JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fatty Acids and Fat-Soluble Vitamins in Salted Herring (*Clupea harengus*) Products

TARJA L. ARO,† PETRA S. LARMO,† CHRISTINA H. BÄCKMAN,‡ HEIKKI P. KALLIO,† AND RAIJA L. TAHVONEN*,†

Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland, and National Veterinary and Food Research Institute, P.O. Box 45, FIN-00581, Helsinki, Finland

The fatty acid composition and contents of fat and fat-soluble vitamins of three salted products prepared from Icelandic herring were analyzed. The effects of storage on the products over their shelf life, 6 or 12 months, were investigated. The average oil content of salted, gutted herring and salted fillets in vacuum remained constant, 17 and 12% of wet weight, respectively. In the pickled product the oil content decreased during the 12 months of storage from 13 to 12%. The composition of the products was typical for herring, the most abundant fatty acids being oleic (18:1*n*−9), palmitic (16:0), cetoleic (22:1*n*−11), and gadoleic (20:1*n*−9) acids. Monounsaturated acids constituted clearly the main group with a proportion of >50% of all fatty acids. Eicosapentaenoic acid (EPA, 20:5*n*−3) and docosahexaenoic acid (DHA, 22:6*n*−3) comprised together >12% of all fatty acids. During storage, some hydrolysis of triacylglycerol (TAG) occurred, causing a slight reduction in practically all esterified fatty acids. In none of the three products was the loss of polyunsaturated fatty acids from TAG greater than the loss of saturated ones, indicating that the loss of EPA and DHA was not due to oxidation. After packing, the average content of vitamins A, D, and E in the products varied between 27 and 87 μg/100 g (wet weight), between 17−28 μg/100 g (wet weight), and between 77−120 μg/100 g (wet weight), respectively. During storage, the level of vitamin A decreased significantly, whereas no loss of vitamin D was observed. The content of vitamin E was low in all products and showed wide variation. When compared to the recommended daily intake, it could be concluded that the products investigated were good and stable sources of long-chain *n*−3 fatty acids (EPA, DHA) and vitamin D.

KEYWORDS: Herring; fish product; fatty acids; vitamins; storage

INTRODUCTION

The nutritional importance of fish is universally acknowledged. Especially fatty fish is rich in polyunsaturated long-chain *n*−3 fatty acids (PUFA). The PUFA in fish seem to have a beneficial effect on health by, for example, decreasing the risk of stroke, reducing serum triacylglycerol levels, reducing blood pressure, and insulin resistance and modulating the glucose metabolism (*1*). Moreover, fatty fish is an important source of fat-soluble vitamins, especially vitamin D. The principal physiological function of vitamin D is to maintain calcium concentrations within a physiologically acceptable range (*2*). In addition, vitamin D has a role in the activity of many enzymes (*3*), proliferation of colon cells (*4*), and function of the nervous system (*5*).

One of the fattiest fish in northern waters is the Atlantic herring (*Clupea harengus*) (*6*). It is caught in the North Atlantic from Iceland, southern Greenland and northern Norway to northern Spain and the coastal areas of North America (*7*). Due to the large living area and seasonal variation, its lipid content can exceed 20% or be <8% (*8*−*10*). A characteristic feature of herring oil is the high amount of long-chain polyunsaturated fatty acids, which makes herring susceptible to oxidation (*11*) and may limit processing and storage.

Atlantic herring is not normally consumed as round fish or fillets as most of the catch is processed before sale. Salting, spice-salting, and marinating are typical ways to process herring in Europe (*11*). The primary purpose of salting is preservation but, at the same time, the ripening continues, giving the product its typical taste, odor, and texture (*12*). Fully matured salted herring can be used, for example, as a raw material for pickled products. In Finland, ∼1500−1900 tons of salted Atlantic herring is processed annually. The major amount of herring is caught and salted in Iceland and imported in barrels to Finland (*13*, *14*). In Finland, the herring is further processed into many types of semipreserves, the shelf lives of which can be several months.

The ripening process of herring during salting has been widely investigated (*12*, *15*, *16*). However, there is a lack of information concerning the chemical composition of salted and marinated

* Author to whom correspondence should be addressed (telephone +358-2-333 6844; fax +358−333 6860; e-mail raija.tahvonen@utu.fi).
† University of Turku.
‡ National Veterinary and Food Research Institute.

10.1021/jf0401221 CCC: $30.25
Published on Web 02/11/2005

Table 1. Fat and Moisture Contents in Salted Herring Products[a]

	gutted herring		fillets		pickled herring	
sample	fat (w/w %)	moisture (%)	fat (w/w %)	moisture (%)	fat (w/w %)	moisture (%)
raw material	16.4 ± 1.0 ab	57.7 ± 1.2 a	12.0 ± 1.4 a	62.2 ± 1.2 a	13.8 ± 0.7 a	57.5 ± 0.6 a
0 months	16.0 ± 0.5 a	58.0 ± 0.8 a	12.0 ± 1.0 a	62.3 ± 0.9 a	13.1 ± 0.6 a	57.0 ± 1.4 ab
3 months	17.2 ± 1.0 b	57.3 ± 1.0 a	11.8 ± 0.3 a	62.1 ± 0.1 a		
6 months	17.1 ± 0.6 b	58.3 ± 2.9 a	11.9 ± 0.6 a	62.0 ± 0.8 a	11.8 ± 1.3 b	56.3 ± 0.7 b
12 months					11.5 ± 0.4 b	60.1 ± 0.2 c

[a] For each parameter, different letters in the same column denote significant differences among samples ($p < 0.05$).

herring products. Thus, the aim of this study was to investigate the fatty acid composition and content of fat-soluble vitamins in three salted herring products. In addition, the effect of storage on the products over their shelf lives, 6 and 12 months, was investigated.

