Universidad de Navarra

FACULTAD DE FARMACIA Y NUTRICIÓN

# Impact of heat treatment on selected vegetables: bioaccessibility of (poly)phenolic compounds after *in vitro* gastrointestinal digestion and colonic microbiota action, and furan occurrence

# Impacto del tratamiento térmico en vegetales seleccionados: bioaccesibilidad de compuestos fenólicos -tras digestión gastrointestinal *in vitro* y acción de la microbiota-, y generación de furano

**Isabel Juániz Zurbano**

Pamplona, Junio de 2017

Universidad de Navarra

# Impact of heat treatment on the selected vegetables: bioaccessibility of (poly)phenolic compounds after *in vitro* gastrointestinal digestion and colonic microbiota action, and furan occurrence

# Impacto del tratamiento térmico en vegetales seleccionados: bioaccesibilidad de compuestos fenólicos -tras digestión gastrointestinal *in vitro* y acción de la microbiota-, y generación de furano

Memoria presentada por Dña. Isabel Juániz Zurbano para aspirar al grado de Doctora por la Universidad de Navarra.

El presente trabajo ha sido realizado bajo la dirección de la Dra. Mª Paz de Peña Fariza y la co-dirección de la Dra. Mª de la Concepción Cid Canda en el Departamento de Ciencias de la Alimentación y Fisiología y autorizamos su presentación ante el Tribunal que lo ha de juzgar.

En Pamplona, Junio de 2017

Dra. Mª Paz de Peña Fariza

Dra. Mª de la Concepción Cid Canda

Universidad de Navarra
Facultad de Farmacia y Nutrición
Departamento de Ciencias de la Alimentación y Fisiología

La directora del Departamento, Dra. Diana Ansorena Artieda, CERTIFICA que el presente trabajo de investigación ha sido realizado por la graduada Dña. Isabel Juániz Zurbano, en el Departamento de Ciencias de la Alimentación y Fisiología de la Facultad de Farmacia y Nutrición de la Universidad de Navarra.

Dra. Diana Ansorena Artieda

En Pamplona, a 2 de Junio de 2017

Los datos presentados en este trabajo son parte de las investigaciones realizadas en el ámbito de dos proyectos de investigación financiados:

**"Capacidad antioxidante de alimentos e ingredientes alimenticios de origen vegetal. Influencia de la presencia de lípidos y de procesos de digestión gastrointestinal."**

Entidad financiadora: Plan de Investigación de la Universidad de Navarra-Fundación Universitaria de Navarra (PIUNA).

IP: Dra. Diana Ansorena Artieda

Duración: 1 Septiembre 2013 - 31 Agosto 2015

**"Matrices alimentarias de origen vegetal con potencial actividad antioxidante sometidas a diferentes tecnologías: evaluación química y biológica."**

Entidad financiadora: Ministerio de Economía y Competitividad

Referencia del Proyecto: (AGL2014-52636-P).

IP1: Dra. Mª Paz de Peña Fariza

IP2: Dra. Diana Ansorena Artieda

Duración: 1 Enero 2015 - 31 Diciembre 2017

# AGRADECIMIENTOS /ACKNOWLEDGEMENTS

Porque esta tesis es fruto del trabajo y esfuerzo de mucha gente, de todos y todas los que de una forma u otra habéis participado en su realización en el laboratorio, en la cocina, o frente al ordenador, pero también de todos y todas los que nunca habéis dejado de creer en mí y me habéis apoyado siempre incluso cuando he querido tirar la toalla. Gracias a todos y todas y en especial:

A las directoras de este trabajo, Mª Paz de Peña y Mª de la Concepción Cid, por su interés y apoyo y por haberme ayudado a descubrir el mundo de la investigación permitiéndome formar parte de un extraordinario grupo.

A la Universidad de Navarra, la Facultad de Farmacia y Nutrición y al Departamento de Ciencias de la Alimentación y Fisiología por haberme proporcionado los medios técnicos y humanos necesarios para mi formación investigadora y haberme dado la oportunidad de seguir formándome profesional y personalmente.

A la Asociación de Amigos de la Universidad de Navarra por las becas concedidas a lo largo de estos años para poder llevar a cabo la tesis.

Al Dr. José Manuel Moreno Rojas y la Dra. Gema Pereira Caro del centro IFAPA Alameda del Obispo, de la Junta de Andalucía, por su total disponibilidad a la hora de acogerme en su laboratorio y por el apoyo e interés mostrados en todo momento.

Al Dipartimento di Scienze degli Alimenti e del Farmaco della Università di Parma (Italia), in particolare al Dr. Del Rio per la cogglienza nel suo laboratorio. A Margherita e Letizia per la vostra dedicazione e aiuto con il mio progetto. A Donatto, Marta, Michele, Luca e Federico, ma soprattuto a Daniela e Alice, grazie per avermi fatto sentire di più della vostra "famiglia" e aver reso il mio soggiorno a Parma una grande esperienza. Non posso dimenticare a Pedro, grazie per tutto.

A mis compañeras de "Broma", a las que continúan y las que ya no están, gracias por esos momentos inolvidables durante los cafés. Al "Coffee Team", por aquellos maravillosos años en CIFA trabajando codo con codo y compartiendo aventuras y desventuras más allá del laboratorio. Y especial agradecimiento a Gwen, la súper técnico.

A toda mi familia, por hacerme ser la persona que soy. Especialmente a mi madre y mi hermana, porque me habéis enseñado que en la vida hay que luchar por los sueños que uno tiene y no hay que tener miedo a afrontar nuevos retos. Y a mi padre, estrella fugaz que allá donde brillas seguro te enorgullece que tu apellido deje una pequeña huella en la ciencia.

Por último a vosotros, Pepe e Irati, por ser los pilares de mi vida y por iluminar con una sonrisa cada día. Pepe, gracias por tu apoyo incondicional, por creer en mí siempre, por tu infinita paciencia y por ser el mejor compañero de aventuras y sueños que puedo tener. Esta tesis también es tuya.

**No te conformes con el qué, sino que logra saber el porqué y el cómo.**

*Robert Baden-Powell*

# ABSTRACT

Plant foods are the main source of dietary antioxidants, including (poly)phenolic compounds, with health properties. However, their bioaccessibility might be affected by culinary processes, as well as by gastrointestinal digestion and microbiota. Additionally, heat treatment induces the formation of volatile compounds, among them furan which has been classified as a possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer. Therefore, the main aim of the present research was to investigate the influence of heat treatment (frying and griddling) on the antioxidant capacity, nutritional composition and (poly)phenolic compounds in selected vegetables (yellow onion (*Allium cepa*), Italian green pepper (*Capsicum annuum*) and cardoon stalks (*Cynara. cardunculus* L.)), as well as their bioaccessibility after a simulated gastrointestinal digestion and colonic microbiota action. Moreover, the presence of furan in heat treated vegetables in comparison with that formed during deep-frying in bread coated frozen foods was also investigated.

A total of 7, 24 and 25 free and bound (poly)phenolic compounds were identified and quantified in both raw and cooked onion, green pepper and cardoon, respectively. The main (poly)phenolic compounds were quercetin glucosides (approx. 90%) in onion, quercetin and luteolin derivates (>90%) in green pepper, and chlorogenic and other phenolic acids (approx. 99%) in cardoon. Heat treatments, especially griddling process, tended to increase the total (poly)phenolic compounds in vegetables, and consequently their antioxidant capacity, since DPPH and (poly)phenols were correlated ($r=0.70$, $p<0.001$). Although bound (poly)phenolic compounds decreased higher than the free ones during heat treatments, they were less affected during griddling process (19-22%) than during frying (35-80%) due to the higher temperature applied during griddling (150°C) than during frying (115°C).

Heat treatments exerted a positive effect on the bioaccessibility (%) of (poly)phenolic compounds in both green pepper (>82% in cooked pepper vs 48% in raw one) and cardoon (60-67% in cooked cardoon vs 2% in raw one). After *in vitro* gastrointestinal digestion, both griddled green pepper and griddled cardoon still maintained the highest amount of (poly)phenolic compounds (9.447 and 41.853 μmol (poly)phenolic compounds/g dm, respectively). Gut microbiota exerted a high metabolic activity resulting in a large and rapid degradation of (poly)phenolic compounds into new metabolites, being 3-(3'-hydroxyphenyl)propionic acid by far the most abundant catabolite in all samples after 24 h of fecal incubation. Catabolic pathways for colonic microbial degradation of flavonoids and chlorogenic acids have been proposed. Griddled vegetables were the samples with the highest amount of bioaccessible (poly)phenolic compounds even after the fecal fermentation.

Finally, frying process did not result in the formation of furan in vegetables, while low amount (3.5 μg $kg^{-1}$ in gridlled onion) or traces of this compound were formed in vegetables during griddling. Therefore, cooked vegetables do not represent a high exposure to furan. In contrast, deep-frying process induced the formation of considerable amounts of furan in bread-coated foods (12 μg $kg^{-1}$ (tuna pasties)-172 μg $kg^{-1}$ (onion rings)), implying a health risk in Spanish population groups with a high consumption of frozen precooked products.

# RESUMEN

Los alimentos vegetales constituyen la principal fuente de antioxidantes en la dieta, incluyendo los compuestos (poli)fenólicos, con propiedades saludables. Sin embargo, su bioaccesibilidad puede verse afectada por procesos culinarios, así como por la digestión gastrointestinal y la microbiota. Además, el tratamiento térmico induce la formación de compuestos volátiles, entre ellos el furano que ha sido clasificado como posible carcinógeno en humanos (grupo 2B) por la Agencia Internacional para la Investigación del Cáncer. Por todo ello, el objetivo general de esta tesis doctoral fue investigar la influencia de los tratamientos térmicos (fritura y plancha) en la composición nutricional, la capacidad antioxidante, y los compuestos potencialmente responsables de la misma (compuestos (poli)fenólicos) en alimentos vegetales seleccionados (cebolla (*Allium cepa*), pimiento verde (*Capsicum annuum*) y cardo (*Cynara cardunculus L.*)), así como su bioaccesibilidad después de un proceso *in vitro* de digestión gastrointestinal y acción de la microbiota del colon. Así mismo, se planteó evaluar la presencia de furano en dichos alimentos y compararla con el generado durante la fritura de alimentos precocinados empanados.

Se identificaron y cuantificaron un total de 7, 24 y 25 compuestos (poli)fenólicos libres y unidos en muestras crudas y cocinadas de cebolla, pimiento verde y cardo, respectivamente. Los principales compuestos (poli)fenólicos fueron los glucósidos de quercetina (aprox. 90%) en cebolla, derivados de quercetina y luteolina (>90%) en pimiento verde, y ácidos clorogénicos y otros ácidos fenólicos (aprox. 99%) en cardo. Los tratamientos térmicos, en especial el tratamiento a la plancha, tendieron a aumentar el total de compuestos (poli)fenólicos en los vegetales y, en consecuencia, su capacidad antioxidante, ya que el DPPH y los (poli)fenoles están correlacionados ($r=0,70$, $p<0,001$). Aunque los compuestos (poli) fenólicos unidos disminuyeron más que los libres durante los tratamientos térmicos, se vieron menos afectados cuando se aplicó un tratamiento a la plancha (19-22%) que durante la fritura (35-80%) debido a la mayor temperatura aplicada durante la plancha (150°C) que durante la fritura (115°C).

Los tratamientos térmicos ejercieron un efecto positivo en la bioaccesibilidad (%) de los compuestos (poli)fenólicos tanto en el pimiento verde (>82% en pimiento cocinado frente al 48% en el crudo) como en el cardo (60-67% en cardo cocinado frente al 2% en el crudo). Después de la digestión gastrointestinal *in vitro*, tanto el pimiento verde como el cardo sometidos a tratamiento a la plancha continuaron manteniendo la mayor cantidad de compuestos (poli)fenólicos (9,447 y 41,853 µmol (poli) fenólicos/g dm, respectivamente). La microbiota intestinal ejerció una alta actividad metabólica, que resultó en una intensa y rápida degradación de los compuestos (poli)fenólicos en nuevos metabolitos, siendo el ácido 3-(3'-hidroxifenil) propiónico el catabolito más abundante en todas las muestras después de 24 h de incubación fecal. Se han propuesto rutas catabólicas para la degradación microbiana en el colon de flavonoides y ácidos clorogénicos. Los vegetales tratados a la plancha fueron los que presentaron la mayor cantidad de compuestos (poli)fenólicos bioaccesibles, incluso después de la fermentación fecal.

Por último, el proceso de fritura no dio lugar a la formación de furano en los vegetales, mientras que en los vegetales a la plancha se formaron pequeñas cantidades (3,5 μg $kg^{-1}$ en cebolla a la parrilla) o trazas de este compuesto. Por lo tanto, los vegetales cocinados no representan una alta exposición al furano. Por el contrario, el proceso de fritura indujo la formación de cantidades considerables de furano en los alimentos precocinados empanados (12 μg $kg^{-1}$ (empanadillas de atún)-172 μg kg-1 (aros de cebolla)), implicando un riesgo para la salud en los grupos de población españoles con un alto consumo de productos precocinados empanados.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| $ABTS^{\bullet+}$ | 2,2′-azinobis (3-ethylbenzothiazonile-6-sulfonic acid) diammonium salt |
| AECOSAN | Agencia Española de Consumo, Seguridad Alimentaria y Nutrición |
| ANOVA | Analysis of Variance |
| AOAC | Association of Analytical Communities |
| BMDL 10 | Benchmark Dose Lower Confidence Limit of 10% |
| CAR/PDMS | Carboxen/Polydimethylxilosane |
| CQA | Caffeoylquinic Acid |
| CVD | Cardiovascular Diseases |
| DiCQA | Dicaffeoylquinic Acid |
| DM | Dry Matter |
| $DPPH^{\bullet}$ | 2,2- diphenyl-1-picrylhydrazyl |
| EFSA | European Food Safety Authority |
| FAME | Fatty Acid Methyl Esters |
| FAO | Food and Agriculture Organization |
| FID | Flame Ionization Detector |
| GA | Gallic Acid |
| GC | Gas Chromatography |
| HPLC | High Performance Liquid Chromatography |
| IARC | International Agency for Research on Cancer |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LC | Liquid Chromatography |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| MAGRAMA | Ministry of Agriculture, Food and Environment |
| MOE | Margin of Exposure |

| | |
|---|---|
| MS | Mass Spectrometry |
| MUFA | Monounsaturated Fatty Acids |
| nd | Not Detected |
| P95 | 95th Percentile |
| P99 | 99th Percentile |
| PBS | Phosphate Buffered Saline |
| PC | Principal Components |
| PCA | Principal Component Analysis |
| PDA | Photodiode Array |
| PUFA | Polyunsaturated Fatty Acids |
| SD | Standard Deviation |
| SFA | Saturated Fatty Acids |
| SPME | Solid-Phase Microextraction |
| SuccinyldiCQA | Succinyldicaffeoylquinic |
| TPC | Total Phenolic Compounds |
| TFC | Total Flavonoid Compounds |
| tr | Traces |
| Trolox | 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid |
| UHPLC | Ultra High Performance Liquid Chromatography |
| US FDA | United States Food and Drug Administration |
| WHO | World Health Organization |

# CONTENTS

# INTRODUCTION

Mediterranean Diet is characterized by a wide variety of products such as cereals (whole grain), plenty of fruit, vegetables and nuts, olive oil, legumes, herbs and spices, fish and seafood, and moderate amounts of meat and wine (Gerber & Hoffman, 2015). In general Europe produces a broad range of fruits and vegetables thanks to its varied climatic and topographic conditions, and it is one of the main global producers of some plant foods, being the Mediterranean countries such as Turkey, Spain or Italy the largest producers (Eurostat, 2016). Specifically, in 2015 Europe produced 17.6 million tonnes of tomatoes, 6.1 million tonnes of onions and 5.1 million tonnes of carrots (Eurostat, 2016). Spain is one of the main producer countries and this high production also favors its high consumption of vegetables. Recent data indicate that the consumption of fresh vegetables, without taking into account fresh potatoes, in Spanish households in 2015 was 163.58 g/capita/day (MAGRAMA, 2015). This consumption was increased around 13% in the last decades, since in 2001 the intake of fresh vegetables was 144.93 g/capita/day (MAGRAMA, 2001). Specifically, the consumption of tomatoes among Spanish population reached 38.30 g/capita/day in 2015, resulting the most consumed vegetable, followed by onion and peppers with a consumption of 20.13 and 12.90 g/capita/day respectively (MAGRAMA, 2015). Furthermore, in Spain and, in general, in Europe, there are many local vegetables as cardoon, chard, borage or cabbage which have also a high acceptability among population depending on the region (AECOSAN, 2011).

Usually vegetables, like other foods, are consumed after a cooking process, which provides food with specific organoleptic characteristics, allowing them to be consumed as a basis in different recipes or as a garnish. But vegetables are not only interesting from a culinary and gastronomic point of view, but especially from the health point of view. Plant foods are the main source of dietary antioxidants, including phenolic compounds, which have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer, probably due to their ability to protect against oxidative damage in cells (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014). Additionally, vegetables are rich in minerals and vitamins and contain a large amount of water in its composition, implying a low caloric intake in the diet. Therefore, vegetables are of a great interest from the nutritional point of view and the World Health Organization and other international agencies urge for a higher consumption of plant foods (WHO, 2004).

# 1. Heat treatment

Many dietary vegetables are usually eaten both crude or after cooking in different ways. Culinary processes induce significant changes in foods such as water loss, changes in the total fat content, degradation of thermolabile compounds, and formation of others due to heat-induced chemical reactions (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Miranda et al., 2010). Since frying is one of the most common culinary techniques in Mediterranean cuisine, especially in countries like Spain, particularly its study could be of great interest. Vegetables are often subjected to a frying process to be further consumed directly, be used as a basis in different recipes or as a garnish. Frying process induces significant changes in foods, most of them related to fat such as increase of total fat content, and changes in the fatty acid profile due to the mass exchange between frying media and the fat of food (Romero, Cuesta, & Sánchez-Muniz, 2000; Miranda et al., 2010). Healthy properties of foods partially depends on the total amount of fat and the unsaturated/saturated fatty acids ratio, and consequently on the type of fat used during cooking. In Spain, olive oil is the most consumed culinary fat (around 23 ml/capita/day) followed by sunflower oil (8.5 ml/capita/day) (MAGRAMA, 2015). In addition, fried food palatability is related to unique sensory characteristics, including brown color, crunchy texture and other desired flavor and taste, mainly due to Maillard reactions (Rossell, 2001). Furthermore, Maillard reactions favor the formation of high molecular weight end-products, such as melanoidins, which could retain several compounds, like (poly)phenolic compounds into their structures (Nunes & Coimbra, 2010). Thus, the bioaccessibility of those bioactive compounds could be affected by frying processes. Some studies confirmed that higher roasting temperature induced higher formation of melanoidins and that melanoidins content also depends on the extent of roasting (Bekedam, Loots, Schols, Van Boekel, & Smit, 2008; Sacchetti et al., 2016). Therefore, not only frying process, but also other intense culinary techniques could influence (poly)phenols bioaccessibility. (Poly)phenolic compounds can be either degraded or released from plant tissue structures by thermal processes, depending on the cooking methods and their time and temperature conditions, as well as the type of vegetable (Turkmen, Sari, & Velioglu, 2005; Miglio, 2008; Pellegrini et al., 2009; Palermo, Pellegrini, & Fogliano, 2014; Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015; Guillén, Mir-Bel, Oria, & Salvador, 2017).

There are some studies that report the effect of heat treatment on the antioxidant activity and (poly)phenolic compounds in vegetables, however, results are still unclear. While boiling is the most investigated cooking method, few studies are about frying process, both deep frying and pan frying (Palermo et al., 2014) and as far as we know, only one study was found about the effect of griddling on the antioxidant capacity of vegetables, (Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009), but none of them focused on the (poly)phenol profile. In general, cooking methods without addition of water, such as microwave, or frying, seem to ensure the maximum retention of antioxidant compounds in vegetables and, consequently, result in higher levels of antioxidant capacity (Chuah et al., 2008; Jiménez-Monreal et al., 2009; Pellegrini et al., 2010). Onion is one of the most studied

vegetables, and both losses and gains in (poly)phenolic compounds after heat treatment are reported in the literature (Crozier, Lean, McDonald, & Black, 1997; Price & Rhodes, 1997; Ewald, Fjelkner-Modig, Johansson, Sjöholm, & Åkesson, 1999; Ioku et al., 2001; Lombard, Peffley, Geoffriau, Thompson, & Herring, 2005; Rohn, Buchner, Driemel, Rauser, & Kroh, 2007; Pellegrini et al., 2009; Fu et al., 2010; Rodrigues, Pérez-Gregorio, García-Falcón, & Simal-Gándara, 2009; Siddiq, Roidoung, Sogi, & Dolan, 2013; Harris, Brunton, Tiwari, & Cummins, 2015). Influence of heat treatment on phenolic compounds and antioxidant capacity on Brassica vegetables have also been well studied (Miglio et al., 2008; Wachtel-Galor, Wong, & Benzie, 2008; Gorinstein et al., 2009; Pellegrini et al., 2010; Girgin & El Nehir, 2015; Kapusta-Duch, Kusznierewicz, Leszczyńska, & Borczak, 2016; Guillén et al., 2017). Most of them are referred to broccoli and cauliflower, but few are focused on other brassica vegetables like cabbage. Despite the high consumption and the great variety of peppers, few studies about the effect of heat treatment on pepper, and specifically green pepper, have been found, and none of them focused on (poly)phenolic compound profile changes (Turkmen et al., 2005; Chuah et al., 2008; Gorinstein et al., 2009; Jiménez-Monreal et al., 2009). The influence of the heat treatment on the antioxidant capacity and phenolics of other highly consumed vegetables, such as tomatoes and artichokes, has also been reported in the literature (Dewanto, Wu, Adom, & Liu, 2002; Ferracane et al., 2008; Pellegrini et al., 2009; Lutz, Henríquez, & Escobar, 2011; Kamiloglu et al., 2014; Guillén et al., 2017).

On the other hand, Maillard reaction also induces the formation of volatile compounds that provide the characteristic aroma and flavor of roasted and fried foods. Among them, furan and furanic compounds can significantly contribute to the sensory properties of heat treated foods (Maga, 1979; Anese & Suman, 2013). However, furan is a highly volatile compound, which has been classified as a possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC, 1995).

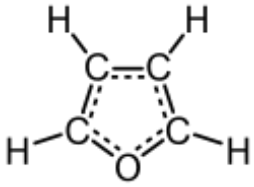



**Figure 1.** Furan

Cooking conditions such as temperature or time are important parameters on the formation of furan. Generally, higher temperatures produced higher levels of furan, especially above 120°C (Hasnip, Crews, & Castle, 2006; Senyuva & Gokmen, 2007). A study carried out by Fromberg et al. (2009) showed a positive correlation between the frying temperature and the furan content in homemade crisps, and between the degree of browning of bread slices and the level of furan formed. A previous study about bread observed how a higher temperature applied during the thermal process (170-200 °C versus 140-160°C) resulted in

a higher amount of furan concentration (Hasnip et al., 2006). Culinary techniques in general, and frying process in particular, are not only applied to vegetables, but also to other highly consumed foods. Nowadays, innovation in the food industry with the development of new food products associated to social changes in Western countries have increased the consumption of a great variety of time-saving "ready to eat" frozen foods. Many of these foods require the application of culinary techniques before their consumption, being frying process one of the most common. In Spain, the consumption of pre-cooked frozen foods, most of them bread-coated, has increased in the last years. When considering both household and catering and institutions consumption, recent data indicate that around 12.9 kg per capita per year of ready-to serve foods (including pre-cooked frozen foods) were consumed in Spain, increasing every year (4.6% higher in 2015 than in 2014) (MAGRAMA, 2015). Thus, the study of the frying process on this kind of foods could also be of interest.

Despite thermal treatment, other factors like different precursors and food composition appear to induce many pathways to furan formation. Carbohydrate degradation, pyrolysis of sugars, decomposition of ascorbic acid and oxidation of polyunsaturated fatty acids during heat treatment can promote furan generation (Perez Locas & Yaylayan, 2004; Becalski & Seaman, 2005; Märk, Pollien, Lindinger, Blank, & Marrk, 2006; Limacher, Kerler, Conde-Petit, & Blank, 2007; Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008; Owczarek-Fendor et al., 2011). Some authors suggest that carbohydrate foods are more prone to the formation of furan, probably due to the Maillard reaction, and that the retention of furan in foods is mainly caused by the lipid fraction, especially polyunsaturated fatty acids (Fromberg et al., 2009; Arisseto, Vicente, Ueno, Tfouni, & Toledo, 2011). So that, it might be expected that the content of furan in foods subjected to a frying process, especially those rich in carbohydrates, could be high.

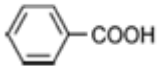
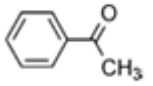
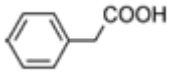
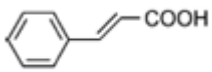
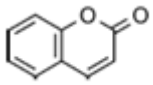
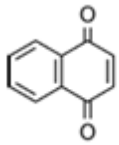
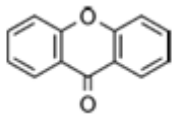
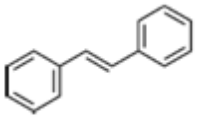
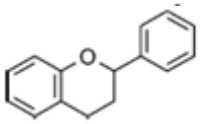
Taking into account final amount of furan in foods and different exposure scenarios, the risk assessment should be calculated. Nevertheless, there is not a consensus on what is the best form to assess the risk of substances that are both genotoxic and carcinogenic. Usually, due to all the uncertainties around the formation of furan in foods and to reduce the exposure, ALARA (as-low-as-reasonably-achievable) concept is used to apply for furan exposure. Nowadays, the Scientific Committee from EFSA (2005) recommends the use of a different approach, the margin of exposure (MOE) approach. The MOE is the ratio between a defined reference point on the dose response curve for the adverse effect and the human intake, and makes no implicit assumptions about a "safe" intake (EFSA, 2005). For calculating the dose for the adverse effect, the Scientific Committee recommends the use of the benchmark dose (BDM) to obtain the MOE. Specifically, it is advisable to use the BMDL10 which is an estimation of the lowest dose which is 95% certain to cause no more than a 10% cancer incidence in rodents (EFSA, 2005). Then, to estimate the human intake of furan, different exposure scenarios should be provided including intakes from highly exposed individuals as represented by the $90^{th}$, $95^{th}$, or $99^{th}$ percentiles (due both to high consumption of foods or to average consumption of highly contaminated foods). According to the EFSA (2005), a MOE of 10,000 or higher would be considered as a low public health concern and reasonably as a low priority for risk management actions. Coffee (for adults) and commercial baby foods (for

infants) have been proposed as the major contributors to furan exposure (Fromberg, Fagt, & Granby, 2009). Some authors have studied the risk assessment of furan in these products (Lachenmeier, Reusch, & Kuballa, 2009; Waizenegger et al., 2012). However other cooked foods could also contribute in a high extent to furan exposure due to the fact that furan formation can be influenced by the heat treatment conditions (Fromberg et al., 2009). Additionally, the global diet should be taken into account. In 2011, WHO estimated the risk assessment of furan obtaining MOEs of furan of 960 and 480 for average and high dietary exposures, respectively (WHO, 2011). Therefore, the Committee considered that these MOEs indicate a human health concern for furan. Nevertheless, an EFSA report highlights that only 8% of the furan data were reported after food preparation and it claims that future testing of furan should preferably analyzed both as purchased and as consumed indicating the exact cooking preparation conditions (time, temperature and handling label information) (EFSA, 2010).

## 2. (Poly)phenolic compounds

(Poly)phenolic compounds are plant secondary metabolites commonly found in vegetables and fruits. They are compounds with at least one aromatic ring with one or more hydroxyl groups attached and represent a wide variety of compounds which are divided into several classes. They can be classified by the number and arrangement of their carbon atoms (Table 1).

**Table 1.** Basic structural skeletons of (poly)phenolic compounds (Adapted from Crozier et al., 2009)

| Classification | Skeleton | Basic Structure |
|---|---|---|
| Phenolic acids | $C_6$-$C_1$ |  |
| Acetophenones | $C_6$-$C_2$ |  |
| Phenylacetic acid | $C_6$-$C_2$ |  |
| Hydroxycinnamic acids | $C_6$-$C_3$ |  |
| Coumarins | $C_6$-$C_3$ |  |
| Naphthoquinones | $C_6$-$C_4$ |  |
| Xantones | $C_6$-$C_1$-$C_6$ |  |
| Stilbenes | $C_6$-$C_2$-$C_6$ |  |
| Flavonoids | $C_6$-$C_3$-$C_6$ |  |

Usually phenolics are classified into two groups: flavonoids and non-flavonoids. Flavonoids are (poly)phenolic compounds comprising fifteen carbons, with two aromatic rings connected by three-carbon bridge (Figure 1). The basic flavonoid skeleton can have numerous substituents, hydroxyl groups as well as sugars are very common. The majority of flavonoids exist naturally as glycosides. Flavonols including quercetin, isorhamnetin, myricetin or kaempferol, are the most widespread of the flavonoids. Most of them are present in plants as

O-glycoside, such as occurs in onion where the main phenolic compounds are quercetin-glycosides, mainly those sugars at the 4-position (Murota & Terao, 2003; Bonaccorsi, Caristi, Gargiulli, & Leuzzi, 2005; Lombard et al., 2005). With a similar structure to flavonols, the flavones are found, including apigenin or luteolin. A wide range of substitutions are also possible with flavones, being 7-O-glycoside the most common. Unlike flavonols, flavones are not as abundant, but they are the major compounds among some of the most consumed vegetables. Luteolin derivates are the most abundant phenolic compounds in green peppers, accounting for around 80% of total phenolics (Marín, Ferreres, Tomás-Barberán, & Gil, 2004).

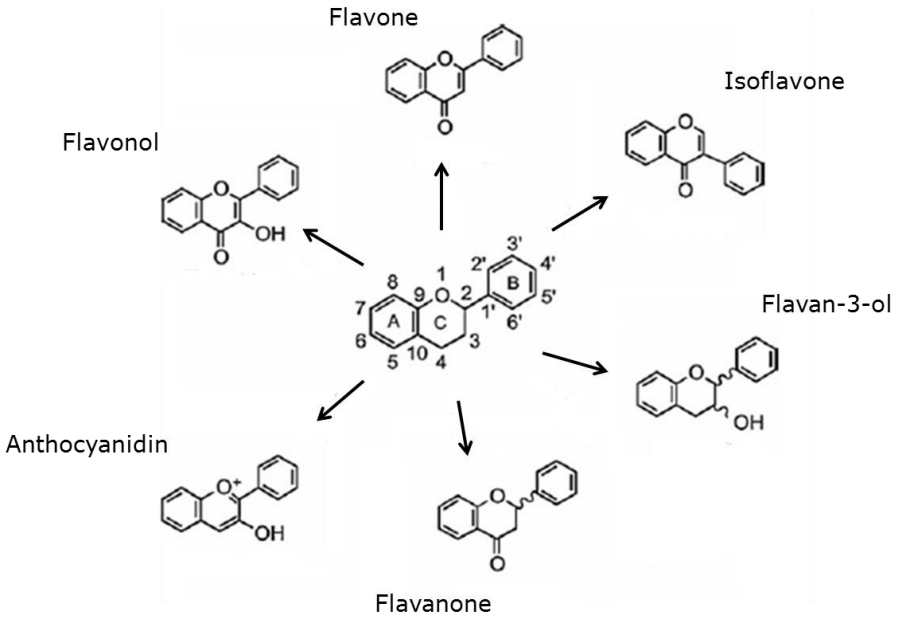



**Figure 2.** Generic structure of the major flavonoids (Adapted from Del Rio et al., 2013)

Among non-flavonoid compounds, phenolic acids ($C_6$-$C_1$) are the most abundant, however other non-flavonoid compounds such as hydroxycinnamic acids are also a good source of antioxidants. Chlorogenic acids and derivates are probably, some of the most known and most studied compounds. They are the main (poly)phenolic compounds in coffee (Bravo et al., 2012; Ludwig et al., 2012) but they are also present in other vegetables, such as *Cynara cardunculus* (Pinelli et al., 2007; Ramos et al., 2014). This kind of vegetables is commonly consumed in Spain and includes different vegetables, such as artichoke and cardoon. Major (poly)phenols of artichoke are caffeic acid derivatives, which mainly occur as esters with quinic acid (Häusler, Ganzera, Abel, Popp, & Stuppner, 2002; Wang et al., 2003; Mulinacci et al., 2004; Schütz, Kammerer, Carle, & Schieber, 2004;). However, only a few studies about stalks (cardoon) composition have been reported (Velez et al., 2012; Ramos et al., 2014), and only one of them provided information on the (poly)phenolic profile by high-performance liquid chromatographic (HPLC) (Ramos et al., 2014).

Phenolic compounds can also be divided into free, esterified and insoluble-bound forms, depending on they occur in the free form or covalently bound to other molecules such as fatty acids (soluble esters) or insoluble macromolecules (insoluble-bound phenolics). Most insoluble-bound phenolics chemically form covalent bonds with cell wall substances including pectin, cellulose, arabinoxylan and structural proteins. Bound (poly)phenolics fraction in vegetables can account up to 60% depending on the food matrix (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014; Nayak, Liu, & Tang, 2015; Shahidi & Yeo, 2016). Free, esterified and bound (poly)phenolic estimation has been carried out since the 1980s (Krygier, Sosulski, & Hogge, 1982a, 1982b; Sosulski, Krygier, & Hogge, 1982; Naczk & Shahidi, 1989) applying different alkaline, acid and enzymatic hydrolyses conditions in order to measure their content in food (Acosta-Estrada et al., 2014; Shahidi et al., 2016). Moreover, some authors have reported that food processing such as thermal processing and fermentation, can release bound phenolic acids from food matrix (Shahidi et al., 2016).

## 2.1 (Poly)phenolic compounds and health

Despite the high variety, (poly)phenolic compounds in general have been reported to exhibit a wide range of protective effects against several diseases, particularly neurodegenerative diseases, cardiovascular diseases (CVD), and cancer, probably due to their ability to protect against oxidative damage in cells (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014). Some studies showed the pathophysiological role of inflammation in the development of neurological disorders (Rodriguez-Mateos et al., 2014). In addition, some studies indicated that (poly)phenols may inhibit the development of Alzheimer's disease-like pathology and counteract age-related cognitive declines (Vauzour, 2012). However, there is no consistent information regarding (poly)phenols ability to reach the brain (Rendeiro, Rhodes, & Spencer, 2015). (Poly)phenols must first cross a tightly regulated, selectively permeable endothelial cell layer which can excludes many substances. Animal studies detected some compounds, such as naringenin and some epicatechins, in the brain after (poly)phenolic compounds consumption (Abd El Mohsen et al., 2002). Nevertheless, other (poly)phenolic compounds such as quercetin-4'-*O*-glucoside or caffeic acid did not result in the accumulation in the brain (Mullen et al., 2008; Omar et al., 2012). Regarding human studies, there are limitations for the study *in vivo* and most of them are epidemiological studies, which have reported the possible relationship between (poly)phenols rich foods and neurodegenerative disorders, concluding positive effects against Alzheimer disease, Parkinson and dementia (Rodriguez-Mateos et al., 2014).

With regard to CVDs, two recent reviews collected numerous epidemiological studies concluding that there are epidemiological evidences indicating that a diet rich in fruit and vegetables is associated with a low risk of cardiovascular diseases (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014; Martínez-González et al., 2015). Specifically in Spain, recent results of an epidemiological study (PREDIMED) suggested that intake of (poly)phenols provides protection against CVDs throughout the inversely association found with plasma glucose, plasma triglycerides and systolic blood pressure (Guo et al., 2016). The same study

observed also an inverse association of fruits and vegetables consumption with CVD in an elderly Mediterranean population at high cardiovascular risk (Buil-Cosiales et al., 2016). Despite the large epidemiological evidence, it has to be taken into account some limitations of this kind of studies. There is a lack of reliable databases on the (poly)phenol content of foods, even though each day there are advances in analytical techniques to identify and quantify (poly)phenols levels in foods. In addition, how to collect consumption data for the estimation of dietary intakes is usually subjected to a high degree of error and bias since they are usually self-reported through food frequency questionnaires (Rodriguez-Mateos et al., 2014).

It has also been reported that a higher consumption of fruits, vegetables and legumes, rich in (poly)phenolic compound, is inversely associated with blood inflammation markers (Nanri et al., 2008; Salas-Salvadó et al., 2008) and it can be concluded that antioxidant phytochemicals present anti-inflammatory effects in the body (Hoffman & Gerber, 2015). This anti-inflammatory effects might be related in turn with the influence of (poly)phenolic compounds on carcinogenesis and tumor development. Many epidemiological studies have been performed to study the relationship between fruit and vegetables consumption and some types of cancer; however the conclusions are not as clear as in other diseases. Some authors confirmed a negative correlation between regular fruit and vegetables consumption and the development of cancer (Benetou et al., 2008; Brown et al., 2014), especially stomach cancer where (poly)phenolic compounds will be at highest concentration (Brown et al., 2014). Contrarily, other studies observed no reduction in the incidences of pancreatic and stomach cancer, or even reported the absence of evidence about the association between consumption of fruits and vegetables and cancer risk in overall (Larsson, 2006; Boffetta et al., 2010). There are few clinical intervention studies focused on cancer prevention, incidence and mortality related to foods, due to ethical aspects. The majority of clinical evidences have been shown with green tea (poly)phenols or curcumin, so that further studies including other foods and focusing in other compounds should be developed (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014).

## 2.2 Bioaccessibility of (poly)phenolic compounds

Bioavailability is a term that describes the proportion of a nutrient or bioactive compound in foods that after ingestion is used or accumulated for normal physiological functions. The bioavailability of nutrients can be subdivided into three phases: 1) Availability in the intestinal lumen for absorption, 2) Absorption and/or retention in the body, and 3) Utilization and metabolization by the organism.

The fraction of a compound that is released from its food matrix in the gastrointestinal tract and thus become available for intestinal absorption is usually named bioaccessibility. Otherwise, bioactivity includes events linked to how the bioactive compounds are transported and reached the target tissue, as well as the metabolism and generation of biomarkers and the subsequent physiological response in the organism. From a rigorous viewpoint,

bioavailability includes both bioaccessibility and bioactivity (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009) (Figure 2).

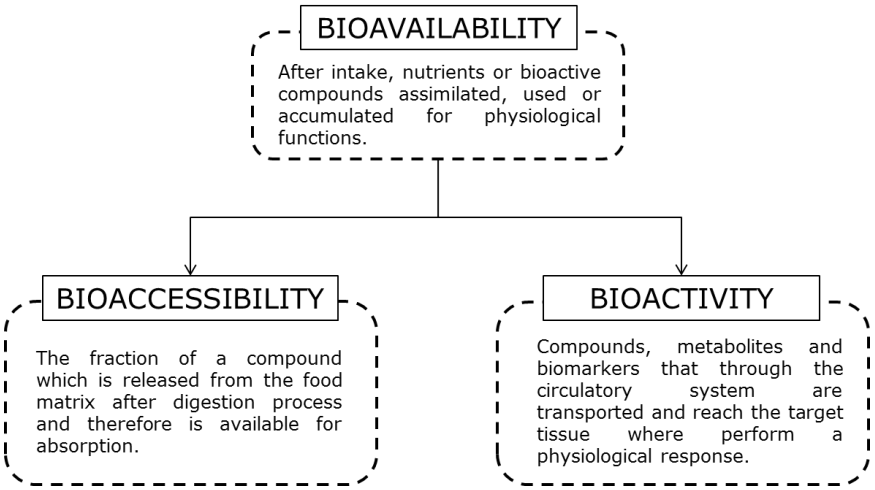



**Figure 3.** Bioavailability, bioaccessibility and bioactivity.

Vegetables are usually eaten cooked, and the softening effect of the cooking process due to heat-induced wall and cell ruptures can affect (poly)phenolic extractability (Palermo et al., 2014), possibly favouring the release of these compounds from the food matrix. Some studies focused on the bioaccessibility of (poly)phenolic compounds demonstrated a higher bioaccessibility after thermal treatment of some vegetable foods, such as tomatoes and cauliflower (Bugianesi et al., 2004; Girgin & El Nehir, 2015).

After ingestion, (poly)phenols can also be modified in the gastrointestinal tract by digestive enzymes and, consequently, their bioaccessibility might be affected. The stomach reduces the particle size of food, in turn potentially enhancing the release of phenolic compounds (Scalbert, Morand, Manach, & Rémésy, 2002). Most of the (poly)phenolic compounds, mainly flavonoids, occur in foods as glycosides and it is in particular the glycosylation one of the factors that may influence the absorption through the gut barrier. In fact, glycosides are absent from plasma after nutritional doses (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). Deconjugation can take place in the lumen by the action of membrane-bound lactase phlorizin-hydrolase (LPH) and aglycones may then be absorbed passively through the epithelium. The epithelial cells can also hydrolyze the glycosides by the action of cytosolic β-glucosidase, and consequently aglycones may be formed after absorption by the active sodium-dependent glucose transporter, SGLT-1 (Day et al., 2000; Németh et al., 2003). Nevertheless, the levels of flavonoids in plasma after dietary intake are low (Aura, 2008), which is probably related to their limited absorption. Chlorogenic acids are also characterised by a low absorption at small intestinal level (Erk et al., 2014). Coffee is one of the main source of chlorogenic acids in the diet (Clifford, 2000) and the bioavailability and limited absorption of its (poly)phenolic compounds is well known (Stalmach et al., 2009; Stalmach, Steiling, Williamson, & Crozier, 2010; Ludwig, de Peña, Cid, & Crozier, 2013;

Monente et al., 2015). However, other foods rich in chlorogenic acids, such as *Cynara cardunculus* L. species, have hardly been studied (Azzini et al., 2007; Garbetta et al., 2014; D'Antuono, Garbetta, Linsalata, Minervini, & Cardinali, 2015).

Absorbed compounds undergo phase II enzymatic metabolism and they can be conjugated with glucuronic acid, sulphate and methyl groups in the liver and enterohepatic recirculation may result in some recycling back to the small intestine through bile excretion, so parent compounds could not be detected in plasma (Aura, 2008; Del Rio et al., 2013). A large part of the ingested (poly)phenols could then reach the colon where they could be transformed by the local microbiota to smaller and more absorbable molecules (Ludwig et al., 2013). Gut microbiota metabolism can also modulate the health effects of dietary (poly)phenolic compounds by altering absorption, bioavailability, and biological activity. The bioactive properties of the newly formed metabolites can be different from that of their parent compounds (Duda-Chodak, Tarko, Satora, & Sroka, 2015), making the studies investigating the biological effects of vegetables phenolics *in vitro* without taking this transformation into account of very limited scientific relevance.

Trying to standardize this process, current research has proposed *in vitro* digestion models to simulate gastrointestinal conditions, using commercial digestive enzymes of human, porcine or bovine origin and bile salts, and also regulating the pH, temperature, substrate and incubation time (Hur, Lim, Decker, & McClements, 2011; Minekus et al., 2014). Figure 3 shows the characteristics of a simulated gastrointestinal digestion, including the last part of the intestine where take place the fecal fermentation by colonic microbiota and the subsequent formation of metabolites.

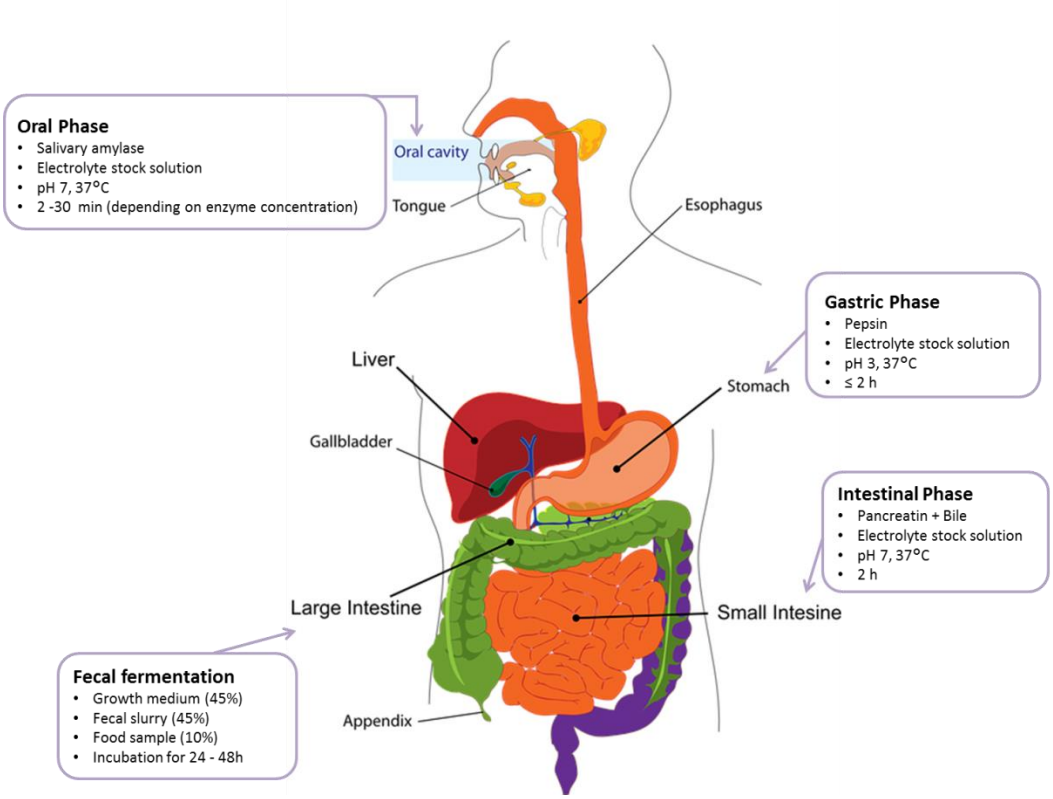



**Figure 4.** Diagram of a simulated gastrointestinal digestion.

To complete the *in vitro* digestion procedure and to study the transepithelial transport, a cell culture model of human intestinal absorption has been proven to be a good alternative to predict intestinal absorption of various substances. Some authors combine the gastrointestinal digestion with the Caco-2 cell culture model to assess globally the bioaccessibility of phenolic compounds (Dupas, Marsset Baglieri, Ordonaud, Tomé, & Maillard, 2006; Kosińska-Cagnazzo, Diering, Prim, & Andlauer, 2015; Monente et al., 2015). The absorption of (poly)phenolic compounds is still unclearly known. Some authors reported that one third of phenolic compounds was absorbed intact in human small intestine by using and ileostomy model (Olthof, Hollman, & Katan, 2001; Bugianesi et al., 2004) while other authors showed that phenolic compounds were poorly absorbed even with an absorption below 0.3% by using Caco-2 cells models (Dupas et al., 2006; Farrell, Dew, Poquet, Hanson, & Williamson, 2011; Monente et al., 2015). In general, polyphenols bioavailability in the first gastrointestinal tract has been estimated around 10%, ranging from 2 to 20% (Hu, 2007). This implies in general that part of (poly)phenolic compounds will be absorbed, but most of them will reach the colon where will be further metabolized by the microbiota where could be transformed by intestinal microbiota to smaller and more absorbable molecules. The amount of (poly)phenolic compounds that reach the colon and consequently the catabolites generated might depend on the food matrix, culinary processes, as well as the specific (poly)phenolic composition of each food (Erk et al., 2014).

