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Intermediate moisture breaded, deep-fried catfish

Chang-Cheng Chen

To the Graduate Council:

I am submitting herewith a thesis written by Chang-Cheng Chen entitled "Intermediate moisture breaded, deep-fried catfish." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Melvin R. Johnston, Major Professor

We have read this thesis and recommend its acceptance:

Jimmie L. Collins, James A. Corrick Jr

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(Original signatures are on file with official student records.)

March 1, 1970

To the Graduate Council:

I am submitting herewith a thesis written by Chang-Cheng Chen entitled "Intermediate Moisture Breaded, Deep-fried Catfish." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

Major Professor

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Accepted for the Council:

Vice Chancellor for
Graduate Studies and Research

INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Chang-Cheng Chen

March 1970

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ABSTRACT

This study was conducted to determine the effect of high temperature (100°F) storage on quality attributes of catfish fillets prepared to an intermediate moisture level by cooking in a solution containing glycerol, NaCl, K-sorbate and propylene glycol, followed by breading and deep-frying.

A preliminary experiment was designed to study the effect of different levels of glycerol in the cooking solution on the moisture content and water activity of the cooked samples. Subsequently, breaded fillets were deep-fried to determine the effect of frying time on moisture content and water activity. The samples previously cooked in the glycerol containing solution (30 percent and 40 percent) were fried for one, two, three, four and five minutes. Additional samples were prepared for storage and further analyses. Methods for preparation were based upon analyses of samples prepared in preliminary experiments. A cooking solution containing 35 percent glycerol, 5 percent NaCl, 0.5 percent K-sorbate and 1 percent propylene glycol and a frying time of three minutes were selected to produce fillets with a desirable water activity (A_w = 0.80).

The product contained 38.9 percent moisture, 21.2 percent glycerol, 4.29 percent salt, 19.2 percent crude fat and exhibited a water activity of 0.80. A relative humidity of 76 percent was necessary to establish the isotherm point.

Samples stored at 100°F were analyzed at weekly intervals for five weeks for moisture content, firmness, color, pH, rancidity and

iodine value. From this study the following results were found:

1. The moisture content decreased from 38.9 percent to 37.8 percent.
2. The shear values increased from 400 to 475.
3. The breaded fillets retained their light yellow color throughout storage.
4. The pH was lowered from 6.64 to 6.41.
5. The TBA value increased from 0.953 to 1.502.
6. The iodine values increased from 141.1 to 154.4.
7. The samples were contaminated after frying and when the prepared samples were stored at 100°F, the water activity was increased from 0.8 (at 70°-75°F) to near 1.0. In further research of this nature, care must be undertaken to adjust the water activity so that at the higher temperature the activity will be 0.8 to avoid spoilage.

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CHAPTER I

INTRODUCTION

Foods of intermediate moisture, identified as having a water activity between 0.6 and 0.9, were prepared on a limited basis long before the dawn of record history (9). The increasing need for food with a high caloric density has stimulated interest in the development of intermediate moisture foods for special military, manned space-flight and small animal needs. These foods have the advantages of being readily available for consumption without the need for preparation and having a more familiar mouthfeel and flavor than the dehydrated foods.

Foods of a high solute content, produced either naturally or induced, have been brought into the intermediate moisture range by partial dehydration. This dehydration may be attained through equilibrium of water and solute between external and internal aqueous phases. A number of intermediate moisture meat products, vegetable and combination items have been prepared by equilibration with a glycerol solution to produce the desired water activity. The soft-moist pet food has been the most notable example of a food item that was prepared commercially as an intermediate moisture food. Fruit filled toast products have been developed for human consumption. Extension of the technology needed to make pet food and toast products has been limited because such products must be comminuted prior to final preparation. Also the

problems of flavor, color retention, and resistance to enzymatic change remain to be solved.

There has been considerable interest in the possibility of exploiting the ocean as a source of food. Although some intermediate moisture foods have been developed, the use of seafood in this manner has been nil. Catfish is an important part of the human diet, therefore, a study was initiated to convert catfish fillets into an intermediate moisture food.

This study was conducted to develop a product with an intermediate level of moisture by cooking the catfish fillets in a glycerol solution followed by breading and deep-frying. Chemical, physical and microbiological tests were conducted on the finished products.

CHAPTER II

REVIEW OF LITERATURES

I. WATER ACTIVITY

The Concept of Water Activity

Water is the only ubiquitious ingredient in foodstuffs and is by far the most dominant constituent of foods. The behavior of water is directly related to the ultimate quality of the foods and a loss or gain in moisture content during processing or storage often accounts for major changes in the characteristics of foods (38). Changes in water distribution may lead to significant quality changes in food products.

A key factor concerning the effect of water on food stability is water activity, defined by Christian (15), Scott (49) and Matz (38) as:

$$A_w = P/P_o$$

where A_w = water activity;

P = partial pressure of water in food; and

P_o = saturated pressure of water at the specified temperature.

Christian (15) and Scott (49) considered water activity also as being numerically equal to the corresponding relative humidity (RH) which they expressed as RH/100. By this definition water activity is the property of the aqueous solution and refers to a substrate but RH is the property of the atmosphere surrounding the materials. Under an equilibrium

condition, RH = Aw x 100. Christian (15) discussed the lowering of water vapor pressure by addition of solutes, a property defined by Raoult's Law for ideal solutions. This relationship may be written in the following equation:

$$P/P_o = n_1 / n_1 + n_2$$

where n_1 and n_2 refer to the molarity of the solute and solvent in the solution, respectfully.

By this definition the water activity of a solution is equal to $n_1 / n_1 + n_2$.

Controlling Water Activity

According to Raoult's Law, since the reduction of water activity results from the concentration of solute in a solution, it may be achieved either by adding solutes or by removing water (15). Snow et al. (52) described a method to control water activity by equilibrium with a solution in a closed container. The kinds of the solutions and the strength of the solutions provide the desired water activity. Sulfuric acid has been used widely to produce an equilibrium relative humidity (40). The effect of solute, such as sucrose, salt, potassium chloride and calcium chloride to control water activity on the growth of mold has been studied by Scott (48), Heintzeler (21) and Burick (12).

Van Arsdel and Copley (54) pointed out that changes in food as affected by moisture content and temperature are related to changes in the water vapor pressure of the substance. The water sorption isotherm of a substance is a graphic representation of the equilibrium water

content at a specified temperature (48). The determination of water activity from the water sorption isotherm was described by Scott (48), Snow et al. (52), and Christian and Scott (16). They indicated that for foods these curves are sigmoid which start at zero water content at A 0.0 and rise to infinite water content at A 1.0. At intermediate water activity the position of the curve varies considerably according to the composition of the food.

In general, a moist food held at a constant temperature will display a vapor pressure which approaches a steady state equilibrium which is characteristic of the materials, its moisture content and the temperature. According to the experiment of Gane (19), the isotherm for a food varied with temperature but in general the variation is small compared to that between different foods. Charm (14) concluded that water sorption isotherms which show the relationship between moisture and percentage relative humidity for a series of temperatures are much more informative than the moisture content alone.

II. WATER ACTIVITY IN RELATION TO FOODS

Water Activity and the Growth of Microorganisms

Microorganisms require an aqueous environment in which to carry on the solute exchanges accompanying growth and reproduction. Scott (49) and Christian (15) indicated that the water requirements for growth of many microorganisms are best considered in terms of the activity of water in the immediate environment of the organism. According to

Acker (1), control of the absolute water content is not as desirable as control of the relative humidity of the air in which the food is in hygroscopic equilibrium.

Scott (49) and Christian (15) indicated that microbial growth has been reported at water activity levels ranging from very close to 1.0 to about 0.62. Each strain of organism will exhibit an optimum water activity requirement for growth, which usually lies between 1.0 and 0.9. As the water activity is increased above the optimum, the rate of growth falls sharply; as it is reduced below the optimum, the decrease in growth rate is usually less abrupt. Reduction in water activity leads also to an increase in the lag phase and for spores, it leads to an increase in the time required for germination.

Scott (49) pointed out that the general agreement among researchers concerning the minimum water activity for growth of certain molds on a variety of substrates supports the contention that it is the water activity rather than water content or particular solutes that is most important in retarding growth in dry or concentrated environments. There are many molds that germinate and grow only above A_w0.8, but some of these species, which are therm Xerophilic exhibit extremely low water requirements and grow very slowly at water activity below 0.65 (15).

Yeast has been divided into osmophilic and nonosmophilic groups. Some osmophils will multiply in syrups of high water activity, some will not (15). Von Schelohorn (46) deduced that the water activity requirements for osmophils is similar in both electrolytes and non-electrolytes. The lowest water activity at which growth of osmophilic

yeast has been observed is about 0.62 which is similar to the minimum for Xerophilic molds. Christian (15) stated that although some osmophilic yeast grow in liquid substrates below A_w0.65, yeast growth has been observed on solid substrate of A_w below 0.75.

Bacteria may be divided into halophilic and non-halophilic strains. It appears that the effect of water on the non-halophilic strain is largely independent of the solute used to control water activity (15). Of this type of bacteria the great majority, which includes most coliform organisms, will not grow at water activity below 0.94; many Bacillus species have lower limits of about A_w0.9 and Cocci grow at well below A_w0.9 (15). The usefulness of the water activity concept to describe the water requirements of bacteria was demonstrated by Scott (48) with Staphylococcus aureaus. He indicated that the lower limits of water activity for growth was not appreciably affected by the nature of the solutes used to obtain it. The halophilic group includes those bacteria which have a demonstrable requirement for sodium chloride and a considerable tolerance of it. He pointed out that the lowest level at which bacterial growth has been observed is A_w0.75, the water activity of a saturated sodium chloride solution. The water requirements of some bacteria found in various foods have been studied; these include Salmonella by Christian and Scott (16), Staphylococcus aureaus by Scott (48), Chlostridium botulimum by Williams and Purnell (57) and Bacterial coliform by Ware et al. (55).

Scott (1957) reviewed the factors that affect water requirement during growth of microorganisms. He reiterated the principle that as

the nutrients increase the rate of growth, they also increase the range of water activity. Snow et al. (52) studied food spoilage by mold. They found that the variations in the nutrient status of foodstuffs may affect the onset and severity of mold spoilage to an extent which is of practical importance at relatively low water activities. Christian (15) pointed out that all microorganisms exhibit optimum growth within specific temperature ranges. In general, they tend to be mostly resistant to the inhibitory effects of low water activity at temperatures close to the optimum growth temperatures. A similar situation exists in respect of pH. In an experiment with four strains of osmophilic yeast, Scarr (45) found the greater rates of fermentation between pH 4 and 5 than at pH 3 and 6 at a common water activity level. Chemical inhibitors appear to affect microbial water relationships in much the same way as unfavorable physical agents in that they exhibit the least inhibitor effect at a water activity near the optimum pH for growth. In this respect it is likely that many naturally occurring inhibitors whose preservative action is slight at high water activity may exert an appreciable effect when the water activity may be low (15). Other factors such as oxygen requirements and adaption also affect water requirement in the growth of microorganisms (8, 15, 16, 48).

Water Activity in Relation to Food and Food Constituents

Hamm (23) studied the effect of water activity in relation to the protein content of the products. He stated (24) that the water is immobolized within the meashes of the protein network. With large

macromolecules, water sorption occurs as a result of the interaction between the field of force at the surface of solids and that emanating from the molecule of water vapor. The amount adsorbed at given pressure and especially at the low pressure end of the isotherm is proporation to the extent of the solid surface exposed to the vapor and, therefore, to the specific surface area. Rhodin (39) found that in general at water activity values below 0.25 the adsorbed layer is only a single molecule in thickness, and a multilayer is formed as the saturated vapor is approached. Other work on adsorption vapor by protein has been reported by Dunford and Morrison (17) and Bull (11).

Starch is a carbohydrate system which has a potential for imbibing large amounts of water. The various functions of water in flour, dough and bread have been reviewed with particular reference concerning the type of bounds by which the water molecules are attached (7). Water associated with starch is believed to exist as both bound and free moisture. The bound water is held mainly by hydrogen bounding to the hydroxyl group to the carbohydrate molecules. The moisture is present in two states as indicated by the wide reversible variations in starch gelatinization temperature inducible by drying or soaking (33). A more recent discussion of moisture relationships in bread may be found in the review by Herta (22).

Water Activity in Relation to Food Deterioration

The manifestation of water as a solvent as well as a reactant has been presented in the work of Labura et al. (29). They indicated that even at low water activity sucrose may be hydrolyzed to reducing

sugars which have a potential for browning. They stated that water has a dominate influence on the rate of browning on all carbonyl-containing systems. Goldblith et al. (20) studied dehydrated orange crystals and found that a moisture as low as 1 percent on a dry basis is not low enough to completely eliminate browning and ascorbic acid destruction. They concluded that complete inhibition of this type of reaction required a complete absence of water.