MATERIALS AND METHODS

Sample Collection and Preparation. Three salted herring products and their raw materials were analyzed. The raw materials (autumn herring) were caught and placed into barrels in Iceland and further processed at a local fish factory in Finland (Boyfood Oy, Rymättylä). All of the raw materials had passed a long ripening process (~6 months) in barrels and were fully matured. The products were light-salted, gutted herring in vacuum (raw material: sugar-salted, gutted herring in barrel), light-salted herring fillets in vacuum (raw material: sugar-salted fillet in barrel), and pickled herring pieces in glass jars (raw material: spice-salted fillet in barrel). The salt contents of the products were 7.5, 7.0, and 7.0%, respectively. The gutted herring was headless but contained backbone and skin, which are typically removed before eating. The shelf lives defined by the fish factory were 6 months for gutted herring and fillets and 12 months for pickled herring. Three batches of each product were analyzed. Samples were taken from the raw material as well as from all of the products immediately after packing, at 3 and 6 months (gutted herring and fillets) or at 6 and 12 months (pickled herring).

Before analyses, the pickle was removed from the products. The fish were then placed on a sieve, and the fluid was allowed to flow through. After that, the samples were prehomogenized using a Robot Coupe R8 (Robot Coupe S.A., Bourgogne, France), and the final homogenizing was done using a Moulinette (Moulinex, Ecully Cedex, France) homogenizer. For each product two samples of every batch at different stages of storage were homogenized. One sample consisted of 5−10 gutted herring (~1500−3000 g), 7−20 fillets (~600−1800 g), or 12 jars of pickled herring (~1080 g) depending on the samples available. The homogenized samples were stored at −70 °C (lipid and vitamin samples) or at −20 °C (moisture samples) until analyzed.

Extraction of Lipids. Lipids were extracted according to the modified Folch method (*17*) as described by Aro et al. (*18*). After gravimetric quantifying, the extracted lipid fraction was totally dissolved in hexane containing 0.02% butylated hydroxytoluene. The headspace volume of the sample vials was replaced with nitrogen, and the vials were stored at −70 °C for esterification. Two subsamples of each homogenized sample were extracted. The accuracy of the lipid content analysis was checked by using a commercial reference material (Mackerel Paste, Certified Reference Material LGC7101, Laboratory of the Government Chemists, Teddington, U.K.).

Fatty Acid Analysis. Fatty acid methyl esters were prepared by using a base-catalyzed transesterification according to that of Christie (*19*). For quantitative analysis an internal standard glyceryl trinonadecanoate (Larodan, Malmö, Sweden) was added to the samples prior to transesterification. Fatty acid methyl esters were analyzed using a Perkin-Elmer AutoSystem gas chromatograph (Norwalk, CT) equipped with a flame ionization detector, a split injector, and a fused silica capillary column (Supelcowax-10, 30 m × 0.32 mm i.d., 0.25 μm film thickness, Supelco, Bellefonte, PA). The temperature program was 2 min at 120 °C, raised at 4 °C/min to 200 °C, 55 min at 200 °C, raised at 10 °C/min to 240 °C, and 10 min at 240 °C. The temperatures of the injector and detector were 225 and 240 °C, respectively. Helium was used as the carrier gas with a split ratio of 40:1, and the flow rate through the column was 1.2 mL/min. Fatty acids were identified using mixtures of commercial fatty acid methyl esters (68 D, Nu Chek Prep, Elysian, MN; and Supelco 37 Component FAME Mix, Supelco, Bellefonte, PA) and single methyl ester reference compounds (methyl *cis*-11-eicosenoate and methyl *cis*-13-docosenoate: Larodan, Malmö, Sweden). To follow the possible hydrolysis of fatty acids from triacylglycerols (TAG) the fatty acids were also analyzed by the boron trifluoride method (*20*) using the same analytical procedure as above.

Moisture Analysis. Moisture was determined by drying homogenized fish samples (6 g) at 105 °C for 16−18 h.

Vitamin Analysis. Saponification and extraction of vitamins A and E were performed according to the method of Ollilainen et al. (*21*). The determination of vitamin D was performed using internal standard according to NMKL Method 167 (*22*). This method includes saponification, extraction, purification using straight phase HPLC, and finally separation of vitamin D with reversed-phase HPLC. Standard solutions were freshly made for each sample set.

Vitamins A and E were determined simultaneously using a Waters 510 HPLC pump (Milford, MA), a Waters 717 autosampler, a Waters 486 UV detector operated at 325 nm, and a Waters 996 fluorescence detector programmed for excitation at 295 and emission at 330 nm. Signals from both detectors were recorded by the Waters Empower chromatographic data system. The chromatographic separation was performed with a Merck Lichrosorb Si60 column (5 μm. 4.6 mm × 25 mm, Merck, Darmstadt, Germany). The solvent consisted of *n*-hexane/2-propanol (97.5:2.5). The flow rate was 1 mL/min.

Vitamin D was determined with a UV detector (Milford, MA) set at 265 nm. Fractions from a Waters μPoracil normal phase column (3.9 mm × 300 mm, Milford, MA) were collected with a Gilson FC203 fraction collector (Gilson Medical Electronics, Milwaukee, WI). The solvent consisted of *n*-hexane/2-propanol/tetrahydrofuran (98:1:1). The flow rate was 1 mL/min. The final separation of vitamin D was performed using a Vydac TP 201TP54 reversed phase column (4.7 mm × 250 mm, Grace Vydac, Hesperia, CA) with a solvent consisting of methanol/water (97:6) and the flow rate set to 1 mL/min.

Intratest reproducibilities calculated as CV% were 4.1 for vitamin E, 3.3 for vitamin A, and 6.6 for vitamin D. The limits of detection were 0.04 μg/100 g, 20 μg/100 g, and 0.2 mg/100 g for vitamins D, A and E, respectively.

Statistical Analysis. Statistical analysis was performed using SPSS software, version 10.0. Comparisons of fat content, fatty acid compositions, and moisture content between different stages of storage were tested using an ANOVA test. The differences in vitamin contents between unstored and stored products were tested using independent samples *t* tests. When the assumptions for the ANOVA test were not fulfilled, Kruskal−Wallis and Mann−Whitney *U* tests were used.

RESULTS AND DISCUSSION

Moisture and Fat Contents. The water content ranged between 57 and 60% in the gutted herring and pickled product (**Table 1**). In fillets, the content was slightly higher, ~62%. The average fat contents of gutted herring and fillets were 17 and 12% of wet weight, respectively, the contents remaining quite constant during storage. The contents were similar to those reported for salted Icelandic herring caught in autumn (*12*, *15*).

