COST STSM Scientific Report- Adriana Guerreiro

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HPLC analyses for quality evaluation of Arbutus unedo L. fruits

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Host:	**Prof. Dr. Terry Leon**, Cranfield University (UK) *l.a.terry@cranfield.ac.uk*

1. Background and Purpose

Fresh fruits and vegetable consumption has increased in the last years due to more awareness of consumers for healthy food. However, those products are highly perishable, and losses can be of great importance if postharvest correct measures are not provided. Low temperatures in combination with other factors such as edible coatings (EC) have the objective to reduce metabolic processes, delaying senescence. EC are made of polysaccharide/lipid bilayer, proteins and fatty acids and are suitable for increasing fresh fruit and vegetable storage life and be carriers of many functional ingredients, such as essential oils and/or their constituents which have antimicrobial and antioxidant activities.

The strawberry tree (Arbutus unedo L.) belongs to the family Ericacea and is native to the Mediterranean region. Its plant parts have been used in traditional medicine, namely the leaves, berries, bark and roots. There is also the economic and ecological interest of this plant, since it re-growth easier than other species after forest fire. Its fruits are conspicuous, globular, orange-red, rough, up to 2 cm in diameter, are tasty when fully ripe and are normally used in the production of an alcoholic distillate, a very aromatic and appreciated drink (containing40–60% alcohol/volume).

A. unedo fruits are very good dietary source of antioxidants, including phenolics compounds (e.g. anthocyanins and other flavonoids, gallic acid derivatives and tannins), vitamins C and E, and carotenoids. Nevertheless, the consumption as fresh fruit is the great concern of producers and supermarkets because of its rapid degradation.

Recently it has been growing the interest in cultivating *A. unedo* in the forest fields as a mean to obtain other, non-wood products from forests.

The objective of this Short Term Scientific Mission (STSM) is to have a period of training in a recognized scientific laboratory, to gain knowledge about the HPLC analyses on Cranfield University, under the supervision of Professor Leon Terry.

2. Description of the work carried out during the visit

The fruits were harvested in the mountain “Caldeirão”, in Algarve Region, Portugal, in mid-November, when they were ripe, and immediately transported to the postharvest laboratory at the University of Algarve, where they were selected for uniformity of size and freedom from defects, for the experiments.

Food grade Sodium alginate (AL) (Sigma-Aldrich Chemic, Steinhein, Germany) was the biopolymer used for coating formulations. Calcium chloride (Sigma-Aldrich Chemic, Steinhein, Germany) was used to induce cross linking reaction and ascorbic acid (AA) (Scharlau, Barcelona, Spain) was added as anti-browning agent.

Citral and eugenol were purchased from Sigma-Aldrich Chemic, Steinhein, Germany and strawberry tree honey from a local producers association.

Edible Coatings

The coating forming solutions based on sodium alginate were formulated as described by Rojas-Graü, Raybaudi-Massilia, Soliva-Fortuny, Avena-

Bustillos, McHugh, & Martín-Belloso (2007). AA 1% was added as anti-browning agent in the edible coating solutions according to previous work (Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martín-Belloso, 2009) .

Three different formulations of edible coatings were prepared. The treatments were: Control; AL 1% + Eugenol 0. 2%; AL 1% + Citral 0.15% + Eugenol 0.1%.

Each treatment was performed in two steps: first arbutus berries were dipped into the edible coating solution + AA for 2 min; the excess of coating material was allowed to drip off for 30 sec before the second dip in the calcium chloride solution for 1 min. Then, 10 fruits were placed in polypropylene plastic trays (8 cm x 10 cm x 4 cm), perforated in the cover, and stored at 0.5 ºC ± 1 ºC until analysis. On days 0, 14 and 28, three trays per treatment were taken to perform the analyses.

Fruit samples for sugars and organic acids measurements were freeze dried and stored at -80ºC till transport to Cranfield University.

Extraction and quantification of sugars

Extraction and quantification of sugars was based on a method described by Terry et al., 2007 and modified as described in Magwaza et al., 2012. Briefly, a 150 ± 0.5 mg of fruit powder was extracted in 3 mL 62.5% (v/v) aqueous methanol. Following extraction, the concentrations of fructose, glucose and sucrose were determined in an HPLC binary pump system (1200 series, Agilent Technologies, UK). Twenty micro litres (20 µL) of a diluted sample

solution (1:10) was injected into a Rezex RCM monosaccharide Ca+(8%) column of 7.8 mm diameter × 300 mm (Phenomenex, Torrance, CA, USA) with a Carbo-Ca2+guard column of 3 mm × 4 mm (Phenomenex). The thermostated column compartment (G1316A,Agilent) temperature was set at 80◦C. The mobile phase used was HPLC-grade water at a flow rate of 0.6 mL/min and the presence of carbohydrates was detected on a refractive index detector (RID,G1362A, Agilent Technologies). Sugars were quantified from a linear standard curve (0.05–1.25 mg/mL; average R^2= 0.99).

