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BIOCHEMICAL COMPOSITION OF TANGERINE FRUITS UNDER MICROFERTILIZERS

Yuliya Abilfazova, Oksana Belous

ABSTRACT

The paper presents the long-term research and its results in the field of biochemical composition and mechanical analysis of dwarf tangerine fruits (‘*Miagava-Vase*’) growing in the subtropical zone of the Black Sea coast, Krasnodar region. The given results have been obtained after treatments with the following micro fertilizers: H_3BO_3 (at a concentration of 0.06%), $MnSO_4$ H_2O (0.4%), $ZnSO_4$ H_2O (0.3%) and $CuSO_4$ H_2O (0.06%), an option with foliar water spraying served as a control. It is shown that treatments with copper, zinc and boron significantly (at 2.37 – 3.66 mg.100g^{-1}) increase the amount of vitamin C in fruits compared to the control option. The total amount of sugars in fruits was slightly increased under foliar application of manganese salts. The results show an effect of manganese and zinc to the ratio of total sugars and titratable acids and consequently, on the sugar-acid index value. We found that the content of ascorbic acid in tangerine depends on harvesting term, fruit location on the crown, light level, etc. Equally important is the fact that if storage makes up 3 months the loss of ascorbic acid is not more than 12%. Micro fertilizers had a positive effect on market quality: heavier fruits with thin peel were recorded on options with boric acid and manganese treatments. Treatments with boron and copper sulphate increased juice output from the pulp (74 and 76%, respectively, which is 3 – 7% higher than the control option). A higher dry substance accumulation was observed in the option with foliar application of zinc (13.93%). The despite differences in climatic characteristics of research years and annual adverse periods, foliar feeding with micro fertilizers had a positive effect not only on the accumulation of assimilates in the fruit, but also on the preservation of their presentation. The studies have shown that foliar treatments with micronutrients are promising for tangerine crop, which allowed us to develop guidelines for their use as an agro technical method on dwarf tangerine plantations.

Keywords: tangerines; foliar fertilizing; microelements; sugars; acidity; ascorbic acid; sugar-acid index; market quality.

INTRODUCTION

The Black Sea coast of Russia, where Sochi is located, refers to the humid subtropics, where it is possible to grow a large number of tropical and subtropical crops, including citrus fruits – the most frost-resistant and very popular among local citizens and visitors. Citrus crops are more popular and include a large group of evergreen woody plants belonging to the botanical genus *Citrus*, *Aurantioideae* subfamily, *Rutaceae* family **(Vorontsov, 1979)**. Tangerines are playing a leading role on the territory of Russian humid subtropics. Due to their rich chemical composition tangerine fruits are special for their excellent taste, as well as great nutritional, dietary and medicinal values **(Metlitskiy, 1955)**.

Russian humid subtropics are northern and their natural and climatic characteristics are slightly different from the classic subtropical regions with a bit lower winter temperatures. Dwarf tangerines of ‘*Miagava-Vase*’ were the object of our study, being highly competitive with frost-resistant ‘*Unshiu*’. However, some biological features of dwarf cultivars are significantly different, and, therefore, their biological potential, their adaptability, productivity and fruit quality parameters in terms of the region, as well as probable use of biogenic microelements as regulators and listed characteristics represent great interest.

The role of microelements is extremely high, not only in various physiological (photosynthesis, respiration, synthesis of proteins), but also in biochemical processes that underlie fructification **(Arnon, 1962)**. Despite the fact that research of mineral nutrition physiology has been developed for more than 100 years, the main issues of controlling microelemental plant nutrition processes are relevant today **(Sennovskaya, 2006; Shchukin et al., 2006; Mohseni et al., 2006; Kashina et al., 2008)**. This is not only due to the complexity of the given issues under study, but due to the change in varietal composition as well as increasing demands on their productivity and product quality.

