# SHRIMP-WASTE MEAL: EFFECT OF STORAGE VARIABLES ON PIGMENT CONTENT

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ABSTRACT

One of the potential uses of shrimp-waste meal is as a supplement in the ration of hatchery-raised trout. The astaxanthin present in the meal gives trout a desirable color. This paper reports (1) a study of a method of analysis of the pigment and (2) the effect of storage variables on pigment losses and the development of oxidative rancidity. The benefits to be derived from addition of antioxidants were investigated.

## INTRODUCTION

Astaxanthin, a carotenoid pigment present in certain insects and crustacea, has been identified as being one of the principal dietary pigments causing coloration in trout (Goodwin 1954). Hatchery-raised trout usually lack this natural and desirable coloration unless a source of the pigment--for example, salmon eggs or shrimp--is included in the diet. The use of such supplements in production-type rations, however, has not been a common practice because of limited supply or prohibitive cost. Recent feeding tests now have shown that meal prepared from Alaska shrimp waste also would impart a natural coloration to young rainbow trout (Sinnhuber 1955[1/]). This finding indicated a potential outlet in trout-hatchery feeds for the considerable amount of waste being discarded annually by the Alaska shrimp industry.

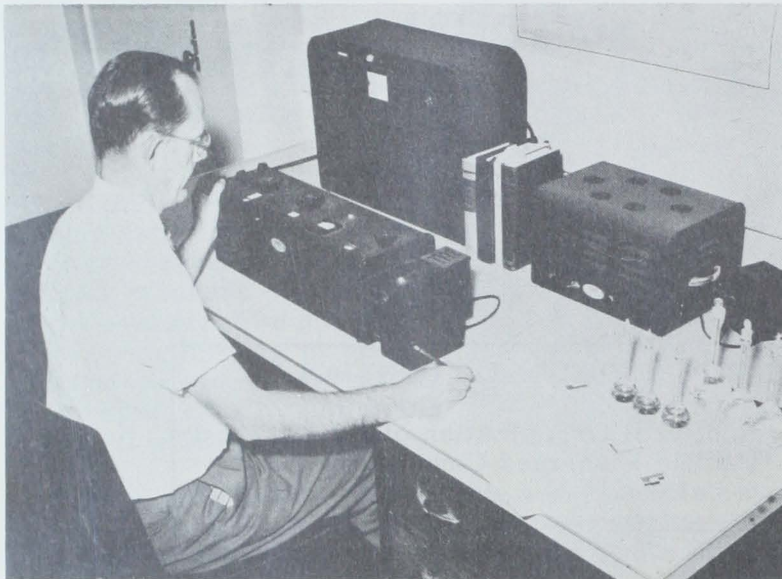

Fig. 1 - Spectrophotometric determination of astaxanthin in Alaska shrimp-waste meal.

Astaxanthin, in common with other carotenoids, is destroyed readily by heat, air, and light. The pigment thus may become a limiting factor in the value of meal when used for the purpose of coloring trout. There is consequently a need for information as to the effect of storage variables on the destruction of the pigment. A problem exists also in the analytical determination of the astaxanthin.

The purposes of the work reported here therefore were as follows:

1. To study the method of analysis of the pigment.

2. To determine the effect of storage on loss of the pigment and the development of oxidative rancidity in Alaska shrimp-waste meal, which has a relatively high oil content (Brown 1959), and to compare concurrently the effectiveness of antioxidants in retarding these changes.

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## METHOD OF PIGMENT ANALYSIS

The primary interest in the work reported in the present paper was the effect of storage on the pigment in Alaska shrimp-waste meal. As often happens in studies of this kind, however, a suitable method of analysis must be available before the principle objectives can be attained. The procedure used in the analysis of the pigment is described, and the suitability of the procedure is discussed.

PROCEDURE: The pigment in shrimp-waste meal is determined as follows:

1. Place an accurately weighed sample of shrimp-waste meal of about 2.5 grams in a 100-milliliter centrifuge tube.

2. Add 50 milliliters of acetone. (Baker's analyzed reagent was used in the present work.)

3. Shake the tube mechanically for 15 minutes.

4. Centrifuge the resulting mixture at about 650 times gravity for 5 minutes.

5. Dilute an aliquot suitably for spectrophotometric absorption measurement.

6. Pipette a sample into a 1-centimeter silica absorption cell.

7. Determine the absorption of the acetone extract at a wavelength of 470 milimicrons. (A Model DU Beckman spectrophotometer was used.)

