



GLUCOSINOLATES FROM FLOWER BUDS OF PORTUGUESE BRASSICA CROPS

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Abstract—Samples of green flower buds from Portuguese cabbage (*Brassica oleracea* var. *trunchuda*) and Portuguese kale (*B. oleracea* var. *acephala*) and *B. rapa* var. *rapa* were obtained from a local market and analysed for individual and total glucosinolate composition. In the *B. oleracea* types, the major glucosinolates were 2-propenyl-, 3-methylsulphinylpropyl- and indol-3-ylmethyl-, which accounted for an average of 35, 25 and 29%, respectively, of the total glucosinolate content, while in *B. rapa*, but-3-enyl- represented 86% of the total, with pent-4-enyl and 2-phenylethyl- being the other major glucosinolates. The average total glucosinolate content of the flower buds was between 2518 $\mu\text{mol } 100 \text{ g}^{-1}$ dry wt in Troncha (*B. oleracea* var. *trunchuda*) and 4979 $\mu\text{mol } 100 \text{ g}^{-1}$ dry wt in Nabo (*B. rapa*), which is much higher than the highest amounts reported for broccoli (*B. oleracea* var. *italica*), the florets of which are harvested at a similar stage in the plant's development. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In Portugal, the production of *Brassica* crops ranks in third place after potatoes and tomatoes, with a consumption of 65 kg capita⁻¹ year⁻¹ (one of the highest in the world). In the Iberian Peninsula, the high consumption of *Brassica* crops is reflected by a large consumption of flower buds of several of these species, which are similar in nature to broccoli; however, no information is available on the glucosinolate content of these products. In addition to *Brassic*as typically grown in the more northern regions of Europe, Portuguese cabbage (*B. oleracea* var. *trunchuda*) is grown for its leaves and loose heads and the leaves of Portuguese kale (*B. oleracea* var. *acephala*) are harvested by 'picking over'. They are still considered as primitive cultivars, being well adapted to the soil and climate conditions in the country and are grown the whole year round. Other species, such as *B. rapa*, are commonly used for their leaves and roots. In all of these species, bolting occurs in late winter followed by the formation of flower buds which, before opening and while still green, are used for human consumption, being normally eaten as a boiled vegetable. In the coldest regions of Portugal and Spain, they constitute a unique supply of vegetables during the winter.

Glucosinolates are sulphur-containing compounds that occur naturally in the *Brassicaceae* and which during processing may undergo enzymatic hydrolysis

by the enzyme myrosinase (thioglucoside glucosylhydrolase EC 3.2.3.1.) to yield a variety of biologically active products, including isothiocyanates, nitriles and oxazolidine-2-thiones. The nature and amount of these products and, hence, their biological activities, depend upon the original glucosinolates present in the foodstuff, and also upon the conditions of processing or cooking. The role of glucosinolates and the nature and properties of compounds derived therefrom, have been detailed in many reviews [1–3] and include toxic, antinutritional and goitrogenic effects in animals, in addition to contributing to the desirable sensory properties of brassicas and other crucifers. Other studies have indicated that glucosinolates appear to be effective in inhibiting the development of cancer [4–8]. The recently isolated isothiocyanate, sulphoraphane (1-isothiocyanate-(4*R*)-(methylsulphinyl)butane) was shown to inhibit 9,10-dimethyl-1,2-benzanthracene-induced mammary tumours, when administered 3 hr before the carcinogen [9]. These results suggested that when consumed shortly before exposure to carcinogens, these naturally occurring compounds can inhibit carcinogenesis and, thus, have a special role in cancer prevention. Current nutritional recommendations suggest an increase in the total consumption of leafy green vegetables [5, 10]. Interest has been shown in the production of novel *Brassic*as, including green-curded cauliflowers and different types of broccoli [9]. Data on glucosinolate

content from these crops have recently been published [11].

The object of the present work was to determine the nature and quantity of glucosinolates present in samples of flower buds of Portuguese *Brassica* types collected in the market at peak season (middle of January to middle of March) in the northern region of Portugal.

RESULTS AND DISCUSSION

The glucosinolates identified in the Portuguese species (*B. oleracea* var. *trunchuda* ('Troncha'), *B. oleracea* var. *acephala* ('Galega') and *B. rapa*), in the order which they were eluted from the HPLC column, were: 3-methylsulphinylpropyl- (glucoiberin), 2-hydroxybut-3-enyl- (progoitrin), 2-propenyl- (sinigrin), but-3-enyl- (gluconapin), 4-hydroxyindol-3-ylmethyl- (glucobrassicin), 2-phenylethyl- (gluconasturtiin), 4-methoxyindol-3-ylmethyl- and 1-methoxyindol-3-ylmethyl- (neoglucobrassicin). Pent-4-enyl glucosinolate was exclusive to *B. rapa*, while 4-methoxyindol-3-ylmethyl was found only in *B. oleracea*. This difference suggests the possibility of using glucosinolates as genetic markers.

