Universidad Católica de Valencia San Vicente Mártir

MEJORA DE LA ESTABILIDAD OXIDATIVA DE ACEITES Y GRASAS CON PRODUCTOS NATURALES

IMPROVING THE OXIDATIVE STABILITY OF OILS AND FATS WITH NATURAL PRODUCTS

TESIS DOCTORAL

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ABSTRACT

Lipid oxidation has been identified as the major deterioration process of oils and fats affecting food quality, nutritional value, safety, colour, development of off-flavours and consumers' acceptance. The aim of this research was, firstly, to elucidate the complex relationship between composition and oxidative stability of oils and fats and, secondly, identify natural product(s) of known phytochemical profile with documented antioxidant properties that can be used to increase the oxidative stability of the most commonly used oils used for industrial and domestic applications.

A detailed composition analysis and oxidative stability determination was performed for 22 diverse oils and fats. Five ground spices (black pepper, ginger, turmeric, rosemary and oregano) were assessed for their antioxidant capacity, phenolic content and ability to improve the oxidative stability of vegetable oils. The most potent spice (rosemary) was incorporated in ground form in olive, rapeseed and sunflower oil formulations which were subjected to consecutive frying cycles monitoring lipid oxidation. Moreover, the protective effect of rosemary against oxidative deterioration was assessed in rapeseed oil following 24 hours maceration and filtration to remove solid residues.

The total phenolic compounds and saturated fatty acids were the main individual factors that correlated positively with oxidative stability ($r^2=0.417$, $r^2=0.321$ respectively, $P<0.01$). Saturated, monounsaturated and polyunsaturated fatty acids together accounted for 67% of variability and are considered the most important parameters to affect oxidative stability. Rosemary powder was the most effective antioxidant among the ones tested. Rosemary powder effectively improved the oxidative stability of sunflower (128.91%), olive (55.61%) and rapeseed (73.20%) oil during deep-frying and prevented the formation of conjugated dienes/trienes in rapeseed oil. The use of rosemary powder filtrate after maceration was also effective for preventing lipid oxidation of rapeseed oil. The beneficial effects of rosemary as a natural antioxidant were more profound at concentrations ≥0.5% (w/w).

Results of this Thesis provide a better understanding of the complex relationship between oil and fat composition and their oxidative stability, which is an essential step for designing strategies to increase stability of culinary oils. Rosemary is a natural product that effectively protects vegetable oils from oxidation, incorporated either as ground spice

in powdered form or powder filtrate. This could lead to alternative reformulation strategies for oil manufacturers, with potential domestic or industrial applications.

KEYWORDS: Oxidative stability; Rancimat; Oil; Fat; Antioxidants; Frying; Lipid oxidation; Natural products; Spices; Rosemary; Rapeseed oil

RESUMEN

La oxidación lipídica es el principal proceso por el que se deterioran los aceites y grasas, viéndose afectados, la calidad de los alimentos, el valor nutricional, la seguridad, el color, el desarrollo de sabores no deseados, así como la aceptación por parte de los consumidores. El objetivo de esta investigación fue, en primer lugar, analizar la compleja relación que existen entre la composición química y la estabilidad oxidativa de aceites y grasas y, en segundo lugar, identificar productos naturales que puedan aumentar de forma efectiva la estabilidad oxidativa de los aceites más comúnmente empleados para uso doméstico.

Se realizó un análisis detallado de la composición de 22 aceites y grasas diferentes, y se determinó su estabilidad oxidativa. Cinco especias trituradas (pimienta negra, jengibre, cúrcuma, romero y orégano) fueron estudiadas en relación a su capacidad antioxidante, el contenido en fenoles y la capacidad para mejorar la estabilidad oxidativa de aceites vegetales. La especia más potente (el romero) se incorporó al aceite de oliva, de girasol y de colza, y una vez hecha la mezcla, se utilizaron los aceites para freír patatas, controlando la oxidación de lípidos. Además, el efecto protector del romero contra el deterioro oxidativo se evaluó en el aceite de colza después de un filtrarlo tras una maceración de 24 horas.

Los principales factores individuales que se correlacionaron positivamente con la estabilidad oxidativa fueron los compuestos fenólicos y los ácidos grasos saturados (r^2=0,417, r^2=0,321 respectivamente, $P<0.01$). Los ácidos grasos saturados, monoinsaturados y poliinsaturados combinados, representaron el 67% de la variabilidad y se consideran los parámetros más importantes que afectan a la estabilidad oxidativa. El romero triturado fue el antioxidante más eficaz entre los evaluados. El romero mejoró la estabilidad oxidativa del aceite de girasol (128,91%), oliva (55,61%) y colza (73,20%) durante la fritura, e impidió la formación de dienos y trienos conjugados en el aceite de colza. El uso de romero filtrado tras una maceración también fue efectivo para prevenir la oxidación de lípidos en el aceite de colza. Los efectos beneficiosos del romero como antioxidante natural fueron mayores a concentraciones ≥0,5% (p/p).

Los resultados de esta Tesis proporcionan una mejor comprensión de la compleja relación que existe entre la composición de aceites y grasas y su estabilidad oxidativa, que es un

paso esencial para diseñar estrategias que permitan aumentar la estabilidad de los aceites. El romero es un producto natural capaz de proteger a los aceites vegetales frente a la oxidación, tanto añadido directamente en polvo, como filtrado tras una maceración. Esto podría ayudar a los fabricantes de aceites a generar estrategias alternativas que podrían utilizarse tanto para uso doméstico como industrial.

PALABRAS CLAVE: Estabilidad oxidativa; Rancimat; Aceite; Grasa; Antioxidantes; Fritura; Oxidación lipídica; Productos naturales; Especias; Romero; Aceite de colza

RESUMEN LARGO

INTRODUCCIÓN

Lípidos es un término que hace referencia a un conjunto de compuestos entre los que destacan los triglicéridos, los fosfolípidos y los esteroles y, que se caracterizan por ser solubles en disolventes apolares. A los triglicéridos, también se les conoce con el nombre de grasas, y están formados por una molécula de glicerol y tres ácidos grasos. Existen diferentes tipos de ácidos grasos que pueden clasificarse atendiendo a dos características: según el número de átomos de carbono (ácidos grasos de cadena corta, media o larga) y según la presencia o no de dobles enlaces y del número de ellos (ácidos grasos saturados, monoinsaturados o poliinsaturados).

Los **aceites y grasas comestibles** son alimentos formados principalmente por triglicéridos y pequeñas cantidades de otros lípidos como fosfolípidos y compuestos no saponificables. La diferencia entre aceites y grasas reside en su punto de fusión: a temperatura ambiente, los aceites son líquidos y las grasas sólidas. Los aceites y grasas se comercializan en diferentes formatos dependiendo del procesado al que se ven sometidos: refinados, parcialmente refinados, vírgenes o extraídos en frío. Durante el proceso de refinado se forman compuestos no deseables para la salud como los carbonilos, dímeros de triglicéridos, ácidos grasos *trans* y, el que se considera más tóxico, el 3-MCPD (3-Monochloropropane-1,2-diol). Por lo tanto, tener en cuenta el proceso al que se haya sometido el aceite o grasa antes de comercializarlo es fundamental a la hora de valorar su calidad.

La **oxidación lipídica** es el proceso principal por el que se deterioran los aceites y grasas, viéndose afectados, entre otros, el valor nutricional, el color y el sabor. La auto-oxidación, la foto-oxidación y la oxidación enzimática son los tres mecanismos responsables de la oxidación lipídica. Aunque tradicionalmente se ha considerado que las reacciones de auto-oxidación (las más habituales) se producen en tres fases consecutivas llamadas iniciación, propagación y terminación, actualmente se ha observado que estas reacciones son más complejas y no cumplen exactamente dichas fases. Este es un hecho importante a la hora de utilizar o interpretar métodos analíticos que determinen la oxidación de un alimento. Durante los procesos de **fritura** se producen reacciones químicas aún más complejas; esto se debe a las altas temperaturas, a la humedad del alimento y al contacto con el oxígeno. Durante la fritura, además de la oxidación lipídica, también tiene lugar la

hidrólisis de triglicéridos, así como reacciones de polimerización, ciclación e isomerización.

La **estabilidad oxidativa** es un parámetro utilizado para evaluar la resistencia de aceites y grasas a oxidarse. El **Rancimat**, es el método más utilizado para valorar este parámetro, y se basa en la determinación de compuestos volátiles (productos de oxidación secundaria) formados en condiciones de oxidación acelerada en las que se calientan las muestras mientras se hace pasar una corriente continua de aire. El tiempo transcurrido hasta la formación de estos productos de oxidación secundaria es lo que se denomina "tiempo de inducción", "periodo de inducción" o "índice de estabilidad oxidativa" (OSI, por sus siglas en inglés). En general, los aceites y grasas más insaturados tienen una menor estabilidad oxidativa que los más saturados, no obstante, el grado en el que influyen los ácidos grasos, así como otros componentes minoritarios presentes en aceites y grasas, no se conoce con exactitud.

Durante la oxidación lipídica se forman numerosos compuestos que pueden tener consecuencias negativas sobre la **salud** de los consumidores, siendo los más importantes: 4-hidroxinonenal, crotonaldehído, acrilamida y acroleína, malonaldehído y ácidos grasos *trans*. Además, el consumo de alimentos fritos se ha asociado con diabetes, enfermedades cardiovasculares, obesidad, hipertensión, cáncer y alteraciones de la microbiota intestinal.

Los **antioxidantes**, son compuestos que pueden ayudar a reducir la oxidación de aceites y grasas. Los antioxidantes sintéticos como el hidroxitolueno butilado (BHT), el hidroxianisol butilado (BHA) o la terbutilhidroquinona (TBHQ) se usan con frecuencia para retrasar la oxidación de los lípidos en los alimentos. Sin embargo, no debemos pasar por alto que son compuestos con baja estabilidad térmica y, que su uso, cuenta cada vez con más detractores por la creciente preocupación sobre sus efectos a largo plazo en la salud humana. Todo esto, sumando a la mayor demanda y preocupación de los consumidores por utilizar y consumir productos naturales, sitúa a los extractos vegetales como excelentes alternativas a los antioxidantes sintéticos.

Ahora bien, en la práctica, los extractos vegetales son adecuados principalmente para la fabricación de alimentos a escala industrial, mientras que, el uso directo de **productos vegetales triturados** (en polvo) podría ser una alternativa válida tanto para la industria alimentaria como para uso doméstico. Es más, el uso de estos productos vegetales triturados aporta una mayor actividad antioxidante que sus correspondientes extractos preparados a partir de una cantidad equivalente. Esto sugiere que, durante el proceso de

extracción, se pierde una gran cantidad de compuestos antioxidantes que podrían ser decisivos, puesto que aportan un efecto sinérgico para la estabilidad oxidativa del producto final.

HIPÓTESIS Y OBJETIVOS

Según la **hipótesis** de la presente Tesis: la estabilidad oxidativa de aceites y grasas está determinada por su contenido en ácidos grasos, tocoferoles, beta-carotenos, clorofila y/o compuestos fenólicos. Y, el uso de productos naturales con reconocido efecto antioxidante, como son las especias pimienta negra, jengibre, cúrcuma, romero y orégano, usadas directamente en polvo o filtradas después de una maceración, protegen la oxidación lipídica de aceites y grasas.

Para dar respuesta a la hipótesis planteada, los **objetivos** marcados en esta investigación son los siguientes:

1. Estudiar una gran variedad de aceites y grasas, incluyendo muestras tanto refinadas como vírgenes, con el fin de valorar su estabilidad oxidativa y determinar su composición en ácidos grasos, tocoferoles, beta-carotenos, clorofila y compuestos fenólicos. Y, analizar, mediante un análisis estadístico, si los componentes estudiados influyen o no, y en qué grado lo hacen, sobre la estabilidad oxidativa de los aceites y grasas.
2. Seleccionar un producto natural que, o bien utilizado directamente en polvo, o bien filtrado después de una maceración, pueda aumentar la estabilidad oxidativa de los aceites más comúnmente empleados para uso doméstico.

MATERIAL Y MÉTODOS

Se estudiaron 22 aceites y grasas, incluyendo muestras de diferentes calidades y variedades: aceite de maíz desprovisto de tocoferoles, aceite de oliva, aceite de oliva virgen (3 tipos), aceite de oliva virgen extra (4 tipos), aceite de girasol refinado, aceite de girasol virgen, aceite de girasol alto oleico refinado, aceite de colza refinado, aceite de colza virgen, aceite de sésamo refinado, aceite de sésamo virgen, aceite de sésamo tostado virgen, aceite de coco refinado, aceite de coco virgen, aceite de palma virgen, mantequilla y ghee (mantequilla clarificada).

Se realizó un análisis detallado de la composición de cada aceite o grasa mediante la determinación de: ácidos grasos (analizados con cromatografía de gases), tocoferoles y carotenoides (con HPLC), contenido total de compuestos fenólicos (con el método de Folin-Ciocalteu) y clorofila (con espectofotómetro). Además, se determinó la estabilidad oxidativa de cada muestra con el equipo Rancimat 743, a 120ºC de temperatura y una corriente de aire continua de 20 L/h.

Cinco especias fueron seleccionadas para el estudio en base a su conocida capacidad antioxidante: pimienta negra, jengibre, cúrcuma, romero y orégano. De cada una de ellas se evaluó la capacidad antioxidante (utilizando el análisis del Potencial Reductor Férrico), el contenido total de compuestos fenólicos (con el método de Folin-Ciocalteu) y la capacidad para mejorar la estabilidad oxidativa de aceites vegetales (con el equipo Rancimat 743). El efecto de las especias en la estabilidad oxidativa de los aceites fue comparada con el del BHT. La especia más potente (el romero) se incorporó en forma triturada al aceite de oliva, de girasol y de colza; el de oliva y girasol por ser los aceites más utilizados de uso doméstico en España, y el de colza por ser el más utilizado en Escocia. Tras mezclar la planta con cada aceite durante 1 hora, los aceites se utilizaron para freír patatas en una freidora (Tesco 3L Pro Fryer). 2,5 litros de aceite se calentaron a 180ºC durante 10 minutos, después se llevaron a cabo 4 inmersiones de 150 gramos de patatas cada 5 minutos. Tras la fritura, se determinó el contenido de dienos y trienos conjugados (con espectofotómetro), así como el contenido en tocoferoles (con HPLC).

Además, el efecto protector del romero contra el deterioro oxidativo se evaluó en el aceite de colza después de una maceración de 24 horas y de pasarlo por un filtro (Whatman filter paper no. 1) con la finalidad de eliminar residuos sólidos. El aceite de colza fue seleccionado por ser el más consumido en Escocia y tener una baja estabilidad oxidativa. Por un lado, se determinó la estabilidad oxidativa y se evaluó la capacidad antioxidante del aceite macerado y filtrado (utilizando el Potencial Reductor Férrico). Mientras que, por otro lado, se llevó a cabo un test de oxidación acelerada con el Rancimat en el que las muestras se calentaron a 120ºC y se les hizo pasar una corriente de aire continua de 20 L/h durante 1,5 y 3 horas. Tras estas condiciones de oxidación acelerada, se evaluó la conductividad con el equipo Rancimat, el contenido en tocoferoles y carotenoides (con HPLC), se realizó un análisis del color (con un colorímetro) y se analizó el contenido de malondialdehído (mediante el análisis de sustancias reactivas al ácido tiobarbitúrico, TBARS por sus siglas en inglés) y dienos conjugados (a través del espectofotómetro).

Con tal de poder analizar los resultados obtenidos en los múltiples ensayos llevados a cabo y, de poder verificar o refutar nuestra hipótesis, se llevó a cabo un análisis estadístico de todos los resultados. Para evaluar la relación entre la composición química y la estabilidad oxidativa de los 22 aceites y grasas, se llevó a cabo un análisis de componentes principales (PCA, por sus siglas en inglés), así como modelos de regresión linear simple y múltiple. Las diferencias significativas de los valores se determinaron con análisis de varianza (ANOVA), prueba t (para variables independientes) y prueba t de dos colas (para variables dependientes).

Los resultados se expresan como media con desviaciones estándar y los análisis y determinaciones fueron llevados a cabo por duplicado, triplicado o cuadriplicado, según el caso. Los análisis estadísticos se realizaron con el programa SPSS (v.21.0 software, IBM Corporation, USA) y Minitab (17.1.0 para Windows), asumiendo el nivel de significancia como $P < 0,05$.

RESULTADOS

El análisis de la composición de los 22 aceites y grasas estudiados, muestra que existen grandes diferencias en relación a la presencia y concentración de los diferentes compuestos analizados: perfil de ácidos grasos, tocoferoles, beta-carotenos, clorofila y compuestos fenólicos, así como en la estabilidad oxidativa. Con estos resultados, se llevó a cabo un análisis de componentes principales (PCA, por sus siglas en inglés), con tal de poder reducir todas las variables estudiadas a un número menor, tener una visión más clara de las muestras de aceites y grasas y poder identificar patrones de comportamiento. A través de la representación bidimensional de los dos componentes principales, las muestras se distribuyeron en 3 grupos diferenciados: el grupo 1 incluyó muestras caracterizadas por un alto contenido en ácidos grasos saturados (AGS), compuestos fenólicos y estabilidad oxidativa, al mismo tiempo que, un bajo contenido en ácidos grasos insaturados (AGI). Todas las muestras del grupo 1 fueron sólidas a temperatura ambiente por su elevado contenido en AGS. Dentro del grupo 2 se incluyeron los diferentes tipos de aceite de oliva y el aceite de girasol alto oleico; todos ellos ricos en ácidos grasos monoinsaturados (AGMI). Por último, en el grupo 3, se incluyeron los aceites de semillas (excepto el de girasol alto oleico), caracterizados por ser ricos en ácidos grasos poliinsaturados (AGPI) y en tocoferoles (especialmente delta-tocoferol).

El análisis de regresión lineal simple realizado, mostró que: compuestos fenólicos, tocoferoles totales, AGS, AGI y AGPI fueron los principales contribuyentes a la estabilidad oxidativa. Los compuestos fenólicos y los AGS se correlacionaron positivamente (r^2=0,417, r^2=0,321 respectivamente, P<0,01), mientras que los ÁGI, ÁGPI y el total de tocoferoles se correlacionaron negativamente (r^2=0,304, r^2=0,264 y r^2=0,223 respectivamente) con la estabilidad oxidativa. El análisis de regresión lineal múltiple, mostró que la mayor parte de la variabilidad en la estabilidad oxidativa (el 67%) puede explicarse por las diferencias en el contenido y combinación de los AGS, AGMI y AGPI presentes en los distintos tipos de aceites y grasas.

Periodo de inducción = -904 + 9.49 [AGS] + 9.16 [AGMI] + 8.94 [AGPI], r^2 = 0.670

El **romero** fue, de todos los productos naturales estudiados, el que mostró mejores resultados en cuanto a: capacidad antioxidante, contenido en compuestos fenólicos y, mejora de la estabilidad oxidativa cuando se añade a aceite de maíz sin tocoferoles (en una concentración de 0,5% p/v). Además, el romero triturado mejoró la estabilidad oxidativa del aceite de girasol (128,91%), de oliva (55,61%) y de colza (73,20%) durante el proceso de fritura; e impidió, de forma significativa, la formación de dienos y trienos conjugados en el aceite de colza.

Mientras que los dienos y trienos conjugados aumentaron un 29,25% y un 211,76% respectivamente tras la fritura del aceite de colza, al añadir romero, el aumento se vio fuertemente reducido y fue de tan solo un 7,71% y un 60,56% respectivamente. Por último, el contenido en tocoferoles no sufrió cambios significativos ni durante la fritura ni al añadir el romero.

El uso de **romero filtrado** tras una maceración también fue efectivo para prevenir la oxidación de lípidos en el aceite de colza. Tanto la estabilidad oxidativa como la capacidad antioxidante del aceite aumentaron al añadir romero, especialmente a partir de una concentración igual o superior al 0,5% (p/p). Además, tras la aceleración de las condiciones de oxidación en el Rancimat durante 1,5 y 3 horas, la adición de romero en concentraciones de 1 y 2 % p/p, redujo la conductividad un 80% y un 75%, respectivamente. El romero también fue capaz de reducir los cambios de color, la formación de malondialdehído y de dienos conjugados, así como de proteger la

degradación de tocoferoles y carotenoides al someter a condiciones de oxidación acelerada al aceite de colza.

CONCLUSIONES

Las principales conclusiones de la presente Tesis son las siguientes:

1. Los 22 aceites y grasas estudiados (incluyendo refinados, no refinados y prensados en frío) muestran una gran diversidad en su composición en relación a la presencia de tocoferoles, beta-carotenos, clorofila, compuestos fenólicos y composición de ácidos grasos. Las muestras estudiadas fueron: aceite de maíz sin tocoferoles, aceite de oliva, siete tipos de aceites de oliva virgen, aceite de girasol (tanto refinado como prensado en frío), aceite de girasol alto en oleico, aceite de colza (refinado y prensado en frío), aceite de sésamo (refinado y prensado en frío), aceite de sésamo tostado, aceite de coco (refinado y prensado en frío), aceite de palma virgen, mantequilla y ghee.
2. **Los aceites de coco y de oliva son los que muestran mayor estabilidad oxidativa**, mientras que los aceites de maíz sin tocoferoles, girasol y sésamo son los que muestran una menor estabilidad. La estabilidad oxidativa del aceite de girasol y sésamo, según estudios previos, se asocia con una concentración de compuestos polares que excede el límite legal (24%), motivo por el cual no serían aptos para frituras. El aceite de maíz sin tocoferoles también está por debajo del límite; sin embargo, este aceite no está destinado al consumo humano o animal y se suministra únicamente para uso en el laboratorio.
3. El análisis de componentes principales nos permite agrupar los 22 aceites y grasas estudiados en **tres grupos diferentes**:
    a. Ricos en ácidos grasos saturados: las grasas (sólidas a temperatura ambiente).
    b. Ricos en ácidos grasos monoinsaturados: los aceites de oliva y el aceite de girasol alto oleico.
    c. Ricos en ácidos grasos poliinsaturados: los aceites de semillas.
4. Los aceites y grasas contienen compuestos naturales que pueden actuar como antioxidantes o pro-oxidantes, sin embargo, **no existe un compuesto único que**

pueda explicar en un alto porcentaje la mayor o menor estabilidad oxidativa. Los **compuestos fenólicos totales** y los **ácidos grasos saturados** son factores individuales que se correlacionan positivamente con la estabilidad oxidativa, y que pueden explicar el 41.7% y el 32.1% de variabilidad la estabilidad oxidativa, respectivamente. Cuando se tienen en cuenta todos los parámetros, nueve componentes (ácidos grasos saturados, monoinsaturados y poliinsaturados, clorofila, beta-carotenos, compuestos fenólicos y, alfa, delta y gamma tocoferol) explican el 76.5% de la variabilidad total, mientras que la diferente combinación de los ácidos grasos saturados, monoinsaturados y poliinsaturados explica el 67% de la variabilidad. Por lo tanto, **el perfil de ácidos grasos es clave en la estabilidad oxidativa de aceites y grasas**.

5. El **romero en polvo** es el producto natural que mejor protege a los vegetales de la oxidación, en comparación con la pimienta negra, el jengibre, la cúrcuma y el orégano. El romero muestra la mayor capacidad antioxidante y tiene la mayor concentración de compuestos fenólicos. Añadir romero (0,5% p/v) es la opción más eficaz de las estudiadas para aumentar la estabilidad oxidativa del aceite de maíz sin tocoferoles; incluso es más eficaz que las formulaciones con BHT a niveles permitidos.
6. Añadir **romero en polvo** a los aceites de girasol, oliva y colza durante un **proceso de fritura**, conduce a un aumento significativo de la estabilidad oxidativa de éstos. La adición de romero triturado tiene un efecto beneficioso adicional cuando se incorpora al aceite de colza, puesto que previene la formación de dienos y trienos conjugados. Añadir romero en polvo a aceites vegetales puede inhibir, o retrasar, la oxidación de ciertos lípidos durante la fritura; sin embargo, se necesitan más estudios que aborden la oxidación lipídica con tal de esclarecer los mecanismos que puedan explicar el efecto beneficioso observado al añadir romero en polvo durante la fritura con aceites vegetales.
7. La maceración de aceite de colza con **romero en polvo** seguido de un **filtrado,** mejora la estabilidad oxidativa del aceite. El efecto protector se observa por menores cambios de conductividad, menos diferencias de color y una menor producción de malondialdehído y dienos conjugados después de la exposición durante 1,5 y 3 horas a condiciones de oxidación acelerada en el equipo Rancimat. Además, el aceite con romero en polvo muestra una mayor capacidad antioxidante

y un efecto protector contra la degradación de tocoferoles y carotenoides durante el proceso de oxidación acelerada. Los efectos beneficiosos del romero en polvo son más notables a **concentraciones superiores al 0,5%** (p/p).

8. El romero es un producto natural capaz de proteger a los aceites vegetales frente a la oxidación, tanto añadido directamente en polvo, como filtrado tras una maceración. Esto podría ayudar a los fabricantes de aceites a generar estrategias alternativas que podrían utilizarse tanto para uso doméstico como industrial.

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ABBREVIATIONS

BHA, butylated hydroxyanisole

BHT, butylated hydroxytoluene

CD, conjugated diene

CT, conjugated triene

GAE, gallic acid equivalent

FRP, ferric reducing power

IP, induction period

IT, induction time

MDA, malondialdehyde

MUFA, monounsaturated fatty acid

NEFA, non-esterified fatty acids

OSI, oil stability index

PC, principal component

PUFA, polyunsaturated fatty acid

SFA, saturated fatty acid

TAG, triacylglycerides

TBARS, thiobarbituric acid reactive substances

TBHQ, tertbutylhydroquinone

TPC, total phenolic content

UFA, unsaturated fatty acid

Introduction

CHAPTER 1. INTRODUCTION

1.1. LIPID OXIDATION

1.1.1. ¿WHAT ARE LIPIDS?

Food lipids are principally triacylglycerides, phospholipids and sterols found naturally in natural food sources and added as functional ingredients in many processed foods. The common feature of these molecules is that they are soluble in non-polar solvents but not in water. Triacylglycerides (a sub-group of lipids), that consist of three fatty acids and a glycerol, are also known as fats.

From a nutritional perspective, fats are a concentrated caloric source, providing nine calories per gram, which makes them the most energy rich component of food. A certain amount of dietary fat is essential for maintaining body functions, particularly the unsaturated omega 3 and omega 6 fatty acids. Moreover, fats serve as vehicle for fat-soluble vitamins (A, D, E and K) and other lipid components of food, facilitating their transport and absorption. The presence of fat significantly enhances the organoleptic properties of foods by contributing to the texture, taste and flavor, which is commonly perceived as creamy or oily sensation.

