

Spatial distribution of low molecular weight compounds in orange carrot root revealed by mass spectrometry imaging

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ABSTRACT

Carrot (*Daucus carota* L. subsp. *sativus* Thell.) is a root vegetable of fundamental importance in global food production. Its economic significance is attributed to its versatile applications, high nutritional value, and the presence of numerous phytochemicals with health-promoting properties. In this study, the spatial distribution of low-molecular-weight metabolites - including amino acids, sugars, organic acids, fatty acids and phytosterols, vitamins, carotenoids, phenols, flavonoids, volatile compounds (aldehyde and monoterpenes), and oxylipin - within cross-sections of orange-coloured carrot root was investigated using mass spectrometry imaging (MSI) with the ^{109}Ag nanoparticle-enhanced target ($^{109}\text{AgNPET}$). In addition, the biological roles and potential health effects of the identified compounds are reviewed.

Keywords: carrot, *Daucus carota*, mass spectrometry imaging, silver nanoparticles, low molecular weight compounds

1. Introduction

Carrot (*Daucus carota* L. subsp. *sativus* Thell.) is a root vegetable belonging to the celery family (*Apiaceae*). It plays a significant role in global agricultural production. According to data from the Food and Agriculture Organization (FAO), global carrot production in 2023 reached 41.39 million tonnes (FAO commodity code: 0426 - carrots and turnips) [1]. China is the world's largest producer, with 18.38 million tonnes, followed by Uzbekistan (3.44 million tonnes) and the Russian Federation (1.40 million tonnes). Within the European Union, the leading producers are Germany (0.79 million tonnes), France (0.62 million tonnes), and Poland (0.57 million tonnes). Notably, Poland ranks as the thirteenth-largest producer of fresh carrots globally.

Orange carrots are the most widely cultivated variety; however, other colour variants - including yellow, white, pink, red, purple, and black - are also grown. The colour of carrots results from the presence of various pigmented compounds. For example, carotenoids such as α -carotene and β -carotene give carrots their orange colour, xanthophylls contribute a yellow tint, and anthocyanins are responsible for a purple colour. In contrast, white carrots lack these pigment compounds [2]. Carrots are used in various culinary applications, including consumption in raw form or after roasting, steaming, blanching, or incorporating into stews, soups, cakes, and pies. Furthermore, they are extensively processed into products such as juices and baby food. The market also features various fresh-cut carrot products, including shredded carrots, slices, and snack-sized forms such as baby or mini carrots [3].

The carrot root typically consists of 86-88.8% moisture, 6-10.6% carbohydrates, 0.7-1% protein, 0.2-0.5% fat, and 1.2-2.4% dietary fibre. Carrots are also a valuable source of essential minerals, including calcium, magnesium, potassium, phosphorus, sodium, and various trace elements [4]. Carrot is a nutritionally important root vegetable

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rich in bioactive compounds, such as carotenoids (α - and β -carotene, lutein, lycopene, and zeaxanthin), phenolic compounds (including flavonoids), polyacetylenes (falcarinol, falcarindiol, and falcarindiol-3-acetate), and vitamins (C, E, K, thiamine, and choline). These compounds are known for their nutraceutical potential and associated health benefits. Phytochemicals present in carrots exhibit antioxidant, anticarcinogenic and immunomodulatory properties. Moreover, carrots have demonstrated anti-diabetic, cholesterol-lowering, cardioprotective, antihypertensive, hepatoprotective, antibacterial, antifungal, anti-inflammatory, and analgesic effects [5].

Conventional analytical methods, i.e. high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) and gas chromatography coupled with mass spectrometry (GC-MS), do not offer a reliable means of localising and imaging primary and secondary metabolites in plant tissues, including carrot storage root. Imaging mass spectrometry (MSI) can effectively address this limitation. For instance, Xiang et al. [6], using matrix-assisted laser desorption/ionisation imaging mass spectrometry (MALDI-IMS), visualised the spatial distribution of phthalate esters and plant metabolites such as sucrose, hexose sugars, β -carotene, stigmastrol, and lanostane-3-yl acetate in radish root. Wang et al. [7] demonstrated a new method based on laser desorption/ionisation mass spectrometry imaging (LDPI-MSI) for the *in situ* detection of carbendazim (CBZ) pesticide residues in carrot tubers.

In this study, LDI-MS imaging was used to analyse the spatial distribution of low molecular weight compounds in the storage root of the orange-coloured carrot, employing a silver isotope (^{109}Ag) nanoparticle-enhanced target. LDI-MS imaging with a ^{109}Ag nanoparticle-enhanced target provides high spatial resolution for mapping low molecular weight compounds in complex tissues such as carrot storage roots. The silver nanoparticles enhance ionisation efficiency, increasing sensitivity and enabling the detection of a broad range of metabolites. This label-free technique requires minimal sample preparation, preserving the native chemical distribution and allowing comprehensive, multiplexed metabolic profiling. A significant part of this work focused on describing the biological function of the detected compounds in relation to their localisation on the carrot cross-section surface. This exploratory study aimed to map the spatial distribution of low-molecular-weight metabolites in orange carrot root using MSI with a ^{109}Ag nanoparticle-enhanced target and to assess their biological significance.

2. Experimental

2.1. Plant material

The material studied was the storage root of the carrot (*Daucus carota*), purchased in April 2017 from a local grocery store in Rzeszów, Poland, and transported to the Laboratory of the Department of Inorganic and Analytical Chemistry at Rzeszów University of Technology. The orange-colored root was selected for its small size, relative juiciness, and softness. Mass spectrometry imaging was conducted immediately upon arrival at the laboratory, without any further storage.

2.2. Methods

2.2.1 ^{109}Ag NPET preparation

^{109}Ag NPET target plate was prepared as previously described in the publication by Nizioł et al. [8]. All solvents used in this work were of HPLC quality.

2.2.2 LDI MS imaging

The washed and dried carrot root was placed on blotting paper and transversely cut using a sterile razor blade. Optical photograph of cross-sectional fragment of the carrot root (Fig. 1A) was made with the use of an Olympus SZ10 microscope equipped with an 8-megapixel Olympus digital camera. The exposed surface of the section was gently but firmly pressed onto a steel plate coated with ^{109}Ag silver nanoparticles in such a way that the imprint faithfully reflected the carrot's cross-section (Fig. 1B). The plate was then inserted into a Bruker Autoflex Speed/ToF mass spectrometer equipped with a positive-ion reflectron, and the imaging area was defined. FlexImaging 4.0 software was used for data processing and analysis. The apparatus was equipped with a SmartBeam II 1000 Hz 355 nm laser. Laser impulse energy was approximately 100-190 μJ , laser repetition rate was 1000 Hz, and deflection was set on m/z lower than 80 Da. The m/z range was 80-1500 Da, spatial resolution of $50 \times 50 \mu\text{m}$. The experiments were made with 500 laser shots per individual spot with a default random walk applied. All spectra were calibrated with the use of silver ions ($^{109}\text{Ag}^+$, 15 calibration points). All ion images were generated within a $\pm 0.05\%$ m/z window using Cardinal MSI 1.6.

3. Results and discussion

Carrots develop thickened storage roots in which three main anatomical regions can be distinguished: the epidermis, the cortex, and the vascular cylinder. The epidermis, also referred to as the skin, externally covers the cortex. The cortex accounts for approximately 70% of the total root weight and serves primarily as a storage tissue. The central part of the root is the vascular cylinder, which is dominated by parenchyma cells that, like those in the cortex, store reserve substances. Directly beneath the innermost layer of the primary cortex lies the pericycle, whose cells retain meristematic activity and give rise to lateral roots [9].

