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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 5
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
JUAN PABLO CADENA SARMIENTO

Asunto: Radicación: 12-12071- -5
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 10401
 Folios: 5

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/BR2010/000226				
Fecha de Presentación Internacional	16/07/2010				

1. Documentos estudiados

Descripción:	Rad. N°12-012071-00000-0000	26/Enero/2012
Reivindicaciones:	Rad. N°12-012071-00000-0000	26/Enero/2012
Prioridad:	BR PI0903778-0	17/Julio/2009

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Para el caso de la presente solicitud, al verificar la tasa pagada, todo el ultimo capitulo reivindicatorio presentado se tendrá en cuenta para realizar el examen de patentabilidad.

Título de la solicitud: “compuesto alimenticio de grasa vegetal, proceso de preparación

del compuesto alimenticio de grasa vegetal y uso del compuesto alimenticio de grasa vegetal en la preparación de rellenos”.

El problema planteado en esta solicitud es la necesidad de desarrollar composiciones alimenticias que tengan un contenido reducido de ingredientes no deseables, tales como isómeros trans y ácidos grasos saturados en las formulaciones de grasa, sin perder propiedades de textura, consistencia y palatabilidad, así como el proceso de obtención de dichas grasas de forma simple y con bajo costo.

Para resolver este problema técnico la solicitud en estudio proporciona un proceso de preparación de un compuesto alimenticio con sus etapas debidamente definidas y un compuesto alimenticio debidamente definido (Rad. N°12-012071-00000-0000, Pág. 8).

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 23 D 07/00.

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 22 – 23 no es patentable, de acuerdo con el Art 14, puesto que las reivindicaciones mencionan el uso del compuesto alimenticio.

5. De la Solicitud de Patente

Título

El título no corresponde con el objeto de la solicitud porque se menciona el uso de la composición y esto no es patentable, además porque no se mencionan los ingredientes de manera general que componen el compuesto alimenticio.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 menciona la expresión: “opcional” precediendo una característica por lo tanto no es concisa, porque hace alternativas a las características y no permite delimitar el alcance de la reivindicación. Se sugiere cambiar el término.

La reivindicación 2 menciona un catalizador, pero este es un término general que abarca muchos tipos de compuestos, se debe mencionar de qué tipo de catalizadores o cuales son los de interés a emplear.

En la reivindicación 11 se menciona un compuesto alimenticio definido por unos componentes, pero no se menciona en qué proporción se encuentra cada uno de ellos, por lo tanto se le recuerda al solicitante que las proporciones hacen parte de las características esenciales de dicho compuesto alimenticio y que deben mencionarse en esta reivindicación.

En la reivindicación 11 se menciona el término “antioxidante” el cual es muy amplio puesto que incluye una gran cantidad de compuestos por lo tanto se sugiere mencionar que tipo de antioxidantes o cuales son los de interés.

En las reivindicaciones 11 y 21 se mencionan una serie de variables, tales como un límite máximo de ácidos grasos trans, un contenido de ácidos grasos saturados, un punto de goteo, un índice de peróxido las cuales representan un resultado a alcanzar y no definen al compuesto alimenticio porque la invención no queda definida por el resultado a alcanzar, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere retirar dichos resultados.

En las reivindicaciones 14 y 15 se mencionan los antioxidantes de interés y las proporciones en que se encuentran, pero no es claro el número de antioxidantes que lleva el compuesto alimenticio, puesto que según las realizaciones preferidas son más de uno. Por lo tanto se debe mencionar claramente en la reivindicación 11, que es la independiente y contiene todas las características esenciales de la invención, cuales, cuantos y en que proporciones son los antioxidantes de interés.

Las reivindicaciones 14 y 16 – 19 mencionan las proporciones en que debe ir cada uno de los componentes del compuesto alimenticio, las cuales hacen parte de las características esenciales que definen la invención y deben mencionarse en la reivindicación 11, que es la independiente y contiene todas las características esenciales que definen la invención.

El capítulo reivindicatorio no es claro en cuanto a si el proceso de las primeras reivindicaciones produce el compuesto alimenticio de la reivindicación 1, puesto que en el proceso no se mencionan los mismos ingredientes del compuesto alimenticio, principalmente en el proceso no se menciona alguna etapa donde se agregue el antioxidante. Se sugiere aclarar bien si el proceso es para la obtención del compuesto alimenticio de la reivindicación 11.

Descripción (Art 28 D 486)

En la descripción se menciona que se realizó un prueba de análisis sensorial, pero no es muy claro que tipo de pruebas se realizaron, es decir si se hizo un perfil sensorial que parámetros (sabor, color, aroma y textura) se evaluaron. Además si los panelistas eran catadores y efectivamente se evaluó la palatabilidad y se hizo un análisis comparativo con una muestra sin la invención versus una con la invención o si simplemente lo que se realizó fue una prueba hedónica con consumidores, para determinar la aceptabilidad del producto. Se sugiere aclarar este ensayo.

La descripción menciona que se siguieron las etapas del proceso y que se mezclaron los ingredientes, además de los ensayos químicos, pero no se mencionan las mejoras en la palatabilidad, textura del compuesto alimenticio y el bajo costo y mejor rendimiento del proceso.

6. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la Fecha de Presentación de la Solicitud de Prioridad, Julio 17 de 2009 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de publicación*
D1	WO2009029793	"Polyunsaturated fatty acid-containing solid fat compositions and uses and production thereof"	05/marzo/2009
D2	WO0200815	"Biodegradable vegetable oil compositions"	03/Enero/2002
D3	EP0771531	"Stable edible oil composition"	07/Mayo/1997
D4	WO0505747	"Natural antioxidant system "	02/Marzo/1995

El documento D1^[1] divulga un método de producción de una composición sólida grasa que comprende a) mezclar un aceite que comprende grasas saturadas y un aceite que comprende al menos un LC – PUFA (ácidos grasos polinsaturados de cadena larga) para formar una mezcla y b) solidificar la mezcla. Además comprende una etapa de interesterificación y una etapa de Desodorización (Pág. 4, líneas 19 – 22; Pág.

¹URL: [http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=2009029793A1&KC=A1&FT=D&ND=3&date=20090305&DB=EPODOC&locale=en_EP)

[CC=WO&NR=2009029793A1&KC=A1&FT=D&ND=3&date=20090305&DB=EPODOC&locale=en_EP](http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=2009029793A1&KC=A1&FT=D&ND=3&date=20090305&DB=EPODOC&locale=en_EP)

5, líneas 12, 6 – 22; Pág. 66, reivindicación 9).

El documento D2 ^[2] divulga una composición que comprende: a) un aceite de triglicérido que es un aceite vegetal, tal como aceite de semilla de algodón, c) Una amina antioxidante d) Otros aceites, tal como aceites refinados y sin refinar. Donde la composición provee estabilidad antioxidante (Págs. 1, 3, 5 y 11).

El documento D3 ^[3] divulga una composición de aceite comestible que comprende: Volumen aceite que comprende un tipo de aceites o mezcla, tal como aceite de semilla de algodón y un aceite de semillas de sésamo, junto con un tercer aceite que puede ser aceite con alto contenido en 5- Δ avenaesterol, tal como aceite de germen de avena, salvado de arroz (Pág. 2, líneas 13 y 14; Pág. 7, Línea 28 – 52; Pág. 8, línea 36 a Pág. 9 línea 33).

