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# Research Interchange with the Lab of Food Chemistry from WUR

## Instituto Superior de Agronomia
## Lisbon, Portugal
## 26 October 2023

**BOOK OF ABSTRACTS**

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# Research Interchange with the Lab of Food Chemistry from WUR

Every two years, the PhD candidates of the Laboratory of Food Chemistry of Wageningen University go into an international study trip. These trips are aimed at knowledge exchange and netwroking in the food technology area.

The selected location for the PhD Trip 2023 was the Iberian Peninsula, and included Madrid and Salamanca in Spain, Porto, Aveiro and Lisbon in Portugal.

At LEAF – Linking Landscape, Environment, Agriculture and Food Research Centre from Instituto Superior de Agronomia, PhD candidates from WUR and LEAF had the chance to present their research, discuss findings and future avenues. This was a great opportunity for knowldege exchange and networking among young researchers!

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09:30 – 09:40 | **Welcoming session**
Isabel Sousa, LEAF Coordinator
Anabela Raymundo, Coordinator of LEAF Research Group 'Food & Feed'

09:40 – 09:50 | **LEAF Research Unit**
Isabel Sousa, LEAF Coordinator

09:50 – 10:00 | **G3 - Food & Feed**
Anabela Raymundo, G3 Coordinator

10:00 – 10:20 | **Laboratory of Food Chemistry at WUR and Exchange program**
Jean-Paul Vicken, WUR

10:20 – 10:50 | *Coffee break*

10:50 – 11:10 | **Sustainable plant fractionation: Wet mild separation of potato proteins**
Thore Diefenbach, WUR

11:10 – 11:30 | **Development of Camembert-like cheese fortified with microalgae**
Héctor Hernández, LEAF, ISA-ULisboa

11:30 – 11:50 | **Prenylated isoflavonoids as antimicrobials and potential efflux pump inhibitors in fluoroquinolone resistant *Staphylococcus aureus***
Marina Ika Iriantia, WUR

11:50 – 12:10 | **3D printing technology to develop algae-based snacks: engaging consumers in innovative and sustainable food solutions**
Sónia Oliveira, LEAF, ISA-ULisboa

12:10 – 12:30 | **Discussion**

12:30 – 14:00 | *Lunch break*

14:00 – 14:20 | **Dimethylallyl tryptophan synthase RePT from *Rasamsonia emersonii* catalyzes prenylation on tryptophan, tyrosine, and plant phenolics**
Pimvisuth Chunkruaa, WUR

14:20 – 14:40 | **Effect of Spirulina (*Arthrospira platensis*) dietary inclusion and heat stress on two slow-growing broiler strains, naked neck and fully feathered**
Elisabete Fernandes, LEAF, ISA-ULisboa

14:40 – 15:00 | **Kinetic modelling of lipid hydroperoxide formation in pure triacylglycerides**
Vincent Boerkamp, WUR

15:00 – 15:20 | **Development of new functional plant-based food models from fruit and vegetable pomaces with high nutrition and bioactivity value**
Saeed Salari, LEAF, ISA-ULisboa

15:20 – 15:30 | **Closing session**

15:30 – 17:30 | Visit to Living Labs in Tapada da Ajuda | Visit to Food Science Labs

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# Kinetic modelling of lipid hydroperoxide formation in pure triacylglycerides

**Vincent J.P. Boerkamp[a], Khoa A. Nguyen[a], Jean-Paul Vincken[a], Marie Hennebelle[a], John van Duynhoven[b,c]**

[a] Laboratory of Food Chemistry, Wageningen University & Research, The Netherlands
[b] Biophysics, Wageningen University & Research, The Netherlands
[c] Unilever Global Food Innovation Centre, Wageningen, The Netherlands

Lipid (per)oxidation is studied in various fields, ranging from biology, to pharmaceuticals, and foods. The lipid autoxidation cycle starts with the abstraction of a hydrogen next to a double bond of an unsaturated fatty acid. This radical reacts with oxygen, and a hydrogen donor to form lipid hydroperoxides, which are the first metastable reaction products, and can therefore be detected easily [1]. Our understanding of these chemical reactions can be deepened by kinetic modelling them. However, such approach requires the formation rate constants of hydroperoxides individually, which are not available yet.
In this study, we used NMR spectroscopy for the annotation and quantification of a wide range of (novel) individual hydroperoxides that were formed during the autoxidation of pure triacylglyceride standards at varying temperatures [2,3]. The obtained curves enabled us to kinetically model the formation of hydroperoxides. Our model included the intermediate radical species as well as the degradation of hydroperoxide catalysed by transition metals. The obtained set of pathways and kinetic parameters enables the comprehensive quantitative modelling of lipid oxidation in products with complex oil compositions.

