



## DOES APPLICATION OF NAPHTHENIC ACIDS IN EARLY FRUIT DEVELOPMENT STAGE RESULT IN PROLONGED EFFECT ON COLD STORAGE AND SHELF LIFE OF APRICOT FRUIT?

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**Abstract:** In this experiment, the effects of the application of naphthenic acids (NAs) on apricots in the early fruit development phases on fruit morphological properties, composition and postharvest properties were investigated. Two concentrations of NAs (1 mg/L and 3 mg/L) were applied at two development stages in the apricot cultivar NS-4. The application of NAs at the beginning of the petal fall development phase resulted in the reduction of fruit dimensions at harvest while the application 7 days later increased fruit dimensions. Although there were no significant differences in the most investigated characteristics between fruits treated with NAs and untreated control at harvest or in the postharvest period during 20 days of cold storage ( $1 \pm 1$  °C, RD 80%) and particularly after 3 days of shelf life at room temperature. However, compared to the untreated control, apricots treated with NAs were characterized by higher total soluble solids content accompanied by higher fructose content, and lower titratable acidity accompanied by higher succinic acid content. Apricots treated with NAs showed trends towards improved sensory properties: sweeter and less sour taste, with more expressed apricot aroma accompanied with decreased gumminess and crispiness and more intensive tissue breakdown, but without expressed notes of inappropriate taste.

**Key words:** *naphthenic acids, apricot, cold storage, shelf life, postharvest*

## INTRODUCTION

As a typical climacteric fruit, the apricot is prone to rapid deterioration after reaching a certain maturity stage. This process is most notably reflected in the loss of fruit firmness, which is subsequently one of the key factors limiting apricot distribution to consumers. Actions aimed at improving shelf life of ap-

ricot include storage at low temperatures, packaging in the modified and controlled atmosphere, irradiation, but also chemical treatments including 1-methylcyclopropene, calcium salts, salicylic acid, putrescine and use of edible coatings (Muzzaffar, Bhat, Wani, Wani & Masoodi, 2018). Despite applied postharvest treat-

ments, apricots remain susceptible to disorders during storage including gel breakdown and internal browning, often coupled with microbiological disorders, mainly caused by fungi (Crisosto, Echeverria & Manganaris, 2020). Another very important factor influencing the efficiency of apricot storage is the maturity stage (Stanley, Prakash, Marshall & Schröder, 2013) as well as the cultivation system (Leccese et al., 2010). The duration of apricot storage is highly dependent on temperature. After 17 days at 15 °C almost half of apricots' flesh firmness is lost while at 2 °C similar loss is reached after 29 days (Álvarez-Hernández, Martínez-Hernández, Avalos-Belmontes, Miranda-Molina & Artes-Hernandez, 2002). Despite all the efforts, a significant loss of firmness in apricot occurs very quickly. To improve the production of apricot, but at the same time to promote fruit thinning, plant growth regulators (PGRs) are applied, usually in the early phases of fruit development (Southwick, Yeager & Weis, 1997). These treatments affect fruit size and quality (Canli, Sahin, Temurtas & Pektas, 2014), as well as storability (Lal et al., 2011), suggesting a potentially prolonged effect of PGRs even after harvest.

Naphthenic acids (NAs) could naturally be found in oils and oil sands bitumen. Because of its solubility in water, NAs are present in refinery wastewaters. However, industrial extraction of oil sands bitumen releases NAs directly to tailings pond waters with the estimation of 100 tons per day (Clemente & Fedorak, 2005). Besides being present as a result of anthropogenic activities, NAs could also be found naturally, for example in the Athabasca River with concentrations ranging from 0.1-0.9 mg/l (Schramm, Stasiuk & MacKinnon, 2000). The dual nature of NAs effects on plants was demonstrated on *Arabidopsis thaliana* in the study by Leishman et al. (2013), where the application of high concentrations of NAs had an inhibitory effect on seed germination as well as on the growth of root and primary leaves while application of NAs at low concentration had a stimulatory effect on root and shoot growth. The stimulatory effect of lower doses of NAs is also obtained in the case of coleoptile elongation of wheat, where the highest elongation was recorded with  $10^{-7}$  mmol, and the lowest with  $10^{-5}$  mmol of NAs (Ćirin-Novta, Kuhajda, Kevrešan, Kandrač & Radić, 2002). The stimulatory effect of NAs was successfully used for rooting sunflower cuttings (Kevrešan et al.,

2003), poplar (Kovačević et al., 2008) and black locust (Kovačević et al., 2014). If applied in the latter stages, together with nutrient solution, NAs effected K, Na, Mn, Fe, Zn and Ni content in the root, but only Fe in the stem and Mn in the shoot (Kevrešan et al., 2005). In cucumber, when added to the nutrient solution (treatment through root), NAs elicited both local and systematic antioxidative responses of a different degree, inversely dependent of the applied dosages (Kevrešan et al., 2012). In the same study, foliar application of NAs also caused both types of antioxidative reactions, but those on the local level were much more prominent. Repeated foliar treatment with NAs changed lettuce's fresh green mass and reduced nitrate content in the leaf (Chitu, Lacatus, Gaidau, Ionita & Filipescu, 2010).

Despite being considered a toxic compound in higher concentrations, application of NAs in 500 and 1000 ppm increased 100-grain weight and yield per plant in two varieties of wheat (Ahmed, Fattah & Jahan, 2010), while even higher dose of 1500 ppm NAs was shown to be optimal for cowpea (Ullah, Fattah & Hossain, 2007). In apple cv. "Golden Delicious" application of 2.63 mg/L of NAs when fruit size reached 11 mm, increased number of cortical cell layers within three days after application, lower number of fruit set but increased fruit weight (Milić et al., 2017), suggesting that early NAs application could affect latter fruit development.

This study aimed to determine whether the application of NAs in the early stages of fruit development has a prolonged beneficiary effect on the apricot quality and its storability in the postharvest period, i.e. during cold storage and shelf life?

