Universidade Federal de Pernambuco

Centro de Ciências Biológicas

Departamento de Bioquímica

Aproveitamento Integral do Pescado: Novas Alternativas e Oportunidades Visando Segmentos Industriais de Maior Valor Agregado

Prof. Ranilson de Souza Bezerra

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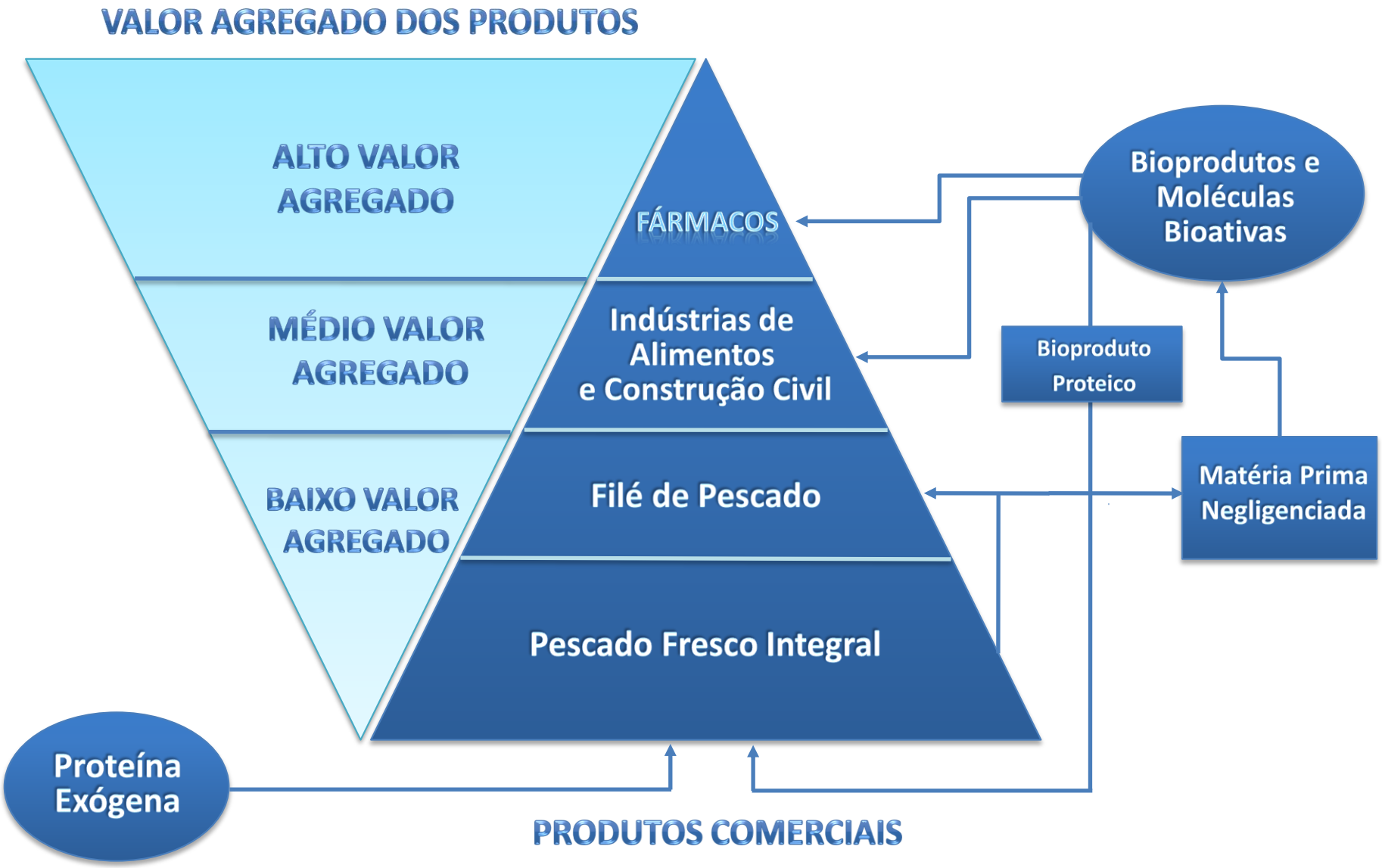
Fortaleza – CE

2014

Antigos e novos conceitos



Recuperação de proteína, quitina, carotenoides e glicosaminoglicanos do resíduo de processamento do camarão branco do Pacífico (*L. vannamei*)

Process Biochemistry 47 (2012) 570–577

Contents lists available at SciVerse ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Recovery of protein, chitin, carotenoids and glycosaminoglycans from Pacific white shrimp (*Litopenaeus vannamei*) processing waste

