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Hypoxanthine Measurement in Assessing Freshness of Chilled Channel Catfish (*Ictalurus punctatus*)

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Existing methods were modified for the inexpensive, objective measurement of hypoxanthine in muscle of channel catfish (*Ictalurus punctatus*) stored at 2° *postmortem* for up to 22 days. Sensory ratings were performed for appearance, color, aroma, texture, and flavor and compared to the rate of hypoxanthine accumulation in the same fish. Measurement of the purine, especially prior to noticeable spoilage, shows potential value as an index in assessing freshness of chill-stored catfish.

Nucleotide degradation in fish muscle commences at death or early *postmortem* and proceeds throughout storage. The autolytic processes involve dephosphorylation and deamination of adenosine triphosphate to form inosine monophosphate, which is degraded to inosine and finally to hypoxanthine. Examination of nucleotide degradation in fish muscle as a means for assessing fish freshness and quality has recently received considerable attention (Hiltz *et al.*, 1971). Measurement of the accumulation of hypoxanthine in particular has shown potential as an index of freshness, especially in marine fishes (Burt *et al.*, 1969; Fraser *et al.*, 1968a,b; Jones *et al.*, 1964; Kassemsarn *et al.*, 1963; Spinelli, 1967). Although information on hypoxanthine formation in freshwater fishes is less abundant, its increased levels during storage have been demonstrated (Dugal, 1967; Kuusi and Aalto, 1968) and its usefulness in assessing freshness has been noted (Bligh, 1971).

Methods of culturing, harvesting, and processing presently employed in the channel catfish (*Ictalurus punctatus*) industry are varied and inconsistent. Commercially available catfish may have been fed one of many diets and cultured in ponds, raceways, or in the wild; they may or may not have been iced at their point of harvest and transported long distances, or they may have been harvested, slaughtered and chilled, iced, or frozen all within a period of minutes. These variations in handling may result in catfish of different body composition and microflora. In any case, the body temperature at harvest can range from 20 to 24°, which demands an immediate reduction in order to combat bacterial growth and autolytic breakdown. It would be highly desirable to rely on a simple, rapid, and preferably inexpensive test to assess the quality of channel catfish as they are received at the processing plant or as they are presented in the market place. Such a test should not be greatly influenced by variation in environmental conditions to which the fish were exposed before death.

The present study involves the measurement of hypoxanthine in stored chilled channel catfish by a procedure derived from previously existing methods (Burt *et al.*, 1968, 1969; Jones *et al.*, 1965). The procedure appears to be valuable in assessing freshness of chilled channel catfish.

EXPERIMENTAL SECTION

Materials. Pond-raised channel catfish averaging 400 g (live weight) were obtained locally and transported in an aerated tank to the Food Science Laboratory, Experiment, Ga., where they were stunned with alternating current, decapitated, eviscerated, and skinned. Fish were then washed, packaged individually in polyethylene bags, and stored at 2° in a constant temperature cabinet until analyses were performed.

Organoleptic Evaluation. Four catfish were removed from storage at 2° after 0, 2, 4, 7, 9, 11, 14, 17, and 22 days. A fillet was removed from one side of each fish and retained for hypoxanthine analysis; the remaining portions of the fish were subjected to sensory evaluation by a trained six-member panel. After ratings were assigned to the raw fish for appearance, color, and aroma, the fish were baked at 350°F for 25 min in sealed aluminum foil pouches and ratings were performed for texture and flavor. A score of 9 on a 9-point hedonic scale indicated extremely good, while a 1 indicated extremely poor quality for the particular organoleptic character being judged.

Reagents for Hypoxanthine Determination. The reagents required are among those described in an earlier publication (Burt *et al.*, 1969) from which this procedure has been adapted. The procedure followed here requires 0.6 *N* perchloric acid, buffered potassium hydroxide [prepared by combining 20% KOH (w/v) with 0.4 *M* potassium phosphate buffer at pH 7.6 in the ratio of 42:56 (v/v)], 0.17 *M* phosphate buffer (pH 7.6) containing 20 μg of 2,6-dichlorophenolindophenol per ml, xanthine oxidase (General Biochemicals, Chagrin Falls, Ohio, I.U.B.