Problems associated with lipid oxidation are presented in many processed foods. Lipid oxidation results in the production of carbonyl compounds that react to produce pigments (43). According to Schults et al. (47), rancidity in freeze-dried foods occur through a free radical reaction between unsaturated lipid and oxygen. Many studies have shown that for freeze-dried and dehydrated foods, storage at low moisture above a monolayer gave maximum resistance to oxidation (43). Maritinez and Laburtz (35) studied the production of peroxides in freeze-dried salmon. They showed that peroxide production decreased as water activity was increased above the monolayer of water content.

Acker (2) stated that stored foods which are protested against microbiological deterioration due to a low water content and water activity undergo, in addition to various chemical reactions, enzymatic reactions if the enzymes in these foods are still active. He noted the remarkable dependence of enzymatic reactions on moisture content and the fact that if enzymes are not inactive, they can play an important role in low moisture foods. This dependence of enzymatic reaction on moisture content cannot be explained by the law of mass action but

can be understood in relation to sorption isotherms of the corresponding food. Accordingly, it seems desirable to study the enzymatic changes in relation to the relative humidity level.

Water Activity in Relation to Food Storage Stability

Hygroscopicity is one of the most important characteristics of dried food. Hygroscopicity is the tendency of a product to attract water vapor from the atmosphere (54). Dehydrated food must be protected from moisture uptake; however, under normal conditions they have a good shelf life if the moisture content is maintained within narrow limits. Many dried foods are extremely hygroscopic and when in contact with an environment of greater relative humidity will absorb moisture rapidly (43).

Rockland (41) showed that the equilibrium relative humidity or water activity is more related to the stability of food products than the total moisture content. The moisture isotherm of heterogeneous biological products represents the integrated hygroscopic properties of numerous constituents which vary in respect to both quality and quantity.

The maximum moisture content that would permit sufficient storage stability in dried vegetables lies below the sorption isotherm point. The monolayer, as calculated by the equation of Brunauer et al. (10) corresponds also to a moisture content that lies below the sorption isotherm point. Salwin (44) found that differences in equilibrium relative humidity relationships are responsible for the migration of moisture vapor from one ingredient to another when several foods containing

several ingredients are packaged together. He contended that the moisture sorption data will serve as a useful guide for processing and packing.

III. INTERMEDIATE MOISTURE FOOD

Definition and Fundamentals of Intermediate Moisture Food

Intermediate moisture food is a heterogerous group of foods which owe their stability to reduce water activity but contain too much water to be regarded as dried foods (9). Kalpow (26) defined an intermediate moisture food as one that can be eaten as is, without rehydration, and yet is shelf stable without refrigeration or thermal processing. According to the definition, jams, jellies, marshmallows and salted fish may be classified as intermediate moisture foods (9, 26).

Depriving microbes of sufficient water is an effective method for prohibiting their growth (15, 49). Massels (37) showed that the limiting water activity for microbial growth was 0.8 for mold, 0.88 for yeast and 0.9 for bacteria. Brockman (9) pointed out that the range of water activity from 0.6 to 0.9 appeared to characterize our common intermediate moisture foods.

Brockman (9) studied the effectiveness of constituents such as sucrose, salt, glucose and glycerol in reduction of the water activity of food. He found that all the compounds have undesirable characteristics at the concentrations required to control water activity. He stated that glycerol had less flavor impact than glucose or sucrose, for all practical purposes is non-volatile, is well tolerated physciologically

and is metabolized to yield 4.3 Kcal/g. Salt should be used only at the normal level for seasoning. In addition to the substances used to depress water activity, a low concentration of an antimycotic such as potassium-sorbate should be introduced to suppress growth of mold and yeast (9). Kalpow (26) suggested the use of propylene glycol which acts as a plasticizing humectant for texture as well as contributes to the water soluble solids of the aqueous phase. Also, propylene glycol protects the product against molds and yeasts. The solution which was prepared for the reduction of water activity in food was called non-dissociated additive solution (9).

The Method for Preparation of Intermediate Moisture Food

An initial approach to the development of intermediate moisture foods was to start with dehydrated pieces of food and infuse them with an aqueous solution containing additives required for preservation and palatability. Kalpow (26) investigated several methods of drying (air, microwave, dielectric, infrared, vacuum with desiccant); all caused shrinkage and toughness in the resultant intermediate moisture food. He stated that intermediate moisture samples previously dried by freeze drying were superior to those dried by other methods. Two methods for preparation of intermediate moisture foods were worked out by Brockman (9) and Kalpow (26). One method consisted of holding food in an infusion solution to a point where, after draining, the food piece would have the proper water content and water activity. However, it was found that if a dehydrated porous, absorptive food was soaked in a solution

whose viscosity was not too high to allow penetration, the food would absorb an amount of solution approximately equal to the amount of water removed. Moist puff-dried carrots have been prepared by this method by Kalpow (26). The second method of infusion consisted of soaking a normal moisture food in a solution so that after draining, the moisture content and the water activity would be reduced to the desired levels. Brockman (9) presented the formulation of typical infusion solutions for chicken, pork, carrots and peas. In preparation of the intermediate moisture food, Brockman (9) showed that vaporation of water from the product consisting of a high solutes content must be carefully controlled to avoid accumulation of solutes at the surface.

Stability of Intermediate Moisture Food

Food is a complex substance consisting of lipids, carbohydrates, protein, metals and water. This combination makes it very difficult to predict the extent of deterioration as a function of water content, especially at the intermediate moisture level (29). Labura et al. (29) reviewed non-enzymatic browning as related water and indicated that the maximum rate of this reaction was in the intermediate moisture range. If intermediate moisture foods can be held to the level of water activity above the point of maximum browning without microbiological deterioration, some increase in storage life could be obtained.

Maloney et al. (35) studied the oxidation of lipids by moisture. They showed the effect of increasing the relative humidity on the microcrystalline cellulose surface. Increasing the concentration of water

slowed the oxidative reaction up to a relative humidity corresponding to that where the intermediate range often begins. Labura et al. (29) studied the mechanisms by which water exerts the protective effect in lipid oxidation. They pointed out that at increasing moisture levels, except at the intermediate moisture level, the above protective effect of water may substantially protect against oxidation. They also concluded that for intermediate moisture foods both oxidation and browning can occur simultaneously as indicated in the model systems studies The other limitation to stability is the growth of microorganisms, which occurs at the higher water activity level (15, 49).

According to the experiment of Brockman (9), a variety of foods in the intermediate moisture range (A_w0.80-0.85) can be prepared by equilibrating (or cooking) with an aqueous solution of glycerol, salt and antimycotic. Except for a slight but recognizable sweet taste, these products have normal sensory properties, no microbiological development or significant chemical, physical or sensory changes after three months storage at 38°C. Intermediate moisture foods have a potential application in military, spaceflight and pet food (9, 26, 51). In addition to providing a margin of safety in the event of package failure, they can provide variety and high caloric density and require no preparation for consumption.

IV. COMPOSITION OF CATFISH FLESH

One hundred grams of raw catfish supplies 10.3 calories and contains 78 percent water, 17.6 percent crude protein, 3.1 percent

crude fat, 1.3 percent ash, 0.4 mg iron, 60 mg sodium, 330 mg potassium, 0.4 mg thiamin, 0.03 mg riboflavin, and 1.7 mg niactin (56).

Borgstrom (6) reviewed the early literature concerning the loss in weight and nutritive materials during cooking. The losses in catfish differ with different cooking methods and losses of weight were greater when the fish were fried in fat than when cooked by boiling or steaming. On the other hand losses of nutrients were lowered considerable by frying. For example, the following percentage of substances lost from catfish due to boiling or steaming are compared with losses by frying, respectively: total nitrogen, 5.6-9.9 and 0.05-0.1; purine nitrogen, 12.7-17.6 and 1.7-2.4; and non-protein nitrogen, 23.6-35.0 and 1.1-2.1. Mineral element losses were considerably small from frying. He concluded that the primary loss of weight with fresh catfish is due to the loss of water, while some losses are due to the removal of nitrogenous constituents, fat and salts.

CHAPTER III

MATERIALS AND METHODS

I. SOURCE AND HANDLING OF CATFISH FILLETS

The catfish fillets were purchased from Travis Meat and Seafood Company, Knoxville, Tennessee. The product was originally supplied by United Maritime Fisherman LTD, Noncton, N. B., Canada. This catfish is a member of the Anarhichadidae family, commonly called Wolffishes. The species are Anarhichas minor known as Spotted Catfish and Anarhichas lupus known as Spotted Wolffish or Lepoardfish. From purchase time until the experiment was begun the catfish was stored at -22°F in a blast freezer in the Food Technology Department.

II. EXPERIMENTAL DESIGN FOR PREPARATION OF THE PRODUCT

The catfish product was prepared according to the procedure in Figure 1. The fillets were removed from storage, washed under tap water and sliced into pieces 1-1/2 inches x 3/4 inches x 3/8 inches. The mean weight of each piece was 15.2 grams. A weighted sample was cooked in the non-dissociated additive solution (NDAS) at 250°F for ten minutes. The NDAS consisted of an aqueous solution of glycerol, K-sorbate and propylene glycol. The weight ratio of the sample to the cooking solution in the cooking process was 1.9:1. The cooked sample was allowed to drain for 15 minutes and breaded with bread and flour. The mean weight ratio of cooked sample to bread and flour was 10.5:1.

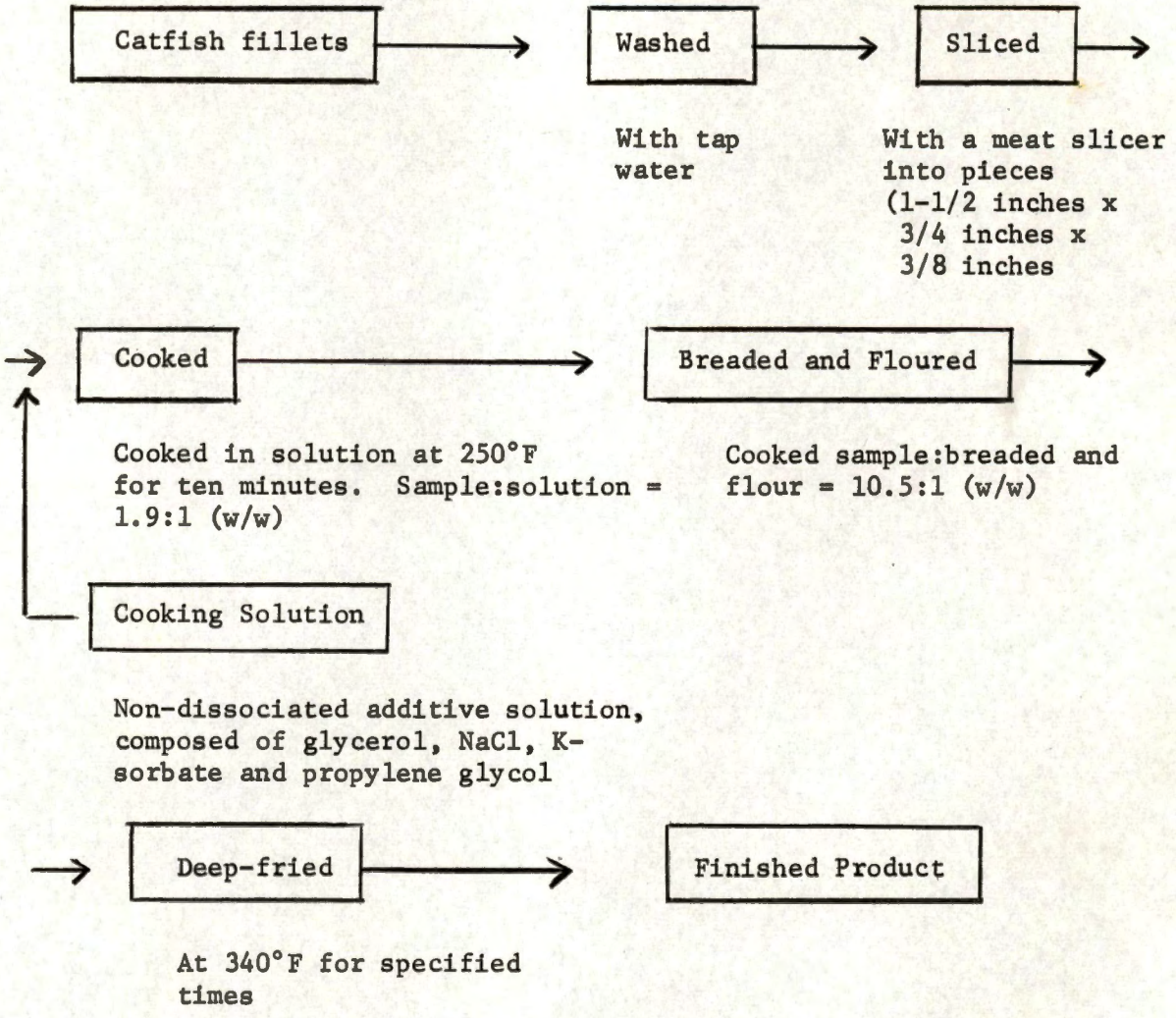


Figure 1. Flow Diagram for Preparation of Breaded Catfish Fillets

The breaded sample was fried for the specified time in corn oil at 340°F and allowed to cool five minutes on absorbant paper. The fried sample constituted the product with which the experiment was conducted. Preparation of the product was divided into three parts and is described in the following experiments.

