

## Bioactive Compounds Content and Total Antioxidant Activity of Two Savoy Cabbages

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### Abstract

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The bioactive compounds, as well as the *in vitro* antioxidant activity of two Savoy cabbage cultivars, Dama and Leticia, grown in west of Spain under similar conditions were identified, quantified and compared. We found that cv. Dama presented in general better results when compared with cv. Leticia. Cv. Dama presented higher concentrations of chlorophyll *a* (2.26 mg/100 g fresh weight), total phenolic content (102.71 mg of chlorogenic acid equivalent/100 g fresh weight) and total intact glucosinolates (195.22  $\mu$ mol of sinigrin equivalent/100 g fresh weight). Thus, cv. Dama exhibited higher values of *in vitro* antioxidant activity.

**Keywords:** *Brassica oleracea* L. convar. *capitata* var. *sabauda*; carotenoids; chlorophylls; glucosinolates; phenolic compounds; vitamin C

In recent years, increasing attention has been paid to the role of diet in human health. Several epidemiological studies have indicated that a high intake of plant products is associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer (TEMPLE 2000). These beneficial effects have been partly attributed to the compounds which possess antioxidant activity. The major antioxidants of vegetables are vitamin C, carotenoids, chlorophylls, phenolic compounds, and glucosinolates. Those antioxidants may act together to reduce reactive oxygen species level more effectively than single dietary antioxidants, because they can act as synergists (PODSEDEK 2007).

*Brassicaceae* is a wide plant family that includes different genera of cultivated plants, collectively called Brassica vegetables. Within the *Brassica oleracea* species, various types of cabbages are comprised (white, red, Savoy, Chinese), cauliflower, broccoli, Brussels sprouts, and kale. These vegetables possess antioxidant and anticarcinogenic properties (CHU *et al.* 2002).

Cabbage is one of the most important vegetables grown worldwide. Different cultivated types of cab-

bage show great variation in respect of the size, shape, and colour of leaves as well as size, shape, colour, and texture of the head (SINGH *et al.* 2006). NIEUWHOF (1969) categorised the different forms of cultivated cabbage into white cabbage, red cabbage, and Savoy cabbage.

The consumption of cabbage has increased in recent years, from 1.64 to 1.99 kg/person from 2006 to 2010 (FEPEX 2012), which may be because the benefits of these vegetables are becoming better known by the consumers. In addition, it is necessary that consumers know that the variation in the antioxidant activity of Brassica vegetables is caused by many factors such as the cultivar, maturity at harvest, growing conditions, and post-harvest storage conditions (SINGH *et al.* 2007). Previous studies, such as by SINGH *et al.* (2006), have analysed antioxidant phytochemicals content in cabbages. However, the study on the two Savoy cabbage cultivars (Dama and Leticia), most produced and consumed in Extremadura, Spain (FEPEX 2012), has not been performed yet. Thus, the new contribution of this study to the scientific knowledge was to quantify the antioxidant

compounds (vitamin C, carotenoids, chlorophylls, phenolic compounds, and glucosinolates) in the two Savoy cabbage cultivars (Dama and Leticia) not previously studied. Having precise information about the influence of the cultivar on the bioactive compounds will be useful for those consumers that demand food products with high nutritional values, health benefits, and high quality standards.

## MATERIAL AND METHODS

**Plant material.** The plants of Savoy cabbages (*Brassica oleracea* L. convar. *capitata* var. *abauda*) cvs Dama and Leticia (Syngenta Seeds BV, Enkhuizen, Netherlands) were grown under the same conditions. The crop was located at the experimental fields of La Orden-Valdesequera placed in Vegas Bajas del Guadiana in Extremadura (Spain). The plants were harvested and rapidly transported to the laboratory. A total of 12 fresh Savoy cabbage heads of each cultivar were analysed. Around 20–30 Savoy cabbage leaves were randomly selected, external, middle, and internal leaves from the cabbage heads. The Savoy cabbage heads were cut manually with a large knife, taking samples from different depths of cabbage to avoid the compositional variation in the different leaves. The leaves were packed in vacuum using plastic bags and stored at  $-80^{\circ}\text{C}$  for further analysis. The samples were analysed immediately after the freezing.

