Journal of Applied Botany and Food Quality 80, 111 - 115 (2006)

[1]Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, Ås, Norway
[2]Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Ås, Norway

Antioxidant activity in commonly grown and consumed vegetables: a screening survey

Anne-Berit Wold[1], Trude Wicklund[2], Karin Haffner[1]

(Received April 20, 2006)

Summary

A diet rich in fruits and vegetables is associated with a reduced risk of developing cardiovascular diseases and certain types of cancer. This positive effect is related to bioactive phytochemicals found in plants.

The vegetables were grown in the field or in greenhouses at the Norwegian University of Life Sciences (59º40'N) during the years 2000-2002. The vegetables were harvested at commercial maturity and analysed for dry matter and antioxidant activity assessed by the FRAP (Ferric Reducing Ability of Plasma) assay. There was a large variation in antioxidant activity both between and within different species. The highest antioxidant activity was observed in kale, red cultivars of cabbage and table beet. The lowest antioxidant activity was observed in lettuce, cucumber, carrots and tomato. The vegetables possessing a red colour showed higher antioxidant activity with the exception of carrots and tomatoes.

Introduction

Epidemiological studies have shown that a diet rich in fruits and vegetables is associated with reduced incidences of several chronic diseases, and a reduced risk of developing cardiovascular disease and certain types of cancer. A review of approximately 200 epidemiological studies examining the relationship between intake of fruits and vegetables and different types of cancer found that in 128 of 156 dietary studies the consumption of fruits and vegetables had a protective effect (Block et al., 1992). This beneficial effect of fruits and vegetables is related to their content of bioactive compounds (phytochemicals), which may be carotenoids, phenolics, alkaloids, nitrogen containing compounds and organosulfur compounds. The most studied are the phenolics and carotenoids (Liu, 2004). Especially the phenolic compounds are effective antioxidants (Rice-Evans et al., 1997).

The combination of phytochemicals from fruits and vegetables was proposed to be responsible for the potent antioxidant and anticarcinogenic activity of these foods. Taken alone the individual antioxidants studied in clinical trials do not appear to have consistent positive/preventive effects. Synergistic activity between antioxidants has been observed. Therefore the consumer should obtain their phytochemicals from a wide variety of fruits and vegetables.

The antioxidant activity in fruits and vegetables may be influenced by cultivar, geographic origin, growing season, and agricultural practices. A systematic screening of total antioxidant activity in dietary plants (Halvorsen et al., 2002) revealed a large variation in the antioxidant content of vegetables. Large variation was also observed within the species. This variation may have several causes, including genetic and environmental influences as well as differences in plant organ and tissue examined. *Brassica* vegetables are important contributors of vitamins and antioxidants in human diets, the major antioxidants being vitamins C and E, carotenoids, and phenolic compounds (Podsedek, in press). Many previous studies have been limited to the use of samples without the knowledge of genotype, growing conditions or post-harvest conditions. The aim of this work was to assess the antioxidant activity in vegetables commonly grown and consumed in Norway. The Nordic growth season is fairly short with long daily light periods and relatively low temperatures. The special climatic conditions may influence the content of secondary plant metabolites including bioactive phytochemincals.

Material and methods

Plant material

Vegetables were grown in the field and in greenhouses at the Norwegian University of Life Sciences (59º40'N) in the years 2000-2002. The vegetables included in the screening are listed in Tab. 1. Fertilisation, irrigation and plant protection corresponded to the standard cultivation procedures for each species. Vegetables were harvested at commercial maturity.

The vegetables were analysed for antioxidant activity by means of the FRAP (Ferric Reducing/Antioxidant Power) assay. Products from at least two seasons were analysed. Fifteen units of product (head, root, bulb or fruit) of each vegetable were harvested. The products were split into three samples each consisting of 5 units. Approximately ¼ of each product unit was used for analyses. Onions were analysed both freshly harvested and dried for 2 weeks at a temperature of 30 ºC the first year and for 4 weeks at 20 ºC the second year. The cured onions were stored at 0 ºC. Tomatoes were harvested mature green and vine ripe and analysed as mature green, vine ripe and post-harvest ripened fruits. Cucumbers were harvested at two different maturities and both small (348 g) and large (576 g) cucumbers were analysed. Samples were frozen at -20 ºC until further analyses.

