doi: 10.17221/35/2015-CJFS

Increasing the Omega-3 Content of Traditional Meat Products by the Addition of an Underutilised By-Product from Fish Processing

Sabine SAMPELS, Tomáš ZAJÍC and Jan MRÁZ

University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocoenoses, Institute of Aquaculture, České Budějovice, Czech Republic

Abstract

Sampels S., Zajíc T., Mráz J. (2015): **Increasing the omega-3 content of traditional meat products by the addition of an underutilised by-product from fish processing**. Czech J. Food Sci., 33: 431–440.

The aim of our study was to find a way to use the minced fish flesh that is separated from the bones of carp after filleting (fish separate). In collaboration with the industry traditional recipes for barbecue sausages, hotdog and Vienna type sausages and liver pâté were modified by replacing a part of the meat with the fish separate. The proportion of nutritionally valuable n-3 fatty acids – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) calculated together was 74, 54, 109, and 77 mg/100 g product in the barbecue sausage, hot dog, Vienna sausage and pâté, respectively. This means 29.6, 21.6, 43.6, and 30.8% of the daily recommended intake according to EFSA.

Keywords: n-3 fatty acids; human nutrition; EPA; DHA; sausages; pâté

A diet rich in n-3 long-chain polyunsaturated fatty acids (LC-PUFA) has shown to beneficially effect the human health (Williams 2000). Polyunsaturated fatty acids (PUFA) of the n-3 series are important in the prevention of atherosclerosis, neurological dysfunction, insulin resistance, and autoimmune disorders (Storlien *et al.* 1997; Connor 2000; Williams 2000; Calder & Grimble 2002; Richardson 2006). In opposite, a high intake of n-6 fatty acids (FA) contributes to the formation of thrombi and atheromas and to inflammatory disorders (Simopoulos 2002). In addition, n-6 FA have been described as adipogenic (Ailhaud & Guesnet 2004). Furthermore, the ratio between n-6 and n-3 PUFA is important for the further transformation of the essential FA (EFA), linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, ALA) to the longer chain PUFA and their derivatives, like prostanoids. Since the different FA series compete for the transforming enzymes, desaturases and elongases, the ratio influences their metabolism. An optimal ratio has been suggested to be about 1–2, or not higher than 4–5 (Eaton *et al.* 2003; Weylandt & Kang 2005). Unfortunately, this ratio in today's Western diet is normally much higher and estimated to range between 15 and 20 or in America even up to 40. In opposite, during earlier stages of human evolution it was close to 1 (Simopoulos 2001, 2002; Ailhaud *et al.* 2006).

Both lean and oily fish are a good source for n-3 PUFA (Pickova & Morkore 2007) and the consumption of fish has been recommended due to beneficial health effects of n-3 (Simopoulos 2006). However, the consumption of fish is quite low in many regions in Europe. A recent study from Sweden showed that 71% of Swedish 4-year-olds in well-educated families never ate fat fish (Garemo *et al.* 2007). In addition, in a national questionnaire it was found out that

Supported by Ministry of Education, Youth and Sports of the Czech Republic, Projects "CENAKVA" (No. CZ.1.05/2.1.00/01.0024), "CENAKVA II" (No. LO1205 under the NPU I program), the pilot project CZ.1.25/3.1.00/11.00270, and GAJU 074/2013/Z.

doi: 10.17221/35/2015-CJFS

young people tended to eat less fish than older adults (Becker & Pearson 2003). In the Czech Republic fish consumption is as low as 5 kg/capita per year (Czech Statistical Office 2013). Even for Flemish adolescents an increase of seafood consumption was recommended (Sioen *et al*. 2007).

In the Czech Republic and central Europe common carp (*Cyprinus carpio*) is the major produced and commonly consumed fish species. Also worldwide carps represent the largest group of cultured fish constituting around 70% of freshwater aquaculture. In 2012, the world production was 3 791 912 t for this species (FAO Fisheries and Aquaculture Department 2014). Currently, most of this fish is available in the form of whole fish, fillets or fillet portions. The skeletons left after the filleting process still carry a lot of flesh which is mechanically removed. This mechanically separated meat (hereinafter the separate) is a source of valuable fish protein, lipids and other nutrients. It is intended to be a raw material for manufacturing other products. However, the utilisation is quite low as this raw material is prone to oxidation and bacterial breakdown. Until now, there have been quite few applications on the market for this nutritional valuable by-product.

The aim of the present study was to create attractive products and thereby promote and increase the consumption of fish flesh. As a base we used some traditional Czech meat products (sausages and pâté) and replaced around 50% of the meat with fish separate. Due to the low fish consumption, meat and meat products can be relatively important sources of LC-PUFA for many people (Howe *et al*. 2006). Hence, ready to eat or fast food products containing fish could improve the n-3 FA intake of today's Czech society. There have been many attempts earlier to produce sausages or other meat products with an increased nutritional value in respect to long-chain n-3 FA. However, most of these studies use either fish oil (Andres *et al*. 2009; Martínez *et al*. 2012; Marchetti *et al*. 2013, 2014) or minced whole fish (Garcia *et al*. 2005). However, fish oil is getting scarce and the price will most probably increase (Pickova & Morkore 2007) and whole fish is a valuable product by itself, so this production is both relatively expensive and not very sustainable in the long run. The use of a nutritional valuable by-product will increase the yield from the produced/captured fish, increase sustainability and decrease unnecessary waste from fish processing. The more efficient use of fish by-products is also encouraged by the FAO (2012).

