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HEALTH-AFFECTING METHYL-DONOR COMPOUNDS IN SOUR CHERRY (*PRUNUS CERASUS* L.) FRUIT PARTS IN THE FRUIT BURGEONING STAGE

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In recent years, numerous studies have confirmed the vital role and therapeutic potential of quaternary ammonium compounds and endogenous formaldehyde in the prevention and treatment of diseases. These compounds participate in the transmethylation processes and play a role in the metabolism and in the regulation of cellular processes. The present research indicates that sour cherry fruit contains large quantities of quaternary ammonium compounds in the early developmental phase (burgeoning). The quantity of methyl-donor compounds (choline, carnitine) and easily mobilizable methyl groups were measured in various fruit parts (stalk, fruit flesh, seed kernel) of five sour cherry cultivars using OPLC technology, and the cultivar dependence of the detected compounds was examined. The results of comparative examinations established the presence of choline in the stalk and seed and of a significant amount of carnitine in the fruit flesh. The clear, significant differences between the genotypes will facilitate the selection of cultivars containing the largest proportion of components beneficial for human health.

Keywords: sour cherry, choline, HCHO, carnitine, OPLC evaluation

Sour cherries are very important dietary sources of vitamins and polyphenols. Most studies focus on the antioxidant properties of the polyphenol components (SASS-KISS et al., 2005). High anthocyanin content has beneficial effect in the case of obesity, inflammatory processes, hyperlipidaemia, type 2 diabetes, and cardiovascular diseases (FORD & MOKDAD, 2001; SEYMOUR et al., 2009). Sour cherry stalks have long been used as an infusion or tea, but compared to the fruit, relatively little information is available regarding the composition and bioactive properties (BASTOS et al., 2015). Based on previous studies, sour cherry stalks make an excellent diuretic (HOOMAN et al., 2009) and, due to its natural antioxidant (polyphenol) content, can also be used as a detoxicant (BURSAL et al., 2013). The extract of sour cherry seed kernel is rich in flavin, flavonoid, and trans-resveratrol that have protective effects in the cardiovascular system (BAK et al., 2006). The oil of seed kernel contains unsaturated fatty acids, oleic acids, α-tocopherol, and tocopherol like components. The combined effect of these compounds is expected to make sour cherry seed kernel oil a valuable dietary supplement (BAK et al., 2010).

In woody plants, compounds participating in transmethylation processes, such as endogenous formaldehyde (HCHO) and quaternary ammonium compounds, have been the subject of less research, though their role in the biotic and abiotic stress tolerance of plants has been demonstrated (GOPAL et al., 1990; SÁRDI & TYIHÁK, 1998; NUCCIO et al., 2001). In transmethylation reactions, S-adenosyl-L-methionine (SAM) is the “methyl donor”, and the

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methyl group of SAM reaches the acceptor molecules through the formation of HCHO as an intermediate product (Huszti & Tyihák, 1986). HCHO precursors (generators, methyl donors) are connected directly or indirectly to the methylation cycle, in which nucleic acids, proteins, peptides, amino acids, biogenic amines, nor-alkaloids, etc. may be involved as acceptor molecules. Transmethylation, i.e. the removal or integration of methyl groups, is a low-energy, fast substitution reaction allowing reversible regulation. The reversible reactions influence the weight, hydrophobicity, and solubility of molecules, thereby affecting their function and enzyme activity (Németh, 2002). The "HCHO" denomination means those methyl or oxidised methyl groups in the biological systems from which potentially HCHO may arise.

Recent research shows that HCHO plays a key role in the antibiotic effects (Burke & Koch, 2001) and may fulfil an essential function in regulating the balance between cell division and cell death, where a defect may lead to diseases through abnormal cell proliferation (Tyihák et al., 2001). The role of methyl-donor compounds (primarily choline and betaine) in the metabolism and in the regulation of cellular processes has been proved from various aspects (Niculescu & Zeisel, 2002; Mudd et al., 2007; Kotsopoulos et al., 2010; Corbin & Zeisel, 2012).

On the one hand, choline inhibits cholesterol accumulation and facilitates metabolism, the utilization of fatty acids, and the detoxification of the body; on the other hand, during brain metabolism, it transmutes into acetylcholine, which participates in signal transduction and in the regulation of the functions of nerves coordinating muscle movement (Yoshimoto et al., 2004). As phosphatidylcholine, it preserves the integrity of the cell membrane, participates in intracellular communication, in the transmission of information, and in the energy supply of cells (Vivekananda et al., 2000). Based on research by Zeisel and co-workers (2003), the most significant food products containing choline are animal source foods. Among plant source foods, seeds or young plant parts are worth mentioning.