Experiment One--Cooking of Fillets in the Different Concentrations of Non-Dissociated Additive Solution

This experiment was designed to study the effect of glycerol content in the NDAS on the relationship between moisture content and water activity in the cooked product. The raw fillets were cooked in five different concentrations of the NDAS, in order to prepare a product with different levels of moisture. The composition of the NDAS is shown in Table I. The frying time was three minutes. Preparation of these products was performed according to the flow diagram. The experiment was replicated.

Experiment Two--Different Times for Deep-frying

This experiment was performed to determine the effect of frying time on moisture content and water activity of the product. The level of glycerol to use in these tests was obtained after the cooked samples, prepared according to the procedures of experiment one, were analyzed for their water activity values. Cooking solutions containing 30 percent and 40 percent glycerol were accepted since these levels of glycerol produced cooked samples with water activity values consistant with those of intermediate moisture foods. Fillets were divided into

TABLE I

PERCENTAGE COMPOSITION OF THE NON-DISSOCIATED ADDITIVE SOLUTION IN WHICH RAW CATFISH FILLETS WERE COOKED

Composition of aqueous solution	Non-dissociated additive solution				
	S-60	S-50	S-40	S-30	S-20
	percent				
Glycerol	60	50	40	30	20
NaCl	5	5	5	5	5
K-sorbate	0.5	0.5	0.5	0.5	0.5
Propylene glycol	1.0	1.0	1.0	1.0	1.0

two groups and cooked in solution, S-30 and S-40 (Table I). Cooked samples of each group were fried at 340°F for one, two, three, four, and five minutes. The product was prepared according to the steps presented in the flow diagram (Figure 1, page 18). The experiment was replicated.

Experiment Three--Preparation of the Samples for Storage

A third experiment was designed to prepare a finished product for storage and further analyses. Conditions of this test were based upon analyses of the sample prepared according to steps in experiment two. These results indicated that the NDAS which contained 35 percent glycerol, 5 percent NaCl, 0.5 percent K-sorbate and 1 percent propylene glycol might be ideal for development of the desired water activity. The samples were cooked at 230°F for ten minutes and subsequently deep-fried at 340°F for three minutes, since the three-minute frying time previously used produced the most desirable product.

Sixty grams of the fried product from each treatment were placed in air-tight jars in duplicate and stored in an incubator at 100°F for five weeks. Samples were removed from storage at weekly intervals for analyses. The experiment was replicated.

III. METHODOLOGY AND INSTRUMENTATION

The finished products of all three experiments were analyzed for moisture content and water activity. Only the samples of experiment three were utilized for determination of composition and quality. The

following tests were performed on the finished product only at the time of preparation: total lipid content, glycerol content, salt content and hygroscopic properties. Stored samples including 0 storage were analyzed for moisture content and water activity, texture, color, pH, rancidity (TBA test), iodine value, and bacterial level (total bacteria, staphylococcus, coliform and salmonella counts).

Determination of Moisture Content

The direct oven method was sued for determination of moisture content of samples from experiments one and two. Five grams of the sample were dried at 105°C to constant weight (about 12 hours). The moisture content was calculated from the weight loss of the sample (31).

The moisture content of stored sample from experiment three was determined by freeze-drying. Five grams of sample were freeze-dried for 50 hours. The moisture content was calculated from the weight loss of the sample. Since the mean moisture content of stored samples at 0 storage was 38.89 percent by determination with direct oven method and 37.41 percent by determination with freeze-drying, in order to compare these two methods, each from freeze-drying was adjusted by adding 1.48 percent to the values of moisture content obtained by oven drying.

The sampling was carried out in duplicate and the number of observations for each value was eight.

Determination of Water Activity

An Electric Hygrometer Indicator, Type 15-3001, was used to determine water activity of the product (Hygrodynamic Inc., Silver Springs, Maryland). The temperature-humidity sensors were utilized.

For analysis, 50 grams of sample were sealed in an air-tight jar in which a temperature-humidity sensor was fitted through the lid. After an equilibrium between the product and surrounding atmosphere was reached (about 30 minutes), the temperature and humidity readings were obtained by connecting the sensing element to the indicator. Equilibrium relative humidity values were obtained from calibrated curves of the percent relative humidity at different temperatures; the curves were supplied by the manufacturer. All the results were converted to water activity values, expressed as A_w = RH/100. The sampling was carried out in duplicate and each replication had three observations.

Determination of Total Lipid Content

The continuous extraction method was used for determination of the crude lipid content. Five grams of sample were weighted into the thimble which was placed in a Soxhlet continuous extractor and extracted for 16 hours with distilled petroleum ether (25). The samples were replicated and each replication had two observations.

Determination of Glycerol Content

The method used to determine the glycerol content of the finished product was the same method employed with meat extracts and similar products. The whole product (breading and tissue) comminuted and one and

one-half grams of the blended constituted a sample. Determinations were duplicated and each replication had two observations. The procedure was the following.

Composition of reagents.

1. Strong potassium dichromate solution: 74.55 grams of dry crystals of K_2Cr_7O were dissolved in distilled water and 150 ml. of concentrated H_2SO_4 was added. The volume was brought to one liter with water. One ml. of this solution was equal to 0.01 g of glycerol in titration.

2. Dilute potassium dichromate solution: 25 ml. of strong potassium dichromate solution was brought to 500 ml. with distilled water. Twenty ml. of this solution were equal to one ml. of strong potassium dichromate solution.

3. Ferrous ammonium sulfate solution: 30 grams of $FeSO_4(NH_4)_2SO_4 \cdot 6\ H_2O$ were dissolved in water and 50 ml. of H_2SO_4 were added. The volume was brought to one liter with distilled water. One ml. of this solution was equal to approximately one ml. of dilute potassium dichromate solution in titration.

4. Retarder: 150 ml. of H_3PO_4 were diluted with 600 ml. of distilled water and 250 ml. of H_2SO_4 were added.

5. Indicator: one gram of diphenylene was dissolved in 100 ml. of H_2SO_4.

Procedure for determination. The sample was placed in a mortar and mixed with ten grams of sand and ten grams of anhydrous Na_2SO_4.

The mixtures were transferred to a Soxhlet apparatus and the entire mass was extracted with distilled anhydrous acetone for ten hours. After extraction, 5 ml. of 10 percent $AgNO_3$ solution were added to the residue and the volume was brought to 100 ml. with distilled water. The distilled residue was allowed to stand overnight and filtered. After filtering, 60 ml. of strong potassium dichromate solution and 24 ml. of H_2SO_4 were added. This mixture was heated in a water bath at 85°C for exactly 20 minutes. The flask was removed from the bath and the solution was cooled to room temperature. This solution contained oxidized glycerol and an excess of strong potassium dichromate solution.

Twenty ml. of ferrous ammonium sulfate solution were pipetted into a beaker with 20 ml. of the retarder and four drops of the indicator. This solution was titrated with the dilute potassium dichromate solution until the liquid developed a dark green color, then dilute potassium dichromate solution was added dropwise, with continuous stirring until the color changed from a blue grey to deep violet. The amount (ml.) of dilute potassium dichromate solution used was designated as (a).

Next, in place of the dilute potassium dichromate solution, 20 ml. of ferrous ammonium sulfate solution with 20 ml. of retarder and four drops of indicator were titrated with the solution containing oxidized glycerol and an excess of strong potassium dichromate solution. The amount (ml.) used was designated as (b) and the glycerol content was calculated by the following formula.

Percent glycerol content = (ml. of strong potassium dichromate solution used to oxidize glycerol - $\frac{250(a)}{20(b)}$) x 0.01 g/sample weight (g) (3).

Determination of Salt Content

The Open Official Method for fish and other marine products was used for determination of salt content. The determinations were duplicated and each value was an average of four observations. The procedure follows:

One hundred fifty ml. of 0.1 N $AgNO_3$ and 20 ml. of HNO_3 were added to ten grams of sample and boiled gently on a hot plate for 15 minutes until all solid materials, except AgCl, were dissolved. The mixture was cooled and 50 ml. of water and 5 ml. of saturated ferric ammonium sulfate were added as the indicator. This solution was titrated with 0.1 N ammonium thiocyanate which was standardized against the 0.1 N $AgNO_3$ solution until a permanent light brown color appeared. The amount (ml.) of 0.1 N $AgNO_3$ added was substracted from the amount of 0.1 N thiocyanate used in titration. The quantity of Cl^- expressed as NaCl in ten grams of sample was calculated (each ml. of 0.1 N $AgNO_3$ = 0.058 percent NaCl) (3).

Determination of Moisture Content-relative Humidity Equilibrium

To determine the moisture content-relative humidity equilibrium, a series of atmospheres were prepared by use of different concentrations of H_2SO_4. Each atmosphere was produced in a unit consisting of a glass crystallizing dish covered with a glass plate. Contacting surfaces between the dish and glass plate were covered with heavy stopcock

grease to insure an air-tight closure. The relative humidity for the atmosphere in each test unit was developed by placing in the bottom of the unit an aqueous solution of 150 ml. which contained the following percentage of H_2SO_4.

RH	H_2SO_4	RH	H_2SO_4
	percent		
100	0	35	50.9
90	18.5	25	55.9
75	30.4	10	64.8
65	36.0	0	100.0
50	43.3		

Two grams of sample consisting of 39.44 percent moisture were placed in metal pans having approximately a three inch diameter. The pan was placed on the glass support platform in the middle of the test units. The sample was equilibrium at 80°F for four hours. After equilibrium, the sample was weighed quickly. The gain or loss in weight of the samples was plotted against the relative humidity of the atmosphere in which they were held. The point at which a true equilibrium existed between relative humidity and the original moisture content of product was that where no gain and loss in weight occurred. This point was the equilibrium or isotherm point (13, 30). There were nine treatments and two replications were made.

Shear Press Measurement

An Allo-Kramer Shear-Press, model SP-12, was used to determine the texture of the product. The 1000-pound proving ring was employed and

a 25-second thrust was used. A sample consisting of 30 grams of the product was placed in the standard shear-compression cell for testing. Texture was expressed as the maximum pounds of force required to shear the sample. There were six treatments and two observations were made for each of the two replications.

Color Measurement

Color measurements were carried out by the Color-Eye (Model D-1, Instrument Development Laboratory, Division of Kollmorgan Company, Attleboro, Massachusetts). To make this measurement, a reference standard (Illuminant "C"-barium sulfate) was placed over the Standard Reference Port. A sample consisting of 6.5 grams was placed in the curvette and fitted over the sample port. The Selector Switch was set on "Hi" and Color Eye values for x, X, Y, and Z were read directly from the microdial. These values were converted to X, Y, and Z values of the CIE system based by the following equations (for tristimulus values and chromaticity coordinates):

$$X_{CIE} = 0.783X_{\text{Color Eye}} + 0.197\,x_{\text{Color Eye}}$$

$$Y_{CIE} = Y_{\text{Color Eye}}$$

$$Z_{CIE} = 0.180Z_{\text{Color Eye}}$$

The x and y were calculated as follows:

$$x = X/\ (X + Y + Z)$$

$$y = Y/\ (X + Y + Z)$$

Hue, purity, dominate wavelength, and luminosity were obtained by reference to the CIE chromaticity diagram. There were six treatments which were replicated and two observations for each were made.

pH Measurement

The pH of each sample was measured with a Beckman Zeromatic pH meter. A ten-gram sample was blended with 100 ml. of deionized water for 30 seconds. The slurry was continuously mixed with a magnetic stirrer as the pH was measured. The study consisted of six treatments in which two observations for each of two replications were made.

Measurement of TBA Value for Rancidity

The red compound of malonaldehyde formed by reaction of 2-thiobarbituric acid (TBA) with oxidized lipids has been demonstrated to be a measure of the extent of oxidative rancidity in fatty products (58). Since malonaldehyde acid hydrolysis of 1, 1, 3, 3, tetrahoxypyrance (TEP) yields malonaldehyde which reacts quantitatively with TBA, TEP was employed as a standard for the TBA determination (50). In this determination the malonaldehyde content of the product was quantitatively measured and the degree of oxidative rancidity expressed in milligrams of malonaldehyde per 1000 grams of sample (TBA Value). Six treatments were studied and two observations for each of two replications were made.

Preparation of reagent.

1. TBA reagent: this reagent was prepared from 1 percent TBA and 0.05 M citrate buffer. The former was obtained by mixing two grams of TBA, 193 ml. of water and 6.6 ml. of 2 N NaOH. After heating for five minutes, a yellow solution was obtained. The citrate buffer contained 59 grams of reagent $Na_3C_6H_5O_7 \cdot 2H_2O$ and 50 ml. of concentrated reagent HCl; the volume was brought to 400 ml. with water. The

completed TBA reagent was made by mixing two parts of TBA solution with one part of citrate buffer (28).

2. Trichloroacetic acid solution: 20 grams of trichloroacetic acid were dissolved with distilled water and the volume was brought to 100 ml.

