VII. ANEXOS

VII.1. Abreviaturas

(24S)-HC: (24S)-hidroxicolesterol

α-CE: colesterol-5α,6α-epóxido

β-CE: colesterol-5β,6β-epóxido

27-HC: 27-hidroxicolesterol.

7α-HC: 7α-hidroxicolesterol

7β-HC: 7β-hidroxicolesterol

7-CC: 7-cetocolesterol

$A^{\bullet}$: radical antioxidante

a.ol.: alto en ácido oleico

AFNOR: Association Française de Normalisation

AG: Ácidos grasos

AGI: Ácidos grasos insaturados

AGL: Ácidos grasos libres

AGPI: Ácido grasos poliinsaturados

AGPI: Ácidos grasos poliinsaturados

AGS: Ácidos grasos saturados

AH: antioxidante

ANOVA: analysis of variance

AOAC: Association of Official Analytical Chemists

AOCS: American Oil Chemists´ Society

AOM: Active oxygen method

ASTM: American Society for Testing and Materials

ATB: Ácido 2-tiobarbitúrico

b.er: bajo en ácido erúcico

BHA: Butilhidroxianisol

BHT: Butilhidroxitolueno

BOE: Boletín Oficial del Estado

BSI: British Standard Internacional

CCF: Cromatografía en capa fina

CD: Constante Dieléctrica

CEE: Comunidad Europea

CG: Cromatografía de gases

CHP: Hidroperóxido de cumeno

CLAE: Cromatografía líquida de alta eficacia

CP: Compuestos Polares

CT: colestantriol

CV: Coeficiente de variación

d.i.: diámetro interno

DC: Dienos conjugados

DG: Diacilgliceroles

DGF: Deutsche Gesellschaft für Fettwissenschaft

Dim: Dímeros

DMSO: Dimetilpolisiloxano

EDTA: ácido etilendiaminotetracético

EM: Ésteres metílicos

ENV: Envasado

ET: Extracto de tocoferol

Euro Fed Lipid: European Federation for the Science and Technology of Lipids

FAME: ésteres metílicos de los ácidos grasos

FID: flame ionization detector

FOS: Food Oil Sensor

FOX: Ferrous oxidation xylenol orange

FTIR: Fourier Transform Infrared Spectroscopy

GC/MS: Cromatografía de gases/Espectrometría de masas

HMG-CoA: Hydroxametilglutaril coenzima A

HPL: Hidroperóxidos lípidicos

GA: grado de acidez

IA: índice de acidez

IAn: índice de p-anisidina

IC: índice de carbonilos

II: índice de yodo

IP: índice de peróxidos

IR: índice de refracción

ISO: Internacional Standard Organization

IUPAC: International Union of Pure and Applied Chemistry

JOCS: Japan Oil Chemists´ Society

$L^{\bullet}$: radical alquilo

LD: Dosis Letal

LDL: Low-density Lipoprotein

LH: molécula lipídica

$LO^{\bullet}$: radical alcoxilo

$LOO^{\bullet}$: radical peroxilo

LOOH: hidroperóxido lipídico

MANOVA: multifactor analysis of variance

MC: Monómeros cíclicos

MDA: Malondialdehído

MG: Monoacilgliceroles

MOE: Margen de exposición

NCPA: National Cottonseed Products Association

NOAEL: Non Observed Adverse Effects Level

OSET: Oxidative stability at elevated temperature

PA: Palmitato de ascorbilo

POL: Polímeros

TA: Tiempo de almacenamineto

TBHQ: tert-butilhidroxiquinona

TC: Trienos conjugados

TCA: Tricloroacético

TEP: 1,1,3,3- tetraethoxypropane

TFRIT: Tiempo de fritura

TG: Triacilgliceroles

THF: Tetrahidrofurano

TPP: Trifenilfosfina

UNE: Una Norma Española

UV: Ultravioleta

Vis: Viscosidad

VII.2. Métodos Oficiales

AOCS Official Method Cd 1-25

Withdrawn (Historical Interest Only)

Iodine Value of Fats and Oils Wijs Method

DEFINITION

The iodine value is a measure of the unsaturation of fats and oils and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample (% iodine absorbed).

SCOPE

Applicable to all normal fats and oils that do not contain conjugated double bonds (see Notes, 1). Applicable to all normal fats and oils with samples having iodine values between 0–15.

APPARATUS

1. Glass-stoppered iodine flasks—500 mL.
2. Glass-stoppered volumetric flasks—1000 mL, for preparing standard solutions.
3. Pipet—25 mL, for accurately dispensing 25.0 mL of Wijs solution.
4. Volumetric dispenser—20 mL, 1-mL adjustability, for 10% potassium iodide (KI) solution.
5. Volumetric dispenser—2 mL, 1-mL adjustability, for starch solution.
6. Volumetric dispenser—50 mL, 1-mL adjustability, for distilled water.
7. Repeater pipet—with filling flask, 20 mL, for cyclohexane.
8. Analytical balance—accurate to ±0.0001 g.
9. Magnetic stirrer.
10. Filter paper—Whatman no. 41H, or equivalent.
11. Beakers—50 mL.
12. Hot air oven.
13. Timer.

REAGENTS

1. Wijs solution—see Notes, 2 and *Caution*.
2. Potassium iodide (KI)—reagent grade.
3. Carbon tetrachloride—reagent grade (see Notes, *Caution* and Recommendations). The absence of oxidizable matter in this reagent is verified by shaking 10 mL of the reagent with 1 mL of saturated aqueous potassium dichromate solution and 2 mL of concentrated sulfuric acid; no green coloration should appear.
4. Soluble starch solution—recently prepared, tested for sensitivity (see Notes, 3). Make a paste with 1 g of natural, soluble starch (see Notes, 4) and a small amount of cold distilled water. Add, while stirring, to 100 mL of boiling water.

   Test for sensitivity—Place 5 mL of starch solution in 100 mL of water and add 0.05 mL of *freshly prepared* 0.1 N KI solution and one drop of a 50 ppm chlorine solution made by diluting 1 mL of a commercial 5% sodium hypochlorite (NaOCl) solution to 1000 mL. The deep blue color produced must be discharged by 0.05 mL of 0.1 N sodium thiosulfate.
5. Potassium dichromate—primary standard grade. The potassium dichromate is finely ground and dried to constant weight at about 110°C before using.

   Note—A standard sample of potassium dichromate with a certificate of analysis may be obtained from the National Bureau of Standards in Washington, D.C., USA. This sample, or equivalent, is strongly recommended for the primary standard for this method. Treat as directed in the certificate of analysis accompanying the sample.
6. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)—reagent grade.

SOLUTIONS

1. Potassium iodide (KI) solution—100 g/L (10% solution), prepared by dissolving 100 g of reagent grade KI in 1000 mL of deionized water.
2. Starch indicator solution—prepared and tested as noted in Reagents, 4. Salicylic acid (1.25 g/L) may be added to preserve the indicator. If long storage is required, the solution must be kept in a refrigerator at 4–10°C (40–50°F). Fresh indicator must be prepared when the end point of the titration from blue to colorless fails to be sharp. If stored under refrigeration, the starch solution should be stable for about 2–3 weeks.
3. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) solution—0.1 N (see Notes, 5), accurately standardized vs. potassium dichromate primary standard as follows:
   (a) Sodium thiosulfate solution 0.1 N, prepared by dissolving 24.9 g of sodium thiosulfate in distilled water and diluting to 1 L.
   (b) Weigh 0.16–0.22 g of finely ground and dried potassium dichromate into a 500-mL flask or bottle by difference from a weighing bottle. Dissolve in 25 mL of water, add 5 mL of concentrated hydrochloric acid, 20 mL of potassium iodide solution and rotate to mix. Allow to stand for 5 min, and then add 100 mL of distilled water. Titrate with sodium thiosulfate solution, shaking continuously until the yellow color has almost disappeared. Add 1–2 mL of starch indicator and continue the titration, adding the thiosulfate solution slowly until the blue color just disappears. The strength of the sodium thiosulfate solution is expressed in terms of its normality.

   Normality of $Na_2S_2O_3$ solution =

   20.394 × mass of $K_2Cr_2O_7$ in g/mL of sodium thiosulfate
4. Wijs solution—see Notes, 2 and *Caution*.

Table 1
Sample weights.

Iodine value	Mass of sample		Weighing accuracy
	100% excess	150% excess	
	g	g	g
<3	10	10	± 0.001
3	10.576	8.4613	0.005
5	6.346	5.0770	0.0005
10	3.1730	2.5384	0.0002
20	1.5865	1.2720	0.0002
40	.7935	.6346	0.0002
60	.5288	.4231	0.0002
80	.3966	.3173	0.0001
100	.3173	.2538	0.0001
120	.2644	.2115	0.0001
140	.2266	.1813	0.0001
160	.1983	.1587	0.0001
180	.1762	.1410	0.0001
200	.1586	.1269	0.0001

PROCEDURE

1. Melt the sample, if it is not already liquid (the temperature during melting should not exceed the melting point of the sample by more than 10°C), and filter through two pieces of filter paper to remove any solid impurities and the last traces of moisture. The filtration may be performed in an air oven at 80–85°C, but should be completed within 5 min ± 30 sec. The sample must be absolutely dry.

   Note—All glassware must be absolutely clean and completely dry.
2. After filtration, allow the filtered sample to achieve a temperature of 68–71 ± 1°C before weighing the sample.
3. Once the sample has achieved a temperature of 68–71 ± 1°C, immediately weigh the sample into a 500-mL iodine flask, using the weights and weighing accuracy noted in Table 1 (see Notes, 6).
4. Add 15 mL of carbon tetrachloride on top of the sample and swirl to ensure that the sample is completely dissolved.
5. Dispense 25 mL of Wijs solution using the pipet (Apparatus, 3) into flask containing the sample, stopper the flask and swirl to ensure an intimate mixture. Immediately set the timer for 30 min.
6. Immediately store the flasks in the dark for the required reaction time at a temperature of 25 ± 5°C.
7. Remove the flasks from storage and add 20 mL of KI solution, followed by 100 mL of distilled water (see Notes, 8 and 9).
8. Titrate with 0.1 N $Na_2S_2O_3$ solution, adding it gradually and with constant and vigorous shaking (see Notes, 10). Continue the titration until the yellow color has almost disappeared. Add 1–2 mL of starch indicator solution and continue the titration until the blue color just disappears.
9. Prepare and conduct at least one blank determination with each group of samples simultaneously and similar in all respects to the samples.

CALCULATIONS

1. The iodine value =

$$\frac{(B - S) \times N \times 12.69}{\text{mass, g of sample}}$$

Where—

B = volume of titrant, mL of blank
S = volume of titrant, mL of sample
N = normality of $Na_2S_2O_3$ solution

NOTES

Caution

Wijs solution causes severe burns, and the vapors can cause lung and eye damage. Use of a fume hood is recommended. Wijs solution without carbon tetrachloride is available commercially.

Carbon tetrachloride is a known carcinogen. It is toxic by ingestion, inhalation and skin absorption. It is a narcotic. It should not be used to extinguish fires. It decomposes to phosgene gas at high temperature. The TLV is 5 ppm in air. A fume hood should be used at all times when using carbon tetrachloride.

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalation and is a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

Chlorine is a poisonous gas. The TLV is 1 ppm in air. It is a strong oxidizing agent and should not be allowed to come in contact with organic materials, hydrogen, powdered metals and reducing agents. A fume hood should be used at all times when using chlorine.

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is an oxidizing agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive spattering. Always add the acid to water, never the reverse.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

Potassium dichromate is toxic by ingestion and inhalation. There is sufficient evidence in humans for the carcinogenicity of chromium [+6], in particular lung cancer. It is a strong oxidizing agent and a dangerous fire risk in contact with organic chemicals.

RECOMMENDATIONS

The most satisfactory replacement found to date for carbon tetrachloride has been cyclohexane + acetic acid, 1:1, v/v (see AOCS Cd 1d-92). Because of environmental concerns, 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113) is not recommended. Acetic acid alone and a mixture of cyclohexane + acetic acid (4:1, v/v), respectively, have been shown to be unsatisfactory replacements for carbon tetrachloride.

NUMBERED NOTES

1. When the iodine value is determined on materials having conjugated systems, the result is not a measure of total unsaturation, but rather is an empirical value indicative of the amount of unsaturation present. Reproducible results are obtained which afford a comparison of total unsaturation. When the iodine value is required on fatty acids, the preparation and separation are performed as directed in AOCS Official Method Cd 6-38.
2. Because the preparation of the Wijs solution is time-consuming and involves the use of both hazardous and toxic chemicals, this solution may be purchased from a chemical supplier. Solutions are available which contain no carbon tetrachloride, and such solutions should be used. All Wijs solutions are sensitive to temperature, moisture and light. Store in a cool and dark place, and never allow to come to a temperature above 25–30°C. The laboratory preparation of Wijs solution is noted for reference purposes:

   Reagents

   (a) Glacial acetic acid—reagent grade (see Notes, *Caution*). The permanganate test should be applied to ensure that this specification is met.

   Permanganate test—Dilute 2 mL of the acid with 10 mL of distilled water and add 0.1 mL of 0.1 N $KMnO_4$. The pink color must not be entirely discharged within 2 hr.

   Alternate test—The absence of oxidizable matter in the reagent may be verified by shaking 10 mL of the reagent with 1 mL of saturated aqueous potassium dichromate solution and 2 mL of concentrated sulfuric acid; no green coloration should appear.

   (b) Chlorine—99.8% (see Notes, *Caution*). Satisfactory commercial grades are available in cylinders, but the gas must be dried by passing through sulfuric acid (sp. gr. 1.84) before introducing into the iodine solution.

   (c) Iodine—reagent grade.

   (d) Hydrochloric acid (HCl)—reagent grade, concentrated, sp. gr. 1.19 (see Notes, *Caution*).

   (e) Sulfuric acid (H_2SO_4)—reagent grade, concentrated, sp. gr. 1.84 (see Notes, *Caution*).

   (f) Iodine monochloride—reagent grade.

LABORATORY PREPARATION

(a) Dissolve 13.0 g of iodine in 1 L of glacial acetic acid. Gentle heat may be necessary to promote solution. Cool, remove a small quantity (100–200 mL) and set aside in a cool place for future use. Pass dry chlorine gas into the iodine solution until the original titration is not quite double. A characteristic color change takes place in the Wijs solution when the desired amount of chlorine has been added. This may be used to assist in judging the end point. A convenient procedure is to add a small excess of chlorine and immediately bring back to the desired titration by addition of some of the original iodine solution which was taken out at the beginning. The original solution and finished Wijs solution are both titrated with $Na_2S_2O_3$ solution as directed in Procedure, 6 and 7.

(b) The Wijs solution may be prepared from commercial iodine monochloride as follows:

Stock solution—Add 317 ± 0.1 g of iodine monochloride to 1 L of glacial acetic acid and filter through Whatman no. 41H filter paper, or equivalent, into a clean and dry actinic glass bottle. Filter rapidly to prevent contamination with moisture and store in a cool place. Discard the solution if a precipitate forms on standing.

Wijs solution—Using a graduate, pour 117.0 ± 0.1 mL of the stock solution into a standard 5-lb bottle of glacial acetic acid and mix well by shaking.

(c) The I/Cl ratio of the Wijs solution should be within the limits of 1.10 ± 0.1. The procedure for determining the ratio is as follows:

Iodine content—Pour 150 mL of saturated chlorine water into a 500-mL Erlenmeyer flask and add a few glass beads. Pipet 5 mL of the Wijs solution into the flask containing the saturated chlorine water. Shake, and heat to boiling. Boil briskly for 10 min, cool and add 30 mL of 2% sulfuric acid and 15 mL of 15% potassium iodide solution. Mix well and titrate immediately with 0.1 N sodium thiosulfate solution to a starch end point.

Total halogen content—Pour 150 mL of recently boiled distilled water into a clean, dry 500-mL Erlenmeyer flask. Add 15 mL of 15% potassium iodide solution. Pipet 20 mL of Wijs solution into the flask and mix well. Titrate with 0.1 N sodium thiosulfate solution to a starch end point.

Calculation of halogen ratio—

$$\text{Halogen ratio } R = \frac{2A}{3B - 2A}$$

Where—

A = volume of titrant, mL of iodine content as mL sodium thiosulfate

B = volume of titrant, mL of total halogen content as mL sodium thiosulfate

3. 1% starch solution may be purchased from a chemical supplier.
4. "Potato Starch for Iodometry" is recommended, because this starch produces a deep blue color in the presence of the iodonium ion. "Soluble Starch" is not recommended because a consistent deep blue color may not be developed when some soluble starches interact with the iodonium ion. The following are suitable starches: Soluble Starch for Iodometry, Fisher S516-100; Soluble Potato Starch, Sigma S-2630; Soluble Potato Starch for Iodometry, J.T. Baker 4006-04.
5. The sodium thiosulfate solution may be purchased from a chemical supplier. However, it still must be accurately standardized in the laboratory.
6. The weight of the sample must be such that there will be an excess of Wijs solution of 50–60% of the amount added; i.e., 100–150% of the amount absorbed. In the case of tung oil, weigh 0.1–0.2 g of sample, using an excess of 55 ± 3% Wijs solution. In the case of dehy-

drated castor oils and dehydrated castor oil fatty acids, weigh 0.11–0.13 g.

