UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ENGENHARIA DE ALIMENTOS

HENRIQUE DI DOMENICO ZIERO

SUBCRITICAL WATER TREATMENT OF ANIMAL PROTEIN BIOMASS FOR THE PRODUCTION AND RECOVERY OF PROTEINS AND AMINO ACIDS

TRATAMENTO EM ÁGUA SUBCRÍTICA DE BIOMASSA PROTÉICA ANIMAL PARA A PRODUÇÃO E RECUPERAÇÃO DE PROTEÍNAS E AMINOÁCIDOS

CAMPINAS
2021

HENRIQUE DI DOMENICO ZIERO

SUBCRITICAL WATER TREATMENT OF ANIMAL PROTEIN BIOMASS FOR THE PRODUCTION AND RECOVERY OF PROTEINS AND AMINO ACIDS

TRATAMENTO EM ÁGUA SUBCRÍTICA DE BIOMASSAS PROTÉICA ANIMAL PARA A PRODUÇÃO E RECUPERAÇÃO DE PROTEÍNAS E AMINOÁCIDOS

Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of master's in food engineering

Dissertação apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestre em Engnharia de Alimentos

Orientadora: Profa. Dra. Tânia Forster Carneiro
Co-orientadora: Prof. Dra. Solange Inês Mussatto Dragone

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELO ALUNO HENRIQUE DI DOMENICO ZIERO, E ORIENTADO PELA PROFA. DRA. TÂNIA FORSTER CARNEIRO

Campinas
2021

Agências de fomento e n°(s) de processo(s): Fundação Brasileira de Ciência e Pesquisa (CNPq) 302473 / 2019-0, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil - Código financeiro 001, Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (2018 / 05999-0; 2018 / 14938-4).

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

Z64t

Ziero, Henrique Di Domenico, 1996-
Tratamento em água subcrítica de biomassa proteica para produção e recuperação de proteínas e aminoácidos / Henrique Di Domenico Ziero. – Campinas, SP : [s.n.], 2021.

Orientador: Tânia Forster-Carneiro.
Coorientador: Solange Inês Mussatto Dragone.
Dissertação (mestrado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Biomassa proteica. 2. Tecnologia hidrotérmica. 3. Aminoácidos. 4. Hidrólise. 5. Água subcrítica. I. Forster-Carneiro, Tânia. II. Dragone, Solange Inês Mussatto. III. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Subcritical water treatment of animal protein biomass for the production and recovery of proteins and amino acids
Palavras-chave em inglês:
Protein biomass
Hydrotermal technology
Amino acids
Hydrolysis
Subcritical water
Área de concentração: Engenharia de Alimentos
Titulação: Mestre em Engenharia de Alimentos
Banca examinadora:
Tânia Forster-Carneiro [Orientador]
Mauro Donizeti Berni
Maria Thereza de Moraes Gomes Rosa
Data de defesa: 19-05-2021
Programa de Pós-Graduação: Engenharia de Alimentos

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0003-0449-5736
- Currículo Lattes do autor: http://lattes.cnpq.br/3456094381847985

Folha de aprovação

Profª. Drª. Tânia Forster Carneiro
ORIENTADORA – DEA/FEA/UNICAMP

Prof. Dr. Mauro Donizeti Berni
NÚCLEO INTERDISCIPLINAR DE PLANEJAMENTO ENERGÉTICO (NIPE)

Profa. Dra. Maria Thereza de Moraes Gomes Rosa
UNIVERSIDADE PRESBITERIANA MACKENZIE

Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade

Dedico esse trabalho à Larissa e minha família. Sem vocês isso jamais seria possível.

AGRADECIMENTOS

A Larissa Castro Ampese, que me suportou, me apoiou, me ensinou, me cuidou e fez dos momentos tensos mais amenos com sua presença. Seu carinho e companhia tornaram meu dia a dia mais feliz e me fazem te amar cada vez mais.

Aos meus pais Claudio Ziero e Neuza Di Domenico Ziero, que me deram todo o suporte necessário para atingir todas as metas da minha vida. Além de me ensinarem que nunca devemos desistir e sempre devemos permanecer de cabeça erguida nos momentos difíceis.

Aos meus irmãos Gabriel e Leonardo, por sempre me lembrarem que tem alguém olhando para minhas atitudes e que pode me usar como exemplo. Servindo sempre de bússula moral para eu tomar as decisões mais assertivas possível para não os decepcionar.

À Profª. Drª. Tânia Forster Carneiro, por me aceitar em seu ambiente de trabalho e permitir a conclusão do mestrado por meio de sua orientação.

À Prof. Dr. Solange Mussato, pela coorientação e pela ajuda no decorrer do curso.

Aos membros da banca, agradeço por aceitarem corrigir e melhorar o meu trabalho.

Aos meus amigos Marcos, Renan, Teresa, Billie, Rúben, Guilherme, Gabriel, Jade&José, Flávio, Kendro, Rafael, Sérgio, Michel, Renan&Luana, Douglas, Hissashi, William, Heloísa, Jonathas, Renata, Eduardo, Cristina, Guilherme&Laura: obrigada por me alegrar e por tornar a vida mais divertida.

Aos amigos e colegas da UNICAMP e à equipe do BIOTAR pelos momentos compartilhados.

Aos professores de toda a minha carreira acadêmica, por despertarem em mim a vontade de aprender cada vez mais.

Finalmente, agradeço a todos os funcionários da FEA e da UNICAMP que ajudaram direta ou indiretamente na realização deste trabalho!

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior – (Brasil) CAPES – Codigo de financiamento 001

RESUMO

Os setores da indústria avícola desempenham papéis essenciais na economia global, entretanto também geram grandes quantidades anuais de resíduo, tais como penas de aves. Uma revisão de literatura mostrou que a extração/hidrólise em água subcrítica e supercrítica a partir de resíduos proteicos podem apresentar alto potencial econômico, evidenciou as principais rotas para extração de proteínas, a influência e interações dos principais parâmetros e, também, as condições de reação. O objetivo deste trabalho foi avaliar a utilização da hidrólise em água subcrítica na valorização de penas de aves *in natura*, nas temperaturas de 210 ° C, 230 º C e 250 ° C, a 15 MPa de pressão, e vazões de 5, 7,5 e 10 mL.min^{-1}. A Metodologia de Resposta de Superfície (SFM) foi usada para identificar os aminoácidos mais expressivos. Os resultados indicam que valores de pH dos hidrolisados ficou entre 6 e 8 e mostrou-se maior que os relatados para hidrólise de biomassa. Análise de demanda química de oxigênio (DQO) mostrou ser um bom indicador da quantidade de matéria orgânica em uma solução, apresentando maiores valores nas primeiras coletas de amostras se comparados com os últimos pontos de coleta. Uma concentração de aminoácidos essenciais foi observada na temperatura 250 °C como valina, metionina, triptofano, fenilalanina, isoleucina, leucina e lisina, enquanto aminoácidos não essenciais foram obtidos nas temperaturas mais baixas e moderadas. Os resultados indicam que ácido aspártico e serina apresentaram melhor hidrólise em alto vazão e em baixa temperatura. Por outro lado, isoleucina e metionina apresentaram comportamento oposto, diferentes condições de vazão e temperatura são necessárias para obtenção de altas concentrações e pode induzir a obtenção de certos aminoácidos em detrimento de outros.

Palavras-chaves: *Biomassa proteica*, *Tecnologia Hidrotérmica, Aminoácidos, Hidrólise, Água subcrítica*

ABSTRACT

The poultry industry sectors play key roles in the global economy, but also generate large annual amounts of waste, such as poultry feathers. A literature review showed that the extraction/hydrolysis in subcritical and supercritical water from protein residues may presents high economic potential, evidenced the main routes for protein extraction, the influence and interactions of the main parameters and also the reaction conditions. The objective of this work was to evaluate the use of hydrolysis in subcritical water in the valorization of *in natura* feathers, at temperatures of 210 ° C, 230 º C and 250 ° C, at 15 MPa of pressure, and flows of 5, 7.5 and 10 $mL.min^{-1}$. The Surface Response Methodology (FMS) was used to identify the most expressive amino acids. The results indicate that pH values of hydrolyzed stayed between 6 and 8 and were higher than those reported for biomass hydrolysis. Analysis of chemical oxygen demand proved to be a good indicator of the amount of organic matter in a solution, presenting higher values in the first sample collections when compared to the last collection points. A concentration of essential amino acids was observed at 250 °C temperature such as valine, methionine, tryptophan, phenylalanine, isoleucine, leucine and lysine, while non-essential amino acids were obtained at the lowest and moderate temperatures. The results indicate that aspartic acid and serine showed better hydrolysis at high flow and at low temperature. On the other hand, isoleucine and methionine showed opposite behavior, different conditions of temperature and flow rate are necessary to obtain high concentrations and may induce obtaining certain amino acids to the detriment of others.

Keywords: *Protein biomass, Hydrothermal Technology, Amino acids, Hidrolysis, Subcritical water*

Sumário

1. CAPÍTULO 1.- Introdução geral, objetivos e estrutura da tese 11

1.1. Introdução Geral 12

1.2. Objetivos..... 16

1.2.1. *Objetivo Geral* 16

1.2.2. *Objetivos específicos* 16

1.3. Estrutura da Tese 17

2. CAPÍTULO 2.- Uma visão geral do tratamento subcrítico e supercrítico da água de diferentes biomassas para a produção e recuperação de proteínas e aminoácidos 19

3. CAPÍTULO 3.- Formação de aminoácidos na hidrólise subcrítica da água de penas de aves... 74

4. CAPÍTULO 4.- Discussões gerais 112

4.1. Discussão sobre o Capítulo 2- Uma visão geral do tratamento subcrítico e supercrítico da água de diferentes biomassas para a produção e recuperação de proteínas e aminoácidos.....110

4.2. Discussão sobre o Capítulo 3- Obtendo aminoácidos recuperados de penas de aves utilizando de hidrólise com água subcritica.....112

5. CAPÍTULO 5.- Conclusões 117

6. CAPÍTULO 6.- Referência Bibliográficas.....117

CAPÍTULO 1 - Introdução, Objetivos e Estrutura da dissertação

1. Introdução

A indústria avícola possui grande importância para o produto interno bruto (PIB) nacional e fonte de empregos e renda para diversas famílias. A indústria avícola contribui com aproximadamente 1,5% do PIB do Brasil além de gerar cerca de 3,6 milhões de empregos para a população brasileira (ABPA, 2020). Como consequência, também se trata de um mercado com alta produção de resíduos gerados no processamento das aves. Os resíduos de penas de frango representam, aproximadamente, 10% da massa do frango e, globalmente, mais de 8,5 milhão de toneladas de penas são geradas por ano (REDDY; SANTOSH, 2016a). Atualmente, a biomassa proteica não possui disposição final ambientalmente adequada em diversas partes do mundo, inclusive no Brasil. As principais formas de disposição final são: incineração, aterros e a produção de farinha de penas. Entretanto, os dois primeiros processos consideram a pena como rejeito da indústria e podem levar a danos ambientais por emissão de gases poluentes e/ou de efeito estufa e por inutilização de áreas onde o aterro se localiza. E, finalmente, possui valor nutricional baixo, devido aos processos térmicos que danificam as proteínas e aminoácidos que compõem as penas (TESFAYE; SITHOLE; RAMJUGERNATH; CHUNILALL, 2017). Com isso, se faz necessária a aplicação de tratamentos não convencionais para valorização desta grande quantidade de resíduos gerados, visando a obtenção de subprodutos com maior valor agregado e amigáveis ao meio ambiente.

A composição da pena de frango consiste em 85-90% de proteína bruta à base de queratinas α e β (PENG; MAO; ZHANG; DU *et al.*, 2020; REDDY; SANTOSH, 2016a). Essa biomassa proteica é amplamente estudada para produção de compostos com alto valor agregado como aminoácidos, compostos fenólicos, ácidos orgânicos, metano, etc., (AZMIR, J.; ZAIDUL, I. S. M.; RAHMAN, M.; SHARIF, K. *et al.*, 2013; CHENG,

HONGBIN; ZHU, XIAN; ZHU, CHAO; QIAN, JING *et al.*, 2008; MARCET; ÁLVAREZ; PAREDES; DÍAZ, 2016; SHITU, A.; IZHAR, S.; TAHIR, T., 2015). Diversas tecnologias poderiam permitir a reinserção no ciclo produtivo industrial, com ênfase nas indústrias farmacêutica, de cosméticos e de biocombustíveis (DENG; SHEN; XU; KUANG; GUO; ZENG; GAO; LIN; XIE; XIA, 2012). Atualmente, existem duas principais rotas tecnológicas para obtenção de aminoácidos a partir de penas de frango: a extração convencional (Soxhlet, métodos físicos, e moagem) (AZMIR, J.; ZAIDUL, I. S. M.; RAHMAN, M.; SHARIF, K. *et al.*, 2013; SHITU, A.; IZHAR, S.; TAHIR, T., 2015) e a não convencional, com principal ênfase na hidrólise enzimática (ALVAREZ; RENDUELES; DIAZ, 2012; ANOOTTHATO, SIRIORN; THERDTHAI, NANTAWAN; RITTHIRUANGDEJ, PITIPORN, 2019; YANG; CAI; LIU; WANG, 2019; ZHANG; JIANG; LI; YU *et al.*, 2019). Ambos os processos apresentam desvantagens quando aplicados em larga escala. A primeira rota apresenta-se limitado devido a baixa eficiência ou à geração de compostos químicos altamente poluentes e custo adicional com tratamento dos mesmos. O segundo, que utiliza enzimas capazes de catalisar reações de quebra da estrutura proteica das penas até seus blocos construtores mais básicos, apresenta também alto custo, tanto para instalação de maquinários quanto pela demanda de reagentes enzimáticos empregados no processo. Tecnologias limpas e sustentáveis de baixo custo poderiam contribuir para este mercado emergente, com processos mais eficientes para a obtenção de aminoácidos proveniente de penas de aves por exemplo.

O processo hidrotérmico é uma tecnologia promissora que consegue extrair ou hidrolisar matéria orgânica de maneira adequada, e obter subprodutos que podem ter um valor agregado para reinserção na indústria. Dentre os processos hidrotérmicos existentes, destaca-se o processo de hidrólise em água subcrítica. A água subcrítica é obtida em uma

faixa de temperatura de 100 a 374 °C com pressão maior que a atmosférica para evitar a transição da fase líquida para a fase gasosa, ou seja, a água permanece líquida a temperaturas acima do ponto de ebulição e adquire propriedades únicas pertencentes a esse estado (BRUNNER, 2005; KING, J., 2000). Nessas condições, a água se comporta como um solvente polar e apolar, sendo capaz de solubilizar compostos apolares também. Além de apresentar um alto produto iônico quando comparado a água em condição ambiente, aumentando consideravelmente sua capacidade de quebra de compostos por reações ácidas ou básicas (OKAJIMA; SAKO, 2014). O processo de hidrólise em água subcrítica da proteína poderia produzir compostos mais simples, como por exemplo os aminoácidos, através do seu principal solvente, a água.

Alguns resíduos vegetais proteicos podem ser usados como fonte de aminoácidos. O uso de farelo de arroz desengordurado, resíduos de feijão e até microalgas foram avaliados para obtenção de aminoácidos. O processamento destes alimentos gera grande quantidade de subprodutos, e diversos estudos utilizaram a hidrólise em água subcrítica para obter proteínas e aminoácidos. Também foi observado que, a quantidade de proteína reduzia com o aumento da temperatura, enquanto a quantidade de aminoácidos aumenta até certa temperatura e depois diminuiu com o aumento da mesma (SEREEWATTHANAWUT; PRAPINTIP; WATCHIRARUJI; GOTO *et al.*, 2008; ZHU; ZHU; FAN; LIU *et al.*, 2010b; ZHU; ZHU; XIAO; ZHOU *et al.*, 2015). Alguns resíduos proteicos de origem animal também podem ser usados como fontes de aminoácidos. Por exemplo, gelatina de peixe, carne de peixe, pele de atum, e colágeno foram usados para a produção de aminoácidos e obteve como resultados aminoácidos tais como, serina, glicina, alanina, arginina (UENO; ICHINOI; ZHAO; FUJII, 2015). Asaduzzaman e Chun (2015) investigaram a hidrólise dos músculos das lulas através da hidrólise subcrítica e os resultados obtidos demonstraram a maior concentração de

aminoácidos livres a 250 ºC, enquanto a maior a concentração de aminoácidos essenciais foi obtida a 220 ºC (ASADUZZAMAN, A.; CHUN, B.-S., 2015). No entanto, dada a variabilidade da composição do substrato e a outras especificidades associadas às condições de reação hidrotérmica, as vias de decomposição e a cinética de cada aminoácido são diferentes de sistema para sistema (ESTEBAN, M.; GARCÍA, A.; RAMOS, P.; MÁRQUEZ, M., 2010; KOOMYART; NAGAMIZU; KHUWIJITJARU; KOBAYASHI *et al.*, 2017b; PINTO; ANTAS; SANTOS; BOWRA *et al.*, 2017; POSMANIK; CANTERO; MALKANI; SILLS *et al.*, 2017).

Considerando o atual cenário de crescente geração de resíduos decorrentes de maiores demandas por alimentos, cresce a preocupação com os resíduos gerados, inclusive com as penas de aves. Adicionalmente, indústrias de diferentes setores já utilizam aminoácidos e, portanto, teriam interesse em obtê-los de maneira econômica e ambientalmente favoráveis. Desta forma, o presente trabalho tem como finalidade analisar os parâmetros de processos que dependem das características intrínsecas de resíduos de penas de aves para a hidrólise em água subcrítica, visando a obtenção de aminoácidos com alto valor agregado.

2. Objetivos

2.1. Objetivo Geral

O objetivo geral deste trabalho é analisar o processo de hidrólise em água subcrítica de penas de aves para produção de subprodutos com maior valor agregado, os aminoácidos.

2.2. Objetivos específicos

- Produzir um artigo de revisão com foco na obtenção de aminoácidos por processos hidrotérmicos com água subcrítica.

- Analisar os principais parâmetros de operação, nas temperaturas de 210 ºC, 230 ºC e 250 ° C, a 15 MPa de pressão, e vazões de 5, 7,5 e 10 $mL.min^{-1}$ do processo de hidrólise com água subcrítica a partir de penas de aves *in natura*;

- Analisar o hidrolisado obtido nas cinéticas quanto ao pH, proteínas, teor de matéria orgânica mediante demanda química de oxigênio, sólidos totais e voláteis e aminoácidos.

3. Estrutura da Tese

O presente documento encontra-se dividido em capítulos. O artigo que apresenta a revisão bibliográfica já se encontra publicado e os resultados experimentais correspondem ao artigo enviado à revista científica internacional The Journal of Supercritical Fluids, da Área de Engenharia de Alimentos.

O **Capítulo 1** é constituído da Introdução, onde são fornecidas informações acerca do tema central da dissertação, apresentando brevemente os pontos mais importantes a serem estudados, o objetivo geral e os específicos do trabalho e a estrutura da dissertação. Inicialmente foi apresentada a matéria-prima, penas de aves, de interesse para o estudo. Em seguida foram discutidos trabalhos similares com extração de proteínas a partir de outros resíduos. Justificou-se o crescente interesse pelo aproveitamento da proteína e obtenção de aminoácidos.

O **Capítulo 2** traz uma contextualização sobre trabalhos de pesquisa relatados entre 2001 e 2020 sobre a hidrólise com água subcrítica para a hidrólise de substratos à base de proteínas para obtenção de aminoácidos. Abordam-se aspectos de relevância de proteínas e aminoácidos, principais vias de extração de proteínas, influência e interações dos principais parâmetros e condições de reação. Desagregação-agregação de proteínas e uma comparação da hidrólise, configurações do reator e, finalmente, as perspectivas de pesquisas futuras.

A revisão apresentada no Capítulo 2 está na forma de artigo científico com título “An overview of subcritical and supercritical water treatment of different biomasses for protein and amino acids production and recovery” e autores “Henrique Di Domenico Ziero, Luz Selene Buller, Ackmez Mudhoo, Larissa Castro Ampese, Solange I. Mussatto,

Tânia Forster Carneiro". Este artigo foi publicado no Journal of Environmental Chemical Engineering 8, 104406, em 2020, doi: https://doi.org/10.1016/j.jece.2020.104406

O **Capítulo 3** apresenta uma análise do processo de hidrólise em água subcrítica de penas de aves para produção de subprodutos com maior valor agregado, os aminoácidos. Nesta análise, os principais parâmetros de operação foram avaliados tais como, temperaturas e vazões de alimentação a partir de penas de aves *in natura*. Finalmente, os resíduos líquidos (hidrolisado) foram analisados quanto ao rendimento de proteínas, teor de matéria orgânica e concentração de aminoácidos.

Os resultados experimentais do Capítulo 3 são apresentados na forma de artigo científico com título "Subcritical water hydrolysis of poltry feathers for amino acid prodution" e autores "Henrique Di Domenico Zieroa; Larissa Castro Ampese; William G. Sganzerla; Paulo C.T. Mayanga, M.T. Timko; Solange I. Mussatto; T. Forster-Carneiro". Este artigo foi submetido no Journal of Supercritical Fluids, em 2021.

O **Capítulo 4** apresenta as discussões gerais da dissertação.

O **Capítulo 5** apresenta as principais conclusões da dissertação, bem como as perspectivas para os trabalhos futuros.

CAPÍTULO 2 - Uma visão geral do tratamento subcrítico e supercrítico da água de diferentes biomassas para a produção e recuperação de proteínas e aminoácidos

Artigo publicado no periódico Journal of Environmental Chemical Engineering
Volume 8, Issue 5, October 2020, 104406

(Impact Factor: 4.3)

An overview of subcritical and supercritical water treatment of different biomasses for protein and amino acids production and recovery

Henrique Di Domenico Ziero[1], Luz Selene Buller[1], Ackmez Mudhoo[2], Larissa Castro Ampese[1], Solange I. Mussatto[3], Tânia Forster Carneiro[1,*]

[1]School of Food Engineering, University of Campinas (UNICAMP), Rua Monteiro Lobato, 80, 13083-862, Campinas, São Paulo, Brazil

[2]Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Mauritius, Réduit 80837, Mauritius

[3]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet, Building 220, 2800, Kongens Lyngby, Denmark

*** Corresponding Author**

Professor Tânia Forster Carneiro

Food Engineering School, University of Campinas (UNICAMP), Rua Monteiro Lobato, 80, 13083-862, Campinas, São Paulo, Brazil

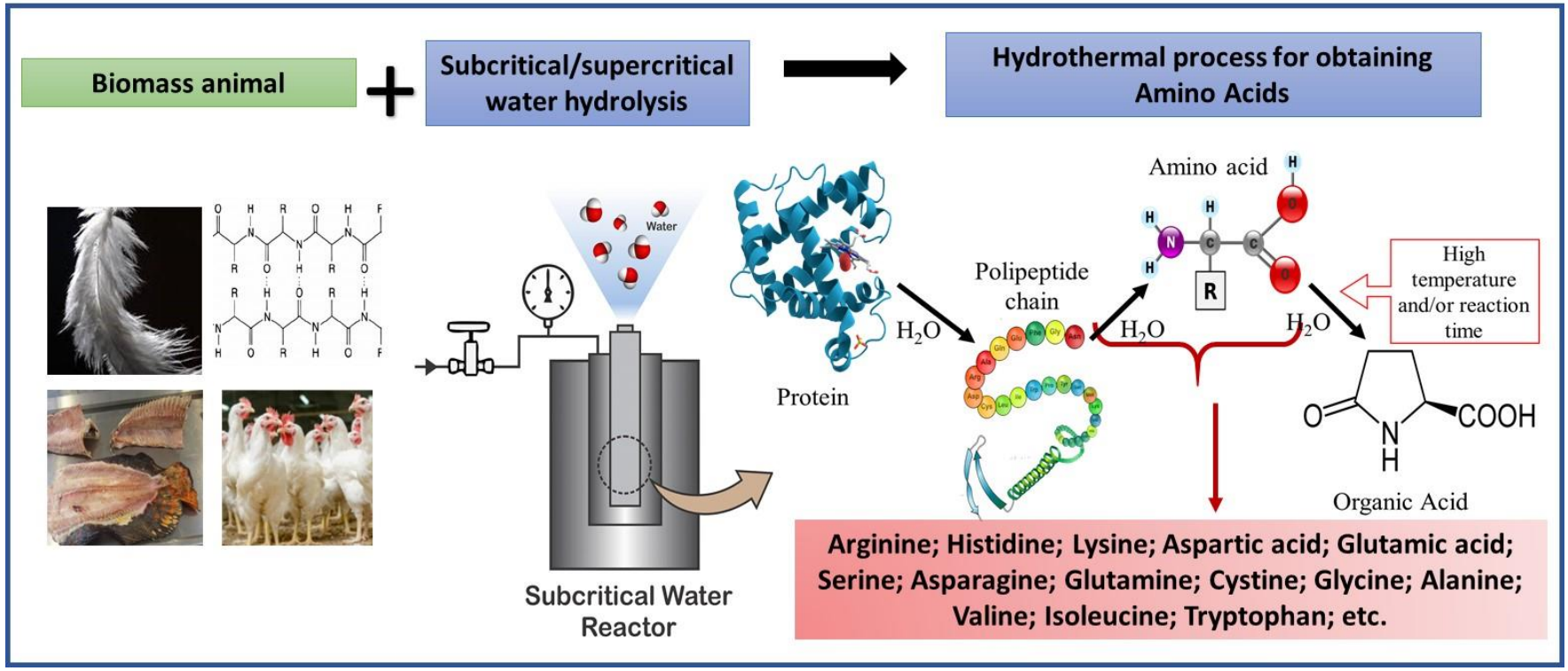
E-mail address: taniafc@unicamp.br

Abstract

The agricultural and food industry sectors play essential roles in the global economy, but also generate significant amounts of wastes every year associated to their activities, which require an appropriate management. As an alternative, agro-industrial by-products can be used for obtaining valuable compounds such as amino acids, phenolic compounds, water-soluble sugars, organic acids, methane and oils, among others. Several processes have been developed for obtaining such compounds from by-products and residues, among of which the subcritical and supercritical water technologies are considered as green alternatives. This review presents a concise assessment of the research findings reported from 2001 to 2020 on the subcritical and supercritical water extraction and hydrolysis of protein-based substrates to obtain amino acids, addressing aspects such as: economic relevance of protein and amino acids, main routes for protein extraction, influence and interactions of the main reaction parameters and conditions. Protein aggregation-disaggregation and a comparison of selected extraction arrangements, reactor configurations, and finally, future research perspectives are also discussed.

Keywords: Biomass; Pressurized Fluid; Proteins; Amino acids; Subcritical Water Hydrolysis

GRAPHICAL ABSTRACT



1. Introduction

Biomass can be defined as "the total mass of living or freshly dead organic matter within a specific milieu" that presents a potential resource for the production of value-added products (LACHOS-PEREZ; BROWN; MUDHOO; TIMKO *et al.*, 2017). Some common biomass sources are molasses, bagasse, and powdered by-products, for instance, which can be obtained at low- or no-cost while being always generated (PEDRAS, 2015). Agro- and food-industry generated solid by-products can be used for the production of valuable compounds such as protein, amino acids, phenolic compounds, water-soluble sugars, organic acids, methane, and oils (AZMIR, J.; ZAIDUL, I. S. M.; RAHMAN, M. M.; SHARIF, K. M. *et al.*, 2013; CHENG, H.; ZHU, X.; ZHU, C.; QIAN, J. *et al.*, 2008; MARCET; ALVAREZ; PAREDES; DIAZ, 2016; SHITU, A.; IZHAR, S.; TAHIR, T. M., 2015). Animal-derived biomass like skin, hair, feathers, bones and entrails are commonly considered as 'wastes' (ALAO; FALOWO; CHULAYO; MUCHENJE, 2017). Since these by-products present limited and low reinsertion efficiency in the productive cycle, the standard disposal routes are landfills or controlled composting systems. However, these materials are composed of several substances that can be extracted to obtain high value-added species such as proteins, amino acids, phenolic compounds, furfurals, essential oils and fatty acids (KNEZ; HRNČIČ; ČOLNIK; ŠKERGET, 2018).

Amino acids contain an alpha carbon linked to a carboxyl group, an amino group, hydrogen, and one distinguishing group from amino acid to amino acid. Peptides, composed of a group of amino acids, can be transformed into proteins or enzymes. Proteins play an important role in sustaining structural and regulatory functions in the cells and the body. At

the same time, enzymes are biocatalysts which enhance several low-speed reactions by lowering the associated activation energy. In addition, amino acids have many metabolic functions, namely, participating in biochemical reactions, essential energy generation, energy transfer, and muscle activity (ZHU; ZHU; XIAO; ZHOU *et al.*, 2015). Some amino acids can be synthesized in the body; however, the essential ones have to be obtained from dietary ingestion. Therefore, amino acids have considerable importance in the medical, food, pharmaceutical, animal, and cosmetic industries.

Several methods have been reported for the extraction of proteins from lignocellulosic biomass (ARAUZO; DU; OLSZEWSKI; ZAVALA *et al.*, 2019; QIN; JOHANSEN; MUSSATTO, 2018). Yet, an efficient and economically attractive protein extraction/recovery from biomass remains challenging (QIN; JOHANSEN; MUSSATTO, 2018), especially from a process engineering perspective. Subcritical water hydrolysis (SWH) is a potential process approach for the 'valorization' of biomass to high-added-value nutrients. The properties of water in subcritical thermodynamic conditions are unique within the subcritical temperature and pressure zone, ranging from 100 to 374 ^{o}C and 1 to 22.1 MPa, respectively (LACHOS-PEREZ; BROWN; MUDHOO; TIMKO *et al.*, 2017; TORRES-MAYANGA; LACHOS-PEREZ; MUDHOO; KUMAR *et al.*, 2019). Within these temperature and pressure ranges, water can dissolve non-polar substances as a result of a decrease in the dielectric constant compared with room temperature water. Under such conditions, water can be used as a clean(er) medium for performing hydrolysis and extraction of organic molecules and biological/bioactive compounds (GBASHI; ADEBO; PIATER; MADALA *et al.*, 2017; KIAMAHALLEH; NAJAFPOUR-DARZI; RAHIMNEJAD; MOGHADAMNIA *et al.*, 2016; SARAVANA; TILAHUN; GERENEW; TRI *et al.*, 2018). In addition, the ionic product of water being then considerably higher than that of water at room temperature (OKAJIMA; SAKO, 2014), it acquires a strong catalytic character

(CHENG, H.; ZHU, X.; ZHU, C.; QIAN, J. *et al.*, 2008; ZHOU; HEARNE; LI, 2019). Subcritical water can also be applied to favor energy and mass transfer due to its density fluctuations (COCERO; CABEZA; ABAD; ADAMOVIC *et al.*, 2018; OKAJIMA; SAKO, 2014) and low dielectric properties (CHENG, H.; ZHU, X.; ZHU, C.; QIAN, J. *et al.*, 2008; OKAJIMA; SAKO, 2014; SARFARAZI; JAFARI; RAJABZADEH; FEIZI, 2019).

In this review, the research findings on the subcritical and supercritical water processes applied for the conversion of different types of protein-rich biomasses into amino acids have been discussed based on studies reported in the period 2001 to 2020. Subcritical water extraction/hydrolysis is an inherently complex process which is dependent on the biomass properties, composition, and cell walls structure. Hence, each specific biomass brings along its unique constraints and challenges related to process development and optimization. Therefore, this review has been organized to discuss the following aspects: economic relevance of proteins and amino acids, main protein extraction routes from biomass, interactions and influence of main reaction parameters and conditions (by considering the temperature-dependent bioprocess dynamics, and protein aggregation-disaggregation and its control on protein and amino acid recovery), comparison of some extraction arrangements and reactors configuration, and finally, some perspectives for future research.