Once the colon is reached, gut bacteria can hydrolyze glycosides, glucuronides, sulfates, amides, esters and lactones. They also carry out ring-cleavage. Gut microbiota metabolism can also modulate the health effects of dietary (poly)phenolics, by altering absorption, bioavailability, and biological activity and resulting to new compounds (Aura, 2008; Ludwig et al., 2013), so biological effects should not be only attributed to the native compounds present in foods but also to their metabolites. Some metabolites are potentially more biological active than the parent compounds. Some studies that take metabolic physiological modifications into consideration, demonstrate beneficial effects, such us antioxidant, anti-inflamatory, antihyperglicemic or neuroprotective activities of (poly)phenolic catabolites (Verzelloni et al., 2011; Masella et al., 2012; Duda-Chodak et al., 2015). Moreover, some compounds present in the diet can modulate the microbiota composition, resulting in changes in the metabolic activity of intestinal bacteria (Selma, Espin, & Tomas-Barberan, 2009; Duda-Chodak et al., 2015). Specific microbiota is involved in the metabolism of (poly)phenolic compounds and different enzymatic reactions are achieved by the human intestinal microbiota (Selma et al., 2009). Furthermore, interindividual variability in gut microbiota ecology could determine the phenolic compound absorption. Overall, fecal metabolomics helps study new products resulting from the interaction among food, individual genetic and gut microbiota.

## REFEERENCES

Abd El Mohsen, M. M., Kuhnle, G., Rechner, A. R., Schroeter, H., Rose, S., Jenner, P., & Rice-Evans, C. A. (2002). Uptake and metabolism of epicatechin and its access to the brain after oral ingestion. *Free Radical Biology and Medicine*, *33*(12), 1693–1702.

Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O. (2014). Bound phenolics in foods, a review. *Food Chemistry*, *152*, 46–55.

AECOSAN (Agencia Española de Consumo, Seguridad Alimentaria y Nutrición. (2011). ENIDE: encuenta nacional de ingesta dietética (2009-2010). Resultados sobre datos de consumo. URL http://www.aesan.mspsi.gob.es/AESAN/docs/docs/evaluacion_riesgos/datos_consumo/ENIDE.pdf. Access 28.09.2015

Anese, M., & Suman, M. (2013). Mitigation strategies of furan and 5-hydroxymethylfurfural in food. *Food Research International*, *51*(1), 257–264.

Arisseto, A. P., Vicente, E., Ueno, M. S., Tfouni, S. A. V., & Toledo, M. C. D. F. (2011). Furan levels in coffee as influenced by species, roast degree, and brewing procedures. *Journal of Agricultural and Food Chemistry*, *59*(7), 3118–24.

Aura, A.-M. (2008). Microbial metabolism of dietary phenolic compounds in the colon. *Phytochemistry Reviews*, *7*(3), 407–429.

Azzini, E., Bugianesi, R., Romano, F., Di Venere, D., Miccadei, S., Durazzo, A., Foddai, M. S., Catasta, G., Linsalata, V., & Maiani, G. (2007). Absorption and metabolism of bioactive molecules after oral consumption of cooked edible heads of *Cynara scolymus* L. (cultivar Violetto di Provenza) in human subjects: a pilot study. *British Journal of Nutrition*, *97*(5), 963.

Becalski, A., & Seaman, S. (2005). Furan precursors in food: a model study and development of a simple headspace method for determination of furan. *Journal of AOAC International*, *88*(1), 102–6.

Bekedam, E. K., Loots, M. J., Schols, H. A., Van Boekel, M. A. J. S., & Smit, G. (2008). Roasting effects on formation mechanisms of coffee brew melanoidins. *Journal of Agricultural and Food Chemistry*, *56*(16), 7138–45.

Benetou, V., Orfanos, P., Lagiou, P., Trichopoulos, D., Boffetta, P., & Trichopoulou, A. (2008). Vegetables and Fruits in Relation to Cancer Risk: Evidence from the Greek EPIC Cohort Study. *Cancer Epidemiology Biomarkers & Prevention*, *17*(2), 387–392.

Boffetta, P., Couto, E., Wichmann, J., Ferrari, P., Trichopoulos, D., Bueno-de-Mesquita, H. B., van Duijnhoven, F. J. B., Buchner, F. L., Key, T., Boeing, H., Nothlings, U., Linseisen, J., Gonzalez, C. A., Overvad, K., Nielsen, M. R. S., Tjonneland, A., Olsen, A., Clavel-Chapelon, F., Boutron-Ruault, M. C., Morois, S., Lagiou, P., Naska, A., Benetou, V., Kaaks, R., Rohrmann, S., Panico, S., Sieri, S., Vineis, P., Palli, D., van Gils, C. H., Peeters, P. H., Lund, E., Brustad, M., Engeset, D., Huerta, J. M., Rodriguez, L., Sanchez, M. J., Dorronsoro, M., Barricarte, A., Hallmans, G., Johansson, I., Manjer, J., Sonestedt, E., Allen, N. E., Bingham, S., Khaw, K. T., Slimani, N., Jenab, M., Mouw, T., Norat, T., Riboli, E., & Trichopoulou, A. (2010). Fruit and Vegetable Intake and Overall Cancer Risk in the European Prospective Investigation Into Cancer and Nutrition (EPIC). *JNCI Journal of the National Cancer Institute*, *102*(8), 529–537.

Bonaccorsi, P., Caristi, C., Gargiulli, C., & Leuzzi, U. (2005). Flavonol glucoside profile of southern Italian red onion (*Allium cepa* L.). *Journal of Agricultural and Food Chemistry*, *53*(7), 2733–40.

Bravo, J., Juániz, I., Monente, C., Caemmerer, B., Kroh, L. W., De Peña, M. P., & Cid, C. (2012). Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds. *Journal of Agricultural and Food Chemistry*, *60*(51), 12565–73.

Brown, E. M., Latimer, C., Allsopp, P., Ternan, N. G., McMullan, G., McDougall, G. J., Stewart, D., Crozier, A., Rowland, I., & Gill, C. I. R. (2014). In vitro and in vivo models of colorectal cancer: antigenotoxic activity of berries. *Journal of Agricultural and Food Chemistry*, *62*(18), 3852–66.

Bugianesi, R., Salucci, M., Leonardi, C., Ferracane, R., Catasta, G., Azzini, E., & Maiani, G. (2004). Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and beta-carotene in cherry tomatoes. *European Journal of Nutrition*, *43*(6), 360–6.

Buil-Cosiales, P., Toledo, E., Salas-Salvadó, J., Zazpe, I., Farràs, M., Basterra-Gortari, F. J., Diez-Espino, J., Estruch, R., Corella, D., Ros, E., Marti, A., Gómez-Gracia, E., Ortega-Calvo, M., Arós, F., Moñino, M., Serra-Majem, L., Pintó, X., Lamuela-Raventós, R. M., Babio, N., Gonzalez, J. I., Fitó, M., Martínez-González, M. A., & PREDIMED investigators. (2016). Association between dietary fibre intake and fruit, vegetable or whole-grain consumption and the risk of CVD: results from the PREvención con DIeta MEDiterránea (PREDIMED) trial. *The British Journal of Nutrition*, *116*(3), 534–46.

Chuah, A. M., Lee, Y.-C., Yamaguchi, T., Takamura, H., Yin, L.-J., & Matoba, T. (2008). Effect of cooking on the antioxidant properties of coloured peppers. *Food Chemistry*, *111*(1), 20–28.

Clifford, M. N. (2000). Chlorogenic acids and other cinnamates – nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture*, *80*(7), 1033–1043.

Crozier, A., Lean, M. E. J., McDonald, M. S., & Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal of Agricultural and Food Chemistry*, *45*(3), 590–595.

D'Antuono, I., Garbetta, A., Linsalata, V., Minervini, F., & Cardinali, A. (2015). Polyphenols from artichoke heads (*Cynara cardunculus* (L.) subsp. scolymus Hayek): in vitro bio-accessibility, intestinal uptake and bioavailability. *Food & Function*, *6*(4), 1268–77.

Day, A. J., Cañada, F. J., Díaz, J. C., Kroon, P. A., Mclauchlan, R., Faulds, C. B., Plumb, G. W., Morgan, M. R., & Williamson, G. (2000). Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Letters*, *468*(2–3), 166–70.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling*, *18*(14), 1818–1892.

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, *50*(10), 3010–3014.

Duda-Chodak, A., Tarko, T., Satora, P., & Sroka, P. (2015). Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. *European Journal of Nutrition*, *54*(3), 325–41.

Dupas, C., Marsset Baglieri, A., Ordonaud, C., Tomé, D., & Maillard, M.-N. (2006). Chlorogenic acid is poorly absorbed, independently of the food matrix: A Caco-2 cells and rat chronic absorption study. *Molecular Nutrition & Food Research*, *50*(11), 1053–60.

EFSA (European Food Safety Authority). (2005). Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *The EFSA Journal*, *282*, 1–31.

EFSA (European Food Safety Authority). (2010). Update of results on the monitoring of furan levels in food.URL http://www.efsa.europa.eu/it/search/doc/1702.pdf. Access 25.04.2017

EFSA (European Food Safety Authority). (2011). Update on furan levels in food from monitoring years 2004-2010 and exposure assessment. URL http://www.efsa.europa.eu/en/efsajournal/doc/2347.pdf. Access 25.04.2017

Erk, T., Renouf, M., Williamson, G., Melcher, R., Steiling, H., & Richling, E. (2014). Absorption and isomerization of caffeoylquinic acids from different foods using ileostomist volunteers. *European Journal of Nutrition*, *53*(1), 159–66.

Eurostat. (2016). Agriculture, forestry and f ishery statistics 2016 edition. URL http://ec.europa.eu/eurostat/documents/3217494/7777899/KS-FK-16-001-EN-N.pdf/cae3c56f-53e2-404a-9e9e-fb5f57ab49e3. Access 25.04.2017

Ewald, C., Fjelkner-Modig, S., Johansson, K., Sjöholm, I., & Åkesson, B. (1999). Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chemistry*, *64*(2), 231–235.

Farrell, T. L., Dew, T. P., Poquet, L., Hanson, P., & Williamson, G. (2011). Absorption and metabolism of chlorogenic acids in cultured gastric epithelial monolayers. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *39*(12), 2338–46.

Fernández-García, E., Carvajal-Lérida, I., & Pérez-Gálvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research*, *29*(11), 751–760.

Ferracane, R., Pellegrini, N., Visconti, A., Graziani, G., Chiavaro, E., Miglio, C., & Fogliano, V. (2008). Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. *Journal of Agricultural and Food Chemistry*, *56*(18), 8601–8608.

Fromberg, A., Fagt, S., & Granby, K. (2009). Furan in heat processed food products including home cooked food products and ready-to-eat products. *EFSA Supporting Publications*, *6*(9).

Fu, L., Xu, B.-T., Xu, X.-R., Qin, X.-S., Gan, R.-Y., & Li, H.-B. (2010). Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. *Molecules*, *15*(12), 8602–17.

Garbetta, A., Capotorto, I., Cardinali, A., D'Antuono, I., Linsalata, V., Pizzi, F., & Mineruini, F. (2014). Antioxidant activity induced by main polyphenols present in edible artichoke heads: influence of in vitro gastro-intestinal digestion. *Journal of Functional Foods*, *10*, 456–464.

Gerber, M., & Hoffman, R. (2015). The Mediterranean diet: health, science and society. *The British Journal of Nutrition*, *113 Suppl*, S4-10.

Girgin, N., & El Nehir, S. (2015). Effects of cooking on in vitro sinigrin bioaccessibility, total phenols, antioxidant and antimutagenic activity of cauliflower (*Brassica oleraceae* L. var. Botrytis). *Journal of Food Composition and Analysis*, *37*, 119–127.

Gorinstein, S., Park, Y. S., Heo, B. G., Namiesnik, J., Leontowicz, H., Leontowicz, M., Ham, K. S., Cho, J. Y., & Kang, S. G. (2009). A comparative study of phenolic compounds and antioxidant and antiproliferative activities in frequently consumed raw vegetables. *European Food Research and Technology*, *228*(6), 903–911.

Guillén, S., Mir-Bel, J., Oria, R., & Salvador, M. L. (2017). Influence of cooking conditions on organoleptic and health-related properties of artichokes, green beans, broccoli and carrots. *Food Chemistry*, *217*, 209–16.

Guo, X., Tresserra-Rimbau, A., Estruch, R., Martínez-González, M. A., Medina-Remón, A., Castañer, O., Corella, D., Salas-Salvadó, J., & Lamuela-Raventós, R. M. (2016). Effects of polyphenol, measured by a biomarker of total polyphenols in urine, on cardiovascular risk factors after a long-term follow-up in the PREDIMED study. *Oxidative Medicine and Cellular Longevity*, *2016*, 2572606.

Harris, S., Brunton, N., Tiwari, U., & Cummins, E. (2015). Human exposure modelling of quercetin in onions (*Allium cepa* L.) following thermal processing. *Food Chemistry*, *187*, 135–9.

Hasnip, S., Crews, C., & Castle, L. (2006). Some factors affecting the formation of furan in heated foods. *Food Additives and Contaminants*, *23*(3), 219–27.

Häusler, M., Ganzera, M., Abel, G., Popp, M., & Stuppner, H. (2002). Determination of caffeoylquinic acids and flavonoids inCynara scolymus L. by high performance liquid chromatography. *Chromatographia*, *56*(7–8), 407–411.

Hoffman, R., & Gerber, M. (2015). Food Processing and the Mediterranean Diet. *Nutrients*, *7*(9), 7925–7964.

Hu, M. (2007). Commentary: bioavailability of flavonoids and polyphenols: call to arms. *Molecular Pharmaceutics*, *4*(6), 803–6.

Hur, S. J., Lim, B. O., Decker, E. A., & McClements, D. J. (2011). In vitro human digestion models for food applications. *Food Chemistry*, *125*(1), 1–12.

IARC (International Agency for Research on Cancer). (1995). Furan. *Monographs on the Evaluation of Carcinogenic Risks to Humans*, *63*, 393–407.

Ioku, K., Aoyama, Y., Tokuno, A., Terao, J., Nakatani, N., & Takei, Y. (2001). Various cooking methods and the flavonoid content in onion. *Journal of Nutritional Science and Vitaminology*, *47*(1), 78–83.

Jiménez-Monreal, A. M., García-Diz, L., Martínez-Tomé, M., Mariscal, M., & Murcia, M. A. (2009). Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Science*, *74*(3), H97–H103.

Kamiloglu, S., Demirci, M., Selen, S., Toydemir, G., Boyacioglu, D., & Capanoglu, E. (2014). Home processing of tomatoes (*Solanum lycopersicum*): effects on in vitro bioaccessibility of total lycopene, phenolics, flavonoids, and antioxidant capacity. *Journal of the Science of Food and Agriculture*, *94*(11), 2225–33.

Kapusta-Duch, J., Kusznierewicz, B., Leszczyńska, T., & Borczak, B. (2016). Effect of cooking on the contents of glucosinolates and their degradation products in selected Brassica vegetables. *Journal of Functional Foods*, *23*, 412–422.

Kosińska-Cagnazzo, A., Diering, S., Prim, D., & Andlauer, W. (2015). Identification of bioaccessible and uptaken phenolic compounds from strawberry fruits in in vitro digestion/Caco-2 absorption model. *Food Chemistry*, *170*, 288–294.

Krygier, K., Sosulski, F., & Hogge, L. (1982a). Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry*, *30*(2), 330–334.

Krygier, K., Sosulski, F., & Hogge, L. (1982b). Free, esterified, and insoluble-bound phenolic acids. 2. Composition of phenolic acids in rapeseed flour and hulls. *Journal of Agricultural and Food Chemistry*, *30*(2), 334–336.

Lachenmeier, D. W., Reusch, H., & Kuballa, T. (2009). Risk assessment of furan in commercially jarred baby foods, including insights into its occurrence and formation in freshly home-cooked foods for infants and young children. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *26*(6), 776–85.

Larsson, S. C. (2006). Fruit and Vegetable Consumption in Relation to Pancreatic Cancer Risk: A Prospective Study. *Cancer Epidemiology Biomarkers & Prevention*, *15*(2), 301–305.

Limacher, A., Kerler, J., Conde-Petit, B., & Blank, I. (2007). Formation of furan and methylfuran from ascorbic acid in model systems and food. *Food Additives and Contaminants*, *24 Suppl 1*, 122–35.

Limacher, A., Kerler, J., Davidek, T., Schmalzried, F., & Blank, I. (2008). Formation of furan and methylfuran by maillard-type reactions in model systems and food. *Journal of Agricultural and Food Chemistry*, *56*(10), 3639–47.

Lombard, K., Peffley, E., Geoffriau, E., Thompson, L., & Herring, A. (2005). Quercetin in onion (*Allium cepa* L.) after heat-treatment simulating home preparation. *Journal of Food Composition and Analysis*, *18*(6), 571–581.

Ludwig, I. A., de Peña, M. P., Cid, C., & Crozier, A. (2013). Catabolism of coffee chlorogenic acids by human colonic microbiota. *BioFactors*, *39*(6), 623–32.

Ludwig, I. A., Sanchez, L., Caemmerer, B., Kroh, L. W., De Peña, M. P., & Cid, C. (2012). Extraction of coffee antioxidants: Impact of brewing time and method. *Food Research International*, *48*(1), 57–64.

Lutz, M., Henríquez, C., & Escobar, M. (2011). Chemical composition and antioxidant properties of mature and baby artichokes (*Cynara scolymus* L.), raw and cooked. *Journal of Food Composition and Analysis*, *24*(1), 49–54.

Maga, J. (1979). Furan in foods. *Critical Reviews in Food Science and Nutrition*, *11*(4), 355.

MAGRAMA (Ministery of Agiculture Food and Environment). (2001). La alimentación mesa mes en Hogares Año 2001 enero/diciembre acumulado. URL http://www.mapama.gob.es/ministerio/pags/Biblioteca/Revistas/pdf_AMM%2Fpdf_AMM_2001_acumuladoalimentaci%F3n_en_Hogares.pdf. Access 25.04.2017

MAGRAMA (Ministery of Agliculture Food and Environment). (2015). Informe del consumo de alimentacion en España 2015. URL http://www.magrama.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/informeconsumoalimentacion2015_tcm7-422694.pdf. Access 25.04.2017

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition*, *81*(1 Suppl), 230S–242S.

Marín, A., Ferreres, F., Tomás-Barberán, F. A., & Gil, M. I. (2004). Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry*, *52*(12), 3861–9.

Märk, J., Pollien, P., Lindinger, C., Blank, I., & Marrk, T. (2006). Quantitation of furan and methylfuran formed in different precursor systems by proton transfer reaction mass spectrometry. *Journal of Agricultural and Food Chemistry*, *54*(7), 2786–2793.

Martínez-González, M. A., Salas-Salvadó, J., Estruch, R., Corella, D., Fitó, M., & Ros, E. (2015). Benefits of the mediterranean diet: Insights from the PREDIMED study. *Progress in Cardiovascular Diseases*, *58*(1), 50–60.

Masella, R., Santangelo, C., D'Archivio, M., Li Volti, G., Giovannini, C., & Galvano, F. (2012). Protocatechuic acid and human disease prevention: biological activities and molecular mechanisms. *Current Medicinal Chemistry*, *19*(18), 2901–17.

Mesías, M., & Morales, F. J. (2014). Reliable estimation of dietary exposure to furan from coffee: An automatic vending machine as a case study. *Food Research International*, *61*, 257–263.

Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2008). Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of Agricultural and Food Chemistry*, *56*(1), 139–47.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carriere, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Menard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function*, *5*(6), 1113–1124.

Miranda, J. M., Martínez, B., Pérez, B., Antón, X., Vázquez, B. I., Fente, C. A., Franco, C. M., Rodríguez, J. L., & Cepeda, A. (2010). The effects of industrial pre-frying and domestic cooking methods on the nutritional compositions and fatty acid profiles of two different frozen breaded foods. *LWT - Food Science and Technology*, *43*(8), 1271–1276.

Monente, C., Ludwig, I. A., Stalmach, A., de Peña, M. P., Cid, C., & Crozier, A. (2015). In vitro studies on the stability in the proximal gastrointestinal tract and bioaccessibility in Caco-2 cells of chlorogenic acids from spent coffee grounds. *International Journal of Food Sciences and Nutrition*, *66*(6), 657–664.

Mulinacci, N., Prucher, D., Peruzzi, M., Romani, A., Pinelli, P., Giaccherini, C., & Vincieri, F. . (2004). Commercial and laboratory extracts from artichoke leaves: estimation of caffeoyl esters and flavonoidic compounds content. *Journal of Pharmaceutical and Biomedical Analysis*, *34*(2), 349–357.

Mullen, W., Rouanet, J.-M., Auger, C., Teissèdre, P.-L., Caldwell, S. T., Hartley, R. C., Lean, M. E. J., Edwards, C. A., & Crozier, A. (2008). Bioavailability of [2- 14 C]Quercetin-4′-glucoside in Rats. *Journal of Agricultural and Food Chemistry*, *56*(24), 12127–12137.

Murota, K., & Terao, J. (2003). Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. *Archives of Biochemistry and Biophysics*, *417*(1), 12–17.

Naczk, M., & Shahidi, F. (1989). The effect of methanol-ammonia-water treatment on the content of phenolic acids of canola. *Food Chemistry*, *31*(2), 159–164.

Nanri, A., Yoshida, D., Yamaji, T., Mizoue, T., Takayanagi, R., & Kono, S. (2008). Dietary patterns and C-reactive protein in Japanese men and women. *The American Journal of Clinical Nutrition*, *87*(5), 1488–96.

Nayak, B., Liu, R. H., & Tang, J. (2015). Effect of Processing on Phenolic Antioxidants of Fruits, Vegetables, and Grains—A Review. *Critical Reviews in Food Science and Nutrition*, *55*(7), 887–918.

Németh, K., Plumb, G. W., Berrin, J.-G., Juge, N., Jacob, R., Naim, H. Y., Williamson, G., Swallow, D. M., & Kroon, P. A. (2003). Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition*, *42*(1), 29–42.

Nunes, F. M., & Coimbra, M. A. (2010). Role of hydroxycinnamates in coffee melanoidin formation. *Phytochemistry Reviews*, *9*(1), 171–185.

Olthof, M. R., Hollman, P. C., & Katan, M. B. (2001). Chlorogenic acid and caffeic acid are absorbed in humans. *The Journal of Nutrition*, *131*(1), 66–71. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11208940

Omar, M. H., Mullen, W., Stalmach, A., Auger, C., Rouanet, J.-M., Teissedre, P.-L., Caldwell, S. T., Hartley, R. C., & Crozier, A. (2012). Absorption, disposition, metabolism, and excretion of [3- 14 C]caffeic acid in rats. *Journal of Agricultural and Food Chemistry*, *60*(20), 5205–5214.

Owczarek-Fendor, A., De Meulenaer, B., Scholl, G., Adams, A., Van Lancker, F., Eppe, G., De Pauw, E., Scippo, M.-L., & De Kimpe, N. (2011). Furan formation from lipids in starch-based model systems, as influenced by interactions with antioxidants and proteins. *Journal of Agricultural and Food Chemistry*, *59*(6), 2368–76.

Palermo, M., Pellegrini, N., & Fogliano, V. (2014). The effect of cooking on the phytochemical content of vegetables. *Journal of the Science of Food and Agriculture*, *94*(6), 1057–70.

Pellegrini, N., Chiavaro, E., Gardana, C., Mazzeo, T., Contino, D., Gallo, M., Riso, P., Fogliano, V., & Porrini, M. (2010). Effect of different cooking methods on color, phytochemical concentration, and antioxidant capacity of raw and frozen brassica vegetables. *Journal of Agricultural and Food Chemistry*, *58*(7), 4310–21.

Pellegrini, N., Miglio, C., Del Rio, D., Salvatore, S., Serafini, M., & Brighenti, F. (2009). Effect of domestic cooking methods on the total antioxidant capacity of vegetables. *International Journal of Food Sciences and Nutrition*, *60 Suppl 2*(s2), 12–22.

Perez Locas, C., & Yaylayan, V. A. (2004). Origin and mechanistic pathways of formation of the parent furan--a food toxicant. *Journal of Agricultural and Food Chemistry*, *52*(22), 6830–6.

Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E., & Romani, A. (2007). Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated cardoon leaves. *Food Chemistry*, *105*(4), 1695–1701.

Price, K. R., & Rhodes, M. J. C. (1997). Analysis of the major flavonol glycosides present in four varieties of onion (*Allium cepa*) and changes in composition resulting from autolysis. *Journal of the Science of Food and Agriculture*, *74*(3), 331–339.

Ramírez-Anaya, J. D. P., Samaniego-Sánchez, C., Castañeda-Saucedo, M. C., Villalón-Mir, M., & de la Serrana, H. L.-G. (2015). Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. *Food Chemistry*, *188*, 430–8.

Ramos, P. A. B., Santos, S. A. O., Guerra, Â. R., Guerreiro, O., Freire, C. S. R., Rocha, S. M., Duarte, M. F., & Silvestre, A. J. D. (2014). Phenolic composition and antioxidant activity of different morphological parts of *Cynara cardunculus* L. var. altilis (DC). *Industrial Crops and Products*, *61*, 460–471.

Rendeiro, C., Rhodes, J. S., & Spencer, J. P. E. (2015). The mechanisms of action of flavonoids in the brain: Direct versus indirect effects. *Neurochemistry International*, *89*, 126–39.

Rodrigues, A. S., Pérez-Gregorio, M. R., García-Falcón, M. S., & Simal-Gándara, J. (2009). Effect of curing and cooking on flavonols and anthocyanins in traditional varieties of onion bulbs. *Food Research International*, *42*(9), 1331–1336.

Rodriguez-Mateos, A., Vauzour, D., Krueger, C. G., Shanmuganayagam, D., Reed, J., Calani, L., Mena, P., Del Rio, D., & Crozier, A. (2014). Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. *Archives of Toxicology*, *88*(10), 1803–1853.

Rohn, S., Buchner, N., Driemel, G., Rauser, M., & Kroh, L. W. (2007). Thermal degradation of onion quercetin glucosides under roasting conditions. *Journal of Agricultural and Food Chemistry*, *55*(4), 1568–73.

Romero, A., Cuesta, C., & Snchez-Muniz, F. J. (2000). Cyclic fatty acid monomers and thermoxidative alteration compounds formed during frying of frozen foods in extra virgin olive oil. *Journal of the American Oil Chemists' Society*, *77*(11), 1169–1175.

Rossell, J. B. (2001). *Frying : improving quality*. CRC Press. Retrieved from https://books.google.es/books?hl=es&lr=&id=XljJMZWJfvYC&oi=fnd&pg=PR9&dq=Frying:+Improving+quality&ots=_MSWylsk_V&sig=zTQQmZyWaE0DFFMplbwR4nZvTPo&redir_esc=y#v=onepage&q=Frying%3A Improving quality&f=false

Sacchetti, G., Ioannone, F., De Gregorio, M., Di Mattia, C., Serafini, M., & Mastrocola, D. (2016). Non enzymatic browning during cocoa roasting as affected by processing time and temperature. *Journal of Food Engineering*, *169*, 44–52.

Salas-Salvadó, J., Garcia-Arellano, A., Estruch, R., Marquez-Sandoval, F., Corella, D., Fiol, M., Gómez-Gracia, E., Viñoles, E., Arós, F., Herrera, C., Lahoz, C., Lapetra, J., Perona, J. S., Muñoz-Aguado, D., Martínez-González, M. a, Ros, E., & PREDIMED Investigators. (2008). Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. *European Journal of Clinical Nutrition*, *62*(5), 651–9.

Scalbert, A., Morand, C., Manach, C., & Rémésy, C. (2002). Absorption and metabolism of polyphenols in the gut and impact on health. *Biomedicine & Pharmacotherapy*, *56*(6), 276–282.

Schütz, K., Kammerer, D., Carle, R., & Schieber, A. (2004). Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice, and pomace by HPLC-DAD-ESI/MS(n). *Journal of Agricultural and Food Chemistry*, *52*(13), 4090–6.

Selma, M. V, Espin, J. C., & Tomas-Barberan, F. A. (2009). Interaction between phenolics and gut microbiota: Role in human health. *Journal of Agricultural and Food Chemistry*, *57*(15), 6485–6501.

Senyuva, H. Z., & Gokmen, V. (2007). Potential of furan formation in hazelnuts during heat treatment. *Food Additives and Contaminants*, *24*, 136–142.

Shahidi, F., & Yeo, J.-D. (2016). Insoluble-Bound Phenolics in Food. *Molecules (Basel, Switzerland)*, *21*(9), 1216.

Siddiq, M., Roidoung, S., Sogi, D. S., & Dolan, K. D. (2013). Total phenolics, antioxidant properties and quality of fresh-cut onions (*Allium cepa* L.) treated with mild-heat. *Food Chemistry*, *136*(2), 803–806.

Sosulski, F., Krygier, K., & Hogge, L. (1982). Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *Journal of Agricultural and Food Chemistry*, *30*(2), 337–340.

Stalmach, A., Mullen, W., Barron, D., Uchida, K., Yokota, T., Cavin, C., Steiling, H., Williamson, G., & Crozier, A. (2009). Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: identification of biomarkers of coffee consumption. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *37*(8), 1749–58.

Stalmach, A., Steiling, H., Williamson, G., & Crozier, A. (2010). Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Archives of Biochemistry and Biophysics*, *501*(1), 98–105.

Turkmen, N., Sari, F., & Velioglu, Y. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry*, *93*(4), 713–718.

Vauzour, D. (2012). Dietary polyphenols as modulators of brain functions: biological actions and molecular mechanisms underpinning their beneficial effects. *Oxidative Medicine and Cellular Longevity*, 1–16.

Velez, Z., Campinho, M., Guerra, Â., García, L., Ramos, P., Guerreiro, O., Felício, L., Schmitt, F., & Duarte, M. (2012). Biological characterization of *Cynara cardunculus* L. methanolic extracts: Antioxidant, anti-proliferative, anti-migratory and anti-angiogenic activities. *Agriculture*, *2*(4), 472–492.

Verzelloni, E., Pellacani, C., Tagliazucchi, D., Tagliaferri, S., Calani, L., Costa, L. G., Brighenti, F., Borges, G., Crozier, A., Conte, A., & Del Rio, D. (2011). Antiglycative and neuroprotective activity of colon-derived polyphenol catabolites. *Molecular Nutrition & Food Research*, *55 Suppl 1*(SUPPL. 1), S35-43.

Wachtel-Galor, S., Wong, K. W., & Benzie, I. F. F. (2008). The effect of cooking on Brassica vegetables. *Food Chemistry*, *110*(3), 706–710.

Waizenegger, J., Winkler, G., Kuballa, T., Ruge, W., Kersting, M., Alexy, U., & Lachenmeier, D. W. (2012). Analysis and risk assessment of furan in coffee products targeted to adolescents. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *29*(1), 19–28.

Wang, M., Simon, J. E., Aviles, I. F., He, K., Zheng, Q.-Y., & Tadmor, Y. (2003). Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *Journal of Agricultural and Food Chemistry*, *51*(3), 601–8.

WHO (World Health Organization). (2011). *Safety evaluation if certain contaminants in food*. *WHO Food Additive Series* (Vol. 62).

WHO (World Health Organization). (2004). Global Strategy on Diet, Physical Activity and Health. *World Health Organization*. URL http://www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf. Access 25.04.2017

Zoller, O., Sager, F., & Reinhard, H. (2007). Furan in food: headspace method and product survey. *Food Additives and Contaminants*, *24 Suppl 1*, 91–107.

# OBJECTIVES / OBJETIVOS

Plant foods are the main source of dietary antioxidants, including (poly)phenolic compounds, which have been reported to exhibit a wide range of health properties. Together with other nutritional properties, the bioaccessibility of (poly)phenolic compounds might be affected by the culinary processes applied to foods. Nevertheless, since both gains and losses are reported in literature, results are still unclear. After ingestion, (poly)phenols could also be modified in the gastrointestinal tract by digestive enzymes and microbiota and, consequently, their bioaccessibility might also be affected. Additionally, heat treatment induces the formation of volatile compounds like furan and furanic compounds, which significantly contribute to the sensory properties of heat treated foods. However, furan is a highly volatile compound, which has been classified as a possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer.

For all these reasons, the **main aim** of this work was to investigate the influence of heat treatment (frying and griddling) on the antioxidant capacity, nutritional composition and (poly)phenolic compounds in selected vegetables, as well as their bioaccessibility after a simulated gastrointestinal digestion and colonic microbiota action. Moreover, the presence of furan in heat treated selected vegetables in comparison with that formed during an intense thermal treatment (deep-frying) in foods with a composition potentially more prone to the formation of furan (bread coated frozen foods) was also investigated.

To achieve this overall objective, the following **partial objectives** were proposed:

1. To investigate the influence of heat treatment on food composition, antioxidant capacity and (poly)phenolic compounds in vegetables. (Papers 1 & 2).
    1.1. Screening and selection of vegetables.
    1.2. Impact of heat treatment on the selected vegetables: onion (*Allium cepa*), green pepper (*Capsicum annuum*) and cardoon (*Cynara cardunculus* L.).
2. To evaluate the bioaccessibility of (poly)phenolic compounds and their metabolites after an *in vitro* gastrointestinal digestion and colonic microbiota fermentation, of green pepper (*Capsicum annuum*) and cardoon (*Cynara cardunculus* L.) before and after heat treatment. (Papers 3 & 4).
3. To evaluate furan occurrence in vegetables and bread coated frozen foods before and after heat treatment. (Paper 5).
    3.1. Development and validation of the methodology for furan determination in foods.
    3.2. Impact of heat treatment on furan occurrence in vegetables and bread coated frozen foods.
    3.3. Evaluation of the risk assessment of furan in foods for the Spanish population.

Los alimentos vegetales constituyen la principal fuente de antioxidantes de la dieta, incluyendo los compuestos (poli)fenólicos que proporcionan una gran variedad de propiedades saludables. Además de las propiedades nutricionales, la bioaccesibilidad de los compuestos (poli)fenólicos podría verse afectada por los procesos culinarios aplicados a los alimentos, encontrándose en la literatura tanto efectos positivos como negativos sobre dichos compuestos, sin poder considerar los resultados concluyentes. Además, después de la ingesta de los alimentos, los (poli)fenoles pueden verse modificados en el tracto gastrointestinal por los enzimas digestivos y la microbiota y, en consecuencia, su bioaccesibilidad también podría verse afectada. Por otro lado, el tratamiento térmico de los alimentos induce la formación de compuestos volátiles como el furano y sus derivados, que contribuyen significativamente a las propiedades sensoriales de los alimentos tratados térmicamente. No obstante, el furano es un compuesto altamente volátil, que ha sido clasificado como posible carcinógeno en humanos (grupo 2B) por la Agencia Internacional para la Investigación del Cáncer.

Por todo ello, el **objetivo general** de este trabajo fue investigar la influencia de los tratamientos térmicos (fritura y plancha) sobre la composición nutricional, la capacidad antioxidante, y los compuestos potencialmente responsables de la misma (compuestos (poli)fenólicos) en alimentos vegetales seleccionados, así como su bioaccesibilidad después de un proceso simulado de digestión gastrointestinal y acción de la microbiota del colon. Así mismo, se planteó evaluar la presencia de furano en dichos alimentos y compararla con el generado durante un tratamiento térmico intenso (fritura en profundidad) en alimentos que por su composición son potencialmente propensos a la formación de furano (alimentos precocinados empanados).

Para lograr este objetivo se propusieron los siguientes **objetivos parciales**:

1. Estudio de la influencia del tratamiento térmico en la composición, capacidad antioxidante y compuestos (poli)fenólicos en alimentos vegetales. (Publicaciones 1 y 2).
   1.1. Screening y selección de alimentos vegetales.
   1.2. Impacto del tratamiento térmico en los alimentos vegetales seleccionados: cebolla (*Allium cepa*), pimiento verde (*Capsicum annuum*) y cardo (*Cynara cardunculus L.*).
2. Evaluación de la bioacccesibilidad de los compuestos (poli)fenólicos y sus metabolitos tras los procesos de digestión gastrointestinal *in vitro* y fermentación por la microbiota del colon, de pimiento verde (*Capsicum annuum*) y cardo (*Cynara cardunculus L.*) tanto crudos como sometidos a tratamientos térmicos. (Publicaciones 3 y 4).
3. Evaluación de la presencia de furano en alimentos vegetales y alimentos precocinados empanados sometidos a tratamientos térmicos. (Publicación 5).
   3.1. Puesta a punto y validación de la metodología para la determinación de furano en alimentos.
   3.2. Impacto del tratamiento térmico en la presencia de furano en alimentos vegetales y alimentos precocinados empanados.
   3.3. Evaluación del riesgo de furano en alimentos para la población española.

# EXPERIMENTAL DESIGN

**VEGETABLES**

| Chicory | Onion | Green pepper | Cardoon | Cabbage |
|---|---|---|---|---|
| (*Cichorium intybus*) | (*Allium cepa*) | (*Capsicum annuum*) | (*Cynara cardunculus L.*) | (*Brassica oleracea*) |

**Heat treatment**

Raw | Fried in olive oil | Fried in sunflower oil | Griddled

**Objective 3**
Next page

**Objective 1**

**1st selection → Antioxidant capacity and phenolic compounds**

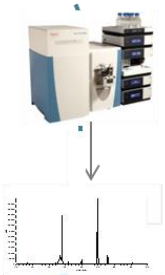
| Onion | Green pepper | Cardoon |
|---|---|---|
| (*Allium cepa*) | (*Capsicum annuum*) | (*Cynara cardunculus L.*) |

**Raw and heat treated vegetables**

**Composition**
Moisture
Proteins
Total fat content
Fatty acids profile
Carbohydrates
Ash
Total phenolic compounds
Total flavonoid compounds
Energy

**Antioxidant capacity**
DPPH
ABTS

**(Poly)phenolic compounds (UHPLC–PDA–HR–MS)**
Flavonoids
Non-flavonoids



Paper 1 (in preparation)

Paper 2

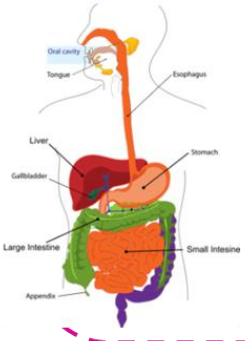
**2nd selection**

| Green pepper | Cardoon |
|---|---|
| (*Capsicum annuum*) | (*Cynara cardunculus L.*) |

**Raw and heat treated vegetables**

**Objective 2**

**Bioaccessibility and metabolomics**

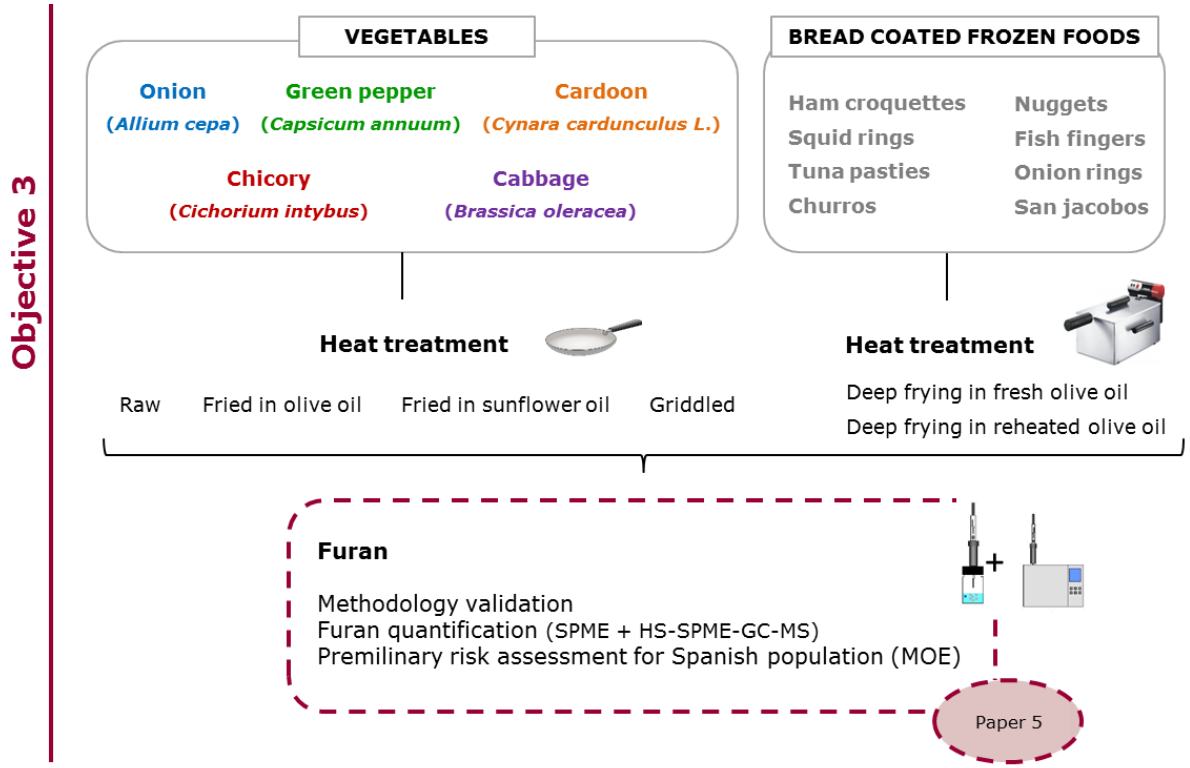



***1. In vitro gastrointestinal* digestion**
**(Poly)phenolic compounds (UHPLC-ESI-MSn)**
Flavonoids — Free
Non-flavonoids — Bounds

***2. Colonic fermentation***
**(Poly)phenolic compounds (UHPLC-ESI-MSn)**
Flavonoids — Free
Non-flavonoids — Bounds
Metabolites

Paper 3

Paper 4


Objective 3
VEGETABLES
Onion
(Allium cepa)
Green pepper
(Capsicum annuum)
Cardoon
(Cynara cardunculus L.)
Chicory
(Cichorium intybus)
Cabbage
(Brassica oleracea)
BREAD COATED FROZEN FOODS
Ham croquettes
Squid rings
Tuna pasties
Churros
Nuggets
Fish fingers
Onion rings
San jacobos
Heat treatment
Raw
Fried in olive oil
Fried in sunflower oil
Griddled
Heat treatment
Deep frying in fresh olive oil
Deep frying in reheated olive oil
Furan
Methodology validation
Furan quantification (SPME + HS-SPME-GC-MS)
Premilinary risk assessment for Spanish population (MOE)
Paper 5

# RESULTS

# Objective 1

**To investigate the influence of heat treatment on food composition, antioxidant capacity and (poly)phenolic compounds in vegetables.**

Estudio de la influencia del tratamiento térmico en la composición, capacidad antioxidante y compuestos (poli)fenólicos en alimentos vegetales.

# Objective 1.1

**Screening and selection of vegetables.**

Screening y selección de alimentos vegetales.

# Screening and selection of vegetables.

Part of these results has been published in the next paper (paper 2 of the present thesis, see page 81):

**Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables**

**I.Juániz**, I.A. Ludwig, E. Huarte, G.Pereira-Caro, J.M. Moreno-Rojas C. Cid, M.P. de Peña (2016). *Food Chemistry*, 197, 466-473. http://dx.doi.org/10.1016/j.foodchem.2015.10.139.

