# Occurrence of Squalene and Cholesterol in Various Species of Czech Freshwater Fish

*Zdenka KOPICOVÁ and Slavomíra VAVREINOVÁ*

*Department of Quality Features and Microbial Products, Food Research Institute Prague, Prague, Czech Republic*

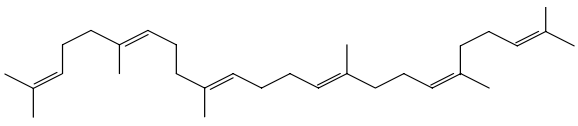
**Abstract**

Kopicová Z., Vavreinová S. (2007): **Occurrence of squalene and cholesterol in various species of Czech freshwater fish**. Czech J. Food Sci., **25**: 195–201.

The triterpenoid hydrocarbon squalene, $C_{30}H_{50}$, abundantly occurring in nature, is known as a substance with a high anti-tumour activity proven by a number of research studies. A high content of squalene was found mainly in shark liver oil and also in olive oil and amaranth seed oil. Our work was aimed at examining squalene contents in fresh water fish. Altogether 20 fish species were analysed. Squalene was determined in the unsaponifiable matter of muscular and visceral fat by a capillary gas chromatography method using a flame ionisation detector; the analysis of the unsaponifiable matter was augmented by cholesterol assay. The quantity of squalene found in muscular and visceral fat of individual fish averaged hundreds of mg/kg, ranging from 98.0 to 1536.8 mg/kg in muscular fat and from 70.1 to 1803.8 mg/kg in visceral fat. Muscular cholesterol amounted from 0.011% to 0.170% and visceral cholesterol from 0.104% to 0.297%.

**Keywords**: squalene; cholesterol; fat; shark liver; olive oil; amaranth, anti-tumour effects; antioxidant; freshwater fish; unsaponifiable matter; GC

The polyunsaturated hydrocarbon squalene ($C_{30}H_{50}$) from the series of isoprenoid polyenes, formed by 6 isoprene units, is classed among the group of triterpenes:



It was first mentioned by Tsujimoto (1906) who found considerable quantities of it in shark liver fat. Kayama *et al.* (1969) determined the hydrocarbon contents in six species of sharks; three of these were found to have 33% to 64% of squalene in liver oil. Olive oil is often mentioned as another rich source of squalene (De Leonardis *et al.* 1998; Ostlund *et al.* 2002), with its content of 112 to 869 mg/100 g oil; the higher values belong to extra virgin oil (De Leonardis *et al.* 1998). Amaranth seed is another commodity known for a high squalene content. Berger *et al.* (2003) declare 2% to 7% of squalene in amaranth oil. Squalene also occurs in other plant materials and also in human tissues; it is always possible to isolate it from the fat constituents. Liu *et al.* (1976 in Heikki Relas 2001) examined squalene concentrations in human tissues. The highest value was found in skin (475 μg/g dry matter) (Heikki Relas 2001). The average daily intake of squalene in the U.S. population is about 30 mg/person, whereas in the Mediterranean countries with a

Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE 0002702201.

high consumption of olive oil this intake reaches up to 200–400 mg (Smith 2000).

The anti-tumour effects of squalene in carcinogenesis seem to be unequivocally established. There exist a number of papers demonstrating the inhibitory effect of squalene after the administration of diverse compounds with established tumorogenicity such as, e.g., sodium arsenite (Shyh-Rong Fan *et al.* 1996), 12-O-tetradecanoylforbol-13-acetate (Murakoshi *et al.* 1992), hydrogen peroxide (O'Sullivan *et al.* 2002), azoxymethane (Rao *et al.* 1998), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanon (Smith 2000) and other substances, or after the application of ionising radiation (Storm *et al.* 1993). These tests were mostly executed using rodents (mice, Norway rats and Chinese hamsters) or model experiments *in vitro*.