Carrot quality is significantly influenced by genotype, soil properties, climate conditions, post-harvest storage, and processing methods. MS imaging using the monoisotopic ^{109}Ag allowed preparation of ion images, providing a spatial distribution of low molecular weight metabolites found in *Daucus carota* root. Data regarding compounds and their ions analysed in this work are presented in Table 1.

Table 1. Ion data from LDI-MSI analysis of a carrot root cross-section imprint using $^{109}\text{AgNPT}$

Compound name	Ion formula	<i>m/z</i>
Oxalic acid	$[\text{C}_2\text{H}_2\text{O}_4+\text{Na}]^+$	112.9845
Taurine	$[\text{C}_2\text{H}_7\text{NO}_3\text{S}+\text{H}]^+$	126.0219
Pinene	$[\text{C}_{10}\text{H}_{16}+\text{Na}]^+$	159.1144
Asparagine	$[\text{C}_4\text{H}_8\text{N}_2\text{O}_3+\text{K}]^+$	171.0167
Camphor	$[\text{C}_{10}\text{H}_{16}\text{O}+\text{Na}]^+$	175.1093
Arginine	$[\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2+\text{Na}]^+$	197.1009
Alanine	$[\text{C}_3\text{H}_7\text{NO}_2+^{109}\text{Ag}]^+$	197.9519
Pyridoxine (vitamin B ₆)	$[\text{C}_8\text{H}_{11}\text{NO}_3+\text{K}]^+$	208.0371
Ascorbic acid (vitamin C)	$[\text{C}_6\text{H}_8\text{O}_6+\text{K}]^+$	214.9953
Citric acid	$[\text{C}_6\text{H}_8\text{O}_7+\text{K}]^+$	230.9902
Octanal	$[\text{C}_8\text{H}_{16}\text{O}+^{109}\text{Ag}]^+$	237.0243
Tryptophan	$[\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2+\text{K}]^+$	243.0531
Lysine	$[\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2+^{109}\text{Ag}]^+$	255.0097
Apigenin	$[\text{C}_{15}\text{H}_{10}\text{O}_5+\text{H}]^+$	271.0601
Glucose/Fructose	$[\text{C}_6\text{H}_{12}\text{O}_6+^{109}\text{Ag}]^+$	288.9676
Linolenic acid	$[\text{C}_{18}\text{H}_{30}\text{O}_2+\text{Na}]^+$	301.2138
Methoxymellein	$[\text{C}_{11}\text{H}_{12}\text{O}_4+^{109}\text{Ag}]^+$	316.9778
Falcarindiol	$[\text{C}_{17}\text{H}_{24}\text{O}_2+^{109}\text{Ag}]^+$	369.0818
Lignoceric acid	$[\text{C}_{24}\text{H}_{48}\text{O}_2+\text{Na}]^+$	391.3546
Quercetin	$[\text{C}_{15}\text{H}_{10}\text{O}_7+^{109}\text{Ag}]^+$	410.9469
Stigmasterol	$[\text{C}_{29}\text{H}_{48}\text{O}+\text{H}]^+$	413.3778
Sucrose	$[\text{C}_{12}\text{H}_{22}\text{O}_{11}+^{109}\text{Ag}]^+$	451.0204
Phylloquinone (vitamin K ₁)	$[\text{C}_{31}\text{H}_{46}\text{O}_2+\text{K}]^+$	489.3130
Campesterol	$[\text{C}_{28}\text{H}_{48}\text{O}+^{109}\text{Ag}]^+$	509.2747
Dicaffeoylquinic acid (DCQ)	$[\text{C}_{25}\text{H}_{24}\text{O}_{12}+\text{K}]^+$	555.0899
Carotene	$[\text{C}_{40}\text{H}_{56}+\text{Na}]^+$	559.4274
Diferuloylquinic acid (DFQ)	$[\text{C}_{27}\text{H}_{28}\text{O}_{12}+\text{K}]^+$	583.1212
Cryptoxanthin	$[\text{C}_{40}\text{H}_{56}\text{O}+\text{K}]^+$	591.3963
Rutin	$[\text{C}_{27}\text{H}_{30}\text{O}_{16}+\text{H}]^+$	611.1607

3.1. Amino acids

Amino acids are of major importance in plant metabolism. In addition to their role in protein synthesis, amino acid metabolism is closely interconnected with energy and carbohydrate metabolism, the carbon-nitrogen balance, hormone and secondary metabolism, and responses to biotic and abiotic stresses [10]. In this study, five proteinogenic amino acids - arginine, alanine, lysine, asparagine, and tryptophan - and one non-proteinogenic amino acid, taurine, were detected in the edible root of carrot. Humans can synthesise several amino acids, including alanine and asparagine, whereas essential amino acids such as arginine, lysine, and tryptophan must be obtained through the diet.

Arginine (Arg) is the only proteinogenic amino acid with the highest nitrogen-to-carbon ratio, which makes it an important compound for nitrogen storage and transport in many plants. It also serves as an alternative source for proline biosynthesis. In addition, arginine is a precursor of nitric oxide (NO) and polyamines, both of which play key roles in regulating developmental processes and mediating responses to biotic and abiotic stresses [11]. Nitric oxide, in particular, is crucial for regulating various physiological functions in humans, including the enhancement of neurotransmitter release and modulation of the immune system [12]. In the carrot analysed, arginine was identified as the potassium adduct with m/z 197.1009 (Table 1, Fig. 1C). It was predominantly localised in the cortex, with markedly lower concentrations observed in the vascular cylinder, lateral root, and just beneath the skin. Alabran and Malbrouk [13], using ion-exchange chromatography, identified 20 nitrogenous compounds in the ethanol extract of fresh *Daucus carota* cv. Imperator, including arginine, which accounted for 5.23% of all detected compounds. Kapuler and Gurusiddiah [14] investigated the concentration of free arginine in eight carrot cultivars and found that the Nantes Fancy and Guerande varieties had the lowest levels of this amino acid (0.02 $\mu\text{mol/ml}$ of juice), whereas the Gelbe Rheinische H cultivar had the highest (0.92 $\mu\text{mol/ml}$ of juice). The application of a biostimulant containing non-pathogenic microorganisms (*Bacillus subtilis*, *Bacillus megaterium*, and *Trichoderma harzianum*) increased the levels of 11 amino acids in the roots of *Daucus carota* cv. Vitaminnaya 6, including arginine (20-fold) and lysine (34.5-fold) [15]. Cubero-Leon et al. [16] demonstrated that the cultivation regime (organic vs. conventional) affects the metabolite composition of carrots. Interestingly, arginine exhibited an increasing trend in organically grown carrots, despite the fact that only 10-15% of nitrogen is directly available from organic fertilisers. Eppendorfer and Eggum [17] reported that with increasing nitrogen concentration, the concentrations of all amino acids (expressed in g/kg dry weight) increased in the roots of *Daucus carota* cv. Nandor. The most pronounced increases were observed for arginine, glutamic acid, and aspartic acid.