Table 2. Fatty Acids in Salted, Gutted Herring[a]

	raw material		storage time of the product					
	sugar-salted, gutted herring		0 months		3 months		6 months	
fatty acid	g/100 g of fat	% of all FAs	g/100 g of fat	%	g/100 g of fat	%	g/100 g of fat	%
14:0	5.3 ± 0.4 a	6.4	5.4 ± 0.4 a	6.5	5.1 ± 0.3 a	6.5	5.2 ± 0.3 a	6.6
16:0	14.3 ± 0.8 a	17.1	14.0 ± 0.9 ab	16.8	13.5 ± 0.6 ab	17.2	13.3 ± 0.7 b	16.8
18:0	1.2 ± 0.1 a	1.4	1.2 ± 0.1 a	1.4	1.1 ± 0.0 ab	1.4	1.1 ± 0.1 b	1.4
others	0.7 ± 0.0	0.8	0.7 ± 0.1	0.8	0.7 ± 0.0	0.8	0.7 ± 0.0	0.8
Σ SAFA	21.5 ± 0.8 a	25.7	21.3 ± 1.3 ab	25.5	20.4 ± 0.8 bc	26.0	20.2 ± 0.8 c	25.7
16:1*n*–7	4.8 ± 0.3 ab	5.8	4.9 ± 0.3 a	5.8	4.6 ± 0.2 b	5.8	4.5 ± 0.2 c	5.7
18:1*n*–9	15.0 ± 1.0 a	17.9	15.0 ± 1.0 a	18.0	14.3 ± 0.7 a	18.1	14.6 ± 1.0 a	18.5
18:1*n*–7	3.0 ± 0.2 a	3.6	2.9 ± 0.2 ab	3.4	2.7 ± 0.1 b	3.4	2.9 ± 0.2 ab	3.6
20:1*n*–9	7.0 ± 0.9 a	8.4	7.4 ± 0.9 a	8.9	6.7 ± 0.5 a	8.5	7.1 ± 0.6 a	9.0
22:1*n*–11	10.4 ± 1.3 a	12.4	11.0 ± 1.5 a	13.2	9.9 ± 0.9 a	12.5	10.5 ± 0.9 a	13.3
others	2.3 ± 0.1	2.7	2.3 ± 0.2	2.8	2.1 ± 0.2	2.7	2.2 ± 0.1	2.8
Σ MUFA	42.6 ± 2.0 a	50.9	43.5 ± 2.8 a	52.1	40.2 ± 1.9 b	51.1	41.7 ± 1.0 ab	53.0
18:2*n*–6	1.1 ± 0.0 a	1.4	1.1 ± 0.1 ab	1.4	1.1 ± 0.1 bc	1.4	1.0 ± 0.0 c	1.3
18:4*n*–3	2.4 ± 0.3 a	2.9	2.2 ± 0.2 ab	2.6	2.3 ± 0.1 a	2.9	2.0 ± 0.2 b	2.6
20:5*n*–3	5.8 ± 0.4 a	7.0	5.5 ± 0.4 ab	6.5	5.3 ± 0.2 bc	6.7	5.0 ± 0.3 c	6.3
22:6*n*–3	6.1 ± 0.5 a	7.3	5.8 ± 0.4 ab	7.0	5.6 ± 0.2 b	7.1	4.9 ± 0.3 c	6.3
others	1.5 ± 0.1	1.8	1.5 ± 0.1	1.8	1.4 ± 0.1	1.8	1.3 ± 0.1	1.7
Σ PUFA	17.1 ± 0.9 a	20.4	16.1 ± 1.1 b	19.3	15.6 ± 0.5 b	19.9	14.3 ± 0.8 c	18.2
unidentified	2.5 ± 0.2 ab	3.0	2.6 ± 0.2 a	3.1	2.4 ± 0.1 b	3.0	2.5 ± 0.1 ab	3.2
Σ all fatty acids	83.7 ± 2.4 a	100	83.6 ± 4.9 a	100	78.7 ± 2.7 b	100	78.7 ± 2.3 b	100
n–3/*n*–6 ratio	9.4 ± 0.7 a		9.0 ± 0.5 ab		9.2 ± 0.5 a		8.6 ± 0.3 b	

[a] For each parameter, different letters in the same row denote significant differences among samples ($p < 0.05$).

Table 3. Fatty Acids in Salted Herring Fillet[a]

	raw material		storage time of the product					
	sugar-salted fillet		0 months		3 months		6 months	
fatty acid	g/100 g of fat	% of all FAs	g/100 g of fat	%	g/100 g of fat	%	g/100 g of fat	%
14:0	5.4 ± 0.5 ab	6.2	5.5 ± 0.3 a	6.3	5.1 ± 0.4 ab	6.2	5.2 ± 0.2 b	6.5
16:0	14.8 ± 1.5 ab	17.1	15.0 ± 0.8 a	17.2	14.2 ± 1.0 ab	17.2	13.5 ± 0.7 b	17.0
18:0	1.2 ± 0.1 a	1.4	1.2 ± 0.1 a	1.4	1.2 ± 0.0 a	1.4	1.1 ± 0.1 b	1.4
others	0.7 ± 0.1	0.8	0.7 ± 0.0	0.8	0.7 ± 0.0	0.8	0.6 ± 0.0	0.8
Σ SAFA	22.1 ± 2.0 ab	25.6	22.4 ± 1.1 a	25.7	21.2 ± 1.5 ab	25.7	20.4 ± 0.8 b	25.7
16:1*n*–7	4.9 ± 0.5 a	5.7	5.0 ± 0.3 a	5.7	4.9 ± 0.4 a	5.9	4.7 ± 0.3 a	5.9
18:1*n*–9	16.4 ± 2.2 a	18.9	16.1 ± 1.3 a	18.4	15.9 ± 0.8 a	19.3	15.1 ± 0.8 a	19.0
18:1*n*–7	3.2 ± 0.5 ab	3.8	3.2 ± 0.3 a	3.7	3.1 ± 0.2 ab	3.7	2.9 ± 0.2 b	3.6
20:1*n*–9	7.6 ± 0.7 a	8.8	7.7 ± 0.5 a	8.8	7.3 ± 0.3 a	8.9	7.3 ± 0.4 a	9.2
22:1*n*–11	11.4 ± 1.2 a	13.3	11.6 ± 0.6 a	13.2	10.9 ± 0.4 a	13.3	11.0 ± 0.7 a	13.9
others	2.4 ± 0.1	2.8	2.4 ± 0.1	2.8	2.3 ± 0.1	2.8	2.3 ± 0.1	2.9
Σ MUFA	45.9 ± 2.8 a	53.3	46.0 ± 2.6 a	52.6	44.4 ± 2.0 ab	53.9	43.2 ± 1.5 b	54.5
18:2*n*–6	1.1 ± 0.1 ab	1.3	1.1 ± 0.0 b	1.3	1.0 ± 0.1 ac	1.3	1.0 ± 0.0 c	1.3
18:4*n*–3	2.0 ± 0.3 ab	2.4	2.2 ± 0.2 b	2.5	1.9 ± 0.1 ac	2.2	1.7 ± 0.1 c	2.2
20:5*n*–3	5.1 ± 0.6 ab	6.0	5.3 ± 0.3 b	6.1	4.8 ± 0.3 ac	5.8	4.4 ± 0.3 d	5.6
22:6*n*–3	5.7 ± 0.5 a	6.7	6.2 ± 0.3 b	7.2	5.2 ± 0.3 c	6.3	4.8 ± 0.4 c	6.0
others	1.3 ± 0.1	1.5	1.4 ± 0.1	1.6	1.3 ± 0.1	1.5	1.2 ± 0.1	1.5
Σ PUFA	15.3 ± 1.2 a	17.8	16.2 ± 0.7 a	18.6	14.2 ± 0.7 b	17.2	13.1 ± 0.9 c	16.5
unidentified	2.8 ± 0.3 a	3.3	2.7 ± 0.2 ab	3.1	2.6 ± 0.1 ab	3.2	2.6 ± 0.1 b	3.3
Σ all fatty acids	86.1 ± 5.1 ab	100	87.4 ± 4.1 b	100	82.4 ± 4.0 ac	100	79.3 ± 2.6 c	100
n–3/*n*–6 ratio	9.1 ± 1.0 abc		9.3 ± 0.6 a		8.6 ± 0.4 bc		8.4 ± 0.3 c	