7. The indicated reaction times are those specified in the International Union of Pure and Applied Chemistry (IUPAC) Iodine Value Method 2.205 (References, 1) and were the reaction times used in the IUPAC/ISO validation study of the cyclohexane + acetic acid method (AOCS Official Method Cd 1d-92). Previous AOCS versions of iodine value methods specified a reaction time of 0.5 hr, regardless of the iodine value, but noted that "a longer reaction time may be necessary for oils with high iodine value." The longer reaction times appear to be particularly critical when cyclohexane is used as a replacement for carbon tetrachloride.
8. If the reaction is not terminated within 3 min after the reaction time, the sample must be discarded.
9. The sample must be titrated within 30 min of reaction termination, after which the analysis is invalid.
10. Mechanical stirring is recommended for agitation during the addition of thiosulfate.

REFERENCES

1. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 2.205.

AOCS Official Method Cd 3d-63
Formerly Cd 3a-63 • Reapproved 1997 • Revised 1999

Acid Value

DEFINITION

The acid value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids in 1 gram of sample. With samples that contain virtually no free acids other than fatty acids, the acid value may be directly converted by means of a suitable factor to percent free fatty acids.

SCOPE

Applicable to crude and refined animal, vegetable and marine fats and oils, and various products derived from them.

APPARATUS

1. Erlenmeyer flasks—250 or 300 mL.
2. Magnetic stirring device.
3. Burette—10mL, graduated in 0.05-mL divisions with a tip drawn to a fine opening and extending at least 10 cm below the stopcock.

REAGENTS

1. Potassium hydroxide (KOH), 0.1 N—Reagent Grade KOH having a carbonate specification of 0.5% Max, or 0.1 N KOH with NIST traceable standardization to ±1 part in 1000 in water, methanol or ethanol.
2. Solvent mixture consisting of equal parts by volume of isopropyl alcohol (AOCS Specification H 18-58) and toluene (AOCS Specification H 19-58). See Notes, *Caution*. The mixture must give a distinct and sharp end point with phenolphthalein in the titration as noted in Procedure, 5.
3. Phenolphthalein indicator solution—1.0% in isopropyl alcohol.

PROCEDURE

1. Add indicator solution to the required amount of solvent in ratio of 2 mL to 125 mL and neutralize with alkali to a faint but permanent pink color.
2. Determine the sample size from the following table:

Acid value	Mass, g of sample (± 10%), g	Weighing accuracy, ± g
0–1	20	0.05
1–4	10	0.02
4–15	2.5	0.01
15–75	0.5	0.001
75 and over	0.1	0.0002

3. Weigh the specified amount of well-mixed liquid sample into an Erlenmeyer flask.
4. Add 125 mL of the neutralized solvent mixture. Be sure that the sample is completely dissolved before titrating. Warming may be necessary in some cases.
5. Shake the sample vigorously while titrating with standard alkali to the first permanent pink color of the same intensity as that of the neutralized solvent before the latter was added to the sample. The color must persist for 30 sec.
6. Perform a blank titration using 125 mL of the neutralized solvent mixture.

CALCULATIONS

1. Acid value, mg KOH/g of sample =

$$\frac{(A - B) \times N \times 56.1}{W}$$

Where—

A = volume, mL of standard alkali used in the titration
B = volume, mL of standard alkali used in titrating the blank
N = normality of standard alkali
W = mass, grams of sample

To express in terms of free fatty acids as percent oleic, lauric or palmitic, divide the acid value by 1.99, 2.81 or 2.19, respectively.

PRECISION

1. Single determinations performed in two different laboratories should not differ by more than 0.22 for values less than 4 nor by more than 0.36 for values in the range 4–20.

ALTERNATE PROCEDURE FOR HIGHLY COLORED SAMPLES

APPARATUS

1. Glass electrode—calomel electrode pH meter for electrometric titration. A sleeve-type calomel electrode should be used (see Notes, 2).
2. Variable-speed mechanical stirrer—with glass stirring paddle.
3. Burette—10 mL, graduated in 0.05-mL divisions with a tip drawn to a fine opening and extending at least 10 cm below the stopcock.
4. Beakers—250 mL.
5. Stand and mountings for electrodes, stirrer and burette.
6. Magnetic stirring device.

REAGENTS

1. Same as for the phenolphthalein titrimetric procedure, except that the standard alkali should be standardized by electrometric titration of pure potassium acid phthalate and no indicator solution is necessary.

PROCEDURE

1. Determine the sample size from Procedure, 2 and weigh the sample into a 250-mL beaker.

2. Add 125 mL of solvent mixture.
3. Mount the beaker in the titration assembly so that the electrodes are half immersed. Start the stirrer and operate at speeds that will give vigorous agitation without splattering. Immerse the tip of the burette to 1 cm below the surface of the sample.
4. Titrate with suitable increments of alkali. After each addition of alkali, wait until the meter reading is essentially constant (usually within 2 min), then record burette and meter readings graphically. Limit increments of alkali so that changes in meter readings are 0.5 pH units (0.03 volts) or less; when inflections in titration curve occur, add alkali in 0.05-mL portions.
5. Remove titrated solution, rinse electrodes with isopropyl alcohol and immerse in distilled water.
6. Perform a blank titration, using 125 mL of solvent mixture.

CALCULATIONS

1. Acid value, mg KOH/g of sample =

$$\frac{(A - B) \times N \times 56.1}{W}$$

Where—

A = volume, mL of standard alkali used in titrating to middle of inflection in titration curve of sample
B = volume, mL of standard alkali used in titrating to same pH meter reading for the blank
N = normality of standard alkali
W = mass, grams of sample

To express in terms of free fatty acids as percent oleic, lauric or palmitic, divide the acid value by 1.99, 2.81 or 2.19, respectively.

NOTES

Caution

Isopropyl alcohol is flammable and a dangerous fire risk. The explosive limits in air are 2–12%. It is toxic by ingestion and inhalation. The TLV in air is 400 ppm.

Toluene is flammable and a dangerous fire risk. Explosive limits in air are 1.27–7%. It is toxic by ingestion, inhalation and skin absorption. The TLV is 100 ppm in air. A fume hood should be used at all times when using toluene.

NUMBERED NOTES

1. A standard methanolic potassium hydroxide (0.1 N) solution (see AOCS Specification H 15-52) may be used as an alternate titrant in place of the standard aqueous solution. The methanolic potassium hydroxide is reported to provide a complete solvent system, having a distinct, clear end point. See References, 1 regarding the ruggedness of the acid value method.
2. The pH meter should be standardized to pH 4.0 with standard buffer solution. Immediately before using, wipe the electrodes thoroughly with clean cloth or tissue and soak for several minutes in distilled water. At weekly intervals, or more often if necessary, clean the glass electrode in a suitable cleaning solution. Also, drain calomel electrode and refill with fresh potassium chloride (KCl) electrolyte at weekly intervals. Both electrodes should be stored in distilled water when not in use.

REFERENCES

1. *J. Assoc. Off. Anal. Chem. 59:*658 (1976).

▼B

ANEXO IX

PRUEBA ESPECTROFOTOMÉTRICA EN EL ULTRAVIOLETA

INTRODUCCIÓN

La prueba espectrofotométrica en el ultravioleta puede proporcionar indicaciones sobre la calidad de una materia grasa, su estado de conservación y las modificaciones inducidas por los procesos tecnológicos.

Las absorciones en las longitudes de onda indicadas en el método se deben a la presencia de sistemas diénicos y triénicos ►C1 conjugados ◄. Los valores de estas absorciones se expresan en extinción específica $E_{1cm}^{1\%}$ (extinción de una solución de la materia grasa al 1 % en el disolvente determinado, en un espesor de 1 cm) que se expresará convencionalmente como K, también denominado coeficiente de extinción.

1. OBJETO

El método describe el procedimiento de ejecución de la prueba espectrofotométrica en el ultravioleta de las materias grasas.

2. PRINCIPIO

La materia grasa se disuelve en el disolvente requerido y se determina la extinción de la solución a las longitudes de onda prescritas, respecto al disolvente puro. A partir de los valores espectrofotométricos se calculan las extinciones específicas.

3. MATERIAL Y APARATOS

3.1. Espectrofotómetro para medidas de extinción en el ultravioleta entre 220 y 360 nm, con posibilidad de lectura para cada unidad nanométrica.

3.2. Cubetas de cuarzo, con tapadera, con paso óptico de 1 cm. Las cubetas, llenas de agua o de otro disolvente adecuado, no deben presentar entre ellas diferencias superiores a 0,01 unidades de extinción.

3.3. Matraces aforados de 25 ml.

▼M6

3.4. Columna de cromatografía de 270 mm de longitud y 35 mm de diámetro en la parte superior: de 270 mm de longitud y 10 mm de diámetro en la parte inferior.

▼B

4. REACTIVOS

4.1. Isooctano (2,2,4-trimetilpentano) de calidad para espectrofotometría: debe tener, respecto al agua destilada, una transmitancia del 60 % como mínimo a 220 nm y del 95 % como mínimo a 250 nm; o

— ciclohexano de calidad para espectrofotometría: debe tener, respecto al agua destilada, una transmitancia del 40 % como mínimo a 220 nm y del 95 % como mínimo a 250 nm.

▼M6

▼B

4.2. Alúmina básica para cromatografía en columna, preparada y controlada como se describe en el apéndice I.

4.3. n-Hexano para cromatografía.

5. PROCEDIMIENTO

5.1. La muestra debe ser perfectamente homogénea y estar exenta de impurezas en suspensión. Los aceites líquidos a temperatura ambiente se filtran con papel de filtro a una temperatura aproximada de 30 °C, las grasas sólidas se homogeneizan y se filtran a una temperatura superior en 10 °C como máximo a su temperatura de fusión.

5.2. Se pesan con precisión 0,25 g aproximadamente de la muestra preparada y se colocan en un matraz aforado de 25 ml, se completa con el disolvente adecuado y se homogeneiza. La solución resultante debe estar perfectamente clara. Si presenta opalescencia o turbidez, se filtrará rápidamente con papel de filtro.

▼B

5.3. Se llena una cubeta con la solución obtenida y se miden las extinciones, usando como referencia el disolvente empleado, a las longitudes de onda comprendidas entre 232 y 276 nm. Los valores de extinción obtenidos deben estar comprendidos en el intervalo entre 0,1 y 0,8; en caso contrario es necesario repetir la medida utilizando soluciones más concentradas o más diluidas según el caso.

5.4. Cuando se quiera determinar la extinción específica después del tratamiento con alúmina se procederá del siguiente modo: en la columna para cromatografía se introducen 30 g de alúmina básica en suspensión en hexano; después de asentarse el absorbente se elimina el exceso de hexano, hasta 1 cm aproximadamente sobre el nivel superior de la alúmina.

Se disuelven 10 g de materia grasa, homogeneizada y filtrada tal como se describe en el punto 5.1, en 100 ml de hexano y se vierte esta solución en la columna. Se recoge el líquido eluido y se evapora totalmente el disolvente en vacío a una temperatura inferior a 25 °C.

Con la materia grasa así obtenida se procede inmediatamente tal como se indica en el punto 5.2.

6. EXPRESIÓN DE LOS RESULTADOS

6.1. Se expresan las extinciones específicas o coeficientes de extinción a las diversas longitudes de onda, calculadas como sigue:

$$K_\lambda = \frac{E_\lambda}{c.e.}$$

siendo:

K_λ = extinción específica a la longitud de onda lambda,

E_λ = extinción medida a la longitud de onda lambda,

c = concentración de la disolución en g por 100 ml,

e = espesor de la cubeta en cm.

Los resultados deben expresarse con dos cifras decimales.

6.2. La prueba espectrofotométrica del aceite de oliva según el método oficial de los Reglamentos de la CEE requiere la determinación de la extinción específica, en solución en isooctano, a las longitudes de onda de 232 y 270 nm, y la determinación de Δ E definido como:

$$\Delta K = K_m - \frac{K_{m-4} + K_{m+4}}{2}$$

donde K_m es la extinción específica a la longitud de onda m, longitud de onda de máxima absorción alrededor de 270 nm.

▼B

APÉNDICE I

Preparación de la alúmina y control de su actividad

A.1.1. Preparación de la alúmina

En un recipiente que pueda cerrarse herméticamente se echa la alúmina previamente desecada en horno a 380-400 °C durante tres horas, se añade agua destilada en una proporción de 5 ml por 100 g de alúmina, se cierra rápidamente el recipiente, se agita repetidas veces y se deja reposar durante 12 horas como mínimo antes del uso.

A.1.2. Control de la actividad de la alúmina

Se prepara una columna para cromatografía con 30 g de alúmina. Se opera tal como se describe en el apartado 5.4. Se hace pasar a través de la columna una mezcla formada por:

— 95 % de aceite de oliva virgen, con extinción específica a 268 nm menor que 0,18,

— 5 % de aceite de cacahuete tratado con tierras decolorantes en el proceso de refinado, con una extinción específica a 268 nm mayor o igual que 4.

Si, después del paso por la columna, la mezcla presenta una extinción específica a 268 nm mayor que 0,11, la alúmina es aceptable; en otro caso se debe aumentar el porcentaje de hidratación.

AOCS Official Method Cd 18-90

Reapproved 1997

p-Anisidine Value

DEFINITION

The p-anisidine value is defined by convention as 100 times the optical density measured at 350 nm in a 1-cm cuvette of a solution containing 1.00 g of the oil in 100 mL of a mixture of solvent and reagent according to the method described.

SCOPE

This method determines the amount of aldehydes (principally 2-alkenals and 2,4-dienals) in animal and vegetable fats and oils, by reaction in an acetic acid solution of the aldehydic compounds in an oil and the p-anisidine (see Notes, 1), and then measuring the absorbance at 350 nm.

APPARATUS

1. Test tubes—10 mL min. with either ground-glass stoppers or Teflon™-lined screw caps.
2. Volumetric flasks—25 mL.
3. Automatic pipet or automatic burette.

   Note—Any pipette and/or burette capable of delivering exactly 1 mL and 5 mL is satisfactory.
4. Spectrophotometer suitable for observation at 350 nm.
5. Glass cuvettes—1.00 ± 0.01 cm, the two cuvettes of each pair must be identical.

REAGENTS

1. Isooctane (2,2,4-trimethylpentane)—optically clear (see Notes, *Caution* and 2).
2. Glacial acetic acid—analytical reagent quality (see Notes, 3).
3. p-Anisidine—analytical reagent quality (see Notes, *Caution* and 4) 0.25 g/100 mL solution in glacial acetic acid (Reagents, 2) (see Notes, 5).

PROCEDURE

Note—The sample should be perfectly clear and dry (see Notes, 3).

1. Weigh 0.5–4.0 ± 0.001 g of the sample into a 25-mL volumetric flask. Dissolve and dilute to volume with isooctane.
2. Measure the absorbance (Ab) of the solution at 350 nm in a cuvette with the spectrophotometer, using the reference cuvette filled with solvent as a blank.
3. Pipet exactly 5 mL of the fat solution into one test tube (Apparatus, 1) and exactly 5 mL of the solvent into a second test tube. By means of an automatic pipet (Apparatus, 3) add exactly 1 mL of the p-anisidine reagent (Reagents, 3) to each tube, and shake (see Notes, 6).
4. After exactly 10 min measure the absorbance (As) of the solvent in the first test tube in a cuvette (Apparatus, 5) at 350 nm, using the solution from the second test tube as a blank in the reference cuvette.

CALCULATIONS

The p-anisidine value (p-A.V.) is given by the formula

$$\text{p-A.V.} = \frac{25 \times (1.2\text{As} - \text{Ab})}{\text{m}}$$

Where—

As = absorbance of the fat solution after reaction with the p-anisidine reagent (Reagents, 3)

Ab = absorbance of the fat solution

m = mass of the test portion, g

PRECISION (see References, 2)

	Crude Rapeseed Oil		Refined Palm Oil	
	Sample 1	Sample 2	Sample 1	Sample 2
No. of labs	20	20	20	20
Mean value	2.0	2.0	2.3	2.3
Repeatability, CV, %	4.0	5.8	4.8	4.6
Reproducibility, CV, %	35	37	30	31

NOTES

Caution

Isooctane is flammable and a fire risk. Explosive limits in air are 1.1–6.0%. It is toxic by ingestion and inhalation. A properly operating fume hood should be used when working with this solvent.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

p-Anisidine is an irritant and should be handled with care, preferably in a fume hood. p-anisidine is an aromatic amine, a class of toxic and possibly carcinogenic chemicals. o-Anisidine is a carcinogen in rats and mice, causing urinary carcinomas or papillomas. [Fourth Annual Report on Carcinogens, NTP 85-002, 1985, p. 2; *Chem. Res. Toxicol. 4*:474 (1991)]. The TLV is 0.1 ppm.

NUMBERED NOTES

1. In the presence of acetic acid, p-anisidine reacts with aldehydic compounds in oils or fats. The intensity of color of the yellowish reaction products formed depends not only on the amount of aldehydic compounds present but also on their structure. It has been found that a double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance four to five times. This means that 2-alkenals and dienals, especially, will contribute substantially to the value found.