Sample preparation for the FRAP assay

Samples were homogenized in a food processor, and 3 g of the homogenised material were dissolved in 30 mL of methanol. Three replicates were prepared from each sample. The bottles were flushed with nitrogen before closing, the samples were then mixed and sonicated in a water-bath at 0 °C for 15 min. The extracts were stored at -20 °C until analysis. Three samples of 1.5 mL of the extract were centrifuged at 12,402 x *g* for 2 min at 4 °C. The concentration of total antioxidants in the supernatant was measured in triplicate.

FRAP assay

The Ferric Reducing Ability of Plasma (FRAP) assay was used to measure the concentration of total antioxidants. FRAP was determined in extracts by the method of Benzie and Strain (1996), with the exception that the sample was not diluted with water (Halvorsen et al., 2002). A Technicon RA 1000 system (Technicon Instruments Corporation, New York, USA) was used for the measurements of absorption changes that appear when the TPTZ-Fe^{3+} (2,4,6-tri-pyridyl-s-triazine) complex is reduced to the TPTZ-

Tab. 1: Vegetables included in the screening survey.

Vegetable	Botanical name	Cultivar	2000	2001	2002
Cucumber*	*Cucumis sativus*	Ventura	x	x	
Early cabbage	*Brassica oleracea* var. *capitata*	Ladi	x	x	
White cabbage	*Brassica oleracea* var. *capitata*	Bartolo	x	x	x
Red cabbage	*Brassica oleracea* var. *capitata*	Autoro	x	x	x
		Rovite		x	x
		Primero		x	x
Savoy	*Brassica oleracea* var. *sabauda*	Taler	x	x	x
Cauliflower (white)	*Brassica oleracea* var. *botrytis*	Fremont	x	x	x
Cauliflower (green)		Alverda		x	x
Cauliflower (red)		Grafitti	x		
Brussels sprouts	*Brassica oleracea* var. *gemmifera*	Content	x	x	x
Kale	*Brassica oleracea* var. *sabellica*	Ekstra moskruset	x	x	x
Red kale	*Brassica oleracea* var. *sabellica*	Redbor		x	x
Iceberg salad	*Lactua sativa* var. *capitata*	Set	x		
		Match		x	
		Roxet		x	x
		Brandon		x	x
Lollo rosso	*Lactua sativa* var. *foliosum*	Malibu	x	x	
		Anthony		x	x
		Concorde		x	x
Radiccio rosso	*Cihcorium intybus* var. *foliosum*	Indigo	x	x	
Spinach	*Spinacea oleracea*	Viking 290	x	x	
Broccoli	*Brassica oleracea* var. *itallica*	Maraton	x	x	x
		Montop		x	x
		Lord		x	x
Red broccoli	*Brassica oleracea* var. *itallica*	BE 1891		x	x
Swede	*Brassica napus* var. *rapifera*	Vige	x	x	
Carrot	*Daucus carota* var. *sativus*	Nantes Duke	x	x	x
		Yukon	x	x	x
Onion	*Allium cepa*	Jumbo	x		x
Red onion	*Allium cepa*	Red baron	x		x
Rødbete	*Beta vulgar* var. *conditiva*	Boltardy			x
		Kosak			x
Tomato*	*Lycopersicon esculentum*	Liberto		x	x
		Favorita (Cherry)		x	x
		Durinta (Truss)		x	x

* Vegetables grown in greenhouses

Fe^{2+} form in the presence of antioxidants. An intense blue colour with the absorption maximum at 593 nm develops. The measurements were performed at 600 nm. An aqueous solution of 500 µM $FeSO_4$ x 7 H_2O was used for calibration of the instrument.

Dry matter

Dry matter was determined by drying approximately 5 g of homogenized material for 24 h at 104 ºC, followed by stabilising at room temperature in a dessicator and weighing the final product.

Results and discussion

Kale was found to have the highest antioxidant activity followed by red cabbage, table beet, red broccoli, red cauliflower and Lollo Rosso lettuce (Tab. 2). The lowest antioxidant activity was found in iceberg lettuce, carrots (Tab. 2), cucumber and tomatoes (Tab. 3) with the other vegetables at intermediate values. Low antioxidant activity in tomato, carrot and lettuce was also observed by BAHORUN et al. (2004).

