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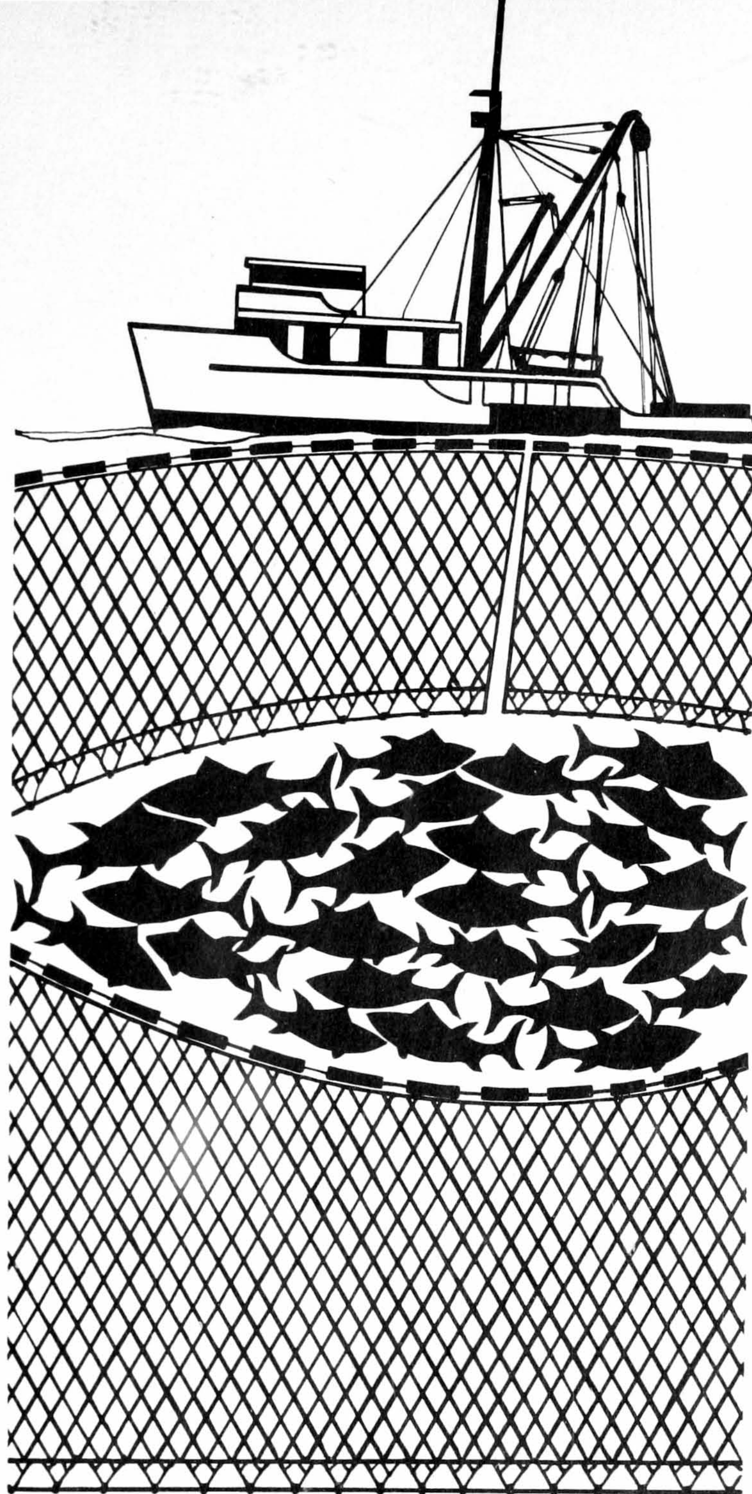

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VOLUME 5 NO. 3

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FISHERY INDUSTRIAL RESEARCH

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AUGUST 1969

As the Nation's principal conservation agency, the Department of the Interior has basic responsibilities for water, fish, wildlife, mineral, land, park, and recreational resources. Indian and Territorial affairs are other major concerns of America's "Department of Natural Resources."

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USE OF SODIUM TRIPOLYPHOSPHATE TO CONTROL FISH SHRINKAGE DURING HOT-SMOKING

by

Harold J. Barnett, Richard W. Nelson, and John A. Dassow

ABSTRACT

Loss of moisture in the hot-smoking (kippering) of thawed halibut, salmon, and black cod results in an economic loss as well as in a loss of quality. Because sodium tripolyphosphate effectively reduces loss of moisture in other foods, including fresh fish, it was tried with these smoked products. Use of this substance effectively aided the retention of moisture in halibut and salmon during smoking but was less effective in aiding the retention of moisture in black cod.

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INTRODUCTION

In the commercial production of hot-smoked fish -- such as kippered halibut, salmon, and black cod (sablefish) -- significant shrinkage due to loss of moisture occurs during the smoking. Kippered fish, unlike the partially dried hard-smoked fish, is prepared as a moist product that has a distinctive color and flavor. Therefore, from the standpoint of both acceptability and yield, the loss of moisture during smoking should be minimized.

In general, the kippering process consists of: (1) brining, (2) applying dye, and (3) hot-smoking. The thawed fish are usually split

Authors: Harold J. Barnett, *Research Chemist;* Richard W. Nelson, *Research Chemical Engineer;* and John A. Dassow, *Supervisory Research Chemist;* Bureau of Commercial Fisheries Technology Laboratory, 2725 Montlake Boulevard East, Seattle, Washington 98102.
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into two sides, with skin on, and are cut into pieces. The pieces are then soaked for one-half to 2 hours, depending on their size and weight, in a solution of concentrated brine (22 to 26 percent NaCl by weight). After the pieces have been brined, they are often dipped in a solution of orange or red food coloring[1] and are drained for a short period. The fish are then smoked at a temperature of about 80° F. for 7 to 15 hours. During the last hour, the fish are hot smoked at a temperature of 180° F. In some instances, the fish are hot smoked for a shorter time but at a higher temperature -- that is, for one-half hour at 250° F. (Jarvis, 1950).

Sodium tripolyphosphate is commonly used to control the loss of moisture in fresh fish fillets and in other food products; therefore, its application to smoked fish seemed appropriate. The purpose of the work reported here was to determine its effect in controlling the loss of moisture in kippered fish. In this experiment, we did not use sodium tripolyphosphate as an inhibitor of microorganisms of public health significance. In addition, we made no attempt to study the chemical relations between sodium tripolyphosphate and the smoked (kippered) fish.

Halibut (*Hippoglossus stenolepis*), silver salmon (*Oncorhynchus kisutch*), and black cod (*Anoplopoma fimbria*) represent major species of fish used on the Pacific Coast for kippering. Accordingly, these three species were chosen for study here.

I. HALIBUT

A. PROCEDURE

Halibut stored at −10° F. were thawed for 48 hours at 35° F. and were cut into 6- to 8-ounce pieces with the skin being retained on one side. The pieces were then divided into three lots of about equal size and weight (Table 1).

Table 1.—Smoked halibut -- effects of TPP (sodium tripolyphosphate) treatment on loss of weight

Sample	Lot number	Brined weight prior to smoking	Weight after smoking	Loss of weight
		Pounds	*Pounds*	*Percent*
Control (concentrated brine)	1	10.5	7.4	29.5
7.5% TPP + 2% NaCl	2	9.2	6.8	26.0
2.0% TPP + concentrated brine	3	9.7	7.3	24.8

The first lot was soaked at a 2:1 ratio (weight of brine to fish) for 1 hour in concentrated brine, which is a commercial procedure. This lot was used as a control.

The second lot was treated as was just described above. Then it was dipped for 1 minute in a solution containing 7.5 percent sodium tripolyphosphate and 2 percent sodium chloride. This method was found effective for treating fresh fillets in earlier tests at this laboratory (Spinelli, Pelroy, and Miyauchi, 1967).

The third lot was soaked for 1 hour in a concentrated brine solution containing 2 percent sodium tripolyphosphate.

In each of the above three treatments, the lots of fish were given a short drain of 10 to 15 minutes at room temperature. The individual lots were then weighed to determine any changes in weight due to brining. Under the brining conditions just described, no significant changes in weight were observed.

The three lots of fish were smoked overnight (14 hours) in an electrically heated smokehouse at a temperature of 70° F. Smoke was introduced into the smokehouse via an electrically operated smoke generator equipped with a blower fan. The production of smoke was controlled by an adjustable timer. Smokehouse temperatures were controlled by an electric thermostat.

During the final hour of processing, the fish were hot smoked at a maximum smokehouse temperature of 235° F. In a previous study made at this laboratory, using similar experimental procedures, we had found that this

[1] Only colorings approved by the U.S. Food and Drug Administration for use in food should be used.

procedure gave internal temperatures ranging from 180° F. in large pieces of fish (10 ounces) to 195° F. in small pieces of fish (6 ounces).

These temperatures meet interim Federal recommendations (Patashnik, Lee, Seagran, and Sanford, 1964) for time-temperature conditions in the hot-smoking of fish. Temperatures were measured by thermocouples imbedded in the flesh of the fish and connected to a multipoint recording thermometer. After being processed, the fish were cooled and weighed.

B. RESULTS

Table 1 shows that the treatment with sodium tripolyphosphate dissolved in brine effectively reduced the loss of weight in the smoked halibut as compared with the treatment with concentrated brine alone. Use of the short 1-minute dip in the solution containing 7.5 percent sodium tripolyphosphate and 2 percent sodium chloride reduced the loss of weight from 29.5 percent to 26 percent. Use of the 2 percent sodium tripolyphosphate and brine reduced the loss of weight from 29.5 percent to 25 percent. Thus, the weaker sodium tripolyphosphate treatment was the more effective.

Brining of the pieces of fish as described above gave concentrations of salt (Horwitz, 1960) in the water phase[2] of the smoked fish that ranged from 2.5 percent to 5.0 percent, depending on the size of the fish chunks.

II. SILVER SALMON

The silver salmon were treated like the halibut.

Table 2.—Smoked silver salmon -- effects of TPP (sodium tripolyphosphate) treatment on loss of weight

Sample	Lot number	Brined weight prior to smoking	Weight after smoking	Loss of weight
		Pounds	*Pounds*	*Percent*
Control (concentrated brine)	1	9.37	6.68	28.7
7.5% TPP + 2% NaCl	2	9.50	6.75	29.0
2.0% TPP + concentrated brine	3	9.44	7.06	25.2

Dipping the thawed salmon in the 7.5-percent sodium tripolyphosphate solution resulted in no significant decrease in shrinkage. Soaking the thawed salmon in the 2-percent sodium tripolyphosphate solution, however, reduced the loss of weight by 3.5 percent (Table 2). Thus, again, the weaker sodium tripolyphosphate treatment was the more effective.