2. Survey methodology

Data have been collected from the Web of Science© (**Web of Science**, 2020) and Elsevier's Scopus (**SCOPUS**) database. The search was done for title, abstract, author's keywords and Keywords Plus and by applying a "basic search" using the following terms: "Subcritical water hydrolysis", "Amino acids hydrolysis", "Subcritical water extraction", "Supercritical protein extraction", "Hot compressed water protein hydrolysis", "Hot

compressed water protein extraction". The timespan considered for articles was from 2001 to 2020. The output of each data search was analyzed to select the results that fitted the aim of this review. After this initial screening, the selected documents provided data and information for further analysis.

3. Economic relevance of proteins and amino acids

Proteins are essential nutrients for proper human and animal metabolism and, therefore have high economic value (FARMER; MASCAL, 2015). Within the growth of the biodiesel and bioethanol markets, protein-containing co-products have gradually risen and have become more abundant, making proteins more accessible for other uses than solely nutrition (LAMMENS; FRANSSEN; SCOTT; SANDERS, 2012). Therefore, there is a growing interest in protein recovery from natural resources and agro-industrial by-products for further utilization in different industrial areas (DRAGONE; KERSSEMAKERS; DRIESSEN; YAMAKAWA *et al.*, 2020; FARMER; MASCAL, 2015; TUCK, CHRISTOPHER O; PÉREZ, EDUARDO; HORVÁTH, ISTVÁN T; SHELDON, ROGER A *et al.*, 2012). The food and beverage industries, for example, generate a large amount of protein-containing by-products (220 billion tonnes per year in 2007 (ZHANG; FAN; XING; PAN *et al.*, 2007)) such as distiller's grains (from maize or wheat), vinasse (from sugar beet or sugarcane), fish silage, cakes (from oil seeds like rapeseed or palm), materials from coffee and tea processing and other agricultural by-products (TUCK, CHRISTOPHER O; PÉREZ, EDUARDO; HORVÁTH, ISTVÁN T; SHELDON, ROGER A *et al.*, 2012). Feathers, composed of more than 90% of proteins (keratin), are an example of high-protein biomass, generated mainly from poultry slaughterhouses (TUCK, CHRISTOPHER O; PÉREZ, EDUARDO; HORVÁTH, ISTVÁN T; SHELDON, ROGER A *et al.*, 2012). Only the

chicken feathers, approximately 8.5 million tons, are produced every year (REDDY; SANTOSH, 2016b). As feathers are produced, they are considered as a renewable source of proteins (REDDY; SANTOSH, 2016b). The significant amino acids extracted from feather keratin are alanine (29.5%), cysteine (14.4%), proline (10.1%), and serine (7.0%) (YIN; LI; HE; WANG *et al.*, 2013).

Amino acids, mainly produced by biotechnological methods, are employed as flavor enhancers, animal feed additives and specialty nutrients in the medical field, and as cosmetic and pharmaceutical ingredients (IVANOV; IVANOVA; GEORGIEVA; ATANASOV, 2014). Along with this, the increasing availability of agro-industrial by-products opens a wide field for extraction technologies, which became essential for new markets thriving on biomass conversion into new biomaterials. Proteins/peptides from these by-products' flows can, in many cases, be used as valuable starting materials for food and feed applications when extracted at the start of biomass processing (DRAGONE; KERSSEMAKERS; DRIESSEN; YAMAKAWA *et al.*, 2020). The high value per unit weight of amino acids (**Table 1**) can positively add value to the general economy of biorefining systems (SOLATI; MANEVSKI; JØRGENSEN; LABOURIAU *et al.*, 2018) and for the application of an effective high-yield extraction technology that makes possible and promising their reinsertion in the productive cycle (DENG; SHEN; XU; KUANG; GUO; ZENG; GAO; LIN; XIE; XIA *et al.*, 2012).

Table 1. Major amino acids market prices.

Major amino acids	Market prices and assay level [a] (USD/g)	Reference
Cystine	0.53 – 1.02 (≥98% TLC, crystalline)	Sigma-Aldrich (SIGMA-ALDRICH, 2020b)
Glutamic acid	0.10 – 0.20 (ReagentPlus®, ≥99% HPLC)	Sigma-Aldrich (SIGMA-ALDRICH)
Histidine	0.64 – 1.04 (ReagentPlus®, ≥99% TLC)	Sigma-Aldrich (SIGMA-ALDRICH, 2020a)

Isoleucine	0.88 – 1.52 (Reagent grade, ≥98% HPLC)	Sigma-Aldrich (SIGMA-ALDRICH)
Lysine[b]	**3.09** (≥98% TLC)	Sigma-Aldrich (SIGMA-ALDRICH)
Tryptophan	0.68 – 1.16 (Reagent grade, ≥98% HPLC)	Sigma-Aldrich (SIGMA-ALDRICH)

[a] Free on Board (FOB) Prices depending on packaging size (varying from 100g to 1000g) for assay levels from ≥98.0% to ≥99.0%

[b]Except for Lysine where packaging size is 100g

A straightforward route to obtain platform chemicals from protein-rich materials is by peptide bond hydrolysis, which results in a mixture of amino acids. Once this mixture is produced, it is possible to isolate each amino acid by employing separation processes involving electrodialysis (LIN; PAN; ZHOU; XU *et al.*, 2016) and chromatography (HARADA; KARAKAWA; YAMADA; MIYANO *et al.*, 2019), further converting the amino acids into bulk organic compounds, which can serve as pools of industrial monomers. The hydrolysis results in a complex potpourri of useful molecules (**Figure 1**) (FARMER; MASCAL, 2015; TUCK, CHRISTOPHER O; PÉREZ, EDUARDO; HORVÁTH, ISTVÁN T; SHELDON, ROGER A *et al.*, 2012): the amino acids obtained through this route have diverse applications and functionalities, and represent a good source of nitrogen-containing platform chemicals (FARMER; MASCAL, 2015).

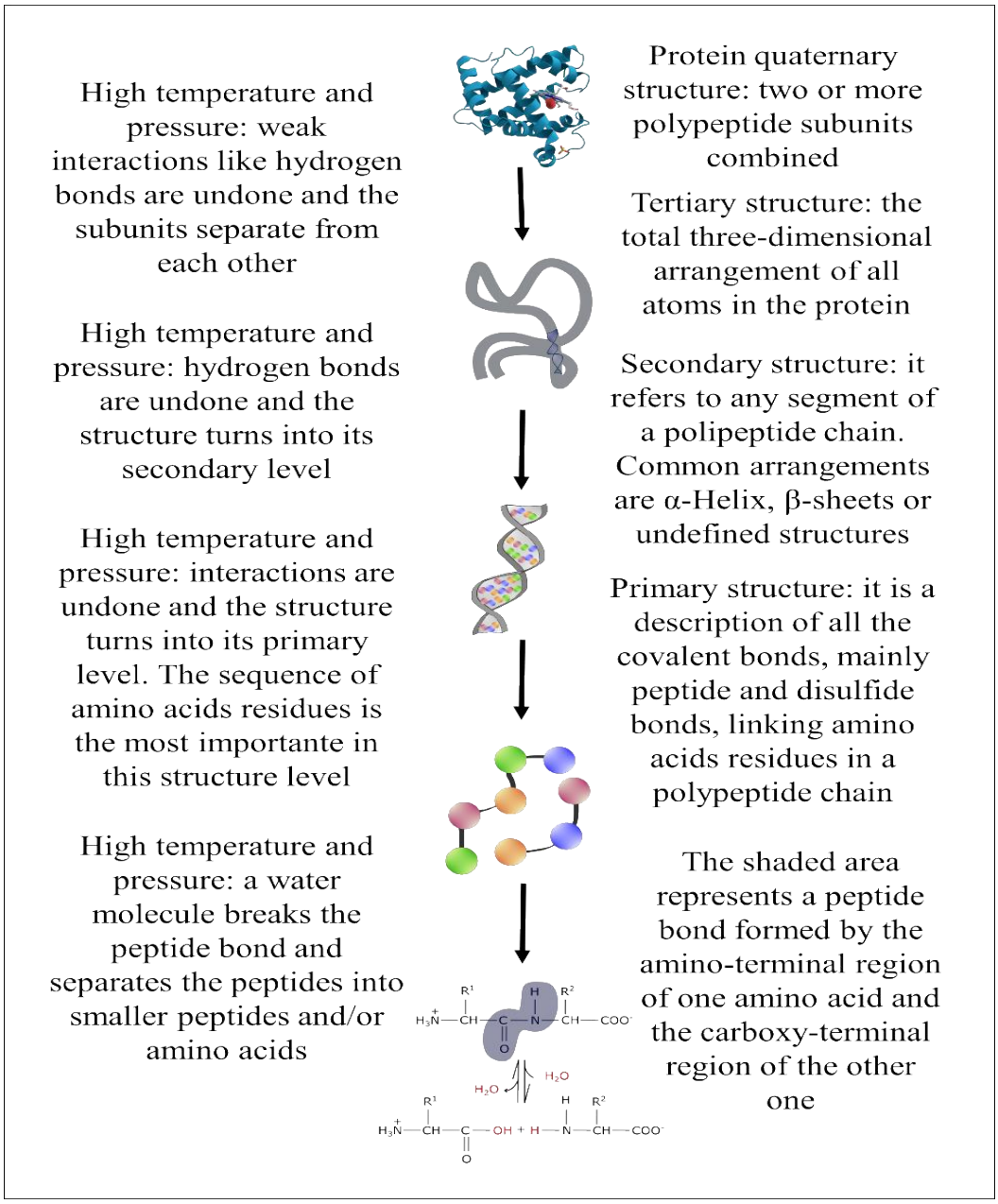


Figure 1. Representation of generic protein decomposition in subcritical hydrolysis process.

Figure 1 shows a simplified representation of the hydrolysis and protein extraction pathways. As a treatment route, subcritical water hydrolysis can promote the extraction and decomposition of proteins and their constituent amino acids. However, in some cases, hydrolysis can be applied with the purpose of promoting a pre-treatment of the material for subsequent enzymatic hydrolysis. The initial reactions that lead to the production of platform

chemicals are deamination, decarboxylation, and hydrolysis (LI; NG; CHEN; CHIANG *et al.*, 2018). For example, glutamic acid is an abundant non-essential amino acid that can be obtained from the hydrolysis of diverse plants and animal proteins. Accordingly, glutamic acid turns out to be an interesting starting material for chemical products synthesizing, from which a variety of commoditized chemicals could be produced. Via an initial decarboxylation reaction catalyzed by glutamate decarboxylase, glutamic acid can be converted to γ-aminobutyric acid. The latter molecule can be a source material for the synthesis of bio-based pyrrolidone-derivates, such as N-vinylpyrrolidone (NVP) and N-methylpyrrolidone (NMP). From glutamic it is possible to obtain the polyamide precursor succinonitrile (precursor of 1,4-diaminobutane) via the intermediate 3-cyanopropanoic amide. Additionally, glutamic acid can be converted to acrylonitrile via oxidative decarboxylation in water to 3-cyanopropanoic acid and then a decarbonylation-elimination reaction using Pd catalyst. Also glutamic acid can be converted to α-ketoglutaric acid alpha-ketoglutaric acid production from L-glutamic acid via L-amino acid deaminase (HOSSAIN; LI; SHIN; CHEN *et al.*, 2014; INFORMATION, 2004; LAMMENS; FRANSSEN; SCOTT; SANDERS, 2012; LAMMENS; LE NÔTRE; FRANSSEN; SCOTT *et al.*, 2011; LE NÔTRE; SCOTT; FRANSSEN; SANDERS, 2011; LI; NG; CHEN; CHIANG *et al.*, 2018; TUCK, CHRISTOPHER O; PÉREZ, EDUARDO; HORVÁTH, ISTVÁN T; SHELDON, ROGER A *et al.*, 2012).

Some amino acids such as arginine, phenylalanine and lysine are of interest to produce different other chemicals. Arginine can be obtained from jatropha seed meal, while phenylalanine is found on cassava leaves and soybean meal, the latter being also a source of lysine (only considering agricultural residues). Arginine can be converted into ornithine, which takes part in Nylon-4,6 production that can be produced through the decarboxylation

of ornithine to 1,4-diaminobutane using ornithine decarboxylase (LI; NG; CHEN; CHIANG *et al.*, 2018). Phenylalanine can be used for the production of styrene via deamination and decarboxylation or for the production of both styrene and acrylates via ethenolysis, while lysine can be used to obtain 1,5-diaminopentane, caprolactam (a Nylon 6 precursor) and 5-amino valeric acid (FARMER; MASCAL, 2015; LI; NG; CHEN; CHIANG *et al.*, 2018; TUCK, CHRISTOPHER O; PÉREZ, EDUARDO; HORVÁTH, ISTVÁN T; SHELDON, ROGER A *et al.*, 2012). Other products derived from amino acids through enzymatic pathways include β-alanine (aspartic acid), α-ketoglutaric acid (glutamic acid), primary amines (alcohol and alanine) and enantio-compounds (L-amino acids) (LI; NG; CHEN; CHIANG *et al.*, 2018). In order to be competitive with building block chemicals produced from the petrochemical industry, alternative synthetic routes which are as simple as possible and involving as little as possible functional group changes should be developed (TUCK, CHRISTOPHER O; PÉREZ, EDUARDO; HORVÁTH, ISTVÁN T; SHELDON, ROGER A *et al.*, 2012). Such simpler and greener synthetic production routes will aim at reducing the synthetic complexity and also alleviating the burden of parameter control and optimization at larger production scales. Animal-derived wastes or by-products tend to offer limited reinsertion within a productive cycle and are therefore commonly disposed of without energy and/or material recovery. Keeping in mind the merits and limitations of the different typical waste management and treatment options, such biomass-type materials contain untapped substances that could be eventually recovered as high value-added products.

4. Protein extraction routes

The efficiencies of amino acid recovery and protein extraction depend on the characteristics of the substrate namely, chemical composition, cell wall rigidity, protein structure, and protein storage location (DE SCHOUWER; CLAES; VANDEKERKHOVE;

198 VERDUYCKT *et al.*, 2019; DRAGONE; KERSSEMAKERS; DRIESSEN; YAMAKAWA
199 *et al.*, 2020). The processing of protein-rich materials requires special conditions because of
200 the high sensitivity of proteins to pressure and temperature. High pressure degrades the
201 quaternary structure of proteins (LULLIEN-PELLERIN; BALNY, 2002). In contrast, the
202 tertiary structure can be sequentially disjointed under high temperature and pressure
203 conditions. Proteins could be denatured through exposure to high pressure at room
204 temperature without breaking the covalent bond and not changing their protein size
205 (RODILES-LOPEZ; ARROYO-MAYA; JARAMILLO-FLORES; GUTIERREZ-LOPEZ *et*
206 *al.*, 2010). The same occurs when exposed to high heat under atmospheric pressure (no
207 covalent bond are undone under this situation) (SMELLER, 2001). Only the effects of
208 pressure and temperature can collectively hydrolyze protein into small fragments and
209 subsequently into free amino acids. Furthermore, when the reaction time is long enough, the
210 amino acids will be reduced to organic acids (ROGALINSKI; HERRMANN; BRUNNER,
211 2005).

212 Conventional and advanced chemical routes have been used to obtain valuable
213 compounds from waste materials (AZMIR, J.; ZAIDUL, I. S. M.; RAHMAN, M. M.;
214 SHARIF, K. M. *et al.*, 2013; SHITU, A.; IZHAR, S.; TAHIR, T. M., 2015). A widely
215 employed technique includes maceration and Soxhlet extraction. Physical methods
216 (SANTAMARIA-FERNANDEZ; MOLINUEVO-SALCES; LÜBECK; UELLENDAHL,
217 2018) and a combination of ultrafine milling with electrostatic separation (BARAKAT;
218 JÉRÔME; ROUAU, 2015) have equally been reported for the isolation of proteins from
219 biomass. However, both methods could be disadvantageous because of the excessive use of
220 organic solvents, generation of final polluting and undesirable moieties, and low selectivity
221 (AZMIR, J.; ZAIDUL, I. S. M.; RAHMAN, M. M.; SHARIF, K. M. *et al.*, 2013). After
222 appropriate synthetic route optimization and scale-up, non-conventional methods could be

useful. Some of the upcoming green routes are supercritical fluid extraction (SFE) (AKALıN; TEKIN; KARAGÖZ, 2016; LI; SAKURAGI; MAKINO, 2019), subcritical water treatment (MOHD THANI; MUSTAPA KAMAL; TAIP; SULAIMAN *et al.*, 2019), pulsed electric field assisted extraction (JAESCHKE; MERCALI; MARCZAK; MULLER *et al.*, 2019), microwave-assisted extraction (POPESCU; ASTANEI; BURLICA; POPESCU *et al.*, 2019), pressurized liquid extraction (DERWENSKUS; METZ; GILLE; SCHMID-STAIGER *et al.*, 2019), enzyme-assisted extraction (ANOOTTHATO, S.; THERDTHAI, N.; RITTHIRUANGDEJ, P., 2019) and ultrasound-assisted extraction (TUTUNCHI; ROUFEGARINEJAD; HAMISHEHKAR; ALIZADEH, 2019). However, selectivity is a significant parameter to be considered when selecting the most suitable approach to be used for protein recovery from biomass (QIN; JOHANSEN; MUSSATTO, 2018).

Enzymatic hydrolysis (ANOOTTHATO, S.; THERDTHAI, N.; RITTHIRUANGDEJ, P., 2019; PEREIRA; JUSTUS; FALCÃO; ROCHA *et al.*, 2019b; YANG; CAI; LIU; WANG, 2019; ZHANG; JIANG; LI; YU *et al.*, 2019; ZHENG; HAO; WENG; REN, 2019; ZULUAGA-DOMÍNGUEZ; CASTRO-MERCADO; CECILIA QUICAZÁN, 2019) presents the potential to cleave proteins into smaller fragments and moieties, and could help bring in process standardization because the addition of alkali to for keeping a constant pH is moderate in limiting amino acids degradation (MARCET; ALVAREZ; PAREDES; DIAZ, 2016). Nevertheless, the comparison of this chemical route with other enzyme extraction techniques places its reagents as relatively expensive, and make the method economically challenging to sustain. An alternative approach for protein cleavage is chemical hydrolysis. Although it is a relatively cheaper method which can be readily integrated in the industry, it still requires relatively high temperatures and high acid(s)/alkalis concentrations. Despite that chemical hydrolysis could cause the degradation of some amino acids, it has been applied for amino acid extraction (ALVAREZ;

248 RENDUELES; DIAZ, 2012). The major disadvantage of this route is its chemical footprint
249 (SALA; GORALCZYK, 2013), which is associated with the dangerous/hazardous chemicals
250 employed.

251 The supercritical water process involves two thermodynamic conditions for water.
252 Firstly, the subcritical part, which covers temperatures between 100-374 °C and pressures
253 higher than the water saturation pressure at the selected temperature; secondly, the
254 supercritical part, which covers water temperatures and pressures higher than 374 °C and
255 22.1 MPa, respectively (conditions over the critical point of water) (BRUNNER, 2009;
256 KING, J. W., 2000). This approach presents a high potential in the extraction of high value-
257 added compounds like amino acids. Besides, it can be relatively cheap given the requirements
258 for organic reagents are relatively low, and it can also avoid the generation of undesirable
259 residues that could be of environmental concern (MARCET; ALVAREZ; PAREDES; DIAZ,
260 2016). The physicochemical properties of subcritical water make it an excellent solvent for
261 polar and ionic compounds. When temperature and pressure rise, water can also dissolve
262 non-polar molecules (MARCET; ALVAREZ; PAREDES; DIAZ, 2016; SHITU, A.; IZHAR,
263 S.; TAHIR, T. M., 2015). At subcritical conditions, water can interact with non-polar
264 molecules such as organic compounds because its electrochemical dielectric continually
265 decreases from 78 Fm^{-1} (at 25 °C and 1 atm or 0.1 MPa) to 14.08 Fm^{-1} at 350 °C and 20 MPa
266 (CHEIGH; YOO; KO; CHANG *et al.*, 2015; UEMATSU; FRANK, 1980). Besides, the ionic
267 product of water, defined as $K_w = [H^+]\,[OH]$, increases from 10^{-14} to 10^{-12}, increasing the H^+
268 and OH^- concentrations. Under such conditions, the chemical catalytic activity of acid- or
269 base-like compounds tends to increase (MARCET; ALVAREZ; PAREDES; DIAZ, 2016).

270 Subcritical water hydrolysis (SWH) presents considerable potential for liquefaction
271 of protein-rich substrates. However, because of the high-pressure requirement and,
272 consequently, high energy demand, this method still requires a techno-economic assessment

to support the selection of the best operational conditions for implementation. Different SWH arrangements can be used, namely: batch, semi-batch, and continuous mode operation. The batch system, where substrate and water are fed in a closed reactor, is the arrangement most used in a lab-scale. The semi-batch and continuous arrangements, where the substrate is fed into a continuous flow of solvent, or both substrate and solvent are constantly fed together in the reactor, respectively, are the most employed for scale-up purposes. After supercritical extraction or hydrolysis of the substrate, a purification step is necessary to obtain amino acids suitable for commercialization (**Table 1**). Purification could be undertaken by techniques such as ultra or nanofiltration (DE MOURA; GONÇALVES; SARMENTO; PETRUS, 2007), exclusion chromatography coupled with hydrogen-deuterium exchange (SEC-HDX) technology, or Hydrophobic Interaction Chromatography (GOVENDER; NAICKER; BAIJNATH; CHUTURGOON *et al.*, 2020). These methods can give high yields and purity, and are used for protein purification (DE ANDRADE LIMA; CHARALAMPOPOULOS; CHATZIFRAGKOU, 2018).

5. Sub/supercritical water applications for amino acids extraction

Due to the growing concern with regards to the quality and safety of food products and the strict regulations on solvent residues, the subcritical and supercritical fluid process routes have become attractive options for extraction, fractionation, and isolation of active ingredients (ARNÁIZ; BERNAL; MARTÍN; NOZAL *et al.*, 2012; CASTRO-VARGAS; RODRÍGUEZ-VARELA; FERREIRA; PARADA-ALFONSO, 2010; HERRERO; CIFUENTES; IBAÑEZ, 2006; MUSSATTO, 2016). In fact, SWH presents a variety of possible applications and can be applied in different types of substrates. **Table 2** summarizes several examples where subcritical water conditions applied for the synthesis of amino acids from different types of biomass.

298 **Table 2.** Amino acid production from different biomass/residues treated under different
299 subcritical conditions
300

Reference	Biomass Source/ By-product	Subcritical Reaction System	Best Subcritical water parameters	Yield of amino acids obtained	Major amino acids found
Sereewatthanawut et al. **(SEREEWATTHANAWUT; PRAPINTIP; WATCHIRARUJI; GOTO *et al.*, 2008)**	Defatted rice bran	Batch reactor Volume: 8.8 mL	Temperature: 200 °C Pressure of saturated steam Residence time: 30 min	0.8±0.16 g/100 g raw material	---
Zhu et al. **(ZHU; ZHU; FAN; LIU *et al.*, 2010a)**	Beans dregs	Batch reactor Volume: 300 mL Water+CO_2	Temperature: 330 °C Pressure of saturated steam Reaction time: 20 minutes	22 g/100 g of raw material	Arginine Lysine Alanine
Park et al. (PARK; JEONG; CHUN, 2019)	Laver (*Pyropia yezoensis*)	Batch reactor Volume: 1000 mL	Temperature: 120 °C Pressure: 3 MPa Reaction time: 30 minutes	6.2733 g/100 g of raw material	Taurine Alanine Glutamic acid

Garcia-Moscoso et al. (GARCIA-MOSCOSO; TEYMOURI; KUMAR, 2015)	Microalgae (*Scenedesmus* sp.)	Flash Hydrolyze	Temperature: 320 °C Pressure: 20.7 MPa Reaction time: 6 s	12.3 g of arginine/100 g of protein	Arginine
Ueno et al. (UENO; ICHINOI; ZHAO; FUJII, 2015)	Fish gelatin	Tubular reactor Volume: 15.7 mL	Temperature: 220 °C Pressure: 2 MPa Retention time: 157 minutes	---	Serine Glycine Alanine Arginine Valine
Asaduzzaman and Chun) **(ASADUZZAMAN, A. K.; CHUN, B. S., 2015)**	Squid muscle (*Todarodes pacificus*)	Batch reactor Volume: 200 mL	Temperature: 250 °C Pressure of saturated steam Reaction time: 3 minutes	0.42153 g/100 g of raw material	Glycine Leucine Valine Phenylalanine
Ahmed and Chun (AHMED; CHUN, 2018)	Tuna's collagen	Batch reactor Volume: 200 mL	Temperature: 250 °C Pressure: 5-8 MPa Reaction time: 5 minutes	0.05746 g/100 g of raw material	Glycine Proline Alanine Glutamic acid

Esteban et al. (ESTEBAN; GARCIA; RAMOS; MARQUEZ, 2010)	Hog hair	Batch reactor Volume: 500 mL	Temperature: 250 °C Pressure of saturated steam Reaction time: 60 minutes	32.5 g/100 g of protein	Alanine Glycine Arginine

301

Some proteinaceous vegetable wastes can be used as source of amino acids. **Table 2** shows, for example, the use of defatted rice bran, beans dregs, and even microalgae to obtain amino acids. Soybean is an essential food for human nourishment, especially in the eastern part of the globe (ZHU; ZHU; FAN; WAN, 2011). Asian countries like China produce 80,000 tons per year of bean dregs, mostly soybean (ZHU; ZHU; FAN; WAN, 2011). It is the same for rice, one of the most consumed food in the world, mainly in Asian countries, from which a high amount of by-products are generated during the rice recovery process, and representing about 8% of milled rice (ZHU; ZHU; XIAO; ZHOU *et al.*, 2015). Sereewatthanawut et al. (SEREEWATTHANAWUT; PRAPINTIP; WATCHIRARUJI; GOTO *et al.*, 2008) used SWH to process defatted rice bran to obtain protein and amino acids, and observed that the amount of protein produced increased with the increase in temperature, while the amount of amino acids decreased with the increase in temperature. Focused on the efficiency of subcritical technology compared to alkalis, Lu et al. (LU; CHEN; WANG; YANG *et al.*, 2016) compared conventional alkali extraction to enzyme-assisted subcritical water extraction of isolated soy protein. The results indicated that the enzyme-assisted subcritical water extraction contained a high quantity of proteins with uncharged and hydrophobic amino acids (LU; CHEN; WANG; YANG *et al.*, 2016). In that same work, it was observed that the protein quality, after the enzymatic process, had risen significantly compared to the alkali extraction. In another work, Zhu et al. (ZHU; ZHU; FAN; LIU *et al.*, 2010a) studied the recovery of amino acids from soybean dregs under SWH, and reported a relatively high yield of amino acids at 330 °C and 20 minutes with a significant amount of arginine, lysine, and alanine. Another vegetal waste of interest is *laver,* which is rich in carbohydrates and proteins, and is in high demand in the cosmetics, foods, and pharmaceutical industries, Park et al.

(PARK; JEONG; CHUN, 2019) employed SWH to hydrolyze *Pyropia yezoensis* (a species of laver found in Japan) and produced taurine, alanine, and glutamic acid.

Microalgae can also be a source of amino acids (GARCIA-MOSCOSO; TEYMOURI; KUMAR, 2015). Microalgae are microorganisms that engage in photosynthesis and can be cultivated in different water systems, including wastewater, for the potential recovery of nutrients and pollutant removal. Garcia-Moscoso et al. (GARCIA-MOSCOSO; TEYMOURI; KUMAR, 2015) focused on the recovery of peptides and arginine from *Scenedesmus* sp. biomass using flash hydrolysis, and reported having obtained a higher content of arginine) in the microalgae protein fractionated. It has been reported that arginine concentration rises with the reaction temperature, and there are some studies which have associated the latter behavior with the stability of arginine at high temperatures and pressure (GARCIA-MOSCOSO; TEYMOURI; KUMAR, 2015; ZHU; ZHU; FAN; LIU *et al.*, 2010a).

Some proteinaceous animal wastes can also be used as sources of amino acids. **Table 2** shows samples of fish gelatin, fish meat, tuna skin, and collagen used towards amino acid production. Ueno et al. (UENO; ICHINOI; ZHAO; FUJII, 2015) used fish gelatin to produce serine, glycine, alanine, arginine, and valine in SWH at higher temperatures (240 ºC), and the number of amino acids produced had drastically decreased. Asaduzzaman and Chun (ASADUZZAMAN, A. K.; CHUN, B. S., 2015) had investigated the hydrolysis of squid muscles through SWH and the results obtained thereof demonstrated the production of an average highest concentration of free amino acids at 250 °C, while the highest concentration of essential amino acids was obtained at 220 °C. Ahmed and Chun (AHMED; CHUN, 2018) have assessed the hydrolysis of tuna skin and collagen with SWH. Thereof, it was found that fish hydrolysate presented antioxidant and antimicrobial properties, along with a giving a good yield of amino acids

(**Table 2**). However, at higher temperatures, a decline in amino acid yield was observed due to its decomposition into organic acids (AHMED; CHUN, 2018). In the study of Esteban et al. (ESTEBAN; GARCIA; RAMOS; MARQUEZ, 2010) where hog hair was processed under SWH conditions, it was inferred that keratin could be thereafter effectively converted into amino acids, mainly alanine and glycine.

Hydrothermal processing of biomass for value-added bioactive molecules recovery is a complex process because of its several physicals, chemical, and biological control parameters. These parameters can also have a significant influence on each other and the overall dynamics of the ongoing reactions and processes during the hydrolysis. It has been observed that temperature, pressure, reaction conditions, and concentration bear significant influences on the yield of the target compounds [6, 82, 83]. In view to better understand the process dynamics from a more holistic perspective, the severity factor has been employed in several studies to assess the combined influence of temperature and residence time in hot water treatment processes using different types of biomass (GETACHEW; CHUN, 2017; KOOMYART; NAGAMIZU; KHUWIJITJARU; KOBAYASHI *et al.*, 2017a; KUMAR; GUPTA; LEE; GUPTA, 2010; PIŃKOWSKA; OLIVEROS, 2014; PINTO; ANTAS; SANTOS; BOWRA *et al.*, 2017; POSMANIK; CANTERO; MALKANI; SILLS *et al.*, 2017). According to Posmanik et al. (POSMANIK; CANTERO; MALKANI; SILLS *et al.*, 2017), severity factor provides a measure of the reaction extent in a specific time duration at a particular temperature, where the latter temperature is considered as the reference (i.e. datum). Similar severity factors would theoretically induce similar influences on the reaction dynamics than the studied conditions, but it will always be necessary to perform actual experiment-based validations.