**Chemicals.** All solvents used (acetone, acetonitrile, methanol, ethanol, Folin-Ciocalteu reagent, formic acid, and hydrochloric acid) were of analytical grade quality and were purchased from Panreac (Córdoba, Spain). All standards (ascorbic acid,  $\beta$ -carotene, lutein, chlorophyll *a*, chlorophyll *b*, chlorogenic acid, gallic acid, sinapic acid, quercetin, kaempferol, sinigrin, radical chromophore ABTS, and Trolox) were purchased from Sigma-Aldrich (Madrid, Spain).

**Ascorbic acid.** Ascorbic acid was determined by HPLC-DAD following a previously published method of our group (BERNALTE *et al.* 2007). The quantification was carried out by the external standard calibration method and the results were expressed as mg of ascorbic acid/100 g of fresh weight (FW).

**Carotenoid and chlorophyll pigments determination.** Carotenoid pigments were determined by HPLC-DAD according to MÍNGUEZ-MOSQUERA and HORNERO-MÉNDEZ (1993) method. The pigments were quantified by external standard calibration, and the results were expressed as mg of  $\beta$ -carotene and mg of lutein/100 g of fresh weight (GONZÁLEZ-GÓMEZ *et al.* 2011).

Chlorophyll *a* and *b* contents were determined using multivariate calibration by means of Partial Least Squares (PLS) (FERNÁNDEZ-LEÓN *et al.* 2010). The results were expressed as mg/100 g FW.

**Total and individual phenolic compounds determination.** The determination of total phenolic content (TPC) was performed by spectrophotometry according to BERNALTE *et al.* (2007). The results were expressed as mg of chlorogenic acid equivalent/100 g FW.

Individual phenolic compounds were determined by liquid chromatography combined with mass spectrometry (HPLC-MS) according to FERNÁNDEZ-LEÓN *et al.* (2012). Standard calibration curves were made for the quantification and the results were expressed in mg/100 g FW, for each identified compound.

**Intact glucosinolates determination.** Intact glucosinolates were extracted following VALLEJO *et al.* (2003) method using HPLC-MS. The chromatographic and spectrometric conditions used were described by TIAN *et al.* (2005) and FERNÁNDEZ-LEÓN *et al.* (2012). Eleven individual glucosinolates were identified, which were quantified using the calibration curve of sinigrin as an external standard and expressed as  $\mu\text{mol}$  of sinigrin equivalent/100 g FW.

**Antioxidant activity.** Antioxidant activity (AA) was measured spectrophotometrically at 750 nm by the reaction of the plant extract with the radical chromophore ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (CANO *et al.* 1998). The results were expressed as mg of Trolox/100 g FW.

**Statistical analysis.** For the statistical studies, SPSS 15.0 software was used (SPSS Inc., Chicago, USA). The correlations were estimated with the Pearson's test at the significance levels  $P < 0.05$  and  $P < 0.01$ . The data are expressed as means  $\pm$  SD of six independent analysis and samples. Mean values were analysed by Student's test.

## RESULTS AND DISCUSSION

**Ascorbic acid.** The content of vitamin C among Brassica vegetables varies significantly between and within their subspecies (PODSEDEK 2007). In this study, vitamin C has been quantified as ascorbic acid in the two Savoy cabbage cultivars, and its contents are summarised in Table 1. No significant differences were observed between both cultivars studied regarding the ascorbic acid contents. The values obtained were higher than those measured before in Savoy cabbage by MARTÍNEZ *et al.* (2010) and similar to

Table 1. Mean values  $\pm$  standard deviations of the bioactive compounds found in Dama and Leticia Savoy cabbage cultivars