The results concerning processing temperatures and salt concentrations for the smoked halibut are applicable to smoked silver salmon.

III. BLACK COD

The black cod were treated like the halibut and salmon.

Table 3.—Smoked black cod -- effects of TPP (sodium tripolyphosphate) treatment on loss of weight

Sample	Lot number	Brined weight prior to smoking	Weight after smoking	Loss of weight
		Pounds	*Pounds*	*Percent*
Control (concentrated brine)	1	9.75	7.25	25.6
7.5% TPP + 2% NaCl	2	9.50	7.19	24.6
2.0% TPP + concentrated brine	3	10.12	7.67	24.0

Treatment of the black cod with sodium tripolyphosphate, although reducing shrinkage, was not as effective as was this treatment with halibut. The reductions in loss of weight in black cod with the two solutions were 1.0 percent and 1.6 percent, respectively (Table 3).

[2] Salt concentration in the water phase of the fish is defined as:

$$\text{Percent} = \frac{\text{grams of NaCl in sample} \times 100}{\text{grams of NaCl} + \text{grams of } H_2O \text{ in sample}}$$

(Weckel and Wosje, 1966).

Again, as with halibut and salmon, the treatment with 2-percent sodium tripolyphosphate and brine was the more effective.

The results concerning processing temperatures and salt concentrations for the smoked halibut are applicable to smoked black cod.

RECOMMENDATIONS FOR INDUSTRY TRIAL

Each processor of smoked fish who plans to use the sodium tripolyphosphate treatment should determine the application and usefulness of the treatment under his own processing conditions. The following is suggested for such a plant trial with frozen dressed fish:

1. Thaw the fish at about 35° F.
2. Split the fish as required, and cut them into 6-ounce pieces. Divide the pieces into three lots of about equal weight.
3. Treat the individual lots as is described in the text. (Note: because tripolyphosphate is difficult to get into solution, the solution should be prepared at least 24 hours prior to use.)
4. Weigh each lot accurately, place it in the smokehouse, and smoke it.
5. Remove the smoked pieces of fish, let them cool, and reweigh them.
6. Calculate shrinkage by use of the following formula:

$$\frac{\text{weight loss}}{\text{weight prior to smoking}} \times 100 = \text{percent loss.}$$

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USE OF ELECTRON PARAMAGNETISM IN RESEARCH ON FISH LIPIDS

by

William T. Roubal

ABSTRACT

The products of lipid oxidation cause undesirable alterations, not only in lipids themselves, but also in the quality of associated proteins, enzymes, and other biomolecules. Fundamental studies of oxidizing lipids in biochemical systems are needed if these undesirable changes are to be minimized.

Measuring the paramagnetic properties of these systems has great potential for elucidating the mechanism of the undesirable changes and thereby giving us a possible way of finding how to control them. Unfortunately, this technique of measurement is not widely understood.

This paper explains the technique and gives examples of how the measurement of paramagnetic properties can be applied in research related to fish lipids, particularly in the relation of free-radicals to the loss of nutritive value and in the study of antioxidant functions.

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Author: William T. Roubal, *Research Chemist*, Bureau of Commercial Fisheries Food Science Pioneer Research Laboratory, 2725 Montlake Boulevard East, Seattle, Washington 98102.
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INTRODUCTION

Lipids of edible fishery products are more highly unsaturated than are those derived from other food sources. Because of the high unsaturation, fish lipids undergo extensive oxidation that often is rapid (Lundberg, 1967; Roubal, 1967).

The products of lipid oxidation cause undesirable biochemical alterations. These alterations include a reduction in the overall quality of the lipids themselves as well as a reduction in the quality of associated proteins, enzymes, and other biomolecules (Roubal and Tappel, 1966 and 1967). Fundamental studies of oxidizing lipids in biochemical systems are needed if we are to develop methods for minimizing these undesirable changes.

A relatively new technique that has great potential and that is admirably suited for studying systems containing oxidizing lipid is the measurement of their paramagnetic properties. Unfortunately, this technique is based on complex concepts that are not commonly understood. As a result, the potentialities of the technique are not recognized widely, particularly by the people in business and government who are responsible for the development of our fisheries and who do not have the time to study the complex mathematics underlying the technique. The purpose of this paper therefore is to explain, in nonmathematical language, how this technique can be applied to research involving fish lipids.

In our discussion of the technique, we consider first the theory and then the application.

I. THEORY

As will be seen, a molecule is paramagnetic when some of the electrons in the molecule are not paired. In this section, we look into the relation of electrons to magnetism and then into the measurement of the magnetic character associated with molecules containing unpaired electrons.

A. ELECTRONS AND MAGNETISM

Of major concern in this paper are free-radicals, because they are involved in the undesirable biochemical alterations caused by lipid oxidation. In this subsection, we consider first the interrelations among electrons, chemical bonds, and free-radicals, and then consider the interrelations among electrons, free-radicals, and magnetism. Our ultimate aim is to see how the interaction of electromagnetic fields with electrons maintained in a static magnetic field will enable us to detect and characterize free-radicals in oxidizing lipids.

1. Electrons, Chemical Bonds, and Free-Radicals

In general, the bonding forces that maintain the integrity of atomic combinations, or molecules, arise with pairs of electrons shared between atoms. The electrons are in motion, and, under certain conditions, they interact with one another. During some interactions, pairs of atoms split apart. Two atoms held together by electrons can break apart in one of two ways: by unsymmetrical cleavage (ionization) or by symmetrical cleavage (homolysis).

a. Unsymmetrical cleavage (ionization.)— If we use dots to represent electrons in the particular pair of shared electrons that we are considering at the moment, and the letters A and B to represent different atoms, we can illustrate unsymmetrical cleavage as follows:

$$A : B \longrightarrow A:^{-} + B^{+}$$

Unsymmetrical cleavage places both electrons of the original atomic combination with just one of the cleavage products. This process is familiar to the chemist and is called ionization.

b. Symmetrical cleavage (homolysis). — In symmetrical cleavage, called homolysis, the bonding electrons are distributed equally among the reaction products, say, as follows:

$$A : B \longrightarrow A^{\cdot} + B^{\cdot}$$

Homolysis results in the formation of free-radicals. Free-radicals thus are molecules that contain one or more unpaired electrons.

2. Electrons, Free-Radicals, and Magnetism

What is the relation between electrons and magnetism? And what is the relation of electron's magnetic field and an external magnetic field? We need to know the answers to these questions if we are to use an external magnetic field to probe into the structure of a molecule; molecules are so small that they offer great difficulty in direct observation. We therefore need some method of probing into the molecule that will produce a response that will, in turn, give us insight into the structure of the molecule. A magnetic field furnishes us with such a probe.

a. Electron electromagnetic fields. — Electrons are spinning charged particles. As a result of this rotation (spin) of the charge a tiny electromagnetic field exists about each electron.

b. Interaction of electron electromagnetic fields with external magnetic fields.—Under certain circumstances (diamagnetism), a molecule exhibits no external magnetism -- that is, the net magnetism of the molecule is zero. As a result, these molecules do not react with an external magnetic field. Under other circumstances (paramagnetism), the molecules do not react with an external magnetic field. Under other circumstances (paramagnetism), the molecules do exhibit external magnetism, and they do react with an external magnetic field.

(1) Diamagnetism.—The electrons in most molecules occur together in pairs. One electron is spinning in one direction, and the other electron in the pair is spinning in the opposite direction. Under these conditions the magnetic fields due to the rotation of the charge on each electron counteract one another, so the net magnetism in the molecule as a whole is zero. Molecules with electrons coupled in this manner are diamagnetic--that is, they are not influenced by an external magnetic field. With the exception of molecular oxygen and certain common inorganic materials, most of the chemical compounds familiar to us are of this nature.

(2) Paramagnetism.—In free-radicals, however, the magnetism of the single electron remains uncompensated. Molecules with unpaired electrons thus are paramagnetic -- that is, they are influenced by an external magnetic field.

B. MEASUREMENT OF PARAMAGNETIC PROPERTIES OF MATTER

We are now approaching the point where we can see how paramagnetism can be used in the detection and identification of free-radicals. First, however, we need briefly to

consider the precession of electrons, because electron precession is what gives rise to the phenomena that we use in our measurements.

1. Precession of Electrons

Suppose that we now place free-radicals between the poles of an electromagnet. When the magnetism of this external source interacts with the magnetism of the spinning unpaired electron of the free-radical, the electron begins to wobble in the same way that a spinning top will wobble when we push it. The frequency of the wobble, or precession, of the electron depends on the strength of the electromagnet. The stronger the magnet, the faster the precession.

If we now direct microwave energy of proper frequency onto a material containing such a precessing electron, the electron will extract energy from the radio frequency field if this field has the same frequency as that of the precession. We can show mathematically that, at the strength of the magnetic field suitable for use in our measurements, energy will be absorbed if we employ high-frequency microwaves having a wave length of about 3 centimeters.

2. Detecting and Identifying Free-Radicals

We can measure the amount of microwave energy absorbed by precessing electrons by means of an electron paramagnetic resonance spectrometer. The electron paramagnetic resonance spectrometer, or EPR spectrometer (also called the electron spin resonance spectrometer; ESR), thus is a radio spectroscope (Figure 1) for the characterization of free-radicals.

How are the data recorded in the spectrometer? As Figure 1 indicates, this instrument presents the data in graphical form. The y-axis shows the relative signal strength (amount of energy absorbed by the precessing electrons), and the x-axis shows the magnetic field strength in gauss.

