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Determination of fully N-methylated compound in different cabbage and beetroot varieties

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ABSTRACT It is proved that antioxidants have an important role in preventing cell damage - cancer, senescence and many other alterations leading to diseases. Supplementing the first pieces of experience and observations with scientific investigations has shown that food contains several components which may strengthen the common physiological functions, enhance the natural protecting systems of the body and the elimination of reactive radicals. The pyramid illustrating the "antioxidant" nutritional recommendations is based on vegetables and fruits. It is a proven fact that consuming these vegetables can restrict the absorption of carcinogenous substances and inhibit cell mutation. Cabbage and beetroot have long been used in folk medicine due to their widely known beneficial effects on human health. We carried out investigations comparing different varieties of beetroot and cabbage grown in identical circumstances. During the comparison of the quantity of quaternary ammonium compounds in cabbage varieties we have found the highest concentration of choline, while in beetroot varieties we have measured significant amount of betaine. Our results reveal well-detectable, significant differences among the analysed varieties (of both vegetable plants) which may be used in selecting the varieties most valuable with regard to human nutrition. **Acta Biol Szeged 49(1-2):43-45 (2005)**

KEY WORDS

cabbage (*Brassica oleracea* L. convar. capitata provar. capitata)
beetroot (*Beta vulgaris* L. ssp. esculenta convar. crassa provar. conditiva ALEF.)
quaternary ammonium compounds
choline
betaine

The latest investigations have proved that besides genetical disposition, lifestyle and nutrition are also of crucial importance in human health care and they inhibit the occurrence of many diseases. It has also been established that certain quaternary ammonium compounds play an important role in human health care and (disease) prevention.

Vegetables of Brassica genus belong to the most frequently consumed vegetables, and have been proved to restrict the absorption of carcinogenous substances and inhibit cell mutation.

According to the lately published research results, the vegetables of Brassica genus are the finest nutriment against cancer (Bonnesen et al. 2001).

The latest investigations highlight the important role of choline among quaternary ammonium compounds, because on the one hand it takes part in the process of hindering the accumulation of cholesterol, helping metabolism, consuming fatty acids, detoxicating the body and on the other hand during brain metabolism it is transformed into acetyl-choline that is the stimulus carrier substance of the nervous system (Zeisel and Blusztajn 1994; Busby et al. 2004). With regard to human health care, choline, or its metabolites (is needed for the structural integrity and signaling functions of cell membranes; it is a major source of methyl groups in the diet and it directly affects cholinergic neurotransmission, transmembrane signaling, and lipid transport/metabolism.
The major function of L-carnitin is to facilitate the passage of long-chain fatty acids through the mitochondrial membrane for subsequent β-oxidation and ketone synthesis, which is very important for human health (Hoppel 2003). Dietary intakes of betaine and choline are estimated to be in the 1–2 g amounts each per day (Frits 2005).

The significance of quaternary ammonium compounds in biotic and abiotic stress reactions (and in plant resistance) is also proven (William et al. 1996). The efficiency of the elimination of free radicals that are hazardous substances for living organisms is characterized by a total antioxidant capacity. Numerous publications prove the outstanding antioxidant effects of the Brassicaceae species (Kurilich et al. 2002; Llorach et al. 2003; Ninfali and Bacchiocca 2003).

Among the numerous useful components found in beetroot, betain, which takes part in homocysteine (Hcy) metabolism (in the reduction of the homocysteine level in the blood), has the most significant positive effect on human health care. Betaine and betaine analogues have multiple effects on homocysteine (Hcy) metabolism, which may have several implications for medicine and nutrition. Homocysteine is the toxic by-product of amino acid metabolism and may damage blood vessels thus leading to cardiac infarct and peripheral vascular disease as well as arthritis and osteoporosis. The increased plasma Hcy concentrations in humans correlates with increased risk of coronary, cerebral and peripheral vascular disease. Betaine and choline status have recently been identified as strong determinants of plasma Hcy clearance. Increased plasma Hcy levels are nowadays widely recognized

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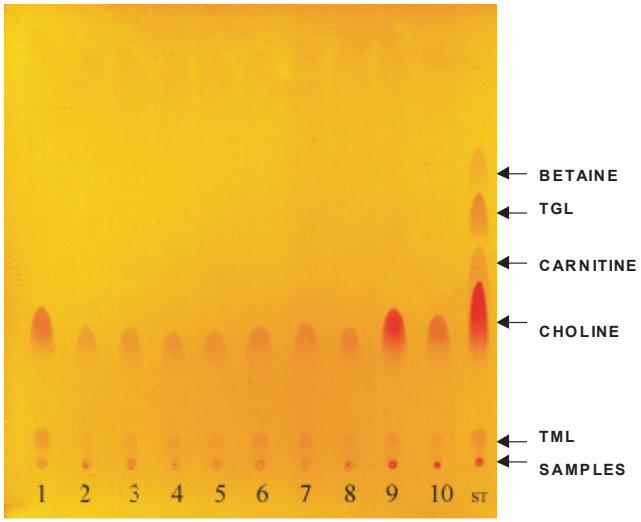

Figure1. Example for the separation of the fully N-methylated compounds: N^{E}-trimethyl-L-lizine (TML), choline, carnitine, trigonelline(TGL), betaine.

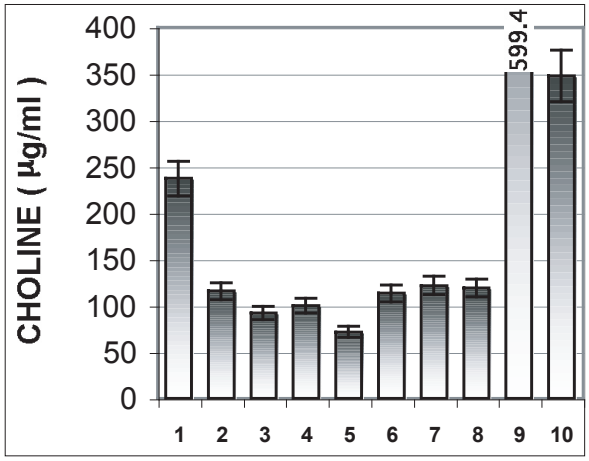

Figure 2. The concentration of quaternary ammonium compounds in cabbage cultivars. The codes from 1 to 10 represent the different cabbage cultivars, which were grown under identical growing circumstances.

as potential risk factor for atherothrombotic vascular disease (Barak et al. 1996; Eikelboom et al. 1999). Betain is used in the treatment of (hyperhomocysteinemia) HHcy, (also called hereditary homocystinuria), which is an inborn error that is clearly related to thromboembolic events that respond favorably to Hcy lowering, and it may also help to prevent the liver from becoming fatty as a result of chronic alcoholism, protein production problems or neglected diabetes. (Wilcken et al. 1983; Devlin et al. 2004; Slow et al. 2004).

Betain is important for proper liver function and the replication of liver cells as well as in the detoxification process. Betain takes part in carnitin production, which helps to defend the kidneys as well. It plays a role in binding harmful free radicals, which makes it potentially important in cancer research.

In accordance with our goals, we have examined a group of compounds, which, apart from playing a role in the disease resistance of plants, significantly improve human health conditions (when the plants are consumed) by means of their bioactivity and importance in human health care. The results of our experiments may be used on the one hand in order to breed, grow and commercially distribute varieties (hybrids) that are more resistant to diseases, *i.e.* which are rich in natural immune stimulating factors and on the other hand to produce varieties with especially high active agent contents, which have a positive effect on health conditions.

Materials and Methods

The compared cabbage and beetroot varieties were grown in identical agricultural land (identical growing circumstances), therefore the quantitative differences between detected quaternary ammonium compounds with various approaches reflected truly variety-dependent differences.

The leaves and roots were frozen with liquid nitrogen, powered and suspended in dimedone solution (0,05% dimedone in methanol). This suspension was centrifuged at 1500 g for 10 minutes at 4°C. The clear supernatants were used to OPLC separations (Gersbeck et al. 1989).

The separation was carried out on OPLC silica gel 80 F254 precoated chromatoplates using a chloroform-methylenechloride mixture (35:65, V/V) for quaternary ammonium compounds. Calibration curves were made by means of authentic substances (at λ=265 nm for formaldemethone and at λ=525 nm for quaternary ammonium compounds which were detected by Dragendorff reagent). Densitograms were taken with a Shimadzu CS-930 scanner. Samples were applied with a NANOMAT sample applicator.

Results

Between the fully N-methylated compounds (N^{E}-trimethyl-

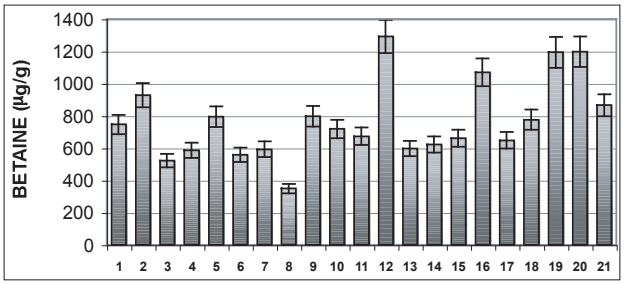

Figure 3. The concentration of betain in various beetroot cutivars which were grown on the same plot under identical growing circumstances.

L-lizine, choline, carnitine, trigonelline betaine), the cabbage varieties showed a prominently high choline content. The nutritionally significant choline quantity in the studied cabbage varieties varies between 200 and 1040 μg/g per fresh mass. N^E-trimethyl-L-lizine and carnitine can also be found in the samples but their concentration is significantly lower and was not clearly detectable under the prevailing conditions.

Among the quaternary ammonium compounds, in the beetroot samples, a high concentration of betain can be found in significant concentration. 28,5% of the varieties fall under the 0,6 mg/g level, another 28,5% are above the 0,8 mg/g level and 43% can be characterised by a betain concentration of 0,6-0,8 mg/g per fresh mass. In the lyophilised samples significantly higher concentration was measured. Fresh samples contained betain in a concentration of 0,35-1,3 mg/g, while the concentration of lyophilised ones was between 10,3-18 mg/g of dry mass.

Discussion

Our measurements were aimed at answering the question what variability the inner characteristics playing a supposed or proven role in the biaoactive effect of cabbage and beetroot show in the varieties grown in identical circumstances but with different genetical backgrounds.

On the basis of the measurements of quaternary ammonium compounds, every studied biochemical parameter was clearly separable, indicated well-definable, quantitative differences among varieties, which is new evidence in favour of the significant variety-dependence of the quantity of the natural compounds with positive effects found in plants.

The experience and the results of our investigations may on the long run provide help to plant breeders, producers and distributors with selecting the cabbage and beetroot varieties which are the most valuable from a human health point of view.

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