UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

MARIANA BARRETO CARVALHAL PINTO

A NOVEL PERSPECTIVE ON HOPS: ENHANCEMENTS FROM FARM TO BREWERY FOR GREATER EFFICIENCY

UMA PERSPECTIVA INOVADORA SOBRE O LÚPULO: MELHORAMENTOS DESDE A FAZENDA ATÉ A CERVEJARIA PARA MAIOR EFICIÊNCIA

CAMPINAS
2024

MARIANA BARRETO CARVALHAL PINTO

A NOVEL PERSPECTIVE ON HOPS: ENHANCEMENTS FROM FARM TO BREWERY FOR GREATER EFFICIENCY

UMA PERSPECTIVA INOVADORA SOBRE O LÚPULO: MELHORAMENTOS DESDE A FAZENDA ATÉ A CERVEJARIA PARA MAIOR EFICIÊNCIA

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Tecnologia de Alimentos.
Esta tese foi produzida no âmbito de um Acordo de Cotutela firmado entre a Unicamp e a Universidade Técnica de Berlim

Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food Technology. This thesis was conducted as part of a Cotutelle Agreement between the University of Campinas and Technische Universität Berlin

Supervisor/Orientador: Flávio Luis Schmidt
Co-supervisor/Coorientador: Brian Gibson

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA MARIANA BARRETO CARVALHAL PINTO, E ORIENTADA PELO PROF. DR. FLÁVIO LUIS SCHMIDT E PELO PROF. DR. BRIAN GIBSON

CAMPINAS

2024

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

P658n

Pinto, Mariana Barreto Carvalhal, 1992-
A novel perspective on hops : enhancements from farm to brewery for greater efficiency / Mariana Barreto Carvalhal Pinto. – Campinas, SP : [s.n.], 2024.

Orientador(es): Flavio Luis Schmidt e Brian Richard Gibson.
Tese (doutorado) – Universidade Estadual de Campinas (UNICAMP), Faculdade de Engenharia de Alimentos.
Em cotutela com: Technische Universität Berlin, Alemanha.

1. Lúpulos. 2. Sustentabilidade. 3. Economia circular. 4. Isomerização. 5. Proteômica - Análise. I. Schmidt, Flavio Luis. II. Gibson, Brian Richard. III. Universidade Estadual de Campinas (UNICAMP). Faculdade de Engenharia de Alimentos. IV. Título.

Informações complementares

Título em outro idioma: Uma perspectiva inovadora sobre o lúpulo : melhoramentos desde a fazenda até a cervejaria para maior eficiência
Palavras-chave em inglês:
Hops
Sustentability
Circular economy
Isomerization
Proteomic - Analysis
Área de concentração: Tecnologia de Alimentos
Titulação: Doutora em Tecnologia de Alimentos
Banca examinadora:
Brian Richard Gibson [Orientador]
Flavio Luis Schmidt
Marianne Nissen Lund
Christina Schönberger
Martina Gastl
Guilherme Miranda Tavares
Data de defesa: 27-09-2024
Programa de Pós-Graduação: Tecnologia de Alimentos

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-3761-8731
- Currículo Lattes do autor: http://lattes.cnpq.br/0282704099096648

TU Berlin | Straße des 17. Juni 135 | 10623 Berlin, H 88

Fakultät III Prozesswissenschaften
Fakultäts-Service-Center III

Der Dekan
Univ.-Prof. Dr. Frank Behrendt

Der Vorsitzende des Promotionsausschusses
Prof. Dr. Sascha Rohn

Sachbearbeiterin
Beate Neumann

Raum H 8157/8158
Telefon +49 (0)30 314-25330
Telefax +49 (0)30 314-25965
Beate.Neumann@tu-berlin.de

Unser Zeichen:
H 88

Berlin, den 27. September 2024

PROTOKOLL

über die wissenschaftliche Aussprache im Rahmen des Promotionsverfahrens

mit Frau Mariana Barreto Carvalhal Pinto

im Institut für Lebensmitteltechnologie und Lebensmittelchemie

zur Erlangung des akademischen Grades „Doktor der Ingenieurwissenschaften" (Dr.-Ing.)

Titel der Dissertation:

"A NOVEL PERSPECTIVE ON HOPS: ENHANCEMENTS FROM FARM TO BREWERY FOR GREATER EFFICIENCY"

Die Dissertation wurde von den Gutachtern

Prof. Dr. Gibson	mit "sehr gut"
Prof. Dr. Nissen Lund	mit „gut"
Prof. Dr. Gastl	mit "gut"
Dr.-Ing. Schönberger	mit "gut"
Prof. Dr. Tavares	mit "sehr gut"
Prof. Dr. Schmidt	mit "sehr gut"

beurteilt.

Gemeinsames Urteil über die Dissertation: Gut

Gemeinsames Urteil über die wiss. Aussprache: Sehr Gut

Gesamturteil über das Promotionsverfahren: Gut

Anmerkungen:

Die wissenschaftliche Aussprache fand über das Videokonferenztool https://tu-berlin.zoom.us/_statt. Für den öffentlichen und den nicht-öffentlichen Teil der wissenschaftlichen Aussprache sowie die Ergebnisverkündung wurden drei separate Besprechungsräume erstellt.

Der*Die Kandidat*in und der gesamte Promotionsausschuss erklären sich mit der Durchführung der wissenschaftlichen Aussprache über tu https://tu-berlin.zoom.us/ einverstanden und verfügen über die notwendigen technischen Voraussetzungen.

Technische Universität Berlin

TU Berlin | Straße des 17. Juni 135 | 10623 Berlin, H 88

Änderungen im Einvernehmen mit Frau Barreto Carvalhal Pinto

in der Dissertation	ja ☒	nein ☐ (detaillierte Liste ist beigefügt)*
im Titel	ja ☐	nein ☒
beim Doktorgrad:	ja ☐	nein ☒

neuer Doktorgrad: .....

Neuer Titel: **(Bitte in Blockbuchstaben)**

.....

.....

.....

Fakultät III Prozesswissenschaften
Fakultäts-Service-Center III

Der Dekan
Univ.-Prof. Dr. Frank Behrendt

Der Vorsitzende des Promotionsausschusses
Prof. Dr. Sascha Rohn

Sachbearbeiterin
Beate Neumann

Raum H 8157/8158
Telefon +49 (0)30 314-25330
Telefax +49 (0)30 314-25965
Beate.Neumann@tu-berlin.de

Unser Zeichen:
H 88

Berlin, den 27. September 2024

* Eine detaillierte Liste mit den Änderungswünschen ist der Promovendin während der Überreichung der Vorläufigen Bescheinigung auszuhändigen, eine Kopie der Liste wird den Protokollunterlagen für das FSC beigefügt. Ohne detaillierte Liste gelten keine Änderungen als vereinbart.

Bei vereinbarten Änderungen ist die Dissertation vor Drucklegung der Betreuerin/dem Betreuer vorzulegen.

Wissenschaftliche Aussprache

Vortrag

Beginn: 14:04

Ende: 14:28

Diskussion über die Dissertation

Beginn: 14:29

Ende: 15:39

Prof. Gibson

Prof. Gastl

Prof. Nissen Lund

Prof. Schönberger

Prof. Tavares

Prof. Schmidt

TU Berlin | Straße des 17. Juni 135 | 10623 Berlin, H 88

Fakultät III Prozesswissenschaften
Fakultäts-Service-Center III

Der Dekan
Univ.-Prof. Dr. Frank Behrendt

Der Vorsitzende des Promotionsausschusses
Prof. Dr. Sascha Rohn

Sachbearbeiterin
Beate Neumann

Raum H 8157/8158
Telefon +49 (0)30 314-25330
Telefax +49 (0)30 314-25965
Beate.Neumann@tu-berlin.de

Unser Zeichen:
H 88

Berlin, den 27. September 2024

Änderungen im Einvernehmen mit Frau Barreto Carvalhal Pinto

in der Dissertation	ja ☒	nein ☐ (detaillierte Liste ist beigefügt)*
im Titel	ja ☐	nein ☒
beim Doktorgrad:	ja ☐	nein ☒

neuer Doktorgrad: .....

Neuer Titel: **(Bitte in Blockbuchstaben)**

.....

.....

.....

* Eine detaillierte Liste mit den Änderungswünschen ist der Promovendin während der Überreichung der Vorläufigen Bescheinigung auszuhändigen, eine Kopie der Liste wird den Protokollunterlagen für das FSC beigefügt. Ohne detaillierte Liste gelten keine Änderungen als vereinbart.

Bei vereinbarten Änderungen ist die Dissertation vor Drucklegung der Betreuerin/dem Betreuer vorzulegen.

Wissenschaftliche Aussprache

Vortrag

Beginn: 14:04

Ende: 14:28

Diskussion über die Dissertation

Beginn: 14:29

Ende: 15:39

Prof. Gibson

Prof. Gastl

Prof. Nissen Lund

Prof. Schönberger

Prof. Tavares

Prof. Schmidt

TU Berlin | Straße des 17. Juni 135 | 10623 Berlin, H 88

Fakultät III Prozesswissenschaften
Fakultäts-Service-Center III

Der Dekan
Univ.-Prof. Dr. Frank Behrendt

Der Vorsitzende des Promotionsausschusses
Prof. Dr. Sascha Rohn

Sachbearbeiterin
Beate Neumann

Raum H 8157/8158
Telefon +49 (0)30 314-25330
Telefax +49 (0)30 314-25965
Beate.Neumann@tu-berlin.de

Unser Zeichen:
H 88

Berlin, den 27. September 2024

Änderungen im Einvernehmen mit Frau Barreto Carvalhal Pinto

in der Dissertation	ja ☒	nein ☐	(detaillierte Liste ist beigefügt)*
im Titel	ja ☐	nein ☒	
beim Doktorgrad:	ja ☐	nein ☒	

neuer Doktorgrad: .....

Neuer Titel: **(Bitte in Blockbuchstaben)**

.....

.....

.....

* Eine detaillierte Liste mit den Änderungswünschen ist der Promovendin während der Überreichung der Vorläufigen Bescheinigung auszuhändigen, eine Kopie der Liste wird den Protokollunterlagen für das FSC beigefügt. Ohne detaillierte Liste gelten keine Änderungen als vereinbart.

Bei vereinbarten Änderungen ist die Dissertation vor Drucklegung der Betreuerin/dem Betreuer vorzulegen.

Wissenschaftliche Aussprache

Vortrag

Beginn: 14:04

Ende: 14:28

Diskussion über die Dissertation

Beginn: 14:29

Ende: 15:39

Prof. Gibson

Prof. Gastl

Prof. Nissen Lund

Prof. Schönberger

Documento assinado digitalmente
gov.br GUILHERME MIRANDA TAVARES
Data: 01/10/2024 11:09:41 0300
Verifique em https://validar.iti.gov.br

Prof. Tavares

Prof. Schmidt

TU Berlin | Straße des 17. Juni 135 | 10623 Berlin, H 88

Fakultät III Prozesswissenschaften
Fakultäts-Service-Center III

Der Dekan
Univ.-Prof. Dr. Frank Behrendt

Der Vorsitzende des Promotionsausschusses
Prof. Dr. Sascha Rohn

Sachbearbeiterin
Beate Neumann

Raum H 8157/8158
Telefon +49 (0)30 314-25330
Telefax +49 (0)30 314-25965
Beate.Neumann@tu-berlin.de

Unser Zeichen:
H 88

Berlin, den 27. September 2024

Wie in der Einladung erbeten, meldeten sich Gäste bis zum 26.09.2024 um 9.00 Uhr an, um an der wissenschaftlichen Aussprache teilzunehmen. Der Zugang zum Besprechungsraum für den öffentlichen Teil wurde ihnen mitgeteilt.

Der*die Kandidat*in hat versichert, prüfungsfähig zu sein.

Der*die Kandidat*in war über die gesamte Dauer der wissenschaftlichen Aussprache per Video zugeschaltet.

Bei technischen Störungen ist die Aussprache zu unterbrechen oder ggf. abzubrechen. Dies ist auf dem Protokoll zu vermerken.

Ort: https://tu-berlin.zoom.us/

Öffentlicher Teil für den/die Kandidat*in, den Promotionsausschuss und Gäste:
https://tu-berlin.zoom.us/j/65324505056?pwd=CcDMGG4eNzIOmM6Exo174aN6k6S2es.1

Für die nicht-öffentlichen Teile der Aussprache wird der Vorsitz Unterräume einrichten bzw. Gäste in die Lobby verschieben.

Anmerkungen zum Verlauf der Aussprache:

Keine besonderen Vorkommnisse

Prof. Dr. Sascha Rohn
Vorsitzender des Promotionsausschusses

Technische Universität Berlin

TU Berlin | Straße des 17. Juni 135 | 10623 Berlin, H 88

Fakultät III Prozesswissenschaften
Fakultäts-Service-Center III

Der Dekan
Univ.-Prof. Dr. Frank Behrendt

Der Vorsitzende des Promotionsausschusses
Prof. Dr. Sascha Rohn

Sachbearbeiterin
Beate Neumann

Raum H 8157/8158
Telefon +49 (0)30 314-25330
Telefax +49 (0)30 314-25965
Beate.Neumann@tu-berlin.de

Unser Zeichen:
H 88

Berlin, den 27. September 2024

Preliminary Certificate - **Dieses Exemplar bitte zurück an H 88!**

This is to confirm that Ms. Mariana Barreto Carvalhal Pinto

due to the joint assessment of the doctoral thesis submitted by her and the thesis defense that took place with her on 27th September 2024 the doctoral procedure of Ms. Mariana Barreto Carvalhal Pinto received an overall assessment of

- gut - *)

It should be noted that the Doctorate is only awarded by delivery of the doctoral certificate to Ms. Mariana Barreto Carvalhal Pinto.

This can take place as soon as the University Library confirms that Ms. Mariana Barreto Carvalhal Pinto

has submitted the documentation that must be prepared in accordance with § 10 of the Doctoral Regulations in order to be awarded the Doctor of Science/Doctor of Engineering Science by Technische Universität Berlin to the University Library.

(Submission Deadline: 27th September 2025)

27.09.2024

The following assessments are possible:
*) passed with distinction (or summa cum laude),
passed very good (or magna cum laude),
passed good (or cum laude),
passed (or rite)

“Eu fico com a pureza
Da resposta das crianças
É a vida, é bonita
E é bonita

Viver e não ter a vergonha
De ser feliz
Cantar, e cantar, e cantar
A beleza de ser um eterno aprendiz”

Música “O que é o que é”, Gonzaguinha

"I stick with the purity
Of children's answers
Life is beautiful
And it's beautiful

To live and not be ashamed
To be happy
To sing, and to sing, and to sing
The beauty of being an eternal learner"

Song "O que é o que é" by Gonzaguinha

AGRADECIMENTOS

Nessa vida, nós nunca estamos sós se quisermos caminhar mais longe. E, eu posso dizer que na minha jornada durante este doutorado, eu estive muito bem acompanhada por pessoas que me seguraram nas minhas quedas, me reergueram e me impulsionaram. Ao longo do tempo, pessoas que estavam mais perto, ficaram mais longe (fisicamente) e outras entraram nessa estrada. Eu só posso agradecer a todas elas e a todas as oportunidades que tive e que conquistei.

Começando com os nomes, os primeiros não poderiam deixar de ser a minha família amada. Meus pais, Sílvia e Manoel que são a razão por eu estar nesse mundo e a razão por eu ter conseguido ir tão longe. Eles me deram a seguridade material e emocional para que isso acontecesse. As minhas irmãs também, Lívia e Vânia, são as minhas melhores amigas e a melhor companhia pra essa vida quer eu poderia ter, elas são minha inspiração de mulheres e meus orgulhos. Elas também trouxeram a esse mundo as minhas pequenas jóias que são meus sobrinhos, Luiz Augusto, Pedro Felipe e Matteo (Felipe, como eu sempre chamo). Essas crianças me ensinaram a leveza da vida e me trouxeram felicidade em diversos momentos difíceis. Eles também me ensinaram a definição de amor incondicional. Também não poderia esquecer quem me apoiou desde sempre, como minhas primas Lilian e Letícia, minha tia Olinda e minha eterna vozinha Olinda.

Aos meus amigos, e esperem que a lista é longa, porque esses também são como uma família para mim. Aos de longa data, Conguinhas, Steffani, Zorbinha, Vicky, Lê, Tefinha, Lu e Thi, que ao longo de todos esses anos, desde os primórdios de Unicamp, me ouvem, me aconselham, me ensinam coisas sobre mim e a vida, me inspiram, me enchem de orgulho, me aturam na minha bebedeira, cuidam de mim e me trazem muita alegria. Aos répteis que sempre me acolheram naquela casa (e que saudades da sala 2) com muitas conversas longas e inspiradoras de seres humanos inpiradores, como Aninha, Livinho, David, Mago, Má. Não vou contar aqui todos os papos engraçados e as especulações da vida dos outros.

Aos amigos que surgiram na pós, Nariê, Cyntia, Curi e Filipe (Bb), que me ouviram muitas vezes reclamar sobre meus artigos, sobre o doutorado e que me ajudaram de tantas formas que sem vocês eu definitivamente não teria terminado essa tese. Além disso, vocês são

amigos que me trouxeram tantas reflexões, tantos papos bons, com ou sem cerveja, tantos momentos felizes que foram essenciais pra aliviar o peso de um doutorado. E também tem o time do lab frutas que me aguentou por muito tempo falando e muito e fazendo o horóscopo de todos e justificando sempre com um mercúrio retrógrado ou sol em tal signo. Ana Valéria, Túlio, Isa, Aline, Yuri, e Pri como vocês são sensacionais e que saudades dos nossos cafezinhos regados de conversa boa. Com vocês ao meu lado, meu doutorado com certeza ficou mais leve e também muito melhor. Eu também quero agradecer ao meu orientador Flávio que permitiu que tudo isso começasse e que me apoiou em todas as decisões (loucas ou não) e que é como um paizão para seus alunos.

Bom, como tudo muda, eu também mudei e literalmente, para um lugar muito mais longe da minha querida terra tropical em busca de algo desconhecido, mas que eu sabia que era o meu caminho e que seria um crescimento enorme pessoal e profissional. Mas nada disso seria possível se em julho de 2020 no meio de uma pandemia, Philip e Natalia não tivessem acreditado no potencial de uma brasileira doida que queria mudar para Berlim no meio do inverno e no meio de uma pandemia para fazer 6 meses de um doutorado sanduíche. Eu serei eternamente grata à vocês que permitiram que minha vida mudasse e que eu pudesse ter todas essas experiências que ainda estou vivendo. E falando especialmente do Philip, essa pessoa com um coração gigante e uma mente brilhante que me ensinou exatamente como ser uma cientista e uma pesquisadora melhor. Muito obrigada por todo o apoio nesses últimos 3 anos e que certamente fizeram diferença para que essa tese tivesse uma qualidade muito superior. Com isso, também agradeço ao Brian que ainda acredita em mim e que também me ensina como ser pesquisadora, que me abre várias oportunidades e me proporcionou continuar no time da TU Berlin. E um time que também trouxe um dos meus melhores companheiros Fred que me faz rir, que podemos contar piadas, reclamar e curtir a vida juntos.

Voltando ao começo de 2021, queria agradecer ao Vitor por ser um amigo incrível e me acolher em sua casa por um ano e por ser minha melhor companhia em Berlim, junto com minha amiga linda Flá que me enche de felicidade a cada encontro. Aos amigos de Berlim, eu agradeço ao Jonas por todas as conversas que tivemos naquele tempo em 2021, por me ajudar e muito

com a burocracia da CAPES por me apresentar a Sessa que é uma das minhas melhores companhias nessa big B que traz seu jeitinho meigo e meio doido ao mesmo tempo, mas que me ensina muito sobre a vida. À Marina que foi o melhor reencontro nesses tempos de Berlim e que me ouviu reclamando tanto de tanta coisa e que me acompanhou nessas reclamações dessa Alemanha.

E Berlim também me proporcionou um dos melhores encontros da minha vida com meu companheiro Conne. Eu nunca imaginaria que num verão de 2021 eu conheceria essa pessoa tão especial que é também uma das maiores razões por várias conquistas nesses últimos 3 anos. Sem o seu apoio eu com certeza não estaria caminhando tão longe e não teria conquistado tantas coisas nesses últimos tempos, principalmente esse duplo diploma. Você me ensinou como é ter um relacionamento leve e o que é alguém acreditar incondicionalmente em mim e estar sempre torcendo pelo meu sucesso. Junto com ele veio uma família incrível que se tornou a minha família de Berlim. O Klaus e a Christine (que nos faz muita falta) e que se tornaram meus segundos pais nessa Alemanha e que cuidam e cuidaram de mim, também acreditam em mim e comemoram e comemoraram por todo meu sucesso.

Aos que não estão listados nominalmente, mas que também tiveram um papel importante nessa etapa da minha vida, eu agradeço vocês imensamente e saibam que vocês todos tem um lugar cativo no meu coração e na minha mente.

Com isso, eu fecho meus agradecimentos para todas essas pessoas especiais que contribuíram e muito para que tudo isso fosse possível porque fazer um doutorado e escrever uma tese não é só sobre fazer pesquisa e ter um título. É sobre um crescimento pessoal e uma mudança interna em direção a melhor versão de nós mesmos. E isso não dá pra fazer só! A vida é muito mais bem acompanhada e com as melhores companhias.

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de financiamento 001.

ACKNOWLEDGMENTS

In this life, we are never alone if we want to go further. I can say that on my journey through this Ph.D., I was accompanied by people who supported me during my falls, lifted me back up, and pushed me forward. Over time, once close people became physically distant, and others joined this path. I can only thank all of them and all the opportunities I had and earned.

Starting with names, the first must be my beloved family. My parents, Sílvia and Manoel, are the reason I am in this world and the reason I have come this far. They provided me with the material and emotional security needed to make this happen. My sisters, Lívia and Vânia, are my best friends and the best companions I could have in this life. They are my inspiration and my pride. They also brought into this world my little jewels, my nephews Luiz Augusto, Pedro Felipe, and Matteo (whom I always call Felipe). These children taught me the lightness of life and brought me happiness in many difficult moments. They also taught me the definition of unconditional love. I can't forget those who have always supported me, like my cousins Lilian and Letícia, my aunt Olinda, and my eternal grandma Olinda.

To my friends, bear with me because the list is long, as they are like family to me. To those long-standing friends, Conguinhas, Steffani, Zorbinha, Vicky, Lê, Tefinha, Lu, and Thi, who have been listening to me, advising me, teaching me about myself and life, inspiring me, filling me with pride, putting up with me when I drink, taking care of me, and bringing me joy since the early days at Unicamp. To the “Répteis” who always welcomed me in that house (and how I miss room 2) with many long and inspiring conversations among inspiring humans like Aninha, Livinho, David, Mago, Will and Má. I won't recount all the funny talks and speculations about others' lives here.

To the friends I met during my post-graduation, Nariê, Cyntia, Curi, and Filipe (Bb), who often listened to me complain about my papers and the Ph.D. and helped me in so many ways that I definitely wouldn't have finished this thesis without you. Besides, you brought me many reflections, great conversations with or without beer, and happy moments that were essential to relieving the weight of a Ph.D. And also, the lab Frutas team who put up with me talking a lot daily and doing everyone's horoscope, always justifying it with a retrograde

Mercury or Sun in some sign. Ana Valéria, Túlio, Isa, Aline, Yuri, and Pri, you are amazing, and I miss our coffee breaks filled with good conversations. With you by my side, my Ph.D. was undoubtedly lighter and much better. I also want to thank my advisor Flávio, who allowed this all to begin, supported all my decisions (crazy or not), and is like a big father figure to his students.

Well, as everything changes, so did I, literally moving far from my beloved tropical land in search of something unknown but which I knew was my path and would be a huge personal and professional growth. But none of this would have been possible if in July 2020, in the middle of a pandemic, Philip and Natalia hadn't believed in the potential of a crazy Brazilian who wanted to move to Berlin in the middle of winter and a pandemic for six months of a sandwich Ph.D. I will be eternally grateful to you for allowing my life to change and for all the experiences I am still having. Speaking especially of Philip, this person with a giant heart and brilliant mind taught me exactly how to be a better scientist and researcher. Thank you very much for all the support over the last three years, which certainly made a difference in the quality of this thesis. I also thank Brian, who still believes in me, teaches me how to be a researcher, opens up many opportunities for me, and makes it possible for me to continue with the TU Berlin team. And a team that also brought me one of my best friends, Fred, who makes me laugh, and with whom I can joke, complain, and enjoy life together.

Going back to the beginning of 2021, I want to thank Vitor for being an incredible friend, welcoming me into his home for a year, and being my best company in Berlin, along with my beautiful friend Flá, who fills me with happiness every time we meet. To my friends in Berlin, I thank Jonas for all the conversations we had back in 2021, for helping me a lot with CAPES bureaucracy, and for introducing me to Sessa, who is one of my best friends in this big B, bringing her sweet and slightly crazy way but teaching me a lot about life. To Marina, who was the best meeting during these Berlin times, who listened to me complain about so many things and accompanied me in these complaints about Germany.

Berlin also brought me one of the best encounters of my life with my partner Conne. I would never have imagined that in the summer of 2021, I would meet such a special person

who is also one of the main reasons for many achievements in the last three years. Without your support, I certainly wouldn't have come this far or achieved so much recently, especially with this dual degree. You taught me what it means to have a light relationship and to have someone believe unconditionally in me, always cheering for my success. Along with him came an incredible family that became my Berliner family. Klaus and Christine (whom we miss dearly) became my second parents in Germany, taking care of me, believing in me, and celebrating all my successes.

To those not listed by name but who also played an important role in this stage of my life, I thank you immensely and know that you all have a special place in my heart and mind.

With this, I conclude my thanks to all these special people who contributed so much to making all this possible. Doing a Ph.D. and writing a thesis is not just about conducting research and earning a title. It's about personal growth and an internal shift towards becoming the best version of ourselves. And this cannot be done alone! Life is much better accompanied and with the best companions.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001

RESUMO

A crescente ameaça das mudanças climáticas exige ações urgentes em vários setores, incluindo a indústria cervejeira, a qual contribui significativamente para as emissões de gases de efeito estufa (GEE). Esta tese investiga estratégias para melhorar operações de fabricação de cerveja, com foco particular na otimização do processamento e uso do lúpulo no processo de fabricação para um processo mais sustentável. Este estudo teve como objetivo destacar as possibilidades de melhorar o processamento e o uso do lúpulo na fabricação de cerveja por meio de uma abordagem proteômica inovadora. Ele examina também o potencial para aprimorar técnicas de processamento de lúpulo, como secagem e extração supercrítica de CO_2, para minimizar o consumo de energia e a geração de resíduos, ao mesmo tempo em que maximiza a qualidade e o rendimento do produto. Além disso, esta pesquisa explora os mecanismos bioquímicos subjacentes à utilização do lúpulo durante a fabricação de cerveja, empregando análises proteômicas avançadas para elucidar os mecanismos moleculares que governam as interações entre proteínas e lúpulo. As percepções obtidas a partir dessas investigações oferecem insights valiosos para otimizar as práticas de fabricação de cerveja e reduzir o impacto ambiental. Além das abordagens tradicionais, esta tese adota avanços tecnológicos, incluindo aprendizado de máquina, para informar a tomada de decisões e a otimização de processos. Ao usar o poder de insights baseados em dados, este estudo abre caminho para soluções inovadoras aos desafios de sustentabilidade dentro da indústria cervejeira. Em geral, esta tese destaca a importância de abordagens interdisciplinares e inovação tecnológica na fabricação de cerveja para uma maior sustentabilidade.

ABSTRACT

The escalating threat of climate change necessitates urgent action across various sectors, including the brewing industry, a significant contributor to greenhouse gas emissions (GHG). This thesis investigates strategies to enhance the sustainability of brewing operations, with a particular focus on optimizing hop processing and hop usage in the brewing process. This study aimed to highlight the possibilities to improve hop processing and usage in brewing through an innovative proteomic approach. It examines the potential for improving processing techniques, such as drying and supercritical CO_2 extraction, to minimize energy consumption and waste generation while maximizing product quality and yield. Furthermore, this research explores the biochemical dynamics underlying hop utilization during brewing, employing advanced proteomic analyses to elucidate the molecular mechanisms governing hop-protein interactions. Insights gained from these investigations offer valuable insights into optimizing brewing practices and reducing environmental impact. In addition to traditional approaches, this thesis embraces technological advancements, including machine learning, to inform decision-making and process optimization. By using the power of data-driven insights, this study paves the way for innovative solutions to sustainability challenges within the brewing industry. Overall, this thesis highlights the importance of interdisciplinary approaches and technological innovation in brewing for greater sustainability.

ZUSAMMENFASSUNG

Die zunehmende Bedrohung durch den Klimawandel erfordert dringende Maßnahmen in verschiedenen Sektoren, einschließlich der Brauindustrie, einem bedeutenden Verursacher von Treibhausgasemissionen (THG). Diese Arbeit untersucht Strategien zur Verbesserung der Nachhaltigkeit von Brauereibetrieben, mit besonderem Fokus auf die Optimierung der Hopfenverarbeitung und des Hopfeneinsatzes im Brauprozess. Ziel dieser Studie war es, die Möglichkeiten zur Verbesserung der Hopfenverarbeitung und des Hopfeneinsatzes im Brauwesen durch einen innovativen proteomischen Ansatz hervorzuheben. Sie untersucht das Potenzial zur Verbesserung der Verarbeitungstechniken, wie Trocknung und superkritische CO2-Extraktion, um den Energieverbrauch und die Abfallproduktion zu minimieren und gleichzeitig die Produktqualität und den Ertrag zu maximieren. Darüber hinaus erforscht diese Forschung die biochemischen Dynamiken, die der Hopfennutzung beim Brauen zugrunde liegen, und verwendet fortschrittliche proteomische Analysen, um die molekularen Mechanismen der Hopfen-Protein-Interaktionen zu erläutern. Die gewonnenen Erkenntnisse bieten wertvolle Einblicke in die Optimierung der Braupraktiken und die Reduzierung der Umweltbelastung. Neben traditionellen Ansätzen greift diese Arbeit auch auf technologische Fortschritte, einschließlich maschinellen Lernens, zurück, um Entscheidungsprozesse und Prozessoptimierungen zu unterstützen. Durch die Nutzung datenbasierter Erkenntnisse ebnet diese Studie den Weg für innovative Lösungen zu Nachhaltigkeitsherausforderungen in der Brauindustrie. Insgesamt hebt diese Arbeit die Bedeutung interdisziplinärer Ansätze und technologischer Innovationen im Brauwesen für eine größere Nachhaltigkeit hervor.

TABLE OF CONTENTS

Chapter 1

General introduction, objectives, and thesis structure

1.1. Introduction

Climate change has rapidly transformed from a distant concern to an urgent reality confronting humanity. In 2023, the World Meteorological Organization (WMO) confirmed that the year marked the hottest period since the Industrial Revolution, with the global average temperature surpassing pre-industrial levels by 1.45°C(Carissa Wong, 2023; World Meteorological Organization, 2024). The Intergovernmental Panel on Climate Change (IPCC) underscores the criticality of immediate mitigation and adaptation efforts, emphasizing the pivotal role of reducing greenhouse gas (GHG) emissions to attain net-zero emissions (Calvin et al., 2023). Despite these warnings, global GHG emissions persistently arise, reaching 57.4 gigatons of CO_2 equivalent in 2022. Among the contributors to this environmental degradation is the brewing industry, a cornerstone of the global beverage sector, which in 2022 alone produced 1.89 billion hectoliters of beer, emitting approximately 30,172.15 kilotons of CO_2 equivalent and generating 4,352,000 tons of waste annually (Silva et al., 2023). Furthermore, the industry's heavy reliance on hops, a critical ingredient in beer production, exacerbates its environmental footprint, with hop cultivation contributing significantly to GHG emissions, estimated at 78,481,800 kilotons of CO_2 equivalent in 2021.

The hop plant is essential to the brewing industry, which uses the inflorescences, or cones, of the female plants for flavoring. Inside female strobiles, positioned under the bracts are found yellow glands called lupulins, which produce two resin fractions, hard and soft (Biendl et al., 2014). The main compounds used in the brewing process to provide bitterness and aroma to the beer are located in soft resins, which contain bitter acids and essential oils. The bitter acids are divided into two groups comprised of α- and β-acids, the first is related to the bitter taste in beer and is one of the most important compounds in the beer industry (Almaguer et al., 2014).

Hops are rarely used in their raw cone form in the brewing process. Instead, hop-derived products are favored for their high quality and efficiency (Arruda et al., 2022). Therefore, hop processing is a crucial component of the brewing supply chain, beginning on the farms with the drying step after harvest to reduce moisture and avoid spoilage (Biendl et al., 2014). Among the

core hop products, extracts are particularly valuable for mass beer production, as they offer superior quality control and higher yields. These extracts are primarily produced using supercritical CO_2 extraction, which isolates soft resins through fluid polarity and achieves higher recovery of bitter acids (Briggs et al., 2004). Though necessary, hop processing contributes significantly to CO_2 emissions, especially during the drying and extraction steps which involve substantial solvent consumption (Hauser & Shellhammer, 2019). Although well-established in hop-producing countries, there remains considerable potential for optimization in the main steps of hop-extract production. This optimization is increasingly urgent to reduce GHG emissions and mitigate the effects of climate change.

The brewing process is also far from optimal in terms of waste production, generating a vast quantity of by-products. During wort boiling, compounds such as proteins, iso-α-acids, and polyphenols precipitate, forming a residue known as trub. This trub accounts for between 0.2 and 0.4 kg of waste per 100 liters of beer, which amounted to approximately 364 to 728 thousand tons of waste generated by the global beer industry in 2020 (Barth-Haas Group, 2021; Mathias et al., 2014). This residue is also a rich source of proteins, comprising about 50% of its content (Mathias et al., 2014). Additionally, around 50% of hop bitter compounds are lost during wort boiling, significantly impacting profitability (Jaskula-goiris et al., 2010). The utilization of α-acids, the percentage of this compound converted into its isomer, in the brewing industry remains a major challenge due to the significant loss of bitter compounds to trub. According to Gänz et al. (2021), proteins originating from barley malt deplete the iso-α-acids content, further complicating the efficient use of hops.

Wort proteins play a crucial role in stabilizing beer foam due to the formation of a viscoelastic layer, which consists of a continuous liquid phase and a discontinuous gas phase (Briggs et al., 2004). Hydrophobic proteins, particularly Lipid Transfer Protein (LTP) and protein Z, are key to this stabilization (Steiner et al., 2011). The study by Lu et al. (2020) highlighted the significant influence of protein Z and LTP1 on the surface layer properties, showing that these proteins interact strongly with iso-α-acids to form spherical aggregates, indicating a robust bond, especially with protein Z (Lu, Osmark, Bergenståhl, & Lars, 2020). These proteins also constitute

a major portion of the proteins that precipitate as wort trub during the boiling process, due to their binding with protein-derived polypeptides (Iimure et al., 2012a). However, most research has focused on the interaction between proteins and hop bitter compounds in the foam, with insufficient attention to the role of these compounds in protein precipitation during boiling.

Efforts to improve hop utilization rates have predominantly focused on technological upgrades. However, addressing this challenge requires a multifaceted approach that considers both biochemical interactions and brewing practices. Understanding the intricate interplay between hop bitter compounds and barley proteins during the boiling step could offer valuable insights into enhancing hop utilization efficiency. By elucidating the mechanisms underlying these interactions, researchers can inform breeding programs, optimize malting and brewing parameters, and ultimately, advance sustainability within the brewing industry.

1.2. General Objectives

Towards the goal of a more sustainable and cost-effective brewing process, the present work's primary objective is an in-depth understanding of the main steps in hop-extract processing and barley malt protein and hop bitter acids linkage throughout wort boiling. This understanding is essential to improve hop processing and bitter acids utilization in beer production.

1.3. Research questions

- ? How does hop drying affect hop quality?
- ? Could hop extract be produced from a Brazilian hop variety?
- ? How can hop extract production be optimized?
- ? Do hop bitter acids affect protein precipitation as trub?
- ? Do hop bitter acids interact with specific proteins during wort boiling?
- ? Does barley cultivar influence hop bitter acids isomerization during wort boiling?

1.4. Specific Objectives

The study-specific objectives are:

- ✓ Determination of the effect of drying temperature on hop quality traits.
- ✓ Investigation of hop-drying performance and kinetic parameters.
- ✓ Obtention of supercritical CO_2 extraction kinetics.
- ✓ Enhancement of hop drying and supercritical CO_2 extraction steps to establish a low-energy and cost-effective process.
- ✓ Elucidation of the effect of hop extract addition on the protein profile during wort boiling.
- ✓ Identification of protein groups likely to interact with hop bitter acids.
- ✓ Understanding of how barley malt protein profile affects hop bitter acid utilization.

1.5. Thesis Structure

This thesis is divided into 8 chapters as demonstrated in Figure 1.1. The journey through this study begins in the **first chapter** ***(Chapter 1: General introduction, objectives, and thesis structure)*** with a brief introduction to the topic with current information about the global situation, the role of hop in the brewing industry, hop processing, and the challenge of hop utilization in brewing. Furthermore, the readers will find in this chapter the research questions and objectives of this study.

In **Chapter 2** ***(Literature review),*** readers will find an extended review of the literature to highlight the state of the art in hop processing, the current global demand for circular economy adaptations, the role of hop in beer, and a way to improve its utilization during wort boiling.

In **Chapter 3** ***(Is it possible to improve further hop processing?)***, the first experimental paper is presented with the results from an extended study of the drying step's influence on hop quality. Furthermore, this paper presents the results from an optimized hop supercritical CO_2 extraction using a Brazilian hop variety called Mantiqueira. The possibility of extracting hop bitter acids from hop dried at higher temperatures is presented. This approach may lead to a shorter and more sustainable process.

Chapter 4 ***(Could AI also be used for hop processing?)*** presents the second experimental paper, which aims to demonstrate the suitability of using machine learning models to predict hop drying time, thereby enhancing process control. This chapter also presents results on the effect of the drying step on hop quality and structure.

Chapter 5 ***(Do hop bitter acids influence wort protein profile during boiling?)*** contains the third experimental paper, which contains results on the interaction between hop bitter acids and protein from barley malt. This outcome highlights also the role of those compounds in the formation of protein aggregates during wort boiling.

The last experimental paper is presented in **Chapter 6** ***(Does barley cultivar play a role in hop bitter acids utilization during wort boiling?)***. This paper shows the influence of barley proteome on hop bitter acids isomerization rate during wort boiling. The outcomes include the protein profile of two barley cultivars and the protein modification during wort boiling, leading to precipitation.

Chapter 7 ***(What is the outcome of this thesis?)*** presents the general discussion of the thesis. This chapter contains the main observed results that answered the research questions, moreover, the core to which this thesis contributed to scientific development.

In the last chapter ***(Chapter 9: Which conclusions can be drawn?)***, the thesis journey finishes with a brief general conclusion of the results chapters. In this chapter, is addressed the responses to the general objective of this thesis.

Figure 1.1. Flowchart containing the thesis's structure according to the chapters presented in the document

Chapter 2

Literature review

The world of hops: state of the art and room for innovation toward a circular economy

1.1.1. Current global demand for industry reform

Climate change is no longer a distant scenario; it is a reality we are facing today. In 2023, the World Meteorological Organization (WMO) confirmed it as the hottest year since the Industrial Revolution (World Meteorological Organization, 2024). The global average temperature achieved 1.45°C above pre-industrial levels, edging close to the 1.5°C limit set in the Paris Agreement (Sanderson, 2023; United Nations - Climate Change, 2024). This surge is primarily fueled by greenhouse gas (GHG) emissions, resulting in severe climate impacts like heatwaves, heavy rainfall, and droughts (United Nations - Climate Action, 2024). In the same year, the Amazon Forest experienced its driest season on record, devastating the world's richest biodiversity and affecting thousands of indigenous people (Meghie Rodrigues, 2023). These extreme droughts, coupled with heatwaves, fueled wildfires across the globe, signaling the irreversible consequences of climate change (Nasa - earth observatory, 2024). The Intergovernmental Panel on Climate Change (IPCC) emphasizes that the extent and speed of climate change and its associated risks pivot greatly on immediate mitigation and adaptation efforts. Scientists emphasize the urgency of reducing GHG emissions to achieve net-zero emissions (Calvin et al., 2023). However, despite these warnings, global GHG emissions continue to break records, reaching a new high of 57.4 gigatons of CO_2 equivalent in 2022 (United Nations, 2023).

Beer, the largest segment of alcoholic beverages, has a long history and is a principal drink globally. In 2022, the brewing industry produced 1.89 billion hectoliters of beer worldwide (Statista, 2024). This production also generates significant waste and GHG emissions. In 2023, the industry emitted approximately 30,172.15 kilotons of CO_2 equivalent, generating 4,352,000 tons of waste annually from various stages of the process (Silva et al., 2023). This represents around 1% of total global GHG emissions. The primary wastes include Brewer's Spent Grain (BSG), hot trub, and spent yeast, often repurposed as animal feed. Additionally, beer production requires substantial energy input, and its raw materials, principally barley, and hops, also contribute to an environmental footprint. Furthermore, energy consumption during brewing and raw material processing significantly impacts product quality. For instance, the boiling step in brewing is one

of the most energy-intensive stages, yet it is essential for enhancing beer color and flavor through the Maillard reaction, facilitating DMS volatilization, and ensuring wort clarification.

The brewing industry stands as the keystone of the hop sector, consuming 97% of its output for beer production (Biendl et al., 2014). Hop cultivation has seen steady growth in recent decades, particularly in the production of bitter acids, reaching around 14,000 metric tons of alpha-acids in 2021 (Barth-Haas Group, 2021). Hops play a vital role in beer-making, imparting distinct flavors, especially the bitter taste provided by isomerized alpha-acid. However, they also represent the most expensive raw material, with a market value of USD 1741.05 million in 2023 (Skyquest, 2024). Nevertheless, hop production in 2021 resulted in a substantial estimated GHG emission of 78,481,800 kilotons of CO_2 equivalent (BarthHaas Europe, 2021; Hopfenverwertungsgenossenschaft, 2021). According to the sustainability report of Hopfenverwertungsgenossenschaft (HVG) (2021), counting only energy consumption for harvesting, drying, and irrigation, hop cultivation in Germany emitted 2,340 kg of CO_2 equivalent per hectare.

Like many others since the Industrial Revolution, these industries have adhered to the linear economy model, which revolves around production, consumption, and disposal (Liu & Ramakrishna, 2021). However, this model has proven to be unsustainable, as it entails extracting resources in an unlimited manner from a planet with finite resources. This unsustainable approach has significantly contributed to the global climate crisis over the past century (Calvin et al., 2023). Thus, transitioning from a linear economy model to a circular one could be pivotal in achieving sustainable production and contributing to the attainment of UN sustainable development and net-zero goals (United Nations, 2024).

The principles of the circular economy model revolve around minimizing waste and energy consumption through reuse, reduction, and recycling (Deutz, 2019). However, this concept is not entirely new; it has been practiced for millennia by ancient civilizations such as the indigenous societies of the Amazon Forest (Peripato et al., 2023). During the pre-Columbian era, these societies developed their civilizations based on circularity, as evidenced by the construction of recently discovered cities in the region (McMichael et al., 2012; Peripato et al., 2023). For

example, in the Equatorial Amazon, an urban center was recently unearthed in the Upano Valley. Its circular layout is characterized by integrating agriculture into urban landscapes, showcasing sustainable food production practices. These societies exemplify the potential for growth in harmony with natural cycles (Rostain et al., 2024).

The circular economy is built on concepts like industrial symbiosis, where one industry's waste becomes another's raw material, and sustainable development, which aims to harmonize economic growth with environmental and social welfare (Liu & Ramakrishna, 2021). The challenge lies in moving from theory to action, particularly in ensuring that circular economy practices benefit all parts of society and align with global sustainability objectives. In the brewing and hop industry, circularity could involve reusing nutrient-rich by-products to create new food and cosmetic items, as well as enhancing efficiency and reducing energy consumption by optimizing the process (Cimini & Moresi, 2021; Petiti et al., 2022).

The brewing industry holds significant potential for transitioning into a biorefinery, supplying raw materials for food production through a circular model (Claudia Salanță et al., 2023). For instance, one such by-product, hot trub, contains valuable protein, polyphenols, and hop bitter acids (Silva et al., 2023). Over the past decades, hot trub has been combined with brewer's spent grain for livestock feed, a low-value use for these potentially valuable compounds. On the other hand, the modern food market has a clear demand for plant-based protein, driven by trends in vegan and high-protein diets, which could be fulfilled by hot trub protein. Moreover, hop bitter acids, highly valued in brewing, are largely found in hot trub, resulting in a substantial loss of approximately US$ 8,368,740 worth of hop bitter acids in the brewing industry (Kunze, 2019; Mathias et al., 2014). This not only represents a financial loss but also carries a significant environmental impact due to the high CO_2 emissions associated with hop production. It underscores the urgent need to optimize the utilization of hop products within the brewing process.

An integral part of reusing agro-industrial residues involves adding value to by-products, thus lessening their environmental impact. For example, a recent study by Cerqueira e Silva (2022) proposed extracting hop bitter acids from hot trub using membrane technology. This method effectively eliminated solids, mainly protein aggregates, due to their size and electrophoretic

profile, presenting a potential solution for purifying these compounds. By embracing a circular economic model, the brewing industry could utilize new technologies to increase the value of its by-products and diminish its environmental footprint.

Over the past decade, there has been a substantial focus on developing innovative technologies for recovering and reusing brewing by-products, as represented in Figure 2.1, which illustrates the increasing number of publications related to these keywords. However, it is crucial to note that the circular economic model extends beyond mere reuse and recovery; it also emphasizes the significant reduction of waste production and energy consumption throughout the production process (Geng, 2019). By adopting more efficient resource utilization strategies, such as optimizing raw material usage and implementing sustainable production practices, not only can environmental benefits be achieved, but the overall environmental footprint can also be significantly reduced. This holistic approach ensures that the brewing industry not only minimizes waste but also operates in a more sustainable and environmentally responsible manner.

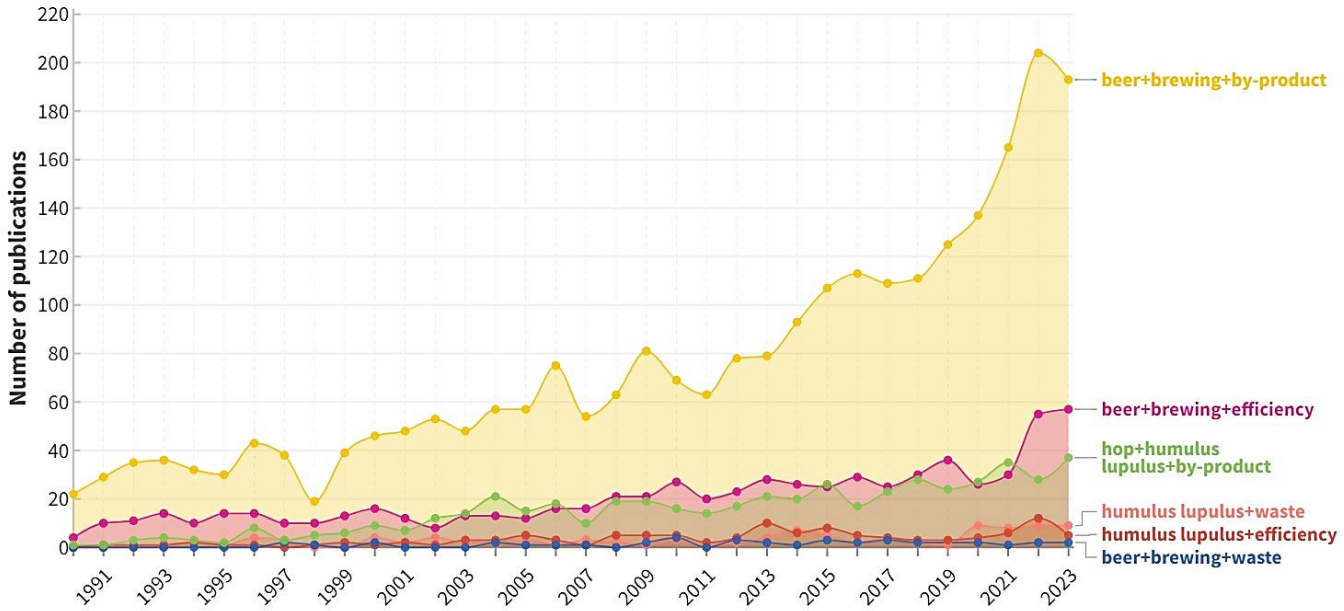


Figure 2.1. Evolution of paper publication related to hops and brewing by-product in comparison with efficiency of process. The mentioned numbers represent the absolute number of publications by year.

As illustrated in Figure 2.1, there has been a noticeable lack of focus on improving process efficiency within the brewing and hop industry. This oversight becomes especially critical in light of the energy crises that Europe has faced since 2022, rooted in the Russian conflict with Ukraine which led to natural gas shortages in Europe. This situation has urged the industry to reconsider

its energy consumption practices and develop new strategies to conserve energy. Consequently, the brewing and hop sectors in Europe have begun taking initial steps towards transitioning to renewable energy sources in alignment with the European Union's directives (European Commission, 2024). However, to further reduce the industry's environmental footprint without significant loss of production, there is a pressing need for additional research into process parameters and optimization. By fine-adjusting these aspects, the industry can achieve greater energy efficiency and sustainability, contributing to the broader goals of reducing GHG emissions and environmental impact.

In this regard, this review aims to provide updated information on recent developments in optimized hop production and its enhanced utilization in the brewing process. The review methodology involved synthesizing information regarding hop processing, potential improvements in processing techniques, and applications in the brewing industry. Selection criteria for relevant studies included their quality, data availability, and relevance to the topic, with a focus on publications from the past three decades. To gather this information, the metadata tool "Scholarly Works," available on the Lens platform, was utilized. Studies within the Agricultural and Food Science fields were targeted, and a combination of keywords such as "hops," "*Humulus lupulus,"* "process," "drying," "extraction," "by-products," "waste," "beer," and "brewing" was used to search. This comprehensive approach ensured a thorough exploration of recent advancements and insights into optimizing hop production and its integration into the brewing process.

1.1.2. Why process hops?

Botanically, hops are described as climbing, perennial, and dioecious plants belonging to the *Cannabinacea* family, specifically in the species *Humulus lupulus* or *Humulus japonicus* (Verzele & Keukeleire, 1991). However, only *H. lupulus* holds commercial value in the brewing industry, utilizing the inflorescence of female plants known as hop cones (Figure 2.2). These cones comprise glandular trichomes called lupulin glands, containing compounds crucial to brewing and the hop industry, including bitter acids and hop essential oils. However, these bitter acids are susceptible to oxidation when exposed to light and oxygen, as well as the loss of essential oils

through volatilization (Dierckens & Verzele, 1969; Verzele, 1986). Consequently, over 95% of hop cones undergo processing into hop products to extend their shelf-life (Schönberger, 2006).

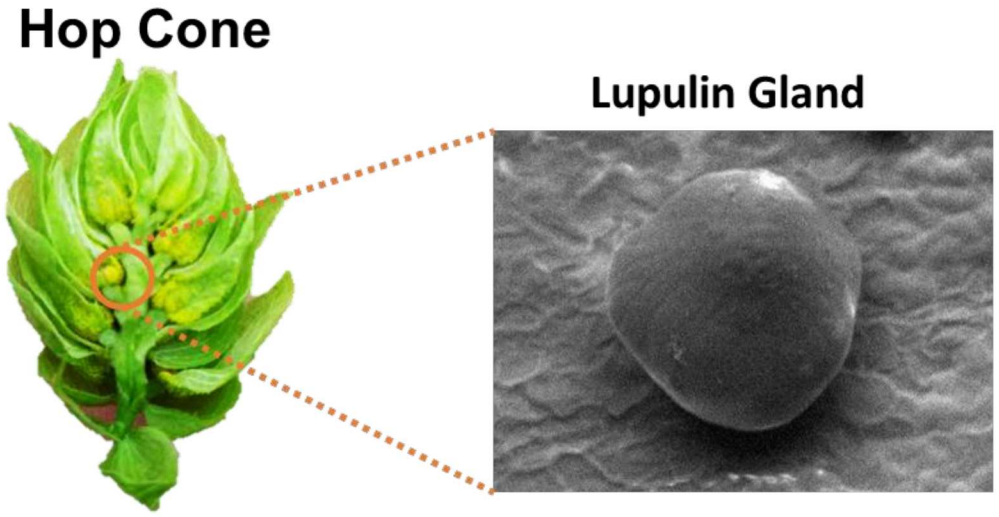


Figure 2.2. Representation of hop female inflorescence and the glandular trichome (lupulin gland)

Presently, the primary hop products are hop pellets and extracts, offering enhanced efficiency due to their ease of handling and homogeneity. Given the higher moisture content of hop cones after harvesting, drying occurs on-site at farms to prevent spoilage and microbial degradation. Subsequently, the dried cones undergo further processing steps, such as pelletization or extraction. Hop pellets may be enriched (type 45) or non-enriched (type 90), with the enriched pellet production involving an additional step of milling and sieving at low temperatures to crystallize the lupulin glands. This process separates less desirable components like cone leaves, stems, and stalks, resulting in pellets with a higher concentration of lupulin and desirable brewing compounds like bitter acids (Hopfenveredlung, 2024).

Hop extracts are derived from pre-pelletized hops, commonly through supercritical CO_2 extraction, utilizing CO_2 as a solvent under high pressure to achieve a supercritical state. This method is highly selective, leading to increased extraction yields (Hopfenveredlung, 2024). Extracts are more stable and can be stored for up to ten years under refrigeration, reducing transportation and storage volume compared to whole cones and pellets (HVG, 2024). Additionally, it enhances efficiency in the brewing process. Therefore, hop processing plays a crucial role in improving utilization during brewing and storage logistics.

1.1.3. Hop processing

Hop processing involves several steps, starting with harvesting at the hop farms. Figure 2.3 presents a simplified flowchart of the hop processing stages, highlighting the main steps to produce hop pellets and hop extract through supercritical CO_2 extraction. This review will explore the advances in the drying and extraction steps, which are the most energy- and time-consuming aspects of hop processing.

