STEMS: A SWEET CHERRY BY-PRODUCT WITH HIGH POTENTIAL

IECAG 2021 The 1st International Electronic Conference on Agronomy
03–17 MAY 2021 | ONLINE

Sílvia Afonso[1*], Ivo Oliveira[1], Anne S. Meyer[2], Alfredo Aires[1], Maria José Saavedra[1], Berta Gonçalves[1]

[1] Centre for the Research and Technology of Agro-Environmental and Biological Sciences- CITAB, University of Trás-os-Montes and Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal

[2] Technical University of Denmark, Department of Biotechnology and Biomedicine, DTU Building 221, DK-2800 Kgs. Lyngby, Denmark

*safonso@utad.pt

ABSTRACT

During sweet cherry processing, large amounts of by-products are generated. There is no substantial use of this waste, which increase environmental and managements costs each year to deal with the excess of such residues. These by-products only recently received attention and this new interest is focused in finding ways to achieve their valorization.

Therefore, we conducted a study in which chemical composition, phenolic profile, antioxidant activity of stems of four sweet cherry cultivars (*Early Bigi* (grown under net cover (C) and without net cover (NC)), *Burlat*, *Lapins*, and *Van*) and antibacterial activities against important Gram negative and Gram positive bacterial human isolates, were examined.

Extracts from stems of cv. *Lapins* presented high levels of total phenolics, flavonoids, *ortho*-diphenols and saponins. Regarding DPPH and FRAP methods, higher overall results were also recorded for cv. *Lapins*, while for ß-carotene method, results were higher for cv. *Van*.

Apart from cv. *Early Bigi* NC, major phenolic compound identified in stems was sakuranetin. In cv. *Early Bigi* NC the most abundant compound was ellagic acid. In all extracts, antioxidant activities showed a positive correlation with the increments in phenolic compounds. Antimicrobial activity assays showed that stem's extracts were capable of inhibiting the growth of Gram positive isolates.

This new data is intended to provide new possibilities of valorization of these by-products and their valuable properties.

INTRODUCTION

Sweet cherry is one of the fresh fruits most appreciated by consumers in the temperate areas of Europe, not only due to its organoleptic characteristics, such as color, brightness, flavor, aroma and texture, but also for consumers' awareness of its benefits for human health. Worldwide sweet cherry production has been increasing in the last years from 2 to 2.60 million tons, with Turkey, USA, Chile as the main producers accounting for about 50% of the total world production, and Chile, China and USA the main exporters. Portugal is also well-known producer of sweet cherries, with latest data referring 19563 tons of cherries annually, providing some of the first cherries of Europe, produced in the municipality of Resende.

