Nature Biocative Foods

Desenvolvimento de produtos e ingredientes alimentares bioativos através de recursos agrícolas endógenos portugueses para uma alimentação saudável

Entregável 6.1 "Conferências e congressos" e 6.2 "Papers e artigos científicos"

Parceiros envolvidos nas tarefas 6.1 e 6.2	I&TEC-CAPS (responsável pela execução da Tarefa 5.1) e BLC3, VN, UCP e iBET (colaboração)
Data de Início previsto das tarefas 6.1 e 6.2:	01 de abril de 2020
Data de Conclusão das tarefas 6.1 e 6.2:	31 de dezembro de 2022 – prolongamento do projeto aceite até 30 de dezembro de 2022
Data dos Entregáveis 6.1 e 6.2:	31 de dezembro de 2022

Índice

1. Promoção e Divulgação da Iniciativa 2

1.1 Grupos focais de divulgação: Participação em feiras do setor 3

1.2. Colóquios ou congressos 8

1.3. Publicações técnicas 19

1.4. Ambiente web 23

1.5. Estágios realizados no âmbito da Iniciativa Nature Bioactive Food 24

1. Promoção e Divulgação da Iniciativa

O presente relatório refere-se à Atividade 6, mais concretamente às Tarefas 6.1 e 6.2 que são relativas à Promoção e divulgação da iniciativa que engloba os **Entregáveis 6.1 e 6.2**, respetivamente. Neste sentido, a disseminação dos resultados foi feita através de 5 tipologias de difusão, nomeadamente, (1.1) Grupos focais de divulgação: Participação em feiras do setor, (1.2) Colóquios ou congressos, (1.3) Publicações técnicas, (1.4) Ambiente web com a divulgação do projeto, (1.5) Estágios realizados no âmbito da Iniciativa Nature Bioactive Food.

Este entregável pretende demonstrar a divulgação ampla dos conhecimentos que foi efetuada no âmbito da Iniciativa Nature Bioactive Foods, quer ao nível da comunidade científica, quer ao nível das empresas do setor e dos potenciais clientes. Para a concretização desta atividade foram estabelecidos dois *deliverables* (6.1 e 6.2), que correspondem à divulgação no formato de (i) Participação em conferências e congressos e (ii) Publicação de papers e artigos científicos em revistas da especialidade.

No total foram contabilizadas **27 ações de difusão** da Iniciativa Nature Bioactive Foods. De seguida são apresentadas as evidências das diferentes ações de promoção e divulgação da Iniciativa Nature Bioactive Foods.

1.1 Grupos focais de divulgação: Participação em feiras do setor

O consórcio da Iniciativa Nature Bioactive Foods marcou presença em sessões de divulgação no setor agroalimentar, nomeadamente:

1) *Agri Innovation Summit 2017*

Nos dias 11 e 12 de outubro de 2017, a *Agri Innovation Summit* 2017 reuniu mais de 600 agricultores, empresas rurais, investigadores, organizações não governamentais e outros agentes de inovação em Lisboa. Esta foi uma iniciativa conjunta entre um Consórcio Português, o Governo Português, a rede EIP-AGRI e a Rede Europeia para o Desenvolvimento Rural, com o objetivo de: (i) Promover a fertilização cruzada entre iniciativas de inovação multi-atores, tanto no âmbito de Programas de Desenvolvimento Rural como no Horizonte 2020; (ii) Sensibilizar para as oportunidades de inovação e digitalização da agricultura e das economias rurais e para a forma como estas podem ser apoiadas no âmbito dos Programas de Desenvolvimento Rural em curso; e (iii) Dar contributos para as políticas de inovação da UE para a agricultura e as zonas rurais após 2020, dando seguimento ao ponto 7 da Declaração *Cork 2.0.* Investigadores da I&TEC-CAPS e BLC3 participaram nesta cimeira, com a apresentação da iniciativa Nature Bioactive Foods (Figura 1).

Figura 1 - Poster apresentado no âmbito da Iniciativa Nature Bioactive Foods, no Agro Innovation Summit 2017.

2) *Agri Innovation 2018*

No dia de 29 de outubro de 2019, a *Agri Innovation 2018* reuniu centenas agricultores, empresas rurais, investigadores, organizações não governamentais e outros agentes de inovação em Lisboa. Esta cimeira teve como objetivo principal a promoção e o intercâmbio de boas práticas, designadamente através da identificação de desafios comuns e da exploração de potenciais soluções, do desenvolvimento de sinergias entre equipas que tratam temas comuns e da apresentação de linhas orientadoras para o futuro da inovação no setor.

A Agro Inovação 2018 surgiu na sequência da sessão "Competitividade e Sustentabilidade do Complexo Agroflorestal", realizada em 2017 pelo MAFDR para apresentação dos Grupos Operacionais (GO), aprovados no âmbito da Medida do PDR 2020. Nessa sessão o Ministro da Agricultura afirmou que o MAFDR se comprometia a organizar anualmente, um evento de divulgação dos resultados deste GO. Investigadores da I-TEC&CAPS e BLC3 participaram nesta cimeira, com a apresentação da iniciativa Nature Bioactive Foods (Figura 2).

Figura 2 - Poster apresentado no âmbito da Iniciativa Nature Bioactive Foods, na Agro Innovation 2018.

3) *Cimeira Nacional AgroInovação 2022*

Nos dias 11 e 12 de outubro de 2022, realizou-se a *Cimeira Nacional de AgroInovação 2022*, que resultou de uma iniciativa do Ministério da Agricultura e Alimentação, em colaboração com os parceiros do setor e com o apoio do Crédito Agrícola. Com este evento pretendeu-se: (i) Promover a disseminação do conhecimento e da inovação no setor e nos territórios rurais, apresentando os resultados de projetos de inovação em parceria; (2) Promover o encontro entre parceiros do sistema nacional de conhecimento do setor agrícola (AKIS Nacional) e de outros setores, tendo em vista estimular diferentes abordagens inovadoras; (iii) Aumentar a consciencialização da importância das oportunidades disponibilizadas pela inovação, digitalização e realidade virtual ou aumentada; e (iv) Reconhecer empresas e projetos inovadores. Investigadores da I&TEC-CAPS e BLC3 participaram nesta Cimeira, com a apresentação da Iniciativa Nature Bioactive Foods e dos resultados alcançados (Figura 3 e Figura 4).

Figura 3 - Poster apresentado no âmbito da Iniciativa Nature Bioactive Foods, na Cimeira Nacional da Agroinovação de 2022.

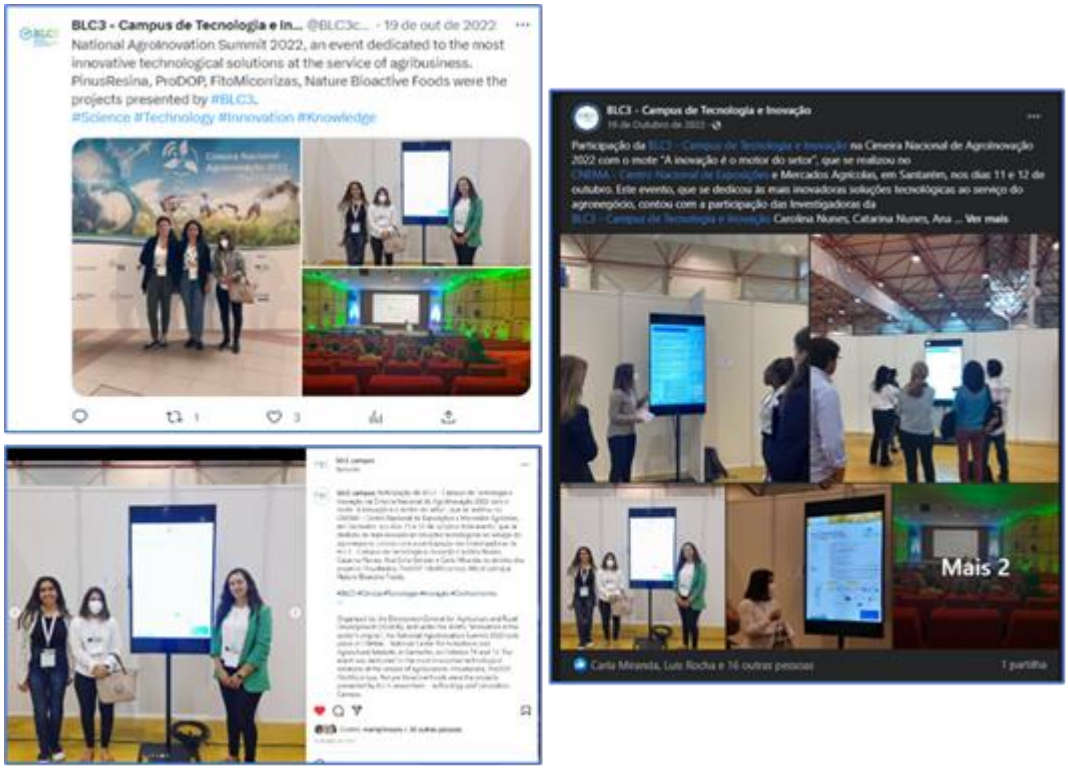


Figura 4 - Participação das investigadoras BLC3 na Cimeira Nacional AgroInovação 2022, com a apresentação da iniciativa Nature Bioactive Foods.