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ABSTRACT

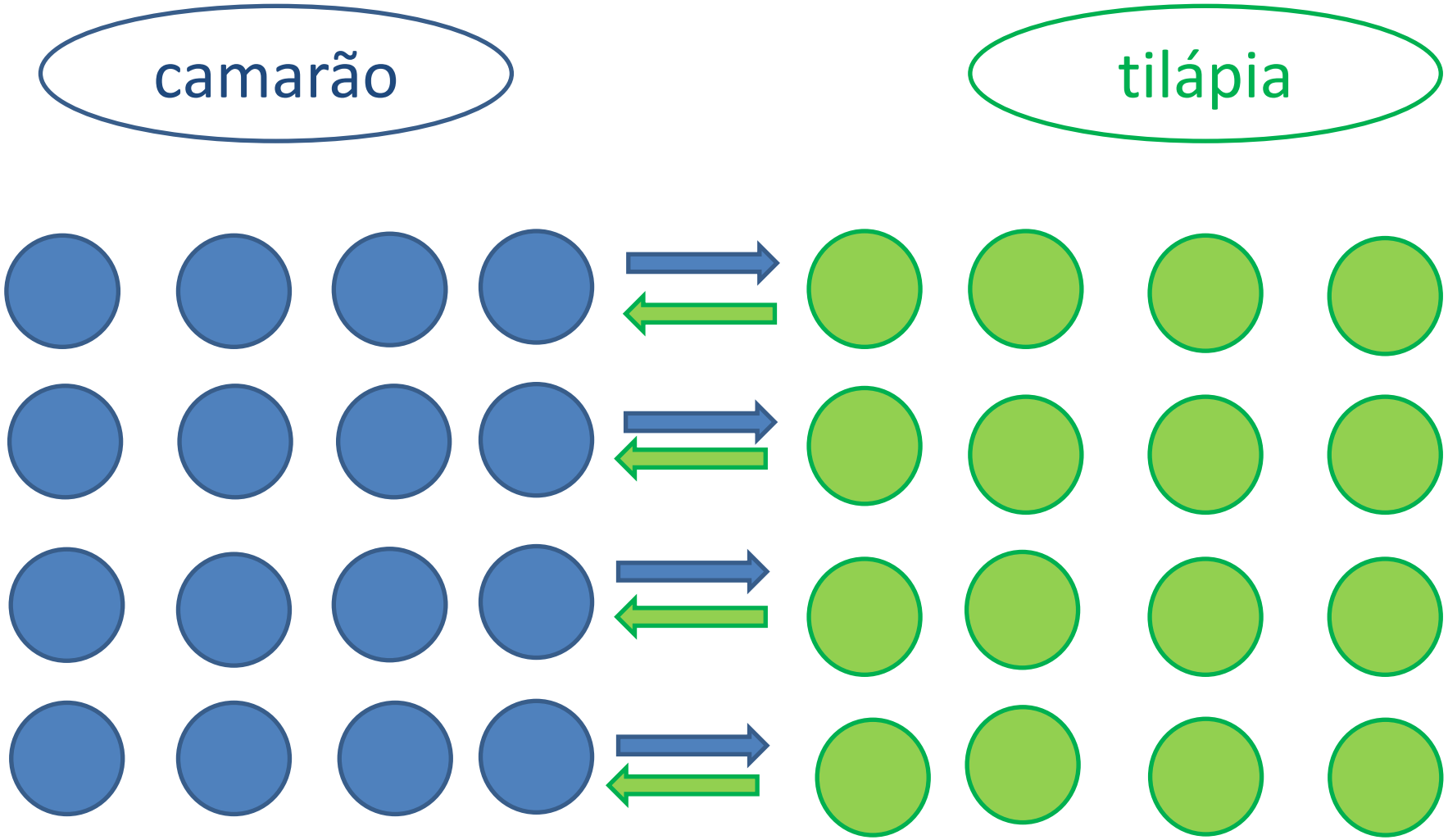
Shrimp head waste is a major byproduct of crustacean processing in North-eastern Brazil and represents an interesting source of bioactive molecules. Additionally, its use increases the sustainability of processing fishery products. The present study reports a process developed for recovering bioactive molecules from shrimp heads through autolysis. A protein hydrolysate (120 ± 0.4 g) formed by a 9% (w/v) solution was recovered and lyophilized from 1 kg of shrimp heads. Approximately 195 ± 0.5 mg of carotenoids was recovered as an ethanolic extract. The recovery of chitin and chitosan were 25 ± 2 g kg^{-1} and 17 ± 4 g kg^{-1} wet processing waste, respectively. Chitosans were characterized by ^{13}C NMR, and FT-IR analysis and exhibited a variable degree of deacetylation (60–80%). Sulfated glycosaminoglycans that exhibited electrophoretic migration similar to mammalian standards were also recovered (79 ± 2 mg kg^{-1} wet processing waste), and their degradation products suggested the presence of C6-sulfated heparan sulfate. These data point to the feasibility of an integrated process for isolating highly bioactive molecules, such as sulfated- and amino-polysaccharides, with a broad spectrum of applications from shrimp processing waste.

Tabela 1. Composição centesimal e perfil lipídico dos bioprodutos de hidrolisados de camarão.

	Farinha de soja	SPH33	SPH50	SPH67
Gordura saturada $g.kg^{-1}$	12,1	20,09	27,85	28,3
Gordura monoinsaturada $g.kg^{-1}$	8,376	16,18	23,64	24,22
Gordura poliinssaturada $g.kg^{-1}$	28,718	40,20	51,42	52,03
Gordura Trans $g.kg^{-1}$	0,002	0,07	0,14	0,14
Gordura insaturada $g.kg^{-1}$	37,094	56,38	75,06	76,25
Proteína bruta $g.kg^{-1}$	436,762	540,44	605,11	647,26
Fibras $g.kg^{-1}$	53,342	38,88	29,2	23,98
Extrato Etéreo $g.kg^{-1}$	49,226	76,47	102,91	104,54

Tabela 2. Perfil de aminoácidos essenciais dos bioprodutos de hidrolisados de camarão.

	Soybean Meal	SPH33	SPH50	SPH67	SPH100
Soma dos aminoácidos totais $g.kg^{-1}$	519,75	583,94	608,00	650,07	714,22
Arginina $g.kg^{-1}$	39,37	43,80	45,74	48,36	52,78
Histidina $g.kg^{-1}$	13,04	13,89	14,01	14,76	15,60
Isoleucina $g.kg^{-1}$	19,50	21,50	22,30	23,56	25,55
Leucina $g.kg^{-1}$	38,87	42,14	42,76	45,50	48,76
Lisina $g.kg^{-1}$	33,90	39,23	40,98	44,72	50,04
Metionina $g.kg^{-1}$	3,29	7,00	9,77	10,82	14,52
Fenialanina $g.kg^{-1}$	24,52	26,04	26,34	27,62	29,14
Treonina $g.kg^{-1}$	17,34	19,73	21,00	22,19	24,57
Triptofano $g.kg^{-1}$	4,43	5,05	5,61	5,68	6,29
Valina $g.kg^{-1}$	22,76	26,44	28,32	30,23	33,90