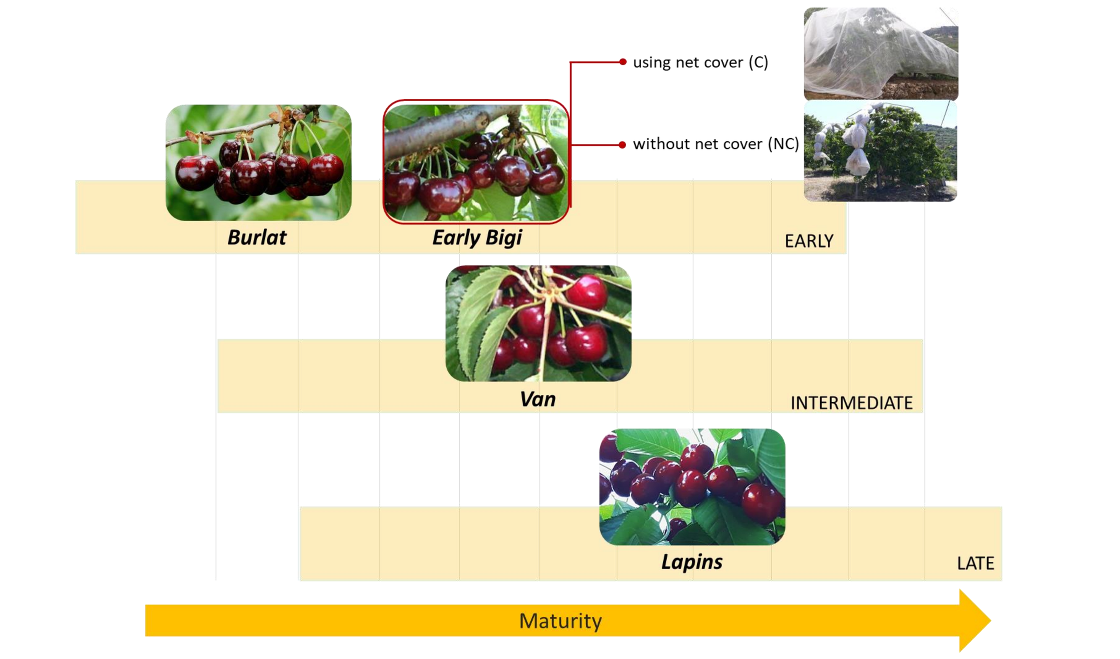
Although sweet cherry is mainly commercialized as fresh fruit, a considerable quantity is used after processing as jam, jelly or juice, that generates large amounts of by-products, namely stems. There is no substantial use of this waste, which increase environmental and managements costs each year to deal with the excess of such residues.

Therefore, a solution to achieve valorization of the excess of this material is urgent. While cherry stems are known by traditional medicine and widely used in infusions and decoctions, due to their claimed sedative, diuretic and anti-inflammatory properties, their study, only recently have received attention. Various hydroxycinnamic acids, such as *p*-cumaric, ferulic, caffeic, chlorogenic and neochlorogenic acids, have been reported in stems of *P. avium*.

In this context, the objective of the current work is to evaluate the biological potential of such less used residues, providing a detailed study on their phenolic profile and antimicrobial activities.

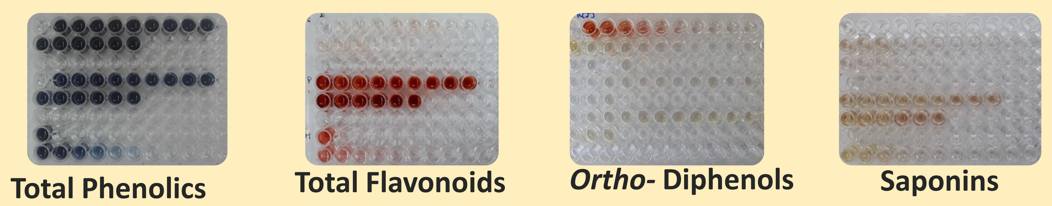
MATERIALS

Selected 4 sweet cherry cultivars grown in an orchard located in Resende.

