Validation of a Hygenikx air purification unit as an aid to prolong the shelf-life of refrigerated fresh foodstuff and to improve environmental conditions

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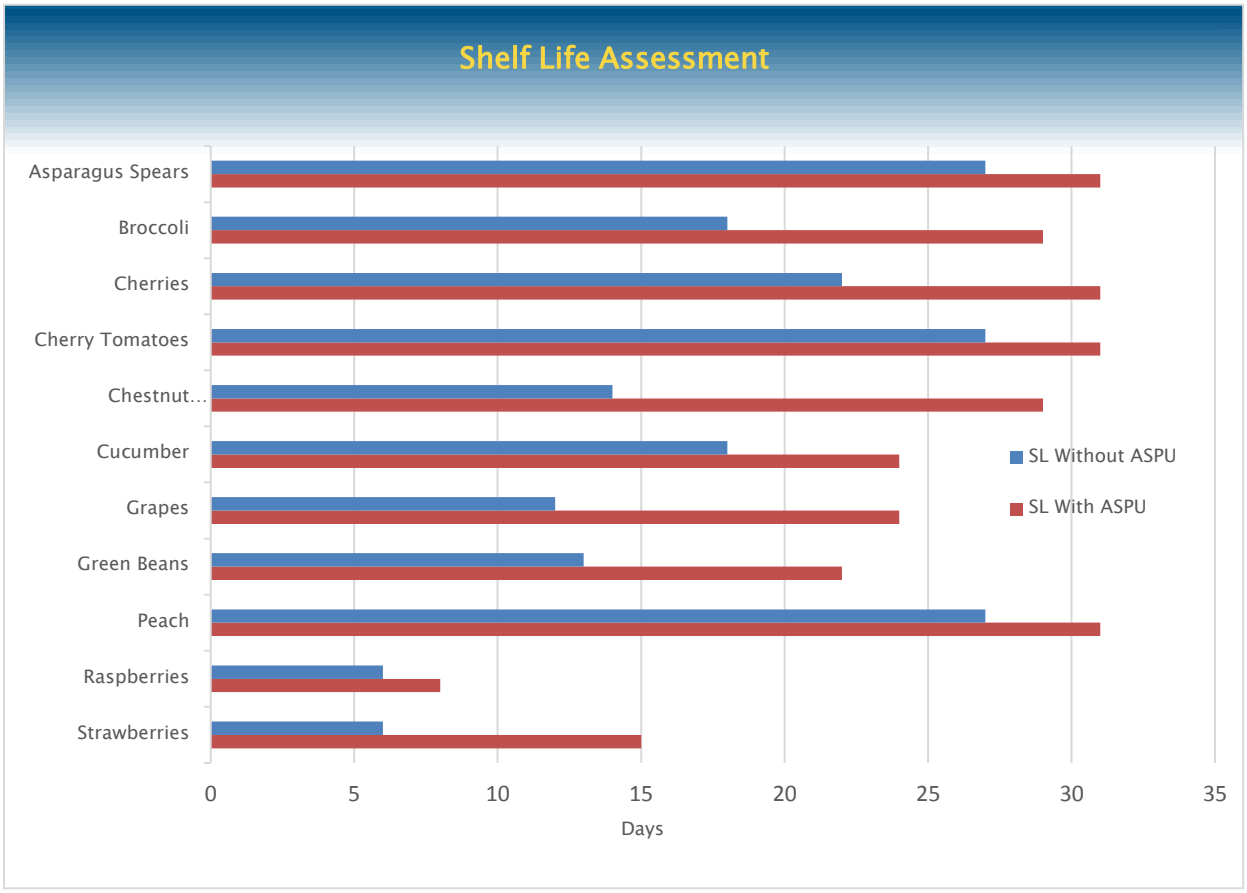
1. Executive Summary

A validation project was carried out to establish the ability of a Hygenikx air and surface purification unit (ASPU) to prolong the shelf life of produce stored in a cold room and to improve environmental conditions.

The trial was structured in two phases, each identical in all aspects with the exception of the introduction of the ASPU in the cold room at the beginning of the second phase. During the 31 days of each of the two phases, the 11 matrices chosen for the trial were examined to establish whether they were still within their shelf-life (this assessment being based on objective guidelines), the surface hygiene and air quality of the cold store were also monitored.

The overall results showed a consistent increase in the shelf-life of the produce during the second phase of the trial (with the ASPU) with an average increase of the shelf-life of 58.1%.



The surface hygiene was found improved by ~45% and levels of airborne contamination were found significantly reduced with the use of the ASPU.

2. Rationale and Overview

The client has requested that a validation project is completed on a method to prolong the life of produce in a cold store by aid of a Hygenikx air and surface purification unit (referred to throughout this document as ASPU), with the aim to extend its shelf life and improve the air and environmental quality by abating levels of microorganisms.

The trial was run in two phases, each lasting a total of four 31 days.

- Phase 1 was the portion of the trial run without the ASPU
- Phase 2 was the portion of the trial run with the ASPU

The foodstuffs listed in the table below were held under one temperature regime until deemed past their "display by" (saleable shelf life) date. The assessment of what a "pass" or "fail" for this test constituted has necessarily had to be a subjective assessment however this was based on objective criteria (please see point 3.2 below) and agreed by the Specialist Microbiology Team. The main criteria used to identify items that had failed the test are set out below:

- Visible mould/growth
- Detectable spoilage (appearance or smell)
- Whether the item would be considered unacceptable to buy or use

Product
Asparagus Spears
Broccoli
Cherries
Cherry Tomatoes
Chestnut Mushrooms
Cucumber
Grapes
Green Beans
Peach
Raspberries
Strawberries

Table 1 – *table listing the foodstuffs chosen for the trial*

The products were stored in a cold store at 5±1°C until deemed failed but no longer than 31 days.

The following tests were also carried out during the two phases of the trial.

- Monitoring of the cold store's environment through swabs and air plates
- Microbiological monitoring of the foodstuffs at their start of life (SOL) and end of life (EOL)

This report details the Validation of a Hygenikx air purification unit as an aid to prolong the shelf-life of refrigerated fresh foodstuff products and environmental conditions over a period of 31 days, performed at ALS Rotherham, which operates under ISO 17025 regulations. The project was carried out between 16th August 2018 and 16th October 2018 on the request from Peter Galliford, Director, on behalf of Mechline Developments Limited.

3. Analytical Procedure

3.1 Sample Sourcing

The foodstuffs listed in Table 1 above were sourced in duplicate for each of the two phases of the trial through a retailer, taking care of maintaining the shelf life and other variables as similar as possible (e.g. same shelf-life, same weights, same transport method and handling, same storage conditions etc.).

3.2 Shelf Life Assessment

The foodstuffs listed in Table 1 were assessed and photographed on the dates reported in Table 2 for signs of spoilage until deemed "failed". Although this is a subjective test, the visual testing was based on recommendations extracted from Carpenter Roland P., Lyon David H. and Hasdell Terry A., Guidelines for Sensory Analysis In Food Product Development and Quality Control, Second Edition, Aspen Publishers, Inc., Gaithersburg, Maryland, 2000, Print. Testing was carried out consistently throughout the trial.

