18

Phytochemical and Antioxidant Characterization of *Brassica oleracea* Var. *Costata* Extracts

Carla Sousa[1], Patrícia Valentão[1], David M. Pereira[1], Marcos Taveiraa[1], Federico Ferreres[2], José A. Pereira[3], Albino Bento[3], Rosa M. Seabra[1] and Paula B. Andrade[1*]

Abstract

The genus Brassica is related with the prevention of carcinomas, especially of stomach, colon and recto and with the prevention of cardiovascular diseases. The most consumed Brassica species in Portugal is the tronchuda cabbage or Portuguese cabbage (Brassica oleracea var. costata DC). This study concerned the characterization of chemical composition (phenolics and organic acids) and the evaluation of the antioxidant potential of tronchuda cabbage. Seeds, sprouts, internal and external leaves were analysed. To evaluate the antioxidant potential, the ability of tronchuda cabbage materials to act as a scavenger of reactive oxygen species (superoxide radical, hydroxyl radical and hypochlorous acid) was investigated. Additionally, it was found that Pieris brassicae, a plague infesting Brassica cultures, exhibits interesting antioxidant potential, once it sequesters, metabolizes and accumulates phenolics. The possibility of commercialization of standardized aqueous extracts of Brassica oleracea var. costata DC seeds, sprouts, internal and external leaves, with nutritional and healthy potential benefits, is discussed.

Key words: *Brassica oleracea* var. *costata* DC, Phenolics, Organic acids, Antioxidant activity.

1. REQUIMTE/Serviço de Farmacognosia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 164, 4050-047 Porto, Portugal.
2. Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University of Espinardo, Murcia, Spain.
3. CIMO//Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Sta Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.

* *Corresponding author* : E-mail : pandrade@ff.up.pt

Introduction

An increasing amount of evidence shows that the consumption of fruits and vegetables is, in general, beneficial to health due to the protection provided by the antioxidant compounds contained in them (Kahkonen *et al*., 1999). In fact, the presence of phytochemicals, in addition to vitamins and provitamins, has been considered of great nutritional interest in the prevention of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, rheumatism, ischemic and cardiovascular diseases and also in the aging process, in which oxidants or free radicals are involved (Chu *et al*., 2002; Pulido *et al*., 2000; Behl & Moosmann, 2002).

Brassica species are reported to possess cancer preventive properties (Beecher, 1994) that have been attributed to the glucosinolates and their derived products (Stoewsand, 1995). Flavonoids and other phenolics also contribute to this capacity (Marchand, 2002; Galati & O'Brien, 2004). Although essentially temperate, *Brassica oleracea* forms are now grown in other regions all over the world (Vaughan & Geissler, 1997). Tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) (Fig 1) is still considered to be a primitive cultivar, being high yielding, less susceptible to pests and diseases and well adapted to a wide range of climates (Rosa, 1997).

The objectives of this study were to define the phenolics and organic acids composition of the different tronchuda cabbage materials and to evaluate the antioxidant potential of their different aqueous extracts.

Fig 1. *Brassica oleracea* var. *costata* DC

Phytochemical study

Phenolic compounds

Internal and external leaves

The phenolic compounds of internal and external leaves of *Brassica oleracea* L. var. *costata* DC were characterized and quantified by reversed-phase HPLC-DAD-ESI-MSn and HPLC/DAD. There are found remarkable

differences between them (Ferreres *et al.*, ~~2005 a~~): the internal leaves present phenolic acid derivatives as the main compounds and small amounts of flavonol glycosides, whereas external leaves exhibit only flavonol derivatives.

So, internal leaves (Ferreres *et al.*, ~~2005 a, b~~) presented a phenolic profile (Fig 2) with seventeen phenolic compounds: quercetin 3-*O*-sophoroside-7-*O*-glucoside, 3-p-coumaroylquinic acid, kaempferol 3-*O*-sophoroside-7-*O*-glucoside, kaempferol 3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside, sinapoyl glucoside acid, kaempferol 3-*O*-(sinapoyl)-sophoroside-7-*O*-glucoside, kaempferol 3-*O*-(feruloyl)-sophoroside-7-*O*-glucoside, kaempferol3-*O*-(p-coumaroyl)-sophoroside-7-*O*-glucoside, 4-p-coumaroylquinic acid, sinapic acid, kaempferol 3-*O*-sophoroside, 3 isomeric forms of 1,2-disinapoylgentiobiose, 1-sinapoyl-2-feruloylgentiobiose, 1,2,2'-trisinapoylgentiobiose and 1,2'-disinapoyl-2-feruloylgentiobiose.

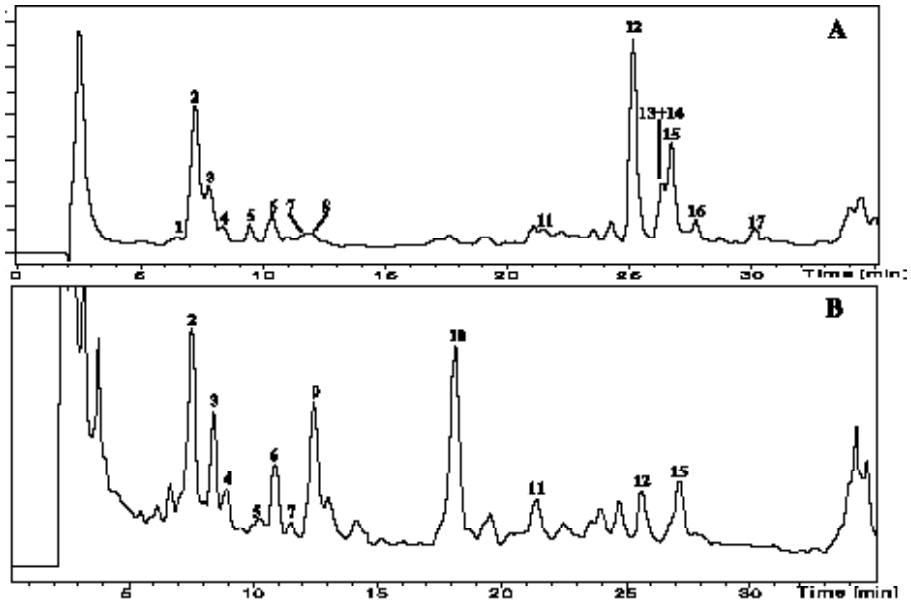


Fig 2. HPLC-DAD phenolic profile of tronchuda cabbage internal leaves (A) hydromethanolic extract and (B) aqueous lyophilized extract.. Detection at 330 nm. Peaks: **(1)** quercetin 3-*O*-sophoroside-7-*O*-glucoside; **(2)** 3-p-coumaroylquinic acid; **(3)** kaempferol 3-*O*-sophoroside-7-*O*-glucoside; **(4)** kaempferol 3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside; **(5)** sinapoyl glucoside acid; **(6)** kaempferol 3-*O*-(sinapoyl)-sophoroside-7-*O*-glucoside; **(7)** kaempferol 3-*O*-(feruloyl)-sophoroside-7-*O*-glucoside; **(8)** kaempferol 3-*O*-(p-coumaroyl)-sophoroside-7-*O*-glucoside; **(9)** 4-p-coumaroylquinic acid; **(10)** sinapic acid; **(11)** kaempferol 3-*O*-sophoroside; **(12)** 1,2-disinapoylgentiobiose; **(13)** 1-sinapoyl-feruloylgentiobiose; **(14)** isomer of 1,2-disinapoylgentiobiose; **(15)** 1,2,2`-trisinapoylgentiobiose; **(16)** 1,2`-disinapoyl-2-feruloylgentiobiose; **(17)** isomer of 1,2-disinapoylgentiobiose (Ferreres *et al.*, ~~2005a~~).

External leaves (Ferreres *et al*., ~~2005 a, b~~) presented a phenolic profile (Fig 3) with kaempferol 3-*O*-sophorotrioside-7-*O*-glucoside, kaempferol 3-*O*-(methoxycaffeoyl/caffeoyl)sophoroside-7-*O*-glucoside, kaempferol 3-*O*-sophoroside-7-*O*-glucoside, kaempferol 3-*O*-sophorotrioside-7-*O*-sophoroside, kaempferol 3-*O*-sophoroside-7-*O*-sophoroside, kaempferol 3-*O*-tetraglucoside-7-*O*-sophoroside, kaempferol 3-*O*-(sinapoyl/caffeoyl) sophoroside-7-*O*-glucoside, kaempferol 3-*O*-(feruloyl/caffeoyl) sophoroside-7-*O*-glucoside, kaempferol 3-*O*-sophorotrioside, kaempferol 3-*O*-(sinapoyl) sophoroside, kaempferol 3-*O*-(feruloyl) sophorotrioside, kaempferol 3-*O*-(feruloyl) sophoroside, kaempferol 3-*O*-sophoroside and kaempferol 3-*O*-glucoside. Chemical structures of phenolics of tronchuda cabbage internal and external leaves are represented in Figs 4 and 5, respectively.

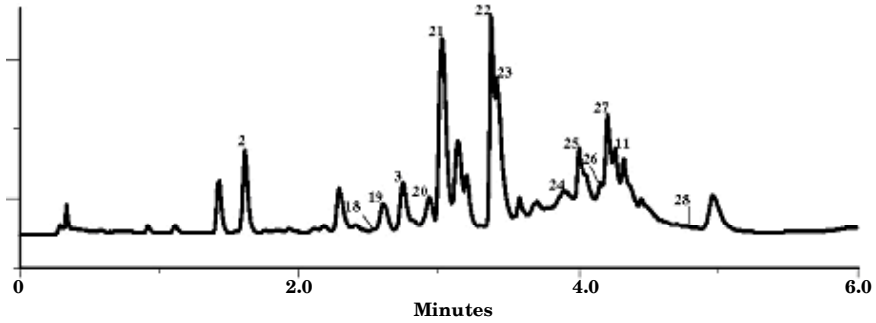


Fig 3. HPLC-DAD phenolic profile of tronchuda cabbage external leaves aqueous lyophilized extract. Detection at 330 nm. Peaks: **(2)** 3-p-coumaroylquinic acid; **(3)** kaempferol 3-*O*-sophoroside-7-*O*-glucoside; **(11)** kaempferol 3-*O*-sophoroside; **(18)** kaempferol 3-*O*-sophorotrioside-7-*O*-glucoside; **(19)** kaempferol 3-*O*-(methoxycaffeoyl/caffeoyl)-sophoroside-7-*O*-glucoside; **(20)** kaempferol 3-*O*-sophorotrioside-7-*O*-sophoroside; **(21)** kaempferol 3-*O*-sophoroside-7-*O*-sophoroside; **(22)** kaempferol 3-*O*-(sinapoyl/caffeoyl)-sophoroside-7-*O*-glucoside; **(23)** kaempferol 3-*O*-(feruloyl/caffeoyl)-sophoroside-7-*O*-glucoside; **(24)** kaempferol 3-*O*-sophorotrioside; **(25)** kaempferol 3-*O*-(sinapoyl)-sophoroside; **(26)** kaempferol 3-*O*-(feruloyl)-sophorotrioside; **(27)** kaempferol 3-*O*-(feruloyl)-sophoroside; **(28)** kaempferol 3-*O*-glucoside (Ferreres *et al*., ~~2005 a~~)

With the purpose to evaluate the influence of two fertilization regimens and collection date on the organic acids and phenolic compounds profiles of tronchuda cabbage leaves, a study was carried out in Mirandela, northeastern Portugal (U.T.M. 29 PG5602), according to two different agronomic practices (Sousa *et al*., 2005). Briefly, in one of the fields the production followed the organic status and in the other field the production was developed according to the standard cultural practices of the region (conventional production). Plant material was sown by the end of June 2002 and transplanted to the fields at the end of August. In the organic field only organic fertilization was applied with sheep manure. In the conventional field, organic fertilization was made during the

