Effect of consumption of green tea extracts on the plasma molecular signature

Rúben A.D. Araújo, *Member*, *IEEE*; Luís M. Ramalhete, *Member*, *IEEE*; Edna Ribeiro; Cecília R.C. Calado, *Member*, *IEEE*

Abstract **–** Epigallocatechin-3-gallate (EGCG), the major catechin present in green tea, displays diverse biological activities as anti-oxidation, anti-inflammation, anti-proliferation and anti-microbial among others. In the present work it was evaluated the effect of the consumption of EGCG along 90 days on healthy human volunteers (n=30) on plasma molecular signature acquired by mid-infrared (MIR) spectroscopy. It was observed by principal component analysis of spectra that plasma samples presented a significant different molecular profile after 90 days of EGCG consumption. Based on the corresponding loading vector, it was observed that EGCG consumption affected the profile of the major molecules as proteins and lipids. Were identified diverse ratios of spectral bands statistically different ($p < 0.01$) after EGCG consumption, according to a high impact of EGCG on the general metabolism. MIR spectroscopy enabled to acquire the plasma whole molecular signature in a highly sensitive and specific mode. Since the MIR spectra is also acquired in an economic, simple, fast and high-throughput mode, the technique presents promising characteristics to acquire information in large-scale epidemiological studies towards a better understand of the *in vivo* effect of EGCG.

Keywords: Green tea, epigallocatechin, polyphenols, metabolomics

I. INTRODUCTION

Tea, namely black and green tea, is the most consumed beverage in the world after water [1]. From the bioactive compounds present in green tea, diverse studies have focused their attention in epigallocatechin-3-gallate (EGCG), the most abundant polyphenols isolated from it. EGCG presents diverse biological activities as anti-oxidant, anti-inflammatory, anti-carcinogenic and anti-microbial agent, leading in recent years to an increase of new products supplemented with extracts of green tea (reviewed in Hu et al. [2]).

R. A. D. Araújo is with ISEL, Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Rua Conselheiro Emídio Navarro, 1, 1959-007 Lisboa, Portugal (email: rubenalexandredinisaraujo@gmail.com).

L. Ramalhete is with ISEL, Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Rua Conselheiro Emídio Navarro, 1, 1959-007 Lisboa, Portugal and CSTL-T-Centro de Sangue e Transplantação de Lisboa – Área Funcional da Transplantação, Instituto Português do Sangue e da Transplantação, I.P., Alameda das Linhas de Torres, n° 117, 1769-001 Lisboa, Portugal (email: luis.m.ramalhete@gmail.com).

E. Ribeiro is with H&TRC-Health & Technology Research Centre, ESTeSL-Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Av. D. João II, lote 4.69.01, Parque das Nações, 1990-096 Lisboa, Portugal (email: edna.ribeiro@estesl.ipl.pt).

C. R. C. Calado is with ISEL, Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Rua Conselheiro Emídio Navarro, 1, 1959-007 Lisboa, Portugal (email: ccalado@deq.isel.pt).

EGCG has been associated to regulate mitochondria biogenesis, metabolism as bioenergetic and even mitochondria-mediated cell cycle and apoptosis (reviewed in Shi et al. [3]). EGCG inhibits Toll-Like receptors 4 (TLR4) [4] which induces the production of pro-inflammatory cytokines, chemokine and is associated with cancer progression. Therefore, the EGCG inhibition of TLR4 will act as anti-inflammatory while reducing cancer progression. Diverse *in vivo* studies, focusing animal models, have also pointed the EGCG activity in inhibiting cancer cells proliferation and migration, as conducted by Luo et al. [5], in *in vitro* bladder cancer cells, showing that EGCG induced apoptosis via caspases-8, -9 and -3, Bax, Bcl-2 and PARP pathways, and *in vivo* on mice, showing a decrease in tumour volume, by down-regulation of nuclear factor-kappa B and matrix metalloproteinase-9.

EGCG might present opposite activities in function of the cells metabolic status, e.g., may present anti-oxidative activity in "normal" cells and pro-oxidative to counteract cancer cells [3]. One method to direct the EGCG activity is for example to target it to specific cell receptors, as conducted by Sanna et al. [6] that developed polymeric EGCG-encapsulated nanoparticles targeting prostate specific membrane antigens. These researchers observed an enhanced anti-proliferative activity in prostate cancer (PCa) cell lines.