Detecting free-radicals by means of this instrument is simple. Identifying them, however, is not.

a. Detection. — The problem of detecting free-radicals is straightforward. The number

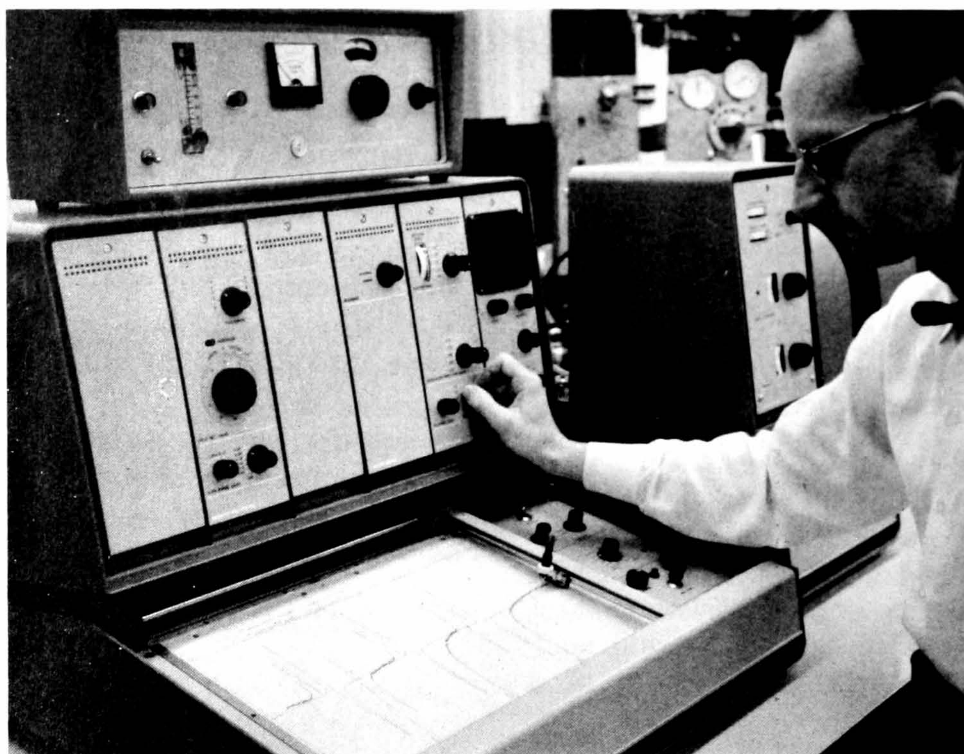


Figure 1.—Electron paramagnetic resonance spectrometer. The microwave generator and electromagnet are located in the module at the far right.

of radicals must be sufficiently large, so that they will absorb enough energy that the electron paramagnetic resonance spectrometer can detect them. The number of radicals, or more precisely the number of unpaired electrons, needed for detection is of the order of 10 billion, which is not a large number when you work at the molecular level.

b. Identification. — The problem of identifying a radical, once it is detected, is far from simple. What you observe is not correlated directly with the identity of the free-radical.

The most important means for identifying different free-radicals is called the "hyperfine interaction." Hyperfine interaction arises because the molecule contains other magnetic components in addition to unpaired electrons. Inasmuch as protons, nitrogen-14, and certain isotopes of oxygen, sulfur, and carbon exhibit, to a lesser extent, intrinsic magnetic properties, the spectrum of a free-radical may take the form of a series of absorption lines which may, or may not, vary in both spacing and intensity (amplitude) (see the curve in Figure 2). Thus, the problem of identifying free-radicals becomes one of decoding, by means of a quantum mechanical treatment, the information portrayed by the recorded collection of lines.

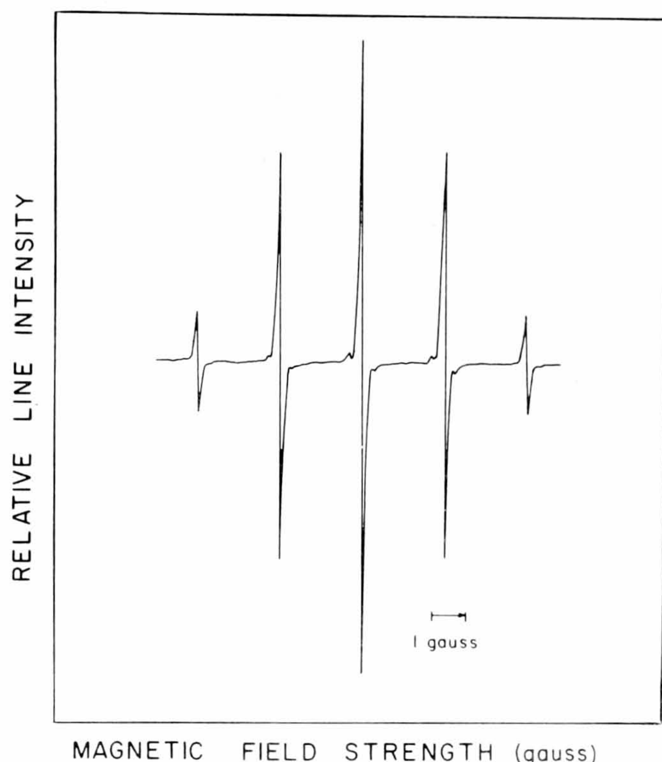


Figure 2.—Electron paramagnetic resonance spectrum of hydroquinone radical ion in solution. Major lines are identically spaced 2.37 gauss apart. The magnetic field of the center of the spectrum has a strength of 3,379.5 gauss.

II. APPLICATION

A. GENERAL APPLICATIONS

By decoding free-radical spectra, we can ascertain upon which atom or atoms the unpaired electron resides. We also can ascertain the degree of interaction of the unpaired electrons with other magnetic nuclei in a molecule. In addition, we can detect paramagnetic metal ions in chemical and biological samples and can study certain complex properties of chemical bonds.

By studying spectral changes in a dynamic system, we can obtain information as to the stability of a free-radical. Many radicals have very short lifetimes, whereas others are so stable that they can be stored indefinitely at room temperature.

We can attach stable radicals to diamagnetic biological materials. From this association, we can investigate various properties of the host diamagnetic biomolecule that would otherwise be difficult to study. This investigation of properties is possible because the spectrum of the radical is strongly affected by the environment of the radical within, or on, the framework of the biomolecule (Barrett, Green, and Chapman, 1968; Roubal, unpublished results[1]).

[1] Roubal, William T. 1968. Spin-labelling in studies of lipid peroxide protein polymerization. Unpublished manuscript filed at the Bureau of Commercial Fisheries Food Science Pioneer Research Laboratory, Seattle, Washington 98102.

B. SPECIFIC APPLICATIONS TO FISHERY RESEARCH

We now have briefly considered the general applications of free-radical spectra, but what are the specific applications to fishery research? We shall first consider the relations of free-radicals to loss of nutritive value and then the use of electron paramagnetic resonance effects in the study of antioxidants used in fishery products.

1. Relation of Free-Radicals to Loss of Nutritive Value

Proteins and amino acids in systems of oxidizing lipid are altered in such a way that they become incompletely available nutritionally (Osner and Johnson, 1968; Roubal and Tappel, 1966). This reduction in nutritive value probably continues as long as radicals are formed by the oxidation of the lipid.

Normally, free-radicals in oxidizing fishery products have a short life expectancy; but, under certain conditions, they may be trapped in a cage or matrix, which prevents them from reacting rapidly. Thus the adverse effects are merely slowed, not stopped. Our basic problem therefore is to prevent oxidizing reactions from occurring by minimizing the concentration of free-radicals. To accomplish this goal, we have to know the variables affecting their concentration.

a. Life expectancy of free-radicals.—Free-radicals associated with oxidizing lipids in fish ordinarily exhibit only a fleeting existence before they undergo a further reaction that ends with a product that is not a radical.

b. Matrixing of free-radicals. — In dried fishery products such as fish meal and fish protein concentrate, radicals apparently are trapped or caged in a matrix, inasmuch as concentrations of free-radicals are readily detected even though rapid lipid oxidation has stopped. An electron paramagnetic resonance measurement of such fishery products will reveal the extent to which the radicals persist.

Although the free-radicals trapped in a matrix do not react rapidly, they nevertheless can be expected to react eventually in the course of time. Hence, regardless of the fact that trapping the free-radicals in a matrix slows them down, this approach is not the true solution to the problem. Hence, the use of electron paramagnetic resonance is valuable in revealing the persistence of the free-radicals and can thereby aid us in research directed toward eliminating them from the product.

c. Variables affecting the concentration of free-radicals. — In our laboratory, we are investigating the effects of moisture, lipid content, and storage conditions on the level of radical concentration in dried fishery products. Certain combinations of moisture and lipid will probably favor low levels of radicals. In turn, this low level may well reflect an improvement in nutritional properties of the stored material.

2. Study of Antioxidant Functions Using Electron-Paramagnetic-Resonance Techniques

As we have indicated, free-radicals and their reaction products decrease the nutritional value of proteins and amino acids. As is well known, antioxidants help to prevent this damage. Paradoxically, in preventing the damage, some antioxidants give rise to free-radical intermediates. We would like to characterize the intermediates in order to gain insight into how these antioxidants function. Fortunately, electron paramagnetic resonance provides a basis for such a study. The aim of this section is to indicate how electron paramagnetic resonance can be used for this purpose.

In our discussion, we first consider the antioxidant hydroquinone in some detail, because using it for the purpose of illustration furnishes a relatively simple example of the relation of molecular structure to electron paramagnetic resonance spectra. With that background, we then can briefly consider the antioxidant properties of vitamin E, vitamin K, coenzyme Q, and related substances.

a. Hydroquinone. — Hydroquinone is probably the simplest commercial antioxidant of the quinoid type. It reacts with air in basic media to form a short-lived free-radical inter-

mediate, which quickly becomes a dark-colored nonradical end product.

The following phenomena are observed:

1. In the presence of air, the colorless solution of hydroquinone quickly turns bright yellow before it fades to a muddy brown color.

2. The only way that an electron paramagnetic resonance spectrum of hydroquinone free-radical can be recorded is by flowing a solution of hydroquinone in water together with a solution of dilute caustic soda and quickly passing the reacting mixture through the part of the electron paramagnetic resonance instrument that holds the sample (Figure 3). If the flowing solution is interrupted, the signal disappears.

