Food quality and safety activities in the EDEN ISS project
Pre mission results.

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The Project

HORIZON 2020

EDEN ISS

- COMPET 7 - 2014: Space exploration - Life support
- 14.5 Points out of 15
- EU Contribution ~4.5 M€ (plus partner contributions; total over 5.5 M€)
- 13 Partners from Industry, Academia and research Institutes
- Germany, Ireland, Italy, Netherlands, Sweden, Austria, and Canada

2015-2018

http://eden-iss.net/

DLR, ThalesAlenia Space, University of Guelph, Liquifer Systems Group, Airbus Defence & Space, heliospectra, EnginSoft, Aero Sekur, Consiglio Nazionale delle Ricerche, AWI, Wageningen University, Limerick Institute of Technology, Telespazio

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Kick-off Meeting

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Food quality and safety activities.

We want to produce

good, nutritious and safe food

to fulfill astronauts needs

Overall Work Plan

- **Preparative Stage**
  - **Procedures**
  - **Plant growth under Controlled Environmental Conditions to modulate Quality Parameters (Quality Driving Attributes)**
  - **Sample selected species to develop and test analysis protocols and data output**
    - Destructive Quality Analysis Protocols
    - Organoleptic Analysis Protocols
    - Food Safety Protocols
    - Storage Protocols (Analytical and Organoleptic Samples)
    - Post Harvest Procedures
    - Non-destructive Quality Assessment Protocols
  - **Food Safety and Processing Manual (D4.3)**
- **Test Campaign (MTF)**
  - Organoleptic Crew Surveys
  - Non-destructive Quality and Safety Assessment (Toolbox)
  - Post-harvest Procedures
  - Sample harvesting and storage for post-mission analysis
- **Post-mission analysis and data processing**
  - Chemical Composition, Microbial & Organoleptic Analysis
  - **Food Safety and Processing Results (D5.7)**
  - **Palatability & Contamination Report (D5.8)**

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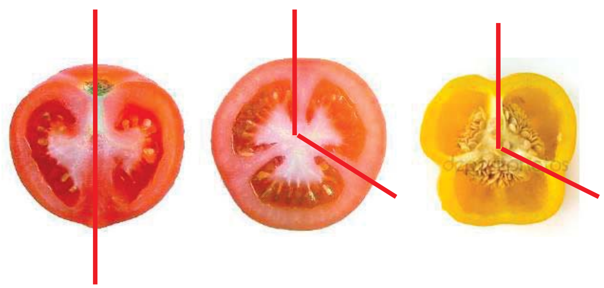
Preparative Stage

Procedures

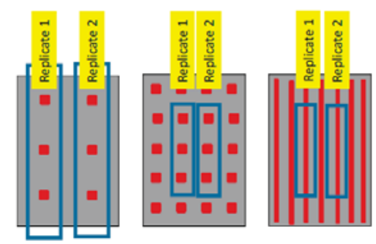
SAMPLING: Sub sampling 100 g of produce

Large sized plants

Fruit with NON homogeneous composition



Replicates and Experimental design



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Preparative Stage

Procedures

Nitrate measurement at the NMIII

SOP 011 - Nitrate Ion Meter

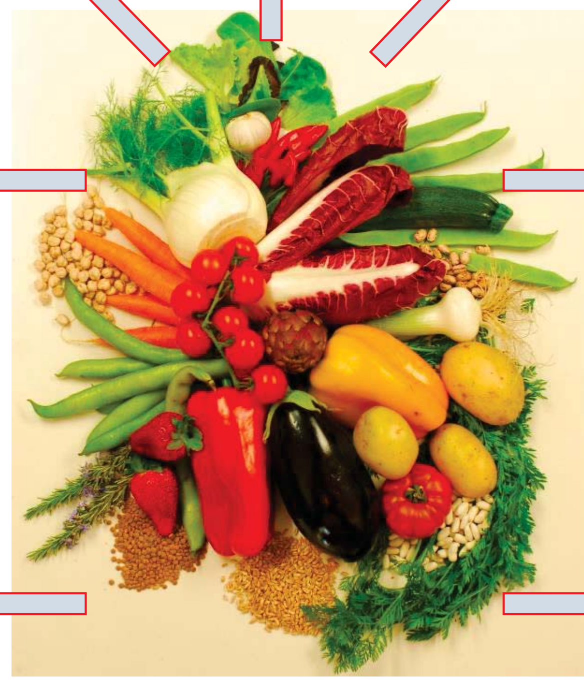
EDEN ISS / LIT LIMERICK INSTITUTE OF TECHNOLOGY	Identification Number: **S.O.P. 011**	Revision Number: **2.0**	
	Equipment Model: Horiba LAQUA Nitrate NO3- Meter (B-743)	Serial Number:	
	Document Owner: **Limerick Institute of Technology/EDEN-ISS**		
	Document Status: **Approved**	No. of Pages:	Language: **English**
	Document Type: **Standard Operating Procedure (Internal)**	Contact/Creator: **Ms. Michelle McKeon-Bennett**	