El documento D4 ^[4] divulga una composición natural de antioxidantes que comprende Tocoferoles, β -caroteno, ácido cítrico, ácido ascórbico y Fosfolípidos tal como lecitina de soya. Donde las proporciones son preferiblemente 0,001% a 0,005% de tocoferoles, 0,001% a 0,05% de β -caroteno; 0,001 a cerca de 0,005% de ácido cítrico y cerca de 0,1% a 0,2 % de ácido ascórbico (Pág. 4, líneas 15 – 22; Pág. 5, líneas 1 – 5; Pág. 6, líneas 16 – 24)

7. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La **reivindicación 1** consiste en un proceso de preparación debidamente definido (Rad. N°12-012071-00000-0000, Págs. 19 – 20).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
Proceso de preparación:	Método de producción:
a)hidrogenación total de un aceite de semilla de algodón clarificado	Mezclar una estearina (estearina de semilla de algodón) que comprende un LC-PUFA(ácidos grasos polinsaturados de cadena larga) con un segundo aceite (aceite de semilla de algodón no fraccionada, oleína de semillas de algodón, mezcla de aceite de palma interesterificada, mezcla de aceite de semilla de algodón interesterificada y mezcla de estas) que comprende una grasa saturada para formar una mezcla
b)Mezcla del aceite de semilla de algodón hidrogenado que resulto de la etapa a) con aceite de semilla de algodón liquido	
c) interesterificación de la mezcla obtenida en la etapa b)	
d)adición de aceite de palma completamente hidrogenado a la base interesterificada producida en la etapa c)	
Adición opcional de aceite de palmiste completamente hidrogenado al producto obtenido en la etapa d)	La mezcla es interesterificada
Desodorización del producto obtenido en cada una de las etapas d) o e)	La mezcla es desodorizada
Enfriamiento del producto obtenido en el etapa f)	No se menciona

2^oURL:[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=0200815A2&KC=A2&FT=D&ND=3&date=20020103&DB=EPODOC&locale=en_EP)

CC=WO&NR=0200815A2&KC=A2&FT=D&ND=3&date=20020103&DB=EPODOC&locale=en_EP

3^oURL:[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19970507&CC=EP&NR=0771531A2&KC=A2)

DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19970507&CC=EP&NR=0771531A2&KC=A2

4^oURL:[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19950302&CC=WO&NR=9505747A1&KC=A1)

DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19950302&CC=WO&NR=9505747A1&KC=A1

El documento D1 es considerado el estado de la técnica cercano a la invención definida en la reivindicación 1, porque divulga un método de producción de una composición solida grasa que comprende a) mezclar un aceite que comprende grasas saturadas y un aceite que comprende al menos un LC – PUFA (ácidos grasos polinsaturados de cadena larga) para formar una mezcla y b) solidificar la mezcla (Pág. 4, líneas 19 – 22). Además comprende una etapa de interesterificación (Pág. 5, línea 12). Donde el aceite de grasas saturadas incluye estearina de semillas de algodón y donde el segundo aceite puede ser seleccionado de aceite de semilla de algodón no fraccionada, oleína de semillas de algodón, mezcla de aceite de palma interesterificada, mezcla de aceite de semilla de algodón interesterificada y mezcla de estas (Pág. 5, líneas 6 – 22). Además el método comprende una etapa de Desodorización (Pág. 66, reivindicación 9)

La diferencia entre la invención, definida en la reivindicación 1 y el método de preparación del documento D1 consiste en que D1 no especifica la etapa de enfriamiento, ni el orden de las etapas, mientras que la solicitud menciona un orden en las etapas y la etapa de enfriamiento.

El efecto que lograría la diferencia de especificar el orden y de mencionar la etapa de enfriamiento, en cuanto a rendimiento y al bajo costo, antes mencionadas en el proceso de la reivindicación 1, no puede ser determinado toda vez que no existe alguna evidencia que indique que dicha diferencia en cuanto a especificar el orden de las etapas y mencionar la etapa de enfriamiento, otorgan al proceso ahora reivindicado una ventaja técnica sobre el método ya conocido en la anterioridad D1, puesto que el método de D1 proporciona un producto con una alta cantidad de LC-PUFA (ácidos grasos poliinsaturados de cadena larga).

Por lo tanto, el problema técnico objetivo a resolver consiste en plantear una alternativa al método conocido según D1 para que sea más eficiente en el rendimiento de la grasa, la simplicidad del proceso y el bajo costo.

Teniendo en cuenta lo expuesto anteriormente, la persona de nivel medio versada en la materia derivara de manera obvia el proceso descrito en la reivindicación 1 de la presente invención a partir de las enseñanzas del documento D1 toda vez que las diferencias se limitan en especificar un orden de las etapas y agregar una etapa de enfriamiento.

Puesto que las reivindicaciones dependientes 6 – 9 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

Conclusión:

Las reivindicaciones 1, 6 – 9 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

La **reivindicación 11** consiste en un compuesto alimenticio de grasa vegetal debidamente definido (Rad. N°12-012071-00000-0000, Págs. 20 – 21).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D2	D3	D4
Compuesto alimenticio que incluye:	Una composición que comprende:	Composición de aceite comestible que comprende:	Una composición que comprende:
Una base interesterificada formada de aceite de semilla de algodón líquido y aceite	Aceite de triglicérido que es un aceite vegetal, tal como aceite de semilla de	Volumen aceite: un tipo de aceites o mezcla, tal como aceite de semilla de	Fosfolípidos tal como lecitina de soya

de semilla hidrogenado	algodón	algodón	
Aceite de palma hidrogenado	Otros aceites, tal como aceites refinados y sin refinar	Aceite con alto contenido en 5 – Δ avenaesterol, tal como aceite de germen de avena, salvado de arroz	
Antioxidante	Una amina antioxidante	Aceite de semillas de sésamo	Tocoferoles, β-caroteno, ácido cítrico y ácido ascórbico

Los documentos D2, D3 y D4 son considerados el estado de la técnica cercano a la invención definida en la reivindicación 8, porque:

El documento D2 divulga una composición que comprende: a) un aceite de triglicérido que es un aceite vegetal, tal como aceite de semilla de algodón, c) Una amina antioxidante (Págs. 3, 5) d) Otros aceites, tal como aceites refinados y sin refinar (Pág. 11). Donde la composición provee estabilidad antioxidante (Pág. 1).

El documento D3 divulga una composición de aceite comestible que comprende: Volumen aceite que comprende un tipo de aceites o mezcla, tal como aceite de semilla de algodón y un aceite de semillas de sésamo, junto con un tercer aceite que puede ser aceite con alto contenido en 5-Δavenaesterol, tal como aceite de germen de avena, salvado de arroz (Pág. 7, Línea 28 – 52; Pág. 8, línea 36 a Pág. 9 línea 33). Donde dichas composiciones proporcionan estabilidad térmica (Pág. 2, líneas 13 y 14)

El documento D4 divulga una composición natural de antioxidantes que comprende Tocoferoles, β-caroteno, ácido cítrico, ácido ascórbico y Fosfolípidos tal como lecitina de soya. Donde las proporciones son preferiblemente 0,001% a 0,005% de tocoferoles, 0,001% a 0,05% de β-caroteno; 0,001 a cerca de 0,005% de ácido cítrico y cerca de 0,1% a 0,2 % de ácido ascórbico (Pág. 4, líneas 15 – 22; Pág. 5, líneas 1 – 5; Pág. 6, líneas 16 – 24)

La diferencia entre la invención, definida en la reivindicación 11 y la composición del documento D2 consiste en que D2 no especifica que la composición comprende un aceite de palma hidrogenado sino que menciona aceites refinados o sin refinar.

La diferencia entre la invención, definida en la reivindicación 11 y la composición del documento D3 consiste en que D3 no especifica que el segundo aceite sea de palma sino que menciona que es un aceite proveniente de germen de avena o de salvado de arroz, entre otros.

La diferencia entre la invención, definida en la reivindicación 11 y la composición del documento D4 consiste en que D4 no especifica la presencia de dos aceites sino que simplemente menciona la presencia de fosfolípidos.

