Experiencias de proyectos de investigación en el campo del tratamiento de aguas en la industria: Proyecto: DESARROLLO Y EVALUACIÓN DE NUEVOS PROCESOS FOTOQUIMICOS Y BIOLOGICOS PARA EL TRATAMIENTO Y LA REUTILIZACION DE AGUAS EN INDUSTRIAS ALIMENTARIAS (WATER4FOOD)"

Almería, 4 Febrero 2021

Cristina Pablos

Universidad
Rey Juan Carlos

Facultad de Ciencias Experimentales

Outline

Project

WATER4FOOD

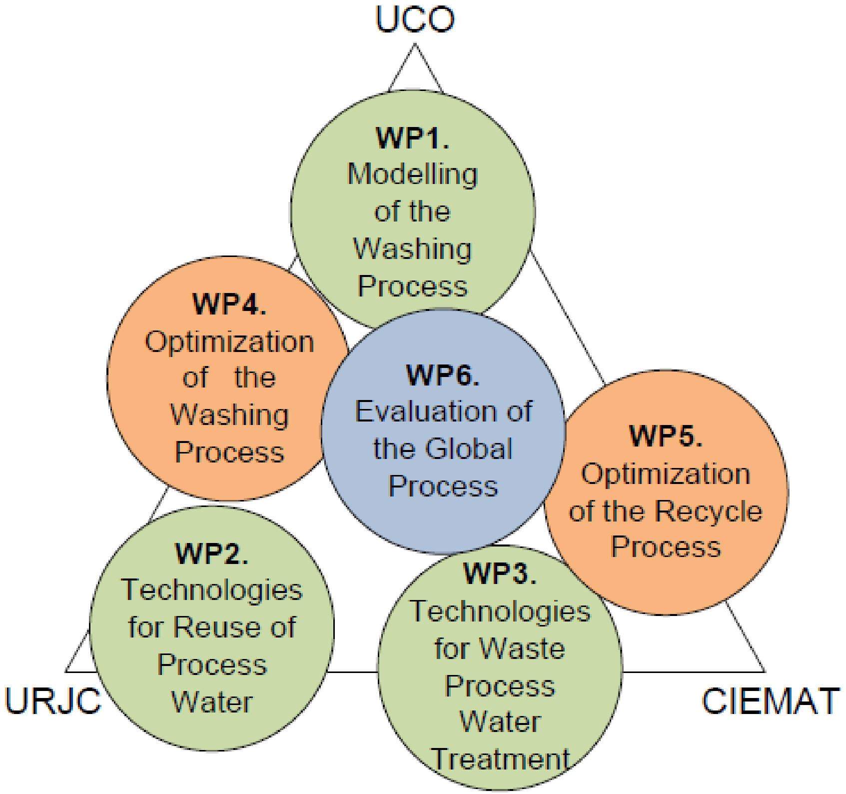
2015-2017, Financial support: Ministerio de Economía y Competitividad: MINECO: 220,000 €

PARTNERS

URJC: Experts on Advanced Oxidation processes (AOP’s) and biological processes for wastewater (WW) treatment. Javier Marugán & Fernando Martínez

CIEMAT-PSA: Experts on pilot plant development for solar AOP’s WW and reuse. Pilar Fernández Ibáñez & Inmaculada Polo López

UCO: Modelling and prediction of risks in food security. Fernando Pérez Rodríguez



Universidad Rey Juan Carlos

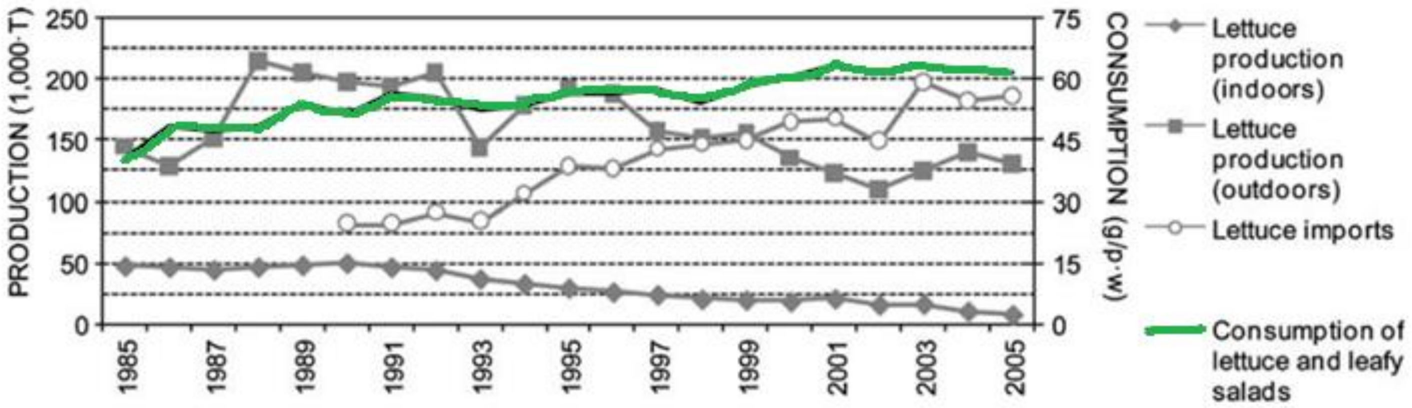
UNIVERSIDAD D CÓRDOBA

Ciemat PSA

Motivation

Fast increase in the market of ready-to-eat (RTE)/minimally-processed vegetables (MPV)

Trends in Production and Consumption of lettuce in the UK (DEFRA 2006, 2007)



A. Hospido et al., Int J Life Cycle Assess (2009) 14:381–391

Primaflor

Dimmidisì
El bienestar nace de la frescura

Florette

Verdifresh

freshfel
EUROPEAN FRESH PRODUCE ASSOCIATION

Motivation

Fresh produce microbial food safety concerns in EU and USA

Number of Outbreaks (illnesses) (2000 -2009) (USA) linked with fresh produce items (CDC website)

Produce item	Bacterial agents: *Salmonella* spp.	*Escherichia coli* O157:H7[c]	*Shigella* spp.	*Campylobacter jejuni*	Other[d]
Cabbage	1 (8)	1 (41)			2 (68)
Lettuce	10 (456)	14 (364)	1 (4)	2 (16)	3 (114)
Spinach		2 (223)			1 (6)
Sprouts	12 (441)	4 (46)			1 (20)
Herbs	3 (70)				
Leafy green salads	23 (997)	15 (280)	7 (190)	7 (42)	10 (145)
Coleslaw	1 (26)				4 (22)
Peppers	4 (1,643)			1 (5)	2 (17)
Tomatoes	25 (1,867)		1 (886)	1 (13)	2 (10)
Cantaloupe/melons	19 (1,180)	1 (5)	1 (56)		1 (55)

E. R. Choffnes et al., The National Academy Press (2012) Washington, DC

Introduction

Importance of water in the fresh produce supply chain.