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# Dimethylallyl tryptophan synthase RePT from *Rasamsonia emersonii* catalyzes prenylation on tryptophan, tyrosine, and plant phenolics

**Pimvisuth Chunkrua**[a], Kai Leschonski[a], Alejandro A. Gran-Scheuch[b], Gijs J. C. Vreeke[a], Jean-Paul Vincken[a], Marco W. Fraaije[b], Willem. J. H. van Berkel[a], Wouter J. C. de Bruijn[a], Mirjam A. Kabel[a]

[a] Laboratory of Food Chemistry, Wageningen University & Research, The Netherlands

[b] Molecular Enzymology Group, University of Groningen, The Netherlands

Dimethylallyl tryptophan synthases (DMATS) are aromatic prenyltransferases that catalyze the transfer of a prenyl moiety from a prenyl donor to an aromatic acceptor. Aside from their natural role in prenylating aromatics in the biosynthesis of microbial secondary metabolites, DMATSs also act on structurally diverse aromatic substrates [1]. This capability makes DMATSs a potential biotechnological tool to produce biologically active compounds in a wide range of applications, such as antimicrobial plant phenolics [2,3].

Our study explored the substrate scope and product profile of a recombinant RePT, a novel DMATS from a thermophilic fungus *Rasamsonia emersonii*. RePT was successfully produced with His6- SUMO-RePT construct, showing a molecular weight of 66,405 ± 2 Da. Among a variety of (plant) aromatic substrates, RePT showed the highest substrate conversion for L-tryptophan and Ltyrosine (>90%), both yielding two mono-prenylated products. Eight phenolics from diverse phenolic subclasses were accepted with a noticeable conversion (>10%). Three stilbenes, namely oxyresveratrol, pinostilbene, and resveratrol showed the highest conversion (37-55%). Other phenolics were (+)-catechin, (□)-epicatechin, coumestrol, (±)-equol, and phloretin, showing 11-25% conversion. The structures of prenylated L tryptophan were determined using NMR to be either normal C7-prenylation (major product) or reverse N1-prenylation (minor product). For plant phenolics, the position of prenylation as annotated using MS2 fragmentation pattern, showed the main products to be mono-O prenylated. Moreover, RePT was tolerant to organic solvents, yielding higher than 90% L-tryptophan conversion in the presence of 20% (v/v) methanol or DMSO. RePT may be a promising biocatalyst for generating valuable bioactive prenylated aromatics for food, cosmetic, and pharmaceutical industries.

## REFERENCES

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# Sustainable plant fractionation: Wet mild separation of potato proteins

**Thore Diefenbach**[a], Peter Wierenga[a], Robin Spelbrink[b], Gijs Vreeke[a], Jean-Paul Vincken[a]
[a] Laboratory of Food Chemistry, Wageningen University & Research, The Netherlands
[b] AVEBE UA, The Netherlands

Plant-based proteins are being increasingly used in foods. However, enzymatic activities in the protein isolate pose a great challenge due to off-taste and off-colour development, which impede the quality of the food product. These enzymatic activities comprise (1) lipid oxidation, catalysed by lipases and lipoxygenases and (2) oxidation of phenolics, catalysed by polyphenol oxidases. Approaches to reduce off-taste/colour formation often deteriorates techno-functional properties of the protein isolate and thus limits the range of its applications. Off-taste/colour generating enzymes in potato protein isolates are only poorly studied to date. This study aims to investigate off-taste/colour generating enzymes in potato protein isolates and explore strategies to mitigate their action. Presence of enzymes and their variants in the potato protein isolate will be determined using ULPLCPDA-MS techniques and the results will be linked to the enzyme activity in isolates from different cultivars/batches. Moreover, the activity of the potato lipase patatin towards different substrates will be investigated under different processing conditions (pH, temperature, oxygen levels) relevant for isolation of potato proteins. Based on this, possible strategies to mitigate enzymatic activity will be tested and the influence on techno-functional properties will be analysed.