[a] For each parameter, different letters in the same row denote significant differences among samples ($p < 0.05$).

In the pickled product, the fat content was ~13% just after manufacturing but decreased during 12 months of storage to 12%, with some oil possibly being released into the pickling sauce. Engvang and Nielsen (*23*) reported that the fat content of sugar-salted herring decreased from 18.9 to 15.3% during 26 weeks of storage.

In general, the oil content of herring is highly dependent on the catching area, season, and sexual maturation. Herring stores large quantities of lipids during the feeding season and uses these as an energy source when the food supply is at minimum (*24*, *25*). Usually the lowest oil values occur at spawning time or thereafter due to the increased consumption of fat reserves (*9*, *26*, *27*). In contrast to lean fishes, which store fat in the liver, herring stores fat in the flesh and under the skin (*28*). This explains the higher fat values measured in gutted herring compared to other products made from skinless raw material.

Fatty Acid Composition. The composition of esterified fatty acids of raw materials and the three products are shown in **Tables 2**−**4**.

Raw Materials and Nonstored Products. The most abundant fatty acids were oleic (18:1*n*−9), palmitic (16:0), cetoleic (22:1*n*−11), and gadoleic (20:1*n*−9) acids. Monounsaturated

Table 4. Fatty Acids in Pickled Herring[a]

	raw material		storage time of the product					
	spice-salted fillet		0 months		6 months		12 months	
fatty acid	g/100 g of fat	% of all FAs	g/100 g of fat	%	g/100 g of fat	%	g/100 g of fat	%
14:0	6.3 ± 0.5 a	7.1	5.8 ± 0.5 ab	7.3	5.5 ± 0.5 b	7.2	4.4 ± 0.3 c	6.0
16:0	14.0 ± 1.0 a	16.0	12.5 ± 1.0 bc	15.7	12.4 ± 0.5 b	16.3	11.8 ± 0.5 c	16.1
18:0	1.0 ± 0.1 ac	1.2	0.9 ± 0.1 b	1.2	1.0 ± 0.1 ab	1.3	1.1 ± 0.0 c	1.5
others	0.7 ± 0.1	0.8	0.6 ± 0.1	0.8	0.7 ± 0.0	0.9	0.6 ± 0.0	0.9
Σ SAFA	22.0 ± 1.5 a	25.1	19.9 ± 1.6 b	25.0	19.5 ± 1.6 b	25.5	17.9 ± 0.8 c	24.4
16:1*n*–7	5.0 ± 0.4 a	5.7	4.5 ± 0.4 b	5.6	4.3 ± 0.1 b	5.7	3.9 ± 0.2 c	5.4
18:1*n*–9	12.6 ± 1.2 a	14.3	11.0 ± 1.0 b	13.7	12.3 ± 0.6 ab	16.2	13.5 ± 0.6 a	18.4
18:1*n*–7	2.8 ± 0.2 a	3.2	2.4 ± 0.3 b	3.0	2.3 ± 0.2 b	3.1	2.5 ± 0.1 b	3.4
20:1*n*–9	9.4 ± 0.7 a	10.7	8.9 ± 1.0 a	11.1	7.7 ± 0.9 b	10.1	7.0 ± 0.2 b	9.5
22:1*n*–11	15.2 ± 1.5 a	17.3	14.2 ± 1.8 a	17.8	11.9 ± 1.4 b	15.6	10.8 ± 0.4 b	14.8
others	2.5 ± 0.2	2.8	2.3 ± 0.2	2.9	2.1 ± 0.1	2.8	2.1 ± 0.1	2.9
Σ MUFA	47.4 ± 3.5 a	54.0	43.3 ± 3.9 ab	54.1	40.8 ± 1.3 b	53.3	39.8 ± 0.8 b	54.4
18:2*n*–6	1.1 ± 0.1 a	1.3	1.0 ± 0.1 b	1.2	1.0 ± 0.0 b	1.3	1.0 ± 0.0 b	1.3
18:4*n*–3	2.2 ± 0.2 a	2.6	2.0 ± 0.2 ab	2.6	2.0 ± 0.2 b	2.6	1.7 ± 0.1 c	2.3
20:5*n*–3	5.4 ± 0.4 a	6.2	4.9 ± 0.5 ab	6.1	4.4 ± 0.3 b	5.8	4.4 ± 0.1 b	6.0
22:6*n*–3	5.4 ± 0.4 a	6.2	4.9 ± 0.4 b	6.1	4.8 ± 0.3 b	6.3	4.8 ± 0.3 b	6.5
others	1.3 ± 0.1	1.4	1.2 ± 0.1	1.5	1.3 ± 0.1	1.7	1.2 ± 0.1	1.7
Σ PUFA	15.5 ± 1.1 a	17.6	14.0 ± 1.3 b	17.5	13.5 ± 0.5 b	17.7	13.1 ± 0.5 b	17.9
unidentified	2.9 ± 0.3 a	3.3	2.7 ± 0.3 ab	3.4	2.5 ± 0.1 bc	3.3	2.4 ± 0.1 c	3.2
Σ all fatty acids	87.9 ± 6.1 a	100	79.9 ± 7.0 b	100	76.2 ± 2.3 b	100	73.0 ± 1.9 c	100
n–3/*n*–6 ratio	9.3 ± 0.4 a		9.1 ± 0.3 a		8.6 ± 0.5 b		8.3 ± 0.2 b	

