Phytochemical Composition and Antioxidant Properties of Portuguese Kale and Portuguese Tronchuda Cabbage Produced in a Sustainable Agriculture Production System are Affected by Climate Conditions.

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Summary

In this study we evaluate the biological role of Portuguese kale and tronchuda cabbage, tow of major *Brassica* plants most consumed in Portugal, based on the determination of average levels of Glucosinolates (individual and totals), total phenolics, total flavonoids, L-ascorbic acid (vitamin C), and minerals (Fe, Zn and Se) of plants produced in two different climate seasons, Spring-Summer (SS) and Summer-Winter (SW) during two consecutive years. The antioxidant activity was also evaluated and respectively IC_{50} values were estimated by a curve dose-response. In addition we used broccoli inflorescences, one of the *Brassicacea* plants with biological role well established, for results comparison.

Keywords: *Brassicacea*; phytochemicals; antiradical and antioxidant activity; climate.

Objectives

I. Evaluate the biological role of Portuguese kale and Portugese tronchuda cabbage in contrast with broccoli inflorescences.

II. Understand how the different climate conditions affect the average levels of bioactive components.

III. What is the antioxidant potential of Portuguese kale and tronchuda cabbage.

III. Understand which components are directly associated with their antioxidant activity and how it is affected by climate modifications.

IV. Can *Brassica* plants can provide considerable amounts of bioactive components and may constitute an important source of natural dietary antioxidants? Is the major goal of the current work.

Material and Methods

I. The experimental was conducted in two consecutive years during the period 2005-2007 in two consecutive growing seasons Spring-Summer (SS) and Summer-Winter (SW).

II. The study was conducted in the field of Universidade de Trás-os-Montes e Alto Douro (UTAD) "campus" placed in Vila Real, Northern Portugal (460 m altitude, 41º 17´N and 7º44´W).

III. The study was carried out on three *Brassica* vegetables divided in two botanical groups :

A. one group formed exclusively by broccoli inflorescences

(*Brassica oleracea* L. var. *italica* Plenck cv. Marathon)

B. the second group formed by brassica leaves namely, Portuguese kale and tronchuda cabbage

(*Brassica oleracea* L. var. *acephala* D.C cv. Galega portuguesa)

(*Brassica oleracea* L. var. *costata* D.C. cv. tronchuda).

IV. The plants were sowed in a greenhouse in pots with a mixture of sterilized peat and sand in proportion of 3:1 ($^v/_v$), and transplanted at 3-4 true leaf stage to the field. Immediately to the transplantation the plants were watered and the irrigation was performed, always it was consider necessary. No fertilizations or pesticide applications were made.

V. The sowing dates were 24 March 2005 and 18 April 2005 for Spring-Summer (SS) seasons and 19 August 2005 and 14 August 2006 for Summer-Winter seasons (SW).

VI. The experiment was set in a randomized block system with three replicates of 25 plants each.

VII. Plant material was harvested at commercial maturity stage:

In the SS:

- 67-68 days after transplantation for broccoli inflorescences
- 52-54 days after transplantation for Portuguese kale
- 66-67 days after transplantation for Portuguese tronchuda cabbage

In the SW:

- 95-98 days after transplantation for broccoli inflorescences
- 68-80 days after transplantation for Portuguese kale
- 87-95 days for Portuguese tronchuda cabbage

Five plants from each replicate were harvested, always at same time at the same day to avoid diurnal influence

VIII. The plant material of each replicate was divided in two subsamples:

1st-Half part was immediately deep frozen in liquid nitrogen and fine-grounded in a mortar, and a homogenous subsample was freeze-dried (Dura-Dr™ µP-FTS Systems) for further analysis

2nd-The remaining half material was dried in a forced-air oven at 60 ºC (Memmert UL 80, Germany), to determine the dry weight (DW)

IX. During the entire production period the meteorological parameters (average temperatures, precipitation, light duration, frost) were systematic and periodically measured (Figure 1).

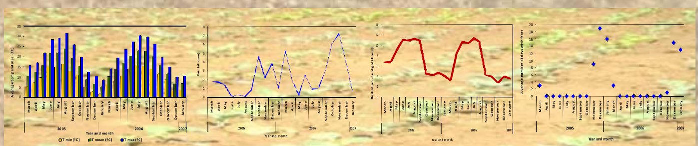

Figure 1. Evolution of climate parameters during the entire cycle of *Brassica* production in Vila Real region (Northern Portugal). Average minimum (T_{min}), maximum (T_{max}) and mean temperatures (T_{mean}), average humidity (H), average rainfall (mm), average sunlight (hours), average number of days with frost and growing degree days (GDD).

