



PROFILING OF DIFFERENT PHENOLIC COMPOUNDS IN *Myrciaria cauliflora* FRUIT BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Iker Chasco-Llorente, Charlotta Turner, Merichel Plaza
Lund University, Department of Chemistry, Centre for Analysis and Synthesis, P.O. Box 124, SE-22100, Lund, Sweden.

Merichel.Plaza@organic.lu.se

INTRODUCTION



Myrciaria cauliflora commonly called Jabuticaba (Brazilian fruit)

The skin present high content of anthocyanins

Anthocyanins promote several health benefits: reduction in the risk of cardiovascular diseases, diabetes and cancer; a protective effect against hepatic and gastric damage and collagen degradation; and an increase in cognitive performance.



Lyophilized peel of the Brazilian fruit Jabuticaba

OBJECTIVE to develop a HPLC method (using diode array detection) for the simultaneous separation, detection and quantification of different phenolic compound groups like phenols, flavonoids and anthocyanins from *Myrciaria cauliflora* in a single run.

HPLC CONDITIONS

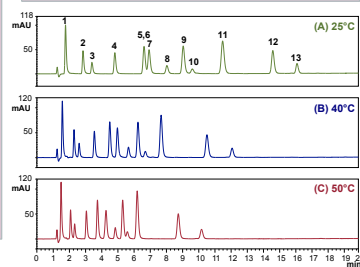
A Dionex Ultimate 3000 system was used (Dionex Corp.) equipped with a Ultimate 3000 photodiode array detector.
Column: Zorbax SB-C18 3.5 μm (100 x 2.1 mm i.d.) from Agilent Technologies
Column temperature: 20 / 40 / 50 $^{\circ}\text{C}$
Flow: 0.2 / 0.3 / 0.4 ml/min
Sampler temperature: 4 $^{\circ}\text{C}$
Injection volume: 5 μl
Mobile phases:
• (A) Milli-Q water (5% formic acid); (B) Methanol (5% formic acid)
Gradient:

t (min)	%B
0	15
8	20
40	70
42	15

Peak assignment:

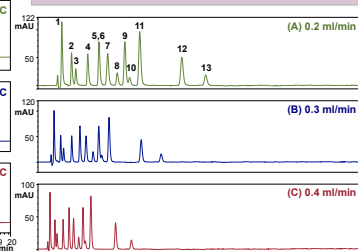
Phenolic acids: 1, gallic acid; 2, protocatechuic acid; 3, 3,4-dihydroxyphenylacetic acid; 4, *p*-hydroxybenzoic acid; 5, syringic acid; 6, vanillic acid; 7, 3-hydroxyphenylacetic acid; 8, caffeic acid; 9, homovanillic acid; 10, *m*-hydroxyphenylpropionic acid; 11, *p*-coumaric acid; 12, ferulic acid; 13, sinapinic acid.
Flavonols: 14, quercetin-3,4'-diglucoside; 15, ellagic acid; 16, hyperoside; 17, resveratrol; 18, isoquercitrin; 19, rutin; 20, morin; 21, spiraeoside; 22, quercitrin; 23, kaempferol-7-O-neohesperoside; 24, quercetin; 25, kaempferol.
Anthocyanins: 26, myrtillin; 27, kuromanin; 28, cyanidin; 29, pelargonidin.

Column temperature: 25 / 40 / 50 $^{\circ}\text{C}$



HPLC-DAD chromatograms (280 nm) at different temperatures (A) 25, (B) 40 and (C) 50 $^{\circ}\text{C}$.

Temperature 50 $^{\circ}\text{C}$ and different flows: 0.2 / 0.3 / 0.4 ml/min

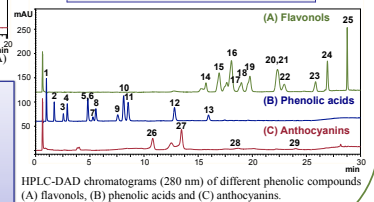


HPLC-DAD chromatograms (280 nm) at 50 $^{\circ}\text{C}$ with different flow (A) 0.2, (B) 0.3 and (C) 0.4 ml/min.

Optimized method:

Temperature: 50 $^{\circ}\text{C}$
Flow: 0.4 ml/min
Mobile phases:
• (A) Milli-Q water (5% formic acid); (B) Methanol (5% formic acid)
Gradient:

t (min)	%B
0	1
17	16.3
24	20
30	80
32	100



HPLC-DAD chromatograms (280 nm) of different phenolic compounds (A) flavonols, (B) phenolic acids and (C) anthocyanins.

Advantages of using HIGHER temperature in HPLC are:

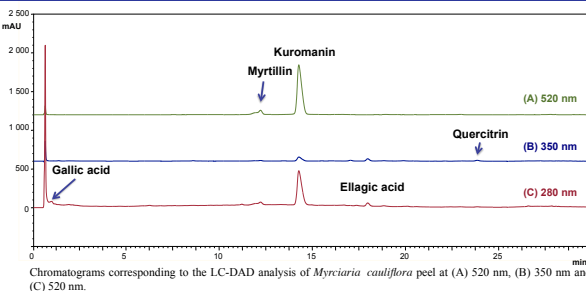
- Higher speed.
- At high temperature flow rate can be increased to reduce analysis time without exceeding the pressure limits of the system.
- Improvements in resolution and higher sensitivity, as it enhances peak height and peak shape.
- Lower consumption of organic solvents.

ANALYSIS OF PHENOLIC COMPOUNDS IN THE FRUIT

Extraction from lyophilized peel powder capsules:
2 g of peel powder was extracted in darkness for 48 h at 4 $^{\circ}\text{C}$ with 30 mL of Milli-Q water : ethanol : formic acid (94:5:1 v/v/v) mixture.

Quantification of the phenolic compounds in *Myrciaria cauliflora* peel extract. Concentration indicated as mg/g of solid lyophilized peel powder \pm sd. Values are the mean of three replicates.

Compound	Concentration \pm sd
Gallic acid	0.630 \pm 0.240
Myrtillin	0.500 \pm 0.060
Kuromanin	1.700 \pm 0.090
Ellagic acid	0.540 \pm 0.001
Quercitrin	0.130 \pm 0.010



Chromatograms corresponding to the LC-DAD analysis of *Myrciaria cauliflora* peel at (A) 520 nm, (B) 350 nm and (C) 280 nm.

The purple peel of *Myrciaria cauliflora* had high amount of kuromanin followed by gallic acid, ellagic acid and myrtillin. Quercitrin was the phenolic found in the lowest concentration. This edible fruit is a rich source of biologically active phenolic compounds, similar to other well studied berries and fruits.

CONCLUSIONS

- ⚡ The results obtained for the simultaneous determination of different kind of phenolic compounds (phenolic acids, anthocyanins and flavonols) using higher temperature in HPLC-DAD confirm that the proposed method can be successfully applied to routine analysis of these kind of compounds.
- ⚡ *M. cauliflora* peel capsules presented high content in anthocyanins and others phenolics comparable to berries and other fruits.

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