Revision History

Revision No.	Date	Comments
1.0	25th April 2017	• Document creation • Establishment of procedure
2.0	29th May 2017	• Addition of Control Record • Procedure update

Compounds relevant for the nutritional QUALITY of produce.

Carbohydrate **Protein** **Fat**



VITAMINS
Pro-vitamin A (β-carotene)
Vitamins B group
- B1-Thiamin
- B2: Riboflavin
- B3: Niacin amide
- B5: Pantothenic acid
- B6: Pyridoxine
- B7: Biotin
- B9: Folic acid

Vitamin C (ascorbic acid)
Vitamin E (α-tocophrol)
Vitamin K1 (phyllochinon)

Fiber
Cellulose
Hemicellulose
Lignin
Pectin
Fructans
- Inulin
- Oligofructosides

Raffinose series sugars
Resistant starch

Other compounds with nutraceutical value:
Antioxidants,
Phytosterols,
Flavonoids,
Glucosonolates
.....

Negative compounds:
Nitrate,
Oxalic acid
Pollutants
....

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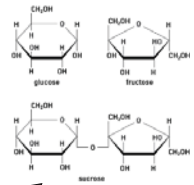
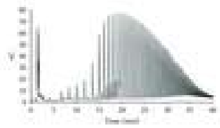
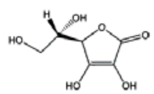
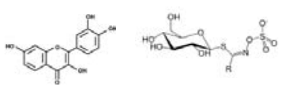




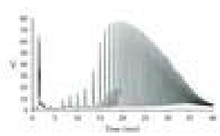
Carbohydrates + Fibre

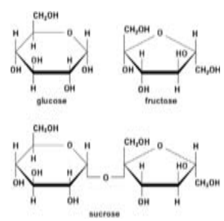


Ascorbic Acid

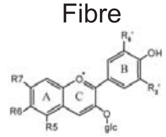


Polyphenols + Glucisinolates

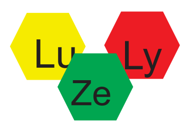




Carbohydrates + Fibre



Anthocyanin



Carotenoids

Type of analysis	CODE
Dry matter percentage	DM%
Nitrate ion content	NO_3
Chlorophylls	CHL
Total carotenoids	TCARO
Total proteins	TPROT
Carotenoid identification	SCARO
Soluble proteins	SPROT
Ascorbic acid	ASCO
Non-structural carbohydrate (Glucose fructose, sucrose and others)	NSC
Ash content	ASH
Fructans	FRUCS
Oligo-Fructosides	FOS
Mineral composition	MIN
Antocyanidin	ANTO
Organic acid	ORGA
Oxalic Acid	OXA
Lycopene	LYCO
Total polyphenols	TPOLY
Polyphenols	SPOLY
Carotenoid identification	SCARO
Total Glucosinolate	TGLUC
Glucosinolate identification	SGLUC
Antioxidant Capacity	ORAC
Antioxidant identification	SANTHO
Oils/Fats	FATS
Hardiness	PTRO
Soluble solids	BRIX

