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Free radical generation in uncooked carrot (*Daucus carota*) root tissue after cell disruption – A model for chemical reactions during mastication

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ABSTRACT

Free radical processes resulting from cell disruption of carrot hypocotyl root stock have been investigated at various developmental stages and positions using EPR spectroscopy in combination with chemical spin traps, in order to simulate reactions that might occur in the mastication process when eating raw carrot tissue. With the spin trap PBN, EPR spectra corresponded to a carbon-centred radical adduct of the spin trap and the *N*-(*t*-butyl)aminoxyl radical, an oxidative breakdown product of the spin trap. When 4-POBN was used, an oxidation product of the spin trap was observed along with the *N*-(*t*-butyl)aminoxyl radical. These oxidation reactions were strongest in the root tips. Additional measurements with spin traps DEPMPO and DPPMPO showed the formation of hydroxyl radical adducts. Thus oxidative free radical production occurs on cell disruption, especially in rapidly developing tissue, a result which suggests that free radicals might not be detrimental components of foods.

1. Introduction

Carrots are important horticultural crops and make contributions to a healthy diet, which are believed to be at least partly because of their contents of beneficial antioxidant molecules, especially carotenoids (Britton, Liaaen-Jensen, & Pfander, 1995). One function of these molecules *in planta* is to control free radical reactions which are generated during normal metabolic processes (Smirnoff, 1993). Although free radicals are generally considered to have undesirable consequences in biological systems, they also have important functions, especially in developing tissues. For example, free radicals have been reported to be involved in cell wall softening during the growth of roots (Liszkay, van der Zalm, & Schopfer, 2004) and shoots (Schopfer, Liszkay, Bechtold, Frahry, & Wagner, 2002). Schopfer (2001) suggested that cell-wall polysaccharides are cleaved by hydroxyl (·OH) radicals generated in a Fenton-type reaction. This reaction involves the oxidative breakdown of H_2O_2 by a low oxidation state transition metal ion (e.g. Fe(II)) (Cohen, 1985; Symons & Gutteridge, 1998). In plants, H_2O_2 is generated in various biochemical reactions, one of the most important being dismutation of the superoxide anion radical ($O_2^{\cdot-}$) (Jiang & Zhang, 2002), which is catalysed by the enzyme superoxide dismutase (SOD) (McCord & Fridovich, 1969). There are several potential sources of ($O_2^{\cdot-}$) in cells, including cell wall bound peroxidases, or NADPH oxidases in the plasmalemma (Neil, Desikan, & Hancock, 2002). These produce ($O_2^{\cdot-}$) by transfer of an electron from cytoplasmic NADPH to extracellular oxygen. Thus free radicals are produced extensively in plant tissues, especially those that have not been processed. Furthermore, cell disruption leads to the mixing of intra- and inter-cellular components, which have the potential for the initiation of additional free radical reactions. Such reactions in carrot are investigated in the present paper, and correspond to the reactions that would be expected to occur during the chewing process as well as those that occur on cutting during food preparation.

Previous studies of free radical processes in carrot hypocotyl root stock (Goodman et al., 2002) found major differences in behaviour between different specimens with respect to the products of their reaction with the spin trap α-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone (4-POBN). Those electron paramagnetic resonance (EPR) spectroscopy measurements were based on commercially-grown carrot samples of unknown age and origin. It was thus unclear whether these differences were related to the plant genetics, storage or growth history, or position in the carrot from which the sample was taken. The present experiments were undertaken with the objective of answering (at least some of) these questions. Any effects related to genetic variations were eliminated by using seeds of just one variety from a single seed lot. Plants were grown under carefully controlled conditions using an automated irrigation system to avoid water stress. Harvests were made at three distinct developmental stages and samples from different positions in the root tissue were investigated.

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The reactions that occurred in the carrot tissue on and after cell disruption were investigated using a number of spin traps with different chemical structure and reactivity. Two separate experiments were performed in order to investigate the generation of these short lived radicals during cell disruption. In one experiment, samples were ground in a liquid nitrogen slurry at 77 K and the frozen powder was then mixed with the spin traps 4-POBN or phenyl-*N*-*t*-butylnitrone (PBN). In another experiment, fresh samples were ground at room temperature in the presence of each of the spin traps 4-POBN, PBN, 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-*N*-oxide (DEPMPO) and 5-(dipropoxyphosphoryl)-5-methyl-1-pyrroline-*N*-oxide (DPPMPO).