Asparagine (Asn) has a nitrogen-to-carbon (N:C) ratio of 2:4, making it an efficient molecule for nitrogen storage and transport in living organisms. This amino acid accumulates in all plant organs during periods of low protein synthesis and high availability of reduced nitrogen. It also accumulates during normal physiological processes such as seed germination and nitrogen translocation. Furthermore, the accumulation of asparagine can be induced by both biotic and abiotic stresses, including pathogen attack, mineral deficiencies, drought, salinity, and exposure to toxic metals [18]. MS imaging revealed that the localisation of asparagine (m/z 171.0167; Table 1, Fig. 1C) in the analysed carrot tissue is similar to that of arginine, although slightly higher levels are observed in the vascular cylinder and slightly lower levels in the cortex. According to the literature, in fresh carrot roots grown organically with green manure, the concentrations of twelve individual amino acids, including asparagine and glutamine, increased linearly with the concentration of soil mineral nitrogen at the time of carrot emergence [19]. Stolz and Struble [20] identified twenty amino acids in carrots cv. Rodelika, with their percentage content in fresh matter ranging from 0.01% to 0.1%. The highest levels were recorded for asparagine and glutamine, accounting for 0.052% and 0.090%, respectively.

Alanine (Ala) is one of the simplest naturally occurring α -amino acids, formed via a transamination reaction between glutamate and pyruvate, catalysed by alanine aminotransferase. It plays a role in both carbon and nitrogen metabolism and is associated with chlorophyll synthesis and photosynthetic activity [21]. Alanine was identified as the silver-109 adduct with m/z 197.9519 (Table 1, Fig. 1C). It was present in very low abundance and exhibited an uneven distribution within the vascular cylinder. Alanine in the vascular cylinder plays a crucial role as a nitrogen carrier and energy source, enabling efficient transport and metabolism essential for the growth and division of lateral root cells. Through its conversion to pyruvate, it supports energy production and supplies substrates necessary for protein synthesis, which are vital for the proper development and differentiation of new roots. Alanine was also detected in purple carrots by Sciubba et al. [22] using high-resolution ^1H NMR spectroscopy. They observed that alanine levels in the carrot root more than doubled between November and December, increasing from 1.19 to 2.61 $\mu\text{mol/g}$ fresh weight. This rise may reflect the activation of the plant's defence mechanisms in response to abiotic stress, as waterlogging caused by heavy rainfall reduced oxygen availability in the soil. Alanine synthesis facilitates the removal of excess pyruvate generated under anaerobic conditions, thereby preventing the accumulation of lactic acid and ethanol during hypoxia. Zamana et al. [15] reported that the alanine concentration in the roots of *Daucus carota* cv. Vitaminnaya 6 was 2.12 g/kg dry weight (DW). According to the literature, a 30-day fermentation process significantly increases the alanine content in carrot slices; in the case of the Berlikum cultivar, the concentration reached 5.22 mg/g DW [23].

Lysine (Lys) is an essential amino acid for mammals but is typically present in low amounts in plants, thereby limiting their nutritional value. In plants, lysine is primarily catabolised via the saccharopine pathway, which is involved in responses to both abiotic and biotic stresses. Degradation intermediates of lysine also contribute to various metabolic processes, including tryptophan metabolism, the tricarboxylic acid (TCA) cycle, starch metabolism, and the unfolded protein response [24]. Lysine was detected as the adduct with the $^{109}\text{Ag}^+$ ion (m/z 255.0097; Table 1, Fig. 1C). Its localisation pattern resembles that of alanine; however, within the vascular cylinder, lysine is present at higher concentrations and forms more pronounced point-like clusters. Lysine in the vascular cylinder supports lateral root development by supplying essential nitrogen and participating in the

metabolism required for cell division and differentiation. The presence of lysine in carrots has been confirmed by previous studies. Nandula et al. [25], using HPLC, reported significantly lower concentrations of free lysine in roots of the Nantes Coreless variety parasitised by *Orobancha aegyptiaca* (0.4 $\mu\text{mol/g}$ dry weight) compared to non-parasitised roots (1.8 $\mu\text{mol/g}$ dry weight), while levels of other amino acids such as arginine, alanine, and histidine remained unaffected. Gao et al. [26], in a UPLC-MS/MS-based metabolomic study, detected 26 amino acids across three developmental stages in the Tianhong No.1 orange-red carrot cultivar, with L-lysine and L-glutamine showing rapid accumulation from root formation (stage S) through to maturity (stage H).

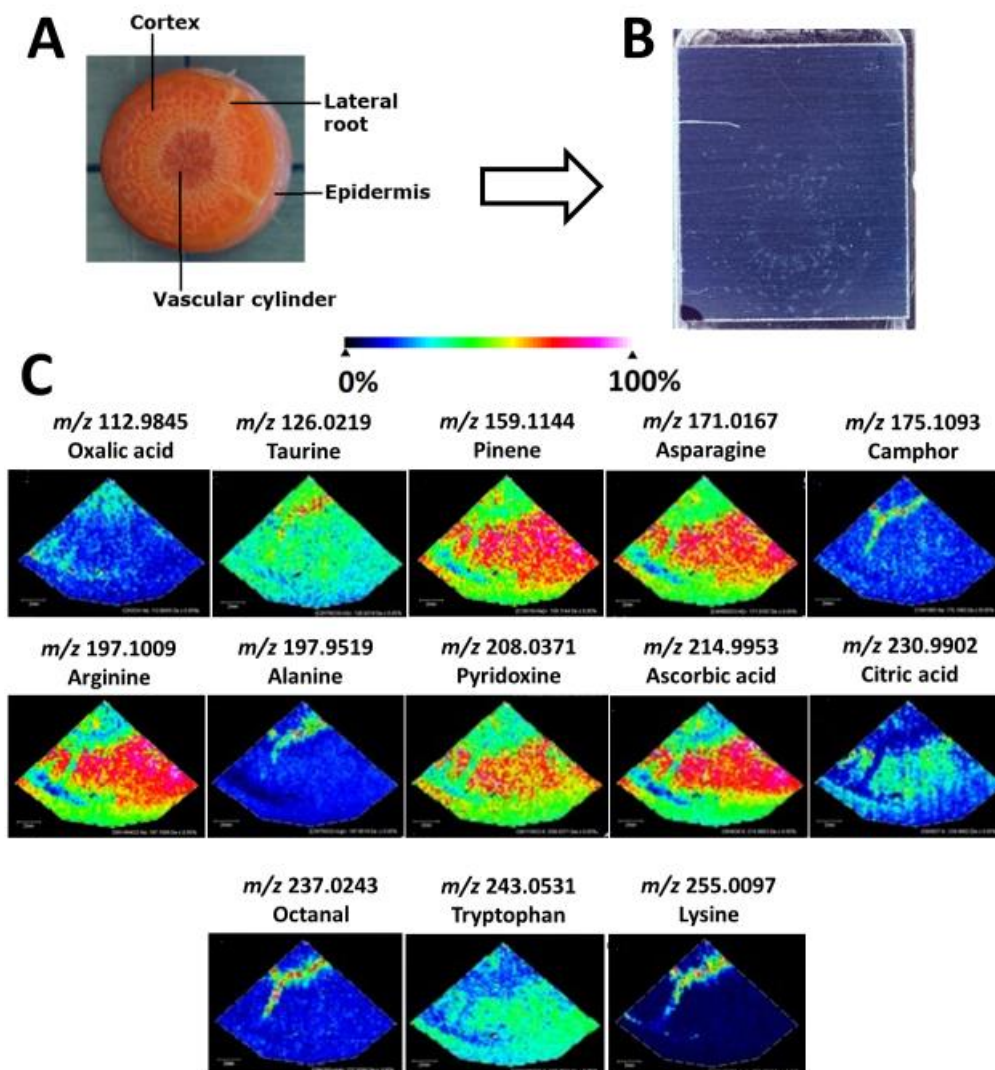


Fig. 1. Optical photograph of a cross-sectional fragment of carrot root (A). Panel B shows an optical image of the imprint of the analysed cross-section on a steel plate coated with a pre-prepared suspension of monoisotopic silver nanoparticles. C. Ion images (spatial resolution: 50 $\mu\text{m} \times 50 \mu\text{m}$) of the analysed carrot cross-section. Nominal m/z values and the names of compounds assigned to the ions of interest are displayed above each image.

Tryptophan (Trp) is an aromatic amino acid synthesised via the shikimate/chorismate pathway. Beyond its role as a protein constituent, Trp is especially important as a precursor in secondary metabolism, including the biosynthesis of key molecules such as auxin (indole-3-acetic acid), serotonin, and melatonin. These compounds participate in a wide range of physiological processes in higher plants, such as seed germination, root growth and development, senescence, flowering, fruit ripening, and responses to both biotic and abiotic stresses [27]. In this study, tryptophan was identified as the potassium adduct with the m/z of 243.0531 (Table 1, Fig. 1C). Its spatial localisation in the carrot is similar to that of histidine, although its concentration in the root cortex is lower. Notably, tryptophan was the only amino acid whose content in carrots of the Vitaminnaya 6 cultivar remained unaffected by biostimulant treatment, maintaining a concentration of 0.25 g/kg dry matter (DM) [15].

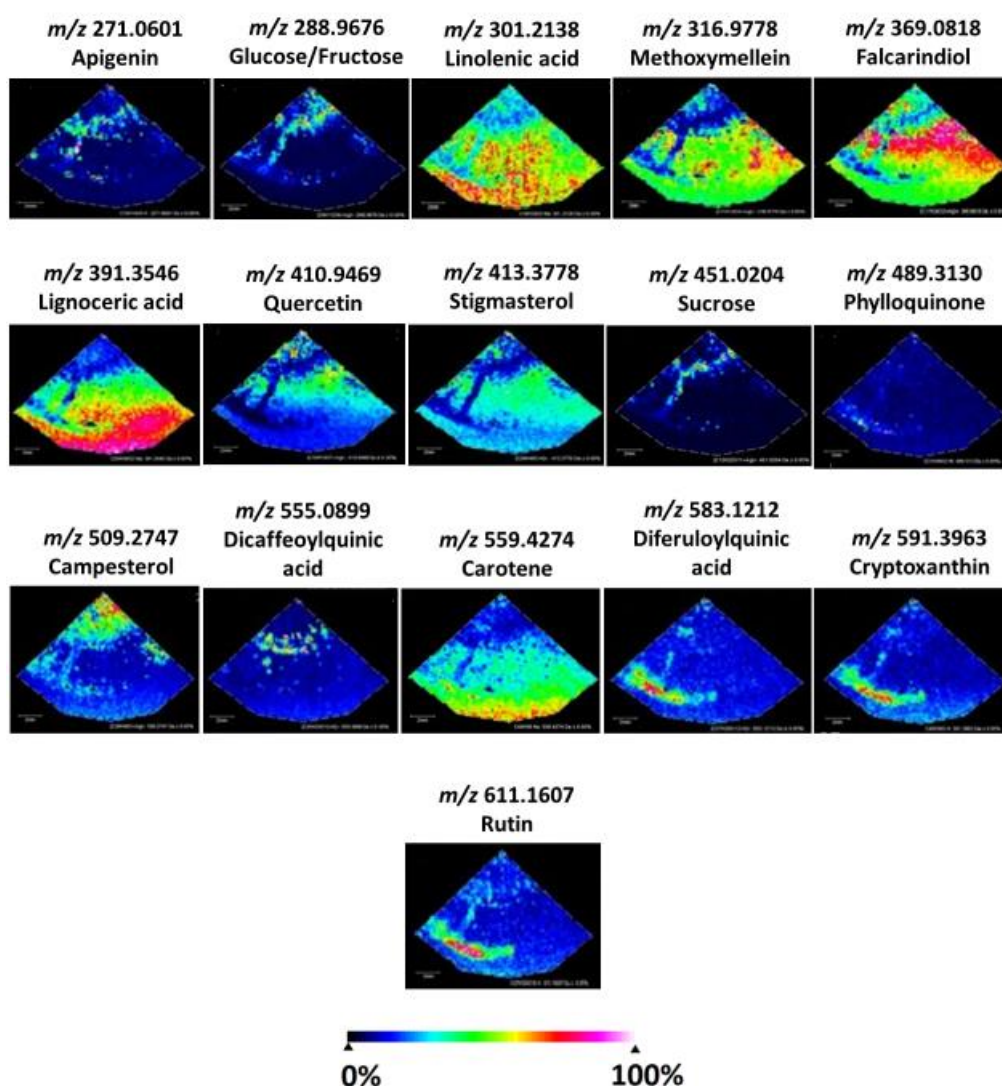


Fig. 2. Ion images (spatial resolution: $50 \mu\text{m} \times 50 \mu\text{m}$) of the analysed carrot cross-section. Nominal m/z values and the names of compounds assigned to the detected ions are indicated above each image.

Taurine is a sulphur-containing non-protein amino acid that acts as a natural regulator of the antioxidant defense network. In animals, taurine is involved in osmoregulation, membrane stabilization, maintenance of mitochondrial integrity, calcium homeostasis, and acts as an antioxidant. Taurine reduces oxidative damage primarily by enhancing the detoxification of reactive oxygen species (ROS). However, its physiological roles in plants remain largely unknown [28]. Reports in the literature suggest that exogenous taurine can enhance plant tolerance to abiotic stress [29]. In this study, taurine was detected as the proton adduct with the m/z of 126.0219 (Table 1, Fig. 1C). It is distributed throughout the carrot root, although its concentration is lower in the region near the peel compared to the rest of the cortex. In contrast, higher concentrations are observed in the peripheral zone of the vascular cylinder adjacent to the cortex, where taurine forms distinct clusters of very high intensity. Otsuka and Take [30] identified 18 proteinogenic amino acids and taurine in hot water extracts of carrot (carrot soup), using an automatic amino acid analyser. The concentration of taurine was reported to be 20.30 mg/100 ml of carrot soup.

3.2. Sugars

In cultivated carrots, carbohydrate reserves consist mainly of glucose, fructose, sucrose, and occasionally small amounts of starch. The disaccharide sucrose is the primary product of photosynthesis and is translocated from source tissues (e.g., mature leaves) to sink organs such as seeds, flowers, and roots, where it supports growth and development and may be stored as starch. In addition to its metabolic role, sucrose functions as a signalling molecule, regulating various physiological processes, including carbohydrate metabolism, storage protein

accumulation, sucrose transport, anthocyanin biosynthesis, and the induction of flowering [31]. The sweetness of carrots is primarily determined by the presence of sucrose and the two reducing sugars, glucose and fructose. Moreover, the perception of sweetness is further influenced by volatile compounds and soluble phenolic compounds.

In this study, two sugars were detected: sucrose and glucose/fructose. Sucrose was identified as an adduct with the $^{109}\text{Ag}^+$ ion at m/z 451.0204 (Table 1, Fig. 2), while glucose/fructose was assigned to the $^{109}\text{Ag}^+$ adduct at m/z 288.9676 (Table 1, Fig. 2). Both compounds exhibited a similar spatial distribution within the carrot root, being localised in the vascular cylinder and lateral roots; however, glucose/fructose was more abundant in the vascular cylinder than sucrose. The localisation of glucose/fructose and sucrose in these regions likely reflects their roles in energy supply and carbon transport, supporting cell division and growth during lateral root development. Notably, the localisation of glucose/fructose aligns with the findings of Xiang et al. [6], who, using MALDI-IMS, observed fructose/glucose accumulation in the central region of the vascular cylinder, identified as the protoxylem.

Studies on carrots of the Nantes demi-long cultivar have shown that the carbohydrate composition of the root changes significantly during ripening: sucrose levels double, while glucose and fructose concentrations decrease by approximately 40% and 65%, respectively. After the ripening phase, carbohydrate reserves continue to accumulate steadily, with sucrose increasing by about 35% and reducing sugars by 30% [32]. Kouřimská et al. [33] demonstrated that cropping systems (organic and integrated), as well as factors such as cultivar and plant density, significantly influence carrot quality parameters. Organic carrots of both cultivars (Aftalon F1 and Cortina F1) exhibited higher concentrations of sucrose, glucose, and fructose compared to those grown under integrated systems. More recently, Sitkey et al. [34] reported significant differences in the chemical composition of 24 carrot varieties cultivated in Slovakia in 2022. The Katlen variety contained the highest concentration of reducing sugars, followed by Vanda, Maestro F1, and Sugarsnax 54 F1. These findings may offer valuable insights for both carrot producers and consumers.

3.3. Organic acids

In the carrot analysed, two organic acids were detected and spatially localised: oxalic acid and citric acid. Oxalic acid is a naturally occurring dicarboxylic acid found in various organisms, including plants, algae, fungi, lichens, and animals. In plants, oxalic acid and its salts (oxalates) are present in all tissues, including leaves, petioles, flowers, tubers, and roots. Owing to its high acidity, redox activity, and metal-chelating properties, oxalic acid plays key roles in intracellular pH regulation, ion homeostasis, detoxification, redox balance, and plant defence. However, excessive accumulation - due to metabolic imbalance or environmental conditions - can impair plant growth, reduce yield, and increase pathogen susceptibility [35]. In humans, dietary oxalic acid may hinder mineral absorption, particularly calcium, and is associated with disorders such as hyperoxaluria and systemic oxalosis. As a result, it is considered a potential anti-nutrient. Various mitigation strategies - such as thermal processing, fermentation, or biotechnological approaches - have been developed to reduce its content in foods [36].

In this study, oxalic acid was detected as the sodium adduct at m/z 112.9845 (Table 1, Fig. 1C). It was localised in both the vascular cylinder and the cortex of the carrot root; however, its distribution within the cortex was heterogeneous, with clusters observed in the central cortical region. Kim et al. [37] analysed the oxalic acid content of 32 raw vegetables commonly consumed in Korea using HPLC and reported that carrots contained 16.2 mg of soluble oxalates per 100 g fresh weight, accounting for 99.1% of the total oxalate content. According to Singh [38], the oxalic acid content in red (Gajar Lal) and yellow (Gajar Pili) carrot varieties was 200.7 mg and 118.6 mg per 100 g fresh weight, respectively. Chai and Liebman [39] investigated the effects of various cooking methods on oxalate content in vegetables and found that steaming reduced the soluble and insoluble oxalate levels in carrots by 53% and 42%, respectively.

Citric acid (CA) is a naturally occurring six-carbon tricarboxylic acid present in plant cells. It is biosynthesised via the tricarboxylic acid (TCA) cycle in mitochondria or through the glyoxylate cycle in glyoxysomes. Once transported into the cytosol, CA can be either immediately utilised in cellular metabolism or stored in the vacuole to help maintain cytosolic pH [40]. Plants growing in alkaline soils secrete citric acid and malate from their roots, acidifying the rhizosphere and thereby enhancing the availability of essential nutrients such as phosphorus and iron. Moreover, exogenous application of CA has been associated with improved tolerance to various abiotic stresses, including salinity, drought, mineral deficiency or excess, alkalinity, and temperature extremes [41].

The potassium adduct with the m/z of 230.9902 was assigned to citric acid (Table 1, Fig. 1C). Unlike oxalic acid, citric acid was predominantly localised in the cortex of the analysed carrot. Local carrots from Tiggiano (southern Italy), with yellow, orange, and purple epidermis, contained lower citric acid levels compared to the commercial orange variety used as a control, which may influence their organoleptic properties, including the perception of astringency [42]. According to Latvian researchers [43], steam treatment and drying affected the concentrations of organic acids, including citric acid, in carrot roots. Among the 11 organic acids detected, citric acid exhibited the highest concentration in fresh carrots, reaching 1074.49 mg/100 g dry weight (DW). Interestingly, a 3-minute steam blanching reduced the citric acid content to 215.49 mg/100 g DW, while drying at

45 °C led to a nearly fourfold increase, reaching 4163.34 mg/100 g DW. In addition, the aluminium-tolerant carrot cell line TA-1 (*Daucus carota* L. cv. MS Yonsun) was found to release greater amounts of citric acid into the medium compared with the parental cell line SO-1. The secreted citric acid was capable of chelating aluminium ions, thereby contributing to enhanced aluminium tolerance [44].

3.4. Fatty acids and phytosterols

Fatty acids (FAs) are essential for the normal functioning of all living organisms. They serve as key components of plasma membranes, act as energy storage molecules, and function as signaling mediators involved in the regulation of cell growth, differentiation, and gene expression. In higher plants, the most common unsaturated fatty acids (UFAs) are the three 18-carbon species: oleic acid (C18:1), linoleic acid (C18:2), and α -linolenic acid (C18:3). UFAs play a crucial role in plant defense against a wide range of biotic and abiotic stresses, including pathogens, drought, extreme temperatures, and exposure to heavy metals [45]. One of the primary adaptive responses to such stress factors involves modifications in membrane lipid composition. Notably, the profiles of very long-chain fatty acids (VLCFAs), including lignoceric acid (C24:0), are known to undergo both qualitative and quantitative changes under stress conditions [46].

Plant sterols, or phytosterols, are essential components of membrane lipids, contributing to membrane fluidity and permeability and playing roles in signal transduction. Phytosterols are present in small amounts in a wide variety of fruits, vegetables, nuts, seeds, cereals, and legumes. These compounds are recognized for their pharmacological properties, including anti-inflammatory, anti-diabetic, anti-cancer, and cholesterol-lowering effects [47].

In this study, the spatial localisation of two fatty acids - linolenic acid and lignoceric acid - and two plant sterols, stigmasterol and campesterol, was identified. Ions assigned as sodium adducts of linolenic acid (m/z 301.2138; Table 1, Fig. 2) and lignoceric acid (m/z 391.3546; Table 1, Fig. 2) were detected in the highest amounts in the cortex of the analysed root. Among the identified fatty acids, lignoceric acid was found at the highest concentration. The protonated stigmasterol adduct at m/z 413.3778 (Table 1, Fig. 2) and the campesterol adduct with $^{109}\text{Ag}^+$ at m/z 509.2747 (Table 1, Fig. 2) exhibited distinct spatial distributions in carrot tissue. Specifically, stigmasterol was predominantly localised in the cortex, whereas campesterol was mainly found in the vascular cylinder.