El efecto que lograría la diferencia de especificar el origen de uno de los aceites

presentes en el compuesto alimenticio, en cuanto a no perder propiedades de textura, consistencia y palatabilidad, antes mencionadas en el compuesto alimenticio de la reivindicación 11, no puede ser determinado toda vez que no existe alguna evidencia que indique que dicha diferencia de especificar el origen de un aceite, otorgan al compuesto alimenticio ahora reivindicado una ventaja técnica sobre las composiciones ya conocidas en la anterioridad D2 o D3 o D4, puesto que la composición de D2 proporciona estabilidad antioxidante o D3 proporciona estabilidad térmica, principalmente para alimentos fritos o D4 proporciona la reducción de la oxidación de grasas y aceites en alimentos.

Por lo tanto, el problema técnico objetivo a resolver consiste en plantear una alternativa a las composiciones conocidas según D2 o D3 o D4 para que sean más eficientes en cuanto a no perder propiedades de textura, consistencia y palatabilidad.

Además es conocida la importancia de emplear antioxidantes en la industria alimentaria, principalmente en comidas lipídicas, por su actividad en la emulsión y los efectos en el procesamiento de aceites comestibles⁵, al igual que el efecto de los tocoferoles en los aceites vegetales refinados y en los procesos de fritura⁶

Teniendo en cuenta lo expuesto anteriormente, la persona de nivel medio versada en la materia derivara de manera obvia el compuesto alimenticio descrito en la reivindicación 11 de la presente invención a partir de las enseñanzas del documento D2 o D3 o D4 toda vez que las diferencias se limitan en especificar el origen de un aceite y pues cada uno de los documentos ya mencionan las mejoras de un producto con aceites con menor contenido de grasas saturadas y con la presencia de antioxidantes.

Puesto que las reivindicaciones dependientes 13, 14 y 19 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas

Conclusión:

La reivindicaciones 11, 13, 14 y 19 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2009029793	1, 6 – 9	Nivel inventivo
D2	WO0200815	11, 13, 14 y 19	Nivel inventivo
D3	EP0771531	11, 13, 14 y 19	Nivel inventivo
D4	WO9505747	11, 13, 14 y 19	Nivel inventivo

5 Frankel. Antioxidants in lipid foods and their impact on food quality. Food Chemistry, vol 57, No1, pp 51 – 55, 1996.

6 Rossi et Al. Tocopherols and tocotrienols as free radical-scavengers in refined vegetable oils and their stability during deep-fat frying. Food chemistry 102, p 812 – 817, 2007.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-06-18

Elaboró: Mayra Alejandra Quintero
Revisó: Gloria Jacqueline Alfonso Roza

Antioxidants in lipid foods and their impact on food quality

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This paper reviews the antioxidant properties of tocopherols and ascorbic acid in edible oils, and the impact of interfacial phenomena on their activities in emulsions and the effects of edible oil processing. Tocopherols are the most important natural antioxidants found in vegetable oil-derived foods. These antioxidants can interrupt lipid autoxidation by interfering with either the chain propagation or the decomposition processes. α -Tocopherol at high concentrations inhibits hydroperoxide decomposition but promotes hydroperoxide formation. The effect of antioxidants in inhibiting hydroperoxide decomposition may thus be critical in preserving food quality by reducing rancidity due to aldehyde formation. Ascorbic acid can regenerate α -tocopherol, inactivate metal initiators and reduce hydroperoxides. The activity of natural antioxidants is greatly affected by complex interfacial phenomena in emulsions and multi-component foods. In an oil-in-water emulsion system, the lipophilic antioxidants α -tocopherol and ascorbyl palmitate are more effective than in bulk oil, while the opposite trend is found for the hydrophilic antioxidants Trolox and ascorbic acid. The methodology to evaluate natural antioxidants must be carefully interpreted depending on whether oxidation is carried out in bulk oils or in emulsions, and what method is used to measure lipid oxidation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The development of rancidity in edible oils is a serious problem in some sectors of the food industry because of increasing emphasis on the use of polyunsaturated vegetable and fish oils, discontinuing the use of synthetic antioxidants and fortification of cereal foods with iron. Oxidation of lipids not only produces rancid odors and flavors, but can decrease the nutritional quality and safety by the formation of secondary products in foods after cooking and processing.

Effective control methods against lipid oxidation include processing by minimizing the loss of natural tocopherols, by eliminating metal contamination and by using antioxidants. In the last 15–20 years, special attention has been given to the use of natural antioxidants because of the worldwide trend to avoid or minimize the use of synthetic food additives. The interest in natural antioxidants continues to grow because they are presumed to be safe since they occur in foods and have been used for centuries, and the question of safety of synthetic compounds can thus be avoided. Furthermore, evidence is accumulating that natural antioxidants in foods may have clear benefits because they have anticarcinogenic effects and inhibit biologically

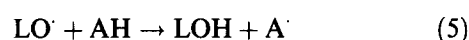
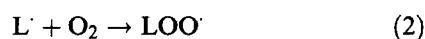
harmful oxidation reactions in the body (Simic & Karel, 1980).

Much research has been carried out to better understand the basic processes of oxidation of polyunsaturated fatty acids, antioxidant action and the effects of decomposition products of lipid oxidation. In our research we recently found that interfacial phenomena are keys to a better understanding of antioxidant action in heterogeneous foods and biological systems. To learn about the real effects of antioxidants, it is important to obtain specific chemical information about what products of lipid oxidation are inhibited. Several specific assays are needed to elucidate how lipid oxidation products act in the complex multi-step mechanism of lipid oxidative deterioration in foods.

This paper reviews the stabilizing effects of tocopherols and ascorbic acid and esters in edible oils, and the effects of oil processing on their antioxidant properties. New work is reviewed on the effect of complex interfacial phenomena affecting the activity of natural antioxidants in emulsions and multi-component foods. The methodology to evaluate natural antioxidants must be carefully interpreted depending on whether oxidation is carried out in bulk oils or in emulsions, and what analytical method is used to determine extent and end-point of oxidation.

α -TOCOPHEROL ACTION

Polyunsaturated lipids (LH) can form alkyl radicals (L $\cdot$) when they become oxidized in the presence of an initiator (X $\cdot$), generally an alkoxy radical (LO $\cdot$) produced by decomposition of hydroperoxides in the presence of trace metals (eqn 1). These alkyl radicals react very rapidly with oxygen to form peroxy radicals (LOO $\cdot$) (eqn 2), which react with more lipids to produce hydroperoxides (LOOH) (eqn 3). α -Tocopherol inhibits this free radical oxidation by reacting with the peroxy radicals to stop chain propagation (eqn 4), and with the alkoxy radicals to inhibit the decomposition of the hydroperoxides and decrease the formation of aldehydes (eqn 5). Thus, α -tocopherol behaves as a chain-breaking antioxidant by competing with the substrate (LH) for the chain-carrying peroxy radicals, normally present in the highest concentration in the system (eqn 3):



The tocopherol radical can form non-radical products, including dimers, stable peroxides, alkyl or unsaturated derivatives, whereby the antioxidant is regenerated.

Tocopherol mixtures are generally regarded as poor antioxidants in edible oils, especially those containing polyunsaturated fatty acids. With soybean oil containing 1500 $\mu\text{g/g}$ mixed tocopherols, we found that the oxidative stability is markedly increased by removing a portion of the natural tocopherols with carbon-black (Frankel *et al.*, 1959a). The oxidative stability of soybean oil was optimum at concentrations between 400 and 600 $\mu\text{g/g}$. This observation has now been confirmed in studies with soybean oil-tocopherol mixtures added to stripped corn oil (Huang *et al.*, 1995).

The relative antioxidant activity of different tocopherol homologs has received much attention in the literature. On the basis of induction period measurements of oxidation of methyl linoleate, lard, cottonseed, and linseed esters, γ - and δ -tocopherols have been reported to be relatively more effective than β - and α -tocopherols (Lea, 1960; Lea & Ward, 1959). On the basis of initial rates of oxidation of styrene in chlorobenzene solution in the presence of azo-bis(isobutyronitrile) as chain initiator, the relative activities of tocopherol isomers increased in the same order as their vitamin E activities: $\alpha > \beta > \gamma > \delta$ (Burton & Ingold, 1981).