3. Pyridine hydrochloride solution: 30 ml. of pyridine were mixed with 70 ml. of six N hydrochloric acid.

4. Mixing solution: 650 ml. of six N hydrochloric acid were mized with 50 ml. of trichloroacetic acid solution and 50 ml. of pyridine hydrochloride solution (50).

Preparation of standard curve for the absorbance of hydrolysis product of TEP versus concentration of TBA. TEP in amounts of 0.0002 to 0.001 moles per liter (dissolved in 40 percent ethanol) were weighted into a flask and 4 ml. of distilled water, 5 ml. of pyridine hydrochloride solution, 10 ml. of trichloroacetic acid solution, 6 ml. of TBA solution were added. The flask containing the mixture was connected to a condenser and placed in vigorously boiling water. The contents were refluxed for 30 mintues followed by adding of 75 ml. of 0.6 N HCl through the top of the condenser. Refluxing was continued ten minutes. The contents of the flask were cooled to room temperature in a water bath and the condenser was disconnected. Fifty ml. of the solution were transferred to 25 ml. centrifuge tube and centrifuged for five minutes at 3000 r.p.m. Ten ml. of the clear solution were pipetted to a test tube and 10 ml. of petroleum ether were added. The solution was agitated

vigorously for one minute and centrifuged for three minutes at 4000 r.p.m. The clear solution was drawn into a colorimetry tube, and the absorbance was determination at 535 nanometer (nm) by the Spectronic-20 colorimeter. The reagent blank gave an absorbance value of 0.003 which was used to make corrections for the samples. The resultant absorbance was expressed by plotting the percentage absorbance versus concentration of a series of TEP solutions (50).

Determination of TBA number of sample. One gram of the fried fillet sample was used for determination of the TBA value. The procedure was similar to the one described above except that a mixing solution was used in place of 0.6 N hydrochloric acid. After 30 minutes under reflux 75 ml. of the mixing solution were added to the reaction mixture through the top of the condenser. The results were expressed in milligrams of malonaldehyde per 1000 grams of sample (TBA value) from Figure 2 (50).

Wijs Iodine Method for Determination of Iodine Value

The unsaturated glycerides of an oil or fat have the ability to absorb a definite amount of iodine. The quality of iodine absorbed is a measure of the degree of unsaturation of an oil and fat (25). The Wijs Iodine Method was used for this determination. Two observations for each replication and two replications for each of six treatments were made. The procedure is described below.

Fat and oil extraction. Ten grams of the tissue from each sample were cut into small cubes and mascerated in a blender with 250 ml. of

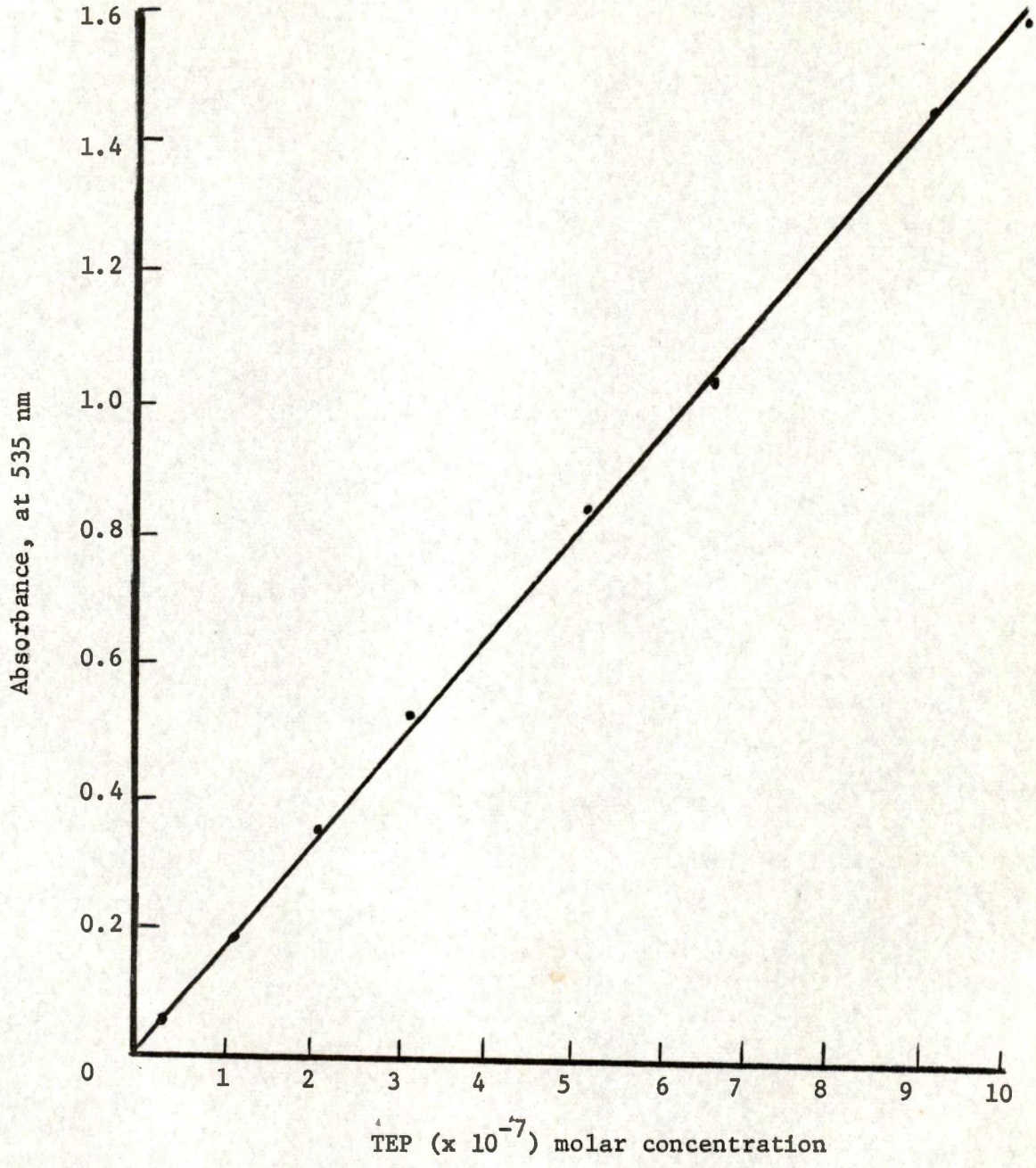


Figure 2. Relationship of absorbance of the hydrolysis product of TEP (malonaldehyde) with TBA versus concentration of TEP

chloroform for 30 seconds. The mixture was filtered immediately through filter paper into a beaker and four, 25 ml. portions of the filtered solution were withdrawn for analysis. Two of the 25 ml. portions of solution were placed in a previously tared beaker and analyzed for the iodine value. The two other 25 ml. portions were placed in Erlemeyer flasks where the chloroform was evaporated over a water bath, then the residues were dried for ten minutes in an oven at 101°C. The weight of the sample was used for calculation of the iodine value (42).

Procedures for the iodine value determination. Fifteen ml. of carbon tetrachloride were added to dissolve the oil which was previously prepared and then 25 ml. of the Wijs iodine solution were added. The mixture was placed in the dark and allowed to stand for two hours. Twenty ml. of 15 percent KI solution were added and the mixture was titrated with 0.1 N sodium thiosulfate solution. Two ml. of 1 percent starch solution were added as the indicator. The number of milliliters of 0.1 N sodium thiosulfate solution (b) and the reagent blank (c) was recorded. The iodine value was calculated by using the following formula (25).

$$\text{Iodine value} = \frac{(b - c) \times 0.12692 \times 100}{\text{sample weight (g)}}$$

Determination of Bacterial Counts

Twenty grams of stored sample in duplicate were used for determination of the bacterial population of the sample. The sample was placed in a sterile blender jar and 180 ml. of phosphate buffer was added to

dilute the sample. This provided a dilution of 1/10. After blending, one ml. of the material was pipetted into 99 ml. of phosphate buffer. This process, using the progressing increasing dilute, was utilized to prepare dilutions of 1/10, 1/100, 1/1000 and 1/10000. The dilution solution was used for the following determinations (18).

Total bacterial count determination. The plate count agar method was used in the total bacterial count determination. One ml. aliquots from 1/100, 1/1000 and 1/100000 dilutions were poured into petric dishes each containing 10 ml. of standard agar. The petric dish was inverted. The plate was incubated at 35°C for 48 hours. A colony counter and tally register was used to count all colonies on the plate. The result was expressed by the total bacterial count per gram of sample (18).

Staphylococcus count determination. The blood agar-surface plating method was used for this determination. One ml. aliquots from 1/100, 1/1000 and 1/100000 dilutions were poured into the petric dishes containing ten ml. of blood agar. The plate was incubated at 37°C for 24 hours. The number of green hemolytic staphylococcus colonies on the blood agar plate was counted. The result was expressed as staphylococcus count per gram of sample (53).

Coliform count determination. Brilliant-green lactose agar was used for the determination of coliform count. One ml. each of 1/10, 1/100, 1/1000 dilutions was pipetted into each of five separate tubes of 2 percent brilliant-green lactose broth, and the tubes were incubated

at 35°C for 24 hours. After incubation, the tubes showing gas production were recorded and the tubes not displaying gas were returned to the incubator for an additional 24 hours. After an additional 24 hours, the tubes showing gas production in each dilution that were confirmed as positive for coliform organism were recorded. To obtain the Most Probable Number (MPN) of coliform organism of sample, the following formula was used:

$$\frac{\text{MPN from table}}{\text{Sample weight}} \times \text{Dilution factor of middle of tube} = \text{MPN/gram of sample} \quad (53).$$

Salmonella-shigella count determination. Xylose Lysine Deoxychloate agar was used for the Salmonella-shigella determination. The agar was air dried at room temperature and one ml. aliquots of 1/10, 1/100 dilutions were pipetted onto the surface of the dried plate. After drying, the plate was incubated at 37°C for 24 hours, and colonies in the plate that appeared to be of a uniform red color were counted. These colonies were considered to be prosumptive Shigella. The prosumptive Shigella count was calculated from the dilution used and was expressed as Salmonella-Shigella count per gram of sample (53).

Statistical Analyses

The ANOVAR program adapted for the IBM Model 7040 Computer at The University Computer Center was used to perform the analysis of variance calculations. The moisture and water activity data in experiments one and two, and the equilibrium of moisture content and relative of humidity of the atmosphere with stored samples, and the effect of storage on firmness, pH, TBA value and iodine value of the product were analyzed.

The data were analyzed as a factorial arrangement of a completely randomized design.

Duncan's New Multiple Range Test was used to determine significant differences at the 0.05 level among means (32).

CHAPTER IV

RESULTS AND DISCUSSION

I. EFFECT OF CONCENTRATION OF GLYCEROL IN NON-DISSOCIATED ADDITIVE SOLUTION AND GLYCEROL SOLUTION ON WATER ACTIVITY

Changing the concentration of glycerol had an effect on the water activity of the non-dissociated additive solution (NDAS) as presented by the summary of the analysis of variance of Table II.

The experimental values of water activities of the NDAS and the theoretical water activites of the glycerol solutions calculated according to Raoult's Law are shown in Figure 3. The mean water activity values in the NDAS were significantly lower by 0.05 to 0.06 units than values of solutions containing the same concentration of glycerol only. This lower activity was due primarily to the presence of NaCl, K-sorbate and propylene glycol. The change in water activity of the NDAS showed a linear relationship to the values calculated by Raoult's Law.