Fresh-cut processing water is used for

- **Cooling**
- **Rehydration**
- **Sorting/Transport**
- **Washing**
- **Cleaning contact surfaces as conveyor belts, etc.**

Problems of chlorination to reduce load of microbial contamination

Wash water of inadequate quality shows the potential to be a direct source of contamination and a way for spreading bacterial contamination.

Simulated Wash Water composition

Compound	Concentration (mg/L)
F^-	0.14
Cl^-	282
NO_2^-	0.030
Br-	10.2
NO_3^-	51.6
SO_4^{2-}	51.0
Na^+	87.7
NH_4^+	1.24
K^+	108.0
Mg^{2+}	9.55
Ca^{2+}	47.1

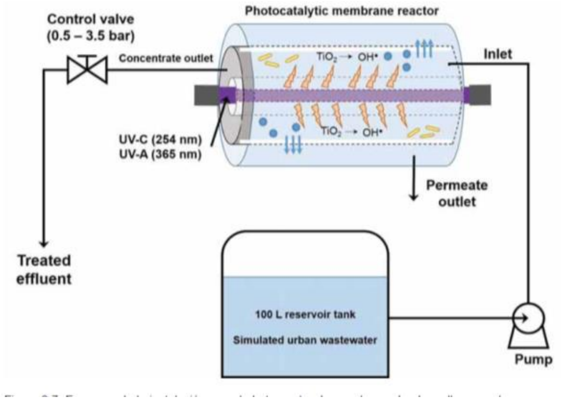
Physicochemical characterization	
TOC (mg/L)	150
Turbidity (NTU)	100
pH	6.3
Conductivity (µS/cm)	1206

Pesticides characterization

		$T_{muestreo}$			
Espinacas	Pesticida (ng/L)	6:20	13:20	19:20	22:00
	Acetamiprid	6	14	416	395
	Azoxystrobin	1	3	58	74
	Imidacloprid	-	-	66	167
	Iprodione	-	1008	1583	1392
	Metalaxyl	-	35	21	32
	Propamocarb	-	10	773	2696
	Simazine	-	-	159	124
	Pirimicarb	-	555	2	3

		$T_{muestreo}$			
Lechuga	Pesticida (ng/L)	6:20	13:20	19:20	22:00
	Acetamiprid	61	262	354	385
	Imidacloprid	72	323	398	281
	Iprodione	-	-	413	59
	Metalaxyl	17	104	148	97
	Simazine	-	-	82	10

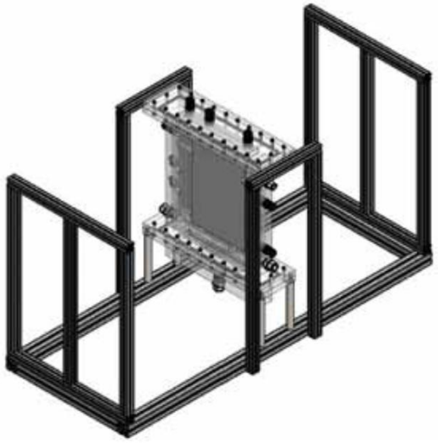
(1) Energy-efficient & sustainable water treatment

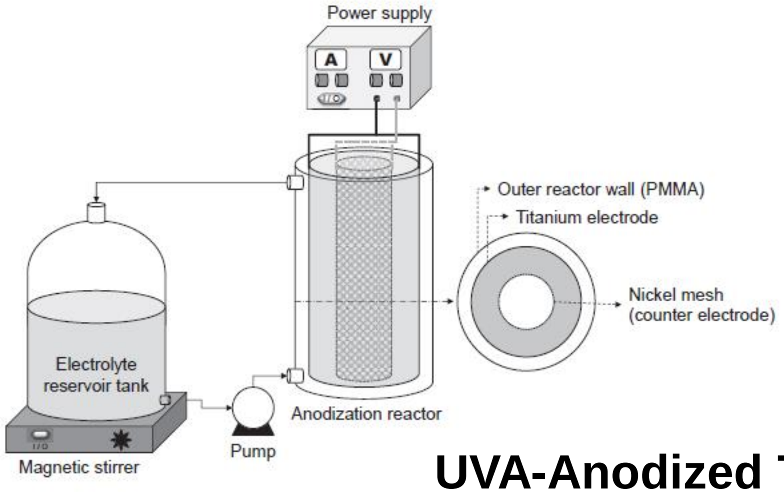


UVC/MicrofiltratIon

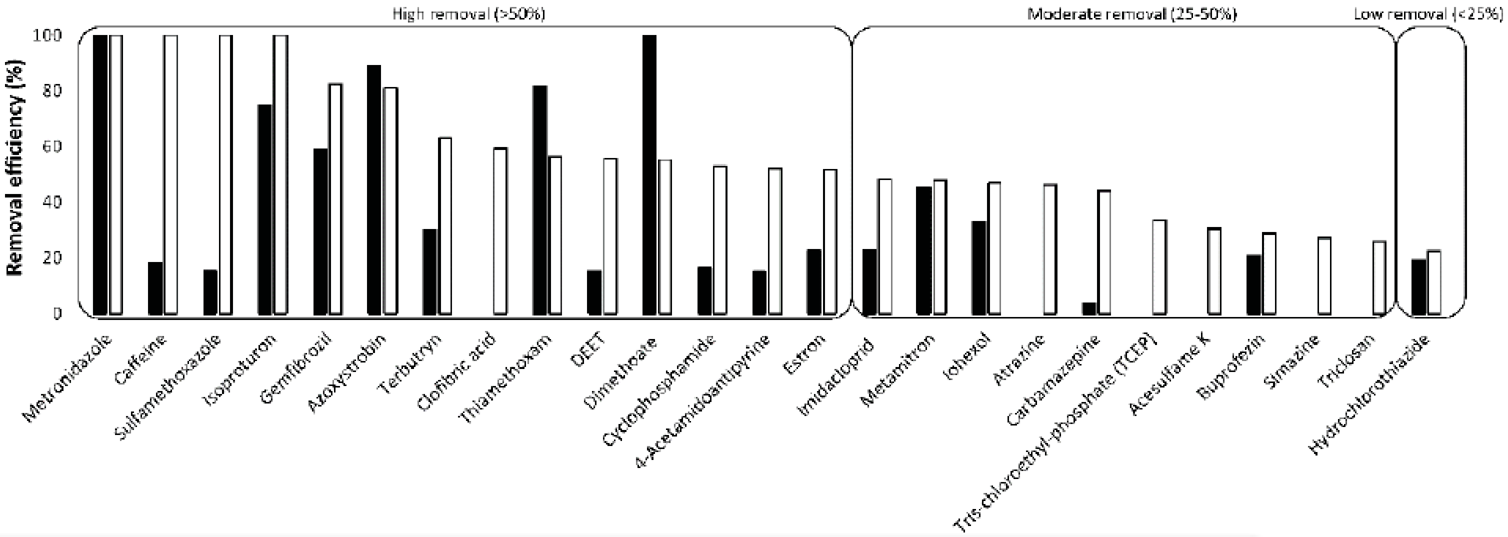
PAnMBR
Phototrophic purple bacteria (PPB)

