University of Reading

Influence of harvest maturity on the aroma quality of two celery (Apium graveolens) genotypes

Article

Accepted Version

Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0

Turner, L., Dawda, D., Wagstaff, C. ORCID: https://orcid.org/0000-0001-9400-8641, Gawthrop, F. and Lignou, S. ORCID: https://orcid.org/0000-0001-6971-2258 (2021) Influence of harvest maturity on the aroma quality of two celery (Apium graveolens) genotypes. Food Chemistry, 365. 130515. ISSN 0308-8146 doi: 10.1016/j.foodchem.2021.130515 Available at https://centaur.reading.ac.uk/98987/

It is advisable to refer to the publisher's version if you intend to cite from the work. See Guidance on citing.
Published version at: https://doi.org/10.1016/j.foodchem.2021.130515
To link to this article DOI: http://dx.doi.org/10.1016/j.foodchem.2021.130515

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

University of Reading

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Influence of harvest maturity on the aroma quality of two celery (*Apium graveolens*) genotypes

Lucy TURNER[1], Dhriti DAWDA[1], Carol WAGSTAFF[1], Frances GAWTHROP[2], Stella LIGNOU[1]

[1]Department of Food and Nutritional Sciences, University of Reading, Harry Nursten Building, Pepper Lane, Whiteknights, Reading, RG6 6DZ.

[2]A.L. Tozer Ltd, Pyports, Downside Bridge Road, Cobham, KT11 3EH.

Email:

Lucy TURNER (l.turner@pgr.reading.ac.uk)

Dhriti DAWDA (dhritidtz@gmail.com)

Stella LIGNOU (s.lignou@reading.ac.uk)

Frances GAWTHROP (frances.gawthrop@tozerseeds.com)

Carol WAGSTAFF (c.wagstaff@reading.ac.uk)

*Corresponding author: Dr Stella Lignou

Phone number: +44 (0) 118 378 8717

Highlights

- Genotype determined the crop's ability to maintain optimal aroma profile
- Maturity had a stronger influence over different compound groups than genotype
- Monoterpenes, sesquiterpenes and phthalides are key compound groups to celery aroma
- Odours produced during the maturity process are determined strongly by genotype

Abstract

Celery is a fibrous horticultural vegetable grown globally and widely consumed due to its health benefits, distinct flavours and culinary versatility. Currently, few datasets examine its aroma development across maturity which could help guide growers towards optimising harvest times whilst identifying potential consequences of harvesting outside commercial maturity. Freeze-dried celery of two genotypes, selected for biochemical and sensory differences, were harvested at three time-points and investigated using solid-phase microextraction gas chromatography/mass spectrometry (SPME GC/MS) and gas chromatography/olfactometry (GC/O). Both maturity and genotype showed significant (P<0.05) interactions between compounds, and harvest stage exhibited greater impact upon aroma quality than plant genotype. Thus, indicating that agronomic practice is key in determining crop quality. Monoterpenes, sesquiterpenes and phthalides begun to decrease once commercial maturity was reached, whereas alcohols were more prominent in post-mature celery. GC/O results confirmed the importance of phthalides to mature celery aroma and aroma differences caused by genotype.

Key words: Celery, aroma, volatile compounds, GC-O, SPME GC-MS, maturity, phthalides, terpenes

1. *Introduction*

Apium graveolens, is a popular biennial crop that is grown and consumed globally; in salads as a raw ingredient or in cooking, whereby it forms the base of many soups, stocks and sauces (Rozėk, 2007). Celery has a distinct flavour profile that has been investigated extensively, with studies looking at the aroma profile of various cultivars in a variety of forms, such as fresh, dried or as an essential oil. Regardless of the material under investigation, a wide

range of compounds that contribute to its strong flavour, including alcohols, aldehydes, monoterpenes, sesquiterpenes and phthalides have been identified (Gold & Wilson, 1963; van Wassenhove, Dirinck, Vulsteke & Schamp, 1990). The latter are seen as characteristic compounds. Phthalides are mainly found in members of the Apiaceace family, predominantly *Ligusticum* and *Angelica* (Karmakar, Pahari, & Mal, 2014). Phthalides including 3-*n*-butylphthalide, sedanenolide and *cis* and *trans*- ligustilide have been identified in celery, possessing odour descriptors such as "celery", "herbal" and "green" (Macleod & Ames, 1989; Kurobayashi, Kouno, Fujita, Morimitsu & Kubota, 2006).

Sellami, Bettaieb, Bourgou, Dahmani, Limam & Marzouk (2012) identified more than 25 volatile compounds in the roots, petioles and leaves of celery in the form of essential oil. Although more compounds were identified in the roots, the leaves exhibited a high concentration of aroma compounds, including phthalides. Similarly, Kurobayashi et al. (2006) utilised a combination of analytical techniques including gas chromatography olfactometry (GC/O) to analyse the odorants that characterise the aroma in raw and boiled celery and identified a much higher proportion of phthalides in the leaves rather than the petioles. Using GC/O, Kurobayashi et al. (2006) stated that sedanenolide, 3-*n*-butylphthalide and *cis*- and *trans*-sedanolide were the most distinguishing components of the celery aroma and through aroma extract dilution analysis (AEDA) quantified these compounds (3,200, 140 and 78 µg/kg respectively) to be the most abundant odour active compounds in raw celery petioles. Through sensory analysis and GC/O, these compounds were found to contribute odour characteristics such as "fragrant", "green" and "spicy" to celery.

Being such a widely consumed horticultural crop, research into the development across maturity of these key odour active compounds with celery is unexpectedly low. Yommi, Di Gerónimo, Carrozzi, Quillehauquy, Goñi & Roura (2013) monitored the quality changes (structural and textural) of self-blanching celery every seven days from day 80 (after

transplanting) until day 129. It was concluded that the optimum yield and quality balance of the cultivar was attained at 122 days after transplanting, noting that a later harvest was strongly associated with lower quality due to textural changes. Ultimately, there has been inadequate focus on the internal quality aspects of celery during maturity and a possibility for this could simply be the flavour attribute labelled as 'characteristic flavour' as used by Yommi et al. (2013). This is not an appropriate descriptor as the flavour profile is more complex than this. Furthermore, a more analytical method such as solid-phase microextraction (SPME) or solvent-assisted flavour extraction (SAFE) could be required to monitor changes in the volatile content across maturity.

While quality standards are usually based on visual evaluation (petiole shape, appearance, health) (Raffo, Sinesio, Moneta, Nardo, Peparaio & Paoletti, 2006), it can be argued that aroma and, therefore, flavour are attributes that should be considered when determining quality, as these also play an important role in consumer product acceptance. The purpose of this study was to investigate the development of aroma over maturity by utilising two different genotypes of *A. graveolens*, harvested at three different time points during plant development. The relationship between genotype and odour as well as maturity and odour were investigated using SPME and gas chromatography/mass spectrometry (GC/MS) and GC/O. From this, time points during maturation when key families of compounds were at their most abundant, such as monoterpenes that contribute fresh and citrus notes or phthalides that give the strong, characteristic herbal and celery odour could be recognised. Eventually, this could help guide the fresh produce industry to introduce more flavour variation for celery and other vegetable products.

2. *Materials & Methods*

2.1 Celery material and Minimum Information About a Plant Aroma Experiment (MIAPAE) standard[1]

2.1.1 Sample information

The two varieties used in this experiment were chosen due to their vast differences in physical and chemical attributes. Although commercial confidentiality precludes revealing the exact genetic identity of each line in this paper, the sensory properties of these genotypes can be revealed as these (along with others) were evaluated by the trained panel at the Sensory Science Centre (n = 12) (University of Reading, UK) using Quantitative Descriptive Analysis (QDA). Prior to GC/MS and GC/O analysis, celery material was freeze-dried to ensure consistent aroma quality throughout instrumental analysis.

The first genotype, coded as line 12, has United Kingdom origins. Green and pink in colour with long, narrow petioles and ribs that appear compact and very prominent (Supplementary data, Figure S1). This genotype is characterised by a fibrous physiology, revealing strings of vascular tissue when a petiole is snapped, and bitter tasting.

The second genotype, coded as line 22, has North American origins with light green, compact petioles (Supplementary data, Figure S2). This genotype had a more typical celery appearance and is less bitter than the line above. It is not stringy, and the petiole breaks cleanly in half when snapped.

2.1.2 Timing, Location, and Environment

Celery seed (*Apium graveolens*) of two parental lines supplied by Tozer Seeds Ltd (Pyports, United Kingdom) were grown in commercial conditions and harvested in Cambridgeshire,

[1] MIAPAE standards refer to Minimum Information About a Plant Aroma Experiment as described in Turner et al. (2021) Food Chemistry 345: 128673.

United Kingdom by G's Fresh Ltd (Barway, United Kingdom) (52°21'12.9"N 0°17'15.6"E) during spring/summer 2018. Celery was grown in a field with commercial celery products and treated to the same agronomic techniques and conditions as commercial celery.

Plants were transplanted after 26 days of growing in the nursery. The first harvest occurred on day 63 after transplanting, in late July 2018 (premature, M1), the second harvest occurred on day 76 after transplanting, in mid-August 2018 (mature, M2) and the final harvest occurred on day 89 after transplanting, in late August 2018 (post-mature, M3). Average climate conditions from day one of transplanting to day 89 after transplanting were as follows: air temperature was 18 °C, average soil temperature was 22 °C and average rainfall was 0.04 mm. 20 to 25 mm of overheard irrigation was used and standard commercial fertiliser, pest and disease control regimes were applied.

2.1.3 Raw material collection, processing and storage

Within the field, the celery was grown in three randomised blocks (10 plants m^{-2}) and were harvested using a celery knife. M1 celery were cut to 10 cm from the base, M2 and M3 were cut to 13 cm from the base, ensuring that no knuckles or leaves were included in the petiole cuttings. Three biological replicates were harvested from each block. Once cut, the petioles were sealed in labelled bags for immediate transportation to the University of Reading (United Kingdom). Celery for aroma analysis was frozen at -80 °C and freeze-dried for five days. Celery was then milled to a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ) and stored in an airtight container out of sunlight exposure at room temperature for a maximum of 2 weeks before instrumental analysis.

2.2 Chemical reagents

For GC/MS analysis, calcium chloride solution was prepared with HPLC-grade water and added to the sample with 100 ppm propyl propanoate in methanol, as the internal standard. For GC/O analysis, HPLC-grade water was used to rehydrate the samples and dry ice obtained from the University of Reading. The alkane standards C_6-C_{25} in diethyl ether was used for both GC/MS and GC/O analysis. All reagents were purchased from Sigma Aldrich (Gillingham, United Kingdom).

2.3 Solid-phase microextraction followed by GC/MS to identify changes in the aroma profile of different celery maturities and genotypes

Celery (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution and filled to 5 mL using HPLC-grade water with 50 µL of 100 ppm propyl propanoate (internal standard) in a 15 mL SPME vial fitted with a screw cap. Analysis was carried out by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA) as described by Turner, Lignou, Gawthrop & Wagstaff (2021).

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading), or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C_6–C_{25} *n*-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions. The approximate quantification (AU) of volatiles collected from the headspace were calculated from GC peak areas, by comparison with the peak area of the propyl propanoate standard.