camarão
tilápia

Aquicultura Cíclica Regionalizada

Unidade de beneficiamento de pescado

Produtos primários

Produção

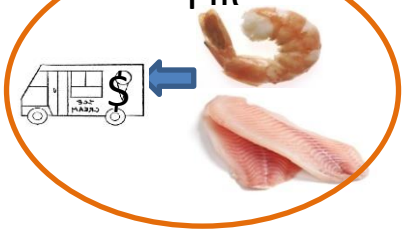
Produção

Cabeças
Cascas
Carcaças
vísceras

Unidade de processamento de Co-produtos

Proteínas
Polissacarídeos
Hidrolisados

Filé



Insumos

Unidade de produção de ração

Ração regionalizada

Aeração

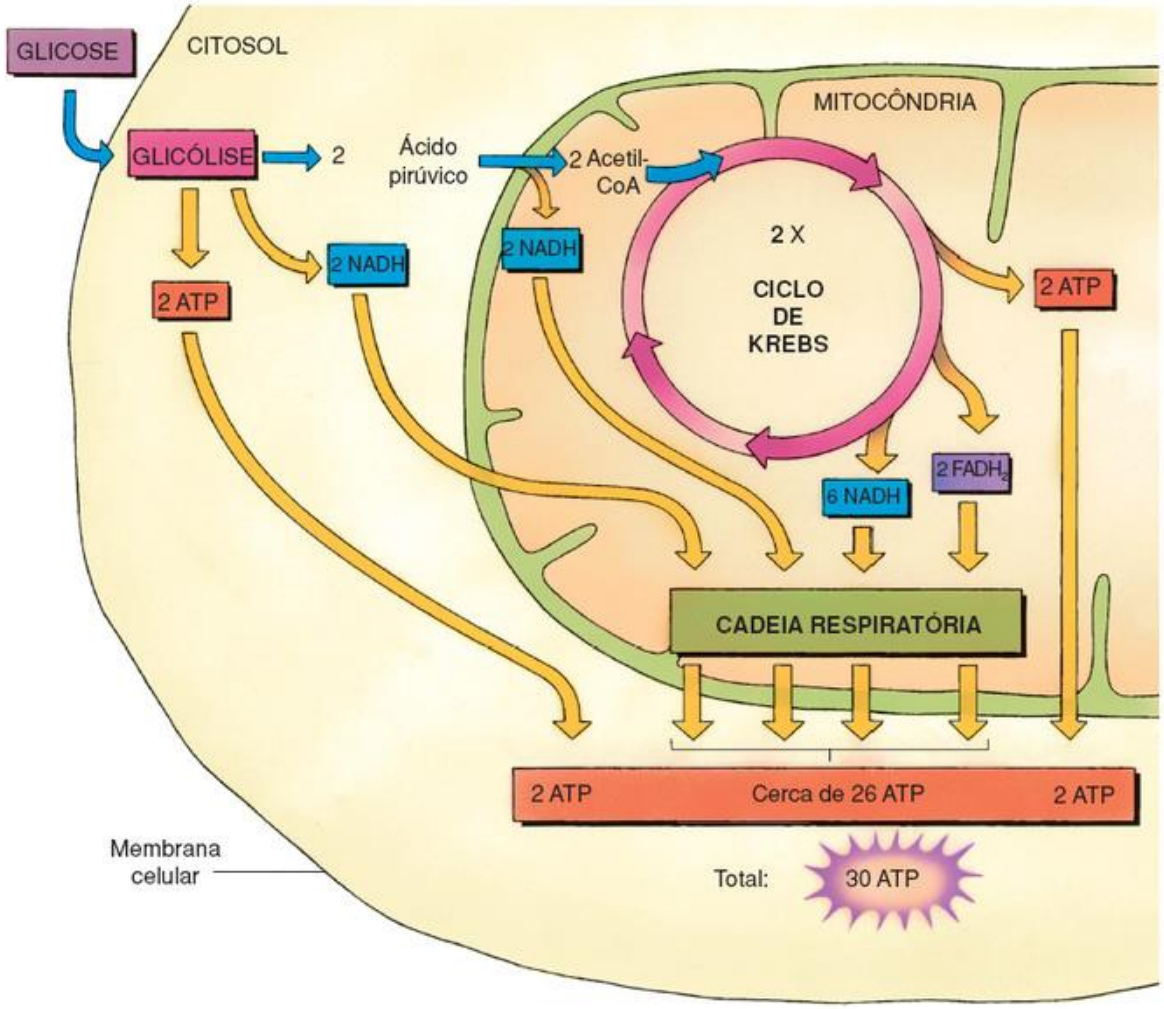
Aeração

Unidade de aeração

Tanques de camarão

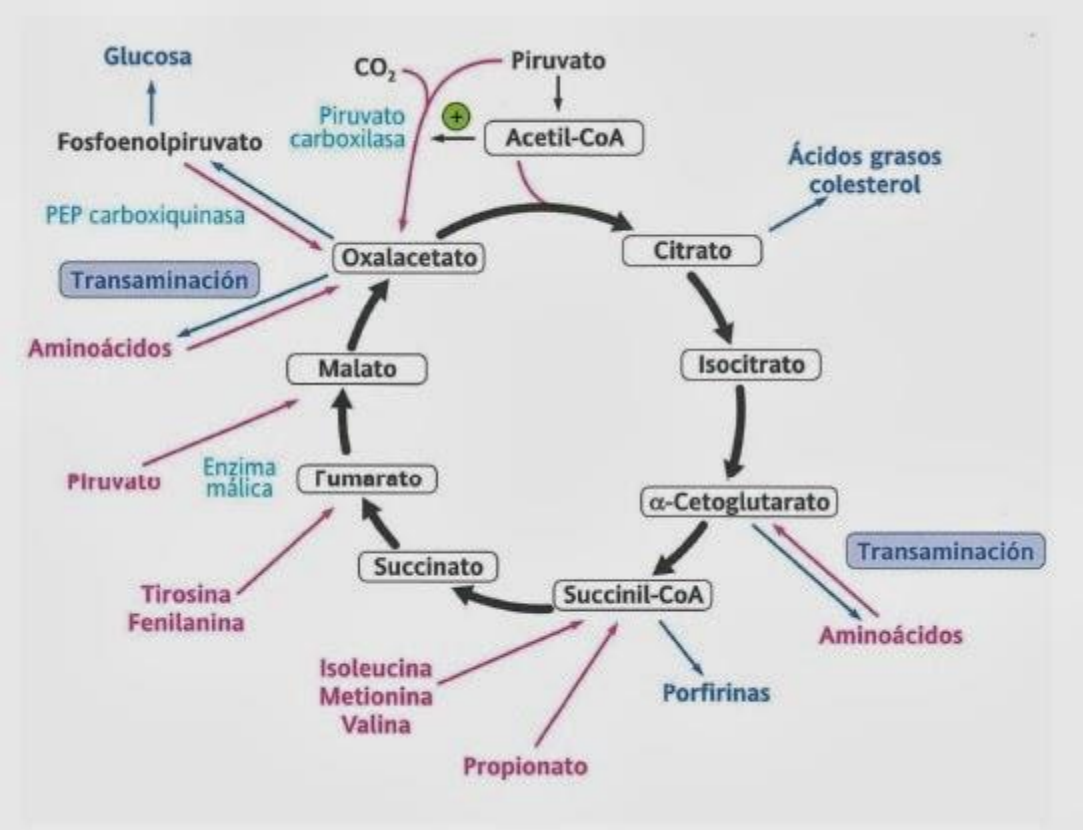
Tanques de tilápia

Ciclo de Krebs
Uma Via Chave do metabolismo aeróbico



Uma via conservativa, regenerativa e anfibólica

Ciclo de Krebs
Uma Via Regenerativa e Anfibólica

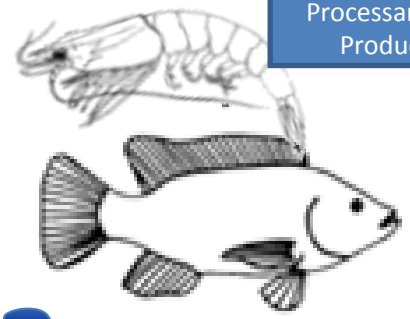


Aquicultura Cíclica Regionalizada

Alimento

Rações para aquicultura

Cultivo de peixes e camarões



Processamento
Produção

Co-produtos

Resíduos do processamento

Pigmentos

Proteína animal

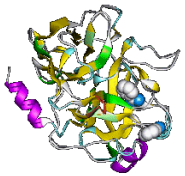
Quitosana

Quitina

Proteínas e nutrientes

Matéria prima para produção de insumos visando outros segmentos industriais

Portfólio Científico-Tecnológico visando novos segmentos industriais de maior valor agregado



