VARIABILITY OF ANTHOCYANIN CONTENT AND DRY MATTER AMOUNT IN FRUITS OF SOME *LONICERA CAERULEA* SELECTIONS DEPENDING ON STORAGE CONDITIONS

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Abstract. *Lonicera caerulea* var. *kamtschatica* is a species with multiple uses mainly due to the valuable biologically active substances with high antioxidative potential. The anthocianins occupy an important place in inducing the health-protective benefits of the berries of this species. The aim of our work was to determine the total anthocyanin and dry matter amounts in the fruits collected from Romanian selections of blue honeysuckle, preserved by freezing (three months in refrigerator) and drying at 60°C (to constant weight). The obtained results showed that the storage conditions have generally influenced the anthocyanin content. Thus, in freezing storage conditions, the anthocyanin amount either decreased (C, SL6, SL8, SL15) or increased (SL17, SL57), depending on the biological material, whereas the drying preservation declined the anthocyanin level with ~ 80%, also varying with the selections of blue honeysuckle.

INTRODUCTION

Lonicera caerulea L. *var. kamtschatica* (blue honeysuckle) (Caprifoliaceae family) is a species of shrub largely used in folk medicine because of its rich phytochemical content and which grows across the Northern Hemisphere (Russia, China and Japan) (Palíková *et al*., 2008). From northern Russia, the plant was introduced and cultivated, although on smaller surfaces, in Central Russia, in Urals, Siberia, and more recently in Canada and USA (Mladin *et al*., 2011) where the blue honeysuckle was little known for the edible properties of its berries (Svarcova *et al*., 2007). In Europe, the species occurs rarely in the Alps and Scandinavia (Hummer *et al*., cf. Svarcova *et al.,* 2007). In Romania, this plant - one of the few species with edible fruits of the *Lonicera* genus – was introduced ~ 30 years ago, at the Research Institute for Fruit Growing, Pitesti – Maracineni, where it was subjected to complex breeding and selection activities with the aim to enlarge the range of the phenotype variability concerning the bio productive and the biochemical traits and to select the forms with the best adaptability to the conditions of Romania (Mladin *et al*., 2011).

The *Lonicera caerulea* berries have multiple edible and medicinal uses due to the complex biochemical constitution and in literature there are numerous studies dedicated to the knowledge of the biochemical profile of various provenances belonging to this species (Svarcova *et al*., 2007; Ochmian *et al*., 2010; Rop *et al*., 2011; Jurikova *et al*., 2012). The main group of the polyphenolic compounds showing high antioxidant properties and which confer to the blue honeysuckle berries beneficial effects for human health are the anthocyanins (Oprea *et al*., 2002; Jordheim *et al*., 2007). They are secondary plant metabolites, namely water-soluble flavonoids, generally found as glycosidic forms (3-glycosides and 3,5-diglycosides), which are generally linked to glucose, galactose, rhamnose, and arabinose (Svarcova *et al.*, 2007). In ecosystems, the anthocyanins have crucial role; together tannins, they play a major role in the expression of fruit colour and taste, with repercussions on the fruit quality and edible valences (Mladin *et al*., 2011). Coloured petals of the flowers have role in attraction of the insects during pollination, while the ripe fruits serve as food for animals (distributors of seeds), and for humans, so being contributors to their diet. The maximum anthocyanin content was evidenced during fruit ripening, and it decreased towards the end of this period. In early stage of fruit ripening, the anthocyanins are masked by chlorophyll (Vlahov, 1992). Some authors consider that anthocyanins are protective factors for the photochemical system and contribute to the increase of photosynthesis yield (Lee *et al*., 2003). Out of the anthocyanins, the fruits of *Lonicera* are rich sources of ascorbic acid, sugars (5 – 10%), lipids (1.52%), organic acids (1.5 – 4%, the most important being citric acid – 90% of the total), and variable quantities of minerals (K, Mg, P, Ca), carotenoids, other phenolic compounds such as proanthocyanidins or phenolic acids which determines their multiple biological activities. Into the anthocyanin level, cyanidin-3-glucoside generally dominates in the berries of most blue honeysuckle berries, with ~84-90% from total amount, followed by peonidin, delphinidin and pelargonidin (Malodobry *et al*., 2010; Mladin *et al*., 2011 Kusznierewicz *et al*., 2012).

