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Amino acids, phenolic and flavonoid contents in two diverse extracts of *Spinacia Oleracea L.*: evaluation of *in vitro* antioxidant and enzyme inhibitory activity

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INTRODUCTION & AIM

Spinach (*Spinacia oleracea L.*) is a green herbaceous annual leafy vegetable cultivated in many parts of the world, characterized by low cost and widely used in many traditional dishes. It is considered a functional food for its nutritional composition, phytochemicals and bioactive compounds that contribute to reduce oxidative stress, inducing secretion of satiety hormones, helping to promote protection mechanisms against hypoglycaemia, cancer and obesity[1].

The aim of this work is to identify and quantify amino acids in two extracts of *Spinacia Oleracea L.*, following their preliminary biological *in vitro* evaluation.

METHOD

Plant materials were collected for the protein extraction process. Two extraction processes were conducted in which one involved the use of $CaCl_2$ (e.g. sample S2), while the other one was extracted as such (e.g. sample S1)(Figure 1). Bradford colorimetric assay was used to quantify the soluble protein in each sample (Figure 2 and Table 1) and an SDS-PAGE analysis was used for separation of RuBisCo subunits in samples S1 and S2.

Then the amino acid content was determined in both samples using HPLC-DAD technique with the aim to investigate the phytochemical profile, together with the phenolics and flavonoids compounds (Table 4). To allow the identification and quantification of amino acids using HPLC-DAD, derivatization with the fluorenylmethyloxycarbonyl (Fmoc) group was carried out following a previously described procedure[2]. The amino acids profile can be observed in Table 2 and Table 3. Further biological assays were done to determine the antioxidant and enzyme inhibition activity of two samples (Tables 5 and 6).

Fig.1: Samples S1 and S2 after centrifugation and pH regulation.

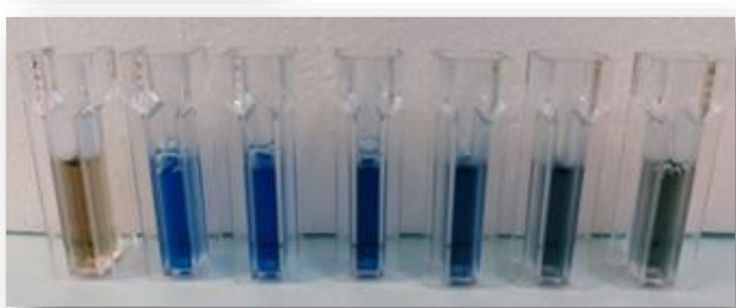

Fig.2: A six-level calibration plot based on least-squares linear regression within the range of 0, 0.033, 0.066, 0.132, 0.265, 0.53, 1.06 ppm BSA using 1000 μL of Coomassie Brillant Blue G-250 + 20 μL of protein samples.

Sample	mg/mL protein
S1	2.46
S2	2.56

Tab.1: Protein contents in samples S1 and S2.

RESULTS & DISCUSSION

Data reveal that Sample S1 contains Lysine (40,28 mg/g DE) and Tyrosine (0,86 mg/g DE) as principal amino acids while in sample S2 none of them were detected. Both of them, present a comparable total phenolic content instead of the total flavonoid content in which sample S1 shows the best result (3.65 mg RE/g). Biological assays show a higher antioxidant activity in sample S1 by ABTS (21,35 mg TE/g) and metal chelating (32,30 mg EDTAE/g) assays, than sample S2 in antioxidant tests by CUPRAC (21,61 mg TE/g) and FRAP (13,65 mg TE/g). Finally, sample S2 exhibits greater inhibition of tyrosinase, than sample S1. Sample S1 exhibits greater inhibition of the glucosidase enzyme than sample S2.

Amino acid	Wavelength (nm)	mg/g DE ± S.D.
FmocGlyOH	265	underLOQ
FmocAlaOH	265	underLOQ
FmocProOH	265	underLOQ
FmocTryOH	265	0,86 ± 0,05
FmocMetOH	265	ND
FmocValOH	265	ND
FmocIleOH	265	ND
FmocPheOH	265	underLOQ
FmocLeuOH	265	underLOQ
FmocLys(fmoc)OH	265	40,28 ± 5,43

Tab.2: Amino acid content in sample S1.

Amino acid	Wavelength (nm)	mg/g DE ± S.D.
FmocGlyOH	265	ND
FmocAlaOH	265	ND
FmocProOH	265	ND
FmocTryOH	265	ND
FmocMetOH	265	ND
FmocValOH	265	ND
FmocIleOH	265	ND
FmocPheOH	265	ND
FmocLeuOH	265	ND
FmocLys(fmoc)OH	265	ND

Tab.3: Amino acid content in sample S2.

Sample	Total Phenolic content mg GAE/g	Total Flavonoid content mg RE/g
S1	7,12 ± 1,28	3,65 ± 0,07
S2	7,38 ± 0,94	1,31 ± 0,81

Tab.4: Determination of phenolics and flavonoids in samples S1 and S2.

Sample	DPPH mg TE/g	ABTS mg TE/g	CUPRAC mg TE/g	FRAP mg TE/g	Phophomolibdenum mmol TE/g	Metal chelating mg EDTAE/g
S1	na	21,35 ± 2,28	21,51 ± 4,96	12,16 ± 1,21	0,50 ± 0,06	32,30 ± 6,93
S2	na	11,97 ± 10,76	21,61 ± 31,88	13,65 ± 4,02	0,43 ± 0,09	20,11 ± 2,51

Tab.5: Biological assay for evaluation of antioxidant activity in samples S1 and S2.

Sample	AChE inhibition mg GALAE/g	BChE inhibition mg GALAE/g	Tyrosinase inhibition mg KAE/g	Amylase inhibition mmol ACAE/g	Glucosidase inhibition mmol ACAE/g
S1	2,46 ± 0,24	3,50 ± 0,86	47,45 ± 7,77	0,29 ± 0,03	8,15 ± 0,02
S2	2,63 ± 0,10	4,69 ± 0,41	49,90 ± 3,86	0,21 ± 0,06	8,06 ± 0,07

Tab.6: Biological assay for evaluation of enzyme inhibitory activity in samples S1 and S2.

CONCLUSION

Sample S1 reveals better amino acid content, antioxidant activity and enzyme inhibitory activity than S2. Further studies are due to improve the protein extraction method promoting the development of enriched foods and beverages.

FUTURE WORK / REFERENCES

Further studies are due to improve the protein extraction method promoting the development of enriched foods and beverages.

1. Roberts, Joseph L. *et al.* *Food & function* 7.8 (2016): 3337-3353.
2. Stefanucci, Azzurra *et al.* *Antioxidants* 11.8 (2022): 1474.

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