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Species	Cultivar /type	N° of harvest sampled	N° samples per harvest	Type of sample	Tissue (and total amount of samples to transport in g)	stabilisation	Storage	Analysis type	Site of analysis
Lactuca sativa L.	TBD	3	4	Fresh material (FM)	Leaves (100 g)	Freezing	-20°C	CHL.; TCARO; DM; TPROT.; SPROT NSC; ASH; FRUCS; FOS OXA ASCO FIBRE	CNR IBAF
								MIN	LIT
								NO_3	NM-III
Lactuca sativa L.	TBD	1	5	FM	Roots (100 g)	Freezing	-20°C	TCARO; DM; TPROT.; SPROT NSC; ASH; FRUCS; FOS OXA ASCO	LIT
								NO_3	NM-III
Eruca sativa Mill.	Cultivated	3	4	FM	Leaves (100 g)	Freezing	-20°C	CHL.; TCARO; SCARO; ASCO; DM; TPROT.; SPROT NSC; ASH TPOLY FIBRE	CNR IBAF LIT
								TGLUC; SGLUC; ORAC; SANTHO MIN SPOLY	LIT CNR

We defined the

Quality driving attributes

(QDA) as those nutritional and nutraceutical quality attributes that are the most relevant for each species, for acceptance by the NM-III crew, by astronauts and for general consumers….

We selected up to three QDA for each of the adopted species and varieties

Species	Cultivar /type	QDA 1	QDA 2	QDA 3	Comments
Lactuca sativa L.	TBD	NO_3	OXA	FOS	Nitrate levels are monitored. Oxalic acid should be low due to threat to human health.
Eruca sativa Mill.	Cultivated	ASCO	TGLUC	FIBRE	Methods to quantify polymeric composition of rocket produce were tested. Original data are available. Glucosinolates method development is complete.
Raphanus sativus L.	TBD	TGLUC	ANTIOX	NSC	Nitrate levels are monitored.

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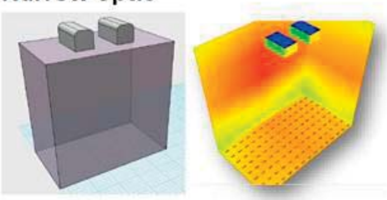
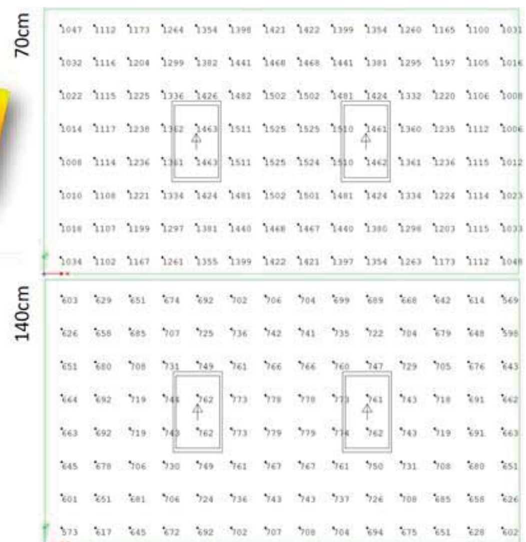
Upgraded in-house growth facilities for pre-mission activity

Modelling light intensity and distribution

Simulation – 2 lamps
Narrow optic





Two lamps with the narrow optic provide an even distribution at both 140cm and 70cm. The separation of the lamps could likely be increased a more to further improve uniformity.

Intensities over 700umol/m2/s at floor level and over 1400umol/m2/s at 70cm can be expected.

heliospectra

CELLS M12 & EGC M48 Growth Chambers

Testing light intensity and distribution

	Licor PAR (μ mol quanta m^{-2} s^{-1}) [80 cm from lamp]				
LED setting led	450+660 +5700k	5700 K	660	450	660 + 450
1000	825	200	570	70	633
800	711	177	481	60	538
600	584	147	392	51	440
400	451	115	295	41	334
200	315	80	200	35	234

Started the Pre-Mission activities with in-house experiments

- **Food Quality-composition**
- **Food Safety-composition**
- **Food Quality-organoleptic**
- **Food safety-microbial contamination**

OUR DRIVING IDEA:
to increase the efficiency of agriculture we need to maximize the positive sides of the plant-environment interaction while avoiding the negative ones.

This can be done by controlling the plant growth environment (and using renewable energy)

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Pre-Mission activities

- **Food Quality-composition**

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ENERGY: A KEY ASPECT

Light quantity and lamp setting

Photoperiod and PPFD value	Treatment	Blue (450nm)	Red (660nm)	FR (730nm)	Cool white 5700K
12h/12h PPFD= 600 µmol m^{-2} s^{-1}	A-12h	15%	71%	2%	12%
24h (CN) PPFD= 300 µmol m^{-2} s^{-1}	B-CL	0	0	0	100%
24h (CN) PPFD= 300 µmol m^{-2} s^{-1}	C-CL	27%	73%	0	0

Cool white lamps 5700K have

25% Blue 450 nm;

45% Green ; 563 nm

27% Red 660 nm

3% Far-Red730 nm.