Fatty acids are monocarboxylic acids, constituents of triacylglycerides and other saponifiable lipids; the latter are easily hydrolyzed by sodium hydroxide and those generally have ester linkages. Fatty acids can be classified according to their carbon chain length (Gil, 2013); short-chain fatty acids (contain less than 6 carbon atoms), medium-chain fatty acids (8-12 carbon atoms), long-chain fatty acids (14-18 carbon atoms), and very-long-chain fatty acids (more or equal than 20 carbon atoms). Moreover, fatty acids can be classified by their number of double bonds: **saturated fatty acids** (SFA), if no double bond is present; **monounsaturated fatty acids** (MUFA), if only one double bond is present; and **polyunsaturated fatty acids** (PUFA), if two or more double bonds are present. Both MUFA and PUFA can be classified into families depending on the position of the double bond on the methyl terminal end. The most common families present in foods are omega 9, omega 6 and omega 3 fatty acids (Figure 1.1). Moreover, the configuration of each double bond in an unsaturated fatty acid can take two forms

(formerly known as *isomers*): the predominant form naturally occurring is *cis*, in which both hydrogen atoms are on the same side of the chain; a *trans* isomer may also occur, in which the hydrogen atoms are located on opposite sides of the double bond.

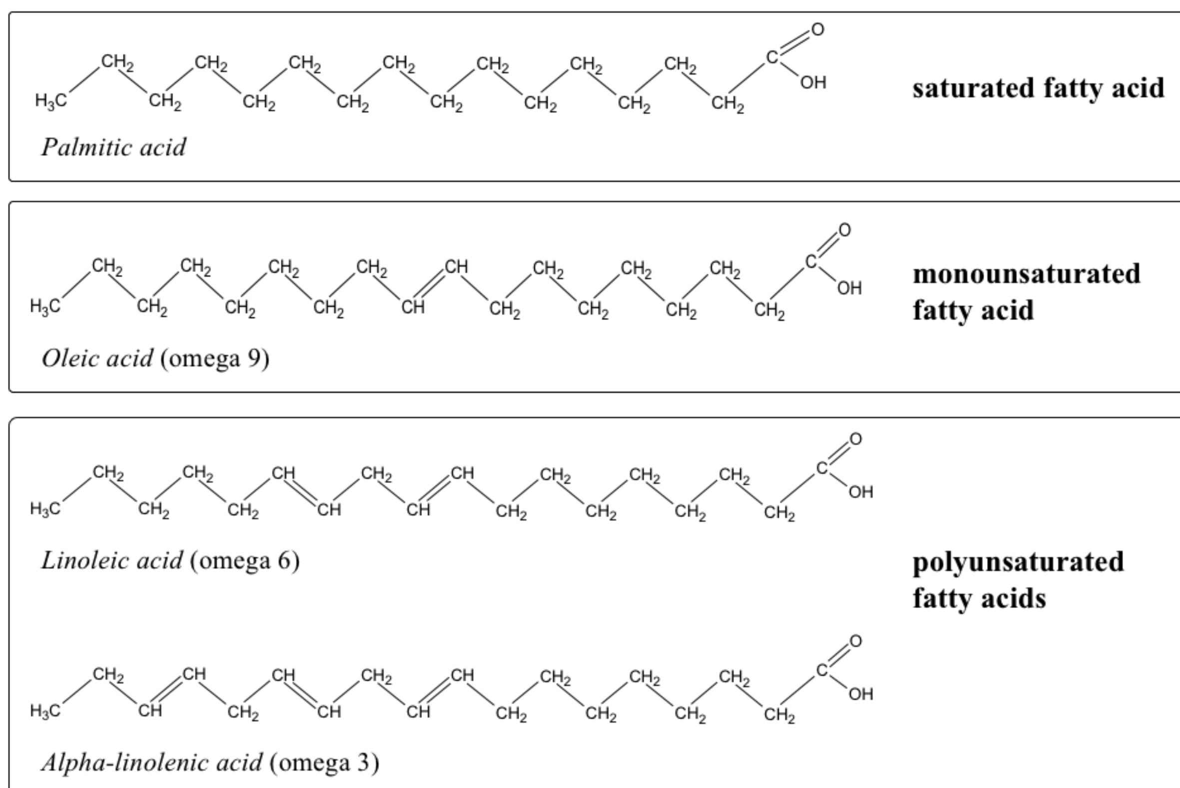


Figure 1.1. Examples of saturated, monounsaturated and polyunsaturated fatty acids.

The most common fatty acids present in foods are listed in Table 1.1 Dietary fat is a **complex mixture** of many different types of fatty acids. For example, butter provides 22 g of palmitic acid, 17 g of oleic acid, 7 g of myristic acid, 3 g of butyric acid and smaller quantities of other fatty acids (U.S. Department of Agriculture and Agricultural Research Service, 2016). Moreover, the fatty acid composition in food is affected by numerous factors; in the case of dairy, by feed, animal (breed, lactation stage, body condition) and environmental (especially cold and heat stress) factors (Samkova *et al.*, 2012).

Table 1.1. Types of fatty acids.

Name	Number of carbon atoms	Carbon atoms: double blonds	Food source
Saturated fatty acids			
Butyric acid	4 C	4:0	Dairy
Caproic acid	6 C	6:0	Dairy
Caprylic acid	8 C	8:0	Dairy
Capric acid	10 C	10:0	Dairy
Lauric acid	12 C	12:0	Coconut oil
Myristic acid	14 C	14:0	Dairy and coconut oil
Palmitic acid	16 C	16:0	Dairy, palm oil and cocoa fat
Stearic acid	18 C	18:0	Meat fat and cocoa fat
Unsaturated fatty acids			
Oleic acid (OA)	18 C	18:1ω9	Olive oil, avocado, nuts
Linoleic acid (LA)	18 C	18:2ω6	Seeds oils, nuts and seed
Alpha-linolenic acid (ALA)	18 C	18:3ω3	Flax and chia seeds, soy and walnuts
Gamma-linolenic acid (GLA)	18 C	18:3ω6	Evening primrose and borage oil
Dihommo-gamma-linolenic acid (DGLA)	20 C	20:3ω6	Meat
Arachidonic acid (AA)	20 C	20:4ω6	Meat
Eicosapentaenoic acid (EPA)	20 C	20:5ω3	Fatty fish
Docosahexaenoic acid (DHA)	22 C	22:6ω3	Fatty fish

1.1.2. EDIBLE OILS AND FATS

According to Codex Alimentarius (FAO/WHO Codex Alimentarius Commission, 1981a), edible oils and fats are foodstuffs which are composed of glycerides of fatty acids, and they can be of vegetable, animal or marine origin. They may contain small amounts of other lipids such as phosphatides, of unsaponifiable constituents and of free fatty acids naturally present in the oil or fat. Oils and fats are currently used for "dressing" food, cooking or frying. Moreover, they are found in products such as margarines, shortenings and in bakery foods, infant formulas, and dairy products and some sweets.

The difference between oils and fats is their melting point. **Oils** tend to be liquid at ambient temperature; **fats** tend to be solid at ambient temperature. To turn a fat into an oil, the temperature needs to be raised above its melting point. In general, those containing a greater proportion of unsaturated or short fatty acids are liquid at room temperature, whereas those with higher amounts of saturated (or unsaturated *trans* isomers) and long fatty acids will be solid.

There are many different types of edible oils and fats available for human consumption. Tennant and Gosling (2015) developed an innovative model to estimate the consumption of vegetable oils and fats by European Union consumers. Oils and fats used for dressing or cooking, as well as those incorporated as ingredients in products, were taken into account. The consumption of oils for Europeans follows the order: sunflower oil > palm oil > rapeseed oil > olive oil > soybean oil > coconut oil > palm kernel oil > maize germ oil > groundnut oil. However, there are noticeable differences between countries, for example, in Spain **olive oil** is the main oil consumed, followed by sunflower oil.

In addition to the type of oil or fat used, it is important to consider the quality of the foodstuff. Edible oils and fats are commercially available as fully refined, partially refined, virgin or cold-pressed, depending on the processing steps involved in oil/fat production. According to Codex Alimentarius (FAO/WHO Codex Alimentarius Commission, 1981a), **virgin oils and fats** are produced by mechanical procedures (e.g. expelling or pressing) and the application of heat only. **Cold-pressed oils and fats** are obtained by mechanical procedures only without the application of heat. Both types (virgin or cold-pressed) may have been clarified by washing with water, settling, filtering and centrifuging. No food additives are permitted in virgin or cold-pressed oils/fats. And

finally, **refined oils and fats** are typically subjected to several chemical and physical refining processes including degumming, neutralisation, bleaching and deodorization.

Regarding refined oils and fats, a reduction in oxidizable compounds such as free fatty acids and oxidised triglyceride monomers and total polar compounds is typically observed (Farhoosh, Einafshar and Sharayei, 2009). On the other hand, refined oils/fats suffer losses of phenolic compounds, tocopherols, phytosterols, and carotenoids and as a result are more susceptible to oxidative deterioration (Chaiyasit *et al.*, 2007). Moreover, an increase of compounds derived from oxidation reactions have been observed during various refining processes. Carbonyl compounds, triglyceride dimers and *trans* fatty acids formation increased during this manufacturing process. 3-Monochloropropane-1,2-diol (3-MCPD) is the most toxic compound formed during the deodorization step of the refining process. Refined palm oil presents the highest levels of this toxic compound (EFSA Panel on Contaminants in the Food, 2016). 3-MCPD is classified as a class 2B carcinogen (possibly carcinogenic to human) by the International Agency for Research on Cancer (IARC). Moreover, 3-MCPD can induce kidney toxicity in animals, infertility in rats and suppression of the immune function.

Therefore, the quality of the oil or fat should be determined holistically by simultaneously considering the **industrial process involved**, the chemical composition of the product and its resulting oxidative stability (Castelo-Branco *et al.*, 2016).

1.1.3. THE PROBLEM OF LIPID OXIDATION

Lipid oxidation has been identified as the major deterioration process of oils and fats affecting food quality, nutritional value, safety, colour, development of off-flavours and consumers' acceptance. Moreover, some of the compounds formed during oxidation may have undesirable consequences on consumers' health (Hosseini *et al.*, 2016), as will be described in 1.1.7. section of the present Thesis. The control of lipid oxidation remains an ongoing challenge.

For more than four decades, nutritional recommendations have promoted high-carbohydrate, low-fat diets, and lipid oxidation was overlooked as a risk factor for human health. Currently, scientific evidence suggests that the consumption of certain types of fat especially those rich in unsaturated fatty acids, is beneficial for human health. On the

other hand, vegetable oils rich in unsaturated fatty acids, are more prone to oxidation. A better understanding of the lipid oxidation processes is therefore essential for making dietary recommendations in relation to oil or fat consumption as well as for developing new food products with acceptable oxidative stability and shelf-life.

1.1.4. CHEMISTRY OF LIPID OXIDATION

Three different mechanisms responsible for lipid oxidation are widely recognised, yielding different oxidation products: auto-oxidation, photo-oxidation and enzymatic oxidation:

1) **Auto-oxidation** is the most common process leading to oxidative deterioration and is defined as the spontaneous radical reaction of atmospheric oxygen with lipids. The classical scheme of lipid auto-oxidation involves three stages: initiation, propagation, and termination (Figure 1.2).

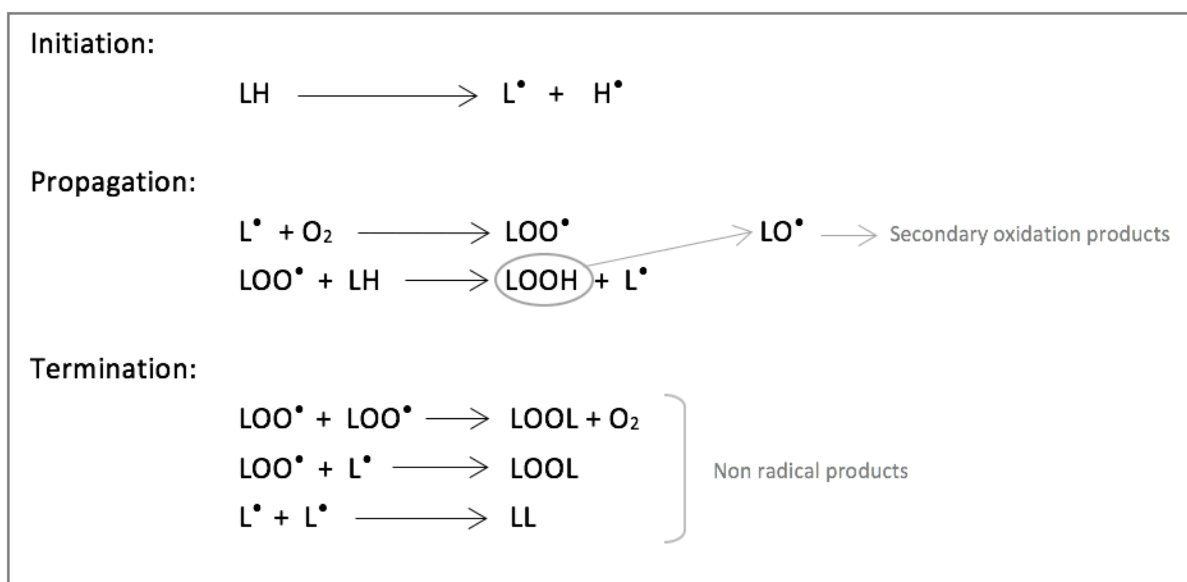


Figure 1.2. Simplified classical theory explaining the mechanism of lipid auto-oxidation. Abbreviations: LH, lipid; L•, alkyl radical; LOO•, peroxyl radical; LOOH, lipid hidroperoxide.

The theory of the three stages of lipid oxidation was developed in the 1940s (Budilarto and Kamal-Eldin, 2015). However, scientific research on this subject has been

inconsistent with the traditional reaction scheme; clearly, lipid oxidation is a much more complex process than originally thought. Karen M. Schaich described in 2005 a proposed integrated reaction scheme for lipid oxidation (Figure 1.3), which compliments the classical theory. The existence of simultaneous alternate pathways must be considered when analyzing lipid oxidation. For example, according to the original theory for lipid oxidation, secondary products do not form until after lipid hydroperoxides (LOOH) decompose. However, free radical chemistry has shown that, peroxyl radicals (LOO•) can generate dimers, epoxides and aldehydes (secondary oxidation products) from the beginning of oxidation; so, secondary products can be generated without the formation of hydroperoxide (LOOH) intermediates.

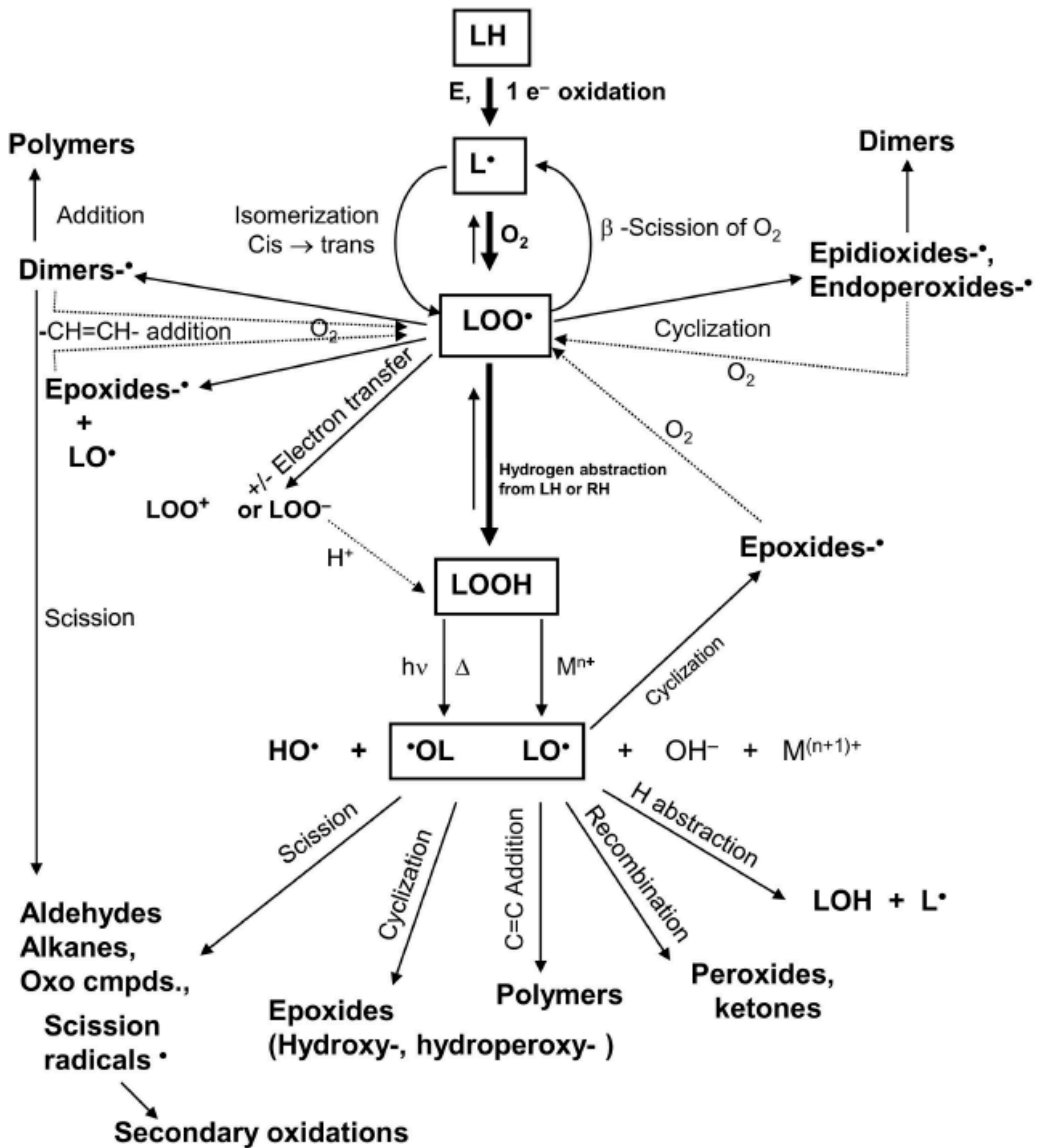


Figure 1.3. Proposed reaction scheme for integrating alternate pathways of lipid oxidation with traditional chain reaction driven by hydrogen abstractions (Schaich, 2012).

Lipid oxidation is initiated (LH → L•) in the presence of heat, **metals** (iron, copper) or **reactive oxygen species** (ROS). The initiator removes a hydrogen atom from a fatty acid to from an alkyl radical (L•). This radical formation in fatty acids occurs at the carbon atom that requires the least energy for hydrogen removal; the hydrogen atom adjacent to a double bond, especially hydrogen attached to the carbon between two double bonds, is easily removed (Choe and Min, 2006b). When applied to an oil or fat, the oxidation process becomes more complex, since the unsaponificable fraction can modulate this reaction (increase or decrease); moreover, other factors in the same lipids can affect the process, for example, an unsaturated fatty acid at the position one or three of the triacylglycerol oxidizes more quickly than the same fatty acid at position two, where is more protected against the oxidation process.

The lipid radicals then react with **oxygen** (specifically with triplet oxygen) to form peroxyl radicals (LOO•), which act by "attacking" a new lipid molecule, generating at the same time lipid hydroperoxides (LOOH), which in turn are very unstable and tend to degrade rapidly in a variety of sub-products. As described in the new scheme for lipid oxidation (Figure 1.3), peroxyl radicals (LOO•) can also generate directly compounds such as dimers, epoxides and aldehydes.

Several products can appear at the end of lipid oxidation; these are known as the **secondary oxidation products**. On one hand, dimers and polymers can be formed, which are responsible for altering viscosity, colour changes and foaming in oils. On the other hand, breakdown products can arise, including aldehydes, ketones, alcohols, alkanes, epoxy compounds and volatile organic acids. Secondary oxidation products are potentially harmful to human health; these effects will be detailed in 1.1.7. section of the present Thesis. Moreover, they contribute to flavour and as a result significantly impact the sensory quality of oils/fats and oils/fats-containing foods.

2) Photo-oxidation occurs when an oil or a fat is exposed to **light** in the presence of sensitizers and atmospheric oxygen (Choe and Min, 2009). Light can trigger lipid oxidation in two different ways, both mediated by small amounts of compounds called photosensitizers. Photosensitizers such as chlorophyll, pheophytine, and riboflavin absorb light in the visible or near-UV region and become electronically excited. Depending on the oxygen concentration, the electronically excited sensitizer can cause photosensitized oxidation by two distinct mechanisms (Johnson and Decker, 2015):

- **Type I** photosensitized oxidation reaction; once the sensitizer is activated by light, it reacts directly with the fatty acid, generating radicals which act as initiators in the auto-oxidation process. Type I occurs under lower oxygen concentrations.
- **Type II** photosensitized oxidation reactions; once the sensitizer is activated by light, the energy is transferred to molecular oxygen, converting it to its excited singlet state ($^3O_2 \rightarrow ^1O_2$). Singlet oxygen is very unstable, and can quickly abstract hydrogen atoms from unsaturated fatty acids and promote lipid oxidation. Type II reactions occur when oxygen is readily available and involves the transfer of energy from the photosensitizer to oxygen.

3) **Enzymatic oxidation** of lipids in oils and fats is caused primarily by lipoxygenases. These enzymes are detected in raw virgin oils, such as virgin olive oil. Plant lipoxygenases can incorporate oxygen into fatty acids to yield lipid hydroperoxides (LOOH), so they can initiate the early stages of the oxidation process.

1.1.5. OXIDATIVE STABILITY

Oxidative stability is an important parameter for evaluating the quality of oils and fats; it refers to the ability to resist oxidative rancidity (or deterioration) over processing and storage periods (Hu and Jacobsen, 2016).

Several accelerated methods have been developed to test the resistance of edible oils and fats to oxidation. All these accelerated methods involve the use of elevated temperatures because it is known that the reaction rate is exponentially related to temperature. Among these methods, nowadays the **Rancimat method** is very popular and it is frequently used and reviewed due to its ease of use and reproducibility. Based on induction times from Rancimat measurements it is very easy to rank the oxidative stability of oils and fats.

The Rancimat method is based on the conductometric determination of volatile compounds formed as a result of lipid oxidation. Oxidation is accelerated by means of heating up (usually at 100–130°C) the reaction vessel and by passing air continuously through the sample. This process causes the fatty acid molecules in the sample to oxidize. In due time, the fatty acids are degraded and secondary oxidation products are formed, including volatile low-molecular organic acids such as, acetic acid and formic acid. These are transported by the airstream to a second vessel containing distilled water, where

conductivity is measured continuously. The readings of the conductivity measurements draw a curve whose inflection point marks the period of induction from which a drastic increase of the same is produced (Figure 1.4), linked to the increase of volatile oxidation products. This measure is called "**induction period (IP)**", "**induction time (IT)**" or "**Oil Stability Index (OSI)**" (Figure 1.5). This value characterizes the resistance of the sample to oxidation. The longer the induction time, the more stable a sample is.

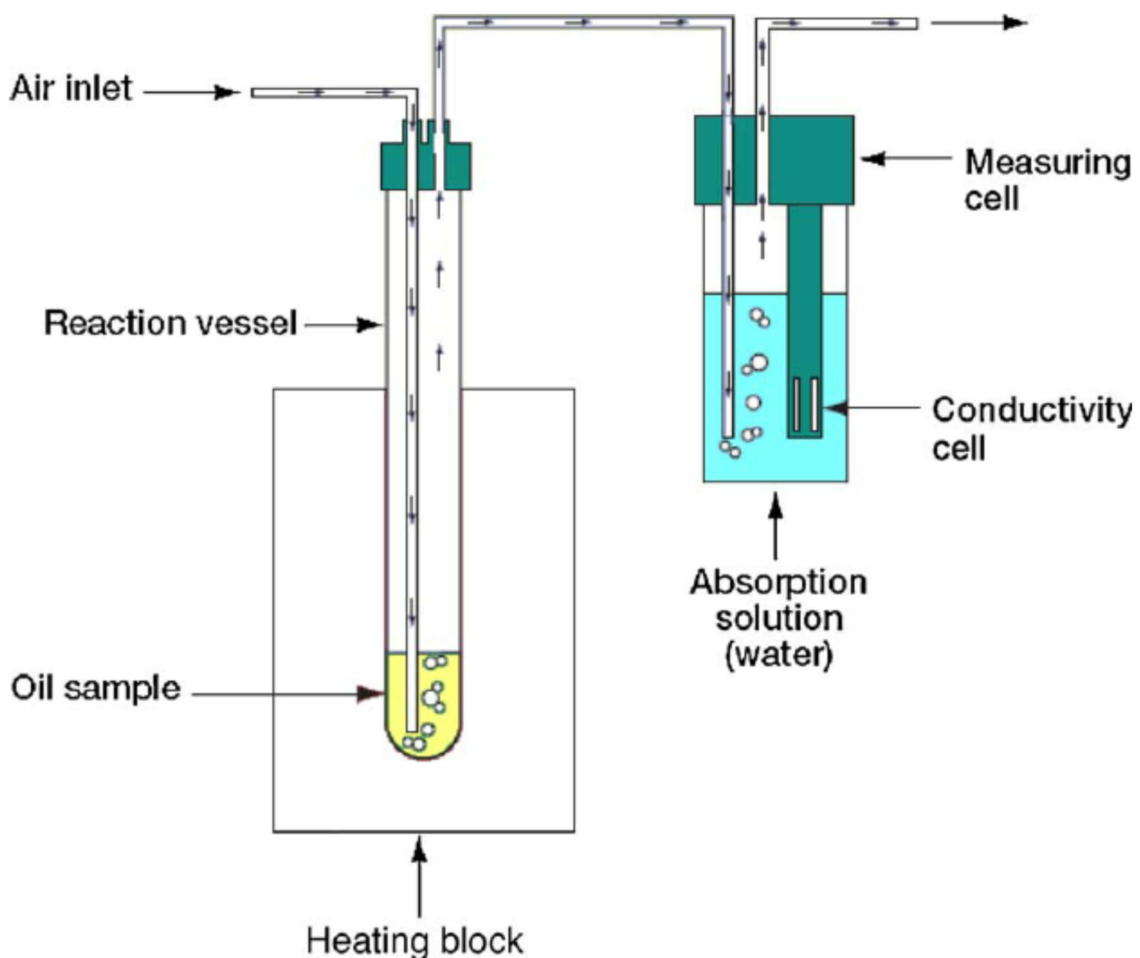


Figure 1.4. Schematic representation of the Rancimat method (Jain and Sharma, 2010).