Our hypothesis was that if we could create a meat product still tasting like a meat product but containing a significant proportion of fish flesh, we could increase the proportion of long-chain n-3 FA in these products and produce a nutritionally valuable, ready to eat product. As ready to eat products are increasingly consumed, such products could effectively increase the uptake of long-chain n-3 FA also for consumers who do not prepare nor like fish. We analysed the FA composition of the final products in comparison with literature data and our own analyses of similar meat products. In addition, we performed analyses of the proximate composition, oxidation, microbiology and sensory properties of the newly developed products to ensure high quality.

MATERIAL AND METHODS

Meat products. We produced four different types of traditional meat products where a part of the meat was replaced with fish separate: barbecue sausage, Vienna type sausage and hotdog type sausage, and liver pâté. A representative collection of similar sausages produced with meat only was obtained from the local supermarket and analysed for fatty acid composition. For the processing of our products with fish separate, pork meat, salt, and breadcrumbs were bought at a local supermarket. Spices and other additives were obtained from the company Fimex s.r.o. (Prague, Czech Republic). Fish separate was obtained from the company Rybníkářství Pohořelice a.s. (Czech Republic). The experimental processing of the product was done at the processing plant Mauz výroba s.r.o. (Hluboká nad Vltavou, Czech Republic). Final recipes were chosen after sensory testing by the panel of volunteers from the Faculty of Fisheries and Protection of Waters at the University of South Bohemia. Finally used recipes are shown in Table 1. After processing the samples were stored at +4°C for storage tests or frozen at –20°C for fatty acid analyses. Analyses of all samples were performed within 2 weeks.

Proximate composition and microbial analyses. For analyses of proximate composition and microbiological analyses, fresh samples (a pooled sample of 3 pieces for each product) were sent to the State Veterinary Institute, Prague, Czech Republic. Proximate composition [energy (kJ/100 g), carbohydrates (g/100 g), fat (g/100 g), protein (g/100 g), ash (g/100 g), and dry matter (g/100 g)] was analysed according to

doi: 10.17221/35/2015-CJFS

Table 1. A list of ingredients (in %) of the studied products

	Sausage with carp separate			Pâté
	Frankfurter type	Vienna type	Barbecue	
Fish separate	46.5	46.5	53.5	31.8
Pork meat	19.2	19.4	25.3	24.7[a]
Pork skin	11.5	9.7	2.92	
Pork liver				14. 1
Ice	19.2	19.4	11.7	
Broth				24.7
Salt (NaCl)			1.94	
Nitrite salt	2	2		1.6
Breadcrumbs			1.94	
Starch				2.1
Spices	1	1	0.83	0.5
Pro pork protein[b]	0.6	2	1.94	
Livemal (emulsifier)[b]				0.5

[a]cooked, from pork head; [b]from the Fimex company

the certified ISO methods: ISO 936:1998 (determination of total ash by combustion), ISO 1442:1997 (moisture content gravimetrically after drying), ISO 1443:1973 (determination of lipid content gravimetrically after extraction with solvent), ISO 1871:2009 (determination of protein nitrogen by Kjeldahl), and ISO 13965:1998 (enzymatic determination of sugar and starch content). Total energy content was calculated from the obtained analytical data.

Microbiological analyses (total colony-forming units (CFU) and presence and amount of *Salmonella* ssp., *Escherichia coli*, and *Listeria monocytogenes*) were done according to the certificated methods ISO 4833:2013 (Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 degrees C by the pour plate technique), ISO 16649-2:2001 (Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* – Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide), and ISO 6579:2002 (Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.).

FA composition. The lipid extraction of the different products, sausages and the raw fish separate was performed according to Hara and Radin (1978), with slight modification. The samples were semi-thawed, and a sub-sample of approx. 1 g was taken for extraction from fish and pork. The samples were homogenised for 3 × 30 s in 10 ml of a mixture of hexane/isopropanol (3 : 2, v/v) using an Ultra Turrax (T25; IKA Werke, Staufen im Breisgau, Germany), and 6.5 ml of Na_2SO_4 solution (0.47 M) was added. The homogenate was left to separate at 4°C for 20 min and the upper phase was then transferred to a new tube and evaporated under N_2. The lipid content of the samples was determined gravimetrically from this total extracted lipid, which was then dissolved in 1 ml 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 (BF3) (Appelqvist 1968). To each sample, 2 ml of a 0.01 M solution of NaOH in dry methanol was added, and the samples were then heated at 60°C for 10 minutes. Next, 3 ml of BF_3 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 3.42 M NaCl solution in water was added to all tubes. The FA methyl esters (FAME) were extracted with 2 ml hexane, the upper layer was transferred to a new tube and evaporated under nitrogen gas to dryness. The lipids were dissolved in 0.5 ml 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, 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 and a second ramp with a rate of 2°C/min until 220°C and a final constant time of 11 min at 220°C. 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 25 ml/minutes. 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. 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

doi: 10.17221/35/2015-CJFS

530 nm against the sample blank using a UV-visual spectrophotometer (Specord 210; Analytik Jena, Germany). Results were expressed as equivalents to malonaldehyde (MDA) in µg/g.