Day	Phase 1	Phase 2
0	16/08/2018	17/09/2018
2	18/08/2018	19/09/2018
4	20/08/2018	21/09/2018
6	22/08/2018	24/09/2018
11	27/08/2018	26/09/2018
13	29/08/2018	28/09/2018
15	31/08/2018	30/09/2018
18	03/09/2018	03/10/2018
20	05/09/2018	05/10/2018

22	07/09/2018	07/10/2018
25	10/09/2018	10/10/2018
27	12/09/2018	12/10/2018
29	n/a	14/10/2018
31	n/a	16/10/2018

Table 2 – *table listing the dates the analysis was carried out on the samples. Please note the shorter span of Phase 1, due to the samples all having been deemed failed by day 27.*

The foodstuffs were analysed for the tests reported in Table 3 at their SOL and EOL, this to aid the evaluation process in assessing their Shelf Life. The results of this testing have been included in the report however not discussed in particular.

3.3 Environmental Monitoring

On the days listed in Table 2, the walls of the cold store were swabbed and the air quality assessed according to ALS's internal environmental monitoring methods (see Table 3).

The methods used for all microbiological testing are listed below

Target Organism	Method	Method Details	ALS method reference
Aerobic Colony Count (ACC)	Colony counts @ 22°c, 30°c, 37°c and 55°c in food products, animal feedingstuffs and environmental swabs	In-House Method, partially based on BS EN ISO 4833:1 2013, Marks & Spencer Manual of Microbiological Methods, Method 1.1 (June 2015).	ESGMM300
Enterobacteriaceae (enumeration)	Enumeration and confirmation of *enterobacteriaceae* in food, animal feedingstuffs and swabs	Method derived from BS ISO 21528-2:2017 and Marks & Spencer Manual of Microbiological Methods (on-line), Method 3.1 June 2015. Method in accordance with Animal By Product Regulations (ABPR): 2011.	ESGMM303
Lactic Acid Bacteria (enumeration)	Enumeration of lactic acid bacteria and lactobacillus spp (presumptive) at 30°c by the pour plate technique	Method derived from BS ISO 15214:1998 and Marks & Spencer Manual of Microbiological Methods, Method 3.11 (June 2015).	ESGMM320
Escherichia coli (enumeration)	Enumeration of *Escherichia coli* (β-glucuronidase positive) in food, animal feeding stuffs and swabs by the pour plate method	Based on BS ISO 16649-2:2001	ESGMM304

Listeria monocytogenes (enumeration)	Enumeration of *Listeria monocytogenes* and *Listeria* spp. in food, animal feeding stuffs and swabs	Based on BS ENO ISO 11290-2:2017, and Marks & Spencer Manual of Microbiological Methods, June 2015 , Method 4.11	ESGMM321
Listeria monocytogenes (presence/absence)	Detection of *Listeria* spp using Solus *Listeria* ELISA method	Based on SOLUS Listeria Elisa test, AFNOR (Association Française de Normalisation) Validation certificate SOL – 37/02 – 06/13 and M&S proprietary list method ELISA SOLUS Listeria (December 2016)	ESGMM523
Pseudomonas spp. (enumeration)	Enumeration of presumptive *Pseudomonas* spp in food, animal feedingstuffs and swabs	Method based on BS EN ISO 13720:2010 and Marks & Spencer Manual of Microbiological Methods, Method 3.6 (June 2015).	ESGMM312
Salmonella spp. (presence/absence)	Detection of *Salmonella* spp. using SOLUS *Salmonella* ELISA method	Base on SOLUS *Salmonella* ELISA, AFNOR (Association Française de Normalisation) Validation certificate SOL – 37/01 – 06/13 and M&S proprietary list method ELISA SOLUS *Salmonella* (December 2016)	ESGMM515
Staphylococcus aureus (enumeration)	Enumeration of *Staphylococcus aureus* (coagulase positive Staphylococci) in food, animal feeding stuffs and swabs	Based on BS ENO ISO 6888-1:1999, and Marks & Spencer Manual of Microbiological Methods, Method 4.1 (June 2015)	ESGMM307
Yeasts and Moulds (enumeration)	Enumeration of yeasts and moulds in food, animal feeding stuffs and swabs, for samples with aw >0.95 (dRBCA method)	Method derived from the ISO 21527-1:2008 and is compliant with Marks & Spencer Manual of Microbiological Methods, Method 3.8, June 2015	ESGMM308
Aerobic colony count and Yeasts and Moulds settle plates	Enumeration of microorganisms isolated on the surface of contact plates, settle plates and plates from air samplers	Developed and Validated in-house	ESGMM322
Environmental Monitoring	Environmental Monitoring (microbiology laboratories)	Developed and Validated in-house	ESGMM012

Table 3 – *Methods used for the trial their details and method references (all ESGMM method references with the exception of ESGMM322 and ESGMM012 are UKAS accredited)*

The details and method extracts for the methods used have not been included in this report however they are available on request.

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4. Results

4.1 Environmental Monitoring

4.1.1 Air Quality

4.1.1.1. Day 0

Day 0 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	1	Trailer Front	16	Trailer Front	0
	Trailer Back	4	Trailer Back	7	Trailer Back	0
Phase 2	Trailer Front	18	Trailer Front	47	Trailer Front	0
	Trailer Back	32	Trailer Back	3	Trailer Back	0

4.1.1.2. Day 2

Day 2 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	0	Trailer Front	1	Trailer Front	0
	Trailer Back	1	Trailer Back	25	Trailer Back	0
Phase 2	Trailer Front	1	Trailer Front	3	Trailer Front	0
	Trailer Back	1	Trailer Back	4	Trailer Back	0

4.1.1.3. Day 4

Day 4 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	3	Trailer Front	0	Trailer Front	0
	Trailer Back	2	Trailer Back	3	Trailer Back	0
Phase 2	Trailer Front	1	Trailer Front	0	Trailer Front	0
	Trailer Back	1	Trailer Back	0	Trailer Back	0

4.1.1.4. Day 6

Day 6 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	3	Trailer Front	30	Trailer Front	0
	Trailer Back	11	Trailer Back	16	Trailer Back	0
Phase 2	Trailer Front	0	Trailer Front	1	Trailer Front	0
	Trailer Back	1	Trailer Back	1	Trailer Back	0

4.1.1.5. Day 11

Day 11 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	n/a	Trailer Front	n/a	Trailer Front	n/a
	Trailer Back	n/a	Trailer Back	n/a	Trailer Back	n/a
Phase 2	Trailer Front	1	Trailer Front	3	Trailer Front	0
	Trailer Back	0	Trailer Back	0	Trailer Back	0

4.1.1.6. Day 13

Day 13 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	n/a	Trailer Front	n/a	Trailer Front	0
	Trailer Back	n/a	Trailer Back	n/a	Trailer Back	0
Phase 2	Trailer Front	1	Trailer Front	0	Trailer Front	0
	Trailer Back	0	Trailer Back	0	Trailer Back	0

4.1.1.7. Day 15

Day 15 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	2	Trailer Front	0	Trailer Front	0
	Trailer Back	4	Trailer Back	1	Trailer Back	0
Phase 2	Trailer Front	0	Trailer Front	2	Trailer Front	0
	Trailer Back	2	Trailer Back	0	Trailer Back	0

4.1.1.8. Day 18

Day 18 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	1	Trailer Front	2	Trailer Front	0
	Trailer Back	5	Trailer Back	4	Trailer Back	0
Phase 2	Trailer Front	0	Trailer Front	0	Trailer Front	0
	Trailer Back	0	Trailer Back	0	Trailer Back	0

