International Conference and Exhibition on Nutraceuticals and Functional Foods

2018

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Sheraton Hotel (Richmond)

Vancouver, BC, Canada

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O48. Impact of elicitors in the metabolism of bioactive compounds and sensory properties of butterhead lettuce (*Lactuca sativa* var. *capitata*) cultivated by hydroponic method

Jesús Omar Moreno-Escamilla, Emilio Alvarez-Parrilla, Laura A. de la Rosa, Nina del R. Martínez-Ruiz, José Alberto Núñez-Gastélum, Gustavo A. González-Aguilar, Joaquín Rodrigo-García - Universidad Autónoma de Ciudad Juarez (Mexico)

The use of different strategies on vegetable crops, such as elicitor application, with the aim of increasing the content of bioactive compounds has taken great importance in recent investigations. Different concentrations of arachidonic (AA) and salicylic acid (SA), methyl jasmonate (MJ) and Harpin protein (HP) were used as elicitors in green and red butterhead lettuce. Highest increase for polyphenols was observed in green and red lettuce after treatment with MJ (90 µM). Carotenoids were raised by HP (60 mg/L) in green lettuce, and by AA (45 µM) in red lettuce. Overall, MJ (90 µM) was the elicitor with the highest effect on phytochemical concentration. HPLC-MS analysis indicated that in green lettuce the main effect of elicitation was observed in the increase of hydroxycinnamic acids and in red lettuce in flavonoids. Descriptive sensory analysis of lettuce showed that elicitation did not significantly affect the organoleptic characteristics of the lettuce samples.

O49. Flaxseed: Comprehensive processing and high value products

Nam Fong Han, Huihuang Ding, Steve W. Cui - Natunola Health Inc. (Canada)

Flaxseed (*Linum usitatissimum* L.), one of the most economically important oilseed crops, is also rich in dietary fibres, protein and lignans. The brown Canadian flaxseed has an average of 41% oil, 28% total dietary fibre, 20% protein and 1% lignans. Flaxseed is gluten free, a low GI food ingredient, and is among the richest vegan source of alpha-linolenic acid in the North American diet. Flaxseed dehulling process into several value-added products and functionality of flaxseed dietary fibres as prebiotics will be presented. The results showed that flaxseed dietary fibres are comparable with psyllium fibre, which can promote gut health and enhance the immune systems.

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The International Society for Nutraceuticals & Functional Foods
certifies that

Joaquín Rodrigo-García

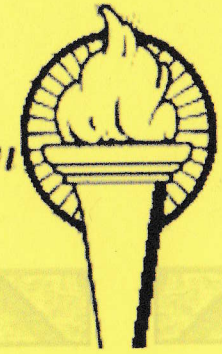
Delivered a presentation entitled:

Impact of elicitors in the metabolism of bioactive compounds and sensory properties of butterhead lettuce

at the:

2018 *Annual Conference & Exhibition*

Vancouver Airport Sheraton
October 14-17, 2018



J. Shahidi

Fereidoon Shahidi
Conference Organizer

IMPACT OF DIFFERENT ELICITORS ON BIOACTIVE COMPOUNDS AND SENSORY PROPERTIES IN HYDROPONIC BUTTERHEAD LETTUCES (*LACTUCA SATIVA* VAR CAPITATA)

Dr Joaquin Rodrigo-Garcia

Dr Jesus Omar Moreno Escamilla
Dr Emilio Alvarez Parrilla
Dra Laura A de la Rosa
Dr. Gustavo González Aguilar
Dra Nina del Rocio Martinez Ruiz

CONACYT

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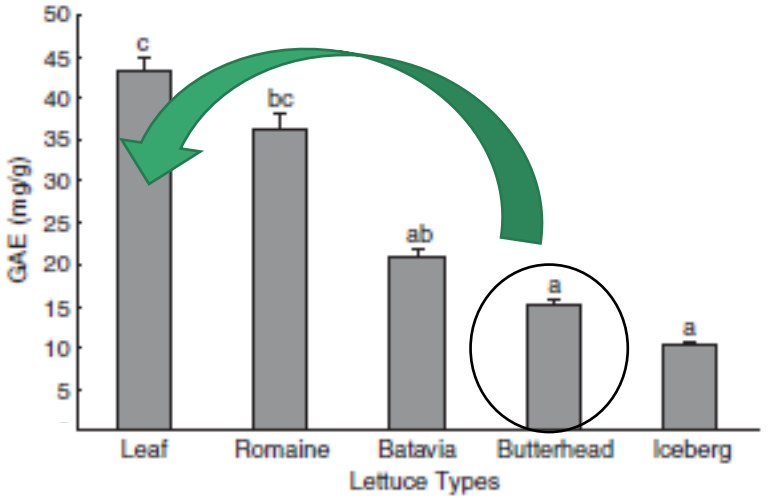
Highlights

Lettuce

Production 23 millions global

Main ingredient in salads

Butterhead lettuce= soft texture and most appreciated sensory properties by consumers

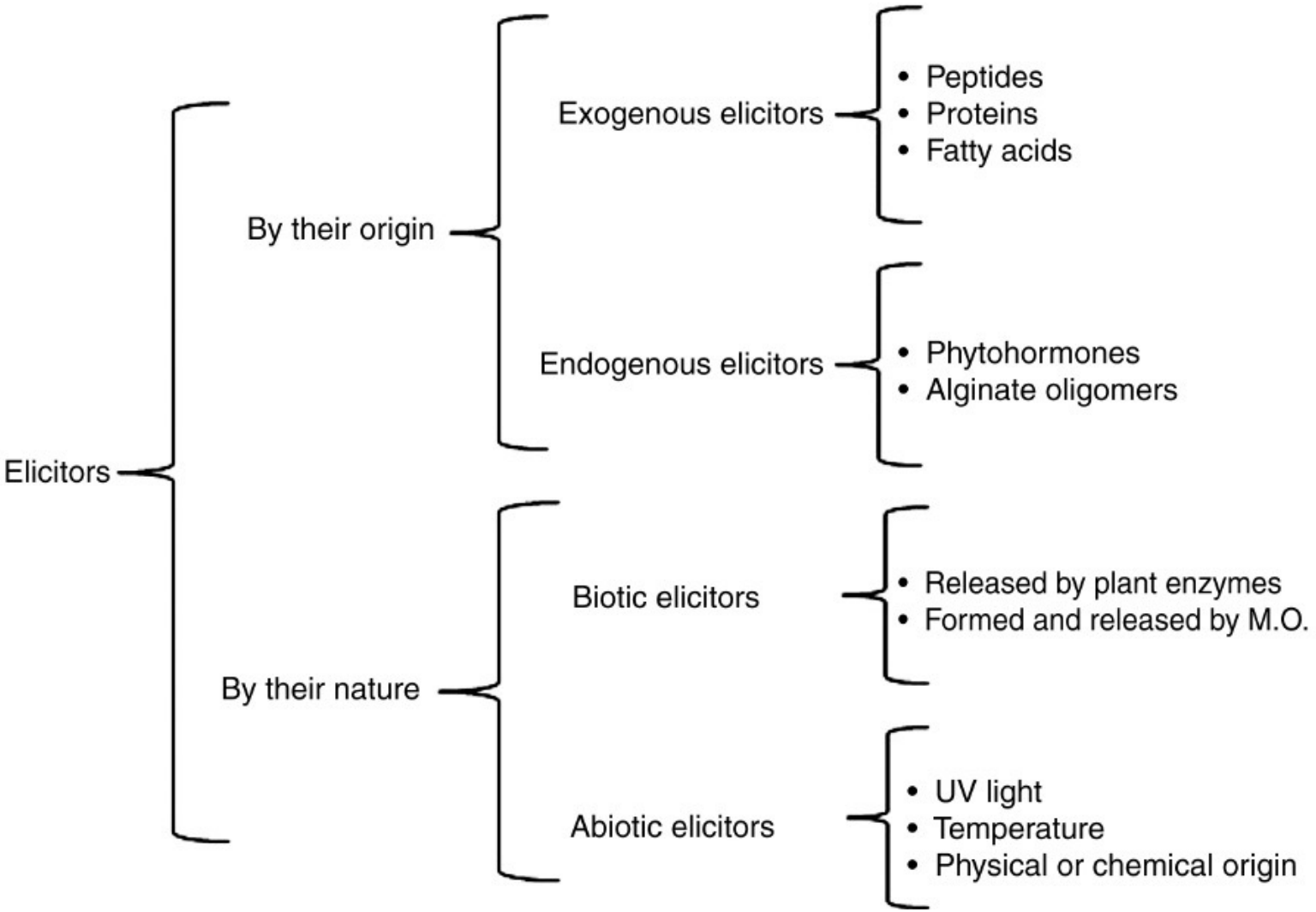


Genetic modified

Elicitors

(Yosof, 2015; FAO, 2010; Moreno-Escamilla *et al., 2018;* Liu *et al*., 2007; Llorach *et al*., 2008; Chu *et al*., 2002; Arzu *et al*., 2009; Zlotek et al., 2014)

Introduction



(Schreiner *et al*, 2005; Ramakrishna & Ravishankar, 2011; Moreno Escamilla et al., 2018)

Introduction

- Efeccts reported of some elicitors

Elicitor	Plant response
Salicilyc acid	↑ Flavonoids (Perez-Balibrea, 2011) ↑ Phenolic, PAL activity (Jian-ye, 2006)
Metil jasmonate	↑Antocianins (Saavedra, 2016) ↑ Flavonoids (Perez-Balibrea, 2011)
Harpin protein	↑ Ascorbic acid. Antioxidant enzymes (Bestwick, 2011) ↑ Phenolic content antioxidant activity, PAL expression gene (Navarrete, 2014)
Arachidonic acid	↑ Phenolic, Ascobic acid, Antioxidant activity (Zlotek, 2014; Zlotek, 2011)
Hydric stress	↑ PAL, ɣ-TMT gene expression (Oh, 2009)
Temperature	↑ Antocianins, Flavonoids y Ascorbic acid. Antioxidant activity (Boo, 2011)

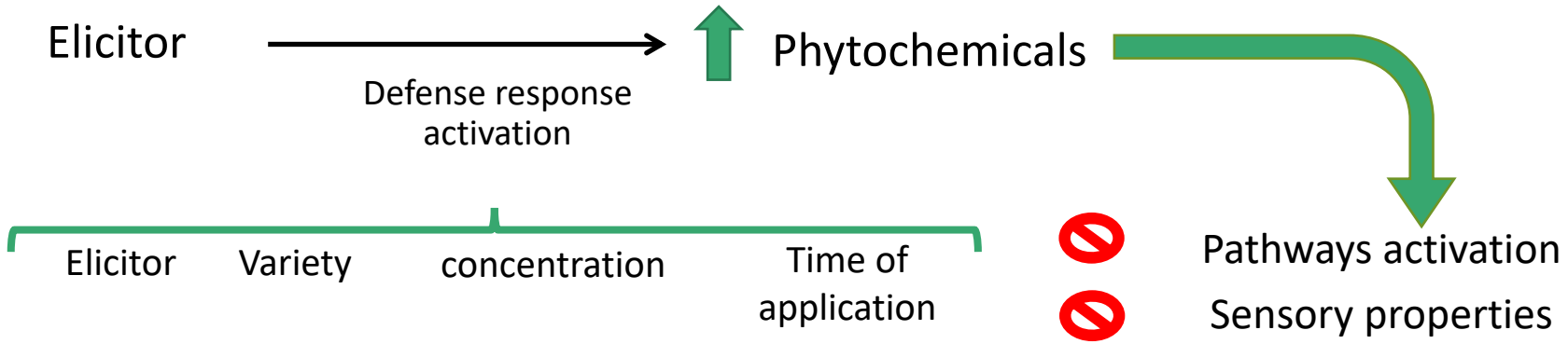
Introduction

Preharvest Modulation of Postharvest Fruit and Vegetable Quality
2018, Pages 71–91

CHAPTER 3

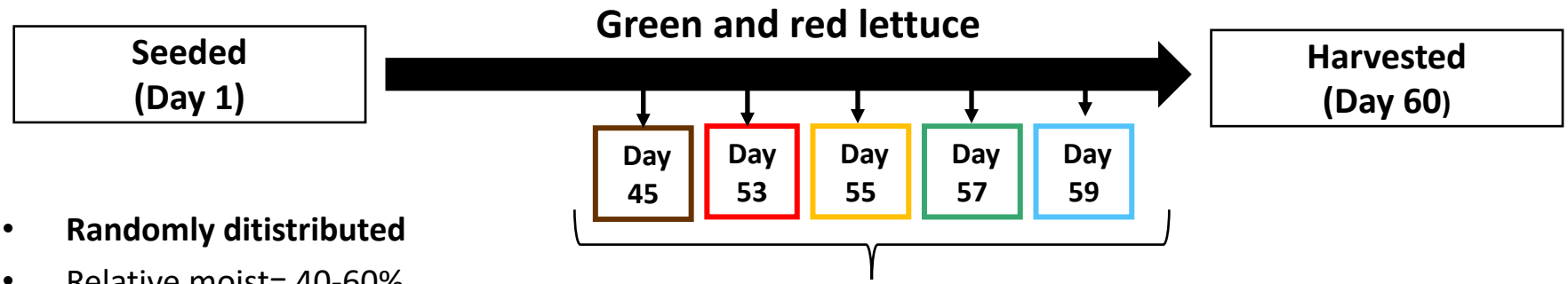
Effect of Elicitors in the Nutritional and Sensorial Quality of Fruits and Vegetables