Plant foods are the main source of dietary antioxidants, including phenolic compounds. (Poly)phenols rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer, probably due to their ability to protect against oxidative damage in cells (Del Rio et al., 2013; Rodríguez-Mateos et al., 2014). In order to carry out a first screening and select potential antioxidant vegetables, five different vegetables (yellow onion (*Allium cepa*), Italian green pepper (*Capsicum annuum*), cardoon stalks (*Cynara cardunculus* L.), cabbage (*Brassica oleracea*) and chicory (*Cichorium intybus*) were chosen to their study antioxidant properties. These five vegetables have a great consumer acceptance among the Spanish population; specifically onion and pepper are two of the most consumed vegetables (MAGRAMA, 2015). Moreover, all of them can be consumed both raw and after being subjected to different heat treatments. Culinary processes induce significant changes in foods such as modifications in the phenolic compounds profile and therefore in the antioxidant capacity (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015). Thus total phenolic compounds (TPC), total flavonoid compounds (TFC) and antioxidant capacity measured by DPPH and ABTS assays were analyzed in both raw and cooked vegetables.

## 1. Material and methods

### 1.1 Chemical and reagents

Yellow onion (*Allium cepa*), Italian green pepper (*Capsicum annuum*), cardoon stalks (*Cynara cardunculus* L.), cabbage (*Brassica oleracea*), chicory (*Cichorium intybus*), olive oil (refined and virgin olive oil blend) and sunflower oil were obtained from local stores.

The methanol and ethanol were of analytical grade from Panreac (Barcelona, Spain). Folin−Ciocalteu reagent and sodium carbonate were also from Panreac (Barcelona, Spain). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2′-azinobis (3-ethylbenzothiazonile-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH·), rutin, gallic acid and aluminum chloride hexahydrate were purchased from Sigma-Aldrich (Steinheim, Germany).

### 1.2. Samples preparation

Chopped vegetables (yellow onion, green pepper, cardoon stalks, cabbage and chicory) (300 g) were fried with olive or sunflower oils (30 mL) at 115 ºC for 10 minutes in a non-stick frying pan. Then, temperature was decreased to 108 ºC for 5 minutes. Chopped vegetables were also submitted to heating at 150 ºC for 10 minutes and then at 110 ºC for 5 minutes in a non-stick griddle without oil addition. Then, raw and cooked vegetables were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain), and stored at -18ºC until analysis.

1.3. Vegetables extracts

Vegetables extracts were prepared according to Siddiq et al. (2013) with some modifications. The same procedure was applied to all vegetables. Briefly, thirty mL of ethanol/water (80/20) was added to 2 grams of lyophilized vegetables. The content was mixed on a mechanical shaker for 1 hour at room temperature and then centrifuged at 4000 rpm for 10 minutes. Supernatant was collected and residue was re-extracted twice using 10 mL of ethanol 80% by vortexing (1 minute) and centrifuged at 4000 rpm for 5 minutes. All three supernatants were combined and frozen at -18ºC for further analysis.

1.4. Total phenolic compounds (TPC)

Total phenolic compounds were measured using the Folin–Ciocalteu reagent according to Singleton's method (Singleton & Rossi, 1965). Each vegetable extract was properly diluted in demineralized water. A volume of 500 µL of Folin–Ciocalteu reagent was added to a mixture of 100 µL of the extract sample and 7.9 mL of demineralized water. After a 2 min delay, 1.5 mL of a 7.5% sodium carbonate solution was added. Next, the sample was incubated in darkness at room temperature for 90 min. The absorbance of the sample was measured at 765 nm in a spectrophotometer Lambda 25 UV/VIS (Perkin Elmer Instruments, Madrid, Spain). Gallic Acid (GA) was used as reference, and the results were expressed as milligrams of GA equivalent per gram of dry matter sample (mg GA/ g dm).

1.5. Total flavonoid content (TFC)

The aluminium chloride method (Lamaison & Carnet, 1990) was used estimating the total flavonoids content of the extracted samples. An aliquot of 100 µL of each vegetable extract properly diluted was added to 1 mL of a 2% $AlCl3{\cdot}6H2O$ methanol solution. The mixture was vigorously shaken, and after 10 min of incubation at room temperature, the absorbance was

read at 430 nm in a spectrophotometer Lambda 25 UV/VIS (Perkin Elmer). Total flavonoid content was calculated from the calibration curve of rutin standard solutions, and expressed as milligram of rutin equivalent per gram of dry matter sample (mg rutin/ g dm).

1.6. Antioxidant capacity by ABTS assay

The ABTS antioxidant capacity was performed according to the method of Re et al. (1999). The radicals ABTS•+ were generated by the addition of 0.36 mM potassium persulfate to a 0.9 mM ABTS solution prepared in phosphate buffered saline (PBS) (pH 7.4), and the ABTS•+solution was stored in darkness for 12 h. The ABTS•+ solution was adjusted with PBS to an absorbance of 0.700 (±0.020) at 734 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). An aliquot of 100 µL of each vegetable extract sample properly diluted in demineralized water, was added to 2 mL of ABTS•+ solution. The absorbance was measured spectrophotometrically at 734 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analog), and the antioxidant capacity was expressed as micromoles of Trolox equivalent per gram of dry matter sample (µmol Trolox/g dm).

1.7. Antioxidant capacity by DPPH assay

The antioxidant capacity was also measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) decolorization assay (Brand-Williams, Cuvelier, & Berset, 1995) with some modifications. A 6.1×10−5 M DPPH• methanolic solution was prepared immediately before use. The DPPH• solution was adjusted with methanol to an absorbance of 0.700 (±0.020) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). Vegetable extracts were properly diluted in demineralized water prior to analysis. Samples (50 µL) were added to 1.95 mL of the DPPH• solution. After mixing, the absorbance was measured at 515 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analog). The antioxidant capacity was expressed as micromoles of Trolox equivalent per gram of dry matter sample (µmol Trolox/g dm).

1.8. Statistical analysis

Each parameter was analysed in triplicate. Results are shown as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied for each parameter. A Tukey test was applied as a posteriori test with a level of significance of 95%. Principal Component Analysis (PCA), based on Pearson's correlation matrix, was applied in order to study the effect of the heat treatment. All statistical analyses were performed using the STATA v.12.0 software package.

**2. Results and discussion**

Figure 1 shows total phenolic and total flavonoid compounds of selected vegetables. Among raw samples, pepper and onion were the vegetables with the highest content of phenolic compounds (16.27 ± 0.26 and 10.06 ± 0.39mg g.a/g dm respectively), following by chicory (8.45 ± 0.30 mg g.a/g dm) and cardoon (7.72 ± 0.28 mg g.a/g dm). The vegetable with the lowest amount of phenolic compounds was cabbage (3.05 ± 0.40 mg g.a/g dm). In all cases the total amount of phenolic compounds was influenced by the different heat treatments applied. Heat-induced wall and cells ruptures can affect (poly)phenolic extractability (Harris, Brunton, Tiwari, & Cummins, 2015; Palermo, Pellegrini, & Fogliano, 2014). Generally, frying degraded phenolic compounds while griddling increased them. The higher temperature applied during the griddling treatment than that applied during frying, might favor the release of these compounds from the food matrix. Griddled cardoon and griddled pepper were the samples with the highest amount of phenolic compounds (around 20 mg g.a/g dm in both samples).

Regarding total flavonoid, the raw vegetables with the highest amount of these compounds were green pepper and chicory (5.95 ± 0.07 and 5.89 ± 0.03 mg rutin/g dm, respectively). In literature green pepper has been described as a vegetable rich in flavonoids, specifically luteolin glycosides (Marín, Ferreres, Tomás-Barberán, & Gil, 2004; Morales-Soto, Gómez-Caravaca, García-Salas, Segura-Carretero, & Fernández-Gutiérrez, 2013). In cabbage also a large number of flavonoids have been identified by different authors, including myricetin, quercetin, kaempferol and luteolin (Franke, Custer, Arakaki, & Murphy, 2004; Park et al., 2014). On the contrary, cardoon, which belongs to the same family of *Cynara cardunculus* L., hardly presents flavonoids in its composition. *Cynara cardunculus* L. is a family of vegetables characterized by phenolic compounds, specifically caffeoylquinic acids such as 5-CQA (Pinelli et al., 2007; Ramos et al., 2014). Total flavonoid compounds were also

influenced by the heat treatment in all samples. There was a tendency to increase flavonoid compounds after cooking. Griddled chicory resulted in the sample with the highest amount of flavonoids (9.27 ± 0.78 mg rutin/g dm).

Figure 2 shows antioxidant capacity (ABTS and DPPH) of raw and cooked vegetables. In general antioxidant activity was affected by the different heat treatments and it depended on the vegetable analyzed and it composition. A significant correlation between antioxidant capacity, measured both with ABTS and DPPH assays, and TPC was observed (Table 1). However a correlation with TFC was not observed, so flavonoids might not be as relevant as TPC with regard to antioxidant properties in vegetables. Some authors had also previously reported a correlation between phenolic compounds and antioxidant capacity measured by DPPH (Ramírez-Anaya et al., 2015). Regarding ABTS assay, fried samples showed less antioxidant capacity (ABTS) than raw ones, except for sunflower oil fried cardoon. In general, griddled vegetables also showed less antioxidant capacity (ABTS), nevertheless this decline was no so great as in fried samples. Raw pepper presented the highest antioxidant activity (ABTS) (257.96 ± 0.75 μmol Trolox/g dm) following by griddled cardoon, griddled pepper and raw onion (192.12 ± 4.91, 173.03 ± 3.15 and 162.10 ±1.33 μmol Trolox/g dm, respectively). Antioxidant activity measured by DPPH assay was also affected by the heat treatment, however in this case some cooking processes seemed to increase antioxidant capacity of some vegetables. Specific, griddling and frying with sunflower oil increased significantly antioxidant capacity (DPPH) of pepper and cardoon. Griddled cardoon, which was the vegetable with the highest amount of TPC, showed also the highest antioxidant capacity by DPPH (90.73 ± 1.07 μmol Trolox/g dm) followed by griddled pepper (57.36 ± 1.88 μmol Trolox/g dm). Griddled onions also showed a higher antioxidant capacity by DPPH than the raw ones. Contrary, cooked cabbage and chicory presented less antioxidant capacity (DPPH) than their raw vegetables. In general, cabbage and chicory were no relevant in terms of their antioxidant capacity measured both by ABTS and DPPH assays, probably due to their lower content of TPC.

Additionally, Principal Component Analysis (PCA) has been applied to evaluate at a glance the most interesting vegetables to be subsequently studied in order to follow the objectives outlined in the thesis. Figure 3 shows the bidimensional representation of all the variables (Figure 3A) and vegetables samples (Figure 3B) according to the two selected Principal

Components (PC). PC1 (59.42% of the total variance) was mainly characterized by TPC as well as the scavenging activity measured by DPPH and ABTS on the right half graphic. According to this, griddled cardoon as well as raw onion and pepper samples, with the exception of olive oil fried pepper, are characterized by the presence of a high amount of TPC and a high antioxidant capacity. Therefore onion, pepper and cardoon could result of interest for further studies. PC2, which explained 26.42% of the total variance, is characterized by TFC. In fact, chicory samples, specifically griddled ones, which are the samples with the highest amount of flavonoids, are situated at the top of the graphic. Since it was previously described, flavonoids were not correlated with antioxidant capacity, so chicory was discarded for further studies. It was also observed how cooked onion and cooked cabbage tended to be placed at the top of the graphic, contrary to their corresponding raw samples, indicating an increase of flavonoid compounds after cooking.

In summary, chicory was the sample with the highest amount of flavonoids, nevertheless there was not a correlation between flavonoids and antioxidant capacity, so chicory was not selected for further studies. In the same way, cabbage was not selected because it did not show a high antioxidant capacity by DDPH and ABTS, moreover the antioxidant capacity of cabbage was lower in the cooked samples than the in raw ones. On the other hand, pepper and cardoon seem to have more relevance in antioxidant capacity due to the higher amount of TPC, specially griddled samples so these two vegetables were selected to further analysis. At last, onion was also selected since its antioxidant capacity (DPPH) was higher in the cooked samples than in the raw ones and it could help us to understand the effect of the heat treatment on the antioxidant properties of vegetables.

## 3. References

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30.

Franke, A. A., Custer, L. J., Arakaki, C., & Murphy, S. P. (2004). Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. *Journal of Food Composition and Analysis*, *17*(1), 1–35.

Harris, S., Brunton, N., Tiwari, U., & Cummins, E. (2015). Human exposure modelling of quercetin in onions (Allium cepa L.) following thermal processing. *Food Chemistry*, *187*, 135–139.

Lamaison, J. L. C., & Carnet, A. (1990). Teneurs en principaux flavonoids des fleurs de Crataegeus monogyna Jacq et de Crataegeus laevigata (Poiret D. C) en fonction de la vegetation. Pharm. Acta. Helv. 65: 315-320. *Pharmaceutica Acta Helvetiae*, *65*, 315–320.

MAGRAMA (Ministry of Agriculture, Food and Enviroment). (2015). Informe del consumo de alimentación en España 2015. URL http://www.mapama.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/informeconsumoalimentacion2015_tcm7-422694.pdf. Accessed 16.03.2017

Marín, A., Ferreres, F., Tomás-Barberán, F. A., & Gil, M. I. (2004). Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry*, *52*(12), 3861–9.

Morales-Soto, A., Gómez-Caravaca, A. M., García-Salas, P., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2013). High-performance liquid chromatography coupled to diode array and electrospray time-of-flight mass spectrometry detectors for a comprehensive characterization of phenolic and other polar compounds in three pepper (Capsicum annuum L.) samples. *Food Research International*, *51*(2), 977–984.

Palermo, M., Pellegrini, N., & Fogliano, V. (2014). The effect of cooking on the phytochemical content of vegetables. *Journal of the Science of Food and Agriculture*, *94*(6), 1057–1070.

Park, S., Arasu, M. V., Jiang, N., Choi, S. H., Lim, Y. P., Park, J. T., … Kim, S. J. (2014). Metabolite profiling of phenolics, anthocyanins and flavonols in cabbage (Brassica oleracea var. capitata). *Industrial Crops and Products*, *60*, 8–14.

Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E., & Romani, A. (2007). Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated cardoon leaves. *Food Chemistry*, *105*(4), 1695–1701.

Ramírez-Anaya, J. D. P., Samaniego-Sánchez, C., Castañeda-Saucedo, M. C., Villalón-Mir, M., & de la Serrana, H. L.-G. (2015). Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. *Food Chemistry*, *188*, 430–8.

Ramos, P. A. B., Santos, S. A. O., Guerra, Â. R., Guerreiro, O., Freire, C. S. R., Rocha, S. M., … Silvestre, A. J. D. (2014). Phenolic composition and antioxidant activity of different

morphological parts of Cynara cardunculus L. var. altilis (DC). *Industrial Crops and Products*, *61*, 460–471.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237.

Siddiq, M., Roidoung, S., Sogi, D. S., & Dolan, K. D. (2013). Total phenolics, antioxidant properties and quality of fresh-cut onions (*Allium cepa* L.) treated with mild-heat. *Food Chemistry*, *136*(2), 803–806.

Singleton, V., & Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16(3)*, 144–158.

**Figure 1.** Total Phenolic Compounds and Total Flavonoid Compounds of raw and cooked vegetables. Different letters indicate significant differences ($p \leq 0.05$).

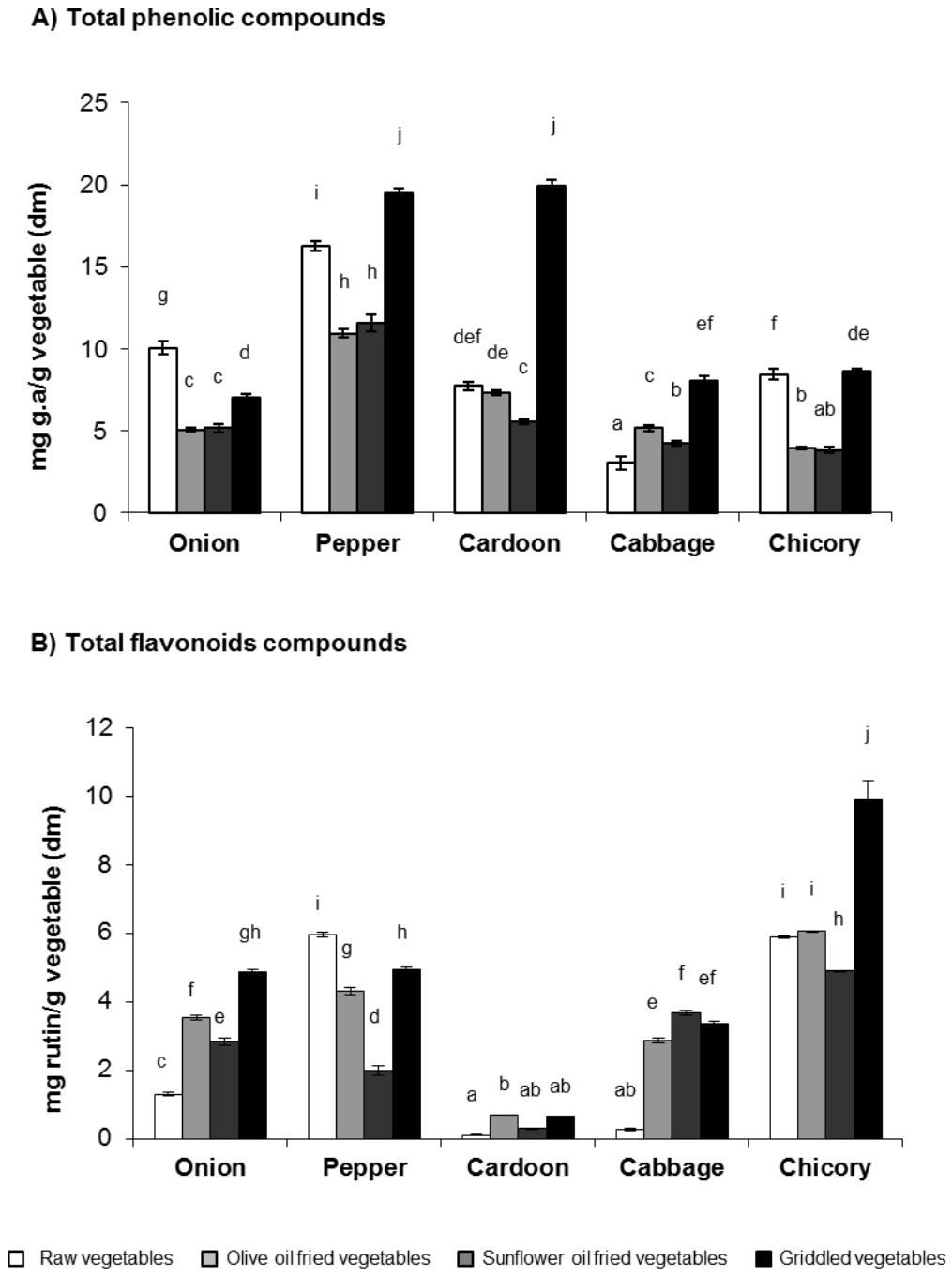


**Figure 2.** Antioxidant capacity (DPPH and ABTS) of raw and cooked vegetables. Different letters indicate significant differences ($p \leq 0.05$).

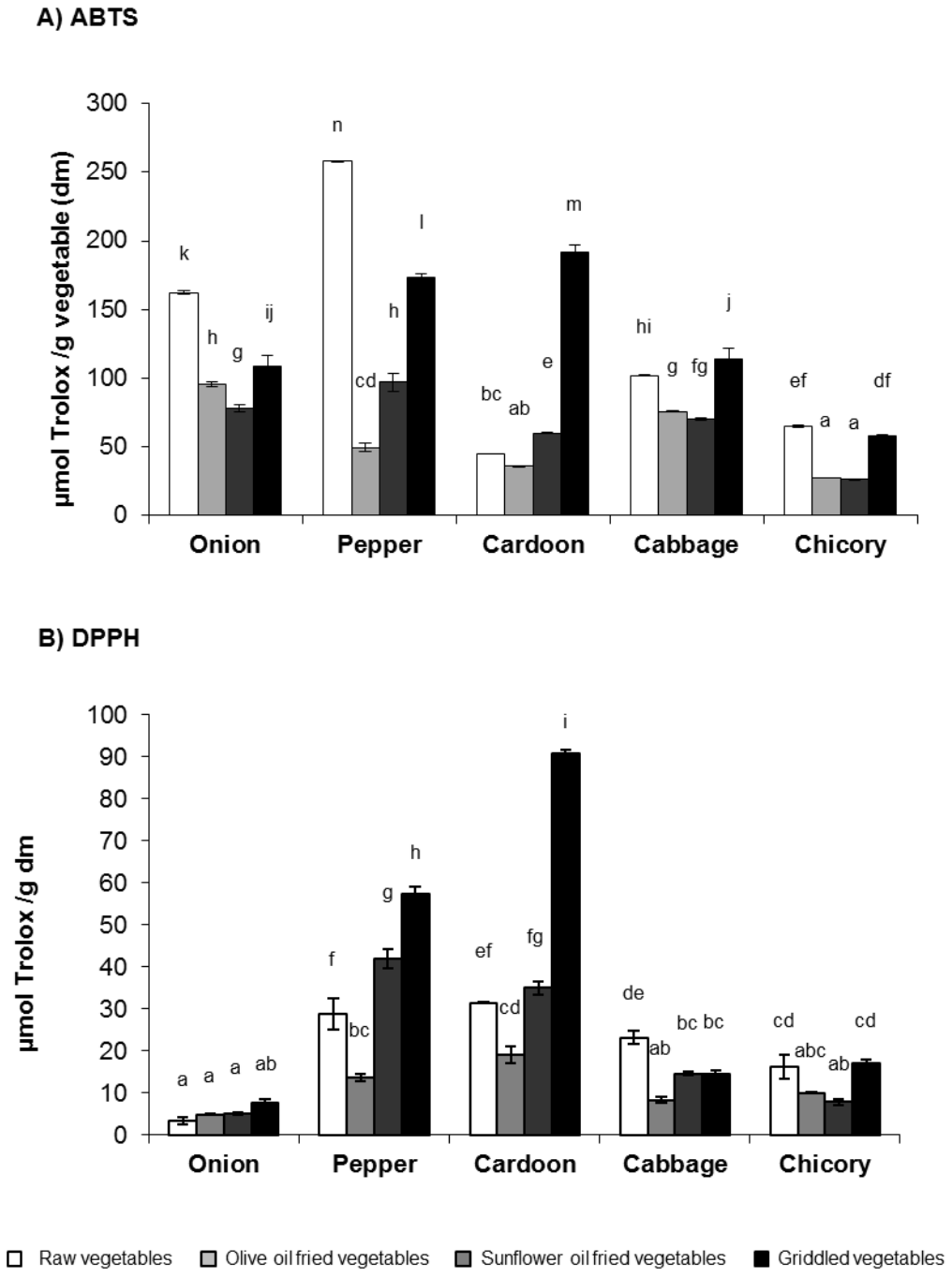
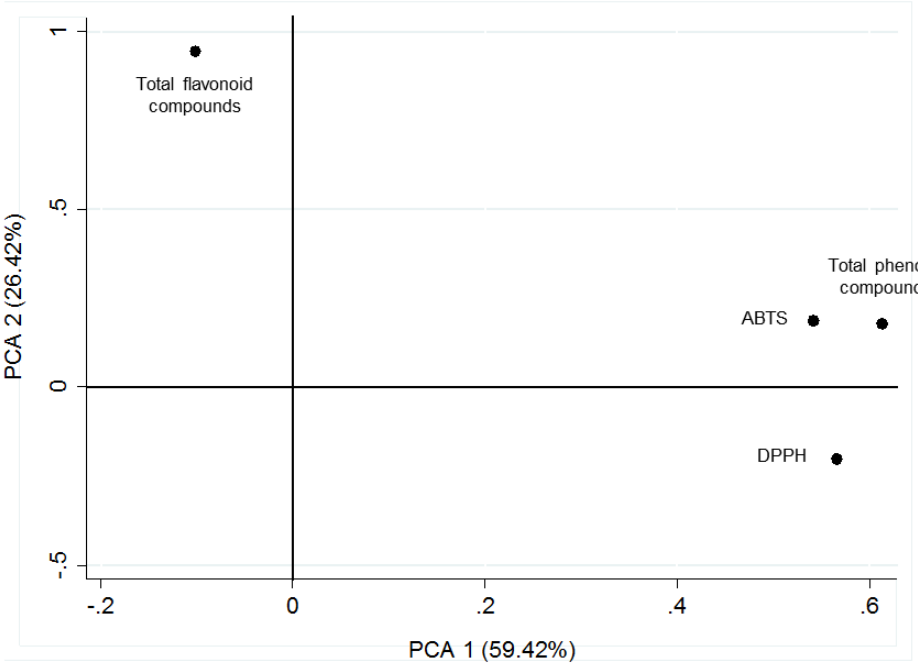


**Figure 3.** Principal Component Analysis (PCA) of the raw and cooked vegetables. (a) Parameter loadings. (b) Sample scores.

A) Parameter loadings




B) Sample scores

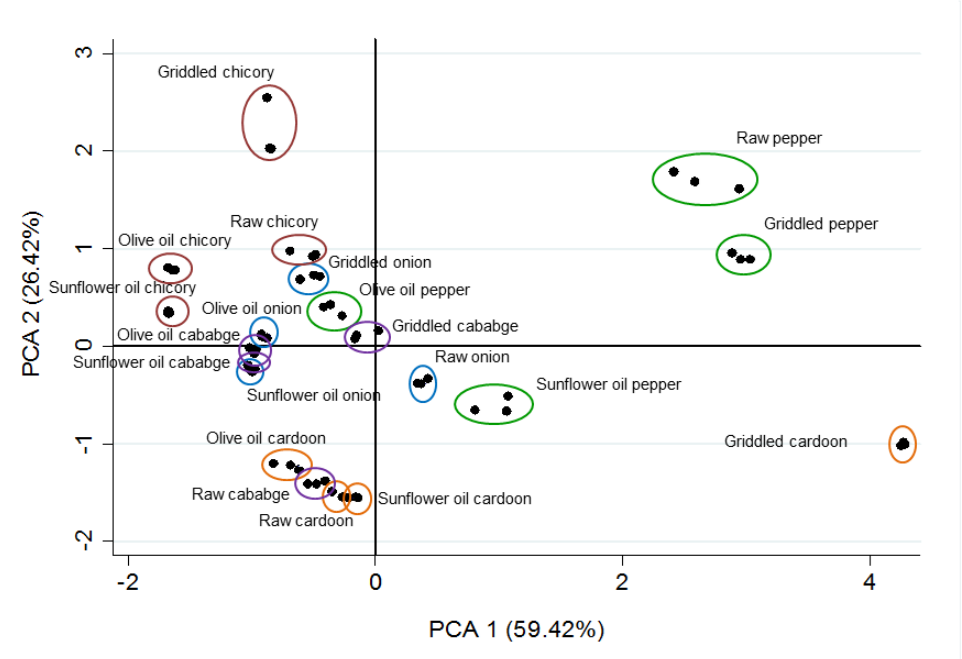


**Table 1**. Pearson correlation coefficients among the different variables.

| | **Total phenolic compounds** | **Total flavonoid compounds** | **DPPH** | **ABTS** |
|---|---|---|---|---|
| **Total phenolic compounds** | r= 1 | | | |
| **Total flavonoid compounds** | r= 0.0300 ns | r=1 | | |
| **DPPH** | **r= 7919 ***** | r= - 0.2594 ns | r=1 | |
| **ABTS** | **r= 0.7467 ***** | r= - 0.0212 ns | **r= 0.5048 ***** | r=1 |

*P < 0.05; **P < 0.01; ***P < 0.001; ns no significant correlation

# Objective 1.2

**Impact of heat treatment on the selected vegetables: onion (*Allium cepa*), green pepper (*Capsicum annuum*) and cardoon (*Cynara cardunculus* L.).**

Impacto del tratamiento térmico en los alimentos vegetales seleccionados: cebolla (*Allium cepa*), pimiento verde (*Capsicum annuum*) y cardo (*Cynara cardunculus L*.).

# Paper 1

## Impact of several cooking processes on nutritional composition and antioxidants of selected vegetables

**I.Juániz**, E. Huarte, C.B. de Gorostiza, C. Cid, M.P. de Peña. (In preparation)

**TITLE:**

**Impact of several cooking processes on nutritional composition and antioxidants of selected vegetables**

**AUTHORS: Isabel Juániz, Estibaliz Huarte, Carlos Borja de Gorostiza, Concepción Cid and María-Paz De Peña***

Universidad de Navarra, Facultad de Farmacia y Nutrición, Departamento de Ciencias de la Alimentación y Fisiología, C/ Irunlarrea 1, E-31008 Pamplona, España. IdiSNA, Navarra Institute for Health Research.

*Corresponding author: María-Paz de Peña. Tel: +34 948 425600 (806580); Fax: +34 948 425740. E-mail address: mpdepena@unav.es

**ABSTRACT**

The impact of cooking heat treatments (frying in olive or sunflower oil, and griddling) on the proximate and fat composition, (poly)phenols and antioxidant properties of onion, green pepper and cardoon was evaluated. Only fried vegetables showed relevant amounts of fat, with higher ω-3 fatty acids content and lower ω-6/ω-3 PUFA ratio when olive oil was used. Thus, olive oil should be the preferable frying oil. Raw cardoon had the highest total phenolics content (167.59 ± 4.05 mg GA/100g) and raw green pepper the highest flavonoids amount (44.02 ± 0.71 mg rutin/100g). Heat treatment, particularly griddling, tends to increase (poly)phenols and flavonoids, and antioxidant capacity, being griddled cardoon the vegetable with both the highest total phenolics content (334.32 ± 6.94 mg GA/100g) and antioxidant capacity (1524.20 ± 17.94 μmol Trolox/100g). Therefore, griddling process is suggested as the best heat treatment to substantially increase (poly)phenols and antioxidant capacity of vegetables, without fat addition.

**KEYWORDS:** Vegetables, Heat treatment, Nutritional value, Fatty acids, Phenolics, Flavonoids, Antioxidant capacity

## 1. Introduction

Mediterranean countries such as Turkey, Spain, Italy, Greece or France are the largest producers of vegetables in Europe (Eurostat, 2015). Mediterranean Diet is characterized by the consumption of a wide variety of products such as cereals (whole grain), plenty of fruit, vegetables and nuts, olive oil, legumes, herbs and spices, fish and seafood and moderate amounts of meat and wine (Gerber & Hoffman, 2015). Despite the high variety of foods, many of the benefits attributed to a Mediterranean diet are due to the antioxidant phytochemicals of fruits and vegetables. Plants foods are the main source of dietary antioxidants, including phenolic compounds, which have been reported to exhibit a wide range of biological effects such as anti-inflamatory effects or protective effects against cardiovascular diseases, neurodegenerative diseases and cancer, probably due to their ability to protect against oxidative damage in cells (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014). Additionally, vegetables are rich in minerals and vitamins and contain a large amount of water in its composition, implying a low caloric intake in the diet. Therefore, vegetables are of a great interest from the nutritional point of view and the World Health Organization and other international agencies urge for a higher consumption of plant foods (WHO, 2004).

In Spain, an increase in the consumption of vegetables has been observed in the last decades. In 2015, the consumption of fresh vegetables (without potatoes), was 163.58 g/capita/day (MAGRAMA, 2015), a 13% higher than in 2001 when the consumption of vegetables in Spanish households was 144.93 g/capita/day (MAGRAMA, 2001). Specifically, onion and pepper are two of the most consumed vegetables in Spain (MAGRAMA, 2015), however there are lots of local vegetables as cardoon, chard or chicory which also have a high acceptability among population depending on the region.

Many dietary vegetables are usually eaten both crude or after cooking in different ways. Among the high variety of cooking processes, frying is a very common culinary technique applied to foods to develop their typical sensorial properties or in order to be used as base ingredients in the Mediterranean cuisine. Also griddling is quite common in this cuisine. Culinary processes generally induce significant changes in foods such as water loss, changes in fat content, degradation of thermolabile compounds, and formation of others due to heat-induced chemical reactions (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Miranda et al., 2010). Furthermore, phytochemical composition of consumed vegetables, specifically (poly)phenolic compounds, can also be affected by thermal processes and, consequently

their antioxidant capacity (Juániz, et al., 2016a; Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015). Aditionally, healthy properties of foods also depends on the total amount of fat and the unsaturated/saturated fatty acids ratio, and consequently on the type of fat used during cooking. In Spain, olive oil is the most consumed culinary fat (around 23 ml/capita/day) followed by sunflower oil (8.5 ml/capita/day) (MAGRAMA, 2015).

Therefore, the aim of this work was to study the impact of three cooking heat treatments (frying in olive oil, frying in sunflower oil and griddled) on the proximate composition, fatty acids profile, total phenolic and flavonoid compounds and antioxidant properties of different vegetables commonly consumed as crude in salads and cooked in several ways in the mediterranean diet in order to suggest the best culinary technique.

## 2. Material and methods

### 2.1 Chemical and reagents

Yellow onion (*Allium cepa*), sweet Italian green pepper (*Capsicum annuum*), cardoon stalks (*Cynara cardunculus L*), olive oil and sunflower oil were obtained from local stores.

Different solvents of analytical grade were from Panreac (Barcelona, Spain) as well as potassium chloride, sodium chloride, sodium hydroxide, and Folin–Ciocalteau reagent. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH·), gallic acid, rutin and aluminum chloride hexahydrate were purchased from Sigma-Aldrich (Steinheim, Germany). All fatty acid methyl esters (FAME) were also purchased from Sigma-Aldrich (Steinheim, Germany).

### 2.2 Samples preparation

Chopped vegetables (yellow onion, green pepper and cardoon) (300 g) were fried with olive or sunflower oils (30 mL) at 115 ºC for 10 minutes in a non-stick frying pan. Then, temperature was decreased to 108 ºC for 5 minutes. Chopped vegetables were also submitted to heating at 150 ºC for 10 minutes and then at 110 ºC for 5 minutes in a non-stick griddle without oil addition. Then, raw and cooked vegetables were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa), and stored at -18ºC until further analysis.

### 2.3 Nutritional composition

The samples were analyzed for moisture, protein, ash, fat and carbohydrates using official methods (AOAC, 2002a). Moisture of each sample was determined drying the samples in an oven at 102 ± 3 °C until constant weight. Total protein content of the samples was

estimated by the Kjeldahl method. The ash content was determined by incineration at 550°C ± 10°C. Total fat content was extracted with petroleum ether from previously dried samples by Soxhlet extraction system (Extraction Unit B-811 Standard BUCHI, Flawil, Switzerland). Finally total carbohydrates were calculated by difference. Energy was calculated according to the following equation:

Energy (Kcal/100g) = 4 x (g protein/100g) + 4 x (g carbohydrates/100g) + 9 x (g fat/100g)

Fatty acid profile was determined in the lipid extract by gas chromatography. Lipids were extracted using the method of Folch et al. (1957) and boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (FAME) (AOAC, 2002b). An Agilent HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) fitted with a capillary column SPTM-2560 (100m × 0.25mm × 0.2 μm) and flame ionization detection was used. The injector temperature was 250°C and the carrier gas was helium at a flow of 1 ml $min^{-1}$. The oven temperature was programmed at 175°C for 10min and increased to 200°C at a rate of 10°C $min^{-1}$, then increased at 4°C $min^{-1}$ up to 220°C, which was kept for 15min. The FID detector temperature was 260°C. Fatty acid methyl esters were identified by comparison of the retention times of the peaks in the sample with those of standard pure compounds. Individual fatty acids were quantified using heptadecanoic acid methyl ester as an internal standard. After the quantification of the individual fatty acids, the sums of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), ω-3, ω-6 and trans fatty acids and their corresponding ratios were calculated.

2.4 Vegetables extracts

Vegetables extracts were prepared using the Siddiq method (Siddiq, Roidoung, Sogi, & Dolan, 2013) with some modifications (Juániz et al., 2016a). Briefly, 30 mL of Ethanol/Water (80/20) were added to 2 grams of lyophilized vegetables. The content were mixed on a mechanical shaker for 1 hour at room temperature and then centrifuged at 4000 rpm for 10 minutes. Supernatant was collected and residues were re-extracted twice using 10 mL of ethanol 80% by vortexing (1 minute) and centrifugation at 4000 rpm for 5 minutes. All three supernatants were combined and freezer at -18ºC for subsequent analyses.

2.5 Total phenolic compounds (TPC)

Total phenolic compounds were measured using the Folin–Ciocalteu reagent according to Singleton's method (Singleton & Rossi, 1965). Each vegetable extract was properly diluted in

demineralized water. A volume of 500 µL of Folin–Ciocalteu reagent was added to a mixture of 100 µL of the extract sample and 7.9 mL of demineralized water. After a 2 min delay, 1.5 mL of a 7.5% sodium carbonate solution was added. Next, the sample was incubated in darkness at room temperature for 90 min. The absorbance of the sample was measured at 765 nm in a spectrophotometer Lambda 25 UV/VIS (Perkin Elmer Instruments, Madrid, Spain). Gallic Acid (GA) was used as reference, and the results were expressed as milligrams of GA equivalent per 100 gram of sample (mg GA/100 g vegetable).

2.6 Total flavonoid content (TFC)

The aluminium chloride method (Lamaison & Carnet, 1990) was used for estimation of the total flavonoids content of the extracted samples. An aliquot of 100 µL of each vegetable extract properly diluted was added to 1 mL of a 2% $AlCl_3 \cdot 6H_2O$ methanol solution. The mixture was vigorously shaken, and after 10 min of incubation at room temperature, the absorbance was read at 430 nm in a spectrophotometer Lambda 25 UV/VIS (Perkin Elmer Instruments, Madrid, Spain). Total flavonoid content was calculated from the calibration curve of rutin standard solutions, and expressed as milligram of rutin equivalent per 100 grams of sample (mg rutin/100 g vegetable).

2.7 Antioxidant capacity by DPPH assay

The antioxidant capacity was measured using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) decolorization assay (Brand-Williams, Cuvelier, & Berset, 1995) with some modifications. A $6.1 \times 10^{-5}$ M $DPPH^{\bullet}$ methanolic solution was prepared immediately before use. The $DPPH^{\bullet}$ solution was adjusted with methanol to an absorbance of 0.700 (±0.020) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). Vegetable extracts were properly diluted in demineralized water prior to analysis. Samples (50 µL) were added to 1.95 mL of the $DPPH^{\bullet}$ solution. After mixing, the absorbance was measured at 515 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analog). The antioxidant capacity was expressed as micromoles of Trolox equivalent per 100 gram of samnple (µmol Trolox/100 g).

2.8 Statistical analysis

Each parameter was analysed in triplicate. Results are shown as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied for each parameter. A Tukey test was applied as *a posteriori test* with a level of significance of 95%. Within each

type of fried sample, the differences in fatty acid profile between frying with olive oil and frying with sunflower oil were evaluated by Student t-test. All statistical analyses were performed using the STATA v.12.0 software package.

## 3. Results and discussion

### 3.1 Nutritional composition

Table 1 shows the effect of heat treatment on proximate composition of the selected vegetables (onion, green pepper and cardoon). All raw vegetables showed a high content of water in their composition, specifically cardoon was the vegetable with the lowest moisture level (83.2 g/100g) followed by onion (90.1 g/100g) and green pepper (92.6 g/100g). Moisture levels were significantly decreased after cooking process due in general to the temperature applied and, in the case of fried samples, also to the relative gain of fat during heat treatment. Fried samples, and specifically sunflower oil fried cardoon (76.4 g/100g), showed the lowest moisture levels.

Vegetables are characterized by their low fat content and, as expected, only fried vegetables showed relevant amounts of fat on their composition (6.0 – 7.7 g/100g). No significant differences on the total fat content between vegetables fried in olive oil or in sunflower oil were observed. Nevertheless, differences on the fatty acid profile were detected depending on the oil employed during the process (Table 2). Oleic acid was the most abundant fatty acid in those olive oil fried vegetables, whereas linoleic acid was the main one in that sunflower oil fried. Therefore, the main fatty acids in olive oil fried samples were monounsaturated fatty acids (MUFA). High MUFA diets reduce fasting glucose in patients with type 2 diabetes and show a beneficial effect on important risk factors as fat mass and systolic and diastolic blood pressure (Schwingshackl & Strasser, 2012; Schwingshackl, Strasser, & Hoffmann, 2011). Some studies report that olive oil consumption is associated with reduced risks of cardiovascular disease, and mortality (Buckland et al., 2012; Guasch-Ferré et al., 2014; Soriguer et al., 2013). In contrast, sunflower oil fried vegetables presented higher content polyunsaturated fatty acids (PUFA). As expected, olive oil fried vegetables shown a higher content of ω-3 fatty acids and a lower ω-6/ω-3 PUFA ratio than those fried with sunflower oil. A low ω-6/ω-3 PUFA ratio has been recommended since a high intake of ω-6 fatty acids may reduce the formation of anti-inflammatory mediators by ω-3 fatty acids (Aranceta & Pérez-Rodrigo, 2012). Consequently, different health authorities and scientific organizations now recommend an increase in the dietary intake of ω-3 PUFAs to

achieve nutrient adequacy and to prevent cardiovascular diseases (Gebauer, Psota, Harris, & Kris-Etherton, 2006).

Heat treatments favored the occurrence of trans fatty acids in fried vegetables, especially in those fried in sunflower oil. In fact, trans fatty acids were no present in the fresh oil samples (Table 3). Among olive oil fried samples, cardoon was the vegetable with the highest amount of trans fatty acids (0.036g/100g), whereas green pepper presented the highest level of trans fatty acids when sunflower oil was used for frying (0.054 g/100g). In all samples the major trans fatty acid was elaidic acid (C18:1Δ9t) which probably could be formed during heat treatment by changes from cis to trans configuration of the double bond of oleic acid (C18:1). However, the sum of all trans linoleic acids (t-linoleic, c-t-linoleic and t-c-linoleic acids) represented the most abundant trans fatty acids fraction in those sunflower oil fried vegetables, while they are hardly present in olive oil fried samples, and totally absent in fresh oils. Wolff (1994) reported that heating at high temperature (240°C) affects mainly octadecatrienoic acids such as linoleic and linolenic acids, which undergo cis-trans isomerization of their double bonds. Probably, the lower temperature applied during the frying process of vegetable samples (115°C) induces the formation of low amount of trans fatty acids. These results are in agreement with the little impact on trans fatty acids observed when unhydrogenated edible oils are used in ordinary frying process (Tsuzuki, Matsuoka, & Ushida, 2010). The intake of trans fatty acids has been associated with health disorders such as cardiovascular diseases, breast cancer or attention-deficit/hyperactivity disorder (Chajes et al., 2008; Kim et al., 2012; Mensink & Katan, 1990; Ruano et al., 2011; Willett et al., 1993). Then, the intake of trans fatty acids has to be as low as possible (EFSA, 2010). Therefore, according to their lower ω-6/ω-3 PUFA ratio, their higher ω-3 fatty acids, and their lower trans fatty acids, it could be preferable the use of olive oil instead of sunflower oil for frying vegetables. Nevertheless, the global diet and not only the fat provided by one individual food should be considered for health recommendations.

Protein content was not significantly affected by thermal treatment, with the exception of both, olive oil or sunflower oil fried cardoon samples, which showed less protein content than raw and griddled cardoon (Table 1). Similarly, ash or mineral content was no significantly affected by cooking process. Finally, energy values were calculated (Table 1). Raw vegetables implied a low caloric intake in the diet, which is significantly affected by the addition of oil during thermal treatment. As expected, fried vegetables showed the highest

caloric intake contribution (more than 100 Kcal/100g in all fried samples) due to the fat content. Despite the absence of fat in griddled vegetables, their energy value was significantly higher than that of the raw ones, due to water losses and the subsequently relative increase of the carbohydrates. However, griddled vegetables provided around the half of the energy of fried samples, so they could be of a high interest from a nutritional point of view in order to increase the variety of the culinary techniques that can be used in low caloric diets.

3.2 Phenolic content and antioxidant capacity

The amount of total phenolic content in the analyzed raw and cooked vegetables is shown in Figure 1A. *Cynara cardunculus* L. stalks (cardoon) was the raw vegetable with the highest amount of total phenolic content (167.59 ± 4.05 mg GA/100g). This result expressed in dry matter (7.72 ± 0.28 mg GA/g dm) is substantially higher than those reported previously by other authors in *C. cardunculus* L. stalks (0.66 - 2.88 mg GA/g dm) (Velez et al., 2012). On the other hand, onion was the vegetables with the lowest amount of phenolic compounds (96.52 ± 2.15 mg GA/100g). This value is in the range between 16.8 and 114.7 mg GA/100g, found by Yang, Meyers, Van der Heide, & Liu (2004) for different types of onion. In general, differences found in total phenolic content could be due to differences in the vegetable variety, as well as other factors like genetic differences, climate, madurity or harvest season variation (Lu, Ross, Powers, & Rasco, 2011; Rodríguez Galdón, Rodríguez Rodríguez, & Díaz Romero, 2008; Sellappan & Akoh, 2002; Yang et al., 2004). Figure 1B shows the total flavonoid content of raw and cooked vegetables. Cardoon had the lowest amounts of flavonoids (2.21 ± 0.27 mg rutin/100g). In agreement with literature, some authors reported that the most abundant compound in *Cynara cardunculus* L., and specifically in the stalks part (Cardoon), are chlorogenic acids while only traces of some flavonoids have been previously identified in this vegetable (Juániz et al., 2017; Juániz et al., 2016b; Pinelli et al., 2007). Contrary to cardoon, green pepper is the vegetable with the highest amount of flavonoids before cooking process (44.02 ± 0.71 mg rutin/100g). Some authors reported that quercetin and luteolin derivates were the main (poly)phenols in green pepper samples, accounting for more than 90% of total phenolic compounds in all samples (Juániz, et al., 2016a; Juániz, et al., 2016b; Marín, Ferreres, Tomás-Barberán, & Gil, 2004). Quercetin derivates, as well as other flavonoids such as isorhamnetin and kaempferol derivates, are also reported as the main (poly)phenolic compounds in onion (Lanzotti, 2006;

Lombard, Peffley, Geoffriau, Thompson, & Herring, 2005; Lu et al., 2011; Rodríguez Galdón et al., 2008; Simin et al., 2013).

