POLYPODIUM VULGARE LINN (*POLYPODIACEAE*), AS A SOURCE OF BIOACTIVE COMPOUNDS

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Abstract

The *pteridophytes*, popularly known as ferns, due to their independent evolution with respect to the rest of the species, constitute a source of new phytoconstituents [1,2]. In the last decades, studies published on the aqueous extract of the fronds of the fern *Polypodium leucotomos* Linn (*Polypodiaceae*) have highlighted the potential of new applications of this fern as a nutraceutical and cosmeceutical agent due to photoprotective properties [3,4].

The European Medicines Agency (EMA) has also published a monograph on the therapeutic use of the *Polypodium vulgare* Linn (*Polypodiaceae*) rhizome for its beneficial expectorant properties in cough and cold as well as for short-term use in cases of occasional constipation. However, other uses and potential applications are here explored.

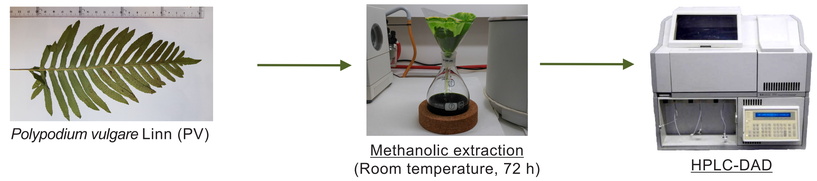
In the present study we focused on the phytocharacterization and *in vitro* bioactivity of the methanol extract from the fronds of *Polypodium vulgare* (PVM) in the cytotoxicity and phototoxicity assays and the evaluation of the cytoprotective and neutralization capacity of ROS against H_2O_2 as an oxidative stress agent. The polyphenolic profile obtained by HPLC-DAD has revealed a high content of polyphenolic compounds (77823.7 mg/kg) with a predominance of 3-O-caffeoylquinic acid (58778.3 mg/kg) over the rest of the phytoconstituents analyzed. The extract did not induced cytotoxicity against tumoral cell lines, HaCaT being the cell line that has experienced a greater decrease in cell viability ($LC_{50,\ HaCaT}$ = 0.390 mg/mL). The 3T3 cell line has experienced better results than HaCaT in terms of cytoprotection using different models of toxic agents.

Introduction

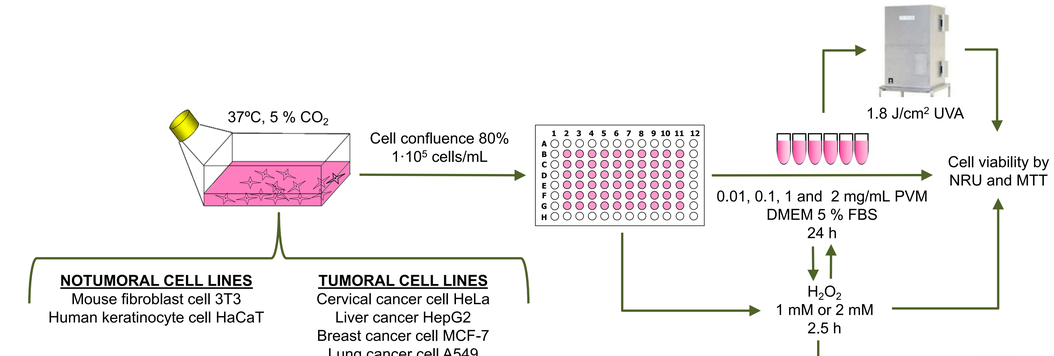
Polyphenols have been described as fundamental agents to prevent the imbalance of the homeostasis of pro-oxidant agents. The lack of control of the oxidative state has been associated with a damage to the fundamental biological molecules of organisms such as DNA. The antioxidant and anti-tyrosinase activity of the PVM extract has already been described previously by our research [5].

Materials and methodology

Polyphenol characterization



Cytotoxicity, cytoprotective, cellular repair, phototoxicity and ROS activity



Results*

*Statistical differences were considered as follows: *$p \leq 0.05$ and **$p \leq 0.01$, ***$p \leq 0.001$ and ****$p \leq 0.0001$.

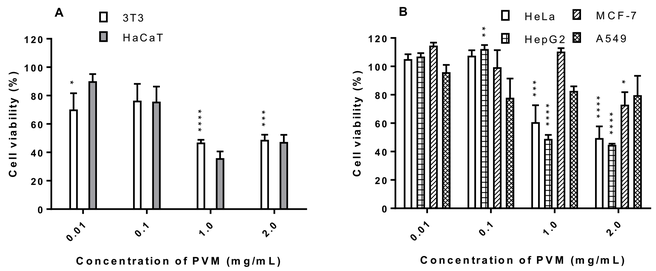


Figure 1. Cytotoxic activity of PVM at different concentrations on no tumoral (A) and tumoral (B) cell lines by MTT assay. Results are expressed as mean ± standard error of n=3 exception for Fig.1A for HaCaT that's n=2. Control cells only with culture medium. A two-way analysis of variance (ANOVA) and a Bonferroni's *post hoc* assay have been performed.

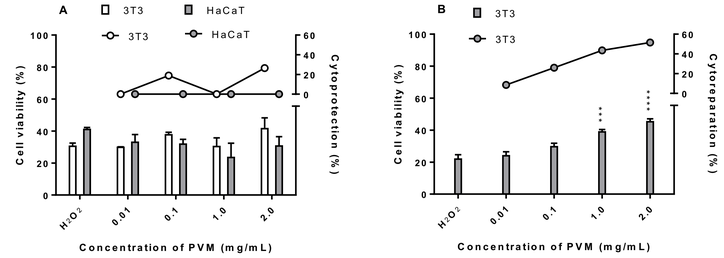


Figure 2. Protective activity of PVM at different concentrations on 3T3 and/or HaCaT by MTT assay for cytoprotective (A) and cellular repair (B) activity. Hydrogen peroxide (H_2O_2) cell viability was used as positive control. Results are expressed as mean ± standard error of n=3. A two-way analysis of variance (ANOVA) and a Bonferroni's *post hoc* assay have been performed.

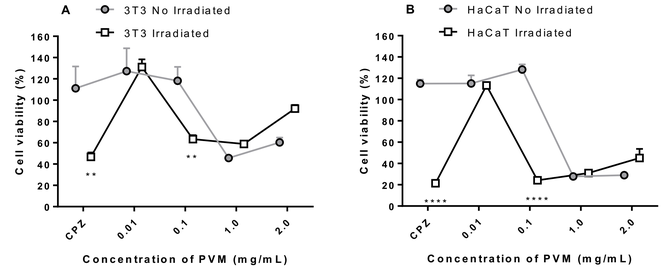


Figure 3. Phototoxicity activity at 1.8 J/cm² UVA of PVM at different concentrations on 3T3 (A) and HaCaT (B) cell lines by MTT assay. Chloropromazine (CPZ) cell viability was used as positive control. Results are expressed as mean ± standard error of n=3. Control cells only with culture medium. A two-way analysis of variance (ANOVA) and a Bonferroni's *post hoc* assay have been performed.

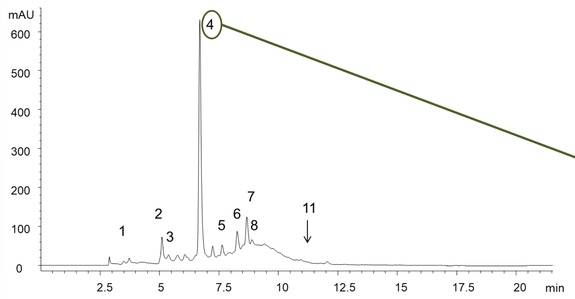
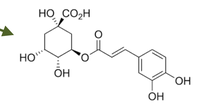


Figure 4. HPLC-DAD chromatogram reported at 272 nm for PVM. List of compounds: 1: shikimic acid, 2: gallic acid, 3: 5-O-caffeoylquinic acid, 4: 3-O-caffeoylquinic acid, 5: catechin, 6: epicatechin, 7: rutin, 8: hyperoside, 9: naringin, 10: quercitrin, 11: 3,5-di-O-caffeoylquinic acid, 12: rosmarinic acid, 13: cinnamic acid, 14: eugenol, 15: trans-cinnamaldheyde.



3-O-caffeoylquinic acid

Major phytoconstituent determined

Conclusions

1. No marked cytotoxic effect was found for PVM in no tumoral and tumoral cell lines at physiologic concentrations (0.01 – 0.1 mg/mL) by the MTT assay.
2. PVM had cytoprotective and cellular reparation effects in 3T3 cell line at tested conditions.
3. There is no phototoxic effect of PVM in the UVA condition tested and it even has a slight photoprotective effect at the highest concentrations of the extract.
4. Regarding the content of polyphenols by HPLC-DAD, the major compound was 3-O-caffeoylquinic acid.

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Bioactive food colorants obtained from *Lonicera caerulea* L., *Morus nigra* L., and *Rubus fruticosus* fruits

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Introduction

Lonicera caerulea L., *Morus nigra* L., and *Rubus fruticosus* L. fruits are widely known for their nutritional and bioactive properties. Their richness in anthocyanins, which are the main responsible compounds for the reported beneficial properties, justify their exploitation not only as functional foods but also as sources of natural colorants, in alternative to some artificial compounds with reported adverse effects to human health [1,2]

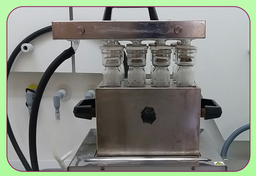
M. nigra

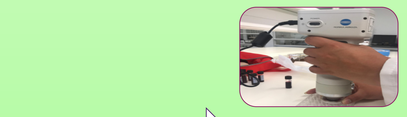
L. caerulea

R. fruticosus

Methodology

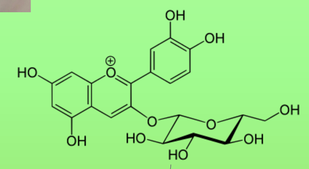
The fruits were characterized in terms of anthocyanin and non-anthocyanin compounds, by HPLC-DAD/ESI-MS, and two solid colouring formulations were prepared through the spray-drying technique with maltodextrin and mixtures of Arabic gum and maltodextrin in different proportions, according to the characteristics of each fruit juice and the efficiency of the process. The stability of the prepared colorants was assessed over three months of storage at room and refrigerated temperature. For that purpose, the microbial load, the cytotoxicity, and the bioactive properties (antioxidant and antimicrobial) were evaluated, along with their anthocyanin concentration and colouring capacity.

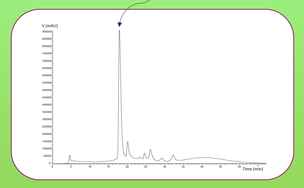




Results and conclusions

Different phenolic compounds were detected in the three fruits, among which, some anthocyanins as cyanidin-3-*O*-glucoside and cyanidin-*O*-hexose, as the most abundant ones. All the formulations revealed great colouring, antioxidant, and antimicrobial properties, with a slight variation of anthocyanin concentration along the three months of storage at room and refrigerated temperature, which validate their application for colouring purposes. None of these formulations revealed cytotoxic properties, being, then, considered safe for food application.





Stable colour
Stable anthocyanin concentration
Microbial load < allowed limits
Bioactivity

Cytotoxicity X

Future works

This bench-scale application study can also support the scale-up of natural colorants production, which is of interest for several sectors such as food, pharmaceutical, and cosmetic, among others. With these promising results, the next step would be the optimization of the incorporation of these colorants into food matrices, in order to validate their colouring and bioactive potential.

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Acknowledgments

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PROSPECTION FOR THE BEHAVIOR OF ENZYMATIC ACTIVITY FOR POLYPHENOLOXIDASE (PPO), PEROXIDASE (PO), PECTINAMETHYLESTERASE (PME) IN THE ASSAYS OF THE FERMENTATIONS OF CACAO (THEOBROMA CACAO L).

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Abstract

This work presents the behavior of the enzymes polyphenoloxidase (PPO), peroxidase (PO) and pectinamethylesterase (PME) extracted from cocoa beans during the conventional fermentation (F1) and a prospecting of this activity for the fermentation with inoculum start (F2). The seeds were monitored through temperature (º C), pH and Total Soluble Solids (º Brix). It can be inferred from the prospecting, that when reaching the peak of maximum temperature for all enzymes, these activities remain constant, in the graph of absorbance and soluble solids it is possible to observe a projection of a larger area for the activity of the enzyme (PO), in the graph for pH and ABS it is possible to observe a more pronounced interval before beginning the decline of activity for all enzymes analyzed.

Introduction

Enzymes are protein molecules, but not all protein matrices are enzymes, have high molecular mass, catalyze reactions in biological systems, increase the speed of chemical reaction, are associated with biomolecules because they contain high catalytic power, participate in digestion, respiration, metabolism and tissue maintenance (BRASIL, 2011), (LIMA, 2001). The pectic enzymes break the pectic chain by depolymerization reactions (hydrolases and liases) or desesterification (sterases) (CHAICOUSKI et al., 2016). The enzyme Polyphenoloxidase (PPO) is found in plant tissues, apple, banana, coffee, etc., when desirable in food the catalyzed reactions accelerate the pigmentation process, accentuating the final coloring of food. The enzyme (PO) is related to the appearance of foreign flavors in food and its inactivation occurs through heat treatments (ARAÚJO, 1999) capable of oxidizing phenolic compounds only in the presence of H2O2 and its detection is based on the development of orange-red color in the presence of substrate-water-oxygenate (colorless) (NESPOLO, OLIVEIRA, *et al.*, 2015).

Materials

The work presents the analyses of the monitored enzymatic activity in cocoa samples, collected during the 7-day time interval, totaling 168 hours of assay for inoculum fermentation (f2) and without inoculum start (f1). The enzymes analyzed were polyphenoloxidase (PPO), pectinamethylesterase (PME) and peroxidase (PO). Held at the Chemistry Laboratory of the State University of Pará (UEPA).

Methodology

Extraction solution

It was comminuted 5 g to 10 g of sample, vacuum filtered, homogenized in 40 mL of 0.1 M potassium phosphate buffer solution, pH 6.0. The extract was stirred in ice bath for 1 hour. http://www.nadp.ufc.br/assets/proc-anbiol-enzima-pme.pdf).

Determination of activity (PME)

In large tubes 0.16 mL of extract, 1 mL of pectin at 0.5%, 0.3 mL of bromothymol blue solution at 0.01% and 0.4% of NaCl at 1M were inserted, the tubes were heated 80º C for 1 min and read at 620 nm.

Determination of activity (PPO)

In tubes 1.7 mL of 0.1 M sodium phosphate buffer solution, pH 6, 1.2 mL of 0.4 % catechol and 0.2 mL of extract in final volume of maximum 3.0 mL were inserted, the test tubes were shaken and read in the spectrophotometer at 420 nm.

Determination of activity (PO)

In tubes were inserted 1.5 mL of 1% guide, 1.2 mL of 0.1 M.pH 6 phosphate buffer, 0.1 mL of extract and 0.4 mL of H2O2 at 0.4%. The enzymatic activity was measured with spectrophotometric readings of absorbances at 470 nm (LIMA, 2001).

Calculation for enzymatic activity

$$\frac{U}{mL} = A \times FD \times 1000 \div E \times Ve \times T$$

A is the absorbance of the enzyme (PPO = 26.9/ PO = 26.6) and V the Volume used and T the Time. Dilution factor, E is the

Results

The table 1 presents the results to the activity of the analyzed enzymes. The PPO reached the highest activity 2497 (U/g) occurred on the 1st day, at 30º C, the PO enzyme reached maximum absorbance 5608 (U/g) on the 2nd day of Fermentation, when the pH of the seeds were at 4.5, (WILLIAN FLURKEY, 1978) found activity for the PPO of 900 (U/g) in the cocoa seed. SAKHAROV (1999) reported that the almond and cocoa bean had activity for PO of 5,700 (U/g).

Table 1 - Quantitative Analysis of Enzymes (PPO), (PO) and (SME) during the Fermentation Test (f1).

Tempo (dias)	Temperatura (⁰C)	pH	PPO (U/g)	PO (U/g)	PME (U/mL)
Af.°	29	4,2	1765	1322	946
Af.1	30	3,8	2497	1708	756
Af.2	39	4.5	1412	5608	646
Af.3	38	3,9	1228	4639	673
Af.4	38	4,1	1151	3198	526
Af.5	38	4,5	945	2312	459
Af.6	31	4,6	692	860	287
Af.7	31	5,2	554	716	256

The prospecting of enzymatic activity in fermentation (f2), when reaching the maximum temperature peak for all enzymes, these activities remain constant, figure 1.

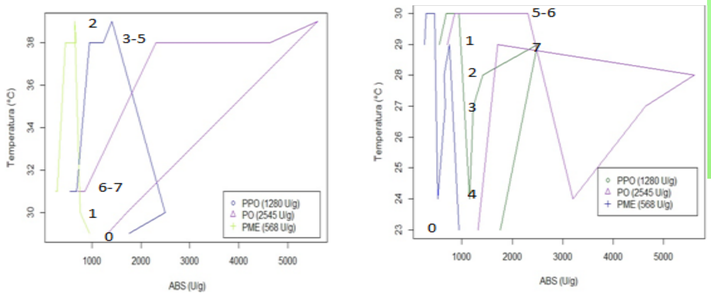

Figure 1 - Temperature and ABS (absorbance) of the enzymatic activity in the fermentation monitored assay (f1) and (f2).

In figure 2, it is possible to observe a projection of a larger area for enzyme activity (PO).

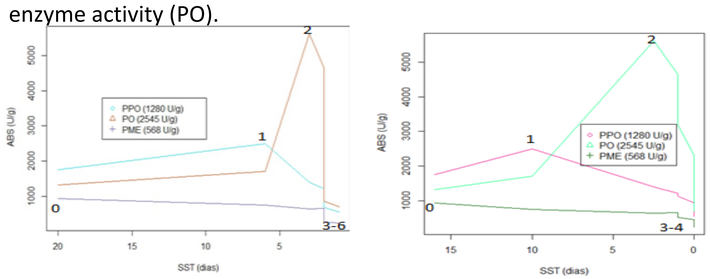

Figure 2 - Relationship of TSS and ABS of enzymatic activity in the fermentation assay (f1) and (f2).

For pH and ABS in figure 3 it is possible to observe a more pronounced interval before starting the decline of activity for all enzymes analyzed.

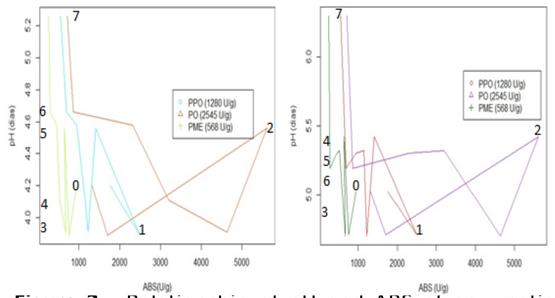

Figure 3 - Relationship of pH and ABS of enzymatic activities in the assay with fermentation (f1) and (f2).

Conclusion

The prospecting of the enzymatic activity for the monitoring of fermentation (F2) proved to be potentially more intense for all the enzymes analyzed when, at maximum temperature, during the decrease of the SST showed higher activity for the enzyme PO, it can also be observed with greater clarity the decrease of these activities with increased pH. Therefore, it can be concluded that the fermentation (F2) improved not only the physical-chemical and biochemical parameters of the final cocoa products but also directed to the best behavior of the enzymatic activity.

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By-Products of Agri-Food Industry as Tannin-Rich Sources: A Review of Tannins' Biological Activities and Their Potential for Valorisation

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B. A. Carreira-Casais [1], L. Cassani [1,3], M. A. Prieto [1,2,*] and J. Simal-Gandara[1,*]

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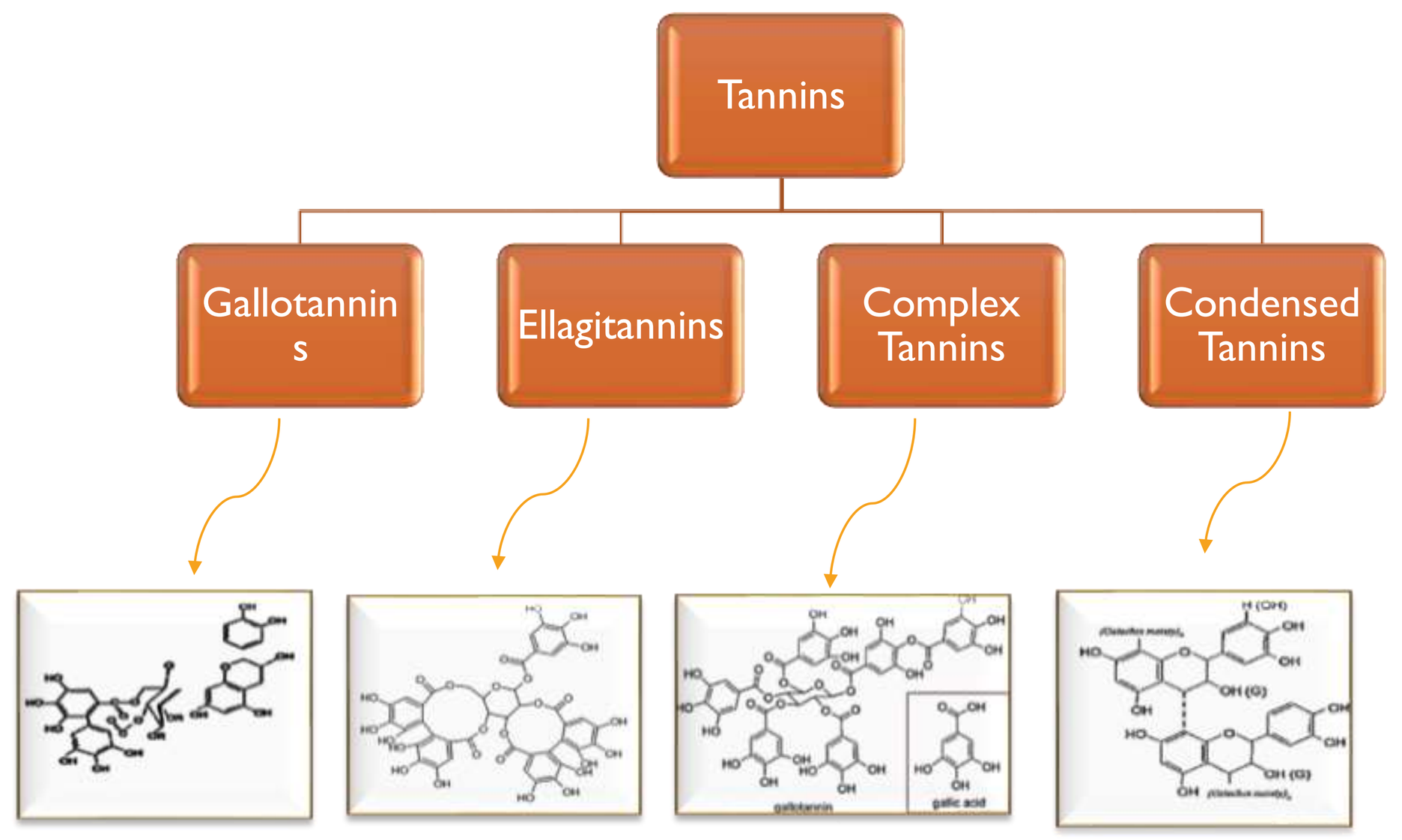
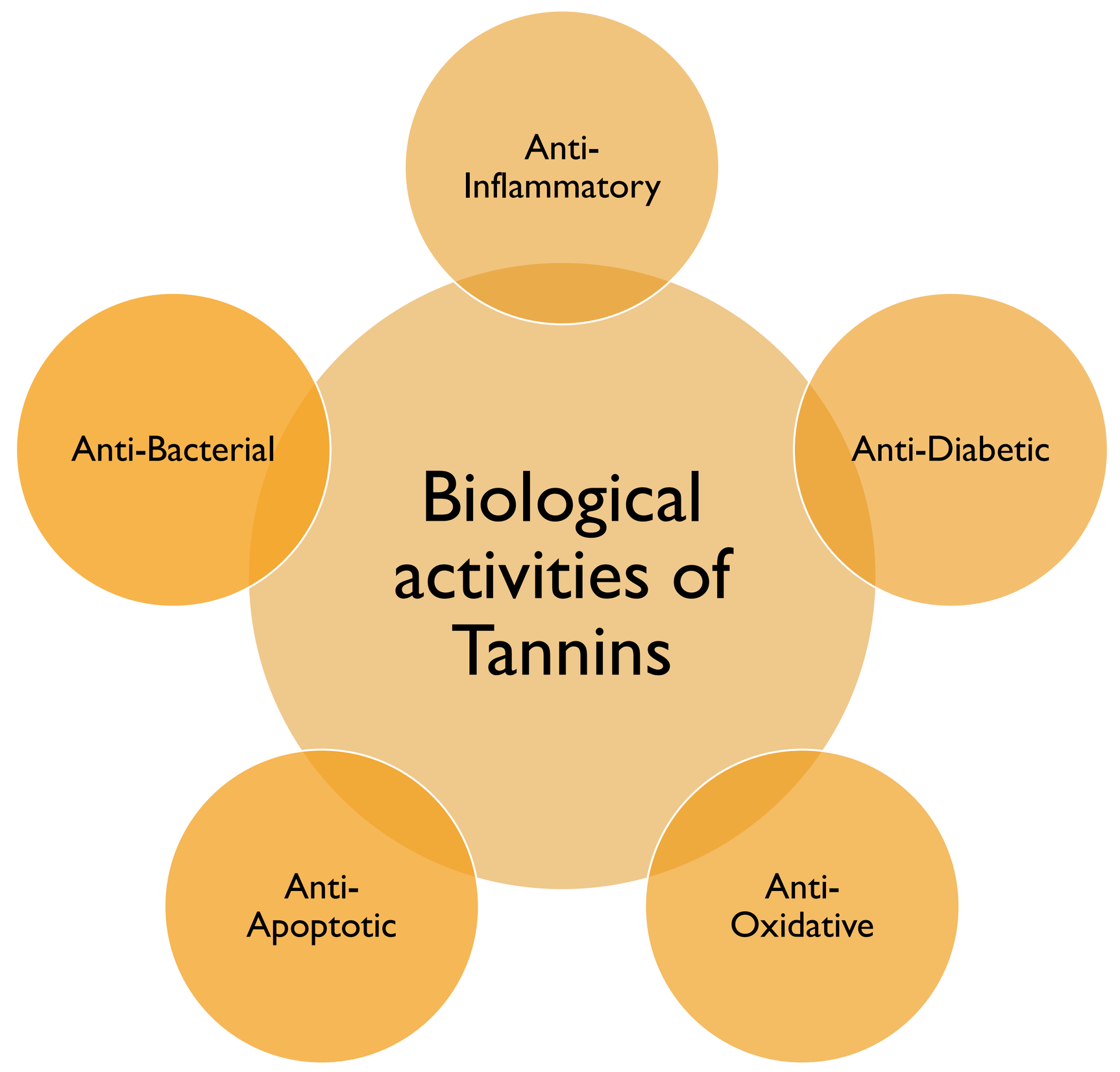
Introduction

Tannins are a diverse group within phenolic compounds widely distributed in nature. They are secondary metabolites of plants usually produced as a result of stress and they exert a protective role, including photoprotection against UV rays and free radicals or defense against other organisms and environmental conditions, such as dryness. Moreover, Tannins are a heterogeneous group, having molecular weights between 500 and 20,000 Da and very different chemical structures. Tannins have been demonstrated to exert different biological activities, such as antioxidant activity. This property is related to their chemical structure as they possess phenolic rings able to bind to a wide range of molecules and act as electron scavengers to trap ions and radicals Regarding tannin classification, they have been historically classified into hydrolysable tannins (HTs) and condensed tannins (CTs), and the latter are also called proanthocyanins. Nowadays, the classification according to their chemical characteristic and structural properties has been updated. Thus, tannins can be grouped into gallotannins, ellagitannins, CTs, complex tannins (CoTs) and phlorotannin (PTs, an exclusive class of tannins found in the algal species of the Phaeophycean class). A schematic representation of tannin structural classification is presented in Figure 1 .

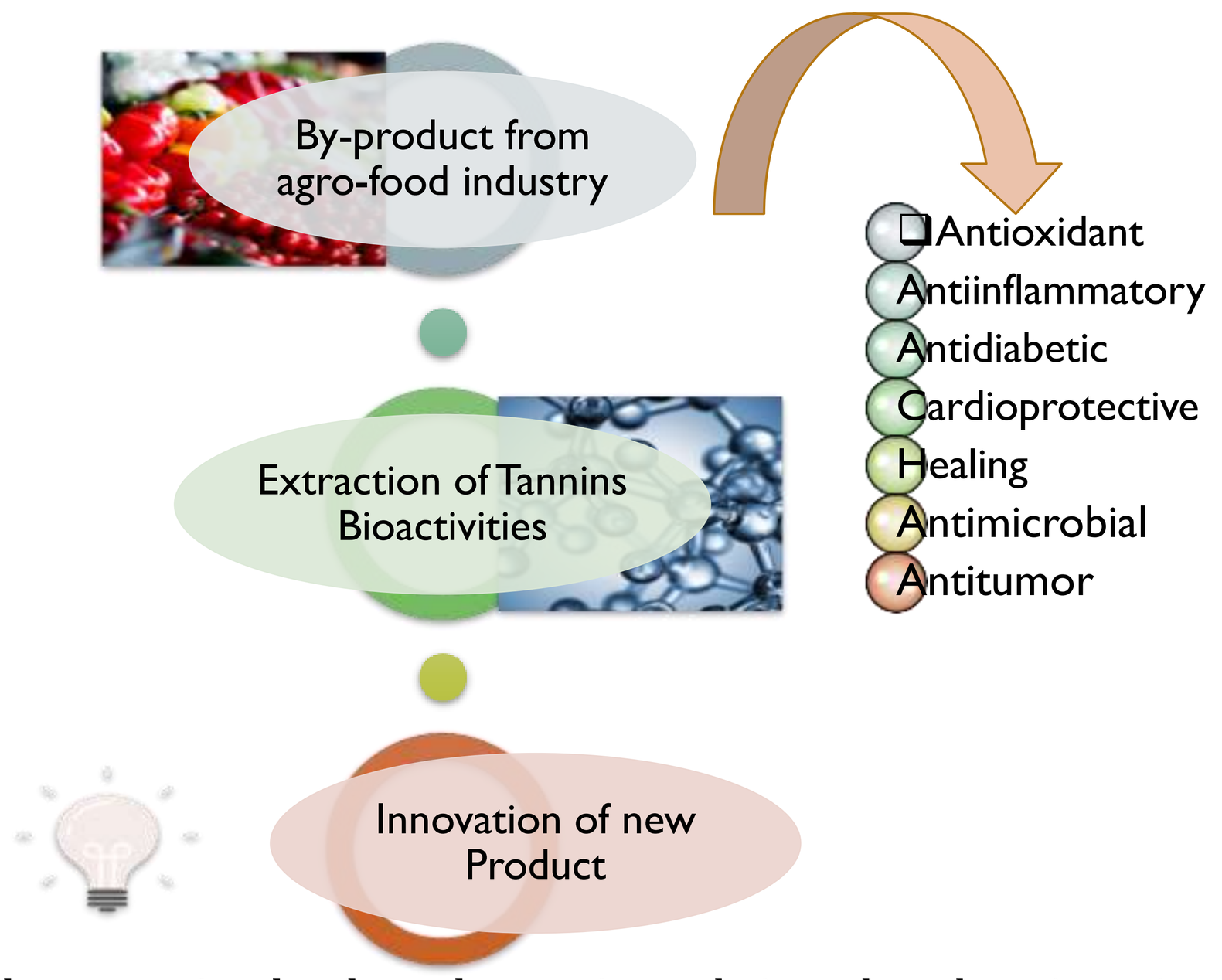


Able to enhance the safety and the shelf life of products and as clarification agents in drinks Although tannins have been sometimes linked to unpleasant organoleptic properties, they have also shown plenty of properties and applications. Some of these properties are antioxidant, antimicrobial or anti-inflammatory, among others, which have given rise to their use in the food, nutraceutical and pharmaceutical industry . Additionally, their toxic effects have been assessed. Particularly, they have been proposed as natural food additives [2].



Methodology

This Work is directed towards the description of the biological activities exerted by tannins as they could be further extracted from by-products of the agri-food industry to produce high-added-value products,



Lately, conventional and novel extraction techniques have been investigated to optimize the extraction parameters of tannin recovery from different by-products.

Table 1 : Examples of tannin extraction techniques from different agri-food by-products.

Species	Tannin	By-Product	Extraction Techniques	Experimental Conditions	Activity
Trapa quadrispinosa	HT	Pericarps	UAE	Et/W (60/40, *v/v*), 30 min, 40 °C, L/S ratio 40 mL/g	Antioxidant (DPPH)
Cupressus lusitanica and *Cistus ladanife*	TTC	Waste distilled after steam distillation	UAE	Et S/L ratio 1:20, 30 min, 30 °C, 70% A	Antioxidant (ABTS)
Coffee (*Coffea arabica*)	Procyanidins (CT)	Pulp	UAE	W/A extract, 20 min, RT	-
Pomegranate (*P. granatum* var. *Gabsi*)	TTC	Peels	UAE	2.63 g/100g dw, 55.46% E, 30 min	Antioxidant (DPPH and ABTS)
Red grape variety (*Vitis vinifera*	CT	Pomace	HAE	NaOH, Na_2CO_3 or $NaHCO_3$) and Na_2SO_3 (2.5% or 5% (*w/w*). S/L ratio 1:8, 120 min, 100 °C	Production of environmentally friendly wood adhesive; Silver NPs, antimicrobial and apoptotic potential
Chestnut (*Castanea sativa*)	TTC	Shells	Maceration	Et (20 mL × 3 days × 3 times) or Et/W 7:3 *v/v* (20 mL × 3 days × 3 times)	Antioxidant (DPPH and TEAC)
Pomegranate (*Punica granatum* L.)	TTC	Peels	HAE	W, 2% SS and 0.5% SB, S/L ratio 1:5, 7 h, 80 ± 5 °C	-
Tea (*Camellia sinensis* L.)	TTC	Leaves	SFC-CO_2	Supercritical CO_2 flow rate 8 g/min, 188 bar, 50 °C, co-solvent flow rate 2.94 g/min	Antioxidant (ABTS)
Acacia mollissima	HT and CT	Bark	HAE and MAE	HAE: M (2h, 20 °C and 60 °C). MAE (1 min, 300W or 5 min, 150W)	-
Myrtus communis L.	TTC	Leaves	MAE	Et 42% (60 s, 500 W, S/L ratio 32 mL/g)	Antioxidant (DPPH, TEAC and ORAC)
Endopleura uchi	TTC	Bark	Maceration	Et/W 50%	Antimicrobial, cytotoxic and antioxidant
Norway spruce (*Picea abies*)	CT	Bark	Hot water extraction	10% solid content, 2% SS, 0.5% SC, (75 °C, 120 min)	-
Spruce (*Picea abies*)	TTC	Bark	SFC-CO_2	Solvent consumption 2.5 kg CO_2/kg product and 24.94 kg Et 70/kg product, 100 bar, 40 °C	Antioxidant (DPPH)

The interest in a circular economy and food or agricultural waste valorization is increasing. Therefore, biorefinery approaches for recovering bioactive molecules with target biological properties are consequently growing to face the current challenge: moving towards a circular system production model for this reason Further applications are focused on obtaining other products from tannin-based sources. For instance, coffee pulp (rich in tannins) was submitted to solid state fermentation by Penicillium verrucosum to produce tannase.

Results

As aforementioned, tannins represent a chemical defense barrier for plants and algae that improve the response against pathological attacks and adverse abiotic conditions. The biological activity in plants and algae has prompted their utilization as traditional remedies to treat numerous diseases or infections. Currently, the biological effects of purified tannins or tannin-rich extracts (containing additional biomolecules) have been evaluated in vitro and in vivo using animal models, and more recently by clinical trials performed on humans. Most of these research works have been focused on the study of the bioactivities of plants containing high amounts of tannins or, less commonly, purified tannins, to disclose their potential for developing innovative applications in the field of medicine, pharmacology, cosmetics, botany and/or veterinary medicine **[1]**

Among the biological activities of tannins, the most relevant ones are antioxidant, ***anti-inflammatory, anti-diabetic, cardioprotective, healing and antimicrobial (antiviral and antibacterial)***

Table 2 : some representatives of Tannin-rich and their tannin chemical profile with major compounds and their reported bioactivities

Source	Species	Classification	Compounds	Bioactivities	Ref.
Acacia sp.	*A. mearnsii*	CT	Epi-FIS derivatives	Antioxidant, anti-inflammatory, antimicrobial	[33,34]
	A. nilotica	CT	PoGG, EA, GA, diGA, epi/gallocatechin, dicatechin derivatives	Antinociceptive, anti-inflammatory and antipyretic	[35,36]
Castanea sp.	*C. sativa*	HT	CAST, VES, EA, chestanin	Antioxidant, anti-inflammatory, antidiabetic, cardioprotective, antimicrobial, antifungal, antidiarrheal (vet.)	[37–45]
Juglans sp.	*J. regia*	HT	EA, pedunculagin, casuariin	Antiplatelet, cardioprotective, antiatherogenic and anti-inflammatory	[46–48]
Lotus sp.	*L. corniculatus* *L. pedunculatus*	CT CT	Heteropolymers PC: PD	Improvement of animal performance	[49–51]
Picea sp.	*P. abies*	CT	-	Antioxidant (food preservative)	[52]
Punica sp.	*P. granatum*	HT	Punicalagin, punicalin, geraniin	Antiviral (herpes simplex-2, hepatitis B)	[53,54]
Quercus sp.	*Q. robur*	HT	Castalin, vescalin, CAST, VES, GA, EA, PoGG	Antioxidant, antidiabetic	[55–57]
Rhus sp.	*R. coriaria*	CT HT	GA, QUERG, CYANG derivatives	Antimicrobial, anti-inflammatory, immunomodulatory, antiapoptotic and healing	[58,59]
Rubus sp	*R. fruticosus*	CT	CYANG, GA, malvidin-3-galactoside, vanillic acid	Antioxidant, anti-inflammatory, antidiabetic and gastroprotective	[60,61]
Sargassum sp.	*S. fusiforme*	PT	Eckol, dieckol, fuhalols	Antioxidant	[62]
	S. muticum	PT	PG, diphlorethol, bi- and tri-fuhalol A, B	Antioxidant, antibacterial, antiproliferative, anti-inflammatory	[63]
Schinopsis sp.	*S. lorentzii*	CT HT	FIS-catechin polymers TGG, PGG, quinic acid-GA esters	Antioxidant, antimicrobial, anthelmintic	[64–67]
	S. balansae	CT	ProFIS polymers	Antioxidant, antimicrobial, anthelmintic	[1,64–108]
Terminalia sp.	*T. chebula*	HT	Chebulinic acid, TGG	Anti-inflammatory	[68]
Vitis sp.	*V. vinifera*	CT	Galloylated PC, PC, PD	Antioxidant, anti-inflammatory, antiobesity	[69]

Despite the antioxidant mechanism of tannins has been frequently investigated, deeper studies about the concrete mechanism of action are needed, particularly considering the administration as well as the variability of the tannin metabolic profile associated with each species. Furthermore, it is worth mentioning that the antioxidant capacity is the basis for triggering further systematic and beneficial effects, such as anti-inflammatory responses and wound healing [2]

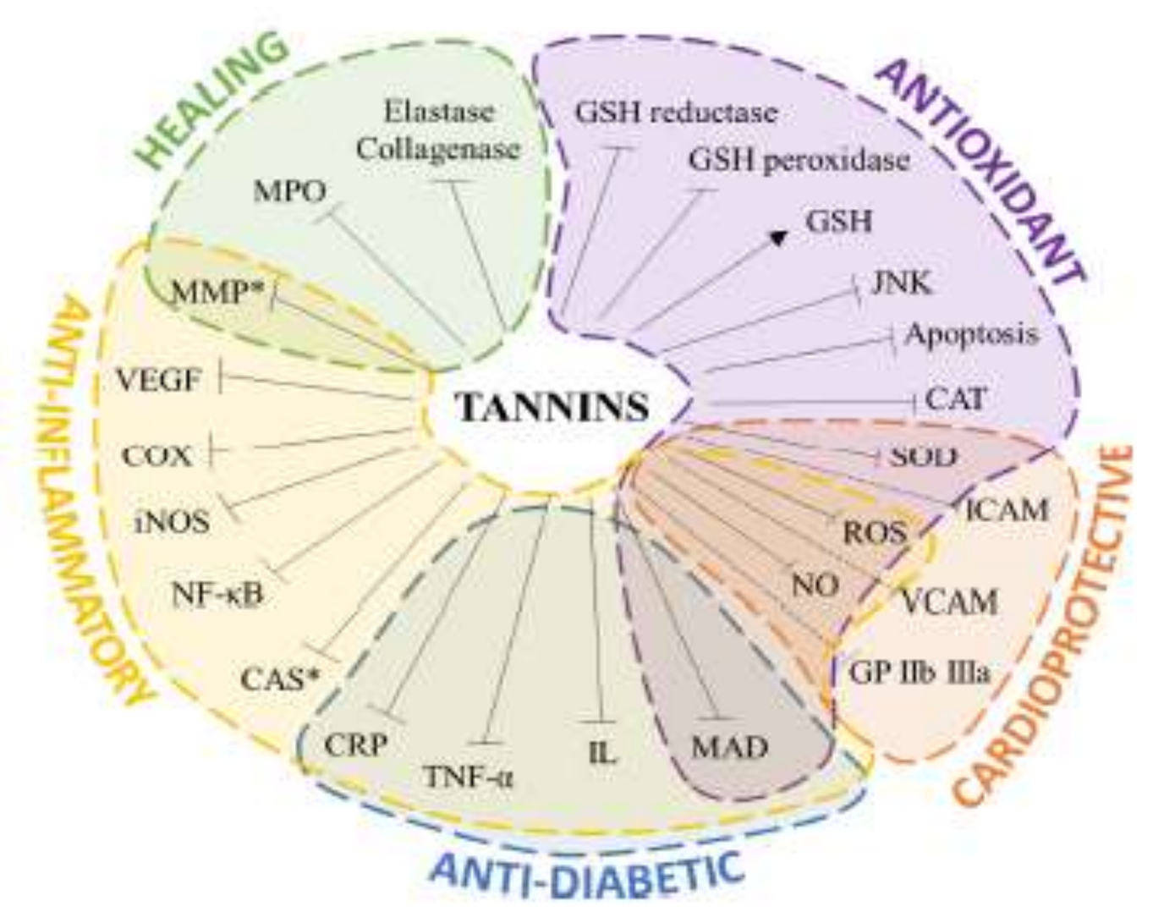
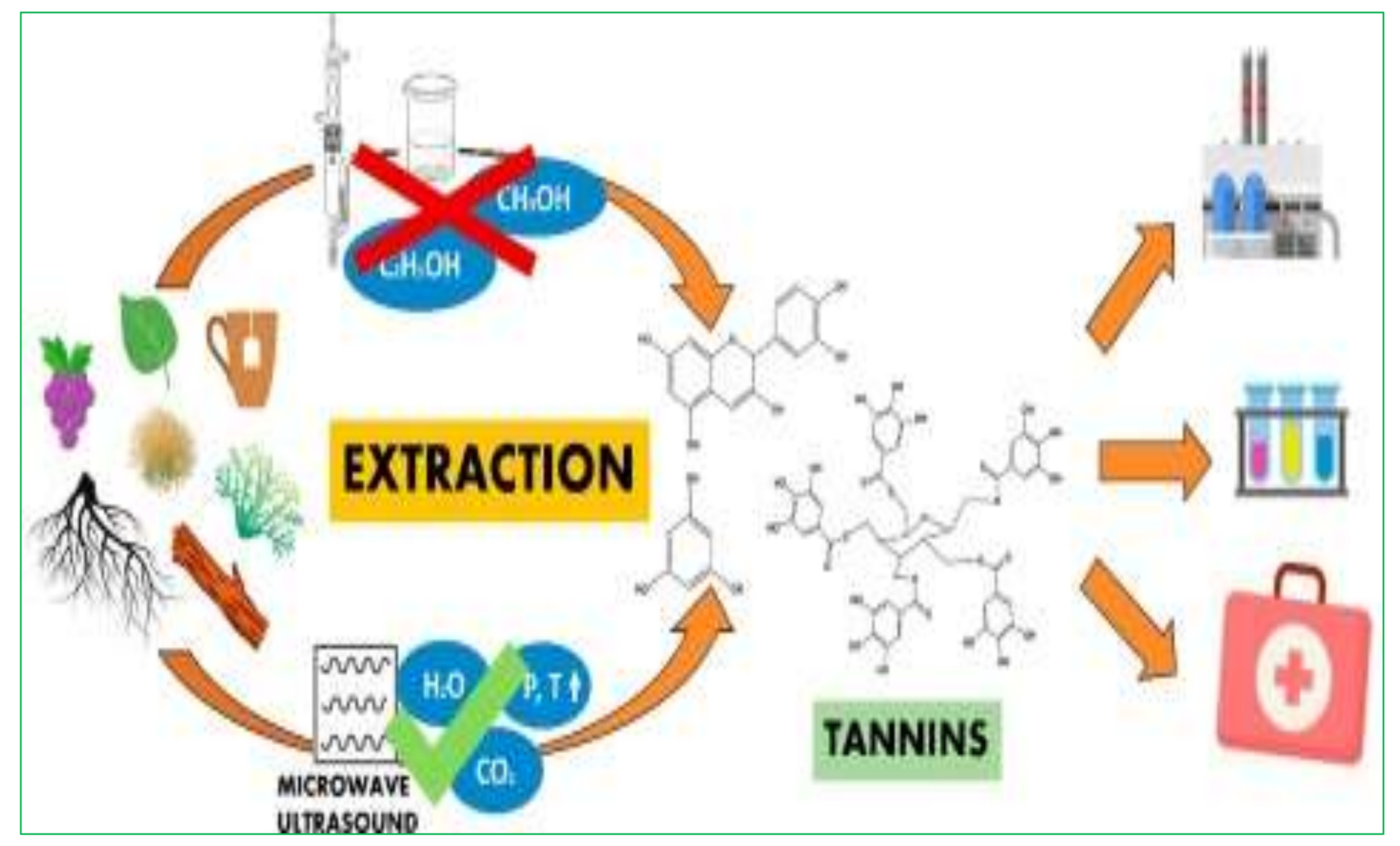


Figure 1 Visual representation of the suggested mechanisms involved in the biological properties of tannins. Lines show decrease in or inhibition of biomarkers, whereas arrows show an increase in or promotion of reduced glutathione (GSH). (* = antioxidant activity; MAD: malondialdehyde; IL: interleukin; TNF-α: tumor necrosis factor-α; CRP: c-reactive protein; CAS: caspase; NF-κB: nuclear factor-κB; iNOS: nitric oxide synthase; COX: cyclooxygenase; VEGF: vascular endothelial growth factor; MMP: matrix metalloproteinase; JNK: C-Jun N-terminal kinase; MPO: myeloperoxidase; CAT: catalase; SOD: superoxide dismutase; ROS: reactive oxygen species; NO: nitric oxide; VCAM: vascular cell adhesion protein; ICAM: intercellular adhesion molecule; GP IIb
IIIa: glycoprotein IIb/IIIa).2.2. Anti-Inflammatory **[1]**

Conclusion

Regarding all the biological properties described in tannins obtained from natural sources, valorization could be an efficient approach to re valorize agri-food by-products. Nevertheless, further researches are still necessary to completely ***clarify the mechanisms of action of the biological activities and improve the extraction methods and conditions to obtain tannins in an optimal way.***



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Blueberry juice as a nutritious and bioactive beverage to be included in novel food products

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Introduction

Blueberry (*Vaccinium myrtillus* L.) is a very popular fruit, native from the northern hemisphere and consumed worldwide. It has been widely studied for being a rich source of bioactive compounds with recognized beneficial properties for Human health [1]. Therefore, several industrialized products, such as juices and derivatives, have been developed from blueberry fruit, aiming at most practical forms of consumption.

Vaccinium myrtillus L.

Methodology

Blueberry juice

Nutritional value

Chemical composition

- Free sugars
- Organic acids
- Phenolic compounds

Bioactive properties

- Lipid peroxidation inhibition
- Oxidative hemolysis inhibition

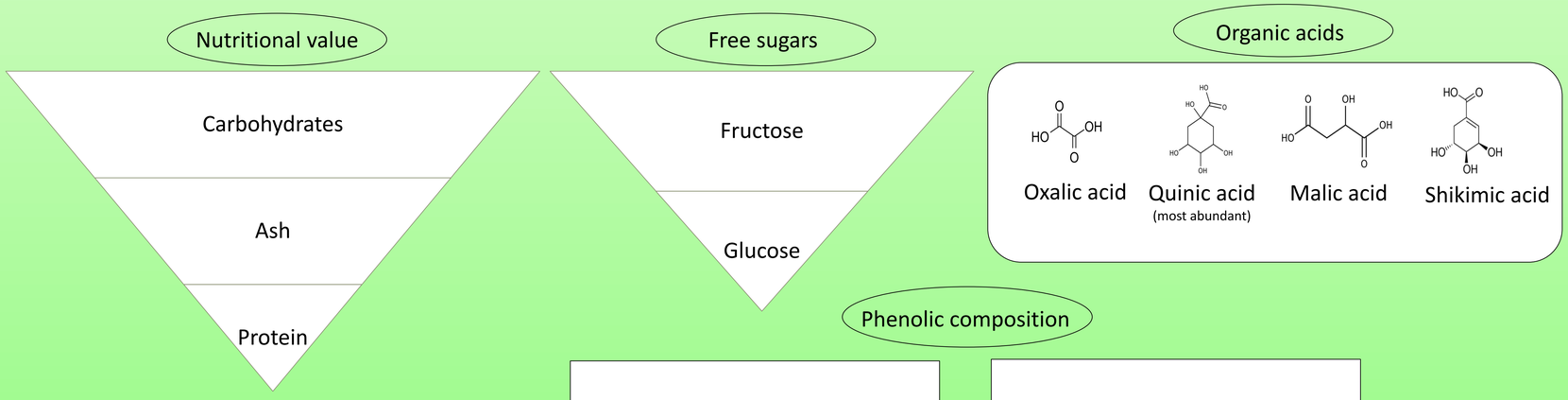
AOAC

HPLC-RI
UFLC-PDA
HPLC-DAD/ESI-MS

TBARS
OxHLIA

Results and conclusions

The results obtained in the present study validate the nutritional and bioactive quality of the juice obtained from *Vaccinium myrtillus* L., justifying its application in the development of novel foodstuff.



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SUBCRITICAL WATER EXTRACTION OF *ACTINIDIA ARGUTA* LEAVES: EVALUATION OF TEMPERATURE EFFECTS ON BIOACTIVITY AND RADICAL SCAVENGING ACTIVITY

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Abstract

Actinidia arguta

Bioactive compounds and biological properties

Subcritical Water Extraction (SWE)

Green and eco-friendly extraction technique

Food Industry Sustainable Applications

Introduction

- *Actinidia arguta* is a plant originally from Asian [1,2];
- *A. arguta* fruit is considered an excellent source of bioactive compounds, such as antioxidants, contributing to human's health [3,4];
- By-products of this vine, such as leaves, were also associated to different health benefits, such as antioxidant, anti-inflammatory, antimicrobial and radical scavenging activities [5,6,7,8];

Actinidia arguta [5]

- SWE technique uses water at temperatures above the boiling point (100°C-374°C) to maintain its liquid state [9,10];
- Clean, cheap and easily available solvent [10];
- Green and sustainable extraction method [10];
- Excellent extraction efficiency in less time without associated toxicity [10].

Materials and Methods

❖**Extraction**

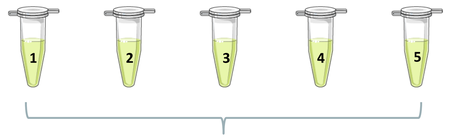
A. Arguta leaves dehydrated

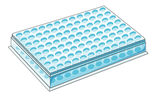
Subcritical Water Extraction
- Temperature: 110°C-160°C
- Pressure: 20 bars
- Time: 30 minutes

Lyophilization

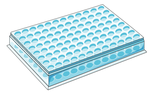
- **Condition 1: 110°C**
- **Condition 2: 123°C**
- **Condition 3: 135°C**
- **Condition 4: 148°C**
- **Condition 5: 160°C**

❖**Assays**

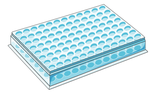




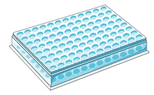
Total Polyphenolic Content (TPC)



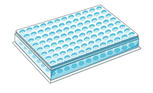
Total Flavonoids Content (TFC)



DPPH· radical scavenging activity (DPPH)



Superoxide ($O_2^{\cdot-}$) radical scavenging capacity



Hypochlorous acid (HOCl) radical scavenging capacity

Results/Discussion

Table 1: TPC and TFC results of *A. arguta* leaves extracts by SWE. Values are expressed as mean ± standard deviation (n = 3). Different letters in the same column mean significant differences (p<0.05) between samples.

A. arguta extracts	TPC (mg GAE/g dw)	TFC (mg CE/g dw)
110°C	106.48 ± 4.71^{a}	46.07 ± 4.11^{b}
123°C	109.72 ± 4.98^{a}	53.11 ± 4.52^{a}
135°C	68.78 ± 2.72^{c}	33.68 ± 3.38^{c}
148°C	72.92 ±1.18^{b,c}	32.69 ± 1.60^{c}
160°C	77.37 ± 3.01^{b}	32.72 ± 1.27^{c}

dw: dry weight; GAE: gallic acid equivalents; CE: catechin equivalents

- The highest TPC result was achieved for condition 1 (106.48 mg GAE/g dw) and 2 (109.72 mg GAE/g dw) without statistical differences among them (p>0.05);
- For TFC assay, the best result belongs to condition 2 (53.11 mg CE/g dw).

Table 2: DPPH free radical scavenging, $O_2^{\cdot-}$ and HOCl scavenging capacity results of *A. arguta* leaves extracts by SWE (n = 3). Different letters in the same column mean significant differences (p<0.05) between samples.

A. arguta extracts	IC_{50} (µg/mL)		
	DPPH·	HOCl	$O_2^{\cdot-}$
110°C	583.43 ± 17.02^{a,b}	18.61 ± 0.72^{b}	344.53 ± 23.09^{c}
123°C	497.13 ± 22.78^{b}	17.06 ± 0.92^{b}	335.23 ± 11.71^{c}
135°C	539.63 ± 23.17^{a,b}	20.56 ± 0.11^{b}	440.67 ± 2.51^{b}
148°C	625.60 ± 28.71^{a}	20.28 ± 1.41^{b}	473.07 ± 6.57^{b}
160°C	574.73 ± 11.28^{a,b}	26.93 ± 1.34^{a}	563.73 ± 24.13^{a}
Positive controls			
Trolox	30.57 ± 1.20^{c}	-	-
Gallic acid	-	11.06 ± 0.40^{c}	99.46 ± 2.12^{d}
Catechin	-	0.95 ± 0.03^{d}	137.67 ± 7.19^{d}

IC_{50} = *in vitro* concentration required to decrease in 50% the reactivity of the studied reactive species in the tested media

- In DPPH assay, the results ranged between 497.13 µg/mL and 625.60 µg/mL;
- For HOCl assay, no statistical diferences were found between condition 1, 2, 3 or 4. These results were better than the ones reported by Almeida *et al.* (2018) [7];
- For $O_2^{\cdot-}$ assay, condition 1 and 2 revealed better results than the others and no statistical differences between them were observed (p>0.05).

Conclusion

- *A. arguta* is a valuable source of bioactive compounds with recognized biological properties;
- SWE technique showed to be an effective extraction method to recover high-value compounds from *A. arguta*;
- The temperature of 123°C is the best condition according to the obtained results. Higher temperatures may lead to polyphenols degradation [11];
- Further assays should be performed to identify and quantify the bioactive compounds present in these extracts as well as toxicological studies to ensure their safety.

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Acknowledgements

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ANTIOXIDANT ACTIVITY OF A NEWLY DEVELOPED BIOPOLYMER-BASED COATING

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NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

Introduction

A green and sustainable economy requires that a revolution occurs in the use of raw materials. Ultimately it needs to substitute the overwhelming consumption of fossil fuel-based materials with processes and products obtained from plant-based and other renewable resources. Solar and wind power could likely drive the change in the energy supply sector, while the change will be driven within the manufacturing industry, by bio-based materials and their composites in different applications such as food packaging, textile fibrous materials, geotextile, plastics industry. Bio-based materials have gained attractiveness in the last decades due to both sustainable and economic concerns. Bio-based materials can be synthesized mainly by the production of polymers directly from naturally occurring compounds or the production of bio-based monomers and their subsequent (bio)chemical polymerization, through processes such as fermentation. Whatever the pathway for production of bio-based materials, the main problem is to control its composition and reproducibility.

Objectives

The main objective of this study it is to explore the antioxidant and antimicrobial activity of the spray solution to define its shelf-life.

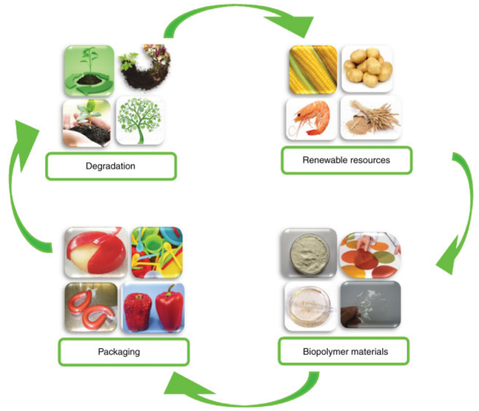
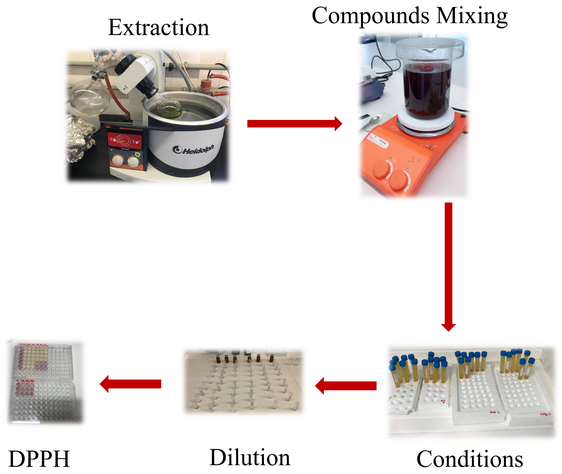


Fig. 1. Natural polymer cycle

Materials and Methods

The spray solution is composed of a natural biopolymer and a mixture of different natural antioxidants extracted from specific herbs.

The analysis was made by varying conditions, namely **% of ethanol** (80 and 100%), **storage time** (0, 7, 15 days, 1, 2, 6 and 12 months), **temperature** (room temperature and 3 ºC) and **exposure to light** (exposed and in the dark).



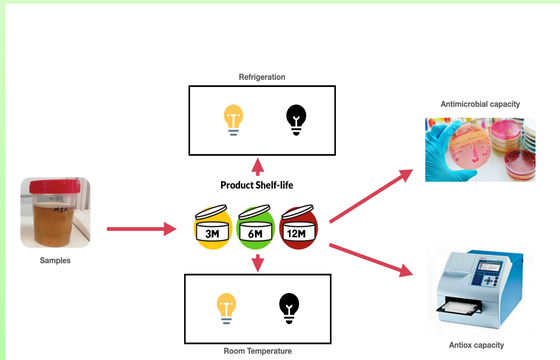


Fig. 2. Shelf-life analysis diagram

Conclusions

The work is currently underway, with promising results in terms of the maintenance of the antioxidant activity for at least 3 months. Definite results are expected to be published in the coming months

The application of biopolymers as alternatives to plastic and especially single use plastic is a trend that has been increasing around the world due to the the high pollution associated with plastic. Thus, the use of biodegradable, edible and sustainable biopolymers will be the future of food packaging.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to the CIMO (UIDB/00690/2020). L. Barros thanks the national funding by FCT – Foundation for Science and Technology, P.I., through the institutional scientific employment program-contract for her contract, and M. Carocho for his individual contract CEECIND/00831/2018.

INTESTINAL MORPHOLOGICAL CHANGES PRODUCED BY MALNUTRITION: EVALUATION OF A SUPPLEMENT BASED ON BURITI FLOUR AND MILK DERIVATIVES

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Abstract

Malnutrition induces a series of changes that affect several body parameters, including the integrity of the intestinal mucosa. Thus, we evaluated the effects of a supplement based on buriti flour and whey, on the recovery of the intestinal mucosa of malnourished Swiss mice, focusing on histological parameters. Two dietary supplements were used: one already commercialized and another, test supplement. There was a phase of induction of malnutrition and another of renutrition: control group, two isocaloric groups for control (commercial supplement and test), one malnourished group and two isolaloric groups for malnourished. It was observed, after renutrition, that the intestinal villi of the groups in the test supplement were thicker and higher. The nutritional quality of the ingredients of the test supplement, whey and buriti flour may have contributed to these results. Our work presents a new tested and effective supplement composition in the treatment of malnutrition.

Introduction

Malnutrition induces a series of changes that affect several body parameters [1], including the integrity of the intestinal mucosa [2]. Different studies show the benefits of dietary supplementation to improve malnutrition. In this sense, interest in the consumption of native fruits with functional activity and nutritional properties has increased, and research on these foods has become very important due to their contribution to health and the public economy. The fruits of the Cerrado region have been the subject of scientific research [1,3]. Among them, we highlight the buriti (Mauritia flexuosa L. f.), Mainly fruit flour. Buriti flour can be added to various food products, adding nutritional value as it is considered a potential source of dietary fiber, carotenoids and antioxidants [3]. In addition, there are by-products of the dairy industry, such as whey and its proteins, which have applications as functional, nutritional and therapeutic products[4]. Thus, the development of a powdered food supplement containing by-products from the Cerrado dairy and fruit industry can improve the health of people who are malnourished or at nutritional risk. In this context, the objective of the present study was to evaluate its effectiveness in recovering the intestinal mucosa of malnourished mice with a focus on histological parameters.