3. A five-line spectrum consisting of equally spaced lines that have an intensity ratio (relative amplitude) of 1:4:6:4:1 is recorded (Figure 2). The fact that we have no trouble recording an electron paramagnetic resonance spectrum tells us that we are dealing with a radical produced in high concentration. The yellow color, however, was our first clue, inasmuch as many radicals are deeply colored, whereas reactants may or may not be colored. Thus, for the partial oxidation of hydroquinone in which a free-radical form could be generated, we write:

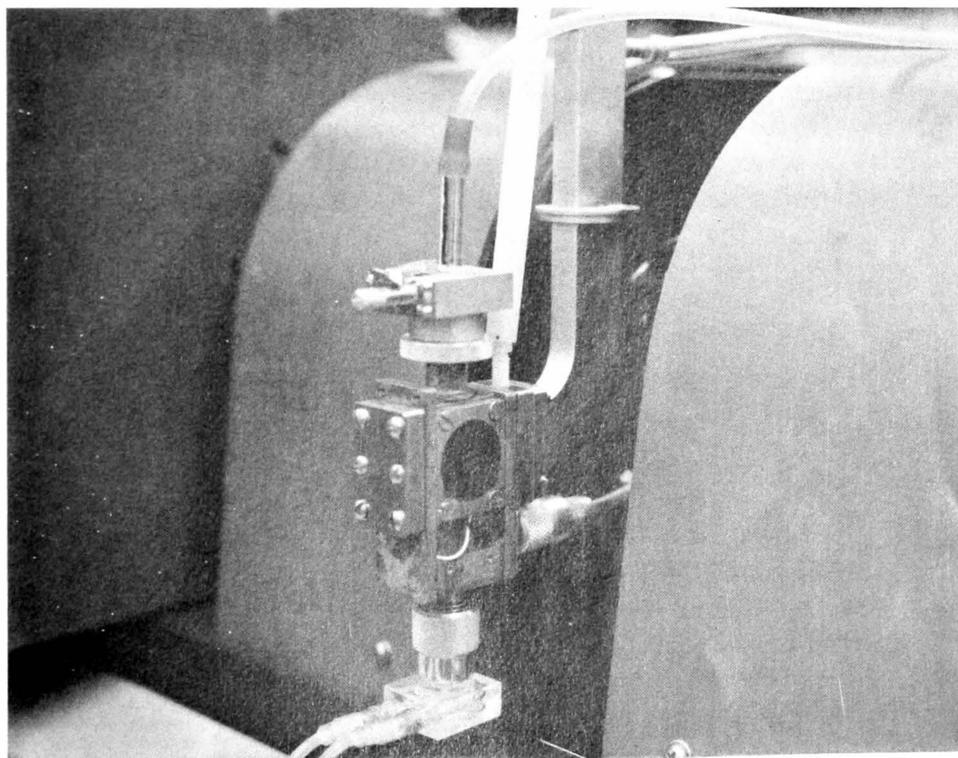
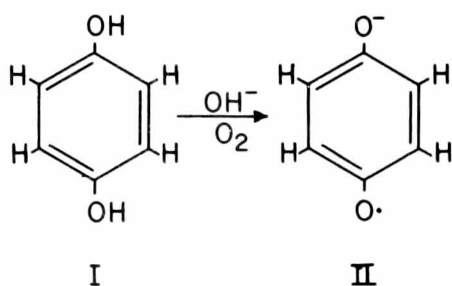
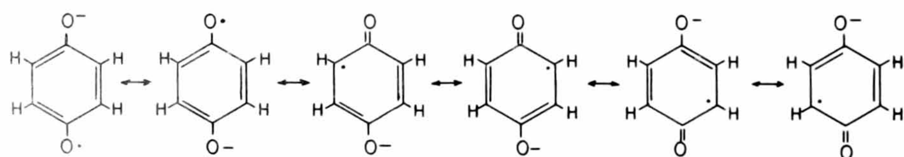


Figure 3.—Sample holder or cavity for the electron paramagnetic resonance spectrometer. For the purpose of showing the arrangement of the liquid-flow sampling cell, the cavity has been moved forward from its normal position between the poles of the electromagnet. The jet mixer, which ensures rapid mixing and delivery of reactants into the flow cell, is at the bottom of the cavity. Mixed reacted liquids exits from the top. Microwave energy is directed onto the sample by means of the rectangular waveguide connected to the rear of the cavity.



Note the unpaired electron on the oxygen atom in Structure II.

One of the protons (that is, one of the hydrogen atoms) of the two phenolic (OH) groups is hydrolyzed and provides relative stability for the intermediate. Structure II, however, does not explain other stabilizing factors or the existence of the five-line spectrum. If the unpaired electron resided solely on oxygen, only a single line would be recorded inasmuch as oxygen-16 (the major isotope of oxygen) has no spin. So, for an explanation of the five lines, we turn to the principle that no structure can be written to represent all of the observed properties of the intermediate. In actuality, the unpaired electron moves continually over the framework of the molecule; the best we can do to reveal the actual phenomenon structurally is to write as many contributing molecular structures as possible to represent this overlap, such as the following which shows the various structures possible with a single molecule:



Because the four protons of the benzene ring are equivalent, resonance theory dictates equal interaction of the electron with all four protons. The symmetrical recorded spectrum in Figure 2 bears this out perfectly.

To explain the relative amplitudes of the peaks in Figure 2, we would have to go into a mathematical discussion involving statistics, which is beyond the scope of this report. The point that we want to make here is that we can couple electron paramagnetic spectra with the appropriate mathematics to gain insight into the structure of chemical intermediates that decompose rapidly and that therefore are difficult, if not impossible, to investigate by other methods.

b. Vitamin E and related substances. — Vitamin E, vitamin K, coenzyme Q (ubiquinone), and related substances exist either in the quinoid or quinol forms, or they are capable of being converted into one of these forms under relatively mild conditions. Evidence suggests that the quinol form is the form that produces free-radicals in an oxidative environment and that it is the reactive form. Because these substances are reactive under these conditions, some of them have been used as antioxidants for nonbiological applications.

The biological antioxidative character of others have drawn considerable attention; but, for many substances, their exact function is still unknown. In model systems (that is, in artificial systems in which the researcher attempts to duplicate what he believes are the essential components of the more complex natural material), however, the antioxidative character has been fairly well defined (Gruger and Tappel, unpublished results[2]).

[2] Gruger, Edward H., and A. L. Tappel. 1968. Reactions of biological antioxidants I. Ferric iron-catalyzed dissociations of methyl linoleate hydroperoxide with α-tocopherol.
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SUMMARY AND CONCLUSIONS

Spinning electrons in molecules create tiny electromagnetic fields. Although most electrons in molecules occur in pairs with closed magnetic fields, other molecules, called free-radicals, contain one or more unpaired electrons. These molecules are paramagnetic -- that is, they interact in a predictable way with an external magnetic field. When a sufficient number of paramagnetic molecules, or free-radicals, are present, they can be detected by means of an electron paramagnetic resonance spectrometer.

Electron paramagnetic resonance spectra tell important things about the nature of the free-radical. Electron paramagnetic resonance applied to fishery research provides a powerful new tool and gives new understanding of how prooxidants and antioxidants function. Not only does electron paramagnetic resonance enable the researcher to characterize reactions at the molecular level, it also provides him with new data required in the development of better processing methods.

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MS #1927

EVALUATION OF MUSCLE HYPOXANTHINE AND VOLATILE BASES AS POTENTIAL QUALITY INDICES FOR INDUSTRIAL BOTTOMFISHES FROM THE GULF OF MEXICO

by

Enrique J. Guardia and Gerhard J. Haas

ABSTRACT

Croaker and spot are the two species of fishes found most commonly in catches of industrial bottomfishes in the Gulf of Mexico. Hypoxanthine increased linearly in both species during the first 2 weeks that these fishes were stored in ice. This test for hypoxanthine could thus indicate the quality of both croaker and spot and presumably that of the whole catch. Only after the fish had been stored 1 week in ice, however, did the total volatile bases increase. Consequently, this latter test could not be used as an index of freshness, although it might be used as an index of spoilage.

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Authors: Enrique J. Guardia and Gerhard J. Haas, Corporate Research Department, General Food Corporation, Tarrytown, New York 10591.
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INTRODUCTION

Since 1952, the annual production of industrial bottomfishes from the North-Central Gulf of Mexico has increased greatly. The annual catch in 1952, for example, was only about 2,000 or 3,000 tons, whereas that in the early 1960's was about 40,000 tons (Roithmayr, 1965).

The species of fishes taken in these catches vary markedly. In fact, the catches may contain as many as 177 species. The normal catch, however, usually contains only 20 to 30 species. Of this number, two species -- croaker (*Micropogon undulatus*) and spot (*Leiostomus xanthurus*) -- make up about 80 to 90 percent of the total. Croakers average about 65 percent of the catch (Roithmayr, 1965).

The purpose of our study was to develop an objective index of quality for these catches of bottomfishes. Levels of hypoxanthine and of total volatile bases in the muscle were studied as potential indices. The work was done on the assumption that the quality of croaker and of spot can be indicative of the quality of the whole catch.

I. HYPOXANTHINE

A. MATERIALS AND METHODS

1. Samples

The samples of fish were obtained and handled in the following manner. The fish were caught by a trawler about 5 miles off Pascagoula, Mississippi. They were iced on board the vessel and were transported to land, where they were placed in wooden boxes, washed, and repacked with fresh ice. Daily samples of croaker and spot were withdrawn, frozen in liquid nitrogen, and kept frozen in packs of solid carbon dioxide. The last sample was taken after the fish had been stored for 2 weeks. At that time, spoilage was so evident that no further sampling was needed. The samples of fish were then transported frozen to Tarrytown, New York, where they were kept at −30° C. until we analyzed them.

2. Chemical Analysis

Hypoxanthine was determined by the xanthine oxidase method of Kalckar (1947) as modified for fish by Spinelli, Eklund, and Miyauchi (1964) and by Jones, Murray, Livingston, and Murray (1964). Briefly, in this method, the fish muscle is extracted with diluted perchloric acid, filtered, and neutralized; and the anions are removed by treatment with a resin column. The effluent, which contains the nucleosides and free bases, is analyzed by the addition of xanthine oxidase. This enzyme catalyzes the conversion of hypoxanthine to uric acid. The amount of hypoxanthine present can be then determined by a decrease in absorption at 250 μ or by the concomitant increase in absorption at 290 μ due to uric acid.

B. RESULTS

As Figures 1 and 2 show, the accumulation of muscle hypoxanthine in both croaker and spot during storage in ice was nearly linear with time.

Because of impurities in the available enzyme, the analysis of hypoxanthine by means of xanthine oxidase usually requires the fractionation of neutralized perchloric acid extract by ion-exchange resins to separate the nucleotides from the fraction containing the nucleotides and free bases. In this study, we found no differences in hypoxanthine levels whether the enzyme reaction was carried out using the neutralized extract before or after fractionation (Figure 1). The close agreement in our results is probably due to the high purity of the enzyme that we used (xanthine oxidase was purchased from Worthington Biochemical Corporation, Freehold, New Jersey[1]). This agreement is important from a practical point of view, because eliminating the ion-exchange treatment can markedly reduce the time of analysis.

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[1] Trade names are mentioned merely to facilitate descriptions; no endorsement of products is implied.