## MATERIALS AND METHODS

### Fruit production and treatment

Fruits of apricot (*Prunus armeniaca* L.) cultivar NS-4 were obtained from the trees grown at the Experimental field for fruit growing, Faculty of Agriculture, Novi Sad, located at Rimski šančevi (45°3382' N and 19°8445 ' E, 86 m a.s.l.), Serbia. Cultivar NS-4 used Myrobalan (*P. cerasifera* Ehrh.) seedlings as a rootstock with blackthorn (*Prunus spinosa* L.) as an interstock. The orchard was established under black anti-hail net and equipped with drip irrigation. The lanes were covered with grass, while the space under the trees within

each row was treated with herbicides. The standard agro-technical procedures were performed during the experimental year. The experiment was set up by using 5-years-old apricot trees grown at  $4 \times 2$  m planting distance ( $1250$  trees  $\text{ha}^{-1}$ ). The trial was set up in a completely randomized design and six single trees were used per treatment.

NAs used in the experiment are described in details starting from the isolation procedure (Ćirin-Novta et al., 2003) and their physico-chemical characteristic (Ćirin-Novta et al., 2003, Kevrešan et al., 2005) in the previous publications. Two concentrations of NAs (1 mg/L and 3 mg/L) were applied at two different development phases: (I) when the green ovary was surrounded by a dying sepal crown, sepals beginning to fall (stone fruit, principal grown stage 7: development of fruit, code 72), according to Meier (1997) (T1) and (II) 7 days latter (T2).

The treatments were applied with a backpack sprayer (Stihl SR-420) with 4 L used per treatment, each containing 6 representative trees. After harvest, fruits from all six trees were combined and used further in the experiment to select appropriate ripening stages.

The prolonged effects of NAs on apricot fruit weight, height, width, thickness, surface and flesh/pit ratio were examined in two ripening stages determined by DA-meter (TR Turoni, Bologna, Italy): commercial ripeness ( $I_{ad}$  0.4-0.8) and full maturity ( $I_{ad}$  0). Fruit weight (g) was measured on 30 fruits, using a technical balance (Kern 572-35, Kern & Sohn, GmbH, Balingen, Germany).

Fruit size (mm) was determined by measuring three linear dimensions (length, width, thickness) for each fruit with a digital caliper gauge (0.01 mm) (Mitutoyo, CD-6"CX, Japan). Fruit volume was calculated using the formula  $4/(3\pi r)^3$ , where  $r = (\text{length} + \text{width} + \text{thickness})/6$ ; flesh ratio was calculated using the formula  $100 - ((\text{stone weight} * 100) / \text{fruit weight})$ , the fruit surface area is calculated according to formulae:  $S = \pi Dg^2$ ,  $Dg = (\text{length} \times \text{width} \times \text{thickness})^{0.333}$ ,  $Dg$  (geometric mean diameter).

### Postharvest analyses

The **postharvest experiment** was performed on apricots at the commercial maturity stage ( $I_{ad}$  0.4-0.8). Approximately 40 kg of fruits per treatment were distributed in wooden crates

( $50 \times 30 \times 8$  cm, 8 crates per treatment) and placed in a cooling chamber for 21 days ( $1 \pm 1$  °C;  $80 \pm 10$  % RH). After 21 days the apricots were removed from cold storage and exposed to 3 days of shelf life at room temperature ( $24 \pm 2$  °C). Pomological properties were analyzed at harvest, while physicochemical properties were carried out after harvest, after cold storage and after shelf-life. For each sampling period, the sample for chemical analysis was prepared from the quarters of 20 apricots, which were homogenized and immediately frozen in dry ice.

**Fruit color** was determined on 20 randomly selected apricot fruits, with two measurements on the opposite sides at equatorial region, using CR-400 Chroma Meter (Konica-Minolta, Osaka, Japan).

**Flesh firmness** was measured for 20 apricots, after peeling off a small circle of skin at equatorial region of each fruit. The penetration test was performed with an 8-mm diameter stainless steel rounded cylinder probe with TA.XT Plus Texture Analyzer (Stable Micro Systems, England, UK). Flesh firmness, defined as the force (N) needed for penetration of the probe into fruit flesh, was determined using parameters proposed by Stanley, Prakash, Marshall and Schröder (2013).

**Ethylene production and respiration rate** were determined in triplicates, on approximately 250-300 g of fruits placed in a 770 ml container, hermetically sealed with multilayer foil. Samples were taken at harvest and after cold storage, and on the same samples, analyses were performed for four consecutive days. The analysis was performed at 24 °C ( $\pm 2$  °C). Ethylene content was analyzed from 2 ml of gas sampled by plastic syringe and injected into a 10 ml headspace vial sealed with silicone septa. Ethylene content was determined by gas chromatography (GC 7890, Agilent, USA), equipped with an FID detector (Agilent, USA) and autosampler (COMBIPAL, CTCAnalytics AG, Switzerland).  $\text{CO}_2$  measurement was performed by direct puncturing of sealed foil via sampling needle of OXYBABY® 6.0 (WIT-Gasetechnik GmbH & Co KGT, Germany).

**Total soluble solids** (TSS; %) were determined by digital refractometer ATR-ST plus (Schmidt + Haensch, Germany) on previously homogenized apricot samples at 20 °C.

**Titration acidity** (TA, g malic acid/100 g) was measured from 3 g of sample dissolved in 30 ml of deionized water. After homogenization, the sample was centrifuged (Centrifuge 5804R, Ependorf, Germany) at 13,776 g for 5 min and 10 ml of supernatant was used for titration with 0.1M NaOH (Kovač et al., 2022).

**Carotenoid** content was analyzed according to Costache, Campeanu and Neata (2012).

**Phenols and flavonoids** were extracted according to Larrauri, Rupérez and Saura-Calixto (1997), phenol content was determined according to Folin-Ciocalteu method (Singleton, Orthofer & Lamuela-Raventos, 1999), while for flavonoids procedure proposed by Pękal and Pyrzyńska (2014) was used.

**Fructose, glucose, sucrose, citric, malic and succinic acid** were extracted according to the procedure described by Milenković et al. (2020). Separation was performed on HPLC Agilent 1200 series, (Agilent, USA). For sugar analysis Zorbax Carbohydrate 4.6 × 250 mm, 5 μm column (Agilent Technologies), evaporative light scattering detector (ELSD) was used while for acid NUCLEOGEL SUGAR 810 H (MACHEREY-NAGEL) column with diode array detector (DAD) was used.