4.1.1.9. Day 20

Day 20 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	2	Trailer Front	3	Trailer Front	0
	Trailer Back	1	Trailer Back	1	Trailer Back	0
Phase 2	Trailer Front	0	Trailer Front	3	Trailer Front	0
	Trailer Back	2	Trailer Back	1	Trailer Back	0

4.1.1.10. Day 22

Day 22 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	0	Trailer Front	4	Trailer Front	0
	Trailer Back	1	Trailer Back	1	Trailer Back	0
Phase 2	Trailer Front	1	Trailer Front	1	Trailer Front	0
	Trailer Back	0	Trailer Back	1	Trailer Back	0

4.1.1.11. Day 25

Day 25 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	3	Trailer Front	3	Trailer Front	0
	Trailer Back	1	Trailer Back	0	Trailer Back	0
Phase 2	Trailer Front	4	Trailer Front	5	Trailer Front	0
	Trailer Back	4	Trailer Back	2	Trailer Back	1

4.1.1.12. Day 27

Day 27 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	4	Trailer Front	12	Trailer Front	0
	Trailer Back	4	Trailer Back	6	Trailer Back	0
Phase 2	Trailer Front	1	Trailer Front	0	Trailer Front	0
	Trailer Back	3	Trailer Back	0	Trailer Back	0

4.1.1.13. Day 29

Day 29 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	/	Trailer Front	/	Trailer Front	/
	Trailer Back	/	Trailer Back	/	Trailer Back	/
Phase 2	Trailer Front	2	Trailer Front	1	Trailer Front	0
	Trailer Back	0	Trailer Back	0	Trailer Back	0

4.1.1.14. Day 31

Day 31 (ACC, cfu/plate)						
	Air Plates- ACC		*Air Plates- Mould*		*Air Plates- Yeasts*	
Phase 1	Trailer Front	/	Trailer Front	/	Trailer Front	/
	Trailer Back	/	Trailer Back	/	Trailer Back	/
Phase 2	Trailer Front	1	Trailer Front	0	Trailer Front	0
	Trailer Back	0	Trailer Back	0	Trailer Back	0

4.1.2 Surface Hygiene, Aerobic Colony Count (ACC)

4.1.2.1. Day 0

Day 0 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	184	72
Back Wall	0	0
Door	1	2
Shelf	0	0

4.1.2.2. Day 2

Day 2 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	22	126
Back Wall	6	0
Door	10	4
Shelf	35	0

4.1.2.3. Day 4

Day 4 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	>300	/
Back Wall	0	/
Door	0	/
Shelf	1	/

4.1.2.4. Day 6

Day 6 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	58	7
Back Wall	0	0
Door	4	1
Shelf	1	4

4.1.2.5. Day 11

Day 11 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	>300	>300
Back Wall	0	0
Door	3	9
Shelf	2	20

4.1.2.6. Day 13

Day 13 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	74	5
Back Wall	0	0
Door	0	1
Shelf	3	8

4.1.2.7. Day 15

Day 15 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	52	3
Back Wall	0	0
Door	2	1
Shelf	0	2

4.1.2.8. Day 18

Day 18 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	18	12
Back Wall	1	1
Door	26	2
Shelf	0	4

4.1.2.9. Day 20

Day 20 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	7	3
Back Wall	0	2
Door	0	3
Shelf	6	0

4.1.2.10. Day 22

Day 22 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	19	4
Back Wall	0	0
Door	6	0
Shelf	0	0

4.1.2.11. Day 25

Day 25 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	3	121
Back Wall	0	0
Door	9	1
Shelf	2	0

4.1.2.12. Day 27

Day 27 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	32	4
Back Wall	0	0
Door	0	1
Shelf	0	0

4.1.2.13. Day 29

Day 29 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	/	15
Back Wall	/	1
Door	/	1
Shelf	/	1

4.1.2.14. Day 31

Day 31 (ACC, cfu/swab)		
Area	*Phase 1*	*Phase 2*
Floor	/	5
Back Wall	/	0
Door	/	1
Shelf	/	0

4.2 Microbiological Testing

4.2.1 Start of Life

4.2.1.1. Aerobic Colony Count

ACC (cfu/g)		
SOL	Phase 1	Phase 2
Strawberries	1.88E+04	2.10E+03
Raspberries	2.40E+04	3.00E+04
Grapes	1.50E+03	0.00E+00

Cherries	5.20E+04	1.51E+06
Peach	4.90E+03	3.20E+03
Cherry Tomatoes	2.12E+04	1.10E+04
Chestnut Mushrooms	1.08E+05	1.02E+07
Asparagus Spears	3.40E+03	3.92E+06
Broccoli	2.50E+05	7.90E+05
Cucumber	1.60E+04	6.70E+04
Green Beans	2.12E+04	2.48E+06

4.2.1.2. Enterobacteriaceae

Enterobacteriaceae (cfu/g)		
SOL	Phase 1	Phase 2
Strawberries	1.88E+04	1.50E+02
Raspberries	2.40E+04	0.00E+00
Grapes	1.50E+03	0.00E+00
Cherries	5.20E+04	3.30E+03
Peach	4.90E+03	0.00E+00
Cherry Tomatoes	2.12E+04	2.40E+02
Chestnut Mushrooms	1.08E+05	0.00E+00
Asparagus Spears	3.40E+03	2.20E+02
Broccoli	2.50E+05	6.40E+03
Cucumber	1.60E+04	2.20E+02
Green Beans	2.12E+04	1.50E+04

4.2.1.3. Yeasts

Yeasts (cfu/g)		
SOL	Phase 1	Phase 2
Strawberries	n/a	8.00E+02
Raspberries	n/a	1.00E+03
Grapes	8.00E+01	6.00E+01
Cherries	2.60E+02	1.07E+03
Peach	6.30E+02	3.90E+02
Cherry Tomatoes	3.50E+02	6.00E+02
Chestnut Mushrooms	1.50E+03	0.00E+00
Asparagus Spears	1.20E+02	7.00E+04
Broccoli	1.50E+03	2.30E+02
Cucumber	5.00E+01	1.00E+01
Green Beans	1.50E+03	5.90E+03

4.2.1.4. Moulds

Moulds (cfu/g)		
SOL	Phase 1	Phase 2
Strawberries	1.50E+03	9.10E+03
Raspberries	1.50E+03	4.60E+04
Grapes	1.90E+02	1.00E+01
Cherries	0.00E+00	0.00E+00
Peach	9.00E+01	8.00E+01
Cherry Tomatoes	4.70E+02	3.20E+03
Chestnut Mushrooms	0.00E+00	1.00E+01
Asparagus Spears	1.00E+01	0.00E+00
Broccoli	1.50E+03	4.20E+02
Cucumber	2.00E+01	0.00E+00
Green Beans	5.00E+02	4.00E+02

4.2.1.5. *Escherichia coli*

Escherichia coli (cfu/g)		
SOL	Phase 1	Phase 2
Strawberries	0.00E+00	0.00E+00
Raspberries	0.00E+00	0.00E+00
Grapes	0.00E+00	0.00E+00
Cherries	0.00E+00	0.00E+00
Peach	0.00E+00	0.00E+00
Cherry Tomatoes	0.00E+00	0.00E+00
Chestnut Mushrooms	0.00E+00	0.00E+00
Asparagus Spears	0.00E+00	0.00E+00
Broccoli	0.00E+00	0.00E+00
Cucumber	0.00E+00	0.00E+00
Green Beans	0.00E+00	0.00E+00

4.2.1.6. Lactic Acid Bacteria

Lactic Acid Bacteria (cfu/g)		
SOL	Phase 1	Phase 2
Strawberries	4.40E+02	1.00E+02
Raspberries	3.05E+03	2.40E+03
Grapes	0.00E+00	0.00E+00
Cherries	1.10E+02	3.00E+04
Peach	1.00E+01	3.00E+02
Cherry Tomatoes	3.60E+02	1.00E+03
Chestnut Mushrooms	0.00E+00	0.00E+00
Asparagus Spears	1.00E+01	2.00E+01

Broccoli	1.50E+03	3.00E+04
Cucumber	0.00E+00	0.00E+00
Green Beans	2.94E+03	3.00E+04

4.2.1.7. *Pseudomonas* spp.

