UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA E FISIOLOGIA

DISSERTAÇÃO DE MESTRADO

EXTRAÇÃO E CARACTERIZAÇÃO DE COLÁGENO OBTIDO A PARTIR DAS ESCAMAS OBTIDAS NO PROCESSAMENTO DO PEIXE CIOBA (*Lutjanus analis*)

ROBSON COELHO DE ARAUJO NERI

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Dissertação apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco

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Esta dissertação foi julgada para a obtenção do título de Mestre em Bioquímica e Fisiologia e aprovada em ___/___/_____ pelo Programa de Pós-Graduação em Bioquímica e Fisiologia da Universidade Federal de Pernambuco em sua forma final.

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Recife, 28 de Fevereiro de 2013

O que sabemos é uma gota; o que ignoramos é um oceano.
Isaac Newton

Dedico este trabalho aos meus pais e irmãos por sempre mostrarem que na vida portas e janelas sempre estarão abertas para aqueles que ousam sonhar.

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RESUMO

O organismo animal apresenta-se composto por diversas proteínas fibrosas, sendo o colágeno a que ocorre em maior quantidade equivalendo a 30% do conteúdo proteico total do animal. Bovinos e suínos são os organismos animais que apresentam a maior quantidade proporcional de colágeno na pele e ossos, porém devido ao risco de transmissão de zoonoses como Encefalopatia Espongiforme Bovina (EEB), Encefalopatia Espongiforme Transmissível (EET) e a Febre Aftosa (FA), torna-se necessário buscar fontes alternativas dessa proteína tais como os organismos aquáticos. Os peixes devido a sua disponibilidade e baixo risco de transmitir doenças são bastante relatados em recentes estudos científicos. No presente trabalho objetivou-se extrair e caracterizar parcialmente o colágeno obtido a partir das escamas do peixe cioba (*Lutjanus analis*), e investigar a possibilidade da utilização deste subproduto da indústria pesqueira como fonte alternativa ao colágeno mamífero. Colágeno ácido solúvel (ASC) e pepsino solúvel (PSC) tipo I foram extraídos com rendimentos de 3,85% e 6,15% (peso seco)**,** respectivamente, obtendo um rendimento total da extração de 10%. A SDS-PAGE (7,5%) das amostras de colágeno (ASC e PSC) apresentaram duas cadeias α1 e uma cadeia α2, além de cadeias β. O colágeno PSC foi solúvel na faixa de pH 2 – 6, apresentando máxima solubilidade relativa no pH 3, enquanto que o colágeno ASC foi solúvel na faixa de pH de 1 – 6, apresentando máxima solubilidade relativa no pH 1. Ambos colágenos foram solúveis na faixa de concentração de NaCl de 0-4% (p/v). A temperatura máxima de transição (T_{max}) obtida para as amostras de ASC e PSC (respectivamente) foi 76°C e 77°C. O espectro de absorção de raios ultravioleta (UV) ocorreu na mesma faixa para ambos, sendo 236nm e 239nm os pontos de maior absorção para ASC e PSC respectivamente. Os resultados obtidos neste trabalho indicam a possibilidade do uso de escamas da cioba como fonte de biomoléculas com grande potencial de aplicação biotecnológica e industrial.

Palavras-chave: cioba (*Lutjanus analis*), colágeno, escamas, subprodutos pesqueiros.

ABSTRACT

The animal organism presents composed of several fibrous proteins, being collagen that which occurs in larger amount corresponding to 30% of the total protein content of the animal body. Bovine and porcine are the animals with the greatest proportional amount of collagen in the skin and bones, but due to the risk of transmitting diseases such as Bovine Spongiform Encephalopathy (BSE), Transmissible Spongiform Encephalopathy (TSE) and Foot and Mouth Disease (FMD) it becomes necessary to find alternative sources of this protein such as marine animals. Fishes due to its availability and low risk of transmitting disease are fairly reported in recent scientific studies. The present work aimed to extract and partially characterize the collagen obtained from fish scales of mutton snapper (*Lutjanus analis*) and investigate the possibility of using this fisheries by-product as an alternative source to mammal collagen. Type I acid soluble (ASC) and pepsin soluble (PSC) collagen were extracted with yields of 3.85% and 6.15% **(**dry weight**)**, respectively, with a total yield extraction of 10%. Electrophoresis (SDS-PAGE) pattern showed that both ASC and PSC consisted of two α1 and one α2 chains, well as β chains. The collagen PSC was soluble in the pH range 2 – 6, with maximum relative solubility at pH 3, while collagen ASC was soluble in the pH range 1 – 6, with maximum relative solubility at pH 1. Both collagens were soluble in the range of NaCl concentration 0 – 4% (w/v). The maximal transition temperatures (T_{max}) for ASC and PSC were 76°C and 83°C, respectively. Both collagen samples had the same range of absorption of ultraviolet (UV) spectrum, being 236nm and 239nm which points to greater absorption of ASC and PSC respectively. The results obtained in this study indicate the possibility of using mutton snapper scales as a source of biomolecules with great potential for biotechnological and industrial application.

Keywords: mutton snapper (*Lutjanus analis*), collagen, scales, fisheries by-products.

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1 INTRODUÇÃO

O colágeno trata-se de uma proteína estrutural de origem animal, comum aos vertebrados que constitui cerca de 30% do conteúdo proteico total (Muyonga, et al., 2004). Essa proteína de característica fibrosa possui propriedades como a formação de fibras insolúveis com elevada resistência à tração. Estruturalmente o colágeno é caracterizado por ser constituído de três cadeias polipeptídicas ligadas entre si formando uma tripla hélice com orientação de sentido destrogiro e por possuir um motivo, (Glicina-X-Y)n, comum a todos os vinte e nove tipos de colágeno e que se repete ao longo das cadeias. As posições X e Y são ocupadas com frequência por prolina e hidroxiprolina (Gelse et al., 2003; McCormick, 2009). Principal elemento estrutural dos ossos, cartilagens, pele, tendões, ligamentos, vasos sanguíneos, dentes, córneas e muitas outras estruturas presentes nos vertebrados (Senaratne et al., 2006).

Dos vinte e nove tipos de colágeno, o tipo I é o mais recorrente, sendo encontrado em todos os tecidos conectivos dos vertebrados, possui a função de conferir resistência mecânica aos tecidos e órgãos e também de auxiliar na regulação do meio celular (Nagai et al., 2008; Ikoma et al., 2003; Muyonga et al., 2004).

Amplamente utilizado nas indústrias de alimentos, cosméticos e fármacos devido a propriedades como biocompatibilidade, biodegradabilidade e baixa antigenicidade, o colágeno contido nos subprodutos de animais como bovinos, suínos e aves constitui atualmente a principal fonte desta proteína (Liu et al., 2009). Mas devido aos recorrentes casos de zoonoses como: encefalopatia espongiforme bovina (BSE), encefalopatia espongiforme transmissível (TSE), febre aftosa (FA) e gripe aviária, a busca por fontes alternativas e mais seguras desse composto tornou-se uma opção atrativa (Zhang et al., 2007).

Organismos aquáticos, como os peixes, devido a sua grande disponibilidade, baixos riscos de transmissão de doenças, alto rendimento nos processos de extração e ausência de toxicidade tem ganhado destaque como uma alternativa frente ao colágeno de animais terrestres (Senaratne et al., 2006). O número de estudos objetivando extrair colágeno de peixes tem crescido continuamente. Nos últimos anos diversos trabalhos têm relatado extrações bem sucedidas a partir de pele, ossos e escamas de peixes como *Priacanthus tayenus* e *Priacanthus macracanthus* (Benjakul et al., 2010); *Pagrus major* e *Oreochromis niloticus* (Ikoma et al., 2003); *Lates niloticus* (Muyonga et al., 2004). Tecidos como pele, ossos, nadadeiras e escamas têm o colágeno como seu principal constituinte

proteico, e devido a grande distância evolutiva entre peixes e humanos, os riscos de transmissão de doenças por essa via tornam-se muito baixos (Song et al., 2006).

O processamento de peixes gera uma grande quantidade de resíduos como: pele, ossos, nadadeiras, vísceras e escamas, que são descartados no meio ambiente sem nenhum tratamento, causando sérios problemas relacionados à poluição. Por possuir um processamento sanitário oneroso para a indústria pesqueira, o aproveitamento desses resíduos na obtenção de biomoléculas tem sido relatado como uma via de aproveitamento desse material (Gildberg, 1992).

