THE AROMA PROFILE OF APPLES AS INFLUENCED BY 1-MCP

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ABSTRACT

Treatment with 1-methylcyclopropene (1-MCP), an ethylene inhibitor, considerably prolongs the storage and shelf life of apples, and diminishes the occurrence of some physiological diseases, such as scald. Various fruit quality parameters define fruit acceptance by consumers, while some also determine the storability of fruits, including firmness, acidity and internal ethylene concentration. The aroma of apples is regarded as one of the most important quality parameters. Many factors influence the aroma profile, with ethylene having a key role in the biosynthesis of flavour compounds. 1-MCP is an inhibitor of ethylene synthesis and is now widely used. We tested apples of the three main cultivars grown in Slovenia (‘Idared’, ‘Jonagold’ and ‘Golden Delicious’) following their treatment with 1 µl l^{-1} 1-MCP at +1 °C, with 6 months of storage under standard ultra-low oxygen storage conditions (1.2% O_2, 1.5% CO_2). The fruits were analysed for firmness and ground colour, with headspace analyses of the aroma compounds, before and after storage. Compared with the control fruits, 1-MCP-treated apples had significantly improved firmness and colour parameters (L*, a*, b*), but showed strong inhibition of total aroma production. Among the aroma compounds, the esters were most severely suppressed by the treatment with 1-MCP, with the exception of 2-methylethyl acetate. In general, 1-MCP positively affected most of the quality parameters (fruit firmness, ground colour, acidity), but greatly suppressed the synthesis of the aroma volatiles.

Key words: apple, 1-MCP, aroma compounds, quality parameters, fruit firmness, ground colour

INTRODUCTION

Fruit is an important source of nutritionally important constituents, like vitamins, minerals, dietary fibre and antioxidants (Kader, 2008). Among the various fruits, the apple has a long been regarded as a healthy food due to various cholesterol-lowering compounds, antioxidants, anti-inflammatory substances, blood-pressure-lowering (hypotensive) agents, anti-clotting elements, and cancer prevention constituents. Providing high quality, better-flavoured apples at affordable prices is a key to increase their consumption. Fruit quality parameters, including firmness, juiciness, sugar-/acid ratio, flavour composition, appearance and colour, define fruit acceptance by consumers. From the point of view of the consumer, the aroma of apples is regarded as one of the most important quality parameters. Thus, the fruit flavour depends upon both taste and aroma, with the latter defined by the concentration of odour-active volatile compounds.

Among the sensory attributes, plant breeders have focused more on textural quality, juiciness, turgidity and crispness (Vicente et al., 2006; 2007). The perception of flavour is rather complex, and more sensory parameters are involved. In the past, little or no attention was given to the flavour quality, mainly because many odour-active compounds are involved, but also because other parameters like juiciness, crispness and turgidity influence flavour perception. Among pre-harvest factors, all agricultural practices that are aimed at increasing yield usually result in less than optimal flavour quality (Kader, 2008). Similarly, breeders have given their highest priorities to yield, fruit size and shelf-life, all of which are factors that negatively influence fruit flavour. However, according to Baldwin (2008), the bottom line for flavour quality is still genetic.

The maturity stage at harvest is a factor that, after the genotype, most severely influences flavour quality. Synthesis of aroma volatiles increases during maturation, although apples are usually harvested before reaching full ripeness. The main reason are the higher prices at the beginning, but they also have better storability and a longer shelf-life. On the other hand, when apples are harvested at a pre-climacteric stage, they never reach good eating quality (Fellman et al., 2003). Furthermore, the production of aroma volatiles increases during on-tree ripening because of the greater availability of substrates; thus, early-harvested fruits are always poor in aroma compounds (Villatoro et al., 2007).