8. Express the absorbency of a 100-milliliter acetone extract of 5 grams of the meal as the pigment index, $E^{5\%}_{1\ cm}$.

SUITABILITY: The simple procedure for acetone extraction and spectrophotometric analysis just described is not specific for astaxanthin, since other pigments absorbing in the region of 470 millimicrons also will contribute to the total absorption and be measured as astaxanthin. One of the oxidation products of astaxanthin is another carotenoid, astacin, for example, which has a similar absorption spectrum but which has been shown to be ineffective in producing color when included in the diet of hatchery trout (Goodwin 1954). Tests therefore were carried out to obtain evidence for the identity of the pigment in shrimp-waste meal and, in addition, to estimate what percentage of the pigments extracted by acetone was made up of astaxanthin. In this way the applicability of the simple extraction procedure to the analysis of astaxanthin in shrimp-waste meal could be evaluated.

Procedure: To identify the pigment in shrimp-waste meal, we followed essentially the methods of Goodwin and Srisukh (1949). These methods consisted of a phasic analysis by partitioning the pigment between 90 percent (V/V) aqueous methanol and petroleum ether (b.p. 30° to 60° C.), separation of the pigments on alumina, and determination of the absorption spectra of the chromatographed solutions in carbon disulfide.

Results and Discussion: The pigment was found to be mainly epiphasic a property of astaxanthin when partitioned between 90 percent (V/V) aqueous methanol and petroleum ether (b.p. 30° to 60° C.). Astacin, an artifact of astaxanthin, is hypophasic between these solvents. Chromatography of the pigment in petroleum ether solution on alumina (Merck, acid washed, for chromatography) indicated that the pigment was esterified astaxanthin, since it was eluted readily with 5 percent (V/V) acetone-petroleum ether. A more polar solvent, 2 percent (V/V) glacial acetic acid-ethanol, was required to elute free astaxanthin from alumina (Goodwin and Srisukh 1949). Astacin, which is more strongly absorbed, would not be expected to be eluted

under either of the aforementioned conditions (Goodwin and Srisukh 1949). A decrease in absorbency after chromatography indicated that the astaxanthin fraction constituted approximately 75 percent of the pigment of the unchromatographed extract. The simple acetone-extraction procedure therefore overestimates the astaxanthin content of shrimp-waste meal.

Absorption spectra of the chromatographed extracts exhibited one absorption maximum at a wavelength of 502 millimicrons in carbon disulfide. This compared favorably with the reported absorption maximum for esterified astaxanthin, namely, 503 millimicrons. The absorption maximum for astacin is 510 millimicrons in carbon disulfide (Goodwin and Srisukh 1949). Attempts to obtain additional evidence for the identity of the pigment by means of color tests (Karrer and Jucker 1950) were unsuccessful.

Pigment from shrimp-waste meal that had been heated over an extended period exhibited absorption at 470 millimicrons; the spectra departed, however, from the characteristic spectrum of astaxanthin in this solvent. Although these meals would have a low pigment content, the analyses could be misleading. Consequently, the development of chromatographic techniques for routine analysis would be desirable. The development of such a method would probably take considerable time, so the simple acetone-extraction procedure was adopted to follow the changes in pigment content of the meals.

## STORAGE TESTS

Three experiments were run that had as their objective the determination of the following:

1. Level of antioxidants needed for retention of pigment.

2. Effect of antioxidants and a synergist on pigment retention and retardation of oxidative rancidity.

3. Effect of antioxidants in meals of different moisture content.

Shrimp-waste meals used in these experiments were dried in a forced-convection oven. Fresh, unground shrimp waste was placed on shallow trays and dried for 6 hours at $65^{\circ}$ to $75^{\circ}$ C. ($149^{\circ}$-$167^{\circ}$ F.). The dried material was ground in a Hobart grinder.

DETERMINATION OF EFFECTIVE LEVELS OF ANTIOXIDANTS FOR PIGMENT RETENTION: Procedure: Samples of shrimp-waste meal weighing 10 grams were treated with either BHT (2,6-ditertiarybutyl-4-hydroxytoluene) or Santioquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) at levels of 0, 0.02, 0.08, 0.32, and 0.64 percent in the meal. This was accomplished by spraying and blending into the meal acetone solutions containing the respective concentration of the antioxidant. The latter samples were transferred to shallow dishes and placed in a current of air for 1 hour to evaporate the acetone. The samples then were placed in an oven maintained at $50^{\circ}$ C. ($122^{\circ}$ F.) and analyzed initially and at weekly intervals for content of pigment.

