

During our experiment we measured FRAP and colour content in 4 red cabbage hybrids and 20 beet root varieties. In formation of bioactive substances of vegetables are very important the heritable quality parameters too. In this way we examined not only the different species, and the role of varieties belong to them.

The red pigments were evaluated from diluted samples. A spectrophotometer was used to determine the absorbance of pigments:  $\lambda=538$  nm for red pigments and  $\lambda=476$  nm for yellow ones. For total phenol content the colour reaction to Folin-Denis reagents were evaluated at  $\lambda=760$ nm, by means of a catechin standard (mg catechin/100 ml). Total antioxidant content was expressed in the so-called FRAP values (ferric reducing ability of plasma) in  $\mu\text{M/l}$ . The method is based on the ability of antioxidants to reduce  $\text{Fe(III)}$  ions to  $\text{Fe(II)}$  ions in buffered sour medium (pH 3.6). The produced  $\text{Fe(II)}$  can be measured on photometers. Absorbance is proportional to the quantity of the produced  $\text{Fe(II)}$  ions and the antioxidants, respectively.

Our measurements showed more than threefold differences in total antioxidant activity among varieties, the lowest value being  $171.13 \mu\text{M/l}$  and highest  $702.57 \mu\text{M/l}$ . The corresponding betanin ( $17.18$  and  $57.80 \text{ mg/100 ml}$ ) and total polyphenol ( $37.5$  and  $85.5 \text{ mg/100 ml}$  respectively) contents show similar differences. The highest FRAP values was measured in the *Bonel*, *Pablo* and *Pronto* varieties ( $506.97$ ;  $571.43$ ;  $702.57 \mu\text{M/l}$ ). Based on our results it can be stated that varieties of higher betanin and polyphenol contents have higher antioxidant values as well. With the further measurements we concluded that red cabbage varieties greatly vary in pigment. There is a correlation between the pigment and dry matter content and FRAP. According to our data the highest FRAP parameters were measured in *Sandoro F<sub>1</sub>*, whose colour intensity also proved to be excellent. Lower parameters were shown by *Rendero F<sub>1</sub>*, which also lagged behind regarding dry matter content and pigment content.

Our measurements showed the varieties with higher pigment and polyphenol content have high antioxidant values too. There is a close correlation between red pigments (betanin), total polyphenol contents and FRAP values. The correlation between the quantity of these compounds and the FRAP values ( $r = 0.7799$  and  $r = 0.7435$ , respectively).

Accordingly, the two compounds must have a role in the evolution of antioxidant effects.

## Biological evaluation of volatile oils and aromatic agents by FRAP method

M Then<sup>1</sup>, I Szöllősi Varga<sup>2</sup>, É Héthelyi<sup>1</sup>, É Lemberkovics<sup>1</sup>, K Szentmihályi<sup>3</sup>

<sup>1</sup>Institute of Pharmacognosy, Semmelweis University, Budapest, Hungary, <sup>2</sup>Department of Genetics and Molecular Biology, University of Szeged, Szeged, Hungary, <sup>3</sup>Institute of Materials and Environmental Chemistry, Chemical Research Center of the Hungarian Academy of Sciences, Budapest, Hungary

Biological (antioxidant) values of volatile oils used in medication and basic components used in flavouring and perfumery have not been known so far. For that reason the authors examined the applicability of a chemical method (FRAP) adapted for plant materials. The aim of the work was to study that the volatile oils and essence of perfume of positive effect to biochemical (allergic) processes in the human body may possess any antioxidant properties.

Volatile oils, fragrance compositions (known and unknown combinations, but known tendency) were studied. The measurements were made in 1% solutions. The components of volatile oils and fragrance compositions were identified by GC-MS and FRAP method was used for measurement of antioxidant property.