4) *Grupo focal nas instalações da BLC3 (dezembro 2022)*

A sessão do *FocusGroup* foi realizada na BLC3 (Figura 5), aquando da prova sensorial, para traçar o perfil organolético do concentrado funcional desenvolvido no âmbito do projeto em apreço. Neste sentido, primeiro realizou-se a prova sensorial e depois procedeu-se à sessão do grupo focal contando com os mesmos intervenientes da prova.

Figura 5 – Provas sensoriais e grupo focal do concentrado funcional desenvolvido no âmbito da Iniciativa Nature Bioactive Foods.

1.2. Colóquios ou congressos

Durante a execução da Iniciativa, o consórcio participou em diversos eventos da comunidade científica, com vista à promoção e divulgação da Iniciativa junto deste público alvo. No total foram efetuadas 5 comunicações em painel ("poster") e 3 comunicações orais.

De seguida são apresentadas as evidências correspondestes aos trabalhos desenvolvidos no âmbito desta tarefa.

- ***Posters***

1) FoodWasteNet2018

Rodrigues, L., Pereira C., Gaspar, F., Carvalho, A., Duarte, A., Paiva, A. and Matias, A. Recovery of bioactive ingredients from canned sardine waste streams using green solvents. In: Valorising Field Wastes - the next steps? - FoodWasteNet2018, November 2018, Nottingham, UK. (Figura 6).

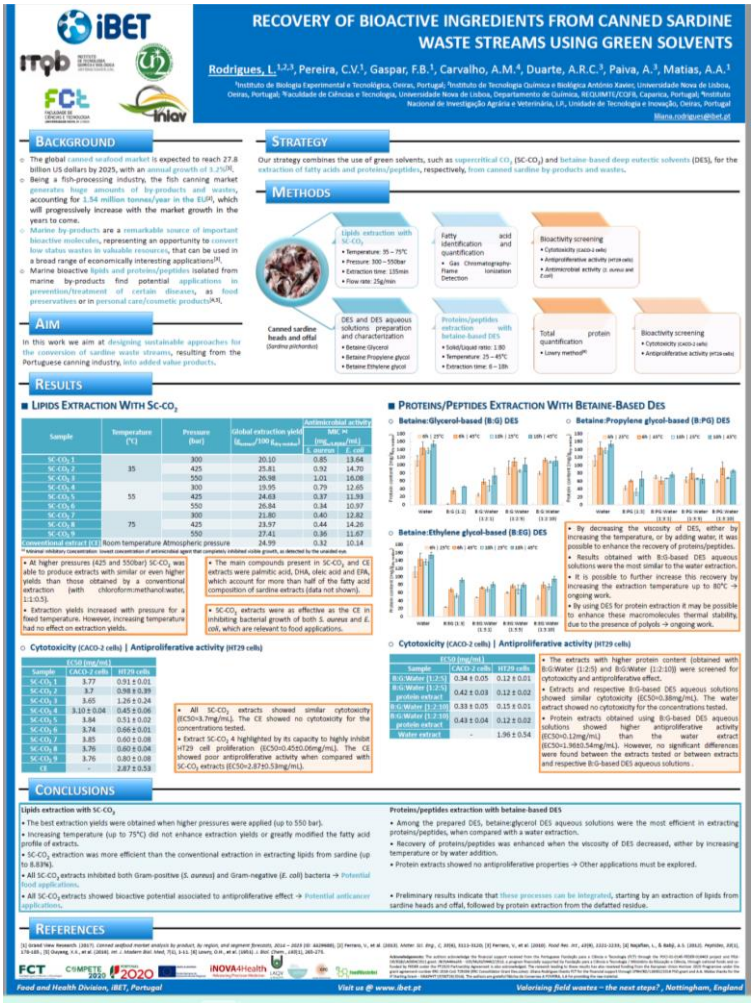


Figura 6 - Participação dos investigadores do iBET no congresso FoodWaste2018, com poster da iniciativa Nature Bioactive Foods.

Armada, R., Matias, A., Villanueva, M. and Aparicio, I. Application of green technologies for the sustainable valorisation of apple by-product. In Valorising Field Wastes - the next steps? - FoodWasteNet2018, November 2018, Nottingham, UK. (Figura 7).

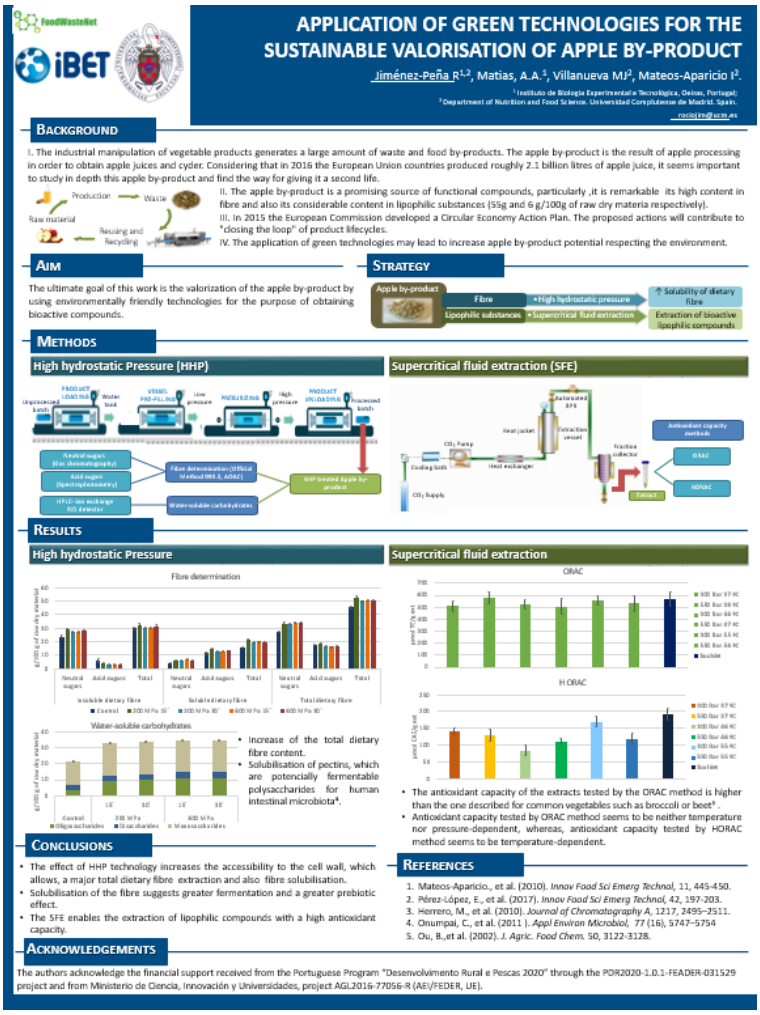


Figura 7 - Participação dos investigadores do iBET no congresso FoodWaste2018, com poster da iniciativa Nature Bioactive Foods.

Rodrigues, L., Serra, T., Romero-Díez, R., Fernández, N., Rodríguez-Rojo, S., Matias, A. and Bronze, M. Food-industry waste stream valorization at iBET: extraction, phytochemical characterization and bioactivity of brassica, citrus and wine industry byproducts. In Valorising Field Wastes - the next steps? - FoodWasteNet2018, November 2018, Nottingham, UK. (Figura 8).