Stage I (Black): PPB acclimation
Stage II (White): Stabilized PPB

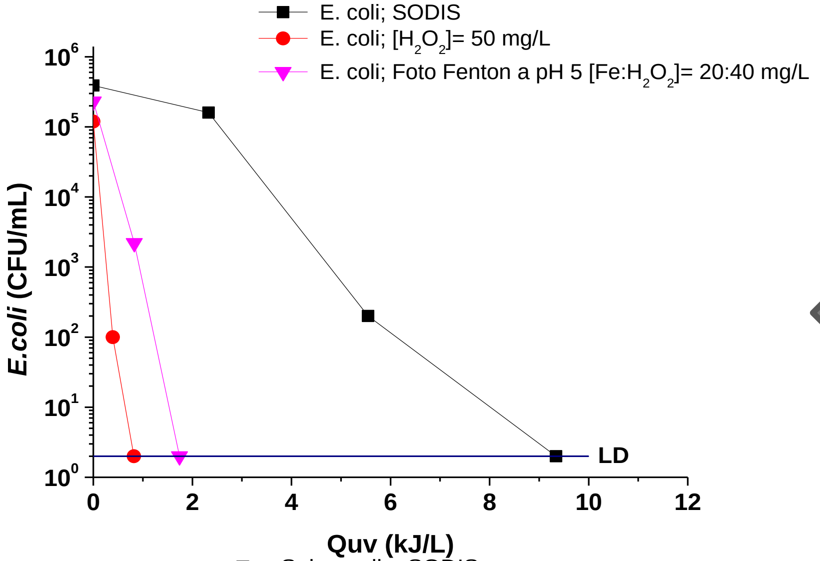




UVA-Anodized Ti



(1) Energy-efficient & sustainable water treatment



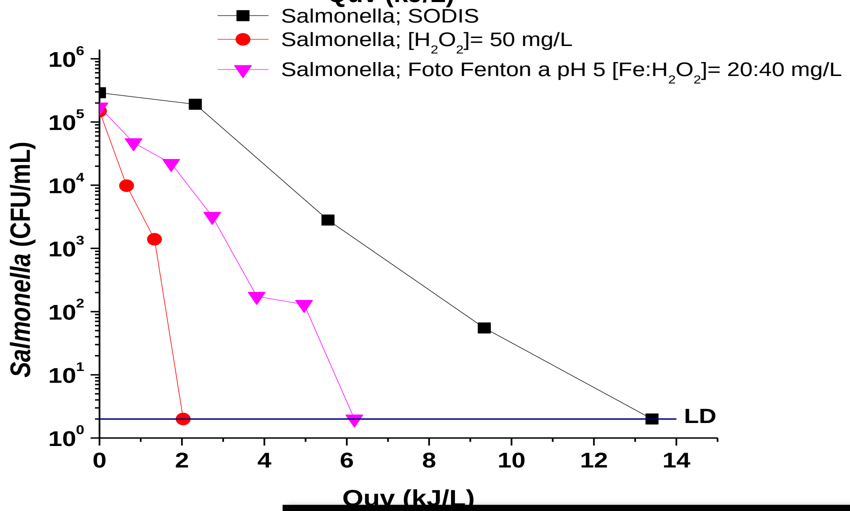


Photo-Fenton solar

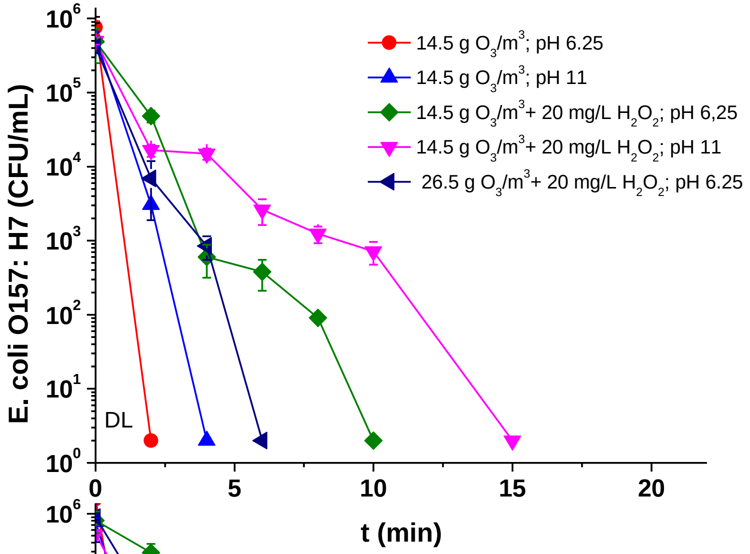
H_2O_2/Solar

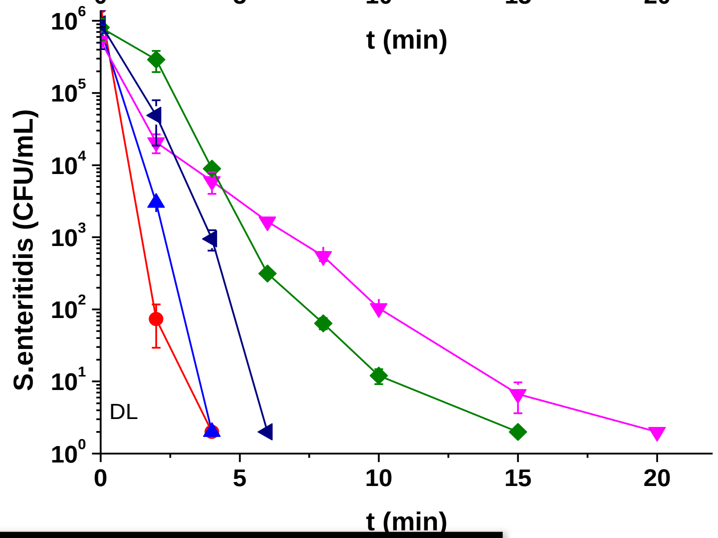
200 mL
Distilled water

Ozonation

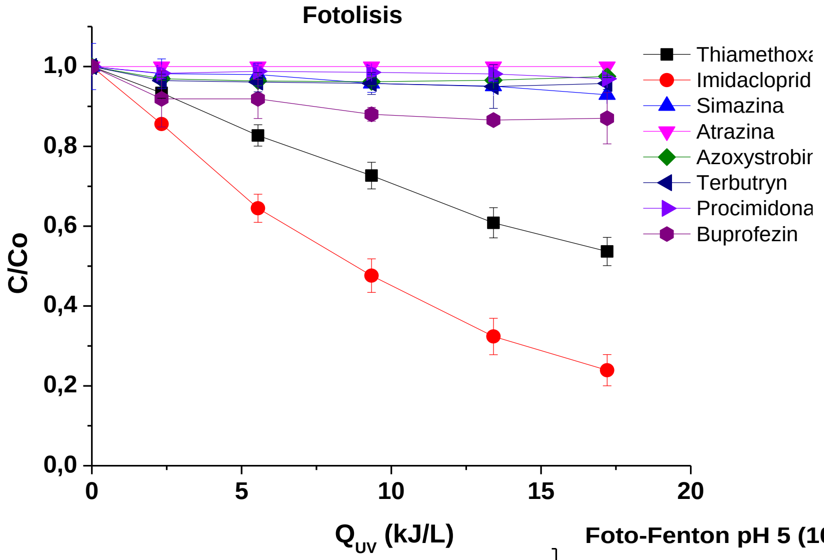
10 L
Distilled water

C_0: 10^6 CFU/mL

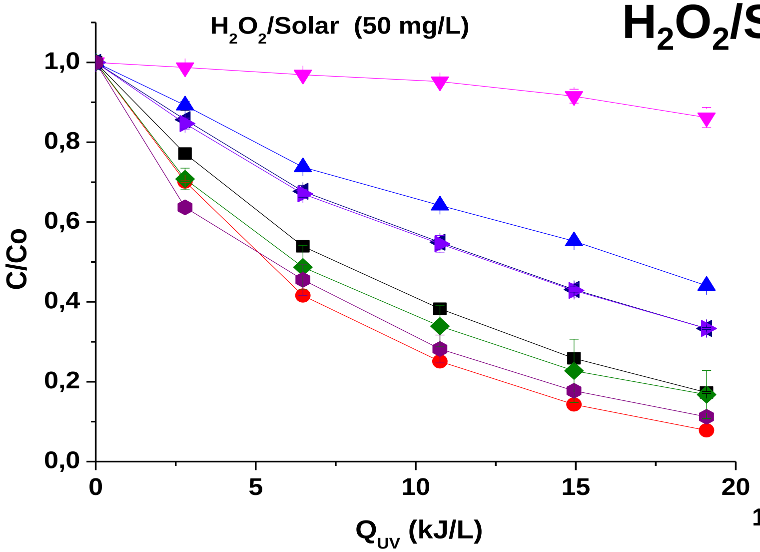




(1) Energy-efficient & sustainable water treatment



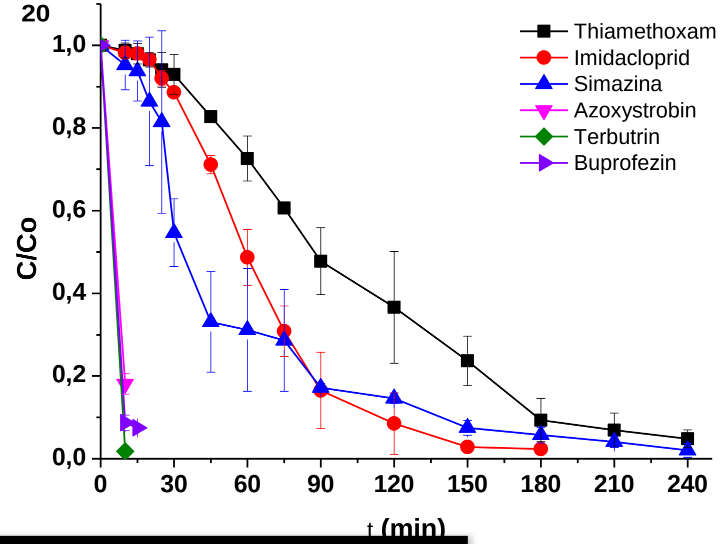
H_2O_2/Solar



C_0: 100 µg/L

Ozonation

$14.5\ g\ O_3/m^3$ pH:6.25



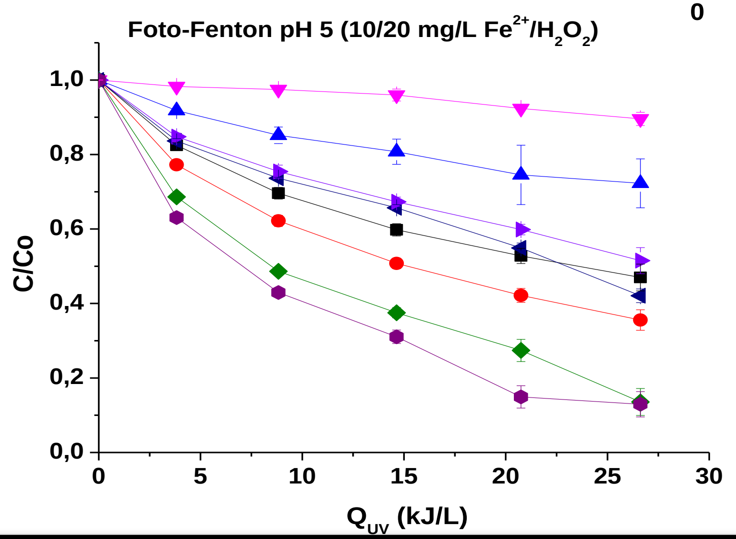


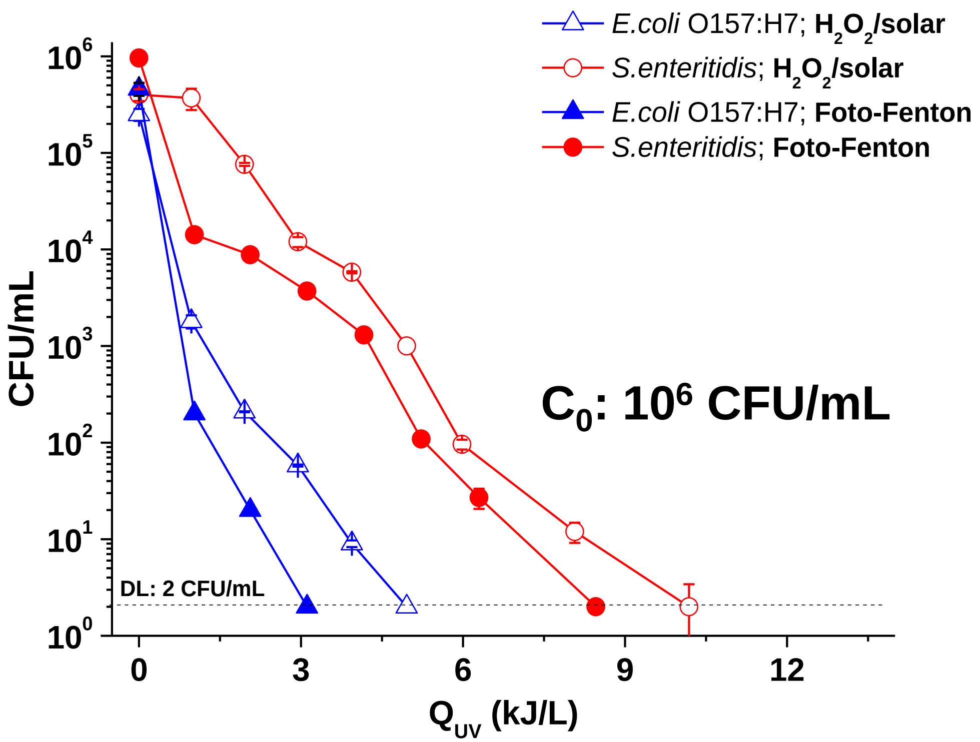
Photo-Fenton solar

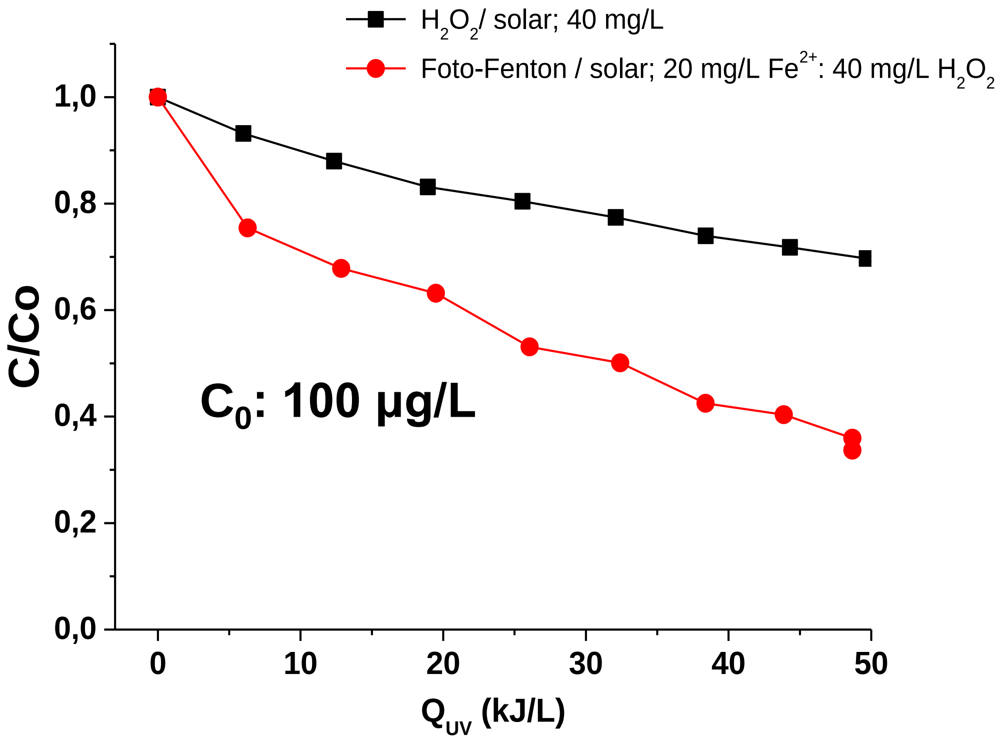
(1) Energy-efficient & sustainable water treatment

H_2O_2/Solar (40 mg/L)

