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# **Analyse par spectrométrie d'absorption atomique des échanges d'ions sodium entre des racines de carottes (*Daucus carota* L.) et une solution aqueuse où elles sont traitées thermiquement.**

**Using atomic absorption spectrometry to determine how sodium ions exchange between plant tissues (roots of *Daucus carota* L.) and a liquid aqueous environment during thermal treatments.**

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## **Abstract**

In order to characterize the transport of sodium ions that takes place in roots of carrots *Daucus carota* L. during thermal treatment, without or with

sodium chloride added to the liquid environment in which the roots are thermally processed, the sodium concentration of

different parts of roots was measured by flame atomic absorption spectrometry (after prior mineralization). Much of the sodium lost during a treatment in pure water, or absorbed during a treatment in a solution of sodium chloride, is likely to be transferred both through lateral surfaces of roots and by the conductive channels (xylem, phloem). These results can help to determine how much salt is consumed, with traditional culinary preparations.

### Résumé

Afin d'explorer le transport des ions sodium dans les racines de carottes *Daucus carota* L. pendant un traitement thermique, sans ou avec du chlorure de sodium ajouté à l'environnement liquide où les racines sont traitées thermiquement, la concentration en sodium de différentes parties des racines a été mesurée par spectrométrie d'absorption atomique en mode flamme (après minéralisation). Le sodium perdu lors d'un traitement dans l'eau pure, ou absorbé lors d'un traitement dans une solution de chlorure de sodium, semble être transféré à la fois par les surfaces latérales des racines et par les canaux conducteurs (xylème, phloème) de la racine. Ces résultats peuvent contribuer à déterminer la quantité de sel absorbée lors de la consommation de carottes préparées selon des méthodes culinaires traditionnelles.

### Key words

carrot, *Daucus carota* L., sodium, atomic absorption spectrometry, exchange, diffusion, osmosis

### Mots clés

carotte, *Daucus carota* L., sodium, spectrométrie d'absorption atomique, échange, diffusion, osmose

### 1. Introduction

To understand the chemical and physical effects of processes on food ingredients, one strategy is to study particular cases before trying to find general laws, and moving from simple to more complex cases (Polya, 1990). Most foods include plant or animal tissues, that can be considered as gels (IUPAC, 2019). These gels are anisotropic: for example, in plant tissues such as the roots of carrots *Daucus carota* L. ("carrots"), the xylem and phloem channels are mainly parallel to the axis of the root (Katou and Furumoto, 1988; Mizuno and Mizuno, 2002); for animal tissues, muscular fibres are aligned in bundles that are themselves grouped in aligned super-bundles (Picard and Gagaoua, 2020). In such cases, the exchange of molecular and ionic species is anisotropic, *i.e.*, different in the direction of the main axis (the axis of the root for plant tissues, or the direction of muscular fibres for animal tissues) and in directions perpendicular to this axis (Havis, 1939). To assess this anisotropy, it is necessary to determine the exchanges along the axis and also perpendicular to it.

Moreover the animal and plant tissues are heterogeneous (Campbell, 1995), and the study of the exchanges of biochemical molecules or ions inside them, and also between them and their environment, has to take into account their particularities. For example, a cross cut perpendicular to the axis of a carrot root shows different zones, which are the primary xylem, the secondary xylem, the cambium, the secondary phloem, the cortex, and the epidermis (Bowes, 1996). Accordingly a question is to understand how these structures can determine the exchanges of chemicals species, either molecular (various metabolites) (Passos *et al.*, 2004; Cazor *et al.*, 2006) or ionic (Gao *et al.*, 2022).

In particular, sodium ions ( $\text{Na}^+$ ) can be present in different amounts within the plant tissues before and after thermal treatments. These ions are especially important for public health, because in Western countries, the salt consumption is often more than that recommended by the World Health Organization (5 g/day), with potential

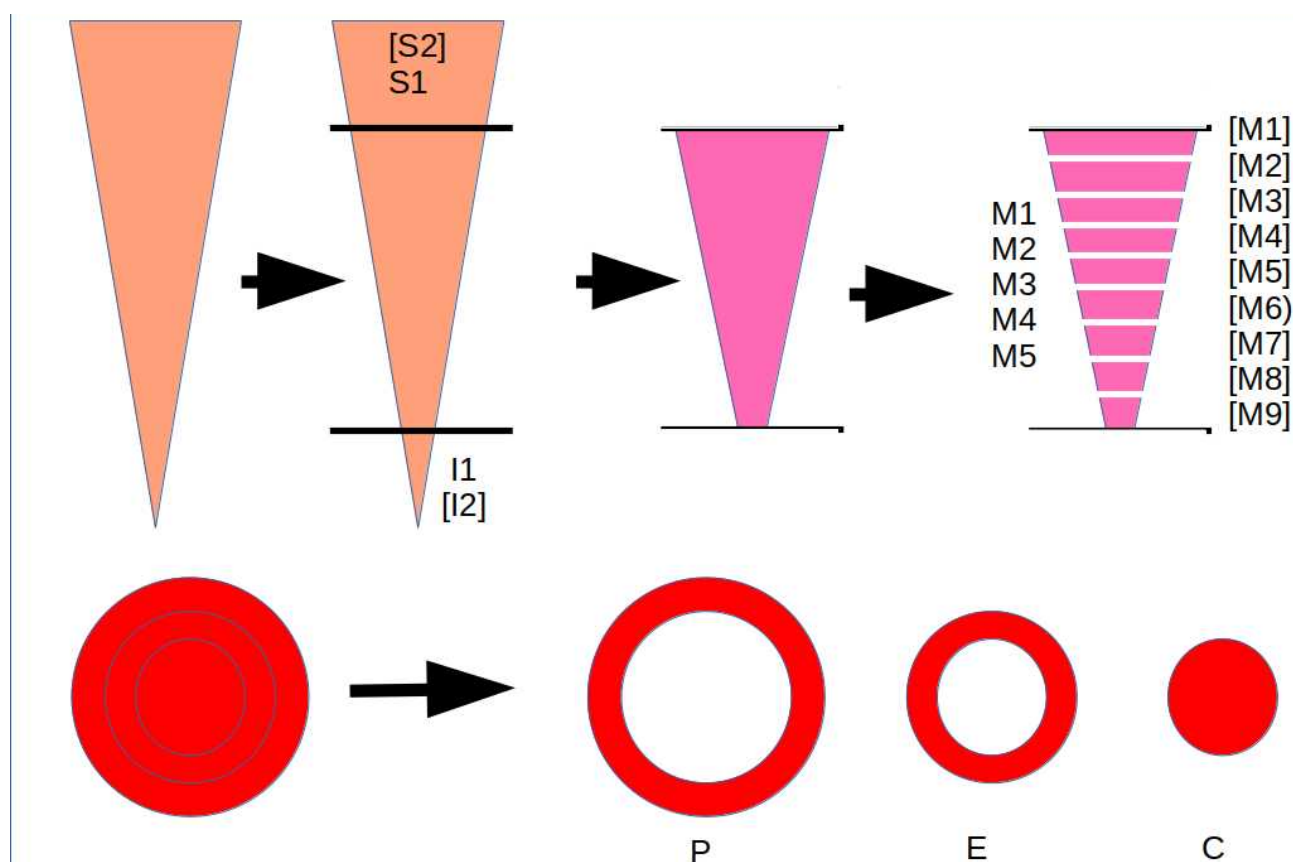


Figure 1. Preparation of samples: after cleaning, the carrot roots were peeled, and the upper and lower (respectively S and I) parts were separated and analyzed as raw tissue (orange); when possible, two slices were cut from the two ends (S1, S2, and I1, I2, respectively away from the top of the M segment or from the bottom of the M segment). Then the middle segment (M) was thermally processed, before being divided into 5 slices (purple, M1 to M5); when possible (last two experiments with a longer root), 9 slices were analyzed (M1 to M9). For the analysis, each slice was divided into three parts ("core" C, "endoderm" E, and "parenchyma" P).

health issues (WHO, 2022). For years, the food industry has been trying to reduce the salt content in its products (Santos *et al.*, 2021; Moran *et al.*, 2022), but consumers have preferred products rich in sodium chloride (NaCl), and they often add "discretionary" salt to the already prepared food, sometimes reaching higher salt content than would have been present before the efforts of the companies (Bhat *et al.*, 2020).

Today the origin of the Na<sup>+</sup> consumed through food is not sufficiently documented, and it is a goal of technical or educational programs in the world to

envision how to reduce the consumption of table salt, based on a better knowledge of the origin of salt: from the food ingredients, from their processing environment, or discretionary salt (WHO, 2013; 2020; Thomas-Danguin, 2018).