According to the reviews by Christian (15) and Scott (49), water activity must be lowered to at least 0.65 to prevent mold and yeast growth. It is evident from Figure 3 that to reduct the water activity to this level, a very high concentration of glycerol would be required. Such high concentrations of glycerol would have an undesirable influence on flavor. However, if an antimycotic and propylene glycol were introduced into the solution, less glycerol would be required, yet the

TABLE II

F-TABLE SHOWING THE EFFECT OF GLYCEROL CONTENT OF NON-DISSOCIATED ADDITIVE SOLUTION ON WATER ACTIVITY

Source	D.F.	M.S.
Solution concentration	4	119.42**
Replication	1	0.11
Residual error	4	0.04

**Significant at the 0.05 level of probability.

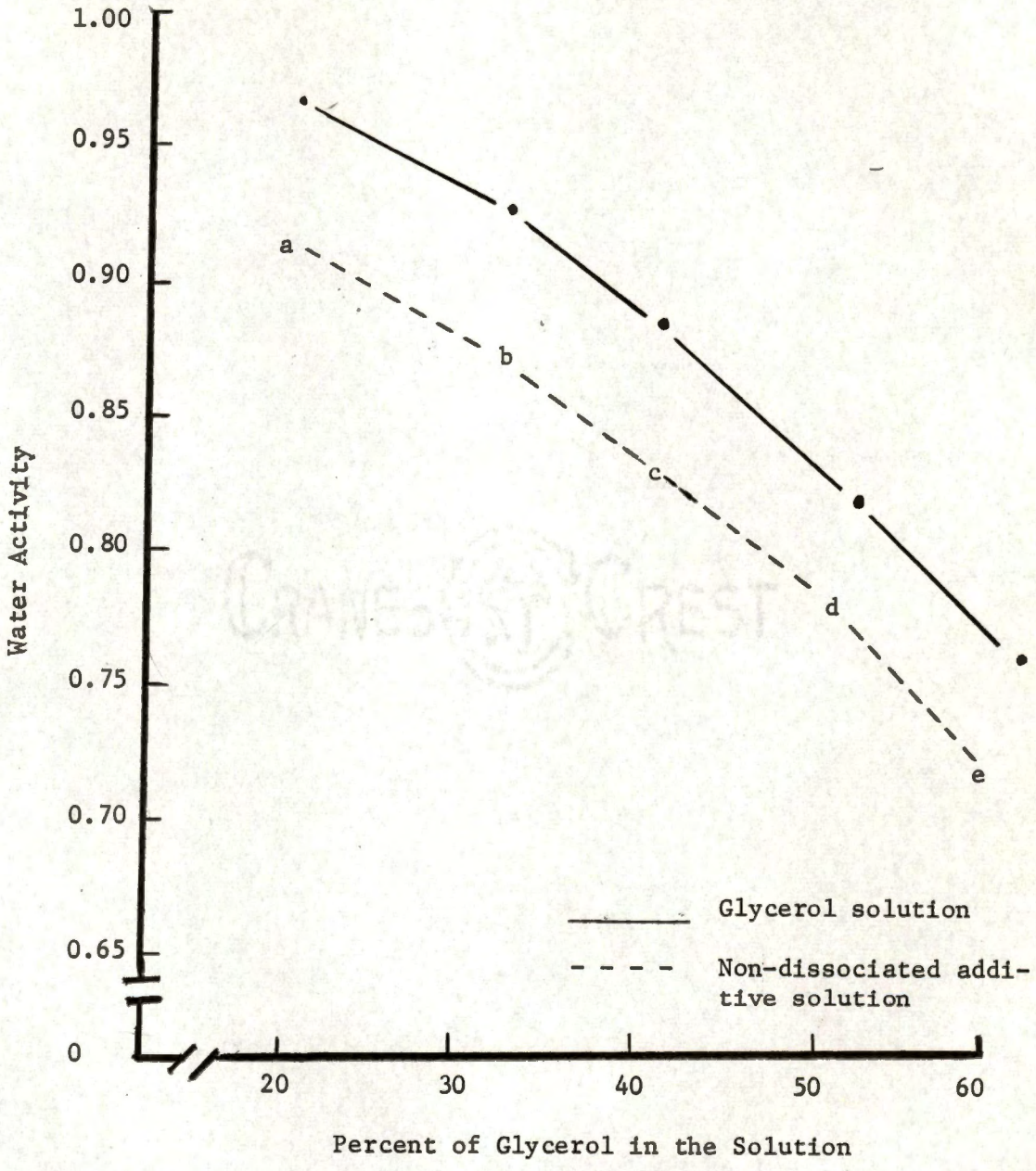


Figure 3. The effect of glycerol at different concentrations in solution alone and in non-dissociated additive solution on water activity.

Within the variable (- - -), means indicated by different letters are significantly different at the 0.05 level of probability.

growth of mold and yeast would be suppressed. This practice was suggested by Brockman (9) and Kaplow (26).

II. EFFECT OF CONCENTRATION OF GLYCEROL IN NON-DISSOCIATED ADDITIVE SOLUTION ON WATER ACTIVITY AND MOISTURE CONTENT OF TREATED CATFISH

The summary of the analysis of variance for the effect of concentration of glycerol in the NDAS on water activity and moisture content of the cooked and deep-fried catfish fillets is presented in Table III. The concentration of glycerol influenced water activity and moisture content of the catfish after both methods of cooking.

Table IV shows the moisture content and water activity of the product as influenced by concentrations of glycerol from 20 percent to 60 percent in NDAS. Mean values for moisture content and water activity in fillets cooked in the NDAS and deep-fried are presented. The mean weight of sliced fish sample was 15.27 $\pm$ 0.31 grams. As indicated an increase in the concentration of glycerol in NDAS lowered the water activity and moisture content of the product. According to the Kaplow (26), food can absorb an amount of solution approximately equal to the amount of water remove. Deep-frying reduced the amount of moisture and lowered the water activity in addition to that resulting from the cooking in the NDAS.

Fillets cooked in the NDAS with 20 percent glycerol had a water activity of 0.94. When the glycerol comprised 60 percent of the solution,

TABLE III

F-TABLE SHOWING THE EFFECT OF CONCENTRATION OF GLYCEROL OF THE NON-DISSOCIATED ADDITIVE SOLUTION ON WATER ACTIVITY AND MOISTURE CONTENT OF TREATED CATFISH FILLETS

		M.S.			
		After Cooking		After Frying	
Source	D.F.	Moisture	Water activity	Moisture	Water activity
		-percent-		-percent-	
Solution concentration	4	88.34**	84.86**	141.22*	225.70**
Replication	1	0.08	0.04	0.22	0.00
Residual error	4	0.43	0.81	0.23	0.11

**Significant at the 0.01 level of probability.

TABLE IV

EFFECT OF CONCENTRATION OF GLYCEROL OF NON-DISSOCIATED ADDITIVE SOLUTION ON WATER ACTIVITY AND MOISTURE CONTENT OF BREADED, DEEP-FRIED CATFISH[1/]

Sample	Non-dissociated additive solution (percent glycerol in solution)	After Cooking		After Frying	
		Moisture[2/]	Water activity[3/]	Moisture[2/]	Water activity[3/]
		-percent-		-percent-	
I	60	51.7^{e}	0.785^{j}	23.6^{p}	0.624^{x}
II	50	56.1^{d}	0.815^{i}	30.5^{n}	0.668^{w}
III	40	61.0^{c}	0.870^{h}	36.2^{m}	0.765^{v}
VI	30	64.4^{b}	0.908^{g}	40.1^{l}	0.832^{u}
V	20	68.4^{a}	0.942^{f}	44.6^{k}	0.874^{t}

[1/] Moisture content of fresh catfish was 77.6 percent.

[2/] Means of eight observations.

[3/] Means of six observations.

Within each column, means followed by the same letter are not significantly different at the 0.05 level of probability.

the water activity was lowered further to 0.79. The corresponding activity values for the cooked fillets which were breaded and deep-fried were 0.87 and 0.62, respectively.

According to Brockman (9), a variety of foods in the intermediate moisture range (A_w 0.80 to 0.85) have no microbiological growth or significant chemical and physical changes during storage. To produce catfish fillets with water activity at the 0.80 level, the fillets must be cooked in NDAS with glycerol concentration between 30 percent and 40 percent, and subsequently fried three minutes. Consequently, the NDAS selected for further use consisted of 30 percent and 40 percent glycerol.

III. EFFECT OF FRYING TIME ON MOISTURE CONTENT AND WATER ACTIVITY OF CATFISH FILLETS COOKED IN NON-DISSOCIATED ADDITIVE SOLUTIONS FOLLOWED BY BREADING AND DEEP-FRYING

Table V shows the summary of the analysis of the variance for the effect of frying time on moisture content and water activity of breaded catfish previously cooked in NDAS containing 30 percent and 40 percent glycerol. Time and concentration had a significant effect on the finished product. The interaction of time and glycerol concentration had a significant effect on moisture content of the fried product.

The change in moisture content and water activity of the product resulting from one to five minutes frying times are shown in Table VI. Merely cooking the fillets in solutions of 30 percent and 40 percent glycerol did not lower the water activity to the acceptable level (0.80

TABLE V

F-TABLE SHOWING THE EFFECT OF FRYING TIME ON MOISTURE CONTENT AND WATER ACTIVITY OF BREADED CATFISH FILLETS PREVIOUSLY COOKED IN NON-DISSOCIATED ADDITIVE SOLUTION

Source	D.F.		M.S.	
	Moisture	Water activity	Moisture	Water activity
	-percent-		-percent-	
Time	4	4	1,406.63**	1,189.77**
Concentration	1	1	124.98**	537.06**
Time x Conc.	4	4	5.93**	60.92
Replication	1	1	0.94	96.57
Residual error	69	49	0.36	95.55

**Significant at the 0.01 level of probability.

TABLE VI

EFFECT OF FRYING TIME ON MOISTURE CONTENT AND WATER ACTIVITY OF BREADED CATFISH FILLETS[1/] PREVIOUSLY COOKED IN NON-DISSOCIATED ADDITIVE SOLUTION

Sample	Non-dissociated additive solution (percent glycerol in solution)	After Cooking		Frying time (minutes)	After Frying	
		Moisture[2/]	Water activity[3/]		Moisture[2/]	Water activity[3/]
		-percent-			-percent-	
I	40	60.68[b]	0.867[d]	1	49.68[f]	0.920
				2	44.20[h]	0.864
				3	36.21[j]	0.759
				4	31.04[l]	0.678
				5	25.60[n]	0.620
II	30	64.85[a]	0.910[c]	1	51.59[e]	0.926
				2	45.50[g]	0.880
				3	40.01[i]	0.826
				4	33.14[k]	0.720
				5	29.34[m]	0.663

[1/] The moisture content of fresh catfish was 77.89 percent.

[2/] Means of eight observations.

[3/] Means of six observations.

Within each column, means followed by the same letter are not significantly different at the 0.05 level of probability.

to 0.85). However, when the cooked fillets were breaded and deep-fried for a period of three minutes the water activity was lower to this level. The solution containing 40 percent glycerol were slightly more effective than the solution containing 30 percent glycerol, thus the intermediate concentration of 35 percent was chosen for further studies.

When the appearance of the fried product and water activity were considered, a frying time of three minutes was selected for frying the sample for study of storage effects. Fillets prepared for storage studies were cooked in NDAS of 35 percent glycerol and subsequently fried three minutes.

IV. THE COMPOSITION OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH PRESIOUVLY COOKED IN NDAS OF 35 PERCENT GLYCEROL COMPARED WITH THAT OF OTHER INTERMEDIATE MOISTURE PRODUCTS

The composition of the breaded, deep-fried catfish fillets (35 percent glycerol, fried three minutes) and other intermediate moisture products is shown in Table VII. The fillets contained 38.9 percent moisture, 21.2 percent glycerol, 4.29 percent salt, 19.2 percent crude fat and exhibited a water activity 0.80.

The water activity values for the intermediate moisture products are those listed by Brockman (9) and by Kalpow (26). The water activity of the fillets (0.80) was almost the same as that of moist pork (0.81) but higher than that of infused freeze-dried moist carrots (0.77) and infused puff-dried moist carrots (0.76) and lower than that of infused moist

TABLE VII

THE COMPOSITION OF INTERMEDIATE MOISTURE BREADED DEEP-FRIED CATFISH AND OTHER INTERMEDIATE MOISTURE FOODS

Sample	Water activity	Moisture content	Glycerol content	Salt content	Crude fat content
		percent			
Intermediate moisture breaded deep-fried catfish	0.80	38.9	21.2	4.29	19.2
Infused moist carrots[1/]	0.81	51.5	----	----	----
Infused freeze-dried carrots[2/]	0.77	39.3	51.1	2.10	----
Infused puff-dried carrots[2/]	0.76	27.5	35.9	1.50	----
Moist peas[1/]	0.83	50.2	----	----	----
Moist pork[1/]	0.80	46.6	22.7	4.18	----
Moist chicken[1/]	0.84	42.6	----	----	----
Moisture beef[1/]	0.83	40.3	----	----	----

[1/] List of products described by M. C. Brockman, 1969, Development of intermediate moisture food for military use. Presented at 29th annual meeting of Institute of Food Technologist, Chicago.

[2/] List of products described by M. Kalpow, 1969, Commercial development of intermediate moisture foods. Presented at 29th annual meeting of the Institute of Food Technologist, Chicago.

carrots (0.82), moist chicken (0.84), moist beef (0.83) and moist peas (0.83). According to Brockman (9) and Kalpow (26) products developed at water activities in the range of 0.80 to 0.85 possessed acceptable sensory properties, no microbiological developments or significant chemical, physical or sensory changes during storage.

The glycerol content was comparable to that in the moist pork product, but was considerably lower than that of the carrot products. It was found that the moist vegetable products needed a higher glycerol content than the moist animal products to produce comparable water activity values. This is obviously due to the fact that the moisture holding capacity and adsorption of water vapor by protein (meats) is higher than that of carbohydrates (vegetables).

The moisture content of the deep-fried catfish (38.9 percent) was lower than that of other products tested except infused puff-dried moist carrots (27.5 percent). Also, the salt content of the fried fillets (4.29 percent) was similar to that of moist pork (4.18 percent), but higher than the level in infused freeze-dried moist carrots (2.1 percent) and infused puff-dired moist carrots (1.5 percent).

The product contained 19.2 percent crude fat which was composed of the natural oil and the oil adsorbed from the fryer. Rancidity is a serious problem concerning storage of fish products because of oxidation of fatty acid; therefore, strict attention is required to guard against development of off-flavors.

V. EFFECT OF DIFFERENT RELATIVE HUMIDITIES ON MOISTURE CONTENT OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Table VIII shows the analysis of variance summary for the effect of differnet relative humidities on the moisture content of the intermediate moisture breaded, deep-fried catfish. The relative humidity factor had a significant effect at the 0.01 level of probability.