The present work aims to evaluate the effect of the consumption of EGCG on the plasma whole molecular signature based on mid-infrared (MIR) spectroscopy. This analytical technique detects the fundamental vibration modes present in biomolecules of a high diversity of functional groups in biological samples, such as C-C, C=C, C-O, C=O, CN, CP, CH, PO, CP, OH, NH, among others. The acquisition of the complete molecular signature based on this technique benefits from a high sensitivity and specificity, making it a prime candidate for research in the field of diagnosis and prognosis of diverse diseases as infectious [7], neurodegenerative and cancer [8,9].

II. MATERIALS AND METHODS

A. Biological assay

All participants (n=30) provided a signed written informed consent before enrolment in the study approved by a research ethics committee. Previous studies established that 400 mg to 800 mg of EGCG intake are considered safe doses previously utilized in human clinical trials [10]. These doses result in peak serum concentrations in the range of 100 to 400 ng/ml [11]. In this study, participants took 225mg of EGCG present in commercial capsules each day for 90 days. Blood samples were taken just before the start of the assay (T-0) and at the end of the 90 days. Plasma was obtained by centrifugation fresh blood sample 3500 rpm for 10 min.

B. MIR spectra acquisition

Triplicates of 25 µL of plasma diluted at 1/10 were transferred to a 96-wells Si plate and then dehydrated for about 2.5 h, in a desiccator under vacuum. Spectral data was collected using a FTIR spectrometer (Vertex 70, Bruker) equipped with an HTS-XT (Bruker) accessory. Each spectrum represented 64 coadded scans, with a 2cm^{-1} resolution, and was collected in transmission mode, between 400 and 4000 cm^{-1}. The first well of the 96-wells plate did not contain a sample and the corresponding spectra was acquired and used as background, according to the HTS-XT manufacturer.

C. Spectra pre-processing and processing

Spectra were pre-processed by atmospheric correction, baseline correction and normalization to amide I peak. Second derivative spectra were computed from raw spectra using a Savitzky-Golay filter, and a 2rd order polynomial over a 15-point window.

Atmospheric and baseline corrections were conducted with OPUS® software, version 6.5 (Bruker, Germany) and all remaining pre-processing work and principal component analysis (PCA) was conducted with Matlab (Matworks, Natick, MA, USA).

Absorbance ratios were determined based on spectra pre-processed with atmospheric and baseline correction and normalization. To evaluate possible differences in the selected absorbance ratios a paired *t*-test was performed at 1% significance level.

III. RESULTS AND DISCUSSION

It was observed that 90 days of a daily consumption of 225 mg of EGCG affected diverse clinical outputs as the hematocrit, hemoglobin, reticulocyte hemoglobin and fetal hemoglobin content at 1% level of significance.