Parameters	Dama	Leticia	Significance	
Ascorbic acid <sup>1</sup>	49.06 $\pm$ 7.52	43.69 $\pm$ 4.86	ns	
$\beta$ -Carotene <sup>1</sup>	0.37 $\pm$ 0.05	0.33 $\pm$ 0.08	ns	
Lutein <sup>1</sup>	0.18 $\pm$ 0.02	0.48 $\pm$ 0.03	**	
Chlorophyll <i>a</i> <sup>1</sup>	2.26 $\pm$ 0.18	1.16 $\pm$ 0.02	**	
Chlorophyll <i>b</i> <sup>1</sup>	0.85 $\pm$ 0.04	0.50 $\pm$ 0.01	**	
TPC	102.71 $\pm$ 4.10	50.00 $\pm$ 6.51	**	
Phenolic acids	gallic <sup>1</sup>	0.89 $\pm$ 0.14	0.22 $\pm$ 0.01	**
	chlorogenic <sup>1</sup>	0.20 $\pm$ 0.01	0.54 $\pm$ 0.08	**
	sinapic <sup>1</sup>	1.59 $\pm$ 0.27	0.46 $\pm$ 0.07	**
Flavonoids	Quercetin <sup>1</sup>	1.21 $\pm$ 0.03	1.27 $\pm$ 0.16	ns
	Kaempferol <sup>1</sup>	1.47 $\pm$ 0.03	1.45 $\pm$ 0.15	ns
AA	56.62 $\pm$ 2.72	43.53 $\pm$ 5.02	**	

<sup>1</sup>expressed as mg/100 g FW; TPC – total phenolic compounds expressed as mg of chlorogenic acid equivalent/100 g FW; AA – antioxidant activity expressed as mg Trolox/100 g FW; \*\*significantly differences among the values ( $P < 0.01$ ); ns – no significance

those obtained by PODSEDEK *et al.* (2006). Generally, among Brassica vegetables, white cabbage and Savoy cabbage are the poorest source of vitamin C, however, in many countries are these the most popular species of Brassica vegetables (PODSEDEK 2007).

**Carotenoid and chlorophyll pigments.** The contents of carotenoid and chlorophyll pigments are summarised in Table 1. The results obtained were comparable to other reported values (SINGH *et al.* 2006, 2007; FERNÁNDEZ-LEÓN *et al.* 2010; MARTÍNEZ *et al.* 2010).

No significant differences were observed between cvs Dama and Leticia Savoy cabbage cultivars in  $\beta$ -carotene concentration. However, lutein concentration was significantly higher in cv. Leticia (0.48 mg lutein/100 g FW) than in cv. Dama (0.18 mg lutein/100 g FW).

With regard to the chlorophyll pigments (Table 1), chlorophyll *a* was the most abundant pigment found in the Savoy cabbage studied, its amount being higher in cv. Dama than in cv. Leticia (2.26 and 1.16 mg chlorophyll *a*/100 g FW, respectively). There were also significant differences between the two cultivars of Savoy cabbage studied in chlorophyll *b* content when also revealed a higher value in cv. Dama (0.85 mg chlorophyll *b*/100 g FW) than in cv. Leticia (0.50 mg chlorophyll *b*/100 g FW). Similar

concentrations were reported by other authors for other green vegetables (KHACHIK *et al.* 1986; GARCÍA *et al.* 2005; NOICHINDA *et al.* 2007).

**Total and individual phenolic compounds.** The measured of amounts of total phenolic compounds (TPC) were statistically different between both cultivars, the TPC content being higher in cv. Dama than in cv. Leticia (102.71 and 50.00 mg of chlorogenic acid equivalents/100 g FW, respectively) (Table 1). The values obtained in this study were similar to those obtained by PODSEDEK *et al.* (2006) and MARTÍNEZ *et al.* (2010) for Savoy cabbage, and similar to those obtained for Chinese cabbage by BAHORUN *et al.* (2004), the phenolic contents ranging from 15.3 mg/100 g FW in white cabbage to 337.0 mg/100 g FW in broccoli (CHU *et al.* 2002; WU *et al.* 2004).

Considering the individual phenolic compounds (Table 1), our findings are consistent with the previously published works (MARTÍNEZ *et al.* 2010). Significant differences were observed between the contents of phenolic acids with higher values of gallic and sinapic acids in cv. Dama (0.89 mg gallic acid/100 g FW and 1.59 mg sinapic acid/100 g FW) than in cv. Leticia (0.22 mg gallic acid/100 g FW and 0.46 mg sinapic acid/100 g FW). However, the chlorogenic acid content was higher in cv. Leticia than in cv. Dama (0.54 and 0.20 mg chlorogenic acid/100 g FW, respectively). With flavonoids concentrations no significant differences were observed between both cultivars of Savoy cabbage.