Jesús O. Moreno-Escamilla*, Emilio Alvarez-Parrilla*, Laura A. de la Rosa*, José A. Núñez-Gastélum*, Gustavo A. González-Aguilar**, Joaquín Rodrigo-García*



(Yosof, 2015; FAO, 2010; Moreno-Escamilla *et al., 2018;* Liu *et al*., 2007; Llorach *et al*., 2008; Chu *et al*., 2002; Arzu *et al*., 2009; Zlotek et al., 2014)

Selection day of application of elicitor



- **Randomly ditistributed**
- Relative moist= 40-60%
- Temperature= 25°C

Green Butterhead lettuce

Red Butterhead lettuce

- Control
- Ácido araquidónico (90 µM)
- Metil jasmonato (90 µM)
- Ácido salicílico (90 µM)
- Proteína Harpin (120 mg/L)

- **Phenolic compounds**
- **Total flavonoids**
- **Ascorbic acid**
- **Carotenoids**
- **Antocianins**
- **Antioxidant activity**

(Kováčik et al., 2009; Zhu & Zhang, 2016; Złotek et al., 2014)

COLORIMETRIC ANALYSIS

1. Fenolic compounds (Folin-Ciocalteu)
2. Flavonoids ($AlCl_3$)
3. Ascorbic acid (DNPH)
4. Total carotenoids (Acetona)
5. Anthocyanins (pH)
6. Antioxidant capacity (DPPH)
7. Antioxidant capacity (FRAP)
8. Antioxidant capacity (ABTS)

CHROMATOGRAPHIC ANALYSIS

- **HPLC-ESI-QTOF-MS**

- Bomba cuaternaria
- Ionización por electrospray (ESI)
- Ionización negativa
- (A) water (0.1% formic acid)
- (B) Acetonitrile
  - 0–4 min, 90% A,
  - 4–6 min, 70% A,
  - 6–8 min, 62% A,
  - 8–8.5 min, 40%A
  - 8.5–9.5 min, 90% A.
- 0.4 mL/min
- Mass spectra was measure from m/z 100 to m/z 1700.

Table 1. Phytochemical content related to application day of elicitor in butterhead lettuce samples.

Treatment		Total Phenolics (mg EAG /g*)	Flavonoids (mg EC/g*)	Ascorbic acid (mg ASC/g*)	Carotenoids (mg β-carotene/g*)
Day PH 15	AA	37.61 ± 5.44^{a}	21.39 ± 1.04^{a}	3.58 ± 0.19^{b}	3.38 ± 0.36^{a}
	AS	32.41 ± 4.33^{a}	18.54 ± 2.15^{a}	3.85 ± 0.12^{ab}	2.82 ± 0.10^{a}
	MJ	33.41 ± 3.70^{a}	21.69 ± 3.94^{a}	3.67 ± 0.04^{ab}	2.88 ± 0.60^{a}
	PH	33.37 ± 1.77^{a}	22.16 ± 2.72^{a}	3.98 ± 0.11^{a}	3.26 ± 0.47^{a}
	C	35.57 ± 1.99^{a}	22.92 ± 2.24^{a}	3.47 ± 0.19^{b}	3.08 ± 0.38^{a}
Day PH 7	AA	**37.74 ± 1.91^{a}**	**21.25 ± 0.80^{b}**	3.67 ± 0.28^{ab}	**3.19 ± 0.30^{ab}**
	AS	**35.91 ± 1.81^{a}**	**21.01 ± 0.38^{b}**	3.45 ± 0.27^{ab}	**3.52 ± 0.11^{a}**
	MJ	**31.96 ± 1.56^{b}**	**19.09 ± 2.80^{bc}**	3.03 ± 0.25^{b}	**3.22 ± 0.19^{ab}**
	PH	**35.41 ± 1.21^{ab}**	**36.66 ± 1.71^{a}**	4.13 ± 0.25^{a}	**2.49 ± 0.35^{b}**
	C	**32.04 ± 1.31^{b}**	**14.62 ± 2.23^{c}**	4.03 ± 0.28^{a}	**3.02 ± 0.15^{ab}**
Day PH 5	AA	34.09 ± 1.10^{a}	38.36 ± 0.52^{a}	3.35 ± 0.08^{b}	3.34 ± 0.44^{a}
	AS	31.81 ± 0.87^{a}	19.63 ± 2.03^{c}	3.81 ± 0.16^{ab}	2.61 ± 0.21^{a}
	MJ	31.65 ± 1.48^{ab}	26.28 ± 1.66^{b}	4.06 ± 0.14^{a}	2.94 ± 0.28^{a}
	PH	31.24 ± 0.80^{b}	28.68 ± 2.01^{b}	3.98 ± 0.25^{a}	2.87 ± 0.55^{a}
	C	30.57 ± 0.38^{b}	17.82 ± 0.48^{c}	4.03 ± 0.27^{a}	2.83 ± 0.15^{a}
Day PH 3	AA	34.84 ± 0.72^{a}	31.26 ± 2.88^{a}	3.72 ± 0.23^{b}	2.62 ± 0.19^{a}
	AS	33.26 ± 2.11^{a}	16.67 ± 0.50^{c}	3.95 ± 0.32^{b}	2.56 ± 0.26^{a}
	MJ	31.41 ± 3.02^{a}	15.96 ± 1.52^{c}	3.95 ± 0.09^{b}	2.57 ± 0.34^{a}
	PH	34.08 ± 2.38^{a}	19.38 ± 0.73^{c}	4.32 ± 0.42^{ab}	3.22 ± 0.19^{a}
	C	33.77 ± 2.73^{a}	25.91 ± 2.36^{b}	4.89 ± 0.22^{a}	2.93 ± 0.45^{a}
Day PH 1	AA	32.31 ± 2.53^{a}	34.79 ± 4.09^{a}	3.42 ± 0.13^{c}	3.05 ± 0.67^{a}
	AS	32.21 ± 0.22^{a}	19.22 ± 1.58^{b}	3.60 ± 0.09^{bc}	2.31 ± 0.17^{a}
	MJ	31.02 ± 2.89^{a}	30.79 ± 0.43^{a}	4.34 ± 0.34^{ab}	2.49 ± 0.16^{a}
	PH	29.66 ± 0.12^{a}	18.49 ± 2.36^{b}	3.89 ± 0.08^{abc}	2.99 ± 0.47^{a}
	C	31.51 ± 1.95^{a}	26.82 ± 5.53^{ab}	4.47 ± 0.53^{a}	2.35 ± 0.39^{a}

Los valores representan la media ± DE de tres repeticiones. AA, ácido araquidónico; AS, ácido salicílico; ASC, ácido ascórbico; EAG, equivalentes de ácido gálico; EC, equivalentes de catequina; MJ, metil jasmonato; PH, proteína Harpin; C, control; PC, precosecha; * g de muestra PS. Letras diferentes indica la diferencia entre el tratamiento y el control (rociado de agua destilada) en el mismo día de aplicación previo a la cosecha ($p<0.05$).

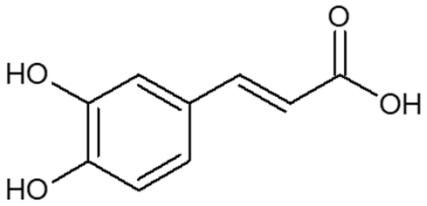
Table 2. Phytochemicals content related to application day of elicitor in red Butterhead lettuce samples.

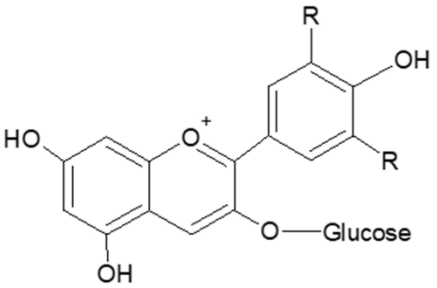
Treatment		Total phenolics (mg EAG /g*)	Flavonoids (mg EC/g*)	Antocianins#	Ascorbic acido (mg ASC/g*)	Carotenoids (mg β-carotene/g*)
Day PH 15	AA	**53.34 ± 2.12^{a}**	**36.56 ± 1.16^{a}**	**3.85 ± 0.22^{a}**	6.05 ± 0.26^{a}	**6.16 ± 0.17^{a}**
	AS	**51.17 ± 0.88^{a}**	**38.26 ± 3.94^{a}**	**4.99 ± 0.19^{a}**	5.59 ± 0.35^{ab}	**6.15 ± 0.25^{a}**
	MJ	**44.81 ± 3.31^{bc}**	**31.10 ± 0.94^{b}**	**2.57 ± 0.30^{b}**	5.72 ± 0.92^{ab}	**6.91 ± 0.64^{a}**
	PH	**49.70 ± 2.11^{ab}**	**33.96 ± 0.04^{ab}**	**3.25 ± 0.25^{b}**	4.90 ± 0.33^{ab}	**6.73 ± 0.59^{a}**
	C	**39.89 ± 2.68^{c}**	**30.64 ± 1.65^{b}**	**2.95 ± 0.18^{b}**	4.36 ± 0.39^{b}	**5.59 ± 0.06^{b}**
Day PH 7	AA	42.19 ± 2.01^{a}	33.88 ± 1.81^{a}	4.07 ± 0.28^{b}	5.26 ± 0.58^{a}	6.88 ± 0.11^{a}
	AS	44.08 ± 1.19^{a}	34.63 ± 3.95^{a}	4.07 ± 0.58^{b}	5.43 ± 0.27^{a}	6.35 ± 0.36^{a}
	MJ	46.51 ± 3.82^{a}	33.89 ± 3.00^{a}	4.09 ± 0.55^{b}	5.20 ± 0.58^{a}	7.18 ± 0.49^{a}
	PH	47.98 ± 1.88^{a}	36.34 ± 1.56^{a}	5.27 ± 0.27^{a}	5.34 ± 0.29^{a}	6.80 ± 0.25^{a}
	C	46.05 ± 4.14^{a}	36.15 ± 5.67^{a}	4.27 ± 0.25^{ab}	6.11 ± 0.42^{a}	6.38 ± 0.25^{a}
Day PH 5	AA	48.23 ± 2.36^{a}	38.98 ± 1.70^{a}	3.84 ± 0.13^{ab}	4.54 ± 0.40^{b}	7.71 ± 0.11^{a}
	AS	46.24 ± 1.69^{a}	40.98 ± 5.06^{a}	4.66 ± 0.44^{a}	6.10 ± 0.15^{a}	7.54 ± 0.39^{a}
	MJ	44.54 ± 0.75^{a}	41.14 ± 2.94^{a}	2.75 ± 0.33^{b}	4.60 ± 0.45^{b}	6.60 ± 0.57^{a}
	PH	36.65 ± 3.92^{ab}	34.48 ± 0.74^{a}	3.12 ± 0.71^{b}	4.52 ± 0.23^{b}	6.61 ± 0.59^{a}
	C	42.37 ± 4.00^{a}	41.97 ± 2.02^{a}	3.06 ± 0.23^{b}	5.02 ± 0.19^{b}	7.07 ± 0.06^{a}
Day PH 3	AA	44.25 ± 2.10^{a}	37.03 ± 4.18^{a}	3.61 ± 0.28^{a}	5.38 ± 0.18^{b}	5.89 ± 0.62^{ab}
	AS	45.83 ± 1.54^{a}	41.21 ± 1.88^{a}	3.82 ± 0.15^{a}	5.10 ± 0.31^{b}	6.71 ± 0.37^{a}
	MJ	44.71 ± 2.22^{a}	36.31 ± 4.59^{a}	4.41 ± 0.48^{a}	6.65 ± 0.53^{a}	4.97 ± 0.40^{b}
	PH	40.94 ± 3.88^{a}	37.46 ± 2.60^{a}	3.71 ± 0.73^{a}	5.50 ± 0.47^{ab}	5.47 ± 0.50^{ab}
	C	42.11 ± 3.69^{a}	37.22 ±1.37^{a}	4.27 ± 0.20^{a}	5.12 ± 0.55^{b}	6.75 ± 0.57^{a}
Day PH 1	AA	45.07 ± 3.70^{a}	45.48 ± 3.05^{a}	4.09 ± 0.08^{b}	5.53 ± 0.32^{b}	5.42 ± 0.16^{b}
	AS	45.21 ± 8.02^{a}	35.45 ± 2.14^{b}	3.91 ± 0.69^{b}	6.89 ± 0.21^{a}	5.65 ± 0.35^{ab}
	MJ	48.58 ± 7.24^{a}	51.69 ± 3.51^{a}	4.85 ± 0.35^{ab}	5.94 ± 0.30^{ab}	6.46 ± 0.30^{a}
	PH	48.97 ± 4.26^{a}	51.80 ± 5.30^{a}	5.91 ± 0.63^{a}	7.08 ± 0.79^{a}	5.86 ± 0.46^{ab}
	C	44.21 ± 4.35^{a}	43.65 ± 2.14^{ab}	4.46 ± 0.65^{b}	5.18 ± 0.46^{b}	6.24 ± 0.33^{ab}

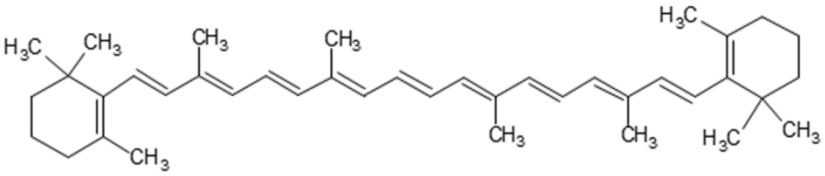
Los valores representan la media ± DE de tres repeticiones. AA, ácido araquidónico; AS, ácido salicílico; ASC, ácido ascórbico; EAG, equivalentes de ácido gálico; EC, equivalentes de catequina; MJ, metil jasmonato; PH, proteína Harpin; C, control; PC, precosecha; * g de muestra PS. # mg de cianidin 3-rutinosido/g de PS. Letras diferentes indica la diferencia entre el tratamiento y el control (rociado de agua destilada) en el mismo día de aplicación previo a la cosecha ($p<0.05$).

