



# Spent Coffee grounds as a source of bioactive compounds

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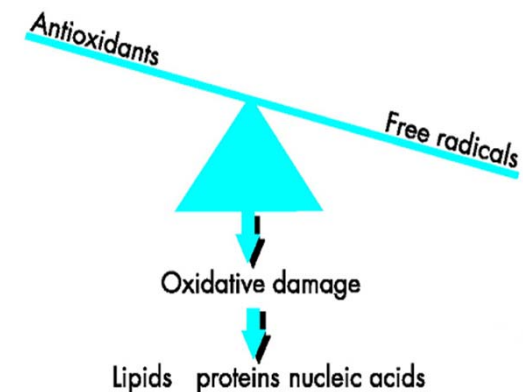


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de Navarra

Millions of coffee cups are consumed every day around the World (ICO, 2013)

Coffee may reduce the risk of :

- Cancer (liver, kidney, oral, breast and colorectal) (Nkondjock, 2012)
- Neurodegenerative diseases:
  - Parkinson (Ascherio et al., 2001)
  - Alzheimer (Barranco Quintana et al., 2007)
  - Amyotrophic Lateral Sclerosis (Beghi et al., 2011)
- Type 2 diabetes (van Dam & Hu, 2005; Matusheski et al., 2012)
- Cardiovascular diseases (Bonita et al., 2007)
- ...



**Coffee is one of the main source of antioxidants in diet** (Pulido et al., 2003; Pellegrini et al., 2003; Svilaas et al., 2004)

## Coffee antioxidant compounds

### Natural

#### Phenolic compounds Chlorogenic acids

- *Chemical-based assays:*  
Antiradical activity (Daglia et al., 2004)
- *In vitro assays:*  
Protection against oxidative stress  
(Pavlica & Gebhardt, 2005; Cho et al., 2009)
- *In vivo assays:*  
Protection against oxidative DNA damage  
(Bichler et al., 2007; Hoelzl et al., 2010)

### Caffeine

- *Chemical-based assays:*  
Scavenger of hydroxyl radicals (Daglia et al., 2004)
- *In vitro assays:*  
Prevention of LDL oxidation (Lee et al., 2000)  
Protection against oxidative DNA damage  
(Faustmann et al., 2009)

### Formed during roasting

#### Maillard Reaction Products (MRPs) Volatiles Non-volatiles: Melanoidins

- *Chemical-based assays:*  
Antiradical activity (Nicoli et al., 1997; Daglia et al., 2000;  
Del Castillo et al., 2002; Morales & Jimenez-Perez, 2004)  
Metal chelating agents  
(Borrelli et al., 2002; Delgado-Andrade et al., 2005)
- *In vitro assays:*  
Protection against oxidative stress (Goya et al., 2007)  
Genoprotective effect (Del Pino-García et al., 2012)
- *In vivo assays:*  
Increase plasma total antioxidant capacity (Somoza et al., 2003)  
Protection of LDL oxidation (Dittrich et al., 2009)



## Coffee compounds extraction

Solubles

Non solubles

Chlorogenic acids  
Caffeine  
Soluble Melanoidins  
Hydrophilic volatile compounds

Lipids  
Melanoidins  
Polisacharides

- Extraction method (Bell et al., 2000; Andueza et al., 2003a; Bravo et al., 2012; Ludwig et al., 2007)
- Grinding (Spiro & Selwod, 1984; Lingle, 1996; Barberá, 2000; Andueza et al., 2003a; Illy & Viani, 2005)
- Coffee/water ratio (Andueza et al., 2007)
- Water pressure and Temperature (Andueza et al., 2002, 2003b, 2007)

# Spent coffee grounds as a source of bioactive compounds



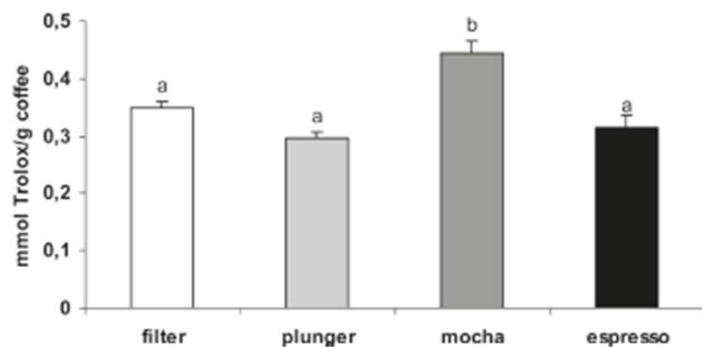
## Brew Antioxidant capacity

(Pérez-Martínez et al., 2010; Bravo et al., 2012)

Per g roasted coffee

Per mL coffee brew

b) ABTS antioxidant capacity



Pérez-Martínez et al., 2010

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**Spent coffee grounds:  
Source of natural antioxidants?**

Millions of coffee cups are consumed every day around the World (ICO, 2013)