Photo-Fenton solar (20 mg/L Fe^{2+}/40 mg/L H_2O_2)

CPC (60 L), SWW





(2) Water disinfection & irrigation of crops

Cultivo Lechuga

Germinación de semillas.

3 meses de riego.

Cosechado de muestras.

Cultivo Rábano

Germinación de semillas.

1.5 meses de riego.

Cosechado de muestras.

Análisis de transferencia de microorganismos (Protocolo A) y contaminantes (Protocolo B)

3 gr Lechuga

7-9 gr Rábanos

+ 10 mL solución salina

Stomaquer

Agitación y mezcla
5 minutos a 260 rpm.

Siembra de 0.5 mL – 5 mL extracto en medios de cultivo selectivos:

- -*E. coli* (Chromocult)
- -*Salmonella sp* (Salmonella-Shigella agar)

Incubación placas 24 h, 37 ºC

Recuento de colonias y análisis de resultados

10 g Muestra → + 10 mL MeCN 1% acido acetico → Agitación fuerte (5 min) → + 6 g $MgSO_4$ anhidro + 1.5 g NaOAc → Agitación fuerte (5 min) Centrifugación (3500 rpm, 5 min)

5 mL de extracto obtenido → + 750 mg $MgSO_4$ + 125 mg C18 + 125 mg PSA → Agitación fuerte (5 min) Centrifugación (3500 rpm, 5 min) → Evaporación hasta secado y reconstitución en MeCN/H2O (10/90) (v/v)

30 m², 23ºC, RH:50%

100 plantas/cultivo. Riego 50 mL (3-4 riegos/semana)

Análisis 30 muestras: turba y tejidos vegetales: hoja (lechuga), fruto (rábano)

(2) Water disinfection & irrigation of crops

	LECHUGA		RÁBANO	
Contaminantes químicos	% Recuperación	Concentración (µg/Kg)	% Recuperación	Concentración (µg/Kg)
Atrazine	93	132,52	65	25,66
Azoxystrobin	99	56,96	79	25,66
Buprofezin	92	46,02	42	28,59
Imidacloprid	100	91,52	106	11,75
Methiocarb	96	5,87	71	2,89
Procymidone	95	No detectado	62	No detectado
Simazine	102	137,70	84	75,96
Terbutryn	93	25,61	103	5,34
Thiamethoxam	53	172,28	40	21,32
Microorganismos	CFU/mL	CFU/g	CFU/mL	CFU/g
E. coli O157:H7	60	200	296	370
Salmonella	75	250	754	942

Control (+):

