

## Effects of Frying Fat and Preparation on Carp (*Cyprinus carpio*) Fillet Lipid Composition and Oxidation

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### Abstract

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We investigated the changes in omega-3 enriched carp fillets caused by pan frying. The investigated characteristics were fat uptake, fatty acid (FA) composition, and oxidation. Four different fats were used and fillets were fried plain or battered. The fillet fat content increased during frying and FA composition in the fillets reflected the composition of the frying fat. Frying with sunflower oil negatively influenced the nutritional value by decreasing the n-3/n-6 ratio in the fillets. Frying with rapeseed oil preserved the favourable n-3/n-6 ratio without increasing the saturated fatty acids (SFA). Frying with lard and butter preserved the n-3/n-6 ratio but increased the SFA content. No increased oxidation occurred with the use of rapeseed oil. We concluded that using rapeseed oil for fish seemed to preserve the nutritionally valuable composition best.

**Keywords:** DHA; EPA; TBARS; nutritional quality

**List of abbreviations** (in the order of appearance): FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; omega 6 = n-6; omega 3 = n-3; FAME = fatty acid methyl esters; TBARS = thiobarbituric acid reactive substances; MDA = malondialdehyde; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DPA = docosapentaenoic acid

The inclusion of fish and fish products in the human diet at least twice a week is recommended from the nutritional point of view, due to the high content of omega 3 polyunsaturated fatty acids (n-3 PUFA) in marine and fresh water fishes (STEFFENS 1997; EFSA 2009; EFSA Panel on Dietetic Products 2010). The n-3 PUFA are well known to have positive effects on different health aspects such as, for example, metabolic syndrome, obesity, diabetes, arteriosclerosis, neural and brain development (STORLIEN *et al.* 1997; CONNOR 2000; WILLIAMS 2000; CALDER & GRIMBLE 2002; RICHARDSON 2006). However, today's Western diet contains an increasing content of omega 6 (n-6) PUFA, leading to increased

occurrence of cardiovascular and atherosclerotic diseases, type 2 diabetes and obesity (SIMOPOULOS 1999; AILHAUD *et al.* 2006).

Both n-6 and n-3 PUFA are precursors of a variety of divers chemical messengers, regulating factors, and eicosanoids like prostaglandins, leukotriens and related substances, which play important roles in the inflammation and regulation of immunity (KINSELLA 1988; HORROBIN 1995; CALDER 1997, 2001). Since metabolites of n-3 and n-6 PUFA have different, often opposing biological actions and potencies, the intake ratio between n-3 and n-6 PUFA is important (CALDER 2001; SCHMITZ & ECKER 2008). There is a competition between the enzymes involved in the

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elongation and desaturation of n-3 and n-6 FA as well as for the production of their respective metabolites, which will lead to increased n-6 metabolites if n-6 FA are present in a much higher proportion than the n-3 ones. In addition, high amounts of 18:2n-6 decrease the conversion of 18:3n-3 to 20:5n-3 (SIMOPOULOS 2000). The values of 1 to 4 for n-6/n-3 in the diet have been recommended while in today's Western diets this ratio is often 15 to 20 (SIMOPOULOS 2002a).

It is known from earlier studies that the type of preparation has some influence on the final fatty acid (FA) composition of the product and that especially during frying the fat used will have some influence on the FA composition of the final product (ÅGREN & HÄNNINEN 1993; RAMIREZ *et al.* 2005; SIOEN *et al.* 2006; WEBER *et al.* 2008). The effects are partly due to the heat which can cause oxidation, but also due to the fact that during frying the exchange of water and fat will occur in which some water from the surface layer of the fried object is exchanged with the frying fat (CANDELA *et al.* 1998; DOBARGANES *et al.* 2000). In addition, when dealing with an object like fatty fish whose fat, due to its high unsaturation, has a very low melting point and a higher liquidity than the frying fat, a loss may also happen of the original fat due to leakage. Furthermore, the possibility of fat leakage may also depend on the fish fat content (SIOEN *et al.* 2006).

Most of the work until now has been done in deep-fat frying whereas only few researchers investigate pan frying (MAI *et al.* 1978; AL-SAGHIR *et al.* 2004; SIOEN *et al.* 2006; HAAK *et al.* 2007). On the other hand, pan-frying is one of the most frequently used ways to prepare fish or fish products at home. Therefore, the knowledge of what happens during this process is important when giving nutritional recommendations to consumers.

Many investigators have fried fish in sunflower oil and found increased proportions of 18:2n-6 influencing the n-3/n-6 ratio enormously (GLADYSHEV *et al.* 2006, 2007). On the other hand, in a study on catfish fried in canola oil 18:3n-3 was increased and thereby also the n-3/n-6 ratio leading to a more valuable nutritional composition (WEBER *et al.* 2008).

In the Czech Republic and central Europe, the carp is a commonly consumed fish species and recently an omega 3 rich carp has been patented and is sold on the market as a product with positive effects on health, based on the results from a clinical study (ADÁMKOVÁ *et al.* 2011; MRÁZ *et al.* 2011). The intake of two 200 g portions of this carp rich in n-3 per week improved the blood lipid values of patients

recovering from ischemic heart disease (ADÁMKOVÁ *et al.* 2011).

There are only very few papers concerning the preparation of carp before the consumption at home. Some authors investigated cooking methods without adding any oil or fat and only one reference was found to the investigation of deep fat frying of whole carp fillets. (TOTHMARUS & SASSKISS 1993; DE CASTRO *et al.* 2007; NASERI *et al.* 2010). A very recent publication described for the first time shallow fat frying of silver carp with olive, sunflower, and canola oils (RAHIMABADI & DAD 2012).

Especially with the new developed n-3 rich carp is it important to know what types of changes can occur in the fish's FA composition when using different types of frying fat. This is why we chose butter and lard besides sunflower and rapeseed oils as the most commonly used oils in Czech homes. In addition, we fried the fillets plain and battered, which is also a typical way of preparation in Czech homes. Besides FA composition, we also monitored the oxidation due to frying with different fats.