In order to complement the observation of the salt consumption in households and the studies of the perception of salt, the analysis of the chemical and physical phenomena associated with the transfers of salt in foodstuffs during food preparation is useful, allowing to envision novel strategies for salt use during food preparation. As

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*Table 1. Overall organization of the experiments and masses of the samples from top to bottom of the roots (the mass of the whole slice is given first, before the masses of the C, E, P parts).*

Experiment number	Processing medium	Commentaries	Mass of carrot used, and mass of slices (in the order: total, and C, E, P parts) (g)
1	Water	1st treatment in pure water (Exp-)	Carrot (34.85) S1 (5.6273: 1.3553, 1.3836, 2.5372) M1 (1.9414: 0.4573, 0.2451, 1.2388) M2 (1.1085: 0.3127, 0.2404, 0.5519), M3 (2.1233: 0.4604, 0.2661, 1.3967), M4 (1.5359: 0.2861, 0.2173, 1.0323), M5 (1.1742: 0.2797, 0.4953, 1.0157) I1 (2.5078: 0.4052, 0.5256, 1.3036)
2	Water	Repetition of experiment 1, Further exploration of the raw parts (Exp-)	Carrot (53.493) S1 (8.4481: 1.22147, 2.6369, 4.5370) M1 (2.8588: 0.2641, 0.5367, 1.8006), M2 (2.7342: 0.1801, 0.5553, 1.8218), M3 (2.5449: 0.1762, 0.3080, 1.9793), M4 (1.6506: 0.1196, 0.2499, 1.1876), M5 (1.2666: 0.0720, 0.2221, 0.8385) I1 (2.1832: 0.2640, 0.6109, 1.2674)
3	Sodium chloride solution	1st treatment in a solution containing Na <sup>+</sup> and validation of the distribution of Na <sup>+</sup> in the raw parts (Exp+). Possibility of having two raw slices at each end.	Carrot (46.417) S2 (5.0695: 0.6126, 1.2771, 3.0427), S1 (4.9081: 0.5530, 1.3595, 2.9295), M1 (2.6881: 0.3071, 0.8494, 1.4613), M2 (2.8498: 0.3770, 0.6017, 1.8708), M3 (2.4188: 0.3809, 0.6055, 1.4322), M4 (2.1262: 0.3064, 0.4419, 1.3773), M5 (1.6163: 0.1151, 0.4338, 1.0673) I1 (1.9771: 0.2498, 0.6889, 0.9874), I2 (1.6390: 0.2174, 0.4272, 0.9110)
4	Sodium chloride solution	Repetition of experiment 3 (Exp+)	Carrot (41.498) S1 (4.6641: 0.6930, 1.2700, 2.5367) M1 (3.2374: 0.3924, 0.5106, 2.2385) M2 (2.6536: 0.3661, 0.6709, 1.5394) M3 (2.6161: 0.2557, 0.6439, 1.6110) M4 (2.0328: 0.2215, 0.3430, 1.3493) M5 (2.2414: 0.2154, 0.4678, 1.3999) I1 (1.9253: 0.1118, 0.5171, 1.2235)
5	Sodium chloride solution	Second repetition of experiment 3 (Exp+). Possibility of analyzing two raw slices at each end.	Carrot (63.017) S2 (4.6267: 0.3333, 1.3972, 2.8028), S1 (3.7283: 0.2054, 1.1170, 1.8102), M1 (2.4910: 0.0801, 0.6675, 1.7027), M2 (3.3167: 0.2195, 0.7853, 2.3296), M3 (2.5927: 0.0982, 0.6619, 1.7782), M4 (2.1380: 0.1576, 0.4315, 1.5076), M5 (1.3030: 0.0877, 0.2897, 0.8955) I1 (1.8840: 0.1454, 0.2977, 1.3534), I2 (1.2603: 0.0673, 0.2988, 0.8443)
6	Water	Repetition of experiment 1, Determination of Na <sup>+</sup> in a longer segment (Exp-), with 9 slices thermally processed section	Carrot (81.2323) S1 (5.9877: 0.5205, 0.3950, 1.4297) M1 (5.2545: 0.4408, 0.6710, 1.4149) M2 (4.3976: 0.3724, 0.7033, 1.3966) M3 (4.0954: 0.3983, 0.5427, 1.3453) M4 (3.9792: 0.3367, 0.3654, 1.5286) M5 (3.7071: 0.2385, 0.7196, 1.0281) M6 (3.3219: 0.2961, 0.3742, 1.3027) M7 (2.4180: 0.0952, 0.3226, 0.9818) M8 (3.3460: 0.2482, 0.3642, 1.5174) M9 (2.7504: 0.1773, 0.4490, 0.9478) I1 (4.1333: 0.2504, 0.2580, 1.2348)
7	Sodium chloride solution	Repetition of experiment 3, Determination of Na <sup>+</sup> in a longer segment (Exp+) with 9 slices in the thermally processed section.	Carrot (94.8846) S1 (7.7690, 1.1655, 1.0443, 1.6506), M1 (6.0238: 0.8256, 0.6692, 2.8885), M2 (5.3806: 1.3998, 1.1935, 1.9373), M3 (4.8327: 0.4687, 0.7054, 1.9083), M4 (3.9190: 0.3775, 0.6404, 1.9617), M5 (3.2849: 0.2443, 0.6613, 1.2301), M6 (3.8654: 0.4431, 0.4181, 1.8448), M7 (4.0105: 0.5124, 0.4882, 1.8001), M8 (3.2868: 0.2500, 0.7392, 1.5870), M9 (3.3577: 0.3283, 0.4277, 2.5266), I1 (7.6447: 0.5763, 0.4562, 2.3577)

an increase in the consumption of plant products is recommended in view of healthy diet, the study of the distribution of salt in plant tissues being processed is needed first. In this study, the processing of carrots was investigated, because these roots are major food ingredients throughout the world (Food and Agriculture Organization of the United Nations, 2021).

In the past, most studies about exchanges of mineral ions and of organic compounds in living tissues have been performed at room temperature, *i.e.*, when proteins (such as transporters) remain active (Brooks, 1916; Koefoed-Johnsen and Ussing, 1953; Birt and Hird, 1958; Philip, 1966; Tukey, 1970; Molz, 1981; Oliveira and Silva, 1992; Ma and Peterson, 2000; Kocsis *et al.*, 2018; Aubry *et al.*, 2019). To the best of our knowledge the exchange of Na<sup>+</sup> between plant tissues and a liquid environment during thermal processing at 100 °C ("boiling") has not been studied, and different mechanisms could come into play, as various compounds important for the metabolism are then chemically modified (*e.g.*, pectins through beta elimination) or inactivated (for enzymes such as transporters) (Anthon and Barrett, 2002; Vu *et al.*, 2006).

We decided first to do 7 experiments: 3 thermal treatments in pure water (Exp-) and 4 experiments with NaCl dissolved in the liquid solution in which the carrot samples were thermally processed (Exp+). The concentrations in Na<sup>+</sup> were measured in various parts of carrots before and after thermal processing. The determination of the evolution of calcium (Ca<sup>2+</sup>) was also studied during two experiments (one Exp- and one Exp+) in order to interpret the Na<sup>+</sup> variations: Ca<sup>2+</sup> was chosen because the mechanisms for its transfers have reasons to be different from the mechanisms for Na<sup>+</sup>, as it has structural roles in the cell wall and membranes, and it is a counter-cation for inorganic and organic anions in the vacuole, and an intracellular messenger in the cytosol (Marschner, 1995).

## 2. Materials and methods

### 2.1. Materials

Each experiment, including the preparation of a root to be analyzed, thermal processing and sample preparation prior to analysis, was performed within the same day. Roots of carrot *Daucus carota* L. were bought at a local grocer (origin France, SAS Gosselin; growing season 2020), but all had different origins. The mass of the carrots used in the 6th and 7th experiments was almost twice (about 80 g after preparation of the roots in view of experiments) the mass of the carrots used in the first 5 experiments, which made possible to get more samples from them (see below and Table 1).

The water used for all experiments was ultrapure (type I) Milli-Q water, for which the content in Na<sup>+</sup> and Ca<sup>2+</sup> ions was checked (using atomic absorption spectrometry) to be below the detection limits of the analytical method that was used.

NaCl was from VWR chemicals, Rectapur 99.5%. For mineralizations in a digestion block DigiPREP Jr (SCP Science), nitric acid HNO<sub>3</sub> PlasmaPure (67-69%) and 50 mL graduated polypropylene tubes were used.

For analysis, standard Na<sup>+</sup> and Ca<sup>2+</sup> solutions 1000 mg/L (SCP Science) were used. The following products were also used: hydrochloric acid HCl (SCP Science, Ultra pur PlasmaPURE, 34-37%), potassium chloride KCl (Fluka Biochimica, purity > 99.5%), lanthanum (III) chloride heptahydrate LaCl<sub>3</sub> (VWR Chemicals Rectapur). The atomic absorption spectrometer used was a model ContrAA 800D (Analytik Jena), type HR-CS-AAS, with the AspectCS software for control (List *et al.*, 1971; Welz, 2005). The flame mode was used for Na<sup>+</sup>, but the oven mode was used for Ca<sup>2+</sup> (see below).

### 2.1. Methods

For the experiments described here, all materials, products and reagents were weighed three times

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at each experimental step using a Mettler Toledo AG135 scale (precision 0.0001 g) or a Mettler Toledo PJ300 scale (precision 0.001 g) for the largest masses.

#### *Preparing samples for the determination of Na<sup>+</sup> in raw tissues:*

After washing and rinsing the roots with Milli-Q water, they were weighed and geometrically characterized using a caliper rule with Vernier (0.1 mm). Then they were peeled using a home peeler (maximum thickness of cut parts 1.3 mm), because one goal of this study was also to determine the quantity of Na<sup>+</sup> consumed: the experiments were designed to mimic culinary preparations. After washing with Milli-Q water, the peeled roots were blotted with absorbent paper, weighed again and geometrically characterised.