Materials

Two dietary supplements were tested: one already sold and the other, developed in partnership with the food engineering school at UFMG - Campus Montes Claros, MG, Brazil. The amounts of each ingredient in the developed supplement, referred to here as the test supplement, were determined to be close to the macro and micronutrient nutritional composition of a commercially available dietary supplement. The commercial supplement consisted of the following ingredients: skimmed-milk powder, maltodextrin, isolated protein from bovine whey, calcium caseinate obtained from bovine milk, milk fat, fructooligosaccharides, inulin, minerals, vitamins and soy lecithin emulsifier. The test supplement consisted of powdered whey, whole milk powder, buriti flour, maltodextrin, bovine whey protein isolate, minerals, vitamins and xanthan gum. For the in vivo experiment, 48 animals (male Swiss mice aged 15 weeks) were divided into 6 groups (n = 8 each). All procedures followed ethical standards.

Methodology

Malnutrition protocol and renutrition diets: after the adaptation period, the animals were submitted to two treatment phases: 20% food restriction in relation to the control group, in order to induce malnutrition and the 30-day renutrition phase. The groups with their respective nutrition diets were: Control-ST (Standard Chow); Isocaloric Commercial Supplement to Control - CS-ST (Standard Chow + Commercial Supplement); Isocaloric test supplement to the S-ST control (Standard Chow + Test Supplement); FR 20% malnourished (standard food); Commercial isocaloric supplement to the malnourished CS-FR20% (Standard Chow + Commercial Supplement); and Isocaloric Test Supplement to malnourished S - FR20% (Standard Chow + Test Supplement).

Morphology of the small intestine: fragments of the intestinal mucosa were collected for microscopic evaluation. The materials were fixed in neutral buffered formalin and included in paraffin, sectioned at 5 µm and later stained with Hematoxylin and Eosin (H&E). The morphometry included the height of the villi, the depth of the crypt and the total thickness of the intestinal mucosa. All images were captured in an Evolution LC optical microscope with a color photographic camera (Media Cybernetics®, USA).

Results

Our results indicate that the new supplement recovered damage to the intestinal mucosa caused by malnutrition in animal models, presenting results similar or superior to the commercial reference supplement in the market. It was observed, after renutrition, that the intestinal villi of the groups in the test supplement were thicker and higher (Figure 1). It is important to highlight that the nutritional composition of the two supplements was almost similar in macro and micronutrients. The nutritional quality of the ingredients of the test supplement, whey and buriti flour may have contributed to these results. Buriti (Mauritia flexuosa L. f.) it is a species native to South America. The fruits are rich in vitamins A, C and E, antioxidants, unsaturated oils and dietary fibers. Dehydration of the pulp after pressing gives rise to buriti flour, one of the main ingredients of the test supplement. The fiber content of buriti flour stimulates intestinal fermentation and the production of short-chain fatty acids, with beneficial effects on this organ [3]. This fact corroborates and reinforces some of our findings related to the recovery of intestinal structure. The dairy by-products added to the test supplement may also have contributed to these results. The high bioavailability of whey proteins and the higher leucine content stimulate protein synthesis and renewal [4].

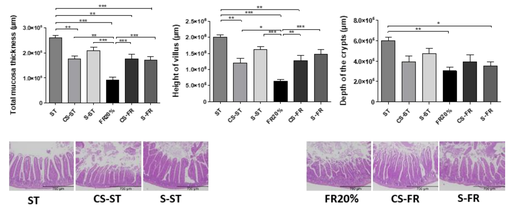

Fig. 1 Intestinal villi of mice submitted to different diets: ST (Standard Chow), CS-ST (Standard Chow + Commercial Supplement), S-ST (Standard Chow + Test Supplement), FR20% (Standard Chow - 20% food restriction), CS-FR (Standard Chow + Commercial Supplement) and S-FR (Standard Chow + Test Supplement). Data presented as mean ± SEM; * p <0.05, * p <0.01; ** p <0.001 versus indicated group

Conclusion

In conclusion, we show in the present study that dietary supplementation was successful in recovering damage to the intestinal mucosa related to malnutrition, in an animal model, presenting similar or superior results compared to the commercial reference supplement in the market. Our work presents a new composition of food supplement based on buriti flour and milk derivatives, tested and proven to be effective in the treatment of malnutrition.

Recommendations

It is recommended to deepen the studies related to the intestinal mucosa of the animals: histopathological evaluations, RT-qPCR Analysis, among others. The new supplement can be tested alone or in combination with fruits typical of each region. It can also be adapted and tested for use in different life cycles, such as adducts, pregnant women, nursing mothers, childhood, among others.

Acknowledgements

This work was supported by the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico (CNPQ) and Fundacão de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

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Bixa orellana L. pods and seeds: nutritional and chemical characterization, bioactivity studies, and development of a carotenoid-based food colorant

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Introduction

Bixa orellana L. is worldwide known as a source of bixin, a carotenoid compound with high colorant capacity [1]. The growing tendency in the food industry to use new and safer colorant compounds with higher stability coupled to the high demand of consumers concern, became of the upmost importance. Moreover, sustainable extraction technologies coupled with greener solvents are increasingly required to maximize the recovery of high added value compounds [2].

Methodology

The present work intended to deepen the study of the nutritional and chemical profile and bioactive potential of *B. orellana* seeds. Furthermore, to maximize the extraction of bixin from seeds ultrasound-assisted technologies combined with RSM were used. As an externality of this work, the pods of this plant (bio-residues resulting from the processing of seeds) were also studied, in relation to its bioactive properties and profile in phenolic compounds.

RAPID, NON-THERMAL AND EFFICIENT EXTRACTION

AGING OF THE ULTRASONIC PROBE SURFACE CAN CHANGE THE EXTRACTION EFFICIENCY

RSM

ULTRASOUND-ASSISTED EXTRACTION (UAE)

Results

The most abundant macronutrients found in seeds were carbohydrates, followed by fat, proteins, and ash. Sucrose and trehalose were the only sugars found; and malic acid and α-tocopherol were the main organic acid and tocopherols present, respectively. Monounsaturated fatty acids (eicosenoic acid) were found in higher amounts. Pods hydroethanolic extract presented lower IC50 values for antioxidant activity; while seeds sample revealed lower IC50 values for Δt = 60 min and Δt = 120 min for OxHLIA assay, and lower GI50 values for cytotoxic and hepatotoxic assays. Both samples presented lower MIC, MBC, and MFC in comparison to the two positive controls, against all bacterial and fungal strains. Pods presented the highest amounts of phenolic compounds (due to protocatechuic acid).

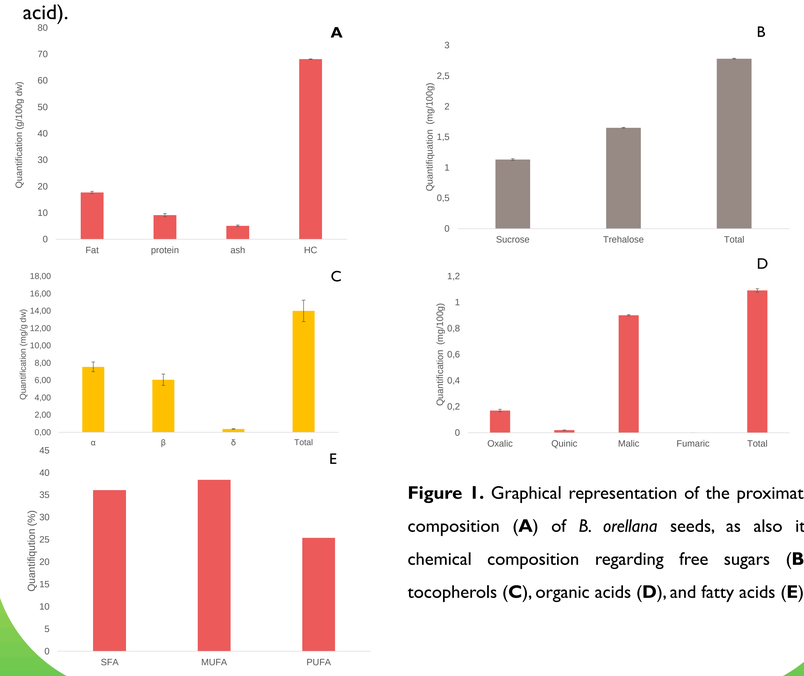

Figure 1. Graphical representation of the proximate composition (**A**) of *B. orellana* seeds, as also its chemical composition regarding free sugars (**B**), tocopherols (**C**), organic acids (**D**), and fatty acids (**E**).

Results

The model developed for the extraction process was validated, with optimal processing conditions (sonication - 348 W, 6 min, 79 % (*v*/*v*) ethanol), being possible to obtain 27.1 mg of bixin per g of extract (**Figures 2** and **3**).

Increasing the ethanol proportion, increases the amount of carotenoids extracted

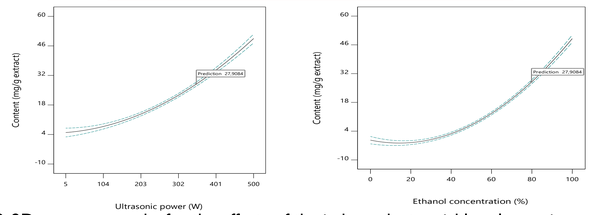

Figure 2. 2D response graphs for the effects of the independent variables ultrasonic power and ethanol concentration on the carotenoids content (mg/g extract) extracted from achiote. In each graph, the excluded variables were fixed at their optimal value.

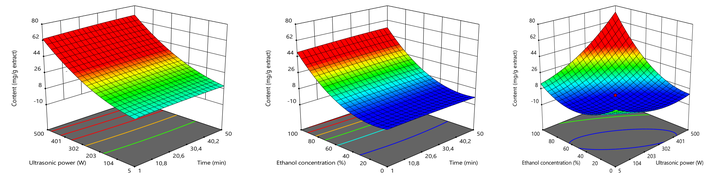

Figure 3. Response surface graphs for the combined effects of the independent variables on the carotenoids content (mg/g extract) obtained from B. Orellana. In each graph, the excluded variable was positioned at its optimal value.

Conclusion

Overall, this study allowed to present innovative results in relation to the nutritional, chemical and bioactive properties of the seeds, and as an externality the great potential of the pods regarding its biological activity. Considering the good results obtained in the optimization procedure, it is worth mentioning the sustainable way in which this extract can be obtained.

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Acknowledgments

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PRODUCTION OF NATIVE TREE PLANTS FOR FOOD, RESTORATION AND CONSERVATION PURPOSES

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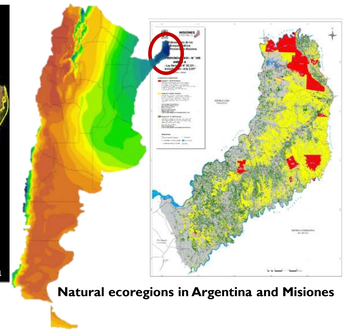
Abstract

The Selva Paranaense in the province of Misiones, contains the greatest biodiversity for Argentina. Strategies for restoration of degraded forest areas, recommends the inclusion of species for food purposes. Native species of arboreal habit of the Myrtaceae family as: *Eugenia involucrata, Eugenia uniflora*, *Eugenia myrcianthes*, *Eugenia pyriformis*, *Plinia peruviana*, *Plinia rivularis* and *Myrcianthes pungens*, are considered for plant production in nurseries. Trees are previously registered, the ripe fruits are harvested, and the fresh seeds are sown in containers. If stored, the seeds should be placed in wet sand and under cold temperature. Field planting requires moist soils and shady environments. The payment for the products as seeds, fruits and plants as a regional and natural product, allows to recognize the conservation effort of private owners.

Key Words: edible fuits, non-timber, organic certification

Introduction

The Upper Parana Atlantic Forest, a hotspot of highly threatened biodiversity, extends to northwestern in Argentina in the province of Misiones is known as the Selva Misionera or Selva Paranaense. National and provincial laws establish and promote the restoration of degraded forest areas, mainly linked to protective forests of natural water sources. This is a crucial process necessary to the establishment of biodiversity islands, which requires plant propagation material of native species to ensure their viability and diversity. As part of our previous work on restoration of degraded lands with native tree species that can be useful for productive purposes, the local diversity contains more than 100 species of edible fruits, whose attributes or properties are just being known. These species are actually of interest, but their plants are not available in the nurseries, if required for plantations. This proposal describes the required conditions for the production of plants in nurseries, for a group of known tree native species of edible fruits.



Natural ecoregions in Argentina and Misiones

Materials

Mother trees, fruits and seeds from native species of arboreal habit of the Myrtaceae family as: *Eugenia involucrata, Eugenia uniflora*, *Eugenia myrcianthes*, *Eugenia pyriformis*, *Plinia peruviana*, *Plinia rivularis*, *Myrcianthes pungens,* considered locally among the best known, due to its delicious edible fruits. Several of them are already recognized by the food code and considered in the gourmet dishes of regional cousine.

Methodology

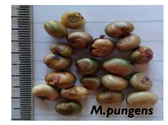
In order to provide seeds for the nursery and production of plants, trees are previously registered in every landscape where they are naturally present, whether they are part of forests, riverine strips or growing in fields. The harvest should be done when the fruits are ripe on the tree or in the ground at the time of dispersal. The immediate separation of the seed from the pulp must be carried out and can be used for the production of juices, liqueurs, vinegars and frozen pulp.

Results

The seeds, due to their recalcitrance, must be sown directly in the nurseries, for the production of plants. If is necessary to be stored, the humidity should be generally maintained above 35%, and they should be preserved in moist sand under cold temperatures (4 to 8°C), conditions to which the seeds can survive for more than one year. The sowing is done directly in containers with composted pine bark substrate and slow release fertilizer and after 12 to 24 months (depending on the species) the plants are ready to be planted in the field, preferably in humid places and medium shade. The economic valuation of the goods and services produced by the native forest includes fruits for pulp and seeds for their propagation in nurseries. Being an incipient market to develop it is suggested, to apply the methodology of the cost - price ratio, as a way to cover the costs incurred and reasonable for the market which includes the cost of conservation.

Conclusion

Trees registered in natural areas provide fruits and seeds. Fruits and seeds are considered an economic good.
The availability of plants in the nurseries, facilitate the implementation of restauration / production programs.
The payment for a natural product at the price that the producer defines, allows to recognize the conservation effort to maintain the remnants o natural forests.



Recommendations

It is important to garantee the availability of plants, for a non-timber multiple-use purpose, as: medicine, ornamental, landscape, shade, carbon fixation, restoration, conservation, honey, food.
It is necessary to register trees for the harvest of fruits in the different regions of their natural distribution.
Generate protocols for plant production of more native species of edible fruits. Learn about the use of native species for environmental restoration.
Include conservation costs in final price of natural products.
Work for knowledge through communication in Botanic Gardens about the productive use of native species.
Include more species in the food code.

Acknowledgements

Experiences carried out in the seed laboratory and nurserie of the Forestry School, of the University National of Misiones. Private native plants nurseries and the Mesopotamia Node of the Botanic Garden in Argentina, RAJB.

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Phenolics compounds from Amaranthaceae family: extraction and biological properties

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Abstract

Species of the Amaranthaceae family have become a potential group of plants for their latent beneficial properties. Their use in traditional medicine and potential biological properties can be considered as the basis to guide further investigations about their characteristics and beneficial uses. In this work, three species of the Amaranthaceae family traditional from China (*Alternanthera sessilis*, *Dicliptera chinensis* and *Dysphania ambrosioides*) were proposed as an alternative source of bioactive compounds, namely phenolic compounds (Adegbola et al., 2020).

The study was aimed to extract and characterize the phenolic compounds of the three species and search for possible antitumor, antimicrobial, antioxidant and anti-inflammatory activities. For this purpose, the antioxidant activity was assessed by two *in vitro* assays: TBARS (thiobarbituric acid reactive species) and OxHLIA (oxidative hemolysis inhibition). In the case of antimicrobial activity, it was tested against Gram (-), Gram (+) pathogenic bacteria. The antitumor properties were assessed on *in vitro* studies to assess the inhibition of the growth of several tumor cell lines. The anti-inflammatory activity was evaluated on Raw 264.7 (Mouse lipopolysaccharide (LPS)-stimulated macrophage-like cell line). Thus, Amaranthaceae family could be an alternative source of bioactive compounds to formulate new innovative products and incorporate them into the food, cosmetic and pharmaceutical industry.

Alternanthera sessilis

Dicliptera chinensis

Dysphania ambrosioides

Introduction

The search of new bioactive compounds to produce drugs is a necessity promoted by the increasement of resistant bacteria, new viruses and numerous pathologies without treatment. The production of drugs produced, in 2016, 250 billion euros, data that situates the pharmacological industry in one of the most rentable markets. One of the most used sources of bioactive compounds are the natural products. Since 1981 was approved 1881 new drugs of which the 25% came from natural sources and the 41.9 % came from no synthetic sources(McKerrow, 2015) (Figure 1).

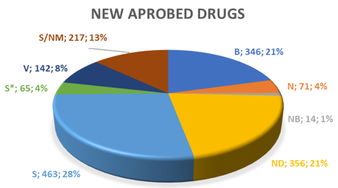
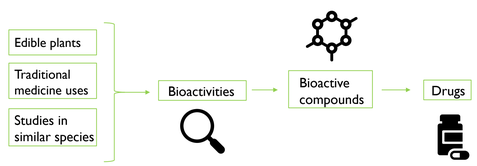


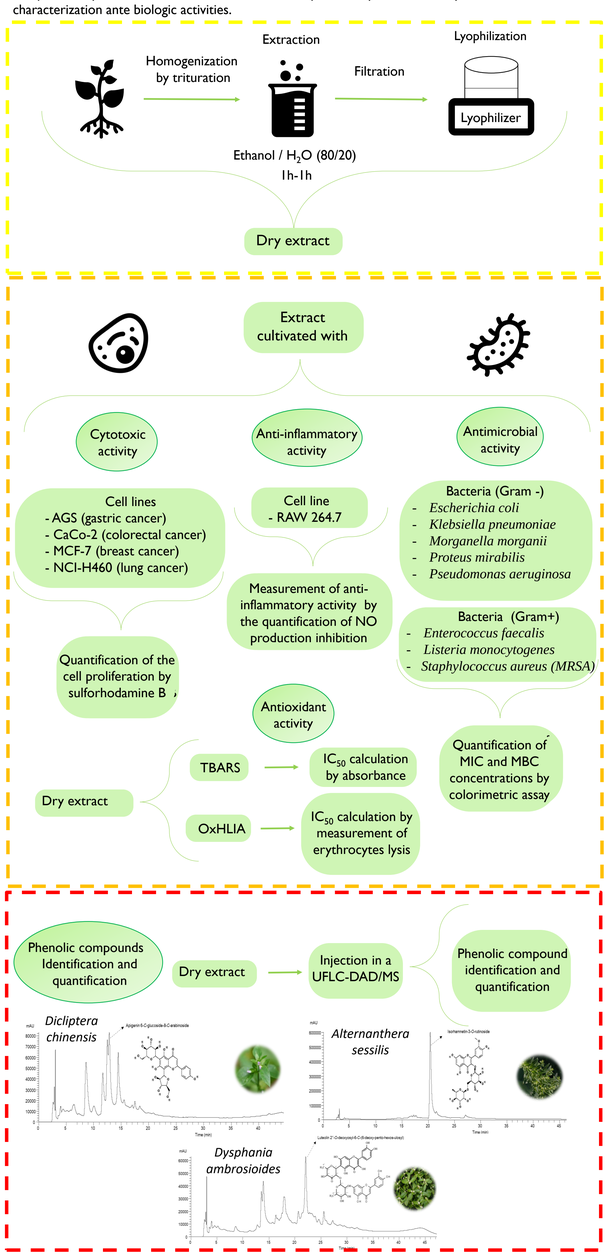
Figure 1. Drugs approved since 1981 (n = 1881) classified by their source. B: biological macromolecule, N: unaltered natural product, NB: botanical drug, ND: natural product derivative, S:synthetic drug, S*: Synthetic drug (NP pharmacophore), V: vaccine, S/NM: mimic of natural producto.

One of the biggest sources of natural drugs is the secondary metabolites of the plants. Inside this big group, phenolic compounds are the most important for their bioactivities, diversity and abundance. The three species under study have been present in the diets of Asian and middle East countries. Moreover, these plants have been used in traditional medicine for treat different pathologies an illness (skin diseases, ocular diseases, wound healing, animal bites etc.). This use could be a clue of the presence of bioactive compounds. Therefore, this work consisted in the identification of the phenolic compounds present in the species and their bioactivities.



Methodology

This work can be separated in three sections. First of all, was made the extraction of the phenolic compounds by maceration. The result of this step is a dry extract ready to continue with the characterization ante biologic activities.



Results

The data obtained showed interesting results in the antioxidant (Table 1), cytotoxic (Table 3) and antimicrobial activity (Table 3). Moreover, in table 4 is represented all the compounds identified and their concentration in the samples.

Table 1. Antioxidant activity results expressed as EC_{50} of the antioxidant response (μg/mL)

Antioxidative activity	TBARS	OxHLIA
Dicliptera chinensis	458±15	109±7
Alternanthera sessilis	138±20	109±9
Dysphania ambrosioides	66±15	104±7

Table 2. The cytotoxic and the anti-inflammatory results expressed as the GI_{50} of the different cell lines tested and IC_{50} respectively

Cytotoxic and anti-inflammatory activity	AGS	CaCo	MCF-7	NCI-H460	RAW
Dicliptera chinensis	>400	>400	>400	>400	>400
Alternanthera sessilis	>400	>400	>400	>400	>400
Dysphania ambrosioides	>400	188±14	245±13	263±12	>400

Table 3. The antimicrobial activity expressed in MIC and MBC (mg/mL) of the different microorganism tested

Antimicrobial activity	*Dicliptera chinensis*		*Alternanthera sessilis*		*Dysphania ambrosioides*	
	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria						
Escherichia coli	20	>20	10	>20	10	>20
Klebsiella pneumoniae	>20	>20	20	>20	>20	>20
Morganella morganii	10	>20	5	>20	5	>20
Proteus mirabilis	>20	>20	>20	>20	>20	>20
Pseudomonas aeruginosa	>20	>20	>20	>20	>20	>20
Gram-positive bacteria						
Enterococcus faecalis	10	>20	20	>20	10	>20
Listeria monocytogenes	10	>20	>20	>20	10	>20
MRSA	10	>20	5	>20	10	>20

Table 4. Tentative identification of the phenolic profile and their quantification (mg/g of dry extract)

Dicliptera chinensis		*Dysphania ambrosioides*	
Tentative identification	Concentration	Tentative identification	Concentration
3-*p*-Coumarouylquinic acid	1.2±0.1	5-Hydroxy-3,4′ 7 trimethoxy-flavone	0.93±0.01
Sulfo-caffeic acid	1.18±0.05	Eriodictyol-*O*-glucuronide	0.0005±0.00004
Apigenin-6,8-di-*C*-glucoside (vicenin-2)	2±0.2	Isorhamnetin-3-*O*-neohesperidoside	1.14±0.01
Luteolin-6-*C*-hexosyl-8-*C*-pentosyl	0.606±0.004	Kaempferol dirhamnoside-*O*-hexoside	0.98±0.06
Apigenin-6-*C*-xyloside-8-*C*-glucoside	1.3±0.1	Quercetin-3-*O*-rutinoside	0.721±0.002
Apigenin 2″-*O*-xyloside-8-*C*-hexoside	1.9±0.1	Lignan-*O*-coumaroylglucoside	0.337±0.005
Apigenin 6-*C*-glucoside-8-*C*-arabinoside (Schaftoside)	2.41±0.04	Quercetin-*O*-rhamnosyl-pentoside	0.888±0.001
Apigenin-6-*C*-glucoside-8-*C*-arabinoside	2.0±0.1	Kaempferol-*O*- rhamnosyl-*O*-pentoside	0.84±0.01
Apigenin 6-*C*-pentosyl-8-*C*-hexoside	0.36±0.01	Isorhamnetin-3-*O*-neohesperidoside	0.680±0.003
Apigenin-6-*C*-hexoside-8-*C*-rhamnoside	0.33±0.02	Luteolin-7-*O*-Rhamnosyl(1→2)Hexoside	0.68±0.01
Apigenin-6-*C*-arabinoside-8-*C*-glucoside	0.29±0.02	Isorhamnetin-3-*O*-rutinoside	5.76±0.04
Apigenin-6-*C*-glucoside-8-*C*-xyloside	0.09±0.01	Kaempferol-*O*-rhamnosyl-*O*-pentoside	0.587±0.002
		Kaempferol-*O*-hexose-*O*-gallic acid	0.707±0.003
		Acetylated luteolin pentosyl-rhamnoside	0.82±0.03
Alternanthera sessilis			
Tentative identification	Concentration	Tentative identification	Concentration
p-Coumaroyl pentoside acid	0.34±0.01	Kaempferol-*O*-rhamnoside-*O*-hexoside	0.78±0.02
Caffeic acid acetylhexoside	0.75±0.04	Apigenin 8-*C*-rhamnoside-6-*C*-glucoside	0.24±0.03
Kaempferol-*O*-rhamnoside-*O*-hexoside	0.94±0.02	Luteolin 7-*O*-neohesperoside	0.7±0.1
Luteolin-6-*C*-glucoside-7-*O*-glucoside	0.31±0.03	Luteolin-*O*-rutinoside	0.75±0.02
Quercetin-3-*O*-glucosyl-pentoside-7-*O*-glucuronide	0.54±0.01	Luteolin 2″-*O*-deoxyosyl-6-*C*-(6-deoxy-pentohexoside-ulosyl)	0.70±0.04
Dihydroxyl methyl quercetin-chalcone	0.57±0.01	Methyl-luteolin 2″-*O*- deoxyhexosyl-6-*C*-hexoside	0.8±0.1
Luteolin 2″-*O*-deoxyhexosyl-6-*C*-glucoside	0.60±0.04	Luteolin-8-*C*-(rhamnosyl)ketodeoxihexoside	2.02±0.05
Luteolin-6-*C*-glucoside	1.6±0.1	Luteolin-8-*C*-(rhamnosyl)ketodeoxihexoside	0.29±0.01
Luteolin 2″-*O*-deoxyhexosyl-*C*-pentoside	0.247±0.004	Luteolin-*O*-deoxyosyl-*C*-deoxy-pento-hexosulosyl	1.0±0.1
Quercetin-*O*-rutinoside	0.56±0.01	Luteolin-*O*-deoxyosyl-*C*-deoxy-pento-hexos-ulosyl	0.916±0.003
Apigenin-6-*C*-glucoside	0.17±0.02	Apigenin 6-*C*-glucoside-2″-*O*-rhamnoside	0.42±0.02
Chrysoeriol-8-*C*-(2-rhamnosyl)hexoside	0.0059±0.0002	Apigenin 4′-*O*-hexoside-*D*-deoxyhexoside	0.91±0.02

Conclusion

The results showed significant results in antimicrobial, cytotoxic and antioxidant activity.

The antimicrobial activity is not strong enough to think in a father investigation in terms of search a possible antibiotic in this species. Nevertheless, the MIC and the antioxidant activity of *Dicliptera chinensis* and *Alternanthera sessilis* are interesting to use these extracts as a possible natural food preservative. Both activities could increase the shelf life of the food thanks to the inhibition of oxidation and microbial growth.

Dysphania ambrosioides present a low IC_{50} against CaCo, MCF-7 and NCI-H460 cell lines. This activity could be useful for find new bioactive compounds for new treatments against different cancers.

Further investigations are needed to complete this study and found what are the compounds in this species that give to this species their biologic activities.

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Acknowledgements

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WINTER SAVORY (*SATUREJA MONTANA*) ESSENTIAL OIL AS A NATURAL ANTIMICROBIAL FOR MEET PRESERVATION

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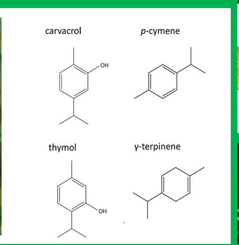
Abstract

Microbial contamination is considered one of the most important causes of food spoilage. In order to control pathogens, numerous preservatives and procedures are used. However, due to possible side effects of the artificial food additives, the natural ones, including essential oils (EOs), are enthusiastically accepted. In addition, marinating process that is commonly used in food preparation could also contribute to better conservation. Assessing different foods, fresh meat could be considered as an extremelly subjected to microbial contamination. Among its contaminants, *Listeria monocytogenes*, a well-known foodborne pathogen that causes listeriosis, is listed as one of dominant. Taking into account all abovementioned, the aim of this study was to chemically characterized and examine antibacterial effect of winter savory (*Satureja montana*) EO, both *in vitro* and *in situ* in wine-marinated beef meat. *In vitro* antibacterial effect was screened in microdilution and time kill assays, both performed on *L. monocytogenes* strains (reference ATCC 19111 and three isolates originated from meat industry: LMB, LMS and LMT). *In situ* analysis involved monitoring of antilisterial effect, as well as activity against meat spoilage bacteria: aerobic heterotrophic mesophyll bacteria (AHMB), Enterobacteriaceae (ENT) and lactic acid bacteria (LAB).
GC-MS analysis revealed carvacrol (30.7%) and thymol (18.0%) as the most abundant among identified constituents. In microdilution assay EO induced antilisterial effect against all tested strains, with MIC values determined at 0.5 % for referent strain and 1 % for isolates, and MBC values determined at 1 % for all the strains. *In vitro* time kill assay was performed on selected listerial strians (ATCC 19111 and LMB) and pointed out the dynamics of growth inhibition. Additinally, it enabled us to estimate curve MIC values, which indicated a higher bacterial sensitivity under aerobic than hypoxic conditions. Considering in situ analysis, red wine-marination of a beef reduced the growth of previously inoculated listeria (ATCC 19111 and LMB isolate), as well as pre-existing meat spoiling contaminants AHMB, ENT and LAB. Furthermore, addition of winter savory EO notably enhanced antimicrobial effect of marinade.
In conclusion, obtained results suggest possible application of winter savory EO in the form of a natural preservative in beef and recommend further research directed to other foodstuffs.

Introduction

Since ancient times, herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. The concept of food that combines nutritional and medicinal benefits is especially popular today. Winter savory (*Satureja montana* L.) is well-known as a medicinal and aromatic plant which is used as a spice, tea and food additive. Due to presence of phenolic compounds, *S. montana* is known to posses a few pharmacological activities such as antimicrobial, antiviral and antioxidative.
Almost all groups of microorganisms can contribute to spoilage of food under certain conditions. The psychrotolerant behavior and relatively high resistance to various environmental factors increases the incdence of *Listeria monocytogenes* in food and consequently it is consider as a major foodborne pathogen. Thus, there are different food preservation techniques and one of the most common, effective and cheap process for preserving food is marination. Taking into account the antimicrobial potentional of marinade constituents, this process could be applied as an efficient way to control *L. monocytogenes*. Furthermore, the addition of *S.montana* essential oil (EO) into marinades could enhance the biocontrol of microbial contamination of meat, including *L. monocytogenes*.
The aim of this study was to determine chemical composition of *S. montana* EO, as well as to monitor its antilisterial effect both *in vitro*, and *in situ* in red wine marinated beef. In addition, *in situ* screening of antibacterial potential included inhibitory potential of marinades against meat spoilage bacteria.



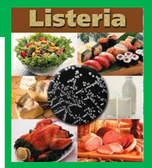


Table 1. MIC and MBC values of EO determined in microdilution assay

Satureja montana	MIC assay		Time kill assay
Listeria monocytogenes strains	MIC [%]	MBC [%]	cMIC [%]
ATCC 19111	0.5	1	0.03
LMB (beef carcass isolate)	1	1	0.04
LMS (salmon isolate)	1	1	
LMT (tunnel slaughterhouse isolate)	1	1	

Concentration 1 % corresponds to 8.6 mg/mL of *S.montana* EO

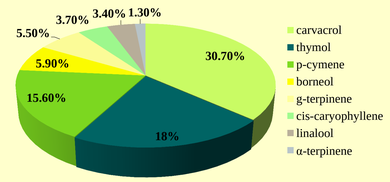


Figure 1. Chemical composition of *S. montana* EO

Table 2. Effect of *S. montana* EO against ATCC 19111 strain in time-kill assay *in vitro*

Population (log CFU/mL)	sub-I(S)$_{0.25}$	sub-I(S)$_{0.375}$	sub-I(S)$_{0.5}$	sub-I(S)$_{0.75}$	I(S)
h	**Control**	**0.031**	**0.050**	**0.062**	**0.094**	**0.125**
0	5.11±0.29	4.81±0.20	4.91±0.23	4.95±0.24	4.31±0.24	4.77±0.23
4	6.43±0.29	4.46±0.26^C	4.28±0.19^C	3.83±0.21^C	3.78±0.18^C	4.83±0.18^C
8	7.35±0.29	4.40±0.26^C	4.01±0.27^C	2.60±0.16^C	2.48±0.20^C	2.68±0.15^C
12	8.66±0.35	5.08±0.10^C	4.16±0.34^C	2.18±0.19^C	2.10±0.15^C	1.41±0.13^C
24	9.19±0.31	8.19±0.23^C	5.61±0.21^C	2.07±0.21^C	1.88±0.13^C	<1
28	9.40±0.26	9.16±0.24	6.36±0.23^C	3.15±0.21^C	<1	<1
32	9.33±0.35	9.42±0.26	6.23±0.24^C	3.66±0.27^C	<1	<1

Item denoted with capital letter C are significantly different from control (p<0.05)
<1 – obtained results were below the level of detection
I – inhibitory concentration
sub-I: subinhibitory concentration (subscript number indicates factor of reduction)

Table 3. Effect of *S. montana* EO against LMB strain in time-kill assay *in vitro*

Population (log CFU/mL)	sub-I(S)$_{0.25}$	sub-I(S)$_{0.375}$	sub-I(S)$_{0.5}$	sub-I(S)$_{0.75}$	I(S)
h	**Control**	**0.031**	**0.050**	**0.062**	**0.094**	**0.125**
0	4.42±0.17	4.83±0.27^C	4.67±0.22^C	4.50±0.25	4.37±0.24	4.46±0.23
4	5.54±0.28	3.99±0.22^C	4.12±0.20^C	4.06±0.27^C	3.97±0.23^C	4.21±0.15^C
8	6.26±0.24	5.27±0.21^C	3.96±0.21^C	3.19±0.16^C	2.97±0.23^C	2.18±0.18^C
12	8.79±0.32	6.19±0.33^C	3.98±0.20^C	3.04±0.24^C	2.65±0.25^C	1.18±0.12^C
24	9.26±0.24	8.37±0.23^C	4.48±0.26^C	2.34±0.19^C	2.20±0.15^C	<1
28	9.29±0.31	9.19±0.29	4.13±0.29^C	2.65±0.18^C	2.15±0.16^C	<1
32	9.15±0.36	9.09±0.30	4.34±0.27^C	2.14±0.20^C	2.05±0.12^C	<1

Item denoted with capital letter C are significantly different from control (p<0.05)
<1 – obtained results were below the level of detection
I – inhibitory concentration
sub-I: subinhibitory concentration (subscript number indicates factor of reduction)

Results

GC-MS analysis showed that *S. montana* EO contained dominantly oxygenated monoterpenes (61.2 %) with carvacrol (30.7%), thymol (18.0%) and borneol (5.9 %) as the most abundant constituents. In the case of monoterpene hydrocarbons (29.1 %), *p*-cymene and γ-terpinene (5.5 %) were the most dominant components (Figure 1). Obtained MIC and MBC values indicated the moderate antilisterial effect: MIC values of EO were pointed at 1 % in case of isolates and 0.5 % in case of referent strain, while the MBC values were 1 % for all tested strains (Table 1). Isolate LMB and referent strain were selected for further research, and *in vitro* time-kill assay performed on them showed strong inhibitory potential (Tables 2,3). Moreover, the results obtained indicated higher bacterial sensitivity in the time-kill than in the microdilution assay. This result could be ascribed to different experimental conditions: hypoxia in the case of microdilution assay and high partial presssure of oxygen in the case of time-kill assay. In order to quantify different sensibility of bacteria, time-kill curve analyses was performed and cMIC values were estimated (Figure 2). The obtained cMIC values were considerably lower than the MIC values determined in the microdilution assay (cMIC for ATCC 19111 was estimated at 0.03 % and 0.04 % in the case of LMB, Table 1). Obtained results of sensory evaluation showed that samples marinated with EO concentrations 0.0625 % and 0.125 % were sensory acceptable, while the highest tested (0.25 %) was sensory unacceptable (Figure 3). *In situ* time-kill assay was performed on marinated beef using the sensory acceptable concentration (0.125 %). The monitored strains/groups were: inoculated ATCC19111 or LMB strain, and pre-existing meat spoilage contaminants (AHMB, ENT and LAB) Obtained results indicated that the basic red wine marinade, and especially the marinades containing EO, remarkably decreased the counts of all monitored bacteria comparing to saline control (Figures 5-8). For all monitored targets, the bactericidal effect during marination was followed by bacteriostatic effect during subsequent meat storage.

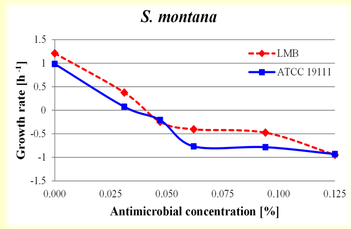


Figure 2. Growth rate curve in function of concentration of *S. montana* EO

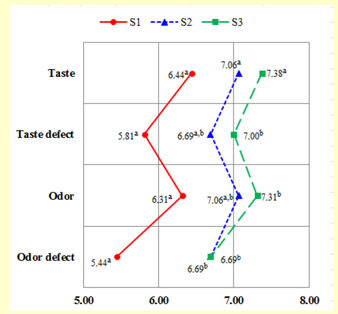


Figure 3. Sensory evaluation of beef marinated with red wine containing *S.montana* EO. S1- 0.0625 %; S2-0.125 %; S3-0.25 % Mean values within tha same row with different superscripts are significantly different (p<0.05)

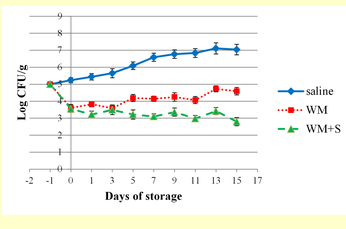


Figure 4. Growth inhibition of *L. monocytogenes* ATCC 19111 strain in marinated beef.
Saline – negative control; WM – basic wine marinade; WM+S – marinade containing 0.125 % of EO

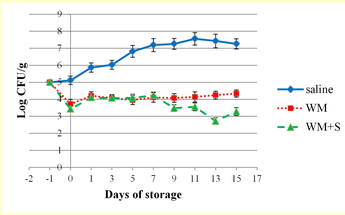


Figure 5. Growth inhibition of *L. monocytogenes* LMB strain in marinated beef.
Saline – negative control; WM – basic wine marinade; WM+S – marinade containing 0.125 % of EO

Figure 6. Growth inhibition of AHMB in marinated beef.
Saline – negative control; WM – basic wine marinade; WM+S – marinade containing 0.125 % of EO

Figure 7. Growth inhibition of Enterobacteriaceae in marinated beef.
Saline – negative control; WM – basic wine marinade; WM+S – marinade containing 0.125 % of EO

Figure 8. Growth inhibition of LAB in marinated beef.
Saline – negative control; WM – basic wine marinade; WM+S – marinade containing 0.125 % of EO

Methodology

Commercially provided *S. montana* EO was analyzed by gas chromatography/mass spectrometry (GC-MS). Antibacterial effect of EO was determined in microdilution assay, performed on *L. monocytogenes* strains: referent ATCC 19111 strain, and isolates from beef carcass (LMB), salmon (LMS) and from slaughterhouse water drainage tunnel (LMT). Both minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations have been assigned. In order to monitor the effect on dynamics of growth inhibition against selected listerial strains (ATCC 19111 and LMB), time kill assay was used. The same test was performed to calculate the curve MIC values (cMIC). *In situ* antibacterial effect of *S. montana* EO on red wine marinated beef was investigated in time kill assay, too, and the targeted microorganisms were: inoculated listerial strains ATCC 19111 and LMB, as well as groups of meat spoilage bacteria, i.e. aerobic heterotrophic mesophilic bacteria (AHMB), Enterobacteriaceae (ENT) and lactic acid bacteria (LAB). In order to measure the taste and odor difference between marinated beef samples, sensory evaluation was determined.

Conclusion

Taken together, obtained results indicate potential of *Satureja montana* EO to control bacterial growth of meet spoilage bacteria, especially of *L. monocytogenes*. It could receive particular attention as a potential natural agent for food preservation.

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia; grant number 451-03-68/2020-14/200178. We thank Dr Branko Velebit, Institute of Meat Hygiene and Technology for obtained *Listeria monocytogenes* isolates.

ANANAS COMOSUS L. BIO-WASTE AS A SOURCE OF BIOACTIVE COMPOUNDS WITH HEALTH BENEFITS

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Abstract

Annually, tons of bio-waste are wasted by the food industry, causing environmental and economic concerns. This concern has prompted studies that aim at the valorisation and exploitation of bio-waste with a view to their exploitation. In this sense, this work focuses on circular economy and waste recovery, aiming at the characterization of pineapple bio-residues and the study of its potential for industrial application as a natural ingredient.

Introduction

Pineapple (*Ananas comosus* L.) is a fruit appreciated and consumed worldwide not only because it is recognized for nutritional properties, but also for the beneficial characteristics that help in the development of the organism [1]. Although only the pulp is consumed, several studies have been exploring different parts of the fruit, as they have high amounts of bioactive compounds of interest. Thus, and since the food industry annually produces tons of waste that are not properly used [2], this work aimed at the characterization of the pineapple peel and crown in order to enhance this bio-waste and a circular bioeconomy.

Materials

After receiving the bio-waste, it was frozen and lyophilized. The lyophilized samples were crushed and reduced to a fine powder that was stored protected from light and moisture (Fig. 1).

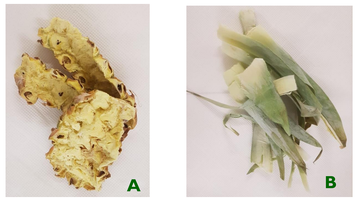

Fig.1 –Pineapple crown (A) and peel (B) samples.

Methodology

Heat-assisted hydroethanolic extraction was used to recover compounds subsequently identified and quantified by High-Performance Liquid Chromatography coupled with a diode array detector and electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS). Regarding bioactivities, the following activities were evaluated: antioxidant, anti-inflammatory activity and cytotoxicity (Fig. 2). The antioxidant activity of the extracts was tested and proved through two *in vitro* tests: the lipid peroxidation inhibition test (TBARS) and the oxidative hemolysis inhibition test (OxHLIA). The antiproliferative activity of both extracts was evaluated in tumor and non-tumor cell lines using the sulforhodamine B method, and the anti-inflammatory activity in lipopolysaccharide-activated RAW 264.7 macrophages by the ability to inhibit NO production.

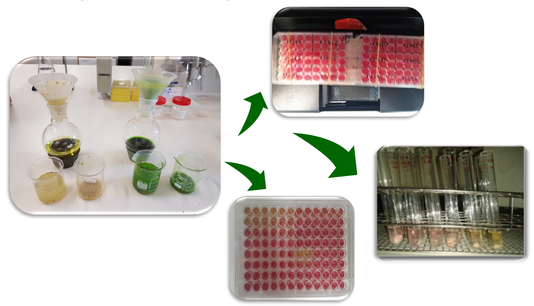

Fig.2 –Bioactivity assyas of pineapple crown and peel extracts.

Results

Twenty phenolic compounds were identified in both peel and crown extracts, among them, phenolic acids and flavonoids. The main detected compounds were caffeic acid derivatives and flavones such as apigenin 6,8-*C*-diglucoside (Fig. 3).

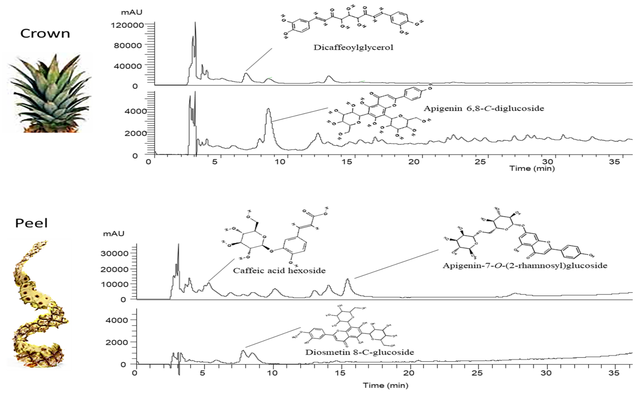

Fig. 3 – Phenolic profile of pineapple crown and peel recorded at 280 nm and 320 nm.

Table 1 – Bioactivities of pineapple crown (PC) and peel (PP) extracts.

Antioxidant Activity (IC_{50} values, µg/mL)				
		PP	PC	Trolox
TBARS		4.3±0.1	6.6±0.3	5.4±0.3
OxHLIA	Δt 60 min	190±7	395±19	21.8±0.3
	Δt 120 min	333±9	714±33	43.5±0.8
Anti-inflammatory (GI_{50} values; µg/mL)				
		PP	PC	Dexametasona (uM)
RAW264,7		>400	>400	16 ± 1
Cytotoxicity (GI_{50} values; µg/mL)				
		PP	PC	Ellipticine (uM)
Tumoral cell lines	AGS	>400	>400	0.9±0.1
	CaCo	378±7	>400	0.8±0.1
	MCF_7	322±3	>400	1.020 ± 0.004
	NCI-H460	>400	>400	1.01±0.01
Non-tumoral culture	VERO	>400	>400	0.6±0.1

Four human tumour cell lines: gastric adenocarcinoma (AGS), colorectal adenocarcinoma (CaCo), breast carcinoma (MCF7), non-small cell lung carcinoma (NCI-H460), and a non-tumoral culture from African green monkey (VERO)

The results showed that both extracts had an excellent performance in the cell-based tests of antioxidant activity, highlighting the lower IC_{50} values and consequently greater activity for the bark extract (**Table 1**). The same trend was seen in the tests of anti-tumor activity, with none of the extracts showing toxicity up to the maximum concentration tested (GI_{50} > 400 µg/mL).

Conclusion

This study confirms the potential application of pineapple bio-residues, especially the peel, in the food industry as a source of compounds with bioactive properties, contributing to the valorisation of this bio-waste.

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ANTIOXIDANT COMPOUNDS FROM *UNDARIA PINNATIFIDA*: MICROWAVE-ASSISTED EXTRACTION AND OPTIMIZATION USING RESPONSE SURFACE METHODOLOGY

C. Lourenço-Lopes [1], L. Cassani [1,4], F. Chamorro [1], A. Carreira-Casais [1], P. Garcia-Oliveira [1,2], Aurora Silva[1,3], M. Fraga-Corral [1,2], P. Otero [1], M. Fátima Barroso [3], Miguel A. Prieto [1,*] and J. Simal-Gándara [1,*]

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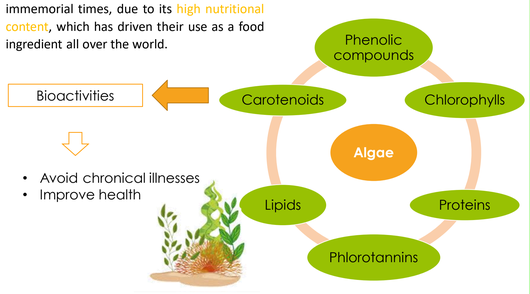
INTRODUCTION

Algae are a rich source of diverse secondary metabolites such as phenolic compounds and carotenes with health-related benefits. These compounds have attracted special attention in both, scientific and industrial fields, since they could be included in nutritional supplements for the prevention and treatment of a wide variety of pathologies.

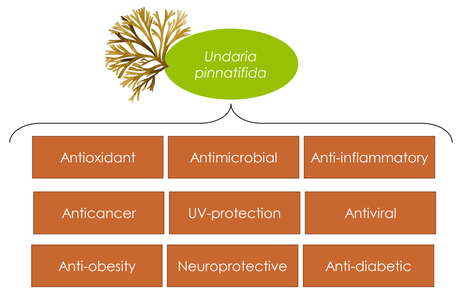
Algae have been part of the Eastern diet since immemorial times, due to its high nutritional content, which has driven their use as a food ingredient all over the world.



In the eastern countries, *Undaria* is one of the most used algae for this purpose, and it is colloquially known as Wakame. The inclusion of algae in the diet has been generalized and its consumption has triggered the controlled production of species of interest which are also considered prolific and sustainable organisms. Nevertheless, wakame is an invasive species all around the world and it needs to be controlled in many ecosystems.

In addition to its ancestral use in food, algae contain other compounds, which have been widely applied in pharmaceutical and/or cosmetic industries.

In the last decades, numerous studies have reported the wide range of biological activities of *Undaria pinnatifida* extracts.



OBJECTIVES

- Obtain extracts rich in compounds with potential functional properties.
- Use "green" technologies to produce the macroalgae extracts, contributing to a sustainable exploitation.
- Optimize critical parameters (time, pressure and ethanol concentration) affecting the extraction of antioxidants from *Undaria pinnatifida* using Response Surface Methodology (RSM) in order to simultaneously maximize the radical scavenging activity and the total phenolics content.

METHODOLOGY

Table 1. Matrix of the experimental design indicating the coded and uncoded values of the independent variables (X1, X2 and X3).

	Independet variables		
	X_1: t (min)	X_2: P(bar)	X_3: Et (% v/v)
1	-1 (7,5)	-1 (5,6)	-1 (20,3)
2	-1 (7,5)	-1 (5,6)	1 (79,7)
3	-1 (7,5)	1 (16,4)	-1 (20,3)
4	-1 (7,5)	1 (16,4)	1 (79,7)
5	1 (20,5)	-1 (5,6)	-1 (20,3)
6	1 (20,5)	-1 (5,6)	1 (79,7)
7	1 (20,5)	1 (16,4)	-1 (20,3)
8	1 (20,5)	1 (16,4)	1 (79,7)
9	-1,68 (3)	0 (11)	0 (50)
10	1,68 (25)	0 (11)	0 (50)
11	0 (14)	-1,68 (2)	0 (50)
12	0 (14)	1,68 (20)	0 (50)
13	0 (14)	0 (11)	-1,68 (0)
14	0 (14)	0 (11)	1,68 (100)
15	-1,68 (3)	-1,68 (2)	-1,68 (0)
16	-1,68 (3)	-1,68 (2)	1,68 (100)
17	-1,68 (3)	1,68 (20)	-1,68 (0)
18	-1,68 (3)	1,68 (20)	1,68 (100)
19	1,68 (25)	-1,68 (2)	-1,68 (0)
20	1,68 (25)	-1,68 (2)	1,68 (100)
21	1,68 (25)	1,68 (20)	-1,68 (0)
22	1,68 (25)	1,68 (20)	1,68 (100)
23	0 (14)	0 (11)	0 (50)
24	0 (14)	0 (11)	0 (50)
25	0 (14)	0 (11)	0 (50)
26	0 (14)	0 (11)	0 (50)
27	0 (14)	0 (11)	0 (50)
28	0 (14)	0 (11)	0 (50)

Response Surface Methodology (RSM)

Intercept

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2$$

Linear effect | Interactive effect | Quadratic effect

Response variables (dependent):

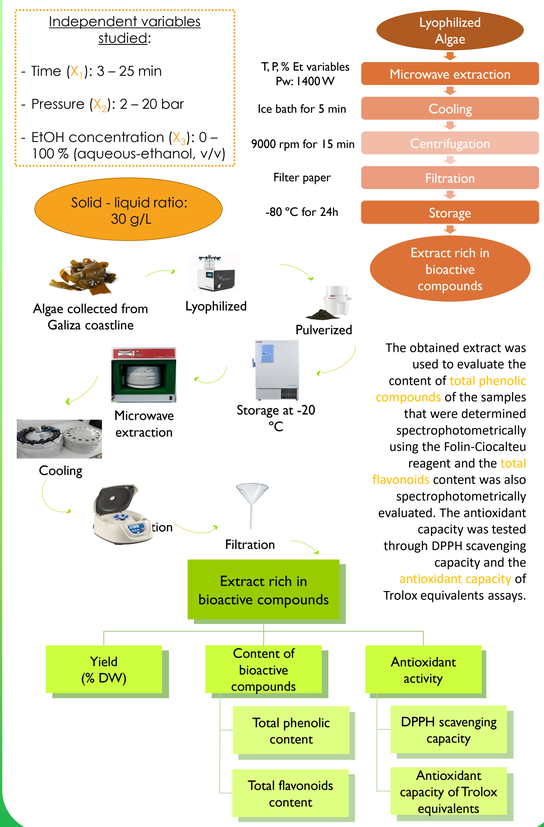
Y_1: % of extraction yield
Y_2: mg of total phenolics per g of dried seaweed

The response surface methodology with a five-level central composite design was used to study the influence of the operational conditions during extraction of antioxidants from *Undaria*. The method of least squares regression was used to fit data to a quadratic model, showed in the equation.
The mathematical solutions produced, allow to control the complete extraction process and can be used by the industry to select the conditions that makes the process more profitable.

Independent variables studied:

- Time (X_1): 3 – 25 min
- Pressure (X_2): 2 – 20 bar
- EtOH concentration (X_3): 0 – 100 % (aqueous-ethanol, v/v)

Solid - liquid ratio: 30 g/L



The obtained extract was used to evaluate the content of total phenolic compounds of the samples that were determined spectrophotometrically using the Folin-Ciocalteu reagent and the total flavonoids content was also spectrophotometrically evaluated. The antioxidant capacity was tested through DPPH scavenging capacity and the antioxidant capacity of Trolox equivalents assays.

RESULTS

The data obtained can be seen in Table 2. We obtained a great variety of values for the different response variables due to the effect of the factors considered in the analysis.

Table 2. Matrix of the experimental design indicating the coded and uncoded values of the independent variables (X1, X2 and X3) and values of the response variables for the treated sample under different experimental conditions.

	Response variables				
	DW (mg/g dw)	TPC (mg PGE/ g dw)	TFC (mg QE/ g dw)	DPPH (mg Trolox/ g dw)	TEAC (mg Trolox/ g dw)
1	446.29	7.70	0.73	4.91	5.14
2	384.97	15.36	0.41	17.66	37.08
3	596.97	48.44	5.74	56.81	41.20
4	427.51	18.23	1.95	20.44	27.86
5	524.91	13.53	1.16	7.34	9.26
6	400.93	16.12	0.64	21.29	28.89
7	531.55	46.63	6.88	46.17	38.68
8	416.36	17.10	2.61	15.62	32.63
9	453.32	16.99	2.25	12.62	49.70
10	378.50	11.82	1.17	12.71	32.33
11	379.37	9.82	1.13	15.42	31.19
12	512.74	28.07	3.45	27.60	53.23
13	481.85	22.57	8.39	30.73	40.35
14	103.88	1.84	3.13	8.38	19.50
15	410.97	2.63	0.54	6.73	9.99
16	71.21	3.54	0.25	12.43	19.16
17	489.33	22.39	5.79	23.31	49.40
18	137.70	1.92	4.80	2.57	5.36
19	462.68	2.47	0.94	2.42	13.36
20	93.68	0.37	0.97	5.29	5.41
21	489.80	17.66	5.04	24.70	42.31
22	134.47	1.74	7.19	2.44	2.86
23	436.77	25.31	2.65	22.32	47.98
24	429.76	14.91	1.20	30.50	61.09
25	587.41	18.21	2.02	11.34	34.23
26	434.65	12.64	0.85	16.37	35.87
27	423.22	14.06	1.76	9.72	27.75
28	433.15	11.60	1.47	12.84	20.75

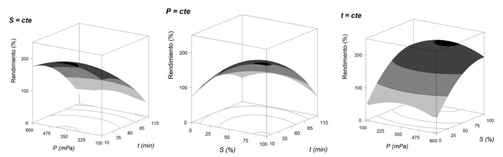
Each response was individually optimized and compared with a global optimization that allowed to obtain the optimum extraction conditions which maximize all responses at the same time. Analysis of variance of each response variable model showed that they were all significant, suggesting that these models could be used to describe the effects of the selected independent variables on Total Phenolic Compounds, Total Flavonoid Content, Extraction Yield and antioxidant activities tested of *Undaria pinnatifida*.

So, the optimum conditions for extracting bioactive compounds with antioxidant activity from the studied alga were approximately 10 minutes, 20 bar and 0% of ethanol, which means that water was a more receptive polar solvent for microwave energy absorption than ethanol. In the graphics we can see the optimum conditions represented in black.

Table 3. The optimal conditions of processing parameters (extraction time, pressure and ethanol concentration) for the microwave-assisted extraction of antioxidant compounds from *Undaria pinnatifida* under study.

Algae Extract	Time	Pressure	Ethanol Concentration
UP	10.25 ± 0.34	20.00 ± 4.00	0.00 ± 0.00

Data are shown as LS means values ± standard error. UP: *Undaria pinnatifida*



CONCLUSION

After that we used the determined optimum conditions to performed all the analyses again to ensure the validation of the study and verify a close agreement between experimental and predicted values observed. These results indicate the success of the model employed and of RSM in modelling responses to characterize their dependence with the extraction conditions under evaluation.

Table 4. Effect of *Undaria pinnatifida* extracted at optimal conditions on antioxidant capacity parameters.

Algae Extract	Total Phenolic Content (mg PGE/ g DW)	Total Flavonoid Content (Mg QE/ G Dw)	Antioxidant Capacity (mg TE/ g DW)		Extraction Yield
			DPPH	TEAC	
UP	29.073 ± 3.643 (21.800; 36.345)	11.276 ± 0.829 (9.607; 12.945)	26.420 ± 6.728 (12.986; 39.854)	51.421 ± 16.656 (18.166; 84.675)	0.650 ± 0.010 (0.628; 0.673)

Data are shown as LS means values ± standard error. 95% confidence interval is also presented. (**UP**: *Undaria pinnafitida*). PGE: phloroglucinol equivalent, QE: quercetine equivalent, TE: Trolox equivalent.

In conclusion, an aqueous microwave-assisted extraction (20 bar, 10 min) appears to be the optimum processing approach for the extraction of polyphenols with antioxidant activity from *Undaria pinnatifida* with no need for the use of organic solvents.

These are promising results because it was possible to maximize the polyphenol content through a technique that is friendly to the environment and free of organic solvents.

The validation study was performed and a close agreement between experimental and predicted values was observed, indicating the suitability of the model employed and the success of RSM in modelling responses to characterize their dependence with the extraction conditions under evaluation.

Currently, we are working on the purification of the extract and on the identification and quantification of the phenolic compounds soluble in water.

ACKNOWLEDGEMENTS

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Development of energy bars with bee pollen and bee bread

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Abstract

Bee pollen and bee bread are two beehive products with high nutritional value, result of the presence of proteins, amino acids, fatty acids, carbohydrates, vitamins, minerals and phenolic compounds. Therefore, these products can be considered as excellent ingredients for energy bars.

The study focused on the formulation of different energy bars with compositional variation of pollen and bee bread. Additionally, the composition included almonds, walnuts, hazelnuts, common in Trás-os-Montes region, and ingredients such as white quinoa, sesame and oat flake.

Introduction

Energy bars are food products with high energy and nutritional value, with high consumption rates in our days. As a rule, they are purchased by consumers of dietary supplements, particularly by athletes in situations of effort, who need fast assimilation foods and a concentrate source of energy. These bars are usually the result of pressing mixtures of cereals, fruits and nuts, which are added through a syrup of glucose and honey, among others. The objective of this work focused on the development of different formulations of energy bars that incorporate bee products with characteristics that respond to the nutritional needs of consumers in situations of intense physical effort, integrating high protein contents and carbohydrates of rapid assimilation, such as bee pollen and bee bread, rich in protein and fiber, and honey, rich in fructose and glucose. The first stage of the work, namely, the nutritional analysis of the ingredients and the sensorial acceptability of the bars are presented in this poster.

Materials

- Infra-red balance
- Muffle
- Soxhlet method
- Kjedahl
- Enzymatic-gravimetric method
- HPLC - RI

Raw material

- Bee pollen
- Bee bread
- Honey
- Dry fruits (almond, walnut, hazelnut)
- Oat flake
- Sesame seed
- White quinoa

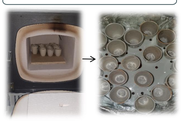
Solvents and reagents

- Petroleum ether;
- Ethanol;
- Diethyl ether;
- Acetonitrile (HPLC quality);
- Sulfuric acid
- Boric acid
- Hydrochloric acid
- Anhydrous disodium phosphate
- Monobasic disodium phosphate monohydrate
- Acetone
- Sodium hydroxide
- Green bromocresol indicator
- Methyl red
- Metal catalyst

Methodology

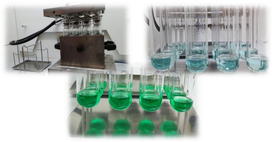
Moisture content — Infra-red at 105ºC

Ash — Incineration at 550ºC



Fat — Soxhlet method

Protein content — Quantification of total N by Kjeldahl method →



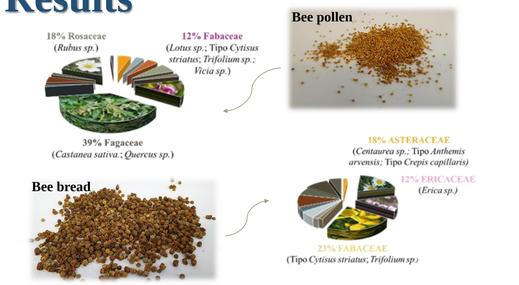
Carbohydrate content → 100 − (% Ash + % Protein + % Fat)

Energy → 4 × (% Protein + % Carbohydrate) + 9 × (% Fat)

Fiber — Enzymatic-gravimetric method

Sugars profile — HPLC - RI

Results



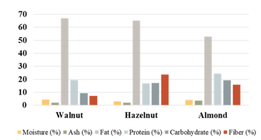


Figure 1. Nutritional parameters of dry fruits.

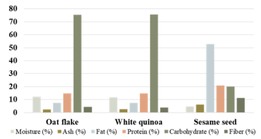


Figure 2. Nutritional parameters of cereal/seeds.

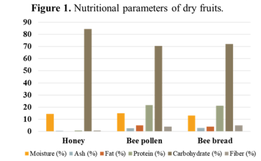


Figure 3. Nutritional parameters of beehive products.

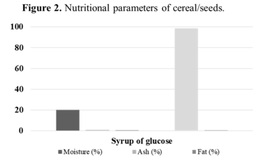


Figure 4. Nutritional parameters of syrup of glucose.

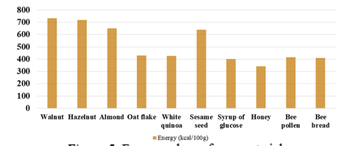


Figure 5. Energy values of raw materials

Table 1. Sugar profile of raw materials used for the preparation of energy bars (values expressed as a percentage on a dry basis).