Sensory evaluation. Final sensory evaluation was done by a sensory panel of ten members of university employees from the University of South Bohemia, Faculty of Fisheries and Protection of Waters (Vodňany, Czech Republic) with an experience in sensory evaluation of fish and fish products. The panel members received samples of the different products and a questionnaire, refereeing to taste and texture properties. The attributes to evaluate were: odour intensity, odour acceptability, texture, juiciness, flavour intensity, flavour acceptability and appearance. The scale of the various properties was from 1 to 100 with 1 meaning the worst and 100 the best evaluation for each attribute. The scale was subjective, meaning that the panellists gave their personal evaluation. If the product was not acceptable at all, the panel members were asked to state this and the sample would be classified as unacceptable. However this was not the case in any of the products.

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

RESULTS AND DISCUSSION

Together with the industry it was evaluated what type of products would be accepted by the general customers and modifications of traditional recipes for barbecue, Vienna and Frankfurter type sausages as well as liver pâté, by replacing a part of meat with the fish separate (53.5, 46.5, 46.5, and 31.8%, respectively) were produced. To ensure high quality, sensory, microbial and storage stability analyses were carried out besides the general composition and FA composition to determine the final proportion of nutritionally valuable n-3 PUFA.

The results of the sensory evaluation are shown in Figure 1. In general, the products received quite a high ranking. Odour intensity of the Vienna type sausages was below 50, however the odour acceptability was rated 61 points. In addition, both the texture and appearance of the Frankfurter type sausages were rated below 50, but flavour and odour qualities as well as juiciness got high rankings above 60. Similarly to our results, Park *et al*. (1989) showed that Frankfurters produced with the addition of fish oil had some lower texture properties. This could be due to the fact that fish oil is more liquid than lard or the fat of pork in general. Another reason in our case can be the fact that fish protein has different collagen composition resulting in different gelling properties from those of meat from mammals (Ramírez *et al*. 2011). It was already shown earlier that the replacement of meat and connective tissue by fish flesh or added fish oil in this type of sausages leads to increased softness, decreased hardness and shear force (Murphy *et al*. 2004; Intarasirisawat *et al*. 2014; Marchetti *et al*. 2014).

However, our main concern was that the products would have some off-flavour or odour due to the use of the fish separate. In earlier studies where fish or fish-oil were added to meat products a risk of off-flavour increased (Intarasirisawat *et al*. 2014).

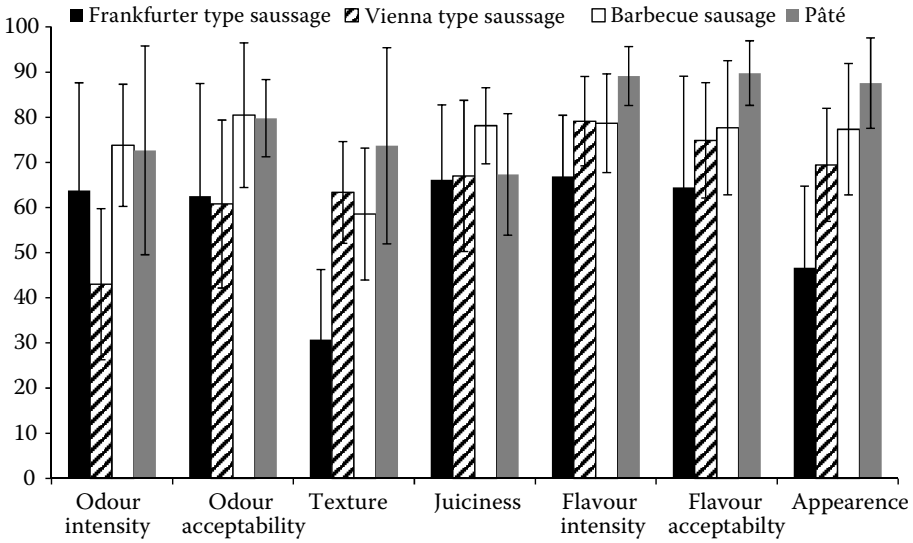

Figure 1. Sensory evaluation of the sausages and pâté produced with carp separate