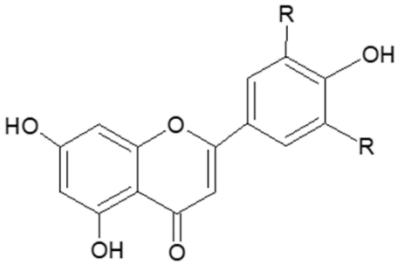
HIGHER IMPACT

PHENOLIC COMPOUNDS
CAROTENOIDS









HIGHER IMPACT OF ELICITORS ON PREHARVEST DAY 7 FOR GREEN LETTUCE SAMPLES

HIGHER IMPACT OF ELICITORS ON PREHARVEST DAY 15 FOR RED LETTUCE SAMPLES

WOULD IT BE REFLECTED ON ANTIOXIDANT ACTIVITY?

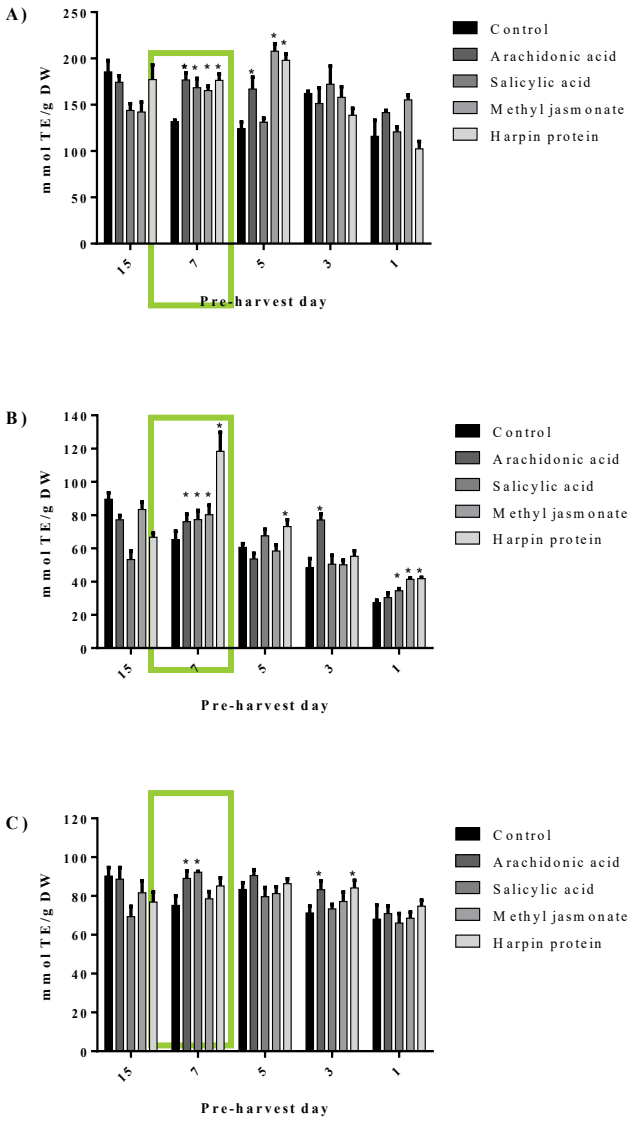


Table 3. Correlations between phytochemicals and antioxidant capacity for green lettuce on preharvest day 7.

Variables	TPC	TF	ASC	CAR	TEAC	DPPH	FRAP
CFT	**1**	0.410	0.189	0.061	0.714	0.251	**0.893**
FT	0.410	**1**	0.418	-0.724	0.649	**0.984**	0.363
ASC	0.189	0.418	**1**	-0.707	-0.234	0.360	-0.077
CAR	0.061	-0.724	-0.707	**1**	-0.063	-0.762	0.302
TEAC	0.714	0.649	-0.234	-0.063	**1**	0.591	0.745
DPPH	0.251	**0.984**	0.360	-0.762	0.591	**1**	0.227
FRAP	**0.893**	0.363	-0.077	0.302	0.745	0.227	**1**

Los valores en negrita son diferentes de 0 con un nivel de significación alfa=0.05

HIGH CORRELATION BETWEEN PHENOLIC CONTENT AND DPPH, FRAP

SAME BEHAVIOUR BETWEEN TOTAL FLAVONOIDS AND DPPH

Figure 1. Antioxidant capacity for green butterhead lettuces after elicitors treatment. ABTS (A), DPPH (B) and FRAP (C). * means significance differences between treated sample and control.

Table 4. Correlations between phytochemicals and antioxidant capacity for red lettuce on preharvest day 15.

Variables	TPC	TF	ASC	CAR	ANT	TEAC	DPPH	FRAP
CFT	**1**	**0.884**	0.715	0.294	0.719	**0.881**	0.774	0.259
FT	**0.884**	**1**	0.564	-0.055	**0.940**	0.748	0.692	0.650
ASC	0.715	0.564	**1**	0.420	0.278	0.669	0.723	0.077
CAR	0.294	-0.055	0.420	**1**	-0.178	0.004	0.639	-0.300
ANT	0.719	**0.940**	0.278	-0.178	**1**	0.523	0.589	0.810
TEAC	**0.881**	0.748	0.669	0.004	0.523	**1**	0.421	0.009
DPPH	0.774	0.692	0.723	0.639	0.589	0.421	**1**	0.424
FRAP	0.259	0.650	0.077	-0.300	0.810	0.009	0.424	**1**

Los valores en negrita son diferentes de 0 con un nivel de significación alfa=0.05

HIGH CORRELATION BETWEEN PHENOLIC CONTENT AND TEAC

ANTIOXIDANT CAPACITY EXPLAINED BY PHENOLIC CONTENT

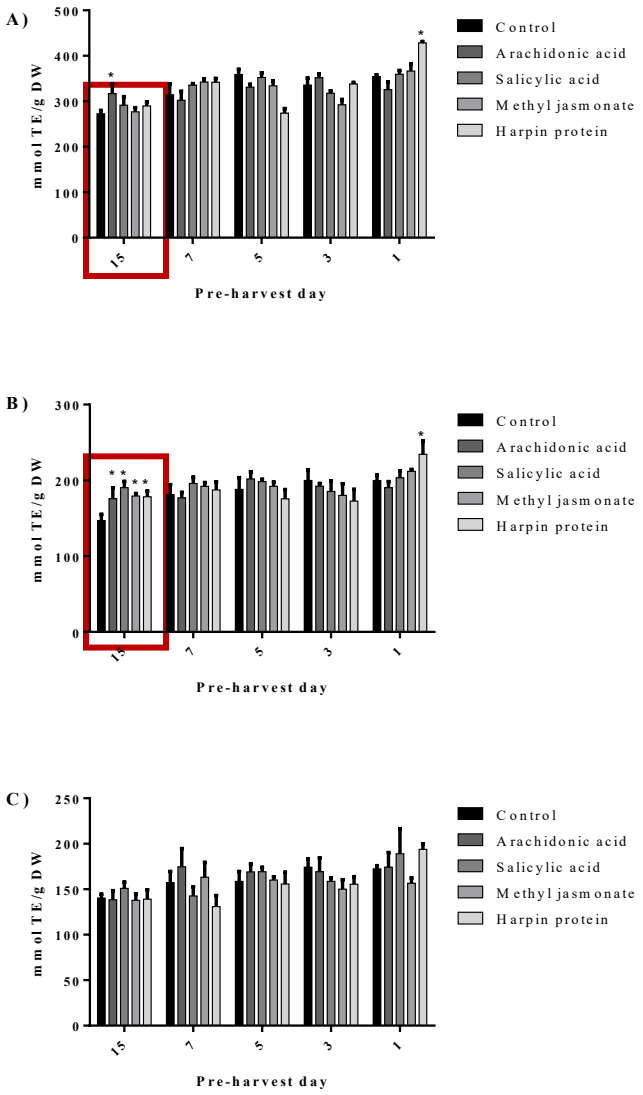


Figure 2. Antioxidant activity for red butterhead lettuce after elicitor treatment. ABTS (A), DPPH (B) and FRAP (C). * means significant differences between treated samples and control.

Phenolic compounds profile

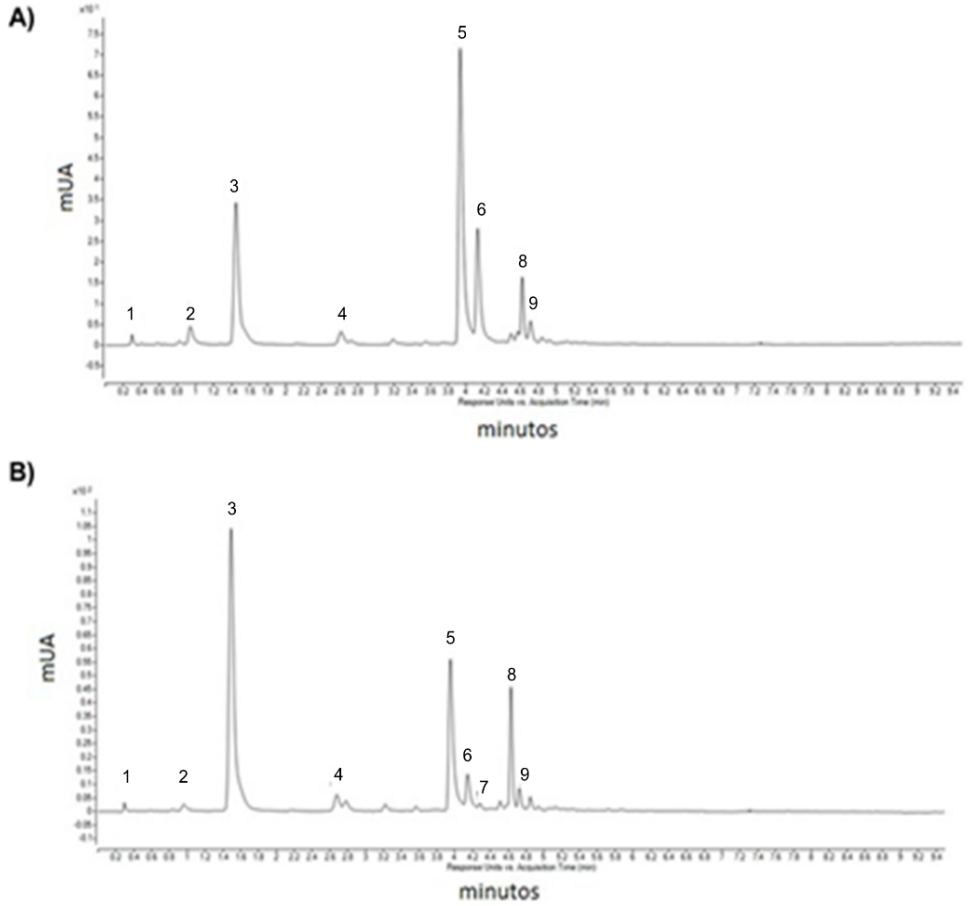


Figure 2. Chromatograph (HPLC-MS) of phenolic compounds detected at 320 nm in butterhead lettuces control green (A) and red (B).

