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Research Report No. 3. Application of Controlled Atmospheric Storage to Extending the Shelf Life to Whole Processed Fresh Fish

Application of Controlled Atmospheric Storage to Extending
the Shelf Life of Whole Processed Fresh Fish

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ABSTRACT

Present packaging and handling methods extensively limit refrigerated storage life of freshwater fish. Consequently a large portion of fish reaches the consumer in a frozen state or in an inferior-quality fresh form. Improved quality could increase consumption and expand markets for fresh fish.

Our objective was to determine the feasibility of extending the shelf life of refrigerated fish through advanced storage technology. This included modifications of the vacuum packaging concept and controlled atmospheric storage utilizing different mixtures of CO_2, N_2, and O_2. We also wanted to assess the physical and microbiological qualities of freshwater smelt and herring fillets from Lake Superior at 1°C, using pH, aerobic, anaerobic and psychrotrophic bacterial counts, rancidity and sensory analyses as parameters of quality measurements.

Within the limits of the experiments, the following observations and data were obtained:

Vacuum-packaged fish retained color and texture to a greater extent than did fish stored in CO_2. However, fish stored in mixtures of gases were more stable both in color and odor than fish stored in 100 percent CO_2. Low concentrations of CO_2 resulted in a more acceptable product with respect to odor and appearance after 20 days of storage.

Microbiologically, a concentration of 100 percent CO_2 was most effective in inhibiting growth of aerobes and anaerobes as well as psychrotrophs. This closely paralleled the data obtained on frozen, vacuum-packaged samples.

Changes in pH were negligible for the duration of the storage period, indicating little absorption of CO_2 by the fish and essentially no production of carbonic acid.

Oxidative rancidity, as measured by formation of malonaldehyde, was minimal. Changes in gas composition by measurement of headspace were apparent in some treatments. For example, the CO_2 concentration increased from less than 1 percent to 7 to 27 percent, and the concentration of O_2 decreased from 20 to 15 percent to less than 1 percent.

We were pleased that, within the limits of the study, we were able to identify and test a very useful new approach to measure the hypoxanthine content of the stored product through the use of HPLC (high pressure liquid chromatography) techniques. This procedure has great potential as a possible measurement of the index of freshness of chill-stored fish.

APPLICATIONS AND CONCLUSION

Storage life of freshwater fish held at refrigeration temperatures is short and limited by current packaging and handling methods. These methods have remained essentially unchanged since the inauguration of the use of ice to preserve fish. The handling and storage of fresh fish have not been studied extensively. Consequently they have not advanced technologically in comparison to the handling of other raw and perishable food products such as meats and fruits. Most fish reach consumers in the frozen form or in a less than maximal quality fresh form, not encouraging strong interest from consumers. Improved quality through advanced technology would increase consumption and markets for fresh fish.

Interest in new handling and storage technology is evident among the Lake Superior fish processors, such as Sivertson of Duluth and Bodin of Bayfield. Others, too, have expressed the immediate need for extending shelf life of fresh fish. Fish distributors and retailers in Chicago and Minneapolis, representatives of the National Marine Fishery Service, and other scientists and technologists working in the area share the concern. Dr. Robert C. Lindsay, professor of food science, University of Wisconsin (Madison) stated, "Increasing worldwide demands for protein have put a lot of pressure on the fishing industry to supply more fishery products. Packaging that prevents deterioration during storage will help industry meet this demand."

The economic advantages of extended shelf life of refrigerated fresh fish are evident in several ways. Ice is expensive considering transportation costs of current fresh fish shipped with 25 to 30 percent ice. Also, in some situations the product could be shipped by less expensive means--by truck instead of air. The elimination of the wet ice packing would aid in the prevention of cross-contamination, thereby yielding a much cleaner operation. The added cost of packaging materials and processing equipment would be outweighed by reduced losses during shipment and premium prices for improved quality. The extension of the shelf life of fresh fish would enable the product to be shipped over longer distances, thereby increasing the marketing possibilities.

Presently, most of the advances in fish packaging technology have been limited to vacuum packaging of fresh fish. This was discussed in a recent article in Food Engineering (1978). The major hazard in vacuum packaging of fish is the establishment of a suitable environment for the organism that causes botulism food poisoning. The University of Wisconsin (Madison) investigated the safety of vacuum-packed pouches of fresh fish which had been inoculated with activated botulism spores and stored at various temperatures. Even after storage periods of up to 30 days, no botulism toxin was formed before the product had spoiled beyond palatability.

A Gulf Coast area fish processor has been vacuum packaging fish for almost a year. The improvised method is to place fresh fish on a plastic tray, over-wrap them with a shrink film, and place them in a bulk-size bag holding up to 40 pounds of fresh fish. A vacuum is drawn, the bag is backflushed with carbon dioxide, and the bag is heat sealed. When the retailer receives the bulk-packed fish, he simply opens the bag. The individual packages are ready for the display case. This processor stated that he was able to cut his deliveries from twice to once a week. His major gain was new customers who previously would never handle fresh fish.

There is an immediate need for further studies to examine the possibility of extending the shelf life of fresh fish with new technologies such as controlled atmospheric (CA) storage. The CA storage of various fresh food products has been extremely successful. However, little useful information is available on freshwater fish stored at refrigeration temperatures.

Vacuum packaging has indicated much promise, but CA systems have been used mainly with salmon in limited experimental studies. Fresh salmon packaged in gas-permeable plastic bags and stored at 28° to 31°F in a controlled atmosphere were acceptable after 19 days (Liston et al., 1974). The oxygen content of the atmosphere was reduced to approximately 2 percent with varying amounts of carbon dioxide (0 to 90 percent) and nitrogen. A study also was conducted by the Northwest and Alaska Fisheries Center - Utilization Research Division on preserving fresh, chilled salmon held for 21 days at 33°F in a controlled atmosphere containing CO_2 (U.S. Dept. Commerce, 1979). Bacterial counts did not exceed 10^4 organisms per gram and the salmon was considered to be of very good quality at the end of the 21-day storage period. Normally, the shelf-life of fresh fish is six to eight days.

Work on red meat and poultry products also indicates that a significant increase in shelf life can be anticipated with selected CA storage. For example, the average storage and retail shelf-life of fresh pork is approximately 10 to 14 days. The limiting factors are bacterial spoilage and deterioration of the desired color. Controlled atmospheric storage has been shown to reduce significantly microbial growth on fresh pork chops stored at temperatures above freezing (Huffman, 1974). Gas treatments that have been investigated include: air, O_2, N_2, CO_2, and a gas mixture of 70 percent N_2, 24 percent CO_2 and 5 percent O_2. Pork stored in CO_2 at -1.1°C had significantly lower aerobic counts (approximately $10^8/cm^2$) during a 5-week storage. The anaerobic bacterial growth was inhibited by CO_2 throughout the 35-day storage period, never rising above 10 organisms per cm^2. The object of using the gas mixture was to take advantage of the bacteriostatic effect of CO_2 and provide sufficient O_2 in the atmosphere to retain the desired color of pork.

Another recent announcement of an application of controlled atmospheric storage indicated that shelf life of pork loins was increased "two to three times" and the meat retained its normal color longer (Food Eng., 1978). The use of CA storage for extending the shelf life of prepackaged beef has also been investigated on experimental bases (Clark and Lentz, 1972; Huffman et al., 1975) and has had some limited application by Trans FRESH Corp. (Wolfe et al., 1976). An atmosphere of 15 percent CO_2 was the most beneficial in terms of extending the shelf life. Color shelf life was extended from 5 to 8 days and odor shelf life from 6 to 16 days. Bacterial inhibition was mainly attributable to an increase in the lag phase of growth. Since these results show that a change in color rather than development of off-odor limits shelf life in prepackaged fresh beef, the full advantage of the adverse effect of CO_2 on microbial growth was not realized in terms of total storage extension.

Limited information on saltwater fish indicates similar good potential for CA storage. The costs involved are sufficiently low and the technology sufficiently simple to permit adoption by small operators who process Lake Superior and Lake Michigan species. Therefore, it is appropriate that the CA storage approach to extending shelf life of freshwater fish be investigated.

Our findings in this study suggested that using a high percentage of CO_2 was not directly applicable in the process of storing fish and maintaining its quality for consumption. Further storage studies need to be conducted to determine the best gas (or mixture) capable of keeping fishes' physical characteristics suitable for consumption.

METHODS AND MATERIALS

Our study's methods were divided into three parts, described in detail below:

I. Monitoring pH of fish samples in storage at 1°C

The pH change of fish during storage was monitored because carbon dioxide was expected to be absorbed by the fish flesh, yielding carbonic acid and thus lowering the pH.

Fresh herring fillets (with and without skin) and fresh smelt (minus gut and head) were obtained and stored at 1°C until packaging. Individual samples of fish were placed in Nylon/SURLYN pouches and sealed on a Pac-Vac Model 10V sealer (Packaging-Aids Corporation, San Francisco, California). Two variables were studied: vacuum and CO_2 (>95 percent). The dimensions of the pouches varied with the fish samples; the size of the pouch was not critical since the pouches were "overflushed" with CO_2. The pH of the samples was taken using an Orion Model 601 pH meter and a Corning Model #476115 electrode. The sample was prepared by blending the fish with 10 ml distilled water in a 8-ounce blender jar using high speed

Osterizer blender. A uniform slurry was obtained using 30- to 60-second blending time. As shown in Table 1, the reduction in pH was minimal. For example, with the smelt, the initial pH was 6.87 and after 21 days storage at 1°C, the pH was reduced to 6.59.

II. Appearance and odor of fish fillets stored with various gas mixtures.

This study was conducted to evaluate different gas mixtures varying in the amount of carbon dioxide and oxygen to determine which gas mixture(s) would be suitable for a scale-up run. The physical characteristics of odor, color and texture (subjective) were used to evaluate the different environments.