Aremu et al. [48] analysed the lipid profile of dried carrot (*Daucus carota* L.) samples and reported the presence of several fatty acids, including linolenic acid (6.26%) and lignoceric acid (0.08%). The carrots analysed also contained notable levels of phytosterols, with stigmasterol and campesterol present at concentrations of 118.42 mg and 34.48 mg per 100 g of sample, respectively. Similarly, carrot root oil obtained by supercritical carbon dioxide (SC-CO₂) extraction was found to contain 4.9% linolenic acid and 0.2% lignoceric acid of total fatty acids. This oil was also rich in sterols, particularly campesterol and stigmasterol, with concentrations of 4159.8 mg/kg and 3203.8 mg/kg, respectively, among individual sterols and triterpene dialcohols [49].

Campesterol and stigmasterol were also detected in carrots consumed in Sweden, with concentrations of 2.2 mg/100 g and 2.8 mg/100 g of the edible portion, respectively [50]. Interestingly, carrot juice was found to be particularly rich in plant sterols, containing two to three times more sterols than other vegetable juices, with stigmasterol and campesterol concentrations of 1270 and 677 $\mu\text{g}/100$ ml, respectively. However, fresh carrots are not especially rich in plant sterols, suggesting that juice processing technology and the quantity of carrots used per millilitre play a significant role in determining sterol content [51].

3.5. Vitamins

Another group of compounds identified in carrots was vitamins, both water-soluble (ascorbic acid, pyridoxine) and fat-soluble (phyloquinone). L-ascorbic acid (vitamin C) is a water-soluble, low-molecular-weight compound that is highly abundant in plants. It occurs in all cell compartments, including the cell wall. Vitamin C is known to play a crucial role in regulating the redox potential in cells [52, 53]. It acts as an antioxidant, enzyme cofactor, and precursor in the synthesis of tartrate and oxalate. It is involved in many processes, including photosynthesis, photoprotection, resistance to environmental stresses, cell wall growth and expansion, and the synthesis of gibberellins, anthocyanins, hydroxyproline and ethylene. Humans are unable to synthesize vitamin C, so plants are the primary dietary source of this vitamin [54].

Vitamin C accumulation within the same plant species can vary depending on the cultivar, tissue type, and developmental stage [55, 56]. Despite this variability, vitamin C levels are tightly regulated through net biosynthesis, recycling, degradation/oxidation, and inter- and intracellular transport. In this study, vitamin C was identified as the potassium adduct at m/z 214.9953 (Table 1, Fig. 1C). It was distributed across the entire cross-sectional area of the carrot root, with the highest concentration observed in the central cortex. Lower levels were detected in the vascular cylinder, lateral root, and in the outer cortex just beneath the peel. According to the literature, differences in vitamin C content exist among carrot varieties. Matějková and Petříková [57] reported that the vitamin C content in six carrot varieties ranged from 54 mg/kg to 132 mg/kg, with significantly higher

levels found in the late cultivars Olympia and Tinga. Additionally, a 30-day storage period resulted in a substantial reduction in vitamin C content, averaging 47%. L-glutamic acid, recognized as an important biostimulant, was shown to increase ascorbic acid levels in carrot storage roots compared to the control following both foliar application and combined soil and foliar treatments [58].

Pyridoxine (vitamin B₆) is an important regulator of cellular metabolism in plants, functioning as a cofactor in various enzymatic reactions and acting as a potent antioxidant involved in responses to both abiotic and biotic stresses. In addition, it plays a crucial role in numerous metabolic, physiological, and developmental processes. Plants are capable of synthesizing vitamin B₆ *de novo* and serve as one of the primary dietary sources of this vitamin for humans. The potassium adduct at *m/z* 208.0371 was attributed to vitamin B₆ (Table 1, Fig. 1C). Its spatial distribution in the analysed carrot samples was similar to that of ascorbic acid; however, comparison of the two MS images indicates that vitamin B₆ is present at lower levels. Overall, the vitamin B₆ content in carrots is relatively low, amounting to just 0.12 mg per 100 grams [59]. Gürbüz et al. [60] reported a pyridoxine content of 0.93 mg/kg in carrots, with a bioavailability of 37%, as estimated through digestive modelling. More recently, the vitamin B₆ content in locally grown carrots of the Farovon variety was found to be 0.405 mg per 100 g of sample [61].

Phylloquinone is a prenylated naphthoquinone synthesised exclusively by plants, green algae, and certain cyanobacteria, where it functions as an essential electron carrier in photosystem I and serves as an electron acceptor in the formation of protein disulfide bonds. In humans and other vertebrates, phyloquinone acts as a vitamin (vitamin K₁), which is required for blood clotting as well as bone and vascular metabolism. Green leafy vegetables and vegetable oils are the primary dietary sources of phyloquinone for humans. Its biosynthesis in plants occurs in plastids and peroxisomes, and the intermediates of this pathway represent key metabolic branching points shared with other plastid-synthesised metabolites, such as chlorophylls, tocopherols, and salicylates [62]. In this study, phyloquinone was detected as the potassium adduct at *m/z* 489.3130 (Table 1, Fig. 1C) in very low amounts. It was localised in the cortex near the peel, appearing as a small clustered region. Aresta et al. [63] used gas chromatography-mass spectrometry (GC-MS) to determine phyloquinone concentrations in fresh and dried carrot extracts, reporting values of 2.6 µg/g and 8.1 µg/g of sample, respectively.

3.6. Carotenoids

Carotenoids are the main bioactive compounds in carrots [64]. These valuable phytochemicals vary in type depending on their chemical structure. For instance, carotenes - such as α-carotene and β-carotene - consist solely of carbon atoms, whereas xanthophylls - such as β-cryptoxanthin, lutein, and zeaxanthin - contain oxygen-bearing functional groups. Importantly, carotenoids serve as precursors of vitamin A, which is essential for vision and cellular regulation. In addition, carotenoids contribute to the prevention of cancer, bone-related diseases, oxidative cell damage, diabetes, obesity, and cardiovascular conditions.

In this study, the spatial localisation of two carotenoids, carotene and cryptoxanthin, was identified and mapped. In the orange carrot analysed, the sodium adduct at *m/z* 559.4274 (Table 1, Fig. 2) was assigned to carotene. It was predominantly localised in the cortex, with the highest concentration observed just beneath the peel. According to the literature, α- and β-carotene consistently represent the predominant carotenoids in orange carrots, although the proportion of α-carotene varies considerably among different varieties, ranging from 5% to 50% of the total carotenoid content [65]. Simon and Wolff [66] reported that 94-97% of the total carotenoid content in seven carrot lines grown across different years and locations was attributable to β-, α-, and ζ-carotene, while only 3-6% was due to β-zeacarotene, γ-carotene, and lycopene. Carrot extract obtained from a traditional market in Indonesia contained a total carotene content of 147 mg/100 g [67]. Using HPLC, Mech-Nowak et al. [68] demonstrated that, among the seventeen carrot varieties tested, the highest total carotenoid content (including all carotenoid types) and the highest β-carotene content were found in orange carrots (14 varieties). The most valuable varieties in terms of carotenoid content included Korund F1, Salsa F1, Afro F1, Kazan F1, Polka F1, Kongo F1, and Niland F1.