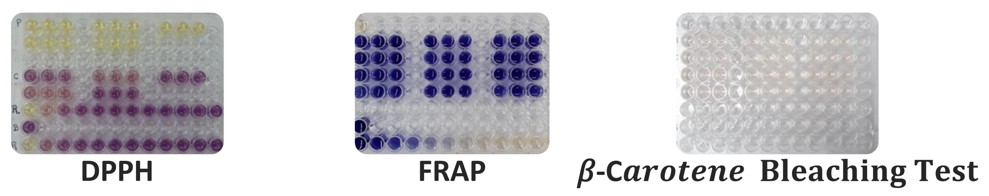


METHODOLOGY

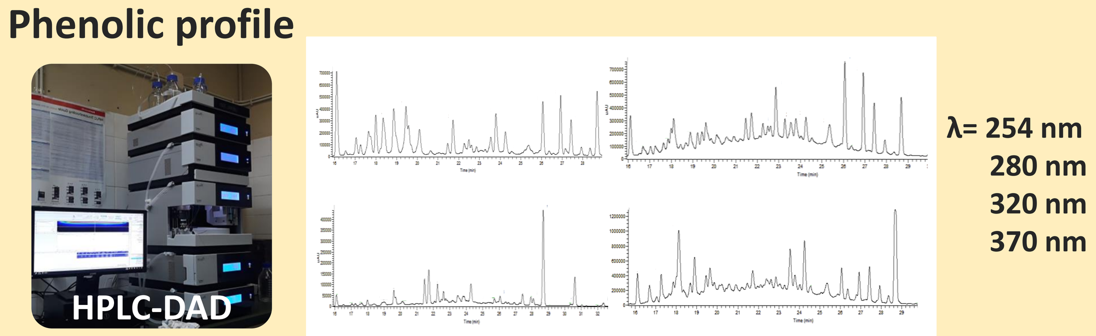
Chemical Characterization



Antioxidant Activity

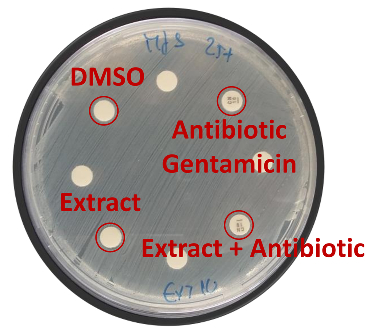


Phenolic profile



Antimicrobial activity

	Gram +	Gram -	
Faecal sample	*Enterococcus faecalis*	*Klebsiella pneumoniae*	Urine sample
	Staphylococcus aureus	*Enterobacter aerogenes*	
	Staphylococcus aureus ATCC	*Escherichia coli* ATCC	



$$\% \text{ RIZD} = \frac{IZD\ sample - IZD\ negative\ control}{IZD\ antibiotic\ standard} \times 100$$

where IZD is the inhibition zone diameter (mm)

Statistical analysis

Statistical Package for Social Sciences (SPSS) software, version 25.0 (IBM Corporation, New York, USA).

Comparison of averages performed using the Tukey test with a significance level of 5%.

RESULTS

Regarding the chemical characterization of stems, cv. ***Lapins*** presented higher levels of total **phenolics**, total **flavonoids**, ***o*-diphenols** and **saponins** (Table 1). Interestingly, the values for chemical characterization of cv. *Early Bigi* show significant differences between samples from covered and non covered fruits.

No previously data is available regarding saponin content in sweet cherry stems, being these the first data about the presence of those compounds in this samples.

Table 1- Chemical characterization (average ± SD) of stems. Different letters indicate significant differences ($p < 0.05$).

Cultivar	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	*Ortho*-diphenols (mg CAE/g)	Saponins (mg DE/g)
***Early Bigi* C**	23.59±0.14b	13.06±1.97b	3.88±0.15	42.45±2.97c
***Early Bigi* NC**	31.30±2.15a	19.71±3.43ab	5.15±0.97	101.79±8.35a
Lapins	32.49±5.23a	24.75±1.14a	5.65±0.75	181.12±6.92a
Burlat	26.63±1.65ab	14.67±3.23b	3.75±0.85	38.05±3.94c
Van	30.56±1.29ab	21.18±4.75ab	4.24±0.70	98.66±13.44b
P- value	0.012	0.006	0.113	0.000