Segundo o Boletim Estatístico da Pesca e Aquicultura (MPA), em 2010 o Brasil produziu cerca de 1.264.765 toneladas, figurando entre os vinte maiores produtores de pescado do mundo. Neste cenário o estado de Pernambuco aparece como sendo o 15° maior produtor nacional.

A cioba (*Lutjanus analis*) é uma espécie de peixe demersal da família Lutjanidae que habita águas costeiras de mares tropicais e subtropicais, próximos de recifes e fundos rochosos em profundidades de até 650 metros (Resende et al., 2003). Os indivíduos adultos formam grandes cardumes aderidos às rochas, ocorrendo em águas litorâneas mais rasas unidas aos arrecifes (Frédou & Ferreira, 2005). Devido a sua biodisponibilidade a cioba apresenta um significativo valor para o comércio pesqueiro nas regiões do Nordeste brasileiro (Resende et al., 2003). Sua produção é bastante rentável e no ano de 2010 alcançou a cifra de 2987 toneladas (MPA, 2010).

O presente trabalho objetivou extrair e isolar o colágeno ácido solúvel (ASC) e pepsino solúvel (PSC) a partir das escamas da cioba assim como também caracterizar e sugerir sua utilização como fonte alternativa ao colágeno de animais terrestres.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Colágeno

A matriz extracelular dos tecidos conjuntivos é formada por diversos tipos de proteínas que definem sua estrutura e funções fisiológicas. As suas características biofísicas são definidas pela disposição supramolecular de elementos fibrilares, redes microfibrilares, como também de proteínas, glicoproteínas e uma grande variedade de outras moléculas solúveis. Entre os diferentes tipos de tecido conjuntivo podemos encontrar variações na sua composição e estrutura. Quanto ao

conteúdo proteico, as proteínas mais abundantes na matriz extracelular pertencem à família do colágeno, proteínas com estrutura molecular característica e que contribuem para a sustentação extracelular (Gelse et al., 2003).

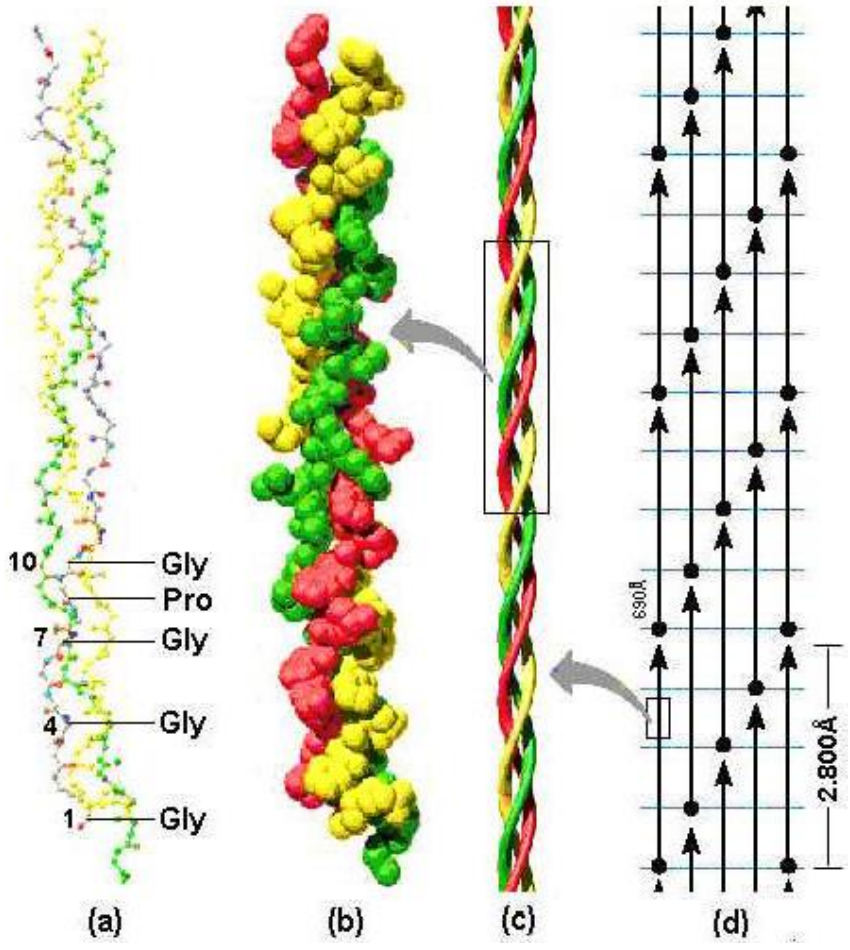
Proteína de origem animal de maior ocorrência chegando a perfazer cerca de 30% do conteúdo proteico total, em humanos o colágeno representa 6% do seu peso total (Tonhi & Plepis, 2002). Principal elemento estrutural de composições corporais como ossos, cartilagens, pele, tendões, ligamentos, vasos sanguíneos, córneas, dentes e demais órgãos (Senaratne et al., 2006), o colágeno se caracteriza por formar fibras insolúveis e elásticas, que modulam forças externas e internas exercidas dentro do organismo. Mas seu papel não se restringe apenas ao de um elemento estrutural, pois o colágeno é também uma proteína capaz de orientar tecidos em desenvolvimento (Kubota 1997). Conclui-se que essa proteína é um importante elemento estrutural de todos os tecidos conectivos e está presente em praticamente todos os tecidos intersticiais e todos os órgãos parenquimatosos (Gelse et al., 2003).

A estrutura molecular do colágeno se caracteriza por ser constituído de três cadeias alfa com polipeptídeos ligados entre si formando uma tripla hélice que é a unidade básica dessa proteína e denomina-se tropocolágeno. Fibras ligadas por pontes de hidrogênio que ocorrem entre grupos -NH de glicina e grupos carbonila C=O de resíduos localizados em outra cadeia polipeptídica ou pontes de hidrogênio com moléculas de água são responsáveis por manter consolidada a estrutura em tripla hélice. Outra característica estrutural inerente a esta proteína trata-se da repetição do arcabouço, (Gli-X-Y)n, caracterizando um motivo comum a todos os tipos de colágeno. Além da glicina este arranjo costuma ter presente prolina e hidroxiprolina ocupando as posições X e Y (Figueiró, 2002; Gelse et al., 2003). A estrutura em tripla hélice tem sua importância ligada a funções celulares como aderência e ativação da matriz extracelular, assim como também a funções enzimáticas como a hidroxilação dos resíduos lisina e prolina do colágeno (Fields, 1995).

O arcabouço em tripla hélice do colágeno é uma estrutura altamente conservada que se encontra presente em todas as variações dessa proteína. Mas em contraste com este fato, é possível evidenciar a presença de domínios não colagenosos entre os diferentes tipos de colágeno existente caracterizando uma diversidade estrutural e funcional as diferentes variações da proteína. Essa variedade contribui com a geração de estruturas que exigem a ação de enzimas específicas (colagenases) para a clivagem proteolítica. Um arranjo em tripla hélice com interrupções, por exemplo, contribui para que essa situação ocorra. As triplas hélices nativas são resistentes a ação de proteases como pepsina, tripsina e quimotripsina, sendo degradadas pelos diferentes tipos de colagenases específicas (Bruckner & Prockop, 1981; Goldberg, 1986).

Na literatura são relatados 29 tipos de colágeno classificados de I-XXIX que diferem de forma considerável quanto à estrutura, sequência e função. Esses diferentes tipos de colágeno são caracterizados por possuírem considerável complexidade, diversidade estrutural e variações na ocorrência de domínios não helicoidais. Os tipos I, II, III, V e XI aparecem com maior frequência, perfazendo cerca de 90% do conteúdo total, e são os colágenos formadores de fibrilas. O tipo I é o mais abundante e se faz presente em todos os tecidos conectivos, incluindo pele e ossos, conferindo resistência mecânica aos tecidos e órgãos e auxiliando na regulação do meio celular. Quanto à estrutura, o colágeno tipo I trata-se de um heteropolímero formado por dois tipo de cadeias α, duas α_1 e uma α_2, em que a glicina constitui um terço do seu conteúdo de aminoácidos e possui baixos níveis de tirosina e histidina (McCormick, 2009; Ottani et al., 2004; Bailey et al.,1998; Ikoma et al., 1998; Muyonga et al., 2004). A figura 1 esquematiza as características estruturais comuns a todos os tipos de colágeno além de mostrar seus possíveis arranjos. Sua via biossintética é constituída por oito enzimas específicas (Myllyharju & Kivirikko, 2001).

