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## Introduction

- **Lentils** are nutrient-dense legumes with a **low glycemic index**, **high phenolic** content and **antioxidant** properties.
- Epidemiological data suggest they may help prevent **metabolic disorders**, but the mechanisms by which their phenolics influence inflammation and metabolism remain poorly understood.

### Objectives:

- ✓ Identify the lentil variety with the highest phenolic/flavonoid content and profile;
- ✓ Assess immunomodulatory and gene expression effects in an *in vitro* intestinal inflammation model.

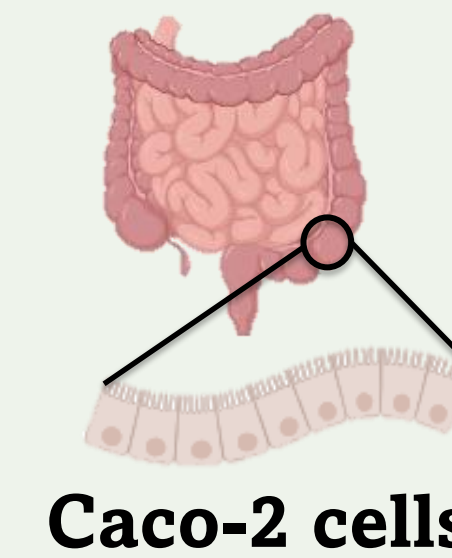
## Methods

- Raw and cooked *Lens culinaris* L. varieties;
- Determination of total phenolics, flavonoids and antioxidant activity;
- Quantification and identification of phenolic compounds;
- Immunomodulatory activity tested on Caco-2 cells with inflammation induced by pro-inflammatory IL-1 $\beta$ .



Inflammatory markers:  
IL-6 & IL-8

ELISA  
Quantified by



Genes related to:

Inflammation (*IL-8*, *IL-6*, *IL-1 $\beta$* , *TGF $\beta$* )  
Intestinal barrier integrity (*Occludin*, *CDH1*)  
Metabolic regulation (*PPAR $\gamma$* , *SIRT1*)  
Oxidative defense (*HO1*)