There are studies that attempted to provide insights into the dynamics of amino acid decomposition under hydrothermal processing conditions (MEKONNEN; MUSSONE; EL-THAHER; CHOI *et al.*, 2015). For example, in the study of Klingler et al. (KLINGLER; BERG; VOGEL, 2007) which probed conversion trends and products yields from alanine and glycine in subcritical and supercritical water under the specific conditions (solution concentrations of 1.0 and 2.0% (g g^{-1}) amino acid, temperatures ranging 250–450 °C, residence times of 2.5–35 seconds, and pressures of 24 MPa and 34 MPa), it has been noted that alanine conversion at 34 MPa for a feed solution of 1.0% (g g^{-1}) proceeded faster at temperature increases. The latter process dynamics was set on account of plausible decarboxylation to produce ethylamine, and also deamination to produce lactic acid (with an accumulation of the lactic acid at subcritical temperature and its decomposition to produce acetaldehyde at supercritical temperature. Earlier, Kang et al. (KANG; QUITAIN; DAIMON; NODA *et al.*, 2001) reported a maximum yield of 137 mg g^{-1} dry matter of total amino acids from waste fish entrails hydrolyzed in batch mode at 250 °C and 4 MPa for 60 minutes, but under supercritical conditions (380 °C and 45 MPa), the yield was reduced as a result of the fast amino acid decomposition. Mekonnen et al. (MEKONNEN; MUSSONE; EL-THAHER; CHOI *et al.*, 2015) studied the subcritical hydrolysis of cattle waste tissues and observed that protein recovery and cleavage, production, and degradation of free and total amino acids, as well as the formation of organic acids, were dependent on the temperature.

However, given the variability of substrate composition and the further specificities associated with the hydrothermal reaction conditions, the decomposition pathways and kinetics thereof of each amino acid are expected to be different from system to system. Sato et al. (SATO; QUITAIN; KANG; DAIMON *et al.*, 2004) studied the decomposition dynamics of serine, aspartic acid, leucine, phenylalanine, and alanine at

different temperatures (200−340 °C) at 20 MPa using a continuous-flow tubular reactor configuration, and reported that alanine could be decomposed into pyruvic and lactic acids, before being eventually mineralized to carbon dioxide. In that same work, the degradation rate increased in the following order: alanine, leucine, phenylalanine, serine, and aspartic acid (deamination resulted in ammonia and organic acid production, while decarboxylation led to the production of carbonic acid and amines). It has also been indicated that decarboxylation, deamination, transamination and oxidation could be possible secondary reactions which could account for the different trends in amino acids yield before and after hydrothermolysis (PIŃKOWSKA; OLIVEROS, 2014). In a batch-mode synthesis of amino acids from *Nannochloropsis sp.* microalgae biomass in subcritical water, Zainan et al. (ZAINAN; SAPARDI; HO; SIAJAM *et al.*, 2019) inferred that amino acids production and decomposition could occur rapidly during the process, and based on thermodynamics considerations, the endothermic process was not spontaneous, and thus would require a constant energy supply to maintain the chemica1 reaction proceeding.

Lamoolphak et al. (LAMOOLPHAK; DE-EKNAMKUL; SHOTIPRUK, 2008) demonstrated that in the absence of toxic chemicals, silk waste could be decomposed into protein and amino acids under hydrothermal reaction conditions, and that protein (0.455 mg mg^{-1} silk fiber) and amino acids (0.755 mg mg^{-1} silk fiber) yield of fibroin rose for rising temperature and time with the best reaction temperature of 220 ^{o}C for both; but in the case of sericin, the best protein production (0.466 mg mg^{-1} raw silk) occurred at 120 ^{o}C, while amino acids were best produced (0.203 mg mg^{-1} raw silk) at 160 ^{o}C. When examining the processing of water lettuce biomass under hydrothermal conditions, Luo et al. (LUO; SHI; CHEN; NI *et al.*, 2011) reported that a good protein yield at lower temperature and longer reaction time, and this behavior indicated that higher solubility

had been most plausibly overridden by thermal degradation, and that reaction time, instead of the temperature, had influenced the yields more probably because of lower stability and susceptibility of soluble amino acids for thermal decomposition with time. Another interesting observation made was that alkalis addition (sodium carbonate) had increased the protein yield but decreased amino acids (LUO; SHI; CHEN; NI *et al.*, 2011). By nearing the behavior and influence of the sodium carbonate to that induced by longer reaction time or higher temperature, Luo et al. (LUO; SHI; CHEN; NI *et al.*, 2011) suggested that the use of the alkali catalyst could be expected to reduce the costs associated to energy requirements.

Espinoza and Morawicki (ESPINOZA; MORAWICKI, 2012) reported that sodium bicarbonate, compared to water only had improved the extent of hydrolysis of whey protein isolate four-folds and amino acids production by 44%, but with a lowering in the molecular weight of peptides. Later, in another work, it had been observed that free amino acids' concentrations had been significantly lower when ethanol was added during the subcritical water extraction from porcine placenta (PARK; KIM; MIN; JO *et al.*, 2015). More recently, in the work of Hao et al. (HAO; CAO; LI; CHEN *et al.*, 2019), it was reported that the extraction yield of abalone viscera in subcritical water at 140 °C and 0.36 MPa was in agreement with that obtained by the enzymatic hydrolysis of the same biomass, and that a high subcritical water temperature could promote the dissolution of proteins at the expense of polysaccharide deconstruction. Based on the results of a subcritical water extraction process employed to recover phenolic species from kiwifruit pomace, it was observed that at more prolonged extraction reactions, the decomposition of total free amino acids was favored over their production (KHEIRKHAH; BAROUTIAN; QUEK, 2019). Lamp et al. (LAMP; KALTSCHMITT; LÜDTKE, 2020)

have recently carried a comprehensive study to investigate the effect of temperature, time, and pH during the subcritical water treatment of thin stillage insoluble (TSI), and it was thereof inferred that it is considerably important to perform process optimization of future biological systems' design so that the targeted process outcomes are achieved.

6. Protein aggregation-disaggregation

The aggregation dynamics of proteins is a complex phenomenon generally influenced by environmental factors (JACOBS; GRACE; BLUMLEIN; MCMANUS, 2019; SASAHARA; YAMAGUCHI; SO; GOTO, 2019; TREUHEIT; KOSKY; BREMS, 2002; WANG; NEMA; TEAGARDEN, 2010), occurring in different bioprocesses such as biopharmaceuticals (SHAH, 2018) or enzymatic saccharification of biomass (GOMAA; HIFNEY; FAWZY; ABDEL-GAWAD, 2017; VAID; BAJAJ, 2017). Protein aggregation is a barrier when it comes to the production and purification of bioactive molecules (CROMWELL; HILARIO; JACOBSON, 2006), and it can have impacts on the product quality and performance (ROBERTS, 2014). In several instances, protein aggregation is influenced by temperature, pH, ionic strength, and concentration of excipients (WANG; NEMA; TEAGARDEN, 2010).

According to Wang et al. (WANG; NEMA; TEAGARDEN, 2010), protein aggregation can be a result of unfolding intermediates and unfolded states, protein self-association or chemical linkages, and/or a consequence of chemical degradation. Earlier, Philo and Arakawa (PHILO; ARAKAWA, 2009) described five common aggregation mechanisms for proteins: (i) a reversible association of the natural protein form, (ii) aggregation of conformationally-modified protein, (iii) aggregation of chemically-altered product, (iv) nucleation-controlled aggregation, and (v) surface-induced aggregation which begins with the binding of the native protein to a surface. Wang et al. (WANG;

NEMA; TEAGARDEN, 2010) also indicated that pH plays an essential part in determining the dominant form of the aggregates, because pH acts on the type and distribution of surface charges of proteins and on the extent of the structural disruption. Concerning the effects of the concentration of proteins, a possible aggregation decrease could be the result of a '*crowding*' effect, and be associated to the higher likelihood of proteins association, or precipitates formation emanating from a solubility limit reached with a high protein concentration (WANG; NEMA; TEAGARDEN, 2010).

In hydrothermal systems designed to produce proteins and/or amino acids, mechanical agitation or mixing is sometimes employed to assure the reaction mixture homogeneity. Both shaking and shearing can potentially give rise to protein aggregation, and the aggregation dynamics depends on the intensity and exposure time to the stress resulting from the mechanical forces developed (WANG; NEMA; TEAGARDEN, 2010). It has been observed that the influence of protein concentration was related to the regime of agitation, whereby under motionless (quiescent) shelf-life incubation, aggregation was promoted when protein concentration was increased; whilst agitation had led to more aggregation when the concentration of protein decreased (TREUHEIT; KOSKY; BREMS, 2002). In another work, it was also explained that protein degradation could be induced due to exposure to agitation (BAI; BEE; BIDDLECOMBE; CHEN *et al.*, 2012). Torisu et al. (TORISU; MARUNO; HAMAJI; OHKUBO *et al.*, 2017) reported that dropping could induce the aggregation of protein as a result of cavitation, and also that shaking had a crucial part to play in the dynamics of protein aggregation because of combination stress.

When considering the effects of pressure, pressurization above an absolute pressure can induce protein unfolding and promote its aggregation as a result of more hydrophobic interactions (WANG; NEMA; TEAGARDEN, 2010). In their study, Patel

et al. (PATEL; SINGH; HAVEA; CONSIDINE *et al.*, 2005) have pressure-treated whey protein (WhP) concentrate solutions (12% w v^{-1}, pH 6.65±0.05) at 800 MPa for 20-120 minutes, and observed, that the pressure-treated samples had a time-dependent loss of native WhPs. It was noted that the protein aggregates had affected the samples' viscosity and opacity, and that the aggregates were crosslinked by intermolecular disulphide bonds and by non-covalent interactions (PATEL; SINGH; HAVEA; CONSIDINE *et al.*, 2005).

Based on findings in a study analyzing the influence of dynamic high pressure on whey protein aggregation, it was concluded that aggregation was induced through the collective effect of mechanical forces and heating phenomena arising from short-time thermal treatment (GRÁCIA-JULIÁ; RENÉ; CORTÉS-MUÑOZ; PICART *et al.*, 2008). Changes in the viscosity and non-specific protein–polymer interactions could plausibly affect the structure and dynamics of protein in 'crowded' environments ('crowded' means a simultaneous presence of proteins, carbohydrates and nucleic acids) (BREYDO; REDDY; PIAI; FELLI *et al.*, 2014). Based on their results, Gong et al. (GONG; CHEN; XIA; DAI *et al.*, 2019) inferred that: (i) sulfhydryl/disulfide bonds interchange reactions, (ii) hydrophobic interactions, (iii) electrostatic interactions (analyzed based on ζ–potential data) and (iv) hydrogen bonds (H-bonds), cannot singly cause changes in peanut protein isolates; instead; a combination of these interactions could determine the disaggregation and reaggregation dynamics in aqueous dispersion in high-pressure micro fluidization. Interestingly, it has also been found that plant globular protein aggregation had a consequential bearing on both the solubility and functionality of protein, and also that high-pressure homogenization can be a valuable technique for modifying protein functionality by modulating the aggregation (YANG; LIU; ZENG; CHEN, 2018).

Protein aggregation could affect amino acids recovery in SWH because a medley of competitive interplay of high temperature, high pressure, pH, short reaction time,

variable concentration of bioactive species and different modes of agitation are commonly found in crowded hydrothermal biomass processing systems designed for protein and amino acids production and recovery. Some studies examined the dynamics of protein aggregation and disaggregation during the extraction of specific biomolecules from biomass hydrolyzed under subcritical water conditions. Abdelmoez et al. (ABDELMOEZ; NAKAHASI; YOSHIDA, 2007) found that the co-existence of many amino acids in a mixture had lowered the overall balance because the activation energy for the decomposition of each amino acid had decreased by much, and that the amino acids turned out labile at acidic and near-natural pH, while more stable at highly alkaline pH. Such a priority consideration of the yield of biomass-derived bioactive materials will inherently be of significant practical relevance when determining process parameters and optimal conditions to large-scale proteins-amino acids recovery systems (ABDELMOEZ; NAKAHASI; YOSHIDA, 2007). Sunphorka et al. (SUNPHORKA; CHAVASIRI; OSHIMA; NGAMPRASERTSITH, 2012) studied the production of amino acids from rice bran in subcritical water, and the rate of protein aggregation and protein disaggregation with polypeptide generation, and made a number of important observations: (i) protein aggregation was influenced by temperature and initial concentration of the rice bran, whereby the sharp drop in protein concentration with time risen at higher temperatures (225 °C and 250 °C) being accompanied by a complete aggregation of protein before five minutes, (ii) protein/polypeptides concentration raised after protein aggregation because the aggregated protein thereafter disaggregated could be partially hydrolyzed under subcritical water conditions to obtain polypeptides and amino acids, whose yields were prominently influenced by reaction time and temperature, and (iii) amino acids obtained at higher temperatures were more significant in comparison to lower temperatures because of their thermolabile characteristics.

Aggregation-disaggregation of proteins after subcritical processing can also have a significant influence on the specific properties of the species involved. Such a behavior can be potentially harnessed and adapted to meet the particular requirements of specific applications. Zhang et al. (ZHANG; TU; WANG; HUANG *et al.*, 2015) treated soybean protein isolate (SPI) with subcritical water at different temperatures for 20 minutes, and observed the following outcomes: (i) the treated SPI solution changed from colorless to slight yellow at temperatures of 120 and 160 °C, and eventually turned dark brown at the highest temperature of 200 °C, (ii) the average particle size of SPI was reduced from 263.7 nm to 116.8 nm at a temperature of 120 °C and after that attained a peak average particle size of 1446.1 nm when the temperature was raised to 160 °C as a result of protein aggregation, but declined to 722.9 nm at a higher temperature of 200 °C due to degradation of proteins, and (iii) the subcritical treatment prominently enhanced the emulsifying, solubility and foaming properties (assessed by determining the forming capacity and forming stability) of the SPI.

7. Reactors configuration

Efficient and effective reactors designs are core elements for robust chemical processes, optimization, intensification and scaling up purposes. The relative influences and roles of each component within the reactor configuration have direct bearings on the overall performance, resilience, stability, and greenness of the process. The objective of selecting and putting to use one type of reactor configuration is to maximize the fruitful conversion of reactants and promote selectivity of the species sought as useful, with a high-purity level and marketable; concomitantly, reducing the occurrence of secondary reactions or undesired chemical routes to almost null, if so is practicable. The latter goals remain the central challenge for reactors design and operation, but might not be fully met

as the desired, due to a certain number of limitations. These constraints do crop up and hence require continual investigation and retrofitting to keep the designed processes functional and productive. Some of these constraints are related to:

(i) competing side/secondary reactions which reduce the efficiency;

(ii) different kinetics (either chemical, biochemical, or both) which influence both mass and heat transport phenomena;

(iii) net heat of reactions such as a robust temperature control (and, much plausibly pressure and flow controls) become necessary;

(iv) the need to function within certain limits of the operating range of temperature, pressure, concentration of reactants, dosage of catalyst(s), flow regimes and modes of mixing;

(v) the variable extents of ideal mixing/agitation patterns and vortices which can engender the onset of temperature and concentration gradients within different parts of the reactor and affect the planned process dynamics and expected outputs;

(vi) the battery of various risks and hazards associated with the reactor operation and maintenance;

(vii) impacts of impurities, additives, scaling, fouling, corrosion and plugging on the process dynamics, quality and purity of product(s) recovered, and structural integrity and durability of equipment; and finally,

(viii) total cost for the design, construction, commissioning, operation and maintenance, and decommissioning of the reactor units, and the overall productivity and profitability margins of the process.

The latter limitations become more critical to address in reactors design considerations when the scaling up of one particularly useful and promising small-scale (batch or continuous) configuration is being earnestly envisaged. It has been recently

pointed out that one of the strengths of subcritical hydrolysis, when carried out on a large scale, would be its capability to process feedstocks with high moisture levels without the need for prior drying (ZABOT; ABAIDE; TRES; MAZUTTI, 2019).

It has been observed, in several cases, that empirical data garnered from large-scale reactor systems that involve relatively complex mass flows and heat transport under harsh operating conditions, differ from the results obtained from the corresponding small-scale/lab-scale systems. This type of disagreement can be attributed to the difference in the rate at which temperature rises, whereby the heat-up time of the reactant species to the required temperature of reaction in flow-type reactors usually is less in duration than the heat-up time required in a batch reactor system, since the former is always heated as compared to the latter having to be heated each time (OKAJIMA; SAKO, 2014). For example, Abdelmoez and Yoshida (ABDELMOEZ; YOSHIDA, 2006) had studied the types of temperature profiles developed in a tube batch reactor operated under different subcritical water conditions and inferred that both reactors (shaking and salt bath mixing) had a significant effect on the heating-up rate.

In their study, Cheng et al. (CHENG, H.; ZHU, X.; ZHU, C.; QIAN, J. *et al.*, 2008) ensured that the emulsion injected into the experimental vessel was sufficiently emulsion could be heated-up fast to the desired reaction temperature. Zhang et al. (ZHANG; TU; WANG; HUANG *et al.*, 2015) had carried out subcritical water treatment of soybean protein isolate with different heat-up times, and the results showed that different heat-up times may then also connote having to closely control the temperature and heat transfer, pressure and mixing regimes in the reactor such that any competing set of reaction biokinetics eventually yields the desired moieties in their sought quality. Temperature is a highly critical parameter in subcritical/supercritical water processing of biomass for biomolecules extraction purposes and therefore the control of temperature

rise becomes primordial. However, the compositional variability of the biomass being used as substrates and the specific operational limits and process operation protocols imposed by a particular configuration of reactor result in the need to practice different heat-up times (ZHANG; TU; WANG; HUANG *et al.*, 2015). Over the last ten years, efforts have been made to design, study and improve different types of lab-scale subcritical/supercritical water reactors intended for processing/hydrolyzing biomass to recover proteins and amino acids

7.1. Reactors design

Tracking from 2005 to date, some of the salient reactor design features are following reported. Rogalinski et al. (ROGALINSKI; HERRMANN; BRUNNER, 2005) have conducted sub-critical water hydrolysis of bovine serum albumin for protein production in a continuous-flow unit with the piping and reactor comprising non-corroding and heat-resistant Cr-Ni-stainless steel 1.4404 (3.05mm ID and 6.35 mm OD) under an overpressure of 0.2 MPa with nitrogen, so that the inlet pressure of pump could be increased. Contact between feed air oxygen is prevented. Abdelmoez and Yoshida (ABDELMOEZ; YOSHIDA, 2006; 2013) studied the behavior of fast reactions under subcritical water conditions in a stainless-steel pipe SUS 316 (0.0075 m ID, reactor volume of $9.0\times10^{-}6$ m^3) with Swadgelok caps. Klingler et al. (KLINGLER; BERG; VOGEL, 2007) took measurements of process parameters from a continuous high-pressure plant, which included an electrically heated, gradient-free Inconel 625 jet loop reactor of volume 16 cm^3, and in the reactor studied by Klingler et al. (KLINGLER; BERG; VOGEL, 2007), the negative pressure difference being developed at the jet entrance led the reaction mixture to move downwards again and generated intense mixing by a current flow. Cheng et al. (CHENG, H.; ZHU, X.; ZHU, C.; QIAN, J. *et al.*, 2008)

carried out subcritical water hydrolysis in an experimental vessel pressurized to 2 MPa with nitrogen (air or carbon dioxide). Esteban et al. (ESTEBAN; GARCIA; RAMOS; MARQUEZ, 2010) performed subcritical water hydrolysis of hog hair for the production of amino acid in a 500 mL stirred high temperature/high pressure 316 stainless steel Parr batch reactor mounted in a Harold called heater. Asaduzzaman and Chun (ASADUZZAMAN, A. K.; CHUN, B. S., 2015) treated squid muscle under subcritical water hydrolysis in a 200 mL 276 Hastelloy batch reactor with temperature control. Park et al. (PARK; KIM; MIN; JO *et al.*, 2015) had collaboratively developed a laboratory-scale subcritical water system (SFE SYSTEM, CS-1000), which comprised a control box, the reaction vessel, a water bath, a heater, a pressure controller and a temperature controller. Lu et al. (LU; CHEN; WANG; YANG *et al.*, 2016) processed soy meal dispersions under enzyme-assisted subcritical water conditions in a 20 mL Parr pressure reactor equipped with a thermal control system. Cvetanović et al. (CVETANOVIĆ; ŠVARC-GAJIĆ; GAŠIĆ; TEŠIĆ *et al.*, 2017) investigated a subcritical water extraction process using a homemade high-pressure stainless steel subcritical water extractor/reactor with a total capacity of 1.7 L, a 1500-W heating plate which could allow a heating rate of nearly 10 °C min^{-1}, a vibrating platform installed to promote mass transfer through agitation induced by operating the vibrating platform for 2-6 Hz, and also to reduce local overheating in contact with the heater. Fu et al. (FU; ZHANG; CHEN; LIAO *et al.*, 2018) used an experimental system of a continuous reactor to study the hydrothermal hydrolysis of a microalgae slurry under subcritical conditions (maximum pressure of 2 MPa and maximum temperature of 200 °C). Ahmed and Chun (AHMED; CHUN, 2018) used a 200-mL 276 Hastelloy batch-type laboratory-scale subcritical water hydrolysis unit equipped with temperature control. *Park et al.* (PARK; JEONG; CHUN, 2019) *treated* *Pyropis yezoensis* powder under subcritical water conditions in a 1000 cm^3 Hastelloy 276

batch-type reactor. Hao et al. (HAO; CAO; LI; CHEN *et al.*, 2019) have also used a 2000 cm^3 working volume autoclave consisting of a stirring shaft and 4 rectangular blade impellers to treat abalone viscera under subcritical water. Luo et al. (LUO; SHI; CHEN; NI *et al.*, 2011) had used a reactor (autoclave) consisting of a 3 L cylindrical vessel (ID 0.125 m) equipped with a mixing device and which was held sealed with Swagelok caps during the hydrothermal treatment. Recently, Lamp et al. (LAMP; KALTSCHMITT; LÜDTKE, 2020) have studied the liquid hot treatment of bioethanol stillage in 45 mL stainless steel reactors (High-Pressure Reactor BR-25, Bergh of, Eningen, Germany). Fan et al. (FAN; HU; WANG; YANG *et al.*, 2020) have developed an ultrasound-assisted subcritical water protein/polypeptide extraction system that improved the extraction of kettle configuration to achieve higher extraction efficiency, and the improvement was effected by introducing multilayer-material bags for increasing contact between *Spirulina platensis* samples and the subcritical water. Kheirkhah et al. (KHEIRKHAH; BAROUTIAN; QUEK, 2019) investigated the subcritical water extraction of phenolics from freeze-dried kiwifruit pomace in a 1-L reactor system with the pressure kept at 5 MPa with nitrogen gas.

The literature describes numerous technical considerations of *large-scale* commercial supercritical water oxidation reactor systems specifically for organic wastes processing. However, for the topic of the present analysis, there appears to be a lack of data for subcritical biomass process reactor systems, in particular, for the production and recovery of protein and amino acids at a large scale. However, it is interesting to note that one PDF-version datasheet which provides useful information on the CELABOR's (CELABOR) automated subcritical water lab-scale and pilot-scale plants used in applications such as proteins and insoluble fibers extraction, and some of CELABOR's projects which had used subcritical water for the production of chemicals (e.g.

polyphenols) and energy (e.g. biogas) from certain biomass types. Encouragingly, around the mid of August 2019, Luiz F. Mendes, a chemist and one of the founding partners of BioativosGroup® (**BioativosGroup®**), shared that the firm BioativosGroup® has plans to use subcritical and supercritical fluid extraction technology for the complete conversion of different renewable biomass resources into green intermediate chemicals (FAPESP).

7.2. Kinetic modeling of reactors

The relatively limited pool of experimental work on the application of SWH to produce and recover protein and amino acids from different biomass types is appreciably insightful and diverse in the findings. Besides conducting empirical analyses of hydrothermal biomass processing, which provides a direct way to collect data on process-related system performance, modeling of the processes can also be undertaken. However, a reliable and robust model development of complex processes such as SWH can be confronted with constraints stemming from the complexity and variability of substrates compositions and feed rates; from the influences, competition and predominance of specific reactions; production and decomposition pathways of certain products; and, the interplay and effects of other process parameters e.g. pH, development of parameter gradients and turbulence patterns, and presence of additives. The present literature survey indicated that there are very few studies which have addressed the kinetic modeling of subcritical water hydrolysis of biomass in the specific context of production and recovery of proteins and amino acids. Rogalinski et al. (ROGALINSKI; HERRMANN; BRUNNER, 2005) developed a simplified empirical reaction model with the notion that the substrate was split via the cleavage of peptide bonds within the molecules of the substrate (modeled as a first-order step), and that the amino acids being thus produced were thermally labile and could decompose to give other products. Abdelmoez and

Yoshida (ABDELMOEZ; YOSHIDA, 2006) organized their modeling with the fast reactions occurring in three different stages, viz, heating period, transferring of the reactor from hot salt bath to cooling bath, and cooling down the stage. Therein, in the second stage, the temperature was assumed to be constant. At the same time, it was mainly a careful modeling step when deciding if any reaction continued in the cooling down period. A few years later, Sunphorka et al. (SUNPHORKA; CHAVASIRI; OSHIMA; NGAMPRASERTSITH, 2012) developed a simplified reaction model based on the concept of parallel and consecutive reactions. Recently, in the kinetic model developed by Lamp et al. (LAMP; KALTSCHMITT; LÜDTKE, 2020), the release and degradation of protein were predicted, and the model results were found to be in good agreement with empirical values. Lamp et al. (LAMP; KALTSCHMITT; LÜDTKE, 2020) reported that amino acid degradation had followed a first-order kinetics best.

7.3. Large-scale industrial reactors

Despite the current interest in this specific area of research, the development of large-scale industrial continuous-mode subcritical water processes to recover proteins and amino acids is seemingly slow. There are, however, examples of the application of subcritical conditions for processing biomass to produce other substances. The CatLiq technology has been developed by the Danish company SCF Technologies. In brief, in the CatLiq® process, organic waste is processed to produce oil at subcritical conditions (280–350 °C and 22.5–25 MPa) in the presence of two catalysts (TOOR; ROSENDAHL; NIELSEN; GLASIUS *et al.*, 2012). The ConAgra Butterball Co., USA, conducted the scale-up of a subcritical hydrolysis scheme in 2005 to process 250 tonnes/day of turkey offal and fats (ZABOT; ABAIDE; TRES; MAZUTTI, 2019). The company Renmatix

utilizes water to deconstruct biomass for the conversion of cellulose to cellulosic sugar, in particular (ZABOT; ABAIDE; TRES; MAZUTTI, 2019).

Amongst the pool of articles discussing the features of large-scale commercial supercritical water oxidation reactor systems, the data presented in Xu et al. (XU; WANG; TANG; GONG *et al.*, 2012) and Marrone (MARRONE, 2013) are particularly insightful. Among other aspects, Xu et al. (XU; WANG; TANG; GONG *et al.*, 2012) have indicated that corrosion is a major technical hurdle that limits the commercial application. Toor et al. (TOOR; ROSENDAHL; RUDOLF, 2011) pointed out that corrosion is indeed also a significant issue since acidic and oxidizing conditions can induce fast corrosion. The severity of corrosion is more in subcritical environments than in supercritical conditions because of subcritical water has relatively dense and polar characteristics. However, it is interesting to come across the review of Marrone (MARRONE, 2013), which provided a very insightful account of the status of full-scale commercial supercritical water oxidation reactor systems employed for the destruction of wastes. Moving onto the limits of supercritical water oxidation systems' commercialization, corrosion is notorious for shortening the reactor life and also inducing an adverse treatment effect of the substrate materials as a result of the formation of different corrosion products (RODRIGUEZ; MERWIN; KARMIOL; CHIDAMBARAM, 2017; XU; WANG; TANG; GONG *et al.*, 2012). Hence, to address the critical issues related to the onset and propagation of corrosion in supercritical water oxidation reactor systems, Xu et al. (XU; WANG; TANG; GONG *et al.*, 2012) have highlighted that plugging (which can be described as the agglomeration and deposition of "*sticky salt*" on the internal surfaces of the reactor) is a significant obstacle to supercritical water oxidation commercialization because of subsequent increases in the corrosion rate of reactor which can in turn lead to the inactivation of catalyst(s) and also lower the heat transfer coefficient of the reactor wall.

Moreover, Xu et al. (XU; WANG; TANG; GONG *et al.*, 2012) suggested that heat self-sufficiency is a crucial consideration that has to be adequately addressed if a low running cost is being sought given that heat self-sufficiency will mean that no additional energy will be made available in the operation process and that opportunities to recycle the heat in the reactor effluent into the supercritical water oxidation system should be earnestly envisaged. Later, Marrone (MARRONE, 2013) identified a series of approaches for dealing with corrosion and salt/precipitation/accumulation in supercritical water oxidation systems at the commercial scale: (i) exploring the possibility to use a film-cooled wall reactor or a vortex/circulating flow reactor, (ii) to use high-corrosion resistance materials (e.g. liners, coatings, sacrificial materials) in view to ensure long term operation, (iii) to optimize process operating conditions, in envisaging practicing extreme pressure operation, (iv) to control the type of feeds and their compositions, using additives which can offer surface for nucleation, and (v) to incorporate mechanical brushing or reactor flushing.

8. Perspectives and concluding remarks

Subcritical water processing of biomass for protein and amino acid production and recovery at the laboratory scale has been progressing rather slowly over the last ten years. However, the findings so far reported have provided insights into the several process performance dimensions, dynamics, and obstacles such as corrosion, plugging, and production of inhibitor species. Hampered by many hurdles potentially cropping up, the design and analysis of subcritical water processing systems appear to be very scanty on the largescale, and less so, at the industrial scale. Yet, the plans of no more than a handful of companies, are pretty encouraging. The design and commissioning of some existing

large-scale supercritical water oxidation systems for biomass conversion into biofuel molecules, for example, are clear and encouraging evidence of such promising possibility.

Nevertheless, further research and development efforts are required to sustain a stronger academy-industry cooperation for knowledge creation and sharing. Such collaborations will be set up to address issues related to biomass supply chains and work out cost-effective solutions to troubleshoot the techno-economic subcritical water extraction process-related hurdles. At the heart of research and mandatory development of greener scale-up strategies and potential retrofit schemes, the following aspects will have to be regularly addressed: (i) the elucidation and optimization of the complex biomass-specific reaction mechanisms and the competitions thereof, (ii) the heat transfer dynamics and energy requirements, (iii) the selective production and recovery of specific commercial meaningful moieties, (iv) the delicate control of process parameters such as temperature, residence time, pH, pressure, and presence of additives/impurities, and finally, (v) the computation of the corresponding costs.

Acknowledgments

The authors acknowledge the financial support from the 'Brazilian Science and Research Foundation (CNPq)', the 'Coordination for the Improvement of Higher Education Personnel – Brazil- Finance code 001', and the Novo Nordisk Foundation, Denmark (grant number NNF10CC1016517). Author T.F.C. is also thankful for the financial support received from the São Paulo Research Foundation – FAPESP (2018/05999-0; 2018/14938-4) and CNPq for the productivity grant (302473/2019-0). Author A.M. is grateful to Professor Byung-Soo Chun (Department of Food Science and Technology, Pukyong National University, Republic of Korea) for clarifying a point with regards to the dimension of a reactor design parameter. The manuscript has been checked for similarity in an appropriate software.

References

ABDELMOEZ, W.; NAKAHASI, T.; YOSHIDA, H. Amino acid transformation and decomposition in saturated subcritical water conditions. **Industrial & Engineering Chemistry Research**, 46, n. 16, p. 5286-5294, 2007.

ABDELMOEZ, W.; YOSHIDA, H. Simulation of fast reactions in batch reactors under sub-critical water condition. **AIChE journal**, 52, n. 10, p. 3600-3611, 2006.

ABDELMOEZ, W.; YOSHIDA, H. Production of amino and organic acids from protein using sub-critical water technology. **International Journal of Chemical Reactor Engineering**, 11, n. 1, p. 369-384, 2013.

AHMED, R.; CHUN, B.-S. Subcritical water hydrolysis for the production of bioactive peptides from tuna skin collagen. **The Journal of Supercritical Fluids**, 141, p. 88-96, 2018.