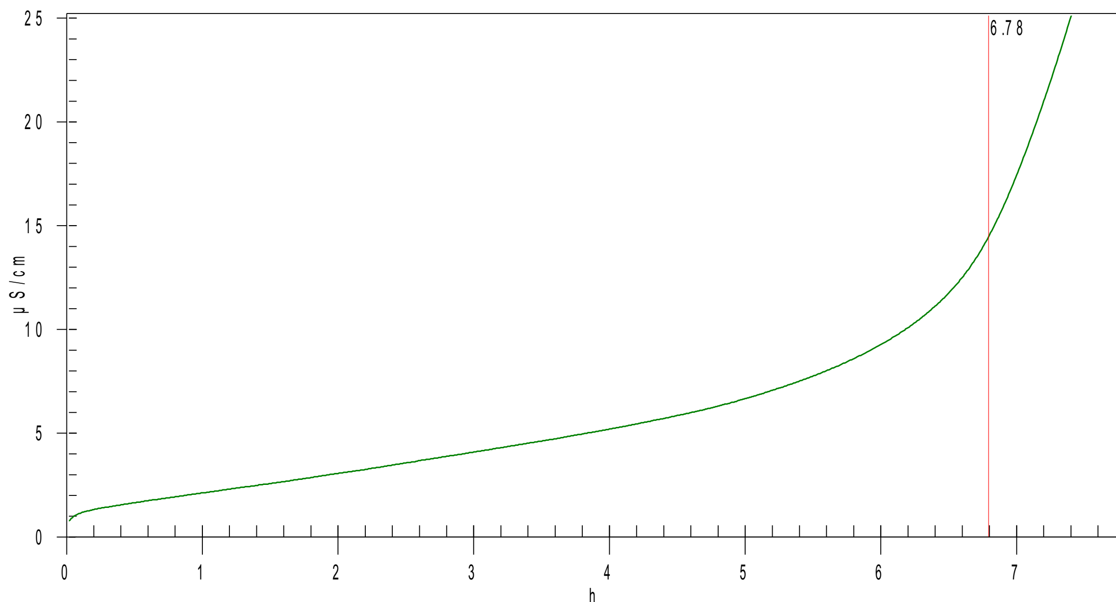


Figure 1.5. Example of conductivity measurement in Rancimat. Horizontal line indicates the induction period.

Sample weight, air flow rate, and temperature are the operational parameters that can be adjusted easily in the Rancimat method and may affect the determination of the induction time (Pawar *et al.*, 2014).

The Rancimat method is included in various national and international standards, such as:

- American Oil Chemists' Society Cd 12b-92, Sampling and analysis of commercial fats and oils: Oil Stability Index.
- International Organization for Standardization ISO 6886 Animal and vegetable fats and oils – Determination of oxidative stability (accelerated oxidation test).
- Japan Oil Chemists' Society 2.4.28.2–93 fat stability test on autoxidation, Conductometric Determination Method 2.4.28.2–93.

Practical applications of Rancimat test:

1. **Compare** the resistance to oxidation of different oils and fats.
2. Examine **the effectiveness of antioxidants** to protect lipid oxidation in oils and fats (Yanishlieva and Marinova, 2001).
3. "Screening" to predict the **frying performance** of oils and fats (Farhoosh and Moosavi, 2007). It cannot guarantee or predict the actual frying performance of the

oil, but it is considered that this method can be useful to act as a "screening" test and eliminate the possibility of using low stability oils or fats for frying. Moreover, Rancimat method can be indicative of quality deterioration of vegetable oils, since a strong correlation with some parameters of oil oxidation and IP has been established:

- → Total polar compounds (TPC) ($r^2 = 0.9949$) determined in seven frying oil samples during frying. Assuming that 24% of TPC is the maximum level permitted in frying oils, the IP of a used frying oil should be ≥ 2.32 h (Farhoosh and Moosavi, 2007).
- → Total polar compounds (TPC) ($r^2 > 0.98$) found in the determination of sunflower oil during 32 h frying (Farhoosh and Tavassoli-Kafrani, 2011).
- → Carbonyl value (CV) ($r^2 = 0.9856$) determined in seven frying oil samples during frying. Assuming that 50 mmol/g of CV is the maximum level permitted in frying oils, the IP of a used frying oil should be ≥ 2.05 h (Farhoosh and Moosavi, 2007).
- → Conjugated diene value was closely related to IP, determined in 15 samples of sunflower blends stabilized with oleoresin rosemary (Upadhyay, Sehwag and Niwas Mishra, 2017).
- → Differential scanning calorimetry (Tan *et al.*, 2002).
- → Peroxide value (Gordon and Mursi, 1994).
- → Electron spin resonance spectroscopy (Velasco, Andersen and Skibsted, 2004).
- → Sensory evaluations of rancid flavours and odours in oils (Coppin and Pike, 2001; Broadbent and Pike, 2003).

4. **Shelf-life.** Attempts to predict the shelflife of oils based on their induction period determined by Rancimat have been documented; however, the validity of the methods has been questioned (Mancebo-Campos, Salvador and Fregapane, 2007). The induction period only gives an indication which must be correlated with the actual shelf-life of a sample using storage tests. The mechanisms of lipid oxidation under Rancimat conditions and at ambient temperature are substantially different. Furthermore, in measurements with the Rancimat, air is continuously fed through the

sample, whereas under normal storage conditions, the sample is generally stored in air tight containers.

Factors that influence oxidative stability

The fatty acid composition, presence of metals such as iron and cupper, antioxidants or photosensitizers (clorophyll) are some of the factors that can influence the oxidative stability of oils and fats.

Even though fatty acid composition and other minor compounds such as antioxidants are known to be of fundamental importance for the process of lipid oxidation, consistent quantifications of the magnitude of their relative contribution to oxidative stability have proved elusive in edible oils and fats. Furthermore, although the association between fatty acid composition (saturated vs unsaturated) and oxidation is well documented, studies have usually demonstrated substantial deviations from the simple expectation that "the higher the content of unsaturated fatty acids the lower the corresponding oxidative stability" (Kerrihard *et al.*, 2015). Non-saponificable components, naturally present in oils and fats such as tocopherols or phenol compounds could also significantly affect oxidative stability (Kamal-Eldin, 2006). Previous studies could not detect correlations between chemical composition and oxidative stability of edible oils (Bozan and Temelli, 2008; Ayyildiz *et al.*, 2015); meanwhile others reported significant differences between refined and cold-pressed oils (Castelo-Branco *et al.*, 2016).

1.1.6. LIPID OXIDATION DURING FRYING PROCESS

The frying process

Frying is one of the most common cooking techniques used in domestic and industrial food preparation. The unique organoleptic and sensory attributes of fried products including flavour, colour, texture and aroma makes this cooking practice popular and desirable among consumers. The frying process includes the immersion of the food pieces in hot oil (150–190 °C); during this process, the simultaneous transfer of heat and mass between the oil and food occurs (Hosseini *et al.*, 2016). While both the process as a cooking method and fried products are popular, there are several key disadvantages

associated with fried foods. Product disadvantages are principally associated with oil deterioration during the process.

Chemistry of frying oils

During frying, a variety of reactions cause a spectrum of physical and chemical changes. In the presence of oxygen (from either air or the product), food moisture and high temperature, the oil undergoes three deleterious reactions: hydrolysis, oxidation, and thermal alterations (polymerization, cyclization and isomerization):

→ **Hydrolysis**. It involves the breakdown of triglycerides in the presence of water and steam. It produces monoglycerides, diglycerides, free fatty acids and glycerol, eventually. Hydrolysis is preferable in oils with short and unsaturated fatty acids because the latter are more soluble in water than long and saturated fatty acids (Choe and Min, 2006a). For example, coconut oil contains high levels of fatty acids with less than C14 carbon chain length and it causes hydrolysis of the triacylglycerides (TAGs) and excessive smoke development (Gertz, 2014). The extent of hydrolysis also depends on the oil temperature, interface area between oil and the aqueous phase, amounts of water and steam.

→ **Oxidation**. The oxygen in deep-fat frying reacts with oil. Three types of oxidation mechanisms take place in deep-fried oil such as autoxidation, thermal oxidation and photosensitized oxidation. These processes have been previously explained in the present Thesis.

→ **Polymerization, cyclization and isomerization**, caused by thermal alteration.

These reactions result in the generation of a variety of compounds. Figure 1.6, shows the main compounds formed by the degradation or oxidation process during frying from TAG: non-esterified fatty acids (NEFA), diacylglycerols, monoacylglycerols, volatile compounds (alcohols, aldehydes, ketones, acids), TAG monomers and TAG polymers (Zhang *et al.*, 2012; Dobarganes and Márquez-Ruiz, 2015). The nature and rate of decomposition products depend, among others, on the composition of the oil (fatty acids pattern, unsaponifiable matter content), the mode of frying (temperature, time, contact

with oxygen, frying capacity, continuous or intermittent frying process), and finally the characteristics of the fried food (Gertz, 2014; Hosseini *et al.*, 2016).

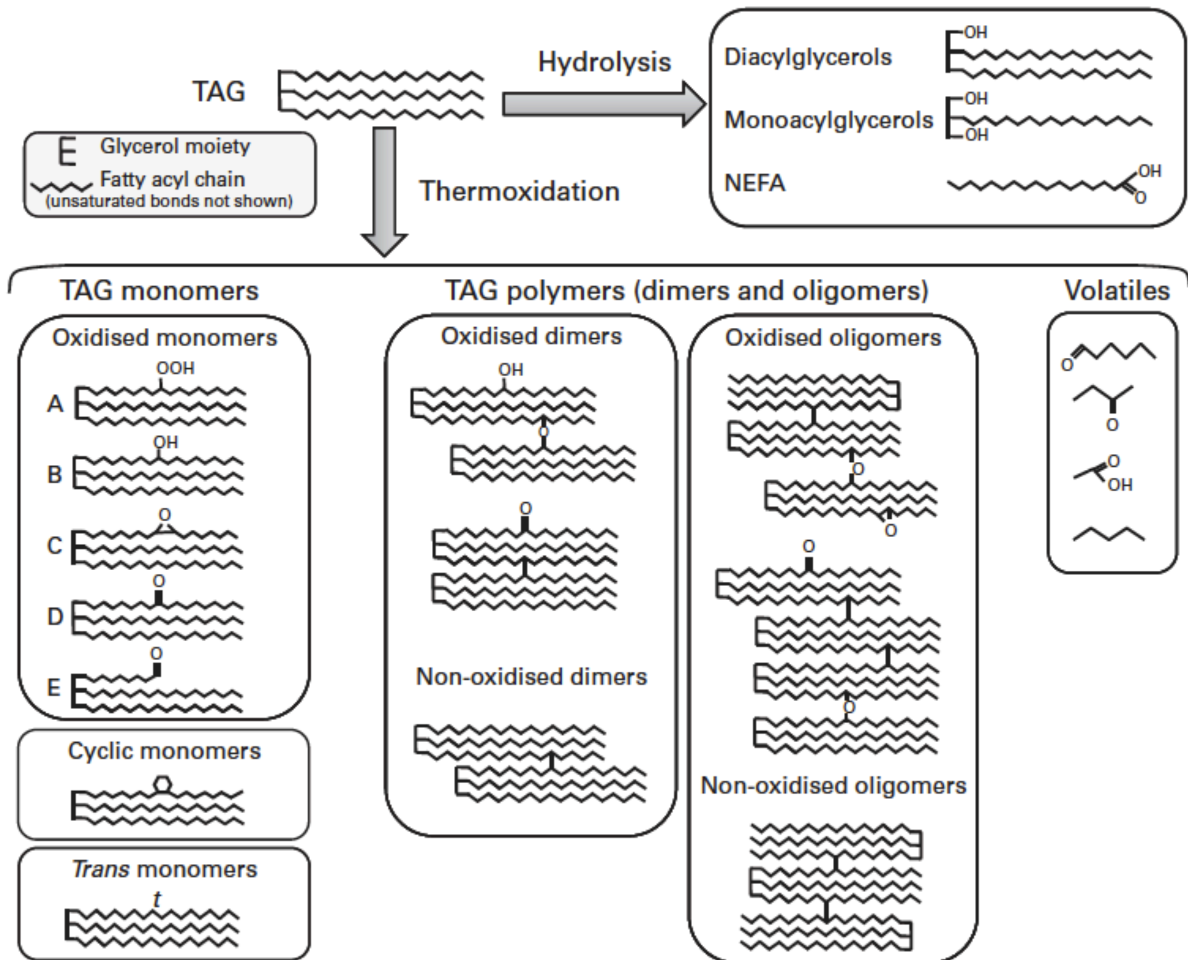


Figure 1.6. Schematic representation of the main groups of alteration compounds formed during frying. A, B, C, D and E are simplified structures of hydroperoxy, hydroxy, epoxy, keto and short-chain n-oxo fatty acyl groups, respectively, in oxidised TAG (Dobarganes and Márquez-Ruiz, 2015).

Common methods for determination of frying oil deterioration

Numerous analytical methods are used for measuring frying oil deterioration. However, as shown in Figure 1.3 and due to the complexity of lipid oxidation reactions (Schaich, 2012), there is a very high risk of **underestimating the process of oxidation when solely one or two molecules are analyzed**. The most important used methods to assess lipid oxidation and oxidative stability of oils and fats are described in Table 1.2.

Table 1.2. Common methods used to assess lipid oxidation and oxidative stability of oils and fats.

METHOD	DESCRIPTION
Fatty acid methyl esters (FAME)	Frequently used for determination of fatty acid (FA) composition. FA composition is affected during the frying process.
Iodine Value	Reflects the degree of unsaturation that could be attributed to oxidation and polymerization reactions involving the double bonds.
Free fatty acids (FFAs)	The increase in FFAs during frying could be attributed to hydrolysis and oxidation.
Peroxide Value (PV)	It measures total hydroperoxide content (primary product of oxidation). It is a classic method to evaluate oil oxidation, but it does not seem to be an accurate tool for the evaluation of heat-treated oils since hydroperoxides are unstable under frying conditions.
Conjugated Dienes (CDs) **and Trienes** (CTs)	Are formed during oxidation of unsaturated fatty acids containing two or more double bonds.
Thiobarbituric Acid (TBA) **Test**	TBA test measures malondialdehyde content. During lipid oxidation, malondialdehyde is formed as a result of the degradation of polyunsaturated fatty acids.
p-Anisidine Value (AV)	AV measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides.
Totox Value	It is a combination of PV and AV: Totox value = 2PV + AV. Is a measure of the total oxidation, including primary and secondary oxidation products.
Carbonyls	The carbonyl compounds, including aldehydes and ketones, are secondary oxidation products generated from degradation of hydroperoxides, and are considered to be the major contributors to off-flavors associated with the rancidity of many food products.

Viscosity	The increase in viscosity of frying oils is due to polymerization of fatty acids that results in the formation of higher molecular weight compounds.
CIE L*a*b* (CIELAB) **colour**	The oil appearance (colour) is one of the traditional methods used for assessing its quality. The L* value is an estimation of the luminosity and indicates dark (0-50) or white (51-100); a positive number indicates red and a negative number indicates green for thea* scale, whereas the b* value is positive for yellowish colors and negative for the bluish ones. With the formation and degradation of polymers in the oxidation process, the color of oil becomes darker.
Total polar compounds (TPC)	Polar compounds represent all the fatty acid breakdowns with higher polarity than TAG, comprising of oxidised and polymerised TAG, DAG and FFA.
Polymer triglycerides (PTG)	Polymers form one of the groups of TPC and, of those originating at the high temperatures of the frying process, are the most representative. Polymers include dimmers, trimers, and tetramers, and may be formed through oxidative and thermal reactions.
Electron spin resonance (ESR)	Relies on the paramagnetic properties of the unpaired electrons in radicals and has been developed for assessing the formation of free radicals originating at the early stages of oxidation and the onset of primary oxidation.
Differential Scanning Calorimetry (DSC)	This technique is used in lipid chemistry to assess the melting and crystallization characteristics of edible oils, heat of fusion, and polymorphism. Furthermore, DSC has been successfully applied for the assessment of the identity of vegetable oils and changes during oxidation.
Infrared spectroscopy	It can be used for identification, characterization, qualitative and quantitative analysis and monitoring the parameters of edible oils.

Nuclear Magnetic Resonance (NMR) **Spectroscopy**	NMR spectroscopy is a well-recognized technique to check the identity and structure of chemical compounds. It can determine the molecular nature of the lipid components, as well as their concentration, in just one fast run without any sample modification.
Smoke point	It is the temperature at which an oil, heated at a rate of 5-6ºC/min, begins to produce a continuous bluish smoke that becomes clearly visible. Smoke point does not predict oil performance when heated (de Alzaa, Guillaume and Ravetti, 2018), but can be considered an indicator of the temperature limit up to which an oil can be heated.
Rancimat method	It evaluates the stability or resistance of an oil or fat to oxidation. It is described in 1.1.5. section of the present Thesis. It is considered that the Rancimat induction period can be useful as a "screening" test to eliminate the possibility of using low-stability oils with undesirable consequences in human heath for cooking or manufacturing purposes.

Each country has its own regulations concerning the quality of frying oils (Stier, 2013), for example, health and safety regulations in Spain determine that polar components should be less than 25% (Boletín Oficial del Estado, 1989). However, there is no uniform and standard method for detecting **all oxidative changes** in oils and fats. For example, a study has found significant concentrations of toxic aldehydes present in the oil albeit the 25% limit for polar compounds is reached in the oil (Guillén and Uriarte, 2012). Some researchers assert that potentially toxic compounds can appear in oil even if the regulation is accomplished (Oke *et al.*, 2017).

1.1.7. OXIDATIVE COMPOUNDS AND THEIR HEALTH IMPACT

Potentially toxic lipid oxidation products formed during frying performance

About 400 types of decomposition products have been identified during the frying process, some of which may be detrimental to health including approximately 50

substances that were identified with a potential risk for cancer (Hosseini *et al.*, 2016). The major toxic compounds that are generated during the frying process are the following (Aladedunye, 2016; Vieira, Zhang and Decker, 2017):

- → **4-hydroxynonenal (HNE)**: is the most important toxic aldehydic compound formed by the decomposition of hydroperoxides, with respect to concentration levels and degree of toxicity. It is formed mainly from thermo-oxidative degradation of linoleic acid and other ω-6 fatty acids. HNE has been shown to exhibit mutagenic, cytotoxic and genotoxic properties, which are related to pathogenesis of several human diseases such as Alzheimer's, atherosclerosis, Parkinson's disease, cataract, and severe malaria anemia (Aladedunye, 2016).
- → **Crotonaldehyde** (2-butenal) is a four carbon α,β-unsaturated aldehyde formed from unsaturated fatty acids. Crotonaldehyde is produced mainly from the decomposition of hydroperoxides resulting from oxidation of ω-3 polyunsaturated fatty acids (Papastergiadis *et al.*, 2014). Crotonaldehyde has a strong reactive electrophillic carbonyl group, which allows it to conjugate with glutathione thus reducing glutathione within the body. The electrophilic nature of crotonaldehyde means that it can form propanodeoxyguanosine adducts in DNA resulting in mutagenic, genotoxic, and carcinogenic activity, and thus it can produce hepatic tumours in rodents (Vieira, Zhang and Decker, 2017).
- → **Acrylamide** (2-propenamide) and **acrolein** (2-propenal). Acrylamide is a toxic compound mainly produced by the Maillard reactions involving asparagines and reducing sugars. Acrylamide can be also formed during frying of oil from acrolein, which reacts with ammonia coming from amino acids. On one hand, acrolein is formed from the degradation of glycerol (when the triacylglycerols are hydrolyzed into glycerol and fatty acids) and, on the other hand, because of the oxidation of polyunsaturated fatty acids (Daniali et al., 2016). Acrylamide is classified as probably carcinogenic to humans and animals (group 2A) by the International Agency for Research on Cancer (IARC), meanwhile acrolein is classified in group 3 (not classifiable as to its carcinogenicity to humans) by the IARC.
- → **Malonaldehyde** (MDA) is one of the most abundant fatty acid decomposition products (aldehydes) in foods. Fatty acids with at least three double bonds are

required for the formation of MDA. MDA is known to be mutagenic to humans because it can form adducts with proteins and DNA.

→ ***Trans* fat**. Although frying at normal temperature (160–185 °C) does not appreciably increase the level of *trans* fats in the frying oils, frying at much higher temperatures or under poor management can significantly increase the *trans* fatty acids contents of fried foods (Aladedunye, 2016). A systematic review and meta-analysis of observational studies revealed that total *trans* fatty acids intake was associated with all-cause mortality, coronary heart disease (CHD) mortality, and total CHD (de Souza *et al.*, 2015). *Trans* fatty acids have also been shown to have pro-inflammatory effects, including aggravating the symptoms of gut inflammation in patients with gut inflammation, such as inflammatory bowel disease.

Health effects of deep-fried oil consumption

It is very complicated to determine the health effects of fried oils, since this depends on the oil used and frying procedure and, as expected, the results of the studies are controversial. As reported by Dobarganes and Márquez-Ruiz (2015), "on the one hand, those researchers who found high levels of toxicity used abusive heating conditions in an attempt to generate sufficient amounts of degradation products, but the level and structures of the compounds thus formed are not representative of those encountered in oils subjected to normal culinary practices. On the other hand, some researchers applied very soft conditions when heating oils disregarding that the use of good practices in the frying process is obviously safe".

It is known that **oxidative stress** is implicated in the pathogenesis of various degenerative diseases including cancer, Alzheimer disease, myocardial infarction, kidney dysfunction, diabetes mellitus, and its complications (Falade, Oboh and Okoh, 2017). Free radicals formed during thermal oxidation in fried oils, could explain the possible link between consumption of fried food and some pathologies (Figure 1.7).

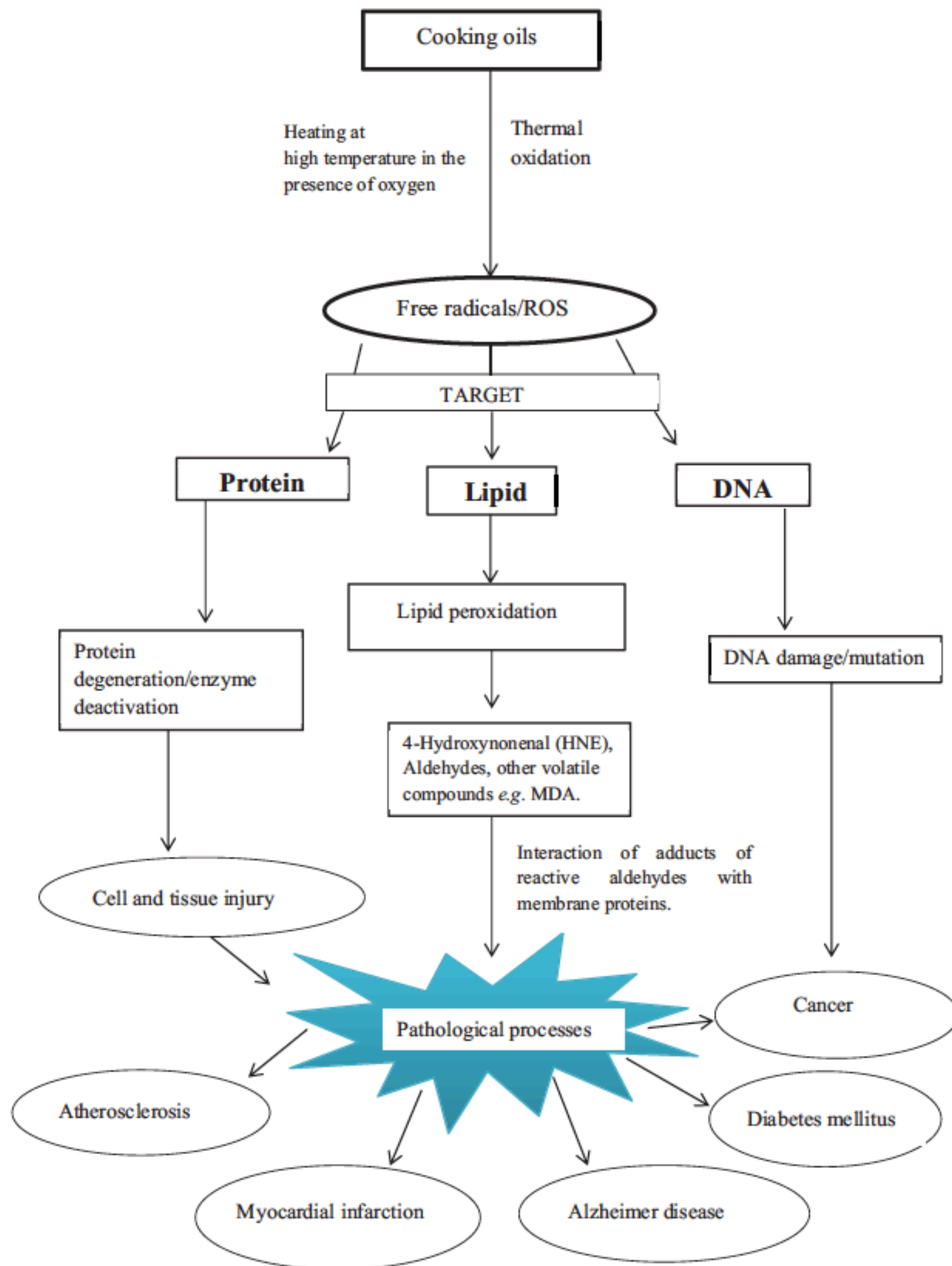


Figure 1.7. Pathway showing the link between consumption of thermally-oxidized cooking oils and some pathology (Falade, Oboh and Okoh, 2017).

Regarding **epidemiological studies**, Gadiraju *et al.* (2015) reviewed fried food consumption and cardiovascular health. According to their findings, frequent consumption of fried foods (i.e., four or more times per week) is associated with a higher

risk of developing **diabetes, heart failure, obesity and hypertension**. Mixed results have been generated regarding the association of fried foods with coronary artery disease; for example, null findings are reported by the consumption of fried food in the context of Mediterranean diet, surely due to the use of olive oil and the context of a healthy diet.

Consumption of heated cooking oils has been associated with **higher blood pressure** (Jaarin, Masbah and Nordin, 2016). For example, the consumption of heated (twice, five times and ten times) palm and soy oil increases blood pressure in rats, meanwhile respective fresh oils had no effect on blood pressure. Heated palm and soy oils cause an increase in aortic vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression; heated corn oil and virgin coconut oil cause an increase in soluble VCAM-1, soluble ICAM-1 and C-reactive protein; and heated canola oil induced presence of nitrotyrosine in the aorta. Moreover, postprandial **inflammatory response** was increased after ingestion of heated sunflower oil as evidenced by increased NFkB and other inflammatory molecules like TNF-α, IL-1β and IL-6 in a randomized crossover study (Jaarin, Masbah and Nordin, 2016). Ganesan, Sukalingam and Xu (2018) confirmed in a review that the repeatedly heated vegetable oils increase the effect of lipid peroxidation and aggravate the development of **cardiovascular diseases**.

Ganesan, Sukalingam and Xu (2017) also reviewed the impact of the consumption of repeatedly heated cooking oils on the incidence of **cancer**. These oils and its cooking fumes has been found to enhance the incidence of aberrant cells, including breaks, fragments, exchanges and multiple chromosomal damages and micronuclei in a dose-dependent manner. Furthermore, consumption of deep-fried foods has been associated with a number of malignancies, including prostate, breast, oral/pharyngeal, oesophageal and laryngeal cancers (Dobarganes and Márquez-Ruiz, 2015).

