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-Review-

Utilization of Crawfish Processing Wastes as Carotenoids, Chitin, and Chitosan Sources

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Abstract

The Louisiana crawfish industry comprises the largest concentration of crustacean aquaculture in the United States. Processing plants throughout the culture region annually generate as much as 80 million pounds of peeling waste during recovery of the 15% (by weight) edible tail meat. A commercial oil extraction process for recovery of carotenoid astaxanthin from crawfish waste has been developed. Crawfish pigment in its various forms finds applications as a source of red intensifying agents for use in aquaculture and poultry industries. Crawfish shell, separated in the initial pigment extraction step, is an excellent source of chitin. Applicable physicochemical procedures for isolation of chitin from crawfish shell and its conversion to chitosan have been developed. Crawfish chitosan has been demonstrated to be both an effective coagulant and ligand-exchange column material, respectively, for recovery of valuable organic compounds from seafood processing wastewater.

Key words : crawfish, waste, carotenoid, chitin, chitosan

INTRODUCTION

The Louisiana crawfish industry comprises the largest concentration of crustacean aquaculture in the world, with an annual production in excess of one hundred million pounds. Following recovery of the tail meat, the residue, representing 85% of the biomass, has been traditionally discarded. As of January, 1985, disposal via landfill dumps was prohibited, creating an urgent and critical need for economically sound alternate methodology, especially product recovery. With emphasis on total biomass utilization, a lengthy series of studies from the Louisiana State University (LSU) Food Science Department[1~3] has demonstrated the commercial feasibility of recovery and utilization of this renewable waste resource for use as a valuable natural source of the carotenoid astaxanthin. Development of a commercial oil extraction process for recovery of carotenoid astaxanthin from the waste has allowed establishment of a viable industry for by-product usages[3].

Apart from the recoverable pigment, crawfish wastes represent a significant and renewable major resource for the biopolymers chitin and its deacetylated form, chitosan[4,5]. Applications of these two functional polymers, especially chitosan, are readily seen in a broad range of scientific areas, including those in biomedical, food, and chemical industries[6~8]. Integration of the chitin/chitosan production with an existing commercial pigment recovery process represents a potentially cost-effective operation with an immediate economic benefit to the rapidly growing crawfish industry, as well as in uti-

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lization of other crustacean wastes.

This paper summarizes experimental data published from the LSU Food Science Department to serve as a guide in other crustacean processing operations leading to economically viable by-product recovery and application.

SOME GENERAL CHARACTERISTICS OF CRAWFISH

Crawfish, like shrimp, crabs and lobsters, belong to the scientific class Crustacea. These animals have a hard shell called an exoskeleton, which provides some protection and gives rigidity to their bodies. The characteristic exoskeleton of the crawfish can be divided into three body regions (Fig. 1). The head and thorax are combined into a head-thorax or cephalothorax. The abdomen is highly segmented[9]. There are two species of crawfish consumed in Louisiana, the red swamp crawfish and the white river crawfish. The red swamp crawfish, *Procambarus clarkii*, is by far the most common, making up more than 60% of the total catch from the Atchafalaya river basin. Live crawfish and fresh tail meat are usually available to Louisiana consumers from December until June. March through May are the months when crawfish are most plentiful and are of the best quality. Crawfish harvested in July, August or September are usually of poor quality, due to extremely hard shells and tough meat[10].

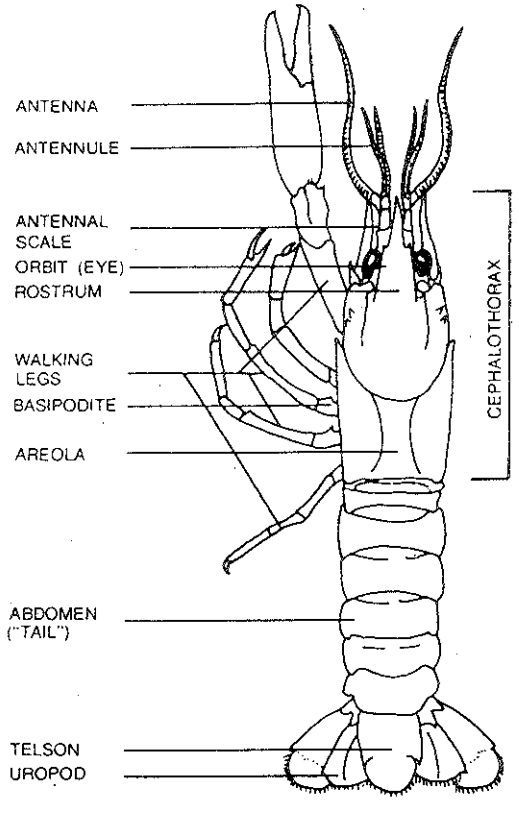

Fig. 1. Dorsal view of generalized crawfish, showing various body parts.