doi: 10.17221/35/2015-CJFS

Table 2. A list of ingredients of sausages bought in the Czech market

	Barbecue sausages				Frankfurter type sausages			Vienna type sausages				
	1	2	3	4	1	2	3	1	2	3	4	5
Fat content max (%)	35	40	24	40	35	34	30	30	34	36	40	28
Meat content (%)		60			60				85	55	60	
Pork meat (%)	5	*	82	35	*	85	8	60	*	*	*	90
Separated poultry meat (%)	56			*			40					
Pork lard and skin (%)	*						28			*		
Pork skin				*				*				
Beef meat					*		*	5		*	*	
Water	*	*	*	*	*	*	*	*	*	*	*	*
Wheat flour							*					
Potato starch	*			*			*	*		*		
Salt	*	*	*	*	*		*	*		*	*	*
Nitrite salt						*			*			
Rice fibre										*		
Stabilisers	*	*	*	*	*	*	*	*	*	*	*	*
Flavour and aroma enhancers				*			*	*		*		*
Antioxidants	*	*		*	*	*	*	*	*	*	*	*
Spice mixture	*	*	*		*	*	*	*	*	*	*	*
Emulsifiers			*							*		
Spice extracts	*			*		*	*	*	*			
Dextrose	*		*		*	*		*	*		*	
Glucose syrup			*		*				*		*	
Amaranth						*			*			
Aroma	*			*		*		*				
Thickener	*			*				*				
Acidity regulator	*							*				
Preservative	*			*				*				
Yeast extract	*											

*ingredients were listed on the package of the respective product; 1, 2, 3, 4, 5 – number of various samples of the type of product analysed in duplicates

Nevertheless, in our trial with fish mince this was not the case. Cáceres *et al.* (2008) discussed that the fat content played an important role in masking the fish taste. A higher total fat content seemed to be related to a less fishy taste in their study. In our case the spicing as well as the combination with a significant proportion of meat most probably created a good mixture masking a possible fish taste.

The ingredients used for our products are presented in Table 1, lists of ingredients of comparable

Table 3. Proximate composition of the fish separate and different products produced with it

	Fish separate	Sausage with carp separate			Pâté
		Frankfurter type	Vienna type	Barbecue	
Energy (kJ/100 g)	925.4	918.4	939.6	827.7	881.7
Carbohydrates (g/100 g) (± 5%)	3.59	6.21	3.82	2.95	5.61
Fat (g/100 g) (± 1.4%)	17.59	17.09	18.36	15.34	14.93
Protein (g/100 g) (± 1.4%)	12.56	10.62	11.49	12.35	13.76
Ash (g/100 g) (± 1.6%)	0.74	2.34	2.79	2.47	2.91
Dry matter (g/100 g) (± 0.2%)	34.48	36.26	36.46	33.11	37.22

doi: 10.17221/35/2015-CJFS

Table 4. Proximate composition of chosen meat products, data taken from Czech Food Database (Czech Centre for Food Composition Database 2013) and from one of the products bought from a local supermarket

	Carp raw	Fine Frankfurters	Pâté	Poultry Frankfurters	Barbecue type sausage[a]
Energy (kJ/100 g)	652	1230	1310	1010	1052
Carbohydrates (g/100 g)	1.3		1.7	3.7	1
Fat (g/100 g)	8.9	26.8	28.0	19.2	22
Protein (g/100 g)	17.7	12.9	14.2	13.9	13

[a]sample bought from a local supermarket

meat products are shown in Table 2. The proximate composition of newly developed products is shown in Table 3. Data on comparable products taken from the Czech Food Database (Czech Centre for Food Composition Database 2013) and from our own analyses is presented in Table 4.

All our products had a lower energy and fat content compared to comparable products on the market without compromising the taste (Tables 3 and 4). This is also an advantage as the proportion of fat intake is also an increasing problem leading to health problems in the western societies (Donahoo *et al*. 2008; Shikany *et al*. 2010).

FA composition and content of EPA and DHA in our products are shown in Table 5. For comparison literature data for pâté and analytical data of a representative collection of similar sausages are shown in Table 6. In traditional only meat products the only present n-3 FA was ALA with a percentage of 1.68–0.59%. We could not detect any EPA and DHA in traditional products. Our data is also supported by both different databases (Czech Centre for Food Composition Database 2013; National Food Administration 2008) as well as earlier analyses (Sampels *et al*. 2009) showing that these LC-PUFA are not usually found in these types of products. In opposite,

Table 5. Fatty acid composition (percentage of the total identified) of different types of meat products produced with the addition of carp separate (mean and standard deviation (n = 5)

