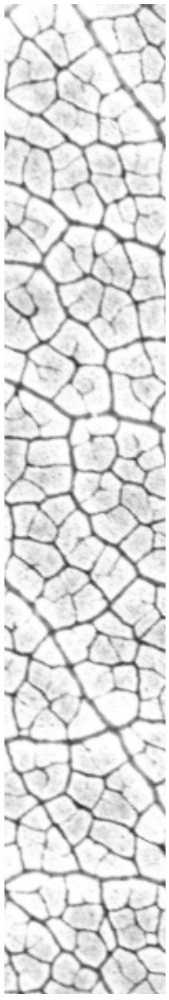

EurAsian Journal of BioSciences
Eurasia J Biosci 13, 1037-1043 (2019)

Phytochemical composition and antioxidant activity in *Brassica oleracea var. Sabellica* under the effect of plant growth regulators

Esraa Almughraby [1*], Marat Ildusovich Kalimullin [1], Olga Arnoldovna Timofeeva [1]

[1] Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Volga Region, RUSSIA
*Corresponding author: esraaalmgrabe@gmail.com

Abstract
The aim of this study is to investigate the effect of natural hormones (gibberellic acid, brassinolide) and Commercial preparations of growth stimulants (ecopain, novosil) on the content of carotenoids, ascorbate, phenols, flavonoids, as well as antioxidant activity in kale *Brassica oleracea var. Sabellica*. Our results demonstrated the possibility of controlling the phytochemical composition of kale through treatment with growth regulators - natural phytohormones and commercial preparations. All the studied compounds increased the content of health-promoting compounds and the antioxidant activity of kale cabbage, but in different degrees. The content of carotenoids was largely influenced by gibberellic acid, ascorbate and flavonoids by brassinolide. While novosil had the greatest effect on increased antioxidant activity.

Keywords: *brassica*, brassinolide, gibberellic acid, carotenoids, ascorbate, flavonoids, antioxidant activity

Almughraby E, Kalimullin MI, Timofeeva OA (2019) Phytochemical composition and antioxidant activity in *Brassica oleracea var. Sabellica* under the effect of plant growth regulators. Eurasia J Biosci 13: 1037-1043.

INTRODUCTION

Kale (*Brassica oleracea var. sabellica*) is a leafy green vegetable belonging to the *Brassicaceae* family, with a high content of health-promoting phytochemicals. Kale plants are robust and can tolerate cold temperatures below freezing. Kale has a high concentration of vitamins, minerals, dietary fiber and antioxidative compounds (Heimler et al. 2006, Olsen et al. 2010, Podsedek 2007, Sikora and Bodziarczyk 2013). These phytochemicals play an important role in the interaction between plants and their environment, e.g. as pollination attractants, compounds which protect against pathogens or various abiotic stresses, antioxidants or signaling molecules (Gill and Tuteja 2010). The *Brassica oleracea* groups are an important source of secondary plant metabolites, especially phenolic compounds (flavonoids and other polyphenols) (Bilyk and Sapers 1985, Schmidt et al. 2010a). Phenolic compounds significantly differ in structure, chemical properties and biological activity; this explains the breadth of their functions - from their involvement in the electron transport chains of photosynthesis and respiration to participation in protecting cells from stressful effects (Bahorun et al. 2004, Chun et al. 2005).

Flavonoids are one of the largest classes of phenolic compounds, they have health-promoting effects on humans. Flavonoids possess a variety of biological activities, which may contribute to protecting humans against chronic diseases. Quercetin, a main aglycone in human nutrition, is a potent free radical scavenger due to the di-hydroxylated B-ring, unsaturation at the C-ring and a 4-oxo function at the C-ring (Williams et al. 2004). Up to now a total number of 71 flavonol glycosides have been identified in kale by HPLC-DAD-MS so far, 27non-acylated, 30 monoacylated and 14 diacylated glycosides have been described based on the flavonol aglyconesquercetin, kaempferol and isorhamnetin (Schmidt et al. 2010b). It has been shown that cabbage kale contains quercetin (Neugart et al. 2016), it has anti-inflammatory properties and inhibits the development of atherosclerosis (Kleemann et al. 2011), anti-carcinogenic activity, initiates apoptosis of human lung cancer cells (Zheng et al. 2011) and suppresses the growth of human bladder cancer cells (Kim et al. 2011). Kempferol, another component of cabbage kale (Neugart et al. 2016), it has a strong antioxidant potential, it increases the resistance of the body to the action of oxidative stress which may lead to the development of carcinogenesis, Kempferol initiates apoptosis of ovarian cancer cells due to activation of the tumor suppressor protein p53 and apoptosis proteins Bad and Bax (Luo et al. 2011). In addition, it was shown