Carnitine (L-carnitine) plays a critical role in intracellular and metabolic functions, such as the transportation of fatty acids to the mitochondria, the stabilization of cell membranes, and the reduction of serum lipid levels. L-carnitine supplement has proved to be advantageous in the case of obesity, type 2 diabetes, and liver cirrhosis (Del Ben et al., 2016). The best sources of carnitine are animal source foods. Vegetables have significantly lower carnitine content and fruit even lower (Knüttel-Gustavsen & Harmeyer, 2007).

Lack of methyl-donor compounds alters DNA methylation, and thus gene expression (Zeisel, 2012). Abnormal DNA methylation can often be found in cancer cells and may be associated with chromosome instability and the inactivity of tumour-inhibiting genes (Davis & Uthus, 2004). The objective of the present experiments was thus to examine compounds participating in endogenous transmethylation processes in different plant parts of sour cherry fruit at a young developmental stage (in spring). The research focused primarily on the comparison of cultivars based on the nutritional and human-health-related significance of quaternary ammonium compounds.

1. Materials and methods

1.1. Plant material

The sour cherry cultivars 'Érdi bőtermő', 'Csengődi', 'Pipacs 1', 'Kántorjánosi', and 'Újfehértói fürtös' were obtained from the Fruitculture Research Institute of the National

Agricultural Research and Innovation Centre. The samples were collected from a commercial orchard. The trees were grafted on *Mahaleb* rootstock and were spaced 5 m apart in the row, with 7 m between rows. The samplings were performed in 2017 in five repetitions. One repetition was prepared by homogenising 15 samples from each fruit part. The fruit were pitted, all flesh and stem were used for analysis. The endocarps of the seeds were removed, and only the kernels were used for analysis. The determination of the optimal times of fruit burgeoning was based on the method elaborated by SZABÓ (2007). All fruit samples (stalk, fruit flesh, and seed kernel) were collected in the same burgeoning state, i.e. when the fruit turn from green to yellowish green.

1.2. Chemicals and OPLC evaluation

The chemicals (N^{ε}-trimethyl-lysine, carnitine, choline, trigonelline, betaine, dimedone, and sodium acetate) were of HPLC grade and were purchased from Sigma-Aldrich Ltd. (Budapest, Hungary). All solvents (methanol, chloroform, methylene chloride, and *i*-propanol) were of HPLC grade and were purchased from Merck Ltd. (Budapest, Hungary).

The OPLC (Overpressured Layer Chromatographic Separation) technique and densitometry evaluation (developed by OPLC-NIT Co., Ltd., Budapest, Hungary) were used for the quantitative and qualitative determination of quaternary ammonium compounds and endogenous formaldehyde. Tissue samples of fruit parts were frozen in liquid nitrogen, powdered, and extracted with dimedone solution (500 mg tissue powder in 800 ml of 0.03% methanolic dimedone solution). After 10 min of ultrasonic cleaning (Titertek Ultrasonic Cleaner), the suspension was centrifuged (ScanSpeed Mini) at 1000 *g* for 10 min at room temperature. The clear supernatants were used for OPLC separation on silica gel plates (Kieselgel 60 F_{254}). The mixture of quaternary ammonium compounds used as standard contained N^{ε}-trimethyl-lysine (TML), carnitine, choline, trigonelline (TGL), and betaine. The OPLC separations were carried out using chloroform – methylene chloride (v/v=35:65) for formaldemethone determination and *i*-propanol – methanol – 0.1 M sodium acetate (v/v=20:3:30) for quaternary ammonium compounds. After development, the formaldemethone and quaternary ammonium compound spots were evaluated using a Shimadzu CS-930 TLC/HPTLC scanner at λ=265 nm for formaldemethone and at λ=525 nm for quaternary ammonium compounds using, in this case, Dragendorff reagent (SÁRDI & TYIHÁK, 1998).

1.3. Determination of dry matter content

From each homogenised fruit sample 5 g was oven-dried at 105 °C to constant weight, and the dry matter content was calculated from the weight loss.

