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Micronutrient-Enriched Rapeseed Oils Improve the Brain Oxidant/Antioxidant System in Rats Fed a High-Fat Diet

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Supporting Information

ABSTRACT: The main proposal of this study was to evaluate in vivo whether micronutrient-enriched rapeseed oils obtained using different crushing and refining procedures and characterized by different quantities and qualities of micronutrients (optimized oils) could have any beneficial effect on the antioxidant status of the brain. Sprague−Dawley rats were fed a high-fat diet for 4 weeks. The lipid source consisted of 20% optimized rapeseed oils with different quantities and qualities of micronutrients. The control group received traditional refined rapeseed oil. The experimental optimized oils decreased lipid peroxidation and increased endogenous antioxidant status in parallel with the enhancement of micronutrients. No alteration in acetylcholinesterase activity was induced by the high-fat diet in any experimental group. These results indicate that a regular intake of optimized rapeseed oils can prevent oxidative stress, providing evidence that optimized rapeseed oils could be a functional food with potentially important neuroprotective properties.

KEYWORDS: rapeseed oil, micronutrients, dietary antioxidants, lipid peroxidation, reduced-glutathione, antioxidant enzymatic activities, high-fat diet, cognitive diseases, acetylcholinesterase activity

INTRODUCTION

The brain is particularly vulnerable to oxidative stress because of its high oxygen utilization, high content of polyunsaturated fatty acids, the presence of redox-active metals (Cu, Fe), and a low reserve of antioxidant defenses.[1] An unbalanced overproduction of reactive oxygen species (ROS) may give rise to oxidative stress, which can lead to neuronal death by apoptosis or necrosis.[2] A large volume of literature indicates a correlation between ROS production, the induction of apoptosis (or necrosis), and the pathogenesis of neurodegenerative disorders.[3] Many epidemiological studies[4,5] report that dietary antioxidants can influence the incidence of neurodegenerative disorders such as dementia, including Alzheimer's (AD) and Parkinson's diseases (PD).

Because the richest food sources of tocopherols and polyphenols are vegetables, seed oils are currently receiving considerable attention for their recently reported health benefits in relation to neurological disorders,[6] as well as cardiovascular diseases.[7] Against this background, the past two decades have seen an expansion in the production of low erucic acid rapeseed oil, which has the potential to improve consumers' health both because of its potent antioxidant effects and because it is an important source of n-3 fatty acids (α-linolenic acid). Rapeseed seeds are characterized by a high content of bioactive compounds such as tocopherol, which is present in all its isomeric structures: α, β, δ, and γ; coenzyme Q (CoQ); phytosterols and phenols.[8−10] These bioactive compounds have a potent antioxidant activity, characterized by their ability to scavenge or neutralize ROS directly. The most effective protection for animal cells may be obtained by a combination of antioxidants. Different bioavailable antioxidants may work in concert to upgrade the complex antioxidant network necessary to sustain cellular function.[11] Previous studies have shown that polyphenols act synergistically with vitamins C and E[12] and β-sitosterol[13] and that α-tocopherol synergizes with γ-tocopherol[14] to produce a larger increase in antioxidant capacity than that provided by each compound separately. Studies in humans indicate that the combined intake of the eight different forms of tocopherol reduces oxidative stress and inflammation to a greater degree than that of α-tocopherol alone.[15] However, the industrial processes currently used in the production of edible oils (extraction and refining) are not optimally suited to the satisfactory preservation of these minor nutritional compounds.

The present investigation is part of the European Union OPTIM'OILS project ("Valorisation of healthy lipidic-micronutrients by optimizing food processing of edible oils and fats"), the main objective of which is to improve the processes currently used for seed oil production to preserve the levels of micronutrients and to develop new healthy oils to be marketed in the European Union (EU). By adopting different extraction procedures three optimized rapeseed oils with increasing micronutrient content were obtained. As part of this EU project nutritional studies have been carried out to evaluate the potential health effects of these optimized oils. The objective of the present study was to assess in vivo whether optimized rapeseed oils could affect the antioxidant defenses of the brain. Diets containing 20% micronutrient-enriched oils as lipid source were administered to rats for 4 weeks. Previous experimental studies have shown that a high-fat diet (HFD) enhanced neuronal oxidative stress and induced cognitive impairment and changes in brain acetylcholinesterase

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Table 1. Micronutrients in Different Rapeseed Oils

	mg/kg oil				
	RAP REF	RAP 1 DH-COOKP	RAP 2 EXT-HEX	RAP 3 TSE-BETH	RAP 4 TSE-BETH+PL
sterols	7913	7629	9003	13511	16059
tocopherols	614	708	750	1677	1677
phospholipids	0	105	428	0	752
phenols (in equiv, caffeic acid)	81	386	352	495	469
CoQ9 + CoQ10	85.4	57.6	269.5	262.9	272.6

activity (AChE).[16,17] AChE is considered to be a good indicator of cholinergic activity essential for the regulation of cognitive function.

The interactions among these factors play an important role in the pathophysiology of age-related neurodegenerative diseases. Enzymatic [superoxide dismutase (SOD), glutathione peroxidase (GPX), and nonenzymatic defense mechanisms (reduced glutathione, GSH)] as well as lipid peroxidation and AChE activities were all measured in rat brain homogenate. The study aimed to investigate whether these micronutrient-enriched oils can play a neuroprotective role by decreasing oxidative stress in the brain.

MATERIALS AND METHODS

Optimized Oils. Rapeseed oils obtained by different technical procedures (optimized oils) were supplied by CREOL and ITERG (Pessac-France). Three rapeseed oil extraction procedures were used to obtain high levels of micronutrients: (1) cooking at 100 °C for 60 min and pressing after dehulling or flaking (DH-COOKP); (2) hexane extraction of the partially defatted cake obtained after cold pressing (EXT-HEX); and (3) ethanol extraction of the partially defatted cake obtained after twin-screw extrusion (TSE-BETH). A fraction of the rapeseed oil obtained by TSE-BETH at 80 °C was enriched with 0.3% gum phospholipids (PL).

Micronutrient Determination. Sterols were determined by Asociacion de Investigacion de la Industria Agroalimentaria (ANIA, Valencia, Spain) using gas chromatography (GC) according to the EN ISO 12228:1999 standard.[18] Briefly, the sample was saponified, and the sterols were isolated by thin-layer chromatography. The sterol fraction was silanized and analyzed by GC (Fison GC 8000 Top Series, Kent, U. K.). A capillary column (50 m × 0.25 mm i.d. × 0.1 μm) with a stationary phase of SE-54 (Supelco Inc., Bellefonte, PA) was used. The temperature of the injector was 280 °C. Chromatographic conditions were 240 °C for 1 min, increased to 260 °C at a rate of 4 °C/min, held for 20 min, then increased to 280 °C at a rate of 5 °C/min, and held for 10 min. Betuline was used as an internal standard.

Tocopherols and phenols were analyzed by AgroParisTech (Massy, France). Tocopherols were quantified according to the official NF ISO method 9936[19] by high-performance liquid chromatography (HPLC) (Waters, Milford, MA) equipped with 2996 UV−vis photodiode array detector. Analysis of tocopherols was performed using a Supelcosil LC-Si column (25 cm × 4.6 mm i.d. × 5 μm) thermostated at 25 °C, with the corresponding analysis guard column (2 cm × 4 mm i.d. × 5 μm, Supelco), and with detection at 298 nm. The HPLC mobile phase consisted of hexane/isopropanol (99.5:0.5, v/v), eluted at a flow rate of 1 mL/min.

Phenol compounds were extracted from oils by solid-phase extraction (SPE) using a sorbent Oasis HLB cartridge. Total phenolic compounds were quantified by the Folin−Ciocalteu method, according to a protocol adapted from Escribano-Bailon et al.[20]

Phospholipids were determined by ULg - Gembloux Agro-Bio Tech (Gembloux, Belgium) using HPLC (Agilent Technologies 1200 series 190,Diegem, Belgium) associated with an Evaporative Light Scattering Detector (ELSD) (Alltech 3300 -Lokeren, Belgium) according to Rombaut et al.[21] The column was a Prevail Silica 3 μm (150 mm × 3 mm) used with a 5 μm (7.5 mm × 3 mm) Prevail Silica guard column (Alltech Associates Inc., Lokeren, Belgium). Analyses were achieved at 40 °C and a flow of 0.5 mL/min, with automatic injection. The elution program was a linear gradient with chloroform/methanol/triethylamine buffer (87.5:12:0.5, v/v/v) at t = 0 min to 28:60:12 (v/v/v) at t = 16 min. The vaporization temperature of the ELSD was set at 85 °C, the gas (air) flow at 1.4 L/min, and the gain at 1 (impactor mode: off).

The coenzyme Q fraction was isolated by SPE on an amino phase eluting with a mixture of heptane/ethyl ether (80:20, v/v). The organic solvent was evaporated under nitrogen, and the residue, dissolved in a mixture of acetonitrile/tetrahydrofuran (90:10, v/v), was analyzed by reverse-phase HPLC (Dionex Ultimate 3000 series, Sunnyvale, CA) with a mass detector as described by Acuña et al.[10] The micronutrient composition of the different oils is shown in Table 1.

Animals and Diets. Sprague−Dawley rats weighing 150−170 g were obtained from Harlan-Nossan (Milano, Italy). The animals were housed in stainless steel cages (two rats per cage) at a controlled room temperature of 24 °C, under a 12:12 light/dark cycle. After 1 week of acclimatization, they were divided into five groups (n = 10 rats per group). They were allowed ad libitum access to food and tap water. All groups received the same basal synthetic diet containing 18% casein, 0.3% *dl*-methionine, 36.5% rice starch, 15% sucrose, 3% fiber, 3.5% salt mixture (AIN-76; see the Supporting Information), 3.5% vitamin mixture (AIN-76; see the Supporting Information), 0.2% choline chloride, 20% fat. The lipid fraction was provided by different rapeseed oils. Experimental diets were administered for 4 weeks. Group 1 received the reference refined rapeseed oil (RAP REF) and was the control group; group 2 received oil obtained by cooking and pressing (DH-COOKP); group 3 received oil obtained by hexane extraction after extrusion (EXT-HEX); group 4 received oil obtained by ethanol extraction after extrusion (TSE-BETH); and group 5 received oil obtained by TSE-BETH but enriched with gum phospholipids. The diets were prepared under vacuum and stored in the dark at 4 °C. Animals were weighed twice each week, and food intake was recorded weekly to monitor the animals' growth rate and the potential effect of diet on food consumption. At the end of the experiment rats were deeply anesthetized with a mixture of medetomidine and ketamine (1:1v/v) and perfused through the left ventricle of the heart with 30 mL of PBS. The brains were immediately excised, weighed, frozen in liquid nitrogen, and stored at −80 °C until further processing. All animal experiments were performed according to European Community Council Directive 86/609/ECC and Italian legislation (DL 116/92) on animal experimentation.

Chemicals and Reagents. Trichloroacetic acid, 2-thiobarbituric acid (TBA), acetylthiocholine (AcThCh), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), betuline, bovine serum albumin, butylated hydroxytoluene, 1-methylimidazole, *N*-methyl-*N*-(trimethylsilyl)heptafluorobutyramide, and sodium phosphate buffer were obtained from Sigma-Aldrich (St. Louis, MO). Chloroform, ethanol, methanol, hexane,

Table 2. Body Weight Gain, Food Intake, and Food Efficiency Ratio of Different Optimized Rapeseed Oil Groups

treatment	body weight gain (g)	food intake (g/day)	food efficiency ratio
group 1 (refined oil)	135.87 ± 14.00	18.1 ± 1.3	0.27 ± 0.05
group 2 (DH-COOKP)	135.00 ± 15.83	18.6 ± 1.6	0.26 ± 0.05
group 3 (EXT-HEX)	133.12 ± 12.91	18.3 ± 1.6	0.26 ± 0.04
group 4 (TSE-BETH)	132.28 ± 21.35	17.9 ± 1.3	0.26 ± 0.03
group 5 (TSE-BETH+PL)	132.44 ± 21.27	18.1 ± 1.8	0.26 ± 0.04

heptane, ethyl ether, acetonitrile, tetrahydrofuran, isopropanol, and acetone were of analytical grade and provided by Merck (Darmstadt, Germany).

Lipid Peroxidation. Brain samples were homogenized in ice-cold 50 mM phosphate buffer (pH 7.4) in a glass homogenizer. The level of lipid peroxidation was evaluated according to the method of Yagi,[22] which determines the thiobarbituric acid reactive substance (TBARS) content in brain homogenates. In general, 0.5 mL of 10% brain homogenate was mixed with 10% (w/v) trichloracetic acid to precipitate the proteins. The supernatant fraction was incubated with thiobarbituric acid and butylated hydroxytoluene solution at 80 °C for 40 min. After cooling, the absorbance was spectrophotometrically determined at 535 nm, and the concentration of TBARS was calculated using extinction coefficient $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$.[22] The data are expressed as micromoles of TBARS per gram of wet weight of brain.

Antioxidant Enzyme Activities. GPX (EC. 1.11.1.9) activity was evaluated in brain indirectly by a coupled reaction with glutathione reductase (GR) using a commercial kit (Cayman, Ann Arbor, MI). Briefly, oxidized glutathione, produced on reduction of hydroperoxide by GPX, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH is accompanied by a decrease in absorbance at 340 nm.

SOD (EC 1.15.1.1) was determined using a commercial kit (Cayman) that utilizes a tetrazolium salt formation monitored at 450 nm for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to achieve 50% dismutation of superoxide radical.

Glutathione Redox Status. Total glutathione (GSX) and oxidized glutathione (GSSG) levels were evaluated at 415 nm by the DTNB−glutathione reductase (GR) recycling assay using a commercial kit (Cayman). Reduced glutathione (GSH) was calculated by subtracting GSSG from the GSX concentration.

AChE Activity Measurement. Total AChE activity was determined by a slight modification of the method of Ellman et al.[23] on brain homogenate (prepared with phosphate buffer, pH 7.4, using tissue-buffer, ratio of 1:20 in a Polytron apparatus (PT3000), for 1 min, at 20000 rpm). The samples (corresponding to 5 μL of homogenate) were incubated in 39 mM phosphate buffer, pH 7.2, containing 0.21 mM DTNB in a final volume of 1.4 mL, at 25 °C for 30 min (in duplicate). AcThCh (0.56 mM) was used as substrate. The absorbance at 412 nm was measured in a Perkin-Elmer λ 2 spectrometer. The enzymatic activity was expressed as nanomoles of AcThCh hydrolyzed per minute per milligram of protein.

Protein Determination. Protein concentration of the brain homogenates was performed according to the method of Lowry et al.[24] using bovine serum albumin as standard.

Statistical Analysis. All data are presented as the mean ± SD. The data were evaluated by one-way ANOVA. Post hoc Tukey's test was performed to evaluate differences between groups. Correlations (r) between different oxidative markers were performed using Pearson's product−moment correlation coefficient. SPSS was used for the statistical analyses. Statistical significance was considered at $p < 0.05$.

RESULTS

Animals and Treatments. The mean body weights of the rats were similar among groups at the beginning of the dietary treatment and increased gradually throughout the experiment. At the end of treatment no significant differences were observed in weight gain and food intake in rats fed diets rich in optimized oils compared with controls, as shown in Table 2. Likewise, the mean brain weight was similar among experimental groups, ranging between 1.03 and 1.14 g (average = 1.06 ± 0.06 g).

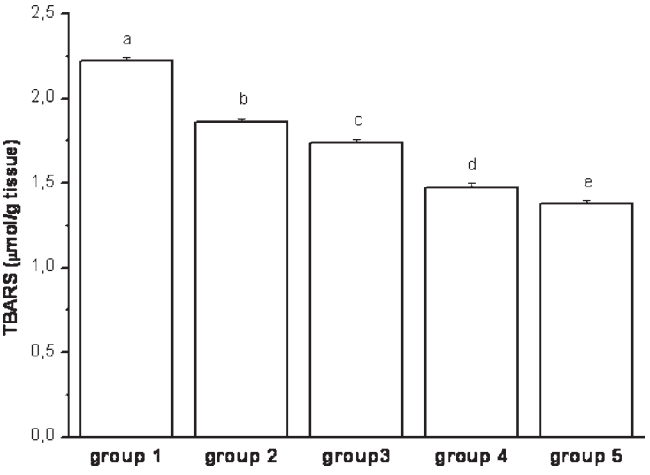

Figure 1. Effect of feeding optimized oils on brain TBARS. Group 1, total refined rapeseed oil; group 2, cooking and pressing after dehulling or flaking (DH-COOKP); group 3, hexane extraction after cold pressing (EXT-HEX); group 4, ethanol extraction after twin-screw extrusion (TSE-BETH); group 5, a fraction of the rapeseed oil obtained by TSE-BETH was enriched with gum phospholipids (PL). Values represent the mean ± SD of five samples. Data were analyzed by one-way ANOVA. Tukey's test was used when differences in treatment were present. Values with different letters are significantly different ($p < 0.05$).

Brain Oxidative Markers. The brain TBARS levels, a biomarker of lipid peroxidation, were significantly influenced by feeding with micronutrient-enriched oils. The experimental diets caused a significant decrease in TBARS values, which was in parallel with the increase in micronutrient levels in the oils. Brain TBARS values were most reduced (~30%) in group 5. PL enrichment affected the TBARS content, with significant differences ($p < 0.01$) being observed between groups 4 and 5, as shown in Figure 1.

In contrast, GSH levels increased with the enhancement of micronutrient oil content, although the increase in optimized oil groups compared with controls began to be significant ($p < 0.001$) from group 3, as shown in Figure 2. No differences were observed between groups 4 and 5, showing that the addition of PL did not affect the brain GSH content. The regression coefficient test indicated a significant correlation between the enhancement of GSH and the decrease in TBARS ($r = -0.93$; $p < 0.01$).

Brain Enzymatic Activities. To study the effect of optimized oils on brain enzymatic activity of rats fed high-fat diets, SOD and

GPX activities were evaluated. As shown in Figure 3 both enzymatic activities were higher in all groups fed optimized rapeseed oils than in controls. However, post hoc comparisons indicated that the increase was significant only in the groups fed optimized rapeseed oils, with the largest content of bioactive compounds, namely, groups 4 and 5. In addition, the increase induced by the addition of PL (group 5 vs group 4) was statistically significant ($p < 0.01$) only for SOD enzymatic activity. A negative correlation was found between TBARS and SOD ($r = -0.90$; $p < 0.01$) and GPX ($r = -0.90$;$p < 0.01$) activities.

AChE Activity in Brain. To evaluate whether the optimized oils had any effects on brain function we measured AChE activity: the results are reported in Table 3. No significant differences were observed between optimized groups and controls, indicating that micronutrient-enriched oils had no effect on AchE activity.

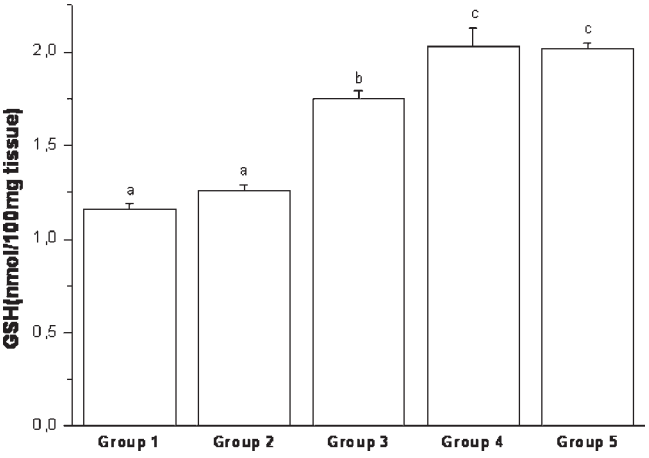

Figure 2. Effect of feeding optimized oils on reduced glutathione (GSH). Group 1, total refined rapeseed oil; group 2, cooking and pressing after dehulling or flaking (DH-COOKP); group 3, hexane extraction after cold pressing (EXT-HEX); group 4, ethanol extraction after twin-screw extrusion (TSE-BETH); group 5, a fraction of the rapeseed oil obtained by TSE-BETH was enriched with gum phospholipids (PL). Values represent the mean ± SD of five samples . Data were analyzed by one-way ANOVA. Tukey's test was used when differences in treatment were present. Values with different letters are significantly different ($p < 0.05$).

DISCUSSION

Many studies indicate that dietary antioxidants may be important for the prevention of age-related neurodegenerative disorders such as AD.[25,26] In our previous paper, we demonstrated that rapeseed oils enriched in micronutrients on account of the different crushing and soft-refining procedures decrease CVD risk factors in rats fed a high-fat diet (HFD).[27] A decrease in lipids and an increase in antioxidant defenses in both plasma and liver have been observed to be correlated with the enhancement of micronutrients in optimized oils.

In the present paper, we report that optimized rapeseed oils also have beneficial effects on oxidative status in the brain.

The intake of micronutrient-enriched oils reduces brain TBARS levels in parallel with the increase in the content of micronutrients, and the reduction in lipid peroxidation occurs in parallel with the increased GSH content. GSH is one of the most common biological antioxidants, and its functions include the removal of free radicals such as superoxide anions and alkoxy radicals and acting as a substrate for GPX and GR. Evidence has been presented that the neuronal defense against H_2O_2, which is the most toxic molecule for the brain, is mediated primarily by the glutathione system.[28] GSH decreases during the aging process and may underlie a number of changes that occur in normal aging and in the onset of various diseases.[29,30] The most valuable evidence of an altered glutathione metabolism as an important factor contributing to the pathogenesis of a neurodegenerative diseases has been found in PD.[31] The increase in GSH and the concomitant decrease in TBARS that we observed indicate that the brain is also a target of micronutrients and that these can affect its oxidative status. Previous studies have reported that in rats CoQ10, vitamin E, and polyphenols cross the blood−brain barrier and that their endogenous content increases in a dose-dependent manner.[32,33] Our data show that PL work as antioxidants, because their addition to TSE-BETH oil (group 5) further and significantly decreased the TBARS content.

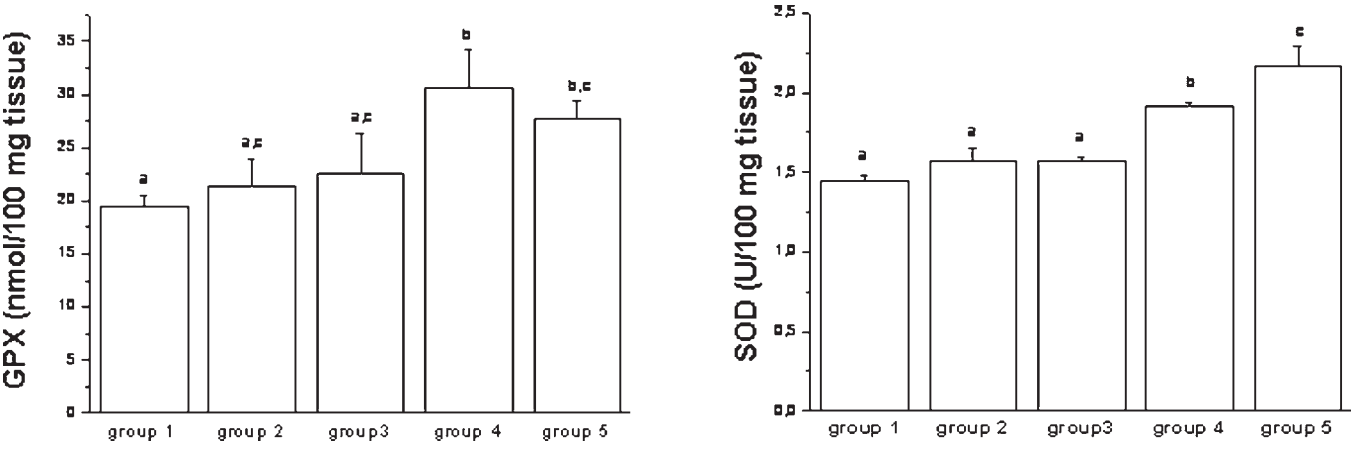

Figure 3. Effect of feeding optimized oils on superoxide dismutase (SOD) and glutathione peroxidise (GPX). Group1, total refined rapeseed oil; group 2, cooking and pressing after dehulling or flaking (DH-COOKP); group 3, hexane extraction after cold pressing (EXT-HEX); group 4, ethanol extraction after twin-screw extrusion (TSE-BETH); group 5, a fraction of the rapeseed oil obtained by TSE-BETH was enriched with gum phospholipids (PL). Values represent the mean ± SD of five samples. Data were analyzed by one-way ANOVA. Tukey's test was used when differences in treatment were present. Values with different letters are significantly different ($p < 0.05$).

Table 3. AChE Activity in the Brain of Rats Fed Different Optimized Rapeseed Oils[a]

	group 1 (refined oil)	group 2 (DH-COOKP)	group 3 (EXT-HEX)	group 4 (TSE-BETH)	group 5 (TSE-BETH + PL)
AChE (nmol/h/mg protein)	45.2 ± 2.5	45.5 ± 2.1	44.7 ± 2.2	47.7 ± 1.6	43.4 ± 2.9

[a] Results represent the mean ± SD of six animals.

Phospholipids could act through two mechanisms: (i) directly as antioxidants or (ii) by stimulating the brain's antioxidant system. The antioxidant activity of PL in the brain has also been reported in a different experimental model. Aabdallah et al. observed that lecithin administration decreased lipid peroxidation in a transient brain ischemia model in rat.[34]

The antioxidant defenses also comprise various antioxidant enzymes such as SOD and GPX. SOD is one of the major enzymes of the endogenous antioxidant defense system that catalyzes the dismutation of superoxide anions, whereas GPX catalyzes the reduction of H_2O_2 and hydroperoxides to nontoxic products.

In our study, all optimized oils tended to enhance the enzymatic antioxidant system, although the enhancement reached significant levels only in the groups with the highest micronutrient levels, namely, groups 4 and 5. The significant increase in SOD and GPX activities probably indicates that optimized oils can either increase the expression and/or activity of SOD and GPX or reduce the extent of oxidative stress, mitigating the depletion of SOD and GPX, or both effects may be present.

The putative relative contribution of each micronutrient is difficult to establish because the optimized oils contain different groups of bioactive compounds. On the other hand, it has also been reported that complex mixtures of phytochemicals can provide more health benefits through a combination of additive and/or synergistic effects than the intake of excessive amounts of a single antioxidant.[35] Furthermore, the intake of natural antioxidants in their own matrix could play an important role in controlling bioactivity.

To evaluate whether optimized oils affect the cognitive system, we measured the activity of AChE. This enzyme hydrolyzes the neurotransmitter acetylcholine (ACh) in the synaptic cleft of cholinergic synapses and neuromuscular junctions. AChE responds to various insults including oxidative stress. Studies have demonstrated that alterations in AChE activity may be induced by an increase in the formation of free radicals, which can provoke lipid peroxidation of cerebral membranes and cause changes in the conformational state of the AChE molecule and consequently in its activity.[36]

Kaizer et al.[19] reported that in rats fed a HFD an increase in plasma lipids and in hepatic lipid peroxidation was accompanied by an alteration of AChE activity in different brain regions. Other experimental models of cognitive disorders associated an increase in brain TBARS levels with an increase in AChE and found that molecules able to reduce oxidative stress reversed the variation in AChE.[36] Although the antioxidant micronutrients present in optimized oils decreased TBARS levels, they did not affect brain AchE activity, which was similar in all experimental groups. On the other hand, despite their intake of a HFD, rats fed traditionally refined oil (group 1) showed no increase in TBARS or AChE compared with rats fed a laboratory chow diet (data not shown). Besides, we previously[34] observed that traditionally refined rapeseed oil induced a hypolipidemic effect accompanied by a decrease in TBARS in the plasma of rats fed a HFD and that the decrease in CVD risk factors such as dyslipidemia and oxidative stress could also contribute indirectly to the beneficial effects of optimized rapeseed oils on the brain. In fact, current evidence indicates an association between several CVD risk factors and cognitive decline.[37]

In conclusion, the present study shows that optimized rapeseed oils that naturally contain high amounts of antioxidants could be a promising nutritional approach for the prevention of cognitive impairment as they improve the antioxidant status in the brain. Further studies will be required to confirm the beneficial effects of optimized rapeseed oils in the prevention of neurodegenerative diseases in which oxidative stress plays a key role in triggering a cascade of events that lead to neuronal malfunctions.

■ ASSOCIATED CONTENT

Supporting Information. The composition of the AIN-76 mixture of salt and vitamins (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVATIONS USED

AD, Alzheimer's disease; AChE, acetylcholinesterase; AcThCh, acetylthiocholine; GPX, glutathione peroxidase; GSH, reduced glutathione; HFD, high-fat diet; SOD, superoxide dismutase; PL, phospholipids; TBARS, thiobarbituric acid reactive substance.

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