### Sensory evaluation

Sensory evaluation of apricot fruit after cold storage and shelf life was conducted by 12 trained panelists (6 women and 6 men), aged 20-65 years, according to the methodology adopted by Melgarejo et al. (2014). The panelists were asked to score visual appearance (tissue breakdown and browning) on halved fruits, and the intensity of 5 fruit attributes (sweetness, acidity, apricot flavor, crispiness and off flavor) on slices of fruits. The evaluation was performed by trained panelists using a continuous scale ranging from 0 (lowest score) to 100 (highest score). The total scale length was 100 mm, and the obtained results were further used for calculations. The process was carried out at room temperature (20 °C) in individual cabins under white lighting. The evaluation was performed in two separate sessions on the same day. All participants received written information about the study, and they signed informed consent to participate.

### Statistical analysis

Obtained results were analyzed using ANOVA. Duncan's multiple range test was

used for testing the significance of differences between the applied treatments, while for respiration and ethylene Tukey's HSD test was used. Statistical calculations were performed using TIBCO Data Science - Workbench (Statistica® 14.0.0) (<http://tibco.com>).

## RESULTS AND DISCUSSION

As expected, ripening stage and treatment time significantly affected almost all examined pomological parameters in apricots (Table 1) while application of NAs affected only flash/fruit ratio in the fruit. Chemical treatment did not change fruit shape index, sphericity, and elongation either (data not shown).

Regarding quality and postharvest properties, storage, as expected influenced significantly all observed properties except citric acid content, the sourness of fruits and the appearance of inappropriate taste. In the presentation of results, the focus was primarily on the influence and differences among applied treatments with NAs. At harvest, NAs exhibited an effect on respiration rate and ethylene production, but after 21 days of cold storage, during shelf life, the difference remained only in the case of ethylene production (Fig. 1).

The color of apricots ( $L^*$ ,  $h$ ) was significantly affected by NAs treatments, while flesh firmness showed dependence on the time of NAs application (Table 2).

Both NAs treatments and application time significantly impacted TSS and TA, but did not influence secondary biomolecules, phenols, flavonoids and carotenoids (Table 3). Glucose and malic acid contents were not affected by any of the applied treatments (data not shown).

On the other hand, fructose content was affected by NAs concentration, while in the case of citric acid application time was crucial. Finally, for sucrose and succinic acid content all experimental factors were significant (Table 4).

Sensory analysis of apricots after storage as well as after shelf life characterized the fruits treated with NAs as significantly sweeter, more aromatic, with less acidity and gumminess, comparing to the control. The time of NAs application proved to be irrelevant (Table 5).

At the same time, tissue breakdown was affected by applied NAs concentrations, while browning proved to be affected by both, NAs concentration and application time (Table 6).

**Table 1.**  
Effects of NAs applied at different fruit growth stages on physical parameters in apricots at harvest

Maturity stage	I <sub>ad</sub>	Fruit weight (g)		Fruit height (mm)		Fruit width (mm)		Fruit thickness (mm)		Fruit volume (cm <sup>3</sup> )		Flesh/fruit ratio (%)		Fruit surface (cm <sup>2</sup> )	
		T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
0.4-0.8	Control	72.4 <sup>ab</sup>		49.3 <sup>ab</sup>		47.3 <sup>abc</sup>		43.4 <sup>abc</sup>		53.3 <sup>ab</sup>		95.9 <sup>a</sup>		67.7 <sup>abc</sup>	
	NAs1	69.0 <sup>ab</sup>	83.3 <sup>bc</sup>	48.5 <sup>a</sup>	52.3 <sup>bc</sup>	46.8 <sup>ab</sup>	50.3 <sup>cd</sup>	41.0 <sup>ab</sup>	46.1 <sup>c</sup>	49.4 <sup>ab</sup>	64.1 <sup>bc</sup>	98.1 <sup>c</sup>	97.7 <sup>c</sup>	64.0 <sup>ab</sup>	76.4 <sup>a</sup>
	NAs3	60.7 <sup>a</sup>	85.0 <sup>bc</sup>	47.3 <sup>a</sup>	52.6 <sup>bc</sup>	44.4 <sup>a</sup>	49.8 <sup>bcd</sup>	39.3 <sup>a</sup>	44.6 <sup>bc</sup>	43.8 <sup>a</sup>	63.6 <sup>bc</sup>	98.3 <sup>c</sup>	98.0 <sup>c</sup>	59.1 <sup>a</sup>	75.2 <sup>bcd</sup>
0	Control	107.0 <sup>d</sup>		57.6 <sup>d</sup>		55.5 <sup>ef</sup>		51.4 <sup>d</sup>		87.6 <sup>de</sup>		97.7 <sup>c</sup>		93.9 <sup>ef</sup>	
	NAs1	86.9 <sup>bc</sup>	94.8 <sup>cd</sup>	52.4 <sup>bc</sup>	58.7 <sup>d</sup>	50.2 <sup>bcd</sup>	59.1 <sup>g</sup>	45.6 <sup>c</sup>	52.4 <sup>d</sup>	64.3 <sup>bc</sup>	96.6 <sup>e</sup>	96.8 <sup>b</sup>	96.6 <sup>bc</sup>	76.1 <sup>cd</sup>	100.1 <sup>f</sup>
	NAs3	96.6 <sup>cd</sup>	92.6 <sup>cd</sup>	54.0 <sup>c</sup>	58.9 <sup>d</sup>	53.0 <sup>de</sup>	57.3 <sup>fg</sup>	50.0 <sup>d</sup>	51.8 <sup>d</sup>	75.7 <sup>cd</sup>	93.1 <sup>e</sup>	96.4 <sup>bc</sup>	96.6 <sup>bc</sup>	85.4 <sup>de</sup>	97.7 <sup>f</sup>
Ripening stage		**		**		**		**		**		*		**	
NAs concentration		NS		NS		NS		NS		NS		**		NS	
Application time		*		**		**		**		**		NS		**	
Ripening stage × NAs concentration		NS		NS		NS		NS		**		**		NS	
Ripening stage × Application time		NS		NS		NS		NS		NS		NS		NS	
NAs concentration × Application time		NS		**		**		**		NS		NS		**	
Ripening stage × NAs concentration × Application time		NS		NS		NS		NS		NS		NS		NS	

<sup>†</sup> statistically significant values are denoted with different letters ( $p < 0.05$ ); \* statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$ ; N.S. - not significant; NAs1 – 1 mg/l naphthenic acids; NAs3 - 3 mg/l naphthenic acids; T1 – NAs applied when sepals began to fall; T2 – NAs applied on 7th day after sepals began to fall