Fish Materials

Fresh white fish fillets were obtained from a local supplier. The fish were stored on ice until filleted; the two fillets from a given fish were placed in an individual bag to facilitate the sampling scheme discussed later in this report. The fish were stored at 1°C.

Packaging Film

The film was a 3.8 ml coextrusion of 1.2 ml Nylon/1.6 ml Plexar/1.0 ml SURLYN (Freshstuff[TM], American Can, Neenah, Wisconsin). The sealed pouch dimensions were approximately 27 x 23 cm.

Gas

The following systems were used--Air compressed: Chemetron Industrial grade; Special mixtures: Ohio Medical Products, Airco, Inc.; CO_2-liquified: University of Minnesota.

Sealer

A mobil Multivac AG 900 chamber machine (Koch, 1411 West 29th Street, Kansas City, MO) was equipped with an inert gas flushing unit and contact vacuum gauge. This gauge portions the gas flushing and serves to control evaluation when products with a high water content are being packaged so that as little liquid as possible evaporates when under vacuum. The sealer used also had a clear plexiglass lid, enabling observation of the samples during the sealing operation.

Head Space (Gas Composition) Analysis

The gas chromatographic procedure for analysis of gas mixtures containing CO_2, N_2 and O_2 used the following:

Gas Chromatograph: Hewlett Packard Model #7620, thermal conductivity
                    detector (TC)

Integrator: Hewlett Packard Model #3380.

Column: 16-3/4 ft x 1/8 in in O.D. stainless steel tubing packed with
120/140 mesh carbosieve B (Supelco, Inc.)

Syringe: Gas lock, 5.0 ml Precision Sampling Corp., P.O. Box 15119,
Baton Rouge, LA 70815, (504) 924-1720

Catalog #050035 Pressure-Lok, series A-2

Sample injection size: 2.5 cc

TC detector: 200C

Bridge current: 150 milliamps

Carrier gas flow: 35 ml/min, helium

Temperature program: 35° to 175°C, 10 min, hold post injection, 30°C/min,
hold final level until CO_2 comes off.

Injection port temperature: 250°C

A septum was applied to sample bags; the septum was composed of a drop of
silicone adhesive (General Electric) which had been allowed to dry for
24 hours prior to use.

Sampling Scheme

The two fillets from a given fish were cut in half and identified. For
each fillet the upper case letter represented the head portion and the
lower case letter represented the tail portion (Table 3a). Two pieces
of fish were then placed in a preformed pouch according to the code
listed in Table 3b. Using the Koch sealer, the pouches were evacuated
and sealed or evacuated and flushed with a given gas mixture (Table 4).
After the pouches were sealed, the samples were stored at 1°C for the
duration of the storage study, 20 days. Visual observations were made
daily, and every 7 days a pouch from each gas environment was opened and
the odor and appearance of the sample was noted (Table 5).

III. Controlled atmosphere storage of herring fillets at 1°C.

Two storage studies were conducted to evaluate different gas environments
on the physical and microbiological qualities of the fish. The physical
parameters measured were: gas headspace changes of the pouch; odor,
color and texture evaluations by a 20-member sensory panel; pH; oxidative
rancidity; nucleotide degradation by measuring hypoxanthine. The micro-
biological quality of the fish was determined by the total plate count
(aerobic and anaerobic) and by the psychrotroph count.

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MATERIALS

Packaging film: A 3.8 ml coextrusion of 1.2 ml Nylon/1.6 ml Plexar/ 1.0 ml Surlyn, designated z-408 was used in the form of "Freshstuff,^{TM}" primal meatbags with dimensions 10 x 15 inches (lot #PB1015, American Can Company, 1915 Marathon Ave., P.O. Box 702, Neenah, Wisc.).

Sealer: A Koch Multivac AG-900 (Koch, 1411 West 29 Street, Kansas City, MO).

Gas: Compressed air and carbon dioxide liquified, Chemetron, University of Minnesota and gas mixtures, Ohio Medical Products, were used.

Gas headspace: The procedure stated previously in Part II was used.

pH meter: Orion Research Model 601, and standard electrodes were used.

pH determination: A convenient amount of pre-weighed fish sample was placed in an 8-ounce blender jar and blended on an Osterizer blender, using short bursts of power at slow speed in order to obtain a homogeneous mixture. The pH of this mixture was taken using a standard electrode.

Oxidative rancidity: Fish (varying with species) contain a large proportion of highly polyunsaturated lipids which can undergo oxidation and lead to the formation of malonaldehyde. The thiobarbituric acid (TBA) test developed by Lemon (1975) was used. Sinnhuber and Yu (1958) considered the test to be applicable, particularly to stored fishery products. More detail is given here and in nucleotide degradation to assist in the explanation of data.

THEORY

TEP (1,1,3,3,-tetraethoxypropane) plus acid hydrolysis yields malonaldehyde, which reacts quantitatively with thiobarbituric acid (TBA) to yield a red-colored compound. Malonaldehyde content can be quantitatively measured and the degree of oxidative rancidity expressed in mg malonaldehyde per 1000 g sample.

REAGENTS

Trichloroacetic acid (Cat #A-322, Fisher Scientific Co., Chem. Manufacturing division, Fair Lawn, NJ)

Propyl gallate (Cat #P5,330-6; Aldrich Chemical Co., Milwaukee, Wisc.)

EDTA, Ethylenediaminetetraacetic acid, disodium salt, dihydrate (Cat #10,631-3; Aldrich Chem. Co.)

2-Thiobarbituric acid (4,6-dihydroxy-2-mercapto-pyrimidine, 98 percent) (Cat #D11,350-6, Aldrich Chem. Co.)

TEP; 1,1,3,3,-tetramethoxypropane (Cat #M01150 Pfaltz & Bauer, Inc., 375 Fairfield Ave., Stanford, CT)

A. Extracting Solution

    7.5 percent trichoroacetic acid (TCA) - (75.0 g/liter, 37.5 g/500 ml)
    0.1 percent propyl gallate - (1 g/liter, 0.5 g/500 ml)
    0.1 percent ethylenediaminetetraacetic acid (EDTA) -

    Mixed together with magnetic stirrer over low heat.

B. TBA Reagent

    0.02M Thiobarbituric Acid - (2.338 g/liter, 0.5845 g/250 ml)

    Dissolved over low heat, using magnetic stirrer. Stored in dark bottle under refrigeration.

C. TEP Standard

    1 μmole/ml TEP (1,1,3,3-tetraethoxypropane) - (0.22 g/liter, 0.055 g/250 ml)

    If refrigerated, the standard was stable for a few weeks. A working standard was made by diluting the TEP solution 1:100.

STANDARD CURVE DETERMINATION

Method

A working standard was prepared by diluting the TEP standard one-hundred-fold. Solutions were then prepared as follows:

Sample	TBA Solution (mls)	H$_2$O (mls)	Working Std TEP (mls)	Malonaldehyde Content (μmoles)
1	5	0	5	0.05
2	5	1	4	0.04
3	5	2	3	0.03
4	5	3	2	0.02
5	5	4	1	0.01
6	5	5	0 (blank)	0.00

These solutions were boiled for 40 minutes simultaneously with the samples, cooled, and absorbance read at 530 nm on the Coleman double-beam Hitachi 124 spectrophotometer against a blank. A straight-line relationship between absorbance and μmoles was required.

STANDARD TBA PROCEDURE

1. A 15 g portion of tissue was blended with 30 mls of extracting solution for 30 seconds in a small Waring blender or similar homogenizer.

   The amount of sample used was not critical but the <u>ratio of one part sample to two parts extracting solution was maintained</u>.

   Sample contained <u>no skin</u>.

2. The homogenate was filtered using Whatman #1 filter paper or centrifuged at 2000 rpm for 15 minutes. A clear filtrate was used for TBA reaction.

3. 5.0 ml TBA reagent was mixed with 5.0 ml of extract in test tubes (Pyrex 120x10 mm) with screw caps. If a high concentration of malonaldehyde was present, if turbidity problems occurred, it was necessary to use less extract, i.e., only 1 to 2 ml and the volume was then brought up to 5 ml with water before adding the 5 ml TBA reagent.

4. Tubes were capped tightly and heated in boiling water for 40 minutes. If tubes were larger than Pyrex 120x10 mm, caps were loosened slightly to allow air to escape during heating.

5. Tubes were cooled under running tap water.

6. Optical density at 530 nm was measured against the blank prepared with standard curve. The absorption curve from 400 to 700 nm was reviewed to confirm that 530 nm was maximum.

7. The TBA value was calculated from the standard curve determination. The moisture content of the tissue was added to the volume of extracting solution in making the calculation. Thus, the total malonaldehyde content in each sample was determined in the following manner (assuming 30 mls of TCA and 5 mls of the extract were used in determination):

$$\frac{\mu\text{moles malonaldehyde}}{\text{sample}} = \frac{\mu\text{moles malonaldehyde}}{\text{in the 5 mls extract}} \times \frac{30+ \text{mls } H_2O \text{ in sample}}{5}$$

Results were expressed as mg of malonaldehyde/1000 gm of tissue (wet basis).

Nucleotide Degradation

Hypoxanthine formation from the degradation of nucleotides may be correlated with flavor loss caused by autolytic activities during early storage and with bacterial spoilage during extended storage (Martin et al., 1978) (Jones et al., 1964). Hypoxanthine is a product of post-mortem nucleotide degradation in fish muscle which proceeds via the following sequence of reactions:

$$\text{ATP} \xrightarrow{\text{Pi}} \text{ADP} \xrightarrow{\text{Pi}} \text{AMP} \xrightarrow[\text{NH}_3]{} \text{IMP} \xrightarrow{\text{Pi}} \text{INO} \longrightarrow \text{Hx} \longrightarrow \text{Xa} \longrightarrow \text{UA}$$

(ATP = adenosine 5-triphosphate; AMP = adenosine 5-monophosphate; IMP = inosine 5-monophosphate; INO = inosine; Hx = hypoxanthine; Xa = xanthine; UA = uric acid)

In this study hypoxanthine was measured by high pressure liquid chromatography (HPLC). The specific parameters in this system were:

Instrument: HPLC Water Associates, Model 440 detector using a 254 nm filter, 660 solvent programmer, M-6000 A pump.