The temperature of oxidation had a marked effect on the relative activities of tocopherol homologs in linseed esters high in linolenate but not in cottonseed esters in which linoleate was the main unsaturated fatty acid (Lea & Ward, 1959). The discrepancies in the literature

concerning the relative antioxidant activities of tocopherol homologs can be attributed to the wide differences in unsaturated substrates tested, the level of oxidation used in the tests and the method used to analyze oxidation.

α -Tocopherol can act as an antioxidant or pro-oxidant depending on the test system, the concentration, the oxidation time and the method used to follow oxidation. On the basis of hydroperoxide formation (measured by conjugated diene formation) in bulk corn oil, α -tocopherol exhibited optimum antioxidant activity at lower concentration (100 $\mu\text{g/g}$) than in the corresponding oil-in-water emulsion (250–500 $\mu\text{g/g}$). However, on the basis of hydroperoxide decomposition (measured by hexanal formation), the antioxidant activity of α -tocopherol increased with concentration and oxidation time in both bulk and emulsified oil (Huang *et al.*, 1994).

The effect of different lipid substrates has a significant impact on the activity of different antioxidants according to their hydrophilic or lipophilic character. We recently compared the antioxidant activities of α -tocopherol and its carboxylic acid analog Trolox in different lipid substrates (Huang *et al.*, 1996). In bulk methyl linoleate and corn oil triglycerides, Trolox was a better antioxidant than α -tocopherol, but the opposite trend was observed in the corresponding oil-in-water emulsions. On the other hand, Trolox was a better antioxidant than α -tocopherol in both bulk and emulsified linoleic acid. Linoleic acid constitutes a unique substrate because of the formation of mixed micelles. For this reason, linoleic acid is not an appropriate lipid to test antioxidants since their behavior in this substrate would be significantly different than that in foods composed mainly of triglycerides. Yet linoleic acid is commonly used to evaluate natural antioxidants (Frankel, 1993).

ASCORBIC ACID ACTION

Ascorbic acid is noted for its complex multi-functional effects. Depending on conditions ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or as an oxygen scavenger. In aqueous systems containing metals, ascorbic acid can act as a pro-oxidant by reducing the metals, which become more active catalysts of oxidation in their lower valence state (Uri, 1961). In the absence of added metals, ascorbic acid is an effective antioxidant at high concentrations (Cort, 1982). In non-aqueous media, ascorbic acid and esters are not good antioxidants (Porter, 1980).

When we removed the tocopherols almost completely from soybean oil by carbon-black treatment, added α -tocopherol or BHT were less effective than citric or ascorbic acids (Frankel *et al.*, 1959a) (Fig. 1). The mixtures of α -tocopherol and citric acid gave the same induction period as citric acid alone. The mixture of BHT and citric acid was as effective as the mixture of α -tocopherol and citric acid. Therefore, in soybean oil

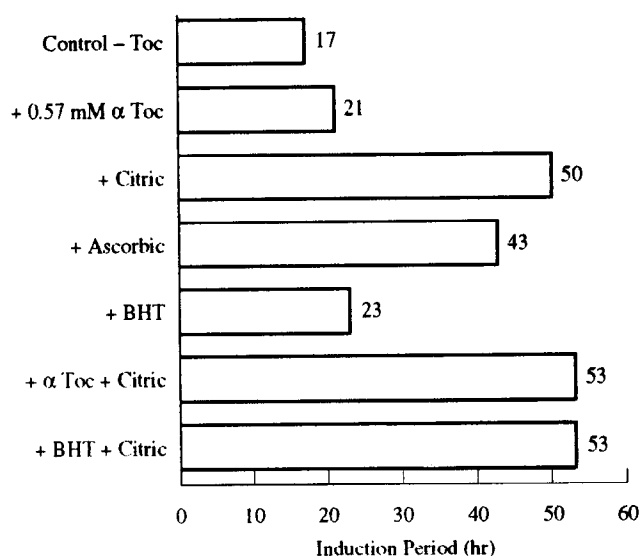


Fig. 1. Effect of tocopherols and antioxidants on oxidative stability of soybean oil at 60°C (Frankel *et al.*, 1959a). Control-Toc is carbon-treated soybean oil containing 45 µg/g tocopherols; 0.57 mM/kg oil equivalent to 0.01% ascorbic acid.

the metal chelation effect by citric acid may be more important than the antioxidant effects of α-tocopherol or BHT.

Cort (1974, 1982) also obtained different antioxidant trends under different oxidation conditions. In soybean oil oxidized at 45°C, ascorbic acid was less effective than ascorbyl palmitate, which was in turn more effective than BHA and BHT. TBHQ was the most active of the antioxidants tested. At 98°C the reverse trend was observed and ascorbic acid was more active than both ascorbyl palmitate and TBHQ.

The mixture of α-tocopherol and ascorbic acid exhibits a strong synergistic effect which is well recognized (Uri, 1961; Tappel *et al.*, 1961). Evidence based on pulse radiolysis (Packer *et al.*, 1979) and electron spin resonance studies (Bascetta *et al.*, 1983; Niki *et al.*, 1984; Löliger *et al.*, 1986) supports a redox mechanism involving reduction of the tocopheroxyl radical intermediate by ascorbic acid to regenerate α-tocopherol. By this synergistic mechanism tocopherols and ascorbic acid can mutually reinforce one another by regenerating the oxidized form of the other. Another mechanism for synergism involves the metal inactivating effect of ascorbic acid. Tocopherols are readily decomposed in the presence of metals and their activity is significantly improved by adding a metal inactivator (Frankel *et al.*, 1959b).

The lipophilic derivative ascorbyl palmitate is also known for its synergistic activity with natural tocopherols in vegetable oils where it is more active than BHT and BHA (Cort, 1974). The mixtures of α-tocopherol, ascorbic acid or ascorbyl palmitate and phospholipids are also known for their good synergistic activities (Bourgeois, 1981; Löliger, 1989a).

The multiple effects of ascorbic acid and ascorbyl palmitate include: (a) hydrogen donation to regenerate

the stable antioxidant radical; (b) metal inactivation to reduce the rate of initiation by metals; (c) hydroperoxide reduction to produce stable alcohols by non-radical processes; and (d) oxygen scavenging.

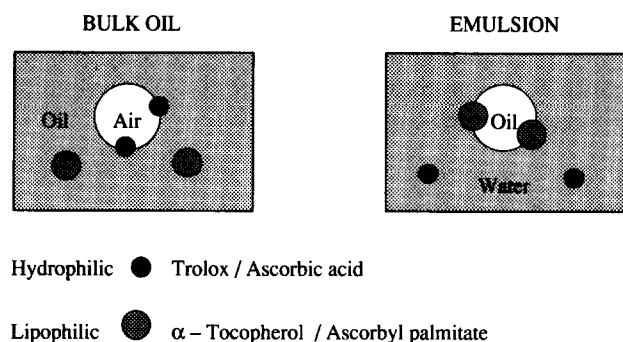
EFFECT OF PROCESSING

Tocopherols are decomposed during vegetable oil processing but a large proportion are left in the finished oils. Mixed tocopherols in refined, bleached and deodorized (RBD) soybean oil range between 550 and 1000 µg/g (Sherwin, 1978). The loss of α-tocopherol in soybean oil was 4.3% after refining, 15% after bleaching and 20–51% after deodorization (Kanematsu *et al.*, 1983b). This loss of tocopherols does not reduce the oxidative stability of the RBD soybean oil because its final tocopherol content, ranging between 700 and 1000 µg/g, is still above the optimum range of 400–600 µg/g for antioxidant activity (Frankel *et al.*, 1959a). The losses of tocopherols reported in other refined vegetable oils were 18% in olive oil, 25% in soybean and rapeseed oils, 32% in corn oil, 36% in cottonseed oil, 37% in sunflower oil and 40% in peanut oil (Kanematsu *et al.*, 1983a). The tocopherol levels in rapeseed, sunflower, cottonseed, soybean and corn oils, ranging from 640 to 1170 µg/g, may be adequate to protect the oils against oxidation under ambient conditions. However, the levels of tocopherols left in the olive oil (110 µg/g) and the peanut oil (200 µg/g) may not be sufficient for optimum oxidative stability, but the level for best antioxidant protection has not been established for these oils.