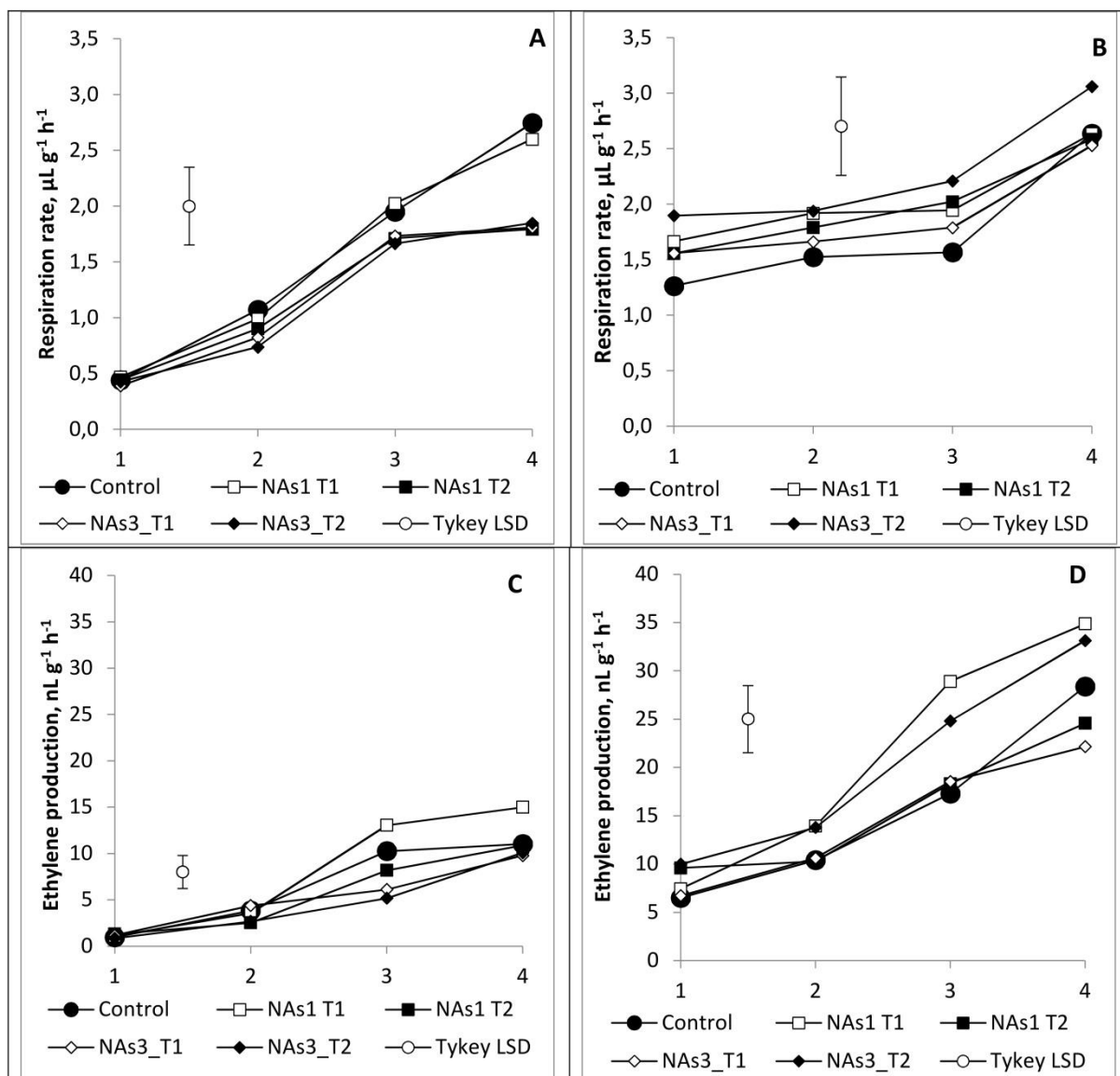


Figure 1. Respiration rate, and ethylene production in apricots treated with NAs at harvest (A, C, E) and after 21 days of cold storage (B, D, F), followed by 4 consecutive days of shelf life, respectively. Control – untreated fruits; NAs1 – 1 mg/l naphthenic acids; NAs3 – 3 mg/l naphthenic acids; T1 – NAs applied when sepals began to fall; T2 – NAs applied on 7<sup>th</sup> day after sepals began to fall. Statistical significance among the treatments was tested by Tukey’s HSD test

Ripening stage and NAs treatment significantly affected fruit weight, however a significant decrease in commercial ripening stage was noted only between fruit treated with NAs3, while in full maturity fruit treated with NAs1 at T1 had lower fruit weight than respective control fruit (Table 1). Above mentioned significant differences are not enough data to conclude NAs effect on fruit weight. Fruit weight, height and width showed similar trends for both ripening stages while no difference was observed between different NAs concentrations. Treatment with NAs at T1 resulted in obtaining fruit with smaller weight,

width, thickness and consequently smaller volume than fruits treated at T2. When compared to control fruits, fruit weight, width, and thickness were not statistically different in commercially ripe fruit while in fully ripe fruit height, thickness and volume were smaller. In general, this effect was more obvious in commercially ripe fruit while in the case of fully ripe fruits this effect was less expressed pointing out at prolonged effect of NAs during the natural ripening process. Increased fruit dimensions were not dependent on the concentration of NAs used for treatment in the case of application of NAs at T2.

**Table 2.**

The color and textural properties of apricot fruit at harvest, after storage and shelf life after being treated with NAs at different fruit growth stages

Treatment		L*		h		Flesh firmness (N)	
Control	0	62.2 <sup>g</sup>		77.5 <sup>f</sup>		21.15 <sup>d</sup>	
	21	59.2 <sup>def</sup>		70.8 <sup>cde</sup>		6.87 <sup>c</sup>	
	21+3	57.0 <sup>bcd</sup>		68.2 <sup>bcd</sup>		2.88 <sup>ab</sup>	
NAs1	0	T1	T2	T1	T2	T1	T2
	21	59.0 <sup>def</sup>	61.4 <sup>fg</sup>	67.6 <sup>abc</sup>	73.7 <sup>def</sup>	20.67 <sup>d</sup>	31.02 <sup>e</sup>
	21+3	55.6 <sup>b</sup>	58.4 <sup>cde</sup>	62.4 <sup>ab</sup>	67.5 <sup>abc</sup>	5.35 <sup>bc</sup>	5.37 <sup>bc</sup>
NAs3	0	52.7 <sup>a</sup>	56.1 <sup>bc</sup>	61.5 <sup>a</sup>	66.4 <sup>abc</sup>	0.88 <sup>a</sup>	2.61 <sup>ab</sup>
	21	62.1 <sup>g</sup>	60.3 <sup>efg</sup>	74.5 <sup>gef</sup>	70.3 <sup>cde</sup>	21.80 <sup>d</sup>	23.78 <sup>d</sup>
	21+3	58.9 <sup>def</sup>	56.6 <sup>bcd</sup>	67.4 <sup>abc</sup>	64.7 <sup>abc</sup>	5.07 <sup>bc</sup>	5.53 <sup>bc</sup>
NAs concentration		**		**		NS	
Application time		NS		NS		**	
Storage		**		**		**	
Storage × Application time		**		**		**	
Storage × NAs concentration		NS		NS		**	
NAs concentration × Application time		NS		NS		**	
NAs concentration × Application time × Storage		NS		NS		**	