Zhou *et al.* (2016) investigated the changes in energy metabolism, **colon histology and gut microbiota** in rats following deep-fried oil consumption. Rats were randomly divided into three groups: group 1 (basal diet without extra oil consumption; control group); group 2 (basal diet supplemented with non-heated canola oil); and group 3 (basal diet supplemented with deep-fried canola oil). Fresh canola oil was heated at 190 ± 5°C for 4 intermittent days (8 h each day) for a total of 32 h. Rats supplemented with deep-fried canola oil had associated inflammation in intestinal mucosa. Moreover, they had the lowest intestinal bacterial community diversity and showed the lower proportion of

Prevotella and the highest proportion of Bacteroides among the three groups. These findings in microbiota composition are associated with negative effects in health.

Other studies in animals have focused in other outcomes. For example, **digestibility** has been generally found to decrease upon the consumption of fried oils or oils heated at frying temperatures; specifically, it has been demonstrated that triacylglycerol polymers are poorly hydrolysed by pancreatic lipase (Dobarganes and Márquez-Ruiz, 2015).

1.2. ANTIOXIDANTS TO REDUCE OXIDATION IN OILS AND FATS

Antioxidants can be used to retard lipid oxidation in fats and oils. An antioxidant is defined as any substance that, when present at low concentrations compared with those of an oxidizable substrate, delays or prevents the oxidation of that substrate (Budilarto and Kamal-Eldin, 2015).

1.2.1. MECHANISMS OF ACTION OF ANTIOXIDANTS IN OILS AND FATS

Two kinds of antioxidants, namely primary and secondary antioxidants, have been classified based on their mechanisms of action in inhibiting lipid oxidation reactions. The **primary antioxidants** act as hydrogen donors or radical scavengers: these react with lipid hydroperoxyl radicals producing lipid hydroperoxides and more stable, low energy, antioxidant radicals, which are significantly much less reactive in propagation reactions. Flavonoids, tocopherols, hydroxytoluene (BHT), butylated hydroxyanisole (BHA) or tertbutylhydroquinone (TBHQ) are examples of primary antioxidants. The **secondary antioxidants** are preventive antioxidants that enhance the inhibitory activity of primary antioxidants. This class of antioxidants can chelate pro-oxidants agents, provide hydrogen to primary antioxidants, decompose LOOH to non-radical species, scavenging ground state and singlet oxygens, or absorbing UV light. Ascorbates, phytic acid, citric acid, EDTA and lecithin are examples of secondary antioxidants (Budilarto and Kamal-Eldin, 2015). Combinations of primary and secondary antioxidants are often found more

effective in retarding lipid oxidation than the sum of their single actions (Budilarto and Kamal-Eldin, 2015).

1.2.2. NATURAL ANTIOXIDANTS

Synthetic antioxidants such as BHT, BHA or TBHQ are often used to retard lipid oxidation in food systems. Due to their low thermal stability, the concern about their long-term effects on human health and the increasing demand by consumers for natural products, plant extracts emerged as good alternatives to synthetic antioxidants (Taghvaei and Jafari, 2015; Şahin *et al.*, 2017). Possible sources of natural antioxidants are spices, herbs, teas, oils, seeds, cereals, cocoa shell, grains, fruits, vegetables. A large number of reports focusing on the antioxidant activity of natural products in edible oils/fats has been published and, in general, their antioxidant capacity is a promising alternative to the use of synthetic compounds (Table 1.3).

Table 1.3. Use of natural products with antioxidant properties in edible oils.

PLANT EXTRACT	APPLIED EDIBLE OIL/FAT	MEASUREMENT CONDITIONS	REFERENCE
Rosemary extract	Rice bran oil	Oxidative stability index (110ºC)	Jennings and Akoh (2009)
Essential oils from rosemary, clove and cinnamon	Hazelnut and poppy oils	50ºC for 14 days	Özcan and Arslan (2011)
Rosemary extract	Mixture of soybean and sunflower oils	180ºC for 30 h	Chammem *et al.* (2015)
Ground rosemary plant (and then filtered), its alcoholic extracts and essential oil	Hazelnut oil	Frying process	Tohma and Turan (2015)

Beetroot, carrot, tomato and swede (powder)	Rapeseed oil	Rancimat method (120ºC)	Tundis *et al.* (2017)
Olive leaf and lemon balm extracts	Corn oil	Rancimat method (130ºC)	Şahin *et al.* (2017)
Rosemary extract	Soybean oil, rice bran oil, and cottonseed oil	Rancimat method (120ºC) and Schaal oven test (62ºC)	Yang *et al.* (2016)
Rosemary and thyme leaves macerated in oil	Soybean oil	180ºC for 24 h	Saoudi *et al.* (2016)
Rosemary extract	Palm oil	Frying process and Schaal oven test	Guo *et al.* (2016)
Rosemary extract and ferulago	Mixture of sunflower oil and palm olein	Frying process	Alizadeh, Nayebzadeh and Mohammadi (2016)

In recent years, a significant body of research literature with respect to the antioxidant activities of plant extracts in edible oils is available (Taghvaei and Jafari, 2015). However, the use of the whole ground plants (instead of extracts) or maceration of this plants in oils has attracted little attention. Commercially available plant extracts are suitable mainly for food manufacturing at industrial scale, whereas the use of the whole ground plants could be a good option both for the food industry and for domestic use. Moreover, there is higher antioxidant activity in whole plants than in their corresponding extracts prepared from an equivalent amount. This suggests that a plethora of antioxidant compounds are removed in the process of extraction, which may have an accumulative or synergistic effect on the oxidative stability of the end-product (Yanishlieva, Marinova and Pokorný, 2006).

Hypothesis and objectives

CHAPTER 2. HYPOTHESIS AND OBJECTIVES

2.1. BACKGROUND

Lipid oxidation has been identified as the major deterioration process of oils and fats affecting food quality, nutrient value, safety, colour, development of off-flavours and consumers' acceptance. Moreover, some of the compounds formed during oxidation may have undesirable consequences on consumers' health (Hosseini *et al.*, 2016).

Even though fatty acid composition and other minor compounds such as antioxidants are known to be of fundamental importance for the process of lipid oxidation, consistent quantifications of the magnitude of their relative contribution to **oxidative stability** have proved elusive in edible oils and fats. Previous studies could not detect correlations between chemical composition and oxidative stability of edible oils (Bozan and Temelli, 2008; Ayyildiz *et al.*, 2015); meanwhile others reported significant differences between refined and cold-pressed oils (Castelo-Branco *et al.*, 2016).

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) or tertbutylhydroquinone (TBHQ) are often used to retard lipid oxidation in food systems. Due to their low thermal stability, the concern about their long-term effects on human health and the increasing demand by consumers for **natural products**, plant extracts emerged as good alternatives to synthetic antioxidants (Taghvaei and Jafari, 2015; Şahin *et al.*, 2017). However, commercially available plant extracts are suitable mainly for food manufacturing at industrial scale, whereas the use of the whole ground plants could be a good option both for the food industry and domestic use. Moreover, there is higher antioxidant activity in whole plants than in their corresponding extracts prepared from an equivalent amount. This suggests that a plethora of antioxidant compounds are removed in the process of extraction, which may have an accumulative or synergistic effect on the oxidative stability of the end-product (Yanishlieva, Marinova and Pokorný, 2006).

2.2. HYPOTHESIS

Hypothesis 1:

The oxidative stability of oils and fats is determined by the levels of fatty acids, tocopherols, carotenoids, chlorophyll and/or total phenol content.

Hypothesis 2:

The use of natural products with established antioxidant effect, incorporated either as ground plants in powdered form or powder filtrate, protect edible fats and oils from oxidative deterioration. Natural products which are rich in phenolic compounds (see Table 2.1), increase the antioxidant capacity of oils and fats and are capable to delay the onset of the oxidation process.

Table 2.1. Antioxidant capacity and total phenolic compounds in spices.

	Antioxidant capacity	**Total phenolic compounds**
	μM Fe^{+2} per g	mg GAE per g
Black pepper	82.78 ± 1.42	50.16 ± 13.89
Ginger	252.04 ± 11.55	121.53 ± 3.88
Turmeric	166.01 ± 0.69	239.96 ± 32.17
Rosemary	573.35 ± 72.74	410.16 ± 22.39
Oregano	290.20 ± 2.73	155.25 ± 17.79

Abbreviations: GAE; gallic acid equivalents.

Data from Redondo-Cuevas, Castellano and Raikos, 2017.

2.3. OBJECTIVES

The main objective of the present Thesis is, on one hand, to elucidate the complex relationship between composition of selected oils and fats and their oxidative stability as determined by experimental procedures. And, on the other hand, to identify natural product(s) of known phytochemical profile with documented antioxidant properties that can be used to increase the oxidative stability of the most commonly used oils used for industrial and domestic applications. These objectives are further detailed in Parts 1 and 2.

PART 1

Main objective:

Determine the main factor(s) which determine the oxidative stability of oils and fats.

Secondary objectives:

- → Investigate the composition of a range of commercially available oils and fats in relation to tocopherols, carotenoids, chlorophyll, total phenol content and fatty acid profile.
- → Determine the oxidative stability of the above mentioned oil and fat samples.
- → Perform statistical analysis to co-relate the composition of oils and fats with their oxidative stability. Apply principal component analysis (PCA) to identify the behaviour of fats and oils, and a simple linear regression model, as well as a multiple linear regression model, in order to determine the factor(s) that account for the measured oxidative stability.

PART 2

Main objective:

To identify a natural product that can be used to increase the oxidative stability of the most commonly used oils used for industrial or domestic applications.

Secondary objectives:

- → Determine the antioxidant capacity and total phenolic content of five selected spices with documented antioxidant properties: black pepper, ginger, turmeric, rosemary and oregano.
- → Assess the quantitative effect of the natural antioxidants (ground plants) on the oxidative stability of tocopherol-stripped corn oil.
- → Select the most effective natural product (rosemary) and assess its effectiveness to confer protection against oxidation in common oils used for domestic cooking purposes (olive, sunflower and rapeseed oil). Investigate the antioxidant effect of the most potent natural product during a deep-frying process for all three selected vegetable oils.
- → Assess the antioxidant effect of rosemary in rapeseed oil following a filtration processing step (to remove solid residues).

2.4. WORK PLAN

To achieve the objectives, the work plan described in Figure 2.1 is developed:

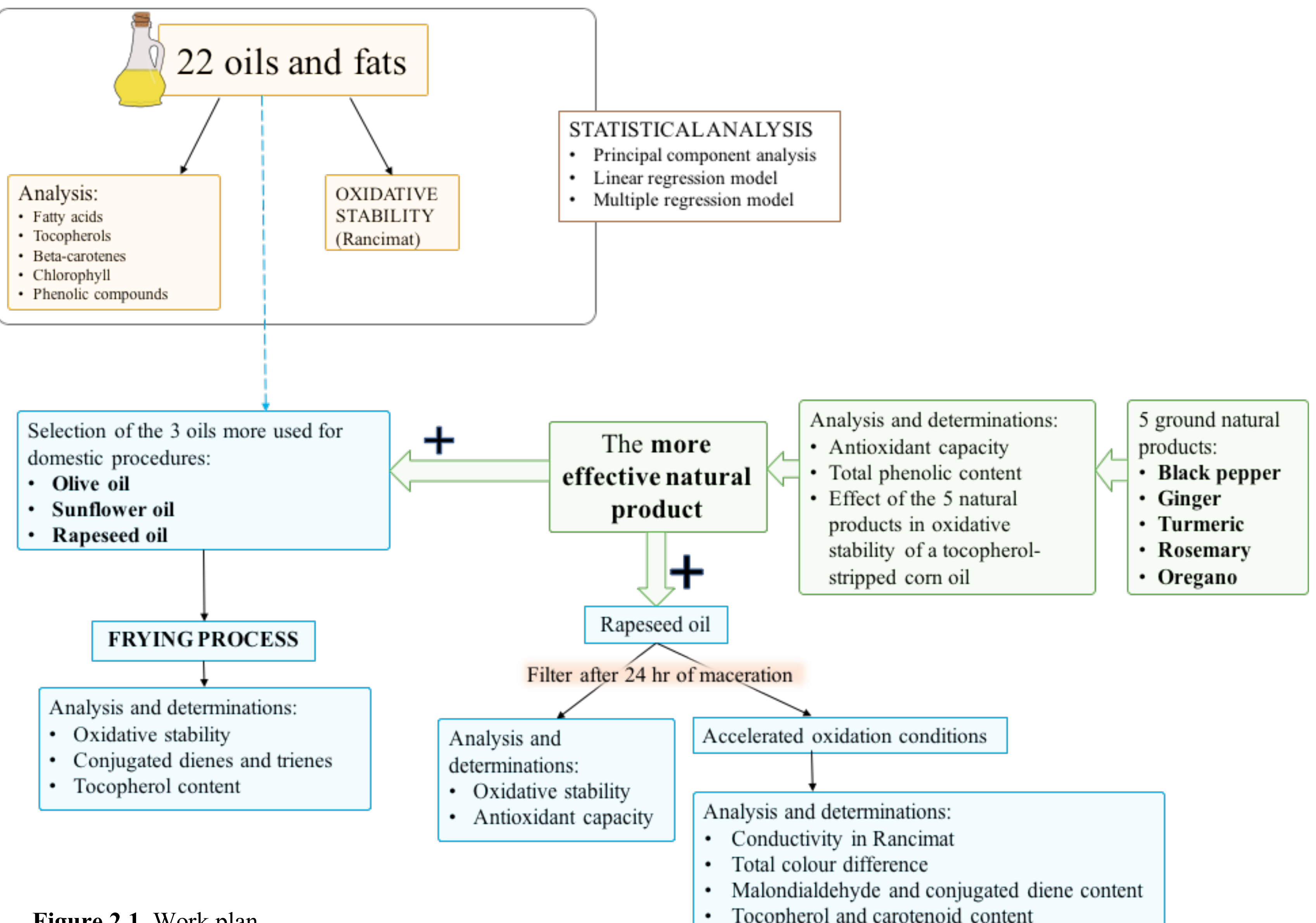
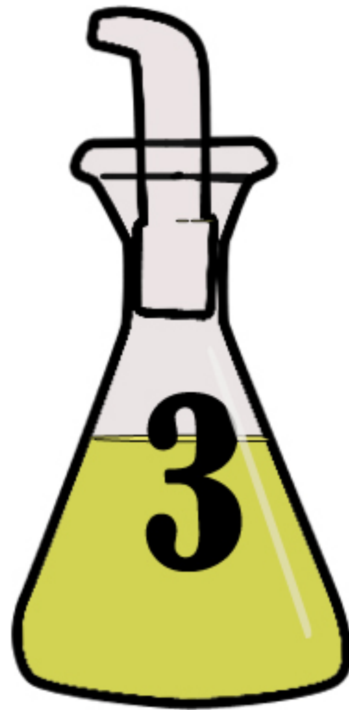


Figure 2.1. Work plan.



Material and methods

CHAPTER 3. MATERIAL AND METHODS

3.1. OILS AND FATS

Twenty-two oils and fats characteristics are detailed in Table 3.1. Products without the addition of tocopherols were selected. Oils and fats were stored in a dark cool place to protect them from oxidation. Butter was stored at -6 ºC to prevent oxidation.

Table 3.1. Abbreviated names, description and origin of oils and fats.

OIL OR FAT	CHARACTERISTICS	BRAND	PLACE OF PURCHASE
Corn oil	Tocopherol stripped, for laboratory use only	MP Biomedicals	Sigma–Aldrich, Co Ltd (Dorset, UK)
Olive oil	Blended (refined and virgin)	Tesco	Tesco supermarket (Aberdeen, Scotland)
Olive oil EV	Extra virgin	Tesco	Tesco supermarket (Aberdeen, Scotland)
Olive oil VC1	Virgin, from Cornicabra olives (100% ripe)	Villajos	Villajos, Agrovillasierra S.L. (Spain)
Olive oil EVC2	Extra virgin, from Cornicabra olives (80% green)	Villajos	Villajos, Agrovillasierra S.L. (Spain)
Olive oil VA1	Virgin, from Arbequina olives (70% green)	Villajos	Villajos, Agrovillasierra S.L. (Spain)
Olive oil VA2	Virgin, from Arbequina olives (10% green)	Villajos	Villajos, Agrovillasierra S.L. (Spain)
Olive oil EVP	Extra virgin, from Picual olives (50% green)	Villajos	Villajos, Agrovillasierra S.L. (Spain)
Olive oil EVH	Extra virgin, from Hojiblanca olives (10% green)	Villajos	Villajos, Agrovillasierra S.L. (Spain)
Sunflower oil	Refined	Tesco	Tesco supermarket (Aberdeen, Scotland)
Sunflower oil VCP	Virgin cold pressed, organic	NaturGreen	Sabor del Monte, Valencia (Spain)
Sunflower oil HO	High oleic, refined, organic	Spectrum	iHerb (USA)
Rapeseed oil	Refined	Goldenfields	Tesco supermarket (Aberdeen, Scotland)

Rapeseed oil VCP	Virgin cold pressed	Tesco	Tesco supermarket (Aberdeen, Scotland)
Sesame oil	Refined	Spectrum	iHerb (USA)
Sesame oil VCP	Virgin cold pressed, organic	Sabor del Monte	Sabor del Monte, Valencia (Spain)
Toasted sesame oil V	With toasted seed, virgin, organic	Spectrum	iHerb (USA)
Coconut oil	Refined	KTC	Tesco supermarket (Aberdeen, Scotland)
Coconut oil VCP	Virgin cold pressed, organic	Dr. Goerg	Sabor del Monte, Valencia (Spain)
Red palm oil V	Virgin, organic	Nutiva	iHerb (USA)
Butter		Tesco	Tesco supermarket (Aberdeen, Scotland)
Ghee		East End	Tesco supermarket (Aberdeen, Scotland)

3.2. NATURAL PRODUCTS (SPICES)

Ground organic black pepper (*Piper nigrum*) produced in India, ground organic ginger (*Zingiber officinale*) produced in India, ground organic turmeric (*Curcuma longa*) produced in India, and ground oregano (*Origanum vulgare*) produced in Turkey were purchased from Justingredients Limited (Monmouthshire, UK). Ground rosemary *(Rosmarinus officinalis)* produced in Spain was purchased in G Baldwin & Co (London, UK).

3.2.1. PREPARATION OF UNFILTERED OILS WITH SPICES

Ground black pepper, ginger, turmeric, oregano and rosemary were used at a concentration of 0.5% (w/v) and BHT at maximum permitted levels of 100 mg/kg (European Commission, 2011). Each of these materials were added to tocopherol-stripped corn oil and then mixed using a tube roller for 1 hr at room temperature before initiating the Rancimat analysis. The most potent plant in terms of increasing the oxidative stability was mixed with "olive oil", "sunflower oil" and "rapeseed oil" (described in Table 3.1) following the same procedure.

3.2.2. PREPARATION OF FILTERED OILS WITH SPICES

Rapeseed oil described in Table 3.1 was used to this preparation. Rapeseed oil was mixed with the following concentrations of the plant powder (rosemary): 0.25%, 0.5%, 1.0% and 2.0% (w/w), and plain oil was used as control. The samples were mixed for 24 hr on a Stuart SRT6 tube roller (Cole-Palmer, Staffordshire, UK) at room temperature and then filtered with Whatman filter paper no. 1 (Sigma-Aldrich, St Louis, MO, USA) to remove solids. Samples were immediately used for Rancimat analysis and the remaining amounts were stored at -20°C for further analysis.

3.3. RANCIMAT APPARATUS

3.3.1. INDUCTION PERIOD DETERMINATION

Oxidative stability of oils and fats were determined by the oxidation induction period (IP) in a 743 Rancimat apparatus (Metrohm Ltd., Herisau, Switzerland; Figure 3.1) according to the AOCS Official Method Cd 12b-92 (American Oil Chemists' Society, 1998). Oil samples (3 g) were heated at 120°C with a constant airflow of 20 L/h. The times required for a sharp increase in water conductivity is calculated automatically by the software and corresponds to the IP in hours. The operating mechanism is described in 1.1.5. section (Introduction). Measurements were taken in quadruplicate.

3.3.2. ACCELERATED OXIDATION TEST

Rancimat apparatus was also used to create temperature-controlled accelerated oxidation conditions for predefined periods of time. For the accelerated oxidation test, the samples were placed into the Rancimat vessels and were heated at 120°C with a constant airflow of 20 L/h for 1.5 and 3 hr. In this case, the water conductivity was manually reported and the samples were removed from the vessels at the specified time intervals for further analysis. Measurements were taken in quadruplicate.

Figure 3.1. 743 Rancimat apparatus.

3.4. FRYING PROTOCOL

The frying process was carried out on commercially available oils namely olive, sunflower and rapeseed oil. Rosemary powder (0.5% w/v) was allowed to mix for 1 hr using a tube roller. Fresh potatoes were purchased in Tesco supermarket (Aberdeen, Scotland) and were peeled, washed and sliced into discs of 3 mm thickness using a Swan SFS102 food slicer (Swan Products Ltd, Staffordshire, UK). After equilibrating 2.5 L of oil for 10 min at 180°C in an electric deep-fryer (Tesco 3L Pro Fryer; Figure 3.2), four batches of potato slices (150 g per batch) were consecutively introduced into the oil for 5 min each; this protocol was used based on Akil *et al.* (2015). The oil was then allowed to cool down for 2 hr at room temperature. Following the frying process, samples were taken immediately for Rancimat analysis and remaining sample was frozen at −20°C for further analyses. Fresh oil (control) was also stored at −20 °C for subsequent analyses.

Figure 3.2. Frying process.

3.5. CHEMICAL ANALYSIS IN OILS AND FATS

3.5.1. FATTY ACID COMPOSITION ANALYSIS

The fatty acid composition of fats and oils was determined by analysing their methyl ester derivatives with gas-liquid chromatography (Liu, 1994). Analysis of the fatty acid methyl esters (FAMEs) was carried out using a gas chromatograph (HP6890, Hewlett Packard, Avondale, PA) using 50 m × 20 mm Chrompac CP7488 CP Sil-88 capillary column (film thickness 0.20 µm). Helium was used as carrier gas at a rate of 0.5 ml/min, and the split/splitless injector was used at a split ratio of 20:1. The injector and detector temperatures were 250°C. The column oven temperature was maintained at 80°C for 1 min after sample injection and was programmed to increase then at 25°C/min to 160°C where it was maintained for 3 min. Temperature was then increased to 190°C at 1°C/min and then to 230°C at 10°C/min. The temperature was maintained at 230°C for 30 min. Separation was recorded with HP GC Chemstation software (Hewlett Packard, Avondale,

PA). The FAMEs were identified by comparison to previously essayed standards. Measurements were taken in duplicate. Results are expressed as % of total fatty acids.

3.5.2. TOCOPHEROL AND CAROTENOID CONTENT

A reverse phase HPLC method was used to quantify beta-carotene, alpha-tocopherol, gamma-tocopherol and delta-tocopherol in the 22 oils and fats using fluorescence and visible detection according to Hess *et al.* (1991). The measurements were taken in triplicate.

Carotenoids and tocopherols were extracted from the oil phase as follows: 20 mg of oil was mixed with 280 μL H_2O and 400 μL ethanol. Each tube was vortexed for 10 seconds and 700 μL of hexane (containing BHT) and 100 μL of echinone were added and the samples were shaken for 10 min in the vortex genie before centrifugation for 5 min. The supernatant hexane layer (600 μL) was removed and dried down on the speed vacuum for 10 min. Each sample was then dissolved in 200 μL of DEA (20 % (v/v) 1,4 dioxan, 20 % (v/v) ethanol, 60 % (v/v) acetonitrile) and was shaken for 5–10 min before injected for HPLC analysis. The HPLC analysis was performed using a Waters 717 plus Autosampler Module (Waters Corporation, Milford, USA) equipped with a Waters 2475 scanning fluorescence detector, a 2487 UV/VIS absorbance detector and a C-18 silica (Beckman Ultrasphere ODS) analytical column (250 × 4.6 mm ID 5 μm particle size). The eluent used was acetonitrile 67.4%, tetrahydrofuran 22%, methanol containing BHT 6.8%, 1% (w/v) and ammonium acetate 3.8%. Elution flow rate was 1.1 ml/min, sample run was 30 min and injection volume was 150 μL. Measurements were determined with mixed standards containing carotenoids and tocopherols at appropriate concentrations and results were expressed in μg/g of oil. Echinone was used as an internal standard.

3.5.3. TOTAL PHENOLIC CONTENT (TPC)

Extraction and testing sample preparation was performed as follows: 1 g of oil was measured into a test tube and then 3 ml of methanol were added. The test tube was vortexed and then centrifuged at 6000 rpm using a CompactStar CS4 centrifuge (VWR International Ltd, West Sussex, UK) for 5 min and the supernatant was collected. The oil residues were re-extracted twice with methanol (3 mL × 2). The three methanol extracts

were combined and the final volume was brought to 10 ml with methanol to obtain the testing sample solutions. The resulting antioxidant solution was then kept at 4°C in dark for one night until the analysis.

The TPCs of the samples were determined using the Folin-Ciocalteu reagent as described by Parry *et al.* (2005). In brief, the reaction mixture contained 50 µL of testing sample solutions, 250 µL of the Folin-Ciocalteu reagent, 0.75 mL of 20% sodium carbonate and 3 mL of pure water. After 2 h of reaction at ambient temperature, the absorbance at 765 nm was measured by a Pye Unicam UV-4 UV-VIS scanning spectrophotometer (Spectronic Camspec Ltd., Leeds, UK) and was used to calculate the phenolic contents of oils using gallic acid as standard. Measurements were taken in triplicate. The results were expressed as gallic acid equivalents (GAEs) in micrograms per gram of oil.

3.5.4. DETERMINATION OF CHLOROPHYLL

The chlorophyll content of oils samples was determined by the method of Minguez-Mosquera *et al.* (1991). Oil (7.5 g) was accurately weighed and dissolved in cyclohexane up to a final volume of 25 ml. Chlorophyll content was calculated from the absorption spectra of the oils at 670 nm, measured by a Pye Unicam UV-4 UV-VIS scanning spectrophotometer (Spectronic Camspec Ltd., Leeds, UK). Absorption at 670 nm is usually considered to be related to the chlorophyll fraction with pheophytin-a being its major component. The chlorophyll content was calculated as follows: Chlorophyll (mg/kg) = (A_{670}) x 106 / (613 x 100 x d), where "A" is the absorbance and "d" is the spectrophotometer cell thickness (1 cm). Data is reported as mg of chlorophyll per kg of oil.

3.6. CHEMICAL ANALYSIS IN FRYING OILS

3.6.1. DETERMINATION OF CONJUGATED DIENES (CDs) AND TRIENES (CTs)

Ultraviolet absorption at 232 nm and 270 nm of fresh and fried oils was determined according to the Regulation (EU) No 299/2013 method (European Commission, 2013)

for measuring the presence of conjugated diene and trienes systems, respectively. Oils were dissolved in the required solvent (cyclohexane) and the absorbance of the solution was measured at the specified wavelength (232 nm or 270 nm) with reference to pure solvent. The results were expressed as Ks values (specific extinction coefficients), which are calculated for a concentration of 1% w/v sample dissolved in cyclohexane in a 10mm cell. Measurements were taken in triplicate by a Pye Unicam UV-4 UV-VIS scanning spectrophotometer (Figure 3.3).