Phenolic compounds profile

Table 5. Phenolic profile identified in butterhead lettuce treated with elicitors

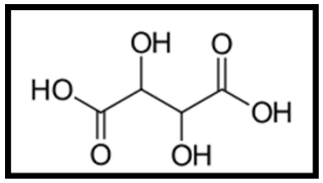
Peak	t_R (min)	$[M-H]^-$	Proposed molecule	Green lettuce				Red lettuce			
				AA	AS	MJ	PH	AA	AS	MJ	PH
1	0.34	133.05	Not identified	-3.5	5.9	-5.9	11.8	150.0	155.6	150.0	163.9
2	1.02	311.11	Caffeoyl tartaric acid	7.5	42.5	-10.0	12.5	-30.4	13.0	-4.3	-4.3
3	1.49	353.16	Chlorogenic acid	-9.1	54.5	-47.3	-9.1	-7.1	28.6	-7.1	14.3
4	2.67	353.16	Chlorogenic acid isomer	-42.9	-28.6	-57.1	-52.4	-22.5	-5.0	-34.0	-5.0
5	4.00	473.11	Chicoric acid	18.2	-9.1	0.0	36.4	0.0	76.5	41.2	29.4
6	4.15	473.11	Meso dicaffeoyl tartaric acid	-28.6	-21.4	-7.1	-28.6	-31.4	0.0	-8.6	-14.3
7	4.35	461.1	Luteolin-7-glucoside	nd	nd	nd	nd	20.0	50.0	0.0	10.0
8	4.60	505.2	Not identified	-20.0	26.7	-20.0	33.3	12.5	37.5	0.0	-12.5
9	4.70	515.22	Isochlorogenic acid	40.0	137.5	0.0	50.0	0.0	55.6	-5.6	22.2

*Aplicación precosecha del inductor: verde 7 días y roja 15 días. Valores negativos representan una disminución respecto al control (l)

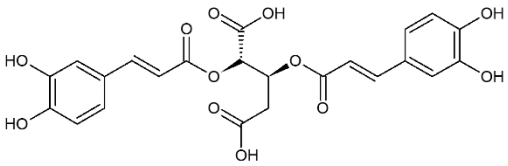
HIGH PRESENCE OF CHLOROGENIC ACID DERIVADED

PRESENCE OF LUTEOLIN IN RED LETTUCES

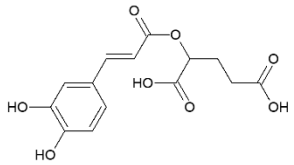
Llorach et al., 2008, Abu-Reidah et el., 2013)



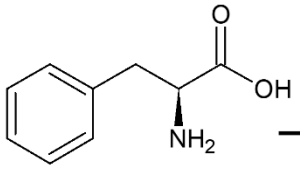
Ácido Tartárico



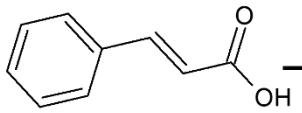
Ácido Chicórico



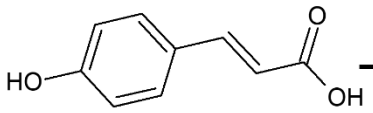
Ácido Cafeoiltratárico



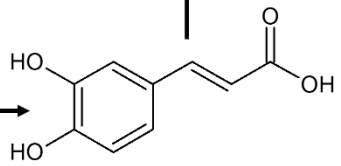
Fenilalanina



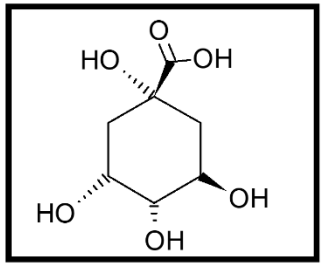
Ácido *Trans*-cinámico



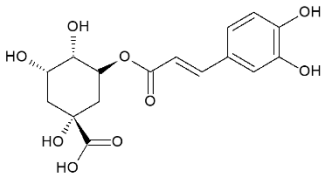
Ácido *p*-cumárico



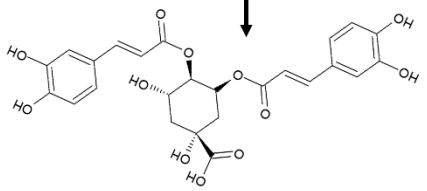
Ácido Caféico



Ácido Quínico



Ácido Clorogénico



Ácido Isoclorogénico

(Tómas-Barberan et al., 1997)

Effect of elicitor concentration

- Seeded, elicitor application and harvest of lettuce

- Random design
- Relative humidity = 40-60%
- Temperature= 25°C

Green Butterhead lettuce
(Pre-harvest day 7)

Red Butterhead lettuce
(Pre-harvest day 14)

- Arachidonic acid
- Salicylic acid
- Methyl jasmonate

(15, 45 and 90 µM)

- Harpin protein

(30, 60 and 120 mg/L)

- Water
- Control

Effect of elicitor in lettuce

Lettuce per group= 5

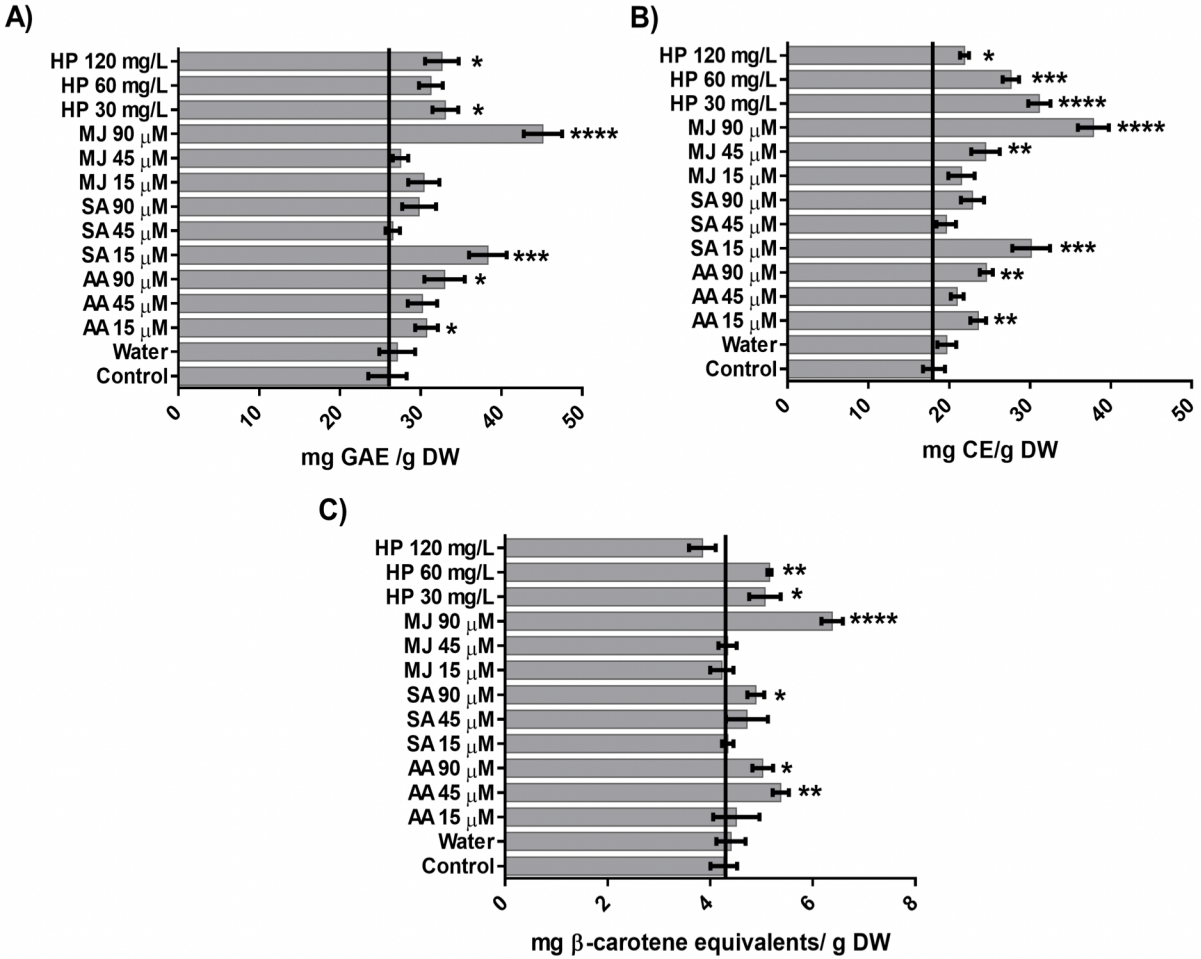
COLORIMETRIC ANALYSIS

1. Phenolic compounds(Folin-Ciocalteu)
2. Flavonoids ($AlCl^3$)
3. Total carotenoids (Acetone)
4. Anthocyanins (pH)

CHROMATOGRAPHIC ANALYSIS

- **HPLC-ESI-QTOF-MS**
  - Binary pump
  - Ionización por electrospray (ESI)
  - Ionización negativa
  - (A) Water (0.1% formic acid)
  - (B) Methanol (0.1% formic acid)
  - Gradient:
    - 0.00= 10% B
    - 8.00= 100% B
    - 9.50= 100% B
    - 9.60= 10% B
  - 0.4 mL/min
  - Specter was measure from m/z 100 to m/z 2000.

Concentration of Phytochemical



MJ 90 µm ENHANCED CFT, FT, CT

HIGHER EFFECT ON FLAVONOIDS

Figure 3. Effect of elicitors on phytochemical content in green Butterhead lettuce.
Phenolic compounds (A), Total flavonoids (B) and total carotenoids (C). AA: Arachidonic acid; AS: Salicylic acid; MJ: Methyl jasmonate; PH: Harpin protein; EAG: Galic acid equivalent; EC: Cathechin equivalent

PCA ANALYSIS FOR GREEN BUTTERHEAD LETTUCE

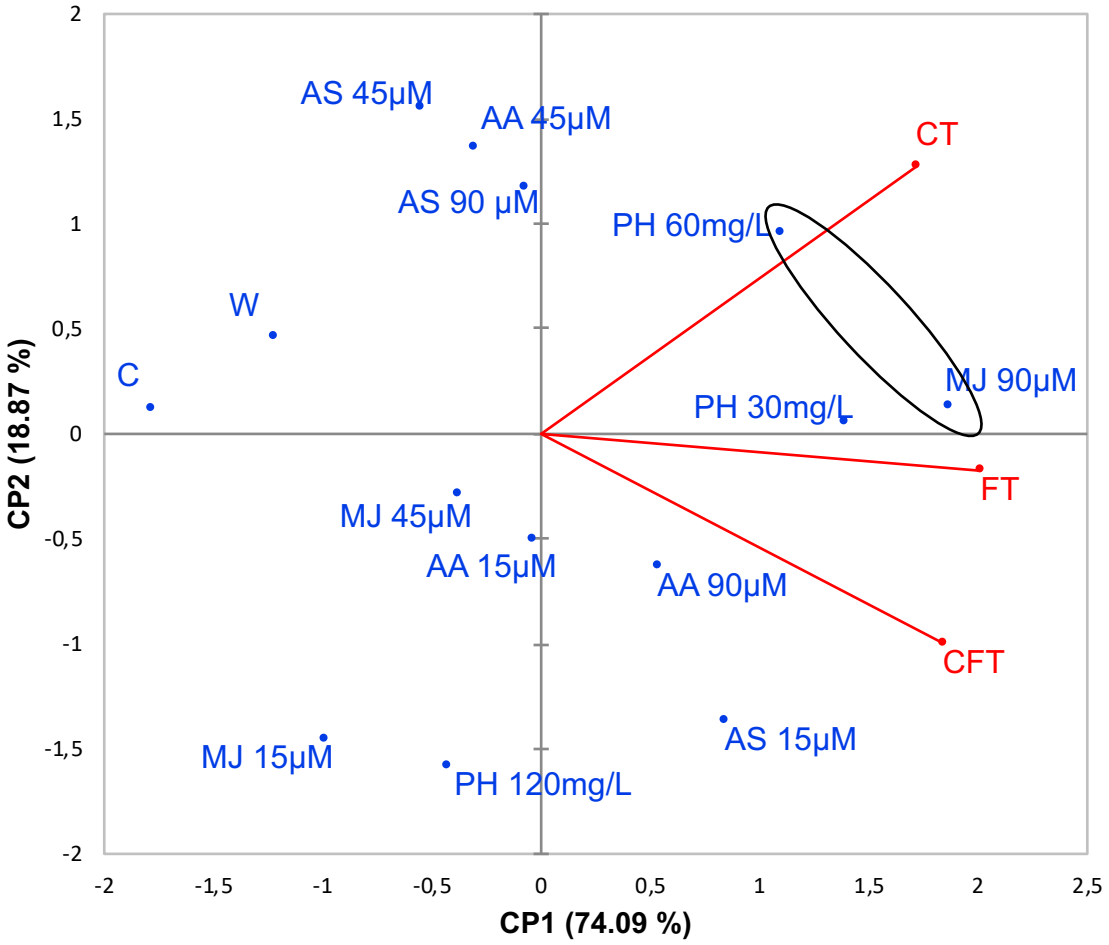


TABLE 6. Proporciones y eje vectores de la varianza explicada por dos componentes principales.