The advantage of squalene occurrence in foodstuffs is diminished by the fact that this compound is an intermediate in the endogenous synthesis of cholesterol, with acetyl-CoA as the starting unit. Mevalonate and farnesylpyrophosphate are the intermediates of this organic synthesis. Squalene is formed of two molecules of farnesylpyrophosphate (Smith 2000). It further cyclises to form lanosterol which changes to cholesterol. The enzymatic conversion of squalene to cholesterol proceeds via a large number of steps and nowadays it is no more possible to accept the overly simplistic statement that a higher intake of squalene results in an increase of cholesterol, e.g. in blood serum. A number of studies have been conducted with contradictory results. In some studies, the consumption of high squalene diet is identified as an unquestionable cause of the increased LDL cholesterol level in blood plasma of humans (Pedersen *et al.* 2000; Ostlund *et al.* 2002) and hamsters (Zhang *et al.* 2002). However, another study claims that high squalene diet does not raise the levels of triacylglycerols and cholesterol in blood serum, whereas the concentrations of faecal cholesterol and its non-polar derivatives, as well as of bile acids, increase dramatically (Strandberg *et al.* 1990). Smith *et al.* (2000) also concludes that the addition of 1% squalene to the diet does not lead to the rise of lipoproteins in hamster plasma. Squalene and foods containing it, like shark liver, are consumed as health foods. In Japan and Southeast Asia they are used as a folk medicine against skin and liver disorders. Antibacterial properties of squalene and the possibilities of its utilisation in the cosmetics industry were also communicated (Dunford 2001). Squalene is known to form a significant constituent of fats in human skin, whereas it is lacking in the skin of furred mammals (Kohno *et al.* 1995). There exist a theory suggesting that squalene contained in human skin blocks lipid peroxidation which can happen due to an oxidative attack induced by solar radiation. This theory is supported by a model experiment, where squalene functioned as a scavenger of peroxide radicals (Kohno *et al.* 1995).

Table 1. List of fish species examined

| Species | Scientific name | Species | Scientific name |
|---|---|---|---|
| **Fish of commercial importance** | | **Fish of minor importance** | |
| Common carp | *Cyprinus carpio* | Carp bream | *Abramis brama* |
| Silver carp | *Hypophthalmichthys molitrix* | European perch | *Perca fluviatilis* |
| Grass carp | *Ctenopharyngodon idellus* | European chub | *Leuciscus cephalus* |
| Brown trout | *Salmo trutta* | Gudgeon | *Gobio gobi* |
| Tench | *Tinca tinc* | Rudd | *Scardinius erythrophthalmus* |
| Wels catfish | *Silurus glanis* | Roach | *Rutilus rutilus* |
| Brook trout | *Salvelinus fontinalis* | Asp | *Aspius aspius* |
| European eel | *Anguilla anguilla* | Crucian carp | *Carassius carassius* |
| Siberian sturgeon | *Acipenser baerii* | Barbel | *Barbus barbus* |
| Atlantic salmon | *Salmo salar* | | |
| Northern pike | *Esox lucius* | | |

The Mediterranean countries, noted for olive oil with a high squalene content as an important constituent of human food, exhibit significantly lower incidence of some types of carcinoma (Smith *et al.* 2000; Stanley 2005) and cardiovascular diseases. A certain favourable influence of the squalene intake on the human organism health status can therefore be accepted as sufficiently proven. In the Central European environment, where the consumption of olive oil is very low, the search for alternative ways of the squalene intake via other kinds of foodstuffs appears appropriate.

Our research was focused on freshwater fish. A number of local fish species were monitored for squalene and cholesterol contents. This task was chosen with the purpose to reflect the inland population's food makeup and to judge the possibilities of its health status betterment by promoting an increased consumption of freshwater fish foods. Another reason consisted in the efforts to compare the published data on squalene contents in marine fauna with the results of experimental analysis of squalene in local fish.

## MATERIAL AND METHODS

Adult fish of varied age and size were employed in these experiments. Most of them were fished out from Czech rivers and fish ponds in the year 2005; two individuals of brown trout, and one of each of grass carp, silver carp, tench and Atlantic salmon, were purchased from retail outlets in Prague. Some fish were obtained from the University of South Bohemia – Research Institute of Fish Culture and Hydrobiology in Vodňany. Altogether 20 species of freshwater fish were examined; they are listed in Tables 1 and 2. Immediately after delivery, the fish samples were stored at –20°C untill processing.

***Sample preparation***. Each sample individual was decapitated and eviscerated, scales and fins were removed. A combined homogenate from muscle and skin was made. Another sample was prepared by homogenising total viscera including the reproductive organs. The homogeniser Ultra Turrax was used. The prepared samples were immediately deep-frozen to –25°C and gradually processed.

***Fat extraction***. Fat was extracted from the homogenate preparations by triple extraction of 20–30 g sample with a mixture of petrolether: acetone (2:1) following the method by Bohačenko and Kopicová (1997), and the final desiccation of the combined extracts was done using anhydrous sodium sulphate and evaporation in a vacuum rotatory evaporator. Subsequently, the contents of squalene and cholesterol were determined in the non-saponifiable fraction of the separated fat.