The effect of heat treatment on total phenolic and total flavonoid compounds was also evaluated (Figure 1). A tendency to increase (poly)phenolic compounds with the heat treatment is observed, but it depends on the analyzed vegetable. In onion, total flavonoid content increased drastically after heat treatment. The increment was 397.18%, 455.38% and 506.75% for sunflower oil fried onion; olive oil fried onion and griddled onion, respectively. However, cooking process did not significantly affect ($p>0.05$) the total phenolic content. These contradictory results might be explained by a substantial loss of other non-flavonoid (poly)phenols during heat treatment that might counterbalance the flavonoids increase. In green pepper, a significant increase in total phenolic content after heat treatment was observed. There were not significant differences ($p>0.05$) between olive and sunflower oil fried samples, while griddling process favored a great release of phenolic compounds, reaching 249.25 ± 0.61 mg g.a /100g vegetable. Total flavonoid content in pepper was also increased after heat treatment; being olive oil fried pepper the sample with the highest levels (82.55 ± 1.59 mg rutin/100g vegetable). In the case of cardoon samples, those fried in olive oil also showed the highest content of flavonoids (15.20 ± 0.08 mg rutin/100g vegetable) followed by griddled cardoon (10.83 ± 0.42 mg rutin/100g vegetable), while only griddled cardoon presented a significantly higher amount of total (poly)phenolic compounds than raw and fried cardoon samples, becoming the sample with the highest content among all the analyzed samples (334.32 ± 6.94 mg GA/100 g vegetable). Overall, the increment of total phenolic and flavonoid contents observed could be caused by the thermal destruction of cell walls and sub cellular compartments during the cooking process that favor the release of these compounds (Palermo, Pellegrini, & Fogliano, 2014). Moreover, the higher temperature during griddling than that applied during frying might favor the release of all these polyphenolic compounds, including flavonoids, from the food matrix (Juániz et al., 2016a). Other authors also reported increases on different phenolic compounds in cooked vegetables as a consequence of the disruption of cell walls, which liberated soluble phenolic compounds from the insoluble ester bound, or also due to the inactivation of polyphenol oxidase enzyme during heat treatment, leading to the inhibition of polyphenols degradation (Chuah et al., 2008; Hwang, Shin, Lee, Lee, & Yoo, 2012; Jiménez-

Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009; Juániz et al., 2016a; Ramírez-Anaya et al., 2015)

Additionally, the antioxidant capacity (DPPH) of the selected vegetables analyzed in the present study is shown in Figure 2. Antioxidant capacity was higher in cooked vegetables than in raw ones, with the exception of olive oil fried cardoon. Griddled cardoon, which was the sample with the highest amount of total phenolic content, also had the highest antioxidant capacity (1524.20 ± 17.94 μmol Trolox/100g). Contrarily, raw and cooked onion had lowest antioxidant capacity, which also correspond with the lower amount of total phenolic content. In agreement with these results, some authors had previously reported good positive correlations between phenolic compounds and the antioxidant capacity measured by DPPH (Juániz et al., 2016a; Ramírez-Anaya et al., 2015).

**4. Conclusion**

In summary, the higher antioxidant capacity due to the presence of higher amounts of phenolic compounds suggests that the consumption of cooked vegetables would be better than in their raw form, from the health point of view. Specifically, griddling process can be recommended as the best heat treatment to cook vegetables in order to substantially increase their total phenolic content, or at least their flavonoid fraction, and consequently their antioxidant capacity, without the addition of fat. On the other hand, when frying process is chosen, olive oil should be the preferable cooking oil because the high presence of MUFA, especially oleic acid, the higher ω-3 fatty acids and the lower amount of trans fatty acids.

**References**

AOAC. (2002a). Chapter 3. Plants. In W. Horwitz (Ed.), Official method of analysis (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists. *Official Methods of Analysis*.

AOAC. (2002b). Methyl esters of fatty acids in oils and fats. 969.33. In W. Horwitz (ed.), official method of analysis (17th ed.) (pp 19-20). Gaithersburg, Maryland: Association of Official Analytical Chemists. *Official methods of analysis*.

Aranceta, J., & Pérez-Rodrigo, C. (2012). Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review.

*British Journal of Nutrition*, *107*(S2), S8–S22.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30.

Buckland, G., Travier, N., Barricarte, A., Ardanaz, E., Moreno-Iribas, C., Sanchez, M.-J., … Gonzalez, C. A. (2012). Olive oil intake and CHD in the European prospective investigation into cancer and nutrition Spanish cohort. *British Journal of Nutrition*, *108*(11), 2075–2082.

Chajes, V., Thiebaut, A. C. M., Rotival, M., Gauthier, E., Maillard, V., Boutron-Ruault, M.-C., … Clavel-Chapelon, F. (2008). Association between serum trans-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC study. *American Journal of Epidemiology*, *167*(11), 1312–1320.

Chuah, A. M., Lee, Y.-C., Yamaguchi, T., Takamura, H., Yin, L.-J., & Matoba, T. (2008). Effect of cooking on the antioxidant properties of coloured peppers. *Food Chemistry*, *111*(1), 20–28.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling*, *18*(14), 1818–1892.

European Food Safety Authority (EFSA). (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, *8*(3), 1461.

Eurostat. (2015). Statistical books. Agriculture, forestry and fishery statistics 2015 edition. URL http://ec.europa.eu/eurostat/documents/3217494/7158355/KS-FK-15-101-EN-N.pdf/79470e8c-abf3-43d3-8cd4-84880962cdd4. Accessed 15.03.2017

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*(1), 497–509.

Gebauer, S. K., Psota, T. L., Harris, W. S., & Kris-Etherton, P. M. (2006). n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular

benefits. *The American Journal of Clinical Nutrition*, *83*(6 Suppl), 1526S–1535S.

Gerber, M., & Hoffman, R. (2015). The Mediterranean diet: health, science and society. *The British Journal of Nutrition*, *113 Suppl*, S4-10.

Guasch-Ferré, M., Hu, F. B., Martínez-González, M. A., Fitó, M., Bulló, M., Estruch, R., … Salas-Salvadó, J. (2014). Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. *BMC Medicine*, *12*, 78.

Hwang, I. G., Shin, Y. J., Lee, S., Lee, J., & Yoo, S. M. (2012). Effects of Different Cooking Methods on the Antioxidant Properties of Red Pepper (*Capsicum annuum* L.). *Preventive Nutrition and Food Science*, *17*(4), 286–292.

Jiménez-Monreal, A. M., García-Diz, L., Martínez-Tomé, M., Mariscal, M., & Murcia, M. A. (2009). Influence of Cooking Methods on Antioxidant Activity of Vegetables. *Journal of Food Science*, *74*(3), H97–H103.

Juániz, I., Ludwig, I. A., Bresciani, L., Dall'Asta, M., Mena, P., Del Rio, D., … de Peña, M.-P. (2017). Bioaccessibility of (poly)phenolic compounds of raw and cooked cardoon (Cynara cardunculus L.) after simulated gastrointestinal digestion and fermentation by human colonic microbiota. *Journal of Functional Foods*, *32*, 195–207.

Juániz, I., Ludwig, I. A., Huarte, E., Pereira-Caro, G., Moreno-Rojas, J. M., Cid, C., & De Peña, M.-P. (2016a). Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables. *Food Chemistry*, *197*(Pt A), 466–73.

Juániz, I., Ludwig, I. A., Bresciani, L., Dall'Asta, M., Mena, P., Del Rio, D., … de Peña, M. P. (2016b). Catabolism of raw and cooked green pepper (*Capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and faecal fermentation. *Journal of Functional Foods*, *27*, 201–213.

Kim, J.-H., Nam, C.-M., Kim, J.-W., Lee, D.-C., Shim, J.-S., & Lee, H.-R. (2012). Relationship between attention-deficit/hyperactivity disorder and trans fatty acids intake in female adolescents. *Acta Paediatrica*, *101*(9), e431–e433.

Lamaison, J. L. C., & Carnet, A. (1990). Teneurs en principaux flavonoids des fleurs de *Crataegeus monogyna* Jacq et de *Crataegeus laevigata* (Poiret D. C) en fonction de la vegetation. Pharm. Acta. Helv. 65: 315-320. *Pharmaceutica Acta Helvetiae*, *65*, 315–320.

Lanzotti, V. (2006). The analysis of onion and garlic. *Plant Analysis*, *1112*(1–2), 3–22.

Lombard, K., Peffley, E., Geoffriau, E., Thompson, L., & Herring, A. (2005). Quercetin in onion (Allium cepa L.) after heat-treatment simulating home preparation. *Journal of Food Composition and Analysis*, *18*(6), 571–581.

Lu, X., Ross, C. F., Powers, J. R., & Rasco, B. A. (2011). Determination of quercetins in onion (Allium cepa) using infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, *59*(12), 6376–82.

MAGRAMA (Ministry of Agriculture, Food and Enviroment). (2001). La alimentación mesa mes en Hogares Año 2001 enero/diciembre acumulado. URL http://www.mapama.gob.es/ministerio/pags/Biblioteca/Revistas/pdf_AMM%2Fpdf_AMM_2001_acumuladoalimentaci%F3n_en_Hogares.pdf. Accessed 15.03.2017.

MAGRAMA (Ministry of Agriculture, Food and Enviroment). (2015). Informe del consumo de alimentación en España 2015. URL http://www.mapama.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/informeconsumoalimentacion2015_tcm7-422694.pdf. Accessed 15.03.2017.

Marín, A., Ferreres, F., Tomás-Barberán, F. A., & Gil, M. I. (2004). Characterization and quantitation of antioxidant constituents of sweet pepper (Capsicum annuum L.). *Journal of Agricultural and Food Chemistry*, *52*(12), 3861–9.

Mensink, R. P., & Katan, M. B. (1990). Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New England Journal of Medicine*, *323*(7), 439–445.

Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2008). Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of Agricultural and Food Chemistry*, *56*(1), 139–147.

Miranda, J. M., Martínez, B., Pérez, B., Antón, X., Vázquez, B. I., Fente, C. A., … Cepeda, A. (2010). The effects of industrial pre-frying and domestic cooking methods on the nutritional compositions and fatty acid profiles of two different frozen breaded foods. *LWT - Food Science and Technology*, *43*(8), 1271–1276.

Palermo, M., Pellegrini, N., & Fogliano, V. (2014). The effect of cooking on the phytochemical

content of vegetables. *Journal of the Science of Food and Agriculture*, *94*(6), 1057–1070.

Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E., & Romani, A. (2007). Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated cardoon leaves. *Food Chemistry*, *105*(4), 1695–1701.

Ramírez-Anaya, J. D. P., Samaniego-Sánchez, C., Castañeda-Saucedo, M. C., Villalón-Mir, M., & de la Serrana, H. L.-G. (2015). Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. *Food Chemistry*, *188*, 430–8.

Rodriguez-Mateos, A., Vauzour, D., Krueger, C. G., Shanmuganayagam, D., Reed, J., Calani, L., … Crozier, A. (2014). Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. *Archives of Toxicology*, *88*(10), 1803–1853.

Rodríguez Galdón, B., Rodríguez Rodríguez, E. M., & Díaz Romero, C. (2008). Flavonoids in Onion Cultivars (*Allium cepa* L.). *Journal of Food Science*, *73*(8), C599–C605.

Ruano, C., Henriquez, P., Bes-Rastrollo, M., Ruiz-Canela, M., Lopez del Burgo, C., & Sanchez-Villegas, A. (2011). Dietary fat intake and quality of life: the SUN project. *Nutrition Journal*, *10*, 121.

Schwingshackl, L., & Strasser, B. (2012). High-MUFA diets reduce fasting glucose in patients with type 2 diabetes. *Annals of Nutrition and Metabolism*, *60*(1), 33–34.

Schwingshackl, L., Strasser, B., & Hoffmann, G. (2011). Effects of monounsaturated fatty acids on cardiovascular risk factors: A systematic review and meta-analysis. *Annals of Nutrition and Metabolism*, *59*(2–4), 176–186.

Sellappan, S., & Akoh, C. C. (2002). Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. *Journal of Agricultural and Food Chemistry*, *50*(19), 5338–5342.

Siddiq, M., Roidoung, S., Sogi, D. S., & Dolan, K. D. (2013). Total phenolics, antioxidant properties and quality of fresh-cut onions (*Allium cepa* L.) treated with mild-heat. *Food Chemistry*, *136*(2), 803–806.

Simin, N., Orcic, D., Cetojevic-Simin, D., Mimica-Dukic, N., Anackov, G., Beara, I., … Bozin, B. (2013). Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of

small yellow onion (*Allium flavum* L. subsp. flavum, Alliaceae). *LWT - Food Science and Technology*, *54*(1), 139–146.

Singleton, V., & Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16(3)*, 144–158.

Soriguer, F., Rojo-Martinez, G., Goday, A., Bosch-Comas, A., Bordiu, E., Caballero-Diaz, F., … Vendrell, J. (2013). Olive oil has a beneficial effect on impaired glucose regulation and other cardiometabolic risk factors. Di@bet.es study. *European Journal of Clinical Nutrition*, *67*(9), 911–916.

Tsuzuki, W., Matsuoka, A., & Ushida, K. (2010). Formation of trans fatty acids in edible oils during the frying and heating process. *Food Chemistry*, *123*(4), 976–982.

Velez, Z., Campinho, M. A., Guerra, Â. R., García, L., Ramos, P., Guerreiro, O., … Duarte, M. (2012). Biological characterization of *Cynara cardunculus* L. methanolic extracts: antioxidant, anti-proliferative, anti-migratory and anti-angiogenic activities. *Agriculture*, *2*, 472–492.

WHO (World Health Organization). (2004). Global strategy on diet, physical activity and health. *World Health Organization*.

Willett, W. C., Stampfer, M. J., Manson, J. E., Colditz, G. A., Speizer, F. E., Rosner, B. A., … Hennekens, C. H. (1993). Intake of trans fatty acids and risk of coronary heart disease among women. *Lancet*, *341*(8845), 581–5.

Wolff, R. L. (1994). Cis-Trans isomerization of octadecatrienoic acids during Heating - Study of pinolenic (Cis-5,cis-9,cis-12-18/3) acid geometrical-isomers in heated pine seed oil. *Journal of the American Oil Chemists Society*, *71*(10), 1129–1134.

Yang, J., Meyers, K. J., van der Heide, J., & Liu, R. H. (2004). Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *Journal of Agriculture and Food Chemistry*, *52*(22), 6787–6793.

**Figure 1.** Total phenolic and flavonoid content of raw and cooked vegetables. Different letters indicate significant differences ($p \leq 0.05$)

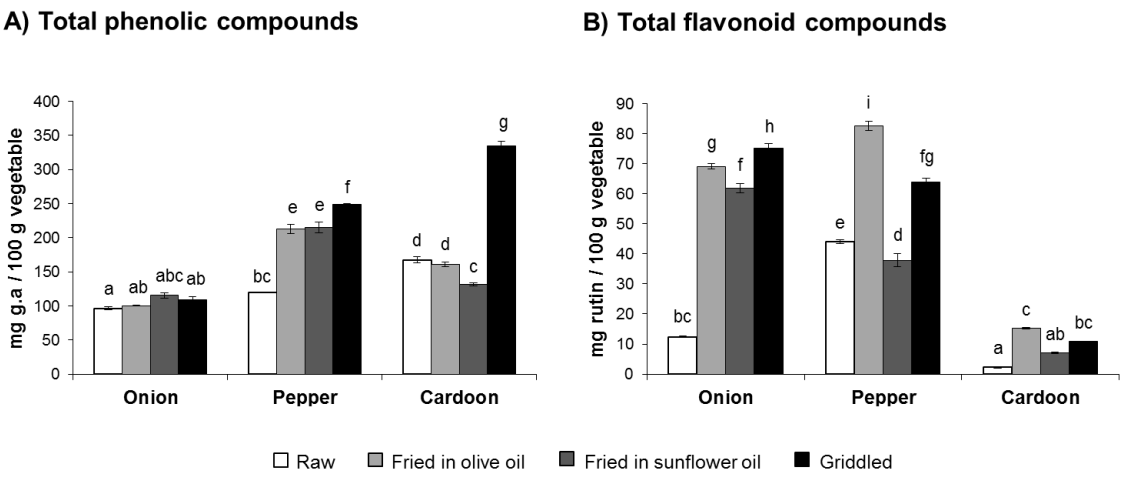


**Figure 2.** Antioxidant capacity (DPPH) of raw and cooked vegetables. Different letters indicate significant differences ($p \leq 0.05$)

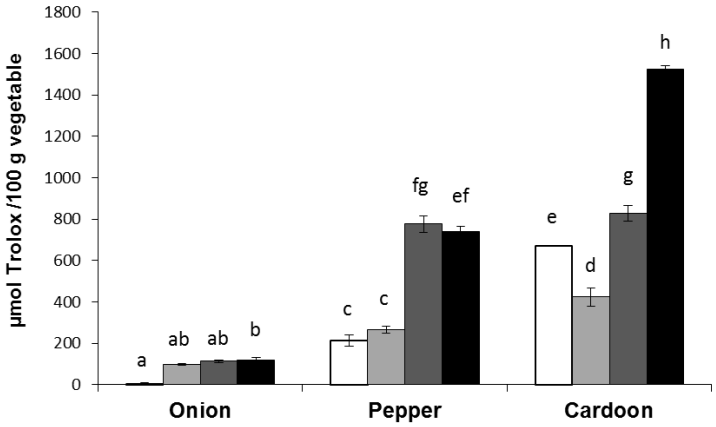


**Table 1.** Proximate composition of selected vegetable both raw and cooked (fried in olive oil, fried in sunflower oil and griddled). Results are expressed as mean ± standard deviation (g per 100 g of vegetable)

| | Onion | Green Pepper | Cardoon |
|---|---|---|---|
| **Moisture** | | | |
| Raw | 90.1 ± 0.2 b B | 92.6 ± 0.1 c B | 83.2 ± 1.6 b A |
| Fried in Olive Oil | 80.4 ± 0.9 a A | 80.6 ± 0.1 a A | 77.9 ± 0.0 a A |
| Fried in Sunflower Oil | 78.0 ± 0.0 a B | 81.5 ± 0.1 a C | 76.4 ± 0.0 a A |
| Griddled | 84.5 ± 2.8 ab B | 87.1 ± 1.0 b B | 78.7 ± 0.2 ab A |
| **Fat** | | | |
| Raw | < 0.1 a | < 0.1 a | < 0.1 a |
| Fried in Olive Oil | 6.3 ± 0.5 b A | 7.7 ± 0.7 b B | 6.0 ± 0.1 b A |
| Fried in Sunflower Oil | 7.5 ± 0.2 c A | 6.8 ± 0.3 b A | 6.5 ± 0.7 b A |
| Griddled | < 0.1 a | < 0.1 a | < 0.1 a |
| **Proteins** | | | |
| Raw | 1.9 ± 0.1 a B | 1.0 ± 0.1 a A | 1.9 ± 0.1 c B |
| Fried in Olive Oil | 1.4 ± 0.0 a B | 1.4 ± 0.0 b B | 0.8 ± 0.1 a A |
| Fried in Sunflower Oil | 1.7 ± 0.2 a B | 1.4 ± 0.1 b B | 0.8 ± 0.0 a A |
| Griddled | 1.7 ± 0.1 a A | 1.5 ± 0.1 b A | 1.6 ± 0.1 b A |
| **Ash** | | | |
| Raw | 0.3 ± 0.0 a A | 0.3 ± 0.0 a A | 1.2 ± 0.1 a B |
| Fried in Olive Oil | 0.3 ± 0.1 ab A | 0.4 ± 0.0 a A | 0.8 ± 0.1 a B |
| Fried in Sunflower Oil | 0.4 ± 0.0 ab A | 0.4 ± 0.0 a A | 0.9 ± 0.1 a B |
| Griddled | 0.5 ± 0.0 b A | 0.4 ± 0.0 a A | 1.2 ± 0.1 a B |
| **Carbohydrates** | | | |
| Raw | 7.6 ± 0.2 a A | 6.0 ± 0.2 a A | 13.6 ± 2.1 a B |
| Fried in Olive Oil | 11.5 ± 1.4 a AB | 9.8 ± 0.6 b A | 14.5 ± 0.1 a B |
| Fried in Sunflower Oil | 12.3 ± 0.2 a AB | 9.9 ± 0.1 b A | 14.7 ± 1.2 a B |
| Griddled | 13.3 ± 4.1 a B | 11.0 ± 1.4 b A | 18.5 ± 0.2 a C |
| **Energy (Kcal/100g)** | | | |
| Raw | 37.9 ± 1.1 a A | 28.5 ± 0.9 a A | 62.3 ± 8.4 a B |
| Fried in Olive Oil | 108.4 ± 4.9 c A | 115.2 ± 2.7 c A | 115.5 ± 1.1 c A |
| Fried in Sunflower Oil | 123.8 ± 0.6 c B | 106.7 ± 0.5 c A | 125.1 ± 2.2 c B |
| Griddled | 59.9 ± 5.9 b B | 50.2 ± 5.5 b A | 80.3 ± 0.1 b C |

In each parameter, different small letters in the same column denote significant differences ($p < 0.05$) among cooking processes.
In each parameter, different capital letters in the same row indicate significant differences ($p \leq 0.05$) among vegetables.

**Table 2.** Fatty acid profile of selected vegetables fried in olive oil and sunflower oil. Results are expressed as mean ± standard deviation (g fatty acid / 100g vegetable).

| Fatty acid | Onion | Green Pepper | Cardoon |
|---|---|---|---|
| **Caprilic C8:0** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Capric C10:0** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Lauric C12:0** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Myristic C14:0** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | 0.013 ± 0.000$^{a}$ | 0.013 ± 0.000$^{a}$ | 0.011 ± 0.001$^{a}$ |
| LS | ** | *** | ** |
| **Palmitic C16:0** | | | |
| Fried in Olive Oil | 0.514 ± 0.004$^{a}$ | 0.712 ± 0.011$^{b}$ | 0.539 ± 0.027$^{a}$ |
| Fried in Sunflower Oil | 0.293 ± 0.004$^{a}$ | 0.352 ± 0.017$^{b}$ | 0.315 ± 0.016$^{ab}$ |
| LS | *** | ** | ** |
| **t-Palmitoleic C16:1 t Δ9t** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Palmitoleic C16:1 (ω-7)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Stearic C18:0** | | | |
| Fried in Olive Oil | 0.149 ± 0.000$^{a}$ | 0.207 ± 0.000$^{b}$ | 0.200 ± 0.006$^{b}$ |
| Fried in Sunflower Oil | 0.196 ± 0.001$^{a}$ | 0.242 ± 0.009$^{b}$ | 0.175 v 0.009$^{a}$ |
| LS | *** | * | ns |
| **Elaidic C18:1Δ9t** | | | |
| Fried in Olive Oil | 0.017 ± 0.000$^{a}$ | 0.015 ± 0.000$^{a}$ | 0.025 ± 0.002$^{b}$ |
| Fried in Sunflower Oil | 0.013 ± 0.003$^{a}$ | 0.015 ± 0.001$^{ab}$ | 0.012 ± 0.002$^{a}$ |
| LS | ns | ns | * |
| **Oleic C18:1 (ω-9)** | | | |
| Fried in Olive Oil | 3.474 ± 0.011$^{b}$ | 4.744 ± 0.009$^{c}$ | 3.184 ± 0.117$^{a}$ |
| Fried in Sunflower Oil | 1.027 ±0.009$^{a}$ | 1.261 ± 0.058$^{b}$ | 1.472 ± 0.070$^{b}$ |
| LS | *** | *** | ** |
| **c-Vaccenic C18:1 (ω-7)** | | | |
| Fried in Olive Oil | 0.065 ± 0.002$^{a}$ | 0.087 ± 0.000$^{b}$ | 0.063 ± 0.002$^{a}$ |
| Fried in Sunflower Oil | 0.016 ± 0.000$^{a}$ | 0.021 ± 0.001$^{b}$ | 0.044 ± 0.002$^{c}$ |
| LS | *** | *** | ** |
| **t-Linoleic C18:2 Δ9t,12t** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | 0.012 ± 0.000$^{a}$ | 0.012 ± 0.001$^{a}$ | 0.009 ± 0.000$^{a}$ |
| LS | ---- | ---- | ---- |
| **c-t-Linoleic C18:2 Δ9c,12t** | | | |
| Fried in Olive Oil | nd | nd | 0.006 ± 0.000 |
| Fried in Sunflower Oil | 0.010 ± 0.000$^{b}$ | 0.011 ± 0.001$^{b}$ | 0.008 ± 0.001$^{a}$ |
| LS | ---- | ---- | ns |
| **t-c-Linoleic C18:2 Δ9t,12c** | | | |
| Fried in Olive Oil | nd | nd | 0.005 ± 0.000 |
| Fried in Sunflower Oil | 0.008 ± 0.000$^{ab}$ | 0.009 ± 0.001$^{b}$ | 0.006 ± 0.001$^{a}$ |
| LS | ---- | ---- | ns |
| **Linoleic C18:2 Δ9c,12c** | | | |
| Fried in Olive Oil | 0.312 ± 0.002$^{a}$ | 0.458 ± 0.000$^{b}$ | 1.235 ± 0.049$^{c}$ |
| Fried in Sunflower Oil | 2.912 ± 0.030$^{b}$ | 3.497 ± 0.152$^{c}$ | 2.176 ± 0.115$^{a}$ |
| LS | *** | ** | ** |
| **Arachidic C:20** | | | |
| Fried in Olive Oil | 0.013 ± 0.000$^{a}$ | 0.019 ± 0.000$^{b}$ | 0.012 ± 0.000$^{c}$ |
| Fried in Sunflower Oil | 0.004 ± 0.000$^{a}$ | 0.009 ± 0.000$^{c}$ | 0.006 ± 0.000$^{b}$ |
| LS | ** | ** | ** |

| | | | |
|---|---|---|---|
| **ϒ-Linoleic C18:3 (ω-6)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Eicosenoic C20:1 (ω-9)** | | | |
| Fried in Olive Oil | 0.008 ± 0.000$^{a}$ | 0.011 ± 0.000$^{b}$ | 0.007 ± 0.000$^{a}$ |
| Fried in Sunflower Oil | 0.002 ± 0.000$^{a}$ | 0.004 ± 0.000$^{b}$ | 0.003 ± 0.000$^{ab}$ |
| LS | ** | ** | * |
| **α-Linolenic C18:3 (ω-3)** | | | |
| Fried in Olive Oil | 0.027 ± 0.000$^{a}$ | 0.054 ± 0.000$^{c}$ | 0.031 ± 0.001$^{b}$ |
| Fried in Sunflower Oil | 0.006 ± 0.000$^{a}$ | 0.020 ± 0.001$^{c}$ | 0.015 ± 0.000$^{b}$ |
| LS | *** | *** | ** |
| **Eicosadienoic C20:2** | | | |
| Fried in Olive Oil | 0.002 ± 0.000$^{a}$ | 0.002 ± 0.000$^{a}$ | 0.003 ± 0.000$^{a}$ |
| Fried in Sunflower Oil | 0.002 ± 0.000$^{a}$ | nd | 0.001 ± 0.001$^{a}$ |
| LS | ns | ---- | ns |
| **Behenic C22:0** | | | |
| Fried in Olive Oil | 0.005 ± 0.000$^{a}$ | 0.008 ± 0.000$^{b}$ | 0.017 ± 0.000$^{c}$ |
| Fried in Sunflower Oil | 0.032 ± 0.001$^{b}$ | 0.043 ± 0.000$^{c}$ | 0.026 ± 0.001$^{a}$ |
| LS | *** | *** | * |
| **Brassidic C20:1Δ13t** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | 0.006 ± 000 | nd |
| LS | ---- | ---- | ---- |
| **Erucic C22:1 (ω-9)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Eicosatrienoic C20:3 (ω-3)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Arachidonic C20:4 (ω-6)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Eicosapentanoic C20:5 (ω-3)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Lignoceric C24:0** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Nervonic C24:1 (ω-9)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Docosapentaenoic C22:5 (ω-6)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Docosapentaenoic C22:5 (ω-3)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **Docosahexaenoic C22:6 (ω-3)** | | | |
| Fried in Olive Oil | nd | nd | nd |
| Fried in Sunflower Oil | nd | nd | nd |
| LS | ---- | ---- | ---- |
| **SFA** | | | |
| Fried in Olive Oil | 0.689 ± 0.003$^{a}$ | 0.946 ± 0.011$^{c}$ | 0.768 ± 0.033$^{b}$ |
| Fried in Sunflower Oil | 0.539 ± 0.004$^{a}$ | 0.658 ± 0.027$^{b}$ | 0.532 ± 0.027$^{a}$ |
| LS | *** | ** | * |
| **MUFA** | | | |
| Fried in Olive Oil | 3.547 ± 0.012$^{b}$ | 4.842 ± 0.009$^{c}$ | 3.255 ± 0.115$^{a}$ |
| Fried in Sunflower Oil | 1.045 ± 0.009$^{a}$ | 1.286 ± 0.059$^{b}$ | 1.519 ± 0.072$^{c}$ |
| LS | *** | *** | ** |
| **PUFA** | | | |
| Fried in Olive Oil | 0.341 ± 0.002$^{a}$ | 0.514 ± 0.001$^{b}$ | 1.280 ± 0.051$^{c}$ |
| Fried in Sunflower Oil | 2.948 ± 0.030$^{b}$ | 3.550 ± 0.153$^{c}$ | 2.214 ± 0.116$^{a}$ |
| LS | *** | ** | ** |

| | | | |
|---|---|---|---|
| **trans** | | | |
| Fried in Olive Oil | 0.017 ± 0.000[a] | 0.015 ± 0.000[a] | 0.036 ± 0.002[b] |
| Fried in Sunflower Oil | 0.042 ± 0.003[a] | 0.054 ± 0.001[b] | 0.035 ± 0.004[a] |
| LS | ** | *** | ns |
| **ω - 3** | | | |
| Fried in Olive Oil | 0.027 ± 0.000[a] | 0.054 ± 0.000[c] | 0.031 ± 0.001[b] |
| Fried in Sunflower Oil | 0.006 ± 0.000[a] | 0.020 ± 0.001[c] | 0.015 ± 0.000[b] |
| LS | *** | *** | ** |
| **ω - 6** | | | |
| Fried in Olive Oil | 0.314 ± 0.002[a] | 0.460 ± 0.001[b] | 1.250 ± 0.050[c] |
| Fried in Sunflower Oil | 2.942 ± 0.030[b] | 3.530 ± 0.153[c] | 2.200 ± 0.116[a] |
| LS | *** | ** | ** |
| **PUFA /SFA** | | | |
| Fried in Olive Oil | 0.501 ± 0.005[a] | 0.544 ± 0.006[b] | 1.668 ± 0.006[c] |
| Fried in Sunflower Oil | 5.471 ± 0.011[c] | 5.392 ± 0.009[b] | 4.159 ± 0.010[a] |
| LS | *** | *** | *** |
| **(PUFA + MUFA) / SFA** | | | |
| Fried in Olive Oil | 5.716 ± 0.048[ab] | 5.665 ± 0.056[a] | 5.908 ± 0.040[b] |
| Fried in Sunflower Oil | 7.410 ± 0.012[b] | 7.345 ± 0.018[b] | 7.011 ± 0.003[a] |
| LS | *** | *** | *** |

nd: no detected
Different letters in the same row indicate significant differences among different vegetables ($P < 0.05$).
LS correspond to Student t test of each vegetable fried in olive oil and fried sunflower oil. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns no significant differences.

**Table 3.** Fatty acid profile of olive oil and sunflower oil. Results are expressed as g fatty acid / 100g oil.

| Fatty acid | Olive oil | Sunflower oil |
|---|---|---|
| **Caprilic C8:0** | nd | nd |
| **Capric C10:0** | nd | nd |
| **Lauric C12:0** | nd | nd |
| **Myristic C14:0** | nd | nd |
| **Palmitic C16:0** | 9.627 ± 0.002 | 5.567 ± 0.001 |
| **t-palmitoleic C16:1 t Δ9t** | nd | nd |
| **Palmitoleic C16:1 (ω-7)** | nd | nd |
| **Stearic C18:0** | 2.940 ± 0.000 | 2.187 ± 0.001 |
| **Elaidic C18:1Δ9t** | nd | nd |
| **Oleic C18:1 (ω-9)** | 65.515 ± 0.015 | 24.722 ± 0.005 |
| **c- Vaccenic C18:1 (ω-7)** | 1.260 ± 0.001 | 0.686± 0.000 |
| **t-Linoleic C18:2 Δ9t,12t** | nd | nd |
| **c-t-Linoleic C18:2 Δ9c,12t** | nd | nd |
| **t-c-Linoleic C18:2 Δ9t,12c** | nd | nd |
| **Linoleic C18:2 Δ9c,12c** | 6.506 ± 0.001 | 45.437± 0.020 |
| **Arachidic C:20** | nd | 0.132 |
| **ϒ-linoleic C18:3 (ω-6)** | nd | nd |
| **Eicosenoic C20:1 (ω-9)** | 0.155± 0.000 | 0.068± 0.000 |
| **α-Linolenic C18:3 (ω-3)** | 0.544± 0.000 | nd |
| **Eicosadienoic C20:2** | nd | nd |
| **Behenic C22:0** | nd | 0.530± 0.001 |
| **Brassidic C20:1Δ13t** | nd | nd |
| **Erucic C22:1 (ω-9)** | 0.320± 0.000 | nd |
| **Eicosatrienoic C20:3 (ω-3)** | nd | nd |
| **Arachidonic C20:4 (ω-6)** | nd | nd |
| **Eicosapentanoic C20:5 (ω-3)** | nd | nd |
| **Lignoceric C24:0** | nd | nd |
| **Nervonic C24:1 (ω-9)** | nd | nd |
| **Docosapentaenoic C22:5 (ω-6)** | nd | nd |
| **Docosapentaenoic C22:5 (ω-3)** | nd | nd |
| **Docosahexaenoic C22:6 (ω-3)** | nd | nd |

nd: no detected

# Paper 2

## Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables

**I.Juániz**, I.A. Ludwig, E. Huarte, G.Pereira-Caro, J.M. Moreno-Rojas C. Cid, M.P. de Peña (2016). *Food Chemistry*, 197, 466-473. http://dx.doi.org/10.1016/j.foodchem.2015.10.139

Quality indices:

- Impact factor (JCR, 2015): 4.052
- Journal Rank in categories:
    - Food Science & Technology: 7/125 (Q1)(D1)
    - Chemistry, applied: 7/72 (Q1)(D1)
    - Nutrition & Dietetics: 12/80 (Q1)

Juániz I, Ludwig I, Huarte E, Pereira-Cabo G, Moreno-Rojas JM, Cid C, De Peña, MP. Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables. Food Chemistry, April 2016, 197:466-473.

## SUPPLEMENTARY INFORMATION

**TITLE: Influence of heat treatment on antioxidant capacity and bioactive compounds of selected vegetables**

**RUNNING TITLE: Heat treatment on antioxidant capacity and phenolics of vegetables**

**AUTHORS: Isabel Juániz [a], Iziar A. Ludwig [b], Estibaliz Huarte [a], Gema Pereira-Caro [b], Jose Manuel Moreno-Rojas [b], Concepción Cid [a] and María-Paz De Peña [a]***

a Department of Nutrition, Food Science and Physiology, School of Pharmacy, University of Navarra. IdiSNA, Navarra Institute for Health Research. E-31080-Pamplona, Spain.

b Postharvest Technology and Agrifood Industry Area. Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA). Alameda del Obispo Centre, Córdoba. Spain

*Corresponding author: María-Paz de Peña. Tel: +34 948 425600 (806580); Fax: +34 948 425740. E-mail address: mpdepena@unav.es

**Table S1. Identification of polyphenols in onion by UPLC-MS**

| Peak no. | Rt (min) | Compound | $[M-H]^-_{theor.}$ (m/z) | $[M-H]^-_{exp.}$ (m/z) | δ (ppm) | Online fragment (m/z) |
|---|---|---|---|---|---|---|
| 1 | 13.46 | Quercetin triglucoside | 787.1927 | 787.1924 | -0.381 | 301.0342 |
| 2 | 23.44 | Quercetin diglucoside I | 625.1399 | 625.1405 | 0.959 | 301.0342 |
| 3 | 24.02 | Quercetin diglucoside II | 625.1399 | 625.1405 | 0.959 | 301.0342 |
| 4 | 25.87 | Isorhamnetin diglucoside | 639.1555 | 639.1560 | 0.782 | 315.0499 |
| 5 | 30.01 | Quercetin glucoside I | 463.0871 | 463.0874 | 0.648 | 301.0342 |
| 6 | 34.82 | Quercetin glucoside II | 463.0871 | 463.0874 | 0.648 | 301.0342 |
| 7 | 37.85 | Isorhamnetin glucoside | 477.1027 | 477.1026 | -0.209 | 315.0499 |

[M-H]-theor.: theoretical exact mass of negatively charged molecular ion; [M-H]-exp.: experimentally measured accurate mass of negatively charged molecular ion; δ: difference between [M-H]-theor and [M-H]-exp; online fragment: daughter ion produced from [M-H]- fragmentation.

**Table S2. Identification of polyphenols in green pepper by UPLC-MS**

| Peak no. | Rt (min) | Compound | $[M-H]^-_{theor.}$ (m/z) | $[M-H]^-_{exp.}$ (m/z) | δ (ppm) | Online fragment (m/z) |
|---|---|---|---|---|---|---|
| 1 | 11.88 | Caffeic acid glucoside I | 341.0867 | 341.0871 | 1.172 | 179.0338 |
| 2 | 15.03 | Caffeic acid glucoside II | 341.0867 | 341.0871 | 1.172 | 179.0338 |
| 3 | 16.51 | CQA | 353.0867 | 353.0873 | 1.699 | 191.055 |
| 4 | 23.44 | Quercetin rhamnoside glucoside I | 609.1450 | 609.1455 | 0.821 | 463.0871 301.0342 |
| 5 | 23.88 | Luteolin hexoside pentoside I | 579.1344 | 579.1347 | 0.518 | 489.1629, 561.1245, 519.114 |
| 6 | 24.46 | Quercetin 3-sambubioside-7-rhamnoside | 741.1872 | 741.1879 | 0.944 | 301.0342, 447.0921, 463.0871, 595.1293 |
| 7 | 25.25 | Luteolin hexoside pentoside II | 579.1344 | 579.1347 | 0.518 | 489.1629, 561.1245, 519.114 |
| 8 | 26.8 | Luteolin hexoside pentoside III | 579.1344 | 579.1347 | 0.518 | 489.1629, 561.1245, 519.114 |
| 9 | 28.86 | Luteolin glucoside I | 447.0921 | 447.0920 | -0.223 | 285.0364 |
| 10 | 29.32 | Luteolin glucoside II | 447.0921 | 447.0920 | -0.223 | 285.0364 |
| 11 | 34.6 | Quercetin rhamnoside glucoside II | 609.1450 | 609.1455 | 0.821 | 301.0342, 463.0871 |
| 12 | 35.61 | Luteolin 7-apiosylglucoside | 579.1347 | 579.1344 | 0.518 | 447.0921, 285.0398 |
| 13 | 35.94 | Quercetin glucoside | 463.0871 | 463.0874 | 0.647 | 301.0342 |
| 14 | 41.75 | Quercetin rhamnoside | 447.0921 | 447.0920 | -0.223 | 301.0342 |
| 15 | 44.22 | Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside | 665.1348 | 665.1360 | 1.804 | 621.1464, 489.1033, 447.0925 |

[M-H]-theor.: theoretical exact mass of negatively charged molecular ion; [M-H]-exp.: experimentally measured accurate mass of negatively charged molecular ion; δ: difference between [M-H]-theor and [M-H]-exp; online fragment: daughter ion produced from [M-H]- fragmentation.

**Table S3. Identification of polyphenols in cardoon by UPLC-MS**

| Peak no. | Rt (min) | Compound | $[M-H]^-_{theor.}$ (m/z) | $[M-H]^-_{exp.}$ (m/z) | δ (ppm) | Online fragment (m/z) |
|---|---|---|---|---|---|---|
| 1 | 8.69 | CQA I | 353.0867 | 353.0873 | 1.699 | 191.0549 |
| 2 | 11.05 | CQA II | 353.0867 | 353.0873 | 1.699 | 191.0549 |
| 3 | 15.23 | 5-CQA | 353.0867 | 353.0873 | 1.699 | 191.0549 |
| 4 | 18.23 | CQA III | 353.0867 | 353.0873 | 1.699 | 191.0549 |
| 5 | 20.39 | 1,3-diCQA I | 515.1184 | 515.1185 | 0.194 | 191.0549 |
| 6 | 30.28 | Luteolin glucoside | 447.0921 | 447.0925 | 0.894 | 285.0364 |
| 7 | 31.52 | 3,4-diCQA II | 515.1184 | 515.1185 | 0.194 | 353.0873, 173.0442 |
| 8 | 32.08 | 1,4-diCQA III | 515.1184 | 515.1185 | 0.194 | 353.0873, 173.0442 |
| 9 | 33.52 | 3,5-diCQA IV | 515.1184 | 515.1185 | 0.194 | 353.0873, 191.0549 |
| 10 | 33.80 | 1,5-diCQA V | 515.1184 | 515.1185 | 0.194 | 353.0873, 191.0549 |
| 11 | 36.34 | 4,5-diCQA VI | 515.1184 | 515.1185 | 0.194 | 353.0873, 173.0442 |
| 12 | 36.55 | succinyldiCQA I | 615.1344 | 615.1351 | 1.138 | 515.1184, 353.0873, 191.0549 |
| 13 | 39.59 | succinyldiCQA II | 615.1344 | 615.1351 | 1.138 | 515.1184, 353.0873, 191.0549 |
| 14 | 40.20 | succinyldiCQA III | 615.1344 | 615.1351 | 1.138 | 515.1184, 353.0873, 191.0549 |
| 15 | 41.60 | Apigenin glucoside | 431.0972 | 431.0974 | 0.464 | 269.0451 |
| 16 | 42.42 | disuccinyldiCQA | 715.1504 | 715.1512 | 1.118 | 615.1351, 515.1184, 353.0873, 191.0549 |

[M-H]-theor.: theoretical exact mass of negatively charged molecular ion; [M-H]-exp.: experimentally measured accurate mass of negatively charged molecular ion; δ: difference between [M-H]-theor and [M-H]-exp; online fragment: daughter ion produced from [M-H]- fragmentation.

# Objective 2

**To evaluate the bioaccessibility of (poly)phenolic compounds and their metabolites after an *in vitro* gastrointestinal digestion and colonic microbiota fermentation, of green pepper (*Capsicum annuum*) and cardoon (*Cynara cardunculus* L.) before and after heat treatment.**

Evaluación de la bioacccesibilidad de los compuestos (poli)fenólicos y sus metabolitos tras los procesos de digestión gastrointestinal *in vitro* y fermentación por la microbiota del colon, de pimiento verde (*Capsicum annuum*) y cardo (*Cynara cardunculus L.*) tanto crudos como sometidos a tratamientos térmicos.

# Paper 3

## Catabolism of raw and cooked green pepper (*capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and faecal fermentation

**I.Juániz**, I.A. Ludwig, M. Dall'Asta, L. Bresciani, P. Mena, D. Del Rio, C. Cid, M.P. de Peña (2016). *Journal of Functional Foods, 27*, 201-213. http://dx.doi.org/10.1016/j.jff.2016.09.006

Quality indices:

- Impact factor (JCR, 2015): 3.973
- Journal Rank in categories:
    - Food Science & Technology: 8/125 (Q1) (D1)

Juániz I, Ludwig I, Dall'Asta M, Bresciani L, Mena P, Del Río D, Cid C, De Peña, MP. Catabolism of raw and cooked green pepper (*Capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and faecal fermentation. Journal of Functional Foods, 2016, 27: 201-213.