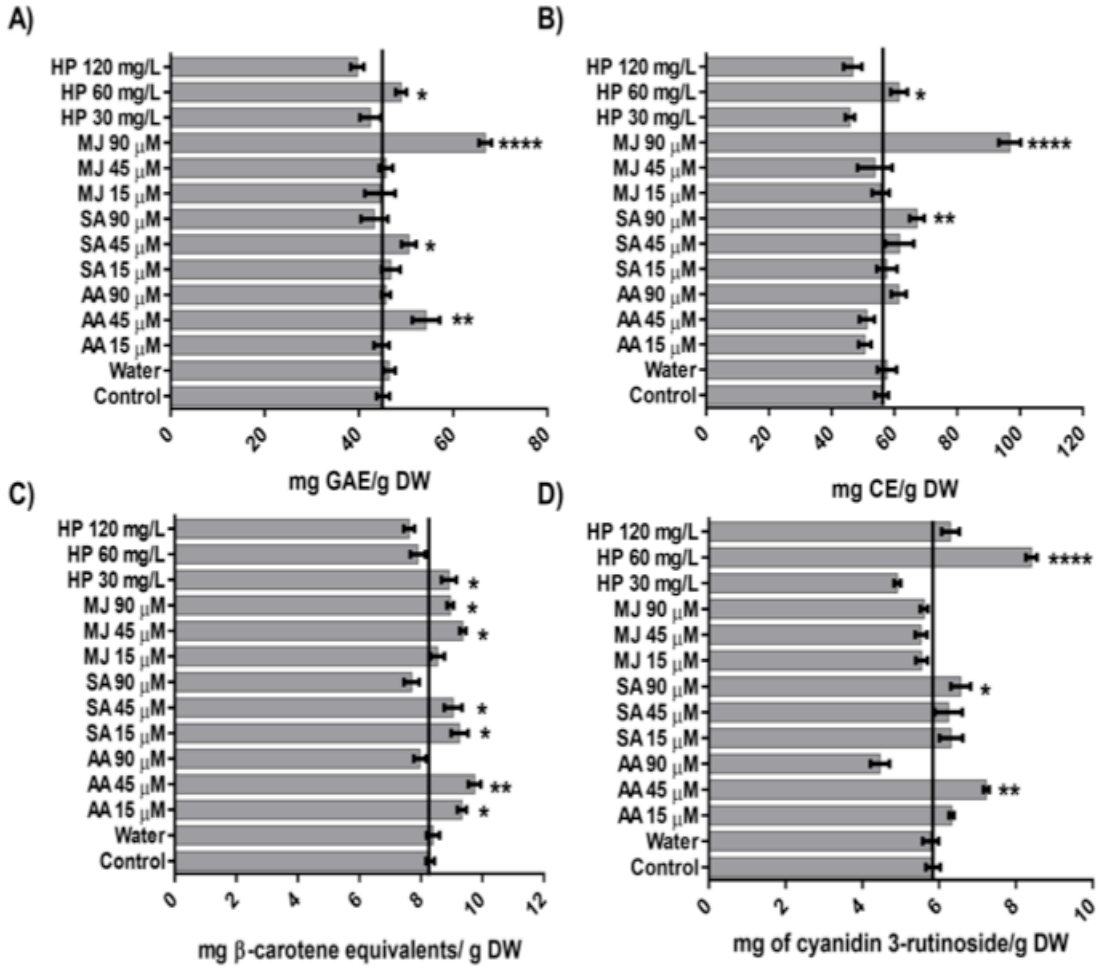
	CP1 (74%)	CP2 (18.8%)
Total phenolics	0.571	-0.613
Flavonoids	**0.625**	-0.108
Carotenoids	0.533	**0.783**

CP1= Hydrophilic compounds
CP2= Lipophilic compounds

PH 60 mg/L MAINLY AFFECT CT

MJ 90 µM IMPACT CFT Y FT

Figure 4. PCA analysis for elicitor effect onto phytochemical in green Butterhead lettuce. AA: Arachidonic acid; AS: Salicylic acid; MJ: Methyl jasmonate; PH: Harpin protein; C: Control; W: water; CFT: Total phenolic compounds; CT: Total carotenoids; FT: Total Flavonoids.



MJ 90 µm ENHANCED CFT, FT, CT

CT WAS RISEN BY AA 45 µm

LOWER RESPONSE THAN GREEN BUTTERHEAD LETTUCES

Figure 5. Effect of elicitors on phytochemical in red Butterhead lettuces.
Total phenolic (A), Total flavonoids (B), Total carotenoids (C) y Total anthocyanins (D). AA: Arachidonic acid; AS: Salicylic acid; MJ: Methyl jasmonate; PH: Harpin protein; EAG: Galic acid equivalent; EC: Catechin equivalent

PCA ANALYSIS FOR RED BUTTERHEAD LETTUCE

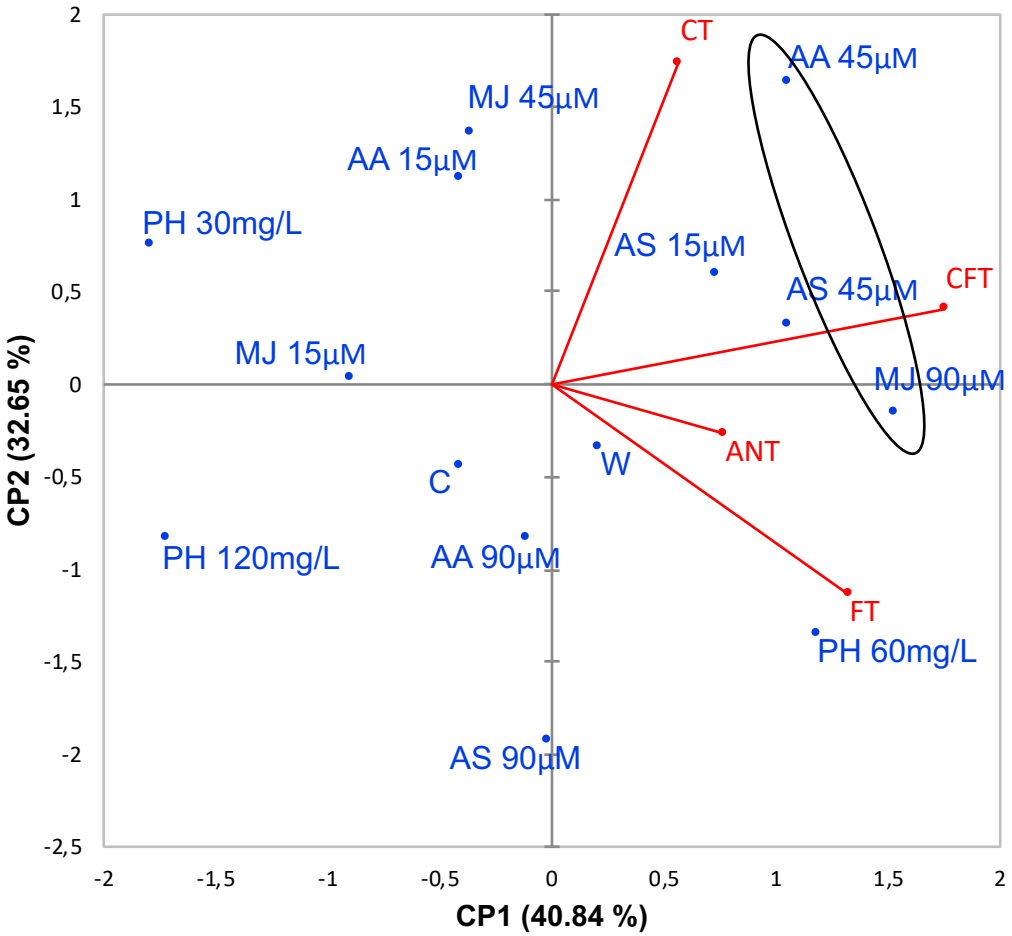


Table 7. Proporciones y eje vectores de la varianza explicada por dos componentes principales.

	CP1 (40.8%)	CP2 (32.6%)	CP3 (23.2%)
Total phenolic	**0.732**	0.190	-0.162
Flavonoids	0.552	-0.530	-0.317
Carotenoids	0.237	**0.817**	-0.026
Anthocyanins	0.321	-0.124	**0.934**

CP1= Hydrophilic compounds
CP2= Lipophylic compounds

AA 45 µM MAINLY ENHANCED CT

MJ 90 µM ENHANCED CFT Y ANT

Figure 6. PCA analysis for elicitor effect onto phytochemical content in red Butterhead lettuce. AA: Arachidonic acid; AS: Salicylic acid; MJ: Methyl jasmonate; PH: Harpin protein; C: Control; W: Water; ANT: Total anthocyanins; CFT: Total phenolic content; CT: Total carotenoids; FT: Total Flavonoids.

Identificación de compuestos fenólicos

A)

B)

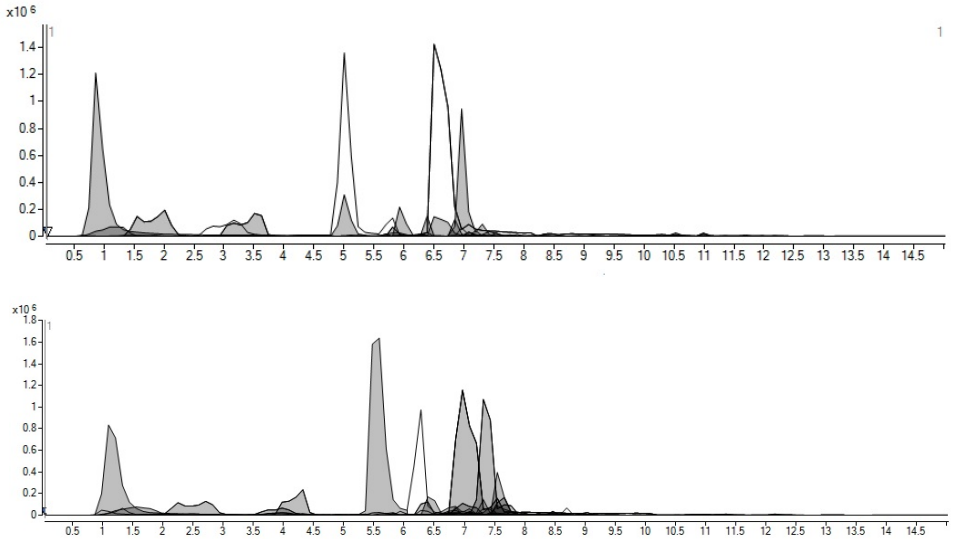


Figura 7. Cromatograma (HPLC-MS) de compuestos fenólicos con detección UV a 320 nm de lechuga mantequilla control verde (A) y roja (B).

ANALISIS SEMICUALIATIVO
MASA EXACTA
100% ABUNDANCIA PICO EN CONTROL

47 COMPUESTOS EN LECHUGA VERDE

58 COMPUESTOS EN LECHUGA ROJA

Cuadro 8. Compuesto fenólicos identificados para cada familia de polifenoles.