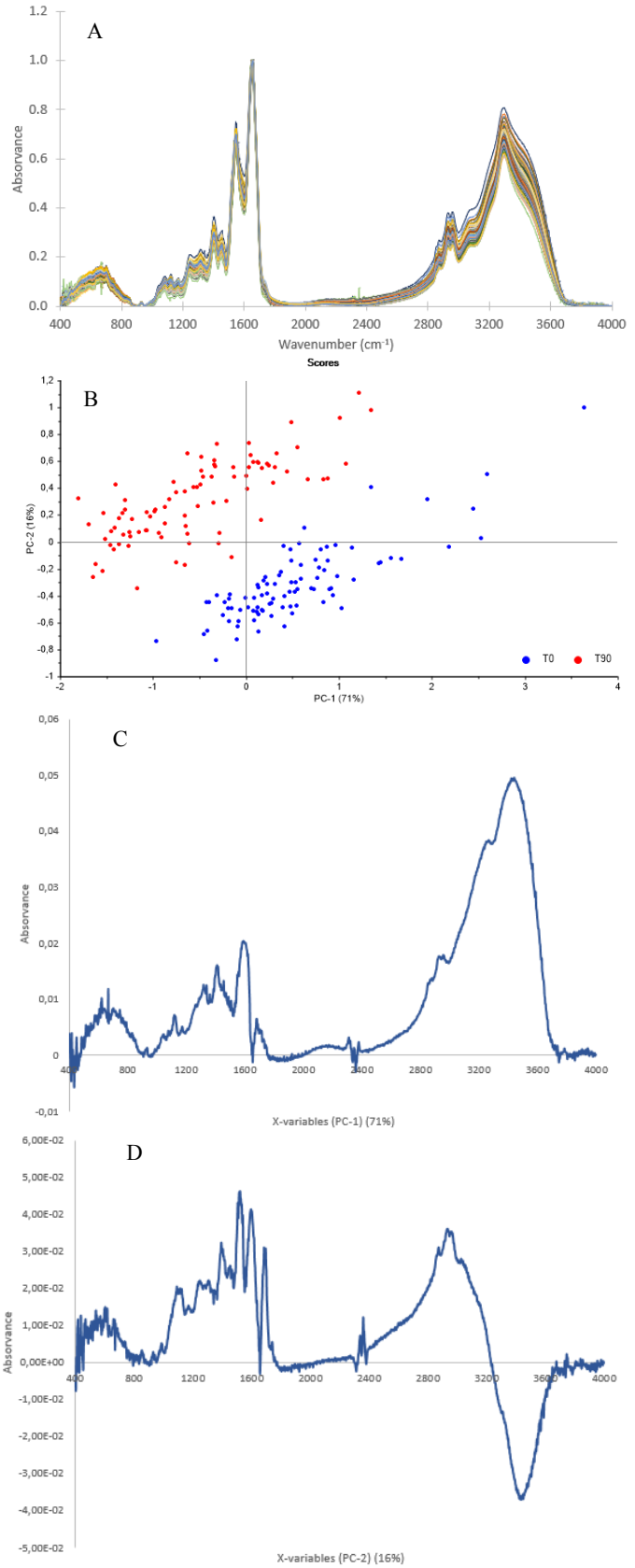

Fig. 2. Spectra from plasma after atmospheric, baseline correction and normalization to amide I (A), the corresponding PC score-plot (B) and the loading vectors of PC-1 (C) and PC-2 (D).

The non-target metabolomics of plasma was acquired by MIR spectroscopy, as this technique retrieves the metabolic fingerprint of biological fluids and cells [8,12]. Fig. 1A represents the MIR spectra of plasma of 30 participants after atmospheric, baseline correction and normalization to amide I (at 1650cm^{-1}), and Fig. 1B the corresponding score-plot of PCA. It was possible to observe in the score-plot of PC-1 *versus* PC-2, which make up for 87% of data variance, a clear separation between all plasma samples taken just before the start of EGCG consumption (T0) when comparing to the plasma samples taken after 90 days (T90) of EGCG consumption. Therefore, the EGCG consumption significantly affected the plasma molecular composition.

The loading vectors of PC-1 and PC-2 (Fig. 1C and 2D) highlight the spectra regions that contributed the most for plasma samples separation between T0 and T90. These were: amide A from proteins (between 3000 and 3500 cm^{-1}); the methyl and methylene groups from lipids (between 2850 and 2970 cm^{-1} and between 1300 to 1450 cm^{-1}); amide I and II (1650 and 1550 cm^{-1}) from proteins; phosphate groups (at 1080 and 1240 cm^{-1}) from nucleic acids, phospholipids and phosphorylated proteins; ribose from nucleic acids (at 1125 and 1170 cm^{-1}); and the fingerprint region between 400 to 800 cm^{-1}, representing combinations of diverse functional groups.

Based on the identification of the most relevant spectral bands on the PCA loading vectors, it was subsequently evaluated diverse ratios of spectral bands, as this dimensionless ratio minimizes the effect of the spectra baseline distortion and sample quantity. For simplicity, the annotation Axx represents the absorbance at the wavenumber xx. Fig. 2 represents some of these absorbance ratios observed before and after 90 days of EGCG consumption.

It was observed that the following spectral ratios discriminate the plasma between T0 and T90 at a significance of 1 %: From the fingerprint region, A455/A478, A594/A621, A664/A700 and A700/A743; from the amide A and lipids region, A3076/A3300, A2966/A3300 and A2966/A3076; the A1170/A1547 representing the ratio between RNA and proteins; and diverse ratios relative to phosphate groups (1083 and 1240 cm^{-1}) in relation to amide I and II as A1240/A1547, A1083/A1547 and A1083/A1650. This highlights the high impact of EGCG consumption on the whole plasma molecular composition. These observations are in accordance with the high impact of EGCG on the general cell metabolism as on metabolic enzymes associated to glycolysis, pentose phosphate pathway and serine biosynthesis [12], mitochondria energetic metabolism [13], lipid metabolism and lipid peroxidation [14,15], cell division and apoptosis [16].