The hydrophilic spin trap 4-POBN and the more lipophilic molecule PBN were chosen because of their abilities to detect carbon (C)-centred radicals, with which they form relatively stable adducts. The half-life ($t_{½}$) of POBN-CH_3 has been reported to be ~2 h (Samuni, Carmichael, Russo, Mitchell, & Riesz, 1986). In contrast, adducts of oxygen (O)-centred radicals are short-lived (e.g. the ·OH radical adduct of PBN has $t_{½}$ of 38 s (Janzen, Kotake, & Hinton, 1992)). Therefore, other spin traps are required to detect the production of O-centred radicals. Spin traps related to dimethylpyrroline-*N*-oxide (DMPO) were used for this purpose. Although ·OH and alkoxyl radical adducts of DMPO are relatively stable, the ($O_2^{\cdot-}$) adduct is not. However, its stability is greatly enhanced with some DMPO derivatives ($t_{½}$ is *c.* 14 min with DEPMPO (Fréjaville et al., 1995)) and, depending on the solvent, 7–28 min with DPPMPO (Stolze, Udilova, & Nohl, 2000), so these latter spin traps were also used to distinguish between these two types of O_2-derived radical.

2. Materials and methods

2.1. Production of carrot tissue

Seeds of carrot (*Daucus carota* cv. Maestro) were sown in two 15-l pots, which were covered with plastic until the plants were *c.* 2 cm high. During this germination stage, the pots were watered from above only; later a home-made self-irrigating system was used. Each pot was placed above a bucket filled with water, to which it was connected by wicks of glass fibre. Samples were harvested at three different developmental stages 49, 55, and 83 days after sowing. Thin slices of each carrot sample were taken from the top, the centre, and the tip of the root (picture in Table 1). They were immediately frozen to 77 K and stored in liquid nitrogen until analysis. In a second set of experiments thin slices of fresh carrot were excised and used immediately.

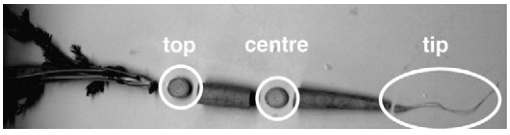
2.2. Chemicals

The spin traps PBN and 4-POBN were purchased from Sigma-Aldrich, whereas DEPMPO and DPPMPO were synthesised at the Molecular Pharmacology and Toxicology Unit at the University of Veterinary Medicine Vienna.

2.3. Preparation of samples for EPR spectroscopy

In one set of experiments, EPR spectroscopy was carried out using the spin traps PBN and 4-POBN to detect short-lived radicals when cells were disrupted. The 4-POBN solution was made with distilled water, the PBN solution was prepared with 10% aqueous ethanol, because of the relatively low solubility of PBN in water. Frozen carrot samples were ground under liquid nitrogen until a homogenous powder was obtained. A small quantity of the powder (*c.* 50 mg) was gently mixed with 800 μl of the spin trap solution (164 mM) in a second mortar. The suspension was filtered through a disposable syringe filter holder (Fa. Sartorius, 0.45 μm), the filtrate was transferred into a flat cell and the EPR spectrum measured immediately. The reproducibility of this procedure was confirmed on one sample by repeating the procedure three times. The standard deviation was found to be ~4%. In a second set of experiments, where the aim was purely the quality of the EPR spectrum, thin slices of fresh carrot were crushed in a mortar together with the spin traps DEPMPO and DPPMPO (~300 mM), PBN and 4-POBN (164 mM). It was necessary to use a higher spin trap concentration with DEPMPO and DPPMPO because the signal intensity of radical adducts is spread over 12 peaks compared to 6 peaks for adducts of PBN and 4-POBN (except for the signal from the oxidation product of the spin trap). The mixture was then centrifuged for 3 min (13000 rpm, 4 °C), after which 500 μl of the supernatant were transferred into cryo-tubes and stored in liquid nitrogen. Immediately prior to running the EPR spectra, the samples were thawed in a water bath (hot water, ~40 °C) and transferred to a flat cell as quickly as possible.