The oxidative stability of virgin olive oils containing high levels of natural phenolic compounds was significantly decreased by their high initial peroxide values compared with an RBD olive oil, which contained much lower concentrations of phenolic compounds and peroxide value (Satué *et al.*, 1995). Phenolic compounds extracted from virgin olive oils increased the oxidative stability when added to the RBD olive oil. α-Tocopherol behaved as a pro-oxidant at concentrations higher than 250 µg/g, on the basis of peroxide value, but was very effective in inhibiting hexanal formation in RBD olive oil. Whether or not determination of hexanal formation is a better index of rancidity than peroxide value remains to be established in vegetable oils of known storage history.

ACTION OF ANTIOXIDANTS

The published data comparing the antioxidant activities of plant extracts and spices are difficult to evaluate because of the diverse testing methods used and the questionable conditions of oxidation (Frankel, 1993). Natural antioxidants exhibit complex interfacial affinities between air–oil and oil–water interfaces that significantly affect their relative activities in different lipid systems. Porter (1980, 1983) and Porter *et al.* (1989)



ANTIOXIDANTS

Fig. 2. Interfacial phenomena to explain the action of antioxidants in bulk oil and oil-in-water emulsion systems (Frankel *et al.*, 1994).

observed that in food systems of low surface-to-volume ratio (e.g. bulk vegetable oils) polar antioxidants are more effective than non-polar lipophilic antioxidants. In contrast, in foods of high surface-to-volume ratio (e.g. emulsified oils) lipophilic antioxidants are strongly favored. Trolox proved to be superior to α -tocopherol under a wide range of conditions and test systems (Cort *et al.*, 1975).

In our research we recently found that interfacial phenomena are keys to a better understanding of antioxidant action in heterogeneous foods and biological systems. Thus, the lipophilic antioxidants α -tocopherol and ascorbyl palmitate were more effective in an oil-in-water emulsion system than in bulk oil, while the opposite trend was found for the hydrophilic antioxidants Trolox and ascorbic acid (Frankel *et al.*, 1994). Mixtures of α -tocopherol and ascorbic acid were more active in bulk oil, while mixtures of α -tocopherol and ascorbyl palmitate were more efficient in emulsion systems. Differences observed in the efficiency of antioxidants were explained by their affinities toward the air-oil interfaces in bulk oil and the oil-water interfaces in emulsions (Fig. 2). In the bulk corn oil system the hydrophilic antioxidants are oriented in the air-oil interface and become more protective against oxidation than the lipophilic antioxidants which remain in solution in the oil. In contrast, in the oil-in-water emulsion system, lipophilic antioxidants are sufficiently surface active to be oriented in the oil-water interface to better protect oil against oxidation. By moving to the water phase the hydrophilic antioxidants become too diluted to adequately protect the oil in the oil-water interface. According to this mechanism, hydroperoxide formation and decomposition in emulsion systems are dependent on the effective concentrations of antioxidants in the oil and the water phases, and the interface.

Rosemary extracts provide a major source of commercial natural antioxidants used in foods (Löliiger, 1989b). In a recent study, significant differences in antioxidant activities were found for a commercial rosemary extract and its components, carnosic acid, carnosol and rosmarinic acid, when they were compared in bulk corn oil stripped of natural tocopherols and in the

corresponding corn oil-in-water emulsions (Frankel *et al.*, 1996). In bulk corn oil, the rosemary extract, carnosic acid, rosmarinic acid and α -tocopherol were significantly more active than carnosol. In contrast, in corn oil-in-water emulsion, the rosemary extract, carnosic acid, carnosol and α -tocopherol were more active than rosmarinic acid. These differences in antioxidant action can be explained by the same interfacial phenomenon as observed with α -tocopherol and Trolox (Frankel *et al.*, 1994). Thus, polar hydrophilic rosemary compounds may be less active in the emulsion system because they partition into the water phase and become less protective than in the bulk oil system.

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Tocopherols and tocotrienols as free radical-scavengers in refined vegetable oils and their stability during deep-fat frying

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Abstract

The aim of this study was to assess the effect of total tocopherols and tocotrienols of refined vegetable oils on oil radical-scavenging activity and to investigate the stability of the various homologues during the deep-fat frying of French fries. Eight different refined vegetable oils were investigated, having variable levels of natural tocopherols and tocotrienols. A direct correlation between the radical-scavenging capacity of the oils, measured by the DPPH test, and the total content of natural tocopherols and tocotrienols was found. Frying experiments showed that the stability of the different tocopherols and tocotrienols present in the refined vegetable oils basically depend on two factors: the fatty acid composition of the oil, in particular polyunsaturated fatty acid content, and the kind of tocopherol and tocotrienol homologues present. The more oxidizable the oil, on the basis of fatty acid composition, the more stable were the tocopherolic antioxidants. Among the different homologues, γ -tocotrienol in palm super olein proved to be the least stable during the deep-fat frying, thus preserving the other homologues.

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Keywords: Antioxidants; Deep-fat frying; DPPH; Radical-scavenging activity; Refined oils; Tocopherols; Tocotrienols

1. Introduction

Deep-fat frying may be defined as the process of cooking foods by immersing them in an edible oil or fat maintained at a temperature of about 150–200 °C (Yamsaengsung & Moreira, 2002). There are many types of oils and fats available for frying. The oil or fat used for frying becomes part of the food being fried; therefore, the quality of the frying oil is of great importance with regard to quality of the fried food (Kochhar, 2001). During frying, a complex series of various chemical reactions, such as thermoxidation, hydrolysis and polymerisation, takes place and the frying oil decomposes to form a variety of volatile compounds as well as monomeric and polymeric products. Several factors affect the overall performance of frying oils (Andrikopoulos, Kalogeropoulos, Falirea, & Barbagianni, 2002). In

particular, each frying oil has a characteristic stability against autoxidation, depending on the composition of fatty acids and the content and composition of minor components. Actually, fatty acid composition alone may not adequately explain the stability of frying oils (Normand, Eskin, & Przybylski, 2001). Crude vegetable oils contain a variety of minor components, such as hydrocarbons, sterols, tocopherols, polyphenols, colour compounds and trace metals. Some of these components, e.g. tocopherols (particularly γ -tocopherol), phospholipids (at less than 100 mg/kg), carotenoids (at low levels), squalene, and certain sterols, are beneficial to oil stability during frying (Kochhar, 2001).

Natural tocopherols and tocotrienols are retained at considerable levels in finished refined vegetable oils (Karabulut, Topcu, Yorulmaz, Tekin, & Ozay, 2005; Norris, 1982; Rossi, Gianazza, Alamprese, & Stanga, 2001; Simone & Eitenmiller, 1998). Since they are relatively thermal-resistant, so that only modest tocopherol losses are registered during deodorization/distillation phase (carried

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out at 220–260 °C) (De Greyt & Kellens, 2000; Rossi et al., 2001), their natural antioxidant activity should protect the refined oils against thermal oxidation (Simonne & Eitenmiller, 1998). Tocopherols and tocotrienols, while protecting the fat, are generally themselves first oxidized to quinones and dimers (Hoffmann, 1989).