	Lechuga verde	Lechuga roja
Antocianinas	**0**	1
Cumarinas	**1**	1
Flavonoles	**9**	18
Flavonas	**0**	1
Flavan-3-oles	**0**	1
Taninos hidrolizables	**1**	**0**
Ac. Hidroxibenzoicos	**11**	**12**
Ac. Hidroxicinámicos	**25**	24

Análisis de componentes principales

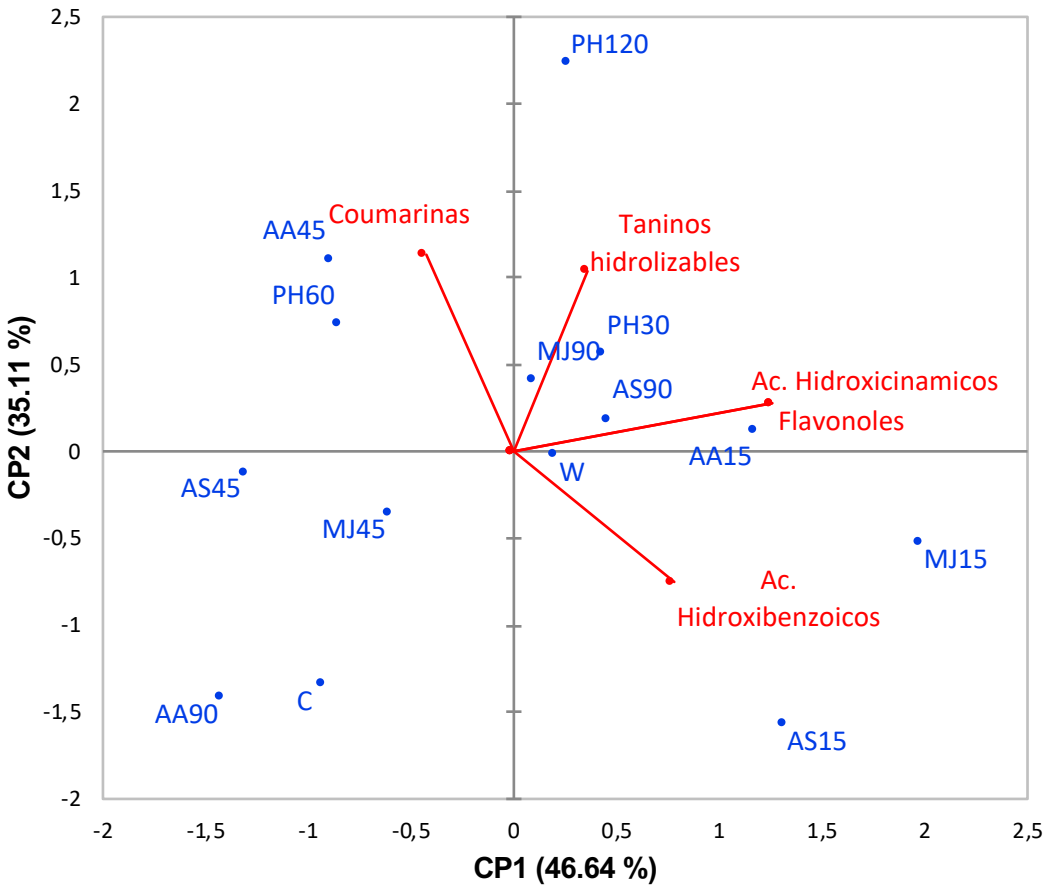


Figura 8. Análisis de componentes principales para evaluar el efecto de inductores de respuesta de estrés sobre el perfil de fenoles en muestras de lechuga roja. AA, Ácido araquidónico; AS, Ácido salicílico; MJ, Metil jasmonato; PH, proteína Harpin; C, Control; W, Agua..

Cuadro 9. Proporciones y eje vectores de la varianza explicada por dos componentes principales.

	CP1 (46.6%)	CP2 (35.1%)
Cumarinas	-0.211	0.646
Flavonoles	**0.622**	0.158
Taninos hidrolizables	0.177	**0.591**
Ac. hidroxibenzoicos	0.386	-0.429
Ac. hidroxicinámicos	**0.622**	0.158

Análisis de componentes principales

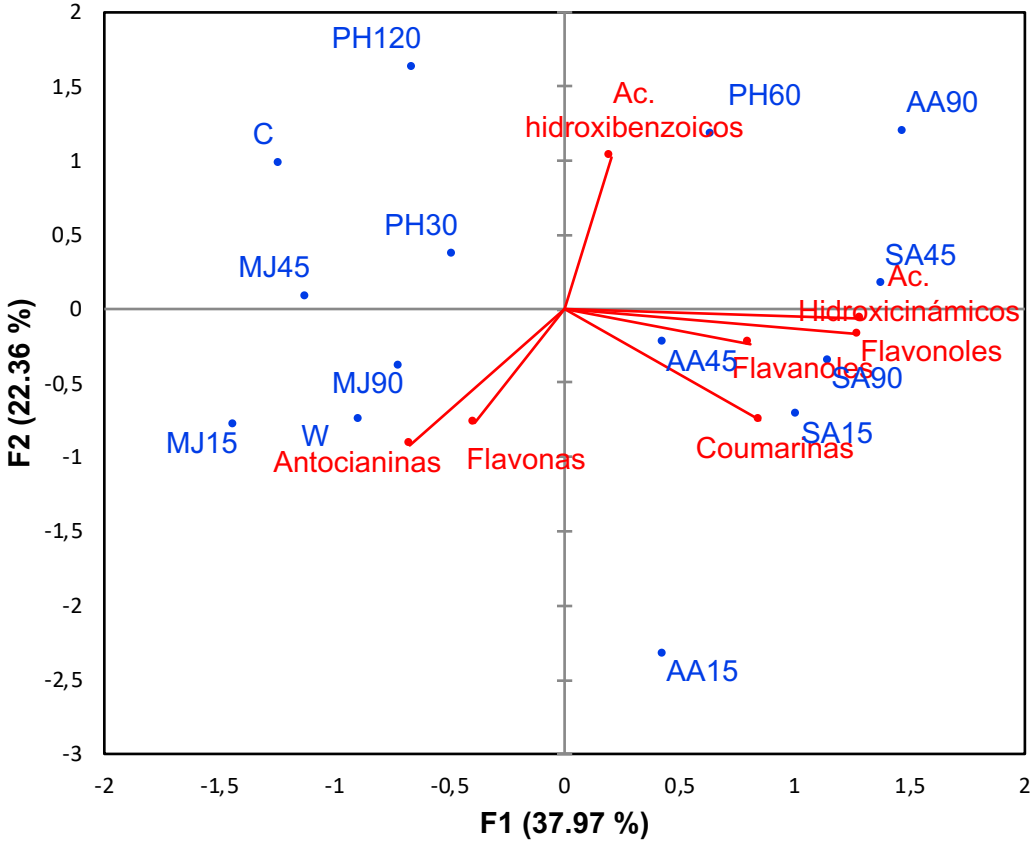


Figura 9. Análisis de componentes principales para evaluar el efecto de inductores de respuesta de estrés sobre compuestos fenólicos en muestras de lechuga roja. AA, Ácido araquidónico; AS, Ácido salicílico; MJ, Metil jasmonato; PH, proteína Harpin; C, Control; W, Agua..

Cuadro 10. Proporciones y eje vectores de la varianza explicada por dos componentes principales.

	CP1 (37.9%)	CP2 (22.3%)	CP3 (17.8%)
Antocianinas	-0.292	-0.520	-0.016
Cumarinas	0.368	-0.424	-0.158
Flavonoles	0.350	-0.134	**0.623**
Flavonas	-0.169	-0.434	-0.555
Flavonoles	**0.553**	-0.096	-0.138
Ac. Hidroxibenzoicos	0.088	**0.577**	-0.442
Ac. Hidroxicinámicos	**0.561**	-0.037	-0.255

Seeded, elicitor application and harvest

Seeded (Day 1) → **Green and red Butterhead** → **Harvest (Day 60)**

Green Butterhead lettuce
(Pre-Harvest day 7)

- Control
- Methyl jasmonate (90 µM)
- Harpin protein (60 mg/L)

Red Butterhead lettuce
(Pre-Harvest day 15)

- Control
- Arachidonic acid(45µM)
- Methyl jasmonate (90 µM)

Effect of elicitors in sensory properties

Phytocemical stability during commercial life (0, 1, 2, y 3 week at 4°C)

- Total phenolic
- Anthocyanins
- Carotenoids

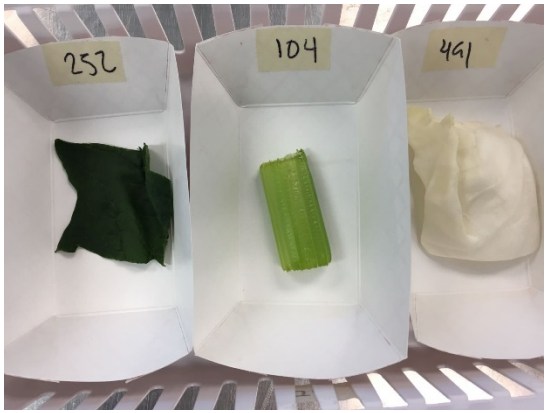
Sensory analysis

- Spectrum method
- 9 trained panelist (Professor from UACJ)

- Atributes
  1. *Odor*
  2. *Colour*
  3. *Sweetness*
  4. *Acidity*
  5. *Bitterness*
  6. *Wettability*
  7. *Crispyness*
  8. *Astringency*
  9. *Freshness*
  10. *Brightness*

- ANOVA

- Lettuce samples



- Standard

Sensory analysis in green Butterhead lettuce

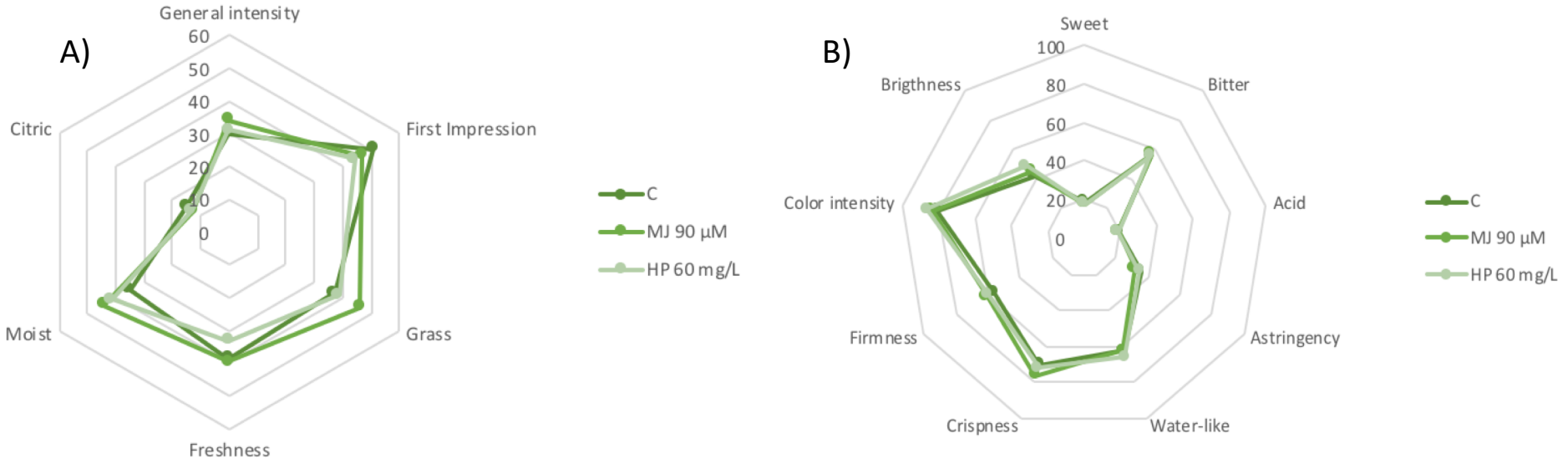


Figure 13. Sensory profile of Green Butterhead lettuce after elicitor treatment. A) Odor and propused atributes B) Flavor, tactile in mouth, tactile, and view sensory attributes (ρ>0.05). MJ (90 µM); PH (60mg/L).

(Park et al,., 2009; Alsadon et al, 1993; Oliveira et al., 2013; Zlotek et al., 2014)

