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9. ABSTRACT

A process was developed for the recovery of shrimp protein as a by-product of a chitin recovery operation. The protein was extracted and precipitated from shrimp waste by acid and heat treatment. The coagulum was found to be 80.5% water and 7.6% ash, 10% calcium and 75% protein on a dry weight basis. It was low in bacteriological counts and odor (sic.) and compared favorably to reported amino acid profiles for other shrimp meals. The meal and pigment extract of the meal was fed to rainbow trout. After two weeks, significant amounts of astaxanthin and its isomers were incorporated into the flesh and skin of the trout. The results show that the shrimp coagulum could be used as a pigment and protein source for salmonids raised in aquaculture.

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UTILIZATION OF RECOVERED SHRIMP PROTEIN AS A PIGMENT SOURCE FOR SALMONIDS

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INTRODUCTION

In terms of dollars, shrimp is an extremely valuable fishery. Current domestic and imported shrimp amount to more than a half billion pounds per year (Meyers and Rutledge, 1971; 1973). In processing shrimp the head and hard carapace are removed during semi-mechanized peeling operations. Waste material accounts for approximately 70% of the whole shrimp and may approach 85% in the processing of other crustaceans such as the Atlantic deep sea red crab. Thus these food processing industries are faced with the problem of disposing of waste products or converting this waste material into economic by-products. These waste products must compete with products that they replace or else create their own market. Shrimp meal is traditionally sold on the basis of its protein content and not on its astaxanthin content. Lambertsen and Braekkan (1971) studied

ABSTRACT

A process was developed for the recovery of shrimp protein as a by-product of a chitin recovery operation. The protein was extracted and precipitated from shrimp waste by acid and heat treatment. The coagulum was found to be 80.5% water and 7.6% ash, 10% calcium and 74% protein on a dry weight basis. It was low in bacteriological counts and ordor and compared favorably to reported amino acid profiles for other shrimp meals. The meal and pigment extract of the meal was fed to rainbow trout. After two weeks, significant amounts of astaxanthin and its isomers were incorporated into the flesh and skin of the trout. The results show that the shrimp coagulum could be used as a pigment and protein source for salmonids raised in aquaculture.

the astaxanthin content of a number of industrial shrimp meals. It was found that the astaxanthin level was generally low or even absent in commercial shrimp meals. The vacuum-dried meal contained three times more astaxanthin than the best industrial sample.

A number of studies have shown that astaxanthin is the typical pigment in salmonids (cf. Amano et al., 1968; Kanemitsu and Aoe, 1958; Crozier, 1974; Steven, 1947). It was also shown from the stomach contents of salmon (Manzer, 1968) and trout (Needham, 1969) that these fish consume crustaceans, insects and other organisms rich in astaxanthin. It is thus not surprising that a number of authors have shown that shrimp and crab waste can be used to pigment trout and salmon (cf. Spinelli et al., 1974; Steel, 1971: Saito and Regier, 1971; Kuo, 1976).

The limiting factor in the use of crustacean meals as protein supplements has been the large proportion of exoskeleton material in the meal and in most cases, the taste of the product. Although the protein level on a dry weight basis varies from 25-40%, the high calcium level may have a deleterious effect on the overall nutrition.

In the present paper, protein was precipitated from shrimp waste. This coagulum was tested for its amino acid content, bacteriological contamination and calcium and astaxanthin levels. The whole coagulum and a pigment extract of the coagulum were fed to trout to test the bioavailability of the pigments and protein.

MATERIALS AND METHODS

Preparation of shrimp protein coagulum

The mechanical separation of the proteins from shrimp processing waste

was accomplished by an initial grinding to produce a partical size no greater than 10 mm. The resulting slurry was passed through a Baäder Fish Bone Separator to remove the shell material. The protein slurry was acidified with acetic acid to the isoelectric point of the protein (pH 4.5), treated with 0.1% TBHQ Tenox (Eastman Chemical) as an anti-oxidant and allowed to coagulate for one hour at 80°C in a stainless steel tank. The coagulum was dewatered by filtering in cloth filter bags or centrifuged in a Sharples P-600 Horizontal Bowl Centrifuge. The coagulum was packaged in air tight bags, frozen and shipped by air freight in dry ice to Rhode Island.

The characteristics of the coagulum were determined by several independent laboratories as well as by MCI and URI. Amino acid analysis was performed by Texas A&M University laboratories (Nickleson, 1976) according to the method of Cobb and Hyder (1972) and employed a fully automatic 120C Spinco Amino Acid Analyzer. Microbial counts were performed by Texas A&M University laboratories (Nickleson, 1976) using the method of Surkiewicz (1966). Moisture, ash and protein were determined by the AOAC methods (1970).

The carotenoids were extracted and identified by the method of Saito and Regier (1971) and Kuo et al. (1976).