2.4 Odour analysis using GC/O to identify changes in the perception of aroma compounds as celery matures

Celery (0.5 g) and 4.5 mL of HPLC grade water was placed in a SPME vial of 15 mL fitted with a screw cap. After equilibration at 37 °C for 10 min, the SPME device (divinylbenzene/Carboxen™ on polydimethylsiloxane) was exposed to the headspace above the sample for 30 minutes. After extraction, the SPME device was inserted into the injection port of an Agilent 7890B Series ODO 2 (SGE) GC/O (Agilent, Santa Clara, CA) system equipped with a HP-5MS column (30 m × 0.25 mm × 0.25 µm). The outlet was split between a flame ionisation detector and a humified sniffing port (1:1). The fibre contents were desorbed for 2 min onto five small loops of the column in a coil, which were cooled in solid carbon dioxide, contained within a 250 mL beaker. The injector and detector temperatures were maintained at 280 °C and 250 °C respectively. The oven was held at 40 °C during desorption. After desorption, the solid carbon dioxide was removed from the oven. The temperature program used was: 40 °C for 2 min isothermal, an increase of 4 °C/min to 200 °C, and an increase at 8 °C/min to 300 °C. Helium was the carrier gas with a flow rate of 2.0 mL/min. A standard of C_6–C_{25} *n*-alkanes was used to collect linear retention index (LRI) values.

Three assessors were used for the detection and verbal description of the aroma compounds. All assessors were subjected to multiple training sessions with different materials on the GC/O prior to scoring using celery material, accounting to seven hours in training. Two assessors were already considered to be well trained on the GC/O. Further training, including odour identification using 12 flavour compounds, threshold and discrimination tests using Sniffin' Sticks (Burghardt®, Wedel, Germany) were also completed prior to assessment. Assessors smelt each sample in duplicate and documented the odour description, time and odour intensity (OI) using a seven-point scale (2-8) where 3 = weak, 5 = medium and 7 =

strong. Each session lasted 40 min and assessors were advised to refrain from drinking coffee and eating at least 30 min before the scoring session.

2.5 Statistical analysis and data pre-treatment

Quantitative data from the SPME GC/MS analysis were analysed by both one- and two-way analysis of variance (ANOVA) and principal component analysis (PCA) following Spearman's correlation, using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine which sample means differed significantly ($P<0.05$) between harvest maturities and the celery parental lines. Only those compounds exhibiting significant differences between maturity, genotype and their interaction (maturity x genotype) were included in the principal component analysis plots.

207 Table 1 – Approximate quantities of volatile compounds identified in the headspace of celery using SPME GCMS harvested at three different maturity stages.
208

				Mean relative abundance (AU) [f]								
				Line 12			**Line 22**			**P-value [g]**		
Code	**Compound**	**LRI expt[a]**	**ID[b]**	**M1[c]**	**M2[d]**	**M3[e]**	**M1**	**M2**	**M3**	**M[h]**	**L[i]**	**MxL[j]**
	Alcohols											
A1	3-methyl-3-buten-1-ol	730	A	n.d. ^{C}	4.6±1.3 ^{A}	8.6±0.91 ^{A}	n.d. ^{C}	3.7±0.40 ^{B}	4.3±0.76 ^{B}	***	***	***
A2	1-pentanol	763	A	0.19±0.03 ^{E}	3.7±0.53 ^{BC}	2.5±0.24 ^{CD}	0.5±0.12 ^{E}	5.7±0.85 ^{AB}	7.9±1.7 ^{A}	***	***	***
A3	1-hepten-3-ol	893	A	n.d. ^{C}	n.d. ^{C}	1.7±0.10 ^{B}	n.d. ^{C}	n.d. ^{C}	5.2±0.45 ^{A}	***	***	***
A4	*(E)*-2-hexen-1-ol	867	A	0.37±0.02 ^{C}	n.d. ^{C}	4.5±0.50 ^{B}	0.68±0.12 ^{C}	n.d. ^{C}	8.1±0.88 ^{A}	***	***	***
A5	(*E*)-2-octen-1-ol	1069	A	n.d.	n.d.	1.8±1.8	n.d.	n.d.	1.7±1.2	***	ns	ns
A6	1-octanol	1073	A	1.5±0.30	n.d.	n.d.	1.8±0.27	n.d.	n.d.	***	*	ns
A7	1-nonanol	1176	A	6.0±1.7 ^{A}	4.1±0.59 ^{AB}	5.1±0.57 ^{AB}	2.1±0.57 ^{AB}	1.4±0.17 ^{B}	3.7±1.0 ^{AB}	***	***	**
A8	1-decanol	1272	A	n.d. ^{C}	2.9±0.64 ^{A}	n.d. ^{C}	n.d. ^{C}	1.6±0.39 ^{B}	n.d. ^{C}	***	*	*
A9	1-dodecanol	1469	A	1.1±0.16 ^{A}	n.d. ^{C}	0.63±0.16 ^{B}	0.65±0.10 ^{B}	n.d. ^{C}	0.83±0.18 ^{AB}	***	ns	**
	Total			**9.2**	**15.3**	**24.8**	**5.7**	**12.4**	**31.7**			
	Aldehydes											
AH1	*(E)*-2-pentenal	754	A	4.7±0.57 ^{C}	4.1±0.99 ^{C}	7.6±1.4 ^{BC}	6.5±2.4 ^{BC}	13.6±3.2 ^{A}	11.3±1.9 ^{AB}	*	***	*
AH2	hexanal	802	A	3.1±0.32 ^{B}	14.3±3.3 ^{B}	7.1±1.1 ^{B}	5.7 ±0.60 ^{B}	134±32.3 ^{A}	153±2.2 ^{A}	***	***	***
AH3	*(Z)*-2-hexenal	855	A	1.3±0.05 ^{B}	1.7±0.10 ^{BC}	n.d. ^{D}	0.39±0.07 ^{CD}	2.5±0.45 ^{A}	n.d. ^{D}	***	**	***
AH4	*(Z)*-4-heptenal	902	A	n.d.	4.1±0.61	n.d.	n.d.	3.7±0.91	n.d.	***	ns	ns
AH5	*n*-octanal	1007	A	8.9±0.47 ^{A}	5.1±1.1 ^{B}	4.9±0.96 ^{B}	4.0±0.72 ^{B}	5.6±1.2 ^{B}	4.3±0.54 ^{B}	*	**	***
AH6	benzeneacetaldehyde	1049	A	6.9±0.92 ^{BC}	4.4±0.57 ^{C}	4.5±0.25 ^{C}	15.8±2.4 ^{A}	8.4±1.9 ^{B}	3.8±0.33 ^{C}	***	***	***
AH7	2-hydroxybenzaldehyde	1056	A	n.d. ^{B}	n.d. ^{B}	4.8±0.05 ^{B}	n.d. ^{B}	n.d. ^{B}	34.6±6.3 ^{A}	***	***	***

AH8	*(E,Z)*-2,6-nonadienal	1156	A	2.1±0.38 ^{A}	n.d. ^{C}	n.d. ^{C}	1.0±0.23 ^{B}	n.d. ^{C}	n.d. ^{C}	***	***	***
AH9	*(E,E)*-2,4-nonadienal	1221	A	3.0±0.41 ^{A}	1.1±0.09 ^{C}	n.d. ^{D}	1.2±0.27 ^{BC}	0.44±0.28 ^{B}	n.d. ^{D}	***	**	*
	Total			**30**	**34.8**	**28.9**	**34.6**	**168.2**	**207**			
	Ketones											
K1	3-hexanone	779	A	n.d. ^{C}	n.d. ^{C}	1.3±0.12 ^{B}	n.d. ^{C}	n.d. ^{C}	2.1±0.45 ^{A}	***	***	**
K2	1-octen-3-one	978	A	n.d. ^{C}	n.d. ^{C}	6.7±1.3 ^{B}	n.d. ^{C}	n.d. ^{C}	4.7±1.0 ^{A}	***	ns	*
K3	2-nonanone	1090	A	2.4±0.14	n.d.	n.d.	1.6±0.51	n.d.	n.d.	***	ns	ns
	Total			**2.4**	**n.d.**	**28.6**	**1.6**	**n.d.**	**6.8**			
	Esters											
E1	methyl butanoate	720	A	n.d. ^{C}	0.53±0.05 ^{B}	n.d. ^{C}	n.d. ^{C}	2.3±0.09 ^{A}	n.d. ^{C}	***	***	***
E2	propyl 3-methylbutanoate	947	A	1.5±0.26 ^{C}	9.8±0.69 ^{C}	8.8±1.2 ^{C}	1.5±0.45 ^{C}	52.5±10.8 ^{A}	23.1±0.31 ^{B}	***	***	***
E3	bornyl acetate	1297	A	0.71±0.15 ^{B}	n.d. ^{B}	n.d. ^{B}	0.41±0.03 ^{B}	n.d. ^{B}	2.4±0.67 ^{A}	***	***	***
E4	(*E)*-pinocarvyl acetate	1304	A	8.3±1.1 ^{A}	n.d. ^{C}	7.9±0.95 ^{A}	4.8±1.2 ^{B}	n.d. ^{C}	7.3±1.7 ^{AB}	***	*	*
E5	carveol acetate	1339	A	8.7±0.54 ^{A}	n.d. ^{C}	10.5±0.47 ^{B}	4.2±1.1 ^{B}	n.d. ^{C}	5.2±1.5 ^{B}	***	***	***
E6	hexyl hexanoate	1385	A	0.36±0.07 ^{CD}	1.5±0.12 ^{B}	n.d. ^{D}	0.92±0.36 ^{BC}	2.6±0.69 ^{A}	n.d. ^{D}	***	**	*
E7	hexyl octanoate	1584	A	0.67±0.15	n.d.	n.d.	0.57±0.12	n.d.	n.d.	***	ns	ns
	Total			**20.2**	**11.8**	**27.2**	**12.4**	**57.4**	**38**			
	Monoterpenes											
M1	α-thujene	932	A	12.5±1.5 ^{A}	4.6±0.34 ^{B}	1.3±0.10 ^{D}	3.4±0.32 ^{BC}	4.3±0.54 ^{B}	1.6±0.36 ^{CD}	***	***	***
M2	α-pinene	939	A	15.8±3.7 ^{A}	8.8±0.86 ^{BC}	11.4±1.3 ^{AB}	5.9±0.60 ^{C}	6.7±1.4 ^{BC}	5.0±0.40 ^{C}	*	***	**
M3	camphene	958	A	3.7±0.64 ^{C}	4.9±1.3 ^{BC}	6.8±0.97 ^{AB}	2.2±0.40 ^{C}	8.0±1.7 ^{A}	7.8±0.76 ^{A}	***	ns	**
M4	dehydrosabinene	960	A	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	0.5±0.14 ^{A}	***	***	***
M5	sabinene	976	A	13.3±2.5 ^{A}	5.5±1.0 ^{B}	4.6±0.17 ^{B}	3.7±0.45 ^{B}	6.7±1.2 ^{B}	3.5± 0.73 ^{B}	***	***	***
M6	β-pinene	980	A	190±37.9 ^{A}	86.9±10.8 ^{B}	14.9±2.4 ^{C}	39.3±5.6 ^{C}	16.9±2.7 ^{C}	17.4±3.2 ^{C}	***	***	***
M7	myrcene	991	A	122±25.7 ^{A}	49.6±11.8 ^{B}	15.1±2.4 ^{C}	20.3± 5.7 ^{BC}	12.3± 2.8 ^{C}	6.9± 2.3 ^{C}	***	**	***