1.4. Statistical analysis

Data were analysed using the ANOVA model and means comparison, and significant differences were calculated using Duncan's multiple range test (P=0.05). Five repetitions were run in all three methods of testing (stem, fruit flesh, and seed kernel) in 2017. The correlations were analysed by Spearman's rank order correlation (P=0.01 and P=0.05). Statistical analysis was carried out using the 22.0 software (SPSS Inc., Chicago, USA).

2. Results and discussion

Among the quaternary ammonium compounds occurring in the standard used for identification under the given measuring circumstances, choline was reproducibly measurable at this developmental stage in the seeds and stalks and carnitine in the fruit flesh.

The quantities of detectable methyl-donor compounds depended on the plant part and were significantly dependent on the cultivar. The quantities of endogenous HCHO and methyl-donor choline showed the least variability in the seed (Fig. 1), where the choline levels were about five times that detected in the stalk (Fig. 2).

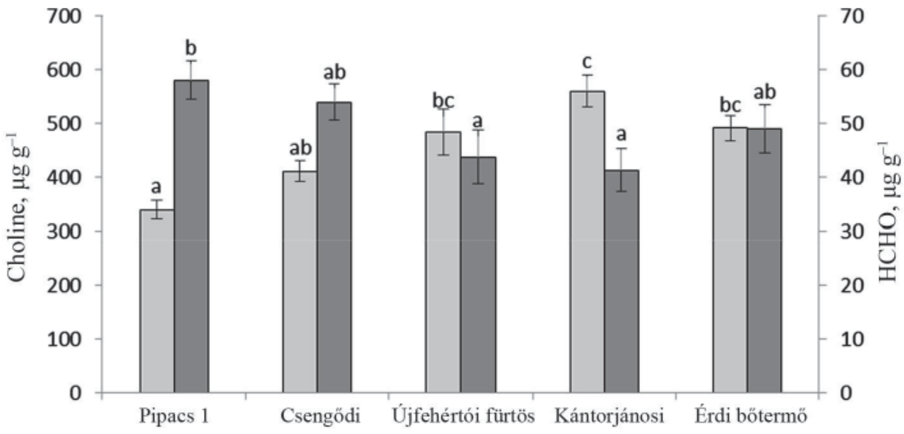

Fig. 1. Choline and endogenous HCHO contents in the seed kernels (Mean values with different letters are significantly different at P=0.05). ■: Choline; □: HCHO

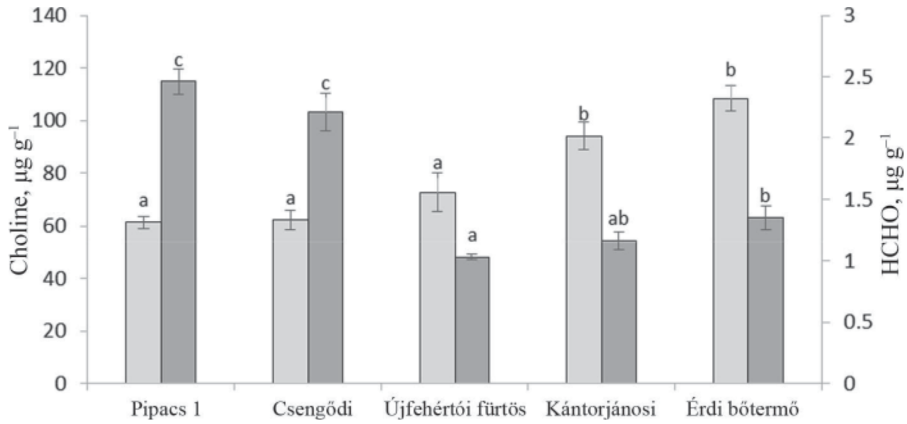

Fig. 2. Choline and endogenous HCHO contents in the stalks (Mean values with different letters are significantly different at the P=0.05). ■: Choline; □: HCHO

Significant differences could also be seen in the HCHO quantities, which were again dependent on plant part and genotype. There was a negative correlation between the choline and HCHO contents: cultivars with higher choline contents contain smaller amounts of "bound" HCHO than those with lower choline contents. However, a statistically verifiable negative correlation between choline and HCHO could only be established in the seed kernel, R^2=0.9006 (Fig. 3). A negative correlation between choline and HCHO was also observed in the fruit stalk, but the correlation was not significant, so further examinations will be needed before conclusions can be drawn.