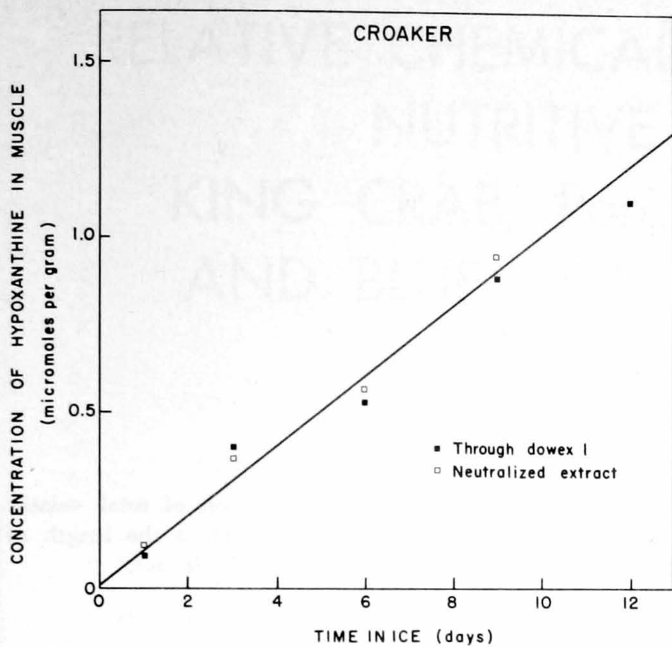

Figure 1.—Relation of the concentration of hypoxanthine in the muscle of croaker to the length of time that the fish were held in ice.

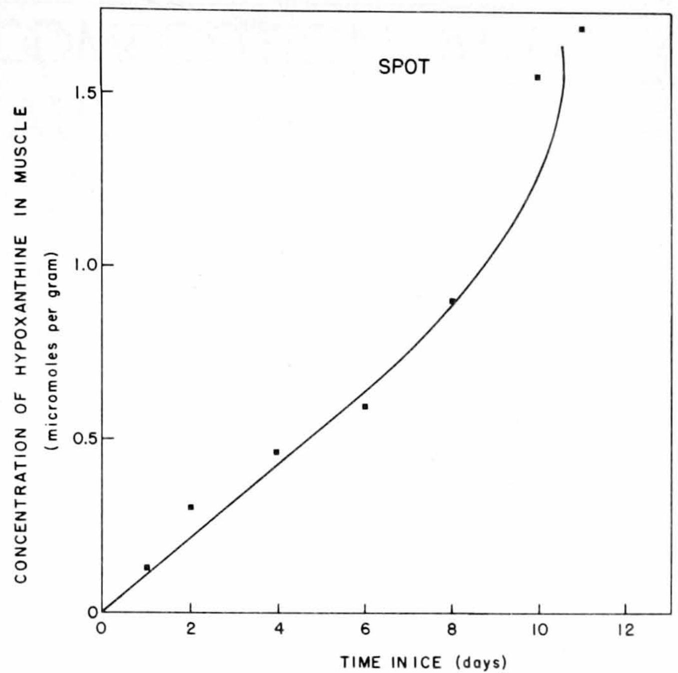

Figure 2.—Relation of the concentration of hypoxanthine in the muscle of spot to the length of time that the fish were held in ice.

II. TOTAL VOLATILE BASES

A. MATERIALS AND METHODS

1. Samples

The samples of fish were the same as those used in the hypoxanthine studies.

2. Chemical Analyses

Total volatile bases were determined by the microdiffusion method described by Stansby, Harrison, Dassow, and Sater (1944). Plastic Conway microdiffusion dishes (Obrink type) were used for this determination.

B. RESULTS

The volatile bases in the muscle of both croaker and spot increased markedly after storage of the fish for a week in ice (Figures 3 and 4). During the early part of storage, however, the level of these bases changed only slightly. Consequently, this test would be of little help in establishing differences in quality during the first days of iced storage, although it might be useful in the determination of spoilage.

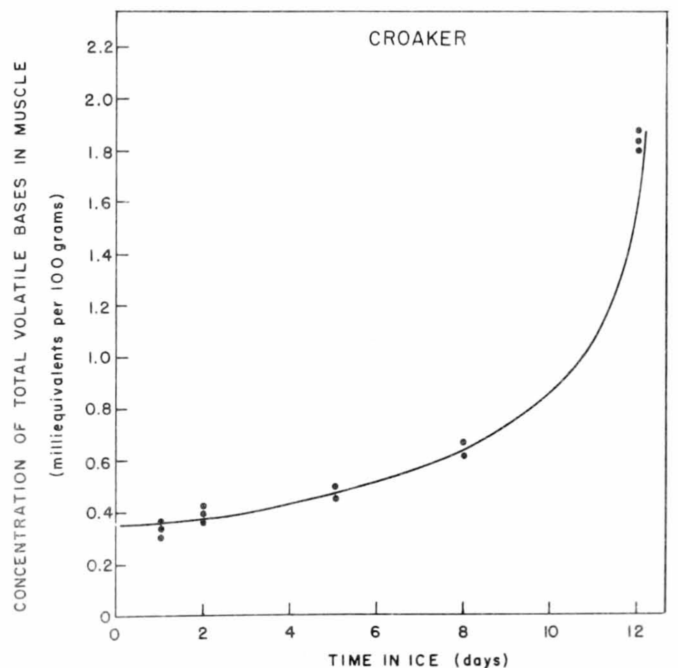

Figure 3.—Relation of the concentration of total volatile bases in the muscle of croaker to the length of time that the fish were held in ice.

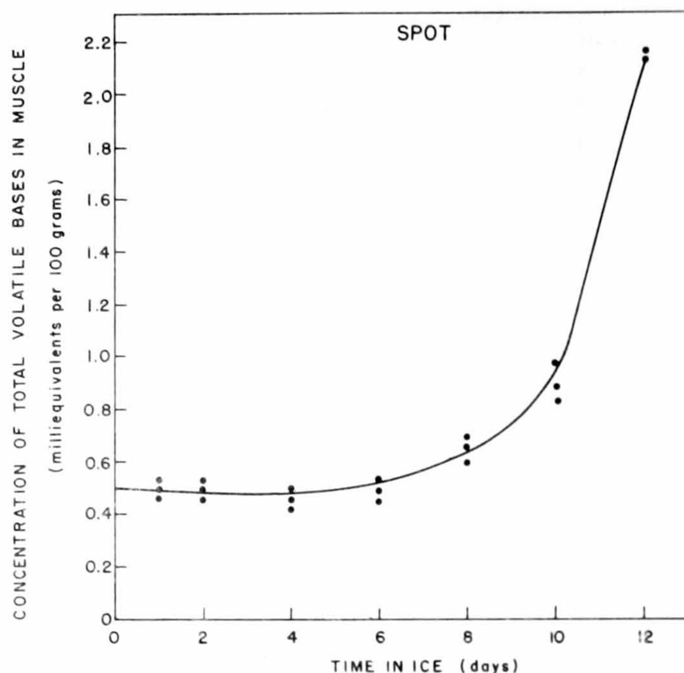

Figure 4.—Relation of the concentration of total volatile bases in the muscle of spot to the length of time that the fish were held in ice.

CONCLUSIONS

The results of this study indicate that the determination of muscle hypoxanthine can be a useful index of freshness for either croaker or spot. Because the quality of these two species is probably representative of the whole catch from which they were obtained, the test can presumably also be used as an indicator of the quality of the catch in which they are taken.

Total volatile bases apparently are produced only as spoilage sets in. Because, however, the bottomfishes of concern here are usually landed only 2 or 3 days after they are caught, no significant spoilage should occur if they are properly iced, so this test would not be a useful index of freshness. As an index of spoilage, however, the determination of volatile bases should be of value.

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MS #1906

RELATIVE CHEMICAL COMPOSITION AND NUTRITIVE VALUES OF KING CRAB, *Paralithodes camtschatica*, AND BLUE CRAB, *Callinectes sapidus*

by

Robert R. Kifer and Paul E. Bauersfeld

ABSTRACT

Alaska king crabs are being harvested in quantity. The question has arisen as to the potential and comparative value of king crab meal in broiler diets. Accordingly, king crab meal and blue crab meal (an established product) were evaluated as to their chemical composition and nutritive value when fed to chicks. Growth rates obtained were about equal when (1) diets containing the various crab meals, (2) a commercial diet, (3) diets containing menhaden meal, or (4) a corn-soybean meal-methionine-supplemented diet were fed.

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Authors: Robert R. Kifer, *Research Animal Husbandman*, and Paul E. Bauersfeld, *Biological Laboratory Technician*, Bureau of Commercial Fisheries Technological Laboratory, P.O. Box 128, College Park, Maryland 20740.
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INTRODUCTION

Blue crab meal has been demonstrated to have a high supplementary nutritive value for poultry when fed in conjunction with other protein supplements. Lubitz, Fellers, and Parkhurst (1943) and Parkhurst, Gutowska, Lubitz, and Fellers (1944) indicated that blue crab meal, when fed as a protein supplement to broilers, supported rapid growth, good feathering, good pigmentation, and high feed efficiency. The crab meal also satisfactorily replaced red fish meal (*Sebastes marinus*) used in some of the rations. Parkhurst, Gutowska, and Fellers (1944) found blue crab meal to be a satisfactory protein concentrate for laying and breeding hens.

The Alaska king crabs are currently being harvested in sufficient volume so that quantities of processing waste are available for reduction into meal. Accordingly, the question has now arisen as to the potential and comparative nutritive value of king crab meal in broiler diets. The purpose of the work reported here therefore was to obtain information on the suitability of the use of king crab meal in the diets of broilers.

The work was divided into two main parts. Part I determined the chemical composition of king crab meal relative to that of certain other feed ingredients; Part II determined its relative nutritional value.

I RELATIVE CHEMICAL COMPOSITION

In addition to king crab meal, blue crab meal, menhaden (*Brevoortia tyrannus*) meal, and soybean meal were analyzed. The following sections give the origin of the samples and the analyses of them.

A. ORIGIN OF SAMPLES

Menhaden meal, soybean meal, and two samples of blue crab meal were purchased from commercial sources.

Two samples of king crab waste (carapaces, blood, viscera, and tails) were obtained from a processing plant and were frozen. The material was then cooked in a steam-jacketed kettle until the protein was coagulated and all visible water was evaporated. The moist crab waste, after being cooked, was passed through a Hercules Meat-Bone Chopper.[1] It was then spread on galvanized wire trays and dried at 150° F. The dried waste was ground through a Hobart Food Chopper twice--once through a coarse plate and once again through a finer plate.

Although our primary interest in this study was in the king crab meal and blue crab meal, we included menhaden meal and soybean meal for comparison, inasmuch as they are commonly used and are well-known feed ingredients.

