Instrumental Analysis of bacterial cells growth under incubation with *Crocus sativus* L. extracts using FT-IR spectroscopy

E. Anastasaki[1], G. Zoumpopoulou[2], K. Papadimitriou[2], P. Tarantilis[1], E. Tsakalidou[2] and M. Polissiou[1]

[1]Laboratory of Chemistry and [2]Laboratory of Dairy Research
Department of Food Science and Human Nutrition, Agricultural University of Athens, 75 Iera Odos Street, 11855, Athens, Greece

Foodborne illness is a threat to public health and challenge for food industry. Very young children, pregnant women, people with compromised immune systems and the elderly are at the most risk. Foodborne illness also known as food poisoning usually arises from improper handling, preparation, or food storage and is associated with microbial pathogens. Bacteria are a common cause of foodborne illness and especially *Salmonella* and *Escherichia coli* [1]. At present food industry uses chemical additives in several processes in order to prevent bacterial growth and extend the shelf life of foods. However, these substances have adverse effects.

In the current study, *Crocus sativus* L. extracts were tested as potential natural antimicrobial agents. The antimicrobial activity of plants extracts was studied towards Gram-negative strains belonging to the above species. Fourier transform infrared spectroscopy (FT-IR) was applied in order to evaluate the changes in the cellular composition of target bacterial cells after their exposure to extracts.

Sample preparation.

Plants were subjected to sequential extraction with petroleum ether, hexane, diethyl ether and methanol, as shown in figure 1.

All extracts were evaporated under reduced pressure and dried using rotary evaporator. Dried extracts were stored in labeled screw capped bottles at -20°C.

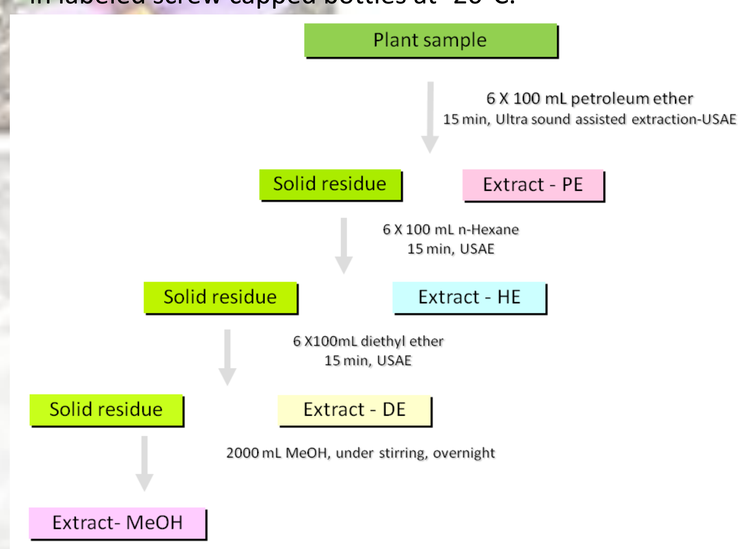

Figure 1. *Schematic plan of extraction procedure*

Screening of plants extracts against pathogens.

Different concentrations of all extracts were tested against *Escherichia coli* CFA-I, *Escherichia coli* C1845 and *Salmonella typhimurium* SL1344 by the well diffusion assay (WDA) as a preliminary screening test.

Only the diethyl ether (DE) extracts had a bactericidal effect against all tested bacteria. (Table 1.)

Table 1*. Antimicrobial activity of Crocus sativus* L. *extracts towards E.coli and Salmonella strains as determined by the well diffusion assay*

Strain	CRO-PE (mg/mL)		CRO-HE(mg/mL)	CRO-DE (mg/mL)		CRO-MeOH (mg/mL)			
	50	25	25	50	25	280	168	84	28
***Escherichia coli* CFA-I**	0	0	0	**24**	**18**	0	0	0	0
***Escherichia coli* C1845**	0	0	0	**25+6**	**18+7**	0	0	0	0
***Salmonella typhimurium* SL1344**	0	0	0	**20**	**15**	0	0	0	0

Time killing studies of diethyl ether extracts against *S. typhimurium* SL1344 and *E. coli* C1845 cells.

Antimicrobial activity was studied (*in vitro* killing assays) against cells of *S. typhimurium* SL1344 and *E. coli* C1845 in the logarithmic phase of bacterial growth.

The viability of bacterial cells was studied for 24 hours of incubation with 5 mg/mL (final concentration) dietyl ether extracts. 99% cell death of was achieved in 0,5 hours for both strains.

FT-IR Analysis.