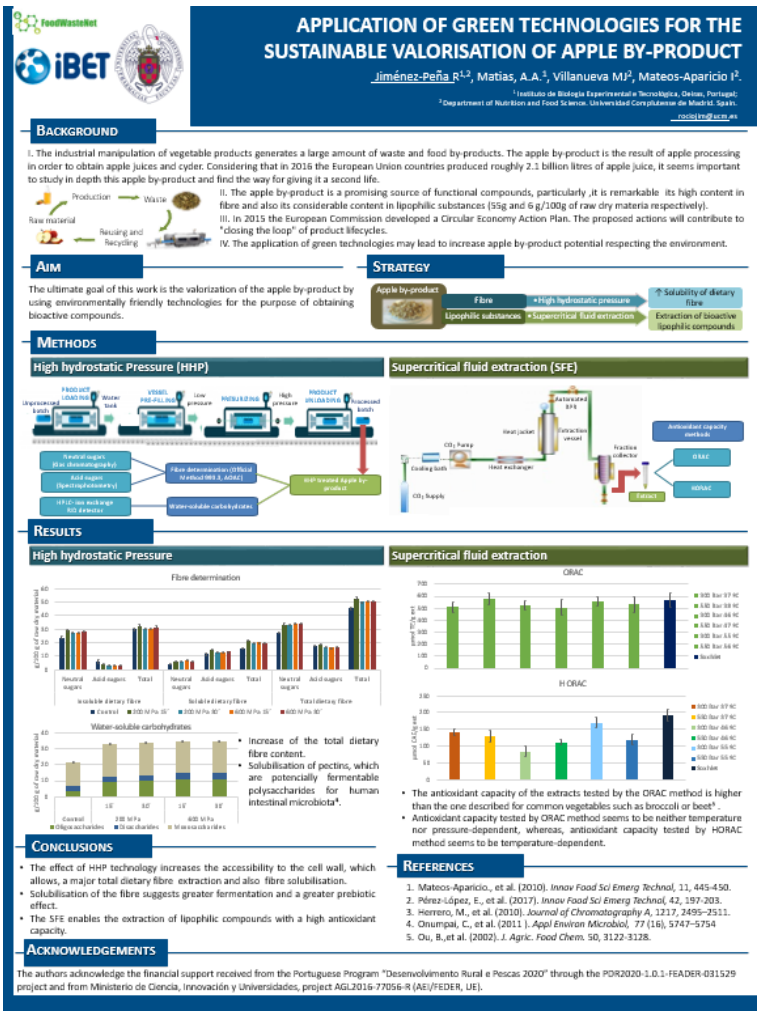


Figura 8 - Participação dos investigadores do iBET no congresso FoodWaste2018, com poster da iniciativa Nature Bioactive Foods.

2) ICPH2019 - International Conference on Polyphenols and Health 2019

Vilas Boas, A., Nunes., C., Ribeiro, T., Nunes, J., Pintado, M. Quince (Cydonia Oblonga Miller) fruit culls: an integral valorisation to develop novel functional ingredients. In: ICPH2019 Kobe, 28 November-01 December 2019, Kobe, Japan. Poster. (Figura 9).

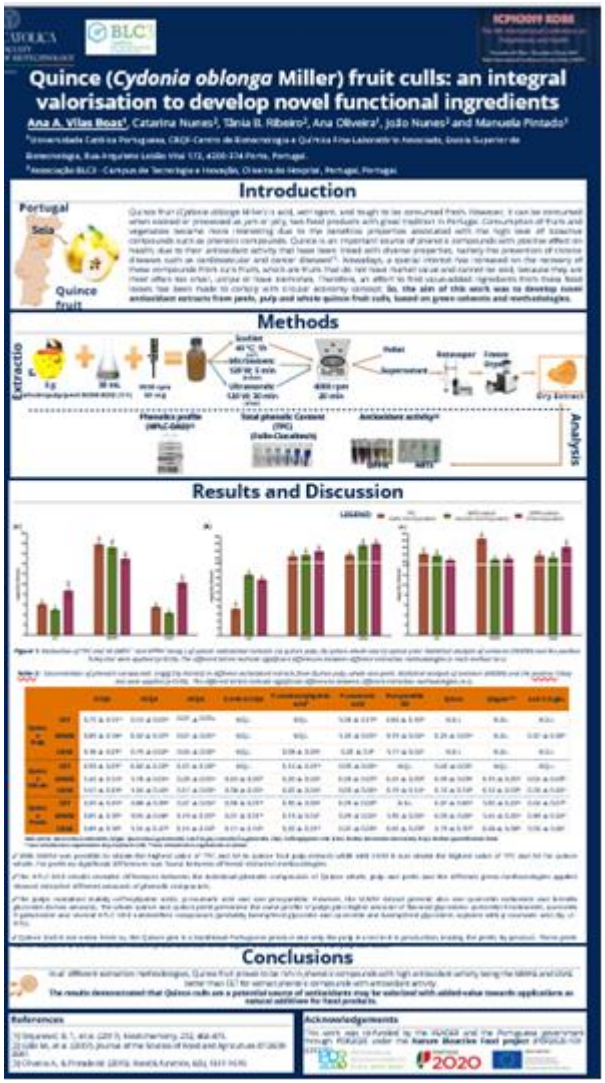


Figura 9 - Participação dos investigadores da UCP no congresso ICPH2019, com o poster da iniciativa Nature Bioactive Foods.

3) Iberphenol 2020

Vilas-Boas, A., Nunes, C., Ribeiro, S., Oliveira, A., Nunes, J., Pintado, M. Are Mentha suaveolens extracts a promising antioxidant additive? In: Iberphenol, Coimbra, 2020. Poster. (Figura 10).

Are Mentha suaveolens extracts a promising antioxidant additive?

Ana A. Vilas-Boas[1], Catarina Nunes[2], Sónia Ribeiro[2], Ana Oliveira[1], João Nunes[2] and Manuela Pintado[1]

[1] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
[2] Associação BLC3 - Campus de Tecnologia e Inovação, Centre Bio R&D Unit, Oliveira do Hospital, Portugal

Nowadays, the demand for plant-derived antioxidant additives by food industries to prevent the food oxidation and growth of food pathogens has increased because they are accepted by consumers as relatively safe and healthy compared to their synthetic antioxidants or preservatives. *Mentha suaveolens* Ehrh. is an aromatic herb native from Portugal used in the traditional medicine. It has an extensive range of biological activities, including antioxidant and antimicrobial, due to the presence of two classes of bioactive compounds: essential oils and phenolic compounds. Effectively, phenolic compounds are considered a major group of compounds that contribute to the antioxidant activities[1]. The aim of this work was to develop a natural antioxidant extract from dry Mentha suaveolens (MS) rich in phenolic compounds using green solvents and methodologies. MS extracts were obtained using leaves and stems with water:ethanol mixtures employing microwave (MAE) or ultrasound (UAE) assisted extraction. The extractive yield was measured by weight, the content of total phenolic compounds (TPC) was evaluated by Folin-Ciocalteu method, antioxidant activity (AA) evaluated through ABTS and DPPH and the phenolic profile of the extracts was assessed by HPLC-DAD.

The best extraction yields were obtained using water:ethanol (50:50 (v/v)) mixture for both green methodologies (MAE: 25.07 ± 1.67 % w/w and UAE: 23.56 ± 1.41 % w/w), and, when comparing with conventional extraction, the extraction yields were 1.7 times higher. When using MAE operating at 500 W during 15 min with water:ethanol (50:50 (v/v)) it was possible to obtain the highest value of TPC (380.15 ± 15.30 mg GAE/g dry extract) and AA (ABTS$^{\bullet}$: 233.50 ± 11.11 mg AAE/g dry extract and DPPH: 299.44 ± 9.78 mg TE/g dry extract). The most abundant phenolic compounds present in MS extracts were rosmarinic acid > eriocitrin > luteolin glucoside > apigenin > caffeoylquinic acids > caffeic acid. The phenolic profile was similar in all the extracts, being the higher concentrations present observed for the MWAE, water:ethanol (50:50 (v/v)) extracts.

Regarding the results obtained from this research study, it is possible conclude that the extraction solvent and methodology influence the recovery of phenolic compounds. This research suggests that MS can be a promising antioxidant additive for application in food products, besides the potential health related effect.

References: [1]Božović, M., Pirolli, A., & Ragno, R. (2015). Molecules, 20(5), 8605-8633.

Acknowledgements: This work was supported by Ministry of Agriculture and Rural Development and co-financed by the European Agricultural Fund for Rural Development (EAFRD), through the partnership agreement Portugal PDR-2020, under the project Nature Bioactive Food (Ref. PDR2020-101-031531) and is a result of the project Trasnfere Empreende, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). We would also to thank the scientific collaboration under the FCT project UID/Multi/50016/2019.