B. ANALYSES

The fishery products used were analyzed chemically for (1) proximates (protein, fat, ash, and water), (2) amino acids, (3) macrominerals (calcium, magnesium, phosphorus, potassium, and sodium) and trace minerals (aluminum, barium, boron, chromium, copper, iron, manganese, strontium, and zinc), and (4) glucosamine.

1. Proximate Compositon

a. Procedure. — Protein (Kjeldahl N x 6.25) was determined according to the methods of analysis (2.042) of the Association of Official Agricultural Chemists (1965). Moisture was obtained by drying a 2-gram sample 16 hours in a forced-air-draft oven at 100° C. Ash was determined on a 2-gram sample in a muffle furnace at 600° C. for 16 hours. Fat was determined by the extraction of samples by ethyl ether for a 4-hour period using a Goldfisch apparatus.

b. Results.—Table 1 gives the results of the analyses. The two king crab meals contained 12 percent more crude protein (corrected for glucosamine) than did the blue crab

[1] The use of trade names is merely to simplify description; no endorsement is implied.

meals. Compared with menhaden and soybean meal, the blue crab meals contained 1/2 to 3/5 the content of protein, and the king crab meals contained 2/3 to 4/5 the content of protein.

Table 1.—Proximate composition of various feedstuffs used in Experiments I and II

Constituent	Concentration of constituents in:					
	King Crab Meal		Blue Crab Meal		Menhaden meal	Soybean meal
	I	II	I	II		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Protein	42.09	45.18	30.72	33.78	62.06	51.30
Corrected protein[1]	38.42	41.31	28.15	30.59	--	--
Fat (ether)	4.68	10.14	1.90	2.15	11.13	0.56
Ash	35.68	26.56	50.30	45.71	16.84	5.80
Moisture	4.59	4.88	4.16	4.60	7.63	9.92

[1] Nitrogen from glucosamine was considered to be nonprotein nitrogen and therefore was subtracted, resulting in a corrected protein value.

Ether-fat values for the king crab meals were also higher than those found for the blue crab meals. All crab-meal fat values except for King Crab Meal II were lower than those found for menhaden meal. The fat content of soybean meal fat was markedly lower than that found in all the fishery products.

The blue crab meals contained about 50 percent more ash than the king crab meals.

2. Amino Acids

a. Procedure. — Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine were determined by the method of Moore, Spackman, and Stein (1958) and Spackman, Stein, and Moore (1958).

Samples were prepared by open hydrolysis of 0.5 gram in 250 milliliters of 6 N HCl for 24 hours and analyzed on either a Beckman/Spinco or Phoenix automatic amino acid analyzer by standard procedures. L-nor-leucine and L-α amino-β-guanadino propionic acid-HCl were used as internal standards.

Cystine was determined microbiologically by the Wisconsin Alumni Research Foundation according to the method of Henderson and Snell (1958).

Tryptophan was determined chemically by the method of Spies and Chambers (1948, 1949). For tryptophan analysis, samples were hydrolyzed with 19 N H_2SO_4 and p-dimethylaminobenzaldehyde and then were reacted with $NaNO_2$; the intensity of color produced was compared with a standard curve. Although many chemists use alkaline hydrolysis, we have found that, for fish meals, the hydrolysis with 19 N H_2SO_4 is more satisfactory and that less tryptophan is destroyed.

b. Results.—Tables 2A and 2B give the results. Data in Table 2A are expressed as a percent of corrected proteins and in Table 2B as a percent of ingredient.

The crab meals did not differ markedly in amino acid concentrations. Menhaden meal contained greater concentrations of the essential amino acids (on a percent-of-protein basis) than did any of the crab meals.

3. Minerals

a. Procedure. — Calcium and phosphorus were determined by the method of Kingsley and Robnett (1958), which was modified and adapted to the Technicon AutoAnalyzer by Smith, Kurtzman, and Ambrose (1966). This method for calcium uses the dye Eriochrome Blue S.E. The calcium ion is determined in that portion of the dye spectrum where absorbance decreases with increasing concentration of the calcium ions. A tolerance for magnesium ions of up to 12 milligrams per 100 milliliters was effected by the use of 1 N NaOH in the preparation of the dye solution. Citric acid used in the HCl diluent for the determination of calcium alone was omitted for the determination of calcium and phosphorus simultaneously.

Phosphorus was determined colorimetrically by the method of Fiske and Subbarow (1925), based upon the reaction of phosphate with molybdic acid to form phosphomolybdic acid. On treatment with 1,2,4-amino-napthol-sulfonic acid, phosphomolybdic acid is selectively reduced, producing a deep blue color (molybdenum blue), which is probably due to a mixture of lower oxides of molybdenum. The resultant blue color, which is proportional to the amount of phosphate present, is compared with that of known phosphate standards.

Table 2A.—Concentration of amino acid and glucosamine in the feedstuffs used in Experiments I and II as a percent of protein

Constituent	King Crab Meal I	King Crab Meal II	Blue Crab Meal I	Blue Crab Meal II	Menhaden meal	Soybean meal
	Percent of corrected protein				*Percent of protein*	
Amino acid						
Alanine	4.69	4.98	4.69	5.01	5.97	4.08
Ammonia[1]	3.36	2.32	3.07	3.93	1.35	2.01
Arginine	6.46	5.57	5.87	6.01	5.98	7.78
Aspartic acid	9.59	9.25	7.55	7.63	8.76	11.76
Cystine	0.54	0.94	0.52	0.72	0.70	--
Glutamic acid	11.96	11.32	9.93	10.45	12.20	17.88
Glycine	6.58	6.34	6.37	6.12	6.58	4.04
Histidine	2.69	2.23	2.29	2.39	2.52	2.56
Isoleucine	4.03	4.00	3.34	3.58	4.28	4.58
Leucine	5.82	5.90	5.12	5.36	7.10	7.39
Lysine	4.43	3.98	4.68	4.86	7.66	5.90
Methionine	1.80	1.87	1.75	1.86	2.89	1.27
Phenylalanine	4.10	4.06	3.59	4.13	3.92	4.88
Proline	4.95	4.64	4.30	4.30	4.52	5.05
Serine	4.17	4.15	3.15	3.00	3.55	4.71
Taurine	2.80	3.80	1.16	0.86	--	--
Threonine	4.06	4.21	3.28	3.32	3.85	3.72
Tryptophan	1.13	1.36	1.28	1.12	1.16	1.06
Tyrosine	4.15	4.10	3.45	3.33	3.25	3.53
Valine	5.14	5.15	4.43	4.53	4.98	4.85
Glucosamine[2]	8.56	8.85	8.37	9.43	--	--

[1] Nonamino acid, which indicates degree of amino acid breakdown.
[2] As percent of uncorrected protein.

Table 2B.—Concentration of amino acid in the feedstuffs used in Experiments I and II as a percent of ingredient

Amino Acid	King Crab Meal I	King Crab Meal II	Blue Crab Meal I	Blue Crab Meal II	Menhaden meal	Soybean meal
	Percent of ingredient					
Alanine	1.80	2.06	1.32	1.53	3.71	2.09
Ammonia	1.29	0.96	0.86	1.20	0.84	1.03
Arginine	2.48	2.30	1.65	1.84	3.71	3.99
Aspartic acid	3.68	3.82	2.13	2.33	5.44	6.03
Cystine	0.21	0.39	0.15	0.22	0.43	0.74
Glutamic acid	4.60	4.68	2.80	3.20	7.57	9.17
Glycine	2.53	2.62	1.79	1.87	4.08	2.07
Histidine	1.03	0.92	0.64	0.73	1.56	1.31
Isoleucine	1.55	1.65	0.94	1.10	2.66	2.35
Leucine	2.24	2.44	1.43	1.64	4.41	3.79
Lysine	1.70	1.64	1.32	1.49	4.75	3.03
Methionine	0.69	0.77	0.49	0.57	1.79	0.65
Phenylalanine	1.58	1.68	1.01	1.26	2.43	2.50
Proline	1.90	1.92	1.21	1.36	2.81	2.59
Serine	1.60	1.71	0.90	0.95	2.20	2.42
Taurine	1.08	1.57	0.33	0.26	--	--
Threonine	1.56	1.74	0.92	1.02	2.39	1.91
Tryptophan	0.43	0.56	0.36	0.34	0.72	0.54
Tyrosine	1.59	1.69	0.97	1.02	2.02	1.81
Valine	1.97	2.13	1.25	1.41	3.09	2.49

Calcium, magnesium, phosphorus, potassium, sodium, aluminum, barium, boron, chromium, copper, iron, manganese, strontium, and zinc were determined by the method of Christensen (1967).[2]

[2] R. E. Christensen. Computer application for direct concentration print out of plant tissue analysis by emission spectroscopy. Paper presented at the Pittsburgh Conference, March 1967.

b. Results.—As we saw earlier, the blue crab meals contained about 50 percent more ash than the king crab meals. Most of this difference is attributable to a higher content of calcium in the blue crab meal. Table 3 shows that phosphorus contents were slightly higher in the blue crab meals. The king crab meals however, contained more sodium, iron, copper,

Table 3.—Concentrations of minerals in the foodstuffs used in Experiments I and II

Mineral	Concentration of constituents in:					
	King Crab Meal		Blue Crab Meal		Menhaden meal	Soybean meal
	I	II	I	II		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Calcium	8.00	5.80	18.00	17.50	5.17	0.35
Magnesium	0.68	0.52	0.61	0.61	0.14	--
Phosphorus	1.23	1.15	1.90	1.58	2.95	0.83
Potassium	0.84	0.81	1.24	1.20	0.60	--
Sodium	4.38	2.65	1.26	1.29	0.31	--
	P.p.m.[1]	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Aluminum	161.00	190.00	350.00	510.00	270.00	--
Barium	7.00	10.90	31.50	36.00	27.00	--
Boron	22.00	22.00	22.00	29.00	12.70	--
Chromium	17.00	13.40	40.00	43.00	9.60	--
Copper	117.00	105.00	28.30	36.50	13.20	--
Iron	365.00	385.00	85.00	225.00	345.00	--
Manganese	13.00	10.50	>400.00	>400.00	25.50	--
Strontium	>200.00	>200.00	>200.00	>200.00	46.00	--
Zinc	257.00	245.00	105.00	98.00	158.50	--

[1] P.p.m. means parts per million.

and zinc; but they contained less potassium, aluminum, barium, manganese, and chromium. The ash content of menhaden meal was 10 to 33 percent lower than that of the crab meals. The phosphorus content, however, was nearly double that of the crab meals. The level of calcium in menhaden meal was about equal to that of King Crab Meal II but less than that of the remaining crab meals. With respect to the remaining mineral content, the menhaden meal contained about equal quantities of iron, zinc, aluminum, and barium and contained less potassium, magnesium, sodium, strontium, boron, chromium, and copper than did all crab meals.