EXPERIMENTAL DATA

Fig. 2 illustrates a total postulated utilization of crawfish waste, i.e., proteinaceous meal, natural carotenoid pigment, chitin/chitosan, and flavor concentrate sources[4,11]. Information such as this is needed before a complete economic overview on multi-product application can be developed. Each of these product areas is discussed in this paper.

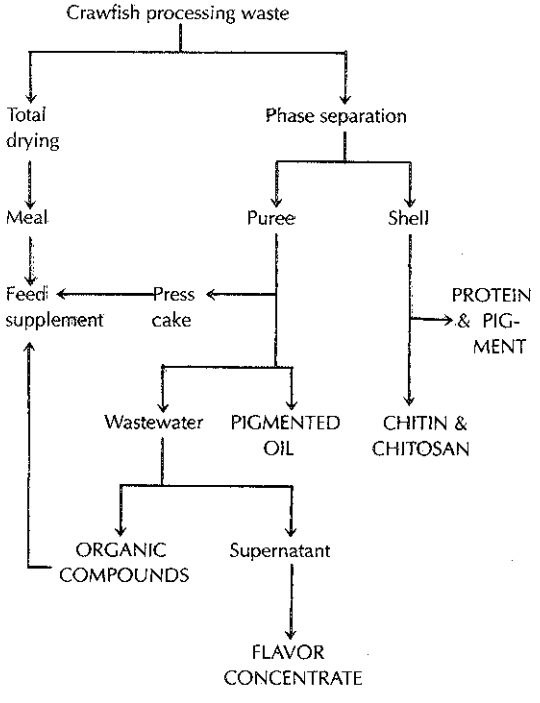

Fig. 2. Total utilization of crawfish waste.

Composition of crawfish meal and shell

The chemical composition of crawfish whole meal and mechanically-separated shell is shown in Table 1[5]. Crawfish meal comprises a composite dried waste of cephalothorax, abdominal exoskeleton, and viscera. Table 1 indicates that both crawfish whole meal and shell are excellent sources of the carotenoid astaxanthin and chitin. The carotenoid profile of crawfish meal consists of astaxanthin ester 49.4%, astaxanthin 40.3%, and astacene 10.3%[12]. The whole meal has application in aquatic or poultry dietary formulations as a source of protein, carotenoids, and chitin[1,2,12-14]. In Louisiana, crawfish whole meal or presscake (Fig. 2) is also included as a feeding attractant in crawfish trap baits[15].

Compared with whole meal, the shell contains a higher chitin and lower protein content. Furthermore, since this shell portion is obtained as a by-product of pigment extraction, prospects of using it as a source of chitin appear promising. Application of the pigmented shell with its high concentration of astaxanthin (108 ppm), as well as its potential value as a source of chitin, will be discussed.

Calcium percentage in both crawfish meal and shell was 12.3% and 24.8%, respectively. If calcium is assumed to be primarily in the form of calcium carbonate, this would represent roughly 31% of the total meal and 62% of the shell.

The amino acid compositions of crawfish meal and shell vary considerably, both in total levels and in the relative abundance of specific amino acids. The major amino acids in both crawfish meal and shell are tyrosine, glutamic acid, and aspartic acid[4].

Table 1. Chemical compositions of crawfish whole meal and shell[1]

Composition	Whole meal	Shell
Crude protein (%)	35.8	16.9
Fat (%)	9.9	0.6
Chitin (%)	15.9	23.5
Ash (%)	38.1	63.6
Minerals		
Ca (%)	12.3	24.8
P (%)	0.8	1.0
K (%)	1.0	0.1
Mg (%)	0.2	0.3
Mn (ppm)	545	200
Fe (ppm)	1611	180
Astaxanthin (ppm)	78	108

[1]Average of three determinations

Pigment-Related Research

Ensilage treatment

Raw crawfish waste is extremely reactive due to high concentrations of proteolytic enzymes present, and must be used fresh or properly preserved to avoid decomposition. Investigation[16] has been conducted to combine ensilage technology with oil extraction techniques for optimal pigment recovery. Implementation of acid ensiling prior to pigment extraction increases concentration of the astaxanthin oil extract by 40~50% and oil recovery by 10%, without qualitatively or adversely affecting astaxanthin profiles in the pigment-enriched oil[17]. Ensilage approaches are now being evaluated on a practical basis to store ground crawfish waste at satellite facilities during the peak season, with subsequent pigment removal at the extraction plant[15].