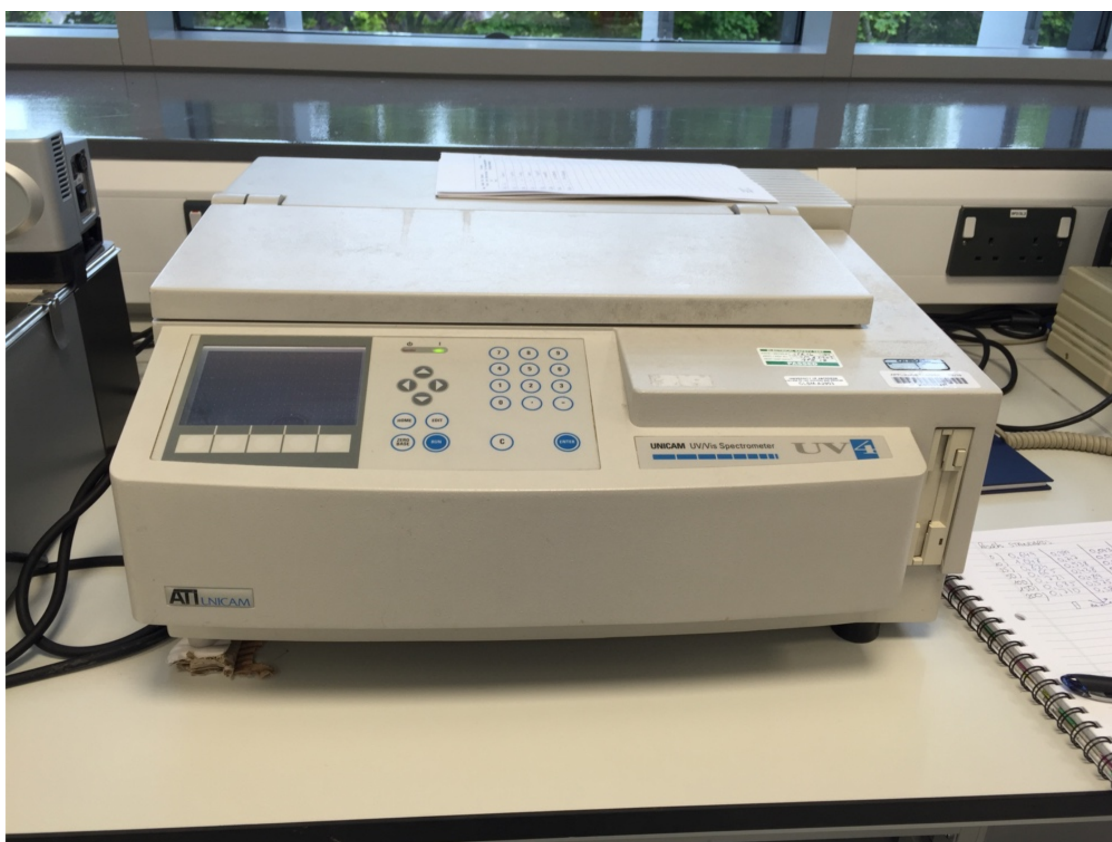


Figure 3.3. Pye Unicam UV-4 UV-VIS scanning spectrophotometer.

3.6.2. TOCOPHEROL CONTENT

Alpha-tocopherol, gamma-tocopherol and delta-tocopherol were determined in both fresh and fried oils. The same procedure was used as described in 3.5.2. section.

3.7. CHEMICAL ANALYSIS IN FILTERED RAPESEED OIL WITH ROSEMARY

3.7.1. FATTY ACID COMPOSITION ANALYSIS

The fatty acid composition of fats and oils was determined as described in 3.5.1. section.

3.7.2. FERRIC REDUCING POWER (FRP) ASSAY

FRP assay was used to determine the antioxidant capacity of filtered rapeseed oil with different concentrations of rosemary. It was measured as described by Siddhuraju and Becker (2003) with slight modifications. 0.1 mL of sample was dissolved in 0.25 ml phosphate buffer (0.2 M, pH 6.6), mixed with 0.25 mL potassium ferricyanide (1% w/v) and incubated at 50ºC for 20 min. Next, 0.25 mL of trichloroacetic acid (10% w/v) was added and mixed thoroughly followed by centrifugation at 650xg for 10 min. 100µl of distilled water and 25µl of ferric chloride (0.1% w/v) were added to 100µl of supernatant on a 96 well plate and mixed for 10s. The absorbance was read spectrophotometrically at 700 nm after 10 min, using a SpectraMax® 190 Absorbance Plate Reader (Molecular Devices, CA, USA). Measurements were taken in quadruplicate. The results are expressed as optical density (OD) at 700 nm. A higher absorbance of the reaction mixture indicated greater reducing power.

3.7.3. TOCOPHEROL AND CAROTENOID CONTENT

Beta-carotene, xanthophyll, alpha-tocopherol, gamma-tocopherol and delta-tocopherol were determined during accelerated oxidation conditions. The same procedure was used as described in 3.5.2. section.

3.7.4. COLOUR ANALYSIS

The colour parameters L* (lightness/darkness), a* (redness/greenness), b* (yellowness/blueness) values were measured by using a Konica Minolta CR1 10 colorimeter (Konica Minolta Solutions Ltd, Basildon, UK). Three replications were conducted. The colour changes during the accelerated oxidation test are expressed as ΔE with the colour of the fresh rapeseed oil (before the oxidation test) used as a reference sample. ΔE is the total colour change calculated from:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

3.7.5. THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)

The TBARS analysis measures malondialdehyde (MDA) present in the samples, which is one of the secondary volatiles oxidation products generated mainly from fatty acids with 3 or more double bonds (Figure 3.4).

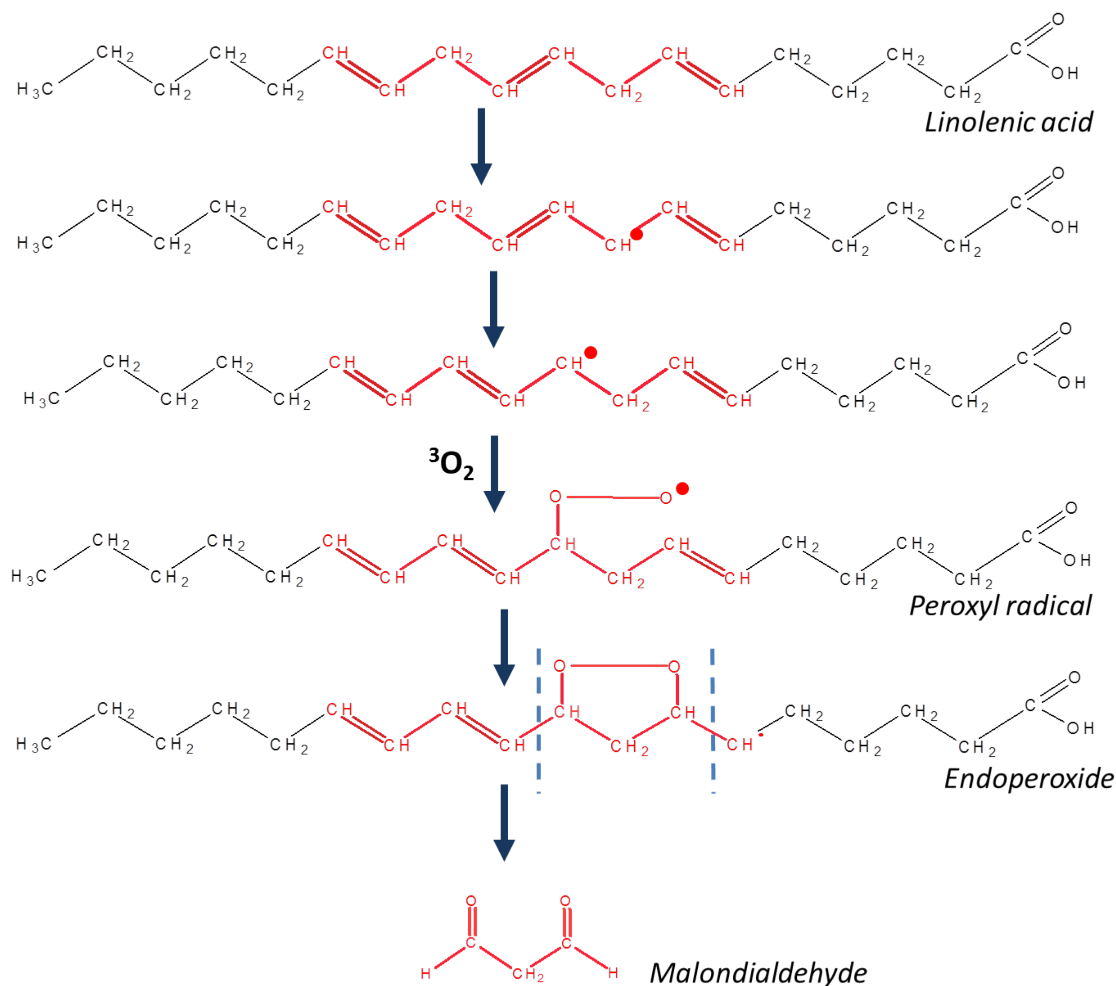


Figure 3.4. Malonaldehyde formation from alpha-linolenic acid.

For the TBARS analysis, samples (150µl, accurately weighed) were mixed with 4 ml distilled water before addition of 1 ml of thiobarbituric acid (Sigma) (0.34% w/v in 50% v/v acetic acid). Samples were boiled for 30 min in a water bath (VWR International Ltd, Leicestershire, UK), allowed to cool and centrifuged at 2500xg for 15 min. Supernatant (200µl) was transferred to a 96 well plate and the absorbance measured at 532nm (SpectraMax190, Molecular Devices Ltd, Wokingham, UK). Concentrations of TBARS were calculated from a standard curve prepared with malonaldehyde bis (dimethyl acetal) standard (Sigma) in the range of 0.1 to 10 µM. The measurements were taken in duplicate.

3.7.6. DETERMINATION OF CONJUGATED DIENES (CDs)

CDs were also assessed during the accelerated oxidation test as another parameter of secondary oxidation. The procedure was according to Kiokias and Gordon (2003). Oil samples were diluted 1:100 ml in ethanol and their oxidative state was determined by monitoring absorbance at 233 nm with a UV–vis Spectrometer (Spectronic Camspec Ltd., Leeds, UK). This solution was further diluted as necessary to achieve spectrophotometric readings in the target absorbance range of 0.2-0.8 at 233 nm. The amount of conjugated dienes per 100 g of sample was calculated using the relative molecular mass (280 g mol^{-1}) and the molar absorptivity of linoleic acid (ε=26,000). Ethanol was used as the blank. CD levels are expressed in units of raw absorbance. The measurements were taken in duplicate.

3.8. CHEMICAL ANALYSIS IN GROUND SPICES

3.8.1. FERRIC REDUCING POWER (FRP) ASSAY

Extraction and testing sample preparation was adapted from Wojdyło, Oszmiański and Czemerys (2007) with some modifications. In brief, 1 gr of each ground spice was weighed into a test tube. A total of 10 mL of 80% aqueous methanol was added and the suspension was gently mixed. Tubes were sonicated twice for 15 min and then centrifuged for 10 min at 1500 g using a CompactStar CS4 centrifuge (VWR International Ltd, West Sussex, UK). Supernatants were collected and stored at 4°C prior to use within 24 h.

The total antioxidant potential of the five ground plants was determined using the FRP assay adapted from Benzie and Strain (1996) as a measure of antioxidant power. The FRP reagent was freshly prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of a solution of 10 mM TPTZ in 40 mM HCl, and 2.5 mL of 20 mM FeCl3 and once prepared was kept in a water bath at 37°C. The following were added directly to each cuvette in a Pye Unicam UV-4 UV-VIS scanning spectrophotometer (Spectronic Camspec Ltd., Leeds, UK) thermostated at 37°C: 90 µL H2O, 900 µL FRP reagent and 30 µL of sample (diluted 25 times), standard or H2O as a blank. Absorbance readings

were taken at 593 nm after 4 minutes at 37°C. A solution of 1 mM FeSO4 was used as standard. All determinations were performed in triplicate. The results were corrected for dilution and expressed in μM Fe^{+2}/gr.

3.8.2. TOTAL PHENOLIC CONTENT (TPC)

Extraction and testing sample preparation was performed as follows: 1 gr of each ground plant was weighed into a test tube and 20 ml of methanol were added. The test tube was vortexed, homogenized for 2 min at 8000 rpm, left at a tube roller mixer for 1 h and then centrifuged at 1100 g for 15 min. The supernatant was collected and the procedure was repeated one more time with another 20 ml of methanol. The two methanol extracts were combined and the final volume was brought up to 40 ml with methanol.

The total phenolic contents of the samples were determined using the Folin-Ciocalteu reagent as described by Parry *et al.* (2005). In brief, the reaction mixture contained 50 μL of testing sample solutions, 250 μL of the Folin-Ciocalteu reagent, 0.75 mL of 20% sodium carbonate and 3 mL of pure water. After 2 h of reaction at ambient temperature, the absorbance at 765 nm was measured by a Pye Unicam UV-4 UV-VIS scanning spectrophotometer (Spectronic Camspec Ltd., Leeds, UK) and was used to calculate the phenolic contents of oils using gallic acid as standard. Measurements were taken in triplicate. The results were expressed as mg gallic acid equivalents (GAE)/gr of powder.

3.9. STATISTICAL ANALYSIS

All sampling and chemical analyses were performed in duplicate, triplicate or quatriplicate. Results of this study were expressed as a mean with standard deviations.

3.9.1. RELATIONSHIP BETWEEN OILS/FATS COMPOSITION AND OXIDATIVE STABILITY

One-way ANOVA followed by Tukey's or Games-Howell post-hoc test was used to compare chemical composition and oxidative stability between the 22 oils and fats

analyzed. These treatments were carried out using SPSS (v.21.0 software, IBM Corporation, USA) and the level of statistical significance was set at $p < 0.05$. Principal component analyses (PCA), simple linear regression and multiple linear regression models were performed by using Minitab (Release 17.1.0 for Windows).

3.9.2. SPICES AS NATURAL ANTIOXIDANTS TO IMPROVE THE OXIDATIVE STABILITY AND FRYING PERFORMANCE OF VEGETABLE OILS

To identify statistical differences between spices characteristics, Factor Analysis was performed and a new variable was obtained (antioxidant power index) from the z-score of the three variables described. Significant differences between mean values were evaluated by variance analysis (ANOVA), Student's t-test (for independent variables) and two-tailed t-tests (for dependent variables). These analyses were performed using SPSS (v.21.0 software, IBM Corporation, USA) and the level of statistical significance was set at $P < 0.05$.

3.9.3. ROSEMARY POWDER FILTRATE AS A NATURAL ANTIOXIDANT OF RAPESEED OIL FOR DOMESTIC COOKING

Regarding the effect of rosemary powder filtrate in rapeseed oil, significant differences between mean values were evaluated by variance analysis (ANOVA). All analyses were performed using SPSS statistical software and the level of statistical significance was set at $P < 0.05$.

Results and discussion

CHAPTER 4. RESULTS AND DISCUSSION

4.1. RELATIONSHIP BETWEEN OILS/FATS COMPOSITION AND OXIDATIVE STABILITY

4.1.1. COMPOSITION OF OILS AND FATS

Details of the studied oils/fats was presented in Table 3.1 (Chapter 3, Material and methods), and their fatty acid composition of 22 oils and fats is presented in Table 4.1. As expected, the fatty acid composition varied considerable between the samples, which is attributed mainly to varietal and genetic differences among plant species, different stages of seed maturity, geographical and climatic conditions as well as processing effects during the production (Kamal-Eldin and Andersson, 1997). The fatty acid composition was in accordance with the *Codex Alimentarius* Standards (FAO/WHO Codex Alimentarius Commission, 1981b, 1999) for olive, coconut, rapeseed, sesame and sunflower (both regular and high oleic acid) oils. The composition of the other oils and fats included in this study is not described in the *Codex Alimentarius*.

Coconut oil showed the highest saturated fatty acid composition (94.3 and 92.7% for virgin cold-pressed and refined respectively), followed by butter (65.2%), ghee (64.1%) and virgin red palm oil (49.3%). Distinctive differences in the distribution of saturated fatty acids were detected between the above products. Coconut oil was rich in lauric acid (C12:0), accounting for 52.2% and 47.2% of the total fatty acid composition for virgin cold-pressed and refined respectively. Meanwhile butter, ghee and red palm oil were rich in palmitic acid (C16:0), with distributions of 32.8%, 31.8% and 41.2% respectively. Rapeseed oils (both refined and VCP) were the richest source of *alpha*-linolenic acid (C18:3ω3) contributing 9.24% and 10.8% to the fatty acid composition respectively. With respect to the polyunsaturated to saturated fatty acids ratio (PUFA/SFA), sunflower VCP and sunflower oil generated the highest values (6.48 and 5.23 respectively), whereas coconut VCP and coconut oil showed the lowest values (0.01 and 0.02 respectively). In addition, butter, ghee, red palm oil and all types of olive oils showed a low PUFA/SFA ratio.

Table 4.1. Fatty acid composition (expressed as % of total fatty acids) of 22 oils and fats.

	OIL/FAT	12:0	14:0	16:0	18:0	18:1ω9	18:2ω6	18:3ω3	MUFA	PUFA	UFA	SFA	PUFA/SFA	UFA/SFA
1	Corn oil	0.0	0.03±0.0	11.1±0.02	1.98±0.0	28.3±0.01	55.8±0.06	1.17±0.01	28.5	57.2	85.7	14	4.09	6.14
2	Olive oil	0.0	0.02±0.0	11.2±0.02	3.67±0.01	74.7±0.03	7.36±0.05	0.94±0.0	75.8	8.59	84.3	15.6	0.55	5.40
3	Olive oil EV	0.0	0.01±0.0	10.5±0.01	3.80±0.01	76.6±0.01	6.05±0.01	0.92±0.0	77.7	7.22	84.9	15.0	0.48	5.65
4	Olive oil VC1	0.0	0.01±0.0	7.24±0.18	3.08±0.09	82.7±0.43	4.40±0.11	0.84±0.03	83.8	5.61	88.8	11.4	0.49	8.00
5	Olive oil EVC2	0.0	0.01±0.0	10.6±0.01	3.17±0.0	79.4±0.10	3.57±0.05	0.88±0.0	80.5	4.81	85.1	14.7	0.33	5.81
6	Olive oil VA1	0.0	0.02±0.0	12.8±0.03	2.21±0.01	74.3±0.01	7.30±0.01	0.75±0.0	75.7	8.28	84	15.8	0.53	5.34
7	Olive oil VA2	0.0	0.02±0.0	12.4±0.01	2.09±0.0	74.5±0.05	7.61±0.02	0.77±0.0	75.9	8.65	84.5	15.3	0.57	5.55
8	Olive oil EVP	0.0	0.01±0.0	11.0±0.03	3.61±0.01	78.2±0.01	4.21±0.0	0.84±0.0	79.2	5.31	84.5	15.4	0.35	5.51
9	Olive oil EVH	0.0	0.02±0.0	13.2±0.01	3.02±0.01	70.9±0.02	9.24±0.03	0.93±0.0	72.4	10.4	82.8	17.0	0.61	4.87
10	Sunflower oil	0.0	0.08±0.0	6.61±0.04	3.46±0.01	27.8±0.16	60.2±0.25	0.0±0.0	28.2	60.6	88.4	11.6	5.23	7.66
11	Sunflower oil VCP	0.0	0.07±0.0	5.98±0.01	3.50±0.0	19.5±0.04	69.4±0.08	0.0±0.0	19.6	69.5	89.0	10.7	6.48	8.31
12	Sunflower oil HO	0.0	0.05±0.0	4.07±0.01	2.97±0.0	81.7±0.40	8.40±0.40	0.34±0.0	82.4	9.45	91	8.72	1.08	10.5
13	Rapeseed oil	0.0	0.08±0.04	4.97±0.76	1.97±0.43	62.4±0.83	18.4±0.44	9.24±0.23	63.9	28.9	91.1	9.25	3.12	11.4
14	Rapeseed oil VCP	0.0	0.05±0.0	4.38±0.12	1.59±0.02	62.2±0.25	18.5±0.09	10.8±0.06	63	29.8	92.3	7.29	4.09	12.9
15	Sesame oil	0.0	0.04±0.01	10.4±0.06	4.27±0.0	40.8±0.31	41.6±0.76	0.72±0.19	41.4	43.5	83.7	15.7	2.77	5.34
16	Sesame oil VCP	0.0	0.03±0.01	9.63±0.16	5.9±0.03	39.2±0.01	43.2±0.16	0.54±0.0	39.3	44.1	83.2	16.7	2.64	5.04
17	Toasted sesame oil V	0.0	0.04±0.01	10.4±0.07	3.88±0.01	39.1±0.05	43.8±0.10	0.75±0.0	39.4	45	84.3	15.6	2.88	5.44
18	Coconut oil	47.2±1.81	19.2±0.76	10.7±0.48	3.47±0.01	7.99±1.01	1.86±0.13	0.0±0.0	9.25	2.11	10.1	92.7	0.02	0.11
19	Coconut oil VCP	52.2±0.13	20.0±0.2	8.79±0.12	3.39±0.06	5.23±0.04	0.94±0.01	0.0±0.0	5.27	0.95	6.17	94.3	0.01	0.07
20	Red palm oil V	0.51±0.28	0.95±0.09	41.2±0.47	4.97±0.19	40.8±0.45	10.2±0.08	0.0±0.0	41.5	10.6	51.5	49.3	0.21	1.07
21	Butter	3.68±0.0	11.5±0.16	32.8±0.15	11.8±0.14	24.1±0.0	2.09±0.05	0.83±0.0	29.1	4.00	32.4	65.2	0.06	0.50
22	Ghee	3.75±0.04	11.7±0.11	31.8±0.04	11.5±0.08	24.6±0.10	1.79±0.01	0.92±0.04	29.8	3.86	32.9	64.1	0.06	0.52

Results are expressed as mean ± SD (standard deviation). *Abbreviations*: MUFA; monounsaturated fatty acid, PUFA; polyunsaturated fatty acid, UFA; unsaturated fatty acid, SFA; saturated fatty acid. The most abundant fatty acids are selected for presentation in the table.

Tocopherols, *beta*-carotene, TPC and chlorophyll content of oils and fats are shown in Table 4.2. Tocopherol content was in accordance with the *Codex Alimentarius* Standards for coconut, rapeseed, sunflower oils and small differences were observed for sesame and high oleic sunflower oil (FAO/WHO Codex Alimentarius Commission, 1999). Tocopherol content in oils may be affected by climate, genetic variety and is also largely dependent on the production process, as reported for Brazil-nut oil (Funasaki *et al.*, 2013), flaxseed oil (Obranović *et al.*, 2015) and olive oil (Špika, Kraljić and Škevin, 2016). The highest amounts of total tocopherols without significant differences between them were presented by sunflower and rapeseed oil in the following order: sunflower oil, sunflower oil VCP, rapeseed oil and rapeseed oil VCP. Szydłowska-Czerniak *et al.* (2008) analysed the total tocopherol content of rapeseed and olive oils subjected to different grades of refining; data from rapeseed oils were in accordance with our results (between 555 and 690 mg/kg), but not for olive oils, which demonstrated lower levels (80–190 mg/kg) compared to our results. In agreement with Dauqan *et al.* (2011), tocopherols were not detected in coconut oils. Non- surprisingly, the lowest tocopherol levels were detected in stripped corn oil followed by butter and ghee. *Alpha*-tocopherol was the major tocopherol in all olive oils, without significant differences between them, whereas *gamma*-tocopherol was the most abundant in rapeseed oils, sesame oils and corn oil. With respect to vitamin E activity, *alpha*-tocopherol is the most effective form because of the specificity of absorption and distribution within the human body (Galli *et al.*, 2017). However, *gamma*-tocopherol might be more effective in increasing oxidative stability (Seppanen, Song and Saari Csallany, 2010) and antioxidant activity of oils (Castelo-Branco *et al.*, 2016).

Table 4.2. Alpha-tocopherol, gamma-tocopherol, delta-tocopherol, total tocopherol, beta-carotene, total phenolic and chlorophyll content in 22 oils and fats.

	Oil or fat	Alpha-tocopherol	Gamma-tocopherol	Delta-tocopherol	Total tocopherols	Beta-carotene	Total phenolic content	Chlorophyll
		µg/g	µg/g	µg/g	µg/g	µg/g	µg GAE/g	mg/kg
1	Corn oil	2.19±0.07	19.9±0.77	0.68±0.04	22.78±0.29	n.d.	149±37.8	0.00±0.02
2	Olive oil	220±21.1	15.2±1.43	0.83±0.10	236±7.55	0.10±0.02	306±30.8	0.53±0.03
3	Olive oil EV	209±16.8	19.2±1.54	0.88±0.09	229±6.13	0.45±0.01	361±30.2	1.88±0.05
4	Olive oil VC1	168±9.44	10.7±0.57	0.91±0.06	180±3.35	0.23±0.03	344±24.5	1.74±0.04
5	Olive oil EVC2	182±9.49	11.6±0.42	1.27±0.05	195±3.32	1.72±0.13	459±11.3	8.26±0.02
6	Olive oil VA1	177±8.74	4.57±0.26	0.95±0.08	182±3.03	1.24±0.07	457±27.9	7.02±0.01
7	Olive oil VA2	121±10.8	3.93±0.21	0.58±0.05	126±3.70	1.20±0.09	294±37.8	6.78±0.12
8	Olive oil EVP	169±8.80	20.7±1.15	0.89±0.09	190±3.35	0.76±0.07	389±10.1	3.95±0.03
9	Olive oil EVH	244±25.3	16.2±1.72	0.87±0.03	261±9.00	0.81±0.12	411±14.8	3.28±0.05
10	Sunflower oil	578±20.4	25.4±1.01	6.66±1.77	610±7.73	n.d.	309±19.8	0.04±0.02
11	Sunflower oil VCP	449±28.1	22±1.27	8.17±0.56	480±9.99	0.09±0.02	329±14.9	0.22±0.02
12	Sunflower oil HO	387±25.1	17.8±1.10	4.58±0.24	410±8.82	n.d.	179±9.62	0.03±0.02
13	Rapeseed oil	214±7.33	335±12.7	9.28±0.33	558±6.79	n.d.	202±27.4	0.08±0.02
14	Rapeseed oil VCP	179±13.0	374±29.8	14.8±1.21	568±14.7	1.13±0.06	137±22.1	0.39±0.30
15	Sesame oil	78.7±10.1	244±29.3	9.27±0.92	332±13.43	n.d.	269±40.2	0.04±0.03
16	Sesame oil VCP	n.d.	418±10.9	8.90±0.85	427±5.88	n.d.	304±12.8	0.09±0.01
17	Toasted sesame oil V	65±2.78	372±20.1	11.1±0.30	448±7.73	n.d.	501±55.1	1.01±0.03
18	Coconut oil	n.d.	n.d.	n.d.	n.d.	n.d.	881±171	0.13±0.02
19	Coconut oil VCP	n.d.	n.d.	n.d.	n.d.	n.d.	681±100	0.02±0.02
20	Red palm oil V	122±5.52	1.35±0.07	7.14±0.21	131±1.94	133±10.4	499±10.4	0.31±0.03
21	Butter	22.8±2.50	0.40±0.03	n.d.	23.2±1.26	3.50±0.11	226±9.37	3.29±0.31
22	Ghee	42.4±2.74	0.68±0.05	n.d.	43.1±1.90	5.64±0.40	326±35.7	0.44±0.03

Results are expressed as mean ± SD (standard deviation). *Abbreviations*: GAE; gallic acid equivalent, n.d.; not detected.