Each segment to be thermally processed (M, in Figure 1), about 11 cm long for the first 5 experiments and 16 cm for the 2 last experiments, was separated from the upper (S1) and lower (I1) slices using a stainless steel scalpel on a plastic board, washed (Milli-Q water) and dried (absorbent paper), before weighing. The two S1 and I1 slices were dried, weighed and geometrically characterized; they were used to know the Na<sup>+</sup> content in the raw plant tissue of the particular root of which a central segment (M) was thermally treated, assuming that adjacent tissues have equal Na<sup>+</sup> concentrations. For the experiments 2 and 3 (respectively one of type Exp- and one of type Exp+), other slices S2 and I2 were also cut respectively in the upper and in the lower part of the root, adjacent to the S1 and I1 slices (the S2 and I2, slices were used for validating the Na<sup>+</sup> concentration in the S1 and I1 slices and also for possibly showing the distribution of Na<sup>+</sup> in the root studied). After mass measurement and geometrical characterization, the S1, I1, S2 and I2 slices were individually divided into three zones called C, E, P, according to root morphology. Indeed a transverse cut of a root shows more than three zones, but preliminary experiments showed that in order to make the separation of the samples more robust, only three parts could be easily recovered for each slice: a "core" (C), *i.e.*, the

inner part, comprising xylem and phloem; an "endoderm" (E), *i.e.*, a thin layer at the outside of the core, comprising tissues that play a role in growth and division of the root; a "cortical parenchyma" (P), where reserve compounds are stored.

The slices were cut on a polyethylene board using a scalpel, washed with Milli-Q water and dried with absorbent paper, before being weighed. Particular care was taken to minimize unavoidable losses during the division process (these losses explain why the sum of the masses of the C, E, P parts is less than the mass of the slice from which they originate). The samples were stored at 4 °C in cleaned, weighed, polypropylene tubes (50 mL) in view of mineralization and analysis, but the various operations were performed as quickly as possible, in order to avoid enzymatic modifications.

#### *Thermal treatment of the intermediate segment M:*

As indicated above, two cases were studied: processing in pure water (Exp-), and processing in an aqueous solution of NaCl (Exp+) (Vu *et al.*, 2006). The Table 1 summarizes the various treatments, and the masses of samples.

For Exp- experiments, a weighed Pyrex crystalliser, containing 350.0 g of Milli-Q water and a bar magnet, was heated by a RCT Basic® IKA Safety Control Heating System with Pt1000 thermoprobe. When the water temperature reached 100 °C, the M section was immersed, and kept in the water in turbulent conditions (Reynolds number  $Re > 8000$ ) for 1800 s (as for home processing) (Escoffier *et al.*, 1903).

With preliminary experiments, it was observed that the temperature of the core was higher than 90 °C after more than 180 s, for a root diameter of 2.53 cm. When the processing time was reached, the M processed section was immediately taken out of the solution, wiped with absorbent paper, and weighed; then it was immediately cut into slices, and each slice was divided into C, E, P parts; samples were immediately stored at 4 °C (see below). The

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*Table 2. Na<sup>+</sup> concentrations (mg/kg) in the various parts of carrots. The abbreviations are as in Table 1 (nd stands for "not determined").*

Exp +Par	S2	sd S2	S1	sd S1	M1	sd M1	M2	sd M2	M3	sd M3	M4	sd M4	M5	sd M5	M6	sd M6	M7	sd M7	M8	sd M8	M9	sd M9	I1	sd I1	I2	sd I2	
1C	nd	nd	376.3	18.8	165.3	8.3	277.1	13.9	291.2	14.6	389.6	19.0	265.4	13.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
1E	nd	nd	209.0	10.5	187.2	9.4	305.8	15.3	277.9	13.9	328.7	16.4	203.8	10.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	238.7	11.9	nd	nd
1P	nd	nd	196.4	9.82	139.4	7.0	243.1	12.2	227.3	11.4	207.6	10.4	134.2	6.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	217.5	10.9	nd	nd
2C	143.0	7.2	151.9	7.6	93.72	4.7	111.3	5.57	99.78	5.0	83.43	4.2	74.43	3.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	128.2	6.4	135.9	6.8
2E	119.4	6.0	97.01	4.9	83.88	4.2	95.54	4.78	89.41	4.5	79.32	3.4	65.69	3.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	121.5	6.1	121.3	6.1
2P	105.6	5.3	168.2	8.4	66.73	3.34	81.75	4.09	75.68	3.8	73.91	3.7	58.55	2.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.425	114.0	5.700	0.3
3C	230.4	11.5	218.2	10.9	348.2	17.4	294.7	14.7	292.6	14.6	374.3	18.2	412.1	20.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	312.1	174.1	308.9	15.5
3E	213.4	10.7	182.4	9.1	385.0	19.2	338.6	16.9	330.0	16.5	388.0	19.4	417.6	20.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	286.8	14.3	308.9	15.5
3P	282.2	14.1	274.4	13.7	428.8	21.4	381.3	19.0	356.0	17.8	404.7	20.2	467.2	23.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	247.0	12.4	275.5	13.8
4C	nd	nd	934.3	46.7	3555.0	177.8	3137.	156.9	3069.0	153.5	3407.0	170.4	3807.0	190.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	1560.0	78.0	nd	nd
4E	nd	nd	540.5	27.0	3322.0	166.1	3015.0	150.8	3027.0	151.4	3157.0	157.9	3785.0	189.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	844.8	42.2	nd	nd
4P	nd	nd	405.4	20.3	3378.0	168.9	3172.0	158.6	3139.0	157.0	3556.0	177.8	3715.0	185.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	450.7	22.5	nd	nd
5C	205.3	10.3	172.8	8.6	3482.0	174.1	2947.0	147.4	2926.0	146.3	3743.0	187.2	4121.0	206.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	255.4	12.8	236.9	11.9
5E	225.4	11.3	183.0	9.2	3850.0	192.5	3386.0	169.3	3300.0	165.0	3888.0	194.4	4176.0	208.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	87.74	4.4	225.4	14.1
5P	255.4	12.8	283.5	14.2	4288.0	214.4	3813.0	190.7	3560.0	178.0	4047.0	202.4	4672.0	233.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	313.0	15.7	255.4	12.8
6C	nd	nd	500.0	25.0	640.1	32.0	539.2	27.0	1191.	59.6	1362.	68.1	1313.	65.7	1161	58.1	1125	56.3	1168	58.4	766.	38.3	277.3	13.9	nd	nd	
6E	nd	nd	485.3	24.3	1371.0	68.6	518.3	25.9	1059.0	53.0	892.9	44.7	895.6	44.8	817.	40.9	788.5	39.4	636.2	31.8	603.2	30.2	147.7	7.4	nd	nd	
6P	nd	nd	538.8	26.9	367.9	18.4	437.4	21.9	510.1	25.5	485.3	24.3	462.5	23.1	473.	23.7	452.1	22.6	412.8	20.6	355.1	17.8	866.7	43.3	nd	nd	
7C	nd	nd	345.6	17.3	1524.	76.2	466.1	23.3	573.6	28.7	730.	36.5	809.5	40.5	717.	35.9	721.7	36.1	434.3	21.7	1677.	83.9	170.	8.5	nd	nd	
7E	nd	nd	349.8	17.5	2025.	101.3	819.8	41.0	992.9	49.6	1334	66.7	1211.	60.6	1241	62.1	917.1	45.9	741.7	37.1	1644.	82.2	236.	11.8	nd	nd	
7P	nd	nd	215.9	10.8	3097.	154.9	2543.	127.2	2454.	122.7	2286.	114.3	2859.	143.0	3438	171.9	2749.	137.5	2563.	128.2	2983.	149.2	822.5	41.1	nd	nd	

aqueous solution was also recovered, weighed, and stored for analysis. For Exp+, the processing solution was prepared from 3.5000 g (Escoffier *et al.*, 1903) of pure NaCl dissolved in 350.00 g of Milli-Q water, in a weighed Pyrex crystalliser; a bar magnet was added, and the thermal treatment and the sample preparation were as above.

*Preparing samples from the thermally processed segments in view of analysis:*

In order to avoid Na<sup>+</sup> redistribution within slices and between parts of slices, after the end of the thermal process (Clerjon *et al.*, 2022; El Sabbagh *et al.*, 2022), the samples were prepared immediately after thermal processing (in less than 30 s). The M segment was cut into a certain number of slices (5 for the 5 first experiments, and 9 for the last 2 experiments, depending on the possibilities) about 1.0 cm wide (M1 to Mi, i = 5..9), each of them being geometrically characterized and weighed. With a scalpel, the 3 parts (C, E, P) of each slice were separated, and the 15 or 27 samples were weighed and stored in tubes for mineralization (in less than 60 s).

