Physicochemical characterization of five Brassica vegetable species and their plant tissues

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I. Introduction

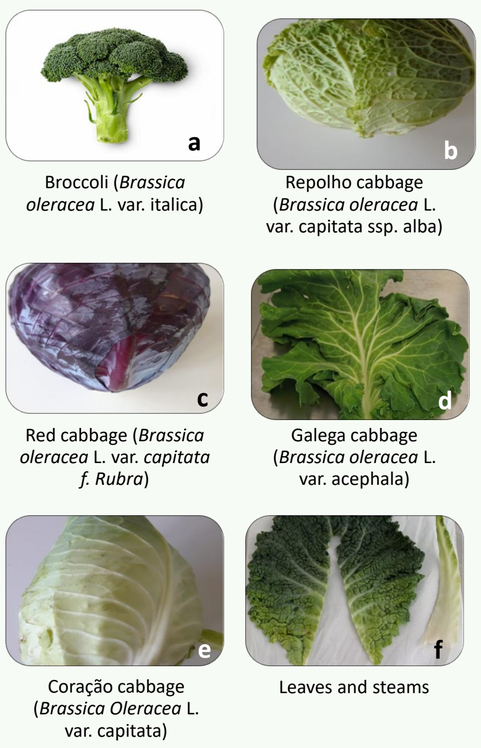
Among vegetable crops, the numerous Brassicaceae family (formerly Cruciferae) holds great socioeconomic relevance in Portugal, showing large genetic variability and botanical diversity, contributing to its enormous versatility regarding commercial presentation and culinary preparation. In recent years, a rising tendency in brassicas crop production and consumption is observed. The growing trend is supported by Brassica vegetables excellent nutritional traits, including vitamins, minerals and fiber, and by its classification as functional foods, as Brassicaceae plants are a rich source of antioxidants, e.g., glucosinolates and polyphenols (plants secondary metabolites), which biological activities actively contribute to improving human health as identified in several publications [1,2].

II. Objective

The aims of this study were:

- The physical-chemical, bioactive and antioxidant characterization of five cultivated brassica species with the highest consumption representativeness in Portugal;
- The characterization of different plant tissues (leaf and stem) and of mixtures of leaves and stems (50:50) of each species.

III. Material & Methods



Broccoli (*Brassica oleracea* L. var. italica)
Repolho cabbage (*Brassica oleracea* L. var. capitata ssp. alba)
Red cabbage (*Brassica oleracea* L. var. *capitata f. Rubra*)
Galega cabbage (*Brassica oleracea* L. var. acephala)
Coração cabbage (*Brassica Oleracea* L. var. capitata)
Leaves and steams

Figure 1. Analyzed samples of (a) Broccoli; (b) Repolho cabbage; (c) Red cabbage; (d) Galega cabbage; (e) Coração cabbage and (f) leaves and stems

The characterization included:

- Determination of pH (Crison, micro pH 2001), titratable acidity (TA; g lactic acid / 100 g DW), moisture (%);
- Determination of total phenolic compounds (TPC, mg GAE / 100 g DW; Folin-Ciocalteu method) and phenolic profile (HPLC-DAD);
- Determination of antioxidants activity by ferric reducing antioxidant power assay (FRAP) and free radical scavenging (DPPH radical) methods (µmol TE /100 g DW).;
- Independent triplicates for each vegetable sample.

Statistical evaluation:

- The results were subjected to analysis of variance (ANOVA) using the Statistic v software. 8.0. Tukey's HSD test was used to assess the existence of significant differences ($P < 0.05$) between samples.

HPLC conditions (adapted from [3]):

- HPLC system: Alliance System (Waters 2690 Separations Module, Milford, MA, USA) equipped with a photodiode array (PDA) detector (Waters 996, Milford, MA, USA);
- Column: Spherisorb ODS2 C18, 5 µm (4.6 mm x 250 mm) (MZ Analysentechnik, Mainz, Germany) operated at room temperature;
- Injection volume: 20 µL;
- Mobile phases: (A) 0.1% formic acid in water; (B) acetonitrile;
- Elution gradient: 0/10, 5/15, 10/25, 20/35, 25/50, 35/50, 40/10, 50/10 (min/% solvent B)
- Flow rate: 0.5 mL.min-1;
- Commercial standards of gallic, chlorogenic, p-coumaric, sinapic, t-cinnamic, ferulic, and p-hydroxybenzoic acids, rutin, quercetin, quercetin-3-b-D-glucoside and cyanidin-3,5-di-O-glucoside were used for peak identification, when possible, by comparing respective retention times and UV–VIS spectra (190-600 nm).

IV. Results

Figure 2 (a, b and c) presents the evaluation of the **pH, titratable acidity and moisture**, respectively. As can be observed, pH was lower for Repolho cabbage's leaves and higher ($p > 0,05$) for broccoli´s steams. Acidity is concededly higher in leaves than in steams in all the vegetables. Coração cabbage presented the highest value of moisture (94%) ($p > 0,05$) in the different parts of the vegetables analyzed.