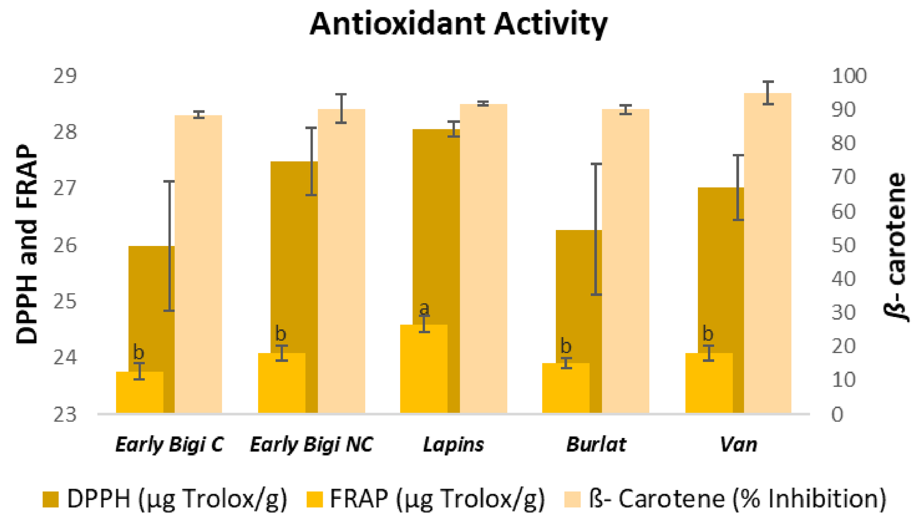


Figure 1- Antioxidant activity (average ± SD) of stems. Different letters indicate significant differences ($p < 0.05$).

The antioxidant activity (Figure 1) only differed between cultivars for the **FRAP** methodology, with enhanced results when using cv. ***Lapins*** stems. For this methodology, results were found to correlate with the content in flavonoids ($y = 0.8899x + 1.5521$, $R^2 = 0.781$) and saponins ($y = 0.0874x + 10.085$, $R^2 = 0.824$).

Sixteen individual compounds were identified in extracts of **stems**. The content of each phenolic compound varied significantly when comparing cultivars, with only syringic acid present in similar amounts in all cultivars. In all samples, the **most abundant** compound was **sakuranetin**, with the exception of stems of cv. *Early Bigi* NC, where the most abundant compound was ellagic acid (Table 2).

Table 2- Phenolic composition (average ± SD) of sweet cherry stems (mg/100 g DW). Different letters indicate significant differences ($p < 0.05$).

	Early Bigi C	*Early Bigi* NC	*Lapins*	*Burlat*	*Van*	*P- value*
Catechin	15.02±2.12b	22.26±0.51a	17.45±0.53b	15.64±0.71b	17.86±0.31b	0.000
Epicatechin	59.05±4.19b	74.88±5.05a	50.23±0.99c	61.82±0.72b	49.39±1.63c	0.000
Naringenin-7-*O*-glucoside	22.78±2.42bc	27.52±1.11a	26.61±0.40ab	26.37±0.44ab	20.77±1.75c	0.000
Hydroxycinnamic acid	18.16±3.89a	19.14±0.20a	8.51±0.08b	18.09±0.75a	9.52±0.40b	0.000
Sakuranetin	139.73±11.06ab	128.31±7.79b	140.88±0.58ab	158.30±10.08a	131.63±0.85b	0.006
Ellagic acid	106.87±14.56b	145.25±4.69a	96.37±5.59b	106.42±3.93b	95.59±5.26b	0.000
Neochlorogenic acid + isomer	19.11±2.09d	25.63±0.28c	40.62±0.20a	26.38±0.79c	33.11±1.25b	0.000
Chlorogenic acid + isomer	43.29±4.90b	51.13±0.57a	23.77±0.41c	19.18±0.45c	25.23±1.69c	0.000
p-Coumaric acid + isomer	18.81±2.58bc	22.47±0.71ab	18.38±0.69c	22.56±0.85a	13.46±1.02c	0.000
Ferulic acid	14.95±1.82a	14.28±0.07a	9.56±0.25bc	10.99±0.57b	7.59±0.65c	0.000
Syringic acid	12.63±1.38	13.89±0.22	9.48±0.29	7.37±0.33	9.15±0.17	0.470
Taxifolin	7.07±1.00a	8.34±0.32a	4.94±0.29b	4.09±0.30b	4.85±0.18b	0.000
Kaempferol-3-*O*-rutinoside	5.94±0.60c	11.69±0.20a	7.60±0.17b	12.92±0.69a	6.28±0.67bc	0.000
Kaempferol-3-*O*-glucoside	3.20±0.41c	5.95±0.07a	Nd	4.29±0.22b	2.70±0.28c	0.000
Genistein	4.93±0.74b	6.63±0.13a	4.97±0.08b	1.53±0.07d	3.25±0.09c	0.000
Quercetin-3-*O*-glucoside	16.17±1.50a	8.61±0.31b	Nd	3.78±0.18c	0.65±0.12d	0.000
Total	508.57±46.57ab	589.99±21.20a	493.02±51.30b	501.71±19.35ab	434.02±9.74b	0.003