Figura 10 - Participação dos investigadores da UCP no congresso IBerphenol 2020, com o poster da iniciativa Nature Bioactive Foods.

4) XV Encontro de Química dos Alimentos 2021

Vilas-Boas, A., Nogueira, M., Oliveira, A., Nunes, C., Ribeiro, S., Nunes, J., Pintado, M. (September 2021). Lavandula pedunculata antioxidant extracts as a new potential food additives. XV Encontro de Química dos Alimentos, Madeira Portugal. Poster. (Figura 11).

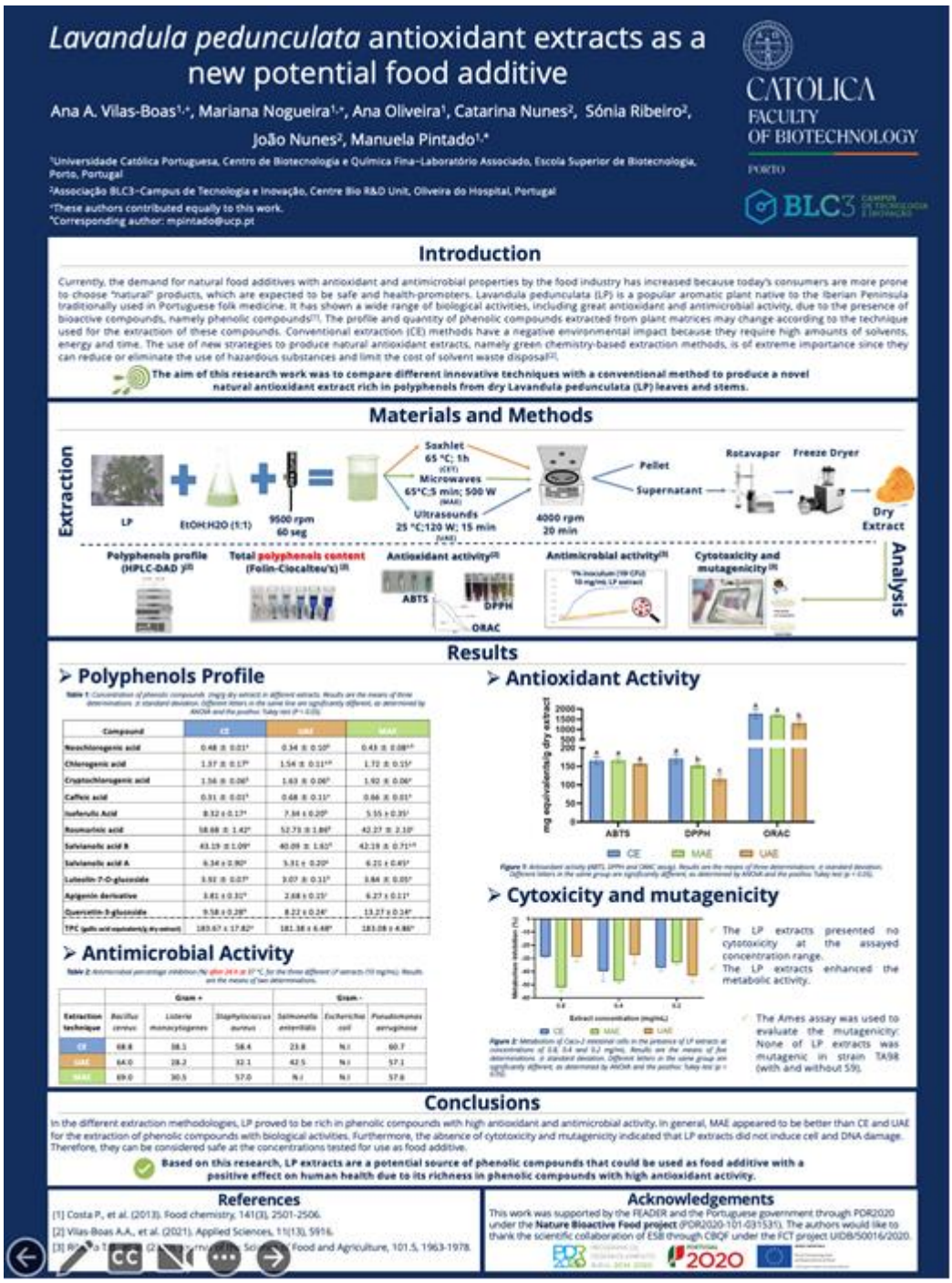


Figura 11 - Participação dos investigadores da UCP no XV Encontro da Química dos Alimentos 2021, com o poster da iniciativa Nature Bioactive Foods.

5) Encontro Ciência'22

Nunes, C., Ribeiro, S. e Nunes, J. Optimization of green extraction methods to obtain Mentha suaveolens extracts with improved biological activity. In: Encontro Ciência'22, 16 May - 18 May 2022, Lisboa, Portugal. Poster. (Figura 12).

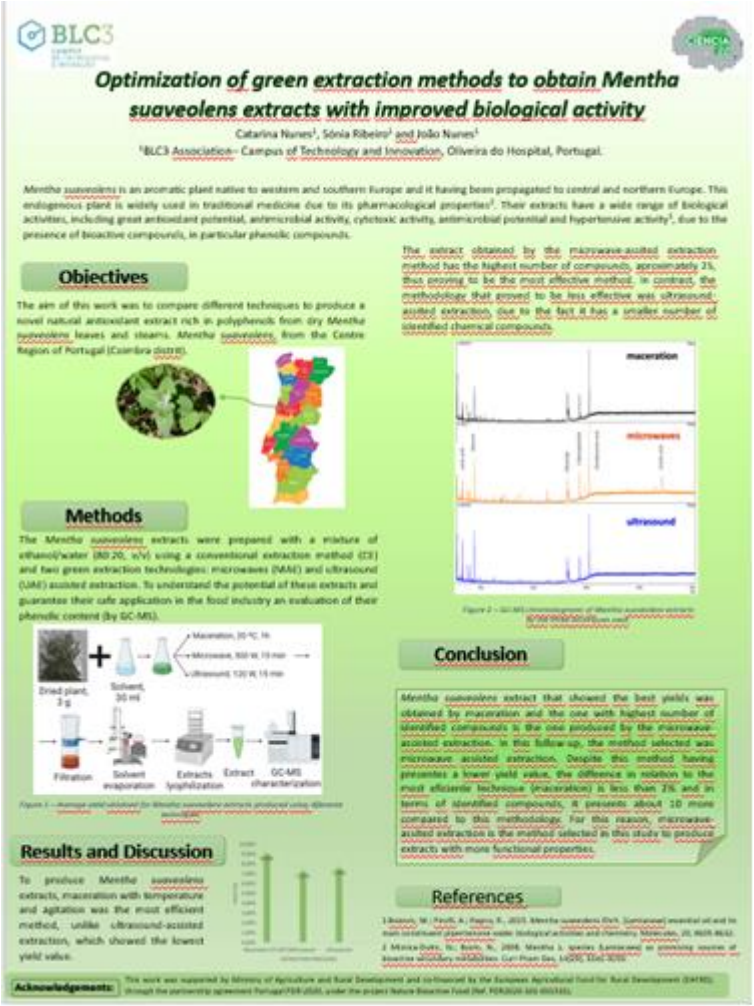


Figura 12 - Participação dos investigadores da BLC3 no Encontro Ciência'22, com o poster da iniciativa Nature Bioactive Foods.

6) XVI Encontro da Química dos Alimentos 2022

Ribeiro, S., Nunes, C., Farinha, D., Nunes, J. Development of bioactive food and ingredients using endogenous Portugal agricultural resources for healthy nutrition. Em: XVI Encontro Química dos Alimentos, 23-26 de outubro de 2022, Castelo Branco, Portugal. Poster. (Figura 13).