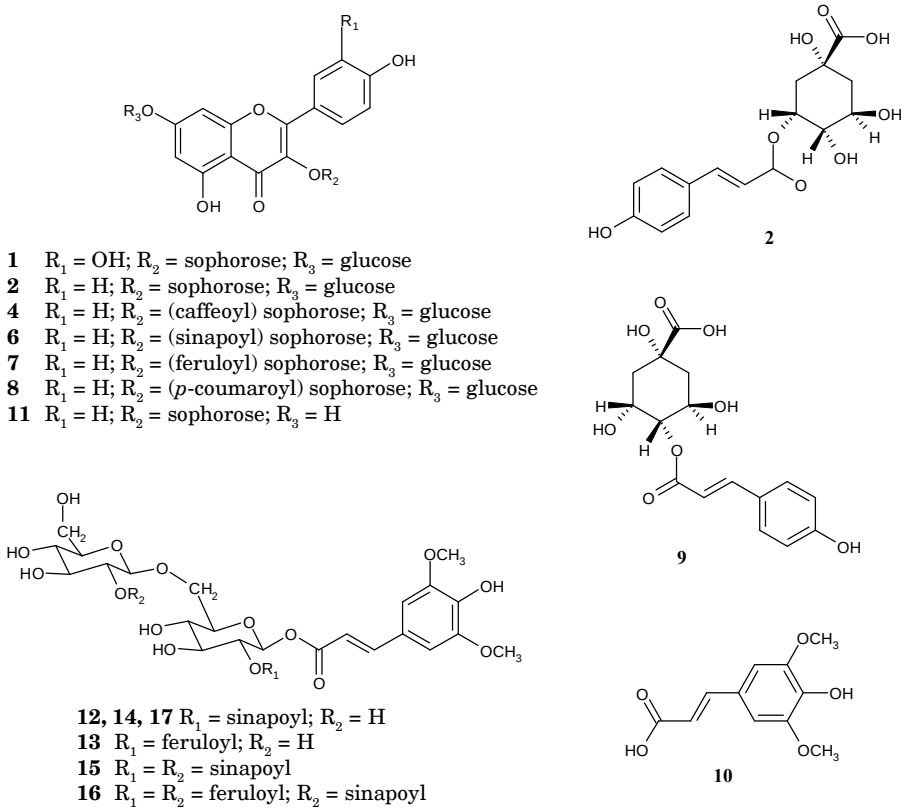
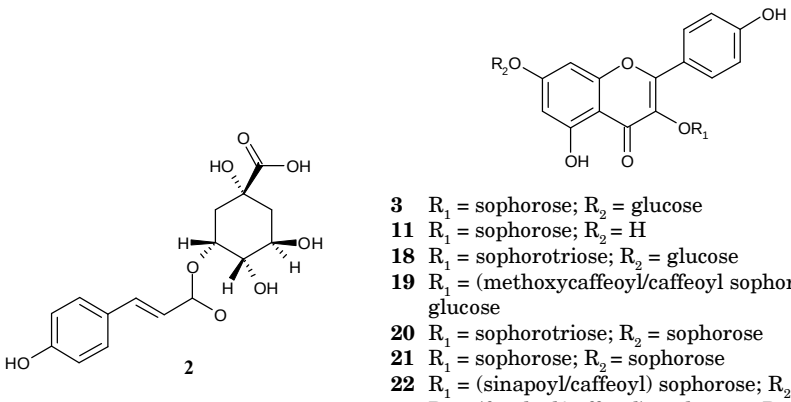


Fig 4. Chemical structures of phenolics from tronchuda cabbage internal leaves. **(1)** quercetin 3-*O*-sophoroside-7-*O*-glucoside; **(2)** 3-p-coumaroylquinic acid; **(3)** kaempferol 3-*O*-sophoroside-7-*O*-glucoside; **(4)** kaempferol 3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside; **(6)** kaempferol 3-*O*-(sinapoyl)-sophoroside-7-*O*-glucoside; **(7)** kaempferol 3-*O*-(feruloyl)-sophoroside-7-*O*-glucoside; **(8)** kaempferol 3-*O*-(p-coumaroyl)-sophoroside-7-*O*-glucoside; **(9)** 4-p-coumaroylquinic acid; **(10)** sinapic acid; **(11)** kaempferol 3-*O*-sophoroside; **(12)** 1,2-disinapoylgentiobiose; **(13)** 1-sinapoyl-feruloylgentiobiose; **(14)** isomer of 1,2-disinapoylgentiobiose; **(15)** 1,2,2`-trisinapoylgentiobiose; **(16)** 1,2`-disinapoyl-2-feruloylgentiobiose; **(17)** isomer of 1,2-disinapoylgentiobiose

transplantation of the plants and, at the beginning of September, a mineral fertilization with ammonium nitrate and CaO (ADP Adubos de Portugal) was applied with a side dress rate of 50 kg of N/ha. At the end of September, this field was subjected to one pesticide treatment with deltamethrin (Decis) (Bayer Crop Science) at a rate of 30 ml/hl.

Data from the quantification of the phenolics internal leaves (Sousa *et al*., 2005) showed that 3-*p*-coumaroylquinic acid and the sinapic acid derivatives, namely, the two isomers of 1,2-disinapoylgentiobiose, 1-sinapoyl-2-feruloylgentiobiose and 1,2,2'-trisinapoylgentiobiose, were the major compounds, representing >79% of total phenolics, with the exception



2

3 R_1 = sophorose; R_2 = glucose
11 R_1 = sophorose; R_2 = H
18 R_1 = sophorotriose; R_2 = glucose
19 R_1 = (methoxycaffeoyl/caffeoyl sophorose; R_2 = glucose
20 R_1 = sophorotriose; R_2 = sophorose
21 R_1 = sophorose; R_2 = sophorose
22 R_1 = (sinapoyl/caffeoyl) sophorose; R_2 = glucose
23 R_1 = (feruloyl/caffeoyl) sophorose; R_2 = glucose
24 R_1 = sophorotriose; R_2 = H
25 R_1 = (sinapoyl) sophorose; R_2 = H
26 R_1 = (feruloyl) sophorotriose; R_2 = H
27 R_1 = (feruloyl) sophorose; R_2 = H
28 R_1 = glucose; R_2 = H

Fig 5. Chemical structures of phenolics from tronchuda cabbage external leaves. **(2)** 3-*p*-coumaroylquinic acid; **(3)** kaempferol 3-*O*-sophoroside-7-*O*-glucoside; **(11)** kaempferol 3-*O*-sophoroside; **(18)** kaempferol 3-*O*-sophorotrioside-7-*O*-glucoside; **(19)** kaempferol 3-*O*-(methoxycaffeoyl/caffeoyl)-sophoroside-7-*O*-glucoside; **(20)** kaempferol 3-*O*-sophorotrioside-7-*O*-sophoroside; **(21)** kaempferol 3-*O*-sophoroside-7-*O*-sophoroside; **(22)** kaempferol 3-*O*-(sinapoyl/caffeoyl)-sophoroside-7-*O*-glucoside; **(23)** kaempferol 3-*O*-(feruloyl/caffeoyl)-sophoroside-7-*O*-glucoside; **(24)** kaempferol 3-*O*-sophorotrioside; **(25)** kaempferol 3-*O*-(sinapoyl)-sophoroside; **(26)** kaempferol 3-*O*-(feruloyl)-sophorotrioside; **(27)** kaempferol 3-*O*-(feruloyl)-sophoroside; **(28)** kaempferol 3-*O*-glucoside

of one sample, collected in January from the conventional culture, in which kaempferol 3-*O*-(sinapoyl)-sophoroside-7-*O*-glucoside was the compound present in highest amount (29% of total phenolics). 1,2'-diSinapoyl-2-feruloylgentiobiose was the minor compound.

The phenolic profile of tronchuda cabbage internal leaves revealed to be more homogeneous than that of the external ones (Ferreres *et al*., 2005), which is not surprising considering that the internal leaves are less exposed to external factors and phenolics are very susceptible to the external environment.

At external leaves (Ferreres *et al*., 2005), in a general way, the highest amounts were found for the pair kaempferol 3-*O*-sophorotrioside-7-*O*-glucoside plus kaempferol 3-*O*-(methoxycaffeoyl/caffeoyl)sophoroside-7-*O*-glucoside. In general, the minor compounds were kaempferol 3-*O*-sophorotrioside-7-*O*-sophoroside and kaempferol 3-*O*-(feruloyl)-sophorotrioside. Kaempferol 3-*O*-glucoside always exists in trace amounts. In general, internal leaf samples from organic culture exhibited higher total phenolics content than those from conventional practice collected in the same period (Sousa *et al*., 2005), as was observed with the external

leaves (Ferreres *et at*., 2005 a), with the exception of the samples from November. The interference of the mineral fertilizers and/or pesticides, used in conventional culture, in the biosynthetic pathway of phenolic compounds could explain the lower amounts presented by those samples.

In what concerns the phenolic composition during winter, we observed a decrease of the total phenolics content until December, which was more evident in samples from conventional culture. A considerable increase of total phenolics in both organic and conventional samples was noticed in January, as was observed before with the external leaves (Ferreres *et al*., 2005). Additionally, the production of flavonoids was higher in January for the two agronomic practices, a fact that could be explained by the very low temperatures registered in Mirandela region during January. In fact, the existence of a positive correlation between high levels of flavonoid glycosides and increased frost resistance is well-known (Swiderski *et al*., 2004). As these compounds contain sugar residues, they can delay water crystallization by the formation of hydrogen bonds between their hydroxyl groups and water molecules. Also, the action of flavonoids as antioxidants can be invoked, as protectors of plant tissues against the adverse effects of low-temperature oxidative stress (Swiderski *et al*., 2004). It seems that phenylalanine ammonia-lyase activity, a key enzyme of phenylpropanoid biosynthesis, is increased under low-temperature conditions (Solecka *et al*., 1999), which may justify the production of flavonoids as defense agents.

The results obtained in this study indicate that, in a general way, tronchuda cabbages from organic culture present higher phenolics contents than those from the conventional one.

Seeds

Phenolic compounds have been considered as UV screens in young seedlings (Gitz III *et al*., 1998) and have been associated with seedling vigor, height and weight (Randhir & Shetty, 2005). Ferreres *et al*. (2007) characterized and quantified in tronchuda cabbage seeds thirteen phenolic compounds (Fig 6 and Table 1) by reversed-phase HPLC-DAD-MS/MS-ESI and HPLC-DAD, respectively: two sinapoylgentiobiose isomers, three sinapoylglucose isomers, kaempferol-3-*O*-(sinapoyl)sophorotrioside-7-*O*-glucoside, sinapoylcholine, kaempferol-3,7-*O*-diglucoside-4'-*O*-(sinapoyl) glucoside, three disinapoylgentiobiose isomers, 1,2,2'-trisinapoylgentiobiose and 1,2-disinapoylglucose (Fig 7). The seeds exhibited a high content of phenolic compounds (6.0 g/kg) (Table 1), 1,2-disinapoylgentiobiose being the compound present in highest amounts, representing 17% of total phenolics. In the phenolic profile of tronchuda cabbage seeds the hydroxycinnamic derivatives are the main phenolics, corresponding to 80% of total compounds. This is clearly distinct from what happened with the internal leaves, in which they represented 46% of total phenolics (Ferreres *et al*., 2006), or with the external leaves, in which only flavonol glycosides were determined (Vrchovská *et al*., 2006).

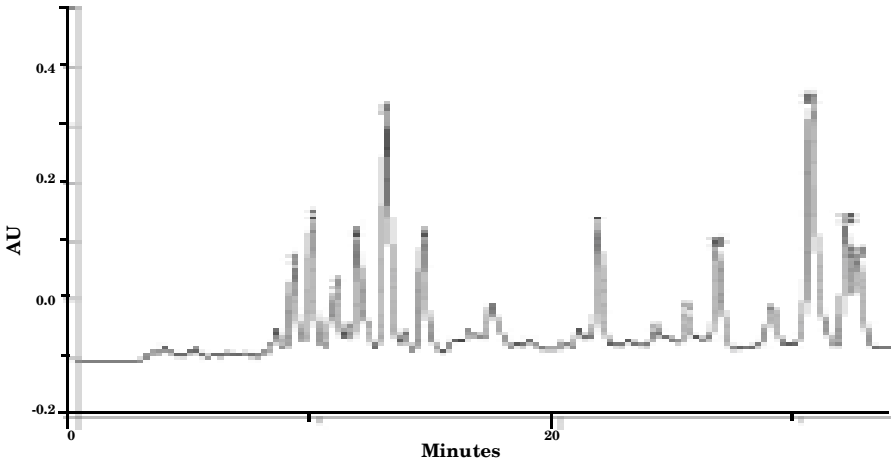


Fig 6. HPLC-DAD phenolic profile of tronchuda cabbage seeds' aqueous lyophilized extract. Detection at 330 nm. Peaks: **(1)** sinapoylgentiobiose; **(2)** 1-sinapoylglucose isomer; **(3)** sinapoylgentiobiose isomer; **(4)** 1-sinapoylglucose isomer; **(5)** 1-sinapoylglucose; **(6)** kaempferol-3-*O*-(sinapoyl)-sophorotrioside-7-*O*-glucoside; **(7)** sinapoylcholine; **(8)** kaempferol-3,7-*O*-diglucoside-4`-*O*-(sinapoyl)-glucoside; **(9)** 1,2-disinapoylgentiobiose isomer; **(10)** 1,2-disinapoylgentiobiose isomer; **(11)** 1,2-disinapoylgentiobiose; **(12)** 1,2,2`-trisinapoylgentiobiose; **(13)** 1,2-disinapoylglucose (Ferreres *et al.*, ~~2007~~).

