VARIABILITATEA CONTINUTULUI DE POLIFENOLI SI DE ANTOCIANI TOTALI DIN FRUCTELE DE LONICERA

VARIABILITY OF THE CONTENT OF POLIFENOLS AND TOTAL ANTOCIANS IN BLUE HONEYSUCKLE FRUITS

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Abstract

The presence of large amounts of free radicals has a negative effect on living organisms. The presence of an unpaired electron or the ability to extract an electron from other molecules, free radicals can react with different molecules-lipids, proteins, DNA, causing their biodegradation. They are associated with the pathology of some diseases (Durackova, 2014). Reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals can cause lesions on cellular structures and functional molecules (DNA, proteins and lipids) (Finkel and Holbrook, 2000). Many researches suggests that oxidative stress is a major cause of many diseases, including aging, cancer, diabetes, cardiovascular disease, Alzheimer's disease and other neurodegenerative disorders (Halliwell, 1994; Durackova, 2014). Antioxidants are considered to be very effective in managing ROS-mediated tissue insufficiency. Antioxidants are molecules that can safely interact with free radicals and end the chain reaction before the vital molecules are damaged. Although there are several enzymatic systems in the body that eliminate free radicals, the main micronutrient antioxidants are vitamin C, polyphenols, anthocyanins. The body cannot produce these micronutrients, so they must be assimilated from the diet.

Cuvinte cheie: substanţă uscată solubilă, substanţă uscată totală, zahăr total, vitamina C, antociani, polifenoli, SOR.
Key words: soluble dry matter, total dry substance, total sugar, vitamin C, anthocyanins, polyphenols, ROS.

1. Introduction

The presence of large amounts of free radicals has a negative effect on living organisms. By the presence of an unpaired electron or the ability to extract an electron from other molecules, free radicals can react with different molecules - lipids, proteins, DNA, causing their biodegradation. They are associated with the pathology of some diseases (Durackova, 2014).

Reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals can cause lesions on cellular structures and functional molecules (DNA, proteins and lipids) (Finkel and Holbrook, 2000). Numerous researches suggests that oxidative stress is a major cause of many diseases, including aging, cancer, diabetes, cardiovascular disease, Alzheimer's disease and other neurodegenerative disorders (Halliwell, 1994; Durackova, 2014). Antioxidants are considered to be very effective in managing ROS-mediated tissue insufficiency. Antioxidants are molecules that can safely interact with free radicals and end the chain reaction before the vital molecules are damaged. Although there are several enzymatic systems in the body that remove free radicals, the main micronutrient antioxidants are vitamin C, polyphenols, and anthocyanins. The body can not produce these micronutrients, so they must be assimilated from food.

Forest fruits are one of the most important sources of biochemically active compounds in the human diet (Fukumoto et al, 2000). They are a rich source of ascorbic acid and phenolic compounds, especially phenolic acids, anthocyanins, proanthocyanidins and other flavonoids. These compounds cause fruit pigmentation and are beneficial to human health (Hummer, 2006, Bagchi 2004). Their biological activities include: protection against cancer risk (Doll, 1990), protection against mortality (Armstrong, 1975) due to ischemic cardiopathy. They have antitumoral role (Bingham, 1990), antimicrobial (Puupponen-Pimia, 2001), anti-inflammatory and antiallergenic (Middleton et al., 1992) and antimutagenic properties (Edenharder, 1993).

Numerous results show that Lonicera selections differ greatly in ascorbic acid (67.7 - 186.6 mg / 100 g), and these values can be considered higher than in other fruit species well appreciated for their high ascorbic acid content (Tunde Jurikova, 2012).

The purpose of this paper was to determine the total levels of biochemically active compounds in *Lonicera* fruit produced by RIFG Pitesti Maracineni. Our objectives are to demonstrate their potential in

maintaining human health by establishing the biochemical composition for their introduction into culture on large surfaces.