[a] For each parameter, different letters in the same row denote significant differences among samples ($p < 0.05$).

acids clearly constituted the main group with proportions of 51−54% of all fatty acids. The high amount of monounsaturated fatty acids and the especially high level of cetoleic acid are typical for Atlantic herring (*29*−*33*). It has been estimated that herring obtains 20:1 and 22:1 acids from small zooplanktonic crustacea, which are rich in the corresponding fatty alcohols in their wax esters (*34*−*36*).

The total amount of polyunsaturated fatty acids was ∼17−20% of all fatty acids. Within this group, the major fatty acids were eicosapentaenoic acid (EPA, 20:5*n*−3) and docosahexaenoic acid (DHA, 22:6*n*−3). According to previous studies, the proportions of EPA and DHA in fillets of Atlantic herring may vary from 3.7 to 8.0% and from 3.9−9.6%, respectively (*6*, *9*, *37*). Thus, the proportions (6−7%) measured in this study fitted well within this range.

In the case of gutted herring and salted fillets, the fatty acid compositions of raw materials and nonstored products were quite similar. During the processing of pickled herring the total levels of fatty acids decreased significantly. Slicing the fillets and placing them into glass jars seemed to have a significant effect on fatty acid composition.

Some differences could be noted in the fatty acid compositions among the products. Pickled herring pieces contained less oleic acid and more cetoleic acid than salted fillets. Both products were prepared from herring fillets, and the difference was already observed in the raw material. The total amount of monounsaturates was slightly lower and polyunsaturates slightly higher in gutted fish than in the fillets or pickled fish. The same phenomena have been previously found in Baltic herring (*18*). The level of PUFA in herring skin is not higher than in light or dark muscles (*28*), but the parts removed during filleting may be rich in polyunsaturates. Differences may also in part be due to a natural variation between two raw materials not of the same lot.

Storage. In all products, the total amount of esterified fatty acids analyzed decreased during storage. Esterified fatty acids comprised ∼80−87% of total lipids just after packing, but at the end of the storage the proportion was ∼79% in gutted herring and fillets and ∼73% in the pickled herring. In gutted herring and fillets the change was most pronounced in the group of polyunsaturated fatty acids: Decreases of 11% in gutted herring and 19% in fillets were observed when calculated using the grams per 100 g of fat values. However, these values did not differ significantly from the corresponding values among the saturated fatty acids. In the pickled product, again, the decrease was greatest in saturated fatty acid (10%), whereas the amount of polyunsaturates decreased by ∼7%. When all three products were compared, no clear trend could be observed in individual fatty acids. For example, the level of EPA decreased in salted fillets after 3 months of storage and in gutted herring after 6 months storage, whereas in pickled pieces no decrease was observed during the entire shelf life. The decrease in the absolute amounts indicates that some hydrolysis has occurred during storage. The results of the boron trifluoride method showed that the total amounts and proportions of fatty acids remained unchanged throughout the whole 6 or 12 month period. Also, the amounts of EPA and DHA were quite stable. Hernández-Herrero et al. (*38*) found that free fatty acid contents increased gradually during the ripening of salted anchovies. They stated that salting did not inhibit the action of lipases responsible for the liberation of free fatty acids.

Oxidation analyses were not done from the stored products, and we cannot completely exclude the possibility that some oxidation of the polyunsaturated fatty acids has occurred. It is known that salting is an operation which promotes oxidation in fish (*39*, *40*). It is supposed that the pro-oxidant effect of NaCl is based on its capability to disturb the interactions between iron ions and proteins and, therefore, to leave more free iron ions to interact with lipid fraction (*41*). Metal ions accelerate lipid oxidation, and the effect of Fe^{2+} is the highest among them (*39*). In herring the iron and other pro-oxidants are especially concentrated in dark muscle (*28*). Aubourg and Ugliano (*42*) have studied the effect of brine pretreatment on the lipid stability of frozen horse mackerel and found that the higher the NaCl content in the brine, the higher the rancidity in fish. On the other hand, the same study showed that salting inhibited lipid

Table 5. Vitamins A, D, and E Contents[a] in Salted Herring Products

product	storage time (months)	vitamin A	vitamin D	vitamin E
gutted herring	0	32–159[b]	26–30	80–157
	6	nd[c]	23–34	nd–230
fillets	0	23–29[b]	16–17[b]	89–99
	6	nd	21–24	nd–295
pickled herring	0	26–30[b]	17–19	68–84
	12	nd–20	12–25	60–170

[a] Range of three pooled samples (micrograms per 100 g of wet weight). [b] Significant difference between 0 and 6/12 months stored products ($p < 0.05$). [c] Not detected.

hydrolysis during long-term frozen storage but could not prevent it. In our investigation, oxidation can by no means be the major reason for changes in the fatty acids, because typically different profiles in the disappearance of mono- and polyunsaturates were not observed.