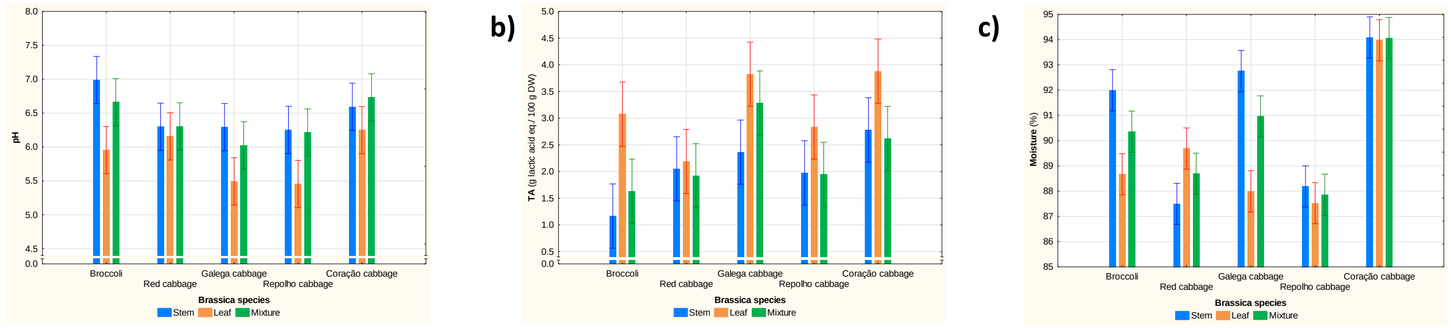


Figure 2. Evaluation of pH (a), TA (b) and moisture (c) of the different samples of Broccoli, Red cabbage, Galega cabbage, Repolho cabbage and Coração cabbage.

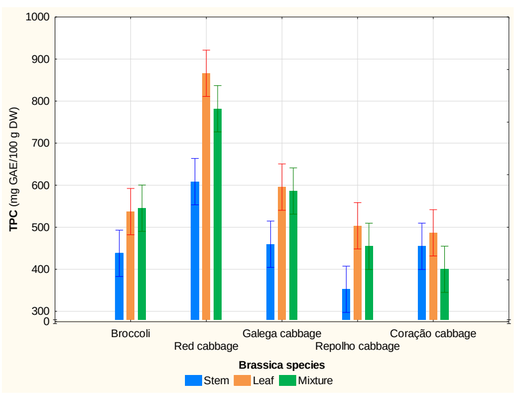


Figure 3 presents the evaluation of **total phenolic content (TPC)** in the different five brassicas analyzed. The TPC of the various vegetable extracts varied between 353 ± 5 to 608 ± 107 mg GAE / 100 g DW in stems extracts and 487 ± 40 to 866 ± 60 mg GAE / 100 g DW in leaf extracts, with the Red cabbage exhibiting the highest values. As expected, all the leaves presented significantly higher ($p < 0.05$) values than the stems.

Figure 3. Total phenolic content of Broccoli, Red cabbage, Galega cabbage, Repolho cabbage and Coração cabbage.

Figure 4 (a and b) shows the determination of **antioxidants activity** by ferric reducing antioxidant power assay (FRAP) and free radical scavenging (DPPH radical) methods (µmol TE /100 g DW), respectively, for all the vegetables species analyzed.

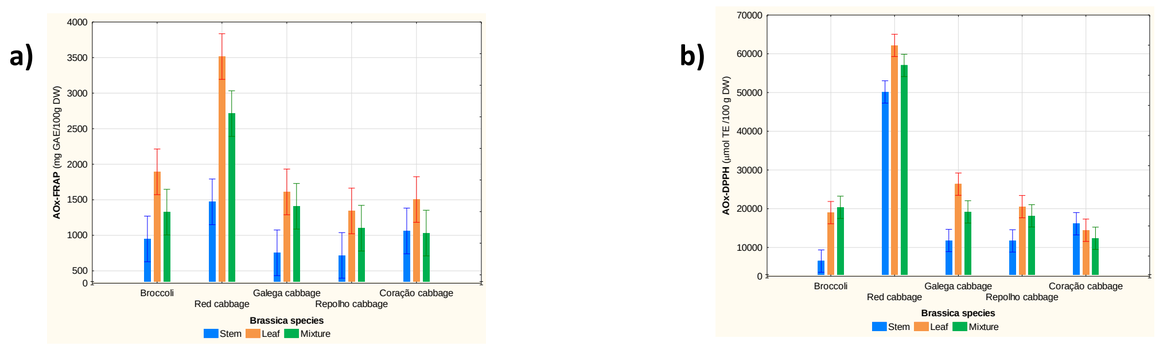


Figure 4. Determination of antioxidants activity by ferric reducing antioxidant power assay (FRAP) (a) and free radical scavenging (DPPH radical) (b) methods (µmol TE /100 g DW) for all analyzed vegetables.