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# Effect of Spirulina (*Arthrospira platensis*) dietary inclusion and heat stress on two slow-growing broiler strains, naked neck and fully feathered

**Elisabete Fernandes**[a], Cátia F. Martins[a,b,c], Daniela Carvalho[a], Luisa L. Martins[a], Anabela Raymundo[a], Madalena Lordelo[a], André M. Almeida[a]
[a] LEAF – Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal.
[b] CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisbon, Portugal.
[c] AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Portugal.

In tropical regions, poultry production plays a very important role in providing a valuable source of protein. However, it faces two obstacles: heat stress and difficult access to feedstuffs on international markets. Strains tolerant to high temperatures as well as microalgae may represent a possible solution to these two challenges.
The aims of this study were: 1) to evaluate the effect of a diet with 15% Spirulina (**SP**) as alternative feed and 2) to evaluate the impact of heat stress in two slow-growing strains, naked neck (**NN**) and fully feathered (**FF**) fed with SP dietary inclusion.
For this purpose, two independent trials were conducted. In both trials, a total of 40 one-day-old male broilers were randomly allotted into 4 groups with *ad libitum* access to water and feed. Each group received either a Control diet (**C**) or a diet with 15% Spirulina (**SP**) inclusion. Weekly, body weight (**BW**) and feed intake (**FI**) were monitored to calculate feed conversion ratio (**FCR**) and average daily gain (**ADG**). In the heat stress (**HS**) trial, the animals were kept at 30° C, while in the thermoneutral (**TN**) trial the animals were kept at standard temperature. At the end of the experiment, all broilers were slaughtered, and organs were collected, weighed, and measured.
The final BW of the animals, in the TN trail ranged from 3360 g (**FFC**) to 2622 g (**NNSP**). In HS trial ranged from 2741 g (**NNC**) to 2141 g (**FFSP**). This parameter was negatively influenced by SP diet incorporation ($P < 0.05$) in both strains.
At the end of the trials, in the TN trial, FI, FCR, and ADG were not significantly affected ($P > 0.05$) by diet or strain. However, in the HS trial FI was higher in NN in comparison to FF broilers ($P < 0.05$). The incorporation of SP increased ADG in comparison to the control animals ($P < 0.05$) but neither strain nor diet influenced FCR ($P > 0.05$).
The incorporation of SP in the diet increased the relative length (cm/kg) of the gastrointestinal tract, ($P< 0.05$) in ileum, in an average of 17%, in both trials.
In the HS trial, carcass yield was negatively influenced ($P < 0.05$) by SP incorporation in FF broilers, but this effect was not observed for the NN broilers. In the TN trial, FFC animals had the highest carcass yield (78.9%) while NNC animals had the lowest (76.8%). Regardless of the strain or diet breast muscle yield was not significantly affected ($P > 0.05$) in both trials.
The incorporation of SP and HS negatively influenced the growth performance of broilers. Future information provided by digestibility coefficients and meat quality will complement these results, allowing a better knowledge of the nutritional values of SP for broilers.

**FUNDING**
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**KEYWORDS**
Heat Stress, Naked Neck, Fully Feathers, Spirulina, Poultry

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# Development of camembert-like cheese fortified with microalgae

**Héctor Hernández**[a], M. Cristiana Nunes[a], Catarina Prista[a], Anabela Raymundo[a]
[a] LEAF – Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal.

