Grower Summary

FV 386

Use of gaseous ozone to prevent microbial post-harvest spoilage and reduce pesticide residue levels

Annual 2012

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Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

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Project Number:	FV 386
Project Title:	Use of gaseous ozone to prevent microbial post-harvest spoilage and reduce pesticide residue levels
Project Leader:	Ian Singleton and Shreya Wani (Student)
Contractor:	Newcastle University
Industry Representative:	Thane Goodrich, Intercrop Ltd Steve Rothwell, Vitacress Salads Ltd
Report:	Annual Report 2012
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Previous report/(s):	None
Start Date:	01 October 2011
End Date:	30 September 2014
Project Cost (Total project Cost):	£83,150 (£103,150)

Headline

In vitro studies suggest microorganisms associated with post-harvest spoilage are sensitive to ozone gas, but in vivo studies have not demonstrated a significant reduction of microbial load.

Background

The shelf-life of the fresh leafy produce is affected by the presence of spoilage microbes. Also, where field crops are sprayed with pesticides, residue issues maybe an issue for growers. Hence, there is a need to develop residue free alternatives to reduce both microbial load and pesticide residue levels; particularly in the climate of potential changes in EU pesticide regulation. Successful development of a residue free crop storage method will enhance the healthy image of the sector, enhance marketability of fresh produce and improve sales. The technology should create a market lead for UK produce and develop an expertise base in the UK.

The aims of the year 1 work were to explore the potential afforded by ozone treatment to reduce post-harvest spoilage and extend the shelf-life of leafy salads and herbs and specifically:

- To characterize microbial population over the shelf-life of targeted leafy produce
- To determine the impacts of ozone on key elements of the surface microflora
- Optimise the concentration & duration of ozone exposure for fresh and processed product
- Visualize bacteria on spinach leaves by using Confocal Scanning Laser microscopy.

Summary

This project focuses on the use of gaseous ozone treatment administered during pre-packaging to reduce post-harvest contamination, spoilage and pesticide residue levels in targeted produce (leafy salads and root vegetables). Initial work has focused on laboratory and pilot-scale optimization of ozone exposure treatments (level*duration) to reduce spoilage and enhance shelf-life of leafy salads. Depending on findings, the study may be extended to explore the impacts of ozone treatment on pesticide residues and/or a wider range of produce (especially root vegetables). It is anticipated that commercial-scale trials will be conducted during the course of the study to test the efficacy of the technology in an industry environment.

Objective 1: To characterize the principle microbial population of shelf-life of targeted leafy produce

Two packets of organic Italian style salad (lettuce, wild rocket & spinach), watercress & rocket, organic spinach & coriander were purchased and the microflora over the shelf-life of the product examined. Products were stored at 4^{o}C in the dark as directed on the packaging and tested at the start of life (SOL) and sell-by-date i.e. end of life (EOL). A range of micro-organisms were present and microbial numbers ranged from 10^5 to 10^9 CFU/g. An increase in microbiological counts was observed as the duration of incubation increased (i.e. with the passage of shelf-life). The most numerous microbial genera were *Pseudomonas spp., Debaryomyces spp. and Cryptococcus spp.*

Objective 2: To determine the ozone sensitivity of key classes of microbial shelf-life determinants in artificial culture

Pseudomonas spp., Yeasts and Moulds were isolated from Coriander samples and cultured on agar plates. These plates were then exposed to 1, 10, & 50 ppm ozone or 'clean air' (controls) for 10 minutes. The number of colonies propagated on control plates (non-ozone exposed) was compared to the numbers found on ozone-treated plates. Colony numbers (CFU) of *Pseudomonas fluorescens* (bacterial model species), *Alternaria alernata* (fungal model species) and *Debaryomyces hansenii* (yeast model species) *in vitro* were significantly reduced by ozone treatment. Direct ozone-treatment of plates prior to introduction of microbes delivered similar results. There was no significant difference between the treatments so direct impacts of ozone on the media used to culture the organisms was not the reason for the observed effects (data shown in the full report).