The aim was to observe the combined effects of fat exchange and oxidation for us to be able to make suggestions on the preferable way to prepare carp in order to retain its nutritional value. As the effects may be similar to those with other oily fish species, the results can also have a more general use.

## MATERIAL AND METHODS

**Fish samples.** Twelve 4-year-old market scaly carp (*Cyprinus carpio*) were used, reared for one season (April–September) with a rapeseed/linseed pellet supplementation for an enriched content of n-3 FA.

After harvesting and purging, the fish were killed and filleted. Fillets were frozen in  $-20^{\circ}\text{C}$  until the frying experiment. All samples were stored frozen for the same period of time.

Lard, butter, rapeseed oil, and sunflower oil obtained in the local supermarket were used for frying. Before frying, the fish fillets were cut in three similar parts. Front and back parts of the fillets were cut making about 100 g sized pieces to be fried, while the minor middle part was used as raw control for each fillet. From each corresponding fillet one back and one front part were used to be fried in the same fat source, either with or without a batter. The batter was prepared according to the traditional recipe by first turning the filet piece in wheat flour, then in beaten egg and finally in breadcrumbs. No spices or salt were added. For frying, 50 ml of the fat (butter

and lard were carefully melted before use) were used to fry the two pieces of the same fish and the same treatment (plain or battered) in a Teflon pan with 24 cm in diameter. The amount of fat was chosen so that the bottom of the pan was covered with liquid fat. The fat was heated to approximately 130°C and then the pieces were fried for 4 + 4 min on each side, and then for additional 2 min on each side until reaching a core temperature of 65°C, measured with a meat thermometer. After frying and cooling, the whole pieces were separately minced in a table blender to assure that all edible parts were included equally in the sample. The 2 stripes of raw samples from one fish were combined and minced together. For each fat type, three fish were used resulting in 3 duplicate plain fried pieces, 3 duplicate battered fried pieces, and 3 raw samples. The minced samples were frozen and kept at –80°C until further analysis.

**Total fat content and FA composition.** The lipid extraction was performed according to HARA and RADIN (1978), with a slight modification. The samples were semi-thawed, and sub-samples of approx. 1 g were taken for the extraction from the fish, and of 200 mg from the fats. The samples were homogenised for 3 × 30 s in 10 ml of hexane : isopropanol (3 : 2 v/v) using an Ultra Turrax (T25; IKA-Werke Janke & Kunkel GmbH & Co, Staufen, Germany), and 6.5 ml Na<sub>2</sub>SO<sub>4</sub> solution (0.47M) was added. The homogenate was left to separate in 4°C for 20 min and the upper phase was then transferred to a new tube and evaporated under N<sub>2</sub>. The lipid content of the fillet pieces was determined gravimetrically from this total extracted lipid, which was then dissolved in 1 ml of hexane. The samples were stored at –80°C in normal atmosphere until further analyses.

FA from the total lipids were methylated with boron trifluoride-methanol complex (BF<sub>3</sub>) (APPELQVIST 1968). To each sample, 2 ml of a 0.01M solution of NaOH in dry methanol was added, and the samples were then heated for 10 min at 60°C. Further, 3 ml of BF<sub>3</sub> reagent were added and the samples were reheated at 60°C for 10 minutes. Thereafter, the tubes were cooled in ice water and 2 ml of a 3.42M NaCl solution in water was added to all tubes. The FA methyl esters (FAME) were extracted with 2 ml of hexane, the upper layer was transferred to a new tube and evaporated under nitrogen to dryness. The lipids were dissolved in 0.5 ml of hexane and stored under normal atmosphere at –80°C until gas chromatography analysis.

The FAME were then analysed with a gas chromatograph (Trace Ultra FID; Thermo Scientific, Milan,

Italy) equipped with a flame ionisation detector and PVT injector, using a BPX 70 column (SGE Inc., Austin, USA), length 50 m, *i.d.* 0.22 mm, and film thickness 0.25 µm. The GC was programmed with a constant gas flow of 1.2 ml/min and a temperature program which started at 70°C for 0.5 min, followed by a ramp of 30°C/min up to 150°C, a second ramp with a rate of 2°C/min up to 220°C, and a final constant time of 11 min at 220°C. The injector and detector temperatures were programmed at 150 and 250°C, respectively. The injector was programmed in splitless mode, with a splitless time of 0.8 min and a split flow of 25 ml/minute. The peaks were identified by comparing their retention times with those of the standard mixture GLC-68D (Nu-Chek Prep, Elysian, USA) and other authentic standards (Nu-Chek Prep, Elysian, USA; Larodan, Sweden).

**Oxidation.** The analysis of thiobarbituric acid reactive substances (TBARS) was conducted in the fresh and fried fillets according to a method described by MILLER (1998). After reaction in darkness for 15–20 h (overnight) at room temperature (20°C), the reaction complex was detected at a wavelength of 530 nm against the sample blank using a UV-visual spectrophotometer (Specord 210; Analytik Jena, Germany). The results were expressed as malonaldehyde equivalents (MDA) in µg/g.

**Statistics and calculation.** The averages and standard deviations were calculated in Excel and the statistical evaluation was performed using the Mixed Procedure in SAS (Version 9.1; SAS Institute Inc., Cary, USA). The changes in FA percentages were calculated in Excel.

## RESULTS

**Total fat content.** Fat content is presented in Table 1. The fat content increased significantly due to frying and even more when the fillet was battered before frying. The increase difference between the plain fried and battered fried fillets was significant for lard.