There is some difficulty in adequately evaluating the antioxidant activity of tocopherols and tocotrienols, because this activity is frequently influenced by their concentration, the types of oils and fats used as substrates, and the method of evaluation (Yoshida, Kajimoto, & Emura, 1993). Moreover, the relative stabilities of the individual homologues, under simulated frying conditions, vary according to the oils under study (Simonne & Eitenmiller, 1998).

Antioxidant activity can be evaluated as total free radical-scavenging capacity, by spectrophotometrically measuring the disappearance of the free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The DPPH[•] test has previously been successfully employed in assessing the antioxidant activity of crude oils (Ramadan, Kroh, & Morsel, 2003), in particular virgin olive oil (Gomez-Alonso, Fregapane, Salvador, & Gordon, 2003; Lavelli, 2002; Rotondi et al., 2004), but little is known about refined vegetable oils (Espín, Soler-Rivas, & Wichers, 2000).

In the literature, much information is available on the deterioration of frying oils (Andrikopoulos et al., 2002; Du Plessis & Meredith, 1999; Gomez-Alonso et al., 2003; Negishi, Nishida, Endo, & Fujimoto, 2003; Saguy & Dana, 2003; Singh & Tyagi, 2001), whereas few studies (Andrikopoulos et al., 2002; Barrera-Arellano, Ruiz-Mendez, Velasco, Marquez-Ruiz, & Dobarganes, 2002; Du Plessis & Meredith, 1999; Gordon & Kourimska, 1995; Normand et al., 2001; Simonne & Eitenmiller, 1998) have been done on the stability of tocopherols and tocotrienols during deep-fat frying. Besides, some studies have been carried out under simulated frying conditions, in the absence of food (Barrera-Arellano et al., 2002; Simonne & Eitenmiller, 1998).

The aim of this study was to assess the effect of the total tocopherol and tocotrienol contents of selected refined vegetable oils on their radical-scavenging activity, measured by the DPPH test, and to investigate the stability of the various homologues during the deep-fat frying of French fries.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade hexane and stabilized 2,2-diphenyl-1-picrylhydrazyl, an artificial free radical, were purchased from Sigma-Aldrich (Steinheim, Germany); α -, γ - and δ -tocopherols were purchased from Calbiochem-Merck KGaA (Darmstadt, Germany); HPLC-grade ethyl acetate was purchased from Merck.

2.2. Materials

Samples of eight different refined vegetable oils were investigated in this work: three palm oils (PO1, PO2 and PO3) and two palm super oleins (double fractionated; PSO1 and PSO2) directly provided by a local refinery (Industria Grassi e Oli Raffinati, Orzinuovi, Italy); one olive oil (OO), one sunflower oil (SO) and one organic frying oil (SHO) made of a blend of sunflower and hazelnut oils, all purchased at local supermarkets. Samples PO1, PO3, PSO1 and OO were only analysed as fresh oils, while PO2, SO, PSO2 and SHO were also used for frying experiments. Two different retail-bought varieties of potato were used for frying experiments: “Vittoria” (from Belgium) and “Bintje” (from Holland). One hour before use, potatoes were manually peeled and cut into sticks (9 mm × 9 mm × 60 mm). Subsequently, the sticks were washed for 5 min in flowing water and then dried for 5 min between two sheets of blotting paper. Finally, they were stored in an air-tight polystyrene container to avoid moisture loss before frying.

2.3. Frying experiments

Two groups of frying experiments were carried out. In the first group, PO2 and SO oils were used for 12 h, frying Vittoria potatoes; in the second group, PSO2 and SHO oils were used for 18 h, frying Bintje potatoes. Experiments were carried out using a domestic deep-fat fryer equipped with a removable stainless steel wire basket and a thermostat. The operative conditions were as follows: actual frying temperature, 175 °C; potato cooking time, 8 min; break time between two successive fryings, 7 min. A low and constant potatoes to oil ratio of 25 g:1 l (Simonne & Eitenmiller, 1998) was used, in order to avoid excessive cooling of the oil at the moment of food immersion and to have enough oil for all sampling, also taking into account the oil uptake by food. Frying sessions lasted continuously for 6 h/day with no oil make up. After each frying session, the fryer was switched off and the oil covered and left at room temperature until the next session.

2.4. Sampling

Oil sampling for analysis (100 ml) was performed during frying every 3 h, for both groups of experiments. For the second group, oil was also sampled after 1 and 2 h of frying. Samples were stored at –20 °C prior to analysis.

2.5. Fatty acid composition

The relative percentage of fatty acids in the fresh oils was determined by capillary gas chromatography on fatty acid methyl esters (FAMES) obtained according to Liebich, Wirth, and Jakober (1991). Gas chromatographic analysis

of FAMES was carried out as reported by Colonello, Rossi, and Amelotti (1996), using a Carlo Erba HRGC 5160 instrument equipped with a Supelco SP-2340 capillary column (60 m length, 0.25 mm internal diameter, and 0.25 μ m phase thickness). The operative conditions were as follows: carrier, H₂ (90 kPa); oven temperature, 177 °C for 18 min, increased to 205 °C (2.3 °C/min), 205 °C for 2 min, increased to 210 °C (2 °C/min), 210 °C for 30 min; injection temperature was 240 °C; flame ionisation detector temperature, was 250 °C.

2.6. Tocopherol and tocotrienol content

The concentrations of the different tocopherol and tocotrienol homologues in fresh and fried oils were determined by normal phase HPLC analysis, as reported by Rossi et al. (2001). Oil samples were diluted with HPLC-grade hexane using a dilution ratio of 1:15 (w/v), and filtered through a 0.22 μ m GV membrane (Millipore Co., Bedford, MA). Calibration curves were prepared by analysing standard solutions in hexane of the following: α -tocopherol in the range 9–53 mg/l, γ -tocopherol in the range 4–45 mg/l, and δ -tocopherol in the range 8–40 mg/l. The curves were linear within the concentration range considered ($r \geq 0.998$). α -, γ - and δ -tocotrienols were identified by comparing retention times with the previous study (Rossi et al., 2001) and quantified using the same response factors as the corresponding tocopherol homologues according to the AOCS Ce 8-89 method (2001).

2.7. Radical-scavenging capacity

The total free radical-scavenging capacity of fresh oils was evaluated by measuring the disappearance of the stabilized 2,2-diphenyl-1-picrylhydrazyl artificial free radical (DPPH $\cdot$) at 515 nm according to the method of Espín et al. (2000), with minor modifications. A 6.1×10^{-5} M DPPH $\cdot$ ethyl acetate solution, adjusted to an absorbance of 0.670 AU, was used. A dose–response curve was built, using, for each point, a mixture of 1900 μ l DPPH $\cdot$ solution and 100 μ l sample diluted with HPLC-grade ethyl acetate at five different ratios. Mixture absorbance was read after 30 min of reaction at 25 ± 1 °C. Results are reported as $1/I_{50}$, where I_{50} , extrapolated from the rectilinear part of the dose–response curve, is the volume of fresh oil (in μ l) required to lower the initial DPPH $\cdot$ concentration by 50%.

All the above analyses were performed in duplicate.

2.8. Calculated oxidizability (Cox) of oils

The Cox value of oils was calculated by the percentage of unsaturated C18 fatty acids, applying the formula proposed by Fatemi and Hammond (1980):

$$\text{Cox} = [1(18:1\%) + 10.3(18:2\%) + 21.6(18:3\%)]/100.$$

2.9. Statistical analysis

A Pearson correlation matrix was performed using Systat 5.03 software for Windows (Systat Inc., USA).