Received: March 2019
Accepted: June 2019
Printed: August 2019

that kaempferol has anti-inflammatory and antiallergic effect and it prevents the development of atherosclerosis (Xiao et al. 2011). In animal experiments, kaempferol prevents obesity and osteoporosis (Byun et al. 2012). Nevertheless, the change in the content of both general phenols and flavonoids depends on genetics and on the environment, including methods of crop management, collection, storage, processing and cooking. In connection with this, it is of interest to identify factors which can enhance synthesis of these compounds. It is shown that the content of flavonoids can change under the influence of temperature and UV radiation (Neugart et al. 2013).

Plant hormones also have an effect on the synthesis of secondary metabolites in plants. The EBR treatment significantly increases total phenolic content, total tannin content, total flavonoid content and total anthocyanin content of grape skins and wines made from grapes. The antioxidant capacities of the wines were increased by EBR treatment. There was a good correlation between the antioxidant capacity and phenolic content (Xi et al. 2013). Also the results on HPLC and UV spectrophotometry showed that exogenous application of both GA3 and IAA increased the content of flavonoids in the hairy roots. The content of flavonoids and apigenin in the hormone-treated hairy roots and regenerates were higher in comparison with those in the untreated hairy roots and the regenerates (Qiao et al. 2011). EBR increased the free radical scavenging capacity, flavonoid content and the activity and transcription of secondary metabolism related enzymes in Cucumissativus L. (Ahammed et al. 2017). Despite the fact that there has been a lot of research in the use of phytohormones in different cultures, further studies are needed, as different horticultural cultures contain different phytochemicals and react differently to treatment with different phytohormones. It is of interest to clarify the role of natural growth regulators in inducing healthy compounds in cabbage kale. This work is devoted to study the influence of natural (gibberellic acid and brassinolide) and commercial hormones (ecopain and novosil) on the antioxidant activity and the content of phenolic compounds, vitamin C, flavonoids and carotenoids in cabbage kale plants.

METHODS

Seeds *Brassica oleracea var. sabellica* were grown in the field in May 2018 in sandy loam soil with a spacing of 45 / 45 cm. after two months in stage 3-4 leaves, plants were treated with hormones by spraying in the following concentrations: Gibberellic acid 10^{-6} M, Novosil 5 g/hectare, Ecopain 0.25g/hectare and Brassinolid 10^{-6} M. At the end of October (after the first frost) some of the plants were transferred to laboratory conditions and left to grow for 2 weeks at a temperature of 20 °C. We determined the content of total flavonoids, total phenolic. The antioxidant activity, total carotenoid and Vitamin C. Phenolic compounds were determined by the modified Folin-Ciocalteu method (Velioglu et al. 1998). Optical density was measured on a spectrophotometer at 725 nm. The content of phenolic compounds was determined in terms of gallic acid. Flavonoids were determined by the reaction with AlCl3 by the colorimetric method (Zhishen et al. 1999) at a wavelength of 420 nm. The content of vitamin C was determined as the sum of ascorbic acid (AA) and dehydroascorbic acid (DHAA) using the spectrophotometric method (Sokolovskiy et al. 1974). The antioxidant activity of the plant material studied was measured by their ability to inhibit the autooxidation of adrenaline in vitro and thereby prevent the formation of reactive oxygen species (Sirota n.d.). Definition of the content carotenoids was carried out by the spectrophotometric method (Musienko et al. 2001).