2. Material and methods

The study was conducted in the Small Fruits experimental field of the Research Institute for Fruit Growing for a two-year period (2016 and 2017). The experience consisted in rows of *Lonicera*, arranged in three replicates. The soil is part of the protisol class, wet aluviosoil type, formed on fluvial deposits, clayey and sandy granulometric composition. The land on which the experience was placed is a meadow terrace of the Argeş River. As for the physical and chemical properties of the soil, it is characterized by an acidic reaction (pH = 5.8), a medium humus provision (humus% = 2.48) in the arable horizon and a low assimilable phosphorus (P2O5 ppm = 14.5). Ensuring the soil with total nitrogen is poor (Total nitrogen % = 0.1).

13 blue honeysuckles were evaluated. Samples (approximately 200 g) were harvested at the beginning of the month June, early in the morning. The water and total dry substance were determined by the gravimetric method by measuring the water loss at heating at 105°C. The total % sugar content was determined by the Fehling-Solet method (1964).

The method consists of extracting the sugar from the fruit by boiling them in the distilled water, inverting the sugar and titrating the Fehling reagent with the sugar solution obtained.

The determination of anthocyanins was done by the spectrophotometric method. The principle of this method is to measure absorption at the wavelength λ = 535 nm (Fuleki and Francis, 1968). The extraction of anthocyanins from the fruits was done using as the solvent extraction the ethanol ethoxylated with hydrochloric acid. The extinctions corresponding to the extracts were read at wavelength λ = 535 nm, using a Zeiss Jena spectrophotometer. Expression of the results was made in mg of anthocyanin / 100 g of fresh fruit.

Vitamin C expressed in mg / 100 g fresh fruit was determined by the titrimetric method, after extraction of fruit with 2% hydrochloric acid.

The total polyphenol content, expressed as mg of gallic acid per kg of fresh fruit, was determined spectrophotometrically using a Zeiss Jena spectrophotometer. Extraction of the polyphenols was carried out using methanol: water = 80: water as solvent.

3. Results and discussions

Analyzing the values of the quality biochemical indicators of *Lonicera* fruit, differences in the values between the two years, 2016 and 2017 are observed. Increases in total sugar, vitamin C, anthocyanins and polyphenols in 2017 as compared to 2016 are observed. Accumulations of biochemically active substances were influenced by the different rainfall and temperature during the fruit coagulation during the two years of study.

The water and dry substance correlate negatively: the water content decreased from 7B 85.67 to 83.85 at clone 30B in the series of clones analyzed. In the case of the total dry substance content (determined gravimetrically), we notice contents that rise from 12.95 for clone 41B, for clone at 16.15 for clone 30B.

Total sugar content decreases from 8.63 to clone 7B to 3.00 at clone 41B, correlating negatively with total dry substance.

Fruit vitamin C correlates negatively with dry substance in 2017 when decreases are seen from 88.42 to clone 3B at values of vitamin C content from 88.0 at clone 3B to 61.6 at clone 5B, 32B, 30B, 39B.

The anthocyanin content of lone fruit grows in 2017 from 595.10 mg / kg in clone 7B to 610.99 mg / kg at clone 38B. In 2016, the total anthocyanin levels of 570 at clone 7B at 692.46 at clone 5B.

Concerning the total polyphenol content, the content values increase from 8100 mg / kg to clone 30 B to 14900.80 mg / kg for clone 5B in 2016.

In 2017, increases in values from 8032.10 mg / kg to clone 31B were observed at 14980.00 for clone 3B.

Analyzing the data in Figure 1, which re-mains the mean values of the dry substance content in the 13 *Lonicera* genotypes, we find that the highest value (16.15) was recorded at the clone 30B and the lowest value (12.95) was recorded at clone 41B.

4. Conclusions

1. *Lonicera* fruits are very rich in total anthocyanins, total polyphenols and vitamin C. The high levels of antioxidants in *Lonicera* fruits show that it has a very high antioxidant capacity (TAC), being particularly effective in counteracting the destructive effects of free radicals and in maintaining health.