<sup>†</sup>statistically significant values are denoted with different letters ( $p < 0.05$ ); \* statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$ ; N.S. - not significant; NAs1 – 1 mg/l naphthenic acids; NAs3 – 3 mg/l naphthenic acids; 0 – at harvest; 21 – 21 days of cold storage; 21+3 – 21 days of cold storage + 3 days shelf-life; T1 – NAs applied when sepals began to fall; T2 – NAs applied on 7<sup>th</sup> day after sepals began to fall

**Table 3.**

The contents of carotenoids, phenols and flavonoids in apricots at harvest, after storage and shelf life after treatment with NAs at T1 and T2

Treatment		TSS		TA		Carotenoids		Phenols		Flavonoids	
Control	0	10.0 <sup>c</sup>		1.21 <sup>efg</sup>		1.47 <sup>a</sup>		45 <sup>a</sup>		7.6 <sup>cd</sup>	
	21	11.0 <sup>de</sup>		1.19 <sup>def</sup>		2.85 <sup>fg</sup>		55 <sup>abcd</sup>		8.0 <sup>cd</sup>	
	21+3	10.8 <sup>d</sup>		1.36 <sup>h</sup>		3.21 <sup>gh</sup>		49 <sup>bcd</sup>		5.6 <sup>a</sup>	
NAs1	0	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
	21	10.2 <sup>c</sup>	9.5 <sup>b</sup>	1.25 <sup>fg</sup>	1.19 <sup>def</sup>	2.04 <sup>bcd</sup>	1.63 <sup>ab</sup>	53 <sup>abc</sup>	53 <sup>abc</sup>	9.6 <sup>ef</sup>	10.6 <sup>f</sup>
	21+3	11.4 <sup>fg</sup>	10.9 <sup>de</sup>	1.15 <sup>bcd</sup>	1.10 <sup>ab</sup>	2.28 <sup>cde</sup>	2.92 <sup>fg</sup>	50 <sup>abc</sup>	51 <sup>abc</sup>	5.7 <sup>a</sup>	5.6 <sup>a</sup>
NAs3	0	13.9 <sup>i</sup>	11.2 <sup>ef</sup>	1.27 <sup>eg</sup>	1.10 <sup>abc</sup>	3.60 <sup>h</sup>	2.75 <sup>efg</sup>	56 <sup>bcd</sup>	57 <sup>cd</sup>	4.9 <sup>a</sup>	5.8 <sup>a</sup>
	21	9.3 <sup>ab</sup>	9.2 <sup>a</sup>	1.06 <sup>a</sup>	1.13 <sup>abcd</sup>	2.17 <sup>cd</sup>	1.89 <sup>abc</sup>	47 <sup>ab</sup>	47 <sup>ab</sup>	8.6 <sup>cde</sup>	8.7 <sup>de</sup>
	21+3	11.5 <sup>g</sup>	11.6 <sup>g</sup>	1.18 <sup>cdef</sup>	1.09 <sup>ab</sup>	2.09 <sup>bcd</sup>	2.98 <sup>fg</sup>	53 <sup>abc</sup>	56 <sup>bcd</sup>	5.0 <sup>a</sup>	7.2 <sup>bc</sup>
NAs concentration		**		**		NS		NS		**	
Application time		**		NS		NS		NS		NS	
Storage		**		NS		**		**		**	
Storage × Application time		**		NS		NS		NS		NS	
Storage × NAs concentration		**		**		**		*		**	
NAs concentration × Application time		**		**		**		*		**	
NAs concentration × Application time × Storage		**		**		**		*		**	

<sup>†</sup>statistically significant values are denoted with different letters ( $p < 0.05$ ); \* statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$ ; N.S. - not significant; NAs1 – 1 mg/l naphthenic acids; NAs3 – 3 mg/l naphthenic acids; 0 – at harvest; 21 – 21 days of cold storage; 21+3 – 21 days of cold storage + 3 days shelf-life; T1 – NAs applied when sepals began to fall; T2 – NAs applied on 7<sup>th</sup> day after sepals began to fall

**Table 4.**

Sugar and organic acid composition in apricots at harvest, after cold storage and shelf life after being treated with NAs at different fruit growth stages