2. In most cases n-hexane can be substituted for isooctane as a solvent. However, oils containing high amounts of oxidized fatty acids will not dissolve completely in n-hexane. For such oils isooctane should be used as the solvent. The absorbance of the solvent used (isooctane or n-hexane), measured in a 1.00-cm cuvette between 300 and 380 nm, must be nil or nearly nil. The commercial product can be freed from absorptive material by percolating it through a glass column (3–5 cm i.d., and 100 cm long) filled with silica gel.
3. The reaction between p-anisidine and aldehydes involves the formation of water. Hence, the presence of moisture in any of the reagents or in the sample leads to incomplete reaction and, consequently, low values. Since glacial acetic acid is highly hygroscopic, it is essential to check its moisture content by a Karl Fischer determination. If the content exceeds 0.1 percent, the acetic acid must be discarded.
4. In storage, p-anisidine tends to darken as a result of oxidation. The p-anisidine crystals, which should be cream colored, should be stored at 0–4°C in a dark bottle. The crystals should not be exposed to strong light and should be used before any color change is observed. A discolored reagent can be reduced and decolorized in the following way. Dissolve 4.0 g of p-anisidine in 100 mL of water at 75°C. Add 0.2 g of sodium sulphite and 2.0 g of active carbon and stir for 5 min. Then filter through a double filter paper. If carbon passes through, repeat filtration. Cool the filtered solution to about 0°C, allow to stand at this temperature for at least 4 hr, or, preferably, overnight. Filter off the crystallized p-anisidine and wash with a small amount of water at a temperature of about 0°C. After drying in a vacuum desiccator, transfer the crystals into a brown glass bottle. If stored in the dark and at low temperature, the crystals obtained should not darken appreciably for 1 yr.
5. Reagent solutions having an absorbance greater than 0.200 when measured in a 1.00-cm cuvette at 350 nm against isooctane or n-hexane as a blank should be discarded.
6. The mixture should be completely homogenized with minimum shaking and then allowed to react for 10 min before proceeding with the absorbance measurement (References, 4).

REFERENCES

1. IUPAC, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed., Method Number 2.504 Determination of the p-anisidine value (p-A.V.), Blackwell Scientific Publications, Boston, MA and Oxford, UK (1987).
2. FOSFA International Collaborative Study #P15, May 1986, Document No. 384, ISO/TC 34/SC 11, February 12, 1987.
3. *JAOCS 51:*17 (1974).
4. Hamilton, R.J., and S. Hamilton, *Lipid Analysis*, Oxford University Press, New York, 1992, pp. 45–47.

2.508 Determination of polymerized triglycerides in oils and fats by high performance liquid chromatography

1 Scope

This Standard describes a method to determine the polymerized triglycerides content in oils and fats which contain not less than 3 per cent (m/m) of these polymers.

2 Field of application

This method is applicable to any kind of fats and oils, heated or not. It can also be applied to the determination of polymers in animal-feed fats.

3 Principle

Dissolution of the sample in a suitable solvent and separation of the polymerized triglycerides by gel permeation chromatography according to molecular size.

4 Apparatus

4.1 Solvent reservoir of about 250 ml of capacity with a mobile-phase liner filter (pore size 1 μm).

4.2 HPLC pump, pulseless, with a flow of 0.7–1.5 ml/min.

4.3 Injection valve with a 10-μl loop and a suitable syringe with a volume of 50–100 μl (Rheodyne or Valco).

4.4 Stainless steel column: 300 mm in length 7.7 mm internal diameter, packed with a high performance spherical gel made of styrene–divinylbenzene co-polymer; diameter of the particles: 5 μm; pore size: 100 Å (0.1 nm) of the equivalent in terms of exclusion power and resolution.

The storage of such column must be achieved in toluene.

4.5 Detector: refractive index detector with a sensitivity at full scale at least 1×10^{-4} of refractive index.

4.6 Recorder and/or integrator: to allow display and accurate quantification of the peak areas.

5 Reagents

5.1 Tetrahydrofuran, analytical grade.

5.2 Toluene, analytical grade.

6 Procedure

6.1 Starting up HPLC equipment

It is advisable to follow carefully the manufacturer's recommendations. Switch on the system and pump tetrahydrofuran at a rate of 1 ml/min to purge the whole system up to the injection valve. Connect the column to the injection valve and wash it with about 30 ml of tetrahydrofuran. Connect the column to the sample cell of the detector. Fill the reference cell with the tetrahydrofuran. Adjust the mobile phase flow to 0.8–1.0 ml/min. Wait until a convenient stabilization of the system (no appreciable deviation of the baseline) is obtained (*note 1*).

6.2 Preparation of the samples and analysis

As the samples may contain suspended particles, resulting in the blockage of the porous fritted filter at the top of the column, it is advisable to filter them before injection. Filtration can be quickly and effectively realized with a 1-μm pore size filter (*note 2*).

The samples must be anhydrous. If not, they must first be dried (*note 3*).

Weigh about 50 mg of fat and add 1 ml of tetrahydrofuran. Homogenize. Take with the

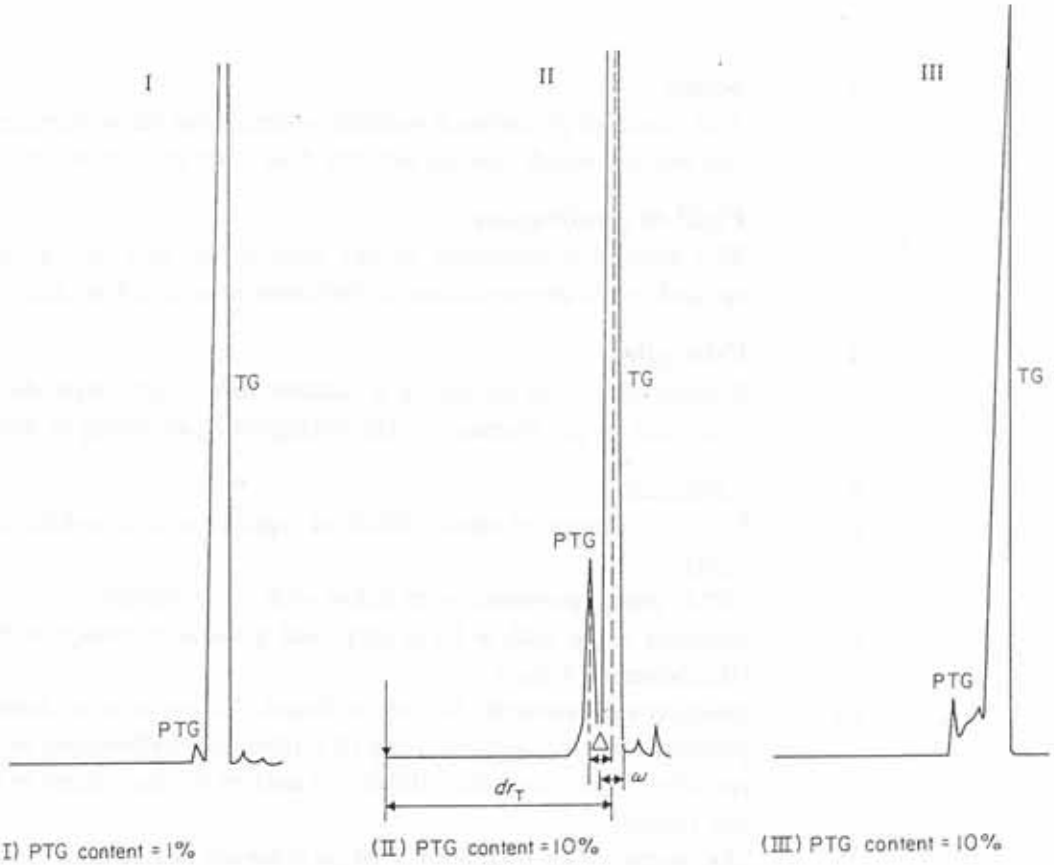

Fig. 2.508.1. HPLC of triglycerides (TG) and polymerized triglycerides (PTG).

syringe 50–100 μl of that solution. Fill the injection loop. Inject and switch on the integrator.

With a mobile phase flow of 1 ml/min, the analysis time is about 10 minutes.

7 Results

7.1 Qualitative analysis

The chromatographic pattern of the determination may show a main peak representative of monomeric triglycerides (mol. wt. about 900) and one or several smaller peaks with a shorter pattern retention distance than triglycerides, representative of polymerized triglycerides (dimers and upper oligomers).

Reference should be made to the three typical chromatograms presented in Fig. 2.508.1. Under suitable conditions, triglycerides and polymerized triglycerides can be separated with good resolution (I and II) even at low levels of polymerized triglycerides (I). However, in some cases, which seem to be connected to the complex degradation phenomena (hydrolysis), the peak pattern preceding the triglycerides peaks may be less clear (III), with consequent difficulties in calculation.

7.2 Quantitative analysis

The calculation is achieved by the internal standard method, assuming that all components of the sample are eluted.

The polymerized triglycerides content can be calculated using the formula:

$$\%\text{PTG} = \frac{A_{\text{PTG}}}{\Sigma A} \times 100$$

where:
%PTG is the percentage of polymerized triglycerides
A_{PTG} is the sum of areas of the polymerized triglycerides peaks
ΣA is the sum of areas of all peaks.
For calculating A_{PTG}, two cases are possible:
(a) *Good resolution between peaks* ($R \geq 1$) (similar to I and II). The general methods of integration (manual and electronic) can be used to calculate individual and total areas.
(b) *Poor resolution between peaks* ($R < 1$) (similar to III).
It is assumed that all components eluted before dr_T (see below) are polymerized triglycerides.
The resolution is calculated from:

$$R = \frac{\Delta}{\omega}$$

where:
Δ is the distance, in mm, between the peak maxima of the non-polymerized triglycerides peak (TG) and the adjacent polymerized triglycerides peak
ω is the width, in mm, of the triglycerides peak at the baseline, measured between the points of the intersection between tangents and baseline
dr_T is the retention distance, in mm, from the beginning of the chromatogram to the peak maximum for triglycerides.
Using electronic integration, the integrator has to be carefully adjusted (backward horizontal integration) to integrate all the surfaces included between the curve and the baseline. If a manual technique is used, it is necessary to determine the triglycerides peak area by triangulation.
Express the results to one decimal place.

8 Quality assurance

8.1 For *general principles* of analytical quality control, see the section on *Quality Assurance* in the introductory part of the *Compendium of Standard Methods*.

8.2 For *specific applications* of analytical quality control, see the Annexe to this standard method.

9 Notes

1 If the composition of the column is as indicated, an acceptable stabilization of the system should be obtained in about 15 minutes. With other column packings, the stabilization of the system may be more difficult: for example, changing the mobile phase should be done stepwise from toluene to tetrahydrofuran, with different mixtures, each time 25 per cent higher in tetrahydrofuran. Acceptable stabilization is normally obtained in about 12 hours.

2 The sample can be filtered with a 1-μm pore size filter made of polytetrafluoroethylene or cellulose esters. Syringe disposable filter units are available from manufacturers of HPLC equipment.

3 Samples that may contain water must first be dried: dissolve 50–100 mg of the sample in 1 ml of tetrahydrofuran. Add 50 mg of anhydrous sodium sulphate, shake, and leave for about 2 minutes. Filter upper layer through a 1-μm pore size filter.

Annexe

1 Repeatability

When the mean of the values obtained from two single determinations carried out in rapid succession by the same operator, using the same apparatus under the same conditions for the analysis of the same laboratory sample, lies within the range of the mean values cited in the table below, the difference between the two values obtained should not be greater than the repeatability limit (r), which can generally be deduced by linear interpolation from the values in the table below.

2 Reproducibility

When the values for the final result, obtained by operators in different laboratories using different apparatus under the same conditions for the analysis of the same laboratory sample, lie within the range of the mean values cited in the table below, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility limit (R), which can generally be deduced by linear interpolation from the values in the table below.

3 Results of the interlaboratory tests

Two interlaboratory tests carried out at an international level in 1986–87 by the IUPAC Commission on Oils, Fats and Derivatives, in which 10 and 17 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with *ISO 5725-1986*) summarized in Table 2.508.1.

Table 2.508.1.

	Sample							
	A	B	C	D	E	F	G	H
Number of laboratories	17	10	10	10	17	16	10	10
Number of laboratories retained after eliminating outliers	15	9	10	16	10	16	15	10
Number of accepted results	30	18	20	32	20	32	30	20
Mean value (percentage of total triglycerides)	1.8	2.4	3.6	5.2	7.2	9.7	10.0	22.0
Repeatability standard deviation (S_r)*	0.1	0.07	0.12	0.1	0.37	0.3	0.2	0.18
Repeatability relative standard deviation†	0.1	2.8	3.3	2.3	5.2	3.2	2.2	0.8
Repeatability limit (r)* ($2.8 \times S_r$)	0.3	0.19	0.34	0.3	1.06	0.9	0.6	0.51
Reproducibility standard deviation (S_R)*	0.4	0.61	0.45	0.3	2.14	0.4	1.2	1.48
Reproducibility relative standard deviation†	23.7	25.9	12.5	6.7	29.8	4.6	12.2	6.8
Reproducibility limit (R)* ($2.8 \times S_R$)	1.2	1.7	1.25	1.0	6.05	1.3	3.5	4.2

* Expressed as percentage of total triglycerides; † coefficient of variation.

Zertifikat

Certificate

Die Deutsche Gesellschaft für Fettwissenschaft e.V. (DGF) bescheinigt dem Institut
The DGF herewith certifies that the institute

University of Barcelona, Food Science and Nutrition
Department
Barcelona

hiermit die Teilnahme an der Laborvergleichsuntersuchung
took part in the

9. DGF LVU 2003 (9th DGF Proficiency Test 2003)

bei der folgende Parameter* getestet werden konnten:
The following analytical parameters could be checked:

Säurezahl (Acid Value)
Buttersäure (Butyric Acid)
Anisidinzahl(Anisidine Value)/ Peroxidzahl (Peroxid Value)
Lovibond-Farbzahl (Lovibond Colour Index)
Stigmastadien (Stigmastadiene)
Sterine (Sterols)
Gesamtsterin-Gehalt (Total Sterol Content)
Fettsäureverteilung (Fatty Acid Composition)
Transfettsäuren (Trans Fatty Acids)
Tokopherolverteilung und – Gehalt (Tocopherol Composition and content)
Vitamin A and E Gehalt (Vitamin A and E Content)
Polymere Triglyceride (Polymeric Triglycerides)
Polare Anteile (Polar compounds)
PAKs (PAHs)
Gesamtfettgehalt (Total Fat Content)

*Der Umfang und Erfolg der Teilnahme sind dem Testreport zur 9. DGF LVU 2003 zu entnehmen.
The extent of individual participation is given in the enclosed final report.

Ihre Labor-Kenn-Nummer : 15

(Your laboratory has been assigned the code)

Frankfurt, 5.12.2003

Dr. Christian Gertz
Vorsitzender der DGF-Fachgruppe
"Analyse und Einheitsmethoden"

Dr. Frank Amoneit
Geschäftsführer der DGF

VII.3. Comunicaciones en congresos

LIPID HYDROPEROXIDE DETERMINATION IN SNACKS FRIED IN VEGETABLE OILS THROUGH A MODIFIED FERROUS OXIDATION-XYLENOL ORANGE METHOD

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INTRODUCTION

The stability of fried foods is affected by lipid oxidation, which yields both primary and secondary oxidation compounds. Determination of lipid hydroperoxides (primary oxidation compounds) is used to monitor lipid oxidation in fried foods. Several ferrous-xylenol orange (FOX) methods have been used to measure lipid hydroperoxide (LHP) content in foods and biological samples. In these methods, ferrous ions are oxidized by LHPs to ferric ions, which bind xylenol orange (XO) and develop a blue-purple complex with an absorbance maximum of 550-600 nm. In certain conditions, the FOX methods that use alcohol-based reaction media show turbidity when applied to vegetable oils and lipid extracts. Turbidity occurs as a consequence of the precipitation of certain lipids when the sample is added to the FOX reaction medium. In some cases, a centrifugation step is used to clarify the reaction mixture prior to the spectrophotometric reading; however, this is a time-consuming procedure. If the polarity of the reaction medium decreases the lipids do not precipitate. Thus, when using chloroform:methanol (7:3, v/v) as the main solvents in the FOX reaction medium, Shantha and Decker (1994) did not observe turbidity when the method was applied to fats and oils. However, the response obtained using alcohol-based media is 1.5 times greater than when chloroform:methanol (7:3, v/v) is used as the base for the medium.

OBJECTIVES

The main objective of this study is to set up a FOX method that will determine LHP in lipid extracts from snacks fried in vegetable oils, but which, unlike methods that rely on alcohol reaction media, avoids turbidity without losing sensitivity.

MATERIAL AND METHODS

Reagents and standards

Xylenol orange (XO) (ACS grade) and ethanol (96%, HPLC grade) were purchased from Scharlau (Barcelona, Spain). Cumene hydroperoxide (CHP) was from Sigma (St. Louis, MO, USA). Triphenilphosphine (TPP) was from Aldrich (Steinheim, Germany), and hydrochloric acid (35%), sulfuric acid (96%) and ammonium ferrous sulfate hexahydrate (analytical grade) were from Panreac (Barcelona, Spain). All other solvents used (HPLC grade) were from SDS (Peypin, France). Distilled deionized water was used throughout.

Lipid extraction

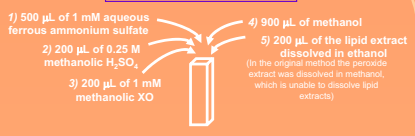
LHPs were determined in lipid extracts from several snack foods, which had been fried in soybean oil, sunflower oil, palm olein, palm olein/sunflower oil blends, and palm olein/soybean oil blends. Lipid extraction was performed using a high-speed homogenizator with chloroform:methanol (2:1, v/v).