Sensory analysis in red Butterhead lettuce

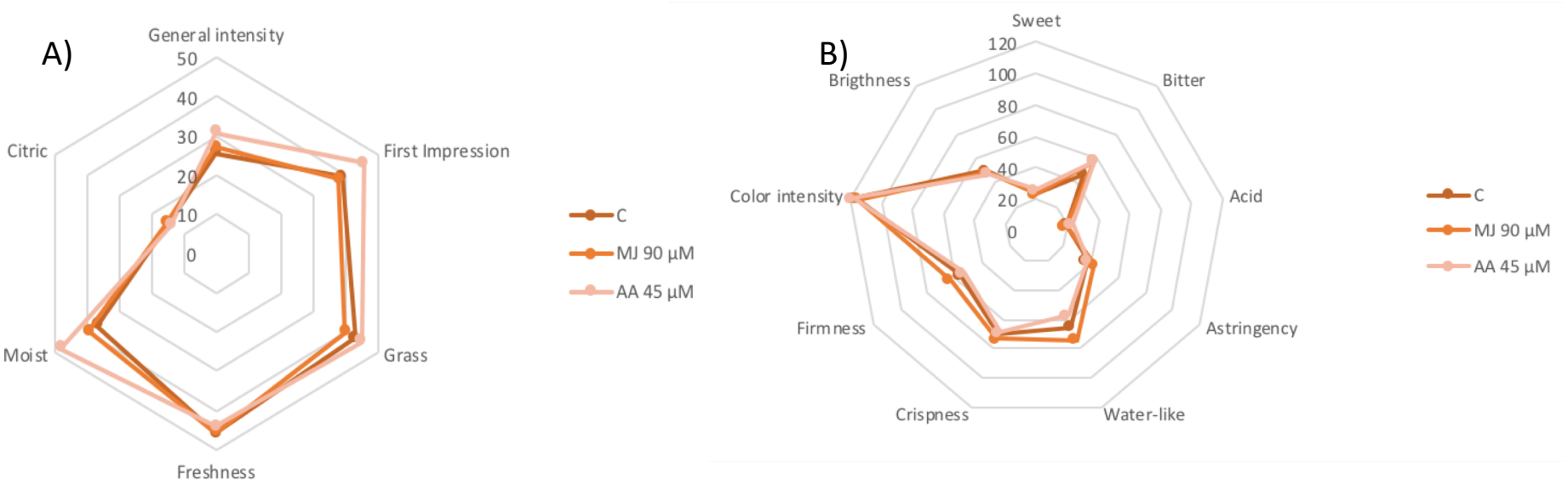
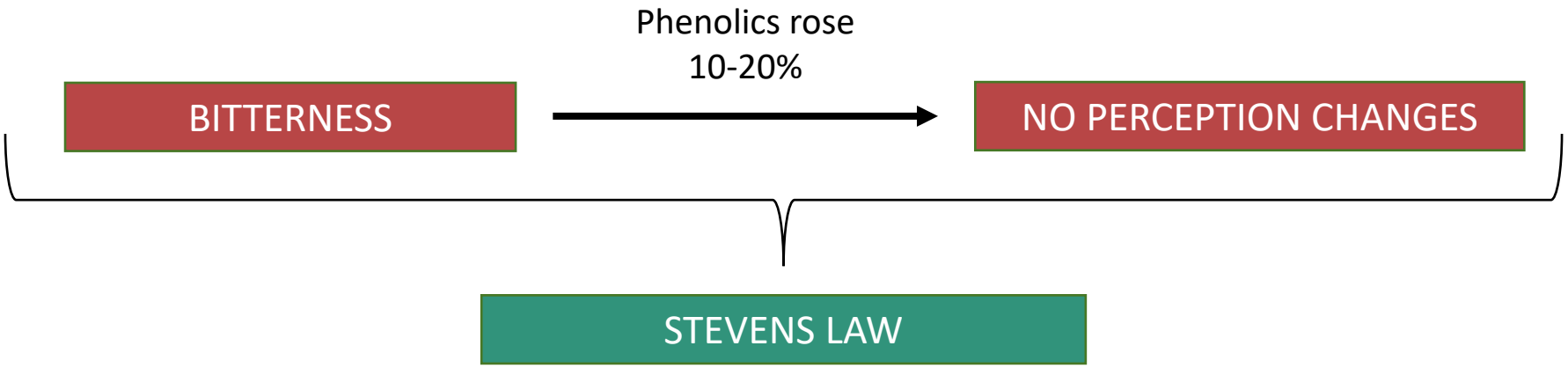


Figure 14. Sensory profile of Red Butterhead lettuce after treatments. Odor and propused attributes (A) and attributes not related with odor(B) ($\rho>0.05$). MJ (90 µM); AA (45 µM).

(Park et al,., 2009; Alsadon et al, 1993; Oliveira et al., 2013; Zlotek et al., 2014)

- Main attributes

BITTERNESS
WATERLIKE
CRISPYNESS



Our results suggested that these elicitor may be used to enhance phytochemicals witout any changes in sensory perception:
Green Butterhead lettuce (MJ 90 µM y PH 60 mg/L)
Red Butterhead lettuce (MJ 90 µM y AA 45 µM)

(Stevens, 1970; Soto-Vaca et al., 2012; Soares et al., 2013; Gutiérrez-Rosales et al., 2003)

Stability of phytochemical during shelflife green lettuce

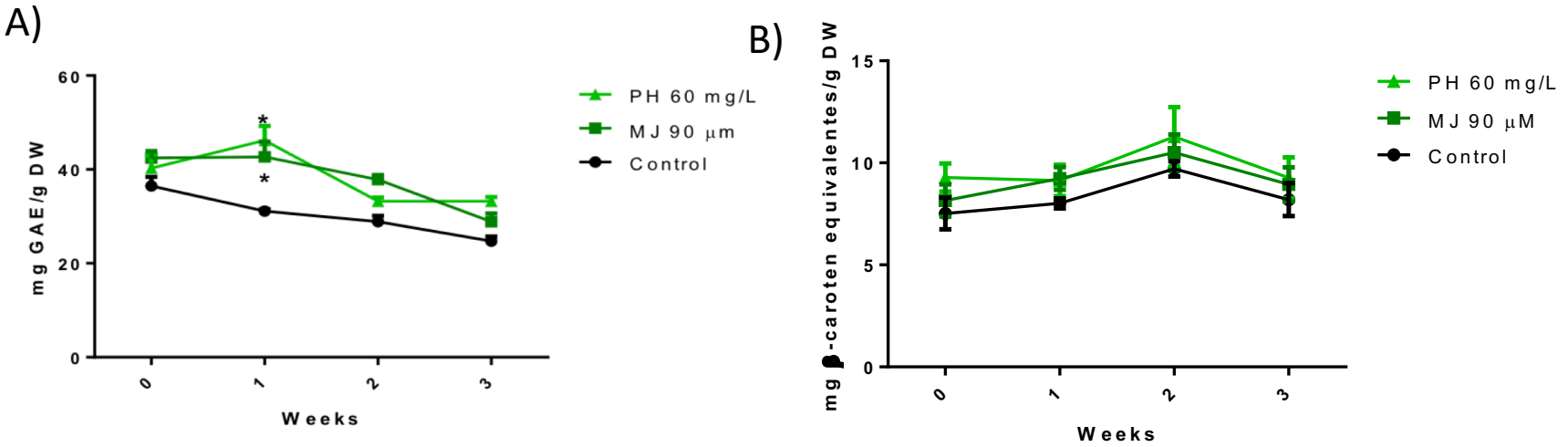


Figure 15. Efecto de inductores de respuesta de estrés en la estabilidad de fitoquímicos en lechuga verde. Efecto en el contenido de compuestos fenólicos totales (A) y carotenoides totales.

PHENOL CONTENT WAS KEPT ABOVE CONTROL LEVELS

NO SIGNIFICANT CHANGES FOR CAROTENOIDS

(Moreno-Escamilla *et al., 2017; Kim et al*., 2007; Llorach *et al*., 2008; Chu *et al*., 2002; Arzu *et al*., 2009)

Stability of phytochemical during shelflife red lettuce

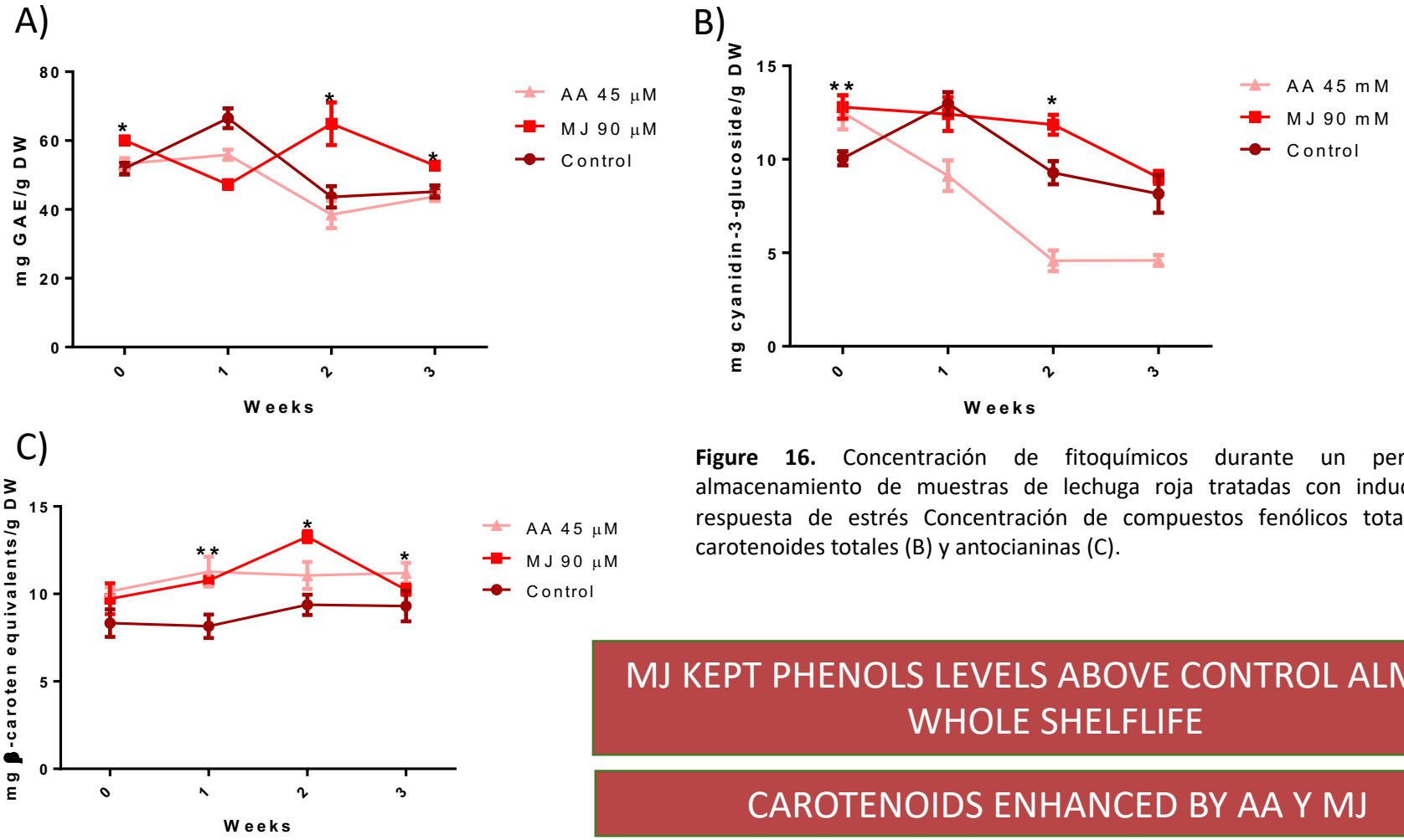


Figure 16. Concentración de fitoquímicos durante un periodo de almacenamiento de muestras de lechuga roja tratadas con inductores de respuesta de estrés Concentración de compuestos fenólicos totales (A) y carotenoides totales (B) y antocianinas (C).

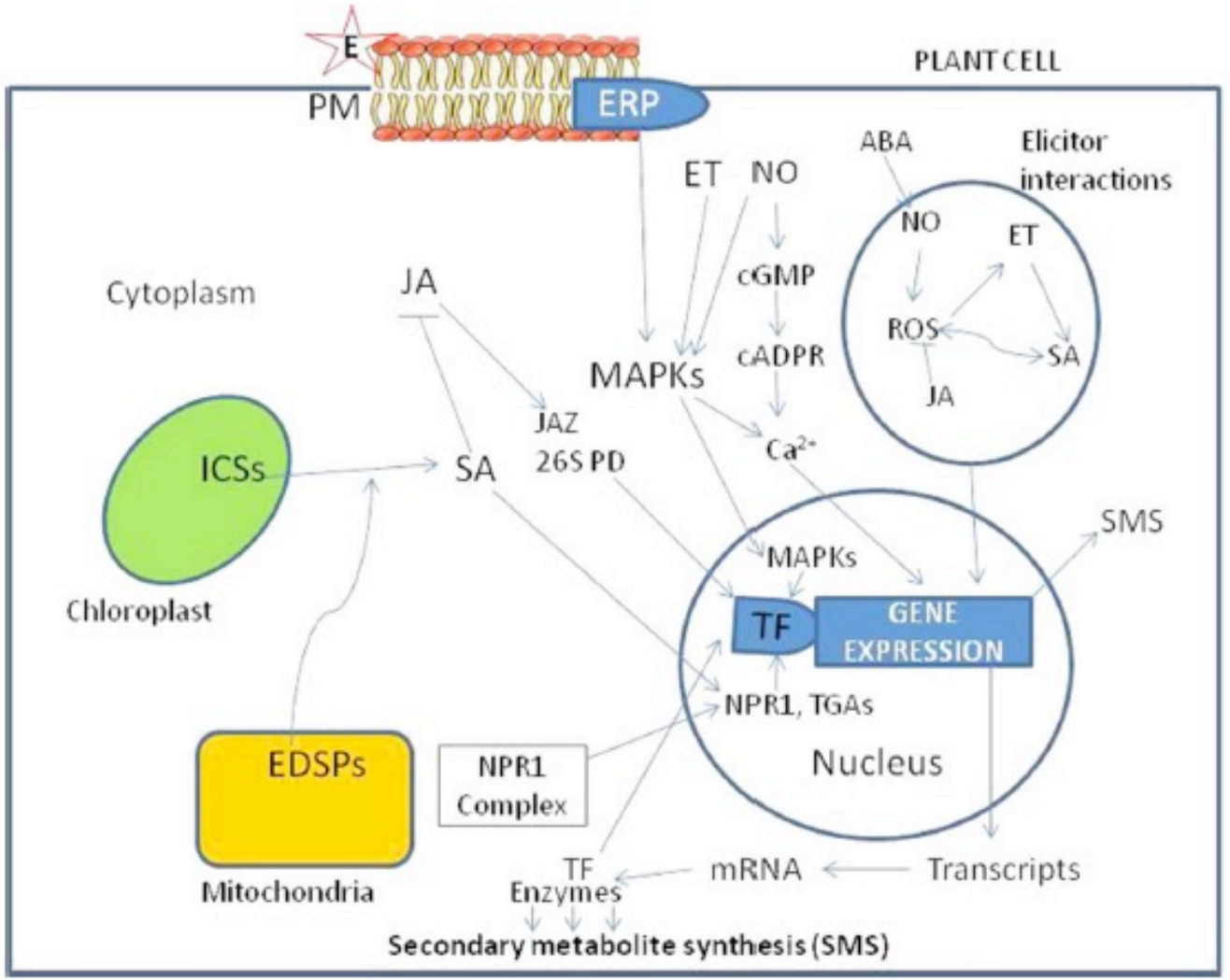
MJ KEPT PHENOLS LEVELS ABOVE CONTROL ALMOST WHOLE SHELFLIFE

CAROTENOIDS ENHANCED BY AA Y MJ

(Moreno-Escamilla *et al., 2017; Kim et al*., 2007; Llorach *et al*., 2008; Chu *et al*., 2002; Arzu *et al*., 2009)