C_0: 10^6 CFU/mL

C_0: 100 µg/L

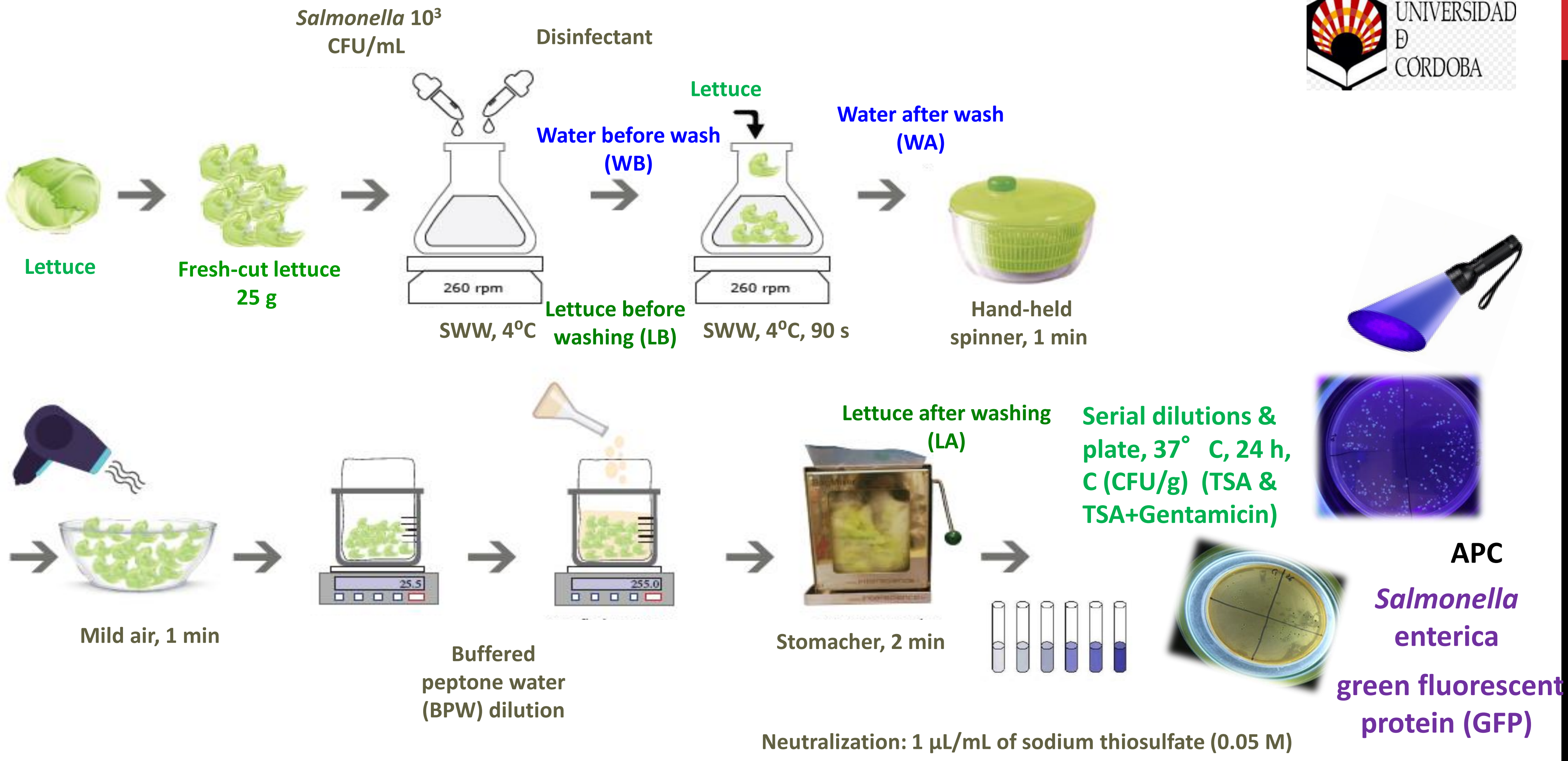
SWW

H_2O_2/Solar (0.22 €/m^3)

Photo-Fenton solar (0.25 €/m^3)

Ozonation (0.78 €/m^3)

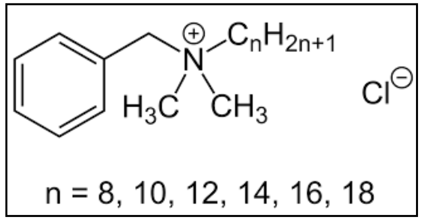
(3) Washing Process Optimization



(3) Disinfectants as alternative to chlorine

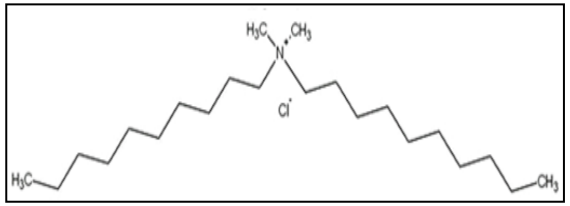
Family	Antimicrobial	Formula
Quaternary ammonium compounds (QACs)	Benzalkonium chloride (BZK)	$C_9H_{13}N\text{-}RCl$
	Didecyldimethylammonium chloride (DDAC)	$C_{22}H_{48}ClN$
Isothiazolinones	Kathon®	CMIT: C_4H_4ClNOS
	Predator 8000®	MIT: C_4H_5NOS
Monoterpenes (essential oils)	Carvacrol	$C_6H_3CH_3(OH)(C_3H_7)$
Sodium hypochlorite	Free chlorine	NaClO

BZK

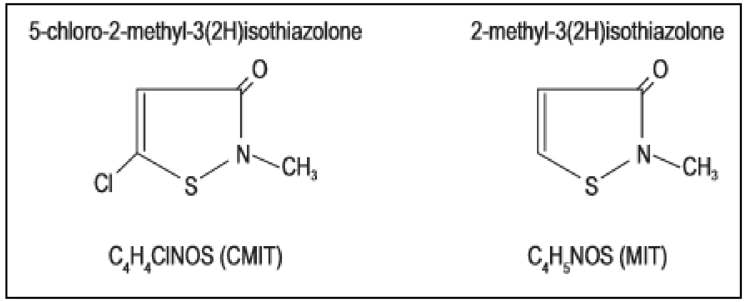


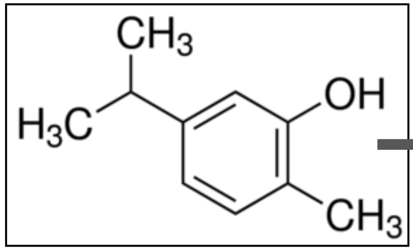
positively charged molecules bind to anionic sites on bacterial cell walls

DDAC



inhibition of key enzymes ← Kathon® & Predator 8000® (3:1)