β-Cryptoxanthin is an oxidised carotenoid with a chemical structure similar to β-carotene, but it is more polar. It occurs in various fruits and vegetables and plays several important roles for human health. β-Cryptoxanthin exhibits antioxidant properties *in vitro* and may contribute to reducing the risk of certain cancers and degenerative diseases. In addition, several studies in animal models and humans suggest that β-cryptoxanthin-rich foods may exert anabolic effects on bone, potentially helping to delay the onset of osteoporosis. In this study, cryptoxanthin was assigned to the potassium adduct at *m/z* 591.3963 (Table 1, Fig. 2). It was present at a significantly lower concentration than carotene. In the MS image, it appears as a cluster near the peel, while in the vascular cylinder it is observed as small, scattered accumulations. The results obtained are consistent with literature data, which indicate that β-cryptoxanthin is present in carrots at relatively low concentrations - approximately 199 µg per 100 g of edible portion - compared to other dietary sources of this carotenoid, such as butternut squash (3471 µg/100 g) or persimmon (1447 µg/100 g) [69].

3.7. Phenols

Phenolic compounds are widely distributed in most fruits and vegetables. They exhibit antioxidant activity by scavenging reactive oxygen species and electrophiles, inhibiting nitrosation, and chelating metal ions. In addition, phenolics can undergo autooxidation and modulate the activity of certain cellular enzymes [70].

In this study, dicaffeoylquinic acid and diferuloylquinic acid were detected as potassium adducts at m/z 555.0899 and 583.1212, respectively (Table 1, Fig. 2). Dicaffeoylquinic acid was localised in the outer region of the vascular cylinder, appearing as clusters with varying concentrations, whereas diferuloylquinic acid was predominantly found in the cortex tissue, forming a cluster near the epidermis. The higher abundance of dicaffeoylquinic acid in the vascular cylinder may indicate its role in protecting against oxidative stress and supporting the metabolism and development of transport tissues. Using HPLC, Zhang and Hamazu [71] identified phenolic acids and their derivatives - including 3',4'-dicaffeoylquinic acid and 3',5'-dicaffeoylquinic acid - in two carrot cultivars, Chibagosun and Hitomigosun. Notably, the Hitomigosun cultivar contained nearly three times the amount of both dicaffeoylquinic acid isomers (6.97 mg/100 g FW) compared to Chibagosun (2.19 mg/100 g FW). However, in both cultivars, the concentration of 3,4-DCQ was substantially lower, representing approximately one-fifth of the 3,5-DCQ level. High hydrostatic pressure (HHP) treatment of whole carrots significantly increased the levels of dicaffeoylquinic and diferuloylquinic acids, with the most pronounced rise observed for 3,4-di-*O*-feruloylquinic acid (up to 466.1%) following treatment at 100 MPa. These findings highlight HHP as a promising tool for enhancing the phenolic profile of carrots [72].

6-Methoxymellein (6-MM), a dihydroisocoumarin and phenolic compound found in carrots and carrot purées, contributes to their bitter taste [73]. The silver-109 adduct at m/z 316.9778 was assigned to methoxymellein (Table 1, Fig. 2). MS imaging revealed that this compound is primarily localised in the cortex, forming clusters of high concentration, while it is scarce in the vascular cylinder and present only in trace amounts in the lateral root. It functions as a phytoalexin that is induced in carrot tissue by UV-C irradiation, enhancing resistance to *Botrytis cinerea* and other microbial pathogens [74-76].

3.8. Flavonoids

According to the literature, quercetin, luteolin, myricetin, and kaempferol have been identified as the main flavonoids present in carrots [77]. These compounds are of particular interest due to their biochemical and pharmacological activities, including antioxidant, anti-inflammatory, anticancer, anti-atherosclerotic, anti-aggregative, anti-allergic, and antimicrobial properties [78]. Apigenin, quercetin, and rutin (a flavonoid glucoside), all detected in our study, belong to this group of bioactive flavonoids.

Apigenin (4',5,7-trihydroxyflavone) is a natural flavonoid compound from the flavone subclass, found in a variety of fruits, vegetables, and medicinal plants. It has been reported to exhibit strong antioxidant, anti-inflammatory, antimutagenic, and antiviral properties. Furthermore, both in vitro and in vivo studies have demonstrated that apigenin exerts beneficial effects by inhibiting tumour growth and progression. It is also known to reduce oxidative stress, enhance the activity of detoxification enzymes, inhibit cell cycle progression, induce apoptosis, and stimulate the immune response. These biological activities suggest that apigenin may serve as a health-promoting and disease-preventing agent [79].

The ion at m/z 271.0601 $[M+H]^+$, assigned to apigenin (Table 1, Fig. 2), was detected in the outer region of the vascular cylinder as larger clusters, and as smaller, individual clusters in the central part of the cortex. Lugasi and Hóvári [80] examined the flavonoid content, including apigenin, in thirty-one fresh vegetables using RP-HPLC with UV detection. They reported that the apigenin content in carrot root was below the detection limit. Similarly, apigenin was not detected in a methanol extract of violet carrot juice, a popular beverage in Turkey [81]. Apigenin typically occurs in nature in glycosylated forms, which are more soluble than its aglycone form, as the latter is relatively unstable and poorly soluble in both water and organic solvents [82]. Interestingly, apigenin was identified in root exudates from carrot seedlings of *Daucus carota* cv. Nantaise by RT-HPLC [83], where it exhibited an inhibitory effect on the growth of the mycorrhizal fungus *Gigaspora margarita*.

Quercetin is a plant pigment that belongs to the flavonol group. Its content in plants is regulated by fluctuations in photosynthetic photon flux density ($43\text{-}230 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) [84]. Quercetin plays a key role as both an antioxidant and an antimicrobial compound. In addition, it is involved in physiological processes such as seed germination, photosynthesis, plant growth, and yield characteristics under both healthy and stressful environments. This compound also exhibits various medicinal properties, including anti-inflammatory, antiviral, anti-allergic, anticancer, cardiovascular-protective, anti-tumour, anti-diabetic, immunomodulatory, anti-hypertensive and gastroprotective effects [85]. In this study, quercetin was detected as the $^{109}\text{Ag}^+$ adduct with m/z 410.9469 (Table 1, Fig. 2). It is present in the cortex adjacent to the vascular cylinder and forms distinct clusters with higher concentrations in the central part of the cylinder, while it is absent from the lateral root. According to the literature, quercetin was the main flavonoid identified in the methanolic extracts of 62 edible tropical plants, with its content

in carrots measured at 55 mg/kg dry weight [86]. Quercetin was the most abundant flavonoid in extracts and exudates of Ri T-DNA-transformed *Daucus carota* roots, which are involved in mycorrhizal symbiosis [87].

Rutin (quercetin-3-O-rutinoside) is a quercetin derivative with the disaccharide rutinose (α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranose) attached at the 3-OH position. In this study, the proton adduct with m/z 611.1607 was assigned to rutin (Table 1, Fig. 2). It was localised in the analysed carrot as a high-concentration cluster located in the cortex near the epidermis, present in the lateral root, and forming scattered low-concentration clusters in the vascular cylinder. Kyslychenko et al. [88] determined the rutin content in the fruits of three carrot varieties - Yaskrava, Olenka, and Nantska Kharkivska - using the UPLC-ESI-MS/MS method, reporting the rutin levels of 14.91, 11.61, and 11.50 $\mu\text{g/g}$ of absolutely dry matter, respectively. Konar et al. [89], employing triple quadrupole liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), quantitatively identified rutin in the edible roots of fresh carrots subjected to different pretreatments. The rutin content in the methanolic extract was 5.72 $\mu\text{g}/100$ g fresh weight, whereas in samples subjected to enzymatic and acid hydrolysis, it was 2.81 $\mu\text{g}/100$ g fresh weight.