***Preparation of non-saponifiable fraction***. The assay method according to Bohačenko and Kopicová (1999) was applied. The fat sample was saponified by boiling in aqueous/ethanol KOH solution (1/5), KOH:$H_2O$ = 40:60 g, and the unsaponifiable matter was extracted into *n*-hexane, desiccated with anhydrous sodium sulphate, and the solvent was then evaporated. The residue was silanisated with the 300 µl mixture of Pyridin:HMDS:TMCS = 9:3:1 and made up to 1.0 ml with hexane. The samples for GC analysis were analysed for the contents of squalene and free cholesterol by a capillary gas chromatography method. The method is minutely described in the paper Bohačenko and Kopicová (1999).

Conditions of squalene and cholesterol determination using capillary GC:
Gas chromatograph: HP5890 II
Detector: FID, $T_{detector}$ = 360°C
Injector: SPLIT 20:1, $T_{injector}$ = 350°C
Carrier gas: nitrogen, purity = 5.0
Column: DB-5HT, length 30 m, i.d. 0.25 mm, 1 µm
Temperature programme: 200°C 1 min, 5°C/min to 280°C, final time 15 min, 20°C/min to 360°C, final time 5 min
Total time of analysis = 45 min
Flow rate = 3.24 ml/min at 30°C
Injection: 1 µl

***Method reproducibility***. To validate the precision of the assays, a reproducibility test was conducted consisting in the determination of squalene in 7 sub-samples prepared from a single sample of fish muscle fat, including the preparation of unsaponifiable matter. Standard deviation achieved from seven assays amounted to 0.008.

## RESULTS AND DISCUSSION

Note: viscera of Brown trout, Brook trout, European eel, Atlantic salmon, European chub, and gudgeon were not available.

Twenty species of Czech freshwater fish were examined for squalene and cholesterol contents in lipids extracted from muscle and viscera samples. Muscular fat values are shown in Tables 3 and 4, visceral fat values in Tables 5 and 6. Both squalene

Table 2. Properties of processed fish

| Species | Weight (g) | History of sample |
|---|---|---|
| Common carp | 1040 | drew out of pond in Dubeč 26. 10. 2005 |
| Silver carp | 3140 | purchased in a retail outlet 26. 1. 2005 |
| Grass carp | 1305 | purchased in a retail outlet 26. 1. 2005 |
| Brown trout | 479 | purchased in a retail outlet 26. 1. 2005 |
| Tench | 347 | purchased in a retail outlet 29. 4. 2005 |
| Wels catfish | 1404 | received from the RIFCH 9. 9. 2005 |
| Brook trout | 632 | received from RIFCH 9. 9. 2005 |
| European eel | 128 | fished out of River Elbe 3. 6. 2005 |
| Siberian sturgeon | 1120 | received from RIFCH 9. 9. 2005 |
| Atlantic salmon | 510 | purchased in a retail outlet 29. 4. 2005 |
| Northern pike | 1400 | fished out of River Elbe 3. 6. 2005 |
| Carp bream | 351 | fished out of River Elbe 21. 7. 2005 |
| European perch, homogenate of 5 pieces | 349 | received from RIFCH 9. 9. 2005 |
| European chub | 547 | drew out of pond in Dubeč 26. 10. 2005 |
| Gudgeon, homogenate of 2 pieces | 16 and 22 | fished out of stream in Úvaly by Prague 5. 8. 2005 |
| Rudd | 88 | fished out of pond in Úvaly by Prague 15. 4. 2005 |
| Roach | 80 | fished out of River Elbe 21. 7. 2005 |
| Asp | 538 | fished out of River Elbe 27. 4. 2005 |
| Crucian carp, homogenate of 4 pieces | 215 | drew out of pond in Dubeč 26. 10. 2005 |
| Barbel, homogenate of 16 pieces | 380 | received from RIFCH 9. 9. 2005 |