RESULTS AND DISCUSSION

"Methanol modified method"

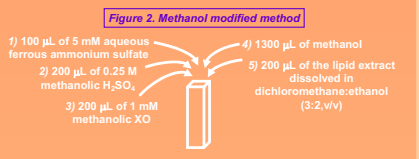
We first applied a slightly modified FOX method, as described by Grau et al. (2000) to the lipid extracts from various snacks; however when the sample was added to the reaction medium, turbidity became evident at very low lipid extract concentrations.

Figure 1. Grau et al. (2000) method



In order to avoid precipitation of the lipid extracts in the reaction medium, water content of the medium was reduced to 100µL, whilst the same concentration of ferrous ions in the final reaction mixture was maintained. Any further reduction of the water content produced a loss of sensitivity.

Figure 2. Methanol modified method



However, turbidity still occurred at concentrations of 0.5 and 0.6 mg of lipid extract/mL reaction medium (lipid extracts from snacks fried in palm olein and sunflower oil, respectively) (Figure 6). The concentration of LHP in these quantities of lipid extracts was not enough to ensure optimal absorbance [0.7-0.8, as recommended by Grau et al. (2000)]. Thus, a further decrease of the polarity of the reaction medium was necessary to avoid the precipitation of certain lipids.

For all the FOX reaction conditions assayed in this study, incubation was carried out in glass cuvettes with Teflon caps for 30 minutes.

Other solvents assayed

In a further attempt to avoid turbidity we decreased the polarity of the medium by experimenting with less polar solvents as substitutes for methanol. None of the several solvents used [dichloromethane:methanol (7:3, v/v) (D/M 7:3), dichloromethane:ethanol (3:2, v/v) (D/E 3:2) and chloroform:methanol (7:3, v/v) (C/M 7:3)], showed problems of turbidity at a concentration of 20 mg of lipids extracted from a snack fried in palm olein/ mL reaction medium. However, for some of these solvents a great reduction in response was observed. We thus tested the response obtained with the same solvents using a known concentration of CHP and using methanol (M) and ethanol (E) as controls for the response (Figure 3).

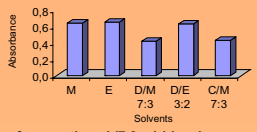


Figure 3. Absorbance obtained using FOX media with different solvent compositions. Reaction media consisted of 100 µL of 5 mM aqueous ferrous ammonium sulfate, 200µL of 0.25 M methanolic H_2SO_4, 200µL of 1mM methanolic XO, 1300µL of solvent and 200µL of a solution of CHP in the corresponding solvent (final CHP concentration was 11nmols/ mL reaction medium).

Chloroform:methanol (7:3, v/v) has been used in some FOX methods (e.g., Shantha and Decker, 1994). Although this solvent did not cause turbidity problems, the response obtained for CHP and for lipid extracts was greater for methanol and dichloromethane:ethanol (3:2, v/v) (Figure 3).

Shantha and Decker (1994) also proposed using HCl instead of H_2SO_4 in the FOX reaction medium.

FOX reaction medium	Absorbance at 560nm/ sample concentration in mg/mL reaction medium	Precision C.V.(%)
with H_2SO_4	0.88	1.4
with HCl	0.56	3.6

Table 1. Precision (CV) obtained for lipid extracts from snacks fried in sunflower oil (n=8). The reaction medium consisted of 100µL of 5 mM aqueous ferrous ammonium sulfate, 200µL of 0.5M HCl or 0.25M H_2SO_4 in chloroform:methanol (7:3, v/v), 200µL of 1mM methanolic XO, 1300µL chloroform:methanol (7:3, v/v) and 200 µL of sample in chloroform:methanol (7:3, v/v).

This would indicate that not only was sensitivity improved using sulfuric acid but greater precision was obtained.

Hydroperoxide presence in solvents

When ethyl acetate, butanol or 2-propanol were used instead of methanol, blank cuvettes turned blue after 30 minutes incubation. When these solvents were incubated for 30 minutes with TPP prior to the FOX reaction, the blue colour disappeared. Thus, this blue colour developed as a consequence of non-H_2O_2 hydroperoxides. This would seem to indicate that even if some authors (e.g., Nourooz-Zadeh et al. 1995, Södergren et al. 1998, Yildiz et al. 2003) have used them, these three solvents are not suitable for FOX reactions.

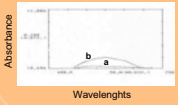


Figure 4. Absorbance spectra obtained using the FOX method after 30 minutes incubation of 2-propanol with (a) and without (b) TPP. (a) 350µL of 2-propanol and 150µL of 10 mM methanolic TPP and (b) 350µL of 2-propanol and 150µL methanol were incubated for 30 minutes. After incubation, 1500µL of FOX reagent was added to both solutions (100µL of 5 mM aqueous ferrous ammonium sulfate, 200µL of 0.25M methanolic H_2SO_4, 200µL of 1mM methanolic XO, 1000µL of methanol), which were incubated for a further 30 minutes. Blank cuvettes contained 500µL of methanol without TPP.

Comparison of dichloromethane:ethanol and dichloromethane:methanol reaction media

Several mixtures of dichloromethane:methanol and dichloromethane:ethanol at different proportions were assayed. When proportions of dichloromethane:ethanol at 2:3 and 1:1 (v/v) were used, the response to CHP was greater than when methanol was used (Figure 5). However, these solvent mixtures were not able to prevent the precipitation of some lipids in the reaction medium (20 and 25 mg lipids extracted from snacks fried in palm olein/mL reaction mixture of dichloromethane:ethanol at 2:3 and 1:1 (v/v) respectively).

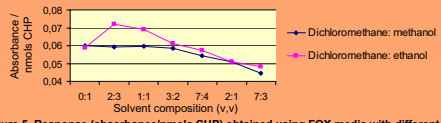


Figure 5. Response (absorbance/nmols CHP) obtained using FOX media with different solvent compositions. Reaction media consisted of 100µL of 5 mM aqueous ferrous ammonium sulfate, 200µL of 0.25 M methanolic H_2SO_4, 200µL of 1mM methanolic XO, 1300µL of the solvent mixture and 200µL of a CHP solution in the corresponding solvent mixture (final CHP concentration was 11nmols/mL reaction medium)

When dichloromethane:ethanol (3:2, v/v) was used, the response to CHP was of the same order as when methanol was used (Figures 3 and 5); furthermore, no precipitation problems occurred at the concentrations experimented with (up to 25 mg/ml) for any of the lipid extracts assayed (lipid extracts from snacks fried in sunflower oil, palm olein, soybean oil palm olein/sunflower oil blends and palm olein/soybean oil blends) (Figure 6). Figure 5 shows how media richer in dichloromethane responded more poorly than methanol.

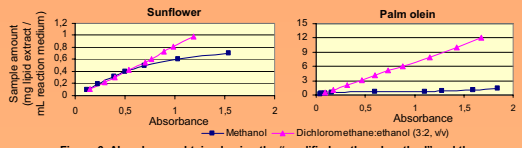
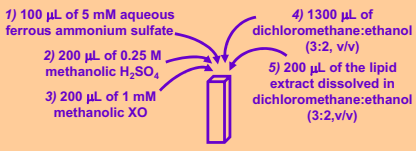


Figure 6. Absorbance obtained using the "modified methanol method" and the "dichloromethane:ethanol (3:2, v/v) method" based on different quantities of lipid extracts from snacks fried in sunflower oil and palm olein. Note that in the "methanol modified method" turbidity occurred at concentrations of 0.6 mg (sunflower oil) and 0.5 mg (palm olein) of lipid extract/mL reaction medium.

"Dichloromethane:ethanol (3:2, v/v) method"

From the previous results it can be concluded that the reaction medium containing dichloromethane:ethanol (3:2, v/v) as the main solvents shows good sensitivity and avoids lipid precipitation even when large quantities of sample are used. Thus, the FOX reaction medium finally chosen to determine the LHPs in lipid extracts from snacks fried in vegetable oils consisted of:

Figure 7. Dichloromethane:ethanol (3:2, v/v) method



Lipid extract from snacks fried in:	LHP value (µmols CHP/ mg lipid extract)	Precision C.V.(%)
Sunflower oil	20	1.0
Palm olein	2.4	1.1

Table 2. Precision (CV) under these conditions (n=8)

Absorbance should be measured between 0.3 and 0.8 at 560 nm, otherwise the maximum appears at other wavelengths (Figure 8).

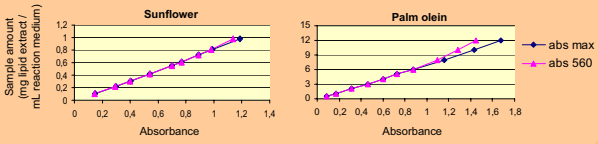


Figure 8. Comparison of maximum absorbance and absorbance at 560 nm for the "dichloromethane:ethanol (3:2, v/v) method" using different quantities of lipid extracts from snacks fried in sunflower oil and palm olein.

CONCLUSION

The proposed "dichloromethane:ethanol (3:2, v/v) method" is suitable for determining LHP in lipid extracts from snacks fried in vegetable oils. This reaction medium shows a high sensitivity which permits nanomolar concentrations of LHP to be determined. It is therefore particularly useful for samples with a low LHP content, since in this case large quantities of lipid extract (we have assayed up to 25 mg of lipid extract/mL of reaction medium) are needed to obtain an absorbance value of between 0.3 and 0.8.

This method gives similar results for vegetable oils.

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INFLUENCE OF VARIOUS ANTIOXIDANT COMBINATIONS ON THE QUALITY OF OILS AND SNACKS PRODUCED BY CONTINUOUS DEEP FRYING

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INTRODUCTION

Oxidative deterioration of edible oils and fatty foods is of great concern because it affects the development of rancidity as well as the decay of nutritional value and food safety. For these reasons, antioxidants are added to fats and oils to prevent lipid oxidation during deep frying.
Therefore, in this study, three antioxidants have been chosen according to their different action mechanism. Citric acid prolongs the induction period of free radical formation by chelating metals present in fats and oils, tocopherols inhibit free radical chain reactions because they are capable of accepting a radical from oxidizing free radical species, while ascorbyl palmitate lengthen the induction period by its oxygen scavenging ability (Gwo, et al., 1985; Yanishlieva and Marinova, 2001).

OBJECTIVES

The main objective of this study is to evaluate the effect of several antioxidants on the alteration of the frying medium, and the oxidative stability and sensory acceptability of the fried products through several quality variables, which, in our previous studies, have been shown to be the most suitable for the quality control of the continuous frying of snacks.

METHODS

Lipid extraction

Several alteration variables were determined in the frying medium and lipids extracted from snack foods, which had been fried in palm olein. Lipid extraction was performed using a high-speed homogenizer with chloroform:methanol (2:1, v/v).

Determinations

Acid Value (AV) and anisidine value (AnV) were determined by AOCS official methods, Cd 3d-63 and Cd 18-90 respectively. Lipid ultraviolet absorption was measured at 232, 270 and 280 nm according to the by a double-beam spectrophotometer (Shimadzu UV-160 A; Kyoto, Japan). Triacylglycerol polymers (POL) were determined by HPLC according to the IUPAC standard method 2.508. Lipid hydroperoxides (LHP) content was determined by a FOX method according to Navas et al. (2003). Fatty acid profile of the oil was determined according to Guardiola et al. (1994) with addition of methyl pentadecanoate as internal standard. The ratio $C_{18:2n-6}/C_{16:0}$ was calculated from fatty acid composition with the aid of experimental calibration curves. Consumer acceptability was tested after 0, 8 and 16 months of storage at room temperature. Panellists were asked to rank the acceptability of the product using a 9-point scale (1=very bad; 9= very good).

EXPERIMENTAL DESIGN

The effect of various antioxidant combinations on the quality of frying medium (palm olein) and snacks (potato pellets) produced by continuous industrial deep frying was studied through the following factorial design (2 x 2 x 3 x 2 or 3): citric acid (0 or 200 mg/L), ascorbyl palmitate (0 or 500 mg/L), tocopherol extract (0, 250 or 500 mg/L) and frying time for palm olein (3 or 45 min) or storage time at room temperature for fried snacks (0, 8 or 16 months). The antioxidants were added to the frying medium at 120 ºC and rotatory shovel helped to reach complete homogeneity of antioxidants in frying oil. Afterwards, the temperature was increased up to 195ºC (frying temperature). Palm olein samples were taken after 3 and 45 minutes of deep fat frying. Snacks products produced between 10 and 12 min of frying were packed into bags of metallic polypropylene (Polibol, Zaragoza, Spain).

STATISTICAL ANALYSIS

Multifactor analyses of variance (MANOVAs) were performed to determine whether any significant effects were produced by the studied factors (antioxidants and frying or storage times) on the responses ($C_{18:2n-6}/C_{16:0}$, AV, lipid UV absorption , AnV, POL, LHP, sensory acceptability). In all cases, P values ≤0.05 were considered significant.

FRYING CONDITONS

Fryer	Continuous electric fryer Foodtech
Heating system	Direct
Capacity	500 L
Frying temperature	200 ºC
Production rate of the snacks	350 kg/h
Oil consumption	81 kg/h
Turnover rate	6 h 12'
Frying time	33 seconds

RESULTS AND DISCUSSION

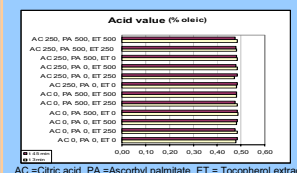
Frying medium

Oxidation variables such as anisidine value, lipid UV absorptions and triacylglycerol polymer content increased during frying (P≤ 0.05) (Table 1). Anisidine value and polymer content clearly decreased (P≤0.05) in palm olein when the ascorbyl palmitate was added to the frying medium (Table 1). In fact, other authors (Gordon and Kourimska, 1995) reported that this antioxidant prevents oxidation and dimmer formation during deep fat frying.

Table 1. Effect of the studied factors on the alteration of the frying medium.

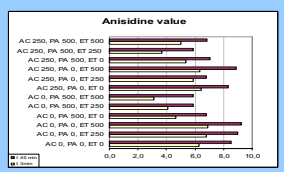
	Citric Acid (mg/mL)		Ascorbyl palmitate (mg/mL)		Tocopherol extract (mg/mL)			Frying Time (min)	
	0	250	0	500	0	250	500	3	45
$C_{18:2\ n-6}/C_{16:0}$ [4]	0.48[2]	0.48	0.48	0.48	0.48	0.48	0,48	0.48	0.48
AV	0.11a[3]	0.13b	0.10a	0.14b	0.12	0.12	0,12	0.12	0.12
K_{232}	3.18	3.14	3.16	3.16	3.13a	3.14ab	3,20b	3.12a	3.20b
K_{270}	0.66	0.66	0.67	065	0.65a	0.65a	0,67b	0.63a	0.68b
K_{280}	0.53	0.53	0.54	0.52	0.52	0.52	0,55	0.51a	0.55b
AnV[4]	6.42	6.36	7.44a	5.34b	6.68b	5.98a	6,52b	5.37a	7.42b
POL	1.01	1.02	1.07a	0.96b	1.01	1.00	1,04	0.97	1.07

[1] C18:2n-6/C16:0 (linoleic acid/palmitic acid ratio), AV (acid value), K_{232}, K_{270}, K_{280} (specific absorbances at 232, 270 y 280 nm), AnV (anisidine value), POL (% triacylglycerol polymer content),
[2] Values given in this table correspond to least-squares means obtained from MANOVA (n=24).
[3] Means corresponding to a certain factor with different letters differ significantly (P≤0.05). Means for the factors having a significant effect were separated using the Scheffé´s test.
[4] Interaction between ascorbyl palmitate and tocopherol extract significant at P≤0.05.



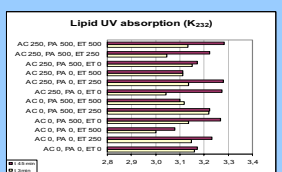
AC =Citric acid, PA =Ascorbyl palmitate, ET = Tocopherol extract
Concentrations of antioxidants: 0 mg/L, 250 mg/L, 500 mg/L

Figure 1. Influence of antioxidants on acid value of palm olein.



AC =Citric acid, PA =Ascorbyl palmitate, ET = Tocopherol extract
Concentrations of antioxidants: 0 mg/L, 250 mg/L, 500 mg/L

Figure 2. Influence of antioxidants on anisidine value of palm olein.



AC =Citric acid, PA =Ascorbyl palmitate, ET = Tocopherol extract
Concentrations of antioxidants: 0 mg/L, 250 mg/L, 500 mg/L

Figure 3. Influence of antioxidants on K_{232} of palm olein.

Figure 1 shows that no differences were found for the acid value between initial (t=3min) and final times (t=45min). This could be due to the fact that extruded snack products used in this study contained much lower moisture before frying (10% w/w) than fresh potatoes (70% w/w). Low sensitivity of acid value could be explained because possibly hydrolytic reactions are less important than oxidative reactions in the frying medium at the tested conditions. Moreover, Figures 2 and 3 show the suitability of anisidine value and lipid absorption at 232 nm to evaluate alteration in our experimental conditions. Both oxidation variables increased with frying time (Table 1). From this results and previous studies it can be concluded that oxidation variables are more reliable than acid value to monitor alteration in continuous frying.