Samples	Fructose (%)	Glucose (%)	Sucrose (%)	Trehalose (%)	Turanose (%)	Maltulose (%)	Maltose (%)
Walnut	n/d	n/d	5.59 ± 0.28	n/d	n/d	n/d	n/d
Hazelnut	n/d	n/d	10.65 ± 0.70	n/d	n/d	n/d	n/d
Almond	n/d	n/d	9.44 ± 0.30	n/d	n/d	n/d	n/d
Oat flake	n/d	n/d	3.07 ± 0.14	n/d	n/d	n/d	n/d
White quinoa	n/d	n/d	8.08 ± 0.75	n/d	n/d	n/d	n/d
Sesame seed	n/d	n/d	1.44 ± 0.01	n/d	n/d	n/d	n/d
Syrup of glucose	0.41 ± 0.00	39.12 ± 0.00	n/d	19.19 ± 0.00	n/d	n/d	n/d
Honey	48.36 ± 0.20	40.54 ± 0.17	n/d	1.93 ± 0.09	3.51 ± 0.70	3.50 ± 0.53	4.61 ± 0.80
Bee pollen	37.69 ± 0.16	26.89 ± 0.37	18.15 ± 0.09	0.81 ± 0.18	1.52 ± 0.11	n/d	n/d
Bee bread	41.45 ± 0.08	20.28 ± 0.31	2.30 ± 0.19	n/d	n/d	n/d	n/d

n/d – not detected

Table 2. Sensorial analysis for the different energetic bars formulations, measure as general acceptancy.

Formulations of energy bars	Minimum	Maximum	Average	Formulations of energy bars	Minimum	Maximum	Average
Oat flake, pollen, walnut	3	8	4.9	Oat flake, bee bread, walnut	3	7	5.5
Oat flake, pollen, hazelnut	4	9	5.8	Oat flake, bee bread, hazelnut	4	8	6.7
Oat flake, pollen, almond	5	8	6.3	Oat flake, bee bread, almond	5	8	5.9
Sesame seed, pollen, walnut	3	8	6.0	Sesame seed, bee bread, walnut	4	9	6.4
Sesame seed, pollen, hazelnut	4	9	6.2	Sesame seed, bee bread, hazelnut	4	9	6.6
Sesame seed, pollen, almond	4	9	7.1	Sesame seed, bee bread, almond	4	9	6.4
White quinoa, pollen, walnut	3	9	6.3	White quinoa, bee bread, walnut	4	8	5.9
White quinoa, pollen, hazelnut	5	8	7.0	White quinoa, bee bread, hazelnut	4	8	6.7
White quinoa, pollen, almond	4	8	6.5	White quinoa, bee bread, almond	4	7	5.5

Conclusion

The results of the pollen analysis for bee pollen and bee bread, identified as the predominant origin the *Fabaceae* family (21%) and *Fagaceae* family (39%), respectively. In nutritional terms, bee pollen showed a higher content of moisture, proteins, fat, and energy when compared to bee bread. In opposition, the bee bread had a higher content of ash, fiber, and carbohydrates. The sugar profile for bee bread highlighted the high fructose content when compared to pollen. For the dry fruits, walnuts showed a high fat content, while almonds have presented high levels of protein and carbohydrates. The hazelnut, on the other hand, was characterized by its rich fiber and sucrose contents. Regarding seeds, the sesame seed exhibited the highest contents of ash, fat, protein, energy, and fiber. For the sugar profile, the seeds only contained sucrose, with the highest value found in white quinoa. According to a sensory analysis (**Table 2**), the panel acceptability was good for all the energy bars, with the two highest scores registered for the formulations containing pollen, particularly those with white quinoa/hazelnut and sesame seed/almond combinations.

Recommendations

- ✓ Use new cereal or seeds in order to obtain a more nutritious and tasty cereal bar;
- ✓ Modify the percentages and types of sweeteners used in order to study how they influence the structure of the cereal bar;
- ✓ Evaluate how the formulation of energy bars can affect their chemical stability.
- ✓ Introduce our bars to a company so that they can enter the market, and thus diversifying the consumption of these beehive products.

Acknowledgements

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CHEMICAL COMPOSITION AND BIOACTIVE PROPERTIES OF PUMPKIN SEEDS AND SEED CAKES

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Introduction

- Pumpkin, (*Cucurbita pepo* L.) is one of the most important vegetables of the Cucurbitaceae family which is widely used for its edible fleshy fruit, especially fruit pulp.
- Cucurbitaceae family, includes 130 genera and more than 800 species (Perez-Gutierrez, 2016). The different species offer a diversity of fruit characteristics such as shape, size, color, taste, and seeds (Gong et al., 2012).
- The popularity of the pumpkin for use in various traditional medicines for several ailments (antidiabetic, antihypertensive, antitumour, immunomodulation, antibacterial, antalgic, antihypercholesterolemia, intestinal antiparasitia, anti-inflammation) has attracted scientific attention to this plant (Fu et al., 2006).
- Pumpkin oil press-cake has a substantial amount of residual oil, which is rich in omega-6 fatty acids, tocopherols, minerals and proteins, and as such, could have different applications in the development of functional food products (Radočaj et al., 2011a).
- In the present work, the chemical composition and bioactive properties of pumpkin seeds and seed cakes were evaluated.

Materials and Methods

- Pumpkin seeds (*Cucurbita pepo* L.) of the local landrace "Nychaki" were sown directly in soil on 27/7/2020 at the University of Thessaly during the summer-autumn growing period of 2020.
- Plant distances were 2.5 m between rows and 0.80 m within rows (4705 plants/ha).
- Fruit were collected at marketable maturity on 20/11/2020 and seeds were removed from 15 randomly selected after cutting each fruit at the equatorial axis.
- The seeds were air-dried at room temperature and pressed with a cold-press to obtain the seed cakes, while whole air-dried seeds were ground to fine powder.
- Nutritional value was assessed according to AOAC (2016).
- Tocopherols, free sugars and organic acids were analyzed with high performance liquid chromatography (HPLC).
- Fatty acids obtained with Soxhlet apparatus were analyzed by gas liquid chromatography after transesterification of the lipid fraction.
- Cytotoxicity was determined on a non-tumour primary culture of porcine liver cells (PLP2 cells).
- For chemical composition analyses three batch samples from the collected seeds were used (n=3). Data were evaluated by a one-way ANOVA, while the means of values were compared with Tukey's HSD test (p=0.05).

Image 1. Pumpkins (*Cucurbita pepo* L.)

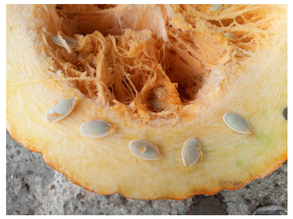

Image 2. Pumpkin seeds

Results

Table 1. Nutritional value (g/100 g dw) and energetic value (kcal/100 g dw) of the studied cucurbit ground seeds and seedcake (mean ± SD).

	Fat	Proteins	Ash	Carbohydrates	Energy
Ground seeds	42.74±0.09	37.7±0.2	3.52±0.09	16.07±0.03	599.6±0.1
seedcake	7.62±0.08	58.6±0.3	5.40±0.05	28.4±0.2	416.5±0.4

Table 2. Composition in tocopherols (mg/100 g dw) of the studied cucurbit ground seeds and seedcake (mean ± SD).

	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total Tocopherols
Ground seeds	0.075±0.004	0.011±0.001	6.59±0.03	0.28±0.01	6.96±0.02
seedcake	0.018±0.001	0.079±0.002	1.07±0.04	0.016±0.002	1.18±0.04

Table 3. Composition in sugar (g/100 g dw) of the studied cucurbit ground seeds and seedcake (mean ± SD).

	Fructose	Glucose	Sucrose	Trehalose	Total Sugars
Ground seeds	0.20±0.01	0.21±0.01	1.97±0.04	0.26±0.01	2.6±0.1
seedcake	0.34±0.01	0.19±0.01	2.9±0.1	0.25±0.01	3.7±0.1

Table 4. Composition in organic acids (g/100 g dw) of the studied cucurbit ground seeds and seedcake (mean ± SD).

	Oxalic acid	Malic acid	Total organic acids
Cucurbit seeds ground	tr	tr	-
Cucurbit cake	0.006±0.001	tr	0.006±0.001

tr- traces

Table 5. Cytotoxicity of the studied cucurbit ground seeds and seedcake (GI_{50} values μg/mL).

	Hepatotoxicity PLP2 (non-tumor cells)
Cucurbit seeds ground	>400
Cucurbit cake	>400

Positive control (Ellipticine). GI_{50} values (3.2±0.7 μg/mL), corresponds to the sample concentration achieving 50% in liver primary culture PLP2.

Table 6. Fatty acids composition (%) of the studied cucurbit ground seeds and seedcake (mean ± SD).

	Cucurbit seeds ground	Cucurbit cake
C6:0	0.015±0.001	0.168±0.006
C8:0	0.002±0.001	0.022±0.001
C10:0	0.006±0.001	0.011±0.001
C12:0	0.020±0.001	0.039±0.001
C14:0	0.117±0.004	0.230±0.006
C15:0	0.020±0.001	0.043±0.001
C16:0	12.20±0.04	14.0±0.4
C16:1	0.119±0.004	0.162±0.001
C17:0	0.094±0.004	0.092±0.003
C18:0	4.83±0.08	5.46±0.02
C18:1n9c+t	37.0±0.1	36.27±0.02
C18:2n6c	43.89±0.01	41.5±0.3
C18:3n3	0.242±0.004	0.585±0.001
C20:0	0.359±0.004	0.400±0.002
C20:1	0.192±0.001	0.242±0.005
C20:3n3+C21:0	0.27±0.01	0.154±0.005
C20:5n3	0.11±0.01	0.063±0.001
C22:0	0.294±0.009	0.43±0.02
C22:1n9	0.048±0.003	0.016±0.001
C23:0	0.027±0.001	0.064±0.001
C24:0	0.118±0.003	0.062±0.001
Total SFA (% of total FA)	18.10±0.03	21.0±0.3
Total MUFA (% of total FA)	37.4±0.1	36.69±0.01
Total PUFA (% of total FA)	44.51±0.09	42.3±0.3

- The ground seeds were rich in fat and proteins while seed cakes contained a high amount of protein and carbohydrates.
- Ground seeds and seed cakes contained all the four vitamin E isoforms with γ-tocopherol being the most abundant isomer in both samples.
- The main detected free sugar in ground seeds and seed cakes were sucrose, followed by trehalose, fructose and glucose, while seed cakes contained a higher amount of sucrose and total free sugars than ground seeds.
- Oxalic acid content was the only detected compound in seed cakes, whereas no organic acids were detected in ground seeds.
- The main detected fatty acids were linoleic acid (43.9% and 41.5% in ground seeds and seed cakes, respectively) and oleic acid (37.0% and 36.3% in ground seeds and seed cakes, respectively), followed by stearic acid (4.83% and 5.46% in ground seeds and seed cakes, respectively). Polyunsaturated and monounsaturated fatty acids were the main fatty acids class and accounted for 81.9% and 79.0% of total fatty acids in ground seeds and seed cakes, respectively.

Conclusion

- Both pumpkin seeds and seed cakes showed no toxic effects against non-tumor PLP2 cell lines indicating that they are safe for human consumption.
- The presented results highlighted the nutritional value of the pumpkin seeds and seed cakes which could be considered a rich source of protein.
- The high content in polyunsaturated fatty acids and tocopherols due to results could be further valorized for pharmaceutical and nutraceutical purposes and increase the added value of pumpkin crop.

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Acknowledgements

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Effect of Pre- and Post-Harvest Treatments on Quality, Organoleptic, and Nutraceutical Properties of Wild Edible Plants

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Abstract

The Mediterranean basin is a biodiversity hotspot of wild edible plant species, and their therapeutic and culinary uses have long been documented Owing to the growing demand for wild edible species, there are increasing concerns about the safety, standardization, quality, and availability of fresh or stored products derived from these species collected in the wild. Moreover, the maintenance of the nutraceutical profile of wild edible species in the same domesticated species is another request by consumers. After a literature screening of the most interesting Mediterranean wild edible species, *S. minor* samples were analyzed as fresh and then exposed to different preservation processes (oven-drying at 60 °C until constant weight or freeze-drying until constant weight), and studied for their content in phenolic compounds, antioxidant, antimicrobial, cytotoxic and anti-inflammatory properties. In most of the cases, the oven-dried samples showed higher bioactive properties and higher content in phenolic compounds than the freeze-dried samples. The most abundant phenolic compounds in both samples were kaempferol-3-*O*-glucoside and caffeoyl ester, with some differences between wild and domesticated samples. This study provides important information to choose the most adequate methodology to maintain secondary metabolites and bioactive properties of *S. minor*. Further researches are occurring to introduce S. minor extract in fresh pasta, trying, in this way, to enhance the nutraceutical profile of a common food.

Introduction

Nowadays, the research of a dietary vegetable variety is increasing, especially in developed countries. For this reason, the cultivation of wild edible herbs has been studied. Different growing techniques have been applied to wild edible species, in order to evaluate their response to cultivation. These results were significant in terms of nutraceutical properties and phenolic compound content as well as in terms of availability of plant material for an yearly marketability.

Recently, the consumers, as well as the food industry, have started to concern about the quality of foodstuff. Since the use of nutraceutical ingredients is continuously increasing, the drying method as a storage method is resulting of extreme importance in the quality of these ingredients and in the quality of the final food product. It has been noted that the drying methods can inhibit the enzymatic activity, which can lead to the senescence of plant material over variable periods and to the loss of phenolic compounds and nutraceutical properties. The drying method is considered the oldest food storage technique; thus, **different drying methods have been developed to assure the retention of the food constituents**. In this perspective, scientific research has been conducted on different wild edible plants, including green tea, *Anoectochilus roxburghii* (Wall.) Lindl. and *Dendrobium nobile* Lindl. aiming to analyze the effects of different drying methods such as sun drying, hot air drying or oven-drying at different temperatures, vacuum drying, infrared radiation drying, microwave drying and freeze-drying methods, concluding that **different drying methods can affect differently the content in phenolic compounds and nutraceutical properties**.

***Sanguisorba minor* Scop.** is an evergreen perennial species belonging to the Rosaceae family, native from Europe, western Asia and Siberia, and northern Africa. During the ancient times, in famine periods, this species was included in the dishes, mainly in mixtures and soups, and more recently, this herb has been introduced in traditional and folk recipes. Nowadays, this species is also considered as a nutraceutical and functional species with health-promoting effects to the human health. Some studies stated that ***S. minor* had interesting nutraceutical properties such as anti-inflammatory activity in over-producing ROS (Radical Oxygen Species) processes**.

Aim:
Given the importance that new wild edible species could have in human health and the introduction of a new source of bioactive compounds and nutraceutical properties in the current monotonous diet, the present work aimed at comparing the nutraceutical properties (i.e., inhibition of lipid peroxidation, antibacterial, anti-inflammatory and cytotoxic properties in a range of tumor cell lines) and the phenolic compound's composition of freeze-dried and oven-dried *S. minor* plants collected as wild or domesticated by using three different growing systems that were analysed separately. Indeed, the retention of phenolic compounds in *S. minor* resulted in fundamental importance in introducing this species as a new functional ingredient in typical food such as pasta or bread.

Materials

Wild plant material (W) (about twenty plants) was collected in the Pisa area (Italy, 43°44'39.6"N 10°31'53.39"E) during spring 2019 (April-May). The *S. minor* plant material was provided by Tirrenofruit S.r.l (Florence, Italy). This local wholesaler selected three local farms (F1, F2, and F3) for providing the material to be used for the present experiment.

Methodology

After the applied drying methods, the plant material was ground at 20 µm filters and kept in a desiccator protected from light and humidity. The extraction procedure for the determination of the nutraceutical properties and the phenolic compounds was performed according to Bessada et al. (2016).

Determination of phenolic compounds
The phenolic compounds were determined according to Bessada et al. (2016). A Dionex Ultimate 3000 HPLC-DAD-ESI/MS was used to analyse the samples. The acquisition and the processing of data were carried out with the Xcalibur® data system. Authentic standards (Extrasynthèse S.A., Genay, France) and data available from the literature were used to identify individual phenolic compounds.

Antioxidant activity
The antioxidant activity was evaluated by the inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS) as described by Mandim et al. (2020). The obtained extracts were dissolved in a methanol solution (80% *v/v*) to obtain a stock solution of 5 mg/mL. Afterward, successive dilutions were made to obtain a range of concentrations of 40 to 0.3 µg/mL. The antioxidant activity resulted from the reduction of TBARS, resulting in the formation of the malondialdehyde-thiobarbituric acid complex (MDA-TBA).

Antibacterial Activity
The bacterial strains were isolated from patients hospitalised in various departments at the North-eastern local health unit (Bragança, Portugal) and Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal). Five Gram-negative bacteria: *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Morganella morganii*; and three Gram-positive bacteria: *Enterococcus faecalis*, *Listeria Monocytogenes*, and methicillin-resistant *Staphylococcus aureus (*MRSA) were tested. The determination of the Minimal Inhibitory Concentration (MIC) was conducted by the microdilution method and the colourimetric method using p-iodonitrotetrazolium chloride (INT) (Panreac Applichem-Barcelona, Spain), according to Pires et al. (2018).

Cytotoxic activity
The cytotoxic activity was evaluated in four different human tumour cell lines (HeLa: cervical carcinoma; HepG2: hepatocellular carcinoma; MCF-7: breast adenocarcinoma; and NCI-H460: non-small-cell lung cancer) using the sulphorhodamine B (SRB) assay as described by Guimarães et al. (2013).

Statistical analysis
The experimental layout was arranged according to the completely randomized design. The statistical analysis was carried out by using SPSS v. 23.0 software and using the one-way analysis of variance (ANOVA), while means were compared with Tukey's HSD test ($p < 0.05$). Three samples were analyzed for each treatment, and all the assays were carried out in triplicate. Data were expressed as mean ± standard deviation.

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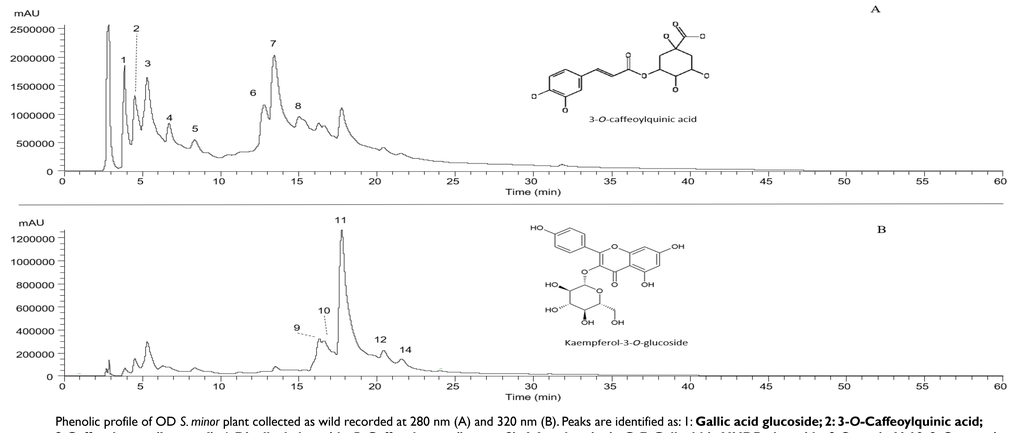
Results



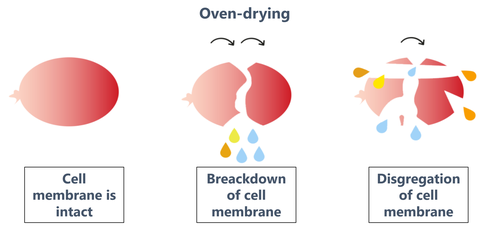
Phenolic profile of OD *S. minor* plant collected as wild recorded at 280 nm (A) and 320 nm (B). Peaks are identified as: 1: **Gallic acid glucoside; 2: 3-O-Caffeoylquinic acid; 3:Caffeoyl ester (Isomer I); 4: Digalloyl glucoside; 5: Caffeoyl ester (isomer 2); 6: Lambertianin C; 7: Galloyl-bis-HHDP-glucoside; 8: Sanguin H-10; 9: Quercetin-O-hexoside gallate (isomer 1); 10: Quercetin-O-hexoside gallate (isomer 2); 11: Quercetin-3-O-glucuronide; 12: Kaempferol-3-O-glucoside; 13: Quercetin-3-O-rutinoside; 14: Kaempferol-O-hexoside**

The W plants reported a higher total phenolic content than the cultivated plants (F1, F2, F3)

The OD samples reported a higher total phenolic content and higher nutraceutical properties (antioxidant, antibacterial, anti-inflammatory and cytotoxic activities) than the FD samples

The 60 °C temperature of the OD method retained the nutraceutical properties of the plant

The most abundant phenolic compounds in both samples were **kaempferol-3-O-glucoside** and **caffeoyl ester**, with some differences between wild and cultivated plants



The obtained results suggested that phenolic compounds deterioration may be affected by many factors other than temperature treatments. These factors may include **the activity of polyphenol oxidase (PPO),** drying times, and plant moisture. PPO catalyzes the oxidation of phenols into quinones, which subsequently deteriorate and polymerize into brown pigments.

Cellular destruction due to extensive drying times promotes phenolic compounds' loss by increasing their polarity in water. In this way, water allows phenolic compounds to dissolve, being dragged to the surface.

Moreover, the higher phenolic content of OD samples compared to FD samples could be linked to more effective extraction of the insoluble phenolic compounds such as phenolic acids or condensed tannins linked to cell wall polysaccharides or, more specifically, proteins

Conclusion

A renewed interest in *S. minor* species has been showed by scientific literature for its edibility and its nutraceutical properties due to the high content in phenolic compounds. Appropriate methods must be applied to preserve the nutraceutical properties of this species during the storage.

The present work showed that OD samples presented a higher antioxidant and antimicrobial activity and a higher content in phenolic compounds when compared with FD samples, showing that the OD resulted the most indicated drying method to preserve and retain these bioactive compounds.

Further researches are necessary to standardise the cultivation and to verify the highest efficiency of OD storage of *S. minor* species.

The use of wild/cultivated species as functional ingredient in common food may be an alternative to introduce nutraceutical properties in the human diet

Acknowledgements

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CHITIN ISOLATION AND CHITOSAN PRODUCTION FROM CRAYFISH PRESENT IN PORTUGUESE FRESHWATERS

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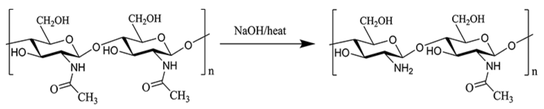
Abstract

In this study, crayfish shells were used for the extraction of chitin and production of chitosan using a classical methodology which includes demineralization, deproteinization, decolorization, and deacetylation. Chitin and chitosan structures were characterized by FTIR. The FTIR results showed that the obtained chitin from the organisms was α-chitin.

Introduction

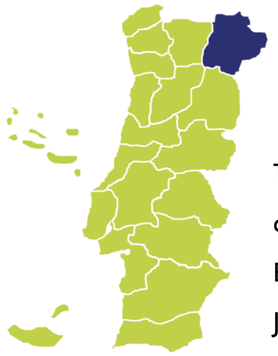
Chitin, which has *N*-acetyl-d-glucosamine structure, is the second most abundant biopolymer in nature after cellulose and may lead to chitosan by deacetylation [1].

Chitin and chitosan are effectively used in cosmetics, medicine, and food industry because of their biodegradability and biocompatibility as well as their antimicrobial and antioxidant properties [2].



This study examined crayfish species (*Procambarus clarkii*) as a potential source of chitin.

Materials



The crayfish were collected from Bragança river's on July 2020.

Methodology

Demineralization (HCl)

Desproteinization (NaOH)

Decoloration (water, chloroform, methanol)

Chitin

Deacetylation (NaOH/heat)

Chitosan

Results

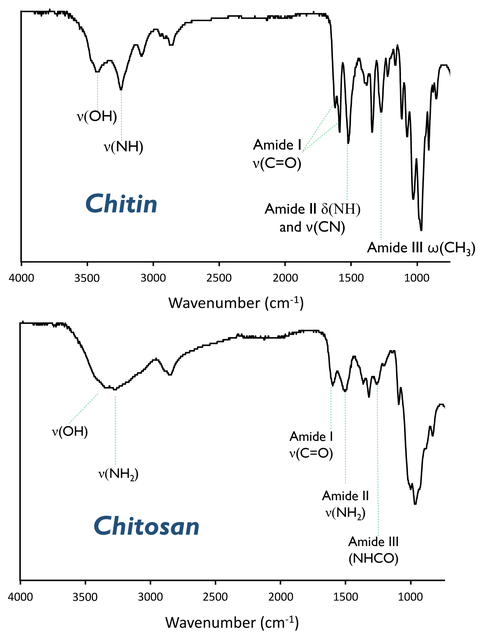
Chitin content of crayfish dry weight

17%

Chitosan productivity from the crayfish chitin

82%

FTIR spectra



ν= stretching, δ= bending, ω= wagging.

Conclusion and Future perspective

- Chitin and chitosan were successfully obtained from crayfish, an invasive species found in Portuguese freshwater.
- Extracted crawfish chitin can be used in the development of chitosan-based edible films reinforced with phenolic extracts for applications in food preservation.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020). National funding by FCT- Foundation for Science and Technology, through the institutional scientific employment program-contract with Soraia I. Falcão

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Amaranthus caudatus L. flowers: recovering of added value colouring molecules, Chemical and bioactive characterization

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Introduction

The vibrant colors of many plants are due to secondary metabolites, such as nitrogen-containing compounds, where betacyanins are included. *Amaranthus caudatus* L., a plant that has aroused enormous interest due to the nutritional profile of its seeds [1,2], present a very strong pink colour on its flowers, disclosing the interest for the exploitation of these flowers in terms of natural colourants. In this perspective, and due to an overproduction of this crop that results in the accumulation of large amounts of bio-residues, including flowers without any economic value or subsequent destination, these flowers can be exploited as sources of betacyanins for industrial application. In addition to an excellent colouring power, these compounds (betacyanins), also present strong bioactivities [3].

Objectives

The present work aimed at exploiting the flowers of *A. caudatus* L. as a source of bioactive (tocopherols, organic acids), but specially colouring compounds (betacyanins), and its bioactivities.

Methodology

An ultrasound assisted extraction (UAE) was applied and optimized through the Response surface methodology (RSM), a statistical tool widely used in the optimization of extraction processes, that allows the evaluation of several factors that affect the extractability and stability of the target molecules. These extracts were chemically characterized through different HPLC techniques regarding the betacyanins, tocopherols, and organic acids contents. Moreover, the bioactive potential of these extracts was also evaluated for the antioxidant activity trough the OxHLIA assay, the antimicrobial potential by the microdilution method, as also the hepatotoxicity for normal cells using the sulphorrodamine B assay.

Results

From the obtained results, three isoforms of tocopherols were detected, being β-tocopherol (0.884± 0.003 mg/100 g dw) the most abundant one. Regarding the organic acids, oxalic (2.48±0.05 mg/100g dw), shikimic (0.170± 0.003 mg/100 g dw) and traces of fumaric acid were found in the extract (Table 1.).

Concerning the colouring agents, four betacyanins were identified and quantified, namely: amaranthine (171±1 mg/g extract), isomaranthine (38±1 mg/g), betanin (1.6 ± 0.1 mg/g), and isobetanin (1.3 ± 0.1 mg/g)(Table 2.). The obtained extract also presented antioxidant activity with IC_{50} values of 29.0±0.4 μg/mL and 114±4 μg/mL for Δ*t* of 60 min and 120 min, respectively in the OxHLIA assay. An interesting antibacterial activity was also verified, with minimum inhibitory concentrations ranging from 5-20 mg/mL against pathogenic bacteria (Table 3.); and no toxicity for normal cells was observed at the maximum tested concentration of 400 μg/mL.

Table 1. Tocopherols and organic acids composition of *A. caudatus* flowers

Tocopherols	Content (mg/100 g dw)
α-Tocopherol	0.469 ± 0.010
β-Tocopherol	0.884± 0.003
γ-Tocopherol	nd
δ-Tocophero	0.599 ± 0.057
Total	1.952 ± 0.064
Organic acids	**Content (g/100g dw)**
Oxalic acid	2.48 ± 0.05
Shiquimic acid	0.170 ± 0.003
Fumaric acid	tr
Total	2.65± 0.03

tr: traces; nd: non-detected

Table 2. Betacyanin tentative identification and quantification. Indicar o significado de Rt, etc.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	Tentative identification	Content (mg/g extract)
1	18.68	536	727	Amaranthine	171±1
2	20.02	536	727	Isoamaranthine	38±1
3	22.04	536	551	Betanin	1.6±0.1
4	23.23	536	551	Isobetanin	1.3±0.1
				Total	212±1

Table 3. Antihaemolytic (IC_{50} values, μg/mL) and antibacterial activity (MIC and MBC values, mg/mL) of the extracts, and positive controls (trolox, ampicillin, imipenem and vancomycin).

Antihaemolytic activity	IC_{50} (μg/mL)		Antibacterial activity	*A. caudatus* .		Ampicillin (20mg/mL)		Imipenem (1mg/mL)		Vancomycin (1mg/mL)	
	OxHLIA, Δt=60 min	OxHLIA, Δt=120 min		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A. caudatus ..	29.0 ± 0.4	113 ± 13	**Gram-negative bacteria**								
			Escherichia coli	10	>20	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
			Klebsiella pneumoniae	5	>20	10	20	<0.0078	<0.0078	n.t.	n.t.
Trolox	20.4 ± 0.5	44 ± 1	*Morganella morganii*	5	>20	20	>20	<0.0078	<0.0078	n.t.	n.t.
			Proteus mirabilis	20	>20	<015	<0.15	<0.0078	<0.0078	n.t.	n.t.
			Pseudomonas aeruginosa	20	>20	>20	>20	0.5	1	n.t.	n.t.
			Gram-positive bacteria								
			Enterococcus faecalis	5	>20	<0.15	<0.15	n.t.	n.t.	<0.0078	<0.0078
			Listeria monocytogenes	>20	>20	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
			MRSA	5	>20	<0.15	<0.15	n.t.	n.t.	0.25	0.5

nt: not tested

Conclusion

After such promising results, this plant can be a viable alternative to obtain natural colorant ingredients.

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Acknowledgements

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DEVELOPMENT AND CHARACTERIZATION OF POLYMERIC NANOPARTICLES WITH SILYMARIN BY SOLID DISPERSION

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Abstract

- Silymarin: active principle extracted from the plant *S. marianum*
- Mixture of flavonolignans presenting antioxidant activity.
- Nanoencapsulation is an alternative to preserve and protect bioactive compounds from unfavorable environmental conditions, in addition to increasing their bioavailability and stability. The present study aimed to obtain and characterize silymarin-loaded nanoparticles by the solid dispersion.
- Kolliphor® Poloxamer 407 polymer was used an encapsulant, ethanol was used as solvent, Tween 80 PS as surfactant and silymarin was acquired from Sigma-Aldrich.
- The nanoencapsulated compound was characterized by Differential Scanning Calorimetry and Infrared Spectroscopy Fourier Transform.
- Characterization analyzes showed that silymarin was efficiently encapsulated in the polymeric matrix, going from a crystalline state to a solid amorphous solution, forming the nanoparticles.

Introduction

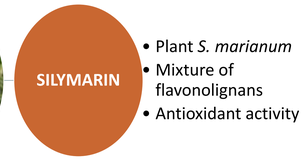

Figure 1: Plant *Silybum marianum* . Abouzid; Ahmed, (2016).

SILYMARIN

• LOW WATER SOLUBILITY AND LESS BIOAVAILABILITY;

NANOENCAPSULATION

SOLID DISPERSION

Objective

The present study aimed to obtain and characterize silymarin-loaded nanoparticles by the solid dispersion.

Materials

The materials and equipment that was used to carry out the present work are available in the laboratories of da Central Analítica Multiusuário (CAMulti-CM) and Post-Graduation Program of Food Technology (PPGTA).

NANOPARTICLES

- Silymarin (Sigma-Aldrich)
- Poloxamer 407 (Sigma-Aldrich)
- Surfactant Tween 80 P.S. (Dinâmica)

FTIR

- Potassium bromide (KBr, Sigma-Aldrich)

Methodology

➢ **DEVELOPMENT OF NANOPARTICLES**

The nanoparticles were obtained by the solid dispersion technique described by De Almeida et al. (2018), with modifications.

➢ **NANOPARTICLES CHARACTERIZATION**

Differential Scanning Calorimetry (DSC, Perkin Elmer 4000)

Heated from 0 to 400 ° C with a rate of 10 ° C.min^{-1} and nitrogen flow of 50 mL.min^{-1}

Figure 2: DSC

Infrared Spectroscopy Fourier Transform (FTIR, Frontier Perkin Elmer

2 mg of compound were weighed to produce potassium bromide tablets conducted with a resolution of 1 cm $^{-1}$ from 4000 to 400 cm $^{-1}$

Figure 3: FTIR

Results

➢ **Differential Scanning Calorimetry (DSC)**

DSC thermogram demonstrated that the melting peak of silymarin was not detected in the physical mixture or in the nanoparticles, being able to suggest its conversion from the crystalline physical state to the amorphous, this fact being indicative of the efficient encapsulation of the compound inside the encapsulation matrix (Figure 4).

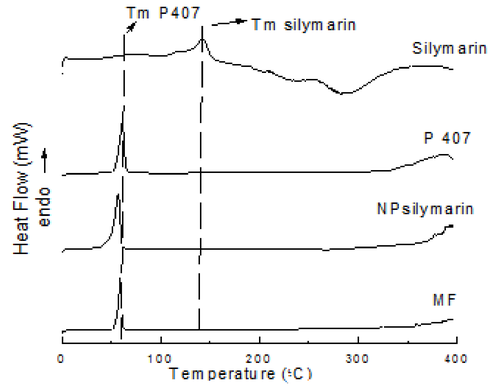

Figure 4: DSC thermograms for silymarin, P 407, NP, MF

➢ **Infrared Spectroscopy Fourier Transform (FTIR)**

FTIR was used to investigate possible chemical interactions between the reagents used in the production process of silymarin nanoparticles.

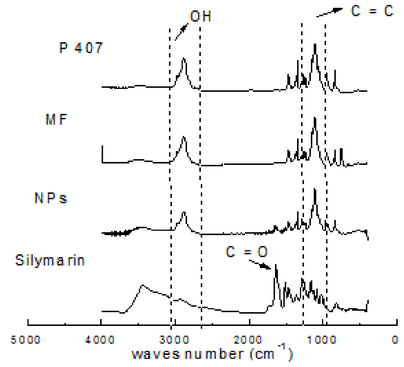

Figure 5: FTIR spectrum.

The FTIR spectrum of silymarin showed a peak at approximately 1600 cm-1, a characteristic peak of silymarin at approximately 3000 cm-1, and between 1460-1500 cm^{-1} (Figure 5).

Conclusions

- Characterization analyzes showed that silymarin was efficiently encapsulated in the polymeric matrix, going from a crystalline state to a solid amorphous solution, forming the nanoparticles.
- Further works in the group will focus on the bioactivity of the nanoencapsulated silymarin in *in silico, in vitro, in vivo* and *ex vivo* tests.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. Authors also thank “Central Analítica Multiusuário da UTFPR Campo Mourão (CAMulti-CM) for the characterization analyses”.

CHEMICAL AND BIOACTIVE CHARACTERIZATION OF *IMPATIENS BALSAMINA* L. PINK FLOWERS AND THEIR APPLICATION IN A PORTUGUESE PASTRY PRODUCT

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Abstract

The rose petals of the species *Impatiens balsamina* L. were investigated. In this way the nutritional composition, phenolic profile and its bioactive composition were evaluated. Next, the extract obtained was applied to a product of the Portuguese pastry "*bombocas*". Proving to be a possible promising natural colour for the food industry.

Keywords: *Impatiens*, Bioactivities, Natural ingredients.

Introduction

Edible flowers have been exploited and applied in cosmetics, pharmaceuticals and especially gastronomy, in line with the growing demand for safer and healthier foods [2]. The genus Impatiens is popularly known for two attractive flowers, and preliminary studies have demonstrated the bioactive potential of these plants [3]. Thus, the present work focused on the nutritional properties of *Impatiens balsamina* flowers, followed by phenolic characterization and the study of bioactivity. Finally, the extract was tested as a natural dye for the "bombocas" filling (**Fig.1**).

Materials

Pink flowers of the *I. balsamina* were collected in a public park and identified in the herbarium FLOR (Brazil). The petals were carefully removed, frozen, lyophilized and crushed. The samples were stored in a cool, dry place and protected from light.

Methods

The nutritional value (ash, protein, fat, and carbohydrate content, and energy value, by AOAC methodology) and the phenolic compounds profile (by High-Performance Liquid Chromatography coupled with a diode array detector and mass spectrometry by electrospray ionization - HPLC-DAD-ESI/MS) of *I. balsamina* L. pink petals were determined. Moreover, the antioxidant, antimicrobial, cytotoxic, and anti-inflammatory evaluation (by the oxidative hemolysis inhibition assay - OxHLIA, microdilution method with ATCC strains, the sulforhodamine B method in four human tumour cell lines, and analysis in macrophage cells of rats (RAW 264.7), to inhibit the production of NO, respectively) in the hydroethanolic extracts was also accessed. Finally, the enriched-coloured extract was applied as a colorant in a cake filling called "bombocas", and its colorant capacity was compared with an artificial additive (E163).

Results

In the pink petals, proteins stood out as the main macronutrient, and only fructose and glucose were found in sugars profile. As for the phenolic composition, eighteen compounds were tentatively identified, five non-anthocyanin compounds (caffeic and coumaric acids, and eryodictiol-*O*-hexoside) and ten anthocyanin compounds (mainly acylated *O*-glycosylated malvidin, pelargonidin, and peonidin derivatives).

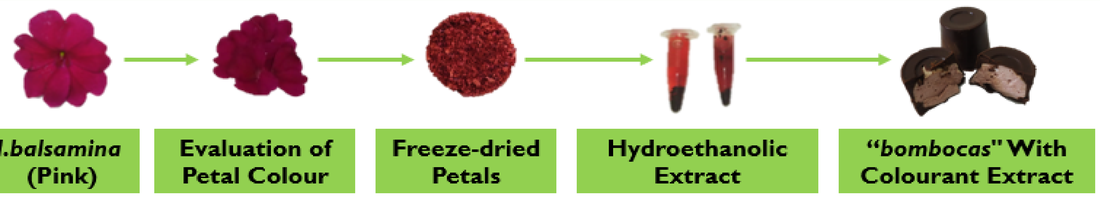

Fig.1 Elaboration stages of the colourant extract of *Impatiens balsamina* flowers.

In addition, the hydroethanolic extracts demonstrated anti-inflammatory and cytotoxicity for all cell lines studied, presenting also a remarkable antifungal activity (**Table 1**).

Table 1. Bioactivities of petal extract.

	Petals Extract
Tumour cell lines (GI_{50} values; µg/mL)	
HeLa	90.4 ± 5.5
HepG2	134.9 ± 9.2
MCF7	154.9 ± 14.5
NCI-H460	167.2 ± 12.5
Non-tumour cell lines (GI_{50} values; µg/mL)	
PLP2	>400
Anti-inflammatory (GI_{50} values; µg/mL)	
RAW264.7	163.5 ± 6.8
Antioxidant activity (IC_{50} values; µg/mL)	
Oxidative hemolysis inhibition assay(OxHLIA)	29 ± 2

Finally, the coloured extract applied in the formulations conferred a more natural colour to the "bombocas" (**Table 2**), as also functional properties such as antioxidant activity.

Table 2. Shelf life of the bombocas.

Formulations	DAYS AFTER PREPARATION		
	First Day	Third Day	Seventh Day
Control (BC) (No added colorants)	 BC-T0	 BC-T3	 BC-T7
Strawberry (BS) (With E163 colorant)	 BS-T0	 BS-T3	 BS-T7
Impatiens (BI) (With *I. balsamina* extract)	 BI-T0	 BI-T3	 BI-T7

Conclusion

The hydroethanolic extract of the pink flowers of the species *I. balsamina*, showed auspicious characteristics as a source of bioactive compounds, particularly anthocyanins. Furthermore, its incorporation as an alternative colouring agent in pastry formulations has been able to contribute to a more natural aspect of the product. Indicating that these flowers, can be exploited in the future by the food industry as a natural colouring agent. However, for this to be possible, an in-depth study of the best extraction methods is required, as well as optimum conditions for the yield and stabilization of its phenolic compounds, in particular anthocyanins.

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Acknowledgements

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ANTIMICROBIAL SENSITIVITY OF *Escherichia coli* TO COFFEE EXTRACTS (*Coffea arabica* AND *Coffea canephora*)

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Abstract

Plant extracts as coffee is recognized by its bioactive potential. In this research, the antimicrobial activity of extracts from *Coffea arabica* roasted coffee (AR), *Coffea canephora* roasted coffee (CR) and *Coffea canephora* green coffee (CG) were evaluated against the growth of *Escherichia coli* ATCC 43888 (reactivated in BHI broth, 35 ± 1 °C/12 h). Strain suspension were standardized at 10^8 CFU mL^{-1} for sensitivity testing by agar diffusion [1] [2]. Coffee extract solutions were prepared in sterilized distilled water (0.5, 1.5, 2.0, 2.5 and 5.0% (m v^{-1})), which was impregnated in sterile disks (20 µL). Sterile commercial antibiotics disks (amoxicillin clavulanic acid, trimethoprim and ampicillin) were tested as a positive control. The strain suspension was inoculated (100 µL) on the surface of Mueller-Hinton agar in Petri dishes and spread. The sterile disks impregnated with coffee extracts and sterile antibiotics disks were deposited in the inoculated agar; the inverted plates were incubated at 35 ± 1 ° C for 16 to 20 h. Sequentially, the plates were stored under refrigeration from zero to twenty days. The presence of a translucent inhibition halo around the deposited disks suggests growth inhibition. *E. coli* was sensitive against all coffee extract from 0.5% to 5.0%, but in concentrations greater than 1.5%, larger halos were obtained for all coffee extracts, and no differences were observed in the concentration range from 1.5% to 5.0%. Comparing the studied coffee extracts, the CR had a greater inhibition evidenced by greater inhibition halos below 5.0% coffee extract. Moreover, *Escherichia coli* were sensitivity to the positive control. The results observed for CR is probably associated with the effect of melanoidins, produced during roasting. The antimicrobial action of coffee extracts against *E. coli* opens new possibilities for the use of these extracts as a compound to microbial growth control in foods.

Introduction

Coffea arabica roasted coffee (AR), *Coffea canephora* roasted coffee (CR) and *Coffea canephora* green coffee (CG)

Antimicrobial sensitivity of *Escherichia coli*

https://www.laboratoriogeditec.com/bacteria-escherichia-coli/

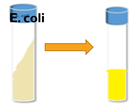
- Brazil is recognized as the main worldwide coffee producer and exporter, and the second greater coffee consumer.
- Plant extracts ingredients as coffee have the characteristic of being natural, safe, and healthy, mainly due its antioxidant activity ensured by the presence of bioactive compounds as chlorogenic acids, caffeine, and melanoidins, which may also have antibacterial activity.
- Microorganisms as *Escherichia coli*, of great concern to the food industry and involved in food infections, have been tested against coffee extracts.
- It is important to explore the coffee extracts effect against strains as *Escherichia coli*, providing identification of bioactive molecules responsible for the antimicrobial activity.
- This study evaluated the effect of roasted *Coffea arabica* and roasted and green *Coffea canephora* extracts on the inhibition of *Escherichia coli*.

Materials

- Culture media: Brain Heart Infusion broth (BHI); Plate Count agar (PCA); Mueller-Hinton agar (MH);
- Sterile disks (6,3-6,4 mm of diameter and 320-350 g m^{2-1} of grammage; Kaj Lab, São Paulo, Brasil);
- Sterile antibiotic impregnated disks [(5 µg of amoxacillin clavulanic acid (Sensibiodisc, Norbrook, Brazil), 5 µg of trimethoprim and 10 µg Ampicillin (Laborclin, Paraná, Brazil)];
- 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Darmstadt, Germany) was used as redox indicator for colony differentiator on Petri dishes;
- Ultrapure water was obtained with an ultra-purifier system (18.2 MΩ cm resistivity, Master System®, Gehaka, São Paulo, Brazil);
- Strain of *Escherichia coli* ATCC 43888.

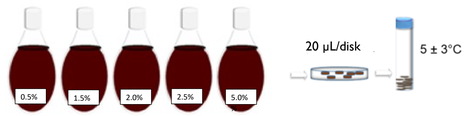
Methodology

1. Strain activation (10^8 CFU mL^{-1})

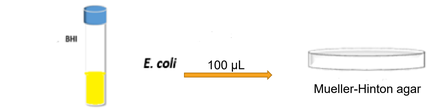


BHI broth, 35 ± 1 °C/12 h

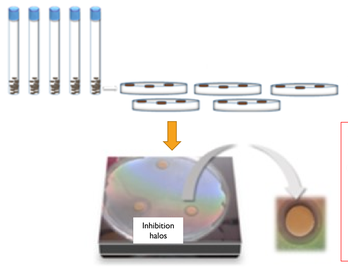
2. Coffee extract solutions and sterile disks impregnation



3. Strain Inoculation



4. Disks impregnated deposition in the inoculated agar.



Translucent inhibition halo around the deposited disks suggests a microbial growth inhibition. The inhibitory effect was evaluated at zero and twenty days of storage under refrigeration.

Results

Table 1. Coffee extracts characterization.

Coffee extract	Caffeine (g 100 g^{-1})	5-Caffeoylquinic acid (g 100 g^{-1})	Melanoidins (AU)	pH	Luminosity (L*)	Hue (h°)
AR	$2.82^{b} \pm 0.04$	$1.35^{b} \pm 0.07$	$0.638^{b} \pm 0.001$	$4.68^{b} \pm 0.01$	$40.97^{b} \pm 0.16$	$71.04^{c} \pm 0.09$
CR	$3.30^{a} \pm 0.06$	$0.67^{c} \pm 0.04$	$0.686^{a} \pm 0.001$	$4.65^{c} \pm 0.01$	$40.00^{c} \pm 0.20$	$71.32^{b} \pm 0.06$
CG	$3.45^{a} \pm 0.15$	$6.76^{a} \pm 0.26$	$0.302^{c} \pm 0.001$	$4.73^{a} \pm 0.01$	$48.97^{a} \pm 0.12$	$74.69^{a} \pm 0.05$

AR: *Coffea arabica* roasted; CR: *Coffea canephora* roasted; CG: *Coffea canephora* green; results expressed by mean ± standard deviation (n = 3); different lowercase superscript letters in the same column indicate difference by Tukey test ($p \leq 0.05$).

- CR had higher content of caffeine and melanoidins, with a darker color, and a lower content o 5-CQA.
- CG had lower content of melanoidins and a lighter color, and the higher content of 5-CQA.
- AR had the lower content of caffeine, a higher content of melanoidins with a dark color, and an intermediate content of 5-CQA.

Table 1- Results of halo diameter (in mm) for *Escherichia coli* against the coffee extracts.

Coffee extract	Concentration (%; w v^{-1})				
	0.5%	1.5%	2.0%	2.5%	5.0%
AR	9.1 ± 0.1^{bB}	11.5 ± 0.6^{aA}	11.6 ± 0.4^{aA}	10.8 ± 1.0^{bAB}	11.2 ± 0.8^{aA}
CR	11.1 ± 0.4^{aB}	11.8 ± 0.8^{aAB}	12.3 ± 0.7^{aAB}	12.7 ± 0.5^{aA}	12.5 ± 0.2^{aAB}
CG	7.8 ± 0.2^{cB}	11.5 ± 0.8^{aA}	10.3 ± 0.6^{bA}	10.3 ± 0.3^{bA}	11.4 ± 0.8^{aA}

AR: *Coffea arabica* roasted; CR: *Coffea canephora* roasted; CG: *Coffea canephora* green; results expressed by mean ± standard deviation (n = 3); different lowercase superscript letters (same column) indicate difference among the coffee extracts in the same concentration (Tukey test, $p \leq 0.05$); different capitalized superscript letters (same line) indicate difference among concentrations of the same extract (Tukey test ($p \leq 0.05$).

- E. coli was sensitive against all coffee extract from 0.5% to 5.0%, but in concentrations greater than 1.5%, larger halos were obtained for all coffee extracts, and no differences were observed in the concentration range from 1.5% to 5.0%.
- Comparing the coffee extracts, the CR had a greater inhibition evidenced by greater halos below 5.0% coffee extract. This extract presented the highest content of melanoidins, produced during roasting, indicating that the inhibitory activity may be associated with these compounds.

❖ *Escherichia coli* were sensitivity to the positive control.

Inhibition halos of 16.64 ± 3.01 mm, 26.83 ± 2.60 mm, and 24.11 ± 3.36 mm were obtained for *Escherichia coli* against the antibiotic ampicillin, trimethoprim and amoxicillin clavulanic acid.

Conclusion

The antimicrobial sensitivity of *Escherichia coli* to coffee extracts were evidenced in this work and was more pronounced for *Coffea canephora* roasted coffee (CR). These extract presented the highest content of melanoidins, produced during roasting, indicating that the inhibitory biological activity is probably associated with these compounds.

The antimicrobial action of coffee extracts against *E. coli* opens new possibilities for the use of these extracts as a compound to microbial growth control in foods.

Recommendations

- Explore the coffee extracts effect against other strains, providing identification of bioactive molecules responsible for the antimicrobial activity;
- Evaluate growth kinetics in culture media, after the strain's sensitivity evaluation;
- Study the effect of coffee extracts under kinetic growth parameters using predictive models;
- After evaluating the strain sensitivity and growing kinetics tests in culture media, perform validation in a food matrix.

Acknowledgements

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Application of guabijú powder (*Myrcianthes pungens*) in ice cream development: Physico chemical approach

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Abstract

The *guabijú* (*Myrcianthes pungens*) is a fruit native from Brazil, characterized by its pleasant sweet flavor, yellow pulp and purple skin in ripe stage. The fruit has potential for application in food products, such as ice cream. In this study three ice cream formulations were developed with *guabijú* lyophilized in powder. The formulations were characterized in terms of proximal and bioactive composition. The results demonstrated a potential use of *guabijú* in product development, since it has natural antioxidants.

Introduction

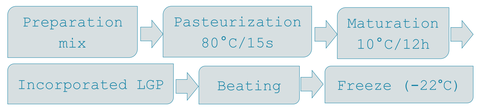
The use of natural ingredients in the development of food products is of interest to industries to meet the consumers demand for healthier foods. Fruits have essential health elements, such as bioactive compounds and may improve the sensory characteristics of products developed. Some native species are little known, such as the *guabijú* berry fruit (*Myrcianthes pungens*). Native from Brazil, *guabijú* is characterized by its pleasant sweet taste, yellowish and juicy pulp. The ripe fruit has velvety bark with dark purple color (figure 01). The berry is also reported as a source of bioactive compounds, such as anthocyanins, which are associated with antioxidant activity. Additionally, ice cream is a popularly consumed milk product, which has a suitable matrix for adding healthier ingredients, making it more nutritious. The objective of this study was developed ice cream formulations with lyophilized guabijú powder (LGP), and determine the proximal composition and bioactive compounds of the formulations.

Figure 01: Ripe Guabijú

Materials

Guabijú fruits were collected in the city of Francisco Beltrão, PR (Brazil). Ripe fruits with no skin defects were selected, which were submitted to a freeze drying process at -40°C for 48h. For application in ice cream, the dried fruits milled until a fine powder was obtained.
The ingredientes for ice cream, UHT whole milk, whole powder milk, crystal sugar, vegetable fat, glucose syrup, stabilizer and emulsifier, were purchased at the local store. Three ice cream formulations were developed with 4%, 8% and 12% LGP concentration, according to figure 02.

Figure 02: Ice cream processing



Methodology

Chemical physical parameters

Parameters of moisture, ash, total lipids, crude proteins and carbohydrates were determined according to the methodology official.
The antioxidant activity was determined using the 2,2-diphenyl-1-picryl-hydrazil free radical capture method (DPPH). The absorbance of a DPPH solution and the extracts from the ice cream samples were measured in a UV-Vis spectrophotometer at 515 nanometers (nm). The result was expressed as EC50.
The total anthocyanin was determined by measured the absorbance of the samples of the ice cream extracts in a UV-Vis spectrophotometer at 535 nm. The result was expressed in mg of anthocyanin 100 g^{-1} of fruit.
The phenolic compounds were determined by the Folin-Ciocalteau method. The process consisted of reading the absorbance of a Gallic acid solution and the samples of the ice cream extracts in UV-Vis spectrophotometer at 765 nm. The result was expressed in mg of Gallic acid 100 g^{-1} of fruit
The data were evaluated by Analysis of Variance (ANOVA) and Tukey test ($p < 0.05$) using the software Statistica version 8.0.

Results

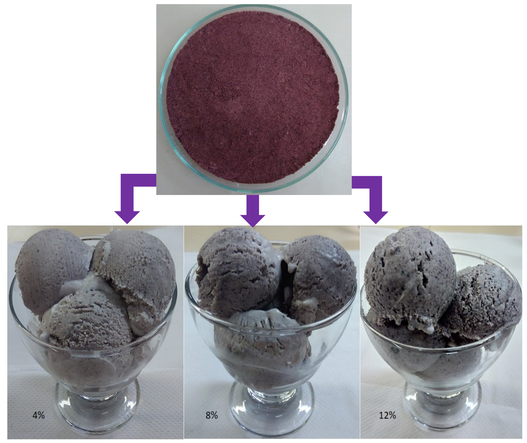
According to Table 01, the formulation with 12% LGP had the lowest content of moisture, ash and lipids and the highest content of carbohydrates. The proteins were not significantly different among the formulations. The amount of added LGP influenced the bioactive content of the samples. The formulation with 12% LGP had a higher bioactive content and consequently greater antioxidant activity compared with other formulations. Moreover, the ice cream developed with the LGP maintained a characteristic purple color of the fruit and powder obtained, as visually verified in figure 03.

Table 01: Physical chemical and bioactive characterization of ice cream with LGP

Parameters	4%	8%	12%
Moisture (g 100 g^{-1})	63.47 ± 2.12^{a}	62.08 ± 2.17^{ab}	59.24 ± 1.87^{b}
Ash (g 100 g^{-1})	1.05 ± 0.06^{a}	1.03 ± 0.03^{a}	0.85 ± 0.06^{b}
Total lipids (g 100 g^{-1})	9.47 ± 0.10^{a}	7.40 ± 0.17^{b}	6.92 ± 0.15^{b}
Crude protein (g 100 g^{-1})	3.93 ± 0.04^{a}	3.98 ± 0.11^{a}	4.00 ± 0.20^{a}
Carbohydrates (g 100 g^{-1})	13.78 ± 0.29^{c}	25.48 ± 0.41^{b}	27.02 ± 0.15^{a}
Anthocyanin (mg 100 g^{-1})	7.41 ± 1.18^{c}	10.43 ± 0.19^{b}	11.41 ± 0.66^{a}
EC_{50} (g g^{-1} DPPH)	1829.72 ± 48.27^{a}	528.82 ± 0.48^{b}	472.03 ± 3.34^{c}
PC (mg EGA 100 g^{-1})	215.36 ± 0,00^{c}	280.12 ± 5.90^{b}	317.81 ± 10.22^{a}

PC: Phenolic compounds; EGA: Equal gallic acid; Means ± standard deviations (n = 3); ^{a,b,c}: different superscript letters in the lines indicate a significant difference by the Tukey ($p < 0,05$). Source: the authors (2021).

Figure 03: LGP and formulations ice cream



Conclusion

The addition of 4%, 8% and 12% of LGP allowed obtaining ice creams with desired characteristics with as the predominant purple color of the fruit and natural antioxidants. Thus, the LGP emerging as a possibility of use in food preparations, especially in the production of ice creams.

Recommendations

As a future recommendation, different techniques can be used to extract bioactive compounds and promote improvements in the formulation of ice cream, encouraging the use of *guabijú* in the food industry.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) -Finance Code 001. The authors are thankful to the Multi-User Analytics Center of Universidade Tecnologica Federal do Paraná.

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Laccase production by *Trametes versicolor* using food and non-food wastes as substrates

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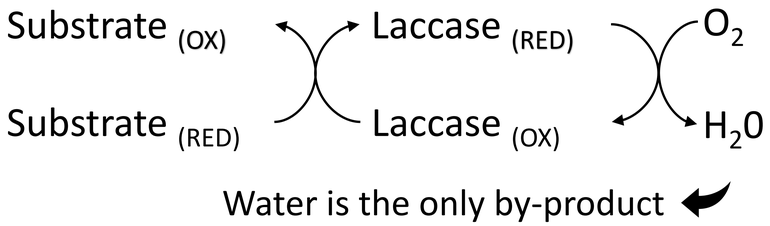
BACKGROUND

Why is the food industry interested in laccases?

✓ They promote green catalysis;



✓ Oxidize a wide range of substrates;

✓ Improve technological and sensorial properties of several food products:

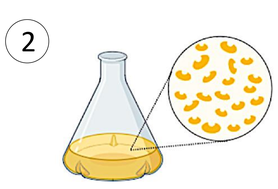
Problem and aim

Application of enzymes is hampered by their high production costs;
Hence, various wastes were evaluated as possible cheap and renewable substrates (carbon sources) for enzyme production.

PROCEDURE

Trametes versicolor cultivation conditions:

Solid-state fermentation
5 days
28 ± 2 °C
80% initial moisture



Laccase extraction and measurement

Laccase activity was expressed in U per gram of substrate.

U: the amount of enzyme that catalyzes the transformation of 1 µmol of substrate per minute.

Carbon sources (substrates) used

Pineapple wreath (**PW**); pineapple peel (**PP**); wheat bran (**WB**); passion fruit peel (**PFP**); orange bagasse (**OB**); eucalyptus sawdust (**ES**); corn cob (**CC**); sugarcane bagasse (**SB**); soy peel (**SP**).

FINDINGS

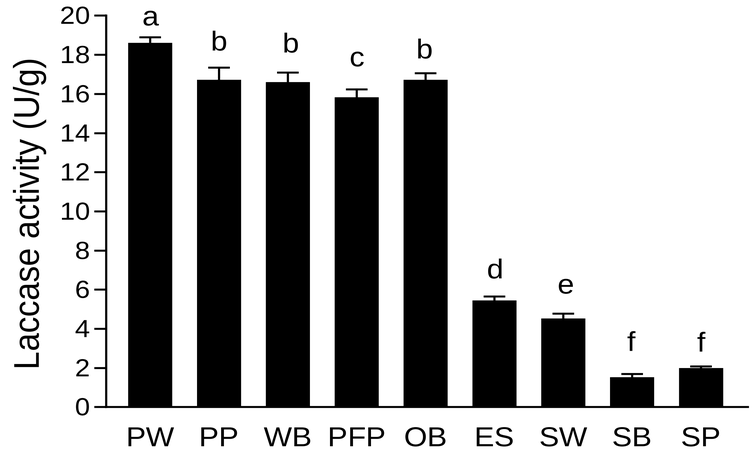


Fig. 1: Influence of the substrates used for growing *T. versicolor* on laccase production.
Columns with the same letter are not significantly different at the 5% level (Tukey's test).

PW was the most effective substrate;

Slight changes in the carbon source composition can affect the capacity of inducing laccase production.

ACKNOWLEDGEMENT

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Berry pomace: Chemical composition and applications

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Politécnico de Viseu Agrária

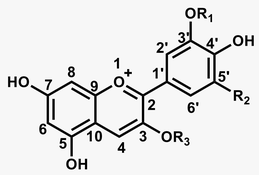
CITAB Centre for the Research and Technology of Agro-Environment and Biological Sciences

The problem

The berries juice industry produces very large amounts of pomace. Berries are a great source of a wide variety of bioactive compounds such as phenolic compounds, including anthocyanins, flavonols, phenolic acids, stilbenes, flavanols and tannins, as well as sugars, essential oils, carotenoids, vitamins, and minerals [1]. Some of those compounds are still present in high amounts in berry pomace. Despite this, large amounts of berry pomace continue to be discarded as waste or used for composting and animal feed. However, a simple way of pomace management seems to be its use in bakery and confectionery products as it can brings some evident benefits [2]. This work intends to summarize the last developments on berry pomace valorization with focus laid on the extraction of bioactive compounds and their potential food applications.

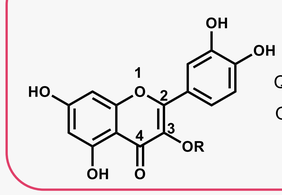
Phenolic compounds present in berries

Anthocyanins



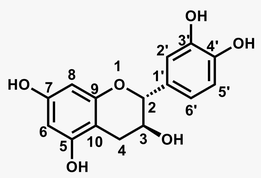
	R_1	R_2	R_3	Berry
Cyanidin-3-*O*-glucoside	H	H	glucose	Elderberry, Blackberry
Cyanidin-3-*O*-sambubioside	H	H	sambubiose	Elderberry
Cyanidin-3-*O*-rutinoside	H	H	rutinose	Blackberry, Blackcurrant
Cyanidin-3-*O*-arabinoside	H	H	arabinose	Cranberry
Cyanidin-3-*O*-sophoroside	H	H	sophorose	Raspberry
Delphidin-3-*O*-galactoside	H	OH	galactose	Blueberry
Delphidin-3-*O*-rutinoside	H	OH	rutinose	Blackcurrant
Petunidin-3-*O*-glucoside	CH_3	OH	glucose	Blueberry

Flavonols

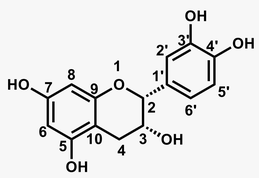


	R
Quercetin-3-*O*-glucoside	glucose
Quercetin-3-*O*-rutinoside (Rutin)	rutinose

Flavanols

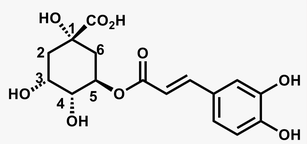


Catechin

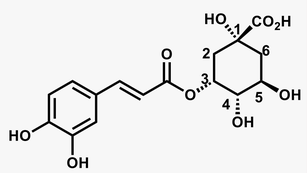


Epicatechin

Phenolic acids



5-*O*-caffeoylquinic acid (Chlorogenic acid)



3-*O*-caffeoylquinic acid (Neoclorogenic acid)

Processes

Enzymes + Heating → Mashing → Pressing → Berry Juice / **Pomace**

Peels; Pulp; Stalk and Leaves; Seeds

Food Applications

- Food fortification to enhance nutritional value and oxidative stability;
- Novel funtional food ingredientes.