Figura 1: Estrutura do colágeno: (a) forma de tríplete presente nas matrizes colagênicas; (b) tropocolágeno; (c) hélice tripla; (d) modelo do quarto alternado pentafibrilar proposto por Smith 7,14.



Fonte: http://www.freedom.inf.br/artigo_tecnicos/21082007/biomateriais.asp

Geralmente os vários tipos de colágeno estão relacionados aos aspectos biomecânicos, mas além dessa função básica, essas proteínas possuem outras atribuições. Podendo atuar na sinalização celular; contribuir no armazenamento local de fatores de crescimento e citocinas, tendo assim um papel fundamental no desenvolvimento de órgãos, envolvimento em processos de cicatrização e reparo de tecidos (Yamaguchi & Ruoslathi, 1990). Essa capacidade de se vincular a fatores de crescimento e citocinas credencia estas moléculas como veículos de transporte com potencial para fins terapêuticos e farmacológicos como entrega de fatores (Gelse et al., 2003).

O colágeno responde de forma sensível a variações de temperatura, fato que o caracteriza como uma proteína termoinstável. A sua estrutura química está ligada a esta sensibilidade, sendo o conteúdo de hidroxiprolina um fator determinante da sua estabilidade térmica. O conteúdo de hidroxiprolina e a estabilidade térmica são fatores de uma equação em que o primeiro é diretamente proporcional ao segundo, pois quanto maior é a presença de hidroxiprolina, uma maior estabilidade térmica é conferida ao colágeno. Isso se deve ao fato deste aminoácido atuar formando ligações intercadeias por meio de pontes de hidrogênio tendo como resultado a estabilização da estrutura em tripla hélice (Gudmundsson & Hafsteinsson, 1997).

Propriedades como biodegradabilidade, baixa antigenicidade e ampla capacidade de adesão celular credenciam o colágeno tipo I como um importante biomaterial que é amplamente utilizado nas indústrias de cosméticos, alimentos, farmacêutica, cultura de células, produção de gelatina fotográfica, indústria de couro, síntese de filmes para embalar alimentos e nas áreas de engenharia biomédica e tecidos (Ikoma et al., 2003; Liu et al., 2010). Na área farmacêutica o colágeno é utilizado na fabricação de implantes vítreos, carreadores de drogas, suporte para enzimas, produção de compostos biologicamente ativos. Na área médica essa proteína pode ser aplicada no tratamento de doenças angiogênicas, hipertensão, incontinência urinária e osteoartrite (Zhang et al., 2006). Dentre os biopolímeros, o colágeno é o material de origem animal mais abundante e fornecedor de uma ótima base para biomateriais. Depois de extraído, o colágeno pode ser processado para obtenção de filmes, membranas e fibras. Quanto à produção de filmes o colágeno é o material, de natureza proteica, mais empregado. Sua conversão em gelatina envolve a hidrólise catalisada por ácido, base ou aquecimento. Isso se deve ao fato da abundância dessa matéria prima que tem um baixo custo e possui excelentes propriedades funcionais (Poppe, 1997; Carvalho & Grosso, 2006; Kokoszka et al., 2010).

Recentemente tem se desenvolvido materiais biológicos que combinam diferentes biopolímeros e materiais inorgânicos ao colágeno e a outros compostos biológicos, como a quitosana, isso visando gerar compostos que cumpram requisitos funcionais específicos. Filmes

compostos por proteínas apresentam excelente adequação mecânica, mas se caracterizam por serem sensíveis a umidade representando uma barreira ineficaz em ambientes úmidos (Guilbert et al., 1996). Filmes compostos por lipídeos apresentam maior resistência à umidade, mas em geral são opacos, rígidos e vulneráveis a oxidação. Por esta razão é que nos últimos anos a concepção de biomateriais prima por combinar diferentes biopolímeros (Lee et al., 2004; Li et al., 2006; Longares et al., 2005).

Para obtenção do colágeno com certo grau de pureza são utilizadas várias etapas de pré-tratamento visando remover proteínas solúveis. Sua remoção dos tecidos pode ser feita com o uso de ácidos orgânicos (acido cítrico, por exemplo) para fazer o processo de extração ou com ácidos inorgânicos (HCL) (Sadowska et al., 2003; Skierka & Sadowska, 2007). Devido ao seu rendimento de extração o ácido acético é o mais utilizado nestes processos. Entretanto, as ligações covalentes cruzadas que aparecem nas regiões telopeptídicas e as ligações cruzadas intermoleculares não são solubilizadas pelo ácido acético sendo então necessário o uso da pepsina que é capaz de clivar essas ligações sem comprometer a integridade da estrutura em tripla hélice do colágeno. Outros fatores como espécie alvo da extração, idade e parâmetros adotados para a extração terão uma influência direta no seu rendimento (Jongjareonrak et al., 2005; Nalinanon et al., 2007).

Pré-tratamentos químicos, enzimáticos e mecânicos podem aumentar o rendimento de extração do colágeno (Skierka & Sadowska, 2007). Um exemplo disso é o uso de enzimas proteolíticas não específicas que atuam removendo os telopeptídeos do colágeno, permitindo a clivagem das ligações intermoleculares (Bailey & Light, 1989; Nishihara, 1962; Hickman et al., 2000).

Um dos produtos obtidos a partir do colágeno que é o mais comercializado trata-se da gelatina. Em 2007 a produção mundial de gelatina foi de 326.000 toneladas. Grande parte das fontes deste material tem como matriz tecidos e órgãos de animais como: pele de porco (46%), couro bovino (29,4%) e ossos desses mesmos animais que correspondem a 23,1% da produção (GME Market, 2007). No entanto a existência de zoonoses além das barreiras religiosas existentes em grupos como Judaísmo e Islamismo ao uso de colágeno de porco (Zhang et al., 2007) e alguns grupos cristãos como os Adventistas que impõem barreiras a qualquer produto de origem suína; representam restrições que têm impulsionado diversos estudos numa busca por fontes alternativas de colágeno.

Neste cenário, animais como os peixes tem angariado cada vez mais a atenção como uma possível fonte de colágeno capaz de atender as demandas da indústria, pois cerca de 30% dos resíduos de filetagem gerados pela indústria pesqueira correspondem à pele e ossos, tecidos ricos em colágeno (Muyonga et al., 2004). Além disso, esses animais apresentam vantagens como a

ausência de restrições religiosas, grande disponibilidade, baixos riscos de transmissão de doenças, elevados rendimentos de extração e ausência de toxicidade (Senaratne et al., 2006). Em 2007 a produção de gelatina oriunda de peixe equivalia a 1,5% da produção total de gelatina. Mesmo sendo um percentual pequeno, quando comparado à produção a partir de animais terrestres, esse número já representa um aumento de 100% em comparação aos dados de mercado obtidos em 2002. Isso indica que a produção de gelatina a partir de fontes alternativas aos mamíferos terrestres está crescendo em importância (GME Market, 2007).

O colágeno encontrado na maioria dos peixes possui um peso molecular de aproximadamente 95.000 Da podendo ser evidenciado em SDS-PAGE (Kubo & Takagi, 1984). O colágeno de peixes costuma apresentar uma variação no conteúdo de aminoácidos, em especial nos níveis de prolina e hidroxiprolina, essa variação ocorre de espécie para espécie (Gudmundsson & Hafsteinsson, 1997). Para extrair o colágeno a partir dos tecidos de peixes, pode-se fazer o uso de ácidos orgânicos e inorgânicos, além do uso de extrações enzimáticas. Estudos com diferentes animais marinhos têm encontrado ampla variação no rendimento de extração. Kittiphattanabawon et al., 2005 obtiveram um rendimento de 1,59% para *Priacanthus tayenus*, enquanto que Wang et al., 2007 encontrou um rendimento de 92,2% para *Sebastes mentella*.

2.2 Produção mundial e nacional de produtos pesqueiros

A produção mundial de pescado, proveniente tanto de atividades extrativas como da aquicultura, alcançou a cifra de 148 milhões de toneladas no ano de 2010, garantindo um ganho de 217, bilhões de dólares americanos. Desse valor cerca de 128 milhões de toneladas foram usadas para consumo humano, sendo o restante utilizado na geração de produtos não alimentícios como farinha e óleos. Nas últimas cinco décadas, devido a melhorias nos canais de distribuição, tem se evidenciado um crescimento do abastecimento mundial do mercado pesqueiro a uma taxa de 3,2 % ao ano no período 1961-2009, ultrapassando a taxa de crescimento da população mundial (1,7% ao ano). Ainda durante o ano de 2010 cerca de 45,9% da produção mundial passou por processamento (FAO, 2012). A sustentabilidade da atividade depende da observação de cuidados que evitem a repetição de erros que levarão a diversos danos ambientais (Abdallah, 1998).