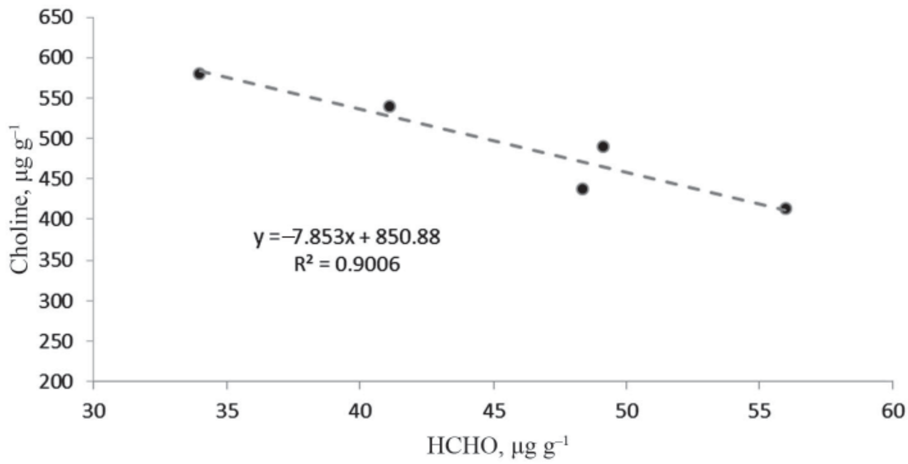

Fig. 3. Correlation between the average choline and HCHO quantities in the seed kernel (P=0.01)

Surprisingly high quantities of carnitine were detected in the fruit flesh, but considerable cultivar dependence was again observed. With the exception of one cultivar ('Érdi bőtermő'), higher carnitine contents were associated with lower "bound" HCHO quantities (Fig. 4). The negative correlation was statistically verifiable, R^2=0.6131 (Fig. 5).

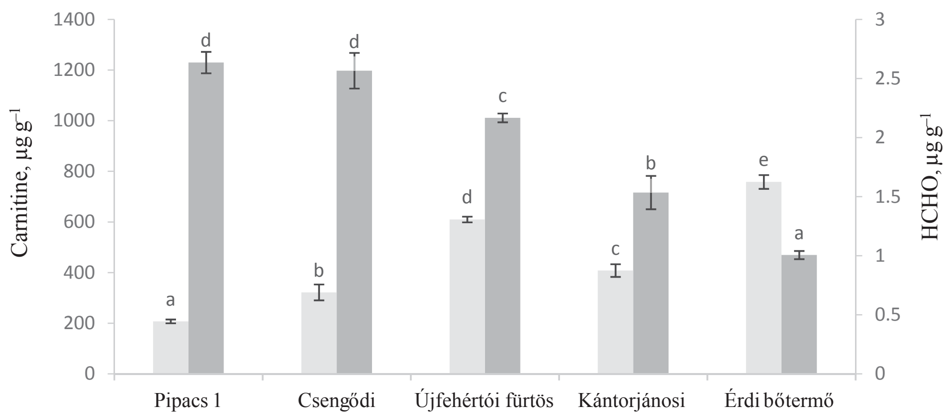

Fig. 4. Carnitine and endogenous HCHO contents in the fruit flesh (Mean values with the different letters are significantly different at P=0.05) ■: Carnitine; ■: HCHO

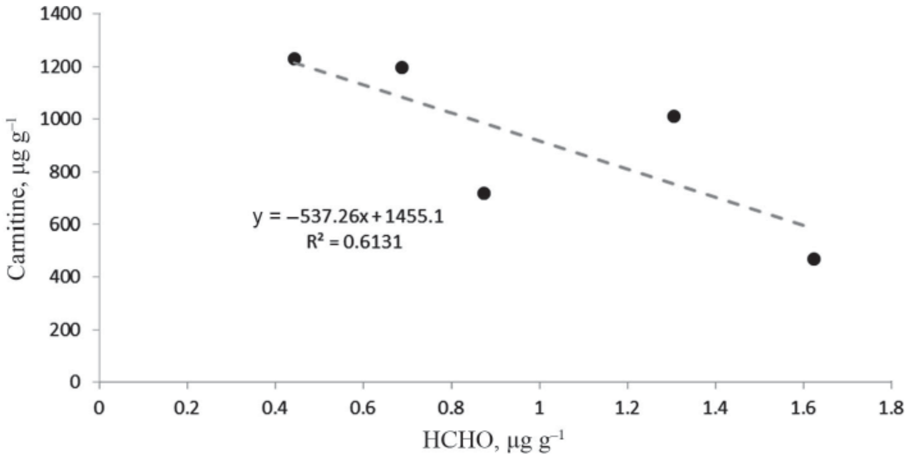

Fig. 5. Correlation between the average quantities of carnitine and HCHO in the fruit flesh (P=0.05)

On the basis of dry matter content, the quantities of methyl-donor compounds were converted into dry weight in order to determine the chemical quality of the fruit parts as possible components of dried or lyophilised products (Table 1).