The fortification of dairy products with microalgae biomass has been growing expectations as it is possible to obtain more nutritious, sustainable and healthy fermented products. Microalgae biomass is a potential source of bioactive compounds, which can affect the mechanical behavior and the sensory properties of food[1-3]. This work aimed to study the effect of adding *Chlorella vulgaris* microalgae biomass on the rheology, texture, nutritive value and sensory properties of Camembert-like cheese. Temperature and frequency sweep tests (oscillatory rheology) were performed at 20 °C and 90 °C, the impact microalgae addition was noticed for the studied levels (2% to 7%). The cheeses are viscoelastic solids (G´ > G´´), despite a higher frequency or temperature dependence when microalgae biomass is added (Fig.1a). Texture analysis of the cheeses, performed by using a puncture test, showed a hardness decrease ($p<0.05$) in 5% and 7% *Chlorella*-fortified cheeses (16.1 N and 16.5 N respectively), when compared to the control sample (21.1 N, without algae addition). Nutritional composition (namely lipids and ash) was significantly ($p<0.05$) affected by the addition of microalgal biomass, showing a decrease of 8.7% in lipids and 10.7% in ash for 7% *Chlorella*-fortified cheese, when compared to the control. Regarding the bioactivity of the cheese, the results of the antioxidant activity analysis and total phenolic compounds indicated an increase ($p<0.05$) in the antioxidant activity and phenolic compound concentration, specifically in treatments fortified with 5% and 7% of *C. vulgaris.* Sensory analysis showed that panelists preferred the control and 7% *Chlorella*-fortified cheese, both with an overall acceptability score of 5.6 (1 to 7 hedonic scale) according to Fig. 1b. In addition, it was observed that 30% of the tasters "would always buy" the cheese supplemented with 5% of *C. vulgaris* and 40% “would buy very frequently” the cheese with 7% of *C. vulgaris*, indicating a high probability of consumption of these innovative and hybrid cheeses, made from animal and vegetable sources.

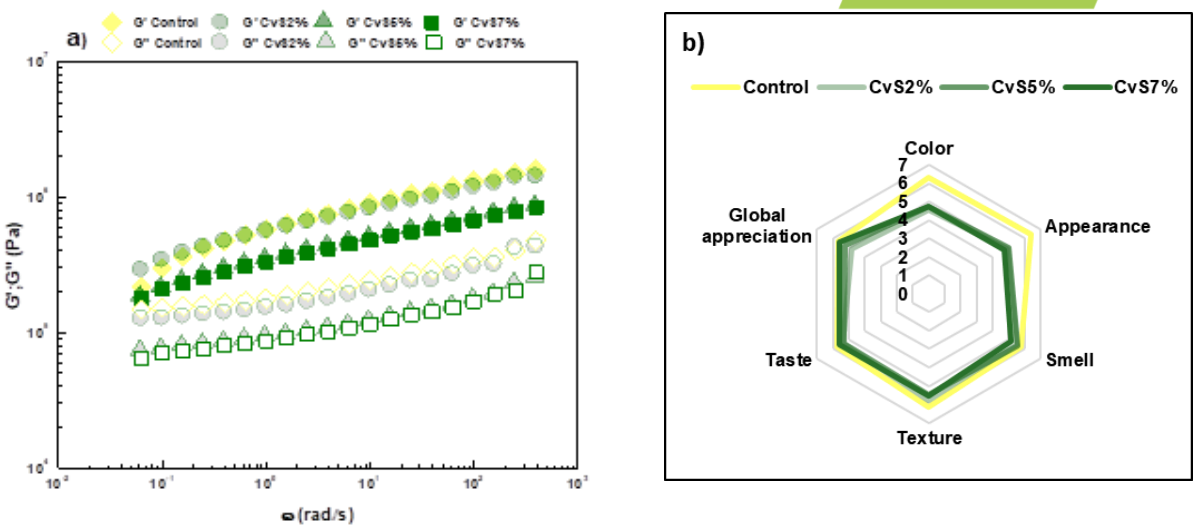


**Fig 1.** Mechanical spectra at 20 °C for cheeses fortified with *C. vulgaris* (2, 5 and 7%) and the control sample (a) and Responses of the sensory analysis (n = 35) (b).

**KEYWORDS**

Microalgae Biomass, Cheese, Rheology, Bioactives, Digestibility, Sensory Properties.