O processamento de peixes gera uma grande quantidade de resíduos líquidos (águas residuais) e sólidos (pele, ossos, vísceras e nadadeiras). Esses em geral são descartados no meio ambiente sem nenhum tratamento causando poluição ambiental (Gildberg, 1992). A quantidade de insumos produzidos a partir do processamento de peixes tende a aumentar anualmente (Shahide,

1994). Aplicar esses resíduos na produção de materiais como o colágeno, sulfato de condroitina e gelatina tem significativos benefícios ambientais (Woo et al., 2008).

Nas duas últimas décadas tem se observado um crescente interesse nos aspectos econômicos, sociais e ambientais do gerenciamento da atividade pesqueira além de agregar valor e comercializar estes insumos gerados pela indústria durante o processamento de peixes. A utilização de resíduos pesqueiros tornou-se um importante setor da industrial em muitos países, sendo a sua manipulação uma atividade que gera cada vez mais interesse. Além da obtenção de farinha de peixe esses resíduos são utilizados com outros propósitos. A partir de resíduos de crustáceos, por exemplo, pode-se obter astaxantinas, quitosana e carotenoides. Colágeno pode ser isolado a partir da pele e escamas, descartados durante o processamento de peixes (FAO 2012).

O setor pesqueiro representa um segmento importante para a economia brasileira e existe um grande interesse na exploração dos recursos marinhos (Diegues, 2006). No ano de 2009 o Brasil foi o 18° maior produtor mundial de pescado com uma produção de 1.240.813 toneladas, sendo 825.164 toneladas provenientes da pesca extrativa e 415.649 toneladas provenientes das atividades de cultivo. Em 2010 a Região Nordeste foi mais uma vez a que apresentou a maior produção de pescado do país, com 410.532 toneladas, respondendo por 32,5% da produção nacional. O estado de Pernambuco apresenta-se neste cenário como sendo o 15° maior produtor a nível nacional (MPA 2010).

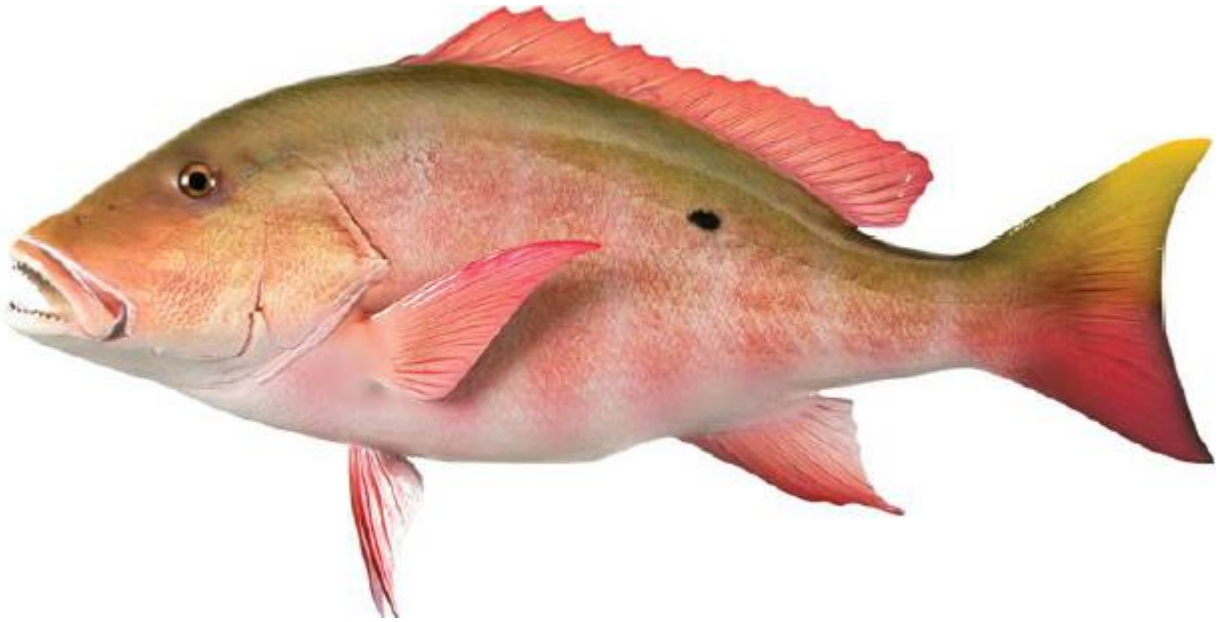
2.3 Cioba

A espécie conhecida popularmente como cioba (*Lutjanus analis*) trata-se de um peixe demersal da família Lutjanidae pertencente à ordem Perciforme que é encontrada principalmente em águas costeiras de mares tropicais e subtropicais. Morfologicamente se caracteriza por possuir uma nadadeira dorsal com 10 espinhos e 14 raios, além de ter uma mancha preta lateral abaixo dos primeiros raios da nadadeira dorsal e características listras, de cor azul clara, ocorrendo abaixo dos olhos (Menezes & Figueiredo, 1980), como mostra a Figura 2. Os indivíduos vivem próximos aos recifes e fundos rochosos, sendo encontrados em profundidades de até 650 metros (Resende et al., 2003). Os representantes adultos desta espécie formam grandes cardumes próximos às rochas, sendo possível encontrá-los em águas profundas da plataforma continental interna e externa. Os mais jovens se agrupam em águas litorâneas mais rasas, aderidas aos arrecifes (Manooch & Drennon, 1987; Fredou & Ferreira, 2005). Sua alimentação baseia-se no consumo de peixes, camarões, caranguejos, cefalópodes e gastrópodes (Froese & Pauly, 2006).

Quanto à ocorrência, os membros da espécie *L. analis*, são encontrados ocupando um área que se estende desde o litoral do estado de Massachusetts, Estados Unidos, até a regiões litorâneas do sudeste brasileiro, sendo mais abundante no entorno das Antilhas, Bahamas e sul da Flórida (Acero & Garzón, 1985; Allen, 1985; Cervigón et al, 1992).

Peixes ocorrentes em áreas de recife da família Lutjanidae são importantes para a pesca em diversas regiões do mundo. No Brasil o gênero Lutjanus inclui mais de 70 espécies (Starck & Shroeder, 1971). Esses peixes têm sido capturados de forma intensa pela atividade pesqueira realizada na região Nordeste do Brasil (Begossi et al., 2011; Frédou et al., 2009).

Figura 2: Exemplar de Cioba L. analis que apresenta as principais características relatadas na literatura.



Fonte: http://graytaxidermy.com/mutton-snapper-fish-mount.html

Os peixes identificados como *L. analis* caracterizam-se por serem bastante biodisponíveis, devido ao fato de estarem presentes em diferentes faixas de profundidade, e também por apresentarem intensa migração vertical devido a fatores bióticos e abióticos (Paiva, 1997). Dentre os peixes demersais a cioba está entre os seis principais peixes provindos do desembarque pesqueiro constituindo 75% do conteúdo junto a peixes como saramunete, budião, sapuruna, boca-torta e guarajuba (Lessa et al., 2011). No ano de 2005 o estado de Pernambuco contribuiu com 283,5 toneladas de cioba arrecadando um valor total em torna de R$ 2.055.375,00 (IBAMA, 2007), demonstrando que as atividades relacionadas à produção deste peixe são atrativas e rentáveis.

3 OBJETIVO

3.1 Objetivo Geral

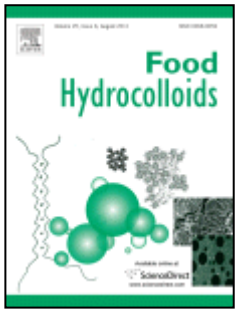
Extrair o colágeno obtido a partir das escamas do peixe Cioba (*Lutjanus analis*), e investigar sua aplicação, assim como sugerir sua utilização como fonte alternativa ao colágeno animal.