3. Results and discussion

Table 1 lists the fatty acid composition, the ratio between unsaturated and saturated fatty acids and the Cox value of the refined vegetable oils considered. It can be observed that the palm oils (PO1, PO2 and PO3) and the palm super oleins (PSO1 and PSO2) can be distinguished from the other oils due to their high concentration of saturated fatty acids, palmitic acid (C16:0) in particular. Consequently, the ratio between unsaturated and saturated proves to be the lowest in these oils. SO stands out for its high linoleic acid content (C18:2) and its low saturated fatty acid content, which make it particularly prone to oxidation. Indeed, of the oils considered, SO shows the highest Cox value. OO and SHO are characterized by a very high concentration of oleic acid (C18:1) and a low content of saturated fatty acids. SHO is a blend of sunflower oil (primary ingredient) and hazelnut oil; given the fatty acid composition of the blend, it can be hypothesized that a sunflower oil high in oleic fatty acid was used in its formulation, although this was not stated on the label. Even though SHO presents the highest ratio of unsaturated to saturated fatty acids, its Cox value is not particularly high, as the predominant unsaturated acid is the monounsaturated oleic acid which has a lower weight, in the Cox calculation, than the polyunsaturated linoleic and linolenic acids (C18:3), since its oxidation rate is far below those of the other C18 unsaturated acids (Gunstone & Hilditch, 1946).

In Table 2, the composition and the total content (as the sum of all the identified homologues) of tocopherols and tocotrienols of the various refined oils considered are shown, together with their radical-scavenging capacities expressed as $1/I_{50}$ multiplied by 100.

SO, OO and SHO principally contain α -tocopherol (α -T), whereas the palm-derived oils are also characterized by the presence of α -tocotrienol (α -T3), γ -tocotrienol (γ -T3) and δ -tocotrienol (δ -T3). More specifically, γ -T3 proved to be the most abundant, while the other homologues were found in different ratios according to the oil considered. The tocopherol and tocotrienol contents were fairly high, confirming the resistance of these natural antioxidants to refining operations. Actually, the average tocopherol and tocotrienol contents of crude olive oil, sunflower oil, hazelnut oil and palm oil are 199, 1008, 455, and 800 mg/kg, respectively (O'Brien, 2004; Souci, Fachmann, & Kraut, 2000). Therefore, disappearance due to degradation and/or stripping in the refining processes ranges from 25% to 65% of the total content. The oils richest in tocopherols and tocotrienols were the two palm super oleins, which actually also showed the highest values for antiradical activity. The weakest radical-scavenging activity was

Table 1
Fatty acid composition (%) of the refined vegetable oils considered

Fatty acids	OO	SO	SHO	PO1	PO2	PO3	PSO1	PSO2
C8:0	Absent	Absent	Absent	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	Traces
C10:0	Absent	Absent	Absent	Traces	Absent	Absent	Traces	Traces
C12:0	Traces	Absent	Absent	0.43 ± 0.02	0.32 ± 0.07	0.30 ± 0.07	0.41 ± 0.02	0.27 ± 0.02
C14:0	Traces	0.07 ± 0.01	0.04 ± 0.01	1.19 ± 0.03	1.23 ± 0.18	1.07 ± 0.17	1.11 ± 0.02	0.96 ± 0.06
C15:0	Absent	Absent	Traces	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
C16:0	12.8 ± 0.3	6.07 ± 0.03	3.99 ± 0.01	46.3 ± 0.50	47.2 ± 2.02	45.0 ± 2.05	35.8 ± 0.15	33.9 ± 0.51
C16:1n9	0.11 ± 0.01	Traces	0.03 ± 0.01	Traces	Traces	Traces	0.04 ± 0.01	0.04 ± 0.01
C16:1n7	1.01 ± 0.02	0.08 ± 0.01	0.11 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.22 ± 0.01	0.20 ± 0.01
C17:0	0.07 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
C17:1	0.13 ± 0.01	Traces	0.04 ± 0.01	Traces	Traces	Traces	Traces	0.03 ± 0.01
C18:0	2.49 ± 0.04	3.12 ± 0.01	2.66 ± 0.02	3.99 ± 0.05	3.93 ± 0.20	3.71 ± 0.08	3.41 ± 0.06	3.42 ± 0.01
C18:1n9 <i>trans</i> + C18:1n7 <i>trans</i>	Absent	Absent	Traces	0.10 ± 0.02	0.20 ± 0.01	0.18 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
C18:1n9 + C18:1n7	72.5 ± 0.25	24.5 ± 0.11	79.0 ± 0.02	38.2 ± 0.33	37.4 ± 1.50	39.8 ± 1.78	45.0 ± 0.18	47.4 ± 0.42
C18:2n6 <i>cis-trans</i>	0.04 ± 0.01	0.48 ± 0.01	Traces	0.31 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.17 ± 0.01	0.11 ± 0.01
C18:2n6 <i>trans-cis</i>	Traces	0.41 ± 0.01	Traces	0.30 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.16 ± 0.01	0.10 ± 0.01
C18:2n6	9.51 ± 0.11	64.1 ± 0.15	12.7 ± 0.10	8.16 ± 0.07	8.61 ± 0.31	8.94 ± 0.42	12.7 ± 0.07	12.6 ± 0.19
C20:0	0.37 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.30 ± 0.01	0.28 ± 0.04	0.27 ± 0.03	0.29 ± 0.01	0.31 ± 0.01
C18:3n3 + C20:1n11	0.83 ± 0.01	0.19 ± 0.02	0.40 ± 0.01	0.19 ± 0.01	0.24 ± 0.02	0.24 ± 0.02	0.37 ± 0.01	0.40 ± 0.01
C22:0	0.10 ± 0.01	0.52 ± 0.01	0.60 ± 0.03	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
C24:0	0.04 ± 0.01	0.19 ± 0.02	0.23 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
Unsaturated/saturated	5.30	8.79	11.85	0.90	0.88	0.98	1.42	1.56
Cox value	1.89	6.89	2.18	1.27	1.31	1.37	1.84	1.86

Traces = ≤ 0.02%.

Table 2
Tocopherol and tocotrienol contents and radical-scavenging activity (average ± s.d.) of the different oil samples analyzed

Sample	α-T (mg/kg)	α-T3 (mg/kg)	γ-T3 (mg/kg)	δ-T3 (mg/kg)	Total tocol-derived compounds (mg/kg)	1/I ₅₀ × 10 ² (μL ⁻¹)
OO	148 ± 6	–	–	–	148	1.67 ± 0.07
SO	475 ± 11	–	–	–	475	5.12 ± 0.04
SHO	393 ± 14	–	–	–	393	2.79 ± 0.14
PO1	63.7 ± 0.7	62.8 ± 1.2	167 ± 2	62.8 ± 4.8	356	1.65 ± 0.09
PO2	63.4 ± 11.5	74.9 ± 2.7	138 ± 7	86.8 ± 5.4	276	1.89 ± 0.08
PO3	134 ± 9	163 ± 8	295 ± 14	72.0 ± 3.4	688	4.99 ± 0.13
PSO1	200 ± 9	192 ± 3	390 ± 14	99.8 ± 12.9	881	9.79 ± 1.01
PSO2	246 ± 10	259 ± 5	438 ± 6	122 ± 2	1064	9.08 ± 0.16

found for OO and PO1, where it was almost six times lower than the average value for the two super oleins.

The radical-scavenging activity proved to be significantly correlated ($P < 0.001$) with the total content of tocopherols and tocotrienols (Fig. 1). This proves that these compounds play a predominant role in determining the antioxidant characteristics of refined vegetable oils in which other kinds of natural antioxidants (e.g. polyphenols and carotenoids) have been removed or destroyed by the refining treatments (Andrikopoulos et al., 2002; Nergiz, 1993; Rossi et al., 2001). Moreover, as regards the various homologues, significant correlations were obtained between oil radical-scavenging activity and the contents of γ-T3 ($P < 0.01$) and α-T3 ($P < 0.05$), whilst no correlation was found for α-T and δ-T3. Indeed, in the literature, it is reported that α-T has the lowest antioxidant activity, even though its biological activity, as vitamin E, is the greatest (Belitz & Grosch, 1999), whereas γ-T3 has the highest antioxidant activity (Therault, Chao, Wang, Gapor, & Adeli, 1999).