Column: Water Associates, Micro-bonda-pak C18 (Cat #47957)

Filter for sample prep: Metricell membrane filter, 0.2 um (Cat #TCM-200) Gelman Instrument Company, Ann Arbor, Mich.

Regents: Hypoxanthine (6-hydroxypurine) (Cat #H-9377) Sigma Chemical Co., P.O. Box 14508, St. Louis, MO.

  Potassium phosphate monobasic (KH_2PO_4)
  Trichloroacetic acid

Primary Extraction Procedure for Hypoxanthine

1. With scissors to cut the flesh, 15.0 gm (NO SKIN) of fish were weighed into an 8-ounce blender jar.

2. 30 ml of 7.5 percent trichloroacetic acid solution were added and the suspension was blended at high speed for one minute.

3. The mixture was transferred to funnel with Whatman #1 filter paper and the filtrate was collected in 100 ml stoppered volumetric flask.

4. After transfer to funnel, 10 ml (2x) 7.5 percent TCA solution was used to wash blender jar (put on blade and cap, shake about 25 times) and transferred to funnel.

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5. The filter paper was washed twice with 10 ml (each time) with 7.5 percent TCA.

6. The pH of samples in 100 ml volumetric flask was adjusted using 1.0 N NaOH, checking pH with pH indicator paper or sticks. A final pH 4 to 5 was used.

7. The volume was adjusted with mobile phase for liquid chromatograph; i.e., 4 percent KH_2PO_4 (pH 4.40).

8. An aliquot of each sample was filtered through a metricell filter and collected in screw-cap test tube.

9. The sample was stored under refrigeration until assayed.

Steps in Analysis

1. The hypoxanthine standard (ca 1 mg/ml) was prepared using heat to get into solution.

2. 2 ml portion of hypoxanthine standard was injected.

3. The sample injected size was usually 10 ml.

4. Figure 1 shows the typical response on the HPLC using the stated procedure for hypoxanthine in fish extract.

5. Calculations for hypoxanthine were based on the measurement of peak heights for the standard and samples.

Sensory Evaluation

A 20-member panel evaluated samples for odor, appearance, and texture. Samples were presented to the panel members in coded (random number) plastic petri dishes (100 x 15 mm) with tops. The criterion for a sample being unacceptable is that the panel member would not cook and eat the fish. To evaluate the texture, the panelists were asked to use their finger and press gently on the surface of the fish; if the fish felt firm it was acceptable, if a slight depression remained (felt soft) it was unacceptable. The sensory evaluation is given in Table 6.

MICROBIOLOGICAL ANALYSIS

Standard plate count agar (Difco) was prepared in accordance with instructions on the bottle and used as the enumeration media. The diluent was 0.1 percent peptone, Bacto-Peptone (Difco). The sample was prepared in the Stomacher Lab Blender [400 (Model #BA 6021) A.J. Seward, UAC House, Blackfriar Road, London SE19U6, Dynatech Laboratories, Inc., 900 Slaters Lane, Alexandria, VA].

Sampling Preparation

1. 100 g of fish were weighed into stomacher bag

2. 100 ml of sterile 0.1 percent peptone diluent were added

3. Sample was blended in the stomacher approximately 1 minute (1:2 dilution)

4. 20 g of above sample were weighed into another stomacher bag using sterile tongue depressor for transfer; and 80 ml of sterile 0.1 percent peptone water were added.

5. Sample was blended approximately 30 seconds (1:10 dilution)

6. Further appropriate dilutions were made

Plating Procedure

1. A 1.0 ml portion of the proper dilution was dispensed onto sterile petri dishes for aerobic and anaerobic analysis (usually 4 dilutions in duplicate = 8 plates).

2. A 0.1 ml portion of the proper dilution was dispensed onto prepoured SPC (Standard Plate Count) for psychrotroph analysis (4 dilutions in duplicate = 8 plates).

3. For aerobic and anaerobic plates, about 10 ml of tempered (45° to 47°C) medium were added and swirled to uniformly distribute the inoculum. For anaerobic analysis, the plate was overlaid with an additional 10 ml of SPC agar.

4. Psychrotroph prepoured plated required the 0.1 inoculum distributed over the surface of the agar with a sterile bent glass rod until dry.

5. Aerobic plates were incubated at room temperature (25°C); anaerobe plates were placed in BBL anaerobic GasPak jars, evacuated 3 times and flushed 3 times with N_2 (5# and 15 psi vacuum). Anaerobic jars were then incubated at room temperature (25°C) with aerobic plates.

6. Psychrotroph plates were incubated at 7°C.

7. Plates were counted after 48 hours of incubation.

Sampling Procedure

Approximately 250 g of fish were placed in each pouch. On the day of analysis all the samples of a specific gas environment for the various tests were from the same pouch. The pouch was opened using a sterile

scissor and using aseptic technique, 100 g were weighed into a stomacher bag for microbiological analysis. A portion of the fish was placed in a plastic petri dish (covered) for sensory testing and stored at 1°C until evaluated. Samples for TBA, hypoxanthine and pH were weighed into respective 8-ounce blender jars and stored at 1°C until sample processing for the given analysis.

STORAGE STUDY

Fish: Herring fillets ranging in weight from 140 to 200 g per fillet were obtained from Lake Superior Fresh Fish Market, Duluth, MN. Approximately 23 pounds of fillets were contained in a plastic bag and placed in a styrofoam cooler with crushed ice. The coolers were transported by car to the University of Minnesota and stored at 1°C until sample packaging. The elapsed time from transporting the fish to initiation of the packaging was about 16 hours.

Packaging: Fillets were randomly taken from the coolers using sterile gloves and placed in preformed pouches of Nylon/Surlyn and sealed on the Koch AG 900 Multivac sealer. The various gas environments are listed in Table 7. The sample pouches were stored horizontally on wire shelves at 1°C for the duration of the storage period. Single samples per gas environment were analyzed after indicated days storage at 1°C. On specified days (0, 8 and 14 in Trial I) triplicate samples of treatments 5 and 6 (100 percent CO_2, vacuum) were analyzed.

Storage Study - Trial I

For the discussion of results the following key will be used:

Treatment No.	Theoretical Percentage			Actual Percentage (GC Analysis)		
	CO_2	N_2	O_2	CO_2	N_2	O_2
1 (air)		78	21	--	79.7	20.3
2	20	79	1	29.5	69.6	0.9
3	30	69	1	41.3	57.8	0.9
4	30	70	--	41.8	58.0	0.2
5	100	--	--	100.0	--	--
6	Vacuum 1°C					
7	Vacuum - frozen					

The initial counts on colony forming units (cfu/g) were ca 10^6/g for the fresh fillets. Samples from treatment #1 (air) showed increased growth in both aerobes (Table 8 and Figure 2) and anaerobes (Table 9 and Figure 3) to ca 10^9/g after 19 days in storage at 1°C. Samples from treatments 2, 3, 4 (30 to 40 percent CO_2, 58 to 70 percent N_2, 0.2 to 0.9 percent O_2) showed a gradual increase from 10^5 to 10^7/g for both aerobes and anaerobes during the storage period. In both cases treatment #4 increased at 12 days storage to ca 10^8/g and then declined to a final level of ca 10^7/g. Treatment #5 (100 percent CO_2) resulted in little growth for both aerobes and anaerobes. Of all the treatments investigated, treatment #5 (100 percent CO_2) most closely approximated treatment #7 (vacuum, frozen). The data for the aerobes and anaerobes are quite similar, indicating the presence of facultative anaerobes. The data for the psychrotrophs are given in Table 10 and Figure 4; the same trends were observed.

Oxidative rancidity as calculated by the amount of malonaldehyde formed was minimal in all treatments (Table 11).

The pH of fish versus days in storage at 1°C is given in Table 12. For all the treatments studied, there was little change in pH during the 19 days of storage.

The concentration of hypoxanthine (μmoles/g fish) in the samples during the storage period is given in Table 13. During the chill-storage (1°C) of fish, the rate of formation of hypoxanthine typically increased at 10 to 12 days (Jones et al., 1964). The data demonstrate that between 8 to 12 days of storage at 1°C there was an increase in the concentration of hypoxanthine in all treatments except #5 (100 percent CO_2) and #7 (vacuum, -20°C). The concentration of hypoxanthine in treatment #1 (air) is comparable to the levels in treatments 2, 3, 4 (30 to 42 percent CO_2)m 58 to 70 percent N_2, <1 percent O_2). Based on these limited data, it was difficult to speculate whether the higher concentration of carbon dioxide had a direct effect on retarding the formation of hypoxanthine.

Headspace analysis of the samples was made routinely throughout the storage period (Table 14). Samples of treatment #1 showed the greatest amount of change in the gas composition; the percentage of CO_2 increased to ca 27 percent and the percentage of O_2 was reduced to less than 1 percent.

RESULTS AND DISCUSSION

Because our study's methods were divided into three parts, results also were separated into three sections corresponding to each method:

I. Monitoring pH of fish samples in storage at 1°C

In evaluating the odor of the samples upon opening the pouches, dramatic differences were noted on 21 days in storage. All vacuum samples had an

extremely strong odor indicating proteolysis, whereas the carbon dioxide samples had only a slight grassy odor, no proteolytic odor.

A more significant finding was the change in the physical appearance of the fish which was most dramatic in the herring fillets (Table 2). This subjective evaluation showed that in vacuum samples the flesh was slightly pink and relatively firm to the touch. The samples stored in carbon dioxide were grey. This was a surface phenomenon extending approximately 1/8 to 1/4-inch into the flesh while the interior region remained pink. The grey flesh was mushy to the touch.