**Fatty acid composition.** FA composition in raw and fried samples is presented in Table 1. The main FA of the fats are shown in Table 2. After frying the fillets showed an increase in the major FA from the different fats and oils used for frying. When using butter for frying, SFA significantly increased from raw to plain fried and to fried in batter, while MUFA and PUFA decreased. The difference, however, was significant only when the fillets were fried battered. The FA that increased most were C14:0 and C16:0

Table 1. Fat content (% in whole fillet with skin and with batter) and fatty acid composition (% of total identified) in raw and fried fillets of carp ( $n = 3$  in each treatment), fried in different fats, plain or covered with a bread-batter with each fish as their own raw control (averages  $\pm$  standard deviation)

	Butter			Lard			Rapeseed oil			Sunflower oil		
	raw	fried plain	fried with batter	raw	fried plain	fried with batter	raw	fried plain	fried with batter	raw	fried plain	fried with batter
Total fat	4.69 $\pm$ 2.53 <sup>D</sup>	10.5 $\pm$ 3.16 <sup>E</sup>	14.1 $\pm$ 2.65 <sup>E</sup>	3.07 $\pm$ 2.65 <sup>D</sup>	9.82 $\pm$ 1.76 <sup>E</sup>	14.0 $\pm$ 4.08 <sup>F</sup>	3.74 $\pm$ 1.05 <sup>D</sup>	9.47 $\pm$ 1.39 <sup>E</sup>	13.1 $\pm$ 1.34 <sup>E</sup>	5.07 $\pm$ 4.31 <sup>D</sup>	11.9 $\pm$ 5.05 <sup>E</sup>	13.9 $\pm$ 3.32 <sup>E</sup>
C14:0	1.19 $\pm$ 0.09 <sup>D</sup>	5.31 $\pm$ 1.08 <sup>Ea</sup>	8.11 $\pm$ 1.02 <sup>Ea</sup>	1.04 $\pm$ 0.26	1.48 $\pm$ 0.08 <sup>b</sup>	1.43 $\pm$ 0.05 <sup>b</sup>	1.04 $\pm$ 0.17	0.54 $\pm$ 0.09 <sup>c</sup>	0.40 $\pm$ 0.21 <sup>c</sup>	1.33 $\pm$ 0.51 <sup>D</sup>	0.60 $\pm$ 0.17 <sup>cDE</sup>	0.42 $\pm$ 0.24 <sup>Ec</sup>
C16:0	19.0 $\pm$ 1.88 <sup>D</sup>	25.3 $\pm$ 1.08 <sup>Ea</sup>	30.3 $\pm$ 1.87 <sup>Ea</sup>	18.7 $\pm$ 0.22 <sup>D</sup>	23.95 $\pm$ 1.52 <sup>Ea</sup>	25.21 $\pm$ 0.84 <sup>Eb</sup>	16.8 $\pm$ 0.79 <sup>D</sup>	10.4 $\pm$ 0.90 <sup>Eb</sup>	10.1 $\pm$ 3.30 <sup>Ec</sup>	18.12 $\pm$ 1.50 <sup>D</sup>	12.8 $\pm$ 1.90 <sup>Eb</sup>	12.3 $\pm$ 4.45 <sup>Ec</sup>
C16:1tr	1.14 $\pm$ 0.10 <sup>Dab</sup>	0.83 $\pm$ 0.07 <sup>D</sup>	0.65 $\pm$ 0.11 <sup>Eab</sup>	1.35 $\pm$ 0.49 <sup>Dabc</sup>	0.87 $\pm$ 0.26 <sup>E</sup>	0.66 $\pm$ 0.23 <sup>Ea</sup>	1.01 $\pm$ 0.29 <sup>Db</sup>	0.59 $\pm$ 0.05 <sup>E</sup>	0.42 $\pm$ 0.23 <sup>Eb</sup>	1.59 $\pm$ 0.45 <sup>Dc</sup>	0.63 $\pm$ 0.27 <sup>E</sup>	0.41 $\pm$ 0.29 <sup>Eab</sup>
C16:1	7.72 $\pm$ 1.88 <sup>Da</sup>	6.12 $\pm$ 1.73 <sup>DEa</sup>	4.66 $\pm$ 1.24 <sup>Ea</sup>	4.03 $\pm$ 2.60 <sup>b</sup>	3.72 $\pm$ 1.03 <sup>b</sup>	3.14 $\pm$ 0.85 <sup>ab</sup>	4.53 $\pm$ 1.42 <sup>b</sup>	2.87 $\pm$ 0.48 <sup>b</sup>	2.18 $\pm$ 0.9 <sup>b</sup>	6.75 $\pm$ 5.67 <sup>Dab</sup>	3.15 $\pm$ 1.80 <sup>Eb</sup>	2.14 $\pm$ 2.04 <sup>Fb</sup>
C18:0	5.12 $\pm$ 0.27	6.21 $\pm$ 1.44 <sup>a</sup>	7.41 $\pm$ 1.80 <sup>a</sup>	4.33 $\pm$ 1.95 <sup>D</sup>	11.9 $\pm$ 2.44 <sup>Eb</sup>	12.3 $\pm$ 3.62 <sup>Eb</sup>	5.12 $\pm$ 0.51	3.17 $\pm$ 0.32 <sup>c</sup>	3.10 $\pm$ 0.84 <sup>c</sup>	6.51 $\pm$ 2.16 <sup>D</sup>	4.47 $\pm$ 0.74 <sup>DEac</sup>	3.84 $\pm$ 1.00 <sup>Ec</sup>