The antioxidant activity in *Brassica* vegetables examined ranged from 0,11 - 2,44 mmol/100g fresh weight. Kale showed the highest values followed by cultivars of red cabbage, red broccoli, red cauliflower, Brussels sprouts, broccoli cultivars, Savoy cabbage, yellow cauliflower, early cabbage, cauliflower and white cabbage. Examining carotene, tocopherol and ascorbate contents in subspecies of *Brassica oleracea*, KURILICH et al. (1999) found that kale had the highest level of vitamins, followed by broccoli and Brussels sprouts with intermediate levels, and cabbage and cauliflower containing the lowest concentrations.

Tab. 2: Antioxidant activity (FRAP) in field grown vegetable species and cultivars. The letter n indicates number of replicates. n = 3 means three replicates for one growing season, n = 6 means three replicates for two growing seasons and n = 9 means three replicate for three growing seasons.

Vegetable	Cultivar	Sample	Dry matter %	FRAP mmol/100g FW		
				FRAP	min-max	Mean
Red kale	Redbor	n=3	14.5	2.44	2.30-2.55	2.44
Kale	Ekstra moskruset	n=9	15.9	2.38	1.95-2.94	2.38
Red cabbage	Autoro	n=9	8.7	1.90	1.52-2.15	1.60
	Rovite	n=6	9.0	1.82	1.68-1.98	
	Primero	n=6	7.1	1.08	0.99-1.20	
Table Beet	Boltardy	n=3	14.6	1.56	1.52-1.61	1.46
	Kosak	n=3	14.0	1.36	1.28-1.43	
Red broccoli	BE1891	n=3	15.5	1.34	1.29-1.41	1.34
Broccoli	Lord	n=6	9.5	0.41	0.37-0.46	0.36
	Montop	n=6	9.6	0.34	0.32-0.39	
	Marathon	n=9	9.6	0.34	0.26-0.48	
Lollo Rosso	Malibu	n=6	6.1	1.16	0.37-2.34	0.95
	Anthony	n=6	4.8	1.05	0.66-1.40	
	Concorde	n=6	4.1	0.65	0.42-1.11	
Radiccio Rosso	Indigo	n=6	6.3	0.47	0.16-0.85	0.47
Red cauliflower	Grafitti	n=3	7.2	1.11	1.05-1.23	1.11
Red onion/cured	Red Baron	n=6	12.1	0.89	0.47-1.48	0.89
Spinach	Viking 290	n=6	8.4	0.77	0.66-1.12	0.77
Red onion/fresh	Red Baron	n=6	15.3	0.75	0.48-1.20	0.75
Onion/cured	Jumbo	n=6	12.1	0.71	0.33-1.48	0.71
Brussels Sprouts	Content	n=9	18.0	0.65	0.54-0.72	0.65
Onion/fresh	Jumbo	n=6	11.7	0.60	0.33-1.12	0.60
Savoy cabbage	Thaler	n=9	11.0	0.26	0.19-0.40	0.26
Swede	Vige	n=6	9.1	0.26	0.18-0.38	0.26
Yellow cauliflower	Alverda	n=6	9.4	0.21	0.17-0.24	0.21
Early cabbage	Ladi	n=6	5.7	0.12	0.11-0.14	0.12
Cauliflower	Fremont	n=9	7.2	0.12	0.11-0.13	0.12
White cabbage	Bartolo	n=9	10.2	0.11	0.07-0.17	0.11
Carrot	Nantes Duke	n=9	10.4	0.04	0.02-0.05	0.04
	Yukon	n=9	10.4	0.04	0.02-0.05	
Iceberg lettuce	Zet	n=3	4.0	0.05	0.032-0.067	0.038
	Match	n=3	3.3	0.03	0.028-0.031	
	Brandon	n=6	3.6	0.05	0.004-0.08	
	Roxett	n=6	3.3	0.02	0.009-0.032	

The antioxidant activity in salads ranged from 0,02 - 1,16 mmol/100 g fresh weight. The Lollo Rosso cultivars had the highest FRAP values followed by Radiccio Rosso whereas the iceberg cultivars showed the lowest antioxidant activity. Red-pigmented oak leaf lettuce has been found to have higher antioxidant activity than green cultivars of lettuce including butter and Batavia salads (Nicolle et al., 2004). Red oak leaf lettuce had 3.5 times higher antioxidant capacity than green lettuce. Analysing phenolic acids in Lollo Rosso, Ferreres et al. (1997) found the red tissue to contain more than the green and white tissue. The red tissue also showed higher flavonoid content than green tissue while white tissue had the smallest amount.