The range of the therapeutic effects manifested by the compounds extracted from blue honeysuckle includes the lowering of blood pressure, protection against the risk of heart attack, prevention of osteoporosis and anaemia, diminution of child hyperactivity, amelioration of healthy state in malaria, gastrointestinal disorders and some eye disorders, slowing of the aging processes, modulation of glycemic responses *etc*. (Tarabasanu-Mihaila *et al*., 1997; Svarcova *et al*., 2007; Palíková *et al*., 2009; Hanhineva *et al*., 2010; Jiao and Wang, 2010; Malodobry *et al*., 2010; Jurikova *et al*., 2012). Out of the above mentioned benefits, the antioxidative action of the anthocyanins induces a good protection against serious diseases such as diabetes mellitus, neurodegenerative conditions and certain types of cancers (Zhao *et al*., 2004; Gruia *et al*., 2008). The anthocyanins can be also utilized as natural food colorants (Vendramini and Trugo, 2004), while Palíková *et al*. (2008) reported that freeze-dried fruits of blue honeysuckle and their purified phenolic fraction reduced biofilm formation and adhesion of some pathogenic microorganisms. The impressive variability in biochemical pattern evidenced in blue honeysuckle fruits - both quantitatively and qualitatively - which determines their nutritional valences and health benefits, is strongly affected by the environmental conditions, harvest date, storage conditions and genotype (Hoppula *et al*., 2006; Ochmian *et al*., 2010; Truta *et al*., 2012).

The aim of our work was to quantify the total anthocyanins and the dry matter amounts in the fresh berries collected from Romanian selections of blue honeysuckle, by comparing to the berries preserved by freezing (three months in refrigerator) and drying at 60°C (to constant weight).

MATERIALS AND METHODS

For the quantitative assessment of the anthocyanins, berries of *Lonicera caerulea* var. *kamtschatica* were used. The fruits have been harvested at biological maturity from the experimental fields of Research Institute for Fruit Growing, Pitesti – Maracineni (geographical coordinates: 44°51'N, 24°54'E). The working variants were constituted by different honeysuckle genotypes, namely control (C) and five selections conventionally noted as SL6, SL8, SL15, SL17, SL57. Biochemical analyses were carried out on fresh berries and on plant material preserved by drying (at 60°C, to constant weight) and freezing (three months in refrigerator).

Dry matter (DM) was determined by gravimetric method, which basically consists in maintenance of the biological material at 105°C to constant weight (Boldor *et al*., 1983). The results are expressed in g dry matter/100g fresh biological material.

The amount of the *total anthocyanins* was estimated by determination of absorbance of the ethanol-acidified extracts at 515 nm. The results are expressed as mg/100g DM (Fuleki and Francis, 1968).

RESULTS AND DISCUSSION

Dry matter content, determined for the experimental model in which the quantification of the biochemical indicator was performed on fresh biological material, is higher than control in all analyzed selections (Fig. 1). The maximum value belongs to SL6 variant (18.03g/100g), while SL57 showed the smallest amount of dry matter (16.05g/100g).

In frozen fruits the behaviour is similar, the selections having amounts of dry matter superior to the control (Fig. 1), with maximum values in SL6 and SL8, and minimum values in SL57.

The drying storage leads to the decrease in water content in the berries harvested from SL6 selection and occurrence of a lower dry matter content (83.9g/100g) as compared to the control, whereas the other selections presented quantities of dry matter higher than the control, with a maximum level of 87.49g/100g registered in SL17.