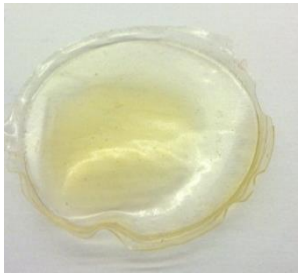
Polimeros de Pescado
Quitina/Quitosana

FILMES DE QUITOSANA

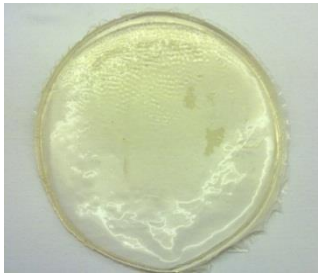
Os filmes podem ser preparados de duas formas:

- Evaporação da solução de quitosana numa placa de Petri (CHATELET *et al*., 2001);
- Congelamento e liofilização da solução de quitosana para obtenção de arcabouço poroso (CHEN *et al.,* 2007).

Quitosana (2%)
e
Glicerol (2%)



Quitosana (2%)
e
Glicerol (6%)



Quitosana (2%)
e
Glicerol (20%)

Quitosana(2%)
e
Ácido Acético (1%)

Quitosana(2%)
e
Gelatina (20%)

Quitosana (2%)

(Liofilizada)

Polímeros de Pescado
Colágeno de pele de peixe

Fig. 1. Amostra de pele de bijupirá (*R. canadum*).

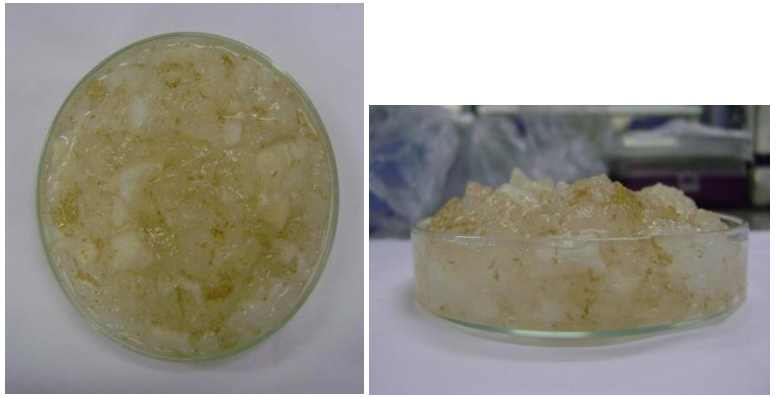


Fig. 2. Amostra da pele de bijupirá após etapas de pré-tratamento e extração de colágeno.

Fig. 3. Colágeno Ácido Solúvel (ASC).

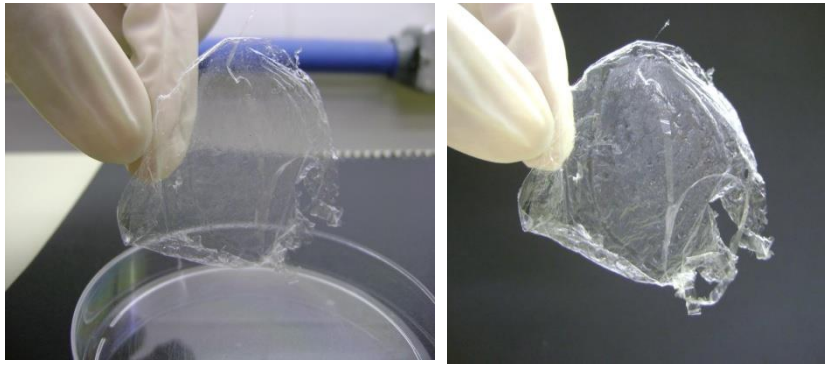
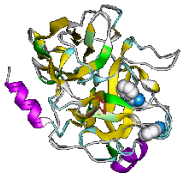


Fig. 4. Filme de colágeno (ASC).

Polímeros de Pescado
Revestimentos de filés de peixe

Filés de *O. niloticus*

Revestimento
Quitosana 1% + 0,1 % Glicerol



Polímeros de Pescado
Revestimentos de filés de peixe

Total volatile base nitrogen (TVB-N)

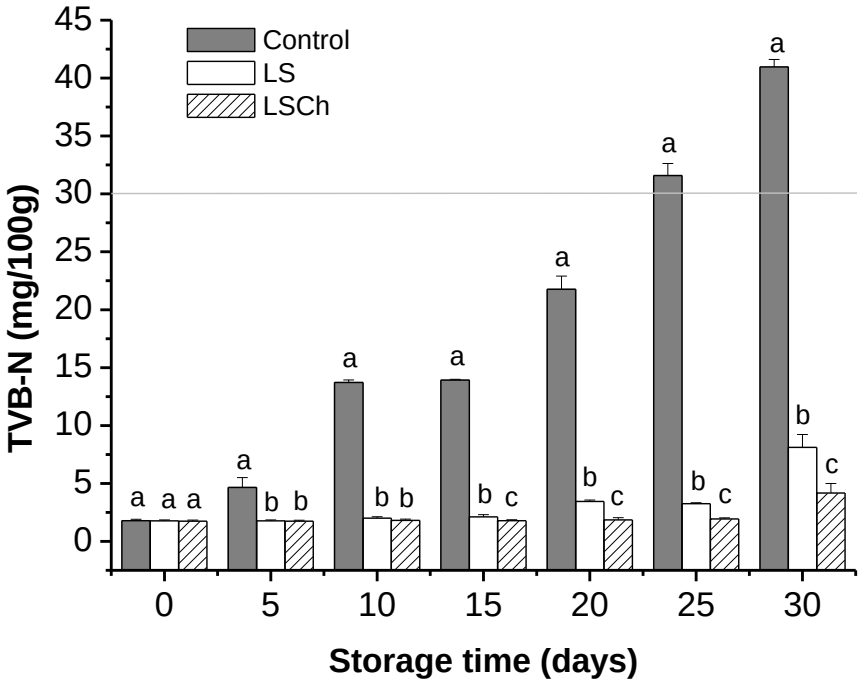


Figure 2. Total volatile base nitrogen (TVB-N) of tilapia fillets stored at 4°C. The horizontal line represents the rejection limit in fishflesh, which is 30 mg of TVB-N/100 g.