ENERGY AS A KEY ASPECT: growth analysis parameters of *E. sativa* Mill. as affected by different light treatments.

Measured Variables
Total fresh matter (g)
Total dry matter (g)
HI%
Leaf fresh weight (g)
Leaf dry weight (g)
DM%
SLDW (g/m^2)
Root fresh weight (g)
Root dry weight (g)
DM%

RESULTS:

- Highest total production in B-CL (+45%)
- Lowest Dry matter production in A-12h
- Highest HI in B-CL
- Highest edible production in B-CL
- Highest DM% and SLDW in C-CL
- Highest root amount in C-CL

Practical Indications:

- Grow under 24 h light regime (if no flowering occurs)
- Use a substantial amount of green light

Questions for future research:

- Why is the "neglected" green light so beneficial?

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ENERGY AS A KEY ASPECT: leaf tissue pigment concentrations on a fresh matter basis (mg 100 g FM-1) of Eruca sativa Mill. as affected by different light treatments.

Measured Variables
Chl a
Chl b
Chl a/b
Total Chl (a+b)
Carotenoids
Lutein
Neoxanthin
Violaxanthin

RESULTS:

- All parameters changed with the light treatment
- C-CL had the lowest Chl content
- Lutein differences did not match those of chlorophyll

Practical Indications:

- Light regime can affect strongly pigment composition

Questions for future research:

- Optimize light recipe for specific pigment accumulation
- How to maximize lutein content?

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ENERGY AS A KEY ASPECT: Chemical composition of cell wall of E. sativa Mill. as affected by different light treatments.

Measured Variables
Ash
Total extractives
Total lignin
Monomers of insoluble polysaccharides
Glucose
Fucose
Arabinose
Galactose
Xylose
Mannose
Rhamnose
Galacturonic acid

RESULTS:

- High extractives in 24h treatments
- Significant changes in lignin content not due to changes in extractives.
- Relatively low xylose amount indicating low amount of hemicelluloses
- Large amount of galacturonic acid (and rhamnose) indicating abundance of pectin.

Practical Indications:

- **35-45% of rocket dry weight can be due to compounds related to dietary fibre.**

Questions for future research:

- Effect of light regime on lignin biosynthesis
- Nutraceutical value of pectin (and hemicelluloses), effect on the gut microbiome.
- Effect of the growth environment on the dietary fiber components of leafy vegetables

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Food quality: quality test on produce grown inside the FEG during the test phase in Bremen

Non Structural Carbohydrate (NSC) content in different plant species grown in the FEG during the test phase in Bremen.
Data are reported as mean ± s.e. (mg · g dry weight $^{-1}$)

Species	Galactose	Glucose	Fructose	Sucrose	RFO (Raf+Stac)	Starch	Total Soluble	Total NSC
Rocket	-	16,1 ± 1,8	6,5 ± 0,7	2,5 ± 0,5	-	12,2 ± 5,6	25,1 ± 2,6	37,3 ± 6,9
Red Giant	-	30,7 ± 1,9	12,0 ± 0,7	2,4 ± 1,5	-	23,6 ± 5,5	45,1 ± 2,8	68,3 ± 11,8
Basil	2,5 ± 0,1	8,5 ± 1,9	6,4 ± 1,3	0,4 ± 0,1	0,7 ± 0,1	48,7 ± 24,5	18,5 ± 3,4	67,2 ± 8,6
Mini-Cucumber	0,6 ± 0,04	145,9 ± 4,6	145,7 ± 10,6	-	-	7,2 ± 1,9	292,1 ± 13,7	282,1 ± 13,7
Mini-Pepper	0,7 ± 0,2	157,1 ± 14,7	154,0 ± 9,5	54,8 ± 31,1	-	155,1 ± 28,3	366,6 ± 16,1	521,6 ± 16,1

Pre-Mission activities

- **Food Safety-composition**

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ENERGY AS A KEY ASPECT: Inorganic anion content (ppm) and organic acid (mg 100 g FW-1) in E. sativa Mill. leaves as affected by different light treatments.