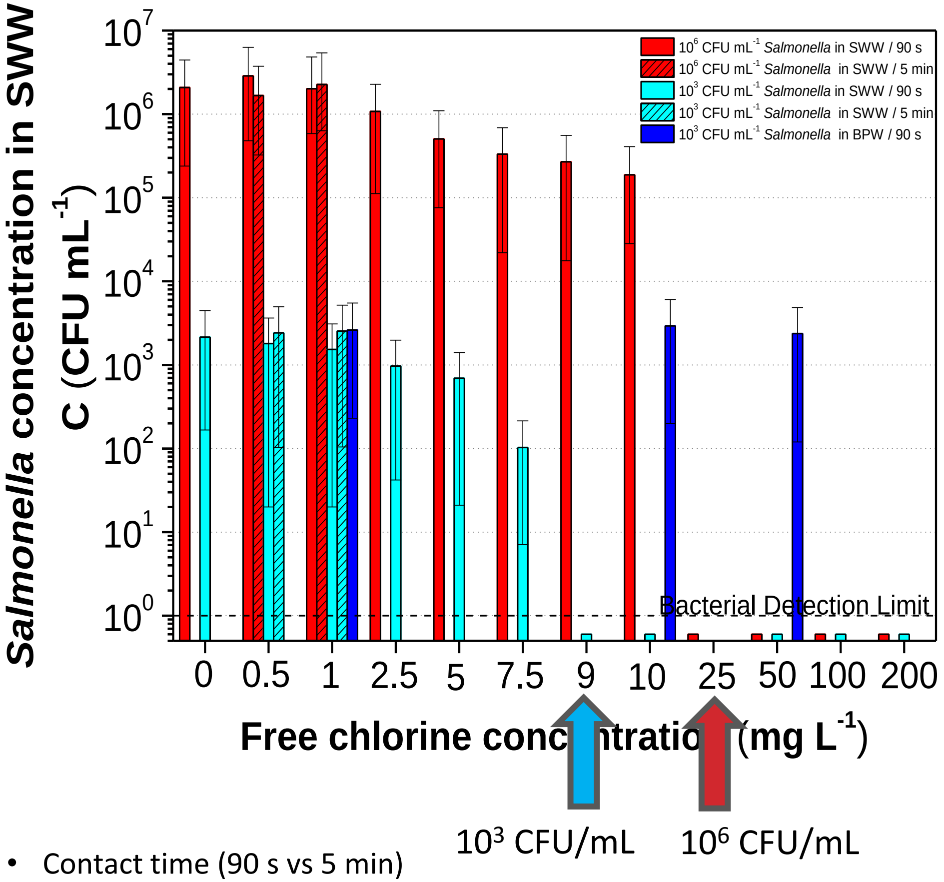


phenolic hydroxyl group acts as a proton exchanger

Carvacrol

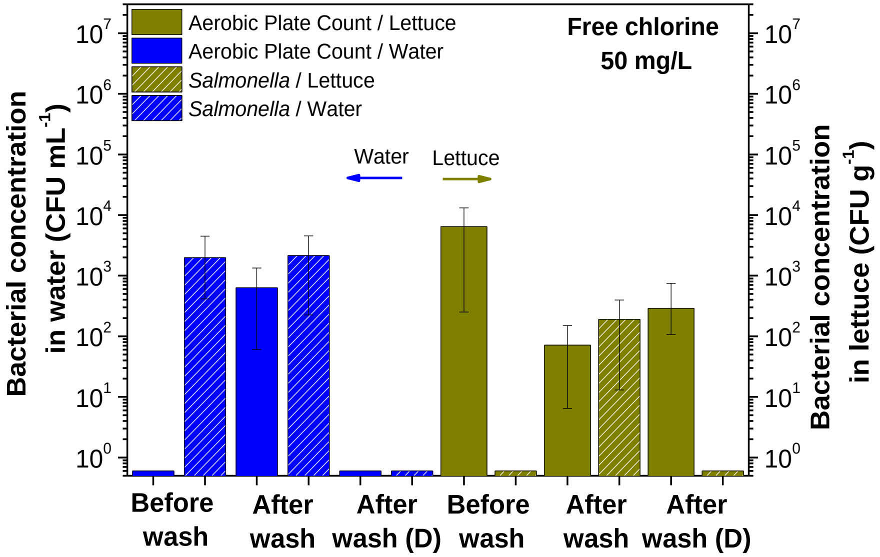
(3) Free chlorine in the washing process

Concentration selection



- Contact time (90 s vs 5 min)
- *Salmonella* concentration: 10^6 and 10^3 CFU/mL
- SWW (TOC: 150 mg/L) vs BPW (TOC: 1200 mg/L) . Organic matter effect.

Effects during washing: 50 mg/L



- Transfer of *Salmonella* from wash water to produce without disinfectant. Transfer of natural microbiota from the produce to wash water.
- Adding disinfectant (D): *Salmonella* is inactivated in wash water and produce. Possible prevention of *Salmonella* to be transferred to the produce. Natural microflora remain on the produce (95 % reduction), and contaminate wash water.

(3) Sensory and microbial quality of fresh-cut produce after washing

25 g washed fresh-cut produce Disinfectant & **25 g washed fresh-cut produce (No disinfectant)**

PACKED in chamber vacuum packing machine 7-14 Days, 4° C

(MAP: 90 % N_2, 5 % CO_2, 5 % O_2)

PA/PE/PA/PE Bags 80 µm

200 x 300 mm O_2 permeability : 50 cc/(m^2/24 h)

CO_2 permeability: 150-250 cc/(m^2/24 h)

Visual appearance (0-5): **1 = extremely poor, 2 = poor, 3 = fair (limit of acceptability), 4= good, 5= excellent.**

Odour (0-5): **1 = severe, 2 = strong, 3 = moderate (limit of consumer acceptability), 4 = slight, 5 = none.**

Browning index *(Palou et al. 1998)*

CIEL*a*b color space

color-difference formula (sensation)

$$\Delta E^* = \sqrt{(L^*_D - L^*_{ND})^2 + (a^*_D - a^*_{ND})^2 + (b^*_D - b^*_{ND})^2}$$

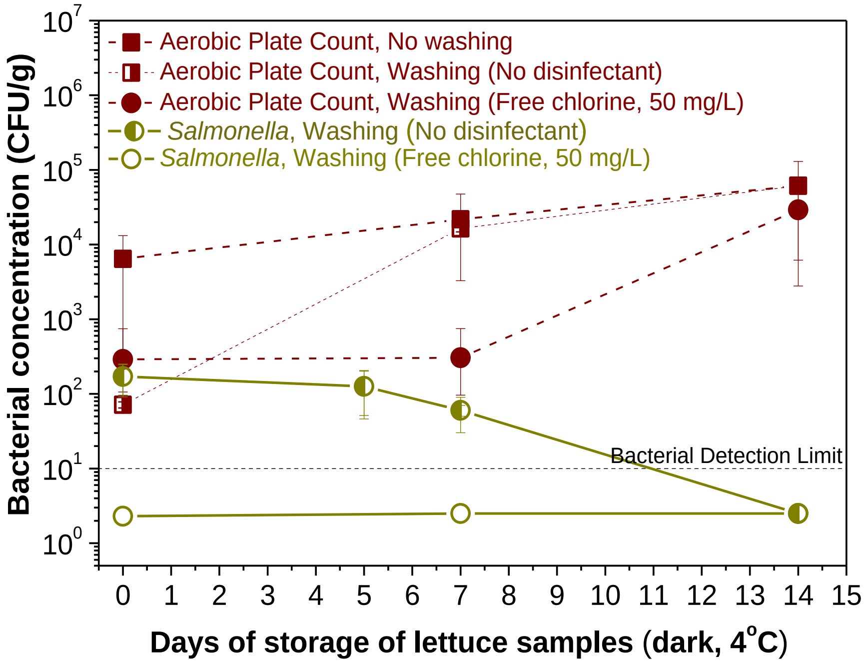
$$BI = \frac{(100 \cdot (x - 0.31))}{0.172}$$

$$X = (a^* + 1.75L^*) / (5.645L^* + a - 3.012b^*)$$

Browning: High values of a*, b*, ΔE*, BI and low values of L*.