RIFCH = Research Institute of Fish Culture and Hydrobiology in Vodňany

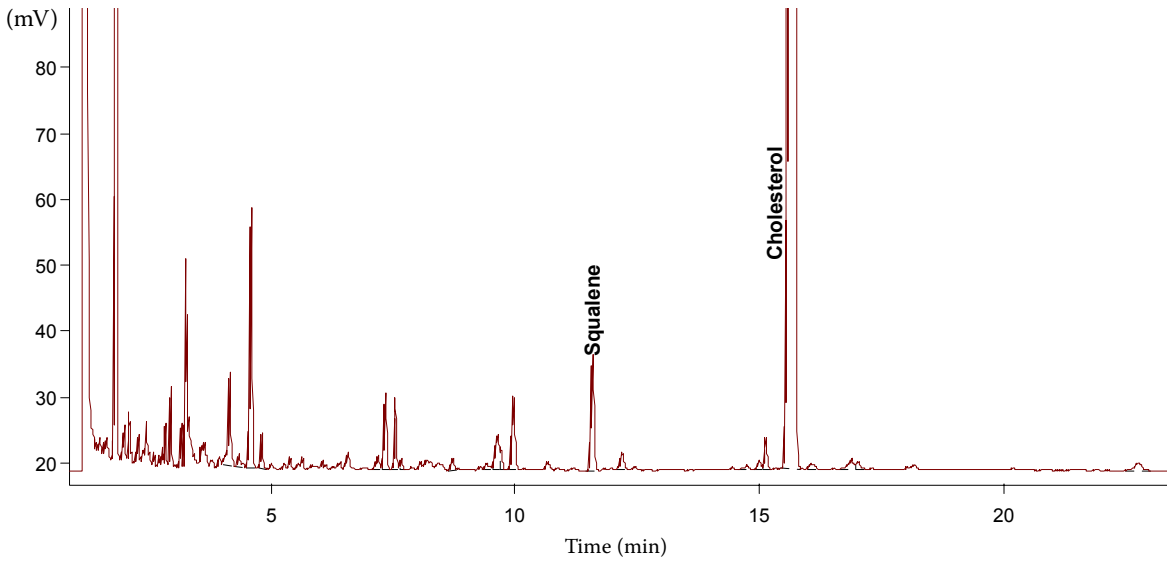



Figure 1. Gas chromatogram of unsaponifiable matter of muscle fat in silver carp

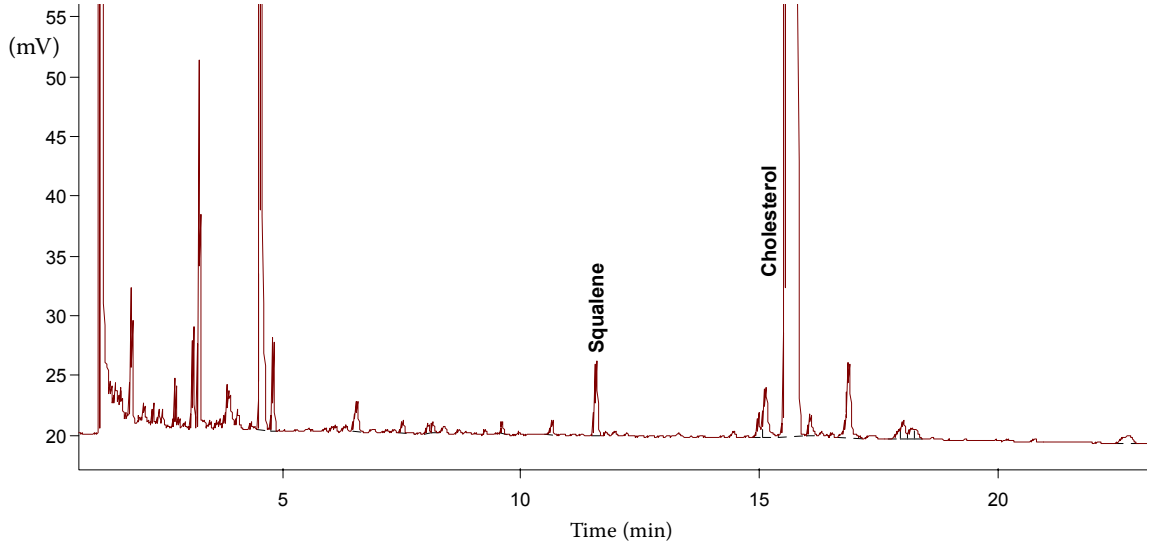



Figure 2. Gas chromatogram of unsaponifiable matter of visceral fat in common carp

Table 3. Squalene contents in muscle fat and in fresh muscle of freshwater fish (in

| Species | Fat (%) | Fat (mg/kg) | Muscle (mg/kg) |
|---|---|---|---|
| **Fish of commercial importance** | | | |
| Common carp | 1.01 | 386.59 | 3.90 |
| Silver carp | 1.67 | 216.49 | 3.62 |
| Grass carp | 7.41 | 217.41 | 16.11 |
| Brown trout | 6.45 | 226.35 | 14.60 |
| Tench | 1.55 | 384.38 | 5.96 |
| Wels catfish | 2.66 | 354.39 | 9.43 |
| Brook trout | 11.79 | 98.02 | 11.56 |
| European eel | 7.55 | 365.13 | 27.57 |
| Siberian sturgeon | 10.44 | 121.66 | 12.70 |
| Atlantic salmon | 8.60 | 176.17 | 15.15 |
| Northern pike | 4.01 | 398.46 | 15.98 |
| **Fish of minor importance** | | | |
| Carp bream | 0.72 | 552.26 | 3.98 |
| European perch | 1.06 | 492.29 | 5.22 |
| European chub | 1.52 | 118.14 | 1.80 |
| Gudgeon | 0.88 | 567.80 | 5.00 |
| Rudd | 0.56 | 1536.76 | 8.61 |
| Roach | 2.83 | 173.70 | 4.92 |
| Asp | 3.45 | 162.10 | 5.59 |
| Crucian carp | 1.32 | 651.03 | 8.59 |
| Barbel | 3.47 | 234.53 | 8.14 |