Table 1. Quantities of methyl-donor compounds and HCHO in terms of dry mass in specific fruit parts

Fruit part	Seed kernel		Stalk		Fruit flesh	
	dry mass, µg g^{-1}		dry mass, µg g^{-1}		dry mass µg g^{-1}	
Cultivar	HCHO	Choline	HCHO	Choline	HCHO	Carnitine
Pipacs 1	54.8±16.8a	937±135.6b	1.4±0.3a	119.2±23.2b	0.5±0.1a	1375.8±103.1d
Csengődi	68.5±13.6ab	898.8±67.1ab	1.4±0.3a	106.6±11b	0.7±0.3a	1298.7±110d
Újfehértói fürtös	83.9±16bc	758.5±224.6ab	1.6±0.4ab	49.9±9.2a	1.5±0.5b	1128.9±125.2c
Kántorjánosi	95.9±26.8c	707.8±166a	2.1±0.6bc	56.1±7.8a	0.9±0.4a	776±157.7b
Érdi bőtermő	78.1±20.6abc	778.4±117.9ab	2.4±0.9c	65.1±13.7a	1.7±0.4	502.4±162.6a

[a,b,c]: Mean values followed by different letters are significantly different at P=0.05

At present, primarily in the field of medical sciences, increasing attention is being paid to the physiological effects of endogenous transmethylation and the methyl-donor compounds participating in the methylation cycle, as well as to the study of their role in the human metabolism, in its regulation, and in the control of various gene functions (Corbin & Zeisel, 2012). Methylation defects are demonstrably linked to an increased risk of numerous diseases (including tumorous diseases). Knowledge of the connection between defects in methylation and demethylation processes and the development of various diseases will enable us to influence and, if necessary, to normalize the “available” methyl pool of the body in order to preserve or, in the case of destabilization, to restore the methyl balance (Tiraboschi et al., 2004; Del Ben et al., 2016). This question can be approached through chemical-biochemical means and via natural resources and nutrition (Sárdi et al., 2009; Blázovics et al., 2012). The quantitative dependence of HCHO and methyl-donor compounds on the age and development of the plant part was previously verified in the case of watermelon (Sárdi & Tyihák, 1998) and bean plants (Sárdi & Stefanovits-Bányai, 2006), larger quantities being observed in younger plant parts than in older ones. The early developmental stage was chosen, because the main purpose was to find plant sources rich in methyl-donor compounds. The quantities of these compounds showed significant cultivar dependence even at the early burgeoning stage and were also significantly influenced by the plan part. A considerable amount of choline was detected in the seeds and high concentrations of carnitine in the fruit flesh.

The average carnitine content in the fruit flesh of the sour cherry genotypes was 91.8 mg/100 g of fresh mass and 101.6 mg/100 g of dry mass, considerably exceeding the published data for the plants (Knüttel-Gustavsen & Harmeyer, 2007). The average choline content in the seed kernels of burgeoning sour cherry fruit was 49.2 mg/100 g of fresh mass and 81.6 mg/100 g of dry mass, which, based on data in the literature (Zeisel et al., 2003), could make them a good plant source for choline. The average choline content of the stalk was 7.68 mg/100 g of fresh mass and 7.95 mg/100 g of dry mass, which could contribute to its well-known health benefits.

3. Conclusions

The results confirm that, by systematically extending the range of plant species examined, it is possible to find new sources of compounds with proved role in human health preservation. The consumption of biologically active compounds of natural origin is less likely to lead to dangerous overdoses than that of synthetic compounds currently available, further emphasising the benefit of natural sources. Because the plants' methyl donor pool is a less researched area, our examination may give new information in the case of any plant origin food recommended for consumption. If the seeds are to be used as food supplement, it is important to examine not only beneficial components, but also possible harmful compounds, such as amygdaline, which occurs in various quantities in drupes and whose beneficial or harmful effect is very controversial (Park et al., 2005; Milazzo et al., 2007).

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