The composition of the product utilized in this study was the following: moisture content, 39.44 percent; glycerol content, 20 percent; salt content, 4.35 percent; crude fat content, 19.4 percent; and water activity, 0.81.

The relative humidity-moisture equilibrium values are shown in Figure 4. The isotherm point, which is a true equilibrium, existed when the fried product was held in an atmosphere of a given relative humidity in which there was no loss or gain in product moisture. For fillets of 39.4 percent moisture, a relative humidity of 76 percent was necessary to establish the isotherm point. The moisture content was reduced when the product was held at relative humidities below 76 percent and increased at relative humidites above 76 percent. This phenomenon of moisture loss or gain at different relative humidities is due to the characteristic hygroscopicity which is the tendency to attract water vapor from the atmosphere.

There was a rapid loss of moisture from the product when the relative humidity was reduced from 35 percent to 0 percent. No significant change occurred in the moisture content resulting from raising

TABLE VIII

F-TABLE SHOWING THE EFFECT OF DIFFERENT RELATIVE HUMIDITIES ON MOISTURE CONTENT OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Source	D.F.	M.S.
Relative humidity	8	13.44**
Replication	1	0.18
Residual error	8	0.06

**Significant at the 0.01 level of probability.

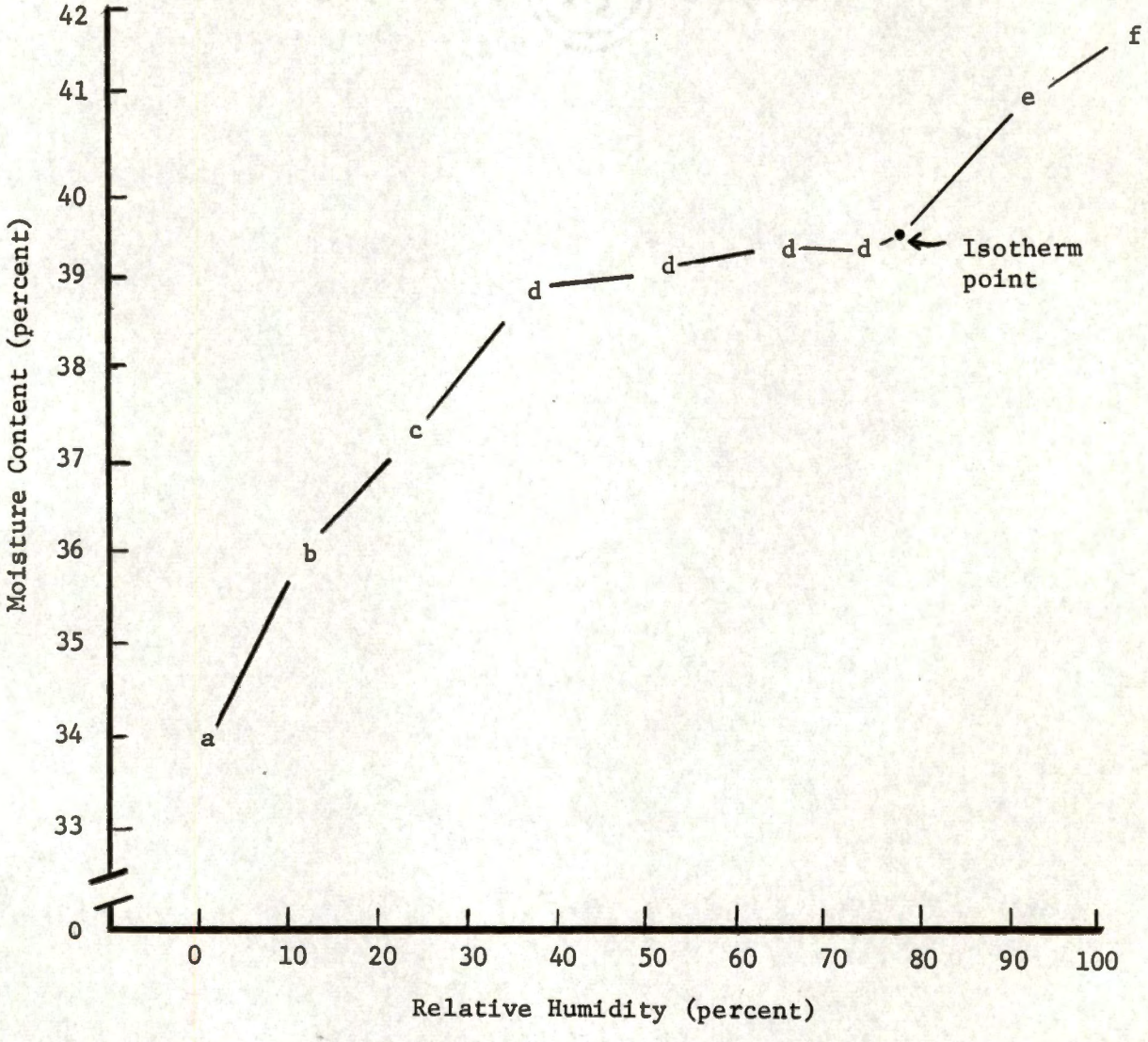


Figure 4. Effect of different relative humidities on moisture content of intermediate moisture breaded, deep-fried catfish after holding for four hours

Means indicated by the same letter are not significantly different at the 0.05 level of probability.

the relative humidity from 35 percent to 75 percent, but the moisture content increased sharply and significantly as the relative humidity was raised from 75 percent to 100 percent.

Since a loss or gain of mositure of stored foods may lead to undesirable quality changes, the moisture content must be maintained within a narrow range for stability (54). This study showed that a gain in moisture may be a problem if the relative humidity surrounding the product is allowed to rise above 76 percent (isotherm point).

VI. EFFECT OF STORAGE TIME ON MOISTURE CONTENT OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

The analysis of variance summary for the effect of storage on moisture content of intermediate moisture breaded, deep-fried catfish is shown in Table IX. The effect of holding time, as well as replication, was significant at 0.01 level of probability.

The loss or gain of moisture in stored foods often accounts for major changes in the characteristics of the foods, thus the behavior of water, hygroscopic properties and moisture sorption are intimately related to the ultimate quality of food (38, 41). Therefore, the moisture content of the catfish product which was sealed in its container was measured over in a period of five weeks at a holding temperature of 100°F. The moisture percentage values are presented in Figure 5. During the first two weeks of storage, there was a significant loss of moisture (1.07 percent); however, there was no loss with subsequent storage to five weeks.

TABLE IX

F-TABLE SHOWING THE EFFECT OF STORAGE TIME ON MOISTURE CONTENT OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Source	D.F.	M.S.
Time	5	0.46**
Replication	1	0.25**
Residual error	5	0.01

**Significant at the 0.01 level of probability.

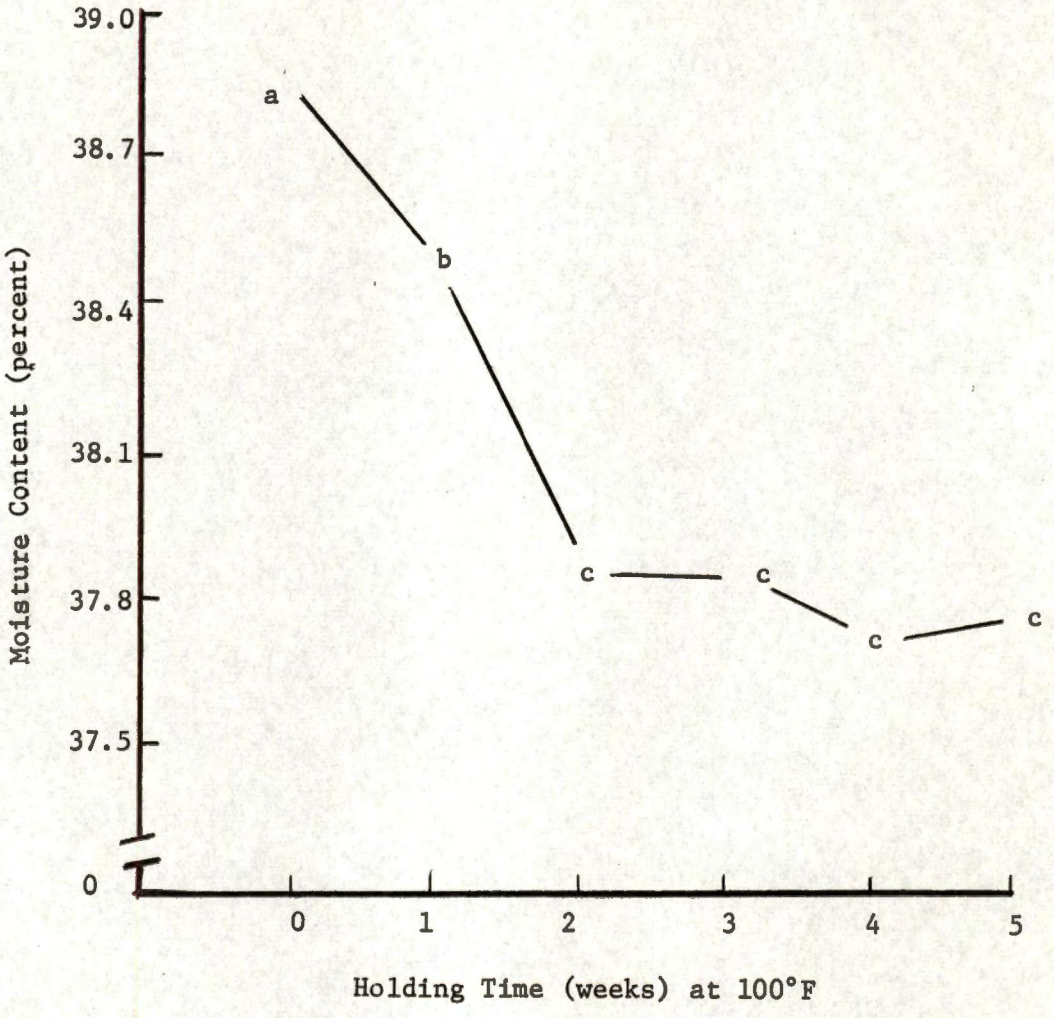


Figure 5. Effect of storage time on moisture content of intermediate moisture breaded, deep-fried catfish held in air-tight containers

Means indicated by the same letter are not significantly different at the 0.05 level of probability.

VII. EFFECT OF STORAGE TIME ON FIRMNESS OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

The analysis of variance summary for the effect of storage time on firmness of intermediate moisture breaded deep-fried catfish is presented in Table X. The storage time had a significant effect on firmness at 0.01 level of probability. The replication factor was significant, also.

The hygroscopic equilibrium is a primary factor which affects the mechanical and sensory textural parameters (27). The change of firmness of the product during storage was studied. The shear value of the samples stored up to five weeks at 100°F (Figure 6) indicated that there was an increase in firmness between one and three weeks.

The increase in firmness should not be due solely to moisture loss since the loss was only slightly over 1.0 percent (Figure 5). Kapsalis (27) reviewed the textural changes occurring with storage as related to the chemical composition, residual moisture, temperature and equilibrium atmosphere. According to his review, textural changes in the product during storage are probably due to many factors and not limited to changes in the moisture content.