**FUNDING**

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# Prenylated isoflavonoids as antimicrobials and potential efflux pump inhibitors in fluoroquinoloneresistant *Staphylococcus aureus*

**Marina Ika Irianti**[a], Jean-Paul Vincken[a], Sarah van Dinteren[a], Ellen ter Beest[a], Klaas Martinus Pos[b], Carla Araya-Cloutier[a]
[a] Laboratory of Food Chemistry, Wageningen University & Research, The Netherlands
[b] Institute of Biochemistry, Goethe-University Frankfurt, Germany

Overexpression of NorA efflux pumps plays a pivotal role in the multidrug-resistance mechanism in *S. aureus*. Here, we investigated the activities of prenylated isoflavonoids, present in the legume plant family (Fabaceae), as natural antimicrobials and efflux pump inhibitors (EPIs) in fluoroquinolone-resistant *S. aureus*. To check the EPI activity towards NorA, we performed ethidium (Eth) accumulation and antibiotic potentiation assays in *S. aureus* norA overexpressing and norA knockout strains. We found that four prenylated isoflavonoids showed efflux pump inhibition in the norA overexpressing *S. aureus*, namely neobavaisoflavone, glabrene, glyceollin I, and glyceollin III. At sub-inhibitory concentrations, neobavaisoflavone (6.25 µg/mL, 19 µM) and glabrene (12.5 µg/mL, 39 µM), showed 4.5 and 5.9 times more Eth accumulation in norA overexpressing *S. aureus* than the control, respectively. In addition, these two compounds boosted the MIC of fluoroquinolones up to 8-fold. No fluoroquinolone potentiation was observed with these isoflavonoids in the norA knockout strain, indicating NorA as the main target of these potential EPIs. In comparison to the reported NorA EPI reserpine, neobavaisoflavone showed similar potentiation of fluoroquinolone activity at 10 µM, higher Eth accumulation, and less cytotoxicity. Moreover, neobavaisoflavone and glabrene did not exhibit membrane permeabilization effects and no cytotoxicity on Caco-2 cells at the concentrations tested as EPIs (up to half MIC). In conclusion, our findings suggest that the prenylated isoflavonoids neobavaisoflavone and glabrene are promising phytochemicals that could be developed as antimicrobials and resistance-modifying agents to treat fluoroquinolone-resistant *S. aureus* strains.

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# 3D printing technology to develop algae-based snacks: engaging consumers in innovative and sustainable food solutions

**Sónia Oliveira**[a], Maria D. Torres Pérez[b], Isabel Sousa[a], Anabela Raymundo[a], Carlos Bengoechea[c]

[a] LEAF – Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal.
[b] Department of Chemical Engineering, Universidade de Vigo (Campus Ourense), Science Faculty, As Lagoas, 32004 Ourense, Spain.
[c] Departamento de ingeniería química, Universidad de Sevilla, España.

Health impact of food has seen a growing attention with consumers demanding more from the agrifood system to support their health and wellbeing. Emergent foods, including algae, have demonstrated their potential as ingredients for functional foods. Algae possess an abundance of valuable nutrients and bioactive compounds like proteins, minerals, dietary fiber, and antioxidants [1]. Consequently, the development of algae-based food solutions holds the potential for a substantial influence in meeting future food needs. Yet, the search for new ingredients is frequently hindered by consumer taste preferences [2]. It is imperative to design strategies to address the unfamiliarity and reluctance to embrace algae-based foods. 3D Food Printing (3DFP) emerges as a promising technology to fulfill the increasing market demands for targeted nutrition, all the while generating appealing multi-layered textured structures that can positively influence consumer perception of algal food [3]. **The goal** of the research work is to develop innovative 3D algae snacks, driving consumers to embrace a plant-based, healthier diet, enriched by the inclusion of algae, ultimately making algae-based foods both delicious and accessible.

So far, our research has resulted in cereal-based snacks with addition of *Chlorella vulgaris* (Cv) and snacks based on seaweed gels. In the case of the cereal-based snacks, the levels of Cv incorporation (2-30%) were determined based on the printability of the raw dough. Furthermore, the research delved into the health impact of baked snacks (170°C, 15 min), assessing their nutritional composition, *in vitro* antioxidant activity, total phenolic compounds, *in vitro* digestibility and bioaccessibility. Lastly, sensory analysis was conducted. The results indicated that higher Cv incorporations promoted an increase of the bioactive compounds, improved antioxidant activity and enhanced mineral's bioaccessibility. Additionally, the inclusion of Cv in the snacks positively influenced their sensory acceptance.