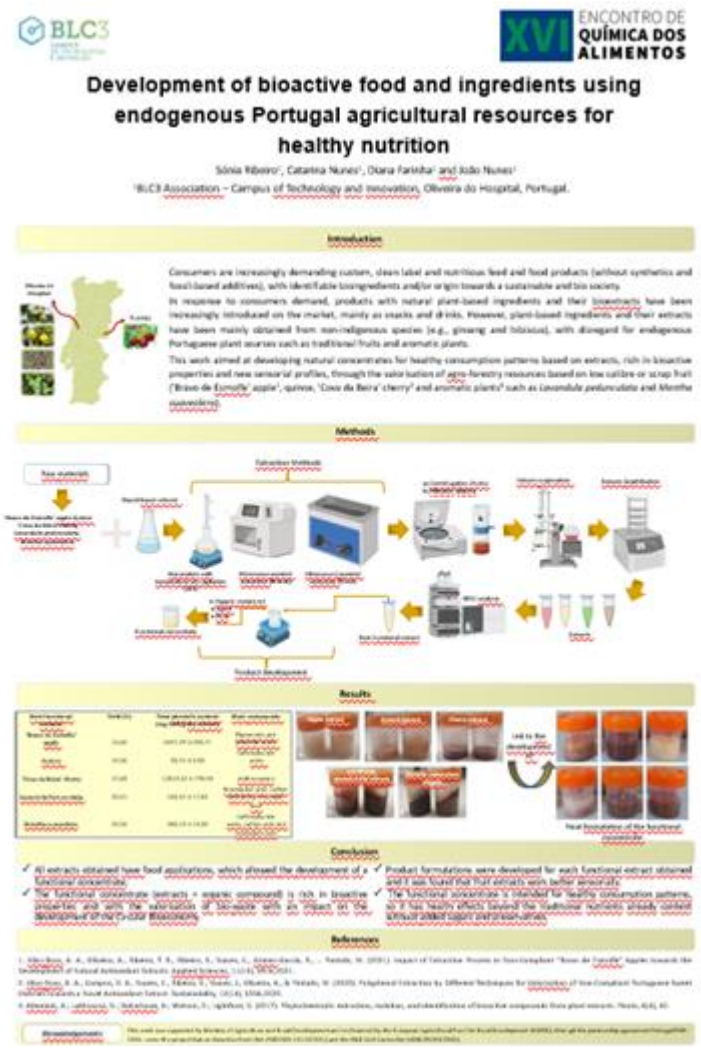


Figura 13 - Participação dos investigadores da BLC3 no XVI Encontro da Química dos Alimentos 2022, com o poster da iniciativa Nature Bioactive Foods.

- ***Comunicações orais***

1) Fernández, N. Efficient and sustainable extraction of bioactives from agro- and food industry waste streams at iBET. In Valorising Field Wastes - the next steps? - FoodWasteNet2018, November 2018, Nottingham, UK. (Figura 14).

FoodWasteNet

Valorising field wastes – the next steps?

Food-industry waste stream valorisation: extraction, phytochemical characterization and bioactivity of brassica, citrus and wine industry by-products

Liliana Rodríguez [1], Teresa Serra[1], Rut Romero-Dies[2], Naiara Fernández [1], Soraya Rodríguez-Rojo[2], Ana Matias[1] & Maria Rosário Bronze [1,3]

[1] Instituto de Biologia Experimental e Tecnológica (iBET), [2]University of Valladolid (UVa) & [3] Faculdade de Farmácia da Universidade de Lisboa (FFUL)

iBET is a private not for profit research centre in the area of biotechnology and life sciences based in Portugal. Food & Health Division has strong competences studying food products, considering aspects related to their quality, safety, authenticity, sensory and functionality.

One of the core areas of the division is the efficient extraction of high added-value products from agro- and food industry related waste. Already manipulated matrices include by products from fisheries wastes (blue shark, crab, shrimp, and codfish), agro-industries (brassicas, citrus, and apples) and wineries (lees, grape pomace) among others.

Focused on the bioactivity of the target extract (antioxidant, anti-inflammatory, anti-cancer, anti-fungal and/or antimicrobial) clean and sustainable processes are designed. Supercritical CO2, pressurized liquids and alternative solvents, such as deep eutectic solvents, are used for the recovery of bioactive compounds.

Chemical and biological properties of the active components are usually assessed by the determination of antioxidant capacity (total phenolic content, ORAC and HORAC values, inhibition of AAPH-induced LDL oxidation.) and activity at cell level (anti-inflammation, anticancer, cytotoxicity, antiobesity.

15.20	REFRESHMENTS & POSTER VIEWING	MURRAYFIELD & TWICKENHAM SUITE
15.50	**Flash presentations on extracting bioactives from field and crop wastes**	
	Effects of LED light on flavonoids and nutritional value in bean sprouts	Xiaoyan Zhang, Nottingham Trent University
	Efficient and sustainable extraction of bioactives from agro- and food industry waste streams at iBET	Naiara Fernández Hernández, iBET

Figura 14 - Participação dos investigadores do iBET no congresso Foodwaste2018, com comunicação oral no âmbito da iniciativa Nature Bioactive Foods.

2) Vilas-Boas, A; Barbosa, L.; Ribeiro, T.; Nunes, C.; Matias, A.; Nunes, J.; Oliveira, A.; Pintado, M. (2019, julho). Valorisation of non-compliant Bravo de Esmolfe Apples and Saco Berries: development of antioxidant extracts. Comunicação oral apresentação no XII IberoAmerican Congress of Food Engineering, Faro, Portugal.

3) Jiménez-Peña, R.; Mateo-Aparicio Villanueva M. J.; Matias, A. A. “Supercritical CO2 as an efficient solvent to extract natural bioactives from apple by-product”. Comunicação oral no 17th European Meeting on Supercritical Fluids (EMSF 2019) - 7th European Meeting on High Pressure Technology (Ciudad Real). (Figura 15).

MONDAY, APRIL 8, 2019					
9:00 - 13:30	Reception and registration desk. Ground floor ITQUIMA.				
12:00 - 12:30	Welcome Ceremony Aula Magna.Biblioteca.				
12:30 - 13:30	Plenary Talk: Gerd Brunner. Aula Magna. Biblioteca. *Developments in the application of supercritical fluids: the way of process materials through reactors*				
13:30 - 15:30	Welcome Lunch. ITQUIMA.				
15:30 - 16:45	Session 1	Aula Magna Biblioteca (Materials) Chair: Pedro Vidinha	Biblioteca 1 (Food, Health and well-being) Chair: Jose P Coelho	Biblioteca 2 (Fundamentals) Chair: Soraya Rodriguez	Sala Grados Letras (High Pressure) Chair: Sebastien Lefevre
15:30 - 16:00	Keynotes	Giuseppe Caputo *Life Cycle Assessment of a combined Oxidation-Gasification system for the waste treatment with supercritical water (SCWG-O)*	Isabel Ferreira *From natural resources to bio-based food additives: a focus on extraction methods*	Tadafumi Adschiri *Supercritical Hydrothermal Synthesis -Solvent effect on kinetics, reaction equilibrium and solubility in supercritical water-*	Martin Sova *Aspects for designing an industrial CO_2 extraction plant*
16:00 - 16:15	Oral presentations	Danielle Barth *Supercritical carbon dioxide drying of monoliths organogels: solvent and geometry sample effects.*	Jasreen Sekhon *Bioactivity of polyphenols recovered by pressurized fluid and supercritical CO_2 extraction of cranberry pomace.*	Dan Feng *Bubble size distribution and gas-liquid interfacial area in a bubble column reactor under high temperature and pressure.*	Marco Furlan *"Precision Cleaning": an enhanced process for impurities removal with supercritical CO_2.*
16:15 - 16:30	Oral presentations	Faheem Ahmad *Flexible Alginate Aerogel Fibers for Textiles*	Rocio Teresa Jiménez-Peña *Supercritical CO_2 as an efficient solvent to extract natural bioactives from apple by-product*	Fan Zhang *A coupled experimental / numerical approach for fluids mixing study under supercritical antisolvent process conditions in microreactors*	Gianluca Morbiato *Optimization of the supercritical CO_2 pasteurization process for the preservation of high nutritional value juice*
16:30 - [illegible]	Oral presentations	Zoran Novak *Polysaccharide aerogels in biomedical*	Petras Rimantas Venskutonis *Recovery and fractionation of lipophilic berry pomace substances by pure* [illegible]	Cara Schwarz *Comparison of the Phase Behaviour of Saturated and Unsaturated Fatty Acid Esters*	Zhanna Cherepanova *Simultaneous cleaning and sterilization of the bacterial cellulose films using the*

Figura 15 - Participação dos investigadores do iBET no congresso 17th European Meeting on Supercritical Fluids (EMSF 2019), com comunicação oral no âmbito da iniciativa Nature Bioactive Foods.