Snack products

Alteration variables measured in the lipid fraction extracted from these fried snacks and its overall acceptability are shown in Table 2. As for the frying medium, ascorbyl palmitate prevented the lipid oxidation of fried snacks. Thus, the addition of this antioxidant significantly decreased (P≤0.05) various oxidation variables (K_{232}, lipid hidroperoxide content and polymer content). Citric acid and tocopherol extract showed no antioxidant activity at the tested conditions, as determined by different measurements (Tables 1 and 2).

On the other hand, K_{232} and LHP values increased significantly during storage at room temperature, while consumer acceptability decreased (Table 2 and Figures 4, 6 and 7). The increase of lipid absorbance at 232 nm and LHP values at room temperature occurs because oxidation of polyunsaturated fatty acids is accompanied by an increase in lipid ultraviolet absorption at 232 nm due to formation of conjugated diene hydroperoxides (Gwo et al., 1985). Both analytical methods are commonly used to evaluate the presence of these primary oxidation products (Dobarganes and Velasco, 2002). Furthermore, the interaction between ascorbyl palmitate addition and storage time (P≤0.05) showed that the K_{232} only increased in fried snacks with the storage time when ascorbyl palmitate was not added (Figure 4).

In addition, anisidine value slightly decreased with storage time, but these differences were not significant (Figure 5). This fact could be due to a decrease of some aldehydes in the fried products, because the α-β-aldehydes (mainly 2-alkenals) can break down to lower molecular weight compounds (Warner and Eskin, 1995).

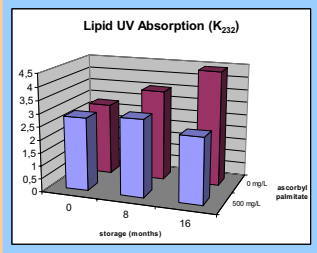


Figure 4. Effect of the Interaction between storage time and ascorbyl palmitate on K_{232} value of fried snacks.

Table 2. Effect of studied factors on the alteration of fried snacks.

	Citric Acid (mg/mL)		Ascorbyl palmitate (mg/mL)		Tocopherol extract (mg/mL)			Storage (months)		
	0	250	0	500	0	250	500	0	8	16
$C_{18:2\ n-6}/C_{16:0}$ [4]	0.49[2]	049	0.49	0.50	0.49	0.50	0.49	0.49	0.49	0.49
K_{232} [4]	3.16	3.19	3.59a[3]	2.77b	3.10	3.23	3.21	2.81a	3.25b	3.48b
K_{270}	0.65	0.66	0.67	0.65	0.64	0.67	0.67	0.66	0.64	0.68
K_{280}	0.57	0.58	0.58	0.57	0.55	0.58	0.59	0.58	0.55	0.59
AnV	5.85	6.02	6.48	5.39	5.82	5.83	6.16	6.11	5.93	5.76
POL	1.05	1.00	1.07a	0.98b	0.99	1.02	1.06	1.03	1.06	0.98
LHP[4]	3737	3689	6805a	621b	3593	3676	3869	446a	2663b	8030c
SA	4.54	4.44	4.64	4.34	4.3	4.5	4.6	5.8a	4.2b	3.5c

[1] C18:2n-6/C16:0 (linoleic acid/palmitic acid ratio), K_{232}, K_{270}, K_{280} (specific absorbance at 232, 270 y 280 nm), AnV (anisidine value), POL (% triacylglycerol polymer content), LHP (lipid hydroperoxide content), SA (Sensory acceptability).
[2] Values given in this table correspond to least-squares means obtained from MANOVA (n=36).
[3] Means corresponding to a certain factor with different letters differ significantly (P≤0.05). Means for the factors having a significant effect were separated using the Scheffé´s test.
[4] Interaction between ascorbyl palmitate and storage time significant at P≤0.05.

Decrease of sensory scores for acceptability agreed with the increase in K_{232} and lipid hydroperoxide content (Figures 4, 6 and 7). The consumer acceptability scores found for freshly produced snacks, were significantly higher (P≤0.05) compared to snacks stored for 8 and 16 months at room temperature. These results could be due to the increase of rancid notes and moisture during storage. Finally, it is interesting to remark that there are no statistically significant first-order interactions between the antioxidants added. Thus, we can conclude that when fried snacks are stored for long periods of time at the tested conditions there is no synergistic effect between these antioxidants.

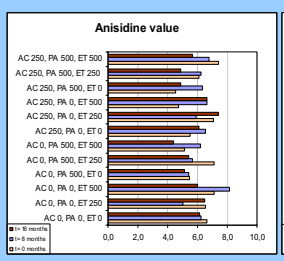


Figure 5. Influence of storage on anisidine value of fried snacks .

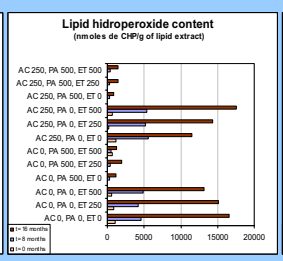


Figure 6. Influence of storage on lipid hidroperoxide content of fried snacks.

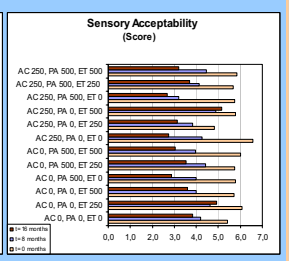


Figure 7. Influence of storage on sesnsory acceptability of fried snacks.

AC =Citric acid, PA =Ascorbyl palmitate, ET = Tocopherol extract. Concentrations of antioxidants: 0 mg/L, 250 mg/L, 500 mg/L

CONCLUSION

Among the assayed antioxidants, ascorbyl palmitate is the only one that prevents the increase of anisidine value and the formation of polymerized triacylglycerols in the frying medium. In addition, this antioxidant prevents the increase of the K_{232} and LHP content in fried snacks stored for long periods of time at room temperature, which is very relevant because these two oxidation values are very useful to monitor the oxidative evolution of the fried products during storage and their increase is accompanied by a decrease in the sensory acceptability scores, assessed by test panels.
Furthermore, when fried snacks are stored for long periods of time, at the tested conditions, there is no synergistic effect between the ascorbyl palmitate and the rest of antioxidants assayed.

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VII.4. Publicaciones en revistas científicas

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Modified ferrous oxidation-xylenol orange method to determine lipid hydroperoxides in fried snacks

A modified ferrous oxidation-xylenol orange (FOX) method was adapted to measure lipid hydroperoxides (LHP) in lipid extracts from snack foods fried in vegetable oils. First, a methanol-based FOX reaction medium was assayed, but this became turbid upon addition of the lipid extracts dissolved in ethanol. To avoid the precipitation of lipids, the polarity of the reaction medium was reduced by lowering its water content and by replacing the methanol as the basis of the medium for less polar solvents. Some of the solvents used instead of methanol yielded a lower FOX reaction response. Of the reaction media assayed, the one based on dichloromethane/ethanol (3:2, vol/vol) was not turbid at high lipid extract concentrations (assayed at up to 25 mg of lipid extract/mL reaction medium) and provided the same response level as the methanol-based medium. Thus, this FOX method shows high sensitivity and is particularly useful for lipid extracts with low LHP content. This method was also successfully applied to edible oils. Solvents such as 2-propanol, ethyl acetate and butanol were discarded, because they easily produce hydroperoxides, which interfere in the FOX reaction. Xylenol orange preparations from a number of suppliers were tested, and some differences affecting the sensitivity of the reaction were observed.

Keywords: Xylenol orange method, lipid hydroperoxide determination, fried snacks in vegetable oils, edible oils.

Research Paper

1 Introduction

The stability of fried foods is affected by lipid oxidation, which yields both primary and secondary oxidation compounds. Primary oxidation compounds, such as lipid hydroperoxides (LHP), are formed in an initial stage of lipid oxidation, increasing in level as oxidation advances, until reaching a plateau, and later decreasing because of degradation into secondary oxidation compounds (such as aldehydes, ketones and hydrocarbons) [1, 2] or interaction with other food components [3]. Thus, lipid oxidation leads to several oxidation compounds with various undesirable biological effects and also implies a loss of nutritional value [4–7] and sensory quality [8–11]. Snack foods are prone to undergo lipid oxidation during storage because of their high lipid content and high surface area and porosity [11].

There are several methods available to monitor lipid oxidation in foods through the determination of LHP [12]. These methods show different performance characteristics, which should be taken into account in order to choose the most adequate method to consistently determine LHP, depending on the analytical situation (*e.g.* kind of sample, number of samples to be analysed, method quality parameters required). In addition, in order to properly assess lipid oxidation in a certain food sample, the LHP measurement should be accompanied by the determination of the appropriate secondary oxidation products [12]. The official techniques of the AOAC [13] and the AOCS [14] to measure LHP, both based on iodometric titrations, are most commonly used, although they present several interferences and a lack of sensitivity [15]. Some potentiometric and iodine spectrophotometric methods have been developed to improve sensitivity [16–18]. Moreover, chromatographic methods (HPLC and GC) have been proposed as alternatives; but despite their high sensitivity and specificity, their application to routine analysis with a large number of samples is not easy [12, 19]. A successful and useful option for routine analysis of edible oils is the Fourier transform infrared (FTIR) spectrometry method described by *van de Voort* et al. [20] and improved by *Ma* et al. [21].

Spectrophotometric techniques such as ferrous oxidation-xylenol orange (FOX) or the International Dairy Federation-ferric thiocyanate method are simple, reproducible and sensitive and can also be successfully applied to routine analysis [15, 22–25]. In these methods, ferrous

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ions are oxidised by LHP to ferric ions, which bind xylenol orange (XO) or thiocyanate and develop a chromophore. The FOX method has been applied to fish fillet, chicken meat, edible oils, butterfat, lipid extracts from meats, plant tissues, and biological samples [15, 23, 25–32]. However, the solvent composition of the reaction media and the extraction steps used differ depending on the author. When *Hermes-Lima* et al. [27], *Grau* et al. [15] and *Eymard* and *Genot* [23] applied the FOX method to animal tissue, chicken meat and fish fillet samples, a methanol- or water-based reaction medium was used, with previous hydroperoxide extraction with methanol. *Nourooz-Zadeh* et al. [29, 31] also used a methanol-based medium to measure hydroperoxides in plasma, with previous extraction with ethyl acetate, and in edible oils, dissolving the sample in 1-propanol. In both cases, a centrifugation step before the spectrophotometric reading was used in order to remove turbidity and flocculated materials; however, this is a time-consuming procedure. Solubility problems were also encountered by *Shantha* and *Decker* [25] when they applied a FOX methanol-based medium to determine hydroperoxides in fats and oils. To overcome this drawback, these authors proposed a chloroform/methanol-based reaction medium; however, the response obtained using alcohol-based media is 1.5 times greater.

This manuscript describes how a highly sensitive FOX method was set up to determine LHP in lipid fractions extracted from snacks fried in vegetable oils, avoiding turbidity in the reaction medium. In addition, the suitability for the FOX method of XO from three suppliers was studied, as some authors have reported differences in behaviour depending on the XO supplier and batch, for instance in sensitivity, colour development, spectrum shape and wavelength of maximum absorbance [15, 33].

2 Materials and methods

2.1 Reagents and standards

XO (ACS grade) was from *Sigma* (St Louis, MO, USA), *Aldrich* (Steinheim, Germany) or *Scharlau* (Barcelona, Spain). The XO supplied by *Scharlau* was used, unless otherwise indicated. Cumene hydroperoxide (CHP) was from *Sigma* and triphenylphosphine (TPP) from *Aldrich*. Ethanol (96%, HPLC grade) was purchased from *Scharlau*. Hydrochloric acid (35%), sulphuric acid (96%), ammonium ferrous sulphate hexahydrate (analytical grade) were purchased from *Panreac Química* (Barcelona, Spain). All other solvents (HPLC grade) were from *SDS* (Peypin, France), and double-deionised water was used throughout.

2.2 Samples

The snack foods (chips, and corn- and potato-extruded snacks) used to optimise the FOX method were provided by six large-scale Spanish producers. These snack foods were fried in soybean oil, sunflower oil, palm olein, palm olein/sunflower oil blend (60:40, vol/vol), or palm oil/soybean oil blend (50:50, vol/vol). Lipid hydroperoxides were also determined in some edible oils (sunflower oil, palm olein and soybean oil) using the FOX method finally proposed.

2.3 Lipid extraction

Lipid extraction was performed as follows: 70 g of snack was weighed out, ground and transferred to a special glass extraction vessel (GS 50, *Kinematica*, Lucerne, Switzerland); 350 mL chloroform/methanol (2:1, vol/vol) was added, and the mixture was homogenised for 40 s at 20,000 rpm using a Polytron PT 3100 (*Kinematica*). The extract was decanted and filtered through Whatman n°1 filter paper, and the residue was re-extracted twice: first with 250 mL chloroform/methanol (2:1, vol/vol) and then with 100 mL chloroform. All the filtrates were pooled, gently shaken and divided into ten 100-mL screw-cap tubes. Then, 10 mL of 8% (wt/vol) aqueous sodium chloride were added to each tube. All tubes were agitated and centrifuged at $400 \times g$ for 20 min. The ten chloroform extracts were pooled, and 100 g anhydrous sodium sulphate was added. After 15 min, the mixture was filtered through Whatman n°1 filter paper into a round-bottom flask, and the solvent was evaporated to near dryness in a vacuum rotatory evaporator at 35 °C. Solvent evaporation was completed by placing the round-bottom flask in a vacuum desiccator at 10 mm Hg overnight. The lipid extract was distributed to various vials, filling them almost completely and leaving a small headspace. Then, the vials were stored at −20 °C for less than 15 d until the FOX value was determined. Later, this frozen lipid extract was used to determine other quality and composition parameters.

2.4 FOX methods

For all the FOX methods assayed, incubation was performed in 1-cm glass cuvettes with Teflon caps for 30 min at room temperature under attenuated light. Absorbance was measured using a Shimadzu UV-160A spectrophotometer (*Shimadzu*, Kyoto, Japan). Absorbance values were measured at 560 and 590 nm. Spectra recording conditions were as follows: spectrum range, 400–700 nm; scan speed, 480 nm/min. LHP content in samples was expressed as μmol CHP equivalents/g lipid

extract, with reference to calibration curves obtained using CHP as standard (2–17 nmol CHP/mL reaction medium) in the corresponding reaction media.

2.4.1 Method 1

The FOX method proposed by *Grau* et al. [15] for dark chicken meat, based on the method described by *Hermes-Lima* et al. [27], was applied with slight modification to the lipid extracts from snacks. The reaction medium consisted of 500 μL 1 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 900 μL methanol, and 200 μL ethanol containing the lipid extract. In the original method, the sample was dissolved in methanol, which does not completely dissolve the lipid extracts.

2.4.2 Method 2

The reaction medium consisted of 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 1300 μL methanol, and 200 μL dichloromethane/ethanol (3:2, vol/vol) containing the lipid extract.

2.4.3 Effect of the acid used in the reaction media

Most authors use sulphuric acid to acidify the FOX reaction medium, but *Shantha* and *Decker* [25] used hydrochloric acid. The effect of the acid was assessed using a reaction medium similar to that described by these authors. The reaction medium consisted of 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.5 M HCl or 0.25 M H_2SO_4 in chloroform/methanol (7:3, vol/vol), 200 μL 1 mM methanolic XO, 1300 μL chloroform/methanol (7:3, vol/vol), and 200 μL chloroform/methanol (7:3, vol/vol) containing the lipid extract. Eight aliquots of a lipid extract from snacks fried in sunflower oil were used to assess the precision (RSD %) and sensitivity of each FOX method.

2.4.4 Effect of the solvent used as the basis of the reaction media

A number of reaction media based on several solvents were assayed: chloroform/methanol (7:3, vol/vol), as previously used by *Burat* and *Bozkurt* [22] and *Shanta* and *Decker* [25]; dichloromethane/methanol (2:3, 1:1, 3:2, 7:4, 2:1, and 7:3; vol/vol); and dichloromethane/ethanol (2:3, 1:1, 3:2, 7:4, 2:1, and 7:3; vol/vol). The capacity of these solvents to prevent lipid precipitation in the medium was tested with lipid extracts from snacks fried in palm olein, the low LHP content of which entailed a larger sample amount to obtain an optimal absorbance value. The response developed in each of these reaction media was evaluated with CHP as standard, in a final concentration of 11 nmol/mL reaction medium. The responses obtained in the methanol- and ethanol-based reaction media were used as controls for the FOX reaction responses obtained in the rest of reaction media. Reaction media consisted of 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 1300 μL solvent, and 200 μL of the corresponding solvent containing CHP or increasing amounts of the lipid extract.

2.4.5 Method 3 (final method proposed)

The reaction medium consisted of 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 1300 μL dichloromethane/ethanol (3:2, vol/vol), and 200 μL dichloromethane/ethanol (3:2, vol/vol) containing the lipid extract.

The linear relationship between sample amount and absorbance value was tested at 560 and 590 nm for lipid extracts from snacks fried in sunflower oil (from 0.1 to 1 mg/mL reaction medium) and in palm olein (from 0.5 to 12 mg/mL reaction medium).

Precision (RSD%) of the method was measured by analysing eight aliquots of lipid extracts from snacks fried in sunflower oil, soybean oil and palm olein. The precision of this method for edible oil analysis was also assessed using sunflower oil, soybean oil and palm olein samples (n = 8).