Cured onion showed higher antioxidant activity than fresh onion, and the red cultivar had higher values than the white one (Tab. 2). Coloured vegetables were found to have a higher antioxidant activity than the paler varieties. Anthocyanin is the abundant flavonoid that causes intense coloration. Anthocyanins are found to have a protective effect against cancer (Cooke et al., 2005). The red colour of red cabbage is caused by anthocyanins belonging to flaovonoids. Red cabbage contains more than 15 different anthocyanins (Dyrby et al., 2001; Mazza and Miniati, 1993). Classifying vegetables according to their major phenols Proteggente et al. (2002) found fruits and vegetables rich in anthocyanins to possess the highest antioxidant capacity, followed by those rich in flavanones and flavonoles (onions, leeks, lettuce, broccoli, spinach and cabbage), while the hydroxycinnamate-rich (tomato) showed lower antioxidant capacity. A strong correlation between antioxidant activity and total phenolics and total flavonoids has been found in vegetables (Bahorun et al., 2004). In this study the tomatoes did not show a high antioxidant activity compared to the other colourful vegetables (Tab. 3). This may be explained by the choice of extraction procedure.

Tab. 3: Antioxidant activity (FRAP) in tomato cultivars and cucumber grown in green houses and harvested at different maturity. The letter n indicates number of replicates. n = 6 means three replicate for two growing seasons.

Vegetable	Cultivar	Maturity	Sample	Dry matter %	FRAP mmol/100g FW	
					min-max	mean
Tomato	Liberto	green	n=6	5.6	0.17-0.22	0.20
	Liberto	post-harvest ripe	n=6	4.9	0.26-0.29	0.28
	Liberto	vine ripe	n=6	6.0	0.24-0.30	0.30
	Favorita	green	n=6	7.4	0.25-0.42	0.32
	Favorita	post-harvest ripe	n=6	6.5	0.39-0.46	0.42
	Favorita	vine ripe	n=6	7.7	0.41-0.52	0.48
	Durinta	green	n=6	5.9	0.11-0.21	0.17
	Durinta	post-harvest ripe	n=6	4.7	0.22-0.28	0.24
	Durinta	vine ripe	n=6	5.5	0.20-0.26	0.26
Cucumber		small	n=6	3.4	0.03-0.06	0.05
		large	n=6	3.2	0.02-0.05	0.04

The methanol extraction prior to the FRAP assay does not dissolve fat-soluble antioxidants such as lycopene. The flavonoids and the glucoside flavonoids are extracted by methanol and measured in the FRAP assay as part of the total antioxidant capacity. This would mean that the potential antioxidant activity in tomato cultivars is underestimated. This would also be the case for carrots, which contain considerable amounts of carotenoids. When estimating the requirement of antioxidants both the antioxidant activity in individual fruits and vegetable species but also the consumption frequency should be considered. CHUN et al. (2005) found asparagus and bell pepper to have high antioxidant activity whereas tomatoes and potatoes were lower. Considering daily consumption potatoes showed the highest antioxidant intake followed by tomatoes. Asparagus and bell peppers were shown to contribute relatively little to the total antioxidant intake due to their low daily consumption.

The post-harvest ripened and vine ripe tomatoes had higher antioxidant activity than mature green fruits. There were no significant differences between vine ripe and post-harvest ripened tomatoes (WOLD et al., 2004), indicating that with respect to tomatoes agronomical practises such as post-harvest ripening did not influence the antioxidant activity. Cucumbers are usually harvested early, as small, immature, dark green fruits. Harvested at a later stage the cucumbers are larger with a lighter green colour. Cucumbers were low in antioxidant activity and there were no differences observed in antioxidant activity between small immature and larger cucumbers.

Following studies should also include total phenolics and the content of individual flavonoids and vitamins to give a better understanding of the contribution of single components to antioxidant activity in fruits and vegetables.

Acknowledgement

The authors wish to thank the Norwegian Research Council for financing the project „Bioactive compounds in fruit, berries and vegetables and their capabilities to regulate disease-associated genes“ (2000-2002), Rune Blomhoff at Institute for Nutrition Research, UiO for use of laboratory equipment, Liv Berge and Karin Svinnset at the Department of Plant and Environmental Sciences and Tor Brun at Department of Chemistry, Biotechnology and Food Science for valuable technical assistance, and Halvard Baugerød for critical reading of the manuscript.