M8	α-terpinene	1018	A	7.2±1.9 ^{A}	4.8±1.0 ^{AB}	0.84±0.02 ^{C}	3.3±0.77 ^{BC}	3.9±0.43 ^{B}	2.5±0.29 ^{BC}	***	*	**
M9	m-cymene	1027	A	185±32.7 ^{A}	71.5±10.6 ^{B}	40.8±9.2 ^{B}	59.1±26.3 ^{B}	59.2±8.0 ^{B}	25.8±0.68 ^{B}	***	***	***
M10	limonene	1034	A	1068±207 ^{A}	598±41.8 ^{B}	264±61.8 ^{C}	581±93.7 ^{B}	605±88.8 ^{B}	264±7.4 ^{C}	***	**	**
M11	γ-terpinene	1063	A	256±34.4 ^{A}	112±20.3 ^{B}	21.7±2.5 ^{C}	63.7±34.6 ^{BC}	54.0±12.9 ^{BC}	42.3±12.8 ^{C}	***	***	***
M12	terpinolene	1093	A	9.6±0.15 ^{B}	8.0±0.89 ^{BC}	15.1±2.0 ^{A}	4.4±0.74 ^{D}	7.3±1.0 ^{BCD}	6.4±1.0 ^{CD}	***	***	***
M13	p-cymene	1099	A	n.d. ^{C}	n.d. ^{C}	3.7±0.35 ^{A}	n.d. ^{C}	n.d. ^{C}	2.9±0.27 ^{B}	***	**	**
M14	β-thujone	1119	A	1.6±0.50	4.2±0.82	0.96±0.20	0.77±0.18	3.0±0.45	0.86±0.13	***	**	ns
M15	*p*-mentha-1,5,8-triene	1113	A	n.d. ^{C}	1.3±0.26 ^{B}	1.9±0.35 ^{A}	n.d. ^{C}	1.4±0.16 ^{B}	1.4±0.05 ^{B}	***	ns	*
M16	citronellal	1159	A	25.4±4.2 ^{A}	9.3±2.4 ^{B}	2.8±0.12 ^{C}	4.2±0.83 ^{BC}	6.5±1.4 ^{BC}	1.2±0.06 ^{C}	***	***	***
M17	(*E*)-dihydrocarvone	1195	A	n.d.	n.d.	2.9±0.64	n.d.	n.d.	2.8±0.18	***	ns	ns
M18	β-cyclocitral	1232	A	1.2±0.27	1.9±0.42	1.8±0.10	0.88±0.28	1.9±0.21	1.1±0.15	***	*	ns
M19	carvone	1246	A	9.2±1.7 ^{B}	18.1±3.3 ^{A}	2.1±0.41 ^{C}	7.0±1.5 ^{BC}	10.2±1.7 ^{B}	4.1±1.2 ^{C}	***	*	*
M20	L-carvone	1257	A	n.d. ^{C}	3.6±0.74 ^{B}	4.9±0.93 ^{B}	n.d. ^{C}	4.4±0.80 ^{B}	7.1±0.84 ^{A}	***	**	**
	Total			**1921**	**993**	**418**	**799**	**812**	**405**			
	Monoterpenoid alcohols											
MA1	linalool	1103	A	1.3±0.23 ^{CD}	1.6±0.34 ^{CD}	1.7±0.36 ^{C}	0.84±0.13 ^{D}	3.7±0.35 ^{A}	2.8±0.19 ^{B}	***	***	***
MA2	*p*-mentha-2,8-dien-1-ol	1122	A	n.d.	1.2±0.15	0.8±0.15	n.d.	1.1±0.20	1.1±0.29	***	ns	ns
MA3	fenchol	1127	A	16.9±1.5 ^{A}	5.6±1.0 ^{B}	1.8±0.27 ^{B}	22.5±5.5 ^{A}	1.9±0.27 ^{B}	3.9±0.86 ^{B}	***	ns	*
MA4	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	1129	A	6.8±1.6^{AB}	9.7±1.9 ^{AB}	1.8±0.35 ^{B}	7.5±1.6 ^{A}	9.3±1.1 ^{B}	1.7±0.13 ^{B}	***	ns	ns
MA5	dihydrolinalool	1136	A	n.d. ^{B}	n.d. ^{B}	6.3±1.0 ^{AB}	n.d. ^{B}	n.d. ^{B}	5.0±1.7 ^{A}	***	ns	ns
MA6	pinocarveol	1152	A	3.1±0.68 ^{B}	4.0±0.84 ^{AB}	4.2±0.22 ^{AB}	1.2±0.35 ^{C}	1.1±0.05 ^{C}	5.4±0.43 ^{A}	***	***	***
MA7	terpinen-4-ol	1184	B^{A}	n.d. ^{C}	1.7±0.30 ^{B}	2.9±0.68 ^{A}	n.d. ^{C}	n.d. ^{C}	2.7±0.61 ^{AB}	***	***	**
MA8	p-cymen-8-ol	1202	A	4.1±0.79	3.8±0.03	4.2±0.91	2.0±0.63	2.8±0.29	2.7±0.78	ns	***	ns
MA9	γ-terpineol	1210	A	2.6±0.71 ^{A}	n.d. ^{C}	1.8±0.40 ^{AB}	1.2±0.44 ^{A}	2.0±0.19 ^{AB}	2.5±0.42 ^{A}	***	ns	***
MA10	*(Z)*-carveol	1220	A	n.d.	7.5±1.5	5.8±0.92	n.d.	4.9±1.0	4.2±1.1	***	**	ns

MA11	thymol	1290	A	0.87±0.15 ^{BC}	2.8±0.30 ^{A}	3.2±0.74 ^{A}	0.31±0.07 ^{C}	n.d.^{C}	1.4±0.37 ^{B}	***	***	**
MA12	carvacrol	1311	A	2.8±0.60 ^{B}	11.2±1.7 ^{A}	13.1±0.78 ^{A}	0.80±0.09 ^{B}	2.8±0.30 ^{B}	2.2±0.38 ^{B}	***	***	***
MA13	*(E)*-8-hydroxylinalool	1342	A	0.90±0.26 ^{A}	n.d. ^{C}	n.d. ^{C}	0.38±0.05 ^{B}	n.d. ^{C}	n.d. ^{C}	***	**	**
	Total			**39.4**	**49.1**	**47.6**	**36.7**	**29.6**	**35.6**			
	Sesquiterpenes											
S1	(+)-cyclosativene	1378	A	n.d. ^{C}	1.1±0.12 ^{B}	n.d. ^{C}	n.d. ^{C}	3.8±0.75 ^{A}	n.d. ^{C}	***	***	***
S2	α-copaene	1389	A	0.36±0.10 ^{B}	1.6±0.43 ^{B}	n.d. ^{B}	2.1±0.30 ^{B}	10.5±1.9 ^{A}	n.d. ^{B}	***	***	***
S3	β-caryophyllene	1440	A	35.9±12.1 ^{A}	46.5±11.4 ^{AB}	12.8±3.3 ^{B}	15.9±3.8 ^{B}	25.6±1.1 ^{B}	6.6±2.1 ^{B}	***	***	ns
S4	α-humulene	1475	A	9.8±2.3 ^{A}	8.5±1.1 ^{BC}	5.2±1.6 ^{B}	2.2±0.29 ^{BCD}	2.0±0.41 ^{D}	1.3±0.17^{CD}	**	***	ns
S5	(+)-aromadendrene	1447	A	1.1±0.18 ^{ABC}	1.5±0.16 ^{A}	0.60±0.10 ^{C}	0.66±0.11 ^{C}	1.3±0.33 ^{AB}	0.97±0.18 ^{BC}	***	ns	**
S6	curcumene	1486	A	2.0±0.21 ^{A}	n.d. ^{C}	n.d. ^{C}	1.0±0.11 ^{B}	n.d. ^{C}	n.d. ^{C}	***	***	***
S7	β-selinene	1505	B^{C}	57.0±13.3	79.2± 14.6	26.4±4.5	21.6±4.2	50.5±11.5	15.0±2.0	***	***	ns
S8	valencene	1516	A	n.d. ^{B}	54.5±9.7 ^{A}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	***	***	***
S9	α-selinene	1518	A	8.3±1.6	14.2±2.4	4.0±0.72	3.5±0.12	9.3±2.1	3.3±0.84	***	***	ns
S10	(*Z*)-β-nerolidol	1535	A	n.d.	n.d.	3.2±0.34	n.d.	n.d.	3.4±0.56	***	ns	ns
S11	kessane	1554	A	60.3±7.8 ^{A}	n.d. ^{B}	n.d. ^{B}	0.64±0.23 ^{B}	n.d. ^{B}	n.d. ^{B}	*******	***	***
	Total			**175**	**207**	**52.2**	**47.5**	**103**	**30.6**			
	Phthalides											
P1	3-propylidene phthalide	1600	A	1.4±0.23	2.1±0.29	1.3±0.36	0.4±0.03	1.4±0.32	0.17±0.03	***	***	ns
P2	3-*n*-butylphthalide	1658	A	37.2±4.5 ^{C}	124±20.2 ^{A}	103±5.5 ^{AB}	26.8±6.7 ^{C}	148±27.3 ^{A}	68.0±22.9 ^{BC}	***	ns	*
P3	*(Z)*-butylidenephthalide	1685	B^{C}	n.d. ^{C}	2.9±0.60 ^{B}	1.5±0.28 ^{C}	n.d. ^{C}	4.3±0.84 ^{A}	0.84±0.07 ^{CD}	***	ns	**
P4	sedanenolide	1730	A	102±16.1 ^{C}	279±21.3 ^{A}	221±42.2 ^{AB}	56.8±12.3 ^{CD}	202±27.1 ^{B}	18.1±4.0 ^{D}	***	***	***
P5	neocnidilide	1753	B^{c}	1.1±0.13 ^{C}	2.9±0.53 ^{BC}	3.2±0.63 ^{BC}	3.0±0.62 ^{BC}	10.0±1.8 ^{A}	3.8±0.52 ^{B}	***	***	***
P6	(*E*)-ligustilide	1758	B^{B}	1.4±0.25 ^{B}	3.8±0.61 ^{A}	3.0±0.55 ^{A}	0.89±0.20 ^{B}	2.9±0.56 ^{A}	0.42±0.07 ^{B}	***	***	**
	Total			**143**	**415**	**333**	**87.9**	**369**	**91.3**			