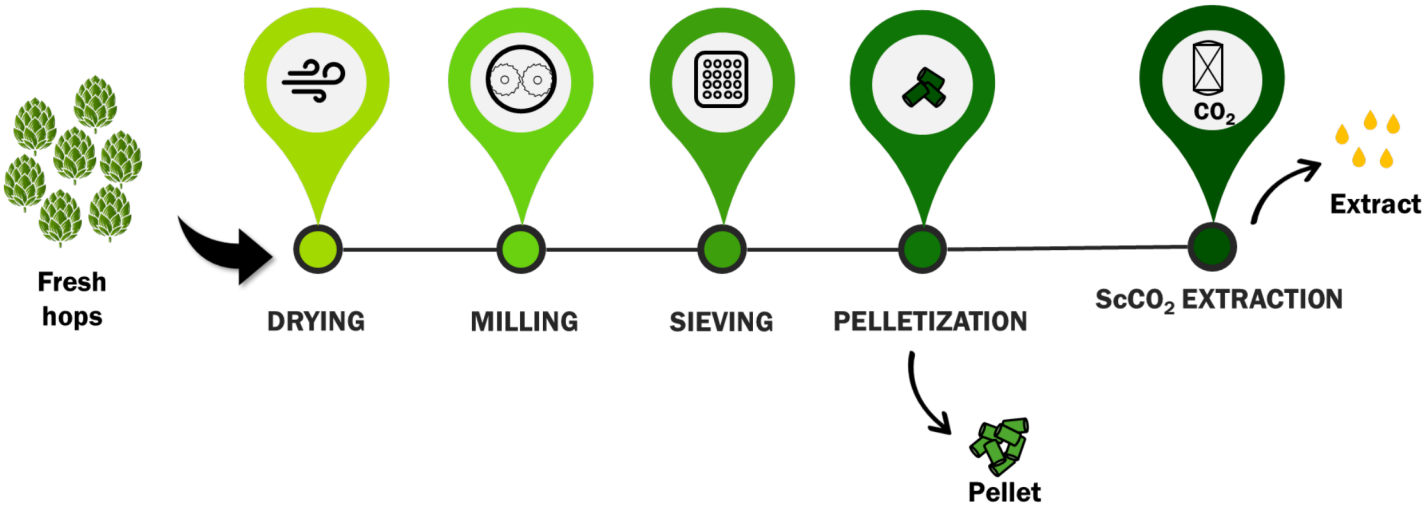


Figure 2.3. Simplified hop processing flowchart

1.1.3.1. Hop drying advances

The first step in hop processing involves drying the flowers, typically done on the farm after harvest to reduce moisture content (Biendl et al., 2014). At harvest, hops usually have around 80% moisture, which is relatively high and can promote degradation reactions such as oxidation, enzymatic breakdown, and microbial spoilage (Neve, 1991). Historically, water removal has been a traditional method of food preservation, offering benefits such as reduced transportation and storage costs. Drying is a common technique used to decrease water content in food, thereby lowering water activity (Kerr, 2013a). This process is crucial for hops, as they are often stored in bales for months before processing, and effective water removal is essential for maintaining quality during storage. Typically, hops are dried after harvest to achieve a final moisture content of 9 to 11%, followed by a conditioning step (Biendl et al., 2014).

For decades, research into hop drying remained largely unexplored, resulting in a lack of innovation in this area. However, since 2020, scientists have increasingly focused on studying the hop drying process and developing alternatives to optimize drying efficiency and enhance hop quality. The urgency of improving energy efficiency in hop drying, particularly in Europe, was highlighted by the war in Ukraine in 2022. This drying phase is often considered the bottleneck in hop processing, as the timing of hop harvesting is dependent on the availability of dryers to prevent the deterioration of hop cones.

Longer storage of hops before drying leads not only to spoilage and deterioration but also to chemical changes, which decrease quality even after drying. Raut et al. (2020) conducted an experiment where fresh hop cones were stored for different periods (5 or 24 hours) before being dried at 65°C for 210 minutes. The study found that the content of linalool, β-caryophyllene, humulene, and geraniol in hops increased depending on the storage period. However, myrcene, a key component of hop essential oils, decreased with longer storage periods. The research also noted a significant color difference (ΔE values) between fresh and stored hops, indicating discoloration during storage. By understanding how storage duration and freezing affect the essential oil content and overall quality of hops, farmers can optimize their storage practices to preserve the best qualities of these essential beer ingredients.

Drying processes have long been employed in the agricultural industry due to their simplicity, primarily relying on the thermal energy from the warm surrounding air to remove free water from products (Kerr, 2013b). However, controlling this process can be challenging, often depending on the operator's experience, and leading to over-drying (Ziegler et al., 2021). To improve the efficiency of hop drying in belt driers, Rybka et al. (2017) examined the impact of drying parameters on over-drying. Their research revealed that hops dried at the end of the second belt in a three-belt drier. This resulted in over-drying the hops at the end of the third belt, ultimately compromising product quality and process efficiency. Further investigation conducted by the same group involved monitoring the moisture content of different hop components, such as strings and bracts, throughout the drying process in a belt drier (Heřmánek et al., 2018). They found that the optimal moisture content for strings is between 6-8%, ensuring thorough drying without becoming

brittle. Conversely, bracts are dried to a level that would typically make pressing the hops impossible. Therefore, a conditioning process is employed where they are re-moistened to achieve an optimal moisture content of 8-11% (Heřmánek et al., 2018). Thus, the main challenge in hop drying lies in achieving uniform drying of the hop cones without over-drying.

The conditioning phase, a period typically characterized by reduced temperature and increased humidity, aims to equalize the moisture content of hops after drying phase. In a prior study conducted by Mejzr & Hanousek (2007) focusing on hop quality in a belt drier, the research suggests optimizing the drying process by eliminating the conditioning phase of the kiln and halting drying at a moisture content below 10%. This approach is proposed to potentially reduce energy and costs while expediting the process. However, this proposal has been controversial. Other researchers, such as Rybka et al. (2017), emphasize the importance of the conditioning step to achieve an optimal moisture level and prevent over-drying.

The performance of the drying process is heavily reliant on the equipment utilized, as it directly impacts the efficiency of moisture removal. Therefore, in-depth attention is required in drying research, considering the specific processes employed and their adaptation to the equipment being used. For example, in a study by Rybka et al. (2018), a comprehensive approach was taken to compare the drying process in chamber dryers with that of belt dryers. This research utilized a combination of dataloggers, fixed sensors, and laboratory analyses. The findings highlighted significant differences in the drying air temperature and relative humidity between the two methods. In chamber dryers, it was observed that as the hops progressed from one slat box to another, the drying air temperature decreased while the relative humidity increased. This gradual change suggested a more controlled and gentle drying process, ultimately resulting in a more uniform moisture content in the hops. By emphasizing these nuances in drying equipment and processes, researchers can better understand and optimize the drying process for improved efficiency and quality outcomes.

Hop drying efficiency depends significantly on various process parameters, including temperature, airflow, bed height, and air humidity. These parameters play crucial roles in determining the velocity of dehydration and the rate of water transfer from the product to the

surrounding air (Kerr, 2013b). In a study by Ziegler & Teodorov (2021), the airflow resistance of hop cones in different drying scenarios was investigated, considering variables such as varying air velocities and bed heights. This resistance, influenced by factors like cone size, shape, and moisture content, directly impacts the efficiency of the drying process. The researchers observed significant differences in airflow resistance among hop varieties, noting a non-linear increase in pressure with bed height, particularly evident in fresh hops. Moreover, the study underscored the importance of bulk density, which refers to the mass of hops per unit volume, in the drying process, thereby influencing drying efficiency. Overall, the findings highlight the distinct bulk densities and airflow resistances exhibited by different hop varieties, emphasizing the necessity of a designed approach to drying based on the specific characteristics of the hops being processed.

The impact of bulk weight was also investigated in a study conducted by Raut et al. (2020). The research involved experiments with hops dried in batches of 15, 25, and 35 kg/m² at 60°C and 0.35 m/s, with additional tests conducted at 65°C and 0.45 m/s for the 25 kg/m² bulk. The findings revealed a significant influence of bulk weight on drying behavior. Specifically, the 15 kg/m² bulk exhibited the highest specific energy consumption and color change (ΔE value) of 6.3, indicating a more pronounced alteration in chemical composition. By taking into account factors such as bulk weight, drying temperature, air velocity, and hop cone size, farmers can refine the drying process to improve both efficiency and product quality.

One key aspect of drying is the temperature at which it is conducted, impacting the hop quality concerning chemical and sensory properties. However, there is no clear consensus on the ideal temperature for preserving hop quality. Rybka et al. (2018) discovered that drying hops at a lower temperature of 40°C retained 90% of their flavor compounds compared to traditional drying at 55°C. In a subsequent study, the impact of drying temperature on essential oil retention in Saaz hop cones was investigated (2021). Results showed a significant retention of essential oil when drying at 40°C in comparison to the conventional 60°C. Conversely, Rubottom et al. (2021) demonstrated that higher drying temperatures can lower the activity of dextrin-reducing enzymes in hops, which could affect beer fermentation and quality. Focusing on two major aroma cultivars, Amarillo and Simcoe, Rubottom et al. (2022) examined how different drying temperatures

(ranging from 49-82°C) affected these enzymes. Additionally, Rubottom et al. (2024) conducted a study over two harvest years, analyzing six popular American aroma hop varieties: Amarillo, Cascade, Centennial, Citra, Mosaic, and Simcoe. Their findings revealed that drying temperature did not significantly impact the chemical and sensory properties of the hops. Interestingly, the authors suggest that hop growers could potentially employ higher drying temperatures without compromising aroma quality, offering potential benefits in terms of sustainability (reduced energy consumption) and operational flexibility.

Innovation in the drying process remains scarce despite its pivotal role in enhancing efficiency and sustainability. Addressing this gap, Hofmann et al. (2013) conducted research on energy recovery during hop drying, a significant step towards fostering more sustainable and efficient beer production. The study aimed to assess the efficacy of heat recovery from the exhaust air generated during the hop drying process. Furthermore, it studied the volatile hop oil components recovery, as these compounds play a vital role in the brewing process and are susceptible to loss during traditional drying methods. The research project involved the implementation of a heat exchanger in an industrial-scale hop drier to capture energy from the exhaust air, which typically contains reusable heat. The heat recovery process demonstrated an efficiency of approximately 57%, resulting in a notable 9-degree increase in the temperature of the fresh air utilized in the drying process. Furthermore, the study analyzed the condensate generated during heat recovery, revealing low concentrations of hop oil components. To enhance the recovery of these volatile compounds, an activated carbon filter was installed, leading to a significant improvement in the retrieval of hop oil components. Overall, this research represents a significant advancement in the quest for more sustainable and efficient beer production practices through innovative approaches to energy utilization and preservation of hop quality.

The adoption of innovative drying techniques holds the potential to revolutionize the preservation of agricultural products, ensuring their chemical and sensory qualities remain at high standards. This not only benefits consumers with superior products but also has broader implications for the industry, making production more cost-effective and environmentally sustainable. Traditionally, hot-air drying has been the standard method for drying hops. However,

recent research has explored innovative techniques like freeze drying and microwave vacuum drying (MVD). Ferenczi et al. (2018) found that freeze-drying stood out for preserving the highest amount of ß-myrcene, a crucial aromatic compound. Yet, this preservation came with a higher energy consumption compared to other methods. On the other hand, MVD, while slightly more energy-intensive than hot-air drying, offered a promising middle ground. It effectively retained aroma compounds and produced a desirable porous and crunchy texture in dried hops, potentially enhancing brewing efficiency. Additionally, Addo et al. (2022) concluded that pre-freezing, especially when followed by microwave-assisted hot air drying, is highly effective, striking a balance between efficiency and quality. However, the application of these innovative techniques presents a challenge for hop farmers due to the high initial costs involved. Despite this obstacle, the long-term benefits in terms of improved product quality and operational efficiency may outweigh the initial investment, making it a worthwhile consideration for the industry's future.

Artificial intelligence (AI) offers promising applications in optimizing hop drying processes through real-time monitoring and control. Sturm et al. (2020) explored the use of visual sensors to achieve this goal, aiming to enhance product quality and performance. The study integrated three types of visual sensors into the drying system: Vis-VNIR hyperspectral and RGB cameras, along with a pyrometer. These sensors facilitated continuous and non-invasive monitoring of hop-drying behavior, generating valuable data for process optimization. By employing chemometric analyses, which utilize statistical methods to analyze chemical data, the researchers accurately predicted moisture content and color changes in the hops. This highlights the potential for real-time quality control during the drying process, enabling adjustments to be made promptly to optimize product quality and efficiency. Incorporating AI-driven monitoring and control systems into hop-drying processes holds promise for improving overall efficiency and product quality, ultimately benefiting both producers and consumers.

1.1.3.2. What is new in hop extraction?

Lupulin glands, constituting approximately one-fifth of the dry hops' weight, are pivotal in hop products like pellets or extracts. They concentrate the lupulin by separating it from the

vegetative matter (Verzele & Keukeleire, 1991). While hops extracts were traditionally produced through solvent extraction methods such as ethanol and hexane, residues from these solvents may persist in the final beer product. Consequently, the hop industry predominantly utilizes supercritical CO_2 extraction due to its fluid polarity and superior yields in recovering bitter acids (Briggs et al., 2004; Lewis & Young, 2002). The resulting extract is typically stored in cans at room temperature, as this environment offers optimal stability against oxidation, facilitated by both processing and packaging methods. Moreover, in brewhouses, precise dosage control of hop extracts is essential for achieving consistent bitter flavor quality and minimizing raw material wastage (Briggs et al., 1982; Brunner, 2005; Verzele & Keukeleire, 1991). Therefore, hop processing plays a vital role in enhancing beer quality and industry efficiency by maximizing the utilization of bitter acids and ensuring a balanced bitter taste in the final product.

The first industrial plant for supercritical fluid extraction (SFE) was established for the extraction of caffeine from coffee beans and resins from hop flowers (Meireles, 2008). Even today, these remain the primary processes utilizing SFE, with CO_2 being the preferred solvent due to its variable solvent power based on pressure and temperature, as well as its low viscosity (Sanz et al., 2019; Sovová & Stateva, 2011). Hop extraction plants typically consist of extractors filled with milled hop pellets, through which CO_2 passes, carrying bitter compounds. Before extraction, liquid CO_2 undergoes a series of cooling, pumping, heating, and compression steps to achieve the supercritical state (Biendl et al., 2014), as explained in Figure 2.4. This process allows for mild extraction conditions with low operating temperatures, resulting in an extract closely resembling the natural material compared to extracts obtained through steam distillation or conventional solvent extraction methods (Herrero et al., 2010; Sanz et al., 2019; Sovová & Stateva, 2011). Liquid CO_2 transitions into the supercritical state when conditions surpass the critical point (31°C and 73 bar), where the fluid exhibits properties similar to both liquids and gases (Herrero et al., 2010). These properties, coupled with CO_2's non-polarity, enable the easy dissolution of both α- and β-acids, resulting in extraction yields of up to 99% of bitter acids (Verzele & Keukeleire, 1991).

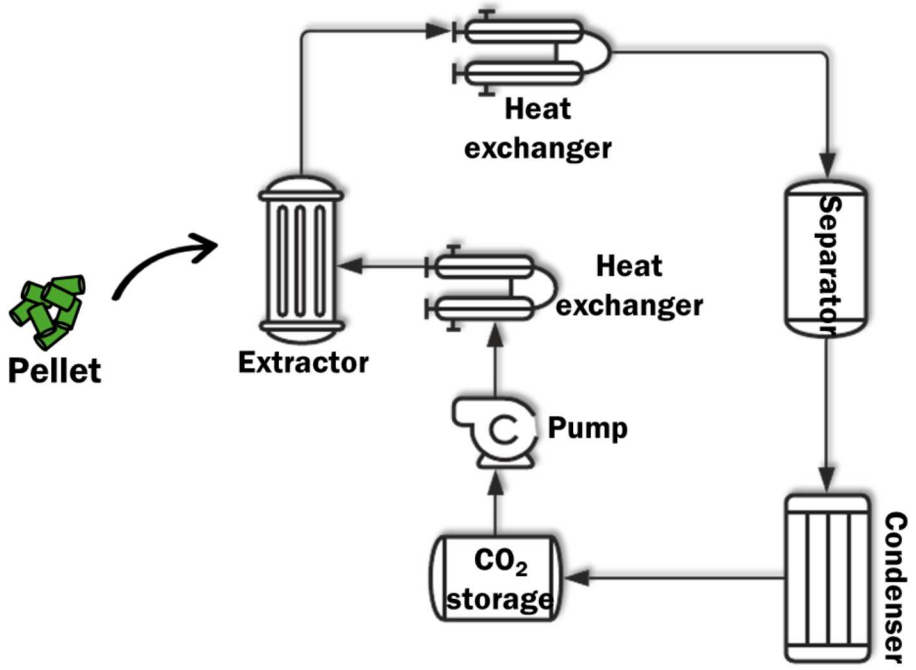


Figure 2.4. Representation of supercritical CO_2 hop extraction

Optimizing supercritical CO_2 extraction conditions, regarding temperature and pressure, is vital for efficiently obtaining the desired compounds from hops. In that matter, Valle et al. (2003) began optimizing the supercritical CO_2 extraction process using Nugget hops as a benchmark. They found that a pressure of 20 MPa and a temperature of 40°C were the most effective conditions. Higher pressures did not significantly improve extraction rates and could lead to the co-extraction of undesirable compounds. Further research by Nagybákay et al. (2021) produced extracts rich in α- and β-bitter acids under conditions of 37 MPa pressure, 43°C temperature, and an 80-minute extraction time. Compared to the conventional one-stage SFE-CO_2 process, these optimized conditions resulted in a three-fold increase in extraction yield and bitter acids recovery, all achieved in a significantly shorter time. The extract's composition was also notable for its negligible content of chlorophyll and carotenoids, which can be undesirable due to their color and taste. This research underscores the potential of supercritical CO_2 extraction to improve both the efficiency and quality of hop extracts, which is crucial for enhancing beer production.

The extraction efficiency can be enhanced by using other solvents in addition to supercritical CO_2. Bizaj et al. (2021) underscore the importance of solvent selection and operating conditions in supercritical fluid extraction, particularly for extracting bitter acids from hops.

Solvents such as CO_2, propane, SF6, and dimethyl ether (DME) were tested at various densities, temperatures (ranging from 20°C to 80°C), and pressures (50 to 150 bar). This study highlights the superior efficiency of DME in extracting these compounds. For instance, the optimal conditions for DME extraction were found to be 40°C and 10 MPa for α-acids, 60°C and 50 MPa for β-acids, and 60°C and 15 MPa for overall extraction yield. These conditions led to the highest concentrations of bitter acids in the extracts, demonstrating DME's superior dissolution power for hop compounds. Veiga et al. (2021) explored the use of supercritical CO_2 and compressed propane enriched with ethyl acetate as a cosolvent to extract phenolic compounds from hops. The results highlight a significant enhancement in extraction yield and efficiency, particularly in reducing extraction time by 71%.

The use of counter-current extraction, a technique where the solvent and the feed move in opposite directions, is particularly favored for large-scale operations due to its potential for higher productivity. Hoshino et al. (2018) explored the use of high-pressure counter-current extraction with dense CO_2 to achieve maximum flavor purity and recovery. This study demonstrates the efficacy of this approach for the fractionation of flavor and bitter compounds in hop-ethanol solutions. By carefully controlling the temperature, pressure, and solvent-to-feed ratio, it is possible to achieve both high purity and recovery of flavor compounds, which is essential for enhancing the quality of beer.

Innovations in SFE involve not only optimizing process parameters and solvents but also refining process conduction. Klimek et al. (2021) employed a modified two-step SFE process to enhance compound extraction. In the first step, the supercritical fluid extraction yielded a crude extract (E1) containing a mixture of α-acids, β-acids, and terpene derivatives, along with some impurities. This extract exhibited a higher yield compared to other extraction methods. The second step involved re-extracting the post-extraction residues to obtain a sample enriched in xanthohumol, termed extract E2.

Another extensive research effort by Van Opstaele (2012a, 2012b, 2013) developed a two-step extraction process to extract and fractionate hop oil essences from hop pellets. In the first step, most volatile compounds were extracted from T-90 hop pellets using CO_2 at a specific density and

temperature. In the second step, the hop residue from the first extraction was subjected to another round of SFE, this time at a higher CO_2 density (Van Opstaele et al., 2012b). This step targeted the less volatile, yet equally important, "spicy" compounds. The researchers found that a CO_2 density of 0.50 g/mL (at 50°C and 110 atm) was the optimal condition, ensuring a balance between selectivity and yield (Van Opstaele et al., 2012a).

To enhance beer flavors and aromas, researchers have turned to SFE using CO_2 as a solvent. This method efficiently separates desirable aromatic compounds from the bitter ones in hops. The challenge in the hop industry is to extract aromatic compounds while minimizing bitterness. Although SFE is well-established in the hop industry, researchers are exploring ways to optimize the process, particularly for hop aroma compounds. Dzingelevičius et al. (2011) applied SFE using supercritical CO_2 to extract essential oils from "Marynka" hops, finding that a trap temperature of 5°C, an extraction pressure of 8.5 MPa, and an extraction time of 10 minutes yielded the highest oil recovery. Bizaj et al. (2022) employed a semi-continuous extraction method using sub- and supercritical fluids with varying polarity—CO_2 and propane as non-polar solvents, and dimethyl ether (DME) as a polar solvent. They highlighted the importance of extraction methods in obtaining higher yields of xanthohumol and essential oils, noting optimal results at 40°C and 5 MPa. Pannush et al. (2023) found that lower temperatures and higher pressures generally led to higher extraction yields, with sensitivity to pressure varying among oil components. For instance, β-myrcene showed greater sensitivity to pressure changes compared to α-humulene.

The extraction might also be influenced by the hop variety used. Therefore, a study by Zeković et al. (2007) focused on five different hop cultivars, with the Magnum variety standing out for its high essential oil content and extract yield. The extraction process was conducted in two steps: first at 15 MPa and 40°C for 2.5 hours, and then at 30 MPa and 40°C for an additional 2.5 hours. The choice of pressure and temperature in each step is crucial, as it determines which compounds are extracted. The Magnum cultivar, known for its bitterness, showed the highest content of α-acids, the compounds responsible for the bitter taste in beer, in the second step of

extraction. This indicates that the SFE-CO_2 method was effective in selectively extracting the bitter compounds from this variety.

As the beer industry continues to evolve, the exploration of extraction methods is crucial for meeting the demand for high-quality, flavorful beers. Formato et al. (2013) focused on extracting hop bitter acids using supercritical fluid extraction (SFE) compared to a cyclically pressurized solid-liquid extractor known as the Naviglio Extractor. The study revealed that using supercritical CO_2 without modifiers was particularly effective in isolating β-acids from hops. However, the addition of ethanol as a co-solvent, a common practice to enhance the extraction of polar compounds, resulted in a heterogeneous extract, indicating a less selective extraction process. In contrast, the Naviglio Extractor, a cyclically pressurized solid-liquid extraction system, demonstrated a higher extraction capacity for α-acids. This method involves the repeated pressurization and depressurization of the extraction vessel, enhancing the penetration of the solvent into the plant material and the subsequent extraction of target compounds. The results suggest that this technique could be particularly useful for extracting specific compounds that are less soluble or more challenging to extract using SFE.

Conventional extraction methods, while effective to some extent, have limitations, prompting the exploration of innovative techniques like ultrasound-assisted extraction (UAE), high hydrostatic pressure (HHP) extraction, and microwave-assisted extraction (MAE). These methods aim to improve extraction efficiency while minimizing the presence of unwanted substances, aligning with the modern industry's focus on environmentally friendly practices. Santarelli et al. (2022) conducted a study comparing traditional extraction methods with innovative approaches such as ultrasound and high-pressure techniques to determine the most efficient way to obtain valuable compounds. Their findings revealed that the choice of extraction method and conditions (temperature and time) significantly influenced the phenolic profile and the content of various bioactive compounds in hop extracts. However, the optimal method and conditions varied depending on the specific bioactive compound being targeted for extraction. In a recent study by Kljakić et al. (2024), SFE was employed for lipophilic extracts and hydrodistillation for essential oils, while hydrophilic extracts were obtained using ultrasound and microwave techniques. The

results indicated that the extraction technique had a more significant influence on these activities than the hop variety. This suggests that the method of extraction plays a crucial role in preserving or enhancing the beneficial properties of hops.

Traditional methods, such as hydrodistillation, have long been employed for essential oil extraction, but they can be time-consuming and may not yield the highest concentrations of desired compounds. A solution could be the innovative technique of microwave-assisted hydrodistillation (MAHD), which offers the potential for higher efficiency and better quality of essential oils. In a study by Tyśkiewicz et al. (2018), two extraction methods were compared: microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD). The researchers identified the optimal conditions for MAHD as 335 W of microwave power for 30 minutes, using a water-to-raw material ratio of 8:3. These conditions resulted in essential oils with a high concentration of β-myrcene, ranging from 74.13% to 89.32% by weight, a key indicator of oil quality and aroma.

Another innovative technique to improve the extraction yield of volatile compounds involves utilizing CO_2 in a subcritical state. This has garnered attention for its ability to preserve volatile compounds and increase extraction yields, making it a promising technique for obtaining high-quality hop extracts. Fisher et al. (2023) conducted a study to compare products obtained through hydrodistillation and subcritical CO_2 extraction methods, assessing their chemical compositions and potential applications, particularly in the brewing industry. The extraction yield varied significantly between the methods, with subcritical CO_2 extraction showing higher yields (ranging from 8.76% to 15.35% m/m) compared to hydrodistillation (0.38% to 1.97% m/m) for both essential oils and extracts. This research highlights the potential of hop extracts, particularly those obtained through subcritical CO_2 extraction, as valuable products with applications in the brewing industry.

The researchers also explored simpler extraction techniques. Recently, García et al. (2024) demonstrated the effectiveness of ten organic solvents for the initial extraction of compounds from hop pellets. Among these, a mixture of methanol and dichloromethane stood out, paving the way for further optimization. The extraction variables, including temperature and solvent concentration, were fine-tuned to achieve the highest recovery rates of the target compounds. The

optimized conditions, involving the use of 19.7% (v/v) methanol at room temperature for 89 minutes, resulted in impressive recovery rates: 86.57% for α-acids, 89.14% for β-acids in soft resins, 78.48% for xanthohumol in hard resins, and 67.10% for phenolics in spent solids. These conditions were then successfully validated across six different hop varieties, highlighting the robustness and versatility of the optimized extraction process. However, most of those solvents are not food-grade, which prevents their usage in hop extraction.

Traditionally, optimization has relied on offline analysis methods, which, while effective, are time-consuming and may not provide real-time insights. This is where the innovation of online monitoring through optical metrology techniques, such as Raman spectroscopy with shifted excitation, comes into play. These techniques offer the potential to enhance the efficiency and quality of SFE processes by providing continuous, real-time data on the extraction process. The core of the research developed by Schuster et al. (2018) lies in the development and application of an in-situ Raman measurement system for monitoring the supercritical CO_2 extraction of hops. This measurement method is a spectroscopic quantitative technique, that successfully measures compounds in a mixture. The system's effectiveness was demonstrated by monitoring the extraction of two different hop varieties under varying conditions of temperature and pressure. This real-time monitoring capability not only optimizes the extraction process by allowing for adjustments in the flow but also reduces the need for costly calibration, making it a practical and efficient tool for various SFE applications.

Also towards a circular economy, researchers have recently been exploring the reuse of spent hops from the brewing process, which are a rich source of bioactive compounds. A study conducted by Inumaro et al.(2023) focused on hop cone residues from craft breweries, aiming to extract and evaluate the bioactive potential of the compounds, particularly in terms of their antioxidant, antimicrobial, and cytotoxic activities. The results were multifaceted, showing that lower temperatures and pressures, along with higher ethanol percentages, led to increased concentrations of certain compounds and higher antioxidant activity in both chemical and cell-based assays. Conversely, other compounds and antioxidant activities were favored by lower temperatures and pressures but higher ethanol percentages. These findings not only contribute to

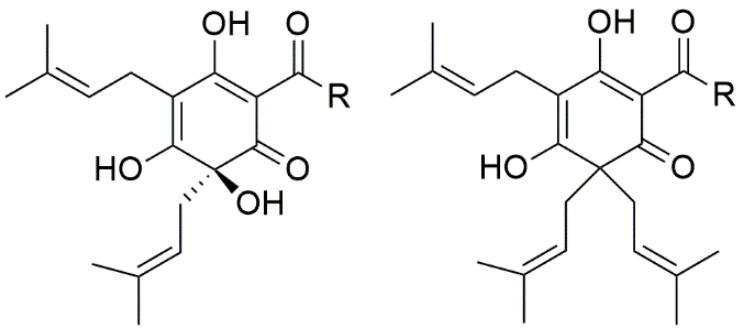
the sustainable utilization of brewing by-products but also open up new avenues for the development of multifunctional ingredients for various industries.

1.2. Hop bitter acids in beer: is it possible to enhance its utilization?

1.2.1. Explaining hop bitter acids

Inside female strobiles, positioned under the bracts, are found the yellow glands called lupulins which are composed of two resins fractions, hard and soft (Biendl et al., 2014). The main compounds used in the brewing process to provide bitterness and aroma to the beer are located on the soft resins, which contain the bitter acids and essential oil. The bitter acids are belong to two groups comprised of α- and β-acid, the first series is related to the bitter taste in beer and is one of the most important compounds in the beer industry (Almaguer et al., 2014).

The α-acids include five constituents that differ by the side chain, they are humulone, adhumulone, cohumulone, posthumulone and prehumulone as shown in Figure 2.5 (Durello et al., 2019; Verzele & Keukeleire, 1991). The β-acids are distinguished from α-acids by the prenyl chain at carbon atom-6. Moreover, they also comprise five homologues named lupulone, adlupulone, colupulone, postlupulone and prelupulone (Figure 2.5) (Briggs et al., 2004; Durello et al., 2019). The compounds are biosynthesized and accumulated during flowering in the female inflorescence and in the leaves. Furthermore, the acyl chain which distinguishes the homologue molecules is obtained in the leucine-biosynthetic pathway (Goese et al., 1999; Keukeleire et al., 2003). Although the reason to use hops α-acids compounds in wort boiling is to provide bitterness in beer, the bitter taste is not offered by α-acid directly, due to low solubility in aqueous solutions (Keukeleire et al., 2003). Rather, the α-acids must be isomerized to form more soluble compounds. This is achieved in the brewing process when high temperatures are applied (Cooman & Goiris, 2018).



R=	Alpha-acids:	Beta-acids:
$-CH_2-CH(CH_3)_2$	Humulone	Lupulone
$-CH(CH_3)_2$	Cohumulone	Colupulone
$-CH(CH_3)-CH_2-CH_3$	Adhumulone	Adlupulone
$-CH_2-CH_3$	Posthumulone	Postlupulone
$-CH_2-CH_2-CH(CH_3)_2$	Prehumulone	Prelupulone

Figure 2.5. Alpha- and beta-acids homologues chemical structure

The key bitter compounds in beer are the iso-α-acids generated by the isomerization reaction that follows first-order kinetics (Huang et al., 2013; Jaskula et al., 2008; Malowicki & Shellhammer, 2005). The humulones are converted into isohumulones via an acyloin-type ring contraction, building two epimeric isomers cis- and trans-isohumulones during wort boiling process step (Clippeleer et al., 2014; Verzele & van Boven, 1971). The reaction is influenced by process parameters like temperature, pH, divalent metal ions, and wort density, which could affect both isomerization reaction and utilization (Bastgen et al., 2019).

1.2.2. What is hop utilization?

The α-acids utilization is defined as the yield of the iso-α-acids present in beer compared to the initial content of α-acids added (Jaskula-goiris et al., 2010). It represents a massive challenge in the brewing industry, considering the extensive loss of bitter compounds during the processes. Only around 30-50% of bitter acids will be found in the finished beer, which translates to a reduction in beer production profitability, especially for highly hopped beverages (Kappler et al., 2010). Barth-Haas Group stands among the largest hop producers in the world. According to the Group (2019), a 10% increase in bitter acid utilization - from 30% to 40% - in a batch of 200,000 hl of beer with the addition of 30 mg of iso-α-acids/L, achieves around 15,000 euros savings. Therefore, iso-α-acids utilization is an essential factor for industry, not only for the environment by reducing hop usage footprint, but also for economic reasons.

In the past decades, the brewing industry has sought alternatives to increase hop α-acid utilization. However, this remains a profitability issue since current options mainly involve acquiring new types of equipment. Improving the utilization of iso-α-acids by around 5% would significantly enhance brewery profitability and reduce GHG emissions. For instance, in a mid-size brewery with an annual beer output of 300,000 hL, such improved utilization could result in savings of approximately €20,000 per year and a reduction in CO_2 emissions of about 16 kg per year. For the German beer industry, this would translate to savings of around €5.61 million and a reduction in CO_2 emissions of about 4.5 tons. Therefore, optimizing the utilization of raw materials, such as hops, would benefit the industry economically and enhance its sustainability.

1.2.3. Hop compound-protein interaction

As mentioned above, the α-acids are converted into iso-α-acids during the wort boiling process (Clippeleer et al., 2014; Verzele & van Boven, 1971). According to Gänz et al. (Gänz et al., 2021), the iso-α-acids content is depleted by the proteins originating from the barley malt. The role of iso-α-acids in beer foam stability due to interaction with foam-active proteins has been reported by several researchers (Clark et al., 1991; Evans et al., 2018; Ferreira et al., 2005; Kapp & Bamforth, 2002; Smith et al., 1998). They are the most abundant proteins in the wort along with the haze-active proteins (Iimure et al., 2012b). However, there is still a lack of knowledge about minor proteins, such as enzymes and barley defense proteins, and hop bitter acids interaction.

It is well established that wort protein plays an important role in foam stabilization and quality due to the formation of a viscoelastic layer with a linkage comprising a continuous liquid phase and discontinuous gas phase (Briggs et al., 2004). Hydrophobic proteins are mainly responsible for stabilizing the foam layer, especially those known as LTP and protein Z (Steiner et al., 2011). Recently, Lu et al. (Lu, Osmark, Bergenståhl, & Lars, 2020) studies showed the influence of these two proteins on the surface layer and the property's dependence upon iso-α-acids and protein interactions (Lu, Osmark, Bergenståhl, & Lars, 2020). They observed formation of spherical aggregates of iso-α-acids and proteins, which suggests a strong bond between these compounds, preferentially with protein Z (Lu, Osmark, Bergenståhl, & Lars, 2020). These protein

fractions were also found to comprise the major fraction of proteins precipitated into wort trub during the boiling step due to binding with protein-derived polypeptides (Iimure et al., 2012a).

However, much of the research up to now has been focused on the protein-hop bitter compounds interaction in the foam and little attention has been paid to the equally important role of hop bitter compounds in protein precipitation during boiling. The mechanism of hop bitter substance interaction with barley proteins is unknown as well as the barley protein fraction that binds to them. Understanding the protein-hop bitter substance binding capacity will help improve hop utilization rates by reducing the reactive protein fractions by selecting appropriate barley cultivars, setting breeding goals, and/or modifying malting and brewing parameters.

1.2.4. Hop bitter acids beyond bitterness

Hop bitter acids, especially iso-α-acids, are well-known for giving beer its bitter taste. However, these compounds also play important technological roles in beer production. Researchers have discovered that these acids have significant antioxidant properties. According to Wietstock and Shellhammer (2011), hop humulones and isohumulones can reduce the formation of hydroxyl radicals due to their metal chelation and scavenging abilities. Oxidative reactions, which commonly occur during beer storage, can degrade the quality of the beer. The antioxidant capacity of hop bitter acids helps maintain the flavor stability of beer during storage by preventing oxidative changes and the formation of aldehydes (Karabín et al., 2014; Mertens et al., 2022).

Beer can undergo changes in flavor and quality over time, especially during storage. A major challenge in maintaining beer quality is the development of stale flavors due to chemical reactions, particularly the degradation of iso-α-acids. As beer ages, these acids break down, reducing bitterness and forming off-flavor aldehydes (Caballero et al., 2012; Intelmann et al., 2011). Clippeleer et al. (de Clippeleer et al., 2010) studied how trans-iso-α-acid degradation affects aldehyde formation during aging. They found that aging increases cis-iso-α-acids due to the reverse isomerization of trans-iso-α-acids. However, aldehyde formation was not directly linked to iso-α-acid degradation, suggesting that factors like malt quality and brewing processes are more significant for flavor stability.

One of the main traits of beer quality is foam stability, which contributes to a visually pleasing appearance for consumers. The impact of iso-α-acids and their chemically modified counterparts on foam stability was highlighted by Hughes (2000). The study demonstrated that iso-α-acids, especially the modified versions called hexahydro- and tetrahydro-iso-α-acids, act as effective foam stabilizers. This stabilization likely occurs via hydrophobic interactions with amphipathic polypeptides. Further research confirmed these findings, elucidating the role of iso-α-acids in beer foam stabilization, particularly through interactions with protein Z and LTP (Evans et al., 2018; Lu, Bergenståhl, et al., 2020).

1.2.5. Innovation and Future Perspectives

There is a growing global demand for greener processes that use resources efficiently and reduce waste generation. To achieve more sustainable production, the circular economy approach is key to reaching net-zero emissions. However, innovating in the hop and brewing process is more challenging than in newer food production methods due to its long-established practices.

The improvement of these processes involves the application of new technologies, such as AI and other emergent technologies. The former has become a popular tool for optimizing daily tasks and processes. While algorithms can be implemented relatively easily, the challenge lies in acquiring sufficient data. To develop reliable AI models, a substantial amount of data is required to enhance accuracy and predictive capabilities. However, storing ‘big data’, particularly on supercomputers, demands significant energy. Despite its promise for process optimization, AI should be used cautiously, with a critical perspective on the models generated to ensure higher prediction accuracy.

Emergent technologies have been extensively explored by researchers and innovative food industries. For instance, microwave-assisted extraction has been implemented for hop compound extraction, enhancing the utilization of this raw material in brewing (Milestone Srl, 2024). These technologies can improve both process efficiency and environmental footprint by optimizing resource usage and reducing energy consumption.

Additionally, understanding the biochemical mechanisms of hop bitter acids binding to proteins could open new possibilities for use in plant-based foods. This market has grown substantially in recent years, with significant investments from both public and private sectors. By comprehending the relationship between proteins and hop bitter acids, these compounds could be used in alternative products to improve emulsion stability and texture in extruded foods. Notably, β-acids offer a neutral taste and anti-microbiological activity, making them particularly valuable in these applications.

Chapter 3

Is it possible to improve further hop processing?

Novel Brazilian hop (*Humulus Lupulus L.*) extracts through supercritical CO_2 extraction: Enhancing hop processing for greater sustainability

Mariana Barreto Carvalhal Pinto[a]*, Renata Vardanega[b,c], Grazielle Náthia-Neves[b], Pedro Renann Lopes de França[d], Louise Emy Kurozawa[d], Maria Angela Almeida Meireles[b], Flavio Luis Schmidt[a]*

[a] *Fruit, Vegetable and Confectionery Products Laboratory, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil*

[b] *Laboratory of Supercritical Technology: Extraction Fractionation and Identification of Extracts, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil.*

[c] *Food Innovation & Technology Research Group, Centre of Biological Engineering, University of Minho, 4710-057, Braga, Portugal.*

[d] *Laboratory of Food Innovation, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil*

Author Contributions:
Mariana Barreto Carvalhal Pinto: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Renata Vardanega:** Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Grazielle Náthia-Neves:** Data curation, Writing - original draft, Writing - review & editing. **Pedro Renann Lopes de França:** Writing - review & editing. **Louise Emy Kurozawa:** Writing - review & editing. **Maria Angela Almeida Meireles:** Conceptualization, Methodology, Writing - review & editing, project administration, and funding acquisition. **Flavio Luis Schmidt:** Conceptualization, Methodology, Writing - review & editing supervision, project administration, and funding acquisition.

Paper published in Food Research International

Reprint with permission from (see Appendix):
https://www.sciencedirect.com/science/article/abs/pii/S0963996923007147

Food Research International 172 (2023) 113169

Contents lists available at ScienceDirect

Food Research International

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

Novel Brazilian hop (*Humulus lupulus L.*) extracts through supercritical CO_2 extraction: Enhancing hop processing for greater sustainability

Mariana Barreto Carvalhal Pinto [a,*], Renata Vardanega [b,c], Grazielle Náthia-Neves [b], Pedro Renann Lopes de França [d], Louise Emy Kurozawa [d], Maria Angela A. Meireles [b], Flavio Luis Schmidt [a,*]

[a] Fruit, Vegetable and Confectionery Products Laboratory, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil
[b] Laboratory of Supercritical Technology: Extraction Fractionation and Identification of Extracts, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil
[c] Food Innovation & Technology Research Group, Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal
[d] Laboratory of Food Innovation, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil

ARTICLE INFO

Keywords:
Alpha-acid
Beta-acid
Drying kinetic
Humulus lupulus L.
Supercritical fluid
Emergent technology

ABSTRACT

Hop cultivation has been increasing in the past decade in Brazil, demanding a better understanding of how the processing influences the national hop varieties. Despite the hop process being well-established in the producer countries, there is still room for optimization to reduce energy consumption for a more sustainable process. This study's main purpose was to understand the influence of drying and supercritical CO_2 extraction on the quality of hop extracts. The hop quality during drying was evaluated regarding color, bitter acids, xanthohumol, total essential oil content, and volatile profile. Supercritical CO_2 extraction yields, and bitter acid recovery were assessed by HPLC in a range of different temperatures (40 or 60 °C) and pressure (15, 20, 25, or 30 MPa) conditions. Hop processing was optimized to produce a greater extract quality from a Brazilian hop variety, saving energy and solvent consumption, and consequently, reducing the process footprint. Furthermore, this study established supercritical CO_2 extraction conditions for Brazilian hop extract production, offering the national beer industry an alternative to overpriced products.

1. Introduction

The hop plant (*Humulus lupulus L.*) naturally grows in temperate climates, with wide daily sunlight and large temperature changes between the seasons. These climatic factors are found in the principal hop-producing countries, such as the USA and Germany (Barth & Meier, 2021). Those conditions are claimed to be essential for cultivation and higher cone quality. However, Thomas (1980) and Srečec et al. (2008) demonstrated no significant effects of sunlight quantity on α-acid accumulation during hop flowering. According to the authors (Srečec et al., 2008), the most relevant weather condition for hop flowering and high α-acid yield in cones was the sum of effective temperature and total rainfall. Furthermore, Bauerle (2019) showed that photoperiodism and dormancy are unrelated. These findings imply that a more widely global hop production is possible, even in countries like Brazil which lie outside the temperate climate zone.

Hop is valued due to its unique composition with hundreds of bioactive compounds. Those are placed within the lupulin gland which is found inside the female inflorescence (Biendl et al., 2014). Among them, the α- and β-acids are the most important and present the highest content ranging between 2-15 % and 2–10 % in dry weight, respectively (Zhang et al., 2021). Furthermore, hop contains around 4 % (dry weight) of phenolic compounds with a wide range of compounds (Durello et al., 2019). Xanthohumol is a particular polyphenol from hops that represents 0.1 to 1 % (dry weight), including a high anti-inflammatory and antioxidant capacity (Stevens & Page, 2004).

Hop production in Brazil is still incipient, summing up to 50 ha cultivated over several regions with different microclimates (Jastrombek et al., 2022). Nevertheless, the crop demonstrates enormous potential, unique terroir, and competitive quality compared with the

* Corresponding authors.
E-mail addresses: maribcarvalhal@gmail.com (M.B.C. Pinto), schmidt@unicamp.br (F.L. Schmidt).

https://doi.org/10.1016/j.foodres.2023.113169
Received 24 March 2023; Received in revised form 14 June 2023; Accepted 17 June 2023
Available online 26 June 2023

main producers (Pontes Guimarães & Ferreira Guesti, 2020; Santos et al., 2022). In addition, Arruda et al. (2021) found a high content of α- and β-acids, and great antioxidant potential, in hydroethanolic extracts prepared from varieties cultivated in Brazil.

Brazil stands out amongst the most important beer markets worldwide, being the third largest producer (Kirin Company, 2019). Also, the Brazilian craft beer market increased rapidly over the past decades. In 2019, for example, 320 new microbreweries were founded, increasing the national total to 1209 (Van Eynde, 2018). The current situation demands greater amounts of raw material supplies, like hops. Currently, the country imports hop products, like pellets or extracts, used in the beer industry contributing to the cost of beer production.

Hop is rarely used as a cone in the brewing process. Rather, hop-derived products are used to enhance beer quality due to their high quality and efficiency (Arruda, Pinheiro, Silva, & Bernardes, 2022). However, hop processing is responsible for high CO_2 emissions, especially the drying step (Hauser & Shellhammer, 2019). Although the process is well-established in hop-producing countries, there is still room for optimization across the main steps of hop extract production. Hop cultivation has been gradually expanding in Brazil, which demands an investigation into the process conditions to reach a high-quality and sustainable product. Thus, more studies on hop processing are necessary to facilitate the development of an efficient and sustainable hop and brewing industry in Brazil, offering excellent hop products at a competitive price. Furthermore, no previous research has evaluated supercritical CO_2 extracts enriched in bitter acids using a Brazilian hop variety as raw material. Therefore, this study's main purpose was to understand the effects of hop process parameters on hop quality, especially drying and supercritical fluid extraction steps. This study optimized the process by saving energy consumption towards more sustainable production.

2. Materials and methods

2.1. Sample material

Fresh Brazilian hop (*Humulus lupulus L.*) variety 'Mantiqueira' provided by Van Den Berg company, containing 3.36 % of α-acids, 2.67 % of β-acids, and around 1.52 mL of total essential oil/100 g of hops, was used in the present study. The hop cones were stored at −18 °C until the drying trials and physicochemical analysis. Furthermore, prior to supercritical fluid extraction, the hop samples were dried at 70 °C for 2.5 h to a final moisture content of 12.03 %.

2.2. Drying process

The fresh cones were dried in triplicate at 70, 55, and 40 °C with an air velocity of 6.6 m/s and volumetric airflow of 0.013 m^3/s. As shown in Fig. 1, the trials were carried out in three perforated (mesh 14, wires 30 mm) 314 stainless steel (30 × 20 × 10 cm) baskets placed in an oven-drier (Model MA035, Marconi, Piracicaba, Brazil). Each batch was performed with three baskets containing 100 g of hops each. The hops' weight was measured during the drying process through one of the baskets linked to a weight balance connected to a PC, acquiring data every minute with the aid of self-developed software. The samples for α- and β-acid analyses were randomly collected from the other two baskets every 30 min during the 70 °C and 55 °C assays, and every 60 min during the 40 °C assays.

The moisture rate (MR) is a dimensionless value calculated according to Equation (1).

$$MR = \frac{X - X_{eq}}{X_0 - X_{eq}} \quad (1)$$

Where, X, X_{eq} and X_0 correspond to average moisture content at weighing time (g/g_{solids}), average equilibrium moisture content (g/g_{solids}), and average initial moisture content (g/g_{solids}), respectively (Berk, 2018). The equilibrium moisture content was determined by drying a hop batch until a constant weight was achieved, characterized as equilibrium.

The drying rate (DR) was calculated according to Equation (2) and expressed as g_{water}/g_{solids}*min.

$$DR = \frac{X_{t1} - X_{t2}}{t_2 - t_1} \quad (2)$$

Where, t_1 and t_2 were at different times during the drying process, while X_{t1} and X_{t2} were the moisture content at time t_1 and t_2, respectively (Berk, 2018).

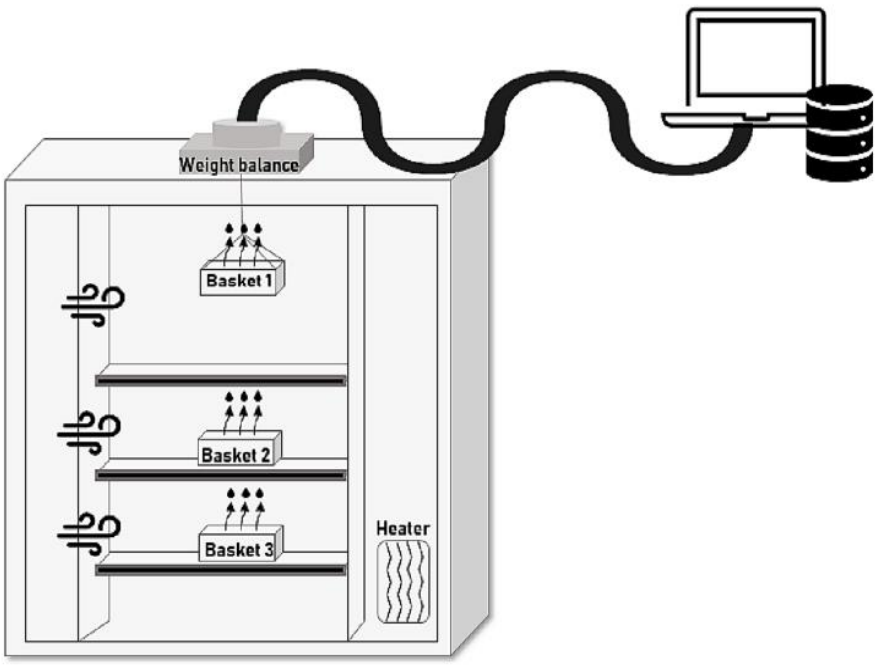


Fig. 1. Diagram of data collection during the drying experiment.

2.3. Moisture content analysis

The moisture content was analyzed following the Hops-4 ASBC (American Society of Brewing and Chemists) (ASBC Methods of Analysis, 1958) methodology adapted to 1 g of sample dried in an oven drier for 2 h at 105 °C.

2.4. Supercritical fluid extraction (X_0)

The dried hops were ground in a knife mill (Model MA340, Marconi, Piracicaba, Brazil). After milling, the mean particle diameter (D_p) was calculated according to ASAE Standards through particle size classification using a vibratory system (Model 1868, Bertel, Caieiras, Brazil), and assembled with 10 to 80 mesh sieves (WS Tyler, Wheeling, USA), resulting in mean particle diameter (D_p) of 0.83 ± 0.03 mm. The apparent density of the bed (ρ_a) was calculated by dividing the sample mass loaded into the extraction cell by the cell's internal volume. The particles' real density (ρ_r) was conducted in an Automatic Pycnometer Ultrapyc 1200 (Quantachrome Instruments, Boynton Beach, USA) by Pictometry, and using the helium gas method, resulting in 0.94 ± 0.01 g/cm^3. The total porosity of the bed (ε) was calculated using Equation (3), resulting in a bed density of 0.4 g/mL and bed porosity (ε) of 0.74 ± 0.01.

$$\varepsilon = 1 - (\rho_a/\rho_r) \quad (3)$$

Supercritical CO_2 extracts were produced in a Spe-ed system (Model 7071, Applied Separations, Allentown, USA). The system also contains a cooling bath (Model MA184, Marconi, São Paulo, Brazil), a pneumatic pump for CO_2 pressurization, an electrical oven to control the extraction temperature, and a flow totalizer (LAO, G0, São Paulo, Brazil).

Initially, the effects of temperature (40 or 60 °C) and pressure (15, 20, 25, or 30 MPa) were evaluated. These conditions were selected based on previous studies (Zekovic et al., 2007; Meyer et al., 2012). Thus, an extraction vessel of 25 mL (Ø 2.0 × 13.0 cm) was filled with 10 g of ground hops. The vessel was assembled in the oven and heated to the selected temperature. The system was pressurized with CO_2 until the desired pressure and maintained for 5 min (static period). The CO_2 flow rate remained constant at 5.0 g/min and the solvent (S) to feed (F) ratio was S/F = 20. The extracts were collected in glass flasks, weighed using an analytical balance, and stored at −18 °C for further analysis.

2.5. Supercritical fluid extraction kinetic study

From the kinetic study, an overall extraction curve (OEC) was obtained at 40 °C and 20 MPa, demonstrating a higher extraction yield. These experiments were performed in the same extraction unit Spe-ed system (Model 7071, Applied Separations, Allentown, USA), using a 300 mL extraction vessel (Ø 5.0 × 21.0 cm). The extraction vessel was filled in with 30 g of milled hops with a bed porosity (ε) of 0.92 ± 0.01. After reaching the desired extraction conditions, the bed was maintained for 15 min (static period). The CO_2 flow rate was 15.0 g/min. A total extraction time of 90 min was adopted to ensure the diffusion-controlled period in the overall extraction curve was achieved. These experiments were performed in duplicate.

2.6. Extraction yield

The extraction yield ($g_{extract}$/100 g of hops) was calculated as the ratio of the total extract mass (M_{EXT}) to the mass of raw material used to feed the system on a dry basis (F), according to Equation (4). The results were expressed as the mean ± standard deviation.

$$\text{Extraction yield}\,(\%) = \left(\frac{M_{ext}}{F}\right) * 100 \quad (4)$$

2.7. Bitter acid analyses

2.7.1. Sample preparation

Samples of fresh and dried cones were lyophilized for 24 h to eliminate residual water and milled using liquid nitrogen to avoid oxidation reactions. The analysis was carried out in triplicate for each sample. For bitter acid extraction, 20 mL methanol was added to 250 mg of hop powder (acidified with 0.01 % formic acid) in a falcon tube and agitated for 1 h. The samples were centrifuged (Laborline, Barueri, Brazil) for 5 min at 700 × *g* and an aliquot of 5 mL of supernatant was withdrawn and transferred to an amber glass tube. The samples were filtered using a 0.45 μm cellulose filter supplied by Sinergia Científica (Campinas, Brazil) and followed by HPLC (High-Pressure Liquid Chromatography) analysis.

For the supercritical CO_2 extract analyses, 10 mg of extract was diluted with 10 mL methanol (acidified with 0.01 % formic acid). The samples were filtered using a 0.45 μm cellulose filter supplied by Sinergia Científica (Campinas, Brazil).

2.7.2. HPLC analysis

The α- and β-acid contents were determined by HPLC (Waters Corporation, Milford, USA), according to the method adapted from Keukeleire et al. (2003) and Hops-14 ASBC (American Society of Brewing and Chemists, 2008). The column used for compound separation was a C-18 column (SunFire, 250 × 4.6 mm, 5 μm; Water Corporation, Milford, USA). Chromatographic conditions consisted of an isocratic gradient composed of 15 % eluent A (milli-Q water acidified with 1.47 % (v/v) phosphoric acid 85 %) and 85 % of eluent B (HPLC-grade methanol). The injection volume was 10 μL using a Waters 717 plus autosampler. The flow rate was 1 mL/min, the run time was 50 min, and the column temperature was 25 °C. The detection was at 314 nm for α- and β-acids and 370 nm for xanthohumol using the Waters 996 photodiode array detector. The retention time comparison with an external standard (ICE-4 and xanthohumol 60 % Standard), also applied for quantification of α-, β-acids, and xanthohumol determined the identification of the peaks.

