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## EFFECT OF N, Ca, MAP, AND 1-MCP TREATMENTS ON ETHYLENE- $I_{AD}$ INDEX INTERACTIONS DURING APRICOT STORAGE AND SHELF LIFE

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**Abstract:** Aim of this study is to investigate the interrelation between ethylene production, DA index ( $I_{AD}$ ), and postharvest treatments in four apricot (*Prunus armeniaca* L.) cultivars: 'Buda', 'NS Kasnocvetna', 'NS Rodna', and 'NS6'. Apricots were harvested with  $I_{AD}$  values between 0.41–0.80, stored at 1 °C for 15 days, followed by 3 days of shelf life at room temperature. Ethylene production and  $I_{AD}$  were measured at harvest, after cold storage, and after shelf life. Preharvest treatments included nitrogen (N) and calcium (Ca), while postharvest treatments included modified atmosphere packaging (MAP) and 1-methylcyclopropene (MCP). Results showed cultivar-specific ethylene responses and  $I_{AD}$ , with 'Buda' exhibited the most rapid decline of  $I_{AD}$  and highest ethylene levels, and 'NS6' showing a lower decrease of  $I_{AD}$ , especially under MAP and Ca treatments. Postharvest treatments effectively reduced a decrease of  $I_{AD}$  for all tested cultivars. The findings highlight the need for cultivar-specific calibration of the  $I_{AD}$  index to optimize the management of apricots at a given ripeness stage, thereby maintaining their overall quality and storage capability.

**Key words:** stone fruit, postharvest, DA meter, preharvest treatments

## INTRODUCTION

Apricots (*Prunus armeniaca* L.) are prized not only for their unique flavor and sensory properties but also for their rich content of bio-active chemicals compounds such as phenols and carotenoids, vitamins, fibers, and minerals (Leccese, Bartolini & Viti, 2007; Vardi et al., 2008). These attributes make apricots a valuable food for a healthy diet, offering considerable health benefits (Ayour et al., 2017). However, apricots, while being the most desi-

table in terms of taste and aroma, are characterized by a short shelf life and high susceptibility to mechanical damage, which limits their availability to consumers (Ayour et al., 2017). Another significant challenge in apricot production is related to the uneven ripening of fruits, which additionally complicates the harvesting process and affects fruit storability (Fan, Argenta & Mattheis, 2000; Bartolini, Viti & Zanol, 2006; Infante, Meneses & Defilippi,

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2008; Defilippi et al., 2009; Stanley, Prakash, Marshall & Schröder, 2013). Harvesting fruits at varying ripeness stages can result in different quality and postharvest behavior, changing storage potential and shelf life. Furthermore, climacteric fruits, such as apricots, experience a rapid increase in ethylene production when moved from cold storage to room temperature. This accelerates ripening and potential tissue breakdown, leading to a significant reduction in shelf life and increased losses (Kan, Che, Xiem & Jinm, 2011; Stanley et al., 2013; Fan et al, 2018). Therefore, selecting the optimal ripening phase for harvesting apricots is crucial for this highly perishable fruit to achieve optimal overall quality and storability, and meet consumer preferences.

Various non-destructive methods have been employed to classify apricots by ripeness (Vanoli & Buccheri, 2012), including the DA-meter, which measures the Index of Absorbance Difference ( $I_{AD}$ ). The  $I_{AD}$  quantifies the difference in light absorbance at 670 nm (chlorophyll a) and 720 nm (baseline), reflecting chlorophyll content. As the fruit ripens, the  $I_{AD}$  value decreases, with higher values indicating less ripeness. Ziosi et al. (2008) stated that determining the  $I_{AD}$  index is a more reliable method than the usual physicochemical parameters such as firmness, soluble solids content, and total acids. Sadar and Zanella (2019) confirmed a strong inverse relationship between  $I_{AD}$  and fruit ethylene production, validating the DA-meter as a reliable tool for assessing fruit maturity. A recent study also supports using the DA-meter for assessing fruit maturity, demonstrating a positive correlation between the  $I_{AD}$  and firmness in five peach cultivars (Zhang et al., 2019). Accordingly, our previous study (Kovač et al., 2022) used a DA-meter to measure and test the correlation of the content of chlorophyll in flesh and ethylene production in apricot cultivar “NS4”, to describe the maturation process in fruit (Ziosi et al., 2008; Costa, Noferini, Fiori & Torrigiani, 2009). The obtained results suggested the reliability of the DA-meter as a useful tool for successfully determining the maturity of the apricot cultivar ‘NS4’. Findings also revealed that ethylene production was markedly higher and initiated earlier in fruits subjected to cold storage compared to fresh fruits (Kovač et al., 2022). Namely, cold storage influenced ethylene production dynamics, particularly increasing its production in commercially ripe and

unripe apricots, thereby potentially affecting their ripening behavior and postharvest quality. The highest ethylene peak was observed in commercially ripe apricots ( $I_{AD}$  0.41-0.80), followed by unripe fruits ( $I_{AD} > 0.81$ ), compared to ripe apricots ( $I_{AD}$  0.00-0.40).