Treatment		Fructose		Sucrose		Citric acid		Succinic acid	
Control	0	1.10 <sup>a</sup>		5.57 <sup>c</sup>		1.98 <sup>abcde</sup>		0.63 <sup>b</sup>	
	21	1.50 <sup>b</sup>		4.64 <sup>bcd</sup>		1.95 <sup>abcde</sup>		0.65 <sup>bc</sup>	
	21+3	1.52 <sup>b</sup>		4.36 <sup>abc</sup>		2.14 <sup>bcdef</sup>		0.97 <sup>g</sup>	
		T1	T2	T1	T2	T1	T2	T1	T2
NAs1	0	1.20 <sup>a</sup>	1.15 <sup>a</sup>	4.73 <sup>cd</sup>	4.64 <sup>bcd</sup>	2.23 <sup>ef</sup>	1.91 <sup>abc</sup>	0.53 <sup>a</sup>	0.76 <sup>ef</sup>
	21	1.73 <sup>cd</sup>	1.68 <sup>bcd</sup>	3.79 <sup>a</sup>	4.02 <sup>ab</sup>	2.15 <sup>def</sup>	1.98 <sup>abcde</sup>	0.81 <sup>f</sup>	0.77 <sup>ef</sup>
	21+3	1.60 <sup>bcd</sup>	1.58 <sup>bcd</sup>	4.33 <sup>abc</sup>	3.78 <sup>a</sup>	2.04 <sup>abcde</sup>	1.83 <sup>a</sup>	0.98 <sup>gh</sup>	1.08 <sup>i</sup>
NAs3	0	1.23 <sup>a</sup>	1.18 <sup>a</sup>	4.88 <sup>cde</sup>	4.85 <sup>cde</sup>	1.83 <sup>a</sup>	1.85 <sup>abc</sup>	0.57 <sup>a</sup>	0.70 <sup>cd</sup>
	21	1.74 <sup>d</sup>	1.70 <sup>cd</sup>	4.16 <sup>abc</sup>	4.18 <sup>abc</sup>	2.22 <sup>def</sup>	1.92 <sup>abcd</sup>	0.80 <sup>ef</sup>	0.75 <sup>de</sup>
	21+3	1.57 <sup>bcd</sup>	1.57 <sup>bcd</sup>	5.34 <sup>de</sup>	4.69 <sup>bcd</sup>	2.35 <sup>f</sup>	2.06 <sup>abcdef</sup>	1.03 <sup>hi</sup>	0.93 <sup>g</sup>
NAs concentration				**		NS		**	
Application time		NS		**		**		**	
Storage		**		*		NS		**	
Storage × Application time		NS		**		NS		**	
Storage × NAs concentration		NS		NS		*		**	
NAs concentration × Application time		NS		NS		NS		**	
NAs concentration × Application time × Storage		NS		NS		NS		**	

<sup>+</sup>statistically significant values are denoted with different letters ( $p < 0.05$ ); \* statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$ ; N.S. - not significant; NAs1 – 1 mg/l naphthenic acids; NAs3 - 3 mg/l naphthenic acids; 0 – at harvest; 21 – 21 days of cold storage; 21+3 - 21 days of cold storage + 3 days shelf-life; T1 – NAs applied when sepals began to fall; T2 – NAs applied on 7<sup>th</sup> day after sepals began to fall

**Table 5.**

Sensory properties of apricots after cold storage and shelf life following the treatment with NAs at different fruit growth stages

Treatment		Sweetness		Sourness		Aroma		Crispiness		Gumminess		Inappropriate taste	
Control	21	44 <sup>a</sup>		31 <sup>a</sup>		46 <sup>a</sup>		31 <sup>c</sup>		39 <sup>b</sup>		2	
	21+3	53 <sup>ab</sup>		21 <sup>ab</sup>		61 <sup>ab</sup>		8 <sup>ab</sup>		10 <sup>a</sup>		2	
		T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
NAs1	21	57 <sup>ab</sup>	52 <sup>ab</sup>	15 <sup>ab</sup>	15 <sup>ab</sup>	60 <sup>ab</sup>	57 <sup>ab</sup>	22 <sup>bc</sup>	28 <sup>c</sup>	26 <sup>b</sup>	34 <sup>b</sup>	2	2
	21+3	62 <sup>ab</sup>	56 <sup>ab</sup>	10 <sup>b</sup>	18 <sup>ab</sup>	75 <sup>b</sup>	63 <sup>ab</sup>	2 <sup>a</sup>	7 <sup>ab</sup>	4 <sup>a</sup>	9 <sup>a</sup>	3	0
NAs3	21	54 <sup>a</sup>	53 <sup>ab</sup>	21 <sup>ab</sup>	21 <sup>ab</sup>	49 <sup>a</sup>	53 <sup>a</sup>	27 <sup>c</sup>	29 <sup>c</sup>	28 <sup>b</sup>	34 <sup>b</sup>	1	0
	21+3	57 <sup>ab</sup>	67 <sup>b</sup>	18 <sup>ab</sup>	18 <sup>ab</sup>	65 <sup>ab</sup>	74 <sup>b</sup>	8 <sup>ab</sup>	5 <sup>a</sup>	9 <sup>a</sup>	8 <sup>a</sup>	0	0
NAs concentration		*		**		*		NS		*		NS	
Application time		NS		NS		NS		NS		NS		NS	
Storage		*		NS		**		**		**		NS	
Storage × Application time		NS		NS		NS		NS		NS		NS	
Storage × NAs concentration		NS		NS		NS		NS		NS		NS	
NAs concentration × Application time		NS		NS		NS		NS		NS		NS	
NAs concentration × Application time × Storage		NS		NS		NS		NS		NS		NS	

<sup>+</sup>statistically significant values are denoted with different letters ( $p < 0.05$ ); \* statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$ ; NS - not significant; NAs1 – 1 mg/l naphthenic acids; NAs3 - 3 mg/l naphthenic acids; 21 – 21 days of cold storage; 21+3 - 21 days of cold storage + 3 days of shelf-life; T1 – NAs applied when sepals began to fall; T2 – NAs applied on 7<sup>th</sup> day after sepals began to fall



**Table 6.**

Apricot tissue breakdown and browning during cold storage and shelf life caused by NAs application at different fruit growth stages

Treatment	Tissue breakdown		Browning		
	21	30 <sup>ab</sup>	T1	T2	
Control	21	30 <sup>ab</sup>		2.5 <sup>ab</sup>	
	21+3	58 <sup>c</sup>		9.0 <sup>bc</sup>	
		T1	T2	T1	T2
NAs1	21	70 <sup>c</sup>	28 <sup>a</sup>	0.0 <sup>a</sup>	8.0 <sup>abc</sup>
	21+3	100 <sup>d</sup>	78 <sup>cd</sup>	9.0 <sup>bc</sup>	11.1 <sup>c</sup>
NAs3	21	53 <sup>bc</sup>	65 <sup>c</sup>	6.0 <sup>abc</sup>	7.5 <sup>abc</sup>
	21+3	75 <sup>c</sup>	100 <sup>d</sup>	9.0 <sup>bc</sup>	18.6 <sup>d</sup>
<b>NAs concentration</b>		**		*	
<b>Application time</b>		NS		*	
<b>Storage</b>		**		*	
<b>Storage × Application time</b>		NS		*	
<b>Storage × NAs concentration</b>		NS		**	
<b>NAs concentration × Application time</b>		**		NS	
<b>NAs concentration × Application time × Storage</b>		NS		NS	

<sup>+</sup>statistically significant values are denoted with different letters ( $p < 0.05$ ); \* statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$ ; NS - not significant; NAs1 – 1 mg/l naphthenic acids; NAs3 - 3 mg/l naphthenic acids; 21 – 21 days of cold storage; 21+3 -21 days of cold storage + 3 days of shelf-life; T1 – NAs applied when sepals began to fall; T2 – NAs applied on 7th day after sepals began to fall

The influence of the application of NAs in early fruit development phases was confirmed also in our previous work. Namely, the application of NAs increased fruit weight and diameter in “Golden Delicious” apples similarly to plant hormone auxin (NAA) used in the same experiment (Milić et al., 2017). The influence of low concentrations of NAA on the increase of fruit weight and volume with effects depending on concentration was reported also by Devrari, Negi and Thakur (2017), while Son (2004) reported the same effect for the application of auxin (NAA) at pit hardening stage. Analysis of NAs distilled in the same way as NAs used in our experiment shows the presence of compounds with condensed rings with 5 and 6 C-atoms (Ćirin-Novta et al., 2002), which resembles an auxin structure.