Carotenoid extract diet

A commercial trout feed manufactured by Zeigler Bros. Feed Mills, Inc. of Pennsylvania was used to feed the control fish. This diet was found to contain 35.3% crude protein, 6.3% crude lipid, 4.9% fiber, 10.4% ash, 10.0% H_2O and 33.1% nitrogen free extract (Kuo et al., 1976), plus amounts of β-carotene (1μg), α-cryptoxanthin (0.4μg), zeaxanthin (0.1μg) and

lutein (0.8μg). Astaxanthin was not isolated from this diet (Kuo et al., 1976). The fish were fed this feed at the hatchery prior to our purchase and the feed was continued as a control.

The desired amount of carotenoids extracted from the frozen shrimp protein was dissolved in a small amount of petroleum ether (PE) and this was added to the control pellets. The PE was removed under vacuum in a flash evaporator at 40°C. The pigmented diet was kept in a dark refrigerator prior to feeding.

Shrimp protein diet

The shrimp protein was mixed with other nutrients to constitute 10% of the mixture by dry weight. The diet used was based on the Oregon Test Diet (Lee et al., 1967) and is given in Table 1. The procedure was similar to that used by Kuo et al. (1976).

Fish culture

Rainbow trout (Salmo gairdneri) were purchased from American Fish Hatchery, Rhode Island and held in 125 gallon fiberglass tanks (Sims Fiberglass, Corvallis, Oregon) with flowing water (2 gal/min) and supplemental aeration. The water temperature was maintained at 11°C and the O_2 level was maintained at 8 ppm (cf. Lee et al., 1967; Kuo et al., 1976). Sixty fish of 140 g average size were distributed evenly between the four tanks. After 30 days, the fish were fed the diets at the rate of 3% of body weight per day.

Analysis of carotenoids in fish

The fish were sacrificed after the feeding periods. The fish flesh

was cut into pieces and ground in a Waring Blender with acetone. The acetone extract was transferred to PE by the addition of water and dried over anhydrous sodium sulfate. The pigments were separated on Microcel-C column with PE and 1-4% acetone in PE as the developing solvent. Each fraction was saponified by adding an equal amount of 20% KOH in methanol. The mixture was heated on a steam table for 10 minutes in the dark. After saponification the pigments were transferred to diethyl ether by the addition of water. Acetic acid was added to bring astacene to the epiphase. The pigment solution was dried over sodium sulfate and rechromatographed on Microcel-C and MgO:Hyflo Supercel columns. The individual bands were collected and purified on silica gel G sheets. Identification of the pigments was the same as described above (Kuo *et al.*, 1976).

RESULTS

The protein coagulum was found to have the following analysis: moisture 80.5%; ash 1.5%; calcium 0.2%; and protein 14.4% (2.2% N x 6.25 uncorrected for chitin N). Table 2 shows the amino acid profile of the protein coagulum compared to casein and to other crustacean meals. The protein coagulum is marginally different from other shrimp meals. The shrimp protein is higher than casein in arginine, aspartic acid, glycine; lower in valine, glutamic acid and proline; and similar in the other amino acids. Tryptophan and cystine were not analyzed by this method. The protein coagulum gave a calculated PER of 2.709.

Bacteriological analyses on the coagulum were conducted by Texas A&M University laboratories on similarly prepared products. These results are

shown in Table 3.

The carotenoid content of the shrimp meal as received from MCI was determined on the frozen samples and on a tray dried sample (Table 4). The frozen sample contained trace amounts of the typical plant pigments lutein and zeaxanthin. These pigments were the main pigments in the fish as a result of the commercial feed that was used. Other minor pigments were isolated which are characteristic of crustacea (Simpson and Chichester, 1976). Astaxanthin and astaxanthin ester together constituted the main pigments.

A rather large amount of astacene was also isolated presumably as a result of the various handling and processing steps prior to freezing. It can be seen that the tray drying (70°C, 12 hrs.) of the coagulum resulted in a great loss of pigmentation. While the dried protein was red it contained only small amounts of astaxanthin and astacene.

A pigment extract was made of the frozen shrimp meal and this was fed together with a commercial ration. Table 5 shows the results of feeding the shrimp protein extract for two weeks. Astaxanthin, astaxanthin ester and astacene were absorbed in about equal amounts. The higher levels of the feed pigments (lutein and zeaxanthin) found in the fish were unexpected and may represent an increased absorption of pigments due to higher lipid levels.

Table 6 shows the results of feeding a 10% shrimp protein diet for two weeks. This diet contained less than 1/10th the amount of pigment contained in the 20 mg % diet. These results show that the fish also contained somewhat less than 1/10th the amount of pigment. We expected this level to be far lower since some difficulty was experienced in

getting the fish to take either the control or the shrimp meal diet. Initial studies with a diet of corn oil as a lipid source supplemented with ω-3 containing lipids was not eaten by the fish. The results reported here were with a fish diet containing herring oil. The initial mean weight of the fish was 140 g. At the end of the feeding trials the control fish and the fish fed the two pigmented diets were on the average of 174-175 g.

DISCUSSION

The total utilization of crustacean waste has been the subject of the reports of numerous investigators. The waste is composed of calcium carbonate, chitin, protein and carotenoids. Variation in proximate analyses will depend on the species, sex, metabolic state and subsequent processing, handling and storage. The shrimp protein concentrate used in these experiments was prepared by MCI as a by-product of a chitin-chitosan recovery procedure. Normally an alkaline digest is used for the recovery but experiments were conducted with a mild acid coagulation since alkali is known to convert astaxanthin to astacene. The resulting product was found to be low in calcium and bacterial contamination and high in protein quality and astaxanthin ester-astaxanthin content.