Taking into consideration the post-harvest factors, most efforts have been dedicated to reducing the quantitative losses and maintaining the appearance of fruit and vegetables. Ethylene is one of several plant-growth regulators that affect the growth and development processes, including ripening and senescence. The blocking of ethylene synthesis or perception with the aim of extending the shelf-life results in a lower production of aroma compounds

(Fellman et al., 2000). According to Defilippi et al. (2005); the enzyme alcohol acyltransferase is under ethylene regulation and is involved in ester formation.

Of the ethylene inhibitors, 1-methylcyclopropene (1-MCP) is widely used on ornamental plants and edible horticultural products. It provides the potential to maintain fruit and vegetable quality after harvest. 1-MCP is believed to interact with ethylene receptors, and to thereby prevent ethylene-dependent responses (Sisler and Serek, 1997). 1-MCP dramatically inhibits the ripening of apples, the extent of the inhibition being related to cultivar, storage type and duration of storage (Fan et al., 1999; Defilippi et al., 2004). Inhibition by 1-MCP results in reductions in the internal ethylene concentrations, respiration and softening rate. Apples treated with 1-MCP are firmer, maintain a greener ground colour, and preserve better acidity compared with control fruit.

As to the negative effects of 1-MCP, they relate mainly to flavour, which is usually poor in apples treated with 1-MCP. Numerous studies (Fan and Mattheis, 1999; Fan et al., 2001; Saftner et al., 2003; Defilippi et al., 2004) have indicated reduced production of aroma volatiles by 1-MCP-treated apples, compared with untreated apples stored under similar conditions. Fan and Mattheis (2001) showed that there was inhibition of esters and alcohols biosynthesis by 1-MCP. Not only have the effects of 1-MCP treatment on the physiological/ chemical properties of apples been established analytically, but also the sensory characteristics of 1-MCP-treated apples have been investigated. In a study by Lurie et al. (2002), members of the tasting panel were indeed able to distinguish the differences in aroma between apples treated with 1 $\mu l\ l^{-1}$ 1-MCP and the control fruit; however, while the overall intensity of the fruity aroma of the control fruit increased, the panellists still preferred the 1-MCP-treated fruit over the control fruit (Lurie et al., 2002).

In a study by Moya-Leon et al. (2007), both a controlled atmosphere and treatment with 1-MCP were shown to inhibit the aroma volatiles of ‘Gala’ apples, although the fruit retained their firmness. Furthermore, good correlation was found between the analytically determined fruit volatiles and the aroma intensity perceived by members of a tasting panel, although, again, the panellists preferred the 1-MCP-treated and controlled-atmosphere-stored fruit (Moya-Leon et al., 2007). Panellists’ decisions are attributed mostly to greater fruit firmness. Marin et al. (2009) found no significant differences when they used sensorial tests for treated and non-treated ‘Gala’, ’Fuji’ and ‘Red Delicious’ apples.

MATERIAL AND METHODS

1-MCP treatment

‘Idared’, ‘Jonagold’ and ‘Golden Delicious’ apples were harvested at their commercial maturity stage for

Table 1. Maturity parameters (firmness and starch index) of ‘Idared’, ‘Jonagold’ and ‘Golden Delicious’ apples determined at picking time

Parameter	Idared	Jonagold	Golden Delicious
Firmness [kg]	8.23±037	8.12±0.32	7.95±0.41
Starch index (1-10)	5.7±0.43	7.5±0.63	6.2±0.48

long-term storage. The maturity stage was determined on the basis of firmness and starch content (Tab. 1). Some of the apples were left untreated (control) while others were treated with 1 µl l^{-1} 1-MCP in a commercial storage house and stored for 6 months under ultra-low oxygen conditions (1.2% O_2, 1.2% CO_2) at +1 °C. The control samples were stored under the same conditions. After 6 months of storage, 5 randomly selected apples of each cultivar and from both treatments were transferred to standard shelf-life test conditions (7 days, 20 °C, normal atmosphere) before analyses of aroma volatiles were performed.

