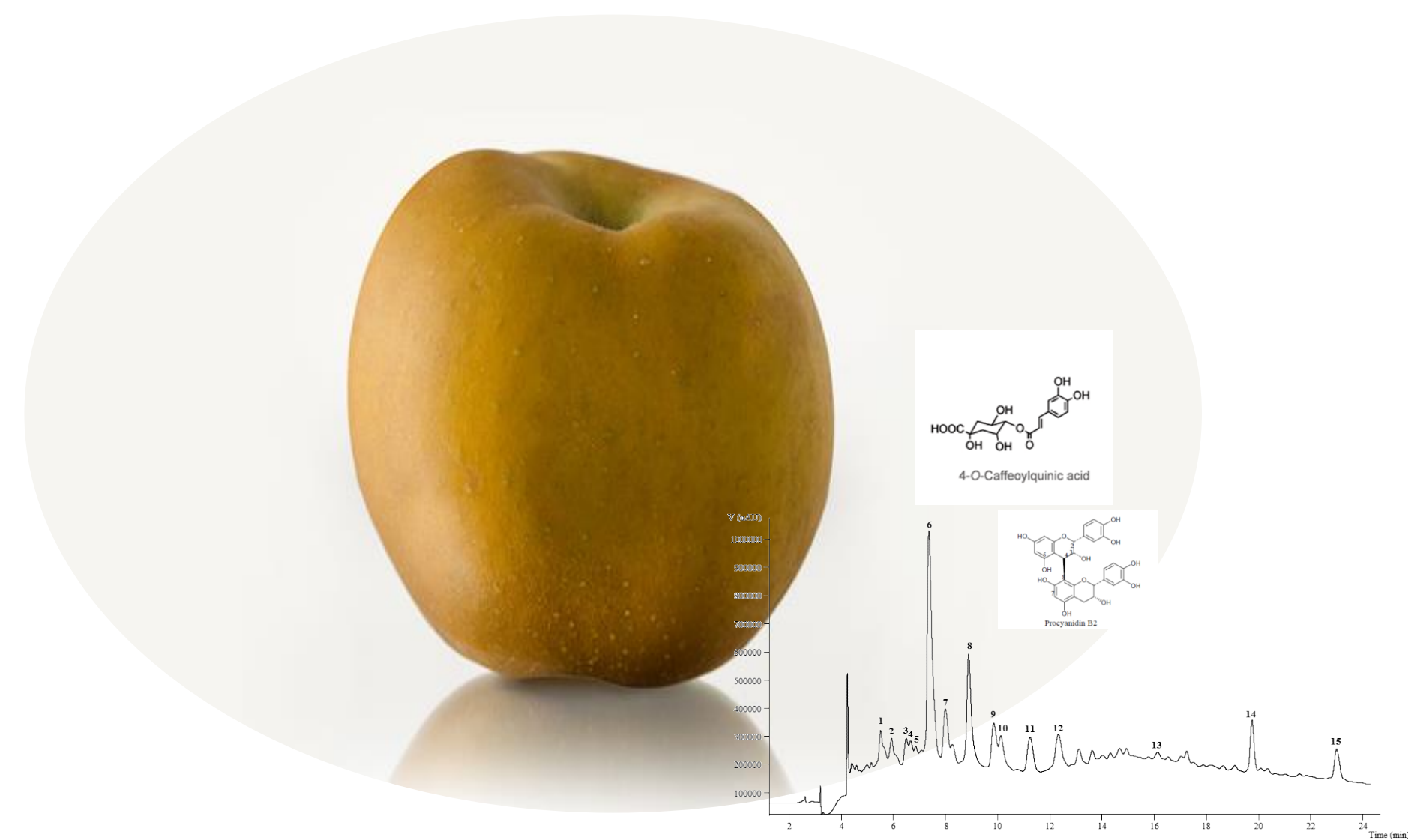


# Phenolic profile and bioactivities of the Portuguese apple of the variety "Bravo de Esmolfe"

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## Introduction

*Malus domestica* Borkh apples are one of the most consumed fruits in the world, due to their sweetness and flavour. This apple is recognised as a product with Protected Designation of Origin (PDO). Its production is carried out in a restricted and small inland region in northern Portugal [1]. Apple fruits have a wide variety and well-balanced composition, being moderately energetic and well-proportioned in sugar and acid contents, giving it a pleasant taste [2].

The regular consumption of fruits and vegetables has been associated with reduced risk of developing chronic diseases [3]. These benefits are often attributed to their high phytochemical content and antioxidant power [4]. The aim of the present work, was to characterize the phenolic compounds of *Malus domestica* Borkh cv 'Bravo de Esmolfe' and study the bioactive properties in terms of antioxidant and antibacterial activities.

## Methodology

**Sample:** *Malus domestica* Borkh. cv 'Bravo de Esmolfe' were kindly supplied by the RBR foods company from Castro Daire (Portugal), in the dry form and without skin [4].

**Extraction:** Methanol/water (80:20, v/v) extracts were prepared by maceration.

**Phenolic compounds:** Determined by HPLC-DAD-ESI/MS, Identification was performed by the fragmentation profile and comparison of the information obtained with data from the literature and standard compounds.

**Antioxidant activity:** Evaluated through DPPH radical-scavenging, reducing power, inhibition of  $\beta$ -carotene bleaching and TBARS inhibition assays.

**Antibacterial activity:** Evaluated using the microdilution method and the rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay to determine minimum inhibitory concentration (MIC).

## Results

The fifteen phenolic compounds identified were described in **Table 1**. The most abundant compounds were 5-*O*-caffeoylquinic acid (52 mg/100 g dry weight), followed by procyanidin B2 (35 mg/100 g dry weight).