Extraction and quantification of non-volatile organic acids

Non-volatile organic acids were extracted and determined using a method described by Crespo et al., 2010 with slight modifications by Magwaza et al., 2013. Briefly, 50 ± 0.5 mg of freeze dried samples were cold extracted for 5 min in 3 mL of HPLC water. The flocculate was filtered through a 0.2 m syringe filter before HPLC analysis. Citric, ascorbic, malic, tartaric and oxalic acid concentrations were determined on a HPLC binary pump system equipped with a diode array detector (DAD) with multiple wavelength detector, degasser and cooled autosampler. The filtered sample extract (20 L) was injected into a Prevail organic acid column (4.6 mm diameter × 250 mm, 5 m particle size; Alltech, UK) with an organic acid guard column (part no. 96429, Alltech). Temperature of the column was set to 35ºC using a thermostated column compartment (G1316A, Agilent). The mobile phase used was 0.2% HPLC-grade aqueous metaphosphoric acid at a flow rate of 1.0 mL/min. Non-volatile organic acids were detected at 210 nm except for ascorbic acid which was

detected at 245 nm and quantified using linear standard curves(0.01–1.25 mg/mL; average R^2= 0.99).

3. Description Of The Main Results Obtained

All analyses proposed in the STSM were performed. Arbutus berries sugars and organic acids are present in Tables 1 and 2. In the case of organic acids citric and tartaric acid, measurements have to be repeated to confirm their presence according to standards retention time.

Analysis of the obtained results and the writing of a paper is still in progress.

All basic techniques that were proposed in the STSM to learn at Cranfield University were acquired and will be applied in the future research in Portugal. Those are basic knowledge very important for the PhD research studies of Adriana Guerreiro.

Table 1. Organic acids (mg/100g DW) of arbutus berries

	Days	Control				Alginato 1% + Eugenol 0.2%				Alginato 1% + Citral 0.15% + Eugenol .0.1%			
		Mean		SD		Mean		SD		Mean		SD	
Oxalic	0	82,96	±	24,14	bA	129,36	±	13,44	aA	140,07	±	12,97	aA
	14	178,90	±	12,18	aA	156,49	±	12,30	aA	167,13	±	11,26	aA
	21	148,96	±	13,87	aA	154,95	±	7,14	aA	132,43	±	6,82	aB
	28	158,63	±	15,19	aA	123,10	±	22,42	aB	140,87	±	28,75	aAB
Malic	0	1750,02	±	381,57	aA	1499,77	±	118,50	aA	1539,81	±	53,44	aA
	14	1296,80	±	135,68	aA	1478,79	±	62,29	aA	1293,60	±	44,12	aA
	21	1592,10	±	80,97	aA	1934,90	±	496,10	aA	1244,61	±	138,20	aA
	28	1830,47	±	600,24	aA	1158,75	±	252,91	aA	1592,80	±	320,40	aA
Ascorbic	0	560,21	±	83,23	aA	701,55	±	58,39	aA	663,36	±	62,81	aA
	14	559,10	±	50,19	aA	616,97	±	55,18	aA	660,14	±	25,77	aA
	21	637,91	±	39,27	aA	684,47	±	56,62	aA	596,23	±	38,67	aA
	28	422,01	±	143,16	aA	620,57	±	87,70	aA	681,19	±	117,05	aA

Table 2. Fructose, Glucose and Sucrose (mg/g DW) of arbutus berries

		Control				Alginato 1% + Eugenol 0.2%				Alginato 1% + Citral 0.15% + Eugenol .0.1%			
	Days	Mean	±	SD		Mean	±	SD		Mean	±	SD	
Fructose	0	59,17	±	19,34	bB	219,11	±	25,52	aA	186,79	±	5,11	aA
	14	267,25	±	82,89	aA	177,02	±	20,61	aAB	107,80	±	48,13	aB
	21	180,14	±	29,05	abA	178,98	±	25,44	aA	172,44	±	19,19	aA
	28	210,27	±	61,51	abA	201,05	±	12,86	aA	164,70	±	56,96	aA
Glucose	0	16,42	±	13,07	bB	80,46	±	1,99	aA	66,86	±	7,25	aA
	14	105,41	±	47,21	aA	69,40	±	7,51	aA	31,59	±	15,09	aB
	21	77,81	±	18,00	aA	78,85	±	10,91	aA	56,13	±	6,79	aA
	28	96,07	±	23,85	aA	89,54	±	6,26	aA	65,03	±	20,92	aA
Sucrose	0	12,87	±	12,87	aA	15,75	±	8,37	aA	14,89	±	3,86	aA
	14	13,11	±	6,71	aA	8,69	±	2,15	aA	3,08	±	3,08	bA
	21	11,77	±	5,36	aA	11,75	±	3,11	aA	5,92	±	0,99	abA
	28	13,26	±	6,15	aA	0,81	±	0,45	aB	8,42	±	4,99	abA

4. Outcomes

Gained results are excellent bases for future research on the whitefly topic in Portugal and good opportunity for Adriana Guerreiro to learn new HPLC and extraction techniques and to get new knowledge and experience from Prof. Dr Terry Leon working group.

In addition, our results will be object of international contributions to conferences, as well as publications in peer-reviewed journals.

5. References

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