Effects of application of plant hormone cytokinin on fruit weight and volume were also widely studied with less consistent results obtained by different authors. Apricot fruit weight and diameter were increased by 100 and 150 ppm BA treatments (Canli et al., 2014), but 50 ppm BA concentration did not have an effect (Abdel-Mohsen & Kamel, 2015). The dependence of applied concen-

trations of BA is also similar to the behavior of applied NAs in our experiment.

Since our results showed the clear prolonged effect of NAs during the natural ripening process, in further text effect of NAs on changes during cold storage and shelf life will be discussed in terms of the influence of applied treatments with NAs on fruit color, firmness, composition and sensory properties and their changes during cold storage and shelf life.

Climacteric fruits, including apricots, after being moved from cold storage to room temperature, are characterized by a rapid increase in ethylene production, which further accelerates ripening, softening, or even some physiological disorders (juiciness loss and mealiness formation) during shelf life (Kan, Che, Xie & Jin, 2011; Stanley et al., 2013; Fan et al, 2018). The prolonged effects of NAs in the postharvest period can be noted in the case of respiration and ethylene production. At harvest all the treatments, except NAs1 at T1, had lower CO<sub>2</sub> production, on the fourth day, versus the control (Fig. 1C), pointing out a lower respiration rate which might be beneficial for the possibility of prolonged storage. Apricots treated with NAs1 at T1 exhibited the highest ethylene production both

after the harvest and after cold storage. After cold storage higher ethylene production in comparison to control was noted also in the case of NAs3 at T2 (Fig. 1E and 1F). All other treatments were characterized with similar or slightly lower ethylene production in comparison to control, non-treated fruits.

The composition of apricot fruits includes besides TSS and TA as basic quality parameters used in practice, also analysis of nutritionally valuable components including total phenols and flavonoids, analysis of the content of carotenoids as the main pigments in apricots fruits (Table 1), as well as the analysis of the composition of sugars and acids as the substances defining the main taste properties of apricot fruits (Table 2).

All NAs treatments, except NAs1 at T1, resulted in lower TSS at harvest if compared to the control, non-treated apricots, but after cold storage, fruits of all treatments were characterized with the higher TSS in comparison to control (Table 1). Higher TSS contributes to the higher commercial value of apricots treated with NAs after storage.

The effect of NAs on TA at harvest was dependent on applied concentration, and only application of higher concentration (NAs3) resulted in lower TA in fruits at harvest, but after cold storage, all fruits treated with NAs exhibited slightly lower TA values in comparison to non-treated control, with the lower TA in the case of NAs application at time T2.

Application of NAs did not exhibit a significant effect on carotenoids, phenols and flavonoids content, although at harvest these parameters were higher in treated apricots than in control fruits (Table 1).

Oppositely, treatments with NAs seem to affect the composition of sugars and acids in apricot fruits. At harvest fructose content in treated fruits was somewhat higher in comparison to non-treated control and a sharper increase of fructose content during cold storage, characterizing treated fruits, resulted in significantly higher fructose content in comparison to control after cold storage. However, after shelf life, fructose level was again similar in all fruits (Table 4). On the other hand, in fruits treated with NAs sucrose content was significantly lower at harvest when compared to control. Investigations reporting trends in sucrose content in apricot fruits during ripe-

ning before harvest (Xi, Zheng, Zhang & Li, 2016) point out an increase in sucrose content during natural ripening on the tree indicating that ripening processes in fruits treated with NAs might have possibly been slowed down regarding sucrose synthesis. After cold storage, sucrose content decreased in all fruits regardless of treatment and remained lower in treated fruits in comparison to control. During shelf life, sucrose content increased, and fruits treated with higher concentration (NAs3) reached higher levels as compared to the control.

The content of citric acid was similar in all fruits, both, at harvest and after cold storage and shelf life, without significant changes in content during the postharvest period (Table 5). Concerning succinic acid, apricots at harvest treated at T1 had significantly lower, whereas those treated at T2 had a significantly higher content of this acid, compared to the respective control. During the development and ripening of fruit, a succession of plant hormones and their crosstalk occurs (Ji, Xu & Wang, 2021) but the external application of NAs at different times, could cause disruption of natural hormone sequence appearance and thus, alter the content of some metabolic products. After cold storage, all applied treatments had significantly higher succinic acid content than control, while after shelf life the differences were diminished (Table 5).

Skin color is among the main traits observed by consumers defining their preferences towards fruits. Regarding fruit skin color, for apricots treated with NAs, it was observed that, in general, there was a trend of lower  $L^*$ , pointing out darker skin color, and lower hue angle ( $h^\circ$ ) values pointing out more orange skin color in comparison to more yellow skin of control apricots. However, the emphasized trend was statistically significant only in the case of treatment with lower concentration (NAs1) at T1 (Table 2). The same observations, considering color, were noted after cold storage as well as after shelf life.

For apricots, loss of flesh firmness is among the main traits defining their shelf life. The effects of different pre- and postharvest treatments on fruit flesh firmness were investigated for different fruits. Canli et al. (2014) reported that, out of six BA treatments, only 100 ppm BA significantly increased fruit firmness at harvest. Treatment with methyl jasmonate and salicylic acid improved firmness during cold

storage and shelf life (Ezzat, Ammar, Szabó & Holb, 2017). Similar to apricots, peaches treated with GA3 retained firmness when compared to control fruits during the first 8 days of storage at 20 °C (Dagar, Weksler, Friedman & Lurie, 2012). Preservation of fruit firmness during cold storage can be achieved by ethylene removal and by modified atmosphere packaging (MAP) (Álvarez-Hernández et al., 2020) or with chitosan coatings (El-Badawy & El-Salhy, 2011). In our experiment application of NAs resulted in an increase of apricot flesh firmness only in the case of treatment with NAs1 at T2 which resulted in significantly higher fruit firmness at harvest but it was not preserved in the postharvest period. Other treatments with NAs did not influence significantly flesh firmness, neither at harvest nor after cold storage. The fact that the firmness of apricots treated with NAs did not deteriorate is also very important because all other achieved improvements in quality and storability might be diminished if the significant deterioration of firmness was registered.