## **SOLUBLE COFFEE TONS OF SPENT COFFEE GROUNDS COFFEE SHOPS AND DOMESTIC INDUSTRY**

- Compost
- Animal feed (Givens & Barber, 1986)
- Biofuel, biodiesel or bioethanol production (Silva et al., 1998; Kondamudi et al., 2008)
- Adsorbent and activated carbon (Namane et al., 2005; Franca et al., 2009)
- Antioxidants extraction (Yen et al., 2005; Ramalakshmi et al., 2009; Mussatto et al., 2011; Murthy & Madhava Naidu, 2012 )



- Compost (Cruz et al., 2014)
- Espresso coffee (Zuorro et al., 2013)



## Spent coffee grounds: Source of natural antioxidants?

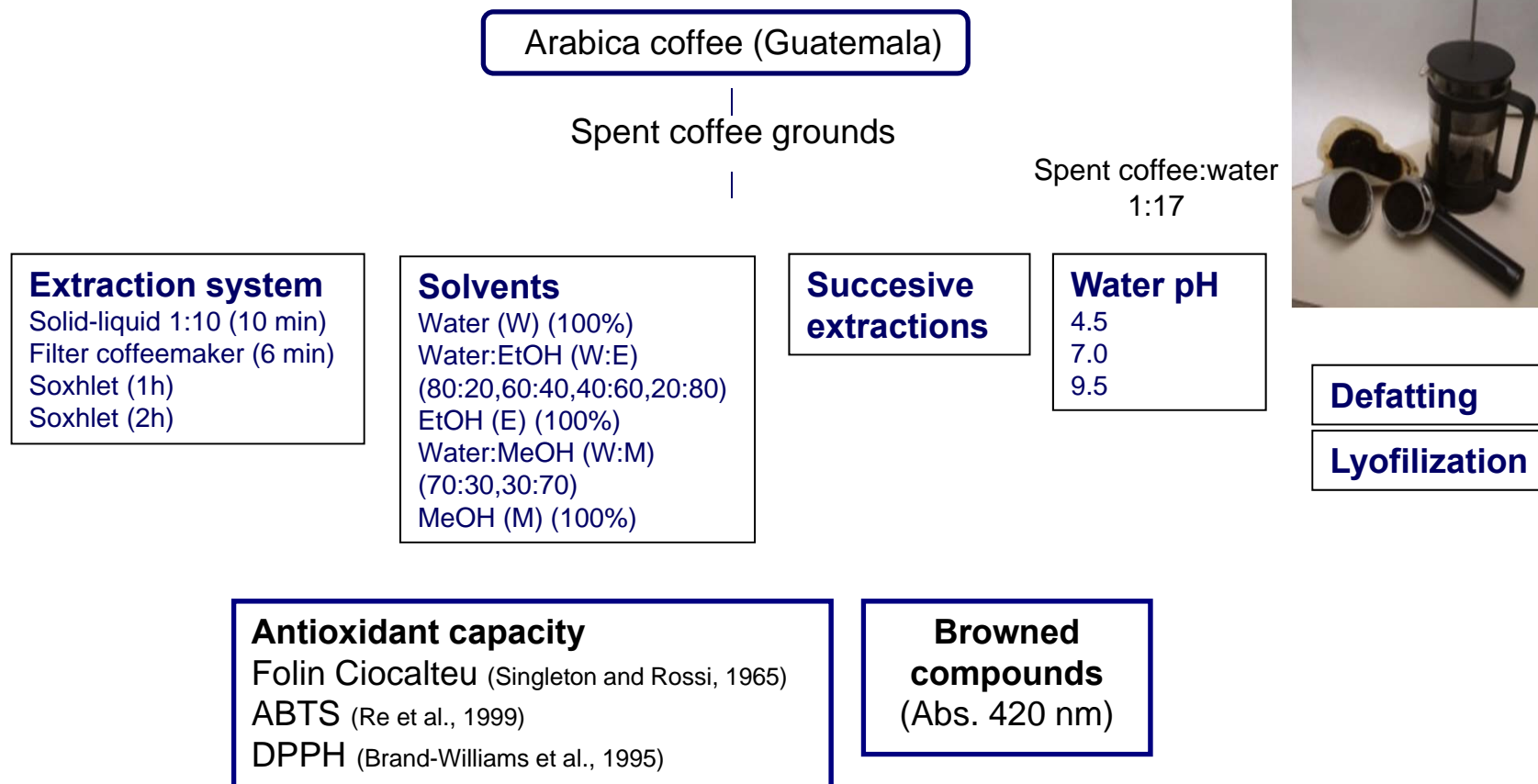


## OBJECTIVES

To explore the possibility to obtain extracts of spent coffee grounds with antioxidant capacity and to evaluate their potential functional properties

1. To develop an **extraction methodology** of spent coffee with antioxidant capacity.
2. To evaluate the **antioxidant** capacity and the main bioactive compounds in spent coffee extracts.
3. To evaluate the **potential functional properties** of spent coffee extracts:
  1. Antioxidant and genoprotective effects in human cells.
  2. Antimutagenic activity
  3. Antimicrobial activity