Therefore, by comparing with the respective controls, with only one exception, the content in dry matter was superior in all analyzed selections, in all experimental models. It is possible that during selection process an increase of the tissue resistance was carried out and, implicitly, the diminution of the diffusion rate of the water vapours. The smallest changes in the dry matter content were observed during freezing storage. The results show that the freezing storage was correctly conducted; as a consequence, the simultaneous alterations supported by the biological material because of freezing of the water present in the cellular solutions were reduced at minimum. According to the results, except for SL8 variant, the other selections – by comparison with the controls – have higher number of intact cells (low variation in dry matter content between experimental variants in fresh condition and after freezing), fact showing an increased capacity of the endocellular colloids to absorb the water.

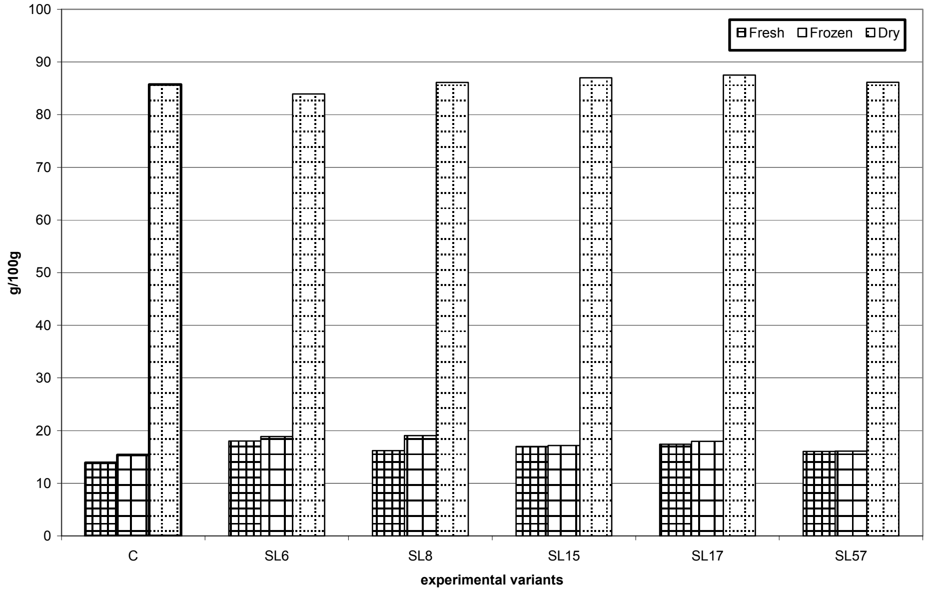

Fig. 1. Variations in the dry matter content in fresh berries, freezing stored berries and drying stored berries harvested from selections of *Lonicera caerulea* var. *kamtschatica*

In literature, Pokorna-Jurikova and Matuskovic (2007) established significant influences of cultivation conditions on dry biomass accumulation, but also variations depending on species and varieties were observed. The amounts of dry matter determined in our selections are generally lower than, for example, those reported by Svarcova *et al.*, 2007) in some Polish varieties (~18.5%).

As concerns the anthocyanins, their amounts in blue honeysuckle fresh berries exceeded the control, with only one exception (SL8) (Fig. 2). The maximum and minimum values belong to the SL15 (2512.71mg/100g DM) and SL8 selections (1784.52mg/100g DM).

The freezing stored fruits had different behaviours by comparing with the controls. So, SL6 and SL8 experimental variants showed diminished anthocyanin levels, while SL15, SL17, and SL57 selections surpassed the control values (Fig. 2).

Regarding the drying stored berries, except for SL8, the other analyzed selections have anthocyanin levels over the control (Fig. 2). SL15 showed the highest content (468.57mg/100g DM), and the SL8 selection had the minimum observed value (296.74mg/100g DM).

The comparative analysis of the obtained results confirms that storage methods exert some influences on anthocyanin content in the tested biological material. Because of their reactivity, the anthocyanins are easily degraded or they interact with other constituents, leading to incoloured or brown-coloured compounds. The instability and the loss of the anthocyanin colour take place as result of their susceptibility to the attack of some nucleophyle agents, the long term exposure to oxygen action, activation of some enzymes or enzymatic systems, temperature, *pH*, light etc. Higher decreases in anthocyanin amounts were observed especially in drying stored berries (generally the decrease rate was over 80%): C - 86.27%; SL6 - 84.10%; SL8 - 83.37%; SL15 - 81.35%; SL17 - 83.80%; SL57 - 79.29%).

