Bioactive properties of blueberry extracts obtained by different drying techniques against *Helicobacter pylori*

Jose Manuel Silvan[1]*, Anna Michalska-Ciechanowska[2], Marisol Villalva[1], Jessica Brzezowska[2], Soledad Díaz[1], Adolfo J. Martinez-Rodriguez[1]

[1] *Microbiology and Biocatalysis group (MICROBIO), Department of Biotechnology and Food Microbiology, Institute of Food Science Research (CIAL, CSIC-UAM), Calle Nicolás Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain*

[2] *Department of Fruit, Vegetable and Plant Nutraceutical Technology, Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, Chełmonskiego 37, 51-630 Wrocław, Poland*

E-mail: *jm.silvan@csic.es*

INTRODUCTION

Helicobacter pylori (*H. pylori*) is one of the most successful and prevalent human pathogens that infect more than 50% of the world's population [1]. Chronic inflammation of the gastric mucosa is one of the main consequences of this infection and is associated with the risk of gastric cancer [2]. Blueberries are rich in different bioactive compounds with antibacterial and anti-inflammatory properties [3] that could contribute to reduce the problems associated with *H. pylori* infection. However, these properties may vary depending on the blueberry variety. Furthermore, the industrial processing of blueberry extracts involves the use of a number of technical procedures that could affect these bioactive properties [4]. For this reason, the main objective of the present work was to evaluate the antibacterial and anti-inflammatory properties of three blueberry extracts from different varieties and obtained by different drying methods against *H. pylori*.

MATERIALS & METHODS

- **Blueberry extracts**
  - ❖ Bluejay
  - ❖ Berkley
  - ❖ Bluecrop

  } cultivars

Blueberry juice preparation
- Frozen fruits (15 kg)
- Pressed by a hydraulic press
- Clarification by centrifugation

Drying of blueberry juices

- **Drying procedures**
  - ✓ Drying blueberry extracts by:
    - ❖ freeze drying (FD)
    - ❖ vacuum drying (VD) → ➢ 50°C, ➢ 70°C, ➢ 90°C
    - ❖ spray drying (SD)

Five blueberry extracts of each cultivar
- FD
- VD50, VD70, VD90
- SD

- **Bacterial strain, growth media and culture conditions**
  - ✓ Strain: *Helicobacter pylori* Hp59 from MICROBIO group's bacterial collection and isolated from gastric biopsy.
  - ✓ Selective growth media: Brucella Broth supplemented with 10% horse serum (BBH) and Mueller-Hinton agar supplemented with 5% sheep blood (MHB).
  - ✓ Growth conditions: strains reactivation into MHB and incubation for 72h at 37°C, in microaerophilic atmosphere in VAIN workstation (85% N_2, 10% CO_2, 5% O_2).

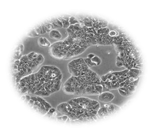
VAIN workstation

- **Antibacterial activity**
  - ✓ Procedure:
    - Bacterial culture mixture:
      1 mL of extracts (2 mg/mL) disolved in BBH (or only BBH for control growth)
      4 mL BB supplemented with 10% horse serum
      100 µL bacterial inoculum (~1x10^8 CFU/mL)

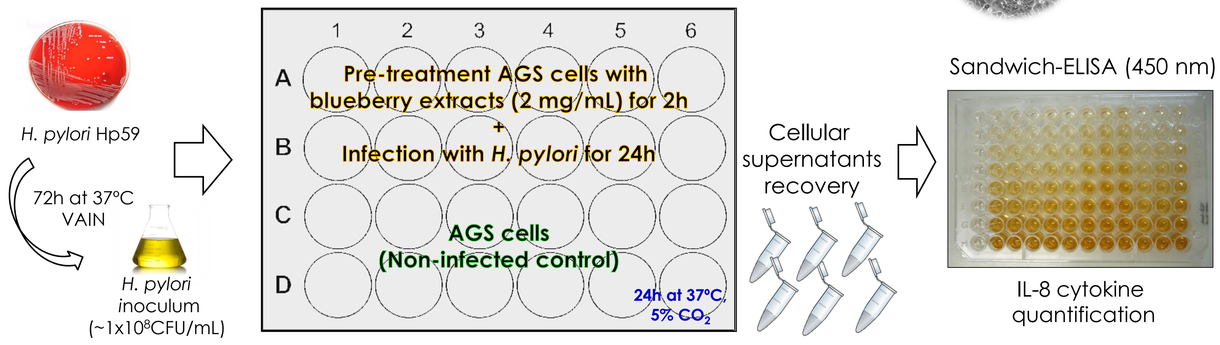
      } **Bacterial culture**

    - Incubation for 24h at 37°C, 150 rpm, in microaerophilic atmosphere (VAIN)
    - Serial decimal dilutions of bacterial cultures were plated onto fresh MHB agar
    - Incubation microaerobically for 72h at 37°C in microaerophilic atmosphere (VAIN)
    - Antibacterial activity determination by CFU counting

- **Anti-inflammatory activity on infected-gastric AGS cells**



Human gastric AGS cells



RESULTS

➢ Antibacterial activity

Table 1. Antibacterial activity of blueberry extracts (2 mg/mL) on the viable counts of *H. pylori* Hp59 after 24 hours of treatment. Results are expressed as CFU/mL (mean ± standard deviation) (n=4).