II. Appearance and odor of fish fillets stored with various gas mixtures.

After six days in storage, most samples had a yeasty odor and all samples were turning white (the original color of the flesh was light pink). After 8 days, the samples in 100 percent CO_2 had a green discoloration. Some major differences were noted after 13 days storage at 1°C. The samples in 100 percent CO_2 were very discolored--grey-green, whereas the other samples were white. The samples stored in air were objectionable in both odor and appearance. After 20 days in storage, all samples except the system stored in the low percentage of carbon dioxide were objectionable based on odor and appearance. Thus, further work needed to be done to assess the effect of the gas environments containing a low percentage of carbon dioxide on the chemical and microbiological qualities of the fish in storage.

III. Controlled atmosphere storage of herring fillets at 1°C

The summary of the sensory data is given in Table 15. No definite trends were observed; the data were erratic especially when one notes the responses for the triplicate samples of treatments #5 and #6 on days 8 and 14. To have more valid sensory data, one could use a trained panel in which each panel member has an individual pouch containing the fish so that the panelist could open the pouch and do the required evaluations.

Storage Study – Trial II

In the discussion of results the following code will be used:

Treatment No.	Theoretical Composition			Actual (GC Analysis)		
	$CO_2\%$	$N_2\%$	$O_2\%$	$CO_2\%$	$N_2\%$	$O_2\%$
1	--	81	19	--	79.7	20.3
2	20	79	1	29.5	69.6	0.9
3	30	69	1	41.3	57.8	0.9
4	30	70	--	41.8	58.0	0.2
5	100	--	--	100.0	--	--
6	Vacuum (1°C)					
7	Vacuum (-20°C)					

The initial level of cfu/g was ca 10^5/g for fresh fillets. Samples from treatment #1 (air) showed increased growth in both aerobes (Table 16, Figure 5) and anaerobes (Table 17, Figure 6) to ca 10^9/g after 11 days storage at 1°C. Treatments #2, 3, and 4 (23 to 34 percent CO_2, 65 to 76 percent N_2, 0 to 0.87 percent O_2) showed a gradual increase in aerobes from 10^5 to 10^6. Essentially the same pattern was shown for the anaerobes (Table 17, Figure 6). Treatment #3 in both cases increased at 7 days storage and then declined to ca 10^6 cfj/g. Treatment #5 (100 percent CO_2) again resulted in very little growth for both the aerobes and anaerobes. Samples from treatment #6 (vacuum) resulted in slightly higher population then the other treatments (excluding #1 = air). As was demonstrated in Trial 1, again, the aerobes and anaerobes followed similar growth trends suggesting the presence of facultative anaerobes. The growth of psychrotrophs closely resembled the aerobes' and anaerobes' growth patterns (Table 18, Figure 7).

The results of the TBA analysis are given in Table 19. The storage study was of short duration (11 days) and consequently there was little increase in the TBA numbers.

The pH of the fish versus days in storage at 1°C is given in Table 20. There was little change in the pH of the fish in the various gas environments during the 11-day storage period.

The concentration of hypoxanthine (μmoles/g fish) in the samples during the 11 day storage at 1°C is given in Table 21. The storage period was so short that no definite trends were noted in the rate of formation of hypoxanthine.

The results of the routine headspace analysis of the pouches during the storage period are given in Table 22. Samples of treatment #1 exhibited a greater change in gas composition than the other treatments. In treatment #1, the percentage of CO_2 increased from <1 percent to ca 7 percent, and the percentage O_2 decreased from 20 percent to 15 percent.

The summary of the sensory data is given in Table 23. As demonstrated in Trial 1, the data again were erratic, suggesting the need for a more sophisticated sensory analysis.

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TABLE 1

pH VS DAYS IN STORAGE AT 1°C

	Initial	11 Days		21 Days	
		Vacuum	CO_2	Vacuum	CO_2
Smelt (-) gut	6.87	6.85	6.49	7.00	6.59
(-) head					
Herring Fillet	6.68	6.62	6.46	6.41	6.34
Herring w/skin	6.72	6.80	6.67	6.62	6.48

TABLE 2

PHYSICAL CHARACTERISTICS

Vacuum samples - Flesh slight pink

- Relatively firm to the touch

CO_2 samples - Flesh: grey color, which was a surface phenomenon, approximately 1/8 to 1/4" in depth. The interior portion was pink.

- The flesh was mushy to the touch

TABLE 3

(a) Coding of fish
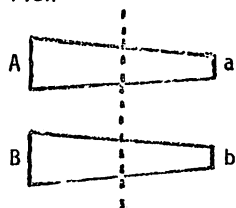
1)	Aa Bb	5)	Jj Kk
2)	Cc Dd	6)	Ll Mm
3)	Ee Ff	7)	Nn Oo
4)	Gg Hh	8)	Pp Qq

(b) Code of fish in pouches

1)	Ah	jQ
2)	aB	Jk
3)	Cb	lK
4)	cD	Lm
5)	Ed	nM
6)	eF	No
7)	Gf	pO
8)	gH	Pq

TABLE 4

GAS MIXTURES

	Theoretical			Actual		
	$\%CO_2$	$\%N_2$	$\%O_2$	$\%CO_2$	$\%N_2$	$\%O_2$
1. (air)	~0.04	~78	~21	0.95	79.13	19.92
2.	10	90	--	16.49	83.34	0.17
3.	20	79	1	29.76	68.44	0.80
4.	30	69	1	42.73	56.48	0.79
5.	30	28	2	41.49	57.19	1.32
6.	30	70	--	42.88	56.87	0.25
7.	100	--	--	100.00	--	--
8.	vacuum					

22

TABLE 5

WHITE FISH FILLETS STORED AT 1°C

Atmosphere	Day 6		Day 13		Day 20	
	Appearance	Odor	Appearance	Odor	Appearance	Odor
Air	White	Yeasty	White, milky film on pkg.	Strong Object.	White, milky	Highly Object.
O_2:0.20%, N_2:83.3%, CO_2:16.50%	White	Yeasty	White	Cooked Fish	White	Old fish
O_2:0.80%, N_2:69.40%, CO_2:29.80%	White	Yeasty	White	Slightly fishy; Initial H_2S	White	Sewage
O_2:1.3%, N_2:57.2%, CO_2:41.5%	White	Yeasty	White	Slightly fishy; Cooked fish	White-greenish	Sewage
O_2:0.30%, N_2:56.8%, CO_2:42.9%	White	Slightly sour	White	Cooked shrimp	Very white	Sewage
CO_2:100%	White(a)	Yeasty	Very grey-green	Slightly cooked fish	Grey-green	Yeasty
Vacuum	White	Slightly fishy	White	Fishy	White-tan	Very fishy

(a) Day 8 - Green discoloration first observed.

TABLE 6

Name _____ Date _____

Please evaluate the samples for odor, appearance and texture as either acceptable or unacceptable. The criteria for a sample being unacceptable is that you would not cook and eat the fish. If you rate any of the characteristics as unacceptable, please try to describe why.

To evaluate texture: use your finger and press gently on the surface of the fish; if it feels firm it is acceptable, if a slight depression remains (or feels soft) it is unacceptable.

1) Sample # _____

    odor: acceptable _____
          unacceptable _____
     description:

    appearance: acceptable _____
            unacceptable_____
     description:

    texture: acceptable _____
          unacceptable _____
     description:

    Would you cook and eat this fish?
       Yes _____   No _____
    _____

2) Sample # _____

    odor: acceptable _____
          unacceptable _____
     description:

    appearance: acceptable _____
            unacceptable _____
     description:

    texture: acceptable _____
          unacceptable _____
     description:

    Would you cook and eat this fish?
       Yes _____   No _____

3) Sample # _____

    odor: acceptable _____
          unacceptable_____
     description:

    appearance: acceptable _____
            unacceptable _____
     description:

    texture: acceptable _____
          unacceptable _____
     description:

    Would you cook and eat this fish?
       Yes _____   No_____
    _____

4) Sample # _____

    odor: acceptable _____
          unacceptable _____
     description:

    appearance: acceptable _____
            unacceptable _____
     description:

    texture: acceptable _____
          unacceptable _____
     description:

    Would you cook and eat this fish?
       Yes_____   No _____

TABLE 7

GAS ENVIRONMENTS

Treatment #	Theoretical (%) CO_2	N_2	O_2	Actual (a) % CO_2	N_2	O_2
1	(air)	~78	~21	0	79.7	20.3
2	20	79	1	29.5	69.6	0.90
3	30	69	1	41.3	57.8	0.90
4	30	70	0	41.8	58.0	0.20
5	100	0	0	100.0	0	0
6	Vacuum (1°C)					
7	Vacuum (-20°C control)					

(a) Analysis of gas mixtures on the gas chromatograph

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TABLE 8

TRIAL I

AEROBIC PLATE COUNT (log cfu/g)

vs.

DAYS OF STORAGE AT 1°C

Treatments

Days in Storage	1	2	3	4	5	6	7
(a) 2	6.24	6.41	6.21	6.04	5.92	6.31	5.99
5	7.92	6.85	6.56	6.41	6.23	6.60	6.33
8	8.41	6.70	6.70	6.53	6.45	7.54	6.05
					6.32	6.79	
					6.71	6.65	
12	7.21	7.24	6.77	8.41	6.61	7.29	5.73
14	9.56 est	7.95	7.79	6.68	5.99	7.62	5.88
					6.26	7.44	
					7.14	7.61	
19	8.36	7.59	7.41	7.62	6.57	8.10	6.41

(a) initial $\overline{X}$ = 5.90
    5.95
    5.77
    6.15
    5.95
    6.01
    5.62

TABLE 9

TRIAL I

ANAEROBIC PLATE COUNT (log cfu/g)

vs.