2.8. Surface color measurement

The surface color of samples was determined by a portable colorimeter MiniScan XE (Hunter Associates Laboratory, Inc., Reston, Virginia, USA), with enlightening D65 and an observation angle of 10°. The CIE Lab color parameters, i.e. L* (whiteness or brightness), a* (redness or greenness), and b* (yellowness or blueness) coordinates, were used to describe the color of samples. Color measurements were taken in triplicate.

2.9. Scanning electron microscopy

Samples were placed onto double-sided carbon tape fixed to an aluminum specimen. A TM-3000 (Hitachi High Technologies America, Inc., Japan) scanning electron microscope was used to analyze the lupulin gland with non-destructively pre-treatment. The crystal lattice was observed in Analytical mode, with a 300 to 500-fold increase.

2.10. Essential oil

2.10.1. Total essential oil content

Total oil content was determined using hydro-distillation, following the methodology described by ASBC (ASBC Methods of Analysis, 1988). 100 g of fresh and dried hops were submitted separately to hydro-distillation for 4 h using a Clevenger apparatus. The volume of oil was observed in the receiver.

2.10.2. Hop oil composition

Samples of fresh and dried cones were lyophilized for 24 h to eliminate residual water and milled using liquid nitrogen to avoid oil

degradation. For sample preparation, extraction was performed with 1 g of hop powder added to 20 mL of ethyl acetate, and a rotary-vacuum evaporator removed the ethyl acetate. The samples were transferred to amber vial glass tubes and stored.

The major essential oil compounds were identified and evaluated following ASBC Hops-17 methodology (ASBC Methods of Analysis, 2004). A sample of 10 % (v/v) hop oil solution was prepared by adding 100 μL of hop oil and 900 μL of hexane in a glass autosampler vial. Hop oil composition was determined by Gas Chromatography (Shimadzu, Kyoto, Japan) coupled with a Flame Ionization Detector (FID). 1 μL of the hop oil solution was directly injected into the injection port held at 200 °C and a split ratio of 1:50. The column used for compound separation was a ZB-5 column (Zebron, 30 m × 0.25 mm, 25 μm), and ultrapure helium was used as a carrier gas with a constant flow rate of 0.6 mL/min. The GC oven temperature program started at 50 °C held for 1 min, followed by a ramp to 260 °C (3 °C/min) and continued at 260 °C for 15 min. The analyte concentrations in hop oil were standardized on a percentual area compared to the standard for each compound (Merk, Darmstadt, Germany).

2.11. Statistical analysis

The temperature (40, 55, and 70 °C) and drying time (30, 60, 90, 120, 150, 180, 210, and 240 min) effects on bitter acid contents were evaluated by analysis of variance (ANOVA), using the software Minitab 16® (Minitab Inc., State College, USA) and with a confidence level of 95 % ($p \leq 0.05$). The effects of the SFE parameter temperatures (40 and 60 °C) and pressure (15, 20, 25, and 30 MPa) were analyzed in a fully randomized factorial design (2 × 4) in duplicate, totaling 16 experiments. The influence of the parameters on the extraction yield and α- and -β acids was evaluated by analysis of variance (ANOVA) and Tukey's Honestly Significant Difference test using the software Minitab 16® (Minitab Inc., State College, USA). Additionally, for the hop oil composition dataset, the unsupervised analysis using principal component analysis (PCA) was performed in RStudio (package: FactomineR) to assess the groupings or trends in the data. The eigenvalue and cumulative percentage of variance were used to determine the number of principal components (Table S1, Supplementary Material).

3. Results and discussion

3.1. Drying curves and kinetics

Fresh hop cones with 79 % (3.77 g_{water}/g_{solids}) initial moisture content were dried until 15 % moisture (0.17 g_{water}/g_{solids}) to avoid overdrying. After, the dried hops were submitted to conditioning, reaching the equilibrium moisture of 12.03 %. Fig. 2a presents the drying curves that express a dimensionless moisture rate (MR) as a function of time. From the graph, the exponential tendency of drying curves (R^2 = 0.9841) can be seen. As expected, the air-drying temperature demonstrated a significant negative correlation with drying time. The drying time increased 2.5-fold with air-drying temperature decreasing from 70 °C (2.5 h) to 40 °C (8 h), and 1.5-fold from 70 °C to 55 °C (4 h). The extended time at low temperatures was expected as a typical time and temperature dependency on food drying. The processing time could also be influenced by air velocity, environmental humidity, and bulk weight; however, this study did not explore those parameters (Kerr, 2013; Márquez et al., 2006).

Fig. 2b provides a kinetic drying curve that expresses the drying rate as a function of moisture content. The drying kinetics presents three phases: initial period, constant rate period, and falling rate period (Kerr, 2013). In the drying kinetics (Fig. 2b), only the initial and falling rate phases are present, representing the main stages. The constant rate period is commonly absent in real food drying processes (Kerr, 2013). The initial drying rate positively correlates with the drying temperature; it reached 0.035 g_{water}/g_{solids}*min at 70 °C. Likewise, the values determined at 55 °C and 40 °C were 0.017 and 0.007 g_{water}/g_{solids}*min, respectively. Critical moisture is defined as the moisture content at the end of the constant rate period. Those values were 3.36, 3.53, and 3.63 g_{water}/g_{solids} for 70, 55, and 40 °C, respectively. The drying temperature affected the critical moisture, which directly influenced the water diffusion rate toward the surface.

Knowledge about the hops' drying profile is paramount to improving the process and product quality and reducing energy consumption. This study's findings offer a wider understanding of the hop drying process, enabling improvement. The drying rate is a key engineering parameter to determine the dryer production capacity and optimize the process (Kerr, 2013). The drying process creates the highest greenhouse gas emission during hop processing (Hauser & Shellhammer, 2019). Therefore, the control of the process, as well as the reduction of the time due to the temperature increase, might lead to a saving in energy consumption.

In this study, the drying curves were obtained for the variety Mantiqueira, although the drying process can be influenced by the size of the cones which is variety-dependent and directly impacts the bed density and the mass flow rate. Therefore, further investigation is needed to provide a complete account of hop drying behavior.

3.2. Evaluation of hop quality during the drying process

3.2.1. Color

Hop color is a relevant quality attribute for product acceptance by the consumer. Fig. 3 shows the color results for the hops during the drying process. As expected, hop color degraded during the drying

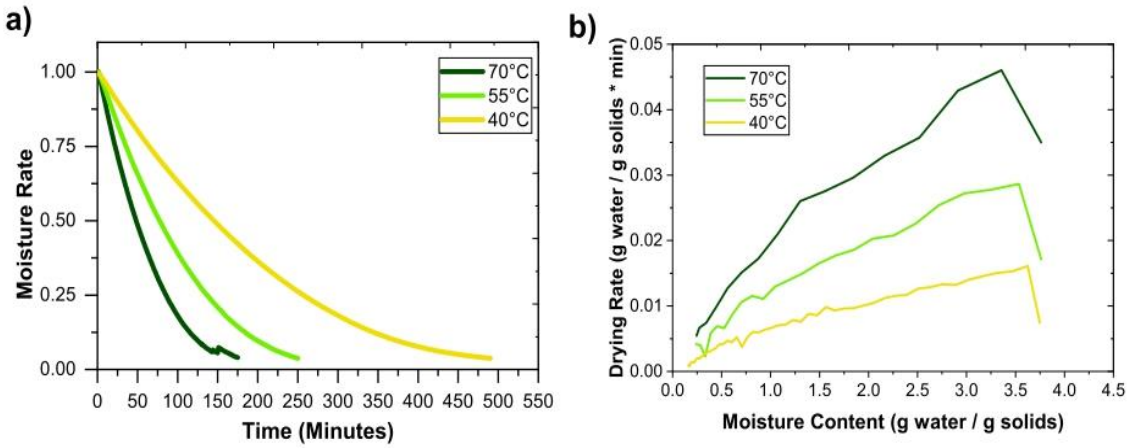


Fig. 2. Kinetic drying curves: **(a)** dimensionless moisture ratio over time at studied temperatures; **(b)** drying rate over moisture content at studied temperatures.

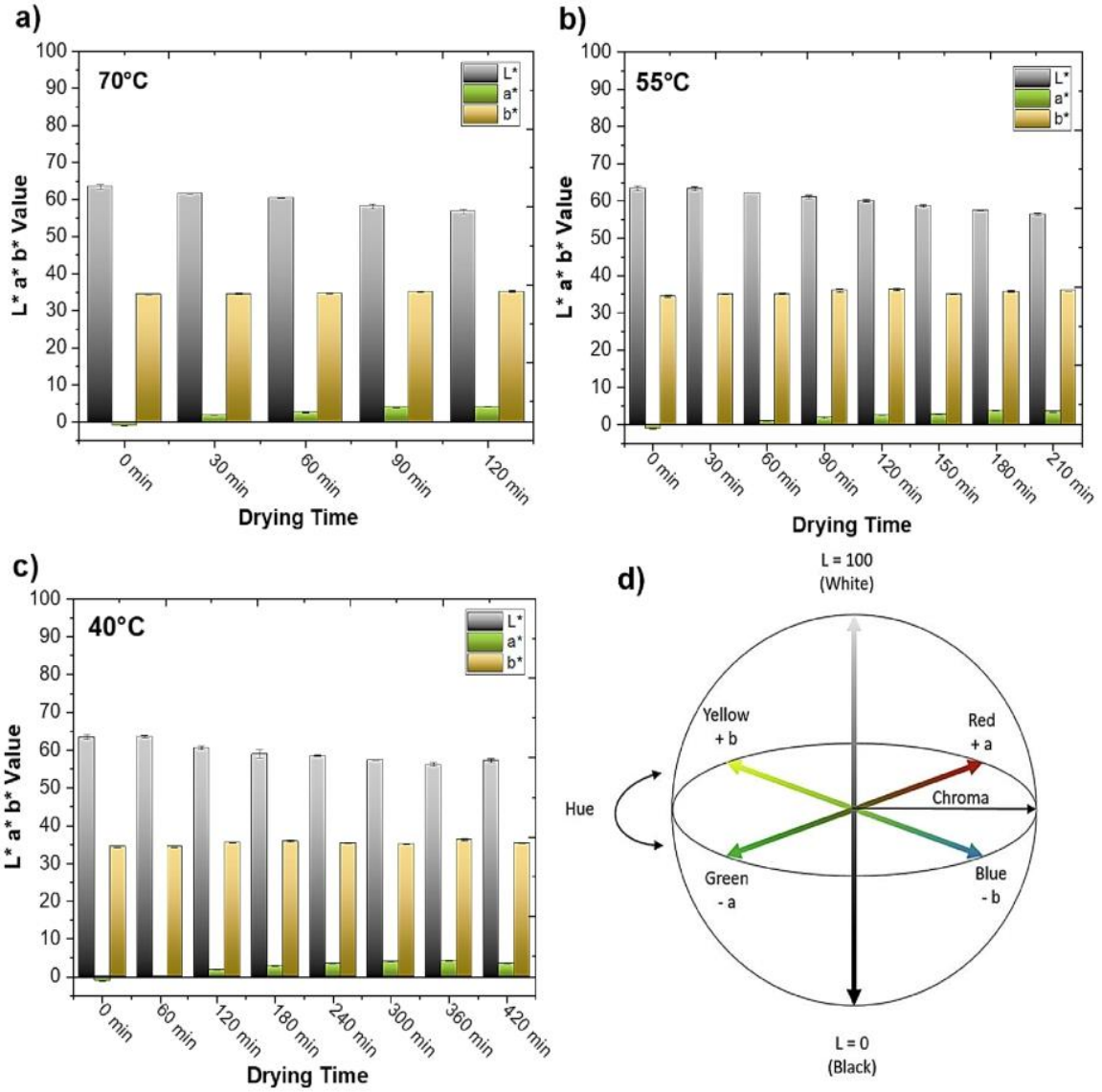


Fig. 3. Hop color measurement during drying process at: (a) 70 °C; (b) 55 °C; (c) 40 °C; (d) CIE L* a* b* color space diagram (Ly et al., 2020) *For detailed statistical information, see Table S4 in the Supplementary Information.

process ranging from green in the fresh cones to green–brown in the dried cones. The CIELAB color space diagram shown in Fig. 3d represents the quantitative relationship between the colors on three axes: L* value indicates lightness, a* and b* are chromaticity coordinates (Ly et al., 2020). By ANOVA (95 % CI), a* value increases significantly (p-value < 0.05) which evidences the green color degradation during drying (Table S4, Supplementary Material). The fresh cone's color contains a* negative value of −1.05, demonstrating the highest green color intensity among the samples. This behavior can be seen in Figure S1 (Supplementary Material) which shows the color of each sample. The L* value significantly (ANOVA, 95 % CI; p-value < 0.05) decreased during drying, indicating the reduction of hop lightness. The overall color difference (ΔE) is a combination of L*, a*, and b* values and reveals the variation of color during processing (Nozad et al., 2016). By ANOVA (95 % CI), the results of ΔE at 55 °C and 40 °C drying (7.34 ± 0.83 and 1.66 ± 0.89) were statistically significantly similar (p-value > 0.05) whereas the values differ significantly from the samples dried at 70 °C. These findings demonstrate that the elevated temperature increased by 70 % of the ΔE with a higher impact on the color change.

This outcome suggests that temperature influenced directly the product quality regarding appearance. The color change in food drying is an unavoidable effect due to the high-temperature exposure of the product. Figure S1 (Supplementary Material) demonstrates the color change of the hop samples during drying. As observed in the analytical results, it can be seen that the green color is the one most affected by the process. Hop is rich in chlorophyll which is degraded to gray-brown compounds, such as pheophytin. Chlorophyll degradation to pheophytin is induced by heat in presence of H^+ (Bonazzi & Dumoulin, 2011). The magnesium atom present in the chlorophyll structure is replaced by two hydrogens, producing pheophytin (Arslan & Özcan, 2012). Previous studies documented the importance of the drying process in the hop quality concerning color (Raut et al., 2020b, 2020a; Rybka et al., 2018; Ziegler et al., 2021). The hop color is an important quality parameter to hop product acceptance, such as pellets due to the remaining amount of vegetal matter. However, it might not greatly affect the hop extract quality (Raut et al., 2020a; Sturm et al., 2020). The supercritical CO_2 extraction removes the vegetal matter, producing an extract comprised of soft resin (Biendl et al., 2014). Therefore, the color modification demonstrated during the drying step does not directly affect the quality of the hop extract which can be produced from hops dried at higher temperatures.

3.2.2. Bitter acids and xanthohumol

Fig. 4 presents the total α-, β-acid, and xanthohumol content in g/

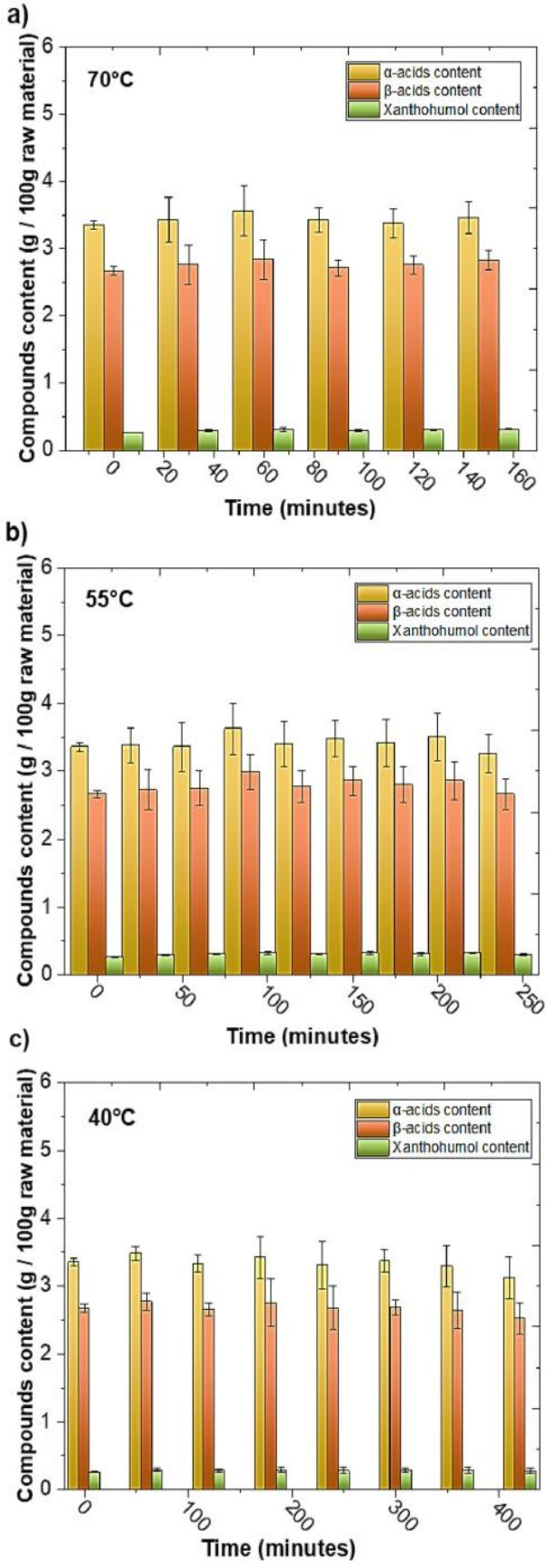


Fig. 4. Hop bitter acids and xanthohumol measured over the drying step at: **(a)** 70 °C; **(b)** 55 °C; **(c)** 40 °C. **Results presented on a dry mass basis (d.b.).*

100 g of hops during drying at 70 °C, 55 °C, and 40 °C. The sampling interval for the 40 °C assay was at 1 h instead of 30 min due to the prolonged process, and the amount of product available for the trials was insufficient to afford higher sampling numbers. Interestingly, this study found no statistical difference (ANOVA, 95 % CI; p-value > 0.05) in the α-, β-acids, and xanthohumol content each time at 40, 55, and 70 °C throughout drying. Furthermore, the data showed no statistical difference due to drying temperature (p-value > 0.05), so the null hypothesis (there was no difference between the averages) was accepted. This finding is in concordance with Sturm et al. (2020) findings, which showed the thermostability of α- and β-acids during the drying regardless of the bulk weight. This behavior is in accordance with this study's outcomes that demonstrate the slight drying temperature influence on hop quality concerning α-acids content. This compound is highly valued in the beer industry, providing bitterness (Almaguer et al., 2014). It is well-established in the literature that loss of bitter acids during hops processing and storage is due to oxidation (Almaguer et al., 2014; Doe & Menary, 1979; Mikyška & Krofta, 2012; Skinner et al., 1977; Srečec et al., 2009). However, the α- and β-acids demonstrate thermostability despite the homologs due to protection against oxidation by the lupulin gland (Table S2, Supplementary Material). The bitter acid content (Fig. 4) endures, regardless of temperature increase. This behavior might not be affected by other drying parameters, such as air velocity or air humidity, which are directly related to drying time (Kerr, 2013).

Lupulin gland structure in fresh and dried hops is shown in Fig. 5a whereas Fig. 5b demonstrates different states in which lupulin could be found during the process. The lupulin gland sheath comprises the secretory cell cuticle and a layer of the primary cell wall which is noticed in the ruptured gland (Fig. 5b) (Oliveira et al., 1988). According to Oliveira et al. (1988), the cuticle is composed of lipids and wax. Lupulin gland morphology constitutes secretory cells, where the secretions accumulate in the space between the cuticle and plasma membrane (Menary & Doe, 1983; Oliveira et al., 1988). In this case, the gland structure persists without ruptures during the drying step, although it shrinks due to dehydration. This behavior can be seen more prominently in the drying at 40 °C, in which the dried gland volume is lower than fresh hop glands (Fig. 5a). Srečec et al. (2009) studying the morphology of the lupulin gland in the hop processing demonstrated that the pelletizing step plays the most important role in damaging the lupulin. The exposure of the bitter acids and polyphenols due to lupulin rupture leads to its oxidation in further processing steps. However, as presented in this study, the compounds are preserved in the drying step by the protection of the lupulin gland. This implies that hops destined to produce bitter-acid enriched extracts could be dried at higher temperatures to reduce processing time without affecting hop bitter acid content.

3.2.3. *Essential oil*

The total essential oil content was measured in fresh and dried hops at each temperature. The results presented in Fig. 6a demonstrate the reduction of the total oil content due to drying. The essential oil content decreases with the increasing temperature, indicating an influence of the drying temperature on this compound. The drying process drastically reduces the amount of essential oil in the hop, decreasing by 51,77 % when dried at 40 °C. Despite the extended time, the lowest temperature maintained the highest total essential oil content. The sample dried at 70 °C contains the lowest content with 0.55 mL/100 g of hops. However, the essential oil profile was not affected by the drying temperature regarding the main essential oil compounds (Table S3, Supplementary Material). The relative percentage of compounds had a low effect, demonstrating that the essential oil is equally lost in all compounds. Rybka et al. (2018) demonstrated that drying hop at 40 °C preserves the total essential oil content compared with hops dried at 55 °C. Hop essential oil is widely used to improve beer's aroma with high usage through dry-hopping techniques (Gomes et al., 2022). However, the essential oil content has only minor relevance in hop bitter acid extract production since the extraction process conditions are specific for bitter

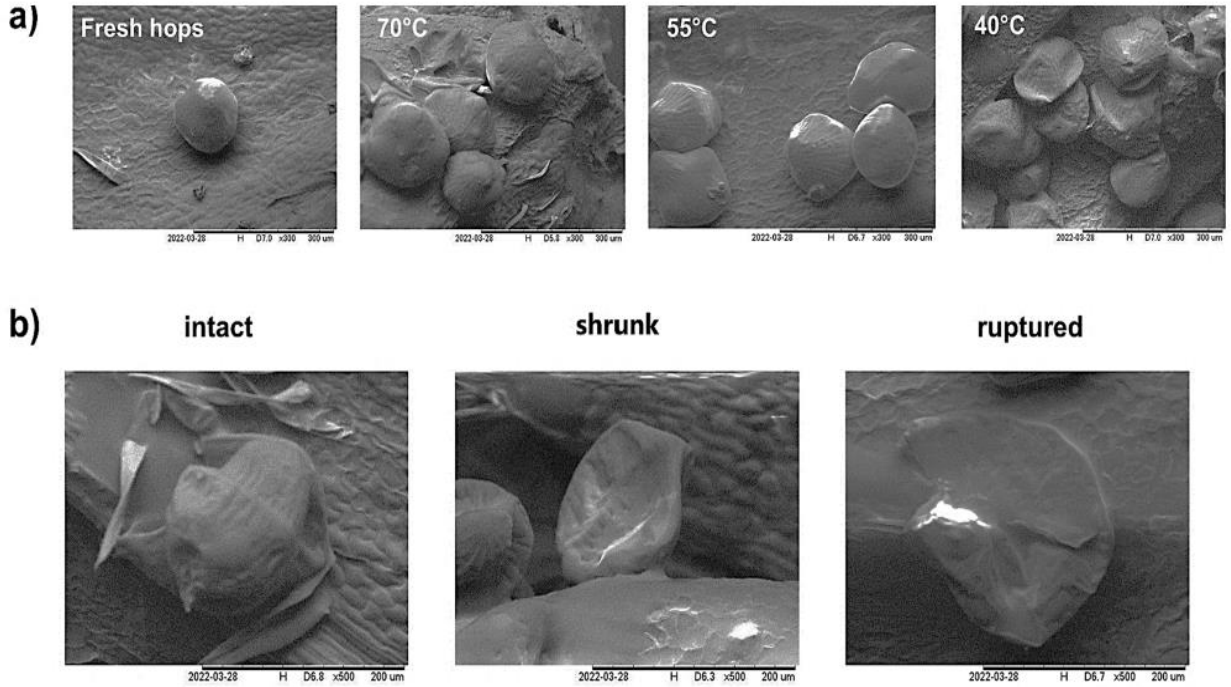


Fig. 5. SEM imaging of lupulin gland: **(a)** image at x300 of fresh and dried hop; **(b)** Illustration of different lupulin gland states.

compounds (van Opstaele et al., 2012).

Hop essential oil comprises a complex mixture of hundreds of volatile compounds. In this study, PCA was used to discriminate the major essential oil compounds in the fresh and dried hops to help to understand the influence of drying temperature on those compounds (Almaguer et al., 2014). The number of principal components (PC) was selected according to the eigenvalues and accumulated variance to describe the results more accurately (Table S1, Supplementary Material). Fig. 6b shows the biplot with the first two PCs, which capture 85.21 % of the accumulated variance (Table S1 Supplementary Material). Notably, the treatments demonstrate a clear separation in the clusters. This behavior indicates the prominent impact of drying temperature on the hop aroma profile, especially the treatment at 55 °C and the fresh hops which were widely distributed.

Interestingly, the close position between the samples dried at 70 °C and 40 °C reveals their similarities in aroma profiles. The PC1 had as the main contributors the compounds alpha-humulene, *trans*-caryophyllene, and myrcene (Figure S2, Supplementary Material). They are responsible for the greatest differentiation between the aroma profile of hop dried at 55 °C and the other treatments. Linalool, beta-pipene, and citronellol represent the higher contribution to the PC2, which separated the samples dried at 70 °C and 40 °C from the fresh hops. A positive correlation was verified between linalool and citronellol and the samples dried at 70 °C and 40 °C by their close location in the plot. Additionally, the fresh hops were positively correlated with the myrcene and beta-pipene.

Myrcene is a monoterpene and is one of the most important hop compounds with notes of herbal and green aroma, signifying the freshness of hops due to their high volatility (Durello et al., 2019; Rettberg et al., 2018). From the results presented in Fig. 6b, the high correlation of this compound with the fresh hop samples and the samples dried at 70 °C and 40 °C can be seen. This indicates that the profile aroma of hops dried either at lower or higher temperatures remains nearer to the fresh aroma, despite the higher temperatures being claimed to damage the aroma profile of hops (Biendl et al., 2014). Similarly, linalool and citronellol are important indicators of hop quality regarding aroma and hop aroma in beer due to their polarity and higher solubility. In this study, the results demonstrate that the increase in the drying temperature from 40 °C to 70 °C had a lower impact on those compounds and the hop quality.

3.3. Effect of temperature and pressure on supercritical CO_2 extraction

Before SFE, the hops were dried at 70 °C for 2 h and 30 min. This condition was selected according to the results of the previous study, which demonstrated that higher temperatures do not greatly impact the hop quality to produce extracts. Those findings are in accordance with the Rubottom et al. (2022) studies which demonstrated that drying temperature had a low impact on the main hop quality parameters. Therefore, the increase in temperature might lead to a reduction in energy consumption due to the considerably reduced process time without greatly affecting the hop quality, especially the hop used to produce extracts rich in bitter acids. Furthermore, shortening the drying step by raising the temperature would benefit earlier stages of hop processing, leading to daily productivity increases in the farms which decrease the losses by over-maturation during harvest as well as reducing energy consumption and greenhouse gas emissions.

Fig. 7 shows the extraction yield from Brazilian hops for two isotherms (40C and 60C) at four pressures (15, 20, 25, and 30 MPa). The maximum yield (8.6 ± 0.3 %) was achieved at 60 °C and 30 MPa (supercritical CO_2 density of 830.33 kg/m^3). Whereas the lowest yield (7.0 ± 0.1 %) was acquired due to the low density of supercritical CO_2 (605.60 kg/m^3) under 60 °C and 15 MP. According to ANOVA (95 % CI), pressure (p-value < 0.001) and the interaction between pressure and temperature (p-value < 0.008) significantly influenced the extraction yield in the studied range.

Elevated pressure at a constant temperature increased the extraction yield. Higher pressures increased the solvation power of CO_2, facilitating the dissolution of the easily accessible extract at the surface of the particles in the fluid phase. This phenomenon also increases CO_2 density, ranging from 781.3 to 910.5 kg/m3 for 40 °C and from 605.6 to 830.3 kg/m3 for 60 °C (Table 1) (De França et al., 1999). Fig. 7 also indicates the crossover effect at a pressure of around 18 MPa which is defined as the point where the vapor pressure of the extract compounds is more pronounced than the effect of the solvent density over the compound's solubility (Brunner, 1994). This means that the effect of

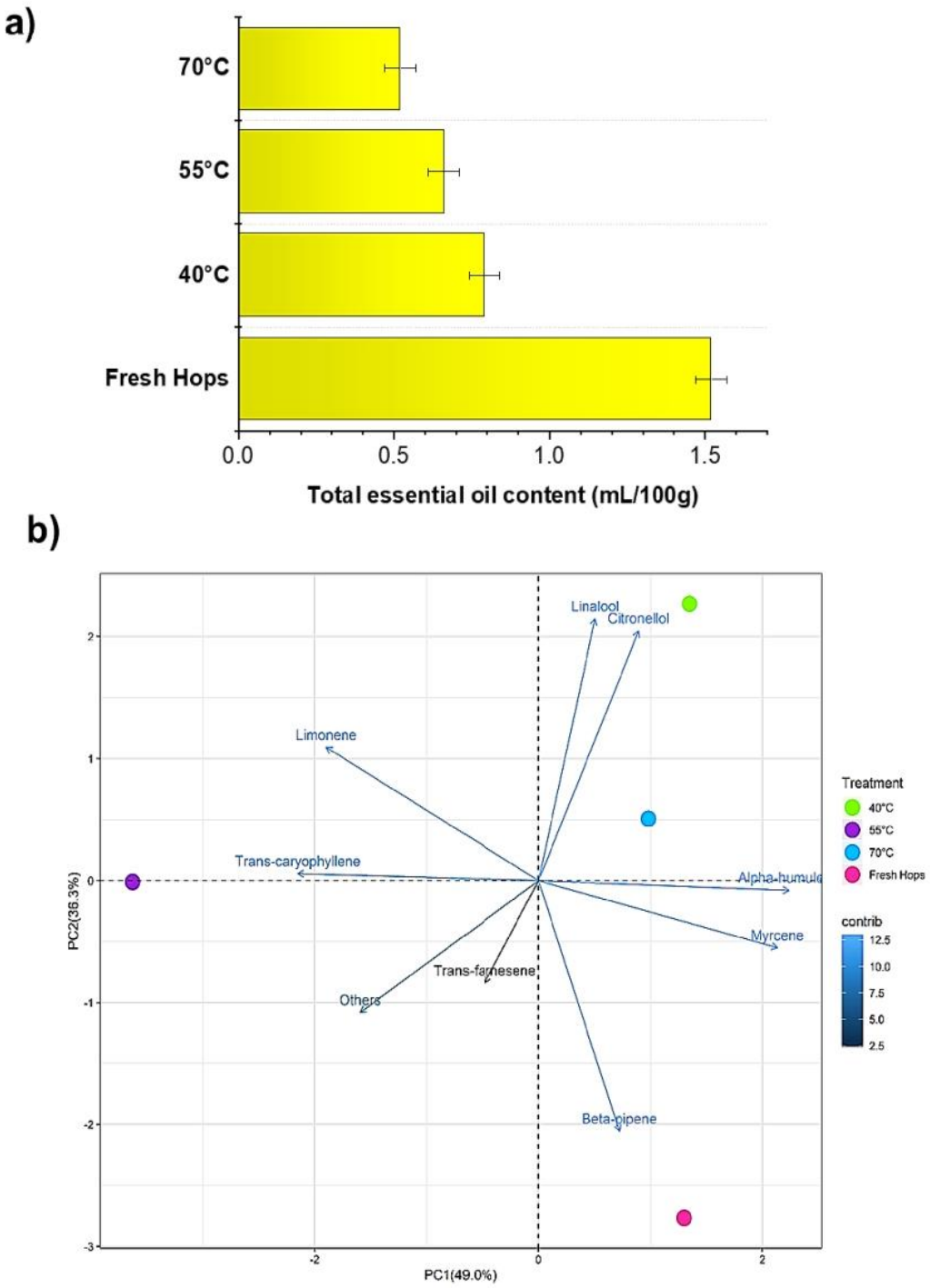


Fig. 6. Hop essential oil evaluation in fresh and dried hops: **(a)** Total oil content; **(b)** Principal Analysis Components of the major essential oil compounds.

CO_2 density is more relevant at pressures below the crossover pressure. On the other hand, the effect of the vapor pressure at determined temperatures is more relevant for increasing the extraction yield when the pressure is above the crossover pressure. This behavior is commonly observed for extracts obtained from vegetable matrices like hops (Mezzomo et al., 2010; Náthia-Neves et al., 2020; Salinas et al., 2020).

Table 1 provides the results of α-acids and β-acids in the extracts, the α-/β-acids purity (g α-/β-acids per g of extract), and the yield of these compounds (expressed as g α-/β-acids per g of raw material). ANOVA (95 % CI) demonstrated that pressure and temperature do not influence the purity and yield of α- and β-acids in the studied range. The α-acid content in the extracts ranged from 46 ± 1 to 52 ± 3 g/100 g of extract, and β-acid content in the extracts ranged from 34 ± 4 to 48 ± 1 g/100 g of extract. The purity for each compound obtained at 40 °C under different pressures studied was not significantly different from each other. Likewise, no difference was observed between these values and those found for 60 °C at pressures of 15, 20, and 25 MPa. The lowest purity, regarding α-acids, was observed under 60 °C and 30 MPa (46 ± 1 g/100 g of extract), while the lowest purity in β-acids was observed under 60 °C and 25 MPa (34 ± 4 g/100 g of extract).

Recently, Veiga et al. (2021) investigated extraction using supercritical CO_2 in hops grown in Brazil. This study used similar conditions, with a higher extraction time (110 min). The authors obtained extraction yields of 6.2 % (20 MPa and 60 °C), 5.6 % (15 MPa and 40 °C), and 6.0 % (25 MPa and 40 °C). Considering conditions and extraction times (40 min) close to this study, Zeković et al. (2007) observed that the

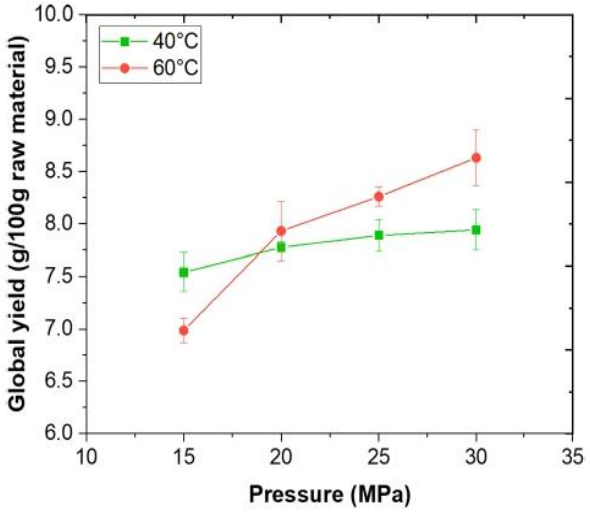


Fig. 7. Isotherms from extraction yield of hops in supercritical carbon dioxide.

extraction yield of the 'Magnum' hop variety at 15 MPa, 40 °C, and 45 min was 6.6 %. A reduced yield (1.5 %) was observed by Formato et al. (2013) using supercritical CO_2 at 40 °C, 35 MPa, and 50 min. The results of those previous studies differ from this study regarding the hops' variety employed in the supercritical CO_2 extraction, which varies widely in composition (Barth & Meier, 2021). It influences the process and consequently the extract constitution (Kupski et al., 2017).

The α-acid yield from the raw material ranged from 3.6 ± 0.3 to 4.1 ± 0.2 g/100 g of raw material, and the β-acids content ranged from 2.8 ± 0.3 to 3.34 ± 0.01 g/100 g of raw material. The α- and β-acid yields were not significantly different. According to the literature (Biendl et al., 2014), the content of α-acids and β-acids in hop cultivars normally range from 4 to 5 % and 2–5 %, respectively. Some varieties present high levels of α- and β-acids (>15 %) and are especially suited to produce hop extracts (Formato et al., 2013). The results obtained in this study show that the Brazilian hop cultivar could be considered an appropriate source for obtaining α-/β-acid-rich extracts, despite the Mantiqueira variety presenting a low α-acid content. The α-acids are mainly responsible for the bitterness of beer, and β-acids act as an antimicrobial agent that assists in preserving beer (Almaguer et al., 2014). β-acids are an essential component in beer production and play an important biological role (Arruda, Pinheiro, Silva, & Bernardes, 2022). This study's findings reveal the possibility of producing an extract from a Brazilian hop variety, potentially benefiting the hop and brewing industry in the country. Furthermore, it explains how supercritical CO_2 extraction parameters influence hop bitter acid obtention, thus enhancing the process.

3.4. Extraction kinetics

Based on previous results, the kinetic behavior was evaluated at 40 °C and 20 MPa. As formerly discussed, the extraction performed at 40 °C and 20 MPa using an S/F of 20 allowed an extraction yield of 7.78 ± 0.05 %, α-acids yield of 4.0 ± 0.2 g/100 g of Brazilian hop, and β-acids yield of 2.9 ± 0.3 g/100 g of Brazilian hop. Furthermore, lower temperature and pressure facilitate equipment management and control as well as demand reduce energy consumption. Fig. 8 presents the extraction yield and the α-/β-acids yield kinetics as a function of extraction time. The extraction curves showed the three distinct regions usually found in the supercritical CO_2 extraction of natural products: constant extraction rate, falling extraction rate, and diffusion-controlled (Kupski et al., 2017).

In the constant extraction rate period (straight line), the mechanism of mass transfer is mainly controlled by convection. This phase occurs in the extraction of the solutes more accessible to the solvent around the superficial layer of the particles. The falling extraction rate period (asymptotic curve) is characterized by the transition period between convection and diffusional extraction. In this period, the solutes on the surface decrease, and the solutes inside the particle diffuse toward the surface. Finally, in the diffusion-controlled period, the mass transfer mechanism is controlled by the diffusion of the solutes inside the particle, decreasing the extraction rates.

From the results obtained, the higher extract and α-/β-acid yields were obtained in the constant extraction rate (15 min) and falling extraction rate (40 min) periods. The curves presented a diffusion-controlled period after 40 min, indicating that maximum extraction from Brazilian hops was achieved. This resulted in a 10.7 % of extraction yield at 90 min with a solvent-to-feed ratio of 44.5. The extraction at the end of the constant extraction rate reached 72 % (extraction yield of 7.70 ± 0.1 %) in comparison with the total yield obtained at 90 min, and the extract comprised 3.5 ± 0.2 g/100 g of raw material of α-acids and 2.9 ± 0.2 g/100 g of raw material of β-acids. Posterior to the falling extraction rate period, the extraction yield represented 90 % of the total yield obtained at 90 min. The extract contained 4.1 ± 0.2 g/100 g of raw material and 3.2 ± 0.2 g/100 g of raw material of α-acids and β-acids, respectively. Thus, after 40 min of process 93 % and 97 % of the total α- and β-acids were extracted compared with the total yield at 90 min.

Valle et al. (2003) reached an extraction yield of approximately 14 %, using a Chilean hop variety at 40 °C, 20 MPa for 240 min. The study yielded 8 g/100 g of raw material and 3.5 g/100 g of raw material for α-acids and β-acids, respectively. Furthermore, Zeković et al. (2007) obtained a yield of 7.54 % using the hop variety 'Magnum' after 150 min of kinetic extraction at 30 MPa and 40 °C. Kupski et al. (2017) extracted 6.0 % of hop extract at 20 MPa, 35 °C, and 170 min. However, a lower yield (<3 %) was obtained at 35 MPa and 50 °C at 250 min of extraction (Formato et al., 2013). The extracts obtained by these authors

Table 1
Results of α-/β-acid content and α-/β-acid yield from extracts obtained from Brazilian hops through supercritical CO_2 extraction at S/F = 20, under different pressures at 40C and 60C.

			α -acids		β -acids	
Temperature (C)	Pressure (MPa)	CO_2 density* (kg/m3)	Purity (g/100 g extract)	Yield (g/100 g raw material)	Purity (g/100 g extract)	Yield (g/100 g raw material)
40.0 ± 0.1	15 ± 1	781.32	50.0 ± 2.0	3.8 ± 0.2^{b}	39.9 ± 0.4	3.0 ± 0.1
	20 ± 1	840.61	52.0 ± 2.0	4.0 ± 0.2^{a}	37.0 ± 5.0	2.9 ± 0.3
	25 ± 1	880.15	50.0 ± 1.0	4.1 ± 0.2 ^{a}	39.4 ± 0.1	3.1 ± 0.1
	30 ± 1	910.47	51.0 ± 1.0	4.1 ± 0.2 ^{a}	38.6 ± 0.5	3.1 ± 0.0
60.0 ± 0.1	15 ± 1	605.60	52.0 ± 3.0^{a}	3.6 ± 0.3^{b}	48.0 ± 1.0^{a}	3.3 ± 0.0
	20 ± 1	724.63	49.3 ± 0.1^{ab}	3.9 ± 0.1 ^{ab}	39.0 ± 1.0^{b}	3.1 ± 0.2
	25 ± 1	787.28	49.0 ± 1.0^{ab}	4.1 ± 0.1 ^{a}	34.0 ± 4^{b}	2.8 ± 0.3
	30 ± 1	830.33	46.0 ± 1.0^{b}	4.0 ± 0.0 ^{a}	35.0 ± 1.0^{b}	3.0 ± 0.0

Results are presented as mean ± standard deviation (n = 3); and different superscript letters in the same column indicate significant differences at $p \leq 0.05$ (the absence of superscript letters means that the results did not show any statistical difference).

* CO_2 density data extracted from Nist (https://webbook.nist.gov/chemistry/fluid/).

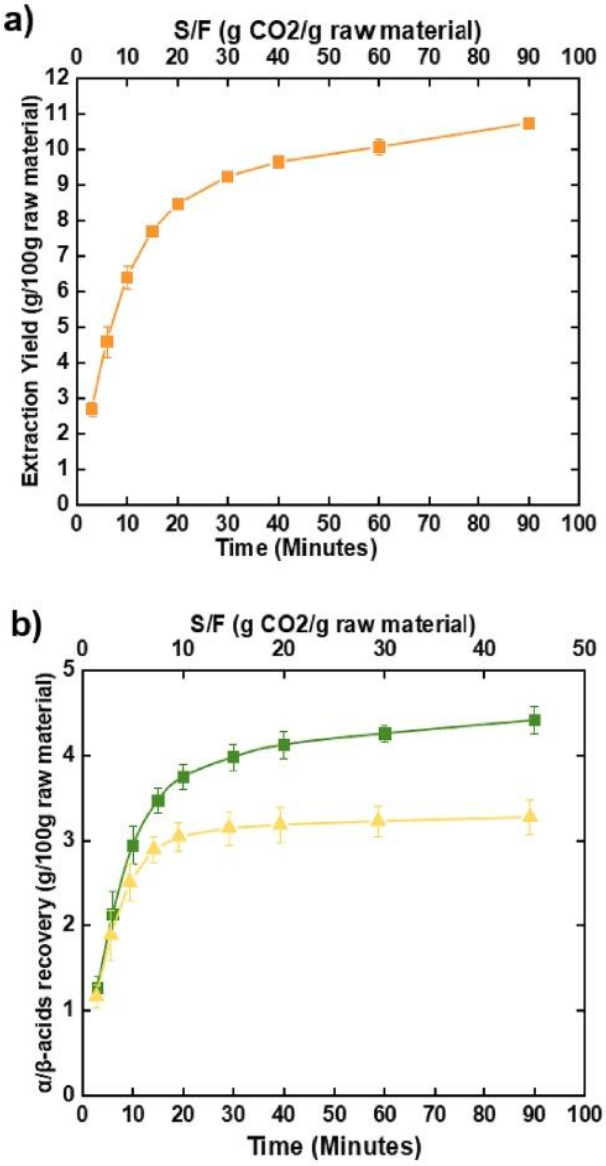


Fig. 8. Overall extraction curves for extractions performed at 40 °C and 20 MPa: **(a)** Extraction yield; **(b)** α-/β-acids recovery yield (green ■ = α-acids) (yellow▲ = β-acids) **Results presented on a dry basis (d.b.)*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

represented 25.6 ± 0.3 % of α-acids.

This study's findings indicate that there would be no reason to extend extraction times up to the end (90 min), based on the drastic increase in solvent cost and process time without significantly increasing extraction and target compound yields. Therefore, it is suggested that the extraction for Brazilian hops could be stopped at 40 min of extraction (S/F = 19.6), as starting a new batch would be more advantageous than continuing with the same extraction. These results are consistent with those reported in the literature, which is preferable to extract during the constant extraction rate period rather than extending over the falling extraction rate period (Culík et al., 2009). Furthermore, in comparison with previous research, this study reached high extraction yields of hop bitter acids in a shorter extraction time. Shortening the extraction process might lead to an economical and sustainable process by reducing unnecessary solvent as well as energy consumption.

Nevertheless, this study used only the Mantiqueira variety, for this reason, a further study utilizing other varieties is required to establish those extraction conditions due to the influence of the hop composition in the process (Formato et al., 2013).

4. Conclusion

This study presents an in-depth understanding of the influence of drying on hop quality as well as supercritical fluid extraction parameters optimization to reach high bitter acids yields. A significant finding to emerge from this study was the maintenance of hop bitter acids during drying despite temperature increases. This implies that higher air-drying temperatures might be a key to shortening this step, reducing loss in hop farms due to hop cone over-maturation and energy saving. Furthermore, rich bitter-acid extracts were obtained through supercritical CO_2 extraction at 40 °C and 20 MPa with a reduced time of 40 min. This optimization of hop processing demonstrates the suitability of producing hop extract from a Brazilian variety by a more sustainable process.

Funding

Mariana Barreto Carvalhal Pinto and Flavio Luis Schmidt appreciate the support of the CNPq – Research Nacional Council - Brazil (Finance code 141485/2018–3). Grazielle Náthia-Neves thanks CAPES - Coordination of Superior Level Staff Improvement - Brazil (Finance Code 001) for Ph.D. assistance. Renata Vardanega thanks European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement N° 101,062,938 for a postdoctoral fellowship and Maria Angela Almeida Meireles thanks CNPq for a productivity grant (309825/2020–2). Pedro Renann Lopes de França acknowledges 2021/06606–4 scholarship funding from FAPESP.

Data availability

The data is included within the article and in the Supporting Information files.

CRediT authorship contribution statement

Mariana Barreto Carvalhal Pinto: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Renata Vardanega:** Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Grazielle Náthia-Neves:** Data curation, Writing – original draft, Writing – review & editing. **Pedro Renann Lopes de França:** Writing – review & editing. **Louise Emy Kurozawa:** Writing – review & editing. **Maria Angela Almeida Meireles:** Conceptualization, Methodology, Writing – review & editing, Project administration, Funding acquisition. **Flavio Luis Schmidt:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

The authors wish to thank the founders Cnpq, CAPES, and FAPESP for the grant provided to develop this study. Also, the authors are grateful to the Van den Bergen company for the donated material.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.foodres.2023.113169.

References

Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). Humulus lupulus - a story that begs to be told. A review. *Journal of the Institute of Brewing, 120* (4), 289–314. https://doi.org/10.1002/jib.160

ASBC Methods of Analysis, online. Hops Method 14. α-Acids and β-Acids in Hops and Hop Extracts by HPLC (International Method). Approved 1990, rev. 2008. American Society of Brewing Chemists, St. Paul, MN, U.S.A. doi: 10.1094/ASBCMethod-Hops14.

ASBC Methods of Analysis, online. Hops Method 4. Moisture. Approved 1958, rev. 2008. American Society of Brewing Chemists, St. Paul, MN, U.S.A. doi: 10.1094/ASBCMethod-Hops4.

ASBC Methods of Analysis, online. Hops Method 13. Total Essential Oils in Hops and Hop Pellets by Steam Distillation. Approved 1988, rev. 2008. American Society of Brewing Chemists, St. Paul, MN, U.S.A. doi: 10.1094/ASBCMethod-Hops13.

ASBC Methods of Analysis, online. Hops Method 17. Hop Essential Oils by Capillary Gas Chromatography-Flame Ionization Detection. Approved 2004. American Society of Brewing Chemists, St. Paul, MN, U.S.A. doi: 10.1094/ASBCMethod-Hops17.

Arruda, T. R., Pinheiro, P. F., Silva, P. I., & Bernardes, P. C. (2021). A new perspective of a well-recognized raw material: Phenolic content, antioxidant and antimicrobial activities and α- and β-acids profile of Brazilian hop (Humulus lupulus L.) extracts. *Lwt, 141*. https://doi.org/10.1016/j.lwt.2021.110905

Arruda, T. R., Pinheiro, P. F., Silva, P. I, & Bernardes, P. C. (2022). Exclusive Raw Material for Beer Production? Addressing Greener Extraction Techniques, the Relevance, and Prospects of Hops (Humulus lupulus L.) for the Food Industry. *Food and Bioprocess Technology, 15*, 275–305. https://doi.org/10.1007/s11947-021-02716-w

Arslan, D., & Özcan, M. M. (2012). Evaluation of Drying Methods with Respect to Drying Kinetics, Mineral Content, and Color Characteristics of Savory Leaves. *Food and Bioprocess Technology, 5*(3), 983–991. https://doi.org/10.1007/s11947-010-0498-y

Bauerle, W. L. (2019). Disentangling photoperiod from hop vernalization and dormancy for global production and speed breeding. *Nature, 9*(1), 1–8. https://doi.org/10.1038/s41598-019-52548-0

Berk, Z. (2018). *Food Process Engineering and Technology*. Elsevier (Chapter 22). https://doi.org/10.1016/C2011-0-05296-1.

Biendl, M., Engelhard, B., Forster, A., Gahr, A., Lutz, A., Mitter, W., … Schönberger, C. (2014). *Hops: Their Cultivation, Composition, an Usage*. Fachverlag Hans Carl GmbH.

Bonazzi, C., & Dumoulin, E. (2011). *Modern Drying Technology*. Wiley-VCH.

Brunner, G. (1994). *Gas Extraction - An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes*. Spring-Verlag Berlin Heidelberg.

Čulík, J., Jurková, M., Horák, T., Čejka, P., Kellner, V., Dvořák, J., … Brew, J. I. (2009). Extraction of bitter acids from hops and hop products using Pressurized Solvent Extraction (PSE). *Journal of the Institute of Brewing, 115*(3), 220–225. https://doi.org/10.1002/j.2050-0416.2009.tb00372.x

De França, L. F., Reber, G., Meireles, M. A. A., Machado, N. T., & Brunner, G. (1999). Supercritical extraction of carotenoids and lipids from buriti (Mauritia flexuosa), a fruit from the Amazon region. *Journal of Supercritical Fluids, 14*(3), 247–256. https://doi.org/10.1016/s0896-8446(98)00122-3

Doe, P. E., & Menary, R. C. (1979). Optimization of the hop drying process with respect to alpha acid content. *Journal of Agricultural Engineering Research, 24*(3), 233–248. https://doi.org/10.1016/0021-8634(79)90066-0

Durello, R. S., Silva, L. M., & Jr, S. B. (2019). Química do Lúpulo. *Química Nova*, 1–20. https://doi.org/10.21577/0100-4042.20170412.

Formato, A., Gallo, M., Ianniello, D., Montesano, D., & Naviglio, D. (2013). Supercritical fluid extraction of α- and β-acids from hops compared to cyclically pressurized solid-liquid extraction. *Journal of Supercritical Fluids, 84*, 113–120. https://doi.org/10.1016/j.supflu.2013.09.021

Gomes, F. D. O., Guimarães, B. P., Ceola, D., & Ghesti, G. F. (2022). Advances in dry hopping for industrial brewing: A review. *Food Science and Technology, 42*. https://doi.org/10.1590/fst.60620

Hauser, D. G., & Shellhammer, T. H. (2019). An Overview of Sustainability Challenges in Beer Production, and the Carbon Footprint of Hops Production. *Technical Quarterly*. https://doi.org/10.1094/tq-56-4-0731-01

Jastrombek, J. M., Faguerazzi, M. M., de Cássio Pierezan, H., Rufato, L., Sato, A. J., da Silva Ricce, W., … Roberto, S. R. (2022). Hop: An Emerging Crop in Subtropical Areas in Brazil. *Horticulturae, 8*. https://doi.org/10.3390/horticulturae8050393

Kerr, W. L. (2013). *Handbook of Farm, Dairy and Food Machinery Engineering* (2nd ed.). Elsevier Inc (Chapter 2). https://doi.org/10.1016/B978-0-12-385881-8.00012-4.

Keukeleire, J. D., Ooms, G., Heyerick, A., Roldan-Ruiz, I., Bockstaele, E. V., & De Keukeleire, D. (2003). Formation and Accumulation of alpha -acids, beta-cids, Desmethylxanthohumol, and Xanthohumol during Flowering of Hops (Humulus lupulus L.). *Journal of Agricultural and Food Chemistry, 51*, 4436–4441. https://doi.org/10.1021/jf034263z

Kirin Company. (2019). Kirin Beer University Report Global Beer Production by Country in 2018. Retrieved from https://www.kirinholdings.com/en/newsroom/release/2019/1003_01.html: Accessed November 23, 2022.

Kupski, S. C., Klein, E. J., da Silva, E. A., Palú, F., Guirardello, R., & Vieira, M. G. A. (2017). Mathematical modeling of supercritical CO2 extraction of hops (Humulus lupulus L.). *The Journal of Supercritical Fluids, 130*, 347–356. https://doi.org/10.1016/j.supflu.2017.06.011

Ly, B. C. K., Dyer, E. B., Feig, J. L., Chien, A. L., & del Bino, S. (2020). Research Techniques Made Simple: Cutaneous Colorimetry: A Reliable Technique for Objective Skin Color Measurement. *In Journal of Investigative Dermatology, 140*(1), 3–12. https://doi.org/10.1016/j.jid.2019.11.003

Márquez, C. A., De Michelis, A., & Giner, S. A. (2006). Drying kinetics of rose hip fruits (Rosa eglanteria L.). *Journal of Food Engineering, 77*(3), 566–574. https://doi.org/10.1016/j.jfoodeng.2005.06.071

Menary, R. C., & Doe, P. E. (1983). Some Morphological and Chemical Changes in Hops During Maturation. *Journal of the Science of Food and Agriculture, 34*, 921–929. https://doi.org/10.1002/jsfa.2740340905

Mezzomo, N., Mileo, B. R., Friedrich, M. T., Martínez, J., & Ferreira, S. R. S. (2010). Supercritical fluid extraction of peach (Prunus persica) almond oil: Process yield and extract composition. *Bioresource Technology, 101*(14), 5622–5632. https://doi.org/10.1016/j.biortech.2010.02.020

Meyer, F., Jaeger, P., Eggers, R., Stamenic, M., Milovanovic, S., & Zizovic, I. (2012). Effect of CO_2 pre-treatment on $scCO_2$ extraction of natural material. *Chemical Engineering and Processing: Process Intensification, 56*, 37–45. https://doi.org/10.1016/j.cep.2012.02.003

Mikyška, A., & Krofta, K. (2012). Assessment of changes in hop resins and polyphenols during long-term storage. *Journal of the Institute of Brewing, 118*(3), 269–279. https://doi.org/10.1002/jib.40

Náthia-Neves, G., Vardanega, R., Hatami, T., & Meireles, M. A. A. (2020). Process integration for recovering high added-value products from Genipa americana L.: Process optimization and economic evaluation. *Journal of Supercritical Fluids, 164*, 1–10. https://doi.org/10.1016/j.supflu.2020.104897

Nozad, M., Khojastehpour, M., Tabasizadeh, M., Azizi, M., Miraei Ashtiani, S. H., & Salarikia, A. (2016). Characterization of hot-air drying and infrared drying of spearmint (Mentha spicata L.) leaves. *Journal of Food Measurement and Characterization, 10*(3), 466–473. https://doi.org/10.1007/s11694-016-9325-0

Oliveira, M. M., Salomk, M., & Pais, S. (1988). Glandular trichomes of Humulus lupulus var. Brewer's Gold: Ontogeny and histochemical characterization of the secretion. *Nordic Journal of Botany, 8*(4), 349–359. https://doi.org/10.1111/j.1756-1051.1988.tb00510.x

Pontes Guimarães, B., & Ferreira Guesti, G. (2020, August). *Physicochemical characterization of hops grown in Brazilian savannah*. Poster session presentation at the meeting of the World Brewing Congress, Minneapolis, Minnesota.