2. The high levels of antioxidant substances in *Lonicera* fruits make this shrub a very valuable plant.

References

1. Armstrong BK, Mann JI, Adelstein AM, Eskin F., 1975. Commodity consumption and ischemic heart-disease mortality, with special reference to dietary practices. J. Chron. Dis. 28:455-469.
2. Bingham SA, 1990. Mechanisms and experimental and epidemiologic evidence relating dietary fi ber (nonstarch polysaccharides) and starch to protection against large-bowel cancer. Proc. Nutr. Soc.; 49:153-171.
3. Bagchi D, Sen CK, Bagchi M, Atalay M., 2004. Anti-angiogenic, antioxidant, and anti-carcinoenic properties of a novel anthocyanin-rich berry extract formula. Biochemistry (Moscow); 69:75.
4. Durackova, Z., 2014. Free radicals and antioxidants for Non Experts. In Systems Biology of Free Radicals and Antioxidants, Laher edit. Springer Verlag, Berlin, Heidelberg.
5. Doll R., 1990. An overview of the epidemiologic evidence linking diet and cancer. Proc. Nutr. Soc. ; 49:119–131.
6. Durackova, Z., 2014. Free radicals and antioxidants for Non Experts. In Systems Biology of Free Radicals and Antioxidants, Laher edit. Springer Verlag, Berlin, Heidelberg.
7. Edenharder R, Vonpetersdorff I, Rauscher R., 1993. Antimutagenic eff ects of fl avonoids, chalcones and structurally related-compounds on the activity of 2-amino-3-methylimidazo[4,5-F]quinoline (Iq) and other heterocyclic amine mutagens from cooked food. Mut. Res.;
8. Finkel, T. and Holbrook, N.J. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408: 239-247.
9. Fukumoto LR, Mazza G., 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. J. Agric. Food Chem.; 48:3597-3604. 287:261-274
10. Halliwell B., 1994. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet,10; 344(8924):721-4.
11. Hummer KE, 2006. Blue honeysuckle: A new berry crop for North America. J.Am.Pomol. Soc. 60:3-8.
12. Middleton E, Kandaswami C., 1992. Effects of flavonoids on immune and inflammatory cell functions. Biochem. Pharmacol; 43:1167-1179.
13. Niki, E. 1991. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *Am. J. Clin. Nutr.*, 1991, 54, 1119S-1124S.
14. Puupponen-Pimia R, Nohynek L, Meier C, Kahkonen M, Heinonen M, Hopia A, et al., 2001. Antimicrobial properties of phenolic compounds from berries. J. Appl. Microb.; 90:494–507.
14. Tunde Jurikova, Jiri Sochor, Otakar Rop, Jiří Mlček, Štefan Balla, Ladislav Szekeres Rastislav Žitný, Ondrej Zitka, Vojtech Adam, Rene Kizek, 2012. Evaluation of Polyphenolic Profile and Nutritional Value of Non-Traditional Fruit Species in the Czech Republic - A Comparative Study., 17, 8968-8981; doi:10.3390/molecules17088968, ISSN 1420-3049 www.mdpi.com/journal/molecules

Figures and Tables:

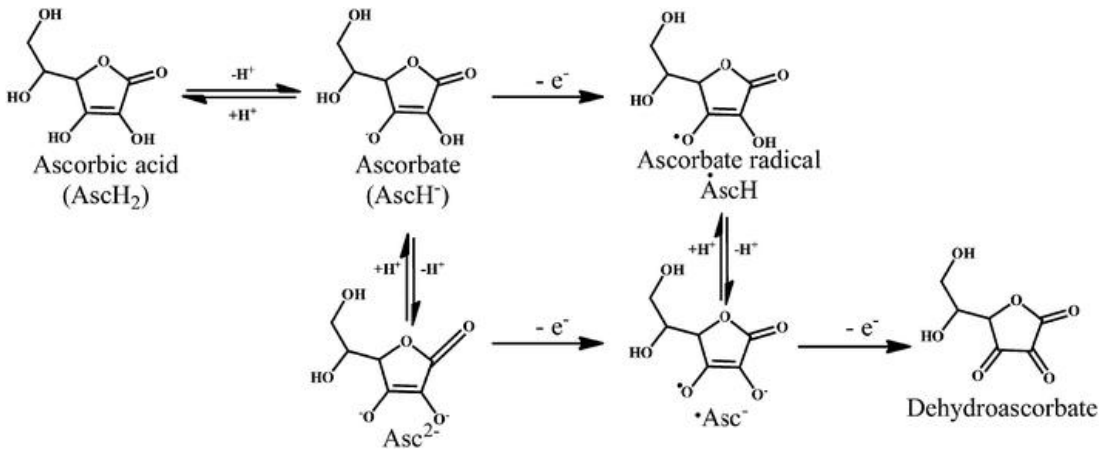


Fig. 1. The mechanism of transformation of ascorbic acid to neutralization of free radicals (E. Niki, 1991)

Table 1 Evaluation of quality biochemical indicators of lonicera fruit, mean values (RIFG Pitesti Romania Lat. 44.513 N, Long. 24.52 E, Alt. 287m

No.	Probe	Water %	Total dry matter %	Soluble dry matter ^{0}Brix	Total sugars %	Vitamin C mg/100g fresh fruit	Antocyans mg/100g fresh fruit	Total Poliphenols mg/kg fresh fruit
1	7B	85,67	14,33	12,0	8,63	79,2	570	11199,10
2	5B	86,30	13,70	11,3	7,66	70,4	692,46	14900,80
3	3B	86,24	13,76	11,5	6,16	88.0	272,9	8229,99
4	6B	86,58	13,42	12,8	8,63	70,4	488,8	10250,00
5	10B	86,73	13,27	10,6	5,65	70,4	488,8	10198,20
6	25B	84,83	15,16	13,0	4,43	70,4	285,13	9020,98
7	28B	85,91	14,09	11,5	5,45	70,4	228,10	8031,99
8	31B	86,51	13,49	12,5	7,66	79,2	285,10	8200,23
9	30B	83,85	16,15	13,2	7,66	61,6	215,88	8100,00
10	32B	86,92	13,08	10,6	3,06	61,6	334,01	10200,41
11	41B	87,05	12,95	11,1	3,00	79,2	285,13	8500,58
12	39B	84,23	15,77	10,0	4,13	61,6	285,13	8502,29
13	38B	84,84	15,16	11,8	2,56	61,6	610,99	13858,62

Table 2. Correlation between biochemical active compounds of Lonicera fruits

		Water (%)	Dry matter (%)	Total sugars (%)	Vitamin C (mg/100g fruit)	Total antocyans (mg/100g fresh fruit)	Total polyphenols (mg/kg)
Water (%)	Pearson Correlation	1	-1,000(**)	,000	,477(**)	,166	-,208
	Sig. (2-tailed)		,000	,999	,000	,146	,068
	N	78	78	78	78	78	78
Dry matter (%)	Pearson Correlation	-1,000(**)	1	,000	-,477(**)	-,166	,208
	Sig. (2-tailed)	,000		,999	,000	,146	,068
	N	78	78	78	78	78	78
Total sugars (%)	Pearson Correlation	,000	,000	1	,350(**)	,215	,123
	Sig. (2-tailed)	,999	,999		,002	,059	,283
	N	78	78	78	78	78	78
Vitamin C (mg/100g fruit)	Pearson Correlation	,477(**)	-,477(**)	,350(**)	1	-,076	-,187
	Sig. (2-tailed)	,000	,000	,002		,506	,100
	N	78	78	78	78	78	78
Total antocyans (mg/100g fresh fruit)	Pearson Correlation	,166	-,166	,215	-,076	1	,125
	Sig. (2-tailed)	,146	,146	,059	,506		,275
	N	78	78	78	78	78	78
Total polyphenols (mg/kg)	Pearson Correlation	-,208	,208	,123	-,187	,125	1
	Sig. (2-tailed)	,068	,068	,283	,100	,275	
	N	78	78	78	78	78	78