GRACIAS POR SU ATENCIÓN

Mecanismo de acción de inductores



Activación de SAR y HR en plantas

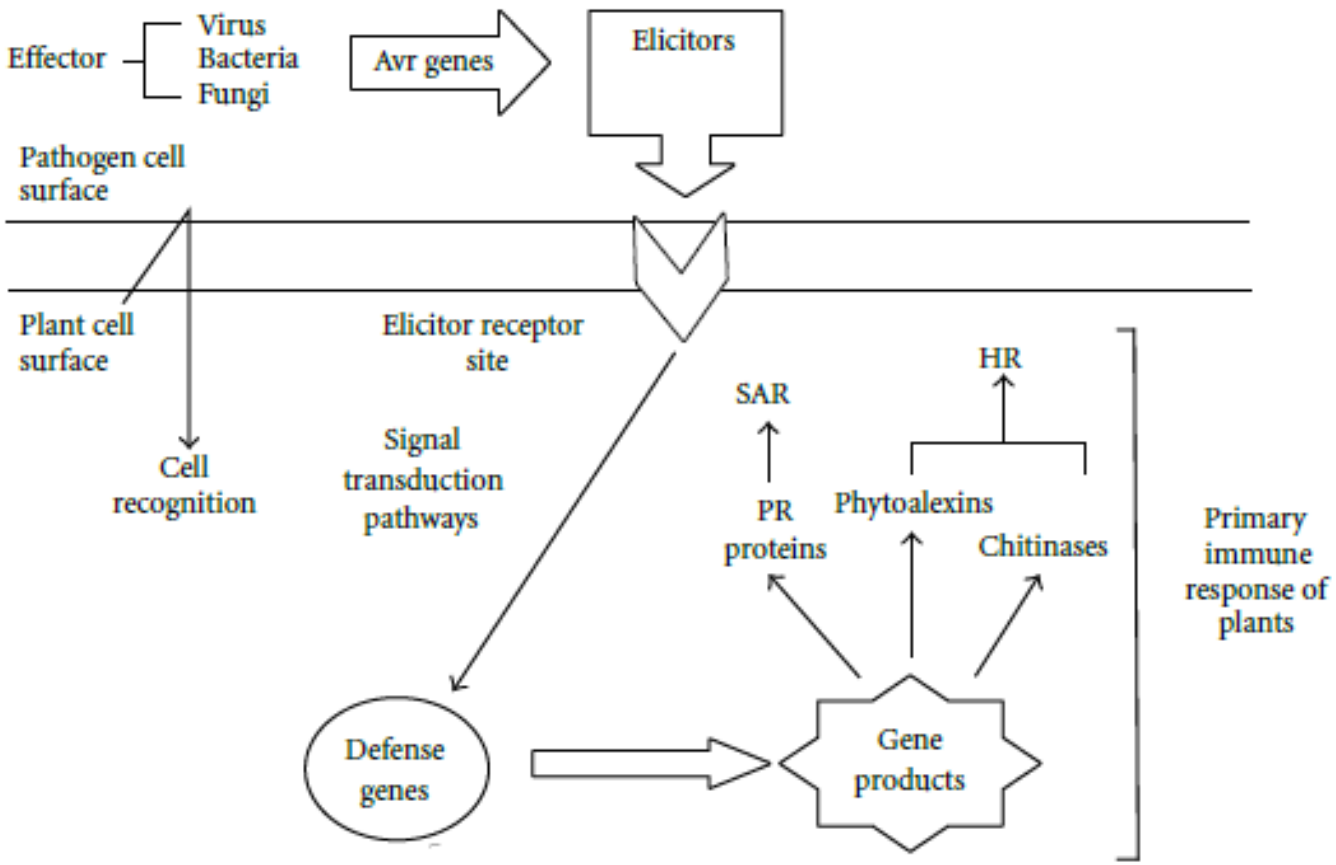


FIGURE 1: Primary immune response of plant in plant-pathogen interaction.

Inductores que activan SAR

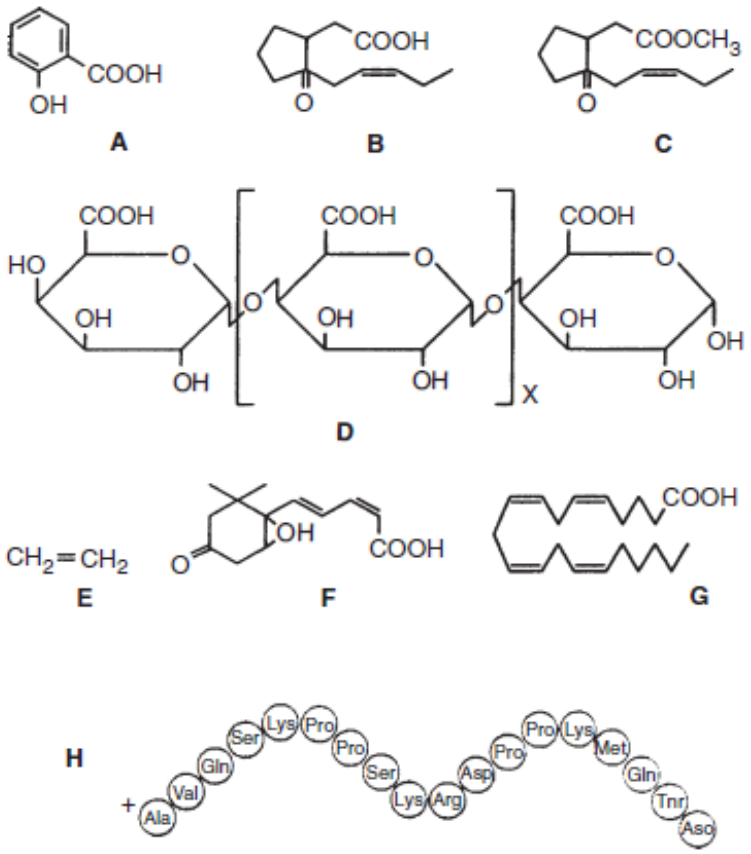
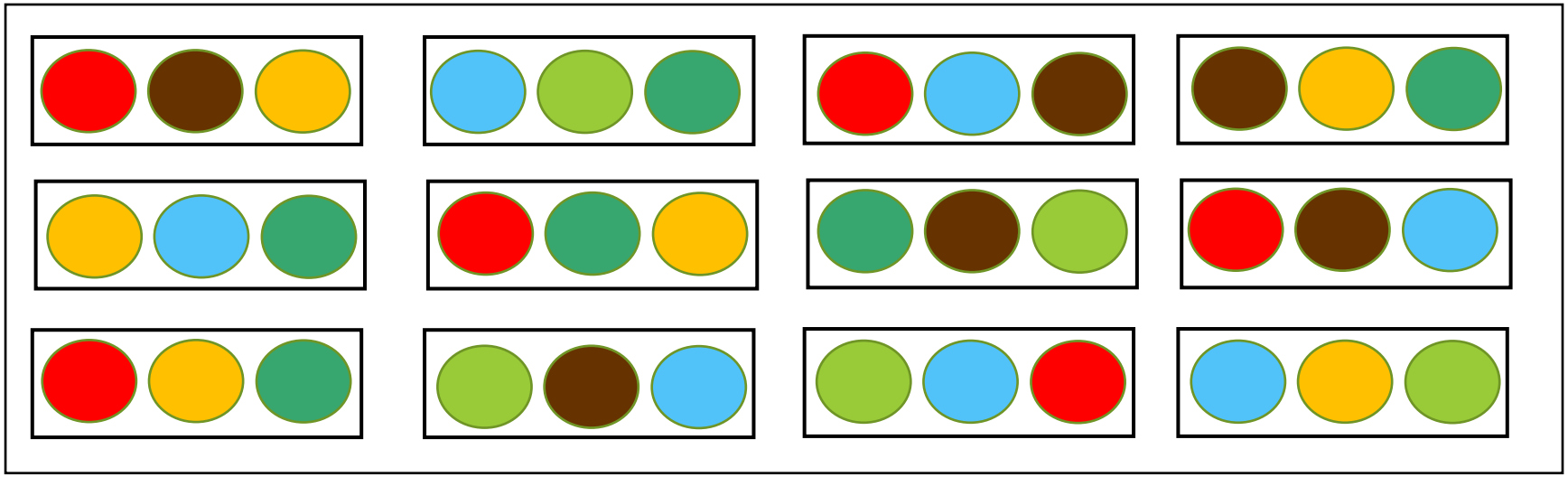


Figure 10.8. Compounds putatively involved in systemic defense signaling. (A) salicylic acid; (B) jasmonic acid; (C) methyl ester of jasmonic acid; (D) oligogalacturonides (X=2–0); (E) ethylene; (F) abscisic acid; (G) arachidonic acid; (H) systemin.

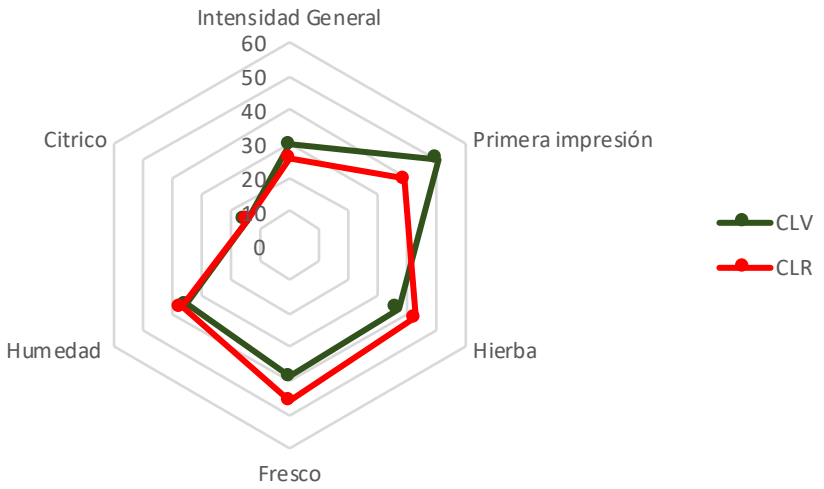
Esquematización de distribución de tratamiento

- Completamente al azar

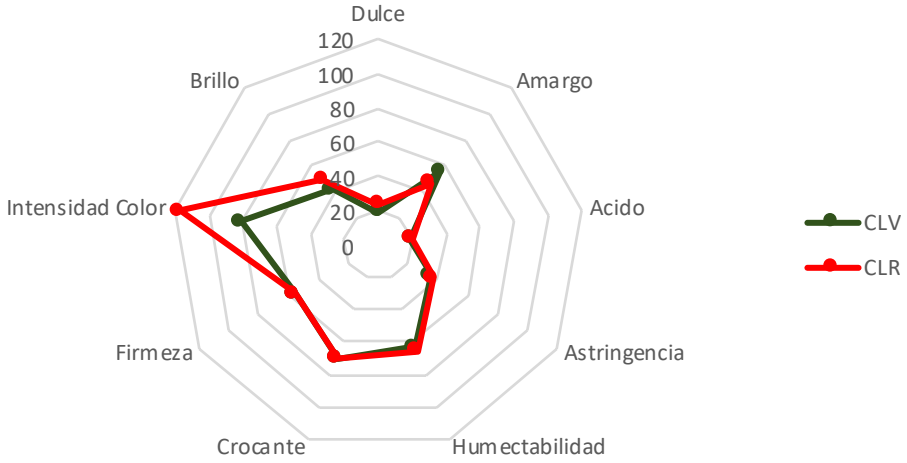


Efecto de inductores en perfil sensorial

A)



B)



Perfil sensorial de lechuga mantequilla verde y roja control. A) Olor y descriptores propuestos y B) atributos sensoriales de sabor, táctil en boca, táctil y vista ($\rho>0.05$).

(Llorach et al. 2008; Park et al,., 2009; Alsadon et al, 1993; Oliveira et al., 2013; Zlotek et al., 2014)