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Phenolic Content and Antioxidant Activity Determination in Broccoli and Lamb's Lettuce

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Abstract**—**Broccoli has been widely recognized as a wealthy vegetable which contains multiple nutrients with potent anti-cancer properties. Lamb's lettuce has been used as food for many centuries but only recently became commercially available and literature is therefore exiguous concerning these vegetables. The aim of this work was to evaluate the influence of the extraction conditions on the yield of phenolic compounds and the corresponding antioxidant capacity of broccoli and lamb's lettuce. The results indicate that lamb's lettuce, compared to broccoli, contains simultaneously a large amount of total polyphenols as well as high antioxidant activity. It is clearly demonstrated that extraction solvent significantly influences the antioxidant activity. Methanol is the solvent that can globally maximize the antioxidant extraction yield. The results presented herein prove lamb's lettuce as a very interesting source of polyphenols, and thus a potential health-promoting food.

Keywords**—**Broccoli, lamb's lettuce, extraction, antioxidant activity, phenolic compounds.

I. Introduction

RECENT studies have demonstrated that the regular consumption of vegetables may reduce the risk of developing chronic diseases, such as cancer, cardiovascular disease, and Type 2 diabetes [1].

Several studies show that *Brassicaceae* species contain very complex flavonoids that occur as complex conjugates, with one to five sugar moieties bound to the aglycone often acylated with hydroxycinnamic acids [2].

Broccoli (*Brassica oleracea* L.) is a good source of health promoting compounds since it contains glucosinolates, flavonoids, hydroxycinnamic acids, vitamin A, C, E, K, folic acid, riboflavin (B_2), iron, calcium, and potassium [3].

Lamb's lettuce (*Valerianella locusta* L.,) a member of the family *Valerianaceae*, also known as corn salad is an annual plant commonly used in salad in France and Germany. It has also been used as food in Britain for many centuries but only became commercially available there in the 1980s.

Literature is very exiguous about these kinds of vegetables, although they are mentioned as a highlight of winter vegetable garden. These vegetables are an important source of vitamin C, B6, B9, E, beta-carotene and omega-3 fatty acids. Grzegorzewski and collaborators [4] worked with this matrix to study the influence of a low-pressure oxygen glow discharge plasma on secondary metabolites. Although their target was not the study of phenolic composition, they reported that the main phenolic compounds present in lamb's lettuce were diosmetin, luteolin, chlorogenic and caffeic acids.

There are many methods available for the measurement of *in vitro* antioxidant capacity. However no single method exists to test the total antioxidant capacity of foods since the antioxidant activity depends on the extraction method as well as on the type of the reactive species in the reaction mixture [5]. This work aimed to study the influence of different extraction conditions on the antioxidant capacity in broccoli and lamb's lettuce.

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II. Experimental Procedure

A. Samples

Broccoli and lamb's lettuce samples were purchased in local markets in Porto. Broccolis were acquired fresh and lamb's lettuces were acquired in a packed form. Afterwards, all the extractions were carried out, in order to prevent degradation of the samples and to ensure that the extraction conditions were the same in all assays. 125g of lamb's lettuce and 120g of broccoli were used for consequent extractions.

B. Extraction Procedure

All the extractions were carried out in two consecutive days in order to prevent degradation problems.

Methanol, acetone and water were the solvents tested in the extraction of compounds in order to optimize the extraction methodologies. As extraction techniques, the gyratory shaker and the ultrasound apparatus were used.

In each case, 5g of sample (broccoli or lamb's lettuce) were extracted with 50mL of solvent using a gyratory shaker (Yellow line, TTS2-IKA) or an ultrasound apparatus (Cole-Parmer, 8891) for 1 hour. The extracts obtained (twelve for each sample) were then filtered (WhatmanTM, 55mm) using a vacuum pump (VWR, PM20405-86) and kept at -20°C until further analysis. All the extractions were carried out in duplicate.

III. Results and Discussion

A. Total Phenolic Content

The total phenolic content of the obtained extracts was determined by the Folin–Ciocalteu method using gallic acid as the standard [6]. 1mL of diluted sample or standard solution

and 5mL of Folin–Ciocalteu reagent (diluted 10-fold in deionized water) were combined in a test tube and then mixed well using a vortex (Yellow Line, TTS 2). The mixture was allowed to react for 5min and then 4mL of sodium carbonate solution (7.5% in deionized water) was added and mixed well. This solution was incubated at room temperature, in the dark for 2 hours. The absorbance was measured at 740nm using a Schimadzu UV- 3101 spectrophotometer (Kyoto, Japan). All the analyses were carried out in duplicate and the results were expressed as mg of gallic acid equivalents per 100 gram of fresh weigh (Table I). Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

TABLE I
TOTAL PHENOLIC CONTENT FOR BROCCOLI AND LAMB'S LETTUCE EXTRACTS, EXPRESSED AS MG OF GALIC ACID EQUIVALENTS PER 100G OF FRESH WEIGHT

	Broccoli		Lamb's lettuce	
	ultrasound	gyratory shaker	ultrasound	gyratory shaker
Methanol	108±6[a]	102.5±0.1[a]	255±8	278±27[a]
Acetone	85±3[a]	89±1[a]	274.3±0.5[a]	280±13[a]
Water	2.5±0.1[a]	2.8±0.7[a]	7±2[a]	6±1[a]

a- Statistically significant between the tested solvents, $p<0.05$

B. Radical Cation Scavenging Activity

The ABTS radical cation decolourisation test is widely used to access antioxidant activity. Reduction in color indicates reduction of ABTS radical [7]. In the present study, it is remarkable the difference of radical scavenging activity between lamb's lettuce and broccoli, 3 to 5-fold higher for lamb's lettuce. One-way ANOVA has showed significant differences between the three different solvents used in broccoli and lamb's lettuce extracts ($P < 0.05$). As observed previously, water extracts showed much lower radical scavenging activity comparatively to methanol and acetone extracts, approximately 14 to 22-fold for lamb's lettuce (Table II). Regarding broccoli, methanol extracts showed higher radical cation activity than acetone and water extracts, 2 to 8-fold respectively. For both vegetables there was no significant difference between the extraction techniques (student t -test, $P > 0.05$).