One commonly used approach to influence fruit ripening involves preharvest applications of nitrogen (N) and calcium (Ca). These nutrients influence various biochemical processes related to fruit development and postharvest behavior. Nitrogen is essential for growth and fruit size (Khasawneh et al., 2021), while calcium plays a pivotal role in cell wall stability, reducing physiological disorders and delaying senescence (Chen et al., 2024). Previous studies have demonstrated that preharvest applications of N and Ca can affect ethylene biosynthesis and modulate postharvest ripening in several fruit crops (Lara, 2013). Postharvest technologies, such as modified atmosphere packaging (MAP) and 1-methylcyclopropene (1-MCP) treatments are widely adopted to further suppress ethylene’s effects during storage (Özkaya, Yildirim, Dündar & Tükel, 2016; Mastilović et al., 2022). MAP modifies the storage atmosphere by reducing oxygen levels and increasing carbon dioxide concentrations, thereby slowing down ethylene action and delaying ripening. On the other hand, 1-MCP, an ethylene receptor inhibitor, prevents ethylene from binding to its receptor, effectively delaying the ripening process and extending fruit shelf life.

This study aims to investigate the combined effects of preharvest nitrogen (N) and calcium (Ca) applications and postharvest treatments, including MAP and 1-MCP, on ethylene production and the  $I_{AD}$  in four apricot cultivars during storage.

## MATERIALS AND METHODS

### Apricot fruit production and sampling

Apricots (*Prunus armeniaca* L.) ‘Buda’, ‘NS Kasnocvetna’, ‘NS Rodna’ and ‘NS6’ were produced at the Experimental field for fruit growing, Faculty of Agriculture, Novi Sad, located at Rimski Šančevi (45°33’820” N and 19°84’450” E, 86 m a.s.l.), Republic of Serbia. Tested apricot cultivars were grafted on Myrobalan seedlings (*Prunus cerasifera* Ehrh.) as a rootstock with blackthorn (*Prunus spinosa* L.) as an interstock. The research was conducted during 2016 and used two foliar pre-

harvest treatments: Urea treatment (N) (46% N in amide form) and Calcium treatment (Ca) (Ca - Wuxal® Calcium), applied three times in a growing season (when average fruit diameter was 11 mm, 21 mm and 30 mm) in concentrations of 1.5% for N and 0.1% for Ca, respectively.

Non-treated trees represented control. The study also included postharvest treatment: MAP (Xtend®, StePac) and 1-MCP (SmartFresh™ 0.14). Postharvest treatments were applied to a sample of chilled fruits without external damage.

Six representative trees of apricots of each cultivar were harvested and only fruits with  $I_{AD}$  = 0.41-0.80 determined by DA-meter (TR Turoni, Bologna, Italy) were selected and immediately transported to the laboratory. Fruit were analyzed at harvest, after cold storage and after 3 days of shelf life at room temperature. Storage in a cooling chamber ( $1 \pm 1$  °C and  $80 \pm 10\%$  of relative humidity) for 15 days was performed in wooden crates  $50 \times 30 \times 8$  cm. After 15 days, the samples of apricots were removed from the cooling chamber and left at room temperature (20 °C) for 3 days.

Ethylene ( $C_2H_4$ ) measurement was performed 12 h following the harvest or cold storage in order for fruit to reach constant room temperature. Its production was determined using approx. 250-300 g of fruit placed in a 770 ml container, hermetically sealed with multilayer foil during 4 h at 24 °C ( $\pm 2$  °C). The content of this plant hormone was analyzed from 2 ml of gas sampled by a plastic syringe and injected into a 10 ml headspace vial sealed with silicone septa.

Determination of ethylene was carried out by gas chromatography (GC 7890, Agilent, USA), equipped with an FID detector (Agilent, USA) and autosampler (COMBIPAL, CTCAnalytics AG, Switzerland). According to Mandić, Kevrešan and Mastilović (2019), separation was performed on the DB-WAX column under the following conditions: temperature gradient set from 60 °C to 150 °C, flow rate set to 30 mL/min, with nitrogen ( $N_2$ ) as a carrier gas, and split mode injection (10:1).

At the same time  $I_{AD}$  index was determined using a DA-meter (TR Turoni, Bologna, Italy). The measurements were carried out on two opposite sides of the fruit and the mean value of

these two readings was taken. The fruits were marked with numbers and concentric circles on both sides of the fruit to ensure that the  $I_{AD}$  value was always read from the same place.

## RESULTS AND DISCUSSION

### Cultivar ‘Buda’

At harvest, ethylene production in ‘Buda’ was quite uniform across treatments, which suggests that the fruits were harvested at a comparable maturity level across treatments (Fig. 1), which is also in line with the presence of chlorophyll ( $I_{AD}$  0.60) for all treatments. After cold storage (15+1), ethylene levels increased in the control ( $5.29 \mu L L^{-1}$ ) and N ( $4.12 \mu L L^{-1}$ ) treatments, while MAP ( $3.99 \mu L L^{-1}$ ) and MCP ( $2.22 \mu L L^{-1}$ ) treatments showed reduced ethylene production.