4. Glucosamine

a. Procedure. — Glucosamine is the product of chitin, the mapor polysaccharide of the shells of crustaceans. Glucosamine values were determined on the four crab meals by the method of Spackman et al. (1958).

b. Results.—Table 2A gives the results. Glucosamine values of the four crab meals ranged between 8.56 and 9.43 percent of the uncorrected protein. Nitrogen from glucosamine was considered to be nonprotein nitrogen and therefore was subtracted, resulting in a corrected protein value.

II. RELATIVE NUTRITIONAL VALUE

King Crab Meals I and II were nutritionally evaluated in two chick-feeding experiments as a protein supplement to a corn-soybean-meal basal diet. In the first experiment, King Crab Meal I was compared with Blue Crab Meal I. In the second experiment, King Crab Meal II was compared with Blue Crab Meal II and with a meal made from menhaden. The control in both experiments was a corn-soybean meal diet. In Experiment II, a corn-soybean meal-methionine supplemented diet and a commercial broiler diet were included as additional controls.

Experiment II was made to verify the results obtained in Experiment I. In addition, the commercial control diet was fed to ensure that the growth of the chicks in both experiments was comparable with that obtained from feeding a practical field-tested diet. The methionine supplemented corn-soybean meal diet was included to establish that the corn-soybean meal control was responsive to the amino acid.

A. EXPERIMENT I: COMPARISON OF KING CRAB MEAL I WITH BLUE CRAB MEAL I

1. Procedure

A corn-soybean meal diet was formulated to contain (1) 22 percent of crude protein, (2) energy-supplying constituents equivalent to 1,400 metabolizable calories per pound, (3) 1.5 percent of calcium, and (4) 1.0 percent of phosphorus. Vitamins and minerals were supplied to meet the National Research Council requirements for the chick. Both crab meals were added to this control diet in a concentration gradient of 2, 4, 6, 8, and 10 percent of the diet. These additions were at the expense of a blend of corn and soybean meal to maintain the diet isonitrogenous, isocaloric, and equalized in calcium and phosphorus content. The incorporation of crab meal at levels of greater than 10 percent was precluded, owing to the restriction on the amount of calcium and phosphorus. Tables 4, 5, and 6 show the diet formulations.

Each test diet was fed to four pens of chicks, each for a 3-week experimental period. The control diet was fed to seven pens; this number was calculated from the square root of the number (rounded to the next highest number) of pens being fed diets other than the control of chicks. Feed and water were supplied ad libitum.

Table 4.—Formulation of diets fed in Experiment I

Ingredient	Concentration of ingredient in diet										
	CS[1] 1	CS+2K[2] 2	CS+4K 3	CS+6K 4	CS+8K 5	CS+10K 6	CS+2B[3] 7	CS+4B 8	CS+6B 9	CS+8B 10	CS+10B 11
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Soybean meal	33.98	32.35	30.90	29.08	27.46	25.83	32.97	31.77	30.59	29.39	28.21
Corn (US #2)	51.00	51.00	50.00	51.00	51.00	51.00	50.00	50.00	50.00	50.00	50.00
King Crab Meal I	--	2.00	4.00	6.00	8.00	10.00	--	--	--	--	--
Blue Crab Meal I	--	--	--	--	--	--	2.00	4.00	6.00	8.00	10.00
Corn oil	6.08	6.14	6.52	6.25	6.30	6.35	6.34	6.26	6.18	6.11	6.02
Dicalcium phosphate	4.94	4.88	4.27	3.65	3.08	2.46	4.08	2.73	1.35	--	--
Limestone	0.38	--	--	--	--	--	--	--	--	--	--
Phosphodic acid	--	--	0.25	0.54	0.84	1.10	0.45	1.07	1.77	2.40	2.34
Vitamin mix[4]	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Mineral mix[5]	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Ethoxyquin premix[6]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Solka floc	0.77	0.78	1.21	0.49	0.49	1.41	1.31	1.32	1.26	1.25	0.58

[1] CS = corn-soybean meal.
[2] 2K = the relative quantity of king crab meal in the respective diet (percent of diet).
[3] 2B = the relative quantity of blue crab meal in the respective diet (percent of diet).
[4] See Table 5.
[5] See Table 6.
[6] One part ethoxyquin; 9 parts cerelose.

Table 5.—Vitamin mix fed in Experiment I

Constituent	Concentration
	Grams/kilogram
Thiamine	0.140
Riboflavin	0.140
Pyridoxine	0.160
Niacin	1.200
Calcium pantothenate	0.600
Folic acid	0.080
Me-napthoquinone	0.040
B_{12} (0.13 percent)	0.580
Biotin	0.004
A (325,000 IU/gram)	0.554
D_3 (15,000 IU/gram)	2.000
E (44 IU/gram)	20.000
Choline chloride	40.000
Corn	934.543
Total	1,000.000

Table 6.—Mineral mix fed in Experiment I

Constituent	Concentration
	Grams/kilogram
Sodium chloride	100.00
Potassium chloride	100.00
Magnesium sulfate	100.00
Ferric citrate	2.38
Manganese sulfate $\cdot H_2O$	6.00
Zinc sulfate $\cdot 7H_2O$	4.00
Sodium silicate	0.80
Copper sulfate $\cdot H_2O$	0.40
Cobalt acetate $\cdot H_2O$	0.20
Ammonium molybdate	0.20
Corn	686.02
Total	1,000.00

The data for gain were submitted to an analysis of variance and a t-test, and those for the utilization of feed were submitted to Duncan's Multiple Range Test (Snedecor, 1957).

2. Results

Table 7 gives the results of Experiment I. The incorporation of either kind of crab meal (king or blue) resulted in significantly improved rates of gain as compared with those obtained with the corn-soybean control diet. Table 8 indicates specific mean comparisons (t-test). King crab meal produced maximum growth at the 6-percent level of supplementation, whereas blue crab meal did not produce maximum growth below the 8-percent level of supplementation. The low gain in weight of 361 grams obtained at the 6-percent level of supplementation with blue crab meal is probably due to one of the four replicates being much lower in weight than the other three replicates were. The mean gain in weight for the remaining three replicates was 378 grams. The inclusion of both crab meals, regardless of the level of incorporation, gave equal or superior performance to that obtained with the control.

Table 7.—Weight gain and utilization of feed obtained from feeding blue or king crab meal to chicks (Experiment I)

Diet designation	Average weight gain[1]	F/G[2]	Relative growth
	Grams		*Percent*
Corn-soybean meal (CS) ...	343	1.43	100
CS + 2% king crab meal .	350	1.42	102
CS + 4% king crab meal .	372	1.41	108
CS + 6% king crab meal .	388	1.40	113
CS + 8% king crab meal .	370	1.39	108
CS + 10% king crab meal .	356	1.38	104
CS + 2% blue crab meal .	348	1.45	101
CS + 4% blue crab meal .	373	1.38	109
CS + 6% blue crab meal .	361	1.44	105
CS + 8% blue crab meal .	396	1.39	115
CS + 10% blue crab meal .	384	1.42	112

[1] t-test comparisons are given in Table 5.
[2] No significant differences were obtained among treatments with respect to F/G = (Feed/Gain).

Table 8.—t-test comparison of rates of gain obtained with the various treatments tested in Experiment I

Diet comparison	Weight gain	t-test
	Grams *Grams*	*Level of significance*
CS versus CS + 2% KC	343 versus 350	NS
CS versus CS + 4% KC	343 versus 372	.05
CS versus CS + 6% KC	343 versus 388	.05
CS versus CS + 8% KC	343 versus 370	.05
CS versus CS + 10% KC	343 versus 356	NS
CS versus CS + 2% BC	343 versus 348	NS
CS versus CS + 4% BC	343 versus 373	.05
CS versus CS + 6% BC	343 versus 361	NS
CS versus CS + 8% BC	343 versus 396	.05
CS versus CS + 10% BC	343 versus 384	.05
CS + 2% KC versus CS + 2% BC	350 versus 348	NS
CS + 4% KC versus CS + 4% BC	372 versus 373	NS
CS + 6% KC versus CS + 6% BC	388 versus 361	.05
CS + 8% KC versus CS + 8% BC	370 versus 396	.05
CS + 10% KC versus CS + 10% BC	356 versus 384	.05

Note: CS means corn plus soybean meal
KC means king crab meal
BC means blue crab meal
NS means not significant

The utilization of king crab meal did not differ significantly from that of corn-soybean meal or of blue crab meal (Tables 7 and 8); however, a numerical improvement is evident as the level of incorporation of either crab meal increased, particularly with the blue crab meal.

B. EXPERIMENT II: COMPARISON OF KING CRAB MEAL I WITH BLUE CRAB MEAL II AND WITH MENHADEN MEAL

The purpose of Experiment II was to verify the response obtained in feeding the crab meals in Experiment I and to include additional control diets.

1. Procedure

The two control diets included were: a commercial broiler diet (24 percent crude protein) and a corn-soybean-methionine supplemented diet (22 percent crude protein). The commercial control diet was fed to ensure that the growth of chicks in both experiments was comparable with that obtained from feeding a practical field-tested diet. The methionine supplemented corn-soybean meal diet was included to establish that the corn-soybean meal control was responsive to the amino acid.