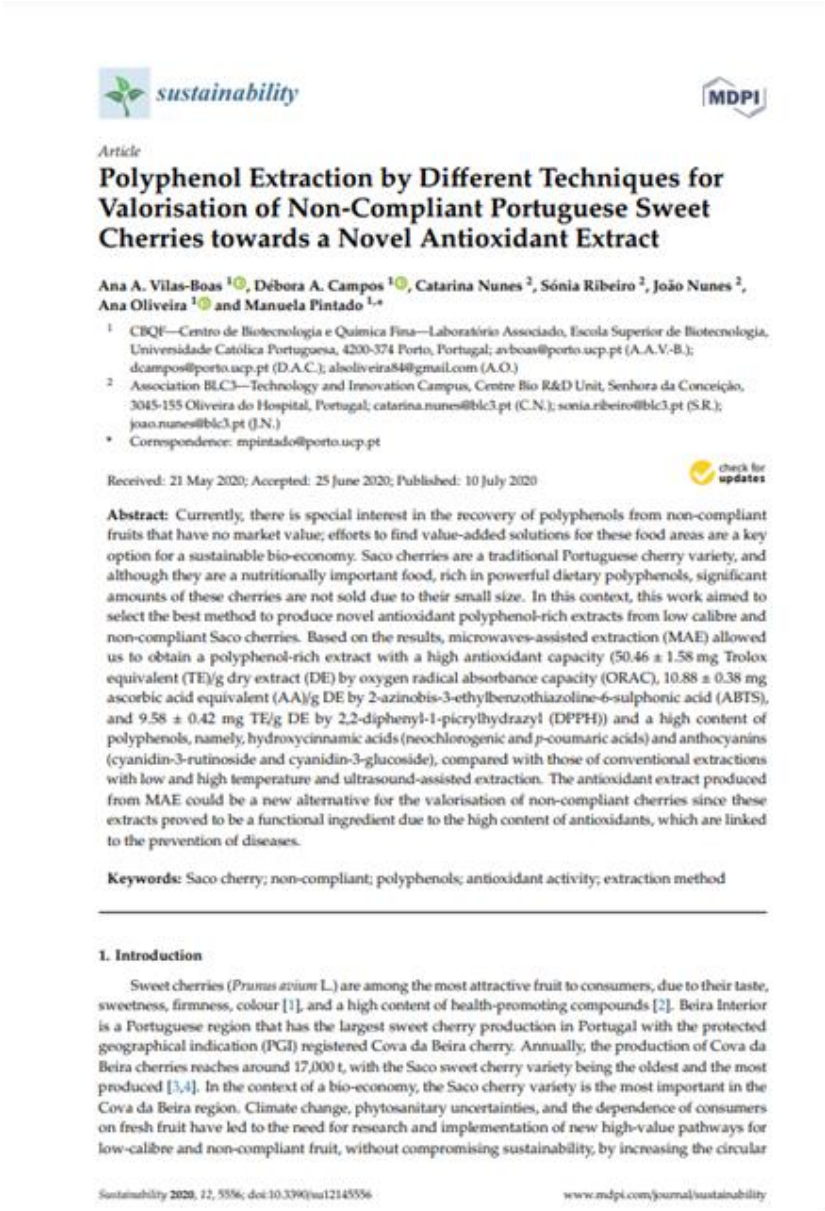
4) Grupo Operacional – Nature Bioactive Foods. Comunicação oral em Agro Inovação 2018 – Cimeira Nacional Inovação na Agricultura, Lisboa, Portugal. (Figura 16).

Figura 16 - Participação dos investigadores do iBET e BLC3 na Agro Inovação 2018 – Cimeira Nacional Inovação na Agricultura, com comunicação oral no âmbito da iniciativa Nature Bioactive Foods.

1.3. Publicações técnicas

O consórcio já publicou **3 artigos**, e ainda 2 em fase de preparação para submissão e escreveu **1 capítulo de um livro**.

1) Vilas-Boas, A.; Campos, D.; Nunes, C.; Ribeiro, S.; Nunes, J.; Oliveira, A., Pintado, M., (2020) Polyphenol Extraction by Different Techniques for Valorisation of Non-Compliant Portuguese Sweet Cherries towards a Novel Antioxidant Extract. Sustainability, 12(4):5556. (Figura 17).



sustainability MDPI

Article

Polyphenol Extraction by Different Techniques for Valorisation of Non-Compliant Portuguese Sweet Cherries towards a Novel Antioxidant Extract

Ana A. Vilas-Boas [1], Débora A. Campos [1], Catarina Nunes [2], Sónia Ribeiro [2], João Nunes [2], Ana Oliveira [1] and Manuela Pintado [1,*]

1 CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, 4200-374 Porto, Portugal; avboas@porto.ucp.pt (A.A.V.-B.); dcampos@porto.ucp.pt (D.A.C.); aloliveira84@gmail.com (A.O.)

2 Association BLC3—Technology and Innovation Campus, Centre Bio R&D Unit, Senhora da Conceição, 3045-155 Oliveira do Hospital, Portugal; catarina.nunes@blc3.pt (C.N.); sonia.ribeiro@blc3.pt (S.R.); joao.nunes@blc3.pt (J.N.)

* Correspondence: mpintado@porto.ucp.pt

Received: 21 May 2020; Accepted: 25 June 2020; Published: 10 July 2020

Abstract: Currently, there is special interest in the recovery of polyphenols from non-compliant fruits that have no market value; efforts to find value-added solutions for these food areas are a key option for a sustainable bio-economy. Saco cherries are a traditional Portuguese cherry variety, and although they are a nutritionally important food, rich in powerful dietary polyphenols, significant amounts of these cherries are not sold due to their small size. In this context, this work aimed to select the best method to produce novel antioxidant polyphenol-rich extracts from low calibre and non-compliant Saco cherries. Based on the results, microwaves-assisted extraction (MAE) allowed us to obtain a polyphenol-rich extract with a high antioxidant capacity (50.46 ± 1.58 mg Trolox equivalent (TE)/g dry extract (DE) by oxygen radical absorbance capacity (ORAC), 10.88 ± 0.38 mg ascorbic acid equivalent (AA)/g DE by 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and 9.58 ± 0.42 mg TE/g DE by 2,2-diphenyl-1-picrylhydrazyl (DPPH)) and a high content of polyphenols, namely, hydroxycinnamic acids (neochlorogenic and *p*-coumaric acids) and anthocyanins (cyanidin-3-rutinoside and cyanidin-3-glucoside), compared with those of conventional extractions with low and high temperature and ultrasound-assisted extraction. The antioxidant extract produced from MAE could be a new alternative for the valorisation of non-compliant cherries since these extracts proved to be a functional ingredient due to the high content of antioxidants, which are linked to the prevention of diseases.

Keywords: Saco cherry; non-compliant; polyphenols; antioxidant activity; extraction method

1. Introduction

Sweet cherries (*Prunus avium* L.) are among the most attractive fruit to consumers, due to their taste, sweetness, firmness, colour [1], and a high content of health-promoting compounds [2]. Beira Interior is a Portuguese region that has the largest sweet cherry production in Portugal with the protected geographical indication (PGI) registered Cova da Beira cherry. Annually, the production of Cova da Beira cherries reaches around 17,000 t, with the Saco sweet cherry variety being the oldest and the most produced [3,4]. In the context of a bio-economy, the Saco cherry variety is the most important in the Cova da Beira region. Climate change, phytosanitary uncertainties, and the dependence of consumers on fresh fruit have led to the need for research and implementation of new high-value pathways for low-calibre and non-compliant fruit, without compromising sustainability, by increasing the circular

Sustainability 2020, 12, 5556; doi:10.3390/su12145556 www.mdpi.com/journal/sustainability

Figura 17 – Artigo publicado na revista *Sustainability* no âmbito da iniciativa Nature Bioactive Foods pelos investigadores da UCP e BLC3.

2) De la Peña Armada, R.; Bronze, R.; Matias, A.; Mateos-Aparicio, I. (2021) "Triterpene-Rich Supercritical CO_2 extracts from apple by-product protect human keratinocytes against ROS". Food and Bioprocess Technology, 14(5), 1-11. (Figura 18).