The  $I_{AD}$  decreased slightly in all treatments, except for MAP (0.33) which showed a marked increase, compared to the control (0.20). After shelf life (15+3), ethylene surged in all treatments, particularly in the control ( $9.90 \mu L L^{-1}$ ) and Ca+MCP ( $10.83 \mu L L^{-1}$ ), while control+MAP ( $4.27 \mu L L^{-1}$ ) suppressed ethylene production when compared to the rest of the treatments. The  $I_{AD}$  index continued to decrease, but the application of MAP and MCP reduced this decrease compared to the control. At the same time, the  $I_{AD}$  index decreased the most with the application of the MAP treatment especially N+MAP (71%) (Fig. 2)

### Cultivar ‘Kasnocvetna’

At harvest there was a notable difference in ethylene production among the preharvest treatments (Fig. 3). After cold storage (15+1), ethylene increased, especially in the N ( $7.89 \mu L L^{-1}$ ) and control+MCP ( $7.17 \mu L L^{-1}$ ) treatments, while MAP suppressed ethylene in all three sets of experimental groups. The  $I_{AD}$  decreased, with MAP and MCP maintaining the highest levels.

Following shelf life (15+3), ethylene production significantly increased in all treatments, particularly in the N+MCP treatment ( $28.29 \mu L L^{-1}$ ).

The  $I_{AD}$  dropped further, with the highest drop detected in the MAP and MCP treatments. In fruits from the control group, the highest decrease in  $I_{AD}$  index (50%) was recorded in the control during the shelf life (Fig. 4).

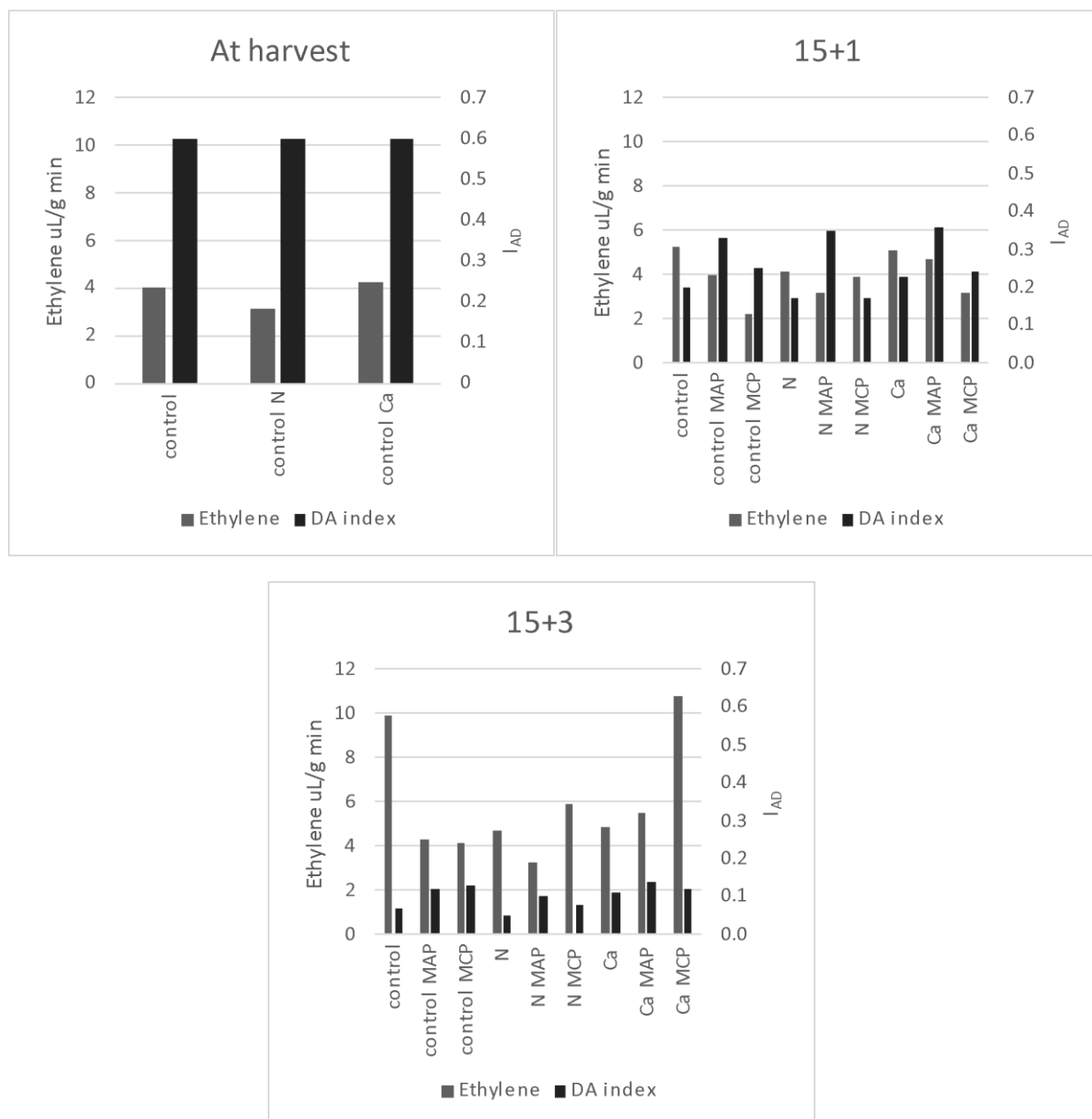


Figure 1. Ethylene and  $I_{AD}$  index at harvest, after storage and after shelf life of apricots “Buda”