*Mineralization of samples:*

AAS was implemented after sample mineralization (Hoenig *et al.*, 1988; Bulska and Ruszcinska, 2017) with 5.0-8.0 mL of HNO<sub>3</sub> 69% added to each sample in a mineralization tube. For the tubes

containing the aqueous solutions in which the thermal treatment was performed, a mineralization was also tested, adding 0.50 mL of HNO<sub>3</sub> to 5 mL of solution. After closing the tubes, a first attack was performed, for 8 hrs, at room temperature. Then the tubes were heated in the digestion block: in order to avoid caramelization of saccharides, the temperature was increased by steps of 10 °C until a temperature of 50 °C was reached; the lids were opened, and the temperature was increased by steps of 10 °C up to 95 °C, kept for 2 hrs. Then Milli-Q water was added until the volume was 50.0 mL, and the content of the tubes was homogenized.

*Preparing the reference samples and "stocks":*

Some tubes were weighed, and prepared, in view of controlling the purity of reagents and determining the Na<sup>+</sup> content of the liquids used for thermal processing ("stocks"). Were analyzed: Milli-Q water, HNO<sub>3</sub> used for mineralization, solutions for thermal treatments, the same solutions after filtration with polypropylene filter on a syringe, Milli-Q water after addition of known quantities of NaCl.

*Atomic absorption analysis (AAS):*

The ContrAA 800 D equipment was used in the HR-CS-AAS mode (high resolution, continuum source). The continuum source has the



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advantage to allow the analysis with only one lamp (xenon, Xe) for all elements, instead of the mono-elementary lamps such as the hollow cathode lamps. With high resolution mode, the selection of narrow emission bands was key to optimal absorption of the energy.

Na<sup>+</sup> was determined in the flame mode (FAAS), at a wavelength of 588.9953 nm, with an acetylene flow rate of 90 L/hr. Standard curves were made from standard solutions at 0, 0.1, 0.2 and 0.4 mg/L (regression coefficient always higher than 0.999 for the calibration lines obtained by linear regression). After tests of absorbance-concentration, cascade dilutions of samples had to be made (Danzer and Currie, 1998), using a solution containing 1% solution of HCl, 0.1% of KCl and 0.1% of LaCl<sub>3</sub> (ionisation buffer). Because of the different masses of samples, various dilution factors (100 times, 200 times, or 1000 times) were used, so that the measured concentrations could lie within the standardized concentration interval, with a final volume of 10 mL.

Ca<sup>2+</sup> was determined for samples produced in experiment # 1 (Exp-) and # 3 (Exp+) using the oven, or electrothermal mode (ETAAS), because the sensitivity with flame mode was insufficient. With the ETAAS, a wavelength of 239.8559 nm had to be used, because of the sensitivity at the principal wavelength at 422.6278 nm was too much, and dilutions would have increased the uncertainties. The calibration curve was established using standard solution (0, 100, 200 µg/L). As for Na<sup>+</sup>, tests were performed before diluting the samples in HNO<sub>3</sub> at 1-2%.

For the analysis of samples, 20 µL of each sample solution were injected in the graphite tube (cuvette) with pyrolytic coating, and a three steps thermal program was applied, involving drying, decomposition and atomization (Dawson *et al.*, 1968). During the process, drying is performed in 3 sub-steps, the last one being at the temperature of 110 °C; the vaporization of the solvent has to be completed in order to avoid losses by projections, because of the fast increase in the temperature at the beginning of the following step (Bradfield and Spincer, 1965).

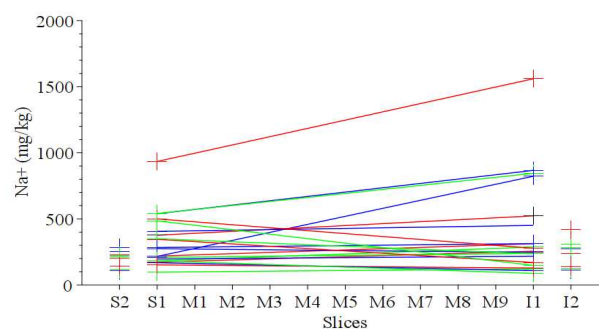


Figure 2. Distribution of Na<sup>+</sup> in samples of raw tissue, for the 7 roots studied. The Na<sup>+</sup> concentration was measured three times for each C ("core"), E ("endoderm") and P ("parenchyma") part of the upper (S1) or lower ends (I1) of the roots. When there was enough material to analyze more than one slice at the ends, the Na<sup>+</sup> concentration was also determined in S2 and I2 slices. For this figure as for all others, the red, green and blue colors respectively stand for C, E and P parts. Lines link Na<sup>+</sup> concentration in parts from a same carrot root. The size of crosses does not correspond to uncertainties, which were estimated to be less than 5%, after propagation of uncertainties taking all steps into account.

The decomposition process (pyrolysis) at 1200 °C achieved mineralisation, simplified the sample matrix, avoided smokes, molecular vapours and the presence of other constituents formed during organic mineralisation. Atomisation at 2550 °C dissociated the residual matrix, generating an atomic fog that included the Na atoms. The elements of interest were then selected by the high resolution monochromator at the specific wavelength used for analysis. Its concentration related to the light attenuation after atomic absorption (Beer-Lambert Law). The analyses were performed under argon flux (Hoenig and De Kersabiec, 1995). Samples were analyzed 3 times. All these methods were validated by the analysis of certified reference materials.

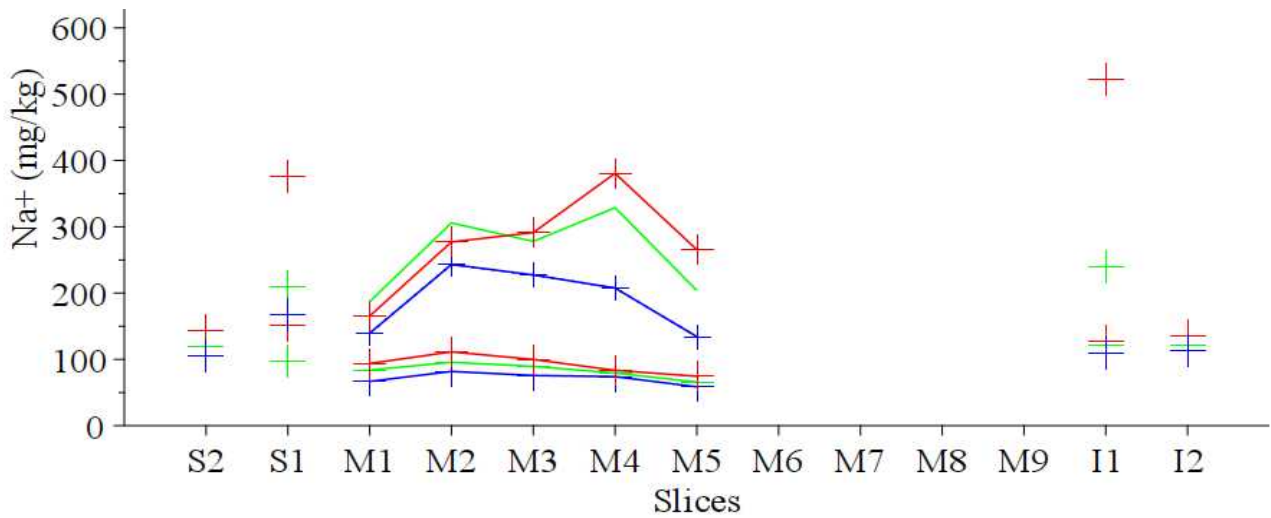


Figure 3. When carrot samples are processed in initially pure water (*Exp-*), the  $\text{Na}^+$  concentration inside the thermally processed roots has an inverted-U profile. Also it is lower in the end parts than for the adjacent raw parts. Here the  $\text{Na}^+$  concentration in the 5 slices made from the thermally treated M segment (abscissa M1 to M5) is given for the three parts: "core" (red), "endoderm" (green), "parenchyma" (blue). The  $\text{Na}^+$  concentration for the raw ends is given for comparison.

