

# Antioxidant Capacity of Different Broccoli Cultivars at Various Harvesting Dates

S. Graeff-Hönninger, J. Pfenning, V. Gutsal, S. Wolf, S. Zikeli, W. Claupein

**Abstract**—Broccoli is considered as being a rich source of AOX like flavonoids, polyphenols, anthocyanins etc. and of major interest especially in the organic sector. However, AOX is environment dependent and often varies between cultivars. Aim of the study was to investigate the impact of cultivar and harvest date on AOX in broccoli. Activity of the AOX was determined using a Photochem®-Analyzer and a kit of reagent solutions for analysis. Results of the study showed that the lipid (ACL) and water-soluble antioxidant potential (AWC) of broccoli heads varied significantly between the four harvesting dates, but not among the different cultivars. The highest concentration of ACL was measured in broccoli heads harvested in September 2011, followed by heads harvested at the beginning of July in 2012. ACW was highest in heads harvested in October 2011. Lowest concentrations of ACW were measured in heads harvested in June 2012. Overall, the study indicated that the harvest date and thus growing conditions seem to be of high importance for final antioxidant capacity of broccoli.

**Keywords**—Antioxidant activity, open pollinating, organic agriculture.

## I. INTRODUCTION

THE concept of functional food is one of the most promising fields of innovations both in nutritional science and food industry. At the same time, a different group of health-conscious consumers is very interested in organic and regionally grown vegetables.

Therefore, horticulture is becoming a very interesting field of study for scientists who look for potential health promoting vegetables with high antioxidant activity. Sufficient epidemiological studies concluded that naturally occurring antioxidants (AOX) of vegetables suppress reaction with free radicals and protect cells against oxidative stress [1]. Vegetables are therefore being widely analyzed to find cultivars and genotypes with highest antioxidant (AOX) amount and activity [2], [3].

Many studies showed convincing evidences that AOX of vegetables effectively neutralize reactive oxygen species (ROS) and protect cells against oxidative stress and cancer [1]. Reactive oxygen species (ROS) play a critical role in cardiovascular diseases, inflammatory diseases, neurodegenerative disorders, cancer and aging because they are highly reactive to biological molecules and can damage DNA, proteins, carbohydrates as well as lipids. Diets rich in foods containing antioxidant compounds, such as Brassica vegetables, could help prevent these pathologies since they

contribute both to the first and second defense lines against oxidative stress [4]. As a result, antioxidant compounds are important for limiting damaging oxidative reactions in cells, which may affect to the development of heart diseases and cancer.

In this context, broccoli was one of the most studied vegetables due to its promising antioxidant characteristics [5]. The main antioxidative components present in Brassica vegetables are water-soluble and include phenolic compounds (mainly flavonoids) and vitamins (mainly ascorbic acid). Other antioxidant constituents are lipid-soluble such as carotenoids and tocopherols. All of these phytochemicals contribute to the reported antioxidant, anti-carcinogenic and cardiovascular protective activities. Antioxidants like flavonoids, glucosinolates, phenolics, vitamin C, A and E, carotenoids (including lutein and zeaxanthin) are known to be superabundant in broccoli plants [6], [7]. However, the amount of AOX depends on cultivar, environment and crop management [8], [9]. These compounds are of major interest for the development of new cultivars with an appropriate antioxidant profile, from which high quality products with an added value can be produced.

The objectives of this study were: (i) to determine the range of antioxidant (AOX) capacity and activity in broccoli cultivars grown under organic farming conditions and, (ii) to determine the influence of growing seasons and harvest date on AOX.

## II. MATERIAL AND METHODS

### A. Field Trial

Eight cultivars of broccoli were grown according to organic farming guidelines on an experimental field of the research station “Kleinhohenheim”, University of Hohenheim, Germany in autumn 2011 and spring 2012. Chosen cultivars consisted of two commercially available open-pollinating varieties (Limba and Miranda), two cultivars from organic breeders (KSV Che-Gre, KSV Th-Can) which are still in breeding process and four different hybrids (Marathon, Monterey, Ironman, Batavia) having a similar phenotype, but different ripening times.