The methanol/water (80:20, v/v) extracts prepared from 'Bravo de Esmolfe' apple demonstrated antioxidant activity through the free radical scavenging activity and inhibition of lipid peroxidation (**Table 2**).

The antibacterial effects (**Table 3**) was more effective against Gram-positive (all the bacterial strains tested) than Gram-negative bacteria, where it was only efficient for *Escherichia coli* and *Morganella morganii*.

Table 1. Phenolic compounds presented in dry apple variety "Bravo de Esmolfe".

| Peak                     | Rt (min) | $\lambda_{max}$ (nm) | [M-H] <sup>-</sup> (m/z) | MS <sup>2</sup> (m/z)  | Tentative identification                                   | Quantification (mg/100 g dw) |
|--------------------------|----------|----------------------|--------------------------|--|--|------------------------------|
| 1                        | 5.56     | 281                  | 577                      | 451(24),425(100),407(21),289(12)                                       | Procyanidin B1   | 11.38 ± 0.04                 |
| 2                        | 5.98     | 271                  | 373                      | 327(17),165(100),121(12),93(14)  | Unknown  | -                            |
| 3                        | 6.54     | 280                  | 865                      | 739(74),713(44),695(100),577(64),575(37),425(10),407(9),289(8),287(7)  | B-type epicatechin trimer <sup>1</sup>                     | 11.3 ± 0.1                   |
| 4                        | 6.7      | 281                  | 865                      | 739(69),713(43),695(100),577(68),575(36),425(11),407(7),289(6),287(8)  | B-type epicatechin trimer <sup>1</sup>                     | 9.6 ± 0.2                    |
| 5                        | 6.96     | 322                  | 353                      | 191(12),179(11),173(100),161(11),135(2)                                | 4- <i>O</i> -Caffeoylquinic acid <sup>2</sup>              | 5.8 ± 0.1                    |
| 6                        | 7.4      | 327                  | 353                      | 191(100),179(6),173(2),161(11),135(1) <sup>2</sup>                     | 5- <i>O</i> -Caffeoylquinic acid <sup>2</sup>              | 51.5 ± 0.5                   |
| 7                        | 8.09     | 280                  | 577                      | 451(17),425(100),407(19),289(7)  | Procyanidin B2   | 34.5 ± 0.3                   |
| 8                        | 8.95     | 311                  | 337                      | 191(3),173(95),163(8),145(4),119(3)                                    | 4- <i>p</i> -Coumaroylquinic acid <sup>3</sup>             | 14.1 ± 0.6                   |
| 9                        | 9.96     | 281                  | 289                      | 245(100),203(5),187(1),161(2),137(2)                                   | Epicatechin <sup>1</sup>                                   | 18.2 ± 0.4                   |
| 10                       | 10.19    | 312                  | 337                      | 191(2),173(100),163(7),119(2)  | 5- <i>p</i> -Coumaroylquinic acid <sup>3</sup>             | 6.93 ± 0.04                  |
| 11                       | 11.34    | 280                  | 865                      | 739(83),713(53),695(100),577(82),575(43),425(14),407(9),289(8),287(12) | Procyanidin C1   | 19.56 ± 0.01                 |
| 12                       | 12.42    | 280                  | 1153                     | 865(19),863(18),577(6),575(11),289(3),287(4)                           | (C4.C8)-epicatechin tetramer <sup>1</sup>                  | 24.77 ± 0.01                 |
| 13                       | 16.2     | 280                  | 579                      | 289(56),245(100),203(9)  | Unknown biflavonoid <sup>1</sup>                           | 16.7 ± 0.4                   |
| 14                       | 19.85    | 285                  | 567                      | 273(100),167(5),123(5)   | Phloretin-2'- <i>O</i> -xyloglucoside <sup>4</sup>         | 7.87 ± 0.02                  |
| 15                       | 23.09    | 285                  | 435                      | 273(100),167(4),123(5)   | Phlorizin (phloretin-2'- <i>O</i> -glucoside) <sup>4</sup> | 4.84 ± 0.01                  |
| Total phenolic compounds |          |                      |                          |  |  | 257 ± 1                      |

Table 2. Antioxidant activity in dry apple variety "Bravo de Esmolfe".

| Antioxidant activity                   | EC <sub>50</sub> values (mg/mL) |
|--|---------------------------------|
| DPPH scavenging activity               | 0.71 ± 0.05                     |
| Reducing power                         | 1.38 ± 0.01                     |
| $\beta$ -carotene bleaching inhibition | 7.19 ± 0.04                     |
| TBARS inhibition                       | 0.45 ± 0.005                    |

Table 3. Antibacterial activity in dry apple variety "Bravo de Esmolfe".

| Gram-negative bacteria            | MIC (mg/mL) |
|-----------------------------------|-------------|
| <i>Acinetobacter baumannii</i>    | >20         |
| <i>Escherichia coli</i>           | 5           |
| <i>Escherichia coli</i> ESBL      | 5           |
| <i>Klebsiella pneumoniae</i>      | >20         |
| <i>Klebsiella pneumoniae</i> ESBL | >20         |
| <i>Morganella morganii</i>        | 5           |
| <i>Pseudomonas aeruginosa</i>     | >20         |
| Gram-positive bacteria            |             |
| <i>Enterococcus faecalis</i>      | 5           |
| <i>Listeria monocytogenes</i>     | 5           |
| MRSA                              | 5           |
| MSSA                              | 2.5         |

## Conclusion

These results show that 'Bravo de Esmolfe' dried apples could be used as snack products with equilibrated nutritional value and could be also useful to prepare nutraceutical formulations with potential antioxidant and antibacterial properties.

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