The proteins isolated from crab waste and shrimp waste were found to be similar to casein in total amino acid content except for a lower cysteine and methionine (Johnson and Peniston, 1981). The total amino acid content of CWPS was found to be similar to SBM in the present experiment, with a lower cysteine, lysine and arginine. The protein recovered from shrimp waste was reported to be higher in quality than that from crab waste (Shahidi and Synowiecki, 1992). The *in vitro* digestibility values obtained for CWPS and SBM were similar, showing that the amino acids are released at the same rate in both proteins.

The feeding value of CWPS was found to be lower than that of SBM when it was evaluated using chicks. The lower feed intake and digestibility were the reasons for the reduced performance. Kino and Okumura (1986) reported that the body weight loss and the reduction in N retention between amino acid deficient diets diminished when the feed intakes were equalized. The reduced performance of chicks in the present experiment even after they were pair-fed, reflects the poor utilization of CWPS diets compared to that of the control. However, the performance of chicks fed CWPS at the 25% level was comparable to the control diet fed birds, indicating that CWPS could be used as a protein supplement in the diet of chicks. Watkins et al. (1982) reported reduced body weight gains and gain / feed ratio when the protein concentrate from crab waste was fed to minks.

Protein efficiency ratio is used to compare specific proteins or protein sources (Maynard et al., 1979). In the present study, PER decreased linearly with increased levels of CWPS in the diets of chicks and the value for 50% CWPS diet fed chicks was comparable to those reported by Shahidi and Synowiecki (1992) for shrimp waste protein and crab waste protein when fed to rats. A lower PER was

reported by Rua et al. (1985) when a mixture of byproducts from shark filleting and from shrimp was fed to weanling rats. Protein efficiency ratio was reported to be the most discriminating method for estimating protein quality of poultry by-product meal (Escalona et al., 1986).

Uric acid excretion was used as an indicator of protein quality since less N is incorporated into body protein and more is excreted as uric acid by chicks fed a poor quality protein (Miles and Featherston, 1976). They obtained good agreement between uric acid excretion and PER as indicators of protein quality. A higher uric acid excretion was reported when low quality of protein was fed (Hevia and Clifford, 1978; Vit et al., 1993). Increased activity of the enzymes, xanthine dehydrogenase and nucleoside phosphorylase, along with high uric acid excretion, were reported in chicks when increasing amounts of gelatin (Millan et al., 1984) or lysine deficient diets (Vit et al., 1993) were fed. In contrast, the results of the present experiment showed lower uric acid excretion with increased levels of CWPS in the diet while the performance of chicks also decreased with increasing levels of CWPS. The uric acid excretion values for the birds fed the control diet were similar to normal values reported in the literature. Since uric acid is produced when the absorbed amino acids are not utilized for body protein synthesis, a lower uric acid excretion of chicks fed CWPS points to a lower rate of amino acid catabolism in the body. This could be due to a lower rate of amino acid absorption or high rate of utilization of absorbed amino acids for growth.

The serum uric acid levels decreased with increased levels of CWPS of pair-fed chicks in the present experiment. The values were higher for the pair-fed than the *ad libitum* fed chicks. This could be because of some body tissue break down to meet their energy requirements when food intake was restricted. Higher

serum uric acid was reported in chicks when fed amino acid deficient diets (Miles and Featherston, 1974; Hevia and Clifford, 1977).

The weight of the pancreas is an indication of the digestibility of the diets fed since the ingestion of less digestible protein can lead to hypertrophy of pancreas. In this study, the weight of pancreas (as % of body weight) increased linearly with increasing levels of CWPS in the diet, indicating that CWPS is less digestible than SBM. Increased pancreatic weights were reported by Miles and Featherston (1976) and Anderson-Haferman et al. (1992) in chicks fed raw soybeans. The N digestibility, calculated using uric acid excretion values, also indicated a decrease in digestibility of protein with increased levels of CWPS in the diets of chicks in the present experiment.

The performance of chicks fed CWPS at 25% level was comparable to that of chicks fed the control diet, indicating the possibility of using CWPS in the diets of birds. The reduced feed intake and lower digestibility of CWPS, especially when incorporated at higher levels, may be due to the contamination of the product during the recovery process. Improved technology to remove the excess fat and minerals will help to isolate protein in more pure form and perhaps get a better response when fed to chicks.

Another possible factor responsible for the reduced performance of chicks fed CWPS is the inactivation of protein by the heat and alkali during the recovery process. Using a lower temperature for the extraction of protein may be beneficial in preventing the denaturation of protein. The quick drying of the protein by spray-drying will also be helpful to retain the quality of the protein. Simultaneous separation of chitin of the discarded waste after protein separation will be helpful to add to the world food supply for animals and man.

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## Vita

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