**SUPPLEMENTARY INFORMATION**

**TITLE:**

**Catabolism of raw and cooked green pepper (*Capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and fecal fermentation.**

**SHORT TITLE: Catabolism of pepper (poly)phenols after digestion and fecal fermentation**

**AUTHORS: Isabel Juániz [a], Iziar Amaia Ludwig [b†], Letizia Bresciani [b], Margherita Dall'Asta [b], Pedro Mena [b], Daniele Del Rio [bc], Concepcion Cid [a], M. Paz de Peña [a*]**

a Department of Nutrition, Food Science and Physiology, School of Pharmacy, University of Navarra. IdiSNA, Navarra Institute for Health Research. E-31080-Pamplona, Spain.

b The Laboratory of Phytochemicals in Physiology, LS9 InterLab Group, Department of Food Science, University of Parma, Parma, Italy.

c The Need for Nutrition Education/Innovation Programme (NNEdPro), University of Cambridge

† Current address: Food Technology Department, Universitat de Lleida-Agrotecnio Center, Lleida, Spain

*Corresponding author: María-Paz de Peña. Tel: +34 948 425600 (806580); Fax: +34 948 425740. E-mail address: mpdepena@unav.es

**Table S1.** Concentrations of electrolytes Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF)

| | stock solution | | SSF (250mL) | SGF (250mL) | SIF (250mL) |
|---|---|---|---|---|---|
| Constituent | g/50mL | mol/L | mL | mL | mL |
| KCl | 1.865 | 0.5 | 9.4375 | 4.3125 | 4.25 |
| $KH_2PO_4$ | 3.4 | 0.5 | 2.3125 | 0.5625 | 0.5 |
| $NaHCO_3$ | 4.2 | 1 | 4.25 | 7.8125 | 26.5625 |
| NaCl | 5.85 | 2 | ----- | 7.375 | 6 |
| $MgCl_2(H_2O)_6$ | 1.525 | 0.15 | 0.3125 | 0.25 | 0.6875 |
| $(NH_4)_2CO_3$ | 2.4 | 0.5 | 0.0375 | 0.3125 | 0.6875 |

**Table S2.** Mass spectrometric characteristics of native (poly)phenolic compounds and their microbial catabolites identified in this study.

| Compound | $R_t$ (min) | $[M-H]^-$ (m/z) | Fragment ions (m/z) |
|---|---|---|---|
| **Quercetin derivaties** | | | |
| Quercetin 3-glucoside-7-rhamnoside | 6.56 | 609 | 447, 463, 301 |
| Quercetin 3-sambubioside-7-rhamnoside | 6.66 | 741 | 595, 301 |
| Quercetin | 7.58 | 301 | 151 |
| Rutin | 7.64 | 609 | 301 |
| Quercetin glucoside | 7.75 | 463 | 301 |
| Rutin isomer | 8.22 | 609 | 301 |
| Quercetin rhamnoside | 8.33 | 447 | 301 |
| **Luteolin derivatives** | | | |
| Luteolin 6,8-di-*C*-glucoside | 6.16 | 609 | 489, 285 |
| Luteolin 6-*C*-hexoside-8-*C*-pentoside | 6.57 | 579 | 459, 489 |
| Luteolin 6-*C*-pentoside-8-*C*-hexoside | 6.86 | 579 | 489, 459 |
| Luteolin 8-*C*-hexoside | 7.06 | 447 | 327, 357 |
| Luteolin | 7.52 | 285 | 133 |
| Luteolin 7-*O*-(2-apiosyl)glucoside | 7.84 | 579 | 447, 285 |
| Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside I | 8.63 | 665 | 621, 489, 579 |
| Luteolin acetylglucoside I | 8.70 | 489 | 285 |
| Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside II | 9.32 | 665 | 621, 489, 579 |
| Luteolin acetylglucoside II | 9.39 | 489 | 285 |
| **Hydroxycinnamic acids** | | | |
| Caffeic acid glucoside I | 1.99 | 341 | 179 |
| Caffeic acid glucoside II | 3.28 | 341 | 179 |
| Caffeic acid | 4.10 | 179 | 135 |
| 5-CQA | 3.72 | 353 | 191 |
| 4-CQA | 5.50 | 353 | 191, 173 |
| Coumaric acid | 6.20 | 163 | 119 |
| **Catabolites** | | | |
| Protocatechuic acid | 3.20 | 153 | 109 |
| Dihydrocaffeic acid | 4.50 | 181 | 137 |
| 3-(3'-Hydroxyphenyl)propionic acid | 5.60 | 165 | 121 |

$R_t$, retention time; m/z, mass-to-charge ratio; $[M-H]^-$, Negatively charged molecular ion

**Figure S1.** (Poly)phenolic compounds profiles of raw green pepper during 24 h fecal fermentation. A) Main (poly)phenolic compounds (flavonoids) degradation profiles and production of their corresponding aglycones. B) Minor (poly)phenolic compounds (hydroxycinnamic acids) degradation profiles. C) Main (poly)phenolic catabolites production profiles after *in vitro* fecal fermentation. C is the control sample before fecal fermentation.

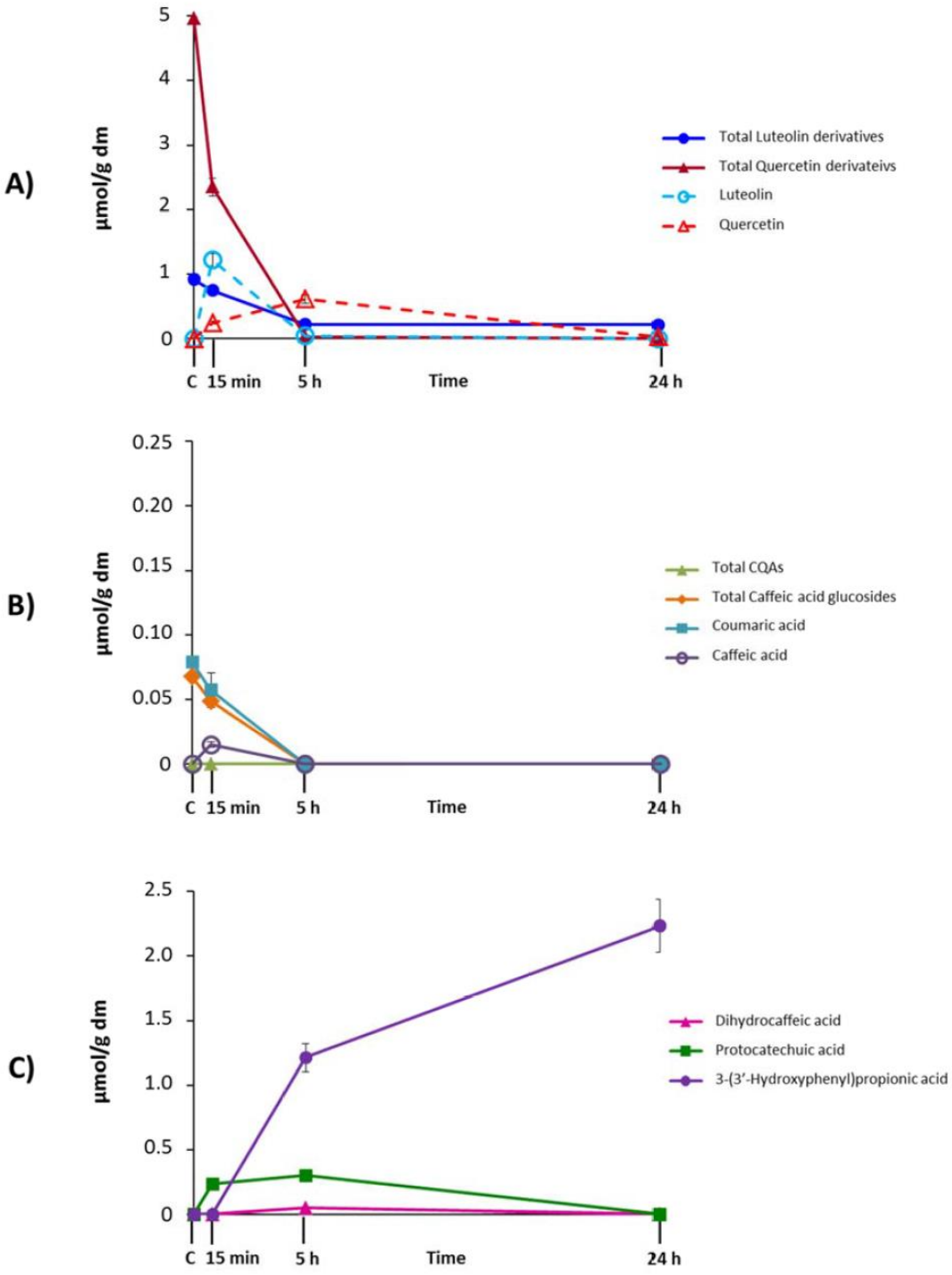


**Figure S2.** (Poly)phenolic compounds profiles of olive oil fried green pepper during 24 h fecal fermentation. A) Main (poly)phenolic compounds (flavonoids) degradation profiles and production of their corresponding aglycones. B) Minor (poly)phenolic compounds (hydroxycinnamic acids) degradation profiles. C) Main (poly)phenolic catabolites production profiles after *in vitro* fecal fermentation. C is the control sample before fecal fermentation.

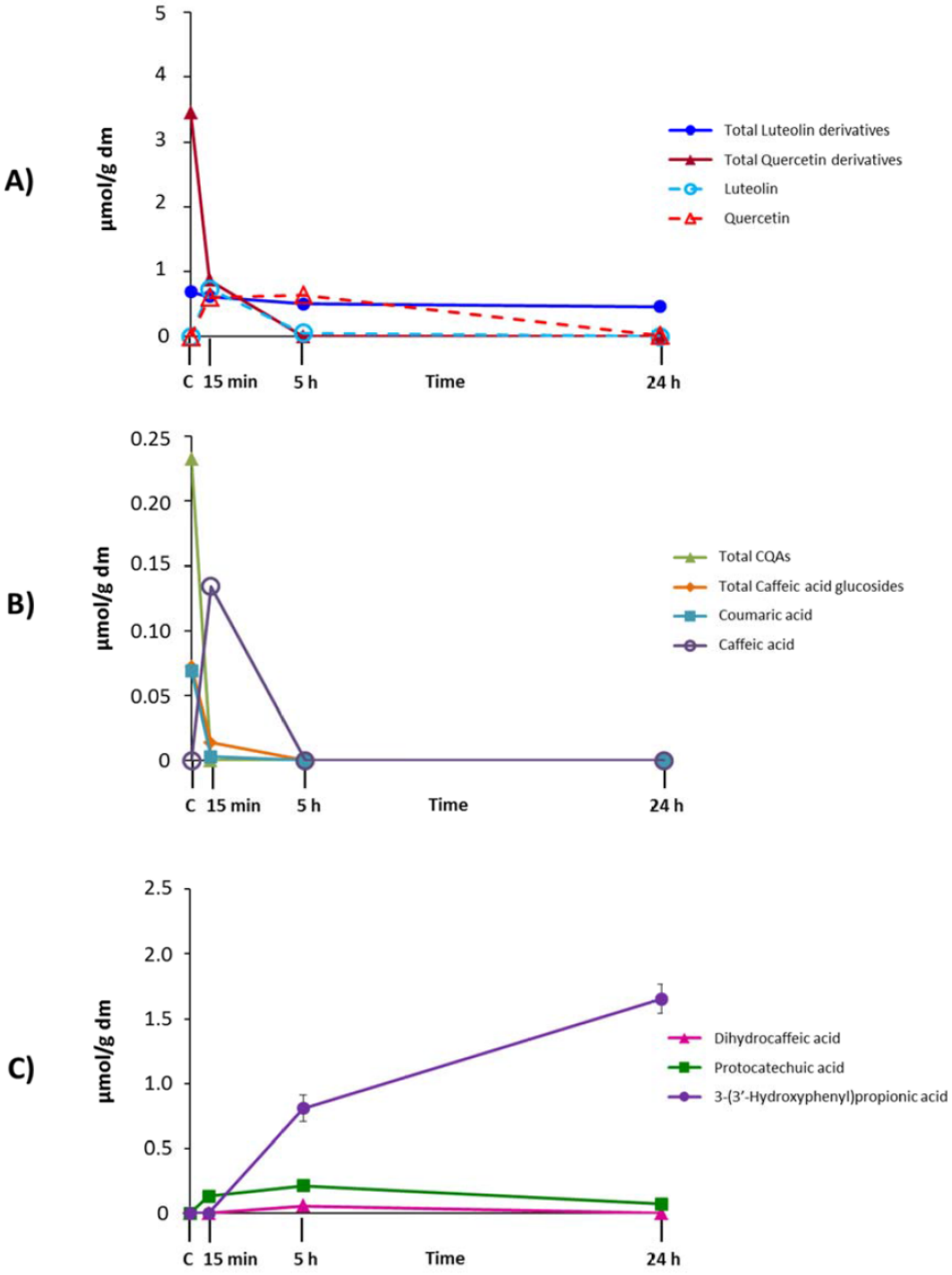
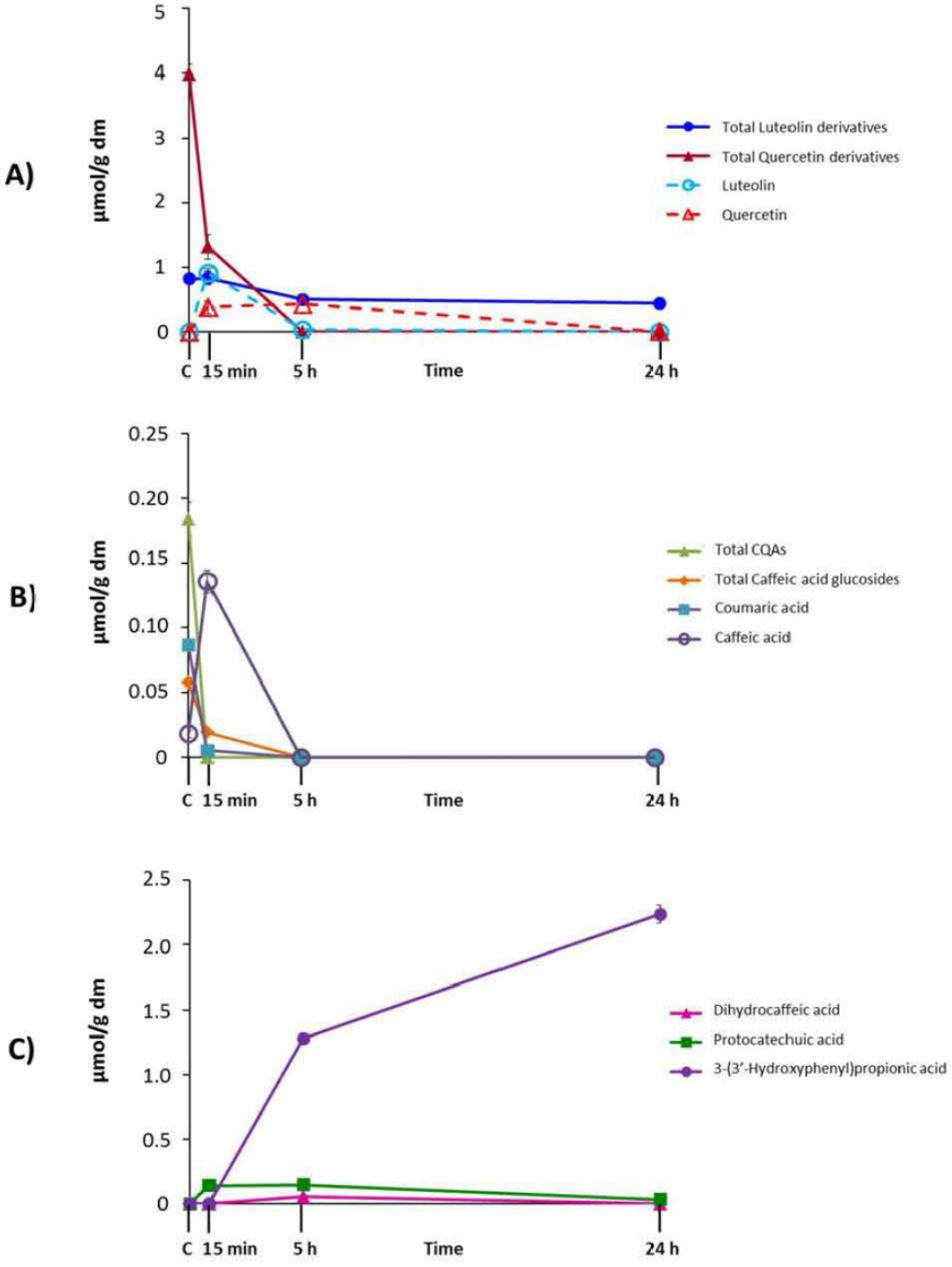


**Figure S3.** (Poly)phenolic compounds profiles of sunflower oil fried green pepper during 24 h fecal fermentation. A) Main (poly)phenolic compounds (flavonoids) degradation profiles and production of their corresponding aglycones. B) Minor (poly)phenolic compounds (hydroxycinnamic acids) degradation profiles. C) Main (poly)phenolic catabolites production profiles after *in vitro* fecal fermentation. C is the control sample before fecal fermentation.



# Paper 4

## Bioaccessibility of (poly)phenolic compounds of raw and cooked cardoon (*Cynara cardunculus* L.) after simulated gastrointestinal digestion and fermentation by human colonic microbiota

**I.Juániz**, I.A. Ludwig, M. Dall'Asta, L. Bresciani, P. Mena, D. Del Rio, C. Cid, M.P. de Peña. (2017). *Journal of Functional Foods,*32, 195-207. http://dx.doi.org/10.1016/j.jff.2017.02.033

Quality indices:

- Impact factor (JCR, 2015): 3.973
- Journal Rank in categories:
    - Food Science & Technology: 8/125 (Q1) (D1)

Juániz I, Ludwig IA, Bresciani L, Dall'Asta M, Mena P, Del Río D, Cid C, De Peña, MP. Bioaccessibility of (poly)phenolic compounds of raw and cooked cardoon (*Cynara cardunculus* L.) after simulated gastrointestinal digestion and fermentation by human colonic microbiota. Journal of Functional Foods, May 2017, 32: 195-207.

## SUPPLEMENTARY INFORMATION

**TITLE:**

**Bioaccessibility of (poly)phenolic compounds of raw and cooked cardoon (Cynara cardunculus L.) after simulated gastrointestinal digestion and fermentation by human colonic microbiota.**

**AUTHORS: Isabel Juániz [a], Iziar Amaia Ludwig [b†], Letizia Bresciani [b], Margherita Dall'Asta [b], Pedro Mena [b], Daniele Del Rio [b], Concepcion Cid [a], María-Paz de Peña [a*]**

[a] Universidad de Navarra, Facultad de Farmacia y Nutrición, Departamento de Ciencias de la Alimentación y Fisiología, C/ Irunlarrea 1, E-31008 Pamplona, España. IdiSNA, Navarra Institute for Health Research.

[b] The Laboratory of Phytochemicals in Physiology, LS9 InterLab Group, Department of Food Science, University of Parma, Parma, Italy.

† Current address: Food Technology Department, Universitat de Lleida-Agrotecnio Center, Lleida, Spain

*Corresponding author: María-Paz de Peña. Tel: +34 948 425600 (806580); Fax: +34 948 425740. E-mail address: mpdepena@unav.es

**Table S1.** Concentrations of electrolytes Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF)

| | stock solution | | SSF (250mL) | SGF (250mL) | SIF (250mL) |
|---|---|---|---|---|---|
| Constituent | g/50mL | mol/L | mL | mL | mL |
| KCl | 1.865 | 0.5 | 9.4375 | 4.3125 | 4.25 |
| $KH_2PO_4$ | 3.4 | 0.5 | 2.3125 | 0.5625 | 0.5 |
| $NaHCO_3$ | 4.2 | 1 | 4.25 | 7.8125 | 26.5625 |
| NaCl | 5.85 | 2 | ----- | 7.375 | 6 |
| $MgCl_2(H_2O)_6$ | 1.525 | 0.15 | 0.3125 | 0.25 | 0.6875 |
| $(NH_4)_2CO_3$ | 2.4 | 0.5 | 0.0375 | 0.3125 | 0.6875 |

**Table S2.** Mass spectrometric characteristics of native phenolic compounds and their microbial catabolites identified in this study.

| Compound | $R_t$ (min) | $[M-H]^-$ (m/z) | Fragment ions (m/z) |
|---|---|---|---|
| **Chlorogenic acid derivates** | | | |
| 3-CQA | 1.3 | 353 | 191 |
| 5-CQA | 3.6 | 353 | 191 |
| 4-CQA | 5.0 | 353 | 191, 173 |
| diCQA I | 6.4 | 515 | 353 |
| diCQA II | 8.2 | 515 | 353 |
| SuccinyldiCQA I | 8.5 | 615 | 453, 353 |
| diCQA III | 8.6 | 515 | 353 |
| SuccinyldiCQA II | 9.1 | 615 | 453, 353 |
| diSuccinyldiCQA | 9.4 | 715 | 615, 453, 353 |
| **Caffeic acid derivates** | | | |
| Caffeic acid glucoside I | 1.8 | 341 | 179 |
| Caffeic acid glucoside II | 3.1 | 341 | 179 |
| Caffeic acid | 4.1 | 179 | 135 |
| **Coumaric acid derivates** | | | |
| Coumaroylquinic acid | 5.9 | 337 | 163 |
| Coumaric acid | 6.2 | 163 | 119 |
| **Luteolin derivates** | | | |
| Luteolin | 7.5 | 285 | 133 |
| Luteolin glucoside | 7.8 | 447 | 285 |
| Luteolin acetylglucoside I | 8.7 | 489 | 285 |
| Luteolin acetylglucoside II | 9.4 | 489 | 285 |
| **Catabolites** | | | |
| Protocatechuic acid | 3.2 | 153 | 109 |
| Dihydrocaffeoylquinic acid | 3.5 | 355 | 191, 181, 137 |
| Dihydrocaffeic acid | 4.5 | 181 | 137 |
| 3-(3'-Hydroxyphenyl)propionic acid | 5.6 | 165 | 121 |

$R_t$, retention time; m/z, mass-to-charge ratio; $[M-H]^-$, Negatively charged molecular ion

**Figure S1.** Degradation profile of phenolic compounds of raw cardoon and their corresponding catabolites produced during fecal incubation. A) Total CQAs degradation profiles of fecal fermentation samples. B) Total diCQAs and caffeic acid degradation profiles of fecal fermentation samples. C) Total succinyldiCQAs, total caffeic acid glucosides, coumaroylquinic acid and coumaric acid degradation profiles of fecal fermentation samples. D) Flavonoids (luteolin derivates) degradation profiles of fecal fermentation samples and production of their corresponding aglycone (luteolin). E) Profiles of main catabolites produced during fecal fermentation over 24 h of incubation. C is the control sample before fecal fermentation.

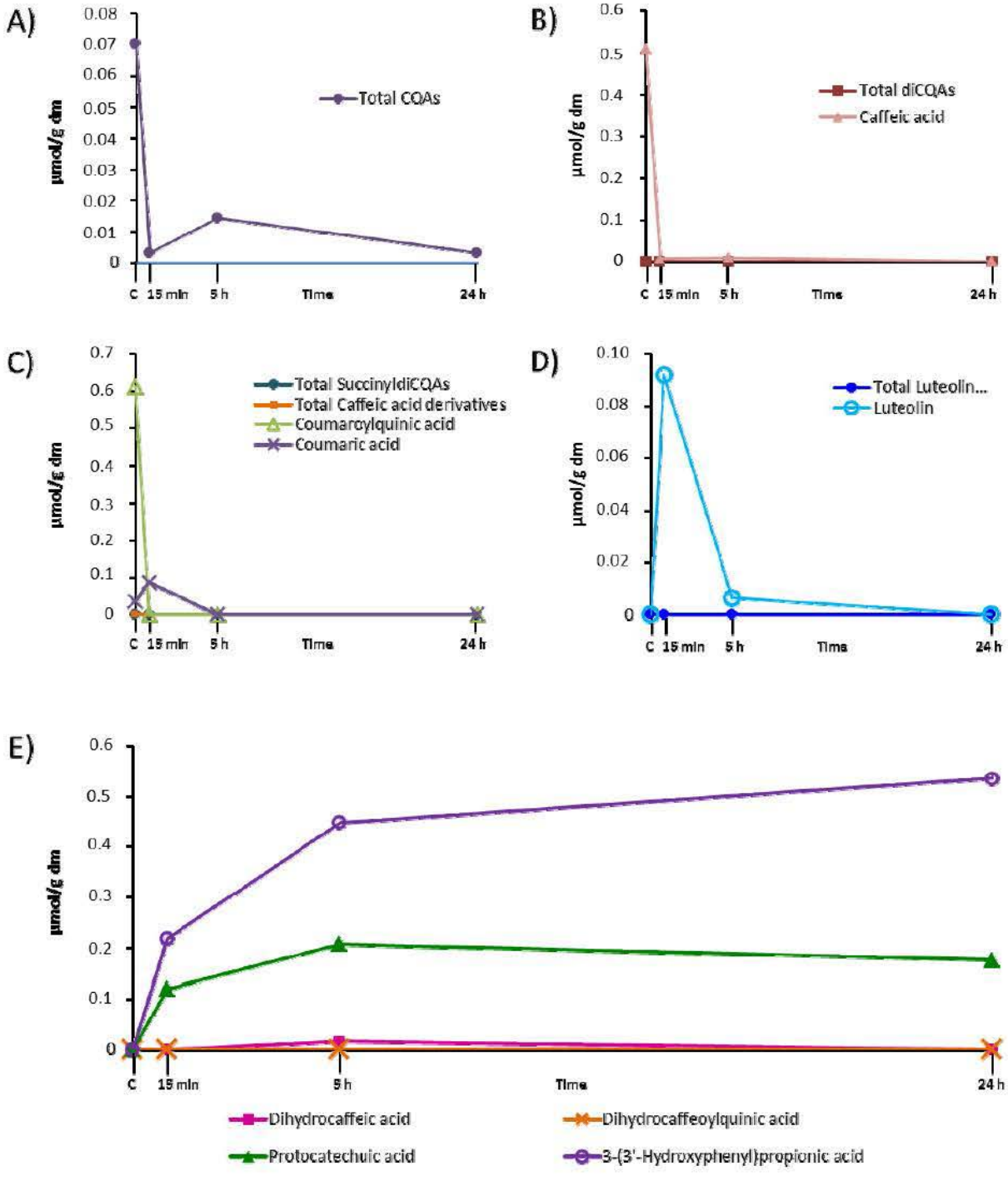


**Figure S2.** Degradation profile of phenolic compounds of olive oil fried cardoon and their corresponding catabolites produced during fecal incubation. A) Total CQAs degradation profiles of fecal fermentation samples. B) Total diCQAs and caffeic acid degradation profiles of fecal fermentation samples. C) Total succinyldiCQAs, total caffeic acid glucosides, coumaroylquinic acid and coumaric acid degradation profiles of fecal fermentation samples. D) Flavonoids (luteolin derivates) degradation profiles of fecal fermentation samples and production of their corresponding aglycone (luteolin). E) Profiles of main catabolites produced during fecal fermentation over 24 h of incubation. C is the control sample before fecal fermentation.

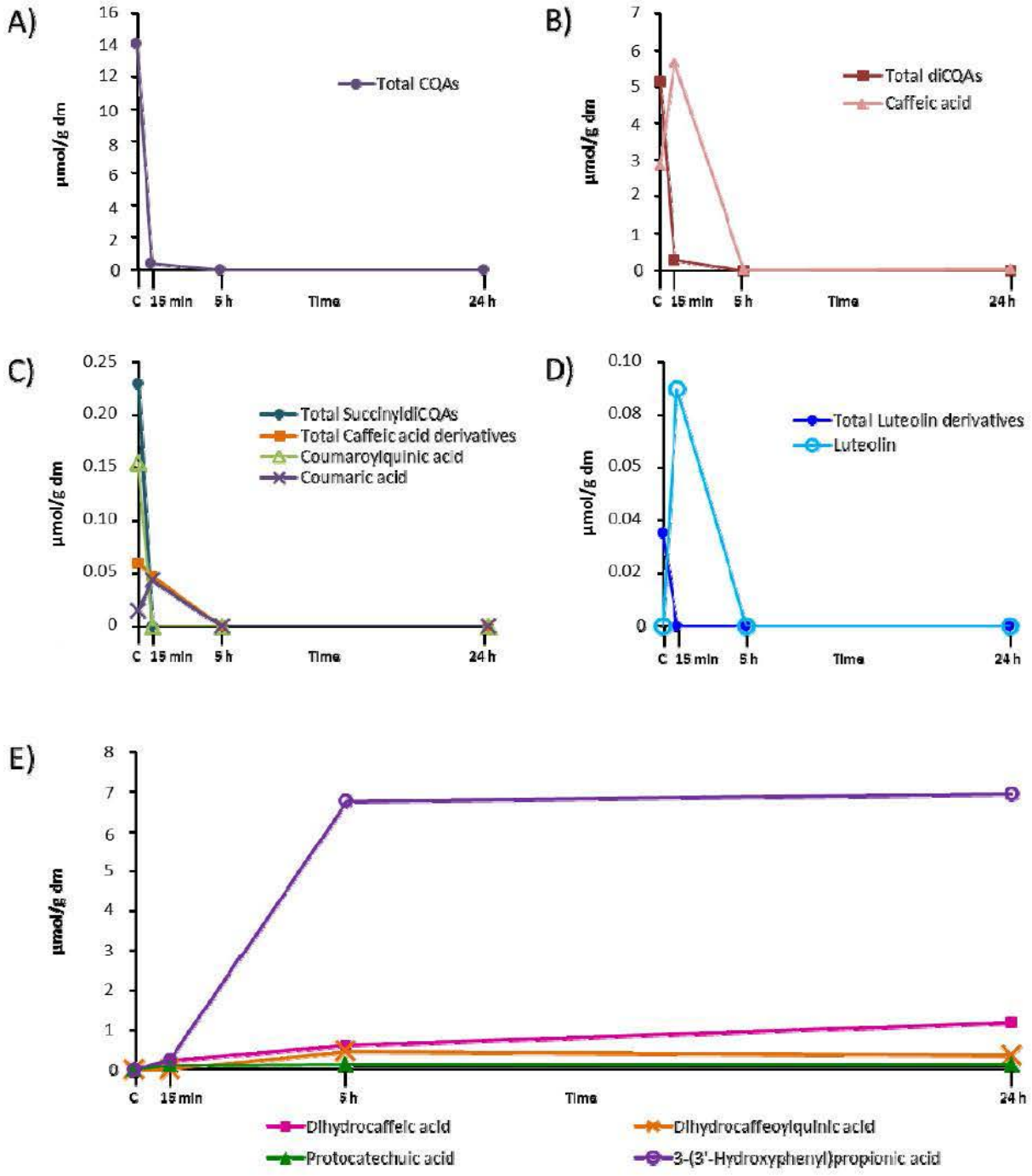
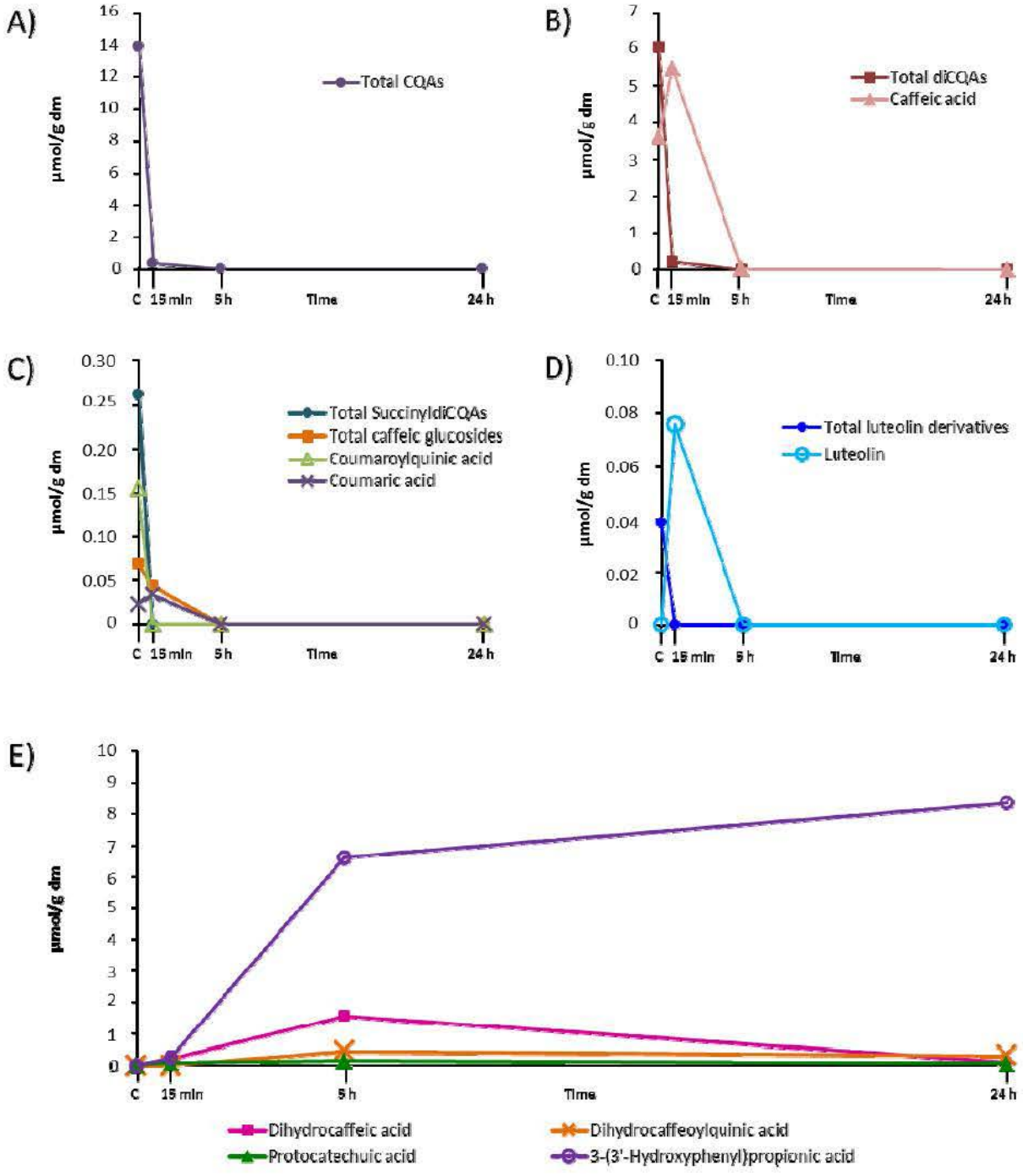


**Figure S3.** Degradation profile of phenolic compounds of sunflower oil fried cardoon and their corresponding catabolites produced during fecal incubation. A) Total CQAs degradation profiles of fecal fermentation samples. B) Total diCQAs and caffeic acid degradation profiles of fecal fermentation samples. C) Total succinyldiCQAs, total caffeic acid glucosides, coumaroylquinic acid and coumaric acid degradation profiles of fecal fermentation samples. D) Flavonoids (luteolin derivates) degradation profiles of fecal fermentation samples and production of their corresponding aglycone (luteolin). E) Profiles of main catabolites produced during fecal fermentation over 24 h of incubation. C is the control sample before fecal fermentation.



# Objective 3

**To evaluate furan occurrence in vegetables and bread coated frozen foods before and after heat treatment.**

Evaluación de la presencia de furano en alimentos vegetales y alimentos precocinados empanados sometidos a tratamientos térmicos.

## Objective 3.1

**Development and validation of the methodology for furan determination in foods.**

Puesta a punto y validación de la metodología para la determinación de furano en alimentos.

## Objective 3.2

**Impact of heat treatment on furan occurrence in vegetables and bread coated frozen foods.**

Impacto del tratamiento térmico en la presencia de furano en alimentos vegetales y alimentos precocinados empanados.

## Objective 3.3

**Evaluation of the risk assessment of furan in foods for the Spanish population.**

Evaluación del riesgo de furano en alimentos para la población española.

# Paper 5

## Effect of frying process on furan content in foods and assessment of furan exposure of Spanish population

**I. Juániz**, C. Zocco, V. Mouro, C. Cid, M.P. de Peña. (2016). *LWT-Food Science and Technology*, 68, 549-555. http://dx.doi.org/10.1016/j.lwt.2015.12.061

Quality indices:

- Impact factor (JCR, 2015): 2.711
- Journal Rank in categories:
    - Food Science & Technology: 23/125 (Q1)

Juániz I, Zocco V, Mouro C, Cid C, De Peña, MP. Effect of frying process on furan content in foods and assessment of furan exposure of Spanish population. LWT – Food Science and Technology, May 2016, 68:549-555.

# GENERAL DISCUSSION

Plant foods are the main source of dietary antioxidants, including phenolic compounds. (Poly)phenols rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer, probably due to their ability to protect against oxidative damage in cells (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014). In order to carry out a first screening and select potential antioxidant vegetables, five different vegetables (yellow onion (*Allium cepa*), Italian green pepper (*Capsicum Annuum*), cardoon stalks (*Cynara cardunculus* L.), cabbage (*Brassica oleracea*) and chicory (*Cichorium intybus*)) were chosen to study antioxidant properties. These five vegetables have a great consumer acceptance among the Spanish population; specifically onion and pepper are two of the most consumed vegetables (MAGRAMA, 2015). Moreover, all of them can be consumed both raw and after being subjected to different heat treatments. Among the high variety of cooking processes, frying is a very common culinary technique applied to foods to develop their typical sensorial properties or in order to be used as base ingredients in the Mediterranean cuisine. Also griddling is quite common in this cuisine. In Spain, olive oil is the most consumed culinary fat (around 23 ml/capita/day) followed by sunflower oil (8.5 ml/capita/day) (MAGRAMA, 2015). Thus, total phenolic compounds, total flavonoid compounds and antioxidant capacity measured by DPPH and ABTS assays were analyzed in both raw and cooked (fried in olive and sunflower oil and griddled) vegetables.

Chicory was the sample with the highest amount of flavonoids, nevertheless no correlation between flavonoids and antioxidant capacity was found. Then, chicory was not selected for further studies. In the same way, cabbage was not selected because it did not show high antioxidant capacity by DDPH and ABTS. Moreover, the antioxidant capacity of cabbage was lower in the cooked samples than in the raw ones. On the other hand, pepper and cardoon seem to have more relevance in antioxidant capacity due to the higher amount of total phenolic compounds, specially griddled samples. Therefore, these two vegetables were selected for further analysis. At last, onion was also selected since antioxidant capacity (DPPH) was higher in cooked samples than in raw one and it could help us to understand the effect of heat treatment on antioxidant properties of vegetables.

Once yellow onion (*Allium cepa*), Italian green pepper (*Capsicum annuum*) and cardoon stalks (*C. cardunculus* L.) were selected, the influence of several **heat treatments** (frying with olive and sunflower oil, and griddling) on proximate composition, fatty acids profile, (poly)phenolic compounds and antioxidant properties was studied.

All vegetables were characterized by high amounts of water in their composition. Green pepper was the vegetable with the highest moisture level (92.6 g/100g) followed by onion (90.1 g/100g) and cardoon (83.2 g/100g). Moisture levels were significantly decreased after cooking process due in general to the temperature applied and, in the case of fried samples, also to the relative gain of fat during heat treatment. Fried samples, and specifically sunflower oil fried cardoon (76.4 g/100g), showed the lowest moisture levels. Protein content was not significantly affected by thermal treatment, with the exception of both, olive oil or sunflower oil fried cardoon samples, which showed less protein content than raw and

griddled cardoon. Similarly, ash or mineral content was no significantly affected by cooking process. As expected, only fried vegetables showed relevant amounts of fat o (6.0–7.7 g/100g). No significant differences on the total fat content between vegetables fried in olive oil or in sunflower oil were observed. Nevertheless, differences on the fatty acid profile were detected depending on the oil employed during the process. The main fatty acids in olive oil fried samples were monounsaturated fatty acids (MUFA), specifically oleic acid. High MUFA diets reduce fasting glucose in patients with type 2 diabetes and show a beneficial effect on important risk factors as fat mass and systolic and diastolic blood pressure (Schwingshackl, Strasser, & Hoffmann, 2011; Schwingshackl & Strasser, 2012). Some studies report that olive oil consumption is associated with reduced risks of cardiovascular disease, and mortality (Buckland et al., 2012; Soriguer et al., 2013; Guasch-Ferré et al., 2014;). In contrast, sunflower oil fried vegetables presented higher content of polyunsaturated fatty acids (PUFA), specifically linoleic acid. As expected, olive oil fried vegetables showed a higher content of ω-3 fatty acids and a lower ω-6/ω-3 PUFA ratio than those fried with sunflower oil. A low ω-6/ω-3 PUFA ratio has been recommended since a high intake of ω-6 fatty acids may reduce the formation of anti-inflammatory mediators by ω-3 fatty acids (Aranceta & Pérez-Rodrigo, 2012). Consequently, different health authorities and scientific organizations now recommend an increase in the dietary intake of ω-3 PUFAs to achieve nutrient adequacy and to prevent cardiovascular diseases (Gebauer, Psota, Harris, & Kris-Etherton, 2006). Additionally, even though heat treatments favored the occurrence of trans fatty acids in fried vegetables, especially in those fried in sunflower oil, the amount of trans fatty acids was very low in all cases. In all samples the major trans fatty acid was elaidic acid (C18:1Δ9t) which probably could be formed during heat treatment by changes from cis to trans configuration of the double bond of oleic acid (C18:1). However, the sum of all trans linoleic acids (t-linoleic, c-t-linoleic and t-c-linoleic acids) represented the most abundant trans fatty acids fraction in those sunflower oil fried vegetables, while they are hardly present in olive oil fried samples, and totally absent in fresh oils. Wolff (1994) reported that heating at high temperature (240°C) affects mainly octadecatrienoic acids such as linoleic and linolenic acids, which undergo cis-trans isomerization of their double bonds. Probably, the lower temperature applied during the frying process of vegetable samples (115°C) induces the formation of low amount of trans fatty acids. These results are in agreement with the little impact on trans fatty acids observed when unhydrogenated edible oils are used in ordinary frying process (Tsuzuki, Matsuoka, & Ushida, 2010). The intake of trans fatty acids has been associated with health disorders such as cardiovascular diseases, breast cancer or attention-deficit/hyperactivity disorder (Mensink & Katan, 1990; Willett et al., 1993; Chajès et al., 2008; Ruano et al., 2011; Kim et al., 2012). Then, the intake of trans fatty acids has to be as low as possible (EFSA, 2010).

Therefore, according to their lower ω-6/ω-3 PUFA ratio, their higher ω-3 fatty acids, and their lower trans fatty acids, it could be preferable the use of olive oil instead of sunflower oil for frying vegetables. Nevertheless, the global diet and not only the fat provided by one individual food should be considered for health recommendations. Fried vegetables were the samples with the highest caloric intake contribution (more than 100 Kcal/100g in all fried

samples) due to the fat content. Despite the absence of fat in griddled vegetables, their energy value was significantly higher than that of the raw ones, due to water losses and the subsequent relative increase of the carbohydrates. However, griddled vegetables provided around half of the energy of fried samples, so they could be of a high interest from a nutritional point of view in order to increase the variety of the culinary techniques that can be used in low caloric diets.

The influence of heat treatment on **antioxidant capacity and (poly)phenolic compounds** of selected vegetables (onion, green pepper and cardoon) was evaluated and the results were published in Food Chemistry journal (Juániz et al., 2016). A total of seven flavonoids were identified and quantified in raw and heat treated onion samples. The most abundant (poly)phenolic compounds in onion were quercetin glucosides, which accounted approximately for 90% of total flavonoids in all samples. This is quite similar to the percentage of quercetin glucosides (83-93% of the total flavonols content) reported by Lombard et al. (2005). Glucosides of isorhamnetin were also detected. Aglycones of these two flavonoids, as well as other minor flavonoids such as kaempferol, luteolin and myricetin derivates, or phenolic acids, previously identified in onion by other authors, were not detected in our samples, probably due to differences in onion varieties, as well as factors like cultivar (Sellappan & Akoh, 2002; Lanzotti, 2006; Rodríguez Galdón, Rodríguez Rodríguez, & Díaz Romero, 2008; Lu et al., 2011; Simin et al., 2013). However, in all studies found, quercetin derivates were also the main compounds in raw onion. All heat treatments applied in the present study resulted in the increase of (poly)phenolic compounds in onion. Griddled onion had the highest amount of flavonoids with an increment of 57.35% compared to raw. Additionally, the use of olive oil for frying resulted in a higher increase of flavonoids (34.55%) than the use of sunflower oil (15.44%). However, in literature, both losses and gains in (poly)phenolic compounds content, especially quercetin derivates, after cooking process of onions have been reported depending on the heat treatment conditions. Quercetin derivates did not significantly change when brown-skinned onion was fried during 5 minutes, whereas at higher frying times (15 minutes) it decreased significantly (Price & Rhodes, 1997). Lombard et al. (2005) also showed how different heat treatments could affect phenolic content; baking or sauteing increased concentrations of predominant quercetin derivates in 25% and 7%, respectively, compared to raw onions, as it occurred in the present study, while boiling decreased total flavonoid concentration in 18%. However, other authors reported that frying process induced 21-39% losses of flavonoids in onion (Crozier, Lean, McDonald, & Black, 1997; Ewald, Fjelkner-Modig, Johansson, Sjöholm, & Åkesson, 1999) while others reported no significant changes in the total levels of quercetin diglucoside and monoglucoside (Rodrigues, Pérez-Gregorio, García-Falcón, & Simal-Gándara, 2009).