Thiobarbituric acid-reactive substances (TBARS)

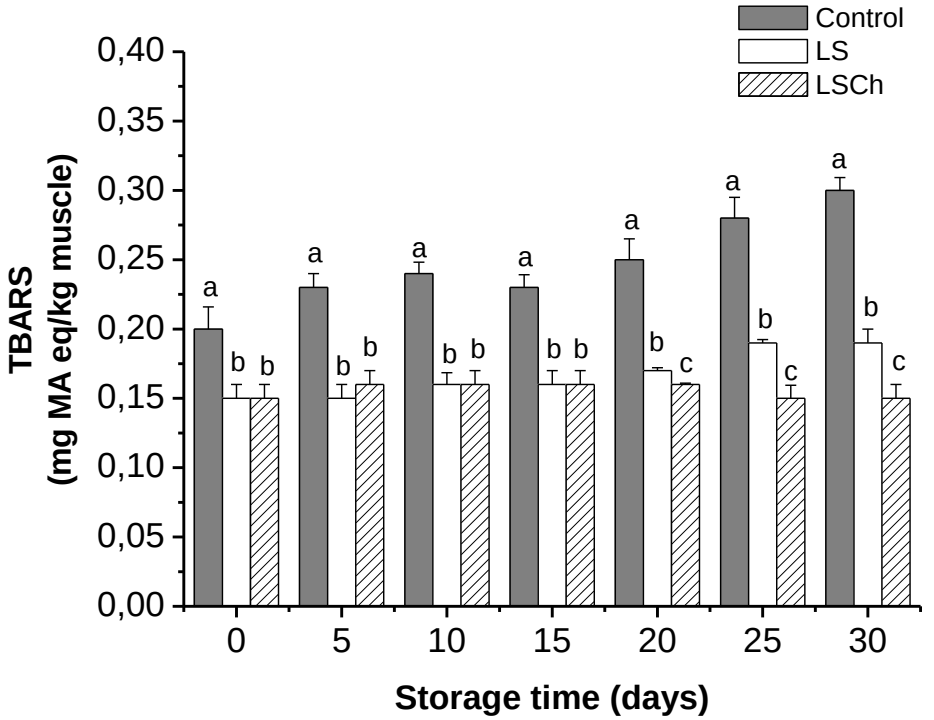
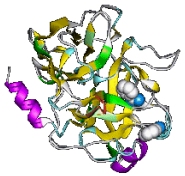


Figure 3. Thiobarbituric acid-reactive substances (TBARS) of *O. niloticus* fillets during cold storage at 4°C.

Polímeros de Pescado
Colágeno de pele de peixe

Aplicação na Construção Civil

Cerca de 1Kg de resíduo para a produção de cola suficiente para cobrir 6-7m² de pastilha de vidro



Compatibilidade das proteases de peixes com detergentes comerciais

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journal homepage: www.elsevier.com/locate/foodchem

Fish processing waste as a source of alkaline proteases for laundry detergent

Talita S. Espósito [a,b], Ian P.G. Amaral [b], Diego S. Buarque [b], Givanildo B. Oliveira [c], Luiz B. Carvalho Jr. [b,c], Ranilson S. Bezerra [a,b,*]

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Alkaline protease
Laundry detergent

ABSTRACT

Proteases were extracted from the viscera of *Colossoma macropomum* and precipitated with ethanol (30–70%, v/v). The enzymatic extract was partially purified with a yield of 75% (2926 U/g of tissue); at least five caseinolytic proteases bands were observed in zymogram. The optimum pH of the preparation was in the alkaline pH range (10–12). The optimum temperature of activity was 60 °C and only about 15% of the initial activity was lost after an incubation period of 30 min at the above mentioned temperature. Both trypsin and chymotrypsin-like enzymes were detected in the proteases, but with a stronger prevalence for the former. These proteolytic enzymes remained stable in the presence of non-ionic (Tween 20 and Tween 80) and ionic surfactants (saponin and sodium choleate). They also revealed high resistance (60% residual activity) when incubated with 10% H_2O_2 for 75 min. Furthermore, the preparation retained approximately 80% of its proteolytic activity after incubation for 1 h at 40 °C with the commercial detergent.

Compatibilidade das proteases de peixes com detergentes comerciais

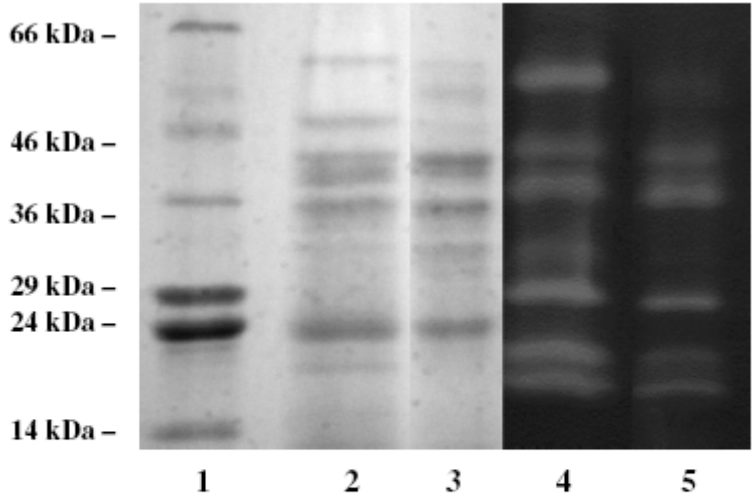
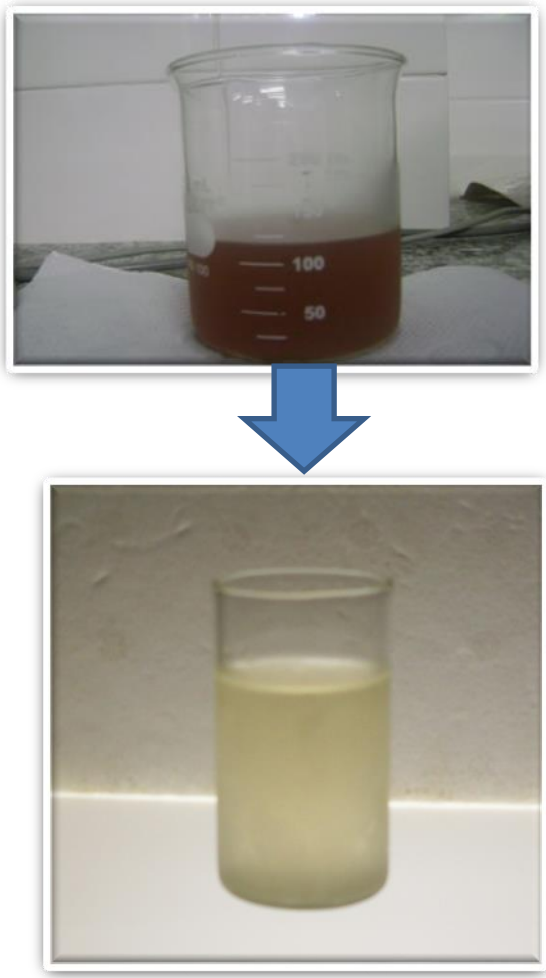
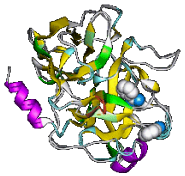