Table 1 - Effect of Level of Antioxidant on Pigment Retention in Shrimp-Waste Meal at $50^{\circ}$ C. ($122^{\circ}$ F.)

| Antioxidant | Level | Retention of Pigment at End of: | | | |
|---|---|---|---|---|---|
| | | 168 Hrs. | 336 Hrs. | 672 Hrs. | 696 Hrs. |
| | | (Percent) | | | |
| BHT | 0 | 77 | 56 | -1/ | -1/ |
| | 0.02 | 75 | 56 | 21 | - |
| | 0.08 | 77 | 58 | 26 | - |
| | 0.32 | 76 | 58 | 30 | - |
| | 0.64 | 81 | 61 | 27 | - |
| Santoquin | 0 | 78 | 64 | -1/ | 38 |
| | 0.02 | 92 | 80 | - | 52 |
| | 0.08 | 94 | 86 | - | 62 |
| | 0.32 | 95 | 89 | - | 71 |
| | 0.64 | 95 | 90 | - | 76 |

1/Not determined.

Results: Results of the test are given in table 1. Santoquin was

effective in retarding pigment losses, whereas BHT-treated samples were similar to controls. The effectiveness of Santoquin appeared to increase with increasing levels of antioxidant in the meal. These results paralleled, in part, previous work on the protection of carotene in alfalfa (Thompson 1950). In the latter study, however, BHT exhibited some antioxidant activity.

EFFECT OF ANTIOXIDANTS AND A SYNERGIST ON PIGMENT RETENTION AND THE RETARDATION OF OXIDATIVE RANCIDITY IN SHRIMP-WASTE MEAL: Procedure: Samples of meal were treated, as before, with acetone solutions of BHT, Santoquin and citric acid, singly and in combinations, to give a level of 0.02 percent each in the meal. Storage tests were conducted at 60° C. (140° F.). Two ovens were used in order to separate samples that contained Santoquin. (Preliminary studies had indicated that when controls and samples treated with Santoquin are placed in the same oven, an antioxidant effect is observed in the untreated controls.) Samples were taken initially and at weekly intervals for pigment analyses and reaction with 2-thiobarbituric acid (TBA), the reaction with TBA to serve as a measure of oxidative rancidity (Yu and Sinnhuber 1957 and Ryan and Stansby 1959).

Table 2 - Retention of Pigment in Shrimp-Waste Meal After Storage at 60° C. (140° F.)

| Antioxidant Added at the 0.02-Percent Level in the Meal | | | Amount of Pigment Retained After | | |
|---|---|---|---|---|---|
| BHT | Citric Acid | Santoquin | 1 Week | 2 Weeks | 3 Weeks |
| | | | (Percent) | | |
| 0 | 0 | 0 | 59 | 29 | 19 |
| + | 0 | 0 | 63 | 33 | 23 |
| 0 | + | 0 | 54 | 30 | 21 |
| + | + | 0 | 62 | 31 | 21 |
| 0 | 0 | + | 78 | 61 | 49 |
| 0 | + | + | 82 | 64 | 50 |
| + | 0 | + | 80 | 59 | 48 |
| + | + | + | 78 | 60 | 48 |

Results: Results of these tests are given in table 2. Only those samples that contained Santoquin exhibited a decrease in the rate of destruction of pigment. In addition, Santoquin, but not BHT, was effective in limiting oxidative rancidity (table 3). Since BHT is reportedly an effective fat antioxidant, these results were not expected. A probable explanation for the ineffectiveness of BHT was suggested by the report (Anonymous 1957) that BHT tends to steam distill at elevated temperatures.

Table 3 - 2-Thiobarbituric Acid Reaction (TBA) of Shrimp-Waste Meal During Storage at 60° C. (140° F.)