Quantitative  
RT-PCR  
Assessed by

## Results & Discussion

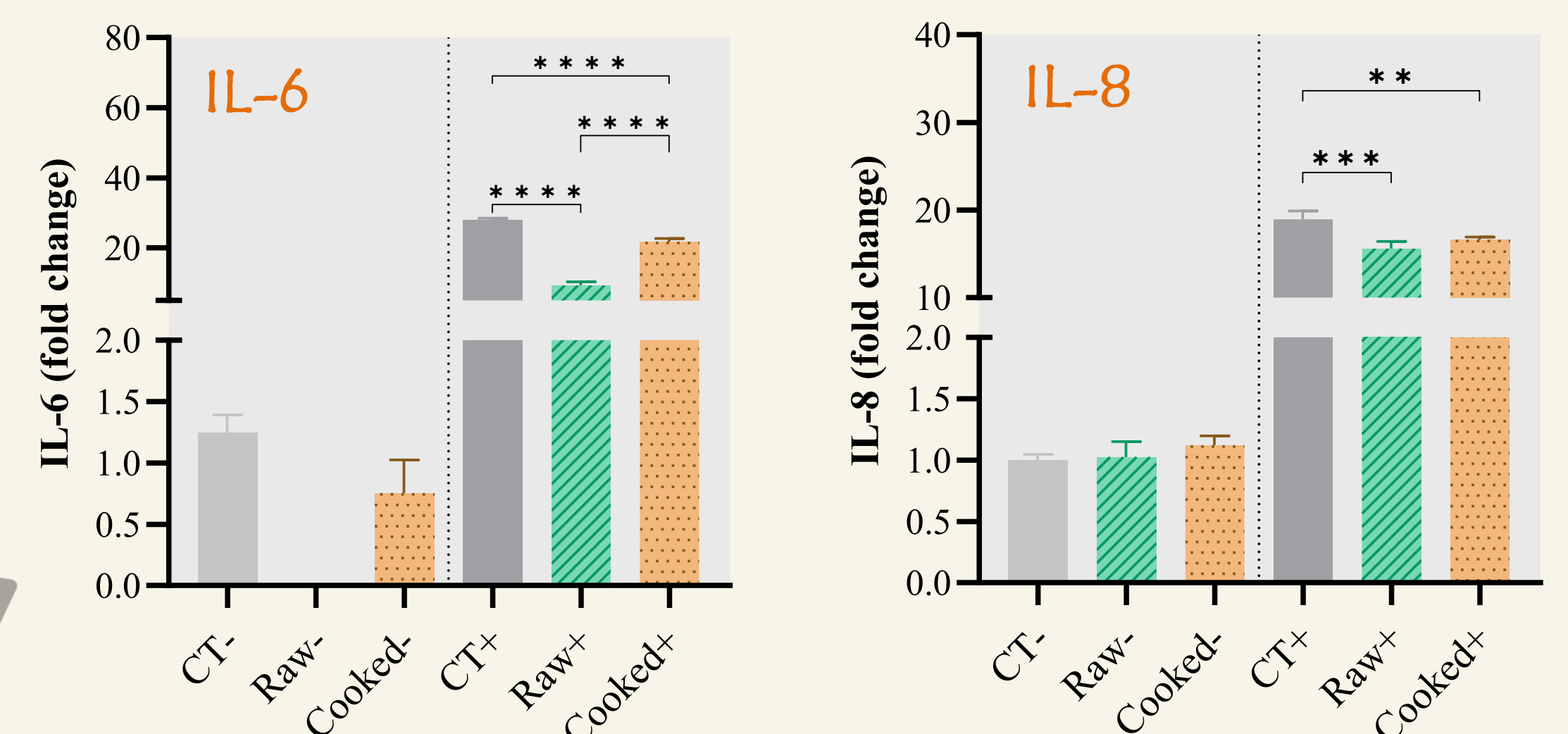
### Phenolic and flavonoid compounds

**Table 1** – Total phenolics, total flavonoids, and antioxidant activity of *Lens culinaris* L. varieties. Values represent mean  $\pm$  SEM in mg of gallic acid, mg of catechin, and  $\mu$ g of Trolox equivalent per g of dry weight (DW), respectively. Different letters indicate significant differences ( $p < 0.05$ ).

		Total Phenolics (mg AG/g DW)	Total Flavonoids (mg CAE/g DW)	Antioxidant activity ( $\mu$ g Trolox/g DW)
L01	Raw	4.0 $\pm$ 0.1 <sup>a</sup>	2.4 $\pm$ 0.0 <sup>a</sup>	26.3 $\pm$ 0.6 <sup>a</sup>
	Cooked	3.3 $\pm$ 0.1 <sup>c</sup>	2.6 $\pm$ 0.0 <sup>a</sup>	23.36 $\pm$ 0.9 <sup>a</sup>
L02	Raw	3.4 $\pm$ 0.1 <sup>b</sup>	2.4 $\pm$ 0.1 <sup>a</sup>	22.5 $\pm$ 0.9 <sup>b</sup>
	Cooked	2.9 $\pm$ 0.0 <sup>d</sup>	2.5 $\pm$ 0.0 <sup>a</sup>	16.4 $\pm$ 0.8 <sup>a</sup>
L03	Raw	2.9 $\pm$ 0.2 <sup>c</sup>	1.7 $\pm$ 0.1 <sup>c</sup>	19.3 $\pm$ 0.8 <sup>c</sup>
	Cooked	2.4 $\pm$ 0.1 <sup>cd</sup>	2.1 $\pm$ 0.0 <sup>b</sup>	18.8 $\pm$ 0.5 <sup>c</sup>
L04	Raw	0.8 $\pm$ 0.0 <sup>f</sup>	0.6 $\pm$ 0.0 <sup>d</sup>	0.8 $\pm$ 0.0 <sup>d</sup>
	Cooked	0.9 $\pm$ 0.0 <sup>f</sup>	0.7 $\pm$ 0.0 <sup>d</sup>	0.7 $\pm$ 0.0 <sup>d</sup>

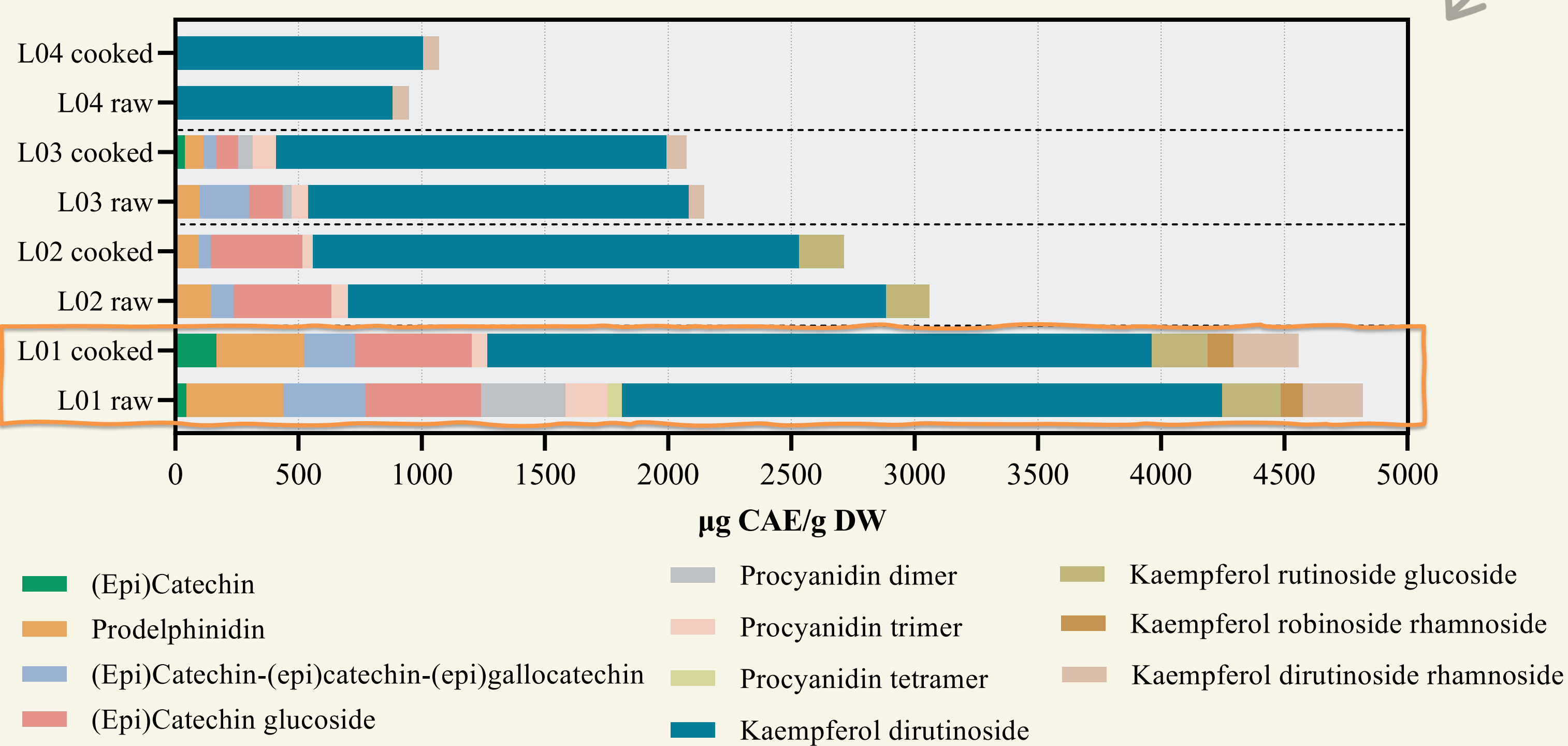
L01 phenolic extracts significantly **reduced IL-6 and IL-8** secretion in IL-1 $\beta$ -stimulated Caco-2 cells

### Immunomodulatory activity



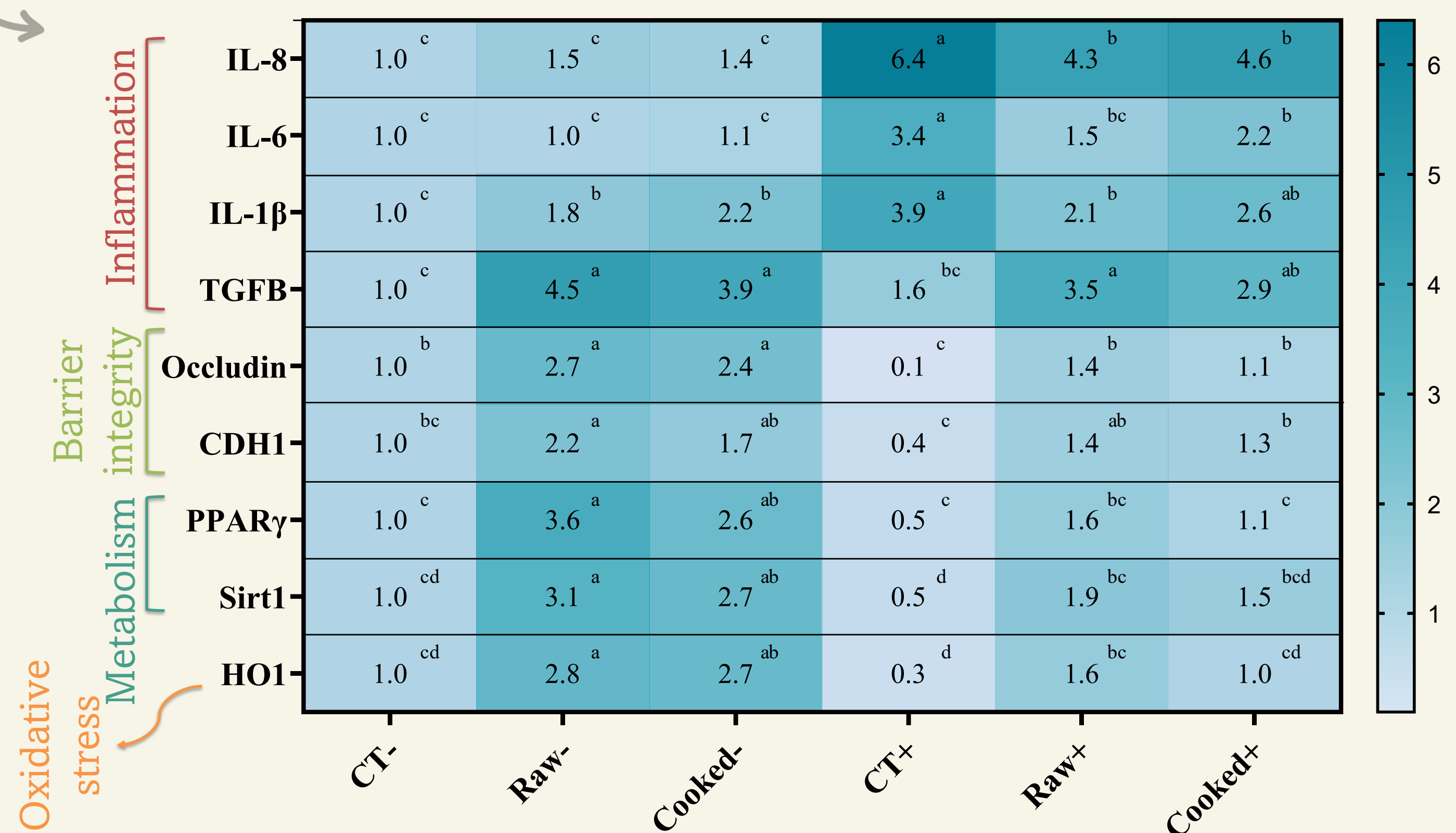
**Figure 2**- Modulation of immune response in Caco-2 cells by L01 phenolic extract (2.50 % m/v) quantified by ELISA. The left part corresponds to the non-stimulated cells (-), and the right to the inflammatory effect following IL-1 $\beta$  (+). Significant differences are indicated as \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

L01 showed the **highest phenolic/flavonoid content**, **antioxidant activity**, and levels of **(epi)catechin, procyanidins, and kaempferol derivatives**. Cooking reduced (epi)catechin and procyanidins but kept kaempferol glycosides stable



**Figure 1** – Concentrations of phenolic compounds [ $\mu$ g catechin equivalent per g of dry weight (DW)] of *Lens culinaris* L. varieties measured using HPLC-DAD-MS.

During inflammation, **L01 extracts downregulate inflammatory genes**, except TGF $\beta$ , which is upregulated in both conditions. Under basal conditions, they **upregulate genes for barrier integrity, metabolism and oxidative stress defense**



**Figure 3**- Fold change of genes related to inflammation, intestinal barrier integrity, metabolic regulation and oxidative stress, assessed by RT-PCR in Caco-2 cells exposed to L01 phenolic extracts. CT- corresponds to the non-stimulated cells (-); CT+ to the inflammatory condition following IL-1 $\beta$  (+). Different letters indicate significant differences within each gene ( $p < 0.05$ ).

## Conclusions

- L01 has the **highest phenolic/ flavonoid content, antioxidant activity, kaempferol and (epi)catechin derivatives quantities**, making it ideal for *in vitro* analyses.
- Under inflammation, L01 phenolics extracts showed: **anti-inflammatory effects** by  $\downarrow$  IL-6 and IL-8 secretion;  $\downarrow$  **inflammatory genes expression**;  $\uparrow$  **CDH1 and Occludin** genes expression. On the contrary, under basal conditions:  $\uparrow$  **genes** involved in **barrier integrity, metabolism, and oxidative stress defense**.
- These findings suggest that **lentil phenolics** may strengthen epithelial tight junctions, modulate metabolic pathways and support the potential use of these as **functional ingredients** for managing intestinal inflammation and metabolic disorders.

## Acknowledgements

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