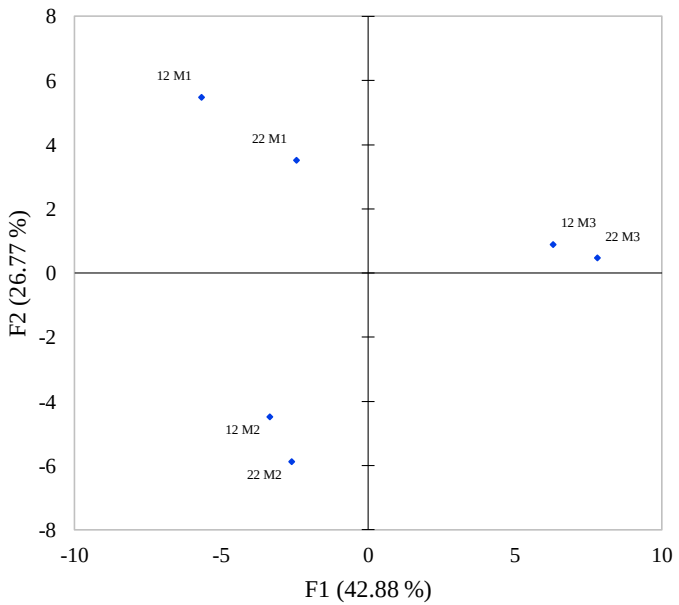
	Alkanes											
ALK1	nonane	900	A	5.9±1.2 ^{AB}	9.7±2.0 ^{A}	6.8±1.1 ^{AB}	5.5±1.9 ^{AB}	n.d. ^{C}	9.3±1.2 ^{AB}	**	**	***
ALK2	decane	1000	A	n.d. ^{D}	6.4±1.2 ^{BC}	5.1±0.74 ^{CD}	n.d. ^{D}	22.5±4.2 ^{A}	11.1±1.6 ^{B}	***	***	***
ALK3	undecane	1100	A	2.4±1.5	2.3±0.17	n.d.	1.7±0.21	3.2±0.76	n.d.	***	ns	ns
ALK4	dodecane	1200	A	0.56±0.08 ^{D}	6.2±1.6 ^{A}	5.5±0.79 ^{A}	1.7±0.21 ^{CD}	4.6±1.0 ^{AB}	3.0±0.60 ^{BC}	***	*	*
ALK5	tridecane	1300	A	n.d. ^{B}	n.d. ^{B}	3.1±0.57 ^{A}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	***	***	***
ALK6	tetradecane	1400	A	0.51±0.13 ^{C}	0.99±0.21 ^{B}	n.d. ^{D}	0.39±0.04 ^{C}	2.0±0.14 ^{A}	n.d. ^{D}	***	***	***
	Total			**9.4**	**25.6**	**20.5**	**9.3**	**32.3**	**23.4**			
	Ether											
ET1	dill ether	1184	A	n.d. ^{C}	n.d. ^{C}	3.5±1.4 ^{A}	n.d. ^{C}	n.d. ^{C}	1.6±0.36 ^{B}	***	ns	*
	Oxide											
O1	*(Z)*-limonene oxide	1145	A	12.8±3.4	n.d.	n.d.	10.8±0.53	n.d.	n.d. ^{B}	***	ns	ns
	Phenol											
PH1	eugenol	1363	A	n.d.	1.8±0.22	2.7±0.23	n.d.	2.3±0.29	2.7±0.42	***	ns	ns
	Unknowns											
U1	unknown	935		3.9±0.58 ^{A}	n.d. ^{D}	1.1±0.21 ^{C}	2.1±0.18 ^{B}	n.d. ^{D}	1.6±0.l6 ^{C}	***	***	***
U2	unknown	1009		n.d. ^{C}	n.d. ^{C}	13.6±1.2 ^{A}	n.d. ^{C}	n.d. ^{C}	10.9±1.1 ^{B}	***	*	**
U3	unknown	1133		n.d. ^{B}	n.d. ^{B}	0.72±0.14 ^{B}	n.d. ^{B}	n.d. ^{B}	2.0±0.71 ^{A}	***	*	**
U4	unknown	1239		n.d. ^{B}	n.d. ^{B}	2.1±0.18 ^{B}	n.d. ^{B}	n.d. ^{B}	22.2±4.38 ^{A}	***	***	***
U5	unknown	1277		n.d. ^{B}	1.4±0.34 ^{B}	4.6±2.0 ^{A}	n.d. ^{B}	1.7±0.25 ^{B}	2.1±0.56 ^{B}	***	ns	*
U6	unknown	1466		n.d. ^{C}	2.6±0.57 ^{A}	n.d. ^{C}	n.d. ^{C}	1.5±0.05 ^{B}	n.d. ^{C}	***	**	***
U7	unknown	1698		n.d. ^{B}	51.8±7.7 ^{A}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	***	***	***
	Total			**64.2**	**55.8**	**22.1**	**2.7**	**3.2**	**38.8**			

[a] Linear retention index on a DB-5 column. [b] A – Experimental LRI, identification of compound whereby the mass spectrum and LRI agree with those of authentic compound (A) Identification, mass spectrum agrees with reference spectrum in the NIST/EPA/NIH mass spectra database or (B) LRI agree with those in the literature [(A)] Mévy et al., 2006 [(B)] Asuming et al., 2005, [(C)] Andriamaharavo, 2014, [(D)] Jalali-Heravi et al., 2006. [c] Premature time-point. [d] Commercial maturity time-point. [e] Post-maturity time-point. [f] Estimated quantities (mg) collected in the headspace of celery samples containing 0.5 mL of saturated calcium chloride and filled up to 5 mL with HPLC-grade water, calculated by comparison with of 100 µg/mL propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means

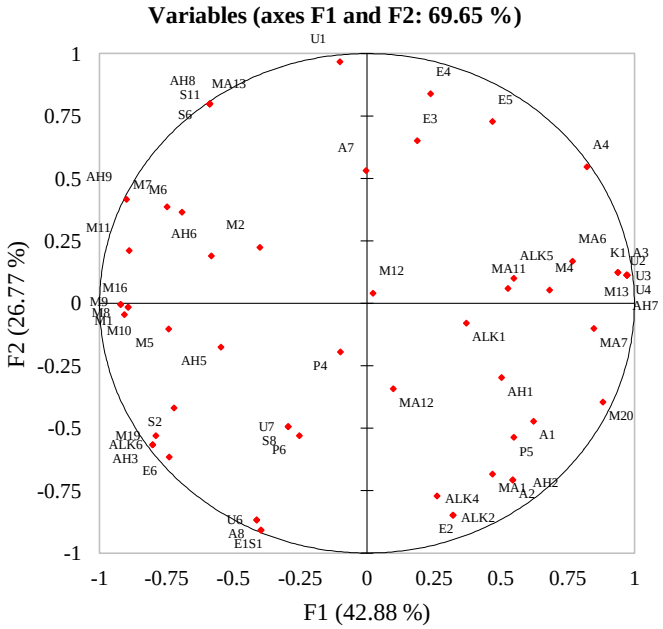
of three replicate samples are shown; n.d. - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. [h] Maturity. [i] Line. [j] Maturity and line interaction. Tukey's HSD - means not labelled with letters are not significantly different ($p < 0.05$) according maturity/line interaction.

Figure 1 – Principal component analysis of two different celery genotypes at three different maturities showing correlations with volatile compounds that are significant according to factors of maturity, genotype and their interaction of maturity x genotype: (A) Projection of samples, (B) Distribution of volatile compounds, (C) Key of compounds used to construct the PCA.

A)



B)



C)

A1	3-methyl-3-buten-1-ol	M12	terpinolene
A2	1-pentanol	M13	p-cymenene
A3	1-hepten-3-ol	M16	citronellal
A4	*(E)*-2-hexen-1-ol	M19	carvone
A7	1-nonanol	M20	L-carvone
A8	1-decanol	MA1	linalool
AH1	*(E)*-2-pentenal	MA6	pinocarveol
AH2	hexanal	MA7	terpinen-4-ol
AH3	*(Z)*-2-hexenal	MA11	carvacrol
AH5	*n*-octanal	MA12	*(E)*-8-hydroxylinalool
AH6	benzeneacetaldehyde	MA13	(+)-cyclosativene
AH7	2-hydroxybenzaldehyde	S1	α-copaene
AH8	*(E,Z)*-2,6-nonadienal	S2	β-caryophyllene
AH9	*(E,E)*-2,4-nonadienal	S6	β-selinene
K1	3-hexanone	S8	α-selinene
E1	methyl butanoate	S11	kessane
E2	propyl 3-methylbutanoate	P4	sedanenolide
E3	bornyl acetate	P5	neocnidilide
E4	*(E)*-pinocarvyl acetate	P6	*(E)*-ligustilide
E5	carveol acetate	ALK1	nonane
E6	hexyl hexanoate	ALK2	decane
M1	α-thujene	ALK4	dodecane
M2	α-pinene	ALK5	tridecane
M4	dehydrosabinene	ALK6	tetradecane
M5	sabinene	U1	unknown
M6	β-pinene	U2	unknown
M7	myrcene	U3	unknown
M8	α-terpinene	U4	unknown
M9	m-cymene	U5	unknown
M10	limonene	U6	unknown
M11	γ-terpinene	U7	unknown

3. Results and Discussion

3.1 Biochemical profile is more influenced by maturity than genotype

In total, 94 compounds were determined in the headspace across two celery parental lines (Table 1) and 91 of these were identified. Ninety-three compounds were shown to be significantly influenced by plant maturity whereas 71 compounds by plant genotype. Identified compounds include 20 monoterpenes, 13 monoterpenoid alcohols, 11 sesquiterpenes, nine alcohols and nine aldehydes, six phthalides and a range of other compounds counting esters and ketones. Monoterpenes, followed by phthalides and sesquiterpenes, comprise the majority of the total volatiles collected from the headspace of the two genotypes and three maturities (Table 1) and are at their highest total volatile content at M1 for line 12 and M2 for line 22. Alcohols displayed an increase as the crop developed and became most abundant at M3; similar trend also observed for the aldehyde content in line 22. Sesquiterpenes and phthalides were at their highest total volatile content at M2.

GC/MS analysis identified groups of compounds that fluctuate throughout maturity and between genotype (Table 1). All compounds apart from *p*-cymen-8-ol, were influenced by maturity and fewer significantly influenced by genotype. Similar patterns can be observed between genotypes as the crop develops, but certain compounds prevent these patterns from occurring consistently between genotypes. For example, hexanal and propyl 3-methylbutanoate dramatically increased in line 22 at M2, causing the total aldehyde and ester content to increase accordingly.

Monoterpene content in line 12 was the highest at M1, with limonene, the most abundant compound, identified across both lines and maturities. Limonene's content decreased as celery developed. Most monoterpenes followed this pattern including γ-terpinene, m-cymene and β-pinene and is most noticeable in line 12. These compounds remained the most abundant monoterpenes in line 22, however, there is less of a noticeable change between M1

and M2. These compounds are known to have odour descriptors that include citrus, pine and sweet. Throughout literature, monoterpenes have been shown to be the most abundant compounds reported in various celery genotypes as shown previously by Turner, Lignou, Gawthrop & Wagstaff (2021). Orav, Kailas & Jegorova (2003) analysed the composition of Estonian grown celery essential oil and similarly, identified monoterpenes to comprise the majority of the flavour profile (85.3%). Likewise, MacLeod & Ames (1989) identified 18 monoterpenes, representing around 46% of the aroma profile of fresh supermarket bought celery and identified limonene as the major component in the celery isolate, similar to this study.

Additional monoterpenes such as p-mentha-1,5,8-triene and L-carvone in M2 and *(E)*-dihydrocarvone and *p*-cymene were identified in both genotypes as maturity developed whereas dehydrosabinene only appeared in line 22 at M3. These compounds could signal the deterioration of the crop through the development of the aroma from fresh and green, to woody and pine. Similarly, further monoterpenoid alcohols such as p-mentha-2,8-dien-1-ol, dihydrolinalool, terpinen-4-ol and (Z)-carveol were identified as maturity developed. Linalool, pinocarveol, thymol and carvacrol exhibited their highest abundance at M3. These compounds are responsible for floral, herbal, pine odours. For both genotypes, fenchol was the most abundant monoterpenoid alcohol with odour descriptors such as minty, medicinal and camphoreous. Compared to M1, fenchol's content at M3 was significantly lower. Monoterpenoid alcohols presented to be least influenced by genotype compared to other compound groups.

Sesquiterpenes, while fewer were identified and with lower relative abundances, contribute woody, herbal and floral notes to celery aroma. Maturity showed to have a significant influence for all sesquiterpenes. Lund, Wagner & Bryan (1973) and MacLeod & Ames (1989) both identified β-selinene to be an important compound to the celery aroma,

although not a characteristic compound. β-Selinene and β-caryophyllene were identified as non-phthalide compounds with the highest concentrations in celery essential oil, however, β-selinene was characterised with a celery-like odour. Using odour evaluation, β-selinene was shown to have a threshold of 1 ppm which is low compared to 3-n-butylphthalide with an odour threshold of 10 ppm (Lund, Wagner & Bryan, 1973). Furthermore, Ehiabhi et al. (2006) reported both β-selinene and β-caryophyllene to be major constituents of Nigerian grown *A. graveolens* and were reported to make up as much as 16.3 and 10.5 % respectively, of the aroma profile.