The glucosinolate pattern in the flower buds of 'Troncha' and 'Galega' is similar to vegetative material of other *B. oleracea* [12, 13], while the pattern of *B. rapa* is characterized by very low amounts of indole glucosinolates (as much as 3% of the total glucosinolate content) and by three major glucosinolates, but-3-enyl- (which represents on average 85% of the total glucosinolate content), pent-4-enyl- and 2-phenylethyl-, the first two are also the major glucosinolates in turnip tops [14]. Although there were significant differences in the contents of individual glucosinolates within the different cultivars, 2-propenyl-, 3-methylsulphinylpropyl- and indol-3-ylmethyl glucosinolates were predominant in all samples of *B. oleracea*, averaging, respectively, 35, 25 and 29% of the total glucosinolate content. Within *B. oleracea*, the average total glucosinolates of bud flowers from the kale type 'Galega' are ca 40% higher than the bud flowers of 'Troncha', mainly due to differences in the two major glucosinolates, 3-methylsulphinylpropyl- and 2-propenyl-. Conversely, between the *B. rapa* types, the average total glucosinolate content in the flower buds of 'Nabo', normally used as roots, is 15% higher than in the flower buds of 'Nabo' used for tops, particularly owing to differences in the major glucosinolate but-3-enyl-.

The average total glucosinolate contents (Tables 1–4) varied between 2518 $\mu\text{mol } 100\text{g}^{-1}$ dry wt (DW) (322 $\mu\text{mol } 100\text{g}^{-1}$ fresh wt (FW)) in 'Troncha' and 4979 $\mu\text{mol } 100\text{g}^{-1}$ DW (578 $\mu\text{mol } 100\text{g}^{-1}$ FW) in 'Nabo' (a variety usually grown for roots), much higher than the highest reported concentrations for broccoli [11].

Broccoli can be considered as a parent plant material of *B. oleracea*, whereas 3-methylsul-

phinylpropyl-, 2-propenyl- and the indole glucosinolates were the major glucosinolates found in the *B. oleracea* varieties examined in this study, 4-methylsulphinylbutyl glucosinolate, reported in broccoli [11, 13], was not found. The breakdown products of the glucosinolates, particularly the isothiocyanates, are of considerable importance for flavour and the large amounts of 2-propenyl glucosinolate have implications for the sensory properties of the material. 2-Propenyl isothiocyanate is derived from 2-propenyl glucosinolate and, as well as contributing to flavour, the compound is associated with pungency, bitterness and lachrymatory effects, properties which would be expected to increase in flower buds when compared with broccoli. It is generally agreed by Iberian consumers that the flower buds of *B. rapa* are slightly bitter. Because in this species, amounts of 2-propenyl are relatively low, the other recognized bitter glucosinolate, but-3-enyl- [1], could be responsible for this characteristic. Because the amounts of the potential goitrogen, 2-hydroxybut-3-enyl glucosinolate, are limited, there does not appear to be a health risk associated with consumption of these vegetables.

EXPERIMENTAL

Plant material. Freshly harvested bunches of flower buds of Portuguese cabbage (*B. oleracea* var. *trunchuda*) traditionally called 'Troncha', of Portuguese kale (*B. oleracea* var. *acephala*) 'Galega' and of (*B. rapa* var. *rapa*) 'Nabo', cultivated for either tops or roots and grown in the northern region of Portugal, were collected in the market of Vila Real and taken to the laboratory for immediate analysis. Plants were grown in a variety of locations around the city of Vila Real (Region of Trás-os-Montes e Alto Douro, North Portugal, altitude of 453 m, Vila Real 41° 17' 18" N and 7° 44' 30" W), and collected over January and February from several retailers. This plant material is widespread in Portugal and, particularly so, in the north of Spain. From each bunch, made from selections from several plants and collected at the same time, material was randomly selected and prepared for cooking by thoroughly shredding in the traditional manner, using only the young parts (bud and leaf tips).

Extraction and isolation. Samples were freeze-dried and ca 200 mg were extracted using 90% boiling MeOH (3 ml plus 0.4 μmol glucotropaeolin as an int. standard), a process which simultaneously inactivates myrosinase. After filtration, the residue was re-extracted twice with 70% boiling MeOH (3 ml) and the extracts combined and the vol. adjusted to 10 ml. An aliquot (3 ml) was evapd to dryness and taken up in H₂O (3 ml), of which 2 ml was applied to small columns of DEAE Sephadex A25 and the absorbed glucosinolates desulphated [14]. Desulphoglucosinolates were analysed using the HPLC procedure described in ref. [15].