Table 1. Quantification of tronchuda cabbage seeds phenolic compounds (mg/kg, dry basis)[a] (Ferreres *et al.*, ~~2005~~)

Phenolic compound	**Mean**	**SD**
Sinapoylgentiobiose	309	0.3
1-Sinapoylglucose isomer	368	11.7
Sinapoylgentiobiose isomer	270	1.7
1-Sinapoylglucose isomer	417	9.8
1-Sinapoylglucose	703	11.5
Kaempferol-3-*O*-(sinapoyl)-sophorotrioside-7-*O*-glucoside	911	17.7
Sinapoylcholine	376	7.1
Kaempferol-3,7-*O*-diglucoside-4'-*O*-(sinapoyl)-glucoside	267	17.6
1,2-Disinapoylgentiobiose isomer	152	3.2
1,2-Disinapoylgentiobiose isomer	345	2.0
1,2-Disinapoylgentiobiose	1023	37.1
1,2,2`-Trisinapoylgentiobiose	448	2.2
1,2-Disinapoylglucose	368	2.4
Σ	5974	

[a]Results are expressed as mean of three determinations. SD standard deviation, Σ, sum of the determined phenolic compounds

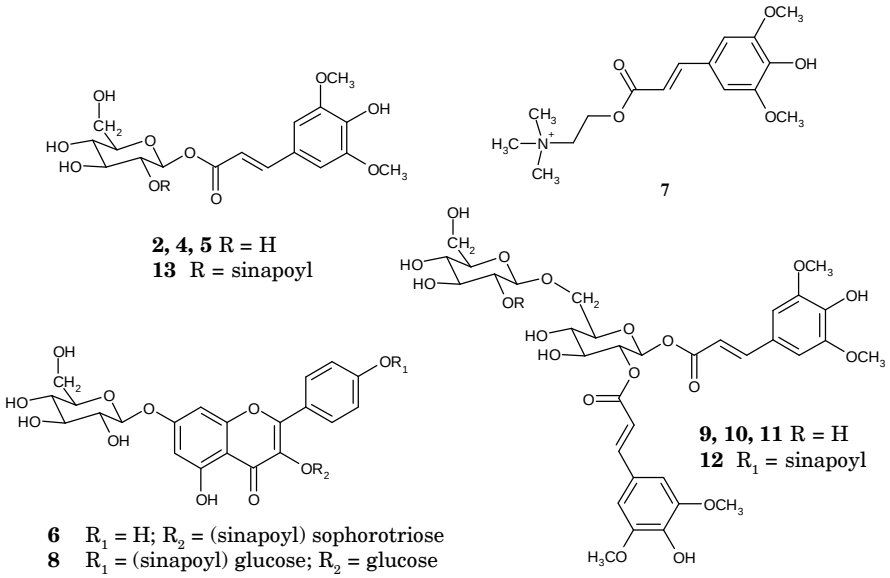


Fig 7. Chemical structures of phenolics from seeds of tronchuda cabbage. **(2)** 1-sinapoylglucose isomer; **(4)** 1-sinapoylglucose isomer; **(5)** 1-sinapoylglucose; **(6)** kaempferol-3-*O*-(sinapoyl)-sophorotrioside-7-*O*-glucoside; **(7)** sinapoylcholine; **(8)** kaempferol 3,7-*O*-diglucoside-4'-*O*-(sinapoyl)-glucoside; **(9)** 1,2-disinapoylgentiobiose isomer; **(10)** 1,2-disinapoylgentiobiose isomer; **(11)** 1,2-disinapoylgentiobiose; **(12)** 1,2,2'-trisinapoylgentiobiose; **(13)** 1,2-disinapoylglucose

Sprouts

The leaves and inflorescences of many Brassicaceae vegetables contribute widely to human diet, especially in winter. Although Brassicaceae seeds can be used in human consumption for its oil (canola seeds) or mixed with some food products (*e.g.*, bread and cake), sprouts, the germinating form of seeds, are favoured for their nutritional value and became a familiar component in salads (Naczk *et al*., 1998; Mwikya *et al*., 2001; Ayaz *et al*., 2006). Besides, there is considerable interest in the use of *Brassica* sprouts for health benefits. For instance, sprouts of brocolli can be more effective for achieving protection against carcinogenesis, mutagenesis and other forms of toxicity of electrophiles and reactive forms of oxygen than mature plants (Fahey *et al*., 1997). Additionally, it has been reported that germination may reduce the content of antinutritional components in the seeds, thus making sprouts safe for the diet (Wanasundara *et al*., 1999).

In order to establish the relationships among primary and secondary metabolism occurring during the developmental processes of young sprouts of *Brassica oleracea* var. *costata* DC (Fig 8), especially in what concerns the changes in organic acids and phenolic compounds, those compounds

were screened by HPLC/DAD and HPLC/UV, respectively, for a twelve days germination period (Sousa *et al*., 2007).

Fig 8. *Brassica oleracea* var. *costata* sprouts with 2 days of germination

During the seedling development, sprouts were screened at time intervals of two days for phenolics. As it was previously shown for the seeds (Ferreres *et al*., ~~2006~~), phenolic acids derivatives were the predominant phenolics found in the sprouts of *B. oleracea* var. *costata* (Figs 9 and 10).

The total phenolics content of sprouts between 2 and 12 days of germination showed significant quantitative changes (Fig 11): the phenolic compounds were depleted throughout the germination period, having a marked decrease between days 2 and 6. The total amount decreased 85%, from ca. 11.1 g/kg in sprouts with 2 days to ca. 1.6 mg/kg in sprouts with 12 days of germination. The decrease in phenolic compounds can be explained by its utilisation for cell wall biosynthesis and as antioxidants (Kubasek *et al*., 1998; Andarwulan *et al*., 1999; Ruegger *et al*., 1999). Some losses in phenolics can also be explained by the leaching of the water-soluble free phenolic acids, upon the imbibitions of dry seeds (Yang *et al*., 2001).

Besides the depletion of the seeds phenolic compounds, the synthesis of the complex kaempferol derivatives, the predominant phenolic compounds in the leaves of mature plants (Ferreres *et al*., 2005, 2006), was not accomplished during this germination period. The lack of induction of flavonoid metabolism in the sprouts may be explained by the fact that the available nutrients are being required for the primary metabolism (Bellani *et al*., 2002).

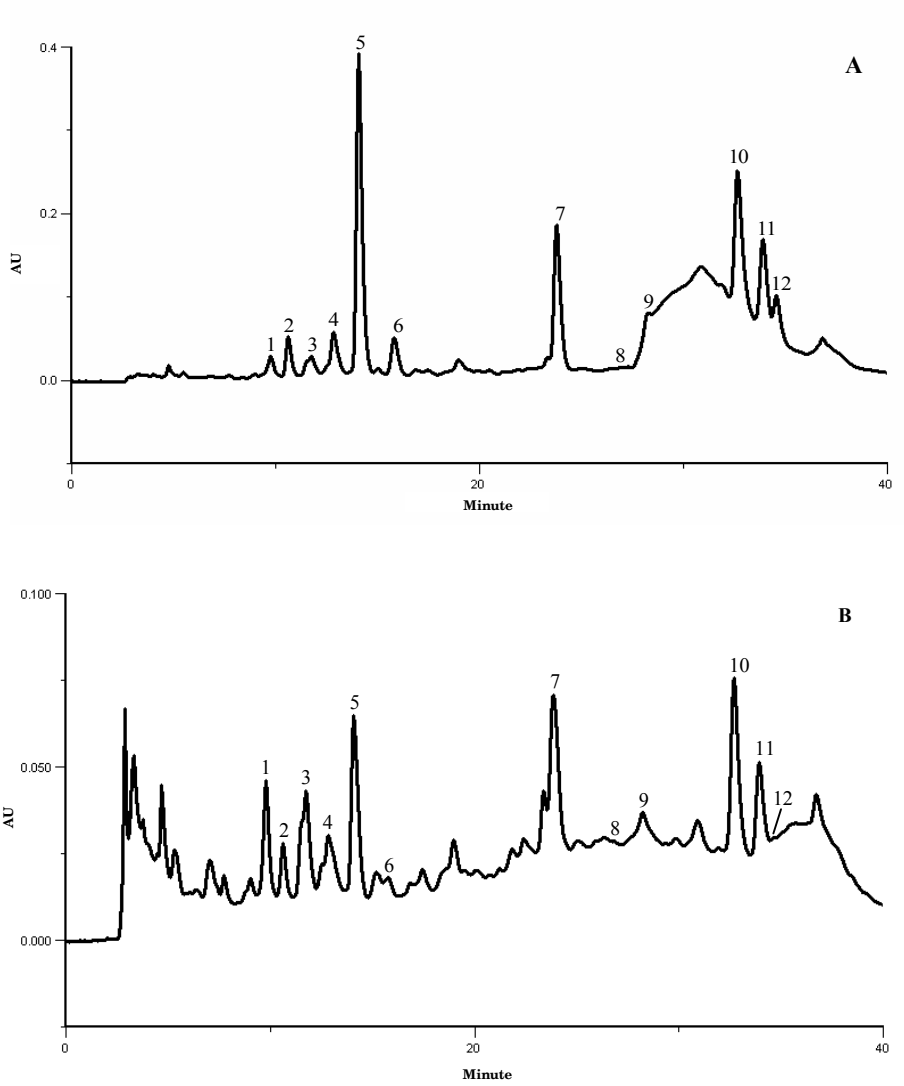


Fig 9. HPLC-DAD phenolic profile of *B. oleracea* var. *costata* sprouts aqueous lyophilized extract. Detection at 320 nm. (A) 2 days germination, **(B)** 12 days germination. Peaks: **(1)** sinapoylgentiobiose; **(2)** 1-sinapoylglucose isomer; **(3)** sinapoylgentiobiose isomer; **(4)** 1-sinapoylglucose isomer; **(5)** 1-sinapoylglucose; **(6)** kaempferol-3-*O*-(sinapoyl)-sophorotrioside-7-*O*-glucoside; **(7)** sinapoylcholine; **(8)** 1,2-disinapoylgentiobiose isomer; **(9)** 1,2-disinapoylgentiobiose isomer; **(10)** 1,2-disinapoylgentiobiose; **(11)** 1,2,2'-trisinapoylgentiobiose; **(12)** 1,2-disinapoylglucose (Sousa *et al.,* 2007)

Despite the diminution of phenolics during the seeds germinations, tronchuda cabbage sprouts can be a source of phenolics with nutritional and health benefits.