Findings in the present study are in agreement with Ehiabhi et al. (2006), β-selinene and β-caryophyllene expressed their highest relative abundance at M2 and decreased once commercial maturity reached (Table 1). A similar pattern was observed for other sesquiterpenes including α-selinene and α-copaene and monoterpenes in line 22. α-humulene was most abundant at M1 with curcumene and kessane only detected at M1. Kessane was also identified by Philippe, Suvarnalatha, Sankar & Suresh (2002) in the essential oil of Indian celery seed. During M3, the abundance of sesquiterpenes remained relatively low compared to monoterpenes and phthalides, however, (Z)-β-nerolidol was only identified at M3 for both genotypes. Kessane, curcumene and (Z)-β-nerolidol were all determined by Nurzyńska-Wierdak, Gruszecki and Kosior (2018) in varying amounts of celery essential oil of two varieties grown in Poland. These had been preserved through various drying techniques and harvested in July and October. Only the July harvest showed the presence of these compounds.

Phthalides exhibited a similar pattern to sesquiterpenes, showing their highest level of abundance at M2. Abundance variation within the phthalides identified were observed between maturities, with line 12 showing a much higher phthalide content than line 22. As shown by both Kurobayashi et al. (2006) and Sellami et al. (2012), phthalide compounds are important contributors to the typical *A. graveolens* aroma and therefore, having a lower abundance of

these compounds at a later maturity may mean that the odour these genotypes exhibit is a much less typical celery odour. Focussing further on the phthalide compounds, a significant difference between the maturities for the majority of these compounds can be observed, with sedanenolide showing the most significant increase from M1 to M2 and then decreasing at M3. Apart from neocnidilide in line 22, all phthalides were at the highest abundance at this time point. 3-*n*-Butylphthalide and (Z)-butylidene phthalide showed no significant difference between genotype, only maturity, and (Z)-butylidene phthalide was not identified at M1.

The relative abundance of alcohols increased as the crop developed for both genotypes. At M3 more alcohols were identified and in most cases at a higher abundance. Compounds 1-nonanol and 1-dodecanol for line 12 were shown to be of lower abundance at M3 when compared to M1 and 1-octanol and 1-decanol were not identified in either genotype at M3. For monoterpenes, sesquiterpenes and phthalides, line 12 has been shown to have the highest abundance of these compounds when compared to line 22. However, for alcohols, aldehydes and esters, line 22 has a significantly higher abundance of these and exhibited a different pattern to line 12. At M1, line 22 expressed a similar aldehyde and ester content to line 12 at M2 and at M3, a much higher abundance of these compounds is observed. The biggest cause of this difference in esters was attributed to the large increase of propyl 3-methylbutanoate, known for its fruity, apple odour. Seven aldehydes were identified at both M1 and M2 compared to the five identified at M3. Compounds contributing to green, fresh odours such as *(Z)*-2-hexenal, (*Z*)-4-heptenal, *(E,Z)*-2,6- and *(E,E)*-2,4-nonadienal were not found in M3. Conversely, 2-hydroxybenzaldehyde was only identified at M3 and at much higher abundance in line 22, again this could possibly be indication for aroma deterioration. Line 22 exhibited a higher abundance in compounds such as hexanal at all maturities, particularly at M3 where hexanal increased in relative abundance, whereas in line 12 this began to decrease after M2.

As these lines were transplanted in the same field at the same time and were grown under the same environmental conditions, minimal significant differences caused by environmental factors were expected. Therefore, any differences observed should be attributed to differences in the genotype and maturity. From the results so far, it seems that maturity has a higher impact on aroma profile differences than genotype however, the difference between genotypes in terms of patterns for different compounds across maturities is apparent. This was expected due to the differences identified by Yommi et al. (2013) and Fellman, Miller and Mattinson (2000). They observed the influence of genetics and harvest maturity on volatile compounds in different apple varieties, stating that the nature and amount of aroma compounds present in apples were cultivar dependent.

Principal component analysis was used to visualise graphically the differences in the volatile compounds in the three maturity stages and the two genotypes and to examine any correlations occurring between maturity, genotype and chemical compounds (Figure 1). Using only the significant compounds for maturity, genotype and their interaction, a clear separation between the maturities and the chemical compounds associated can be observed. Principal component one (F1) and two (F2) explained 69.95 % of the total variation present within the data and it can be observed that the first axis discriminates M3 from M1 and M2, whereas M2 is discriminated from M1 and M3 by the second axis. Predominantly, monoterpene content expresses a strong association with F1 (42.88 %) whereas other compound groups including aldehydes, esters and phthalides are measured through F2 and explaining a lower proportion of the variation present within the data (26.77 %).

Genotype shows a stronger influence upon M1 where a larger separation can be seen between the two genotypes and a stronger association with the volatile compounds associated with line 12 M1. M1 displays a strong positive association with the majority of monoterpenes, such as α-pinene (M2), sabinene (M5), β-pinene (M6), myrcene (M7) and (M11) γ-terpinene,

and aldehydes such as 1-octanol (AH5) benzeneacetaldehyde (AH6), (*E,Z*)-2,6-nonadienal (AH8) and *(E,E)*-2,4-nonadienal (AH9). These are compounds are known to exhibit fresh, waxy, green notes, similar to cucumber odour. The highest number of esters were identified at M1 (Table 1) and these compounds contribute fruity and fresh notes however, these are at low relative abundance compared to the other maturities as seen in Table 1, explaining the low association of these compounds in all PCA plots. Nurzyńska-Wierdak, Gruszecki & Kosior (2018) observed both increases and decreases in the ester content of celery essential oil when comparing freeze-dried with convection drying, however these were not significant differences. Phthalides show no association with M1 in Figure 1 and only sesquiterpenes β-selinene (S6) and kessane (S11) show association with M1.

Developing into M2, the aroma profile shifted, with strong associations with phthalides such as sedanenolide (P4) and *(E)*-ligustilide (P6), and sesquiterpenes such as α-copaene (S1), β-caryophyllene (S2) and α-selinene (S8). The presence of these compounds allows stronger odours that are woodier, herbal and celery-like to seem more apparent, descriptors that are more common when describing *A. graveolens* aroma. At this stage, the highest number of sesquiterpenes and phthalides were observed for both genotypes (Table 1).

Once M3 is reached, the spread of compounds within the quadrant (Figure 1) is much less compared to other maturities, with the compounds more localised. Furthermore, where more obvious groupings of compounds by M1 and M2 can be seen clearly, this is less apparent for M3. Compounds including 2-hydroxybenzaldehyde (AH7), dehydrosabinene (M4), *p*-cymene (M13) and terpinolene (M12) are strongly associated with M3 as well as the monoterpenoid alcohols; pinocarveol (MA6), terpinen-4-ol (MA7), carvacrol (MA11) and (E)-8-hydroxylinalool (MA12). M3 displaying stronger associations with these compounds and weaker associations with monoterpenes, alcohols and phthalides (fresh, green and fruit odours) suggests that the odour of these genotypes are no longer of the same quality as M2 and

368 therefore, deterioration of the crop is beginning. The presence of certain compounds (A3, K1,
369 M4, M13) could act as an indicator of quality decline in celery. Within the same quadrant as
370 M3, esters bornyl acetate (E3), (E)-pinocarvyl acetate (E4), carveol acetate (E5) express a
371 closer association than previous maturities.

372 Furthermore, line 22 shows significantly higher abundances in certain compounds at
373 M3 including AH2, M4 and AH7 whereas line 12, show higher abundances in other compounds
374 at M3 including K2, M13 and MA5 (Table 1). Possibly due to genetic differences or because
375 line 22 may have progressed through developmental stages differently compared to than line
376 12, it is possible that floral transition had occurred, and the plants were preparing to bolt. At
377 the beginning of maturity, line 12 appears to be most aromatic (Figure 1, Table 1) however, as
378 maturity occurs line 22 M2 and M3 progresses into a more aromatic line, showing these two
379 time points to be most significantly different when combined with genotype. Line 12 M1 and
380 line 22 M2 celery share the most similarities in terms of aroma profile and independent of
381 genotype, M1 and M2 appear to be the most similar.

382 Compounds including hexanal and *(E)*-2-hexen-1-ol are known as green leaf volatiles
383 (GLVs); these are released in the early stages of maturity and increase as the plants develop,
384 similar to monoterpenes. Over time, the bolting process begins and the crop invests more
385 resources into reproduction and protecting the developing floral meristem from predatory
386 attack, as shown by Rapparini, Baraldi & Facini, (2001). This is where the concentration of
387 terpenes was highest (Table 1, M1) following flowering and in subsequent reproductive stages.
388 As the plant develops, plant-plant and plant-insect interactions become more important,
389 involving the synthesis of GLVs and other volatile compounds (Spinelli, Cellini, Marchetti,
390 Mudigere & Piovene, 2011). This relationship could explain the increase of monoterpenes from
391 M1 to M2 before the crop focuses on the synthesis of alcohols and aldehydes as maturity
392 develops.

Overall, comparing the odours between the two genotypes and three maturities, it can be seen that line 12 has the highest abundance of volatile compounds and can be assumed to be a more aromatic variety. Harvesting at any time point will result in a crop with a significantly different aroma profile. Harvesting at an earlier, similar to M1 would result in low in phthalide and high monoterpene content, resulting in a more citrus-like profile. Over commercial maturity, phthalide content remains high, maintaining strong celery notes. In order to identify whether there has been aroma quality decline and whether compounds identified in M3 contribute to off-odours, sensory profiling using a trained panel can be completed. The differences support the hypothesis that the time point of harvest does have a significant influence over the aroma of celery as well as the genotype and that genotype will influence the synthesis of odours during deterioration. This relationship is discussed further when considering the GC/O data in section 3.2.

405 Table 2 – Odour description and intensity of the volatile compounds detected by GC-O in the headspace of two celery genotypes harvested at three different maturity stages.