Table 1
Carrot sampling positions, and means and standard deviations for the EPR signal intensities obtained with PBN and 4-POBN for samples from different positions in the carrots



EPR-signal	Position in carrot	Mean ± std	*p*-values		
			Top:centre	Top:tip	Centre:tip
C-centred radical with PBN	Top	7.0 ± 0.8	0.0098	0.0030	0.0014
	Centre	9.9 ± 1.9			
	Tip	4.4 ± 1.1			
N-(*t*-butyl)aminoxyl radical with PBN	Top	7.5 ± 4.3	0.0080	NS	0.0001
	Centre	1.9 ± 1.6			
	Tip	9.0 ± 1.6			
Oxidation product with 4-POBN	Top	55 ± 27	0.0326	NS	0.0146
	Centre	19 ± 22[a]			
	Tip	123 ± 53			

Significant differences were calculated by the *t*-test for paired samples; the *p*-values indicate the level of significance.
[a] High standard deviation due to few replicates.

2.4. EPR spectroscopy

All EPR spectra were recorded using a Bruker ESP300E computer-controlled spectrometer operating at X-band frequencies equipped with an ER4103TM microwave cavity. Microwave generation was by a klystron. Spectral measurements were performed at ~22 °C in a flat cell (Wilmad-Labglass, Buena, NJ, USA). Spectra from the samples with PBN and 4-POBN spin traps were recorded as the sum of 10 scans, (each taking about 42 s) in 1024 points using 100 kHz modulation frequency. A microwave power of 20 mW, modulation amplitude of 0.16 mT and a sweep width of 6 mT were used. Spectra with the other spin traps were recorded as single scans, taking about 84 s in 1024 points using 100 kHz modulation frequency. A microwave power of 20 mW, modulation amplitude of 0.068 mT and a sweep width of 12 or 14 mT were used. Approximate hyperfine couplings were measured manually and then refined by spectral simulation using the Bruker SimFonia software. All values from the current work presented in Table 1 were confirmed by simulation.

For quantification, spectra were baseline corrected and filtered twice using a 15-point polynomial filter. The first peak, doublet or quartet of each spectral component was integrated and its intensity used for the quantitative assessment. One carrot represents one replicate. Two replicates of each of the 3 harvests were investigated with the spin trap PBN whereas two other replicates of the 1st and two of the 3rd harvest were chosen for measurements using 4-POBN. For a better overview and to observe any trends in the graphs the amounts of generated radicals are given for each position of each replicate and harvest separately. The means and the standard deviations are summarised in Table 1.

2.5. Statistical analysis

Differences in the amounts of spin adducts obtained with tissues from various positions in the carrots were determined by the *t*-test with paired samples. STATISTICA software (StatSoft Inc., Tulsa, USA) was used for all calculations of means, standard deviations and tests for significant differences between means.

3. Results

The EPR spectra obtained with PBN were similar with both methods of sample preparation. They consisted of two components (Fig. 1a), a sextet with parameters ($a(^1H) = 0.328$ mT; $a(^{14}N) = 1.590$ mT) similar to those of a C-centred radical adduct of the spin trap, and a quartet with parameters ($a(^1H) = 1.393$ mT; $a(^{14}N) = 1.461$ mT) identical to those reported for the *N*-(*t*-butyl)aminoxyl radical (Atamna, Paler-Martinez, & Ames, 2000; Britigan, Pou, Rosen, Lilleg, & Buettner, 1990; Chamulitrat, Jordan, Mason, Kieko, & Cutler, 1993). The larger linewidths of the high field spectral components are the result of incomplete motional averaging of the spectral anisotropy, a common phenomenon with nitroxide radicals.

The intensity of the sextet signal varied according to the position in the carrot root from which the sample was taken (Fig. 1), the highest intensity being obtained with samples from the centre of the root and the lowest with those from the tip (Fig. 1b). The age/maturity of the tissue did not appear to have much influence on the intensity of this signal. Hence the various data obtained at different times from the tops, centres and tips of the carrots were averaged. These results show significant differences between the different sampling positions (Table 1).

