# P49: DIFFERENT BEHAVIOR OF POLYPHENOLS FROM *THEOBROMA CACAO* IN ENERGY METABOLISM OF LIPOPOLYSACCHARIDE-STIMULATED CELLS

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***Abstract*** – *Theobroma cacao* is worldwide consumed and common ingredient of many food products. Cocoa, containing high-degree of polymerization proanthocyanidins, is a valuable source of bioactive compounds. Mouse embryonic fibroblast (MEF) cell lines, PON-1 KO and MCP-1 KI, were used as potential oxidant and inflammatory scenarios, respectively. Energy metabolism changes drastically when MEF are incubated with LPS. Cacao has an important effect on PON-1 KO incubated with LPS, leading metabolite values to these found in WT (particularly those related to the glycolysis).

***Keywords***: *Theobroma cacao*, proanthocyanidins, antiinflamatory, antioxidant, energy metabolism

## 1. INTRODUCTION

Polyphenols are secondary metabolites naturally synthetized in plants and have demonstrated several biological properties. *Theobroma cacao* (cocoa) is worldwide consumed and common ingredient of many food products. In the last two decades, the food industry has developed new cocoa-based products, e.g. cocoa liquor, cocoa powder, and chocolate, which are consumed worldwide and used as common ingredients of many food products. The cocoa market has remained stable over the last few years [1], and scientific interest in this potential source of bioactive compounds is growing. Indeed, a large number of studies support the health benefits of cocoa consumption, being attributed mainly to the flavanol content [1, 2]. Cocoa, containing high-degree of polymerization proanthocyanidins, is a valuable source of antioxidant compounds [3].

Oxidative stress and inflammation are the basis of the most diseases and their mechanisms are inextricably linked. Chronic inflammation is associated with oxidation, anti-inflammatory cascades are linked to decreased oxidation, increased oxidative stress triggers inflammation, and redox balance inhibits the inflammatory cellular response [4].

In this work, antioxidant and anti-inflammatory properties of procyanidin-rich extract from cocoa were tested on two mouse embrionic fibroblast (MEF) cell lines: 1) knock-out mice for paraoxonaxe-1, an antioxidant endogenous enzyme, as model of oxidation (PON-1 KO mice) and 2) transgenic mice overexpressing the monocyte chemoattractant protein 1 (MCP-1), a proinflammatory cytokine, as model of inflammation (tg-MCP-1 mice). Targeted metabolomics of energy metabolism were performed on gas chromatography coupled to a QTOF mass spectrometer and an electron impact source.

## 2. EXPERIMENTAL

### 2.1. Sample preparation

Concentrated cocoa extract (Nutrafur, Spain) dissolved in DMSO was used in this study.

### 2.2. Instrumentation, chromatographic conditions and QTOF detection

We used a 7890A gas chromatograph coupled with an electron impact source to a 7200 quadrupole time-of-flight mass spectrometer equipped with a 7693 autosampler module and a J&W Scientific HP-5MS column (30 m x 0.25 mm, 0.25 μm) (Agilent Technologies, Santa Clara, USA).

Optimized parameters for chromatographic separation and QTOF detection are explained in *Riera-Borrull et al. (2016)* [5].

Metabolites were quantitate using standard calibration curves.

### 2.3. Cell culture

Cells were grown to 80% confluence in 6-well plates using DMEM medium. *T. cacao* extract (at 100 μg/mL) was tested for 48 hours in PON-1 KO and tg-MCP-1 cells. Prior to the assay, cells were incubated 24 hours with lipopolysaccharide (LPS) from *E. coli* (100 ng/mL) to stimulate the pro-oxidant and pro-inflammatory states. Controls (with or without LPS and with or without plant extracts) were also made.

Cells, then, were scrapped in PBS and centrifuged at 2500 rpm 5 minutes. Supernatant was removed and pellets were stored at -80 ºC until use.

### *2.4. Metabolite extraction*

200 μL of methanol:water (8:2) was added to cell pellets and lysed with three cycles of freezing and thawing using liquid $N_2$. Proteins were precipitated, samples centrifuged at 14000 rpm 10 minutes, supernatant collected and dried under $N_2$ and derivatized using methoxyamine in pyridine (40 mg/mL) and N-methyl-N-(trimethylsilyl)-trifluoroacetamide.

### 2.5. Data analysis

Raw data were processed and compounds were detected and quantified using Qualitative and Quantitative Analysis B.06.00 software (Agilent Technologies), respectively. Statistical analysis was performed using the Kruskal-Wallis test with SPSS 23.0 software (IBM Corporation). Partial least square discriminant analysis (PLS-DA) were made using Metaboanalyst 3.0 (www.metaboanalyst.ca) [6].

## 3. RESULTS AND DISCUSSION

To test the antioxidant and anti-inflammatory properties of this plant, MEF PON-1 KO and tg-MCP-1 cell lines were used as potential oxidant and inflammatory scenarios, respectively. MEF wild-type (WT) were used as lean samples. Figure 1 shows the base peak chromatogram of MEF WT obtained in the GC-EI-QTOF MS.

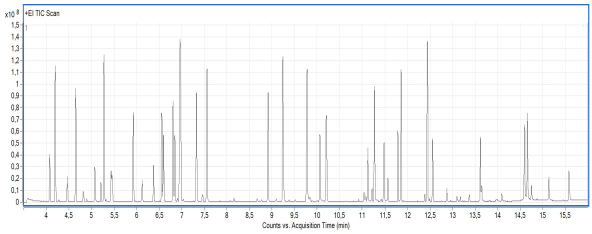


Figure 1. Base peak chromatogram of targeted metabolites of energy metabolism by GC-QTOF-MS.