C18:1n-9	37.2 $\pm$ 4.05 <sup>a</sup>	33.7 $\pm$ 4.08 <sup>a</sup>	31.2 $\pm$ 2.23 <sup>a</sup>	29.5 $\pm$ 3.83 <sup>ab</sup>	33.9 $\pm$ 0.93 <sup>a</sup>	35.9 $\pm$ 0.60 <sup>b</sup>	36.4 $\pm$ 3.20 <sup>Da</sup>	50.5 $\pm$ 1.46 <sup>Eb</sup>	49.7 $\pm$ 8.3 <sup>Ec</sup>	34.3 $\pm$ 8.58 <sup>Db</sup>	34.0 $\pm$ 4.45 <sup>Ea</sup>	35.6 $\pm$ 10.2 <sup>Eab</sup>
C18:1n-7	3.42 $\pm$ 0.15 <sup>D</sup>	2.58 $\pm$ 0.31 <sup>DEab</sup>	2.02 $\pm$ 0.35 <sup>acE</sup>	2.40 $\pm$ 2.08	2.85 $\pm$ 0.22 <sup>ab</sup>	2.72 $\pm$ 0.21 <sup>a</sup>	3.37 $\pm$ 0.33 <sup>D</sup>	3.35 $\pm$ 0.12 <sup>aDE</sup>	3.71 $\pm$ 1.17 <sup>Db</sup>	2.30 $\pm$ 2.01 <sup>D</sup>	2.06 $\pm$ 0.47 <sup>Db</sup>	1.71 $\pm$ 0.65 <sup>Ec</sup>
C18:2n-6	9.0 $\pm$ 2.16	6.8 $\pm$ 1.02 <sup>a</sup>	6.3 $\pm$ 1.41 <sup>a</sup>	11.2 $\pm$ 1.28	10.5 $\pm$ 0.71 <sup>ab</sup>	10.9 $\pm$ 0.4 <sup>a</sup>	11.4 $\pm$ 1.19	15.6 $\pm$ 1.00 <sup>b</sup>	19.2 $\pm$ 5.65 <sup>b</sup>	12.2 $\pm$ 0.78 <sup>D</sup>	32.8 $\pm$ 10.48 <sup>Ec</sup>	40.6 $\pm$ 14.18 <sup>Ec</sup>
C18:3n-3	5.92 $\pm$ 2.30 <sup>Da</sup>	4.17 $\pm$ 1.22 <sup>DEa</sup>	2.96 $\pm$ 1.25 <sup>Ea</sup>	8.21 $\pm$ 2.07 <sup>Dab</sup>	4.34 $\pm$ 1.84 <sup>Ea</sup>	3.08 $\pm$ 1.60 <sup>Ea</sup>	6.74 $\pm$ 0.81 <sup>ab</sup>	7.79 $\pm$ 0.34 <sup>b</sup>	6.99 $\pm$ 1.40 <sup>b</sup>	8.46 $\pm$ 2.18 <sup>Db</sup>	3.61 $\pm$ 1.35 <sup>Ea</sup>	2.41 $\pm$ 1.18 <sup>Ea</sup>
C20:1n-9	2.00 $\pm$ 0.10 <sup>Dab</sup>	1.39 $\pm$ 0.15 <sup>DE</sup>	0.90 $\pm$ 0.25 <sup>Eab</sup>	1.23 $\pm$ 1.06 <sup>a</sup>	1.17 $\pm$ 0.23	0.92 $\pm$ 0.16 <sup>ab</sup>	2.53 $\pm$ 0.39 <sup>Db</sup>	0.99 $\pm$ 0.81 <sup>E</sup>	1.40 $\pm$ 0.29 <sup>Ea</sup>	2.46 $\pm$ 1.58 <sup>Db</sup>	1.08 $\pm$ 0.48 <sup>E</sup>	0.59 $\pm$ 0.17 <sup>Eb</sup>
C20:3n-6	0.28 $\pm$ 0.08 <sup>Da</sup>	0.21 $\pm$ 0.06 <sup>DE</sup>	0.11 $\pm$ 0.04 <sup>E</sup>	0.58 $\pm$ 0.23 <sup>Db</sup>	0.18 $\pm$ 0.07 <sup>E</sup>	0.09 $\pm$ 0.08 <sup>E</sup>	0.48 $\pm$ 0.07 <sup>Db</sup>	0.11 $\pm$ 0.08 <sup>E</sup>	0.05 $\pm$ 0.08 <sup>E</sup>	0.40 $\pm$ 0.22 <sup>Da</sup>	0.13 $\pm$ 0.05 <sup>E</sup>	0.06 $\pm$ 0.07 <sup>E</sup>
C20:4n-6	1.89 $\pm$ 0.50	1.10 $\pm$ 0.18	0.73 $\pm$ 0.14	4.80 $\pm$ 0.96 <sup>D</sup>	1.13 $\pm$ 0.17 <sup>E</sup>	0.88 $\pm$ 0.21 <sup>D</sup>	2.29 $\pm$ 0.63 <sup>D</sup>	0.68 $\pm$ 0.28 <sup>E</sup>	0.42 $\pm$ 0.13 <sup>E</sup>	2.96 $\pm$ 2.07 <sup>D</sup>	0.73 $\pm$ 0.30 <sup>E</sup>	0.45 $\pm$ 0.20 <sup>E</sup>
C20:3n-3	0.28 $\pm$ 0.12 <sup>a</sup>	0.16 $\pm$ 0.07	0.05 $\pm$ 0.04	1.13 $\pm$ 1.12 <sup>Db</sup>	0.18 $\pm$ 0.13 <sup>E</sup>	0.25 $\pm$ 0.29 <sup>E</sup>	0.37 $\pm$ 0.10 <sup>a</sup>	0.15 $\pm$ 0.13	0.06 $\pm$ 0.11	0.29 $\pm$ 0.25 <sup>a</sup>	0.36 $\pm$ 0.19	0.08 $\pm$ 0.08
C22:1	0.32 $\pm$ 0.17 <sup>ab</sup>	0.27 $\pm$ 0.10	0.16 $\pm$ 0.07	0.48 $\pm$ 0.07 <sup>Db</sup>	0.29 $\pm$ 0.18 <sup>DE</sup>	0.15 $\pm$ 0.20 <sup>E</sup>	0.15 $\pm$ 0.01 <sup>a</sup>	0.15 $\pm$ 0.05	0.10 $\pm$ 0.06	0.55 $\pm$ 0.38 <sup>Db</sup>	0.21 $\pm$ 0.08 <sup>E</sup>	0.08 $\pm$ 0.09 <sup>E</sup>