(3) Free chlorine & other disinfectants after washing

Microbial load quality



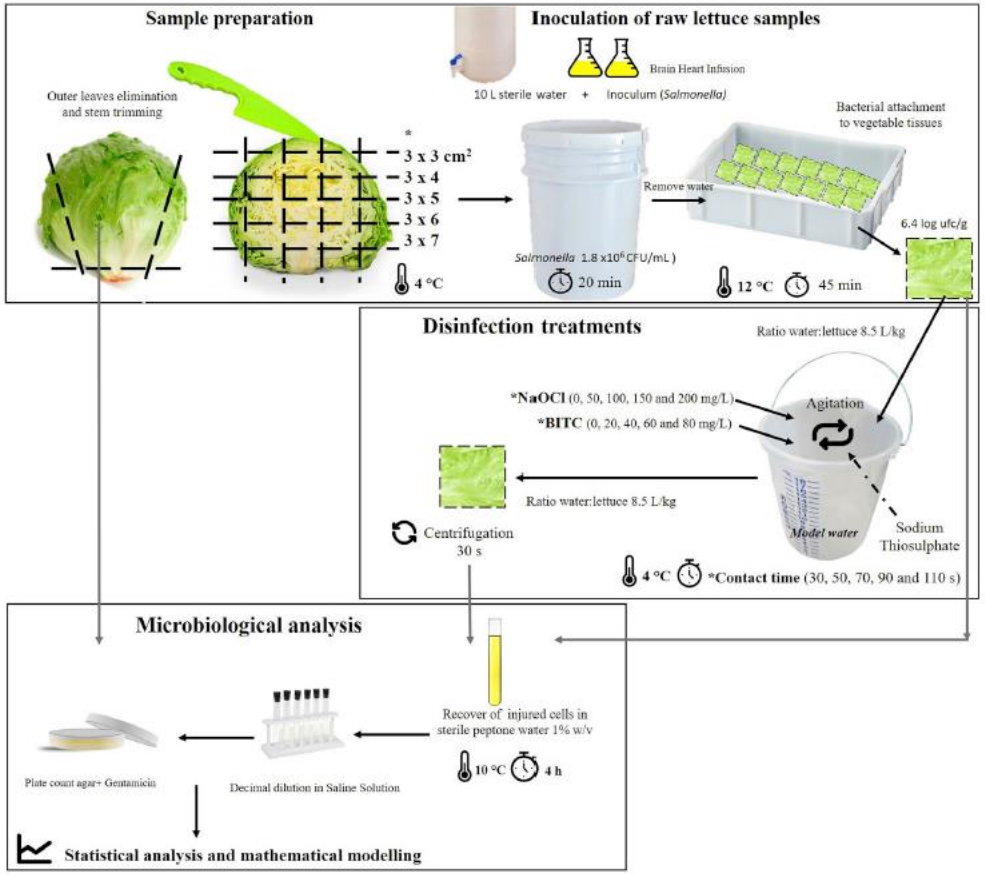
- No *Salmonella* regrowth.
- Aerobic bacteria in fresh-cut vegetables increase and are able to exceed the initial level on the wash water during extended storage.

Microbial growth no affects sensory quality.

Sensory quality

Disinfectant concentration (mg/L)	Reduction of *Salmonella* on lettuce (%)	Reduction Aerobic Plate Count on lettuce (%)	Sensory quality after storage
Free chlorine (50 mg/L)	>99.0	95.1±1.6	✓ after 14 days
CMIT:MIT 3:1 Kathon® (50 mg/L)	82	94.5±7.0	✓ after 14 days
CMIT:MIT 3:1 Predator 8000® (300 mg/L)	96.7	94.7±1.6	✗ < 7 days
DDAC (QACs) (100 mg/L)	98.3	80.8±13	✓ after 14 days
BZK (QACs) (300 mg/L)	66.7	91.3	✓ after 7 days
CAR (300 mg/L)	98.3	89.6±6.1	✗ < 7 days
BZK-CAR (75-200 mg/L)	>99.0	88	✗ < 7 days

(3) Predictive models of the process



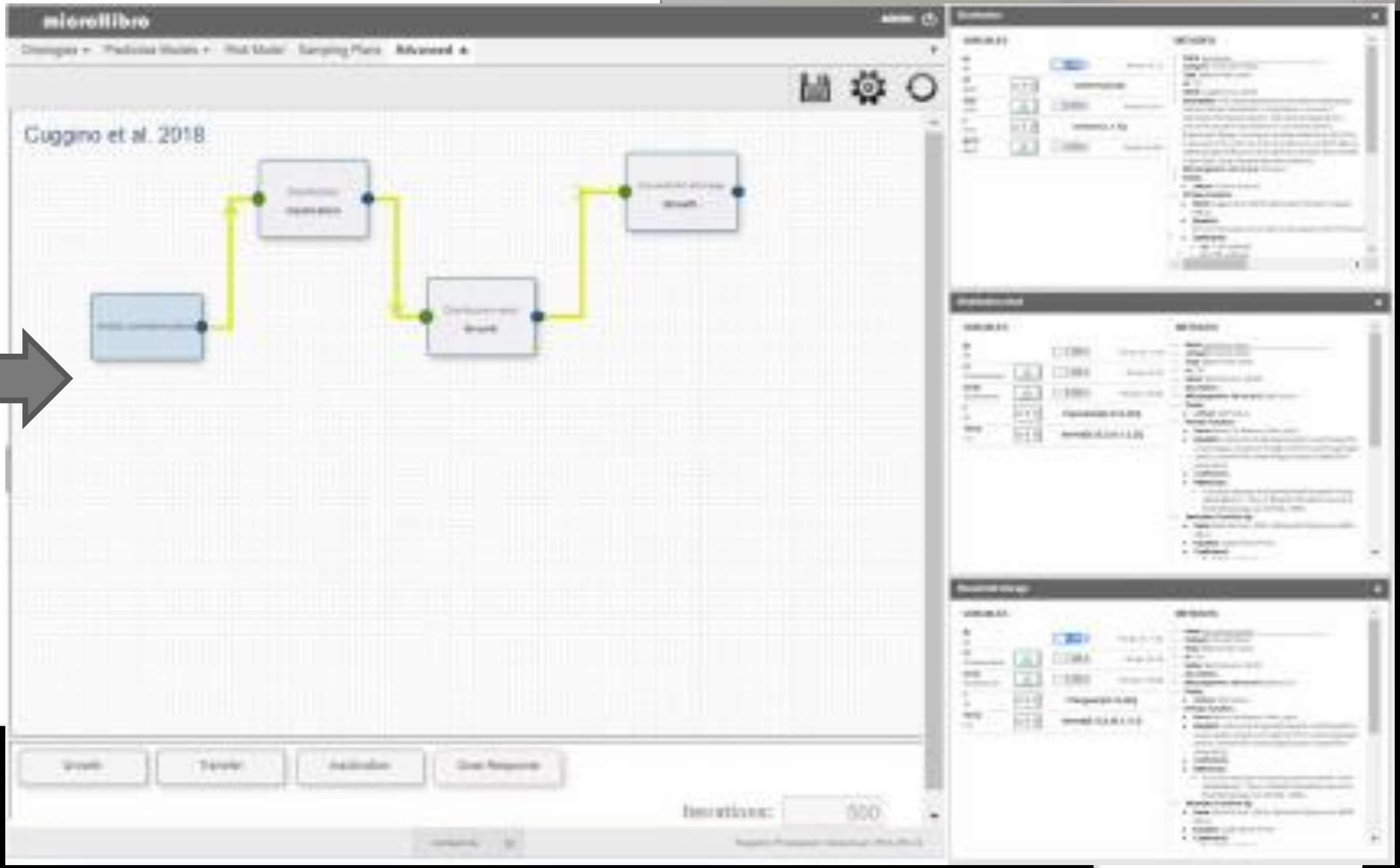
microHibro

Proyecto AT 2017-5686

"Transferencia de un sistema, basado en modelos de microbiología predictiva, para la mejora de la seguridad y calidad microbiológica de los alimentos andaluces."

UNIÓN EUROPEA

Financiados por fondos FEDER.
Una manera de hacer Europa



Acknowledgements

Ministry of Economy and Competitiveness (MINECO) project WATER4FOOD (CTQ2014-54563-C3-1-R)

cristina.pablos@urjc.es

Thank you very much for your attention