Fig. 1. SDS-PAGE of alkaline protease from the viscera of *C. macropomum*. Lane 1: molecular weights of standard protein markers (bovine serum albumin 66 KDa, ovoalbumin 45 KDa, glyceraldehydes 3-phosphate dehydrogenase 36 KDa, carbonic anhydrase 29 KDa, trypsinogen 24 KDa, and α-lactoalbumin 14,2 KDa); lane 2: crude extract; lane 3: precipitate with 30–70% ethanol; lane 4: zymogram of the crude extract and lane 5: zymogram of the precipitate with 30–70% ethanol.



RENDIMENTO: 74,9%

PURIFICAÇÃO: 2,6



Compatibilidade das proteases de peixes com detergentes comerciais

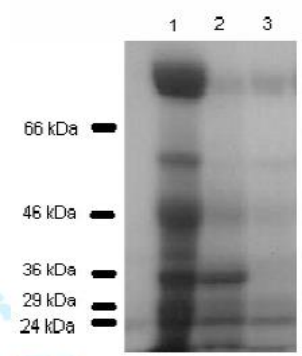


Table 1
Effect of surfactants on proteases of *C. macropomum* pyloric caeca and intestine purified by ethanol precipitation

Surfactants (1% w/v)	Residual activity[a] (%)	
	After 30 min	After 60 min
Saponin	117.5 ± 0.3	118.4 ± 2.1
Sodium choleate	94.2 ± 7.3	107.3 ± 4.4
Tween 20	117.3 ± 5.4	108.2 ± 0.5
Tween 80	112.0 ± 8.1	107.8 ± 4.7
SDS	15.1 ± 1.3	7.3 ± 1.0

[a] Values are expressed in ± standard deviation. $n = 4$. The specific enzyme activity of control sample (100%) was 142.0 U/mg using azocasein as substrate.

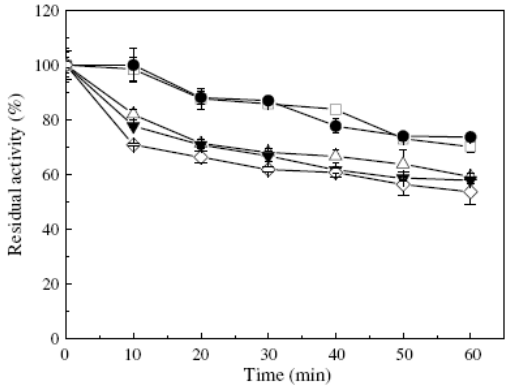


Fig. 5. The stability of protease in commercially available detergents. Protease (0.2 mg mL^{-1}) was incubated at 40 °C in the presence of detergents at 7 mg mL^{-1}. Activity of the control sample devoid of any detergent incubated under similar conditions (●), Surf® (□), Ala® (△), Bem-te-vi® (▼), Omo Multi-Ação® (◇). The specific enzyme activity of the control sample (100%) was 146.0 U/mg using azocasein as substrate.

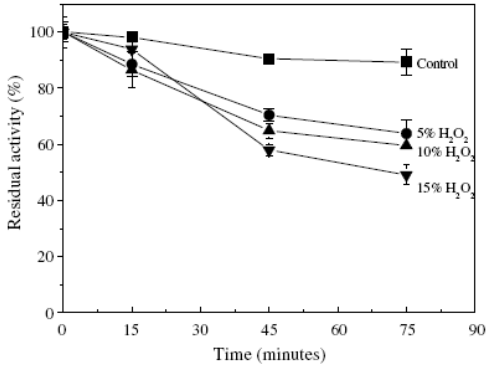
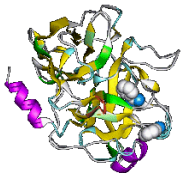


Fig. 4. The inactivation curve of the H_2O_2 of proteases from the *C. macropomum* pyloric caeca and intestine precipitated by 30–70% ethanol. Enzyme preparations were incubated at pH 11.0 and 40 °C with H_2O_2 at the concentrations of 5% (●), 10% (▲), 15% (▼). Samples were withdrawn at time intervals, their activities (duplicates) were established using azocasein as substrate and compared to the non-treated sample (■). The specific enzyme activity of the control sample (100%) was 146.0 U/mg using azocasein as substrate.



Enzima de peixe com biomarcador para pesticidas

Aquatic Toxicology xxx (2012) xxx–xxx

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Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Kinetic and physicochemical properties of brain acetylcholinesterase from the peacock bass (*Cichla ocellaris*) and *in vitro* effect of pesticides and metal ions

Q1 Kaline Catiely Campos Silva, Caio Rodrigo Dias Assis, Vagne Melo Oliveira, Luiz Bezerra Carvalho Jr, Ranilson Souza Bezerra*

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Biomarkers
Cichla ocellaris

ABSTRACT

Brain acetylcholinesterase (AChE; EC 3.1.1.7) from peacock bass (*Cichla ocellaris*) was characterized and the effect of organophosphorus and carbamate pesticides as well as ions and heavy metals was evaluated. The kinetic parameters K_m and V_{max} were determined as 0.769 mM and 0.189 U/mg of protein respectively. Optimal pH and temperature were found to be 8.0 and 45 °C. The enzyme retained approximately half of the activity after incubation at 50 °C for 30 min. Total cholinesterase activity on brain of this species can be ascribed to AChE according to selective inhibitors analysis (neostigmine, eserine and BW284c5 reduced its activity whereas no effect was noticed for Iso-OMPA). Seven pesticides (five organophosphates: dichlorvos, diazinon, chlorpyrifos, temephos, tetraethyl pyrophosphate – TEPP and two carbamates: carbaryl and carbofuran) showed inhibitory effects on *C. ocellaris* AChE. However, the strongest effect was observed with carbofuran (IC_{50} = 0.21 μM and $K_i = 2.57 \times 10^{-3}$ μM). The following ions (1 mM) showed to inhibit its activity (decrescent order): $Hg^{2+} > As^{3+} > Cu^{2+} > Zn^{2+}$. $EDTA^{2-}$ did not affect enzyme activity. The present study provides assay conditions and data to suggest this enzyme as *in vitro* biomarker of organophosphorus and carbamate pesticides in routine environmental screening programs.