AKALıN, M. K.; TEKIN, K.; KARAGÖZ, S. Supercritical fluid extraction of biofuels from biomass. **Environmental Chemistry Letters**, 15, n. 1, p. 29-41, 2016.

ALAO, B. O.; FALOWO, A. B.; CHULAYO, A.; MUCHENJE, V. The potential of animal by-products in food systems: Production, prospects and challenges. **Sustainability**, 9, n. 7, p. 1089, 2017.

ALVAREZ, C.; RENDUELES, M.; DIAZ, M. The yield of peptides and amino acids following acid hydrolysis of haemoglobin from porcine blood. **Animal Production Science**, 52, n. 5, p. 313, 2012.

ANOOTTHATO, S.; THERDTHAI, N.; RITTHIRUANGDEJ, P. Characterization of protein hydrolysate from silkworm pupae (*Bombyx mori*). **Journal of Food Processing and Preservation**, 43, n. 8, 2019.

ARAUZO, P.; DU, L.; OLSZEWSKI, M.; ZAVALA, M. M. *et al.* Effect of protein during hydrothermal carbonization of brewer's spent grain. **Bioresour Technol**, 293, p. 122117, 2019.

ARNÁIZ, E.; BERNAL, J.; MARTÍN, M.; NOZAL, M. *et al.* Supercritical fluid extraction of free amino acids from broccoli leaves. **Journal of Chromatography A**, 1250, p. 49-53, 2012.

ASADUZZAMAN, A. K.; CHUN, B. S. Recovery of functional materials with thermally stable antioxidative properties in squid muscle hydrolyzates by subcritical water. **J Food Sci Technol**, 52, n. 2, p. 793-802, Feb 2015.

AZMIR, J.; ZAIDUL, I. S. M.; RAHMAN, M. M.; SHARIF, K. M. *et al.* Techniques for extraction of bioactive compounds from plant materials: A review. **Journal of Food Engineering**, 117, n. 4, p. 426-436, 2013.

BAI, G.; BEE, J. S.; BIDDLECOMBE, J. G.; CHEN, Q. *et al.* Computational fluid dynamics (CFD) insights into agitation stress methods in biopharmaceutical development. **International journal of pharmaceutics**, 423, n. 2, p. 264-280, 2012.

BARAKAT, A.; JÉRÔME, F.; ROUAU, X. A Dry Platform for Separation of Proteins from Biomass-Containing Polysaccharides, Lignin, and Polyphenols. **ChemSusChem**, 8, n. 7, p. 1161-1166, 2015.

BioativosGroup®. Disponível em: http://bioativosgroup.com.br/.

BREYDO, L.; REDDY, K. D.; PIAI, A.; FELLI, I. C. *et al.* The crowd you're in with: effects of different types of crowding agents on protein aggregation. **Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics**, 1844, n. 2, p. 346-357, 2014.

BRUNNER, G. Near critical and supercritical water. Part I. Hydrolytic and hydrothermal processes. **The Journal of Supercritical Fluids**, 47, n. 3, p. 373-381, 2009.

CASTRO-VARGAS, H. I.; RODRÍGUEZ-VARELA, L. I.; FERREIRA, S. R.; PARADA-ALFONSO, F. Extraction of phenolic fraction from guava seeds (Psidium

guajava L.) using supercritical carbon dioxide and co-solvents. **The Journal of Supercritical Fluids**, 51, n. 3, p. 319-324, 2010.

CELABOR. **CELABOR's automated subcritical water lab-scale and pilot-scale plants**. Disponível em: http://www.celabor.be/site/FCK_STOCK/Media/2016-08%20Eau%20subcritique%20EN.pdf. Acesso em: May 8.

CHEIGH, C. I.; YOO, S. Y.; KO, M. J.; CHANG, P. S. *et al.* Extraction characteristics of subcritical water depending on the number of hydroxyl group in flavonols. **Food Chemistry**, 168, p. 21-26, Feb 1 2015. Research Support, Non-U.S. Gov't.

CHENG, H.; ZHU, X.; ZHU, C.; QIAN, J. *et al.* Hydrolysis technology of biomass waste to produce amino acids in sub-critical water. **Bioresour Technol**, 99, n. 9, p. 3337-3341, Jun 2008. Research Support, Non-U.S. Gov't.

COCERO, M. J.; CABEZA, A.; ABAD, N.; ADAMOVIC, T. *et al.* Understanding biomass fractionation in subcritical & supercritical water. **The Journal of Supercritical Fluids**, 133, p. 550-565, 2018.

CROMWELL, M. E.; HILARIO, E.; JACOBSON, F. Protein aggregation and bioprocessing. **The AAPS journal**, 8, n. 3, p. E572-E579, 2006.

CVETANOVIĆ, A.; ŠVARC-GAJIĆ, J.; GAŠIĆ, U.; TEŠIĆ, Ž. *et al.* Isolation of apigenin from subcritical water extracts: optimization of the process. **The Journal of Supercritical Fluids**, 120, p. 32-42, 2017.

DE ANDRADE LIMA, M.; CHARALAMPOPOULOS, D.; CHATZIFRAGKOU, A. Purification of supercritical-fluid carotenoid-rich extracts by hydrophobic interaction chromatography. **Separation and Purification Technology**, 203, p. 1-10, 2018.

DE MOURA, J. M.; GONÇALVES, L. A.; SARMENTO, L. A.; PETRUS, J. C. C. Purification of structured lipids using SCCO2 and membrane process. **Journal of Membrane Science**, 299, n. 1-2, p. 138-145, 2007.

DE SCHOUWER, F.; CLAES, L.; VANDEKERKHOVE, A.; VERDUYCKT, J. *et al.* Protein-Rich Biomass Waste as a Resource for Future Biorefineries: State of the Art, Challenges, and Opportunities. **ChemSusChem**, 12, n. 7, p. 1272-1303, 2019.

DENG, G.-F.; SHEN, C.; XU, X.-R.; KUANG, R.-D. *et al.* Potential of Fruit Wastes as Natural Resources of Bioactive Compounds. **International Journal of Molecular Sciences**, 13, n. 7, p. 8308-8323, 2012.

DERWENSKUS, F.; METZ, F.; GILLE, A.; SCHMID-STAIGER, U. *et al.* Pressurized extraction of unsaturated fatty acids and carotenoids from wet Chlorella vulgaris and

Phaeodactylum tricornutum biomass using subcritical liquids. **Global Change Biology Bioenergy**, 11, n. 1, p. 335-344, 2019.

DRAGONE, G.; KERSSEMAKERS, A. A.; DRIESSEN, J. L.; YAMAKAWA, C. K. *et al.* Innovation and strategic orientations for the development of advanced biorefineries. **Bioresour Technol**, p. 122847, 2020.

ESPINOZA, A. D.; MORAWICKI, R. O. Effect of additives on subcritical water hydrolysis of whey protein isolate. **J Agric Food Chem**, 60, n. 20, p. 5250-5256, May 23 2012.

ESTEBAN, M. B.; GARCIA, A. J.; RAMOS, P.; MARQUEZ, M. C. Sub-critical water hydrolysis of hog hair for amino acid production. **Bioresour Technol**, 101, n. 7, p. 2472-2476, Apr 2010.

FAN, X.; HU, S.; WANG, K.; YANG, R. *et al.* Coupling of ultrasound and subcritical water for peptides production from Spirulina platensis. **Food and Bioproducts Processing**, 121, p. 105-112, 2020.

FAPESP. **biorefinery will convert renewable biomass into green intermediate chemicals**. Disponível em: https://agencia.fapesp.br/biorefinery-will-convert-renewable-biomass-into-green-intermediate-chemicals/31186/. Acesso em: April 14.

FARMER, T. J.; MASCAL, M. Platform molecules. **Introduction to chemicals from biomass**, 2, 2015.

FU, Q.; ZHANG, H.; CHEN, H.; LIAO, Q. *et al.* Hydrothermal hydrolysis pretreatment of microalgae slurries in a continuous reactor under subcritical conditions for large–scale application. **Bioresour Technol**, 266, p. 306-314, 2018.

GARCIA-MOSCOSO, J. L.; TEYMOURI, A.; KUMAR, S. Kinetics of Peptides and Arginine Production from Microalgae (Scenedesmus sp.) by Flash Hydrolysis. **Industrial & Engineering Chemistry Research**, 54, n. 7, p. 2048-2058, 2015.

GBASHI, S.; ADEBO, O. A.; PIATER, L.; MADALA, N. E. *et al.* Subcritical water extraction of biological materials. **Separation & Purification Reviews**, 46, n. 1, p. 21-34, 2017.

GETACHEW, A. T.; CHUN, B. S. Influence of pretreatment and modifiers on subcritical water liquefaction of spent coffee grounds: A green waste valorization approach. **Journal of Cleaner Production**, 142, p. 3719-3727, 2017.

GOMAA, M.; HIFNEY, A. F.; FAWZY, M. A.; ABDEL-GAWAD, K. M. Statistical optimization of culture variables for enhancing agarase production by Dendryphiella

arenaria utilizing Palisada perforata (Rhodophyta) and enzymatic saccharification of the macroalgal biomass. **Marine Biotechnology**, 19, n. 6, p. 592-600, 2017.

GONG, K.; CHEN, L.; XIA, H.; DAI, H. *et al.* Driving forces of disaggregation and reaggregation of peanut protein isolates in aqueous dispersion induced by high-pressure microfluidization. **International journal of biological macromolecules**, 130, p. 915-921, 2019.

GOVENDER, K.; NAICKER, T.; BAIJNATH, S.; CHUTURGOON, A. A. *et al.* The development of a supercritical fluid chromatography (SFC) based method for the purification of biosynthesised human insulin. **Journal of Chromatography B**, p. 122126, 2020.

GRÁCIA-JULIÁ, A.; RENÉ, M.; CORTÉS-MUÑOZ, M.; PICART, L. *et al.* Effect of dynamic high pressure on whey protein aggregation: A comparison with the effect of continuous short-time thermal treatments. **Food hydrocolloids**, 22, n. 6, p. 1014-1032, 2008.

HAO, G.; CAO, W.; LI, T.; CHEN, J. *et al.* Effect of temperature on chemical properties and antioxidant activities of abalone viscera subcritical water extract. **The Journal of Supercritical Fluids**, 147, p. 17-23, 2019.

HARADA, M.; KARAKAWA, S.; YAMADA, N.; MIYANO, H. *et al.* Biaryl axially chiral derivatizing agent for simultaneous separation and sensitive detection of proteinogenic amino acid enantiomers using liquid chromatography–tandem mass spectrometry. **Journal of Chromatography A**, 1593, p. 91-101, 2019.

HERRERO, M.; CIFUENTES, A.; IBAÑEZ, E. Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. **Food Chemistry**, 98, n. 1, p. 136-148, 2006.

HOSSAIN, G. S.; LI, J.; SHIN, H.-D.; CHEN, R. R. *et al.* Bioconversion of l-glutamic acid to α-ketoglutaric acid by an immobilized whole-cell biocatalyst expressing l-amino acid deaminase from Proteus mirabilis. **Journal of biotechnology**, 169, p. 112-120, 2014.

INFORMATION, N. C. F. B. **PubChem Compound Summary for CID 138, 5-Aminovaleric acid**. 2004. Disponível em: https://pubchem.ncbi.nlm.nih.gov/compound/5-Aminovaleric-acid. Acesso em: 31 July.

IVANOV, K.; IVANOVA, S.; GEORGIEVA, M.; ATANASOV, P. Production and regulatory analytical control of amino acids include in food additives. **Pharmacia**, 61, n. 2, p. 48-54, 2014.

JACOBS, M. R.; GRACE, M.; BLUMLEIN, A.; MCMANUS, J. J. Differential Scanning Calorimetry to Quantify Heat-Induced Aggregation in Concentrated Protein Solutions. *In*: **Protein Self-Assembly**: Springer, 2019. p. 117-129.

JAESCHKE, D. P.; MERCALI, G. D.; MARCZAK, L. D. F.; MULLER, G. *et al.* Extraction of valuable compounds from Arthrospira platensis using pulsed electric field treatment. **Bioresour Technol**, 283, p. 207-212, Jul 2019.

KANG, K.; QUITAIN, A. T.; DAIMON, H.; NODA, R. *et al.* Optimization of amino acids production from waste fish entrails by hydrolysis in sub and supercritical water. **The Canadian Journal of Chemical Engineering**, 79, n. 1, p. 65-70, 2001.

KHEIRKHAH, H.; BAROUTIAN, S.; QUEK, S. Y. Evaluation of bioactive compounds extracted from Hayward kiwifruit pomace by subcritical water extraction. **Food and Bioproducts Processing**, 115, p. 143-153, 2019.

KIAMAHALLEH, M. V.; NAJAFPOUR-DARZI, G.; RAHIMNEJAD, M.; MOGHADAMNIA, A. A. *et al.* High performance curcumin subcritical water extraction from turmeric (Curcuma longa L.). **Journal of Chromatography B**, 1022, p. 191-198, 2016.

KING, J. W. Advances in critical fluid technology for food processing. **Food science & technology today : the information journal of the Institute of Food Science & Technology (UK)**, 2000 v.14 no.4, n. no. 4, p. pp. 186-191, 2000 2000. Article.

KLINGLER, D.; BERG, J.; VOGEL, H. Hydrothermal reactions of alanine and glycine in sub-and supercritical water. **The Journal of Supercritical Fluids**, 43, n. 1, p. 112-119, 2007.

KNEZ, Ž.; HRNČIČ, M. K.; ČOLNIK, M.; ŠKERGET, M. Chemicals and value added compounds from biomass using sub- and supercritical water. **The Journal of Supercritical Fluids**, 133, p. 591-602, 2018.

KOOMYART, I.; NAGAMIZU, H.; KHUWIJITJARU, P.; KOBAYASHI, T. *et al.* Astaxanthin stability and color change of krill during subcritical water treatment. **J Food Sci Technol**, 54, n. 10, p. 3065-3072, 2017.

KUMAR, S.; GUPTA, R.; LEE, Y.; GUPTA, R. B. Cellulose pretreatment in subcritical water: effect of temperature on molecular structure and enzymatic reactivity. **Bioresour Technol**, 101, n. 4, p. 1337-1347, 2010.

LACHOS-PEREZ, D.; BROWN, A. B.; MUDHOO, A.; TIMKO, M. T. *et al.* Applications of subcritical and supercritical water conditions for extraction, hydrolysis,

gasification, and carbonization of biomass: a critical review. **Biofuel Research Journal**, 4, n. 2, p. 611-626, 2017.

LAMMENS, T. M.; FRANSSEN, M. C. R.; SCOTT, E. L.; SANDERS, J. P. M. Availability of protein-derived amino acids as feedstock for the production of bio-based chemicals. **Biomass and Bioenergy**, 44, p. 168-181, 2012.

LAMMENS, T. M.; LE NÔTRE, J.; FRANSSEN, M. C.; SCOTT, E. L. *et al.* Synthesis of biobased succinonitrile from glutamic acid and glutamine. **ChemSusChem**, 4, n. 6, p. 785, 2011.

LAMOOLPHAK, W.; DE-EKNAMKUL, W.; SHOTIPRUK, A. Hydrothermal production and characterization of protein and amino acids from silk waste. **Bioresour Technol**, 99, n. 16, p. 7678-7685, Nov 2008. Research Support, Non-U.S. Gov't.

LAMP, A.; KALTSCHMITT, M.; LÜDTKE, O. Protein recovery from bioethanol stillage by liquid hot water treatment. **The Journal of Supercritical Fluids**, 155, p. 104624, 2020.

LE NÔTRE, J.; SCOTT, E. L.; FRANSSEN, M. C.; SANDERS, J. P. Biobased synthesis of acrylonitrile from glutamic acid. **Green Chemistry**, 13, n. 4, p. 807-809, 2011.

LI, P.; SAKURAGI, K.; MAKINO, H. Extraction techniques in sustainable biofuel production: A concise review. **Fuel Processing Technology**, 193, p. 295-303, 2019.

LI, S. Y.; NG, I. S.; CHEN, P. T.; CHIANG, C. J. *et al.* Biorefining of protein waste for production of sustainable fuels and chemicals. **Biotechnol Biofuels**, 11, p. 256, 2018. Review.

LIN, X.; PAN, J.; ZHOU, M.; XU, Y. *et al.* Extraction of amphoteric amino acid by bipolar membrane electrodialysis: methionine acid as a case study. **Industrial & Engineering Chemistry Research**, 55, n. 10, p. 2813-2820, 2016.

LU, W.; CHEN, X.-W.; WANG, J.-M.; YANG, X.-Q. *et al.* Enzyme-assisted subcritical water extraction and characterization of soy protein from heat-denatured meal. **Journal of Food Engineering**, 169, p. 250-258, 2016.

LULLIEN-PELLERIN, V.; BALNY, C. High-pressure as a tool to study some proteins' properties: conformational modification, activity and oligomeric dissociation. **Innovative Food Science and Emerging Technologies**, 3, p. 209-221, 2002.

LUO, G. E.; SHI, W.; CHEN, X.; NI, W. *et al.* Hydrothermal conversion of water lettuce biomass at 473 or 523 K. **Biomass and Bioenergy**, 35, n. 12, p. 4855-4861, 2011.

MARCET, I.; ALVAREZ, C.; PAREDES, B.; DIAZ, M. The use of sub-critical water hydrolysis for the recovery of peptides and free amino acids from food processing wastes. Review of sources and main parameters. **Waste Manag**, 49, p. 364-371, Mar 2016. Research Support, Non-U.S. Gov't

Review.

MARRONE, P. A. Supercritical water oxidation—current status of full-scale commercial activity for waste destruction. **The Journal of Supercritical Fluids**, 79, p. 283-288, 2013.

MEKONNEN, T. H.; MUSSONE, P. G.; EL-THAHER, N.; CHOI, P. *et al.* Subcritical hydrolysis and characterization of waste proteinaceous biomass for value added applications. **Journal of Chemical Technology & Biotechnology**, 90, n. 3, p. 476-483, 2015.

MOHD THANI, N.; MUSTAPA KAMAL, S. M.; TAIP, F. S.; SULAIMAN, A. *et al.* Effect of sub-critical water hydrolysis on sugar recovery from bakery leftovers. **Food and Bioproducts Processing**, 117, p. 105-112, 2019.

MUSSATTO, S. I. **Biomass fractionation technologies for a lignocellulosic feedstock based biorefinery**. Elsevier, 2016. 0128025611.

OKAJIMA, I.; SAKO, T. Energy conversion of biomass with supercritical and subcritical water using large-scale plants. **Journal of bioscience and bioengineering**, 117, n. 1, p. 1-9, 2014.

PARK, J.-S.; JEONG, Y.-R.; CHUN, B.-S. Physiological activities and bioactive compound from laver (Pyropia yezoensis) hydrolysates by using subcritical water hydrolysis. **The Journal of Supercritical Fluids**, 148, p. 130-136, 2019.

PARK, S. H.; KIM, J. H.; MIN, S. G.; JO, Y. J. *et al.* Effects of Ethanol Addition on the Efficiency of Subcritical Water Extraction of Proteins and Amino Acids from Porcine Placenta. **Korean J Food Sci Anim Resour**, 35, n. 2, p. 265-271, 2015.

PATEL, H. A.; SINGH, H.; HAVEA, P.; CONSIDINE, T. *et al.* Pressure-induced unfolding and aggregation of the proteins in whey protein concentrate solutions. **J Agric Food Chem**, 53, n. 24, p. 9590-9601, 2005.

PEDRAS, B. M. S. Valorization of grape pomace through hot compressed water extraction/hydrolysis. 2015.

PEREIRA, D. G.; JUSTUS, A.; FALCÃO, H. G.; ROCHA, T. D. S. *et al.* Enzymatic hydrolysis of okara protein concentrate by mixture of endo and exopeptidase. **Journal of Food Processing and Preservation**, 2019.

PHILO, J. S.; ARAKAWA, T. Mechanisms of protein aggregation. **Current pharmaceutical biotechnology**, 10, n. 4, p. 348-351, 2009.

PIŃKOWSKA, H.; OLIVEROS, E. Application of the Doehlert Matrix for the Determination of the Optimal Conditions of Hydrolysis of Soybean Protein in Subcritical Water. **Industrial & Engineering Chemistry Research**, 53, n. 4, p. 1320-1326, 2014.

PIŃKOWSKA, H.; OLIVEROS, E. Application of the doehlert matrix for the determination of the optimal conditions of hydrolysis of soybean protein in subcritical water. **Industrial & Engineering Chemistry Research**, 53, n. 4, p. 1320-1326, 2014.

PINTO, A. R. R.; ANTAS, F.; SANTOS, R. C.; BOWRA, S. *et al.* Effect of reactor configuration on the subcritical water hydrolysis of recycled paper mill sludge. **Journal of Analytical and Applied Pyrolysis**, 127, p. 68-74, 2017.

POPESCU, V.; ASTANEI, D.-G.; BURLICA, R.; POPESCU, A. *et al.* Sustainable and cleaner microwave-assisted dyeing process for obtaining eco-friendly and fluorescent acrylic knitted fabrics. **Journal of Cleaner Production**, 232, p. 451-461, 2019.

POSMANIK, R.; CANTERO, D. A.; MALKANI, A.; SILLS, D. L. *et al.* Biomass conversion to bio-oil using sub-critical water: Study of model compounds for food processing waste. **The Journal of Supercritical Fluids**, 119, p. 26-35, 2017.

QIN, F.; JOHANSEN, A. Z.; MUSSATTO, S. I. Evaluation of different pretreatment strategies for protein extraction from brewer's spent grains. **Industrial Crops and Products**, 125, p. 443-453, 2018.

REDDY, N.; SANTOSH, M. Recovery and Applications of Feather Proteins. *In*: **Protein Byproducts**: Elsevier, 2016. p. 255-274.

ROBERTS, C. J. Protein aggregation and its impact on product quality. **Current opinion in biotechnology**, 30, p. 211-217, 2014.

RODILES-LOPEZ, J. O.; ARROYO-MAYA, I. J.; JARAMILLO-FLORES, M. E.; GUTIERREZ-LOPEZ, G. F. *et al.* Effects of high hydrostatic pressure on the structure of bovine alpha-lactalbumin. **J Dairy Sci**, 93, n. 4, p. 1420-1428, Apr 2010. Research Support, Non-U.S. Gov't.

RODRIGUEZ, D.; MERWIN, A.; KARMIOL, Z.; CHIDAMBARAM, D. Surface chemistry and corrosion behavior of Inconel 625 and 718 in subcritical, supercritical, and ultrasupercritical water. **Applied Surface Science**, 404, p. 443-451, 2017.

ROGALINSKI, T.; HERRMANN, S.; BRUNNER, G. Production of amino acids from bovine serum albumin by continuous sub-critical water hydrolysis. **The Journal of Supercritical Fluids**, 36, n. 1, p. 49-58, 2005.

SALA, S.; GORALCZYK, M. Chemical footprint: A methodological framework for bridging life cycle assessment and planetary boundaries for chemical pollution. **Integrated environmental assessment and management**, 9, n. 4, p. 623-632, 2013.

SANTAMARIA-FERNANDEZ, M.; MOLINUEVO-SALCES, B.; LÜBECK, M.; UELLENDAHL, H. Biogas potential of green biomass after protein extraction in an organic biorefinery concept for feed, fuel and fertilizer production. **Renewable energy**, 129, p. 769-775, 2018.

SARAVANA, P. S.; TILAHUN, A.; GERENEW, C.; TRI, V. D. *et al.* Subcritical water extraction of fucoidan from Saccharina japonica: optimization, characterization and biological studies. **Journal of Applied Phycology**, 30, n. 1, p. 579-590, 2018.

SARFARAZI, M.; JAFARI, S. M.; RAJABZADEH, G.; FEIZI, J. Development of an environmentally-friendly solvent-free extraction of saffron bioactives using subcritical water. **LWT**, 114, p. 108428, 2019.

SASAHARA, K.; YAMAGUCHI, K.; SO, M.; GOTO, Y. Polyphosphates diminish solubility of a globular protein and thereby promote amyloid aggregation. **Journal of Biological Chemistry**, 294, n. 42, p. 15318-15329, 2019.

SATO, N.; QUITAIN, A. T.; KANG, K.; DAIMON, H. *et al.* Reaction kinetics of amino acid decomposition in high-temperature and high-pressure water. **Industrial & Engineering Chemistry Research**, 43, n. 13, p. 3217-3222, 2004.

SCOPUS. 2020. Disponível em: https://www.scopus.com/. Acesso em: july 17.

SEREEWATTHANAWUT, I.; PRAPINTIP, S.; WATCHIRARUJI, K.; GOTO, M. *et al.* Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. **Bioresour Technol**, 99, n. 3, p. 555-561, Feb 2008. Research Support, Non-U.S. Gov't.

SHAH, M. Commentary: New perspectives on protein aggregation during Biopharmaceutical development. **International journal of pharmaceutics**, 552, n. 1-2, p. 1-6, 2018.

SHITU, A.; IZHAR, S.; TAHIR, T. M. Sub-critical water as a green solvent for production of valuable materials from agricultural waste biomass: A review of recent work. **Global Journal of Environmental Science and Management**, p. 255-264, 2015.

SIGMA-ALDRICH. **Glutamic acid price table**. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Glutamic+acid&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **Isoleucine price table**. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Isoleucine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **Lysine price table**. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Lysine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **Tryptophan**. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Tryptophan&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **Histidine price table**. 2020a. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Histidine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **L-Cystine price table**. 2020b. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Cystine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SMELLER, L. Pressure-temperature phase diagrams of biomolecules. **Biochimica et Biophysica Acta**, 1595, p. 11-29, 2001.

SOLATI, Z.; MANEVSKI, K.; JØRGENSEN, U.; LABOURIAU, R. *et al.* Crude protein yield and theoretical extractable true protein of potential biorefinery feedstocks. **Industrial Crops and Products**, 115, p. 214-226, 2018.

SUNPHORKA, S.; CHAVASIRI, W.; OSHIMA, Y.; NGAMPRASERTSITH, S. Kinetic studies on rice bran protein hydrolysis in subcritical water. **The Journal of Supercritical Fluids**, 65, p. 54-60, 2012.

TOOR, S. S.; ROSENDAHL, L.; NIELSEN, M. P.; GLASIUS, M. *et al.* Continuous production of bio-oil by catalytic liquefaction from wet distiller's grain with solubles (WDGS) from bio-ethanol production. **Biomass and Bioenergy**, 36, p. 327-332, 2012.

TOOR, S. S.; ROSENDAHL, L.; RUDOLF, A. Hydrothermal liquefaction of biomass: A review of subcritical water technologies. **Energy**, 36, n. 5, p. 2328-2342, 2011.

TORISU, T.; MARUNO, T.; HAMAJI, Y.; OHKUBO, T. *et al.* Synergistic effect of cavitation and agitation on protein aggregation. **Journal of pharmaceutical sciences**, 106, n. 2, p. 521-529, 2017.

TORRES-MAYANGA, P. C.; LACHOS-PEREZ, D.; MUDHOO, A.; KUMAR, S. *et al.* Production of biofuel precursors and value-added chemicals from hydrolysates resulting from hydrothermal processing of biomass: A review. **Biomass and Bioenergy**, 130, p. 105397, 2019.

TREUHEIT, M. J.; KOSKY, A. A.; BREMS, D. N. Inverse relationship of protein concentration and aggregation. **Pharmaceutical research**, 19, n. 4, p. 511-516, 2002.

TUCK, C. O.; PÉREZ, E.; HORVÁTH, I. T.; SHELDON, R. A. *et al.* Valorization of biomass: deriving more value from waste. **Science**, 337, n. 6095, p. 695-699, 2012.

TUTUNCHI, P.; ROUFEGARINEJAD, L.; HAMISHEHKAR, H.; ALIZADEH, A. Extraction of red beet extract with beta-cyclodextrin-enhanced ultrasound assisted extraction: A strategy for enhancing the extraction efficacy of bioactive compounds and their stability in food models. **Food Chemistry**, 297, Nov 2019. Article.

UEMATSU, M.; FRANK, E. U. Static Dielectric Constant of Water and Steam. **Journal of Physical and Chemical Reference Data**, 9, n. 4, p. 1291-1306, 1980.

UENO, S.; ICHINOI, H.; ZHAO, J.; FUJII, T. Degradation of fish gelatin using hot-compressed water and the properties of the degradation products. **High Pressure Research**, 35, n. 2, p. 203-213, 2015.

VAID, S.; BAJAJ, B. K. Production of ionic liquid tolerant cellulase from Bacillus subtilis G 2 using agroindustrial residues with application potential for saccharification of biomass under one pot consolidated bioprocess. **Waste and biomass valorization**, 8, n. 3, p. 949-964, 2017.

WANG, W.; NEMA, S.; TEAGARDEN, D. Protein aggregation—pathways and influencing factors. **International journal of pharmaceutics**, 390, n. 2, p. 89-99, 2010.

Web of Science. 2020. Disponível em: https://webofknowledge.com/. Acesso em: july 17.

XU, D.; WANG, S.; TANG, X.; GONG, Y. *et al.* Design of the first pilot scale plant of China for supercritical water oxidation of sewage sludge. **Chemical Engineering Research and Design**, 90, n. 2, p. 288-297, 2012.

YANG, J.; LIU, G.; ZENG, H.; CHEN, L. Effects of high pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties. **Food hydrocolloids**, 83, p. 275-286, 2018.

YANG, Q.; CAI, X.; LIU, Z.; WANG, S. Antioxidant Assessment of Schizochytrium Meal Protein Enzymatic Hydrolysate and Its Potential Application. **Journal of Aquatic Food Product Technology**, 28, n. 4, p. 413-426, 2019.

YIN, X.-C.; LI, F.-Y.; HE, Y.-F.; WANG, Y. *et al.* Study on effective extraction of chicken feather keratins and their films for controlling drug release. **Biomaterials Science**, 1, n. 5, p. 528-536, 2013.

ZABOT, G. L.; ABAIDE, E. R.; TRES, M. V.; MAZUTTI, M. A. Subcritical hydrolysis contribution in the holistic biorefinery concept: obtaining bioproducts and biofuels from renewable natural resources for a novel bioeconomy. *In*: **Advanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts**: Elsevier, 2019. p. 35-57.

ZAINAN, N. H.; SAPARDI, M. A. M.; HO, B. C. H.; SIAJAM, S. I. *et al.* Kinetic and thermodynamic characterization of amino acids generation via subcritical water reaction of microalgae Nannochloropsis sp. biomass. **Biomass Conversion and Biorefinery**, p. 1-14, 2019.

ZHANG, M.-L.; FAN, Y.-T.; XING, Y.; PAN, C.-M. *et al.* Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures. **Biomass and Bioenergy**, 31, n. 4, p. 250-254, 2007.

ZHANG, Q.-T.; TU, Z.-C.; WANG, H.; HUANG, X.-Q. *et al.* Functional properties and structure changes of soybean protein isolate after subcritical water treatment. **J Food Sci Technol**, 52, n. 6, p. 3412-3421, 2015.

ZHANG, X.; JIANG, D.; LI, D.; YU, C. *et al.* Characterization of a seafood-flavoring enzymatic hydrolysate from brown alga Laminaria japonica. **Journal of Food Measurement and Characterization**, 13, n. 2, p. 1185-1194, 2019.

ZHENG, P.; HAO, G.; WENG, W.; REN, H. Antioxidant Activities of Hydrolysates from Abalone Viscera Using Subcritical Water-Assisted Enzymatic Hydrolysis. **Food and Bioprocess Technology**, 12, n. 6, p. 910-918, 2019.