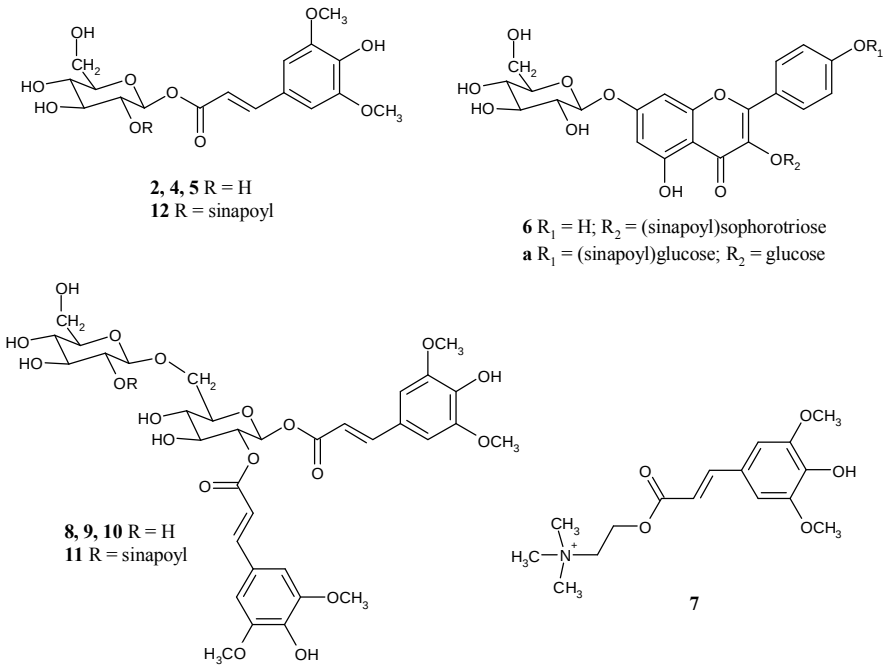


Fig 10. Chemical structures of phenolic compounds quantified in *B. oleracea* var. *costata* sprouts. **(2)** 1-sinapoylglucose isomer; **(4)** 1-sinapoylglucose isomer; **(5)** 1-sinapoylglucose; **(6)** kaempferol-3-*O*-(sinapoyl)-sophorotrioside-7-*O*-glucoside; **(7)** sinapoylcholine; **(8)** 1,2-disinapoylgentiobiose isomer; **(9)** 1,2-disinapoylgentiobiose isomer; **(10)** 1,2-disinapoylgentiobiose; **(11)** 1,2,2'-trisinapoylgentiobiose; **(12)** 1,2-disinapoylglucose; **(a)** kaempferol 3,7-*O*-diglucoside-4'-*O*-(sinapoyl)-glucoside (Sousa *et al.,* 2007)

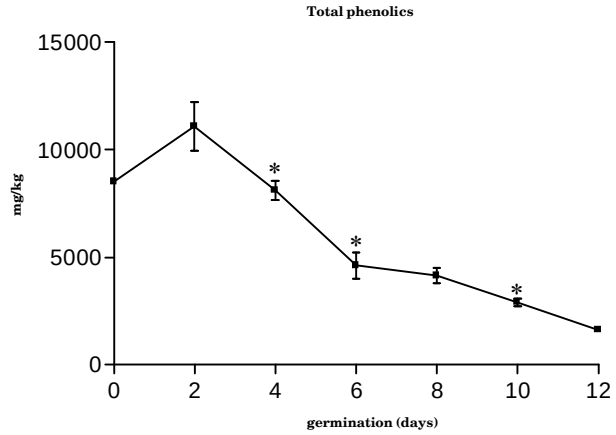


Fig 11. Evolution in total phenolic compounds content *B. oleracea* var. *costata* sprouts with germination time. * $p<0.05$, compared with the previous germination time (Sousa *et al.,* 2007)

Tronchuda cabbage flavonoids uptake by Pieris brassicae

Larvae (Fig 12) of *Pieris brassicae* L. (Lepidoptera: Pieridae) are specialists on crucifers, whereas adults (Fig 12) feed of the nectar of a variety of plants. The larvae can feed on various species of Brassicaceae, namely, cauliflower, cabbage, turnip, nasturtium and more rarely, on red cabbage and radish.

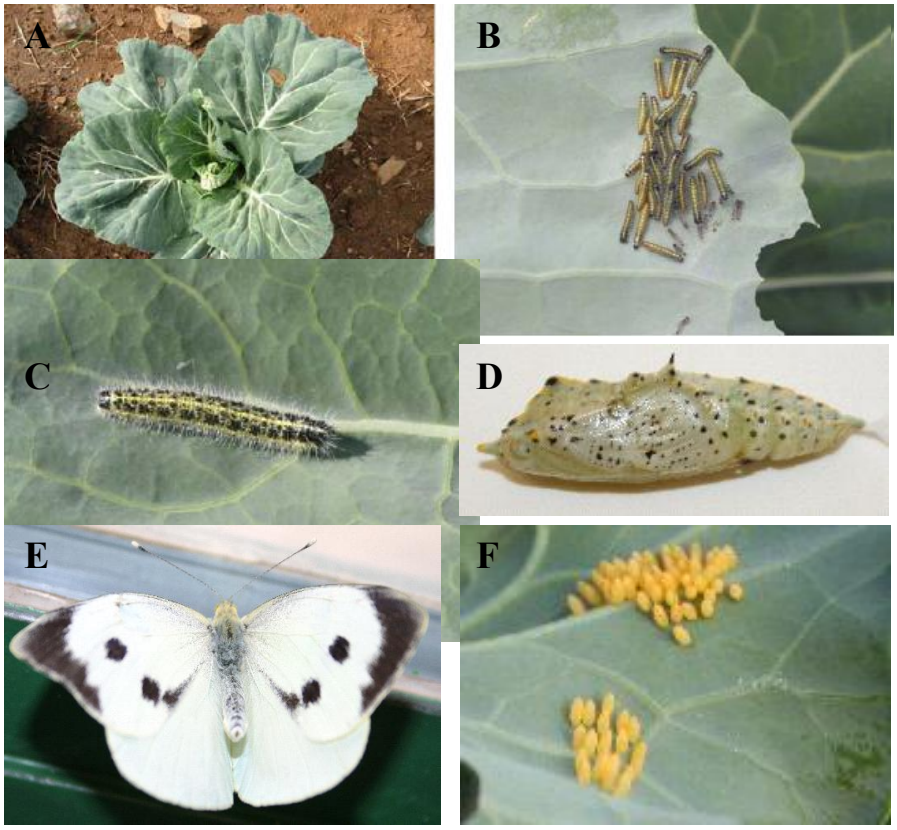


Fig 12. *Brassica oleracea* var. *costata* plant material **(A)**, *Brassica oleracea* var. *costata* infested with *Pieris brassicae* larvae **(B)**, *Pieris brassicae* larvae **(C)**, *Pieris brassicae* pupae **(D)**, *Pieris brassicae* butterfly **(E)**, *Pieris brassicae* eggs **(F)**

The flavonoid pattern of this larvae reared on the leaves of tronchuda cabbage was analyzed by HPLC-DAD-MS/MS-ESI (Fig 13). Twenty flavonoids were identified or characterized (Table 2), namely sixteen kaempferol and four quercetin derivatives. Kaempferol 3-*O*-sophoroside, a minor component of tronchuda cabbage (Table 3), was found to be the main component in *P. brassicae* (15.8%). Apart from this, only two other flavonoids present in significant amounts in tronchuda

cabbage (kaempferol 3-*O*-sophoroside-7-*O*-glucoside and kaempferol 3-*O*-sophoroside-7-*O*-sophoroside) were found in the larvae. The larvae have high amounts of quercetin derivatives (18.5%), which were present only in trace amounts in tronchuda cabbage extracts, suggesting that *P. brassicae* is able to selectively sequester these flavonoids. The occurrence of a high content of flavonoids not detectable in tronchuda cabbage extracts indicates that *P. brassicae* larvae are able to metabolize dietary flavonoids.

Table 2. Phenolic composition of *Pieris brassicae* larvae (Ferreres *et al.*, ~~2007~~)

Compound	%
1 Quercetin 3-*O*-sophoroside-7-*O*-glucoside	8.7
2 Kaempferol 3-*O*-sophoroside-7-*O*-glucoside	10.0
3 Kaempferol 3-*O*-sophoroside-7-*O*-sophoroside	6.6
4 Quercetin 3-*O*-(feruloyl)-triglucoside-7-*O*-glucoside	4.5
5 Kaempferol 3-*O*-(sinapoyl)-triglucoside-7-*O*-glucoside	5.0
6 Kaempferol 3-*O*-(feruloyl)-triglucoside-7-*O*-glucoside	5.6
7 Kaempferol 3-*O*-(*p*-coumaroyl)-triglucoside-7-*O*-glucoside	2.6
8 Kaempferol 3-*O*-(methoxycaffeoyl)-sophoroside-7-*O*-glucoside	0.5
9 Kaempferol 3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside	1.8
10 Quercetin 3-*O*-(*p*-coumaroyl)-sophoroside	3.4
11 Kaempferol 3-*O*-(*p*-coumaroyl)-triglucoside	3.3
12 Kaempferol 3-*O*-(*p*-coumaroyl)-sophoroside	13.4
13 + Kaempferol 3-*O*-(methoxycaffeoyl)-sophoroside	9.2
14 Quercetin 3-*O*-sophoroside	
15 Kaempferol 3-*O*-sophoroside	15.8
16 Kaempferol 3-*O*-(*p*-coumaroyl)-sophoroside (isomer)	2.4
17 Kaempferol 3-*O*-(disinapoyl)-triglucoside-7-*O*-glucoside	2.1
18 Kaempferol 3-*O*-(feruloyl/sinapoyl)-triglucoside-7-*O*-glucoside	1.3
19 Quercetin 3-*O*-(feruloyl)-triglucoside	1.9
20 Kaempferol 3-*O*-glucoside	1.9

Table 3. Phenolic composition of tronchuda cabbage host external leaves (Ferreres *et al.*, ~~2007~~)

Compound	%
21 + Kaempferol 3-*O*-sophorotrioside-7-*O*-glucoside	7.6
22 Kaempferol 3-*O*-(methoxycaffeoyl/caffeoyl)-sophoroside-7-*O*-glucoside	
2 Kaempferol 3-*O*-sophoroside-7-*O*-glucoside	22.9
23 Kaempferol 3-*O*-sophorotrioside-7-*O*-sophoroside	1.4
3 + Kaempferol 3-*O*-sophoroside-7-*O*-sophoroside	11.4
24 Kaempferol 3-*O*-tetraglucoside-7-*O*-sophoroside	
25 Kaempferol 3-*O*-(sinapoyl/caffeoyl)-sophoroside-7-*O*-glucoside	17.1
26 Kaempferol 3-*O*-(feruloyl/caffeoyl)-sophoroside-7-*O*-glucoside	27.8
27 + Kaempferol 3-*O*-sophorotrioside	5.1
28 Kaempferol 3-*O*-(sinapoyl)sophoroside	
29 Kaempferol 3-*O*-(feruloyl)sophorotrioside	0.4
30 Kaempferol 3-*O*-(feruloyl)sophoroside	1.1
15 Kaempferol 3-*O*-sophoroside	5.2

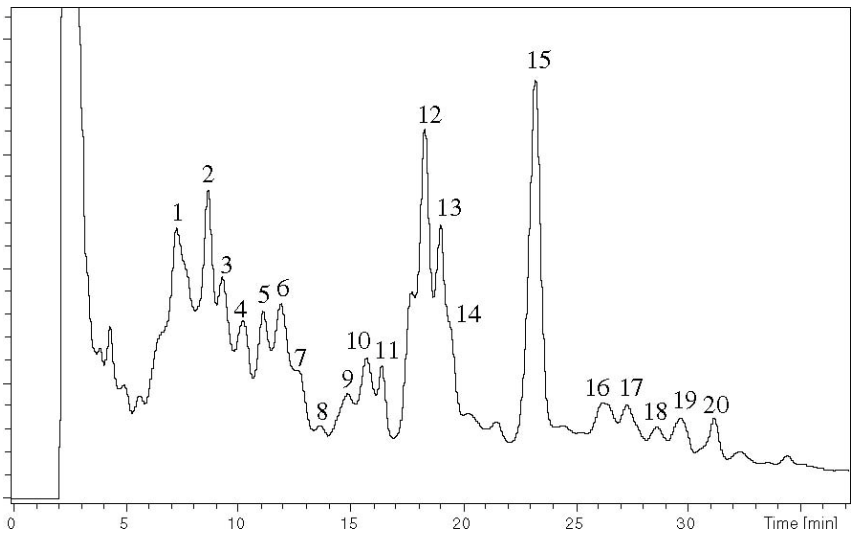


Fig 13. HPLC-DAD phenolic profile of *Pieris brassicae* larvae hydromethanolic extract. Detection at 330 nm. Peaks: **(1)** quercetin 3-*O*-sophoroside-7-*O*-glucoside; **(2)** kaempferol 3-*O*-sophoroside-7-*O*-glucoside; **(3)** kaempferol 3-*O*-sophoroside-7-*O*-sophoroside; **(4)** quercetin 3-*O*-(feruloyl)-triglucoside-7-*O*-glucoside; **(5)** kaempferol 3-*O*-(sinapoyl)-triglucoside-7-*O*-glucoside; **(6)** kaempferol 3-*O*-(feruloyl)-triglucoside-7-*O*-glucoside; **(7)** kaempferol 3-*O*-(p-coumaroyl)-triglucoside-7-*O*-glucoside; **(8)** kaempferol 3-*O*-(methoxycaffeoyl)-sophoroside-7-*O*-glucoside; **(9)** kaempferol 3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside; **(10)** quercetin 3-*O*-(p-coumaroyl)-sophoroside; **(11)** kaempferol 3-*O*-(p-coumaroyl)-triglucoside; **(12)** kaempferol 3-*O*-(p-coumaroyl)-sophoroside; **(13)** kaempferol 3-*O*-(methoxycaffeoyl)-sophoroside; **(14)** quercetin 3-*O*-sophoroside; **(15)** kaempferol 3-*O*-sophoroside; **(16)** kaempferol 3-*O*-(p-coumaroyl)-sophoroside (isomer); **(17)** kaempferol 3-*O*-(disinapoyl)-triglucoside-7-*O*-glucoside; **(18)** kaempferol 3-*O*-(feruloyl/sinapoyl)-triglucoside-7-*O*-glucoside; **(19)** quercetin 3-*O*-(feruloyl)-triglucoside; **(20)** kaempferol 3-*O*-glucoside

The results suggest that *P. brassicae* may have interest for the synthesis and/or accumulation of potential health promoting compounds, which are rather unusual in nature.