Broccoli was planted beginning of July (2011) and mid of May (2012) at a rate of 4.5 plants per m<sup>2</sup>. The trial set up was a randomized block design with three replications. Soil N<sub>min</sub> content was determined in all plots at planting and after final harvest. If necessary, broccoli plants were irrigated to ensure a sufficient water supply throughout the main growing period. To ensure optimum nitrogen supply of broccoli plants, the trials were integrated into the crop rotation after one year of clover grass and additionally fertilized at planting with

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Bioilsa® (7 % N, 7 % P, 7 % K) to achieve a total N supply of 300 kg N ha<sup>-1</sup>.

Harvest of broccoli florets started beginning of September (2011) and mid of June (2012) as soon as 50 % of the plants of a plot had reached a minimum marketable head size of Ø 7 cm or tended to show enhanced flower development. Harvests were accomplished in a two week interval. Florets were trimmed to market specifications for broccoli and graded for diameter, weight, discoloration, and hollow stem.

### B. Analysis

After harvest, each broccoli floret was manually chopped, cooled down with liquid nitrogen and freeze-dried. After freeze drying each sample was homogenized to a powder and stored at room temperature until analyzed.

For the determination of antioxidant capacity ~0,015 g of the freeze-dried samples were mixed with 5 ml of bidistilled water. The resulting suspension was mixed for 30 s and centrifuged at 15.000g for 15 min. Only clean and transparent supernatant was used for analysis.

Activity of AOX in the sample was determined using a Photochem®-Analyzer (photochemiluminescence measuring device) and a kit of reagent solutions for analysis. The instrument compares the sample solution with standard solutions and presents the activity of AOX in equivalent units of the standard. To measure activity of water soluble AOX (ACW value) the calibration curve was plotted using ascorbic acid as a standard. ACL (activity of lipid soluble AOX) values were obtained by comparing sample activity with Trolox® standard.

### C. Statistics

The statistical analysis was done with SAS 9.3 using analyses of variance ("proc mixed") under the prediction of  $p \leq 0.05\%$ . Date repetitions were taken into account with the command "random". Data sets were proved for interactions between genotype and date. If there was no significant interaction, the factors date and genotype were analyzed separately.

## III. RESULTS AND DISCUSSION

The lipid- (ACL) and water-soluble (ACW) antioxidant potential of broccoli florets varied significantly between the four harvesting dates (Figs. 1 and 2), but not among the tested cultivars (Table I). The cultivar 'Batavia' showed the highest concentration of ACW over all tested cultivars with 14.1 mg g<sup>-1</sup> dry matter (DM), while 'Ironman' had the lowest concentration with 9.5 mg g<sup>-1</sup> DM. High concentrations in

ACW, but rather low concentrations in ACL could be observed for the open pollinating cultivar 'Miranda'.

In general, ACL values ranged between 5.7 and 8.5 mg g<sup>-1</sup> DM. Determined values of ACL and ACW were in accordance to literature. However, some studies [10]-[12] also noted genotypic variation for antioxidant potential among cultivars, which was not the case in our experiments. Based on the overall amount of ACL and ACW all tested cultivars could be recommended.

In a second step, the impact of harvest date and growing season on ACL and ACW was investigated. The highest concentration of ACL was measured in broccoli florets harvested in September 2011 with 8.5 mg g<sup>-1</sup> DM (expressed as trolox equivalents), followed by florets harvested at the beginning of July in 2012 with 7.8 mg g<sup>-1</sup> DM (Fig. 1).