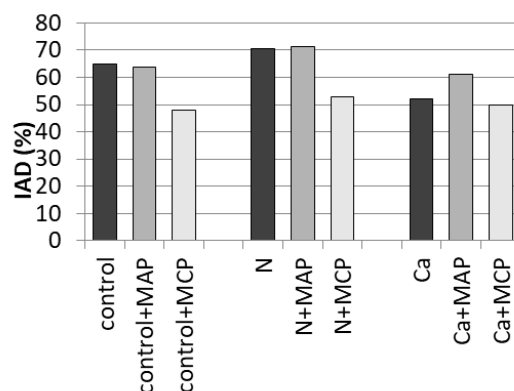


Figure 2. Decrease of  $I_{AD}$  value (%) during shelf life of apricots “Buda”

### Cultivar 'NS6'

At harvest, ethylene production varied, with Ca treatment showing the lowest levels ( $2.41 \mu\text{L L}^{-1}$ ) than N ( $5.09 \mu\text{L L}^{-1}$ ), while the  $I_{AD}$  was consistent (0.60) (Fig. 5). After cold storage (15+1), ethylene levels decreased in Ca+MAP ( $1.34 \mu\text{L L}^{-1}$ ), with the control ( $3.27 \mu\text{L L}^{-1}$ ) and N+MCP ( $4.00 \mu\text{L L}^{-1}$ ) treatments showing moderate increases. The highest  $I_{AD}$  index value was recorded in fruits treated with Ca+MAP (0.41), followed by Ca MCP (0.36). Post-shelf life (15+3), ethylene levels increased across treatments, with Ca MCP ( $21.06 \mu\text{L L}^{-1}$ ) and N+MCP ( $18.54 \mu\text{L L}^{-1}$ ) exhibiting the highest ethylene accumulation. The  $I_{AD}$  continued to decline, with Ca+MAP, Ca+MCP and N+MCP (0.30) treatments slowing  $I_{AD}$  decrease. The lowest loss during shelf life was recorded in the treatment of Ca and N in combination with 1-MCP (14 and 19%) (Fig. 6.)

### Cultivar 'Rodna'

At harvest, Ca treatment exhibited the highest ethylene levels ( $11.18 \mu\text{L L}^{-1}$ ), while the  $I_{AD}$  remained constant (0.60) (Fig. 7). Following cold storage (15+1), ethylene production increased in the control ( $12.78 \mu\text{L L}^{-1}$ ) and all MCP treatments ( $> 14 \mu\text{L L}^{-1}$ ). At the same time, all MAP treatments reduced ethylene levels compared to the control. The  $I_{AD}$  decreased across all treatments, but the decline was reduced in MAP treatments, especially N+MAP (0.41) and N+MCP (0.35). After shelf life (15+3), ethylene decreased sharply in the control ( $3.14 \mu\text{L L}^{-1}$ ) and all other treatments, except in N, and MCP treatments. The  $I_{AD}$  remained relatively stable in MAP (0.25) and Ca+MCP (0.26) treatments. The smallest decrease in  $I_{AD}$  value was achieved with the application of the MCP treatment across all three sets of experimental groups of apricots (Fig. 8).

The study revealed significant differences in how the four apricot cultivars responded to preharvest (N and Ca) and postharvest treatments (MAP and MCP), concerning ethylene production and  $I_{AD}$  values. In all examined cultivars postharvest treatment maintained  $I_{AD}$  and delayed ripening effectively, as evidenced by higher  $I_{AD}$  after cold storage. Across all cultivars, MAP consistently reduced ethylene levels and preserved  $I_{AD}$  but the extent of this

effect varied. For instance, while MAP effectively controlled ethylene release and maintained  $I_{AD}$  in 'Buda' and 'NS6' post-storage, it was less effective in 'NS Kasnocvetna' and 'NS Rodna', particularly during the shelf life. This suggests a differential sensitivity among cultivars, with 'NS6' being the most responsive to MAP packaging in terms of both ethylene suppressions. Considering the natural variability of chlorophyll content, Ziosi et al. (2008) stated that the  $I_{AD}$  index is specific to each cultivar. Valdes, Pizarro, Campos-Vargas, Infante and Defilippi (2009) also noted that the occurrence of the ethylene peak, in addition to storage duration, is influenced by the cultivar.