Red palm oil was the only sample to contain significant amounts of *beta*-carotene (133 µg/g). Dauqan *et al.* (2011) reported an even higher content of *beta*-carotene in red palm oil (542 µg/g). *Beta*-carotene, a provitamin A molecule, is the main pigment that gives red palm oil its distinctive orange-red colour. Bioavailability of *beta*-carotene from red palm oil is higher compared to other vegetable sources and this type of oil is highly efficacious in improving vitamin A status among populations at risk of vitamin A deficiency (You, Parker and Swanson, 2002).

Surprisingly, refined coconut oil followed by virgin cold-pressed coconut oil showed the highest TPC, compared to all other samples. This is because they are different samples of different soil types. Marina, Che Man and Amin (2009) reviewed coconut composition and reported a higher TPC in virgin coconut oils compared to refined oils of the same source. It was concluded that TPC is highly variable depending on coconut varieties and the oil extraction processes, which could account for the opposing results of the present study. Arlee, Suanphairoch and Pakdeechanuan (2013) reported relatively lower TPC data for cold-pressed coconut oil from six varieties of coconut cultivars (486 – 579 µg GAE/g oil), which are not distinctively different from our results. Toasted sesame oil also showed higher TPC (501±55.1 µg GAE/g oil), compared to untoasted sesame oil. Toasted sesame oil is produced by pressing toasted sesame seeds at approximately 200 °C which gives oil its dark colour and characteristic flavour, mainly because of the generation of Maillard reaction-mediated products. It has been reported that TPC increased significantly with the roasting process of sesame seeds (Jannat *et al.*, 2013). Virgin cold-pressed rapeseed oil (VCP) showed the lowest TPC value (137 µg GAE/g) which was even lower than the one determined for refined rapeseed oil (202 µg GAE/g). Koski *et al.* (2003) reported a decrease in the phenolic content detected in rapeseed oil with increasing the degree of refining. This suggests that our results are most likely attributed to other variability factors (i.e. cultivar). When comparing the TPC of olive oil samples, virgin and extra virgin demonstrated higher values than the blended one with one exception (VA2). Olive oils showed TPC values between 294 and 459 µg GAE/g, which is in agreement with reported values (148 to 1,212 µg GAE/g) measured in 22 commercial extra virgin olive oils (Galvano *et al.*, 2007).

Chlorophyll content is typically high in virgin olive oils and accounts for their distinctive light green colour. Chlorophyll acts as antioxidant when the oils are kept in the dark at low temperatures and may have a pro-oxidant activity when samples are exposed to light

(Giuliani, Cerretani and Cichelli, 2011). Concentration of chlorophyll pigments in virgin olive oil is strongly related to the ripening stage of the fruit at the time of harvest, regardless of the variety of olives. Oils produced from unripe olives show a higher content of chlorophylls than those obtained from fruits harvested when completely ripe (Giuliani, Cerretani and Cichelli, 2011). In the present study, this is noticeably evident when comparing the chlorophyll content of Cornicabra variety made from 80% green olives (ECV2, 8.26 mg/kg) with the one from olive oil 100% ripe olives (VC1, 1.74 mg/kg).

Virgin vegetable oils contain different types of natural antioxidants in variable amounts. During the refining process, vegetable oils suffer a reduction of antioxidants (Chen, Mcclements and Decker, 2011). For example, the overall loss of total tocopherols during refining was reported to be 37.9% for sunflower oil (Naz, Sherazi and Talpur, 2011). In the present study, it was not feasible to directly compare refined and virgin versions of the same vegetable oil since the oils are not produced from the same source. Vegetable oil composition of the finished product including antioxidants, is dependent on several parameters which are summarized by the extraction process and type of refining, olive or seed variety, edaphoclimatic conditions, harvesting period and technique, fruit ripening degree and others (Santos *et al.*, 2013).

4.1.2. OXIDATIVE STABILITY (IP) OF OILS AND FATS

Data from induction period (IP) of 22 fats and oils are presented in Figure 4.1. Virgin cold-pressed coconut oil (VCP) showed the highest oxidative stability, which was significantly different ($p<0.05$) compared to all other oils. The IP of coconut oil VCP was not calculated directly using Rancimat software because the apparatus failed to terminate the measurement automatically within a reasonable period of time. Instead measurements were taken at 140ºC (16.44±0.11h), 160ºC (3.16±0.14h) and 180ºC (0.68±0.01h) and IP at 120ºC was calculated (65.01±2.69) from the temperature acceleration factor, known as Q10. The determination of Q10 that is based on the increase in oxidation rate produced by a 10°C increase in temperature (Frankel, 2012).

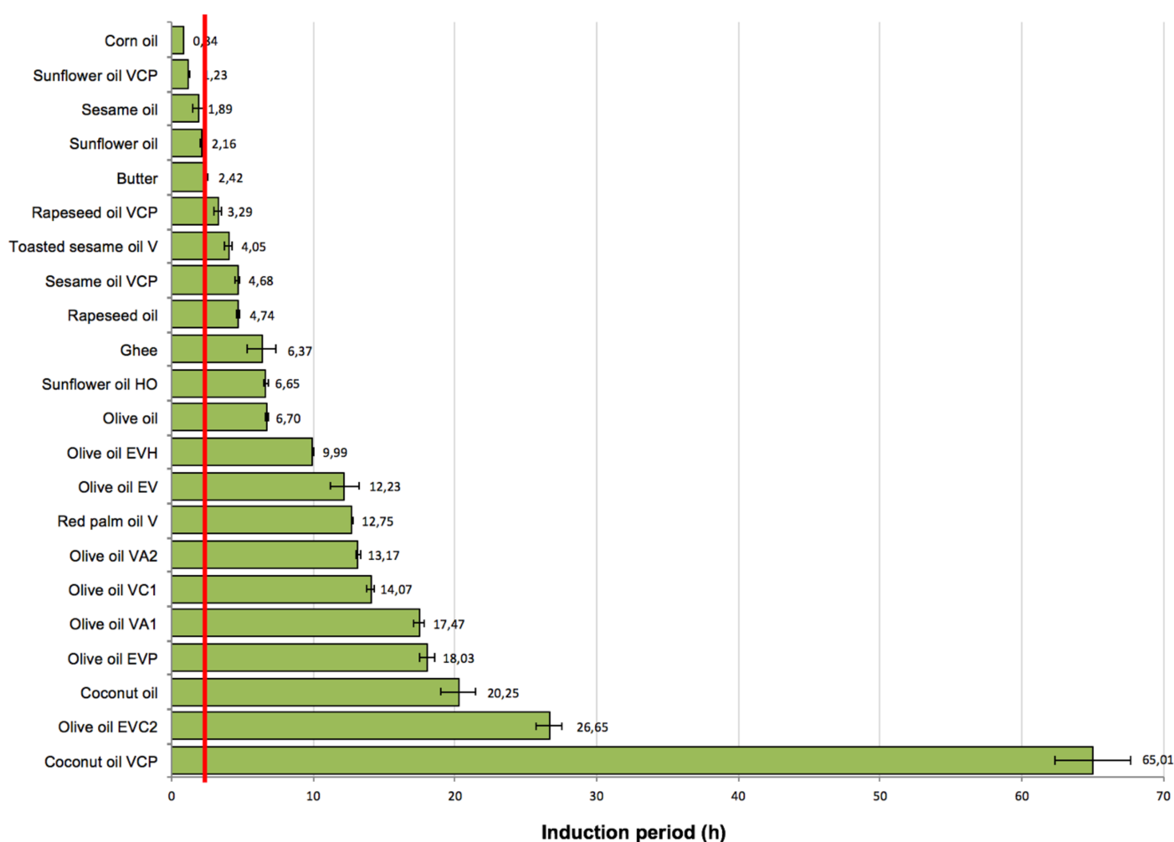


Figure 4.1. Induction period (IP) of 22 oils and fats. Results are presented as mean ± SD. IP of coconut oil VCP was calculated from the empirical Q10 approach, which is based on the observation that the induction time approximately doubles with each 10°C reduction in sample temperature. Vertical line corresponds to the discard point level of oils to be used to frying purposes (according to Farhoosh and Moosavi, 2007).

Virgin olive oil from Cornicabra variety made with 80% green olives (ECV2), generated the second highest IP, which was significantly higher ($P<0.05$) than the IP of all the remaining olive oils and even the one obtained from refined coconut oil. Montaño *et al.* (2016) compared the IP of oils from seven varieties of olives (Arbequina, Cornicabra, Manzanilla Cacereña, Manzanilla de Sevilla, Morisca, Pico Limón and Picual) using a Rancimat apparatus under a different experimental set-up to the one of the present study (100ºC, air flow 15 L/h). The highest IP values ($P<0.05$) of were reported for oils from Picual and Cornicabra olives, whereas Arbequina and Morisca varieties showed the lowest values. In the same study, olives were collected on three harvesting dates from at least three different groves in varied locations; hence, the results are conclusive with respect to the effect of olive variety and not the ripe state. In the present study, the lowest

IP ($P<0.05$) between monovarietal virgin olive oils was found in Hojiblanca and the highest in Cornicabra olive oil.

Previous studies determined the IP oils and fats using the same experimental conditions with the present study (120ºC, air flow 20 L/h) and the results are documented as follows: ghee -10.57 hr (Pawar *et al.*, 2014); refined rapeseed oil - 4.10 hr (Anwar, Bhanger and Kazi, 2003) and 5.9 hr (Kowalski *et al.*, 2004); refined sunflower oil - 1.89 hr (Anwar, Bhanger and Kazi, 2003), 3.05 hr (Ayyildiz *et al.*, 2015) and 3.5 hr (Kowalski *et al.*, 2004); butter - 5.00 to 6.03 hr (Anwar, Bhanger and Kazi, 2003); olive oil - 6.42 hr (Läubli and Bruttel, 1986); virgin olive oils - 3.7 to 48.3 hr (Mateos *et al.*, 2006). As a general rule, virgin oils showed a longer IP due to the presence of minor antioxidant compounds (Chaiyasit *et al.*, 2007); however, refined sunflower and rapeseed oils showed longer IP values than their respective virgin cold-pressed oils. Wroniak, Krygier and Kaczmarczyk (2008) also reported Rancimat data which suggest that cold-pressed rapeseed oils (5.08 hr) were more readily oxidised in comparison with the same oil subjected to a full refining process (5.37 hr).

Farhoosh and Moosavi (2007) confirmed that oxidative stability of oils determined via the Rancimat test, cannot guarantee or predict the actual frying performance of the oil, but it is considered that this method can be useful to act as a "screening" test and eliminate the possibility of introducing lower stability oils into the production area with its consequences. They proposed that assuming a value of 24% of total polar compounds that indicates the maximum permitted levels in frying oils (discard point level), the corresponding IP of the oil should be ≥ 2.32 h for frying oils (Farhoosh and Moosavi, 2007). According to the findings of the present study, sunflower VCP, sesame and sunflower oil are below this point (vertical line in Figure 4.1). Hence it may be concluded that these oils not suitable for frying purposes. Stripped corn oil is also below the limit; however, this product is not intended for human or animal consumption and is supplied for laboratory use only.

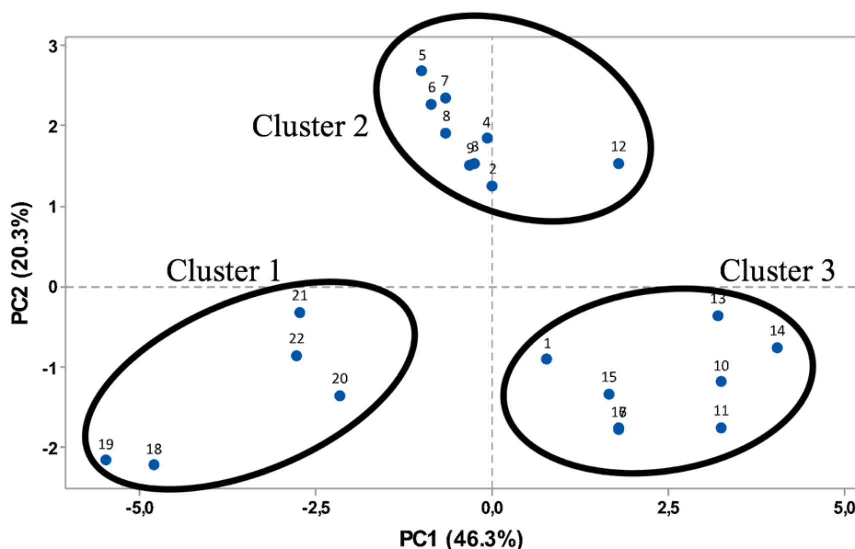
4.1.3. PRINCIPAL COMPONENTS ANALYSIS (PCA)

The following fourteen variables were used for statistical assessment: IP, *alpha*-tocopherol, *gamma*-tocopherol, *delta*-tocopherol, total tocopherols, *beta*-carotene,

chlorophyll, TPC, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (UFA), PUFA/SFA and UFA/SFA ratios.

The PCA was applied to reduce the initial variables to a small number of Principal Components (PCs) in order to obtain an overview of the sample variations and identify behavioural patterns. Figure 4.2 shows the bi-dimensional representation of oils and fats (Figure 4.2A) and all the variables taken into consideration (Figure 4.2B) for the two first ones PCs. The explained variance by PC1 and PC2 is 66.6%. The PC1 (46.3% of the total variance) showed positive loading mainly with total tocopherols, UFA/SFA ratio, UFA and PUFA/SFA ratio, and negative loading with SFA, TPC and IP. The PC2, which explained 20.3% of the total variance, was positively correlated with MUFA and chlorophyll, and negatively with PUFA. Figure 4.2B describe the behaviour of the variables. The most remote properties of the 0.0 point are more important for describing PCs, and those closest to 0.0, as *beta*-carotene, are less important.

A Score Plot



1	Corn oil
2	Olive oil
3	Olive oil EV
4	Olive oil VC1
5	Olive oil EVC2
6	Olive oil VA1
7	Olive oil VA2
8	Olive oil EVP
9	Olive oil EVH
10	Sunflower oil
11	Sunflower oil VCP
12	Sunflower oil HO
13	Rapeseed oil
14	Rapeseed oil VCP
15	Sesame oil
16	Sesame oil VCP
17	Toasted sesame oil V
18	Coconut oil
19	Coconut oil VCP
20	Red palm oil V
21	Butter
22	Ghee

B Loading Plot

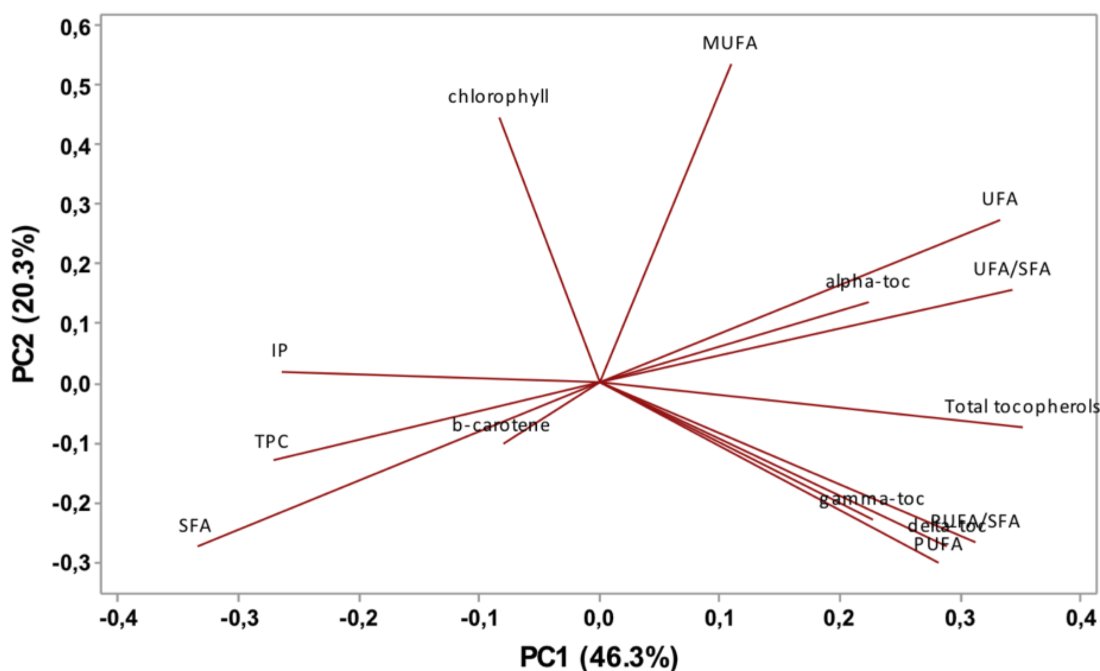


Figure 4.2. Score plot (**A**) and Loading plot (**B**) for oil and fat samples (n=22). Matrix analysis included the following variables: IP, *alpha*-tocopherol (alpha-toc), *gamma*-tocopherol (gamma-toc), *delta*-tocopherol (delta-toc), total tocopherols, *beta*-carotene (b-carotene), chlorophyll, TPC, SFA, MUFA, PUFA, UFA, and PUFA/SFA and UFA/SFA ratios.

Oils and fats are distributed into three major clusters (Figure 4.2A). Cluster 1 exclusively includes samples characterized by the presence of SFA, TPC and IP, and are low in UFA content. It can be shown that coconut oils are more separated from red palm oil, butter and ghee; it is due to the higher values in SFA, TPC and IP. However, all the samples included in Cluster 1 are solids at room temperature due to their relative high content of SFA. Olive oils and high oleic sunflower oil are grouped in Cluster 2, due mainly to their high MUFA content. High oleic sunflower oil showed more distance from olive oils because the differences in chlorophyll content. Finally, seed oils (except high oleic sunflower oil), which are all high in PUFA content and tocopherols (primarily *delta*-tocopherol), are distributed in Cluster 3.

4.1.4. INFLUENCE OF CHEMICAL COMPOSITION ON OXIDATIVE STABILITY

Simple linear regression analysis showed that TPC, SFA, UFA, PUFA and total tocopherols were the major contributors to the oxidative stability (IP) as indicated by the correlation values (Table 4.3). The TPC and SFA were positively correlated with IP. A positive correlation between TPC and IP in virgin olive oils has been extensively documented (Caponio, Gomes and Pasqualone, 2001; Martínez Nieto, Hodaifa and Lozano Peña, 2010; Manai-Djebali *et al.*, 2012; Montaño *et al.*, 2016). On the other hand, Ayyildiz *et al.* (2015) concluded that no correlation between the IP of six fully-refined edible oils and fatty acid composition or tocopherols content could be detected. Furthermore, Bozan and Temelli (2008) reported similar findings (no correlation) between the oxidative stability of flax, safflower and poppy oils and their corresponding fatty acid composition, tocopherols or total phenolic compounds.

Table 4.3. Correlations between chemical composition of oils and fats and oxidative stability (IP) for P < 0.05.

	Correlation coefficient (r)	**Coefficient of determination (r^2)**	**p**
TPCs	+ 0.646	0.417	0.001
SFAs	+ 0.566	0.321	0.006
UFAs	- 0.551	0.304	0.008
PUFAs	- 0.514	0.264	0.014
Total tocopherols	- 0.472	0.223	0.027
UFA/SFA ratio	- 0.424	0.180	0.049

Abbreviations: TPC; total phenolic compound, SFA; saturated fatty acid, UFA; unsaturated fatty acid, PUFA; polyunsaturated fatty acid.

The UFA, PUFA and total tocopherols were negatively correlated with IP (Table 4.3). The antioxidant behaviour of tocopherols in vegetable oils is not yet fully understood and there is evidence that it is manifested in concentration-dependent manner. The "loss of efficacy" of tocopherols at high concentrations, sometimes referred to as a "pro-oxidant effect", is witnessed by an increase in the rate of oxidation during the induction period. This effect is more profound for *alpha*-tocopherol, but is also evident for other tocopherols (Kamal-Eldin, 2006). A positive correlation was observed for tocopherols with UFA and PUFA ($r = 0.665$ and $r = 0.613$, respectively, $p < 0.01$), and a negative correlation with SFA ($r = -0.664$, $p < 0.01$). This suggests that tocopherols are predominantly present in oils or fats with high unsaturated fatty acid content to confer protection against oxidation. A multivariate analysis of 14 vegetable oils showed natural interrelations between *alpha*-tocopherol and linoleic acid ($r = 0.549$, $p < 0.05$) and between *alpha*-linolenic acid and *gamma*-tocopherol (Kamal-Eldin and Andersson, 1997). Kamal-Eldin (2006) suggested that vegetable oils which are more susceptible to oxidation (i.e high unsaturated content), are privileged by nature to contain optimal tocopherol levels for their stabilization.

A multiple linear regression model approach was adopted to determine the quantitative importance of the combined presence of 9 variables (*alpha*-tocopherol, *gamma*-tocopherol, *delta*-tocopherol, *beta*-carotene, chlorophyll, TPC, SFA, MUFA, and PUFA)

on the oxidative stability of the oils and fats. The equation for this model between the summary of analysed compounds and oxidative stability (IP) was as follows:

IP = -1356 + 0.0125[*alpha*-tocopherol] + 0.0161[*gamma*-tocopherol] – 0.02[*delta*-tocopherol] – 0.074[*beta*-carotene] + 1.99[chlorophyll] – 0.0333[TPC] + 14.32[SFA] + 13.66[MUFA] + 13.51[PUFA], $r^2 = 0.765$ **(1)**

The three variables that showed a higher coefficient in the equation (SFA, MUFA and PUFA) are the same that mainly grouped the three clusters in PCA analysis. Taking into account these compounds, the equation was further simplified in the form below:

IP = -904 + 9.49 [SFA] + 9.16 [MUFA] + 8.94 [PUFA], $r^2 = 0.670$ **(2)**

Some notable exceptions for both equations include coconut oil (both refined and VCP) samples. The later samples exhibited a much higher stability which would not fit to the multiple linear regression model signified by the above equations.

Linear regressions suggest that TPC is the most important individual factor (explaining 41.7% of variability); when all the parameters are considered, SFA, MUFA and PUFA in combination, appear to be the most important factors (explaining 67% of variability). These results are in agreement with previously published studies aiming to correlate oxidative stability of 50 refined plant-based oils and fats with fatty acid composition (Kerrihard *et al.*, 2015). According to the findings of this study, the concentrations of MUFA, di and tri-unsatudated fatty acids (DiUFA and TriUFA, respectively) in combination correlate strongly ($r^2 = 0.915$) with the oxidative stability of the samples. The contributions of MUFA, DiUFA and TriUFA in the present study were also assessed by a multiple linear regression model approach, resulting in a lower correlation value ($r^2 = 0.401$). When refined, non-refined and cold-pressed oils and fats are studied, the presence of minor compounds (e.g. phenolic compounds) also impacts on the oxidative stability. This may at least partially explain the lower correlation observed between fatty acid composition and IP in the present study. Castelo-Branco *et al.* (2016) described that *alpha* and *gamma*-tocopherol in combination correlated ($r^2 = 0.916$) with oxidative stability of 17 samples (9 different oils, refined and cold-pressed). In the present study *alpha* and *gamma*-tocopherol explained significantly lower variability ($r^2 = 0.223$).

4.2. SPICES AS NATURAL ANTIOXIDANTS TO IMPROVE THE OXIDATIVE STABILITY AND FRYING PERFORMANCE OF VEGETABLE OILS

4.2.1. CHARACTERIZATION OF SPICES AS NATURAL ANTIOXIDANTS

Antioxidant activity (FRP assay) and total phenolic compounds of black pepper, ginger, turmeric, rosemary and oregano, and also IPs of corn oil with added spices at 0.5% (w/v) are presented in Table 4.4. Tocopherol-stripped corn oil was used as a model system at this stage to identify the most potent antioxidant powder. Rosemary powder was initially incorporated at different concentrations in corn oil in order to determine the optimum formulation for assessing IP: 0.25%, 0.5%, 0.75% and 1% generated an IP of 1.05±0.04, 1.53±0.06, 2.06±0.05 and 2.07±0.09 hr respectively (data not shown). However, at 0.75% and 1% concentration, the powder was not adequately mixed and therefore 0.5% was chosen as optimum for further studies. Moreover, the IP of corn oil mixed with 0.5% w/v rosemary was significantly higher ($P < 0.05$) compared to the control and the oil containing BHT at the legal limit concentration of 100 mg/kg (Table 4.4).

The mean of the score of the "antioxidant power" was significantly higher in rosemary than the other plants, whereas black pepper had a significant lower score compared with the rest of the samples (Table 4.4). There was no significant difference between ginger, turmeric and oregano.

Table 4.4. Antioxidant capacity and total phenolic content of spices, and induction period (IP) of a tocopherol-stripped corn oil (plain oil) formulated with ground spices (0.5% w/v) and BHT (100 mg/kg).

	Antioxidant capacity	**Total phenolic compounds**	**Induction period of plain oil mixed with antioxidants**	**Antioxidant power index***
	μM Fe^{+2} per g	mg GAE per g	h	
Black pepper	82.78 ± 1.42	50.16 ± 13.89	0.79 ± 0.03[a]	**-1.00 ± 0.00[a]**
Ginger	252.04 ± 11.55	121.53 ± 3.88	0.84 ± 0.08[a]	**-0.36 ± 0.02[b]**
Turmeric	166.01 ± 0.69	239.96 ± 32.17	0.86 ± 0.09[a]	**-0.27 ± 0.04[b]**
Rosemary	573.35 ± 72.74	410.16 ± 22.39	1.53 ± 0.06[b]	**1.84 ± 0.07[c]**
Oregano	290.20 ± 2.73	155.25 ± 17.79	0.86 ± 0.06[a]	**-0.21 ± 0.06[b]**
Plain			0.84 ± 0.02[a]	
BHT			1.20 ± 0.03[c]	

Results are expressed as mean ± SD (standard deviation).

*A new variable obtained from a factorial analysis from the z-score of the three variables described. Values from this new variable and induction period with different lower case letter are significantly different (P <0.05). *Abbreviations*: GAE; gallic acid equivalents, BHT; butylated hydroxytoluene.