There is evidence that microelements are localized in organs and parts of plants, most rich in vitamins, in turn, plants rich in vitamins contain more micronutrients, especially manganese. Zinc has a positive effect on the accumulation of vitamin C in plants **(Chvapil, 1973; Stoyanova and Doncheva, 2002)** and Cu **(Inmaculada, 2005)**. Currently it is found that microelements are part of many enzymes that speed up biochemical reactions of synthesis, decomposition and metabolism of organic substances **(Volodko, 1983; Anspok, 1990)**. The data on the occurrence of Cu and Zn in the composition of some enzymes made their role clear in redox processes. A major achievement is the definition of microelements in nitrogen metabolism **(Salyaev at al., 2003; Rak at al., 2005)**. The scientific paper informs about the way microelements influence on the movement and redistribution of mineral elements in plants **(Epstein, 1956; Khomenko at al., 1974; Rybchenko, 1975; Salyaev at al., 2003; Mingkui Zhang at al., 2006)**. A positive influence of microelements on plants ability to withstand adverse conditions was proved **(Bogomaz and Korshuk, 1967; Bozhenko, 1976; Serga, 1998; Gudkovskiy, et al., 2005)**. Application of essential micronutrients, among other agro-technical measures, leads to higher yields and improves the quality of many crops **(Belous, 2006, 2013; Poschenrieder, 2008; Khalid and Khalid, 2012)**. Under their influence, plants can use nitrogen, phosphorus, potash and other fertilizers better **(Rinkis and Nollendorf, 1982)**. Thus, the targeted use of microelements as selective regulators of metabolism is one of the ways to increase the productivity of fruit, vegetable and field crops.

Along with this, there are much less common experimental data on the effect of microelements on subtropical plants, especiallyon tangerines. There is a large prospect in studies to identify the value of microelements for plants belonging to subtropical crops group (mainly tangerine); moreover, these issues still remain poorly studied.

MATERIAL AND METHODOLOGY

The objects of our study were mature fruits of dwarf tangerine *Citrus reticulata* '*Miagava Vase'*. *Poncirus trifoliata* (L.) Raf. served rootstock. Comprehensive research of the effect of microelements on plants functional state was carried out on the plantation (1986) from 1998 till 2008 (the data presented in the paper include three years' period). Soils in pilot area were brown forest soil, slightly unsaturated: it had about 12% humus, fulvic acids exceeds the volume of humic and this soil characterized as sub-acid.

The experiment was laid in the open ground, using randomization method by 4-fold repetition in a covering culture. The plot area was 0.25 hectares and the placement of the plants by scheme 2500 trees per 1 ha correspond to the feeding area 4x1 m^2. Each account of the plot is protected with shelterbelt against accidental damage from strip technique, which also serves to eliminate the interference of neighboring microfertilizers. The experimental scheme included five options: control (spraying with water); H_3BO_3 - at a concentration of 0.06%; $MnSO_4$ H_2O – 0.4%; $ZnSO_4$ H_2O – 0.3%; $CuSO_4$ H_2O – 0.06%. Foliar spraying during the growing season was carried out twice: first spraying at finish blossom and second at beginning of coloring fruits. Agricultural technique was common for tangerine **(Gamkrelidze, 1971)**. Laboratory and field studies were conducted at the Institute, using classical methods. Biochemical and other analyses of tangerine fruits were performed according to the State Cultivar Commission **(Lobanov, 1973)**. The content of ascorbic acid was determined by iodometric method; titratable acidity – by titration with 0.1 N NaOH and sugars amount by refractometers method **(Pochinok, 1978)**. Laboratory analyses were made in triple repetitions: ascorbic acid content was determined by iodometric method with 2% HCl, titrated 0.001 N solution of KIO_3; total acidity - titration with NaOH (0.1 $mol.dm^{-3}$) in the presence of phenolphthalein indicator; dry matter - the method of drying the sample at 105 °C to constant weight The amount of sugar was determined by Bertran`s refractometric method. The method is based on the ability of sugars aldehyde group interact with Fehling's reagent and restore CuO to Cu_2O precipitated as a red solid. Organoleptic analysis of fruits was carried out according to the guidelines and 9 experts took part in fruits testing **(Lobanov, 1973)**.