** Correlation is significant at the 0.01 level (2-tailed).

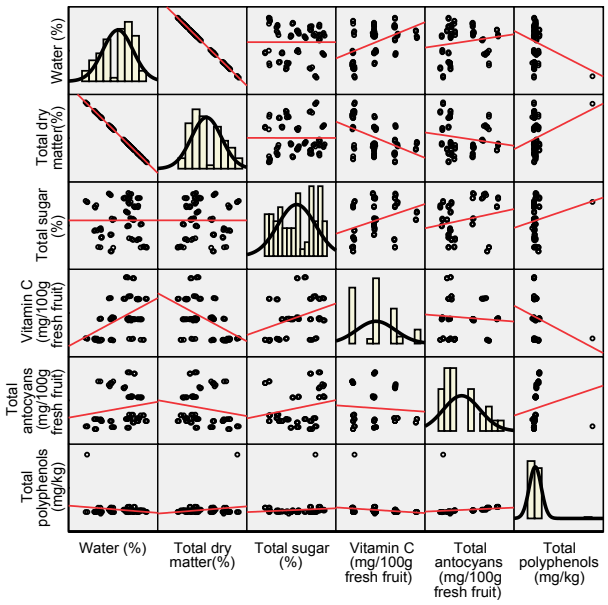
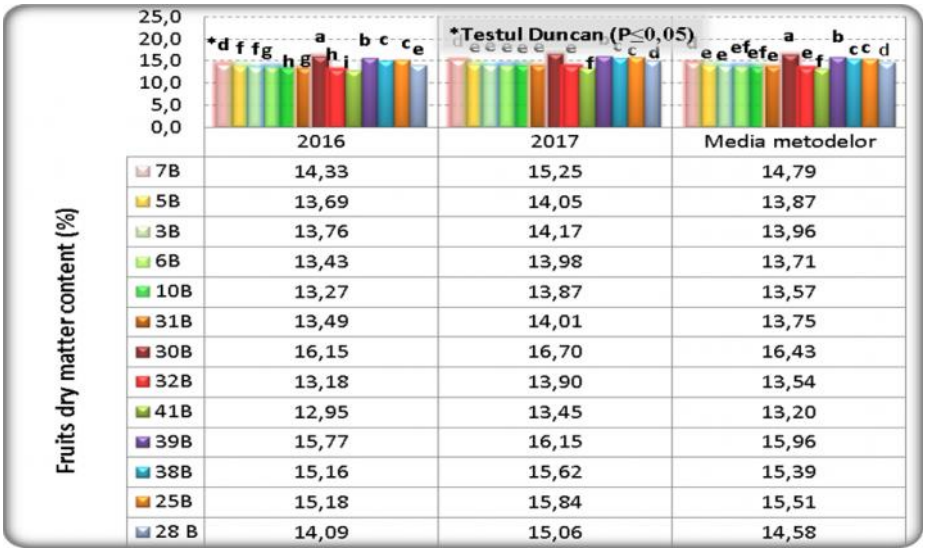
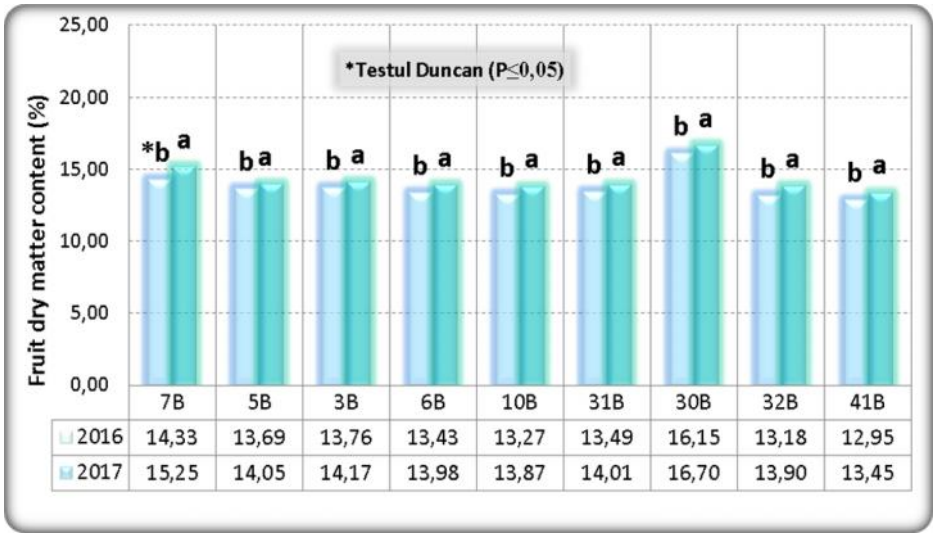