Rosemary powder showed the highest antioxidant capacity and total phenolic content and the best potential to increase the oxidative stability of corn oil of the studied spices. When rosemary powder was mixed with oil, 0.5% w/v concentration was the best option in terms of increasing oxidative stability and also mixing efficiency. In agreement with these findings, when rosemary extract is included in bran, soybean and cottonseed oil the IP values are significantly increased compared to control oils or oils containing synthetic antioxidants (Yang *et al.*, 2016). Rosemary extract is popular as a natural antioxidant in vegetable oils due to its strong antioxidant capacity, which is attributed to the presence of phenolic diterpenes such as carnosic acid and carnosol (Yanishlieva, Marinova and Pokorný, 2006; Terpinc, Bezjak and Abramovič, 2009). In fact, it has been adopted formally into the European regulations as new food additive for use in foodstuffs and was assigned E 392 as its E number (European Commission, 2010a, 2010b).

4.2.2. EFFECT OF DEEP-FRYING ON VEGETABLE OILS

4.2.2.1. OXIDATIVE STABILITY (IP)

Results of the IP of fresh and fried sunflower, olive and rapeseed oils with and without rosemary are shown in Figure 4.3. IP values of fresh plain oils (without rosemary) were in the following order: olive oil (9.02±0.07 hr), rapeseed oil (4.95±0.24 hr) and sunflower oil (2.41±0.06 hr), with significant differences ($P<0.05$) detected between the samples. Frying significantly reduced the oxidative stability of rapeseed and sunflower oil (3.47±0.12 and 1.28±0.65 hr, respectively), whereas the IP of olive oil was not significantly affected by the process of deep-frying (9.35±0.33 hr). According to the nutritional information provided by the manufacturers, olive oil contains 14.3% saturated, 73% monounsaturated and 8.2% polyunsaturated fatty acids; sunflower oil contains 12% saturated, 23% monounsaturated and 65% polyunsaturated fatty acids; and rapeseed oil contains 7% saturated, 57% monounsaturated and 28% polyunsaturated fatty acids. Similar findings indicating that olive oil is more resistant to degradation under domestic frying conditions compared with vegetable oils with high polyunsaturated fatty acid content have been reported (Casal *et al.*, 2010; Marinova *et al.*, 2012).

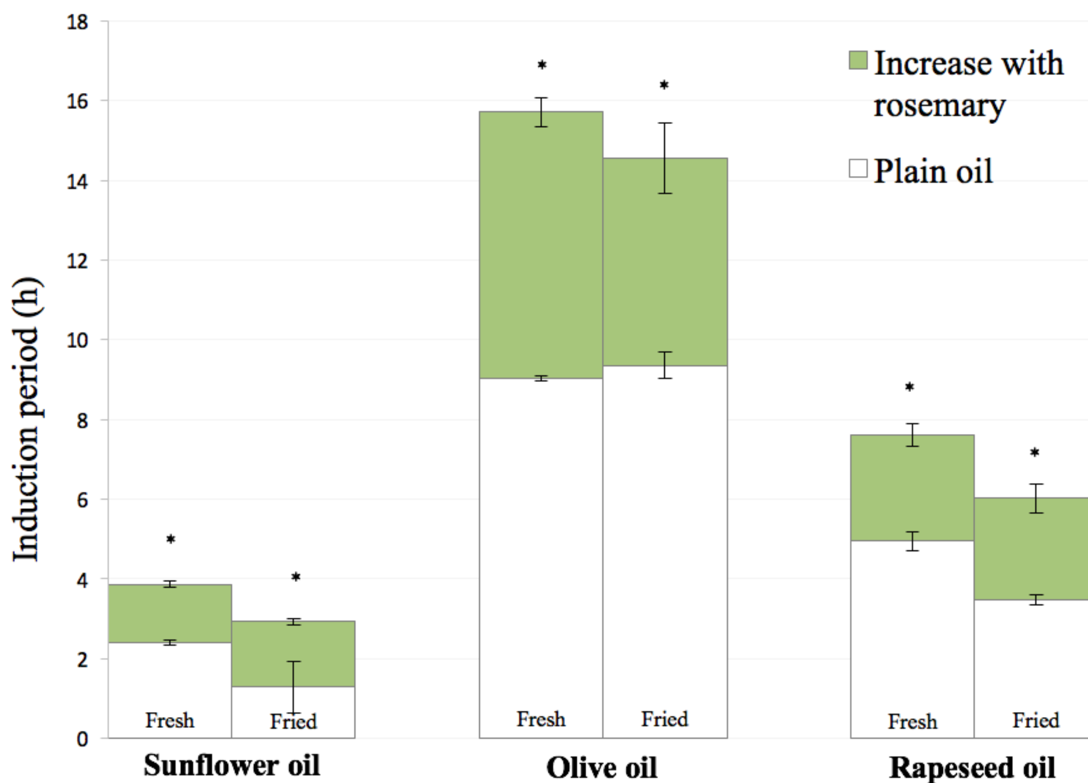


Figure 4.3. Effect of rosemary powder (0.5% w/v) addition on vegetable oil stability (IP) during a deep-frying process. Results are presented as mean ± SD. * denotes significant differences at P < 0.05 for samples before/after rosemary addition.

Adding rosemary powder into the oils significantly (P<0.05) increased the IP of all samples (Figure 4.3). The IP of olive, rapeseed and sunflower oil increased to 15.71±0.37, 7.61±0.29 and 3.87±0.07 hr, respectively after rosemary addition. During frying, IP of these oils was reduced to 14.55±0.89, 6.01±0.37 and 2.93±0.08 hr, respectively. It is worth mentioning that the IP of all fried oils with rosemary powder was higher than the one shown by the plain oils which have not been subjected to the deep-frying process. This indicates the protective effect of rosemary against oxidation. Addition of rosemary into sunflower, olive and rapeseed oil during the frying process led to an increase of IP of 128.91%, 55.61% and 73.20%, respectively. Our results suggest that rosemary powder is more effective in vegetable oils with a high polyunsaturated fatty acid content. Tohma and Turan (2015) investigated the effects of rosemary powder (and its alcoholic extracts) on the oxidative stability of hazelnut oil during deep frying. The rosemary concentration used for the frying procedure were 10 or 50 g/kg and 1000 ppm for the plant powder and its alcoholic extracts respectively. This study indicated that rosemary additives were

effective against lipid oxidation during frying of hazelnut oil. Rosemary additives inhibited the formation of oxidation products, increased the IP and improved the frying performance of hazelnut oil. For rosemary powder this effect was more profound when used at high concentrations (50 g/kg).

The use of rosemary extracts for increasing the oxidative stability of vegetable oils has been extensively documented. The addition of rosemary extracts to frying oils reduced both primary and secondary oxidation products during deep frying of potato slices (Alizadeh, Nayebzadeh and Mohammadi, 2016). Upadhyay, Sehwag and Niwas Mishra (2017) reported that oleoresin rosemary was able to significantly increase the frying stability of sunflower oil blends with ascorbyl palmitate. Moreover, the use of a rosemary extract in soybean oil heated for 10 h at 180ºC led to a significant increase of IP of 75.24%, as reported by Casarotti and Jorge (2014).

Measurement of IP can be indicative of quality deterioration of vegetable oils during a frying process and a linear correlation with some parameters of oil oxidation and IP has been established (Farhoosh and Moosavi, 2007; Farhoosh and Tavassoli-Kafrani, 2011; Upadhyay, Sehwag and Niwas Mishra, 2017). Farhoosh and Moosavi (2007) proposed that assuming 24% of total polar compounds are the maximum permitted levels in frying oils (i.e. discard point level), the IP of used oil should be ≥ 2.32 h. In the present study, fresh sunflower oil had an IP approximating the discard limit and fried sunflower oil was below the minimum recommended value. Adding rosemary powder to sunflower oil increased the IP above the limits, both for fresh and fried oil (Fig. 1). However, additional parameters should be considered when monitoring the oxidative stability of oils during frying, particularly for assessing their safety for human consumption (Nayak *et al.*, 2016).

4.2.2.2. CONJUGATED DIENES (CDs) AND TRIENES (CTs)

CDs and CTs are formed as secondary products from the oxidation of polyunsaturated fatty acid and their levels increase with frying time. Results from changes in CD and CT values in vegetable oils during the deep-frying process and the effect of rosemary powder addition, are shown in Table 4.5. Rapeseed oil showed a significant increase ($P<0.05$) for both CDs and CTs levels during frying. Olive and rapeseed oil showed an increase of CTs (only significant for olive oil) whereas CDs remained stable in these oils during frying.

Rapeseed oil is more susceptible to the formation of secondary products of oxidation during the frying process due to its high polyunsaturated fatty acids content, according to the supplier's information. Olive oil had the lowest CD and CT values compared with sunflower and rapeseed oil before frying. Moreover, the CT value was maintained below the maximum permitted levels (≤0.90) for olive oil (European Commission, 2013).

Table 4.5. Changes in conjugated diene and conjugated triene value of oils during deep-frying and effect of rosemary powder addition to oils (0.5% w/v).

Oils	**Conjugated diene value (K_{232})**		**Conjugated triene value (K_{270})**	
	FRESH OIL	FRIED OIL	FRESH OIL	FRIED OIL
Sunflower oil	$4.62 \pm 0.19^{a,A}$	$4.65 \pm 0.02^{a,A}$	$2.64 \pm 0.08^{a,A}$	$3.23 \pm 0.23^{a,A}$
Sunflower oil + ROS	$4.70 \pm 0.15^{a,A}$	$4.66 \pm 0.23^{a,A}$	$2.79 \pm 0.14^{a,A}$	$3.44 \pm 0.23^{b,A}$
Olive oil	$2.72 \pm 0.18^{a,A}$	$2.65 \pm 0.20^{a,A}$	$0.39 \pm 0.01^{a,A}$	$0.46 \pm 0.01^{b,A}$
Olive oil + ROS	$2.34 \pm 0.19^{a,A}$	$2.26 \pm 0.09^{a,B}$	$0.45 \pm 0.03^{a,B}$	$0.64 \pm 0.07^{a,B}$
Rapeseed oil	$3.59 \pm 0.08^{a,A}$	$4.64 \pm 0.40^{b,A}$	$1.02 \pm 0.29^{a,A}$	$3.18 \pm 0.54^{b,A}$
Rapeseed oil + ROS	$3.89 \pm 0.3^{a,A}$	$4.19 \pm 0.10^{a,A}$	$0.71 \pm 0.08^{a,A}$	$1.14 \pm 0.01^{b,B}$

Results are expressed as mean ± SD (standard deviation). Means followed by the same lower case letter are not significantly (P <0.05) different when comparing fresh and fried oil. Means followed by the same upper case letter are not significantly (P <0.05) different when comparing with and without rosemary. *Abbreviation*: ROS; rosemary.

The addition of rosemary to rapeseed oil prevented the formation of CDs and CTs during deep frying. CDs levels increase by 29.25% for plain rapeseed oil after the frying process; for rapeseed oil containing rosemary the corresponding increase is only 7.71%. A similar effect is observed for CTs where values increase by 211.76% and 60.56% post-processing for plain and rosemary-added rapeseed oil respectively. Furthermore, although the frying process did not affect the CD values of olive oil, fried samples containing rosemary had significantly lower levels compared to plain ones. Rosemary did not prevent CTs formation during frying of olive or sunflower oil. In fact, the addition of rosemary unexpectedly induced CTs formation in fresh and fried olive oil; however, the values obtained were always below the official limits mentioned. The protective effect of

rosemary extract addition to sunflower oil has been documented for longer deep-frying process cycles (up to 20 batches of potatoes) and results indicate that it can perform better than BHA, TBHQ or mixed tocopherols (Urbančič *et al.*, 2014).

4.2.2.3. TOCOPHEROL CONTENT ANALYSIS

Changes in alfa, gamma, delta and total tocopherol content in vegetable oils during the deep-frying process are shown in Table 4.6. The frying process and the addition of rosemary powder seem to have a minor effect on tocopherol levels for the three vegetable oils. Tocopherol content did not significantly decrease after the frying process; surprisingly, tocopherol levels slightly increased in olive oil after frying (alpha and total), but the observed increase was not statistically significant. Previous reports denote that tocopherol content decreases during frying or heating (Barrera-Arellano *et al.*, 2002; Casal *et al.*, 2010; Nayak *et al.*, 2016) and the inclusion of rosemary extract has a protective effect against this degradation (Casarotti and Jorge, 2014; Saoudi *et al.*, 2016). However, all these studies are conducted for longer periods of frying or heating, so it is likely that in the present study exposure to high temperatures was not suffice to induce the chemical breakdown of the antioxidants naturally occurring in vegetable oils.

Table 4.6. Changes in tocopherol levels during deep-frying process and effect of rosemary powder addition to oils (0.5% w/v).

	Tocopherols (μg/ml)							
Oils	**alpha**		**gamma**		**delta**		**TOTAL**	
	FRESH OIL	FRIED OIL	FRESH OIL	FRIED OIL	FRESH OIL	FRIED OIL	FRESH OIL	FRIED OIL
Sunflower oil	565.16 ± 55.71	489.37 ± 58.30	21.92 ± 2.12	19.33 ± 1.64	6.26 ± 0.45	5.28 ± 0.16	593.73 ± 58.36	513.98 ± 60.03
Sunflower oil + ROS	536.80 ± 62.36	488.01 ± 28.93	21.34 ± 2.65	19.19 ± 0.87	5.46 ± 0.33	5.30 ± 0.48	563.60 ± 65.32	512.50 ± 30.27
Olive oil	143.83 ± 16.76	169.88 ± 10.31	11.42 ± 0.97	11.42 ± 0.62	0.56 ± 0.10	0.59 ± 0.02	155.81 ± 17.80	181.89 ± 10.94
Olive oil + ROS	155.97 ±13.97	167.55 ± 26.50	12.17 ± 1.13	11.04 ± 1.86	0.71 ± 0.02	0.63 ± 0.12	168.85 ± 15.12	179.21 ± 28.49
Rapeseed oil	230.27 ± 21.55	213.80 ± 4.98	331.13 ± 30.94	313.97 ± 9.00	9.09 ± 0.90	8.64 ± 0.26	570.49 ± 53.37	536.40 ± 14.24
Rapeseed oil + ROS	199.58 ± 4.38	197.06 ± 13.08	286.88 ± 7.78	283.53 ± 15.79*	7.90 ± 0.28	7.85 ± 0.49	494.35 ± 12.15	488.44 ± 29.28

Results are expressed as mean ± SD (standard deviation). *Means significant differences (P <0.05) when comparing with and without rosemary oils. There were no significant differences in any data at P <0.05 when comparing fresh and fried oil. *Abbreviation*: ROS; rosemary.

4.3. ROSEMARY POWDER FILTRATE AS A NATURAL ANTIOXIDANT OF RAPESEED OIL FOR DOMESTIC COOKING

4.3.1. CHARACTERIZATION OF RAPESEED OIL

The fatty acid profile, tocopherol and carotenoid content and induction period (IP) of fresh rapeseed oil are summarized in Table 4.7. The fatty acid and tocopherol composition is in accordance with the *Codex Alimentarius* Standards (FAO/WHO Codex Alimentarius Commission, 1999) and is indicative of the health benefits associated with its consumption. Rapeseed oil is characterized by low levels of saturated fatty acids (SFA) and high levels of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. The findings of the present study are in close agreement with previously published results, which indicated oleic acid (52.8 % of total fatty acids) as the major fatty acid in rapeseed oil, followed by alpha linoleic acid (18.6 % of total fatty acids) and linolenic acid (7.5 % of total fatty acids) (Vingering *et al.*, 2010). Gamma-tocopherol and xanthophyll were the predominant forms of tocopherol and carotenoid respectively in this type of oil.

Table 4.7. Rapeseed oil characteristics.

Main fatty acids (% of total fatty acids)		Tocopherols (μg/mL)		Carotenoids (μg/mL)		IP (hr)
Palmitic acid (C16:0)	4.63±0.03	Alpha-tocopherol	132±1.41	Xanthophyll	13.0±0.27	3.46±0.17
Stearic acid (C18:0)	1.61±0.02	Gamma-tocopherol	235±6.79	Beta-carotene	1.61±0.22	
Oleic acid (C18:1n9)	61.4±0.01	Delta-tocopherol	4.71±0.32			
Alpha-linoleic acid (C18:2n6)	19.2±0.00					
Linolenic acid (C18:3n3)	11.3±0.00					

Results are expressed as mean ± SD (standard deviation).

Previous studies have reported similar induction periods for rapeseed oil (Tundis *et al.*, 2017). Rapeseed oil has lower oxidative stability compared with other vegetable oils such as olive oil, red palm oil or coconut oil, which is attributed to the high content of unsaturated (mono- and poly-) fatty acids of the former (Redondo-Cuevas *et al.*, 2017). Thus, there is scope to improve the oxidative stability of rapeseed oil, particularly for cooking or processing applications which are likely to deteriorate its quality and involve exposure to high temperature treatments.

4.3.2. EFFECT OF ROSEMARY ADDITION ON OXIDATIVE STABILITY AND ANTIOXIDANT CAPACITY OF RAPESEED OIL FILTRATE

The Rancimat method aims to measure the IP of vegetable oils by the quantitative, conductometric determination of volatile compounds formed as a result of lipid oxidation. The results showing the effect of rosemary addition on the oxidative stability of filtered rapeseed oil are presented in Figure 4.4. The IP values of oils with added rosemary were significantly higher ($P<0.05$) with increasing rosemary concentration. The rosemary concentration range (0.25%-2.0%) was decided based on our findings from previously conducted work and an additional sample (2.0%) was included to compensate for any potential loses in antioxidant activity during the filtration step. In agreement with these findings, previous analysis in our lab indicated that rosemary powder added to a tocopherol-stripped corn oil (unfiltered) at increasing concentrations (0%, 0.25%, 0.5%, 0.75% and 1%), also improved the IP as follows: 0.84±0.02, 1.05±0.04, 1.53±0.06, 2.06±0.05 and 2.07±0.09 hr respectively (Redondo-Cuevas, Castellano and Raikos, 2017). Other studies reported that when rosemary extract is included in bran, soya bean and cottonseed oil, the IP values are significantly increased compared to control oils or oils containing synthetic antioxidants (Yang *et al.*, 2016).

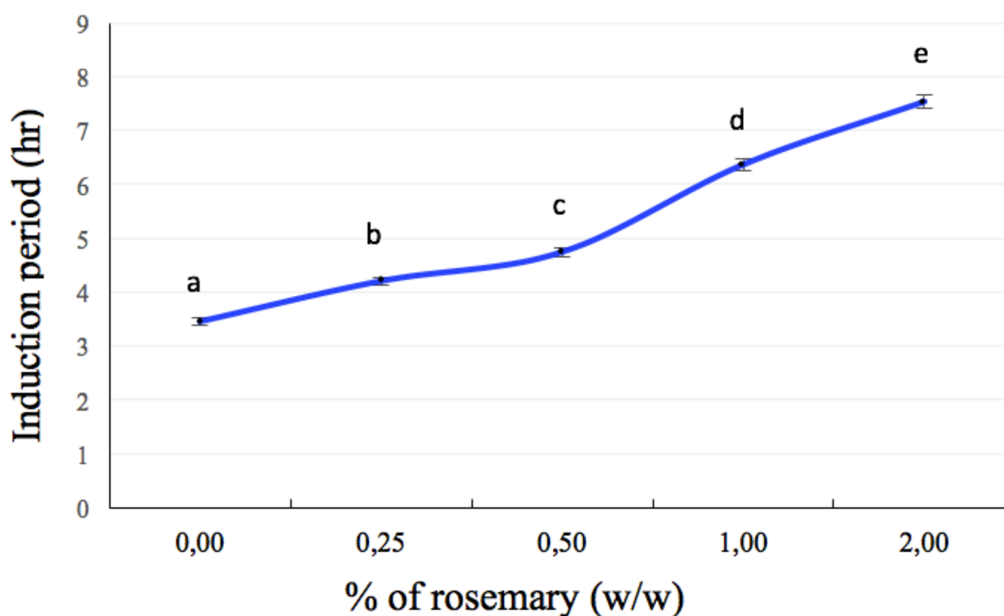


Figure 4.4. Effect of rosemary concentration on the oxidative stability (IP) of filtered rapeseed oil measured by Rancimat. Results are presented as mean ± SD. Values with different lower case letter are significantly different (P<0.05).

Changes in antioxidant capacity of the samples were measured by the Ferric Reducing Power assay (FRP) (Figure 4.5). The FRP value of rapeseed oil with 0.25% (w/w) of rosemary was not significantly different when compared with plain rapeseed oil (control). However, at concentrations 0.5% and above the FRP showed a significant (P<0.05) increase with increasing rosemary concentration.

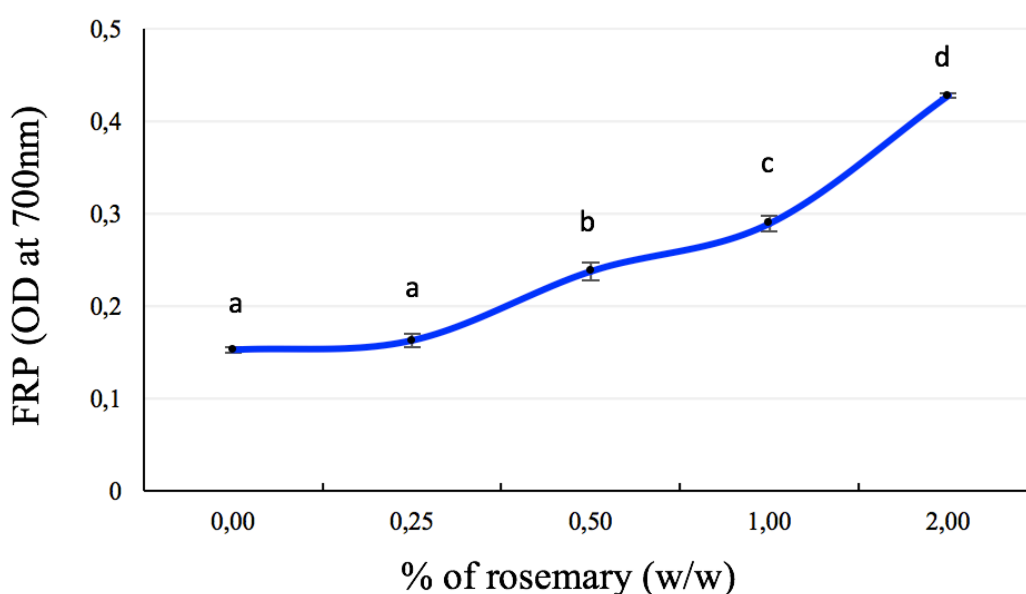


Figure 4.5. Effect of rosemary concentration on rapeseed oil antioxidant capacity measured by FRP. Results are presented as mean ± SD. Values with different lower case letter are significantly different (P <0.05). FRP; ferric reducing power, OD; optical density.

To explore the relationship between the IP values and the antioxidant power of filtered rapeseed oil, a linear regression analysis was performed (Figure 4.6). A significant correlation was observed (r = +0.969, p = 0.006), confirming that a linear correlation is observed between the antioxidant properties of rosemary powder and the oxidative stability of rapeseed oil (as determined by Rancimat analysis). Linear associations of the

oxidative stability of vegetable oils with antioxidant capacity have been previously reported (Ninfali *et al.*, 2002; Arranz *et al.*, 2008; Castelo-Branco *et al.*, 2016). It is known that the antioxidant capacity plays a prominent role in the protection of oil against thermal and oxidative deterioration (Budilarto and Kamal-Eldin, 2015).

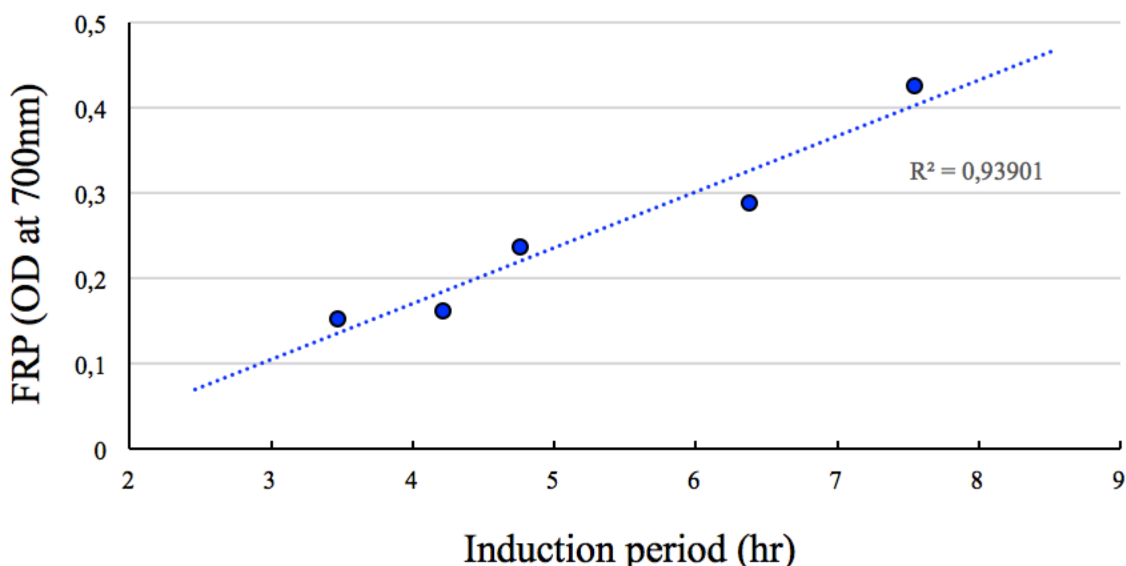


Figure 4.6. Linear regression analysis between FRP value and IP of rapeseed oil samples.

4.3.3. EFFECT OF ROSEMARY DURING ACCELERATED OXIDATION CONDITIONS

4.3.3.1. CONDUCTIVITY

The Rancimat apparatus was used to create accelerated oxidation conditions (120ºC with a constant airflow of 20 L/h) and samples were removed for analysis at 1.5 hr and 3.0 hr intervals. Under the experimental conditions, fatty acids can be degraded and secondary oxidation products are formed, including low-molecular weight, volatile organic acids such as acetic and formic acid. These are transported by the airstream to a vessel containing distilled water, where conductivity is continuously measured.

The recorded conductivity values for plain rapeseed oil after 1.5 hr and 3.0 hr of incubation in accelerated oxidation conditions were 5.08 and 17 µS/cm, respectively. Rosemary addition to rapeseed oil prevented the formation of oxidation products, as

determined by the changes in conductivity at the specified time intervals (Figure 4.7). The more pronounced protective effect of rosemary was shown after 3 hr of the accelerated oxidation process: the addition of 1% and 2% w/w of rosemary was able to reduce conductivity by 80% and 75% respectively. Unexpectedly, a non-significant increase of conductivity was recorded for oil samples containing 2% of rosemary. This result may suggest that optimum formulations for rosemary powder concentration can be within the 1-1.5% range.