C20:5n-3	2.21 $\pm$ 0.80 <sup>Da</sup>	1.33 $\pm$ 0.29 <sup>E</sup>	0.75 $\pm$ 0.28 <sup>E</sup>	4.70 $\pm$ 1.23 <sup>Db</sup>	1.51 $\pm$ 0.51 <sup>E</sup>	0.90 $\pm$ 0.47 <sup>E</sup>	2.63 $\pm$ 0.54 <sup>Da</sup>	1.12 $\pm$ 0.11 <sup>E</sup>	0.67 $\pm$ 0.36 <sup>E</sup>	3.79 $\pm$ 1.34 <sup>Dc</sup>	1.30 $\pm$ 0.37 <sup>E</sup>	0.65 $\pm$ 0.22 <sup>E</sup>
C22:5n-3	0.95 $\pm$ 0.29 <sup>Da</sup>	0.54 $\pm$ 0.09 <sup>DE</sup>	0.34 $\pm$ 0.11 <sup>E</sup>	1.94 $\pm$ 0.78 <sup>Db</sup>	0.56 $\pm$ 0.08 <sup>E</sup>	0.32 $\pm$ 0.09 <sup>E</sup>	1.09 $\pm$ 0.27 <sup>Da</sup>	0.38 $\pm$ 0.06 <sup>E</sup>	0.21 $\pm$ 0.10 <sup>E</sup>	1.63 $\pm$ 0.88 <sup>Db</sup>	0.49 $\pm$ 0.22 <sup>E</sup>	0.20 $\pm$ 0.07 <sup>E</sup>
C22:6n-3	2.03 $\pm$ 0.80 <sup>Da</sup>	0.98 $\pm$ 0.33 <sup>E</sup>	0.58 $\pm$ 0.21 <sup>E</sup>	3.79 $\pm$ 1.26 <sup>Db</sup>	0.99 $\pm$ 0.24 <sup>E</sup>	0.56 $\pm$ 0.23 <sup>E</sup>	2.31 $\pm$ 0.70 <sup>Da</sup>	0.83 $\pm$ 0.14 <sup>E</sup>	0.38 $\pm$ 0.17 <sup>E</sup>	2.41 $\pm$ 2.35 <sup>Da</sup>	0.86 $\pm$ 0.49 <sup>E</sup>	0.40 $\pm$ 0.11 <sup>E</sup>
SFA	25.6 $\pm$ 2.03 <sup>Dab</sup>	40.8 $\pm$ 2.34 <sup>Ea</sup>	51.3 $\pm$ 3.83 <sup>Ea</sup>	24.7 $\pm$ 1.74 <sup>Dab</sup>	37.4 $\pm$ 3.97 <sup>Ea</sup>	39.1 $\pm$ 4.12 <sup>Eb</sup>	23.4 $\pm$ 0.60 <sup>Da</sup>	14.6 $\pm$ 1.08 <sup>Eb</sup>	14.2 $\pm$ 4.62 <sup>Ec</sup>	31.1 $\pm$ 10.75 <sup>Db</sup>	18.4 $\pm$ 2.66 <sup>Eb</sup>	16.5 $\pm$ 3.43 <sup>Ec</sup>
MUFA	52.0 $\pm$ 5.82 <sup>Da</sup>	44.8 $\pm$ 6.46 <sup>DEa</sup>	39.0 $\pm$ 4.09 <sup>Ea</sup>	40.3 $\pm$ 4.95 <sup>bc</sup>	42.8 $\pm$ 1.17 <sup>a</sup>	43.7 $\pm$ 1.57 <sup>a</sup>	48.5 $\pm$ 3.78 <sup>Dab</sup>	58.5 $\pm$ 0.79 <sup>Eb</sup>	57.6 $\pm$ 9.58 <sup>DEb</sup>	35.6 $\pm$ 8.46 <sup>c</sup>	40.9 $\pm$ 7.38 <sup>a</sup>	38.4 $\pm$ 9.37 <sup>a</sup>
PUFA	23.3 $\pm$ 7.12 <sup>Da</sup>	15.8 $\pm$ 2.79 <sup>DEa</sup>	12.1 $\pm$ 3.33 <sup>Ea</sup>	34.9 $\pm$ 3.20 <sup>Db</sup>	19.7 $\pm$ 2.91 <sup>Eab</sup>	17.2 $\pm$ 2.62 <sup>Ea</sup>	28.2 $\pm$ 3.20 <sup>ab</sup>	26.9 $\pm$ 1.17 <sup>b</sup>	28.3 $\pm$ 5.12 <sup>b</sup>	33.4 $\pm$ 3.94 <sup>Dab</sup>	40.6 $\pm$ 9.88 <sup>DEc</sup>	45.1 $\pm$ 12.64 <sup>Ec</sup>
n-3	11.4 $\pm$ 4.29 <sup>Da</sup>	7.18 $\pm$ 1.70 <sup>Ea</sup>	4.69 $\pm$ 1.84 <sup>Ea</sup>	19.8 $\pm$ 1.30 <sup>Db</sup>	7.59 $\pm$ 2.72 <sup>Ea</sup>	5.10 $\pm$ 2.40 <sup>Fa</sup>	13.1 $\pm$ 1.53 <sup>Da</sup>	10.3 $\pm$ 0.35 <sup>DEb</sup>	8.32 $\pm$ 0.71 <sup>Eb</sup>	16.6 $\pm$ 2.63 <sup>Db</sup>	6.61 $\pm$ 1.65 <sup>Ea</sup>	3.75 $\pm$ 1.53 <sup>Fa</sup>
n-6	11.9 $\pm$ 2.90	8.50 $\pm$ 1.18 <sup>a</sup>	7.29 $\pm$ 1.55 <sup>a</sup>	15.1 $\pm$ 2.28	12.1 $\pm$ 0.79 <sup>ab</sup>	12.1 $\pm$ 0.43 <sup>a</sup>	15.0 $\pm$ 1.73	16.7 $\pm$ 0.83 <sup>b</sup>	19.9 $\pm$ 5.79 <sup>b</sup>	16.7 $\pm$ 1.79 <sup>D</sup>	33.9 $\pm$ 10.14 <sup>Ec</sup>	41.2 $\pm$ 13.90 <sup>Ec</sup>
n-3/n-6	0.94 $\pm$ 0.15	0.83 $\pm$ 0.10	0.62 $\pm$ 0.11	1.32 $\pm$ 0.15 <sup>D</sup>	0.63 $\pm$ 0.23 <sup>E</sup>	0.42 $\pm$ 0.19 <sup>F</sup>	0.88 $\pm$ 0.03	0.62 $\pm$ 0.01	0.46 $\pm$ 0.08	0.99 $\pm$ 0.13 <sup>D</sup>	0.41 $\pm$ 0.54 <sup>F</sup>	0.43 $\pm$ 0.85 <sup>E</sup>