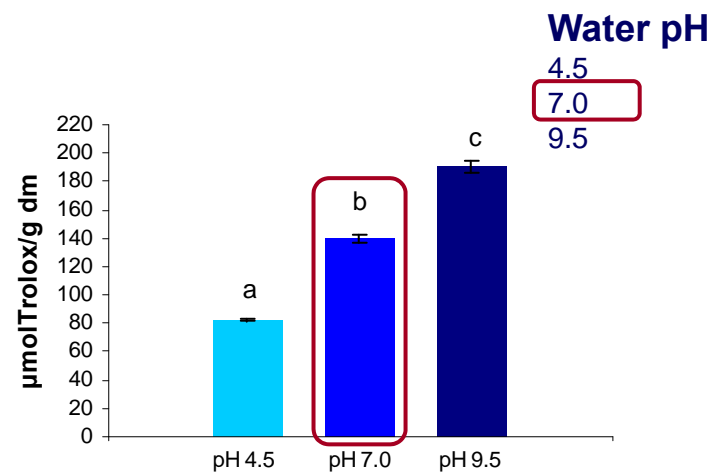
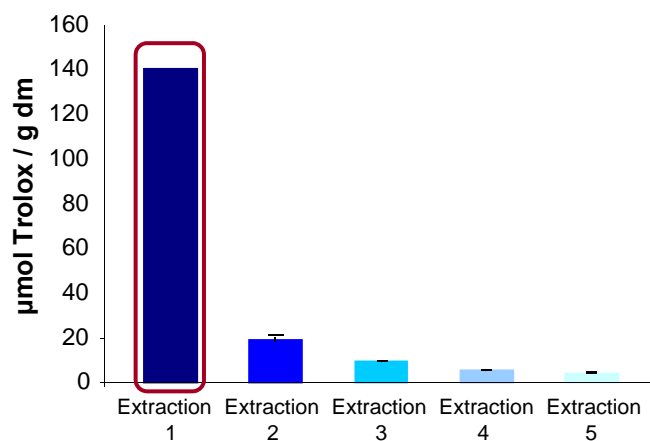
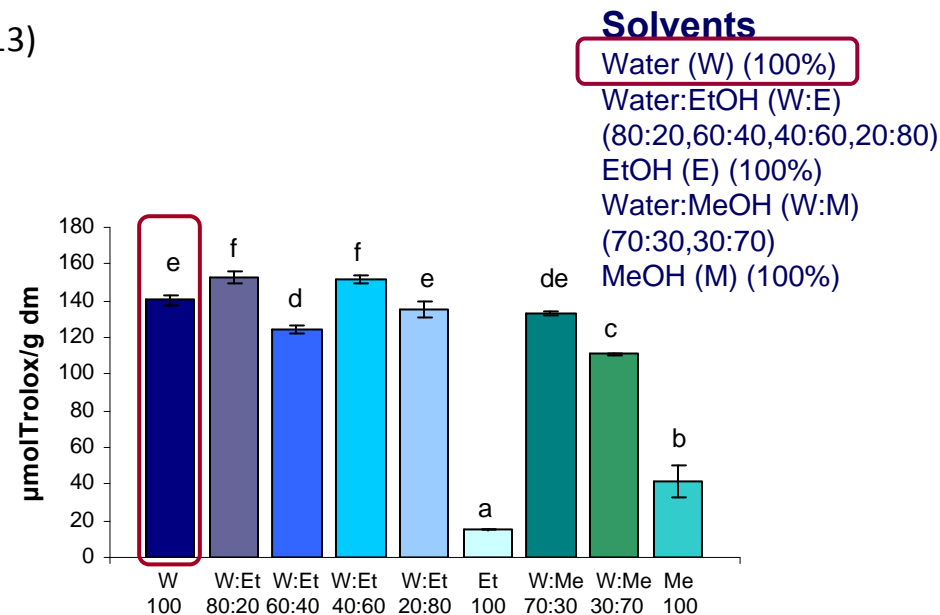
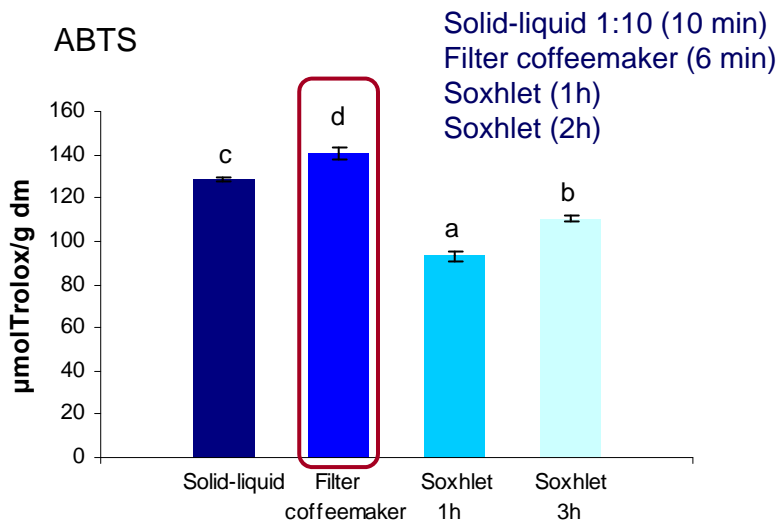
## 1. Extraction methodology (Bravo et al., 2013)