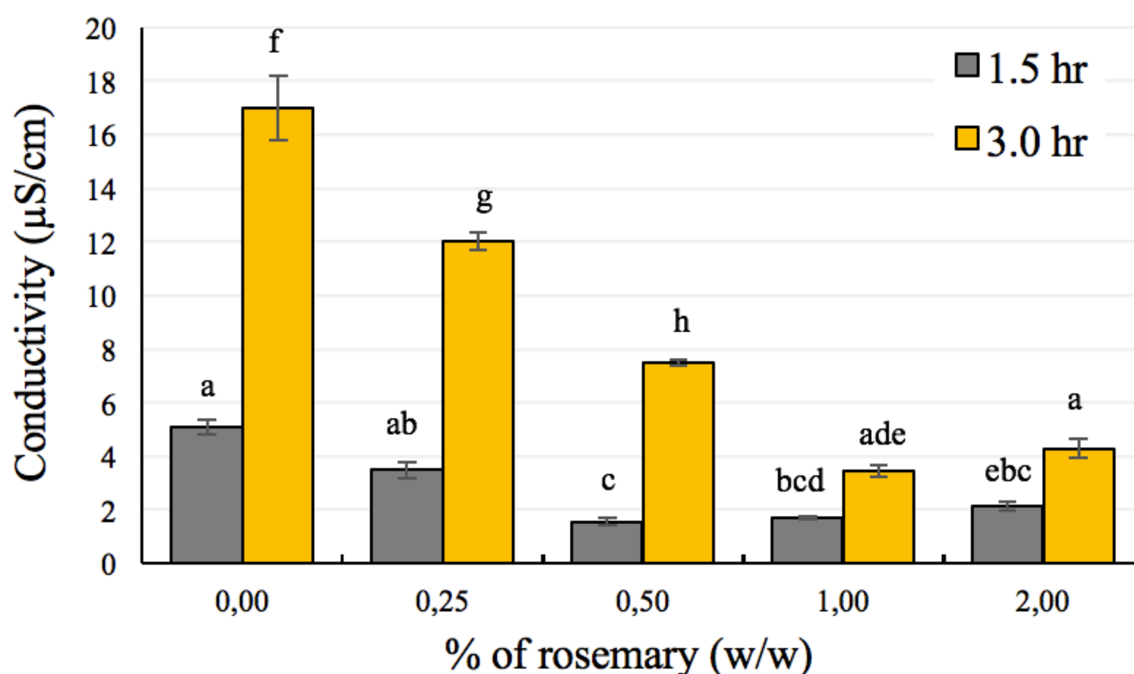


Figure 4.7. Conductivity of samples measured in Rancimat apparatus during accelerated oxidation conditions at 1.5 hr and 3 hr. Results are presented as mean ± SD. Values with different lower case letter are significantly different ($P<0.05$).

The reduction in conductivity measured with Rancimat when rosemary powder was incorporated can be related to the activity of antioxidant compounds. The inclusion of filtered rosemary resulted in the release of active compounds with known antioxidant properties, such as carnosic acid and carnosol, into rapeseed oil (Xie *et al.*, 2017). These compounds can inhibit or retard the oxidative decomposition of rapeseed oil.

4.3.3.2. TOTAL COLOUR DIFFERENCE (ΔE)

The oil appearance (colour) is one of the traditional methods used for assessing its quality. The Hunter scale, which evolved during the 1960s, is based on the opponent-colour theory. This theory assumes that the human eye receptors perceive colour as the following pairs of opposite: the L value is an estimation of the luminosity and indicates dark (0-50) or white (51-100); a positive number indicates red and a negative number indicates green for the a scale, whereas the b value is positive for yellowish colors and negative for the bluish ones.

ΔE is a single value that takes into account the differences between the L, a, and b of the sample and standard. ΔE is a combination of the parameters L, a and b values and is extensively used to characterize the variation of colour in oils during processing. It was calculated from equation described in 3.7.4. section (Materials and Methods) where L_0, a_0 and b_0 correspond to the measurements of the fresh sample and L, a and b to the measurements after 1.5 hr and 3.0 hr of exposure to accelerated oxidation conditions.

The ΔE of the oil after accelerated oxidation conditions are shown in Table 4.8. A remarkable reduction in ΔE was observed with rosemary addition, indicating a protective effect against the oxidative degradation of the oil. It has been reported that rosemary extract can effectively protect oil colour changes during a deep-frying process in hazelnut (Tohma and Turan, 2015) and sunflower oil (Urbančič *et al.*, 2014).

Table 4.8. Total colour differences (ΔE) of the oils after accelerated oxidation conditions.

	ΔE	
% of rosemary (w/w)	**1.5 hr**	**3 hr**
0	5.99±0.08[a]	14.7±0.12[a]
0.25	2.78±0.08[b]	8.69±0.26[b]
0.5	2.42±0.12[c]	7.66±0.28[c]
1.0	0.93±0.11[d]	1.60±0.09[d]
2.0	0.84±0.05[d]	2.79±0.12[e]

Results are expressed as mean ± SD (standard deviation). Means followed by the same lower case letters are not significantly (P>0.05) different between treatments (columns).

4.3.3.3. MALONDIALDEHYDE (MDA) AND CONJUGATED DIENE (CD) CONTENT

Both, MDA and CD are formed as secondary oxidation products as a result of the oxidation of polyunsaturated fatty acids. Results from changes in MDA and CD values in rapeseed oil during the accelerated oxidation process and the effect of rosemary addition are shown in Figure 4.8.

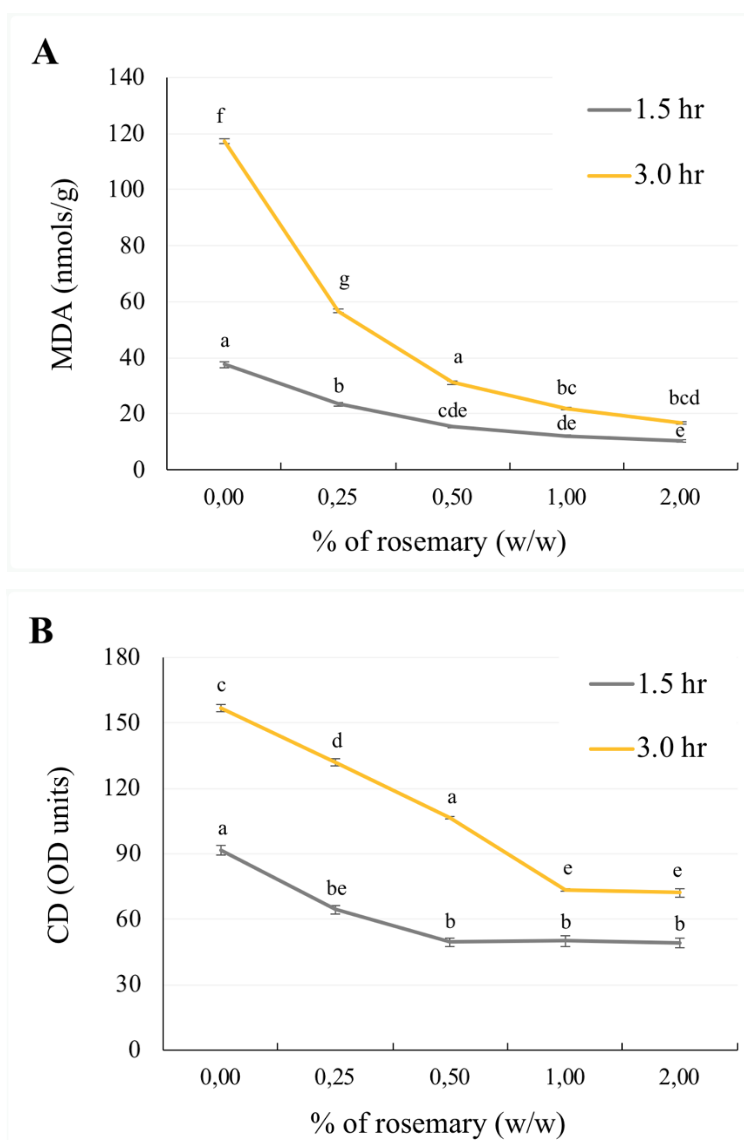


Figure 4.8. Malondialdehyde (MDA) (**A**) and conjugated diene (CD) (**B**) content after exposure to accelerated oxidation conditions in Rancimat apparatus. Results are presented as mean ± SD. Values with different lower case letter are significantly different (P <0.05).

All the rosemary concentrations incorporated in rapeseed oil, exhibited a significant ($P < 0.05$) decrease in MDA formation during the accelerated oxidation conditions. The more pronounced effect was observed for samples incubated with 1% and 2% (w/w) of rosemary powder. These samples showed a decrease of 81% and 86% in the MDA content respectively at the end of the incubation period (3 hr). The effect of rosemary addition on the MDA levels of lipids has been documented previously. Gallego *et al.* (2013) stated that rosemary extract can prevent MDA formation in oil-in-water emulsions. Furthermore, Zhang *et al.* (2010) investigated the effect of carnosinic acid on the stability of sunflower oil incubated at 60°C for a total period of 21 days; carnosinic acid was capable to inhibit the formation of MDA at all concentrations.

Following exposure to accelerated oxidation conditions for 1.5 hr, rapeseed oil samples incubated with 0.5% and 1% (w/w) rosemary showed a decrease of 45% in CD content, meanwhile higher rosemary concentrations (2%) led to a further decrease of 46% (compared to the control). After 3 hr of exposure, the addition of 1% and 2% (w/w) of rosemary powder, decreased the CD content of the rapeseed samples by 53% and 54% respectively compared with the plain oil. The protective effect of rosemary addition against CD formation in vegetable oils has been previously reported. The addition of unfiltered rosemary powder (0.5% w/w) to rapeseed oil prevented the formation of CD during deep frying (Redondo-Cuevas, Castellano and Raikos, 2017). CD levels increased by 29% when the plain rapeseed oil was used for frying; meanwhile, when rosemary powder was added, the corresponding increase was only 8%. Rosemary extract addition in sunflower oil decreased CD formation by 49% after 20 deep-frying cycles (Urbančič *et al.*, 2014). Furthermore, the addition of rosemary extract in soybean oil decreased CD formation by 43% following a heating process at 180ºC for 2 hr (Ravi Kiran *et al.*, 2015).

Antioxidant compounds from rosemary such as rosmarinic acid, carnosic acid and carnosol (Xie *et al.*, 2017), could be responsible for the observed reduction in CD and MDA formation (secondary oxidation products) in rapeseed oil during accelerated oxidation conditions. Carnosic acid and carnosol contain a single aromatic ring with two −OH groups that can serve as H* donors and can also chelate pro-oxidative metals, thus preventing oxidation via two mechanisms (Brewer, 2011). Furthermore, carnosic acid is a reactive oxygen species quencher (Loussouarn *et al.*, 2017). However, a synergistic effect from the antioxidant compounds present in rosemary is more likely since rosemary

extracts demonstrate higher antioxidant activity than do the individual phenolic compounds separately (Brewer, 2011).

4.3.3.4. TOCOPHEROL AND CAROTENOID CONTENT ANALYSIS

Tocopherols and carotenoids are naturally present in most vegetable oils and they may influence the oxidative stability of oils, even if present at minor quantities.

Tocopherols are the most important antioxidants present in rapeseed oil. These compounds exhibit their antioxidant activity by donating a phenolic hydrogen atom to lipid free radicals, thereby retarding the autocatalytic lipid peroxidation processes (Choe and Min, 2009). Heating treatment resulted to a significant decrease ($P<0.05$) of alpha tocopherol levels after 1.5 hr and 3 hr of exposure and the same effect was observed for gamma tocopherol after 3 hr (Figure 4.9). There were no significant changes in gamma tocopherol levels after 1.5 hr of heat treatment and delta tocopherol levels remained unaffected at all treatments. Alpha-tocopherol was the form that degraded faster (66% in the first 1.5 hr and non-detected after 3 hr), meanwhile delta-tocopherol seems to be the most stable of all tocopherols, under the specified oxidation conditions. In agreement with this study, previous reports denote that tocopherol content decreases during heating or frying (Barrera-Arellano *et al.*, 2002; Casal *et al.*, 2010; Nayak *et al.*, 2016; Saoudi *et al.*, 2016) and alpha-tocopherol was shown to be the least stable form (Barrera-Arellano *et al.*, 2002; Choe and Min, 2006b; Márquez-Ruiz, Ruiz-Méndez and Velasco, 2014).

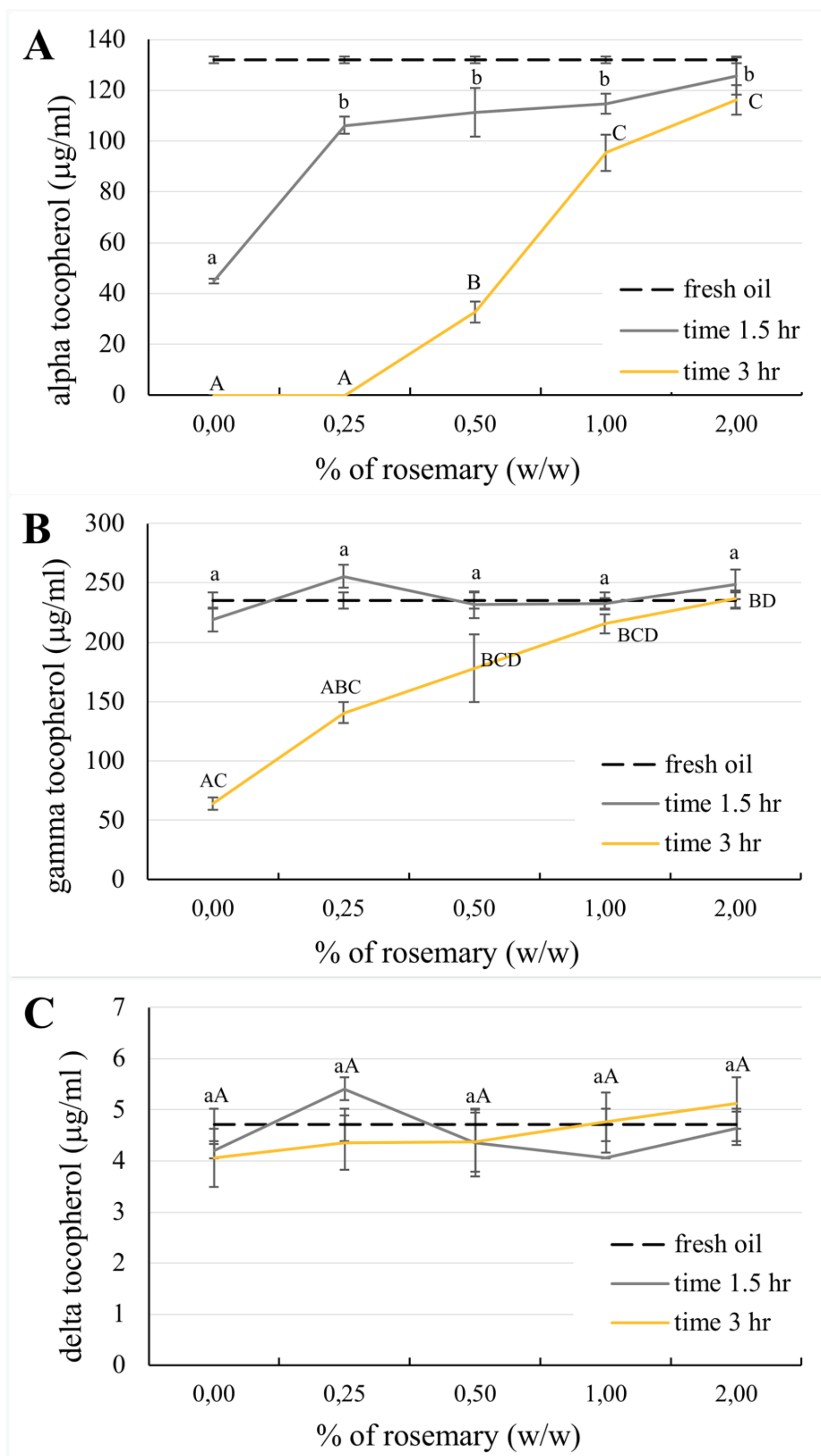


Figure 4.9. Effect of rosemary concentration in rapeseed oil on alpha tocopherol (**A**), gamma tocopherol (**B**) and delta tocopherol (**C**) levels during accelerated oxidation conditions in Rancimat apparatus. Results are presented as mean ± SD. Values with different lower (for values at 1.5 hr) or upper (for values at 3 hr) case letter are significantly different ($P<0.05$).

Alpha-tocopherol levels remained unaffected ($P>0.05$) from the heating process (1.5 hr) regardless the rosemary concentration (0.25%-2.0%). After 3 hr exposure, 0.25% of rosemary powder added to the oil was not sufficient to prevent the total degradation; at 0.5% concentration, the alpha-tocopherol levels were significantly higher, and at concentrations above 1% the alpha-tocopherol was preserved at a level similar to the one detected in the fresh (unheated) oil (Figure 4.9A). With respect to gamma-tocopherol levels, the addition of rosemary at concentrations of 0.5% or above prevented the degradation to not significant levels ($P>0.05$) after 3 hr of heating (Figure 4.9B). It has been previously reported that the inclusion of a rosemary extract has a protective effect against tocopherol degradation (Beddows, Jagait and Kelly, 2000; Casarotti and Jorge, 2014; Saoudi *et al.*, 2016). Specifically, the incorporation of rosemary extract to soybean oil preserved approximately 50% of the total tocopherols when heated at 180ºC for 18 hr (Saoudi *et al.*, 2016). Beddows, Jagait and Kelly (2000) observed that rosemary, thyme, turmeric, sage, oregano and cumin extracts preserved alpha-tocopherol in sunflower oil when subjected to heating at temperatures ranging from 85ºC to 105ºC in a Rancimat apparatus.

Carotenoids are also known to protect lipids from free radical auto-oxidation by reacting with peroxyl radicals, inhibiting their propagation and promoting termination of the oxidation chain reaction (Choe and Min, 2009; Zeb and Murkovic, 2013). The heating treatment significantly decreased ($P<0.05$) both xanthophyll and beta carotene content after 1.5 hr (46% and 47% respectively) and 3 hr (88% for xanthophyll and non-detected for beta carotene) of exposure (Figure 4.10). The present findings are in accordance with previous studies (Zeb and Murkovic, 2013), which reported a thermal degradation of approximately 50% for beta carotene in rapeseed oil when heated at 110ºC for 1 hr.

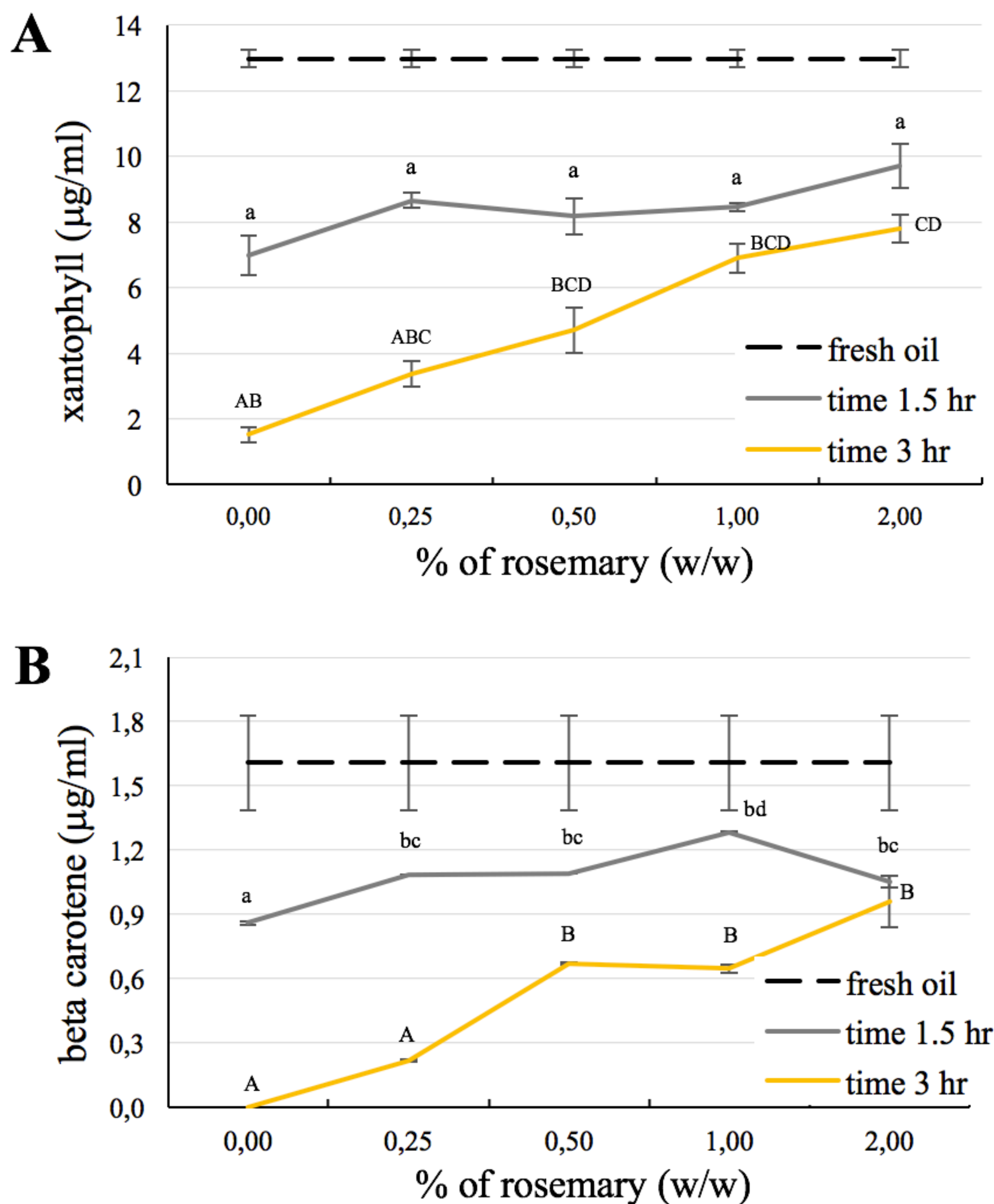


Figure 4.10. Effect of rosemary concentration in rapeseed oil on xanthophyll (**A**) and beta carotene (**B**) levels during accelerated oxidation conditions in Rancimat apparatus. Results are presented as mean ± SD. Values with different lower (for values at 1.5 hr) or upper (for values at 3 hr) case letter are significantly different ($P<0.05$).

On one hand, the addition of rosemary seems to have a protective but non-significant ($P>0.05$) effect against the degradation of xanthophyll after exposure to oxidation conditions for 1.5 hr. On the other hand, under more severe oxidation conditions (3.0 hr) the addition of rosemary to rapeseed oil at concentrations above 0.5% (w/w), has a significant beneficial effect on the stability of xanthophyll (Figure 4.10A). Similarly,

rosemary powder protected beta carotene from degradation after 1.5 hr of exposure at any concentration ($P<0.05$), whereas this effect was significant at concentrations >0.5% (w/w) after 3 hr of exposure (Figure 4.10B).

Phenolic diterpenes (carnosol, carnosic acid) are the main antioxidant compounds found in rosemary plant. These compounds exert their antioxidant activity through different mechanisms (Loussouarn *et al.*, 2017). The findings of the present study suggest that compounds present in rosemary can prevent lipid oxidation of vegetable oils by acting as secondary antioxidants and stabilizing indigenous antioxidants such as tocopherols and carotenoids. Moreover, it has been shown that certain polyphenols exhibit the ability to repair alpha-tocopherol by reducing the alpha tocopheroxyl radical (Brewer, 2011).

Conclusions

CHAPTER 5. CONCLUSIONS

The main conclusions of the present Thesis are:

1. The 22 oils and fats studied (including refined, non-refined and cold-pressed) show a huge diversity in composition in terms of tocopherols, beta-carotenes, chlorophyll, total phenolic compounds and fatty acid composition. The samples studied were: tocopherol-stripped corn oil, olive oil, seven varieties of virgin olive oils, sunflower oil (refined and cold pressed), high oleic sunflower oil, rapeseed oil (refined and cold pressed), sesame oil (refined and cold pressed), toasted sesame oil, coconut oil (refined and cold pressed), red palm oil, butter and ghee.
2. **Coconut and olive oils show the highest oxidative stability**, whereas tocopherol-stripped corn oil, sunflower and sesame oils generated the lowest values. **Sunflower and sesame oils are not suitable for frying purposes** according to the literature (assuming a reference value of 24% of total polar compounds that indicates the maximum permitted levels in frying oils). Stripped corn oil is also below the limit; however, this product is not intended for human or animal consumption and is supplied for laboratory use only.
3. Principal component analysis enable us to group 22 oils and fats into **three different clusters**:
    a. High in SFA, fats (solids at ambient temperature).
    b. High in MUFA, olive oils and high oleic sunflower oil.
    c. High in PUFA, seed oils.
4. Edible oils and fats contain natural compounds that can act as antioxidants or pro-oxidants, however **there is not a unique compound that can account for the oxidative stability in a high percentage**. **Total phenolic compounds** and **saturated fatty acids** are the most important individual factors (explaining 41.7% and 32.1% of variability, respectively) which correlated positively with oxidative stability. When all the parameters were taken in account, nine components (SFA, MUFA, PUFA, chlorophyll, *beta*-carotene, TPC, *delta*-, *gamma*- and *alpha*-tocopherol) explained 76.5% of total variability, whereas SFA, MUFA and PUFA

combined accounted for 67% of variability. Thus, **the type of fatty acids is critically important for the oxidative stability of oils and fats**.

5. **Rosemary powder** has the best potential to protect vegetable oils from oxidation compared with black pepper, ginger, turmeric and oregano. Rosemary shows the highest antioxidant capacity and has the highest concentration of phenolic compounds. Adding rosemary (0.5% w/v) is the most effective means to increase the oxidative stability of tocopherol-stripped corn oil and stability was superior compared to the formulations containing BHT at permitted levels.

6. The addition of **rosemary powder** to sunflower, olive and rapeseed oil during a **deep-frying process**, led to a significant increase of their oxidative stability. Rosemary has an additional beneficial effect when incorporated in rapeseed oil, as it prevents CD and CT formation. Rosemary addition to a vegetable oil can inhibit lipid oxidation during frying, however, further elaborate studies on lipid oxidation are needed to establish the effect of rosemary powder during frying in vegetable oils.

7. The maceration of **rosemary powder** in rapeseed oil followed by **filtration**, improved the oxidative stability of the oil. The protective effect is indicated by lower conductivity changes, total colour differences, and reduced MDA and CD content after exposure for 1.5 hr and 3 hr to accelerated oxidation conditions in Rancimat apparatus. Moreover, the oil filtrate with rosemary powder showed higher antioxidant capacity and a protective effect against the degradation of tocopherol and carotenoid content during the accelerated oxidation process. The beneficial effects of rosemary powder are more profound at **concentrations above 0.5% (w/w)**.

8. **Rosemary** is a natural product that effectively protects vegetable oils from oxidation, incorporated either as ground spice in powdered form or powder filtrate. This could lead to alternative reformulation strategies for oil manufacturers, with potential domestic or industrial applications.

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ANNEXE: SCIENTIFIC PUBLICATIONS

Redondo-Cuevas L, Castellano G, Torrens F, Raikos V. Revealing the relationship between vegetable oil composition and oxidative stability: A multifactorial approach. *Journal of Food Composition and Analysis*. 2018;66:221–229. DOI: 10.1016/j.jfca.2017.12.027

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