The use of MCP also demonstrated different efficacy among the examined cultivars. The ability to absorb and retain 1-MCP may be directly related to the composition of plant tissue (Dauny, Joyce & Gamby, 2003). Palou and Crisosto (2003) as well as Crouch (2003) reported that the effect of this treatment depends on the cultivar and the stage of ripeness. In contrast, 'Buda' showed elevated ethylene levels with MCP and foliar treatment after extended shelf life, indicating a potential cultivar-specific acceleration of ripening post-MCP treatment. Differences in MCP efficacy among apricot cultivars depend on the ripening stage, genetic traits, variability in ethylene receptor, respiration rate, chlorophyll degradation, cuticle properties, etc. The effect of MCP treatment after the shelf life varied significantly depending on the preharvest treatment and cultivar selection. This supports the findings of Fan et al. (2000) that the effect of 1-MCP diminishes as apricot fruits continue to ripen. Although we strived to ensure that the samples were uniform and within an approximate range of  $I_{AD}$  values (0.41-0.80), Dong, Lurie and Zhou (2002) stated that the effectiveness of 1-MCP application largely depends on the ripeness and physiological stage of the fruit.

Preharvest N and Ca treatments further illustrated inter-cultivar differences. N treatment generally increased ethylene production across cultivars after the cold storage, especially in 'NS Kasnocvetna' and 'NS Rodna', high-lighting its role in accelerating ripening. However, the impact of N was mitigated when combined with postharvest treatments like

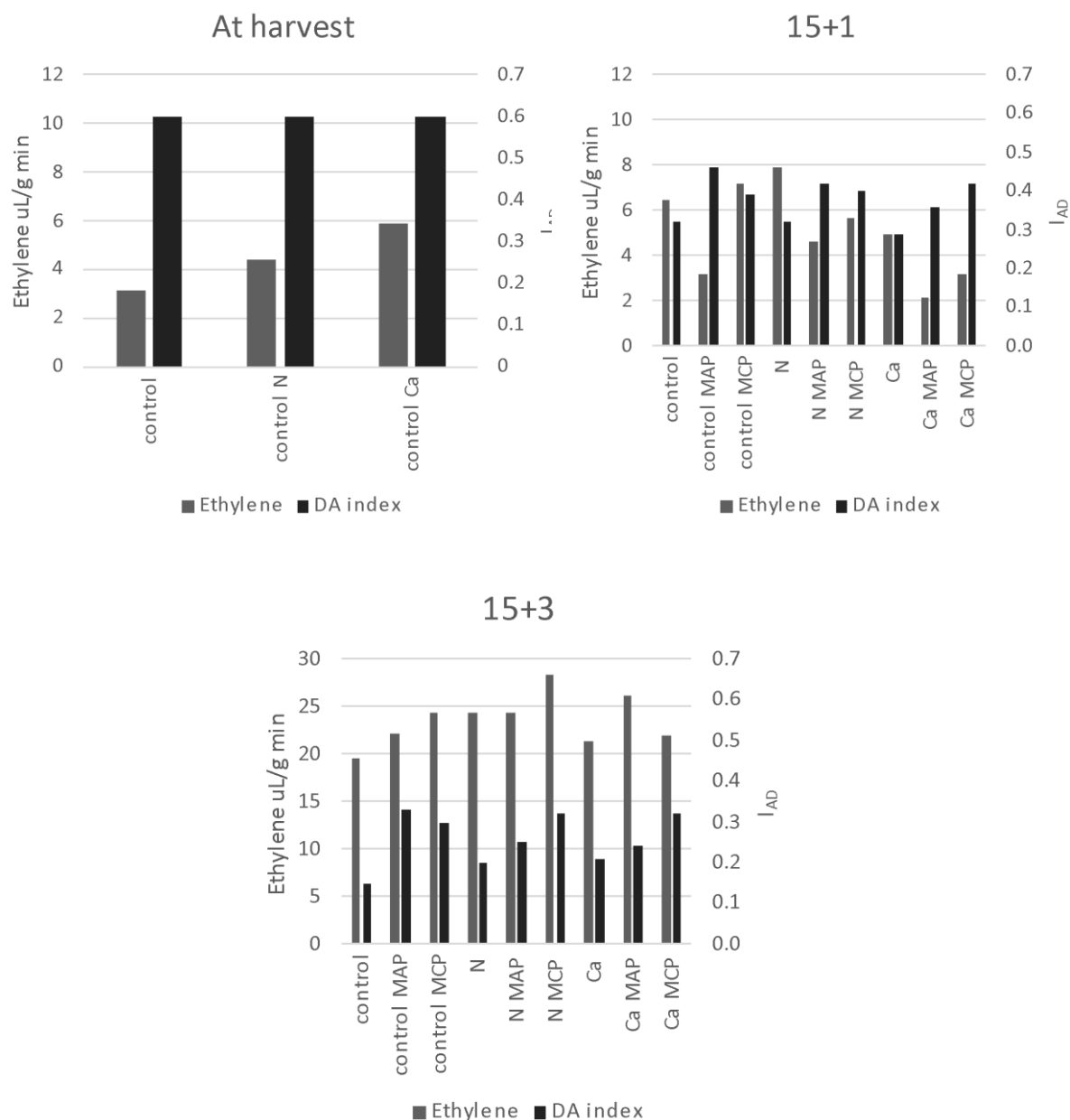


Figure 3. Ethylene and  $I_{AD}$  index at harvest, after storage and after shelf life of apricots 'NS Kasnocvetna'