Higher TSS, accompanied by higher fructose content as well as lower TA accompanied by a higher succinic acid content point out a high probability of change in taste of apricots as a consequence of treatment with NAs. Regarding taste (Table 5), all treated apricots were characterized with increased sweetness after cold storage, when compared to the control (Table 5). This trend continued after shelf life, although none of these differences proved to be significant. The highest sweetness was noted in fruits treated with NAs3 at T2. At the same time, treatment with NAs resulted in a lower intensity of apricot sourness after cold storage, which was additionally reduced by shelf life, if compared to the control, resulting in a similar level of sourness of all treated fruits. The specific, highly desired aroma of apricot was also at a somewhat higher level in all treated fruits in comparison to control, both after cold storage and shelf life. A very important observation is that the application of NAs did not result in the appearance of inappropriate taste in apricot fruits. Despite the absence of statistical significance of differences among obtained results, the sensory analysis points out an improvement in the taste of apricots through the application of NAs in early fruit development phases towards a more sweet and less sour combination of tastes.

Beside taste, an important sensory feature of apricot fruit is its texture characterized as a complex combination of sensations of different texture aspects during apricot consumption. Apricots treated with NAs had slightly less expressed gumminess indicating somewhat lower force needed for manipulation of fruit during consumption prior to its disintegration in the mouth. Another textural property, crispiness, which manifests as the sensation of breaking firm material during chewing, was also slightly lower in apricots treated with NAs. At the same time, tissue breakdown in fruits treated with NAs was higher, in some cases significantly, after storage as well as after shelf life, if compared to the control (Table 6). Such a combination of textural properties points out that NAs treatments, both after cold storage and after shelf life, resulted in apricot fruits with a more tender and soft sensation during consumption.

A minor problem regarding the sensory properties of apricots treated with NAs in early fruit development phases might be the appearance of initial browning of tissue around the pit, particularly in the case of fruits treated with higher NAs concentration (NAs3) at T2.

Having in mind the potential effects of NAs, they are often compared to the effects of auxins and gibberellins which also have an effect on fruit quality at harvest, but initial differences became less expressed as ripening or storage continues. The mode of action for stimulatory effects of NAs has not yet been completely discovered. One possible explanation might include NAs-mediated change in membrane permeability of plant cell (Pavlović et al., 2015). The other is the alteration of NAA effects, since two-ringed NAs have structural similarity to NAA, and may be recognized by auxin receptors (Leishman et al., 2013). The latter might explain increased fruit weight in apples (Keserović, Milić, Kevrešan, Magazin & Dorić, 2016).

## CONCLUSIONS

Application of NAs at early stages and at the commercial stage changed fruit size and shape and those changes remain in fully ripe fruit. Flesh/fruit ratio, fruit color, TA, flavonoids concentration, fructose, sweetness, sourness, aroma, gumminess and tissue breakdown depended on the applied NAs concentration. Fruit weight, height, width, thickness, volume,

surface, flesh firmness, and citric acid depended on time of NAs application while TSS, sucrose, succinic acid and browning depended on both, application time and NAs concentration. Changes caused by NAs are subtle and resemble rather plant growth regulator application than the application of ethylene blockers. Although there were not many significant differences in the composition of fruits treated with NAs in respect to non-treated control at harvest in a postharvest period during 20 days of cold storage and particularly after 3 days of shelf life at room temperature, apricots treated with NAs were characterized with higher total soluble solids content accompanied with higher fructose content, lower titratable acidity accompanied with higher succinic acid content, as well as with trends towards improved sensory properties: sweeter and less sour taste, with more expressed apricot aroma accompanied with decreased gumminess and crispiness and more intensive tissue breakdown, but without expressed notes of inappropriate taste.

Application of NAs could become a useful tool in apricot postharvest, but the time of application, concentration as well as repeated application in further seasons should be investigated. Considering further use of NAs, health aspects should be investigated, especially regarding low NAs concentration effect on soil microorganisms and possible NAs accumulation.

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## DA LI PRIMENA NAFTENSKIH KISELINA U RANOJ FAZI RAZVOJA VOĆA IMA PRODUŽENI EFEKAT NA SKLADIŠNU SPOSOBNOST I ROK TRAJANJA PLODOVA KAJSIJE?

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**Sažetak:** Na osnovu podataka o stimulativnom dejstvu naftenskih kiselina (NAs) na rast i sastav različitih biljaka, sproveden je eksperiment u kome je ispitan uticaj primene NAs na kajsije u ranim fazama razvoja ploda na njihove morfološke karakteristike, sastav i svojstva ploda nakon berbe. Primenjene su dve koncentracije NAs (1 mg/l i 3 mg/l) u dve faze razvoja kod sorte kajsije NS-4. Aplikacija NAs u fazi razvoja kada je začetak ploda bio okružen odumirućim krunicnim listićima, rezultirala je smanjenjem dimenzija ploda pri berbi, dok je aplikacija 7 dana kasnije rezultirala povećanjem dimenzija ploda. Iako pri berbi nije bilo značajnih razlika u sastavu plodova tretiranih NAs u odnosu na netretiranu kontrolu, tokom 20 dana hladnog skladištenja ( $1 \pm 1$  °C, RH 80%), a posebno nakon 3 dana na sobnoj temperaturi, tretirane kajsije su imale veći sadržaj ukupnih rastvorljivih čvrstih materija, veći sadržaj fruktoze, nižu titrabilnu kiselost, veći sadržaj jantarne kiseline, kao i poboljšana senzorna svojstva: slađi i manje kiselkast ukus, izraženiju aromu kajsije praćenu smanjenom gumoznošću i hrskavošću, intenzivnijim razlaganjem tkiva, ali bez izraženog neodgovarajućeg ukusa.

**Ključne reči:** *naftenske kiseline, kajsija, skladištenje, rok trajanja, postharvest*

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