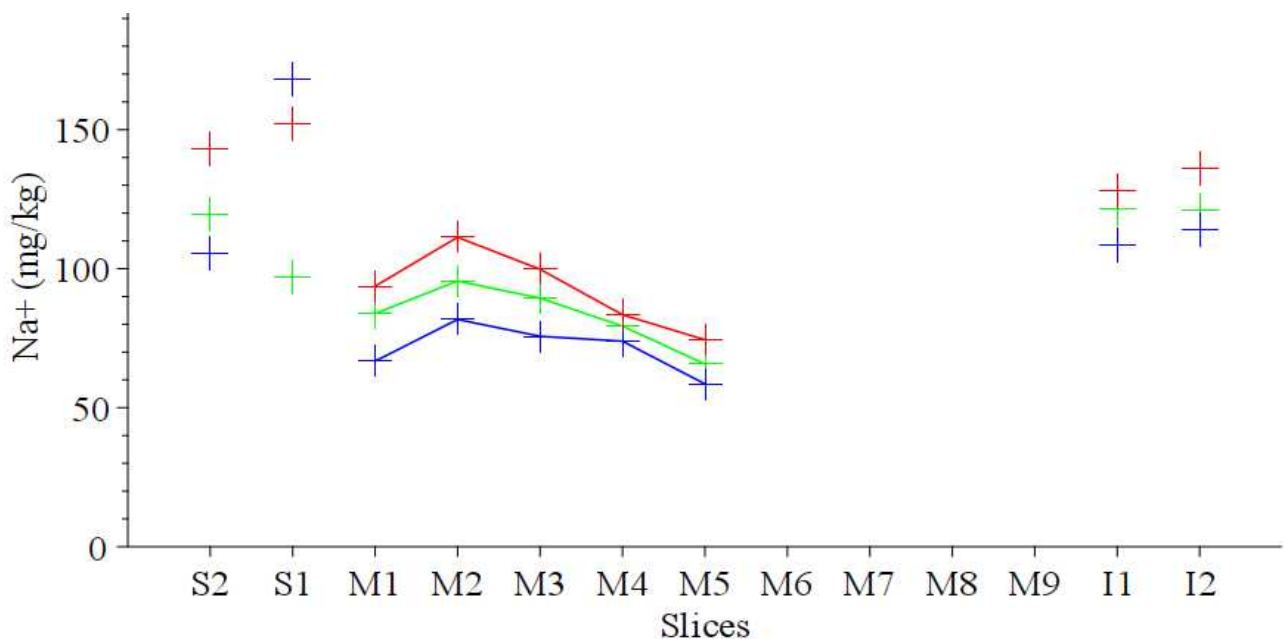


Figure 4.  $\text{Na}^+$  distribution in the 5 slices M1, M2, M3, M4, M5 from a carrot segment thermally treated in pure water (crosses): here the results for one experiment only (# 2) are given, so that the distribution in the various parts ("core" in red, "endoderm" in green, "parenchyma" in blue) appears better. The crosses which are not linked by lines show the  $\text{Na}^+$  concentration in the raw parts of the same carrot (S2 and S1, and I1 and I2), showing that the  $\text{Na}^+$  concentration is reduced in the slices in direct contact with the processing solution.

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Finally the data were analyzed using the software Maple 2021 (Maplesoft/Waterloo Maple Inc), in particular for the calculation of uncertainties and statistical treatments ( $T$  tests for two samples, confidence level = 0.95) (Kanji, 1994) even if, in many cases, the small size of statistical samples did not allow to draw conclusions for significance (Swinscow and Campbell, 1977).

### 3. Results

For all experiments, the overall uncertainty for each concentration measurement was calculated to be less than 5% of the final result, taking into account not only the standard deviation of the three  $\text{Na}^+$  determinations from AAS, but also all previous processes leading to this determination (propagation of uncertainties) (JCGM, 2012). Here the results (concentrations in  $\text{Na}^+$  of  $\text{Ca}^{2+}$ ) are given in relationship with the mass of fresh tissue, because the goal was to observe the actual quantity of consumed  $\text{Na}^+$ , as well as the transport mechanisms within single roots (Table 2).

For raw samples, a wide distribution of  $\text{Na}^+$  concentration can be observed (Figure 2): the coefficient of variation for C, E, P is respectively equal to 0.75, 0.96 and 1.20 for the upper raw slices S1, and 1.10, 1.09 and 1.04 for the lower raw I1 slices. The relative differences between the corresponding upper and lower parts (differences between top and bottom concentrations divided by the mean of these two values) are distributed between 7 and 89%.

Depending on the particular carrots used, the  $\text{Na}^+$  content ranged between 87 and 1560 mg/kg. No obvious coherent distribution can be observed for the  $\text{Na}^+$  concentration in the three C, E, P parts. The  $\text{Na}^+$  concentration in the upper (S) and lower (I) slices can only give an idea of the concentration of  $\text{Na}^+$  in the first (M1) and last (either M5 or M9 depending on the experiments) slices of a particular root before thermal treatment. In particular, no coherent distribution can be found between the upper and the lower parts of row carrots.

For each group of thermal treatments, *i.e.*, without

(Exp-) or with (Exp+) NaCl added to the solution, the same kind of results were obtained. First, without added NaCl in the processing solution (Exp- experiments # 1, 2, 6),  $\text{Na}^+$  is always distributed in the same way in the various slices (Figures 3 and 4): the  $\text{Na}^+$  concentration in the tips of the M segment is less than for adjacent raw sections, and it is also less than in the inner parts, making an inverted U-shape. The average  $\text{Na}^+$  concentration in the entire thermally processed segment is significantly ( $p < 0.01$ ) reduced compared to the raw tissue, as well as the concentration in each part (C, E, P). Accordingly water in which the tissue is processed gets  $\text{Na}^+$  from the plant tissue. For all slices, the external P part of the M segment loses more  $\text{Na}^+$  than the E part, and the C part is the one that retains more  $\text{Na}^+$  than the other two parts (Figure 5).

When carrots were processed in water with NaCl (Exp+ experiments), the tips of the processed segment (slices M1 for the upper part, and M5 or M9 for the lower part, depending on experiments) are more enriched than the inner slices, the  $\text{Na}^+$  concentration of these tips reaching the concentration of the external aqueous solution (U-shape). The solution was depleted in  $\text{Na}^+$  after thermal processing (4270 vs 4560 mg/kg in experiment # 3), but this quantity could not be related to the enrichment, because experimental uncertainties were too large.

A particular analysis has to be made for the experiment # 7, in which a long M segment was processed in a NaCl solution: as shown in Figure 6 (top), the distribution of  $\text{Na}^+$  in the parts still follows the same decreasing order of concentration ( $P > E > C$ ), the concentration in the tips (M1 and M9) being close to the concentration in the processing solution, but here the U-shape that characterized the first Exp+ experiments is not observed.

As a whole, the  $\text{Na}^+$  distribution is very different for Exp- and Exp+ experiments (Figure 7), in spite of much diversity of plant tissues being used for the experiments: the average  $\text{Na}^+$  distribution within the whole tissue processed without NaCl is significantly lower ( $p < 0.05$ ) than for samples

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processed in water with NaCl (average values: 361 (sd 125) mg/kg vs 3114 (sd 415) mg/kg), and this holds for the whole sample, as well as for the three C, E, P parts separately (respectively 584 (sd 484)/2561 (sd 1342), 489 (sd 392)/2684 (sd 1228), 277 (sd 170)/3445 (sd 685), all significantly different.

Finally, for  $\text{Ca}^{2+}$ , the distribution profile is different from the one obtained for sodium (Figure 8). Here, the average value for all concentrations is respectively 281 (14) and 189 (9) mg/kg for Exp- # 1 and Exp+ # 3. One distribution (for the C part in the experiment # 1) is much below the others, with average value of 82 (sd 4) mg/kg. The respectively U-shape and inverted U-shape distributions obtained for  $\text{Na}^+$  are not observed for  $\text{Ca}^{2+}$ .

### 4. Discussion

The sample preparation and the analytical method that were used in this study were first shown to give results from which the  $\text{Na}^+$  exchanges could be followed. However, because these experiments are destructive, changes in  $\text{Na}^+$  concentration over time in the various parts of the plant tissue could not be studied. Other methods, such as  $^{23}\text{Na}$  magnetic resonance imaging, could be used for comparison, but only if the acquisition time is not too long: otherwise transport phenomena (such as the redistribution of  $\text{Na}^+$  within the M segment, axially or radially) will blur the analytical results (Clerjon *et al.*, 2022). Taking this blurring effect into consideration, an important step of the experimental setup implemented here was the division of the various samples in C, E, P parts, from the slices of the M segment, as quickly as possible after the end of the thermal processing of the whole M segment.

As different roots have different  $\text{Na}^+$  content (as shown in the Results section), the repetition of experiments, with a calculation of average parameters was not an option, and this explains why validations within experiments had to be introduced rather than repetitions that would have given different results.

The mineralisation step of samples was shown to give coherent results (Hoenig *et al.*, 1988; Bulska and Ruscinska, 2017), and it appeared to be needed even for the processing solutions, for which the analysis showed higher concentrations when mineralisation of the solutions was performed before AAS analysis (accordingly, the data analysed here were for mineralised solutions). Also the repetition of measurements for the various samples led to the observation that three of the initial analytical results were probably flawed; these samples were analyzed again three times, and the new measurements were checked with another group of three measurements. For the final analysis, only the last three measurements were kept, when it was confirmed that the initial incoherent results were outliers (Grubbs, 1969).

An important step in the analysis was the cutting of each slice (raw or cooked) into three parts C, E and P. The distribution of recovered masses (Table 1) and the regularity of the results for these parts in all the experiments showed that the cuts were consistent.

#### Raw tissues:

Based on the  $\text{Na}^+$  determination of raw tips of carrots (slices S1, I1 for experiments # 1, 2, 4, 6 and slices S1, I1, S2, I2 for experiments # 3 and 6), large differences (coefficients of variation reaching 1.1) were observed in  $\text{Na}^+$  content between different roots. Such differences had been previously recognized in carrots when saccharides and organic acids were analyzed by quantitative nuclear magnetic resonance (q NMR) (Cazor *et al.*, 2006), and especially by *in situ* q NMR, for which the sample size is much reduced (about 1 mm<sup>2</sup> for the section, and 7 cm long) (Bauchard and This, 2015). The carrot variability was also observed by Inal *et al.* (1999), Korolev *et al.* (2000) and Kwiatkowski *et al.* (2015). This high compositional variability of plant tissues was the reason why drawing as much information from each individual root was the strategy chosen in the present work, instead of averaging a large number of repetitions with different roots.