The experiments were carried out in three biological replicas. Statistical data processing was carried out using Microsoft Excel. The reliability of the difference was determined by the Student's t-test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Cabbage kale is a very promising vegetable crop, which can be used not only as a food product, but also to promote health due to its rich phytochemical composition. In this study, phytohormones and their analogues were used for the first time to improve the phytochemical composition of kale. Our results showed the possibility of increasing the content of compounds which are useful for human health with the help of natural growth regulators in kale, similar results were obtained in other species, such as grapes, cucumber, Arabidopsis, buckwheat, Saussure involucrate hairy roots (Ahammed et al. 2017, Park et al. 2017, Peng et al. 2011, Xu et al. 2014). Antioxidants are an essential component in the human diet; they protect body from reactive oxygen species. We investigated the content of vitamin C, carotenoids, total content of phenolic compounds, flavonoids and the general antioxidant activity in the leaves of cabbage kale**.**

Polyphenols are bioactive molecules distributed in different plant species, which play important roles in plant responses, these include the effect of ultraviolet radiation, temperature stress and high concentrations of heavy metals (Bahorun et al. 2004). Phenolic compounds play a huge role in protecting plants from bacterial and fungal infections, from penetration of parasites and damage by insects (Chun et al. 2005). They can also exhibit antioxidant activity, which makes them an indispensable component of many food additives for human medicines (Sun et al. 2012). It is shown that the content of phenolic compounds increases in grapes (Xu et al. 2014) and cucumber (Ahammed et al. 2017) when treated with brassinolide, in buckwheat (Park et al. 2017) when treated with

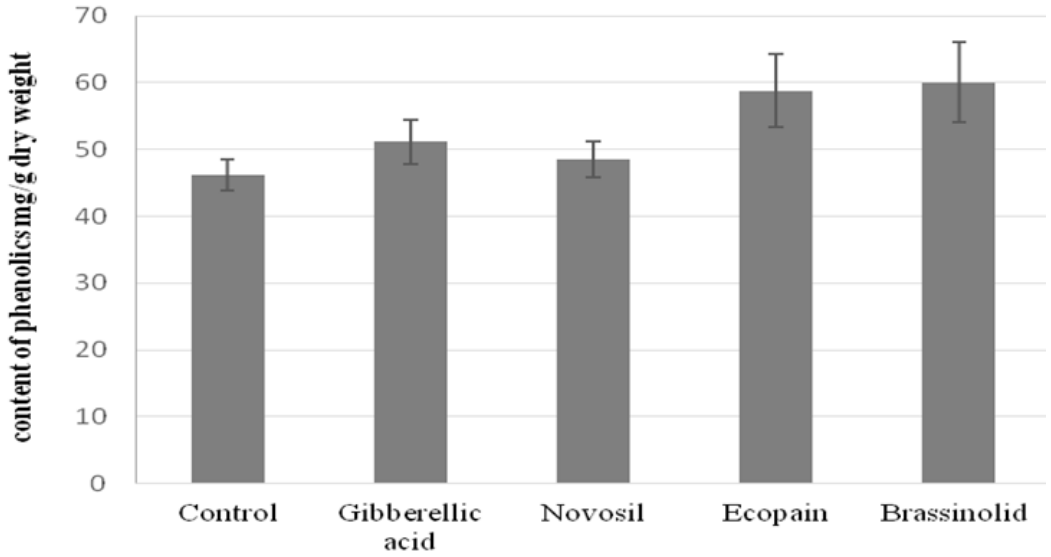

Fig. 1. The effect of growth regulators on the content of phenolic compounds in cabbage kale plants