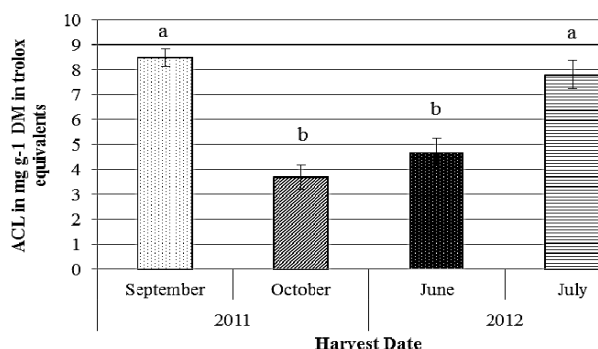


Fig. 1 Lipid soluble antioxidant potential (ACL) of broccoli florets in mg g<sup>-1</sup> DM expressed in trolox equivalents at four harvest dates; means are averaged over all tested cultivars

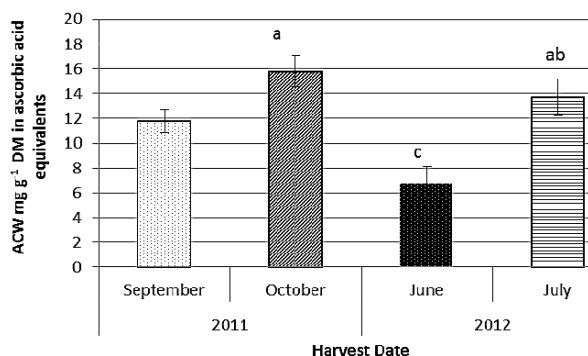


Fig. 2 Water soluble antioxidant potential (ACW) of broccoli florets in mg g<sup>-1</sup> DM expressed in ascorbic acid equivalents at four harvest dates; means are averaged over all tested cultivars

TABLE I

MEAN AVERAGES OF ACL [MG G<sup>-1</sup> DM EXPRESSED IN TROLOX EQUIVALENTS] AND ACW [IN MG G<sup>-1</sup> DM EXPRESSED IN ASCORBIC ACID EQUIVALENTS] OF THE TESTED BROCCOLI CULTIVARS 'LIMBA', 'MIRANDA' (BOTH OPEN-POLLINATING), 'BATAVIA', 'MARATHON', 'MONTEREY', 'IRONMAN' (ALL HYBRIDS), 'KSV-CHE-GRE', AND 'KSV-TH-CAN' (ORGANIC BREEDS) CULTIVATED IN 2011 AND 2012: DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES FOR ACL OR ACW AMONG THE CULTIVARS

	'Limba'	'Miranda'	'Batavia'	'Marathon'	'Monterey'	'Ironman'	'KSV-CHE-GRE'	'KSV-TH-CAN'
ACL	6.1 a	5.8 a	6.5 a	5.7 a	6.8 a	5.4 a	6.4 a	6.6 a
ACW	10.2 ab	13.7 ab	14.1 a	12.9 ab	13.6 ab	9.5 b	10.6 ab	11.5 ab

Lowest concentrations of ACL were observed in florets harvested in October 2011 with 3.7 mg g<sup>-1</sup> DM. Broccoli harvested in June 2012 showed in average 1 mg g<sup>-1</sup> DM higher ACL than florets harvested in October 2011.

ACL values in tested broccoli samples represented the sum of AOX capacities of lipid soluble components such as chlorophyll (a+b), carotenoids (carotene, lutein, zeaxanthin and xanthophylls). Some of these compounds are precursors of vitamin A (i.e. b-carotene, g-carotene, and b-cryptoxanthin), and with a help of conjugated double bonds they have radical quenching ability [13]. Generally, an increase of accumulated light levels, leads to higher activity of photosynthetic pigments (e.g. chlorophyll, carotenoids) and their amount increases. However, after the light saturation point is reached, photosynthetic activity and amount of pigments decreases [14]. This fact might explain why samples of broccoli harvested in October exposed to a longer period of total radiation had lower ACL values due to a decrease in carotenoid and chlorophyll concentrations triggered by a combined effect of pigment structure photodegradation [14].