Organic acids

Internal and external leaves

Like phenolic compounds, organic acids are also known to contribute to the organoleptic characteristics of fruits and vegetables (Vaughan & Geissler, 1997). These compounds have been used in the quality control of several matrices (Silva *et al.,* 2002). In addition, they may also exert a protective role against various diseases due to their antioxidant potential (Silva *et al.,* 2005).

Tronchuda cabbage leaves organic acids were studied by HPLC/UV (Fig 14) (Ferreres *et al.*, ~~2005 a~~; Sousa *et al.*, 2005). Just like phenolic compounds at item 1.1., an organic acids study was undertaken on tronchuda cabbage cultivated under conventional and organic practices and collected at different times (Sousa *et al.*, 2005). Tronchuda cabbage internal and external leaves presented a chemical profile composed by six identified organic acids: aconitic, citric, ascorbic, malic, shikimic and fumaric acids (Fig 15). The external leaves from organic culture exhibited aconitic acid only in December.

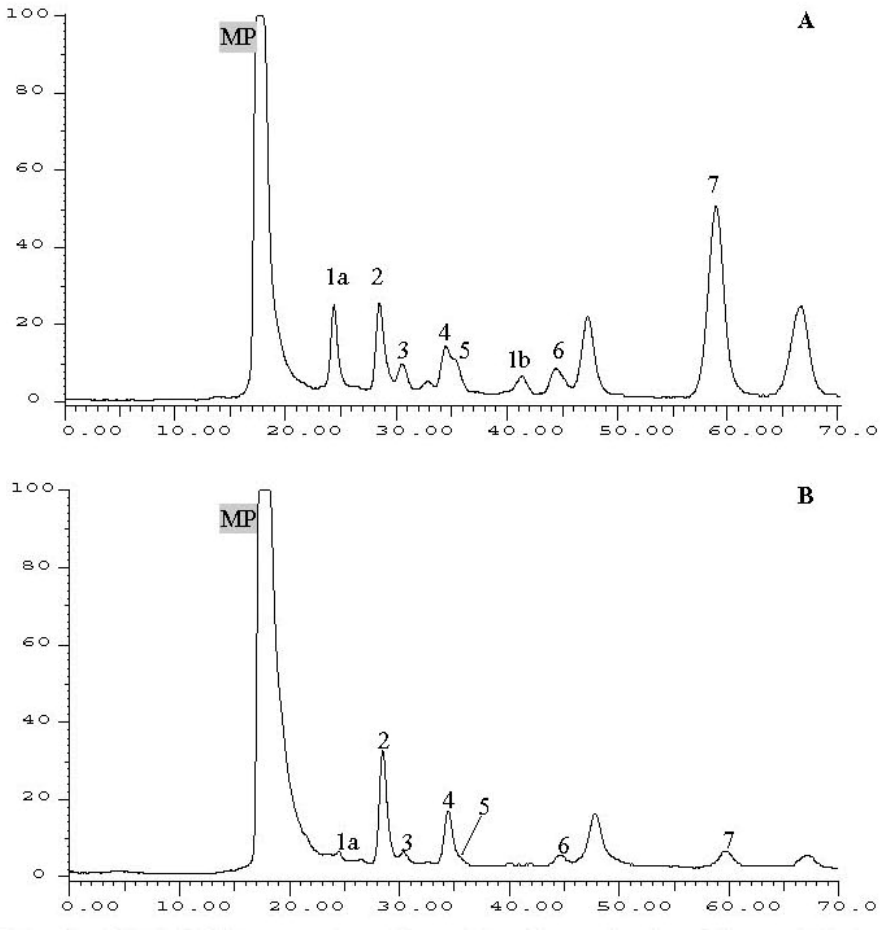


Fig 14. HPLC-UV organic acid profile of tronchuda cabbage **(A)** internal and **(B)** external leaves hot-water extracts. Detection at 214 nm. Peaks: (MP) mobile phase; (**1a** and **1b**) aconitic acid isomers; **(2)** citric acid; **(3)** ascorbic acid; **(4)** malic acid; **(5)** quinic acid; **(6)** shikimic acid; **(7)** fumaric acid (Ferreres *et al.*, ~~2005 a~~)

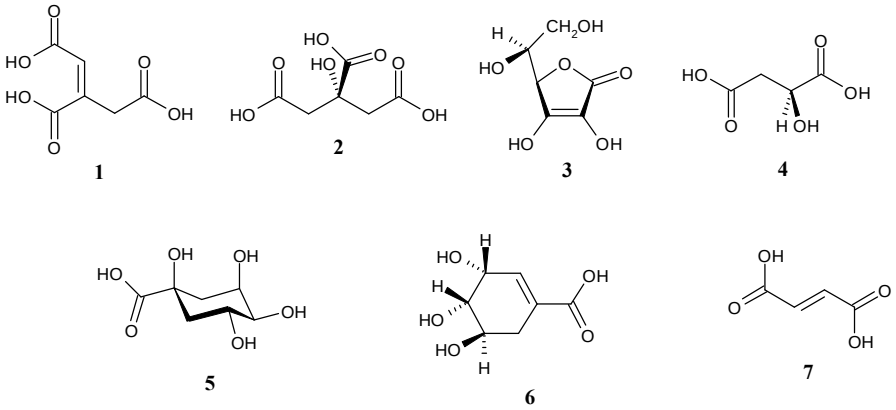


Fig 15. Chemical structures of organic acids from tronchuda cabbage external and internal leaves. **(1)** Aconitic acid; **(2)** citric acid; **(3)** ascorbic acid; **(4)** malic acid; **(5)** quinic acid; **(6)** shikimic acid; **(7)** fumaric acid

The lyophilized extracts showed a high content of organic acids, ranging from ca. 11 to 87 g/kg. In a general way, fumaric acid was the compound present in lower amounts. In the external leaves malic and ascorbic acids were the compounds present in highest amounts, representing from 69 to 93% of total acids, in samples from both organic and conventional culture. The internal leaves exhibited more variety in the relative amounts of each organic acid. Anyway, in these samples malic acid was the major compound until December, accounting for 43-87% of total identified compounds and in January ascorbic acid became the main compound, corresponding to 57-69% of total acids.

With regard to the agronomic procedure both internal and external leaves from organic culture exhibited a similar behavior. The date of collection affects the organic acids profile in the same way, with an increase of ascorbic acid relative amount in January. The highest production of organic acids in the organic samples occurred in December, following the development of the cabbage: organic tronchuda cabbage presented more developed leaves than those of conventional culture in the same period. This is in accordance with previous results (Ferreres *et al.,* ~~2005 a~~), in which the commitment of organic tronchuda cabbage cells to morphogenic developmental pathways was accompanied by the lowest level of secondary metabolites (phenolics) in December. Apparently, the nutrients are mainly used for primary metabolites biosynthesis (Santos-Gomes *et al.,* 2002), which is more related with cabbage growth.

The conventional procedure seems to affect the organic acids profile of tronchuda cabbage, resulting in some discrepancies in the relative amounts of the compounds of external and internal leaves. In the internal leaves ascorbic acid is a vestigial compound until December,

a fact that remains unexplained. October was the month in which the production of citric and malic acids by the samples from conventional practice, subjected to mineral fertilization, was higher. This could be attributed to the existence of nitrate in the fertilizer, which is available in high quantity by that time, leading to higher citric and malic acids contents, as described before (Swiderski *et al.,* 2004).

Seeds

The organic acid profile of tronchuda cabbage seeds was established by HPLC/UV (Fig 16). Tronchuda cabbage seeds presented a chemical profile composed by seven identified organic acids: aconitic, citric, ascorbic, malic, quinic, shikimic and fumaric acids. The total organic acid content of tronchuda cabbage seeds (16g/kg) (Table 4) was similar to that previously found in the leaves (Ferreres *et al.,* 2006; Vrchovska *et al.*, 2006). However, the seeds exhibited a distinct profile, in which ascorbic acid was the main compound, representing 52% of total identified organic acids, followed by citric acid (28% of compounds). As observed with tronchuda cabbage leaves (Ferreres *et al.,* 2006; Vrchovská *et al.*, 2006), shikimic and fumaric acids were minor compounds, accounting for 0.1 and 0.2% of total acids, respectively.

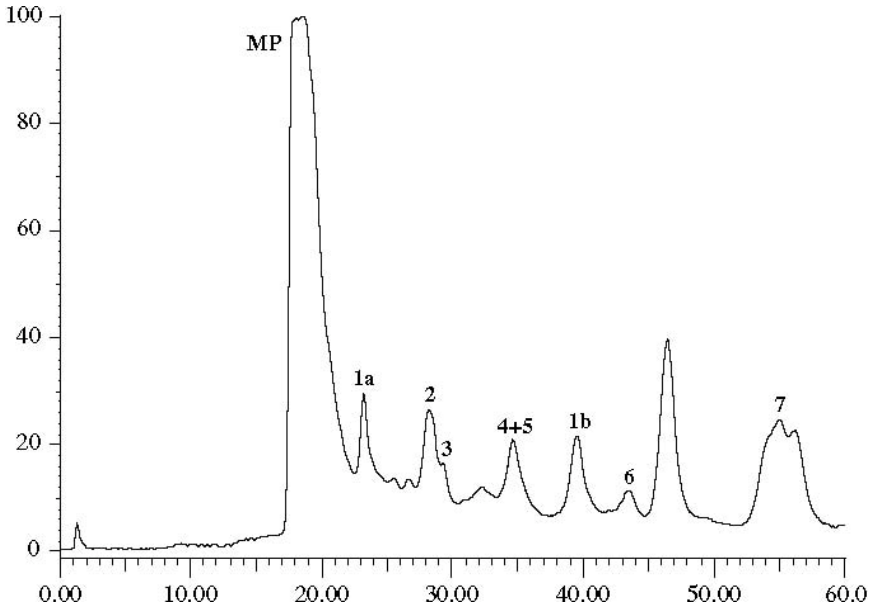


Fig 16. HPLC-UV organic acid profile of tronchuda cabbage seeds. Detection at 214 nm. Peaks: (MP) mobile phase; (**1a** and **1b**) aconitic acid isomers; **(2)** citric acid; **(3)** ascorbic acid; **(4)** malic acid; **(5)** quinic acid; **(6)** shikimic acid; **(7)** fumaric acid (Ferreres *et al.,* ~~2007~~)

Table 4. Quantification of tronchuda cabbage seeds organic acids (mg/kg, dry basis)[a] (Ferreres *et al.,* 2007)

Organic acid		Mean	SD
1a + 1b	Aconitic	170	2.5
2	Citric	4685	197
3	Ascorbic	8546	438
4+5	Malic + quinic	3049	222
6	Shikimic	18.3	0.4
7	Fumaric	39.3	0.5
	Σ	16507	

[a]Results are expressed as mean of three determinations. SD standard deviation, Σ, sum of the determined phenolic compounds

Sprouts

Just like the phenolics compounds the changes in organic acids of *Brassica oleracea* L. var. *costata* DC seeds were monitored during the first twelve days of seedling development. Sprouts were screened at time intervals of two days for organic acids.