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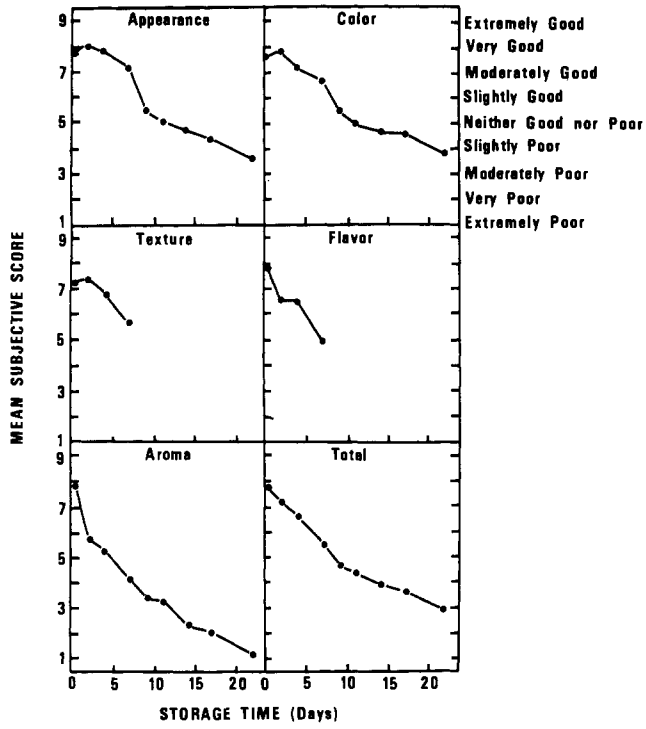

Figure 1. Mean subjective sensory scores assigned to channel catfish stored at 2° for up to 22 days. Appearance, color, texture, flavor, and aroma data represent averaged scores submitted by six panel members for four fish on each evaluation date. Total sensory scores represent the combined average of appearance, color, texture, flavor, and aroma scores.

1.2.3.2.) solution (0.040 IU/ml of chilled deionized water), and standard hypoxanthine solution (20 μg/ml of deionized water). Perchloric acid and buffered KOH may be stored at room temperature indefinitely. Buffered 2,6-dichlorophenolindophenol may be stored for 2 months and hypoxanthine solution may be stored for 3 weeks at 0°. Xanthine oxidase should be prepared daily and kept on ice until used. The temperatures of all other reagents were brought to room temperature (22 to 24°) before analyses were performed in triplicate.

Analytical Procedure for Hypoxanthine Determination. Two separate 10-g samples of muscle were removed from the anterio dorsal region of each of four catfish and homogenized individually in a Waring blender with 50 ml of 0.6 *N* perchloric acid for 1 min. The homogenate was filtered (Whatman No. 2) and 25 ml of filtrate was added to 10 ml of water and 15 ml of buffered KOH. The extract was allowed to stand about 15 min to facilitate crystallization of $KClO_4$. Ten milliliters of the extract are equivalent to 1 g of catfish muscle.

The reaction mixture was prepared by stepwise addition of the following reagents to Klett-Summerson assay tubes: 2.0 ml of 2,6-dichlorophenolindophenol, 0.1 to 1.0 ml of extract or standard hypoxanthine solutions in 0.1-ml increments, water to bring the total volume to 4.0 ml, and 1.0 ml of xanthine oxidase solution. A blank consisted of 4.0 ml of water and 1.0 ml of xanthine oxidase. The temperature of the reaction mixture was 22°. The reactants were mixed immediately and read after a reaction time of exactly 5 min on a Klett-Summerson Photoelectric Colorimeter (Model 800-3; Klett Manufacturing Co., Inc., N. Y.) using filter No. 54.

RESULTS AND DISCUSSION

Organoleptic Evaluation. Spoilage of chilled (0-2°) channel catfish can be generally described as it proceeds through sequential changes in sensory characteristics. The progression is outlined as follows: Phase 1 (0-6 days), no noticeable spoilage; Phase 2 (7-9 days), fruity, aromatic, earthy, marshy aroma, dull appearance, soft texture; Phase 3 (10-13 days), sour, marshy aroma and flavor, slimy appearance, bleached color; and Phase 4 (>13 days), sour, strong putrid, faecal aroma. The earthy, marshy aroma and flavor is thought to be due to geosmin (Gerber, 1968), a compound produced by several strains of actinomycetes and blue-green algae naturally present in inland waters. The characteristic is not generally as intense during the first few days *postmortem*. Sour and putrid odors resulting from products of proteolysis are most likely due to pseudomonads and other psychrophilic bacteria indigenous to fish, as well as autolysis of the fish tissue.

Data obtained from the organoleptic evaluations performed by the six-member trained panel are shown in Figure 1. Sensory ratings were not performed for texture and flavor after 7 days of storage, thus avoiding the risk of food poisoning from the obviously high microbial population. The total sensory evaluation data represent the average of mean scores for all organoleptic characteristics. Appearance, color, and texture, although being considered by a panel member when making a decision to accept or reject a catfish, are not weighed as heavily as are aroma and flavor. A raw fish which is rejected on aroma may be accepted if it is cooked or baked in an open container, as heat apparently destroys some of the undesirable volatiles in raw catfish. Aroma of uncooked catfish, then, is a reliable sensory characteristic when judging its freshness. Such ratings during the first 6 days *postmortem* cannot, however, serve as the entire basis for estimation of time elapsed *postmortem*, due to the variability in environment before and during processing. Some aromas naturally present in catfish at varying intensities may be highly disagreeable to some panel members. Thus, scores obtained early in storage generally fall over a wider range and may not be the best indication of freshness.

Hypoxanthine Analysis. Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and the latter to uric acid. In the presence of a redox indicator such as 2,6-dichlorophenolindophenol, one can monitor the rate and extent of decolorization of the dye colorimetrically, which is dependent upon the hypoxanthine concentration in the test solution. Although measurements presented here will be referred to as hypoxanthine concentration, they might be better described as "apparent hypoxanthine concentrations," since the possibility of positive-reacting materials such as xanthine and aldehydes being initially present in the reaction mixture exists. Concentrations of all other reactants in the mixture are initially the same.

Incorporation of the indicator dye into automated (Burt *et al.*, 1968; Jones *et al.*, 1965) and visual (Burt *et al.*, 1969) procedures for hypoxanthine analysis has resulted in excellent methods for research and quality control purposes. However, the procedures are not without some shortcomings. The automated procedures, although highly accurate, may be too expensive for employment by small-

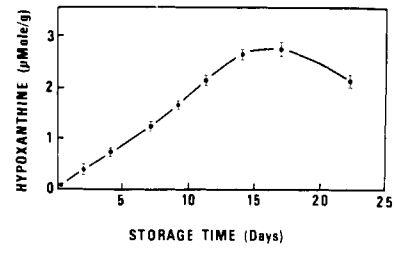

Figure 2. Accumulation of hypoxanthine in channel catfish stored at 2° for 0, 2, 4, 7, 9, 11, 14, 17, and 22 days. Means represent 24 determinations (two individual samples from each of four fish, analyzed in triplicate) for each storage time. Bars (I) indicate standard deviations.

er processors in the fish and fisheries industry. The visual approach may be useful for rapid screening of fish for freshness or spoilage, but it is highly subjective.

The procedure presented here for quantitating hypoxanthine in channel catfish offers a relatively inexpensive, objective, and accurate approach if performed in exact accordance with the directions. Temperatures and concentrations of the reactants must be constant. Just as the rate of hypoxanthine formation in fish tissue is increased at elevated temperatures, so is its rate of oxidation in the test tube. Hypoxanthine concentrations exceeding 10 μg were observed to completely decolorize the 40 μg of dye in the assay mixture after 5 min. Proportionate concentrations of reactants as they are outlined result in a standard curve with a straight line at concentrations ranging from 2 to 7 μg of hypoxanthine in the reaction mixture. These concentrations can be read in Klett units as approximately 92 to 28, respectively, at the end of the 5-min reaction time. All hypoxanthine levels calculated for chilled channel catfish were based on a reading of 60 Klett units, a point approximately midway on the straight-line portion of the standard curve.

The apparent accumulation of hypoxanthine in chill-stored catfish is shown in Figure 2. Values plotted are means of 24 determinations for each evaluation date. Standard deviations are shown. Increased concentrations were demonstrated to occur in a linear fashion, reaching a maximum of 2.7 μmol per g of fish muscle during the first 17 days of storage. Levels subsided somewhat at 22 days, perhaps due to leaching into the drip. The data of greatest interest are those obtained before Phase 2 (7-9 days) of spoilage. As reflected by aroma and total sensory scores (Figure 1), catfish were rated acceptable during a period of up to 6 days *postmortem*. Hypoxanthine analyses resulting in concentrations nearing 1.3 μmol per g in catfish muscle would indicate that the chill-stored catfish are borderline with respect to acceptance based on sensory ratings. Therefore, a catfish processor might employ the objective method of hypoxanthine analysis in combination with sensory ratings (especially aroma) in an attempt to more accurately assess the freshness of catfish which are delivered to his plant.

Little variation in hypoxanthine concentrations among fish analyzed at specific storage times was observed in this study. However, the rates of accumulation may differ in catfish stored at other temperatures. It should also be kept in mind that various conditions of culturing, harvesting, and handling could effect the rate of hypoxanthine accumulation in the stored catfish. Whether or not concentrations will reach approximately 1.3 μmol per g of fish muscle within the same length of time *postmortem* required for rejection of the fish based on sensory evaluation remains to be demonstrated. Nevertheless, no other chemical test holds as much promise as the hypoxanthine test as an index of freshness for freshwater fish. Data presented in this study indicate that measurement of hypoxanthine in chilled channel catfish may be a useful, inexpensive, objective method to assess the degree of freshness prior to incipient spoilage.

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Effects of Coatings on Weight Loss and Ethanol Buildup in Juice of Oranges

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Oranges coated with commercial solvent-type wax lost less weight than those with comparable amounts of water wax or polyethylene coatings. Ethanol buildup in juice and off-flavors occurred after multiple coatings of water wax or solvent-type wax; less ethanol accumulation and no off-flavors were noted in polyethylene-coated fruits. Citrus fruits lose weight at rates not necessarily related to original weight or surface area. Individual fruits lose weight at a constant rate over short time periods and this should be considered in determining the amount of coating by weighing procedures. A method is presented to determine the weight of coating applied.

The use of coatings for citrus fruits to impart gloss and prevent weight loss is well established. Rind disorders, such as "pitting," sunken areas usually associated with cold injury, and "aging," rind tissue collapse near the stem end, may be alleviated with proper coatings. For example, pitting of grapefruit was reduced substantially by polyethylene emulsion coatings (Davis and Harding, 1960) which, in addition, were compatible with fungicides for decay control (Davis and Smoot, 1960). Pitting of Valencia oranges was reduced by preharvest sprays of Pinolene, a polyterpene film former (Albrigo *et al.*, 1970), and by postharvest application of commercial solvent-type wax (Chace *et al.*, 1969).

Coatings also affect the physiology of fruits. Ben-Yehoshua (1967) found that coatings lowered internal O_2 and raised internal CO_2 and suggested that weight loss and in-

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