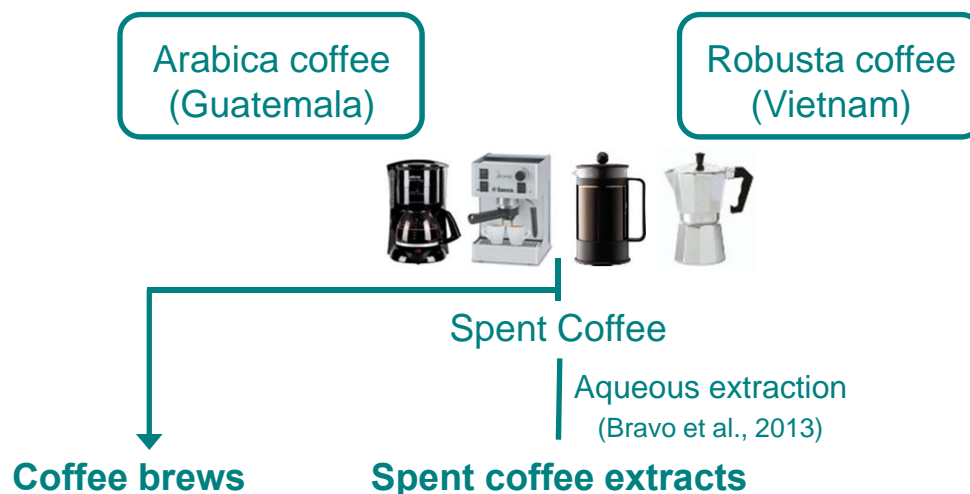
# Spent coffee grounds as a source of bioactive compounds

## 1. Extraction methodology (Bravo et al., 2013)



## Spent coffee grounds as a source of bioactive compounds

### 2. Antioxidants in spent coffee grounds extracts (Bravo et al., 2012)



**HPLC: SPE** (Bicchi et al., 1995) HPLC method (Farah et al., 2005)  
 Caffeoylquinic acids: 3-CQA, 4-CQA, 5-CQA  
 Dicafeoylquinic acids: 3,4-diCQA, 3,5-diCQA, 4,5-diCQA  
 Caffeine

#### Antioxidant capacity

Folin Ciocalteu (Singleton and Rossi, 1965)

ABTS (Re et al., 1999)

DPPH (Brand-Williams et al., 1995)

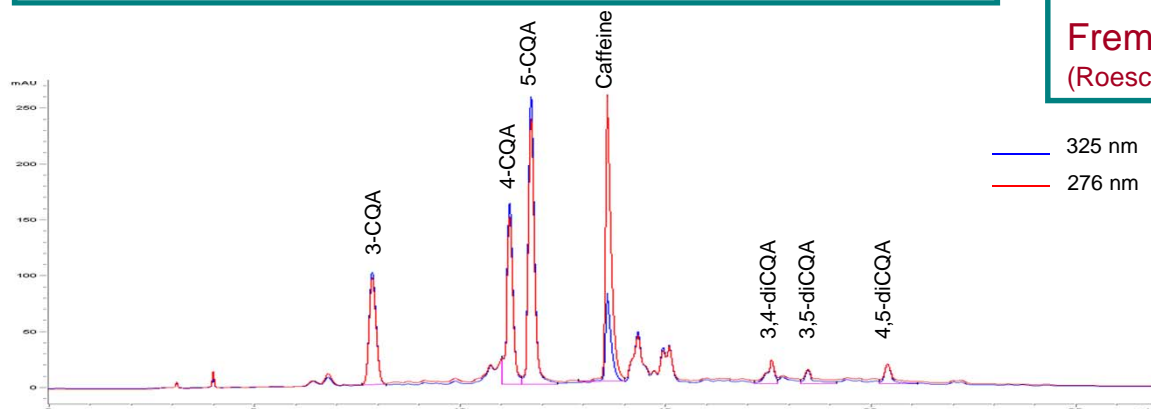
**Fremy's salt & TEMPO**

(Roesch et al., 2003; Caemmerer and Kroh, 2006)