DAYS OF STORAGE AT 1°C

Treatments

Days in Storage	1	2	3	4	5	6	7
(a) 2	6.01	6.31	6.06	5.97	5.54	5.97	5.84
5	7.75	6.80	6.57	6.44	6.43	6.70	6.38
8	8.32	6.45	6.49	6.30	6.36	6.72	6.04
					6.32	7.57	
					6.46	6.60	
12	7.21	7.24	6.67	8.35	6.61	7.27	
14	9.52	7.87	7.64	6.85	5.86	7.65	5.61
						7.46	
						7.49	
19	8.33	7.63	7.49	7.63	6.68	8.15	6.43

(a) initial X̄ = 6.17
6.16
5.88
6.55
6.10
6.05
6.25

TABLE 10

TRIAL I

PSYCHROPHILES (log cfu/g)

VS.

DAYS OF STORAGE AT 1°C

Treatments

Days of Storage	1	2	3	4	5	6	7
(a) 2	6.30	6.29	5.51	5.14	5.11	6.17	5.04
5	8.02	6.85	6.46	6.14	5.59	6.57	5.46
8	8.38	6.61	6.46	6.57	5.72	6.81	5.23
					5.72	8.05	
					6.38	6.74	
12	7.31	7.27	6.92	8.42	6.68	7.41	5.13
14	>8.48	8.08	8.09	7.10	5.97	7.85	5.75
					5.97	7.75	
					7.23	7.77	
19	>8.48	7.69	7.64	7.80	6.65	8.23	5.77

(a) initial X̄ = 5.37
5.06
5.49
5.40
5.10
6.22
4.97

TABLE 11

TRIAL I

TBA RESULTS

MG MALONALDEHYDE/1000 G TISSUE (WET BASIS)

Treatments

Days in Storage	1	2	3	4	5	6	7
(a) 2	0.14	0.14	0.14	0.13	0.11	0.14	0.12
5	0.34	0.13	0.18	0.09	0.11	0.30	0.10
8	0.22	0.15	0.18	0.31	0.23	0.09	0.22
					0.25	0.23	
					0.17	0.28	
12	0.57	0.30	0.67	0.35	0.25	0.14	0.19
14	0.45	0.29	0.24	0.22	0.27	0.11	0.19
					0.34	0.10	
					0.49	0.14	
19	0.50	0.37	0.24	0.86	0.19	0.24	0.21

(a) initial $\bar{X}$ = 0.16
0.11
0.22
0.16
0.13

TABLE 12

TRIAL I

PH OF FISH

VS.

DAYS OF STORAGE AT 1°C

Treatments

Days in Storage	1	2	3	4	5	6	7
(a)							
2	6.31	6.05	6.07	6.30	6.15	6.25	6.44
5	6.44	6.32	6.25	6.25	6.10	6.22	6.33
8	6.22	6.23	6.22	6.27	6.18	6.25	6.44
					6.11	6.35	
					6.16	6.51	
12	6.26	6.45	6.21	6.52	6.10	6.29	6.42
14	6.21	6.29	6.38	6.42	6.18	6.36	6.46
					6.15	6.38	
					6.54	6.39	
19	6.19	6.44	6.27	6.71	6.15	6.35	6.47

(a) initial $\bar{X}$ = 6.36
6.22
6.34
6.49
6.39

TABLE 13

TRIAL I

HYPOXANTHINE: u MOLES/g FISH

(a) initial $\overline{X}$ = 0.28

Treatment

Days in Storage	1	2	3	4	5	6	7
2	0.43	0.43	0.50	0.99	0.28	0.60	0.04
5	0.41	0.80	0.45	0.69	0.35	0.53	0.30
8	0.55	0.72	0.61	0.84	0.61	0.94	0.46
					0.42	1.27	:
					0.16	0.98	
12	1.39	1.27	1.13	2.35	0.68	1.52	0.21
14	2.77	2.40	2.71	0.95	0.55	2.38	1.10
					0.48	1.98	
					0.29	2.27	
19	3.08	2.84	2.99	3.87	3.56	3.87	0.22

(a) 0.23, 0.36, 0.18, 0.35

TABLE 14

TRIAL I

HEADSPACE ANALYSIS - GAS COMPOSITION
VS.
DAYS OF STORAGE AT 1°C

(%) change from initial

Treatment		%CO_2	%N_2	%O_2
#1	0	0	79.70	20.30
	2	1.66 (+ 1.66)	78.94 (- 0.76)	19.40 (- 0.91)
	5	2.19 (+ 2.19)	78.83 (- 0.87)	18.98 (- 1.33)
	7	7.87 (+ 7.87)	77.49 (- 2.21)	14.64 (- 5.67)
	12	18.18 (+18.18)	74.93 (- 4.77)	6.88 (-13.43)
	14	21.79 (+21.79)	77.44 (- 2.26)	0.77 (-19.54)
	19	26.68 (+26.68)	73.16 (- 6.54)	0.17 (-20.14)
#2	0	29.52	69.62	0.86
	2	22.28 (- 7.24)	77.72 (+ 8.10)	0.00 (- 0.86)
	5	22.25 (- 7.27)	76.71 (+ 7.09)	1.04 (+ 0.18)
	7	21.73 (- 7.79)	77.45 (+ 7.83)	0.83 (- 0.03)
	12	18.25 (-11.27)	74.50 (+ 4.88)	7.25 (+ 6.39)
	14	23.39 (- 6.13)	76.17 (+ 6.55)	0.44 (- 0.42)
	19	27.06 (- 2.46)	72.78 (+ 3.16)	0.16 (- 0.70)
#3	0	41.34	57.77	0.89
	2	32.26 (- 9.08)	67.74 (+ 9.97)	0.00 (- 0.89)
	5	31.34 (-10.00)	67.75 (+ 9.97)	0.92 (+ 0.03)
	7	31.18 (-10.16)	67.99 (+10.22)	0.84 (- 0.05)
	12	33.68 (- 7.66)	65.67 (+ 7.90)	0.64 (- 0.25)
	14	31.50 (- 9.84)	67.92 (+10.15)	0.58 (- 0.49)
	19	35.59 (- 5.75)	64.11 (+ 6.34)	0.30 (- 0.59)
#4	0	41.81	58.04	0.15
	2	33.14 (- 8.67)	66.86 (+ 8.82)	0.00 (- 0.15)
	5	32.97 (- 8.84)	66.93 (+ 8.89)	0.10 (- 0.05)
	7	32.61 (- 9.20)	67.24 (+ 9.20)	0.14 (- 0.01)
	12	34.85 (- 6.96)	65.03 (+ 6.99)	0.12 (- 0.03)
	14	32.49 (- 9.32)	67.38 (+ 9.34)	0.14 (- 0.01)
	19	33.42 (- 8.39)	64.37 (+ 6.33)	2.20 (+ 2.05)
#5	0	100.00	0.00	0.00
	2	99.83 (- 0.12)	0.00	0.00
	5	100.00	0.00	0.00
	7	99.78 (- 0.22)	0.00	0.00
	12	99.75 (- 0.25)	0.00	0.00
	14	99.72 (- 0.28)	0.00	0.00
	19	99.71 (- 0.29)	0.00	0.00

TABLE 15

TRIAL I

SUMMARY SENSORY DATA
PERCENTAGE OF PANELISTS RATING SAMPLES ACCEPTABLE

	Treatments 1	2	3	4	5	6	7
Day 2 (22 respondents)							
Odor %A	95.5	90.9	90.9	95.5	90.9	95.5	95.5
Appearance %A	95.5	90.9	95.5	63.6	86.4	90.9	86.4
Texture %A	68.2	90.0	81.8	86.4	90.9	86.4	77.3
%Y(a)	81.8	90.9	86.4	77.3	90.9	90.9	86.4
Day 5 (16 respondents)							
Odor	87.5	87.5	100.0	87.5	87.5	100.0	93.8
Appearance	100.0	100.0	100.0	81.3	68.6	100.0	81.3
Texture	100.0	75.0	93.8	93.8	37.5	93.8	56.3
% Y	93.8	87.5	93.8	97.5	56.3	100.0	68.8
Day 8 (17 respondents							
Odor	64.7	64.7	82.4	82.4	100.0	88.2	94.1
					88.2	76.5	
					94.1	94.1	
Appearance	64.7	82.4	88.2	82.4	82.4	17.0	70.6
					76.5	94.1	
					82.4	88.2	
Texture	70.6	88.2	94.1	70.6	70.6	64.7	88.2
					94.1	88.2	
					58.8	88.2	
%Y	58.8	64.7	88.2	70.6	76.5	76.5	82.4
					82.4	70.6	
					70.6	88.2	
Day 12 (11 respondents)							
Odor	18.2	72.7	90.0	63.6	72.7	45.5	100.0
Appearance	54.5	81.8	81.8	54.5	54.5	72.7	72.7
Texture	63.6	90.9	100.0	100.0	45.5	54.5	100.0
%Y	30.0	72.7	90.9	45.5	36.4	45.5	90.9
Day 14 (14 respondents)							
Odor	35.7	57.1	50.0	100.0	92.9	42.9	100.0
					76.9	57.1	
					57.1	85.7	
Appearance	57.1	86.7	78.6	78.6	92.9	29.9	78.6
					35.7	71.4	
					21.4	78.6	
Texture	57.1	100.0	57.1	100.0	92.9	100.0	78.6
					92.9	78.6	
					57.1	100.0	
%Y	28.6	64.3	50.0	100.0	92.9	57.1	78.6
					57.1	57.1	
					28.6	78.6	
Day 19 (10 respondents)							
Odor	20.0	40.0	40.0	70.0	100.0	60.0	80.0
Appearance	10.0	90.0	60.0	80.0	70.0	60.0	60.0
Texture	30.0	100.0	70.0	80.0	90.0	70.0	60.0
%Y	0.0	60.0	30.0	70.0	80.0	50.0	50.0

(a) %Y = % of panelists who would cook and eat the sample

TABLE 16

TRIAL II

AEROBIC PLATE COUNT (log cfu/g)
VS.
DAYS OF STORAGE AT 1°C

Days in Storage		Treatment 1	2	3	4	5	6	7
(a)	4	6.29	5.21	5.21	5.46	5.08	5.10	5.06
	7	7.61	5.94	6.41	5.67	5.27	5.35	5.09
						5.14	5.77	
						5.28	5.76	
	11	8.89	6.48	5.77	5.95	5.00	6.81	4.63

(a) initial $\overline{X}$ = 5.24
5.26
5.35
5.13
5.16
5.31
5.21

TABLE 17

TRIAL II

ANAEROBIC PLATE COUNT (log cfu/g)

VS.