Volatiles analysis

The analyses were carried out with a gas chromatograph (6890N, Agilent Technologies, USA) equipped with an autosampler (MPS2, Multipurpose Sampler, Gerstel, Germany) and mass ion-selective detector (Hewlett-Packard 5971A, Palo Alto, CA, USA). The gas chromatograph was fitted with a ZB-WAX capillary column, 60 m x 0.32 mm x 0.5 µm (Phenomenex, USA). He 6.0 was used as carrier gas, with a flow rate of 1.2 ml $min.^{-1}$ at 40 °C. The apple aroma volatiles were sampled according to the method described by Jia (2005), but slightly modified. Five apples were enclosed in a glass jar (2.5 l) for 30 min. at 23 °C; aroma volatiles were sampled manually by means of a solid-phase micro-extraction fibre with a carbowax-/polydimethylsiloxane coating (CW-/PDMS, StableFlex, 85 µm thickness). For thermal desorption, the solid-phase micro-extraction fibre remained in the injector for 5 min. The temperature of the injector was set at 270 °C. The oven temperature programme was: 5 min. at 40 °C, then rise from 40 °C to 230 °C at 4 °C $min.^{-1}$, and remain for 5 min. at 230 °C. The mass selective detector was operated at 70 eV, with electron impact ionisation. The transfer line was set to a temperature of 280 °C. Mass spectra were acquired in full-scan mode (30-300 m/z). The peaks were identified by comparing experimental spectra with those in the National Institute for Standards and Technology (USA) database. The relative concentrations of volatiles in the headspace were estimated by comparing the peak areas of the volatiles with that of the external standard, 6-methyl-5-hepten-2-one. In order to check the absorption capacity of each fibre, 10 ml of external standard solution (0.109 µg ml^{-1}) was placed in a 20 ml vial. The fibre was then exposed to the standard headspace for 30 min at 30 °C. The quantification of aroma compounds was carried out using the external standard method.

RESULTS AND DISCUSSION

1-MCP is a well-known fruit-ripening inhibitor that blocks ethylene receptors. It successfully delays the softening of fruit and their colour development, organic acid degradation, and the synthesis of aroma compounds. Aroma compounds are one of the factors that determine apple fruit quality, and therefore affect consumer acceptance of the fruit. According to recent investigations, consumer preference for apples is focused on three characteristics: aroma, firmness and juiciness. Fruit firmness appeared to be cultivar- and treatment-dependent; 1-MCP-treated apples retained their firmness much better compared with the controls. The changes in ground colour were similar. During ripening, the green colour slowly changed until a yellow colour prevailed. The development of the yellow colour was strongly suppressed by the action of 1-MCP. The colour parameters L*, a* and b* showed significant differences between 1-MCP-treated and the control fruit; as to their ground colour, the fruits were later darker, greener and less yellow. The exception was the cultivar 'Idared', which is completely red, so it does not show the same colour development as 'Jonagold' or 'Golden Delicious', both of which are green in colour when immature. These results suggest that aroma compounds are produced in lower amounts by 1-MCP-treated apples compared with the untreated controls.

The aroma profile of apples is characterised by numerous compounds, ten of which were evaluated in this study. The cultivar 'Idared' is regarded as non-aromatic, and the results show that these apples contained half of the total amount of aroma compounds compared with 'Jonagold' and 'Golden Delicious'. The last two cultivars share a very similar aroma profile, which is due to their relationship as 'Jonagold' is a progeny of 'Golden Delicious'.

As can be seen in Table 2, the apple cultivar had a significant effect on fruit firmness and the colour parameters (L*, b*). As to the aroma compounds, most of those appeared to be cultivar-dependent, with the only exception of 3-methybutyl butanoate. Treatment with 1-MCP significantly affected most of the measured parameters, with only the concentration of 3-methybutyl butanoate not influenced significantly by the treatment. In our study, the cultivar × treatment interaction was significant for most of the measured parameters. The exceptions were the colour parameter L* and the aroma compounds, including butyl acetate, 3-methybutyl butanoate and 6-methyl-5-hepten-2-one.