Fig. 1. Correlations trends between biochemical active compounds from *Lonicera* fruits



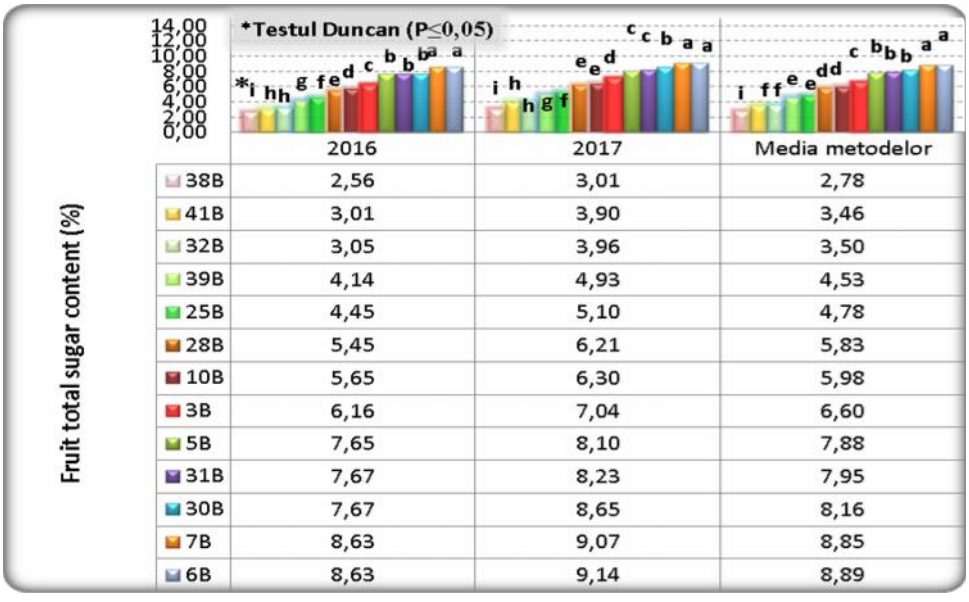
	2016	2017	Media metodelor
7B	14,33	15,25	14,79
5B	13,69	14,05	13,87
3B	13,76	14,17	13,96
6B	13,43	13,98	13,71
10B	13,27	13,87	13,57
31B	13,49	14,01	13,75
30B	16,15	16,70	16,43
32B	13,18	13,90	13,54
41B	12,95	13,45	13,20
39B	15,77	16,15	15,96
38B	15,16	15,62	15,39
25B	15,18	15,84	15,51
28 B	14,09	15,06	14,58

Fig. 2. Influence of annual weather conditions on total dry matter content (%)