Food and Bioprocess Technology (2021) 14:909–919
https://doi.org/10.1007/s11947-021-02615-0

ORIGINAL RESEARCH

Triterpene-Rich Supercritical CO_2 Extracts from Apple By-product Protect Human Keratinocytes Against ROS

Rocío De la Peña Armada[1] · María Rosario Bronze[2,3] · Ana Matias[2] · Inmaculada Mateos-Aparicio[1]

Received: 29 September 2020 / Accepted: 19 February 2021 / Published online: 4 March 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract
Supercritical fluid extraction, employing CO_2 as solvent, was explored to recover high-value lipophilic compounds from apple by-product, the main solid residue generated after apple fruit processing. The experimental conditions were fixed at pressures of 300, 425, and 550 MPa and temperatures of 37, 46, and 55 °C. The total yield from the extractions was calculated and the antioxidant capacity of the different extracts was measured by ORAC and HORAC analytical methods. Furthermore, HPLC-DAD-MS/MS and GLC analyses were performed, respectively, for triterpenic compounds and fatty acid identification. The extracts were tested over human keratinocyte cell (HaCaT) to evaluate their in vitro impact. Supercritical fluid extracts obtained at 46 °C exhibited the greater operational yield and the highest concentration of triterpenic acids, namely, betulinic acid, oleanolic acid, ursolic acid, uvaol/erythrodiol, and lupeol. These results support that apple by-product is a natural source of bioactive compounds with potential cosmeceutical applications.

Keywords Terpenic acids · Fatty acids · Supercritical fluid extraction · Apple

Introduction

Prevention and reduction of food losses and by-product valorization is nowadays a fundamental issue of the European Commission's Circular Economy Package, for ensuring the world's sustainable growth and development (Bourguignon 2016). Considering the vast amount of materials that are discarded in all stages of food processing, the waste disposal represents an environmental and economic problem. Apple by-product is the remaining result of apple manufacturing process to produce juices or cider among other products (Nile et al. 2019). It is estimated that around 3.0 to 4.2 million tons are discarded every year in the world (Jin et al. 2018). However, it has been reported that apple by-product is constituted by several natural compounds such as lipids, phenolics, or terpenoids with an interesting antioxidant activity (Montañés et al. 2018; Waldbauer et al. 2017; Cargnin and Gnoatto 2017). Therefore, the recovery of these bioactive compounds from apple waste may be of relevance for food, cosmetic, or pharmaceutical industries since currently, the preference for natural products over synthetic, as a potential way of preserving the health and safety of consumers, has become a reality (Nyam et al. 2011; Rodrigues et al. 2016; Rebey et al. 2019). Nevertheless, the strategies for the aforementioned recovery process must be in line with the use of environmentally friendly and advanced techniques and technologies (Gallego et al. 2019). In this regard, supercritical fluid extraction (SFE) has been recognized as a promising green process that allows an effective and selective recovery of valuable compounds from natural raw materials (Chemat et al. 2012; Rebey et al. 2019). Furthermore, CO_2 is the most commonly desired solvent for this extraction. Compared to traditional extraction methodologies used for natural products, such as hydrodistillation or Soxhlet extraction, supercritical CO_2 extraction exhibits shorter extraction times and higher selectivity, and does not require the use of organic solvents. Therefore, it promotes health assurance and prevents possible

✉ Inmaculada Mateos-Aparicio
inmateos@ucm.es

1 Departamento de Nutrición y Ciencia de los Alimentos, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain

2 Instituto de Biologia Experimental e Tecnológica, 2780-901 Oeiras, Portugal

3 iMED, Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal

Figura 18 - Artigo publicado na revista *Food and Bioprocess Technology* no âmbito da iniciativa Nature Bioactive Foods pelos investigadores do iBET.

3) Vilas-Boas, A., Oliveira, A., Ribeiro, T., Ribeiro, S., Nunes, C., Gómez-García, R., Nunes, J., Pintado, M. (2021) Impact of extraction process in non-compliant apples towards the development of natural antioxidant extracts. Applied Sciences, 11 (5916), 1-18. (Figura 19).

Article

Impact of Extraction Process in Non-Compliant 'Bravo de Esmolfe' Apples towards the Development of Natural Antioxidant Extracts

Ana A. Vilas-Boas [1], Ana Oliveira [1,*], Tânia B. Ribeiro [1], Sónia Ribeiro [2], Catarina Nunes [2], Ricardo Gómez-García [1], João Nunes [2] and Manuela Pintado [1]

1 Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, CBQF, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal; avboas@porto.ucp.pt (A.A.V.-B.); tribeiro@porto.ucp.pt (T.B.R.); rgarcia@porto.ucp.pt (R.G.-G.); mpintado@porto.ucp.pt (M.P.)
2 Centro Bio R&D Unit, Association BLC3—Technology and Innovation Campus, Senhora da Conceição 2, Oliveira do Hospital, 3045-155 Lagares, Portugal; sonia.ribeiro@blc3.pt (S.R.); catarina.nunes@blc3.pt (C.N.); joao.nunes@blc3.pt (J.N.)
* Correspondence: aoliveira@porto.ucp.pt

check for updates

Citation: Vilas-Boas, A.A.; Oliveira, A.; Ribeiro, T.B.; Ribeiro, S.; Nunes, C.; Gómez-García, R.; Nunes, J.; Pintado, M. Impact of Extraction Process in Non-Compliant 'Bravo de Esmolfe' Apples towards the Development of Natural Antioxidant Extracts. *Appl. Sci.* **2021**, *11*, 5916. https://doi.org/10.3390/app11135916

Academic Editors: Marcos Fallarero Sortica and Lucia Gómez-Coetto

Received: 18 May 2021
Accepted: 21 June 2021
Published: 29 June 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Abstract: The 'Bravo de Esmolfe' apple (BE) is rich in antioxidants and represents one of the most important traditional cultivars in Portugal. A large amount of non-compliant BE, with no market value, it is generated every year. Thus, an effort to find value-added solutions is of utmost importance. For the first time, green extraction (microwave-assisted-extraction (MAE) and ultrasound-assisted-extraction (UAE)) were compared against conventional extraction (with (CET) and without temperature (CE)) to select the most efficient process to produce a natural antioxidant extract from non-compliant BE. The results showed that MAE and CET are good methodologies for developing a BE antioxidant extract. However, MAE is more sustainable than CET, requiring less time to reach high temperatures. The MAE-extract showed great antioxidant activity (14.80 ± 0.70 mg TE/g DE by ORAC, 3.92 ± 0.25 mg AAE/g DE by ABTS, and 5.11 ± 0.13 mg TE/g DE by DPPH). This extract revealed high amount of chlorogenic acid (0.48 ± 0.07 mg/g DE), (−)-epicatechin (0.30 ± 0.02 mg/g DE) and phloridzin (0.13 ± 0.01 mg/g DE). This study shows that non-compliant BE is a useful source of antioxidants, being a sustainable way for the recovery of value-added compounds from the rejected fruit in line with sustainable and circular bioeconomy principles.

Keywords: 'Bravo de Esmolfe' apple; food waste; polyphenols; antioxidant; sustainable extraction methods

1. Introduction

Apples are widely consumed globally [1], being one of the significant sources of antioxidants in the human diet [2]. Several works have reported the potent antioxidant activity of apple extracts that are mainly related to the presence of phenolic compounds, rather than from vitamin C or vitamin E [1–3]. Among apple cultivars, traditional cultivars as the Portuguese variety 'Bravo de Esmolfe' (BE) revealed to be a much richer source of phenolics than exotic varieties consumed worldwide (e.g., Red Delicious, Golden Delicious, Fuji and Granny Smith) [1,2]. Nevertheless, the studies about the potential health-promoting properties of traditional apples, especially from the BE apple, have been limited [2–4]. The BE apple is known by its aroma/flavour properties, and consumers highly appreciate it. This apple was recognized as a product with Protected Designation of Origin (PDO), with great impact on the local and national economy [3,4]. Its production was restricted to a small inland region in northern Portugal ('Beiras'), not exceeding 200 tons per year. Still, commercial demand is now increasing due to its appealing sensory properties [1,3]. However, a substantial percentage of the small production of BE apple does not meet the quality

Appl. Sci. **2021**, *11*, 5916. https://doi.org/10.3390/app11135916 https://www.mdpi.com/journal/applsci

Figura 19 – Artigo publicado na revista *Applied Science* no âmbito da iniciativa Nature Bioactive Foods pelos investigadores da UCP e BLC3.

Em preparação

Vilas-Boas, A., Nogueira, M., Oliveira, A., Nunes, C., Ribeiro, S., Nunes, J., Pintado, M. (2023, in submission). Biological activities and metabolic profile of Lavandula pedunculata polyphenols extracts: comparison between conventional and green extraction.