	Frankfurter type sausages	Vienna type sausages	Barbecue type sausages	Pâté
Total fat	17.2 ± 0.69	19.9 ± 2.52	13.5 ± 2.57	16.4 ± 0.75
C14:0	1.46 ± 0.04	1.39 ± 0.02	1.37 ± 0.18	1.34 ± 0.02
C16:0	24.6 ± 0.40	23.5 ± 0.06	24.3 ± 0.24	23.5 ± 0.06
C16:1*trans*	0.09 ± 0.18	0.43 ± 0.01	0.40 ± 0.01	0.47 ± 0.01
C16:1	5.83 ± 0.13	5.45 ± 0.06	4.39 ± 0.04	4.22 ± 0.02
C18:0	10.9 ± 0.15	10.4 ± 0.09	12.3 ± 0.09	12.7 ± 0.06
C18:1*trans*	0.07 ± 0.09	0.1 ± 0.10	0.11 ± 0.09	0.17 ± 0.03
C18:1n-9	45.0 ± 0.76	43.1 ± 0.12	42.6 ± 0.26	40.7 ± 0.17
C18:1n-7	0.87 ± 1.52	3.41 ± 0.01	3.23 ± 0.04	3.31 ± 0.05
C18:2n-6	8.30 ± 0.17	7.87 ± 0.03	7.53 ± 0.05	9.21 ± 0.04
C18:3n-3	1.15 ± 0.02	1.09 ± 0.01	1.06 ± 0.02	1.05 ± 0.02
C20:0	0.15 ± 0.07	0.17 ± 0.01	0.19 ± 0.02	0.17 ± 0.01
C20:1n-9	0.48 ± 0.59	1.25 ± 0.01	1.13 ± 0.01	0.84 ± 0.42
C20:2n-6	0.38 ± 0.02	0.38 ± 0.01	0.31 ± 0.08	0.41 ± 0.01
C20:4n-6	0.44 ± 0.01	0.42 ± 0.01	0.41 ± 0.02	1.01 ± 0.01
C20:5n-3	0.22 ± 0.18	0.36 ± 0.01	0.33 ± 0.02	0.29 ± 0.01
C22:5n-3	0.00 ± 0.00	0.15 ± 0.01	0.00 ± 0.00	0.05 ± 0.10
C22:6n-3	0.14 ± 0.12	0.28 ± 0.02	0.31 ± 0.02	0.26 ± 0.02
SFA	37.1 ± 0.56	35.5 ± 0.07	38.2 ± 0.13	37.6 ± 0.09
MUFA	52.0 ± 0.48	53.2 ± 0.14	51.4 ± 0.29	49.2 ± 0.19
PUFA	10.7 ± 0.22	10.7 ± 0.02	10.1 ± 0.11	12.5 ± 0.18
n-3	1.56 ± 0.18	2.00 ± 0.03	1.77 ± 0.01	1.75 ± 0.15
n-6	9.12 ± 0.18	8.72 ± 0.02	8.34 ± 0.11	10.8 ± 0.08
EPA+DHA	0.36 ± 0.16	0.65 ± 0.03	0.64 ± 0.04	0.55 ± 0.02
n-6/n-3	5.91 ± 0.68	4.36 ± 0.07	4.72 ± 0.06	6.21 ± 0.49
trans FA	0.16 ± 0.16	0.55 ± 0.09	0.51 ± 0.09	0.65 ± 0.03

doi: 10.17221/35/2015-CJFS

Table 6. Fatty acid composition (%) of a variety of barbecue, Frankfurter and Vienna type sausages bought in local supermarkets

	Barbecue sausages				Frankfurter type sausages			Vienna type sausages				
	1	2	3	4	1	2	3	1	2	3	4	5
C14:0	1.88	1.35	1.38	1.39	1.53	1.44	1.56	1.49	1.48	1.33	1.58	1.40
C16:0	22.5	25.3	24.9	24.5	25.1	24.9	23.2	24.8	24.3	24.5	25.2	24.5
C16:1*trans*	0.42	0.33	0.38	0.38	0.33	0.35	0.46	0.34	0.38	0.37	0.33	0.36
C16:1	3.72	2.13	2.34	3.28	2.57	2.79	3.42	2.61	2.50	2.21	2.64	2.44
C18:0	7.95	15.3	13.8	10.7	13.8	12.7	9.52	13.4	12.4	14.2	13.6	13.8
C18:1n-9	37.8	42.6	40.6	41.4	43.3	41.6	41.1	41.7	39.4	42.4	43.7	42.6
C18:1n-7	2.33	2.92	3.06	3.00	3.23	3.37	3.04	3.11	3.04	3.04	3.31	3.28
C18:2n-6	19.9	7.55	10.5	12.7	7.21	10.0	14.7	9.45	13.5	9.07	7.01	8.94
C18:3n-3	1.68	0.59	0.97	1.03	0.62	0.82	0.97	0.76	1.09	0.80	0.60	0.76
C20:1n-9	0.54	0.90	0.81	0.70	0.84	0.79	0.87	0.86	0.73	0.88	0.84	0.89
C20:4n-6	0.40	0.29	0.33	0.31	0.26	0.32	0.35	0.32	0.39	0.25	0.24	0.31
SFA	32.3	42.0	40.2	36.5	40.4	39.0	34.3	39.7	38.2	40.0	40.4	39.7
MUFA	44.8	48.9	47.2	48.7	50.3	48.9	48.9	48.6	46.1	48.9	50.8	49.6
PUFA	22.0	8.4	11.8	14.0	8.09	11.2	16.1	10.5	14.9	10.1	7.85	10.0
n-3	1.68	0.59	0.97	1.03	0.62	0.82	0.97	0.76	1.09	0.80	0.60	0.76
n-6	20.3	7.84	10.8	13.0	7.47	10.3	15.1	9.77	13.8	9.31	7.25	9.25
n-6/n-3	12.1	13.3	11.2	12.6	12.1	12.6	15.6	12.8	12.8	11.7	12.1	12.2