The intensity of the *N*-(*t*-butyl)aminoxyl radical was also dependent on the position in the carrot root from which the sample was taken. In this case, however, the lowest signal intensity

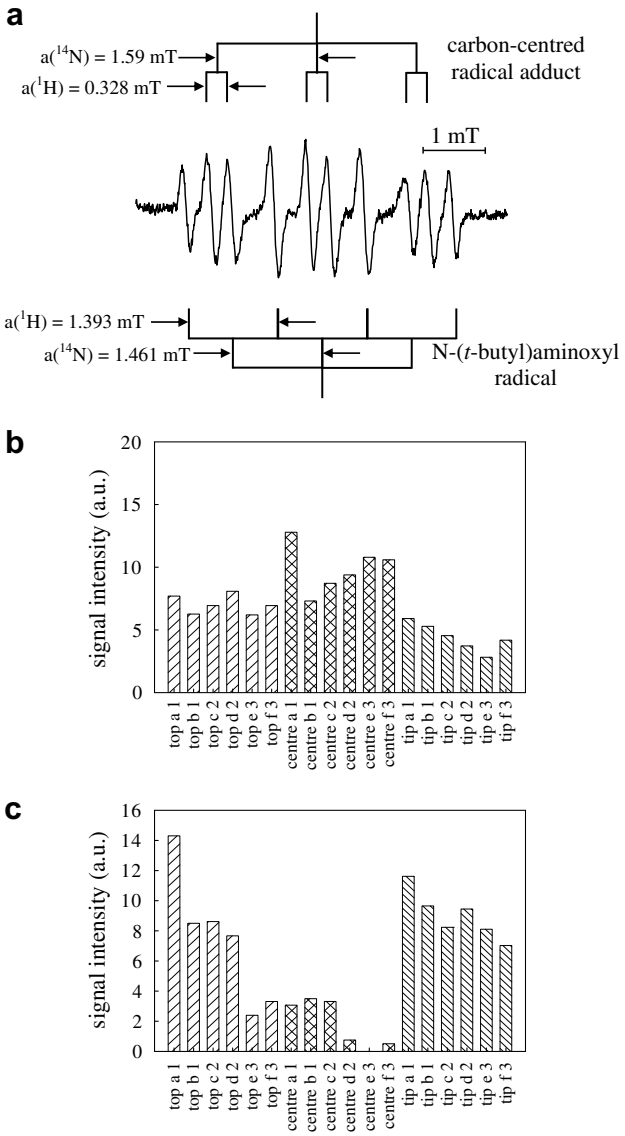


Fig. 1. (a) Typical EPR spectrum of a carrot sample obtained using the spin trap PBN. It consists of a sextet (carbon-centred radical) and a quartet (*N*-(*t*-butyl)aminoxyl radical). Spectral interpretation is shown by the "stick" diagram. (b, c) Signal intensity of the carbon-centred radical trapped with PBN (b) and the *N*-(*t*-butyl)aminoxyl radical from PBN (c) in relation to the position and the maturity of the carrot. a, b, c, d, e, f are replicates of the carrots. Top, centre, tip are the position of the carrot where the samples were taken. 1, 2, 3 are the 1st, 2nd and 3rd harvests. 1, 2, 3 are the 1st, 2nd and 3rd harvests.

was obtained with tissue from the centre of the carrot (Fig. 1c). Also, the intensity of this signal appeared to show a small decrease with increasing age of the samples. However, independent of any age relationship, the mean and standard deviation of the data from the different positions in the carrots showed significant differences between the top and the centre as well as between the centre and the tip of the carrots (Table 1).