Berrie pomace	Application	Reference
Rowanberry (Sorbus aucuparia L.), blackcurrant (*Ribes nigrum* L.) and elderberry (*Sambucus nigra* L.)	Shortbread cookies	[3] Tańska, M., *et al* (2016)
Black currant (Ribes nigrum) and aronia (Aronia melanocarpa)	Replace flour, sugar and fat in sponge cake characteristics.	[4] Quiles, A., et al (2018)
Black currant (*Ribes nigrum*)	Incorporation in wheat dough	[5] Struck, S., *et al* (2018)
Salal berries (*Gaultheria shallon*) and blackcurrant (*Ribes nigrum*)	Fortify a yogurt beverage with polyphenol-rich extracts from plant sources	[6] Raikos, V., *et al* (2018)
Bilberry (*Vaccinium myrtillus* L.)	Cereal based extruded snacks	[7] Höglund, E,. *et al* (2018)

Final Considerations

Valorization of berry pomace through the use of extracts rich in bioactive compounds in the food sector, provides an opportunity for circularity of natural resources and can efficiently reduce environmental stress and system sustainability recovery.

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Acknowledgements

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NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY OF BEE POLLEN SUBMITTED TO DIFFERENT PRESERVATION TECHNIQUES

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Introduction

The moisture content of bee pollen is one of the most important parameters for the preservation and quality of this product, which can vary from 18 to 25%, depending on the technique and the time of collection [1]. These humidity values can contribute to the proliferation of microbiological contamination and changes in the nutritional value of pollen, which can make its consumption and commercialization unfeasible [2 and 4]. In this study, it was intended to evaluate the impact of different preservation techniques on the nutritional value and antioxidant properties of bee pollen.

Methodology

Pollen samples were collected in Bragança (Portugal) and frozen at -20ºC. The frozen samples, which had an initial moisture content of 13.8%, were subsequently subjected to several preservation techniques: oven drying at three different temperatures (35ºC, 40ºC and 45ºC) and freeze drying. The nutritional value (moisture, ash, protein, fat and sugars), total phenolics, antioxidant properties (DPPH and reducing power) were evaluated according to the methodology described by Almeida-Muradian et al., [3] over time for a period of 9 months (1, 3, 6 and 9 months) of storage.

Results

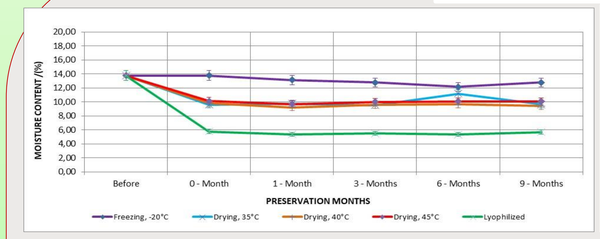

Figure 1. Nutritional value of bee pollen (moisture, ash, protein, fat and total sugars)

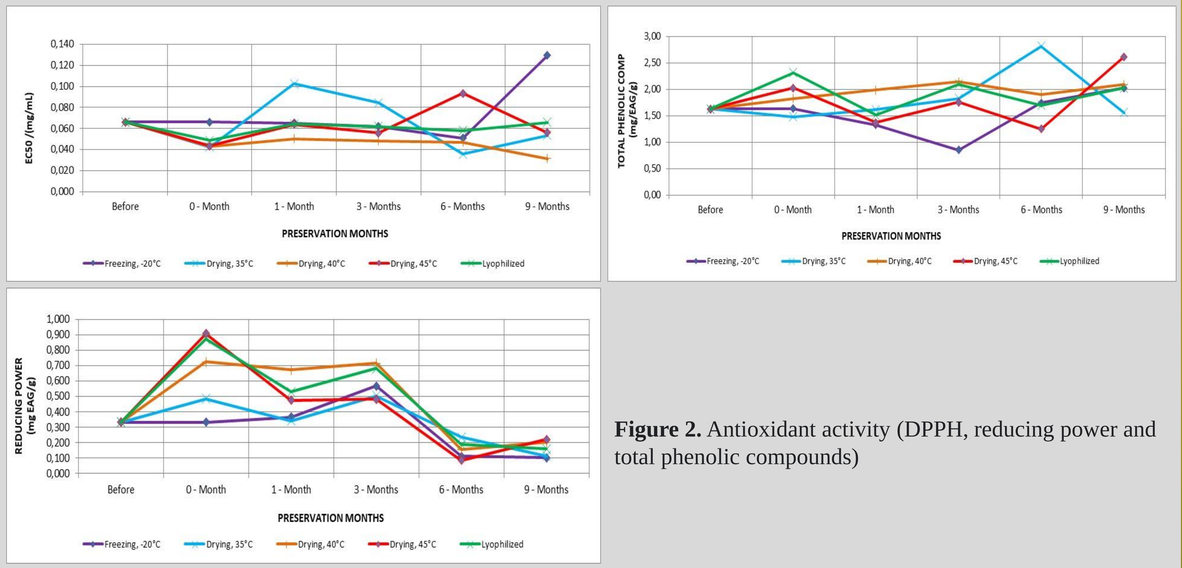

Figure 2. Antioxidant activity (DPPH, reducing power and total phenolic compounds)

Conclusions

Over the 9 month storage period, the lyophilization technique showed the best performance in terms of preserving the nutritional value of the pollen samples. On the other hand, samples preserved using the drying technique in an oven at a temperature of 45ºC showed, after 9 months of storage, a higher content of total phenolic compounds, which was reflected in a greater reducing power of these same samples. Thus, when making the decision on the preservation technique to be used, the beekeeper will have to take into account some aspects, namely: the type of product he intends to present to the consumer, as well as the cost of purchasing the equipment, since in general, the purchase of a freeze dryer will represent an increased cost compared to the purchase of a drying oven.

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KIWI BY-PRODUCTS AS POTENTIAL NEW FOOD ADDITIVES

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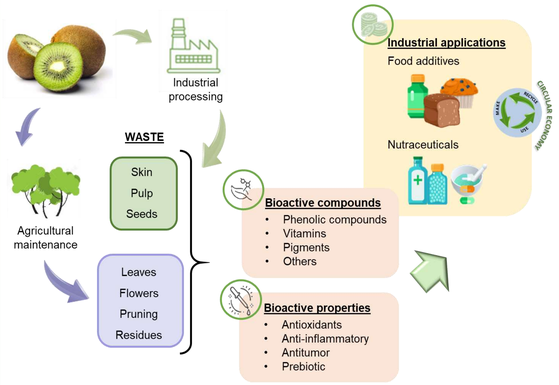
ABSTRACT

The food processing industry generates a large amount of organic waste from both crops and the industrialization of the product itself. The processing of fruits and vegetables constitutes the second generator of waste to the environment, only surpassed by wastewater. These wastes, typically accumulated in landfills, are used for composting, or they are burned, causing environmental problems. However, numerous scientific studies suggest that these residues are rich in bioactive compounds that contain many reusable substances with great economic potential.
The production of kiwi, the main fruit of the Actinidia genus (Actinidiaceae family), generates skin, pulp, seeds and pruning residues, which have been shown to have important bioactive compounds such as phenolic compounds, vitamins and pigments. These molecules possess biological properties like antioxidant, antimicrobial, proteolytic and prebiotic, among others. Therefore, these matrices could be revalued since they are novel, natural and economic sources of flavorings, colorants, proteins, coagulants, dietary fiber, antimicrobials and antioxidant compounds, which can be used in the food industry as natural food additives. Also, the substitution of synthetic additive for natural ones can cause a positive effect on consumer's health and the environment, contributing to the satisfaction of the current consumers' demand of more natural foods. This article reviews the potential of the residues derived from the industrial processing and agricultural maintenance of kiwi, as promising matrices for the development of new food additives, obtaining, at the same time, economic returns and a reduction of the environmental impact of this industry.

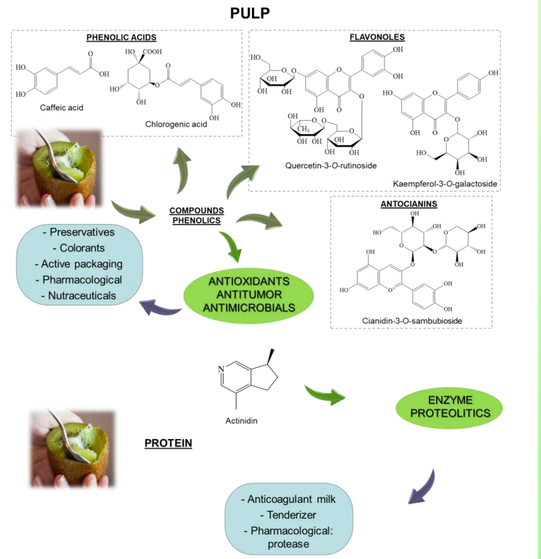
INTRODUCTION

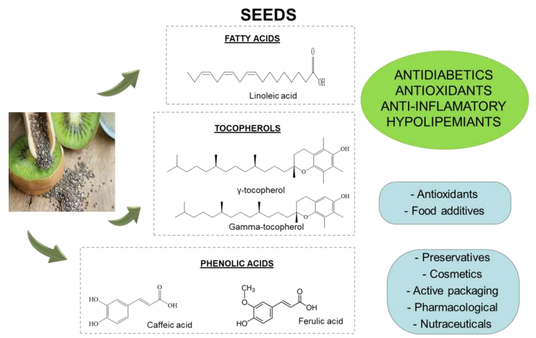
Due to the phytochemical profile and biological properties of kiwi, it can be considered as a food with great potential for valorization, promoting new applications in the food, cosmetic, nutraceutical and pharmaceutical industries [1,2]. To begin with, citric acid can be extracted from kiwi juice, being used in the food and pharmaceutical industry as an acidifying agent and flavor enhancer [3]. As regards natural antioxidants, PC (obtained from the skin of kiwi) can be used in specific concentrations, as an alternative to synthetic antioxidants [4]. Also, several articles have indicated that the injection of a kiwi-based solution (actinidin rich) confers tenderness benefits to the meat, which may be due to modifications in the myofibrillar components [5]. Another potential additive that can be extracted, in this case, from the kiwi skin, are pectins which can be used for commercial purposes thanks to their thickening, texturizing, stabilizing and gelling capacities [6]. Alternatively, BC of kiwi can be used in nutraceutical formulations or to fortify foods or beverages, mainly for its effects as a prebiotic due to its beneficial effects on digestive health [6].
The potential for kiwi waste is much broader. For example, PC have been investigated for their use as antioxidants in novel application forms, such as active packaging. On the other hand, proanthocyanins from kiwi skin have been used as insecticides, food preservatives and cosmetic additives, given their ability to inhibit tyrosinase activity [1].



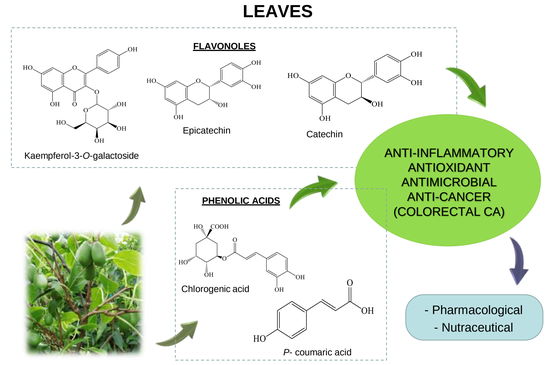
RESULTS

Residues associated with industrial production



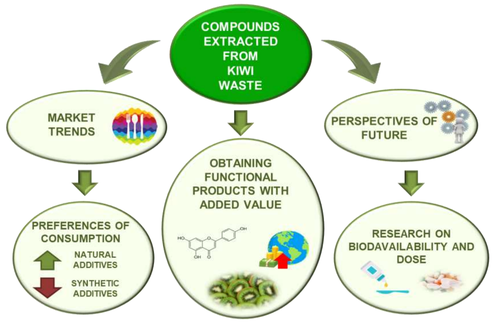


Agricultural maintenance waste



CONCLUSION

This review aims to highlight the possibility of revaluing the residues from the industrial production and agricultural maintenance of kiwifruit. The of chemical components of kiwi show a variety with beneficial health effects and so, kiwi could be considered a natural source of ingredients to develop functional products with applications such as additives or colorants, among others. In this sense, residues from the cultivation and processing of kiwi could be revalued and transformed into new products, favoring the model of a circular economy that contributes to the reduction of the biological and environmental impact and economic of the industrial exploitation of this fruit.



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Acceptability muesli formulations from *Hovenia dulcis* pseudofruits

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Abstract

Hovenia dulcis Thunb., is a tree belonging to the Rhamnaceae family, originally from China, Japan and Korea. Muesli is a very common breakfast cereal in countries like Switzerland and Germany, as well as in other countries in Europe.

As *H. dulcis* is considered as a source of dietary fiber, the present study proposed two formulations of muesli made from sugar and raisins obtained from pseudofruits from this specie. A consumer acceptance study (n=97), FI, FII and FIII formulations were tested by women (69.1%) and men (30.9%), aged 18 to 63 years, using the ordination test (preference). The pseudofruits of *Hovenia dulcis* have a sweet taste and an excellent source of dietary fiber, but it still does not have a good acceptability, when it is incorporated in a muesli formulation. Thus, its consumption in other preparations or the incentive *in natura* must be proposed.

Introduction

Hovenia dulcis Thunb., known as Japanese grape, is a tree belonging to the Rhamnaceae family, originally from China, Japan and Korea. Currently it is also found in Brazil, Argentina, Paraguay, Uruguay, United States, Cuba, Southern Europe and North Africa. Disseminated throughout the southern region, the species has adapted well to the climate and soil of Brazil [1]. The edible part of this species (the peduncle) considered as a pseudofruit, can be eaten fresh or as an ingredient in food products, such as juices, wines, vinegars, sweets and jellies or in use for nutritional fortification of bakery products, such as source of dietary fiber [2]. When dehydrated, pseudofruits can be stored for months and are energy sources that can be used in the form of raisins. Fleshy, juicy and tasty, the pulp has an aroma similar to that of pear [3]. It has functional potential because it contains significant values of polyunsaturated fatty acids, soluble fibers and phenolic compounds [4,5]. Muesli is a very common breakfast cereal in countries like Switzerland and Germany, as well as in other countries in Europe. In addition to other ingredients, muesli is composed of oats, some cereal rich in dietary fiber, which represents the major ingredient in the preparation. The nutritional composition of the muesli may vary according to the ingredients [6].

Materials

Pseudofruits from *Hovenia dulcis* Thunb. were collected in urban forest, after being sanitized and separated from the seeds, they were submitted to dehydration in an oven with forced air circulation (Cienlab, CE-220) at 45 °C for 36 hours, until the final humidity was approximately 25%. Thus, a part of dried peduncles (raisins *Hovenia dulcis* Thunb.) was obtained and another part crushed in multiprocessor (sugar *Hovenia dulcis* Thunb.). The other ingredients, used in the muesli formulations (whole grain oats, pumpkin seed, flaxseed, traditional raisin, milk and honey) were obtained at the local store, both in Pelotas-RS (Brazil.)

Methodology

For the preparation of the two muesli formulations, the dry ingredients listed in percentage in Table 1, were weighed and mixed in order from the lowest to the highest weight, so that better homogenization was then reserved. Sixty minutes before serving (sensory analysis) the wet ingredients were added, so that the grain softened.

Table 1: Tested muesli formulations and energy values.

Ingredients	FI (%)	FII (%)	FIII (%)
Whole grain oats	23.8	23.8	23.8
Pumpkin seed	7.1	7.1	7.1
Flaxseed	7.1	7.1	7.1
Traditional raisin	7.1	-	7.1
Raisin Hovenia dulcis Thunb.	-	9.5	-
Sugar Hovenia dulcis Thunb.	-	-	2.3
Milk	47.6	45.5	45.5
Honey	7.1	4.7	4.7
Energy Value (kcal/100g)	194	196	195

Sensory analysis of the two formulations was performed using pseudofruits from *H. dulcis* Thunb. (dehydrated) Figure 1, together with control to untrained judges.

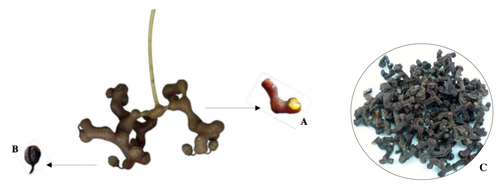

Figure 1: Pseudofruit of *H. dulcis* Thunb., fruit peduncle (A), fruit with seeds (B) and dehydrated peduncle (C).

Results

Muesli is a food that has several beneficial health effects and could be incorporated into the diet and it is a product composed of a mix of dried fruits, dried oil fruits, whole grains such as wheat, oats and rye [7]. It´s daily consumption, combined with healthy habits, can bring benefits such as lowering cholesterol levels, reducing the risk of cardiovascular disease, among others.

A consumer acceptance study (n=97), FI, FII and FII formulations were tested by:

18 to 63 years

ORDINATION TEST (PREFERENCE)

The FI formulation (control) was preferred by the evaluators, with the proposal of formulation FII (raisins) being the second preferred (Figure 2).

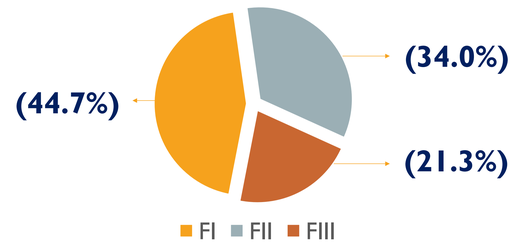

Figure 2: Percentage of preference for different muesli formulations.

Conclusion

Both formulations present as a potential product, however by sensory analysis, the formulation with whole raisins was better accepted, among the two proposals with peduncles, but the control formulation was the most preferred by the test ordination. The pseudofruits of *Hovenia dulcis* Thunb. have a sweet taste and an excellent source of dietary fiber, but it still does not have a good acceptability, when it is incorporated in a muesli formulation. Thus, its consumption in other preparations or the incentive *in natura* must be proposed.

Recommendations

For presenting excellent nutritional and functional attributes, peduncles of the species *Hovenia dulcis* Thunb. are a great choice of ingredient to be incorporated in different preparations, in which there may be a greater acceptance by consumers. Thus, it would assist the population in the adequate intake of nutrients, mainly in the content of dietary fibers and diversification of the diet. The recommendation, after the survey done in the present work, is to improve the muesli formulation, maintaining the incentive of its consumption (*in natura* and as a functional ingredient).

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Valorization of bread waste into a high-value product: Starch

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Abstract

Bread is one of the most wasted products of all food in many countries around the world due its short shelf life. In Tunisia, about 900,000 units of bread are thrown daily, at a cost estimated at $50 million each year. Thereby, bread residues present inexpensive, abundant and underutilized renewable substrate which is highly available for valorization into value-added products. In the present study, the feasibility of starch extraction from white bread waste is investigated. Chemical composition of dried bread waste used as substrate was performed. Initial moisture content, ash content, lipid content, protein content, the carbohydrate content and starch content were assessed. Extraction of starch using several procedures: artisanal, chemical, and enzymatic method with protease and cellulase were investigated. These methods were compared according to their extraction yields and the results provided by spectroscopic analysis: Infrared (IR) and Nuclear Magnetic Resonance (NMR) data. The main results showed that the enzymatic method using cellulase from *Aspergillus niger* (500 units per 10 g of grinded bread) could be an effective alternative for bread waste valorization ensuring an extraction yield of 85%.

Introduction

Bread is considered as an essential component of the human diet in many countries, in Tunisia the average consumption per person is 74 kg per year, according to data from the National Institute of Consumption (INC). On the other hand, study on "consumption of bread by Tunisian families", showed that about 900 thousand loaves are daily thrown , at a cost estimated at $50 million each year.Thereby, bread waste has probably a negative impact on the country's economy leading to relevant economic losses.

Bread Waste is highly accessible raw material for recycling processes, for the following advantages: availability, cost, and reduced contamination. Some bread wastes can be processed into animal feeding when no microbial spoilage has occurred. Numerous investigations were performed for the bioconversion of bread wastes in the purpose of ethanol fuel production, biohydrogen production, lactic acid and succinic acid porduction, hydroxymethylfurfural (HMF) synthesis, glucose fructose syrup production. Bread wastes was also described as a successful material for enzyme production such as protease and amylase using solid-state fermentation. Also as a as cheap and available substrate for baker yeast biomass production.

Starch, the principle carbohydrate constituent of most plant materials. The main starches sources are namely wheat, corn, potato, and rice, due to their ready availability and their extensive utilization in food and non-food applications

The literature contains very little information on isolation and extraction of starches from non-conventional sources such as food residues.

Considering the similar composition of waste bread and wheat, it should be possible to utilise waste bread as a feedstock for the starch extraction using an effective bioprocess.

The present work was focused in the valorization of bread residues into starch by using four extraction procedures: artisanal, chemical, and enzymatic method with protease and cellulase.

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Materials

Chemicals and Reagents

All reagents and solvents used were of analytical reagent grade and were provided by Prolabo (Paris, France). Cellulase from *Aspergillus niger* was purchased from Fluka Chemie Switzerland. Protease from *Aspergillus oryzae,* Azocasein, Carboxymethyl cellulose (CMC) and starch from corn were supplied by Sigma-Aldrich (France).

Raw material conditioning and characterization

White bread residues were kindly supplied by a local bakery and stored at 4°C. The raw material was dried for 24 h at 40 °C in a hot-air oven and then ground to a fine and homogeneous powder (less then 0.5mm). Samples were autoclaved and stored in airtight containers at room temperature

Chemical composition of dried bread waste used as substrate in this study was performed in triplicate according to AOAC standard methods (AOAC 1990). Moisture content was assessed by drying the sample at 105°C to constant weight (AOAC 925.10). Ash content was determined by calcination at 550 °C in a muffle furnace (AOAC 923.03). Lipid content was estimated after extraction with petroleum ether by the Soxhlet technique (AOAC 920.85). Protein content was determined by Kjeldahl Nitrogen Method, (Nx6.25, AOAC 928.08). Starch content was determined spectrophotometrically using a calibration curve of D-glucose. The amount of total sugars (as glucose) was measured using the DNS method after mild acid hydrolysis (70 °C, 10 min) with 80 g /L HCl solution .

Methodology

Starch extraction methods

- **Chemical method**: waste bread powder was mixed with water in a mass ratio of 1:10. The slurry was adjusted to pH 10 using NaOH (1.0M) and stirred for 1 h to solubilize proteins. Subsequently, the slurry was filtered to separate insoluble fiber and was centrifuged at 1600 g for 30 min. The aqueous phase was discarded, whereas the bottom white sediment was collected and recovered as the starch portion. The recovered starch was dried in a forced-air oven at 50 °C overnight.
- **Enzymatic methods: Two enzymes,** protease and cellulase, were used for the enzymatic extarction of starch

Waste bread flour (120 g) was soaked in water (360 ml), then 900 units of protease were added. The slurry was adjusted to pH 7.5 and incubated at 37 °C for a period of 2 h. The slurry was then centrifuged at 1000 g for 10 min. The starch fraction was suspended, washed with water and filtered through. The filtrate was centrifuged. The supernatant and tailings were discarded and the starch dried overnight at 45 °C.

A mixture of waste bread flour (10 g), cellulase (500 units) and water (30 ml) was adjusted to pH 5.0 and 40'C. Digestion was conducted for 3 hours. The mixture was then centrifuged, and the supernatant was discarded. The starch fraction was resuspended and washed with water (100 ml) and then isolated and dried at 45°C.

- **Artisanal method**: 30 g of waste bread flour were mixed with water, after maceration, the slurry was filtered. This operation was repeated many times until the liquid that flows out of the filtration is clear. Obtained slurries were collected and incubated at 4°C for 4 hours. After sedimentation, the white sediment was washed four times with water , then dried overnight at 45 °C.

Extraction yield (%)

In the aim to determine the most suitable extraction procedure, the extraction yield was calculated as follow:

Yield (%) = (Initial weight – Final weight) x 100 / initial weight

Results

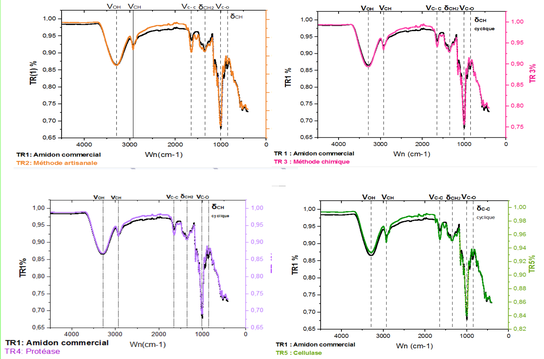
Table 1: Composition of waste bread used in this study (per 100 g)

Moisture (%)	6.77 ± 0,693
Ash (%)	1.83±0,447
Lipid (%)	6±2
Protein (TN x 5.7) (%)	13.61± 0,184
Reducing sugars (UI)	0.4070± 0,0013

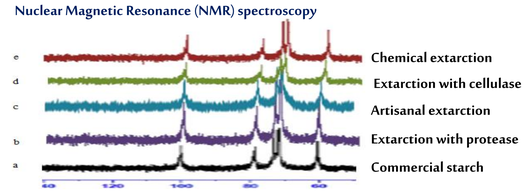
Characterization of starch extracts

Infrared (IR) spectroscopy

The figures below represent the infrared spectra of starch extracts compared with commercial starch used as a reference



Nuclear Magnetic Resonance (NMR) spectroscopy



The reference spectrum of commercial starch gives 6 signals with the corresponding chemical shifts (ppm): 100, 72, 75, 80, 70 and 60 ppm. Obtained spectra showed that starch extracts (b, c, d and e) have the same peaks and chemical shifts of commercial starch (a).

Extraction yield, swelling power and solubility of extracted starches

	Artisanal	With cellulase	With protease	Chemical
Yield (%)	84	85	89	76
swelling power (%)	14.70	14.67	23.56	15.26
Solubility (%)	5 ± 0.05	60 ± 0.6	50 ± 0.707	30 ± 0.424

Conclusion

Results of spectroscopic analysis confirm that it is the starch molecule extracted using the four adopted methods namely chemical, artisanal and enzymatic methods.

Determination of the starch extraction yields allowed us to select the most interesting and profitable method. The enzymatic method using cellulase is found to be the most interesting for recovering starch from bread waste with a yield of around 85% and a solubilizing power of 60%.

The results of this work open perspectives regarding to the transformation of extracted starch into a high added value product.

Recommendations

Declaration of interest:

The authors report no conflicts of interest. The authors alone are responsible for the content of this presentation.

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PHYSICAL PROPERTIES OF 29 COLORED POTATO VARIETIES

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Introduction

This work studied the physical properties of 29 varieties (*Solanum tuberosum* L.) of colored potatoes, analyzing their external color and the different dimensions of texture.

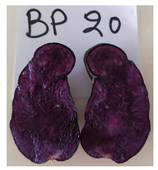
Methodology

- The external color was measured with a portable colorimeter, analyzing the CIELab color space according to the L* (lightness), a* (red-green) and b* (blue-yellow) coordinates.
- The texture was determined using a "texture profile analysis".
- The samples were classified through an analysis of variance using Tukey's test as a post-hoc.

Results

The sample "Blaue ajanhuiri", presented the lightest color in terms of the values of the parameter of a* was the cultivar with a greener tone.

The sample with the darkest color was "Blaue veltlin" variety, with an L* of 27.8.



The analysis of parameter b* showed that "Blaue Neuseeländer" sample was the one with the highest blue tone.

"Purple fiesta" the one with the highest intensity of yellow.

Table 1. Statistical analysisof colored varieties of potatoes (*Solanum tuberosum* L.)

Cultivar	L*	a*	b*	Cultivar	L*	a*	b*
Highland Burgundy Red	64±2^{efg}	9,1±2,1^{abcde}	16,1±0,6^{fg}	Blaue Bamberger Hörnchen	42±6^{abcde}	15,1±2^{cdefgh}	-4,2±1,2^{ab}
Blaue St Galler	30±3^{a}	13,05±0,4^{cdefg}	-4,33±0,5^{ab}	Fleuer Bleue	40,2±11,1^{abcd}	14,3±3,3^{cdefgh}	-3±3,2^{abc}
Hermans Blaue	37,5±1,3^{abc}	15,2±1,1^{cdefgh}	-3±0,1^{abc}	Wildkartoffel	48,3±4,5^{abcdefg}	14,5±0,3^{cdefgh}	2±1,1^{abcde}
Königspurpur	50,5±6,7^{abcdefg}	19,6±7^{fghi}	8±3^{cdef}	Blaue Veltlin	28±2,1^{a}	16,4±1,2^{defghi}	-5,6±0,66^{a}
Königsbau (Valfi)	40±6^{abcd}	15,6±3^{cdefgh}	-5,5±1,4^{a}	Blaue Hindelbank	47±4^{abcdef}	11,6±2,5^{bcdef}	0,7±2,6^{abcde}
Blaue Anneliese	28,4±5,7^{a}	12,5±4,2^{cdefg}	-2,8±0,9^{abc}	Blaue Ajanhuiri	70,4±2^{g}	-0,5±0,7^{a}	15,3±1^{fg}
Black Princess	60±22,3^{abcdefg}	6,7±5,4^{abcd}	6±9^{bcdef}	Blaue Neuseeländer	31,5±2^{a}	18,3±0,8^{efghi}	-6±0,1^{a}
Blue Star	38,3±4,3^{abc}	17,05±1,4^{efghi}	-5,5±0,9^{a}	Kefermarkter Zuchtstamm	57,2±4,2^{bcdefg}	13,1±3,3^{cdefg}	10,1±2^{defg}
Violet Queen	33,6±2,5^{a}	12,8±2,1^{cdefg}	0,1±0,6^{abcd}	Lilly Rose	45,5±2,4^{abcdef}	24,2±2,3^{hi}	14,1±2,2^{fg}
Violine de Boree	40±8^{abcd}	15±0,45^{cdefgh}	-3,5±1,8^{ab}	Black Eye	63,2±4^{defg}	6,4±3,4^{abcd}	8,6±3^{def}
Red Salad Potato	51,2±2^{abcdefg}	22±5^{ghi}	11,1±8,1^{defg}	Purple Rain	28,2±4,4^{a}	15±2,1^{cdefgh}	-3,1±0,4^{abc}
Purple Fiesta	60,6±5,5^{cdefg}	2,42±3^{ab}	20±4,5^{g}	Pink of Bolivia	48,8±4^{abcdefg}	26,1±2,4^{i}	6±1^{bcdef}
Linzer Blaue	63±5,3^{defg}	6,12±5,3^{abc}	11,1±5,5^{efg}	Purple from Congo	33,4±3,3^{a}	14,4±2,03^{cdefgh}	-4,4±0,6^{ab}
Schwarzer Teufel	46,6±19,4^{abcdef}	9±5,03^{abcde}	3±7,6^{abcde}	Blue from Peru	37±8^{ab}	15±2,3^{cdefgh}	-3,5±2,1^{ab}
Blaue Tannenzapfen	68,6±6,2^{fg}	2±5^{ab}	14±5,2^{fg}				

* L* (lightness), a* (red-green) and b* (blue-yellow)

Table 2. Statistical analysis of texture of different potato varieties (*Solanum tuberosum L.)*

Highland Burgundy Red	18158,2±5044,6^{ab}	-68±36,2	Blaue Bamberger Hörnchen	21416,9±6112,6^{abc}	-74,5±62,7
Blaue St Galler	26411,9±7406,2^{abc}	-71,5±44,6	Fleuer Bleue	25986,6±1804,5^{abc}	-109,6±8,1
Hermans Blaue	26411,9±7406,9^{abc}	-78,4±42,2	Wildkartoffel	32689,1±7050,6^{c}	-89,0±50,5
Königspurpur	22904,1±845,1^{abc}	-60,4±34,8	Blaue Veltlin	28947,7±3618,3^{bc}	-83±48
Königsbau (Valfi)	28405,5±2299,4^{bc}	-122,5±21,5	Blaue Hindelbank	22387,2±7154,1^{abc}	-99±17,2
Blaue Anneliese	14687,7±2732,1^{a}	-83±40	Blaue Ajanhuiri	30189,4±715,8^{bc}	-79,1±33,6
Black Princess	30853±5743,3^{bc}	-81,6±29,6	Blaue Neuseeländer	31647,5±4291,2^{c}	-68,8±54,1
Blue Star	30073,2±2310^{bc}	-109,1±6,4	Kefermarkter Zuchtstamm	17855,4±2724,5^{ab}	-78,7±15,8
Violet Queen	25971,9±304,2^{abc}	-110,5±16,5	Lilly Rose	22877,5±362,8^{abc}	-102,7±4,2
Violine de Boree	23689±3728^{abc}	-66,5±8,8	Black Eye	33877,8±2352,1^{c}	-94,2±9,08
Red Salad Potato	23365,9±1271,1^{abc}	-110,05±31,5	Purple Rain	22373,8±1502,8^{abc}	-104,7±17,5
Purple Fiesta	21664,2±5126,0^{abc}	-68,6±33,8	Pink of Bolivia	24052,7±1828,1^{abc}	-115,5±24,9
Linzer Blaue	29449,3±3760,3^{bc}	-22,1±3	Purple from Congo	23683±4674,2^{abc}	-144,1±34,6
Schwarzer Teufel	31815,2±2566,6^{c}	-95,2±29	Blue from Peru	27311,7±3837,3^{abc}	-85,5±11
Blaue Tannenzapfen	28287,1±4373,6^{bc}	-47,2±44,4			

*Concerning the hardness parameter, "Blaue Anneliese" and "Blaue neuseeländer" were the samples with the hardest and softest texture, respectively.

Chewiness had three different levels of significant difference with "Blaue Anneliese" being the least chewy and "Black princess" the most, while for resilience, the least resilient were "Pink from Bolivia" and "Hermanns blaue" and the most resilient was "Linzer blaue".

Figure 1. Samples of the different potato varieties

Figure 2: Powdered samples after freeze-drying of different potato varieties

Conclusion

In conclusion, the presented results showed a great variability among the tested potato varieties indicating that these could dictate different uses of the potatoes in the food industry.

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CALOCYBE GAMBOSA (FR.) DONK WILD GROWING IN SERBIA AS FUNCTIONAL INGREDIENT IN OATMEAL

Jovana Petrović[1], Ângela Fernandes[2], Dejan Stojković[1], Marina Soković[1], Lillian Barros[2], Isabel C.F.R. Ferreira[2], Aditya Shekhar[3], Jasmina Glamočlija[1*]

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Abstract

The fruiting body of edible mushroomse *Calocybe gambosa* (Fr.)Donk, wild growing in Serbia has been chemical characterized (nutritional value, pimary and secondary metabolites) and bioactive properties of its methanolic extract evaluated.

Finally, enrichment of oatmeal cookies with *C. gambosa* flakes not only improved nutritional value of cookies, but was praised among participants in sensory evaluation test.

Introduction

Mushrooms are contain a huge diversity of biomolecules with bioactive properties that should be explored.

The mushroom use
- as pharmaceutical
- as dietary supplements (DS) or nutraceutical.

To check ***Calocybe gambosa*** potential to be incorporated in food, a recipe for oatmeal cookies enriched with *C. gambosa* flakes was developed and assessed among panelist.

Methodology

- In this work the chemical characterization of *C. gambosa* was performed, including bioactive compounds (free sugars, unsaturated fatty acids, tocopherols, organic acids and phenolic compounds). The antioxidant potential of its methanolic extract was evaluated (reducing power, DPPH scavenging activity, β-caroten/linoleic acid and TBARS assay) as also the antimicrobial activity (tested towards Gram positive and Gram negative bacteria and eight microfungi).
- The anti-quorum sensing (AQ) activity (tested using *Pseudomonas aeruginosa* PAO1 as a model system).
- To check potential of *C. gambosa* to be incorporated in food, a recipe for oatmeal cookies enriched with *C. gambosa* flakes was developed and assessed among panelist

Results

This mushroom is a source of carbohydrates and proteins, with low fat content. Sugar analysis revealed presence of trehalose and mannitol. Tocopherol composition revealed presence of α-tocopherol, while fatty acid analysis revealed presence of 24 fatty acids with prevalence of polyunsaturated fatty acids. Comprehensive antioxidant analysis (reducing power, DPPH scavenging activity, β-caroten/linoleic acid and TBARS assay) indicate that mushroom is a perspective antioxidant, whereas its antimicrobial potential turned out to be moderate.

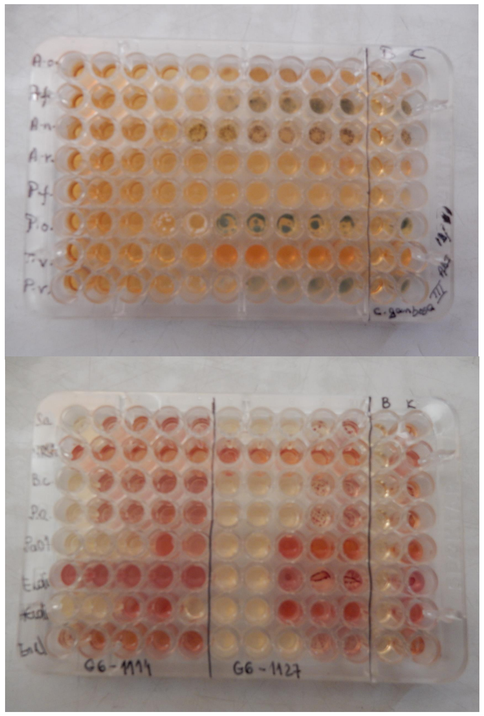

Table: Oatmeal cookies enriched with *C. gambosa* flakes.

Amount per serving (1 cookie ~ 25 g)	Cookie with *C. gambosa*	Cookie without *C. gambosa*
Fat (g/100 g dw)	3.40	3.37
Proteins (g/100 g dw)	1.82	1.47
Carbohydrates (g/100 g dw)	14.95	13.47
Energy(kcal/100g dw)	98.57	90.98
Appearance	4.66	4.25
Smell	4.25	4.25
Taste	4.66	4.25
Consistency	4.83	4.54
Overall acceptability by panelists	4.68	4.24

Conclusion

- Rising number of health issues correlated to poor diet, increased oxidative stress and number of multiresistant pathogenic microorganisms implies that medicine and pharmacy may be encountering a dead end and alternative strategies are a prerequisite for new pharmaceuticals.
- The development of new food products enriched with ingredients of natural origin (St. George mushroom) and demonstrated biological activity has been a trend in both science and food industry.

Acknowledgements

This research was funded by Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2020-14/200007.

Red seaweeds *Grateloupia turuturu* and *Porphyra umbilicalis* as nutraceuticals and functional food: nutritional/chemical composition and immunostimulatory activity

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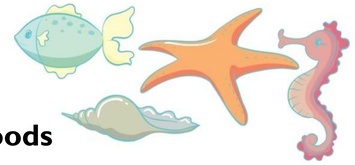
Introduction

The number of…



functional foods

nutraceuticals

…from marine origin is increasing in the worldwide market [1].

Specifically, the red seaweeds…

Grateloupia turuturu

Porphyra umbilicalis

…have shown their potential considering relevant…

bioactive compounds

nutritional value

[1,2].

Nevertheless, it is pertinent to explore knowledge gaps regarding their **nutritional/chemical composition** and **bioactivities**.

The main objectives of this work included the determination of *G. turuturu* and *P. umbilicalis* nutritional/chemical composition and their immunomodulatory potential.

Materials and Methods

For nutritional/chemical analyses

Seaweeds **proximate composition** [3] was determined as:

Dry matter (loss on drying)
Ash (ashing)
Organic matter (calculation)
Crude protein (Kjeldahl method)
Crude lipid (gravimetric method)
Total fibre (TDF), Soluble fibre (SDF), Insoluble fibre (IDF) (enzymatic kit assay)
Ethanol-soluble carbohydrates (ESC) (anthrone method)
[as % dry weight (dw)]

Followed by **lipid class composition analysis** [4] aimed at:

Phospholipids
Sterols
Free fatty acids (FFAs)
(colorimetric methods; as % total lipids)
Tocopherols (RP-HPLC-FLD; as mg/kg dw)

For testing in cell culture and further analyses

Seaweeds were freeze-dried and hydroethanolic and aqueous (infusion and decoction) extracts were prepared for the quantification of:

Mycosporine-like amino acids (MAAs) (RP-HPLC-DAD, LC-DAD-ESI-MS; as mg/g extract) [5].

Total Carbohydrates (phenol-sulfuric acid method; as % extract) [6].

control cells
cells exposed to seaweed
24 h

RAW 264.7 cell line

Immunomodulatory activity
(Griess method; as % •NO production relative to control) [7]

Results and Discussion

Proximate composition and lipid class composition

- Dietary fibre was the most abundant nutritional parameter for both seaweeds, followed by relevant ash and protein contents (Table 1).
- FFAs were the main lipid class in both seaweeds, whereas tocopherols exhibited residual levels (Table 1).

Table 1. Proximate composition and content of lipid classes in *G. turuturu* and *P. umbilicalis*.

	Seaweed	
	Grateloupia turuturu	*Porphyra umbilicalis*
Ash (% dw)*	30.98 ± 0.18	21.61 ± 0.40
Organic matter (% dw)*	69.02 ± 0.18	78.39 ± 0.40
Crude protein (% dw)*	20.16 ± 0.48	22.32 ± 0.24
Crude lipid (% dw)	1.52 ± 0.05	1.52 ± 0.05
TDF (% dw)	**40.15 ± 3.88**	**48.22 ± 3.45**
SDF (% dw)*	27.00 ± 3.40	15.07 ± 2.35
IDF (% dw)*	13.15 ± 0.48	33.14 ± 1.10
ESC (% dw)*	7.77 ± 0.41	18.96 ± 0.61
Phospholipids (% total lipids)*	1.19 ± 0.16	0.64 ± 0.03
Sterols (% total lipids)*	7.34 ± 0.61	5.53 ± 0.22
FFAs (% total lipids)*	**19.39 ± 0.43**	**6.33 ± 0.98**
Tocopherols (mg/kg dw)	0.30 ± 0.05	0.40 ± 0.22

Abbreviations: dw, seaweeds' dry weight; TDF, total dietary fibre; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; ESC, ethanol-soluble carbohydrates; FFAs, free fatty acids. * significant ($p < 0.05$) differences between seaweeds for each parameter. Data in **bold** display the most abundant parameter for proximate composition and lipid classes, for each seaweed ($p < 0.001$).

MAAs and total carbohydrates content in extracts

- *P. umbilicalis* extracts demonstrated greater MAAs content, and porphyra-334 was the main MAA.
- The aqueous extracts of both seaweeds showed higher carbohydrate contents than the hydroethanolic, with *P. umbilicalis* decoction exhibiting the highest level.

Immunostimulatory activity

- For 0.02 mg/mL, the exposure to *P. umbilicalis* aqueous extracts resulted in the most accentuated •NO production, showing immunostimulatory activity (Fig. 1).
- MAAs and carbohydrates are believed to be responsible for the immunostimulatory activity [5,6].

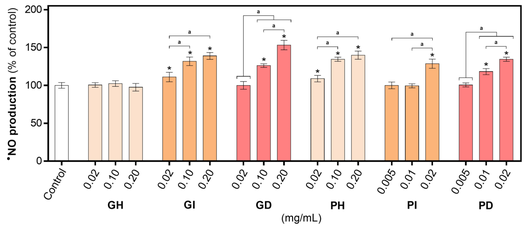


Fig. 1. Immunostimulatory activity of *G. turuturu* and *P. umbilicalis* extracts on RAW 264.7 cells. Extract concentrations were chosen according to cell viability. *G. turuturu* [hydroethanolic (GH), infusion (GI) and decoction (GD)] and *P. umbilicalis* [hydroethanolic (PH), infusion (PI) and decoction (PD)] extracts. * significant ($p < 0.05$) differences in relation to control; [a] significant ($p < 0.001$) differences among concentrations of the same extract.

Conclusions

- The most relevant nutritional parameter for both seaweeds was dietary fibre.
- FFAs were the main lipid class in both seaweeds.
- *P. umbilicalis* extracts showed greater MAAs content.
- The most concentrated MAA was porphyra-334.
- *P. umbilicalis* decoction exhibited the highest content of carbohydrates.
- The greatest immunostimulatory activity was achieved by *P. umbilicalis* aqueous extracts.

***G. turuturu* and *P. umbilicalis* potential as functional food and nutraceuticals is thus reinforced.**

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departamento de biologia universidade de aveiro · CESAM Centre for Environmental and Marine Studies · ALGA+

Controlled fermentation of curly kale juice with the use of autochthonous *Lactobacillus plantarum*

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Introduction

Recently, the interest of the functional properties of fermented fruits and vegetable products is gaining popularity among researchers, food technologist and consumers worldwide for health-related and economic reasons. As the market for probiotic fermented non-dairy products grows, new plant materials rich in biologically active compounds are being sought.

For that reason, the aim of the studies was to evaluate the impact of autochthonous *L. plantarum* JS052, isolated previously during spontaneous fermentation, on the functional properties of curly kale juice. The research included the viability of *L. plantarum* using the standard plate method, pH monitoring, the antimicrobial properties towards Gram-positive and Gram-negative pathogens.

Besides, the focus was set on the evaluation of total phenolic content (TPC) as well as the assessment of antioxidant activity determined by TEAC assay.

Materials and methods

Curly kale juice fermentation

Fresh, packed green curly kale (*Brassica oleracea* L. var *acephala* L.) was purchased in local store in Poznań, Poland. Firstly, curly kale was washed with tap water and left to dry for 3 hours at room temperature. Next, curly kale juice was obtained using low speed squeezer (Hurom, South Korea). The juice was pasteurized at 70°C for 25 minutes and cooled to the room temperature about 22°C.

The previously isolated strains of LAB - *L. plantarum* JS052 (GenBank ID: MT434011) obtained during spontaneous fermentation of curly kale juice were used for controlled fermentation process.

LAB strains were stored at -22°C in MRS medium supplementing with glycerol (20%, v/v). For activation, 1 mL of the culture was added to 9 mL of MRS broth (Biomaxima, Poland) and incubated at 30°C for 48 hours under anaerobic conditions. Then, the suspensions were used as inoculum for 200 mL of MRS broth and incubated at 30°C for 24 hours under anaerobic conditions. The cells were harvested by centrifugation (10.000 rpm, 10 min, 24°C) (Eppendorf 5804R, Germany) and resuspended in pasteurized curly kale juice to obtain final cell density of 8.35±0.05 log CFU/mL.

The fermentation process was carried out at 30°C for 24 hours.

TPC

The total phenolic content (TPC) was examined as described by (Singleton & Rossi, 1965) with modifications for measurements on 48-well microplates according to Włodarska, Pawlak-Lemańska, Górecki, & Sikorska, (2017). This assay is based on absorbance measurement at the wavelength of 765 nm using a Biotek EpochTH (Winooski,VT, USA) microplate spectrophotometer.

Antioxidant activity

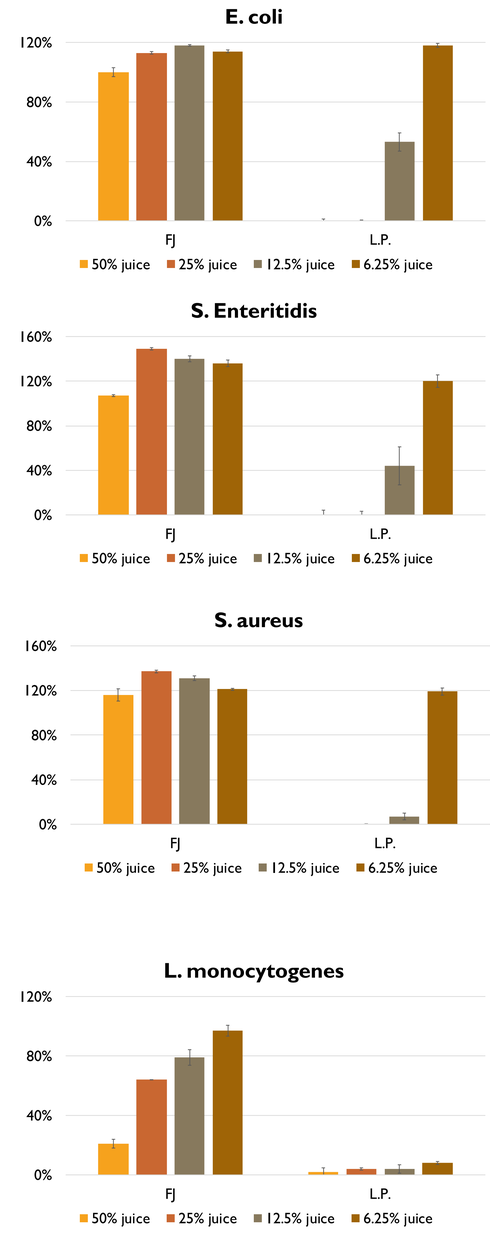
The antioxidant activity assay of fresh and fermented curly kale juice based on TEAC was performed according to the (Re et al., 1999). This method is based on the absorption decay of the $ABTS^{+}$ radical cation in reaction with centrifuged juice samples (14 000 g, 5 min; MiniSpin plus centrifuge, Eppendorf, Hamburg, Germany). The measurements were carried out using a Milton Roy Spectronic Genesys 2 (Houston, TX, USA) spectrophotometer. The TEAC values were expressed in mmol Trolox equivalent in 100 mL of curly kale juice.

Antibacterial activity

The antibacterial activity of fresh and fermented green curly kale juice was analyzed by broth microdilution method using 96-well microplates with slightly modification towards 4 indicator microorganisms - *S. aureus* ATCC 33862, *L. monocytogenes* ATCC 1911, *E. coli* ATCC 35218 and *S. enterica* ser. Enteritidis ATCC 13076. The assay was performed at juice concentration ranged from 50% to 6.25%. The microplates were incubated for 24 hours at 37°C. The optical density of the cultured bacteria was measured at 600 nm wavelength using a BioTek Epoch 2 (United Kingdom) microplate spectrophotometer. The obtained results were expressed as a percentage of positive control (100%).

Results

Firstly, the viability of *L. plantarum* increased from 8.28 log CFU/mL to 10.44 log CFU/mL after 24 hours of incubation and the pH dropped from 5.92 to 3.91. The results showed significant antibacterial properties of fermented juice supernatant towards all tested indicator microorganisms. The 50% and 25% of juice concentration completely inhibits the growth of all indicator microorganisms. In the case of *L. monocytogenes*, even the lower juice concentrations (12.5% and 6.25%) also contributed to a significant inhibition of growth. On the other hand, the lowest fermented juice concentration even stimulated growth of other tested pathogens. What is interesting, 50% of fresh juice concentration inhibited the growth of *L. monocytogenes* and *B. subtilis*, while in relation to *S. aureus* and *S.* Enteritidis the addition of the juice influenced the stimulation of better bacterial growth.
Considering the effect of lactic acid fermentation on TPC it was noticed that the 24-hour process provided a slight increase in TPC, from 119 mg GAE/100 mL for fresh juice to 132 mg GAE/100 mL of fermented juice. In turn, antioxidant activities remained unchanged at the level app. 2.88 mM/100 mL.



FJ – fresh juice, L.P. – *Lactobacillus plantarum juice

	Fresh juice	L. plantarum
pH	5.89±0.04	3.91±0.05
Viable cell counts (log CFU/mL)	x	10.44±0.11
TPC (mg GAE/100 mL)	119.04±2.23	132.01±1.85
TEAC values (mM/100 mL)	2.88±0.15	2.99±0.23

Conclusion

- The viability of *L. plantarum* increased from **8.28 log CFU/mL to 10.44 log CFU/mL** after 24 hours of fermentation.
- The pH dropped from **5.92 to 3.91.**
- The fermentation process contributed to the improved antimicrobial properties in comparison to the fresh juice among all tested pathogens, however it was dependent on the juice concentration. **The 50% and 25% of juice concentration completely inhibits the growth of all indicator microorganisms**. In the case of *L. monocytogenes*, the lower juice concentrations (12.5% and 6.25%) also contributed to a significant inhibition of growth. In relation to other pathogens, the lowest concentration even stimulated their growth.
- Lower fresh juice concentration contributed to the growth stimulation of tested pathogens.
- **According to the FAO and WHO , the antimicrobial activity of fermented products are one of the basic and crucial parameters indicating their potential probiotic properties.**
- Slight increase in TPC during 24-hour fermentation process.
- Antioxidant activity remain unchanged during process.

Acknowledgements

This work was supported by the project: "Integrated Development Program of the Poznań University of Economics and Business" no WND-POWR.03.05.00-00-Z011/17 which is co-financed by the European Union under the Operational Program "Knowledge Education Development 2014-2020".

Characterization of non-conventional food plants seeds *Guizotia abyssinica* (L.F.) Cass., *Panicum miliaceum* L., and *Phalaris canariensis* L. for application in the bakery industry

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Nádia Cristiane Steinmacher[2], Isabel C.F.R. Ferreira[1], Lillian Barros[1]

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NPA Natural products application: Health, Cosmetic and Food
Online Edition
4-5 Feb 2021

Abstract

The present work aimed to study the granulometry and water absorption index of three flour seeds of PANC, *Guizotia abyssinica* (Lf) Cass. (niger), *Panicum miliaceum* L. (millet), and *Phalaris canariensis* L. (birdseed), followed by its nutritional composition (AOAC), and composition in fatty acids (GC-FID), free sugars, organic acids, tocopherols, and phenolic compounds (HPLC-RI, DAD, fluorescence, and DAD/ESI-MS, respectively). The bioactive capacity of the hydroethanolic extracts was also assessed. Finally, bakery products were developed with partial replacement of the wheat flour (20% of the PANC's flour) and an experimental statistical design using the centroid simplex method was applied to understand the effect of applying PANC flours on the final physical-chemical characteristics of the breads.

Introduction

The use of unconventional food plants (PANC, **Figure 1**) has been an asset for the food industry, not only due to its abundance, but also because it does not compete with other vegetable matrices used for human consumption, and for its nutritional properties, chemical, and bioactive potentiality [1-2].

Rumex acetosa L. (Polygonaceae) | *Tropaeolum majus* L. (Tropaeolaceae) | *Acmella oleracea* L. R.K.Jansen (Asteraceae) | *Solanum sessiliflorum* Dun. – (Solanaceae)

Figure 1 – Examples of PANC species.

The study of new food sources and its application for the development of new food products is urgent.

Materials

For the present study, niger, millet and birdseed seeds were used (**Figure 2**).

Figure 2 – *Guizotia abyssinica* (Lf) Cass. (niger, **A**), *Panicum miliaceum* L. (millet, **B**), and *Phalaris canariensis* L. (birdseed, **C**) seeds.

Methodology

Figure 3 illustrates the methodology followed for the present work. After flour obtaining from the seeds, granulometry analysis, water absorption index followed, nutritional composition (AOAC), composition in fatty acids (GC-FID), free sugars, organic acids, tocopherols, and phenolic compounds (HPLC-RI, DAD, fluorescence, and DAD/ESI-MS, respectively) was performed. The bioactive capacity of the hydroethanolic extracts was also assessed.

Finally, bakery products with partial replacement of the wheat flour (20% of the PANC's flour) were developed and an experimental statistical design using the centroid simplex method to understand the effect of applying PANC flours on the final physical-chemical characteristics of the breads was used.

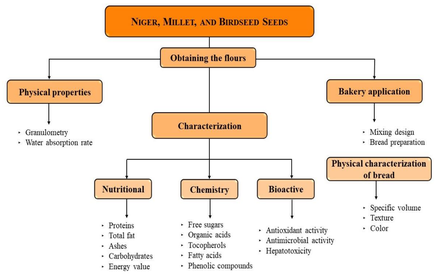

Figure 3 – Work plan.

Results

Birdseed and niger flours showed high granulometry with high water absorption index, indicating that their use in bakery should be supplemented with other flours.

Niger seed revealed higher contents in total fat, PUFA (linoleic acid), sugars, tocopherols (α-tocopherol), and phenolic compounds (derivatives of caffeic acid). All seeds presented relatively low IC_{50} and MIC values for TBARS assay and antimicrobial activity, respectively, and did not show hepatotoxicity.

The hydroethanolic extracts of niger and millet presented lower MIC values for antifungal activity when compared with the positive controls used (E211 and E224).

Breads with 20% of millet and birdseed flour presented highest similarity to the control bread (100% wheat flour), in texture, specific volume and color (**Figure 4** and **5**).

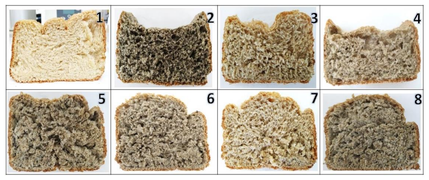

Figure 4 – Different bread formulations. Control bread (1) and loaves of bread with replacement of 20% by flour from: niger (2), millet (3), birdseed (4), niger and millet (5), niger and birdseed (6), millet and birdseed (7), and niger, millet and birdseed (8).

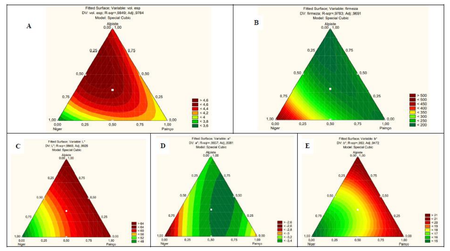

Figure 5 – Area adjusted for specific volume of bread (A), firmness (B) and color parameters for *L** (C), *a** (D) and *b** (E) of bread crumb with different proportions of niger, millet and birdseed flour.

Conclusion

Considering their composition in bioactive compounds, the use of these seeds is highly advisable in the context of a fortified diet, being sources of compounds of high nutritional value and with beneficial effects for the health of the consumer.

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Physico-chemical parameters of Algerian honeys: Rosemary, Tamarisk, Thistle and Multifloral

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NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

Abstract

Even though honey is commonly consumed in Algeria, there is lack of information about its characteristics. The aim of this study was to characterize 10 honey samples (labelled as rosemary, tamarisk thistle and multifloral honey) from two different regions of Algeria (Sidi Belabbes and El Bayedh) based on their physicochemical properties such as: moisture content, color, electrical conductivity, pH and acidity, hydroxymethylfurfural (HMF), diastase activity, and proline content.

Introduction

Honey is a natural food that is highly appreciated by consumers for its nutritional and therapeutic properties. In Algeria, honey is used both for nutritive and healing purposes, and its price reaches quite great levels, while the data of products is still deprived, and the quality control of local and imported honey is totally insufficient. This situation does not allow a safety guaranty to consumers and leads to possible frauds. The aim of this work was to increased the knowledge of Algerian honey, through the quality parameters assessment of monofloral honeys with origin in rosemary, milk thistle, and tamarisk, as well as multifloral honeys, typically found in two highlands regions of Algeria (Sidi Bel Abbès, El-Bayadh) as shown in **figure 1**.

Figura 1- Geographical origin of the honey samples

Materials

- Colorimeter
- Refractometer
- Conductivity meter
- Spectrophotometer

Reagents

- Carrez I
- Carrez II
- Sodium bisulfite
- Sodium hydroxide
- Phadebas Tablets
- Acetate buffer
- Proline
- Formic acid
- Nihinhydrin
- Propan-2-ol

Methodology

Colorimeter – Color assessment

Refractometer – % Humidity

Conductivity meter – Conductivity

Automatic tritrator – Free, Lactonic and Total Acidity

Spectrophotometer – Hydroxymethylfurfural (HMF); Proline; Diastatic Index.

Results

Samples	Color (mm Pfund)	Moisture (%)	Conductivity (mS.cm⁻¹)
Rosemary1	49 ± 0 (Extra white Amber)	13 ± 0	0.09 ± 0.00
Rosemary2	43 ± 0 (Extra white Amber)	14 ± 0	0.10 ± 0.00
Rosemary	42 ± 0 (Extra white Amber)	13 ± 0	0.11 ± 0.00
Tamarisk1	77 ± 0 (Light Amber)	16 ±0	0.11 ± 0.00
Tamarisk2	76 ± 0 (Light Amber)	16 ± 0	0.34 ± 0.05
Tamarisk3	79 ± 0 (Light Amber)	16 ± 0	0.33 ± 0.04
Milk Thisle1	61 ± 0 (Light Amber)	14 ± 0	0.24 ± 0.04
Milk Thisle2	60 ± 0 (Light Amber)	15 ± 0	0.25 ± 0.01
Milk Thisle3	72 ± 0 (Light Amber)	15 ± 0	0.25 ± 0.01
Multifloral	60 ± 0 (Light Amber)	15 ± 0	0.28 ± 0.02

Figure 2. pH, free acidity, lactonic and total aciditiy

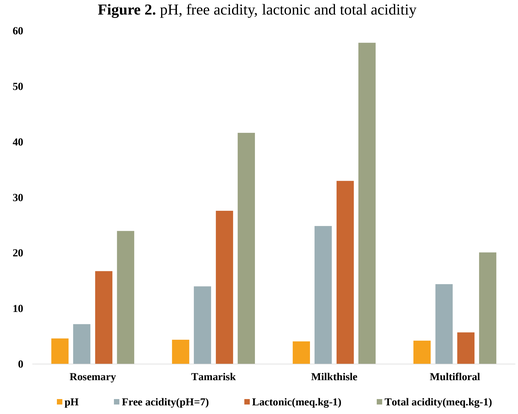
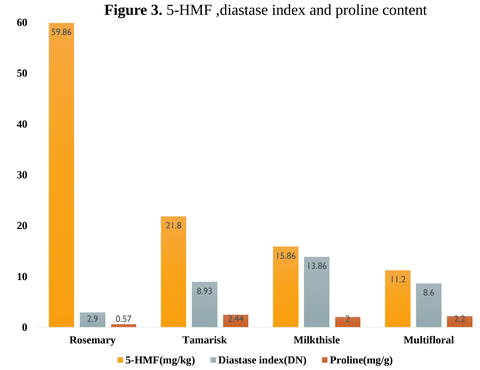


Figure 3. 5-HMF ,diastase index and proline content



Conclusion

The results show that color ranged from extra white to light amber, while the moisture falls in the interval of 13 to 16%, and the conductivity varied from 0.09 to 0.34 mS.cm^{-1}. The free acidity (calculated at pH=7) varied from 7 to 31.2 meq.kg^{-1}, with pH value ranged from 4.0 to 4.7. The amount of HMF range from 11.2 to 79.8 mg.kg^{-1} while the diastase values varied from 2.1 to 14.7 DN. The proline content ranged from 0.4 to 2.8 mg/g. Except for HMF, which in some samples was above the maximum of 40 mg.kg^{-1} permitted on Codex Alimentarius, the physicochemical parameters studied were within the quality standards established for honey.