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; n-3 – sum of omega-3 fatty acids; n-6 – sum of omega-6 fatty acids; 1, 2, 3, 4, 5 – number of various samples of the type of product analysed in duplicates

in our products the percentage of ALA was around 1 but there was between 0.36 and 0.22% of EPA and 0.31 and 0.14% of DHA. In addition, the n-6/n-3 ratio in our products was much lower compared to traditional meat products. In our products the ratio was varying from 4.4 to 6.2 while it was from 11.2 up to 15.6 in traditional products. This can mainly be attributed to a lower content of 18:2 n-6, which was between 7.5 and 9.2% in our products and 7.2–19.9% in traditional products. As the n-6 and n-3 FA are metabolized via the same enzymes (De Henauw *et al.* 2007; Palmquist 2009), a higher intake of n-6 will limit the metabolism of n-3 FA as well as the conversion of ALA to the longer chain n-3 PUFA (Palmquist 2009). Considering the metabolic competition between n-6 and n-3 FA (Palmquist 2009) and their opposing properties (Schmitz & Ecker 2008), an intake ratio of 1–4 is generally recommended (Simopoulos 2001, 2002).

The proportion of combined EPA and DHA was 74, 54, 109, and 77 mg/100g in barbecue sausage, Frankfurter type, Vienna type sausage, and pâté, respectively. This means 29.6, 21.6, 43.6, and 30.8% of the minimum daily recommended intake according to EFSA (2009). Our results show clearly that the addition of the fish separate to meat products

Table 7. Oxidation measured as malondialdehyde equivalents (μg/g, mean and standard deviation) in the different products over the time of 15-day storage at 4°C (n = 5)

	Frankfurter type sausage	Vienna type sausage	Barbecue type sausage	Pâté
Day 0	0.17 ± 0.02^{ab}	0.16 ± 0.01^{a}	0.82 ± 0.04^{a}	0.26 ± 0.02^{a}
Day 3	0.16 ± 0.01^{a}	0.21 ± 0.01^{b}	0.90 ± 0.03^{bc}	0.29 ± 0.02^{ab}
Day 5	0.20 ± 0.03^{bcd}	0.28 ± 0.03^{c}	0.87 ± 0.03^{ac}	0.30 ± 0.02^{bc}
Day 7	0.15 ± 0.05^{abd}	0.23 ± 0.03^{abc}	0.98 ± 0.08^{bd}	0.31 ± 0.05^{abd}
Day 10	0.23 ± 0.03^{c}	0.27 ± 0.03^{c}	0.94 ± 0.03^{d}	0.39 ± 0.03^{e}
Day 12	0.23 ± 0.05^{ce}	0.23 ± 0.04^{bc}	1.11 ± 0.08^{e}	0.34 ± 0.06^{bc}
Day 15	0.20 ± 0.02^{de}	0.23 ± 0.02^{b}	1.26 ± 0.06^{f}	0.33 ± 0.03^{dc}

Different superscript letters show significant differences within one product ($P < 0.05$)

doi: 10.17221/35/2015-CJFS

Table 8. Microbiological analyses of the sausages and pâté produced partially with fish separate

	Fish separate	Frankfurter type sausage			Vienna type sausage			Barbecue type sausage			Pâté		
	Day 0	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Total CFU	1.8×10^4	70	7×10^2	1.5×10^5	80	3.3×10^3	8.5×10^5	7×10^2	1.8×10^3	7.7×10^4	90	6.2×10^2	8.8×10^2
Salmonella ssp. CFU/25 g	negative												
E. coli CFU/g	< 10												
Listeria monocytogenes CFU/25 g	negative												

can result in products with an increased nutritional value, both with respect to the content of EPA and DHA as well as the n-6/n-3 ratio.

However, in general the oxidation of fat or oil is positively correlated to unsaturation. Especially fish, being rich in n-3 PUFA, is susceptible to peroxidation of PUFA resulting in the restriction of storage and processing possibilities (Jacobsen *et al.* 2008). Hence there is a risk of increased oxidation in products containing fish flesh. In spite of this, oxidation in our samples was low (Table 7). Earlier research found that 2 μg/g are noticeable as off-flavour by consumers (Younathan & Watts 1959). The values we found remained below 0.5 during the whole storage time in all products except for the barbecue type sausages where the values were higher already from the beginning (0.8) but remained below 1.5 μg after 15 days of storage. As the sausages were produced in different batches and with different spices, the higher oxidation in barbecue sausages could be explained by a possible higher oxidation in the raw materials. However, considering the low values found overall, we concluded that the products had a sufficient oxidative stability.