3.2 Objetivos Específicos

- Extrair colágeno ácido solúvel (ASC) e pepsino solúvel (PSC) a partir das escamas do peixe cioba (*L. analis*);
- Determinar o rendimento da extração do colágeno ASC e PSC;
- Caracterizar parâmetros de extração e solubilidade do colágeno ASC e PSC;
- Determinar o peso molecular aparente das amostras obtidas de *L. analis* através de SDS-PAGE;
- Caracterizar a temperatura máxima de transição (T_{max}) das amostras de colágeno extraídos;

4 ARTIGO CIENTÍFICO

Isolation and characterization of acid soluble and pepsin soluble collagen obtained from the scales of mutton snapper (*Lutjanus analis*).



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Isolation and characterization of acid soluble and pepsin soluble collagen obtained from the scales of mutton snapper (*Lutjanus analis*).

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Abstract

Acid soluble collagen (ASC) and pepsin soluble collagen (PSC) from mutton snapper (*Lutjanus analis*) scales were isolated and characterized. The yield of ASC and PSC were 3.5% and 6.5% (dry weight), respectively. According to the electrophoretic patterns, both the ASC and PSC consisted of two α1 and one α2 chains, well as β and γ chains, and were characterized to be type I collagen. The PSC had a lower content of high-molecular weight cross-links than ASC. The collagens ultraviolet (UV) absorption spectrum showed that the distinct absorption was between 239 and 236 nm. The collagen PSC was soluble in the pH range 2 – 6, with maximum relative solubility at pH 3, while collagen ASC was soluble in the pH range 1 – 6, with maximum relative solubility at pH 1. Both collagens were soluble in the range of NaCl concentration 0 – 4% (w/v). The transition temperatures (T_{max}) for ASC and PSC were 76°C and 83°C, respectively. The results obtained in this study indicate the possibility of using mutton snapper scales as a source of biomolecules with great potential for biotechnological and industrial application.

Keywords: mutton snapper (*Lutjanus analis*), fish collagen, scales, fisheries by-products.

1. Introduction

Structural protein of animal origin common to the vertebrates, the collagen is equal to 30% of the total content of proteins (Muyonga et al., 2004). That protein of fibrous characteristic possesses the capacity to form insoluble fibers with high resistance the traction. Basically that protein has a structure marked by presenting three chains polypeptide tied forming a triple helix amongst themselves and for possessing a motif, $(Gly\text{-}X\text{-}Y)_n$, common to the twenty-nine types of collagen and that is repeated along the chain. The positions X and Y are frequently busy for proline and hydroxyproline (Gelse et al., 2003; McCormick, 2009). Main structural element of bones, cartilages, skin, tendons, ligaments, blood vessels, teeth, horny and other present structures in the vertebrates (Seneratne et al., 2006).

Collagen type I is the most recurrent, being found in all connective tissues, it possesses the function of checking mechanical resistance to the tissues and assist in regulating the cellular environment (Nagai et al., 2008; Ikoma et al., 2003; Muyonga et al., 2004).

Widely used in food, cosmetics and pharmaceuticals industry, the collagen possesses properties as biocompatibility, biodegradability and low antigenicity. Presently their main source are the byproducts of land animals like cows, pigs and birds (Liu et al., 2009). However recurrent cases of zoonosis such as avian and bovine sponge encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), foot-and-mouth disease (FMD) and avian influenza, have led to the search for alternative and safer this compound an attractive investment (Zhang et al., 2007).

In this scenario, the fish has emerged as an alternative source of collagen front to the use of terrestrial animals. That feels due to advantages as great readiness; low risks of transmitting diseases; high income in the extraction processes; toxicity absence (Senaratne et al., 2006). The number of studies with the objective of extracting collagen from sea fish has been growing continually. In the last years several works have been telling extractions well happened starting from skin, bones and fish scales as *Priacanthus tayenus* and *Priacanthus macracanthus* (Benjakul et al., 2009); *Pagrus major* and *Oreochromis niloticus* (Ikoma et al., 2003); *Lates niloticus* (Muyonga et al., 2004); *Sepia pharaonis* (Aewsiri et al., 2009). Collagen is the major protein constituent of bones, fins, skin and scales present in fish. Due to the large evolutionary distance between fish and humans, the risk of disease transmission by this route becomes very low (Song et al., 2006).

The fish processing generates a lot of waste as skin, bones, fins, scales and guts, which are discarded into the environment without treatment, causing serious problems related to the pollution. For possessing an onerous sanitary processing for the fishing industry, the utilization of these

residues in obtaining biomolecules has been reported as a means of utilization of this material (Gildeberg, 1992).

Accordance to MPA (2010), Brazil produced approximately 1,264,765 tons, being among the twenty larger producing of fish of the world. The mutton snapper (*Lutjanus analis*) is a demersal fish species of the family Lutjanidae that inhabits coastal waters of tropical and subtropical seas, near reefs and rocky bottoms at depths of up to 650 meters (Resende et al., 2003). The adults form big shoals adhered to rocks, occurring in coastal waters shallower attached to the reefs (Fredou & Ferreira, 2005). Because of its bioavailability, the mutton snapper has a significant value to the fish trade in the regions of Northeast Brazil (Resende et al., 2003). Its production is quite cost effective, and in 2010 reached the mark of 2987 tons (MPA, 2010).

The aim of this study was to isolate and extract the acid soluble (ASC) and pepsin soluble (PSC) collagen from the scales of mutton snapper as well as characterize and suggest its use as an alternative source of collagen to land animals.

2. Materials and methods

2.1 Collection and storage of samples

The mutton snapper (*Lutjanus analis*) scales were obtained from the local fishing industry (Terra & Mar Pescados). After collecting, the material were washed with distilled water and stored in polyethylene bags at -20°C prior to collagen extraction.

2.2 Chemicals reagents

All reagents were of analytical grade. Type I collagen from calf skin was purchased from Sigma chemical company (St. Louis, MO, USA).

2.3 Preparation of collagen from scale

The collagens were prepared by the method of Nagai and Suzuki (2000) with a slight modification. All the preparation procedures were performed at 4 ° C.

Fish scales were extracted with 0.1 M NaOH for 12 hours at a sample alkali solution ratio of 1:10 (w/v) to remove non-collagenous proteins, washed fully with cold distilled water. The scales were decalcified with 0.5 M EDTA–2Na (pH 7.5) at sample/EDTA solution ratio of 1:10 (w/v) for 12 hours, and then washed with cold distilled water. The residue was extracted with 0.5 M acetic acid at sample/acid ratio of 1:20 (w/v) for 3 days. The resulting viscous solution was centrifuged at

20,000g for 20 min at 4 °C. The supernatants of the two extracts were combined and salted-out by adding NaCl to give a final concentration of 0.9 M, followed by precipitation of the collagen by the addition of NaCl to the final concentration of 2.5 M in 1.5 M Tris–HCl (pH 7.5). After standing overnight, the resulting precipitate was collected by centrifuging at 20,000g for 60 min and then dissolved in 10 volumes of 0.5 M acetic acid. The solution obtained was dialyzed against 0.1 M acetic acid. Subsequently, the solution was dialyzed against distilled water with changes of water until a neutral pH was obtained. The dialysate was freeze-dried and referred to as acid soluble collagen (ASC). The pepsin soluble collagen (PSC) was obtained through the incubation of the insoluble material obtained in the previous steps with (1:6, w/v) commercial pepsin (EC 3.4.23.1; crystallized and lyophilized, Sigma, MO) with constant homogenization for 24 hours at 4°C. The pepsin-solubilized collagen (PSC) was obtained by the same method as the ASC.

The yield of ASC or PSC was calculated as: Yield (%) = (M/M_0) x 100, where M is the weight of lyophilized collagen (g), and M_0 is the weight of drought scale used (g).

2.4 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS–PAGE was carried out according to the Laemmli (1970), using a 4% (w/v) stacking gel and a 7.5% (w/v) separating gel. The samples of ASC and PSC (25 μg of protein) were mixed with 5% (w/v) SDS and heated in a bath (IKA® Works Inc., China) at 85°C for 1 h and were loaded on to the polyacrylamide gel and subjected to electrophoresis at a constant current of 15 mA/ gel, using a vertical electrophoresis system (Vertical Electrophoresis System Bio-Rad 143 Laboratories, Inc.). After electrophoresis, the gel was stained with 0.05% (w/v) Coomassie Blue R- 250 in 15% (v/v) methanol and 5% (v/v) acetic acid for 10 min and destained with the mixture of 30% (v/v) methanol and 10% (v/v) acetic acid for 12 h. Type I collagen from calf skin (Sigma-Aldrich Co., St. Louis, MO) was also prepared following similar procedure and 10 μl were loaded as standard collagen. High-molecular-weight protein markers (GE Healthcare UK Limited, Buckinghamshire, UK) were used to estimate the molecular weight of proteins.