Pseudomonas spp.(cfu/g)		
SOL	Phase 1	Phase 2
Strawberries	1.00E+01	0.00E+00
Raspberries	0.00E+00	0.00E+00
Grapes	0.00E+00	0.00E+00
Cherries	7.00E+01	3.30E+02
Peach	0.00E+00	0.00E+00
Cherry Tomatoes	2.60E+02	0.00E+00
Chestnut Mushrooms	9.70E+02	1.50E+03
Asparagus Spears	1.40E+02	1.50E+03
Broccoli	0.00E+00	1.30E+02
Cucumber	1.00E+01	1.50E+03
Green Beans	0.00E+00	1.00E+02

4.2.1.8. *Staphylococcus aureus*

Staphylococcus aureus (cfu/g)		
SOL	Phase 1	Phase 2
Strawberries	0.00E+00	0.00E+00
Raspberries	0.00E+00	0.00E+00
Grapes	0.00E+00	0.00E+00
Cherries	0.00E+00	0.00E+00
Peach	0.00E+00	0.00E+00
Cherry Tomatoes	0.00E+00	0.00E+00
Chestnut Mushrooms	0.00E+00	0.00E+00
Asparagus Spears	0.00E+00	0.00E+00
Broccoli	0.00E+00	0.00E+00
Cucumber	0.00E+00	0.00E+00
Green Beans	0.00E+00	0.00E+00

4.2.1.9. *Salmonella* Presence/Absence

Salmonella Presence/Absence (D=DETECTED, ND=NOT DETECTED)		
SOL	Phase 1	Phase 2
Strawberries	ND	ND
Raspberries	ND	ND
Grapes	ND	ND

Cherries	ND	ND
Peach	ND	ND
Cherry Tomatoes	ND	ND
Chestnut Mushrooms	ND	ND
Asparagus Spears	ND	ND
Broccoli	ND	ND
Cucumber	ND	ND
Green Beans	ND	ND

4.2.1.10. *Listeria* Presence/Absence

Listeria Presence/Absence (D=DETECTED, ND=NOT DETECTED)		
SOL	Phase 1	Phase 2
Strawberries	ND	ND
Raspberries	ND	ND
Grapes	ND	ND
Cherries	ND	ND
Peach	D, *Listeria innocua**	ND
Cherry Tomatoes	ND	ND
Chestnut Mushrooms	ND	ND
Asparagus Spears	ND	ND
Broccoli	ND	ND
Cucumber	ND	ND
Green Beans	ND	D, *Listeria innocua**

**Listeria* spp. levels for all samples, including the ones with a Detected result for *Listeria* were <20 cfu/g.

4.2.2 End of Life

4.2.2.1. Aerobic Colony Count

ACC (cfu/g)		
EOL	Phase 1	Phase 2
Strawberries	3.00E+05	2.00E+02
Raspberries	3.50E+04	3.80E+03
Grapes	6.00E+03	3.00E+06
Cherries	1.00E+05	3.00E+06
Peach	1.00E+02	1.10E+02
Cherry Tomatoes	3.00E+03	8.10E+03
Chestnut Mushrooms	3.00E+06	3.00E+06
Asparagus Spears	3.00E+05	3.00E+06
Broccoli	3.00E+05	3.00E+06

Cucumber	3.00E+05	3.00E+06
Green Beans	3.00E+05	2.00E+05

4.2.2.2. Enterobacteriaceae

Enterobacteriaceae (cfu/g)		
EOL	Phase 1	Phase 2
Strawberries	4.00E+02	0.00E+00
Raspberries	0.00E+00	0.00E+00
Grapes	0.00E+00	0.00E+00
Cherries	0.00E+00	1.68E+04
Peach	0.00E+00	0.00E+00
Cherry Tomatoes	0.00E+00	0.00E+00
Chestnut Mushrooms	1.50E+03	0.00E+00
Asparagus Spears	4.00E+01	0.00E+00
Broccoli	1.50E+04	2.60E+03
Cucumber	1.50E+04	1.50E+04
Green Beans	1.50E+03	1.50E+04

4.2.2.3. Yeasts

Yeasts (cfu/g)		
EOL	Phase 1	Phase 2
Strawberries	5.00E+03	1.60E+03
Raspberries	1.26E+05	5.00E+03
Grapes	6.00E+03	2.80E+02
Cherries	2.50E+03	1.50E+05
Peach	9.00E+03	1.20E+02
Cherry Tomatoes	3.00E+03	3.60E+03
Chestnut Mushrooms	3.00E+01	6.00E+01
Asparagus Spears	0.00E+00	7.80E+04
Broccoli	1.02E+04	1.40E+02
Cucumber	1.90E+04	3.20E+02
Green Beans	1.50E+04	2.50E+04

4.2.2.4. Moulds

Moulds (cfu/g)		
EOL	Phase 1	Phase 2
Strawberries	4.10E+04	2.00E+02
Raspberries	1.26E+05	7.00E+03
Grapes	1.40E+03	7.80E+03
Cherries	4.00E+03	0.00E+00
Peach	2.00E+03	2.00E+01

Cherry Tomatoes	9.00E+03	2.00E+02
Chestnut Mushrooms	3.00E+01	7.00E+01
Asparagus Spears	0.00E+00	0.00E+00
Broccoli	5.90E+03	6.70E+02
Cucumber	6.50E+02	2.50E+02
Green Beans	1.24E+04	1.70E+03

4.2.2.5. *Escherichia coli*

Escherichia coli (cfu/g)		
EOL	Phase 1	Phase 2
Strawberries	0.00E+00	0.00E+00
Raspberries	0.00E+00	0.00E+00
Grapes	0.00E+00	0.00E+00
Cherries	0.00E+00	0.00E+00
Peach	0.00E+00	0.00E+00
Cherry Tomatoes	0.00E+00	0.00E+00
Chestnut Mushrooms	0.00E+00	0.00E+00
Asparagus Spears	0.00E+00	0.00E+00
Broccoli	0.00E+00	0.00E+00
Cucumber	0.00E+00	0.00E+00
Green Beans	0.00E+00	0.00E+00

4.2.2.6. Lactic Acid Bacteria

Lactic Acid Bacteria (cfu/g)		
EOL	Phase 1	Phase 2
Strawberries	2.50E+01	0.00E+00
Raspberries	3.00E+03	3.20E+02
Grapes	3.00E+03	0.00E+00
Cherries	4.40E+02	3.00E+04
Peach	0.00E+00	0.00E+00
Cherry Tomatoes	8.00E+02	3.00E+02
Chestnut Mushrooms	0.00E+00	1.00E+01
Asparagus Spears	0.00E+00	0.00E+00
Broccoli	2.81E+03	0.00E+00
Cucumber	0.00E+00	1.12E+04
Green Beans	1.55E+03	3.00E+04

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4.2.2.7. *Pseudomonas* spp.