The identified and frequently occurring basic-, aromatic- and perfumed compounds may be characterized by the following FRAP values: methyl salicylate  $303 \pm 1 \mu\text{mol/L}$ ,  $\alpha$ -amyl acetate  $144 \pm 2 \mu\text{mol/L}$ , borneol  $284 \pm 1 \mu\text{mol/L}$ , camphor  $286 \pm 1 \mu\text{mol/L}$ , carvon  $164 \pm 2 \mu\text{mol/L}$ , menthol  $1.86 \pm 0.23 \mu\text{mol/L}$ , menthon  $1.88 \pm 1.10 \mu\text{mol/L}$ , thymol  $284 \pm 1 \mu\text{mol/L}$ , linalol  $299 \pm 1 \mu\text{mol/L}$ , linalyl acetate  $221 \pm 4 \mu\text{mol/L}$ , limonene  $445 \pm 3 \mu\text{mol/L}$ , terpineol  $142 \pm 1 \mu\text{mol/L}$ , cinnamic aldehyde  $303 \pm 1 \mu\text{mol/L}$ , anethol  $509 \pm 2 \mu\text{mol/L}$ , while the FRAP values of volatile oils were the followings: lemon oil  $198 \pm 1 \mu\text{mol/L}$ , geranium oil  $152 \pm 2 \mu\text{mol/L}$ , sage oil  $313 \pm 3 \mu\text{mol/L}$ , pine oil  $255 \pm 1 \mu\text{mol/L}$ , muscate sage oil  $484 \pm 2 \mu\text{mol/L}$ , patchuli oil  $178 \pm 2 \mu\text{mol/L}$ , petitgrain oil  $174 \pm 3 \mu\text{mol/L}$ , dill seed oil  $323 \pm 1 \mu\text{mol/L}$ , eukalyptus oil  $14 \pm 2 \mu\text{mol/L}$ , clove oil  $197 \pm 1 \mu\text{mol/L}$ , peppermint oil  $167 \pm 1 \mu\text{mol/L}$ , rosemary oil  $35 \pm 1 \mu\text{mol/L}$ . The FRAP values of essence-compositions varied between  $250$ - $1000 \mu\text{mol/L}$  (female  $251 \pm 8 \mu\text{mol/L}$ , male  $409 \pm 13 \mu\text{mol/L}$ , kid  $1093 \pm 7 \mu\text{mol/L}$ ). At the T1-T15 style tendency with unknown compositions the values were between  $3000$ - $9000 \mu\text{mol/L}$  in 10% solutions, while smaller values characterize the american unisex essences (P-18, P-19, P-20):  $243 \pm 2 \mu\text{mol/L}$ ,  $252 \pm 4 \mu\text{mol/L}$  and  $520 \pm 1 \mu\text{mol/L}$ , respectively.

It has been stated that the FRAP method is suitable for measurement of in vitro antioxidant property of complex compositions.



The FRAP values of volatile oils of the knowledge of main component help to estimate the quality of volatile oil with 20-30% deviation. In the case of fragrance compositions with known essence style tendency, the values show the reducing power and they do not contain any other information.

It is recommended to determine the antioxidant values of all aromatic agents, volatile oils and essence-compositions beside physico-chemical characteristics.

## **Modification of fully activated NADPH oxidase activity by antioxidants**

Zs Varga, E Kosaras, M Katkó

Ist. Department of Medicine Health and Medical Science Center, University of Debrecen, Debrecen, Hungary

The first reactive oxygen-derived substance is the superoxide anion produced by NADPH oxidase. This is a multicomponents enzyme containing several cytosolic (phox proteins) and membrane-bound (Cytochrome b558) parts. NADPH oxidase can be activated by receptor-mediated and non-receptor-mediated ways. During the activation the cytosolic components are phosphorylated and translocated to membrane, where they are combined with the membrane-bound part of Cytochrome b558. Cytochrome b558 consists of two parts, gp91phox and p22phox proteins. During the last few years it became clear that this complex is not able to produce superoxide anion, it requires coupling of a Rac 1 or 2 GTPase G protein to complex. When coupling of Rac 1/2 is inhibited the NADPH oxidase enzyme could not produce superoxide anion. Now, it is well demonstrated that NADPH oxidase can be found not only in phagocytes such as neutrophils, monocytes, but many other cells as well, they are called to NOX enzymes. NOX was identified in uterus, renal cells, hepatocytes, endothelial cells, lymphocytes, smooth muscle cells etc. Those non-phagocyte NADPH oxidases (NOX1, NOX3, NOX4) differ from phagocyte one (NOX2) in the structure of gp91 phox subunit. This difference results in that non-phagocyte NADPH oxidase produces smaller amounts of superoxide anion and its activation does not require activation of PKC.

During the last years, our group studied the effects of several natural and synthetic antioxidants on superoxide anion production and activation of PKC in human neutrophils. These examinations involved the study the effects of antioxidants on superoxide anion production by fully activated NADPH oxidase (NOX2) as well. During experiments we have found that some antioxidants can decrease it, while others have not such effects. It was also demonstrated that the effects of antioxidants on fully activated NADPH oxidase was independent on PKC inhibition; and as a consequence, independent from the modification of superoxide anion production in intact neutrophils induced by antioxidants. These differences were the most pronounced in case of tocopherols and their water soluble metabolites (CEHC). Both tocopherols and CEHC-compounds inhibited PKC and superoxide anion production in phorbol-ester stimulated neutrophils, and CEHC were more effective inhibitors as parent tocopherols. In contrast, superoxide anion production by fully activated NADPH oxidase was only decreased by lipid-soluble tocopherols.

On the basis of our observations, we suggest the following mechanism for the action of antioxidants on superoxide anion production by fully activated NADPH oxidase: the bound between the enzyme complex and the small Rac 1/2 protein - which is necessary for superoxide anion production by NOX - might be slack or broken by antioxidants, which is due to the counteraction of lipid-soluble antioxidants and cell membrane. This observation might be useful in those clinical states when activation of NADPH oxidase occurs - in either phagocyte or non-phagocyte cells-, since in these cases we can choose antioxidants which are able to decrease superoxide anion production by fully activated NADPH oxidase(s) (NOX) and prevents development of oxidative stress.

Research was sponsored by Fund of Hungarian Academy of Sciences (T 42550).