| Antioxidant Added at the 0.02-Percent Level in the Meal | | | TBA Color ($E^{1\%}_{1\ cm.}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BHT | Citric Acid | Santoquin | 1 Week | 2 Weeks | 3 Weeks | 4 Weeks | 5 Weeks | 6 Weeks | 7 Weeks |
| 0 | 0 | 0 | 0.405 | 0.475 | 0.562 | 0.636 | 0.671 | 0.730 | 0.762 |
| + | 0 | 0 | 0.373 | 0.449 | 0.546 | 0.634 | 0.690 | 0.703 | 0.765 |
| 0 | + | 0 | 0.406 | 0.486 | 0.560 | 0.620 | 0.697 | 0.708 | 0.765 |
| + | + | 0 | 0.404 | 0.464 | 0.547 | 0.632 | 0.698 | 0.732 | 0.758 |
| 0 | 0 | + | 0.357 | 0.341 | 0.399 | 0.472 | 0.487 | 0.548 | 0.571 |
| 0 | + | + | 0.338 | 0.362 | 0.404 | 0.446 | 0.530 | 0.529 | 0.527 |
| + | 0 | + | 0.328 | 0.363 | 0.409 | 0.532 | 0.542 | 0.592 | 0.594 |
| + | + | + | 0.332 | 0.381 | 0.408 | 0.476 | 0.542 | 0.579 | 0.589 |

EFFECT OF ANTIOXIDANTS AT DIFFERENT MOISTURE LEVELS IN MEALS: A third test was therefore carried out using closed containers and a lower temperature, and included the effect of level of moisture in the meals.

Procedure: Shrimp-waste meals containing 4-percent and 10-percent moisture were treated with either BHT or Santoquin at 0.02-percent level. Samples weighing 20 grams were placed in closed 8-ounce jars and held at 38° C. (100.4° F.). Samples were taken initially and at weekly intervals for pigment and TBA analyses.

Table 4 - Percentage Retention of Pigments in Shrimp-Waste Meals of Different Moisture Contents During Storage at 38° C. (100.4° F.)

| Antioxidant | Moisture Content | Retention of Pigment | | |
|---|---|---|---|---|
| | | 1 Week | 2 Weeks | 3 Weeks |
| | | (Percent) | | |
| None . . . . | 4 | 92 | 88 | 81 |
| Santoquin . | 4 | 96 | 89 | 88 |
| BHT . . . . | 4 | 91 | 84 | 83 |
| None . . . . | 10 | 56 | 39 | 36 |
| Santoquin . | 10 | 79 | 69 | 66 |
| BHT . . . . | 10 | 64 | 48 | 43 |

Results: Results are given in tables 4 and 5. The samples containing 4-percent moisture exhibited lower rates of pigment

loss and less color development in the TBA reaction. The samples containing 10-percent moisture exhibited more rapid rates of decrease in pigment and a marked initial increase in TBA color. After 8 weeks, absorption spectra of the acetone extracts of the meals were obtained. The pigment content of the control and BHT treated 10-percent moisture samples had decreased considerably, and in addition, the spectra in acetone solution were not characteristic of astaxanthin; thus absorbency at 470 millimicrons as an index of astaxanthin content could be misinterpreted. Since extensive destruction of pigment in the controls and BHT-treated samples had occurred at the end of 8 weeks, such meals would be identified visually as nontypical. It nevertheless would appear desirable to employ chromatographic analysis that would allow both a qualitative and quantitative evaluation of the pigment in shrimp-waste meal.

Table 5 - 2-Thiobarbituric Acid (TBA) Reaction for Shrimp-Waste Meal of Different Moisture Contents During Storage at 38° C. (100.4° F.)

| Antioxidant | Moisture Content | TBA Color ($E^{1\%}_{1\ cm.}$) | | | |
|---|---|---|---|---|---|
| | | 0 Week | 1 Week | 2 Weeks | 3 Weeks |
| | Percent | | | | |
| None . . . | 4 | 0.289 | 0.284 | 0.321 | 0.324 |
| Santoquin . | 4 | 0.311 | 0.267 | 0.335 | 0.286 |
| BHT . . . . | 4 | 0.311 | 0.313 | 0.326 | 0.312 |
| None . . . | 10 | 0.419 | 0.694 | 0.812 | 0.806 |
| Santoquin . | 10 | 0.419 | 0.504 | 0.622 | 0.614 |
| BHT . . . | 10 | 0.439 | 0.657 | 0.760 | 0.772 |

## SUMMARY

Details of a method of analysis sufficiently accurate for the present immediate purposes are given, and evidence that the pigment in Alaska shrimp-waste meal is mainly esterified astaxanthin is presented.

Santoquin, but not BHT, was effective in decreasing both the rate of pigment destruction and the development of oxidative rancidity, the latter as measured by the 2-thiobarbituric acid reaction. Citric acid exhibited no synergistic effect with either Santoquin or BHT under the conditions of this study.

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