Pseudomonas spp. (cfu/g)		
EOL	Phase 1	Phase 2
Strawberries	6.00E+01	0.00E+00
Raspberries	0.00E+00	0.00E+00
Grapes	0.00E+00	0.00E+00
Cherries	1.50E+03	2.80E+02
Peach	0.00E+00	0.00E+00
Cherry Tomatoes	1.00E+01	1.30E+03
Chestnut Mushrooms	1.50E+03	1.50E+03
Asparagus Spears	0.00E+00	1.50E+03
Broccoli	0.00E+00	1.50E+03
Cucumber	0.00E+00	1.50E+03
Green Beans	0.00E+00	0.00E+00

4.2.2.8. *Staphylococcus aureus*

Staphylococcus aureus (cfu/g)		
EOL	Phase 1	Phase 2
Strawberries	0.00E+00	0.00E+00
Raspberries	0.00E+00	0.00E+00
Grapes	0.00E+00	0.00E+00
Cherries	0.00E+00	0.00E+00
Peach	0.00E+00	0.00E+00
Cherry Tomatoes	0.00E+00	0.00E+00
Chestnut Mushrooms	0.00E+00	0.00E+00
Asparagus Spears	0.00E+00	0.00E+00
Broccoli	0.00E+00	0.00E+00
Cucumber	0.00E+00	0.00E+00
Green Beans	0.00E+00	0.00E+00

4.2.2.9. *Salmonella* Presence/Absence

Salmonella Presence/Absence (D=DETECTED, ND=NOT DETECTED)		
EOL	Phase 1	Phase 2
Strawberries	ND	ND
Raspberries	ND	ND
Grapes	ND	ND
Cherries	ND	ND
Peach	ND	ND
Cherry Tomatoes	ND	ND
Chestnut Mushrooms	ND	ND

Asparagus Spears	ND	ND
Broccoli	ND	ND
Cucumber	ND	ND
Green Beans	ND	ND

4.2.2.10. *Listeria* Presence/Absence

Listeria Presence/Absence (D=DETECTED, ND=NOT DETECTED)		
EOL	Phase 1	Phase 2
Strawberries	ND	ND
Raspberries	ND	ND
Grapes	ND	ND
Cherries	ND	ND
Peach	ND	ND
Cherry Tomatoes	ND	ND
Chestnut Mushrooms	ND	ND
Asparagus Spears	ND	ND
Broccoli	ND	ND
Cucumber	ND	ND
Green Beans	ND	ND

4.3 Shelf Life Assessment

4.3.1 Shelf Life Results Table

Product	Shelf-Life Phase 1	Shelf-Life Phase 2
Asparagus Spears	27	31
Broccoli	18	29
Cherries	22	31
Cherry Tomatoes	27	31
Chestnut Mushrooms	14	29
Cucumber	18	24
Grapes	12	24
Green Beans	13	22
Peach	27	31
Raspberries	6	8
Strawberries	6	15

Table 4 – *shelf life achieved by the products without and with the ASPU*

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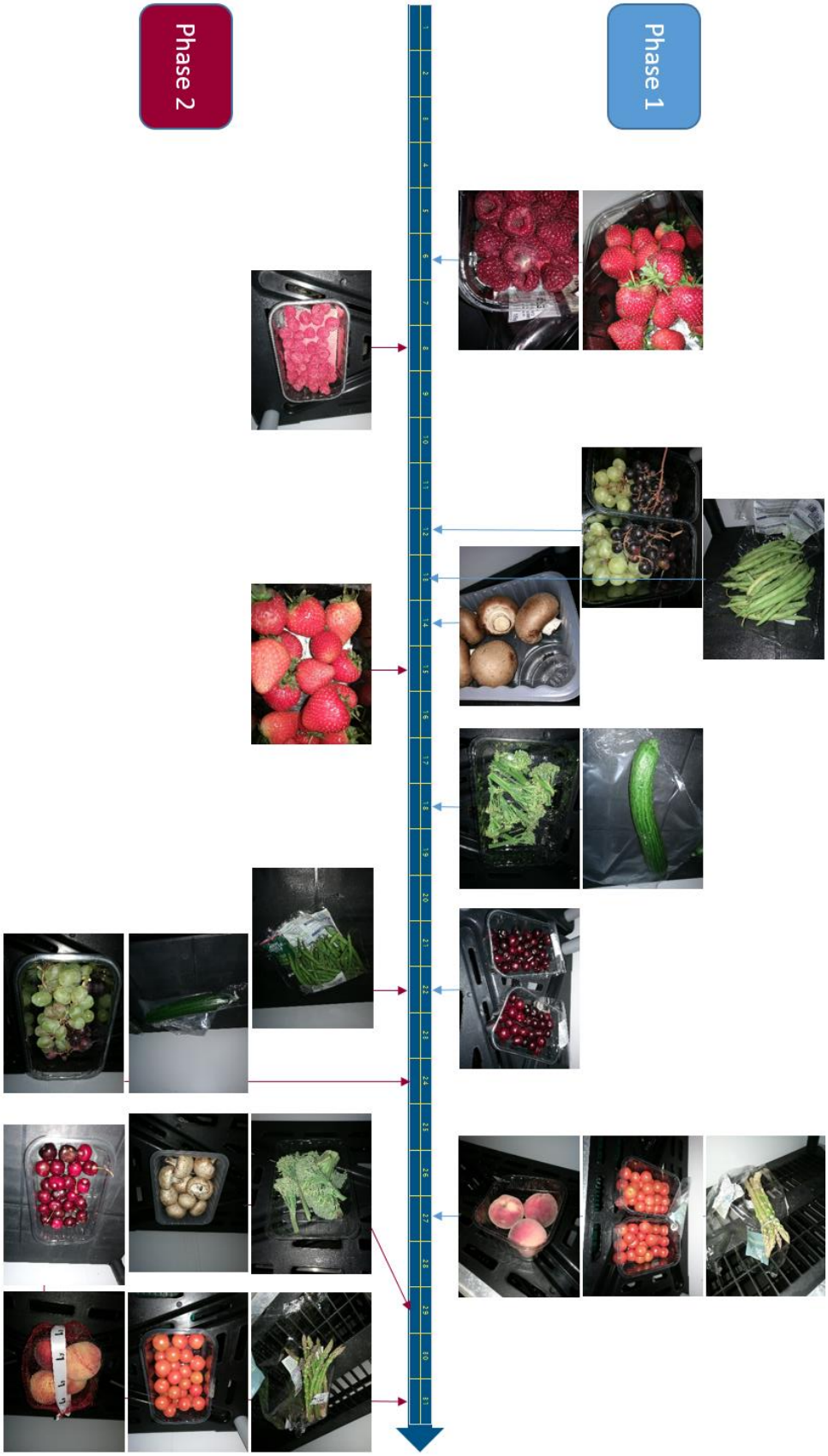
4.3.2 Pictorial Shelf-Life Timeline



5. Discussion

5.1 Environmental Monitoring

5.1.1 Air Quality

The Air Quality readings obtained during Phase 1 of the trial (without ASPU) averaged at 18.8 cfu/plate. The readings ranged from 60 to 6 cfu/plate and excluding Day 0 as the theoretical highest point due to the introduction of contamination with the fresh produce, the average cfu/plate becomes 17.8 cfu/plate and range remains unchanged.