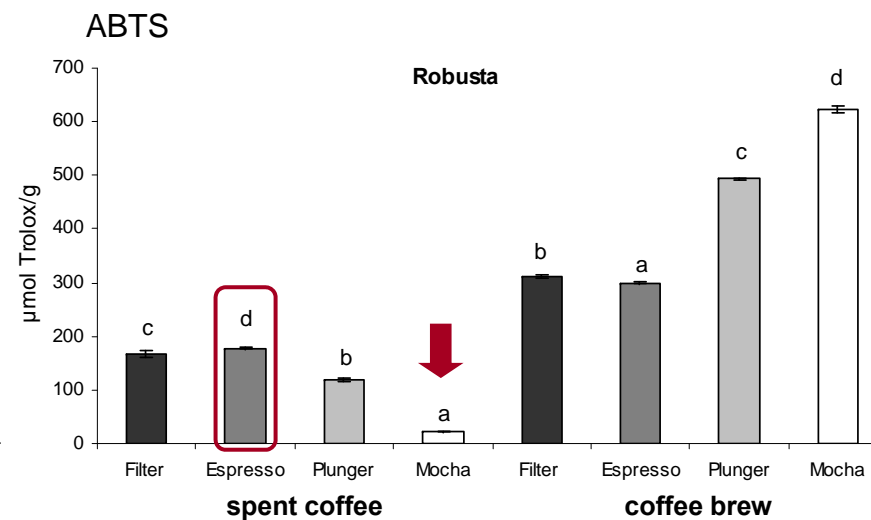
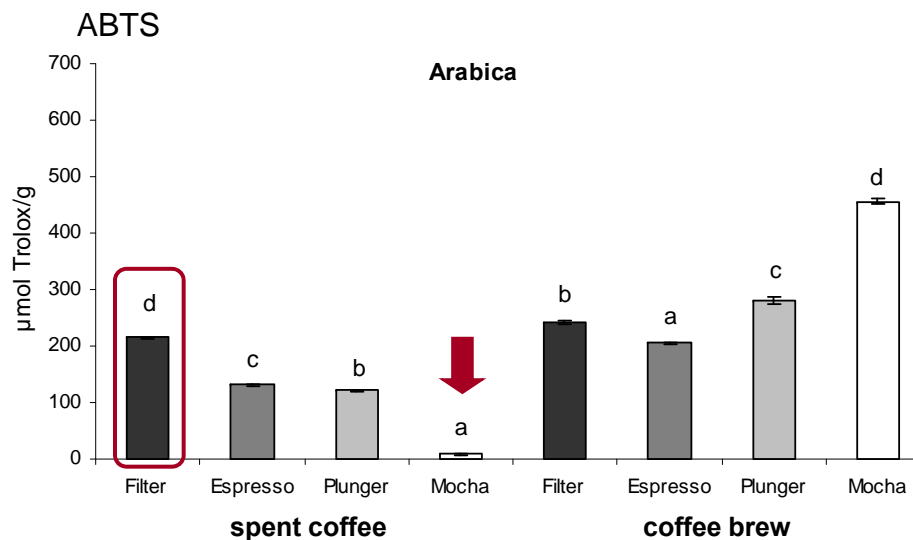
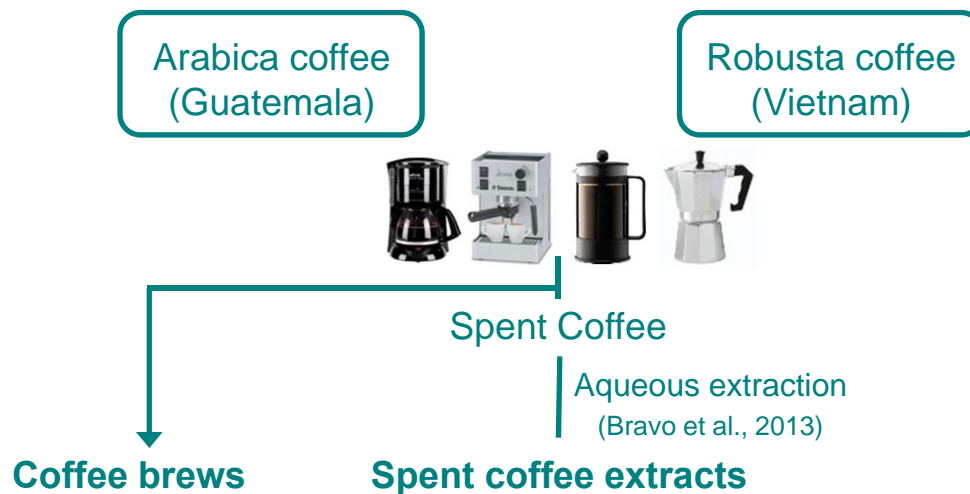
Technische Universität Berlin

**Browned Compounds**  
(Abs. 420 nm)



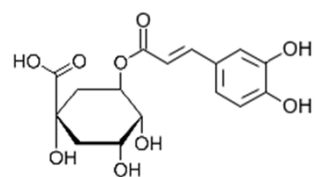
# Spent coffee grounds as a source of bioactive compounds

## 2. Antioxidants in spent coffee grounds extracts (Bravo et al., 2012)

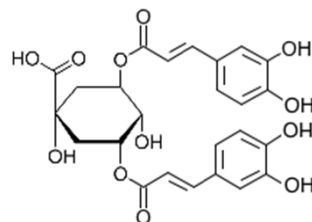


## Spent coffee grounds as a source of bioactive compounds

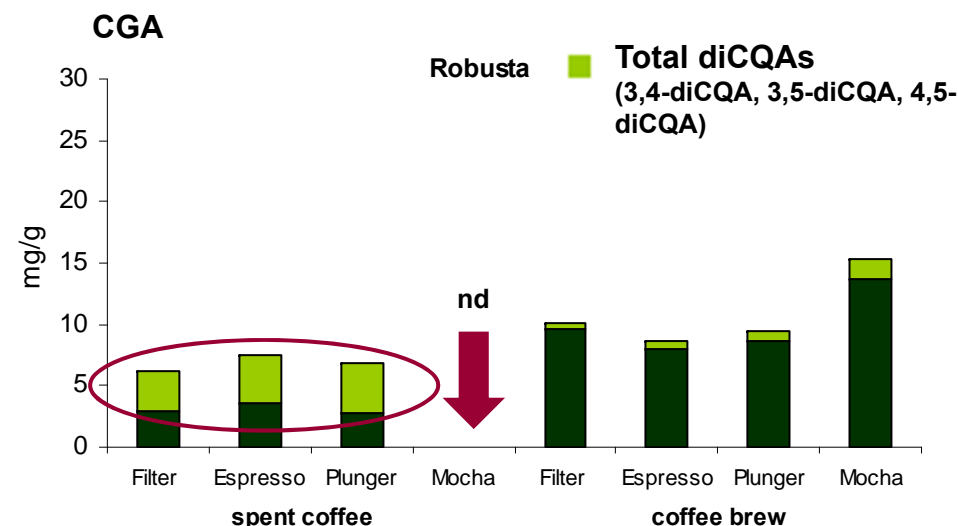
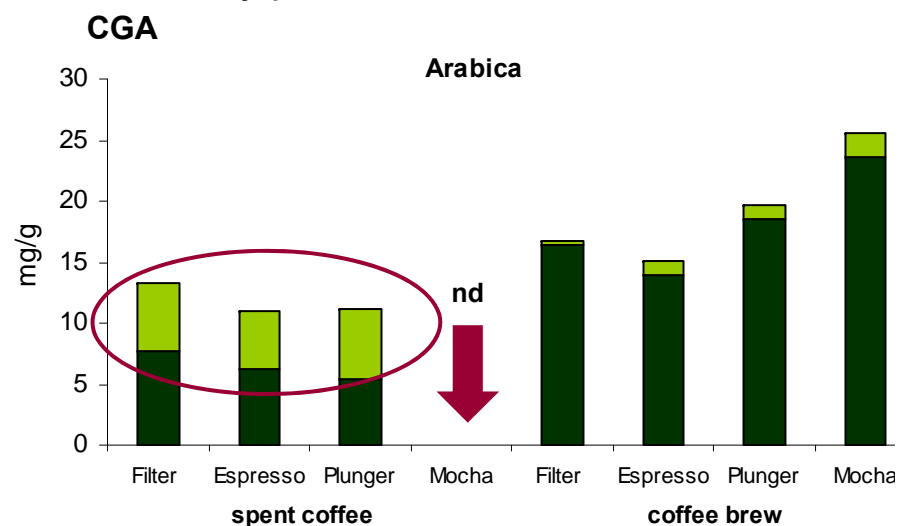
### 2. Antioxidants in spent coffee grounds extracts (Bravo et al., 2012)



caffeoylquinic acid



dicafeoylquinic acid



### 3. Potential Functional properties of spent coffee extracts



1. **Antioxidant (ROS) and genoprotective (Comet assay) effects in human cells** ✓
2. **Antimutagenic activity**
3. **Antimicrobial activity**

## 3. Potential Functional properties of spent coffee extracts

### Antimutagenic activity

Salmonella mutagenicity test (Ames test)

*S. Typhimurium* His<sup>-</sup>, TA98 strain

- Toxicity assay: non-toxic concentration selection

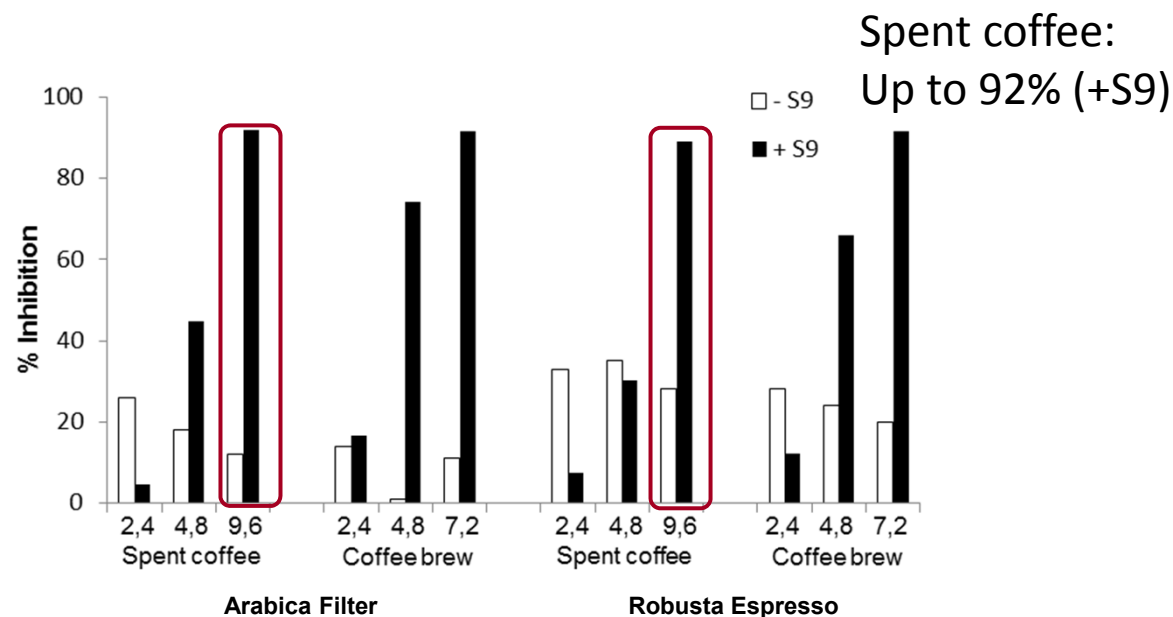
- Antimutagenicity test:

- Without metabolic activation (-S9): sample+phosphate buffer+culture+mutagen (NPD)
- With metabolic activation (+S9): sample+rat liver homogenate (S9mix)+culture+mutagen (NPD)



48h 37°C

Revertant colonies



### 3. Potential Functional properties of spent coffee extracts

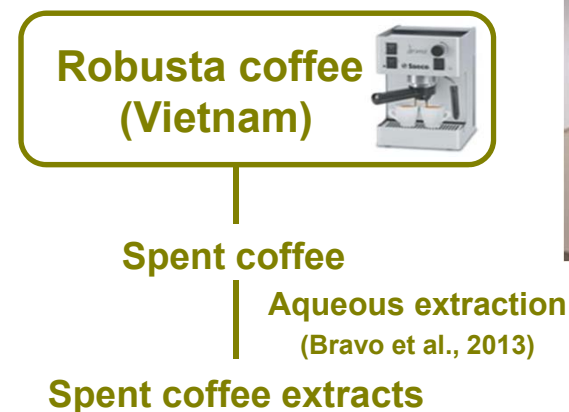


1. Antioxidant (ROS) and genoprotective (Comet assay) effects in human cells
2. Antimutagenic activity
3. Antimicrobial activity



### 3. Potential Functional properties of spent coffee extracts

#### Antimicrobial activity



1. Agar-well diffusion method
2. Minimum inhibitory concentration (MIC)
3. Growth inhibition curves

Gram +  
*Staphylococcus aureus*  
*Listeria monocytogenes*  
*Bacillus subtilis*

Gram -  
*Escherichia coli*  
*Pseudomonas aeruginosa*  
*Salmonella Choleraesuis*

Fungal strains  
*Candida albicans*  
*Aspergillus niger*



### 3. Potential Functional properties of spent coffee extracts

#### Antimicrobial activity

##### 1. Agar-well diffusion method

Organisms	Inhibition zones (mm)			
	Arabica Filter		Robusta espresso	
	Spent coffee	Coffee brew	Spent coffee	Coffee brew
Gram +				
<i>S. aureus</i>	17.3 ± 0.6 <sup>ab</sup>	15.3 ± 0.6 <sup>a</sup>	15.3 ± 1.2 <sup>a</sup>	19.7 ± 1.2 <sup>b</sup>
<i>L. monocytogenes</i>	20.0 ± 3.6 <sup>a</sup>	19.0 ± 2.7 <sup>a</sup>	18.0 ± 2.0 <sup>a</sup>	22.3 ± 3.1 <sup>a</sup>
<i>B. subtilis</i>	8.8 ± 0.3 <sup>a</sup>	9.7 ± 0.6 <sup>a</sup>	ND	11.9 ± 0.2 <sup>a</sup>
<i>E. coli</i>	ND	ND	ND	10.3 ± 1.2
<i>S. Choleraesuis</i>	ND	ND	ND	10.7 ± 0.6
<i>Ps. aeruginosa</i>	ND	ND	ND	10.3 ± 0.6
<i>C. albicans</i>	15.3 ± 1.2 <sup>ab</sup>	13.7 ± 0.6 <sup>a</sup>	15.7 ± 1.5 <sup>ab</sup>	17.7 ± 0.6 <sup>b</sup>
<i>A. niger</i>	ND	ND	ND	ND



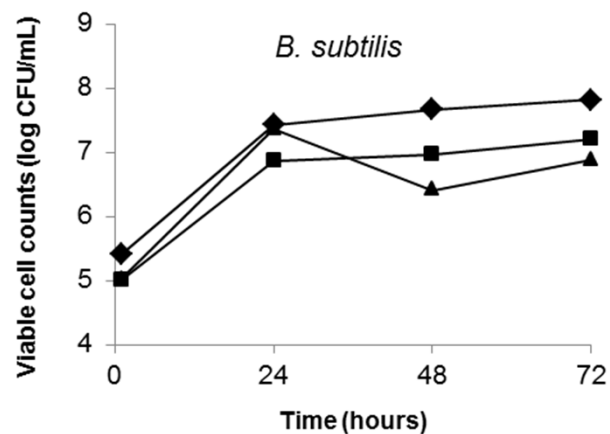
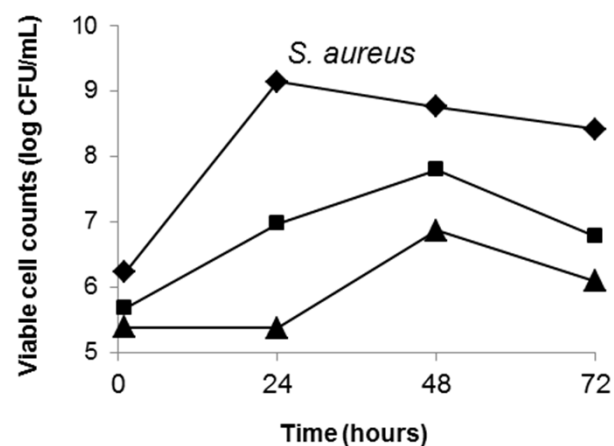
##### 2. Minimum inhibitory concentration (MIC)

Organisms	MIC (mg/mL)			
	Arabica filter		Robusta espresso	
	Spent coffee	Coffee brew	Spent coffee	Coffee brew
Gram +				
<i>S. aureus</i>	5	7.5	10	8.1
<i>L. monocytogenes</i>	20	30	20	16.3
<i>B. subtilis</i>	40	60	40	32.5
<i>E. coli</i>	80	60	160	32.5
<i>S. choleraesuis</i>	40	30	40	16.3
<i>Ps. aeruginosa</i>	40	60	80	16.3
<i>C. albicans</i>	40	60	40	32.5

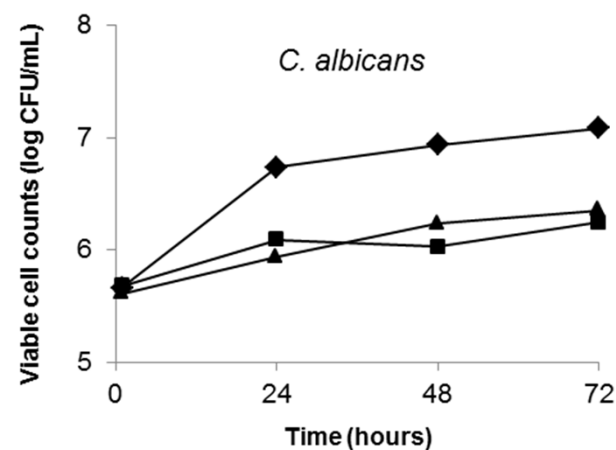
## 3. Potential Functional properties of spent coffee extracts

### Antimicrobial activity

#### 3. Growth inhibition curves



- ◆ Control
- Arabica spent coffee
- ▲ Robusta spent coffee



### 3. Potential Functional properties of spent coffee extracts



1. **Antioxidant (ROS) and genoprotective (Comet assay) effects in human cells**
2. **Antimutagenic activity**
3. **Antimicrobial activity**



## CONCLUSIONS

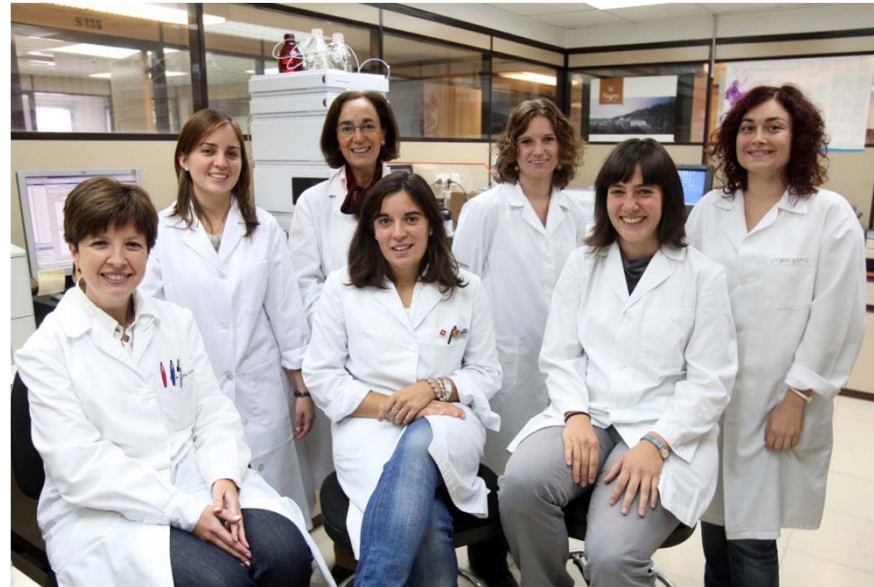
Spent coffee grounds are a **good source of natural bioactive compounds (antioxidants)** and it can be considered as a potential preservative to extend food shelf-life or to be applied to develop functional foods.

### **But... further research are needed:**

1. To optimize technological extraction conditions, mainly at industry level
2. To study the addition of spent coffee grounds to other foods or products to develop functional foods (also in terms of sensorial properties) or other nutraceutical, cosmetic or pharmaceutical products
3. to study the bioavailability of bioactive compounds
4. To know antioxidant activity in animal and human studies

## ACKNOWLEDGMENTS

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  - Toxicology lab
  - Microbiology lab
- University of Navarra



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