A question is to understand why some roots

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contain more Na<sup>+</sup> than others. As different scientific programs from our laboratory, also studying carrots, led to the conjecture that the size of the roots could be correlated with this Na<sup>+</sup> content, the data were used to look for correlations between the average Na<sup>+</sup> concentration in raw tissues and the mass of the roots: the *T* tests (confidence level 0.95) did not provide evidence to conclude that the null hypothesis (equal Na<sup>+</sup> concentration for small and large roots) is false, for the whole raw slices as well as for each part (C, or E, or P). Other studies will be needed to explore such differences in Na<sup>+</sup> content in plants, if any, and to understand the factors responsible for the observed diversity among roots, such as the Na<sup>+</sup> content in the soil, the variety of carrots, etc. (Raddatz *et al.*, 2020).

The results made another test possible, *i.e.*, checking if roots absorb water and ions (raw sap) in proportion of the lateral area of the root, because the total number of the root hairs that can connect with xylem would increase, from the lowest to the highest part of the root (Morot-Gaudry *et al.*, 2017). In this assumption, the higher slices (S2 and S1) should have more Na<sup>+</sup> than the lower slices (I1 and I2). A model for the density of xylem channel as a linear increasing function of the height in the root was tested, but a *T* test (confidence level 0.95) for the whole slices (with the three C, E, P parts together) did not provide enough evidence to conclude that the null hypothesis (equal Na<sup>+</sup> concentrations in the upper and in the lower part) is false; this means that the simplistic model did not hold, confirming that the apoplastic and symplastic pathways have to be taken into account in order to describe Na<sup>+</sup> distribution in roots (Steudle and Peterson, 1998; Amodeo *et al.*, 1999; Mizuno and Mizuno, 2003).

#### *Processed tissues:*

The question of the distribution of chemical species (water, molecular and ionic solutes) between plant tissues, in particular carrots, and a liquid environment has been studied for decades (Koefoed-Johnsen and Ussing, 1953; Birt and Hird, 1958; Philip, 1966; Molz, 1981; Kocsis *et al.*, 2018). As early as 1938, a mechanism was

assumed (Crafts and Broyer, 1938) for the transfer of ions across the root and into the xylem vessels, in which ions actively accumulated by the cortical cells of the root supply the entire plant (Hope, 1953; Briggs *et al.*, 1958). Evapotranspiration accelerates the removal of accumulated ions from the roots to the shoots, but the active, selective transport mechanism acquires the ions initially, whether they are then retained by the absorbing root or passed through it and up to the shoot. Ions accumulated by root tissue appeared to exchange only slowly (time constant: several hours) for other ions of the same ionic species, as indicated in experiments with radioisotopes (Laties, 1959; Sutcliffe, 1959; Rye *et al.*, 2021).

In more details, molecules and ions taken into plant roots must penetrate a series of layers: after entering in the epidermis, they accumulate in the root cells or go through cell layers in cortex, endodermis, and central cylinder to reach the xylem vessels, and then are transported to the above-ground parts. During this transport, the stream is more or less depleted by ions moving into the surrounding tissues. There, the ions are retained or transported into the parenchyma or phloem. In this way, they are able to penetrate into all parts of the organism where they can be accumulated.

The actively absorbing region of the roots is limited (Sandhu *et al.*, 2017). Ions are supposed to penetrate the cell walls of epidermis and cortex of young roots by diffusion and other passive mechanisms such as osmosis and capillarity (Aguilera *et al.*, 2004; Ruan *et al.*, 2010) eventually enhanced by the entering water stream (Shabala *et al.*, 2005) without structural hindrance. Older parts of the roots sometimes show a development of suberised layers which reduce the entrance of ions and water. Because of the presence of protoplasmic connections between the individual cells, the protoplasm from epidermis to xylem vessels is assumed to form a continuum, the symplasm.

For the interpretation of the experiments reported here, one has to observe that the duration of the thermal processing (about 30 min) includes a first

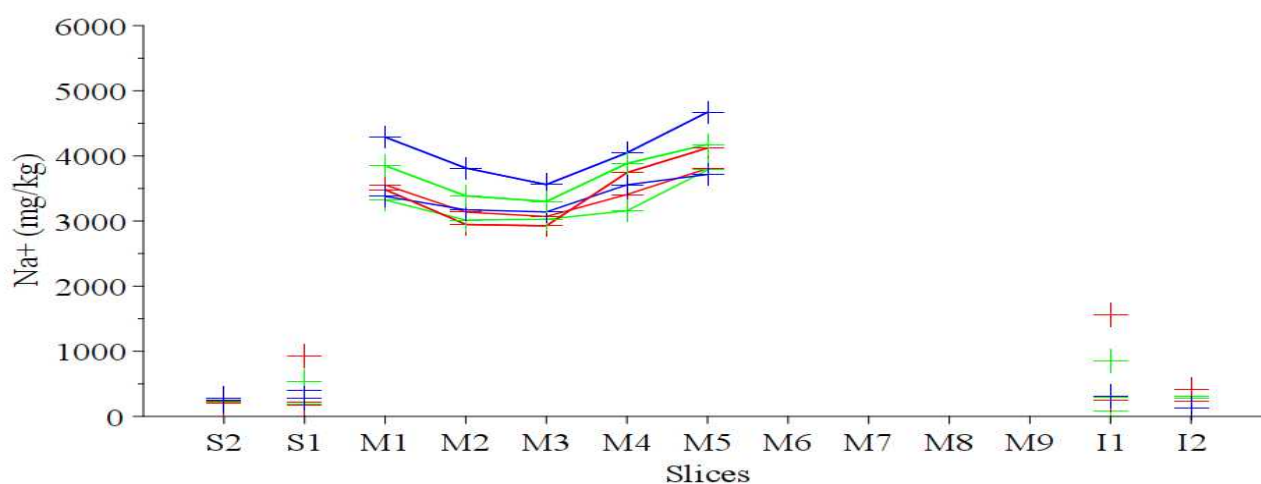


Figure 5. When carrots samples are processed in an aqueous NaCl solution (Exp+), the concentration in Na<sup>+</sup> is increased throughout the segment (slices M1, M2, M3, M4, M5). In this figure as for all others, red, green and blue colors are respectively used for the "core", "endoderm" and "parenchyma". The Na<sup>+</sup> concentration in the slices of the ends of the M segment M1 and M5 is not statistically different from the Na<sup>+</sup> concentration in the solution.

heating step (for about 3 min) during which the temperature inside the roots still allows metabolic processes: Na<sup>+</sup> influx into living cells is mediated mainly by non-selective cation channels, such as the high-affinity K<sup>+</sup> transporters HKT and non-selective cation channels (NSCC) (Uozumi *et al.*, 2000; Ward *et al.*, 2003; Wu, 2018; Gupta *et al.*, 2021), and Na<sup>+</sup> efflux is known to be mediated by antiporters such as SOS1, a Na<sup>+</sup> / H<sup>+</sup> antiporter (Keisham *et al.*, 2018; Gupta *et al.*, 2021).

Inside plants, there are transport mechanisms from the external media into cells and tissues, and across tissues into others, but again one mode of entry of ions into plant tissues is readily reversible and non-metabolic, whereas the other depends on metabolism (Epstein, 1960; Aubry *et al.*, 2019) and could not occur after the initial step of thermal treatment.

The variation of Na<sup>+</sup> concentration over time has also to be discussed. For plant tissues at room temperature, in root samples no thicker than about 1 mm, "equilibration" between the "outer" space and the external solution is usually complete within an hour, and often much less, after immersion of the tissue in the solution

(Epstein, 1960; Ma and Peterson, 2000). When a plant cell or plant tissue is transferred from distilled water to a salt solution, as a rule, a "rapid" initial uptake (1-2 hrs) is followed by a more gradual long-continued absorption (Higinbotham, 1973). The ions taken up during the initial phase are partly released again after transfer to distilled water. As a consequence of the free diffusion of ions to and from a certain space in the tissue, this space has been designated by Briggs (Lv *et al.*, 2012; Ruan *et al.*, 2010) as "free space" and more specifically as "water free space". The initial rapid entrance of ions into cells and tissues is a "diffusion" of ions from the medium to some parts of the cells and is not directly dependent on metabolism. For tissues being thermally processed, molecular and ionic diffusion could be accelerated with temperature (McLaughlin, 1959), and the question is to know if the processing time was or was not higher than the time for reaching the equilibrium state.