	Blueberry extracts		
Cultivars	Bluejay	Berkley	Bluecrop
FD	$1.50 \pm 0.58\times10^2$ *$^{a}_{A}$	$4.00 \pm 0.50\times10^2$ *$^{a}_{B}$	<1.00×10^2 *$^{a}_{A}$
VD50	<1.00×10^2 *$^{a}_{A}$	<1.00×10^2 *$^{a}_{A}$	<1.00×10^2 *$^{a}_{A}$
VD70	$2.25 \pm 1.06\times10^2$ *$^{a}_{A}$	<1.00×10^2 *$^{a}_{A}$	<1.00×10^2 *$^{a}_{A}$
VD90	$2.13 \pm 1.25\times10^2$ *$^{a}_{A}$	<1.00×10^2 *$^{a}_{A}$	<1.00×10^2 *$^{a}_{A}$
SD	<1.00×10^2 *$^{a}_{A}$	$3.75 \pm 0.35\times10^2$ *$^{a}_{B}$	<1.00×10^2 *$^{a}_{A}$

[a] Different superscript letters denote statistical difference within a row (p<0.05 ANOVA Tukey test).
[A,B] Different subscript letters denote statistical difference within a column (p<0.05 ANOVA Tukey test).
* An asterisk denotes statistical difference in comparison with the control growth (p<0.05 ANOVA Tukey test).
Control growth = $1.42 \pm 0.67\times10^8$ CFU/mL.
Growth detection limit = 1.00×10^2 CFU/mL (Bactericidal effect)

➢ Anti-inflammatory activity

Table 1. Effect of blueberry extracts (2 mg/mL) on pro-inflammatory cytokine IL-8 production in AGS cells infected by *H. pylori* Hp59. Results are expressed as % production of IL-8 respect to the untreated infected cells (mean ± standard deviation) (n = 3).

	Blueberry extracts		
Cultivars	Bluejay	Berkley	Bluecrop
FD	78.2 ± 1.2 *$^{a}_{A}$	82.9 ± 3.4 *$^{a}_{A}$	81.5 ± 4.0 *$^{a}_{B}$
VD50	76.8 ± 1.0 *$^{a}_{A}$	84.1 ± 2.2*$^{ab}_{A}$	88.9 ± 2.6 *$^{b}_{B}$
VD70	74.5 ± 0.7 *$^{ab}_{A}$	80.4 ± 4.3 *$^{b}_{A}$	67.3 ± 5.0 *$^{a}_{A}$
VD90	72.9 ± 2.1*$^{a}_{A}$	77.8 ± 4.3 *$^{a}_{A}$	69.2 ± 0.7 *$^{a}_{A}$
SD	78.1 ± 4.8 *$^{a}_{A}$	83.0 ± 1.9 *$^{ab}_{A}$	85.3 ± 0.2 *$^{b}_{B}$

[a,b] Different superscript letters denote statistical difference within a column (p<0.05 ANOVA Tukey test).
[A,B] Different subscript letters denote statistical difference within a row (p<0.05 ANOVA Tukey test).
* Asterisk denotes statistical difference in comparison with the control growth (untreated AGS cells) (p<0.05 ANOVA Tukey test).

CONCLUSIONS

- ❑ All blueberry extracts showed significant antibacterial activity against *H. pylori* Hp59 and some of these extracts showed bactericidal effect.
- ❑ The rest of blueberry extracts without bactericidal effect were able to reduce *H. pylori* Hp59 growth by more than 5 log CFU/mL.
- ❑ Bluecrop extracts showed the most effective antibacterial activity, because all extracts obtained by the different drying methods resulted bactericidal.
- ❑ Vacuum drying (VD) at 50°C were the most effective drying method since the extracts of the three varieties obtained by this method were bactericidal.
- ❑ Regarding anti-inflammatory activity, all blueberry extracts reduced IL-8 production in *H. pylori*-infected AGS cells.
- ❑ Bluecrop extracts obtained by the VD at 70°C and 90°C showed the most active anti-inflammatory effect reducing IL-8 production by 32% and 30%, respectively.
- ❑ These results suggest that the selection of the blueberry variety and drying method can be an effective tools for modulating the antibacterial and anti-inflammatory properties of blueberry extracts.

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REFERENCES: [1] Kusters et al. (2006). *Clin. Microbiol. Rev.*, 19, 449-490.
[2] White et al. (2015). *J. Inflamm. Res.*, 8, 137-147.
[3] Tobar-Bolaños et al. (2021). *J. Food Sci.*, 86, 5062–5077.
[4] Michalska et al. (2015). *Int. J. Mol. Sci.*, 16, 18642-18663.

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