D–E Different capital letters in a row indicate significant difference ( $P < 0.05$ ) between raw fish, plain fried fish, and fish fried in batter in the same fat; a–c different small letters in a row indicate significant differences ( $P < 0.05$ ) between the raw fish or same frying type across different fats ( $n = 12$ ); n-6 = omega 6; n-3 = omega 3; SFA = sum of saturated fatty acids;

MUFA = sum of monounsaturated fatty acids; PUFA = sum of polyunsaturated fatty acids; n-3/n-6 = ratio between the sum of omega 3 and omega 6 fatty acids



Table 2. Fatty acid composition in the oils and fats used for frying (% of total identified)

	butter	lard	Oil	
			rapeseed	sunflower
C10:0	3.11	nd	nd	nd
C12:0	4.35	0.11	nd	nd
C14:0	13.8	1.56	0.07	0.09
C16:0	38.6	27.8	4.58	6.60
C18:0	10.3	17.4	1.59	3.25
C18:1n-9	22.6	35.7	60.5	26.5
C18:1n-7	0.74	2.42	3.32	0.73
C18:2n-6	2.44	10.2	19.0	60.8
C18:3n-3	0.57	0.84	8.46	0.36

nd = not detected

(Figure 1). The highest increase was in 16:0 which was also the major FA in butter (38.6%).

Frying with lard resulted in significant increase of SFA and significant decrease of PUFA and n-3, while n-6 was stable. The main FA increasing were C16:0 and C18:0 while all unsaturated FA decreased more or less significantly. The decrease in n-3 also caused a significant decrease in the n-3/n-6 ratio.

Frying with rapeseed oil led to significantly decreased SFA and significant increase of MUFA. In the battered fillets after frying even the total n-3 FA were significantly decreased as compared to the raw fillets. However, that decrease was not significant in the plain fried fillets. The main FA increased was 18:1n-9 (Figure 1). Frying with rapeseed oil resulted in an increase of 18:1n-9 up to 50% and up to 8% of

18:3n-3, reflecting the proportions of these FA in the oil. In the oil used, the proportions of these FA were 60 and 8.5%, respectively.

The fillets fried in sunflower oil showed a significant increase in total PUFA and n-6 FA, while SFA and n-3 FA decreased significantly. The FA that increased significantly were 18:2n-6 and 18:1n-9. The significant increase in n-6 also caused a significant decrease in the n-3/n-6 ratio.

The total proportion of n-3 was significantly decreased due to the frying process in all samples, however, in the samples fried with rapeseed oil this decrease was the lowest in percentage (36.5% compared to 48.1, 74.2, and 77.4% in battered samples fried with rapeseed oil, butter, lard, and sunflower oil, respectively). At the same time, the proportion of total n-6 increased slightly in the samples fried with butter and lard and significantly in the samples fried with sunflower oil. 20:5n-3 (eicosapentaenoic acid, EPA), 22:5n-3 (docosapentaenoic acid, DPA) and 22:6n-3 (docosahexaenoic acid, DHA) decreased significantly in all frying treatments independently of the fat used.

20:4n-6 (arachidonic acid, AA) decreased significantly due to frying with lard, rapeseed oil, and sunflower oil, however, not when butter was used.

**Oxidation.** TBARS were similar among the raw fish samples were similar. Frying increased TBARS in the fish fried with lard and sunflower oils significantly, while butter did not increase TBARS and frying with rapeseed oil only showed a tendency towards increased TBARS values ( $P = 0.0674$ ). TBARS in the battered fillets were not analysed as the bat-

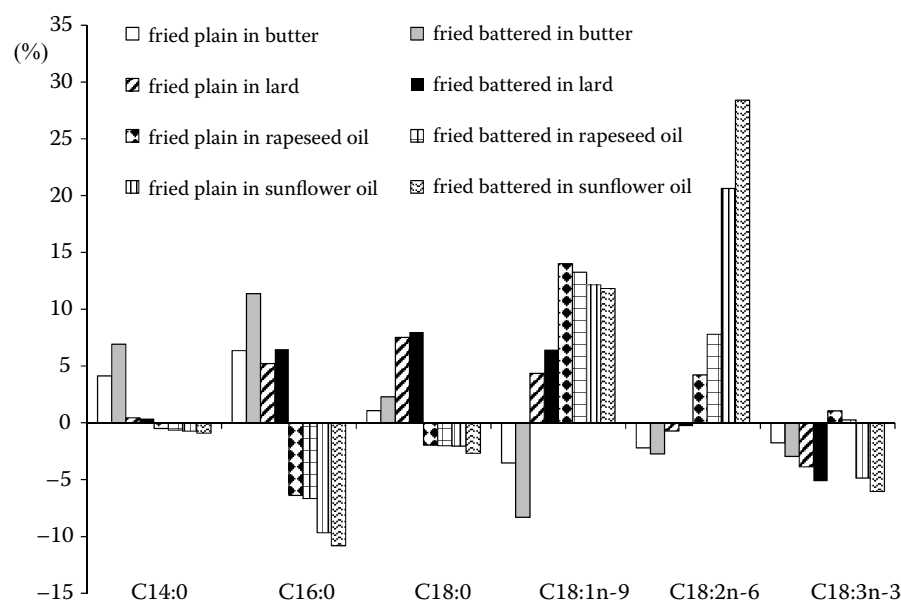


Figure 1. Changes in selected fatty acid percentages in plain or battered carp fillets ( $n = 3$  in each treatment), due to frying in different fats