Enzima de peixe com biomarcador para pesticidas

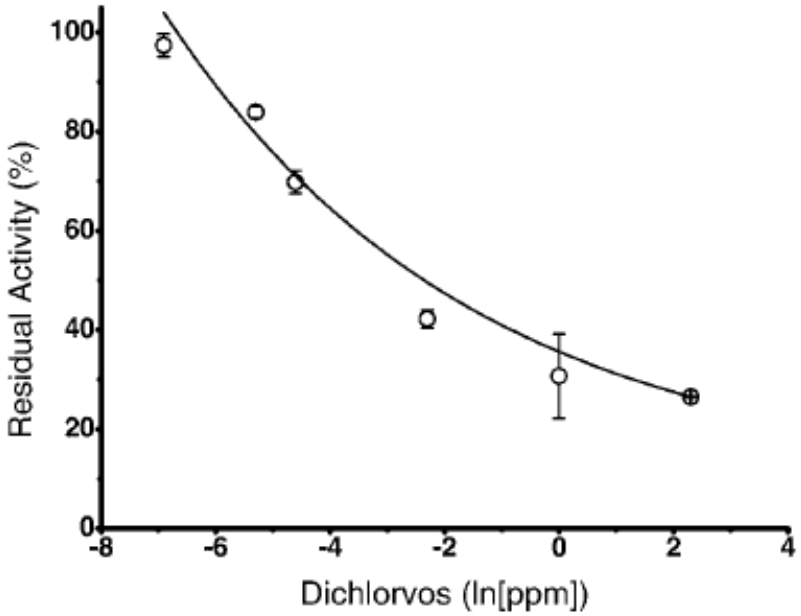
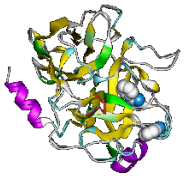


Fig. 1. Effect of increasing concentrations of dichlorvos on acetylcholinesterase (AChE) extracted from brain of juvenile *Colossoma macropomum*. The assay was performed at 25°C as described in the *Materials and Methods* section and the experimental points are the mean ± standard deviation of triplicate of four crude extracts obtained from five brains each ($y = 9.420 + 26.192e^{(-x/5.380)}$; $r^2 = 0.989$).



Carotenoides de camarão Astaxantina

Efeito antagonista ao do etanol crônico no cérebro em desenvolvimento

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Neuroscience Letters 391 (2005) 51–55
Neuroscience Letters
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Shrimp carotenoids protect the developing rat cerebral cortex against the effects of ethanol on cortical spreading depression

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Abstract

Cortical spreading depression is a neural phenomenon present in several animal species. Spreading depression features, like velocity of propagation, depends on several chemical and metabolic factors, as for example, anti-oxidants. Here we studied spreading depression-velocity changes in weaned rat-pups born from dams treated on a daily basis, either during gestation or lactation, with a carotenoid ethanolic extract (30 μg/kg/day) prepared from shrimp waste (heads). These pups were compared with age-mated ones, whose mothers were treated either with the vehicle (ethanol) or with distilled water. Compared to the distilled water-group (mean values, in mm/min, per hour of recording ranging from 3.02 ± 0.26 to 3.15 ± 0.27 [treatment during gestation; $n = 7$], and from 3.03 ± 0.25 to 3.22 ± 0.30 [lactation; $n = 11$]), ethanol-treated rats displayed higher spreading depression-velocities (from 3.74 ± 0.06 to 3.82 ± 0.08 [gestation; $n = 7$], and from 4.26 ± 0.32 to 4.33 ± 0.34 [lactation; $n = 11$]; $p < 0.05$). Compared to the ethanol-group, carotenoid-treatment lead to lower spreading depression-velocities ($p < 0.05$), ranging from 3.38 ± 0.09 to 3.42 ± 0.12, $n = 7$ (gestation) and 3.58 ± 0.13 to 3.62 ± 0.17, $n = 12$ (lactation). Carotenoid-treatment during lactation was shown to be significantly more effective than that during gestation ($p < 0.05$), in lowering spreading depression-velocity. The results suggest a protective action of shrimp carotenoids against the ethanol effects on spreading depression. This protective effect could be related to the carotenoid antioxidant properties, as previously indicated by evidence showing spreading depression-effects of other antioxidants.

Keywords: Spreading depression; Shrimp carotenoids; Ethanol; Brain development; Antioxidants; Free radical injury

Carotenoides de camarão Astaxantina

Efeito antagonista ao do etanol crônico no cérebro em desenvolvimento

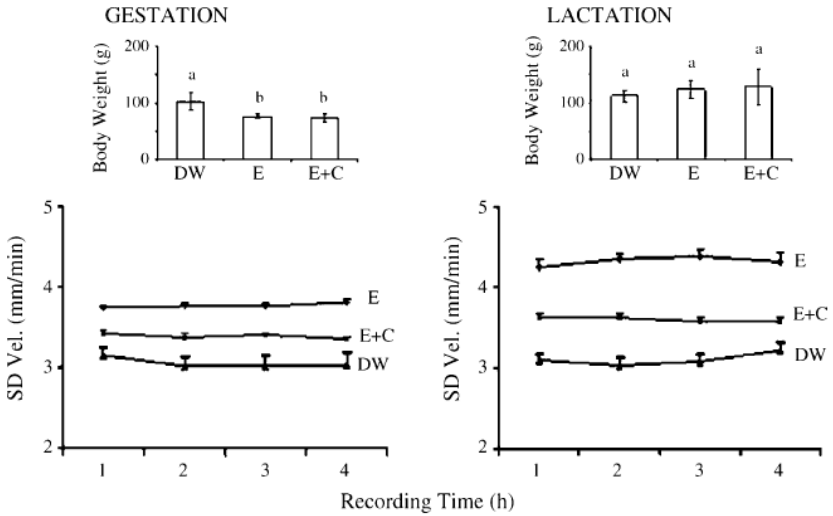
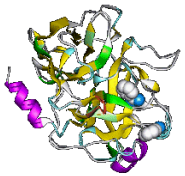