Astaxanthin extraction

The patented process[3] for recovery of the valuable astaxanthin pigment from crawfish processing waste is illustrated in Fig. 3 and is further discussed by Chen and Meyers[1]. In essence this comprises a controlled release of the carotenoid into an oil

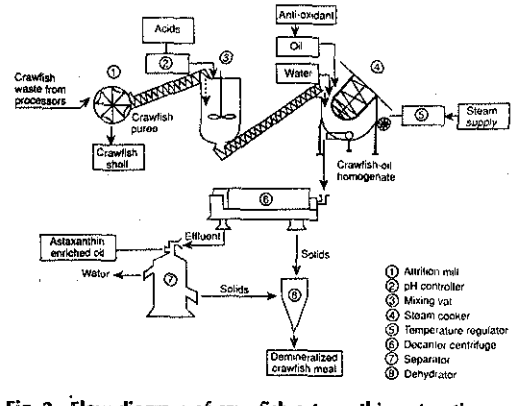

Fig. 3. Flow diagram of crawfish astaxanthin extraction process.

phase, using either a vegetable (i.e., soy) or a fish oil. Use of a vegetable oil in the extraction process further provides a good barrier to oxygen, thus retarding subsequent oxidation.

Initial grinding through a vertical attrition mill is essential to properly size the puree fraction and to remove up to 10% (by weight) of the shell portion. This ratio can be adjusted according to screen size used, and varies with the crustacean species. The efficiency of the process will also vary with the oil used and the stages of extraction. While pigment extraction may be somewhat higher in fish (menhaden) oil, soy oil is being used in the currently produced commercial product as stipulated for the Japanese market. A final terminal polisher has been installed to further enhance stability during storage and shipment overseas[15]. Employment of an antioxidant, ethoxyquin or Endox dry powder, effectively retards autoxidative degradation of the isolated pigment during cold storage[2].

Carotenoid concentrations from single-stage oil extractions of crawfish waste average from 750 to 1,300 ppm. Concentations in the range of 1,500~1,700 ppm are obtainable with two-stage extraction[16]. It does not appear to be economically feasible to proceed beyond the second extraction stage. Pigment concentration is a reflection of seasonal development of the crawfish and use of young vs. older animals, the latter with a deep-reddish, highly calcified exoskeleton.

Analysis of pigmented oil

The primary carotenoids in pigmented oil are mono- and diester astaxanthin[17]. The extraction process results in a pigmented oil rich in ω-3 fatty acids and sterols. Analyses reveal a comparatively high proportion of linolenic acid (18 : 3 ω-3) (8%) and other long-chain polyunsaturated fatty acids, i.e., 20 : 5 ω-3 (2.3%) and 22 : 6 ω-3 (1.5%). Sterol levels of 5.9 μg/mg, mainly cholesterol, have been obtained. Inclusion of sterol in the diet improves growth and survival of aquatic species. Therefore, the sterol content of the pigmented oil can be regarded as a nutritionally advantageous ancillary component in addition to the fatty acid and astaxanthin fractions.

Applications as pigmenting sources

Interest has been shown in use of crawfish processing by-products as a source of pigment in aquatic diets. Carotenoid-rich crawfish meal, in dry form or as extracted astaxanthin pigment in a vegetable oil, has been demonstrated to be an effective dietary supplement for pigmentation of the integument and finnage of the pearl gourami (*Trichogaster leeri* Bleeker) and for enhancement of external coloration of rainbow trout (*Oncorhynchus mykiss*)[19,20].

Continued interest in application of three different crawfish by-products, i.e., whole meal, pigmented oil, and pigmented shell, has focused on their effectiveness as pigmenting agents for imparting a desirable egg yolk color[13]. When supplied to laying hen diets, concentrations of astaxanthin as low as 1 mg /kg imparted consumer-acceptable coloration to egg yolk within 9 to 10 days, allowing replacement of 30% yellow corn, the major traditional pigment source in such a ration. Interestingly, in egg yolk pigmentation, astaxanthin in crawfish shell showed greater efficacy than did the whole meal or pigment-enriched oil at equivalent levels of pigment.

Most recent work[14] has involved utilization of the crawfish chitin-carotenoid complex and the whole meal in controlled broiler diets as potential sources of red intensifying agents. Astaxanthin pigment in the chitin-carotenoid substrate and in the meal was demonstrated to impart significant red intensifying effects to broiler skin and shank pigmentation.