Table 3- Antimicrobial activity (% RIZD) of sweet cherry stems relatively to antibiotic gentamicin.

	Standard bacterial strains	*Early Bigi* C	*Early Bigi* NC	*Lapins*	*Burlat*	*Van*
Gram +	*S. aureus* ATCC	66.6	58.8	56.3	68.8	62.5
	S. aureus MJS241	*	*	*	*	*
	E. faecalis MJS257	*	*	*	*	*
Gram -	*E. coli* ATCC	0	0	0	0	0
	K. pneumoniae MJH812	0	0#	0#	0#	0
	E. aerogenes MJH813	0	0	0	0	0

Means ± SD (n = 3). 0-extract without effect; 0–100– extract less effective than an antibiotic;*-extract effective and antibiotic without effect; #-slight synergistic effect when antibiotic used in combination with extract, with increased inhibition zone comparing to the former.

Extracts from cherry **stems** proved to be **effective**, namely for **Gram positive** bacteria. Indeed, for *S. aureus* ATCC, all samples were able to inhibit its growth, with values of % RIZD above 55 %. More interestingly, for the clinical isolates of ***S. aureus* MJS241** and ***E. faecalis* MJS257**, all the extracts of **stems** were able to **inhibit the growth** of those bacteria that proved to be **resistant** to the **tested antibiotic**. Extracts of cv. ***Lapins*** exhibited the strongest growth suppression for the Gram positive *S. aureus* MJS241 and *E. faecalis* MJS257.

For the tested **Gram negative** bacteria, **no inhibitory effect** was found, although extracts of cvs. *Early Bigi* C and NC and *Lapins* potentiated the antimicrobial effect of when used in combination (Table 3).

Conclusion

This work shows that stems, **sweet cherry by-products,** have very **interesting bioactive properties**, regarding to the content in chemical composition, phenolic profile, antioxidant activity and antimicrobial activities. For the **first time**, **saponins**, known by their pharmacological properties, were **quantified in stems** of sweet cherry. The most **promising extracts** were those from cv. ***Lapins***, as they had overall highest bioactive content, saponins and antioxidant activity and they presented increased % RIZD, probably linked to the higher amount of neochlorogenic acid, known for its recognized antibacterial activity.

These by-products should be **further explored** and **more studies are required**, in order to explore **nutraceutical and pharmacological formulations** or **antioxidant preservatives for the food industry** and their effects on human health. Sweet cherry **stem's extracts** may provide **valuable solutions** to the global problem of **antibiotic resistance** due to their **antimicrobial activity**, however more research should be performed to test its applicability.

Acknowledgements

Sílvia Afonso is grateful to FCT, MCTES and FSE for the PhD Fellowship SFRH/BD/139922/2018. Work supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020. Work funded by "Fundo Europeu Agrícola de Desenvolvimento Rural (FEADER)" and by "Estado Português" in the context of "Ação 1.1 «Grupos Operacionais»", integrated in "Medida 1. «Inovação» do PDR 2020 – Programa de Desenvolvimento Rural do Continente"- Grupo Operacional para a valorização da produção da Cereja de Resende e posicionamento da subfileira nos mercados (iniciativa n. º 362).