					Average Odour Intensity [c]					
					Line 12			Line 22		
Odour Description	LRIexp[a]	Compound	ID[b]	Code[d]	M1[e]	M2[f]	M3[g]	M1	M2	M3
Alcohols										
Burnt, baked, dairy	660	1-butanol	B		-	-	4	3	4	-
Green/chemical	670	1-penten-3-ol	B		4	-	-	-	-	-
Green, plastic, fruity	706	3-pentanol	B		-	3	4	-	-	-
Soapy, green, sharp	733	3-methyl-3-buten-1-ol	A	A1	5	-	5	3	-	-
Fresh, green, fruity	859	(*Z*)-3-hexen-1-ol	B		5	-	4	-	-	-
Musty, moss	867	(*E*)-2-hexen-1-ol	A	A4	-	5	3	-	4	-
Earthy, mushroom, grass	889	1-hepten-3-ol	A	A3	8	-	4	-	5	-
Mushroom	907	2-heptanol	B, C		6	5	-	-	-	3
Mushroom, soil	978	1-octen-3-ol	B, C		7	5	6	4	7	5
Fresh, citrus, waxy	1001	3-octanol	B		7	-	5	5	6	-
Metallic, sweaty	1174	1-nonanol	A	A7	7	-	6	-	4	4
Tomato, herbal, fatty	1274	1-decanol	A	A8	-	-	5	-	5	-
Aldehydes										
Floral, green, waxy	760	(*E*)-2-pentenal	A	AH1	-	4	5	3	3	-
Fresh, green, apple	801	hexanal	A	AH2	5	5	3	6	6	4
Garbage, damp	855	(*E*)-2-hexenal	A	AH3	-	5	-	5	-	-
Biscuit, bread	901	(*Z*)-4-heptenal	A	AH4	5	-	5	4	-	-
Floral, rose, citrus	1005	n-octanal	A	AH5	-	7	-	6	-	3
Rose, honey, floral	1045	benzeneacetaldehyde	A	AH6	7	5	4	5	5	4
Baked, honey, make-up powder	1057	2-hydroxybenzaldehyde	A	AH7	6	-	5	4	4	5

Floral, smoky, cherry	1071	*p*-tolualdehyde	B		-	-	5	3	-	-
Woody, moss, cucumber	1155	(*E*,*E*)-2,6-nonadienal	B, C		6	5	6	7	5	5
Green, cucumber, parsley	1159	(*E*,*Z*)-2,6-nonadienal	A	AH8	6	5	-	7	7	5
Floral, woody	1224	(*E*,*E*)-2,4-nonadienal	A	AH9	-	5	-	-	-	-
Ketones										
Vanilla, creamy, butter	677	1-penten-3-one	B		-	3	-	-	-	-
Bread, floral, grass	687	2-pentanone	B		-	-	4	5	6	3
Green	693	3-pentanone	B		7	-	4	5	-	-
Waxy, green, plastic	776	3-hexanone	A	K1	6	-	-	5	-	-
Green, cut grass, apple	793	2-hexanone	B		7	3	4	4	-	-
Metallic, musty	978	1-octen-3-one	A	K2	-	-	-	4	4	-
Rose, honey, floral	1041	3-octen-2-one	B		7	-	5	-	-	-
Herbal, soil, spicy	1083	2-nonanone	A	K3	-	3	5	-	5	-
Make-up powder, floral, creamy	1146	3-nonen-2-one	B		-	-	6	6	5	-
Make-up powder, baked	1401	*p*-mentha-8-thiol-3-one	B		-	5	4	-	-	-
Esters										
Make-up powder, floral	947	propyl 3-methylbutanoate	A	E2	3	-	6	-	-	-
Woody, pencil shavings, liquorice	1247	linalyl acetate	B		6	-	6	-	5	-
Herbal, woody	1305	bornyl acetate	A	E3	-	-	4	-	-	4
Plastic, green, herbal	1332	carveol acetate	A	E5	-	-	4	7	-	-
Metallic, damp, musty	1381	hexyl hexanoate	A	E6	-	-	4	-	6	4
Monoterpenes										
Pine, minty, floral	931	α-thujene	A	M1	5	-	4	4	4	-
Herbal, citrus, waxy	959	camphene	A	M3	6	4	5	5	5	3

Earthy, mushroom, green	981	sabinene	A	M5	8	-	6	7	7	-
Herbal, earthy, woody	987	β-pinene	A	M6	8	7	4	7	5	5
Lemon, green, waxy	997	β-myrcene	A	M7	-	3	4	6	-	-
Musty, camphoreous	1025	α-terpinene	A	M8	6	-	4	-	-	-
Floral, fresh, mint	1031	limonene	A	M10	6	-	4	4	4	-
Waxy, woody, makeup powder	1062	γ -terpinene	A	M11	6	-	-	-	-	-
Make-up powder, floral, citrus	1094	terpinolene	A	M12	5	3	4	-	5	4
Floral, herbal, violet	1098	*p*-cymene	A	M13	6	-	3	-	-	-
Caramel, honey, floral	1109	*p*-mentha-1,5,8-triene	A	M15	5	-	6	-	-	4
Tomato, spicy	1112	β-thujone	A	M14	-	-	-	5	5	-
Floral, musty, green	1166	citronellal	A	M16	-	7	4	5	6	-
Make-up powder, herbal, floral	1195	(*E*)-dihydrocarvone	A	M17	6	-	4	4	6	5
Floral	1231	β -cyclocitral	A	M18	-	-	6	-	-	-
Spearmint	1245	carvone	A	M19	-	-	6	5	-	3
Herbal, pine, minty	1253	L-carvone	A	M20	-	7	6	6	4	6
Oily, woody	1259	D-carvone	B, C		5	-	5	-	-	-
Monoterpenoid alcohols										
Woody, red fruit	1103	linalool	A	MA1	3	-	-	4	-	-
Herbal, cooked	1116	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	A	MA2	-	-	4	4	-	-
Cucumber, floral, woody	1150	pinocarveol	A	MA6	-	-	6	7	-	4
Mushroom, earthy, metallic	1180	terpinen-4-ol	A	MA7	-	7	3	3	-	-
Herbal	1207	γ -terpineol	A	MA9	-	-	-	4	-	-
Bread, creamy	1214	(*Z*)-carveol	A	MA10	-	-	5	5	4	-
Pine, spicy	1292	thymol	A	MA11	-	3	4	-	-	-

Herbal, starchy	1314	carvacrol	A	MA12	-	-	5	-	-	-
Herbal	1346	*(E)*-8-hydroxylinalool	A	MA13	-	3	-	-	-	-
Sesquiterpenes										
Cucumber skin, fatty	1366	(+)-cyclosativene	A	S1	-	-	3	-	3	-
Damp, bread, woody	1390	α-copaene	A	S2	-	-	4	5	6	4
Sweet, earthy	1443	β-caryophyllene	A	S3	-	-	4	-	-	3
Floral, vegetative, woody	1478	α-humulene	A	S4	-	-	4	-	4	-
Floral, rose, woody	1495	β-selinene	A	S7	-	5	4	5	5	-
Creamy	1513	α-selinene	A	S9	-	3	-	-	-	-
Vegetative	1555	kessane	A	S11	-	-	3	-	-	-
Phthalides										
Celery, vegetables	1603	3-propylidene phthalide	A	PH1	-	3	-	-	-	-
Dried celery, parsley	1660	3-*n*-butylphthalide	A	PH2	-	5	5	-	-	-
Dried celery	1676	(*Z*-butylidenephthalide	A	PH3	-	-	-	4	-	-
Dried celery	1698	*cis*-ligustilide	B, C		5	-	6	4	5	5
Fresh celery	1709	*(E)*-butylidenephthalide	B, C		7	5	6	-	-	3
Cooked celery	1715	sedanolide	B, C		6	6	6	4	5	5
Celery	1731	sedanenolide	A	PH4	6	7	6	5	5	5
Dried celery	1742	neocnidilide	A	PH5	6	7	5	-	-	-
Celery	1752	(*E*)-ligustilide	A	PH6	-	-	4	7	3	-
Furans										
Caramel, rose, strawberry	1081	furaneol	B, C		7	5	5	6	5	5
Unknowns										
Floral, fruity	608	unknown			-	-	3	-	-	-
Floral	625	unknown			-	-	3	-	-	-

Buttery, dairy	632	unknown			-	-	4	4	4	3
Plastic, green, musty	768	unknown			-	-	4	-	5	3
Fresh lime, citrus	808	unknown			4	-	-	-	-	-
Floral, fruity, green	817	unknown			-	-	4	-	6	3
Pungent, cheese	842	unknown			-	-	5	-	4	-
Lemon, soil	913	unknown			-	-	-	-	5	-
Bread	918	unknown			-	-	-	-	-	3
Mushroom, soil	971	unknown			-	-	6	-	-	-
Smokey	1130	unknown	A	UN3	-	-	-	5	-	-
Woody, floral	1284	unknown	A	UN5	-	-	-	5	6	-
Smoked tomato, musty	1324	unknown			-	5	-	-	-	-
Vegetative, woody	1631	unknown			-	5	4	-	-	-
Dried celery	1649	unknown			-	-	5	-	-	-
Fresh celery	1722	unknown			-	6	6	-	5	-
Rotten celery	1765	unknown			-	4	4	-	-	-
Celery	1780	unknown			6	-	4	6	3	-
Celery	1800	unknown			-	-	-	5	3	-
Cooked celery	1816	unknown			5	3	-	-	-	-
Celery	1855	unknown			5	-	-	-	-	-
Total compounds					43	39	77	51	48	31

406
407
408
409
410
411

[a] Linear retention index (LRI) on DB-5 column, calculated from a linear equation between each pair of straight chain n-alkanes C_6-C_{25}. [b] Means of identifying compound (A-Mass Spectrometry B- LRI C- Aroma note recognitions). [c] Average odour intensity recorded by three assessors recording each maturity in duplicate except line 22 where only one was completed. (scoring scale: weak = 3, medium = 5, strong = 7), - = not detected. [d] Code corresponds to compounds identified in Table 1. [e] Prematurity time-point. [f] Commercial maturity time-point. [g] Post-maturity time-point. An average odour intensity was taken by collecting the average scores from the duplicates of each assessor and dividing by the number of GC/O runs completed for the genotype and maturity. The value of average odour intensity was rounded up/down to the nearest whole number.

3.2 Human olfactory analysis using GC-O shows that genotype influences development of off-flavours

In total, 103 different odours were detected in the headspace of the two celery genotypes across three different maturities using GC/O. Out of these, 65 compounds were identified using a combination of GC/MS analysis, LRI comparison to authentic standards and using the aromas they were described with (Table 2). Similarly to the chemistry described by GC/MS (Table 1), differences between genotype as the crop developed is evident in Table 2, with the absence/presence of compounds within genotypes contributing different odours to the overall aroma profile and thus indicating that genotype plays a role in the synthesis of odours that may indicate quality decline.

Within the samples, 18 monoterpenes, 12 alcohols, 11 aldehydes, ten ketones, nine monoterpenoid alcohols and phthalides and other compounds including esters (acetates and non-acetates) and sesquiterpenes were identified respectively. Out of the 103 odours that were identified, only nine of these compounds appeared in both genotypes and across the three maturities (Table 2). Across these compounds, it can be observed that line 12 had the highest recorded intensity for all of these compounds apart from hexanal and *(E,E)*-2,6-nonadienal. In the majority of the cases, the compounds were at their highest intensity at M1 and started to decrease thereafter, with a subset then showing an increase between M2 and M3.

In M1, 43 and 51 compounds were identified in the two genotypes respectively, with the majority of these compounds being monoterpenes (sabinene, β-pinene, limonene and γ -terpinene) and alcohols (1-hepten-3-ol, 1-octen-3-ol and 1-nonanol), all averaging intensity scores of around five and six (Table 2). No sesquiterpenes were not detected in M1 line 12, however, α-copaene and β-selinene were both detected within M1 line 22 at an intensity of five. β-selinene was identified as having a high abundance in GC/MS (Table 1) for both line 12 and 22 across all maturities. The absence of these compounds is with agreement with the

PCA plots, whereby monoterpenes show a high association with M1 with low sesquiterpene association. Aldehydes (benzeneacetaldehyde, (E,E)-2,6- and (E,Z)-2,6-nonadienal), ketones (3-pentanone, 2-hexanone and 3-octen-2-one) were detected to have a high average odour intensity in line 12, contributing cucumber, herbal and green odour notes however, only 2-pentanone was detected in line 22.

Among some of the compounds that were identified with a high average odour intensity, compounds with 'mushroom' and 'earthy' odours were very much apparent. These included 2- and 3-heptanol, 1-octen-3-ol, sabinene and β-pinene. It could be suggested that these mushroom smelling compounds are key contributors to a M1 celery odour. Out of these compounds, sabinene and β-pinene were identified by the GC/MS and exhibited high abundance at M1. In terms of phthalides, *(E)*-3-butylidenephthalide had an odour intensity of seven at M1 line 12 yet *(E)*-3-butylidenepht was not identified in line 22. Sedanenolide and sedanolide were identified throughout maturity and at a high average odour intensity for both genotypes, reflected in Table 1 also.