The Air Quality readings obtained during Phase 2 of the trial (with ASPU) averaged at 11.1 cfu/plate. The readings ranged from 100 to 0 cfu/plate and excluding Day 0 as the theoretical highest point due to the introduction of contamination with the fresh produce, the average cfu/plate becomes 4.3 cfu/plate and range 16 to 0 cfu/plate.

Day	Phase 1 (cfu/plate)	Phase 2 (cfu/plate)
0	28	100
2	27	9
4	8	2
6	60	3
11	–	4
13	–	1
15	7	4
18	12	0
20	7	6
22	6	3
25	7	16
27	26	4
29	–	3
31	–	1

Table 5 – *Air Quality results for the two phases of the trial in cfu/plate. Please note missing readings for Phase 1 on days 11 and 13 due to an analytical issue and again on days 29 and 31 due to all produce having been deemed failed by day 27.*

Variations in the readings obtained during both phases are to be considered not only a result of the presence or absence of the ASPU but also as the results of natural variations occurring in the product, air and conditions tested therefore no single figure should be considered as telling. Notwithstanding trends in the levels of microorganisms recorded are telling and indeed represent a reliable tool to assess the microbiological quality of the air.

The figure below illustrates the results pattern for the two phases.

Considering that the majority of the growth was likely and noted to be caused by fungi, it is significant that the records for Phase 1 peak around day 5-7 and then again towards the end of the trial. Days 5-10 would be the expected time for any fungal contamination introduced on Day 0 and not controlled to have matured and begun producing spores. The end of the trial is the time when produce will begin to deteriorate also impacting the air quality.

In contrast during Phase 2 it is noticeable that the peak occurs on Day 0, as a natural variation of the magnitude of contamination introduced as the fresh produce is brought in however this subsequently decreases quickly to low levels, showing a likely decrease of the airborne spores. Similarly to Phase 1, a peak is noticeable towards the end of the trial however this is again brought to low level by the next testing point, likely due to the action of the ASPU.

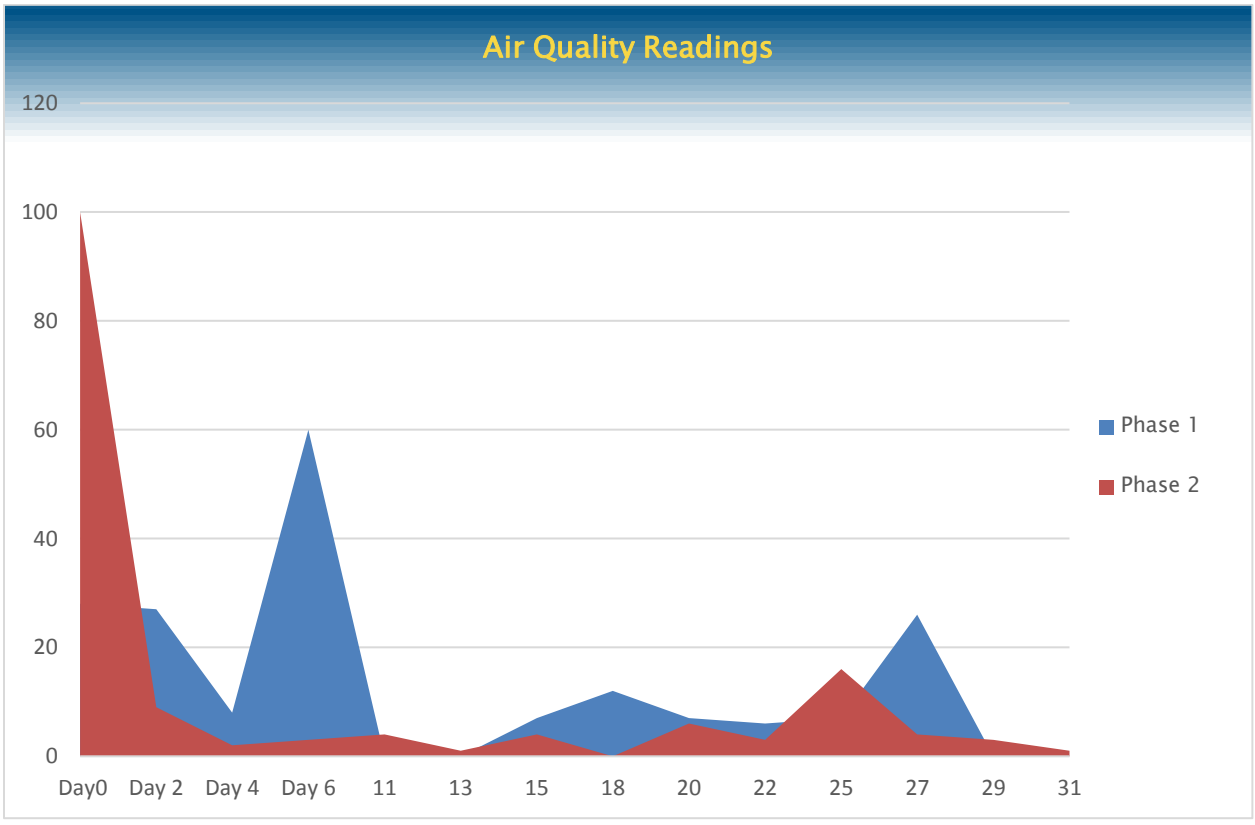


Figure 1 *– graph depicting the sum of all counts for the Air Quality monitoring perfromed during Phases 1 and 2 of the trial.*

In connection to what set out above it is also useful to examine the Air Quality monitoring data pertaining to the first six days of both phases as exemplificative of the expected growth pattern of any fungal contamination introduced with the fresh produce at Day 0.

Day	% Phase 1	% Phase 2
0	100.00%	100.00%
2	96.43%	9.00%
4	28.57%	2.00%
6	214.29%	3.00%

Table 6 – *Sum of all Air Quality monitoring counts for Phases 1 and 2 of the trial expressed as a percentage of the counts obtained on Day 0.*