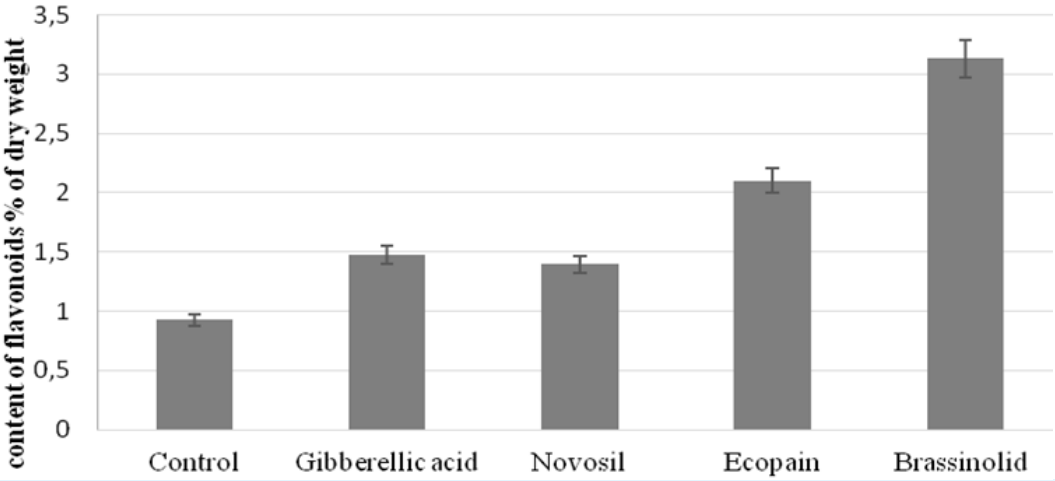

Fig. 2. The effect of growth regulators on the content of flavonoids in cabbage kale plant

gibberellic acid. In our studies, ecopain and brassinolid increased the content of phenolic compounds (**Fig. 1**).

Flavonoids belong to the class of polyphenolic compounds of plant origin. Among secondary products, this group of substances is one of the most notable, Because of its participation in many key processes of plant growth and development. One of the most noticeable functions of flavonoids is their participation in plant protection from oxidative stress .In animal and human cells, flavonoids are not synthesized, and the presence of flavonoids in tissues is completely dependent on the consumption of plant products in food (Mennen et al. 2008) Interestingly flavonoids is caused not only by the possible positive effects of these substances, observed during the consumption of plant products, but also the prospect of obtaining synthetic derivatives of the medicinal substances . Recent studies have shown that derivatives of some flavonoids can be successfully used in the treatment of various diseases of internal organs: these substances are often more effective than known drugs (Garcia et al. 2012) less toxic and exhibit fewer side effects than similar drugs obtained from other sources. All the studied growth regulators in our experiments increased the content of flavonoids. Brassinolide had the greatest effect on the content of flavonoids (**Fig. 2**).

Vitamin C is one of the most important nutritional components in kale as well as in many other horticultural crops. We chose to investigate the contents of vitamin C in kale because of its strong antioxidant capacity, compared with other vegetables (Sun et al. 2012). According to our data, the studied growth regulators reduced the amount of vitamin C, with the exception of brassinolide, which increased its content (**Fig. 3**).

In recent years, experimental data show the relationship between the activity of antioxidant enzymes and the photosynthetic activity of plants under the influence of stresses. One of the biochemical indicators