Vilas-Boas, A., Oliveira, A., Nunes, C., Ribeiro, S., Nunes, J., Pintado, M. (2023, in preparation for submission). Impact of different green extractions on Mentha suaveolens leaves and branches to develop novel antioxidant and antimicrobial extract.

Capítulo de livro

4) Vilas-Boas, A., Goméz-García, R., Campos, D., Oliveira, A., Pintado, M. (2021). Non-compliant fruit as new functional food ingredients. In Sustainable Innovation in Food Product Design (pp- 189-204). Springer Food Engineering Series." (Figura 20).

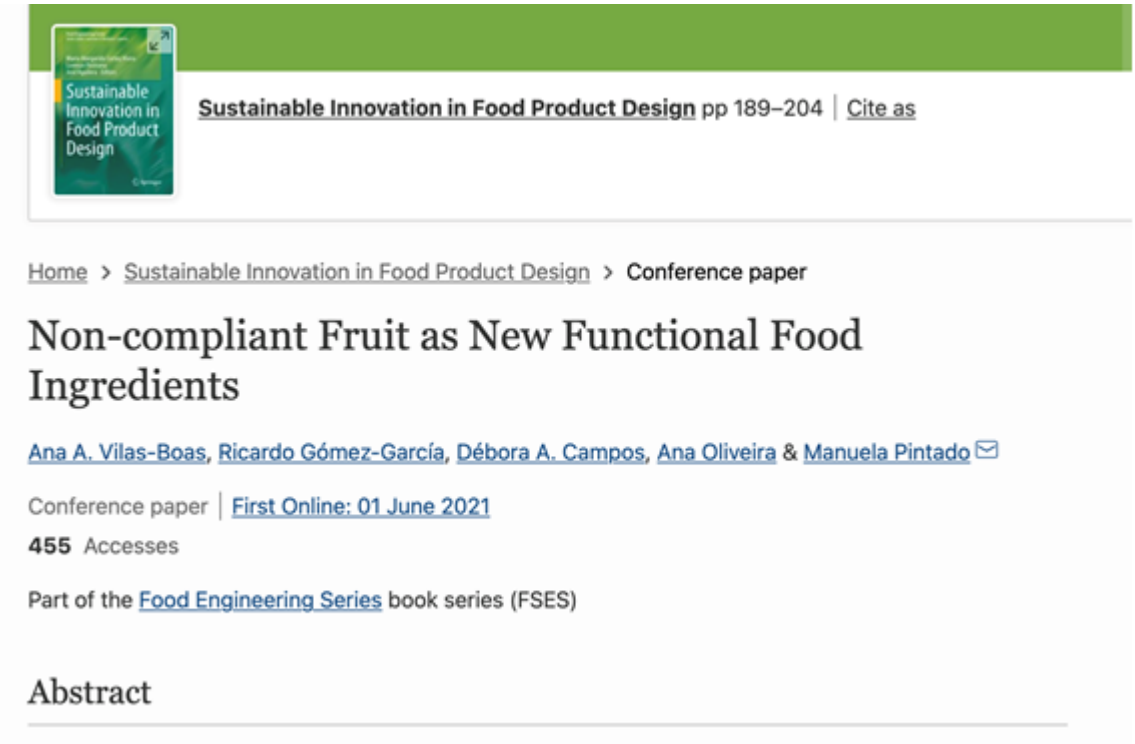


Figura 20 – Capítulo de livro publicado no âmbito da iniciativa Nature Bioactive Foods pelos investigadores da UCP.

1.4. Ambiente web

Relativamente às publicações em ambiente web, a Iniciativa Nature Bioactive Foods foi divulgada através dos websites dos diferentes parceiros:

- https://blc3.pt/blc3_nature-bioactive-foods.pdf
- https://www.cbqf.esb.ucp.pt/sites/default/files/galerias/ficha-projeto-nature-bioactive-food-pdr-2020-pt.pdf
- https://www.ibet.pt/blog/projecto/naturebioactivefood/

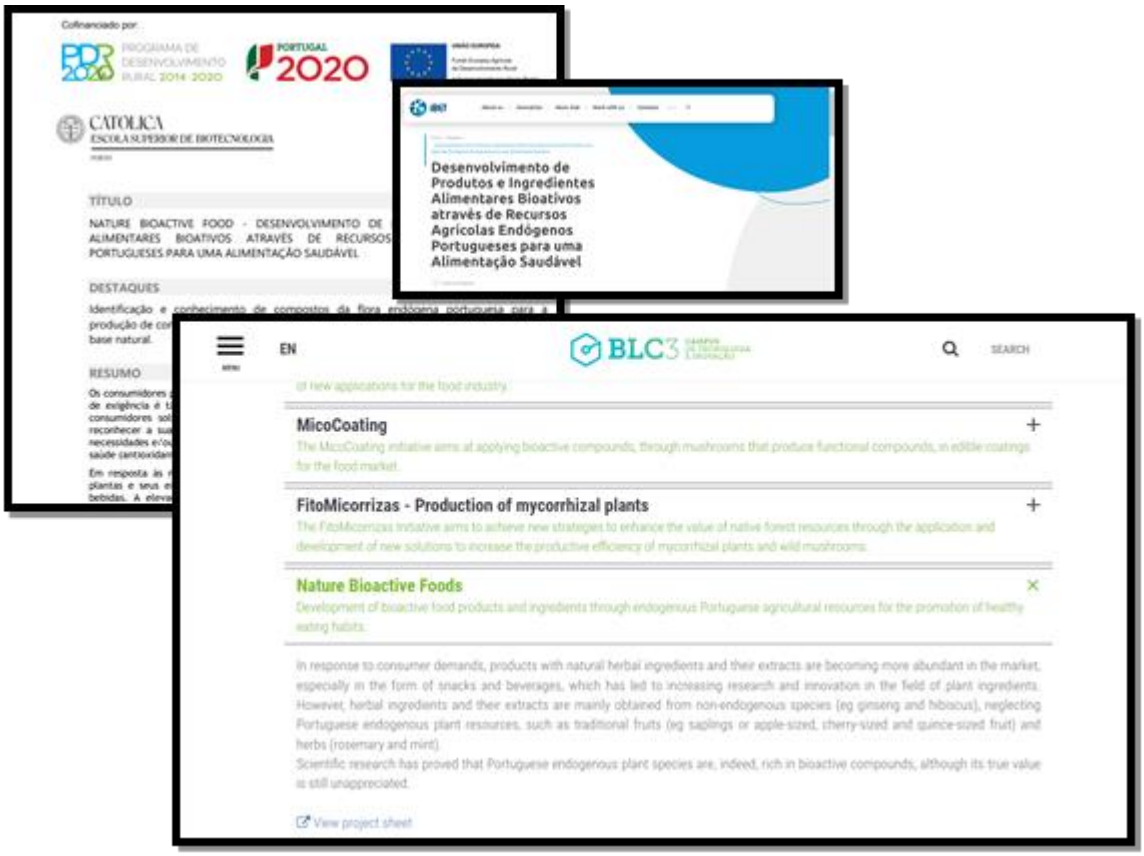


Figura 21 – Divulgação da iniciativa Nature Bioactive Foods em ambiente web.

1.5. Estágios realizados no âmbito da Iniciativa Nature Bioactive Food

Adicionalmente, os resultados da presente Iniciativa foram também disseminados no mundo académico através de um relatório de estágio para a obtenção do grau de licenciatura, duas dissertações para a obtenção do grau de mestre e de uma tese de doutoramento.

Teses

- Leucádio S. J.C. (2018). A influência do método de extração na bioatividade e perfil metabólico de extratos de rosmaninho. (Dissertação de Mestrado). Faculdade de Ciências e Tecnologia Universidade de Coimbra
- Gabriel Pires. (2020). Produção de extratos de plantas. (Relatório de Estágio para obtenção do grau de licenciado). Escola Superior de Tecnologia e Gestão de Oliveira dos Hospital.
- Ana Alexandra Gonçalves Vilas-Boas. (2021). Development and characterization of functional ingredients through the valorization of non-compliant Portuguese fruit: Bravo-de-Esmolfe apple and Saco cherry. Thesis (Master's degree in Food Engineering). Universidade Católica Portuguesa.
- Rocio Jiminez (en desarrollo). Revalorización del subproducto de manzana: Mejora de su funcionalidade mediante la aplicación de altas pressiones hidrostáticas. Thesis doctoral. Universidad Complutense de Madrid.