Vitamins. Vitamin contents were analyzed from the samples taken after packing and at the end of the shelf life (**Table 5**). After packing, the levels of vitamin A ranged from 23 to 159 μg/100 g, and the highest levels were measured in the gutted herring. During storage, the concentrations decreased significantly, being under the limit of determination in most of the fish samples at the end of the storage period. Vitamin A activity in food is based on retinoids and carotenoids, which can be converted into retinol (vitamin A_1) or related compounds in the body. According to the study of Ollilainen et al. (*21*) the main retinoids in sugar-salted herring are *all-trans*-retinol (16.5 $\pm$ 0.4 μg/100 g of fresh weight), 13-*cis*-retinol (9 $\pm$ 0.7 μg/100 g), and 11-*cis*-retinol (6 $\pm$ 0.6 μg/100 g). Among the many fish species and fish products, the two latter compounds have been detected in sugar-salted and marinated herring only. Compared to the present study, the total amount of the three retinols, ~30 μg/100 g, was at the same level as that obtained in salted fillets and pickled herring.

The highest concentrations of vitamin D were also found in the gutted herring, but the difference between gutted and filleted fish was not as clear as with vitamin A. The contents in herring products varied between 12 and 34 μg/100 g, being at the same levels or even slightly higher than previously reported for some unprocessed fish samples (*43*, *44*). In fish the predominant compound is cholecalciferol, vitamin D_3 (*45*). Its concentration varies within different species and also within the same species caught in different areas (*43*, *46*). This difference is typically caused by varying dietary factors of fish (*47*). In the present investigation, the vitamins were analyzed in pooled herring samples caught in the same season, and thus it is assumed that the diet of the fish remained quite constant. According to previous studies, cooking and storage at home cause only minor losses of vitamin D (*44*). In the present study no loss at all of vitamin D was observed during storage.

In freshly packed products, the vitamin E content varied between 80 and 295 μg/100 g. Many of the results were under the limit of determination, but the tendency showed clearly that the concentrations were lower than previously found in raw herring fillets (*8*, *48*), Baltic herring fillets (*49*), or Baltic herring products (*45*). The effect of storage was not clear, mainly due to the high variation among the products. The variation could be related to low values, but according to a previous study, the predominant vitamin E compound in marine animals, α-tocopherol, can show large variation in individual herrings (*28*). The variation has been explained by genetic differences or unequal storage conditions but may also be dependent on the maturation stage and seasonal variation in oil content (*8*, *28*, *50*). However, in our investigation the products were salted, and thus the high variation as well as low contents of vitamin E could also be related to the salting process.

Nutritional Aspect. The average compositions of salted fillets and pickled herring are compared to the nutritional recommendations in **Table 6**. Gutted herring is not included because it is normally deboned and skinned before use, after which the composition is similar to that of the fillet. Recommendations for the supply of long-chain $n-3$ fatty acids, EPA and DHA, vary from 0.3 to 0.5% of daily energy (*51*, *52*). The lower limit can be obtained by eating one meal of herring product, but in order to obtain the higher dose recommended (0.5% of the daily energy), >100 g of salted herring should be eaten. Mainly due to the high salt content, one portion of salted herring is usually only 15−50 g (*53*).

During recent years, special attention has been paid to the ratio of $n-3$ and $n-6$ fatty acids because a very high intake of $n-6$ acids has been recognized to be less desirable (*54*). However, there is no one answer to the question of the optimal ratio of these acids in the diet. Simopoulos (*55*) has stated that

Table 6. Recommended Daily Allowances of Polyunsaturated Fatty Acids and Fat-Soluble Vitamins Compared to Average Intake from Salted Herring Products

	content in a meal (15–50 g)[a]			
	fillets	pickled herring	recommendation	ref
EPA + DHA	0.19–0.62 g	0.19–0.63 g	0.65 g/day	*51*
EPA + DHA	0.07–0.22% of energy[b]	0.07–0.23% of energy[b]	0.5% of energy	*52*
			0.3% of energy	51
EPA	0.09–0.30 g	0.09–0.31 g	minimum 0.22 g/day	*51*
	0.03–0.11% of energy[b]	0.03–0.11% of energy[b]	0.1% of energy	51
DHA	0.1–0.33 g	0.1–0.32 g	minimum 0.22 g/day	*51*
	0.04–0.12% of energy[b]	0.04–0.12 of energy[b]	0.1% of energy	*51*
n–3 PUFA	0.09–0.30% of energy[b]	0.09–0.30% of energy[b]	minimum 0.2% of energy	*56*
n–6 PUFA	0.01–0.04% of energy[b]	0.01–0.04% of energy[b]	minimum 1.0% of energy	*56*
PUFA	0.1–0.33 of energy[b]	0.1–0.33 of energy[b]	5–10% of energy	*57*
			7.5% of energy	*52*
vitamin A	2.0–6.7 μg	2.6–8.6 μg	900 μg/day	*57*
vitamin D	2.9–9.7 μg	2.7–9.1 μg	5 μg/day	*57*
vitamin E	0.020–0.068 mg	0.013–0.043 mg	10 mg/day	*57*

[a] 15–50 g is an average meal of salted herring (*53*). [b] Calculated using the daily energy (10 MJ) requirement of a male, 31–60 years old (*58*).

the ratio was about 1:1 in ancient times, but increased consumption of animal fat has changed the balance. Nowadays, the intake of $n-6$ acids, mainly of linoleic acid, is high, and the ratio of $n-3/n-6$ in the Western diet can be less than 1:10. In our salted products study, the ratio was about 8−9:1 due to the low level of $n-6$ acids and high levels of EPA and DHA.

The recommended daily intake of vitamin D for adults is 5 μg, which is easily available from an average meal of herring products. In our study, the levels of vitamins A and E were low and the contents in a meal below the recommended daily intake.

ABBREVIATIONS USED

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; HPLC, high-performance liquid chromatography; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; TAG, triacylglycerol; UV, ultraviolet.

ACKNOWLEDGMENT

We thank Boyfood Oy for the supply of the herring products. We acknowledge Fanny Henriet for technical assistance.

LITERATURE CITED

(1) Li, D.; Bode, O.; Drummond, H.; Sinclair, A. J. Omega-3 (n-3) fatty acids. In *Lipids for Functional Foods and Nutraceuticals*; Gunstone, F. D., Ed.; The Oily Press: Bridgewater, U.K., 2003; pp 225−262.

(2) Holik, M. F. Vitamin D. In *Modern Nutrition in Healts and Disease*, 8th ed.; Shils, M. E., Olson, J. A., Shike, M., Eds.; Lea and Febinger: Malvern, PA, 1994; pp 308−325.