Recommendations

Some recommendations for future study are set below:

- As future research, the application of statistical analysis will be needed for obtaining a differentiation between monofloral honeys using the chemical characteristics obtained in this research.
- The study of the Algerian honeys can be extended to more samples.
- New monofloral honeys from the Algerian flora can be involved in future research.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020). National funding by FCT- Foundation for Science and Technology, through the institutional scientific employment program-contract with Soraia I. Falcão.

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Extraction of Chlorophylls from natural sources

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INTRODUTION

The growing consumers' concern for possible long-term adverse effects of artificial molecules commonly used in food industry has led to an increased interest in natural products. At the same time, there is a demand for a more eco-sustainable use of natural matrices, which justifies the search for byproducts that have no other application to be explored in the development of novel food products [1,2]. In this context, the present study was designed to exploit natural pigments, more specifically chlorophylls, from bioresidues (aerial parts of carrot and tomato, **figure 1**) for the development of food colorants. These are the most abundant pigments in plants and present, beyond their great coloring capacity, several bioactive properties, which corroborates the importance of their application in foodstuff.

Figure1. Aerial parts of carrot and tomato (bioresidues).

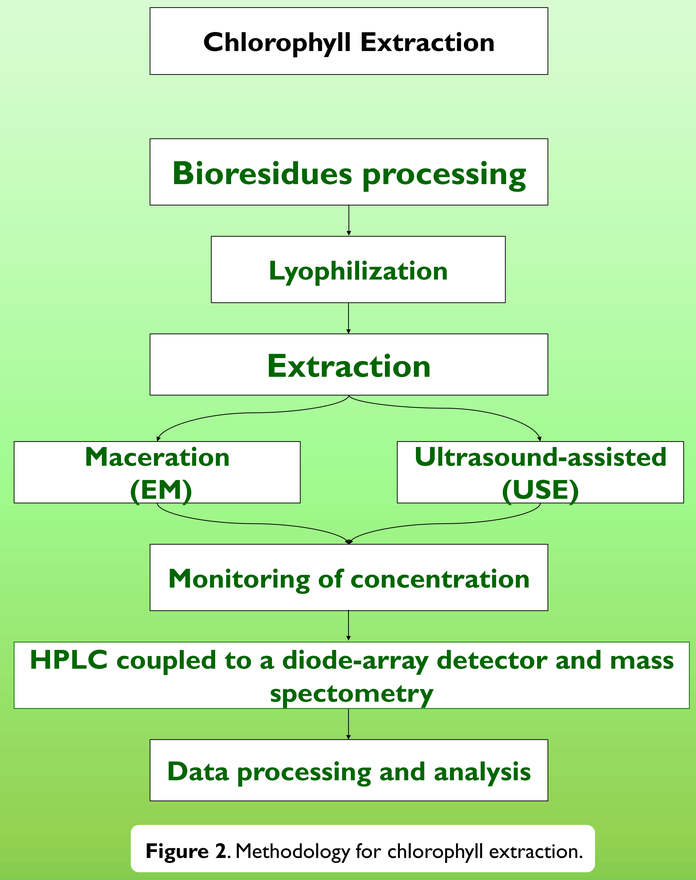

Figure 2. Methodology for chlorophyll extraction.

METHODOLOGY

The method is represented by **figure 2.** For the extraction, green solvents were assigned, namely water, ethanol (90%), and hexane. The parameters affecting the pigments recovery were varied for each technique, namely the time, power, and solvent for USE, and the time and solvent for ME. The extractions were performed protecting the samples from light and the results were monitored through the implementation of a new chromatographic method, HPLC coupled to a diode array detector (DAD) and mass spectrometry (MS), to determine the concentration of chlorophylls and the best procedure to be performed.

RESULTS

Both aerial parts presented chlorophylls and derivatives in significant concentrations and extraction yields up to 88% for the ethanolic extracts. The applied chromatographic method revealed to be appropriate for the analysis of this class of pigments, allowing a good peak resolution and separation, but also characteristic TIC spectrum for the tentative identification of the compounds. Therefore, the results of the present study can be explored for the development of chlorophyll-based colorants from these bioresidues, but also from similar byproducts. **Figure 3** shows the main extraction steps.

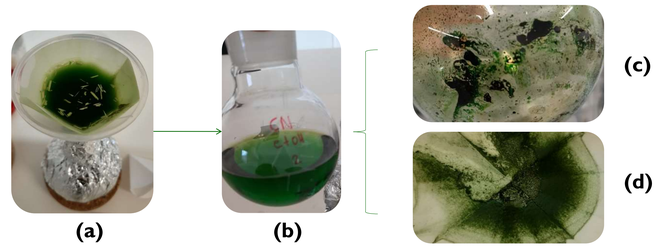

Figure 3. (a) Post-extraction filtration; **(b)** Chloroform extract; **(c)** Dried chloroform extract; **(d)** Extraction residue.

Acknowledgements

To the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020); National funding by FCT, P.I., through the individual scientific employment program-contract for C. Pereira, M.I. Dias, and L. Barros contractsand A.K. MolinaPhD grant (2020.06231.BD).To FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E and TRANSCoLAB 0612_TRANS_CO_LAB_2_P; to the European Regional Development Fund (ERDF) through the RegionalOperational Program North 2020, within the scope of Project Mobilizador Norte-01-0247-FEDER-024479:ValorNatural® and Project GreenHealth - Norte-01-0145-FEDER-000042.

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[2] N. Streit, et al., Ciencia y Tecnología, 8 (2015)27.

BIOLOGICAL CONTROL OF APPLE POST-HARVEST DISEASES BY PORTUGUESE PROPOLIS

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INTRODUCTION

Propolis is a resinous natural product made from plant exudates collected by honeybees[1] used as a building material and a defensive substance[2]. With a complex composition, propolis main components are phenolic compounds which are responsible for its various bioactivities[1], namely antioxidant and antimicrobial[1,2].

The apple culture is globally one of the most important, but diseases arising during fruit conservation - leading up to 50 % of losses[3] and increased food waste - are a big constraint to their marketing and consumption, with negative economic repercussions. Main post-harvest diseases are fungal rots, like blue mold rot, caused by *Penicillium expansum* whose control is currently made by using synthetic fungicides that are highly harmful to the environment and consumers[4]. For this, it is necessary to find equally effective, but safer and more friendly alternatives. Therefore, propolis becomes an excellent candidate for applications other than medicine, a field where it has been widely used for centuries.

References:

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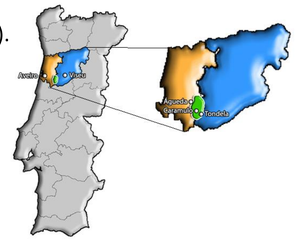
[2]Bankova V. Journal of ApiProduct and ApiMedical Science 1(2) (2009) 23-28.
[3]Marín, A. Atarés, L. & Chiralt A. Biocontrol Science and Technology 27(10) (2017) 1220-1241.
[4]Wenneker M. Doctoral Thesis. Wageningen University (2019).

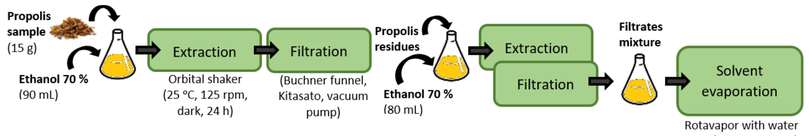
METHODOLOGY

PROPOLIS SAMPLE

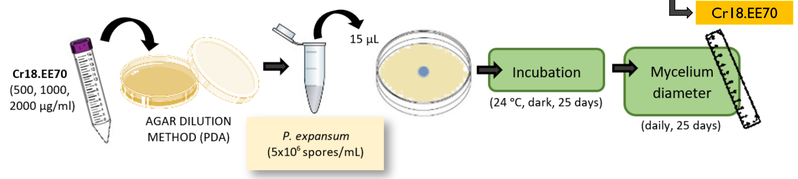
Propolis from Caramulo region, collected in 2018 (Cr.18).



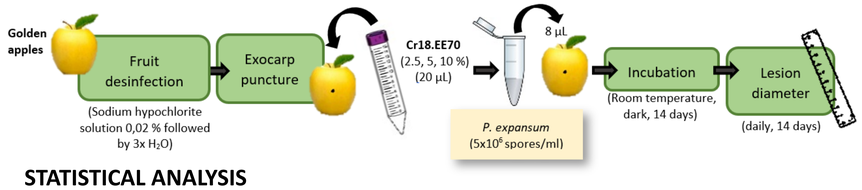
ETHANOL EXTRACTION



IN VITRO ANTIFUNGIC ACTIVITY



IN VIVO ANTIFUNGIC ACTIVITY – STORAGE ASSAY

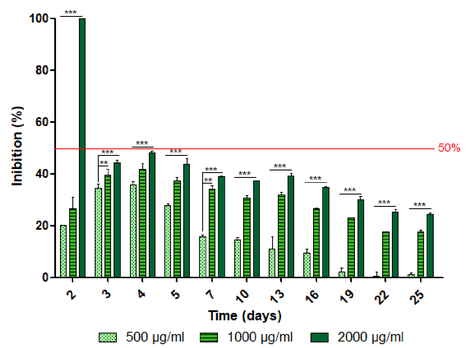


STATISTICAL ANALYSIS

Experiments were done in triplicate and results expressed as mean ± standard deviation (SD). Two-way ANOVA followed by Bonferroni test for multiple comparisons. Differences were considered statistically significant if $p \leq 0.05$, being * if $0.01 < p \leq 0.05$, ** if $0.001 < p \leq 0.01$ or *** if $p \leq 0.001$.

RESULTS

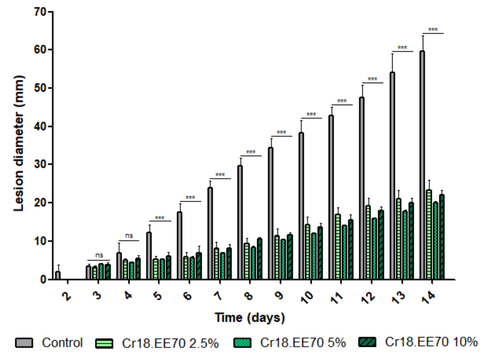
IN VITRO ANTIFUNGIC ACTIVITY

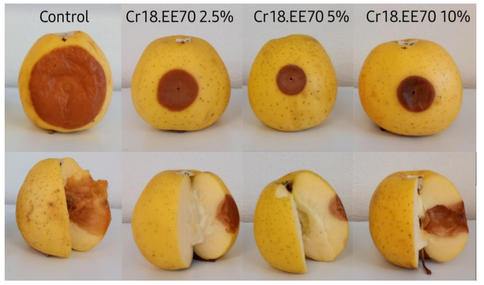


The inhibitory effect of Cr18.EE70 over *P. expansum* mycelial growth is dose-dependent.

Maximum inhibition is observed 4 days after inoculation and stayed below 50 % for 25 days.

IN VIVO ANTIFUNGIC ACTIVITY – STORAGE ASSAY





Treatment with 2.5, 5 and 10 % propolis hydroalchoolic extract (Cr18.EE70) reduced lesion diameter up to 69, 72 and 65 %, respectively, after 8 days, in comparison with the control. Although no significantly differences were found between lesion diameters, the lesion depths were different, being less deep with 5 % propolis extract.

CONCLUSIONS

- ✓ Portuguese propolis hydroalchoolic extract shows both *in vitro* and *in vivo* activity against *P. expansum*. Better results *in vivo* may be due to the higher concentrations used.
- ✓ Overall, the best results were obtained with 5 % propolis hydroalchoolic extract, showing 66.5 % reduction of lesion diameter 14 days after inoculation and a much shallower rot depth comparing to the other concentrations tested.
- ✓ Propolis may be used as a post harvest biocontrol agent in apple and probably other fruits and vegetables.

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Production and fertilization system affects the nutritional, chemical, and bioactive properties of small red fruits

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INSTITUTO POLITÉCNICO DE BRAGANÇA

CIMO Centro de Investigação de Montanha

Introduction

Small red fruits, such as blueberries (*Vaccinium corymbosum* L.) and currants (*Ribes rubrum* L.), are considered as emerging crops in Portugal, with a high growth potential. Although the consumption of these fruits in Portugal is still not very significant, there has been a reasonable increase, compared to the last century, with the growing interest of consumers in functional foods. In this sense, there is an increasing concern for production in more sustainable ways, such as organic and integrated production, replacing the conventional agriculture. This kind of production can enhance the quality of the fruits, rich in added-value antioxidant compounds, allowing to meet the most demanding consumers' expectations [1].

Methodology

Analysis and comparation

V. corymbosum  VS  *R. rubrum*

Different types of production (conventional and applying a biological fertilizer, Ecoser)

Food analysis official methodologies (AOAC) → Nutritional characterization

Fatty acids (GC-FID), free sugars (HPLC-RI), organic acids (UFLC-PDA), tocopherols (HPLC-fluorescence), and phenolic compounds (HPLC-DAD/ESI-MS).

Chemical composition

Antioxidant properties → Inhibition of lipid peroxidation (TBARS) and inhibition of oxidative hemolysis (OxHLIA) assays

Results and conclusions

The method of production was found to influence not only the nutritional parameters, but also the composition of the fruits in free sugars, fatty acids, tocopherols, organic acids, and phenolic compounds.

HIGHER LEVELS	
Conventional	Biological fertilizer Ecoser
Carbohydrates and energy Fructose and glucose Saturated fatty acids, quinic acid, and phenolic compounds	γ- and δ-tocopherol Monounsaturated and polyunsaturated fatty acids, oxalic acid, quinic acid, and malic acid

HIGHER LEVELS	
Conventional	Biological fertilizer Ecoser
Carbohydrates and energy Sucrose Polyunsaturated fatty acids and anthocyanins	Lipids, fructose, glucose, ascorbic acid Saturated and monounsaturated fatty acids, phenolic acid, and flavonoids

- Inhibition of lipid peroxidation → Currants biological way and blueberries conventional way
- Inhibition of oxidative hemolysis → Currants and blueberries cultivated in biological way

The results obtained in the present study may serve as a basis for the definition of production parameters that best fit the culture of each fruit.

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Acknowledgments

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020); national funding by FCT, P.I., through the institutional scientific employment program-contract for C. Pereira, M.I. Dias, and L. Barros contracts and A.K. Molina PhD grant (2020.06231.BD). To FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E and TRANSCoLAB 0612_TRANS_CO_LAB_2_P; to ERDF through the Regional Operational Program North 2020, within the scope of Project GreenHealth - Norte-01-0145-FEDER-000042.

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Stability Analysis of Phenolic Composition and Antioxidant Activity of Pomegranate (*Punica granatum* L.) Leaf Infusion over Long Time Storage

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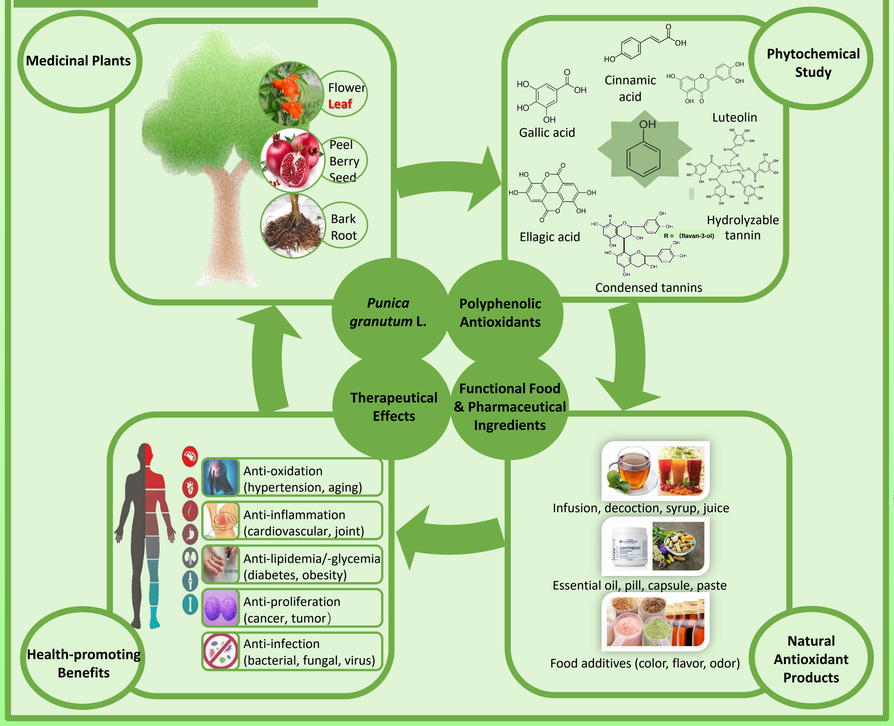
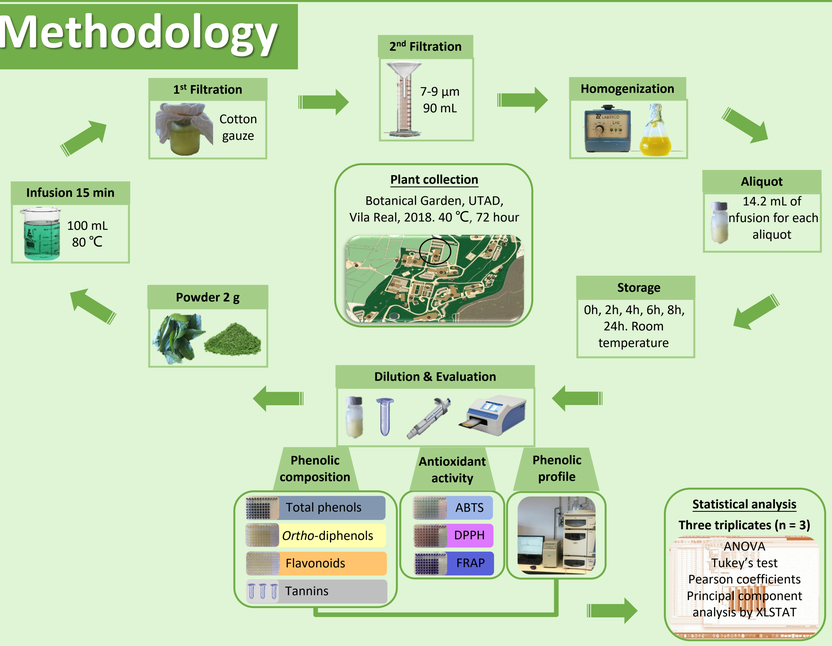
PhD AGRI CHAINS from fork to farm

Introduction

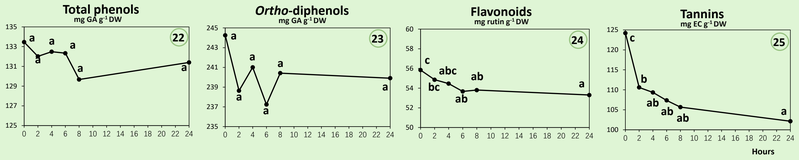


Methodology



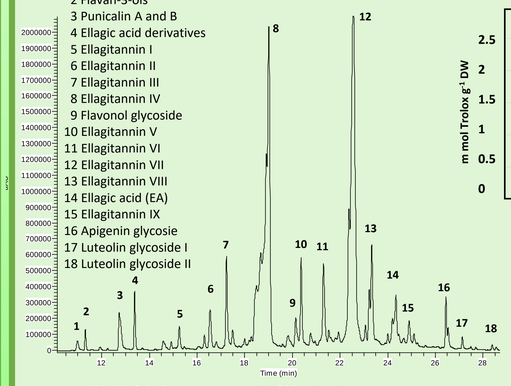
Results & Discussion

Phenolic Content



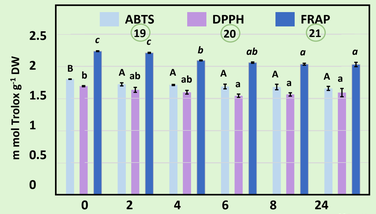
Identified Phenolics

1 Gallic acid (GA)
2 Flavan-3-ols
3 Punicalin A and B
4 Ellagic acid derivatives
5 Ellagitannin I
6 Ellagitannin II
7 Ellagitannin III
8 Ellagitannin IV
9 Flavonol glycoside
10 Ellagitannin V
11 Ellagitannin VI
12 Ellagitannin VII
13 Ellagitannin VIII
14 Ellagic acid (EA)
15 Ellagitannin IX
16 Apigenin glycosie
17 Luteolin glycoside I
18 Luteolin glycoside II



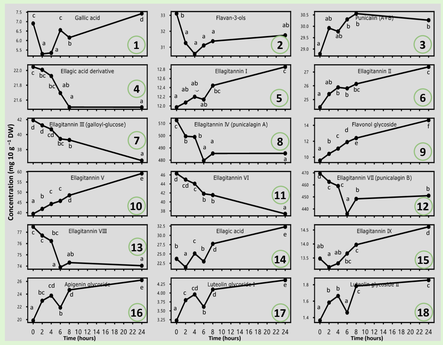
RP-HPLC-DAD chromatogram (at 280 nm) of pomegranate leaf infusion at 0h storage time

Antioxidant Activity

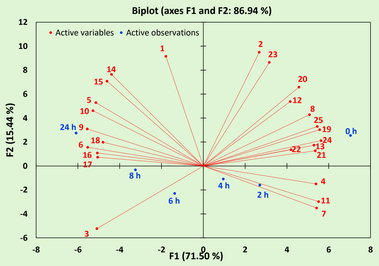


Antioxidant activities (AA) performed by ABTS, DPPH, and FRAP were found to have high retention (> 90%). AA kept stable during 4h-24h, while degraded slightly in the first 2h.

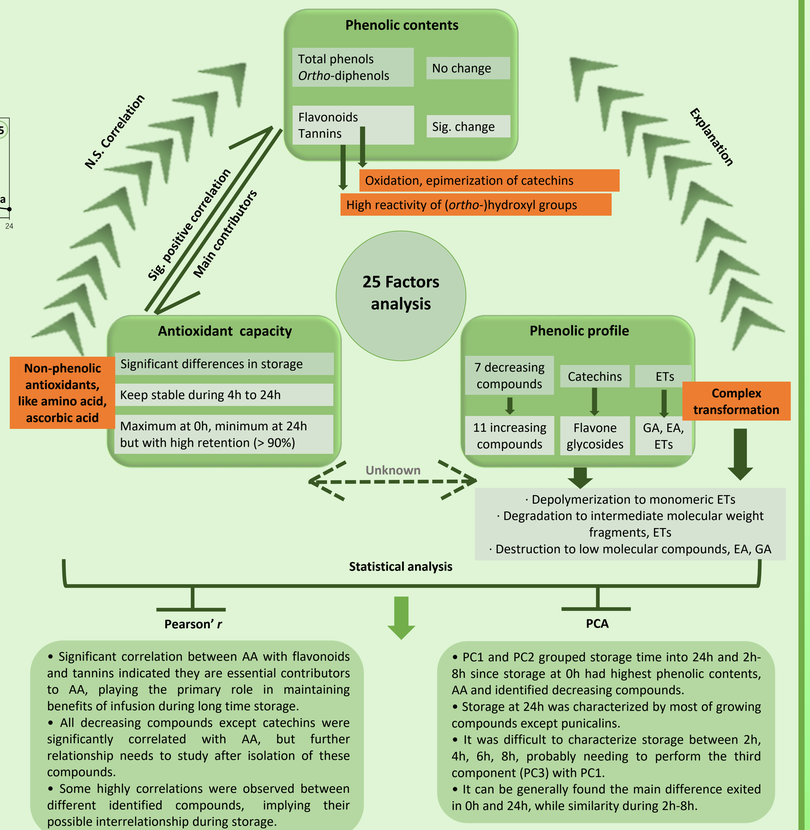
Phenolic Alteration



Principal Component Analysis



Bioplot of PC1 and PC2 describing the variation among 25 factors (vectors) and different storage time (circles) of pomegranate leaf infusion, with 71.50% in PC1 and 15.44% in PC2, both of which explained 86.94% of the total variability. 1-18: compounds identified by HPLC; 19-21: antioxidant activities by ABTS, DPPH, FRAP; 22-25: phenolic contents.



- Significant correlation between AA with flavonoids and tannins indicated they are essential contributors to AA, playing the primary role in maintaining benefits of infusion during long time storage.
- All decreasing compounds except catechins were significantly correlated with AA, but further relationship needs to study after isolation of these compounds.
- Some highly correlations were observed between different identified compounds, implying their possible interrelationship during storage.

- PC1 and PC2 grouped storage time into 24h and 2h-8h since storage at 0h had highest phenolic contents, AA and identified decreasing compounds.
- Storage at 24h was characterized by most of growing compounds except punicalins.
- It was difficult to characterize storage between 2h, 4h, 6h, 8h, probably needing to perform the third component (PC3) with PC1.
- It can be generally found the main difference exited in 0h and 24h, while similarity during 2h-8h.

Conclusion

✓ Pomegranate leaf infusion was found to possess stable total phenolics and *ortho*-diphenols, mainly due to the potential synergistic function by intricate transformation reactions in flavonoids and tannins, such as epimerization, polymerization, depolymerization, and hydrolysis to low molecular weight fragments during storage for 24h. However, advanced techniques are much the agenda for exploiting its underlying mechanisms.

✓ This infusion prepared with potable water could keep relatively higher antioxidant activity during one-day standing under room temperature, although there was slightly degradation in the first 2h, probably attributed to non-phenolic antioxidants, such as amino acid and ascorbic acid.

✓ Administration before storing for 4 hours after fresh preparation of pomegranate leaf infusion was suggested in the present study, in order to amplify the utilization of initial extracted phenolics in the gastrointestinal system *in vivo*.

Pomegranate leaf infusion could be a potential natural source of functional food and pharmaceutical ingredient, due to its high level and relatively stable amount of phenolics and antioxidant capacity.

Acknowledgements

This work was supported by National Funds by the FCT - Portuguese Foundation for Science and Technology under the project UID/AGR/04033/2019, by National Funds from I&D Project Interact - Integrative Research in Environment, Agro-Chains and Technology (NORTE-01-0145-FEDER-000017), and co-founded by the European Regional Development Fund (FEDER) through NORTE-2020 (Programa Operacional Regional do Norte 2014/2020). The first author also acknowledges the financial support provided by the FCT - Portuguese Foundation for Science and Technology (PD/BD/135333/2017), under the Doctoral Programme "Agricultural Production Chains - from fork to farm" (PD/00122/2012).

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Poster Template Provided By NPAC

DEVELOPMENT OF METHODS FOR THE EXTRACTION AND DERIVATIZATION OF CARRAGEENAN FROM CHONDRUS CRISPUS AND ACTIVE COATINGS FORMULATIONS

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Abstract

Plastics present one of the biggest environmental problems on a global scale, due to poor biodegradability. Nevertheless, viable candidates exist for the replacement of conventional plastics. For instance, carrageenans are a class of algal polysaccharides which have the ability to form edible films and coatings.
The extraction method of carrageenans (Carr VI) from *Chondrus crispus* using a Soxhlet device and hot water as extraction solvent, afforded 35.15±0.64% dry weight of yield. The native carrageenan presented some antioxidant activity against DPPH and was capable of suppressing the bleaching of methyl red dye by the NaFeEDTA/acetic acid/H_2O_2 system.
The treatment of native carrageenan with N-(salicyl)-L-cysteine hydrochloride in water, at *ca*. 90 °C, increased the antioxidant activity against DPPH and NaFeEDTA/acetic acid/H_2O_2. No antimicrobial activity against Gram-positive bacteria *Bacillus subtilis* was detected. Structural elucidation studies are being carried to determine nature of the interaction of N-(salicyl)-L-cysteine hydrochloride with carrageenan. Coatings prepared from native and modified carrageenans have shown the ability to adhere to surfaces.

Introduction

Plastic waste is one of the biggest environmental problems on a global scale (Balestri et al., 2017; Yeo et al., 2017) with prospects of worsening (World Economic Forum, 2016).

The industry that uses it most is the food industry due to packaging, with Europe being the second-largest producer of plastics in the world (PlasticsEurope, 2017).

Biodegradable plastics are the main candidates for solving this problem (Shen et al., 2020), and sulfated polysaccharides, such as carrageenan, present themselves as one of the materials with this potential (Yermak et al., 2020).

Cysteine has a high antioxidant potential and can be used in the derivatization of molecules (Önen Bayram et al., 2016; Zeng et al., 2017).

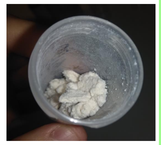
The association of these compounds with these characteristics allows the development of polymers with bioactivity, thus developing active packaging solutions (Han et al., 2018).

The development of this project aims to develop an edible coating of carrageenan derivatized with cysteine salicylaldehyde, with bioactivity potential to increase the shelf life of food products.

Materials and Methodology

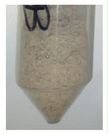
Extraction of carrageenan from *Chondrus crispus* by Soxhlet device (Carr VI)

15 g *Chondrus cripus* dry powder
Discard the first 2 hours
3 cycles of 7 hours
Isopropanol recovery



Derivatized carrageenan with N-(salicyl)-L-cysteine hydrochloride (Carr CysSal V)

Deprotonation with NaOH
Shaking vigorously at 90 ° C for 7 hours
Isopropanol recovery



Chemical Characterization of native and derivatized carrageenan

UV-Vis spectrum
Sulfuric Phenol (carbohydrates)
ρ-Benzoquinone (proteins)

Analysis of the bioactive activities

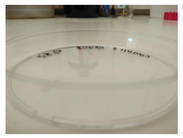
Primary Antioxidation
- H_2O_2 peroxidation

Secondary Antioxidation
- DPPH
- FRAP

Antimicrobial activity
- Agar disk difusion method

Coatings Formulation and Adhesion

1 mg.mL^{-1} Extract in distillated water
Glycerol 0.5%, 1%, 2% and 3%.
Polystyrene surface (Petri dishes) and 90 mm glass
24 hours at room temperature.



Results

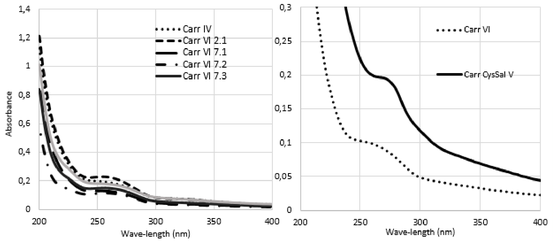


Figure 1 - Absorbance skewers of extracts and fractions of carrageenan extracted by Soxhlet and derivatives.

Without discarding the first two hours, it presents greater contamination by proteins (Porterfield & Zlotnick, 2010), although there is no detection by the p-BQ method. CarrCysSal V has a peak at 260 nm and shoulder at 280 nm not seen in Carr VI.
This is characteristic of cysteine (Siddiqui et al., 2017). This factor is an indicator that carrageenan has been successfully derivatized (Chen et al., 2019).

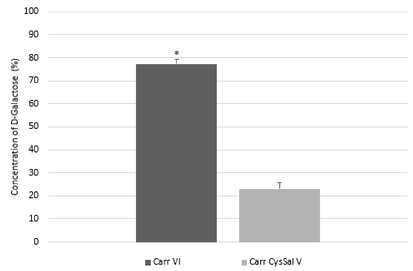


Figure 4 - D-galactose concentration as a percentage of total mass. * Statistically significant difference.

The decrease is due to the change to D-galactose monomers may result in the non-detection of this by the sulfuric phenol method since this method requires calibration for each type of monomer (Nielsen, 2003).

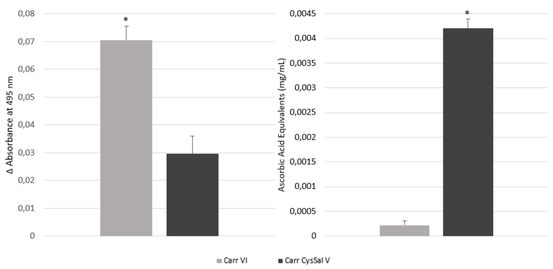


Figure 5 - Variation in absorbance at 495 nm in the HPSA assay (oxidation of NaFeEDTA by H_2O_2, after 15 minutes (A) and antioxidant potential determined by DPPH (B). * Statistically significant difference.

The primary antioxidant potential of native carrageenan (H_2O_2 peroxidation) is recognized and is probably due to its ability to capture oxidizing elements (T. Sun et al., 2010), as well as cysteine (Nelson et al., 2018). DPPH assays demonstrate that Carr VI cannot reduce other compounds (Ferreira et al., 2007; Senevirathne et al., 2006). The potential shown by Carr CysSal V is probably due to cysteine salicylaldehyde (Hwang et al., 2019). This aspect is due to the ability to donate hydrogen electrons to radicals (Spizzirri et al., 2009).

Conclusion

The soxhlet extraction methodology developed shows:
- High yield (35.15 ± 0.64%)
- Low contamination
- Low cost
- Without the production of hazardous waste
- Physical properties like commercial carrageenans

Bioactive properties:

Carr VI	Carr CysSal V
High Primary Antioxidation	High Primary Antioxidation
Very Low Secondary Antioxidation	High Secondary Antioxidation
Without Antimicrobial Potential	Without Antimicrobial Potential

This project allowed the development of extraction protocols and analysis of the composition of semi-refined carrageenan extracts and their physical and bioactive properties. As well as their derivatization methodology and confirms the ability to adhere to surfaces, even hydrophobic ones.

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Anthocyanin-rich extract obtained from *Prunus Spinosa* L. by ultrasound assisted extraction for coloring purposes

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Introduction

Anthocyanins are a group of natural pigments presenting a range of colours between red, blue, and violet that are characteristic of various fruits and vegetables. A complex profile of anthocyanins, predominantly cyanidin 3-rutinoside and peonidin 3-rutinoside, was previously identified in *Prunus spinosa* L. fruit (figure 1), a bitter and astringent fruit from a wild shrub that is poorly commercially exploited [1]

Figure 1: The fruits of *Prunus spinosa* L., known as blackthorn.

Objectives

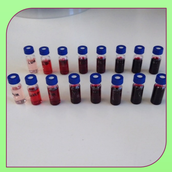
The objective of this work was to develop a natural food colourant based on anthocyanins extracted from the epicarp of *P. spinosa* fruits.

Methodology

A conventional extraction method, maceration, and a rapid and low-cost ultrasound procedure were applied for the extraction of anthocyanins from this matrix.

To achieve the conditions that maximize anthocyanins' extraction, a response surface methodology was applied using a circumscribed central composite design with three variables and five levels, being the variables time, temperature, and ethanol content, in the case of maceration extraction, whereas for ultrasound assisted extraction, temperature was replaced by ultrasound power.
The anthocyanins were identified and quantified by HPLC-DAD-ESI/MS.

The optimized extract was assessed in terms of antioxidant and antimicrobial capacity, and hepatotoxicity.



Results

Ultrasound assisted extraction was the most efficient method, under optimum conditions of 5.00±0.15 min, 400.00±32.00 W and 47.98±2.88% ethanol, where the extraction yield was 68.60±2.06% (v/v), with a total anthocyanin content of 18.17±1.82 mg/g of dry extract and 11.76±0.82 mg/g of dry epicarp (Table 1).

Conditions of optimal global variables			t (min)	T (°C) or P (W)	S(%)	Optimazed responses	
HAE		Yield	49.02±2.94	90.00±7.20	50.00±0.50	50.89±3.05	%
	Y_1	C1				9.71±0.29	mg C1/g R
		C2				4.22±0.13	mg C2/g R
		CT				13.93±0.42	mg CT/g R
	Y_2	C1				5.57±0.11	mg C1/g E dw
		C2				2.36±0.05	mg C2/g E dw
		CT				7.93±0.08	mg CT/g E dw
UAE		Yield	5.00±0.15	400.00±32.00	47.98±2.88	68.60±2.06	%
	Y_1	C1				11.74±0.23	mg C1/g R
		C2				6.43±0.32	mg C2/g R
		CT				18.17±1.82	mg CT/g R
	Y_2	C1				7.81±0.47	mg C1/g E dw
		C2				3.95±0.24	mg C2/g E dw
		CT				11.76±0.82	mg CT/g E dw

Table 1: Variable conditions in natural values that lead to optimal global response values for RSM for each of the extracting techniques assessed (HAE and UAE), for the three response value formats (Y1, mg C/g R; Y2, mg C/g E dw; and Yield, %), for each compound assessed (C1 and C2), and for the total compounds (CT = C1 + C2).

Regarding bioactivity, the optimized extract showed antioxidant and antimicrobial activity and it did not show hepatotoxic effects in a primary culture of porcine liver cells.
To validate its coloring properties, the anthocyanin-rich extract was incorporated into a typical Brazilian confectionery product "beijinho", proving its applicability as food colorant.

Reference

[1] Rafaela Guimarães, Lillian Barros, Montserrat Dueñas, et al., Food Chemistry, 141 (2013) 3721.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020). National funding by FCT, P.I., through the institutional scientific employment program-contract for C. Pereira, M.I. Dias, R.C. Calhelha, and L. Barros contracts and the individual scientific employment program-contract for S.A. Heleno and J.C.M. Barreira contracts. To FEDER-Interreg España-Portugal programme for financial support through the project TRANSCoLAB 0612_TRANS_CO_LAB_2_P; to the European Regional Development Fund (ERDF) through the Regional Operational Program North 2020, within the scope of Project Mobilizador Norte-01-0247-FEDER-024479: ValorNatural® and Project GreenHealth - Norte-01-0145-FEDER-000042. To Xunta de Galicia for M.A. Prieto grant.

Exploring the antioxidant and antibacterial potential of lemon grass

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Introduction

There is an ongoing, extensive search for natural ingredients that either prevent or retard undesirable changes, such as oxidation or microbiological contamination, which take place in food products. Lemon grass (*Cymbopogon citratus* L.) seems to be an interesting plant that may be employed as nutritious, healthy, safe and natural ingredient. Because plant material is rather unstable, a crucial step is to obtain extracts that are rich in phenolic phytochemicals, thus, using different solvents may help to extract valuable compounds. Therefore, this study aimed to investigate the antioxidant and antibacterial activities of lemon grass and to assess the influence of the extraction solvent, that is, ethanol, water, and water:ethanol (50/50 vol/vol), on measured properties.

Materials

Lemon grass grown in a laboratory room at 20°C in natural daylight

→

dried in an air dryer at 50°C for 24 h

Methodology

Preparation of grass extracts (Sielicka-Różyńska & Gwiazdowska, 2020)

Total phenolic content(Singleton & Rossi, 1965)

Antioxidant activity
- Free radical scavenging activity (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998)
- Ferric reducing antioxidant power**(**Benzie & Strain,1996)

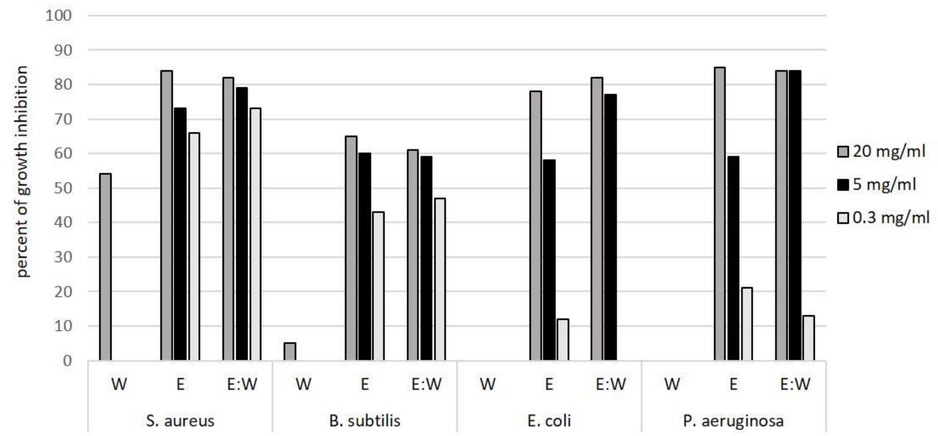
Antimicrobial activity
broth microdilution method (Niemczak, Kaczmarek, Klejdysz, Gwiazdowska, Marchwińska, & Pernak, 2018)

Results

The research revealed the significant influence ($p < .05$) of solvent system on extracts' properties. The water:ethanol extract from lemon grass showed the highest total phenolic content, DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) value.

	Solvent		
	WATER 100%	ETHANOL 100%	WATER:ETHANOL 50:50
Total phenolic content GAE/g d.m. extract)	59.45 ± 1.11^{b}	49.70 ± 0.38^{a}	107.24 ± 2.22^{c}
Free radical scavenging activity (% of	81.2 ± 0.2^{b}	75.1 ± 1.6^{a}	87.8 ± 0.2^{c}
Ferric reducing power (µmol Trolox/g extract)	836.0 ± 4.1^{b}	650.7 ± 9.7^{a}	1445.2 ± 14.1^{c}

Ethanol and water:ethanol extracts revealed high antibacterial activity. Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) bacteria were inhibited by lemon grass extracts at a concentration of 0.3 mg/ml, whereas Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) needed higher extract concentrations (5–20 mg/ml). Water extract inhibited *Staphylococcus aureus* at a level not exceeding 55%.



Conclusion

The results of the research demonstrated potential importance of lemon grass extracts in the food industry, as their implementation in food products, food supplements, and beverages could significantly improve product safety, shelf life, and health-promoting properties.

Extraction of Phenolic compounds from *Artemisia absinthium* L.: optimization through ultrasound assisted extraction using the Response surface methodology

María Sol Terisotto[1,2], Bruno Melgar[1], Márcio Carocho[1], Maria Inês Dias[1], Ema Sabre[2], Sandrina A. Heleno[1*], Isabel C. F. R. Ferreira[1], Lillian Barros[1]

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ABSTRACT

Plants throughout history have been a human resource available to be used for food, feeding and treatment of diseases, thus being considered as natural medicine agents. This is due to the presence of biomolecules such as phenolic compounds, that have been described has having strong antioxidant, antimicrobial, cytotoxic, among other bioactivities. Among the medicinal plants it is possible to find the *Artemisia absinthium L.,* an aromatic plant, that grows in temperate regions of Europe, Asia and North Africa. In addition to the renowned wormwood application in preparation of absinth and related beverages, *A. absinthium* has been used since ancient times for medical purposes. From the ethnopharmacological point of view, this plant has been used for its antihelmintic, stomachic, antibacterial, antifeedant, antifertility, antipyretic, cytostatic, antitumor, and antimalarial actions.

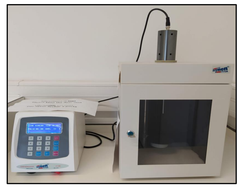
This work intended to optimize the extraction parameters of the ultrasound assisted extraction to obtain highly enriched extracts in phenolic compounds.

METODOLOGY

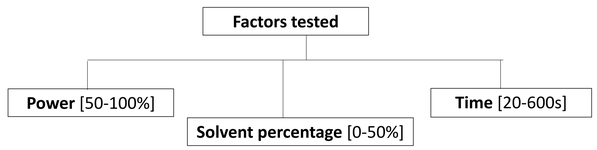
Optimization was possible using the response surface methodology (**RSM**) that is able to model data within certain parameters chosen by the researcher; but prior to start running multiple experiments without knowing the behaviors of certain factors involved, this technique would be only able to visualize certain magnitudes and not all the optimum point, therefore, strong complex and random screening analysis have to be performed in order to work the RSM in the optimal conditions and be able to properly model datasets.

Thus, 4 different screening analyses (Fractional Factorial design, 2 factors multilevel, and 1 factorial design) were developed using a 12-run array mixture which was randomized and codify using statistical software (Design Expert).

Sample *of Artemisia absinthium* in strands



Ultrasound-assisted extraction technique



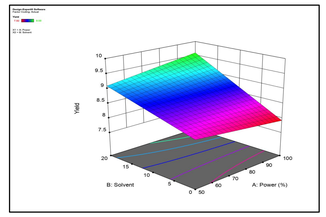
Run	Power (%)	Solv (%EtOH)	Time (s)
1	50	0	600
2	50	0	20
3	50	20	20
4	50	20	240
5	50	0	60
6	50	50	240
7	100	20	240
8	100	0	240
9	50	0	120
10	100	0	20
11	50	0	240
12	50	0	40

Data was processed through an ANOVA analysis employing Pareto plots and the least significant difference (LSD) test. The response analysis was achieved through the phenolic compounds content, analyzed by HPLC-DAD-Ms.

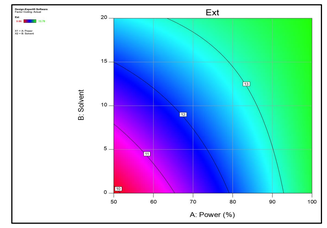
RESULTS

- From the analysis of the multi-level factor of the solvent, it follows that the higher percentage of ethanol more compounds are extracted and the final concentration of the extract is increased.
- An increase in power reflects an increase in extractable compounds and consequently higher concentrations. In addition, the increase in such concentrations with high percentages of solvents is reaffirmed.
- With respect to time, the best result was obtained at 600 seconds, but minimal differences compared to 20 seconds. Values do not follow a trend, so 600 can be randomly the best.

Run order	Power	Solv	Time	Ext	Yield
1	50	20	20	13,25	7,91
2	100	20	240	13,79	9,59
3	100	0	20	10,79	7,56
4	50	0	240	9,88	8,25



Run order	Power	Solv	Time	Ext	Yield
1			20	12,20	9,39
2			40	8,47	7,53
3			60	11,23	8,17
4			120	10,26	7,74
5			600	13,19	7,00



CONCLUSION

According to the obtained results, the UAE ideal conditions for the screening analysis were 100% power, 20% ethanol and 240 s allowing a total phenolic content of 13.79 mg of TPC/g of extract, being chlorogenic acid derivatives, the main compounds.

These results highlight the richness of *A. absinthium* in phenolic compounds and validate the UAE as an efficient extraction technology.

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"ECONÓMICOS" CAKES STRENGTHENED WITH CHESTNUT: EFFECTS ON TEXTURE AND COLOR

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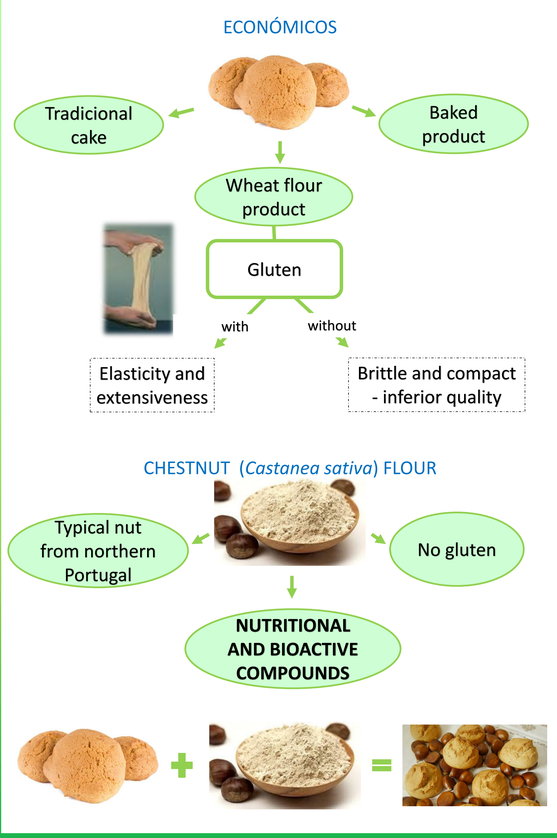
ABSTRACT

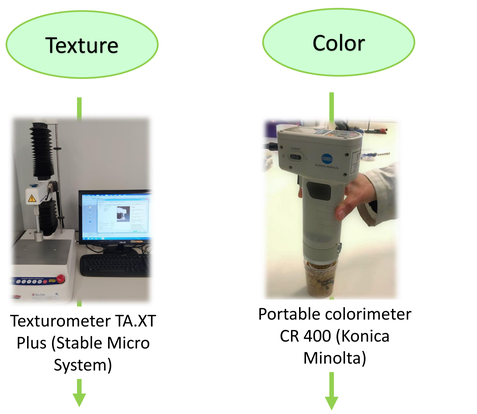
Cereals grains are one of the main sources of nutrients and energy contributors to the human diet. The "económicos" are traditional cakes from Portugal, made with wheat flour and quite appreciated by consumers, because of the good taste. Different sources of flour can be used in pastry products, however, the gluten present in wheat flour, along with starch maintain the union between the ingredients and provide the structure of the baked product. Therefore, with the use of other sources of flour, the presence of gluten becomes minimal and the cake tends to be of inferior quality compared to foods which contain it, namely at the level of low nutritional value, weak coloration, higher tendency to crumble and, mainly, low volume.

Thus, the main objectives of this work were to analyse the texture and color of "económicos" incorporated with chestnut (*Castanea sativa* Mill.) flour, thus strengthening the económicos nutritional value and not fully reducing gluten. The analysis were carried out over the course of 25 days. Overall, significant interaction was sought for all analysis of color and storage time showed, as expected, a higher influence on the texture profile.

INTRODUCTION



METHODS



RESULTS

- Significant interaction for all analysis of color, except for red-green (a^*) outside of the "Económicos" and for lightness (L^*) inside, being the chestnut samples significantly darker than the control ones (**Table 1** and **Figure 1**)

Table 1 - Colorimetric coordinates profile (L^*, a^*, b^*) of outside and inside of the "Económicos" over 25 days with different flours.

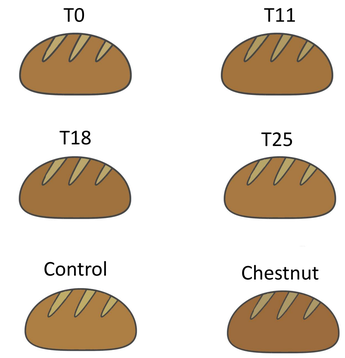
		Outside			Inside		
		L^*	a^*	b^*	L^*	a^*	b^*
Storage Time (ST)	0 Days	55±5	15±1	37±3	70±5	2±2	33±3
	11 Days	53±4	15±1	37±2	65±5	3±2	33±5
	18 Days	52±3	14.5±0.9	36±2	69±4	2±2	33±5
	25 Days	55±3	15±2	37±3	68±3	1.5±1.5	33±5
p-value (n=3)	Tukey test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Type of Flour (TF)	Control	57±3	14±1	39±1	71±3*	0.07±0.75	37±2
	Chestnut	50±2	15.7±0.7	34±1	64±3	3±1	29±1
p-value (n=9)	Tukey test	0.001	0.143	0.005	<0.001	<0.001	0.115
ST×TF (n=27)	*p*-value	<0.007	0.213	0.038	0.051	0.007	<0.001

- Storage time - higher influence on the texture profile (hardness, cohesiveness, springiness, gomosity and chewiness) with statistical differences found from the first to the 11th day (**Table 2**).
- The chestnut flower only influenced the springiness, making thus reducing this dimension (**Table 2**).

Table 2 -Texture profile of the "Económicos" over 25 days with different flours.

		Hardness	Adhesiveness	Resilience	Cohesiveness	Springiness	Gomosity	Chewiness	Firmness
Storage Time (ST)	0 Days	8380±712a	-0.3±0.1	0.21±0.03	0.59±0.05a	0.85±0.02a	4895±293a	4183±314	11478±1072
	11 Days	16961±2328b	-1±1	0.21±0.02	0.56±0.04a	0.79±0.02b	9558±1146b	7518±799	10515±3264
	18 Days	15222±391b	-0.7±0.3	0.21±0.01	0.58±0.03a	0.79±0.01b	8847±380b	7032±295	9842±2136
	25 Days	15822±2787b	-0.4±0.3	0.21±0.02	0.59±0.03a	0.79±0.03b	9286±1606b	7345±1385	11173±4274
p-value (n=3)	Tukey test	<0.001	0.050	0.115	<0.001	<0.001	0.002	0.932	0.971
Type of Flour (TF)	Control	11319±3555	-0.9±0.8	0.22±0.01	0.61±0.02	0.85±0.02*	8127±2084	6604±1540	10775±2745
	Chestnut	14873±4117	-0.5±0.4	0.20±0.01	0.55±0.02	0.79±0.03	8166±2314	6435±1701	10729±3013
p-value (n=9)	Tukey test	<0.001	0.010	0.559	0.194	<0.001	<0.001	<0.001	0.808
ST×TF (n=27)	p-value	0.789	0.263	0.021	0.247	0.670	0.963	0.991	0.654

Figure 1 – Illustrative representation of the resultant of the colorimetric coordinates of outside and inside of the "Económicos" for a) different times and b) different flours



CONCLUSION

- The chestnut flour only influenced the springiness.
- Overall, the chestnut samples were significantly darker than the control ones.
- Chestnut flour can be useful to fortify these cheap snacks, although analysis on the chemical, nutritional and microbial analyses are being processed.

ACKNOWLEDGEMENTS

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020). L. Barros thanks FCT through the institutional scientific employment program-contract for her contract, while M. Carocho and S. Heleno thank FCT through the individual scientific employment program-contracts (CEECIND/00831/2018 and CEECIND/03040/2017); to FEDER-Interreg España-Portugal program for financial support through the project TRANSCoLAB 0612_TRANS_CO_LAB_2_P, to ERDF, through the Incentive System to Research and Technological development, within the Portugal2020 Competitiveness and Internationalization Operational Program for the financial support to BIOMA(POCI_01_0247_FEDER_046112); ERDF through the Regional Operational Program North 2020, within the scope of Project GreenHealth (Norte-01-0145-FEDER-000042).

Eisenia fetida as a biological research model for soy isoflavones

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Abstract

Soy-based foods contain high concentrations of isoflavones which show some chemoprotective effects in cancer. [1]. Animal models of disease have been used; however, several ethical questions are increasing regarding the use of vertebrates. Consequently, it is essential to optimize new biological research models that do not raise ethical issues. *Eisenia fetida* is an earthworm easy to handle and inexpensive, recognized as the most used for ecotoxicology studies, being a useful bioindicator to test soil toxicity [2]. With this work, our team aims to determine the effects of isoflavones on physiological and behavioral parameters of *E. fetida* through soil exposure.

For this study, a toxicity bioassay test was prepared according to the guidelines described by the Organization for Economic Co-operation and Development (OECD, 2016). Briefly, an artificial soil consisting of 10% peat, 20% kaolin, 70% sand mixture was prepared; pH (0.1M KCl, 1:2.5 ratio) was adjusted to 6.0 ± 0.5 by adding $CaCO_3$.and moisture content adjusted to 40% of the maximum water holding capacity. The concentrations of isoflavones selected were 140, 279, and 499 mg/Kg of soil. In addition to exposure groups, three control groups were used: (i) a group with artificial soil; (ii) a group with soil and earthworms and (iii) a group with soil and isoflavones (499 mg/Kg), without earthworms. The worms were acclimatized to the artificial soil for 24 hours, and then they were left to depurate the intestinal contents on a moistened paper filter for 24 hours. For this study, adult earthworms, with a bodyweight of 0.40±0.02 g were used. All procedures were performed at 20±2°C. Each group contained three reactors to which eleven earthworms were randomly allocated (except controls without earthworms). Every week, soil moisture was corrected on a weight basis through the addition of distilled water and earthworms were fed by addicting of 2g ground oats. We evaluated the behavior of earthworms daily, according to their position in the soil. At the end of experimental period (56 days), the worms were weighed and euthanized. No mortality was observed throughout the study and earthworms showed no behavioral changes during the experimental test period. The concentration of isoflavones did not reveal significant effects on the evaluated physiological parameters. However, we found that there is a tendency to increase earthworm weight with the highest concentration (499 mg/kg) and a decrease in weight with the lowest concentration (140 mg /kg) both relative to the control. In all studied treatment, earthworms were able to generate offspring. Our results show that isoflavones did not affect physiological parameters of *Eisenia fetida*, however, the differences observed in weight among exposed groups and the controls suggest that this species is sensitive to this type of compounds. Therefore, further studies must be performed to better understand these changes.

Introduction

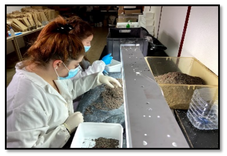
The isoflavones are present in soy-based products and are also important to study their effect on the soil [1}. Earthworms, due to their ability to convert organic products and biodegradable materials into nutrients, play a key role in soil maintenance [2]. Over time, difficulty in carrying out tests on vertebrate animal models has increased due to ethical problems. Given this, there was a different choice of different animal model, *Eisenia fetida*. These are very important organisms, they promote the decomposition of organic matter, the circulation and release of nutrients from the soil and improve the soil structure. Earthworms, such as *E. fetida*, are a great bioindicator for monitoring soil species. In addition to this, they have advantages such as easy handling and the low cost. The main objective of this study is to evaluate the effect of isoflavones on *E. fetida* physiological and behavioral parameters through exposure to soil.

Materials and Methodology

Initially, the artificial soil was made with 10% peat, 20% kaolin and 70% sand. The pH was corrected to 6.0 ± 0.5 by the addition of $CaCO_3$. To calculate the volume of distilled water needed to reach 50% of the maximum water retention capacity, it was measured in the proportion of 25 g of the total substrate to 25 mL of distilled H_2O. The volume initially added was only for 25% of the maximum water retention capacity and then the rest was added with the respective dilutions of the substances to be tested. Isoflavones were acquired in the form of a tablet whose concentration corresponds to 80 mg of isoflavones each. The tablets were broken and diluted in distilled H_2O to the volume corresponding to the remaining 25% of the CMRA. The prepared concentrations were 140 (Group 2), 279 (Group 3) and 499 mg / kg (Group 4) of dry substrate. In addition to exposure groups, three control groups were used: (i) a group with artificial soil; (ii) a group with soil and earthworms and a group with soil and isoflavones (499 mg/Kg) (Group 5), without earthworms.

On arrival, The earthworms, acquired to Ecogrowing, Lda, were placed under a strong light with the soil.

Every 5 min of exposure to light, the soil was gradually removed until there was only one "ball" of earthworms, which were acclimatized to the artificial substrate for 24 hours.



After acclimatization, a first selection was performed, which consisted in separating the adult individuals from the rest. The adult subjects were placed to purify on a moistened filter paper for 24 hours.

Finally, on a second selection phase, the worms were weighed, and ~~they~~ were only selected for testing if their weight was between 300 and 500 mg. Then they were randomly distributed among the groups.

The reactors were opaque, perforated and contained 573.3 g of artificial soil making up about 5 cm in height of the containers. Eleven earthworms were distributed at random by group, each group having 3 replicates.

Acclimatization Depuration

56 days

Every week :

1. Feeding by 2g of crushed organic oats
2. Replacement up to 50% of the maximum water retention capacity
3. Animal behavior assessment
4. Aeration of reactors.

Results

After the first week of testing, we verified the occurrence of fungi derived from oats, the feeding of *E. fetida*. As we can confirm in the figure 2, the reactors that do not have earthworms have a relatively larger quantity of fungi than the respective other groups. It is also possible to verify that Group 1 and Group 4 have a lower quantity of fungi, thus suggesting a higher intake of oats. In addition, we were able to relate this fact through figure 2, as these two groups have a tendency towards greater ponderal gain.

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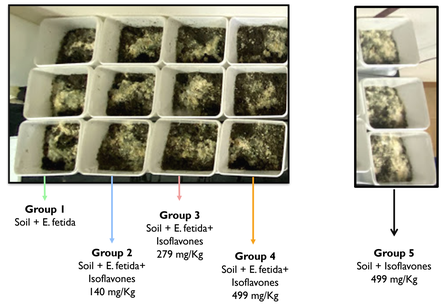

Figure 1- Reactor appearance after the first week of the experimental test

As for the behavioral study throughout the test, normal activity was observed, that is, the ability to dig the soil, we did not find earthworms immobile on the surface, agitated soil, formation of holes / tunnels in the soil. In addition to the fact that there was no mortality, and earthworms were able to generate offspring.

Regarding the body weight of earthworms, we found that there was an increase in weight compared to the beginning of the test. In addition, we found that the higher the dose of isoflavones, the greater the ponderal gain. The group with the highest contraction of isoflavones shows a higher ponderal gain than the control group of the experiment; however, these differences are not statistically significant.

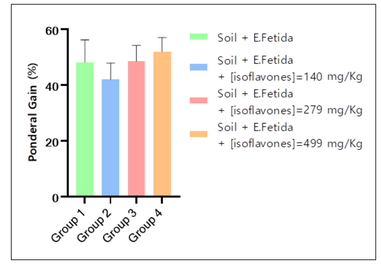

Figure 2- Mean ponderal gain *per* group. All results are presented as mean ± standard error.

Conclusion

As there was a quantity of relevant fungi in all reactors, the feed dose must be adjusted. In addition, we verified that earthworms are sensitive to this compound, therefore new analyzes of histology, oxidative stress and comet assay are being carried out to better understand these changes.

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EVALUATION OF THE CHEMICAL COMPOSITION OF BIOACTIVE COMPOUNDS FROM PLANTS OF THE ASTERACEAE FAMILY

M. Barral-Martínez [1], P. García Oliveira [1,2], B. Nuñez-Estevez [1,2], M. Carpena [1,2], A. Silva [3], M. F Barroso [3,*], M. Fraga-Corral [1,2], J. Simal-Gandara [1] and M.A. Prieto [1,2,*]

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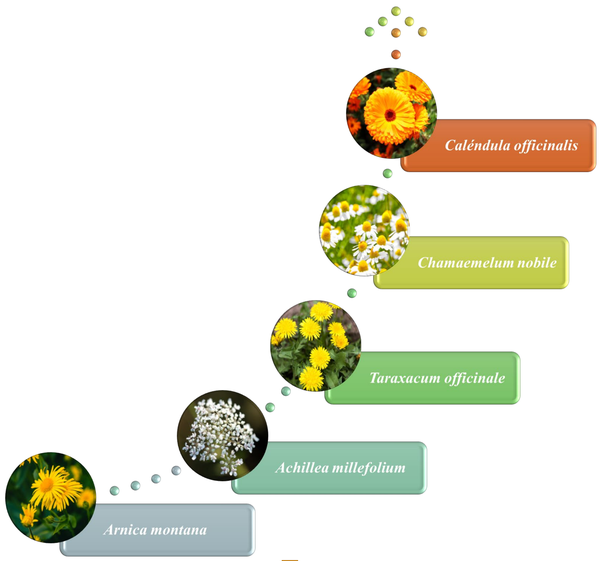
Introduction

Traditional plants have been used in the treatment of disease and pain. Numerous scientific studies have demonstrated their beneficial properties, including antioxidant, anti-inflammation, analgesic, antibiotic, etc., justifying their traditional uses. In several ethnobotanical studies, Asteraceae family is one of the most common groups used in folk medicine. The following plants ***Achillea millefolium***, ***Arnica montana***, ***Calendula officinalis***, ***Chamaemelum nobile***, and ***Taraxacum officinale***, have been used in different remedies in the North West region of Spain.