2.5 Hydroperoxides in solvents

2.5.1 Presence of hydroperoxides in solvents

The FOX reaction was performed with 2-propanol, ethyl acetate or butanol in the reaction medium. Before this reaction, these solvents were incubated (a) with and (b) without TPP as follows: (a) 350 μL solvent and 150 μL 10 mM methanolic TPP or (b) 350 μL solvent and 150 μL methanol were incubated for 30 min at room temperature. After incubation, 1500 μL FOX reagent (100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 1000 μL methanol) was added to both solutions, which were then incubated for a further 30 min at room temperature. Blanks contained 500 μL methanol without TPP and 1500 μL FOX reagent.

2.5.2 Formation of hydroperoxides in solvents

LHP were measured in 2-propanol from different bottles: a bottle that had just been opened (used in blanks), a bottle opened 1 month before, and a bottle opened 3 months before. The first two bottles were from the same batch and the third from an older batch. The FOX reaction was performed as follows: 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M H_2SO_4 in the corresponding 2-propanol, 200 μL 1 mM methanolic XO (from *Sigma*), and 1500 μL of the corresponding 2-propanol.

In addition, the three types of 2-propanol were incubated for 30 min with TPP prior to the FOX reaction, as described above.

2.6 Differences between XO suppliers

XO from three suppliers was used: *Scharlau*, *Sigma* and *Aldrich*. The comparison was done by means of the calibration curves obtained using the corresponding XO and CHP as standard (0.3–10.6 nmol CHP/mL reaction medium for *Sigma* XO; 2–13 nmol CHP/mL reaction medium for *Aldrich* and *Scharlau* XO) under the reaction conditions described in section 2.4.2.

3 Results and discussion

3.1 FOX methods

3.1.1 Methods 1 and 2

First, method 1 was assayed with lipid extracts from snacks fried in vegetable oils; however, upon addition of the sample dissolved in ethanol to the reaction medium, turbidity was evident at very low lipid extract concentrations, causing interference with spectrophotometric measurements. Similar solubility problems were reported by *Shantha* and *Decker* [25] when the methanol-based FOX reaction medium described by *Jiang* et al. [28, 34] was applied to fats and edible oils. *Grau* et al. [15] successfully applied the method 1 to methanolic extracts from poultry meat and did not encounter problems related to turbidity, but the ethanolic solutions used in the present study had a higher lipid content.

In method 2, to prevent precipitation of the lipid extracts in the reaction medium, water content was reduced to 100 μL, whilst the same concentration of ferrous ions in the final reaction mixture was maintained. Any further reduction of water content produced a loss of sensitivity (data not shown). Moreover, in this method, lipid extracts were dissolved in dichloromethane/ethanol (3:2, vol/vol) instead of ethanol and then added to the reaction medium.

Despite this reduction in polarity, turbidity still occurred at concentrations of 0.5 and 0.6 mg lipid extract/mL reaction medium (lipid extracts from snacks fried in palm olein and sunflower oil, respectively), and the concentrations of LHP in these amounts of extracts were sometimes too low to ensure optimal absorbance (0.7–0.8, as recommended by *Grau* et al. [15]). Thus, a further decrease in the polarity of the reaction medium was necessary to prevent the precipitation of certain lipids.

3.1.2 Effect of the acid used in the reaction media

Sulphuric acid [15, 23, 27–30] and hydrochloric acid [22, 25] have been proposed to achieve the conditions required for the development of the reaction.

A higher sensitivity was observed for LHP determination in snacks fried in sunflower oil when sulphuric acid was used instead of hydrochloric acid to acidify the FOX reaction medium, which agrees with previously reported results for LHP determination in chicken meat using a substantially different FOX method [15]. In addition, a better precision was obtained for this acid (RSD = 1.4%, n = 8, absorbance at 560 nm/sample concentration in the reaction medium in $mg \times mL^{-1}$ = 0.88) compared with hydrochloric acid (RSD = 3.6%, n = 8, absorbance at 560 nm/sample concentration in the reaction medium in $mg \times mL^{-1}$ = 0.56).

3.1.3 Effect of the solvent used as the basis of the reaction media

A suitable solvent for the FOX reaction medium should dissolve the lipid extracts from the snacks and allow the development of the colourimetric reaction with high sensitivity. As the decrease of the water volume to 100 μL and the dissolution of the sample in dichloromethane/ethanol (3:2, vol/vol) instead of ethanol did not prevent turbidity, the polarity of the medium was decreased by experimenting with less polar solvents as substitutes for methanol to complete the final volume of the reaction medium at up to 2 mL.

First, dichloromethane/methanol- (7:3, vol/vol), dichloromethane/ethanol- (3:2, vol/vol) and chloroform/methanol-based (7:3, vol/vol) reaction media were assayed, because the latter was reported to prevent precipitation of lipids in the FOX reaction medium when fats and oils were analysed [25]. None of these solvent mixtures showed turbidity at a concentration of 25 mg lipids extracted from a snack fried in palm olein/mL reaction medium. Then, the response obtained with the same solvents using a known concentration of CHP (11 nmol/mL

reaction medium) was tested, and methanol- and ethanol-based reaction media were used as controls for the response. However, only the dichloromethane/ethanol-based (3:2, vol/vol) medium developed a response of the same order as methanol or ethanol (Fig. 1).

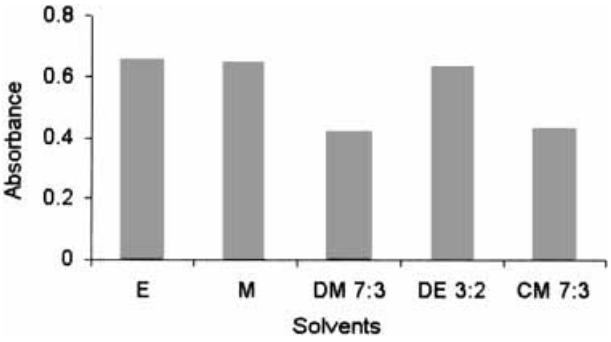

Fig. 1. Absorbance at 560 nm obtained using FOX media with distinct solvent compositions (n = 2). Reaction media consisted of 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 1300 μL solvent and 200 μL of a solution of CHP in the corresponding solvent (the final concentration of CHP was 11 nmol/mL reaction medium). Solvents: ethanol (E), methanol (M), dichloromethane/methanol (7:3, vol/vol) (DM 7:3), dichloromethane/ethanol (3:2, vol/vol) (DE 3:2) and chloroform/methanol (7:3, vol/vol) (CM 7:3).

These results led us to test a set of proportions of dichloromethane/ethanol and dichloromethane/methanol. When proportions of the former at 2:3 and 1:1 (vol/vol) were used, the response to CHP was greater than for ethanol or methanol (Fig. 2). However, these solvent mixtures did not prevent the precipitation of some lipids in the

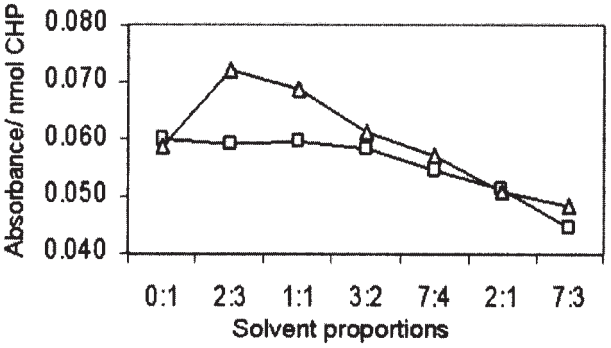

Fig. 2. Response (absorbance at 560 nm/nmol CHP, n = 2) obtained using FOX media with distinct solvent proportions (dichloromethane/methanol in squares, dichloromethane/ethanol in triangles). Reaction media consisted of 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 1300 μL of the solvent mixture and 200 μL of a solution of CHP in the corresponding solvent mixture (final concentration of CHP, 11 nmol/mL reaction medium).

reaction medium at concentrations of 20 and 25 mg lipids extracted from snacks fried in palm olein/mL reaction mixture of dichloromethane/ethanol at 2:3 and 1:1, vol/vol, respectively.

When dichloromethane/ethanol (3:2, vol/vol) was used, the response to CHP was of the same order as for methanol or ethanol (Figs. 1 and 2); furthermore, no precipitation occurred at the concentrations tested (up to 25 mg/mL) for any of the lipid extracts assayed (lipid extracts from snacks fried in sunflower oil, palm olein, soybean oil, palm olein/sunflower oil blend, and palm oil/soybean oil blend). Media richer in dichloromethane yielded a lower response than methanol- or ethanol-based media (Fig. 2).

3.1.4 Method 3 (final method proposed)

On the basis of the previous results, it was concluded that the reaction medium containing dichloromethane/ethanol (3:2, vol/vol) as the main solvent showed good sensitivity and prevented lipid precipitation even when large quantities of sample were used (Fig. 3). Thus, method 3 was chosen to determine the LHP in lipid extracts from snacks fried in vegetable oils.

A linear relationship between the sample amount in the FOX reaction medium and the absorbance value has been previously reported [15, 23, 27]. In this study, for lipid extracts from snack foods fried in sunflower oil or in palm olein, the correlation coefficient (r) between sample amount and absorbance value was higher for readings at 560 nm than at 590 nm. The correlation coefficients (r) were 0.9992 at 560 nm and 0.9988 at 590 nm for sunflower oil samples, and 0.9997 at 560 nm and 0.9962 at 590 nm for palm olein samples. These results are in agreement with those previously reported for fish fillet samples [23]. Moreover, readings of absorbance values above 0.9–1.1 at 560 nm resulted in a loss of linearity, which does not occur if the absorbance values are always measured at the absorbance maximum of the spectra (Fig. 4). However, as the wavelength of the absorbance maximum mainly depends on LHP concentration, but also on sample and reaction media composition (data not shown), the latter option entails to record the spectrum for each sample, which is quite time consuming. Therefore, for method 3, taking into account these results and the results discussed in section 3.3., it is recommended to measure the absorbance under 0.8 at 560 nm.

Precision (RSD %) of method 3 was determined by analysing aliquots of lipid extracts from snacks fried in sunflower oil (RSD = 1.0%, n = 8, LHP value = 20 μmol CHP equivalents/g lipid extract), soybean oil (RSD = 1.0%, n = 8, LHP value = 21 μmol CHP equivalents/g lipid extract),

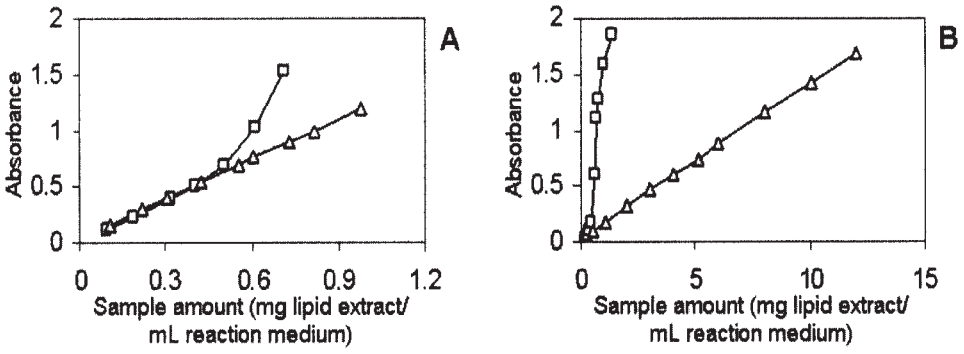

Fig. 3. Absorbance at 560 nm obtained (n = 2) by method 2 (squares) and method 3 (triangles) using a range of amounts of lipid extracts from snacks fried in sunflower oil (**A**) and palm olein (**B**). Reaction media consisted of 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 1300 μL methanol or dichloromethane/ethanol (3:2, vol/vol), and the lipid extracts dissolved in dichloromethane/ethanol (3:2, vol/vol). Note that in method 2, turbidity, which carries a sharp increase of the absorbance, occurred at concentrations of 0.6 mg (sunflower oil) and 0.5 mg (palm olein) lipid extract/mL reaction medium.

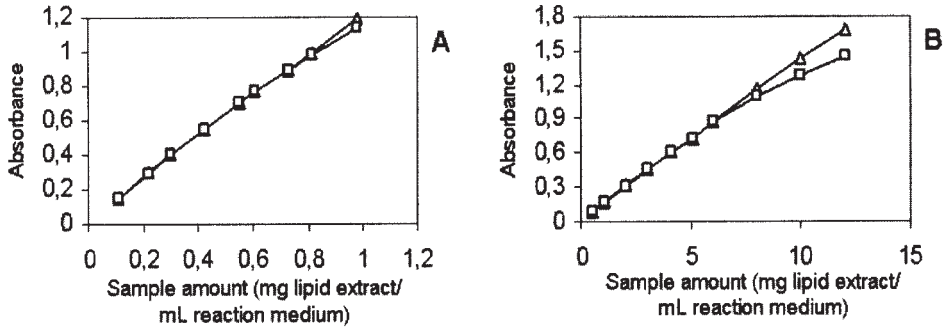

Fig. 4. Comparison of the maximum absorbance (triangles) and the absorbance at 560 nm (squares) obtained (n = 2) with method 3 using different amounts of lipid extracts from snacks fried in sunflower oil (**A**) and palm olein (**B**).

and palm olein (RSD = 1.1%, n = 8, LHP value = 2.4 μmol CHP equivalents/g lipid extract). Precision of this method for edible oil analysis was similar: sunflower oil (RSD = 1.0%, n = 8, LHP value = 4.2 μmol CHP equivalents/g oil), soybean oil (RSD = 1.0%, n = 8, LHP value = 4.4 μmol CHP equivalents/g oil) and palm olein (RSD = 1.1%, n = 8, LHP value = 0.8 μmol CHP equivalents/g oil).The precision of this method is better than the precision reported by other authors determining LHP in fats and oils by various FOX methods [22, 29].

3.2 Hydroperoxides in solvents

3.2.1 Presence of hydroperoxides in solvents

Solvents such as 2-propanol and ethyl acetate have been used in FOX methods that include BHT as antioxidant [29, 31, 35]. However, when these solvents were tested without adding BHT to the medium, as *Grau* et al. [15] and *Eymard* and *Genot* [23] recommended, the blanks turned purple after the FOX reaction instead of remaining yellow as expected. To check whether the purple colour was due to the presence of hydroperoxides in solvents, the solvents were incubated prior to the FOX reaction with TPP, a specific hydroperoxide reductant that has no effect on H_2O_2 [15, 31]. When 2-propanol, ethyl acetate or butanol were incubated with TPP, the mixtures remained yellow after the FOX reaction and no peaks of absorbance were detected at 560 nm (Fig. 5). This result indicates that the purple colour developed using 2-propanol, ethyl acetate or butanol was due to non-H_2O_2 hydroperoxides present in these solvents, which may interfere in the FOX reaction. This drawback was not observed when methanol or ethanol was used as basis of the reaction media.

3.2.2 Formation of hydroperoxides in solvents

The formation of hydroperoxides in 2-propanol increases with the time elapsed since the solvent bottle was opened. Thus, blanks (which contained 2-propanol from a bottle that had just been opened) were still yellow after 30 min, whereas those with 2-propanol from bottles opened 1 or 3 months before were purple and exhibited the absorbance spectra shown in Fig. 6. Absorbance at 560 nm was much higher for the latter.

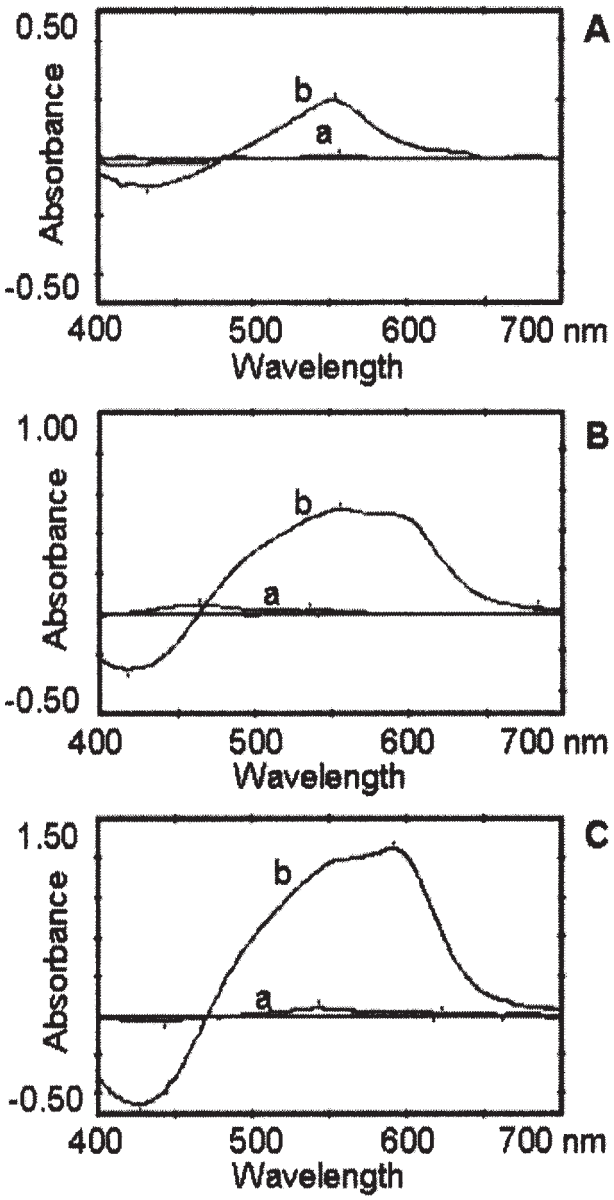

Fig. 5. Absorbance spectra obtained with the FOX method after 30 min of incubation of (**A**) 2-propanol, (**B**) butanol or (**C**) ethyl acetate with (a) or without (b) TPP: (a) 350 μL of the corresponding solvent and 150 μL 10 mM methanolic TPP or (b) 350 μL of the corresponding solvent and 150 μL methanol were incubated for 30 min. After incubation, 1500 μL FOX reagent was added in both cases (100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M methanolic H_2SO_4, 200 μL 1 mM methanolic XO, 1000 μL methanol) and again incubated for 30 min. Blanks contained 500 μL methanol without TPP.