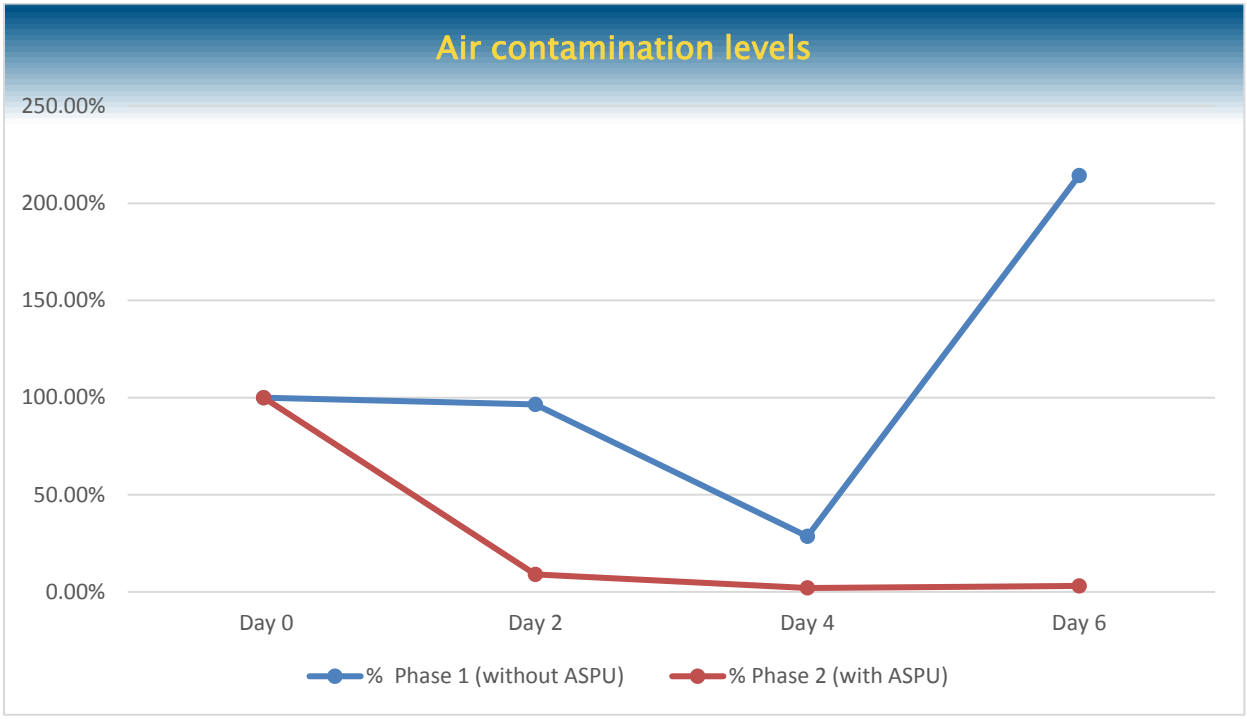


Figure 2 – *Air contamination levels recorded during the first 6 days of Phases 1 and 2 of the trial.*

In normal, unchecked circumstances it would be expected that any contamination (moulds in particular) would yield steady readings from the point of introduction in the environment (i.e. with the fresh produce on Day 0) peaking around days 5–10 which coincides with the maturation and release of spores in the environment of mould colonies following typical fungal growth patterns.

The pattern described above can be observed in the data derived from Phase 1 of the trial, which shows an oscillation as the environmental spores are introduced and settle in the environment between days 0 and 4 and the mature and peak on day 6 with values more than double those recorded on Day 0, indicative of significant growth.

On the other hand the data derived from Phase 2 shows a 91% reduction between Days 0 and 1 and a 97% reduction between Days 0 and Day 6, indicative of an external action influencing the growth pattern of the organisms, likely to be the effects of the ASPU.

5.1.2 Surface Hygiene

The dataset obtained from the Surface Hygiene monitoring displayed great variability and therefore low statistical significance.

The data resulting from the swabbing of the floor was excluded from the analysis as it was though that it was too influenced by the potential contamination introduced by the operator performing the monitoring to be significant. This data is nevertheless reported in the table below for the sake of completeness but has not been analysed. Inclusion of the Floor hygiene monitoring data would not have changed the conclusions drawn from this section's results.

Day	Phase 1	Phase 1 (with Floor data)	Phase 2	Phase 2 (with Floor data)
Day 0	1	185	2	74
Day 2	51	73	4	130
Day 4	1	301	–	–
Day 6	5	63	5	12
Day11	5	305	29	329
Day 13	3	77	9	14
Day 15	2	54	3	6
Day 18	27	45	7	19
Day 20	6	13	5	8
Day 22	6	25	0	4
Day 25	11	14	1	122
Day 27	0	32	1	5
Day 29	–	–	1	18
Day 31	–	–	3	6
Average	9.83	98.92	5.38	57.46

Table 7 – *Surface hygiene monitoring data expressed as total ACC cfu/10cm² recovered from the swabbing of Back Wall, Door and Shelf of the cold room. The same data but with the added counts derived from the swabbing fo the Floor is added in light grey in the adjacent column.*

The total ACC Surface Hygiene readings obtained during Phase 1 of the trial (without ASPU) averaged at 9.83 cfu/10cm² (98.92 cfu/10 cm² with the floor data) and the readings ranged from 51 to 0 cfu/10cm² (305 to 13 cfu/10cm² with the floor data).

The total ACC Surface Hygiene readings obtained during Phase 2 of the trial (with ASPU) averaged at 5.38 cfu/10cm² (57.46 cfu/10 cm² with the floor data) and the readings ranged from 29 to 0 cfu/10cm² (329 to 4 cfu/10cm² with the floor data).

Variations in the readings obtained during both phases are to be considered not only a result of the presence or absence of the ASPU but also as the results of natural variations occurring in the product, air and conditions tested therefore no single figure should be considered as telling. Notwithstanding trends in the levels of microorganisms recorded are telling and indeed represent a reliable tool to assess the microbiological surface hygiene of the environment.

The differences recorded between Phases 1 and 2 of the trial amounted to an average 4.45 cfu/10cm^2 (41.46 cfu/10 cm^2 with the floor data) lower count recorded during Phase 2, amounting to a percentage decrease of the microbial flora recovered during the Surface Hygiene monitoring in Phase 2 of 45.24% (41.91% with the floor data) indicative of the effects of the ASPU.

5.2 Shelf Life Assessment

5.2.1 Shelf Life Duration

The assessment of the shelf life of the products as outlined at point 3.2 showed an increase in the shelf life of all 11 products. The shelf life gains recorded during Phase 2 of the trial are outlined below, together with the % gain to contextualise the figure (*e.g.* in the case of short shelf life items such as raspberries, the two day increase is seemingly small overall however it represents a one third increase in the shelf-life of the product).

Product	SL Without ASPU	SL With ASPU	Δ with ASPU (days)	% Δ with ASPU
Strawberries	6	15	9	+150.0%
Chestnut Mushrooms	14	29	15	+107.1%
Grapes	12	24	12	+100.0%
Green Beans	13	22	9	+69.2%
Broccoli	18	29	11	+61.1%
Cherries	22	31	9	+40.9%
Cucumber	18	24	6	+33.3%
Raspberries	6	8	2	+33.3%
Asparagus Spears	27	31	4	+14.8%
Cherry Tomatoes	27	31	4	+14.8%
Peach	27	31	4	+14.8%

Table 8 – *shelf-life achieved by the products during Phases 1 and 2 of the trial together with the difference in days and as a percentage value. Note all products reported an increase during Phase 2 of the trial (with ASPU).*

Within this dataset, the highest three increases recorded were Strawberries, with +150% (+9 days), Chestnut Mushrooms, with +107.1% (+15 days) and Grapes with a twofold increase (+12 days).

The lowest increase recorded was 14.8% (+4 days) for Asparagus Spears, Cherry Tomatoes and Peaches all of which lasted until day 27 during Phase 1 and outlasted the 31 days of Phase 2 (therefore receiving an arbitrary failed date of Day 31).

The average increase in shelf-life during Phase 2 was recorded as over a week (+7.73 days) and the average percentage increase in shelf-life was recorded as +58.1%.