The main Proprieties of the following Traditional plants

Methodology

- **Materials :**

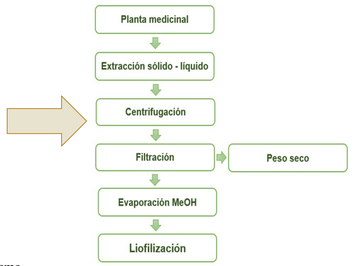
Five different varieties of plants in the family Asteraceae Achillea millefolium, Arnica montana, Calendula officinalis, Chamaemelum nobile and Taraxacum officinale have been used.

All of them were acquired in two companies called Soria natural and Pinisan in 2020.

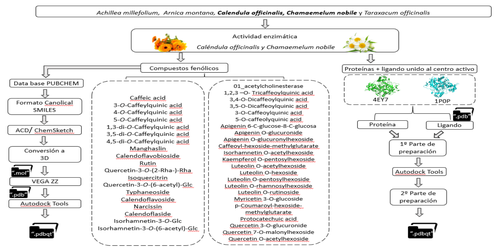
1.1.Conditioning of the raw material | *1.2.Determination of moisture in raw materials* | *1.3.Determination of dry content in raw materials* | *1.4. Conventional array extraction*

105 oC, 24 hours

All the steps to extract an array conventionallysummary form in the following are shown in



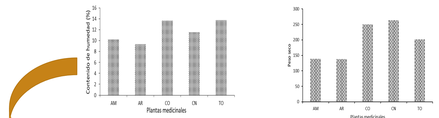
Docking procedure scheme



Results

Moisture content

The five samples of medicinal plants used in this work, after only receiving crushing treatment decreasing in size to a length of approximately 1 cm, have a moisture such as the one shown below



Variation of moisture content and dry weight in the five medicinal plants (AM:*Achillea millefolium, AR:. Arnica montana, CO:Calendula officinalis, CN:Chamaemelum nobile and TO:Taraxacum officinale).*

Antioxidant activity

The evaluation of antioxidant activity is carried out through various processes as there is a response and a quantification of different reaction mechanisms .

That is why different protocols have been determined as the content in phenolic compounds. In this process, an absolute measure of the quantities is not obtained other than a measure of the reducing chemical capacity of the compounds present in the extract in relation to galic acid and is expressed in gálic acid equivalent (GAE)

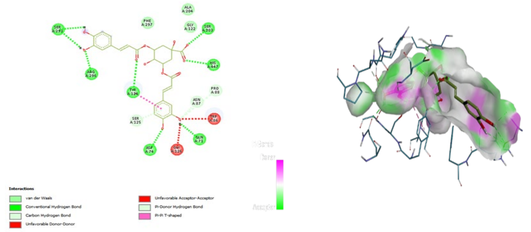
A

MEDICINAL PLANTS	Concentration µg eq. galic acid/mg extract	RSD (%)
ON	80,95 ± 1,36	1,68
AR	170,93 ± 5,05	2,96
CO	45,06 ± 0,99	2,29
CN	77,69 ± 1,03	1,34
TO	42,80 ± 1,05	2,46

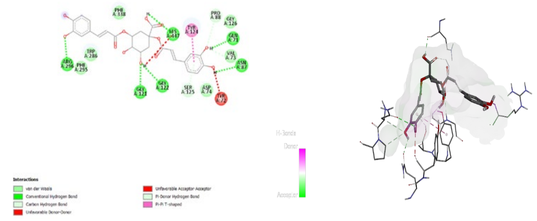
B

MEDICINAL PLANTS	CONCENTRATION µg eq. epicatechin/mg extract	RSD (%)
ON	9,73 ± 0,74	7,58
AR	68,27 ± 0,69	6,62
CO	5,96 ± 0,14	1,76
CN	7,94 ± 0,16	2,61
TO	10,41 ± 6,92	10,14

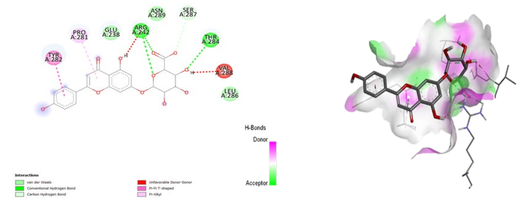
AM: *Achillea millefolium*, AR: *Arnica montana*, CO: *Calendula officinalis*, CN: *Chamaemelum nobile* y TO: *Taraxacum officinale*



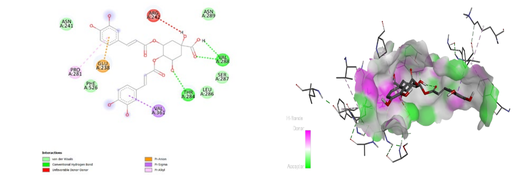
Molecular docking of the compound 3.5-O-Dicaffeoylquinic acid and acetylcholinesterase protein of the plan Chamaemelum nobile 2D and 3D respectively.



Molecular dockning of the compound 1.3-di-o-Caffeylquinic acid and Acetylcholinesterase protein of the plant Calendula Officinalis 2D and 3D respectively.



Molecular docking of the compound Apigenin O-glucuronide and protein butyrycholinesterase of the plant Chamaemlum nobile 2D and 3D respectively.



Acknowledgements

The research leading to these results received institutioanl and financial support from the: Programa de Cooperación Interreg V-A España—Portugal (POCTEP) 2014–2020 (projects Ref.: 0181_NANOEATERS_01_E and Ref: 0377_IBERPHENOL_6_E); MICINN supporting the Ramón&Cajal grant for M.A. Prieto (RYC-2017-22891); Xunta de Galicia and University of Vigo for supporting the post-doctoral grant of María Fraga Corral (ED481B-2019/096) and the pre-doctoral grant of P. García-Oliveira (ED481A-2019/295); to Xunta de Galicia for the program EXCELENCIA-ED431F 2020/12; to Ibero-American Program on Science and Technology (CYTED - AQUA-CIBUS, P317RT0003); by EcoChestnut Project (Erasmus+ KA202) that supports the work of M. Carpena; by the Bio Based Industries Joint Undertaking (JU) under grant agreement No 888003 UP4HEALTH Project (H2020-BBI-JTI-2019), the JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium.

RED RASPBERRY WASTE AS A SOURCE OF ANTHOCYANIN-RICH FOOD COLORANTS: EXTRACTION PROCESS OPTIMIZATION AND FUNCTIONALITY ASSESSMENT

Mikel Añibarro-Ortega, Rosiane Rocha, José Pinela, Tânia C.S.P. Pires, Rui M.V. Abreu, Ana Luísa Saldanha, Maria José Alves, António Nogueira, Isabel C.F.R. Ferreira, Lillian Barros

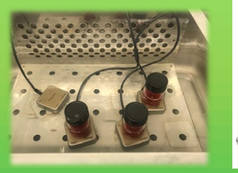
Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal.

INTRODUCTION

Food colorants are increasingly used in the food industry to preserve, improve or change the food color. While the quite controversial artificial colorants are widely used in this sector, the natural counterparts have been less selected in part due to the limited availability of options and stability issues [1]. Within this class, anthocyanins are naturally occurring colorants that can be found in different plant matrices, including berries such as red raspberry (*Rubus idaeus* L.). These water-soluble pigments show attractive colors ranging from red to purple and present health-promoting effects [2,3]. Therefore, this work aimed to develop a novel anthocyanin-rich food colorant from red raspberry waste through the optimization of a sustainable extraction methodology and to characterize this ingredient for its functionality.

METHODOLOGY

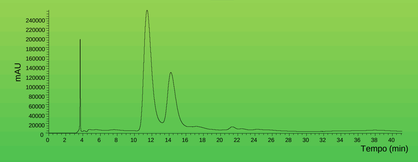
Heat (HAE)- and ultrasound (UAE)-assisted extraction methods were implemented to recover the anthocyanins from red raspberry. Processing time, ethanol concentration, and temperature or ultrasonic power were the independent variables analyzed in a central composite design (CCD) coupled with response surface methodology (RSM) for processes optimization. The extraction yield and levels of anthocyanins (cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside) were monitored gravimetrically and by HPLC-DAD-ESI/MS^n, respectively, and used as response criteria. The constructed theoretical models were successfully fitted to the experimental data and used to determine the optimal extraction conditions. Extracts obtained in optimal conditions were used to evaluate antioxidant and antimicrobial activities.



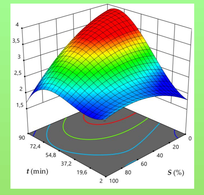
HAE

UAE

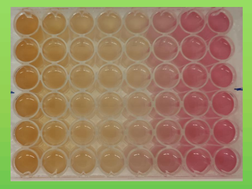
Obtention of anthocyanin-rich extracts under the 20 runs of the five-level CCD design



Quantification of anthocyanins by HPLC-DAD-ESI/MS^n as response criteria



Obtention of optimal conditions by RSM

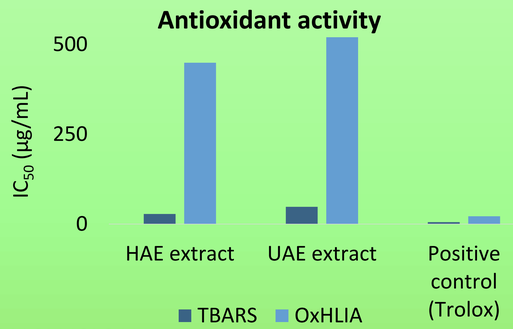


Bioactivity evaluation of the optimal extracts

RESULTS and DISCUSSION

Overall, HAE originated slightly higher response values (61% extract weight and 8.7 mg anthocyanins/g extract) but needed 76 min processing at 38 °C, with 21% ethanol, while the UAE process required 16 min sonication at 466 W, using 38% ethanol, to obtain 58% extract weight and 8.3 mg anthocyanins/g extract. Then, the predictive models were experimentally validated and the purple-red extracts obtained under optimal condition showed antioxidant activity through lipid peroxidation (TBARS) and oxidative hemolysis inhibition (OxHLIA), and antibacterial effects against food-related bacteria, such as *Escherichia coli* and *Enterococcus faecalis* [4]

	Optimal conditions					
	Independent variables				Responses	
	Time (min)	Ethanol (%)	Temperature (°C)	Power (W)	Extraction yield (%)	Anthocyanin content (mg/g)
HAE	76	21	38	-	61	8.7
UAE	16	38	-	466	58	8.3



Antibacterial activity against:

E. coli
E. faecalis

CONCLUSION

These results could be exploited by industries interested in the production of anthocyanin-based ingredients with coloring and bioactive capacity. In future studies, it will be interesting to investigate the stability of the developed anthocyanin-rich extracts when exposed to different factors and in real food matrices. The production of spray-dried red raspberry coloring powders will also be interesting to explore.

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ACKNOWLEDGMENTS

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020). This research was funded by FEDER-Interreg España-Portugal programme through the projects 0377_Iberphenol_6_E and TRANSCoLAB 0612_TRANS_CO_LAB_2_P, and by the European Regional Development Fund (ERDF) through the Regional Operational Program North 2020, within the scope of Project Mobilizador Norte-01-0247-FEDER-024479: ValorNatural®. M. Añibarro-Ortega thanks FCT for his PhD grant (2020.06297.BD). National funding by FCT, P.I., through the individual and institutional scientific employment program-contract for the J. Pinela (CEECIND/01011/2018) and L. Barros contracts, respectively. The authors thank Ponto Agrícola Unipessoal, Lda for the samples.

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Antioxidant and Antihemolytic Activity of Pumpkin By-products: A Contribution Towards Resource-use Efficiency

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Abstract

The antioxidant activity of pumpkin by-products was evaluated *in vitro*. The seed extract was more effective in inhibiting the formation of thiobarbituric acid reactive substances (TBARS), while the peel extract had a better performance in protecting red blood cells from haemolysis. The preservation and/or functionalization of food products could thus be achieved with antioxidant ingredients from pumpkin by-products.

Introduction

The current need to obtain nutritious and healthy foods from alternative and more sustainable sources and to reduce waste in the agri-food sector has led to a great interest in the valorisation of plant parts currently treated as by-product, but which have nutritional value and food-grade potential for inclusion in the human diet. The pumpkin processing industry is a good example because it generates a large amount of biowaste in the form of seeds and peels [1], which can be recycled inside the food chain as bioactive or functional ingredients, since these matrices contain significant amounts of carotenoids, protein, fibre, and tocopherols, among other antioxidants [2]. This study aimed to measure the antioxidant activity of pumpkin by-products, in order to assess their potential for use in novel food formulations.

Methodology

Pumpkin seeds and peels provided by local producers in the northeast of Portugal were lyophilized, ground to a fine powder, and submitted to a solid-liquid extraction using an hydroethanolic mixture as solvent [3]. The obtained liquid extracts were lyophilized, redissolved in distilled water, and successively diluted to different concentrations to evaluate the antioxidant activity. The thiobarbituric acid reactive substances (TBARS) formation inhibition and the oxidative haemolysis inhibition assays were performed in vitro using porcine brain cell homogenates and sheep red blood cells as oxidizable biological substrates, respectively [3]. A Student's t-test was applied to assess the existence of statistical differences between both samples.

Results

Based on the results obtained with the performed cell-based assays, it was possible to conclude that both seed and peel extracts display antioxidant effects. The seed extract was more effective in inhibiting the formation of TBARS, such as the highly reactive malondialdehyde, which results from the peroxidation of polyunsaturated fatty acids that constitute the porcine brain cell membranes. In turn, the peel extract had a better performance in protecting the red blood cells from the haemolytic action of free radicals initially generated in the system by the oxidant 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH), which is thermally activated during incubation at 37 °C.

Conclusions

Overall, these results suggest that the preservation and/or functionalization of food products could be achieved with the addition of bio-based antioxidant ingredients resulting from pumpkin by-products.

Acknowledgments

To FCT (Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020); for the M. Añibarro-Ortega PhD grant (2020.06297.BD) and the J. Pinela (CEECIND/01011/2018) and L. Barros contracts through the individual and institutional scientific employment program-contract, respectively; and to the FEDER-Interreg España-Portugal programme for financial support through the project TRANSCoLAB 0612_TRANS_CO_LAB_2_P and to ERDF through the Regional Operational Program North 2020, within the scope of the Project GreenHealth - Norte-01-0145-FEDER-000042.

FCT Fundação para a Ciência e a Tecnologia · NORTE2020 · PORTUGAL 2020 · UNIÃO EUROPEIA Fundo Europeu de Desenvolvimento Regional · Interreg España - Portugal · TRANSCOLAB

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DEVELOPMENT OF A NEW APPROACH BASED ON REAL-TIME PCR COUPLED WITH HIGH RESOLUTION MELTING (HRM) ANALYSIS TOWARDS THE ENTOMOLOGICAL AUTHENTICATION OF HONEY

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Introduction

Honey is a natural food of high dietary relevance and increasing demand, that has become a target of economically motivated adulteration. According to a 2014 European Parliament report on food fraud, honey is among the 10 food products most prone of being adulterated [1].

Apis mellifera L. is the only native species of honey bees in Europe, where 10 different subspecies from different maternal lineages can be found [2]. In general, honey bees occupy allopatric geographical ranges according to their evolutionary lineages, thus presenting a characteristic natural distribution in Europe. In Portugal, the predominant subspecies is the autochthonous *A. mellifera iberiensis* of mtDNA lineage A. Recently an increased attention has been paid to the entomological origin of honey, since it can indirectly give information about the geographical origin of honey [3].

Therefore, the aim of this work was to develop a novel real-time PCR method coupled with High Resolution Melting (HRM) analysis that allows for the simultaneous differentiation of honeybee from maternal lineages A, M and C, for further application in honey authentication.

Methodology

Primer design

Three primer sets were designe based on previous data of the mitogenomes of a total of 112 honeybees of different lineages were used to design new primer sets aiming to amplify short fragments containing different single nucleotide polymorphisms (SNPs) allowing for HRM application.

- **amsCOI-F/amsCOI-R** targeting Cytochrome oxidase I (COI) gene
- **amsND1-F-amsND1-R** targeting NADH-ubiquinone oxidoreductase chain 1 (ND1) gene
- **amsCox3-F/amsCox3-R** targeting Cytochrome oxidase subunit III (Cox3) gene.

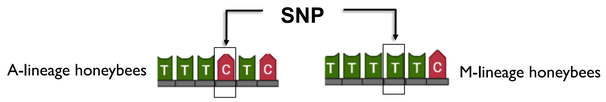

Figure 1. Example of a SNP in the *Cox3* gene of a A-lineage Honeybee and M-lineage honeybee.

DNA extraction

DNA was extracted from the thorax or legs of honeybees from A, M and C mtDNA lineages with the commercial kit Ron's Tissue DNA Mini Kit (®Bioron).

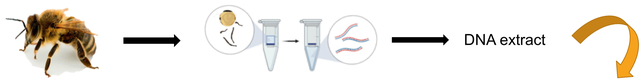

Table 1. *Conditions used in real-time PCR coupled with HRM analysis*

T °C	Time
95.0 °C	5:00
95.0 °C	0:20
52.0 °C [a] / 60.0 °C [b]	0:15
62.0 °C [a] / 72.0 °C [b]	0:10
95.0 °C	1:00
52.0 °C	5:00
52.0 °C to 95.0 °C	Melt curve: Increment of 0.2 °C , 0:05

45 cycles

Real time PCR with HRM analysis

[a], *temperatures used for COX3 and ND1 amplification and* [b] *for COI amplification*

Results

COI gene

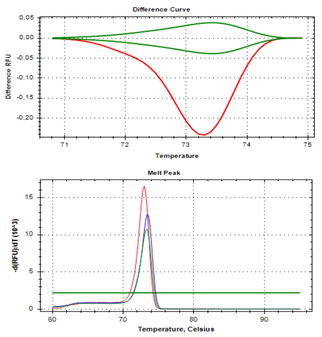

Table 2. *Results of HRM analysis targeting COI gene.*

Lineage of *Apis mellifera*	Cluster	Confidence level (%)	Mealt peak °C
A	1	98.4	73.00
M	2	99.1	73.40
C	2	97.6	73.40

✓ amsCOI-F/amsCOI-R only allowed the separation of the honeybees in two clusters, with lineage C and M clustering together (green cluster), and lineage A in another cluster (red cluster).

Figure 2. Difference curves (A) and melt peak (B) obtained by real-time PCR with EvaGreen dye and HRM analysis.

COX3 gene

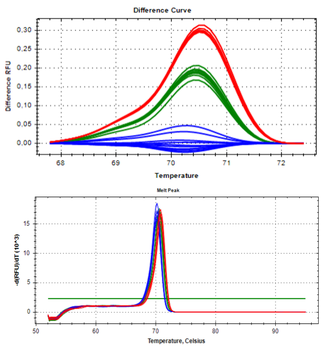

Table 3. *Results of HRM analysis targeting COx3 gene.*

Lineage of *Apis mellifera*	Cluster	Confidence level (%)	Mealt peak °C
A	3	99.6 ± 0.2	70.80
M	1	99.2 ± 0.7	70.20
C	2	99.6 ± 0.4	70.60

✓ amsCox3-F/amsCox3-R allowed to differentiate the three lineages in separate clusters, with high level of confidence.

Figure 3. Difference curves (A) and melt peak (B) obtained by real-time PCR with EvaGreen dye and HRM analysis.

ND1 gene

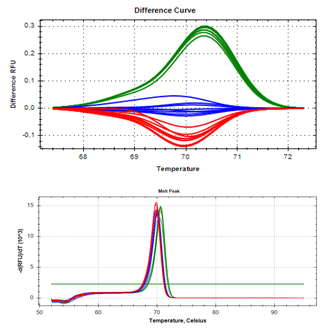

Table 4. *Results of HRM analysis targeting ND1 gene.*

Lineage of *Apis mellifera*	Cluster	Confidence level (%)	Mealt peak °C
A	1	99.0 ± 0.8	69.80
M	2	99.2 ± 0.5	70.00
C	3	99.2 ± 0.7	70.60

✓ amsND1-F/amsND1-R allowed to differentiate the three lineages in separate clusters, with high level of confidence.

Figure 4. Difference curves (A) and melt peak (B) obtained by real-time PCR with EvaGreen dye and HRM analysis.

Conclusions

✓ Different primers sets were tested to differentiate the three mtDNA lineages (A, C and M) of *Apis mellifera*.

✓ Two of them (targeting COX3 and ND1 genes) successfully differentiated the three lineages A, M and C in different clusters with high level of confidence.

✓ In future works, the novel HRM methods developed will be assayed in honey samples aiming to identify the entomological source for authenticity purposes.

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RESPONSE SURFACE ANALYSIS OF ULTRASOUND AND DYNAMIC MACERATION EXTRACTIONS OF *ARBUTUS UNEDO* L.

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ABSTRACT

The use of natural plant extracts in the food industry has been a common practice for many decades, but, due to the pursuit of healthier diets by consumers, has gained a new momentum, in which natural food additives, namely preservatives have been extracted from plants. The most prominent example is the use of rosemary extract (E392) as a food preservative throughout the European Union, paving the way for new extracts to be used for the same purpose, possibly with better results. In this work, the extraction of leafy parts of Arbutus unedo L. is described both through ultrasound assisted extraction (UAE) and dynamic maceration (DM), following an optimization through Response Surface Methodology (RSM) to optimize the extraction yield. For the DM the factors analyzed were (F1) "Time" which varied between 10 and 60 minutes, (F2) "Temperature" which varied between 30 and 80 ºC, and finally (F3) "Solvent" (ethanol) which varied between 0 and 100%. For the UAE, the factors were also (F3) "Solvent" and (F1) "Time", and although the variation in solvent was the same, the time of extraction only varied between 5 and 30 minutes. Finally, the third factor was ultrasonic (F2) "Power" of the equipment that varied between 50 and 500 watts. The analyzed response for both extractions were the dry residue (Y1) which varied between 3 and 65.3 mg for UAE and 10.4 and 99.9 mg for DM. The RSM analysis rendered a quadratic model with an inverse transformation for DM, and a reduced quadratic model with no transformation for UAE. Moreover, to optimize the yield of dry residue (Y1), optimization studies were performed and indicated the optimal points at which a higher yield of dry residue can be obtained, and were F1 – 57 minutes, F2 – 46 ºC and F3 – 52% of ethanol for the DM extraction. For UAE, the optimal points for the same Y1 response were F1 – 17 minutes, F2 – 380 watts and F3 – 39% of ethanol.

In Figure 1, it is clear that for DM a longer time of extraction favors the residue yield, as well as a temperature near 60 ºC, while for the UAE, time did not seem such an important factor, while the power of the ultrasonic probe was quite important.

Overall, due to the lower amount of ethanol needed and the lower extraction time, UAE seems to be the best extraction technique to maximize the yield of dry residue of *A. unedo*, although in terms of mass, DM yielded 99 mg while UAE only 65.3mg. Other responses are currently being studied to determine the best overall extraction technique.

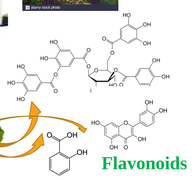
INTRODUCTION

Arbutus unedo L. is a Mediterranean plant

Portugal

Several studies showed the biological proprieties of different parts of *Arbutus unedo* (fruits, roots and leaves

***Arbutus unedo* tree**



Tannins

Flavonoids

Phenolic acids

Antioxidant activity

Antimicrobial activity

The leaf extracts obtained by

Dynamic maceration

Ultrasound assisted extraction

Variation of:
- **time**
- **temperature**
- **ultrasound watts**
- **% ethanol**

Optimization of the extraction through Response Surface Methodology

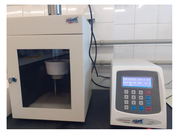
maximum yield

METHODOLOGY

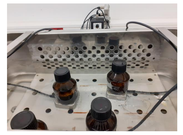
The leaves from Tras os montes region were collected, dried, and grinded

Ultrasound assisted extraction (UAE)

Dynamic maceration (DM)



Ultrassound assisted extraction



Thermostated water bath under continuous electro-magnetic stirring

Table1- Variation intervals of three factors through the dynamic maceration extraction

Factors	F1 Time (min)	F2 Temperature (°C)	F3 Solvent (%)
Interval of variation	10-60	30-80	0-100

Table2- Variation intervals of three factors for the ultrasound assisted extraction

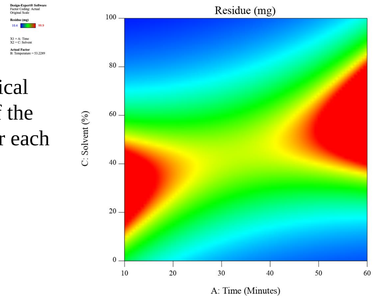
Factors	F1 Time (min)	F2 Ultrasonic(Watts)	F3 Solvent (%)
Interval of variation	5-30	50-100	0-100

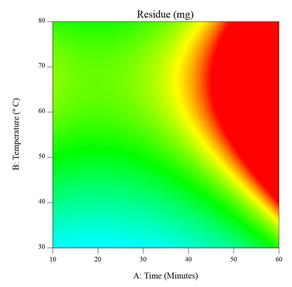
The collected extracts were filtered through a Whatman paper filter

5 ml of filtered extracts were added to weighted crucibles

Drying in the oven for 3 days at 100°C until constant weight

RESULTS





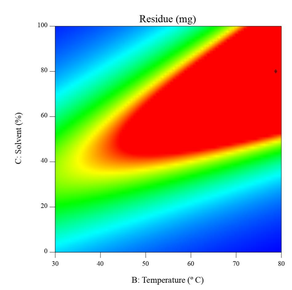
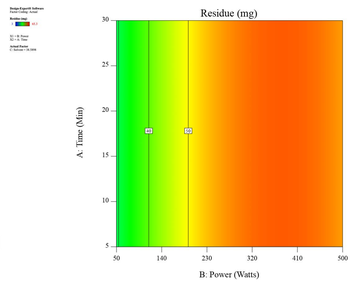


Figure1. Graphical representation of the DM extractions for each parameter



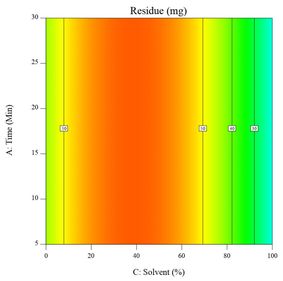


Figure2. Graphical representation of the UAE extractions for each parameter

Figure3. Optimal points for UAE and DM extractions of *A. unedo*

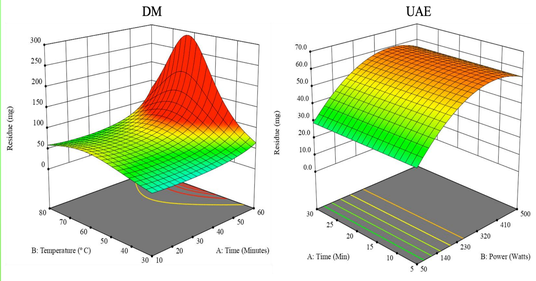


Table3- Optimum points of DM obtained by RSM

Factors	Optimum points
Time (min)	57
Temperature (°C)	46
Solvent (%)	52

Table4- Optimum points of UAE obtained by RSM

Factors	Optimum points
Time (min)	17
Ultrasonic (Watts)	380
Solvent (%)	39

CONCLUSION

- ✓For the DM the longer time of extraction favors the residue yield and is considered as important factor
- ✓ for the UAE, time did not seem such an important factor, while the power of the ultrasonic probe was quite important
- ✓due to the lower amount of ethanol needed and the lower extraction time, UAE seems to be the best extraction technique to maximize the yield of dry residue of *A. unedo*

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020). L. Barros thanks FCT – Foundation through the institutional scientific employment program-contract for her contract, while M. Carocho and S. Heleno thank FCT through the individual scientific employment program-contracts (CEECIND/00831/2018 and CEECIND/03040/2017); to FEDER-Interreg España-Portugal program for financial support through the project TRANSCoLAB 0612_TRANS_CO_LAB_2_P, BIOMA (POCI_01_0247_FEDER_046112) and Green Health (Norte-01-0145-FEDER-000042)).

World market of sunflower oil

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Abstract

The aim of the study is to analyze the current state of the oil market in the world, to consider prospects for market development and to identify ways to adapt producers to modern conditions, to monitor the Ukrainian sunflower oil market to maintain sustainable development and the title of Ukraine as world leader in sunflower oil production and export.
In the course of research the tasks concerning the analysis of the basic indicators of development of branch are solved; identification of the reasons holding back development; finding ways to increase competitiveness.

Introduction

Today, the Ukrainian oil industry has modern production facilities for processing oilseeds, which are constantly growing. Sunflower oil production is one of the most important economic and food components of the agro industrial complex in Ukraine. Every year the volumes of its production and export increase, which makes the country a leader in the domestic and international markets.

Materials

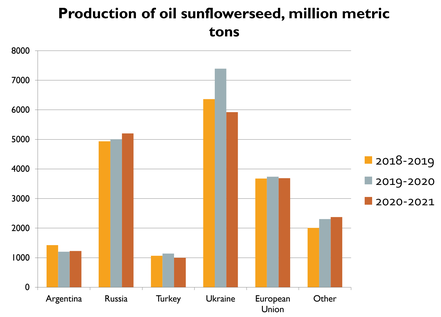
The share of sunflower in the global production of vegetable oils is about 10%. In terms of production volumes, it is the fourth indicator after palm oil (36%), soybean (28%) and rapeseed (13%).
The oil derived from sunflower is used for cooking and frying purpose globally. Sunflower is good for health and it is being used for cooking and frying in industries and food manufacturing services. The sunflower oil market is segmented on the basis of application such as food, pharmaceuticals and cosmetics.

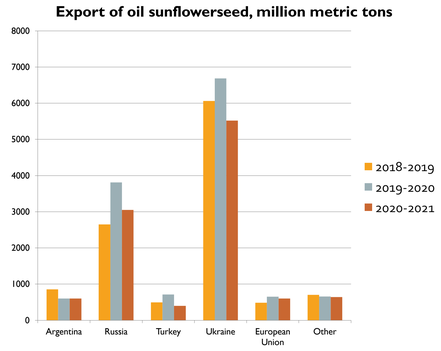
Methodology

To solve the tasks, the following statistical research methods are used to collect statistical information; analytical for forecasting development; inductions, deductions for generalization; graphic for clarity of the obtained results .

Results

Ukraine's share in a separate market of sunflower oil can be considered confidently leading because Ukrainian suppliers account for more than 50% of global product exports. The main buyer is India, followed by China, EU countries and Turkey. Ukraine has become a world leader in the export of sunflower oil in the 2020-2021 marketing year. The demand for sunflower oil is constantly growing, due to its positioning as a product for healthy eating. This is especially true of the HOSO (high oleic sunflower oil) segment. Ukraine has the twenty largest plants, which produce more than 80% of the total production of sunflower oil in the country. Sunflower oil is the main for the Ukrainian market of vegetable oils, an important raw material for many sectors of the food industry. According to Ukroilprom, the TOP-3 producers who produced more than 40% unrefined oil are as follows Kernel – 23,1%, Bunge – 10,7%, Mironovsky bread product – 6,3% .





Conclusion

Currently in Ukraine there is a tendency to increase production capacity for processing oilseeds, new plants are being built. Ukraine's world leadership in the foreign market of sunflower oil also has negative consequences. In particular, serious problems for the country's agricultural sector may be caused by the constant expansion of sown areas. Expansion of areas and non-compliance with cultivation technologies threatens land depletion and reduced yields.

Recommendations

Thus, after assessing the development trends of the sunflower oil market, the priority of further market reform is to expand foreign markets by increasing the competitiveness of sunflower seeds and domestic products and the rapid formation of appropriate customs policy depending on world market conditions.

Acknowledgements

Determination of the phytochemical composition, antioxidant and cytotoxicity profile of *Opuntia ficus-indica*

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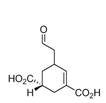
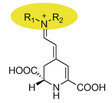
Abstract

Betalains are water-soluble nitrogenous natural colorants consisted of a backbone core, betalamic acid, and have been considered a current trend in food coloring, as has been pointed out to possess a large pharmacological profile. Significantly, the biological activities of betalains seem to play a major role in human health due to their robust antioxidant ability and their capacity to absorb free radicals. In this frame, betalains can be used as ingredients to provide colorful foods that possess enhanced antioxidant ability. Therefore, isolation of betalains and determination of their photophysical properties as also their antioxidant capacity is of importance.



Betalamic acid



General formula of betalain

The plant *Opuntia ficus-indica (L.) Mill.* (cactus or prickly pear) is one of the primary sources of betalains. They are characterized by various colors. Thus, we selected two different plant varieties; purple and orange, in order to isolate betalains and study their pharmacological potency. The purple variety is due to *betacyanins*, and the orange variety due to *betaxanthins*. This study's objective was to identify betalain patterns and content in the fruits of prickly pear cultivars/lines.

Techniques such as column chromatography and HPLC were employed for the separation of the pigments in the extracts. UV-Vis spectroscopy was used for the quantification of the betalains as also the total phenolic in the extracts. For the characterization of betalains in the isolated pigment, both LC-MS/MS and Nuclear Magnetic Resonance (NMR) analyses were performed. Specifically, novel protocols for optimizing the ^{1}H-NMR spectra of the complex mixture were developed to analyze the main pigments present in the extracts. In addition, the photophysical properties, as also their cell uptake capacity, were evaluated using fluorescent spectroscopy. Finally, the antioxidant effect of the extracts and their in vitro cytotoxicity were evaluated.

Introduction

Betalains are considered one of the most important plant pigments in nature, alongside with anthocyanins, chlorophylls and carotenoids. Despite the ubiquitous presence of these pigments in plant kingdom, the occurrence of betalains is only restricted to the order of Caryophyllales, with the exception of Caryo-phyllaceae and Molluginaceae that produce only anthocyanins, as also in some higher fungi such as Amanita muscaria. Moreover, the anthocyanins and betalains have never been reported in the same plant since there is a mutual exclusivity between these two pigment families.

Betalains are water-soluble nitrogenous natural colorants consisted of a backbone core, betalamic acid, whose condensation with imino compounds (cyclo-dopa/its glucosyl derivatives) or amino acids/derivatives forms a wide variety of red-violet betacyanins or yellow-orange betaxanthins, respectively. The chromophore property of betalains is attributed to the presence of the conjugated double bond system of the betalamic acid.

The biological activities (antioxidant, antimicrobial, anticancer etc) of betalains seems to play a significant role in human health as it is described innumerous studies, nonetheless, the use of plant extracts and not purified pigments limit the expectations created from the promising in vivo biological results

One of the main applications of betalains is their use as natural colorant in food processing. The unclear landscape around synthetic dyes about their toxicological safety on the one hand and the valuable properties of betalains on the other hand, has encouraged food industry towards the application of natural pigments such as betalains as food ingredients. In this frame, betalains can be used to provide color in colorless foods, account for color degradation during storage or standardization of raw ingredient colors.

Methodology

Quantification of betalains

To determine the effect of the solvents on the efficiency of the extraction, different solvent systems were used. McIlvaine buffer (pH 6.5) and water was the most efficient. pH adjusted to 6.5 to maintain a slightly acidic environment and avoid decomposition of the pigment. For the extraction 1.0g of the powder of the whole fruit was diluted in 20 mL. The sample was stirred for 1 min at maximum velocity in a Vortex (Thermolyne, USA), and underwent centrifugation at 3500 rpm for 15 min at room temperature. Afterward, the supernatant liquid was filtered through a membrane with 45μm pores (Millipore). The content of the pigment was determined using UV-Vis spectroscopy. The experiments were performed in triplicate.

Colorimetric Determination of Total Phenols

The total phenolic content was determined through the Folin-Ciocalteu method using UV-Vis spectroscopy. Briefly, 0.5g of the powder of the whole fruit was diluted in 10 mL Methanol/Water (80:20). The sample was stirred for 1 min at maximum velocity in a Vortex (Thermolyne, USA), and underwent centrifugation at 3500 rpm for 15 min at room temperature. Afterward, the supernatant liquid was filtered through a membrane with 45μm pores (Millipore). The experiments were performed in triplicate.

Silica gel column chromatography

A silica gel column was packed with ethanol. The extract was dissolved in methanol. Silica gel was added to the solution and the methanol was evaporated to concentrate the pigment in the silica, making sure that no traces of moisture remained that would affect the elution. At once, the pigment was added to the column, forming a thin, uniform layer; the elution was done initially with ethanol at 95%, then with methanol at 95%, and finally with acidified (pH 3.2) methanol/ water (80:20). The fractions obtained from the separation of pigments by column chromatography were analyzed with Nuclear Magnetic Resonance (NMR).

NMR Analysis

NMR and LC NMR spectra were recorded on a Bruker AV-500 spectrometer equipped with a TXI cryoprobe (Bruker BioSpin, Rheinstetten, Germany). The chemical shifts were referenced according to the internal standard, 3-(Trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt (TSP). All 1D (1H,) and 2D NMR (HSQC, HMBC) measurements were performed using standard Bruker pulse sequences 1. For the NMR measurements the betalain pigments were dissolved in D_2O containing 50mM TSP. For the determination of the isolated fractions, we set up novel protocols for optimizing the ^{1}H-NMR spectra of the complex mixture.

LC-HRMS Analysis

The HRMS analysis of the extracts was carried out on the Waters Xevo G2-XS II Q-TOF MS coupled to the Waters Acquity UPLC i-Class Plus system. The lyophilized extracts were dissolved in 50% acetonitrile in water to achieve a concentration of about 0.2mg/ml. A gradient program was applied using 0.1% formic acid in water and acetonitrile as solvents, with a flow rate of 0.25 ml/min. The separation was carried out on a Phenomenex C18, 2.1 x 100 mm RP column at 35°C. An injection volume of 5 μL was used for the analysis. The data acquisition and the data processing were performed using the Waters UNIFI v1.9 software platform. The analysis was performed in positive and negative ESI modes sequentially.

Cytotoxicity- MTT

For the viability assay, DLD1 cells (3000 cells/well) were seeded in 96-well plates. The next day, the cells were treated with Pricky pear cultivaars (10-500 μg/mL) and incubated at 37°C in a humidified atmosphere (5% CO_2). After 72 hrs of the treatment, 10 μL of MTT solution (5 mg/ml in PBS) were added in each well and incubated for 4 hrs. Then, the medium was removed and 100 μL of DMSO were added in each well. The absorbance of the samples was measured via an Elisa plate reader (Awareness Technology Inc). The absorbance was measured at 540 nm while a differential filter was set to 630 nm. Each sample was added in triplicates.

Antioxidant Capacity

To evaluate the free radical scavenging activity, the samples were allowed to react with a stable free radical, 2,2-diphenyl-1-picryl hydrazyl radical (DPPH·). The reduction of (DPPH·) was followed by monitoring the decrease in absorbance at 517 nm until the reaction reached a steady state.

Results

Quantification of betalains

Quantitative Data of Betalains in Prickly Pear Cultivars (n=3)

Sample	Betaxanthins (mg/100g ±SD)		Betacyanins (mg/100g ±SD)		Betalains (mg/100g)	
	Water	McIlvain	Water	McIlvain	Water	McIlvain
Fruit purple color	22.30±1.42	21.13±2.05	37.30±1.92	35.90±1.44	59.60	57.00
Peel purple color	37.27±1.98	34.44±1.59	85.94±2.24	87.68±2.62	123.2	122.12
Sprout purple color	2.53±0.18	2.34±0.25	3.26±0.34	2.98±0.57	5.79	5.32
Fruit orange color	55.81±1.13	57.44±1.13	9.36±0.82	8.18±1.13	65.17	65.62
Peel orange color	51.83±1.52	50.24±0.45	15.54±2.69	13.74±0.66	67.37	63.98
Sprout orange color	1.89±0.43	1.54±0.22	1.68±0.34	1.76±0.39	3.57	3.30

Quantitative Data of Betalains in Juice of Prickly Pear Cultivars (n=3)

Sample	Betaxanthins (mg/100g ±SD)	Betacyanins (mg/100g ±SD)	Betalains (mg/100g)
Orange juice with seeds	105.10±3.27	20.90±2,45	126.00
Orange juice w/o seeds	113.96±2.76	25.11±0.88	139.07
Purple juice with seeds	61.72±3.05	116.23±2.68	177.95
Purple juice w/o seeds	83.67±0.88	147.03±2.06	230.70

Colorimetric Determination of Total Phenols

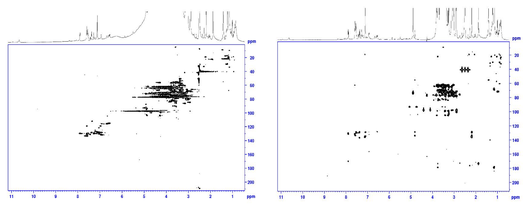
Quantitative Data of total phenolics in Prickly Pear Cultivars (n=3).

Sample	Total phenolics gallic acid mg/100g dry sample (±SD)
Fruit purple color	1.63±0.18
Peel purple color	3.27±0.55
Sprout purple color	9.95±0.84
Fruit orange color	3.24±0.39
Peel orange color	2.27±0.28

Silica Gel Column Chromatography

NMR Analysis

2D-$_1$H-^{13}C HSQC/HMBC spectra of **fraction 5** D_2O (δD_2O=4.8 ppm, T=25 ºC, 500 MHz).



Identified Betalains in *Fraction 5*

Betaxanthins	Betacyanins
Indicaxanthin	Betanin
Miraxanthin	Neobetanin
	Betanidin
	Gomphrenin

Results

LC-HRMS

Identified Betalains in *Prickly Pear* Cultivars

(S)-Tryptophan-Betaxanthin	Bougainvillein R I	Muscaaurin I
15S-betanidin 6-O(6',6''-di-O-E-4-coumaroyl)-β-sophoroside	Celosianin I	Muscaaurin VII
15S-betanidin 6-O-2'-O-β-glucosyl)[6',6''-di-O-E-4-coumaroyl)]-β-sophoroside	Dopaxanthin	Neobetanin
15S-betanidin 6-O--β-sophoroside	Ethanolamine-Betaxanthin	Phenethylamine-Betaxanthin
2-Descarboxy-betanidin	Gomphrenin I	Phenylalanine-Betaxanthin
2-Descarboxy-betanin	Gomphrenin II	Portulacaxanthin I
2'-O-Apiosyl-Betanin	Gomphrenin III	Portulacaxanthin II
2'-O-Apiosyl-Phyllocactin	Gomphrenin V	Portulacaxanthin III
3-Methoxy tyramine-Betaxanthin	Histamine-Betaxanthin	Portulacaxanthin III
4,5-Dihydromuscimol-Betaxanthin	Humilixanthin	Prebetanin
4'-O-Malonyl-betanin	Hylocerenin	Serine-Betaxanthin
5''-O-E-2'-Feruloyl-Apiosyl-Betanin	Indicaxanthin	Sinapoyl-Amaranthin
5''-O-E-sinapoyl-2'-O-Apiosyl-betanin	Isoleucine-Betaxanthin	Valine-Betaxanthin
6'-O-Malonyl-2-descarboxy-betanin	Lysine-Betaxanthin	Vulgaxanthin I
Alanine-Betaxanthin	Methionine-Betaxanthin	Vulgaxanthin II
Amaranthin	Methylated arginine-Betaxanthin	Vulgaxanthin III
Arginine-Betaxanthin	Miraxanthin I	Vulgaxanthin IV
Betanidin	Miraxanthin II	γ-Aminobutyric acid-Betaxanthin
Betanidin 5- O-(4'- O-Malonyl)-B-Sophoroside	Miraxanthin III	Miraxanthin III
Betanin	Miraxanthin V	Miraxanthin V

Cytotoxicity- MTT

Sample	Cytotoxicity of different samples ≤ 500 μg mL^{-1}
Orange Juice	NO
Orange (Pulp)	NO
Purple Juice	NO
Purple Pulp	NO
Orange peel	NO
Purple peel	NO

Antioxidant Capacity

Sample	SC50 (Scavenging Capacity) (μg/ml)
Fraction 5	55.33 ±1.52

Conclusion

We analyzed and reported the betalains in two varieties of *Opuntia Ficus-Indica* from Greece. For first time we present a combined LC-HRMS and NMR platform for their identification. The fruit extracts were also analyzed for their antioxidant capacity and cytotoxicity. They demonstrated significant antioxidant capacity and proved safe for use as colorants in the food industry.

Acknowledgements

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NUTRITIONAL VALUE AND CHEMICAL COMPOSITION OF PURSLANE LEAVES IN RELATION TO HARVESTING STAGE

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INTRODUCTION

- *Portulaca oleracea* L. belonging to the Portulacaceae family is an invasive weed with a widespread distribution through the word
- Its edible plant parts have been acknowledged to the Mediterranean diet due to its high nutritional value especially for their high concentration in omega-3 fatty acids
- Equally, leaves and stems present a valuable mineral and macronutrient profile whereas phenolic compounds and oleracein derivatives of purslane leaves have been attributed with antioxidant properties
- Many researches have pointed out that the effects of cultivation practices, environmental conditions and genetic variation could significantly affect the the nutritional value and chemical composition of the aerial parts of purslane
- In this research, we studied the effect of harvesting stage (29, 43, 52 days after sowing (DAS)) on the nutritional value and chemical composition of purslane edible plant parts

MATERIALS

- The trial was carried out at the experimental field of the University of Thessaly, in Larissa during the summer of 2016. Seeds of common purslane (*Portulaca oleracea* L.) were obtained from Hortus Sementi Srl. (Budrio, Italy) and were sown directly in soil on 06/06/2016
- Prior to sowing, a base dressing of 100 kg/ha with 10-10-10 fertilizer (N-P-K) was applied, whereas irrigation was applied with sprinklers at regular intervals (once a week, starting on the day of sowing) and no pesticides or other agrochemicals were applied during cultivation. Harvesting took place at three different growth stages, namely on 05/07/2016 (29 days after sowing (DAS)), on 19/07/2016 (43 DAS), and on 28/07/2016 (52 DAS)
- After each harvesting stage, the aerial plant parts were divided in stems and leaves in which fresh samples of plant tissues were placed in a forced-air oven, and dry weight was recorded after drying the samples at 70 °C until constant weight
- Batch samples of fresh plant tissues were stored at −80 °C and were then lyophilized. The lyophilized samples were ground to powder with a pestle and mortar, and were put in plastic air-sealed bags and stored at −80 °C until further analysis

METHOLOGY

- Nutritional compounds of the samples were analyzed (moisture, fat, ash, proteins and carbohydrates) following the Association of Analytical Communities (AOAC) procedures
- Tocopherols were determined in the lyophilized samples using a high performance liquid chromatography system coupled to a fluorescence detector using the internal standard method for quantification
- Free sugars composition was evaluated by using a HPLC system coupled with a refraction index detector, organic acid identification was peformed by Ultra-Fast Liquid Chromatography coupled with a Diode-Array Detector (UFLC-DAD) and fatty acids were determined by gas-liquid chromatography with flame ionization detection
- Phenolic compounds and oleracein derivatives were evaluated using an ultra-performance liquid chromatography (UPLC) system equipped with a diode array detector coupled to an electrospray ionization mass spectrometry detector (MS)

Image 1. *Portulaca oleracea* L. cultivation at the experimental field

RESULTS

Table 1. Composition in tocopherols (μg/100 g fw) and sugars (g/100 g fw) of purslane stems and leaves in relation to harvesting stage (mean ± SD)

Harvest Stage (DAS) *	Plant Part	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total Tocopherols
29	Leaves	215 ± 4b	14.0 ± 0.7b	140.7 ± 0.1a	9.6 ± 0.5b	380 ± 4b
43	Leaves	197 ± 3c	12.4 ± 0.2b	87.7 ± 0.2c	5.1 ± 0.2c	302 ± 2c
52	Leaves	327 ± 3a	44 ± 2a	97 ± 8b	13.5 ± 0.5a	481 ± 9a

* DAS: days after sowing; Different Latin letters (a–c) in the same column refer to significant differences between harvest stages for the same plant part (stems or leaves) at $p = 0.05$. ** Comparison of means of different plant parts (stems and leaves) from the same harvest was performed with Student's *t*-test at $p = 0.05$.

Table 2. Composition in organic acids (g/100 g fw) of purslane stems and leaves in relation to harvesting stage (mean ± SD)

Harvest Stage (DAS) *	Plant Part	Oxalic Acid	Quinic Acid	Malic Acid	Citric Acid	Total Organic Acids
29	Leaves	6.2 ± 0.1b	6.82 ± 0.01c	3.00 ± 0.03a	3.26 ± 0.01a	19.2 ± 0.1b
43	Leaves	5.7 ± 0.1c	8.4 ± 0.2b	1.90 ± 0.04b	1.53 ± 0.02b¥	17.6 ± 0.1c
52	Leaves	8.6 ± 0.2a	16.8 ± 0.5a	1.67 ± 0.01c	3.24 ± 0.03a	30.3 ± 0.2a

* DAS: days after sowing; Different Latin letters (a–c) in the same column refer to significant differences between harvest stages for the same plant part (stems or leaves) at $p = 0.05$. ** Comparison of means of different plant parts (stems and leaves) from the same harvest was performed with Student's *t*-test at $p = 0.05$.

Table 3. Composition in sugars (g/100 g fw) of purslane stems and leaves in relation to harvesting stage (mean ± SD)

Harvest Stage (DAS) *	Plant Part	Fructose	Glucose	Sucrose	Trehalose	Total Sugars
29	Leaves	0.11 ± 0.01b	0.041 ± 0.002c	nd	0.012 ± 0.001c	0.160 ± 0.007b
43	Leaves	0.183 ± 0.007a	0.113 ± 0.002a	0.009 ± 0.001a	0.026 ± 0.001b	0.330 ± 0.009a
52	Leaves	0.179 ± 0.007a	0.100 ± 0.001b	0.014 ± 0.001a	0.041 ± 0.001a	0.330 ± 0.008a

nd: not detected; * DAS: days after sowing; Different Latin letters (a–c) in the same column refer to significant differences between harvest stages for the same plant part (stems or leaves) at $p = 0.05$. ** Comparison of means of different plant parts (stems and leaves) from the same harvest was performed with Student's *t*-test at $p = 0.05$.

Table 4. Quantification of phenolic compounds and oleracein derivatives in purslane stems and leaves (mg/100 g dried weight (dw)) in relation to harvesting stage (mean ± SD)

Phenolic compound	Harvest Stage (DAS) *		
	29	43	52
Oleracein C[A]	143 ± 5a	21.2 ± 0.3c	102 ± 2b
Sinapic acid hexoside[C]	22.1 ± 0.7a	nd	nd
Oleracein A[A]	103 ± 2a	8.2 ± 0.1c	34.9 ± 0.8b
TPCOD	268 ± 6a	29.3 ± 0.4c	137 ± 3b

nd: not detected; * DAS: days after sowing; Different Latin letters (a–c) in the same column refer to significant differences between harvest stages for the same plant part (stems or leaves) at $p = 0.05$. ** Comparison of means of different plant parts (stems and leaves) from the same harvest was performed with Student's *t*-test at $p = 0.05$.

- The highest moisture content was recorded at 29 and 43 DAS and decreased at 52 DAS, whereas stems contained more water at 43 DAS. Also, fat content didn't have any significant difference between the harvesting stages and protein content was highest at the last harvest (52 DAS)
- Regarding to the macronutrient content (ash and carbohydrates) and energetic value the highest content was observed at the last harvest, while purslane's stem at 29 DAS presented the highest content in ash, carbohydrates and energetic value
- Leaves had the highest moisture content at the first harvesting stage and contained more fat and proteins at 29 DAS whereas stems had a higher content of carbohydrates, ash and energetic value at the same harvesting stage
- Leaves recorded significantly higher amounts in individual tocopherols and total tocopherols regardless of the harvesting time in comparison with the stems who presented the highest content at the first harvesting stage (29 DAS)
- Purslane's stems part had significantly a higher content of fructose, glucose, sucrose and total free sugars than leaves regardless of the harvesting stage
- Organic acid content in leaves contained mostly Quinic and oxalic acids at all the harvesting stages, whereas stems contained oxalic, Quinic and malic acid at the first harvesting stage (29 DAS)
- Fatty acid content differed significantly between the two plant parts, in particularly leaves had the highest content of palmitic and linoleic acids at 29 DAS, whereas α-linolenic acid contributed the most for the overall fatty acid profile of the stems for the first two harvesting stages
- The highest polyunsaturated fatty acids (PUFA)/saturated fatty acids (SFA) ratio and the lowest n6/n3 ratio were recorded at the first harvesting stage (29 DAS) for both plant parts respectively
- Leaves contained significantly higher amounts of individual and total phenolic compounds and oleracein derivatives compared to stems regardless of the harvesting stage. Also, harvesting at 29 DAS resulted in significantly higher contents of phenolic compounds and olarecein derivatives especially in the case of leaves

Table 5. Fatty acid composition (%) of the studied purslane stems and leaves (mean ± SD) in relation to harvesting stage

Fatty acids	Harvest Stage (DAS) *		
	29	43	52
C6:0	0.024 ± 0.001c	0.067 ± 0.001b	0.220 ± 0.001a
C8:0	0.032 ± 0.003c	0.039 ± 0.001b	0.095 ± 0.007a
C10:0	0.052 ± 0.001b	0.051 ± 0.001b	0.125 ± 0.007a
C12:0	0.81 ± 0.02c	0.867 ± 0.001b	1.37 ± 0.04a
C14:0	0.736 ± 0.002c	0.77 ± 0.01b	1.24 ± 0.01a
C15:0	0.49 ± 0.01b	0.420 ± 0.003c	0.75 ± 0.01a
C16:0	9.8 ± 0.1c	10.83 ± 0.01b	12.39 ± 0.03a
C16:1	0.52 ± 0.01b	0.48 ± 0.01c	0.730 ± 0.001a
C17:0	0.15 ± 0.01c	0.159 ± 0.005b	0.265 ± 0.007a
C18:0	2.52 ± 0.05c	2.72 ± 0.01b	3.89 ± 0.06a
C18:1n9c+t	5.29 ± 0.05b	4.65 ± 0.04c	6.4 ± 0.1a
C18:2n6c	11.40 ± 0.08c	11.63 ± 0.02b	14.81 ± 0.02a
C18:3n3	54.92 ± 0.08a	54.34 ± 0.03a	35.4 ± 0.1b
C20:0	1.79 ± 0.01b	1.80 ± 0.01b	2.95 ± 0.03a
C20:1CIS-11	0.08 ± 0.01c	0.11 ± 0.01b	0.140 ± 0.001a¥
C20:3n3+C21:0	0.155 ± 0.004c	0.195 ± 0.004b	0.32 ± 0.02a
C20:5n3	0.051 ± 0.003a	0.042 ± 0.001b	0.040 ± 0.001b
C22:0	9.0 ± 0.3b	8.62 ± 0.09c	15.0 ± 0.2a
C23:0	0.20 ± 0.01b	0.15 ± 0.01c	0.31 ± 0.01a
C24:0	2.04 ± 0.08b	2.05 ± 0.01b	3.61 ± 0.04a
Total SFA (% of total FA)	27.58 ± 0.06c	28.5 ± 0.1b	42.2 ± 0.3a
Total MUFA (% of total FA)	5.89 ± 0.05b	5.25 ± 0.06c	7.3 ± 0.1a¥
Total PUFA (% of total FA)	66.53 ± 0.01a	66.21 ± 0.04b	50.5 ± 0.2c
PUFA/SFA	2.412 ± 0.003a	2.319 ± 0.007b	1.196 ± 0.009c
n6/n3	0.207 ± 0.001c	0.213 ± 0.001b	0.414 ± 0.002a

* DAS: days after sowing; ¥: no significant difference was observed between plant parts. Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Pentadecylic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Margaric acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9); Linoleic acid (C18:2n6c); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1CIS-11); Eicosatrienoic acid (C20:3n3); Heneicosylic acid (C21:0); Eicosapentaeonic acid (C20:5n3); Behenic acid (C22:0); Tricosylic acid (C23:0); Lignoceric acid (C24:0); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6/n3: omega-6/omega-3 fatty acids. Different Latin letters (a–c) in the same row refer to significant differences between harvest stages for the same plant part (stems or leaves) at $p = 0.05$. ** Comparison of means of different plant parts (stems and leaves) from the same harvest was performed with Student's *t*-test at $p = 0.05$.

Table 6. Nutritional value (g/100 g fresh weight (fw)) and energetic value (kcal/100 g fw) of purslane stems and leaves in relation to harvesting stage (mean ± SD)

Harvest Stage (DAS) *	Plant Part	Moisture (%)	Fat	Proteins	Ash	Carbohydrates	Energy
29	Leaves	91.00 ± 0.49a	0.157 ± 0.001b	1.57 ± 0.02c	2.14 ± 0.05b	5.13 ± 0.02c	43.2 ± 0.1c
43	Leaves	90.81 ± 0.16a	0.148 ± 0.002b	1.91 ± 0.01b	1.89 ± 0.05c	5.25 ± 0.03b	45.70 ± 0.02b
52	Leaves	88.16 ± 0.41b	0.230 ± 0.001a	2.96 ± 0.04a	2.40 ± 0.06a	6.2 ± 0.1a	61.3 ± 0.1a

* DAS: days after sowing. Different Latin letters (a–c) in the same column refer to significant differences between harvest stages for the same plant part (stems or leaves) at $p = 0.05$. ** Comparison of means of different plant parts (stems and leaves) from the same harvest was performed with Student's *t*-test at $p = 0.05$.

CONCLUSIONS

- According to the results of the study, there was recorded a significant interaction between plants part of purslane and the different harvesting stages for all the tested parameters
- Leaves contained higher amounts of macronutrients than stems in case of 52 DAS, while the isoform α-tocopherol increased at 52 DAS resulting in the highest overall tocopherol content
- Related to the oxalic acid and total organic acids content the highest content was recorded in the leaves especially at the last harvesting stage (52 DAS), whereas glucose and fructose were the main sugars detected in which stems had a higher concentration compared to the leaves
- Phenolic compounds and oleracein derivatives were also detected in plant parts with oleraceins A and C being the main compounds regardless of the harvesting stage
- Early harvesting stage could increase the nutritional value through increasing the content of valuable compounds, whereas at the same time contents of anti-nutritional compounds are reduced respectively

RECOMMENDATIONS

- The extensive chemical variation observed in the plant parts of purslane could be a useful indicator for the identification and the classification of different *Portulaca* taxa based on its chemical profile
- The high ratio of polyunsaturated fatty acids (PUFA)/saturated fatty acids (SFA) and the low n6/n3 fatty acid recorded in stems and leaves of purslane could be exploited further as a source of high nutritional value
- Further studies are needed to be carried out in order find out the ideal cultivation practices, environmental conditions and genetic factors attributed to a high nutritional value and an abundant chemical composition of the purslane

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Chemical composition and bioactive properties of vine-cane extracts obtained by subcritical water extraction

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LAQV requimte LABORATÓRIO ASSOCIADO PARA A QUÍMICA VERDE

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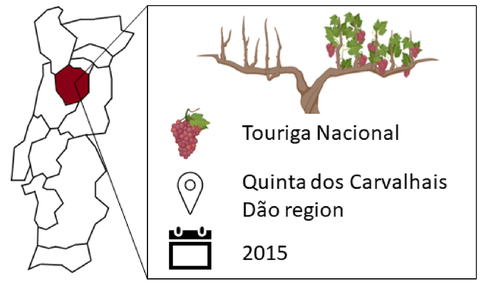
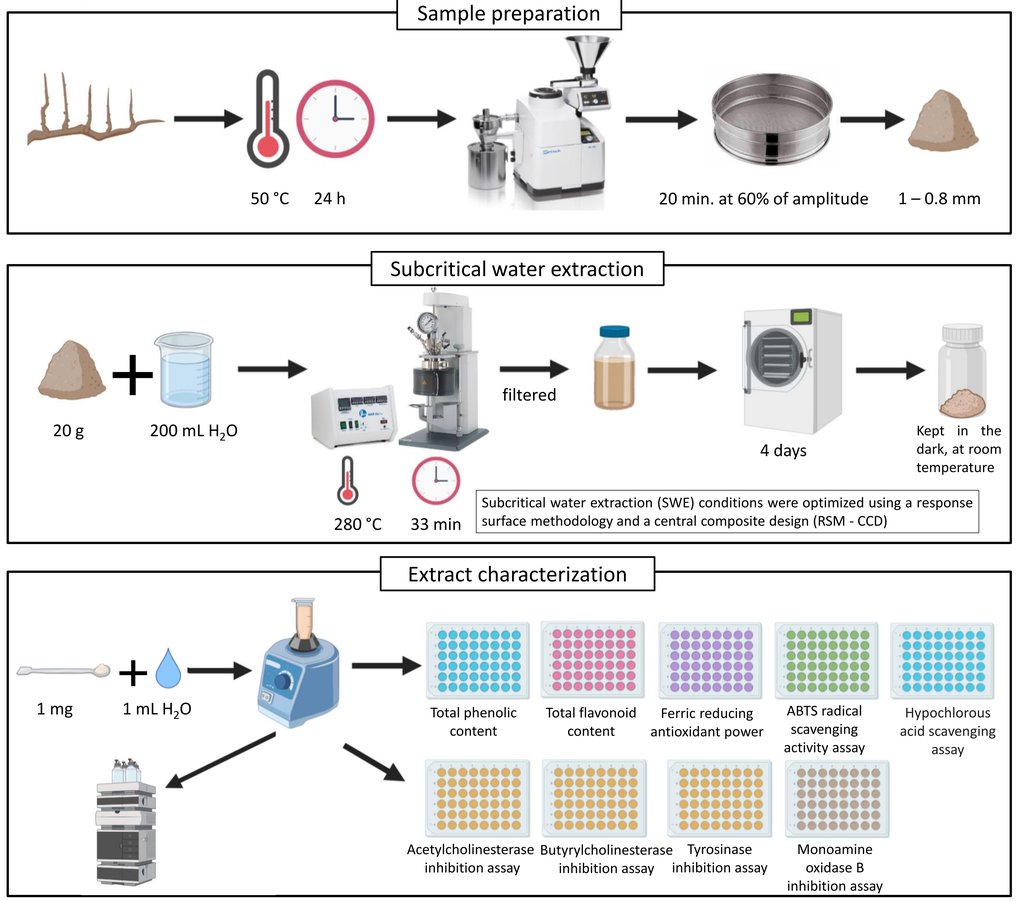
Abstract

Grapes are one of the major fruit crops produced throughout the world due to their high nutritional properties, consumer appreciation and ancient domestication [1]. Vine pruning is crucial to increase the quantity and quality of these fruits. Although, it leads to the generation of large quantities of wastes, that still require a sustainable and profitable destination [2]. Vine-canes have been reported as an attractive natural source of polyphenols that could be employed in many industries; however, one of the biggest challenges is to find an efficient environmentally friendly extraction technique that can be applied at a large scale.