3.9. Volatile compounds (aldehyde, monoterpenes)

The distinctive aroma and flavour of carrots are primarily attributed to their volatile constituents. Both raw and processed carrots possess a complex aromatic profile characterised mainly by „green”, „earthy”, „carrot top”, and „terpene-like” notes, with varying contributions from „fruity”, „spicy”, „woody”, „citrus-like”, and „sweet” notes [90]. Octanal, a long-chain aldehyde formed via autooxidation or thermal degradation of C_{18} unsaturated fatty acids, is known to contribute to the aroma of carrots [91]. In addition, terpenes constitute a major class of volatile compounds that significantly influence the sensory properties of carrots.

In this study, octanal was detected as the $^{109}\text{Ag}^+$ adduct at m/z 337.0243 (Table 1, Fig. 1C). It was localised in the outer part of the vascular cylinder and in the lateral root, where it formed pinpoint clusters of very high concentration. The presence of octanal in these specific root regions may suggest its involvement in local metabolic processes, potentially related to lipid metabolism or stress response. However, its precise biological role beyond contributing to aroma remains unclear and warrants further investigation. According to the literature, this compound occurs in small amounts in carrot root. Buttery et al. [92], using steam distillation at atmospheric pressure, identified several aldehydes derived from linoleic and linolenic acids in *Daucus carota* (type Imperator). They found that 2-nonenal, octanal, 2-decenal, and heptanal were the main components responsible for the odour intensity of carrot root oil. However, octanal contributed only slightly to the overall odour intensity (2%), indicating its relatively low importance in the odour profile of this particular steam-distilled oil. Using GLC-MS, octanal was found in very low amounts in raw carrots (0.02 ppm) and freeze-dried carrots (trace amounts), while its concentration was higher in canned carrots (0.06 ppm) [93]. According to Kjeldsen et al. [90], carrots of the Bolero and Carlo varieties, stored under different temperature conditions (-24°C and $+1^\circ\text{C}$), contained octanal at low concentrations. The authors concluded that octanal does not appear to be an important volatile compound and does not contribute to any noticeable odour sensation.

Two monoterpenes, pinene and camphor, were detected in the analysed carrot. Both compounds were identified as sodium adducts: pinene at m/z 159.1144 and camphor at m/z 175.1093 (Table 1, Fig. 1C). Pinene was detected throughout almost the entire cross-section of the carrot root, with the highest concentration observed in the cortex, while the lateral root and vascular cylinder exhibited lower concentrations. In contrast, camphor was detected at very low concentrations, with its highest levels found in the outer part of the vascular cylinder and in the lateral root, where it appeared as a few discrete clusters with elevated concentration. Present in the vascular cylinder, lateral roots, and to a lesser extent in the cortex, camphor may play a protective and regulatory role during lateral root development and support the defence of peripheral root tissues.

A five-year study involving eight different carrot varieties and breeds investigated the influence of genetic and ecological factors on the composition of essential oils in edible roots. The study found that α -pinene levels were unaffected by cultivar or weather conditions, whereas β -pinene content varied depending on the cultivar. Notably, race IV of the variety „Long red stumpy without core”, known for its excellent taste, exhibited particularly high β -pinene concentrations [94]. Furthermore, the volatile compound composition of seven hybrid F1 carrot cultivars was analysed using static headspace gas chromatography-mass spectrometry (SHS-GC-MS). Mono- and sesquiterpenes constituted approximately 97% of the total volatile compounds identified. α -Pinene was among the predominant volatile compounds in fresh carrots, with concentrations ranging from 0.111 to 1.96 ppm. In addition, camphor was detected for the first time in raw carrots using this method, with concentrations ranging from 0.067 to 0.222 ppm [95].

3.10. Oxylipin

The next compound detected in this study was falcarindiol (3,8-dihydroxyheptadeca-1,9-diene-4,6-diyne), a plant metabolite belonging to the bisacetylenic oxylipin group (fatty acid derivatives containing acetyl groups). Bisacetylenic oxylipins, including falcarinol, falcarindiol, and falcarindiol-3-acetate, are highly bioactive phytochemicals exhibiting cytotoxic activity against microorganisms such as *Mycobacterium tuberculosis* and *Candida albicans* [96-98]. They also demonstrate antifungal effects against pathogens like *Botrytis cinerea* and *Aspergillus niger* [99-101]. Falcarindiol has shown *in vitro* activity against the necrotrophic fungal pathogen *Alternaria dauci*, which causes leaf blight, suggesting a role in plant resistance to this disease [102]. Furthermore, these polyacetylene oxylipins possess antiproliferative properties against leukaemia and other cancer cell lines, both *in vitro* [103, 104] and *in vivo* [105]. Besides their bioactivity, these compounds impart an intense bitter taste and contribute to the bitter aftertaste of fresh and processed carrot products [72, 106].

Falcarindiol was detected as the $^{109}\text{Ag}^+$ adduct at m/z 369.1818 (Table 1, Fig. 2). It was present at high concentrations in the cortex tissue, with considerably lower levels observed in the vascular cylinder and lateral root of the carrot studied. Using gas-liquid chromatography (GLC), Yates and England [107] found the falcarindiol concentration in the root of Red Cored Chantenay carrots to be 80 ppm. Schmiech et al. [108] analysed extracts of *Daucus carota* L. for bisacetylenic oxylipins using LC-MS and 1D/2D NMR spectroscopy. Interestingly, comparative chiral HPLC analysis of synthetic stereoisomers with isolated phytochemicals enabled the unambiguous assignment of the (*Z*)-(3*R*,8*S*)-configuration for falcarindiol in carrot extracts.

4. Conclusions

Using mass spectrometry imaging (MSI) with a ^{109}Ag nanoparticle-enhanced target ($^{109}\text{AgNPET}$), the spatial distribution of 29 metabolites in the edible root of the orange carrot was investigated. For most of the compounds discussed, their spatial localisation is presented here for the first time. Ion images of these primary and secondary metabolites revealed significant variations in intensity.

All detected primary and secondary metabolites were present in the cortex of the storage root, as this tissue primarily functions as a storage tissue; however, the compounds occurred at varying concentrations. High levels were found for compounds responsible for the characteristic aroma and flavour of carrot (pinene, methoxymellein, falcarindiol), which also protect the plant against pathogens and pests. Water-soluble vitamins (ascorbic acid, pyridoxine), amino acids (asparagine, arginine), and fatty acids (linolenic acid, lignoceric acid) were also present at high concentrations in the cortex, and may contribute to its protection against biotic and abiotic stress. Furthermore, carotene, responsible for the orange colour of the carrot, was accumulated in the cortex. Metabolites abundant in the vascular cylinder and lateral roots, including alanine, lysine, octanal, camphor, dicaffeoylquinic acid, and sugars (sucrose and glucose/fructose), collectively support nitrogen and energy transport, lipid metabolism, tissue protection, and cell growth during lateral root development. The identified metabolites show important biological functions that may contribute to both plant physiology and human health.

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