*Exp- experiments:*

For Exp- experiments (Figures 3 and 4), the

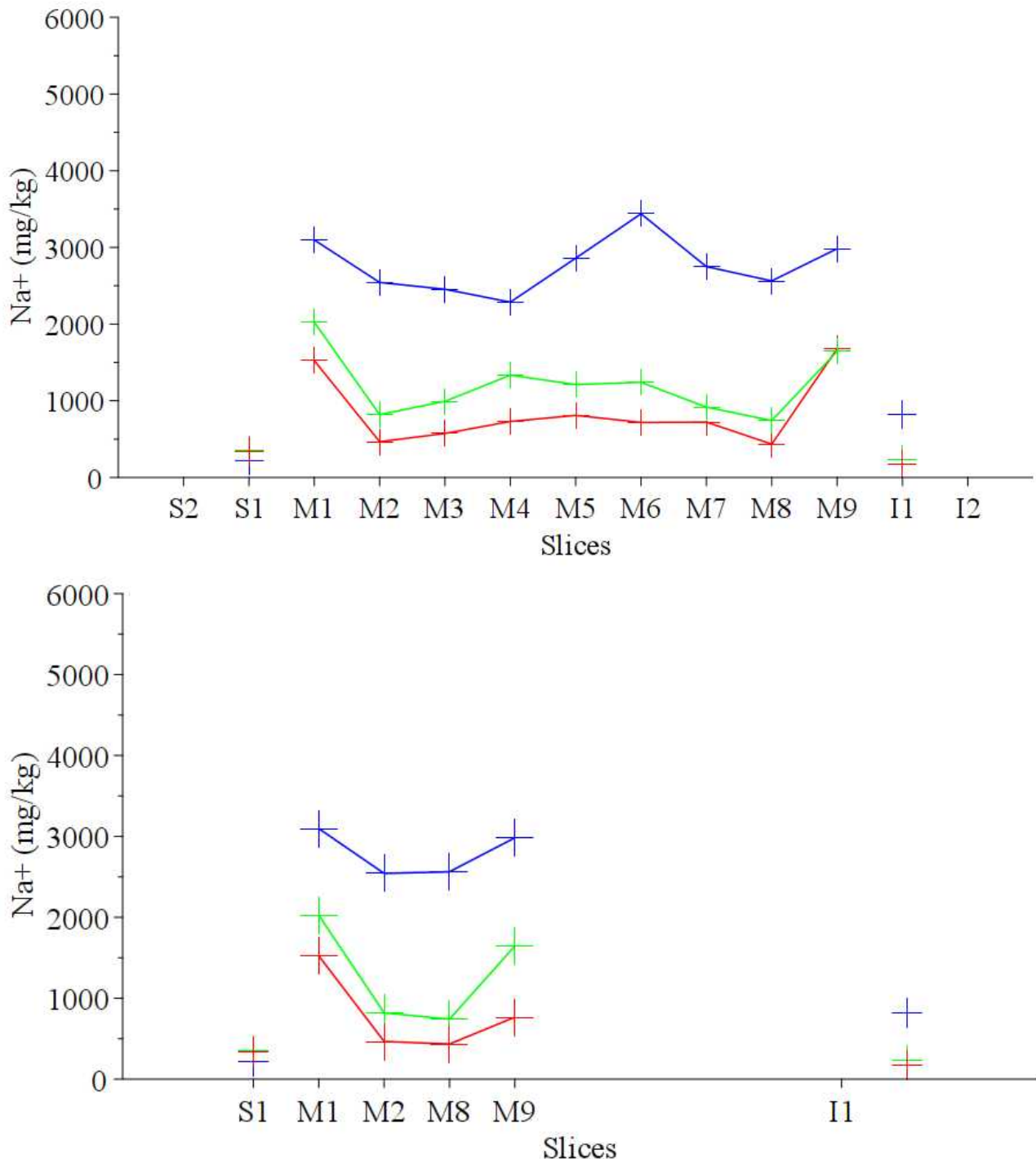


Figure 6. (Top) Na<sup>+</sup> concentration in the M segment, for the 7th experiment, for which a longer carrot segment was processed in a solution of NaCl (3965 mg/kg). The Na<sup>+</sup> distribution is different from the other Exp<sup>+</sup> experiments. But when only the tips are considered (bottom), a U shape is obtained, as for the other Exp<sup>+</sup> experiments.

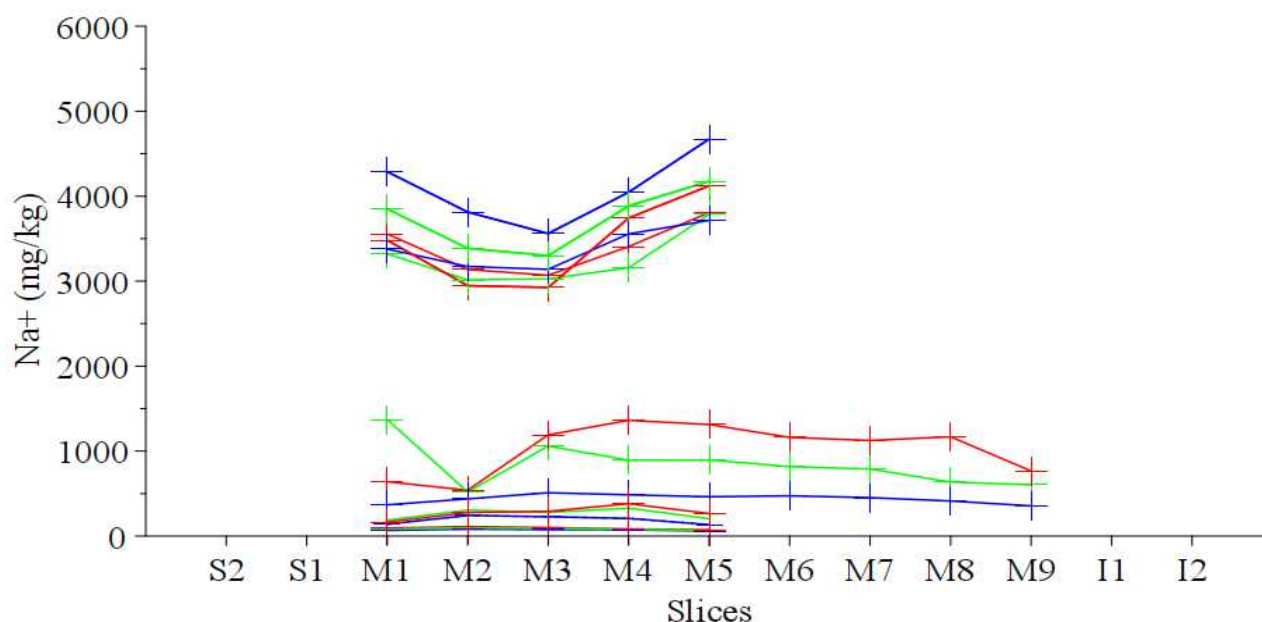


Figure 7. Comparison of Na<sup>+</sup> concentrations in carrot samples thermally processed in initially pure water (lower crosses) and in a NaCl aqueous solution (upper crosses). The color code is as for all other figures. The results for the 7th experiment are omitted in order to show more clearly the separation of the two groups of samples.

resulting Na<sup>+</sup> distribution has the same overall shape as if Fickian diffusion occurred either through channels or through the symplast, from initially loaded tissues towards the outside solution (Crank, 1975), but the complex structure of plant tissue prevents making such a simplistic assumption; “molecular diffusion” can only take place locally, in some compartments, and many interactions of ions can occur with the various charged compounds inside the plant structure, such as proteins, pectins, etc. (Crafts and Broyer, 1938; Hope, 1953; Briggs *et al.*, 1958; Epstein, 1960; Epstein, 1973; Ma and Peterson, 2000).

In particular, the “leaching” of roots has been investigated (Brooks, 1916; Tukey, 1970; Oliveira and Silva, 1992), the transport of various species being followed between plant tissues and the various compartments of the soil, including the liquid one (Kim *et al.*, 2004; Aubry *et al.*, 2019). This phenomenon is similar to the exchange between roots and the aqueous solution in which they are thermally treated. However one should

also consider exchanges between parts, with mechanisms that are not clear.

The fact that the Na<sup>+</sup> concentrations at the end of the thermal treatment are different in the various parts (C, E, P) only points to the conclusion that exchanges between one part and its surrounding is particular, and different than for other parts, and that anisotropic mechanisms are at play. The fact that the Na<sup>+</sup> concentrations are significantly higher in P parts than in C parts, at the end of Exp-, shows that there are likely exchanges perpendicular to the axis of the roots (xylem and phloem being present in the C part, transfers of ions only through them, by Fickian diffusion, would have produced a distribution opposite to the one observed).