Raut, S., von Gersdorff, G. J. E., Münsterer, J., Kammhuber, K., Hensel, O., & Sturm, B. (2020a). Impact of Process Parameters and Bulk Properties on Quality of Dried Hops. *Processes, 8*(11), 1507. https://doi.org/10.3390/pr8111507

Raut, S., Gersdorff, J. E. Von, Münsterer, J., Kammhuber, K., Hensel, O., & Sturm, B. (2020b). Influence of pre-drying storage time on essential oil components in dried hops (Humulus lupulus L.). *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.10844.

Rettberg, N., Biendl, M., & Garbe, L. A. (2018). Hop aroma and hoppy beer flavor: Chemical backgrounds and analytical tools—A review. *Journal of the American Society of Brewing Chemists, 76*, 1–20. https://doi.org/10.1080/03610470.2017.1402574

Rubottom, L. N., Lafontaine, S. R., & Shellhammer, T. H. (2022). Evaluating the impact of kilning temperature on hop quality in American deep bed dryers. *Brewing Science, 75*, 98–108. https://doi.org/10.23763/BrSc22-15rubottom.

Rybka, A., Krofta, K., Heřmánek, P., & Honzík, I. (2018). Effect of drying temperature on the content and composition of hop oils. *Plant, Soil and Environment, 64*(10), 512–516. https://doi.org/10.17221/482/2018-PSE.

Salinas, F., Vardanega, R., Espinosa-Álvarez, C., Jimenéz, D., Muñoz, W. B., Ruiz-Domínguez, M. C., … Cerezal- Mezquita, P. (2020). Supercritical fluid extraction of chañar (Geoffroea decorticans) almond oil: Global yield, kinetics and oil characterization. *Journal of Supercritical Fluids, 161*. https://doi.org/10.1016/j.supflu.2020.104824

Santos, F. C. dos, Santos, M. dos, Huezsmann, R. D., Ceola, D., Souza, E. M. D. de, Junior, C. F. D. S., Guidolin, A. F., & Coimbra, J. L. M. (2022). Phenotypic Variability in the Induction of Alpha Acids in Hops (Humulus lupulus L.) in Brazil. *Journal of Agricultural Science*, 14(6), 198. https://doi.org/10.5539/jas.v14n6p198.

Skinner, R. N., Hildebrand, R. P., & Clarke, B. J. (1977). The Effect of Storage Temperature on the Stability of the Alpha-Acids Content of Baled Hops. *Journal of the Institute of Brewing, 83*, 290–294. https://doi.org/10.1002/j.2050-0416.1977.tb03811.x

Srečec, S., Kvaternjak, I., Kaučić, D., Špoljar, A., & Erhatić, R. (2008). Influence of climatic conditions on accumulation of α-acids in hop cones. *Agriculturae Conspectus Scientificus, 73*(3), 161–166. https://acs.agr.hr/acs/index.php/acs/article/view/38.

Srečec, S., Rezić, T., Šantek, B., & Marić, V. (2009). HOP PELLETS TYPE 90: INFLUENCE OF MANUFACTURE AND STORAGE ON LOSSES OF α -ACIDS. *Acta Alimentaria, 38* (1), 141–147. https://doi.org/10.1556/AAlim.2008.0014

Stephan J. Barth, & Meier, H. (2021). *The Barth Haas Report*. Retrieved from https://www.barthhaas.com/resources/barthhaas-report. Accessed November 26, 2022.

Sturm, B., Raut, S., Kulig, B., Münsterer, J., Kammhuber, K., Hensel, O., & Crichton, S. O. J. (2020). In-process investigation of the dynamics in drying behavior and quality development of hops using visual and environmental sensors combined with chemometrics. *Computers and Electronics. Agriculture, 175*. https://doi.org/10.1016/j.compag.2020.105547

Thomas, G. G. (1980). Weather factors controlling the alpha-acid content of hops (Humulus lupulus L). *Journal of Horticultural Science, 55*(1), 71–77. https://doi.org/10.1080/00221589.1980.11514904

Valle, M., Rivera, O., Teuber, O., & Palma, M. T. (2003). Supercritical CO 2 extraction of Chilean hop (Humulus lupulus) ecotypes. *Journal of the Science of Food and Agriculture*, 1356(April 2002), 1349–1356. https://doi.org/10.1002/jsfa.1547.

Van Eynde, F. (2018). *Flanders Investment & trade market survey*. Retrieved from https://www.flandersinvestmentandtrade.com/export/sites/trade/files/market_studies/850150722161834/850150722161834_4.pdf. Accessed November 23, 2022.

van Opstaele, F., Goiris, K., de Rouck, G., Aerts, G., & de Cooman, L. (2012). Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets - Part 1: Preparation of single variety total hop essential oils and polar hop essences. *Journal of Supercritical Fluids, 69*, 45–56. https://doi.org/10.1016/j.supflu.2012.05.009

Veiga, B. A., Hamerski, F., Clausen, M. P., Errico, M., de Paula Scheer, A., & Corazza, M. L. (2021). Compressed fluids extraction methods, yields, antioxidant activities, total phenolics and flavonoids content for Brazilian Mantiqueira hops. *Journal of Supercritical Fluids, 170*. https://doi.org/10.1016/j.supflu.2020.105155

Zeković, Z., Pfaf-Šovljanski, I., & Grujić, O. (2007). Supercritical fluid extraction of hops. *Journal of the Serbian Chemical Society, 72*(1), 81–87. https://doi.org/10.2298/JSC0701081Z

Ziegler, T., Jubaer, H., & Teodorov, T. (2021). Bottlenecks in continuous hops drying with conveyor-belt dryer. *Drying Technology, 1–19*. https://doi.org/10.1080/07373937.2021.1950168

Chapter 4

Could AI also be used for hop processing?

Machine Learning Approach to Enhancing Drying Efficiency of Hop (*Humulus lupulus L.*)

Pinto, M B C[a]*, Ghion, R A C[a], Schmidt, F L[a]

[a]*Fruit and Vegetables Laboratory - Department of Food Technology, School of Food Engineering, University of Campinas (UNICAMP), R. Monteiro Lobato 80, 13083-862 Campinas, SP, Brazil*

Author Contributions

Mariana B C Pinto: Conceptualization, Methodology, Software, Validation, Formal analysis, Data acquisition, Data interpretation, Writing - original draft, Writing - review & editing. **Rafael do Amaral Campos Ghion:** Methodology, Investigation, Data acquisition, Writing - original draft, **Flavio L Schmidt:** Conceptualization, Methodology, Writing - review & editing supervision, project administration and funding acquisition

Paper published in BrewingScience

Reprint with permission from (see Appendix):
https://doi.org/10.23763/BRSC23-02PINTO

BrewingScience May / June 2023 (Vol. 76) 48

M. B. C. Pinto, R. A. C. Ghion and F. L. Schmidt

Machine Learning Approach to Enhancing Drying Efficiency of Hop (*Humulus lupulus* L.)

Beer is the most produced alcoholic beverage in the world with a production of 1.82 billion hectoliters in 2020, in which hops remain one of the most important raw materials. Hops drying stands as a key step to reduce the moisture right after the harvest, avoiding deterioration. Drying remains an issue due to a lack of process control, high energy demand, and consequently greenhouse gas emissions. This study intends to probe into the viability of computational modeling for time prediction to optimize the drying step of processing. For that, KNN, ANN, and Random Forest algorithms were compared with conventional empirical models according to statistical error and accuracy. From the outcomes, it was constructed a model with high accuracy $R^2 > 0.999$ using the KNN and Random Forest algorithms. It demonstrates higher accuracy in comparison with conventional mathematical models as well as a simple and more rapid time prediction. The new tool developed and tested in this study enables the reduction of drying time by a model using wider process variables. Consequently, the product quality is enhanced, and the drying footprint might be reduced by more effective energy usage.

Descriptors: kilning, machine learning, modeling prediction, artificial neural network

1 Introduction

Beer is the most produced alcoholic beverage in the world, with a production of 1.82 billion hectoliters in 2020 [1]. The main raw materials are barley malt, yeast, water, and hops [2]. Hop is an important ingredient in beer, adding flavor through its unique bitter compounds and essential oils [3]. New hopping techniques, such as dry hopping, and an increase in hoppy beer consumption have led to hop production growth in the past decades, reaching 62,366 ha of hop cultivation in 2020 [4]. Furthermore, higher demand for the addition of hops widely impacts the environment due to greenhouse gas emissions during its cultivation and processing. Hop production is responsible for emitting 3.5 to 5.5 kg of CO_2 per kilogram of hops, as a sum of the footprint of agricultural machinery, pesticides, fertilizers, and drying [5].

The moisture of fresh cones is reduced by drying on the farm right after picking [6]. However, hop drying requires massive energy consumption, impacting greatly on hop processing footprint. This step is crucial to enhance quality during storage, avoiding mold potential, which can lead to microbiological deterioration [5, 7]. Reducing the drying time might be one key to CO_2 mitigation in hop production. Furthermore, an optimized drying process leads to product quality enhancement by avoiding over-maturation of hop cones.

The food drying process involves a complex matrix with physico-chemical transformation and structural changes [8]. Therefore, the drying process optimization is rarely obtained by empirical mathematical models. Several empirical models have been developed in the past decades to predict drying process variables, such as temperature, drying rate, and moisture rate. However, those models are limited regarding time prediction [9–13]. Machine learning application offers several advantages when compared with empirical modeling techniques due to their ability to adjust non-linear functions, learning suitability, and flexibility to numerous systems [14]. Among those, Artificial Neural Networks (ANN), Random Forest, and K-Nearest Neighbor (KNN) have been widely used in food research to optimize food processing, such as fruit drying, detect fraud in food matrices, and control food product quality [15–19].

To date, no studies have been found using the machine learning approach to predict drying time, despite this method being already explored in moisture content prediction [18, 19, 21, 40]. Therefore, this study benefits from the advantages of using computational tools as an alternative to empirical mathematical models. Considering the challenge of hop farms in controlling the drying process, this study aims to propose a novel method to predict the drying time of hops by using machine learning techniques to build a more accurate model including a wider range of drying parameters. Those models were developed using drying absolute temperature, wet-bulb temperature (air temperature measured by a thermometer bulb surrounded by a wet cloth), dry-bulb temperature (air temperature measured by a thermometer bulb), and moisture. They offer an alternative tool for process optimization and control, leading to energy efficiency improvements and, consequently, reduction of greenhouse gas

https://doi.org/10.23763/BrSc23-02pinto

Authors

Mariana Barreto Carvalhal Pinto, Rafael do Amaral Campos Ghion, Flavio Luis Schmidt, Fruit and Vegetables Laboratory – Department of Food Technology, School of Food Engineering, University of Campinas (UNICAMP), Campinas, SP, Brazil; corresponding author: maribcarvalhal@gmail.com

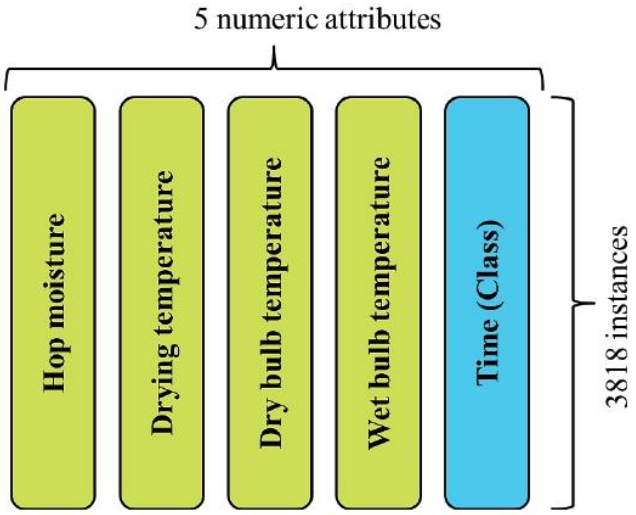


Fig. 1 Machine learning dataset composition

emissions. In addition, more precise drying time control reduces post-harvest waste on the farms due to the faster process.

2 Materials and Methods

This study compares the five classic empirical models used in the drying process with the three most common machine learning algorithms trained and tested with the same experimental dataset. The dataset was randomly split into training (70 %) and testing groups (30 %). It was composed of the 5 numeric attributes (including time as a class) and 3818 instances (Fig. 1). The empirical and machine learning models were compared according to the R^2 and Root Mean Square Error (RMSE). The detailed methodology for data collection, drying experiment, and the machine learning models' parameters are in the following sections.

2.1 Drying experiment

To collect the experimental data on the drying process for the models' construction, Brazilian hops (*Humulus lupulus* L.) variety 'Mantiqueira' was used in the present study. All cones were dried in triplicate at 70, 55, and 40 °C with an air velocity of 6.6 m/s and volumetric airflow of 0.013 m³/s. The air temperatures were measured using fixed-type T thermocouples (Ecklund-Harrison, USA) installed inside and outside of the drier. All thermocouples were connected to a data logger (Almemo 2890-9, Ahlborn, Germany), and data were analyzed using a Microsoft Excel® spreadsheet. As shown in figure 2, the trials were carried out in three perforated (mesh 14, wires 30 mm) 314 stainless steel 30 × 20 × 10 cm baskets placed in model MA035 oven drier (Marconi, Piracicaba, Brazil). Each batch was performed with three baskets containing 100 g of hops each. The hop's weight was measured during the drying process by one of the baskets linked to a weight balance connected to a PC, acquiring the data every minute by self-developed software. The moisture content was analyzed following the Hops-4 ASBC (American Society of Brewing and Chemists) [20] method adapted using 1 g of sample dried in an oven drier for 2 hours at 105 °C.

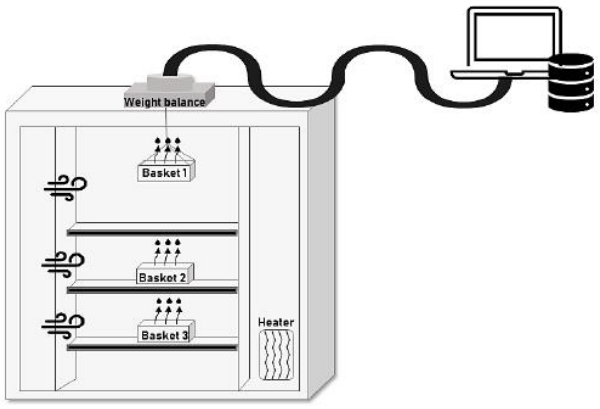


Fig. 2 Diagram of drying experiment with data collection

The moisture rate (MR) is a dimensionless value that was calculated according to equation 1.

$$MR = \frac{X - X_{eq}}{X_0 - X_{eq}} \quad \text{(Eq. 1)}$$

Where, X, X_{eq} an,d X_0 correspond to average moisture content at weighting time (g/g_{solids}), average equilibrium moisture content ($g/_{gsolids}$), and average initial moisture content (g/g_{solids}), respectively [22]. The equilibrium moisture content was determined by drying a hops batch until a constant weight was achieved, which is characterized as in equilibrium.

2.2 Effective Moisture Diffusivity and Activation Energy Determination

The effective moisture diffusivity was calculated by Fick's law, assuming diffusion as the principal mechanism of water transfer [23].

$$\frac{\partial Mt}{\partial t} = \nabla \cdot (D_{eff} \nabla M_t) \quad \text{(Eq. 2)}$$

The equation 3 solution arises with the assumption of uniform initial moisture distribution, constant diffusion, and a spherical shape during drying:

$$MR = \frac{Mt - Me}{Mb - Me} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{2n^2} \exp\left(-n^2 \pi^2 \frac{Deff \cdot t}{r_e^2}\right) \quad \text{(Eq. 3)}$$

where n is the polynomial coefficient, t is the drying time (s), r_e is the sample radius (m), and D_{eff} is the effective moisture diffusivity (m^2/s).

For the extended drying process, Equation 3 could be simplified to the first term, resulting in equation 4.

$$MR = \left(\frac{6}{\pi^2}\right) \exp\left(-\pi^2 \frac{Deff \cdot t}{r_e^2}\right) \quad \text{(Eq. 4)}$$

Equation 5 represents the linear relation of equation 4 after logarithmic transformation.

$$\ln(MR) = \ln\left(\frac{6}{\pi^2}\right) - \left(-\frac{Deff \cdot \pi^2 \cdot t}{r_e^2}\right) \quad \text{(Eq. 5)}$$

The diffusivity coefficient (K_1) was obtained from the ln (MR) × time plotted chart, resulting in equation 6.

$$K_1 = \left(\frac{Deff \cdot \pi^2}{r_e{}^2}\right) \quad \text{(Eq. 6)}$$

The activation energy was calculated based on the Arrhenius equation from the relationship between effective moisture diffusivity and the average temperature.

$$D_{eff} = D_0 \exp\left(\frac{E_a}{R_g \cdot T_a}\right) \quad \text{(Eq. 7)}$$

where T_a is the absolute air temperature (K), R_g is the gas constant (8,3143 kJ/mol), D_0 is the pre-exponential factor, and E_a is the activation energy (kJ/mol). The linear relation of equation 7 results in equation 8.

$$\ln(D_{eff}) = \left(\frac{E_a}{R_g \cdot T_a}\right) + \ln(D_0) \quad \text{(Eq. 8)}$$

The activation energy coefficient (K2) was obtained from the slope of ln (D_{eff}) × $\frac{1}{T_a}$ plotted chart, resulting in equation 9.

$$K_2 = \frac{E_a}{R_g} \quad \text{(Eq. 9)}$$

2.3 Empirical mathematical modeling

Five of the most used mathematical models for drying were selected to compare with the model built by machine learning (Table 1). Those models contain the moisture rate as a function of time and the constants are calculated by fitting the experimental data.

2.4 Machine Learning Methods

Three models of machine learning were tested and compared to the mathematical ones: Artificial Neural Network, Random Forest, and K-Nearest Neighborhood. Here, is proposed their usage to predict the drying time of hops. These algorithms were selected due to their efficiency and malleability in prediction, being widely used for decades in health research for disease development prediction, demonstrating the suitability of the machine learning tools [24–29].

The machine learning models were built as the input attributes of the absolute temperature (°C), wet-bulb temperature (°C), dry-bulb temperature (°C), moisture content (g_{water}/g_{solids}) and drying time (s) as a class. The model was constructed with 3819 instances through cross-validation with 10 folds.

Artificial Neural Network: This model was built using the classifier Multilayer Perceptron at Weka® (version 3.9.4, Hamilton, New Zealand) which uses backpropagation to teach a multi-layer perceptron to classify instances. It is named weka.classifiers.functions.MultilayerPerceptron. The dataset was trained and tested through Cross-validation with 10 folds. Since the class is numeric, the output nodes have non-thresholded linear units. The network parameters were modified to achieve higher model fitting. The algorithm was set to normalize the attributes and the numeric class to improve network performance. The momentum applied to the weight updates was 0.2, the number of decimal places used for the output of numbers in the model was 2, the learning rate for weight updates was 0.3, and the batch size, which is the preferred number of instances to process if the batch prediction is being performed, was 100 instances. The number of seeds was optimized to achieve higher network performance. This model used 4 seeds to initialize the random number generator. The hidden layers of the neural network were set as 't' which means the number of attributes + classes.

Table 1 Empirical Mathematical Drying Model

Model Name	Equation	References
Newton	MR = exp (-kt)	[9]
Page	MR = exp ($-kt^n$)	[10]
Logarithmic	MR = a exp (-kt) + c	[11]
Two terms	MR = a exp ($-k_1t$) + b exp ($-k_2t$)	[12]
Henderson e Pabis modified	MR = a exp (-kt) + b exp (-gt) +c exp (-ht)	[13]

Random Forest: The model was built using the classifier Random Forest at Weka® (version 3.9.4, Hamilton, New Zealand) which is a special case of boosting meta classifier constructed by a forest of random trees [30]. It is named weka.classifiers.trees.RandomForest. The dataset was trained and tested through Cross-validation with 10 folds. The trees parameters were the default of Weka with the random number seed used as 1, the number of execution slots (threads) to use for constructing the ensemble as 1, the size of each bag as 100, and the number of decimal places to be used for the output of numbers in the model as two, the batch size set as 100, the number of trees in the random forest as 100, and the tree depth set as unlimited.

K-Nearest Neighbor: The model was built using the K-nearest neighbors classifier at Weka® (version 3.9.4, Hamilton, New Zealand) which is named weka.classifiers.lazy.IBk [31]. The dataset was trained and tested through Cross-validation with 10 folds. The K value selection was based on cross-validation, using the meta classifier Cross-validation Parameter selection (weka.classifiers.meta.CVParameterSelection) [32]. The classifier parameters were used by the software default with one random seed, 10 folds used for cross-validation, two decimal places to be used for the output of numbers in the model, batch size of 100 instances, and the scheme parameters set to cross-validation "K 1 10 10". The regression with the KNN classifier was performed using the 2 nearest neighbors, as selected by cross-validation, Euclidean distance, batch size of 100 instances, the output of numbers in the model set as two decimal places, and window size set as 0, which means there was no limit to the number of training instances.

3 Bitter acid analyses

3.1 Sample preparation

Samples of fresh and dried cones were lyophilized for 24 hours to eliminate residual water and milled using liquid nitrogen to avoid oxidation reactions. The analysis was carried out in triplicate for each sample. For bitter acid extraction, 20 mL methanol was added to 250 mg of hop powder (acidified with 0.01 % formic acid) in a falcon tube and agitated for 1 hour. The samples were centrifuged (Laborline, Barueri, Brazil) for 5 min at 700 ×g and an aliquot of 5 mL of supernatant was withdrawn and transferred to an amber

glass tube. The samples were filtered using a 0.45 µm cellulose filter supplied by Sinergia Científica (Campinas, Brazil) and followed by HPLC (High-Pressure Liquid Chromatography) analysis.

3.2 HPLC analysis

The α- and β-acid contents were determined by HPLC (Waters Corporation, Milford, USA), according to the method adapted from *Keukeleire* et al. (2003) and Hops-14 ASBC (American Society of Brewing and Chemists, 2008). The column used for compound separation was a C-18 column (SunFire, 250 × 4.6 mm, 5 µm; Water Corporation, Milford, USA). Chromatographic conditions consisted of an isocratic gradient composed of 15 % eluent A (milli-Q water acidified with 1.47 % (v/v) phosphoric acid 85 %) and 85 % of eluent B (HPLC-grade methanol). The injection volume was 10 µL using a Waters 717 plus autosampler. The flow rate was 1 mL/min, the run time was 50 min, and the column temperature was 25 °C. The detection was at 314 nm for α- and β-acids and 370 nm for xanthohumol using the Waters 996 photodiode array detector. The retention time comparison with an external standard (ICE-4 and xanthohumol 60 % Standard), also applied for quantification of α-, β-acids, and xanthohumol determined the identification of the peaks.

3.3 Surface Color Measurement

The surface color of samples was determined by a portable colorimeter MiniScan XE (Hunter Associates Laboratory, Inc., Reston, Virginia, USA), with enlightening D65 and an observation angle of 10°. The CIE Lab color parameters, i.e., L* (whiteness or brightness), a* (redness or greenness), and b* (yellowness or blueness) coordinates, were used to describe the color of samples. Color measurements were taken in triplicate.

3.4 Total Essential Oil Content

Total oil content was determined using hydro-distillation, following the methodology described by ASBC (American Society of Brewing and Chemists, 2011b). 100 g of fresh and dried hops were submitted separately to hydro-distillation for 4 h using a Clevenger apparatus.

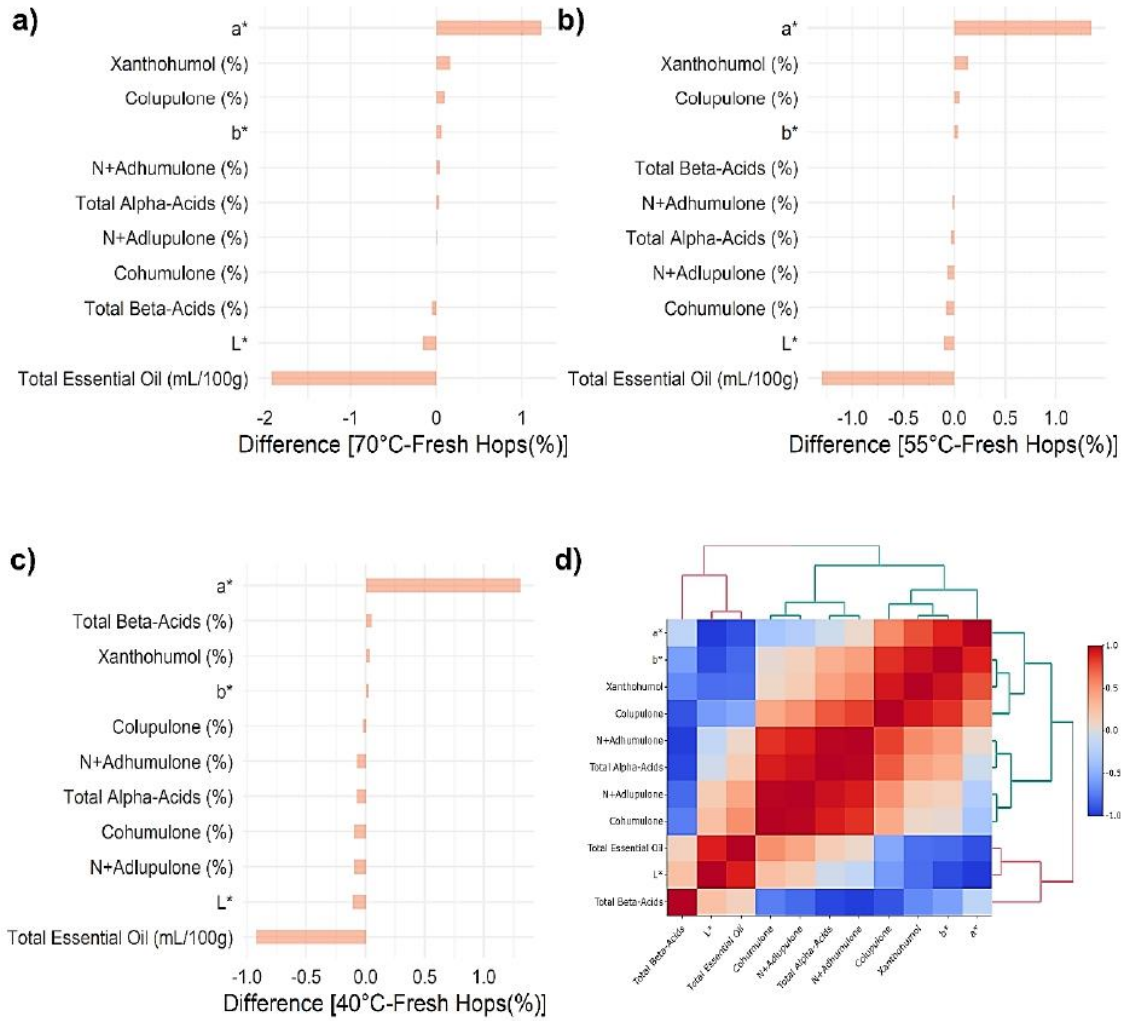


Fig. 3 Overall influence of drying temperature on the main hop quality parameters: a) Difference between fresh hop and dried at 70°C*; b) Difference between fresh hop and dried at 55°C*; c) Difference between fresh hop and dried at 40°C*; d) Correlogram constructed from Pearson's correlation (* L*, a*, and b* corresponding to color CIELAB parameters: L* value indicates lightness, a* and b* are chromaticity coordinates; **Detailed data is available in Supplementary Material (Table S2))**

The volume of oil was observed in the receiver.

3.5 Statistical analysis

The temperature (40, 55, and 70 °C) effects on hop chemical analytes were evaluated by a percentual difference calculated between fresh and dried hops and presented in a bar plot constructed using RStudio (Package: ggplot). Pearson's correlation between the results was performed in RStudio (Package: Heatmaply). The drying curves modeling by the empirical models was performed by non-linear regression analysis using Origin software (version OriginPro 2020b). The experimental data were fitted with the empirical mathematical models, obtaining R^2 and root mean square error (RMSE) values which were used to evaluate the model's fit to the dataset.

4 Results and Discussion

4.1 Effect of drying process on hop quality

The drying process substantially affects food matrices due to higher temperature or processing time applications. Figure 3 shows the overall modification of hop quality during drying at each temperature. The results of the main hop quality parameters were evaluated as a percentual difference in the values between the applied temperature and fresh hops (Fig. a-c) and Pearson's correlation through a correlogram (Fig. d). A percentual difference between the trials was chosen to facilitate the comprehension of the dataset which comprises several compounds and color parameters. Hop color modified greatly in the temperature range with the discoloration more prominent at 70 and 40 °C. The parameter a* is the chromaticity coordinate of the CIELAB color space diagram (Supplementary Material, Fig. S2) that ranges from green (- a) to red (+ a) [33]. a* values increased in all temperatures, representing the green color degradation from fresh green towards green-brown due to the chlorophyll degradation by heat and oxidation [34]. Interestingly, the difference was higher in the lower temperatures (Supplementary File, Table S4) which evidences the great effect of prolonged drying time on green color degradation. Therefore, controlling the termination period of drying could reduce the color degradation in hops and, consequently, enhance the product quality.

Hop total essential oil became one of the main requirements for good quality due to the high demand for hoppy aroma in beer. However, the total essential oil content was greatly affected by the drying process, decreasing by 65.8 % when dried at 70 °C (Supplementary File, Table S3). Figure 3 a-c demonstrates that the total essential oil content decreases considerably with the temperature increase. Despite extended time at 40 °C, this condition represented the lowest difference in the total essential oil content between the fresh and dried hops. This study's finding demonstrates that the processing time has a lower influence on essential oil maintenance in comparison with the temperature. The essential oil components are heat-labile substances, and the higher temperature increases the heat transfer from the surrounding air to the compounds, achieving faster activation energy to volatile [35]. *Rybka* et al. [36, 37] demonstrated the greater effect of drying temperature on the hop essential oil preservation as well as hop quality. In their study, a Saaz variety dried at 40 °C lost 13.6 % of total essential oil content in comparison with 47.0 % when hop was dried at 60 °C. However, *Rubottom* et al. [38] showed that drying temperature impact is variety- and crop-dependent with lower effect on hop oil composition and sensory evaluation.

As demonstrated in the bar chart in figure 3 a-c, the drying process had no effect on the hop bitter acids and xanthohumol content. The difference in those compounds' content between the fresh hops and the dried ones was proximal to zero in all temperatures, highlighting the thermostability of those compounds. This finding is in concordance with *Sturm* et al. [39] results which confirmed the thermostability of α- and β-acids during the drying regardless of the bulk weight.

Figure 3 d presents the correlation between those chemical analytes measured. Interestingly, total essential oil content represented a high correlation with the color parameters which can be certainly explained by the degradation of color alongside the essential oil content decrease during drying. Hop color is a relevant quality attribute for product acceptance by the consumer and it is also an indicator of the overall product quality. In that sense, Sturm et al. [40] developed an in-process predictive model for moisture content and color with the aid of non-invasive optical sensors. This highlights the feasibility of hop quality enhancement through the usage of modern tools as well as process optimization.

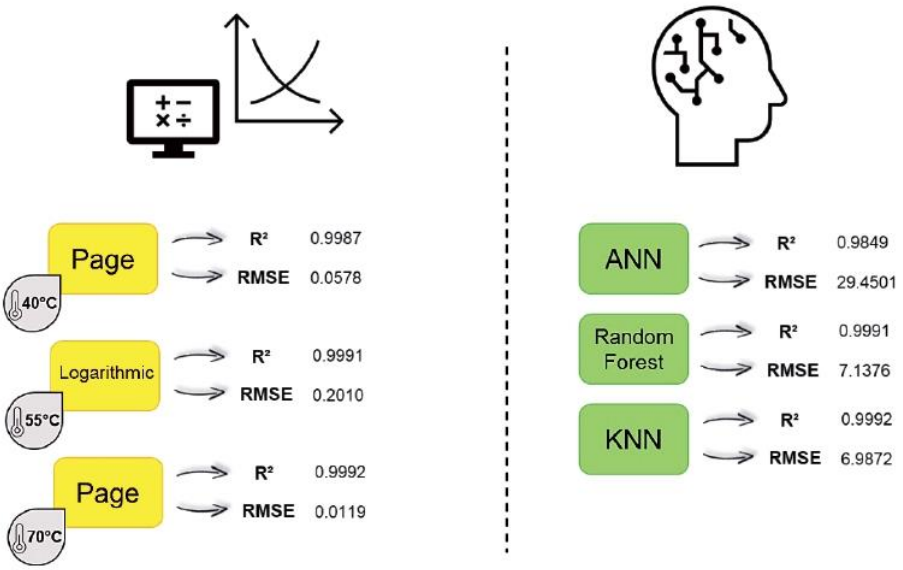


Fig. 4 **R^2 and RMSE values for the best mathematical model fit in each drying temperature[a] (left) and the machine learning models (right); [a]The R^2 and RMSE values for all mathematical models are found in Table S1 (Supplementary Material)**

4.2 Comparison of Conventional Mathematical Models and Machine Learning Models

In figure 4, it can be seen that the Page model sufficiently described the moisture ratio prediction for the temperatures of 40 and 70 °C, with higher R^2 values (> 0.999) and lower RMSE values (< 0.06). While for the temperature of 55 °C the logarithmic model fitted greatly the experimental data, due to the higher values of R^2 (>0.999) and lower RMSE values (<0.03). The findings of the current study are consistent with those of *Demir* et al. [41] study which found suitability in Page's model for predicting the moisture rate in bay leaves drying kinetics. Furthermore, *Darvish* et al. [42] dill leaf drying study demonstrated that the logarithmic model offered the best fit to the experimental data in drying kinetics.

The prediction of drying time presented higher accuracy of the KNN and Random Forest model with a higher R^2 value (>0.999) and lower RMSE (< 7.5) in comparison with the ANN model (R^2 < 0.999) (Fig. 4). As expected, the Random Forest algorithm fitted the experimental data sufficiently due to its capacity to build a randomized decision tree in each iteration of the bagging algorithm, producing excellent predictors [14]. The KNN algorithm is described in the literature as a lazy classifier and is usually slower than Random Forest and Multilayer Perceptron algorithms. However, in this study, the KNN algorithm completed the model in less than 1 second, whereas the Random Forest and ANN algorithms took 1.82 and 3.59 seconds, respectively. Therefore, for this dataset, the KNN algorithm demonstrated reasonable performance regarding computational execution and reduced error rates. The best fit occurred with the Random Forest and KNN models due to the reduced error rate and fewer data dissipation (Fig. 5). Pearson's correlation of these models is 0.9991 and 0.9992 with R^2 values of 0.9982 and 0.9983, respectively. On the other hand, Pearson's correlation for the ANN model stands out at 0.9829 with an R^2 value of 0.9658, demonstrating the model's reduced ability for data fitting and prediction.

Although Page's model at 70 °C and the logarithmic model at 55 °C achieved similar correlation coefficient values to the KNN model, their time prediction demonstrates limited regarding the process parameters used. The empirical mathematical modeling predicts the moisture ratio instead of drying time. The drying time calculation adds complexity to the time prediction through both the Page's and logarithmic models [23]. In this case, would be necessary to integrate several assumptions which could reduce the prediction accuracy as well as adding needless effort. Therefore, the machine learning model is suitable for non-linear datasets and facilitates the construction of a complex model using more attributes. Furthermore, this study used open-source software, to facilitate the user's comprehension, which could be implemented in the computers on the farm. Once the model is constructed, an output (drying time) is generated by the addition of new inputs. However, further investigation must be performed to validate this model to other datasets, since the results obtained in this study are specifically for the dataset used.

4.3 Implications of using machine learning to predict the drying time of hops

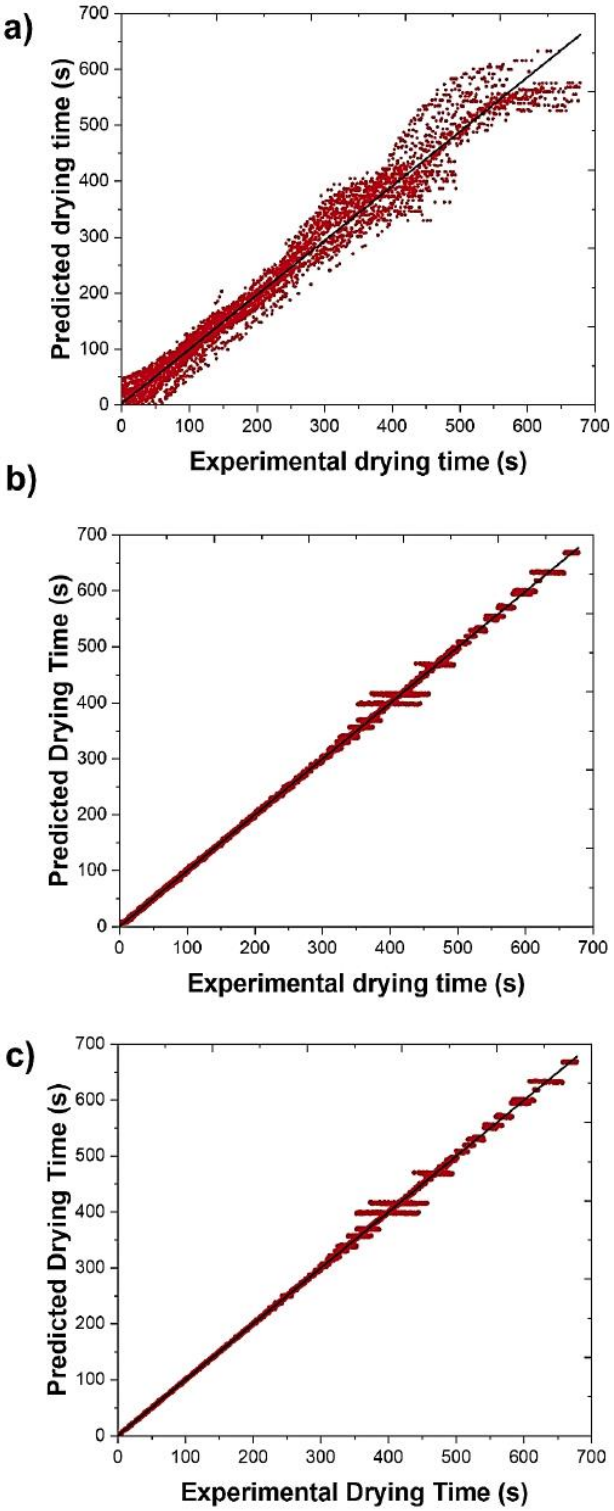


Fig. 5 **Predicted versus experimental drying time using the 10 cross-validation folds: a) ANN model; b) Random Forest model; c) KNN model**

As shown in figure 6, the effective moisture diffusivity coefficient (D_{eff}) has a clear correlation with the air temperature, increasing 3-folds when the temperature shifts from 40 to 70 °C. Generally, the values for agricultural or food products are placed between 10^{-12} and 10^{-8} [43]. A study by *Khaled* et al. [44] found similar results for the persimmon fruit D_{eff}, ranging from 1.3×10^{-9} to 9.2×10^{-9}. There-

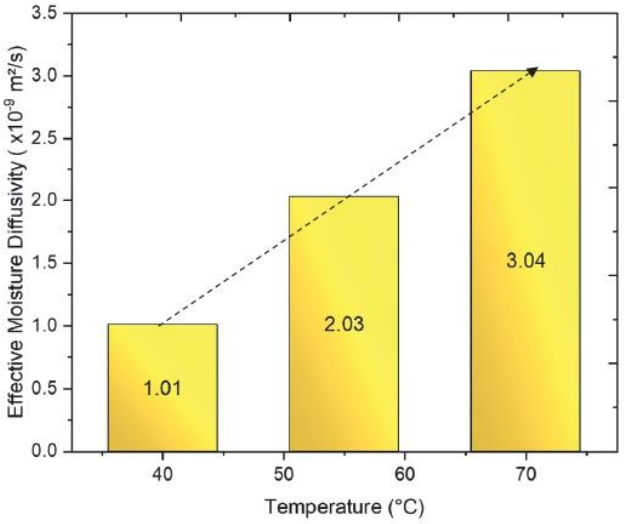


Fig. 6 **Effect of air temperature on the effective moisture diffusion coefficient**

fore, the values found for the hop cones are within the expected. Diffusivity is defined as the moisture transport phenomenon in food and is described by Fick's law [23, 45]. Elevated air temperatures lead to higher heat transfer to inner water which flows rapidly to the surface [23]. The D_{eff} increases at a higher temperature due to the water molecule's diffusional movement over a shorter time. However, high temperatures and increased D_{eff} lead to profound texture changes during the process. In hops, the bracts contain lower moisture content whereas string has higher moisture and is placed inner the cones, coming into less contact with the surrounding air [46]. During drying, bracts dry more rapidly than the string and, consequently, culminate in different final moisture [47]. By extending the exposure time, hop cones undergo over-drying that causes shatter due to the glass transition, especially in the bracts, to a vitreous state [48]. In that case, hop cones tend to lose more lupulin gland along with the main hop compounds, devaluating the product.

The activation energy was obtained by the Arrhenius-type equation. The value for hops was found to be 30.784 kJ/mol, within the expected range of activation energy. For agricultural and food products, an amount of activation energy between 12.7 and 110 kJ/mol is expected [43]. Activation energy is the minimum amount necessary to initiate water flow from the product to the surface and it is directly correlated to diffusivity [49].

Machine learning has been widely used for prediction in the past decades, allowing elevated accuracy along with reduced efforts. During the drying process, time and temperature are the main parameters that influence efficiency and energy expenditure. Nevertheless, climate conditions demonstrate a large effect on the drying performance due to water diffusivity to the outer air as well as the moisture transfer rate from inside the product to the surrounding air, represented by D_{eff}. Therefore, the machine learning models were built using not only the common parameters as attributes (temperature, and moisture rate) but also the environmental measurements (dry- and wet-bulb temperature) in order to provide more accurate results for process improvement at the hop farm. The machine learning model presented in that study could be improved by the addition of further weather conditions and process parameters, such as environmental moisture, inner product temperature, and air velocity. Furthermore, a scale-up study is recommended to validate the machine learning model for real setup conditions.

The machine learning modeling as developed in that study permits drying time reduction due to sharp control using additional process variables, enhancing the product quality. Diminished drying time enables producers to increase daily production, resulting in lower cone mold deterioration after harvesting due to reduced storage time at a higher moisture content [6]. Drying process optimization by machine learning produces high-quality hops with minimal energy expenditure, reducing over-drying. The drying step leads to a higher carbon footprint in the hops processing [50]; therefore, avoiding energy expenses is paramount to reducing greenhouse gas emissions as well as minimizing costs.

5 Conclusion

For the first time, this study proposed using machine learning as a modeling tool for predicting the drying time of hops, which might be a key to improving the process in a more sustainable and economical direction. The performance of machine learning and empirical models was similar; however, the KNN model was able to describe a wider range of experimental data, whereas the application of empirical models is limited to fewer attributes. Machine learning has the advantage of considering not only the common parameters (temperature, and moisture rate) but also environmental measurements (dry- and wet-bulb temperature) which greatly affects hop drying performance. However, there is still room to improve the methodology proposed here, by extensive research using further weather conditions and process parameters that were not considered in this study, as well as exploring the process scale-up with other hop varieties and validation with other datasets.

Acknowledgment

The authors wish to acknowledge the Van den Bergen company for the material donated.

Conflict of interest

The authors have no conflict of interest.

Funding sources

The authors appreciate the support of the CNPq – Research Nacional Council - Brazil (Finance code 141485/2018-3).

6 References

1. Statista: Worldwide beer production, https://www.statista.com/statistics/270275/worldwide-beer-production/#:~:text=In%202020%2C%20the%20global%20beer,and%20yeast%20as%20basic%20ingredients.
2. Lewis, M.J. and Bamforth, C.W.: Essays in Brewing Science, Springer

Science+Business, New York, 2006.
3. Almaguer, C.; Schönberger, C.; Gastl, M.; Arendt, E.K. and Becker, T.: *Humulus lupulus* – a story that begs to be told. A review, Journal of the Institute of Brewing, **120** (2014), no. 4, pp. 289-314.
4. Barth-Haas Group: Barth Haas Report 2021/222021.
5. Hauser, D.G. and Shellhammer, T.H.: An Overview of Sustainability Challenges in Beer Production, and the Carbon Footprint of Hops Production, Technical Quarterly, 2019.
6. Biendl, M.; Engelhard, B.; Forster, A.; Gahr, A.; Lutz, A.; Mitter, W.; Schmidt, R. and Schönberger, C.: Hops: their Cultivation, Composition, and Usage, Fachverlag Hans Carl GmbH, Nuremberg, 2014.
7. Rourke, B.T.O.: Hops and hop products, The brewer international, **3** (2003), no. 1, pp. 21-25.
8. Sun, Q.; Zhang, M. and Mujumdar, A.S.: Recent developments of artificial intelligence in drying of fresh food: A review, Critical Reviews in Food Science and Nutrition, **59** (2019), no. 14, pp. 2258-2275.
9. Lewis, W.K.: The Rate of Drying of Solid Materials, Industrial and Engineering Chemistry, **13** (1921), no. 5, pp. 427-432.
10. Page, G.E.: Factors Influencing the Maximum Rates of Air Drying Shelled Corn in Thin Layers1949.
11. Yagcioglu, A.K.; Degirmencioglu, A. and Cagatay, F.: Drying Characteristics of Laurel Leaves Under Different Drying Conditions, 7th Int Congress Agricultural Mechanisation and Energy, Adana, 1999, pp. 565-569.
12. Henderson, S.M.: Progress in Developing the Thin Layer Drying Equation., Transactions of the American Society of Agricultural Engineers, **17** (1974), no. 6, pp. 1-3.
13. Karathanos, V.T.: Determination of water content of dried fruits by drying kinetics, Journal of Food Engineering, **39** (1999), no. 4, pp. 337-344.
14. Witten, I.H. and Frank, E.: Data Mining: Practical Machine Learning Tools and Techniques, 2nd ed., Elsevier Inc., London, 2011.
15. Jahanbakhshi, A.; Momeny, M.; Mahmoudi, M. and Radeva, P.: Waste management using an automatic sorting system for carrot fruit based on image processing technique and improved deep neural networks, Energy Reports, **7** (2021), pp. 5248-5256.
16. Abbaspour-Gilandeh, Y.; Jahanbakhshi, A. and Kaveh, M.: Prediction kinetic, energy and exergy of quince under hot air dryer using ANNs and ANFIS, Food Science and Nutrition, **8** (2020), no. 1, pp. 594-611.
17. Jahanbakhshi, A.; Abbaspour-Gilandeh, Y.; Heidarbeigi, K. and Momeny, M.: Detection of fraud in ginger powder using an automatic sorting system based on image processing technique and deep learning, Computers in Biology and Medicine, **136** (2021).
18. Golpour, I.; Kaveh, M.; Amiri Chayjan, R. and Guiné, R.P.F.: Optimization of Infrared-convective Drying of White Mulberry Fruit Using Response Surface Methodology and Development of a Predictive Model through Artificial Neural Network, International Journal of Fruit Science, (2020), pp. 1-21.
19. Khaled, A.Y.; Kabutey, A.; Mizera, C.; Hrabe, P. and Herák, D.: Modelling of hot-air and vacuum drying of persimmon fruit (Diospyros kaki) using computational intelligence methods, Agronomy Research, **18** (2020), no. Special Issue 2, pp. 1323-1335.
20. Technical Committe, A.: Hops - 4: Moisture, ASBC Methods of Analysis, 2011, pp. 1-2.
21. Karakaplan, N.; Goz, E.; Tosun, E. and Yuceer, M.: Kinetic and artificial neural network modeling techniques to predict the drying kinetics of Mentha spicata L., Journal of Food Processing and Preservation, **43** (2019), no. 10, pp. 1-10.
22. Berk, Z.: Food Process Engineering and Technology, 3rd ed., Elsevier, 2018.
23. Kerr, W.L.: Handbook of Farm, Dairy and Food Machinery Engineering, 2nd ed., Elsevier Inc., London, 2013.
24. Chen, Y.-C.; Chung, J.-H.; Yeh, Y.-J.; Lou, S.-J.; Lin, H.-F.; Lin, C.-H.; Hsien, H.-H.; Hung, K.-W.; Yeh, S.-C.J. and Shi, H.-Y.: Predicting 30-Day Readmission for Stroke Using Machine Learning Algorithms: A Prospective Cohort Study, Frontiers in Neurology, **13** (2022).
25. Kristinsson, Æ.Ö.; Gu, Y.; Rasmussen, S.M.; Mølgaard, J.; Haahr-Raunkjær, C.; Meyhoff, C.S.; Aasvang, E.K. and Sørensen, H.B.D.: Prediction of serious outcomes based on continuous vital sign monitoring of high-risk patients, Computers in Biology and Medicine, **147** (2022), p. 105559.
26. Chen, S.-D.; You, J.; Yang, X.-M.; Gu, H.-Q.; Huang, X.-Y.; Liu, H.; Feng, J.-F.; Jiang, Y. and Wang, Y.: Machine learning is an effective method to predict the 90-day prognosis of patients with transient ischemic attack and minor stroke, BMC Medical Research Methodology, **22** (2022), no. 1, p. 195.
27. Chen, S.-D.; You, J.; Yang, X.-M.; Gu, H.-Q.; Huang, X.-Y.; Liu, H.; Feng, J.-F.; Jiang, Y. and Wang, Y.: Machine learning is an effective method to predict the 90-day prognosis of patients with transient ischemic attack and minor stroke, BMC Medical Research Methodology, **22** (2022), no. 1, p. 195.
28. Son, J.; Kim, D.; Na, J.Y.; Jung, D.; Ahn, J.-H.; Kim, T.H. and Park, H.-K.: Development of artificial neural networks for early prediction of intestinal perforation in preterm infants, Scientific Reports, **12** (2022), no. 1, p. 12112.
29. Nasser, I.M. and Abu-Naser, S.S.: Lung Cancer Detection Using Artificial Neural Network International Journal of Engineering and Information Systems (IJEAIS), 2019.
30. Breiman, L.: Random forests, Machine Learning, **45** (2001), pp. 5-32.
31. Aha, D.W.; Kibler, D. and Albert, M.K.: Instance-Based Learning Algorithms, Machine Learning, **6** (1991), pp. 37-66.
32. Kohavi, R.: Wrappers for Performance Enhancement and Oblivious Decision Graphs, 1995.
33. Ly, B.C.K.; Dyer, E.B.; Feig, J.L.; Chien, A.L. and Del Bino, S.: Research Techniques Made Simple: Cutaneous Colorimetry: A Reliable Technique for Objective Skin Color Measurement Journal of Investigative Dermatology, 2020.
34. Bonazzi, C. and Dumoulin, E.: Quality Changes in Food Materials as Influenced by Drying Processes, Modern Drying Technology, 2011.
35. Damodaran, Srinivasan.; Parkin, K.L. and Fennema, O.R.: Química de alimentos de Fennema (4a. ed.)., Grupo A - Artmed, 2000.
36. Rybka, A.; Krofta, K.; Heřmánek, P.; Honzík, I. and Pokorný, J.: Effect of drying temperature on the content and composition of hop oils, Plant, Soil and Environment, **64** (2018), no. 10, pp. 512-516.
37. Rybka, A.; Heřmánek, P. and Honzík, I.: Effect of drying temperature in hop dryer on hop quality, **67** (2021), no. 1, pp. 1-7.
38. Rubottom, L.N.; Lafontaine, S.R.; Hauser, D.G.; Pereira, C.; Shellhammer, T.H.: Hop Kilning Temperature Sensitivity of Dextrin- Reducing Enzymes in Hops, Journal of the American Society of Brewing Chemists, **80** (2021), no. 1, pp. 1-13.
39. Raut, S.; Gersdorff, G.J.E. von; Münsterer, J.; Kammhuber, K.; Hensel, O. and Sturm, B.: Impact of Process Parameters and Bulk Properties on Quality of Dried Hops, Processes, **8** (2020), no. 11, p. 1507.
40. Sturm, B.; Raut, S.; Kulig, B.; Münsterer, J.; Kammhuber, K.; Hensel, O. and Crichton, S.O.J.: In-process investigation of the dynamics in drying behavior and quality development of hops using visual and environmental sensors combined with chemometrics, Computers and Electronics in Agriculture, **175** (2020), p. 105547.
41. Demir, V.; Gunhan, T.; Yagcioglu, A.K. and Degirmencioglu, A.: Math-

ematical Modelling and the Determination of Some Quality Parameters of Air-dried Bay Leaves, Biosystems Engineering, **88** (2004), no. 3, pp. 325-335.
42. Darvishi, H.; Farhudi, Z. and Behroozi-Khazaei, N.: Mass Transfer Parameters and Modeling of Hot Air Drying Kinetics of Dill Leaves, Chemical Product and Process Modeling, **12** (2016), no. 2, pp. 1-15.
43. Kaveh, M. and Amiri Chayjan, R.: Modeling Thin-Layer Drying of Turnip Slices Under Semi-Industrial Continuous Band Dryer, Journal of Food Processing and Preservation, **41** (2015), no. 2, pp. 1-14.
44. Khaled, A.Y.; Kabutey, A.; Selvi, K.Ç.; Mizera, Č.; Hrabe, P. and Herák, D.: Application of computational intelligence in describing the drying kinetics of persimmon fruit (*Diospyros kaki*) during vacuum and hot air drying process, Processes, **8** (2020), no. 5.
45. Khan, M.I.H.; Sablani, S.S.; Joardder, M.U.H. and Karim, M.A.: Application of machine learning-based approach in food drying: opportunities and challenges, Drying Technology, **40** (2020), no. 6, pp. 1-17.
46. Münsterer, J.: Optimale Trocknung und Konditionierung von Hopfen, LfL Information2006.
47. Raut, S.; Gersdorff, J.E. Von; Münsterer, J.; Kammhuber, K.; Hensel, O. and Sturm, B.: Influence of pre-drying storage time on essential oil components in dried hops (*Humulus lupulus* L.), Journal of the Science of Food and Agriculture, **101** (2020), no. 6, pp. 2247-2255.
48. Fellows, P.: Food processing technology : principles and practice, Second Edition ed., Woodhead Publishing Limited, Cambridge, England, 2002.
49. Koukouch, A.; Idlimam, A.; Asbik, M.; Sarh, B.; Izrar, B.; Bostyn, S.; Bah, A.; Ansari, O.; Zegaoui, O. and Amine, A.: Experimental determination of the effective moisture diffusivity and activation energy during convective solar drying of olive pomace waste, Renewable Energy, **101** (2017), pp. 565-574.
50. Martynenko, A. and Misra, N.N.: Machine learning in drying, Drying Technology, **38** (2020), no. 5-6, pp. 596-609.