A study completed by Macleod and Ames (1989) identified *(E)*-3-butylidenephthalide, sedanolide and sedanenolide in supermarket purchased celery using GC/MS and GC/O. *(E)*-3-Butylidenephthalide was identified to have an odour of 'cooked celery', *(E)*-sedanolide and sedanenolide were both identified to have an odour of 'celery' as well as being 'pungent'. Although not identified in line 12, *(E)*-ligustilide appeared to be an important compound for line 22, showing a high average odour intensity at M1 with a gradual decrease to not being detected in M3. Neocnidilide exhibited a consistently high odour intensity across the different maturities in line 12, reaching and average odour intensity of seven at M2 before decreasing to five in M3.

At M2, 39 and 48 compounds were identified in line 12 and 22 respectively. A wide variety of compounds were observed at this time point, including a mixture of monoterpenes,

alcohols, aldehydes and phthalides. Key odour descriptors for commercial mature celery include fresh, green, herbal and earthy. These odours are achieved by compounds such as hexanal, β-pinene and phthalides such as neocnidilide and sedanenolide, all scoring at an intensity five and above (Table 2). According to Table 2, the aroma profile of line 22 appeared to be more complex, with more compounds being identified at M2 than line 12 including more alcohols, ketones, esters and monoterpenes. However, more phthalides were detected in line 12 and at a higher average odour intensity. Therefore, although fewer compounds were identified in line 12 M2, it can be hypothesised that this genotype at commercial maturity had a strong celery aroma due to its high phthalide content, whereas line 22 had more odours that are green, grass-like and earthy. Sedanenolide was detected at its highest average odour intensity here and similar to the results reported in Table 1, line 12 reports the highest relative abundance for phthalides when compared to line 22 and is at its highest at M2. Likewise, Kurobayashi et al. (2006) reported sedanenolide, 3-n-butylphthalide, *(E)*- and *(Z)*-sedanolides as having the highest flavour dilution factor upon completion of AEDA. Further stating that odour descriptors of these compounds are similar to the expected celery odour and are possibly the more significant contributors to its odour.

Progressing onto M3, line 12 had the highest number of compounds detected here with 77, conversely line 22 had only 31 compounds detected, the lowest number out of all samples analysed. Here, genotypic differences are very apparent, contradicting Figure 1 whereby M3 showed to have the fewest differences caused by genotype, whereas Table 2 supports the hypothesis that genotype determines how the crop matures. Correspondingly shown in Table 1, the highest number of monoterpenes were identified here and monoterpenoid alcohols such as terpinen-4-ol and (Z)-carveol for line 12. Conversely, these compounds were detected earlier on in maturity in line 22 and not detected at M3, potentially indicating that line 22 was further along maturity that line 12. No odour with an intensity above six was detected for both lines,

showing an obvious decline in aroma quality and intensity. L-Carvone was the compound with the highest intensity in M3 line 12 and 22, with herbal, minty and pine odour descriptors.

Only four phthalides were identified with a relatively low odour intensity and compounds such as 3-n-butylphthalide, neocnidilide and *(E)*- ligustilide were not detected at all in line 22 at M3. The absence of these odour active compounds with odour descriptors such as "celery, fresh celery, dried celery" could possibly imply that M3 line 22 did not have the mature celery odour that line 12 may have. On the other hand, line 12 M3 shows an abundance of these phthalides as well as unknown compounds that express a range of celery odour descriptors from cooked, dried and rotten celery. As line 12 was very abundant in these phthalide compounds (Table 1), it could be that phthalide compounds that could not be detected on GC/MS contributed to off-odours and therefore, aroma quality decline.

Within M3, there were compounds present that were not previously detected by the assessors; these include bornyl acetate, β-caryophyllene and carvacrol (line 12). The odour descriptors that were used to describe the compounds present were 'bread', 'woody', 'sweet' and 'starchy'. The sesquiterpene, α-copaene was identified across all maturities for line 22, yet was only detected in line 12 at M3, with odour descriptors including damp, bread and woody, it is possible that this is an indicator for deterioration in line 22. On the other hand, these compounds have been reported in previous investigations (Pino, Rosado & Fuentes, 1997; Marongui et al., 2013) and identified in GC/MS (Table 1). It could be possible that these compounds with 'starchy' and 'bread' odours could impart a negative odour on the maturity and are synthesised at a higher quantity as the vegetable matures. Due to the nature of GC/O, it is not possible to conclude that these compounds were responsible for off-odours within celery. Using sensory analysis to profile these celery maturities alongside this will help give a better indication of flavour defects within the crop.

Overall, comparing the odours between the three maturity stages and the two genotypes, it was observed that the most odours were identified in line 12 at M3, and a high average odour intensity compared to line 22 and other maturity stages. Despite M2 line 12 expressing a lower number of odours in comparison to M3 line 22, the average odour intensities of these compounds were much higher, particularly for phthalide compounds. From this it can be assumed that at M2 line 12 had a much more distinct odour profile than line 22 and as line 12 matured, it remained aromatic, therefore, having a better field holding capacity and possibly exhibiting a slow bolting trait.

In terms of aroma development, it can be seen that M1 exhibited a high proportion of monoterpenes and alcohols contributing to a fresh, fruity and citrus odour and low intensities of phthalides. The intensity of phthalides increased to M2, whereby a more typical celery odour was observed. Together with monoterpenes, aldehydes, sesquiterpenes and phthalides, the celery odour was present along with subtle floral, woody and herbal notes, whilst remaining fresh and green. As the crop developed beyond commercial maturity these fresh, green notes were at their minimum or not detected. At this stage, the aroma profile was much more herbal and woodier.

Together with 3-*n*-butylphthalide and sedanenolide, neocnidilide could be considered an important compound to the aroma. Although identified in Table 1 at a lower relative abundance, neocnidilide scored a high average odour intensity scored across line 12 in all maturities (Table 2). This is supported by Marongiu et al., (2013), who identified neocnidilide at high abundance across four celery extracts using two varieties grown in Portugal and Spain, extracted using supercritical carbon dioxide extraction as well as hydrodistillation. Despite the two different extraction methods yielding different results, neocnidilide comprised the majority of the aroma profile of both varieties and extraction methods. Furthermore, Shojaei Ebrahimi & Salimi (2011) identified *(E)*-3-butylidenephthalide and *(Z)*-ligustilide as key phthalides in

wild celery, as reflected correspondingly by the GC/O data, whereby these two compounds were scored at a high intensity for line 12 across all maturities. Ligustilide was only identified in M3 for line 12 but more apparent in line 22 (Table 2).

Interestingly, the compound benzeneacetaldehyde, with a characteristic odour of honey, floral and rose, was found at high abundance in M1 line 22 on the GC/MS data and remained high across maturity. A similar observation was made with line 12, albeit at a lower abundance. Conversely on the GC/O, benzeneacetaldehyde was detected in both genotypes across three maturities, with M1 line 12 exhibiting a stronger average odour intensity. Though not commonly identified in *A. graveolens*, Shojaei et al. (2011) identified benzeneacetaldehyde in three ecotypes of wild celery grown in three regions of Iran (0.13 %, 0.03 % and 0.08 % respectively) using GC/MS on essential oil.

As there have been limited studies investigating the development of celery aroma over maturity and that combine both GC/MS and GC/O analytical techniques to investigate celery aroma, comparison with other datasets is difficult. Therefore, studies that have used GC/O or GC/MS separately have been utilised. Although commonly used, SPME may not be able to extract all the compounds present in the isolate due to the low concentrations of some flavour compounds (Lui, Su & Song, 2018). SAFE, as used by Kurobayashi et al. (2006), combined with GC/O, AEDA and sensory profiling would give a more representative aroma profile. Using a method such as AEDA allows for the detection of further compounds that were identified in GC/MS. Due to the abundance of limonene within celery (Table 1) and the multiple terpene compounds that co-elute with limonene (Table 2), the likelihood of assessors missing or not detecting these compounds are high during GC/O. Although multiple training sessions were completed prior to GC/O, the ability for the assessor to separate and determine these compounds presents difficulties and therefore, only compounds with the lowest odour thresholds are detected. Carrying out various dilutions through AEDA will lead to the detection

of compounds with higher odour thresholds that would have been otherwise masked by limonene, building a broadened profile of celery aroma. Furthermore, harvesting vegetable crops at more time points leading up to and after commercial maturity will help to assess the changes in the volatiles profile further. Exploiting different seasons, geographical locations with diverse climates and using different cultivars could help build a better understanding on how celery aroma develops and how is influenced by the various factors.

4. Conclusion

Out of the two genotypes that were used in this experiment, line 12 exhibited a higher abundance for the majority of volatile compounds as well as more odours present when observing the GC/O data. The abundance of these compounds indicated that this genotype may have a more distinctive and complex aroma profile with green, herbal and floral notes along with strong celery notes, contributed from the high abundance of phthalides detected. In contrast, line 22 indicated a more subtle aroma, more similar to cucumber during maturity, but as the crop developed, there was a bigger change in aroma than seen in line 12, with odours developing that suggested a decline in quality. The stability of line 12 in this study shows that genotype could influence field holding capacity.

Monoterpenes contributed to the fresh, piney and earthy notes and were more abundant at prematurity and commercial maturity. The woodier and herbal notes developed as the crop matured and compounds such as sesquiterpenes, monoterpenoid alcohols and most importantly, phthalides were the main contributors to this aroma. Phthalides have been shown in this study, as well as in a plethora of other experiments, to be significant contributors to celery aroma with high relative abundances identified by GC/MS and high average odour intensities from the GC/O; with odour descriptors including ‘celery’ and ‘herbal’.

According to the data presented, the development of the aroma profile of *A. graveolens* changed over time; it commenced as fresh and fruity, progressed to herbal, woody and celery at commercial maturity, and shifted completely away from fresh and fruity towards woody, floral and damp odours at post-maturity. In order to confirm this, the addition of sensory profiling and more sensitive methods of chemical analysis are required. As shown in this study, developmental maturity has a bigger influence over aroma than genotype. However, genotype determined the way in which the flavour profile developed either through driving the synthesis of new compounds, reducing the synthesis of existing compounds, or driving the degradation of existing compounds.

These insights, especially when combined with future consumer preference studies, will provide celery growers with desirable aroma profile targets that will ensure that the crop is harvested at the optimum developmental stage. Growers should avoid taking a late harvest, even though this may improve yield, since the organoleptic profile of the crop will be compromised as overmature celery exhibit odours of lower intensity and compounds that may distort the flavour profile. This information will be useful to guide breeders to develop varieties that maintain an optimal aroma profile over a longer growing period. Furthermore, celery breeders now have access to biochemical information to assist breeding programmes and develop genotypes with improved field holding capacity which retain desirable aroma profiles.

CRediT authorship contribution statement

Conceptualization, L.T., C.W and S.L.; methodology, L.T. and S.L.; software, L.T. and S.L.; validation, L.T. and S.L.; formal analysis, L.T. and D.D.; investigation, L.T., D.D. and S.L.; resources, L.T.; data curation, L.T.; writing - original draft preparation, L.T..; writing - review and editing, L.T., D.D., F.G., C.W. and S.L.; supervision, S.L., F.G. and C.W.; funding

acquisition: F.G. and C.W. All authors have read and agreed to the published version of the manuscript.