Menhaden meal was included as a comparative fishery product. The crab meals (King Crab Meal II and Blue Crab Meal II) and the menhaden meal were incorporated into the diets in the same manner as was done in Experiment I. Table 1 shows the chemical analyses

Table 9.—Formulation and calculated amino acid content of diets fed in Experiment II

Ingredient	Concentration of ingredient in diet:																	
	CS+Meth	CS	COMM	CS + King Crab Meal II					CS + menhaden meal					CS + Blue Crab Meal II				
	1	2	3[1]	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Soybean meal	33.81	33.81	--	32.22	30.46	28.72	26.98	25.22	31.54	29.16	26.76	24.35	21.95	32.65	31.35	30.04	28.74	27.43
Corn (U.S. #2)	51.00	51.00	--	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
King Crab Meal II	--	--	--	2.00	4.00	6.00	8.00	10.00	--	--	--	--	--	--	--	--	--	--
Menhaden meal	--	--	--	--	--	--	--	--	2.00	4.00	6.00	8.00	10.00	--	--	--	--	--
Blue Crab Meal II	--	--	--	--	--	--	--	--	--	--	--	--	--	2.00	4.00	6.00	8.00	10.00
Corn oil	6.65	6.65	--	6.48	6.48	6.47	6.47	6.47	6.51	6.53	6.56	6.59	6.62	6.44	6.39	6.35	6.30	6.26
Dicalcium phosphate	5.00	5.00	--	4.89	4.62	4.15	3.77	3.31	4.67	4.33	4.06	3.72	3.44	4.12	2.81	1.46	0.12	--
Limestone	0.32	0.32	--	0.11	--	--	--	--	0.32	0.24	0.18	0.16	0.11	--	--	--	--	--
Phosphoric acid	--	--	--	--	0.06	0.28	0.44	0.60	--	--	--	--	--	0.41	1.04	1.71	2.40	2.40
Vitamin mix[2]	0.85	0.85	--	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Mineral mix[3]	0.85	0.85	--	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Methionine[4]	0.40	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Sulka floc	0.92	1.32	--	2.40	2.48	2.48	2.44	2.50	3.06	3.83	4.54	5.28	5.98	2.48	2.51	2.54	2.54	2.01
Ethoxyquin premix[5]	0.20	0.20	--	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

[1] Commercial broiler diet.
[2] Vitamin mix--see Table 5.
[3] Mineral mix--see Table 6.
[4] Crystalline dl-methionine hydrochloride.
[5] 1 part ethoxyquin; 9 parts cerelose.

of the products; Table 9 shows the diet formulations.

Each meal test diet and the commercial control diet was fed to quadruplicate groups of 10 chicks. Both soybean-meal control diets (without and with methionine) were fed to eight pens calculated from the square root of the number of treatments of 10 chicks. The experimental period was from the first day to the 21st day of age of the chicks. Feed and water were supplied ad libitum. Data collected on rates of gain and utilization of feed were analyzed by an analysis of variance, a t-test, and Duncan's Multiple Range Test (Snedecor, 1957).

2. Results

Table 10 shows the results of Experiment II. Statistical analysis of the means of the treatments (Table 11) indicates that the incorporation of all three fishery products at all levels significantly improved the rates of gain when these rates were compared with those obtained with the corn-soybean meal diet. Essentially no significant differences were observed, however, between the diets containing

Table 10.—Weight gain and utilization of feed obtained from feeding blue crab, king crab, or menhaden meal to chicks (Experiment II)

Diet designation	Average weight gain[1]	F/G[2]	Relative growth
	Grams		*Percent*
Corn-soybean meal + methionine	412	1.28	113
Corn-soybean meal (CS)	365	1.42	100
Commercial control	419	1.23	115
CS + 2% King Crab Meal II	390	1.41	107
CS + 4% King Crab Meal II	416	1.42	114
CS + 6% King Crab Meal II	414	1.40	113
CS + 8% King Crab Meal II	409	1.39	112
CS + 10% King Crab Meal II	412	1.39	113
CS + 2% menhaden meal ..	395	1.35	108
CS + 4% menhaden meal ..	406	1.33	111
CS + 6% menhaden meal ..	408	1.33	112
CS + 8% menhaden meal ..	423	1.32	116
CS + 10% menhaden meal ..	417	1.29	114
CS + 2% Blue Crab Meal II	399	1.37	109
CS + 4% Blue Crab Meal II	415	1.37	114
CS + 6% Blue Crab Meal II	415	1.37	114
CS + 8% Blue Crab Meal II	418	1.37	115
CS + 10% Blue Crab Meal II	416	1.32	114

[1] t-test comparison of treatment means appears in Tables 11 and 12.
[2] Feed/Gain - Duncan's Multiple Range Test of treatment means appears in Table 13.
Note: CS means corn plus soybean meal.

the fishery products and those containing the corn-soybean-methionine-supplemented control (Table 11). Exceptions were the 2-percent level of supplementation of king crab and menhaden meals, which resulted in significantly slower rates of gain.

Note that the rates of gain obtained with the corn-soybean meal-methionine diet and with most of the diets containing fishery products equalled the rates of gain obtained with a commercial broiler starter diet (Table 10).

The gains in weight obtained with the various fishery products at each dietary level were also compared (Table 12). No significant differences were observed between the gains obtained with the fishery products at each level.

Table 11.—Experiment II: statistical comparison of weight gain obtained between the corn-soybean meal control (CS), corn-soybean meal + methionine, and treatment means.

Dietary level	Diet comparison (weight gain)			t-test
	Grams		*Grams*	*Level of significance*
	Corn-soybean meal control (CS)	versus	King Crab II	
2	365	versus	390	.01
4	365	versus	416	.01
6	365	versus	414	.01
8	365	versus	409	.01
10	365	versus	412	.01
		versus	Blue Crab II	
2	365	versus	399	.01
4	365	versus	415	.01
6	365	versus	415	.01
8	365	versus	418	.01
10	365	versus	416	.01
		versus	menhadden	
2	365	versus	395	.01
4	365	versus	406	.01
6	365	versus	408	.01
8	365	versus	423	.01
10	365	versus	417	.01
	Corn-soybean meal + methionine	versus	King Crab II	
2	412	versus	390	.01
4	412	versus	416	NS
6	412	versus	414	NS
8	412	versus	409	NS
10	412	versus	412	NS
		versus	Blue Crab II	
2	412	versus	399	NS
4	412	versus	415	NS
6	412	versus	415	NS
8	412	versus	418	NS
10	412	versus	416	NS
		versus	menhaden	
2	412	versus	395	.05
4	412	versus	406	NS
6	412	versus	408	NS
8	412	versus	423	NS
10	412	versus	417	NS

Table 13 presents the data on the utilization of feed and the results of statistical analyses of these data (Duncan's Multiple Range Test).

Equal or superior utilization of feed was obtained from incorporation of the three fishery products at all levels as compared with the

Table 12.—Experiment II: comparison of weight gain obtained between meals at each dietary level of incorporation

Dietary level	Diet comparison (weight gain)			t-test
%	*Grams*		*Grams*	*Level of significance*
	King Crab II	versus	Blue Crab II	
2	390	versus	399	NS
4	416	versus	415	NS
6	414	versus	415	NS
8	416	versus	418	NS
10	412	versus	416	NS
	King Crab II	versus	menhaden	
2	390	versus	395	NS
4	416	versus	406	NS
6	414	versus	408	NS
8	409	versus	423	NS
10	412	versus	417	NS
	Blue Crab II	versus	menhaden	
2	399	versus	395	NS
4	415	versus	406	NS
6	415	versus	408	NS
8	418	versus	423	NS
10	416	versus	417	NS

Table 13.—Experiment II: statistical comparison of utilization of feed obtained with the various treatments

Diet designation	Criterion	
	F/G[1]	
Commercial diet	1.23	a
Corn-soybean meal + methionine	1.28	bc
Corn-soybean meal + 10% menhaden	1.29	bcd
Corn-soybean meal + 8% menhaden	1.32	bcde
Corn-soybean meal + 10% Blue Crab II	1.32	bcde
Corn-soybean meal + 6% menhaden	1.33	bcde
Corn-soybean meal + 4% menhaden	1.33	bcde
Corn-soybean meal + 2% menhaden	1.35	cdef
Corn-soybean meal + 6% Blue Crab II	1.37	def
Corn-soybean meal + 8% Blue Crab II	1.37	def
Corn-soybean meal + 4% Blue Crab II	1.37	def
Corn-soybean meal + 2% Blue Crab II	1.37	def
Corn-soybean meal + 10% King Crab II	1.39	ef
Corn-soybean meal + 8% King Crab II	1.39	ef
Corn-soybean meal + 6% King Crab II	1.40	f
Corn-soybean meal + 2% King Crab II	1.41	f
Corn-soybean meal	1.42	f
Corn-soybean meal + 4% King Crab II	1.42	f

[1] Duncan's Multiple Range Tests showed that those means followed by a common letter are not significantly different and that those means with different letters are different at the 5-percent level of significance.

utilization obtained with the corn-soybean meal control diet (Table 13). Significantly greater utilization of feed was obtained from the diet containing 10 percent Blue Crab Meal II and from the diets containing 4 to 10 percent menhaden meal.

Supplementing the corn-soybean meal diet with crystalline methionine, however, resulted in a marked improvement in utilization of feed, which was equal to, or better than, that obtained with the fishery products. Equal utilization of feed values was obtained from the 10-percent blue crab level and from the 4-percent to 10-percent level of menhaden meal and the methionine supplemented corn-soybean meal diets. Significantly poorer results were obtained with the remaining diets.

Table 14 compares the results obtained with the fishery products incorporated at a given dietary level. The only significant difference obtained in the comparison of King Crab Meal II with Blue Crab Meal II was at the 10-percent level of incorporation. No differences were observed in the comparison of Blue Crab Meal II with menhaden meal. However, the utilization of feed obtained from feeding the menhaden-supplemented diets was significantly better than that obtained from feeding King Crab Meal II from the 4-percent to 10-percent levels.

Table 14.—Experiment II: comparison of utilization of feed of the various fishery products at a similar dietary level

Dietary level	Product comparison	
Percent	*F/G*[1]	
	King Crab	versus Blue Crab II
2	1.40	1.37
4	1.42	1.36
6	1.40	1.37
8	1.39	1.37
10	1.39	1.32*
	King Crab	versus menhaden
2	1.41	1.35
4	1.42	1.33*
6	1.40	1.33*
8	1.39	1.32*
10	1.39	1.29*
	Blue Crab	versus menhaden
2	1.37	1.35
4	1.36	1.33
6	1.37	1.33
8	1.37	1.32
10	1.32	1.29

[1] Duncan's Multiple Range Test.
* $P < .05$.

SUMMARY AND DISCUSSION

Under the conditions of the experiments reported here, king crab meal has a relatively high nutritive value and can be used effectively in broiler feeds. Rates of growth obtained from feeding the diets containing crab meal were equivalent to those obtained from a commercial formula, from a menhaden meal, or from a methionine-supplemented corn-soybean meal diet.

The utilization of feed in diets containing king crab meal was equally as efficient as was the utilization of feed in diets containing blue crab meal but was slightly less efficient than was the utilization of feed in the other diets tested.

Although the meals were not tested in breeding or laying diets, the crab meals could apparently be used in these feeds. Such use would take more advantage of their relatively high nutritive value and high mineral contents.

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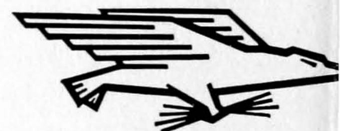

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