The program STATGRAPHICS Centurion XV, Statpoint Technologies, Inc. USA, and mathematical software package MS Excel 7.0 were applied during processing research materials and evaluating experiment results.

RESULTS AND DISCUSSION

Nowadays the scientists are very interest about to natural antioxidants – flavonoids, anthocyanin's, vitamins (and especially vitamin C), which are able to prevent formation of free radicals in human body that usually cause cardiovascular diseases and cancer. Therefore, of a great interest are fruits that are natural sources of these compounds. Not coincidentally, we investigated the amount of ascorbic acid in tangerine fruits and change in its content under the influence of foliar fertilization with microelements.

The studies had found out that the content of ascorbic acid in tangerine fruits under humid subtropics is quite high (within 44 $mg.100g^{-1}$). Thus, treatments with copper, zinc and boron significantly (at 2.37 – 3.66 $mg.100g^{-1}$) increase the amount of vitamin C in fruit as compared to the control one (NCR_{05} = 0.06%). The tendency of ascorbic acid to increase in the fruits of test plants was recorded throughout the study **(Abilfazova, 2009)**. In addition, we found that the content of ascorbic acid in tangerine depends on harvesting term, fruit location on the crown, light level, etc. Equally important is the fact that if storage makes up 3 months the loss of ascorbic acid is not more than 12%.

One of the main fruit quality indicators, interesting to the consumer, is taste, which is largely determined by the ratio of sugars and titratable acids in fruits. As we can see from Table 1, the total amount of sugars in test plants is about 11.02%. A certain increase in this indicator was observed only under manganese salts foliar application. The content of titratable acids ranges among options from 1.88% (in the option with copper sulphate) to 2.33% (for control), and the differences between the options in this case was insignificant. In general, fruits were different in sweet-sour taste (mainly sourish notes), due to the size of sugar-acid index that made up 5.82 relative units. In the ratio of sugars and titratable acids, i.e. sugar-acid index value we observed some influence of microelement treatments; thus, the smallest value was found in the control option, while under foliar application of zinc, especially of manganese, the control was exceeded.

Apart from the qualitative biochemical parameters we were also interested in the influence of micronutrient fertilizing on tangerine mechanical characteristics.

Figure 1 Tangerine experimental plantation.

Figure 2 Tangerine experimental plantation.

Figure 3 Dwarf tangerine *Citrus reticulate* ‘*Miagava Vase*‘ cultivar blossoming.

Figure 4 Dwarf tangerine *Citrus reticulate* ‘*Miagava Vase*‘ cultivar on *Poncirus trifoliata* (L.) Raf. rootstock.

Figure 5 Dwarf tangerine *Citrus reticulate* ‘*Miagava Vase*‘ cultivar fruits.

Figure 6 Dwarf tangerine *Citrus reticulate* ‘*Miagava Vase*‘ cultivar fruits. Control – water spraying.

Figure 7 Dwarf tangerine *Citrus reticulate* ‘*Miagava Vase*‘ cultivar fruits. Spraying - H_3BO_3 (0.06%).

Figure 8 Dwarf tangerine *Citrus reticulate* ‘*Miagava Vase*‘ cultivar fruits. Spraying - $MnSO_4$ (0.4%).