Microbial counts were also below the limits and the products had a comparable storage time to the traditional ones (Table 8). The general initial bacterial load was highest in the barbecue sausages being as low as 7×10^2 CFU (colony-forming units). All products also fulfilled the critical hygienic criteria for *Salmonella, Listeria*, and *E. coli*. According to the legislation the limit for *Salmonella* spp. and *Listeria monocytogenes* in ready to eat products is the absence in 25 g, and the satisfactory limit for *E. coli* in meat products is 500 CFU (European Commission 2005). After 14 days of storage *Salmonella* and *L. monocytogenes* were still negative and *E. coli* was below 10 CFU (Table 8).

CONCLUSIONS

We concluded that the mechanically separated meat of fish is a cheap and easily available raw product to increase the nutritional value of traditional meat products without compromising sensory and textural aspects or storage stability. In addition, the profitable use of the by-product fish separate will increase the total yield from the fish for fish producers and increase the business sustainability.

Acknowledgments. The authors wish to thank Ing. Kateřina Fulinová for her assistance with the chemical analyses and Willem Westra for the professional language correction.

References

Ailhaud G., Guesnet P. (2004): Fatty acid composition of fats is an early determinant of childhood obesity: a short review and an opinion. Obesity Revue, 5: 21–26.

Ailhaud G., Massiera F., Weill P., Legrand P., Alessandri J.-M., Guesnet P. (2006): Temporal changes in dietary fats: Role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Progress in Lipid Research, 45: 203–36.

Andres S.C., Zaritzky N.E., Califano A.N. (2009): Innovations in the development of healthier chicken sausages formulated with different lipid sources. Poultry Science, 88: 1755–1764.

Appelqvist L.-Å. (1968): Rapid methods of lipid extraction and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipid contaminants. Arkiv Kemi, 28: 551–570.

Becker W., Pearson M. (2003): Riksmaten 1997–98 Metod and results analysis. Uppsala, Swedish National Food Administration. (in Swedish)

doi: 10.17221/35/2015-CJFS

Cáceres E., García M.L, Selgas M.D. (2008): Effect of pre-emulsified fish oil – as source of PUFA n-3 – on microstructure and sensory properties of mortadella, a Spanish bologna-type sausage. Meat Science, 80: 183–193.

Calder P.C, Grimble R.F. (2002): Polyunsaturated fatty acids, inflammation and immunity. European Journal of Clinical Nutrition, 56 (Supplement 3): S14–S19.

Connor W.E. (2000). Importance of n-3 fatty acids in health and disease. The American Journla of Clinical Nutrition, 71: 171S–175S.

Cultured Aquatic Species Information Programme *Cyprinus carpio* (Linnaeus, 1758) (2014): Available at www.fao.org/fishery/culturedspecies/Cyprinus_carpio/en. (accessed June 18, 2014).

Czech Centre for Food Composition Database (2013): Online Czech Food Composition Database, Version 4.13. Czech Centre for Food Composition Database. Prague, Institute of Agricultural Economics and Information and Food Research Institute.

Czech Statistical Office (2013): Statistical Yearbook of the Czech Republic. Klatovy, Dragon Press.

De Henauw S., Van Camp J., Sturtewagen G., Matthys C., Bilau M., Warnants N., Raes K., Van Oeckel M., De Smet S. (2007): Simulated changes in fatty acid intake in humans through n-3 fatty acid enrichment of foods from animal origin. Journal of Science of Food Agriculture, 87: 200–211.

Donahoo W., Wyatt H.R., Kriehn J., Stuht J., Dong F., Hosokawa P., Grunwald G.K., Johnson S.L., Peters J.C., Hill J.O. (2008): Dietary fat increases energy intake across the range of typical consumption in the united states. Obesity, 16: 64–69.

Eaton S.B, Eaton I., Stanley B. (2003): An evolutionary perspective on human physical activity: implications for health. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 136: 153–159.

EFSA (2009): Scientific opinion – Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. EFSA Journal, 1176: 1–11.

European Commission (2005): Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, L 338.

FAO (2012): The State of World Fisheries and Aquaculture. Rome.

Garcia A., Izquierdo P., Uzcategui-Bracho S., Faria J.F., Allara M., Garcia A.C. (2005): The tuna and beef formulate sausage: Its life span and acceptability. Revista Cientifica-Facultad De Ciencias Veterinarias, 15: 272–278.

Garemo M., Arvidsson Lenner R., Strandvik B. (2007): Swedish preschool children eat to much junk food and sucrose. Acta Paediatrica, 96: 266–272.

Hara A., Radin N.S. (1978): Lipid extraction of tissues with low toxicity solvent. Analytical Biochemistry, 90: 420–426.