In the present study, all compounds tended to increase after cooking process, but molar ratio of quercetin diglucoside/quercetin glucoside decreased with the frying process. In olive oil fried onion and in sunflower oil fried onion, quercetin diglucoside/glucoside had ratios of 0.74 and 0.72, respectively, lower than that of the raw onion (0.83). These results suggest that during frying process, glucosides of the quercetin diglucosides are thermohydrolyzed producing the corresponding monoglucoside as discussed by several authors (Rohn, Buchner,

Driemel, Rauser, & Kroh, 2007; Rodrigues et al., 2009). However in griddled onion, total onion quercetin diglucosides significantly increased, probably due to the degradation of other quercetin derivates such as quercetin triglucoside, that may be strongly linked to other structures in the food matrix and thus have not been fully extracted. Although some authors reported that quercetin monoglucoside can be also deglycosilated by thermohydrolysis to the corresponding aglycon (Rohn et al., 2007; Rodrigues et al., 2009), no quercetin or isorhamnetin aglycons were detected after the cooking process in the present work, in agreement with Harris et al. (2015), probably due to both heat degradation and their contribution on the formation of Maillard reaction products, like melanoidins (Pérez-Jiménez, Díaz-Rubio, Mesías, Morales, & Saura-Calixto, 2014).

A total of twelve flavonoids and three phenolic acids were identified in green pepper. Flavonoid compounds (quercetin and luteolin derivates) were also the main (poly)phenolic compounds in green pepper, accounting for more than 90% of total phenolic compounds. As well as in onion samples, no aglycone flavonoids were detected. Quercetin rhamnoside and luteolin 7-*O*-(2-apiosyl-6-malonyl) glucoside were the most abundant in all green pepper samples, accounting for around 80% of total phenolic compounds. These results are in agreement with those reported in previous studies in raw green pepper (Marín, Ferreres, Tomás-Barberán, & Gil, 2004). In green pepper, also an increase of the total (poly)phenolic compounds after heat treatment was observed. Griddled pepper had the highest amount of phenolic compounds (0.96 mg (poly)phenolic compounds/ g sample dm). Frying also induced an increase of (poly)phenolic compounds, but the use of olive oil for frying resulted in a lower increase in flavonoids, and therefore in total (poly)phenolic compounds (48.14%) than with sunflower oil (103.70%). Caffeic acid derivates, as well as luteolin hexoside-pentosides and luteolin glucosides, were only found after the heat treatment.

In contrast to the phenolic compounds characteristics of onion and green pepper, the most abundant (poly)phenols of cardoon were chlorogenic acids (four caffeoylquinic acids (CQA), six diccaffeoylquinic acids (diCQA), three succinyldicaffeoylquinic acids (succinyldiCQA) and one disuccinyldicaffeoylquinic acid (disuccinyldiCQA). These last compounds were reported for the first time in raw cardoon leaves (*C. cardunculus L*.) by Pinelli et al. (2007). In the cardoon stalks of the present study, CQAs and diCQAs accounted for 80-90% of total (poly)phenolic compounds. Traces of some flavonoids were also detected in cardoon, mainly in griddled one. As well as in onion and green pepper, the total amount of (poly)phenolic compounds identified by HPLC increased after cardoon heat treatment. The increment in total (poly)phenolic compounds was higher in griddled cardoon (203.06%) than in sunflower oil fried cardoon (44.25%) and in olive oil fried cardoon (25.47%). This could be due to the higher temperature applied during the griddling treatment (150°C) than that applied during the frying processes (115°C), which might favor the release of these compounds from the food matrix, specially 5CQA which increased dramatically after the griddling process probably due to the hydrolysis of diCQAs compounds. On the other hand, the lower increment in fried samples may be due to the slight decrease of 5-CQA after frying processes both with olive and sunflower oils, maybe due to isomerization into other CQAs. These results are in

agreement with Ferracane et al. (2008) which confirmed that cooking practices caused a marked intramolecular transesterification of caffeoylquinic acid.

In summary, all heat treatments, and particularly griddling, tend to increase the (poly)phenols content in the chosen vegetables suggesting a thermal destruction of cell walls and sub cellular compartments during the cooking process that favor the release of these compounds. This increase, especially that observed for chlorogenic acids (CQAs, diCQAs and succinyldiCQAs), was significantly correlated with an increase in the antioxidant capacity measured by DPPH ($r=0.70$, $p<0.001$). Griddled cardoon is the vegetable with both the highest amount of phenolic compounds and the highest scavenging activity (DPPH). Also ABTS increased after the heat treatment in cardoon samples, but decreased in onion and green pepper samples, probably due to the degradation of other thermolabile non-phenolic antioxidants. In onion, this disagrees with the findings of no losses or increases in the ABTS antioxidant activity after frying and griddling (Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009; Pellegrini et al., 2009), probably due to the different heat treatment applied as well as onion varieties. In green pepper, previous studies also reported losses on ABTS scavenging capacity after frying (30-50% losses) and griddling (5-30% losses), but these losses were lower than those found in the present study, probably due to the shorter time of cooking (up to 8 min vs 15 min in the present study) (Jiménez-Monreal et al., 2009).

(Poly)phenolic compounds are present free, esterified and covalently bound to other macromolecules like fiber, carbohydrates and proteins, modulating their bioaccessibility. Up to now, only the free fraction of (poly)phenolic compounds was analyzed in the present study. The increase of this fraction after heat treatment suggests the release of (poly)phenolic compounds from those bound to macromolecules. Then, the next step was the application of an alkaline hydrolysis before (poly)phenols extraction and HPLC analysis, in order to know the (poly)phenol profile of the bound fraction. Simultaneously, the free fraction (extraction without hydrolysis) was also analyzed. Due to the different (poly)phenol profile, green pepper (rich in flavonoids) and cardoon (rich in chlorogenic acids) were chosen to be studied and the obtained results were published in the Journal of Functional Foods (Juániz et al., 2016, 2017). Results were expressed as μmol/g (dm) in order to discuss the gastrointestinal bioaccessibility and catabolism of (poly)phenolic compounds.

In green pepper samples, a total of 21 (poly)phenolic compounds were identified and quantified in the new HPLC-MS analysis. At least 9 of them were not identified in the previous experiment. Similarly, flavonoids, and particularly quercetin rhamnoside, were again the main compounds found both in raw and cooked samples. All flavonoids were mainly detected in the analyzed free fraction, although some of them, as rutin, quercetin glucoside, quercetin rhamnoside, luteolin 8-C-hexoside and luteolin 7-O-(2-apiosyl) glucoside, were also found in the bound fraction. In contrast, some hydroxycinnamic acids, such as caffeic and coumaric acids, were found in the bound fraction but they were not detected in the free one, as in the previous experiment. These compounds could probably be linked to pepper fiber fraction and released from the food matrix only after the hydrolytic

process. In raw green pepper, 66 % of the (poly)phenolic compounds were detected as free compounds while 34% were bound compounds.

In cardoon samples, a total of 17 (poly)phenolic compounds were identified and quantified in the free and bound fraction, being 5-CQA the main (poly)phenolic compound in all samples, accounting for more than 50% of the total compounds in both raw and cooked cardoon, in agreement with the previous experiment. In addition to 5-CQA, other CQAs derivates were also detected as in the previously experiment, specifically two monoCQAs (3-CQA and 4-CQA), three diCQAs, two succinyl-diCQAs and one disuccinyl-diCQA were identified, resulting in CQA derivatives being the most representative compounds in cardoon. As in the previous experiment, small amounts of flavonoids (luteolin derivatives) were also detected, especially in the griddled cardoon. Additionally, two caffeic acid glycosides, caffeic acid, coumaroylquinic acid and coumaric acid were detected in this experiment, but not in the previous one. Most of the (poly)phenolic compounds were detected in both raw and cooked cardoon, although some of them (diCQA I and luteolin acetylglucoside II) were only identified after heat treatment. These compounds were probably strongly bound to the food matrix and were not easily extracted, even after alkaline hydrolysis. However, intensive heat treatment, which can induce wall and cell ruptures, may favor (poly)phenol release and extractability (Palermo, Pellegrini, & Fogliano, 2014; Shahidi & Yeo, 2016). Similarly, diCQA III, caffeic acid glucoside II, and luteolin glucoside, which were found both in the free and bound fractions in raw cardoon, were detected in lower amount or were not detected at all in the bound fraction after the heat treatment, probably due to their release from the food matrix after the heat treatment, with the subsequent increase in the free fraction. In contrast, caffeic and coumaric acids, which were found only in the bound fraction, were not released into the free fraction after heat treatment. These two phenolic acids are probably covalently attached to the cardoon macromolecules.

After the frying process, (poly)phenolic compounds tended to decrease both in green pepper and cardoon samples, mainly due to the decrease of bound compounds. In fact, in green pepper, total (poly)phenolic compounds decreased by more than a half from 12.664 μmol/g dm in raw pepper to 4.715 and 5.113 μmol/g dm in olive and sunflower oil fried green peppers, respectively, resulting in a degradation of 50% of free compounds compared with around 80% of bound (poly)phenolic compounds. In cardoon the degradation of the free (poly)phenolic compounds was around 35-40% after frying, while the percentage of loss of the attached compounds was higher (72% in olive oil fried cardoon and 56% in sunflower oil fried cardoon). In contrast, the amount of (poly)phenolic compounds was not so affected by griddling process. Although the amount of (poly)phenolic compounds of griddled green pepper (11.475 μmol/g dm) was also lower than in raw green pepper, this reduction was much lower than after frying process. In fact, bound compounds decreased only 22%, representing the 29% of the total (poly)phenolic compounds of griddled green pepper. In cardoon, griddling process significantly increased the total amount of (poly)phenols (approximately 12%), resulting the griddled cardoon the sample with the highest content of (poly)phenolic compounds (69.7 μmol (poly)phenolic compounds/g dm), even though the bound fraction of (poly)phenolic compounds was decreased (19%). The decrease of bound

compounds observed in cooked samples with respect to raw ones, could be due to the thermal destruction of cell walls and sub-cellular compartments during the cooking process that induce the release of these compounds. High temperature applied to foods usually enhances the release of bound (poly)phenolic compounds (Shahidi et al., 2016), but also favor Maillard reactions and consequently the formation of typical high molecular weight end-products, such as melanoidins, that could include or retain (poly)phenolic compounds into their structures (Nunes & Coimbra, 2010). Some studies confirmed that higher roasting temperature induced higher formation of melanoidins (Bekedam, Loots, Schols, Van Boekel, & Smit, 2008; Sacchetti et al., 2016). Additionally, some studies about coffee reported the incorporation of chlorogenic acids and other phenolic compounds into melanoidins, which may reach an astounding 54% mainly by non-covalent interactions (Bekedam, Schols, et al., 2008; Nunes et al., 2010; Morales, Somoza, & Fogliano, 2012; Monente, Ludwig, Irigoyen, De Peña, & Cid, 2015). Thus, the higher temperature applied during griddling process (150°C) compared to frying (115°C) could also explain a higher incorporation into melanoidins inducing a lower decrease of bound compounds. Other authors also suggested that part of the bound phenolics were released during lentils boiling, and some of them remained linked to other macromolecules, like proteins, starch and cellulose (Yeo & Shahidi, 2017).

Although there is no direct relationship between chemical extractability and bioaccessibility, it can be hypothesized that the rupture of the plant structures could facilitate the action of gastrointestinal enzymes and might increase the bioavailability of (poly)phenolic compounds and, consequently, their health benefits. Therefore, after the characterization of nutritional and (poly)phenolic compounds (free and bound), the selected vegetables, green pepper and cardoon, both raw and cooked, were submitted to a simulated gastrointestinal digestion and a further fecal fermentation in order to evaluate the **bioaccessibility** of the main free and bound (poly)phenolic compounds and their metabolites.

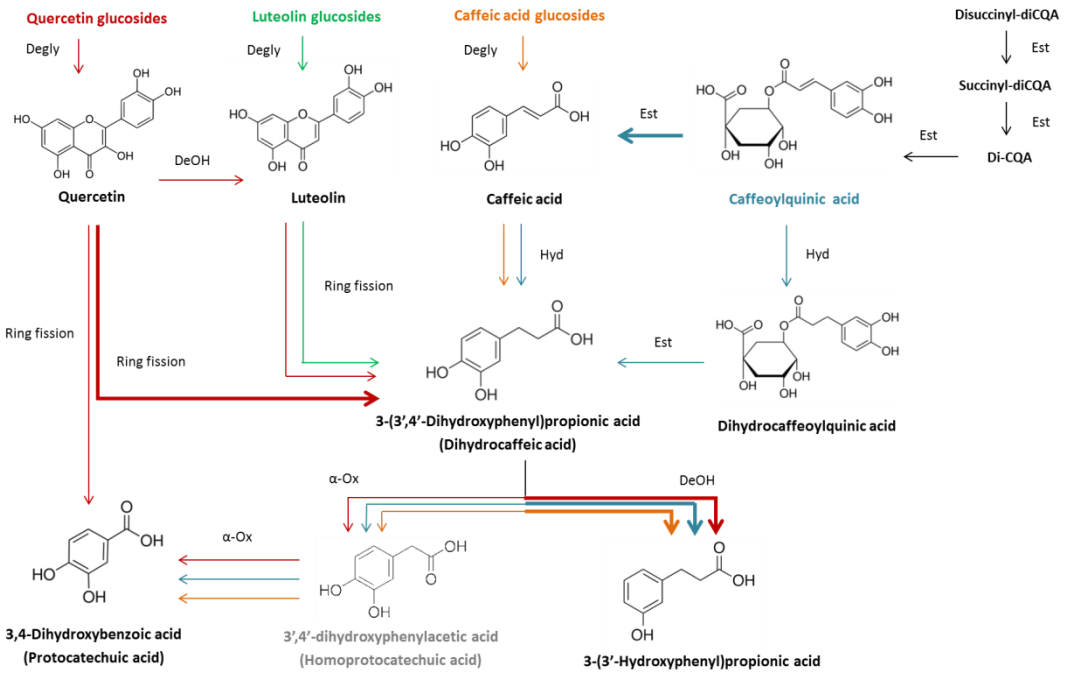
The bioaccessibility of (poly)phenolic compounds of both green pepper and cardoon after a simulated gastrointestinal digestion was highly influenced by the previous heat treatment or its absence. An evident decrease of total (poly)phenolic compounds was observed in raw samples after digestion. In raw green pepper, rich in flavonoids, 52% of total (poly)phenolic compounds were degraded, whereas only 2% of the (poly)phenolic compounds of raw cardoon, whose main compounds were chlorogenic acids, remained bioaccesible after gastrointestinal digestion. Non-flavonoid compounds and specifically chlorogenic acids (in cardoon) seems to be more prone to be degraded by gastrointestinal digestion than flavonoids (in green pepper). The losses in green pepper were mainly due to the decrease of the bound (poly)phenols, through their release from the food matrix into the free fraction as a consequence of the enzymatic action and pH conditions (Shahidi et al., 2016). In cardoon, even bound chlorogenic acids were also degraded or released into the free fraction, free (poly)phenols were degraded at a higher rate than in green pepper. Gastrointestinal digestion also produces losses of total (poly)phenolic compounds in cooked samples, however the bioaccessibility of phenolic compounds after digestion was much higher in cooked samples than in raw ones. In green pepper, the 82%, 96% and 100% of the total

amount of (poly)phenolic compounds of undigested griddled green pepper, olive oil fried green pepper and sunflower oil fried green pepper, respectively, were bioaccessible after gastrointestinal digestion, compared to 48% in raw green pepper. In cooked cardoon samples, between 60 and 67% of the (poly)phenols remained bioaccessible after digestion in comparison with the 2% of the raw cardoon. In other chlorogenic acids-rich foods like coffee, the roasting process has been reported to induce a high decrease (up to 95%) of these (poly)phenolic compounds (Clifford, 1999; Ludwig et al., 2014), but part (23%) of the chlorogenic acids and other phenolic acids are incorporated into the structures of melanoidins formed by Maillard reactions (Coelho et al., 2014) or linked by non-covalent interactions up to 54% (Bekedam, Schols, et al., 2008; Nunes et al., 2010; Morales et al., 2012; Monente, et al., 2015). Coffee chlorogenic acids have shown to be quite stable during gastrointestinal digestion (Olthof, Hollman, & Katan, 2001; Rechner, Spencer, Kuhnle, Hahn, & Rice-Evans, 2001; Monente, Ludwig, Stalmach et al., 2015), probably due to these complex "inclusive" melanoidinic structures. Similarly, in the present study, cooked cardoon showed a higher bioaccessibility of (poly)phenolic compounds after simulated gastrointestinal digestion (60-67%) in comparison with raw cardoon (2%), where no Maillard reaction took place. Fried vegetable samples, both with olive oil and sunflower oil, showed higher bioaccessibility of (poly)phenolic compounds than the griddled ones. This suggests that fat content could modulate and exert a protective effect against enzymatic action. In spite of the higher (poly)phenols bioaccessibility percentages in fried samples, both griddled green pepper and griddled cardoon remained being the samples with the highest amount of (poly)phenolic compounds after gastrointestinal digestion (9.447 and 41.853 μmol (poly)phenolic compounds/g dm, respectively) because of the highest amount of bound compounds. Some studies focused on the bioaccessibility of (poly)phenolic compounds in other vegetables foods, such as tomatoes and cauliflower, also demonstrated a higher bioaccessibility after thermal treatment (Bugianesi et al., 2004; Girgin & El Nehir, 2015).

Considering the individual (poly)phenolic compounds, no new compounds were found after the digestion process in both green pepper and cardoon samples although flavonoid aglyconic forms could be expected. Actually, the loss of the glycosidic moieties is due to membrane-bound glycosylases found on the brush border of the mammalian small intestine (Day et al., 2000; Németh et al., 2003), which are clearly not present under the adopted conditions. However some chemical reactions such as isomerization can be observed, mainly in cardoon chlorogenic acids. Isomerization from 5-CQA to 3-CQA is highly pH dependent and may occur during the intestinal step of the digestion process (alkaline pH) (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007; Erk et al., 2014; Monente, Ludwig, Stalmach et al., 2015; Mawalagedera, Ou, McDowell, & Gould, 2016). This might explain the 5-CQA degradation (59 and 64% bioaccessibility) and the simultaneous 3-CQA increase (191 and 156% bioaccessibility) observed in olive and sunflower oil fried cardoon samples submitted to gastrointestinal digestion. Similarly, other isomerization reactions might take place among diCQAs, increasing 143-377% bioaccessibility of diCQA II. Since this behavior was mainly observed in fried samples, the presence of fat might have a relevant role in these isomerization reactions.

After the intake of vegetables, the levels of flavonoids and non-flavonoids in plasma are low (Aura, 2008), probably related to their limited absorption (Walle, 2004; Manach, Williamson, Morand, Scalbert, & Rémésy, 2005; Dupas, Marsset Baglieri, Ordonaud, Tomé, & Maillard, 2006; Jaganath, Mullen, Edwards, & Crozier, 2006; Monente, Ludwig, Stalmach, et al., 2015;). In fact, (poly)phenols bioavailability in the first gastrointestinal tract has been estimated in less than 20% (Hu, 2007). Therefore, most of the (poly)phenolic compounds are unabsorbed and potentially reach the colon, where the **colonic microbiota** actively participates in their catabolic process. In the present study an important microbial metabolic activity was observed resulting in the degradation of most of the parent (poly)phenolic compounds and, consequently, in the increase of some compounds and the further generation of new metabolites. According to the obtained amounts of native (poly)phenolic compounds and catabolites during 24h fecal fermentation of digested green pepper and cardoon, and based on previous studies in the literature, catabolic pathways for quercetin, luteolin and caffeoylquinic acids derivatives from microbial degradation in the colon were proposed (Figure 1).

**Figure 1**. Proposed catabolic pathways for quercetin, luteolin and caffeoylquinic acids derivatives microbial degradation in the colon after gastrointestinal digestion.




Degly, Deglycosilation; DeOH, Dehydroxylation; Est, Ester hydrolysis; Hyd, Hydrogenation; α-Ox, α-Oxidation. Detected metabolites are in black, non-detected metabolites are in grey. Red arrows show quercetin glucoside catabolic pathway, green arrows correspond to luteolin derivatives colonic pathways, orange arrows evidence caffeic acid derivatives pathways and blue arrows indicate chlorogenic acid metabolic pathways. Bold arrows indicate major pathways.

In flavonoids, an important microbial metabolic activity was observed, which resulted in the deglycosilation of flavonoid glucosides and the consequent formation of aglycones. In green pepper samples, quercetin-based compounds were quickly metabolized and during the first few minutes of colonic biotransformation the amount was halved, while the aglycone increased simultaneously, detecting the highest amount of quercetin after 5 hours of fecal fermentation. However, the recovered amount of quercetin after 5 h did not correspond to the total of the native quercetin derivatives, indicating that also the aglycone, once released, could be rapidly degraded, in agreement with previously reported results (Serra et al., 2012). Similarly, luteolin aglycone was rapidly released at the beginning of the fecal incubation. However, luteolin derivatives were not degraded as fast as luteolin was formed, letting us to hypothesize that luteolin could have also derived from quercetin dehydroxylation. In general, *O*-glucosides of both luteolin and quercetin were almost completely metabolized by the intestinal microbiota while *C*-glucosides were much more slowly degraded, and some of them, like luteolin 8-C-hexoside, still remained present after 24 h of fecal incubation. In cardoon samples, every luteolin derivates were degraded during fecal fermentation, probably because all of them were *O*-glucosides. These results are in agreement with those reported by Hein et al. (2008), who observed a complete metabolism of *O*-glycoside compounds between 20 min and 4 hours of fecal incubation, whereas *C*-glycoside compounds were only partially reduced.

Both luteolin and quercetin are subjected to ring fission, resulting in the formation of dihydrocaffeic acid, which could be further degraded to new catabolites (Serra et al., 2012). Ring fission of quercetin could also result in the formation of protocatechuic acid (Rechner et al., 2004). Hein et al. (2008) reported the complete metabolization of the released aglycones within 8 hours of fecal incubation. Nevertheless, in the present study, luteolin aglycone was substantially metabolized within 5 hours, whereas low amounts of quercetin still remained after 24 h of fecal incubation. The highlighted difference between the compared studies could be linked to the source of native compounds used in the model, as Hein et al. (2008) employed only standard molecules, not a food matrix. Therefore, it could be hypothesized that food matrices used in the present study could have influenced the metabolism of quercetin derivatives, preventing their complete degradation.

Concerning phenolic acids, the compounds detected on the digested fraction of both green pepper and cardoon were quickly metabolized by gut microbiota, and no native compounds were detected after 5 h of fecal fermentation in green pepper whereas in a chlorogenic acids-rich vegetable like cardoon, low amount in 5-CQA and caffeic acid were found especially in the cooked samples. Firstly, successive ester hydrolysis took place in CQAs derivates like diCQAs or succinildiCQAs, resulting in the release of their corresponding CQAs, mainly 5-ÇQA. Next, the major catabolic pathway of CQAs was their transformation into caffeic acid by the action of bacterial esterases (Rechner et al., 2004; Ludwig, de Peña, Cid, & Crozier, 2013; Tomas-Barberan et al., 2014). In addition, caffeic acid could also be formed from caffeic acid glucosides by deglycosilation. Both processes could explain the increase in caffeic acid observed during the first 15 min of fecal incubation, for green pepper and fried cardoon samples, probably modulated by the food matrix, the initial amount of caffeic acid and the

presence of fat. Caffeic acid was then hydrogenated into dihydrocaffeic acid (Rechner et al., 2004; Ludwig et al., 2013; Tomas-Barberan et al., 2014). The high amount of CQAs in cardoon samples gave rise to a greater amount of caffeic acid than green pepper samples, which could not be completely metabolized after 24 hours by colonic microbiota into 3-(3',4'-dihydroxyphenyl)propionic acid (dihydrocaffeic acid). Additionally, hydrogenation was also reported as one of the first catabolic steps of CQAs, yielding dihydrocaffeoylquinic acid, which was further transformed into dihydrocaffeic acid by ester bond hydrolysis reactions (Tomas-Barberan et al., 2014). Since dihydrocaffeoylquinic acid formation is CQA dose dependent (Tomas-Barberan et al., 2014), this catabolite was only detected in cooked cardoon samples, which were the samples with the highest amount of CQAs in their composition in the present study.

Dihydrocaffeic acid should be consider as an intermediate catabolite, since it may subsequently undergo either dehydroxylation, resulting in the production of 3-(3'-hydroxyphenil)propionic acid, or via two α-oxidation steps, resulting in the formation of 3,4-dihydroxybenzoic acid (protocatechuic acid) acting 3',4'-dihydroxyphenylacetic acid (homoprotocatechuic acid) as an intermediate. Actually, 3-(3'-hydroxyphenyl)propionic acid was by far the most abundant catabolite found after 24 h of fecal incubation in both cardoon and green pepper. This compound has also been reported to be one of the main colonic catabolites of 5-CQA found in *in vitro* experiments or after consumption of coffee brew, rich in CQAs (Gonthier et al., 2006; Ludwig et al., 2013). Contrarily, 3',4'-dihydroxyphenylacetic acid was not detected in either cardoon or green pepper samples during fecal fermentation, probably because of its rapid rate of conversion into 3,4-dihydroxybenzoic acid (protocatechuic acid) (Ludwig et al., 2013), which was detected in substantial amounts in 5 h fermented samples, and still remained in considerable amounts after 24 h of fecal incubation. Actually, Aura et al. (Aura et al., 2002) detected the highest concentration of 3',4'-dihydroxyphenylacetic acid within 2 h of fecal incubation, a time point not considered in the present study. Recently, a direct formation of 3-4-dihydroxybenzoic acid (protocatechuic) from dihydrocaffeic acid via β-oxidation has also been suggested (Kay, Pereira-Caro, Ludwig, Clifford, & Crozier, 2017). Like 3',4'-dihydroxyphenylacetic acid, other minor catabolites previously reported as being generated after (poly)phenol compound fecal biotransformation, such as phenylacetic acid, hydroxyphenylacetic acid, hydroxybenzoic acid or benzoic acid (Aura et al., 2002; Serra et al., 2012, Ludwig et al., 2013) were not detected in the present study. This could be due to the different fermentation times applied, as 3-hydroxyphenylacetic acid was formed by dehydroxylation of 3,4-hydroxyphenylacetic acid after 8 h incubation (Aura et al., 2002), while other catabolites presented the maximum amount after 48 h of fecal incubation and only low quantities were detected after 24 h (Serra et al., 2012).

Finally, regarding the influence of heat treatment on fecal metabolism, overall only differences in the total amount of catabolites were observed between raw and cooked samples, whereas no differences were detectable in the number of identified catabolites with the exception of dihydrocaffeoylquinic acid, which was only detected in cooked cardoon samples due to the high amount of CQAs. In green pepper, from the total amount of

(poly)phenolic compounds and catabolites present after digestion, around 41-53% remained bioaccesible after 24 h of fecal incubation, being griddled green pepper the sample with the highest amount of (poly)phenolic compounds and catabolites after fecal fermentation (4.198 μmol (poly)phenolic compounds/g). In the case of cardoon, around 30-40% of (poly)phenolic compounds and catabolites of digested cooked samples were bioaccesible after fecal fermentation. Also griddled cardoon was the sample with the highest amount of (poly)phenolic compounds, including catabolites, after 24 h of fecal incubation (12.489 μmol (poly)phenolic compounds/g). Therefore, griddling process can be suggested as the most appropriate cooking technique to maintain both native (poly)phenolic compounds and catabolites, bioaccesible to be absorbed in the gut, or even to have an influence on the intestinal microbiota (Blaut, Schoefer, & Braune, 2003) and exert health benefits (Verzelloni et al., 2011; Masella et al., 2012; Duda-Chodak, Tarko, Satora, & Sroka, 2015). However, the positive effects of these metabolites on health are still unclearly defined, and further studies are necessary in order to better understand the mechanism of action of these compounds within the human organism, including gut microbiota. Further studies are also clearly needed to investigate the absorption of (poly)phenolic compounds and their subsequent transformation during phase II enzymatic metabolism resulting in glucuronidated, sulphated and methylated derivatives (Aura, 2008; Del Rio et al., 2013).

In summary, it has been demonstrated that heat treatments, especially those carried out at the highest temperature (griddling), of green pepper and cardoon enhances the bioaccessibility of the (poly)phenolic compounds, which remain in greater amount after *in vitro* gastrointestinal digestion and fecal fermentation. Heat treatment also induces other significant changes such as water loss, changes in the total fat content and in the fatty acid profile, as previously demonstrated in the present study, as well as degradation of other thermolabile compounds, and the formation of others due to heat-induced chemical reactions (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Miranda et al., 2010). Additionally, cooking processes, especially those more intense, develop the typical sensorial properties and palatability of roasted and fried foods due to Maillard reactions, including brown color, crunchy texture, and induce the formation of volatile compounds that provide their characteristic aroma and flavor, among which are furanic compounds (Maga, 1979; Anese & Suman, 2013). Since furan has been classified as a possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC, 1995) its formation on different cooking foods including vegetables might be of a great interest to be studied.

In the present study the **occurrence of furan** in five vegetables (yellow onion, green pepper, cardoon, cabbage and chicory) used as base ingredients or garnish in typical dishes in Spanish cuisine after frying with olive or sunflower oil or after griddling was analyzed. These results were compared with those obtained in foods that, due to their composition (bread coated frozen foods) and to the intense heat treatment usually applied before consumption (deep-frying) are very prone to the formation of furan (Fromberg, Fagt, & Granby, 2009; Arisseto, Vicente, Ueno, Tfouni, & Toledo, 2011). The obtained results were published in LW- Food Science and Technology journal (Juániz et al., 2016).

Regarding vegetables, furan was only found in griddled onion at low level (3.5 µg/kg), and in the other griddled vegetables below the limit of quantification of 2.3 µg/kg or the limit of detection of 0.7 µg/kg. Furan was not detected in any of the raw or fried vegetable samples. Furan values in vegetables reported by EFSA (2011) were higher than those resulting on the present study. Since canned and jarred products present high levels of furan (US FDA, 2004) it is probably that EFSA data mainly include this kind of vegetables. The high consumption of onion might contribute up to 368 ng/kg bw/day of furan intake for the worst scenario. However, onion is mainly consumed raw in salads or fried, in which samples furan content was not found. Therefore, only in the case of the consumption of onion treated by griddling, which is less common than the other culinary techniques, it could contribute to furan dietary exposure. The consumption of the other vegetables analysed in the present study did not suppose a significant intake of furan.

As expected, furan levels increased in all bread coated frozen foods (ham croquettes, squid rings, tuna pasties, churros, nuggets, fish fingers, onion rings and san Jacobos) after frying with either fresh or reheated olive oil. In raw foods, furan was only found in tuna pasties (16 µg/kg), probably due to their filling (tuna in tomato sauce) previously cooked. After frying, furan levels increased in all samples. In samples fried in fresh oil, furan levels ranged between 16 µg kg-1 (fish fingers) and 115 µg/kg (onion rings). When reheated oil was used, furan levels tended to be higher in many cases, ranging from 12 µg/kg (tuna pasties) to 172 µg/kg (onion rings). However, no significant differences were found in ham croquettes, tuna pasties, nuggets and fish fingers fried using either fresh or reheated oil. Therefore, the use of reheated oil seemed to have lower influence in furan formation than the frying process itself. Because in the present study the same time (6 min) and temperature (190 ºC) were applied for frying all bread coated frozen foods, the different food composition might explain the different levels of furan. On the other hand, the longer time in comparison to that applied for French fries and homemade crisps (3-3.5min at 190 ºC) (Fromberg et al., 2009) might explain the higher values of furan in the present study. Moreover, the Maillard browning reactions induced by frying at high temperatures in a dough (churros and tuna pasties) or food samples with a carbohydrate-rich external coat, such as bread (ham croquettes, fish fingers, nuggets and san jacobos) or a dough (squid rings and onion rings), might explain the formation of higher furan content in our samples than in toasted bread slices or also in coffee brew, two of the previously reported foods with the higher furan levels (Fromberg et al., 2009; EFSA, 2011). A dietary exposure from 4.7 to 178 ng /kg bw/day of furan was estimated for Spanish population due to the consumption of the fried frozen precooked food analyzed in the present study taking into account the mean and P99 consumption of these foods reported by the Spanish Dietary Intake Survey (AECOSAN, 2011). Unheated olive oil contained low amount of furan (2.5 µg/kg), in agreement with that found in other vegetable oils (EFSA, 2010b, 2011), but lower than that reported for olive oil by Fromberg et al. (2009). However, olive oil after used as frying agent contained between 14 and 17 µg7kg. This may be due to the formation of furan from unsaturated fatty acids at high temperatures (Becalski & Seaman, 2005), and the retention of furan formed in fried foods or in their residues by oil (Van Lancker, Adams, Owczarek, De Meulenaer, & De Kimpe, 2009). Olive oil

has been estimated as one of the main foods which might contribute to furan intake among Spanish population (up to 1463 ng/kg bw/day of furan for the worst scenario) due to its high consumption in Spain. However, it has to be considered that most of the olive oil is consumed unheated, mainly in salads, which present very low furan content (2.5 µg/kg) providing a furan intake of 57 and 192 ng/kg bw/day for those consumers with a mean and P99 food consumption, respectively.

Finally, the total exposure of furan in Spanish population was also estimated based on data of furan content (mean and P95) per main food category reported by EFSA (2011) and the consumption of those foods by Spanish population (AECOSAN, 2011). A total mean dietary exposure of furan of 239 ng/kg bw/day was estimated for Spanish population. This result is in the range reported by EFSA (2011) for European adults (30 and 580 ng/kg bw/day). However, taking into account the worst scenario, that is the high consumption (P99) of different foods with the highest content of furan (P95), total dietary exposure of furan could reach up to 4372 ng/kg bw/day. In European adult populations, coffee brew, with a range of furan levels between 42-45 µg/kg (mean) and 228 µg/kg (P95) (EFSA, 2011), has been proposed as the major contributor to furan intake (Fromberg et al., 2009; EFSA, 2011; Sijia, Enting, & Yuan, 2014). However these values might be overestimated, due to the high consumption of coffee brew in Northern European countries (more than 10 ml/kg bw/day of coffee) that usually has been taken to estimate furan content (Fromberg et al., 2009). In Spain, for example coffee brew consumption is much lower (up to 5.4 ml/kg bw/day) (AECOSAN, 2011), thus coffee is not the main furan contributor in the Spanish population with an average consumption of this brew. The highest furan exposure in the Spanish population is due to cereal products. This could be due to the fact that the category "cereal products" in Spanish survey covers a wide range of products, and consequently its consumption might be overestimated. EFSA report only collected furan data from 4 cereal products samples from Spain, but it is difficult to know what are the specific foods included. Taking into account that typical Spanish fried foods such as churros, croquettes and pasties are included in the category "cereal products" (AECOSAN, 2011), and that as far as we know this is the first study that reports furan content in these products, it is suggested that EFSA data should be revised to include them.

With these data, MOEs of furan were calculated in order to estimate a preliminary approach for risk assessment. According to the EFSA (2005), a MOE of 10,000 or higher would be considered as a low public health concern and reasonably as a low priority for risk management actions. WHO (2011) obtained MOEs of furan of 960 and 480 for average and high dietary exposures, respectively. Therefore, the Committee considered that these MOEs indicate a human health concern for furan. In the case of the total furan exposure in Spanish population, MOEs were also below 10,000 in all scenarios. When MOEs were calculated for fried frozen precooked food samples for Spanish population, results showed that furan could suppose a possible public health risk only in the case of people with the highest consumption of these food products. Regarding vegetables, MOEs were not calculated due to the low levels of furan.

Despite the potential risk of furan in the Spanish population, taking into account the limitation of the low number of samples in the present study, further studies with a higher number of samples from different commercial brands, homemade products, and other ethnic foods should be developed to provide exposure data for a final risk assessment. Additionally, taking into account that 93.6% of the collected furan results in EFSA report (2011) were derived from samples without cooking processes, data should be continuously revised including higher numbers of foods cooked at different conditions. Other aspects, such as the volatilization of furan during the time between cooking and consumption or the reheated process should also be considered. Some authors found a decrease in furan levels (Zoller, Sager, & Reinhard, 2007; EFSA, 2011), while others reported a furan increase during cooling in toasted bread (Fromberg et al., 2009). Additionally, some authors reported furan losses during warming under different times (Goldmann, Périsset, Scanlan, & Stadler, 2005; Zoller et al., 2007; Fromberg et al., 2009) while others have found that furan persist during reheating practice ( Hasnip, Crews, & Castle, 2006; Lachenmeier, Reusch, & Kuballa, 2009). Furthermore, risk assessment of foods should be conducted to consider vulnerable groups, like adolescents and infants, and professionals in restaurants, caterings, etc.

## REFERENCES

AECOSAN (Agencia Española de Consumo, S. A. y N. (2011). ENIDE: encuenta nacional de ingesta dietética (2009-2010). Resultados sobre datos de consumo. URL http://www.aesan.mspsi.gob.es/AESAN/docs/docs/evaluacion_riesgos/datos_consumo/ENIDE.pdf. Access 28.09.2015

Anese, M., & Suman, M. (2013). Mitigation strategies of furan and 5-hydroxymethylfurfural in food. *Food Research International*, *51*(1), 257–264.

Aranceta, J., & Pérez-Rodrigo, C. (2012). Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review. *The British Journal of Nutrition*, *107 Suppl*(S2), S8-22.

Arisseto, A. P., Vicente, E., Ueno, M. S., Tfouni, S. A. V., & Toledo, M. C. D. F. (2011). Furan levels in coffee as influenced by species, roast degree, and brewing procedures. *Journal of Agricultural and Food Chemistry*, *59*(7), 3118–24.

Aura, A.-M. (2008). Microbial metabolism of dietary phenolic compounds in the colon. *Phytochemistry Reviews*, *7*(3), 407–429.

Aura, A.-M., O'Leary, K. A., Williamson, G., Ojala, M., Bailey, M., Puupponen-Pimiä, R., Nuutila, A. M., Oksman-Caldentey, K.-M., & Poutanen, K. (2002). Quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids but not methylated by human fecal flora in vitro. *Journal of Agricultural and Food Chemistry*, *50*(6), 1725–30.

Becalski, A., & Seaman, S. (2005). Furan precursors in food: a model study and development of a simple headspace method for determination of furan. *Journal of AOAC International*, *88*(1), 102–6.

Bekedam, E. K., Loots, M. J., Schols, H. A., Van Boekel, M. A. J. S., & Smit, G. (2008). Roasting effects on formation mechanisms of coffee brew melanoidins. *Journal of Agricultural and Food Chemistry*, *56*(16), 7138–45.

Bekedam, E. K., Schols, H. A., Van Boekel, M. A. J. S., & Smit, G. (2008). Incorporation of chlorogenic acids in coffee brew melanoidins. *Journal of Agricultural and Food Chemistry*, *56*(6), 2055–63.

Bermúdez-Soto, M. J., Tomás-Barberán, F. A., & García-Conesa, M. T. (2007). Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to in vitro gastric and pancreatic digestion. *Food Chemistry*, *102*(3), 865–874.

Blaut, M., Schoefer, L., & Braune, A. (2003). Transformation of flavonoids by intestinal microorganisms. *International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Fur Vitamin- Und Ernahrungsforschung. Journal International de Vitaminologie et de Nutrition*, *73*(2), 79–87.

Buckland, G., Travier, N., Barricarte, A., Ardanaz, E., Moreno-Iribas, C., Sánchez, M.-J., Molina-Montes, E., Chirlaque, M. D., Huerta, J. M., Navarro, C., Redondo, M. L., Amiano, P., Dorronsoro, M., Larrañaga, N., & Gonzalez, C. A. (2012). Olive oil intake and CHD in the European prospective investigation into cancer and nutrition Spanish cohort. *The British Journal of Nutrition*, *108*(11), 2075–82.

Bugianesi, R., Salucci, M., Leonardi, C., Ferracane, R., Catasta, G., Azzini, E., & Maiani, G. (2004). Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and beta-carotene in cherry tomatoes. *European Journal of Nutrition*, *43*(6), 360–6.

Chajès, V., Thiébaut, A. C. M., Rotival, M., Gauthier, E., Maillard, V., Boutron-Ruault, M.-C., Joulin, V., Lenoir, G. M., & Clavel-Chapelon, F. (2008). Association between serum trans-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC Study. *American Journal of Epidemiology*, *167*(11), 1312–20.

Clifford, M. N. (1999). Chlorogenic acids and other cinnamates - nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, *79*(3), 362–372.

Coelho, C., Ribeiro, M., Cruz, A. C. S., Domingues, M. R. M., Coimbra, M. A., Bunzel, M., & Nunes, F. M. (2014). Nature of phenolic compounds in coffee melanoidins. *Journal of Agricultural and Food Chemistry*, *62*(31), 7843–53.

Crozier, A., Lean, M. E. J., McDonald, M. S., & Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal of Agricultural and Food Chemistry*, *45*(3), 590–595.

Day, A. J., Cañada, F. J., Díaz, J. C., Kroon, P. A., Mclauchlan, R., Faulds, C. B., Plumb, G. W., Morgan, M. R., & Williamson, G. (2000). Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Letters*, *468*(2–3), 166–70.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (Poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling*, *18*(14), 1818–1892.

Duda-Chodak, A., Tarko, T., Satora, P., & Sroka, P. (2015). Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. *European Journal of Nutrition*, *54*(3), 325–41.

Dupas, C., Marsset Baglieri, A., Ordonaud, C., Tomé, D., & Maillard, M.-N. (2006). Chlorogenic acid is poorly absorbed, independently of the food matrix: A Caco-2 cells and rat chronic absorption study. *Molecular Nutrition & Food Research*, *50*(11), 1053–60.

EFSA (European Food Safety Authority). (2005). Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic. *The EFSA Journal*, *282*, 1–31.

EFSA (European Food Safety Authority). (2010a). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, *8*(3), 1461.

EFSA (European Food Safety Authority). (2010b). Update of results on the monitoring of furan levels in food. URL http://www.efsa.europa.eu/it/search/doc/1702.pdf. Access 25.04.2017

EFSA (European Food Safety Authority). (2011). Update on furan levels in food from monitoring years 2004-2010 and exposure assessment. URL http://www.efsa.europa.eu/en/efsajournal/doc/2347.pdf. Access 25.04.2017

Erk, T., Renouf, M., Williamson, G., Melcher, R., Steiling, H., & Richling, E. (2014). Absorption and isomerization of caffeoylquinic acids from different foods using ileostomist volunteers. *European Journal of Nutrition*, *53*(1), 159–66.

Ewald, C., Fjelkner-Modig, S., Johansson, K., Sjöholm, I., & Åkesson, B. (1999). Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chemistry*, *64*(2), 231–235.

Ferracane, R., Pellegrini, N., Visconti, A., Graziani, G., Chiavaro, E., Miglio, C., & Fogliano, V. (2008). Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. *Journal of Agricultural and Food Chemistry*, *56*(18), 8601–8608.

Fromberg, A., Fagt, S., & Granby, K. (2009). Furan in heat processed food products including home cooked food products and ready-to-eat products. *EFSA Supporting Publications*, *6*(9).

Gebauer, S. K., Psota, T. L., Harris, W. S., & Kris-Etherton, P. M. (2006). n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *The American Journal of Clinical Nutrition*, *83*(6 Suppl), 1526S–1535S.

Girgin, N., & El Nehir, S. (2015). Effects of cooking on in vitro sinigrin bioaccessibility, total phenols, antioxidant and antimutagenic activity of cauliflower (*Brassica oleraceae* L. var. Botrytis). *Journal of Food Composition and Analysis*, *37*, 119–127.

Goldmann, T., Périsset, A., Scanlan, F., & Stadler, R. H. (2005). Rapid determination of furan in heated foodstuffs by isotope dilution solid phase micro-extraction-gas chromatography--mass spectrometry (SPME-GC-MS). *The Analyst*, *130*(6), 878–83.

Gonthier, M.-P., Remesy, C., Scalbert, A., Cheynier, V., Souquet, J.-M., Poutanen, K., & Aura, A.-M. (2006). Microbial metabolism of caffeic acid and its esters chlorogenic and caftaric acids by human faecal microbiota in vitro. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *60*(9), 536–40.

Guasch-Ferré, M., Hu, F. B., Martínez-González, M. A., Fitó, M., Bulló, M., Estruch, R., Ros, E., Corella, D., Recondo, J., Gómez-Gracia, E., Fiol, M., Lapetra, J., Serra-Majem, L., Muñoz, M. A., Pintó, X., Lamuela-Raventós, R. M., Basora, J., Buil-Cosiales, P., Sorlí, J. V, Ruiz-Gutiérrez, V., Martínez, J. A., & Salas-Salvadó, J. (2014). Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. *BMC Medicine*, *12*, 78.

Hasnip, S., Crews, C., & Castle, L. (2006). Some factors affecting the formation of furan in heated foods. *Food Additives and Contaminants*, *23*(3), 219–27.

Harris, S., Brunton, N., Tiwari, U., & Cummins, E. (2015). Human exposure modelling of quercetin in onions (*Allium cepa* L.) following thermal processing. *Food Chemistry*, *187*, 135–9.

Hein, E.-M., Rose, K., van't Slot, G., Friedrich, A. W., & Humpf, H.-U. (2008). Deconjugation and degradation of flavonol glycosides by pig cecal microbiota characterized by Fluorescence in situ hybridization (FISH). *Journal of Agricultural and Food Chemistry*, *56*(6), 2281–90.

Hu, M. (2007). Commentary: bioavailability of flavonoids and polyphenols: call to arms. *Molecular Pharmaceutics*, *4*(6), 803–6.

IARC (International Agency for Research on Cancer). (1995). Furan. *Monographs on the Evaluation of Carcinogenic Risks to Humans*, *63*, 393–407.