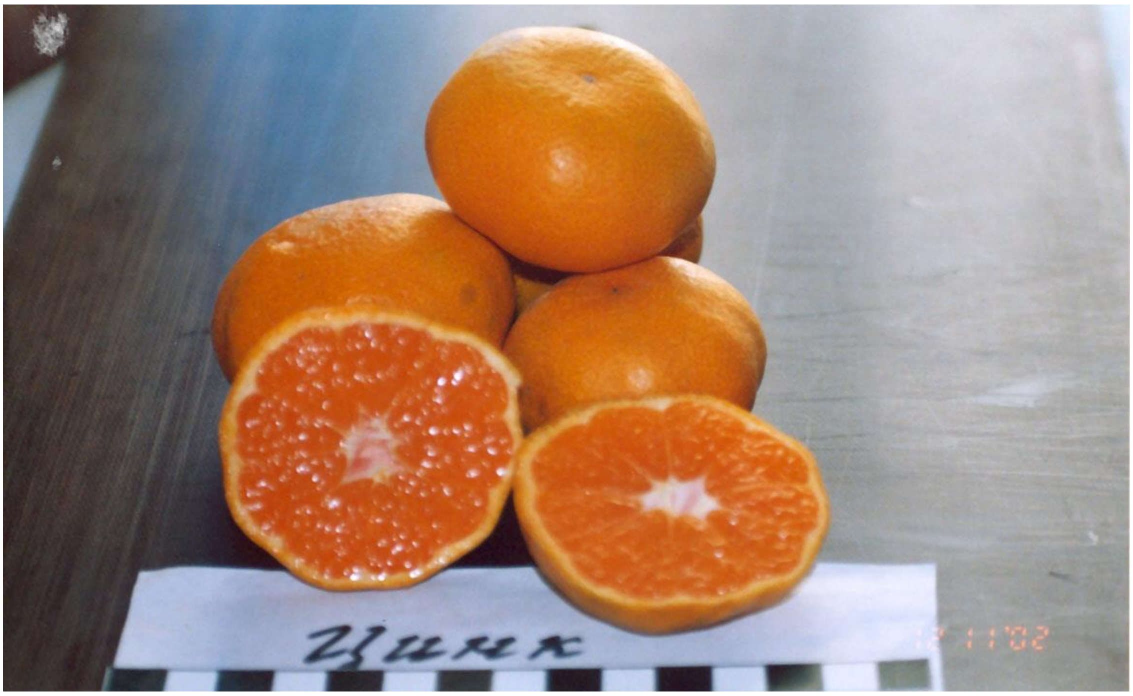

Figure 9 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar fruits. Spraying - $ZnSO_4$ (0.3%).

Figure 10 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar fruits. Spraying - $CuSO_4$ (0.06%).

Table 1 Biochemical composition of tangerine fruits, average for three research years.

Option	Ascorbic acid ($mg.100g^{-1}$)	Titratable acidity (%)	Amount of sugars (%)	Sugar-acid index (relative units)
Control (treatment with water)	41.14 ±4.68	2.33 ±0.85	10.83 ±1.66	5.14 ±2.08
H_3BO_3 (0.06 %)	43.51 ±2.15	2.18 ±0.72	10.70 ±1.56	5.46 ±2.64
$MnSO_4 \cdot H_2O$ (0.4 %)	43.11 ±3.63	1.95 ±0.50	11.87 ±1.27	6.43 ±2.01
$ZnSO_4 \cdot H_2O$ (0.3 %)	43.99 ±3.75	1.96 ±0.60	10.97 ±1.37	6.14 ±2.58
$CuSO_4 \cdot H_2O$ (0.06 %)	44.80 ±4.63	1.88 ±0.40	10.71 ±1.34	5.91 ±1.67

Table 2 Mechanical composition of tangerine fruits, average for three research years (based on 100 g of weight).