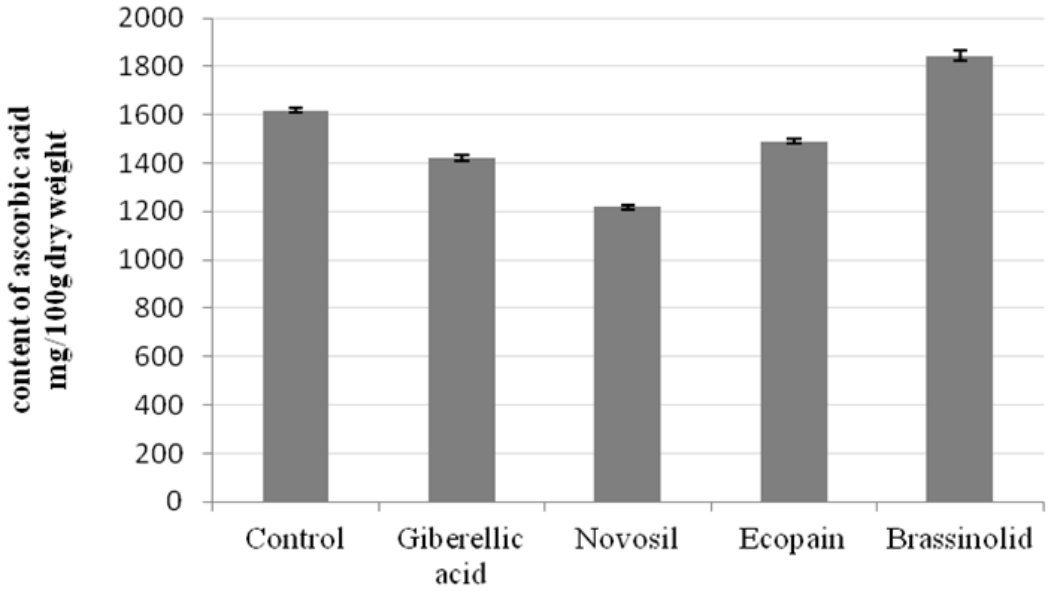

Fig. 3. The effect of growth regulators on the content of ascorbic acid in cabbage kale plants

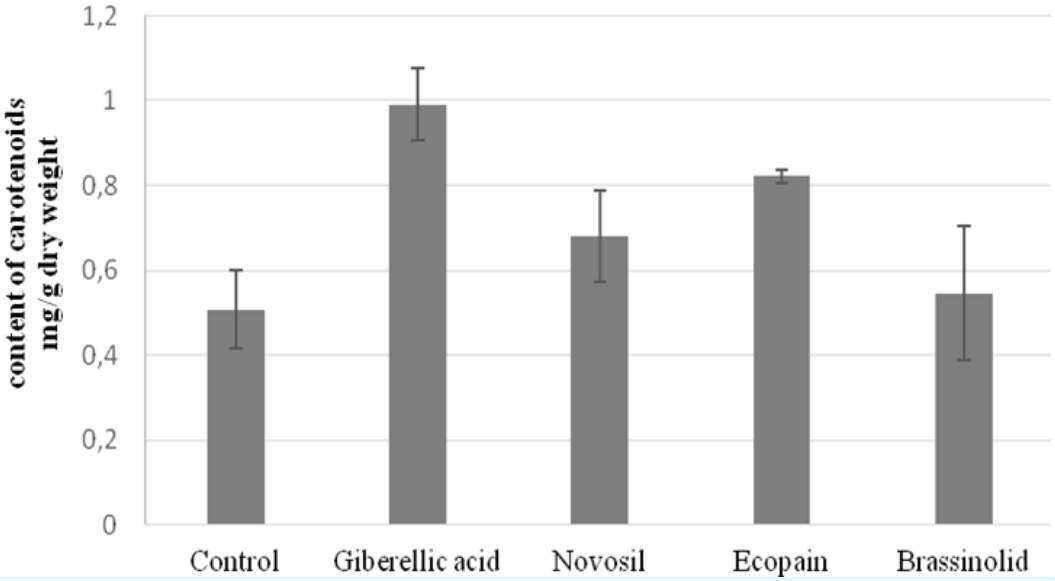

Fig. 4. The effect of growth regulators on the content of carotenoids in cabbage kale plants

of the reaction of plants to changes in environmental factors and the degree of their adaptation to new conditions is the content of chlorophylls and carotenoids. Lipophilic antioxidants include carotenoid, which are considered as one of the factors of plant resistance to various stresses, an increase in their content is due to the antioxidant functions performed by them. In our experiments, the content of carotenoids was increased to a greatest level by gibberellic acid (**Fig. 4**).

Reactive oxygen Species (ROS) are formed in different cell compartments (Fischer et al. 2013) in normal metabolic processes, such as photosynthesis or glycolysis . A high amount of ROS damage membranes, proteins, DNA and other important cell components (Mittler 2002). In plants there are enzymatic and non-enzymatic antioxidant defense systems to reduce the toxicity of ROS, the degree of damage from ROS depends on the balance between their formation and detoxification (Azooz et al. 2009) as can be seen in **Fig. 5**, All the studied growth regulators increased antioxidant activity in the leaves of cabbage kale.

SUMMARY

Generally it was found that different groups of growth regulators increasing the content of health-promoting compounds and the antioxidant activity of kale cabbage, but in different degrees.