VIII. EFFECT OF STORAGE TIME ON COLOR OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Table XI shows the effect of storage on color of intermediate moisture breaded, deep-fried catfish. The product retained its light yellow color throughout storage. The dominant wavelength and luminosity

TABLE X

F-TABLE SHOWING THE EFFECT OF STORAGE TIME ON FIRMNESS OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Source	D.F.	M.S.
Time	5	2,427.94**
Replication	1	129.15**
Residual error	5	11.34

**Significant at the 0.01 level of probability.

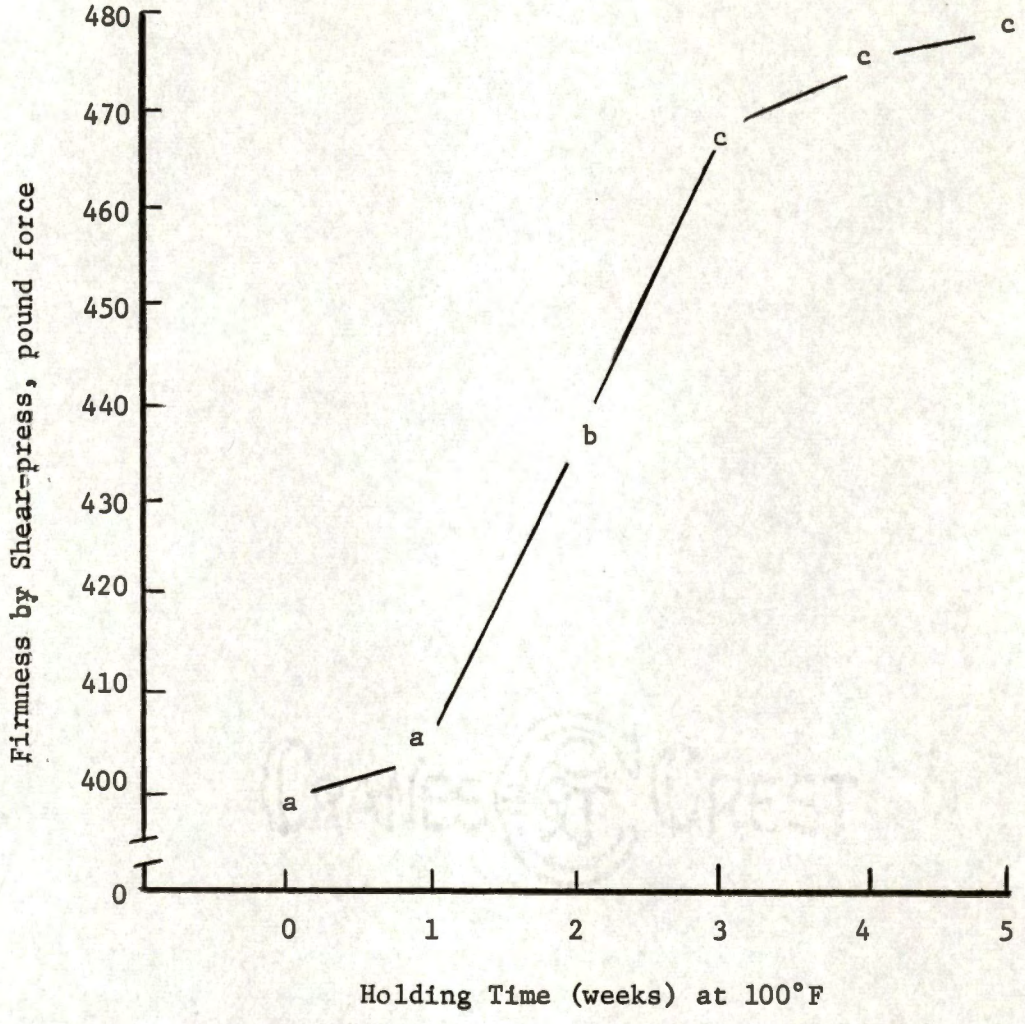


Figure 6. Effect of storage time on firmness of intermediate moisture breaded, deep-fried catfish

Means indicated by the same letter are not significantly different at the 0.05 level of probability.

TABLE XI

EFFECT OF STORAGE TIME ON COLOR OF INTERMEDIATE MOISTURE BREADED DEEP-FRIED CATFISH

Stored sample (weeks)	Color Eye value[1]				Tristimulus value			Chromaticity coordinates		Domni-nant wave-length (nm)	Lu-min-o-sity	Puri-ty	Color
	x	X	Y	Z	X	Y	Z	x	y				
0	19.68	37.33	32.86	19.96	33.09	32.86	23.55	0.372	0.367	575	32.86	34.61	lt. yel.
1	18.78	38.42	33.83	19.08	33.78	33.83	22.60	0.368	0.375	578	33.83	20.00	lt. yel.
2	18.30	38.45	33.59	19.36	33.68	33.59	22.84	0.373	0.372	582	33.59	30.00	lt. yel.
3	15.00	37.05	31.70	16.10	32.25	31.70	19.00	0.386	0.382	581	31.70	38.45	lt. yel.
4	16.21	37.23	32.48	17.28	32.34	32.48	20.39	0.368	0.381	576	32.48	36.54	lt. yel.
5	14.84	37.27	31.66	16.90	31.31	31.66	19.90	0.383	0.383	579	31.66	40.38	lt. yel.

[1] Means of four observations.

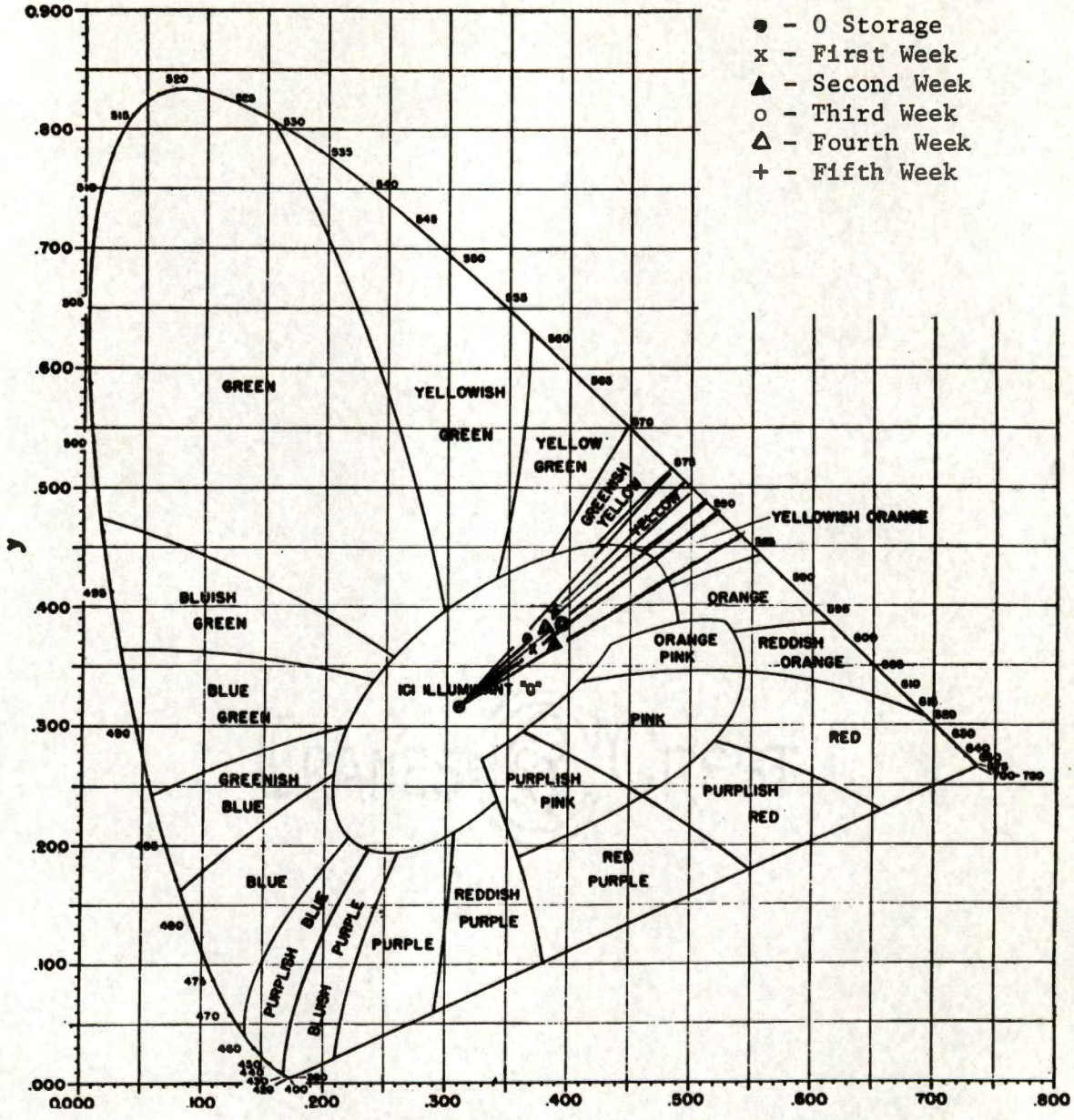
values for the color of the product ranged between 575 and 582 nm, and 31.66 and 33.88, respectively. There were no significant differences among these values. The purity value for the product stored after the first week was considerably lower than that for other samples. The results indicated that there were no significant changes in color during storage. The tristimulus x, y-coordinates for samples of the different storage periods are presented on the chromaticity diagram in Figure 7.

IX. EFFECT OF STORAGE TIME ON pH OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

The summary of the analysis of variance for the effect of storage on pH of intermediate moisture breaded, deep-fried catfish is shown in Table XII. The effect on holding was significant at 0.01 level of probability.

A change in pH of fish products may be due to development of certain substances which cause off-flavors (5). Therefore, the pH was measured for the fried products held from zero to five weeks at 100°F. The pH values are shown in Figure 8. The mean pH values after the first week of storage was lowered from 6.64 to 6.47. With additional storage up to five weeks there was a trend for development of lower pH values, but there was no statistical significant among these means.

According to Brogstrom (5), a lowering of the pH may have a beneficial effect on controlling bacterial activity, since pure faction of fish products is usually found at pH 7.0. Putrefaction is not found below pH 4.5, but autodigestion or autolysis of fish products by



The (x,y)-Chromaticity Diagram of the ICI System

Figure 7. Effect of storage time on the color of intermediate moisture breaded, deep-fried catfish

TABLE XII

F-TABLE SHOWING THE EFFECT OF STORAGE TIME ON pH OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Source	D.F.	M.S.
Time	5	0.01**
Replication	1	0.00
Residual error	5	0.00

**Significant at the 0.01 level of probability.

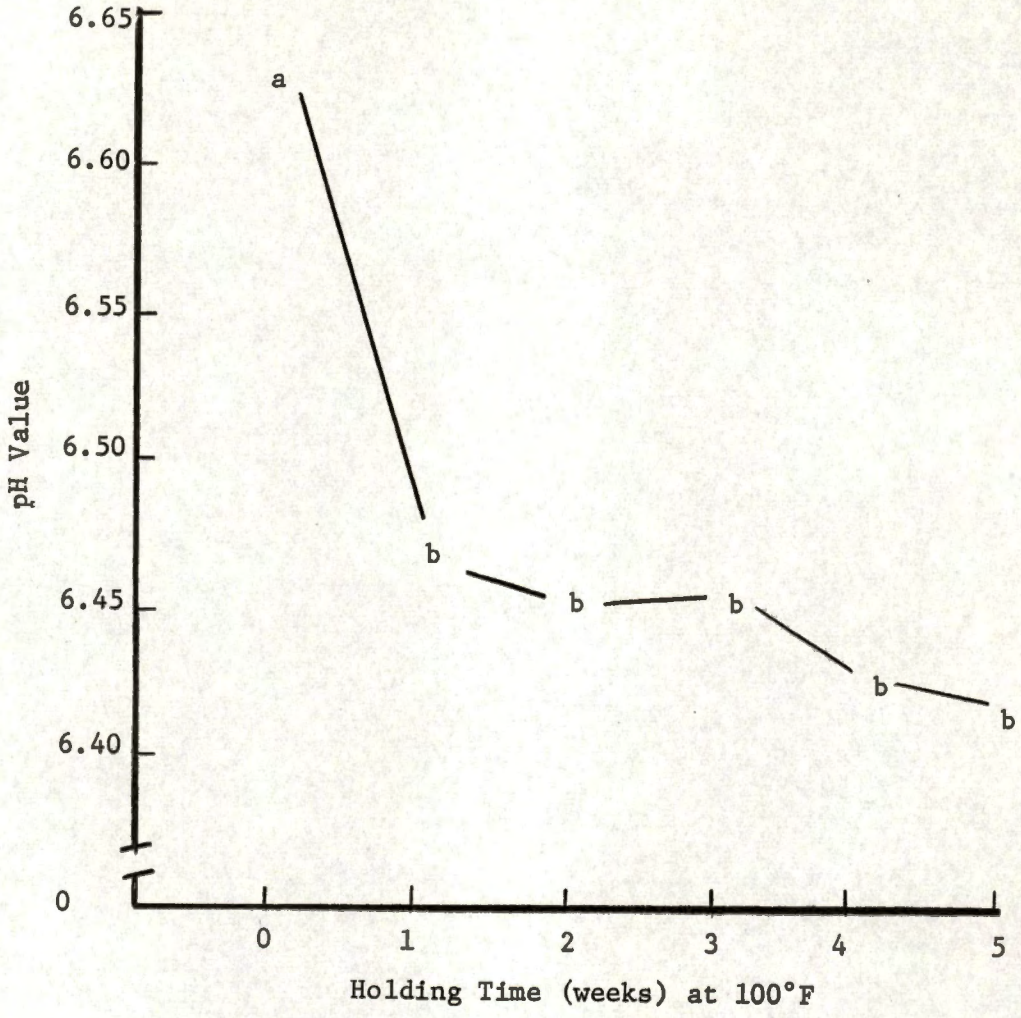


Figure 8. Effect of storage time on pH of intermediate moisture breaded, deep-fried catfish

Means indicated by the same letter are not significantly different at 0.05 level of probability

enzymatic reaction will occur at pH 4 to 4.5. The pH of the fried catfish fillets was well below 7, but higher than 4.5.

X. EFFECT OF STORAGE TIME ON RANCIDITY OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

The summary of the analysis of variance for the effect of storage time on rancidity of intermediate moisture breaded, deep-fried catfish is shown in Table XIII. Rancidity was influenced by the storage time.

Rancidity is a serious problem confronting the fish industry; prolonged storage required for many products often leads to deterioration of quality and flavor (58). Therefore, the mean TBA values (degree of rancidity) were determined for fillets which were stored up to five weeks at 100°F (Figure 9). There was a significant increase in TBA values from 0.935 to 1.485 during the first two weeks of storage; however, there was no significant increase with subsequent storage to five weeks.