When the action of ethylene was reduced by 1-MCP, this resulted in the corresponding changes in firmness and external colour parameters. A marked reduction in the softening was seen in the apples treated with 1-MCP. The differences between the control apples and those treated with 1-MCP were statistically significant in all three cultivars (Tab. 3). This data confirm observations from other studies (Fan et al., 1999; Defilippi

Table 2. Sources of variability and the statistical significance of their influence on the cultivar and treatment, and on the cultivar×treatment interaction

Parameter	Source of variability (p value)		
	cultivar	treatment	cultivar×treatment
Firmness	< 0.0001	< 0.0001	< 0.0001
Colour parameters			
L*	0.0435	0.0027	0.1986
a*	0.1006	0.0081	0.0078
b*	< 0.0001	0.0004	0.0156
Butyl acetate	0.0227	0.0002	0.0860
Butyl butanoate	< 0.0001	< 0.0001	< 0.0001
3-Methybutyl butanoate	0.2875	0.4442	0.3799
Hexyl acetate	0.0165	< 0.0001	0.0483
Hexyl propanoate	0.0027	< 0.0001	0.0022
6-Methyl-5hepten-2-one	0.0073	0.0167	0.1044
1-Hexanol	0.0002	< 0.0001	< 0.0001
Butyl hexanoate	< 0.0001	< 0.0001	< 0.0001
Hexyl butanoate	< 0.0001	< 0.0001	< 0.0001
Hexyl 2-methylbutanoate	< 0.0001	< 0.0001	< 0.0001

Table 3. Comparison of firmness and colour parameters (L, a, b) of the control and 1-MCP-treated 'Idared', 'Jonagold' and 'Golden Delicious' apples

Parameter	Idared		Jonagold		Golden Delicious	
	control	1-MCP	control	1-MCP	control	1-MCP
Firmness	5.27 ±0.36b*	6.3 5±0.39a	3.04 ±0.16b	6.69 ±0.28a	3.92 ±0.33b	6.30 ±0.52a
L*	68.05a	66.80a	68.96a	62.46b	71.47a	67.52b
a*	-9.55a	-8.12a	-8.73a	-11.81b	-5.34a	-10.78b
b*	30.24a	30.29a	34.34a	28.86b	37.27a	33.51b

*Values are means ±SD of 5 replicates. Means followed by different letters in the same row for each cultivar are significantly different at p = 0.05

et al., 2004; Vicente et al., 2006). As to the colour parameter L*, significantly lower values were obtained for 1-MCP-treated 'Jonagold' and 'Golden Delicious' apples. The L* value of 'Idared' was similar because of the intense red colour, while in the case of 'Jonagold' and 'Golden Delicious', the colour measurements were not performed on red-coloured surfaces. The a* value, which represents the red or green colour, was lower in 1-MCP-treated 'Jonagold' and 'Golden Delicious' apples, confirming the slower de-greening of 1-MCP-treated fruit; again, no significant differences were seen for 'Idared' apples. Statistically higher b* values were seen in the non-treated fruits, suggesting that a more intense yellow colour developed in those fruits. Similar colour developments following 1-MCP treatment were seen by Defilippi et al. (2004). As reported by McGuire (1992), a statistical analysis of the colour parameters a* and b* may yield significant results without any practical meaning. In our case, all three colour parameters showed significant differences for 'Jonagold' and 'Golden Delicious' apples, but not for 'Idared' apples. It has to be mentioned, that the differences in ground colour (green to yellow) were also visually perceived.