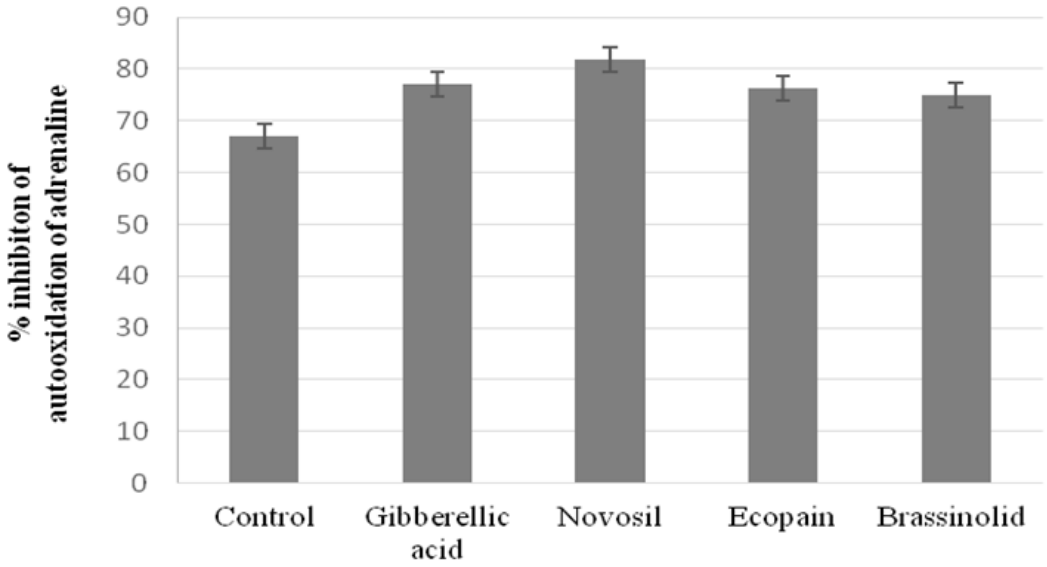

Fig. 5. The effect of growth regulators on the content of flavonoids in cabbage kale plant

CONCLUSION

Thus, our results demonstrated the possibility of controlling the phytochemical composition of kale through treatment with growth regulators - natural phytohormones and commercial preparations, which possesses growth regulating activity, led to an increase in the content of ascorbate, carotenoids, phenolic compounds, essentially flavonoids, as well as antioxidant activity. Thereby different preparations in different degrees stimulated the content of individual groups of compounds. The content of carotenoids was largely influenced by terpene series gibberellic acid and the content of ascorbate, phenols and flavonoids by brasinolide.

ACKNOWLEDGEMENTS

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

REFERENCES

Ahammed GJ, He BB, Qian XJ, Zhou YH, Shi K, Zhou J, Yu JQ, Xia XJ (2017) 24-Epibrassinolide alleviates organic pollutants-retarded root elongation by promoting redox homeostasis and secondary metabolism in Cucumis sativus L, Environmental Pollution, 229: 922-931. https://doi.org/10.1016/j.envpol.2017.07.076

Azooz MM, Ismail AM, Elhamd MF (2009) A Growth, Lipid Peroxidation and Antioxidant Enzyme Activities as a Selection Criterion for Salt Tolerance of Maize Cultivars Grown under Salinity. International Journal of Agriculture and Biology, 11: 21-26.

Bahorun T, Luximon-Ramma A, Crozier A, Aruoma OI (2004) Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. Journal of the Science of Food and Agriculture, 84: 1553–1561. https://doi.org/10.1002/jsfa.1820

Bilyk A, Sapers GM (1985) Distribution of quercetin and kaempferol in lettuce, kale,chive, garlic chive, leek, horseradish, red radish, and red cabbage tissues. Journal of Agricultural and Food Chemistry, 33(2): 226–228. https://doi.org/10.1021/jf00062a017