The EPR spectra obtained with the spin trap 4-POBN were also identical for both preparation procedures. The spectra consisted of two signals (Fig. 2a), one of them being similar and the other different from those obtained with PBN. A minor component corresponds to the *N*-(*t*-butyl)aminoxyl radical as seen with PBN, but the spectra were dominated by a dodecet signal ($a(^1H) =$ 0.145 mT, 0.050 mT; $a(^{14}N) = 1.490$ mT, 0.180 mT), which corresponds to an adduct involving a radical oxidation product of the spin trap (McCormick, Buettner, & Britigan, 1995). Within

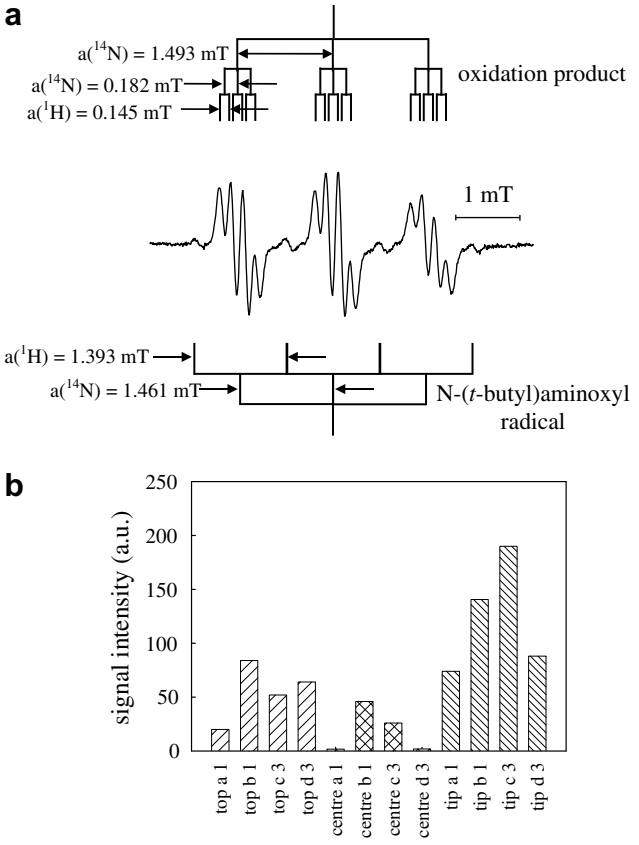


Fig. 2. (a) Typical EPR spectrum from the reaction of a carrot sample with 4-POBN as spin trap. It consists of an oxidation product of the spin trap and the *N*-(*t*-butyl)aminoxyl radical. Spectral interpretation is shown by the "stick" diagram. (b) Signal intensity of the oxidation product from 4-POBN in relation to the position and the maturity of the carrot. a, b, c, d are replicates of the carrots. Top, centre, tip are the position of the carrot where the samples were taken. 1 and 3 are the 1st and 3rd harvests.

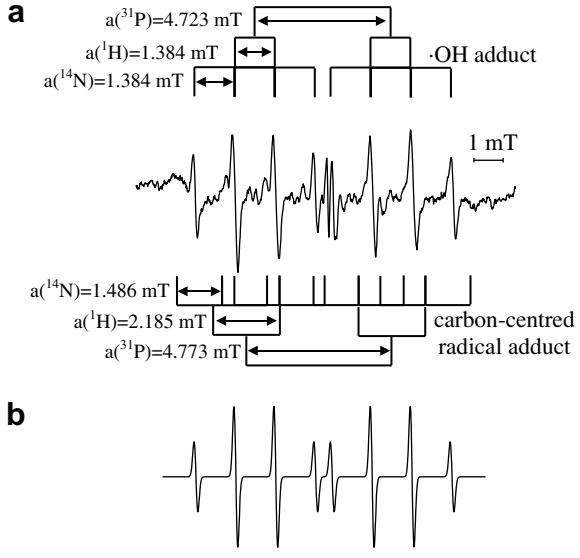


Fig. 3. EPR spectrum of (a) an ·OH-radical from carrot trapped by DEPMPO, (b) simulation of spectrum (c). Spectral interpretation is shown by the "stick" diagram.

individual carrots, there appeared to be a relationship between the intensity of the dodecet signal and the position in the carrot from which the samples were taken, the highest intensity being with