Table 4 summarises the results for the aroma volatiles produced by control and 1-MCP-treated 'Idared', 'Jonagold' and 'Golden Delicious' apples. Over 300 aroma compounds have been identified in apples (Dimick and Hoskin, 1983), although the three esters: butyl acetate, 2-methylbutyl acetate, and hexyl acetate are considered the most important for the characteristic apple-like aroma (Fellman et al., 2000). As can be seen from Table 2, synthesis of the aroma volatiles was cultivar-dependent for most of the compounds. The cultivar 'Idared' is considered non-aromatic; in our study, the 'Idared' apples produced the lowest total aroma volatiles compared with 'Jonagold' and 'Golden Delicious' apples. The latter two are regarded as aromatic and they can produce on average twice as much total aroma volatiles as 'Idared'. 'Jonagold' is a progeny of 'Golden Delicious' (a cross between 'Golden Delicious' and 'Jonathan'), which seems to be the main reason why these two cultivars have similar aroma profiles. The most abundant aroma compounds in both of these cultivars were hexyl propanoate, butyl butanoate, hexyl acetate, butyl hexanoate and hexyl 2-methyl-butanoate. The same principal aroma compounds in 'Golden Delicious' apples were found by Kondo et al. (2005) and by Ferenczi et al. (2006), although with slightly different ratios between them. In 'Idared', the most abundant aroma compound was hexyl acetate, followed by butyl acetate and hexyl 2-methyl butanoate. While the levels of butyl butanoate and hexyl propanoate were considerably higher in 'Jonagold' and 'Golden Delicious', both of these compounds were found in much lower concentrations in 'Idared'. The majority of the aroma compounds were found in significantly lower concentrations in 1-MCP-treated fruits compared with the controls. Our findings are in accordance with those of Mattheis et al. (2005), who showed reduced levels of total volatiles in 1-MCP-treated 'Gala' apples. Our result prove that through its action on ethylene perception, 1-MCP delays the onset of aroma volatiles production and permanently suppresses the maximal rate of the synthesis of aroma compounds (Fernczi et al., 2006). A severe reduction in aroma compounds by 1-MCP may negatively impact on the sensory characteristics of apples, as demonstrated by other authors (Lurie et al., 2002; Moya-Leon et al., 2007; Marin et al., 2009).

T a b l e 4 . Comparison of relative concentrations (mg l^{-1}) of volatile compounds produced by the control and 1-MCP-treated 'Idared', 'Jonagold' and 'Golden Delicious' apples

Compound	Idared		Jonagold		Golden Delicious	
	control	1-MCP	control	1-MCP	control	1-MCP
Butyl acetate	112.25a	29.32b	65.50a	1.26b	42.79a	28.36a
Butyl butanoate	2.89a	0.52b	60.08a	0.29b	92.76a	5.98b
3-Methybutyl butanoate	0.00a	5.15a	0.00a	0.00a	4.75a	3.27a
Hexyl acetate	117.54a	35.86b	87.32a	9.22b	58.97a	32.04a
Hexyl propanoate	9.28a	2.91b	126.52a	0.98b	110.55a	3.30a
6-Methyl-5hepten-2-one	0.75a	0.58a	1.50a	1.32a	1.93a	0.71b
1-Hexanol	13.23a	7.28a	37.95a	1.49b	12.29a	2.94a
Butyl hexanoate	2.19a	0.39b	34.51a	0.56b	71.94a	6.93b
Hexyl butanoate	1.48a	0.39b	25.69a	0.56b	36.92a	3.09a
Hexyl 2-methylbutanoate	28.99a	5.82b	74.64a	2.94b	31.90a	10.30a
Sum of aroma volatiles	288.6a	88.22b	513.71a	18.62b	464.8a	96.92b

*Explanation, see Table 3

T a b l e 5 . Percentage share of aroma compounds (% of total compounds) produced by the control and 1-MCP-treated 'Idared', 'Jonagold' and 'Golden Delicious' apples