ZHOU, F.; HEARNE, Z.; LI, C.-J. Water—the greenest solvent overall. **Current Opinion in Green and Sustainable Chemistry**, 18, p. 118-123, 2019.

ZHU, G.; ZHU, X.; FAN, Q.; LIU, X. *et al.* Study on Production of Amino Acids from Bean Dregs by Hydrolysis in Sub-critical Water. **Chinese Journal of Chemestry**, 28, p. 2033-2038, 2010.

ZHU, G.; ZHU, X.; FAN, Q.; WAN, X. Recovery of biomass wastes by hydrolysis in sub-critical water. **Resources, Conservation and Recycling**, 55, n. 4, p. 409-416, 2011.

ZHU, G.; ZHU, X.; XIAO, Z.; ZHOU, R. *et al.* A review of amino acids extraction from animal waste biomass and reducing sugars extraction from plant waste biomass by a clean method. **Biomass Conversion and Biorefinery**, 5, n. 3, p. 309-320, 2015.

ZULUAGA-DOMÍNGUEZ, C.; CASTRO-MERCADO, L.; CECILIA QUICAZÁN, M. Effect of enzymatic hydrolysis on structural characteristics and bioactive composition of bee-pollen. **Journal of Food Processing and Preservation**, 43, n. 7, 2019.

CAPÍTULO 3 – Hidrólise com água subcrítica de penas de aves para produção de aminoácidos.

Artigo submetido para publicação em 2021 na The Journal of Supercritical Fluids

(Impact Factor: 3.4)

Subcritical water hydrolysis of poultry feathers for amino acids production

Henrique Di Domenico Ziero[a]; Larissa Castro Ampese; William G. Sganzerla; Paulo C.T. Mayanga[a], Michael T. Timko[b]; Solange I. Mussatto[c]; T. Forster-Carneiro[a*]

[a] *School of Food Engineering, University of Campinas (UNICAMP), Rua Monteiro Lobato, n.80, 13083-862 Campinas, SP, Brazil.*

[b] *Department of Chemical Engineering. Worcester Polytechnic Institute. 100 Institute Road, Goddard Hall 123. Worcester MA 01609.*

[c] *Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet, Building 220, 2800, Kongens Lyngby, Denmark.*

* Corresponding author: E-mail address: taniafc@unicamp.br

Highlights

- Subcritical water hydrolysis (SWH) was used to obtained amino acids from poultry feathers
- Hydrolysis temperature and water flow rate were investigated in full factorial design
- The highest amino acids yield was 2.7 ± 0,2 g/L at 250 °C and 5 mL/min
- Alanine, proline, and tryptophan were obtained in the highest yields
- Subcritical hydrolysis is a chemical-free alternative to recover amino acids from protein-rich residues

Abstract

The objective of this study was to assess subcritical water hydrolysis (SWH) to recover amino acids from poultry feathers, a protein-rich waste feed. The semi-continuous reactor (110 mL) was operated over a range of temperatures (210 – 250 °C) and water flow rates (5 – 15 mL/min) at constant mass loading of the feed (10 g). Both non-essential amino acids (serine, aspartic acid, alanine, glycine, proline) and essential ones (tryptophan, isoleucine, methionine, valine) were obtained from SWH. A complete factorial design and response surface methodology (RSM) was used to optimize the amino acid production from poultry feathers. SWH has promise as a chemical-free and economical approach for extracting amino acids from waste protein sources, with significant scope remaining to tune conditions to optimize yields.

Keywords: *Hydrothermal Technology, Fluid Pressurized, Animal Waste, Protein residue;*

Introduction

Responsible disposal and re-use of agro-industrial residues is a critical issue for the economy and the environment (ADRIAN; ELIZABETH, 2021). The circular economy attempts to place an added value on wastes to divert them from environmental disposal, incineration, or landfills, representing a new concept for the re-use and valorization of agro-industrial residues (CENTOBELLI; CERCHIONE; CHIARONI; DEL VECCHIO *et al.*, 2020; UBANDO; FELIX; CHEN, 2020). Successful implementation of the circular economy will require judicious use of existing technologies and the development of new ones, especially for the valorization of wastes with unusual properties (BERGENDAHL; SARKIS; TIMKO, 2018).

Protein-rich residues, including poultry feathers, have potential as a feed for the production of amino acids (VELVIZHI; SHANTHAKUMAR; DAS; PUGAZHENDHI *et al.*, 2020; WAINAINA; AWASTHI; SARSAIYA; CHEN *et al.*, 2020). Poultry feathers are generated in large quantities during chicken and turkey production (CAMPOS; VALENTE; MATOS; MARQUES *et al.*, 2020). Worldwide, approximately 8.5×107 tons of chickens are annually produced in the poultry industry, and around 10% of the chicken mass are feathers, resulting in approximately 8.5×106 tons of chicken feathers (REDDY; SANTOSH, 2016a). Unfortunately, poultry feathers are treated as wastes lacking commercial value. Innovative technologies to waste management must be studied to decrease the environmental impact caused by the generation and disposal of poultry feathers.

Valorizing poultry feathers requires some understanding of their composition (SANTANA; GARDIM; ALMEIDA; BORINI *et al.*, 2020). Feathers are a keratin-based material composed of α-keratin and β-keratin (PENG; MAO; ZHANG; DU *et al.*, 2020). The crude protein content ranges from 85 to 90% (PENG; MAO; ZHANG; DU *et al.*,

2020; REDDY; SANTOSH, 2016a). The substantial protein content of feathers suggests valorization avenues in feed and nutrition applications (KHUMALO; SITHOLE; TESFAYE, 2020; TESFAYE; SITHOLE; RAMJUGERNATH; CHUNILALL, 2017).

Commercial practices handle poultry feathers in several different ways (STINGONE; WING, 2011). Recently, most feathers are landfilled or incinerated; in some cases, part of the feathers is processed into meals for use in the poultry industry and fertilizers (FAGBEMI; SITHOLE; TESFAYE, 2020). Unfortunately, this meal contains low-quality proteins resulting from the hydrolytic and thermal processes that the feed undergoes. Accordingly, converting feathers into meals results in the degradation of amino acids while consuming large amounts of energy (TESFAYE; SITHOLE; RAMJUGERNATH; CHUNILALL, 2017). More energy-efficient methods are required for poultry feather valorization to yield products more valuable than low-quality meals.

Converting the protein in chicken feathers directly into amino acids can solve the problems associated with meal production. Amino acids present several applications for medical, food, pharmaceutical, animal, and cosmetic purposes (ZIERO; BULLER; MUDHOO; AMPESE *et al.*, 2020). Regarding food applications, amino acids can be used as flavor enhancers, specialty nutrients, and animal feed additives (IVANOV; IVANOVA; GEORGIEVA; ATANASOV, 2014). In addition, in the pharmaceutical and cosmetic industries, the main use of amino acids is as an ingredient for the products (IVANOV; IVANOVA; GEORGIEVA; ATANASOV, 2014).

Several technological routes can obtain amino acids from chicken feathers, and the most common methods are chemical and enzymatic extraction (ALVAREZ; RENDUELES; DIAZ, 2012; YANG; CAI; LIU; WANG, 2019). Unfortunately, both methods have substantial disadvantages, including the prohibitive cost of enzymes and the generation of toxic wastes from chemical hydrolysis. Therefore, a new

environmentally friendly approach for amino acid conversion with high efficiency is urgently required. In this context, green techniques using environmentally benign solvents have been studied as an efficient recovery method that eliminates waste generation, inspiring amino acid recovery from poultry feathers (PEREIRA; JUSTUS; FALCÃO; ROCHA *et al.*, 2019a).

Among several plausible solvent options, subcritical water is attractive for transforming protein residues into amino acids. Water at subcritical conditions (typically defined as temperature ranging from 100 to 374 o C and pressures more significant than the corresponding saturation point) has received particular attention as an environmentally benign reaction and extraction solvent (BRUNNER, 2005). Subcritical water behaves as a non-polar solvent that hydrolyzes and extracts organic molecules from complex matrices, including bio-based compounds (GBASHI; ADEBO; PIATER; MADALA *et al.*, 2017; KIAMAHALLEH; NAJAFPOUR-DARZI; RAHIMNEJAD; MOGHADAMNIA *et al.*, 2016; SARAVANA; TILAHUN; GERENEW; TRI *et al.*, 2018). Simultaneously, the ionic product of subcritical water is much greater than in water at ambient temperature and pressure, resulting in the promotion of both acid and base-catalyzed reactions (MOTAVAF; SAVAGE, 2021; OKAJIMA; SAKO, 2014). The result is rapid hydrolysis of the peptide bonds responsible for linking amino acids into proteins without adding acid or base catalysts that would otherwise contribute to waste production (ESPINOZA; MORAWICKI; HAGER, 2012). Subcritical water for simultaneous hydrolysis and extraction can be termed subcritical water hydrolysis (SWH).

A handful of studies have evaluated the use of SWH for the efficient conversion of biomass residues into amino acids. For instance, Park et al. (PARK; JEONG; CHUN, 2019) treated *Pyropia yezoensis* (a type of edible seaweed) with hot water, ethanol, and SWH, finding that SWH treatment formed the most significant number bio-active

compounds, such as amino acids with antioxidant activity. Similarly, Ahmed and Chun (AHMED; CHUN, 2018) reported SWH treatment of tuna skin and collagen to decompose proteins to release peptides and amino acids.

Previous studies on amino acid recovery using SWH demonstrate feasibility but are insufficient for directing research on the poultry feather feed. Fortunately, abundant literature describes the use of subcritical water to hydrolyze vegetable wastes containing recalcitrant biopolymers, such as lignocellulosic byproducts (MACIEL-SILVA; MUSSATTO; FORSTER-CARNEIRO, 2019). Previous studies of SWH of lignocellulosic materials demonstrate that extraction/reaction temperature and water flow rates are critical variables defining performance that must be balanced for optimal performance. Biopolymer hydrolysis rates increase with increasing reaction temperature, thereby releasing soluble monomers for extraction (LACHOS-PEREZ; BASEGGIO; MAYANGA-TORRES; JUNIOR *et al.*, 2018). Unfortunately, monomer degradation rates also increase with increasing temperature, meaning that a balance between hydrolysis and degradation must exist. Minimizing monomer retention in the heated zone – for example, by continuous removal in an extraction flow – can reduce degradation and maximize monomer yields (TORRES-MAYANGA; LACHOS-PEREZ; MUDHOO; KUMAR *et al.*, 2019). On the other hand, operating SWH at high flow rates has the disadvantage of product dilution, complicating product recovery (LACHOS-PEREZ; BASEGGIO; MAYANGA-TORRES; JUNIOR *et al.*, 2018). No theory can predict the tradeoffs between these factors, meaning that the response of each feedstock must be evaluated on its own.

This study evaluated the use of SWH for the recovery of amino acids from poultry feathers. A complete factorial experiment was designed, and the Response Surface Methodology (RSM) was used to examine the effects of temperature and flow rate on

amino acid recovery. The values of temperature and flow rate selected for this analysis were based on inference from previous studies on SWH treatment of lignocellulosic feedstocks. The results presented here can guide future efforts to use SWH for chemical-free poultry feather valorization as amino acids without waste generation, acting as a strategic and sustainable mechanism for the circular economy approach in the poultry industry.

Material and methods

Raw material

Poultry feathers were supplied by the Oriente Company (Videira City, Brazil). Before use, the poultry feathers were shredded in a knife mill (Marconi, model MA 340). The resulting particles were sifted by a Tyler magnetic vibratory stirrer (Bertel, model N.1868). Due to the high surface area to volume ratio, particles between 297 and 710 μm in diameter were selected as the feed to SWH experiments. The milled material was stored at room temperature until use.

Characterization of poultry feathers

The poultry feathers were analyzed for their content of moisture, ash, nitrogen, protein, and chemical oxygen demand (COD), using methods recommended by the Association of Official Analytical Chemistry (AOAC, 2006) (CHEMISTS; HORWITZ, 1975). The moisture (105 °C for 24 h and ash (550 °C for 12 h) content was determined by the gravimetric method (AOAC, 2006). The nitrogen quantification was determined according to the methodology of Micro Kjeldahl (AOAC, 2006). Protein content was quantified based on the nitrogen content, using a conversion factor of 6.25 as recommended by AOAC. The COD was performed according to the Standard Methods

for the Examination of Water and Wastewater (Method 4520 D) (APHA, 1998) (ASSOCIATION; ASSOCIATION; FEDERATION; FEDERATION, 1912).

Experimental design for SWH of poultry feathers

SWH of poultry feathers was performed in a semi-continuous subcritical reactor unit (**Figure 1)**. The system is composed of a high-pressure liquid pump (double piston pump, Model 36 preparation pump, Apple Valley, MN, USA) to pressurize and feed water to the extraction vessel. The extraction vessel is a 316-stainless steel tube with an internal volume of 110 mL. A thermal jacket rated to deliver 1500 W heats the reactor, which is insulated by ceramic fiber. The temperature was controlled using two thermocouples (type K) located in the entrance and outlet of the reactor. The product exiting the extraction vessel is cooled in a shell-and-tube heat exchanger coupled to a thermostatic bath (Marconi, model MA-184). The system's pressure is controlled by a micrometer valve (Autoclave Engineers, model 10VRMM2812) located after the liquid exchanger. The pressure in the system is measured by pressure gauges from WIKA company (0 – 7.500 psi), with an accuracy of up to 0.1 %.

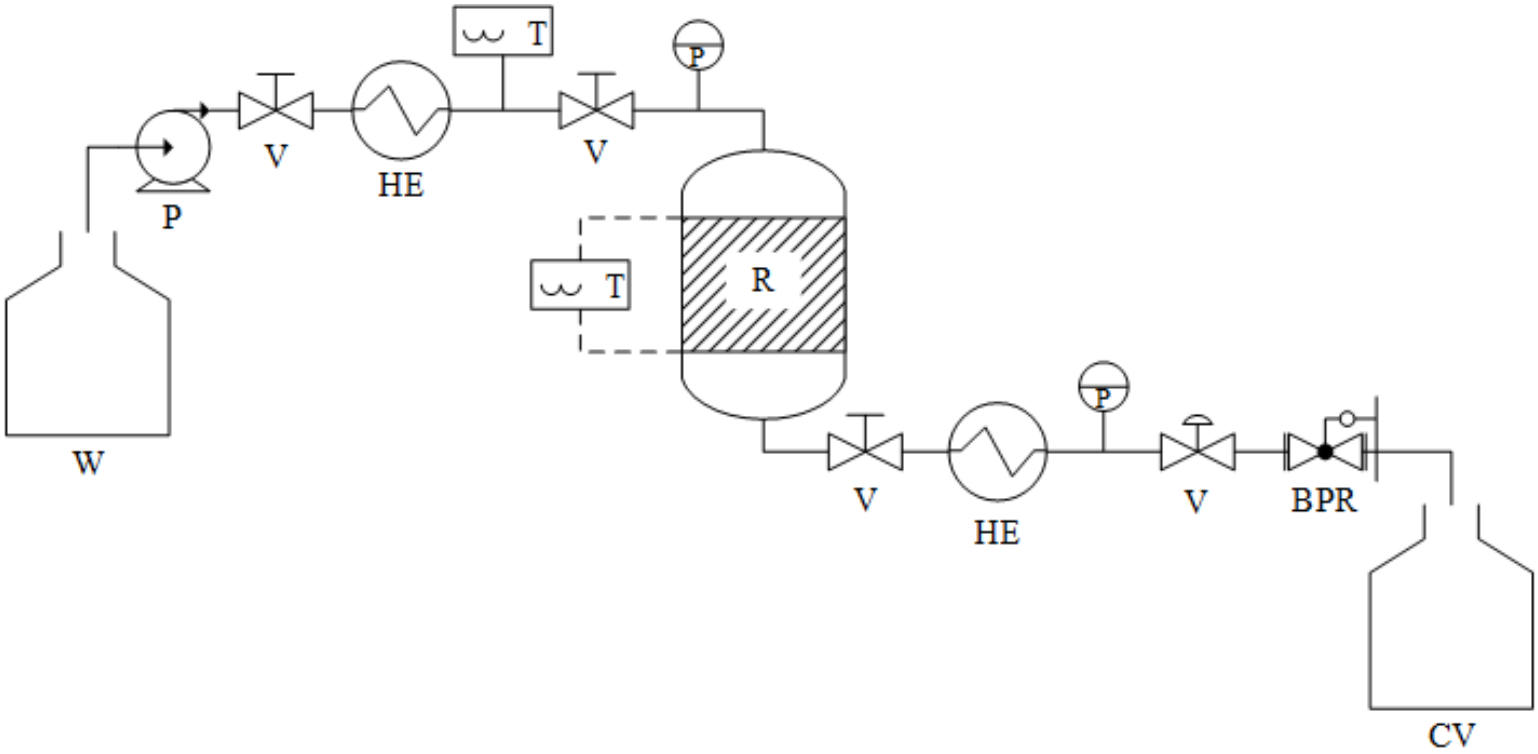


Figure 1. Schematic diagram of experimental apparatus for SWH of poultry feathers: (W) water tank; (P) High-pressure pump; (V) block valves; (P) manometer; (T) thermocouples; (R) subcritical reactor; (HE) heat exchanger; (BPR) back pressure regulator valve; (CV) collecting vessel.

In each experiment, 10 g of milled poultry feathers were loaded into the reactor. The reactor was filled with water from the pump to reach the final pressure, which was held constant at 15 MPa for all experiments. The flow was commenced at the desired rate, and samples were collected at set times. These samples were then stored for further analysis.

The influence of the operating conditions (hydrolysis temperature and flow rate) was studied using a 3^k complete factorial design containing three levels: low (-1), medium (0), and high (+1). Extraction experiments were performed over a range of temperatures (210 to 250 °C) and water flow rates (5 to 10 mL min^{-1}), summarized in **Table 1**. These experimental conditions were selected based on previous studies conducted in the laboratory (LACHOS-PEREZ; BASEGGIO; MAYANGA-TORRES; JUNIOR *et al.*, 2018; LACHOS-PEREZ; MARTINEZ-JIMENEZ; REZENDE; TOMPSETT *et al.*, 2016; LACHOS-PEREZ; TOMPSETT; GUERRA; TIMKO *et al.*, 2017).In total, nine (9) experiments were randomly performed in triplicate to estimate experimental uncertainty and establish reproducibility.

Table 1. Selected variables (factors) and levels in codified and non-codified format.

Variables		Levels		
		Low (-1)	Medium (0)	High (+1)
X_1	Temperature (°C)	210	230	250
X_2	Flow (mL/min)	5	7.5	10

Table 2 presents all the experimental conditions used for SWH of poultry feathers. Samples were collected at regular time intervals to determine the hydrolysis kinetics.

Table 2. Experimental conditions used for SWH of poultry feathers.

Sample	Codified variables		non-codified variables	
	X_1	X_2	Temperature (°C)	Flow (mL/min)
SWH 1	-1	-1	210 (-1)	5 (-1)
SWH 2	-1	0	210 (-1)	7.5 (0)
SWH 3	-1	+1	210 (-1)	10 (+1)
SWH 4	0	-1	230 (0)	5 (-1)
SWH 5	0	0	230 (0)	7.5 (0)
SWH 6	0	+1	230 (0)	10 (+1)
SWH 7	+1	-1	250 (+1)	5 (-1)
SWH 8	+1	0	250 (+1)	7.5 (0)
SWH 9	+1	+1	250 (+1)	10 (+1)

Hydrolysate characterization

The samples were analyzed for pH, COD, total nitrogen content, and crude protein content. The pH (4500-H+ B) was measured at room temperature (Digimed, model DM-22, Brazil). The COD was performed according to the Standard Methods for the Examination of Water and Wastewater (Method 4520 D) (APHA, 1998). Total nitrogen (4500-NorgB) and protein composition were determined through nitrogen Kjeldahl analysis. Protein composition was determined by multiplying the total nitrogen content

(Kjeldahl method) by 6.25 (CUNNIFF, 1997). All the analyses were conducted at least in triplicate.

Quantification of amino acids

The amino acid composition was determined using a Dionex UltiMate 3000 HPLC (temperature: 37 °C, mobile phase: (A) 40mM Na_2HPO_4 with 0.02% NaN_3; and (B) 45% AcN, 45% MeOH and 10% H_2O, flow rate: 1 mL/min, injection volume: 56.5 µL) equipped with a Gemini C18 column (3 µm particle size, 4.6 X 150 mm) from Phenomenex (PN 00F-4439-E0) (USA), Guard column (SecurityGuard Gemini C18, Phenomenex PN: AJO-7597), and UV+fluorescence detector (Thermo Scientific, USA).

Statistical analysis

Analysis of variance (ANOVA) was employed to assess statistically significant factors and interactions between the variables. Significant differences between the samples were evaluated by Tukey's test ($p<0.05$). The linear and quadratic mathematical model was used to determine statistical significance; ANOVA (F-values) was applied to evaluate significance at a probability of 5%. The coefficient of determination (R^2) and the adjusted coefficient of determination (R^2_{adj}) were determined and used to select the most appropriate mathematical model. The selected experimental design is used to evaluate the curvature in the response function. Experimental results were analyzed by response surface methodology (RSM) described by a second-order polynomial function (Eq. 4).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j>i}^{3} \beta_{ij} X_i X_j \quad (4)$$

Where, Y is the studied response (i.e. the dependent variable), β_0 is a constant, β_i are coefficients associated with linear effects, β_{ii} are coefficients linked to quadratic effects, and β_{ij} are coefficients for second-order cross terms.

Finally, the regression coefficients were determined to the variables analyzed by RSM. The significance of the regression coefficients was also confirmed by F-test at $p<0.05$. The statistical analysis was conducted using the Statistica® software (version 10.0, StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

Poultry feathers characterization

Table 3 presents the characterization of poultry feathers used for SWH experiments. The feather showed high protein content (62.68%) and low ash content, demonstrating that this feedstock can be used as a feedstock for amino acid recovery. Other studies showed that feathers generated mainly from poultry slaughterhouses are composed of more than 90% of proteins (keratin) and high-protein biomass (TUCK, CHRISTOPHER O.; PÉREZ, EDUARDO; HORVÁTH, ISTVÁN T.; SHELDON, ROGER A. *et al.*, 2012).

Some proteinaceous vegetable wastes can be used as a source of amino acids because content higher protein concentration, like defatted rice bran, beans dregs, and even microalgae to obtain amino acids (SEREEWATTHANAWUT; PRAPINTIP; WATCHIRARUJI; GOTO *et al.*, 2008; ZHU; ZHU; FAN; WAN, 2011). Also, animal waste has shown more increased interest in the literature for had protein in your composition, for example, fish gelatin (UENO; ICHINOI; ZHAO; FUJII, 2015), squid muscle (*Todarodes pacificus*) (AHMED; CHUN, 2018), hog hair, and tuna's collagen (ESTEBAN, M. B.; GARCÍA, A. J.; RAMOS, P.; MÁRQUEZ, M. C., 2010).

Table 3. Characterization of poultry feathers.

Parameters	Composition	Unit
Moisture	7 ± 2	g 100 g^{-1}
Ashes	2.1 ± 0.2	g 100 g^{-1}
COD	1.9 ± 0.1	g 100 g^{-1}
Total Nitrogen	10 ± 1	g 100 g^{-1}
Proteins	63 ± 1	g 100 g^{-1}

Results expressed as mean ± standard deviation. Analysis conducted in triplicate.

SWH of poultry feathers

SWH experiments were performed to evaluate the effectiveness of flow and hydrolysis temperature on amino acid recovery from poultry feathers. Three levels were evaluated for both independent variables, resulting in 9 experiments. These are labeled SWH 1 through 9, as shown in Table 2. Eight samples were collected during each SWH experiment, and **Figure 2** provides plots of COD measured in the collect points under all tested conditions. In the kinetic analysis, measurements of COD were used as an indication of organic content in the hydrolysate (SCHOMMER; WENZEL; DAROIT, 2020).

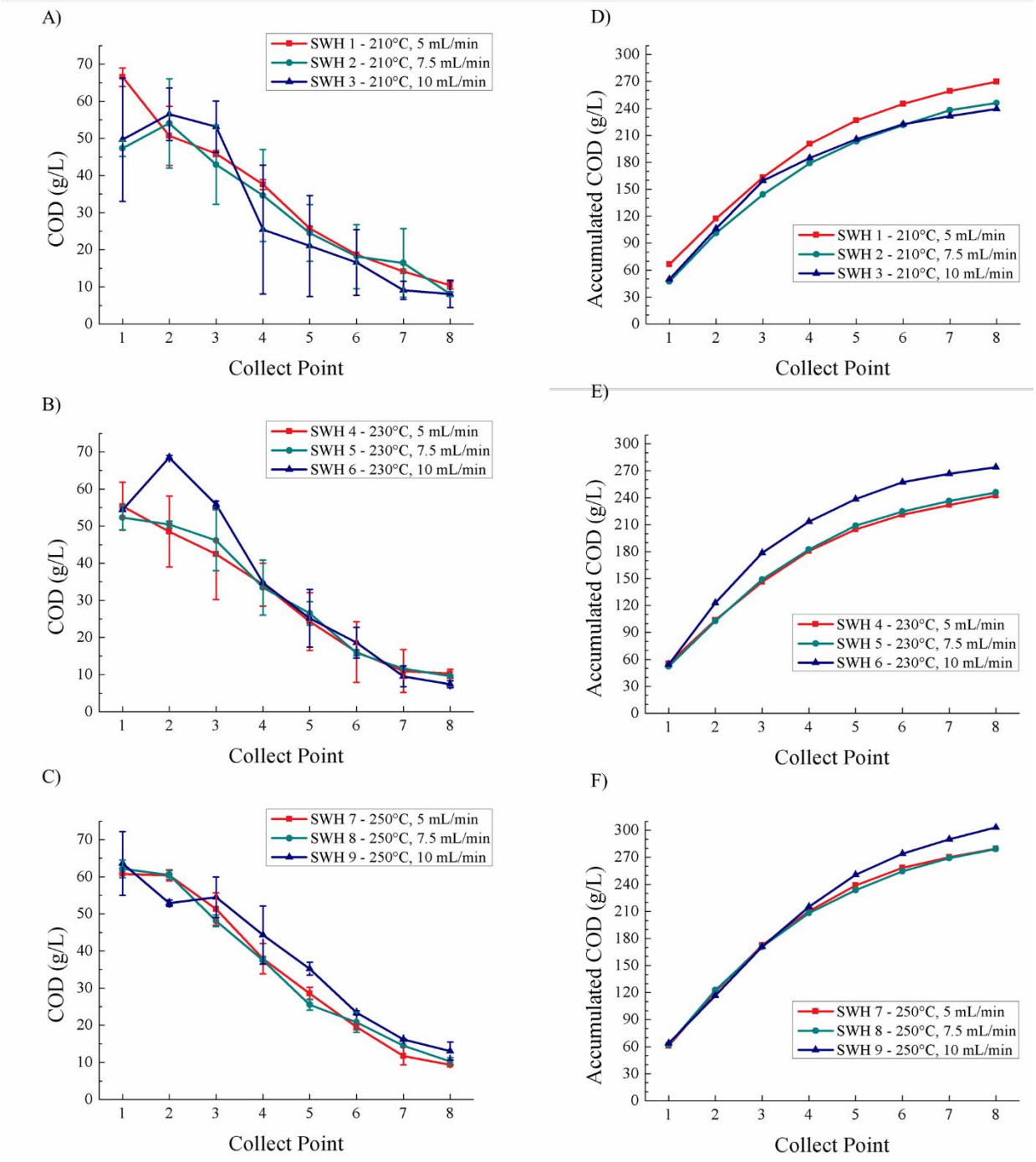


Figure 2. Kinetics of chemical oxygen demand (COD).

Figure 2 shows that COD typically exhibited a maximum value in the first sample and then decreased with increasing extraction time. The lone exception is samples collected at the highest flow rate (10 mL/min). The second sample usually exhibited the

most excellent COD for these samples, after which point COD monotonically decreased. COD indirectly measures the reducing equivalents (elements with a low oxidation number, ie, reduced) present in the sample in question. The COD makes it possible to assess the amount of organic matter in liquid and solid waste regarding the amount of oxygen needed for its total oxidation. In this work, the hydrolysis showed a trend that clearly indicates the importance of balancing protein hydrolysis rates with mass transfer rates to extract the amino acids and other soluble products.

Figure 2 also includes plots of accumulated COD during the hydrolysis of poultry feathers. The final values obtained are in the range of 210 to 290 g/L. Interestingly, at the higher temperatures (230 and 250 °C), the greatest accumulated COD is observed at the highest flow rate (10 mL/min), whereas at the lowest temperature (210 °C), the opposite is observed. Effects of temperature and flow rate indicate a transition from reaction limited to mass transfer limited extraction at a temperature intermediate to 210 and 230 °C, as protein hydrolysis rates are fast enough at 230 and 250 °C that recovery instead becomes mass transport limited.

Composition of the hydrolyzate

3.3.1. pH, COD, crude protein, and total amino acids

Figure 3 provides COD results, total protein, total amino acids, and pH values for all flows and temperatures tested. Samples analyzed correspond to the second collect point of each treatment. Overall, the pH values ranged from 6.62 (SWH 2) to 7.81 (SWH 7). Total protein content was similar for all samples and was in the range of 30-35 g/L. The only notable outlier in this respect was SWH 3, performed at 210 °C and 10 mL/min,

which contained 50,37±0,03 g/L of protein. The combination of low temperature and high flow rate may have maximized partial protein hydrolysis and minimized subsequent degradation, resulting in more effective protein recovery than the other conditions tested here.

COD was measured as a complement to protein analysis. Since COD results indicate the total amount of oxygen needed to oxidize the sample completely, the treatments that resulted in higher COD values should coincide with higher protein and amino acid concentration. This appears to only hold in a very rough sense. For example, COD increases monotonically with increasing flow rate at 230 °C, yet protein increases modestly (or not at all, once uncertainty is included), and amino acid recovery decreases.

The apparent disconnect between COD and protein measurements may be explained by several considerations. First, total protein values include true protein and non-protein nitrogen (TESFAYE; SITHOLE; RAMJUGERNATH; CHUNILALL, 2017). Another possible reason for the incompatibility of COD and total protein comparison may reside in other molecules that affect the COD but do not affect the protein content. For example, considering the degradation of proteins into amino acids and then into carbonic acids and amines (SATO; QUITAIN; KANG; DAIMON *et al.*, 2004), these latter molecules are associated with COD. Still, they are not necessarily accounted for in total protein values. Finally, three different treatments presented a statistically higher value for total amino acid content: SWH 4, SWH 7, and SWH 8. Moderate and higher temperatures may boost amino acid yield, and the lower and medium flow rate may provide sufficient retention time for conversion of protein to amino acids. Consistent with this explanation, the amino acid content of SWH 7 was nearly twice that obtained in SWH 2.