References

BAHORUN, T., LUXIMON-RAMMA, A., CROZIER, A., ARUOMA, O.I., 2004: Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J. Sci. Food Agric. 84, 1553-1561.

BENZIE, I.F.F., STRAIN, J.J., 1996: The Ferric Reducing Ability of Plasma (FRAP) as a measure of „antioxidant power“: the FRAP assay. Anal. Biochem. 239, 70-76.

BLOCK, G., PATTERSON, B. SUBAR, A., 1992: Fruit, vegetable and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer 18, 1-29.

COOKE, D., STEWARD, W.P., GESCHER, A.J., MARCZYLO, T., 2005: Anthocyans from fruits and vegetables – Does bright colour signal cancer chemopreventive activity? Eur. J. Cancer 41, 1931-1940.

CHUN, O.K., KIM, D.-O., SMITH, N., SCHROEDER, D., HAN, J.T., LEE, C.Y., 2005: Daily consumption of phenolics and total antioxidant capacity from fruits and vegetables in the american diet. J. Sci. Food Agric. 85, 1715-1724.

DYRBY, M., WESTERGAARD, N., STAPELFELDT, H., 2001: Light and heat sensitivity of red cabbage extract in soft drink model systems. Food Chem. 72, 431-437.

FERRERES, F., GIL, M.I., CASTANER, M., TOMAS-BARBERAN, F.A., 1997: Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. J. Agric. Food Chem. 45, 4249-4254.

HALVORSEN, B.L., HOLTE, K., MYHRSTAD, M.C.W., BARIKMO, I., HVATTUM, E., REMBERG, S.F., WOLD, A.B., HAFFNER, K., BAUGERØD, H., ANDRESEN, L.F., MOSKHAUG, J.Ø., JACOBS, D.R., BLOMHOFF, R., 2002: A systematic screening of total antioxidants in dietary plants. J. Nutr. 132, 461-471.

KURILICH, A.C., TSAU, G.J., BROWN, A., HOWARD, L., KLEIN, B.P., JEFFERY, E.H., KUSHAD, M., WALLIG, M.A., JUVIK, J.A., 1999: Carotene, tocopherol, and ascorbate contents in subspecies of Brassica oleracea. J. Agric. Food Chem. 47, 1576-1581.

LIU, R.H., 2004: Potential synergy of phytochemicals in cancer prevention: Mechanism of action. J. Nutr. 3479S-3485S.

MAZZA, G., MINIATI, E., 1993: Anthocyanins in fruits, vegetables, and grains. CRC Press, Boca Raton.

NICOLLE, C., CARNAT, A., FRAISSE, D., LAMAISON, J.-L., ROCK, E., MICHEL, H., AMOUROUX, P., REMESY, C., 2004: Characterisation and variation of antioxidant micronutrients in lettuce (*Lactuca sativa* folium). J. Sci. Food Agric. 84, 2061-2069.

PODSEDEK, A., 2005: Natural antioxidant capacity of *Brassica* vegetables: A review. Lebensmittel-Wissenschaft u. -Technologie (in press).

PROTEGGENTE, A.R., PANNALA, A.S., PAGANGA, G., VAN BUREN, L., WAGNER, E., WISMAN, S., VAN DE PUT, F., DACOMBE, C., RICE-EVANS, C.A., 2002: The antioxidant activity of regular consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radic. Res. 36, 217-233.

RICE-EVANS, C.A., MILLER, N.J., PAGANGA, G., 1997: Antioxidant properties of phenolic compounds. Trends Plant Sci. 2, 152-159.

WOLD, A.-B., ROSENFELD, H.J., HOLTE, K., BAUGERØD, H., BLOMHOFF, R., HAFFNER, K., 2004: Colour of post-harvest ripened and vine ripened tomatoes (*Lycopersicon esculentum* Mill.) as related to total antioxidant capacity and chemical composition. Int. J. Food. Sci. Technol. 39, 295-302.

Address of the authors:
Anne-Berit Wold[1]*, Trude Wicklund[2] and Karin Haffner[1]
[1]Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, P. O. Box 5003, N-1432 Ås, Norway, [2]Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, P. O. Box 5003, N-1432 Ås, Norway
*Corresponding author: anne-berit.wold@umb.no