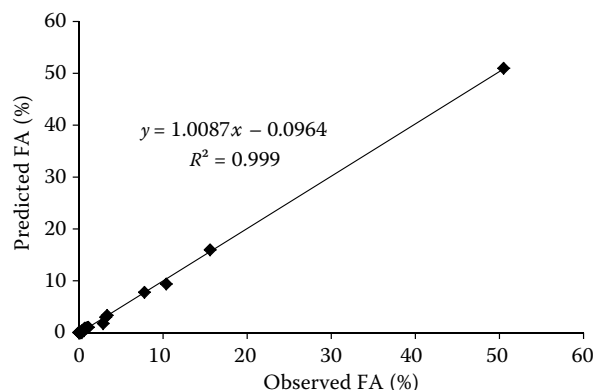


Figure 2. Prediction plot of fillet fatty acid composition (%) according to the dilution model; showing observed versus predicted percentages of fatty acids

ter interfered with the TBA solution leading to a yellowish colour, making the comparison with the standard impossible.

## DISCUSSION

In the present study, the main aim was to investigate the effects of pan frying on the nutritional value of carp fillets. The fact that the carp fillets had an enriched content of n-3 PUFA compared to the fillets from normal production created an additional necessity to investigate the effect of frying on this special product.

**Total lipid content.** During frying, the lipid content in the fillets increased to approximately twice the amount when the fillets were fried plain and up to threefold amount when the fillets were battered. An increase of the lipid content in fish fillets during the preparation has been reported earlier by various researchers with salmon, cod, herring and other fish species (MAI *et al.* 1978; GLADYSHEV *et al.* 2006, 2007; SIOEN *et al.* 2006; WEBER *et al.* 2008). An even higher increase of the lipid content was found during frying with or without breading in bluegill (*Lepomis macrochirus*) and white sucker (*Catostomus commersoni*) fillets, where the lipid content increased nearly 10 times when fried with breading and fourfold in the white sucker when fried without breading (MAI *et al.* 1978). In the same study, the increase of the lipid content in lake trout (*Savelinus namaycush*), however, was not as drastic (from 8.8% to 9.5%), which probably can be ascribed to the higher lipid content in the trout fillets.

Nevertheless, the increase of the lipid content in the fillets in our study was independent of the fat source. This is in line with earlier results reporting

a significant increase in the lipid content in silver catfish (*Rhamdia quelen*) due to panfrying while no variance was found in the lipid content in relation to frying with different oils (WEBER *et al.* 2008). When comparing the different species, it is obvious that in general the lipid content in lean fish like cod increased to a much higher percentage than in fatty fish. Even earlier it was concluded that lean fish take up more fat and the lipid changes are greater compared to more fatty fish (MAI *et al.* 1978).

Consequently, the lipid uptake in fish fillets during pan-frying seems to be negatively correlated with the raw fish fat content. This hypothesis is strengthened by the fact that the fat content in the catfish fillets increased more compared to our carp fillets (WEBER *et al.* 2008). In our trial, the plain fried carp had the fat content from 9.5–11.9% while the reported fat content in the fried catfish was 13–14% (WEBER *et al.* 2008).

**Fatty acid composition.** Several significant changes in the FA composition in the fillets were found after frying. For rapeseed oil, the highest increase was found for 18:1n-9. It was discussed that 18:1n-9 is a FA easily taken up as it has a higher viscosity compared to the more unsaturated FA and a higher adherence to the material to be fried (KALOGEROPOULOS *et al.* 2007). The same authors showed an increased uptake of 18:1n-9 in pan-fried potatoes. Also in catfish an increased uptake of 18:1n-9 from canola oil was found, which is in line with our findings (WEBER *et al.* 2008). However, in the fillets fried with lard, it can be observed in the present study that the SFA seem to have an even stronger effect as the lard we used had 18:1n-9 as the major FA but the main increasing FA was 18:0, of which there was only 17.4% compared to 35.7% of 18:1n-9.

In addition, the increase in 18:0 in the fillets fried with lard was relatively higher than that in 16:0 despite the higher content of 16:0 in lard. This could be due to either the higher liquidity of 18:0 and hence an easier uptake into the fillet or the higher content of 16:0 in the raw fillets, as their ratio of these FA in the fillets was approaching to the ratio in lard (16:0/18:0 from 4.3 to 2.0 in the raw and fried fillets, respectively, compared to 0.8 in lard). Furthermore we found a significant increase of 14:0 and a minor increase of 18:0 in the samples fried with butter, despite the fact that the proportion of 18:1n-9 was higher in butter than the proportion of SFA. This would strengthen the hypothesis that the FA are taken up into the fish fillets depending on the FA composition both of the fat used and of the raw fillet

in a way that the final FA composition in the fried fish reflects the FA proportion in the used oil. This might be influenced in addition by the lipid content of the raw fillets with lean fish facilitating a higher uptake of lipid compared with fatty fish.

This hypothesis is supported by the results showing an increase in 18:3n-3 in catfish when frying in canola oil in opposite to our results with carp (WEBER *et al.* 2008). This can be explained by the fact that the raw catfish fillets had a lower total fat content and a lower percentage of 18:3n-3 (2.5 and 1.4%, respectively) compared to our carp (3.1–5.1 and 5.9–8.5%, respectively), hence leading to an increased overall uptake of fat and also to a relatively increased proportion of 18:3n-3 reflecting the greater discrepancy in these FA between oil and fish.