According to a report by Sinnuber and Yu (50), canned and fresh fish of good quality gave TBA values of less than three, while products of poorer quality gave TBA values of four to 27. The TBA values for fish oil range from 14 in fresh salmon oil to 300 in oxidized samples. Results of this study show there are no TBA values higher than three.

XI. EFFECT OF STORAGE TIME ON IODINE VALUE OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Table XIV shows the analysis of variance summary for the effect of storage time on the iodine values of intermediate moisture breaded,

TABLE XIII

F-TABLE SHOWING THE EFFECT OF STORAGE TIME ON RANCIDITY OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Source	D.F.	M.S.
Time	5	0.00**
Replication	1	0.00
Residual error	5	0.00

**Significant at the 0.01 level of probability.

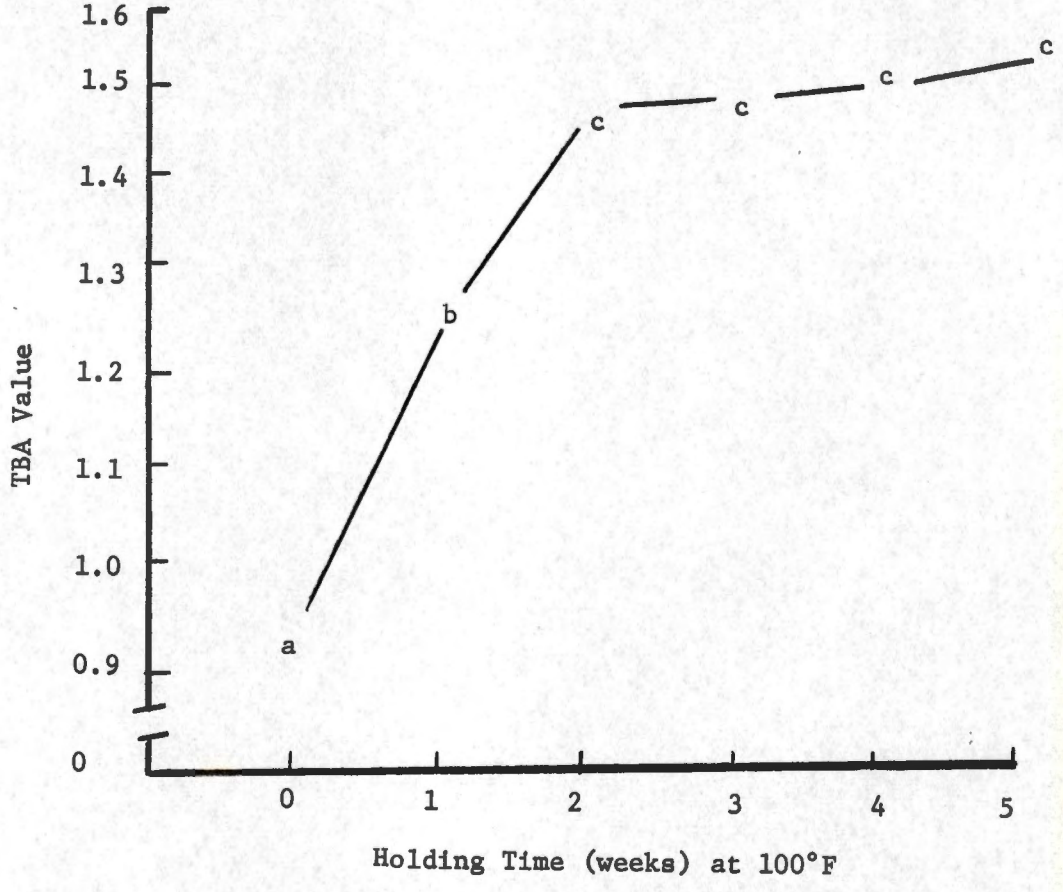


Figure 9. Effect of storage time on rancidity of intermediate moisture breaded, deep-fried catfish

Means indicated by the same letter are not significantly different at the 0.05 level of probability.

TABLE XIV

F-TABLE SHOWING THE EFFECT OF STORAGE TIME ON IODINE VALUE OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH

Source	D.F.	M.S.
Time	5	194.42**
Replication	1	1.86
Residual error	5	24.02

**Significant at the 0.01 level of probability.

deep-fried catfish. The factor of time was significant at the 0.01 level of probability.

The oils of fish are rich in highly unsaturated fatty acid components and are, therefore, susceptible to oxidation. The oxidative deterioration is responsible mainly for the development of rancidity and off-flavors of fish (5). Therefore, the quantity of iodine adsorbed (a measure of the degree of unsaturation of the oil) was studied and the mean iodine values for samples held up to five weeks of storage at 100°F are presented in Figure 10. The data indicated a significant reduction in the iodine value from 154.5 to 147.7 during the first three weeks of storage. Between three and five weeks of holding the iodine value was increased from 140.4 to 141.1, but there was no significant increase.

The range of iodine values (141.1 to 154.4) for the oil under study was higher than that of animal depot fat, such as 50 for swine, 45 for cattle and of seed oils, such as 110 for cotton seed, 130 for soybean and 120 for corn. Conversely, the range was lower than values for fish oil, such as 230 for fresh cod and 165 for fresh salmon (5).

XII. THE BACTERIAL COUNTS OF INTERMEDIATE MOISTURE BREADED, DEEP-FRIED CATFISH DURING STORAGE

Intermediate moisture breaded, deep-fried catfish were analyzed for the bacterial counts of total bacteria, Staphylococci, Coliform and Salmonella-Shigella during the storage at 100°F. The results indicated that at the time of preparation none of the microorganisms were found in the product except mold. In the first week of storage, the total

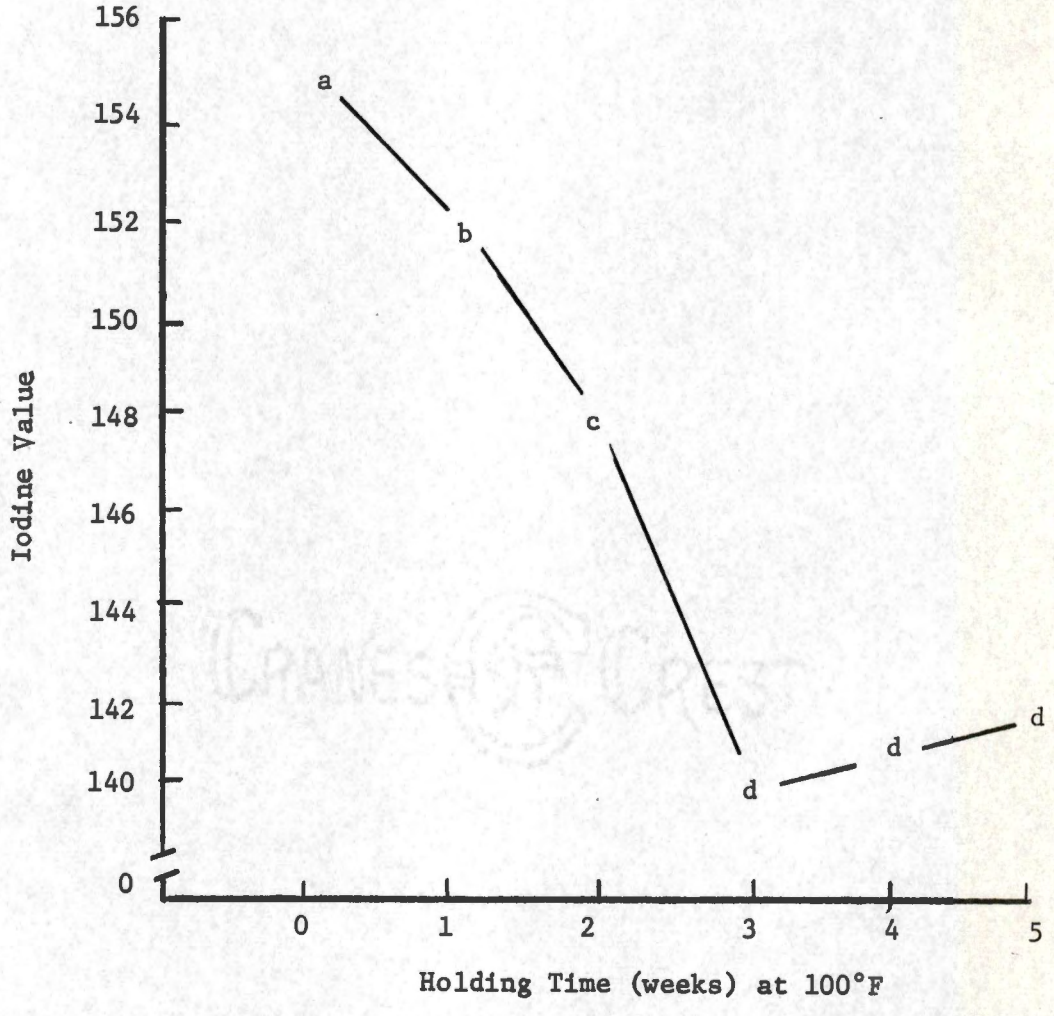


Figure 10. Effect of storage time on the iodine value of intermediate moisture breaded, deep-fried catfish

Means indicated by the same letter are not significantly different at the 0.05 level of probability.

count increased very rapidly from a count of ten molds to 2,000 molds and from no bacteria to a count of 500,000 bacteria. By the end of two weeks, the count was over five million. For the Staphylococci, the rate of growth was very rapid and the count was too high to count (at 1:1,000) after two weeks storage. Coliform was not found in the product in the first weeks but it was found in the second week of storage. For Salmonella-Shigella, this bacterium was not found in the product during two weeks storage. It is believed that contamination of the sample was due to the method of handling. The samples were picked by the hands and were placed in jars that were not sterilized at the temperature of 100°F.

According to the review, the limiting water activity for microbial growth was 0.90 for regular bacteria, 0.88 for regular yeast, 0.80 for regular mold (37), 0.88 for Staphylococci on dried meat (48), 0.86 for Coliform and 0.945 for Salmonella-Shigella on broth media (48). Brockman (9) has reported that a great variety of foods in the intermediate moisture range (A_w = 0.80-0.85) have no microbiological development during storage at 100°F when prepared by equilibrium with aqueous solutions of glycerol, salt and antimycotic. In this experiment the water activity was 0.8 at ambient temperature (ca. 70°-75°F); however, when the product was sealed in jars and held at 100°F, the water activity was increased to the level at which microorganisms thrive. When an NDAS solution containing 47 percent glycerol was prepared, a water activity of 0.785 was found at 78°F, but when the temperature was raised to 100°F, the water activity increased to near 1.0. Consequently, the product held

under similar conditions would become spoiled. For future work at elevated temperatures, the water activity should be adjusted so that at the higher temperatures the activity would be 0.8.

CHAPTER V

SUMMARY

This experiment was conducted to prepare an intermediate moisture product from catfish fillets by cooking them in a solution containing glycerol, NaCl, K-sorbate and propylene glycol, followed by breading and deep-frying. The fried product was analyzed for composition and quality attributes. The following conclusions may be made from the studies:

1. Lowering the concentration of glycerol in the solution had the effect of reducing of the water activity of the non-dissociated additive solution (NDAS).

2. The concentration of glycerol influenced the water activity and moisture content of the fillets which were cooked in the NDAS and fried for three minutes. Catfish fillets cooked in the NDAS with 30 percent to 40 percent glycerol, and breaded and deep-fried three minutes exhibited a water activity of 0.80.

3. Frying time and concentration of glycerol had a significant effect on moisture content and water activity of finished product. When the appearance of the fried product and the water activity were considered, a NDAS containing 35 percent glycerol and a frying time of three minutes were selected as conditions for preparing fillets with the desirable water activity.

4. The finished product (35 percent glycerol in solution, fried three minutes) contained 38.9 percent moisture, 21.2 percent glycerol,

4.29 percent salt, and 19.2 percent crude fat and exhibited a water activity of 0.80.

5. For fillets consisting of 39.4 percent moisture, an atmosphere with a relative humidity of 76 percent was necessary to establish the isotherm point.

The samples (35 percent glycerol in solution, fried three minutes) utilized in the storage test held at 100°F and, were analyzed at weekly intervals for five weeks for moisture content, texture, color, pH, rancidity and iodine value. From this study the following results were found:

1. The range of moisture content during storage was 38.9 percent to 37.8 percent. During the first two weeks of storage, there was a significant loss of moisture.

2. The range of shear values during storage was 400 to 475. There was an increase in firmness between one and three weeks.

3. The breaded fillets retained their light yellow color throughout storage. There was no change in color during storage.

4. The range of pH value during storage was 6.64 to 6.41. There was a significant decrease during the first week of storage only.

5. The range of TBA value during storage was 0.935 to 1.502. There was a significant increase in the TBA value during the first two weeks of storage.

6. The range of iodine value during storage was 141.1 to 154.5 There was a significant reduction in iodine values during the first three weeks of storage.

7. When the product was stored in sealed jars at 100°F, the original water activity of 0.8 was raised to near 1.0. In future research of this nature, care must be undertaken to adjust the water activity so that at the higher temperature the activity will be 0.8 to avoid spoilage.

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