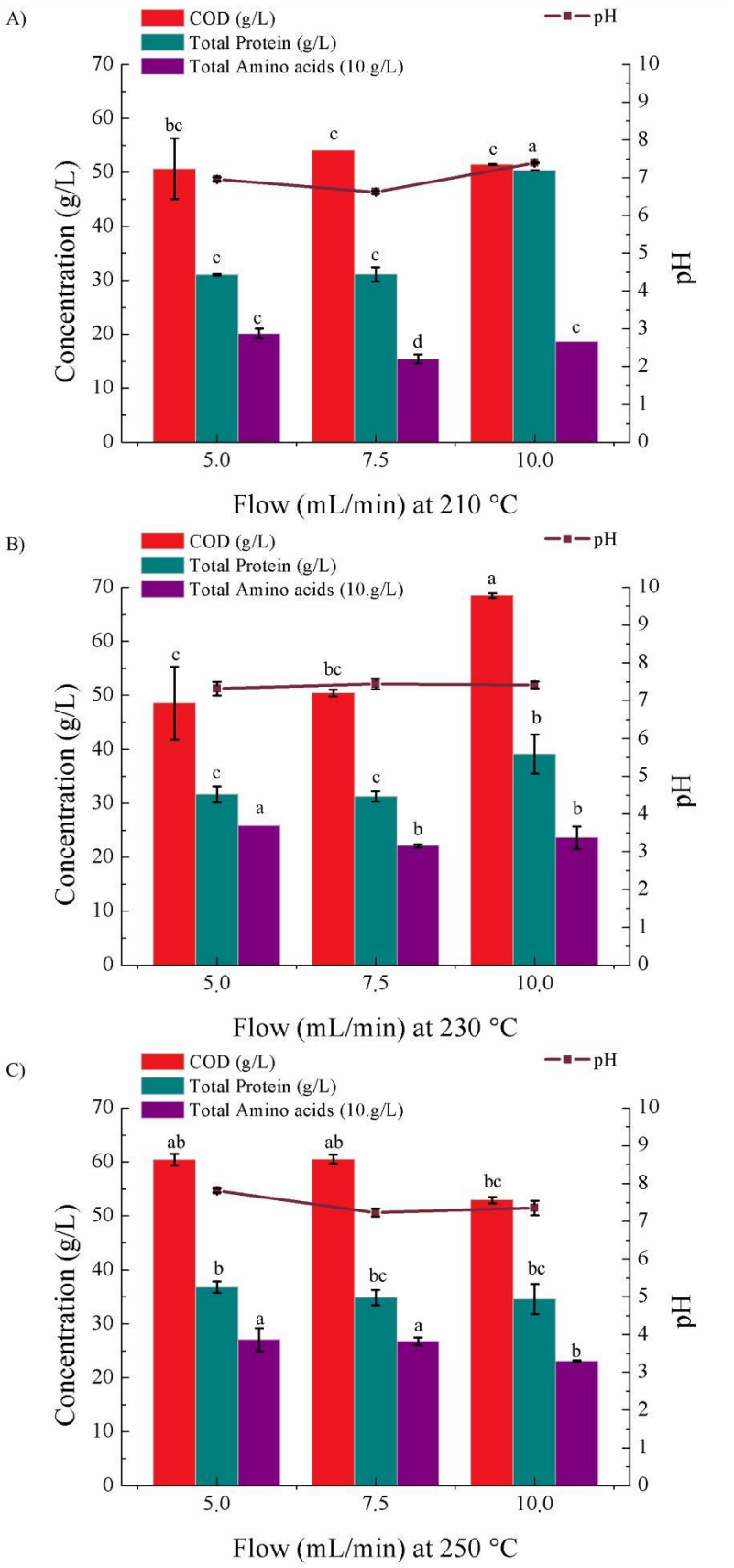

A)
COD (g/L)
Total Protein (g/L)
Total Amino acids (10.g/L)
pH
Concentration (g/L)
pH
Flow (mL/min) at 210 °C
B)
COD (g/L)
Total Protein (g/L)
Total Amino acids (10.g/L)
pH
Concentration (g/L)
pH
Flow (mL/min) at 230 °C
C)
COD (g/L)
Total Protein (g/L)
Total Amino acids (10.g/L)
pH
Concentration (g/L)
pH
Flow (mL/min) at 250 °C

Figure 3. Average value plots of Chemical Oxygen Demand (COD, g/L), total protein (g/L), total amino acids (g/L) and pH values: A) 210 °C and 5.0, 7.5, 10.0 mL/min; B) 230 °C and 5.0, 7.5, 10.0 mL/min; and C) 250 °C and 5.0, 7.5, 10.0 mL/min.

3.3.2. Amino acids composition in the hydrolysate

Table 4 summarizes amino acids composition results obtained at different experimental conditions.

Table 4. Characterization of amino acids obtained from Subcritical water hydrolysis (SWH) of poultry feathers.

Sample	Experimental conditions of SWH								
	SWH 1 210º C/5mL/min	SWH 2 210ºC/7.5mL/min	SWH 3 210ºC/10mL /min	SWH 4 230º C/5mL/min	SWH 5 230ºC/7.5mL/min	SWH 6 230ºC/10mL/min	SWH 7 250º C/5mL/min	SWH 8 250ºC/7.5mL/min	SWH 9 250ºC/10mL/min
Essential amino acids									
Histidine (mg/L)	6.9±0.4^{d}	7.6±0.5^{cd}	7.73±0.01^{cd}	9.1±0.4^{ab}	8.4±0.4^{bc}	7.7±0.1^{cd}	6.7±0.1^{d}	9.8±0.3^{a}	7.3±0.4^{cd}
Threonine (mg/L)	6.8±0.3^{b}	6±5^{b}	12.74±0.01^{a}	2.8±0.2^{d}	3.5±0.2^{d}	4.9±0.7^{c}	0.7±0.6^{e}	2.02±0.06^{d}	1.5±0.9^{e}
Arginine (mg/L)	57±3^{a}	51±1^{b}	40.06±0.01^{c}	45.25±0.03^{c}	52.1±0.2^{ab}	43±7^{c}	7.9±0.2^{f}	18±4^{e}	30±4^{d}
Valine (mg/L)	64±4de	56±2^{e}	60.38±0.01^{de}	79±3^{cd}	72±3^{cde}	82±15^{cd}	135±1^{a}	113±8^{b}	85±3^{c}
Methionine (mg/L)	148±9^{b}	138±1^{b}	145.08±0.01^{b}	151.74±0.04^{b}	151±2^{b}	154±3^{b}	198±30^{a}	166±6^{ab}	159±11^{b}
Tryptophan (mg/L)	172±3^{e}	165±1^{d}	186.14±0.01^{c}	196.59±0.02^{bc}	193±2^{bc}	199±7^{bc}	230±7^{a}	231±13^{a}	209±1^{b}
Phenylalanine (mg/L)	47±3^{cd}	42.3±0.3^{e}	49.13±0.01^{bc} ^{d}	52.2±0.5^{bc}	45±1^{e}	52±8^{bc}	58±2^{a}	54±2^{ab}	47.5±0.7^{cd}
Isoleucine (mg/L)	68±12^{c}	29.1±0.1^{d}	29.76±0.01^{d}	189±2^{bc}	149±7^{bc}	143±32^{bc}	423±166^{a}	418±44^{a}	263±59^{ab}
Leucine (mg/L)	68±5^{cd}	60±3^{d}	68.46±0.01^{cd}	85±3^{b}	69±2^{cd}	78±19^{bc}	115.12±0.06^{a}	105±5^{a}	84±3^{b}
Lysine (mg/L)	6.8±0.5^{bc}	5.5±0.5^{c}	8.65±0.01^{bc}	8.8±0.6^{bc}	7.6±0.5^{bc}	9±3^{b}	13±1^{a}	12.9±0.7^{a}	9.0±0.9^{b}
non-essential amino acids									
Aspartic acid (mg/L)	50.8±0.3^{c}	86±11^{b}	121.43±0.01^{a}	23±2^{def}	34±2^{cde}	41±20^{cd}	10.7±0.4^{f}	15±2^{ef}	20±4^{def}

Glutamic acid (mg/L)	10.1±0.6^{bc}	12±2^{b}	14.50±0.01^{a}	7.6±0.1^{de}	9.0±0.5^{cd}	10.2±0.5^{bc}	6.1±0.1^{e}	7.1±0.8^{de}	8±1^{cd}
Cysteine (mg/L)	3.10±0.01	-	-	-	-	-	-	-	-
Aspargine (mg/L)	4.03±0.05^{b}	8.5±0.5^{a}	8.40±0.01^{a}	2.02±0.02^{cde}	2.5±0.2^{bcd}	3±1^{bc}	0.6±0.5^{2f}	1.24±0.08^{ef}	0.9±0.6^{ef}
Serine (mg/L)	121±5^{ab}	111±38^{bc}	159.97±0.01^{a}	72±4^{cde}	84±3^{bcd}	74±30^{cde}	10.8±0.3^{g}	30±6^{fg}	43±10^{def}
Glutamine (mg/L)	0.23±0.03^{d}	-	-	-	-	0.34±0.04^{c}	-	0.76±0.04^{a}	0.64±0.01^{b}
Glycine (mg/L)	238±39^{cd}	155±6^{d}	168.42±0.01^{d}	392±15^{b}	237±2^{cd}	314±69^{bc}	547±3^{a}	482±33^{a}	366±13^{b}
Alanine (mg/L)	628±38^{c}	402±1^{d}	481.50±0.01^{d}	868±15^{a}	744±9^{b}	772±90^{ab}	680±3^{bc}	736±17^{bc}	694±52^{bc}
Tyrosine (mg/L)	38±3^{b}	40±2^{b}	47.13±0.01^{a}	40.7±0.7^{b}	41.2±0.7^{b}	47±3^{a}	41±1^{b}	40.7±0.9^{b}	36±1^{b}
Proline (mg/L)	275±13^{c}	205±2^{f}	254.70±0.01^{cd}	359±7^{a}	303±1^{b}	321±17^{b}	221±10^{ef}	236±14de	243±24de

Results expressed as mean ± standard deviation. Analysis conducted in triplicate. Different letters in each line represent significant difference by Tukey's test ($p<0.05$).

Table 4 shows that extraction at the highest temperature (250 °C) resulted in the highest concentrations of many different amino acids, such as valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, and lysine. Notwithstanding, some essential amino acids, like threonine (210 °C), histidine, and arginine (230 °C), were extracted more effectively at lower temperatures. This clearly shows that reaction temperature can be tuned for recovery of different amino acids, with the differences attributable either to the thermal stability of the monomer, amino acid solubility, the accessibility of the monomer in the protein itself, or the thermal stability of the peptide bonds associated with a given amino acid.

The extraction performance of a given amino acid is determined by several factors, including abundance in the feathers, reactivity, the water solubility of the amino acid, and stability of the amino acid. Isoleucine was the most abundant amino acid in these samples, and its recovery was maximized at 250 °C. Isoleucine is a moderately soluble amino acid that is highly abundant in poultry feathers (CHERRY; YOUNG; SHEWFELT, 1975). The abundance of isoleucine in poultry feathers is consistent with its recovery; its moderate water solubility may explain why recovery increased with increasing SWH temperature. SWH becomes more capable of dissolving many sparingly soluble molecules with increasing temperature due to the temperature dependence of its dielectric constant, isoleucine has a nonpolar side chain, which should become increasingly water soluble with increasing temperature in the range considered here.

In general, the data show that hydrophobic amino acids more hydrophobic than isoleucine – such as phenylalanine and tryptophan, both of which possess aromatic side

chains – require high temperatures for effective extraction. Again, this observation is consistent with the effects of temperature on the solubility properties of water. The amino acids that are most easily recovered at 210 °C, especially arginine and threonine, possess small, polar side chains. Histidine possesses an ionizable imidazole side chain, which may explain its recovery behavior. The observation of the effects of side chains further establishes the importance of thermodynamic driving force on extraction and recovery.

For non-essential amino acids, lower and moderate temperatures were most effective. SWH 3 contained high concentrations of aspartic acid, glutamic acid, asparagine, serine, alanine, and proline. Similarly, the amino acid concentrations in SWH 4 are much greater than the more aggressive conditions used for SWH 7 and SWH 8. Total amino acid concentrations are consistent with the observation made for individual amino acids, providing a degree of confidence in the two analytical techniques used for total amino acid content and individual amino acid quantification.

Ueno et al. (2015) studied fish gelatin to produce serine, glycine, alanine, arginine, and valine in SWH at higher temperatures (240 °C). The results showed a lower concentration of amino acids compared to the literature. Furthermore, this study also presented different amino acids concentration when the SWH parameters were changed, and amino acids that were recovered in some temperatures were no more present in other conditions (UENO; ICHINOI; ZHAO; FUJII, 2015). Asaduzzaman and Chun (2015) report the use of SWH in squid muscles, and the results obtained demonstrated the production of an average higher concentration of free amino acids at 250 ◦C, while the highest concentration of essential amino acids was obtained at 220 ◦C. Besides, the thermally dried squid muscles present approximately 73.26% protein composition (ASADUZZAMAN, A. K. M.; CHUN, B.-S., 2015), contrasting to chicken feathers crude

protein content, that is about 90%, and comprehends mainly keratin, an insoluble protein (TESFAYE; SITHOLE; RAMJUGERNATH; CHUNILALL, 2017). Ahmed and Chun (2018) evaluated the hydrolysis of tuna and collagen skin with SWH. The study reported the collagen hydrolysate presented higher activity, with glycine, proline, alanine, and glutamic acid as the most abundant structural amino acids for all tested conditions. In addition, the authors reported a higher degradation rate of protein to peptides, and also of peptides to free amino acids when the temperature was increased. However, after a certain point, high temperature caused decomposition of amino acid into organic acids or other product, such as carbonic acids and amines (AHMED; CHUN, 2018; SATO; QUITAIN; KANG; DAIMON *et al.*, 2004).

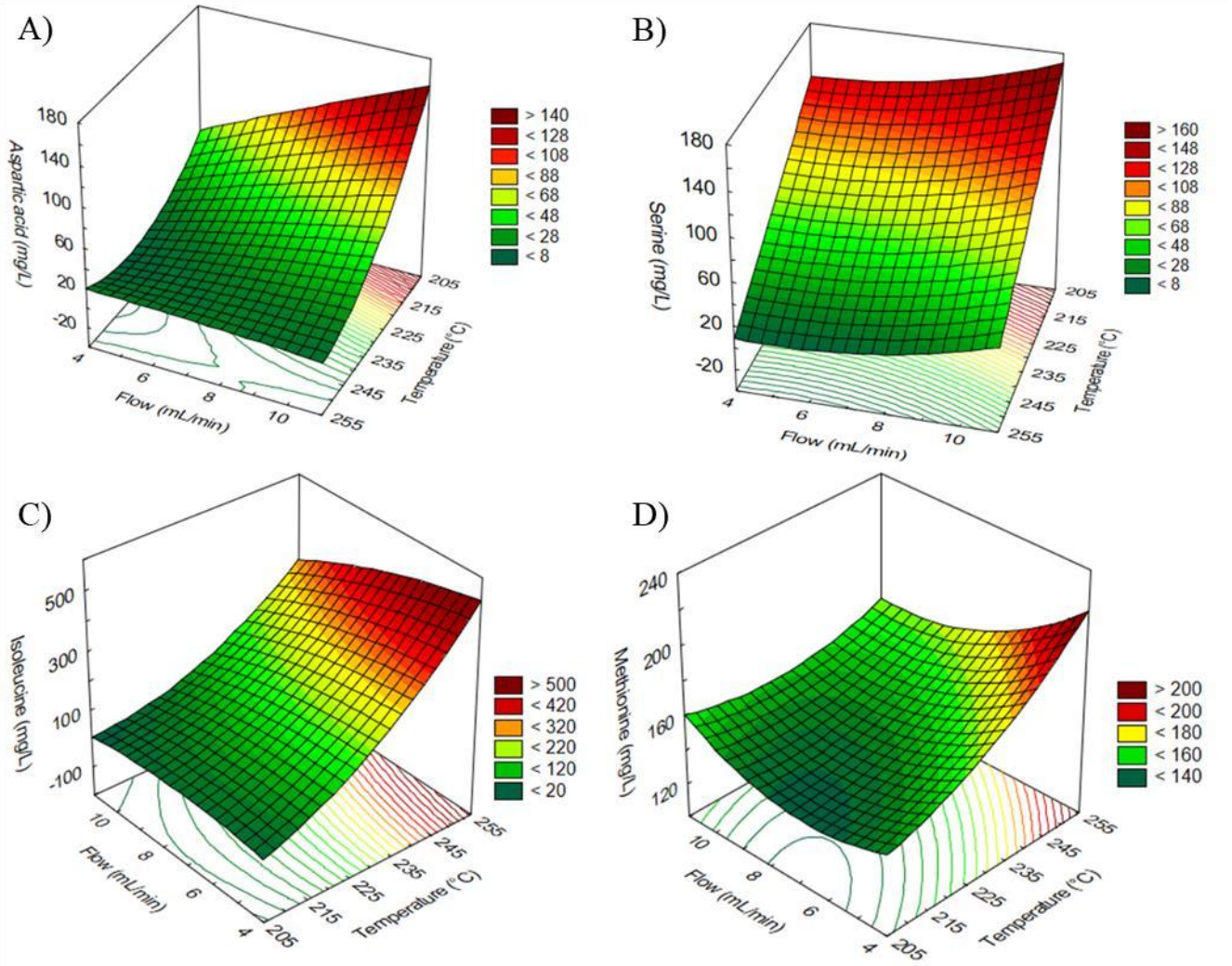


Figure 4. Response surface plots showing the effect of temperature and flow in the production of aspartic acid (A), serine (B), isoleucine (C) and methionine (D).

Statistical modelling and optimization of SWH

The effects of temperature and water flow were investigated by a 3^k full factorial design. Statistical models incorporating both linear and quadratic interactions were evaluated (**Table S1**). Total amino acids, aspartic acid, serine, glycine, alanine, tryptophan, isoleucine, and proline were adjusted in the linear interaction, representing that the equation of the response surface will not be based on quadratic coefficients. Also,

methionine was adjusted by the linear and quadratic models. However, due to the few variations between the models, the linear model was chosen for this variable.

The dataset of the amino acids adjusted in the linear model was analyzed using ANOVA. Based on the ANOVA results (**Table S2**), the lack of fit was significant ($p>0.05$) for most of the independent variables considered in the study, with the exceptions of aspartic acid, serine, isoleucine, and methionine. Similarly, aspartic acid ($R^2_{adj.} = 0.941$), serine ($R^2_{adj.} = 0.831$), and isoleucine ($R^2_{adj.} = 0.831$) presented R^2 value higher than 0.8, an admittedly arbitrary value but one that has been recommended as an indication of a good fit (CHEN; WANG; ZHANG; YANG *et al.*, 2021).

The equations of the adjusted linear regression model for the statistically valid variables follows:

$$Aspartic\ acid\ \left(\frac{mg}{L}\right) = 2258.67 - 20.35T + 0.05T^2 + 70.23Q - 0.12Q^2 - 0.30TQ$$

$$Serine\ \left(\frac{mg}{L}\right) = 936.15 - 5.10T - 0.01T^2 + 0.24Q + 0.83Q^2 - 0.03TQ$$

$$Isoleucine\ \left(\frac{mg}{L}\right) = 3191.02 - 38.90T + 0.11T^2 - 153.85Q - 2.02Q^2 - 0.61TQ$$

$$Methionine\ \left(\frac{mg}{L}\right) = 650.38 - 5.72T + 0.02T^2 + 20.27Q - 1.26Q^2 - 0.18TQ$$

where, T represents the extraction temperature (°C), and Q represents the flow of water (mL/min) used for the SWH process.

Three-dimensional response surfaces were generated for the statistically valid variables (**Figure 4)**. Aspartic acid and serine concentrations were maximized at high flow (around 10 mL/min) and low temperature (around 210 °C). Isoleucine and methionine presented the opposite behavior, with the highest concentrations obtained at low water flow (5 mL/min) and high temperature (250°C).

Figure 5 illustrates the temperature and flow rate, which presented better yields for the mentioned amino acids. These results indicate that different amino acids have a particular condition for their recovery at higher concentration rates. It shows it is possible to induce the obtaining of specific amino acids at the expense of others.

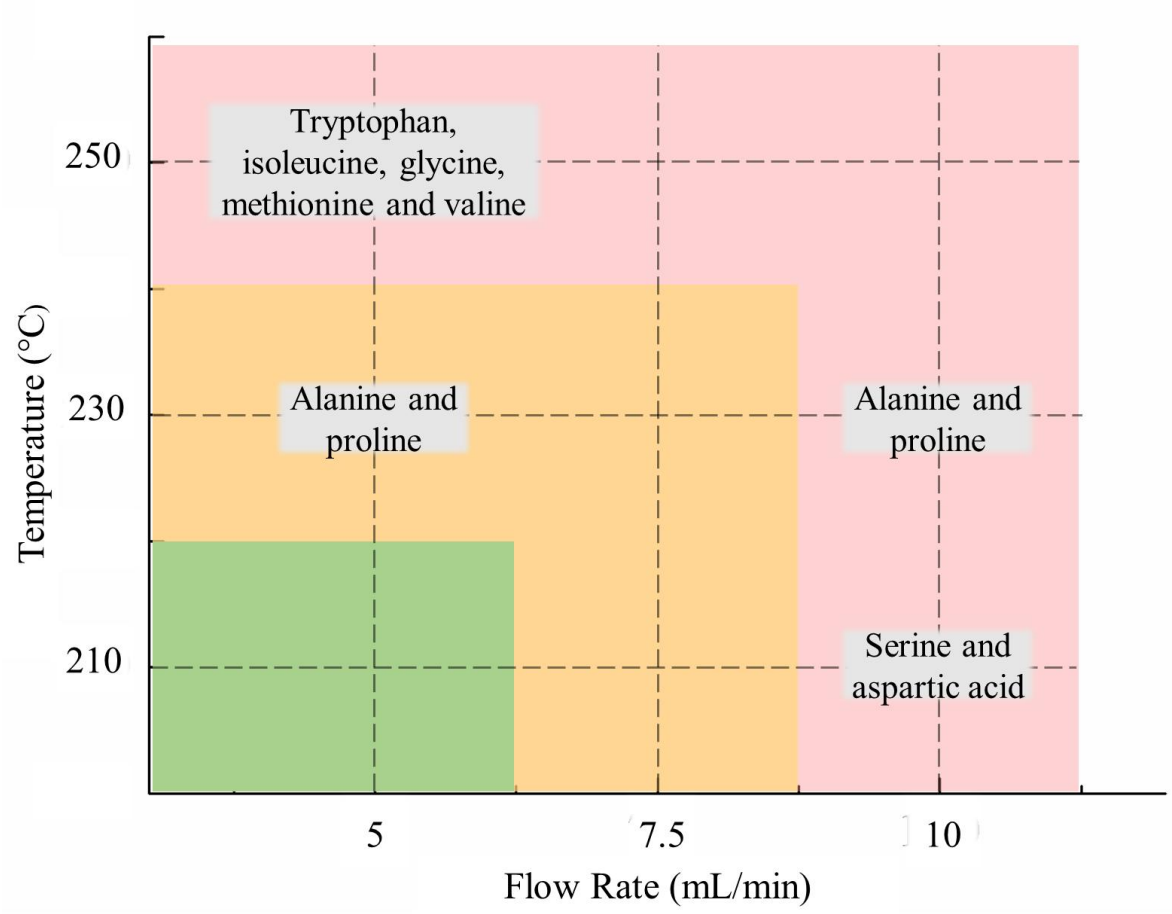


Figure 5. The schematic diagram for amino acids production.

Conclusions

SWH technology was evaluated as a chemical-free technology to obtain amino acids from poultry feathers. The effects of extraction temperature (210, 230, 250 °C) and extraction flow rate (5, 7.5, and 10 mL/min) on amino acid recovery were investigated. Aspartic

acid and serine concentrations were maximized at the highest flow (10 mL/min) and lowest temperature (210 °C); isoleucine and methionine presented were maximized at the opposite extreme (250 °C and 5 mL/min). These observations are broadly consistent with peptide bond hydrolysis to produce water-soluble polypeptides and/or amino acids, followed by extraction of the resulting amino acids. Amino acid recovery broadly followed trends expected from their natural abundance in the poultry feathers and their expected solubility in water at different temperatures. SWH has the potential for chemical-free and economical extraction of amino acids from protein-rich waste streams.

Acknowledgments

The authors thank Dr. Sioji Kuana of Três Fronteiras (Seara/SC) Company (Brazil). The authors acknowledge the financial support from the Brazilian Science and Research Foundation (CNPq) and Higher-Level Personnel Improvement Coordination (CAPES) – Brazil – Finance code 001. The financial support from the São Paulo Research Foundation – FAPESP (2018/05999-0; 2018/14938-4). W.G. Sganzerla thanks FAPESP for the doctorate scholarship (2019/26925-7). T. Forster-Carneiro thanks CNPq for the productivity grant (302473/2019-0). The authors thanks Donna Murillo who performed some of the initial experiments that established the methodology. MTT thanks the U.S. National Science Foundation (ENG #1554283).

REFERENCES

ADRIAN, M.; ELIZABETH, T. Poverty, politics and plastic: Organic waste sorting in Blantyre's public markets. **Journal of Urban Management**, 2021.

AHMED, R.; CHUN, B.-S. Subcritical water hydrolysis for the production of bioactive peptides from tuna skin collagen. **The Journal of Supercritical Fluids**, 141, p. 88-96, 2018/11/01/ 2018.

ALVAREZ, C.; RENDUELES, M.; DIAZ, M. The yield of peptides and amino acids following acid hydrolysis of haemoglobin from porcine blood. **Animal Production Science**, 52, n. 5, p. 313-320, 2012.

ASADUZZAMAN, A. K. M.; CHUN, B.-S. Recovery of functional materials with thermally stable antioxidative properties in squid muscle hydrolyzates by subcritical water. **Journal of Food Science and Technology**, 52, n. 2, p. 793-802, 2015/02/01 2015.

ASSOCIATION, A. P. H.; ASSOCIATION, A. W. W.; FEDERATION, W. P. C.; FEDERATION, W. E. **Standard methods for the examination of water and wastewater**. American Public Health Association., 1912.

BERGENDAHL, J. A.; SARKIS, J.; TIMKO, M. T. Transdisciplinarity and the food energy and water nexus: Ecological modernization and supply chain sustainability perspectives. **Resources, Conservation and Recycling**, 133, p. 309-319, 2018.

BRUNNER, G. Supercritical fluids: technology and application to food processing. **Journal of food engineering**, 67, n. 1-2, p. 21-33, 2005.

CAMPOS, I.; VALENTE, L. M. P.; MATOS, E.; MARQUES, P. *et al.* Life-cycle assessment of animal feed ingredients: Poultry fat, poultry by-product meal and hydrolyzed feather meal. **Journal of Cleaner Production**, 252, p. 119845, 2020.

CENTOBELLI, P.; CERCHIONE, R.; CHIARONI, D.; DEL VECCHIO, P. *et al.* Designing business models in circular economy: A systematic literature review and research agenda. **Business Strategy and the Environment**, 29, n. 4, p. 1734-1749, 2020.

CHEMISTS, A. O. O. A.; HORWITZ, W. **Official methods of analysis**. Association of Official Analytical Chemists Washington, DC, 1975.

CHEN, J.; WANG, X.; ZHANG, B.; YANG, Y. *et al.* Integrating enzymatic hydrolysis into subcritical water pretreatment optimization for bioethanol production from wheat straw. **Science of the Total Environment**, 770, p. 145321, 2021/05/20/ 2021.

CHERRY, J.; YOUNG, C.; SHEWFELT, A. Characterization of protein isolates from keratinous material of poultry feathers. **Journal of food science**, 40, n. 2, p. 331-335, 1975.

CUNNIFF, P. Official methods of analysis of aoac international. **J. AOAC Int**, 80, p. 127A, 1997.

ESPINOZA, A. D.; MORAWICKI, R. O.; HAGER, T. Hydrolysis of whey protein isolate using subcritical water. **Journal of food science**, 77, n. 1, p. C20-C26, 2012.

ESTEBAN, M. B.; GARCÍA, A. J.; RAMOS, P.; MÁRQUEZ, M. C. Sub-critical water hydrolysis of hog hair for amino acid production. **Bioresource technology**, 101, n. 7, p. 2472-2476, 2010/04/01/ 2010.

FAGBEMI, O. D.; SITHOLE, B.; TESFAYE, T. Optimization of keratin protein extraction from waste chicken feathers using hybrid pre-treatment techniques. **Sustainable Chemistry and Pharmacy**, 17, p. 100267, 2020.

GBASHI, S.; ADEBO, O. A.; PIATER, L.; MADALA, N. E. *et al.* Subcritical water extraction of biological materials. **Separation & Purification Reviews**, 46, n. 1, p. 21-34, 2017.

IVANOV, K.; IVANOVA, S.; GEORGIEVA, M.; ATANASOV, P. Production and regulatory analytical control of amino acids include in food additives. **Pharmacia**, 61, n. 2, p. 48-54, 2014.

KHUMALO, M.; SITHOLE, B.; TESFAYE, T. Valorisation of waste chicken feathers: Optimisation of keratin extraction from waste chicken feathers by sodium bisulphite, sodium dodecyl sulphate and urea. **Journal of environmental management**, 262, p. 110329, 2020.

KIAMAHALLEH, M. V.; NAJAFPOUR-DARZI, G.; RAHIMNEJAD, M.; MOGHADAMNIA, A. A. *et al.* High performance curcumin subcritical water extraction from turmeric (Curcuma longa L.). **Journal of Chromatography B**, 1022, p. 191-198, 2016.

LACHOS-PEREZ, D.; BASEGGIO, A. M.; MAYANGA-TORRES, P.; JUNIOR, M. R. M. *et al.* Subcritical water extraction of flavanones from defatted orange peel. **The Journal of Supercritical Fluids**, 138, p. 7-16, 2018.

LACHOS-PEREZ, D.; MARTINEZ-JIMENEZ, F.; REZENDE, C.; TOMPSETT, G. *et al.* Subcritical water hydrolysis of sugarcane bagasse: An approach on solid residues characterization. **The Journal of Supercritical Fluids**, 108, p. 69-78, 2016.

LACHOS-PEREZ, D.; TOMPSETT, G.; GUERRA, P.; TIMKO, M. *et al.* Sugars and char formation on subcritical water hydrolysis of sugarcane straw. **Bioresource technology**, 243, p. 1069-1077, 2017.

MACIEL-SILVA, F. W.; MUSSATTO, S. I.; FORSTER-CARNEIRO, T. Integration of subcritical water pretreatment and anaerobic digestion technologies for valorization of açai processing industries residues. **Journal of Cleaner Production**, 228, p. 1131-1142, 2019.

MOTAVAF, B.; SAVAGE, P. E. Effect of process variables on food waste valorization via hydrothermal liquefaction. **ACS ES&T Engineering**, 1, n. 3, p. 363-374, 2021.

OKAJIMA, I.; SAKO, T. Energy conversion of biomass with supercritical and subcritical water using large-scale plants. **Journal of bioscience and bioengineering**, 117, n. 1, p. 1-9, 2014.

PARK, J.-S.; JEONG, Y.-R.; CHUN, B.-S. Physiological activities and bioactive compound from laver (Pyropia yezoensis) hydrolysates by using subcritical water hydrolysis. **The Journal of Supercritical Fluids**, 148, p. 130-136, 2019.

PENG, Z.; MAO, X.; ZHANG, J.; DU, G. *et al.* Biotransformation of keratin waste to amino acids and active peptides based on cell-free catalysis. **Biotechnology for biofuels**, 13, p. 1-12, 2020.

PEREIRA, D. G.; JUSTUS, A.; FALCÃO, H. G.; ROCHA, T. D. S. *et al.* Enzymatic hydrolysis of okara protein concentrate by mixture of endo and exopeptidase. **Journal of food processing and preservation**, 43, n. 10, p. e14134, 2019.

REDDY, N.; SANTOSH, M. Recovery and Applications of Feather Proteins. **Protein Byproducts**, p. 255-274, 2016.