At present, for the gelled snacks, three red algae species (*Chondrus crispus*, *Gelidium Corneum,* and *Gracilaria gracilis*) were selected to develop gels: after extraction, the biomass was centrifuged and hot supernatants were fed to the printer, leading to gelation as printing took place. Gel's rheological behaviour was analysed and key printing parameters were studied. The addition of locust bean gum (LGB) to supernatants and printing temperature were tested. Results showed that increasing the concentration of LBG and lowering the printing temperature improved the definition for *G. corneum* and *G. gracilis* gels. In contrast, *C. crispus* gels were more stable and less affected by either of these parameters.

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**KEYWORDS**
Algae, 3D Food Printing, Rheology, Digestibility, Sensory Analysis

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# Development of new functional plant-based food models from fruit and vegetable pomaces with high nutrition and bioactivity value

**Saeed Salari[a], Joana Ferreira[a], Ana Lima[a,b], Isabel Sousa[a]**
[a] LEAF – Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal.
[b] Faculty of Veterinary Medicine, Lusophone University of Humanities and Technologies, 376 Campo Grande, 1749-024 Lisbon, Portugal

Health/nutritional claims are effectively used as differentiators to consumers' preferences, and messages related to sustainability concerns are increasingly popular on food labels. Furthermore, the functional food market continues to increase consistently. **Our goal** will be to upcycle a by-product from the juice industry, apple and carrot pomaces, to evaluate their bioactive potential and to develop innovative functional plant-based model foods with excellent nutritional profiles and enriched in bioactive compounds.
**Methods:** Since pomaces contain skins, pulp, seeds, and stems of the fruits, which are rich in different compounds, the pomace samples were separated into five different fractions based on particle size. Nutrient content, including vitamins C, E, K and B6 were evaluated in all fractions by HPLC-UV-Vis. Total phenolic compounds and antioxidant activity were determined by the Folin–Ciocâlteu, DPPH and FRAP methods. Anti-inflammatory activities were determined by evaluating the samples' inhibitory activity towards Matrix metalloprotease-9 (MMP-9), an enzyme related to inflammation and cancer development. Antibacterial activities were evaluated against species related to food contamination, two Gram– (*Escherichia coli* O157 and *Salmonella* sp.) and two Gram+ (*Staphylococcus aureus* and *Listeria monocytogenes*).
**Results** show that both pomaces present a high nutrient content and several bioactivities. Vitamins C, E, K, and B6 were found throughout the fractions. Interestingly, for both apple and carrot pomaces, the fractions with smaller particles (D and E) presented higher amounts of phenolic compounds and antioxidant activity. Anti-MMP-9 activities were found only in the apple pomaces, particularly in the smaller particles as well, suggesting that the phenolics found there may be related to this bioactivity. Additionally, apple pomace presented a high antibacterial activity, which was particularly noticeable against Gram – bacteria, *E. coli* and *Salmonella* sp., reducing their growth down to 31.75 and 36.84% of controls, respectively. Since apple and apple juice are known to be very susceptible to Gram – bacteria contamination and specially to outbreak-inducing *E. coli* strain O157 [1] the discovery that apple pomace reduces these bacteria's growth can be of great potential. However, when added to crackers, these bioactivities were much reduced, suggesting that they may be either affected by processing conditions (e.g., temperature) or by the food matrix. Future work will involve the study of the carrot pomace's activities and further research on the anticancer and anti-inflammatory activity of apple pomace using cell models. We also plan to test the pomaces with different food matrices, the use of Ultrasound to increase extraction and evaluate the effect of digestion upon their bioactivities as well.

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Overall, **our work points out to the high nutritional and bioactive potential of both apple and carrot pomaces**, corroborating their possible use as a functional food additive.

**KEYWORDS**
Plant-based Food Models, Sustainability, Functional Foods, Bioactive Compounds, Health

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**SCIENTIFIC COMMITTEE at LEAF/ISA-ULisboa**
Isabel Sousa
Anabela Raymundo
Cristiana Nunes

**ORGANISING COMMITTEE at LEAF/ISA-ULisboa**
Ândria Viegas
Cristiana Nunes
Francisco Herdeiro
Joana Costa
Lisiane Fernandes
José Matheus Silva
Pedro Coelho
Sónia Oliveira

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