X. Analytical measurements:

1- Glucosinolates content (GS) – desuphoGS method (ISO 9167-1, EEC Regulation No. 9167-1, 1992))

2- Total phenolics content (TPC) – Spectrophotometric method

3- Total Flavonoids content (TFC)- Spectrophotometric method

4- L-ascorbic acid (L-asc) - Spectrophotometric method

5- Micronutrient content (Fe, Zn and Se) –Acid digestion ($HNO_3/HClO_4$ (2:1 $^v/_v$))

6- Antioxidant activity (AA) – DPPH method

* All measurements were made in triplicate.

Methods/procedures based on:

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XI. Statistical analyses were carried out using the SPSS V.17 statistical package program (SPSS, Inc. Chicago, Illinois, USA). Mann Whitney test was used to compare means. Pearson rank correlations were used to determine associations between the different parameters measured. It was also performed a principal component analysis (PCA) in order to identify patterns between antioxidant activity and different growing/climate season. The significance level (α) was set at 0.05.

Results

I. The Portuguese kale and Portuguese tronchuda cabbage present high content of TPC, TF, GSs, L-asc acid and MC, very similar to the average contend in broccoli inflorescences (Tables 1, 2 and 3). These values are within the values presented by other reports (Podsędek 2007), neverthless higher than the average values presented by Souci et al., (1994).

II. Our results showed a variation of bioactive compounds, both profile and content with genotype (Table 1) and climate season (Tables 1, 2 and 3). Effectively, main individual GSs content in each *Brassica* shown significant differences (*P<0.05*) between the two distinct climate seasons SS and SW. (Table 1). These results probably occurred due to genetic differences in GS metabolism or storage, among the botanical groups, as reported by recent studies (Vallejo *et al.*, 2002, 2003).

III. Similar tendency was noted for TPC (*P<0.05*) in broccoli and Portuguese kale, whilst the TPC, L-asc acid, Fe and Se were less affected (*P>0.05*) by climate seasons (Table 2).

Table 1. Mean content of GSs (µmoles.100 g-1 D.W.) in different *Brassica* samples produced in two consecutive years and two different climate seasons [Spring-Summer (SS) and Summer-Winter (SW)].[1,2,3,4]

Cultivar	Climate season	Aliphatic				Indole				Total GSs
		GIB	PROG	SIN	GRAF	4-OH	GBRASS	4-METHOX	NGB	
Broccoli inflorescences	SS	115 ± 9 ᵇ	1 ± 1 ᵃ	ND	1007 ± 202 ᵃ	33 ± 15 ᵃ	290 ± 37 ᵃ	291 ± 177 ᵃ	1129 ± 292 ᵇ	2867 ± 539 ᵃ
	SW	55 ± 26 ᵃ	4 ± 2 ᵃ	ND	1687 ± 221 ᵇ	28 ± 13 ᵃ	559 ± 34 ᵇ	304 ± 59 ᵃ	274 ± 18 ᵃ	2911 ± 114 ᵃ
Portuguese kale	SS	696 ± 42 ᵃ	7 ± 4 ᵃ	320 ± 30 ᵃ	22 ± 10 ᵃ	6 ± 3 ᵃ	414 ± 67 ᵇ	29 ± 14 ᵃ	46 ± 5 ᵇ	1540 ± 91 ᵃ
	SW	100 ± 12ᵇ	8 ± 4 ᵃ	163 ± 65 ᵃ	50 ± 13 ᵃ	16 ± 8 ᵃ	156 ± 21 ᵃ	47 ± 14 ᵃ	20 ± 5 ᵃ	561 ± 74 ᵇ
Portuguese tronchuda cabbage	SS	383 ± 67 ᵃ	10 ± 5 ᵃ	132 ± 20 ᵃ	28 ± 14 ᵃ	10 ± 5 ᵃ	349 ± 93 ᵃ	58 ± 24 ᵃ	82 ± 16 ᵃ	1052 ± 231 ᵃ
	SW	336 ± 83 ᵃ	36 ± 5 ᵇ	210 ± 65 ᵃ	175 ± 59 ᵇ	22 ± 10 ᵃ	527 ± 123 ᵃ	198 ± 90 ᵃ	91 ± 21 ᵃ	1597 ± 310 ᵃ

[1] Data are expressed as mean ± standard error mean (n=12, 6 in each season).
[2] GIB-Glucoiberin; PROG-Progoitrin; SIN-Sinigrin; GRAF-Glucoraphanin; 4-OH-4-hydroxyglucobrassicin; GBRASS-Glucobrassicin; 4-METHOX-4-Methoxyglucobrassicin; NGB-Neoglucobrassicin.
[3] ND-Non detectable.
[4] Values in the same column for diferent climate season but within the same cultivar not followed by the same letter are significantly different at by Man Mann Whitney test, * *P<0.05*.