Results show that energy metabolism changes drastically when MEF are incubated with LPS. *T. cacao* extract has an important effect on energy metabolism in MEF PON-1 KO (Figure 2A) incubated with LPS, leading metabolite values to these found in MEF WT (particularly those related to the glycolysis) (Figure 2B). Although, this effect is not so obvious in MEF tg-MCP-1 metabolism (Figure 3).

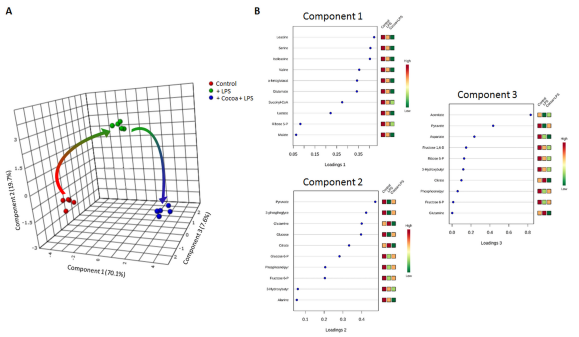


Figure 2. Effect of the treatment with cocoa extract in MEF PON-1 double knock-out treated with LPS. A) Partial least square discriminant analysis (PLS-DA). B) Importance in loading metabolites in each PLS-DA component.

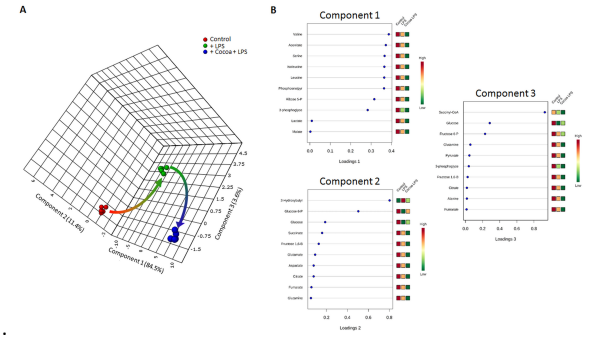


Figure 3. Effect of the treatment with cocoa extract in MEF tg-MCP-1 treated with LPS. A) Partial least square discriminant analysis (PLS-DA). B) Importance in loading metabolites in each PLS-DA component.

Although cocoa has previously demonstrate anti-inflammatory [7] activity, our results are highly related with its antioxidant properties. Procyanidins, the main compounds in cocoa, have an antioxidant capacity strongly studied [8] and can be (among other compounds) the responsible for the antioxidant capacity.

## 4. CONCLUSIONS

In the present study, we highlight the different behaviour of polyphenols as antioxidant and anti-inflammatory molecules. Cocoa extract is responsible to return the impairment in the energy management during a pro-inflammatory and pro-oxidant status in MEF cells to these found in WT at normal conditions. Cocoa polyphenols seems to have a better capacity as antioxidant scavengers than anti-inflammatory activity.

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## REFERENCES

[1] Ellam S, Williamson G (2013) Cocoa and human health. Annu Rev Nutr 33:105–128

[2] Cádiz-Gurrea ML, Fernández-Arroyo S, Joven J, Menendez JA, Micol V, Segura-Carretero A (2015) Proanthocyanidins in Agro-Industrial By-Products: Health Benefits. In: Motohashi N (ed) Occur. Struct. Biosynthesis, Heal. Benefits Based Their Evidences Med. Phytochem. Veg. Fruits. Vol. 3. pp 63–114

[3] Cádiz-Gurrea ML, Lozano-Sánchez J, Contreras-Gámez MM, Legeai-Mallet L, Fernández-Arroyo S, Segura-Carretero A (2014) Isolation, comprehensive characterization and antioxidant activities of Theobroma cacao extract. J Funct Foods. doi: 10.1016/j.jff.2014.07.016

[4] Camps J, Rodríguez-Gallego E, García-Heredia A, Triguero I, Riera-Borrull M, Hernández-Aguilera A, Luciano-Mateo F, Fernández-Arroyo S, Joven J (2014) Paraoxonases and chemokine (C-C motif) ligand-2 in noncommunicable diseases. Adv Clin Chem 63:247–308

[5] Riera-Borrull M, Rodríguez-Gallego E, Hernández-Aguilera A, Luciano F, Ras R, Cuyàs E, Camps J, Corbí AL, Segura-Carretero A, Menendez JA, Joven J, Fernández-Arroyo S (2016) Exploring the process of energy generation in pathophysiology by targeted metabolomics: Performance of a simple and quantitative method. J. Am. Soc. Mass Spectrom 27:168-177

[6] Xia J, Sinelnikov IV, Han B, Wishart DS (2015) MetaboAnalyst 3.0 - making metabolomics more meaningful. Nucleic Acids Res 43:W251-W257

[7] Selmi C, Mao TK, Keen CL, Schmitz HH, Eric Gershwin M (2006) The anti-inflammatory properties of cocoa flavanols. J Cardiovasc Pharmacol 47:S163-S171

[8] Li S, Xu M, Niu Q, Xu S, Ding Y, Yan Y, Guo S, Li F (2015) Efficacy of Procyanidins against In Vivo Cellular Oxidative Damage: A Systematic Review and Meta-Analysis. PLoS One 10: e0139455