Received 11 January 2023, accepted 24 May 2023

Supplementary Material

Table S1 Correlation coefficient and Root Mean Squared Error values to the fitted model

Absolute Temperature	Model	R^2	RMSE
40 °C	Newton	0.9867	0.6078
	Page	0.9987	0.0578
	Logarithmic	0.9979	0.0927
	Two terms	0.9919	0.3685
	Henderson e Pabis modified	0.9919	0.3685
55 °C	Newton	0.9837	0.3701
	Page	0.9989	0.0230
	Logarithmic	0.9991	0.0210
	Two terms	0.9914	0.1949
	Henderson e Pabis modified	0.9914	0.1949
70 °C	Newton	0.9877	0.1910
	Page	0.9992	0.0119
	Logarithmic	0.9975	0.0383
	Two terms	0.9942	0.0901
	Henderson e Pabis modified	0.9942	0.0901

Fig. S1 The system used for the drying trials

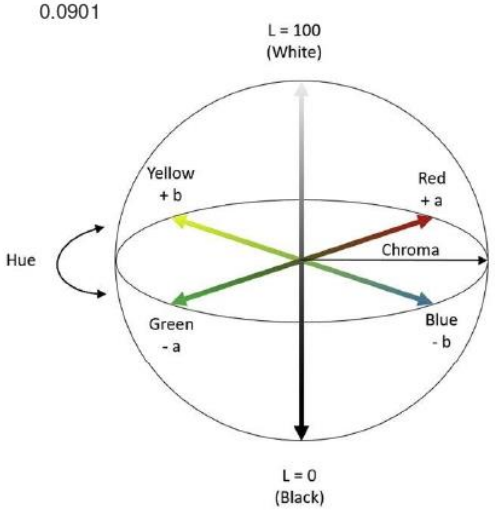


Fig. S2 CIE L* a* b* color space diagram (Ly et al., 2020)[1]

Table S2 Correlogram's dataset calculated with Pearson's correlation

Person's correlation calculated with hop quality dataset											
a*	– 0.17	– 0.99	– 0.92	– 0.35	– 0.26	– 0.05	0.04	0.55	0.77	0.90	1.00
b*	– 0.58	– 0.93	– 0.83	0.02	0.13	0.37	0.46	0.86	0.95	1.00	-
Xanthohumol	– 0.69	– 0.81	– 0.80	0.05	0.18	0.45	0.55	0.93	1.00	-	-
Colupulone	– 0.90	– 0.61	– 0.53	0.41	0.52	0.74	0.81	1.00	-	-	-
N+Adhumulone	– 0.98	– 0.12	0.05	0.85	0.91	0.99	1.00	-	-	-	-
Total Alpha-Acids	– 0.95	– 0.03	0.17	0.91	0.96	1.00	-	-	-	-	-
N+Adlupulone	– 0.82	0.18	0.42	0.99	1.00	-	-	-	-	-	-
Cohumulone	– 0.74	0.27	0.53	1.00	-	-	-	-	-	-	-
Total Essential Oil	0.12	0.90	1.00	-	-	-	-	-	-	-	
L*	0.26	1.00	-	-	-	-	-	-	-	-	-
Total Betal-Acids	1.00	-	-	-	-	-	-	-	-	-	-
	Total Beta-Acids	L*	Total Essential Oil	Cohumulone	N+Adlupulone	Total Alpha-Acids	N+Adhumulone	Colupulone	Xanthohumol	b*	a*

The parameters L, a*, and b* corresponding to color CIELAB parameters: L* value indicates lightness, a* and b* are chromaticity coordinates

Table S3 Average of hop composition and color parameters of fresh and dried hops

Treatment	Color			Bitter Acids						Xanthohumol (%)	Essential Oil
	L*	a*	b*	Total Alpha-Acids	Cohumulone	N+Adhumulone	Total Beta-Acids	Colupulone	N+Adlupulone		Total Essential Oil (mL/100 g)
Fresh Hops	63,41	-1,05	34,47	3,36	0,79	2,57	2,67	1,37	1,30	0,26	1,52
40°C	57,26	3,39	35,34	3,12	0,72	2,40	2,83	1,34	1,18	0,27	0,79
55°C	57,49	3,02	35,88	3,26	0,73	2,52	2,66	1,45	1,22	0,3	0,66
70°C	54,77	4,53	36,72	3,46	0,79	2,67	2,52	1,51	1,32	0,31	0,52

Table S4 Hop quality evaluation by the difference between fresh and dried hops

Treatment		Fresh Hops	40°C	55°C	70°C	Difference (40°C – Fresh Hops)	Difference (40°C – Fresh Hops) %	Difference (55°C – Fresh Hops)	Difference (55°C – Fresh Hops) %	Difference (70°C – Fresh Hops)	Difference (70°C – Fresh Hops) %
Color	L*	63,41	57,26	57,49	54,77	– 6,15	– 0,11	– 5,92	– 0,10	– 8,64	– 0,16
	a*	– 1,05	3,39	3,02	4,53	4,44	1,31	4,07	1,35	5,58	1,23
	b*	34,47	35,34	35,88	36,72	0,87	0,02	1,41	0,04	2,25	0,06
Bitter Acids	Total Alpha-Acids	3,36	3,12	3,26	3,46	– 0,23	– 0,07	– 0,10	– 0,03	0,10	0,03
	Cohumulone	0,79	0,72	0,73	0,79	– 0,07	– 0,10	– 0,06	– 0,08	0,00	0,00
	N+Adhumulone	2,57	2,40	2,52	2,67	– 0,17	– 0,07	– 0,05	– 0,02	0,10	0,04
	Total Beta-Acids	2,67	2,83	2,66	2,52	0,16	0,06	– 0,01	0,00	– 0,15	– 0,06
	Colupulone	1,37	1,34	1,45	1,51	– 0,03	– 0,02	0,08	0,05	0,15	0,10
	N+Adlupulone	1,30	1,18	1,22	1,32	– 0,12	– 0,10	– 0,09	– 0,07	0,01	0,01
	Xanthohumol (%)	0,26	0,27	0,30	0,31	0,01	0,04	0,04	0,13	0,05	0,16
Essential Oil	Total Essential Oil (mL/100g)	1,52	0,79	0,66	0,52	– 0,73	– 0,92	– 0,86	– 1,30	– 1,00	– 1,92

*Difference calculated from the values at 70 °C-FH, 55 °C-FH, and 40 °C-FH

Chapter 5

Do hop bitter acids influence wort protein profile during wort boiling?

Addition of hop (*Humulus lupulus L.*) bitter acids yields modification of malt protein aggregate profiles during wort boiling

Mariana B. C. Pinto[ab], Flavio L. Schmidt[a], Juri Rappsilber[c], Brian Gibson[b], Philip C. Wietstock[b]*

[a] *Fruit, Vegetable and Confectionery Products Laboratory, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil*

[b] *Chair of Brewing and Beverage Technology, Department of Food Technology and Food Chemistry, Technische Universität Berlin, Ackerstraße 76, 13355, Berlin, Germany*

[c] *Bioanalytics, Institute of Biotechnology, Department of Food Technology and Food Chemistry, Technische Universität Berlin,* Gustav-Meyer-Allee 25, 13355, *Berlin, Germany.*

Author Contributions

Mariana B C Pinto: Conceptualization, Methodology, Formal analysis, Data acquisition, Data interpretation, Writing - original draft, Writing - review & editing; **Flavio L Schmidt:** Conceptualization, Methodology, Writing - review & editing supervision, project administration and funding acquisition; **Juri Rappsilber:** Conceptualization, Methodology, Writing - review & editing supervision, and funding acquisition**; Brian Gibson:** Conceptualization, Writing - review & editing supervision, project administration and funding acquisition; **Philip C. Wietstock**: Conceptualization, Methodology, Writing - review & editing supervision, project administration and funding acquisition

Paper published in Journal of Agricultural and Food Chemistry

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC Article

Addition of Hop (*Humulus Lupulus* L.) Bitter Acids Yields Modification of Malt Protein Aggregate Profiles during Wort Boiling

Mariana B. C. Pinto, Flavio L. Schmidt, Juri Rappsilber, Brian Gibson, and Philip C. Wietstock*

Cite This: *J. Agric. Food Chem.* 2023, 71, 5700−5711 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Hop bitter acids are used in the brewing industry to give beer bitterness. However, much of this bitterness is lost during processing, specifically during the wort boiling step. One of the major causes might be the interaction with protein−protein complexes. Therefore, the aim of this study was to clarify the role of hop bitter acids in protein aggregate formation using a proteomic approach. The effect of hop addition on protein composition was analyzed by liquid chromatography−mass spectrometry/MS (LC-MS/MS), and further analyses were performed to characterize the wort before and after boiling. Addition of hop bitter acids yielded a change in wort protein profiles, and hop bitter acids were found to bind primarily to less abundant proteins which are not related to beer quality traits, such as foam or haze. Wort protein aggregate profiles were revealed, and findings from this study suggested the precipitation of particular proteins in the aggregates during boiling when hops were added.

KEYWORDS: *Humulus lupulus L, proteomics, isohumulones, humulones, protein*

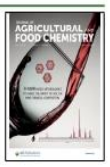
INTRODUCTION

Beer is an ancient beverage produced typically with barley malt, hops, yeast, and water. The brewing process begins with mashing, filtration, and wort boiling, producing a syrup (wort) rich in sugar and other compounds. The wort is fermented to convert sugar into ethanol and byproducts, which characterize the beer. The process generates a vast amount of waste. For instance, during wort boiling, there is a precipitation of compounds, like proteins, *iso*-α-acids, and polyphenols, which are removed from the system. This residue (trub) represents between 0.2 and 0.4 kg of waste per 100 L of beer. This equated to between 364 and 728 thousand tons of waste generated by the world beer industry in 2020.[1,2] Trub contains a high level of protein (approx. 50%).[3] Moreover, according to Kunze,[4] it is estimated that 50% of the hop bitter compounds' losses occur during wort boiling, representing a significant effect on the brewing industry's profitability. Hops are proportionally the highest-priced raw material in the brewing industry and are valued by the content of the bitter compounds. Furthermore, higher demand for hop addition impacts the environment due to the greenhouse gas emission during its cultivation and processing. Hop production is responsible for the emission of 3.5 to 5.5 kg of CO_2 per kilogram of hops, summed by the footprint of agricultural machinery, pesticides, fertilizers, and kilning.[5]

Hop provides the key bittering compounds in beer. These are the isomers of *iso*-α-acids generated by the isomerization reaction that follows first-order kinetics.[6] The α-acids are converted into *iso*-α-acids via an acyloin-type ring contraction, resulting in two epimeric isomers *cis*- and *trans-iso*-α-acids during the wort boiling process step.[7,8] In the brewing industry, utilization of α-acids represents an immense challenge considering the enormous loss of bittering compounds to the trub. According to Gänz et al.,[9] the *iso*-α-acids content is depleted by the proteins originating from the barley malt. The role of *iso*-α-acids in beer foam stability due to interaction with foam-active proteins has been reported by several researchers.[10−14] They are the most abundant proteins in the wort as well as the haze-active proteins.[15] However, there is still a lack of knowledge about minor proteins, such as enzymes and barley defense protein, and hop bitter acid interaction.

It is well established that wort protein plays an important role in foam stabilization and quality due to the formation of a viscoelastic layer with a linkage comprising a continuous liquid phase and discontinuous gas phase.[16] Hydrophobic proteins are mainly responsible for stabilizing the foam layer, especially those known as lipid transfer proteins (LTP) and protein Z.[17] Recently, Lu et al.[17] studies showed the influence of protein Z and LTP1 in the surface layer and the properties depend upon *iso*-α-acids and protein interactions.[18] They observed a formation of spherical aggregates of *iso*-α-acids and proteins, which suggests a strong bond between these compounds, preferentially with protein Z.[18] These protein fractions were also found to comprise the major fraction of proteins precipitated into wort trub during the boiling step due to binding with protein-derived polypeptides.[15] However, much of the research up to now has been focusing on the interaction of protein−hop bitter compounds in the foam and little attention has been paid to the equally important role of hop bitter compounds in protein precipitation during the boiling

Received: January 10, 2023
Revised: March 15, 2023
Accepted: March 21, 2023
Published: March 29, 2023



https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

step. Therefore, the main purpose of this study was to develop an understanding of the hop bitter acids' effect on the barley malt protein−protein complex formation during wort boiling. This was done in an effort to uncover the cause of the bitter compounds to the trub.

MATERIALS AND METHODS

Experimental Design and Wort Preparation. For this study, the design of the experiment (Figure 1) was used to explore the effect

Figure 1. Design of experiment.

of hop bitter acid addition on the wort boiled. The trials were conducted in triplicate, and the samples of sweet wort (SW), hopped wort (HW), and unhopped wort (UW) were collected to proceed with further analysis and characterization. The wort was produced using 100% Pilsner barley malt (Weyermann GMbH, Bamberg) ground to 1.2 mm and mixed with distilled water (1:4 w/w). The mashing was performed in a mash bath following the mashing scheme presented in Figure S1 (Supporting Information), which contains details of the time and temperature used. The sweet wort was separated from the brewer's spent grain by filtration using paper filter. To proceed to the wort boiling, the sweet wort was divided into two portions of 1 L. Hop extract obtained from the Hopsteiner Company (Nuremberg, Germany), containing 46.9% of α-acids, was added to one of these 1 L portions. The boiling lasted 60 min, and the wort was cooled down to 20 °C. Prior to sampling, the wort was filtered to separate the trub formed during boiling. The samples were stored in a freezer at −18 °C until analyzed. All experiments were conducted in triplicate.

Wort Characterization. Table 1 shows the analyses performed for wort characterization. The methodologies were according to the Mitteleuropäische Brautechnische Analysenkommission e.V. (MEBAK). The complete wort characterization data are present in Table S1 (Supporting Information).

Table 1. Wort Characterization Methodologies According to MEBAK

analysis	MEBAK method	MEBAK online reference
density[a]	B-590.08.904	19
real extract[a]	B-590.10.181	20
apparent extract[a]	B-590.09.900	21
pH[a]	B-590.00.040	22
original gravity[a]	B-590.10.181	20
calorie[a]	B-590.78.999	23
total nitrogen	B-400.07.003	24
free amino nitrogen (FAN)	B-400.11.111	25
total polyphenols	B-590.41.111	26
β-glucan	B-400.26.900	27
bitter units (BU)	B-400.17.110	28

[a]Analyses performed on the Density Meter DMA 4500M equipment (Anton Paar GmbH, Graz, Austria).

Bitter Acids by HPLC Analysis. The α- and *iso*-α-acids homologues were analyzed according to ASBC-Methods of Analysis Beer 23-C using an Agilent 1200 series HPLC system (Agilent Technologies, Böblingen, Germany) at a flow rate of 1.0 mL min^{-1} with a 5 μL injection volume. Two mobile phases were used: mobile phase B was 100% methanol, and mobile phase A was 59% methanol, 40% water, and 1% phosphoric acid (85%). The elution began with 100% of mobile phase A for the first 28 min and changed to 100% B over the next 12 min. The last 15 min of analysis corresponded to 100% of phase A. A Purosphere Star LC-18 5 μm silica column (250 mm × 4.6 mm, Merck, Darmstadt, Germany) was used for separation. Absorbance was measured at 270 and 314 nm. ASBC International Calibration Extracts ICS-I4 (*iso*-α-acids) and an ICE4 standardized hop extract containing α- and β-acids were used as standards for all measurements.

Hydropathy and Isoelectric Point Determination. To evaluate the physicochemical profile of the proteins, it was calculated the grand average of the protein classes' hydropathic value (GRAVY) profile by the gravy calculator (https://www.bioinformatics.org/sms2/protein_gravy.html) using the protein sequence in FASTA format obtained from the Uniprot database. The isoelectric point (PI) was calculated using the isoelectric point calculator (https://www.bioinformatics.org/sms2/protein_iep.html).[29]

Protein Extraction and Digestion. Wort proteins were extracted by acetone precipitation. 200 μL of wort was transferred to an Eppendorf tube, and 800 μL of cold acetone (at −20 °C) was added. The mixture was incubated for 90 min at −20 °C, followed by 10 min centrifugation (Concentrator 5305 plus, Eppendorf, Germany) at 13,000× *g*. The supernatant was removed, and the pellet was air-dried for 30 min at room temperature. The protein pellets were resuspended with sodium dodecyl sulfate (SDS) sample buffer and boiled for 5 min at 95 °C. The samples were centrifuged at 17,500× *g* for 10 min. The proteins were separated by SDS-PAGE gel using a Tris-Glycine SDS running buffer. The gels were stained with colloidal Coomassie blue, followed by overnight washing. The SDS-PAGE gel was cut into gel pieces. 250 μL of 50 mM ammonium bicarbonate (ABC) was added and shaken for 30 min at 37 °C. The gel washing was performed with the subsequent addition of acetonitrile (ACN) and 50 mM ABC solution. The protein reduction took place in a shaker at 37 °C for 30 min with 150 μL of reduction buffer (10 mM dithiothreitol (DTT) in 50 mM ABC solution). This was followed by the addition of 150 μL of alkylation buffer (55 mM iodoacetamide (IAA) in 55 mM ABC buffer) and incubated in the darkness at room temperature for 20 min. The alkylation buffer was removed, and 150 μL of ACN was added to shrink the gel pieces for 5 min. The digestion was performed with the addition of 150 μL of trypsin buffer (0.005 μg μL^{-1} trypsin in 50 mM ABC, 5% ACN (v/v)), and the digestion proceeded overnight at 37 °C. The digestion was terminated by acidification with 6 μL of 10% trifluoroacetic acid (TFA), shaking for 15 min. Peptides were desalted using C18 StageTips as described by Rappsilber et al.[30] and then were eluted from StageTips with 2 × 10 μL of 80% acetonitrile and 0.1% TFA. The samples were dried by vacuum centrifugation (Concentrator 5305 plus, Eppendorf, Germany) to be dissolved in 10 μL of 1.6% acetonitrile and 0.1% formic acid (FA) subsequently.

LC-MS/MS Analysis. Liquid chromatography coupled with mass spectrometry (LC-MS/MS) analyses were performed on a Thermo Scientific Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer coupled online to Ultimate 3000 RSLCnano Systems (Dionex, Thermo Fisher Scientific). The analytical column was a temperature-controlled EASY-Spray C18 LC column with a particle size of 2 μm and a length of 500 mm (Thermo Fisher Scientific, Germany) operated at 45 °C. Mobile phase A consisted of 0.1% FA in water; mobile phase B consisted of 80% acetonitrile and 0.1% FA.

Peptides were loaded onto the column and eluted at a flow rate of 0.3 μL min^{-1}. Peptides were separated by a series of linear gradients: 2−38% buffer B in 40 min, 38−52.5% in 4 min, and then 90% in 1 min. Eluted peptides were ionized by an EASY-Spray source (Thermo Scientific, Germany) and introduced directly into the mass spectrometer.

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

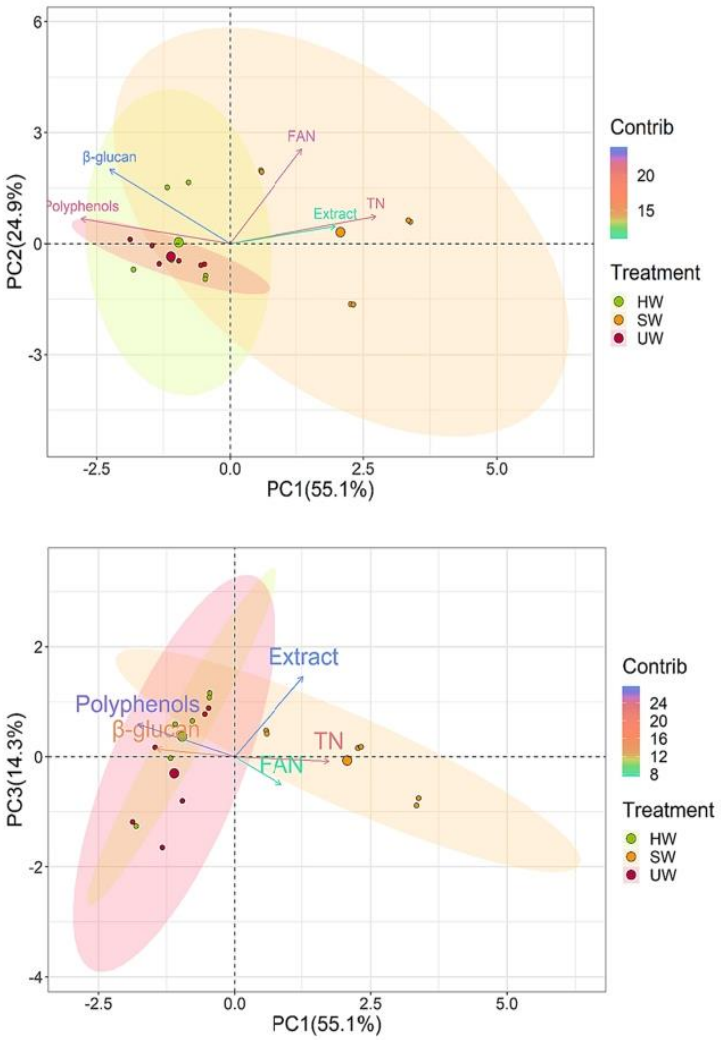


Figure 2. Wort composition showed in a principal component analysis: (a) PCA biplot components 1 and 2; (b) PCA biplot components 1 and 3; HW—hopped wort, SW—sweet wort, and UW—unhopped wort. *The large sample dots represent the centroid of the ellipse, whereas the small dots represent each replicate.

The MS data were acquired in the data-dependent mode. MS1 spectra were recorded at 120,000 resolution (scan range 350−1600 m/z). In each acquisition cycle, the 10 most intense peaks with a charge ≥2 were individually isolated with a 1.6 m/z window. The isolated ions were fragmented using higher-energy collisional dissociation (HCD) with stepped collision energy (27, 29, and 31%). The maximum injection time for MS1 scans was set to 50 ms and the automatic gain control (AGC) to 3.0E6 ions. The MS2 spectra were recorded at 15,000 resolution, maximum injection time of 80 ms, and AGC set to 1.0E5 ions. Dynamic exclusion was enabled with a single repeat count and 60 s exclusion duration.

Data Processing and Protein Identification. Protein identification was conducted using MaxQuant[30] version 1.6.12.0. The mass spectrometry data were searched against reference proteome: UP000011116 (downloaded from Uniprot). The default parameters in MaxQuant were applied. Label-free quantitation was performed with at least two peptides per protein. The final dataset contained the proteins quantified in three replicates of each treatment. The data were pretreated by filtering the noise and $\log_2$ transformation in Perseus 1.6 software.[31] Each protein was analyzed by Student's t-test using Perseus's default parameters. The resultant matrix was used for the protein class analysis in Microsoft Excel (Supporting Information File). The protein groups that belonged to the same class (Panther database) or family (Uniprot) were merged due to the lack of information regarding gene names. The protein relative abundance matrix was built by summation of MaxLFQ normalized protein intensities of protein groups that map to the same class/family. The replicate's mean abundance was calculated (missing values were discarded). The percentual difference was calculated using the absolute difference between the treatments for HW. The enrichment analysis was conducted in Microsoft Excel with the protein classes matching the gene ontology (GO) terms extracted from the Uniprot database.

Statistical Analysis. The wort composition dataset was statistically evaluated by one-way ANOVA with post hoc Tukey's test using Minitab software. Additionally, for this dataset, the unsupervised analysis using principal component analysis (PCA) was performed in RStudio (package: FactomineR) to assess the groupings or trends in the data. The plots of scores and loadings were used to determine the number of principal components (Supporting Table S2).

RESULTS AND DISCUSSION

Effect of Hop Bitter Acids on Wort Composition and Hop Bitter Acid Utilization. Wort is a complex mixture of soluble and suspended proteins, carbohydrates, and small organic molecules. To understand the hop addition influence on the wort composition, an unsupervised PCA was

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

performed. The principal components (PC) were selected according to the eigenvalues for PC since the eigenvalues under 0.7 demonstrate lower significance.[32] The first three and most important components can be seen in the biplot of Figure 2a,b, which captures 94.33% of the accumulated variance (Supporting Table S2). The plot shows the clusters of the treatments, and notably, the cluster of the SW samples demonstrated a separation in comparison with the clusters of HW and UW samples. This clustering indicates that the higher influence was the wort boiling step. The polyphenol content and total nitrogen (TN) represent the higher contribution (Supporting Figure S3b) to the PC1 (first component), which was able to separate the samples before and after the wort boiling. The PC2 was explained mainly by the variables FAN (free amino nitrogen) and β-glucan (Supporting Figure S3c). The plot PC1 vs PC2 demonstrates a negative correlation between the β-glucan and polyphenol content with the extract, FAN, and TN. From Table S3, it can be seen that the TN, FAN, and extract content decreased due to the boiling step.

The TN content is commonly determined to measure the dissolved nitrogenous materials in the wort, which are in good correlation with the protein content.[16] It is well known that the protein thermally denatures during wort boiling, leading to the exposure of the hydrophobic sites.[33] To promote the thermodynamic stabilization of the protein molecule, they form a colloidal structure through hydrophobic interactions.[34] This phenomenon can be clearly seen in the TN content that shows a significant ($p < 0.05$) decrease in the samples after boiling. However, there were no significant differences between the TN in the HW and UW treatments, demonstrating that the hop bitter acid addition had a slight effect on the total protein content of the wort. Proteins play a fundamental role in determining beer quality, especially with respect to foam and haze stability. Therefore, protein precipitation directly affects the sensorial appearance of the beer and consumer acceptance.[17] A negative correlation of −0.72 of TN and polyphenol content was observed (Supporting Figure S3), suggesting an intermolecular interaction between them, i.e., an increase in TN leads to a reduction in polyphenol content, thereby promoting their precipitation with the trub. This is also in accordance with the literature.[35]

As shown in Figure 2a, it is noticed that there was no significant separation in the clusters in the second dimension, indicating that the hop addition and the boiling step had a lower influence on the FAN and β-glucan content. A slight decrease in the FAN content in HW and UW in comparison to SW (Supporting Table S3) was observed. FAN is correlated with the measurement of the amino acid in the wort, being an important malt quality parameter.[16] In addition, amino acids are the minor subunit of protein that are involved in the fermentation performance.[36] The amino acids also cause the buffering of the wort and react with reducing sugar through Maillard reactions.[37,38] Therefore, the FAN content decreased, as expected, due to the Maillard reaction, forming melanoidins that contribute to the brown color of the wort.[38] Furthermore, no significant difference ($p < 0.05$) was observed with the hop addition. These results confirm the Gänz et al.[9] findings, which demonstrated that there was no interaction between FAN and hop bitter acids.

Hop bitter acid analysis by HPLC was performed only in the HW samples since no hops were added to the UW samples, and therefore, no hop bitter acids were expected to be present. The results of the main α- and *iso*-α-acid homologues and the total content are set out in Figure 3. 23.32% of the total α-acids added were converted into *iso*-α-acids. Accordingly, 56% of the

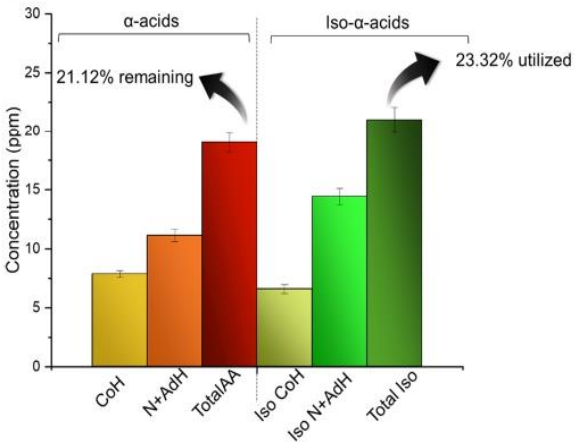


Figure 3. Hop α- and *iso*-α-acid homologue concentration in the hopped wort; CoH: cohumulone, N + AdH: N- and adhumulone, Total AA: total α-acids, Iso CoH: isocohumulone, Iso N + AdH: *iso*-N- and *iso*-adhumulone, and Total Iso: total *Iso*-α-acids.

bitter compounds added were lost, which may be explained by linkages with particular proteins in the aggregates and concomitant precipitation with the trub.[9] Consequently, a higher proportion of hops need to be added to achieve a certain bitterness in the finished beer, thus representing higher production costs. The loss of bitter compounds during wort boiling is a common issue for the brewing industry.

Effect of Hop Compounds on the Barley Malt Protein Profile. Barley is generally composed of 8−15% protein that becomes modified throughout the malting and brewing process.[39] The proteins play an important role in beer quality parameters, such as mouthfeel, foam formation, and haze activity.[17] In this study, proteomic analysis was performed to identify the global proteome of the boiled wort to investigate the influence of hop bitter compounds. For that, LC-MS/MS analysis was used, identifying 83 protein groups. The label-free quantitation (LFQ) data were preprocessed using Perseus software, and the resulting annotation matrix was extracted for further data analysis (Supporting Information File). To investigate the biophysical properties of the boiled wort proteome, the isoelectric point (PI) and grand average of hydropathicity (GRAVY) index were calculated by the FASTA protein sequence in the PI and GRAVY calculator for each protein group identified from HW and UW treatments.[29] The barplot (Figure 4) provides the intercorrelations among the PI and GRAVY index for the protein groups found in both treatments. The GRAVY index demonstrates the hydrophobicity of the proteins, with the positive values representing the hydrophobic proteins, whereas the negative values correlate with the hydrophilic proteins. Many proteins show negative scores, ranging from −1.7 to 0.0 (Figure 4). This indicates that the proteins of the wort in both treatments are more hydrophilic. The study of Mahalingam et al.[40] showed a hydrophilicity tendency of the proteins in dry matured seeds, which demonstrated an inner characteristic of the barley proteomic. In the current study, the boiled wort's higher hydrophilicity is explained by the extraction of the water-soluble proteins after the mashing step.

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

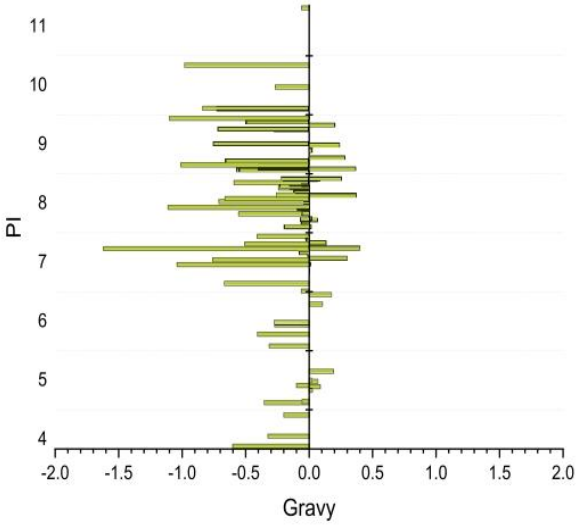


Figure 4. HW and UW protein classes isoelectric point (PI) vs grand average hydrophaticity (GRAVY). *More detailed information about the protein groups is found in the Supporting Information File.

The PI directly affects the solubility of a protein molecule at a certain pH. The protein contains a neutral net charge at the PI, and it is likely to precipitate due to the lack of electrostatic repulsion and the reduction of the solvation by water molecules, promoting aggregation and precipitation by hydrophobic interactions.[41] The PI of protein groups found in the boiled wort range from 3.8 to 11.31, whereas the plant proteome PI normally stands out between 1.99 and 13.96.[42] Despite the barley proteins mainly having an acidic PI, the peak was observed in the alkaline PI with 56 protein groups within the 7.0−10.0 range (Supporting Information File) in contrast to 17 protein groups in the acidic range (3.5−6.0).[42] The wort pH after boiling was at 5.6, which explains the diminished content of proteins in the acidic pH due to lower solubility, resulting in protein aggregation and consequently precipitation. The protein location in a cellular environment demonstrated a correlation with the PI, influencing the protein's molecular function and biological processes.[43] Of the protein groups with alkaline PI, the lipid process-related and storage proteins increase significantly (Supporting Table S4) in comparison to the acidic PI range. The alkaline protein is represented mainly by lipid transfer proteins (LTPs) and hordeins.

The GO terms for the molecular function (MF) and biological process (BP) were matched from the Uniprot *Hordeum vulgare* vulgaris database in Perseus software by an annotation tool, and the enrichment analysis presented in the donut chart in Figure 5a,b was performed in a Microsoft Excel datasheet (Supporting Information File). The labels of MF or BP with a percentage under 2% were removed from the chart, which can be seen in the Supporting Information File for further details. It is noticed that the hop addition had a low influence on the protein profile regarding the most abundant MF and BP in comparison with the wort without hop compounds. The most abundant proteins have the following molecular functions: protease inhibitor (28%), other or undefined (15%), nutrient reservoir activity (15%), lipid binding (13%), and metal binding (7%) functions. Likewise, the main biological process in the remaining proteins of the wort is related to the plant defense, which is also reflected in the molecular function. However, due to the lack of

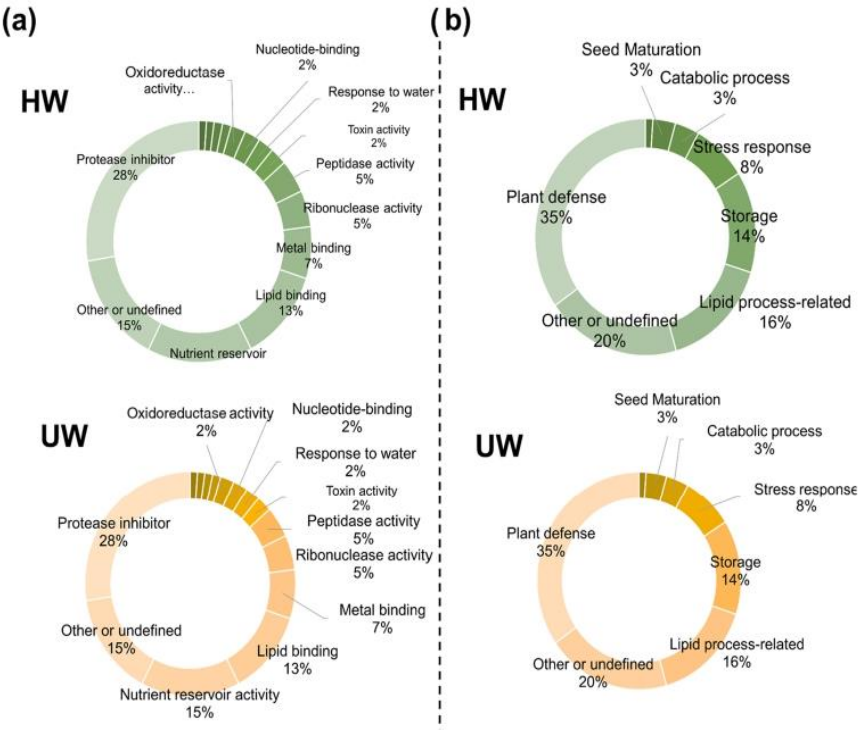


Figure 5. Biophysical profile of protein classes: (a) HW and UW protein class molecular function; (b) HW and UW protein class biological function.

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

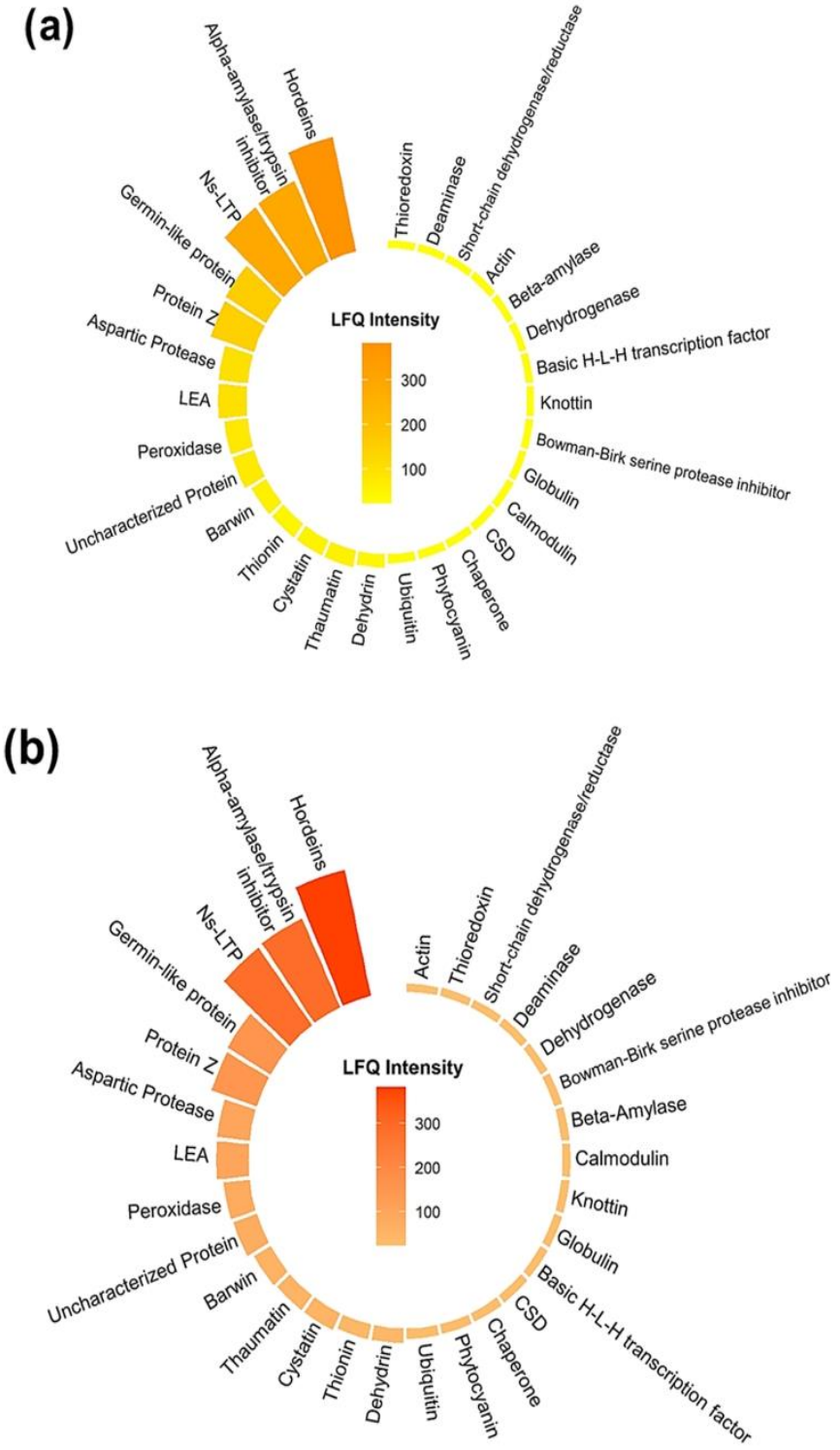


Figure 6. Summary of the HW and UW proteomic analysis: (a) circular barplot of the relative label-free quantitation (LFQ) abundance of the protein classes of HW treatment; (b) circular barplot of the relative LFQ abundance of the protein classes of UW treatment. *The color of each chart is merely a representation of treatment difference.

information in the database, 20% of the proteins belonged to the other or undefined category. Also, the lipid process-related and storage biological processes stand out as the main ones that can be seen in the molecular function. The barley proteome is the largest one known in the plant kingdom, containing proteins for several molecular functions and biological processes, which play an important role in the organism's survival.[42] In that way, the plants evolved along their evolution as a mechanism for defense against microbial attacks. In this study, numerous defense proteins were

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

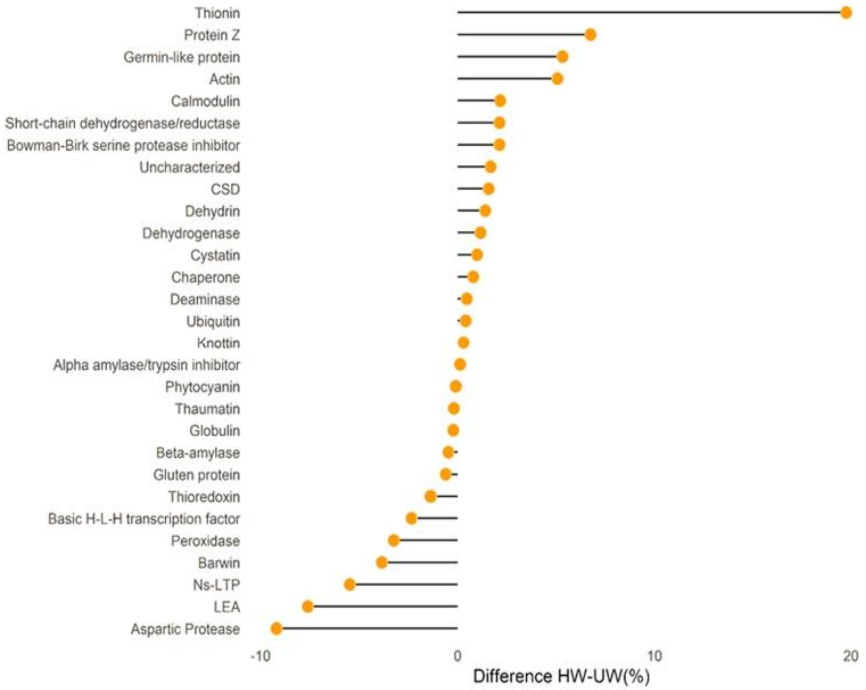


Figure 7. Summary of the HW and UW proteomic analysis: lollipop chart of the percentage difference of the protein class abundance between HW and UW treatments. *The difference HW−UW was calculated according to the equation: difference HW − UW (%) = $\frac{(\overline{HW} - \overline{UW})}{\overline{HW}}$

identified, specifically protease inhibitors, such as amylase and trypsin inhibitors, serine protease inhibitors, and thionins. Normally, those proteins correlate with the exposure of the barley to pathogens and pests, which is mainly dependent on the growing conditions. They act by inhibiting subtilisin-type serine proteases of pathogens and pests, despite their biological mechanisms being still unknown.[44] Hence, reducing contamination might deplete the production of those proteins.

The MF and BP results are correlated with protein abundance. Amongst the most abundant proteins were those that play a role in beer haze and foam formation. The most abundant proteins are represented by the hordeins, which are storage proteins responsible for the haze activity in beer (Figure 6a,b). Due to the incompleteness of entries in the Uniprot database for barley proteins, the protein groups were identified by their homologues from the wheat proteins, namely, gliadin and glutenin, which are present in gluten (Supporting Information File). This protein class count with a complex polymorphic mixture of proteins. The haze-active protein classes of α-amylase/trypsin inhibitors and germin-like proteins are also the most found in wort and beer. Moreover, it can be seen that the foam-related LTP and protein Z are present in higher amounts in the wort. These data are consistent with the wort and beer proteome coverage.[45−48]

The protein modifications are common in the malting and brewing process. To understand the influence of hop bitter acids on protein−protein interactions and the complex formation, the difference in the abundance of each protein class among the treatments was calculated, and it is presented in a lollipop chart in Figure 7. These results did not show a significant difference with the hop addition regarding the most abundant haze-active protein classes, such as hordeins and α-amylase/trypsin inhibitors. In addition, the foam-active protein classes (LTP and protein Z) responded inversely to the hop bitter acids addition. The LTP was more abundant in the presence of hop compounds than protein Z.

However, the minor protein classes changed considerably, as shown in the chart (Figure 7), whereas those proteins are not related to the beer quality. Amongst them, thionin shows the highest difference between the treatments, being more abundant in the HW than in the UW.[44,49−57] On the other hand, the aspartic protease and late embryogenesis abundant protein (LEA) summed up 17% of the difference, being more abundant in the UW, i.e., they were precipitated in the presence of hop bitter compounds. Thionin has been reported as a defense protein, presenting high toxicity toward pathogens, specifically fungi, and bacteria.[58] They are classified as small and cysteine-rich proteins, forming disulfide bridges to stabilize the tertiary structure,[59] whereas the LEA protein family is related to stress response to water deficit and salt stress.[60]

Protein folding is based on the hydrophobic effect in which the molecules undergo hydrophobic interactions with the hydrophobic side chains of the amino acids and isolate themselves within the protein structure to increase the stability in an aqueous solution.[34,61] Moreover, the protein commonly rises its tertiary structure stability by disulfide bonds among cysteine residues.[62] However, the wort boiling induces thermal denaturation, leading to the protein unfolding.[61] The Jin et al.[63] study demonstrated that barley malt proteins unfold during the wort boiling, exposing the inner hydrophobic groups. The unfolded proteins form protein−protein aggregates due to the exposed hydrophobic groups' interaction to promote thermodynamical stabilization.[33,63] Furthermore, the protein denaturation leads to the reduction of the disulfide bonds, which affects the protein stability and the tertiary structure of the cysteine residues exposed.[63,64]

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

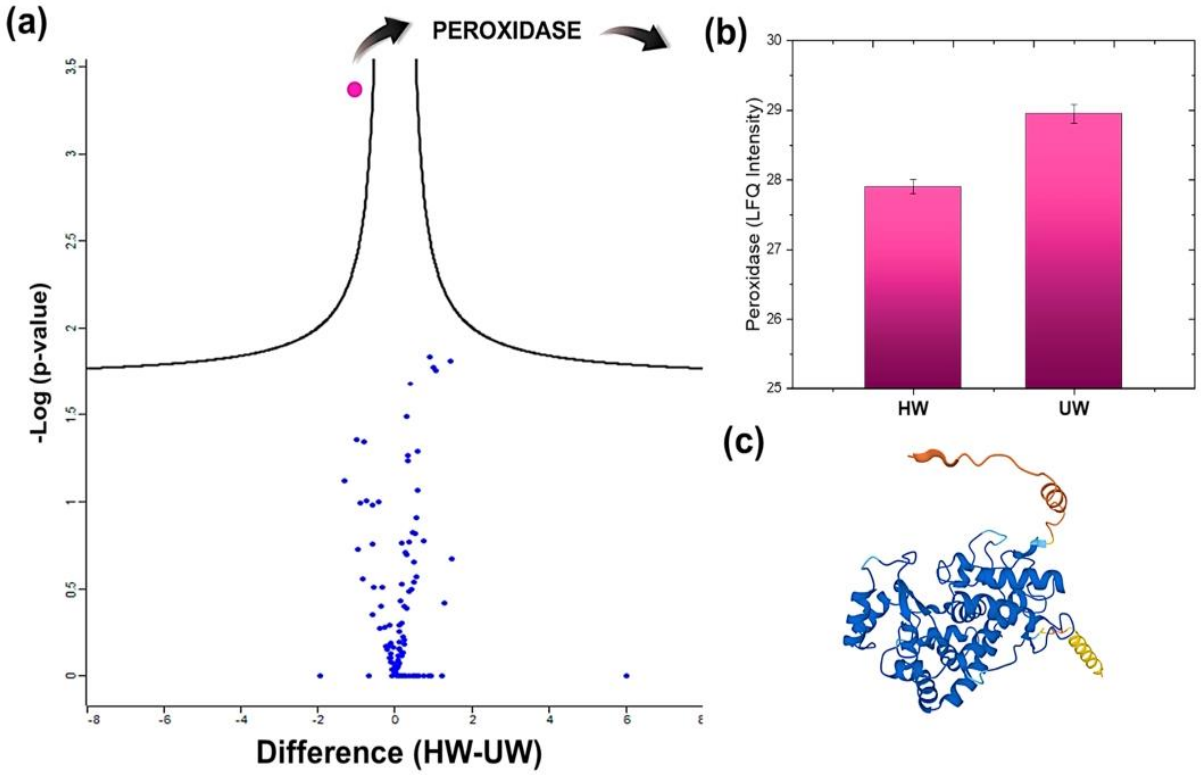


Figure 8. Statistical analysis of the protein groups: (a) volcano plot constructed from Student's *t*-test ($p > 0.05$); (b) peroxidase label-free quantitation (LFQ) intensity in HW and UW treatments; (c) peroxidase structure extracted from the AlphaFold database.[74,75] *Difference (HW−UW) is between the LFQ abundance of the average of each treatment.

This study showed that the composition of the protein−protein complex changes due to the addition of hop bitter acids (Figure 7). Since the abundance of the haze-active proteins remains similar in both treatments, it is suggested that those proteins form the backbone of the protein−protein complex.[65] The lower-molecular-weight protein interacts by hydrophobic interaction and/or a disulfide bond with the hydrophobic domains and cysteine residues, respectively. This commonly occurs in food protein cross-linking to promote protein stabilization in the medium by the formation of layers in the aggregate.[66] On the other hand, it is interesting to note that the protein−protein complex formation is altered due to the influence of hop bitter acids on protein cross-linking. As mentioned in the literature review, the *iso*-α-acids form a vesicular structure with LTP and protein Z. It was proposed by Lu et al.[67] that the protein Z plays a major role in the formation of the aggregates with *iso*-α-acids in comparison to LTP due to the alternating hydrophilic and hydrophobic regions distributed along the polypeptide chain, and a vesicular structure is formed to avoid the exposure of the hydrophobic domains. As suggested by Lu et al.,[68] the *iso*-α-acids act as a cross-linker within the protein layer adsorbed. It is already known that protein−protein adsorption is influenced by the order in which the protein adsorbs onto a pre-adsorbed layer, as was demonstrated by the Lu et al.[68] study. The protein Z adsorbs to the LTP surface due to the positive charge given to the surface by LTP, creating an electrostatic attraction, whereas the LTP does not adsorb to the surface pre-adsorbed by protein Z. This phenomenon was also described by Örnebro et al.,[69] the gliadin fraction adsorption relies on the aggregation order. Also, a study of Lu et al.[70] demonstrated that *iso*-α-acids are prone to adsorb in thick layers at hydrophobic interfaces through hydrophobic interactions, forming colloidal particles. They also showed that the presence of additional ions influences the aggregation properties of *iso*-α-acids.

Over the past decades, less attention has been paid to the less abundant and non-foam-active protein interactions with hop bitter acids. Surprisingly, this study demonstrated that they might play a role in the hop bitter acids' linkage to the protein in the aggregates due to hydrophobic domains as well as cysteine residues. Cysteine is known as a biological nucleophile that is able to react with electrophiles and form a covalent bond, despite its selective reaction.[71] The role of cysteine binding to aldehydes was uncovered by the studies of Baert et al.[72,73] They demonstrated that cysteine interacts with aldehydes due to an increased nucleophilic character of the sulfhydryl group. Similarly, this effect might occur between the hop bitter acids and the exposed cysteine groups during the wort boiling. The carbonyl carbon atom becomes more electrophilic due to the higher electronegativity of the oxygen, being prone to react with the free thiol group of the cysteine.[71] Therefore, hop bitter acids could act as a cross-linker, binding to the protein and facilitating the aggregation of a certain fraction of proteins instead of others. Furthermore, the proteins that were most affected by the hop addition are not involved directly in beer quality. Their production might be suppressed by barley breeding. However, the protein cross-linking and modification due to thermal energy should be more comprehensively investigated to uncover the mechanism of protein aggregate formation.

Single Protein Group(s) Likely to Precipitate in the Presence of Hops. The dataset of the protein groups was

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

statistically analyzed by Student's *t*-test to evaluate the differential significance of the hop addition. To further explore the proteome measurements, a volcano plot was generated to compare the protein abundance differences between the HW and UW treatments (Figure 8a). The differential abundance was plotted against $\log_2$ (*p*-value), and the results are refined according to the significance *p*-value > 0.05. The results revealed that peroxidase abundance was significantly modified by the hop addition, being present in higher abundance in the wort without a hop compound. This suggests that this protein group is likely to precipitate with the addition of hop bitter acids and therefore is prone to react with them.

Peroxidase is present in almost all living organisms. In plants, they participate in myriad biological processes, such as stress response, lignification, and most importantly, the defense against pathogens.[59,76,77] This oxidoreductase enzyme performs a protective activity due to its capability to catalyze the oxidative cross-linking of proteins and phenolics in the plant cell wall, protecting the plant from degradation.[59,78] The peroxidase superfamily comprises three classes in which class III contains the most representative barley grain peroxidase group, BP1. The structure of the peroxidase BP1 (Figure 8c) was determined by Henriksen et al.,[79] revealing a heme group sandwiched between a distal N-terminal domain and a proximal C-terminal domain. The molecule comprises α-helix structures, which are common in peroxidases. The active site structure contains a heme iron surrounded by histidine bound by a hydrogen bond to aspartate, and the heme is embedded in a hydrophobic environment. In addition, the protein comprises cation binding sites composed of Ca^{2+}, which is important for enzyme activity, carbohydrate structure to increase the structure stability, and cysteine residues that form disulfide bonds and stabilize the β-sheet site.[79]

The heme-containing peroxidase reacts with hydrogen peroxide by iron oxidation, forming a radical, which indicates its ability to react with species through an oxidoreduction reaction.[59] It is already known that peroxidase causes oxidation of polyphenols in beer, increasing the turbidity.[74] During the wort boiling, the protein thermally denatures, causing its unfolding and the exposing of the active sites containing the heme group as well as the hydrophobic sites and cysteine residues. The transition-metal ions are involved in several reactions to deplete the flavor stability in beer due to their capacity to form oxidative radicals.[75] Within the compounds that are reactive to transition-metal ions, specifically ferrous iron stands the hop bitter acids.[80] Wietstock et al.[81] elucidated the antioxidant activity of hop α- and *iso*-α-acids by the formation of complexes with Fe^{2+}. Those hop bitter acids chelate the iron ion, demonstrating the ability to bind covalently to form the complex hop bitter acid-metal.[80] Comparably, this linkage might occur between the exposed heme group of the unfolded peroxidase with hop bitter acids throughout wort boiling. Aside from the hop bitter acid interaction with the protein classes discussed previously, peroxidase is suggested to play a major role in the hop bitter acid interaction in the protein aggregate formation. Clearly, the situation is complex, with several possible interactions. However, it might lead to the hop bitter acids binding to the protein−protein complex, which is precipitated to the trub. Further investigation about the mechanism of hop bitter acid linkage to the protein sites should take place to uncover the cause of protein aggregates and hop bitter acid interactions.