Table 1. Glucosinolate concentration ($\mu\text{mol } 100\text{g}^{-1}$ dry wt) in flower buds of 'Galega' kale

Sample	Glucosinolates								Total	Dry matter (%)
	1	2	3	4	5	6	7	8		
1	1278	148	1910	49	1381	136	44	183	5126	13.1
2	790	113	1789	11	733	91	31	58	3617	14.7
3	767	28	1213	0	847	80	82	50	3066	13.5
4	812	55	1695	53	987	129	139	65	3934	12.7
5	726	70	1685	49	929	201	74	45	3780	12.8
6	846	164	1178	58	694	228	0	65	3232	12.7
7	825	24	1070	0	757	165	9	29	2879	12.3
8	1039	43	816	30	910	184	8	48	3077	11.5
9	881	140	1766	38	343	71	34	34	3307	15.5
10	1068	145	1140	23	471	124	15	23	3009	14.2
11	1484	17	1947	16	687	92	14	28	4283	14.0
12	554	578	827	56	1062	333	0	43	3453	10.4
13	869	40	1664	18	998	149	0	70	3808	13.8
14	1066	48	1758	6	1388	173	0	54	4492	13.8
15	925	341	1305	15	408	84	0	26	3104	15.7
16	1052	62	1105	127	271	47	0	32	2696	16.2
17	1597	161	1349	70	462	125	0	13	3776	15.0
18	541	54	882	57	1051	160	0	69	2813	14.2
19	915	106	1527	86	904	77	0	34	3648	14.4
20	1236	69	2021	60	711	149	0	58	4303	14.7
Average	964	120	1432	41	800	140	23	51	3570	
STD	274	131	390	32	311	66	37	35	634	

1, 3-Methylsulphinylpropyl; 2, 2-hydroxybut-3-enyl; 3, 2-propenyl; 4, but-3-enyl; 5, indol-3-ylmethyl; 6, 2-phenylethyl; 7, 4-methoxyindol-3-ylmethyl; 8, 1-methoxyindol-3-ylmethyl.

Table 2. Glucosinolate concentration ($\mu\text{mol } 100\text{g}^{-1}$ dry wt) in flower buds of 'Troncha' cabbage

Sample	Glucosinolates								Total	Dry matter (%)
	1	2	3	4	5	6	7	8		
1	609	52	697	6	1013	146	60	23	2605	11.6
2	293	44	484	0	774	138	118	27	1879	13.1
3	692	59	889	18	534	81	58	15	2345	13.0
4	497	134	907	30	888	151	0	73	2680	13.5
5	535	70	492	20	1092	151	116	70	2547	13.1
6	609	249	1284	26	2351	246	50	151	4967	11.4
7	533	205	1117	19	1928	205	332	123	4460	11.8
8	903	385	1524	54	772	63	0	14	3716	13.1
9	831	443	1911	37	1108	238	71	17	4656	11.4
10	1102	165	1211	45	347	64	0	17	2951	12.8
11	683	344	1129	35	340	88	0	17	2636	12.7
12	385	206	547	12	235	56	0	8	1448	12.1
13	237	90	592	13	513	121	32	26	1624	12.4
14	492	30	844	18	893	266	0	45	2588	11.1
15	1431	14	451	25	662	146	116	33	2878	12.5
16	805	67	168	13	314	63	0	8	1437	15.8
17	182	250	319	23	397	121	0	24	1316	11.4
18	334	45	490	19	425	68	0	13	1393	15.4
19	361	73	446	12	286	87	0	8	1273	15.8
20	151	96	157	7	397	137	0	9	954	12.6
Average	583	151	783	22	764	132	48	36	2518	
STD	320	127	464	13	551	65	80	39	1179	

1, 3-Methylsulphinylpropyl; 2, 2-hydroxybut-3-enyl; 3, 2-propenyl; 4, but-3-enyl; 5, indol-3-ylmethyl; 6, 2-phenylethyl; 7, 4-methoxyindol-3-ylmethyl; 8, 1-methoxyindol-3-ylmethyl.

Table 3. Glucosinolate concentration ($\mu\text{mol } 100\text{g}^{-1}$ dry wt) in flower buds of 'Nabo' used for tops

Sample	Glucosinolates									Total	Dry matter (%)
	1	2	3	4	5	6	7	8	9		
1	20	0	8	1919	9	222	102	269	24	2575	10.4
2	23	6	9	3578	0	298	148	273	76	4412	10.2
3	30	17	0	5690	8	340	164	393	41	6683	12.2
4	40	13	0	5836	22	375	162	407	100	6954	9.9
5	101	36	0	4437	39	161	133	231	32	5169	11.2
6	84	12	0	4391	18	585	161	321	58	5630	9.7
7	0	22	0	8679	21	134	126	317	32	9330	12.4
8	0	9	0	4950	5	177	51	184	17	5391	12.4
9	9	13	14	157	7	161	45	138	8	552	11.5
10	0	19	8	2141	8	6	78	132	9	2402	11.2
11	15	17	11	1391	13	42	89	103	8	1689	11.9
12	8	12	9	1799	8	54	87	119	6	2101	11.9
13	5	12	0	4037	3	539	150	255	88	5090	11.4
14	34	0	22	5492	8	247	154	357	124	6438	11.2
15	38	11	24	4072	20	122	130	224	45	4685	12.5
16	107	7	11	2299	9	36	24	27	7	2527	12.0
17	9	8	0	3918	3	332	65	79	9	4423	11.5
18	11	41	11	3677	4	152	25	52	37	4009	12.0
19	19	0	5	2345	4	539	106	178	24	3220	10.7
20	7	3	11	2234	7	352	87	117	12	2831	10.9
Average	28	13	7	3652	11	244	104	209	38	4306	
STD	33	11	7	1952	9	173	46	114	35	2380	

1, 3-Methylsulphinylpropyl; 2, 2-hydroxybut-3-enyl; 3, 2-propenyl; 4, but-3-enyl; 5, 4-hydroxyindol-3-ylmethyl; 6, pent-4-enyl; 7, indol-3-ylmethyl; 8, 2-phenylethyl; 9, 1-methoxyindol-3-ylmethyl.

Table 4. Glucosinolate concentration ($\mu\text{mol } 100\text{g}^{-1}$ dry wt) in flower buds of 'Nabo' used for roots

Sample	Glucosinolates									Total	Dry matter (%)
	1	2	3	4	5	6	7	8	9		
1	18	16	30	955	27	14	125	138	54	1376	14.2
2	22	11	36	907	18	25	107	101	33	1260	14.6
3	22	35	29	3541	24	66	76	153	14	3960	13.7
4	27	56	36	3901	37	45	78	157	14	4350	13.6
5	24	17	24	3982	18	523	100	231	29	4948	12.4
6	29	16	37	5337	11	172	134	244	15	5995	14.8
7	25	41	47	3800	27	59	64	315	16	4394	14.0
8	36	27	40	3872	10	339	89	98	27	4538	13.3
9	128	8	21	3284	9	444	88	307	53	4342	13.1
10	103	11	14	3643	18	266	106	235	62	4456	12.5
11	18	39	28	5083	17	165	83	301	29	5762	13.5
12	16	18	27	4258	17	123	80	200	43	4780	12.8
13	0	0	0	4742	18	142	77	205	33	5216	12.8
14	16	22	26	4417	22	151	104	221	23	5003	13.5
15	0	0	27	4999	43	140	84	228	0	5521	13.1
16	28	15	28	4947	192	124	101	365	43	5842	12.2
17	24	27	31	3153	22	58	75	196	21	3608	12.1
18	26	32	33	2558	18	56	60	152	16	2949	12.3
19	22	23	24	5118	10	159	105	168	34	5662	11.2
20	27	54	24	4816	15	147	90	148	47	5369	10.9
21	19	25	0	4627	53	368	127	319	54	5592	10.8
22	0	15	0	3616	17	254	106	222	39	4268	11.1
23	18	16	15	3263	28	165	89	288	48	3928	10.6
24	17	12	15	3298	22	148	83	113	61	3768	10.7
25	23	87	20	4527	29	129	128	108	37	5087	9.5
26	0	70	17	5330	39	98	93	215	11	5873	11.9
27	18	24	17	4863	20	99	81	206	13	5341	10.5
28	25	13	20	4256	26	208	85	235	16	4883	10.7
29	18	59	19	5264	32	359	161	210	26	6147	10.4
Average	17	29	19	4375	34	163	95	216	31	4979	
STD	10	23	10	814	40	84	23	67	16	886	

1, 3-Methylsulphinylpropyl; 2, 2-hydroxybut-3-enyl; 3, 2-propenyl; 4, but-3-enyl; 5, 4-hydroxyindol-3-ylmethyl; 6, pent-4-enyl; 7, indol-3-ylmethyl; 8, 2-phenylethyl; 9, 1-methoxyindol-3-ylmethyl.

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