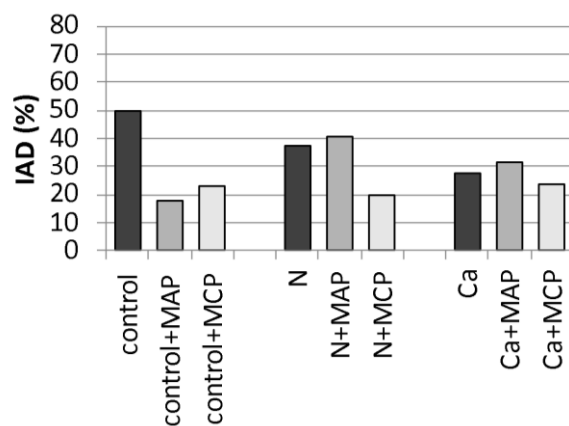


Figure 4. Decrease of  $I_{AD}$  value (%) during shelf life of apricots 'NS Kasnocvetna'

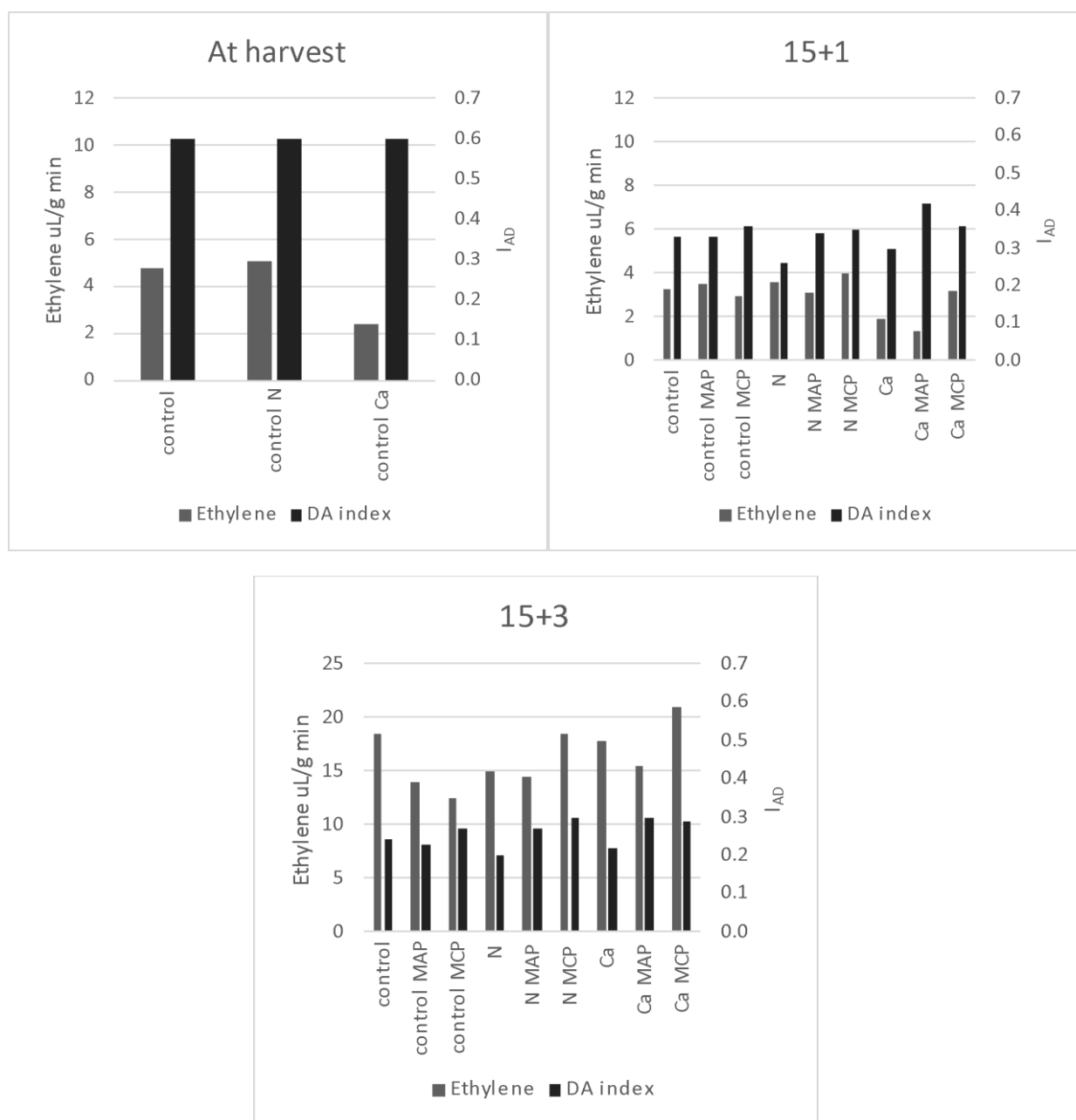


Figure 5. Ethylene and  $I_{AD}$  index at harvest, after storage and after shelf life of apricots 'NS6'