Materials



Methodology



Results

Total phenolic content:
229 ± 23 mg of gallic acid equivalents per g of dry extract

Total flavonoid content:
91 ± 4 mg of epicatechin equivalents per g of dry extract

Ferric reducing antioxidant power:
227 ± 20 mg of ascorbic acid equivalents per g of dry extract

ABTS radical scavenging activity assay:
236 ± 10 mg of ascorbic acid equivalents per g of dry extract

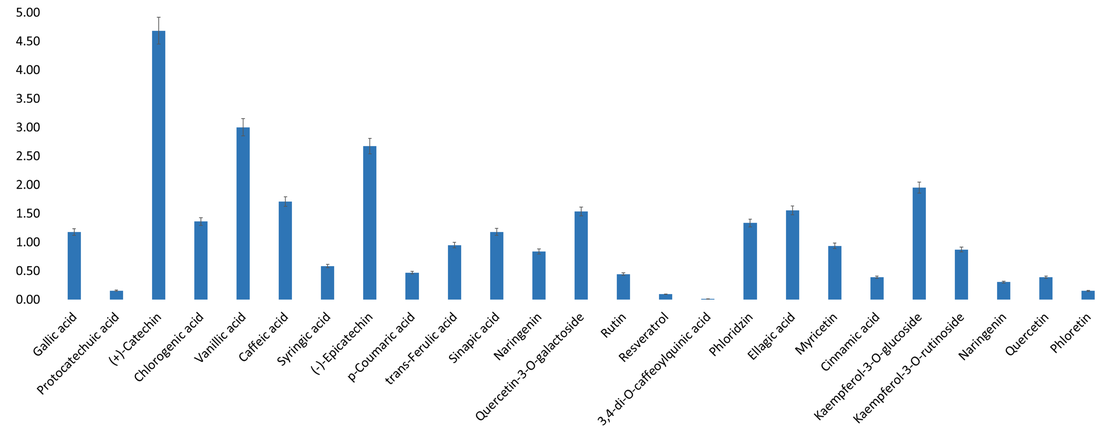
Hypochlorous acid scavenging assay:
IC_{50}= 53 ± 6 µg/mL

Acetylcholinesterase inhibition assay:
IC_{50} = 290.5 µg/mL

Butyrylcholinesterase inhibition assay:
IC_{50} = 244.0 µg/mL

Tyrosinase inhibition assay:
IC_{50} = 1459.0 µg/mL

Monoamine oxidase B inhibition assay:
≅ 53.4 % of inhibition at 1000 µg/mL



Content of the identified phenolic compounds in Touriga Nacional subcritical water extract by high performance liquid chromatography with diode array detection; results expressed in mg compound/g dry extract.

Conclusion

The presented work confirms the potential of vine-canes being used as a natural source of phenolic compounds.

SWE reveal to be an interesting technique to be employed at larger scale to recover high yields of bioactive compounds as it is environmentally friendly and time-saving.

(+)-Catechin, vanillic acid, (-)-epicatechin and kaempferol-3-*O*-glucoside were the major compounds contributing to the Touriga Nacional antioxidant and inhibitory activities.

Considering the potential of this extract for the nutraceutical and food additive industries, further research regarding its safety is undergoing.

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Carotenoid-based solutions for the replacement of artificial colorants in pastry products

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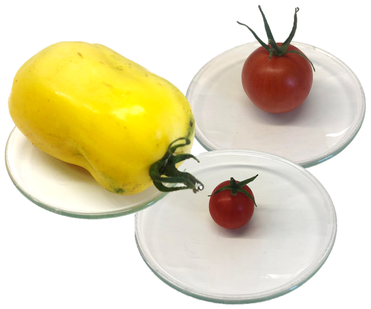
1. Introduction

Colour has a great importance in the first consumers' impression, allowing to infer about the overall quality, the taste, the smell, the texture, and even the safety of foodstuff [1]. For these reasons, there is a massive use of colorants in food products. Nevertheless, the most applied compounds are of artificial origin and some of them have been increasingly associated to health issues, with allergic reactions, children attention deficit, and cancer pointed out as the most common consequences [2].

2. *Solanum* sp.

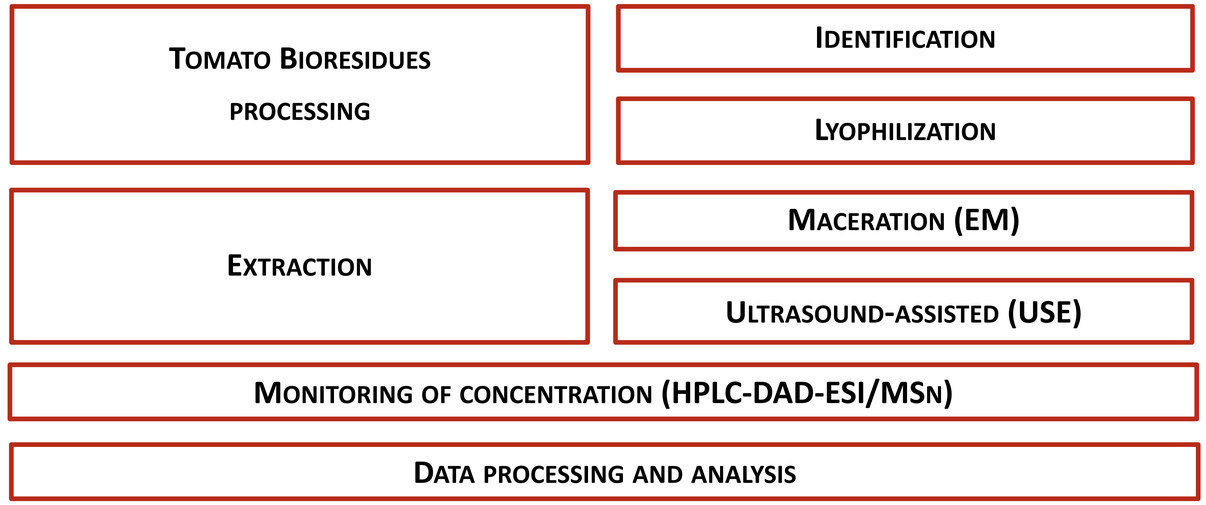
These facts have been driving new research in this field, through the exploitation of natural sources of colouring molecules to be applied in detriment of artificial colorants. Among the numerous natural matrices potentially used for the extraction of colouring compounds, the fruits from the genus *Solanum* represent promising sources of pigments, namely carotenoids [3].

Together with the fact that large amounts of fresh tomato wastes (resulting from crop growing, packaging, processing, storage, and sale) are discarded worldwide, the recovery of valuable colorant biomolecules from these agri-food wastes represents a crucial step of the circular economy by re-introducing them into the food chain as ingredients [3].



3 . Extraction technologies

The need to process these bio-wastes for the recovery of coloring molecules, has led to the use of more eco-sustainable extraction methodologies in detriment of more conventional techniques, such as maceration. Ultrasound-assisted extraction methodology arises as one of the most promising alternatives, with lower extraction times, use of greener solvents, and higher recovery yields, but also with the possibility to be scaled-up to respond to the high demands of the industrialized world [3].



4. Conclusion

Carotenoid compounds are lipophilic pigments responsible for the yellow, orange, and red colours of certain plant matrices, with a vast structural diversity, but prone to isomerization and oxidation [4]. However, the colouring capacity of these molecules overcomes any instability problem (that can be solved with stabilization strategies) and, therefore, carotenoid-based colorants appear as a valid solution for application in the pastry sector, that greatly relies on yellow/orange artificial colorants.

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STUDY OF AROMATIC AND MEDICINAL PLANTS AS POTENTIAL NATURAL INGREDIENTS FOR THE FOOD INDUSTRY

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Introduction

Considering the association of plants consumption and utilization with the treatment of some illnesses and diseases, several aromatic and medicinal plants have been used in traditional and contemporary medicine. This capacity has been proven scientifically and there are numerous studies describing their therapeutic properties in the treatment of inflammatory and cardiovascular disorders, diabetes, among other conditions [1]. The antioxidant and antimicrobial activities have already been studied in a wide variety of plants, which have been shown to contain bioactive molecules effective against pathogenic microorganisms and capable of removing reactive oxygen species formed in cells [2,3].

In this sense, ten aromatic and medicinal plants (*Eucalyptus globulus* Labill., *Olea europaea* L., *Melissa officinalis* L., *Origanum vulgare* L., *Glycyrrhiza glabra* L., *Arbutus unedo* L., *Matricaria recutita* L., *Thymus vulgaris* L., *Ocimum vulgare* L. *and Salvia officinalis* L.) were selected to perform an initial screening for the exploration of natural ingredients with bioactive potential.

Materials

The samples were kindly provided by the company Deifil (Fig. 1). They were oven dried, ground and stored in a cool, dry place, protected from light until further analysis.

Fig. 1- Aromatic and medicinal plants.

Methodology

The plant extracts were obtained after ethanolic extraction. The antioxidant activity was evaluated through two in vitro cell-based assays, namely the lipid peroxidation inhibition test (TBARS) and the oxidative hemolysis inhibition test (OxHLIA), and antimicrobial activity was tested by the broth microdilution method, against a panel of bacteria and fungi, selected according to their public health importance.

Results

These analyses presented very promising results, showing a high bioactive potential for all plant extracts. In the TBARS assay, *E. globulus* and *O. vulgare* stood out with lower EC_{50} values, followed by *T. vulgaris* and *S. officinalis*. In turn, in the OxHLIA assay, for $\Delta t_{60\ min}$ and $\Delta t_{120\ min}$, the excellent antioxidant capacity of *S. officinalis* and *T. vulgaris* was also evidenced, respectively (Table 1).

Table 1. Antioxidant activity of aromatic and medicinal plants extracts.

Sample	OxHLIA (IC_{50} values, µg/mL) Δt 60 min	OxHLIA (IC_{50} values, µg/mL) Δt 120 min	TBARS (IC_{50} values, µg/mL)
Bio_EU	43 ± 2	90 ± 4	1.91 ± 0.01
Bio_OLIV	10.8 ± 0.3	34.1 ± 0.8	26 ± 1
Bio_CID	8.9 ± 0.1	27.1 ± 0.4	25 ± 1
Bio_OREG	16.0 ± 0.3	39.4 ± 0.4	5 ± 1
Bio_ALCA	10.6 ± 0.3	50 ± 3	21.7 ± 0.3
Bio_MED	209 ± 3	365 ± 4	106 ± 1
Bio_CAM	67 ± 1	118 ± 2	26 ± 1
Bio_TOM	5.2 ± 0.1	16.6 ± 0.9	11.8 ± 0.3
Bio_MANJ	29.2 ± 0.8	105 ± 4	23 ± 1
Bio_SALV	3.4 ± 0.2	21 ± 2	14 ± 1
Trolox	19.6 ± 0.7	41 ± 1	5.4±0.3

In addition, the evaluation of the antimicrobial activity highlighted the sample of *G. glabra*, both at the bacterial and fungal levels (Fig. 2).

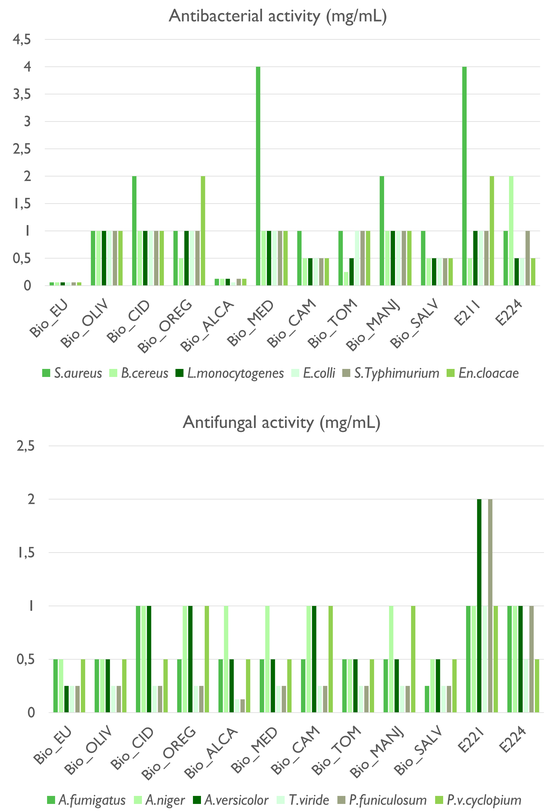

Fig. 2. Antimicrobial activity of aromatic and medicinal plants extracts.

Conclusions

Overall, it was possible to prove the great bioactive potential of the *E. globulus*, *O. vulgare*, *S. officinalis*, *T. vulgaris* and *G. glabra* extracts, and to highlight them as promising options for exploitation in the food industry.

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Effects of Black-Eyed-Beans in an Animal Model of Colorectal Cancer

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Abstract

Several studies revealed a possible protective effect on the intake of vegetables concerning colorectal cancer (CRC) development. Black-eye-bean (*Vigna unguiculata* (L.) Walp) is ingested as a high-quality plant protein source, presenting a better option to red meat [1]. Black-eye-beans have a high antioxidant capacity and high flavonoids content [2]. Flavonoids are among the compounds with the most important anticancer properties of vegetables, especially isoflavones [3]. So, CRC development might be reduced by introducing black-eye-bean in the diet. This work aimed to evaluate the effects of supplementation of black-eyed-beans on an animal model of chemically induced CRC, through the sequential administration of azoxymethane (AOM) and sodium dextran-sulfate (DSS).

Forty-eight female FBV/N strain mice, aged 5-6 months, were randomly divided into four groups: control group (n=9) , induced group (n=13), induced group / 20% (m/m) black-eyed-bean flour (BEBF (n=12) and induced group / 50% (m/m) BEBF (n=14). AOM was administered intraperitoneally only once (7.5 mg/kg). One week later, the animals were exposed to drinking water with 1.5% DSS for seven consecutive days over three cycles, with 7-day intervals between them. The animals had *ad libitum* access to food and water. Weekly, the animals were individually weighed. After 13 weeks, all animals were humanely euthanized. Blood and organs were collected for further analysis: histopathology, immunohistochemistry, biochemical markers, and comet assay.

No differences were observed in initial and final animal weights between groups. Regarding histopathological analysis, 50% (n=6) of the animals in the induced group + 20% BEBF presented epithelial dysplasia in the colon, 16.67% (n=2) presented adenocarcinomas in the rectum, being the group with more lesions. In the three groups induced with AOM/DSS, mild to moderate inflammation was observed in the colon. Regarding the expression of the cell proliferation marker Ki-67 in the colon mucosa, there was a higher immunostaining in the + 20% BEBF induced group. Data showed no significant variations of circulating C-reactive protein, IL-6, TWEAK, MMP-9, MMP-2, and myostatin levels. The comet assay revealed no statistically significant differences between groups.

To conclude, black-eyed-beans did not show negative or positive effects on this model of CRC. New studies must be carried out using different doses of the carcinogenic compound, time of exposure to the inflammatory agent, the moment of sacrifice, and/or concentration of the used black-eyed-beans.

Introduction

In the last few decades, the incidence of cancer has increased worldwide [4]. CRC is the most common malignant neoplasm of the gastrointestinal tract and due to its increased incidence and associated mortality, the costs involved in its diagnosis and treatment have made this disease a serious public health problem [5].

Animal models provide invaluable information to better understand the various aspects involved in the onset and development of the disease, as well as in the discovery and evaluation of new pharmacological and non-pharmacological treatments [6]. The chemically induced models of CRC are animal models that have been used for a long time and are characterized by exposure to chemical carcinogens, such as 1,2-dimethylhydrazine (DMH) or azoxymethane (AOM), among others. These chemical compounds are considered ideal for the study of CRC in laboratory animals [7].

The Back-Eyed-Bean is a legume present in the Mediterranean diet, with low-fat content, rich in iron and low-calorie content, constituting a good alternative source of protein. This legume is a rich source of bioactive compounds, peptides, starch, fiber, phytochemicals and antioxidants, as well as certain vitamins and minerals, having specific characteristics that benefit human health in various ways [1].

Therefore, this study aimed to evaluate the effects of the introduction of Black-Eyed-Beans in the diet of female FVB/n mice to which CRC was induced.

Materials and Methodology

The experimental protocol used for this study is summarized in **figure 1**.

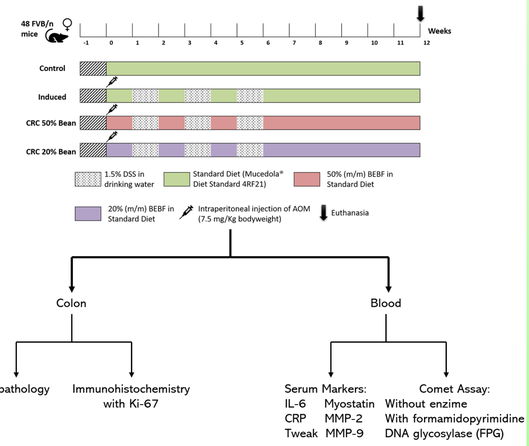


Figure 1. Experimental protocol.

The animals had *ad libitum* access to food and water. Weekly, the animals were individually weighed. All ethical issues followed the guidelines of the Portuguese *Direção Geral de Alimentação e Veterinária* (approval number 010535).

Results

As shown in **figure 2**, there were no differences between groups regarding body weight, both at the beginning of the protocol and at the end.

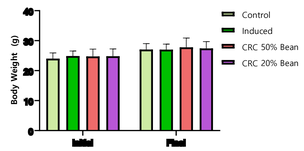


Figure 2. Initial and final animal mean body weight. Data is presented as mean ± standard error.

Control group did not present any type of proliferative lesion (**Figure 3 (a)**), while the induced group presented animals with dysplasia (38.46%) (**figure 3 (b)**) and one animal (7.69%) with adenocarcinoma in the rectum (**figure 3 (c)**). The CRC 20% Bean group had the highest percentage of injuries compared to the other groups with 50% of animals showing dysplastic lesions and 16.67% animals showing adenocarcinomas, also in the rectum. The CRC 50% Bean group had 35.72% of animals with epithelial dysplasia and only one (7.14%) with colon adenocarcinoma (**figure 3 (d)**).

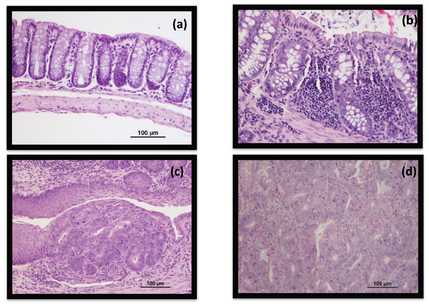


Figure 3. Mouse colon without lesions, control group **(a)**; Mouse colon with moderate inflammatory infiltrate, associated with dysplasia, induced group **(b)**; Mouse rectum: adenocarcinoma, induced group **(c)**; mouse colon: adenocarcinoma, CRC 50% BEBF group (HE, 200x) **(d)**.

The groups induced with supplementation showed the highest proliferation rate, especially the CRC 20% BEBF group (Figure 26), with a mean of 2.24% (standard error of 0.5997.) However, there were no statistically differences between groups **(figure 4 (a))**.

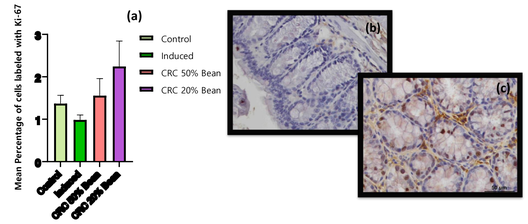
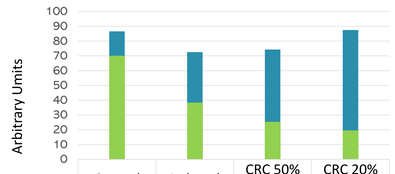


Figure 4. Mean percentage of cells labeled with *Ki-67 per* mouse **(a)**; Mouse colon: *Ki-67* negative immunostaining, control group **(b)**; Mouse colon: positive immunostaining for *Ki-67*, CRC 20% Bean group (Gill's hematoxylin, 400x) **(c)**.

Figure 5 corresponds to the results obtained by the comet assay with and without the use of the FPG enzyme. The use of the enzyme allows to determine the oxidative damage (blue) through the difference of the result of the comet assay with enzyme and without enzyme. There are no significant differences between groups regarding oxidative damage. However, in the results obtained without FPG (basal damage) there are significant differences between the control group in relation to CRC 50% Bean group (p<0.05) and in relation to the CRC 20% Bean group (p<0.05).



	Control	Induced	CRC 50% Bean	CRC 20% Bean
Oxidative Damage	17	34,2	48,9	67,9
Basal Damage	70	38,4	25,4	19,6

Figure 5. Representation of the mean basal damage and oxidative damage per group in arbitrary units (AU).

Data showed no significant variations of circulating C-reactive protein, IL-6, TWEAK, MMP-9, MMP-2, and myostatin levels.

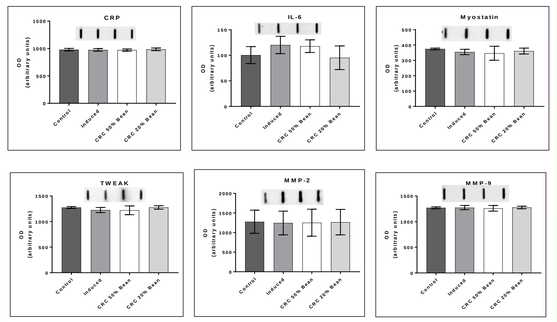


Figure 6. Serum levels of IL-6 and TWEAK, C-reactive proteins, Myostatin, MMPs-2 and -9 in the control and induced groups.

Conclusion

In general, this work allowed us to conclude that the supplementation of BEBF to the mice's diet had no significant effects, either positive or negative, on the progression of the CRC. We consider that further investigations are necessary in this animal model, possibly changing doses of the carcinogenic compound, time of exposure to the inflammatory agent, moment of sacrifice or the concentration of BEBF used.

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The authors declare no conflicts of interest.

PHENOLIC AND BIOACTIVE PROFILES OF TEN COLORED POTATO PEELS

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Abstract

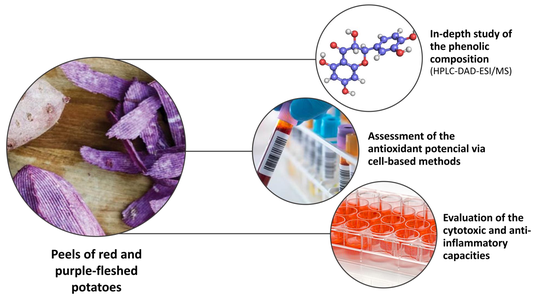

Figure 1. Graphical abstract containing the design of the presented work.

Introduction

The pharmaceutical and food industries have been exhaustively prospecting the use of natural products as sources of bioactive molecules to substitute synthetic drugs and food additives. Withal, the valorization of bio-residues abundant in bioactive phytochemicals that are commonly rejected, like potato peels, could pitch in to the development of more sustainable, both economically and socially, productive chains. Potato peels are rich sources of phytochemicals with known biological activities, such as phenolic acids and flavonoids (Figure 2). Moreover, purple-fleshed potatoes are rich sources of anthocyanins, and the amounts stored in peels are usually higher than the flesh when both flesh and peels are colored. In this study an unprecedented in-depth characterization of the non-anthocyanin and anthocyanin phenolic compounds of potato peels from ten colored potato varieties was performed. Beyond that, the antioxidant, cytotoxic and anti-inflammatory potentials of the samples were explored for the first time.

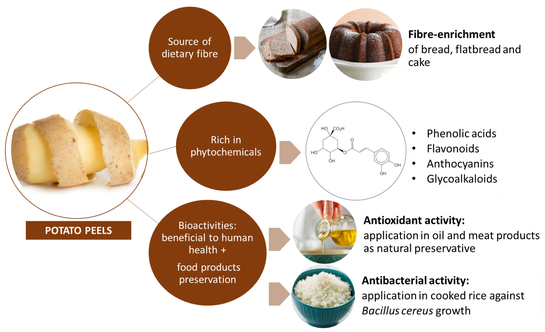

Figure 2. Nutritional features of potato peels and their potential applications.

Materials and Methods

Plant material: red- and purple-skinned potato tubers (*Solanum tuberosum* L.) of ten genotypes from five different countries of origin (Chile, Germany, Austria, United Kingdom and Finland) were cultivated in the experimental farm of the University of Thessaly, Greece. Fresh harvested tubers were transported to the Polytechnic Institute of Bragança, Portugal, where a color assessment of the samples was carried out before peels were manually separated from the flesh and freeze-dried.

Extracts preparation: powdered freeze-dried peels were extracted twice with ethanol:water (80:20 *v/v*) under stirring for 1h and filtered (Figure 3).

Figure 3. Phenolic-rich extract obtainment.

Profile in phenolic compounds: HPLC-DAD-ESI/MS was performed using online double detection, diode array detector (DAD, 280 and 370 nm as preferred wavelengths) and a mass spectrometer (MS). Phenolic compounds were characterized according to their UV and mass spectra and retention times, and compared to authentic standards when available. For quantitative analysis, a calibration curve was obtained by injection of known concentrations of different standards.

Cell-based antioxidant activity: it was evaluated in terms of the potential to inhibit the production of thiobarbituric acid reactive substances (TBARS) in porcine (*Sus scrofa*) brain homogenates and by the oxidative haemolysis inhibition assay (OxHLIA). Trolox was used as a positive control.

Anti-inflammatory potential: it was evaluated by lipopolysaccharide (LPS)-induced nitric oxide (NO) production by mouse macrophages RAW 264.7. Dexamethasone (50 µM) was used as positive control, and a negative control was prepared without the addition of LPS to observe possible effects on the basal levels of NO.

Antiproliferative potential: it was tested against four human tumor cell lines, MCF-7 (breast carcinoma), NCI-H460 (lung carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma) through the Sulforodamine B test, in which Elipticin was used as a positive control. Finally, the toxicity of the extracts was evaluated in primary pig liver cells.

Results

The colored potato peels extracts contained non-anthocyanin and anthocyanin phenolic compounds. Caffeic and caffeoylquinic acid were found in the highest concentrations among non-anthocyanin phenolics in all the samples, while *O*-glycosylated flavonoid derivatives and polyamine derivatives were also detected. Regarding anthocyanins, all the tentatively identified compounds were acylated with a hydroxycinnamic acid. In the red-skinned varieties, pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside isomer, peonidin-3-*p*-coumaroylrutinoside-5-glucoside, and malvidin-3-*p*-coumaroylrutinoside-5-glucoside presented the highest content; while in the purple-skinned varieties, petunidin-3-*p*-coumaroylrutinoside-5-glucoside was the most abundant compound. The concentration of total phenolic compounds and derivatives among the samples showed a great variation and ranged from 0.27 (Red Emmalie) to 1.76 (UACH 0917) mg/g dw.

Comparative data regarding the bioactive evaluation of the samples are displayed in Tables 1 and 2.

Table 1. Antioxidant activities of the studied potato peels (mean ± SD).

	Antioxidant activity (IC_{50} values)	
Potato varieties	TBARS	OxHLIA
Rosemary	26±3^{h}	54±2^{c}
Red Cardinal	187±12^{c}	123±7^{b}
Rote Emmalie	46±2^{g}	n.a.
Purple	114±8^{f}	16±1^{c,d}
Kefermarkter Blaue	208±12^{b}	32±2^{c,d}
UACH 0917	127±7^{e}	24±2^{c,d}
Salad Blue	230±6^{a}	294±8^{a}
Blaue aus Finnland	154±2^{d}	122±4^{b}
Shetland Black	190±6^{c}	53±2^{c}
Violetta (Blaue Elise)	144±5^{d}	16±1^{c,d}

IC_{50}: extract concentration corresponding to 50% of antioxidant activity against lipid peroxidation (TBARS) and haemolysis of sheep blood cells (OxHLIA; Δt = 60 min). In each column, different Latin letters are significantly different according to Tukey's HSD test (p=0.05), for each potato group peels.

Table 2. Antiproliferative potentials of the studied potato peels (mean ± SD).

	Antiproliferative activity (GI_{50} values)			
Potato varieties	NCI-H460	HepG2	MCF-7	HeLa
Rosemary	69±1^{e}	79±4^{f}	51±2^{f}	49±3^{f}
Red Cardinal	241±3^{c}	305±11^{d}	315±10^{a}	333±17^{b}
Rote Emmalie	248±6^{c}	301±21^{d}	244±17^{c}	227±11^{e}
Purple	268±17^{b}	365±18^{a}	188±11^{e}	330 ±2^{b}
Kefermarkter Blaue	217±12^{d}	302±9^{d}	279±12^{b}	313±33^{c}
UACH 0917	280±7^{a}	319±1^{c}	224±10^{d}	346±10^{a}
Salad Blue	281±6^{a}	286±12^{e}	215±12^{d}	292±19^{d}
Blaue aus Finnland	276±2^{a,b}	281±10^{e}	281±66^{b}	333±12^{b}
Shetland Black	269±11^{b}	343±9^{b}	210±10^{d}	233±3^{e}
Violetta (Blaue Elise)	271±19^{b}	317±11^{c}	216±17^{d}	335±11^{a,b}

GI_{50}: values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2; maximum tested concentration: 400 µg/mL. In each column, different Latin letters are significantly different according to Tukey's HSD test (p=0.05), for each potato group peels.

Results

Positive results for the antioxidant capacity were recorded in the extracts of all the assayed potato peels (Table 1), as well as significant antitumor activity (Table 2). Particularly, the peels from the red-skinned variety *Rosemary* presented the best antioxidant and antitumor results.

Regarding the anti-inflammatory assay, *Rosemary* was the only variety that presented significant capacity to inhibit the growth of RAW 264.7 mouse macrophages (IC_{50} = 141 µg/mL); the maximum tested concentration of the extracts was 400 µg/mL.

Considering that potato peels may be rich in potentially toxic compounds such as glycoalkaloids assessing its toxicity is essential prior to suggesting the use of peel extracts for food purposes. The studied potato peels showed no hepatotoxic effects up to the maximum tested concentration of extract (400 µg/mL), except for the *Rosemary* peels that showed toxicity for concentrations above 304 µg/mL. Therefore, our finding highlights the safety of potato peel extracts to be used as natural additives in food formulations in low concentrations which may exert antioxidant activity without cytotoxic effects to non-tumor cell lines.

Conclusion

The herein presented results would support the use of potato peels as valuable sources of bioactive compounds and as natural additives in functional food formulations. However, further studies with more varieties are needed to reveal the genotypic variability, as well as to identify the effect of pre- and post-harvest factors on bioactive compounds content and bioactivities of potato processing bio-residues.

Acknowledgements

EVALUATION OF THE PHENOLIC COMPOUNDS PRESENT IN MONOFLORAL BEE POLLEN FROM PORTUGAL

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Abstract

Bee pollen is composed of hundreds and even thousands of plant pollen that have been harvested by bees. Beekeepers can collect this pollen loads at the hive entrance, with the use of appropriated pollen traps. In the present study, the phenolic profile of seven bee pollen samples, from Nisa and Bragança, were characterized. Bee pollen phenolic extracts were characterized according to their total phenolic and flavonoid content and also the characterization of their phenolic profile by HPLC-DAD-ESI/MS.

The results classified all bee pollen samples as monofloral with the presence of high quantities of important flavonoid and phenylamide content. In terms of quantification, most of the samples contained more than 50% phenylamides, ranging from 7.48±0.71 mg/g for the sample P5, *Echium* sp., to 36.12±1.73 mg/g for P7, *Castanea* sp.

In conclusion, some of the detected phenolic compounds can be considered as floral biomarkers such as methyl herbacetin-*O*-malonyl-hexosyl-deoxyhexoside, which was only detected in two *Rubus* sp. samples.

Introduction

In the course of evolution, a special relationship has been established between plants and bees. Bee pollen is composed of hundreds and even thousands of plant pollen that have been harvested by bees. The insects use their saliva secretions and plant nectar to agglomerate the grains and then transport it to their hives (De-Melo and de Almeida-Muradian, 2017).

Phenolic compounds are a large class of phytochemicals that are abundant micronutrients in the human diet and play a major role in plant protection against biotic and abiotic stresses (Kostić *et al.*, 2019). They stand for a wide range of similarly structured compounds, around 8000 molecules, that have at least one aromatic ring with at a minimum one hydroxyl group, Figure 1 (Garcia-Salas et al., 2010).

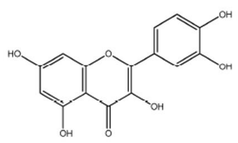


Figure 1: Chemical structure of quercetin

The most extensive phenolics in the human diet are flavonoids as well as phenolic acids (cinnamic and benzoic acids). Flavonoids may be detected in plants in the form of aglycones or glycosides which provide the color of leaves, fruits, and flowers (orange, carmine, blue) (Garcia-Salas et al., 2010).

Methodology

Palynological analysis

Spectrophotometric analysis

- Phenolic compounds extraction
- Total phenolic content using Folin-Ciocalteu method, gallic acid used as standard: results expressed in mg GAE/g of bee pollen
- Total flavonoid content using Aluminum Chloride method, quercetin used as standard: results expressed in mg QE/ g of bee pollen

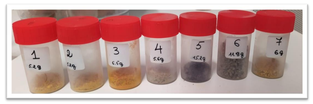
Phenolic compounds profiling and quantification using HPLC-DAD-ESI/MS

- Gradient elution of the mobile phase: A (Water +0.1% Formic Acid) and B (Acetonitrile + 0.1% Formic Acid)
- MS in the negative ion mode
- Identification using chromatographic behavior, UV spectra and MS information
- Quantification using standards: results expressed in mg/g bee pollen

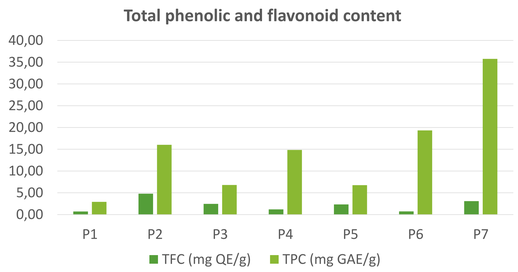
Results

Palynological analysis

Sample code	Dominant botanical family	Predominant pollen type (%)	Other pollen type present	Sample classification
P1	Asteraceae	*Carduus* sp. (>80%)	*Echium* sp., *Asphodelus* sp.	monofloral
P2	Oleaceae	*Ligustrum/Olea* sp. (>80%)	*Eucalyptus* sp.	monofloral
P3	Cistaceae	Cistaceae (100%)		monofloral
P4	Rosaceae	*Rubus* sp. (100%)		monofloral
P5	Boraginaceae	*Echium* sp. (100%)		monofloral
P6	Rosaceae	*Rubus* sp. (100%)		monofloral
P7	Fagaceae	*Castanea* sp. (100%)		monofloral



Spectrophotometric analysis



Phenolic compounds profiling and quantification using HPLC-DAD-ESI/MS

76 compounds were identified and the most abundant compounds were:

- **P1 (*Carduus*)** : Methyl herbacetin glycosides isomers
- **P2 *(Ligustrum)*** : Quercetin-*O*-diglucoside
- **P3 (Cistaceae)** : Myricetin and laricitrin glycosides
- **P4 and P6 (*Rubus*)** : methyl herbacetin glycosides and N^1, N^5, N^{10}-tri-*p*-coumaroylspermidine
- **P5 (*Echium*)** : Feruloyl dicoumaroyl spermidine isomers
- **P7 *(Castanea)*** : Phenylamide glycosides isomers

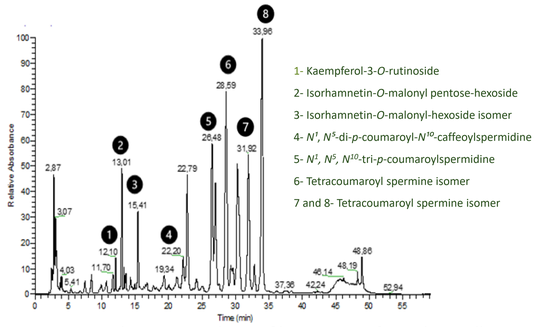
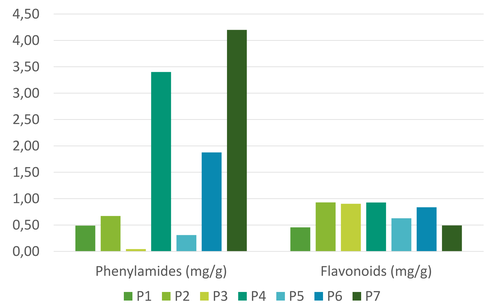


Figure 2: Chromatographic profile at 280 nm for P1 bee pollen



Conclusion

- The floral origin of the 7 bee pollen samples was determined with palynological analysis, being the samples classified as monofloral bee pollen (predominant pollen type ≥ 80%).
- The highest total phenolic and flavonoid content were shown by P7 and P6.
- P2, P3 and P4 displayed comparable amounts of flavonoids, around 7 mg/g.
- *Castanea sp.* bee pollen revealed the richest composition of phenolic compounds, mainly, phenylamides with a quantity of 30.1 ± 1.7 mg/g.
- A single flavonone, naringenin, was only detected in P2, *Ligustrum* bee pollen.
- Some phenolic compounds were only detected in specific botanical origin, such as methyl herbacetin-*O*-malonyl-hexosyl-deoxyhexoside in *Rubus* samples which make of it a potential botanical marker.

Recommendations

- The phenolic extraction needs to be optimized in order to achieve a better phenolic compounds recovery.
- Unidentified compounds require nuclear magnetic resonance analysis to fully recognize their structure.
- Principal Component Analysis (PCA) can provide a better floral origins discrimination result.

Acknowledgements

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PHYSICO-CHEMICAL EVALUATION OF ALGERIAN HONEYS: EUCALYPTUS, JUJUBE, SPURGE AND MULTIFLORAL

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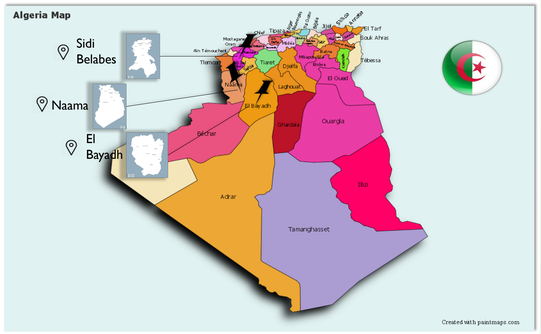
Abstract

The aim of the present study was to evaluate the quality of semi-arid Algerian honeys and verify its compliance with the established honey standards. For that, ten samples with different botanical and geographical origin, Eucalyptus (EC), Jujube (J), Euphorbia (EF) and multifloral (MF), were analyzed regarding the following physicochemical parameters: moisture, color, pH, free acidity, electrical conductivity, hydroxymethylfurfural (HMF), diastase index and proline. Concerning the moisture content, the samples presented values below the 20 % allowed by European Community regulations, ranging from 13.6% (EF) to 18.3% (EC). Eucalyptus honeys showed a darker color when comparing to the other samples. All honey samples presented conductivity values lower than 0.8 which are in accordance with the standard results for nectar honeys. The honeys pH values varied between 4.2 (MF) and 5.1 (J) with an average value equal to 4.6. For free acidity, tested at pH 8.3, the values where between 12.2 meq.kg^{-1} (EC) and 43.9 meq.kg^{-1} (EF). The HMF levels observed for the samples had a minimum of 0.53 (J) and a maximum of 36.5 (EC) mg.kg^{-1}, while diastase values ranged between 8.8 DN and 14.3 DN, being in accordance with the required by the European legislation (<40 mg.kg^{-1} and not less than 8 DN). For proline, the values ranged between 2.2 and 4.7 mg/g indicating the maturity of the honeys and absence of adulteration. Generally, the samples were found to meet the requirements of the international honey standards and were within those found in previous studies about physicochemical properties of Algerian and Moroccan honeys.

Introduction

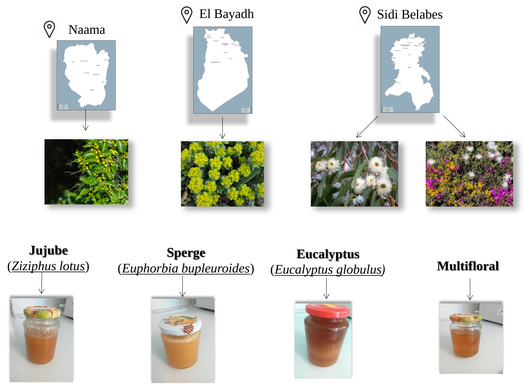
Algeria has a rich and varied melliferous vegetation, which is distributed in different bioclimatic and geographic zones. A large part of Algeria consists of arid and semi-arid regions, with only 4% of the land corresponding to the Mediterranean area. The interest of Algerian consumer in honeys from semi-arid regions is in increasing. Despite this commercial interest, semi arid honey has been scarcely described likewise Algerian beekeepers who have constantly attempted to rescue and guarantee the common characteristics of honey hope to discover different markets from local ones. For that, an extensive study of the Algerian honey is needed.

The aim of the present study was to evaluate the quality of Algerian honey and verify its compliance with the quality standards previously established (International Honey Commission, 2009).



Materials

10 Honey samples, obtained from local beekeeper and harvested in 2019.



Methodology

The evaluation of the physicochemical parameters of our samples were done according to the International Honey Commission (IHC) 2009.

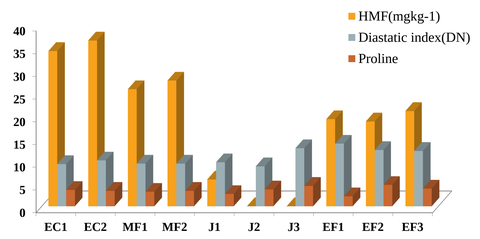
Parameters	Instruments	Methods
Color	Colorimeter	The color is determined using the pfund scale.
Moisture	Refractometer	The moisture conten was determined by means of a refractometer
Electrical conductivity	Conductivimeter	Conductivity was expressed in mS.cm^{-1}.
pH, free, lactonic and total acidity	Automatic Tritrator	Four diferente parameters were performed, namely the pH value of the initial honey solution, and free, lactonic and total acidity obtained by titration.
HMF	Spectrophotometer	The analysis of the contente of 5-hidroxymethyllfurfural was performed by spectrophotometry . Absorbance was read at 284 and 336 nm.
Proline	Spectrophotometer	The proline analysis was performed by spectrometry. The absorbance was read at 510nm
Diastase index	Spectrophotometer	The diastase activity was performed by the Phadebas method. Absorvance was read at 620 nm.

Results

Table 1. Electrical conductivity, moisture and color.

Samples	Color (mm Pfund)	Moisture (%)	Conductivity (mS.cm^{-1})
EC1	89 ± 0 (Amber)	18 ± 0	0.41 ± 0.02
EC2	88 ± 0 (Amber)	18 ± 0	0.41 ± 0.02
MF1	79 ± 0 (Light Amber)	15 ± 0	0.27 ± 0.01
MF2	77 ± 0 (Light Amber)	15 ±0	0.30 ± 0.06
J1	55 ± 0 (Extra Light Amber)	15 ± 0	0.37 ± 0.01
J2	55 ± 0 (Extra Light Amber)	15 ± 0	0.37 ± 0.01
J3	55 ± 0 (Extra Light Amber)	15 ± 0	0.37 ± 0.01
EF1	51 ± 0 (Extra Light Amber)	14 ± 0	0.36 ± 0.01
EF2	52 ± 0 (Extra Light Amber)	14 ± 0	0.36 ± 0.01
EF3	51 ± 0 (Extra Light Amber)	14 ± 0	0.36 ± 0.00

Figure 2. 5-HMF ,diastase index and proline content



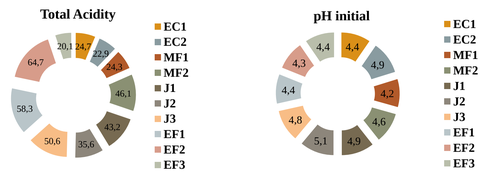
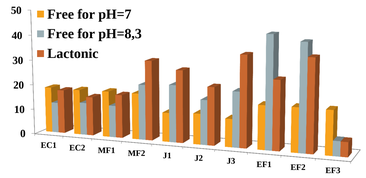


Figure 3. Free acidity, lactonic



Conclusions

The physico-chemical parameters studied showed:

1. All the honeys analyzed have a nectar-bearing origin.
2. The water contents show that all the honeys analyzed comply with the standard proposed by the codex Less than 20%, disabling fermentation.
3. The pH and acidity results show that all the honeys have high acidity but within the standard limit.
4. HMF results indicated that all honeys examined met the standard required the Codex.
5. Diastase index being in accordance with the required by the European legislation (not less than 8 DN).

Recommendations

Some recommendations for future study are set below:

- The application of statistical analysis to acess differentiation between monofloral honeys using the chemical characteristics;
- Increase the number of honey samples for confirmation of the pattern which was recorded in the Algerian honeys;
- New monofloral honeys from the Algerian flora can be involved in future research.

Acknowledgements

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Natural colourants from purple and red potatoes: application in a soft drink formulation and sensory analysis

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Abstract

Aqueous extracts from seven coloured potato varieties (three red-fleshed, three-purple fleshed, and one marble-fleshed genotype) were studied for

1. their anthocyanin content;
2. in vitro biological activities;
3. colouring properties and
4. their potential application in the food industry.

Acylated glycosides or pelargonidin and petunidin aglycones were identified as the main anthocyanin forms in the red and purple varieties, respectively. The total anthocyanin content among varieties ranged from 478.3 to 886.2 mg/100 g extract. All the extracts presented in vitro antioxidant, antibacterial and antifungal activities, whereas no toxic effects were detected. Finally, two selected extracts were tested as colourants in a soft drink formulation and presented suitable sensory profiles as well as high colour stability during a 30-day shelf-life when compared with the commercial colourant E163. Therefore, the tested extracts could be used as natural food colourants.

Introduction

Coloured root vegetable products can be alternative sources of colouring and bioactive compounds. Among root vegetables, potato also presents the highest genetic diversity among all cultivated species, with approximately 5000 registered varieties and a broad phenological variation in terms of flesh and skin colour.

Red and purple-fleshed potatoes are rich in phenolic compounds, particularly in anthocyanins (**Figure 1**), presenting about three times higher amounts of total phenolic compounds content than the widely consumed white- and yellow-fleshed tubers, as well as two to three times higher antioxidant activity. Anthocyanins are suitable to be used as natural colouring compounds due to their bright attractive red and purple colours and water solubility that allows for their easy incorporation into aqueous food systems.

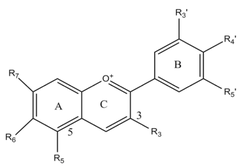


Figure 1. The core structure of anthocyanins with two aromatic benzyl rings (A and B rings) and a portion cyclized with oxygen (C ring).

Plant material

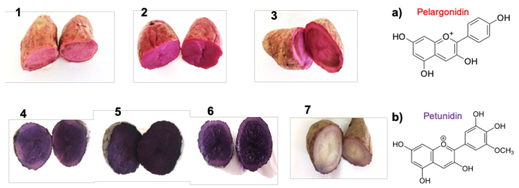


Figure 2. Fresh potato tubers of the genotypes: 1 – Rosemary; 2 - Red Emmalie; 3 - Red Cardinal; 4 – Purple; 5 – Violetta; 6 - Kefermarkter Blaue; 7 – Shetland Black, and the main class of anthocyanidins found in a) red-fleshed varieties and b) purple-fleshed varieties.

Methodology

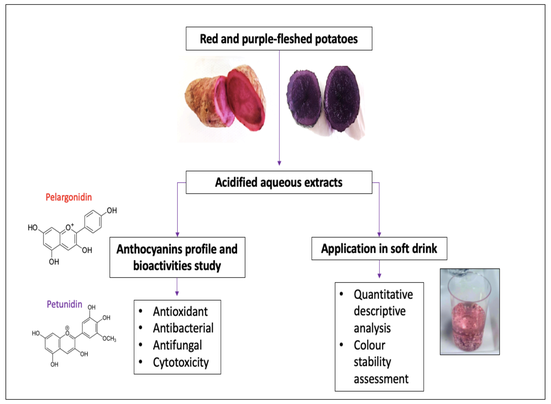
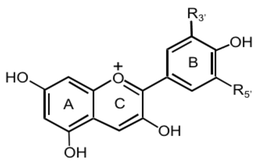


Figure 3. Working plan for the study of the coloured potato genotypes.

Results



	R3'	R5'
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 4. The main anthocyanidins detected in coloured potatoes.

Results

Table 1. In-vitro antioxidant and cytotoxicity activity (μg/mL) of aqueous extracts from red, purple and marble (yellow/purple) potato varieties (mean ± SD).

Potato varieties	Antioxidant activity (TBARS) IC_{50} values	Cytotoxicity (PLP2) GI_{50} values
Red flesh		
Rosemary	$416.9 \pm 4.9^{e,f}$	>400
Red Emmalie	669.4 ± 4.2^{a}	>400
Red Cardinal	591.79 ± 8.1^{b}	>400
Purple fleshed		
Purple	426.2 ± 2.6^{e}	>400
Violetta	380.1 ± 6.2^{f}	>400
Kefermarkter Blaue	484.5 ± 2.1^{d}	>400
Marble-fleshed (purple/yellow)		
Shetland Black	547.6 ± 6.4^{c}	>400

IC_{50}: extract concentration corresponding to 50% of antioxidant activity (against lipid peroxidation). GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2; maximum tested concentration: 400 μg/mL. Positive control: Trolox IC_{50} value - 139±5 μg/mL (TBARS); Ellipticine GI_{50} value - 3.2±0.7 μg/mL (PLP2). In each line different Latin letters are significantly different according to Tukey's HSD test (p=0.05).

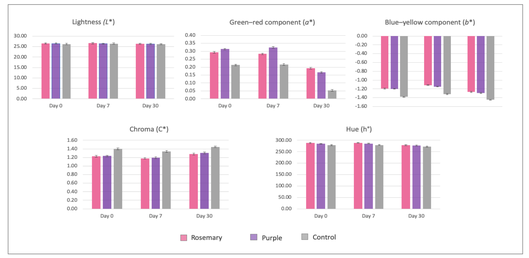


Figure 5. Fresh potato tubers of the genotypes: 1 – Rosemary; 2 - Red Emmalie; 3 - Red Cardinal; 4 – Purple; 5 – Violetta; 6 - Kefermarkter Blaue; 7 – Shetland Black, and the main class of anthocyanidins found in a) red-fleshed varieties and b) purple-fleshed varieties.

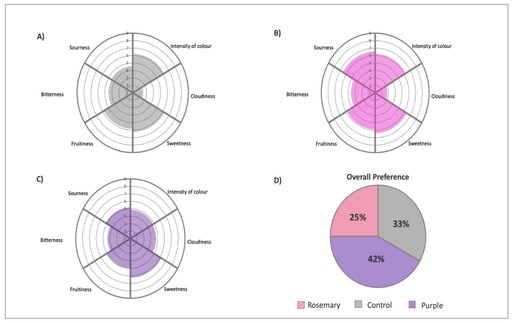


Figure 6.. CIELab colour values (L*, a* and b*) and cylindrical coordinates (C* and h°) of the soft drink formulations Rosemary (red potato extract), Purple (purple potato extract) and Control (E163 commercial colouring), over a 30 days shelf-life period. L*: lightness from black (0) to white (100); a*: green (−) to red (+), b*: blue (−) to yellow (+); C*: chroma, relative saturation; and h°: angle of the hue in the CIELab colour space.

Conclusion

The seven aqueous potato extracts tested presented

- high anthocyanin content and
- high antioxidant, antibacterial and antifungal properties.

Acylated pelargonidin glycosides were the main compounds found in the red varieties and acylated petunidin glycosides in the purple ones. Additionally, no cytotoxic effect was detected in the extracts up to the maximum tested concentration (400 μg/mL), indicating their safety to be incorporated in food formulations.

The two extracts selected to be applied in a soft drink formulation showed suitable profiles in the sensory and shelf-life assessments when compared with the control commercial colourant E163.

The aqueous extracts herein obtained by a simple one-step and cost-effective extraction method could be used as alternative natural food colourants, substituting synthetic colouring agents.

Future perspectives

Future studies in pre- and post-harvest level could support the selection of colour-fleshed potatoes with high anthocyanin content. Furthermore, the assessment of different agronomic practices may increase the concentration of anthocyanins and the added value of this important vegetable crop.

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Chemical Profile and Bioactive Properties of Green- and Red-Colored Basil Cultivars as Affected by Nitrogen Fertilization

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INTRODUCTION

- *Ocimum basilicum* L. belonging to the Lamiaceae family is considered to be an important herb with great commercial interest due to its wide range of industrial uses
- Its phytochemical profile presents a great variation and could be affected by the growing conditions and the genotype characterized by different plant morphology, color of leaves and chemotypes
- Defining the optimal N application rate even for aromatic herbs like basil must be considered not only for the agronomic performance but also for the effects on chemical profile and bioactive properties of the crop
- The objectives of this study are the evaluation of the chemical composition and bioactive properties of green and red-coloured cultivars in relation to nitrogen fertilizer application rate. Three red coloured (Dark Opal, Basilico Rosso and Red Basil) and one green-coloured landrace (Mitikas) of basil were grown under four nitrogen regimes, namely Control (no fertilizer added), 200 ppm, 400 ppm and 600 ppm of nitrogen (N)

MATERIALS

- Seeds from three colored basil cultivars, namely Red Basil, Dark Opal and Basilico Rosso and one green local landrace (Mitikas) were sown in seed trays containing peat on 04/04/2019. Young seedlings were transferred at the stage of 3-4 true leaves in 2 L plastic pots containing peat and perlite (1:1, v/v) on 23/04/2019
- Four nitrogen fertilization rates were applied, namely Control (0 ppm N), 200 ppm, 400 ppm and 600 ppm of nitrogen. Plants were fertigated with similar amount of 50-300 mL per pot. For each treatment, 15 pots were used with one plant per pot (60 pots in total).
- Harvest took place on 14/06/2019, the aerial parts of the plants were weighed in order to estimate total fresh weight per plant and samples of fresh leaves from each treatment were pooled in batch samples and lyophilized, ground to powder and put at -80 °C for chemical analyses

METHODS

- Organic acids were determined by ultra-fast liquid chromatography coupled to a diode-array detector and free sugars were determined with the implementation of a high-performance liquid chromatography (HPLC) system
- Tocopherols were analyzed using the HPLC system coupled to a fluorescence detector, whereas the profile of fatty acids was evaluated by gas-liquid chromatography coupled to a flame ionization detector
- Related to the phenolic compound determination the hydroethanolic extracts were redissolved in ethanol/water (80:20, v/v) to achieve the final concentration of 10 mg/mL and were analyzed in a HPLC system coupled with a diode-array detector (DAD) and a Linear Ion Trap (LTQ XL) mass spectrometer (MS)
- Antioxidant properties properties were evaluated following two ex-vivo procedures: a) the thiobarbituric acid reactive substances (TBARS) assay and b) the oxidative haemolysis (OxHLIA) assay evaluated for Δt of 60 and 120 min

Image 1. Tested basil genotypes under different N rates fertilization

RESULTS

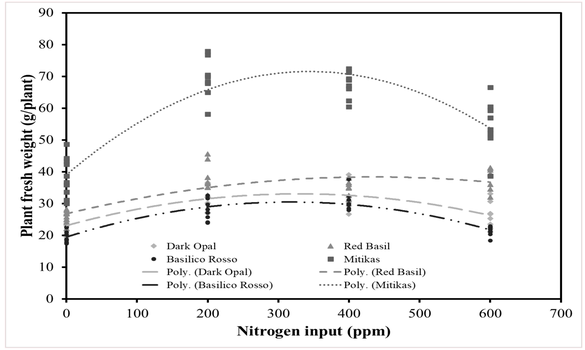

Fig. 1. Above-ground plant fresh weight in response to nitrogen input in Dark Opal, Red Basil, Basilico Rosso, and Mitikas

- Basil fresh yield was significantly affected by both genotype and N application rate to the four tested genotypes, whereas green basil (Mitikas) presented the highest yield regardless the level of N input
- The three red basil genotypes presented a significantly higher content to the total organic acids compared to the green one, while the increasing input of N increased the concentration on each organic acid and the total organic acids in all four genotypes
- Regarding the profile of free sugars detected (glucose, fructose and sucrose) the unfertilized plants had the lower content of glucose and the higher proportion of fructose in all the genotypes except for Basilico Rosso, while the total free sugars content was increased by the increasing N input rate
- The plants fertigated with 200 ppm of N achieved the highest tocopherol content in the leaves especially in the case of Dark Opal and Red Basil and the increased rate of N decreased the tocopherol concentration respectively
- The n-6/n-3 polyunsaturated fatty acids (PUFAs) ratio was affected by the N application rate and the lowest value was recorded in the leaves of plants fertigated with 200 ppm of N with the exception of Dark Opal variety, while the Red Basil genotype presented the lowest n-6/n-3 (PUFAs) ratio profile
- Red Basil and Dark Opal basil presented the highest content of total phenolic acids and total flavonoids and green basil had the lowest content of total phenolic compounds
- Dark Opal and Red basil fertigated at the N application rate of 200 ppm recorded the highest antioxidant activity, while the green basil genotype had the lowest content respectively

Table 1. Organic acids (g/100 g dw) of the studied basil genotypes in relation to nitrogen input (mean ± SD, *n* = 3

Organic acids						
Cultivar	**ppm**	Oxalic acid	Quinic acid	Shikimic acid	Ascorbic acid	Total organic acids
Dark Opal		**5.7±0.8 A***	**9±3 B**	**0.11±0.01 A**		**15±4 A,B**
	0	4.67±0.01 i	4.45±0.01 n	0.120±0.001 b	tr	9.24±0.01 m
	200	5.27±0.05 h	9.80±0.08 h	0.100±0.003 e,f	tr	15.18±0.02 i
	400	6.01±0.09 c,d	10.57±0.06 f	0.100±0.001 e	tr	16.68±0.03 g
	600	6.78±0.07 a	12.41±0.02 b	0.100±0.001 e,f	tr	19.29±0.05 b
Red Basil		**5±1 B**	**10±3 A,B**	**0.09±0.04 B**		**15±4 A,B**
	0	3.17±0.01 i	4.50±0.03 m	0.020±0.001 i	tr	7.69±0.03 n
	200	5.33±0.02 g	10.37±0.02 g	0.100±0.007 f	tr	15.80±0.05 h
	400	5.76±0.06 f	11.07±0.02 e	0.110±0.001 d	tr	16.94±0.04 f
	600	6.04±0.01 c	12.41±0.04 b	0.130±0.001 a	tr	18.59±0.05 c
Basilico Rosso		**5±2 B**	**11±4 A**	**0.09±0.04 B**		**16±5 A**
	0	2.41±0.01 l	4.95±0.06 l	0.030±0.001 i	tr	7.39±0.06 o
	200	5.97±0.01 d,e	11.40±0.02 d	0.110±0.002 c	tr	17.48±0.02 e
	400	6.31±0.09 b	11.50±0.05 c	0.110±0.001 c,d	tr	17.92±0.04 d
	600	6.78±0.01 a	14.90±0.06 a	0.120±0.001 b	tr	21.80±0.07 a
Mitikas		**5±1 B**	**6±1 C**	**0.04±0.02 C**		**12±3 C**
	0	2.90±0.02 k	4.14±0.04 o	0.020±0.001 i	tr	7.06±0.01 p
	200	5.25±0.01 h	6.81±0.02 k	0.030±0.001 i	tr	12.09±0.02 l
	400	5.77±0.03 f	7.14±0.02 j	0.060±0.003 h	tr	12.97±0.02 k
	600	5.95±0.07 e	7.89±0.01 i	0.070±0.003 g	tr	13.92±0.09 j

tr – traces; *Different capital letters within each column represent significant differences between the means of the four types of cultivars. Different small letters within each column represent significant differences between the means of each level of nitrogen input and cultivar. Using Tukey's HSD test at $p = 0.05$.

Table 2. Composition in tocopherols (mg/100 g dw) of the studied basil genotypes in relation to nitrogen input (mean ± SD, *n* = 3).

Tocopherols					
Cultivar	**ppm**	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total Tocopherols
Dark Opal		**4±1 A***	**0.8±0.3 A**	**0.8±0.3 A**	**5±2 A**
	0	3.60±0.03 d	0.44±0.03 f	0.325±0.008 h	4.37±0.05 d
	200	6.07±0.03 b	1.32±0.01 a	1.18±0.03 a	8.58±0.01 b
	400	2.71±0.01 g	0.94±0.02 c	0.97±0.02 c	4.63±0.01 c
	600	2.41±0.02 h	0.60±0.03 e	0.76±0.03 e	3.77±0.03 f
Red Basil		**4±2 A,B**	**0.6±0.3 B**	**0.5±0.4 B**	**5±3 A**
	0	3.14±0.02 e	0.447±0.001 f	0.31±0.02 h	3.90±0.01 e
	200	7.09±0.02 a	1.13±0.01 b	1.12±0.01 b	9.34±0.02 a
	400	3.80±0.03 c	0.41±0.01 g	0.40±0.02 f	4.61±0.01 c
	600	1.71±0.01 i	0.340±0.002 h	0.259±0.003 i	2.31±0.01 j
Basilico Rosso		**0.8±0.3 D**	**0.5±0.1 B,C**	**0.5±0.3 B**	**1.8±0.6 C**
	0	0.55±0.02 o	0.44±0.01 f	0.313±0.004 h	1.30±0.01 n
	200	1.257±0.001 l	0.49±0.02 e	0.91±0.04 d	2.66±0.01 i
	400	0.87±0.02 m	0.67±0.03 d	0.370±0.002 g	1.92±0.02 l
	600	0.66±0.02 n	0.35±0.04 h	0.32±0.02 h	1.33±0.08 n
Mitikas		**1.9±0.7 C**	**0.36±0.07 D**	**0.27±0.06 C**	**2.6±0.8 B**
	0	2.36±0.04 i	0.445±0.003 f	0.315±0.002 h	3.13±0.05 h
	200	2.76±0.05 f	0.396±0.004 g	0.325±0.001 h	3.48±0.04 g
	400	1.55±0.01 k	0.321±0.005 i	0.255±0.004 i	2.12±0.01 k
	600	1.13±0.01 m	0.278±0.004 j	0.171±0.002 j	1.58±0.01 m

*Different capital letters within each column represent significant differences between the means of the four types of cultivars. Different small letters within each column represent significant differences between the means of each level of nitrogen input and cultivar. Using Tukey's HSD test at $p = 0.05$.