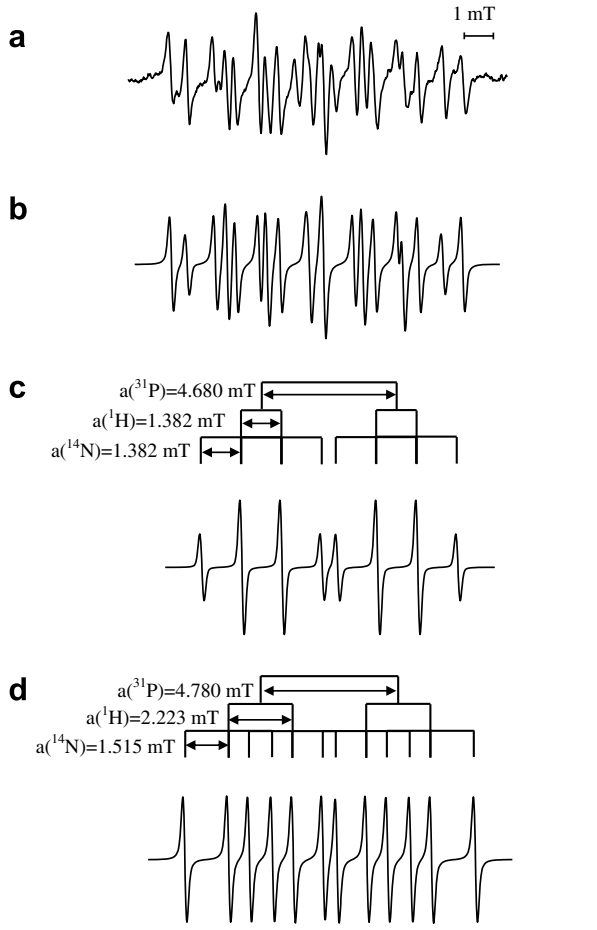


Fig. 4. EPR spectra from carrot and DPPMPO (a) Experimental spectrum, and simulations of (b) weighted sum of C-centred and ·OH-radical adducts, (c) the ·OH-radical adduct, (d) the C-centred radical adduct. Spectral interpretation is shown by the "stick" diagrams.

samples from the tips and the lowest from the centres (Fig. 2b, Table 1). As with the PBN measurements, there was no correlation with age/maturity of the carrot.

The EPR spectra obtained with carrots and two different DMPO-related spin traps are shown in Figs. 3 and 4. The main feature in the spectra with DEPMPO (Fig. 3) corresponds to a ·OH radical adduct of the spin trap ($a(^{31}P) = 4.723$ mT; $a(^{1}H) = 1.384$ mT; $a(^{14}N) = 1.384$ mT). There is an additional peak near the centre of the spectrum which probably corresponds to one half of a doublet from the (monodehydro)ascorbate radical ($a(^{1}H) = 1.8$ G) (Buettner & Jurkewicz, 1993), with the other half being obscured by the ·OH-radical adduct signal. There are also broader features in positions characteristic of C-centred radical adducts ($a(^{31}P) = 4.773$ mT; $a(^{1}H) = 2.185$ mT; $a(^{14}N) = 1.486$mT). The relatively large width of these peaks suggests either that there is a small range of spectral values, consistent with the trapping of a number of similar, but not identical radicals, or that the trapped radical has a relatively high molecular mass, so that its motion in solution is not quite sufficient to completely average the anisotropic parameters.

With DPPMPO (Fig. 4) the spectra also contained a mixture of components from the ·OH radical (Fig. 4c) and C-centred radical adducts ($a(^{31}P) = 4.780$ mT; $a(^{1}H) = 2.223$ mT; $a(^{14}N) = 1.515$ mT) (Fig. 4d), but the contribution of the latter component was appreciably greater than with DEPMPO. When a simulation consisting of a weighted sum of these two components (Fig. 4b) was compared with the original spectrum it could be seen that there must also be

an additional component in the experimental spectrum. This signal is probably the same as that observed by Goodman et al. (2002), who assigned it to a second C-centred radical adduct.

4. Discussion

The carrot samples from different positions in the root all showed two distinct EPR signals after reaction with the spin traps PBN and 4-POBN. One of the components, with parameters identical to those reported for the *N*-(*t*-butyl)aminoxyl radical, was common to the reactions with both spin traps and must be derived either from fragmentation of the spin traps or their adducts (Atamna et al., 2000; Britigan et al., 1990; Chamulitrat et al., 1993), or from a common impurity (Dikalov, Vitek, Maples, & Mason, 1999).