2.5 UV absorption spectrum

UV absorption spectra of ASC and PSC from mutton snapper (*Lutjanus analis*) scales was carried out according to Zeng et al. (2012), using a SmartSpec Plus spectrophotometer Bio-Rad. The collagen samples were dissolved in 0.5 M acetic acid solution with a sample/solution ratio of 1:1000 (w/v). The solutions were then placed into a quartz cell with a path length of 1 mm. UV spectra were measured at wavelength 200 – 600 nm.

2.6 Solubility

The solubilities of collagen samples were determined by the method of Montero, Jiménez-Colmenero, and Borderias (1991) with a slight modification. All samples were dissolved in 0.5 M acetic acid to obtain a final concentration of 3 mg/ml and the mixtures were stirred at 4 °C for 24 h. Thereafter, all the mixtures were centrifuged at 20,000 g for 60 min at 4 °C, and the supernatants were used for solubility study.

2.6.1 Effect of pH on solubility

Sample solution (0.8 ml) was transferred to a 1.5 ml centrifuge tube and the pH was adjusted with either 6 M NaOH or 6 M HCl to obtain the final pH ranging from 1 to 12. The volume of solution was made up to 1 ml by deionized water previously adjusted to the same pH as the sample solution. The solution was centrifuged at 20,000 g for 60 min at 4 °C. For all the samples, protein content in the supernatant was measured. Then the relative solubility was calculated in the comparison with that of be obtained at the pH giving the highest solubility.

2.6.2 Effect of NaCl on solubility

Sample solution (0.5 ml) was mixed with 0.5 ml of NaCl in 0.5 M acetic acid at various concentrations to give the final concentrations of 0%,1%, 2%, 3%, 4%, 5% and 6% (w/v). The mixture was stirred continuously at 4 °C for 60 min, followed by centrifuging at 20,000 g for 60 min at 4 °C. Protein content in the supernatant was measured and the relative solubility was calculated as previously described.

2.7 Thermal transition measurement

Differential scanning calorimetry (DSC) of ASC and PSC was conducted using a Differential Scanning Calorimeter DSC-60 (Shimadzu Corporation, Chiyoda-ku, Tokyo, Japan) to analyze the thermal stability of collagen. The lyophilized collagen samples were rehydrated in a 0.05 M acetic acid solution with a sample/solution ratio of 1:40 (w/v). The mixtures were allowed to stand for 2 days at 4°C. The rehydrated samples (2 mg) were accurately weighed into aluminum pans, sealed, and scanned over a range of 25 – 100°C at a heating rate of 1°C/min, with a nitrogen gas with flow rate of 40 mL/min. A pan with 2 mg alumina was used as a reference. The collagens were applied to the DSC, and the maximum temperatures (T_{max}) were calculated from the triplicate samples.

2.8 Statistical analysis

All experiments were performed in triplicate and expressed in means ± standard deviation and a probability value of <0.05 was considered significant. The mean values were evaluated by analysis of variance (ANOVA) followed by Tukey test. The analyzes were performed using the statistical program MicroCal® Origin® Version 8.0 (MicroCal, Northampton, MA, USA).

3. Results and discussion

3.1 Extraction yield of collagen ASC and PSC

The total yield of collagen extracted from the flakes of Cioba was 10% dry weight, and 3.15% ASC and 6.85% of PSC. This result shows that collagen present in the flakes, as well as in other tissues, is not completely solubilised in 0.5 M acetic acid. The fact that the PSC showed a greater extraction yield, when compared to ASC, can be due to possibility of the collagen molecules in the L. analis scale are linked through crossed covalent bonds that they happen through the condensation of groups aldehydes in the telopeptides areas and intermolecular of the collagen fact that causes a decrease in solubility of the protein in acetic acid (Foegeding et al, 1996,.. Zhang et al, 2007). When submitting the residue of the acid acetic extraction to the pepsin action, the material obtained is a result of cleavage of the telopeptide regions and crossed covalent bonds (Balian & Bowes, 1977).

In other works were found the following total yields for the collagen extraction from fish scales: *Hypophthalmichthys molitrix* (1,45%) (Zhang, Duan Ye, e Konno, 2010), *Cololabis saira* (15%) (Mori et al., 2012), *Hypophthalmichthys nobilis* (2,7%) (Liu et al., 2012); from fish skin: *Lutjanus lutjanus* (10,9%) (Kittiphattanabawon et al., 2005), *Aluterus monóceros* (7,6%) (Ahmad & Benjakul, 2010), *Priacanthus tayenus* e *Priancanthus macracanthus* (7,7% e 7,1%) (Benjakul et al., 2010); from fish bones: *Hypophthalmichthys nobilis* (2,9%) (Liu et al., 2012), *Lutjanus lutjanus* (1,6%) (Kittiphattanabawon et al., 2005).

3.2 Gel electrophoresis

The electrophoretic profiles of ASC and PSC are shown in Figure 3. It can be observed a similar distribution of the bands observed in type I collagen obtained from bovine skin, used as standard, and in most collagen obtained from skin and fish scales related in the literature (Singh et al, 2011;. Li et al, 2013, Zeng et al .., 2012; Motowidło et al, 2008). Some subunits characteristics of type I collagen can be observed in the electrophoretic profile, as the α subunits (α1 and α2), which have molecular mass between 100 and 120 kDa, and β and γ subunits with molecular mass above 200 kDa (Foegeding et al. 1996; Kimura, 1992). In the profile found for ASC and PSC was observed that the intensity of the band α1 is larger than α2. According to Singh et al (2011), one of the characteristics observed in the electrophoretic profile of type I collagen is a 2:1 ratio in the intensity of bands α1 and α2, respectively. Moreover, when it ASC is compared with PSC can observe the presence of large amounts of crossed bond protein by the difference in size of the bands corresponding to the β chain (Sato et al., 2000) This may explain

the better extraction yield using pepsin, because the hydrolysis of the telopeptide and crosslinks regions present in the β chain increases the acid solubility of collagen (Miller, 1972).

3.3 UV-vis spectra

Figures 2 and 3 show the absorption spectrum of UV collagen obtained by the scale of *L. analis*. It can be observed that both BSA as PSC exhibited a higher absorption rate of ultraviolet rays in the range 236-239 nm. A similar result was found for the scale of the collagen Diodon Holocanthus, 210-240 nm (Huang et al., 2011); collagen of frog skin, 236nm (Li et al, 2004); Ictalurus punctaus 236nm (Liu et al, 2007), Theragra chalcogramma, 220 nm (Yan et al, 2008). The absorption maximum in the ultraviolet region to the proteins occurs at wavelengths near 280 nm (Duan et al. 2009). The absorption spectrum of ultraviolet rays can measure the amount of tyrosine and phenylalanine, in addition to being able to measure the integrity of the non-helical telopeptides (Na, 1988). The phenylalanine and tyrosine are sensitive chromophores and absorb ultraviolet rays in a range between 251 and 253nm (Liu & Liu, 2006). Most work on processes of extraction and characterization of collagen reports a small amount of these amino acids in this protein (Singh et al, 2011;. Huang et al, 2011;. Yan et al, 2008., Lin & Liu, 2006). Furthermore, according to Liu and Liu 2006 due to the characteristics of collagen, can be expressed the integrity of the non-helical telopeptides regions and to verify the presence of protein contaminants. Based on this information it may be suggested that collagenous material extracted in this work in both cases is type I collagen and has no large amount of contaminating proteins collagen solubility

3.4 Effect of pH on collagen solubility

The solubility of ASC and PSC at different pH was measured and the result is shown in figure 4. It was observed that both showed higher solubility in acidic range of pH. The maximum solubility has been obtained in the range pH 1.0 to 3.0 for both. It can be observed in both, ASC and PSC, a marked loss of solubility when the pH reaches the neutrality (pH 7.0). Futhermore, a minimum solubility was observed at pH 9.0 to ASC and pH 8.0 to PSC. When the pH of the environment in which collagen is reaches a value equal or close to the isoelectric point (pI), there is a decrease in solubility caused by a reduction in the amount of charges molecular (Vojdani, 1996). Foegeding et al., (1996) report the type I collagen pI ranging between pH6,0 and pH9,0. At this point, the net charge of the molecules constituting the protein is zero and there causes an increase in hydrophobic interactions resulting in aggregation and precipitation of protein (Singh et al., 2011). Profiles similar to collagens extracted from byproducts of other fish processing have been found in the literature (Li et al., 2013; Jongjareonrak et al., 2005, Huang et al., 2011; Nalinanon et al., 2007).