Objective 3: To optimise the concentration and duration of ozone exposure for fresh and processed leafy product

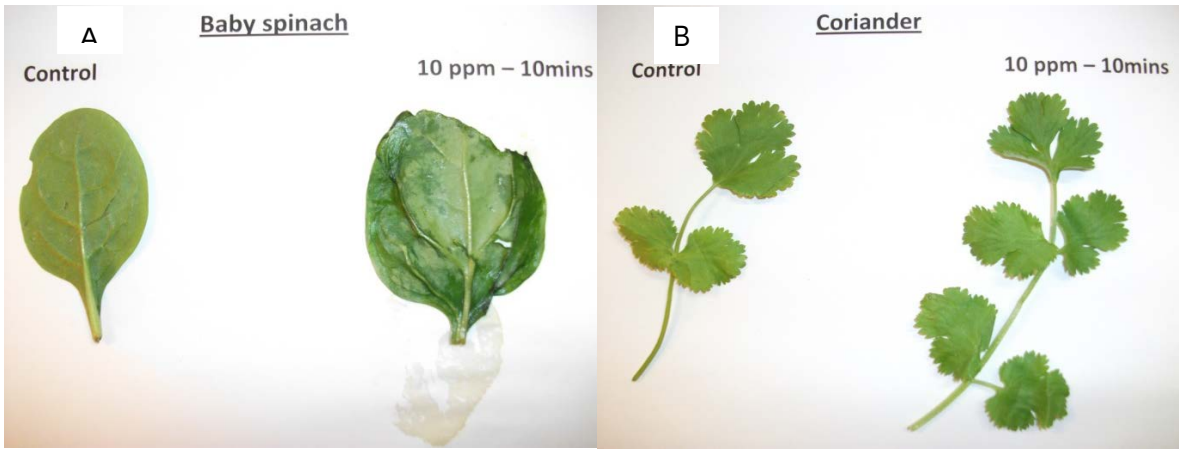
No visual ozone damage was observed when leafy produce was exposed to 1 ppm ozone for shorter time periods.

Impact of high ozone concentration on visual quality of fresh produce

Target produce	**Concentration (ppm)**	**1**						**10**		
	Time (ppm.min)	**0.18**	**2.30**	**7.26**	**12.26**	**27.29**	**57.89**	**1.93**	**21.02**	**69.88**
Watercress		**+**	**+**	**+**	**−**	**−**	**−**	**−**	**−**	**−**
Baby spinach		**+**	**+**	**+**	**+**	**+**	**−**	**−**	**−**	**−**
Coriander		**+**	**+**	**+**	**+**	**+**	**−**	**+**	**+**	**+**
Lettuce		**+**	**+**	**+**	**+**	**+**	**−**	**−**	**−**	**−**

Key: '**+**' indicates positive effect/no visible damage on target produce when exposed to particular ozone concentration. '**–**' indicates negative effect/visible damage on target produce when exposed to particular ozone concentration

In contrast, only coriander retained its appearance when exposed to 10 ppm ozone for any significant period of time (see the photograph below). Ozone injury/visible damage was observed on all produce when exposed to 25 and 50 ppm ozone concentration suggesting there is no 'window of opportunity' at these concentrations.



Impacts of gaseous ozone (10 ppm) on A) baby spinach and B) coriander when exposed for 10 minutes

Objective 4: To explore the ozone sensitivity of microbes present on surface of leafy produce in vivo

Packs of coriander were exposed to either 10 ppm ozone or 'clean' air for 10 minutes and then maintained at 4°C in cold storage room (dark conditions). The total viable count of *Pseudomonas spp*, Yeasts and Moulds were made on day 0 (labeled as 'SOL' – start of life) and on the sell-by-date (labeled as 'EOL' – end of life). The total viable count, were enumerated after growth on agar plates.

There was no significant reduction in Total Viable Counts (TVC), *P. fluorescens,* moulds and yeasts after exposure of fresh produce to similar ozone treatments as the in vitro studies described in Objective 3, where significant reductions in microbial load could be seen. This is an interesting observation and suggests strongly that there are fundamental differences between the phenotype of the microbes under investigation in culture and on the surface of produce.

The next stage of this work will seek to clarify the hypothesis that *P. fluorescens* is in fact the key determinant of the shelf-life of leafy salads and herbs. Future work will also explore, in

some depth, the nature of the difference in sensitivity to ozone of *P fluorescens in vivo* and *in vitro*. It will attempt to probe the physiological and biochemical basis for the differential sensitivity to ozone.

Financial Benefits

At present it is too early in the project to deliver financial benefits. Some very interesting results on the apparent resistance of spoilage bacteria to ozone exposure on a leaf surface mean that fundamental work is required to dissect the observation. This understanding may lead to methods that can overcome resistance. An alternative approach may be the manipulation of the microbial leaf population to reduce spoilage. Such methods could deliver clear financial benefits for the leafy salads industry.