In contrast, ACW was highest in florets of October 2011 with 15.8 mg g<sup>-1</sup> DM (expressed as ascorbic acid equivalents), followed by 13.7 mg g<sup>-1</sup> DM determined in florets harvested in July 2012. Lowest concentrations of ACW were measured in florets harvested in June 2012 with 6.6 mg g<sup>-1</sup> DM (Fig. 2). A significant increase in ACW values was observed from September to October and from June to July. ACW value includes AOX capacity of secondary metabolites such as flavonoids, phenolics and other water soluble components. In broccoli these substances are involved in different processes including plant-pest and plant-pathogen interactions, pollination, and seed development [15]. Many genes that are responsible for AOX are activated under stress conditions and, according to [16] AOX amount increases during exposure to biotic and abiotic factors such as UV-irradiation, drought, pest and pathogen infestation. It is concluded that a later harvest date increased ACW values due to a possible longer exposure of broccoli plants to biotic and abiotic stress factors.

Overall, obtained results indicated that antioxidant activity was highly influenced by harvest date. This might be due to temperature changes as temperature has an important influence on antioxidant activity [17], [19]. In addition, [18] studied the antioxidant potential of cauliflower harvested in three years and in two different dates. There were significant differences among years, but samples showed no clear trend between early and late harvest. Generally speaking, there is an increase of antioxidant activity of Brassica crops during the growing period, starting when samples are sprouts, until it reaches its maximum three months after sowing; after that, antioxidant activity decreases again, which means that crops reach their maximum antioxidant activity when leaves are young. This result is in agreement to [19] who measured the antioxidant activity of white cabbage and Chinese cabbage leaves periodically between transplanting until full maturation (head harvesting) and observed that there was an increase in the antioxidant activity in the first 8 to 12 weeks and afterwards there was a gradual decrease, probably as a

consequence of a more active plant metabolism which accompanies active/rapid growth in the first few months. In conclusion, one has to carefully select the harvest date when high antioxidant activity of broccoli is of major interest.

#### IV. CONCLUSION

All findings reported here demonstrated that broccoli contains bioactive substances with a certain health potential which could explain their protective role on some widespread diseases. In this study, we identified changes in antioxidant activity depending on different growing seasons and on harvest dates, displaying a higher impact on average antioxidant activity than the chosen cultivar.