3.5 Effect of NaCl concentration on collagen solubility

In figure 5 is shown the effect of NaCl on the solubility of ASC and PSC extracted from Cioba. For ASC diluted in 0.5 M acetic acid, the protein starts to precipitate at a concentration of 1% and has a large percentage precipitate when the NaCl concentration reaches 4%. The PSC remained stable under large part of the variations in NaCl concentration, having a subtle increase in precipitation at a concentration of 3% NaCl and precipitation of most of the its content when the salt concentration achieves 5% in the solution. Once the NaCl concentration in solution reaches high values in solution, the "salt out" is triggered contributing to reduction in collagen solubility (Asghar & Henrickson, 1982). Another important factor is related to the increase in ionic strength generated by NaCl, which has as effect of reducing the solubility of proteins due to increased hydrophobic interactions between their chains and increased competition for water with ionic salts (Vojdani, 1996). Trend curves following a pattern where the ASC is more susceptible variations in the concentration of NaCl of what the PSC are commonly reported in the literature, striped catfish (Singh et al., 2011); brown striped snapper fish (Jongjareonrak et al., 2005); cobia (Zeng et al., 2012), mackerel (Li et al., 2013); fish balloon (Huang et al., 2011). The PSC can be more soluble by varying the concentration of NaCl due to hydrolysis promoted by pepsin in regions where crosslinking occurs between molecules of high molecular weight (Singh et al., 2011).

3.6 Thermal stability

Figure 6 shows the DSC patterns following for the samples of ASC and PSC of the Cioba which exhibited endothermic peaks at 76 °C and 77 °C respectively. Factors as the physiologic temperature of the fish may play an important role in determining thermal stability of collagen, but the main factor that has a direct influence on the thermal stability is the amino acid content, with the hydroxyproline being the most important amino acid, because it maintains the stability of the collagen trimers. This makes it possible to establish a linear relationship between the maximum temperature transition (T_{max}) and the hydroxyproline percentage in the total content of amino acids (Sikorski, Scott, & Buisson, 1984). In this experiment, the T_{max} found went similar for both types of collagen. Indicating that digestion by pepsin probably does not affect the structure of the collagen triple helix (Hickman et al., 2000). Comparing with other results in the literature, *Lutjanus vitta* 30.52 and 30.46 ° C (Jongjareonrak et al. 2005); *Rachycentron canadum* 38.13 and 36.03 ° C (Zeng et al. Jan 2012); *Theragra chalcogramma* 46.96 ° C (Yan et al. 2008), it was observed that T_{max} was superior to found it in most of the aquatic organisms. These results also demonstrated a collagen with Tmax higher than from terrestrial animals, Bovine skin collagen 36.3 ° C (Ogawa et al., 2003). The levels of hydroxyproline may be linked to this thermal stability (Kimura et al., 1993). In studies

like: Collagen films from swim bladders (Fernandes et al., 2008); *Stichopus japonicus* (Cui et al., 2007); characterization of collagen Sipunculida (Rong Su et al., 2009), found similar results to the present work, being that Fernandes et al., found the following T_{max}: of 65.9 ° C, 70.9 ° and 74.8 ° C for films collagen obtained from fish, Pescada Amarela, Guarijuba e Pescada Branca respectively.

4 Conclusion

Collagen ASC and PSC were successfully extracted from scales of Cioba. Both showed some differences in relation to physical and chemical standards, because showed different behaviors of precipitation while there was a variation in pH and NaCl concentration of the environment. The extraction achieved a high degree of efficiency evidenced by the income derived from the extraction of the scales, which are an abundant feedstock, and the purity of the extracted protein. The use of pepsin proved useful due to the yield achieved and also to degree of preservation of structures, proving it caused little changes in collagen. With the results obtained both in electrophoresis and in the test with ultraviolet, we can state that collagen obtained in this process is the type I, commercially most important. Showed a high T_{max} even compared to terrestrial animals. These results show that the extraction of collagen from scales of Cioba (*L. analis*) for generating biomaterials is an attractive investment for the state of Pernambuco.

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444 **Figure captions**

445 Figure 1: SDS-PAGE patterns of acid soluble collagen (ASC) and pepsin soluble collagen (PSC)
446 from the mutton snapper scale. M, high-molecular weight markers; I, type I collagen from calf skin.

447 Figure 2: Ultraviolet spectra of acid soluble (A) and pepsin soluble collagen (B) from mutton
448 snapper scale.

449 Figure 3: Relative solubility (%) of ASC and PSC from mutton snapper scale in 0.5 M acetic acid at
450 different pHs. Bars represent the standard deviation (n = 3).

451 Figure 4: Relative solubility (%) of ASC and PSC from mutton snapper scale in 0.5 M acetic acid
452 with different NaCl concentrations. Bars represent the standard deviation (n = 3).

453 Figure 5: DSC thermogram of ASC and PSC from the mutton snapper scale dispersed in 0.05 M
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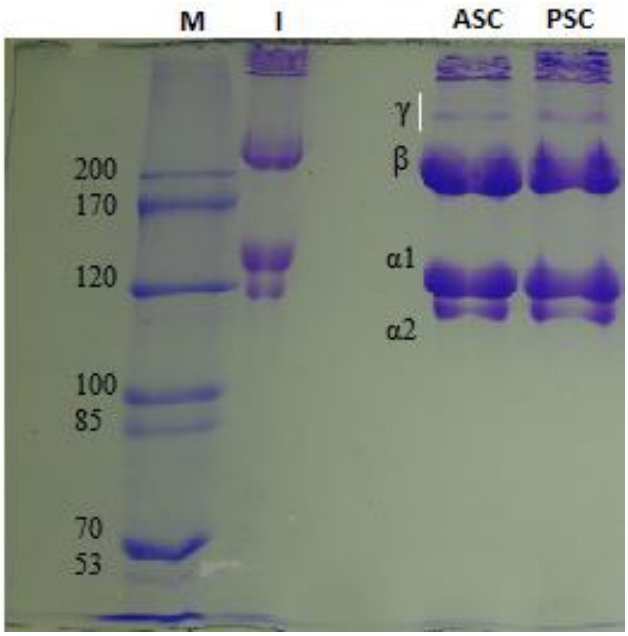