The screening of organic acids belonging to glycolysis, tricarboxylic acid and glyoxylate cycles showed the presence of oxalic, aconitic, citric, pyruvic, malic, shiquimic and fumaric acids (Fig 17). These acids were previously described in *B. oleracea* leaves and seeds (Milkowski *et al.,* 2004; Vrchovská *et al.,* 2006; Ferreres *et al.,* 2007), with the exception of pyruvic acid that was reported for the first time in its sprouts.

The total organic acids content (Fig 18) increased rapidly during the first four days, with less significant variations thereafter. Malic acid, the major organic acid found in sprouts, greatly contributes to this result though oxalic, pyruvic and fumaric acids also increased in the same manner. Once malic acid registered the highest raise, this can indicate that besides β-oxidation, the glyoxylate cycle in which fatty acids are converted to sugars having malate as an intermediate product, was active (Eastmond *et al.,* 2001). Pyruvic acid, which was not detected in seeds and still not quantifiable in sprouts with 2 days, represented ca. 4% of the total organic acids thereafter. This may be due to its production during glycolysis, as a consequence of the increased respiration rate (Salon *et al.,* 1988).

On the other hand, aconitic, citric and shikimic acids showed a decrease between days two and twelve of germination.

Antioxidant activity

The occurrence of oxidative agents in the body arises from (i) normal intracellular biological functions, (ii) inflammatory processes and (iii) the

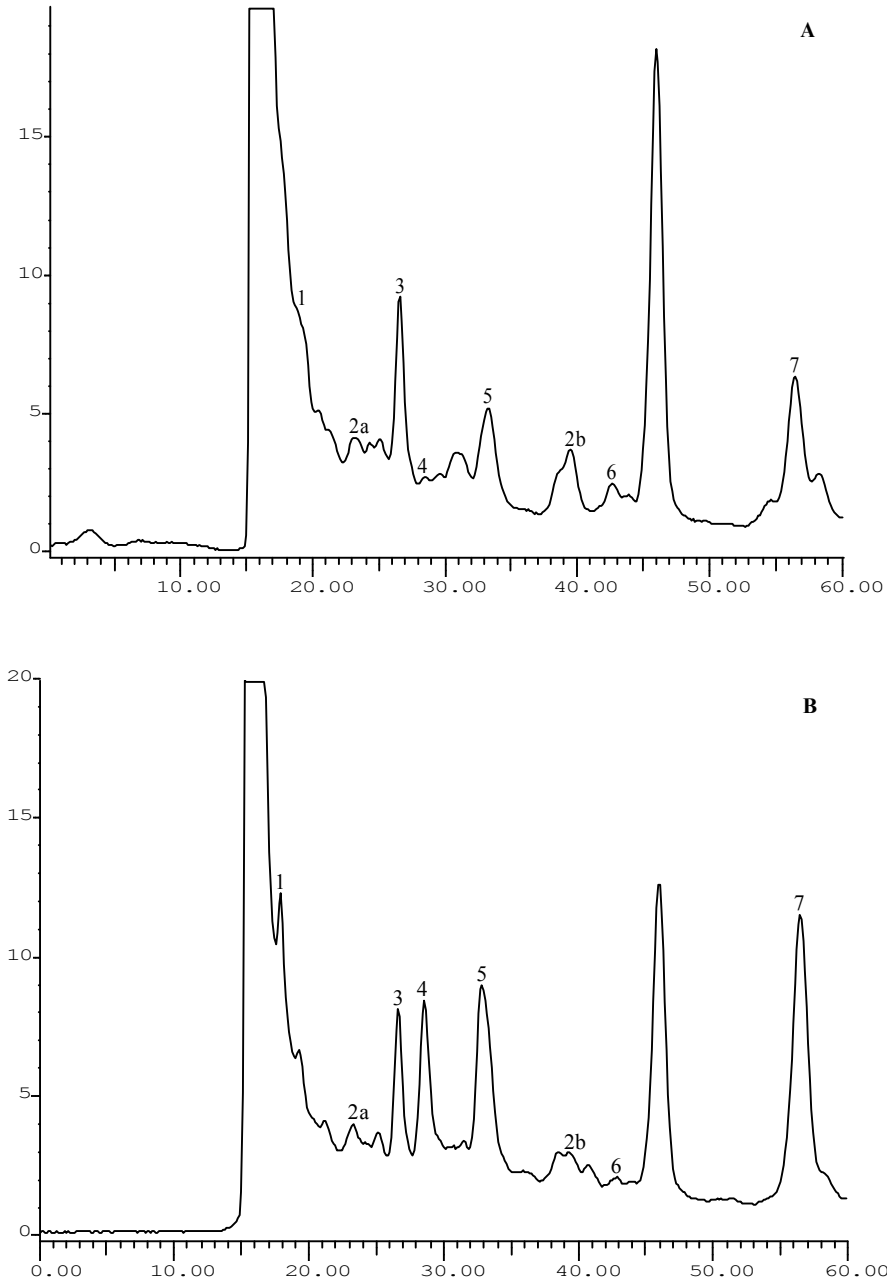


Fig 17. HPLC-UV organic acid profile of *B. oleracea* sprouts. Detection at 214 nm. **(A)** 2 days germination, **(B)** 12 days germination. Peaks: **(1)** oxalic acid; (**2a** and **2b**) aconitic acid isomers; **(3)** citric acid; **(4)** pyruvic acid; **(5)** malic acid; **(6)** shikimic acid; **(7)** fumaric acid (Sousa *et al.,* 2007)

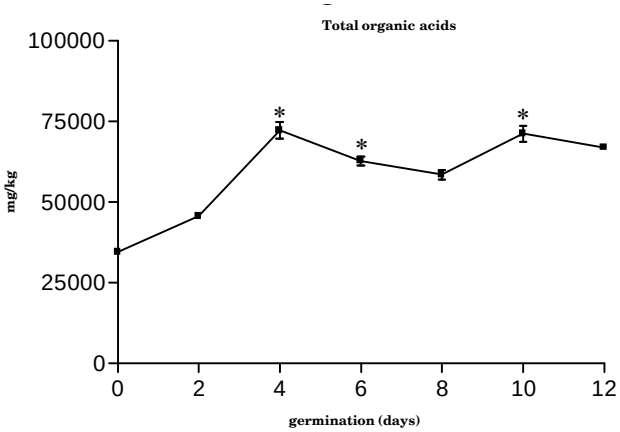


Fig 18. Evolution in total organic acids content *B. oleracea* sprouts with germination time. * $p<0.05$, compared with the previous germination time (Sousa *et al.,* 2007)

presence of xenobiotics, which can have pro-oxidant activity, but also because they induce the formation of other oxidative agents in cell (Crystal, 1991). Aerobic metabolism entails the formation of reactive oxygen species (ROS), resulting in a permanent necessity of their inactivation to maintain the homeostasis. The impairment of the pro-oxidant/antioxidant balance in favour of the former leads to an oxidative stress condition. The loss of control of endogenous processes related with cells oxygen use is a key factor for the damage caused. Such damage can affect all types of molecules, such as nucleic acids, lipids, proteins and carbohydrates. By this way, oxidative stress is involved in mutagenesis, carcinogenesis, inflammation, ageing, atherosclerosis, membrane damage and protein modification (Sies, 1991, 1993; Pulido *et al.*, 2000).

Thus, antioxidative agents are relevant in preventive medicine and therapeutics. Their presence in food is also important, not only for food preservation but also because their intake constitutes an additional defence for the organism. The antioxidant activity of tronchuda cabbage internal and external leaves and seeds aqueous lyophilised extracts was assessed by the capacity to act as scavengers of DPPH radical and ROS (superoxide radical, hydroxyl radical and hypochlorous acid) (Ferreres *et al.,* 2006, ~~2007~~).

Internal and external leaves

Both internal and external leaves displayed a concentration-dependent scavenging activity against all tested radicals (Figs 19 and 20). In addition, once in the xanthine/xanthine oxidase (X/XO) system an inhibitory effect on the enzyme itself would lead to a decrease of superoxide generation (Valentão *et al.,* 2001), the effect of the lyophilised extracts on the enzyme was checked. The results demonstrated that the

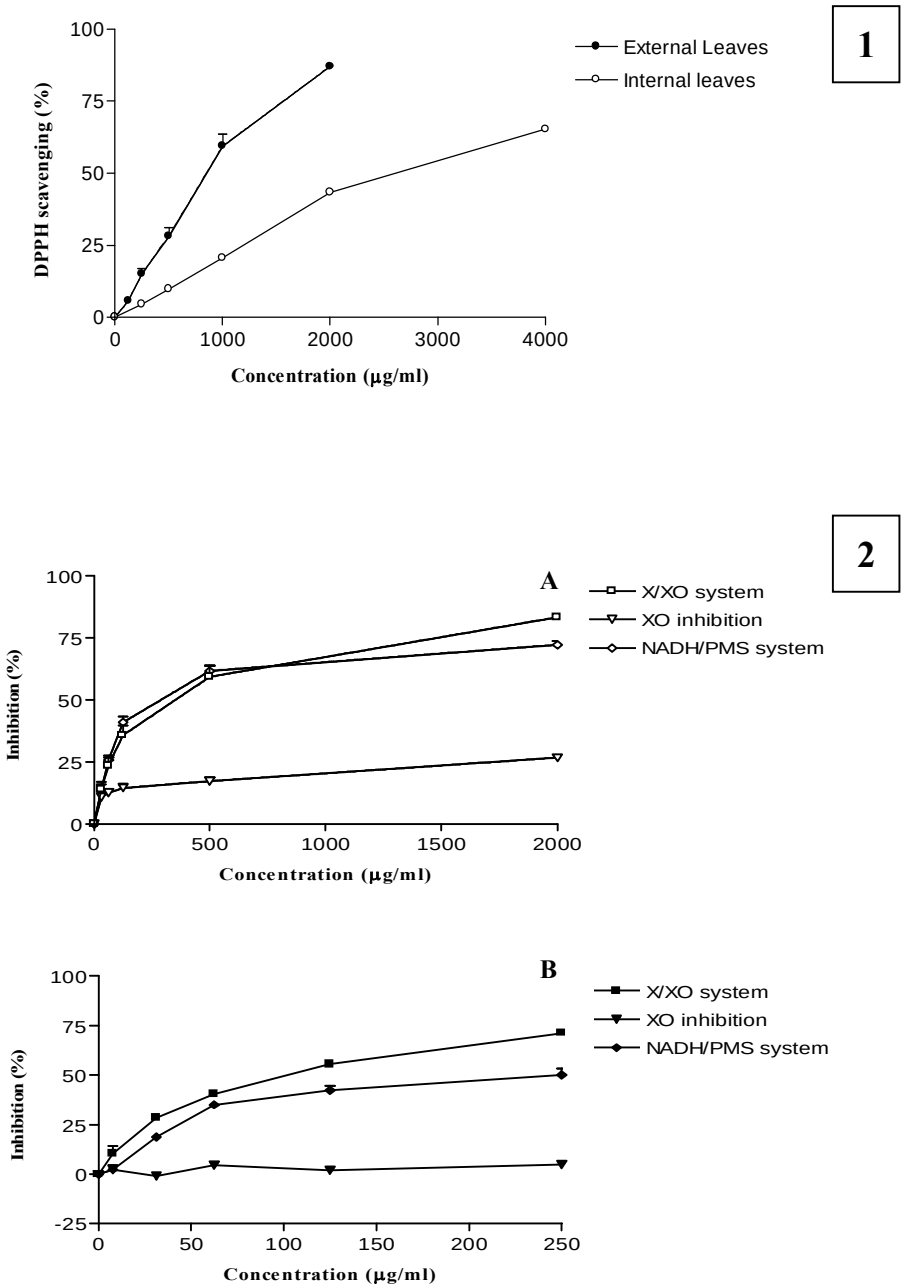


Fig 19. Antioxidant activity of tronchuda cabbage. **(1)** Effect on DPPH reduction. **(2)** Effect of tronchuda cabbage **(A)** internal and **(B)** external leaves against superoxide radical generated in an enzymatic and non-enzymatic systems and on XO activity (Ferreres *et al.,* 2006)

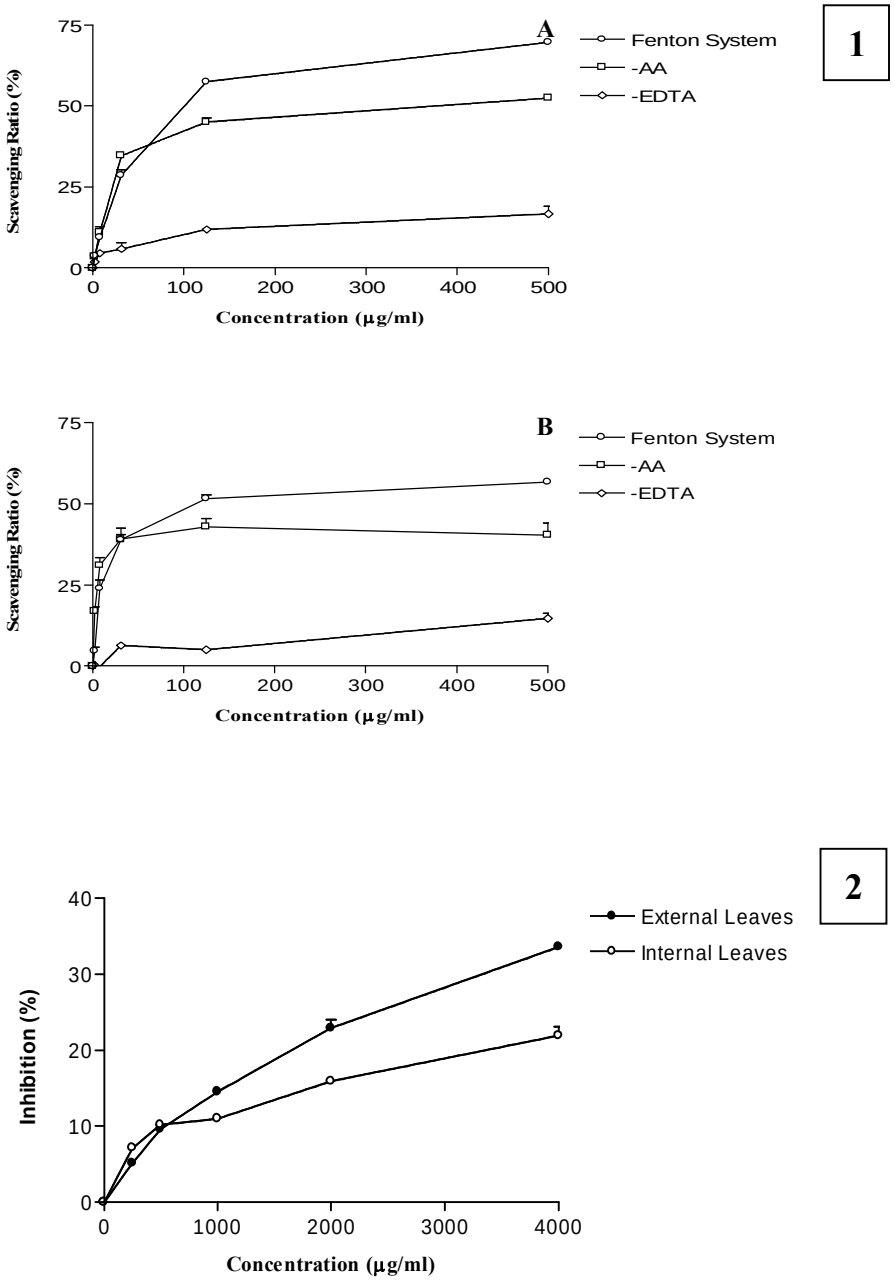


Fig 20. Antioxidant activity of tronchuda cabbage leaves. **(1)** Tronchuda cabbage **(A)** internal and **(B)** external leaves nonspecific hydroxyl radical scavenging activity, pro-oxidant activity (-AA) and specific hydroxyl radical scavenging (-EDTA). **(2)** Effect on the oxidation by HOCl (Ferreres *et al.,* 2006)

internal leaves had a weak inhibitory effect on XO, in a concentration dependent manner, while external leaves had no effect on this enzyme (Fig 19-2). Both internal an external leaves did not revealed to be effective substitutes of ascorbic acid in the Fenton system used to generate hydroxyl radicals, thus, no pro-oxidant activity was attributed to them (Fig 20-1). Attending to the fact that compounds presenting ion-binding capacity can withdraw the iron ions and render them inactive or poorly active in Fenton reactions, leading to a decrease of hydroxyl radical (Payá *et al.,* 1992), this assay was also performed in the absence of EDTA. Under these conditions both internal and external leaves had a similar behaviour, exhibiting a weak ability to chelate iron ions (Fig 20-1).

Despite the antioxidant capacity exhibited by tronchuda cabbage internal leaves, in general terms and according to the results obtained in all assays, they revealed to have a lower antioxidant potential than the external ones (Ferreres *et al.,* 2006). This can be attributed to the higher content of both phenolics and organic acids in the external leaves, which are known for their antioxidant capacity (Madhavi *et al.,* 1996). Moreover, the qualitative phenolic composition is also distinct: 3-p-coumaroylquinic acid, kaempferol 3-*O*-sophoroside-7-*O*-glucoside and kaempferol 3-*O*-sophoroside are the only common compounds in both kinds of leaves and the external leaves extract presented several flavonol glycosides, different from those detected in the internal leaves (Figs 2 and 3). Both flavonol glycosides (Tang *et al.,* 2001; Braca *et al.,* 2003) and hydroxycinnamic esters (Plumb *et al.,* 1997) are referred as having antioxidant activity. However, as the content of flavonol glycosides in external leaves is higher than that of the internal ones (95 and 54% of total phenolic compounds, respectively), this class of phenolics may represent the main contribution for the effects observed. Additionally, the higher amount of acylated flavonols in the external leaves, namely caffeoyl derivatives, can also justify their potent antioxidant capacity. In fact, the presence of an *O*-dihydroxy structure in the caffeoyl moiety, conferring great stability to the radical form and participating in the electron delocalisation, explains their high scavenging ability (Tang *et al.,* 2001). In what respects to organic acids, despite the presence of the previously mentioned seven compounds (Fig 14), quantitative differences were observed between internal and external leaves, which may condition their antioxidant capacity. Citric acid was the main compound in the external leaves, corresponding to 43% of total identified acids, while internal leaves presented ascorbic acid and the pair malic plus quinic acids in the highest amount (34%, each). Thus, it seems that citric acid may exert a relevant role in the antioxidant potential of tronchuda cabbage, as can be seen by the higher capacity exhibited by the external leaves. In fact, the ability that this acid presents to protect ascorbic acid from metal-catalysed oxidation and to function as a synergist with other antioxidants is well known (Madhavi *et al.,* 1996).

Seeds

As for the leaves, the aqueous extract of tronchuda cabbage seeds was also investigated for its capacity to act as a scavenger of DPPH radical, superoxide radical, hydroxyl radical and hypochlorous acid, exhibiting antioxidant capacity in a concentration-dependent way against all radicals (Figs 21 and 22) (Ferreres *et al.,* ~~2007~~). Like external leaves, no effect on XO was observed (Fig 21-2), but a concentration-dependent ability to chelate iron ions was noticed (Fig 22-1). Despite the antioxidant capacity, tronchuda cabbage seeds showed pro-oxidant activity for concentrations higher than 1.9 µg/ml (Fig 22-1), which can be attributed to the high amount of ascorbic acid in this matrix.

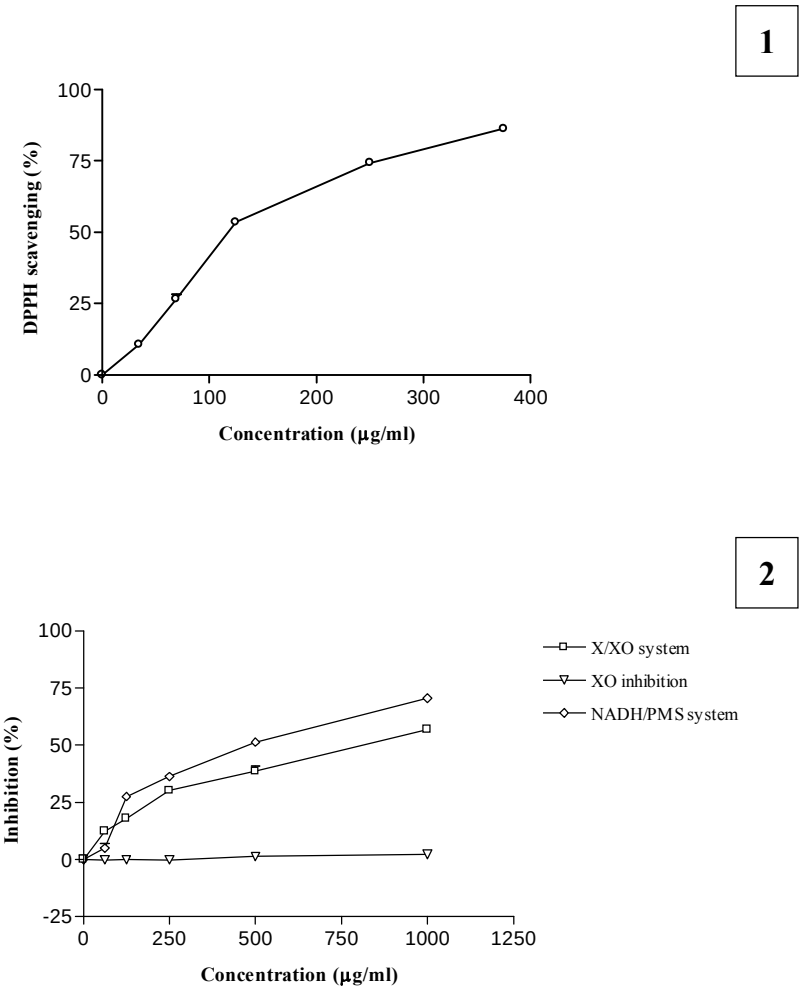


Fig 21. Antioxidant activity of tronchuda cabbage seeds. **(1)** Effect on DPPH reduction. **(2)** Effect against superoxide radical generated in enzymatic (X/XO) and chemical (NADH/PMS) systems and on XO activity (Ferreres *et al.*, ~~2007~~).

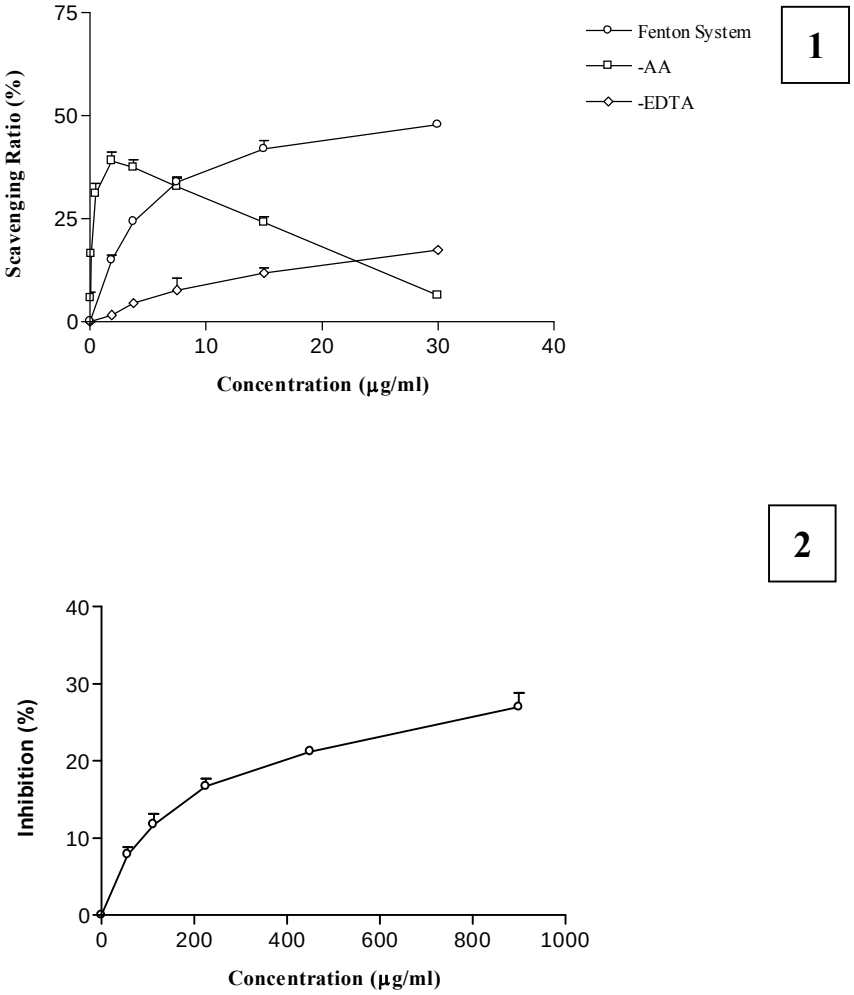


Fig 22. Antioxidant activity of tronchuda cabbage seeds. **(1)** Non-specific hydroxyl radical-scavenging activity, pro-oxidant activity (-AA) and specific hydroxyl radical scavenging (-EDTA). **(2)** Effect on the oxidation by HOCl (Ferreres *et al.,* ~~2007~~)

According to the results obtained in all assays and in comparison with data from both tronchuda cabbage internal and external leaves (Ferreres *et al.,* 2006; Vrchovská *et al.*, 2006), it could be concluded that, in general terms, tronchuda cabbage seeds exhibit higher antioxidant potential than do its leaves (Figs 19 and 20). This is not surprising, since seeds often contain the highest amount of lipids than any plant tissue, with high content of polyunsaturated fatty acids.

The existence of high levels of phenolic compounds, particularly of hydroxycinnamic derivatives and organic acids, namely ascorbic acid, in

tronchuda cabbage seeds, indicate that these compounds protect storage lipids from oxidation, as observed with tocopherols (Sattler et al., 2004), contributing to the viability of seeds and their rapid germination once oxygen demand during germination is high (Andarwulan *et al.,* 1999; Randhir & Shetty, 2003; Sattler *et al.,* 2004). In fact, either hydroxycinnamic esters (Plumb *et al.,* 1997), flavonol glycosides (Braca *et al.,* 2003; Tang *et al.,* 2001) or organic acids (Silva *et al.,* 2004) are known to possess antioxidant activity.

The results obtained in this study suggest that tronchuda cabbage seeds may constitute a good source of health-promoting compounds, namely phenolic compounds and organic acids.

Conclusions

Tronchuda cabbage may constitute a good source of health-promoting compounds, namely, organic acids and phenolic compounds. It should be emphasized that internal and external leaves supply distinct phenolics. This could be of great relevance when biological activities are considered and deserves further studies. Nevertheless, the antioxidant capacity of the different tronchuda cabbage materials follows the order: seeds > external leaves > internal leaves. The distinct chemical composition of these plant materials has a great influence in their antioxidative properties. Thus, the commercialization of standardized aqueous extracts of *B. oleraceae* var. *costata* to be used as antioxidants may be regarded as a possibility for both food and pharmaceutical industries.

Acknowledgements

The authors are grateful to Fundação para a Ciência e a Tecnologia (POCI/AGR/57399/2004 and PTDC/AGR-AAM/64150/2006) for financial support of this work.

References

Andarwulan, N., D. Fardiaz, G.A. Wattimena and K. Shetty. 1999. Antioxidant activity associated with lipid and phenolic mobilization during seed germination of *Pangium edule* Reinw, *J. Agric. Food Chem.* **47**: 3158-3163.

Ayaz, F.A., R.H. Glew, M. Millson, H.S. Huang, L.T. Chuang, C. Sanz and S.H. Ayaz. 2006. Nutrient contents of kale (*Brassica oleracea* L. var. *acephala* DC.), *Food Chem.* **96**: 572-579.

Beecher, C.W. 1994. Cancer preventive properties of varieties of *Brassica oleracea*: a review, *Am. J. Clin. Nut.* **59**: 1166S-1170S.

Behl, C. and B. Moosmann. 2002. Antioxidant neuroprotection in Alzheimer's disease as preventive and therapeutic approach, *Free Radic. Biol. Med.* **33**: 182-191.

Bellani, L.M., M. Guarnieri and A. Scialabba. 2002. Differences in the activity and distribution of peroxidases from three different portions of germinating *Brassica oleracea* seeds, *Physiol. Plant,* **114**: 102-108.

Braca, A., G. Fico, I. Morelli, F. De Simone, F. Tomé and N. De Tommasi. 2003. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species, *J. Ethnopharmacol.* **86**: 63-67.

Chu, Y.F., J. Sun, X. Wu and R.H. Liu. 2002. Antioxidant and antiproliferative activities of common vegetables, *J. Agric. Food Chem.* **50**: 6910-6916.

Crystal, R.G. 1991. Biology of free radicals - Introduction, *Am. J. Med.* **91(suppl 3C)**: 1S

Eastmond, P.J. and I.A. Graham. 2001. Re-examining the role of the glyoxylate cycle in oilseeds, *Trends Plant Sci.* **6**: 72-77.

Fahey, J.W., Y. Zhang and P. Talalay. 1997. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens, *Proc. Natl. Acad. Sci. U.S.A.* **94**: 10367-10372.

Ferreres, F., C. Sousa, P. Valentão, R.M. Seabra, J.A. Pereira and P.B. Andrade. 2007. Tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) seeds: Phytochemical characterization and antioxidant potential, *Food Chem.* **101**: 549-558.

Ferreres, F., C. Sousa, V. Vrchovská, P. Valentão, J.A. Pereira, R.M. Seabra and P.B. Andrade. 2006. Chemical composition and antioxidant activity of tronchuda cabbage internal leaves, *Eur. Food Res. Technol.* **222**: 88-98.

Ferreres, F., P. Valentão, R. Llorach, C. Pinheiro, L. Cardoso, J.A. Pereira, C. Sousa, R.M. Seabra and P.B. Andrade. 2005. Phenolic compounds in external leaves of tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC), *J. Agric. Food Chem.* **53**: 2901-2907.

Galati, G. and P.J. O'Brien. 2004. Potential toxicity of flavonoids and other dietary phenolics: Significance for their chemopreventive and anticancer properties, *Free Radical Biol. Med.* **37**: 287-303.

Gitz III, D.C., L. Liu and J.W. McClure. 1998. Phenolic metabolism, growth and UV-B tolerance in phenylalanine ammonia-lyase-inhibited red cabbage seedlings, *Phytochemistry* **49**: 377-386

Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen. 1999. Antioxidant activity of plant extracts containing phenolic compounds, *J. Agric. Food Chem.* **47**: 3952-3962.

Kubasek, W.L., F.M. Ausubel and B.W. Shirley. 1998. A light-independent developmental mechanism potentiates flavonoid gene expression in *Arabidopsis seedlings, Plant Mol. Biol.* **37**: 217-223.

Le Marchand, L. 2002. Cancer preventive effects of flavonoids: a review, *Biomed. Pharmacother.* **56**: 296-301.

Madhavi, D.L., R.S. Singhal and P.R. Kulkarni. 1996. Natural antioxidants. *In*: D.L. Madhavi, S.S. Deshpande and D.K. Salunkhe, *eds.*, Food antioxidants-Technological, toxicological and health perspectives. Marcel Dekker, New York, U.S.A., pp. 73-76.

Milkowski, C., A. Baumert, D. Schmidt, L. Nehlin and D. Strack. 2004. Molecular regulation of sinapate ester metabolism in *Brassica napus*: Expression of genes, properties of the encoded proteins and correlation of enzyme activities with metabolite accumulation, *Plant J.* **38**: 80-92.

Mwikya, S.M., J.V. Camp, R. Rodriguez and A. Huyghebaert. 2001. Effects of sprouting on nutrient and antinutrient composition of kidney beans (*Phaseolus vulgaris* var. *Rose coco*), *Eur. Food. Res. Technol.* **212**: 188-191.

Naczk, M., R. Amarowicz, A. Sullivan and F. Shahidi. 1998. Current research developments on polyphenolics of rapeseed/canola: a review, *Food Chem.* **62**: 489-502.

Payá, M., B. Halliwell and J.R.S. Hoult. 1992. Interactions of a series of coumarins with reactive oxygen species: scavenging of superoxide, hypochlorous acid and hydroxyl radicals, *Biochem. Pharmacol.* **44**: 205-214.

Plumb, G.W., K.R. Price, M.J. Rhodes and G. Williamson. 1997. Antioxidant properties of the major polyphenolic compounds in broccoli, *Free Radic. Res.* **27**: 429-435.

Pulido, R., L. Bravo and F. Saura-Calixto. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay, *J. Agric. Food Chem.* **48**: 3396-3402.

Randhir, R. and K. Shetty. 2005. Developmental stimulation of total phenolics and related antioxidant activity in light-and dark-germinated corn by natural elicitors, *Process Biochem.* **40**: 1721-1732.

Rosa, E.A.S. 1997. Glucosinolates from flower buds of Portuguese *Brassica* crops, *Phytochemistry* **44**: 1415-1419.

Ruegger, M., K. Meyer, J.C. Cusumano and C. Chapple. 1999. Regulation of ferulate-5-hydroxylase expression in *Arabidopsis* in the context of sinapate ester biosynthesis, *Plant Physiol.* **119**: 101-110.

Salon, C., P. Raymond and A.J. Pradet. 1988. Quantification of carbon fluxes through the tricarboxylic acid cycle in early germinating lettuce embryos, *Biol. Chem.* **263**: 12278-12287.

Santos-Gomes, P.C., R.M. Seabra, P.B. Andrade and M. Fernandes-Ferreira. 2002. Phenolic antioxidant compounds produced by *in vitro* shoots of sage (*Salvia officinalis* L.), *Plant Sci.* **162**: 981-987.

Sattler, S.E., L.U. Gilliland, M. Magallanes-Lundback, M. Pollard and D. DellaPenna. 2004. Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination, *Plant Cell* **16**: 1419-1432.

Sies, H. 1991. Oxidative stress: from basic research to clinical application, *Am. J. Med.* **91 (suppl 3C)**: 31S-38S.

Sies, H. 1993. Strategies of antioxidant defense, *Eur. J. Biochem.* **215**: 213-219.

Silva, B.M. P.B. Andrade, G.C. Mendes, R.M. Seabra and M.A. Ferreira. 2002. Study of the organic acids composition of quince (*Cydonia oblonga* Miller) fruit and jam, *J. Agric. Food Chem.* **50**: 2313-2317.

Silva, B.M., P.B. Andrade, P. Valentão, F. Ferreres, R.M. Seabra and M.A. Ferreira. 2004. Quince (*Cydonia oblonga* Miller) fruit (pulp, peel and seed) and jam: antioxidant activity, *J. Agric. Food Chem.* **52**: 4705-4712.

Solecka, D., A.M. Boudet and A. Kacperska. 1999. Phenylpropanoid and anthocyanin changes in low-temperature treated winter oilseed rape leaves, *Plant Physiol. Biochem.* **37**: 491-496.

Stoewsand, G.S. 1995. Bioactive organosulfur phytochemicals in *Brassica oleracea* vegetables: A review, *Food Chem. Toxicol.* **33**: 537-543.

Swiderski, A., P. Muras and H. Koloczek. 2004. Flavonoid composition in frost-resistant rhododendron cultivars grown in Poland, *Sci. Hortic.* **100**: 139-151.

Tang, Y., F. Lou, J. Wang, Y. Li and S. Zhuang. 2001. Coumaroyl flavonol glycosides from the leaves of *Ginkgo biloba*, *Phytochemistry* **58**: 1251-1256.

Valentão, P., E. Fernandes, F. Carvalho, P.B. Andrade, R.M. Seabra and M.L. Bastos. 2001. Antioxidant activity of *Centaurium erythraea* infusion evidenced by its superoxide radical scavenging and xanthine oxidase inhibitory activity, *J. Agric. Food Chem.* **49**: 3476-3479.

Vaughan, J.G. and C.A. Geissler. 1997. The New Oxford Book of Food Plants. Oxford University Press, New York, U.S.A., pp. 166-167.

Vrchovská, V., C. Sousa, P. Valentão, F. Ferreres, J.A. Pereira, R.M. Seabra and P.B. Andrade. 2006. Antioxidative properties of tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) external leaves against DPPH, superoxide radical, hydroxyl radical and hypochlorous acid, *Food Chem.* **98**: 416-425.

Wanasundara, P.K.J.P.D., F. Shahidi and M.E. Brosnan. 1999. Changes in flax (*Linum usitatissmum*) seed nitrogenous compounds during germination, *Food Chem.* **65**: 289-295.

Yang, F., T.K. Basu and B. Ooraikul. 2001. Studies on germination conditions and antioxidant contents of wheat grain, *Int. J. Food Sci. Nutr.* **52**: 319-330.