In summary, this study presented for the first time the correlation between the hop bitter acid addition and barley malt protein profile. Although the hop addition did not show a significant influence on the overall wort composition, it could be seen that the protein−protein complex formation was modified due to the addition of hop compounds. These findings suggest that hop α- and *iso*-α-acids might interact with the cysteine and/or the active sites of peroxidase during the boiling step. The empirical findings of this study provide a new understanding of the role of barley malt protein in the brewing process. Further research is needed to explore the mechanism of those protein−ligand bindings.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.3c00185.

Additional details of the methodology and data analysis (PDF)

Additional details of the proteomics dataset (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Philip C. Wietstock − *Chair of Brewing and Beverage Technology, Department of Food Technology and Food Chemistry, Technische Universität Berlin, 13355 Berlin, Germany*; Email: philip.wietstock@tu-berlin.de

Authors

Mariana B. C. Pinto − *Fruit, Vegetable and Confectionery Products Laboratory, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, 13083-862 Campinas, São Paulo, Brazil; Chair of Brewing and Beverage Technology, Department of Food Technology and Food Chemistry, Technische Universität Berlin, 13355 Berlin, Germany*; orcid.org/0000-0002-3761-8731

Flavio L. Schmidt − *Fruit, Vegetable and Confectionery Products Laboratory, Department of Food Engineering and Technology, School of Food Engineering, Universidade Estadual de Campinas, 13083-862 Campinas, São Paulo, Brazil*

Juri Rappsilber − *Bioanalytics, Institute of Biotechnology, Technische Universität Berlin, 13355 Berlin, Germany*

Brian Gibson − *Chair of Brewing and Beverage Technology, Department of Food Technology and Food Chemistry, Technische Universität Berlin, 13355 Berlin, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.3c00185

Funding

The authors appreciate the support of the CNPq, Research National Council, Brazil (Finance code 141485/2018-3) and CAPES, Coordination of Superior Level Staff Improvement, Brazil (Finance code 88887.575986/2020-00) for Ph.D. CAPES-PRINT Sandwich Program.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge the companies IREKS and HOPSTEINER for the material donations.

■ ABBREVIATIONS USED

SW, sweet wort; HW, hopped wort; UW, unhopped wort; MEBAK, Mitteleuropäische Brautechnische Analysenkommission; HPLC, high-pressure liquid chromatography; GRAVY, grand average hydropathic value; PI, isoelectric point; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; ABC, ammonium bicarbonate; ACN, acetonitrile; DTT, dithiothreitol; IAA, iodoacetamide; TFA, trifluoroacetic acid; FA, formic acid; LC-MS/MS, liquid chromatography coupled with mass spectrometry; MS, mass spectrometry; HCD, higher-energy collisional dissociation; AGC, automatic gain control; GO, gene ontology; PCA, principal component analysis; PC, principal component; TN, total nitrogen; FAN, free amino nitrogen; LFQ, label-free quantitation; LTP, lipid transfer proteins; MF, molecular function; BP, biological process; LEA, late embryogenesis abundant protein

■ REFERENCES

(1) Lewis, M. J.; Bamforth, C. W. *Essays in Brewing Science*; Springer Science+Business: New York, 2006.

(2) Barth, Stephan J.; Meier, H. *The Barth Haas Report*; Nuremberg2021.

(3) dos Santos Mathias, T. R.; Alexandre, V. M. F.; Cammarota, M. C.; de Mello, P. P. M.; Sérvulo, E. F. C. Characterization and Determination of Brewer's Solid Wastes Composition. *J. Inst. Brew.* **2015**, *121*, 400−404.

(4) Kunze, W. *Technology Brewing and Malting*, 6th ed.; Hendel, O., Ed.; VLB Berlin: Berlin, 2019.

(5) Hauser, D. G.; Shellhammer, T. H. An Overview of Sustainability Challenges in Beer Production, and the Carbon Footprint of Hops Production. *Tech. Q.* **2019**, *56*, 95−100.

(6) Jaskula, B.; Kafarski, P.; Aerts, G.; De Cooman, L. A Kinetic Study on the Isomerization of Hop Alpha-Acids. *J. Agric. Food Chem.* **2008**, *56*, 6408−6415.

(7) de Clippeleer, J.; de Cooman, L.; Aerts, G. Beer's Bitter Compounds − A Detailed Review on Iso-α-Acids: Current Knowledge of the Mechanisms for Their Formation and Degradation. *Brew. Sci.* **2014**, *67*, 167−182.

(8) Verzele, M.; van Boven, M. The Isomerization Mechanism of Humulone. *Bull. Soc. Chim. Belg.* **1971**, *80*, 677−682.

(9) Gänz, N.; Becher, T.; Drusch, S.; Titze, J. Interaction of Proteins and Amino Acids with Iso-α-Acids during Wort Preparation in the Brewhouse. *Eur. Food Res. Technol.* **2021**, *248*, 741−750.

(10) Kapp, G. R.; Bamforth, C. W. The Foaming Properties of Proteins Isolated from Barley. *J. Sci. Food Agric.* **2002**, *82*, 1276−1281.

(11) Clark, D. C.; Wilde, P. J.; Wilson, D. R. The Effect of Pre-Isomerised Hop Extract on the Properties of Model Protein Stabilized Foams. *J. Inst. Brew.* **1991**, *97*, 169−172.

(12) Evans, D. E.; Surrel, A.; Sheehy, M.; Stewart, D. C.; Robinson, L. H. Comparison of Foam Quality and the Influence of Hop α-Acids and Proteins Using Five Foam Analysis Methods. *J. Am. Soc. Brew. Chem.* **2018**, *66*, 1−10.

(13) Smith, R. J.; Davidson, D.; Wilson, R. J. H. Natural Foam Stabilizing and Bittering Compounds Derived from Hops. *J. Am. Soc. Brew. Chem.* **1998**, *56*, 52−57.

(14) Ferreira, I. M. P. L. V. O.; Jorge, K.; Nogueira, L. C.; Silva, F.; Trugo, L. C. Effects of the Combination of Hydrophobic Polypeptides, Iso-α Acids, and Malto-Oligosaccharides on Beer Foam Stability. *J. Agric. Food Chem.* **2005**, *53*, 4976−4981.

(15) Iimure, T.; Nankaku, N.; Kihara, M.; Yamada, S.; Sato, K. Proteome Analysis of the Wort Boiling Process. *Food Res. Int.* **2012**, *45*, 262−271.

(16) Briggs, D. E.; Boulton, C. A.; Brookes, P. A.; Stevens, R.*Brewing Science and Practice*; Woodhead Publishing, 2004.

(17) Steiner, E.; Gastl, M.; Becker, T. Protein Changes during Malting and Brewing with Focus on Haze and Foam Formation: A Review. *Eur. Food Res. Technol.* **2011**, *232*, 191−204.

(18) Lu, Y.; Osmark, P.; Bergenståhl, B.; Lars. Vesicular Structures Formed from Barley Wort Proteins and Iso-Humulone. *Food Hydrocolloids* **2020**, *105*, No. 105788.

(19) MEBAK online. *Method B-590.08.904. Dichte − Biegeschwinger.* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising, BY, Germany. https://www.mebak.org/methode/b-590-08-904/dichte-biegeschwinger/771. Accessed August 31,2022.

(20) MEBAK online. *Methode B-590.10.181. Stammwürze, Extrakt und Alkohol − Biegeschwinger und NIR.* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-590-10-181/stammwuerze-extrakt-und-alkohol-biegeschwinger-und-nir/776. Accessed August 31,2022.

(21) MEBAK online. *Methode B-590.09.900. Scheinbarer Extrakt.* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-590-09-900/scheinbarer-extrakt/774. Accessed August 31,2022.

(22) MEBAK online. *Methode B-590.00.040. pH-Messung.* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-590-00-040/ph-messung/762. Accessed August 31,2022.

(23) MEBAK online. *Methode B-590.78.999. Physiologischer Brennwert − exakte Methode.* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-590-78-999/physiologischer-brennwert-exakte-methode/2295. Accessed August 31,2022.

(24) MEBAK online. *Methode B-400.07.003. Gesamtstickstoff in Würze und Bier − KJELDAHL.* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-400-07-003/gesamtstickstoff-in-wuerze-und-bier-kjeldahl/649. Accessed August 31,2022.

(25) MEBAK online. *Methode B-400.11.111. Freier Amino-Stickstoff (FAN) − Ninhydrin-Methode (spektralphotometrisch).* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-400-11-111/freier-amino-stickstoff-fan-ninhydrin-methode-spektralphotometrisch/655.Accessed August 31,2022.

(26) MEBAK online. *Methode B-590.41.111. Gesamtpolyphenole.* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-590-41-111/gesamtpolyphenole/842. Accessed August 31,2022.

(27) MEBAK online. *Methode B-400.26.900. Hochmolekulares β-Glucan − fluorimetrisch. Rev. 2020-10. Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising.* https://www.mebak.org/methode/b-400-26-900/hochmolekulares-glucan-fluorimetrisch/677. Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-400-26-900/hochmolekulares-glucan-fluorimetrisch/677. Accessed August 31,2022.

(28) MEBAK online. *Methode B-400.17.110. Bittereinheiten.* Mitteleuropäische Brautechnische Analysenkommission (MEBAK) e.V., Freising. https://www.mebak.org/methode/b-400-17-110/bittereinheiten/663. Accessed August 31,2022.

(29) Stothard, P. Internet On-Ramp The Sequence Manipulation Suite: JavaScript Programs for Analyzing and Formatting Protein and DNA Sequences. *Biotechniques* **2000**, *28*, 1102−1104.

(30) Rappsilber, J.; Ishihama, Y.; Mann, M. Stop and Go Extraction Tips for Matrix-Assisted Laser Desorption/Ionization, Nanoelectrospray, and LC/MS Sample Pretreatment in Proteomics. *Anal. Chem.* **2003**, *75*, 663−670.

(31) Tyanova, S.; Temu, T.; Sinitcyn, P.; Carlson, A.; Hein, M. Y.; Geiger, T.; Mann, M.; Cox, J. The Perseus Computational Platform for Comprehensive Analysis of (Prote)Omics Data. *Nat. Methods* **2016**, *13*, 731−740.

(32) Jolliffe, I. T. Discarding Variables in a Principal Component Analysis. I: Artificial Data. *Appl. Stat.* **1972**, *21*, No. 160.

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

(33) Rayment, I. *Encyclopedia of Physical Science and Technology*, 3rd ed.; Meyes, Robert A., Ed.; Elsevier Science Ltd: Tarzana, California, USA, 2001.
(34) Dyson, H. J.; Wright, P. E.; Scheraga, H. A. The Role of Hydrophobic Interactions in Initiation and Propagation of Protein Folding. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13057−13061.
(35) dos Santos Mathias, T. R.; de Mello, P. P. M.; Sérvulo, E. F. C. Solid Wastes in Brewing Process: A Review. *J. Brew. Distill.* **2014**, *5*, 1−19.
(36) Hill, A. E.; Stewart, G. G. Free Amino Nitrogen in Brewing. *Fermentation.* **2019**, *5*, No. 22.
(37) Ribeiro-Filho, N.; Ayed, C.; Akepach, P. The Buffering Capacity of Single Amino Acids in Brewing Wort. *Int. J. Food Sci. Technol.* **2022**, *58*, 1611−1620.
(38) Martins, S. I. F. S.; Jongen, W. M. F.; van Boekel, M. A. J. S. A Review of Maillard Reaction in Food and Implications to Kinetic Modelling. *Trends Food Sci. Technol.* **2000**, *11*, 364−373.
(39) Bahmani, M.; O'Lone, C. E.; Juhász, A.; Nye-Wood, M.; Dunn, H.; Edwards, I. B.; Colgrave, M. L. Application of Mass Spectrometry-Based Proteomics to Barley Research. *J. Agric. Food Chem.* **2021**, *69*, 8591−8609.
(40) Mahalingam, R. Shotgun Proteomics of the Barley Seed Proteome. *BMC Genomics* **2017**, *18*, No. 44.
(41) Damodaran, S.; Parkin, K. L.; Fennema, O. R. *Química de Alimentos de Fennema*, 4th ed.; Grupo A - Artmed, 2000.
(42) Mohanta, T. K.; Khan, A.; Hashem, A.; Abdallah, E. F.; Al-Harrasi, A. The Molecular Mass and Isoelectric Point of Plant Proteomes. *BMC Genomics* **2019**, *20*, No. 631.
(43) Tokmakov, A. A.; Kurotani, A.; Sato, K. I. Protein PI and Intracellular Localization. *Front. Mol. Biosci.* **2021**, *8*, No. 1179.
(44) Nielsen, P. K.; Bønsager, B. C.; Fukuda, K.; Svensson, B. Barley α-Amylase/Subtilisin Inhibitor: Structure, Biophysics and Protein Engineering. *Biochim. Biophys. Acta, Proteins Proteomics* **2004**, *1696*, 157−164.
(45) Grochalová, M.; Konečná, H.; Stejskal, K.; Potěšil, D.; Fridrichová, D.; Srbová, E.; Ornerová, K.; Zdráhal, Z. Deep Coverage of the Beer Proteome. *J. Proteomics* **2017**, *162*, 119−124.
(46) Colgrave, M. L.; Goswami, H.; Howitt, C. A.; Tanner, G. J. What Is in a Beer? Proteomic Characterization and Relative Quantification of Hordein (Gluten) in Beer. *J. Proteome Res.* **2012**, *11*, 386−396.
(47) Iimure, T.; Sato, K. Beer Proteomics Analysis for Beer Quality Control and Malting Barley Breeding. *Food Res. Int.* **2013**, *54*, 1013−1020.
(48) Colgrave, M. L.; Goswami, H.; Howitt, C. A.; Tanner, G. J. Proteomics as a Tool to Understand the Complexity of Beer. *Food Res. Int.* **2013**, *54*, 1001−1012.
(49) Willows, R. D.; Worden, P.; Mirzaei, M. Barley Grain Proteomics. In *Proteomics in Food Science: From Farm to Fork*; Elsevier Inc., 2017; pp 75−88.
(50) Wang, Y.; Ye, L. Haze in Beer: Its Formation and Alleviating Strategies, from a Protein−Polyphenol Complex Angle. *Foods* **2021**, *10*, No. 3114.
(51) Ye, L.; Huang, Y.; Li, M.; Li, C.; Zhang, G. The Chemical Components in Malt Associated with Haze Formation in Beer. *J. Inst. Brew.* **2016**, *122*, 524−529.
(52) Iimure, T.; Nankaku, N.; Watanabe-Sugimoto, M.; Hirota, N.; Tiansu, Z.; Kihara, M.; Hayashi, K.; Ito, K.; Sato, K. Identification of Novel Haze-Active Beer Proteins by Proteome Analysis. *J. Cereal Sci.* **2009**, *49*, 141−147.
(53) Perrocheau, L.; Rogniaux, H.; Boivin, P.; Marion, D. Probing Heat-Stable Water-Soluble Proteins from Barley to Malt and Beer. *J. Agric. Food Chem.* **2005**, *5*, 2849−2858.
(54) Perrocheau, L.; Bakan, B.; Boivin, P.; Marion, D. Stability of Barley and Malt Lipid Transfer Protein 1 (LTP1) toward Heating and Reducing Agents: Relationships with the Brewing Process. *J. Agric. Food Chem.* **2006**, *54*, 3108−3113.
(55) Jégou, S.; Douliez, J. P.; Mollé, D.; Boivin, P.; Marion, D. Evidence of the Glycation and Denaturation of LTP1 during the Malting and Brewing Process. *J. Agric. Food Chem.* **2001**, *10*, 4942−4949.
(56) Stanislava, G. Barley Grain Non-Specific Lipid-Transfer Proteins (Ns-LTPs) in Beer Production and Quality. *J. Inst. Brew.* **2007**, *113*, 310−324.
(57) Cohen, M.; Davydov, O.; Fluhr, R. Plant Serpin Protease Inhibitors: Specificity and Duality of Function. *J. Exp. Bot.* **2019**, *70*, 2077−2085.
(58) García-Olmedo, F.; Salcedo, G.; Sanchez-Monge, R.; Hemandez-Lucas, C.; Carmona, M. J.; Lopez-Fando, J. J.; Fernandez, J. A.; Gómez, L.; Royo, J.et al. Trypsin/α-Amylase Inhibitors and Thionins: Possible Defence Proteins from Barley. In *Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology*; CAB International: Wallingford, U.K., 1992; pp 335−350.
(59) Gorjanović, S. A Review: Biological and Technological Functions of Barley Seed Pathogenesis- Related Proteins (PRS). *J. Inst. Brew.* **2009**, *115*, 334−360.
(60) Xu, D.; Duan, X.; Wang, B.; Hong, B.; Ho, T.-H. D.; Wu, R. Expression of a Late Embryogenesis Abundant Protein Cene, HVA7, from Barley Confers Tolerance to Water Deficit and Salt Stress in Transgenic Rice'. *Plant Physiol.* **1996**, *110*, 249−257.
(61) Camilloni, C.; Bonetti, D.; Morrone, A.; Giri, R.; Dobson, C. M.; Brunori, M.; Gianni, S.; Vendruscolo, M. Towards a Structural Biology of the Hydrophobic Effect in Protein Folding. *Sci. Rep.* **2016**, *6*, No. 28285.
(62) Wiedemann, C.; Kumar, A.; Lang, A.; Ohlenschläger, O. Cysteines and Disulfide Bonds as Structure-Forming Units: Insights From Different Domains of Life and the Potential for Characterization by NMR. *Front. Chem.* **2020**, *8*, No. 280.
(63) Jin, B.; Li, L.; Liu, G.; Li, B.; Zhu, Y.; Liao, L. Structural Changes of Malt Proteins During Boiling. *Molecules* **2009**, *14*, 1081−1097.
(64) Qin, M.; Wang, W.; Thirumalai, D. Protein Folding Guides Disulfide Bond Formation. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 11241−11246.
(65) Houde, M.; Khodaei, N.; Benkerroum, N.; Karboune, S. Barley Protein Concentrates: Extraction, Structural and Functional Properties. *Food Chem.* **2018**, *254*, 367−376.
(66) Gerrard, J. A. Protein-Protein Crosslinking in Food: Methods, Consequences, Applications. *Trends Food Sci. Technol.* **2002**, *13*, 391−399.
(67) Lu, Y.; Osmark, P.; Bergenståhl, B.; Nilsson, L. Vesicular Structures Formed from Barley Wort Proteins and Iso-Humulone. *Food Hydrocolloids* **2020**, *105*, No. 105788.
(68) Lu, Y.; Bergenståhl, B. B.; Nilsson, L. Interfacial Properties and Interaction between Beer Wort Protein Fractions and Iso-Humulone. *Food Hydrocolloids* **2020**, *103*, No. 105648.
(69) Örnebro, J.; Wahlgren, M.; Eliasson, A.-C.; Fido, R. J.; Tatham, A. S. Adsorption of α-, β-, γ- and ω-Gliadins onto Hydrophobic Surfaces. *J. Cereal Sci.* **1999**, *30*, 105−114.
(70) Lu, Y.; Bergenståhl, B. Condensation of Iso-Humulone in Solution and at Hydrophobic Surfaces. *Colloids Surf., A* **2021**, *613*, No. 126102.
(71) LoPachin, R. M.; Gavin, T. Reactions of Electrophiles with Nucleophilic Thiolate Sites: Relevance to Pathophysiological Mechanisms and Remediation. *Free Radical Res.* **2016**, *50*, 195−205.
(72) Baert, J. J.; de Clippeleer, J.; Jaskula-Goiris, B.; van Opstaele, F.; de Rouck, G.; Aerts, G.; de Cooman, L. Further Elucidation of Beer Flavor Instability: The Potential Role of Cysteine-Bound Aldehydes. *J. Am. Soc. Brew. Chem.* **2015**, *73*, 243−252.
(73) Baert, J. J.; de Clippeleer, J.; Trueba, P. B.; Jaskula-Goiris, B.; de Rouck, G.; Aerts, G.; de Cooman, L. Exploring Aldehyde Release in Beer by 4-Vinylpyridine and the Effect of Cysteine Addition on the Beer's Pool of Bound Aldehydes. *J. Am. Soc. Brew. Chem.* **2018**, *76*, 257−271.
(74) Stephenson, W. H.; Biawa, J. P.; Miracle, R. E.; Bamforth, C. W. Laboratory-Scale Studies of the Impact of Oxygen on Mashing. *J. Inst. Brew.* **2003**, *109*, 273−283.

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

(75) Mertens, T.; Kunz, T.; Gibson, B. R. Transition Metals in Brewing and Their Role in Wort and Beer Oxidative Stability: A Review. *J. Inst. Brew.* **2022**, *128*, 77−95.
(76) González, A. M.; Marcel, T. C.; Kohutova, Z.; Stam, P.; van der Linden, C. G.; Niks, R. E. Peroxidase Profiling Reveals Genetic Linkage between Peroxidase Gene Clusters and Basal Host and Non-Host Resistance to Rusts and Mildew in Barley. *PLoS One* **2010**, *5*, No. e10495.
(77) Begović, L.; Lepeduš, H.; Lalić, A.; Štolfa, I.; Jurković, Z.; Kovačević, J.; Cesar, V. Involvement of Peroxidases in Structural Changes of Barley Stem. *Bragantia* **2017**, *76*, 352−359.
(78) Johrde, A.; Schweizer, P. A Class III Peroxidase Specifically Expressed in Pathogen-Attacked Barley Epidermis Contributes to Basal Resistance. *Mol. Plant Pathol.* **2008**, *9*, 687−696.
(79) Henriksen, A.; Welinder, K. G.; Gajhede, M. Structure of Barley Grain Peroxidase Refined at 1.9-Å Resolution. *J. Biol. Chem.* **1998**, *273*, 2241−2248.
(80) Wietstock, P. C.; Shellhammer, T. H. Chelating Properties and Hydroxyl-Scavenging Activities of Hop A- and Iso-α-Acids. *J. Am. Soc. Brew. Chem.* **2011**, *69*, 133−138.
(81) Wietstock, P.; Kunz, T.; Perreira, F.; Methner, F.-J. Metal Chelation Behavior of Hop Acids in Buffered Model Systems. *BrewingScience* **2016**, *69*, No. 56.

https://doi.org/10.1021/acs.jafc.3c00185
J. Agric. Food Chem. 2023, 71, 5700−5711

Chapter 6

Does barley cultivar play a role in hop bitter acids utilization during wort boiling?

The influence of barley proteome on hop bitter acid yield during brewing

Mariana B. C. Pinto[ab], Flavio L. Schmidt[a], Zhuo Chen[c], Juri Rappsilber[c], Brian Gibson[b], Philip Wietstock[b]

[a] *Fruit and Vegetables Laboratory - Department of Food Technology, School of Food Engineering, University of Campinas (UNICAMP), R. Monteiro Lobato 80, 13083-862 Campinas, SP, Brazil*

[b] *Department of Food Technology and Food Chemistry, Chair of Brewing and Beverage Technology, Technische Universität Berlin, Berlin, Germany*

[c] *Bioanalytics, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany.*

Author Contributions

Mariana B C Pinto: Conceptualization, Methodology, Formal analysis, Data acquisition, Data interpretation, Writing - original draft, Writing - review & editing; **Flavio L Schmidt:** Conceptualization, Methodology, Writing - review & editing supervision, project administration and funding acquisition; **Zhuo Chen:** Methodology, Formal analysis, Data acquisition, Data interpretation, Writing - review & editing; **Juri Rappsilber:** Conceptualization, Methodology, Writing - review & editing supervision, and funding acquisition**; Brian Gibson:** Conceptualization, Writing - review & editing supervision, project administration and funding acquisition; **Philip C. Wietstock**: Conceptualization, Methodology, Writing - review & editing supervision, project administration and funding acquisition

Paper published in Journal of Agricultural and Food Chemistry

https://pubs.acs.org/doi/10.1021/acs.jafc.4c04396

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC
Article

The Influence of Barley Proteome on Hop Bitter Acid Yield during Brewing

Mariana B. C. Pinto, Flavio L. Schmidt, Zhuo Chen, Juri Rappsilber, Brian Gibson, and Philip C. Wietstock*

Cite This: https://doi.org/10.1021/acs.jafc.4c04396

ABSTRACT: A persistent challenge in brewing is the efficient utilization of hop bitter acids, with about 50% of these compounds precipitating with trub during wort boiling. This study aims to uncover the correlation between the barley cultivar proteome and hop bitter acid utilization during wort boiling. Therefore, comparative experiments were conducted using two cultivars, Liga and Solist, with varying proteomes to identify specific proteins' role in hop bitter acids precipitation. High-performance liquid chromatography (HPLC) was used to measure hop bitter acid content, while liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify and identify proteins. The 107 protein groups, particularly enzymes linked to barley metabolic defense mechanisms, exhibited significant differences between the two cultivars. Results revealed significantly lower α- and *iso*-α-acid content in wort produced from the barley cultivar Liga. This study highlights the critical role of the barley proteome in optimizing process efficiency by enhancing hop utilization through barley cultivar selection.

KEYWORDS: *Humulus lupulus, proteomics, isohumulones, circular economy, process efficiency*

INTRODUCTION

The global brewing industry holds a significant position as a primary producer of alcoholic beverages, yielding an impressive 1.89 billion hectoliters in 2022.[1] However, this achievement comes with a notable environmental cost, marked by a substantial greenhouse gas (GHG) emission of approximately 30,172.15 kilotons of CO_2 equivalent, with 22% originating from the supply chain, particularly raw materials.[2] Moreover, the industry faces the generation of considerable quantities of by-products, amounting to 4352 kilotons of solid waste in 2021.[3] In light of the pressing global challenge of climate change, the brewing industry faces a pivotal moment, inducing a change from a linear business model to one rooted in the principles of a circular economy. The circular economy, characterized by maximizing resource utilization and minimizing waste generation, serves as a strategic framework for reusing, reducing, and recycling materials, energy, and resources to foster sustainability and mitigate environmental impacts.[4,5]

In the brewing industry, optimizing the extraction of bitter compounds from hops (*Humulus lupulus L.*) presents a significant challenge, as these compounds are crucial for contributing the desired bitterness to the final product. Studies indicate that only a modest portion, typically between 30% and 50%, of the bitter acids are retained in the finished beer.[6] This inefficiency establishes a considerable obstacle to optimizing beer production, especially for highly hopped beverages. The essential bitter compounds in beer are the *iso*-α-acids, generated by the precursor α-acids during wort boiling.[7] However, the formation of *iso*-α-acids is reduced by factors such as protein coagulation and precipitation with trub during the boiling process. Elevated temperatures during boiling induce protein denaturation, facilitating the formation of protein−protein complexes.[8,9] Yet, the precise interaction between hop bitter compounds and barley-derived proteins remains poorly understood.

Barley malt serves as the primary source of proteinaceous material in both wort and beer. However, a significant portion of these proteins coagulate as trub, constituting approximately 50−60% of the protein content in this by-product.[10] The composition of the barley proteome is inherently influenced by genotype, with slight modifications occurring during the malting process.[11] In a recent study, we explored the impact of hop bitter acids addition on the formation of protein−protein complexes during boiling, revealing alterations in protein aggregation mechanisms and protein profiles of resulting aggregates.[12] While existing research has predominantly focused on the influence of barley genotype and proteome on beer quality traits like foam stability and haze, little attention has been paid to their role in protein precipitation during boiling and subsequent hop bitter acid utilization.[13] Hence, this study represents a pioneering effort to elucidate the influence of barley genotype on protein coagulation during boiling and, consequently, its effect on the hop bitter acid yield. Unraveling these complex interactions will enable the brewing industry to use hop products and barley malt more efficiently, thereby promoting sustainability

Received: May 18, 2024
Revised: August 17, 2024
Accepted: September 6, 2024

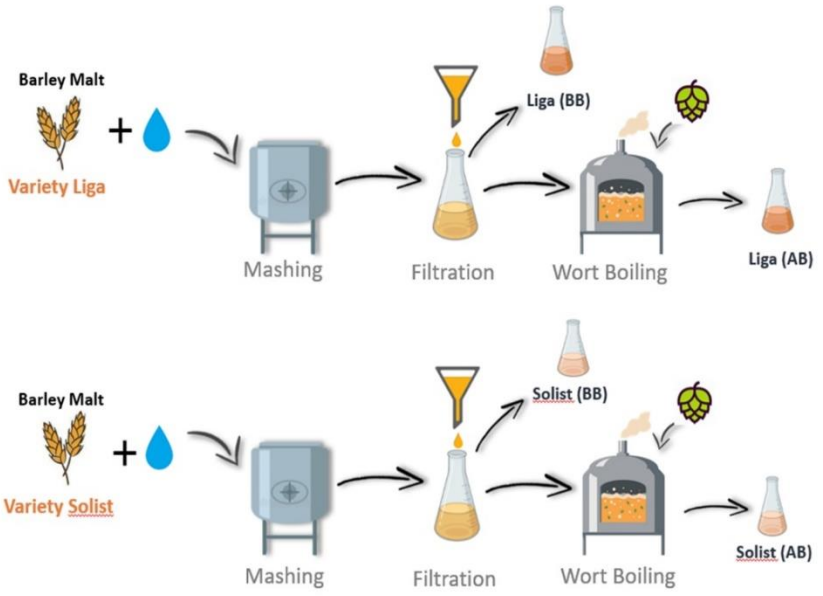


Figure 1. Design of experiment

goals such as GHG mitigation, aligning with the United Nations' sustainable development agenda.[14]

MATERIALS AND METHODS

Experimental Design and Wort Preparation. For this study, experiments were designed (Figure 1) to explore the effect of commercial barley cultivars on hop bitter acid utilization. The wort was produced using 100% Pilsner barley malt from the Liga or Solist barley varieties (Ireks, Kulmbach, Germany) ground at 1.2 mm and mixed with distilled water (1:4 w/w). Those barley cultivars were chosen for this study based on their wider usage in the brewing industry and their proteome variation. This design of the experiment focused on comparing cultivars that were processed similarly into Pilsner malt. The same barley malt batch was used for all trials to avoid variability. The trials were conducted in triplicate, and the samples before boiling (Liga-BB/Solist-BB), Liga wort after boiling (Liga-AB), and Solist wort after boiling (Solist-AB) were collected for further analysis and characterization. The mashing was performed in a mash bath following the mashing scheme presented in Figure S1 (Supporting Information) which contains details of the time and temperature used. The sweet wort was separated from the brewer's spent grain by filtration using a folded paper filter with a diameter of 320 mm (Whatman, Dassel, Germany). Hop CO_2 extract obtained from Hopsteiner Company (Nürenberg, Germany) was added to achieve 90 mg/L of α-acids. Hop CO_2 extract was used in this experiment to avoid the addition of hop polyphenols and, therefore, ensure a more controlled and consistent experimental setup. The boiling was done under reflux and lasted 60 min until the wort was cooled to 20 °C. Prior to the Liga-AB and Solist-AB sample collection, the wort was filtered to separate the trub formed during the boiling. The samples were then stored in a freezer at −18 °C until the analysis.

Wort Characterization. Table 1 shows the analysis performed on the wort. The methodologies were according to the Mitteleuropäische Brautechnische Analysenkommission eV (MEBAK). The complete wort characterization data are present in Table S1 (Supporting Information).

Bitter Acid Analysis by HPLC. Hop α- and *iso*-α-acid homologues were analyzed according to ASBC-Methods of Analysis Beer 23-C in a high-pressure liquid chromatography (HPLC) system Agilent 1100 series HPLC system (Agilent Technologies, Böblingen, Germany) at a flow rate of 1.0 mL/min with a 5 μL injection volume. Two mobile phases were used. Mobile phase B was 100% methanol, and mobile phase A was 59% methanol, 40% water, and 1% phosphoric acid (85%). The elution began with 100% of mobile phase A for the first 28 min and changed to 100% B over the next 12 min. The last 15 min of analysis corresponded to 100% of phase A. The ASBC method was adapted regarding the mobile phase gradient, flow rate, and run time to improve the analysis of the equipment used. A Purosphere Star LC-18 5 μm silica column (250 mm × 4.6 mm, Merck, Darmstadt, Germany) was used for separation. Absorbance was measured at 270 and 314 nm. The international calibration standards ICS-I4 and ICS-A1 obtained from Labor Veritas (Zürich, Switzlerand) were used as standards for all measurements.

Table 1. Wort Characterization Methodologies According to MEBAK

analysis	MEBAK method	MEBAK online ref
density[a]	B-590.08.904	15
real extract[a]	B-590.10.181	16
apparent extract[a]	B-590.09.900	17
pH[a]	B-590.00.040	18
original gravity[a]	B-590.10.181	16
calorie[a]	B-590.78.999	19
total nitrogen	B-400.07.003	20
free amino nitrogen (FAN)	B-400.11.111	21
total polyphenols	B-590.41.111	22
β-glucan	B-400.26.900	23
bitter units (BU)	B-400.17.110	24

[a]Analyses performed on the density meter DMA 4500 M equipment (Anton Paar GmbH, Graz, Austria).

Hydropathy and Isoelectric Point Determination. The grand average of the protein classes' hydropathic value (GRAVY) profile was calculated by the gravy calculator (https://www.bioinformatics.org/sms2/protein_gravy.html) using the protein sequence in FASTA Format obtained from the Uniprot database. The isoelectric point (p*I*) was calculated using the isoelectric point calculator (https://www.bioinformatics.org/sms2/protein_iep.html).[25]

Protein Extraction and Digestion. Wort proteins were extracted by acetone precipitation due to high efficiency, removal of contaminants, simplicity, and speed. Then 200 μL of wort was transferred to an Eppendorf tube, and 800 μL of cold acetone (at −20 °C) was added. It was incubated for 90 min at −20 °C, followed by a 10 min centrifugation (Concentrator 5305 plus, Eppendorf, Germany) at 13 000*g*. The supernatant was removed, and the pellet

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

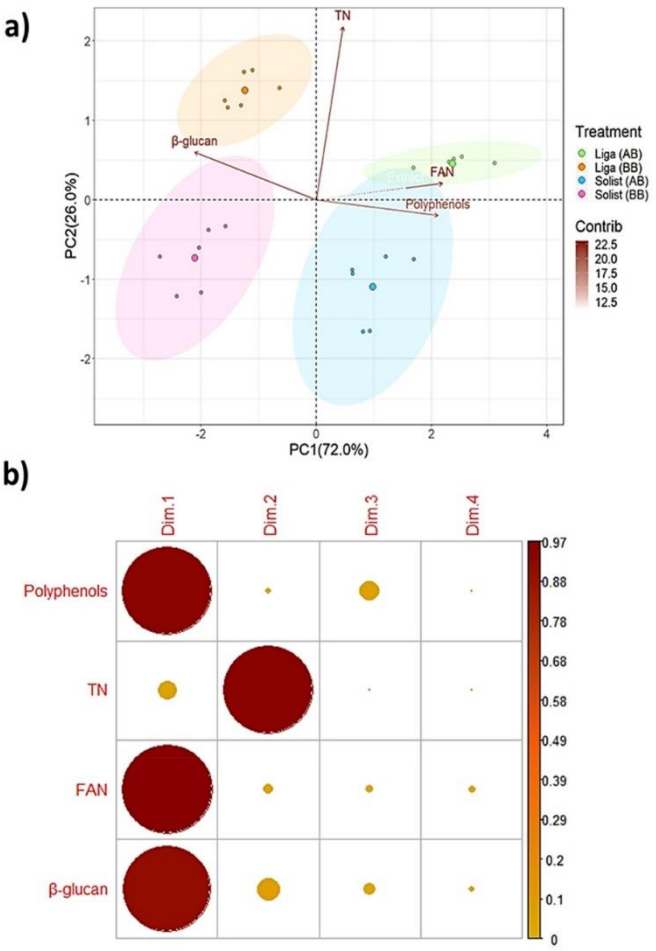


Figure 2. Wort composition showed in a principal component analysis: (a) PCA biplot of components 1 and 2 (the large sample dots represent the centroid of the ellipse, whereas the small dots represent each individual replicate. The "contrib" metric indicates how much each variable contributes to the formation of the principal components). (b) Correlation plot of variables in each dimension calculated by Cos^2: Liga (BB), cultivar Liga before boiling; Liga (AB), cultivar Liga after boiling; Solist (BB), cultivar Solist before boiling; Liga (AB), cultivar Liga after boiling.

was dried for 30 min at room temperature. The protein pellets were resuspended with SDS sample buffer and boiled for 5 min at 95 °C. The samples were centrifuged at 17 500*g* for 10 min. The proteins were separated by SDS-PAGE gel using a Tris-Glycine SDS running buffer and a molecular weight marker to verify the protein separation. The gels were stained with colloidal Coomassie Blue followed by overnight washing. To the gel pieces were added 250 μL of 50 mM ammonium bicarbonate (ABC) before shaking for 30 min at 37 °C. The gel washing was performed with the subsequent addition of acetonitrile (ACN) and 50 mM ABC solution. The protein reduction took place in a shaker at 37 °C for 30 min with 150 μL of reduction buffer (10 mM dithiothreitol, DTT, in 50 mM ABC solution). This was followed by the addition of 150 μL of alkylation buffer (55 mM iodoacetamide, IAA, in 55 mM ABC Puffer) and incubated in darkness at room temperature for 20 min. The alkylation buffer was removed, and 150 μL of ACN was added to shrink the gel pieces for 5 min. The digestion was performed with the addition of 150 μL of trypsin buffer (0.005 $\mu g\ \mu L^{-1}$ trypsin in 50 mM ABC, 5% ACN (v/v)), and the digestion proceeded overnight at 37 °C. The digestion was terminated by acidification with 6 μL of 10% trifluoroacetic acid (TFA), shaking for 15 min.

As described by Rappsilber et al. (2003), peptides were eluted from the StageTips with 2 × 10 μL of 80% acetonitrile and 0.1% TFA. The samples were dried by vacuum centrifugation (Concentrator 5305 Plus, Eppendorf, Germany) to be dissolved in 10 μL of 1.6% acetonitrile and 0.1% formic acid (FA) subsequently.

Protein Identification. Liquid chromatography coupled with mass spectrophotometer (LC-MS/MS) analyses were performed on a Thermo Scientific Q Exactive HF hybrid quadrupole−Orbitrap mass spectrometer coupled online to Ultimate 3000 RSLCnano Systems (Dionex, Thermo Fisher Scientific). The analytical column was a temperature-controlled EASY-Spray C18 LC column with a particle size of 2 μm and a length of 500 mm (Thermo Fisher Scientific, Germany) operated at 45 °C. Mobile phase A consisted of 0.1% FA in water; mobile phase B consisted of 80% acetonitrile and 0.1% FA.

Peptides were loaded onto the column and eluted at a flow rate of 0.3 μL/min. Peptides were separated by a series of linear gradients: 2% to 38% buffer B in 40 min, 38% to 52.5% in 4 min, and then 90% in 1 min. Eluted peptides were ionized by an EASY-Spray source (Thermo Scientific, Germany) and introduced directly into the mass spectrometer.

The MS data were acquired in data-dependent mode. MS1 spectra were recorded at 60 000 resolution (scan range 350−1400 m/z) to ensure less than 5 ppm error for precursor ions and a mass range of 700−8400 Da (approximately 7−84 amino acid residues). In each acquisition cycle, the 10 most intense peaks with a charge ≥2 were

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

individually isolated with a 1.6 m/z window. The isolated ions were fragmented using higher-energy collisional dissociation (HCD) with stepped collision energy (27%, 29%, and 31%). The maximum injection time for MS1 scans was set to 50 ms and the automatic gain control (AGC) was set to 3.0×10^6 ions. The MS2 spectra were recorded at 15 000 resolution to maintain less than 10 ppm error for fragment ions while minimizing scan time and maximizing the number of sampled precursors, maximum injection time of 80 ms, and AGC set to 1.0×10^5 ions. Dynamic exclusion was enabled with a single repeat count and 60 s exclusion duration. Parameters related to hardware performance, such as injection times and automatic gain control (AGC) settings, were configured according to the optimal settings recommended by the manufacturer.

Data Processing. The unsupervised analysis using principal component analysis (PCA) was performed in R (package: FactomineR and ggplot) to assess the groupings or trends in the data. The data was automatically standardized by default of the PCA function from the FactorMineR package. Each variable was centered and scaled to have a mean of zero and a standard deviation of one before PCA was conducted. The scores and loadings plots were used to determine the number of principal components (Supporting Information, Table S2). Protein identification was conducted using MaxQuant[26] version 1.6.12.0. The mass spectrometry data was searched against reference proteome: UP000011116 (downloaded from Uniprot). The default parameters in MaxQuant were applied. Label-free quantification was performed with at least two peptides per protein. The final data set contained the proteins quantified in three replicates of each treatment. The data was pretreated by filtering the noise and $\log_2$ transformation in Perseus 1.6 software.[27] Each protein was analyzed by a student's t test using Perseus's default parameters. The resultant matrix was used for data preprocessing in Microsoft Excel (Table S2 in Supporting Information Excel file). The missing values were imputed by the average and the LFQ intensity was adjusted based on the injection ratio. The enrichment analysis was conducted in Microsoft Excel with the protein classes matching the gene ontology (GO) terms extracted from the Uniprot database. The relative abundance of each GO term was transformed in percentage to highlight the most prominent GO terms.

■ RESULTS AND DISCUSSION

Effect of Barley Cultivar on Wort Composition. Barley malt, in addition to water, is the most prominent raw material in brewing by weight. It is essential in providing important macronutrients for fermentation performance and beer quality such as fermentable sugars, free amino acids, proteins, and polyphenols. The biplot graph presented in Figure 2a, resulting from the principal component analysis (PCA) offers a comprehensive visual representation of the relationships between variables and observations in the data set. The principal components (PC) were selected according to the eigenvalues for PC because the eigenvalues under 0.7 demonstrate lower significance.[28] In this biplot, the principal components PC1 and PC2 collectively explain 97.94% of the total variance in the data set (Supporting Information, Table S2). The distribution of variables along PC1 indicates a strong positive correlation between free amino nitrogen (FAN) and polyphenols, while β-glucan appears to be negatively correlated with both FAN and polyphenols. Furthermore, total nitrogen (TN) is strongly positively correlated with PC2. Also, Figure 2b shows the correlation between variables and dimensions (PCs). This insight into variable relationships and their contributions to principal components facilitate a deeper understanding of the inherent structure within the data set and aid in identifying key variables driving the observed pattern.

The direction, magnitude, and color of the variable vectors in the biplot elucidate their contribution to the principal components. For instance, free amino nitrogen (FAN) appears to have the most significant influence on the direction of PC1, followed by polyphenol. Conversely, β-glucan has a minor impact on the orientation of PC1. Observations clustered toward the positive end of PC1 exhibit a higher correlation with FAN and polyphenols, represented by the post-boiling samples. FAN is normally formed during malting and mashing and its content in wort plays an important role in the fermentation performance of yeast by offering nutrition.[29] Lower levels of FAN during fermentation can impact beer quality due to higher diacetyl production during fermentation.[30] Surprisingly, the samples prepared with Solist cultivar were highly correlated with FAN. This cultivar protein profile might lead to an increase in FAN content during malting due to more available protein for proteolysis, although it contains lower soluble protein content. Furthermore, a statistically significant ($p < 0.05$) decrease in FAN content in the samples after boiling (AB) in comparison with before boiling (BB) was observed (Supporting Information, Table S3). This confirms the phenomenon observed in the previous study which demonstrated the reduction of FAN content in wort boiling due to Maillard reaction by melanoidin formation as well as precipitation to the trub.[12]

The Solist cultivar also showed a stronger correlation with the polyphenol content, as evidenced by the proximity of the clusters to this variable. Polyphenols typically originate from malt or hops. However, in this experiment, only hop extract, which contains no polyphenols, was used.[31] Therefore, it can be inferred that the polyphenol content in these samples is directly related to barley malt polyphenols, which can significantly influence beer quality, particularly in terms of flavor and haze stability.[32] Polyphenols contribute to trub formation by interacting with proteins from malt, forming colloidal particles that precipitate after boiling.[33] The wort prepared with the Solist cultivar contained 20% more polyphenols than did the wort prepared with the Liga cultivar.

Additionally, the biplot reveals distinct clusters of observations based on their similarities and differences in the data set. As expected, the clusters of the Liga cultivar, located toward the positive end of PC2, comprise observations characterized by high values of TN. Because TN measurement demonstrates a great correlation with protein content, it can be seen that this barley cultivar contains greater protein content in comparison with the Solist cultivar even in the post-boil samples, demonstrating that the barley varieties differ greatly in protein content even during brewing.[34] Furthermore, a clear reduction ($p < 0.05$) in TN of samples after boiling was observed. The precipitation of protein during boiling is paramount to improving beer quality regarding haze formation. Haze-active proteins interact with polyphenols during wort boiling, forming colloidal particles with a higher molecular weight, which, consequently, leads to precipitation to the trub.[35] This haze-active protein removal avoids colloidal instability in beer during storage.[36]

These findings emphasize the necessity of controlling boiling conditions to optimize the utilization of hop bitter acids, thereby enhancing the final product's quality. By elucidating the role of protein precipitation during boiling, this study provides a foundation for developing targeted strategies to increase hop bitter acid content, enhancing beer quality and bitterness. The significant reduction in total nitrogen (TN) post-boiling highlights the role of boiling in removing proteins that might interact with those compounds, consequently,

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

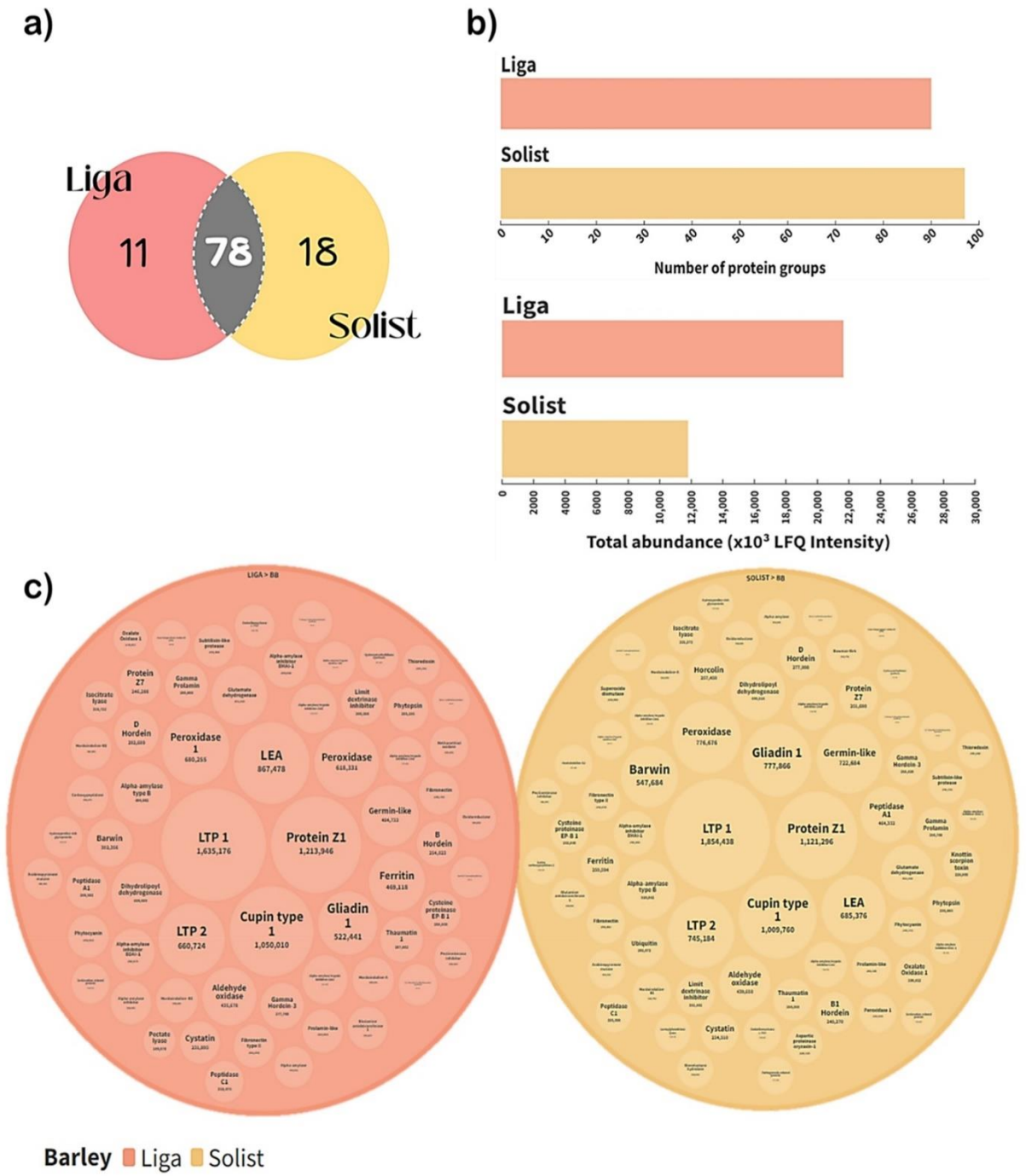


Figure 3. Barley cultivar protein profile: (a) Venn diagram representation of the number of proteins of each cultivar and in common among them. (b) Bar plot with the total number of proteins and total abundance in LFQ Intensity for each cultivar. (c) Tree map representation of protein families found in each cultivar with the respective LFQ Intensity.

carrying them to the trub. These findings shed light on brewing practices, allowing for the optimization of boiling conditions and the selection of barley cultivars that contribute to improved hop utilization in brewing.

Barley Cultivar Protein Profile. Barley typically comprises 8−15% protein, with variability influenced by factors such as cultivar type and growing conditions. As mentioned, this research utilized malt from two distinct German barley cultivars, namely, Liga and Solist, to produce wort. The proteomic analysis of the wort aimed to characterize its overall protein composition. Initially, 118 protein groups were identified, but 11 were considered noise as they were detected exclusively in the post-boiling sample. Thus, the resulting data set comprised 107 protein groups (Table S4 in the Supporting Information Excel file). Among these, 78 protein groups were shared between both cultivars, as shown in the Venn diagram in Figure 3a. Additionally, 11 protein groups were unique to the Liga cultivar, while 18 were specific to the Solist cultivar.

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

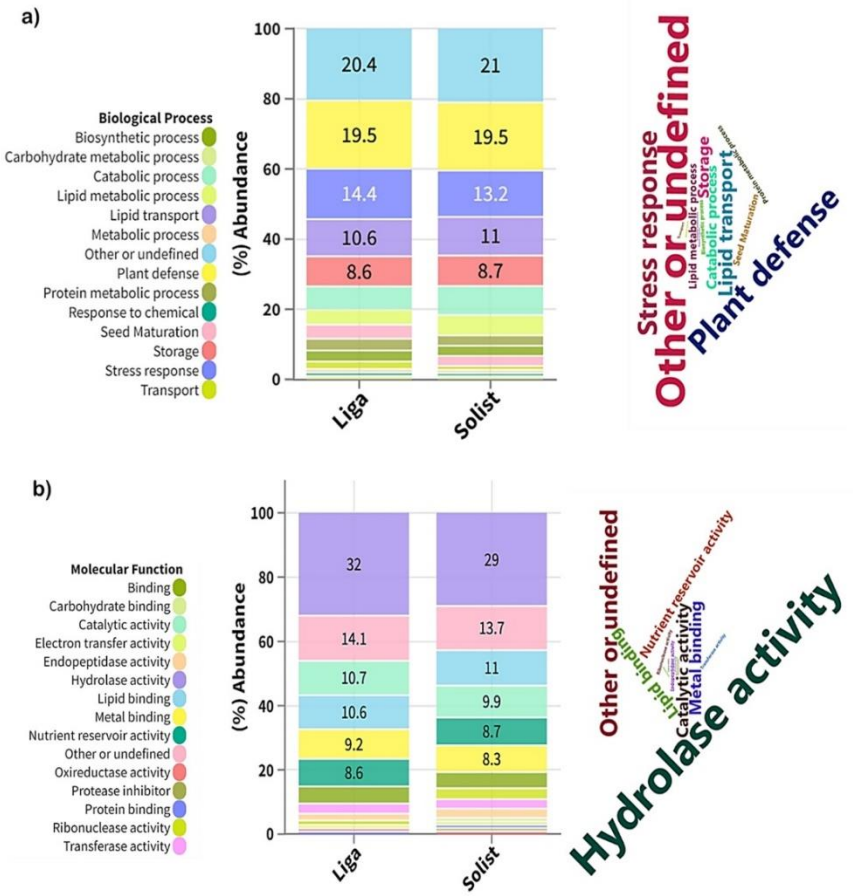


Figure 4. Barley cultivar biological protein profile: (a) biological process of proteins represented in a word cloud and a stacked bar plot in percentage. (b) Molecular function of proteins represented in a word cloud and a stacked bar plot in percentage.

Interestingly, despite Liga having a total protein abundance in LFQ intensity twice that of Solist, it contained a lower number of identified proteins (Figure 3b).

These findings suggest that the genetic characteristics of each barley cultivar led to distinct protein expression profiles, which, in turn, impact the brewing process and final beer quality. For the Liga cultivar, proteins such as α-amylase inhibitor, B hordein, carboxypeptidase, ferritin, and peroxidase 1 are unique. These proteins play distinct roles in the brewing process. For instance, α-amylase inhibitors can affect the breakdown of starches, while ferritin and peroxidase 1 are involved in oxidation−reduction reactions which can influence beer stability and flavor.[37−39] In contrast, the Solist cultivar has unique proteins, such as aspartic proteinase oryzalin-1, B1 hordein, barwin, bowman−birk, gliadin 1, and ubiquitin. Bowman−birk inhibitors are known to affect proteolytic activities, which can influence protein stability and clarity in beer. This highlights the role of cultivar genetics in protein variability, in this case, for water-soluble protein.

Illustrated in Figure 3c is a circle chart showing the distribution of the protein families. Notably, the predominant protein families identified are LTP and protein Z, recognized for their contribution to beer foam stability.[40] These proteins constitute a significant portion of the water-soluble fraction in wort, as confirmed in a previous investigation.[12] While LTP, protein Z, cupin type, and peroxidases emerge as the most abundant protein families in similar proportions for both cultivars, the specific isoforms contributing to these abundances differ substantially between them. Despite the shared presence of many protein isoforms across barley cultivars, their ranking varies, highlighting distinct water-soluble protein profiles (Table S4 in the Supporting Information Excel file).

Genetic variation among cultivars is a well-known biological phenomenon that plays a crucial role in the barley proteome. This variation is significant during grain development and at later stages during malting.[41] Inside cells, genes encode proteins, directly influencing their amino acid sequence and primary structure.[42] Each protein family contains multiple isoforms with differing primary structures, indicating that they were encoded by distinct genes. Therefore, the barley cultivar's genotype directly impacts protein profiles, particularly regarding protein isoforms. The findings of this study validate this phenomenon by highlighting differences in the proteome of the examined barley varieties. Researchers have extensively explored this variation over the past decades, emphasizing its importance in barley breeding programs and the malting process.[43−47] For example, Wang et al.[43] conducted a proteomic analysis of two hulless barley cultivars, identifying 4603 proteins, with 326 showing differential expression between the cultivars.

Environmental factors, including growing conditions and agricultural practices, also play a significant role in shaping the protein profiles of barley cultivars. Aside from the genetic variation among barley cultivars, the proteomic differences

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

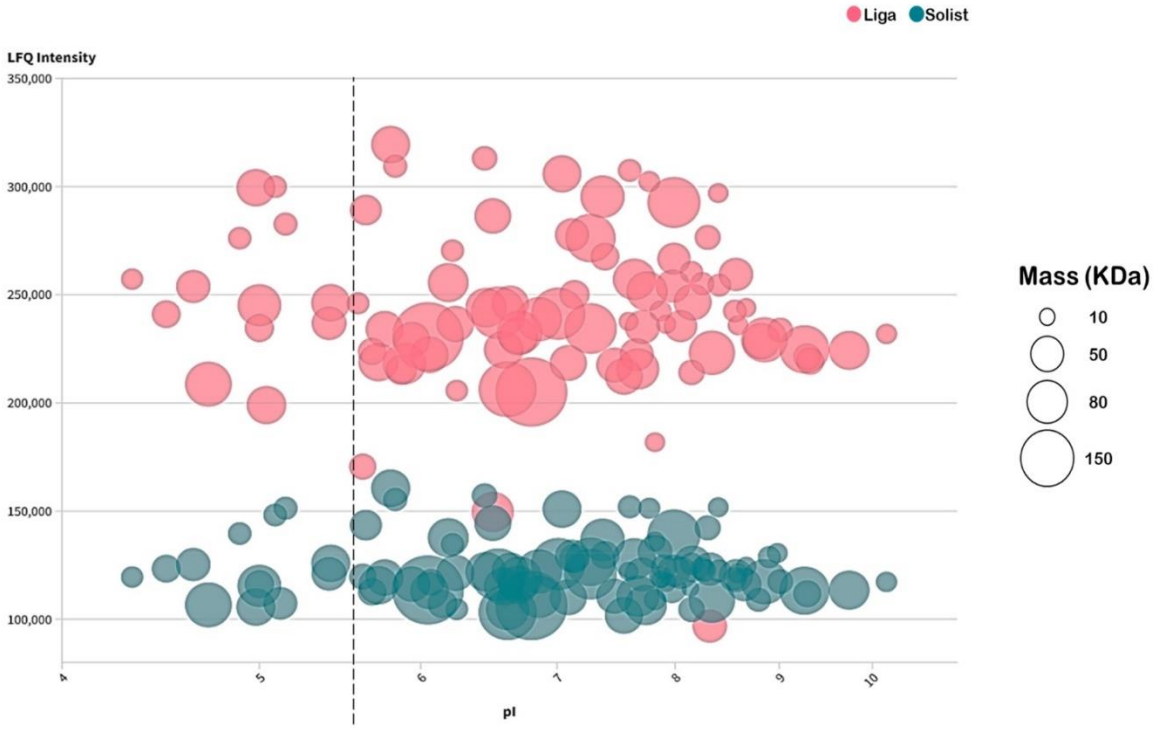


Figure 5. Barley cultivar protein chemical profile: bubble plot representation of the isoelectric point × LFQ intensity. The circle size represents the protein mass in kDa.

observed in this study can be partly attributed to environmental factors. This was evidenced by Halstead et al.[48] study, which conducted a comprehensive evaluation of five fall-planted malting barley lines across three distinct locations in the Pacific Northwest, each representing a unique growing environment. Notably, the nitrogen treatment (N_2) that included an extra application at the heading led to an increase in the level of grain protein. Another study conducted by Luo et al.[49] highlighted the role of nitrogen fertilizer and the environmental impact on the barley protein profile, although the study suggests that the genetic variation of the plant has a more significant impact on the protein content and proteome of malt.

Proteins are characterized as polypeptides fulfilling biological roles. In this investigation, they underwent annotation into gene ontology (GO) category enrichment analysis, aiding in the comprehension of proteome data, biological process (BP), and molecular function (MF) terms extracted from the UniProt database are used for annotating the proteins. The distribution of term frequencies among the 107 proteins in the samples is visualized in the word cloud presented in Figure 4. Nevertheless, due to incomplete entries in the UniProt database concerning barley proteins, a considerable portion of BP and MF is labeled as "other or unidentified", constituting 21.5% and 15.0%, respectively, of the total proteins.

Notably, hydrolase activity emerges as the predominant molecular function, accounting for 30% of the frequency, indicating the prevalence of enzymes within the samples, representing nearly half (46.7%) of the proteins. This observation is expected due to enzymes' higher solubility in aqueous media, as they require a certain level of water for their enzymatic activity and are likely to interact with surrounding water molecules.[50,51] Furthermore, barley seed germination is induced during the malting process. In this step, enzymes are synthesized in the aleurone layer to degrade the nutrient reservoir and initiate the plant growth metabolism.[42] For instance, in this study, a 6,7-dimethyl-8-ribityllumazine synthase enzyme was found. It is involved in the biosynthesis of riboflavin in plants and, consequently, essential for biosynthetic metabolism (Table S5 in the Supporting Information Excel file).[52] In addition, metal binding was a significant molecular function, accounting for 11% of the frequency. These proteins may play an important role in metal chelation, potentially enhancing beer flavor stability by reducing the availability of metal ions in wort.[53]

Additionally, the word cloud in Figure 4a highlights that the most common BPs are plant defense and stress response, accounting for 30%. In a related study by Kerr et al.,[54] the barley proteome was investigated under pathogen infection conditions, indicating modifications in the plant protein profile in response to fungal contamination. Remarkably, oxalate oxidase was found to be more abundant in infected seeds, and this protein was also present in both barley cultivars examined in this study. These findings further underline the environmental influence on the barley proteome, highlighting its crucial role in shaping brewing quality traits and the brewing process.

The column plot presented in Figure 4 provides a detailed breakdown of the percentage abundance (LFQ intensity) of BP and MF terms for each barley cultivar. Despite notable differences in protein profiles among these cultivars regarding their protein isoforms, there is a clear similarity observed in both the BP and MF categories. This similarity suggests that several proteins may acquire similar functions within the organism's metabolism through distinct pathways that are intricately linked to their structural characteristics. In that

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

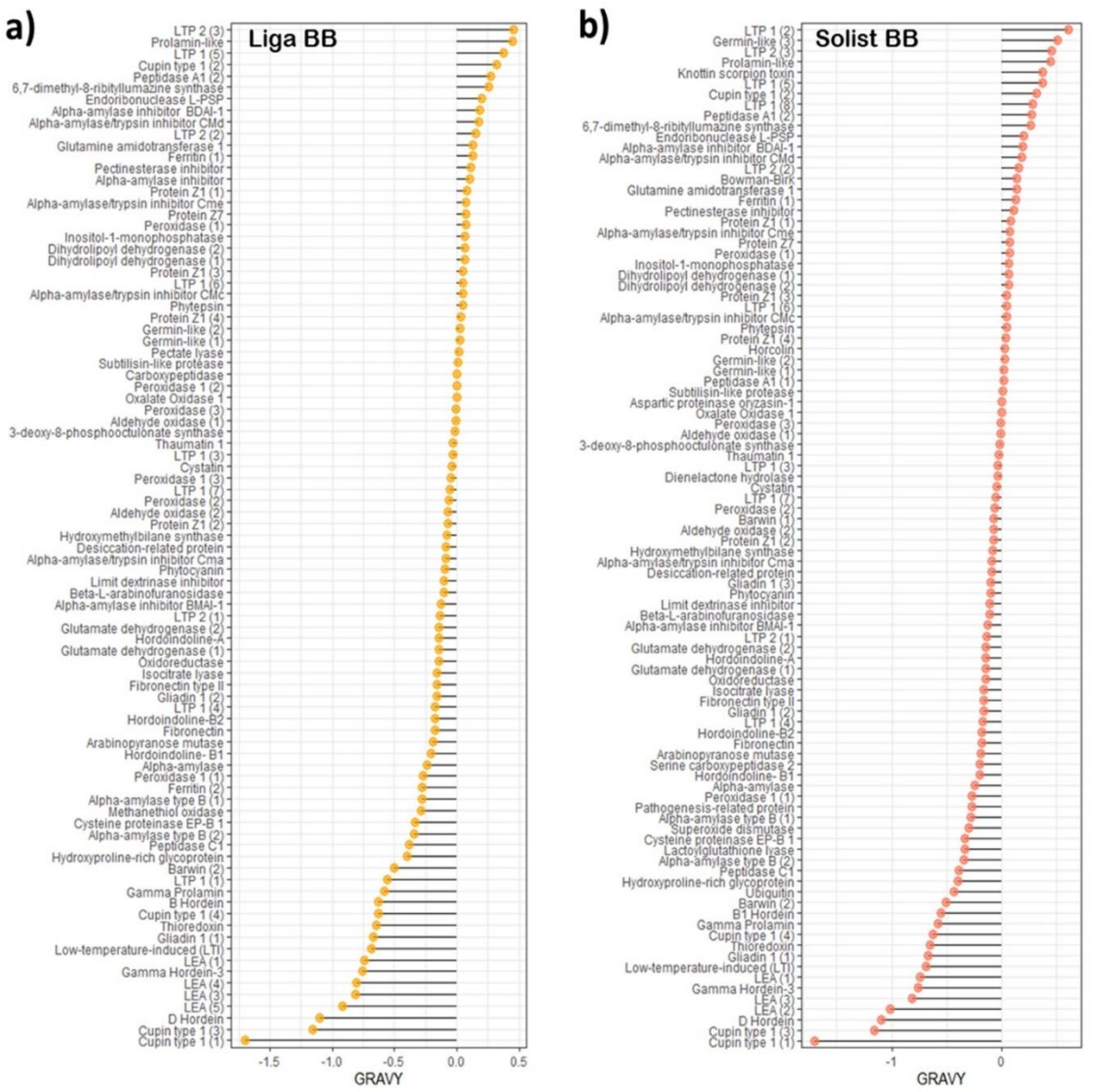


Figure 6. Barley cultivar chemical protein profile: lollipop chart of the grand average hydrophobicity (GRAVY) of (a) the cultivar Liga before boiling; (b) the cultivar Solist before boiling. The circle size represents the abundance in LFQ intensity of each protein.

sense, it is clear that the malting process plays a major role in determining the barley proteome, specifically due to the germination of barley seeds. As described in the study by Jin et al.,[55] the proteome of green barley, i.e., barley seed before germination, varies noticeably, however, some differences disappeared after the malting process. Moreover, the main proteins involved in the variability across the cultivars are pathogen-related proteins and hydrolases, as shown in this study.

Each protein's isoelectric point (p*I*) was determined using a bioinformatics tool that utilizes the FASTA index and protein sequence.[25] This analysis aimed to explore the biophysical properties of water-soluble proteins sourced from different barley cultivars. The bubble chart in Figure 5 demonstrates the p*I* of proteins with the circle size indicating their molecular weight in kilodaltons (kDa). The distribution of proteins across the p*I* range reflects a typical pattern observed in biological systems, following a normal distribution with a peak in the neutral pI (Figure S5 in Supporting Information).[56]

The chart indicates a clustering of proteins with higher molecular weights around the neutral p*I*, suggesting that larger water-soluble barley proteins often possess a net-zero charge at neutral pH. Evidence supports the idea that larger proteins tend to exhibit a neutral or nearly neutral p*I*, whereas smaller proteins tend to show more extreme p*I* values.[57] Despite the barley proteome reaching up to 9857470.16 kDa, the water-soluble proteins from the analyzed samples had <150 kDa mass due to mashing and filtration steps, separating the molecules with higher mass from the wort.[58] For instance, both barley cultivars show two isoforms of aldehyde oxidase with the highest molecular weights, measuring 146.3 and 145.5 kDa, respectively. Conversely, the smallest proteins vary between cultivars, with 11.2 kDa (α-amylase/trypsin inhibitor CMc) for

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

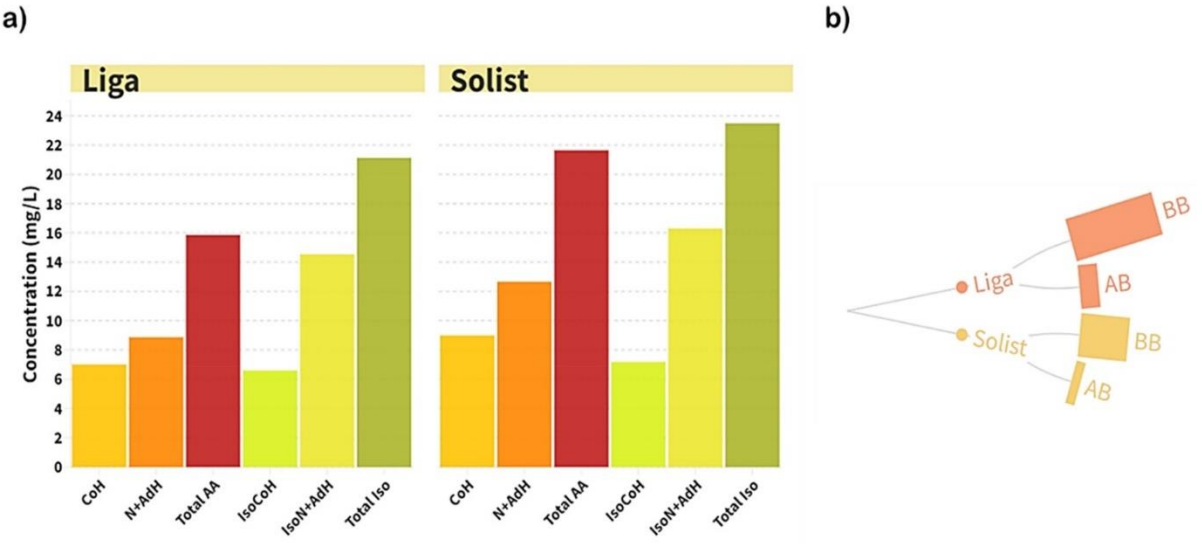


Figure 7. Protein precipitation and hop bitter acids: (a) hop α- and iso-α-acid homologue concentration in the hopped wort. (b) Bar plot representing the total abundance of proteins in LFQ intensity of each treatment; CoH, cohumulone; N + AdH, N− and adhumulone; total AA, total α-acids; iso CoH, isocohumulone; Iso N + AdH, *iso*-N− and *iso*-adhumulone; total iso, total *iso*-α-acids; BB, before boiling; AB, after boiling.

Liga and 8.8 kDa (Knottin scorpion toxin) for Solist, as detailed in Table S4 in the Supporting Information Excel file.

Interestingly, both cultivars share a similar p*I* range from 4.33 to 10.16, even with differences in the protein profile and protein distribution within this range. The Liga cultivar shows a more balanced distribution, with 52.8% of proteins falling in the alkaline range and 47.2% in the acidic range. In contrast, the Solist cultivar contains a higher proportion of alkaline proteins, with 56.2% in that range. Normally, the plant proteome comprises larger numbers of acidic p*I* proteins rather than basic p*I*. However, in this study, most of the proteins are found in the basic p*I* range due to the acidic pH of the wort. The protein's solubility is usually lower at its p*I*, which explains the lack of acidic pI proteins in those samples.[59]

Similarly to p*I*, the grand average hydrophobicity (GRAVY) index was computed using a bioinformatics tool that utilizes the FASTA index and protein sequence, revealing insights into the hydrophobic nature of the proteins (Figure 6).[25] Positive GRAVY values indicate hydrophobic proteins, while negative values suggest hydrophilic proteins. Both barley cultivars predominantly exhibited proteins with negative GRAVY scores ranging from −1.7 to 0.0, consistent with previous findings from wort-boiled samples.[12] A slight variance in hydrophobicity was observed between these cultivars, with Liga containing 65.56% hydrophilic proteins compared to 62.89% for Solist. This trend toward hydrophilicity within barley proteomics appears to be an inherent characteristic.[44] In this study, this property is emphasized through the extraction of water-soluble proteins during the mashing step, facilitating the solubility of hydrophilic proteins.

Effect of Barley Proteome on Hop Bitter Acid Utilization. To investigate how different barley malt cultivars affect the utilization of hop bitter acids, two worts with malt from the different barley cultivars Liga and Solist were prepared, and hop CO_2 extract containing 90 mg/L of α-acids in each one was added to reproduce typical brewing practices and industry standards to achieve a realistic concentration of *iso*-α-acids in the final beer. Further, hop bitter acid content in the samples post-boiling was analyzed by HPLC. The data are presented in the bar plot in Figure 7a. Clearly, the content of all homologues of α- and *iso*-α-acids, as well as the total content, was higher in the wort prepared with the Solist cultivar ($p < 0.05$). The wort prepared with Liga cultivar contained 15.86 mg/L of α-acids and 21.13 mg/L of *iso*-α-acids after boiling, whereas the wort produced from Solist cultivar had 21.65 and 23.49 mg/L, respectively.

To impart bitterness to beer, α-acids undergo a thermal isomerization reaction, converting into the more soluble *iso*-α-acid.[60] This transformation is affected by various factors, particularly pH and original gravity.[61] In this investigation, all worts were standardized to a pH of 5.6 and an original gravity of 16° Plato (Supporting Information, Table S1). Therefore, the only variable considered is the barley malt cultivar and, consequently, the barley proteome. The Liga cultivar exhibited a 45.51% higher total protein abundance (measured by LFQ intensity) compared with the Solist cultivar (Figure 7b). Additionally, wort derived from Liga showed a 10% reduction in *iso*-α-acid content post-boiling compared to that from Solist. This implies that the protein content and proteomic profile of the barley cultivar influence the precipitation of hop bitter compounds to the trub during wort boiling.

Protein aggregates primarily form during the heating-up phase and initial boiling, with a consistent particle size thereafter until boiling terminates.[62] Intriguingly, this study observed a significantly higher difference in total α-acid content compared to *iso*-α-acids, showing a 26.75% higher in the wort produced from the Solist barley cultivar in comparison with the Liga cultivar (see Table S6 in the Supporting Information Excel file). This discrepancy suggests that either the protein profile or the protein content of barley malt directly influences the availability of α-acids for the isomerization reaction. It is well-known that the α-acids' solubility is limited in aqueous solutions and acidic pH conditions, which can impact the rate of isomerization.[63] To eliminate this factor, as stated before, the pH of all wort samples was consistently maintained across the trials. Consequently, the reduced α-acid content and the utilization

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

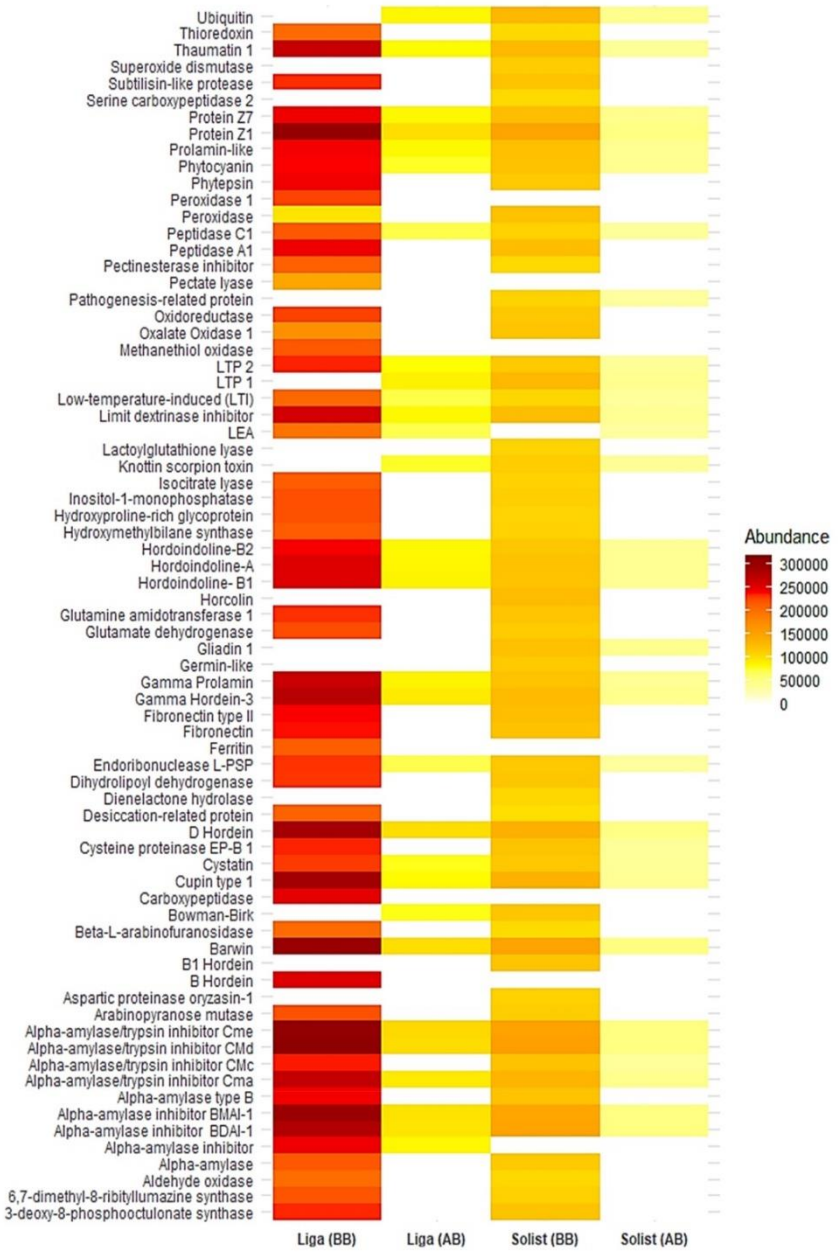


Figure 8. Heatmap plot representation of barley malt protein precipitation during the wort boiling step. For detailed information, see Supporting Information, Excel file.

of wort prepared with the Liga barley cultivar may result from the interaction with proteins, leading to precipitation.

The interaction between hop bitter acids and proteins is revealed in various beer properties, including foam stability and haze formation.[64] During colloid formation at the beginning of the boiling step, α-acids interact with denatured proteins, possibly forming cross-links via hydrogen bonds, metal chelation, or hydrophobic interactions.[65] A higher protein content, as observed in the Liga wort, could intensify interactions between specific proteins and α-acids by greater accessibility of soluble protein, resulting in the encapsulation of these compounds and, consequently, limiting their potential for isomerization into *iso*-α-acids. This phenomenon mirrors a mechanism detailed by Jongberg et al.[66] for protein−polyphenol complexation, where polyphenols react with cysteine proteins' thiol groups exposed during unfolding, forming covalent bonds. Furthermore, the unfolding of proteins, notably LTP1, during wort boiling may expose hydrophobic sites, promoting noncovalent hydrophobic interactions with polyphenols. This analogous mechanism could apply to hop bitter acids, especially α-acids, binding to proteins and protein complexes, as discussed in previous research.[12] However, this hypothesis requires confirmation in further studies.

Protein precipitation as trub is generally considered beneficial in brewing, enhancing beer quality by eliminating haze-active proteins.[67] However, a significant amount of water-soluble protein ends up in this residue. Only one-fifth of the initial protein content was detected in the boiled wort prepared from the Liga or Solist cultivar, representing 21.47% and

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

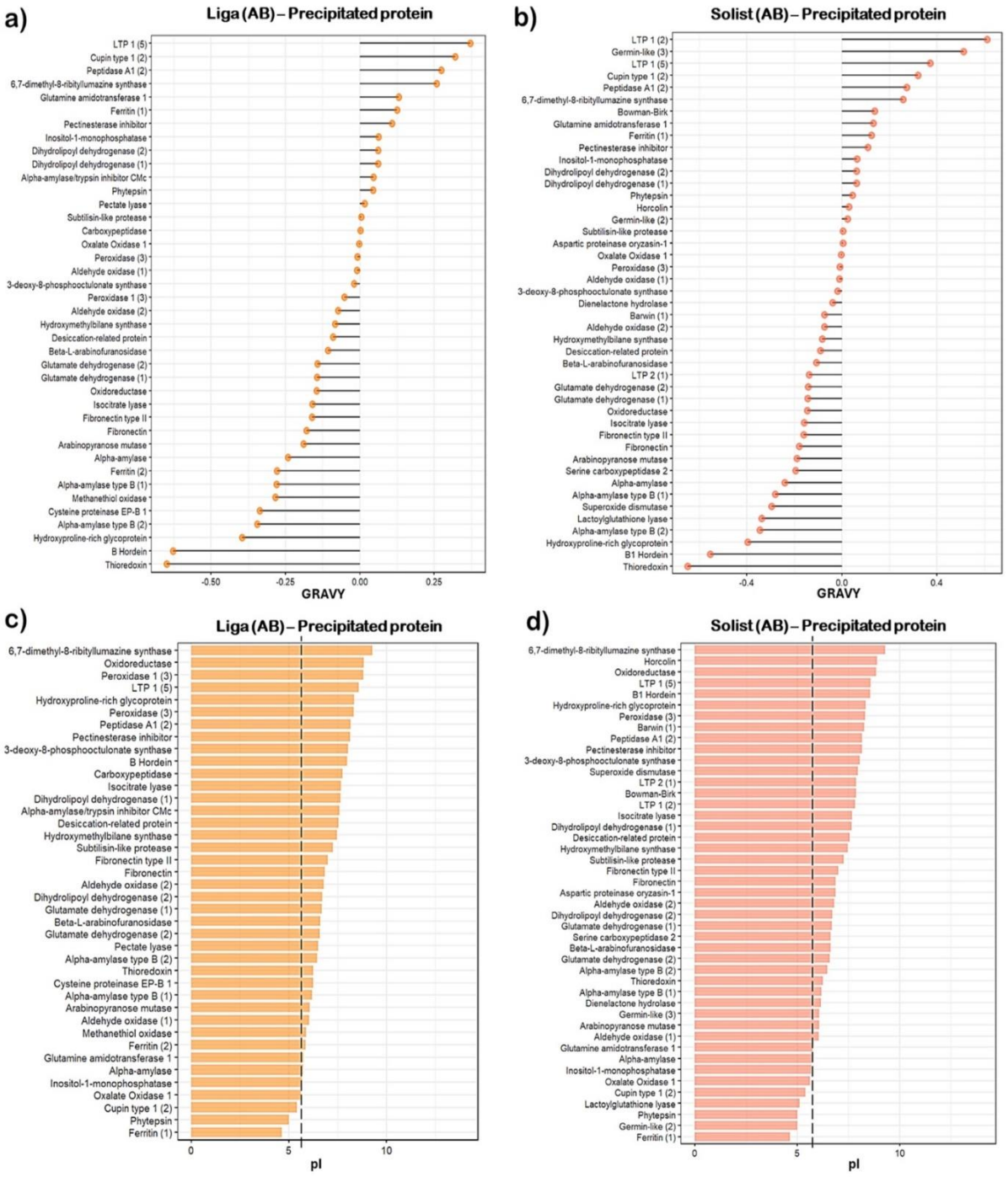


Figure 9. Physicochemical profile of protein completely precipitated during the boiling step: (a) Lollipop chart of the grand average hydrophobicity (GRAVY) of the precipitated protein of the cultivar Liga. (b) Lollipop chart of the grand average hydrophobicity (GRAVY) of the precipitated protein of the cultivar Solist. (c) Bar plot of the Isoelectric Point (p*I*) of the precipitated protein of the cultivar Liga. (d) Bar plot of the isoelectric point (p*I*) of the precipitated protein of the cultivar Solist.

19.16% of the total abundance (LFQ intensity), respectively. Figure 8 shows a heatmap presenting the individual protein abundances (LFQ intensity) for each sample. The 40 proteins in the wort from the Liga cultivar precipitated entirely due to boiling. From the Solist cultivar, 45 proteins precipitated after the same treatment. Of the precipitated proteins, both cultivars shared 32 proteins, primarily comprising enzymes like α-amylase, aldehyde oxidase, glutamate dehydrogenase, and thioredoxin. A notable observation was greater precipitation of higher molecular weight proteins (Supporting Information, Table S7). In the wort from the Solist cultivar, 51.11% of the total precipitated protein had a molecular weight greater than 40 kDa, compared to only 25.49% in the boiled wort. In contrast, the wort from the Liga cultivar exhibited an even higher proportion, with 65% of the total precipitated protein more than 40 kDa, in comparison to 24.49% of the soluble

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

protein in the boiled wort. This trend was also observed in the study developed by Schultz et al.,[68] which showed a considerable decrease in the abundance of high molecular weight proteins during wort boiling.

It is well-known that boiling wort induces protein unfolding via thermal denaturation, exposing inner hydrophobic sites that favor enthalpic interactions.[69] Furthermore, evidence confirms that free cysteines are commonly surrounded by hydrophobic residues.[70] Consequently, unfolded proteins interact via their hydrophobic sites and thiol groups, leading to the formation of protein−protein complexes. Notably, Jin et al.[71] demonstrated the dependence of barley cultivars on the aggregation of unfolded proteins and chemical interactions, especially modification of sulfhydryl content. In this study, we observed a high degree of similarity in the protein profiles of precipitated proteins between cultivars, with the main discrepancies attributed to genotype-specific proteins. However, among the nongenotype-specific proteins, α-amylase/trypsin inhibitor CMc, cysteine proteinase EP-B1, and peroxidase 1 were exclusively precipitated in the boiled wort of the Liga cultivar. These proteins share a remarkable number of cysteine residues in their primary structure (Supporting Information, Figure S6), alongside hydrolase and binding activities. Additionally, Ilimure et al.[72] suggested that α-amylase/trypsin inhibitor CMc is likely to precipitate with the trub, whereas in a previous study peroxidase emerged as an important protein likely to bind to hop bitter acids.[12] Therefore, it is suggested that those proteins might play an important role in protein aggregation through the highly reactive thiol groups as well as the hydrophobic sites that might increase interactions among proteins and other compounds, such as polyphenols and hop bitter acids. While considerable attention has been paid to foam- and haze-active proteins in the brewing process, the current findings underscore the significance of minor proteins not directly linked to beer quality traits. Such proteins can indirectly influence process efficiency and flavor by forming interactions during boiling and further precipitation, thereby carrying important compounds, such as hop bitter acids. A previous study highlighted the specific proteins identified and their roles in the precipitation process and protein aggregate formation.[12]

To understand the physicochemical pattern of the entirely precipitated protein during the boiling step, GRAVY and p*I* values were determined as previously described. The results are presented in Figure 9. It is noteworthy that the observed trends in protein properties appear to be largely consistent across different barley varieties. The hydrophobicity (GRAVY) values of the precipitated proteins in the post-boiled wort show slight variations, ranging from −0.65 to 0.37 for the Liga cultivar and from −0.65 to 0.61 for the Solist cultivar. Notably, after boiling, the precipitated proteins tend to shift toward a higher hydrophobic range compared to their preboiling values (Figure 9a,b). The lowest value preboiling was −1.704 while after boiling stands at −0.65. This observation supports the idea that a protein's primary structure, along with its physicochemical properties, can significantly influence its tendency to precipitate. Böhm et al.[73] also found that hot trub contains elevated levels of hydrophobic amino acids. The author suggests that these higher levels of hydrophobic amino acids reduce hydration, affecting solubility and subsequently leading to aggregation through hydrophobic interactions postdenaturation. This phenomenon is evident in the findings of this study, which demonstrate a higher number of hydrophobic proteins that have been completely precipitated.

Protein p*I* is intrinsically correlated with protein solubility according to pH due to a neutral net charge of protein at pI. Once reaching this point, the protein experiences reduced hydration and electrostatic repulsion, leading to aggregation and subsequent precipitation via hydrophobic interactions.[59] Figure 9c,d illustrate the p*I* values of completely precipitated proteins for each barley cultivar. These p*I* values align closely with the wort pH of 5.6 (indicated by the dashed line). Surprisingly, the p*I* range (4.64−9.29) remains consistent across both barley varieties, suggesting that wort pH has a more significant impact on protein precipitation than the specific barley malt used. Protein precipitation by pH is widely used in the food industry and is also applicable in the brewing process, contributing to enhanced beer quality.[36] In a study by Ilimure et al.,[72] analysis of sweet wort, boiled wort, and trub revealed that proteins in the trub primarily fall within the p*I* range of 5.0−8.0. This study highlights the influence of barley genotype on the utilization of hop bitter acids, although the impact on protein precipitation is comparatively minor concerning physicochemical properties.

In summary, this study presented for the first time the influence of barley genotype on interactions between proteins during boiling and its subsequent effect on the hop bitter acid yield. By examining barley malt's protein composition, genetic variation, and physicochemical properties, this research provides insights into how different barley cultivars affect the utilization of hop bitter compounds and protein precipitation during wort boiling. The findings suggest that barley cultivars influence the availability of hop bitter acids for isomerization, with implications for beer bitterness and quality. Additionally, the study highlights the role of interactions between proteins and physicochemical properties in protein precipitation, contributing to a deeper understanding of the brewing process and its environmental impact.

Based on the desired beer characteristics, brewers can select barley cultivars that align with their quality goals. For clear, stable beer, choosing a low-protein cultivar, such as Solist, might be advantageous. Conversely, if the goal is to enhance yeast fermentation performance, then a cultivar with higher FAN content should be considered. Brewers can optimize the boiling process to enhance protein precipitation. By closely monitoring and adjusting the boiling parameters to target protein precipitation, brewers can achieve better clarity and stability in their beer. Furthermore, the hop bitter acid utilization in brewing could be improved by the investigation of target proteins interaction and the chemical mechanism behind it.

Furthermore, additional studies are recommended to investigate the role of specific amino acids and protein domains in interactions with hop bitter acids. Future research could also be performed with additional commercial barley cultivars processed in different malt products to screen the role of barley genetic variation, malting process, and environmental conditions on hop bitter acids utilization.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.4c04396.

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Additional details of the methodology and data analysis (PDF)

Additional details of the proteomics data set (XLSX)

AUTHOR INFORMATION

Corresponding Author

Philip C. Wietstock − *Institut of Food Technology and Food Chemistry, Chair of Brewing and Beverage Technology, Technische Universität Berlin, 13353 Berlin, Germany*; Email: philip.wietstock@tu-berlin.de

Authors

Mariana B. C. Pinto − *Fruit and Vegetables Laboratory−Department of Food Technology, School of Food Engineering, University of Campinas (UNICAMP), 13083-862 Campinas, São Paulo, Brazil; Institut of Food Technology and Food Chemistry, Chair of Brewing and Beverage Technology, Technische Universität Berlin, 13353 Berlin, Germany*; orcid.org/0000-0002-3761-8731

Flavio L. Schmidt − *Fruit and Vegetables Laboratory−Department of Food Technology, School of Food Engineering, University of Campinas (UNICAMP), 13083-862 Campinas, São Paulo, Brazil*

Zhuo Chen − *Bioanalytics, Institute of Biotechnology, Technische Universität Berlin, 10623 Berlin, Germany*

Juri Rappsilber − *Bioanalytics, Institute of Biotechnology, Technische Universität Berlin, 10623 Berlin, Germany*; orcid.org/0000-0001-5999-1310

Brian Gibson − *Institut of Food Technology and Food Chemistry, Chair of Brewing and Beverage Technology, Technische Universität Berlin, 13353 Berlin, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.4c04396

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge the companies IREKS and HOPSTEINER for the material donation. We appreciate the support of the CNPq−Research Nacional Council−Brazil (finance code 141485/2018-3) and CAPES−Coordination of Superior Level Staff Improvement−Brazil (finance code 88887.575986/2020-00) for Ph.D. CAPES-PRINT Sandwich Program.

ABBREVIATIONS

MEBAK, Mitteleuropäische Brautechnische Analysenkommission; HPLC, high-pressure liquid chromatography; GRAVY, grand average hydropathic value; pI, isoelectric point; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; ABC, ammonium bicarbonate; ACN, acetonitrile; DTT, dithiothreitol; IAA, iodoacetamide; TFA, tridluoroacetic acid; FA, formic acid; LC-MS/MS, liquid chromatography coupled with mass spectrophotometry; MD, mass spectrometry; HCD, higher-energy collisional dissociation; AGC, automatic gain control; GO, gene ontology; PCA, principal component analysis; PC, principal component; TN, total nitrogen; FAN, free amino nitrogen; LFQ, label-free quantitation; LTP, lipid transfer proteins; MF, molecular function; BP, biological process; LEA, late embryogenesis abundant protein

REFERENCES

(1) *Beer World Production*; Statista, 2022; https://www.statista.com/statistics/270275/worldwide-beer-production/.

(2) Heller, M. *Food Product Environmental Footprint Literature Summary: Beer*; State of Oregon Department of Environmental Quality, 2017.

(3) Cerquiera e Silva, K. F.; Martins Strieder, M.; Carvalhal Pinto, M.; Rostagno, M. A.; Hubinger, M. D. Processing Strategies for Extraction and Concentration of Bitter Acids and Polyphenols from Brewing By-Products: A Comprehensive Review. *Processes* **2023**, *11*, 921.

(4) Fabian, M.; Kummer, S.; Bischoff, B.; Ebert, T.; Keßler, H. *Nine Principles for a Circular Economy*; Dessau-Roßlau, 2020; www.umweltbundesamt.de/publikationen.

(5) Deutz, P. Circular Economy. In *International Encyclopedia of Human Geography*, 2nd ed.; Elsevier, 2019; pp 193−201.

(6) Kappler, S.; Schönberger, C.; Krottenthaler, M.; Becker, T. Isohumulones − a Review. *Brewing Science* **2010**, *63*, 105−111.

(7) Jaskula, B.; Kafarski, P.; Aerts, G.; de Cooman, L. A Kinetic Study on the Isomerization of Hop α-Acids. *J. Agric. Food Chem.* **2008**, *56* (15), 6408−6415.

(8) Gänz, N.; Becher, T.; Drusch, S.; Titze, J. Interaction of Proteins and Amino Acids with Iso-α-Acids during Wort Preparation in the Brewhouse. *European Food Research and Technology* **2022**, *248* (3), 741−750.

(9) Lu, Y.; Osmark, P.; Bergenståhl, B.; Nilsson, L. Vesicular Structures Formed from Barley Wort Proteins and Iso-Humulone. *Food Hydrocoll* **2020**, *105*, 105788.

(10) Bamforth, C. W. *Brewing Materials and Processes : A Practical Approach to Beer Excellence*; Academic Press, 2016.

(11) Mahalingam, R. Temporal Analyses of Barley Malting Stages Using Shotgun Proteomics. *Proteomics* **2018**, *18*, 25.

(12) Pinto, M. B. C.; Schmidt, F. L.; Rappsilber, J.; Gibson, B.; Wietstock, P. C. Addition of Hop (Humulus Lupulus L.) Bitter Acids Yields Modification of Malt Protein Aggregate Profiles during Wort Boiling. *J. Agric. Food Chem.* **2023**, *71* (14), 5700−5711.

(13) Bettenhausen, H. M.; Barr, L.; Broeckling, C. D.; Chaparro, J. M.; Holbrook, C.; Sedin, D.; Heuberger, A. L. Influence of Malt Source on Beer Chemistry, Flavor, and Flavor Stability. *Food Research International* **2018**, *113*, 487−504.

(14) United Nations. *The 17 Goals*; United Nations Department of Economic and Social Affairs, 2023; https://sdgs.un.org/goals.

(15) *Method B-590.08.904. Dichte−Biegeschwinger*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, BY, Germany, 2022; https://www.mebak.org/methode/b-590-08-904/dichte-biegeschwinger/771 (Accessed 2022-08-31).

(16) MEBAK online. *Methode B-590.10.181. Stammwürze, Extrakt und Alkohol−Biegeschwinger und NIR*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany, 2022; https://www.mebak.org/methode/b-590-10-181/stammwuerze-extrakt-und-alkohol-biegeschwinger-und-nir/776. (Accessed 2022-08-31).

(17) *Methode B-590.09.900. Scheinbarer Extrakt*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany, 2022; https://www.mebak.org/methode/b-590-09-900/scheinbarer-extrakt/774 (Accessed 2022-08-31).

(18) *Methode B-590.00.040. pH-Messung*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany, 2022; https://www.mebak.org/methode/b-590-00-040/ph-messung/762 (Accessed 2022-08-31.08).

(19) *Methode B-590.78.999. Physiologischer Brennwert−exakte Methode*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany, 2022; https://www.mebak.org/methode/b-590-78-999/physiologischer-brennwert-exakte-methode/2295 (Accessed 2022-08-31).

(20) *Methode B-400.07.003. Gesamtstickstoff in Würze und Bier−KJELDAHL*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany; 2022; https://www.mebak.org/

methode/b-400-07-003/gesamtstickstoff-in-wuerze-und-bier-kjeldahl/649 (Accessed 2022-08-31).
(21) *Methode B-400.11.111. Freier Amino-Stickstoff (FAN) − Ninhydrin-Methode (spektralphotometrisch)*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany, 2022; https://www.mebak.org/methode/b-400-11-111/freier-amino-stickstoff-fan-ninhydrin-methode-spektralphotometrisch/655 (Accessed 2022-08-31).
(22) *Methode B-590.41.111. Gesamtpolyphenole*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany, 2022; https://www.mebak.org/methode/b-590-41-111/gesamtpolyphenole/842 (Accessed 2022-08-31).
(23) *Methode B-400.26.900. Hochmolekulares β-Glucan−fluorimetrisch, Rev. 2020−10*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany, 2022; https://www.mebak.org/methode/b-400-26-900/hochmolekulares-glucan-fluorimetrisch/677 (Accessed 2022-08-31).
(24) *Methode B-400.17.110. Bittereinheiten*; Mitteleuropäische Brautechnische Analysenkommission (MEBAK) eV: Freising, Germany, 2022; https://www.mebak.org/methode/b-400-17-110/bittereinheiten/663 (Accessed 2022-08-31).
(25) Stothard, P. Internet On-Ramp The Sequence Manipulation Suite: JavaScript Programs for Analyzing and Formatting Protein and DNA Sequences. *Biotechniques* **2000**, *28* (6), 1102−1104.
(26) Rappsilber, J.; Ishihama, Y.; Mann, M. Stop and Go Extraction Tips for Matrix-Assisted Laser Desorption/Ionization, Nanoelectrospray, and LC/MS Sample Pretreatment in Proteomics. *Anal. Chem.* **2003**, *75* (3), 663−670.
(27) Tyanova, S.; Temu, T.; Sinitcyn, P.; Carlson, A.; Hein, M. Y.; Geiger, T.; Mann, M.; Cox, J. The Perseus Computational Platform for Comprehensive Analysis of (Prote)Omics Data. *Nature Methods* **2016**, *13*, 731−740.
(28) Jolliffe, I. T. Discarding Variables in a Principal Component Analysis. I: Artificial Data. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* **1972**, *21*, 160.
(29) Rani, H.; Bhardwaj, R. D. Quality Attributes for Barley Malt: "The Backbone of Beer. *Journal of Food Science* **2021**, *86*, 3322−3340.
(30) Castro, L. F.; Affonso, A. D.; Lehman, R. M. Impact of Specialty Malts on Wort and Beer Characteristics. *Fermentation* **2021**, *7*, 137.
(31) Mikyška, A.; Dušek, M. How Wort Boiling Process Affects Flavonoid Polyphenols in Beer. *KVASNY PRUMYSL* **2019**, *65*, 192.
(32) Mikyška, A.; Hrabák, M.; Hašková, D.; Šrogl, J. The Role of Malt and Hop Polyphenols in Beer Quality, Flavour and Haze Stability. *Journal of the Institute of Brewing* **2002**, *108* (1), 78−85.
(33) Leitao, C.; Marchioni, E.; Bergaentzlé, M.; Zhao, M.; Didierjean, L.; Miesch, L.; Holder, E.; Miesch, M.; Ennahar, S. Fate of Polyphenols and Antioxidant Activity of Barley throughout Malting and Brewing. *J. Cereal Sci.* **2012**, *55* (3), 318−322.
(34) Pitts, E.; Witrick, K. Using Malt Total Nitrogen (TN) as a Malt Quality Metric. *ACS Food Science and Technology* **2021**, *1* (10), 1764−1769.
(35) Steiner, E.; Becker, T.; Gastl, M. Turbidity and Haze Formation in Beer − Insights and Overview. *Journal of the Institute of Brewing2* **2010**, *116* (4), 360−368.
(36) Bamforth, C.; Russell, I.; Stewart, G. *Beer: A Quality Perspective*; Academic Press, 2008.
(37) Nielsen, P. K.; Bønsager, B. C.; Fukuda, K.; Svensson, B. Barley α-Amylase/Subtilisin Inhibitor: Structure, Biophysics and Protein Engineering. *Biochimica et Biophysica Acta - Proteins and Proteomics* **2004**, *1696*, 157−164.
(38) Iimure, T.; Takoi, K.; Kaneko, T.; Kihara, M.; Hayashi, K.; Ito, K.; Sato, K.; Takeda, K. Novel Prediction Method of Beer Foam Stability Using Protein Z, Barley Dimeric α-Amylase Inhibitor-1 (BDAI-1) and Yeast Thioredoxin. *J. Agric. Food Chem.* **2008**, *56* (18), 8664−8671.
(39) Okada, Y.; Iimure, T.; Takoi, K.; Kaneko, T.; Kihara, M.; Hayashi, K.; Ito, K.; Sato, K.; Takeda, K. The Influence of Barley Malt Protein Modification on Beer Foam Stability and Their Relationship to the Barley Dimeric α-Amylase Inhibitor-1 (BDAI-1) as a Possible Foam-Promoting Protein. *J. Agric. Food Chem.* **2008**, *56*, 1458−1464.
(40) Niu, C.; Han, Y.; Wang, J.; Zheng, F.; Liu, C.; Li, Y.; Li, Q. Malt Derived Proteins: Effect of Protein Z on Beer Foam Stability. *Food Biosci* **2018**, *25* (July), 21−27.
(41) Finnie, C.; Svensson, B.. Barley Grain Proteins. In *Barley Chemistry and Technologuey*; Shewry, P. R., Ullrich, S. E.,, Eds.; AACC International, Inc, 2014; pp 123−168.
(42) *The Barley Genome*; Stein, N., Muehlbauer, G. J.,, Eds.; Springer, 2018.
(43) Wang, Y.; Yuan, H.; Zha, S.; Jabu, T.; Xu, Q. Comparative Proteomic Based Analysis of Grain Nutrients in Two Hulless Barley Cultivars. *Agron J.* **2021**, *113* (5), 3886−3899.
(44) Mahalingam, R. Shotgun Proteomics of the Barley Seed Proteome. *BMC Genomics* **2017**, *18*, 44.
(45) Taylor, J. P.; Arendt, E. K. A Fundamental Study on the Relationship between Barley Cultivar and Hordeins in Single Cultivar Beers. *Journal of the Institute of Brewing* **2016**, *122* (2), 243−250.
(46) Jin, Z.; Li, X. M.; Gao, F.; Sun, J. Y.; Mu, Y. W.; Lu, J. Proteomic Analysis of Differences in Barley (Hordeum Vulgare) Malts with Distinct Filterability by DIGE. *J. Proteomics* **2013**, *93*, 93−106.
(47) Guo, B.; Luan, H.; Lin, S.; Lv, C.; Zhang, X.; Xu, R. Comparative Proteomic Analysis of Two Barley Cultivars (Hordeum Vulgare L.) with Contrasting Grain Protein Content. *Front Plant Sci.* **2016**, *7*, 542.
(48) Halstead, M.; Morrissy, C.; Fisk, S.; Fox, G.; Hayes, P.; Carrijo, D. Barley Grain Protein Is Influenced by Genotype, Environment, and Nitrogen Management and Is the Major Driver of Malting Quality. *Crop Sci.* **2023**, *63* (1), 115−127.
(49) Luo, H.; Harasymow, S.; Paynter, B.; MacLeod, A.; Izydorczyk, M. S.; O'Donovan, J. T.; Li, C. Genetic and Environmental Impact on Protein Profiles in Barley and Malt. *Journal of the Institute of Brewing* **2019**, *125* (1), 28−38.
(50) Zaks, A.; Klibanov, A. M. *The Effect of Water on Enzyme Action in Organic Media* **1988**, *263*, 8017.
(51) Rezaei, K.; Jenab, E.; Temelli, F. Effects of Water on Enzyme Performance with an Emphasis on the Reactions in Supercritical Fluids. *Critical Reviews in Biotechnologuey* **2007**, *27*, 183−195.
(52) Tian, Q.; Wang, G.; Ma, X.; Shen, Q.; Ding, M.; Yang, X.; Luo, X.; Li, R.; Wang, Z.; Wang, X.; Fu, Z.; Yang, Q.; Tang, J.; Wang, G. Riboflavin Integrates Cellular Energetics and Cell Cycle to Regulate Maize Seed Development. *Plant Biotechnol J.* **2022**, *20* (8), 1487−1501.
(53) Mertens, T.; Kunz, T.; Gibson, B. R. Transition Metals in Brewing and Their Role in Wort and Beer Oxidative Stability: A Review. *Journal of the Institute of Brewing* **2022**, *128* (3), 77−95.
(54) Kerr, E. D.; Phung, T. K.; Caboche, C. H.; Fox, G. P.; Platz, G. J.; Schulz, B. L. The Intrinsic and Regulated Proteomes of Barley Seeds in Response to Fungal Infection. *Anal. Biochem.* **2019**, *580*, 30−35.
(55) Jin, Z.; Cai, G. L.; Li, X. M.; Gao, F.; Yang, J. J.; Lu, J.; Dong, J. J. Comparative Proteomic Analysis of Green Malts between Barley (Hordeum Vulgare) Cultivars. *Food Chem.* **2014**, *151*, 266−270.
(56) Cardinal, L. J. Central Tendency and Variability in Biologueical Systems: Part 2. *J. Community Hosp Intern Med. Perspect* **2015**, *5* (5), 28972.
(57) Kiraga, J.; Mackiewicz, P.; Mackiewicz, D.; Kowalczuk, M.; Biecek, P.; Polak, N.; Smolarczyk, K.; Dudek, M. R.; Cebrat, S. The Relationships between the Isoelectric Point and: Length of Proteins, Taxonomy and Ecologuey of Organisms. *BMC Genomics* **2007**, *8*, 163.
(58) Mohanta, T. K.; Khan, A.; Hashem, A.; Abdallah, E. F.; Al-Harrasi, A. The Molecular Mass and Isoelectric Point of Plant Proteomes. *BMC Genomics* **2019**, *20*, 631.
(59) Damodaran, S.; Parkin, K. L.; Fennema, O. R. *Química de Alimentos de Fennema (4a. Ed.).*; Grupo A - Artmed, 2000.
(60) Jaskula-Goiris, B.; de Cooman, L.; Goiris, K. Humulus Lupulus: Hop Alpha-Acids Isomerization - A Review. *BrewingScience* **2018**, *71*, 85−95.

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

(61) Bastgen, N.; Becher, T.; Titze, J. Influencing Factors on Hop Isomerization Beyond the Conventional Range Influencing Factors on Hop Isomerization Beyond the Conventional Range. *Journal of the American Society of Brewing Chemists* **2019**, *77* (2), 126−133.
(62) Ku, F.; Back, W.; Krottenthaler, M.; Freising, D. Particle Size Distribution in Wort During Large Scale Brewhouse Operations. *AICHe J* **2007**, *53*, 1373.
(63) Malowicki, M. G.; Shellhammer, T. H. Factors Affecting Hop Bitter Acid Isomerization Kinetics in a Model Wort Boiling System. *J. Am. Chem. Soc.* **2006**, *64* (1), 29−32.
(64) Lu, Y.; Bergenståhl, B. Condensation of Iso-Humulone in Solution and at Hydrophobic Surfaces. *Colloids Surf. A Physicochem Eng. Asp* **2021**, *613*, 126102.
(65) Gribkova, I. N.; Eliseev, M. N.; Belkin, Y. D.; Zakharov, M. A.; Kosareva, O. A. The Influence of Biomolecule Composition on Colloidal Beer Structure. *Biomolecules* **2022**, *12*, 24.
(66) Jongberg, S.; Andersen, M. L.; Lund, M. N. Covalent Protein-Polyphenol Bonding as Initial Steps of Haze Formation in Beer. *Journal of the American Society of Brewing Chemists* **2020**, *78* (2), 153−164.
(67) Iimure, T.; Nankaku, N.; Watanabe-Sugimoto, M.; Hirota, N.; Tiansu, Z.; Kihara, M.; Hayashi, K.; Ito, K.; Sato, K. Identification of Novel Haze-Active Beer Proteins by Proteome Analysis. *J. Cereal Sci.* **2009**, *49* (1), 141−147.
(68) Schulz, B. L.; Phung, T. K.; Bruschi, M.; Janusz, A.; Stewart, J.; Meehan, J.; Healy, P.; Nouwens, A. S.; Fox, G. P.; Vickers, C. E. Process Proteomics of Beer Reveals a Dynamic Proteome with Extensive Modifications. *J. Proteome Res.* **2018**, *17* (4), 1647−1653.
(69) Kerr, E. D.; Caboche, C. H.; Schulz, B. L. Posttranslational Modifications Drive Protein Stability to Control the Dynamic Beer Brewing Proteome. *Mol. Cell. Proteomics* **2019**, *18* (9), 1721−1731.
(70) Ho, S. L.; Wang, A. H. J. Structural Bioinformatics Analysis of Free Cysteines in Protein Environments. *J. Taiwan Inst Chem. Eng.* **2009**, *40* (2), 123−129.
(71) Jin, B.; Li, L.; Liu, G.; Li, B.; Zhu, Y.; Liao, L. Structural Changes of Malt Proteins During Boiling. *Molecules* **2009**, *14* (3), 1081−1097.
(72) Iimure, T.; Nankaku, N.; Kihara, M.; Yamada, S.; Sato, K. Proteome Analysis of the Wort Boiling Process. *Food Research International* **2012**, *45* (1), 262−271.
(73) Böhm, W.; Stegmann, R.; Gulbis, O.; Henle, T. Amino Acids and Glycation Compounds in Hot Trub Formed during Wort Boiling. *European Food Research and Technologuey* **2023**, *249* (1), 119−131.

https://doi.org/10.1021/acs.jafc.4c04396
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Chapter 7

What is the outcome of this thesis?