Jaganath, I. B., Mullen, W., Edwards, C. A., & Crozier, A. (2006). The relative contribution of the small and large intestine to the absorption and metabolism of rutin in man. *Free Radical Research*, *40*(10), 1035–46.

Jiménez-Monreal, A. M., García-Diz, L., Martínez-Tomé, M., Mariscal, M., & Murcia, M. A. (2009). Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Science*, *74*(3), H97–H103.

Juániz, I. Ludwig, I.A., Huarte, E., Pereira-Caro, G., Moreno-Rojas, J.M., Cid, C., & de Peña, M.P. (2016). Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables. Food Chemistry, 197, 466-473. (Q1) (D1)

Juániz, I., Zocco, C., Mouro, V., Cid, C., & de Peña, M.P. (2016). Effect of frying process on furan content in foods and assessment of furan exposure of Spanish population. LWT-Food Science and Technology, 68, 549-555. (Q1)

Juániz, I., Ludwig, I.A., Dall'Asta, M., Bresciani, L., Mena, P., Del Rio, D., Cid, C., & de Peña, M.P. (2016). Catabolism of raw and cooked green pepper (capsicum annuum) (poly)phenolic compounds after simulated gastrointestinal digestion and faecal fermentation. Journal of Functional Foods, 27, 201-213. (Q1) (D1)

Juániz, I., Ludwig, I.A., Dall'Asta, M., Bresciani, L., Mena, P., Del Rio, D., Cid, C., & de Peña, M.P. (2017). Bioaccessibility of (poly)phenolic compounds of raw and cooked cardoon (Cynara cardunculus L.) after simulated gastrointestinal digestion and fermentation by human colonic microbiota. *Journal of Functional Foods*, 32, 195-207. (Q1) (D1)

Kay, C. D., Pereira-Caro, G., Ludwig, I. A., Clifford, M. N., & Crozier, A. (2017). Anthocyanins and flavanones are more bioavailable than previously perceived: A review of recent evidence. *Annual Review of Food Science and Technology*, *8*, 155–180.

Kim, J.-H., Nam, C.-M., Kim, J.-W., Lee, D.-C., Shim, J.-S., & Lee, H.-R. (2012). Relationship between attention-deficit/hyperactivity disorder and trans fatty acids intake in female adolescents. *Acta Paediatrica (Oslo, Norway : 1992)*, *101*(9), e431-3.

Lachenmeier, D. W., Reusch, H., & Kuballa, T. (2009). Risk assessment of furan in commercially jarred baby foods, including insights into its occurrence and formation in freshly home-cooked foods for infants and young children. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *26*(6), 776–85.

Lanzotti, V. (2006). The analysis of onion and garlic. *Plant Analysis*, *1112*(1–2), 3–22.

Lombard, K., Peffley, E., Geoffriau, E., Thompson, L., & Herring, A. (2005). Quercetin in onion (*Allium cepa* L.) after heat-treatment simulating home preparation. *Journal of Food Composition and Analysis*, *18*(6), 571–581.

Lu, X., Wang, J., Al-Qadiri, H. M., Ross, C. F., Powers, J. R., Tang, J., & Rasco, B. A. (2011). Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. *Food Chemistry*, *129*(2), 637–644.

Ludwig, I. A., Mena, P., Calani, L., Cid, C., Del Rio, D., Lean, M. E. J., & Crozier, A. (2014). Variations in caffeine and chlorogenic acid contents of coffees: what are we drinking? *Food & Function*, *5*(8), 1718–26.

Ludwig, I. A., Paz de Peña, M., Concepción, C., & Alan, C. (2013). Catabolism of coffee chlorogenic acids by human colonic microbiota. *BioFactors*, *39*(6), 623–32.

Maga, J. (1979). Furan in foods. *Critical Reviews in Food Science and Nutrition*, *11*(4), 355.

MAGRAMA (Ministery of Agliculture Food and Environment). (2015). Informe del consumo de alimentacion en España 2015. URL http://www.magrama.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/informeconsumoalimentacion2015_tcm7-422694.pdf. Access 25.04.2017

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition*, *81*(1 Suppl), 230S–242S.

Marín, A., Ferreres, F., Tomás-Barberán, F. A., & Gil, M. I. (2004). Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry*, *52*(12), 3861–9.

Masella, R., Santangelo, C., D'Archivio, M., Li Volti, G., Giovannini, C., & Galvano, F. (2012). Protocatechuic acid and human disease prevention: biological activities and molecular mechanisms. *Current Medicinal Chemistry*, *19*(18), 2901–17.

Mawalagedera, S. M. M. R., Ou, Z.-Q., McDowell, A., & Gould, K. S. (2016). Effects of boiling and in vitro gastrointestinal digestion on the antioxidant activity of Sonchus oleraceus leaves. *Food & Function*, *7*(3), 1515–22.

Mensink, R. P., & Katan, M. B. (1990). Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *The New England Journal of Medicine*, *323*(7), 439–45.

Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2008). Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of Agricultural and Food Chemistry*, *56*(1), 139–47.

Miranda, J. M., Martínez, B., Pérez, B., Antón, X., Vázquez, B. I., Fente, C. A., Franco, C. M., Rodríguez, J. L., & Cepeda, A. (2010). The effects of industrial pre-frying and domestic cooking methods on the nutritional compositions and fatty acid profiles of two different frozen breaded foods. *LWT - Food Science and Technology*, *43*(8), 1271–1276.

Monente, C., Ludwig, I. A., Irigoyen, A., De Peña, M.-P., & Cid, C. (2015). Assessment of Total (free and bound) phenolic compounds in spent coffee extracts. *Journal of Agricultural and Food Chemistry*, *63*(17), 4327–4334.

Monente, C., Ludwig, I. A., Stalmach, A., de Peña, M. P., Cid, C., & Crozier, A. (2015). In vitro studies on the stability in the proximal gastrointestinal tract and bioaccessibility in Caco-2 cells of chlorogenic acids from spent coffee grounds. *International Journal of Food Sciences and Nutrition*, *66*(6), 657–664.

Morales, F. J., Somoza, V., & Fogliano, V. (2012). Physiological relevance of dietary melanoidins. *Amino Acids*, *42*(4), 1097–1109.

Németh, K., Plumb, G. W., Berrin, J.-G., Juge, N., Jacob, R., Naim, H. Y., Williamson, G., Swallow, D. M., & Kroon, P. A. (2003). Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition*, *42*(1), 29–42.

Nunes, F. M., & Coimbra, M. A. (2010). Role of hydroxycinnamates in coffee melanoidin formation. *Phytochemistry Reviews*, *9*(1), 171–185.

Olthof, M. R., Hollman, P. C., & Katan, M. B. (2001). Chlorogenic acid and caffeic acid are absorbed in humans. *The Journal of Nutrition*, *131*(1), 66–71.

Palermo, M., Pellegrini, N., & Fogliano, V. (2014). The effect of cooking on the phytochemical content of vegetables. *Journal of the Science of Food and Agriculture*, *94*(6), 1057–70.

Pellegrini, N., Miglio, C., Del Rio, D., Salvatore, S., Serafini, M., & Brighenti, F. (2009). Effect of domestic cooking methods on the total antioxidant capacity of vegetables. *International Journal of Food Sciences and Nutrition*, *60 Suppl 2*(s2), 12–22.

Pérez-Jiménez, J., Díaz-Rubio, M. E., Mesías, M., Morales, F. J., & Saura-Calixto, F. (2014). Evidence for the formation of maillardized insoluble dietary fiber in bread: A specific kind of dietary fiber in thermally processed food. *Food Research International*, *55*(0), 391–396.

Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E., & Romani, A. (2007). Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated cardoon leaves. *Food Chemistry*, *105*(4), 1695–1701.

Price, K. R., & Rhodes, M. J. C. (1997). Analysis of the major flavonol glycosides present in four varieties of onion (*Allium cepa*) and changes in composition resulting from autolysis. *Journal of the Science of Food and Agriculture*, *74*(3), 331–339.

Rechner, A. R., Smith, M. A., Kuhnle, G., Gibson, G. R., Debnam, E. S., Srai, S. K. S., Moore, K. P., & Rice-Evans, C. A. (2004). Colonic metabolism of dietary polyphenols: influence of structure on microbial fermentation products. *Free Radical Biology & Medicine*, *36*(2), 212–25.

Rechner, A. R., Spencer, J. P., Kuhnle, G., Hahn, U., & Rice-Evans, C. A. (2001). Novel biomarkers of the metabolism of caffeic acid derivatives in vivo. *Free Radical Biology & Medicine*, *30*(11), 1213–22.

Rodrigues, A. S., Pérez-Gregorio, M. R., García-Falcón, M. S., & Simal-Gándara, J. (2009). Effect of curing and cooking on flavonols and anthocyanins in traditional varieties of onion bulbs. *Food Research International*, *42*(9), 1331–1336.

Rodriguez-Mateos, A., Vauzour, D., Krueger, C. G., Shanmuganayagam, D., Reed, J., Calani, L., Mena, P., Del Rio, D., & Crozier, A. (2014). Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. *Archives of Toxicology*, *88*(10), 1803–1853.

Rodríguez Galdón, B., Rodríguez Rodríguez, E. M., & Díaz Romero, C. (2008). Flavonoids in onion cultivars (*Allium cepa* L.). *Journal of Food Science*, *73*(8), C599–C605.

Rohn, S., Buchner, N., Driemel, G., Rauser, M., & Kroh, L. W. (2007). Thermal degradation of onion quercetin glucosides under roasting conditions. *Journal of Agricultural and Food Chemistry*, *55*(4), 1568–73.

Ruano, C., Henriquez, P., Bes-Rastrollo, M., Ruiz-Canela, M., del Burgo, C. L., & Sánchez-Villegas, A. (2011). Dietary fat intake and quality of life: the SUN project. *Nutrition Journal*, *10*, 121.

Sacchetti, G., Ioannone, F., De Gregorio, M., Di Mattia, C., Serafini, M., & Mastrocola, D. (2016). Non enzymatic browning during cocoa roasting as affected by processing time and temperature. *Journal of Food Engineering*, *169*, 44–52.

Schwingshackl, L., & Strasser, B. (2012). High-MUFA diets reduce fasting glucose in patients with type 2 diabetes. *Annals of Nutrition & Metabolism*, *60*(1), 33–4.

Schwingshackl, L., Strasser, B., & Hoffmann, G. (2011). Effects of monounsaturated fatty acids on cardiovascularriskfactors: A systematic review andmeta-analysis. *Annals of Nutrition and Metabolism*, *59*(2–4), 176–186.

Sellappan, S., & Akoh, C. C. (2002). Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. *Journal of Agricultural and Food Chemistry*, *50*(19), 5338–5342.

Serra, A., Macia, A., Romero, M.-P., Reguant, J., Ortega, N., & Motilva, M.-J. (2012). Metabolic pathways of the colonic metabolism of flavonoids (flavonols, flavones and flavanones) and phenolic acids. *Food Chemistry*, *130*(2), 383–393.

Shahidi, F., & Yeo, J.-D. (2016). Insoluble-Bound Phenolics in Food. *Molecules*, *21*(9), 1216.

Sijia, W., Enting, W., & Yuan, Y. (2014). Detection of furan levels in select Chinese foods by solid phase microextraction-gas chromatography/mass spectrometry method and dietary exposure estimation of furan in the Chinese population. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *64*, 34–40.

Simin, N., Orcic, D., Cetojevic-Simin, D., Mimica-Dukic, N., Anackov, G., Beara, I., Mitic-Culafic, D., & Bozin, B. (2013). Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of small yellow onion (*Allium flavum* L. subsp. flavum, Alliaceae). *LWT - Food Science and Technology*, *54*(1), 139–146.

Soriguer, F., Rojo-Martínez, G., Goday, A., Bosch-Comas, A., Bordiú, E., Caballero-Díaz, F., Calle-Pascual, A., Carmena, R., Casamitjana, R., Castaño, L., Castell, C., Catalá, M., Delgado, E., Franch, J., Gaztambide, S., Girbés, J., Gomis, R., Gutiérrez, G., López-Alba, A., Teresa Martínez-Larrad, M., Menéndez, E., Mora-Peces, I., Ortega, E., Pascual-Manich, G., Serrano-Rios, M., Urrutia, I., Valdés, S., Antonio Vázquez, J., & Vendrell, J. (2013). Olive oil has a beneficial effect on impaired glucose regulation and other cardiometabolic risk factors. Di@bet.es study. *European Journal of Clinical Nutrition*, *67*(9), 911–6.

Tomás-Barberán, F., García-Villalba, R., Quartieri, A., Raimondi, S., Amaretti, A., Leonardi, A., & Rossi, M. (2014). In vitro transformation of chlorogenic acid by human gut microbiota. *Molecular Nutrition & Food Research*, *58*(5), 1122–31.

Tsuzuki, W., Matsuoka, A., & Ushida, K. (2010). Formation of trans fatty acids in edible oils during the frying and heating process. *Food Chemistry*, *123*(4), 976–982.

US FDA (United States Food and Drug Administration). (2004). Exploratory Data on Furan in Food. URL https://www.fda.gov/ohrms/dockets/ac/04/briefing/4045b2_09_furan data.pdf. Access 25.04.2017

Van Lancker, F., Adams, A., Owczarek, A., De Meulenaer, B., & De Kimpe, N. (2009). Impact of various food ingredients on the retention of furan in foods. *Molecular Nutrition & Food Research*, *53*(12), 1505–11.

Verzelloni, E., Pellacani, C., Tagliazucchi, D., Tagliaferri, S., Calani, L., Costa, L. G., Brighenti, F., Borges, G., Crozier, A., Conte, A., & Del Rio, D. (2011). Antiglycative and neuroprotective activity of colon-derived polyphenol catabolites. *Molecular Nutrition & Food Research*, *55 Suppl 1*(SUPPL. 1), S35-43.

Walle, T. (2004). Absorption and metabolism of flavonoids. *Free Radical Biology and Medicine*, *36*(7), 829–837.

WHO (World health organization). (2011). *Safety evaluation if certain contaminants in food*. *WHO Food Additive Series* (Vol. 62).

Willett, W. C., Stampfer, M. J., Manson, J. E., Colditz, G. A., Speizer, F. E., Rosner, B. A., Sampson, L. A., & Hennekens, C. H. (1993). Intake of trans fatty acids and risk of coronary heart disease among women. *Lancet 341*(8845), 581–5.

Wolff, R. L. (1994). Cis-Trans isomerization of octadecatrienoic acids during heating - study of pinolenic (Cis-5,cis-9,cis-12-18/3) acid geometrical-isomers in heated pine seed oil. *Journal of the American Oil Chemists Society*, *71*(10), 1129–1134.

Yeo, J., & Shahidi, F. (2017). Effect of hydrothermal processing on changes of insoluble-bound phenolics of lentils. *Journal of Functional Foods*, *In Press*.

Zoller, O., Sager, F., & Reinhard, H. (2007). Furan in food: headspace method and product survey. *Food Additives and Contaminants*, *24 Suppl 1*, 91–107.

# CONCLUSIONS / CONCLUSIONES

1. In addition to the decrease in water, and the changes in fatty acid profile when fat is added, heat treatments (frying and griddling) tend to increase the total (poly)phenolic compounds in selected vegetables (yellow onion (*Allium cepa*), Italian green pepper (*Capsicum annuum*) and cardoon stalks (*Cynara. cardunculus* L.)), and consequently their antioxidant capacity, since DPPH and (poly)phenols are correlated ($r = 0.70$, $p < 0.001$).

2. Heat treatments, especially griddling, favor the release of (poly)phenolic compounds increasing or at least preserving the main (poly)phenols: quercetin glucosides in yellow onion, quercetin and luteolin derivates in green pepper and chlorogenic and other phenolic acids in cardoon.

3. Although bound (poly)phenolic compounds decrease higher than the free ones during the heat treatments, they are less affected by griddling (19-22%) than by frying process (35-80%), probably due to a higher formation of melanoidins that could include or retain (poly)phenolic compounds into their structures at higher temperature (150°C vs 115°C).

4. Heat treatments exert a positive effect on the bioaccessibility (%) of (poly)phenolic compounds in both green pepper (>82% in cooked pepper vs 48% in raw one) and cardoon (60-67% in cooked cardoon vs 2% in raw one). Therefore, non-flavonoids compounds and specifically chlorogenic acids (in cardoon) seem to be more prone to be degraded by gastrointestinal digestion than flavonoids (in green pepper).

5. After *in vitro* gastrointestinal digestion, both griddled green pepper and griddled cardoon still maintain the highest amount of (poly)phenolic compounds (9.447 and 41.853 μmol (poly)phenolic compounds/g dm, respectively).

6. Gut microbiota exert a high metabolic activity resulting in a large and rapid (<5h) degradation of quercetin and luteolin derivatives into their corresponding aglycones, as well as caffeoylquinic acids derivatives into caffeic acid. The highest bioaccessibility of (poly)phenolic compounds and catabolites after fecal fermentation is again found in both griddled green pepper and cardoon.

7. Catabolic pathways are proposed based on the different catabolites formed during fecal fermentation of green pepper and cardoon: dihydrocaffeic acid, protocatechuic acid and 3-(3'-hydroxyphenil)propionic acid, as well as dihydrocaffeoylquinic acid, which is only detected in cooked cardoon samples due to the high amount of CQAs. 3-(3'-hydroxyphenyl)propionic acid is by far the most abundant catabolite in both raw and cooked green pepper and cardoon after 24 h of fecal incubation.

8. Frying process does not result in the formation of furan in vegetables, while low amount (3.5 μg $kg^{-1}$ in griddled onion) or traces of this compound are formed in vegetables after griddling. Therefore, cooked vegetables do not represent a high exposure to furan. In contrast, deep-frying process induces the formation of considerable amounts of furan in bread-coated foods (from 12 μg $kg^{-1}$ for tuna pasties to 172 μg $kg^{-1}$for onion rings), with

a furan increase tendency when reheated oil is used, implying a health risk in Spanish population groups with a high consumption of these frozen precooked products.

9. A total mean dietary exposure of furan of 239 ng/kg bw/day is estimated for Spanish population, resulting in MOEs below 10,000 in all scenarios, which indicate a human health concern for furan.

In summary, although low amount of furan is formed, griddling process can be suggested as the most appropriated cooking technique applied to vegetables to maintain both native (poly)phenolic compounds and catabolites bioaccesible to be absorbed in the gut, or even to have a positive influence on the intestinal microbiota. In order to increase the variety of culinary techniques, when frying process is chosen, olive oil should be the preferable cooking oil because the high presence of MUFA, especially oleic acid, the higher ω-3 fatty acids and the lower amount of trans fatty acids.

1. Además de la disminución del contenido en agua, y de los cambios en el perfil de ácidos grasos cuando se emplea aceite, los tratamientos térmicos (fritura y plancha) tienden a aumentar los compuestos (poli)fenólicos en los vegetales seleccionados (cebolla (*Allium cepa*), pimiento verde (*Capsicum annuum*) y cardo (*Cynara. cardunculus* L.), y por lo tanto su capacidad antioxidante, ya que el DPPH y los (poli)fenoles están correlacionados ($r=0,70$, $p < 0,001$).

2. Los tratamientos térmicos, especialmente la plancha, favorecen la liberación de los compuestos (poli)fenólicos, incrementando o al menos manteniendo los principales (poli)fenoles: glucósidos de quercetina en la cebolla, derivados de quercetina y luteolina en el pimiento verde y ácidos clorogénicos y otros ácidos fenólicos en el cardo.

3. Aunque los compuestos (poli)fenólicos unidos disminuyen más que los libres durante los tratamientos térmicos, se ven menos afectados cuando se aplica un tratamiento a la plancha (19-22%) que cuando se aplica la fritura (35-80%), debido probablemente a una mayor formación de melanoidinas que podrían incluir o retener compuestos (poli)fenólicos en sus estructuras (150°C vs 115°C).

4. Los tratamientos térmicos ejercen un efecto positivo en la bioaccesibilidad (%) de los compuestos (poli)fenólicos tanto en el pimiento verde (>82% en pimiento cocinado frente al 48% en el crudo) como en el cardo (60-67% en cardo cocinado frente al 2% en el crudo). Por tanto, los compuestos no flavonoides y específicamente los ácidos clorogénicos (en cardo) parecen ser más propensos a ser degradados por la digestión gastrointestinal que los flavonoides (en el pimiento verde).

5. Después de la digestión gastrointestinal *in vitro*, tanto el pimiento verde como el cardo cocinados a la plancha continúan manteniendo la mayor cantidad de compuestos (poli)fenólicos (9,447 y 41,853 µmol (poli)fenólicos / g ss, respectivamente).

6. La microbiota intestinal presenta una alta actividad metabólica originando una intensa y rápida degradación (<5h) de los derivados de quercetina y luteolina en sus correspondientes agliconas, así como de los derivados de los ácidos cafeoilquínicos a ácido cafeico. La mayor bioaccesibilidad de compuestos (poli)fenólicos y catabolitos después de la fermentación fecal se presenta nuevamente tanto en el pimiento verde como en el cardo cocinados a la plancha.

7. Se han propuesto diversas rutas catabólicas basadas en los diferentes catabolitos formados durante la fermentación fecal del pimiento verde y cardo: ácido dihidrocafeico, ácido protocatecuico y ácido 3-(3'-hidroxifenil)propiónico, así como ácido dihidrocafeoilquínico -sólo detectado en las muestras de cardo cocinado por la gran cantidad de CQAs que contienen-. El ácido 3-(3'-hidroxifenil)propiónico es, con diferencia, el catabolito más abundante después de 24 h de incubación fecal tanto en el pimiento verde como en el cardo crudos y cocinados.

8. El proceso de fritura no da lugar a la formación de furano en los vegetales seleccionados, mientras que en los vegetales a la plancha se forman pequeñas cantidades (3,5 μg $kg^{-1}$ en cebolla a la plancha) o trazas de este compuesto. Por lo tanto, los vegetales cocinados no representan una alta exposición al furano. Por el contrario, el proceso de fritura en profundidad induce la formación de cantidades considerables de furano en alimentos precocinados empanados (de 12 μg $kg^{-1}$ en las empanadillas de atún a 172 μg $kg^{-1}$ en los aros de cebolla), con una tendencia a aumentar cuando se emplea aceite reutilizado. Todo esto implica un riesgo para la salud en grupos de población españoles con un elevado consumo de estos productos precocinados empanados.

9. Se estima una exposición media total de furano de 239 ng/kg pc/día en la población española, lo cual se traduce en un MOE inferior a 10.000 en todos los escenarios, lo que indica que el furano presenta un riesgo para la salud humana.

En resumen, aunque se forme una cantidad baja de furano, se sugiere el proceso de cocinado a la plancha como la técnica de cocción más apropiada aplicada a los vegetales para mantener tanto los compuestos (poli)fenólicos nativos como los catabolitos bioaccesibles para ser absorbidos en el intestino o al menos para ejercer una influencia positiva en la microbiota intestinal. Con el fin de aumentar la variedad de técnicas culinarias, cuando se elige el proceso de fritura, el aceite de oliva debe ser el aceite elegido debido a la alta presencia de ácidos grasos monoinsaturados, especialmente ácido oleico, una mayor cantidad de ácidos grasos ω-3 y un menor contenido de ácidos grasos trans.

# RESEARCH DISSEMINATION

**Publications**

**I.Juániz**, I.A. Ludwig, E. Huarte, G.Pereira-Caro, J.M. Moreno-Rojas C. Cid, M.P. de Peña. (2016). Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables. *Food Chemistry*, 197, 466-473. (Q1) (D1)

**I. Juániz**, C. Zocco, V. Mouro, C. Cid, M.P. de Peña. (2016). Effect of frying process on furan content in foods and assessment of furan exposure of Spanish population. *LWT-Food Science and Technology*, 68, 549-555. (Q1)

**I. Juániz**, I.A. Ludwig, M. Dall'Asta, L. Bresciani, P. Mena, D. Del Rio, C. Cid, M.P. de Peña (2016). Catabolism of raw and cooked green pepper (*Capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and faecal fermentation. *Journal of Functional Foods*, 27, 201-213. (Q1) (D1)

**I.Juániz**, I.A. Ludwig, M. Dall'Asta, L. Bresciani, P. Mena, D. Del Rio, C. Cid, M.P. de Peña. (2017). Bioaccessibility of (poly)phenolic compounds of raw and cooked cardoon (*Cynara cardunculus* L.) after simulated gastrointestinal digestion and fermentation by human colonic microbiota. *Journal of Functional Foods*, 32, 195-207. (Q1) (D1)

**I.Juániz**, E. Huarte, C.B. de Gorostiza, C. Cid, M.P. de Peña. Impact of heat treatment on the composition and antioxidant capacity of selected vegetables. In preparation.

**Conferences communications**

**I. Juániz**, M.P. De Peña, C. Cid. (2014). Poster: "Influence of frying on the formation of furan in Spanish precooked breaded foods". In 28th EFFoST Conference. Innovations in Attractive and Sustainable Food for Health. Uppsala, Sweden. Delegate abstract book P1.029. (See annex).

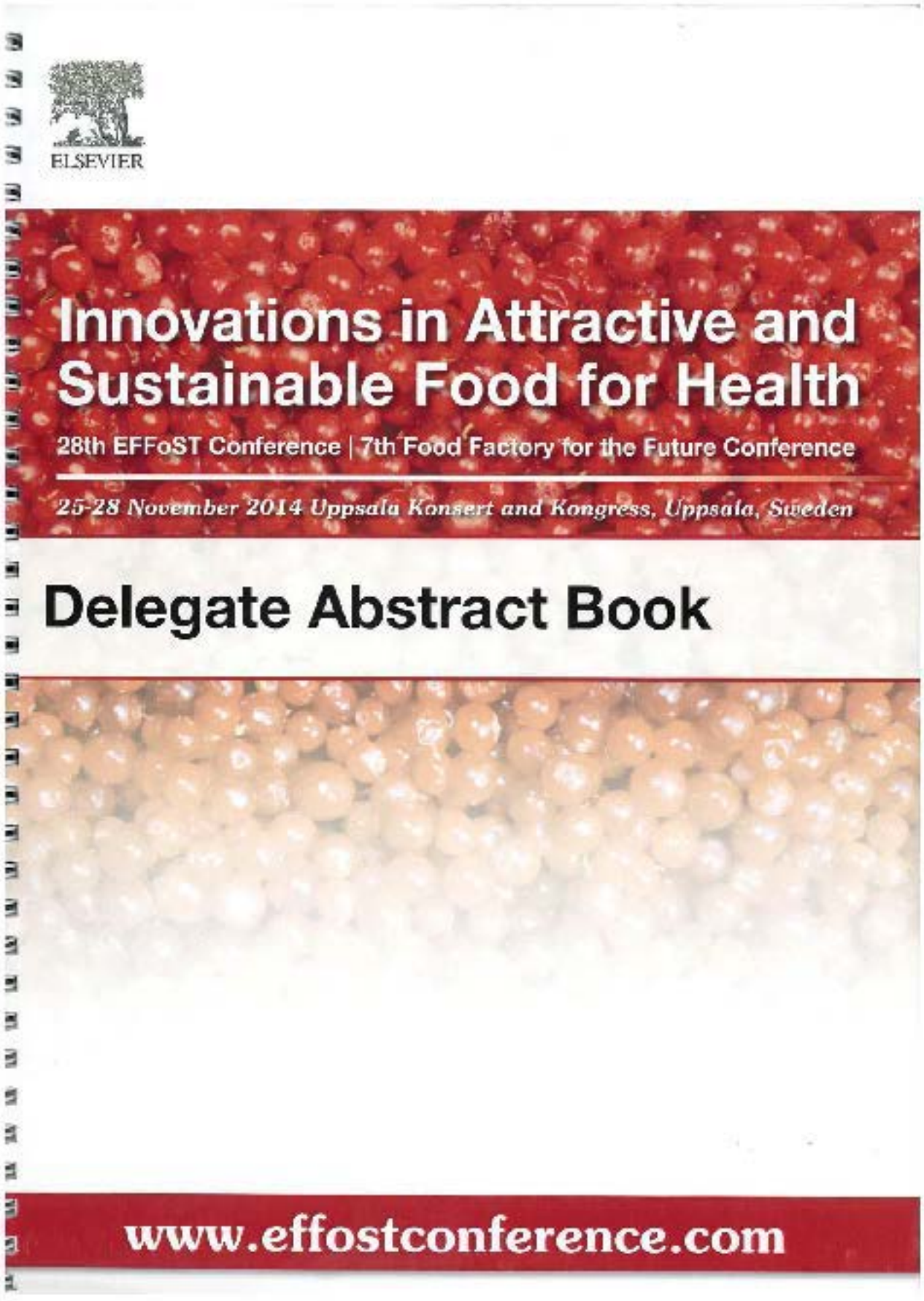
**I. Juániz**, M.P. De Peña, C. Cid. (2014). Poster: "Influence of heat treatment (frying and grilling) on antioxidants of some common consumed vegetables (onion and green pepper)". In 28th EFFoST Conference. Innovations in Attractive and Sustainable Food for Health. Uppsala, Sweden. Delegate abstract book P1.030. (See annex).

**I. Juániz**, I.A. Ludwig, G. Pereira-Caro, J.M. Moreno-Rojas, C. Cid, M.P. de Peña. (2015). Poster: "Heat treatment increase polyphenols levels in onion". In Future Trends in phytochemistry in the global era of agri-food and health II. San Pedro del Pinatar, Murcia, Spain. Book of abstracts 2015; pages 138-139. ISBN: 978-0-9565472-6-2. (See annex).

**I. Juániz**, I.A. Ludwig, G. Pereira-Caro, J.M. Moreno-Rojas, C. Cid, M.P. de Peña. (2015). Poster: "Culinary heat treatments increase polyphenols in selected vegetables of Mediterranean diet". In 7th International Conference on Polyphenols and Health. Tours, France. Book of Abstracts 2015; pages 264. (See annex).

**I. Juániz**, M. Dall'Asta, L. Bresciani, P. Mena, D. Del Rio, C. Cid, M.P. de Peña. (2015). Poster: "Effect of a simulated gastrointestinal digestion on the (poly)phenolic fraction of raw and cooked vegetables (green pepper and cardoon)". In 7th International Conference on Polyphenols and Health. Tours, France. Book of Abstracts 2015; pages 270-271. (See annex).

# ANNEX


ELSEVIER
Innovations in Attractive and Sustainable Food for Health
28th EFFoST Conference | 7th Food Factory for the Future Conference
25-28 November 2014 Uppsala Konsert and Kongress, Uppsala, Sweden
Delegate Abstract Book
www.effostconference.com

**[P1.029]**

**Influence of frying on the formation of furan in Spanish precooked breaded foods**

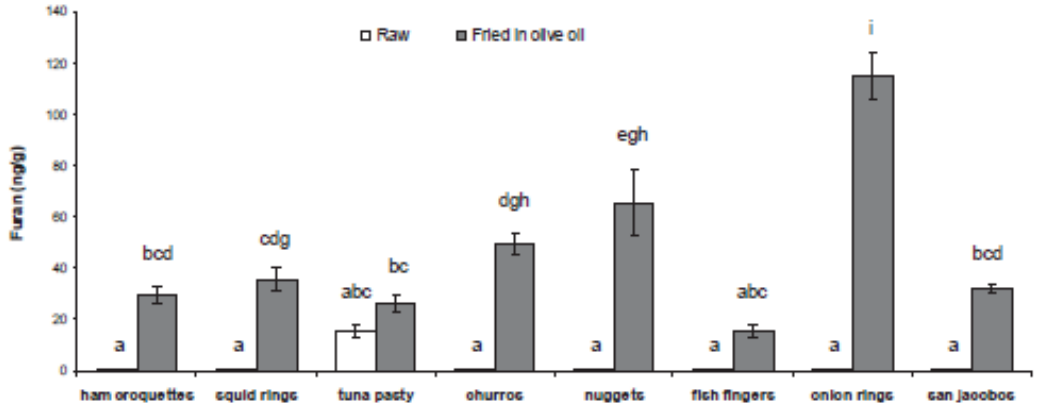
I. Juániz*, M.P. De Peña, C. Cid
*University of Navarra, Spain*

In the last years, consumption of precooked foods that require frying before eating has increased. Frying process produces changes in foods including the formation of volatile compounds such as furan which is potentially carcinogenic. The presence of carbohydrates also promotes the formation of furan that can be retained in lipid fraction. Furthermore, linoleic acid is one of the main precursors of the furan formation. Then, typical Spanish breaded foods commonly fried are a good target to study the furan content.

Eight frozen breaded foods acquired in local supermarkets were deep fried in olive oil (190ºC, 6min) and furan content was immediately measured by GC-MS. Also, total fat, oleic and linoleic acids content were evaluated to study the influence of lipid fraction and potential precursors.

In raw foods, furan was only found in tuna pasty (15.66ng/g) probably due to its filling previously cooked. After frying, furan levels increase significantly. This increment is not the same for all the foods, maybe due to differences in their food composition. Onion rings (114.87ng/g), followed by nuggets (65.57ng/g) and churros (49.60ng/g), were those with high amounts of furan.

Figure 1 Furan (ng/g food) in Spanish precooked breaded foods both raw and fried in olive oil.




Different letters indicate significant differences ($p \leq 0.05$)

Total fat content was significantly higher in fried foods (13.4-29.3 g/100g). A significantly increase in oleic acid was observed in all fried samples, except in san jacobos due to the use of olive oil. However, linoleic acid content is low in all samples. In fact, no significant correlations have been found between furan and these fatty acids.

In summary, daily consumption of fried precooked foods should be limited in diet because of both the content of furan and total fat. However, because furan is a volatile compound, further studies about the losses before consumption at the adequate temperature should be needed.

This research was funded by the University of Navarra (PIUNA) and its Association of Friends.

Keywords: furan, olive oil, frozen foods, frying

# INFLUENCE OF FRYING ON THE FORMATION OF FURAN IN SPANISH PRECOOKED BREADED FOODS

I. Juániz, M.P. De Peña *, C. Cid
Department of Nutrition, Food Science and Physiology, Faculty of Pharmacy.
University of Navarra, Spain. * mpdepena@unav.es

## INTRODUCTION

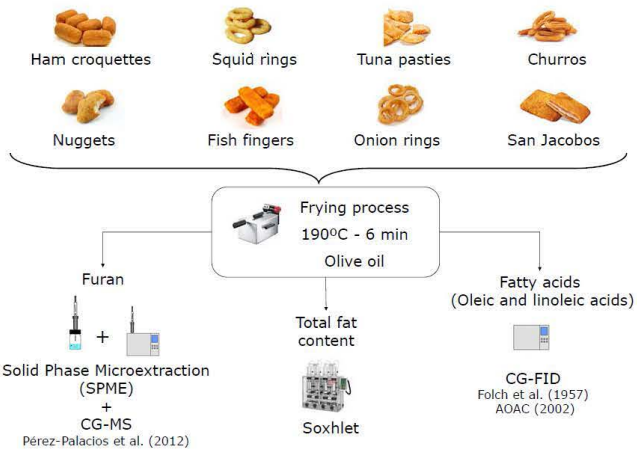
In the last few years, consumption of precooked foods that require frying before eating has increased. Frying process induces changes in foods including the formation of volatile compounds such as furan which has been classified as a possible human carcinogen by the International Agency for Research on Cancer (IARC, 1995)

Furan levels in foods can be influenced by several factors, such as heat treatment conditions and food composition. Carbohydrate foods are more prone to the formation of furan, that can be retained in lipid fraction. Furthermore, linoleic acid is one of the main precursors of the furan formation. Then, typical Spanish breaded foods commonly fried are a good target to study the furan content.

## OBJECTIVE

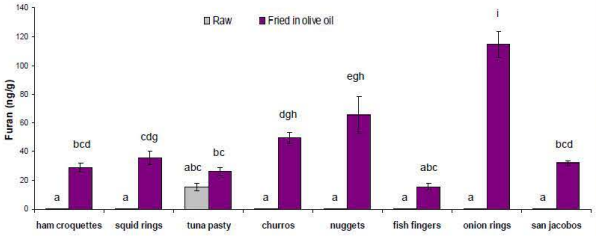
The main aim of this work was to assess the formation of furan in Spanish precooked breaded foods after a frying process, as well as to calculate Spanish population furan exposition due to the consumption of these products. Additionally, the influence of lipid fraction on furan formation and fat intake have been evaluated.

## MATERIAL AND METHODS




## RESULTS AND DISCUSSION

**Figure 1** Furan (ng/g food) in Spanish precooked breaded foods both raw and fried in olive oil.




Different letters indicate significant differences ($p \leq 0.05$)
Values are expressed as mean ± SD

In raw foods, furan was only found in tuna pasties (15.66ng/g) probably due to their filling (tuna in tomato sauce) previously cooked.

After frying, furan levels increase significantly. This increment is not the same for all the foods, maybe due to differences in their food composition. Onion rings (114.87ng/g), followed by nuggets (65.57ng/g) and churros (49.60ng/g), were those with high amounts of furan.

**Table 1.** Total fat content and fatty acids profile (oleic acid and linoleic acid) of precooked breaded foods before and after fried treatment with olive oil.

| Food | total fat g/100g food | fatty acid g/ 100 g food: oleic acid | fatty acid g/ 100 g food: linoleic acid |
|---|---|---|---|
| Ham croquettes | | | |
| Raw | 5.70 ± 0.00 [a] | 1.57 ± 0.01 [a] | 1.98 ± 0.00 [a] |
| Fried olive oil | 19.32 ± 1.54 [b] | 7.02 ± 0.01 [b] | 2.03 ± 0.00 [b] |
| Squid rings | | | |
| Raw | 11.20 ± 0.00 [a] | 2.87 ± 0.02 [a] | 5.69 ± 0.01 [b] |
| Fried olive oil | 23.93 ± 0.06 [b] | 14.31 ± 0.12 [b] | 1.79 ± 0.01 [a] |
| Tuna pasties | | | |
| Raw | 11.30 ± 0.00 [a] | 3.07 ± 0.00 [a] | 1.56 ± 0.00 [a] |
| Fried olive oil | 29.29 ± 0.58 [b] | 10.97 ± 0.01 [b] | 2.26 ± 0.01 [b] |
| Churros | | | |
| Raw | 0.70 ± 0.00 [a] | 0.09 ± 0.00 [a] | 0.31 ± 0.00 [a] |
| Fried olive oil | 13.48 ± 0.85 [b] | 11.84 ± 0.03 [b] | 1.33 ± 0.00 [b] |
| Nuggets | | | |
| Raw | 10.80 ± 0.00 [a] | 3.49 ± 0.03 [a] | 0.40 ± 0.00 [a] |
| Fried olive oil | 23.86 ± 0.00 [b] | 11.17 ± 0.06 [b] | 1.21 ± 0.00 [b] |
| Fish fingers | | | |
| Raw | 6.60 ± 0.00 [a] | 1.31 ± 0.00 [a] | 2.39 ± 0.00 [b] |
| Fried olive oil | 13.38 ± 0.86 [b] | 4.65 ± 0.00 [b] | 0.52 ± 0.00 [a] |
| Onion rings | | | |
| Raw | 12.00 ± 0.00 [a] | 1.88 ± 0.06 [a] | 3.38 ± 0.04 [b] |
| Fried olive oil | 26.34 ± 0.88 [b] | 8.88 ± 0.05 [b] | 1.15 ± 0.01 [a] |
| San jacobos | | | |
| Raw | 16.30 ± 0.00 [a] | 9.46 ± 0.04 [b] | 1.15 ± 0.01 [b] |
| Fried olive oil | 20.33 ± 0.19 [a] | 6.11 ± 0.01 [a] | 0.64 ± 0.00 [a] |

Different letters for each column and each food indicate significant differences. ($p \leq 0.05$)
Values are expressed as mean ± SD

**Table 2.** Mean food consumption, furan levels, total fat content and exposure of furan (ng/capita/day) and fat (g/capita/day) per different foods frying in the Spanish population

| Food | Average consumption[a] (g/capita/day) | Furan min - max (ng/g) | Range of furan intake min - max (ng/capita/day) | Total fat (g/100g) | Fat intake (g/capita/day) |
|---|---|---|---|---|---|
| Olive oil | 19.80 | 2.49 – 16.91[c] | 49.30 – 334.18 | 100 | 19.80 |
| Ham croquettes | 0.74 | 29.24 | 21.63 | 19.32 | 0.14 |
| Squid rings | 0.62[b] | 35.47 | 21.99 | 23.93 | 0.15 |
| Tuna pasties | 0.59 | 26.24 | 15.48 | 29.29 | 0.17 |
| Churros | 1.32 | 49.60 | 65.47 | 13.48 | 0.18 |
| Nuggets | 0.33 | 65.57 | 21.64 | 23.86 | 0.07 |
| Fish fingers | 0.11 | 15.70 | 1.72 | 13.38 | 0.01 |
| Onion rings | 0.62[b] | 114.87 | 71.22 | 26.34 | 0.16 |
| San Jacobos | 0.62[b] | 32.19 | 19.95 | 20.33 | 0.12 |
| **Total** | | | **288.84 – 573.28** | | **20.8** |

a, mean food consumption in the Spanish population as recorded by the Ministry of Agriculture, Food and Environment. ENIDE, 2011.
b, Mean value of fried foods is taken because data are not available.
c, the range of values corresponds to crude oil and fried oil

Olive oil is one of the main foods which could contribute to furan intake due to its high consumption in Spain. However, it is usually consumed as crude oil, mainly in salads, which present the lowest furan content (2.49 ng/g). The highest furan intake are due to onion rings (71.22 ng/capita/day) and churros (65.47 ng/capita/day). Nevertheless, furan intake from analysed foods represents less than the 0.5% of the acceptable daily intake (ADI) of 2 mg/kg bw/day (Kuballa et al, 2005).

Fat intake from foods analysed is also high (20.8g/capita/day) and its consumption should be moderate to prevent overweight, obesity and associated diseases.

Total fat content was significantly higher in fried foods with an increment between 4 and 18 g/100g.

A significantly increase in oleic acid was observed in all fried samples due to the use of olive oil, except in san jacobos. Linoleic acid tends to decrease in those fried foods with higher content in raw samples, maybe due to losses in frying oil and thermal degradation.

No significant correlations between furan and oleic and linoleic acids have been found.

## REFERENCES

IARC International Agency for Research on Cancer. 1995
Perez-Palacios et al. (2012). Food Chem, 135(3):1337-1343.
Folch et al. (1957). J. Biol Chem, 226:497-509.
AOAC. (2002). Official methods of analysis 19-20.
Kuballa et al. (2005). Deut Lebensm-Rundsch, 101(6):229-235.
Ministry of Agriculture, Food and Environment. ENIDE, 2011.

## ACKNOWLEDGEMENTS

We thank the PIUNA (Plan de Investigación de la Universidad de Navarra) for their contribution to the financial support of this work. I.Juániz is grateful to "Asociación de Amigos de la Universidad de Navarra" for the grant received.

## CONCLUSIONS

Frying process induces the formation of furan in precooked breaded foods and increases total fat content.

Although furan intake due to breaded fried foods in Spanish population in less than 0.5% ADI, daily consumption should be limited in diet because of both the content of furan and total fat. However, because furan is a volatile compound, further studies about the losses before consumption at the adequate temperature should be needed.

[P1.030]

**Influence of heat treatment (frying and grilling) on antioxidants of some common consumed vegetables (onion and green pepper)**

I. Juániz*, M.P. De Peña, C. Cid
*University of Navarra, Spain*

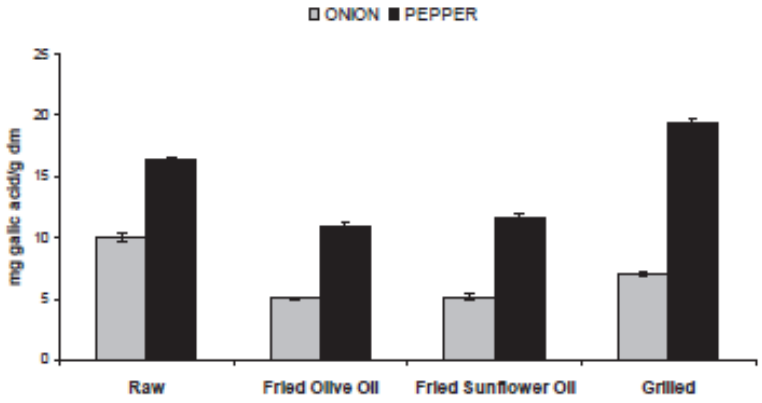
The World Health Organization promotes higher consumption of vegetables, rich in antioxidant compounds, to prevent oxidative stress related diseases. Then, the antioxidant capacity could be influenced by several factors, such as culinary processing. The aim of this work was to study the impact of cooking heat treatments on the antioxidant capacity and bioactive compounds of onions and green peppers, commonly consumed as crude in salads and cooked in several ways in mediterranean diet.

Ethanolic (80%) extracts were prepared from raw and cooked vegetables (grilled, fried in olive and sunflower oils), and antioxidant capacity was measured by DPPH and ABTS assays. Total phenolic and flavonoid compounds were also determined. The results (in dry matter) showed that green peppers have more antioxidant capacity than onions, probably due to the presence of higher amounts of phenolic compounds (figure 1) including flavonoids. Frying process significantly decreased total phenolic compounds inducing a decrease in antioxidant capacity (ABTS). However, antioxidant capacity was hardly affected when it was measured by DPPH, especially in onions maybe because in this case, total flavonoids increased. On the other hand, grilling significantly decreased total phenolic compounds in onions but flavonoids increased sugesting that non-flavonoids phenolic compounds were those mainly degraded. In green peppers an increase of total phenolic compounds and antioxidant capacity was observed, maybe due to the release of those phenolics bound to cell structures damaged during heating. However in terms of consumption (per 100g of fresh vegetable) cooked vegetables both fried and grilled had higher levels of phenolic compounds, including flavonoids, and consequently antioxidant capacity.