Fourier transform infrared spectroscopy (FT-IR) was applied in the respective time period, where 99% of cell death was achieved, in order to evaluate the changes in the cellular composition of cells.

The FTIR spectrum of a biological system like bacteria is complex and consists of broad bands (Figure 2) that arise from the superposition of absorption peaks of various contributing macromolecules (proteins, lipids, polysaccharides, and nucleic acids) [2].

The FT-IR spectra of control cells were compared with the spectra of incubated with extracts cells in four different regions

Region I- 3000– 2800 cm^{-1} related to CH from fatty acids of the bacterial cell membrane

Region II- 1800– 1500 cm^{-1} related to C=O and N–H from proteins

Region III- 1500– 1200 cm^{-1} related to PO_2 from nucleic acids, as well as proteins and fatty acids

Region IV- 1200– 900 cm^{-1}: related to various absorptions of polysaccharides of the cell wall

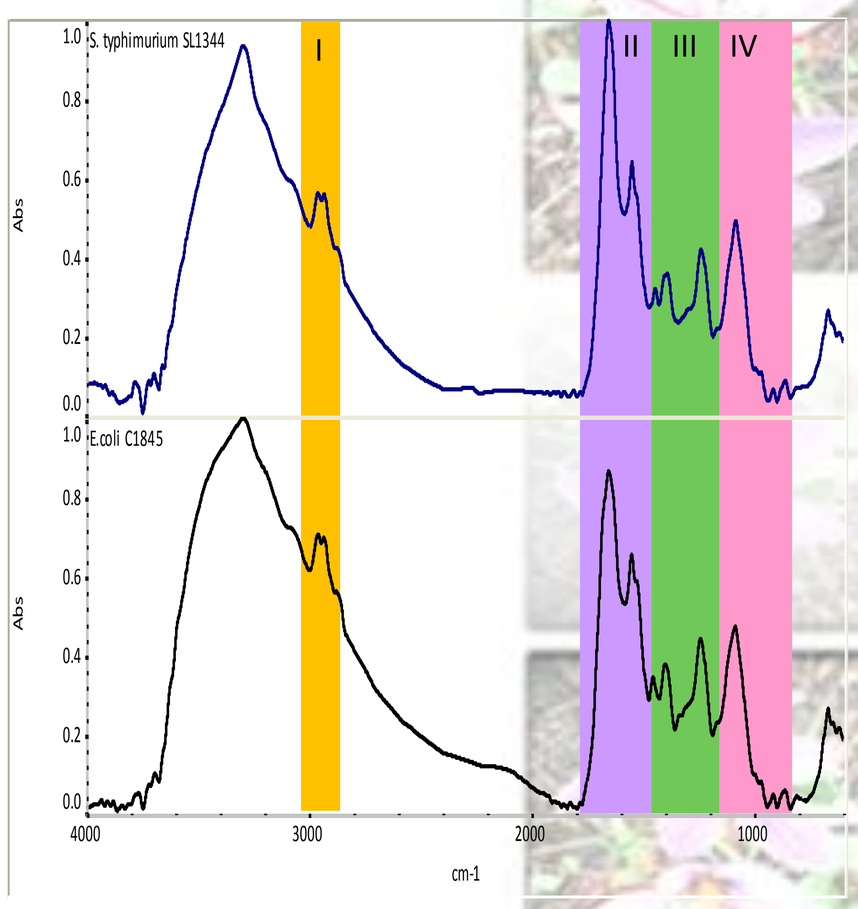

Figure 2. *FT-IR spectra of S. typhimurium* SL1344 *(A) and E. coli* C1845 *(B) cells and the characteristic regions used for comparison*

Principal component analysis (PCA) of the second derivative transformed spectra was performed for each characteristic spectral region (Figure 3) [3] .