DAYS OF STORAGE AT 1°C

Treatments

Days in Storage	1	2	3	4	5	6	7
(a) 4	5.63	5.21	5.15	5.48	4.75	4.51	5.00
7	7.53	5.98	6.90	5.58	5.26	5.43	5.09
					5.14	5.95	
					5.29	5.79	
11	8.89	6.51	5.69	5.88	4.90	6.96	5.44

(a) initial $\overline{X}$ = 5.08
    5.27
    5.07
    4.91
    4.82
    5.21
    5.22

TABLE 18

TRIAL II

PSYCHROPHILES (log cfu/g)

VS.

DAYS OF STORAGE AT 1°C

Treatments

Days in storage	1	2	3	4	5	6	7
(a) 4	7.47 est	4.84 est	4.89	5.86	4.83	4.86	4.83
7	7.56	5.83	5.08	5.18	4.94	5.41	4.61
					5.17	5.66	
					5.12	5.69	
11	8.68 est	6.71	5.83	5.92	4.87	6.80	4.18 est

(a) initial $\overline{X}$ =
    4.86
    4.85
    4.54
    4.80
    4.90
    4.69

TABLE 19

TRIAL II

TBA RESULTS

mg MALONALDEHYDE/1000 G TISSUE (WET BASIS)

Days in storage	Treatments						
	1	2	3	4	5	6	7
(a) 4	0.12	0.12	0.06	0.08	0.08	0.09	0.29
7	0.35	0.12	0.21	0.10	0.15	0.11	0.12
					0.19	0.09	!
					0.12	0.08	
11	0.19	0.13	0.08	0.07	0.14	0.09	0.09

(a) initial $\overline{X}$ = 0.065
    0.06
    0.07

TABLE 20

TRIAL II

pH OF FISH

VS.

DAYS OF STORAGE AT 1°C

Days in storage	Treatments						
	1	2	3	4	5	6	7
(a) 4	6.59	6.77	6.71	6.92	6.51·	6.74	6.82
7	6.63	6.62	6.73	6.69	6.72	6.65	6.82
					6.66	6.95	!
					6.69	6.76	
11	6.71	6.63	6.90	6.76	6.71	6.85	6.89

(a) initial $\overline{X}$ = 6.56
    6.49
    6.62

TABLE 21

TRIAL II

HYPOXANTHINE:u MOLES/G FISH

(a) initial $\overline{X}$ = 0.23

Treatment

Days in storage	1	2	3	4	5	6	7
4	0.43	0.33	0.57	0.77		0.69	0.22
7	0.47	0.69	0.86	0.89	0.75	0.81	0.34
					0.56	0.83	:
					0.66	0.71	
11	1.07	0.93	1.20	0.86	0.57	0.89	0.19

(a) 0.24, 0.22

TABLE 22

TRIAL II

HEADSPACE ANALYSIS - GAS COMPOSITION
VS.
DAYS OF STORAGE AT 1°C
(%) change from initial

Treatment		$\%CO_2$	$\%N_2$	$\%O_2$
#1	Day 0	0.63	79.56	19.81
	4	0.00 (- 0.63)	80.04 (+ 0.48)	19.96 (+ 0.15)
	7	2.41 (+ 1.78)	79.46 (- 0.10)	18.12 (- 1.69)
	11	6.88 (+ 6.25)	78.47 (- 1.09)	14.65 (- 5.16)
#2	Day 0	22.95	76.24	0.81
	4	25.80 (+ 2.85)	73.47 (- 2.77)	0.74 (- 0.07)
	7	21.71 (- 1.24)	77.56 (+ 1.32)	0.73 (- 0.08)
	11	20.46 (- 2.49)	78.75 (+ 2.51)	0.80 (- 0.01)
#3	Day 0	34.36	64.77	0.87
	4	13.24 (-21.12)	71.86 (+ 7.09)	14.90 (+14.03)
	7	32.96 (- 1.40)	66.22 (+ 1.45)	0.82 (- 0.05)
	11	31.50 (- 2.86)	67.71 (+ 2.94)	0.79 (- 0.08)
#4	Day 0	34.07	65.93	0.00
	4	2.47 (-31.60)	78.93 (+13.00)	18.60 (+18.60)
	7	32.11 (- 1.96)	67.48 (+ 1.55)	0.41 (+ 0.41)
	11	30.05 (- 4.02)	68.94 (+ 3.01)	1.01 (+ 1.01)
#5	Day 0	100.00	0.00	0.00
	4	95.24 (- 4.75)	3.81 (+ 3.81)	0.95 (+ 0.95)
	7	100.00	0.00	0.00
	11	100.00	0.00	0.00

TABLE 23

TRIAL II

SUMMARY SENSORY DATA

PERCENTAGE OF PANELISTS RATING SAMPLES ACCEPTABLE

	Treatments						
	1	2	3	4	5	6	7
Day 4 (14 respondents)							
Odor %	92.9	92.9	100.0	92.9	92.9	100.0	71.4
Appearance %	71.4	78.6	57.1	71.4	85.7	78.6	35.7
Texture %	78.6	85.7	71.4	78.6	92.9	78.6	57.1
%Y	71.4	78.6	78.6	78.6	85.7	85.7	50.0
Day 7 (13 respondents)							
Odor	92.3	92.3	84.6	100.0	76.9	92.3	76.9
					92.3	92.3	
					100.0	92.3	
Appearance	69.2	53.8	30.8	84.6	46.2	100.0	38.5
					76.9	76.9	
					84.6	84.6	
Texture	69.2	84.6	61.5	100.0	53.8	92.3	69.2
					92.3	100.0	
					84.6	69.2	
%Y	69.2	53.8	46.2	92.3	53.8	92.3	53.8
					69.2	84.6	
					84.6	69.2	
Day 11 (13 respondents)							
Odor	61.5	84.6	84.6	100.0	84.6	76.9	92.3
Appearance	61.5	61.5	61.5	76.9	92.3	38.5	69.2
Texture	53.8	61.5	61.5	84.6	100.0	46.2	100.0
%Y	53.8	61.5	76.9	84.6	84.6	38.5	84.6

FIGURE 1

High Pressure Liquid Chromatography Analysis of Fish Extract

uv 254 nm
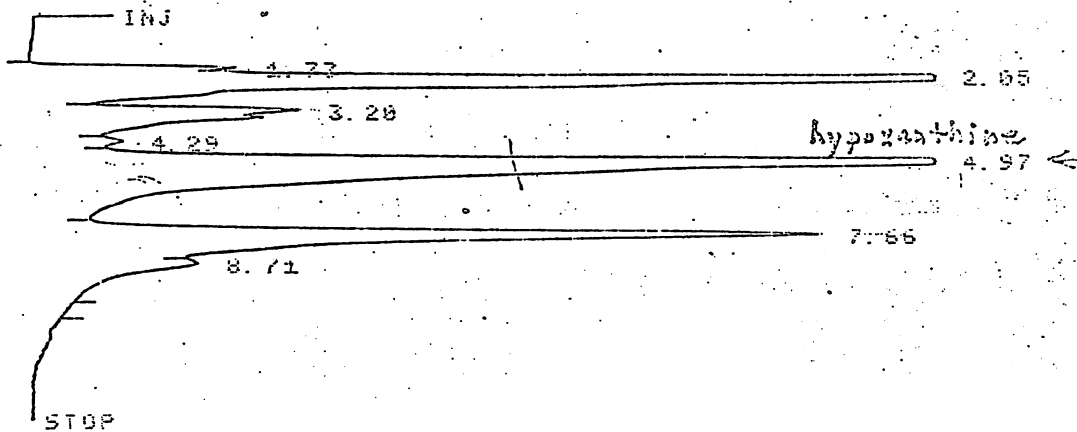
Mobile Phase: 4% KH_2PO_4 (pH 4.40)   Flow: 1.5 ml/min