Measured Variables
Inorganic anions (ppm)
Nitrate
Sulfate
Fosfate
Organic acid (mg 100 g FW^{-1})
Malic acid
Citric acid

RESULTS:

- High content of nitrate in A-12h
- No significant changes in other anions
- Indication of increase of Malate under 24h light

Practical Indications:

- The crew will be allowed to consume Rocket since nitrate in leaves grown under fully controlled environment was lower than the legal limits for commercialization.
- Long photoperiod might help reducing nitrate content more than high light intensity with short photoperiod.

Questions for future research:

- Is nitrate bad or good?
- Light action spectrum on Nitrate reduction (Nitrate reductase activation)

LED TECHNOLOGY- NEGATIVE QUALITY ASPECTS

Effect of the light spectrum on nitrate

	Blue (400-500nm) (%)	Green (500-600 nm) (%)	Red (600-700 nm) (%)
Reference	17	12	72
50% blue	50	12	38

Nitrate content in Red Mustard and Rocket leaves grown under 17% or 50% LED Blue light.

RESULTS:

- Much higher Nitrate content in Red Mustard than in Rocket
- Opposite effect of high blue on two different species, it decreased Nitrate in Red Mustard and increases it in Rocket.

Practical Indications:

- **Nitrate in Red Mustard leaves grown under fully controlled environment was higher than the legal limits for commercialization (of salad). It would be not safe for the crew to consume.**

Questions for future research:

- **Nitrate use efficiency under Controlled environment conditions**
- **Light action spectrum on Nitrate reduction (Nitrate reductase activation)**

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Pre-Mission activities

- **Food Quality organoleptic**

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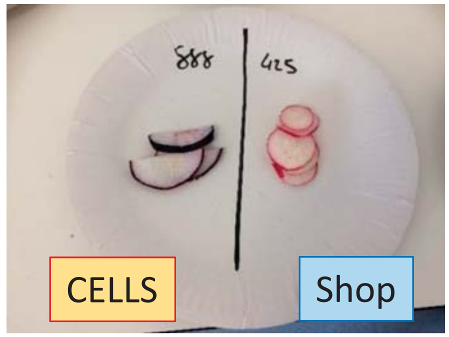
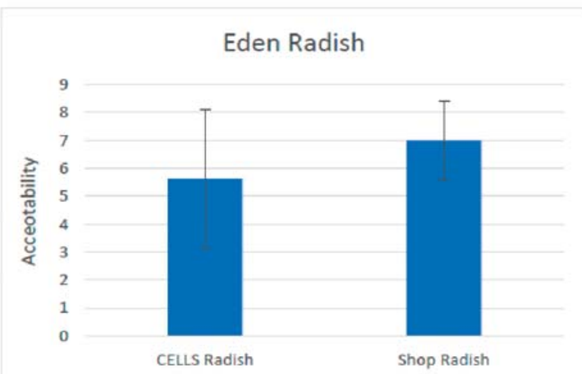
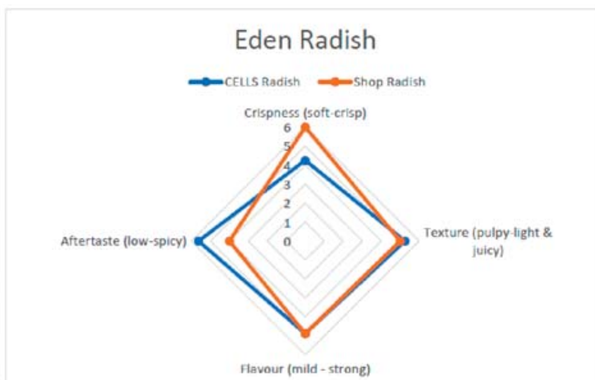
Pre-Mission activities-Food Quality-organoleptic







RESULTS:

- Size and color were different in Shop and CELLS radish
- Crispness was insufficient in CELL radish
- CELLS radish was too bitter

Practical Indications:

- **Variety selection to meet crew expectation in terms of taste and appearance**

Questions for future research:

- Evaluate the role of growth light intensity and spectrum on crispness (osmotics and cell wall composition)
- Evaluate the role of growth light intensity and spectrum on tissue composition (bitterness, glucosinolates)