(3) Drocourt, L.; Ourlin, J.-C.; Pascussi, J.-M.; Maurel, P.; Vilarem, M.-J. Expression of *CYP3A4*, *CYP2B6*, and *CYP2C9* is regulated by the vitamin D receptor pathway in primary human hepatocytes. *J. Biol. Chem.* **2002**, *277*, 25125−25132.

(4) Kállay, E.; Bareis, P.; Bajna, E.; Kriwanek, S.; Bonner, E.; Toyokuni, S.; Cross, H. S. Vitamin D receptor activity and prevention of colonic hyperproliferation and oxidative stress. *Food Chem. Toxicol.* **2002**, *40*, 1191−1196.

(5) Garcion, E.; Wion-Barbot, N.; Montero-Menei, C.; Berger, F.; Wion, D. New clues about vitamin D functions in the nervous system. *Trends Endocrinol. Metab.* **2002**, *13*, 100−105.

(6) Sigurgisladóttir, S.; Pálmadóttir, H. Fatty acid composition of thirty-five Icelandic fish species. *J. Am. Oil Chem. Soc.* **1993**, *70*, 1081−1087.

(7) Wheeler, A.; Newman, C. *The Pocket to Guide Saltwater Fishes of Britain and Europe*; Pargate Books: London, U.K., 1997.

(8) Hamre, K.; Lie, Ø.; Sandnes, K. Seasonal development of nutrient composition, lipid oxidation and colour of fillets from Norwegian spring-spawning herring (*Clupea harengus* L.). *Food Chem.* **2003**, *82*, 441−446.

(9) Aidos, I.; van der Padt, A.; Luten, J.; Boom, R. Seasonal changes in crude and lipid composition of herring fillets, byproducts, and respective produced oils. *J. Agric. Food Chem.* **2002**, *50*, 4589−4599.

(10) Molloy, J.; Cullen, A. The fat content of Irish herring. *Fish. Leaflet* **1981**, *107*, 1−7.

(11) Undeland, I. Lipid oxidation in fillets of herring (*Clupea harengus*) during processing and storage. Thesis, Department of Food Science, Chalmers University of Technology, Göteborg, Sweden, and SIK, The Swedish Institute for Food and Biotechnology, 1998.

(12) Stefánsson, G.; Nielsen, H.; Skåra, T.; Schubring, R.; Oehlenschläger, J.; Luten, J.; Derrick, S.; Gudmunsdóttir, G. Frozen herring as raw material for spice-salting. *J. Sci. Food Agric.* **2000**, *80*, 1319−1324.

(13) Finnish Game and Fisheries Research Institute. Imports and exports of fish in 1997 (in Finnish). *Ympäristö-Miljö* **1998**, 8.

(14) Finnish Game and Fisheries Research Institute. Foreign trade in fish 2001 (in Finnish). *Off. Stat. Finland, Agric., For. Fish.* **2002**, 58.

(15) Gudmunsdóttir, G.; Stefánsson, G. Sensory and chemical changes in spice-salted herring as affected by handling. *J. Food Sci.* **1997**, *62*, 894−897.

(16) Schubring, R.; Oehlenschläger, J. Comparison of the ripening process in salted Baltic and North Sea herring as measured by instrumental and sensory methods. *Z. Lebensm. Unters. Forsch. A* **1997**, *205*, 89−92.

(17) Folch, J.; Lees, M.; Sloane Stanley, G. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.

(18) Aro, T.; Tahvonen, R.; Mattila, T.; Nurmi, J.; Sivonen, T.; Kallio, H. Effects of season and processing on oil content and fatty acids of Baltic herring (*Clupea harengus membras*). *J. Agric. Food Chem.* **2000**, *48*, 6085−6093.

(19) Christie, W. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* **1982**, *23*, 1072−1075.

(20) Ågren, J.; Julkunen, A.; Penttilä, I. Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. *J. Lipid Res.* **1992**, *33*, 1871−1876.

(21) Ollilainen, V.; Heinonen, M.; Linkola, E.; Varo, P.; Koivistoinen, P. Retinoids and carotenoids in Finnish foods: Fish and fish products. *J. Food Compos. Anal.* **1989**, *2*, 93−103.

(22) NMKL 167. Cholecalciferol (vitamin D_3) and Ergocalciferol (vitamin D_2) Determination by HPLC in foodstuffs. 2000.

(23) Envang, K.; Nielsen, H. H. *In situ* activity of chymotrypsin in sugar-salted herring during cold storage. *J. Sci. Food Agric.* **2000**, *80*, 1277−1283.

(24) Bradford, R. Differential utilization of storage lipids and storage proteins by Northwest Atlantic herring (*Clupea harengus harengus*). *J. Fish Biol.* **1993**, *43*, 811−824.

(25) Iles, T.; Wood, R. The fat/water relationship in North Sea herring (*Clupea harengus*), and its possible significance. *J. Mar. Biol. Assoc. U. K.* **1965**, *45*, 353−366.

(26) Henderson, R.; Almatar, S. Seasonal changes in the lipid composition of herring (*Clupea harengus*) in relation to gonad maturation. *J. Mar. Biol. Assoc. U. K.* **1989**, *69*, 323−334.

(27) Wood, R. Fat cycles of North Sea herring. *J. Conseil* **1958**, *23*, 390−398.

(28) Undeland, I.; Ekstrand, B.; Lingnert, H. Lipid oxidation in herring (*Clupea harengus*) light muscle, dark muscle, and skin, stored separately or as intact fillets. *J. Am. Oil Chem. Soc.* **1998**, *75*, 581−590.

(29) Ackman, R. Fish lipids I. In *Advances in Fish Science and Technology*; Connell, J., Ed.; Fishing News Books: Farnham, U.K., 1980; pp 86−103.

(30) deGeus, H.-J.; Aidos, I.; de Boer, J.; Luten, J.; Brinkman, U. Characterisation of fatty acids in biological oil samples using comprehensive multidimensional gas chromatography. *J. Chromatogr. A* **2001**, *910*, 95−103.

(31) Hyvönen, L.; Koivistoinen, P. Fatty acid analysis, TAG equivalents as net fat value, and nutritional attributes of fish and fish products. *J. Food Compos. Anal.* **1994**, *7*, 44−58.