Also in the study on shallow- and deep-fat frying of silver carp from Iran the FA composition in the fried fillets reflected the FA composition of the frying fats to a great extent (RAHIMABADI & DAD 2012). However, as these authors did not report the total fat content before and after frying, it is difficult to estimate the influence of the fat content.

The significant decrease of EPA, DPA, and DHA in all frying treatments independent of the fat used depends most probably on the fact that these FA were not present in the frying fats and hence could not be taken up. As our results are expressed in proportions of FA and not in the absolute amounts, the decrease is derived only relatively and consequentially from the increase of the major FA from the frying fats.

Concerning the nutritional value of fish in general and especially of our n-3 enriched carp fillets in the present study, it can be stated that frying with lard and sunflower oil resulted in a significant decrease of the n-3/n-6 ratio, while the ratio was stable when the fillets were fried with butter or rapeseed oil. However, the desaturase systems for the metabolism of the parental n-3 and n-6 FA ALA and LA are the same for both n-3 and n-6 FA (DE HENAUW *et al.* 2007; PALMQUIST 2009). Even though delta 6 desaturase has a higher affinity for ALA than for LA, due to the much higher dietary intake the n-6 PUFA have been

suggested to limit the conversion of LA to eicosapentaenoic acid = 20:5 n-3 (EPA) and DHA) (PALMQUIST 2009). Thus especially the use of lard and sunflower oil decreased the nutritional value of the fish by changing the n-3/n-6 ratio, as the higher intake of n-6 will shift the human metabolic pathways of n-3 and n-6 FA more towards the n-6 products, which are connected to increased inflammation and platelet aggregation (SIMOPOULOS 2002b). For this reason, a balanced intake of n-6 and n-3 FA is important. This makes butter and rapeseed oil better candidates for fish frying fats. However, as frying with butter resulted in increased SFA in the fillets, the use of butter will also decrease the nutritional value of fish. SFA are also well-known for their negative effects on human health, such as, for example, increased blood cholesterol and prevalence of coronary heart disease (WILLIAMS 2000). This general conclusion has been discussed more recently as it is not valid for stearic acid, for example, and has not been well investigated for the medium chain FA (GRUNDY 1997). However, it is true for palmitic acid 16:0 which was the major FA increased after frying with butter. Following this rationale, as concerns the final FA composition of the fried fish fillets, rapeseed oil showed to be the best choice, preserving the n-3/n-6 ratio in the fillets without increasing the content of SFA at the same time.

**Oxidation.** Unexpectedly, rapeseed oil proved to have a similar good stability against oxidation as butter. In general, oxidation of oil is positively correlated to the number of double bonds from 18:2n-6 and 18:3n-3 and negatively correlated with 18:1n-9 (KAMAL-ELDIN 2006). Therefore the question arises if the use of an oil rich in 18:3n-3 will lead to increased oxidation in the final product. In our present study, we could not find an increased oxidation with rapeseed oil compared to sunflower oil (Table 3). Considering the conclusions drawn by Kamal Eldin, this could be explained by the high proportion of 18:1n-9 in the rapeseed oil used in the present study (KAMAL-ELDIN 2006). As this was 60%, it could have protected the rapeseed oil during frying as it has been

Table 3. TBARS in raw and plain fried pieces from carp fillets ( $n = 3$  in each treatment) fried in different oils ( $\mu\text{g}$  malondialdehyd/g fish; average  $\pm$  SD)

	Butter	Lard	Rapeseed oil	Dunflower oil
Raw	0.37 $\pm$ 0.05	0.61 $\pm$ 0.49 <sup>A</sup>	0.44 $\pm$ 0.21	0.42 $\pm$ 0.31 <sup>A</sup>
Fried plain	0.58 $\pm$ 0.22 <sup>a</sup>	2.03 $\pm$ 0.55 <sup>Bb</sup>	0.97 $\pm$ 0.23 <sup>ac</sup>	1.42 $\pm$ 0.40 <sup>Bc</sup>

Different capital letters in a column indicate significant differences ( $P < 0.5$ ) between raw and fried fish ( $n = 12$ ); different small letters in a row indicate significant difference between fillets fried in different fats

shown that 18:1n-9 could act as an inert (ROMERO & MORTON 1977). Also other researchers showed an increased stability of canola, peanut and sunflower oils with increased proportions of 18:1n-9 (O'KEEFE *et al.* 1993; MARTÍ-POLVILLO *et al.* 1996; PETUKHOV *et al.* 1999). Consequently, we hypothesise that the high percentage of 18:1n-9 in the rapeseed oil compared to the used sunflower oil and the other fats leads to the good oxidative stability in comparison to the other fats.

The significant increase of oxidation after frying with lard was unexpected as the lard was very rich in SFA that are relatively stable during oxidation. One reason could be that the lard was more oxidised from the beginning due to the processing procedure, which includes heating. Our results of MDA contents suggest that lard was the most oxidised among the fats used in the present trial. However, as described above, the measurement was heavily influenced by the high background absorbance, which we did not manage to extinguish. Further investigation is needed in this area.

## CONCLUSIONS

We conclude that frying n-3 rich carp fillets with rapeseed oil preserved the favourable n-3/n-6 ratio without increasing the saturated fats content. It seems that FA uptake in fish is negatively correlated with the raw fish fat content and positively correlated with the FA composition in the frying fat used. Comparing our results with earlier studies in the literature, we expect similar changes of FA composition in other fish species with similar fat contents. However, the concrete mechanisms for the uptake and leakage of FA into and from fish fillets need to be investigated additionally, in order to be able to give recommendations to the public and official institutions dealing with human nutrition for the appropriate frying fat for different species. The frying with rapeseed oil did not increase oxidation compared to frying with sunflower oil, which confirms the suitability of rapeseed oil for pan frying of fish.

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