Byun MR, Jeong H, Bae SJ, Kim AR, Hwang ES, Hong JH (2012) TAZ is required for the osteogenic and anti-adipogenic activities of kaempferol. Bone, 50: 364-372. https://doi.org/10.1016/j.bone.2011.10.035

Chun OK, Kim DO, Smith N, Schroeder D, Han JT, Lee CY (2005) Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. Journal of the Science of Food and Agriculture, 85: 1715-1724. https://doi.org/10.1002/jsfa.2176

Fischer BB, Hideg E, Krieger-Liszkay A (2013) Production, detection, and signaling of singlet oxygen in photosynthetic organisms. Antioxid. Redox Signal, 18: 2145-2162. https://doi.org/10.1089/ars.2012.5124

Garcia A, Bocanegra-Garcia V, Palma-Nicolas JP, Rivera G (2012) Recent advances in antitubercular natural products. Eur. J. Med. Chem., 49: 1-23. https://doi.org/10.1016/j.ejmech.2011.12.029

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48: 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Heimler D, Vignolini P, Dini MG, Vincieri FF, Romani A (2006) Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chemistry, 99: 464-469. https://doi.org/10.1016/j.foodchem.2005.07.057

Kim Y, Kim WJ, Cha EJ (2011) Quercetin-induced Growth Inhibition in Human Bladder Cancer Cells Is Associated with an Increase in Ca-activated K-Channels. Korean J. Physiol Pharmacol., 15: 279-283. https://doi.org/10.4196/kjpp.2011.15.5.279

Kleemann R, Verschuren L, Morrison M, Zadelaar S, van Erk MJ, Wielinga PY, Kooistra T (2011) Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models. Atherosclerosis, 218: 44-52. https://doi.org/10.1016/j.atherosclerosis.2011.04.023

Luo H, Rankin GO, Li Z, Depriest L, Chen YC (2011) Kaempferol induces apoptosis in ovarian cancer cells through activating p53 in the intrinsic pathway. Food Chemistry, 128: 513–519. https://doi.org/10.1016/j.foodchem.2011.03.073

Mennen LI, Sapinho D, Ito H, Galan P, Hercberg S, Scalbert A (2008) Urinary excretion of 13 dietary flavonoids and phenolic acids in free-living healthy subjects – variability and possible use as biomarkers of polyphenol intake. Eur. J. Clin. Nutr., 62: 519-525. https://doi.org/10.1038/sj.ejcn.1602744

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science, 7, 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

Musienko MM, Parshykova TV, Slavnyi PS (2001) Spectrophotometric methods in plant physiology,biochemistry and ecology practice. Kyiv: Phytosociocentr, 200 p. In Russian.

Neugart S, Fiol M, Schreiner M, Rohn S, Zrenner R, Kroh L, Krumbein A (2013) Low and moderate photosynthetically active radiation affects the flavonol glycosides and hydroxycinnamic acid derivatives in kale (Brassica oleracea var. sabellica) dependent on two low temperatures. Plant Physiology and Biochemistry, 72: 161-168. https://doi.org/10.1016/j.plaphy.2013.04.002

Neugart S, Krumbein A, Zrenner R (2016) Influence of Light and Temperature on Gene Expression Leading to Accumulation of Specific Flavonol Glycosides and Hydroxy cinnamic Acid Derivatives in Kale (Brassica oleracea var. sabellica). Frontiers in Plant Science, 7: 1-16. https://doi.org/10.3389/fpls.2016.00326

Olsen H, Aaby K, Borge GIA (2010) Characterization, quantification, and yearly variation of the naturally occurring polyphenols in a common red variety of curly kale (Brassica oleracea L. convar. acephala var. sabellica cv. 'Redbor'). Journal of Agricultural and Food Chemistry, 58: 11346-11354. https://doi.org/10.1021/jf102131g

Park CH, Yeo HJ, Park YJ, Morgan AM, Valan Arasu M, Al-Dhabi NA, Park SU (2017) Influence of Indole-3-Acetic Acid and Gibberellic Acid on Phenylpropanoid Accumulation in Common Buckwheat (Fagopyrum esculentum Moench) Sprouts. Molecules, 22: 374. https://doi.org/10.3390/molecules22030374