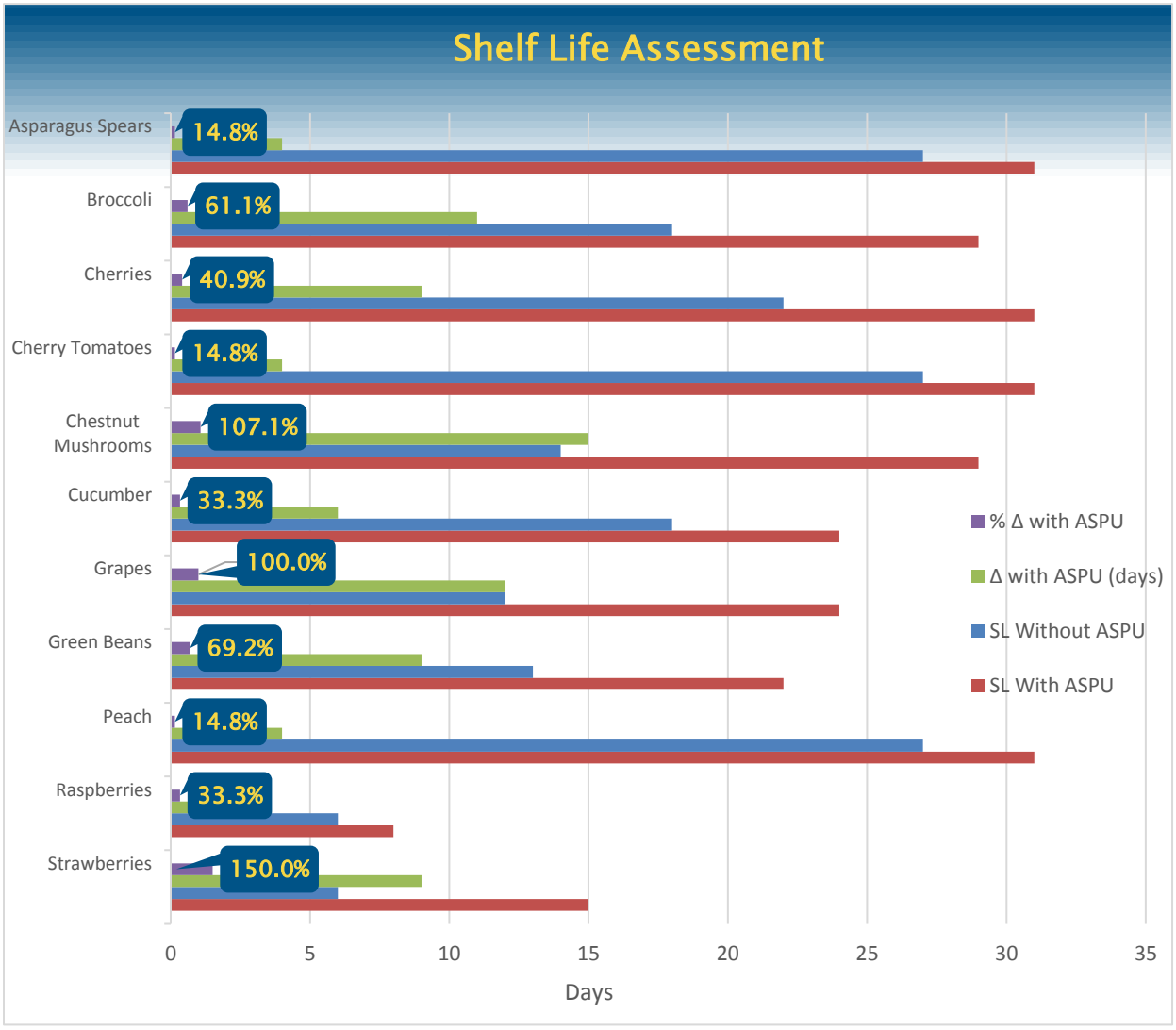


Figure 3– *graph depicting the shelf life (SL) achieved by the various products with and without the ASPU and the differences in days and percentage of shelf-life. Products are ordered from the top highest to smallest percentage increase.*

5.2.2 ACC growth rate

As a further indicator of the effect of the ASPU on the produce, the growth rate (δ) of the Aerobic Colony Count test (ACC, which assesses all recoverable aerobic microorganisms), expressed as Log10(cfu/g)/day was also examined. This to serve as an indicator for the speed at which the microbial flora on the produce is able to grow under normal circumstances and with the ASPU. To do this the Log10 cfu/g of the ACC tests for the SOL and EOL points of all products on both phases were calculated and the difference (Δ) between EOL and SOL calculated. The growth rate (δ) was then calculated by dividing Δ by the shelf-life achieved.

Product	Log10 SOL Phase 1	Log10 EOL Phase 1	Log10 SOL Phase 2	Log10 EOL Phase 2	Δ Phase 1	Δ Phase 2	δ Phase 1	δ Phase 2
Asparagus Spears	3.53	5.48	6.59	6.48	1.95	-0.12	0.07	0.00
Broccoli	5.40	5.48	5.90	6.48	0.08	0.58	0.00	0.02
Cherries	4.72	5.00	6.18	6.48	0.28	0.30	0.01	0.01
Cherry Tomatoes	4.33	3.48	4.04	3.91	-0.85	-0.13	-0.03	0.00
Chestnut Mushrooms	5.03	6.48	7.01	6.48	1.44	-0.53	0.10	-0.02
Cucumber	4.20	5.48	4.83	6.48	1.27	1.65	0.07	0.07
Grapes	3.18	3.78	0.00	6.48	0.60	6.48	0.05	0.27
Green Beans	4.33	5.48	6.39	5.30	1.15	-1.09	0.09	-0.05
Peach	3.69	2.00	3.51	2.04	-1.69	-1.46	-0.06	-0.05
Raspberries	4.38	4.54	4.48	3.58	0.16	-0.90	0.03	-0.11
Strawberries	4.27	5.48	3.32	2.30	1.20	-1.02	0.20	-0.07

Table 9 – *calculated δ values for all produce during both phases of the trial. The data used to calculate this (Log10(cfu/g)) at SOL and EOL is also included.*

The averages for δ on both phases were recorded as 0.05 Log10 (cfu/g)/day for phase 1 and 0.01 Log10 (cfu/g)/day for phase 2.

Although no statistically significant differences were noted (*i.e.* for a change to be statistically significant, it typically needs to be a greater than 0.5Log10 difference) and therefore no single value can be considered telling, analysis of the averages within Phases 1 and 2 reported a modest overall decrease of δ during Phase 2 of the trial.

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6. Conclusions

The validation project described in this report aimed at demonstrating the ability of a Hygenikx Air and Surface Purification Unit (ASPU) to prolong the life of produce stored in controlled conditions in a cold room and to improve its air and environmental conditions by abating levels of present microorganisms.

The results of the trial have shown a consistent increase of the shelf-life achieved by the 11 products chosen when the ASPU was employed, this increase ranged from 14% to 150% with an average increase in shelf-life of 58.1% (or ~7.5 days).

The above increase in the shelf life of the produce was matched by a decrease in the recorded levels of surface and air contamination, with surface contamination displaying a ~45% decrease with the use of the ASPU and air quality showing a marked decrease of microbial levels when compared with the data gathered from the cold room without the ASPU.

Based on the results above the ASPU was found effective in achieving a longer shelf-life for the chosen produce and improved environmental conditions of the cold room during the trial discussed in this report.

7. Final Details

This report was created by Matteo Capocefalo, Technical Project Manager, Specialist Microbiology Team at ALS Rotherham, Units 7&8 Aspen Court, Bessemer Way, Rotherham S60 1FB UK. This report was finalised as dated on the front page of this report and a copy will be held on file.

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