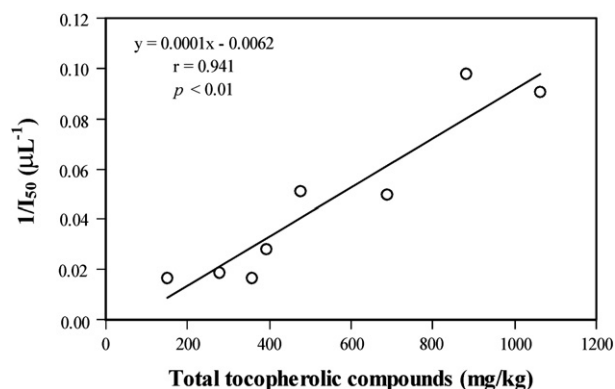


Fig. 1. Correlation between the total tocopherol and tocotrienol content and the radical-scavenging activity of the refined vegetable oils analyzed.

SO, PO2, PSO2 and SHO were utilized to perform the deep-fat frying experiments for the cooking of French fries, over a period of 12 or 18 h. During the frying experiments, the degradation kinetics of tocopherols and tocotrienols in the oils considered were studied.

Table 3
Tocopherol and tocotrienol contents (average $\pm$ s.d.) of PSO2 at different frying times

Time (h)	α -T (mg/kg)	α -T3 (mg/kg)	γ -T3 (mg/kg)	δ -T3 (mg/kg)
0	246 $\pm$ 10	259 $\pm$ 5	438 $\pm$ 6	122 $\pm$ 2
1	245 $\pm$ 10	258 $\pm$ 5	415 $\pm$ 11	122 $\pm$ 2
2	231 $\pm$ 1	236 $\pm$ 1	404 $\pm$ 26	118 $\pm$ 0
3	193 $\pm$ 2	212 $\pm$ 7	322 $\pm$ 3	113 $\pm$ 4
6	205 $\pm$ 9	214 $\pm$ 1	245 $\pm$ 2	121 $\pm$ 8
9	205 $\pm$ 7	214 $\pm$ 24	152 $\pm$ 5	112 $\pm$ 5
12	195 $\pm$ 8	199 $\pm$ 10	75.3 $\pm$ 2.8	100 $\pm$ 2
15	182 $\pm$ 2	174 $\pm$ 2	n.d.	82.5 $\pm$ 2.2
18	66.7 $\pm$ 4.7	n.d.	n.d.	n.d.

n.d., not detectable as a consequence of peak deformation due to superposition of newly-formed degradation compounds.

Table 3 shows contents of the various homologues in PSO2 at different frying times. As can be seen, in the first 2 h of frying, all the tocopherols and tocotrienols were resistant to thermoxidation, with negligible decreases recorded. Instead, starting from the third hour, a more significant reduction was observed, in particular for γ -T3, whose relatively rapid disappearance could be related to its greater antioxidant activity. Indeed, since the mechanism of the radical-scavenging reaction of tocopherols and tocotrienols requires that they lose their mobile hydrogen atoms in the hydroxyl group, forming more stable free radicals than those of the fatty acids, it follows that a faster oxidation of tocopherols and tocotrienols corresponds to a greater antioxidant power (Hoffmann, 1989).

As far as the relative stability of the various homologues is concerned, in the case of PSO2 the following order was observed (comparing the residual percentages after 9 h of frying): δ -T3 $>$ α -T $\geq$ α -T3 $>$ γ -T3. This order should correspond to an inverse order of antioxidant activity. Simone and Eitenmiller (1998) reported a slightly different order of stability (α -T $>$ δ -T3 $>$ α -T3 $>$ γ -T3) for palm olein, in simulated frying tests, and a different oxidizability for tocopherols and tocotrienols, according to the type of oil considered. The same phenomenon was also observed in this study and was brought out particularly by the data relating to SHO and SO. These two oils, which principally contain α -T, showed totally different degradation kinetics (Fig. 2). In SO, α -T proved to have a very high stability;

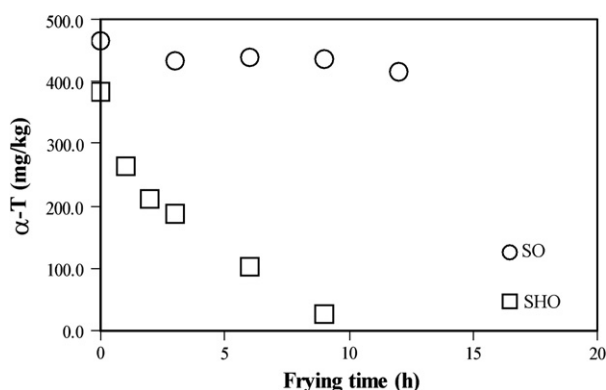


Fig. 2. α -Tocopherol content in SO and SHO during frying.

after 12 h of frying, 90% of it was still present. Instead, in SHO, the α -T content plummeted during the 18 h of frying. Already, after 3 h, it had fallen by approximately 50% and, after 9 h of frying, only 6.6% remained. The considerable difference between the behaviours of α -T in SHO and SO may be attributable to the different Cox values (Table 1) (approximately three times higher in SO). As already observed by Simone and Eitenmiller (1998), tocopherols and tocotrienols seem to degrade more quickly in the oils with a high saturated fatty acid content. Actually, one could suppose that, in the case of oils containing higher levels of polyunsaturated fatty acids, the double bonds which determine unsaturation compete with the tocopherols and tocotrienols as substrates for oxidation, determining a less rapid decrease of these antioxidants. Instead, in the case of oils low in polyunsaturated fatty acids, tocopherols and tocotrienols constitute the substrates that more easily react with oxygen. Indeed, it is reported in the literature that, in the propagation phase of the reaction, the fatty acid peroxy free radicals react preferentially with the phenolic hydrogen of the tocopherol molecule (Hoffmann, 1989).

The degradation rate of α -T in SHO is far greater even than that observed in PSO2, even though the two oils have similar Cox values (1.86 for PSO2 and 2.18 for SHO). Actually, the half time for α -T in SHO (calculated by interpolation of the curve in Fig. 2) is 2.6 h while it is 17.1 h in PSO2 (calculated by interpolation of the data in Table 3). In this case, since the Cox value cannot justify such a different degradation speed for α -T, the quality and quantity of the other homologues present in the oils take on a prominent role. In the case of PSO2, the high levels of tocotrienols, in particular γ -T3, proved to be of fundamental importance in preserving the α -T from degradation. Indeed, the tocotrienols are better antioxidants than are the corresponding tocopherols, devoid of double bonds in the side chain (Theriault et al., 1999). In the case of SHO, the α -T degrades so rapidly because it is the only tocopherol present and, thus, the only one able to act as an antioxidant.

During the frying of PO2, which has a low Cox value, slightly below that of PSO2, and a lower tocopherol and tocotrienol content, a rapid degradation of all the homologues takes place. All of them already disappear after 3–6 h of frying (data not shown).

This study has shown that, in actual frying conditions, the stability of the different homologues of the natural tocopherols and tocotrienols present in the refined vegetable oils basically depends on two factors: the oxidizability of the oil, which is connected with the polyunsaturated fatty acid content, and the kind of tocopherolic compounds present. In particular, tocopherols and tocotrienols, acting as radical scavengers, play an important antioxidant role, since the presence of other antioxidants is negligible. The more oxidizable the oil, on the basis of fatty acid composition, the more stable are the natural tocopherols and tocotrienols present in the oil. As regards the effect of the

different tocopherolic compounds, the presence of γ -T3 preserves the other homologues, in particular α -T, with which the greatest vitamin E activity is associated.

The DPPH[•] test, carried out on different refined vegetable oils, showed the existence of a direct correlation between the radical-scavenging capacity of the oils and the total content of natural tocopherols and tocotrienols.

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