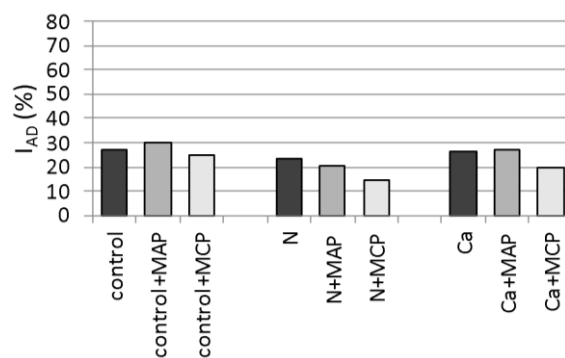


Figure 6. Decrease of  $I_{AD}$  value (%) during shelf life of apricots 'NS6'

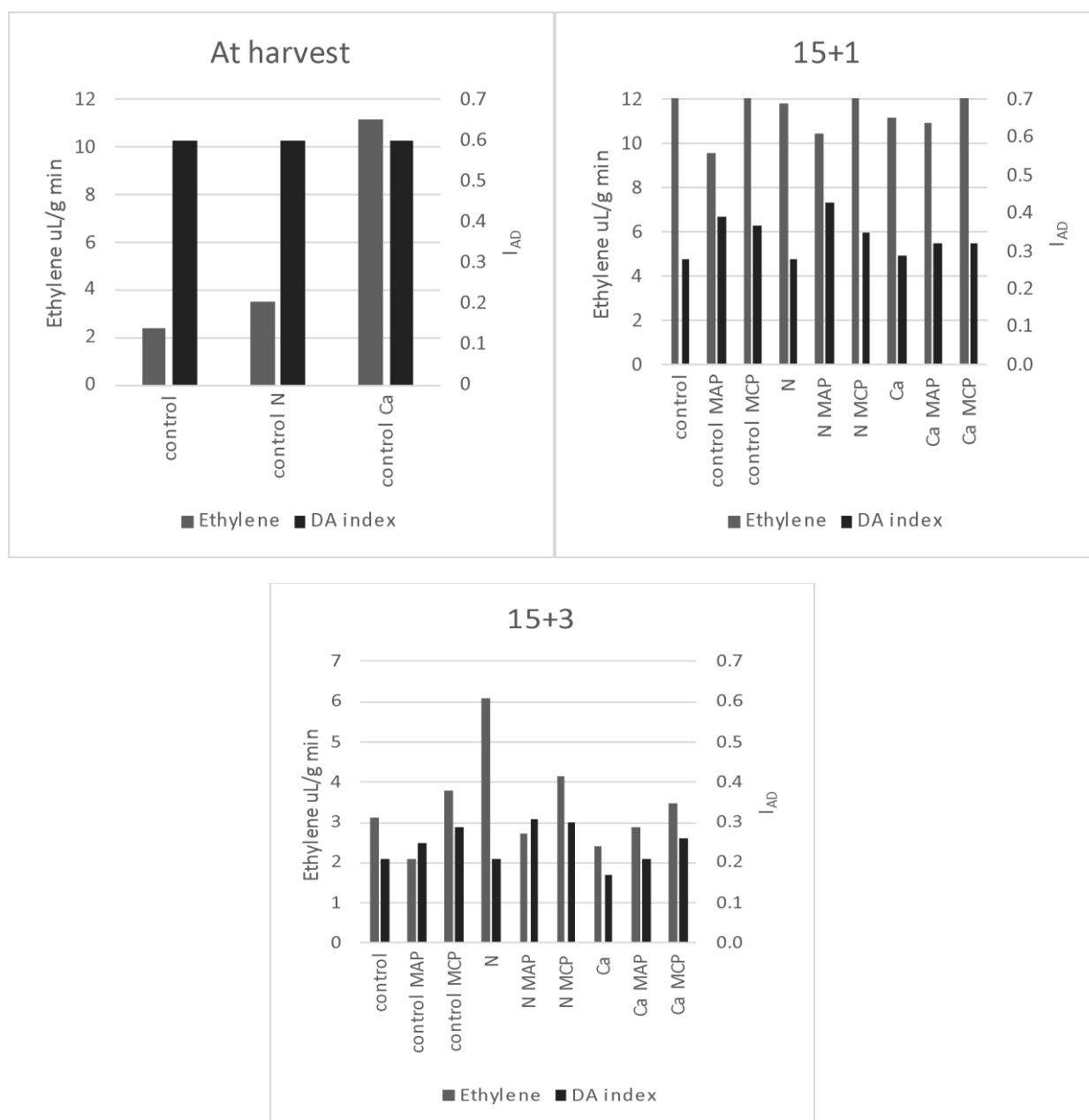


Figure 7. Ethylene and  $I_{AD}$  index at harvest, after storage and after shelf life of apricots 'NS Rodna'