Fig. 2 presents data from some ratios of absorbance observed before and after 90 days of EGCG consumption, statistically different at 1% significance: The A2966/A3076 representing the ratio between methyl group of lipids and amide A of proteins; A1083/A1650, representing the ratio between phosphate groups and amide I of proteins; A1083/A1240, representing the ratio between symmetric and asymmetrical vibrations of phosphate groups and A594/A621 from the fingerprint region.

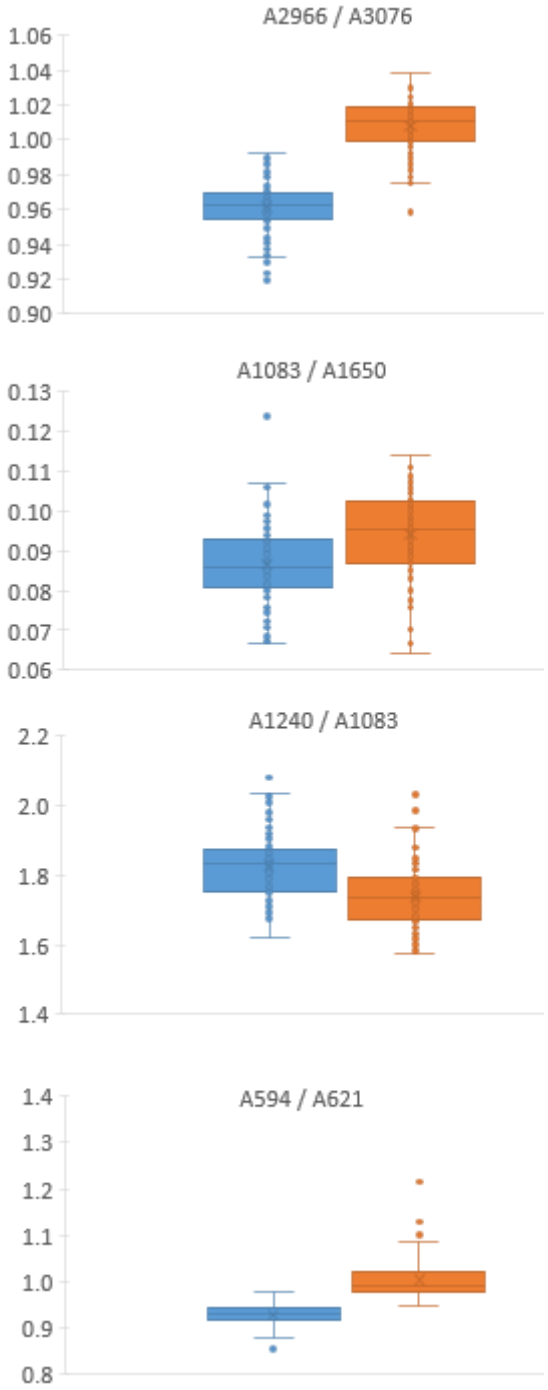

Fig. 2. Box-whiskers representation of median and other quartiles of some ratios of absorbance bands observed before the EGCG consumption (blue boxes) and after 90 days of consumption (orange boxes).

In resume, the presented method, based on MIR spectroscopy, enabled to capture the whole plasma molecular signature in a highly specific and sensitive mode. Since the MIR spectra is acquired based on an economic, simple, fast and high-throughput technique, it can be applied *in vivo* large-scale studies in order to further understand the metabolic impact of EGCG consumption.

V. ACKNOWLEDGEMENTS

This work was supported by *Instituto Politécnico de Lisboa* under grants IDI&CA/IPL/2017/GenTox/ESTeSL and IDI&CA/IPL/2018/RenalProg/ISEL. The present work was partially conducted in *Escola Superior de Tecnologia da Saúde de Lisboa*, *Instituto Politécnico de Lisboa* and in the Engineering & Health Laboratory through a collaboration protocol established between *Universidade Católica Portuguesa* and *Instituto Politécnico de Lisboa*.

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