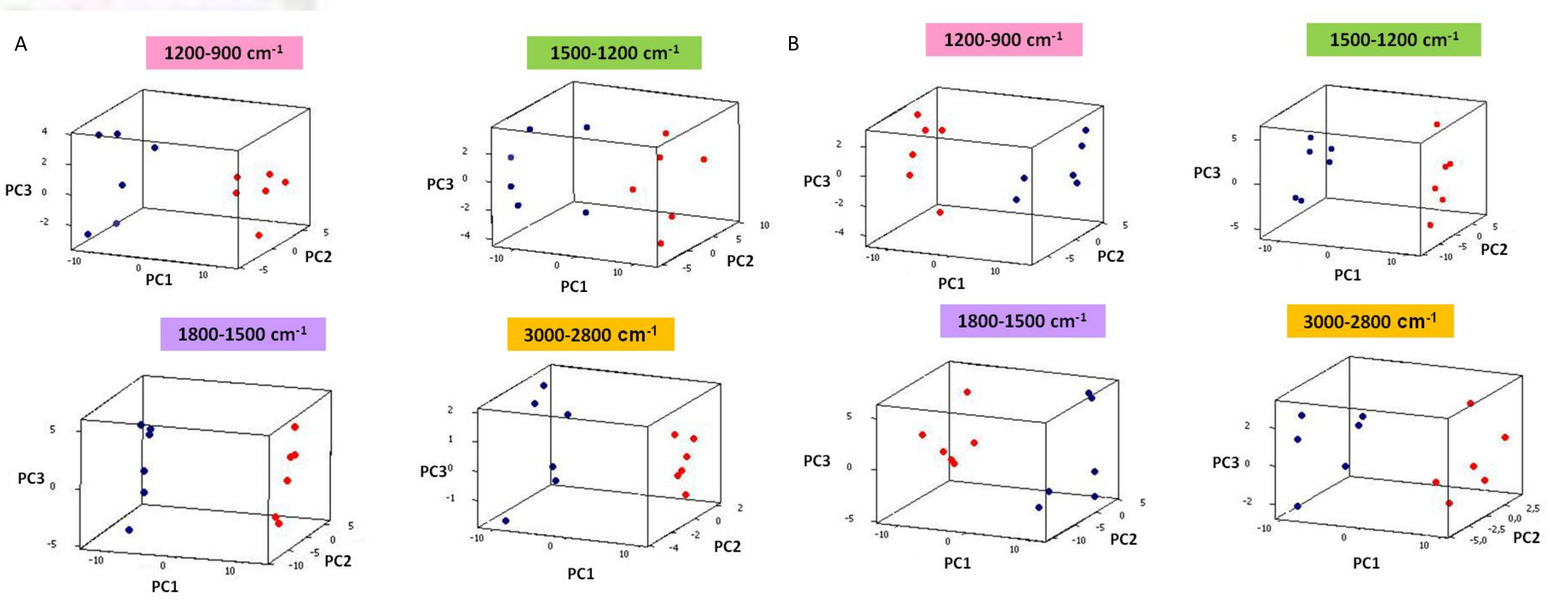

Figure 3. *PCA of second derivative transformed FT-IR spectra of S. typhimurium* SL1344 *(A) and E. coli* C1845 *(B) cells in the four characteristic spectral regions after their incubation with the control sample (MeOH) (●) and 5mg/mL diethyl ether extract of Crocus sativus* L. *(●).*

PCA revealed structural changes among cells treated with the extracts or the control sample . When *Salmonella typhimurium* SL1344 and *E. coli* C1845 cells are exposed to the antimicrobial compounds in diethyl ether *Crocus sativus* L. extracts all spectral regions, related to the major cellular structural components, are affected. (Figure 3).

Conclusions.

The results have shown that *Crocus sativus* L. diethyl ether extracts consist of important secondary metabolites in the search for new effective antibacterial agents against the pathogens responsible for foodborne illness.

FT-IR analysis along with chemometric analysis (PCA) of incubated *E.coli* and *Salmonella* cells revealed significant differences in all regions of spectra that correspond to cellular structural components.

Further studies in order to identify the antimicrobial compound(s) and to elucidate the exact mechanism of action involved in the antimicrobial activity are now been performed.

[1] R.V. Tauxe, M.P. Doyle, T. Kuchenmüller, J. Schlundt, and C.E. Stein, International Journal of Food Microbiology 139 (2010) S16- S28
[2] M. Kansiz, P. Heraud, B. Wood, F. Burden, J. Beardall and D. McNaughton, Phytochemistry 52 (1999) 407–417.
[3] K. Papadimitriou, E. Boutou, G. Zoumpopoulou, P.A. Tarantilis, M. Polissiou, C.E. Vorgias, and E. Tsakalidou, Applied and Environmental Microbiology 74 (2008) 6068–6076.

IMA-2013

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο
ΕΠΙΧΕΙΡΗΣΙΑΚΟ ΠΡΟΓΡΑΜΜΑ
ΕΚΠΑΙΔΕΥΣΗ ΚΑΙ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗ
επένδυση στην κοινωνία της γνώσης
ΥΠΟΥΡΓΕΙΟ ΠΑΙΔΕΙΑΣ, ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗΣ ΚΑΙ ΘΡΗΣΚΕΥΜΑΤΩΝ
ΕΙΔΙΚΗ ΥΠΗΡΕΣΙΑ ΔΙΑΧΕΙΡΙΣΗΣ
ΕΣΠΑ 2007-2013
Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης