Food Chemistry 136 (2013) 576–584

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Short-term comparative study of the influence of fried edible oils intake on the metabolism of essential fatty acids in obese individuals

Carlos Ferreiro-Vera [a,b], Feliciano Priego-Capote [a,b,*], José M. Mata-Granados [a,b,c], María D. Luque de Castro [a,b]

[a] Department of Analytical Chemistry, Annex C-3, Campus of Rabanales, University of Córdoba, E-14071 Córdoba, Spain
[b] Institute of Biomedical Research Maimónides (IMIBIC), Reina Sofía Hospital, University of Córdoba, E-14071 Córdoba, Spain
[c] Sanyres I+D+i Department, Sanyres Group, E-14012 Córdoba, Spain

ARTICLE INFO

Article history:
Received 16 August 2011
Received in revised form 1 March 2012
Accepted 31 August 2012
Available online 8 September 2012

Keywords:
Deep frying
Dietary intervention
Eicosanoids
Essential fatty acids
Inflammation biomarkers

ABSTRACT

The effect of breakfast intake of fried oils containing natural antioxidants or a synthetic autooxidation inhibitor on the metabolism of essential fatty acids focused on obese individuals. Serum levels of eicosanoids were compared in individuals before and after intake of different breakfasts. Univariate descriptive analysis was used to characterise the cohort selected for this study and multivariate analysis to reveal statistical differences of normalised eicosanoids concentrations (determined by solid-phase extraction coupled to LC–MS/MS) depending on the edible oil used for breakfast preparation. The results showed that the intake of breakfast prepared with pure sunflower oil subjected to deep frying causes an effect over the eicosanoids profile that enables discrimination *versus* the rest of individuals. The effect was a significant increase in the concentration of hydroxyoctadecadienoic acid (HODE) metabolites, indicative markers of the intake of fried oils. The concentration of HODE metabolites was lower when the oil contained either natural antioxidants from olive-oil pomace or a synthetic autooxidation inhibitor as dimethylsiloxane. The comparison of the effect of fried sunflower oils with fried extra virgin olive oil shows the benefits associated to the consumption of the latter.

1. Introduction

Dietary fat plays a major nutritional role as a key source of energy despite the concerns about fat intake in developed countries (fat provides 9 kcal/g of metabolizable energy *versus* an average energy of 4 kcal/g for carbohydrates and proteins). Dietary fat is also the source of essential fatty acids (EFAs) and thus, it must be present in the diet. EFAs derive from two families of fatty acids, namely, n-6 and n-3 unsaturated fatty acids.

Linoleic acid (LA; 18:2 n-6), which accounts for more than 50% of the fatty acids in many vegetable oils (*e.g.* sunflower oil), is the main n-6 fatty acid in the diet. LA accounts for between 85% and 90% of the n-6 fatty acids in the diet with the balance coming from arachidonic acid (AA; 20:4 n-6) and γ-linolenic acid (18:3 n-6). By contrast, α-linolenic acid (LNA; 18:3 n-3) is a member of the n-3 family of fatty acids. Like LA, α-linolenic acid is the main n-3 fatty acid in the diet, although common vegetable oils contain LNA concentrations below 1%.

Essential fatty acids are important constituents of biological membranes, which surround cells and subcellular particles (McDonald & Eskin, 2006). EFAs also serve as precursors of a variety of biologically active compounds referred to collectively as eicosanoids (*e.g.* prostaglandins, thromboxanes, and leukotrienes), which are key regulators of a host of physiological reactions, including constriction and dilation of blood vessels, contraction of smooth muscle, platelet aggregation and regulation of immune and inflammatory functions (Wang & DuBois, 2007). Eicosanoids are formed through the action of a set of oxygenase-type enzymes such as cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450 monooxygenases (CYP450s). Thus, metabolism of C-20 fatty acids by COX enzymes leads to the formation of prostanoids, including prostaglandins (PGs) and thromboxanes (TXs), generating three series of compounds depending on the original fatty acid (Fitzpatrick & Soberman, 2001; Peters-Golden & Brock, 2003; Yang et al., 2006) (Supplementary Fig. 1). Eicosanoids of the 2- and 3-series are of clinical interest because they derive from

Abbreviations: AA, arachidonic acid; ASO, refined high-oleic sunflower oil enriched with an olive-pomace extract of phenols; BI, before intake; COX, cyclooxygenase; CYP450, cytochrome P450 monooxygenase; DSO, refined high-oleic sunflower oil enriched with 400 μg/mL of dimethylsiloxane; EFA, essential fatty acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LNA, α-linolenic acid; LOX, lipoxygenase; LT, leukotriene; MANOVA, multivariate analysis of variance; PC, principal component; PCA, principal component analysis; PG, prostaglandin; SFA, saturated fatty acid; SO, refined sunflower oil; SRM, selected reaction monitoring; TX, thromboxane; VOO, extra-virgin olive-oil.

* Corresponding author at: Department of Analytical Chemistry, Annex C-3, Campus of Rabanales, University of Córdoba, E-14071 Córdoba, Spain. Tel./fax: +34 957218615.
E-mail address: q72prcaf@uco.es (F. Priego-Capote).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.08.081

two competitive pathways (n-3 and n-6), which could justify their opposite functions, as suggested by Schmitz and Ecker (2008). Thus, 2-series eicosanoids produced from arachidonic acid possess pro-inflammatory, pro-aggregating, vasoconstriction action and immunosuppressive properties, as recently reported by Wang and DuBois (2010). On the other hand, eicosanoids of the 3-series produced from eicosapentaenoic acid have anti-inflammatory, anti-aggregating, vasodilatory and anti-arythmic actions and immunomodulating properties (González-Périz & Clària, 2010; Groeger et al., 2010).

Lipoxygenases convert AA, LA and other PUFAs into bioactive metabolites such as leukotrienes (LTs), hydroxyeicosatetraenoic acids (HETEs) and hydroxyoctadecadienoic acids (HODEs). LOX-catalysed products, LTs, HETEs, and HODEs also exert profound biological effects on inflammation processes being involved in the development and progression of specific human cancers such as colorectal or pancreatic cancer (Xian-Zhong, Wei-Gang, & Thomas, 2001).

Deep fat frying is one of the most common processes used worldwide for preparation of cooked food. Complex patterns of oxidative and thermolytic reactions take place in fats and oils during heating and deep-fat frying, including polymerisation, hydrolysis, isomerization, and cyclisation (Dobson, Christie, Brechany, Sebedio, & Le Quere, 1995; White, 1991; William & Dobson, 2000). Secondary oxidation products are mainly oxidised triglyceride monomers, dimers, and polymers that define the thermal oxidised compounds of the polar material fraction (Dobarganes, Márquez-Ruiz, & Velasco, 2000; Romero, Bastida, & Sánchez-Muniz, 2006). Oxidation modifies the organoleptic properties of oils and affects their shelf life, mainly owing to formation of oxidation products of cholesterol and phytosterols. Degradation results in loss of nutritional value of food as well as changes in its physiological properties (Saguy & Danaa, 2003; Tyagi & Vasishtha, 1996), which cause rejection from the consumers and losses to the target industries, as a result.

The presence of antioxidants, naturally existing in (or added to) oils, exerts beneficial effects by avoiding or delaying oxidation during frying of compounds such as sterols, fatty alcohols, triterpenic dialcohols and unsaturated fatty acids. As happens with other food additives, natural antioxidants such as phenol compounds have demonstrated an antioxidant activity superior to that of synthetic oxidation inhibitors; therefore, there is an increased trend to replace the latter with natural antioxidants. The enrichment of edible oils with phenols protects them, for example, against oxidation that means better oil quality and prevention from the formation of toxic products such as cholesterol oxides (Dobarganes & Márquez-Ruiz, 2006).

The aim of the present research was to evaluate the nutritional impact of the intake of four breakfasts prepared with oils subjected to deep frying on the profile of eicosanoids in human serum. The target metabolites were selected taking into account the direct implication of their metabolism in the inflammatory cascade. Vegetable edible oils with natural or added content of antioxidants were selected for this purpose. Breakfast muffins made with these oils were ingested by 26 obese volunteers who consumed the different muffins throughout eight weeks. Liquid-chromatography coupled to mass detection was used to monitor eicosanoids profile in human serum extracted from all individuals at three different sampling times.

2. Materials and methods

2.1. Oils and heating procedure

The edible oils were: (1) extra-virgin olive oil (VOO) as such with a total natural phenols concentration of 400 mg/L, expressed as caffeic acid, 70.5% monounsaturated fatty acids (MUFAs), 11.1% PUFAs, 18.4% saturated fatty acids (SFAs); (2) refined high-oleic sunflower oil enriched with an olive–pomace extract of phenols (ASO) up to 400 mg/L, also expressed as caffeic acid, 76.7% MUFAs, 17.6% PUFAs and 5.8% SFAs; (3) refined high-oleic sunflower oil enriched with 400 mg/L of dimethylsiloxane as a synthetic oxidation inhibitor (DSO), 71.8% MUFAs, 18.0% PUFAs and 10.2% SFAs; and (4) pure refined sunflower oil (SO), 34.3% MUFAs, 58.3% PUFAs and 7.3% SFAs. Koipesol (SOS Cuétara S.A., Madrid) provided the oils for subsequent enrichment in the laboratory. The composition of phenolic compounds in VOO and in the extract from olive pomace is listed in Supplementary Table 1 (Girón, Ruiz-Jimenéz, & Luque de Castro, 2009).

Two litres of the target oil was placed in a stainless-steel deep fryer without cover (Fagor F-206, Barcelona, Spain). The oil was heated at 180 ± 5 °C for 5 min 10 times every day for two days (total heating cycles: 20) with 30 min cooling intervals between heating cycles. This protocol simulates a conventional use of oils for frying.

2.2. Subjects and samples

Twenty six obese individuals with a body mass index between 30 and 40 kg/m^2 formed the cohort in this study. All of them gave their informed consent and underwent a comprehensive medical history, physical examination and clinical chemistry analysis before enrolment. Participants with evidence of kidney, pancreas, lung, liver or thyroid disease were excluded. All subjects were non-diabetics, non-smokers, without clinical manifestations of cardiovascular disease and off treatment. The target cohort was composed by 17 post-menopausal women, age 48–70 years, and 9 men, age 39–70 years. None of the subjects was taking medication or supplementary vitamins with influential effect on serum lipidome.

All volunteers received four breakfasts in muffin format prepared with the four different oils (0.45 mL of oil per kilogram of body weight), previously subjected to the simulated frying process. The administration of each breakfast was held at randomization and cross following a Latin square design, which increased the power of the study. The volunteers ate each breakfast every two weeks (4 oils, 8 weeks). All steps from blood extraction to analysis were performed in compliance with the guidelines dictated by the World Medical Association Declaration of Helsinki (2004), which were supervised by the ethical review board of Reina Sofia Hospital (Córdoba, Spain) that approved the experiments.

2.3. Serum extraction

Blood extraction was planned just before breakfast intake and 2 and 4 h after it. Venous blood was collected into evacuated sterile tubes for whole blood haematology determination Vacutainer® (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 1500*g* 4 °C for 10 min to isolate the serum fraction (processing within 2 h after collection), which was placed in a plastic ware tube and stored at −80 °C until analysis.

2.4. Analytical method

A fast and automatic method for quantitative analysis of PGE_1, PGE_2, PGE_3, PGD_2, $PGF_{2\alpha}$, 15keto-$PGF_{2\alpha}$, TXB_2, 9-HODE, 13-HODE, 5-HETE, 8-HETE, 11-HETE, 12-HETE and 15-HETE in serum samples was developed. The approach is based on a hyphenated system composed by an SPE workstation (Prospekt-2 unit) on-line coupled to a liquid chromatograph–triple quadrupole-tandem mass-spectrometer with an ESI source (6410 QqQ Agilent, Palo Alto, CA, USA). Briefly, 100 μL of human serum is injected in the analytical