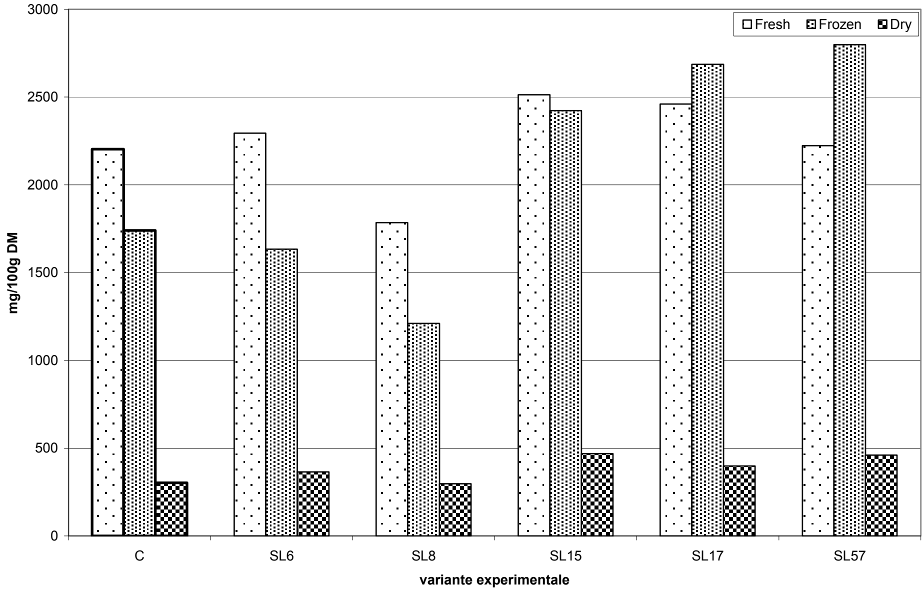

Fig. 2. Variations in the anthocyanin content in fresh berries, freezing stored berries and drying stored berries, harvested from selections of *Lonicera caerulea* var. *kamtschatica*

Although the anthocyanin amount decreased by drying, the fruit colour not greatly changed. This behaviour is due to the co-pigmentation reactions in which the anthocyanins are condensed with each other as well as with other organic molecules, fact resulting in the formation of new pigments.

The method of freezing storage induced different behaviours of the investigated biological material, in that in some experimental variants the anthocyanin content decreased in variable range (C - 21.06%; SL6 - 28.83%; SL8 - 32.16%; SL15 - 3.56%), and in other increased (SL17 - 9.17; SL57 - 25.88).

Mladin *et al*. (2011) also found considerable variations in the anthocyanin content of fresh blue honeysuckle berries, from 206.0mg% to 579.0mg%. In dried fruits, they determined contents by~6.6-6.8 much higher than in fresh fruits. The content in anthocyanins is subjected to intensive degradation during long storage of the berries, the temperature and the light being decisive factors. Kalisz *et al*. (2013) noted the highest losses occurring at the initial period of storage.

CONCLUSIONS

In conclusion, the results obtained in the present study support the existence of a relatively large extent in the phenotypisation of the studied biochemical quantitative characters, as a reflection of the genotype expression of each variety in the respective environmental conditions. Despite the uniformity of the chromosome features established in our previous studies (Truta *et al*., 2013) in the selections of blue honeysuckle, the present work shows enough large variability in dry matter content and anthocyanin levels. It must also note that *Lonicera* berries were harvested from ecotypes having the same geographical provenance (Maracineni-Pitesti Agricultural Station) and growing in the same environmental conditions. Considering this facts, we can sustain that these differences in biochemical patterns are the consequence of the differences existing at genic level. For this reason, further detailed researches at molecular levels are needed in order to clarify the relationship between genetic constitution and the range of phenotypisation of some traits of economical interest.

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