Fig. 2. Reduction of spreading depression-velocity in just-weaned rat pups (36–40 days of life) whose mothers had been treated with an ethanolic extract of shrimp carotenoids (30 μg/kg/day), either during the gestation or during the lactation period, as compared to corresponding vehicle- (ethanol; 3.8 mL/kg) and distilled water-treated controls. Data are expressed as mean ± standard deviation, per hour of recording. All groups were shown to be statistically different from each other (ANOVA plus Tukey test; $p < 0.05$). Insets show body weights. Values marked with distinct letters are significantly different (ANOVA plus Tukey-test; $p < 0.05$). E, E + C and DW refers to ethanol-, ethanol plus carotenoid- and distilled water-treatment, respectively.



Carotenoides de camarão Astaxantina

Efeito antagonista ao do etanol crônico no cérebro adulto

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Dose-Dependent Effects of Astaxanthin on Cortical Spreading Depression in Chronically Ethanol-Treated Adult Rats

Ricardo Abadie-Guedes, Suzan Diniz Santos, Thiago Barbosa Cahú, Rubem Carlos Araújo Guedes, and Ranilson de Souza Bezerra

Background: The consumption of alcoholic drinks is a frequent drug-abuse situation, which is associated to a wide variety of pathological disturbances affecting several organs, including the brain. We have previously shown in the developing rat brain that ethanol intake facilitates the propagation of cortical spreading depression (CSD), an excitability-related neural phenomenon present in several animal species. This electrophysiological effect was attenuated by a shrimp (*Litopenaeus vannamei)* carotenoids extract. Here we investigated the effects of pure astaxanthin, the main carotenoid found in shrimp, on CSD.

Methods: Adult Wistar rats were treated per gavage, during 18 days, with 2.5, 10 or 90 μg/kg/d astaxanthin dissolved in ethanol (3 g/kg) and CSD was recorded on the cortical surface 1 to 3 days thereafter. Four groups, treated respectively with ethanol, distilled water and soybean oil with- and without astaxanthin were also studied for comparison with the ethanol + astaxanthin groups.

Results: Ethanol-treated rats displayed higher CSD-velocities (mean values, in mm/min, per hour of recording ranging from 4.08 ± 0.09 to 4.12 ± 0.16), compared to the distilled water-group (from 3.19 ± 0.13 to 3.27 ± 0.06). Addition of astaxanthin to ethanol lead to lower CSD-velocities in a dose-dependent manner, ranging from 3.68 ± 0.09 to 3.97 ± 0.22 for the 2.5 μg/kg/d-dose, from 3.29 ± 0.09 to 3.32 ± 0.07 for the 10 μg/kg/d-dose, and from 2.89 ± 0.13 to 2.92 ± 0.11 for the 90 μg/kg/d-dose. The velocities of the soybean oil groups (with and without astaxanthin) were not statistically different from the 10 μg/kg/d astaxanthin + ethanol and distilled water groups.

Conclusion: The results demonstrate the antagonistic effect of astaxanthin against the ethanol-induced facilitation of CSD propagation. Probably carotenoid antioxidant properties are involved in such effects.

Key Words: Ethanol, Cortical Spreading Depression, Antioxidants, Carotenoids, Astaxanthin.

Carotenoides de camarão
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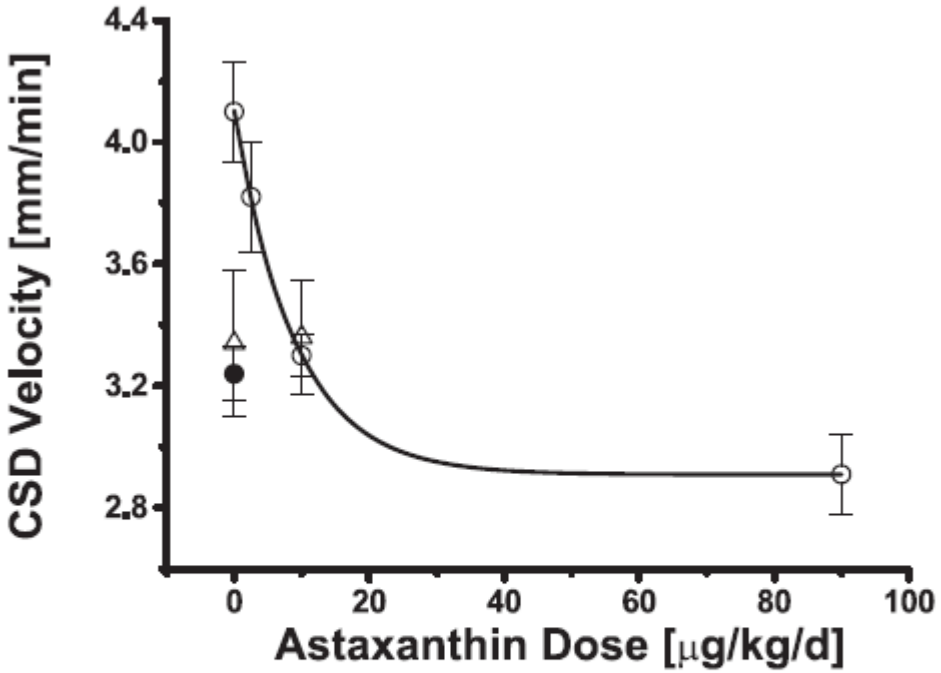
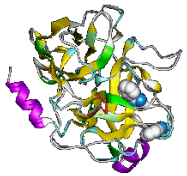


Fig. 3. Dose–response effects of astaxanthin dissolved in ethanol on cortical spreading depression velocity in the rat cortex (open circles). The curve resulted from plotting the mean CSD velocities (during the entire 4-h recording period) as a function of the astaxanthin doses. Each point represents the average CSD velocity for the respective group. Note that the values for the soybean oil groups (with and without astaxanthin; open triangles) are not different from that of the distilled water control group (black dot).

Dedicatória

"Um Médico/Bioquímico que não entendia nada de pescado, mas que teve a coragem de orientar um Engenheiro de Pesca que não entendia nada de Bioquímica"

Prof. Luiz Bezerra de Carvalho Jr



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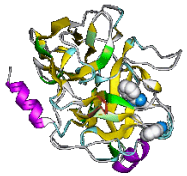

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