TABLE II
ANTIOXIDANT ACTIVITY OBTAINED FOR BROCCOLI AND LAMB'S LETTUCE EXTRACTS

	Broccoli		Lamb's lettuce	
	ultrasound	gyratory shaker	ultrasound	gyratory shaker
Methanol	201±4[a]	193±9[a]	571±10	617±82
Acetone	104±1[a]	113±13[a]	546±19	540±50
Water	26.3±0.3[a]	23±1[a]	28±2[a]	39±22[a]

a-Statistically significant between the tested solvents, $p<0.05$

C. Free Radical Scavenging by the Use of the DPPH Radical

The ability of broccoli and lamb's lettuce to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity assay. As a kind of stable free radical, DPPH can accept an electron or hydrogen radical to become a stable diamagnetic molecule, which is widely used to investigate radical scavenging activity. The antioxidants can react with DPPH, a deep-violet coloured stable free radical, converting it into a yellow coloured α,α-diphenyl-β-picrylhydrazine [8].

It can be concluded (Table III) that lamb´s lettuce extracts showed higher antioxidant capacity comparatively with broccoli extracts (4 to 22-fold). In case of broccoli, one-way ANOVA showed significant difference between methanol and acetone extracts, approximately 2 to 3-fold higher for methanol ($P < 0.05$). For lamb's lettuce, the acetone extracts (ultrasound) showed higher antioxidant capacity than methanol and water extracts, therefore the extracting solvents significantly affect the antioxidant activity of the obtained extracts ($P < 0.05$). Sun and collaborators [9] have also demonstrated that the acetone extract of broccoli had the highest radical scavenging activity determined by the DPPH assay. In the present study, water extracts did not show DPPH scavenging activity in both vegetables. As reported by Sun and collaborators [9] methanol and acetone extracts showed higher antioxidant activity than water extracts, possibly because the phenolic compounds present in both plants show higher solubility in acetone and methanol than in water. Student t-test showed that there were no significant differences between the extraction techniques used ($P > 0.05$). There are several factors influencing the antioxidant activity of polyphenols, such as the colloidal properties of the substrates, the conditions and stages of oxidation, the formation and stability of radicals, as well as the possible location of antioxidants [10]. According to the results were reported, it is recommended to use more than one method to evaluate the antioxidant activity.

TABLE III
FREE RADICAL SCAVENGING ACTIVITY BY THE DPPH ASSAY, EXPRESSED AS PERCENTAGE OF ANTI-RADICAL POWER (% ARP)

	Broccoli		Lamb's lettuce	
	ultrasound	gyratory shaker	ultrasound	gyratory shaker
Methanol	11.3±0.1[a]	9.4±0.7[a]	48.53±0.03 [a]	56±8[a]
Acetone	4.44±0.07[a]	4.0±0.5[a]	86±12[a]	58±5[a]
Water	ND	ND	ND	ND

a-Statistically significant between the tested solvents, p<0.05. ND- Not detected

D.Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power assay is the commonly used method for measuring the total antioxidant capacity of extracts from food and plants. This method is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} and measures directly the reducing capacity of the substance [11]. Increasing absorbance of the reaction mixture represents stronger reducing/antioxidant power. As can be seen in Table IV, once again the lamb´s lettuce has higher antioxidant capacity when compared with the broccoli extracts, between 3-fold (methanol) and 6-fold (acetone). In agreement with previous results, one-way ANOVA showed significant differences between water extracts and acetone or methanol extracts, approximately 40-fold (acetone) and 70-fold (methanol) lower for broccoli and 90-fold (acetone and methanol) lower for lamb's lettuce. Methanol was the solvent showing the highest efficiency, proving that it is the best solvent, probably due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenols. Once more, student t- test showed no significant differences between extraction techniques (P > 0.05) for both vegetables.

TABLE IV
FERRIC REDUCING POWER BY THE FRAP ASSAY, EXPRESSED AS MG OF TROLOX EQUIVALENTS PER 100 G OF FRESH WEIGHT

	Broccoli		Lamb's lettuce	
	ultrasound	gyratory shaker	ultrasound	gyratory shaker
Methanol	117±7[a]	104±6[a]	316±7	341±41
Acetone	62±2[a]	54±4[a]	321±15	327.2±0.7
Water	1.76±0.08[a]	2.9±0.2[a]	7±2[a]	4±1[a]

a-Statistically significant between the tested solvents, p<0.05. ND- Not detected

E. Statistical Analysis

A significance t-test was employed in order to decide whether if the differences between the extraction techniques were significant. One-way analysis of variance (ANOVA, P<0.05) was applied for the comparison of the type of solvent.

IV. CONCLUSIONS

In this study, antioxidants present in broccoli and lamb's lettuce were evaluated using different extraction conditions. Methanol was the solvent that showed the better yield on the extraction of antioxidant compounds, except for the DPPH assay. When compared to broccoli, lamb's lettuce showed 3-fold higher total phenolic content as well as 4 to 22-fold higher free radical scavenging activity. It has been shown that sonication effects made some barley cell walls break down, leading to the release of some bound phenolic compounds although in the present work it was not observed significant differences between ultrasound and gyratory shaker for both vegetables. The results presented herein clearly demonstrate that lamb's lettuce must be regarded as a very interesting source of polyphenols, and thus a potential health-promoting food.

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