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FIGURE 2
Run I
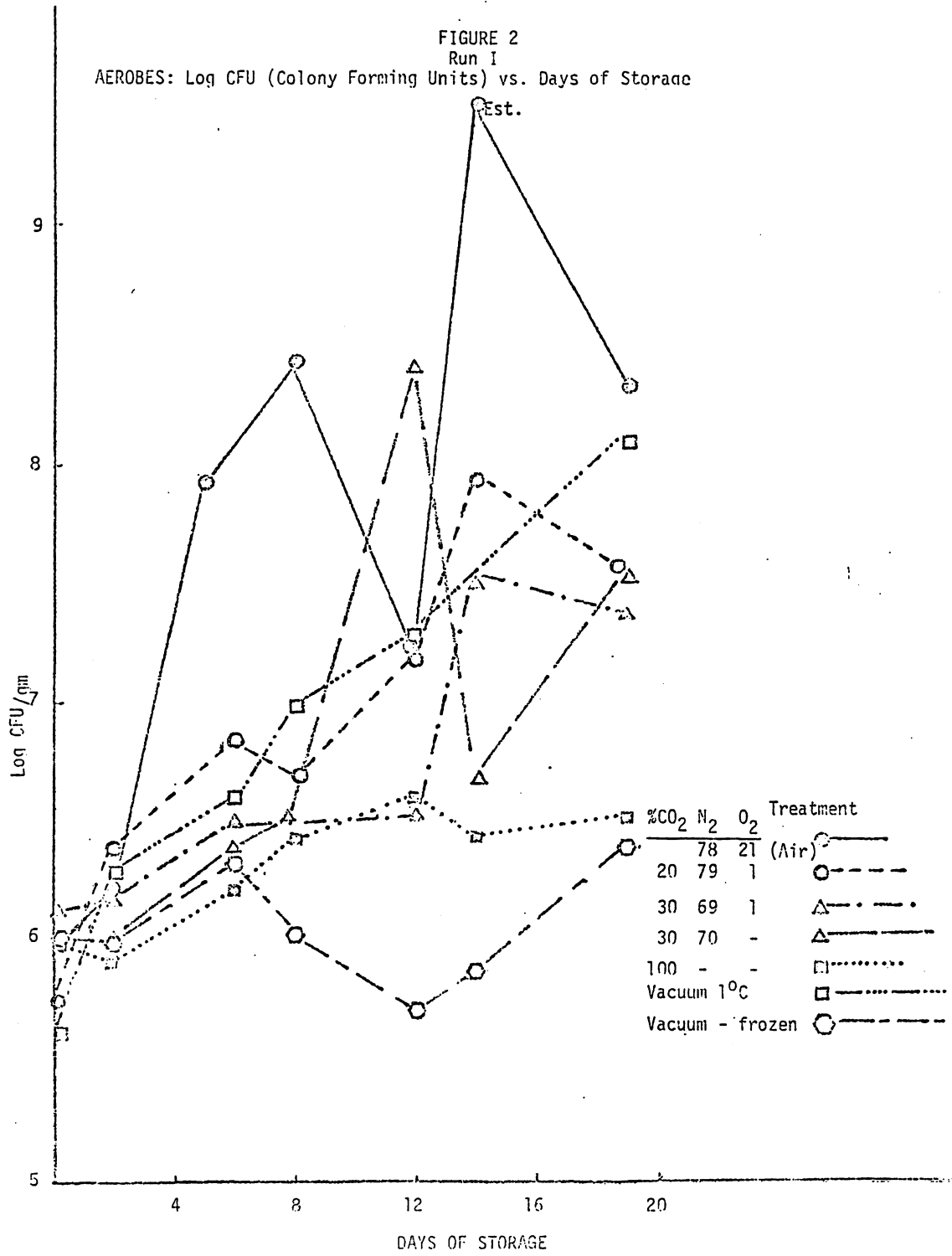AEROBES: Log CFU (Colony Forming Units) vs. Days of Storage

%CO₂	N₂	O₂	Treatment

%CO₂ N₂ O₂ Treatment
78 21 (Air)
20 79 1
30 69 1
30 70 -
100 - -
Vacuum 1°C
Vacuum - frozen

Log CFU/gm

DAYS OF STORAGE

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FIGURE 3
Run I

ANAEROBES: Log CFU (Colony Forming Units)/g vs. Days of Storage

%CO$_2$	N$_2$	O$_2$	Treatment	
	78	21	(Air)	○———
20	79	1		○---
30	69	1		△—·—·
30	70	–		△—··—
100	–	–		☐········
Vacuum	1ºC			☐—···—
Vacuum – frozen				○————
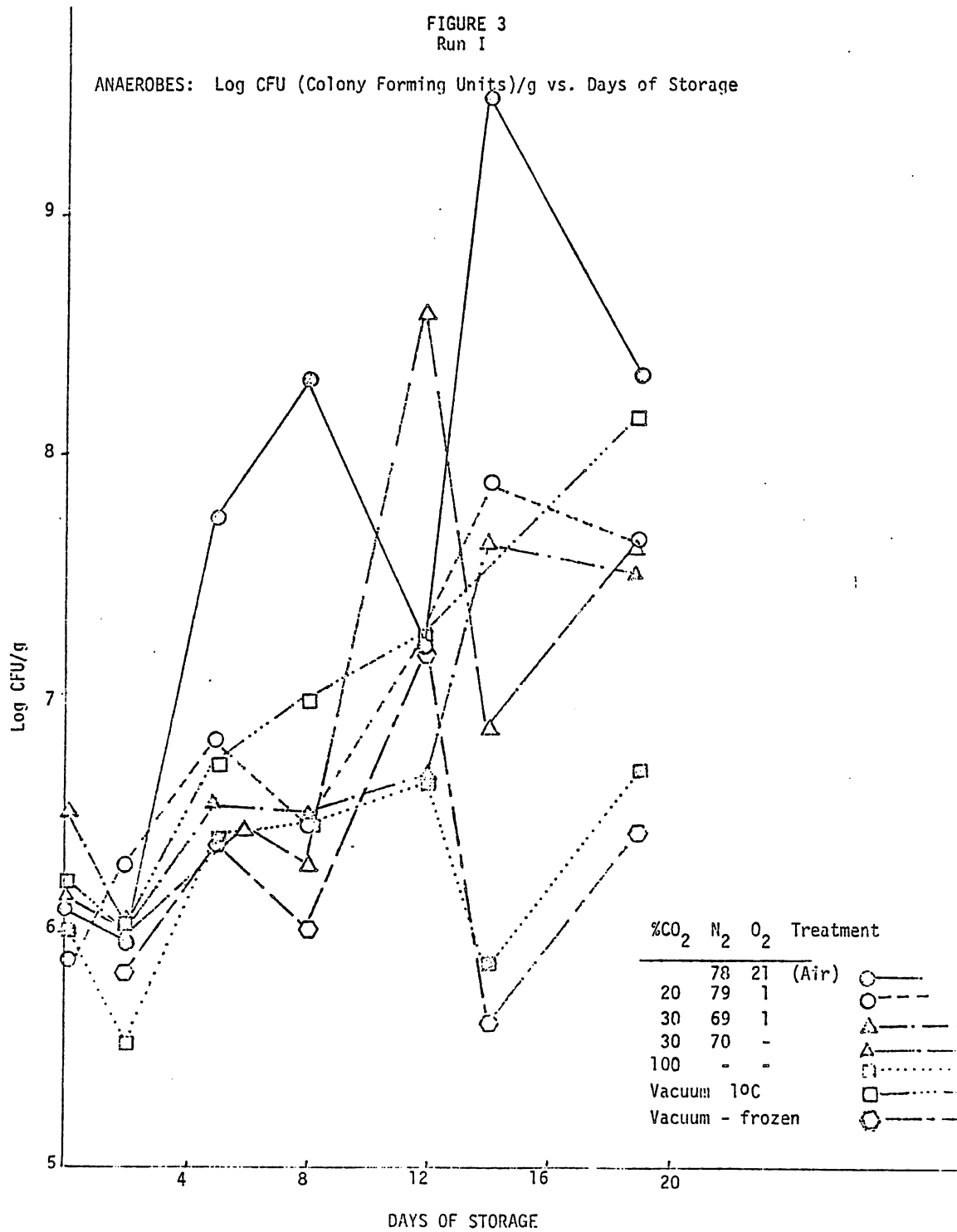
DAYS OF STORAGE

FIGURE 4
Run I

PSYCHROPHILES: Log CFU (Colony Forming Units)/g vs. Days of Storage

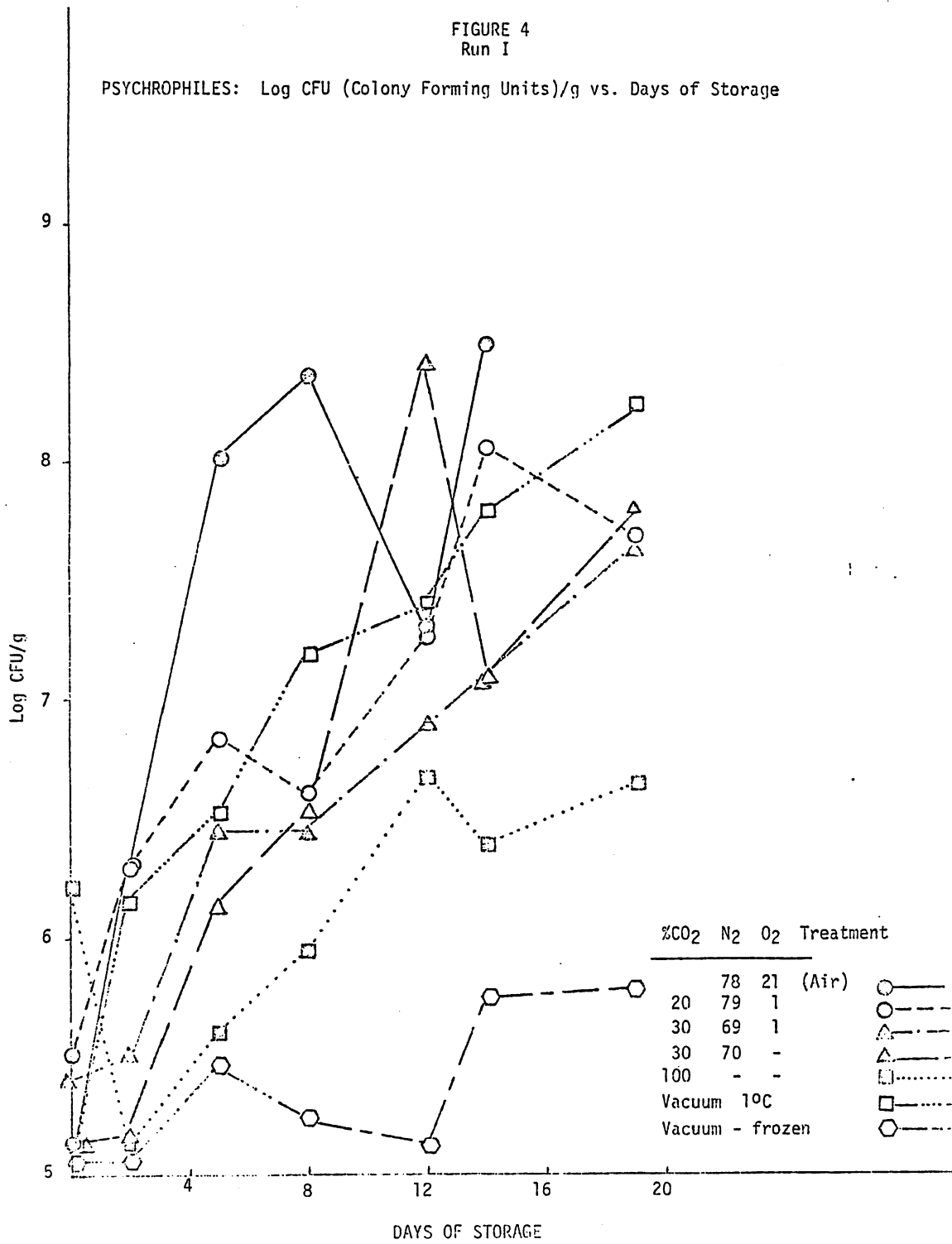| %CO2	N2	O2	Treatment	
	78	21	(Air)	○———
20	79	1		○— — —
30	69	1		△—·—·—
30	70	–		△———
100	–	–		□·········
Vacuum 1°C				□—····—
Vacuum – frozen				⬡—— —