Table 2. Mean content of total phenolics, total flavonoids and L-ascorbic acid in different *Brassica* samples produced in two consecutive years and two different climate seasons [Spring-Summer (SS) and Summer-Winter (SW)].[1,2]

Cultivar	Climate season	Total phenolic content (mg.g-1 GAE)	Total flavonoid content (mg.g-1 CAE)	L-ascorbic acid content (mg.g-1 LACE)
Broccoli inflorescences	SS	18.2 ± 1.4 ᵃ	9.9 ± 1.0 ᵇ	97.2 ± 0.5 ᵃ
	SW	18.9 ± 2.5 ᵃ	4.1 ± 0.4 ᵃ	96.4 ± 0.4 ᵃ
Portuguese kale	SS	25.2 ± 1.9 ᵇ	10.7 ± 0.6 ᵇ	139.3 ± 0.1 ᵃ
	SW	19.7 ± 0.6 ᵃ	7.8 ± 0.3 ᵃ	139.1 ± 0.1 ᵃ
Portuguese tronchuda cabbage	SS	20.2 ± 2.1 ᵃ	6.3 ± 1.9 ᵃ	138.7 ± 0.1 ᵃ
	SW	18.8 ± 0.5 ᵃ	6.0 ± 0.4 ᵃ	138.8 ± 0.2 ᵃ

[1] Data are expressed as mean of 3 replicates ± error mean.
[2] Values in the same column for diferent climate season but within the same cultivar not followed by the same letter are significantly different at by Man Mann Whitney test, * *P<0.05*.

Table 3. Mean content micronutrients (Fe, Zn and Se), in different *Brassica* samples produced in two consecutive years and two different climate seasons [Spring-Summer (SS) and Summer-Winter (SW)].[1,2]

Cultivar	Climate season	$\mu g.100 g^{-1}$ DW		
		Fe	Zn	Se
Broccoli inflorecences	SS	91.7 ± 3.8 ᵃ	17.9 ± 1.8 ᵃ	5.9 ± 1.0 ᵃ
	SW	103.3 ± 1.2 ᵇ	33.1 ± 1.0 ᵇ	6.5 ± 0.7 ᵃ
Portuguese kale	SS	92.7 ± 2.2 ᵃ	26.3 ± 1.0 ᵇ	4.0 ± 0.8 ᵃ
	SW	89.3 ± 3.4 ᵃ	14.0 ± 0.5 ᵃ	3.8 ± 0.8 ᵃ
Portuguese tronchuda cabbage	SS	104.3 ± 4.6 ᵃ	19.5 ± 1.7 ᵃ	4.5 ± 0.7 ᵃ
	SW	107.9 ± 3.1 ᵃ	19.2 ± 1.2 ᵃ	6.3 ± 0.8 ᵃ

[1] Data are expressed as mean ± standard error mean (n=12, 6 in each season).
[2] Values in the same column for diferent climate season but within the same cultivar not followed by the same letter are significantly different at by Man Mann Whitney test, * *P<0.05*.

IV. Our results showed that TF, a group of important secondary metabolites, increased during the SS seasons in all *Brassica* studied (Table 2). These result could be explained by high temperatures and radiation, lower precipitation and therefore higher water stress (Figure 1), particularly in the days next to the harvest, which was noted also in previous reports (Yao *et al.*, 2005 and Sikora *et al.*, 2008).

V. The high levels of TPC, TFC and L-asc acid in SS season could explained by morphological parameters. Portuguese kale and Portuguese tronchuda cabbage generates typical leaves with high surface exposure to climate factors, such as high temperature and lower precipitation (Figure 1) as occurs in SS seasons, as well high radiation, achieving in this way a physiological condition of increase synthesis of phenolics, flavonoids and L-asc acid.

VI. Based on these results it seems acceptable that SS seasonal offers to this botanical group an opportunity to accumulate higher content of these three bioactive compounds.

VII. The curve dose response showed and AA higher in SS seasons, also our results showed that Portuguese kale and Portuguese tronchuda cabbage exhibited AA very similar to the AA showed by broccoli inflorescences (Figure 2). The average levels of % inhibition of DPPH radicals were for 5mg.mL^{-1}, one of the highest extract concentration was in average 80.6 , 82.2 and 81.9 for Portuguese kale, tronchuda cabbage and broccoli inflorescences respectively. Moreover was always higher in SS seasons (Figure 2).

