EFFECT OF NITRIC OXIDE ON METABOLISM OF FRESH-CUT APPLES AND LETTUCES IN RELATION TO SURFACE BROWNING

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DECLARATION

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution

__________________________
Roksana Huque
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<tr>
<td>AAP</td>
<td>ascorbic acid-2-phosphate</td>
</tr>
<tr>
<td>AATP</td>
<td>ascorbic acid-3-triphosphate</td>
</tr>
<tr>
<td>ABA</td>
<td>abscisic acid</td>
</tr>
<tr>
<td>ACC</td>
<td>1-aminocyclopropane-1-carboxylic acid</td>
</tr>
<tr>
<td>DETA</td>
<td>diethylenetriamine</td>
</tr>
<tr>
<td>DETANO</td>
<td>diethylenetriamine nitric oxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDRF</td>
<td>endothelium-derived relaxing factor</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>FAD</td>
<td>flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FMN</td>
<td>flavin mononucleotide</td>
</tr>
<tr>
<td>HO’</td>
<td>hydroxyl radical</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>HR</td>
<td>hypersensitivity response</td>
</tr>
<tr>
<td>IFPA</td>
<td>International Fresh-cut Produce Association</td>
</tr>
<tr>
<td>Inos</td>
<td>inducible NOS</td>
</tr>
<tr>
<td>LOX</td>
<td>lipoxygenase</td>
</tr>
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<td>messenger RNA</td>
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<tr>
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<td>Malondialdehyde</td>
</tr>
<tr>
<td>NiR</td>
<td>nitrite reductase</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NO’</td>
<td>free radical nitric oxide</td>
</tr>
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<td>NO$^+$</td>
<td>nitrosonium cation</td>
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<tr>
<td>NO$^-$</td>
<td>nitroxy anion</td>
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<tr>
<td>NO$_2$</td>
<td>nitrogen dioxide, nitrogen peroxide</td>
</tr>
<tr>
<td>N$_2$O</td>
<td>nitrous oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>NR</td>
<td>nitrate reductase</td>
</tr>
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<td>Abbreviation</td>
<td>Full Name</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>1-MCP</td>
<td>1-methylcyclopropene</td>
</tr>
<tr>
<td>O₂</td>
<td>oxygen</td>
</tr>
<tr>
<td>O₂⁻</td>
<td>superoxide anion</td>
</tr>
<tr>
<td>OONO⁻</td>
<td>peroxynitrite ion</td>
</tr>
<tr>
<td>PAL</td>
<td>phenylalanine ammonia lyase</td>
</tr>
<tr>
<td>PBN</td>
<td>N-tert-butyl-α-phenylnitrone</td>
</tr>
<tr>
<td>POD</td>
<td>peroxidase</td>
</tr>
<tr>
<td>PPO</td>
<td>polyphenol oxidase</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SNAP</td>
<td>S-nitroso-N-acetylpenicillamine</td>
</tr>
<tr>
<td>Sin⁻¹</td>
<td>3-morpholinosyl-nonomone</td>
</tr>
<tr>
<td>SNP</td>
<td>sodium nitroprusside</td>
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ABSTRACT

Surface browning is an important cause of deterioration of fresh-cut produce during postharvest handling. Nitric oxide (NO) has recently been found to delay the onset of surface browning in fresh-cut apples and iceberg lettuce.

Effectiveness of NO applied as NO gas and the NO donor compound 2,2’-(hydroxynitrosohydrazino)-bisethanamine (diethylenetriamine nitric oxide, DETANO) dissolved in phosphate buffer (pH 6.5) solution on the physiological parameters of ethylene production, respiration and water loss, and biochemical parameters of total phenol content, PPO activity, ion leakage and lipid peroxide level were investigated. Granny Smith apple slices treated with 10 µl.l⁻¹ NO gas and 10 mg.l⁻¹ DETANO showed delayed development of surface browning and also resulted in a lower total phenol content, inhibition of PPO activity, reduced ion leakage and reduced rate of respiration but had no significant effect on ethylene production, water loss or lipid peroxide level as measured by malondialdehyde and hydrogen peroxide levels. The two control treatments of phosphate buffer (pH 6.5) and water dips also had significant effects on all parameters compared to untreated slices. The relative effectiveness treatments on postharvest life, apple physiology and biochemistry was DETANO > NO gas > phosphate buffer > water > untreated. The NO donors, sodium nitroprusside (SNP) and Piloty’s acid dissolved in water also inhibited development of surface browning but were not as effective as DETANO.

Apple slices dipped in chlorogenic acid dissolved in water showed surface browning within an hour of treatment. Dipping in DETANO solution negated the effect of chlorogenic acid whether applied before or after dipping in chlorogenic acid solution while the buffer and NO
gas were also effective. A UV-scan of chlorogenic acid dissolved in water showed a marked decrease in absorbance over the eight day storage period suggesting that chlorogenic acid was oxidised by aerial oxygen. The addition of NO gas and DETANO accelerated the loss of chlorogenic acid.

It is suggested that browning development of fresh-cut produce can be inhibited by action taken soon after cutting. The concentration of phenols on the surface could be the rate limiting steps in browning development with non-enzymatic oxidation of phenols by atmospheric oxygen a contributor to browning.

NO gas, DETANO and SNP inhibited the surface browning of green oak lettuce slices. The optimum concentration of DETANO or SNP (500 mg.l⁻¹) and NO gas (100 μl.l⁻¹) resulted in approximately 60% and 30% increase in postharvest life over untreated slices respectively.