In order to investigate Na<sup>+</sup> transport mechanisms, the concentration of calcium (Ca<sup>2+</sup>) was also determined in the same plant tissues, with the assumption that similar transfer mechanisms would give the same distribution of Ca<sup>2+</sup> concentration after thermal processing. Ca<sup>2+</sup> is an



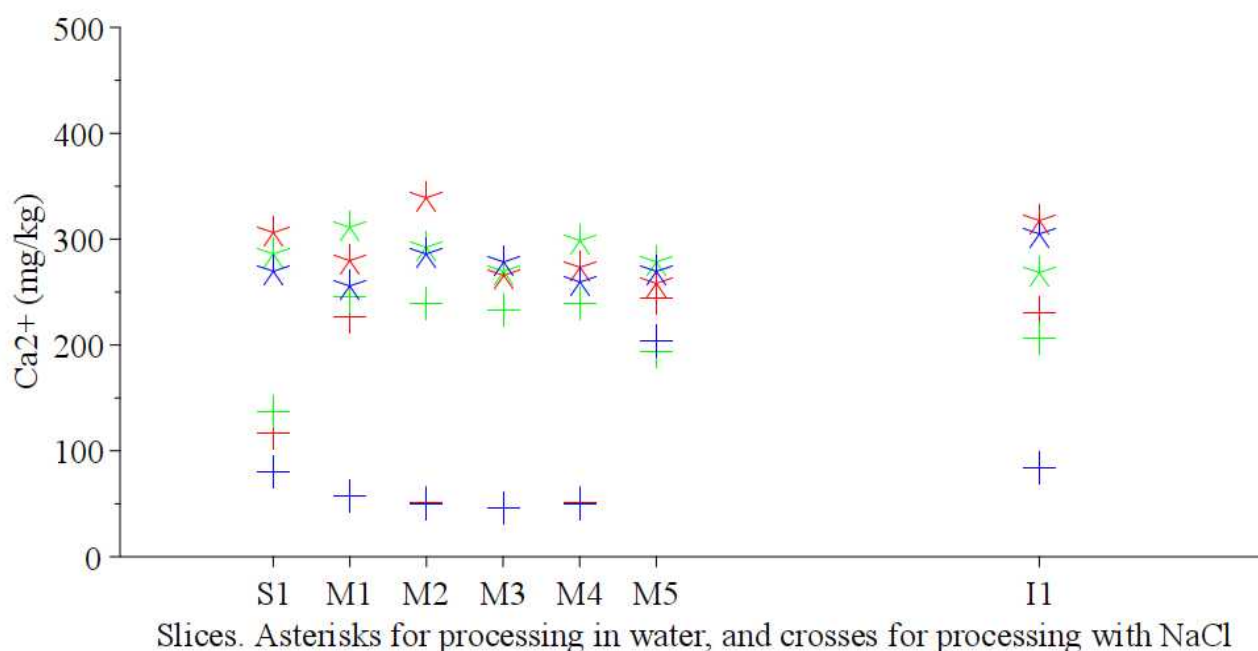


Figure 8. Distribution of Ca<sup>2+</sup> ions in carrot segments thermally processed in aqueous solutions without (asterisks) or with (crosses) NaCl. Numbering and colors are as in other pictures: C parts are depicted in blue, E parts in green and P parts in red.

essential plant nutrient: it is required for structural roles in the cell wall and membranes, as a counter-cation for inorganic and organic anions in the vacuole, and as an intracellular messenger in the cytosol (Marschner, 1995; Yang and Jie, 2005). Ca<sup>2+</sup> is taken up by roots from the soil solution and delivered to the shoot via the xylem (White and Broadley, 2003). It may traverse the root either through the cytoplasm of cells linked by plasmodesmata (the symplast) or through the spaces between cells (the apoplast). The relative contributions of the apoplastic and symplastic pathways to the delivery of Ca<sup>2+</sup> to the xylem are unknown (White, 2001; Kadirimangalam *et al.*, 2022). In living tissues, Ca<sup>2+</sup> enters plant cells through Ca<sup>2+</sup>-permeable ion channels in the plasma membranes (White, 2001), a submicromolar Ca<sup>2+</sup> concentration being maintained in unstimulated cells by Ca<sup>2+</sup>-ATPases and H<sup>+</sup> / Ca<sup>2+</sup>-antiporters (Sze *et al.*, 2000; Hirschi, 2001): these enzymes remove cytosolic Ca<sup>2+</sup> to

either the apoplast or the lumen of intracellular organelles, such as the vacuole or endoplasmic reticulum.

However, as for Na<sup>+</sup>, such systems should not be efficient in the experiments reported here, as the denaturation temperature is reached in the core after about 3 min, as compared to 30 min, for the overall thermal treatment. Moreover very different results were obtained for Ca<sup>2+</sup> (Figure 8): the curved profile that appeared for Na<sup>+</sup> was not observed (as measured from a mean curvature radius), showing that the mechanisms for Na<sup>+</sup> and Ca<sup>2+</sup> transport inside the plant tissue are probably different.

As ions such as Na<sup>+</sup> do not evaporate nor disappear from the solution (excepts through possible sputtering), the total quantity of this ions in the liquid and the root had to remain constant, and a balance was calculated, the variation of Na<sup>+</sup> content in the processing liquid before and after treatment being compared to an estimation

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of losses from the plant tissue. These losses were in turn calculated from the difference between the concentration of a slice and the concentration of the nearest raw slice (for the middle slice, the difference was either calculated with the S1 slice, or with an average value for S1 and I1). With this calculation method, it was observed that no lateral transfer is needed to explain the experiment # 1 (Exp-), but no such conclusion can be made with experiment # 2 (Exp-), because the recovery of the liquid was not sufficient; for experiment # 3 (Exp+), the liquid got more Na<sup>+</sup> than what was calculated to go out from the plant tissue, so that lateral transfer cannot be excluded. And finally the fact that the P parts are the more depleted in Na<sup>+</sup> than the two others shows that this kind of calculation is not relevant.

### *Exp+ experiments:*

In the presence of elevated levels of external Na<sup>+</sup>, under saline conditions, Na<sup>+</sup> efflux from plant cells is an active process (Blumwald, 2000; Apse and Blumwald, 2007 ; Kronzucker and Britto, 2011). But for most of the thermal treatments implemented here, proteins (including transporters) are denatured, so that ions carriers are no longer active; only passive transfers can occur.

For thermal processing in aqueous NaCl solution, all Exp+ experiments showed that all parts of the whole M segments absorbs Na<sup>+</sup>, but there was a difference depending on the size of carrots: for the experiments with small segments (# 3, 4, 5), the upper and lower ends take in more Na<sup>+</sup> than the inner parts with much differences between the various (C, E, P) parts of the root. However, for the last Exp+ experiment (# 7), the distribution of Na<sup>+</sup> inside the tissue does not show the U shape that is observed for small roots. In Figure 6 (bottom), it can be seen that if only the external parts of the distribution are considered (as for small roots) and joined together, then the U-shape is recovered, as if more time were needed to change the Na<sup>+</sup> concentration in the inner parts of carrots. This would be in favor of the diffusion (Fickian) mechanism, but the Na<sup>+</sup> distribution between the various C, E, P parts also shows that the P part is

more loaded with sodium, indicating also a lateral transfer, as for Exp- experiments.

To further analyse the relative contributions of longitudinal and lateral transfers, the quantity of Na<sup>+</sup> that was lost (during Exp- 1, 2, 6) or absorbed (during Exp+ 3, 4, 5, 7) was compared for the adjacent raw parts, assuming that the Na<sup>+</sup> distribution would be the same in neighbouring parts) and with the processing solution, but in spite of much care (e.g., weighing the funnel in which the plant tissue is recovered, weighing the paper with which the plant tissue is cleaned, etc.), the heterogeneity of the parts was too large to get meaningful results. Other experimental setups need to be implemented for answering this question.

### *Comparison of Exp- and Exp+ experiments:*

In Figures 3 and 4, it cannot be seen which part of the carrot contains more Na<sup>+</sup>, so that the difference of concentrations between the P and C part was calculated, and the result is clear: for Exp- experiments, the difference is negative, whereas it is positive for Exp+ experiments. Also, for Exp- experiments, the difference is negative for P and E parts, whereas it is positive for Exp+ experiments. And the E part is in between, both for Exp- and Exp+ experiments. This corroborates a radial transfer of Na<sup>+</sup>, not only from the outside liquid toward the P part of the roots, but also from P parts to E parts, and to C parts.

Finally, the content in salt was estimated for all carrots, using the masses and the concentration for all parts of all slices. For Exp- and Exp+ experiments, it was respectively 0.68 and 4.14 mg/g of fresh product, which is in accordance with the results by Dysko and Kaniszewski (2007), Smolen *et al.* (2012) and Kwiatkosky *et al.* (2015).

## 5. Conclusions and perspectives

Using AAS, it was first shown that, for a thermal treatment longer than is usually done by cooks, the Na<sup>+</sup> concentration can be very different in the

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various parts of raw carrot roots, with no coherent distribution. Na<sup>+</sup> is released from carrot tissues when thermally treated in pure water, with a reduced concentration in the parts in contact with the liquid (tips and "parenchyma"). When the plant tissues are treated in aqueous NaCl solutions, the Na<sup>+</sup> concentration increases throughout the plant tissue, with more Na<sup>+</sup> in the external parts (again tips and "parenchyma"). Some exchanges arise through the cut tips, but also through the lateral surface. Further studies in which the lateral side of carrots are protected against Na<sup>+</sup> transfer are needed to test this assumption. In order to better investigate the mechanisms of Na<sup>+</sup> uptake, unpeeled carrot and peeled carrots could be thermally processed, and compared. Finally, in order to check the reversibility of Na<sup>+</sup> transfers inside plant tissues, it would be interesting to first process plant tissues in a NaCl solution, before performing a second thermal treatment of the same tissue in pure water; perhaps an initial hardening protection of the tissues against disintegration (using prior Ca<sup>2+</sup> impregnation) would be needed, as the 30 min processing time used for the studies reported here led to the softening of the tissues, through the beta elimination of pectins (This, 2009). Anyway, even at this stage, the data obtained in our experiments give a clear basis for interpreting the current culinary practices and advising consumers about salt uses.

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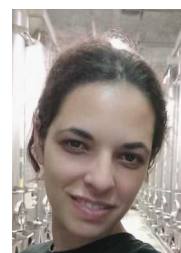
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