In all cases, when each type of 2-propanol was incubated with TPP before application of the FOX method, no absorbance at 560 nm was obtained after the reaction.

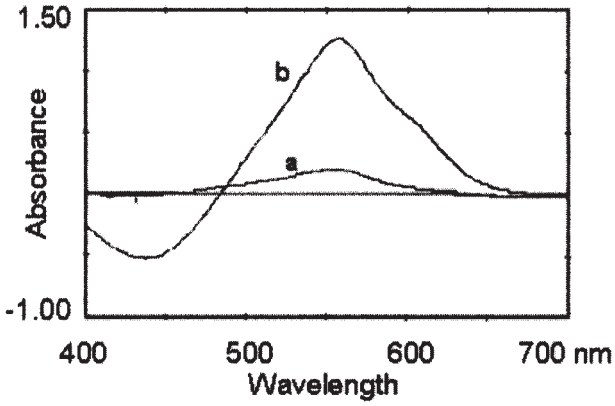

Fig. 6. Absorbance spectra obtained with the FOX method applied to (a) 2-propanol from a bottle opened 1 month before, and (b) 2-propanol from a bottle opened 3 months before. FOX reaction medium was as follows: 100 μL 5 mM aqueous ferrous ammonium sulphate, 200 μL 0.25 M H_2SO_4 in the corresponding 2-propanol, 200 μL 1 mM methanolic XO (from *Sigma*), and 1500 μL of the corresponding 2-propanol. The 2-propanol used to prepare blank reagents was from a bottle that had just been opened.

Thus, the presence of non-H_2O_2 hydroperoxides in solvents such as 2-propanol depends on the time elapsed since the solvent bottle was opened.

Therefore, the use of 2-propanol, butanol or ethyl acetate is not recommended in FOX reactions.

3.3 Differences between XO suppliers

In this study, differences in behaviour between XO sources and batches were observed, as reported by other authors [15, 33]. Depending on the hydroperoxide concentration, when using *Sigma* XO, the colour turned from red to purple, and only one absorption maximum appeared at 560 nm. However, the XO purchased from *Aldrich* or *Scharlau* gave a series of colours ranging from brown to blue, depending on the hydroperoxide concentration, and two absorption peaks at 560 nm and at 590 nm. Thus, the shape of the spectrum given by XO from *Sigma* differed from that given by XO from *Aldrich* or *Scharlau* (Fig. 7).

Moreover, with XO from *Aldrich* and *Scharlau* (method 2), concentrations of CHP under 13 nmol/mL reaction medium (absorbance approximately 0.8) led to a greater absorbance at 560 nm than at 590 nm, but at higher CHP concentrations, absorbance at 560 nm was lower than at 590 nm. This is in agreement with previous findings of *Eymard* and *Genot* [23]. In addition, as found by these authors, the calibration curves with CHP showed higher

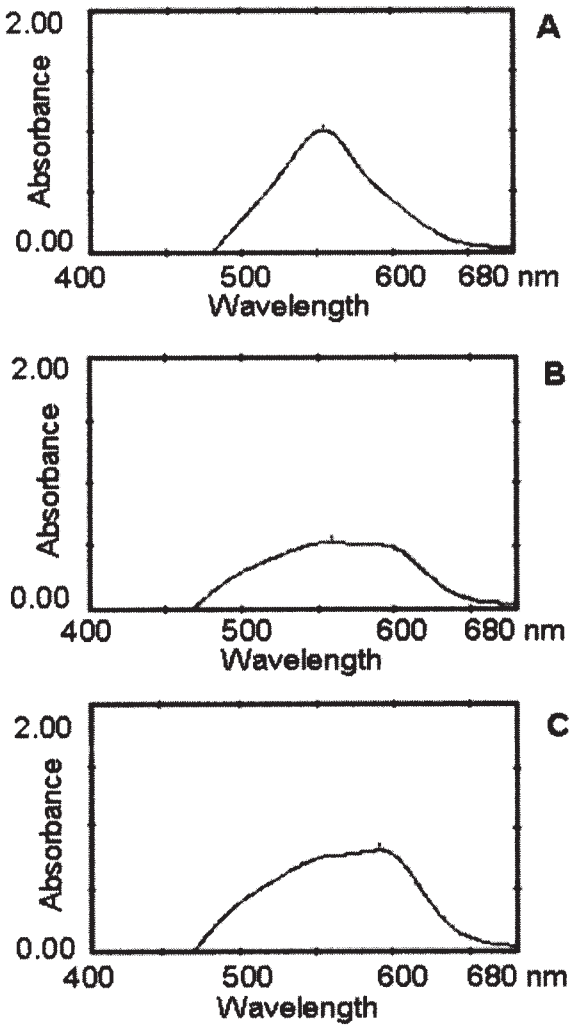

Fig. 7. Absorbance spectra obtained after FOX reaction using XO from three suppliers: (**A**) *Sigma* XO (10.6 nmol CHP/mL reaction medium), (**B**) *Scharlau* XO (8.8 nmol CHP/mL reaction medium), and (**C**) *Aldrich* XO (13.3 nmnol CHP/mL reaction medium). Method 2 was used in all cases.

determination coefficients (r^2) when absorbance was measured at 560 nm than at 590 nm for methods 2 and 3 (Tab. 1). Therefore, it is recommended to use XO from the same supplier and batch in order to obtain comparative results, and to measure the absorbance at 560 nm under 0.8 when the XO from *Aldrich* or *Scharlau* is used. However, reaction conditions and wavelength of reading should be checked for each kind of sample. The XO from Sigma gave a higher sensitivity at 560 nm than that purchased from *Aldrich* and *Scharlau* (Tab. 1), but this product is unfortunately no longer available.

4 Conclusion

Method 3, which uses dichloromethane/ethanol 3:2 (vol/vol) as the main solvent in the reaction medium, is suitable for determining LHP in lipid extracts from snacks fried in vegetable oils. This method shows high sensitivity and is particularly useful for samples with a low LHP content, because the reaction medium allows the dissolution of large amounts of lipid extract (it has been assayed at up to 25 mg lipid extract/mL reaction medium), which are sometimes required to obtain an optimum absorbance value. This method was also successfully applied to edible oils.

Acknowledgements

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Tab. 1. Calibration curves obtained for XO from distinct suppliers at two wavelengths (X = nmol CHP/mL reaction medium; Y = absorbance).

XO supplier	Reaction medium	Wavelength reading	Calibration curve	r^2
Sigma	Method 2	560 nm	$Y = 9.24 \times 10^{-2} X + 3.04 \times 10^{-2}$	0.9946
Aldrich	Method 2	560 nm	$Y = 5.88 \times 10^{-2} X + 4.72 \times 10^{-3}$	0.9998
Aldrich	Method 2	590 nm	$Y = 6.27 \times 10^{-2} X - 3.39 \times 10^{-2}$	0.9928
Scharlau	Method 2	560 nm	$Y = 5.85 \times 10^{-2} X + 8.63 \times 10^{-4}$	0.9998
Scharlau	Method 2	590 nm	$Y = 6.32 \times 10^{-2} X - 4.83 \times 10^{-2}$	0.9929
Scharlau	Method 3	560 nm	$Y = 5.86 \times 10^{-2} X + 4.70 \times 10^{-3}$	0.9993
Scharlau	Method 3	590 nm	$Y = 5.40 \times 10^{-2} X - 2.57 \times 10^{-2}$	0.9939

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Polycyclic Aromatic Hydrocarbons in Frying Oils and Snacks

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ABSTRACT

?1 The high incidence of lung cancer observed among Chinese women has been associated with exposure to fumes from cooking oil. Polycyclic aromatic hydrocarbons (PAHs) are a class of potentially mutagenic substances emitted from cooking oils heated at high temperatures. The objective of this study was to investigate whether deep frying with different oils under different conditions leads to the development of PAHs either in the oil or in the fried product (snacks). PAH analysis was carried out with solid-phase extraction followed by reverse-phase high-performance liquid chromatography and spectrofluorometric detection. Different oils were used to fry chips and extruded snacks in different industrial plants (continuous frying) at temperatures between 170 and 205°C, and peanut oil was used to fry French fries and fish (discontinuous frying) at temperatures between 160 and 185°C. No appreciable differences in PAH load was observed in the same oil before and after frying. Both before and after frying, the benzo[a]pyrene concentration in oils ranged from trace to 0.7 ppb. All the analyzed samples, including oils from fried snacks, had benzo[a]pyrene concentrations well below the 2 ppb limit recently proposed by the European Community.

Polycyclic aromatic hydrocarbons (PAHs) are an important class of contaminants formed through incomplete combustion of organic matter *(15).* Heavy PAHs, formed by four to six benzene fused rings, are carcinogenic and can be assimilated by humans through ingestion, inhalation, or skin contact *(11).*

PAHs can contaminate food through two main routes: food processing at high temperatures (e.g., grilling, roasting, and smoking) and atmospheric deposition of dust and particulates containing large quantities of pyrolitically generated PAHs on the surface of foods *(16).* Because of the lipophilic nature of PAHs, oils and lipid matrices can contain high concentrations of these compounds *(18).* The major contributors to PAH intake in the average diet are oils and fats, cereals, and vegetables *(7).*

Commission regulation (EC) 208/2005 *(8)* has recently set a maximum level of 2 ppb for benzo[a]pyrene (BaP) in oils and fats intended for direct consumption or for use as an ingredient in foods. BaP is used as a marker of the occurrence and effect (toxic potential) of the whole class of genotoxic heavy PAHs.

The optimal temperatures for the formation of PAHs are between 660 to 740°C *(2)*; however, several authors *(17, 21, 25)* have studied the PAH concentration in cooking oil fumes (180 to 270°C). Frying is an important cooking practice that leads to the formation of substances that improve food taste and smell and of compounds that negatively affect the quality of foods *(9).* The production of these compounds is influenced by a number of variables, including the kind of oil or fat, frying temperature, frying method (continuous or discontinuous), and type of vessel. Continuous frying is used for large volumes of product. The process usually is automated, and fresh oil is added continuously to the frying kettle while the food is moved through the cooking oil on a conveyor. The oil is heated continuously, and the heat supply is regulated by a thermostat so that the oil does not cool when food or fresh oil is added. Oil always circulates, and food particles that burn during the process are eliminated continuously by filtering. In discontinuous (batch) frying, which is used for smaller quantities of product, fresh oil is added periodically, and burnt fragments stay in the oil longer because the oil is filtered only once at the end of the process day.

Several epidemiological studies conducted in Taiwan and China revealed that Asian women ranked highest in the world for lung cancer, probably because of the exposure to fumes from cooking oil *(10, 14, 24).* Ko et al. *(14)* found that the risk of lung cancer was higher from stir frying than from deep frying. Some authors *(3, 6)* suggested that the increased cancer risk observed among people exposed to oil fumes is correlated with the presence of PAHs in the fumes of heated oils. Heterocyclic aromatic amines *(5, 22)* and volatile decomposition products such as 1,3 butadiene, benzene, acrolein, and formaldehyde have been suggested to be responsible for the mutagenic properties of the fumes from cooking oils *(20).* Chen and Chen *(4),* in a study of model lipids and different vegetable oils (soybean, canola, and sunflower oils), found that the degree of unsaturation of fatty acids affects the variety and amount of PAHs formed in the fumes; soybean oil, which had the highest degree of unsaturation, produced the most PAHs.

Most studies of PAHs have been focused on oil cook-

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TABLE 1. *PAH concentrations in oil samples from continuous and discontinuous frying*

Sample code[a]	Frying oil[b]	Fried product	PHA concentration (ppb)[c]																
			Na	Ac	F	Pa	A	Fl	P	L-PAH	BaA	Ch	BeP	BbF	BkF	BaP	DBahA	BghiP	IP	H-PAH
CF1(b)	po/so (60/40)	Potato chips	6.1	tr	0.7	2.2	0.1	0.2	1.1	4.3	0.3	0.5		0.2	0.1	0.2		0.2	0.1	1.5
CF1(a)			3.5	tr	0.6	1.7	0.1	0.7	0.9	4.0	0.3	0.3		0.2	0.1	0.2	tr	0.3	0.1	1.4
CF1(p)			13.5	0.2	2.0	12.2	0.3	1.9	3.2	19.5	0.5	tr		0.3	0.1	0.2		tr	0.1	1.2
CF2(b)	pa	Corn-extruded snacks	7.8	0.3	1.0	4.5	0.6	2.7	3.6	12.4	1.1	0.1		0.4	0.3	0.7		0.4	0.3	3.4
CF2(a)			4.2	0.1	1.0	3.9	0.5	2.6	4.2	12.3	1.3	0.2	0.1	0.6	0.2	0.7		0.4	0.2	3.6
CF2(p)			9.3	tr	1.2	4.8	0.4	2.3	4.4	13.2	1.0	0.3		0.5	0.2	0.6	tr	0.5	0.3	3.5
CF3(b)	so	Potato chips	7.2	tr	1.5	8.4	1.0	3.9	5.3	20.1	0.8	0.2	0.1	0.9	0.2	0.3			0.1	2.7
CF3(a)			4.3	tr	0.4	5.0	0.5	3.1	4.7	13.6	0.7	0.6		0.7	0.2	0.3			0.2	2.6
CF3(p)			NQ	0.1	1.5	5.7	0.4	2.6	5.0	15.3	1.2	0.7	tr	0.8	0.3	0.5	tr		0.4	3.9
CF4(b)	sbo	Potato chips	5.9	tr	0.6	2.3	0.2	2.0	3.7	8.7	0.3	0.1	0.1	0.1	0.1	0.1	tr		0.1	0.8
CF4(a)			4.7	tr	0.5	1.8	0.1	1.5	2.6	6.5	0.2	0.1	tr	tr	0.1	tr		tr		0.6
CF4(p)			NQ	0.1	4.2	7.8	0.3	2.1	4.5	19.0	0.3	0.3		0.4	0.1	0.1	0.1	0.1		1.4
CF5(b)	sbo	Potato-extruded snacks	4.2	tr	0.9	1.8	0.1	2.1	2.6	7.5	0.2	0.1	0.1	0.1	tr	tr	tr			0.5
CF5(a)			4.3	tr	1.7	0.9	0.1	2.2	4.3	9.3	0.2	0.1	NQ	0.2	0.1	NQ	0.2			0.7
CF5(p)			20.1	tr	2.4	5.7	0.3	2.8	5.8	17.1	0.4	0.2	NQ	0.4	0.2	0.2	0.1			1.4
CF6(b)	po/sbo (50/50)	Potato chips	5.4	tr	0.6	2.3	0.1	0.8	1.9	5.7	0.2	0.1		0.1	0.1	0.1			0.1	0.6
CF6(a)			5.8	tr	1.3	3.0	tr	0.8	2.2	7.3	0.4	tr		0.2	0.1	0.1	tr			0.9
CF6(p)			16.9	tr	1.8	5.8	0.3	1.1	2.3	11.3	0.2	0.1		0.1	0.1	0.1	0.1	tr		0.6
DF1(b)	pno	French fries, fish	tr	tr	0.0	0.6	0.1	1.1	1.1	2.9	0.3	0.3	0.1	0.3	0.2	0.2	tr	0.3	0.2	1.9
DF1(a) t = 2 h)			0.8	tr	0.5	2.1	0.1	1.0	1.7	5.4	0.3	0.1	0.1	0.4	0.1	0.2	0.1	0.4	0.3	2.1
DF1(a) (t = 2 h)			2.2	0.0	0.4	0.8	0.1	0.9	1.5	3.6	0.3	0.1		0.4	0.1	0.2		0.2	0.3	1.7
DF2(b)	pno	French fries	2.3	tr	0.2	0.6	tr	0.5	0.5	1.8	0.2				tr	tr				0.2
DF(2)(a) (t = 8h)			1.7	tr	0.1	0.9	tr	0.3	0.3	1.6	0.2				tr	tr				0.2
DF3(b)	pno	French fries, fish	4.4	tr	0.6	1.8	0.1	0.9	1.3	4.7	0.4	tr	0.1	0.2	0.1	0.1		tr	0.1	1.0
DF3(a) (t = 6h)			3.6	tr	0.7	3.2	0.1	0.9	1.0	5.8	0.3	tr	tr	0.2	0.3	0.2		tr	0.3	1.4
DF3(a) (t = 2 h)			7.2	0.3	2.0	4.5	0.2	1.1	1.1	8.9	0.1	0.2	0.1	0.3	0.1	0.1	tr	tr	0.2	1.2

[a] CF, continuous frying; DF, discontinuous frying; b, oil before frying; a, oil after frying; p, oil from fried product; t, duration (h) of frying. DF1 and DF2 were taken from the same University canteen.