The crawfish-derived astaxanthin is currently being utilized in Japan as a natural red colorant for red sea bream culture[15]. Natural astaxanthin is especially desired in Japan where the natural crawfish-derived carotenoid has been a ready market because of regulatory restrictions on use of synthetic colorants in food products. However, increasing attention also has been directed toward incorporating synthetic astaxanthin or canthaxanthin into various feed formulations for effective coloration of salmonids in other world aquaculture[21,22].

Chitin/Chitosan-Related Research

Preparation and characterization of chitin/chitosan

Considerable attention has been given to development of a composite process for effective isolation of chitin from crawfish shell, its conversion to chitosan, and characterization of the physicochemical properties of the chitin and chitosan products[4]. To date, the majority of attempts to isolate chitin from crustacean processing operations have been with shrimp and crab waste shell[23]. While chitin and chitosan are currently not being produced in the commercial crawfish pigment recovery process, combination of chitin/chitosan production with an existing pigment recovery process represents a total *by-product utilization concept with realistic implication* in other crustacean waste recovery industries.

The procedure developed for isolation of crawfish chitin is shown in Fig. 4, and its characterization

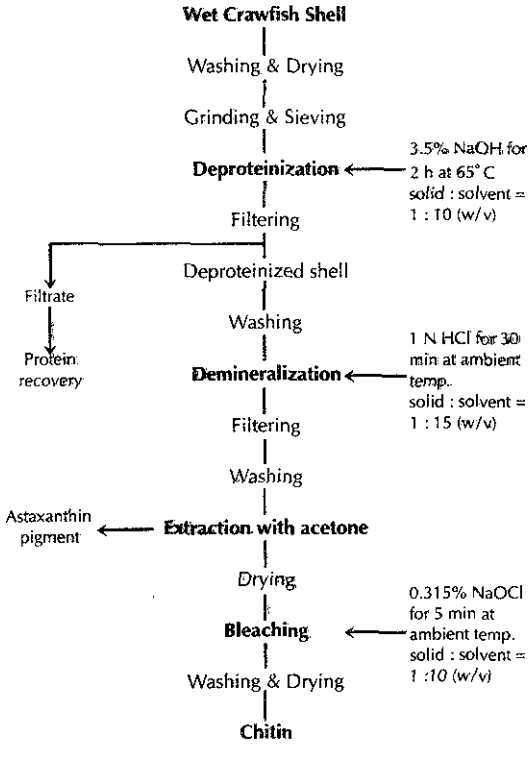

Fig. 4. Overall process for preparation of chitin from crawfish shell.

noted in Table 2[5]. The relatively high acetyl value of the chitin is indicative of the mild alkali treatment used. The presence of small residual amino acids in the final product indicates that protein is bound to chitin by covalent bonds, forming a stable complex.

Protein recovery reduces a considerable portion of the manufacturing cost for chitin and chitosan, and thus contributes significantly to the profitability of a potential chitin/chitosan enterprise[23]. The protein recovered from crawfish shell by alkaline extraction and isoelectric precipitation has an excellent amino acid profile, with all essential amino acids present. The recovered protein product has considerable applications as a protein source in various feed applications[4].

For preparation of chitosan, various conditions have been considered that will sufficiently deacetylate the chitin to yield a non-degraded chitosan product, soluble in dilute acetic acid in minimal time[4]. Chitosan can be prepared by reaction of the crawfish chitin with 50% NaOH (w/w) solution at 100°C for 30min in air using a solid to solvent ratio of 1 : 10 (w/v)[24].

Application of chitosan

A significant concentration of potentially recoverable organics is present in discharge streams from the crawfish pigment extraction process (Fig. 2).

Table 2. Characterization of crawfish chitin[1]

Specification	Description
Nitrogen[2] (%)	7.01
Fat (%)	ND[3]
Ash (%)	0.1
Acetyl (%)	19.6[4]
Deacetylation (%)	7.5
Solubility[5] (%)	26.4
Viscosity (cps)	112[6]
Color	white
Residual amino acids (mg/g)	6.5

[1]Average of three determinations
[2]Calculated on moisture- and ash-free basis
[3]ND = Not detectable
[4]Theoretical value = 21.19%
[5]N,N-Dimethylacetamide containing 5% LiCl (DMAc-5% LiCl)
[6]1% solution on moisture- and ash-free basis in DMAc-5% LiCl

This organically-rich effluent is characterized by high chemical oxygen demand (COD), or biochemical oxygen demand (BOD), and total suspended solids. Effective recovery of organic compounds present in such discharges is realistic in terms of their potential utilization, as well as eventual reductions that can be achieved in effluent discharge loads.