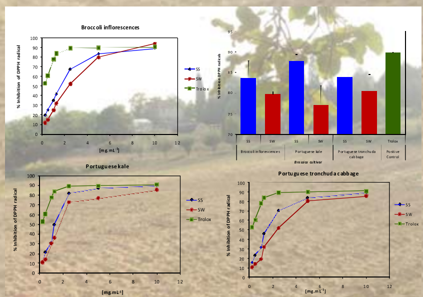

Figura 2. % Inhibition of DPPH radicals at 5 mg.mL^{-1} values determined from the dose-curve response for each plant extract in two consecutive years and two different climate seasons [Spring-Summer (SS) and Summer-Winter (SW)].[1,2]

Table 4. IC50 (mg.mL^{-1}) values determined from the dose-curve response for each plant extract in two consecutive years and two different climate seasons [Spring-Summer (SS) and Summer-Winter (SW)].[1,2]

Plant extract	Climate season	IC_{50} (mg.mL^{-1})
Broccoli inflorescences	SS	1.67 ± 0.1 ᵃ
	SW	2.36 ± 0.8 ᵇ
Portuguese kale	SS	1.26 ± 0.2 ᵃ
	SW	1.73 ± 1.5 ᵇ
Portuguese tronchuda cabbage	SS	1.44 ± 0.3 ᵃ
	SW	2.38 ± 1.8 ᵃ
Positive control (Trolox)		<0.25 mg.mL^{-1}

[1] Data are expressed as mean ± standard error mean (n=12, 6 in each season).
[2] Values in the same column for diferent climate season but within the same cultivar not followed by the same letter are significantly different at by Man Mann Whitney test, * *P<0.05*.

VIII. The principal components analysis (PCA) performed for the different *Brassica* samples showed that not all the components have a critical effect on IC_{50} values and therefore on the AA (Figure 5 and Table 5).

IX. Accordingly our results, in broccoli inflorescences, Portuguese kale and Portuguese tronchuda cabbage, the lower average levels of IC50 obtained in SS season were mainly caused by the high concentrations of total flavonoids (in broccoli), total flavonoids + glucobrassicin + total GSs (in Portuguese kale) and total phenolics + total flavonoids + total GS + glucoiberin (in Portuguese tronchuda cabbage) (Figure 2 and Table 5).

X. Our results also shown, that AA is often associated to a combined effect of several components and not to a specific one.

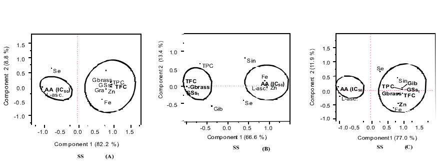

Figure 3. Principal component analysis (PCA) for the different bioactive components and antioxidant activity [AA (IC50)] of each plant extracts from the three *Brassica* samples (**A- Broccoli inflorescences, B- Portuguese kale, C- Portuguese tronchuda cabbage**) produced and harvested in the whole period of production (2005-2007) in two different climate seasons [Spring-Summer (SS) and Summer-Winter (SW)].

Table 5. Significant correlations between antioxidant activity [AA (IC50)] and selected bioactive components from principal component analysis (PCA) in each *Brassica* extract.

Plant extract	Climate season	Variables [1]	
Broccoli inflorescences	SS		AA (IC_{50})
		Total phenolic content (TPC)	-0.9629**
		Total flavonoid content (TFC)	-0.9999**
Portuguese kale	SS		AA (IC_{50})
		Total flavonoid content (TFC)	-0.9998**
		Glucobrasscin (GBRASS)	-0.8918**
		Total GSs	-0.8479*
Portuguese tronchuda cabbage	SS		AA (EC_{50})
		Total phenolic content (TPC)	-0.9584**
		Total flavonoid content (TFC)	-0.9999**
		Glucoiberin (GIB)	-0.9382**
		Total GSs	-0.9890**

[1] *, **, Variables correlated in a significant way at P<0.05 and P<0.01.

Conclusions

We can conclude that Portuguese Kale and tronchuda cabbage shown an interesting antioxidant potential and therefore these Brassica can constitute an important natural source of antioxidants. The climate conditions during the growth are extremely important and different climate factors such as temperature, precipitation and/or radiation can influence the accumulation of several chemical components, affecting thereby the antioxidant activity.

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