This study was divided into two work packages, each further subdivided into two experimental designs, as represented by the experimental papers included in this thesis. The first work package's experiments and analyses were carried out in Brazil at the Universidade Estadual de Campinas, while the second work package's studies were conducted in Germany at the Technische Universität Berlin.

The first work package investigated drying and supercritical CO_2 extraction methods to identify opportunities for process optimization. This study pioneered the production of hop supercritical CO_2 extract using a Brazilian hop variety. Prior to this, no extract from this raw material had been produced in Brazil, underscoring the innovative nature of this work. The fresh Brazilian hop "Mantiqueira" used in this study was donated by the company Van den Berg. The hops contained 79% moisture, 3.36% α-acids, 2.67% β-acids, and approximately 1.52 mL of total essential oil per 100 grams of hops.

To optimize this step, it was necessary to understand the drying performance of hops and the effect of temperature on hop quality. Therefore, the first experimental paper in Chapter 3 presents results from the study on the drying behavior of hops and the influence of drying temperatures on hop quality traits, such as color, bitter acids content, and essential oil profile. As expected in food drying processes, temperature significantly affects the process time. The drying time decreased by 2.5-fold when the temperature increased from 40ºC to 70ºC. As discussed in the literature review in Chapter 2, the drying step is the bottleneck in hop processing due to the lack of on-farm equipment, which makes harvesting dependent on the availability of dryers. Therefore, reducing drying time could prevent the over-maturation of hops. Furthermore, Rubottom et al. (2022) observed similar drying behavior in commercial dryers and noted that higher temperatures might reduce energy consumption.

Drying hop at higher temperatures also promotes an advantage due to the degradation of amylolytic enzymes which causes the phenomenon called 'hop creep'. Hop creep is associated with the hydrolysis of unfermentable into fermentable sugars by the addition of hops in the cold step, such as fermentation or maturation. The yeast consumes those newly released sugars, producing alcohol and CO_2, reducing the product quality and promoting issues during beer storage.

Rubottom et al. (2021) study highlighted the importance of higher hop drying temperature for the depletion of the hop creep effect on beer. Therefore, not only the hop farmers but also the brewer might benefit from hop air drying temperature.

The drying process is crucial for determining hop quality, influencing several key traits. For more detailed results and discussion, refer to the papers in Chapters 3 and 4. Interestingly, it was observed that drying temperature primarily affects the total essential oil content and the color parameter a*, which represents the range between green (a-) and red (a+) colors.

The decrease in essential oil content was explored by Rybka et al. (2021;2018), whose studies demonstrated that drying hops at 40ºC resulted in only a 10% decrease in total oil content, whereas drying at 55ºC led to a 36% decrease for the Saaz variety. Despite the impact of drying temperature on total oil content, the study presented in Chapter 3 shows that the essential oil profile remains unaltered. This finding is corroborated by Rubottom et al. (2024), who demonstrated that hop sensory quality was similar regardless of the drying temperature. Therefore, the hop-drying process could be enhanced by increasing the air temperature, leading to economic and environmental benefits with a lower effect on hop sensory quality.

As expected, the color of hops changes during drying from green in the fresh cones to green-brown in the dried cones. Lower temperatures result in higher degradation due to prolonged exposure during the process. This degradation affects the chlorophyll present in hops, leading to an undesirable brownish color. In a study by Rybka et al. (2018), it was suggested that drying hops at lower temperatures, such as 40ºC, might be preferable for aromatic hops to preserve essential oil compounds. However, the extended drying time associated with lower temperatures poses several disadvantages, including color changes, increased energy consumption, and longer drier occupancy (Sturm et al., 2020). This undesirable drying color is particularly noticeable in hop products like pellets, which still contain vegetal matter. Therefore, drying at lower temperatures might compromise product value despite preserving essential oil compounds.

Considering the lesser impact of higher temperatures on hop quality during drying compared to lower temperatures, it was determined in this study that drying hops at 70ºC could be advantageous for farms to reduce energy and time consumption. Specifically, hops dried at higher

temperatures could be used to produce hop extract, as the extraction process removes vegetal matter, mitigating the effects of color degradation. Thus, the next step involved producing hop extract through SFE using hops dried at higher temperatures to evaluate the suitability of this optimization. This step began with screening process parameters such as pressure and temperature to understand the extraction phenomenon and establish optimal conditions. Subsequently, extraction kinetics were evaluated to optimize extraction time.

Despite extracts being produced from high α-acid hop varieties, this study successfully produced supercritical CO_2 extracts from the Mantiqueira variety, which initially had low α-acid content. The condition of 30 MPa and 60ºC yielded the maximum extraction yield of 8.6%. However, the condition of 20 MPa and 40ºC was preferred due to lower energy consumption and better utilization of the equipment which is provided by the lower temperature and pressure. In this condition, a yield of 7.78% was achieved. Similar studies have been conducted to extract hop bitter acids using supercritical CO_2; however, the extraction yield was considerably lower than that presented in this study (Formato et al., 2013; Veiga et al., 2021; Zeković et al., 2007).

The kinetic curve is an important parameter for evaluating hop extraction behavior. The diffusional-controlled period, which decreases the extraction rate, was observed after 40 minutes. This indicates a higher extraction yield for bitter acids, resulting in 93% and 97% of the total α- and β-acids being extracted compared to the total yield at 90 minutes. While Valle et al. (2003) achieved approximately 14% extraction yield using a Chilean hop variety, it required a prolonged extraction time of 240 minutes, resulting in higher solvent and energy consumption. Therefore, this study optimized the extraction process by employing mild extraction conditions and reducing the extraction time.

With the rise of artificial intelligence (AI) tools for daily use, this technique could also be applied to optimize processes like hop processing. Therefore, for the first time, the experimental work presented in Chapter 4 tested the suitability of machine learning models to predict drying time. Better control of this process might benefit farmers by reducing hops over-drying as well as energy consumption. Data acquired during the drying process were modeled by both conventional mathematical models used for drying and by machine learning models (such as Artificial Neural

Network, K-Nearest Neighbor, and Random Forest). Comparing these models sheds light on the possibility of using higher-performance models, such as KNN and Random Forest. These models permit higher prediction accuracy compared to conventional methods due to their ability to incorporate more significant inputs for the process, such as air humidity. However, these models should continually be enhanced by adding new datasets with hops from other varieties that have been dried.

The use of AI tools is breaking the walls of the informatic technology field and becoming more accessible to our daily lives. However, the usage in the brewing field is still insipient, representing room for expansion. A ground-breaking study developed by Schreurs et al. (2023) recently uncovered the complexity of flavor and consumer perception linkage with beer chemistry. In this study, machine learning predicting models were performed in an attempt to identify compounds that influence beer flavor and acceptance. AI could be used in several ways, such as product design, process optimization, and logistics (Schlechter, 2024). For instance, a company called NotCo which produces vegan products, uses a platform named Giuseppe that consists of an AI system that supports the scientists within the company to screen the plant kingdom and design a combination of those ingredients to create a new product (NotCO Company, 2024). Similarly, an AI tool could be implemented in the brewing sector to improve product development.

The second work package of this thesis involved a proteomic study aimed at uncovering the relationship between hop bitter acids losses and protein precipitation with the trub, to improve hop utilization and reduce waste generation. This phase began with the study presented in the experimental paper in Chapter 5, which investigated the influence of hop addition on protein precipitation profiles. For this study, a sweet wort was prepared and divided into two portions, with hop extract added to one during boiling. Protein profile quantification and identification were conducted using an innovative approach employing Liquid-Chromatography coupled with Mass-Spectrometry (LC-MS/MS).

Through proteomic analysis, it was possible to gain in-depth insight into the role of hop bitter acids in the formation of protein aggregates. The foam-active protein LTP precipitated in the presence of hop compounds, whereas Protein Z exhibited the opposite behavior. Interestingly, the

less abundant proteins Late Embryogenesis Abundant (LEA), Aspartic Proteases, and Thionin were highly affected by hop addition. This study demonstrated that minor proteins may play an important role in hop bitter acids losses, but the interactions between proteins and hop bitter acids remain unknown.

Protein folding relies on hydrophobic interactions and disulfide bonds among cysteine residues to maintain stability in aqueous solutions (Camilloni et al., 2016). During wort boiling, proteins denature, exposing inner hydrophobic groups, which form aggregates for thermodynamic stability (Jin et al., 2009). This process also reduces disulfide bonds, affecting protein stability. The addition of hop bitter acids alters protein-protein complex formation, with proteins interacting through hydrophobic and disulfide bonds (Qin et al., 2015). Studies show that iso-α-acids influence aggregation by acting as cross-linkers, particularly involving protein Z, which forms vesicular structures to protect hydrophobic domains (Lu, Osmark, Bergenståhl, Nilsson, et al., 2020).

The outcomes from the study presented in Chapter 5 were groundbreaking in demonstrating the influence of hop bitter compounds on protein interactions during wort boiling, especially with lesser-studied proteins. Furthermore, these interactions might occur during beer haze formation, an important quality parameter for beer stability. Hop bitter acids could also play a role in haze formation alongside proteins other than hordeins. Beyond the brewing field, the interaction between hop bitter acids and plant proteins could be further explored to enhance the quality traits of vegan products, such as texture. Similar to how polyphenols are being explored, hop bitter acids could act as cross-linkers to promote emulsion stabilization in products such as plant-based yogurt or improve texture in extruded alternative protein meat.

This research also highlights the role of minor proteins in the interaction between hop bitter acids and proteins. Peroxidase exhibited significant differences in abundance with the addition of hops. Peroxidase, found in nearly all living organisms, plays a crucial role in plant defense by catalyzing the oxidative cross-linking of proteins and phenolics in cell walls. In barley, the main peroxidase, BP1, contains a heme group crucial for its function. During wort boiling in beer production, peroxidase denatures, exposing its heme and other reactive sites. This exposure leads

to interactions with hop bitter acids, forming complexes that may contribute to protein aggregation and increased precipitation. Those proteins might impact several quality traits, such as foam, haze, and flavor stability. For instance, the exposed metal active site could lead to oxidation reactions which produces undesirable aldehydes, affecting the beer shelf-life. Therefore, hop bitter acids could act as an antioxidant by reacting primarily to those proteins, improving beer flavor stability.

Barley malt protein could hold the key to improving hop bitter acids utilization by reducing precipitation to the trub. Hence, the second experimental paper of this work package (Chapter 6) was developed to investigate the effect of barley malt protein content on hop bitter acids utilization. A sweet wort was prepared using two barley varieties named Liga and Solist. Proteomic analyses were conducted using LC-MS/MS, similar to the previous work.

The protein profiles of the barley cultivars differed, as revealed by proteomic analysis of wort before boiling, identifying 107 protein groups. Among these, 78 were shared between the two barley cultivars, Liga and Solist, while 11 were unique to Liga and 18 to Solist. Despite Liga exhibiting higher total protein abundance, it had fewer identified proteins, highlighting the impact of genetic variation on protein profiles. This genetic diversity affects protein isoforms and carries significant implications for barley breeding and the malting process. The malting process modifies the protein profile due to the germination which produces protease and amylase enzymes, hydrolyzing the storage proteins. Furthermore, the process parameters play a role in the enzymes production, posttranslational modification, and reduction of storage proteins.

Surprisingly, this study revealed the role of barley proteome in hop utilization which presents the total α- and iso-α-acids concentration in samples prepared with each barley malt variety. It is evident that the total α-acid content significantly decreased with the increase in protein content of the barley. This can be attributed to the formation of protein aggregates, which primarily occurs during the heating and initial boiling phases, with particle size stabilizing afterward (Ku et al., 2007). This study found a 26.75% higher total α-acid content in wort from the Solist barley cultivar compared to Liga, suggesting that barley's protein profile or content affects α-acid availability for isomerization. Maintaining consistent pH across trials eliminated pH as a factor, indicating that Liga's lower α-acid content might result from protein interactions leading to

precipitation. These interactions, involving hydrogen bonds, metal chelation, or hydrophobic interactions, can encapsulate α-acids, reducing their isomerization into iso-α-acids (Gribkova et al., 2022). It is suggested that the hydrophobic sites of α-acids might interact with the inner hydrophobic sites of proteins by hydrophobic interactions and/or hydrogen bonds formed between the hydroxyl group of α-acids and polar amino acids. Another possible mechanism of interactions might be through covalent bonds formed between the thiol group of cysteine amino acids and the electrophilic site of α-acids, such as hydroxyl and carbonyl groups. This mirrors known protein-polyphenol complexation mechanisms and suggests similar processes for hop bitter acids, particularly α-acids (Jongberg et al., 2020). However, further studies are needed for this mechanism confirmation.

The protein content of barley is influenced by environmental factors. Therefore, climate change is also a threat to barley composition and quality, leading to unpredictable variations in crops (Meng et al., 2023). By studying the influence of the protein content of barley on brewing, as presented in Chapter 6, might offer support to the brewing industry to avoid greater interference in the process due to protein content variation. Furthermore, this study's outcomes could be expanded to the use of further malt and cereals influence on hop bitter acid utilization. For instance, oats or wheat which contain higher protein content might lead to lower hop bitter acids utilization and consequently, reduced bitterness in beer.

The brewing industry has long sought alternatives to increase hop α-acids utilization, yet profitability remains a challenge, as current options mainly involve acquiring new technologies. In an attempt to address this issue, an additional experiment testing the use of pure β-acids in the wort boiling before hop extract addition was conducted. In this trial, a sweet wort was prepared and divided into two portions, with one portion having pure β-acids added at the beginning of boiling. The initial results from these trials have already shown that pre-dosing hop β-acids before the addition of hop extract for bittering significantly improved the utilization of iso-α-acids by approximately 4.2%. This underscores the benefits of using this approach to enhance the conversion of iso-α-acids by depleting the proteins likely to bind with α- and iso-α-acids.

Chapter 8

Which conclusions can be drawn?

In conclusion, this research screened possibilities for optimization of hop usage from the hop farm to the brewery with a focus on bitterness and, consequently, reducing energy consumption and waste production. This research has made significant developments toward enhancing the hop and brewing processes using innovative approaches, such as artificial intelligence on hop processing and proteomic analysis on hop-protein interactions. The optimization of hop processing and its utilization in brewing emerged as a central theme due to the urgency for more sustainable processes. Empirical investigations shed light on opportunities to successfully produce hop extract from hop dried at higher temperatures using mild extraction conditions and reduced solvent consumption. Furthermore, this study confirmed that the main effect of drying temperatures lies in hop color which degrades throughout the process. This research also explored the intricate biochemical dynamics governing hop-protein interactions during brewing, offering novel insights into the mechanisms underlying hop utilization efficiency and waste generation. For the first time, the role of hop bitter acids on protein aggregates formation during wort boiling was uncovered. Through advanced proteomic analyses, this study has elucidated the role of genetic variation in barley malt and its implications for hop utilization. The barley proteome directly influences the hop bitter acids yield during wort boiling. The findings from this research open the way to explore in more detail the interaction between hop bitter acids and proteins which play a role in several biochemical reactions throughout the brewing process. The integration of cutting-edge technologies such as machine learning has further advanced our understanding of brewing processes, enabling data-driven decision-making and process optimization. Overall, this thesis highlights the possibility of improving hop and brewing processes to achieve more sustainable and economically viable products.

Chapter 9

References

Addo, P. W., Taylor, N., MacPherson, S., Raghavan, V., Orsat, V., & Lefsrud, M. (2022). Impact of pre-freezing and microwaves on drying behavior and terpenes in hops (Humulus lupulus). *Journal of Applied Research on Medicinal and Aromatic Plants*, *31*. https://doi.org/10.1016/j.jarmap.2022.100436

Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). Humulus lupulus - a story that begs to be told. A review. *Journal of the Institute of Brewing*, *120*(4), 289–314. https://doi.org/10.1002/jib.160

BarthHaas Europe. (2021). *Sustainability Report 2020/21*.

Barth-Haas Group. (2021). *Barth Haas Report 2021/22*.

Bastgen, N., Becher, T., & Titze, J. (2019). Influencing Factors on Hop Isomerization Beyond the Conventional Range Influencing Factors on Hop Isomerization Beyond the Conventional Range. *Journal of the American Society of Brewing Chemists*, *77*(2), 126–133. https://doi.org/10.1080/03610470.2019.1587734

Biendl, M., Engelhard, B., Forster, A., Gahr, A., Lutz, A., Mitter, W., Schmidt, R., & Schönberger, C. (2014). *Hops: their Cultivation, Composition, and Usage*. Fachverlag Hans Carl GmbH.

Bizaj, K., Škerget, M., Košir, I. J., & Knez, Ž. (2021). Sub- and supercritical extraction of slovenian hops (Humulus lupulus l.) aurora variety using different solvents. *Plants*, *10*(6). https://doi.org/10.3390/plants10061137

Bizaj, K., Škerget, M., Košir, I. J., & Knez, Ž. (2022). Hop (Humulus lupulus L.) Essential Oils and Xanthohumol Derived from Extraction Process Using Solvents of Different Polarity. *Horticulturae*, *8*(5). https://doi.org/10.3390/horticulturae8050368

Briggs, D. E., Boulton, C. A., Brookes, P. A., & Stevens, R. (2004). *Brewing Science and practice*.

Briggs, D. E., Hough, J. S., Stevens, R., & Young, T. W. (1982). *Malting and Brewing Science* (2nd ed., Vol. 2, Issue 5).

Brunner, G. (2005). Supercritical fluids : technology and application to food processing. *Journal of Food Engineering*, *67*, 21–33. https://doi.org/10.1016/j.jfoodeng.2004.05.060

Caballero, I., Blanco, C. A., & Porras, M. (2012). Iso-α-acids, bitterness and loss of beer quality during storage. *Trends in Food Science and Technology*, *26*(1), 21–30. https://doi.org/10.1016/j.tifs.2012.01.001

Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P. W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., Blanco, G., Cheung, W. W. L., Connors, S., Denton, F., Diongue-Niang, A., Dodman, D., Garschagen, M., Geden, O., Hayward, B., Jones, C., … Ha, M. (2023). *IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.* (P. Arias, M. Bustamante, I. Elgizouli, G. Flato, M. Howden, C. Méndez-Vallejo, J. J. Pereira, R. Pichs-Madruga, S. K. Rose, Y. Saheb, R. Sánchez Rodríguez, D. Ürge-Vorsatz, C. Xiao, N. Yassaa, J. Romero, J. Kim, E. F. Haites, Y. Jung, R. Stavins, … C. Péan, Eds.). https://doi.org/10.59327/IPCC/AR6-9789291691647

Camilloni, C., Bonetti, D., Morrone, A., Giri, R., Dobson, C. M., Brunori, M., Gianni, S., & Vendruscolo, M. (2016). Towards a structural biology of the hydrophobic effect in protein folding. *Scientific Reports*, *6*. https://doi.org/10.1038/srep28285

Carissa Wong. (2023). EARTH'S HOTTEST YEAR ON RECORD: CLIMATE CHANGE IS TO BLAME. *Nature*, 674–675.

Cerqueira e Silva, K. F., Rabelo, R. S., Feltre, G., & Hubinger, M. (2022). Bitter substances recovery from hot trub: A study of polymeric membranes performance in a sequential mode with fouling investigation. *Separation and Purification Technology*, *303*. https://doi.org/10.1016/j.seppur.2022.122241

Cimini, A., & Moresi, M. (2021). Circular economy in the brewing chain. In *Italian Journal of Food Science* (Vol. 33, Issue 3, pp. 47–69). Codon Publications. https://doi.org/10.15586/ijfs.v33i3.2123

Clark, D. C., Wilde, P. J., & Wilson, D. R. (1991). The Effect of Pre-Isomerised Hop Extract on the Properties of Model Protein Stabilized Foams. *Journal of the Institute of Brewing*, *97*(3), 169–172. https://doi.org/10.1002/j.2050-0416.1991.tb01062.x

Claudia Salanță, L., Corina Fărcaș, A., Borșa, A., & Rodica Pop, C. (2023). Current strategies for the management of valuable compounds from hops waste for a circular economy. In *Food Chemistry: X* (Vol. 19). Elsevier Ltd. https://doi.org/10.1016/j.fochx.2023.100876

Clippeleer, J. de, Cooman, L. de, & Aerts, G. (2014). Beer's Bitter Compounds – A Detailed Review on Iso-α-acids : Current Knowledge of the Mechanisms for their Formation and Degradation. *Brewing Science*, *67*(December), 167–182.

Cooman, L. De, & Goiris, K. (2018). Humulus Lupulus : Hop Alpha-acids Isomerization – A Review. *Brewing Science*, *71*(December), 85–95.

Cvetanović Kljakić, A., Ocvirk, M., Rutnik, K., Košir, I. J., Pavlić, B., Mašković, P., Mašković, J., Teslić, N., Stupar, A., Uba, A. I., & Zengin, G. (2024). Exploring the composition and potential uses of four hops varieties through different extraction techniques. *Food Chemistry*, *447*. https://doi.org/10.1016/j.foodchem.2024.138910

de Clippeleer, J., de Rouck, G., de Cooman, L., & Aerts, G. (2010). Influence of the hopping technology on the storage-induced appearance of staling aldehydes in beer. *Journal of the Institute of Brewing*, *116*(4), 381–398. https://doi.org/10.1002/j.2050-0416.2010.tb00789.x

Deutz, P. (2019). Circular Economy. In *International Encyclopedia of Human Geography, Second Edition* (pp. 193–201). Elsevier. https://doi.org/10.1016/B978-0-08-102295-5.10630-4

Dierckens, J., & Verzele, M. (1969). OXIDATION PRODUCTS OF HUMULONE AND THEIR STEREO- ISOMERISM. *Journal of the Institute of Brewing*, *75*, 453–456.

Durello, R. S., Silva, L. M., & Jr, S. B. (2019). Química do Lúpulo. *Química Nova*, *XY*(00), 1–20.

Dzingelevičius, N., Maruška, A., Ragažinskienė, O., & Obelevičius, K. (2011). *Optimization of hop essential oil extraction by means of supercritical CO 2* (Vol. 57, Issue 2).

European Comission. (2024). *Renewable Energy Directive*. Https://Energy.Ec.Europa.Eu/Topics/Renewable-Energy/Renewable-Energy-Directive-Targets-and-Rules/Renewable-Energy-Directive_en.

Evans, D. E., Surrel, A., Sheehy, M., Stewart, D. C., Louise, H., Evans, D. E., Surrel, A., Sheehy, M., Stewart, D. C., Louise, H., Evans, D. E., Surrel, A., Sheehy, M., Stewart, D. C., White, J., & Louise, H. (2018). Comparison of Foam Quality and the Influence of Hop α-acids and Proteins Using Five Foam Analysis Methods. *Journal of the American Society of Brewing Chemists*, *0470*. https://doi.org/10.1094/ASBCJ-2007-1129-01

Ferenczi, S., Molnár, H., Adányi, N., & Cserhalmi, Z. (2018). Comparison of Microwave Vacuum-, Freeze- and Hot-Air Drying by Energy Efficiency and Aroma Composition of Dried Hop (Humulus lupulus). *International Journal of Food Engineering*, *4*(2), 136–139. https://doi.org/10.18178/ijfe.4.2.136-139

Ferreira, I. M. P. L. V. O., Jorge, K., Nogueira, L. C., Silva, F., & Trugo, L. C. (2005). Effects of the combination of hydrophobic polypeptides, iso-α acids, and malto-oligosaccharides on beer foam stability. *Journal of Agricultural and Food Chemistry*, *53*(12), 4976–4981. https://doi.org/10.1021/jf047796w

Fischer, B., Gevinski, E. V., da Silva, D. M., Júnior, P. A. L., Bandiera, V. J., Lohmann, A. M., Rigo, D., Duarte, P. F., Franceschi, E., Zandoná, G. P., Rombaldi, C. V., Cansian, R. L., Paroul, N., & Junges, A. (2023). Extraction of hops pelletized (Humulus lupulus) with subcritical CO2 and hydrodistillation: Chemical composition identification, kinetic model, and evaluation of antioxidant and antimicrobial activity. *Food Research International*, *167*. https://doi.org/10.1016/j.foodres.2023.112712

Formato, A., Gallo, M., Ianniello, D., Montesano, D., & Naviglio, D. (2013). Supercritical fluid extraction of α- and β-acids from hops compared to cyclically pressurized solid-liquid extraction. *Journal of Supercritical Fluids*, *84*, 113–120. https://doi.org/10.1016/j.supflu.2013.09.021

Gänz, N., Becher, T., Drusch, S., & Titze, J. (2021). Interaction of proteins and amino acids with iso-α-acids during wort preparation in the brewhouse. *European Food Research and Technology*, *248*(3), 741–750. https://doi.org/10.1007/s00217-021-03926-4

Geng, Y. (2019, February 1). The Relevance of Circular Economy Practices to the Sustainable Development Goals. *Nature*, *23*(1), 153–155. https://doi.org/10.1111/jiec.12732

Goese, M., Kammhuber, K., Bacher, A., Zenk, M. H., & Eisenreich, W. (1999). Biosynthesis of bitter acids in hops: A 13C-NMR and 2H-NMR study on the building blocks of humulone. *European Journal of Biochemistry*, *263*(2), 447–454. https://doi.org/10.1046/j.1432-1327.1999.00518.x

Gribkova, I. N., Eliseev, M. N., Belkin, Y. D., Zakharov, M. A., & Kosareva, O. A. (2022). The influence of biomolecule composition on colloidal beer structure. In *Biomolecules* (Vol. 12, Issue 1). MDPI. https://doi.org/10.3390/biom12010024

Group, B. (2019). Humulus lupulus … … and other bitter truths The challenge of utilisation. *Www.Barthhaasgroup.Com*, 2.

Hauser, D. G., & Shellhammer, T. H. (2019). An Overview of Sustainability Challenges in Beer Production, and the Carbon Footprint of Hops Production. *Technical Quarterly*. https://doi.org/10.1094/tq-56-4-0731-01

Heřmánek, P., Rybka, A., & Honzík, I. (2018). Determination of moisture ratio in parts of the hop cone during the drying process in belt dryer. *Agronomy Research*, *16*(3), 723–727.

Herrero, M., Mendiola, J. A., Cifuentes, A., & Ibá, E. (2010). Supercritical fluid extraction : Recent advances and applications. *Journal of Chromatography A*, *1217*, 2495–2511. https://doi.org/10.1016/j.chroma.2009.12.019

Hofmann, R., Garbe, L., & Rettberg, N. (2013). Optimization of the Hop Kilning Process to Improve Energy Efficiency and Recover Hop Oils. *BrewingScience*. https://www.researchgate.net/publication/263817940

Hopfenveredlung. (2024). *Hop processing*. Https://Energy.Ec.Europa.Eu/Topics/Renewable-Energy/Renewable-Energy-Directive-Targets-and-Rules/Renewable-Energy-Directive_en.

Hopfenverwertungsgenossenschaft, H. G. (2021). *Sustainability Report 2021*.

Hoshino, Y., Ota, M., Sato, Y., Smith, R. L., & Inomata, H. (2018). Fractionation of hops-extract–ethanol solutions using dense CO2 with a counter-current extraction column. *Journal of Supercritical Fluids*, *136*, 37–43. https://doi.org/10.1016/j.supflu.2018.02.005

Huang, Y., Tippmann, J., & Becker, T. (2013). Kinetic Modeling of Hop Acids during Wort Boiling. *International Journal of Bioscience, Biochemistry and Bioinformatics*, *3*(1), 47–52. https://doi.org/10.7763/IJBBB.2013.V3.161

Hughes, P. (2000). The significance of iso-α-acids for beer quality cambridge prize paper. *Journal of the Institute of Brewing*, *106*(5), 271–276. https://doi.org/10.1002/j.2050-0416.2000.tb00066.x

HVG. (2024). *Our products*. Https://Www.Hvg-Germany.de/En/Our-Products/.

Iimure, T., Nankaku, N., Kihara, M., Yamada, S., & Sato, K. (2012a). Proteome analysis of the wort boiling process. *Food Research International*, *45*(1), 262–271. https://doi.org/10.1016/j.foodres.2011.10.033

Iimure, T., Nankaku, N., Kihara, M., Yamada, S., & Sato, K. (2012b). Proteome analysis of the wort boiling process. *Food Research International*, *45*(1), 262–271. https://doi.org/10.1016/j.foodres.2011.10.033

Intelmann, D., Haseleu, G., Dunkel, A., Lagemann, A., Stephan, A., & Hofmann, T. (2011). *Comprehensive Sensomics Analysis of Hop-Derived Bitter Compounds during Storage of Beer*. 1939–1953. https://doi.org/10.1021/jf104392y

Inumaro, R. S., Casalvara, R. F. A., Corrêa, R. C. G., Barros, L., Pereira, C., Calhelha, R. M., Heleno, S. A., Pimentel, T. C., Gonçalves, J. E., & Cardozo-Filho, L. (2023). Supercritical fluid-assisted extraction of hop cone residue from craft breweries. *Journal of CO2 Utilization*, *72*. https://doi.org/10.1016/j.jcou.2023.102498

Jaskula, B., Kafarski, P., Aerts, G., & De Cooman, L. (2008). A Kinetic Study on the Isomerization of Hop Alpha-acids. *Journal of Agricultural and Food Chemistry*, *56*, 6408–6415.

Jaskula-goiris, B., Aerts, G., & Cooman, L. De. (2010). Hop alpha-acids isomerisation and utilisation: an experimental review. *Cerevisia*, *35*, 57–70. https://doi.org/10.1016/j.cervis.2010.09.004

Jin, B., Li, L., Liu, G., Li, B., Zhu, Y., & Liao, L. (2009). Structural Changes of Malt Proteins During Boiling. *Molecules*, *14*(3), 1081–1097. https://doi.org/10.3390/molecules14031081

Jongberg, S., Andersen, M. L., & Lund, M. N. (2020). Covalent Protein-Polyphenol Bonding as Initial Steps of Haze Formation in Beer. *Journal of the American Society of Brewing Chemists*, *78*(2), 153–164. https://doi.org/10.1080/03610470.2019.1705045

Kapp, G. R., & Bamforth, C. W. (2002). The foaming properties of proteins isolated from barley. *Journal of the Science of Food and Agriculture*, *82*(11), 1276–1281. https://doi.org/10.1002/jsfa.1177

Kappler, S., Krahl, M., Geissinger, C., Becker, T., & Krottenthaler, M. (2010). Degradation of Iso-α-Acids During Wort Boiling. *Journal of the Institute of Brewing*, *116*(4), 332–338. https://doi.org/10.1002/j.2050-0416.2010.tb00783.x

Karabín, M., Rýparová, A., Jelínek, L., Kunz, T., Wietstock, P., Methner, F., & Dostálek, P. (2014). *Relationship of iso- α -acid content and endogenous antioxidative potential during storage of lager beer*. *January*, 212–219. https://doi.org/10.1002/jib.140

Kerr, W. L. (2013a). Food Drying and Evaporation Processing Operations. In *Handbook of Farm, Dairy and Food Machinery Engineering: Second Edition* (pp. 317–354). Elsevier Inc. https://doi.org/10.1016/B978-0-12-385881-8.00012-4

Kerr, W. L. (2013b). Handbook of Farm, Dairy and Food Machinery Engineering. In *Handbook of Farm, Dairy and Food Machinery Engineering: Second Edition* (2nd ed.). Elsevier Inc. https://doi.org/10.1016/B978-0-12-385881-8.00012-4

Keukeleire, J. De, Ooms, G., Heyerick, A., Roldan-Ruiz, I., Bockstaele, E. Van, & De Keukeleire, D. (2003). Formation and Accumulation of alpha -acids , beta-cids , Desmethylxanthohumol , and Xanthohumol during Flowering of Hops (Humulus lupulus L .). *Journal of Agricultural and Food Chemistry*, *51*, 4436–4441. https://doi.org/10.1021/jf034263z

Klimek, K., Tyśkiewicz, K., Miazga-Karska, M., Dębczak, A., Rój, E., & Ginalska, G. (2021). Bioactive compounds obtained from polish "marynka" hop variety using efficient two-step supercritical fluid extraction and comparison of their antibacterial, cytotoxic, and anti-proliferative activities in vitro. *Molecules*, *26*(8). https://doi.org/10.3390/molecules26082366

Ku, F., Back, W., Krottenthaler, M., & Freising, D.-. (2007). *Particle Size Distribution in Wort During Large Scale Brewhouse Operations*. *53*(5). https://doi.org/10.1002/aic

Kunze, W. (2019). *Technology Brewing and Malting* (O. Hendel, Ed.; 6th ed.). VLB Berlin.

Lewis, M. J., & Young, T. W. (2002). Brewing. In *Kluwer Academic / Plenum Publishers*. https://doi.org/10.1002/9783527620920.ch11

Liu, L., & Ramakrishna, S. (2021). *An Introduction to Circular Economy*.

Lu, Y., Bergenståhl, B. B., & Nilsson, L. (2020). Interfacial properties and interaction between beer wort protein fractions and iso-humulone. *Food Hydrocolloids*, *103*(December 2019). https://doi.org/10.1016/j.foodhyd.2020.105648

Lu, Y., Osmark, P., Bergenståhl, B., & Lars. (2020). Vesicular structures formed from barley wort proteins and iso-humulone. *Food Hydrocolloids*, *105*(February). https://doi.org/10.1016/j.foodhyd.2020.105788

Lu, Y., Osmark, P., Bergenståhl, B., Nilsson, L., & Lars. (2020). Vesicular structures formed from barley wort proteins and iso-humulone. *Food Hydrocolloids*, *105*(February). https://doi.org/10.1016/j.foodhyd.2020.105788

Malowicki, M. G., & Shellhammer, T. H. (2005). Isomerization and Degradation Kinetics of Hop (Humulus. *Journal of Agricultural and Food Chemistry*, *53*(1), 4434–4439.

Mathias, T. R. dos S., Mello, P. P. M. de, & Sérvulo, E. F. C. (2014). Solid wastes in brewing process: A review. *Journal of Brewing and Distilling*, *5*(1), 1–9. https://doi.org/10.5897/jbd2014.0043

McMichael, C. H., Piperno, D. R., Bush, M. B., Silman, M. R., Zimmerman, A. R., Raczka, M. F., & Lobato, L. C. (2012). Sparse Pre-Columbian Human Habitation in Western Amazonia. *Science*, *336*(6087), 1429–1431. https://doi.org/10.1126/science.1219982

Meghie Rodrigues. (2023). THE AMAZON'S RECORDSETTING DROUGHT: HOW BAD WILL IT BE? *Nature*, 675–676. https://doi.org/doi: https://doi.org/10.1038/d41586-023-03469-6

Meireles, M. A. A. (2008). Extraction of Bioactive Compounds from Latin American Plants. In J. L. Martinez (Ed.), *Supercritical Fluid Extraction of Nutraceutical and Bioactive Compounds* (p. 424). CRC Press.

Mejzr, J., & Hanousek, B. (2007). *Drying of hop*. *2007*(4), 155–160.

Meng, G., Rasmussen, S. K., Christensen, C. S. L., Fan, W., & Torp, A. M. (2023). Molecular breeding of barley for quality traits and resilience to climate change. In *Frontiers in Genetics* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fgene.2022.1039996

Mertens, T., Kunz, T., & Gibson, B. R. (2022). Transition metals in brewing and their role in wort and beer oxidative stability: a review. *Journal of the Institute of Brewing*, *128*(3), 77–95. https://doi.org/10.1002/jib.699

Nagybákay, N. E., Syrpas, M., Vilimaitė, V., Tamkutė, L., Pukalskas, A., Venskutonis, P. R., & Kitrytė, V. (2021). Optimized supercritical CO2 extraction enhances the recovery of valuable lipophilic antioxidants and other constituents from dual-purpose hop (Humulus lupulus l.) variety ella. *Antioxidants*, *10*(6). https://doi.org/10.3390/antiox10060918

Nasa - earth observatory. (2024). *2023 Fire Season n the Northern Hemisphere*. Https://Earthobservatory.Nasa.Gov/Images/Event/151148/2023-Fire-Season-in-the-Northern-Hemisphere.

Neve, R. A. (1991). *Hops* (1st ed., Vol. 136, Issue 1). Springer-science+business media.

NotCO Company. (2024). *NotCO AI*. Https://Notco.Ai/.

Paniagua-García, A. I., Ruano-Rosa, D., & Díez-Antolínez, R. (2024). Fractionation of High-Value Compounds from Hops Using an Optimised Sequential Extraction Procedure. *Antioxidants*, *13*(1). https://doi.org/10.3390/antiox13010045

Pannusch, V. B., Viebahn, L., Briesen, H., & Minceva, M. (2023). Predicting the essential oil composition in supercritical carbon dioxide extracts from hop pellets using mathematical modeling. *Heliyon*, *9*(2). https://doi.org/10.1016/j.heliyon.2023.e13030

Peripato, V., Levis, C., Moreira, G. A., Gamerman, D., ter Steege, H., Pitman, N. C. A., de Souza, J. G., Iriarte, J., Robinson, M., Junqueira, A. B., Trindade, T. B., de Almeida, F. O., Moraes, C. de P., Lombardo, U., Tamanaha, E. K., Maezumi, S. Y., Ometto, J. P. H. B., Braga, J. R. G., Campanharo, W. A., … Aragão, L. E. O. C. (2023). More than 10,000 pre-Columbian earthworks are still hidden throughout Amazonia. *Science*, *382*(6666), 103–109. https://doi.org/10.1126/science.ade2541

Petiti, G., Rousselière, S., Duchaine, S., Korbel, E., Cariou, V., Mikhaylin, S., & Audebrand, L. K. (2022). Operationalizing circular economy. Reflections on a by-product upcycling value chain construction in the brewing sector. *International Food and Agribusiness Management Review*, *25*(5), 803–817. https://doi.org/10.22434/IFAMR2021.0154

Qin, M., Wang, W., & Thirumalai, D. (2015). Protein folding guides disulfide bond formation. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(36), 11241–11246. https://doi.org/10.1073/pnas.1503909112

Raut, S., Gersdorff, G. J. E. von, Münsterer, J., Kammhuber, K., Hensel, O., & Sturm, B. (2020a). Impact of Process Parameters and Bulk Properties on Quality of Dried Hops. *Processes*, *8*(11), 1507. https://doi.org/10.3390/pr8111507

Raut, S., Gersdorff, J. E. Von, Münsterer, J., Kammhuber, K., Hensel, O., & Sturm, B. (2020b). Influence of pre-drying storage time on essential oil components in dried hops (Humulus lupulus L.). *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.10844

Rostain, S., Dorison, A., de Saulieu, G., Prümers, H., Le Pennec, J.-L., Mejía Mejía, F., Freire, A. M., Pagán-Jiménez, J. R., & Descola, P. (2024). Two thousand years of garden urbanism in the Upper Amazon. *Science*, *383*(6679), 183–189. https://doi.org/10.1126/science.adi6317

Rubottom, L. N., Lafontaine, S. R., Hauser, D. G., Pereira, C., Shellhammer, T. H., Rubottom, L. N., Lafontaine, S. R., Hauser, D. G., Rubottom, L. N., & Lafontaine, S. R. (2021). Hop Kilning Temperature Sensitivity of Dextrin- Reducing Enzymes in Hops. *Journal of the American Society of Brewing Chemists*, *0*(0), 1–13. https://doi.org/10.1080/03610470.2021.1903290

Rubottom, L. N., Lafontaine, S. R., & Shellhammer, T. H. (2022). Evaluating the impact of kilning temperature on hop quality in American deep bed dryers. *Brewing Science*, *75*, 98. https://doi.org/10.23763/BrSc22-15rubottom

Rubottom, L. N., & Shellhammer, T. H. (2024). Evaluating the Impact of High and Low Kilning Temperatures on Popular American Aroma Hops. *Journal of the American Society of Brewing Chemists*, *82*(1), 39–49. https://doi.org/10.1080/03610470.2023.2194838

Rybka, A., Heřmánek, P., & Honzík, I. (2017). Theoretical analysis of the technological process of hop drying. *Agronomy Research*, *15*(3), 859–865.

Rybka, A., Heřmánek, P., & Honzík, I. (2018). Analysis of hop drying in chamber dryer. *Agronomy Research*, *16*(1), 221–229. https://doi.org/10.15159/AR.18.012

Rybka, A., Heřmánek, P., & Honzík, I. (2021). *Effect of drying temperature in hop dryer on hop quality*. *2021*(1), 1–7.

Rybka, A., Krofta, K., Heřmánek, P., Honzík, I., & Pokorný, J. (2018). Effect of drying temperature on the content and composition of hop oils. *Plant, Soil and Environment*, *64*(10), 512–516. https://doi.org/10.17221/482/2018-PSE

Sanderson, K. (2023). Earth's average 2023 temperature is now likely to reach 1.5 °C of warming. *Nature*. https://doi.org/10.1038/d41586-023-02995-7

Santarelli, V., Neri, L., Carbone, K., Macchioni, V., & Pittia, P. (2022). Use of conventional and innovative technologies for the production of food grade hop extracts: Focus on bioactive compounds and antioxidant activity. *Plants*, *11*(1). https://doi.org/10.3390/plants11010041

Sanz, V., Torres, M. D., Vilariño, J. M. L., & Domínguez, H. (2019). Trends in Food Science & Technology What is new on the hop extraction ? *Trends in Food Science & Technology*, *93*(June), 12–22. https://doi.org/10.1016/j.tifs.2019.08.018

Schlechter, T. (2024). *Impact of AI on the Brewing Industry: A Comprehensive Summary*. https://doi.org/10.23763/BrSc24-01schlechter

Schönberger, C. (2006). The processing of hops. *Brewing: New Technologies*, 123–148. https://doi.org/10.1533/9781845691738.123

Schuster, J. J., Bahr, L. A., Fehr, L., Tippelt, M., Schulmeyr, J., Wuzik, A., & Braeuer, A. S. (2018). Online monitoring of the supercritical CO2 extraction of hop. *Journal of Supercritical Fluids*, *133*, 139–145. https://doi.org/10.1016/j.supflu.2017.10.006

Silva, K. F. C. e., Strieder, M. M., Pinto, M. B. C., Rostagno, M. A., & Hubinger, M. D. (2023). Processing Strategies for Extraction and Concentration of Bitter Acids and Polyphenols from Brewing By-Products: A Comprehensive Review. In *Processes* (Vol. 11, Issue 3). MDPI. https://doi.org/10.3390/pr11030921

Skyquest. (2024). *Hop Market Insights*. Https://Www.Skyquestt.Com/Report/Hops-Market.

Smith, R. J., Davidson, D., & Wilson, R. J. H. (1998). Natural foam stabilizing and bittering compounds derived from hops. *Journal of the American Society of Brewing Chemists*, *56*(2), 52–57. https://doi.org/10.1094/asbcj-56-0052

Sovová, H., & Stateva, R. P. (2011). Supercritical fluid extraction from vegetable materials Supercritical fl uid extraction from vegetable materials. *Reviews in Chemical Engineering*, *2*. https://doi.org/10.1515/REVCE.2011.002

Statista. (2024). *Beer World Production in 2022*. Https://Www.Statista.Com/Statistics/270275/Worldwide-Beer-Production/.

Steiner, E., Gastl, M., & Becker, T. (2011). Protein changes during malting and brewing with focus on haze and foam formation: A review. In *European Food Research and Technology* (Vol. 232, Issue 2, pp. 191–204). https://doi.org/10.1007/s00217-010-1412-6

Sturm, B., Raut, S., Kulig, B., Münsterer, J., Kammhuber, K., Hensel, O., & Crichton, S. O. J. (2020). In-process investigation of the dynamics in drying behavior and quality development of hops using visual and environmental sensors combined with chemometrics.

Computers and Electronics in Agriculture, *175*(October 2019), 105547. https://doi.org/10.1016/j.compag.2020.105547

Tyśkiewicz, K., Gieysztor, R., Konkol, M., Szałas, J., & Rój, E. (2018). Essential oils from Humulus lupulus scCO2 extract by hydrodistillation and microwave-assisted hydrodistillation. *Molecules*, *23*(11). https://doi.org/10.3390/molecules23112866

United Nations. (2023). *Emissions Gap Report 2023*.

United Nations. (2024). *New economics for sustainable development - Circular Economy*.

United Nations - Climate Action. (2024). *Causes and Effects of Climate Change*. Https://Www.Un.Org/En/Climatechange/Science/Causes-Effects-Climate-Change.

United Nations - Climate Change. (2024). *The Paris Agreement*. Https://Unfccc.Int/Process-and-Meetings/the-Paris-Agreement.

Valle, M., Rivera, O., Teuber, O., & Palma, M. T. (2003). Supercritical CO 2 extraction of Chilean hop (Humulus lupulus) ecotypes. *Journal of the Science of Food and Agriculture*, *1356*(April 2002), 1349–1356. https://doi.org/10.1002/jsfa.1547

Van Opstaele, F., Goiris, K., De Rouck, G., Aerts, G., & De Cooman, L. (2012a). Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets - Part 1: Preparation of single variety total hop essential oils and polar hop essences. *Journal of Supercritical Fluids*, *69*, 45–56. https://doi.org/10.1016/j.supflu.2012.05.009

Van Opstaele, F., Goiris, K., De Rouck, G., Aerts, G., & De Cooman, L. (2012b). Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets - Part 2: Preparation of single variety floral, citrus, and spicy hop oil essences by density programmed supercritical fluid extraction. *Journal of Supercritical Fluids*, *71*, 147–161. https://doi.org/10.1016/j.supflu.2012.06.004

Van Opstaele, F., Goiris, K., De Rouck, G., Aerts, G., & De Cooman, L. (2013). Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets. Part 1: Preparation of single variety total hop essential oils and polar hop essences. *Cerevisia*, *37*(4), 97–108. https://doi.org/10.1016/j.cervis.2012.12.002

Veiga, B. A., Hamerski, F., Clausen, M. P., Errico, M., de Paula Scheer, A., & Corazza, M. L. (2021). Compressed fluids extraction methods, yields, antioxidant activities, total phenolics and flavonoids content for Brazilian Mantiqueira hops. *Journal of Supercritical Fluids*, *170*(December 2020). https://doi.org/10.1016/j.supflu.2020.105155

Verzele, M. (1986). 100 years of hop chemistry and its relevance to brewing. *Journal of the Institute of Brewing*, *92*, 32–48.

Verzele, M., & Keukeleire, D. de. (1991). *Chemistry and Analysis of Hop and Beer Bitter Acids* (1st ed.). Elsevier B.V.

Verzele, M., & van Boven, M. (1971). The Isomerization Mechanism of Humulone. *Bulletin Des Sociétés Chimiques Belges*, *80*(5–6), 677–682. https://doi.org/10.1002/bscb.19710800538

Wietstock, P. C., & Shellhammer, T. H. (2011). Chelating properties and hydroxyl-scavenging activities of hop A- and iso-α-acids. *Journal of the American Society of Brewing Chemists*, *69*(3), 133–138. https://doi.org/10.1094/ASBCJ-2011-0718-01

World Meteorological Organization. (2024). *WMO confirms 2023 smashes global temperature record*. Https://Wmo.Int/News/Media-Centre/Wmo-Confirms-2023-Smashes-Global-Temperature-Record.

Zeković, Z., Pfaf-Šovljanski, I., & Grujić, O. (2007). Supercritical fluid extraction of hops. *Journal of the Serbian Chemical Society*, *72*(1), 81–87. https://doi.org/10.2298/JSC0701081Z

Ziegler, T., Jubaer, H., & Teodorov, T. (2021). Bottlenecks in continuous hops drying with conveyor-belt dryer. *Drying Technology*, 1–19. https://doi.org/10.1080/07373937.2021.1950168

Ziegler, T., & Teodorov, T. (2021). Airflow resistance of two hop varieties. *Agronomy Research*, *19*(2), 638–647. https://doi.org/10.15159/AR.21.036

APPENDIX

Supplementary materials

Permissions Helpdesk <permissionshelpdesk@elsevier.com>
para mim

qua., 15 de mai., 07:00 (há 1 dia)

Dear Prof. Mariana Barreto Carvalhal Pinto,

Thank you for contacting us.

RE: Novel Brazilian hop (Humulus lupulus L.) extracts through supercritical CO2 extraction: Enhancing hop processing for greater sustainability, Food Research International, Volume 172, October 2023, 113169.

Proposed use: Thesis/Dissertation.

As an Elsevier journal author, you retain the right to include the article in a thesis or dissertation (provided that this is not to be published commercially) whether in full or in part, subject to proper acknowledgment; see https://www.elsevier.com/about/policies/copyright for more information. As this is a retained right, no written permission from Elsevier is necessary.

As outlined in our permissions licenses, this extends to the posting to your university's digital repository of the thesis provided that if you include the published journal article (PJA) version, it is embedded in your thesis only and not separately downloadable.

Thank you.

Kind regards,

Thomas Rexson Yesudoss
Copyrights Specialist
ELSEVIER | HCM - Health Content Management

Visit Elsevier Permissions

Dr. Lydia Junkersfeld
para mim

qua., 15 de mai., 13:12 (há 22 horas)

Dear Mariana,

We hereby grant you permission for secondary use of the following article in your PhD thesis:

"Machine Learning Approach to Enhancing Drying Efficiency of Hop",
published in
BrewingScience, 76 (May/June 2023), pp. 48-57.

Best regards

Dr. Lydia Junkersfeld
Editor in Chief (she/her)

Fachverlag Hans Carl GmbH
Geschäftsführer: Michael Schmitt
Registergericht: HRB 17388
USt-Id.-Nr.: DE211630158
Andernacher Str. 33a
90411 Nürnberg
Germany

Phone: + 49 911 95285-58
Fax: + 49 911 95285-48
E-Mail: junkersfeld[at]hanscarl.com

BRAUWELT

BRAUWELT
BRAUWELT International
BrewingScience
Bookshop Carllibri
GradPlato

Copyright
para Copyright, mim

ter., 14 de mai., 20:09 (há 2 dias)

Dear Mariana Carvalhal,
In reference to your message below, the following is from the **American Chemical Society's Policy on Theses and Dissertations:**
This policy addresses permission to include **your article**(s) or portions of text from **your article**(s) in your thesis.

Reuse/Republication of the Entire Work in Theses or Collections: Authors may reuse all or part of the Submitted, Accepted or Published Work in a thesis or dissertation that the author writes and is required to submit to satisfy the criteria of degree-granting institutions. Such reuse is permitted subject to the ACS' "Ethical Guidelines to Publication of Chemical Research". Appropriate citation of the Published Work must be made as follows:

"Reprinted with permission from [COMPLETE REFERENCE CITATION]. Copyright [YEAR] American Chemical Society." Insert the appropriate wording in place of the capitalized words. Citation information may be found after the "Cite this:" heading below the title of the online version and at the bottom of **the first page of the pdf or print version of your ACS journal article**.

If the thesis or dissertation to be published is in electronic format, a direct link to the Published Work must also be included using the ACS Articles on Request author-directed link.

If your university requires you to obtain permission for manuscripts in ASAP status or final published articles, you must use the RightsLink permission system. **See RightsLink instructions at http://pubs.acs.org/page/copyright/permissions.html and make requests at the "Rights & Permissions" link under the title of the online version of the article.**

Submission to a Dissertation Distributor: If you plan to submit your thesis to UMI or to another dissertation distributor, you should not include the unpublished ACS paper in your thesis if the thesis will be disseminated electronically, until ACS has published your paper. After publication of the paper by ACS, you may release the entire thesis (**not the individual ACS article by itself**) for electronic dissemination through the distributor; ACS's copyright credit line should be printed on the first page of the ACS paper.

I hope this information is helpful.

Sincerely,

Keri Travis (she/her)
Copyright Specialist
Office of the Secretary & General Counsel
American Chemical Society
1155 Sixteenth Street, NW
Washington, DC 20036
202-872-4368
k_travis@acs.org

Copyright <Copyright@acs.org>
para Copyright, mim

qui., 10 de out., 15:24

Dear Mariana Carvalhal,

In reference to your message below, the following is from the **American Chemical Society's Policy on Theses and Dissertations:**

This policy addresses permission to include **your article**(s) or portions of text from **your article**(s) in your thesis.

Reuse/Republication of the Entire Work in Theses or Collections: Authors may reuse all or part of the Submitted, Accepted or Published Work in a thesis or dissertation that the author writes and is required to submit to satisfy the criteria of degree-granting institutions. Such reuse is permitted subject to the ACS' "Ethical Guidelines to Publication of Chemical Research". Appropriate citation of the Published Work must be made as follows:

"Reprinted with permission from [COMPLETE REFERENCE CITATION]. Copyright [YEAR] American Chemical Society." Insert the appropriate wording in place of the capitalized words. Citation information may be found after the "Cite this:" heading below the title of the online version and at the bottom of **the first page of the pdf or print version of your ACS journal article**.

If the thesis or dissertation to be published is in electronic format, a direct link to the Published Work must also be included using the ACS Articles on Request author-directed link.

Submission to a Dissertation Distributor: If you plan to submit your thesis to UMI or to another dissertation distributor, you should not include the unpublished ACS paper in your thesis if the thesis will be disseminated electronically, until ACS has published your paper. After publication of the paper by ACS, you may release the entire thesis (**not the individual ACS article by itself**) for electronic dissemination through the distributor; ACS's copyright credit line should be printed on the first page of the ACS paper.

As such, please consider this email confirmation that your request to use the material as described is granted.

I hope this information is helpful.

Sincerely,

Keri Travis *(she/her)*
Copyright Specialist
Office of the General Counsel
American Chemical Society
1155 Sixteenth Street, NW
Washington, DC 20036
202-872-4368