(32) Laakso, P.; Christie, W.; Pettersen, J. Analysis of North Atlantic and Baltic fish oil triacylglycerols by high-performance liquid chromatography with a silver ion column. *Lipids* **1990**, *25*, 284−291.

(33) McGill, A.; Moffat, C. A study of the composition of fish liver and body oil triglycerides. *Lipids* **1992**, *27*, 360−370.

(34) Ratnayake, W.; Ackman, R. Fatty alcohols in capelin, herring and mackerel oils and muscle lipids: I. Fatty alcohol details linking dietary copepod fat with certain fish depot fats. *Lipids* **1979**, *14*, 795−803.

(35) Ratnayake, W.; Ackman, R. Fatty alcohols in capelin, herring and mackerel oils and muscle lipids: II. A comparison of fatty

acids from wax esters with those of triglycerides. *Lipids* **1979**, *14*, 804−810.
(36) Sargent, J.; McIntosh, R.; Bauermeister, A.; Blaxter, J. Assimilation of the wax esters of marine zooplankton by herring (*Clupea harengus*) and rainbow trout (*Salmo gairdnerii*). *Mar. Biol*. **1979**, *51*, 203−207.
(37) Ackman, R. Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. *Comp. Biochem. Physiol.* **1967**, *22*, 907−922.
(38) Hernández-Herrero, M.; Roig-Sagués, A.; López-Sabater, E.; Rodríguez-Jerez, J.; Mora-Ventura, M. Total volatile basic nitrogen and other physicochemical and microbiological characteristics as related to ripening of salted anchovies. *J. Food Sci.* **1999**, *64*, 344−347.
(39) Flinck, G.; Hong, G.-P.; Knobl, G. Lipid oxidation of seafood during storage. In *Lipid Oxidation in Food*; St. Angelo, A., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992; pp 183−207.
(40) Hultin, H. Oxidation of lipids in seafoods. In *Seafoods: Chemistry, Processing Technology and Quality*; Shahidi, F., Botta, J., Eds.; Blackie Academic and Professional: London, U.K., 1992; pp 49−74.
(41) Kanner, J.; Harel, S.; Jaffe, R. Lipid peroxidation of muscle food as affected by NaCl. *J. Agric. Food Chem*. **1991**, *39*, 1017−1021.
(42) Aubourg, S.; Ugliano, M. Effect of brine pre-treatment on lipid stability of frozen horse mackerel (*Trachurus trachurus*). *Eur. Food Res. Technol*. **2002**, *215*, 91−95.
(43) Mattila, P.; Piironen, V.; Uusi-Rauva, E.; Koivistoinen, P. Cholecalciferol and 25-hydroxycholecalciferol contents in fish and fish products. *J. Food Compos. Anal.* **1995**, *8*, 232−243.
(44) Mattila, P.; Ronkainen, R.; Lehikoinen, K.; Piironen, V. Effect of household cooking on the vitamin D content in fish, eggs, and wild mushrooms. *J. Food Compos. Anal*. **1999**, *12*, 153−160.
(45) Salo-Väänänen, P.; Ollilainen, V.; Mattila, P.; Lehikoinen, K.; Salmela-Mölsä, E.; Piironen, V. Simultaneous HPLC analysis of fat-soluble vitamins in selected animal products after small-scale extraction. *Food Chem*. **2000**, *71*, 535−543.
(46) Bäckman, C.; Huimasalo, J. Is it safe to eat Nordic fish?. Poster presentation, NMKL Seminar, Mariehamn, Finland, Aug 23, 2000.
(47) Mattila, P.; Piironen, V.; Haapala, R.; Hirvi, T.; Uusi-Rauva, E. Possible factors responsible for the high variation in the cholecalciferol contents of fish. *J. Agric. Food Chem*. **1997**, *45*, 3891−3896.
(48) Hamre, K.; Oyvind, L.; Sandnes, K. Development of lipid oxidation and flesh colour in frozen stored fillets of Norwegian spring-spawning herring (*Clupea harengus* L.). Effects of treatment with ascorbic acid. *Food Chem*. **2003**, 82, 447−453.
(49) Syväoja, E.-L.; Salminen, K.; Piironen, V.; Varo, P.; Kerojoki, O.; Koivistoinen, P. Tocopherols and tocotrienols in Finnish foods: Fish and fish products. *J. Am. Oil Chem. Soc*. **1985**, *62*, 1245−1248.
(50) Aminullah Bhuiyan, A.; Ratnayaje, W.; Ackman, R. Nutritional composition of raw and smoked Atlantic mackerel (*Scomber scombrus*): Oil- and water-soluble vitamins. *J. Food Compos. Anal.* **1993**, *6*, 172−184.
(51) Simopoulos, A.; Leaf, N.; Salem, N., Jr. Workshop statement on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Prostaglandins, Leucotrienes Essent. Fatty Acids* **2000**, *63*, 119−121.
(52) British Nutrition Foundation. Unsaturated Fatty Acids: Nutritional and Physiological Significance. *The Report of the British Nutrition Foundation's Task Force*; British Nutrition Foundation: London, U.K., 1992.
(53) Lallukka, T.; Ovaskainen, M.-L. *Ruokamittoja*; National Public Health Institute: Helsinki, Finland, 2001; p B3.
(54) Horrocks, L.; Yeo, Y. Health benefits of docosahexaenoic acid (DHA). *Pharm. Res*. **1999**, *40*, 211−225.
(55) Simopoulos, A. Nutritional aspects of fish. In *Seafood from Producer to Consumer, Integrated Approach to Quality*; Luten, J., Børresen, T., Oehlenschläger, J., Eds.; Developments in Food Science, Elsevier Science: Amsterdam, The Netherlands, 1997; pp 589−607.
(56) U.K. Department of Health. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. *Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy*; Report on Health and Social Subjects 41; Her Majesty's Stationery Office: London, U.K., 1991.
(57) Kansanterveyslaitos, ravitsemusyksikkö. Fineli. Elintarvikkeiden koostumustietokanta. Helsinki, 2001; http://www.ktl.fi/fineli/
(58) Nordiska Ministerrådet. *Nordiska Näringsrekommendationer*; Köpenhamn, Denmark, 1996. Nord Vol. 28.

Received for review March 10, 2004. Revised manuscript received November 2, 2004. Accepted November 11, 2004.

JF0401221