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#### **DOCTOR OF PHILOSOPHY**

Improved procedures for the transport and storage of fruit and vegetables

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## Improved Procedures for the Transport and Storage of Fruit and Vegetables



A thesis submitted to Bangor University by Karen Harper in candidature for the degree of Doctor of Philosophy

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#### **Abstract**

Natural methods (biocontrol) for the preservation of harvested fruits and vegetables were investigated. This was in order to potentially help the industry overcome the environmental problems and pathogen resistance being encountered with the use of synthetic fungicides.

The main focus of research was the use of volatile compounds found in citrus fruit. These were utilised in *in vivo* studies with citrus fruit artificially inoculated with *Penicillium digitatum* and *P. italicum*, as well as strawberries inoculated with *Botrytis cinerea*. The extent of pathogen growth was measured after exposure to the volatiles for a number of days (7 for citrus fruit and 3 for strawberries) in environmentally-controlled containers. The 'freshness' of the fruit was also ascertained via measurements of water/weight loss. The essential oil of orange itself was highly effective at reducing *Penicillium* growth (> 30% inhibition) and water/weight loss in the fruit (> 20% reduction), as were some of its individual components - (E)-2-hexenal, neryl acetate and linalool. The aldehyde (E)-2-hexenal also had high efficacy against *B. cinerea* and reduced water/weight loss in strawberries, although in these investigations the ketone, (R)-carvone was the most effective overall (> 30% mean pathogen inhibition and > 35% mean reduction in water/weight loss).

Organic and non-organic fruit were utilised in the *in vivo* experiments and discrepancies were observed in the results for each type. *In vitro* studies were therefore conducted with the volatiles, both alone and in combination with commercial fungicides (imazalil, thiabendazole and fludioxonil) in amended-agar investigations. Aldehydes (including (E)-2-hexenal) displayed the highest efficacy against *Penicillium species* and *B. cinerea* (> 90% inhibition), followed by carvone and methyl salicylate (> 40% inhibition). These observations were repeated in the fungicide-amended-agar work, where it was shown that these volatiles could be utilised to supplement the activity of synthetic fungicides and therefore reduce their overall use within the industry.

Chitosan was also investigated as a biological coating for harvested fruit. At 2% concentrations it reduced water/weight loss in oranges compared to uncoated fruit, whilst at 0.1% it reduced *Penicillium* infection in inoculated fruit by almost 50%. Acid and enzyme hydrolysis techniques were performed to obtain chitooligosaccharides with a degree of polymerisation (DP) of 3-10, reported to possess superior antifungal properties than commercially-available chitosan. The hydrolysates were analysed via MALDI-TOF-MS. Chitooligosaccharides produced by one of the acid hydrolysis methods and one obtained using the enzyme Laminarinase were found to be of the desired DP.

Overall, the work revealed the potential of some alternative methods for maintaining the freshness and extending the storage-life of fresh fruits. A number of citrus volatiles were found to be effective at reducing pathogen growth as well as protecting the fruit from water/weight loss. They were also found to operate in parallel with synthetic fungicides, thus implying that the domination of these chemicals in the post-harvest industry could be diminished by combined applications. Chitosan as an edible coating for fresh produce has also been shown to have potential, although additional work is required to elucidate this further.

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Lastly, I would like to dedicate this to my late father, Alexander Harper, whom I miss so very much. I know you'd be proud, Dad.

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	without limitation any related legal fees and court costs (on a full indemnity
	bases), related to any breach by myself of any term of this agreement.

Signature:
Date :

#### **Abbreviations**

ANOVA Analysis of variance

C Carbon

CI Chilling injury
CO<sub>2</sub> Carbon dioxide

COS Chitooligosaccharides
DA Degree of acetylation
DP Degree of polymerization

FLU Fludioxonil

FSA Food Standards Agency

**GlcN** Glucosamine

GlcNAC N-acetylglucosamine

H<sub>2</sub>O Water

HCl Hydrogen chloride
HMW High molecular weight

IMZ Imazalil K Potassium

K2SO4Potassium sulfateKC2H3O2Potassium acetateKCPotassium chlorideLMWLow molecular weightMeJMethyl jasmonateMeSMethyl salicylateMgCl2Magnesium chloride

MIC Minimum inhibitory concentration

m/z Mass-to-charge ratio
NaCl Sodium chloride
NaOH Sodium hydroxide
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> Ammonium sulfate

O2 Oxygen
P Phosphorous
P. italicum/digitatum
PD Potato dextrose agar
PP Polypropylene
RH Relative humidity
TBZ Thiabendazole

VPD Vapour pressure deficit