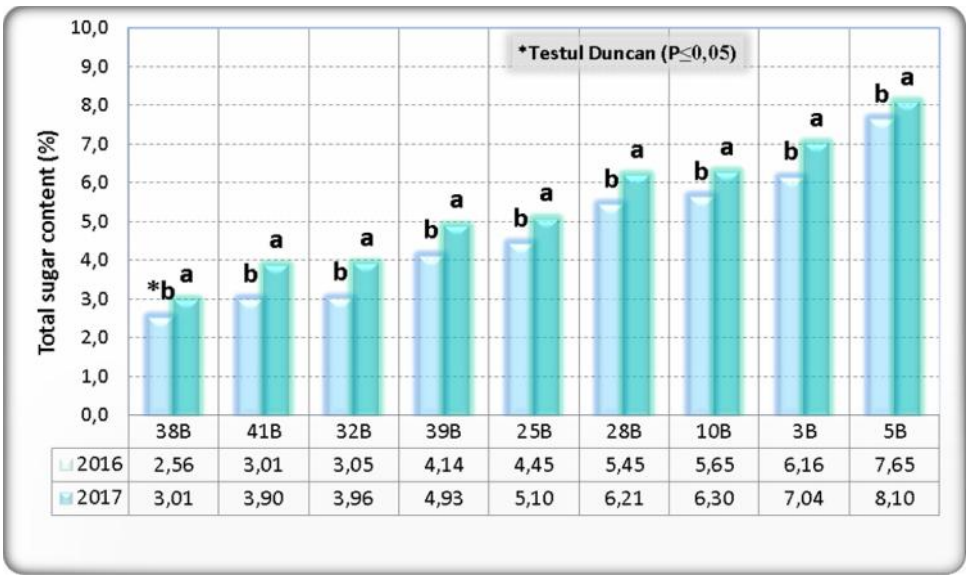
	7B	5B	3B	6B	10B	31B	30B	32B	41B
2016	14,33	13,69	13,76	13,43	13,27	13,49	16,15	13,18	12,95
2017	15,25	14,05	14,17	13,98	13,87	14,01	16,70	13,90	13,45

Fig. 3. Influence of annual weather conditions on total dry substance (%)



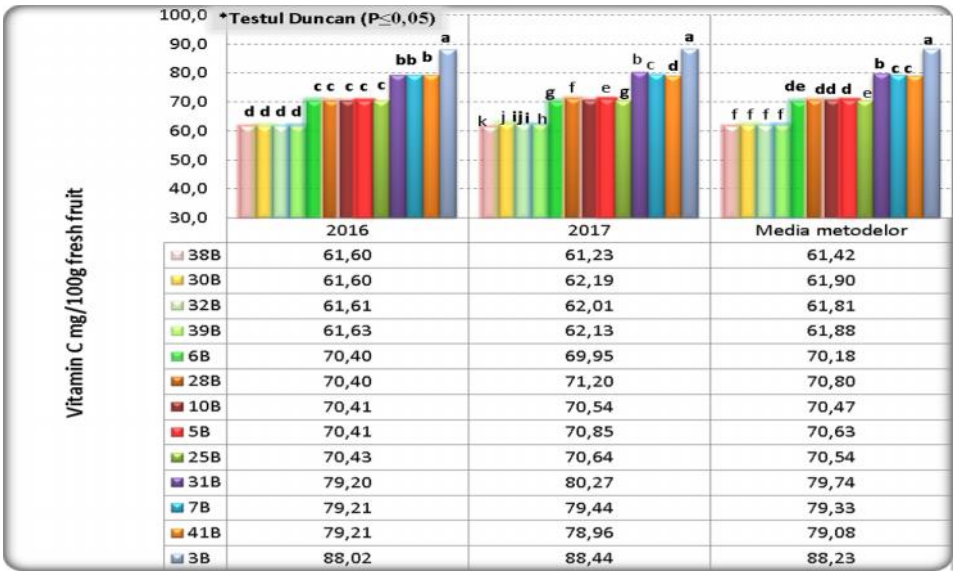
	2016	2017	Media metodelor
38B	2,56	3,01	2,78
41B	3,01	3,90	3,46
32B	3,05	3,96	3,50
39B	4,14	4,93	4,53
25B	4,45	5,10	4,78
28B	5,45	6,21	5,83
10B	5,65	6,30	5,98
3B	6,16	7,04	6,60
5B	7,65	8,10	7,88
31B	7,67	8,23	7,95
30B	7,67	8,65	8,16
7B	8,63	9,07	8,85
6B	8,63	9,14	8,89