All the vegetables extracts were capable of directly reacting with and quenching DPPH radicals. The extracts of Red Cabbage were found to have the highest DPPH radical scavenging capacity, with values of 50152 and 62167 µmol TE /100 g DW in stems and leaves, respectively. The order of antioxidant activities depended on the type of reactive species in the reaction mixture. A correlation analysis was carried out between phenolic compounds and antioxidant capacity (data not shown). Unexpectedly, in broccoli, the correlation only exists between the TPC and DPPH antioxidant capacity.

Figure 5 presents the **HPLC chromatograms** of the phenolic extracts of (a) Broccoli, (b) Red cabbage, (c) Galega cabbage, (d) Coração cabbage and (e) Repolho cabbage recorded at 280 nm and 340 nm.

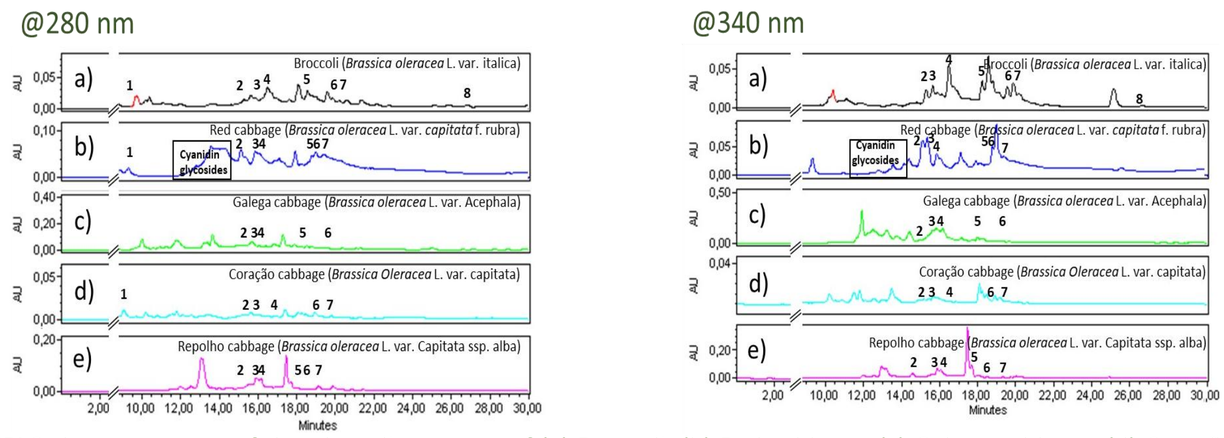


Figure 5. HPLC chromatograms of the phenolic extracts of (a) Broccoli, (b) Red cabbage, (c) Galega cabbage, (d) Coração cabbage and (e) Repolho cabbage recorded at 280 nm and 340 nm. Peak identification: 1 – Gallic acid; 2 – Chlorogenic acid; 3 – Rutin; 4 - quercetin-3-b-D-glucoside; 5 – p-coumaric acid; 6 – Sinapic acid; 7 – Ferulic acid; 8 – (±)-Naringenin.

The main difference between edible parts of the 5 evaluated brassica species is the presence of cyanidin glycosides in red cabbage (highlighted box), most probably acylated with various cinnamic acid derivatives. Moreover, (±)-Naringenin (flavanone) was only identified in broccoli florets. Gallic acid (trihydroxybenzoic acid; peak #1) was only detected/identified in 3 of the 5 brassica species (Broccoli, Red and Coração cabbages). The flavonols rutin (peak #3) and quercetin-3-b-D-glucoside (peak #4) were always found in evaluated brassica species, and it is expected for their contents to differ. Also, several unidentified flavonols glycosides are present, suspect of quercetin and kaempferol glucosides ($15 < t_R < 23$ min). Common identified hydroxycinnamic acids were chlorogenic (peak #2), p-coumaric (peak #5), sinapic (peak #6) and ferulic (peak #7) acids. Exceptions are found in Galega and Coração cabbages, where, respectively, ferulic acid and p-coumaric acid were not detected. Other unidentified hydroxycinnamic acid derivatives are also present, as suggested by the obtained chromatograms at 340 nm, particularly in Galega and Coração cabbages.

V. Conclusions

The comprehensive characterization of the different brassicas and tissues chemical profile revealed important and unexpected results. Red cabbage stands out for its high antioxidant content and phenolic content. However, the distinction between tissues (leaf and stem) is dependent on the type of brassica. The retrieved data allows identifying differential uses for the evaluated brassicas as nutraceuticals for possible incorporation in the human diet as natural food supplements and possible use of their residues, as extraction matrixes for bioactive recovery.

VI. References

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