Peng Z, Han C, Yuan L, Zhang K, Huang H, Ren C (2011) Brassinosteroid enhances jasmonate-induced anthocyanin accumulation in Arabidopsis seedlings. Journal of Integrative Plant Biology, 53(8): 632-640. https://doi.org/10.1111/j.1744-7909.2011.01042.x

Podsedek A (2007) Natural antioxidants and antioxidant capacity of Brassica vegetables: A review LWT— Food Science and Technology, 40: 1-11. https://doi.org/10.1016/j.lwt.2005.07.023

Qiao X, Jiang S, Li X, Li F, Zhao D (2011) Effects of phytohormones on plant regeneration and production of flavonoids in transgenic Saussurea involucrata hairy roots. Sheng Wu Gong Cheng Xue Bao, 27(1): 69-75.

Schmidt S, Zietz M, Schreiner M, Rohn S, Kroh LW, Krumbein A (2010a) Genotypic and climatic influences on the concentration and composition of flavonoids in kale (Brassica oleracea var. sabellica). Food Chemistry, 119: 1293–1299. https://doi.org/10.1016/j.foodchem.2009.09.004

Schmidt S, Zietz M, Schreiner M, Rohn S, Kroh LW, Krumbein A (2010b) Identification of complex ,naturally occurring flavonoid glycosides in kale (Brassicaoleracea var. sabellica) by high- performance liquid chromatography diode- array detection/electro spray ionization multi-stage mass spectrometry. Rapid Communications in Mass Spectrometry, 24: 2009-2022. https://doi.org/10.1002/rcm.4605

Sikora E, Bodziarczyk I (2013) Influence of diet with kale on lipid peroxides and malondialdehyde levels in blood serum of laboratory rats over intoxication with paraquat. Acta Sci. Pol., Technol. Aliment, 12(1): 91–99.

Sirota TV (n.d.) Method determination of antioxidant activity of superoxide dismutase and chemical compounds. Patent for invention №2144674 (In Russian).

Sokolovskiy VV, Lebedeva LV, Lielup TB (1974) On the method of separate determination of AA, DHA, and diketogulonic acid (DKGA) in biological tissues. Laboratory work, 3: 160-162 (In Russian).

Sun B, Yan HZ, Liu N, Wei J, Wang QM 2012() Effect of 1-MCP treatment on postharvest quality characters, antioxidants and glucosinolates of Chinese kale. Food Chemistry, 131: 519–526. https://doi.org/10.1016/j.foodchem.2011.09.016

Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry, 46: 4113-4117. https://doi.org/10.1021/jf9801973

Williams RJ, Spencer JPE, Rice-Evans C (2004) Flavonoids: Antioxidants or signalling molecules? Free Radical Biology and Medicine, 36: 838–849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Xi ZM, Zhang ZW, Huo SS, Luan LY, Gao X, Ma LN (2013) Regulating the secondary metabolism in grape berry using exogenous 24-epibrassinollide for enhanced phenolics content and antioxidant capacity. Food Chem, 141: 3056-3065. https://doi.org/10.1016/j.foodchem.2013.05.137

Xiao HB, Lu XY, Sun ZL, Zhang HB (2011) Kaempferol regulates OPN-CD44 pathway to inhibit the atherogenesis of apolipoprotein E deficient mice Toxicology and Applied Pharmacology, 257: 405–411. https://doi.org/10.1016/j.taap.2011.09.024

Xu F, Luan LY, Zhang ZW, Huo SS, Gao X, Fang YL, Xi ZM (2014) Phenolic profiles and antioxidant properties of young wines made from Yan73 (Vitis vinifera L.) and Cabernet Sauvignon (Vitis vinifera L.) grapes treated by 24-epibrassinolide. Molecules, 19(7): 10189-10207. https://doi.org/10.3390/molecules190710189

Zheng SY, Li Y, Jiang D, Zhao J, Ge JF (2011) Anticancer effect and apoptosis induction by quercetin in the human lung cancer cell line A-549, Molecular Medicine Reports, 5: 822-826. https://doi.org/10.3892/mmr.2011.726

Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem, 64: 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2