Research[24] has focused on utilization of crawfish chitosan as a coagulant for recovery of organic compounds in waste water from the pigment extraction process. Crawfish chitosan, prepared from crawfish shell chitin, has been demonstrated to be equivalent, or superior to, the commercial chitosans and synthetic polyelectrolytes in turbidity reduction. The proximate composition of the coagulated solids was 27.1% crude protein, 51.7% fat, and 3.3% ash. Table 3 shows amino acid composition of coagulated solids compared with shrimp waste protein. A notable feature is the extremely high levels of glutamic and aspartic acid. Together with leucine, arginine, and alanine, the five amino acids account for 62% of those present in the coagulated solids. The use of coagulated solids as valuable feed additives is of particular interest in view of its relevance to organically-rich seafood processing wastes in general. Absence of large levels of inorganics, especially iron and aluminium, in the chitosan-separated solids, and the biodegradable nature of the polymer may be beneficial in view of the potential usage of the chitosan as a feed additive. The supernatant reveals large concentrations of flavor-related free amino acids, including arginine, alanine, glutamic acid, serine, and glycine.

Further chitosan research has involved development of recovery of such flavor-related amino acids from the supernatant[25]. Crawfish chitosan, loaded with copper or amino copper, shows high recovery rates of amino acids at pH 8 (Table 4). Recovery efficiency is pH-dependent, with lower efficiency yield at alkaline pH values. The eluate was completely free of copper ions from the initial copper-chitosan column when treated with a second crawfish chitosan column. Once the second column is saturated with copper ions, it can be used effectively as the initial column for primary sorption of amino acids from the supernatant. The amino acids recovered by this treatment have potential applications as seafood flavors in terms of their sensory attributes.

Table 3. Comparison of amino acid composition of coagulated solids from crawfish wastewater with shrimp waste protein

Amino acid	Content (mg/g)	
	Crawfish	Shrimp[1]
Aspartic acid	61.6	63.4
Threonine	21.1	25.3
Serine	19.1	26.7
Proline	11.5	20.3
Glutamic acid	121.3	91.2
Glycine	17.2	25.3
Alanine	43.0	31.2
Valine	22.7	26.1
Methionine	10.1	16.8
Isoleucine	13.7	19.2
Leucine	48.1	44.6
Tyrosine	16.3	21.4
Phenylalanine	18.8	26.9
Lysine	35.5	36.4
Histidine	8.1	11.2
Arginine	43.5	37.2
Total	511.6	523.2

[1]From Toma and Meyers[26]

Table 4. Percent recovery of amino acids at pH 8

Amino acid	Am-Cu-chitosan[1]	Am-Cu-chitosan & chitosan
Aspartic acid	85	82
Threonine	93	90
Serine	84	77
Proline	21	9
Glutamic acid	77	79
Glycine	15	71
Alanine	47	89
Valine	51	37
Methionine	79	75
Isoleucine	54	57
Leucine	62	63
Tyrosine	86	84
Phenylalanine	86	85
Lysine	49	36
Histidine	59	63
Arginine	73	72

[1]Am-Cu-chitosan = amino-copper-chitosan

SUMMARY

Commercial utilization of these crawfish products suggests that crawfish waste and crustacean waste, as well as organically-rich shellfish processing streams in general, can no longer be considered as disposable "waste" products with minimal economic value. Carefully developed integrated approaches to recovery and utilization of seafood processing wastes, traditionally discarded or used for composting or minimal cost feed or fertilizer, can reveal profitable alternatives leading to valuable products of commerce.

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캐로티노이드, 키틴, 키토산의 원료로서 Crawfish 가공 폐기물의 이용

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요 약

해마다 8천만 파운드에 이르는 막대한 양의 갑각류 폐기물이 미국 루이지애나주 crawfish 가공업체들로 부터 생산되고 있다. 그러나, 환경오염원으로 문제시 되고 있는 이 산업폐기물은 유용한 캐로티노이드 색소뿐 아니라 의학, 식품 및 화학분야 등에 광범위하게 이용될 수 있는 키틴과 키토산의 우수한 원료로서 사용될 수 있다. 본 원고에서는 루이지애나 주립대학교 식품공학과에서 이 폐기물을 캐로티노이드 색소원 및 키틴, 키토산의 원료로서 이용한 연구 결과를 정리하였다. 즉, 이들 폐기물은 양식업 및 축산업 분야에 적색 색소원으로 널리 이용될 수 있고, 또한 폐기물로 부터 분리 제조된 키토산은 수산가공 폐수로부터 유용한 유기물을 회수하는데 훌륭한 응고제 및 기질로서 사용될 수 있음을 증명하였다.