Howe P., Meyer B., Record S., Baghurst K. (2006): Dietary intake of long-chain [omega]-3 polyunsaturated fatty acids: contribution of meat sources. Nutrition, 22: 47–53.

Intarasirisawat R., Benjakul S., Visessanguan W., Wu J. (2014): Effects of skipjack roe protein hydrolysate on properties and oxidative stability of fish emulsion sausage. LWT-Food Science and Technology, 58: 280–286.

Jacobsen C., Undeland I., Storro I., Rustad T., Hedges N., Medina I. (2008): Preventing lipid oxidation in seafood. In: Børresen T. (ed.): Improving Seafood Products for the Consumer. Cambridge, Woodhead Publishing; 426–460.

Marchetti L., Andrés S.C., Califano A.N. (2013): Textural and thermal properties of low-lipid meat emulsions formulated with fish oil and different binders. LWT-Food Science and Technology, 51: 514–523.

Marchetti L., Andres S.C., Califano A.N. (2014). Low-fat meat sausages with fish oil: Optimization of milk proteins and carrageenan contents using response surface methodology. Meat Science, 96: 1297–1303.

Martinez B., Miranda J.M., Vazquez B.I., Fente C.A., Franco C.M., Rodriguez J.L., Cepeda A. (2012): Development of a Hamburger Patty with healthier lipid formulation and study of its nutritional, sensory, and stability properties. Food and Bioprocess Technology, 5: 200–208.

Miller D.D. (1998): Food Chemistry. A Laboratory Manual. New York, Wiley Interscience.

Murphy S.C., Gilroy D., Kerry J.F., Buckley D.J., Kerry J.P. (2004): Evaluation of surimi, fat and water content in a low/no added pork sausage formulation using response surface methodology. Meat Science, 66: 689–701.

National Food Administration (2008): Livsmedelstabeller/Specialtabeller, fatty acids. [Hompage] Uppsala, National Food Administration. Available at http://www.slv.se/upload/dokument/mat/ldb/Fettsyror.xls (accessed July 15, 2008).

Palmquist D.L. (2009): Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. Professional Animal Scientist, 25: 207–249.

Park J., Rhee K.S., Keeton J.T., Rhee K.C. (1989): Properties of low-fat Frankfurters containing monounsaturated and omega-3 polyunsaturated oils. Journal of Food Science, 54: 500–504.

Pickova J., Morkore T. (2007): Alternative oils in fish feeds. European Journal of Lipid Science and Technology, 109: 256–263.

Ramírez J.A., Uresti R.M., Velazquez G., Vázquez M. (2011): Food hydrocolloids as additives to improve the mechanical and functional properties of fish products: a review. Food Hydrocolloids, 25: 1842–1852.

doi: 10.17221/35/2015-CJFS

Richardson A.J. (2006): Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. International Review of Psychiatry, 18: 155–172.

Sampels S., Strandvik B., Pickova J. (2009): Processed animal products with emphasis on polyunsaturated fatty acid content. European Journal of Lipid Science and Technology, 111: 481–488.

Schmitz G., Ecker J. (2008): The opposing effects of n-3 and n-6 fatty acids. Progress in Lipid Research, 47: 147–155.

Shikany J.M., Vaughan L.K., Baskin M.L., Cope M.B., Hill J.O., Allison D.B. (2010): Is dietary fat "fattening"? A comprehensive research synthesis. Critical Reviews in Food Science and Nutrition, 50: 699–715.

Simopoulos A.P. (2001): Evolutionary aspects of diet and essential fatty acids. In: Hamazaki T., Okuyama H. (eds): Fatty Acids and Lipids – New Findings. Basel, Karger: 18–27.

Simopoulos A.P. (2002): The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy, 56: 365–379.

Simopoulos A.P. (2006): Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomedicine & Pharmacotherapy, 60: 502–507.

Sioen I., Matthys C., De Backer G., Van Camp J., De Henauw S. (2007): Importance of seafood as nutrient source in the diet of Belgian adolescents. Journal of Human Nutrition and Dietetics, 20: 580–589.

Storlien L.H., Kriketos A.D., Calvert G.D., Baur L.A., Jenkins A.B. (1997): Fatty acids, triglycerides and syndromes of insulin resistance. Prostaglandins Leukotrienes and Essential Fatty Acids, 57: 379–385.

Weylandt K., Kang J. (2005): Rethinking lipid mediators. Lancet, 366: 618–620.

Williams C.M. (2000): Dietary fatty acids and human health. Annals of Zoology, 49: 165–180.

Younathan M.T., Watts B.M. (1959): Relationship of meat pigments to lipid oxidation. Food Technology, 24: 728–734.

Received: 2015–01–20
Accepted after corrections: 2015–06–22

Corresponding author:

MSc. Sabine Sampels, Ph.D., Jihočeská univerzita v Českých Budějovicích, Fakulta rybářství a ochrany vod, Laboratoř výživy, Husova tř. 458/102, 370 05 České Budějovice, Česká republika; E-mail: sampels@frov.jcu.cz