SANTANA, J. C.; GARDIM, R. B.; ALMEIDA, P. F.; BORINI, G. B. *et al.* Valorization of chicken feet by-product of the poultry industry: high qualities of gelatin and biofilm from extraction of collagen. **Polymers**, 12, n. 3, p. 529, 2020.

SARAVANA, P. S.; TILAHUN, A.; GERENEW, C.; TRI, V. D. *et al.* Subcritical water extraction of fucoidan from Saccharina japonica: optimization, characterization and biological studies. **Journal of Applied Phycology**, 30, n. 1, p. 579-590, 2018.

SATO, N.; QUITAIN, A. T.; KANG, K.; DAIMON, H. *et al.* Reaction kinetics of amino acid decomposition in high-temperature and high-pressure water. **Industrial & Engineering Chemistry Research**, 43, n. 13, p. 3217-3222, 2004.

SCHOMMER, V. A.; WENZEL, B. M.; DAROIT, D. J. Anaerobic co-digestion of swine manure and chicken feathers: Effects of manure maturation and microbial pretreatment of feathers on methane production. **Renewable Energy**, 152, p. 1284-1291, 2020.

SEREEWATTHANAWUT, I.; PRAPINTIP, S.; WATCHIRARUJI, K.; GOTO, M. *et al.* Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. **Bioresource technology**, 99, n. 3, p. 555-561, 2008/02/01/ 2008.

STINGONE, J. A.; WING, S. Poultry litter incineration as a source of energy: reviewing the potential for impacts on environmental health and justice. **New Solutions: A Journal of Environmental and Occupational Health Policy**, 21, n. 1, p. 27-42, 2011.

TESFAYE, T.; SITHOLE, B.; RAMJUGERNATH, D.; CHUNILALL, V. Valorisation of chicken feathers: characterisation of chemical properties. **Waste Management**, 68, p. 626-635, 2017.

TORRES-MAYANGA, P. C.; LACHOS-PEREZ, D.; MUDHOO, A.; KUMAR, S. *et al.* Production of biofuel precursors and value-added chemicals from hydrolysates resulting from hydrothermal processing of biomass: A review. **Biomass and Bioenergy**, 130, p. 105397, 2019.

TUCK, C. O.; PÉREZ, E.; HORVÁTH, I. T.; SHELDON, R. A. *et al.* Valorization of Biomass: Deriving More Value from Waste. **Science**, 337, n. 6095, p. 695, 2012.

UBANDO, A. T.; FELIX, C. B.; CHEN, W.-H. Biorefineries in circular bioeconomy: A comprehensive review. **Bioresource technology**, 299, p. 122585, 2020.

UENO, S.; ICHINOI, H.; ZHAO, J.; FUJII, T. Degradation of fish gelatin using hot-compressed water and the properties of the degradation products. **High Pressure Research**, 35, n. 2, p. 203-213, 2015/04/03 2015.

VELVIZHI, G.; SHANTHAKUMAR, S.; DAS, B.; PUGAZHENDHI, A. *et al.* Biodegradable and non-biodegradable fraction of municipal solid waste for multifaceted applications through a closed loop integrated refinery platform: Paving a path towards circular economy. **Science of the Total Environment**, 731, p. 138049, 2020.

WAINAINA, S.; AWASTHI, M. K.; SARSAIYA, S.; CHEN, H. *et al.* Resource recovery and circular economy from organic solid waste using aerobic and anaerobic digestion technologies. **Bioresource technology**, 301, p. 122778, 2020.

YANG, Q.; CAI, X.; LIU, Z.; WANG, S. Antioxidant Assessment of Schizochytrium Meal Protein Enzymatic Hydrolysate and Its Potential Application. **Journal of Aquatic Food Product Technology**, 28, n. 4, p. 413-426, 2019.

ZHU, G.; ZHU, X.; FAN, Q.; WAN, X. Recovery of biomass wastes by hydrolysis in sub-critical water. **Resources, Conservation and Recycling**, 55, n. 4, p. 409-416, 2011/02/01/ 2011.

ZIERO, H. D. D.; BULLER, L. S.; MUDHOO, A.; AMPESE, L. C. *et al.* An overview of subcritical and supercritical water treatment of different biomasses for protein and amino acids production and recovery. **Journal of Environmental Chemical Engineering**, p. 104406, 2020.

SUPPLEMENTY MATERIAL

Table S1:

Dependent variables	Statistical model	R^2	R^2 adjusted	Dependent variables	Statistical model	R^2	R^2 adjusted
pH	No interaction	0.566	0.487	Threonine	No interaction	0.749	0.703
	L interaction	0.779	0.726		L interaction	0.801	0.753
	L Q interaction	0.921	0.887		L Q interaction	0.854	0.789
COD	No interaction	0.211	0.066	Arginine	No interaction	0.783	0.744
	L interaction	0.254	0.077		L interaction	0.956	0.946
	L Q interaction	0.863	0.803		L Q interaction	0.972	0.960
Crude protein	No interaction	0.506	0.416	Alanine	No interaction	0.836	0.806
	L interaction	0.861	0.827		L interaction	0.872	0.842
	L Q interaction	0.941	0.915		L Q interaction	0.952	0.930
Total aminoacids	No interaction	0.845	0.817	Tyrosine	No interaction	0.344	0.225
	L interaction	0.855	0.823		L interaction	0.703	0.633
	L Q interaction	0.944	0.919		L Q interaction	0.845	0.776
Aspartic acid	No interaction	0.871	0.846	Valine	No interaction	0.800	0.763
	L interaction	0.952	0.941		L interaction	0.895	0.870
	L Q interaction	0.967	0.953		L Q interaction	0.957	0.939
Glutamic acid	No interaction	0.918	0.903	Metionine	No interaction	0.589	0.515
	L interaction	0.936	0.921		L interaction	0.692	0.619
	L Q interaction	0.941	0.915		L Q interaction	0.760	0.654
Cysteine	No interaction	0.285	0.155	Tryptophan	No interaction	0.828	0.797
	L interaction	0.446	0.314		L interaction	0.900	0.876
	L Q interaction	0.571	0.381		L Q interaction	0.955	0.935
Aspargine	No interaction	0.884	0.863	Phenylalanine	No interaction	0.468	0.372
	L interaction	0.939	0.925		L interaction	0.634	0.547
	L Q interaction	0.974	0.961		L Q interaction	0.774	0.674

Serine	No interaction	0.863	0.838	Isoleucine	No interaction	0.845	0.817
	L interaction	0.863	0.831		L interaction	0.863	0.831
	L Q interaction	0.913	0.875		L Q interaction	0.887	0.837
Glutamine	No interaction	0.450	0.350	Leucine	No interaction	0.765	0.723
	L interaction	0.676	0.599		L interaction	0.846	0.812
	L Q interaction	0.864	0.804		L Q interaction	0.897	0.851
Histidine	No interaction	0.405	0.297	Lysine	No interaction	0.591	0.516
	L interaction	0.408	0.266		L interaction	0.735	0.671
	L Q interaction	0.921	0.887		L Q interaction	0.849	0.782
Glycine	No interaction	0.897	0.879	Proline	No interaction	0.845	0.817
	L interaction	0.918	0.898		L interaction	0.865	0.834
	L Q interaction	0.965	0.950		L Q interaction	0.955	0.935

Tabela S2. Análise de Variância (ANOVA) para o modelo matemático “L interaction”.

	MM	DF	Total aminoacids		Aspartic acid		Serine		Glyc
			F	p	F	p	F	p	F
Temperature (°C)	Linear	1	217,79	0,0000	371,60	0,0000	170,18	0,0000	396,7 0
Temperature (°C)	Quadratic	1	18,87	0,0004	32,64	0,0000	0,13	0,7278	0,98
Flow (mL/min)	Linear	1	26,37	0,0001	79,92	0,0000	9,55	0,0063	61,59
Flow (mL/min)	Quadratic	1	11,64	0,0031	0,05	0,8232	0,58	0,4571	14,82
Temperature (°C) by Flow (mL/min)	Linear	1	4,02	0,0601	45,86	0,0000	0,12	0,7287	10,7
Lack of fit	-	3	9,41	0,0006	2,77	0,0718	3,48	0,081	8,43
Temperature (°C)	L+Q	2	118,33	0,0000	202,12	0,0000	85,15	0,000	198,8 4
Flow (mL/min)	L+Q	2	19,00	0,0000	39,98	0,0000	5,07	0,018	38,2
Temperature (°C) by Flow (mL/min)	L+Q	1	4,02	0,0601	45,86	0,0000	0,12	0,729	10,7
			Tryptophan		Isoleucine		Proline		Meth
			F	p	F	p	F	p	F

Temperature (°C)	Linear	1	331,08	0,0000	123,92	0,0000	3,67	0,0715	33,18
Temperature (°C)	Quadratic	1	1,43	0,2478	3,14	0,0933	295,50	0,0000	2,18
Flow (mL/min)	Linear	1	0,47	0,5025	7,78	0,0121	4,11	0,0577	6,15
Flow (mL/min)	Quadratic	1	0,76	0,3962	0,25	0,6235	34,91	0,0000	2,89
Temperature (°C) by Flow (mL/min)	Linear	1	28,68	0,0000	2,88	0,1068	8,19	0,0103	7,71
Lack of fit	-	3	7,41	0,0020	1,26	0,3163	11,87	0,0002	1,71
Temperature (°C)	L+Q	2	166,25	0,0000	63,53	0,000	149,58	0,0000	17,68
Flow (mL/min)	L+Q	2	0,61	0,5533	4,02	0,036	19,51	0,0000	4,52
Temperature (°C) by Flow (mL/min)	L+Q	1	28,68	0,0000	2,88	0,107	8,19	0,0103	7,71

MM, mathematical model; DF, degree of freedom.

CAPÍTULO 4 – DISCUSSÕES GERAIS

Este trabalho buscou verificar as seguintes hipóteses:

- O processo de hidrólise em água subcrítica é uma técnica ambientalmente favorável para a valorização de resíduos proteicos de origem animal para obtenção de altos rendimentos de produtos com alto valor agregado;
- Os aminoácidos obtidos na hidrólise em água subcrítica poderão ter aplicação na indústria alimentícia e farmacêutica;

Neste trabalho, os processos de hidrólise com água subcrítica aplicados para a conversão de biomassa animal rica em proteína, apresentaram alta complexidade e dependência das propriedades, composição e estrutura da biomassa. Consequentemente, cada biomassa específica traz consigo suas restrições e desafios únicos relacionadas ao desenvolvimento e análise de processos. À continuação, uma discussão geral mais detalhada e específica por capítulos é apresentada.

4.1. Discussão sobre o Capítulo 2- Uma visão geral do tratamento subcrítico e supercrítico da água de diferentes biomassas para a produção e recuperação de proteínas e aminoácidos

O trabalho de revisão foi organizado para discutir a relevância econômica de proteínas e aminoácidos, as principais rotas de extração de proteínas oriundas de biomassa, as interações e influência dos principais parâmetros de reação e condições (considerando a dinâmica do bioprocesso dependente da temperatura e agregação-desagregação de proteínas e seu controle na recuperação de proteínas e aminoácidos). Ainda, realizou-se a comparação de diferentes processos de extração, bem como a avaliação de configurações de reatores. Finalmente, realizou-se a abordagem de perspectivas para o desenvolvimento de pesquisas futuras.

Identifica-se que foram desenvolvidos vários processos para a obtenção de aminoácidos a partir de subprodutos e resíduos, com destaque às tecnologias de água subcríticas e supercríticas, consideradas alternativas verdes e sustentáveis. O processamento de biomassa com água subcrítica para a produção e a recuperação de proteínas e aminoácidos em escala laboratorial tem progredido lentamente ao longo dos últimos dez anos. Uma avaliação concisa dos resultados da pesquisa, entre 2001 e 2020, sobre hidrólise com água subcrítica de substratos à base de proteínas para obtenção de aminoácidos, revelaram que, o tema apresenta relevância e interesse acadêmico e econômico.

As principais vias de extração de proteínas, importância econômica, influência e interações dos principais parâmetros e condições de reação também foram descritas no trabalho. Outros pontos abordados foram a desagregação-agregação de proteínas, configurações do reator e, finalmente, as perspectivas de pesquisas futuras.

Os resultados forneceram *insights* sobre as várias dimensões de desempenho de processo, dinâmica e obstáculos a serem superados. Os projetos e sistemas analisados ainda se apresentam muito em escala laboratorial, mas com muito potencial. Também se apresentam escassos em escala piloto, e aparecem menos ainda na escala industrial. O *design* e condicionamento de alguns sistemas de hidrólise com água subcrítica em grande escala para conversão de biomassa em moléculas de biocombustíveis, por exemplo, são evidências mais claras e encorajadoras de tal possibilidade.

4.2. Discussão sobre o Capítulo 3- Obtendo aminoácidos recuperados de penas de aves utilizando de hidrólise com água subcrítica

O descarte e reutilização de alguns tipos de resíduos pode ser considerado uma questão crítica para a economia e o meio ambiente. As penas de aves de corte são um subproduto da produção de derivados de frango, mas seus resíduos são comumente considerados rejeitos. Penas são um material à base de queratina, composto por queratinas α e β, e a porção de proteína bruta na massa total de penas varia de 85 a 90%.

Uma nova abordagem para a recuperação de aminoácidos que obtenham alta eficiência e seja ecologicamente correta se torna necessária. A hidrólise com água subcrítica é uma tecnologia que se encaixa na conversão de materiais orgânicos em nutrientes de alto valor, como resíduos proteicos, em aminoácidos. O trabalho teve como objetivo obter aminoácidos de penas de aves utilizando a água subcrítica como solvente para hidrolisar o teor proteico das penas em um reator semi-contínuo variando a temperatura da hidrólise (210 – 250 °C) e a taxa de vazão de água (5 – 15 $mL.min^{-1}$) e comparar os resultados com técnicas convencionais de hidrólise de proteínas.

Análises de nitrogênio indicaram que as penas de aves *in natura* possuem cerca de 15% nitrogênio, e que é importante notar que nem todo o nitrogênio recuperado é de aminoácidos. As temperaturas estudadas permitiram a recuperação de aminoácidos logo no início do processo de hidrólise.

Análises de DQO mostraram que os valores foram maiores no início das hidrólises, nos primeiros 3 pontos, diminuindo conforme o decorrer da hidrólise em água subcrítica. Isto é válido para as três temperaturas estudadas. Como mencionado, a DQO é um bom indicador da quantidade de conteúdo orgânico em uma solução. Assim, a

concentração de matéria orgânica, durante as primeiras coletas de amostras, é maior do que nos últimos pontos de coleta.

Finalmente, até na análise visual observou-se que, quanto maior a temperatura maior a taxa de hidrólise e, portanto, menor quantidade de resíduo permanece no reator. As análises de proteína bruta indicam resultados favoráveis indicando um rendimento bastante alto. O processo de hidrólise em água subcrítica conseguiu hidrolisar alta porcentagem de proteína ou de complexos proteicos, promovendo a sua quebra e solubilização, resultando na desintegração das cadeias polipeptídicas, que liberaram seus aminoácidos constituintes. A maior temperatura, 250 °C, e menores vazões, 5 e 7,5 $mL.min^{-1}$, proporcionaram maior recuperação de aminoácidos essenciais.

CAPÍTULO 5 – CONCLUSÕES GERAIS

Os setores da indústria agrícola e de alimentos desempenham papéis essenciais na economia global, entretanto, geram grandes quantidades de resíduos associados às suas atividades todos os anos, que, por sua vez, requerem uma gestão adequada. Um destino alternativo para estes subprodutos agroindustriais seria a produção de compostos valiosos, como aminoácidos, compostos fenólicos, açúcares solúveis em água, ácidos orgânicos, metano, óleos, entre outros. Neste sentido, a finalidade deste trabalho foi analisar os parâmetros de processos que dependem das características intrínsecas de resíduos de penas de aves para a hidrólise em água subcrítica, visando a obtenção de aminoácidos com alto valor agregado.

As seguintes conclusões gerais foram selecionadas para os capítulos 2, 3 e 4 deste trabalho:

- ✓ As proteínas são nutrientes essenciais para o adequado metabolismo, portanto, são de alto interesse para a industria;
- ✓ Há interesse crescente na recuperação de proteínas de recursos naturais e subprodutos agroindustriais para posterior utilização em diferentes áreas industriais;
- ✓ Existe rotas diretas para obter produtos químicos de plataforma proveniente de materiais ricos em proteínas através da hidrólise das ligações peptídicas, que resulta em uma mistura de aminoácidos e uma vez que essa mistura é produzida, é possível isolar cada aminoácido empregando processos de separação;
- ✓ Os amoniácidos com maior valor de mercado são: Cistina (0,53 – 1,02 USD/g); Ácido glutâmico (0,10 – 0,20 USD/g); Histidina (0,64 – 1,04 USD/g); Isoleucina (0,88 – 1,52 USD/g); Lisina (3,09 USD/g) e Triptofano (0,68 – 1,16 USD/g);
- ✓ O parecer da Inova sobre a patente aprovou o escopo de proteção de um processo para obtenção de aminoácidos a partir de resíduos industriais de biomassa animal,

mais especialmente penas de aves, utilizando hidrólise em água subcrítica nas condições de 200 a 230ºC, 10 a 14MPa, e fluxo semi-contínuo de 5 a 10 $mL.min^{-1}$;

- ✓ Os ensaios realizados de hidrólise em água subcrítica a partir de penas de aves indicaram maiores rendimentos de proteína e aminoacidos essenciais e não essenciais nas seguintes condições experimentais, 210 °C e 10 $mL.mim^{-1}$, 250 °C e 7,5 $mL.min^{-1}$ e 5 $mL.min^{-1}$, e 230 °C e 10 $mL.min^{-1}$, respecticamente;
- ✓ As condições do processo da presente invenção não foram localizadas em um único documento, portanto, a matéria apresentada na patente possui o requisito de Novidade, no entanto por se tratar de uma questão adaptativa, do ponto de vista da propriedade intelectual, a presente invenção não preencheria particularmente o requisito da atividade inventiva;
- ✓ De maneira geral o valor de pH dos hidrolisados mostrou-se maior (entre 6 e 8) que os relatados na literatura. Indicando que a hidrolisado de penas de aves proporcionou um pH neutro;
- ✓ A análise de nitrogênio total, nas temperaturas estudadas, permitiu perceber a recuperação de nitrogênio logo no início do processo de hidrólise;
- ✓ A DQO mostrou ser um bom indicador da quantidade de matéria orgânica em uma solução, apresentando maiores valores nas primeiras coletas de amostras se comparados com os últimos pontos de coleta;
- ✓ Uma maior concentração de aminoácidos essenciais foram observados na temperatura 250 °C como valina, metionina, triptofano, fenilalanina, isoleucina, leucina e lisina, enquanto aminoácidos não essenciais foram obtidos nas temperaturas mais baixas e moderadas;
- ✓ As análises de proteína bruta mostraram que o processo de água subcrítica conseguiu extrair, degradar ou solubilizar alta porcentagem de proteína ou complexo proteico.

CAPÍTULO 6 – REFERÊNCIAS BIBLIOGRÁFICAS

REFERENCIAS

ABDELMOEZ, W.; NAKAHASI, T.; YOSHIDA, H. Amino acid transformation and decomposition in saturated subcritical water conditions. **Industrial & Engineering Chemistry Research**, 46, n. 16, p. 5286-5294, 2007.

ABDELMOEZ, W.; YOSHIDA, H. Simulation of fast reactions in batch reactors under sub-critical water condition. **AIChE journal**, 52, n. 10, p. 3600-3611, 2006.

ABDELMOEZ, W.; YOSHIDA, H. Production of amino and organic acids from protein using sub-critical water technology. **International Journal of Chemical Reactor Engineering**, 11, n. 1, p. 369-384, 2013.

ABPA. 2020. Disponível em: http://www.brazilianchicken.com.br/pt/poultry-industry/background. Acesso em: 08/04/2021.

ADRIAN, M.; ELIZABETH, T. Poverty, politics and plastic: Organic waste sorting in Blantyre's public markets. **Journal of Urban Management**, 2021.

AHMED, R.; CHUN, B.-S. Subcritical water hydrolysis for the production of bioactive peptides from tuna skin collagen. **The Journal of Supercritical Fluids**, 141, p. 88-96, 2018/11/01/ 2018.

AKALıN, M. K.; TEKIN, K.; KARAGÖZ, S. Supercritical fluid extraction of biofuels from biomass. **Environmental Chemistry Letters**, 15, n. 1, p. 29-41, 2016.

ALAO, B. O.; FALOWO, A. B.; CHULAYO, A.; MUCHENJE, V. The potential of animal by-products in food systems: Production, prospects and challenges. **Sustainability**, 9, n. 7, p. 1089, 2017.

ALVAREZ, C.; RENDUELES, M.; DIAZ, M. The yield of peptides and amino acids following acid hydrolysis of haemoglobin from porcine blood. **Animal Production Science**, 52, n. 5, p. 313-320, 2012.

ANOOTTHATO, S.; THERDTHAI, N.; RITTHIRUANGDEJ, P. Characterization of protein hydrolysate from silkworm pupae (Bombyx mori). **Journal of Food Processing and Preservation**, 43, n. 8, Aug 2019. Article.

ANOOTTHATO, S.; THERDTHAI, N.; RITTHIRUANGDEJ, P. Characterization of protein hydrolysate from silkworm pupae (Bombyx mori). **Journal of Food Processing and Preservation**, 43, n. 8, p. e14021, 2019.

ARAUZO, P.; DU, L.; OLSZEWSKI, M.; ZAVALA, M. M. *et al.* Effect of protein during hydrothermal carbonization of brewer's spent grain. **Bioresour Technol**, 293, p. 122117, 2019.

ARNÁIZ, E.; BERNAL, J.; MARTÍN, M.; NOZAL, M. *et al.* Supercritical fluid extraction of free amino acids from broccoli leaves. **Journal of Chromatography A**, 1250, p. 49-53, 2012.

ASADUZZAMAN, A.; CHUN, B.-S. Recovery of functional materials with thermally stable antioxidative properties in squid muscle hydrolyzates by subcritical water. **Journal of food science and technology**, 52, n. 2, p. 793-802, 2015.

ASADUZZAMAN, A. K.; CHUN, B. S. Recovery of functional materials with thermally stable antioxidative properties in squid muscle hydrolyzates by subcritical water. **J Food Sci Technol**, 52, n. 2, p. 793-802, Feb 2015.

ASADUZZAMAN, A. K. M.; CHUN, B.-S. Recovery of functional materials with thermally stable antioxidative properties in squid muscle hydrolyzates by subcritical water. **Journal of Food Science and Technology**, 52, n. 2, p. 793-802, 2015/02/01 2015.

ASSOCIATION, A. P. H.; ASSOCIATION, A. W. W.; FEDERATION, W. P. C.; FEDERATION, W. E. **Standard methods for the examination of water and wastewater**. American Public Health Association., 1912.

AZMIR, J.; ZAIDUL, I. S. M.; RAHMAN, M.; SHARIF, K. *et al.* Techniques for extraction of bioactive compounds from plant materials: A review. **Journal of food engineering**, 117, n. 4, p. 426-436, 2013.

AZMIR, J.; ZAIDUL, I. S. M.; RAHMAN, M. M.; SHARIF, K. M. *et al.* Techniques for extraction of bioactive compounds from plant materials: A review. **Journal of Food Engineering**, 117, n. 4, p. 426-436, 2013.

BAI, G.; BEE, J. S.; BIDDLECOMBE, J. G.; CHEN, Q. *et al.* Computational fluid dynamics (CFD) insights into agitation stress methods in biopharmaceutical development. **International journal of pharmaceutics**, 423, n. 2, p. 264-280, 2012.

BARAKAT, A.; JÉRÔME, F.; ROUAU, X. A Dry Platform for Separation of Proteins from Biomass-Containing Polysaccharides, Lignin, and Polyphenols. **ChemSusChem**, 8, n. 7, p. 1161-1166, 2015.

BERGENDAHL, J. A.; SARKIS, J.; TIMKO, M. T. Transdisciplinarity and the food energy and water nexus: Ecological modernization and supply chain sustainability perspectives. **Resources, Conservation and Recycling**, 133, p. 309-319, 2018.

BioativosGroup®. Disponível em: http://bioativosgroup.com.br/.

BREYDO, L.; REDDY, K. D.; PIAI, A.; FELLI, I. C. *et al.* The crowd you're in with: effects of different types of crowding agents on protein aggregation. **Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics**, 1844, n. 2, p. 346-357, 2014.

BRUNNER, G. Supercritical fluids: technology and application to food processing. **Journal of food engineering**, 67, n. 1-2, p. 21-33, 2005.

BRUNNER, G. Near critical and supercritical water. Part I. Hydrolytic and hydrothermal processes. **The Journal of Supercritical Fluids**, 47, n. 3, p. 373-381, 2009.

CAMPOS, I.; VALENTE, L. M. P.; MATOS, E.; MARQUES, P. *et al.* Life-cycle assessment of animal feed ingredients: Poultry fat, poultry by-product meal and hydrolyzed feather meal. **Journal of Cleaner Production**, 252, p. 119845, 2020.

CASTRO-VARGAS, H. I.; RODRÍGUEZ-VARELA, L. I.; FERREIRA, S. R.; PARADA-ALFONSO, F. Extraction of phenolic fraction from guava seeds (Psidium guajava L.) using supercritical carbon dioxide and co-solvents. **The Journal of Supercritical Fluids**, 51, n. 3, p. 319-324, 2010.

CELABOR. **CELABOR's automated subcritical water lab-scale and pilot-scale plants**. Disponível em: http://www.celabor.be/site/FCK_STOCK/Media/2016-08%20Eau%20subcritique%20EN.pdf. Acesso em: May 8.

CENTOBELLI, P.; CERCHIONE, R.; CHIARONI, D.; DEL VECCHIO, P. *et al.* Designing business models in circular economy: A systematic literature review and research agenda. **Business Strategy and the Environment**, 29, n. 4, p. 1734-1749, 2020.

CHEIGH, C. I.; YOO, S. Y.; KO, M. J.; CHANG, P. S. *et al.* Extraction characteristics of subcritical water depending on the number of hydroxyl group in flavonols. **Food Chemistry**, 168, p. 21-26, Feb 1 2015. Research Support, Non-U.S. Gov't.

CHEMISTS, A. O. O. A.; HORWITZ, W. **Official methods of analysis**. Association of Official Analytical Chemists Washington, DC, 1975.

CHEN, J.; WANG, X.; ZHANG, B.; YANG, Y. *et al.* Integrating enzymatic hydrolysis into subcritical water pretreatment optimization for bioethanol production from wheat straw. **Science of the Total Environment**, 770, p. 145321, 2021/05/20/ 2021.

CHENG, H.; ZHU, X.; ZHU, C.; QIAN, J. *et al.* Hydrolysis technology of biomass waste to produce amino acids in sub-critical water. **Bioresource technology**, 99, n. 9, p. 3337-3341, 2008.

CHENG, H.; ZHU, X.; ZHU, C.; QIAN, J. *et al.* Hydrolysis technology of biomass waste to produce amino acids in sub-critical water. **Bioresour Technol**, 99, n. 9, p. 3337-3341, Jun 2008. Research Support, Non-U.S. Gov't.

CHERRY, J.; YOUNG, C.; SHEWFELT, A. Characterization of protein isolates from keratinous material of poultry feathers. **Journal of food science**, 40, n. 2, p. 331-335, 1975.

COCERO, M. J.; CABEZA, A.; ABAD, N.; ADAMOVIC, T. *et al.* Understanding biomass fractionation in subcritical & supercritical water. **The Journal of Supercritical Fluids**, 133, p. 550-565, 2018.

CROMWELL, M. E.; HILARIO, E.; JACOBSON, F. Protein aggregation and bioprocessing. **The AAPS journal**, 8, n. 3, p. E572-E579, 2006.

CUNNIFF, P. Official methods of analysis of aoac international. **J. AOAC Int**, 80, p. 127A, 1997.

CVETANOVIĆ, A.; ŠVARC-GAJIĆ, J.; GAŠIĆ, U.; TEŠIĆ, Ž. *et al.* Isolation of apigenin from subcritical water extracts: optimization of the process. **The Journal of Supercritical Fluids**, 120, p. 32-42, 2017.

DE ANDRADE LIMA, M.; CHARALAMPOPOULOS, D.; CHATZIFRAGKOU, A. Purification of supercritical-fluid carotenoid-rich extracts by hydrophobic interaction chromatography. **Separation and Purification Technology**, 203, p. 1-10, 2018.

DE MOURA, J. M.; GONÇALVES, L. A.; SARMENTO, L. A.; PETRUS, J. C. C. Purification of structured lipids using SCCO2 and membrane process. **Journal of Membrane Science**, 299, n. 1-2, p. 138-145, 2007.

DE SCHOUWER, F.; CLAES, L.; VANDEKERKHOVE, A.; VERDUYCKT, J. *et al.* Protein-Rich Biomass Waste as a Resource for Future Biorefineries: State of the Art, Challenges, and Opportunities. **ChemSusChem**, 12, n. 7, p. 1272-1303, 2019.

DENG, G.-F.; SHEN, C.; XU, X.-R.; KUANG, R.-D. *et al.* Potential of fruit wastes as natural resources of bioactive compounds. **International journal of molecular sciences**, 13, n. 7, p. 8308-8323, 2012.

DENG, G.-F.; SHEN, C.; XU, X.-R.; KUANG, R.-D. *et al.* Potential of Fruit Wastes as Natural Resources of Bioactive Compounds. **International Journal of Molecular Sciences**, 13, n. 7, p. 8308-8323, 2012.

DERWENSKUS, F.; METZ, F.; GILLE, A.; SCHMID-STAIGER, U. *et al.* Pressurized extraction of unsaturated fatty acids and carotenoids from wet Chlorella vulgaris and Phaeodactylum tricornutum biomass using subcritical liquids. **Global Change Biology Bioenergy**, 11, n. 1, p. 335-344, 2019.

DRAGONE, G.; KERSSEMAKERS, A. A.; DRIESSEN, J. L.; YAMAKAWA, C. K. *et al.* Innovation and strategic orientations for the development of advanced biorefineries. **Bioresour Technol**, p. 122847, 2020.

ESPINOZA, A. D.; MORAWICKI, R. O. Effect of additives on subcritical water hydrolysis of whey protein isolate. **J Agric Food Chem**, 60, n. 20, p. 5250-5256, May 23 2012.

ESPINOZA, A. D.; MORAWICKI, R. O.; HAGER, T. Hydrolysis of whey protein isolate using subcritical water. **Journal of food science**, 77, n. 1, p. C20-C26, 2012.

ESTEBAN, M.; GARCÍA, A.; RAMOS, P.; MÁRQUEZ, M. Sub-critical water hydrolysis of hog hair for amino acid production. **Bioresource technology**, 101, n. 7, p. 2472-2476, 2010.

ESTEBAN, M. B.; GARCIA, A. J.; RAMOS, P.; MARQUEZ, M. C. Sub-critical water hydrolysis of hog hair for amino acid production. **Bioresour Technol**, 101, n. 7, p. 2472-2476, Apr 2010.

ESTEBAN, M. B.; GARCÍA, A. J.; RAMOS, P.; MÁRQUEZ, M. C. Sub-critical water hydrolysis of hog hair for amino acid production. **Bioresource technology**, 101, n. 7, p. 2472-2476, 2010/04/01/ 2010.

FAGBEMI, O. D.; SITHOLE, B.; TESFAYE, T. Optimization of keratin protein extraction from waste chicken feathers using hybrid pre-treatment techniques. **Sustainable Chemistry and Pharmacy**, 17, p. 100267, 2020.