Compound	Idared		Jonagold		Golden Delicious	
	control	1-MCP	control	1-MCP	control	1-MCP
Butyl acetate	38.89	33.24	12.75	6.77	9.21	29.26
Butyl butanoate	1.00	0.59	11.70	1.56	19.96	6.17
3-Methybutyl butanoate	0.00	5.84	0.00	0.00	1.02	3.37
Hexyl acetate	40.73	40.65	17.00	49.52	12.69	33.06
Hexyl propanoate	3.22	3.30	24.63	5.26	23.78	3.40
6-Methyl-5hepten-2-one	0.26	0.66	0.29	7.09	0.42	0.73
1-Hexanol	4.58	8.25	7.39	8.00	2.64	3.03
Butyl hexanoate	0.76	0.44	6.72	3.01	15.48	7.15
Hexyl butanoate	0.51	0.44	5.00	3.01	7.94	3.19
Hexyl 2-methylbutanoate	10.05	6.60	14.53	15.79	6.86	10.63
Sum of aroma volatiles	100	100	100	100	100	100

The relative proportions of the volatile compounds were different in the control and 1-MCP-treated fruits (Tab. 5). In general, the percentage of the compounds with a fruity character was lower in the 1-MCP-treated fruits compared with the control fruits. The exception was the cultivar 'Idared', which is regarded as non-aromatic. The relative proportions of the aroma compounds in 'Idared' do not differ as much as in the aromatic cultivars 'Jonagold' and 'Golden Delicious'. The percentages for hexyl acetate in the control and treated fruits were similar in 'Idared' but considerably higher in 1-MCP-treated 'Jonagold' and 'Golden Delicious' apples. When evaluated sensorially, hexyl acetate gave a fruity, green apple and sour character. The expected consequence of increased ratios of hexyl acetate is that 1-MCP-treated fruits will have a less fruity, but "more green" character. Another compound that is perceptedas "green" is 1-hexanol, which is also more abundant in 1-MCP-treated apples. Hexyl 2-methylbutanoate is a compound associated with "green", "waxy", "fruity", apple, spicy and tropical aroma, and was found in greater amounts in 1-MCP-treated 'Jonagold' and 'Golden Delicious' apples. The above-mentioned ratios of some of those key compounds were responsible for the "more green" overall sensory impressions of the 1-MCP-treated apples.

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WPŁYW 1-METYLOCYKLOPROPENU NA PROFIL ZAPACHOWY JABŁEK

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STRESZCZENIE

Traktowanie jabłek 1-metylocyklopropenem (1-MCP), inhibitorem etylenu, znacznie wydłuża czas ich przechowywania i trwałość oraz zmniejsza występowanie niektórych chorób fizjologicznych, takich jak oparzelizna powierzchniowa. Różne cechy jakościowe mają wpływ na akceptację jabłek przez konsumentów, a niektóre, takie jak jędrność, kwasowość i wewnętrzne stężenie etylenu, decydują również o ich zdolności przechowalniczej. Aromat jabłek uważany jest za jedną z najważniejszych cech jakościowych. Wiele czynników wpływa na profil zapachowy, przy czym etylen odgrywa główną rolę w regulacji biosyntezy związków smakowych. 1-MCP jest inhibitorem syntezy etylenu i jest obecnie szeroko stosowany. W pracy przetestowano jabłka trzech głównych odmian uprawianych w Słowenii ('Idared', 'Jonagold' i 'Golden Delicious') po potraktowaniu ich 1 µl l^{-1} 1-MCP w temp. +2 °C i przechowywaniu przez 6 miesięcy w warunkach bardzo niskiego stężenia tlenu (1.2% O_2, 1.5% CO_2). Owoce oceniano pod względem jędrności i barwy zasadniczej, a także analizowano lotne związki zapachowe wydzielane przez jabłka przed i po przechowywaniu. W porównaniu z kontrolą jabłka traktowane 1-MCP miały istotnie wyższą jędrność i lepsze parametry zabarwienia (L*, a*, b*), ale ogólnie wytwarzanie aromatu było silnie zahamowane. Traktowanie 1-MCP najsilniej ograniczało wydzielanie estrów, z wyjątkiem octanu 2-metylo-etylowego.

Słowa kluczowe: jabłka, 1-MCP, związki zapachowe, cechy jakościowe, jędrność owocu, barwa zasadnicza