DAYS OF STORAGE

FIGURE 5
Run II

AEROBES: Log CFU (Colony Forming Units)/g vs. Days of Storage

FIGURE 6
Run II

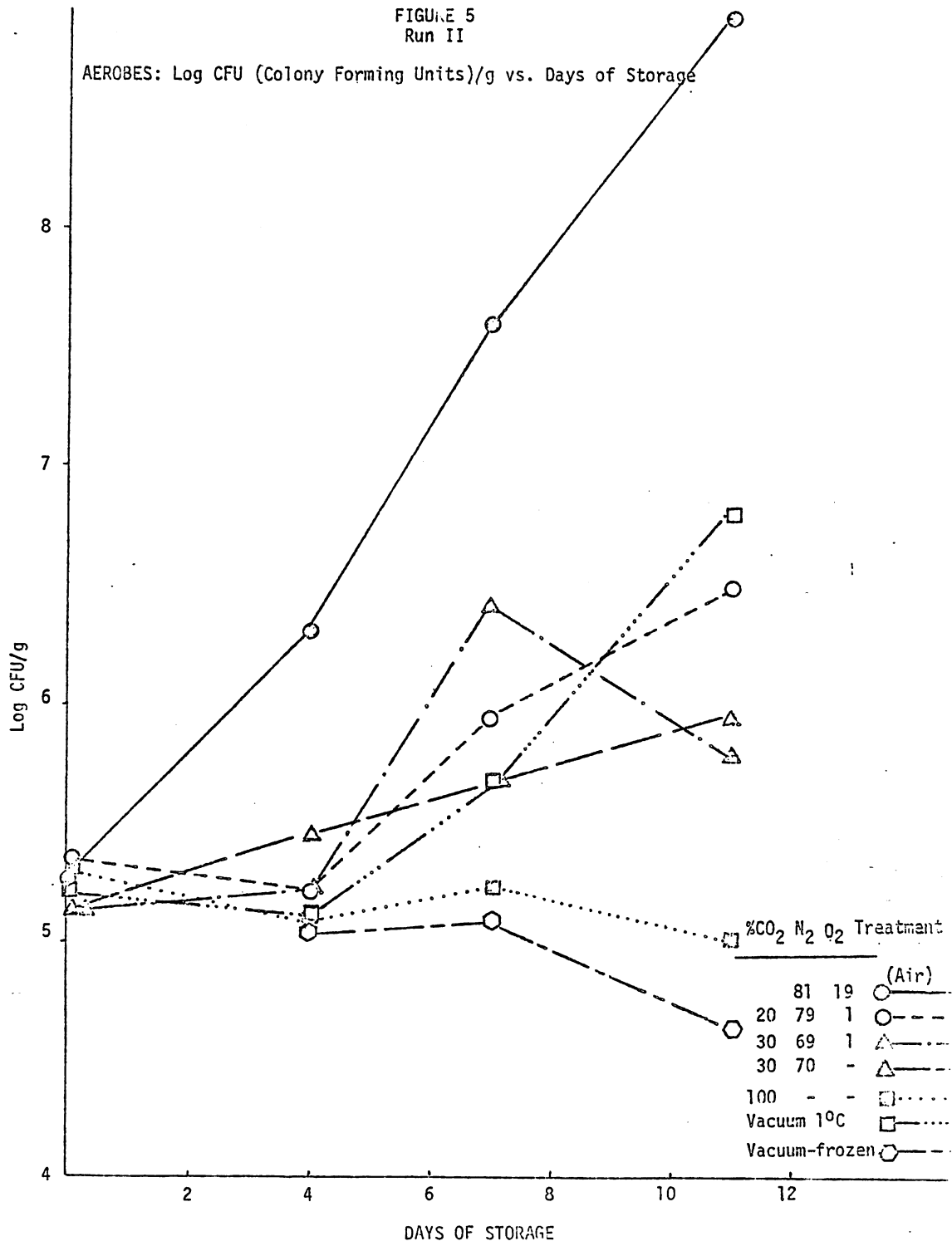ANAEROBES: Log CFU (Colony Forming Units)/g vs. Days of Storage

%CO₂ N₂ O₂ Treatment

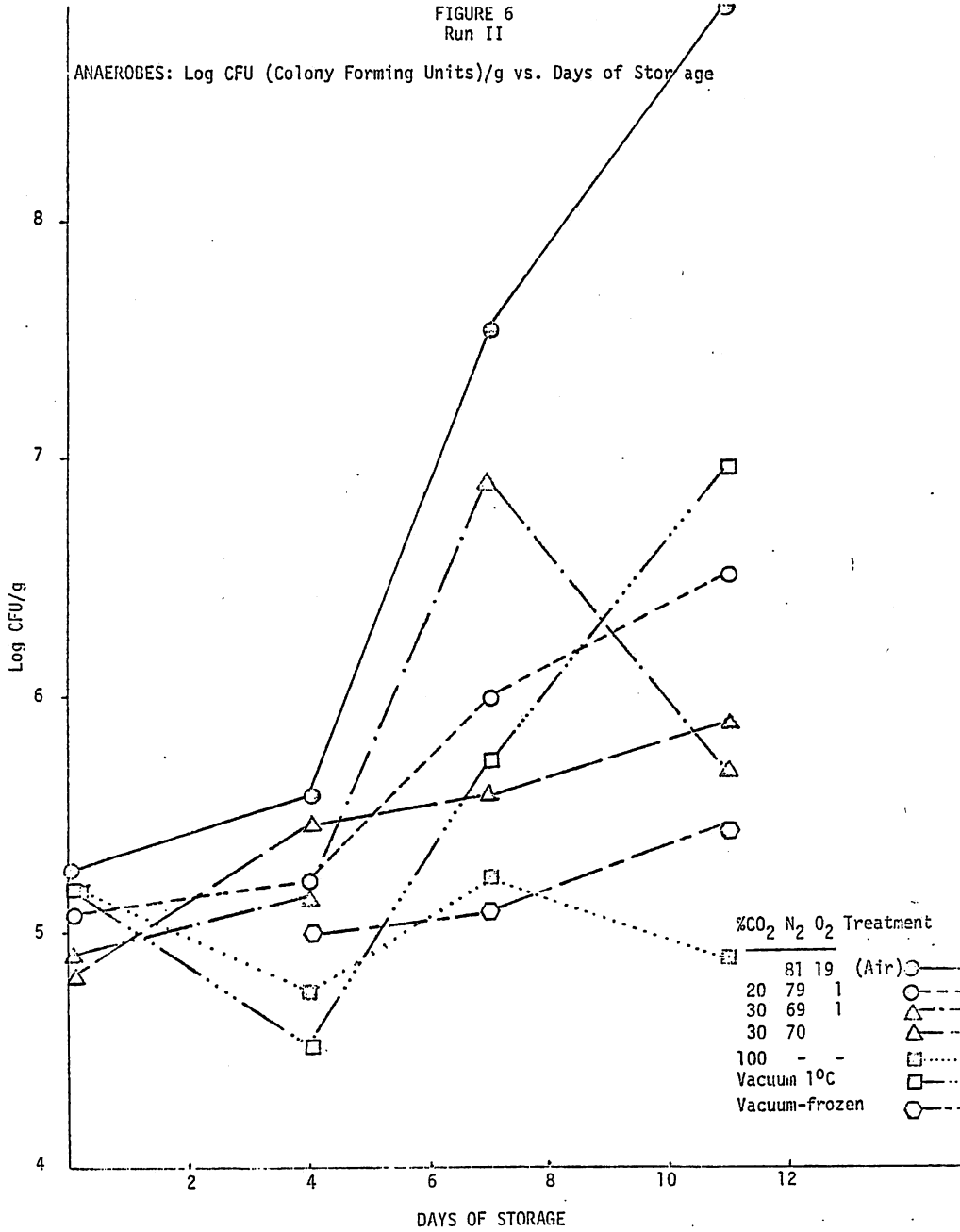| %CO$_2$	N$_2$	O$_2$	Treatment	
	81	19	(Air)	○—
20	79	1		○---
30	69	1		△-·-
30	70			△--
100	-	-		⊡······
Vacuum 1°C				□·····
Vacuum-frozen				○---

DAYS OF STORAGE

Log CFU/g

FIGURE 7
Run II

PSYCHROPHILES: Log CFU (Colony Forming Units)/g vs. Days of Storage

%CO2	N2	O2	Treatment
			(Air)
20	81	19	
	79	1	
30	69	1	
30	70	-	
100	-	-	
Vacuum 1°C			
Vacuum-frozen			