Pre-Mission activities

- **Food Safety-microbial contamination**

Pre-Mission activities-Food safety-microbial contamination

	V1 (SD)	V2 (SD)
Salmonella spp	0 (0)	0
Enterococcus faecalis	65.8 (0.57)	13.15 (0.57)
Total coliforms	0 (0)	0(0)
Proteus vulgaris	106.5 (1.57)	0 (0)
Listeria	0 (0)	0 (0)
St aureus	71.35 (3.05)	160 (17.3)
E coli	0(0)	0(0)
S.thyphi	0(0)	0 (0)
Total count	402 (4.61)	410 (3.21)

Cfu present in 5 grams of sample from V1 and V2 cassettes
The experiments were performed in triplicate; the data are expressed as average (±Standard Deviation)

RESULTS:

- Only for three of the tested species there were counts
- Specific species and total counts were not elevated, still need to be considered as a potential treat for consumers

Practical Indications:

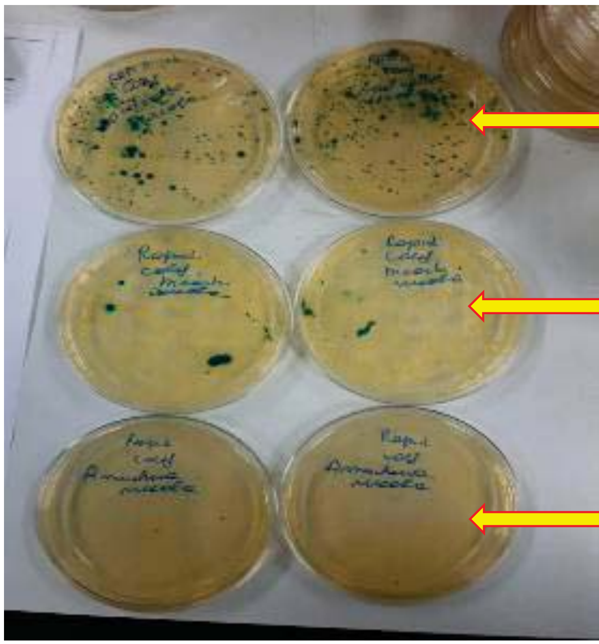
- **Take care of growing environment contamination**
- **Sanitization is necessary**

Questions for future research:

- Evaluate sources of contamination in controlled environments
- Investigate the interaction of plant and pathogenic microorganisms

Pre-Mission activities-Food safety-microbial contamination

Rocket from local market



Sample washed with water

Sample washed with water and treated for 15’ with sodium bicarbonate

Sample washed with water and treated for 15’ with sodium hypochlorite («Amuchina»)

RESULTS:

- Presence of potential pathogens Is common in market produce
- Potential pathogens can be easely removd with commercial producs

Practical Indications:

- **Sanitization necessary**

Questions for future research:

- Evaluate sources of contamination in controlled environments
- Investigate the interaction of plant and pathogenic microorganisms

What to learn for Earth agriculture?

To increase the efficiency of agriculture we need to maximize the positive sides of the plant-environment interaction while avoiding the negative ones.

Controlling the environmental factors we can force plant metabolism to produce useful compounds and to decrease negative compound in plant food, this is a new frontier of agriculture and nutrition

In practice this can be done by controlling the plant growth environment, INTRODUCING VARIABLE DEGREES OF ENVIRONMENTAL CONTROL, UPGRADING EXISTING GREENHOUSES, INTRODUCING NEW ENVIRONMENTAL CONTROL SYSTEMS AND SUB-SYSTEMS IN TRADITIONAL AND NEW AGRICULTURE SYSTEMS.

On Earth agriculture technology should be coupled to the use of RENEWABLE ENERGY, to build modern and SUSTAINABLE agricultural systems.

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What to learn for plant science?

New controlled environment technologies allow testing new growing conditions in terms of single and combined environmental factors, there are important tools for the advancement of plant sciences.

Due the variability of the genetic back ground of plant species and to the interactions between pants and several environmental parameters it is difficult to extrapolate general rules. NOTHING IS SMPLE WITH PLANTS AND ENVIRONMENT INTERACTION

The study of the interactions between plants ad microorganism might produce unexpected and important results in the area of plant production and protection systems and or food safety aspects.

Young, motivated scientist are needed for the academia and to transfer academic knowledge into new agricultural systems and the food industry.

What about the EDEN ISS project?

THE BEST IS YET TO COME!