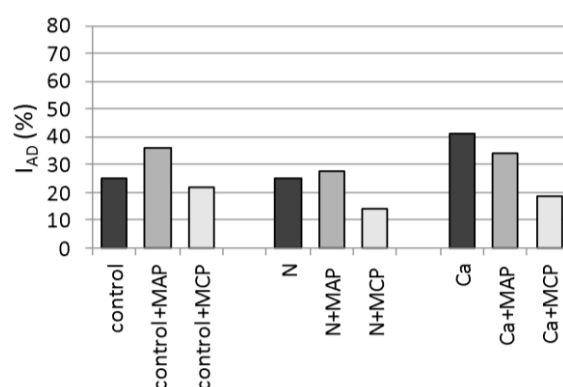


Figure 8. Decrease of  $I_{AD}$  value (%) during shelf life of apricots 'NS Rodna'



MAP and MCP. In contrast, compared to the control, Ca treatment showed more pronounced ethylene suppression in 'Buda', 'NS6' and 'NS Rodna' after cold storage. However, the effect of Ca varied significantly depending on the cultivar and the length of storage.

Comparatively, 'NS Kasnocvetna' and 'NS Rodna' exhibited a stronger ripening response to N treatments, reflected in higher ethylene production, while 'Buda' and 'NS6' were more responsive to Ca and showed better preservation of I<sub>AD</sub> when postharvest treatments were applied.

## CONCLUSIONS

The interrelation between I<sub>AD</sub> and ethylene production regarding different apricot cultivars highlighted the distinct ripening patterns influenced by both pre- and postharvest treatments. Ethylene, a key ripening hormone, typically accelerates the decline in I<sub>AD</sub>. However, the rate of this interaction varies across cultivars. In 'Buda' ethylene production rapidly increased, causing a sharper drop in I<sub>AD</sub>, whereas in 'NS6' and 'NS Rodna', lower ethylene levels corresponded with better I<sub>AD</sub> preservation, particularly under MAP and Ca treatments. Given these differences, the need for I<sub>AD</sub> calibration for each cultivar is evident. Determining the optimal I<sub>AD</sub> index for each cultivar's specific ethylene dynamics allows for more accurate monitoring of firmness and ripeness, ensuring that treatment strategies are tailored to each cultivar's unique ripening behavior.

## AUTHOR CONTRIBUTIONS

Conceptualization, N.M., B.M., J.M.; Methodology, N.M., B.M., Ž.K.; Investigation, M.M., J.K., G.B.; formal analysis, validation, Ž.K., R.K.; writing-original draft preparation, M.M., J.K., R.K., G.B., Ž.K.; Writing-review and editing, Ž.K., M.M., R.K., J.K.; Supervision, N.M., B.M.

## DATA AVAILABILITY STATEMENT

Data contained within the article.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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## UTICAJ TRETMANA N, Ca, MAP I 1-MCP NA INTERAKCIJU ETILENA I $I_{AD}$ TOKOM SKLADIŠTENJA I ČUVANJA PLODOVA KAJSIJE NA SOBNOJ TEMPERATURI

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**Sažetak:** Cilj ove studije je ispitivanje međusobne povezanosti između sinteze etilena, indeksa AD ( $I_{AD}$ ) i tretmana skladištenja kod četiri sorte kajsije (*Prunus armeniaca* L.): ‘Buda’, ‘NS Kasnocvetna’, ‘NS Rodna’ i ‘NS6’. Plodovi kajsije su ubrani sa vrednostima  $I_{AD}$  između 0.41–0.80, skladišteni na 1 °C tokom 15 dana, nakon čega su proveli 3 dana na sobnoj temperaturi. Sinteza etilena i  $I_{AD}$  mereni su pri berbi, nakon skladištenja i nakon tri dana čuvanja na sobnoj temperaturi. Tretmani pre berbe uključivali su primenu azota (N) i kalcijuma (Ca), dok su tretmani nakon berbe obuhvatali pakovanje u modifikovanj atmosferi (MAP) i primenu 1-metilciklopropena (MCP). Rezultati su pokazali specifične odgovore sortimenta sa aspekta sinteze etilena i vrednosti  $I_{AD}$ , pri čemu je sorta ‘Buda’ imala najbrži pad  $I_{AD}$  i najviši nivo sinteze etilena, dok je sorta ‘NS6’ imala manji pad vrednosti  $I_{AD}$ , posebno pod uticajem MAP i Ca tretmana. MCP je efikasno smanjio proizvodnju etilena i pad  $I_{AD}$  kod svih sorti. Nalazi naglašavaju potrebu za sortno specifičnom kalibracijom  $I_{AD}$  indeksa kako bi se optimizovala bolja manipulacija plodovima kajsija određenog stepena zrelosti, a time i održao njihov sveukupni kvalitet i skladišna sposobnost.

‘NS Rodna’

**Ključne reči:** koštičavo voće, hladno skladištenje, DA meter, folijarni tretmani

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