In conclusion, green peppers are richer in phenolics than onions, but both can be used as a good source of antioxidants independently that they are consumed raw or cooked.

This research was funded by the University of Navarra (PIUNA) and its Association of Friends.

Figure1. Total Phenolic Compounds of vegetables both raw and after heat treatment (fried in olive and sunflower oils and grilled)




Different letters in each graphic indicate significant differences ($p \leq 0.05$)

Keywords: Phenolics, Antioxidant capacity, Heating, Vegetables

# INFLUENCE OF HEAT TREATMENT (FRYING AND GRILLING) ON ANTIOXIDANTS OF SOME COMMON CONSUMED VEGETABLES (ONION AND GREEN PEPPER)

Universidad de Navarra

I. Juániz, M.P. De Peña *, C. Cid
Department of Nutrition, Food Science and Physiology, Faculty of Pharmacy.
University of Navarra, Spain. * mpdepena@unav.es

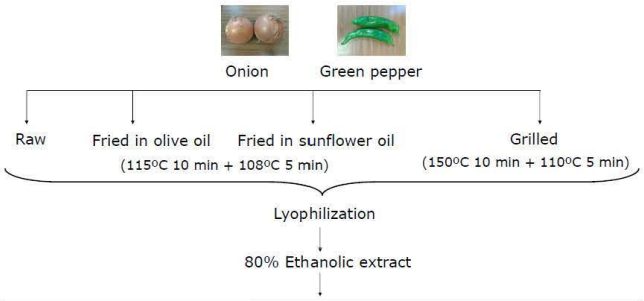
## INTRODUCTION

The World Health Organization and other international agencies urge higher consumption of plant foods to prevent oxidative stress related diseases, such as cancer, diabetes and cardiovascular diseases (OMS, 2004). Dietary antioxidant compounds can be intaked by traditional vegetables and fortified functional foods allowing increase of the diet variety. Bioactivity of these components, in both cases, is influenced by several factors related to their original chemical nature, interaction with other compounds mainly lipids, technological and culinary processing among others.

## OBJECTIVE

The main objective of this work was to evaluate the impact of cooking heat treatments on the antioxidant capacity and bioactive compounds of onions and green peppers, which are commonly consumed as crude in salads and cooked in several ways in mediterranean diet.

## MATERIAL AND METHODS

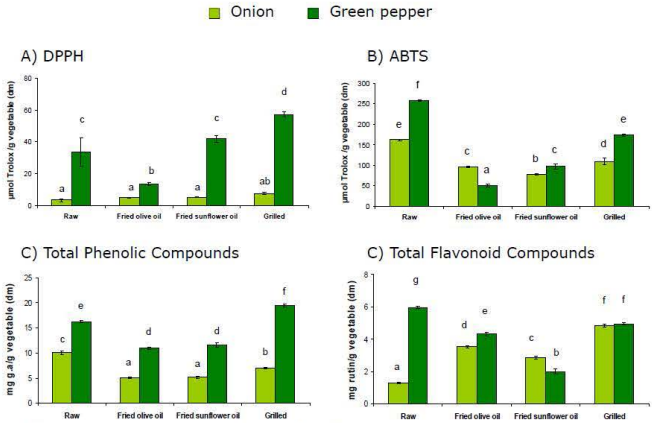



| Antioxidant capacity | Bioactive compounds |
|---|---|
| DPPH (Brand-Williams et al., 1995)<br>ABTS (Re et al., 1999) | Total Phenolic Compounds (Singleton & Rossi, 1995)<br>Total Flavonoid Compounds (Chang et al., 2007) |

## RESULTS AND DISCUSSION

### Impact of heat treatment (per gram of dry matter)

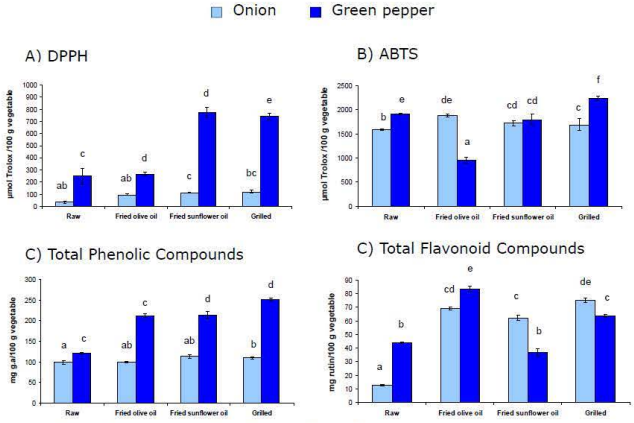
**Figure 1.** Antioxidant Capacity (DPPH and ABTS), Total Phenolic Compounds and Total Flavonoid Compounds of onion and green pepper, both raw and after heat treatment (fried in olive and sunflower oils, and grilled).




Different letters in each graphic indicate significant differences ($p \leq 0.05$)

### Antioxidant in vegetables (per 100g)

**Figure 2.** Antioxidant Capacity (DPPH and ABTS), Total Phenolic Compounds and Total Flavonoid Compounds of onion and green pepper, both raw and after heat treatment (fried in olive and sunflower oils and grilled).




Different letters in each graphic indicate significant differences ($p \leq 0.05$)

**FRYING PROCESS**

Frying process significantly decreased total phenolic compounds inducing a decrease in antioxidant capacity (ABTS). However, antioxidant capacity was hardly affected when it was measured by DPPH, especially in onions, maybe due to total flavonoids increase.

**GRILLED PROCESS**

Grilled process significantly decreased total phenolic compounds in onions but flavonoids increased suggesting that non-flavonoids phenolic compounds were those mainly degraded.

In green peppers increases of total phenolic compounds and antioxidant capacity were observed, maybe due to the release of those phenolics bound to cell structures during heating.

**In terms of consumption (per 100g of fresh vegetable)**

Green peppers both raw and cooked (120.41 –251.36 mg galic acid/100g) show higher levels of total phenolic compounds than onion (98.576 – 113.53 mg galic acid/100g). Antioxidant capacity measured by DPPH was also higher in green pepper.

Globally, cooked vegetables, onion and green pepper, both fried and grilled, had higher levels of phenolic compounds, including flavonoids, and consequently antioxidant capacity than raw vegetables.

## REFERENCES

WHO. World Health Organization (2004).
Singleton & Rosi. (1965). Am J Enol Viticult; 16,144-158.
Chang et al. (2007). Bot Stud; 48, 397-406
Re et al. (1999). Free Radical Bio Med; 26, 1231-1237
Brand-Willians. (1995). Lebensmittel-Wissenschaft und Technologie; 28, 25-30

## ACKNOWLEDGEMENTS

We thank the PIUNA (Plan de Investigación de la Universidad de Navarra) for their contribution to the financial support of this work. I.Juániz is grateful to "Asociación de Amigos de la Universidad de Navarra" for the grant received.

## CONCLUSIONS

Green peppers are richer in phenolics than onions, but both can be used as a good source of antioxidants independently that they are raw or cooked consumed.

In terms of consumption (per 100g of fresh vegetable), cooked vegetables both fried and grilled had higher levels of phenolic compounds, including flavonoids, and consequently antioxidant capacity.

Future Trends in Phytochmistry in the Global Era of

# Agri-Food and Health II

**- A Young Scientists Meeting -**

Bioactive molecules

THE PHYTOCHEMICAL SOCIETY OF EUROPE

27–30 April 2015
San Pedro del Pinatar, Murcia, Spain

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

# Book of Abstracts

**Phytochemical Society of Europe (PSE) and**
**Consejo Superior de Investigaciones Científicas (CSIC)**

## Future Trends in Phytochemistry in the global era of agri-food and health. II: A Young Scientists Meeting

**27–30 April 2015**
**San Pedro del Pinatar, Murcia, Spain**

# Future Trends in Phytochemistry in the Global Era of Agri-Food and Health.II

**First Edition, 2015**



**© Editors:**
Nieves Baenas, Javier Marhuenda, Diego A. Moreno, Cristina García-Viguera

Cover design and artwork by Raúl Dominguez-Perles.

**I.S.B.N.: 978-0-9565472-6-2**

**Printed by:** LIMENCOP S.L., Alicante, Spain

Este trabajo es resultado de la ayuda 19588/OC/14 financiada por la fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia con cargo al Programa "Jiménez de la Espada" de Movilidad, Cooperación e Internacionalidad.

**Contact**:
Cristina García-Viguera
CEBAS-CSIC
Food Sci. & Technol. Dept.
Campus de Espinardo 25, 30100 Espinardo, Murcia, Spain

Young Scientist Meeting
Future Trends in Phytochemistry in the Global Era of Agri-food and Health II

## Heat treatment increases polyphenol levels in onion

I.Juániz[a], I.A. Ludwig[b], G. Pereira-Caro[b], J.M. Moreno-Rojas[b], C. Cid[a], M.P. de Peña[a]

[a]Department of Nutrition, Food Science and Physiology, School of Pharmacy. University of Navarra, 31008, Pamplona, Spain. mpdepena@unav.es
[b]Department of Technology, Postharvest and Food Industry, IFAPA-Alameda del Obispo, 14004, Córdoba, Spain

Onion is one of the most consumed vegetables in Spain (23.3 g/capita/day). It is a rich source of phenolic compounds, specially quercetin and its glycosides. Polyphenol rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer. Because of this, The World Health Organization promotes higher consumption of vegetables. Usually dietary vegetables are eaten after cooking in diferent ways. This process produces significant changes in foods such as water loss, changes in the fatty acid profile, the development of chemical reactions induced by heat, and degradation of thermolabile compounds.

Thus, the aim of this work was to study the impact of different thermal treatments on the polyphenol content in onion, commonly consumed as crude in salads and cooked in several ways in Mediterranean diet.

Onion were cooked by three diferent domestic processes (griddled, fried in olive and sunflower oils). Raw onion and cooked samples were lyophylized and extracted with 80:20 ethanol:water. Ethanolic extracts were analyzed using a UPLC-PDA-HESI-MS to determine polyphenol compounds.

Total of eigth flavonoids were identified and quantified. All of them were glucosides of quercetin and isorhamnetin. However, aglycones of these two flavonoids were not detected. The most abundant polyphenols were three quercetin derivates (one quercetin glucoside and two quercetin diglucosides) which account approx. for 90% of total flavonoids in all samples.

Overall heat treatment resulted in increased polyphenol levels compared to raw onion. The increment was respectively 17.4%, 35.7% and 55.5% for onion fried in sunflower oil; onion fried in olive oil and griddled onion. These changes could be

caused by thermal destruction of cell walls and sub cellular compartments during the cooking process. However, further research is required to investigate if the increase in phenolic compounds, caused by heat treatment, also increases the bioavailability of these compounds after consumption.

We thank the PIUNA (Plan de Investigación de la Universidad de Navarra) for their contribution to the financial support of this work. I.Juániz is grateful to ''Asociación de Amigos de la Universidad de Navarra'' for the grant received.

# HEAT TREATMENT INCREASES POLYPHENOLS LEVELS IN ONION

I. Juániz[a], I.A. Ludwig[b], G. Pereira-Caro[b], J.M. Moreno-Rojas[b], C. Cid[a], M.P. De Peña[a]*
[a] Department of Nutrition, Food Science and Physiology, Faculty of Pharmacy. University of Navarra, Spain
[b] Department of Technology, Postharvest and Food Industry, IFAPA-Alameda del Obispo, 14004, Córdoba, Spain
* mpdepena@unav.es

## INTRODUCTION

Onion is one of the most consumed vegetables in Spain (23.3 g/capita/day) (ENIDE, 2011). It is a rich source of phenolic compounds, specially quercetin and its glycosides. Polyphenol rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer (del Rio et al., 2013). Because of this, the World Health Organization promotes higher consumption of vegetables. Usually dietary vegetables are eaten after cooking in different ways. This process produces significant changes in foods such as water loss, changes in the fatty acid profile, the development of chemical reactions induced by heat, and degradation of thermolabile compounds.

## OBJECTIVE

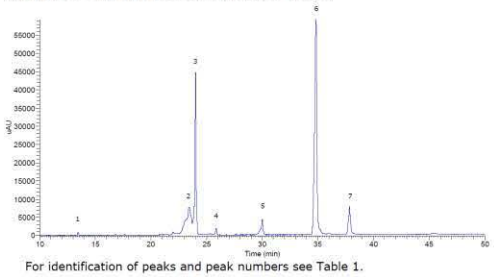
The aim of this work was to study the impact of different thermal treatments on the polyphenol content in onion, commonly consumed as crude in salads and cooked in several ways in Mediterranean diet.

## MATERIAL AND METHODS


## RESULTS AND DISCUSSION

A total of eight flavonoids were identified and quantified (Figure 1, Table 1). All of them were glucosides of quercetin and isorhamnetin. However, aglycones of these two flavonoids were not detected.

**Figure 1.** UPLC-PDA traces of raw onion. λ=360nm




For identification of peaks and peak numbers see Table 1.

**Table 1.** Identification of polyphenols by UPLC-MS

| Peak no. | Rt (min) | Compound | $[M-H]_{theor.}$ | $[M-H]_{exp.}$ (m/z) | δ (ppm) | Online fragment (m/z) |
|---|---|---|---|---|---|---|
| 1 | 13.46 | Quercetin triglucoside | 787.1927 | 787.1924 | -0.381 | 301.0342 |
| 2 | 23.44 | Quercetin diglucoside I | 625.1399 | 625.1405 | 0.959 | 301.0342 |
| 3 | 24.02 | Quercetin diglucoside II | 625.1399 | 625.1405 | 0.959 | 301.0342 |
| 4 | 25.87 | Isorhamnetin diglucoside | 639.1555 | 639.1560 | 0.782 | 315.0499 |
| 5 | 30.01 | Quercetin glucoside I | 463.0871 | 463.0874 | 0.648 | 301.0342 |
| 6 | 34.82 | Quercetin glucoside II | 463.0871 | 463.0874 | 0.648 | 301.0342 |
| 7 | 37.85 | Isorhamnetin glucoside | 477.1027 | 477.1026 | -0.209 | 315.0499 |

[M-H]-theor.: theoritical exact mass of negatively charged molecular ion; [M-H]-exp.: experimentally measured accurate mass of negatively charged molecular ion; δ: difference between [M-H]-theor and [M-H]-exp; online fragment: daughter ion produced from [M-H]- fragmentation.

**Table 2.** Quercetin and Isorhamnetin derivates in raw and cooked onion (fried in olive oil, fried in sunflower oil and griddle). Results are expressed as mean ± standard deviation (mg flavonoid/g onion dm).

| Compound | Raw Onion | Onion fried in olive oil | Onion fried in sunflower oil | Onion griddle |
|---|---|---|---|---|
| Quercetin triglucoside | $0.01 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ |
| Quercetin diglucoside I | $0.22 \pm 0.00^{a}$ | $0.30 \pm 0.01^{a}$ | $0.24 \pm 0.03^{a}$ | $0.35 \pm 0.06^{a}$ |
| Quercetin diglucoside II | $0.33 \pm 0.00^{a}$ | $0.41 \pm 0.01^{b}$ | $0.37 \pm 0.02^{ab}$ | $0.55 \pm 0.03^{c}$ |
| Quercetin diglucoside | $0.02 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ |
| Quercetin glucoside I | $0.04 \pm 0.00^{b}$ | $0.02 \pm 0.00^{a}$ | $0.02 \pm 0.00^{a}$ | $0.03 \pm 0.00^{ab}$ |
| Quercetin glucoside II | $0.64 \pm 0.01^{a}$ | $0.95 \pm 0.01^{c}$ | $0.83 \pm 0.00^{b}$ | $1.02 \pm 0.04^{c}$ |
| **Total Quercetin derivates** | **$1.26 \pm 0.01^{a}$** | **$1.71 \pm 0.03^{bc}$** | **$1.48 \pm 0.05^{ab}$** | **$1.96 \pm 0.12^{c}$** |
| Isorhamnetin diglucoside | $0.02 \pm 0.00^{a}$ | $0.12 \pm 0.02^{bc}$ | $0.09 \pm 0.02^{b}$ | $0.17 \pm 0.02^{c}$ |
| Isorhamnetin glucoside | $0.08 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ |
| **Total Isorhamnetin derivates** | **$0.10 \pm 0.01^{a}$** | **$0.13 \pm 0.02^{ab}$** | **$0.10 \pm 0.02^{a}$** | **$0.18 \pm 0.01^{b}$** |
| **Total flavonoids compounds** | **$1.36 \pm 0.00^{a}$** | **$1.83 \pm 0.05^{bc}$** | **$1.57 \pm 0.07^{ab}$** | **$2.14 \pm 0.14^{c}$** |

Different letters for each row indicate significant differences ($p \leq 0.05$) among onion samples

The most abundant flavonoids were three quercetin derivates (one quercetin glucoside and two quercetin diglucosides) which accounted approximately for 90% of total flavonoids in all samples. In contrast isorhamnetin derivates were minor flavonoids accounting for only 6 to 8% of total flavonoids (Table 2).

Overall an increase of polyphenols levels was observed in samples after heat treatment. The use of olive oil for frying resulted in a higher increase of flavonoids (35.7%) compared to sunflower oil (17.4%). However the griddled onion showed the highest amount of flavonoids with an increment of 55.5% compared to raw onion. The temperature applied during this treatment was higher than the temperature applied during frying processes, which might favor the release of these compounds from the food matrix.

In terms of consumption (per 100g of fresh vegetable), a significant increase of flavonoids content (60%) was also observed after heat treatment. In this case no differences were found between fried in olive oil (35.91mg flavonoids/100g onion), fried in sunflower oil (35.91mg flavonoids/100g onion), or griddled (34.57 mg flavonoids /100g onion).

## CONCLUSION

Heat treatment (frying or griddling) of onion induces significant increases in total quercetin and isorhamnetin derivates and, consequently, total flavonoids content suggesting thermal destruction of cell walls and sub cellular compartments during the cooking process. However, further research is required to investigate if the increase in phenolic compounds caused by heat treatment also increases the bioavailability of these compounds after consumption.

## REFERENCES

Ministry of Agriculture, Food and Environment. ENIDE, 2011.

Del Rio D et al. A. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic diseases. Antioxidants & Redox Signaling. 2013;18(14):1818-1892.

## AKNOWLEDGEMENT

We thank the PIUNA (Plan de Investigación de la Universidad de Navarra) for their contribution to the financial support of this work. I.Juániz is grateful to "Asociación de Amigos de la Universidad de Navarra" for the grant received.

# 7th ICPH International Conference on Polyphenols and Health

## October 27-30, 2015

Congress Center Tours, France

PARIS

TOURS

www.icph2015.com

INRA SCIENCE & IMPACT

Inserm

## P906

### Biological activity of Amazon oils and structured lipids formed by enzymatic interesterification

A. Falcão[1*], T. Ueta[1], V. Nakajima[1], G. Macedo[1], J. Macedo[1]
[1] *Food and Nutrition Department, Faculty of Food Engineering, Campinas State University, Brazil,*
[*] *andrea.ofalcao@gmail.com*

In Amazon, there is a variety of underexplored vegetable oils, with bioactive compounds. These oils have a great potential to apply in cosmetics, drugs and functional foods, attracting the interest of the food industry in obtainment of structured lipids, with better physical chemical and nutritional properties. Enzymatic interesterification, involving the rearrangement of fatty acids among glycerol backbones is proving to be a good alternative. The aim of this work was to evaluate the biological potential, by measuring the minor compounds and the antioxidant capacity, of the selected Amazon oils and the structured lipids generated by enzymatic interesterification in three different enzymatic systems: with non-commercial lipase from Rhizopus sp; with commercial lipase TL-IM of Novozymes® and with the use of this two lipases concurrent. The blends were composed with buriti oil and murumuru fat (7:3 w/w) with 2,5% (w/w) of lipases. The reaction occurred at 40ºC, 150 rpm, for 24 hours. The results showed burity oil as an important source of carotenoids and tocopherols without cytotoxic effects and wit good antioxidant capacity, exceeding murumuru fat in all of these requirements. The enzymatic interesterification originated structured lipids with these same biological potential characteristics of original oil (burity oil), however with improved technological characteristics. The antioxidant capacity of structured lipids was preserved or improved according to the lipase applied. In vitro assays showed the structured lipids as substances capable of modulating the activity of antioxidant enzymes and capable to operate in combating oxidative stress.

Financial support: CNPq/FAEPEX/FAPESP

## P907

### Culinary heat treatments increase polyphenols in selected vegetables of Mediterranean diet

I. Juániz[1], I.A. Ludwig[2], G. Pereira-Caro[2], J.M. Moreno-Rojas[2], C. Cid[1], M.P. de Peña[1*]
[1] *Department of Nutrition, Food Science and Physiology, School of Pharmacy. University of Navarra, 31008, Pamplona, Spain.*
[*] *mpdepena@unav.es*
[2] *Postharvest Technology and Agrifood Industry Area. Andalusian Institute of Agricultural and Fishering Research and Training (IFAPA) Alameda del Obispo, Córdoba. Spain.*

The World Health Organization promotes higher consumption of vegetables, rich in antioxidants, mainly phenolic compounds, to contribute to the prevention of several chronic diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases. However, antioxidant capacity and phenolic compounds could be influenced by many factors, such as culinary processing. The aim of this work was to study the impact of cooking heat treatments on the antioxidant capacity and bioactive compounds of onions, green peppers and cardoon, commonly consumed as crude in salads and cooked in several ways in Mediterranean diet.

Ethanolic (80%) extracts were prepared from raw and cooked vegetables (griddled, fried in olive and sunflower oils). Antioxidant capacity was measured by DPPH and ABTS assays. Total phenolic and flavonoid compounds were determined by spectrophotometric assays. Polyphenol compounds were identified and quantified using a UPLC-PDA-HESI-MS. Principal Component Analysis (PCA), based on Pearson's correlation matrix, was applied in order to study the effect of heat treatment.

Flavonoid compounds were the main polyphenols in onion (quercetin and isorhamnetin derivates) and green pepper (luteolin and quercetin derivates). In contrast, the most abundant polyphenols of cardoon were chlorogenic acids (caffeoylquinic, diccaffeoylquinic and succninyl dicaffeoylquinic acids).

All heat treatments increased polyphenols content in vegetables. This increase, especially that observed for chlorogenic acids, was correlated with an increase in the antioxidant capacity measured by DPPH (r=0.70). The griddled vegetables showed the highest amounts of phenolic compounds with increments of 55.5%, 246% and 203% compared to raw onion, pepper and cardoon, respectively. The higher temperature during griddling than that applied during frying might favor the release of these polyphenol compounds from the food matrix.

Financial support: PIUNA (Plan de Investigación de la Universidad de Navarra) for their contribution to the financial support of this work. I.Juániz is grateful to "Asociación de Amigos de la Universidad de Navarra" for the grant received.

# Culinary heat treatments increase (poly)phenols in selected vegetables of Mediterranean diet

I. Juániz[1], I.A. Ludwig[2], G. Pereira-Caro[2], J.M. Moreno-Rojas[2], C. Cid[1], M.P. de Peña[1*]

1 Department of Nutrition, Food Science and Physiology, School of Pharmacy. University of Navarra. IdiSNA, Navarra Institute for Health Research. Pamplona, Spain * mpdepena@unav.es

2 Postharvest Technology and Agrifood Industry Area. Andalusian Institute of Agricultural and Fishering Research and Training (IFAPA) Alameda del Obispo, Córdoba. Spain.

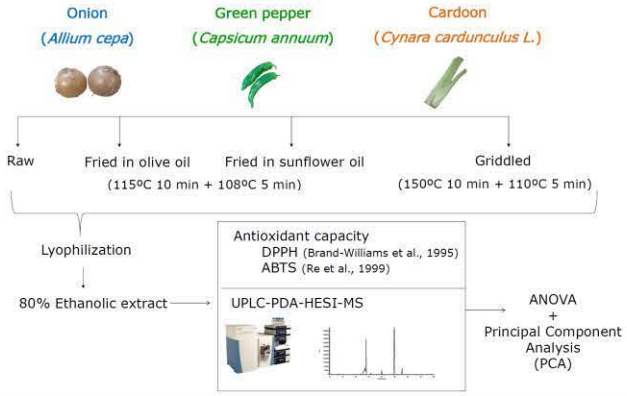
## INTRODUCTION

The World Health Organization promotes higher consumption of vegetables, rich in antioxidants, mainly phenolic compounds, to contribute to the prevention of several chronic diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases (OMS, 2004). However, antioxidant capacity and phenolic compounds could be influenced by many factors, such as culinary processing.

## OBJECTIVE

The aim of this work was to study the impact of cooking heat treatments on the antioxidant capacity and bioactive compounds of onions, green peppers and cardoon, commonly consumed as crude in salads and cooked in several ways in mediterranean diet.

## MATERIAL AND METHODS




## RESULTS AND DISCUSSION

**Table 1.** (Poly)phenolic compounds in raw and cooked onion, green pepper and cardoon (fried in olive oil, fried in sunflower oil and griddled). Results are expressed as mean ± standard deviation (mg/g sample dm).

| | Compound | Raw | Fried in olive oil | Fried in sunflower oil | Griddled |
|---|---|---|---|---|---|
| ONION | Quercetin derivates (5 compounds) | 1.26 ± 0.01 $^{a}$ | 1.71 ± 0.03 $^{bc}$ | 1.48 ± 0.05 $^{ab}$ | 1.96 ± 0.12 $^{c}$ |
| | Isorhamnetin derivates (2 compounds) | 0.10 ± 0.01 $^{a}$ | 0.13 ± 0.02 $^{ab}$ | 0.10 ± 0.02 $^{a}$ | 0.18 ± 0.01 $^{b}$ |
| | Total Flavonoids | 1.36 ± 0.01 $^{a}$ | 1.83 ± 0.05 $^{bc}$ | 1.57 ± 0.07 $^{ab}$ | 2.14 ± 0.14 $^{c}$ |
| GREEN PEPPER | Quercetin derivates (5 compounds) | 0.18 ± 0.41$^{a}$ | 0.23 ± 0.02$^{a}$ | 0.32 ± 0.02$^{b}$ | 0.53 ± 0.01$^{c}$ |
| | Luteolin derivates (7 compounds) | 0.08 ± 0.01$^{a}$ | 0.15 ± 0.02$^{b}$ | 0.20 ± 0.00$^{b}$ | 0.37 ± 0.02$^{c}$ |
| | **Total Flavonoids** | **0.26 ± 0.02$^{a}$** | **0.38 ± 0.04$^{b}$** | **0.52 ± 0.02$^{c}$** | **0.90 ± 0.03$^{d}$** |
| | Caffeic acid derivates (2 compounds) | 0.00 ± 0.00$^{a}$ | 0.00 ± 0.00$^{a}$ | 0.01 ± 0.00$^{a}$ | 0.03 ± 0.00$^{a}$ |
| | CQA derivates (1 compound) | 0.01 ± 0.00$^{a}$ | 0.02 ± 0.00$^{ab}$ | 0.02 ± 0.00$^{ab}$ | 0.03 ± 0.00$^{b}$ |
| | **Total Phenolic acids** | **0.01 ± 0.00$^{a}$** | **0.02 ± 0.00$^{a}$** | **0.03 ± 0.00$^{a}$** | **0.06 ± 0.00$^{b}$** |
| CARDOON | CQAs acids (4 compounds) | 3.46 ± 0.02$^{a}$ | 2.98 ± 0.05$^{a}$ | 3.22 ± 0.05$^{a}$ | 7.46 ± 0.35$^{b}$ |
| | DiCQAs acids (6 compounds) | 1.41 ± 0.06$^{a}$ | 3.12 ± 0.07$^{b}$ | 3.76 ± 0.08$^{c}$ | 7.21 ± 0.18$^{d}$ |
| | SuccinyldiCQAs acids (4 compounds) | 0.35 ± 0.02$^{a}$ | 0.45 ± 0.12$^{b}$ | 0.55 ± 0.00$^{c}$ | 1.14 ± 0.01$^{d}$ |
| | **Total Chlorogenic acids** | **5.22 ± 0.09$^{a}$** | **6.55 ± 0.13$^{b}$** | **7.53 ± 0.14$^{b}$** | **15.82 ± 0.54$^{c}$** |
| | Apigenin derivate (1 compound) | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ | tr$^{a}$ |
| | Luteolin derivate (1 compound) | tr$^{a}$ | tr$^{a}$ | tr$^{a}$ | 0.02 ± 0.00$^{b}$ |
| | **Total Flavonoids** | **tra** | **tra** | **tra** | **0.02 ± 0.00$^{b}$** |

Different letters for each row indicate significant differences ($p \leq 0.05$) among samples.
nd: no detected, tr: traces.

The griddled vegetables showed the highest amounts of phenolic compounds with increments of 55.5%, 246% and 203% compared to raw onion, pepper and cardoon, respectively.

**Figure 1.** Principal Component Analysis (PCA) of the raw and cooked vegetables. (a) Parameter loadings. (b) Sample scores.

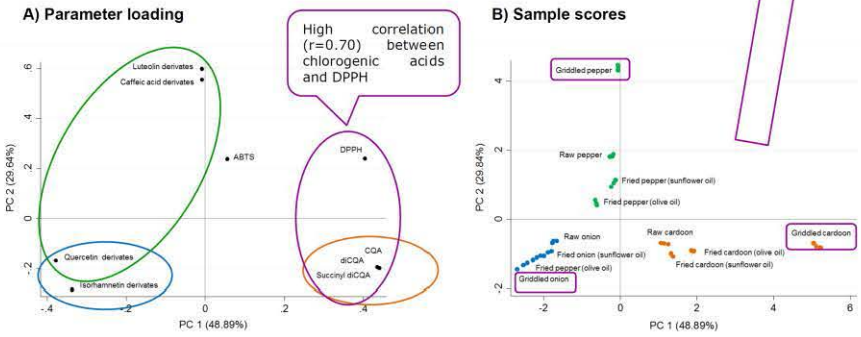
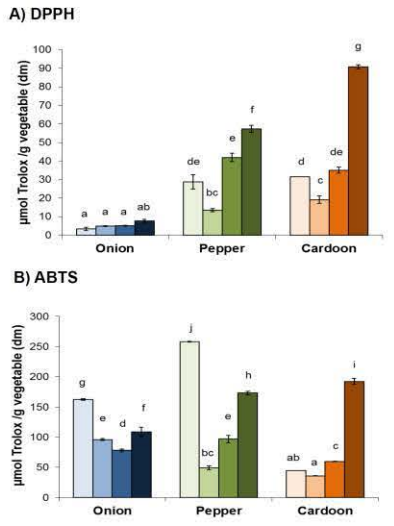



**Figure 2.** Antioxidant capacity (DPPH and ABTS) of raw and cooked vegetables




## CONCLUSION

All heat treatments increased (poly)phenols content in vegetables. The higher temperature during griddling than that applied during frying might favor the release of (poly)phenol compounds from the food matrix.

The increment on (poly)phenolic compounds was correlated with an increase in the antioxidant capacity measured by DPPH.

## REFERENCES

WHO. World Health Organization (2004).
Chang et al. (2007). Bot Stud; 48, 397-406
Re et al. (1999). Free Radical Bio Med; 26, 1231-1237
Brand-Williams et al. (1995). Lebensmittel-Wissenschaft und Technologie; 28, 25-30.

## ACKNOWLEDGEMENTS

We thank Spanish Ministry of Economy and Competitiveness (AGL2014-52636-P) and the PIUNA (Plan de Investigación de la Universidad de Navarra) for their contribution to the financial support of this work. I.Juániz is grateful to "Asociación de Amigos de la Universidad de Navarra" for the grant received.

## P918

### Effect of the irrigation, the cultivation method and the olive variety on the phenolic content of olive oils

M. Colmán-Martínez[1], P. Quifer-Rada[1,2], R. Martínez-Las Heras[3], R. Callejón-Fernández[4], B. Jiménez[5], RM. Lamuela-Raventós[1,2]

[1] *Nutrition and Food Science Department, School of Pharmacy, University of Barcelona, Barcelona, Spain*

[2] *CIBER Physiopathology of obesity and nutrition (CIBERobn), Institute of Health Carlos III, Madrid, Spain*

[3] *Institute of Food Engineering for Development, Universitat Politècnica de València Spain*

[4] *Nutrition and Food Science, Toxicology and Forensic Medicine Department, School of Pharmacy, University of Sevilla, Sevilla, Spain*

[5] *Ministry of Agriculture and Fisheries, Agricultural Research Training Centre, Cordoba, Spain*

Background/Objectives: Olive oil is considered one of the healthiest dietary fats due to its phenolic composition. Nevertheless, the nutritional quality of the olive oils is affected by agronomic and technological factors, the variety of olive, the geographical area of production, the harvest period and the extraction process, as well as the climatic conditions prevailing in the production year. Irrigation positively influences the composition and organoleptic characteristics of olive oil. The aim of this work was to study the phenolic profile of oils produced with two different types of olives harvested in Andalucía, Spain, Hojiblanca and Picual, and to determine whether there are differences in the phenolic content of these oils according to the type of irrigation received and the cultivation method (conventional or organic).

Methods: A double liquid-liquid extraction (LLE) was used to isolate the phenolic fraction of the oils using hexane:methanol (1:2, v/v). Organic solvents were evaporated, then samples were reconstituted with 850µL of MeOH:$H_2O$(20:80)(v/v) and injected to an Acquity UHPLC coupled to an API 3000 triple-quadrupole mass spectrometer with a TurboIon spray source in negative mode for phenolic quantification. Samples were also injected to an UHPLC-LTQ-Orbitrap-MS to identify phenolic compound present in the olive oil samples.

Results and conclusion: Thirty-two phenolic compounds were identified in the two types of olive oils. According to the irrigation received, the Riego technique increases the phenolic content of the two types olives giving oils richest in phenolic compounds. Likewise, organic farming increases the phenolic content of the olive oils. Statistically differences were found mainly for: tyrosol, elenoic acid, ligstroid and derivatives, luteolin, hydroxyoleuropein aglycone and other oleuropein derivatives.

Financial support: CICYT(AGL2013-49083-C3-1-R), Generalitat de Catalunya FI-DGR 2013/FI-DGR 2014,the Instituto de Salud Carlos III and ISCIII (CIBERObn) from the Spanish Ministry of Science and Innovation (MICINN) and Manuel Heredia Halcón, owner of the farm organically grown Suerte Alta.

## P919

### Effect of a simulated gastrointestinal digestion on the (poly)phenolic fraction of raw and cooked vegetables (green pepper and cardoon)

I. Juániz[1], M. Dall'Asta[2], L. Bresciani[2], P. Mena[2], D. DelRio[2], C. Cid[1], M.P. de Peña[1*]

[1] *Department of Nutrition, Food Science and Physiology, School of Pharmacy. University of Navarra. IdiSNA, Navarra Institute for Health Research. Pamplona,Spain.*

**mpdepena@unav.es*

[2] *The Laboratory of Phytochemicals in Physiology, LS9 InterLab Group, Department of Food Science, University of Parma, Parma, Italy*

Plant foods are the main source of dietary bioactive compounds, such as (poly)phenols. Usually dietary vegetables are eaten after cooking in different ways, which can influence their antioxidant capacity and phenolic compounds. Moreover, after ingestion, (poly)phenols can be modified in the gastrointestinal tract by digestive enzymes and, consequently, their bioaccessibility might be affected. The aim of this work was to study the effect of in vitro gastrointestinal digestion on the (poly)phenolic compounds of green pepper and cardoon, both raw and cooked.

Samples of raw and cooked vegetables (griddled, fried in olive and sunflower oils) were submitted to in vitro gastrointestinal digestion and extracted with methanol/acidified water (0.1% formic acid) (80:20 v/v). (Poly)phenol compounds were identified and quantified using a UHPLC-ESI-$MS^n$.

In green pepper, a total of 19 (poly)phenolic compounds were quantified, being flavonoids (luteolin and quercetin derivates) the most abundant. In cardoon, a total of 16 (poly)phenols (mainly phenolic acids) were quantified. Total phenolic compounds decreased in raw samples of green pepper after gastrointestinal digestion. However, digested cooked pepper had higher content of phenolics than non digested samples. In cardoon, a significant decrease in phenolic compounds was observed for all the samples after digestion, although losses were lower in the cooked ones. Additionally, phenolic acids were affected by the in vitro gastrointestinal digestion to a greater extent (losses up to

99%) than flavonoids, which were even increased in some cooked samples. Thus, the higher bioaccesibility of phenolic compounds in cooked vegetables suggests that changes during cooking process, including Maillard Reaction Products formation, might have a protective effect against digestive enzyme activity.

Financial support: Spanish Ministry of Economy and Competitiveness (AGL2014-52636-P) and PIUN(Plan Investigación Universidad de Navarra). I.Juániz is grateful to "Asociación de Amigos de la Universidad de Navarra" for the grant received.

## P920
## Optimization of the methodology for identification and quantification of (poly)phenolic compounds in cactus cladodes (Opuntia ficus-indica)

E. De Santiago[1], I. Juaniz[1], C. Cid[1], M.P. de Peña[1*]
[1] *Department of Nutrition, Food Science and Physiology, School of Pharmacy. University of Navarra. IdiSNA, Navarra Institute for Health Research. 31008,Pamplona, Spain.*
**mpdepena@unav.es*

Consumption of vegetables is related with lower risks of chronic diseases associated with oxidative stress such as cancer, cardiovascular diseases or diabetes. The cactus *Opuntia ficus-indica* is one of the most consumed foods in Mexico and a vegetable rich in antioxidant compounds, like (poly)phenolics. Most (poly)phenolic compounds, particularly flavonoids, are in their glycosides forms, so that for their quantification with HPLC-DAD it is necessary to apply a previous hydrolysis to release their respective aglycones. The acid hydrolysis under continuous heating is the traditional method, but it should be standardized and optimized for each food because it is dependent on the binding sites of the glycosides in the flavonoid nucleus. The aim of this work was to optimize hydrolysis conditions for a better identification and quantification of (poly)phenolic aglycones. Additionally, antioxidant capacity was evaluated in the selected extract.

Cactus cladodes were successive extracted with methanol, acetone and water. Hydrolysis was carried out using HCl at 90ºC at different concentrations (0.6, 1.2, 1. and 1.7M) and times (2 and 3 hours). After hydrolysis, (poly)phenolic aglycones and phenolic acids were identified and quantified using HPLC–DAD. Antioxidant capacity was measured by DPPH and ABTS spectrophotometric assays.

Three flavonoids (isorhamnetin, quercetin and kaempferol) and two phenolic acids (ferulic and hidroxybenzoic acids) were identified and quantified. The 1.5M HCl hydrolisis during 2 hours showed the disappearance of glycosides peaks yielding the highest amount of (poly)phenols, especially flavonoids aglycones. Furthermore, there was an increase in the antioxidant capacity measured by DPPH after hydrolisis, which might release the flavonoids aglycones from their glycosides forms in the food matrix.

Financial support; Spanish Ministry of Economy and Competitiveness (AGL2014-52636-P) and PIUN(Plan Investigación Universidad de Navarra). E. De Santiago and I. Juaniz thank to"Asociación de Amigos de la Universidad de Navarra" for the grants.

# Effect of a simulated gastrointestinal digestion on the (poly)phenolic fraction of raw and cooked vegetables (green pepper and cardoon)

I. Juániz[1], M. Dall'Asta[2], L. Bresciani[2], P. Mena[2], D. Del Rio[2], C. Cid[1], M.P. de Peña[1*]

1 Department of Nutrition, Food Science and Physiology, School of Pharmacy. University of Navarra. IdiSNA, Navarra Institute for Health Research. Pamplona, Spain * mpdepena@unav.es

2 The Laboratory of Phytochemicals in Physiology, LS9 InterLab Group, Department of Food Science, University of Parma, Parma, Italy

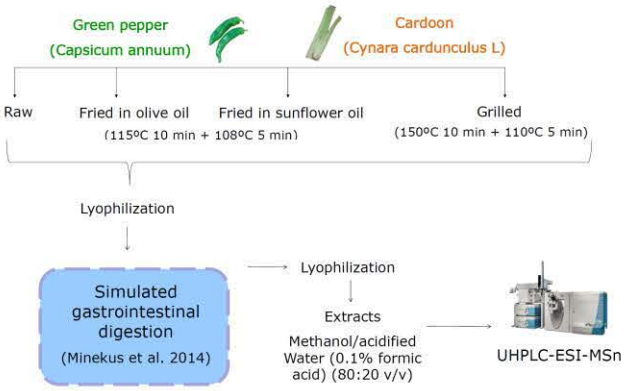
## INTRODUCTION

Plant foods are the main source of dietary antioxidants, including phenolic compounds. (Poly)phenols rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer (Del Rio et al., 2013). Usually dietary vegetables are eaten after cooking in different ways, which can influence their antioxidant capacity and phenolic compounds. Moreover, after ingestion, (poly)phenols can be modified in the gastrointestinal tract by digestive enzymes and, consequently, their bioaccesibility might be affected.

## OBJECTIVE

The aim of this work was to study the effect of *in vitro* gastrointestinal digestion on the (poly)phenolic compounds of green pepper and cardoon, both raw and cooked.

## MATERIAL AND METHODS




## RESULTS AND DISCUSSION

Figure 1. (Poly)phenolic compounds of green pepper, both raw and after heat treatment (fried in olive and sunflower oils, and grilled).

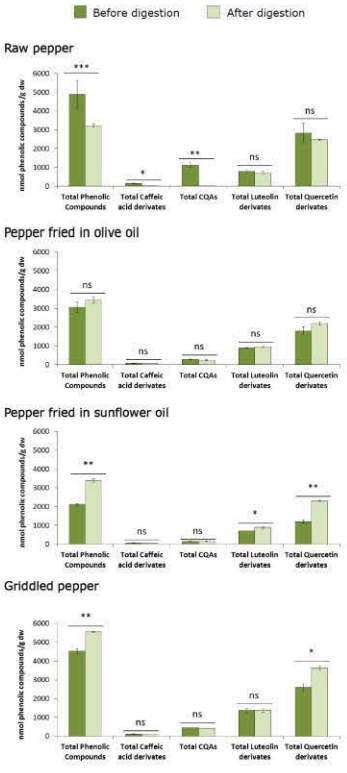
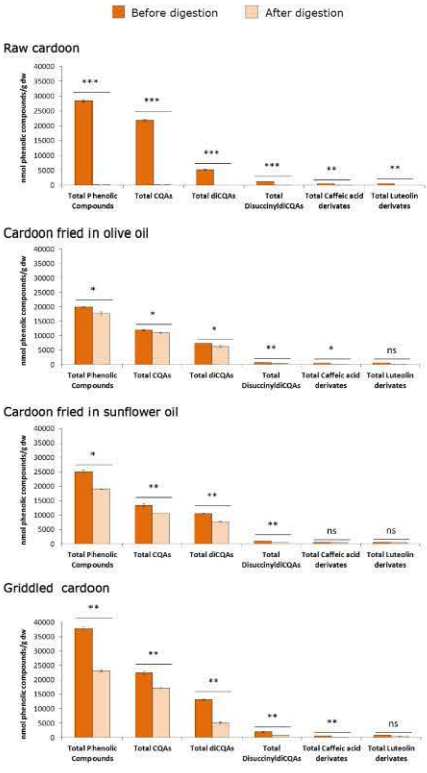



Figure 2. (Poly)phenolic compounds of cardoon, both raw and after heat treatment (fried in olive and sunflower oils, and grilled).




In green pepper, a total of 19 (poly)phenolic compounds were identified and quantified, being flavonoids (luteolin and quercetin derivates) the most abundant. In cardoon, a total of 16 (poly)phenols (mainly phenolic acids) were identified and quantified.

Total phenolic compounds decreased in raw samples of green pepper after gastrointestinal digestion. However, digestion process increased the bioaccessibility of phenolic compounds in cooked pepper samples (12.8%, 60.9% and 22.6%) in pepper fried in olive oil, pepper fried in sunflower oil and griddled pepper, respectively.

In cardoon, a significant decrease in phenolic compounds was observed for all the samples after simulated gastrointestinal digestion, although losses were lower in the cooked ones.

Additionally, phenolic acids were affected by the *in vitro* gastrointestinal digestion to a greater extent (losses up to 99%) than flavonoids, which were even increased in some cooked samples.

## CONCLUSION

The higher bioaccesibility of phenolic compounds in cooked vegetables than in raw ones after gastrointestinal process suggests that changes during cooking process, including Maillard Reaction Products formation, might have a protective effect against digestive enzyme activity.

## REFERENCES

Del Rio D et al. A. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. Antioxidants & Redox Signaling. 2013;18(14):1818-1892.

M. Minekus, et al., A standardised static in vitro digestion method suitable for food – an international consensus. Food Funct., 2014, 5, 1113.

## ACKNOWLEDGEMENTS

We thank Spanish Ministry of Economy and Competitiveness (AGL2014-52636-P) and PIUNA (Plan Investigación Universidad de Navarra) for the financial support. I.Juániz is grateful to "Asociación de Amigos de la Universidad de Navarra" for the grant.