**Intact glucosinolates.** Brassica vegetables are the main source of glucosinolates (GS) in human diet. It is known that particular species, varieties, and cultivars differ with regard to the type and amount of the GS present (FENWICK *et al.* 1983). It should be noted that isothiocyanates are derived from hydrolysis of glucosinolates and have been confirmed to possess protective effects against cancer (FAHEY *et al.* 2001; KECK & FINLEY 2004). Some of them are: sulforaphane derived from glucoraphanin; phenethyl isothiocyanate derived from gluconasturtiin; allyl isothiocyanate from sinigrin; indole-3-carbinol from glucobrassicin; and crambene from progoitrin (KECK & FINLEY 2004).

The determined glucosinolates compounds, in the two Savoy cabbage cultivars, have been arranged in Table 2 taking into account their chemical structure. The reported results are in agreement with previous studies of other Savoy cabbage cultivars (CISKA *et al.* 2000; CARTEA *et al.* 2008).

Significant differences were observed between Savoy cabbage cultivars in terms of total aliphatic glucosinolates (Table 2). For this group of compounds,

Table 2. Mean values  $\pm$  standard deviations of intact glucosinolates contents measured in Dama and Leticia Savoy cabbage cultivars

Glucosinolates	Dama	Leticia	Significance
<b>Alkyl GS</b>			
Glucoraphanin <sup>1</sup>	0.47 $\pm$ 0.10	0.26 $\pm$ 0.08	**
Glucoiberin <sup>1</sup>	3.91 $\pm$ 0.22	3.72 $\pm$ 0.60	ns
Glucoalyssin <sup>1</sup>	1.84 $\pm$ 0.21	2.10 $\pm$ 0.39	ns
<b>Alkenyl GS</b>			
Sinigrin <sup>1</sup>	15.33 $\pm$ 0.46	14.57 $\pm$ 1.06	ns
Gluconapin <sup>1</sup>	8.11 $\pm$ 1.17	16.85 $\pm$ 1.78	**
Glucobrassicinapin <sup>1</sup>	47.34 $\pm$ 1.17	35.32 $\pm$ 2.76	**
<b>Hydroxyalkenyl GS</b>			
Progoitrin <sup>1</sup>	13.81 $\pm$ 1.33	13.79 $\pm$ 1.14	ns
Total aliphatic GS <sup>1</sup>	90.82 $\pm$ 3.04	86.61 $\pm$ 2.71	*
Glucobrassicin <sup>1</sup>	63.62 $\pm$ 2.33	44.02 $\pm$ 3.73	**
4-Methoxyglucobrassicin <sup>1</sup>	13.07 $\pm$ 0.86	7.40 $\pm$ 2.04	**
Neoglucobrassicin <sup>1</sup>	27.72 $\pm$ 0.63	13.76 $\pm$ 0.36	**
Total Indole GS <sup>1</sup>	104.41 $\pm$ 2.24	65.18 $\pm$ 2.19	**
Gluconasturtiin	nd	nd	ns
Total aromatic GS <sup>1</sup>	nd	nd	ns
Total glucosinolates <sup>1</sup>	195.22 $\pm$ 3.09	151.79 $\pm$ 3.93	**

<sup>1</sup>expressed as  $\mu\text{mol}$  of sinigrin equivalent/100 g FW; \* $P < 0.05$ ; \*\* $P < 0.01$ ); ns – no significance; nd – no detected

cv. Dama exhibited higher amounts than cv. Leticia (90.82 and 86.61  $\mu\text{mol}$  sinigrin equivalents/100 g FW, respectively). However, within the aliphatic glucosinolates different behaviour could be observed depending on the alkyl, alkenyl or hydroxyalkenyl chemical structures. Glucoraphanin (alkyl GS) and glucobrassicinapin (alkenyl GS) abundances were higher in cv. Dama than in cv. Leticia. Nevertheless, gluconapin (alkenyl GS) was more abundant in cv. Leticia than in cv. Dama. On the other hand, no significant differences were observed with glucoiberin and glucoalyssin (alkyl GS), sinigrin (alkenyl GS) and progoitrin (hydroxyalkenyl GS).