Fig. 4. Influence of annual weather conditions on total sugar content (%)



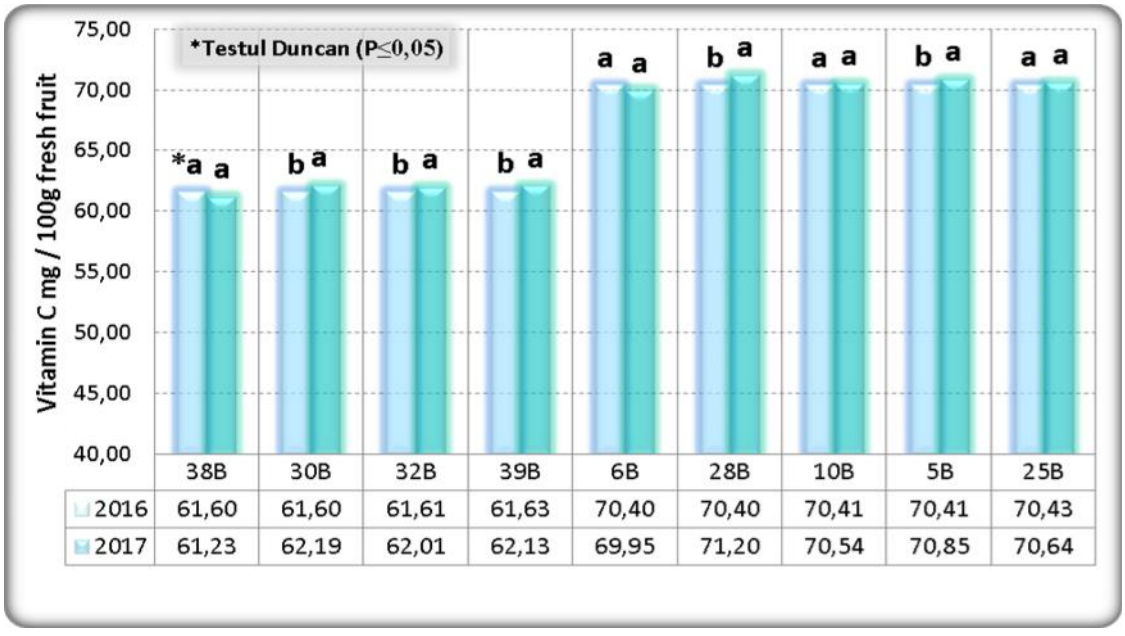
	38B	41B	32B	39B	25B	28B	10B	3B	5B
2016	2,56	3,01	3,05	4,14	4,45	5,45	5,65	6,16	7,65
2017	3,01	3,90	3,96	4,93	5,10	6,21	6,30	7,04	8,10

Fig. 5. Clone influence on total sugar content (%)



	2016	2017	Media metodelor
38B	61,60	61,23	61,42
30B	61,60	62,19	61,90
32B	61,61	62,01	61,81
39B	61,63	62,13	61,88
6B	70,40	69,95	70,18
28B	70,40	71,20	70,80
10B	70,41	70,54	70,47
5B	70,41	70,85	70,63
25B	70,43	70,64	70,54
31B	79,20	80,27	79,74
7B	79,21	79,44	79,33
41B	79,21	78,96	79,08
3B	88,02	88,44	88,23

Fig. 6. Influence of annual weather conditions on vitamin C in *Lonicera* fruits



	38B	30B	32B	39B	6B	28B	10B	5B	25B
2016	61,60	61,60	61,61	61,63	70,40	70,40	70,41	70,41	70,43
2017	61,23	62,19	62,01	62,13	69,95	71,20	70,54	70,85	70,64

Fig. 7. The influence of the clone on the year of study