Option	Average fruit weight (g)	Dry substance (%)	Weight from total weight of fruit (%)		Output of juice (%)
			Pulp	Peel	
Control (treatment with water)	66.70 ±4.11	12.73 ±2.91	77.7 ±0.01	22.3	68.40 ±3.21
H_3BO_3 (0.06%)	84.11 ±1.49	12.83 ±3.69	79.0 ±0.03	21.0	74.03 ±1.72
$MnSO_4 \cdot H_2O$ (0.4%)	73.20 ±7.17	11.70 ±3.87	80.8 ±0.01	19.2	71.93 ±3.82
$ZnSO_4 \cdot H_2O$ (0.3 %)	70.53 ±6.18	13.93 ±5.25	77.9 ±0.00	22.1	71.27 ±5.92
$CuSO_4 \cdot H_2O$ (0.06%)	76.52 ±1.16	12.43 ±3.54	78.3 ±0.03	21.7	75.73 ±1.40

The studies had shown that despite differences in climatic characteristics of research years and annual adverse periods, foliar feeding with microfertilizers had a positive effect not only on the accumulation of assimilates in the fruit, but also on the preservation of their presentation. Thus, high air temperature (above 30 °C) in August was often replaced by long heavy rains with a sharp decrease to +20 to +23 °C in early September. This tends to promote stress cracking of fruits, which was not observed in plants with foliar embodiments. In addition, the average fruit weight ranged from 70.5 to 84.1 g, and heavier fruits were in options with boric acid and manganese treatments, slightly above the control in 1.3 times (Table 2). A similar influence of foliar application of microelements on fruit weight was noted by other researchers **(Bedrikovskaya, 1954**; **Naqvi, Alam and Mumtaz, 1990; Lafer, 2008)**.

But just fruit weight cannot be an indicator of their quality, because large weight may be due to the thick peel. Therefore, every year we took into account the percentages of pulp and peel. When these indicators had a similar value on all options (about 4/1), a little thinner-peeled fruits were recorded among options treated with manganese (19.2%) and boron (21.0%), in which the proportion of edible fruit was much more, compared to inedible. This fact was linked to another indicator which characterizes fruits and represents consumer interest – output of juice from the pulp. Thus, treatments with boron and copper sulphate contributed to more juice output from the pulp, reaching 74 and 76%, respectively, which is 3 – 7% higher than the control option (Table 2).

The accumulation of dry mater in tangerine fruits made up 12.7% on the average for all the options. At the same time, higher accumulation of assimilates was recorded in the option with foliar application of zinc – 13.93%.

As a final assessment, we carried out fruit degustation, which showed that fruits in all options had a good taste and market quality, therefore they received the highest evaluation score corresponding to 4.6 points by a 5-point scale.

At introduction of microelements we were testing their contents, and not only in fruits, but also in leaves. Foliar spraying by manganese caused to increasing this element (by 2.4 point) in leaves. Besides, introduction of manganese promoted accumulation of boron by leaves, contents of element on 15.08 mg/kg exceeded control. Processing of zinc and copper stimulated accumulation of boron and iron at plants (Table 3).

Foliare spraying by microelements influenced accumulation of elements at tangerine pulp and peel (Table 4).

Researches showed that foliar spraing of zinc and manganese influenced strengthening of boron accumulation into fruits. Between introduction of boron and accumulation into fruits of zinc, copper and iron was synergetic effect. Manganese showed antagonistic action in relation to zinc and copper, and zinc – reduced copper and iron accumulation.

The revealed regularities are described by such equations of regression:

$Cu = 13.04 – 3.08\ Zn$; $R^2 = 0.29$;

$Zn = 6.16 + 1.35\ Mn + 5.54\ Zn + 5.40\ Cu$; $R^2 = 0.97$;

$Mn = 1.23 + 1.14\ Mn$; $R^2 = 0.92$;

$FE = 0.32 – 0.52\ Cu – 0.89Zn – 0.92\ Mn$; $R^2 = 0.31$.

Table 3 Contents microelements at tangerine leaves, average for three research years (mg.kg^{-1} of dry weight).