Declaration of interests

Author FG is employed by the company A.L. Tozer Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. LT is funded by a BBSRC CASE PhD studentship reference BB/M016579/1 in partnership with A.L. Tozer Ltd.

References

Asuming, W., Beauchamp, P., Descalzo, J., Dev, B., Dev, V., Frost, S. & Ma, C. (2005). Essential oil composition of four Lomatium Raf. species and their chemotaxonomy. *Biochemical Systematics and Ecology*. *33*(1), 17-26. https://doi.org/10.1016/j.bse.2004.06.005

Ehiabhi, O., Edet, U., Walker, T., Schmidt, J., Setzer, W., Ogunwande, I., Essien, E. & Ekundayo, O. (2006). Constituents of Essential Oils of *Apium graveolens* L., *Allium cepa* L., and *Voacanga africana Staph*. from Nigeria. *Journal of Essential Oil Bearing Plants.* *9*(2), 126-132. https://doi.org/10.1080/0972060X.2006.10643483

Fellman, J., Miller, T. & Mattinson, D. (2000) Factors that influence biosynthesis of volatile flavour compounds in apple fruits. *HortScience. 35*(6). https://doi.org/1026-1033. 10.21273/HORTSCI.35.6.1026

Gold, H., & Wilson, C. (1963). The Volatile Flavor Substances of Celery. *Journal of Food Science*, *28*(4), 484–488. https://doi.org/10.1111/j.1365-2621.1963.tb00231.x

Jalali-Heravi, M., Zekavat, B., & Sereshti, H. (2006). Characterization of essential oil components of Iranian geranium oil using gas chromatography-mass spectrometry combined with chemometric resolution techniques. *Journal of Chromatography A. 1114*(1), 154-163. https://doi.org/10.1016/j.chroma.2006.02.034

Karmakar, R., Pahari, P., & Mal, D. (2014). Phthalides and Phthalans: Synthetic Methodologies and Their Applications in the Total Synthesis, *114*(12), 6213–6284. https://doi.org/10.1021/cr400524q

Kurobayashi, Y., Kouno, E., Fujita, A., Morimitsu, Y., & Kubota, K. (2006). Potent Odorants Characterize the Aroma Quality of Leaves and Stalks in Raw and Boiled Celery. *Bioscience, Biotechnology, and Biochemistry*, *70*(4), 958–965. https://doi.org/10.1271/bbb.70.958

Liu, Y., Su, H., & Song, H. (2018). Comparison of Four Extraction Methods, SPME, DHS, SAFE, Versus SDE, for the Analysis of Flavor Compounds in Natto. *Food Analytical Methods.11*, 343-354. https://doi.org/10.1007/s12161-017-1005-0

Lund, E., Wagner, C. & Bryan, W. (1974). Oils recovered from celery packinghouse waste. *Florida State Horticultural Society.86.* 255-259

MacLeod, G., & Ames, J. (1989). Volatile components of celery and celeriac. *Phytochemistry*, *28*(7), 1817–1824. https://doi.org/10.1016/S0031-9422(00)97866-X

Marongiu, B., Piras, A., Porcedda, S., Falconieri, D., Maxia, A., Frau, M., Gonçalves, M., Cavaleiro, C. & Salgueiro, L. (2013). Isolation of the volatile fraction from *Apium graveolens* L. (Apiaceae) by supercritical carbon dioxide extraction and hydrodistillation: Chemical composition and antifungal activity. *Natural Poduct Research. 27*(17), 1521-1527. https://doi.org/10.1080/14786419.2012.725402

Mévy, J., Bessière Bessière, J., Greff, S., & Zombre, G., (2006). Composition of the volatile oil from the leaves of *Ximenia americana L. Biochemical Systematics and Ecology. 34*(7), 549-555. https://doi.org/10.1016/j.bse.2006.01.007

Nurzyńska-Wierdak, R., Gruszecki, R., & Kosior, M. (2018). Does preservation modify the essential oil content and chemical composition of leaf celery (Apium graveolens L. var. secalinum alef.)? Acta Scientiarum Polonorum, Hortorum Cultus, 17(6), 27–36. https://doi.org/10.24326/asphc.2018.6.3

Orav, A., Kailas, T., & Jegorova, A. (2003). Composition of the essential oil of dill, celery, and parsley from Estonia. *Proceedings of the Estonian Academy of Sciences. Chemistry*, *54*(4), 147–154.

Philippe, J., Suvarnalatha, G., Sankar, R. & Suresh, S. (2002). Kessane in the Indian celery seed oils. *Journal of Essential Oil Research. 14*(4), 276-277. https://doi.org/10.1080/10412905.2002.9699852

Pino, J., Rosado, A. & Fuentes, V. (1997) Leaf oil celery *(Apium graveolens L.)* from Cuba. *Journal of Essential Oil Research. 9.* 719-720. https://doi.org/10.1080/10412905.1997.9700822

Raffo, A., Sinesio, F., Moneta, E., Nardo, N., Peparaio, M., & Paoletti, F. (2006). Internal quality of fresh and cold stored celery petioles described by sensory profile, chemical and instrumental measurements. *European Food Research and Technology*, *222*(5–6), 590–599. https://doi.org/10.1007/s00217-005-0098-7

Rapparini, F., Baraldi, R., & Facini, O. (2001). Seasonal variation of monoterpene emission from *Malus domestica* and *Prunus avium. Phytochemistry. 57*(5). 681-687. https://doi.org/ 10.1016/S0031-9422(01)00124-8

Rozėk, E. (2007). Growth and yielding of leaf celery (Apium graveolens L.var secalinum Alef.) cultivated for two-cut harvests. *Herba Polonica*, *53*(3), 17–21.

Sellami, I., Bettaieb, I., Bourgou, S., Dahmani, R., Limam, F. & Marzouk, B. (2012). Essential oil and aroma composition of leaves, stalks and roots of celery (*Apium graveolens var. dulce*) from Tunisa. *Journal of Essential Oil Research. 24*(6), 513-521. http://dx.doi.org/10.1080/10412905.2012.728093

Shojaei, Z., Ebrahimi, A., & Salimi, M. (2011). Chemical composition of three ecotypes of wild celery (*Kelussia odoratissima*). *Journal of Herbs, Spices and Medicinal Plants*, *17*(1), 62–68. https://doi.org/10.1080/10496475.2011.560089

Spinelli, F., Cellini, A., Marchetti, L., Mudigere Nagest, K. & Piovene, C. (2011). Emission and function of volatile organic compounds in response to abiotic stress. *Abiotic stress in Plants – Mechanisms and Adaptions.* 367-394

Turner, L., Lignou, S., Gawthrop, F., & Wagstaff, C. (2021). Investigating the factors that influence the aroma profile of *Apium graveolens*: a review. *Food Chemistry.* https://doi.org/10.1016/j.foodchem.2020.128673

Turner, L., Lignou, S., Gawthrop, F., & Wagstaff, C. (2021) Investigating the Relationship of Genotype and Climate Conditions on the Volatile Composition and Sensory Profile of Celery (*Apium graveolens*). *Foods*. 10(6). https://doi.org/10.3390/foods10061335

van Wassenhove, F., Dirinck, P., Vulsteke, G., & Schamp, N. (1990). Effect of nitrogen fertilizers on celery volatiles. *Journal of Agricultural and Food Chemistry.38*(1). 220-226. https://doi.org/10.1021/jf00091a049

Yommi, A., Di Gerónimo, N., Carrozzi, L., Quillehauquy, V., Goñi, M., & Roura, S. (2013) Morphological, physicochemical and sensory evaluation of celery harvested from early to late maturity. *Horticultura Brasileira 31*(2), 236-241. https://doi.org/ 10.1590/s0102-05362013000200010

List of Tables and Figures

Table 1: Approximate quantities of volatile compounds identified in the headspace of celery using SPME GCMS harvested at three different maturity stages.

Table 2: Odour description and intensity of the volatile compounds detected by GC-O in the headspace of two celery genotypes harvested at three different maturity stages.

Figure 1: Principal component analysis of two different celery genotypes at three different maturities showing correlations with volatile compounds that are significant according to factors of maturity, genotype and the interaction of maturity x genotype: (A) Projection of samples (B) Distribution of volatile compounds (C) Key of compounds used to construct the PCA.

Supplementary data

Figure S1. Picture shows line 12 at M1, M2 and M3.

M1 M2 M3

Figure S2. Picture shows line 22 at M1, M2 and M3.

M1 M2 M3

715 **Table S1.** Compounds present in the headspace of celery samples as identified by GC/MS and

716 GC/O and LRI values.

Code[a]	Compound	LRI[b]	LRI[c]	Code[a]	Compound	LRI[b]	LRI[c]
A1	3-methyl-3-buten-1-ol	733	730	**M13**	*p*-cymene	1098	1099
A4	(E)-2-hexen-1-ol	867	867	**M14**	β-thujone	1112	1119
A3	1-hepten-3-ol	889	893	**M15**	*p*-mentha-1,5,8-triene	1109	1113
A7	1-nonanol	1174	1176	**M16**	citronellal	1166	1159
A8	1-decanol	1274	1272	**M17**	(*E*)-dihydrocarvone	1195	1195
AH1	(*E*)-2-pentenal	760	754	**M18**	β -cyclocitral	1231	1232
AH2	hexanal	801	802	**M19**	carvone	1245	1246
AH3	(*E*)-2-hexenal	855	855	**M20**	L-carvone	1253	1257
AH4	(*Z*)-4-heptenal	901	902	**MA1**	linalool	1103	1103
AH5	n-octanal	1005	1007	**MA2**	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	1116	1122
AH6	benzeneacetaldehyde	1045	1049	**MA6**	pinocarveol	1150	1152
AH7	2-hydroxybenzaldehyde	1057	1056	**MA7**	terpinen-4-ol	1180	1182
AH8	(*E,Z*)-2,6-nonadienal	1159	1156	**MA9**	γ -terpineol	1207	1210
AH9	(*E,E*)-2,4-nonadienal	1224	1221	**MA10**	(*Z*)-carveol	1214	1220
K1	3-hexanone	776	779	**MA11**	thymol	1292	1290
K2	1-octen-3-one	978	978	**MA12**	carvacrol	1314	1311
K3	2-nonanone	1083	1090	**MA13**	*(E)*-8-hydroxylinalool	1346	1342
E2	propyl 3-methylbutanoate	947	947	**S1**	(+)-cyclosativene	1366	1378
E3	bornyl acetate	1305	1297	**S2**	α-copaene	1390	1389
E5	carveol acetate	1332	1339	**S3**	β-caryophyllene	1443	1440
E6	hexyl hexanoate	1381	1385	**S4**	α-humulene	1478	1475
M1	α-thujene	931	932	**S7**	β-selinene	1495	1505
M3	camphene	959	958	**S9**	α-selinene	1513	1518
M5	sabinene	981	976	**S11**	kessane	1555	1554
M6	β-pinene	987	980	**PH1**	3-propylidene phthalide	1603	1600
M7	β-myrcene	997	991	**PH2**	3-*n*-butylphthalide	1660	1658
M8	α-terpinene	1025	1018	**PH3**	(Z)-butylidenephthalide	1676	1685
M10	limonene	1031	1034	**PH4**	sedanenolide	1731	1730
M11	γ -terpinene	1062	1063	**PH5**	neocnidilide	1742	1753
M12	terpinolene	1094	1093	**PH6**	(*E*)-ligustilide	1752	1758

717 [a]Code refers to compound code from Table 1. [b]LRI of compound detected through GC/O and confirmed through GC/MS analysis, Table 2.
718 [c]LRI of compound identified through GC/MS analysis, confirmed through authentic standards, Table 1.