FAN, X.; HU, S.; WANG, K.; YANG, R. *et al.* Coupling of ultrasound and subcritical water for peptides production from Spirulina platensis. **Food and Bioproducts Processing**, 121, p. 105-112, 2020.

FAPESP. **biorefinery will convert renewable biomass into green intermediate chemicals**. Disponível em: https://agencia.fapesp.br/biorefinery-will-convert-renewable-biomass-into-green-intermediate-chemicals/31186/. Acesso em: April 14.

FARMER, T. J.; MASCAL, M. Platform molecules. **Introduction to chemicals from biomass**, 2, 2015.

FU, Q.; ZHANG, H.; CHEN, H.; LIAO, Q. *et al.* Hydrothermal hydrolysis pretreatment of microalgae slurries in a continuous reactor under subcritical conditions for large–scale application. **Bioresour Technol**, 266, p. 306-314, 2018.

GARCIA-MOSCOSO, J. L.; TEYMOURI, A.; KUMAR, S. Kinetics of Peptides and Arginine Production from Microalgae (Scenedesmus sp.) by Flash Hydrolysis. **Industrial & Engineering Chemistry Research**, 54, n. 7, p. 2048-2058, 2015.

GBASHI, S.; ADEBO, O. A.; PIATER, L.; MADALA, N. E. *et al.* Subcritical water extraction of biological materials. **Separation & Purification Reviews**, 46, n. 1, p. 21-34, 2017.

GETACHEW, A. T.; CHUN, B. S. Influence of pretreatment and modifiers on subcritical water liquefaction of spent coffee grounds: A green waste valorization approach. **Journal of Cleaner Production**, 142, p. 3719-3727, 2017.

GOMAA, M.; HIFNEY, A. F.; FAWZY, M. A.; ABDEL-GAWAD, K. M. Statistical optimization of culture variables for enhancing agarase production by Dendryphiella arenaria utilizing Palisada perforata (Rhodophyta) and enzymatic saccharification of the macroalgal biomass. **Marine Biotechnology**, 19, n. 6, p. 592-600, 2017.

GONG, K.; CHEN, L.; XIA, H.; DAI, H. *et al.* Driving forces of disaggregation and reaggregation of peanut protein isolates in aqueous dispersion induced by high-pressure microfluidization. **International journal of biological macromolecules**, 130, p. 915-921, 2019.

GOVENDER, K.; NAICKER, T.; BAIJNATH, S.; CHUTURGOON, A. A. *et al.* The development of a supercritical fluid chromatography (SFC) based method for the purification of biosynthesised human insulin. **Journal of Chromatography B**, p. 122126, 2020.

GRÁCIA-JULIÁ, A.; RENÉ, M.; CORTÉS-MUÑOZ, M.; PICART, L. *et al.* Effect of dynamic high pressure on whey protein aggregation: A comparison with the effect of continuous short-time thermal treatments. **Food hydrocolloids**, 22, n. 6, p. 1014-1032, 2008.

HAO, G.; CAO, W.; LI, T.; CHEN, J. *et al.* Effect of temperature on chemical properties and antioxidant activities of abalone viscera subcritical water extract. **The Journal of Supercritical Fluids**, 147, p. 17-23, 2019.

HARADA, M.; KARAKAWA, S.; YAMADA, N.; MIYANO, H. *et al.* Biaryl axially chiral derivatizing agent for simultaneous separation and sensitive detection of proteinogenic amino acid enantiomers using liquid chromatography–tandem mass spectrometry. **Journal of Chromatography A**, 1593, p. 91-101, 2019.

HERRERO, M.; CIFUENTES, A.; IBAÑEZ, E. Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. **Food Chemistry**, 98, n. 1, p. 136-148, 2006.

HOSSAIN, G. S.; LI, J.; SHIN, H.-D.; CHEN, R. R. *et al.* Bioconversion of l-glutamic acid to α-ketoglutaric acid by an immobilized whole-cell biocatalyst expressing l-amino acid deaminase from Proteus mirabilis. **Journal of biotechnology**, 169, p. 112-120, 2014.

INFORMATION, N. C. F. B. **PubChem Compound Summary for CID 138, 5-Aminovaleric acid**. 2004. Disponível em: https://pubchem.ncbi.nlm.nih.gov/compound/5-Aminovaleric-acid. Acesso em: 31 July.

IVANOV, K.; IVANOVA, S.; GEORGIEVA, M.; ATANASOV, P. Production and regulatory analytical control of amino acids include in food additives. **Pharmacia**, 61, n. 2, p. 48-54, 2014.

JACOBS, M. R.; GRACE, M.; BLUMLEIN, A.; MCMANUS, J. J. Differential Scanning Calorimetry to Quantify Heat-Induced Aggregation in Concentrated Protein Solutions. *In*: **Protein Self-Assembly**: Springer, 2019. p. 117-129.

JAESCHKE, D. P.; MERCALI, G. D.; MARCZAK, L. D. F.; MULLER, G. *et al.* Extraction of valuable compounds from Arthrospira platensis using pulsed electric field treatment. **Bioresour Technol**, 283, p. 207-212, Jul 2019.

KANG, K.; QUITAIN, A. T.; DAIMON, H.; NODA, R. *et al.* Optimization of amino acids production from waste fish entrails by hydrolysis in sub and supercritical water. **The Canadian Journal of Chemical Engineering**, 79, n. 1, p. 65-70, 2001.

KHEIRKHAH, H.; BAROUTIAN, S.; QUEK, S. Y. Evaluation of bioactive compounds extracted from Hayward kiwifruit pomace by subcritical water extraction. **Food and Bioproducts Processing**, 115, p. 143-153, 2019.

KHUMALO, M.; SITHOLE, B.; TESFAYE, T. Valorisation of waste chicken feathers: Optimisation of keratin extraction from waste chicken feathers by sodium bisulphite, sodium dodecyl sulphate and urea. **Journal of environmental management**, 262, p. 110329, 2020.

KIAMAHALLEH, M. V.; NAJAFPOUR-DARZI, G.; RAHIMNEJAD, M.; MOGHADAMNIA, A. A. *et al.* High performance curcumin subcritical water extraction from turmeric (Curcuma longa L.). **Journal of Chromatography B**, 1022, p. 191-198, 2016.

KING, J. Advances in critical fluid technology for food processing. 2000.

KING, J. W. Advances in critical fluid technology for food processing. **Food science & technology today : the information journal of the Institute of Food Science & Technology (UK)**, 2000 v.14 no.4, n. no. 4, p. pp. 186-191, 2000 2000. Article.

KLINGLER, D.; BERG, J.; VOGEL, H. Hydrothermal reactions of alanine and glycine in sub-and supercritical water. **The Journal of Supercritical Fluids**, 43, n. 1, p. 112-119, 2007.

KNEZ, Ž.; HRNČIČ, M. K.; ČOLNIK, M.; ŠKERGET, M. Chemicals and value added compounds from biomass using sub- and supercritical water. **The Journal of Supercritical Fluids**, 133, p. 591-602, 2018.

KOOMYART, I.; NAGAMIZU, H.; KHUWIJITJARU, P.; KOBAYASHI, T. *et al.* Astaxanthin stability and color change of krill during subcritical water treatment. **J Food Sci Technol**, 54, n. 10, p. 3065-3072, 2017a.

KOOMYART, I.; NAGAMIZU, H.; KHUWIJITJARU, P.; KOBAYASHI, T. *et al.* Astaxanthin stability and color change of krill during subcritical water treatment. **Journal of food science and technology**, 54, n. 10, p. 3065-3072, 2017b.

KUMAR, S.; GUPTA, R.; LEE, Y.; GUPTA, R. B. Cellulose pretreatment in subcritical water: effect of temperature on molecular structure and enzymatic reactivity. **Bioresour Technol**, 101, n. 4, p. 1337-1347, 2010.

LACHOS-PEREZ, D.; BASEGGIO, A. M.; MAYANGA-TORRES, P.; JUNIOR, M. R. M. *et al.* Subcritical water extraction of flavanones from defatted orange peel. **The Journal of Supercritical Fluids**, 138, p. 7-16, 2018.

LACHOS-PEREZ, D.; BROWN, A. B.; MUDHOO, A.; TIMKO, M. T. *et al.* Applications of subcritical and supercritical water conditions for extraction, hydrolysis, gasification, and carbonization of biomass: a critical review. **Biofuel Research Journal**, 4, n. 2, p. 611-626, 2017.

LACHOS-PEREZ, D.; MARTINEZ-JIMENEZ, F.; REZENDE, C.; TOMPSETT, G. *et al.* Subcritical water hydrolysis of sugarcane bagasse: An approach on solid residues characterization. **The Journal of Supercritical Fluids**, 108, p. 69-78, 2016.

LACHOS-PEREZ, D.; TOMPSETT, G.; GUERRA, P.; TIMKO, M. *et al.* Sugars and char formation on subcritical water hydrolysis of sugarcane straw. **Bioresource technology**, 243, p. 1069-1077, 2017.

LAMMENS, T. M.; FRANSSEN, M. C. R.; SCOTT, E. L.; SANDERS, J. P. M. Availability of protein-derived amino acids as feedstock for the production of bio-based chemicals. **Biomass and Bioenergy**, 44, p. 168-181, 2012.

LAMMENS, T. M.; LE NÔTRE, J.; FRANSSEN, M. C.; SCOTT, E. L. *et al.* Synthesis of biobased succinonitrile from glutamic acid and glutamine. **ChemSusChem**, 4, n. 6, p. 785, 2011.

LAMOOLPHAK, W.; DE-EKNAMKUL, W.; SHOTIPRUK, A. Hydrothermal production and characterization of protein and amino acids from silk waste. **Bioresour Technol**, 99, n. 16, p. 7678-7685, Nov 2008. Research Support, Non-U.S. Gov't.

LAMP, A.; KALTSCHMITT, M.; LÜDTKE, O. Protein recovery from bioethanol stillage by liquid hot water treatment. **The Journal of Supercritical Fluids**, 155, p. 104624, 2020.

LE NÔTRE, J.; SCOTT, E. L.; FRANSSEN, M. C.; SANDERS, J. P. Biobased synthesis of acrylonitrile from glutamic acid. **Green chemistry**, 13, n. 4, p. 807-809, 2011.

LI, P.; SAKURAGI, K.; MAKINO, H. Extraction techniques in sustainable biofuel production: A concise review. **Fuel Processing Technology**, 193, p. 295-303, 2019.

LI, S. Y.; NG, I. S.; CHEN, P. T.; CHIANG, C. J. *et al.* Biorefining of protein waste for production of sustainable fuels and chemicals. **Biotechnol Biofuels**, 11, p. 256, 2018. Review.

LIN, X.; PAN, J.; ZHOU, M.; XU, Y. *et al.* Extraction of amphoteric amino acid by bipolar membrane electrodialysis: methionine acid as a case study. **Industrial & Engineering Chemistry Research**, 55, n. 10, p. 2813-2820, 2016.

LU, W.; CHEN, X.-W.; WANG, J.-M.; YANG, X.-Q. *et al.* Enzyme-assisted subcritical water extraction and characterization of soy protein from heat-denatured meal. **Journal of Food Engineering**, 169, p. 250-258, 2016.

LULLIEN-PELLERIN, V.; BALNY, C. High-pressure as a tool to study some proteins' properties: conformational modification, activity and oligomeric dissociation. **Innovative Food Science and Emerging Technologies**, 3, p. 209-221, 2002.

LUO, G. E.; SHI, W.; CHEN, X.; NI, W. *et al.* Hydrothermal conversion of water lettuce biomass at 473 or 523 K. **Biomass and Bioenergy**, 35, n. 12, p. 4855-4861, 2011.

MACIEL-SILVA, F. W.; MUSSATTO, S. I.; FORSTER-CARNEIRO, T. Integration of subcritical water pretreatment and anaerobic digestion technologies for valorization of açai processing industries residues. **Journal of Cleaner Production**, 228, p. 1131-1142, 2019.

MARCET, I.; ALVAREZ, C.; PAREDES, B.; DIAZ, M. The use of sub-critical water hydrolysis for the recovery of peptides and free amino acids from food processing wastes. Review of sources and main parameters. **Waste Manag**, 49, p. 364-371, Mar 2016. Research Support, Non-U.S. Gov't Review.

MARCET, I.; ÁLVAREZ, C.; PAREDES, B.; DÍAZ, M. The use of sub-critical water hydrolysis for the recovery of peptides and free amino acids from food processing wastes. Review of sources and main parameters. **Waste Management**, 49, p. 364-371, 2016.

MARRONE, P. A. Supercritical water oxidation—current status of full-scale commercial activity for waste destruction. **The Journal of Supercritical Fluids**, 79, p. 283-288, 2013.

MEKONNEN, T. H.; MUSSONE, P. G.; EL-THAHER, N.; CHOI, P. *et al.* Subcritical hydrolysis and characterization of waste proteinaceous biomass for value added applications. **Journal of Chemical Technology & Biotechnology**, 90, n. 3, p. 476-483, 2015.

MOHD THANI, N.; MUSTAPA KAMAL, S. M.; TAIP, F. S.; SULAIMAN, A. *et al.* Effect of sub-critical water hydrolysis on sugar recovery from bakery leftovers. **Food and Bioproducts Processing**, 117, p. 105-112, 2019.

MOTAVAF, B.; SAVAGE, P. E. Effect of process variables on food waste valorization via hydrothermal liquefaction. **ACS ES&T Engineering**, 1, n. 3, p. 363-374, 2021.

MUSSATTO, S. I. **Biomass fractionation technologies for a lignocellulosic feedstock based biorefinery**. Elsevier, 2016. 0128025611.

OKAJIMA, I.; SAKO, T. Energy conversion of biomass with supercritical and subcritical water using large-scale plants. **Journal of bioscience and bioengineering**, 117, n. 1, p. 1-9, 2014.

PARK, J.-S.; JEONG, Y.-R.; CHUN, B.-S. Physiological activities and bioactive compound from laver (Pyropia yezoensis) hydrolysates by using subcritical water hydrolysis. **The Journal of Supercritical Fluids**, 148, p. 130-136, 2019.

PARK, S. H.; KIM, J. H.; MIN, S. G.; JO, Y. J. *et al.* Effects of Ethanol Addition on the Efficiency of Subcritical Water Extraction of Proteins and Amino Acids from Porcine Placenta. **Korean J Food Sci Anim Resour**, 35, n. 2, p. 265-271, 2015.

PATEL, H. A.; SINGH, H.; HAVEA, P.; CONSIDINE, T. *et al.* Pressure-induced unfolding and aggregation of the proteins in whey protein concentrate solutions. **J Agric Food Chem**, 53, n. 24, p. 9590-9601, 2005.

PEDRAS, B. M. S. Valorization of grape pomace through hot compressed water extraction/hydrolysis. 2015.

PENG, Z.; MAO, X.; ZHANG, J.; DU, G. *et al.* Biotransformation of keratin waste to amino acids and active peptides based on cell-free catalysis. **Biotechnology for biofuels**, 13, p. 1-12, 2020.

PEREIRA, D. G.; JUSTUS, A.; FALCÃO, H. G.; ROCHA, T. D. S. *et al.* Enzymatic hydrolysis of okara protein concentrate by mixture of endo and exopeptidase. **Journal of food processing and preservation**, 43, n. 10, p. e14134, 2019a.

PEREIRA, D. G.; JUSTUS, A.; FALCÃO, H. G.; ROCHA, T. D. S. *et al.* Enzymatic hydrolysis of okara protein concentrate by mixture of endo and exopeptidase. **Journal of Food Processing and Preservation**, 2019b.

PHILO, J. S.; ARAKAWA, T. Mechanisms of protein aggregation. **Current pharmaceutical biotechnology**, 10, n. 4, p. 348-351, 2009.

PIŃKOWSKA, H.; OLIVEROS, E. Application of the Doehlert Matrix for the Determination of the Optimal Conditions of Hydrolysis of Soybean Protein in Subcritical Water. **Industrial & Engineering Chemistry Research**, 53, n. 4, p. 1320-1326, 2014.

PIŃKOWSKA, H.; OLIVEROS, E. Application of the doehlert matrix for the determination of the optimal conditions of hydrolysis of soybean protein in subcritical water. **Industrial & Engineering Chemistry Research**, 53, n. 4, p. 1320-1326, 2014.

PINTO, A. R. R.; ANTAS, F.; SANTOS, R. C.; BOWRA, S. *et al.* Effect of reactor configuration on the subcritical water hydrolysis of recycled paper mill sludge. **Journal of Analytical and Applied Pyrolysis**, 127, p. 68-74, 2017.

POPESCU, V.; ASTANEI, D.-G.; BURLICA, R.; POPESCU, A. *et al.* Sustainable and cleaner microwave-assisted dyeing process for obtaining eco-friendly and fluorescent acrylic knitted fabrics. **Journal of Cleaner Production**, 232, p. 451-461, 2019.

POSMANIK, R.; CANTERO, D. A.; MALKANI, A.; SILLS, D. L. *et al.* Biomass conversion to bio-oil using sub-critical water: Study of model compounds for food processing waste. **The Journal of Supercritical Fluids**, 119, p. 26-35, 2017.

QIN, F.; JOHANSEN, A. Z.; MUSSATTO, S. I. Evaluation of different pretreatment strategies for protein extraction from brewer's spent grains. **Industrial Crops and Products**, 125, p. 443-453, 2018.

REDDY, N.; SANTOSH, M. Recovery and Applications of Feather Proteins. **Protein Byproducts**, p. 255-274, 2016a.

REDDY, N.; SANTOSH, M. Recovery and Applications of Feather Proteins. *In*: **Protein Byproducts**: Elsevier, 2016b. p. 255-274.

ROBERTS, C. J. Protein aggregation and its impact on product quality. **Current opinion in biotechnology**, 30, p. 211-217, 2014.

RODILES-LOPEZ, J. O.; ARROYO-MAYA, I. J.; JARAMILLO-FLORES, M. E.; GUTIERREZ-LOPEZ, G. F. *et al.* Effects of high hydrostatic pressure on the structure of bovine alpha-lactalbumin. **J Dairy Sci**, 93, n. 4, p. 1420-1428, Apr 2010. Research Support, Non-U.S. Gov't.

RODRIGUEZ, D.; MERWIN, A.; KARMIOL, Z.; CHIDAMBARAM, D. Surface chemistry and corrosion behavior of Inconel 625 and 718 in subcritical, supercritical, and ultrasupercritical water. **Applied Surface Science**, 404, p. 443-451, 2017.

ROGALINSKI, T.; HERRMANN, S.; BRUNNER, G. Production of amino acids from bovine serum albumin by continuous sub-critical water hydrolysis. **The Journal of Supercritical Fluids**, 36, n. 1, p. 49-58, 2005.

SALA, S.; GORALCZYK, M. Chemical footprint: A methodological framework for bridging life cycle assessment and planetary boundaries for chemical pollution. **Integrated environmental assessment and management**, 9, n. 4, p. 623-632, 2013.

SANTAMARIA-FERNANDEZ, M.; MOLINUEVO-SALCES, B.; LÜBECK, M.; UELLENDAHL, H. Biogas potential of green biomass after protein extraction in an organic biorefinery concept for feed, fuel and fertilizer production. **Renewable energy**, 129, p. 769-775, 2018.

SANTANA, J. C.; GARDIM, R. B.; ALMEIDA, P. F.; BORINI, G. B. *et al.* Valorization of chicken feet by-product of the poultry industry: high qualities of gelatin and biofilm from extraction of collagen. **Polymers**, 12, n. 3, p. 529, 2020.

SARAVANA, P. S.; TILAHUN, A.; GERENEW, C.; TRI, V. D. *et al.* Subcritical water extraction of fucoidan from Saccharina japonica: optimization, characterization and biological studies. **Journal of Applied Phycology**, 30, n. 1, p. 579-590, 2018.

SARFARAZI, M.; JAFARI, S. M.; RAJABZADEH, G.; FEIZI, J. Development of an environmentally-friendly solvent-free extraction of saffron bioactives using subcritical water. **LWT**, 114, p. 108428, 2019.

SASAHARA, K.; YAMAGUCHI, K.; SO, M.; GOTO, Y. Polyphosphates diminish solubility of a globular protein and thereby promote amyloid aggregation. **Journal of Biological Chemistry**, 294, n. 42, p. 15318-15329, 2019.

SATO, N.; QUITAIN, A. T.; KANG, K.; DAIMON, H. *et al.* Reaction Kinetics of Amino Acid Decomposition in High-Temperature and High-Pressure Water. **Industrial & Engineering Chemistry Research**, 43, n. 13, p. 3217-3222, 2004/06/01 2004.

SCHOMMER, V. A.; WENZEL, B. M.; DAROIT, D. J. Anaerobic co-digestion of swine manure and chicken feathers: Effects of manure maturation and microbial pretreatment of feathers on methane production. **Renewable Energy**, 152, p. 1284-1291, 2020.

SCOPUS. 2020. Disponível em: https://www.scopus.com/. Acesso em: july 17.

SEREEWATTHANAWUT, I.; PRAPINTIP, S.; WATCHIRARUJI, K.; GOTO, M. *et al.* Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. **Bioresource technology**, 99, n. 3, p. 555-561, 2008/02/01/ 2008.

SHAH, M. Commentary: New perspectives on protein aggregation during Biopharmaceutical development. **International journal of pharmaceutics**, 552, n. 1-2, p. 1-6, 2018.

SHITU, A.; IZHAR, S.; TAHIR, T. Sub-critical water as a green solvent for production of valuable materials from agricultural waste biomass: A review of recent work. 2015.

SHITU, A.; IZHAR, S.; TAHIR, T. M. Sub-critical water as a green solvent for production of valuable materials from agricultural waste biomass: A review of recent work. **Global Journal of Environmental Science and Management**, p. 255-264, 2015.

SIGMA-ALDRICH. **Glutamic acid price table**. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Glutamic+acid&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **Isoleucine price table**. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Isoleucine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **Lysine price table**. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Lysine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **Tryptophan**. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Tryptophan&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **Histidine price table**. 2020a. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Histidine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SIGMA-ALDRICH. **L-Cystine price table**. 2020b. Disponível em: https://www.sigmaaldrich.com/catalog/search?term=Cystine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Acesso em: july 15.

SMELLER, L. Pressure-temperature phase diagrams of biomolecules. **Biochimica et Biophysica Acta**, 1595, p. 11-29, 2001.

SOLATI, Z.; MANEVSKI, K.; JØRGENSEN, U.; LABOURIAU, R. *et al.* Crude protein yield and theoretical extractable true protein of potential biorefinery feedstocks. **Industrial Crops and Products**, 115, p. 214-226, 2018.

STINGONE, J. A.; WING, S. Poultry litter incineration as a source of energy: reviewing the potential for impacts on environmental health and justice. **New Solutions: A Journal of Environmental and Occupational Health Policy**, 21, n. 1, p. 27-42, 2011.

SUNPHORKA, S.; CHAVASIRI, W.; OSHIMA, Y.; NGAMPRASERTSITH, S. Kinetic studies on rice bran protein hydrolysis in subcritical water. **The Journal of Supercritical Fluids**, 65, p. 54-60, 2012.

TESFAYE, T.; SITHOLE, B.; RAMJUGERNATH, D.; CHUNILALL, V. Valorisation of chicken feathers: characterisation of chemical properties. **Waste Management**, 68, p. 626-635, 2017.

TOOR, S. S.; ROSENDAHL, L.; NIELSEN, M. P.; GLASIUS, M. *et al.* Continuous production of bio-oil by catalytic liquefaction from wet distiller's grain with solubles (WDGS) from bio-ethanol production. **Biomass and Bioenergy**, 36, p. 327-332, 2012.

TOOR, S. S.; ROSENDAHL, L.; RUDOLF, A. Hydrothermal liquefaction of biomass: A review of subcritical water technologies. **Energy**, 36, n. 5, p. 2328-2342, 2011.

TORISU, T.; MARUNO, T.; HAMAJI, Y.; OHKUBO, T. *et al.* Synergistic effect of cavitation and agitation on protein aggregation. **Journal of pharmaceutical sciences**, 106, n. 2, p. 521-529, 2017.

TORRES-MAYANGA, P. C.; LACHOS-PEREZ, D.; MUDHOO, A.; KUMAR, S. *et al.* Production of biofuel precursors and value-added chemicals from hydrolysates resulting from hydrothermal processing of biomass: A review. **Biomass and Bioenergy**, 130, p. 105397, 2019.

TREUHEIT, M. J.; KOSKY, A. A.; BREMS, D. N. Inverse relationship of protein concentration and aggregation. **Pharmaceutical research**, 19, n. 4, p. 511-516, 2002.

TUCK, C. O.; PÉREZ, E.; HORVÁTH, I. T.; SHELDON, R. A. *et al.* Valorization of Biomass: Deriving More Value from Waste. **Science**, 337, n. 6095, p. 695, 2012.

TUCK, C. O.; PÉREZ, E.; HORVÁTH, I. T.; SHELDON, R. A. *et al.* Valorization of biomass: deriving more value from waste. **Science**, 337, n. 6095, p. 695-699, 2012.

TUTUNCHI, P.; ROUFEGARINEJAD, L.; HAMISHEHKAR, H.; ALIZADEH, A. Extraction of red beet extract with beta-cyclodextrin-enhanced ultrasound assisted extraction: A strategy for enhancing the extraction efficacy of bioactive compounds and their stability in food models. **Food Chemistry**, 297, Nov 2019. Article.

UBANDO, A. T.; FELIX, C. B.; CHEN, W.-H. Biorefineries in circular bioeconomy: A comprehensive review. **Bioresource technology**, 299, p. 122585, 2020.

UEMATSU, M.; FRANK, E. U. Static Dielectric Constant of Water and Steam. **Journal of Physical and Chemical Reference Data**, 9, n. 4, p. 1291-1306, 1980.

UENO, S.; ICHINOI, H.; ZHAO, J.; FUJII, T. Degradation of fish gelatin using hot-compressed water and the properties of the degradation products. **High Pressure Research**, 35, n. 2, p. 203-213, 2015/04/03 2015.

VAID, S.; BAJAJ, B. K. Production of ionic liquid tolerant cellulase from Bacillus subtilis G 2 using agroindustrial residues with application potential for saccharification of biomass under one pot consolidated bioprocess. **Waste and biomass valorization**, 8, n. 3, p. 949-964, 2017.

VELVIZHI, G.; SHANTHAKUMAR, S.; DAS, B.; PUGAZHENDHI, A. *et al.* Biodegradable and non-biodegradable fraction of municipal solid waste for multifaceted applications through a closed loop integrated refinery platform: Paving a path towards circular economy. **Science of the Total Environment**, 731, p. 138049, 2020.

WAINAINA, S.; AWASTHI, M. K.; SARSAIYA, S.; CHEN, H. *et al.* Resource recovery and circular economy from organic solid waste using aerobic and anaerobic digestion technologies. **Bioresource technology**, 301, p. 122778, 2020.

WANG, W.; NEMA, S.; TEAGARDEN, D. Protein aggregation—pathways and influencing factors. **International journal of pharmaceutics**, 390, n. 2, p. 89-99, 2010.

Web of Science. 2020. Disponível em: https://webofknowledge.com/. Acesso em: july 17.

XU, D.; WANG, S.; TANG, X.; GONG, Y. *et al.* Design of the first pilot scale plant of China for supercritical water oxidation of sewage sludge. **Chemical Engineering Research and Design**, 90, n. 2, p. 288-297, 2012.

YANG, J.; LIU, G.; ZENG, H.; CHEN, L. Effects of high pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties. **Food hydrocolloids**, 83, p. 275-286, 2018.

YANG, Q.; CAI, X.; LIU, Z.; WANG, S. Antioxidant Assessment of Schizochytrium Meal Protein Enzymatic Hydrolysate and Its Potential Application. **Journal of Aquatic Food Product Technology**, 28, n. 4, p. 413-426, 2019.

YIN, X.-C.; LI, F.-Y.; HE, Y.-F.; WANG, Y. *et al.* Study on effective extraction of chicken feather keratins and their films for controlling drug release. **Biomaterials Science**, 1, n. 5, p. 528-536, 2013.

ZABOT, G. L.; ABAIDE, E. R.; TRES, M. V.; MAZUTTI, M. A. Subcritical hydrolysis contribution in the holistic biorefinery concept: obtaining bioproducts and biofuels from renewable natural resources for a novel bioeconomy. *In*: **Advanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts**: Elsevier, 2019. p. 35-57.

ZAINAN, N. H.; SAPARDI, M. A. M.; HO, B. C. H.; SIAJAM, S. I. *et al.* Kinetic and thermodynamic characterization of amino acids generation via subcritical water reaction of microalgae Nannochloropsis sp. biomass. **Biomass Conversion and Biorefinery**, p. 1-14, 2019.

ZHANG, M.-L.; FAN, Y.-T.; XING, Y.; PAN, C.-M. *et al.* Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures. **Biomass and Bioenergy**, 31, n. 4, p. 250-254, 2007.

ZHANG, Q.-T.; TU, Z.-C.; WANG, H.; HUANG, X.-Q. *et al.* Functional properties and structure changes of soybean protein isolate after subcritical water treatment. **J Food Sci Technol**, 52, n. 6, p. 3412-3421, 2015.

ZHANG, X.; JIANG, D.; LI, D.; YU, C. *et al.* Characterization of a seafood-flavoring enzymatic hydrolysate from brown alga Laminaria japonica. **Journal of Food Measurement and Characterization**, 13, n. 2, p. 1185-1194, 2019.

ZHENG, P.; HAO, G.; WENG, W.; REN, H. Antioxidant Activities of Hydrolysates from Abalone Viscera Using Subcritical Water-Assisted Enzymatic Hydrolysis. **Food and Bioprocess Technology**, 12, n. 6, p. 910-918, 2019.

ZHOU, F.; HEARNE, Z.; LI, C.-J. Water—the greenest solvent overall. **Current Opinion in Green and Sustainable Chemistry**, 18, p. 118-123, 2019.

ZHU, G.; ZHU, X.; FAN, Q.; LIU, X. *et al.* Study on Production of Amino Acids from Bean Dregs by Hydrolysis in Sub-critical Water. **Chinese Journal of Chemestry**, 28, p. 2033-2038, 2010a.

ZHU, G.; ZHU, X.; FAN, Q.; LIU, X. *et al.* Study on Production of Amino Acids from Bean Dregs by Hydrolysis in Sub-critical Water. **Chinese Journal of Chemistry**, 28, n. 10, p. 2033-2038, 2010b.

ZHU, G.; ZHU, X.; FAN, Q.; WAN, X. Recovery of biomass wastes by hydrolysis in sub-critical water. **Resources, Conservation and Recycling**, 55, n. 4, p. 409-416, 2011/02/01/ 2011.

ZHU, G.; ZHU, X.; XIAO, Z.; ZHOU, R. *et al.* A review of amino acids extraction from animal waste biomass and reducing sugars extraction from plant waste biomass by a clean method. **Biomass Conversion and Biorefinery**, 5, n. 3, p. 309-320, 2015.

ZIERO, H. D. D.; BULLER, L. S.; MUDHOO, A.; AMPESE, L. C. *et al.* An overview of subcritical and supercritical water treatment of different biomasses for protein and amino acids production and recovery. **Journal of Environmental Chemical Engineering**, p. 104406, 2020.

ZULUAGA-DOMÍNGUEZ, C.; CASTRO-MERCADO, L.; CECILIA QUICAZÁN, M. Effect of enzymatic hydrolysis on structural characteristics and bioactive composition of bee-pollen. **Journal of Food Processing and Preservation**, 43, n. 7, 2019.