Breakdown of PBN to benzaldehyde and *N*-(*t*-butyl)hydroxylamine has been described by Dikalov et al. (1999) (Fig. 5), and these products are considered to be common impurities of commercial samples of PBN. Breakdown of 4-POBN would be expected to yield the same *N*-(*t*-butyl)aminoxyl radical as PBN, which can be formed as a result of metal catalysed oxidation of *N*-(*t*-butyl)hydroxylamine. The most relevant metal in carrot tissue is iron with ~2.1 mg 100 g^{-1} (Carlsson, 2000). Fe(II) catalyses the Fenton reaction, which generates ·OH radicals, and these can then react with *N*-(*t*-butyl)hydroxylamine to form the *N*-(*t*-butyl)aminoxyl radical. Although we do not distinguish whether *N*-(*t*-butyl)aminoxyl radicals are formed as a result of reactions with impurities generated by breakdown of the spin traps during their commercial production, or from breakdown of the spin traps or their adducts during reaction with the carrot tissue, this is not important in the present work. Whatever the mechanism is, the spectral intensities are directly related to the amounts of ·OH radicals generated in the carrot tissue.

The C-centred radical that was seen with PBN is not necessarily a primary radical generated during cell disruption, and it could be a secondary product formed as a result of reactions of ·OH radicals with organic components in the carrot tissue. ·OH reacts indiscriminately with a wide range of organic molecules from which it can extract a H atom, leading to the formation of water and a C-centred radical.

$$R - H + {\cdot}OH \rightarrow R^{\cdot} + H_2O \quad (1)$$

Such C-centred radicals could, therefore, be responsible for part of the EPR signal observed with PBN, although as discussed later this reaction is probably not the main source of C-centred radicals in the present experiments.

Apart from their implication in the formation of *N*-(*t*-butyl)aminoxyl radicals, there is more direct evidence for the presence of ·OH radicals from measurements with the spin traps DEPMPO and DPPMPO. A signal from a C-centred radical adduct was also observed when DEPMPO and DPPMPO were used as spin traps, but as was reported by Goodman et al. (2002), the C-centred radical adduct with DEPMPO was less stable than the ·OH radical adduct. The difference in the distribution of radical adducts seen with PBN and DEPMPO illustrate the selectivity of spin traps for different types of radical.

It may be significant that the spectrum of the ·OH radical adduct with DEPMPO was accompanied by a single peak component

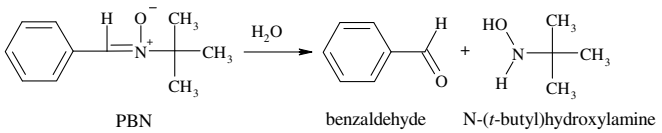


Fig. 5. Decomposition mechanism for the spin trap PBN.

(which is most likely one half of the spectrum from the ascorbate radical). This result indicates that ascorbic acid may also be involved in oxidation reactions in the carrot tissue.

When 4-POBN was used as spin trap, both of the observed EPR signals correspond to reactions that alter the structure of the spin trap. In addition to the *N*-(*t*-butyl)aminoxyl radical, the dodecet signal corresponds to an adduct of a radical formed by oxidation of 4-POBN. Such a radical has been observed previously with carrots (Goodman et al., 2002), and with a number of different types of leaf tissue (Glidewell, Goodman, & Skilling, 1996; Pirker, Reichenauer, Goodman, & Stolze, 2004). The formation of the 4-POBN· radical is dependent on the presence of oxygen, and as suggested earlier (Pirker et al., 2004) 4-POBN may be oxidised by oxidation products that are formed during cellular disruption.