Indole derivatives constitute the most abundant glucosinolates in both cultivars (Table 2). Higher concentrations of total indole glucosinolates were found in cv. Dama (104.41  $\mu\text{mol}$  sinigrin equivalents/100 g FW), than in cv. Leticia, with 65.18  $\mu\text{mol}$  sinigrin equivalents/100 g FW. The same behaviour was observed for the individual compounds, the contents of glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin being significantly higher in

cv. Dama than in cv. Leticia. Glucobrassicin is the most abundant glucosinolate in both cultivars. Glucobrassicin is the dominating GS in all vegetables of *B. oleracea* species and, depending on the cultivar, this GS accounted for 10% to > 65% of the total (CISKA *et al.* 2000). In this study, glucobrassicin GS represented 33% of the total GS with cv. Dama and 29% with the Leticia cultivar.

Among the aromatic glucosinolates, gluconasturtiin was not detected in either cultivar of Savoy cabbage (Table 2), which is the case with other varieties of cabbage (CISKA *et al.* 2000) while, according to the results reported by CARTEA *et al.* (2008), the concentration of this GS in cabbages ranges from 0.00 to 1.38  $\mu\text{mol}$  sinigrin equivalents/100 g FW.

Finally, the results summarised in Table 2 show significant differences in the concentration of total glucosinolates between the cultivars studied, which was higher in cv. Dama (195.22  $\mu\text{mol}$  sinigrin equivalents/100 g FW) than in cv. Leticia (151.79  $\mu\text{mol}$  sinigrin equivalents/100 g FW).

**Antioxidant activity.** Antioxidant activity (AA) found in cv. Dama (56.62 mg Trolox/100 g FW) was statistically higher than in cv. Leticia (43.53 mg Trolox/100 g FW) (Table 1). The values were similar to those obtained by other authors, e.g. PODSEDEK *et al.* (2006).

According to the Pearson's test, AA values correlated with the contents of biocompounds determined in this study (Table 3). This correlation was not found for  $\beta$ -carotene.

Table 3. Correlations among the different bioactive compounds determined and the antioxidant activity, expressed as Pearson's correlation coefficients

Bioactive compounds	Pearson's correlation coefficients
	AA
Ascorbic acid	0.450*
Lutein	0.856**
Chlorophyll <i>a</i>	0.876**
Chlorophyll <i>b</i>	0.894**
TPC	0.913**
Phenolic acids	0.844**
Flavonoids	0.904**
Total glucosinolates	0.884**

AA – antioxidant activity; TPC – total phenolic compounds; phenolic acids – gallic, chlorogenic, and sinapic acids; flavonoids – quercetin and kaempferol; \*correlation is significant at the 0.05 level (2-tailed); \*\*correlation is significant at the 0.01 level (2-tailed)

Regarding phenolic compounds, in many *in vitro* studies demonstrated these compounds a higher antioxidant activity than antioxidant vitamins and carotenoids (VINSON *et al.* 1995). Thus, in this study high values of antioxidant activity were strongly associated (Pearson's correlation coefficient  $r = 0.913$ ,  $P < 0.01$ ) with the content of phenolic compounds in this vegetable (WU *et al.* 2004; PODSEDEK 2007) (Table 3). Among the phenolic compounds, the flavonoid compounds are very abundant in the brassicas and therefore contribute in an important way to the antioxidant activity of these vegetables (CHU *et al.* 2002).

Additionally, glucosinolates are characterised by their high antioxidant capacity (MORENO *et al.* 2006; VERKERK *et al.* 2009). Therefore, as shown in Table 3, a strong correlation exists between the total content of glucosinolates and antioxidant activity ( $r = 0.884$ ,  $P < 0.01$ ).

## CONCLUSIONS

After comparing the results obtained in this research work, we found that the Dama cultivar had higher contents of most of the studied biocompounds than the Leticia Savoy cabbage cultivar. In addition, cv. Dama exhibited higher values of *in vitro* antioxidant activity, because this cultivar contained higher amounts, almost double, of the main antioxidants compounds, such as total phenolic compounds, chlorophyll *a*, and total indole glucosinolates. In addition, remarkable was the elevated amount of glucobrassicin found in cv. Dama. Finally, it was observed that glucoraphanin content (sulforaphane precursor) was about 50% higher in cv. Dama than in Leticia cultivar.

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