[b] po, palm oil; so, sunflower oil; sbo, soybean oil; pno, peanut oil.

[c] Na, naphthalene; Ac, acenaphthene; F, fluorene; Pa, phenanthrene; A, anthracene; Fl, fluoranthene; P, pyrene; L-PAH, sum of light PAHs from F to P; BaA, benzo[a]anthracene; Ch, chrysene; BeP, benzo[e]pyrene; BbF, benzo[b]fluoranthene; BkF, benzo[k]fluoranthene; BaP, benzo[a]pyrene; DBahA, dibenz[a,h]anthracene; BghiP, benzo[g,h,i]perylene; IP, indeno[1,2,3-c,d]pyrene; H-PAH, sum of heavy PAHs from BaA to IP. tr, trace [<0.05 ppb); NQ, not quantified because of interference.

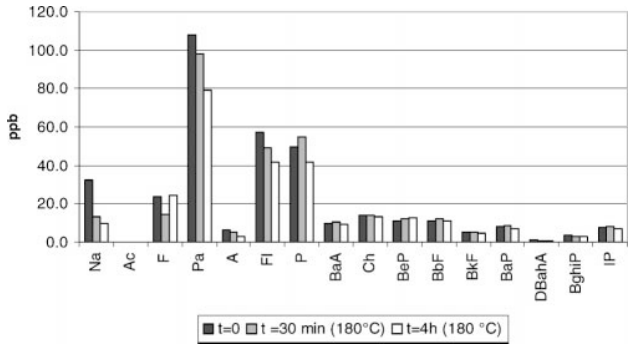

FIGURE 1. *PAH concentrations in olive oil contaminated with both light and heavy PAHs after different frying times at 180°C (oil was used to prepare French fries in an electrically heated fryer). Na, naphthalene; Ac, acenaphthene; F, fluorene; Pa, phenanthrene; A, anthracene; Fl, fluoranthene; P, pyrene; L-PAH, sum of light PAHs from F to P; BaA, benzo[a]anthracene; Ch, chrysene; BeP, benzo[e]pyrene; BbF, benzo[b]fluoranthene; BkF, benzo[k]fluoranthene; BaP, benzo[a]pyrene; DBahA, dibenz[a,h]anthracene; BghiP, benzo[g,h,i]perylene; IP, indeno[1,2,3-c,d]pyrene; H-PAH, sum of heavy PAHs from BaA to IP.*

ing fumes, but only a few have involved PAH concentrations in the frying medium. Siegmann and Sattler *(21)* found that when different vegetable oils were heated up to 260°C, PAH concentrations in the oil aerosol are very high compared with those reported by other authors in liquid oil. PAH concentration in the oil aerosol was estimated to be about 1,000-fold higher than that in the liquid oil. Barranco et al. *(1)* reported increased PAH concentrations in olive oil used to cook eggs, but only a slight increase in light PAHs was observed when peppers were fried. The same authors suggested that fat (cholesterol) present in eggs favored the formation of PAHs.

The aim of this work was to investigate whether deep frying with different frying methods (continuous and batch frying) can lead to formation of important PAHs. Different vegetable oils used for frying snacks, French fries, and fish were collected during the frying process and analyzed. Oil samples extracted from fried snacks were also analyzed to assess PAH contribution from these products. PAH analysis was carried out with a rapid solid phase extraction method previously set up for PAH determination in vegetable oils *(19)* followed by high-performance liquid chromatography (HPLC) and spectrofluorometric detection.

MATERIALS AND METHODS

Reagents and chemicals. Hexane, dichloromethane, acetone, and acetonitrile were HPLC grade (Baker, Deventer, The Netherlands). Water was purified with a Milli-Q System (Millipore, Bedford, Mass.).

The standard 610 M PAH mixture in 1 ml of methanol-dichloromethane (Supelco, Bellefonte, Pa.) consisted of acenaphthene (1,000 μg/ml), fluoranthene (200 μg/ml), naphthalene (1,000 μg/ml), benzo[a]anthracene (100 μg/ml), benzo[b]fluoranthene (200 μg/ml), BaP (100 μg/ml), benzo[k]fluoranthene (100 μg/ml), chrysene (100 μg/ml), acenaphthylene (2,000 μg/ml), anthracene (100 μg/ml), benzo[g,h,i]perylene (200 μg/ml), fluorene (F; 200 μg/ml), phenanthrene (Pa; 100 μg/ml), dibenz[a,h]anthracene

TABLE 2. *PAH concentrations in oil extracted from fried snacks*

Sample code	Frying oil[a]	Fried product	PHA concentration (ppb)[b]																
			Na	Ac	F	Pa	A	Fl	P	L-PAH	BaA	Ch	BeP	BbF	BkF	BaP	DBahA	BghiP	IP	H-PAH
S1	pno	Potato chips	7.9	0.2	1.0	1.3	0.0	0.6	0.8	3.8		0.1	0.2	0.2	0.1	0.1	0.2		0.1	0.9
S2	pno	Potato chips	9.2	tr	0.4	1.5	0.1	2.9	2.2	7.1	0.8	1.0		1.2	0.4	0.6	tr	0.5	0.3	4.8
S3	pno	Potato chips	3.7	tr	0.1	1.2	tr	0.3	0.1	1.7	tr	0.3		tr		tr		tr	tr	0.4
S4	pno	Potato chips	9.6	0.1	0.6	2.1	0.1	0.9	1.4	5.0	tr	tr		0.1	tr	tr	0.1	tr	0.1	0.4
S5	pno	Potato chips	5.6	tr	0.6	4.5	0.2	tr	5.9	11.2	0.3			0.3	tr	0.2	tr		0.4	1.1
S6	pno	Potato chips	5.9	0.1	1.1	5.2	0.1	0.8	1.5	8.8	0.1	0.1	0.2	0.2	0.1	0.4	tr	0.1	0.2	1.3
S7	pno	Potato chips	6.1	tr	0.3	1.6	0.1	1.0	3.9	6.9	0.2			tr		0.1			tr	0.4
S8	pno	Potato chips	3.1	tr	0.5	4.2	0.1	1.7	4.9	11.4	0.7	0.4		0.5	tr	0.5	tr	tr	0.4	2.5
S9	pno	Potato chips	9.2	tr	0.1	1.6	0.1	tr	1.8	3.6	0.2	0.1	0.4	0.8	0.3	0.3	0.6	0.3	0.2	3.1
S10	hvo	Potato chips	24.9	tr	1.3	10.4	0.4	1.9	NQ	14.0	0.4	1.2		0.1	tr	0.3	tr		0.1	2.1
S11	pno	Potato chips	20.4	tr	1.0	4.4	0.3	tr	5.1	10.7	0.8	0.8		0.7	0.3	1.2	tr	1.0	1.3	6.1
S12	pno	Corn extruded	14.3	0.1	1.3	3.3	0.1	0.8	1.3	6.8	0.5	0.3	0.1	0.7	0.2	0.3	tr	0.1	0.8	3.1
S13	pno	Wheat extruded	24.5	tr	2.4	9.5	0.9	1.6	NQ	14.3	0.4	0.5		0.5	0.1	0.5	tr	0.3	0.2	2.4
S14	pno	Cereal mix extruded	4.7	0.3	0.4	1.0	0.1	0.5	tr	2.0	0.2	0.1	tr	0.2	0.1	0.1	0.4		0.2	1.4

[a] pno, peanut oil; hvo, hydrogenated vegetable oil.
[b] Na, naphthalene; Ac, acenaphthene; F, fluorene; Pa, phenanthrene; A, anthracene; Fl, fluoranthene; P, pyrene; L-PAH, sum of light PAHs from F to P; BaA, benzo[a]anthracene; Ch, chrysene; BeP, benzo[e]pyrene; BbF, benzo[b]fluoranthene; BkF, benzo[k]fluoranthene; BaP, benzo[a]pyrene; DBahA, dibenz[a,h]anthracene; BghiP, benzo[g,h,i]perylene; IP, indeno[1,2,3-c,d]pyrene; H-PAH, sum of heavy PAHs from BaA to IP. tr, trace [<0.05 ppb); NQ, not quantified because of interference.

(200 μg/ml), indeno[1,2,3-c,d]pyrene (100 μg/ml), and pyrene (P; 100 μg/ml). This mixture was added to benzo[e]pyrene (Sigma Aldrich, St. Louis, Mo.).

All the glassware was carefully washed and rinsed with distilled solvent (acetone or hexane) before use.

Apparatus. A model 9010 HPLC gradient pump (Varian, Palo Alto, Calif.) equipped with a Rheodyne 7161 injector with a 20-μl loop was used for analytical determination. The C18 reversed phase column (Supelcosil LC-PAH) was 250 by 3 mm inside diameter with 5-μm particle size (Supelco) and was regulated by thermostat to 38°C. The mobile phase consisted of acetonitrile and water at a flow rate of 1 ml/min. The gradient elution program started with 40% acetonitrile (isocratic for 5 min) and continued linearly to 100% acetonitrile (total run time of 40 min).

The eluted compounds were detected with two spectrofluorometric detectors connected on line. The first was a programmable spectrofluorometer (model FP 1520, Jasco, Cremella, Como, Italy), whose wavelength changes have been reported elsewhere *(19),* and the second was a Varian spectrofluorometer (model 9070) set at 280 to 490 nm to improve sensitivity for benzo[e]pyrene.

Sampling. To investigate the effect of temperature (normal temperature reached during frying) on PAH content, four 10-ml aliquots of an olive oil sample rich in both light and heavy PAHs and a peanut oil sample with a low PAH content were heated at 180°C for various times up to 25 h in test tubes with screw caps.

Oils and fried products (chips and corn- or potato-extruded snacks) were collected from six large-scale Spanish frying plants that performed continuous frying. Frying temperature, which was monitored in the frying plants, ranged from 170 to 205°C. Three homogeneous samples were collected from each plant at the middle of the daily productive cycle: 250 ml of fresh oil (sampled at ambient temperature), 250 ml of oil after frying (sampled at frying temperature), and 250 g of the fried product.

Peanut oil samples (about 250 ml) were collected from two University canteens in Udine (Italy) before and after discontinuous frying of potatoes and fish in an electrical fryer (25-liter capacity). Frying temperatures were set at 185°C for potato chips and at 160 to 180°C for fish. About 50 to 60 kg of potatoes and 20 to 30 kg of fish were fried during each frying cycle (2.0 to 2.5 h). For the fish, the oil was discarded immediately after the first cycle of frying, but for the potatoes the oil was filtered and used for a new frying cycle after adding fresh oil. Depending on oil conditions, the oil was discarded after the second frying cycle or was used for a third cycle. Different frying cycles were performed on different days, and the same oil was used for a maximum of 6 to 8 h. Oil samples were collected after different frying cycles 1, 2, and 3, corresponding to different frying times (2 to 8 h). Before samples were removed, the oil was well mixed to assure an homogeneous sample.

The same heavily contaminated olive oil sample previously used for the heating test was also used to fry potato chips with an electrical fryer (1.0 to 1.2 liter capacity). The oil was used to fry four portions of potatoes during 4 h (one 250-g portion per h). The frying time was 8 min, and the oil was held at 180°C during the entire trial period.

Fried products (potato chips and corn- and potato-extruded snacks) were purchased from the Italian market.

Sample preparation. A representative amount of each fried snack (about 50 g) was ground into small pieces with a mortar and pestle. A 10-g sample was then extracted with 30 ml of hexane in an ultrasound bath for 1 h. The mixture was then filtered through paper, and the solvent was evaporated with rotavapor (40°C).

The oil samples so obtained and oil samples collected before and after frying were processed according to the procedure suggested by Moret and Conte *(19).* A 0.250-g sample of oil was loaded into a 5-g silica solid phase extraction cartridge (20 ml; Mega Bond Elut, Varian) previously washed with 20 ml of dichloromethane, dried completely by vacuum, and conditioned with 20 ml of n-hexane. The PAHs were eluted with 8 ml of a mixture of n-hexane and dichloromethane (70:30 vol/vol) after having discharged the first 8-ml fraction containing aliphatic hydrocarbons. The PAH fraction was concentrated to a few microliters with a vacuum evaporator (Uniequip System, Martinsrieder, Munich, Germany) consisting of a centrifuge (Univapo 100 H) and a refrigerated aspirator (Uniject II) system or a nitrogen stream. The residual solvent was allowed to evaporate spontaneously at room temperature to minimize volatile PAH losses. The residue was dissolved in 100 μl of acetonitrile and injected into the HPLC apparatus.

RESULTS AND DISCUSSION

Some preliminary trials were carried out to investigate the fate of PAHs when oil was heated to frying temperatures. Aliquots of olive oil and peanut oil with different PAH loads (8.3 and 0.1 ppb BaP, respectively) were heated at 180°C for various times. No appreciable variations in PAH concentrations were observed for either oil type, even for longer heating times (25 h).

These results are in contrast with those found by Kim et al. *(12),* who reported that when soybean oil was heated to 180 and 200°C, BaP concentration increased about four- to sixfold, reaching a maximum after 10 to 20 h, and then decreased again after 50 h. However, Barranco et al. *(1)* heated olive oil at two different temperatures (150 and 200°C) for different times and found no significant differences even after 45 min of heating (light PAHs decreased slightly, likely by volatilization).

Table 1 lists the concentrations of each PAH and the sums of light PAHs (from F to P) and heavy PAHs (from benzo[a]anthracene to indeno[1,2,3-c,d]pyrene) in oil samples taken from six large-scale Spanish frying plants and from two University canteens (Udine, Italy). Because of their high volatility and consequent low recoveries, naphthalene and acenaphthene were not included in the sum of light PAHs.

All oil samples from the continuous frying plants (CF) had low PAH concentrations, with BaP concentrations well below the European Commission limit of 2 ppb (from trace to 0.7 ppb) and total heavy PAHs of 0.5 to 3.9 ppb. Peanut oil samples taken from two University canteens had a maximum BaP concentration of 0.2 ppb and total heavy PAHs concentrations between 0.2 and 2.1 ppb. Among light PAHs, the highest concentrations were found for Pa (mean, 3.6 ppb) and/or P (mean, 2.7 ppb) for oil samples from both continuous and discontinuous frying. Even though concentrations of heavy PAHs were very low, making it difficult to develop a profile, benzo[a]anthracene and benzo[b]fluoranthene were on average the prevailing heavy PAHs (Table 1).

Heat treatment did not lead to appreciable changes in

concentrations of heavy PAHs. Five different types of oil or mix were used for frying, and none of these showed marked changes in concentrations of heavy PAHs at the end of the frying process. More variability in concentrations of light PAHs (especially F and Pa) were observed before and after frying, but these variations did not follow a general trend (Pa concentration decreased after frying in five of the six continuous frying samples from large-scale plants but increased after discontinuous frying) and could not be directly attributable to frying. No relationship was found between concentrations of light PAHs and the amount of time the oil was used for batch frying (2 to 8 h).

Similar results were obtained when olive oil contaminated with high concentrations of PAHs was used to prepare French fries with a domestic electrical fryer at 180°C. Figure 1 shows PAH concentrations in the olive oil before frying, after the first frying (heating time of 30 min), and after the fourth frying (heating time of 4 h). Concentrations of heavy PAHs remained nearly constant, whereas those of light PAHs decreased slightly as heating time increased (possibly because of volatilization). These results partially confirmed those reported by Barranco et al. *(1)*, who found that when nonfatty foods (peppers) are fried in olive oil, heavy PAHs remained unaltered (even after 6 h), whereas light PAHs increased slightly.

In comparisons made before and after frying, the oil extracted from potato chips and extruded snacks had higher concentrations of light PAHs, in particular Pa and F, and in some cases higher concentrations of heavy PAHs. This slightly higher contamination level probably derives from PAHs present in the air as contaminants (generated from both outdoor and indoor sources) that because of their lipophilic nature can be easily trapped by a fatty product (fried snack). In some trials carried out in our laboratory, the potential of potato chips to easily absorb light PAHs (especially Pa, F, and P) from the atmosphere was clearly demonstrated.

Vegetables can be contaminated by PAHs, in particular Pa, F, and P, from atmospheric pollution and contaminated soil *(13, 23)*. Because potatoes are peeled and washed before processing, contamination of potato products from these environmental sources probably is negligible. Soil and atmospheric pollution probably have a greater effect on cereal grains; in some cases, extruded cereal-based snacks are toasted before frying.

Table 2 lists PAH concentrations in oils extracted from 14 fried snack samples from the Italian market. The results obtained indicate that industrial frying is not of concern for PAH formation in fried products and that vegetable oils used for large-scale frying have relatively low PAH concentrations. Refining processes reduce the concentrations of PAHs; the deodorization step reduces the concentrations of light PAHs, and bleaching with activated carbon removes the most of the heavy PAHs *(1)*. With the exception of sample S1, Pa and/or P were the most abundant light PAHs (means, 3.9 and 2.8 ppb, respectively). BaP concentrations also were below the current European Commission limit. The corn-extruded sample (S11) had the highest BaP concentration (1.2 ppb) and highest total heavy PAH concentration (6.1 ppb).

All results obtained indicate that for nonfatty foods deep frying either in continuous manner at industrial plants or in a discontinuous manner in an electrical fryer (under the conditions described) does not represent a risk for PAH formation in the frying oil or in the fried product. However, we cannot exclude the possibility that the frying process leads to some PAH formation in the fumes of cooking oils, as has been reported by others.

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