Table 3. Composition in free sugars (g/100 g dw) and tocopherols (mg/100 g dw) of the studied basil genotypes in relation to nitrogen input (mean ± SD, *n* = 3).

Free sugars					
Cultivar	**ppm**	Fructose	Glucose	Sucrose	Total free sugars
Dark Opal		**1.7±0.5 A***	**3±1 C**	**0.9±0.2 B**	**5±2 C**
	0	1.65±0.04 f	0.87±0.01 l	0.62±0.01 i	3.14±0.06 j
	200	0.92±0.01 j	2.51±0.01 j	0.95±0.01 g	4.38±0.01 i
	400	2.05±0.09 b	3.25±0.06 h	1.04±0.05 f	6.34±0.07 f
	600	2.32±0.03 a	3.45±0.02 g	1.07±0.01 e	6.84±0.06 c
Red Basil		**1.7±0.4 A**	**3±1 C**	**0.6±0.2 C**	**5±1 B,C**
	0	1.40±0.02 h	0.64±0.02 m	0.53±0.05 j	2.57±0.09 k
	200	1.55±0.01 g	2.69±0.04 i	0.878±0.004 h	5.11±0.04 h
	400	1.40±0.05 h	3.73±0.01 f	0.442±0.006 k	5.57±0.05 g
	600	2.29±0.02 a	3.76±0.02 e	0.51±0.03 j	6.57±0.07 e
Basilico Rosso		**0.9±0.4 B**	**3.5±0.6 A,B**	**2.2±0.1 A**	**7±1 A**
	0	0.55±0.04 k	2.48±0.04 j	2.03±0.04 d	5.06±0.05 h
	200	0.52±0.02 k	3.71±0.03 f	2.09±0.03 c	6.31±0.02 f
	400	1.08±0.01 i	3.96±0.02 d	2.17±0.02 b	7.21±0.05 b
	600	1.40±0.05 h	3.98±0.01 d	2.41±0.01 a	7.79±0.04 a
Mitikas		**1.8±0.2 A**	**4±2 A**	**0.11±0.03 D**	**6±2 A,B**
	0	1.52±0.04 g	0.95±0.06 k	0.096±0.004 m	2.57±0.09 k
	200	1.74±0.05 e	4.48±0.05 c	0.092±0.003 m	6.32±0.01 f
	400	1.85±0.06 d	4.82±0.03 b	0.109±0.001 m	6.78±0.09 d
	600	2.01±0.06 c	4.99±0.01 a	0.160±0.004 l	7.16±0.05 b

*Different capital letters within each column represent significant differences between the means of the four types of cultivars. Different small letters within each column represent significant differences between the means of each level of nitrogen input and cultivar. Using Tukey's HSD test at $p = 0.05$.

Table 4. Antioxidant activity of the leaves' hydroethanolic extracts of the studied basil genotypes in relation to nitrogen input (mean ± SD, *n* = 3).

Cultivar	ppm	TBARS (EC_{50}, μg/mL)	OxHLIA (IC_{50} values, μg/mL)	
			Δt = 60 min	Δt = 120 min
Dark Opal		**32±16 D***	**106±56 B**	**198±89 B**
	0	34±3 d	79±3 g	145±4 f
	200	13.1±0.3 g	30.8±0.9 l	82±1 i
	400	25.7±0.5 f	144±2 d	270±3 d
	600	55.4±0.6 b	171±4 c	293±4 c
Red Basil		**50±15 B**	**68±26 D**	**170±84 C**
	0	55.7±0.2 b	60±2 h	109±2 h
	200	25.2±0.2 f	40.0±0.9 j,k	79±3 i
	400	60±1 a	64±3 h	202±4 e
	600	61±2 a	109±6 f	289±6 c
Basilico Rosso		**43±12 C**	**77±44 C**	**151±76 D**
	0	32±1 d	38±1 k,l	65±2 i
	200	31.0±0.2 e	46±3 k,j	136±3 f,g
	400	54.9±0.7 b	147±4 d	271±7 d
	600	55.1±0.8 b	79±3 g	130±5 g
Mitikas		**57±4 A**	**183±106 A**	**269±254 A**
	0	54.3±0.8 b	51±2 i	105±2 h
	200	52.0±0.9 c	119±7 e	314±15 b
	400	59.7±0.5 a	250±11 b	na
	600	61.2±0.2 a	313±13 a	655±19 a

CONCLUSIONS

- The tested agronomic parameter fresh yield was clearly affected both by genotype and different N rates to the basil genotypes
- The concentration of each organic acid and total organic acids increased with the increasing level of N input in all four genotypes, whereas the highest concentration of total free sugars was consistently observed to the basil genotypes fertigated with the highest level of N input
- Equally, the results of this study highlight the effect of genotype and N application rates to the to tocopherol and fatty acids content, while the lowest n-6/n-3 PUFAs ratio recorded by the Red Basil could be exploited further as a significant indicator for a variety with high nutritional value
- Basil genotype and the different N inputs had a significant interactive effect on the profile of phenolic compounds and antioxidant properties in which both Dark Opal and Red Basil presented the highest content for the two parameters tested
- Even though Red Basil genotype was the less productive genotype regarding to its yield parameter, it presented the finest chemical profile

RECOMMENDATIONS

- Finding out the optimal rates of N according to the needs of each genotype could provide a promising outcome to the agronomic parameters and the phytochemical profile of the crop
- The nowadays requirements for a sustainable agricultural alongside with the environmental and economic concerns lead us to the necessity for the production of food with high nutritional value and bioactive quality
- Further research is demanded in terms of genotypes, environmental conditions and cultivations practices in order to find out the optimum balance between yield, chemical composition and bioactive properties

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GENOPROTECTIVE EFFECT OF BIOLOGICALLY ACTIVE PLANT COMPOUNDS GENTIOPICROSIDE AND MANGIFERIN AGAINST FOODBORNE MUTAGENS IQ AND PHIP

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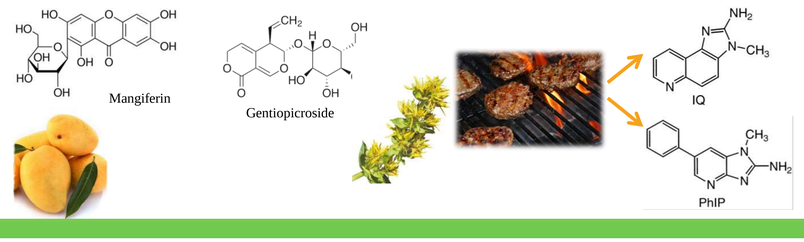
Abstract

During high temperature cooking of protein rich food, particularly red meat and fish, heterocyclic aromatic amines (HAAs) are formed. They represent a class of potent mutagens that can induce serious consequences on human health. Genotoxicity of many HAAs, including IQ and PhIP, can be ascribed to different mechanisms, one of them being the production of reactive oxygen species. To overcome the problem of their genotoxicity, investigation of biologically active genoprotective agents is of a great importance. Taking into account all the above, the main aim of this study was to investigate the antigenotoxic effect of gentiopicroside and mangiferin, known for their numerous biological activities. Antigenotoxicity was screened towards foodborne mutagens IQ and PhIP on human hepatocarcinoma HepG2 cells, by applying alkaline comet assay. In order to analyze the involvement of antioxidative mechanism into possible antigenotoxicity, screening of DPPH radical scavenging activity, expression of Nrf2 transcription factor and glutathione redox status of the cells was included. Nrf2 was selected in accordance with its role in up-regulation of antioxidative enzymes.
Inhibition of IQ/PhIP-induced genotoxicity was recorded for both gentiopicroside and mangiferin. They induced dose-depended response, with the highest effects of gentiopicroside against IQ (up to 68% of DNA damage inhibition, $p<0.001$) and mangiferin against PhIP (up to 67% inhibition, $p<0.001$). Ability of test substances to scavenge DPPH radical revealed moderate antioxidative activity of gentiopicroside and remarkable of mangiferin, with IC_{50} values at 119 μg mL^{-1} and 0.9 μg mL^{-1}, respectively. Further on, mangiferin, and especially gentiopicroside, up-regulated the expression of Nrf2 transcription factor. The protection of glutathione depletion in the cells was demonstrated for both test substances.
In conclusion, the results obtained showed remarkable capacity to reduce HAAs-induced DNA damage. They recommend further and more detailed investigation of gentiopicroside and mangiferin genoprotective effect in *in vitro* and *in vivo* model systems.

Introduction

Due to the fact that food mutagens are considered a serious problem for human health, searching for potent protective agents is a necessity. One of the important classes of food mutagens are heterocyclic aromatic amines (HAAs). They are usually formed during thermal processing of protein rich foods, especially meat and fish, but their formation depends on meat type, temperature and cooking method. Genotoxicity of many HAAs and their carcinogenic effect could be attributed to multiple mechanisms including induction of oxidative damage. To overcome the problem of their genotoxicity different natural products are being explored as a source of potent phyto-antimutagens that can reduce DNA damage.
Gentiopicroside is one of the major active components of the *Gentiana* species of medicinal plants, know for numerous biological activities. Considering mangiferin, it is a predominant componenet of *Mangifera indica* L., and it is known for different pharmacologicall activities, primarily for it's antioxidative potential.

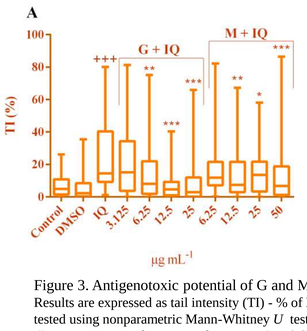


Aim of the study

The aim of this study was to investigate antigenotoxic activity of gentiopicroside (G) and mangiferin (M) against food mutagens **IQ** and **PhIP** on human hepatocarcinoma cells HepG2. In order to analyze possible underlying mechanism, antioxidant activity was also studied by investigating radical scavenging activity, expression of Nrf2 transcription factor responsible for the up-regulation of antioxidant enzymes and glutathione redox status of the cells.

RESULTS

Preliminary research of cytotoxicity revealed that G did not have effect on survival of the cells, while M, IQ and PhIP maximally reduced viability of the cells by 30% (Fig. 1). Considering the fact that threshold of cytotoxicity being acceptable for genotoxicity monitoring was 70% viability, there were no limitations for genotoxicity analysis. Genotoxicity testing showed that G induced DNA damage at the highest tested concentration, and established the genotoxic doses of IQ and PhIP that would be used in antigenotoxicity testing (**200 μg mL^{-1}** and **100 μg mL^{-1}**, respectively, Fig. 2).
Data from antigenotoxicity testing showed that both G and M **significantly reduced IQ- and PhIP-induced genotoxicity** (Fig. 3). DPPH assay revealed moderate and remarkable activity of G and M, respectively (Table 1.). Further on, both G and M up-regulated the expression of *Nrf2* gene (Fig. 4), and significantly increased the content of reduced glutathione after the co-treatment with mutagens (Fig. 5).



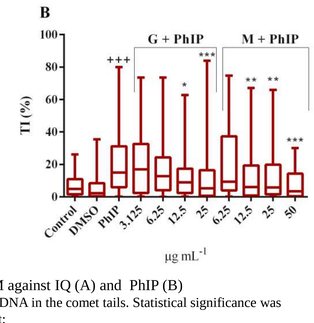


Figure 3. Antigenotoxic potential of G and M against IQ (A) and PhIP (B)
Results are expressed as tail intensity (TI) - % of DNA in the comet tails. Statistical significance was tested using nonparametric Mann-Whitney *U* test:
-Between co-treated groups and mutagen (*p<0.05; **p<0.01; ***p<0.001);
-In regard to solvent control DMSO (+++p<0.001).

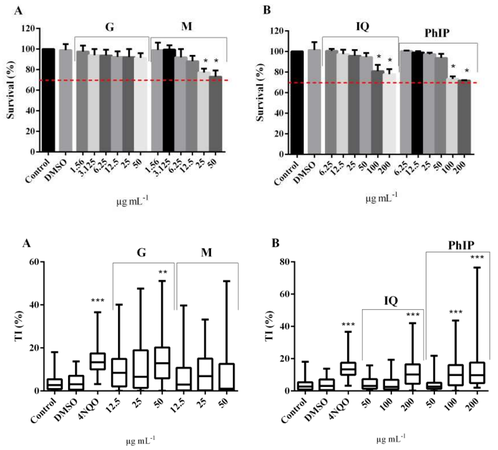


Figure 2. Genotoxicity of G and M (A) and mutagens (B)
Results are expressed as *tail intensity* (TI) -% of DNA in the comet tails. 4NQO was used as positive control. Statistical significance in regard to solvent control DMSO was tested using nonparametric Mann-Whitney *U* test (**p<0.01;***p<0.001).

Figure 1. Cytotoxic potential of G and M (A) and mutagens (B)
Results are expressed as mean values±standard deviation. Statistical significance in regard to solvent control was tested using one-way ANOVA with Dunnet's post hoc test (*p<0.05).

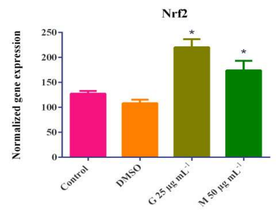


Figure 4. Effect of G and M on the expression of *Nrf2* gene
Results are expressed as mean values±standard deviation. Statistical significance in regard to solvent control DMSO was tested using one-way ANOVA with Dunnet's post hoc test (*p<0.05).

Table 1. Antioxidant activity of gentiopicroside and mangiferin recorded in DPPH assay

Test substances	DPPH IC_{50} inhibition (μg mL^{-1})
G	119±0.7
M	0.9±0.1
Ascorbic acid	12.5±0.3

Ascorbic acid was used as positive control.

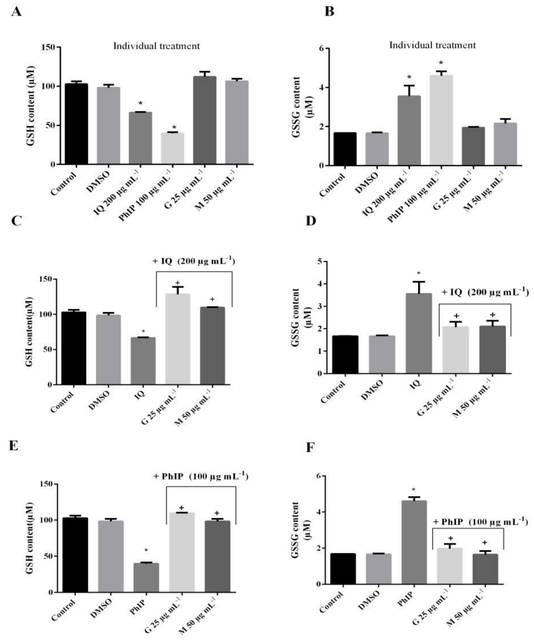


Figure 5. Effect of individual treatments (A, B) and co-treatments with IQ (C, D) and PhIP (E, F) on the content of reduced glutathione (GSH) and oxidized glutathione (GSSG)
Results are expressed as mean values±standard deviation.
Statistical significance was tested using one-way ANOVA with Dunnet's post hoc test:
-In regard to solvent control DMSO (*p<0.05);
-In regard to IQ/PhIP (*p<0.05).

Methodology

- In order to determine non-cytotoxic concentrations of G, M and food mutagens, MTT assay was performed on HepG2 cells.
- To determine non-genotoxic concentrations of G and M and genotoxic concentrations of IQ and PhIP, alkaline comet assay was applied.
- To test antigenotoxicity, alkaline comet assay was applied and cells were co-treated with non-genotoxic concentrations of G and M and selected genotoxic concentrations of IQ and PhIP for 24h.
- Radical-scavenging activity of G and M was tested using DPPH assay.
- Potential of G and M to modulate expression of *Nrf2* gene was analyzed using qRT-PCR on HepG2 cells, previously exposed to 24h treatment of G and M.
- Glutathione redox status in the cells was determined using commercial kit. Prior to performing the assay, cells were subjected to test substances, being applied individually or in co-treatments for 24h.

Conclusion

- **Gentiopicroside and mangiferin showed remarkable capacity to reduce IQ- and PhIP- induced DNA damage.**
- **Antigenotoxic activity might be explained by notable radical scavenging activity of G and M, and by their potential to up-regulate the expression of *Nrf2* gene.**
- **Both tested substances protected cells from glutathione depletion contributing to overall antioxidative defense system.**
- **Obtained results strongly encourage further research of gentiopicroside and mangiferin genoprotective potential.**

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia; grant number 451-03-68/2020-14/200178.

AMONG THE TEA VARIETIES (*Camellia sinensis*) PURPLE TEA IS THE MOST EFFECTIVE INHIBITOR OF THE PANCREATIC α-AMYLASE

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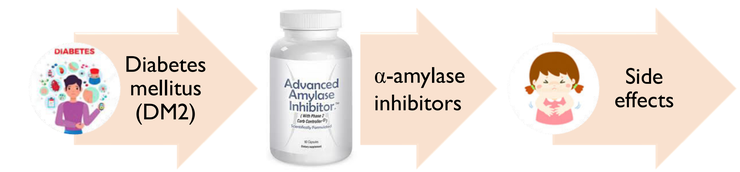
ABSTRACT

INTRODUCTION



- The objective of this study was to compare the in vitro inhibitory activity of purple tea on the pancreatic α-amylase with that of other tea varieties (green, oolong, white and black teas).

MATERIALS

- Porcine pancreatic α-amylase (type IV-B), and potato starch were purchased from Sigma-Aldrich. Acarbose was obtained from local pharmacies. All reagent grade chemicals were from the highest possible degree of purity.

METODOLOGY

- Anthocyanins were evaluated using the pH differential method.

- Pancreatic α-amylase inhibition was assayed by measuring the liberation of reducing sugars.

RESULTS

- Purple tea presented total monomeric anthocyanin levels that were much higher than those of the other teas (0.935±0.047 mg cyanidin-3-O-glucoside equivalents per g extract);
- For samples used in the presented. The sequence of decreasing potency work, different inhibitory capabilities were found was: purple tea > black tea > white tea > oolong tea > green tea;
- As shown by Fig. 1, purple tea did not present stimulation at low concentrations and was much more effective than the other teas as an inhibitor of the α-amylase (≈90% inhibition at the concentration of 4 mg/mL).

RESULTS

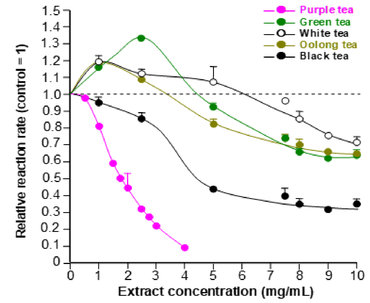


Figure 1. Dependence of the porcine pancreatic α-amylase activity on the concentration of various tea extracts.

CONCLUSION

- It can be concluded that regular ingestion of purple tea is potentially more likely to protect against hyperglycemia than other types of tea.

ACKOWLEDGEMENTS

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The antioxidant potential of wild garlic (*Allium ursinum* L.) plant in shelf-life extension of food lipids

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Introduction

- ✓ Yellow leaves of wild garlic (*Allium ursinum* L.) plant are the source of the sulphur-containing compounds as well as kaempferol derivatives, flavonoid glycosides, phenolic acids and other antioxidants [1,2].
- ✓ Antioxidant potential of *Allium ursinum* L. can be utilized in two ways:
  - as a source of bioactive compounds showing protecting properties against heart disease, cancer, oxidative damage to cells and DNA,
  - as a source of natural antioxidants, showing inhibiting effect against oxidation of food lipids.

The aim of the present study was the evaluation of antioxidant activity of *Allium ursinum* L. extracts in rapeseed oil.

Materials and methods

commercially available dried leaves of *Allium ursinum* L. (A, B, C, D)

↓

extraction (96% ethanol)

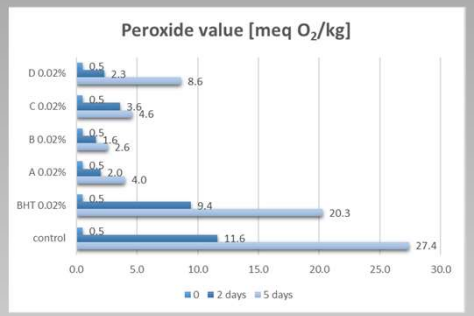
+0.02% + 0.1%

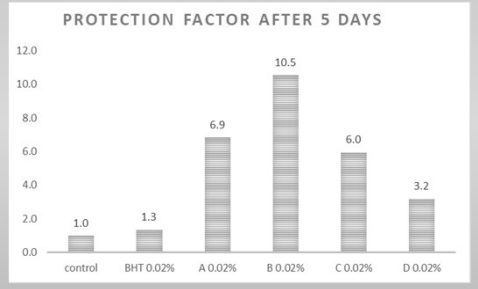
refined rapeseed oil

- ✓ accelerated storage conditions (50 ºC)
- ✓ measurement of hydroperoxides with the use of iodometric method as peroxide value (PV) [3]
- ✓ calculation of protection factor (relative to control)

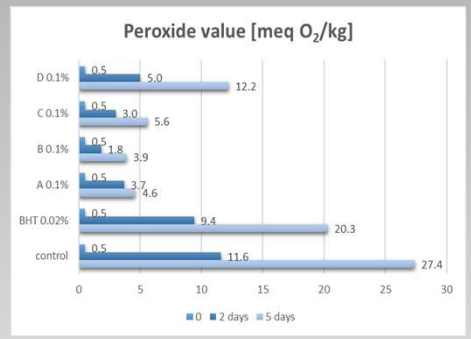
RESULTS

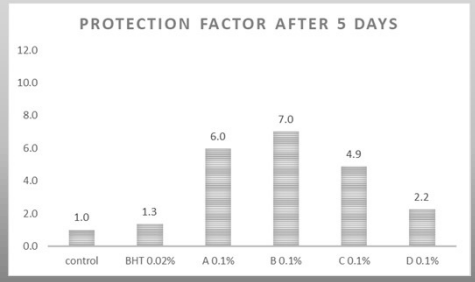
Stability of rapeseed oil in 50 °C with 0.02% extract addition





Stability of rapeseed oil in 50 °C with 0.1% extract addition





Conclusions

1. Concentration of 0.02% is more efficient in rapeseed oil than 0.1%.
2. Extracts of *Allium ursinum* L. can be a good alternative to synthetic antioxidants to extend shelf-life of lipid containing food.
3. Further investigation is necessary in order to confirm consumers' acceptation of sensory attributes of the wild garlic extract.

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Antioxidant properties of Subcritical Water Extracts Derived from Mushroom *Inonotus obliquus*

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Abstract

The medicinal mushroom *Inonotus obliquus* is widespread in Europe, Asia and North America. In many countries, since the 16th century, it has traditionally been used to treat gastrointestinal cancer, cardiovascular disease and diabetes. This study was designed to determine the chemical composition and antioxidant activity of subcritical water extracts obtained from fruiting bodies of *I. obliquus* originating from Mongolia (IM) and from the mountain Vlasina, Serbia (IS). Chemical analysis revealed the total content of proteins, carbohydrates and phenols. High carbohydrate content was found in both extracts, and glucose was the most dominant monosaccharide. In order to identify phenolic acids, the extracts were subjected to a qualitative chemical analysis, and the presence of chlorogenic acid, catechin, p-coumaric acid and cinnamic acid has been confirmed. Chlorogenic acid was detected in the highest concentration, compared to other phenolic acids. DPPH free radical scavenging activity assay was used to measure the antioxidant properties of extracts *in vitro*. Extracts concentrations from 0.156 to 10 mg/mL were tested and a maximum of 93% of scavenging ability was reached. The results indicated that antioxidant activity in both extracts can be achieved through hydrogen atom (HAT) and single electron transfer (SET) as dominant mechanisms .

Introduction

Chaga (*Inonotus obliquus*) is a member of Hymenochaetaceae family. The dark sclerotia are harvested for food and medicinal purposes. It is well known as a non-toxic mushroom with pronounced antimicrobial, antiviral, antioxidative and anti-inflammatory properties. Chemical investigations confirmed that *I. obliquus* produces a diverse range of secondary metabolites, including phenolic compounds, melanins, proteins and carbohydrates. Due to the presence of compounds that have an extremely high ability to capture free radicals, this precious mushroom appears as a significant tool in reducing oxidative stress, which is known to trigger many health disorders in the body. Regarding these facts, the aim of this investigation was to of this investigation was the chemical characterization of phenolic components and determination of antioxidant potential of extracts *in vitro* conditions by determining abilitycapture of DPPH radicals.

Materials

For the purposes of this research, two strains of the mushroom *Inonotus obliquus* (Chaga) originating from the mountain Vlasina (Serbia) and from Mongolia were used.

Subcritical wather extraction was performed in batch-type high-pressure extractor (Parr 4520, USA) and four extracts were obtained under different temperatures (120ºC and 200 ºC) and pressures (bar).

Methodology

Phenolic compounds

Qualitative analysis of phenolic compounds was performed by UHPLC system with a quaternary Accela 600 pump and Accela autosampler connected to LTQ OrbiTrap MS with heated electrospray ionization probe (HESI-II, Thermo Fisher Scientific, Bremen, Germany).

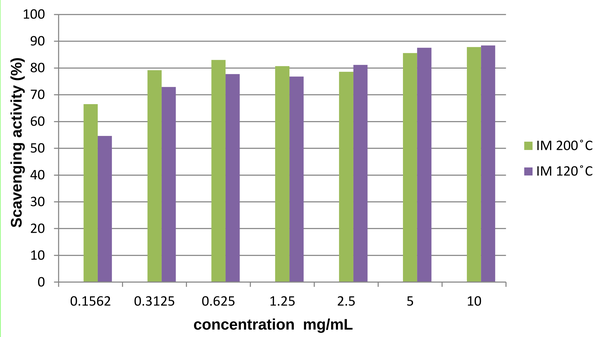
DPPH radical-scavenging activity assay

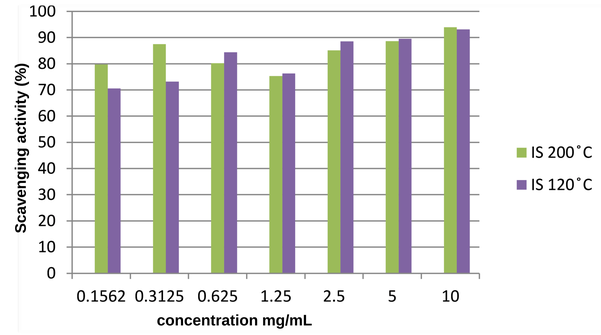
The ability of the extracts to scavenge free DPPH radicals was observed *in vitro*. All extracts were tested at concentrations of 0.156 -10 mg/mL, and the results were expressed as a percentage.

Results

Phenolic acids in subcritical water extracts *Inonotus obliquus.*
IM – Mongolian Chaga; IS- Vlasina Chaga

Sample mg/kg	IS 200 ˚C	IS 120 ˚C	IM 200 ˚C	IM 120 ˚C
Chlorogenic acid	840.347	697.418	741.268	970.559
Catechin	1.799	2.413	2.942	1.512
p-Coumaric acid	1.548	3.070	1.095	0.897
Cinnamic acid	4.325	8.936	5.685	6.425





Conclusion

• Chemical analysis of the subcritical water extract confirmed the presence of chlorogenic acid, catechins, p-coumaric acid and cinnamic acid in all extracts in different concentrations

• The highest concentration of chlorogenic acid was determined in the sample IM 120ºC (970.559 mg/kg)

• P-cumaric acid was found in small concentrations in all samples

Since numerous studies to date indicate the potentially harmful toxic effects of synthetic antioxidants, naturally derived antioxidant agents are becoming increasingly important.

The role of mushrooms in the isolation of such active components is gaining in importance, and this research confirms the presence of an abundance of antioxidants in *I. obliquus*.

Recommendations

The results of this study strongly support the existing scientific data on the use of *I. obliquus* as a powerful antioxidant

Acknowledgements

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DEVELOPMENT OF BIO-BASED INGREDIENTS FROM UNDERUSED TREES AND SHRUB SPECIES FOR INDUSTRIAL APPLICATION

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INTRODUCTION

Around the world, the strategies of companies and governments are increasingly converging around the concept of using biomass in industry. Besides the benefit from moving away from fossil-based raw materials, the use of natural matrices bring health properties and functionalities to the final products and is desirable from a circular economy perspective. This is leading industries like the food, cosmetic and pharmaceutic to look for bio-based ingredients to obtain these bio-based products [1]. To not compete with the current use of biomass, one strategy to obtain these natural ingredients could be the of underutilized species cultivated in marginal lands. This research has different purposes such as the chemical characterization of selected natural matrices from Germany, Spain and Romania to know and improve the contents in the target compounds.

MATERIALS AND METHODOLOGY

Betula pendula L. | *Calluna vulgaris* L. | *Carpinus betulus* L. | *Cistus ladanifer* L. | *Cytisus scoparius* L. | *Cupressus sempervirens* L. | *Euonymus europaeus* L. | *Hippophae rhamnoides* L. | *Juniperus communis* L. | *Populus nigra* L. | *Robinia pseudoacacia* L. | *Rosmarinus officinalis* L. | *Rubus fruticosus* L. | *Spartium junceum* L. | *Ulmus minor* Mill. | *Ulmus pumila* L.

CHEMICAL CHARACTERIZATION

- TERPENOIDS
- PHENOLIC COMPOUNDS
- FLAVONOIDS HYDROSOLUBLE and CONDENSED TANNINS

→ **EXTRACTION OPTIMIZATION**

EXPERIMENTAL DESIGN TOOLS BASED ON RESPONSE SURFACE MODELS

YIELD HPLC-DAD-MS

REFINE → HIGHLY PURE ESSENTIAL OIL AND POLAR EXTRACTS

→ **MEMBRANE TECHNOLOGIES**

ULTRAFILTRATION PROCESSES, SEMI-PREPARATIVE AND PREPARATIVE CHROMATOGRAPHY, SELECTIVE PRECIPITATION, CRYSTALLIZATION AND ADSORBENT BED PURIFICATION

SELECTION OF THE BEST TECHNOLOGY

HPLC-DAD

→ PURIFIED FRACTION ISOLATED COMPOUNDS

→ **BIOACTIVE POTENCIAL**

- ANTIOXIDANT
- ANTIMICROBIAL
- CYTOTOXIC
- ANTI-INFLAMMATORY

→ **APPLICATIONS**

- NUTRACEUTICALS
- PLANT-BASED MEDICAMENTS
- FEED ADDITIVES
- COSMETIC INGREDIENTS

MARKET REQUIREMENTS

REGULATORY DOCUMENTS

RESULTS AND CONCLUSIONS

The results obtained in the present study may serve to add knowledge in the field of valorization of unexploited species through the application of bio-based products in industries like the food and cosmetic as natural-based preservatives and bioactive agents.

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[1] Cho, E. J., Trinh, L. T. P., Song, Y., Lee, Y. G., & Bae, H. J. (2020). Bioconversion of biomass waste into high value chemicals. Bioresource Technology, 298.

AKNOWLEDGMENTS

Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to the CIMO (UIDB/00690/2020). L. Barros and S.A. Heleno (CEECIND/03040/2017) thank the national funding by FCT, P.I., through the institutional and individual scientific employment program-contract for their contracts, respectively. This project has received funding from the Bio Based Industries Joint Undertaking (JU) under grant agreement No 887917 BeonNAT. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium.

BIOACCESSIBILITY OF BIOACTIVE COMPOUNDS FOLLOWING GASTROINTESTINAL DIGESTION OF BEE POLLEN AND BEE BREAD

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NPA Natural products application: Health, Cosmetic and Food Online Edition 4-5 Feb 2021

Abstract

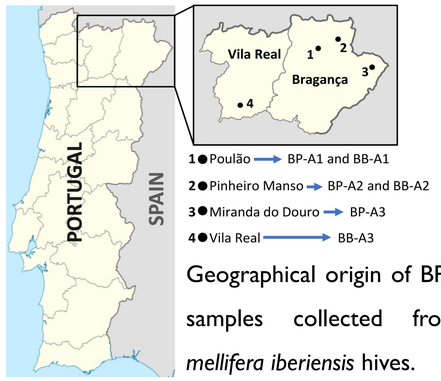
In this study, the bioaccessibility level of bioactive compounds in bee pollen (BP) and bee bread (BB), as well as the mechanisms of action of these compounds in the gastrointestinal tract were investigated. The findings indicated a significant reduction in bioactive compounds in both BP and BB at the end of digestion compared to raw samples. The bioaccessibility level was calculated on average 25% and 33% for BP and BB, respectively.

Introduction

BP is formed as a result of combining plant pollen collected by bees with their own secretions and then are transported to the hive and stored in combs [1]. The stored BP in the combs is mixed with the digestive enzymes secreted by bees and honey and expose to lactic acid fermentation, resulting in the production of BB [1]. Both BP and BB are characterized by high nutritional value, as well as bioactive compounds.

This research evaluates the comparison of the bioavailability properties of BP and BB using an *in vitro* digestive model.

Materials



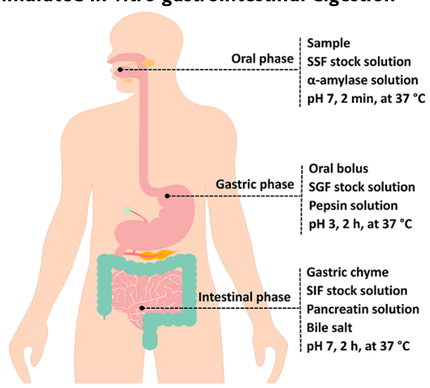
1 ● Poulão → BP-A1 and BB-A1
2 ● Pinheiro Manso → BP-A2 and BB-A2
3 ● Miranda do Douro → BP-A3
4 ● Vila Real → BB-A3

Geographical origin of BP and BB samples collected from *Apis mellifera iberiensis* hives.

Methodology

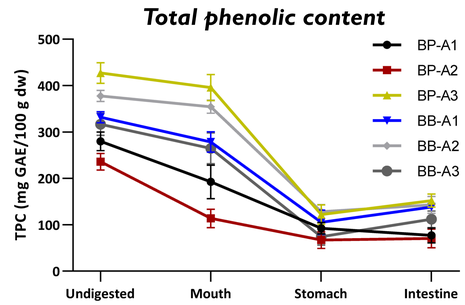
Preparation of extracts and total phenolic (TPC) and flavonoid content (TFC). The extraction process and TPC and TFC were carried out using previously reported methods [2]. Expression of obtained results was done by equivalents of standards - gallic acid (TPC) and quercetin (TFC).

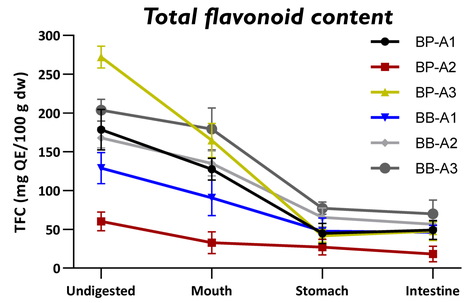
Simulated in vitro gastrointestinal digestion



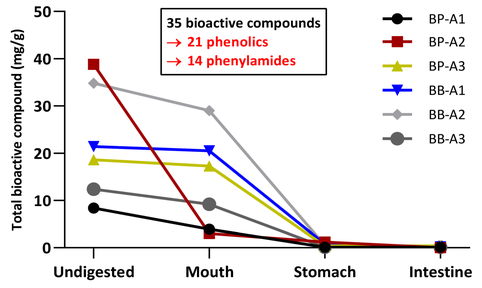
Bioactive compound profile by UPLC-ESI-MS. The bioactive compound profile was determined by an ultra-pressure liquid chromatography, working in negative mode.

Results





The concentrations of phenolic compounds tended to be reduced by the digestive system, whereas phenylamides were completely digested after the intestinal phase.



	Bioaccessibility level	
Analysis	**BP**	**BB**
TPC	**31%**	**38%**
TFC	**25%**	**35%**
UPLC-ESI-MS	**18%**	**27%**

Conclusion and future perspective

- The *in vitro* digestion results revealed that the bioactive compounds in BP and BB generally tended to decrease throughout digestion.
- The gastric phase was found to be an important factor on the bioaccessibility level of bioactive compounds.
- The results showed that BB is either more accessible and richer in bioactive compound content than BP.
- Further studies may support the findings of this study, by implementing a model involving colon microbial digestion with cellular models such as Caco-2 or directly conducting *in vivo* studies.

Acknowledgements

The authors are grateful to FCT, Portugal for financial support to CIMO (UIDB/00690/2020). Thanks to the Project PDR2020-1.0.1-FEADER-031734: "DivInA-Diversification and Innovation on Beekeeping Production". National funding by FCT- Foundation for Science and Technology, through the institutional scientific employment program-contract with Soraia I. Falcão.

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Isolation, purification and *in silico* modeling of halotolerant bacterial endo-β-1,4-glucanase

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Abstract

Current demand for renewable and ecological energy sources has boosted a search for alternatives to replace the use of fossil fossils. One of the most promising innovations to positively impact the scenario energy market is a production of second generation bioethanol (2G-ethanol) from reducing sugars derived from enzymatic degradation of lignocellulosic material that is normally discarded in processes agro-industrial. The present work had as objective the structural modeling *in silico* and the purification of cellulolytic enzymes for the conversion of lignocellulosic material processed into reducing sugars fermentable. Enzymatic purification was able to obtain an endoglucanase with an approximate molecular mass of 37 kDa while *in silico* modeling evidenced the presence of monomeric structure in the main endoglucanase involved in the process. According to the data obtained in this work, it was possible to conclude the possibility of applying this optimized bioprocess in industrial processes for bioethanol generation.

Introduction

Cellulases (or endo-β-1,4-glucanase) are able to hydrolyze the O-glycosidic bonds between glucose residues arranged in a Beta-like isomeric configuration as in the polysaccharide most abundant in the biosphere, which is cellulose. to be degraded only by a tiny portion of organisms that are capable of performing their catalysis on glucose molecules that could then be used in different processes as for the production of ethanol. In addition to this potential employment in the biofuel industry, cellulases are currently widely used in the paper, textile and detergent industries.

Among the genera of cellulolytic bacteria that occur in marine environment, the genus Bacillus presents a ubiquitous distribution in aquatic ecosystems interacting with a wide range of organisms from the benthic regions to the intertidal zones, thus bacteria must be able to withstand extremes of salinity and temperature as well as the fluctuation of these physical-chemical parameters throughout the day what makes this ecological niche a very strong candidate for the prospection of extracellular cellulases of interest for the 2G-Ethanol production industry, replacing the organisms currently available and characterized in the literature. The present work had as objective the structural modeling *in silico* and the purification of cellulolytic enzymes from marine and halotolerant bacteria.

Materials

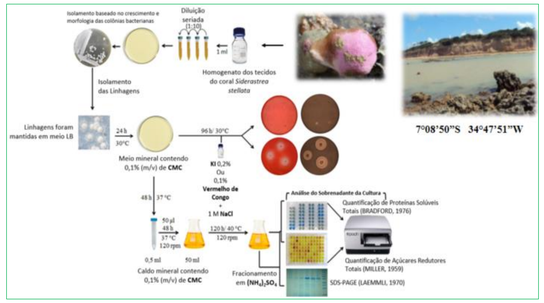
The enzymatic study was carried out using bacterial isolates obtained from tissues collected from the coral *Siderastrea stellata* on the reefs of Cabo Branco, Paraíba, Brazil (7 ° 08'50 "S; 34 ° 47'51" W). For bacterial isolation, tissues of the ecto and anthozoan endoderm were immersed in sterile saline and stirred until homogenized, followed by plating on solid marine agar medium (pH 7.0) until adequate growth.

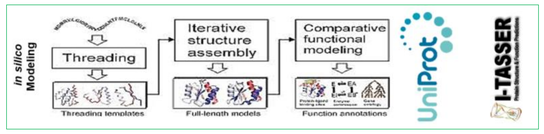
The extracellular proteins secreted from the culture in liquid medium were fractionated and the cellulosic activity detected in the protein fraction precipitated with 60-90% ammonium sulfate. After successive dialysis, the sample was concentrated and applied in molecular exclusion chromatography where cellulolytic activity was detected.

Methodology

In order to predict the structure and biological function of the isolated protein, an alignment was made based on known deposited proteins (PDB 3O5S). An amino acid sequence without the signal peptide was submitted to the ITASSER server of automated comparative modeling of proteins and a 3D model was obtained.

The overlap between the new protein 3D model and structurally similar proteins derived from phylogenetically close microorganisms to the lineage under study was carried out with the aid of VMD software. The following flowcharts illustrates the process of purification (upper) and *in silico* modelling (down) assay:





Results

The purification assay showed a 37 kDa endoglucanase that was named as bc22cel. The protein was purified by ammonium sulphate precipitation, gel filtration chromatography and extraction from the gel after nonreducing sodium dodecylsufate-polyacrylamide gel electrophoresis. The optimal pH value and temperature of bc22cel were 6.5 and 60 °C, respectively. The purified bc22cel showed a considerable halophilic property being able to maintain more than 80% of residual activity even when pre-incubated with 1.5 M NaCl for 1 hour.

The molecular modeling data generated from the comparison with three-dimensional structures obtained by X-ray crystallography and deposited in the Protein Data Bank (PDB), showed that bc22cel in all ways possible three-dimensional dimensions was thermodynamically close to native monomeric structures, which is the natural 3D form for this class of enzymes (Figure 1).

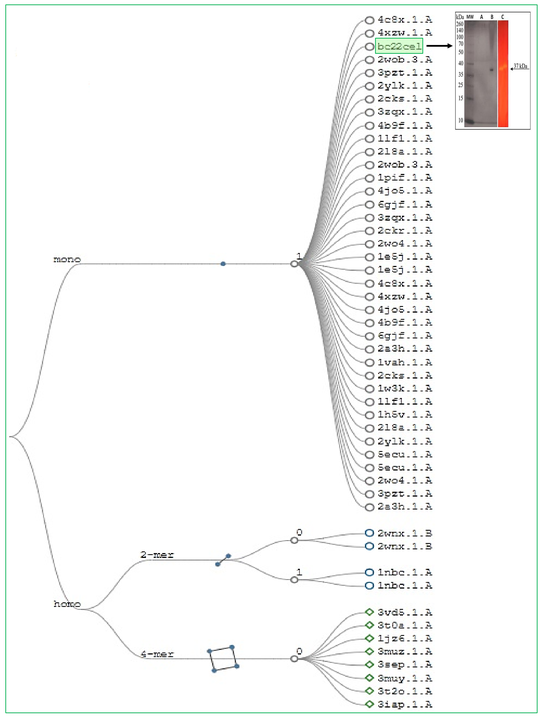


Figure 1. Tree of possible molecular models for purified cellulase (bc22cel). Homotetrameric, homodimeric and monomeric models were generated from data generated from the SWISS-MODEL and I-TASSER servers. In detail, SDS-PAGE with purified bc22cel demonstrating that it is a 37 kDa protein (lane B) and capable of degrading CMC (lane C).

Conclusion

According to the data obtained in this work, it was possible to conclude:

- It was possible to isolate an endoglucanase with an approximate molecular mass of 37 kDa, purified from filtration gel chromatography and presented an optimum temperature of activity at 60 ° C;
- The enzyme maintained activity over a wide pH range (pH 4 to 9), the enzyme showed maximum activity at pH 6.5;
- The enzyme demonstrated considerable stability against NaCl, maintaining more than 80% of its activity in concentrations up to 1.5 M NaCl;
- Computational modeling for major endoglucanase indicated that it is a monomeric enzyme.

Recommendations

As evidenced, bc22cel can be classified as endo-β-1,4-glucanase since it was able to degrade its specific substrate (CMC) and consists of a monomeric enzyme like the vast majority of bacterial endoglucanases. Considering that bc22bel fits into the general pattern of bacterial hydrolases, computational modeling as well as strategies for its production in a heterologous way is facilitated by the high degree of conservation and homology with three-dimensional structures already studied and described in the literature and deposited in the PDB.

In resume, the present work was able to optimize and achieve a considerable yield of induction and production of the cellulolytic enzymes of interest. However, it is still necessary to detail the mechanism through which the enzymes of interest are secreted (channels, efflux systems involved) as well as to expand structural studies via docking in order to corroborate the obtained data.

Acknowledgements

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NANOPARTICLES CONTAINING BERBERINE: PRODUCTION AND EVALUATION OF THERMAL AND SPECTROSCOPIC PROPERTIES

Janmille Monteiro Marcelo[1], Bruna Franzon Rossi[1], Fernanda Vitória Leimann[1], Odinei Hess Gonçalves[1], Rafael Porto Ineu[1]*

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Motivation

- Bioactive compound: **Berberine**
  - Human health benefits;
  - Limitations in the food industry;
- Nanotechnology has been used by industries to overcome important limitations of bioactive compounds.

Introduction

- A berberine (BBR) is a bioactive, specifically an isoquinoline alkaloid of the protoberberine type present in plants.
  - **Species:** *Berberis vulgaris, Berberis aquifolium, Berberis aristata;*
  - **Family:** Berberidaceae;

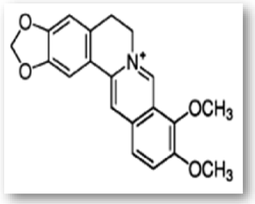
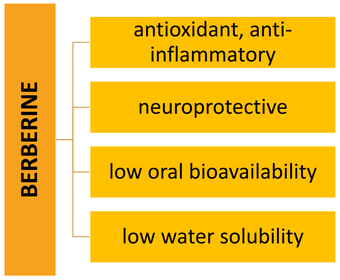


Fig. 1 - Chemical structure of berberine.

Fig. 2 - Berberine species *Berberis vulgaris.*



Numerous nanoencapsulation techniques can be used to improve the dissolution and bioavailability of bioactive compounds that are poorly soluble in water, including **solid dispersion.**

Objectives

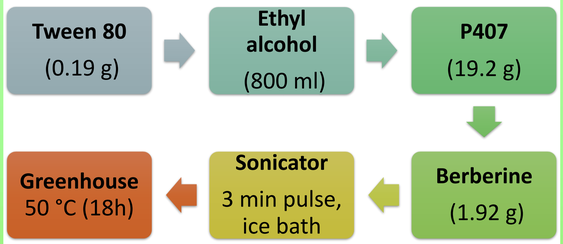
- Nanoencapsulate the berberine;
- Characterize the obtained nanoparticles**.**

Materials and Methodology

The following materials were used:

- Kolliphor® Poloxamer 407 (P407) polymer (12.220 g/mol molar mass, Sigma-Aldrich);
- Berberine chloride (90% pure, Sigma-Aldrich);
- Ethyl alcohol (99.8% pure, Dynamic);
- Tween 80 PS surfactant (Dynamic);
- Potassium bromide (Spectroscopic grade, Sigma-Aldrich).

Berberine nanoencapsulation (solid dispersion):



Characterization:

DSC, 5 to 10 mg of the analyte were placed in closed aluminum sample holders and kept at 0 °C for 5 min and heated to 400 °C to 10 °C min^{-1} under a nitrogen flow of 50 mL min^{-1}.

Fig. 3 - Differential Scanning Calorimetry (DSC) Equipment.

FTIR, potassium bromide tablets analyzed with a resolution of 2 cm^{-1} in the range 4750-450 cm^{-1} and 32 cumulative scans.

Fig. 4 - Fourier Transform Infrared Spectrophotometry (FTIR) analysis equipment.

Results

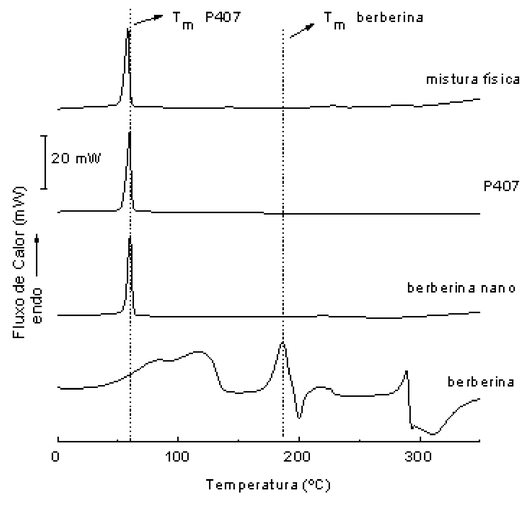


Fig. 5 - Thermograms obtained by berberine DSC, nanoencapsulated BBR, P407 and MF (BBR and P407).

The disappearance of the melting point (Tm) of berberine in the nanoparticles indicates the efficiency of the conversion from the crystalline to the amorphous state.

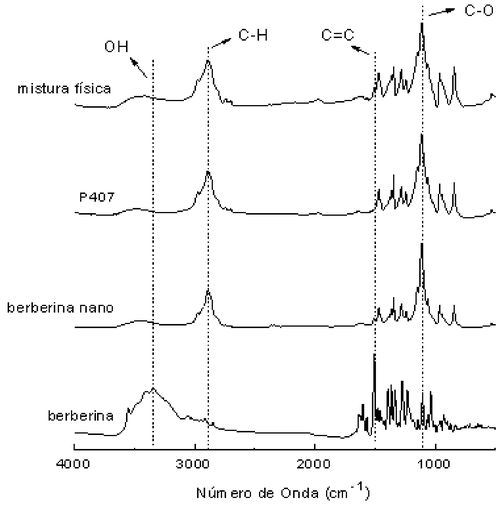


Fig. 6 - Spectra obtained by FTIR of berberine, nanoencapsulated BBR, P407 and MF.

The berberine spectrum revealed peaks at 3300 cm^{-1} (OH), main peak at 1502 cm^{-1} (C = C), suggesting the encapsulation of berberine in the Poloxamer matrix.

Conclusion

- Characterization confirmed the conversion of the physical state of the berberine particles from the crystalline to the amorphous.
- The solid dispersion technique for particle production proved to be efficient for the type of compound studied, thus allowing the characterization of nanoencapsulated berberine.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. The authors also thank "Central Analítica Multiusuário da UTFPR Campo Mourão (CAMulti-CM) for the characterization analyzes".

Caracterização nutricional, química e bioativa do mesocarpo de *Attalea speciosa* Mart. ex Spreng (babaçu) e aplicação da sua farinha em produtos de panificação

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Abstract

Unconventional food plants (UFP) are a viable and efficient alternative for replacing the food products we consume today. Its applicability in food products is practically unlimited due to its potential, but also to its nutritional, chemical, physical, and biological characteristics. The valorization of non-conventional food vegetable represents an important contribution from a cultural, economic, social and nutritional point of view for future consumers and producers. The introduction of UFP on a regular diet basis could lead to a diversification and improvement of nutritional quality considering the current agricultural system, where a small number of plants are produced for commercialization and consumption.

Introduction

The term unconventional food plants (UFP) refers to plants that have one or more parts that can be used in human food, such as: roots, tubers, bulbs, rhizomes and others.

Unconventional food plants have a limited distribution, restricted to certain localities or regions, exerting great influence on the food and traditional culture of populations. The valorization of this type of plants for human food represents a structural change from the cultural, economic, social and nutritional point of view.

Attalea speciose (Mart. ex Spreng – Babassu, **Figure 1**) is a palm tree of the botanical family Arecaceae found in Brazil, considered the largest native oil resource in the world and its production chain is one of the most representative in Brazil.

Figure 1 – Babassu coconut.

The potentialities of babassu range from the use of coconut, as its unfolding in all the primary fractions of the epicarp, mesocarp, endocarp, and almonds. Its economic exploitation is carried out in several ways: human food, folk medicine, cleaning materials, and cosmetics.

The babassu mesocarp represents 20.4% of the fruit, it is the layer below the epicarp, with 0.5 to 1.0 cm thickness (**Figure 2**).

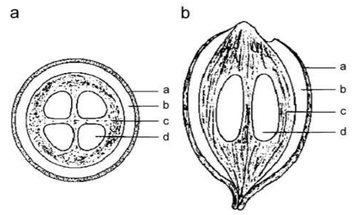

Figure 2 - Cross (a) and longitudinal (b) sections of babassu coconut. Components from external to internal: epicarp-a; mesocarp - b; endocarp - c and core - d

It is a by-product of the babassu oil extraction industry, however much of its application is also for human comsuption It is produced during the separation of coconut almonds, is a source of edible starch, exhibiting antioxidant and antimicrobial properties due to its rich composition in phenolic and flavonoid compounds.

The mesocarp flour (**Figure 3**), in addition to the rich proximate composition, it has also been described as having antioxidant and antimicrobial activity due to the presence of phenolic compounds and flavonoids, as also anti-inflammatory and analgesic properties.

Figure 3 - Flour obtained from babassu mesocarp.

The flour produced from the mesocarp of babassu coconut is also nutritious for its high carbohydrate content and the presence of fibers, which can modify the lipid profile and significantly decrease blood glucose in diets.

The high content of fibers and phytochemicals with biological activity increases the quality of babassu flour for food applications, meeting the increasing demand for healthier food products. With the full potential of UFP, their applicability in food products is limitless as they represent a viable and efficient alternative for replacing the food products we consume today. On the other hand, the non-use of UFP's has always been due to the lack of technology that would enable the obtaining and application of such products, making the industry incipient.

The studies of the nutritional, chemical and bioactive characteristics of the babassu mesocarp flour are very scarce or even non-existing. In addition, its application in bakery products for the substitution of the widely consumed wheat flour are also scarce, making this plant by-product very appealing for the development of new foodstuffs that could be on the vanguard of the next generation of bakery products.

Objectives

The present study aims to deepen the knowledge on the chemical, nutritional and bioactive characteristics of the mesocarp of *Attalea speciosa* Mart. ex Spreng (babassu), and the application of its flour for the development of new bakery products.

Specific objectives of the present work:

- Nutritional evaluation and chemical characterization of flour from babassu mesocarp.
- *In vitro* evaluation of bioactive properties: antioxidant, antimicrobial, anti-inflammatory, cytotoxic activity of hydroethanolic extracts obtained from the flour under study.
- Application of babassu mesocarp flour in bakery products and evaluation of nutritional, chemical, physical and organoleptic properties of the obtained products.

Methodology

- Development of bakery products based on flour of the babassu mesocarp. Evaluation of physical parameters of color, water solubility index and water absorption index, texture, mass and volume, compared to products based on flours of 100% wheat.
- Evaluation of the nutritional value of flour and breads formulated with babassu flour by AOAC methods.
- Determination of the composition in hydrophilic compounds in dry flour and breads formulated with babassu flour: HPLC free sugars coupled to an IR detector and organic acids by UFLC coupled to a DAD detector.
- Determination of the composition in phenolic compounds in hydroethanolic extracts by HPLC-DAD-ESI/MS detector.
- Evaluation of the bioactive properties of hydroethanolic preparations through their:
  - Antioxidant: activities TBARS and OxHLIA;
  - Cytotoxic activities: in four human tumor cell lines; MCF-7 lines (breast adenocarcinoma), NCI-H460 (lung carcinoma), HeLa (cervical carcinoma), HepG2 (liver carcinoma) and a primary culture obtained from pig liver cells - PLP2;
  - Anti-inflammatory: nitric oxide (NO) production in the RAW 264.7 cell line similar to rat macrophages;
  - Antimicrobial: using ATCC strains and food isolates of fungi and bacteria through the microdilution method for further calculation of the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MCB) and minimum fungicidal concentration (MCF).

Acknowledgements

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NPA
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Online Edition
4-5 Feb 2021

ANTIOXIDANT ACTIVITY AND GC-MS CHARACTERIZATION OF *JUNIPERUS COMMUNIS* AND *CISTUS LADANIFER* ESSENTIAL OILS

Virginie Xavier, [1,2] Sandrina Heleno, [1] Miguel A. Prieto[2] , Joana Amaral[1] , Irene Mediavilla Ruiz[3] , Luis Saul Esteban Pascual[3] , Isabel C.F.R. Ferreira[1] , Lillian Barros,[1]*
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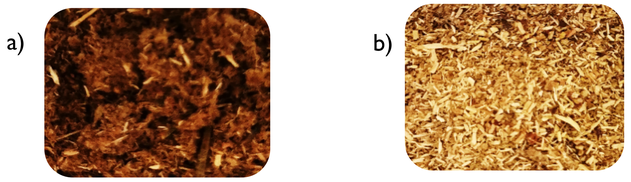
INTRODUCTION

Juniperus communis L. and *Cistus ladanifer* L. are two abundant shrubs in the mountain areas of the Mediterranean basin, particularly in the Iberian Peninsula. Both species are known for their valuable essential oil that can be used in cosmetic, food and pharmaceutical industries for their bioactive properties [1]. Within the scope of the European Project "BeonNat", that aims at developing innovative and bio-based products using the biomass of trees and shrubs growing on marginal and underutilized lands as feedstock for the bio-based industry, these two species were selected for evaluation.

Juniperus communis

Cistus ladanifer

MATERIALS AND METHODOLOGY



Branches milled to <20mm particle size from a) *J. communis* and *b) C. ladanifer* grown in Spain

Steam distillation

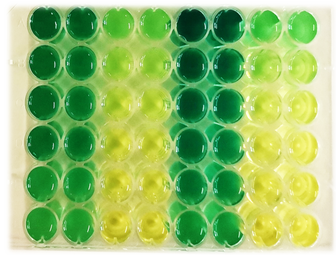
Essential oils

Chemical composition

GC-MS

Antioxidant activity

FERRIC REDUCING POWER ASSAY



RESULTS AND CONCLUSIONS

Yield (% dry basis)	
J. communis	*0.50*
C. ladanifer	*0.08*

Reducing power (EC_{50} mg/mL)	
J. communis	1.35± 0.20
C. ladanifer	1.30±0.07

Chemical composition

J. communis

98.1% identification

Total: 63 identified compounds

α-pinene	32.3%
limonene	15.8%
sabinene	7.6%
germacrene B	4.9%
cis-thujopsene	4.6%
β-myrcene	3.7%
β-caryophyllene	3.6%

C. ladanifer

92.8% identification

Total: 61 identified compounds

viridiflorol	20.7%
α-pinene	19.8%
ledol	8.1%
camphene	7.2%
bornyl acetate	5.6%

The *J. communies* species showed higher extraction yield than *C. ladanifer*. The chemical composition is in good agreement with literature [2] and both oils showed promising results in the reducing power assay.

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Bioactive Potential Of Aromatic And Medicinal Plants Traditionally Used As Condiments

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Introduction

Aromatic and medicinal plants are highly appreciated and used worldwide as condiments, dyes, and preservatives. Given their nutritional value and chemical composition, related to health beneficial properties, their inclusion in the Human diet has gain an increasing expression [1]. Certain mixtures of plants demonstrate greater potential when compared to isolated plants, due to synergistic effects, and these properties make them of great interest in food, pharmaceutical, and cosmetic industries. They have been consumed through direct use in prepared dishes, but also by incorporation into foodstuff, making them bioactive and functional [2].

Materials & Methodology

Four mixtures of aromatic plants used for seasoning poultry, meat, fish, and salads were characterized in terms of phenolic compounds (HPLC-DAD-ESI/MS), organic acids (UFLC-PDA), tocopherols (HPLC-fluorescence), and bioactive properties (antioxidant, antimicrobial, anti-inflammatory, and antitumour).

Mixtures have in their constitution:

Allium schoenoprasum L.

Artemisia dracunculus L.

Origanum vulgare L.

Rosmarinus officinalis L.

Petroselinum crispum L.

Salvia officinalis L.

Thymus × *citriodorus* L.

Satureja montana L.

Thymus mastichina L.

Thymus vulgaris L.

Results

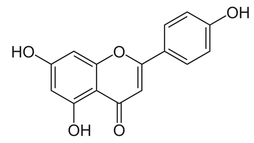


Fig. 1. Chemical structure of Apigenin.

25 phenolic compounds were identified, with apigenin-*O*-malonyl-pentoside-hexoside as the most abundant compound in all extracts.

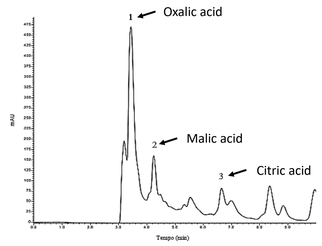
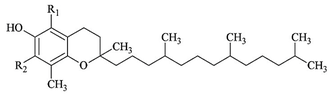


Fig. 2. hromatographic profile of organic acids from extracts of condiment mixtures.

Oxalic, malic, and citric acids were detected in all the samples.



Tocopherol	R_1	R_2
α-	CH_3	CH_3
β-	CH_3	H
γ-	H	CH_3
δ-	H	H

Fig. 3. Chemical structure of vitamin E isomers.

The mixtures also revealed the four isoforms of tocopherols.

Graphic 1. Antioxidant activities

Graphic 2. Antitumour activity

For antioxidant activity, the extracts of the mixtures for meat and salads revealed the best results in the TBARS assay, whereas those from mixtures for meat and poultry stood out in the OxHLIA assay (**Graphic 1**).

The mixture for salad showed the best antitumour properties (**Graphic 2**), and the mixtures for poultry and fish showed the highest anti-inflammatory activity (**Graphic 3**).

Graphic 3. Anti-inflammatory activity

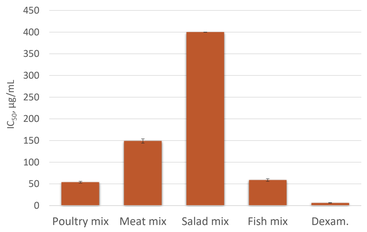


Table 4. Antibacterial activity (minimal inhibition concentration (MIC) and minimal bactericidal concentration ((MBC); mg/mL).

	Poultry mixture		Meat Mixture		Salad mixture		Fish mixture	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	5	>20	10	>20	5	>20	5	>20
K. pneumoniae	>20	>20	>20	>20	>20	>20	>20	>20
M. morganii	5	>20	2.5	>20	5	>20	10	>20
P. mirabilis	10	>20	10	>20	10	>20	20	>20
P. aeruginosa	>20	>20	>20	>20	>20	>20	>20	>20
E. faecalis	5	>20	2.5	>20	2.5	>20	5	>20
L. monocytogenes	20	>20	10	>20	10	>20	10	>20
MRSA	5	>20	2.5	>20	5	>20	10	>20

MRSA: Methicillin resistant *Staphylococcus aureus*.

The mixtures for meat and salad revealed the highest antimicrobial activity.

Conclusion

These seasoning mixtures demonstrated valuable bioactive properties, conferred by their chemical composition and cumulative and synergistic effects observed in the mixtures, which corroborates the importance of their inclusion in the Human diet, either through their consumption as a garnish, or by incorporation into less common foods, and in the conservation and maintenance of food quality.

References

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