In some specimens, there was a clear relationship between the intensities of the various EPR signals and the positions in the carrot from which the samples were taken. The oxidation product observed with 4-POBN and the *N*-(*t*-butyl)aminoxyl radical observed with PBN were highest in the tips and lowest in the central regions, whereas the C-centred radical seen with PBN was highest in the samples from the central regions of the roots and lowest in the tips. On the basis of the positive identification of the ·OH radical with DEPMPO and DPPMPO, it seems likely that this radical is involved in the generation of both the 4-POBN oxidation product and the *N*-(*t*-butyl)aminoxyl radical, which was seen with both PBN and 4-POBN. It has also been reported (Baranska, Baranski, Shulz, & Nothnagel, 2006) that the production of carotenoids is tissue specific and that α-carotene/lutein are formed more rapidly than β-carotene in young carrot cells. There may, therefore, be considerable heterogeneity in the composition of antioxidants in different sections and tissues of carrots and other plant food products and thus differences in the redox status.

All of the evidence discussed above suggests that the ·OH radical plays a fundamental role in the free radical processes in carrot tissue after cell disruption. There is also increasing evidence that ·OH radicals occur extensively in plant root tissues. For example, in experiments using root tips of maize seedlings (*Zea mays*) (Liszkay et al., 2004; Schopfer, 2001; Schopfer et al., 2002), they were considered to play an important role in root elongation by facilitating cell wall loosening in the growing zone. Also, Renew, Heyno, Schopfer, and Liszkay (2005) have reported a method for the localisation of ·OH radicals in cucumber roots by ESR using a spin trap *in vivo*. The higher levels of ·OH radicals and their putative reaction products seen in the present work with the tissue from carrot root tips are thus consistent with these previous observations, and support the idea that this radical is of general importance in plant root development. The higher level of ·OH radical generation at the tips compared to the other tissues after cell disruption of the carrot could, therefore, be the explanation for the higher oxidation potential. Fry (1998) and Schopfer (2001) have implicated ·OH radicals in the oxidative breakdown of plant cell wall polysaccharides, so they may have an important function in the digestive process. Also, ·OH radical production may be associated with reactions involving ascorbic acid. Since this antioxidant can play an important role in the redox cycling of transition metal ions that catalyse the Fenton reaction; this may be (at least part of) the explanation for the observation of the ascorbate radical in the EPR spectrum.

It was indicated above that reactions of ·OH radicals with organic molecules is a potential source of C-centred free radicals. However, the fact that the highest concentrations of the C-centred radical adduct (with PBN) were observed in tissues with the lowest levels of ·OH radical generation suggests that formation of (a sizeable fraction of) the C-centred radicals is independent of ·OH radical generation caused by cell disruption. The observation by Goodman et al. (2002) of two separate C-centred radical adducts with DEPMPO may represent radicals formed by ·OH-independent

and ·OH-dependent mechanisms. In those experiments, production of ·OH radicals was observed to continue over a period of days. The initial C-centred radical was unstable and its spectrum decreased in intensity over a period of hours. The spectrum of a second C-centred radical adduct was formed slowly, and then decayed slowly. Similar results were also observed in the present experiments when measurements were performed over extended periods of time, although they are not reported here.

5. Conclusions

The present experiments demonstrate free radical production in appreciable quantities on cell disruption of raw carrot, and these would also be expected to be formed during the mastication process. However, there was considerable spatial variability in the carrot root tissue, which resulted in differences in the reactions with spin trap molecules. With the PBN and 4-POBN breakdown and oxidation products, respectively, of the spin traps were generated to a much greater extent when it was derived from the rapidly growing root tips, than with tissue from other parts of the root. This is probably related to enzymatic generation of ·OH radicals, maybe indicating higher concentrations of either transition metals or H_2O_2 in this part of the root. In contrast the levels of the C-centred radical adduct of PBN were lowest when tissue from the tips was used, a result which suggests strongly that reaction of organic molecules with ·OH radicals is not the main source of these radicals.

It is commonly assumed that free radicals generated from food are detrimental to health, and that the role of antioxidants is to inhibit/prevent their reactions. On the other hand, it is recommended to eat raw plant products when they are young and fresh, and there is compelling evidence that such foods make a positive contribution to a healthy diet. However, our results show that these are the tissues that generate the highest levels of ·OH radicals. It seems, therefore, that the formation of ·OH radicals in foods, especially those that are biologically viable, is likely to be a common phenomenon, and is unlikely to be detrimental, at least at the eating stage. Consequently, there may be a need to reconsider the dietary effects of free radicals and antioxidant molecules in biologically-viable foods.

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