Figure 1

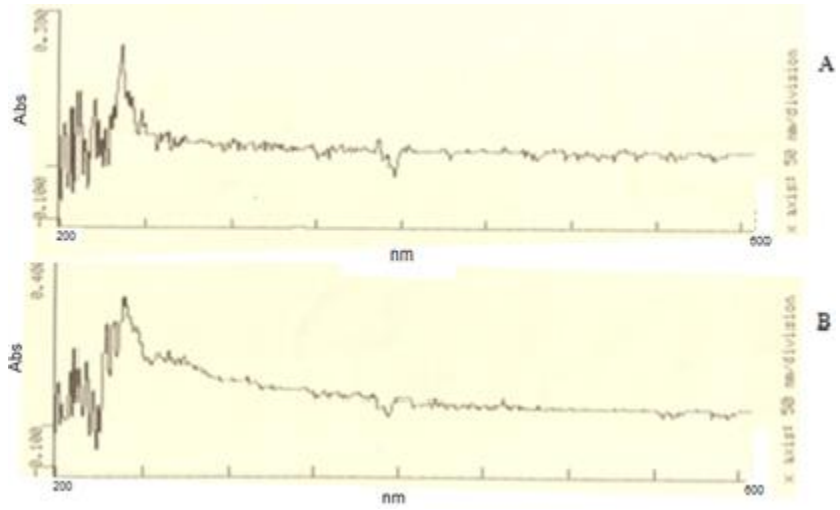


Figure 2

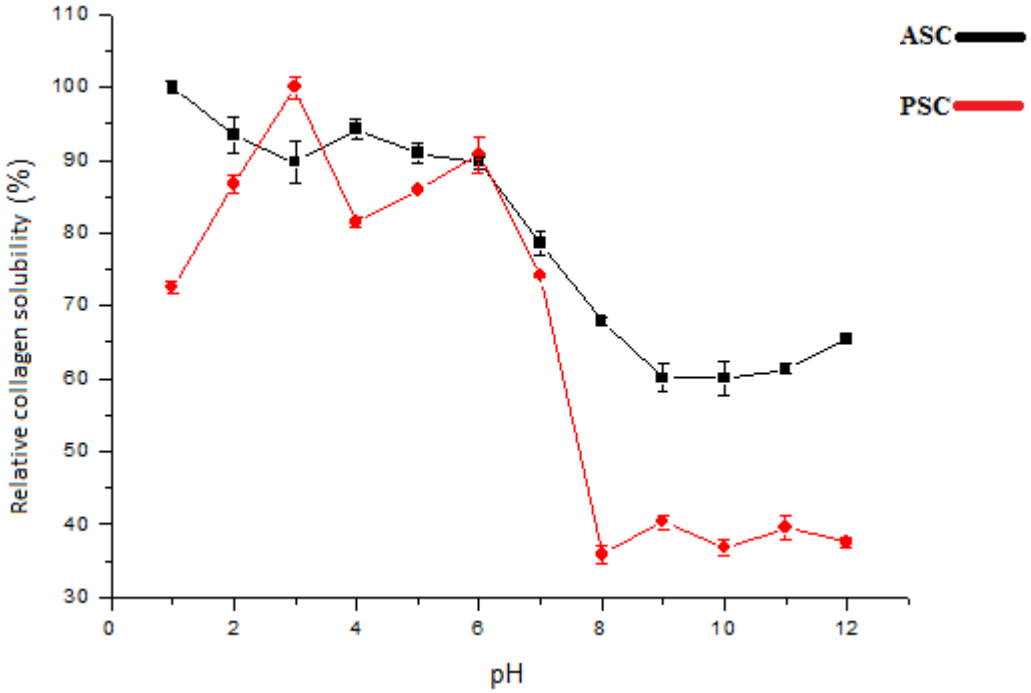


Figure 3

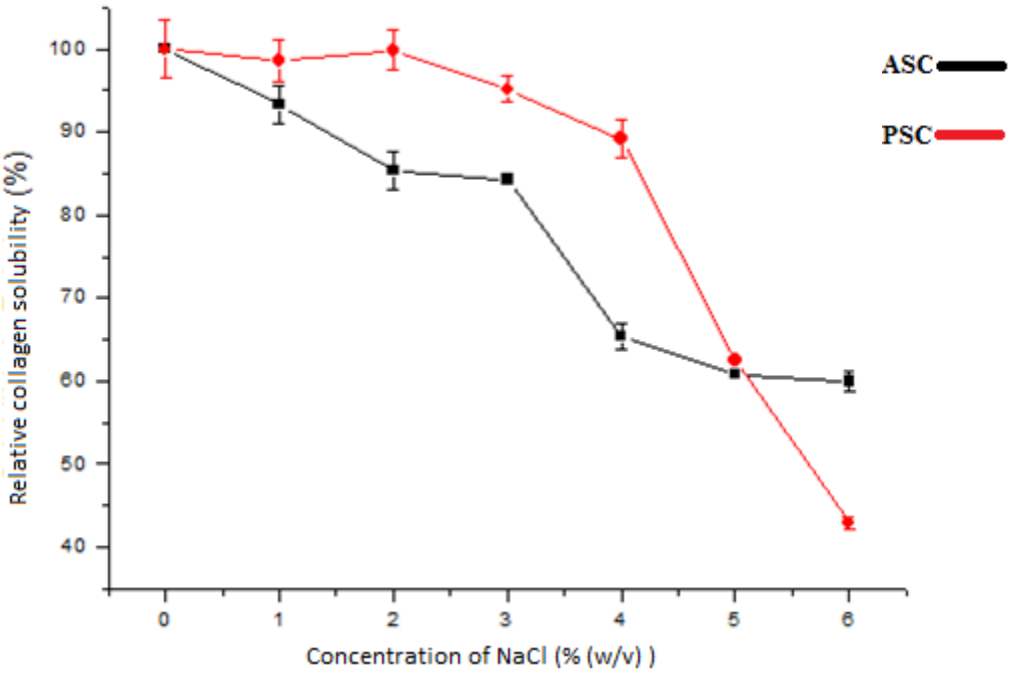
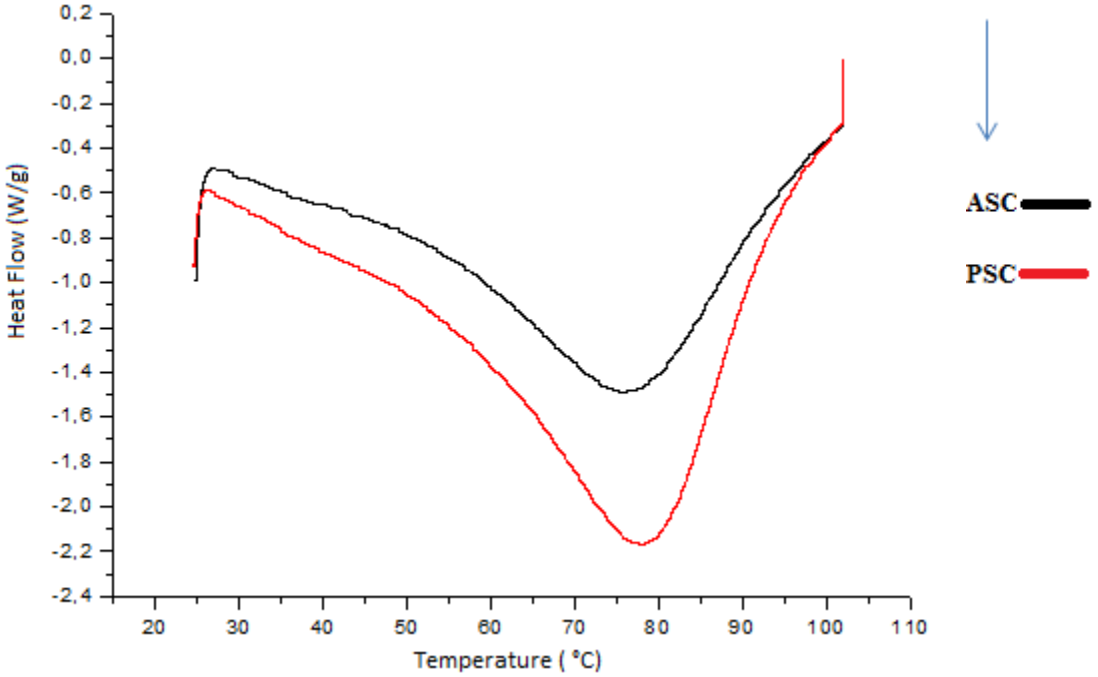


Figure 4


Heat Flow (W/g)
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Temperature (°C)
ASC
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Figure 5

5 CONCLUSÕES

Colágeno ácido solúvel e pepsino solúvel foram extraídos a partir das escamas do peixe cioba (*Lutjanus analis*) com rendimentos de 3,85% e 6,15%, respectivamente. Ambos apresentaram algumas diferenças em relação aos padrões físicos e químicos, pois tiveram comportamentos diferenciados quanto a precipitação mediante variações de pH e da concentração de NaCl.. O uso da pepsina se mostrou útil devido ao rendimento alcançado e também ao grau de preservação das estruturas, comprovando que a mesma provocou poucas alterações no colágeno. Os resultados obtidos tanto na eletroforese como no ensaio com raios ultravioleta, constata que o colágeno obtido no processo trata-se do tipo I, comercialmente mais importante. A temperatura máxima de transição (T_{max}) foi elevada mesmo quando comparada a de animais terrestres. Esses resultados mostram que a extração de colágeno a partir de escamas da cioba (*L. analis*) para a geração de biomateriais é um investimento atrativo para a região do estado de Pernambuco.

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7 ANEXOS

7.1 Normas da revista: FOOD HYDROCOLLOIDS

AUTHOR INFORMATION PACK

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Food Hydrocolloids only publishes original and novel research that is of high scientific quality. Research areas include basic and applied aspects of the characteristics, properties, functionality and use of **macromolecules** in **food systems. Hydrocolloids** in this context include **polysaccharides**, modified polysaccharides and **proteins** acting alone, or in mixture with other food components, as thickening agents, gelling agents, film formers or surface-active agents. Included within the scope of the journal are studies of real and model **food colloids** - dispersions, emulsions and foams – and the associated **physicochemical stability** phenomena - creaming, sedimentation, flocculation and coalescence.

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