#### ACKNOWLEDGMENT

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#### REFERENCES

- [1] P.M. Kris-Etherton, K.D. Hecker, A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert, A.E. Griel and T.D. Etherton, "Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer", *The American Journal of Medicine*, vol. 113, no. 9, pp. 71-88, 2002.
- [2] O. K. Chun, D. O. Kim, N. Smith, D. Schroeder, J. T. Han and C.Y. Lee, "Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet", *Journal of the Science of Food and Agriculture*, vol. 85, no. 10, pp.1715-1724, 2005.
- [3] D. Heimler, P. Vignolini, M.G. Dini, F.F. Vincieri and A. Romani, "Antiradical activity and polyphenol composition of local Brassicaceae edible varieties", *Food Chemistry*, vol. 99, no. 3, pp. 464-469, 2006.
- [4] P. Sogenas, T. Sotelo, P. Velasco, M. and E. Cartea "Antioxidant Properties of Brassica Vegetables" *Functional Plant Science and Biotechnology*5 (Special Issue 2), 43-55, 2011.
- [5] J. Nilsson, K. Olsson, G. Engqvist, J. Ekvall, M. Olsson, M. Nyman, and B. Åkesson, "Variation in the content of glucosinolates, hydroxycinnamic acids, carotenoids, total antioxidant capacity and low-molecular-weight carbohydrates in Brassica vegetables", *Journal of the Science of Food and Agriculture*, vol. 86, no. 4, pp. 528-538, 2006.
- [6] C.W. Beecher "Cancer preventive properties of varieties of Brassica oleracea: a review", *The American Journal of Clinical Nutrition*, vol. 59, no. 5, pp.1166S-1170S, 1994.
- [7] G. Williamson, K. Faulkner, and G.W. Plumb, "Glucosinolates and phenolics as antioxidants from plant foods". *European Journal of Cancer Preview* 7, 17-21, 1998.
- [8] J.T.Guo, H.L. Lee, S.H.Chiang, F. I.Lin and C.Y.Chang, "Antioxidant properties of the extracts from different parts of broccoli in Taiwan". *Journal of Food Drug Analysis*, 9, 96-101, 2001.
- [9] E.H. Jeffery, A.F. Brown, A.C. Kurilich, M.A. Wallig, B.P. Klein, M.M.Kushad and J.A.Juvik, "Content variation in bioactive food components", *Nutrition Today*, vol. 37, no. 5, pp. 208, 2002.
- [10] C. Kaur, K. Kumar, D. Anil, H. C. Kapoor, "Variations in antioxidant activity in broccoli (*Brassica oleracea*L.) cultivars", *Journal of Food Biochemistry* 31, 621-638, 2007.
- [11] Z. Balouchi, G.A. Peyvast, M. Ghasemnezhad and M. Saadatian, "Changes of antioxidant compounds of broccoli (*Brassica oleracea* L. var. *italica*) during storage at low and high temperatures", *South-west. Journal Horticulture Biology Environment* 2: 193-212, 2011.
- [12] A. E. M. M. Naguib, F. K. El-Baz, Z. A.Salama, H. A. E. B.Hanaa, H. F. Ali, and A. A. Gaafar, "Enhancement of phenolics, flavonoids and glucosinolates of broccoli (*Brassica oleracea*, var. *italica*) as antioxidants in response to organic and bio-organic fertilizers", *Journal Saudi Society Agricultural Science*, 11: 135-142. 2012.

- [13] C. A. Rice-Evans, J. Sampson, P. M. Bramley, and D. E. Holloway, "Why do we expect carotenoids to be antioxidants in vivo?", *Free radical research*, vol. 26, no. 4, 381-398, 1997).
- [14] M. G. Lefsrud, D. A. Kopsell, D.E.Kopsell, and J. Curran-Celentano, "Irradiance levels affect growth parameters and carotenoid pigments in kale and spinach grown in a controlled environment", *Physiologia Plantarum*, vol. 127, no. 4, pp. 624-631, 2006.
- [15] B. Winkel-Shirley "Biosynthesis of flavonoids and effects of stress", *Currentopinion in plant biology*, vol. 5, no. 3, pp. 218-223, 2002.
- [16] R. A. Dixon, and N. L. Paiva "Stress-induced phenyl-propanoid metabolism.", *ThePlant Cell*, vol. 7, no. 7, pp. 1085. 1995.
- [17] R. Lo Scalzo, G. Bianchi, A. Genna, C. Summa "Antioxidant properties and lipidic profile as quality indexes of cauliflower (*Brassica oleracea* L. var. botrytis) in relation to harvest time", *Food Chemistry* 100, 1019-1025, 2007.
- [18] H. T. Aldrich, P. Kendall, M. Bunning, F. Stonaker, O. Kulen and C. Stushnoff. "Environmental temperatures influence antioxidant properties and mineral content in broccoli cultivars grown organically and conventionally", *Journal of Agronomy Crop Science*, 2:1–10, 2011.
- [19] D. Samec, J. Piljac-Zegarac, M. Bogovic, K. Habjanic and J.Gruz, "Antioxidant potency of white (*Brassica oleracea* L. var. capitata) and Chinese (*Brassica rapa* L. var. pekinensis (Lour.)) cabbage: The influence of development stage, cultivar choice and seed selection", *Scientia Horticulturae* 128: 78–83, 2011.