Option	Cu Optimum 5 – 10	Zn Optimum 25 – 100	Mn Optimum 25 – 100	B Optimum 50 – 170	Fe Optimum 60 – 120
Control (treatment with water)	12.12 ±1.6	33.86 ±14.4	27.76 ±3.8	38.70 ±3.04	43.30 ±10.2
H_3BO_3 (0.06%)	11.92 ±1.5	35.12 ±14.6	28.61 ±4.4	51.90 ±9.0	47.04 ±9.6
$MnSO_4 \cdot H_2O$ (0.4%)	11.86 ±1.6	35.20 ±14.6	65.42 ±12.6	53.78 ±14.2	46.78 ±10.7
$ZnSO_4 \cdot H_2O$ (0.3%)	11.34 ±2.1	57.50 ±13.1	29.04 ±3.7	44.92 ±6.5	47.12 ±11.9
$CuSO_4 \cdot H_2O$ (0.06%)	18.02 ±4.1	36.46 ±11.7	27.70 ±2.7	44.57 ±7.5	47.68 ±10.8
SSD_{05}^{*}	1.94	3.71	4.97	3.78	2.91

Note: *Smoller significant difference, confidence level – 95%.

Table 4 Contents microelements at tangerine pulp and peel, average for three research years (mg.kg^{-1} of dry weight).

Option	Pulp					Peel				
	B	Mn	Zn	Cu	Fe	B	Mn	Zn	Cu	Fe
Control (treatment with water)	14.6	7.2	2.13	1.27	25.3	33.4	6.5	16.1	12.3	17.5
H_3BO_3 (0.06%)	15.2	6.6	2.32	1.29	30.0	35.1	6.1	16.5	9.9	17.3
$MnSO_4 \cdot H_2O$ (0.4%)	16.3	10.2	1.93	1.05	23.2	37.8	13.8	16.8	11.2	14.4
$ZnSO_4 \cdot H_2O$ (0.3%)	16.2	7.8	2.56	1.24	23.4	38.3	7.0	35.0	9.5	14.8
$CuSO_4 \cdot H_2O$ (0.06%)	15.5	6.4	2.51	1.36	18.0	35.0	6.0	19.4	13.8	11.8
SSD_{05}^{*}	0.7	0.7	0.4	0.2	3.8	1.1	0.5	1.3	1.0	1.4

The analysis showed, that introduction of microelements didn't lead to chemical pollution of fruits pulp, because maximum permissible concentration of such dangerous elements as copper and zinc are much higher (maximum concentration limit Cu – 5.00 mg.kg^{-1} and Zn – 10.00 mg.kg^{-1}). These elements collect to a peel and don't come to pulp.

CONCLUSION

Describing the results of biochemical parameters and composition of fruits, we can claim that dwarf tangerines of '*Miagava Vase*' displayed response to foliar application of boron, manganese, zinc and copper. Microelements, as a whole, had a positive effect on the biochemical indicators of quality, which resulted in higher amount of sugar, as well as accumulation of ascorbic acid and sugar-acid index. At the same time, foliar application of microelements had a certain effect on market quality: the average fruit weight increased together with a high juice output. Such tendencies caused the necessity to study complex influence of microelements on the functional state of dwarf tangerine ('*Miagava Vase*') for a long-term; and that is what exactly was being undertaken by us for ten years (1998 – 2008). As result, our researches influence of foliar spraing microelements at accumulation it's into fruits. Studies were shown prospectively in foliar treatment with micronutrients, which allowed us developed microelements use recommendations as a control method on dwarf tangerine plantations in the humid subtropics of Russia.

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Contact address:
Yuliya Abilfazova**,** senior researcher at Plants Biotechnology, Biochemistry and Physiology Laboratory, Russian Institute of Floriculture and Subtropical Crops, Yana Fabritsiusa st., 2/28, Sochi, Russia, 354002, E-mail: Citrus_Sochi@mail.ru.
Dr. Oksana Belous, Dr. Sci. Biol., professor, head researcher at Plants Biotechnology, Biochemistry and Physiology Laboratory, Russian Institute of Floriculture and Subtropical Crops, Yana Fabritsiusa st., 2/28, Sochi, Russia, 354002, E-mail: oksana191962@mail.ru.