The results of these experiments show that the protein or a pigment extract of the protein would be effective in the pigmentation of salmonids.

It has long been assumed that astacene is an artifact produced during extraction of astaxanthin and does not occur as such in trout. The results shown in Table 5 suggest that astacene may be incorporated into trout. Further work is in progress in the preparation and feeding of pure astacene.

Longer studies would have to be conducted to assess the nutritional

problems, if any, of feeding the coagulum. Its amino acid content would appear to be similar to other shrimp meals.

Longer feeding trials would also be necessary in order to obtain a proper level of pigmentation. The results reported here show that the pigment is readily deposited in the flesh and fins. The pigment fed fish were easily distinguished from the control fish after only two weeks of feeding.

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Table 1. Composition of control and 10% shrimp meal diets

	Control %	10% Shrimp Meal %
Casein (vitamin free)	49.4	42.21
Gelatin	8.7	8.70
Dextrin	15.6	15.60
Carboxymethyl cellulose	1.3	1.30
Mineral mixture	4.0	3.30
Vitamin mixture*	2.0	2.00
Marine oil	10.0	10.00
Vitamin E (250 IU/g)	0.6	0.60
α-Cellulose	6.5	3.75
Choline chloride	1.0	1.00
KH_2PO_4		1.54
$CaCO_3$	0.9	
Shrimp meal		10.00

*Vitamin mix was prepared according to Steel (1971).

Table 2. Amino acid profile (as gAA/16g Nitrogen) of freeze-dried shrimp coagulum and other proteins

Amino Acid	Casein[1]	ISP[1]	Sundried Shrimp meal[2]	Spray-dried Shrimp meal[3]	Freeze-dried Coagulum
Arginine	4.07	7.8	6.31	8.06	6.31
Histidine	3.02	2.5	1.90	2.97	2.09
Isoleucine	6.55	4.9	3.26	5.17	4.68
Leucine	10.05	7.7	7.57	8.14	8.10
Lysine	8.01	6.1	6.17	8.34	7.36
Methionine	3.08	1.1	2.84	2.60	2.62
Phenylalanine	5.39	5.4	4.56	5.05	4.01
Threonine	4.28	3.7	4.28	3.91	4.65
Tryptophan	1.33	1.4	1.26	6.73	
Valine	7.39	4.8	4.42	5.62	5.02
Tyrosine	5.82	3.7	3.64	3.61	3.31
Alanine	3.35	3.9	5.29	7.14	7.11
Aspartic Acid	7.39	11.9	10.74	8.63	11.13
Cystine	0.38	1.2	1.59		
Glutamic Acid	23.05	20.5	15.46	17.8	14.38
Glycine	1.99	4.0	4.29	7.52	6.45
Proline	11.75	5.3	3.44	4.54	7.05
Serine	6.65	5.5	4.53	4.69	5.20

[1]Toma (1971)
[2]Meyers and Rutledge (1973)
[3]Johnson and Peniston (1971)

Table 3. Bacterial analyses of wet and dried coagulum*

Culture type	Wet coagulum	Dry coagulum
APC	1.0×10^3 CFU/g	6.0×10^3 CFU/g
Coliforms	< 3/g	< 3/g
E. coli	< 3/g	< 3/g
Staphylococcus	< 3/g	< 3/g
Salmonella	negative	negative

*Test performed by Texas A&M University, College Station, Texas.

Table 4. Composition of carotenoids in shrimp meal before and after drying

Carotenoids	Before drying (g/g dry basis)	After tray drying
3,3'-Dihydroxy-ε-carotene	1.36	
Echinenone	0.25	
Isocryptoxanthin	0.38	
Canthaxanthin	0.39	
4-Keto-4'-hydroxy-β-carotene	0.74	
Dihydroxypiradixanthin (?)	4.66	
Lutein	trace	
Zeaxanthin	trace	
Astaxanthin ester	66.1	10.3
Astacene	55.4	6.26
Astaxanthin	7.19	trace

Table 5. Composition of carotenoids in fish fed 10% shrimp meal diet (for two weeks)

Carotenoids	Control	10% Shrimp meal
	(g/g dry basis)	
α-Cryptoxanthin	trace	trace
Canthaxanthin	-	trace
Lutein	1.3	1.76
Zeaxanthin	0.16	0.21
Astaxanthin ester	-	0.075
Astacene	-	trace
Astaxanthin	-	trace

Table 6. Composition of carotenoids in fish fed 20 mg % pigmented diet (for two weeks)

Carotenoids	Control	20 mg % Pigmented
	(g/g dry basis)	
α-Cryptoxanthin	0.10	0.18
Canthaxanthin	trace	trace
Lutein	1.79	2.04
Zeaxanthin	0.75	0.9
Astaxanthin ester	-	0.30
Astacene	-	0.28
Astaxanthin	-	0.28