Table 4. Cholesterol contents in fresh muscle of freshwater fish

| Species | Fat (%) | Cholesterol (%) |
|---|---|---|
| **Fish of commercial importance** | | |
| Common carp | 1.01 | 0.047 |
| Silver carp | 1.67 | 0.011 |
| Grass carp | 7.41 | 0.052 |
| Brown trout | 6.45 | 0.041 |
| Tench | 1.55 | 0.064 |
| Wels catfish | 2.66 | 0.037 |
| Brook trout | 11.79 | 0.117 |
| European eel | 7.55 | 0.113 |
| Siberian sturgeon | 10.44 | 0.061 |
| Atlantic salmon | 8.60 | 0.051 |
| Northern pike | 4.01 | 0.086 |
| **Fish of minor importance** | | |
| Carp bream | 0.72 | 0.049 |
| European perch | 1.06 | 0.170 |
| European chub | 1.52 | 0.031 |
| Gudgeon | 0.88 | 0.091 |
| Rudd | 0.56 | 0.047 |
| Roach | 2.83 | 0.072 |
| Asp | 3.45 | 0.045 |
| Crucian carp | 1.32 | 0.029 |
| Barbel | 3.47 | 0.072 |

Table 5. Squalene contents in visceral fat and fresh viscera of freshwater fish

| Species | Fat (%) | Fat (mg/kg) | Raw viscera (mg/kg) |
|---|---|---|---|
| **Fish of commercial importance** | | | |
| Common carp | 1.71 | 259.13 | 4.43 |
| Silver carp | 36.21 | 170.71 | 61.81 |
| Grass carp | 43.98 | 70.10 | 30.83 |
| Tench | 3.42 | 535.40 | 18.31 |
| Wels catfish | 3.11 | 548.99 | 17.07 |
| Siberian sturgeon | 20.29 | 135.38 | 27.47 |
| Northern pike | 1.59 | 358.53 | 5.70 |
| **Fish of minor importance** | | | |
| Carp bream | 2.63 | 279.48 | 7.35 |
| European perch | 12.49 | 283.56 | 35.42 |
| Rudd | 1.15 | 1803.84 | 20.74 |
| Roach | 24.64 | 106.95 | 26.35 |
| Asp | 12.12 | 199.07 | 24.13 |
| Crucian carp | 1.84 | 272.13 | 5.01 |
| Barbel | 9.53 | 221.60 | 21.12 |

Table 6. Cholesterol contents in fresh viscera of freshwater fish

| Species | Fat (%) | Cholesterol (%) |
|---|---|---|
| **Fish of commercial importance** | | |
| Common carp | 1.71 | 0.203 |
| Silver carp | 36.21 | 0.104 |
| Grass carp | 43.98 | 0.156 |
| Tench | 3.42 | 0.181 |
| Wels catfish | 3.11 | 0.105 |
| Siberian sturgeon | 20.29 | 0.269 |
| Northern pike | 1.59 | 0.213 |
| **Fish of minor importance** | | |
| Carp bream | 2.63 | 0.204 |
| European perch | 12.49 | 0.297 |
| Rudd | 1.15 | 0.115 |
| Roach | 24.64 | 0.242 |
| Asp | 12.12 | 0.176 |
| Crucian carp | 1.84 | 0.168 |
| Barbel | 9.53 | 0.236 |

and cholesterol were determined by a capillary gas chromatography method in the unsaponifiable matter prepared from fat extracted from individual species. Examples of gas chromatograms of unsaponifiable matter of muscle and viscera fat are given in Figures 1 and 2.

Squalene quantities in the muscular and visceral fat samples ranged from 70 mg/kg in visceral fat of a grass carp up to 1803 mg/kg in visceral fat of a rudd. Average squalene contents in muscular or visceral fat of the Czech freshwater fish analysed reached hundreds of mg. The average content of commercially important fish like common carp, brown trout, Atlantic salmon, grass carp and silver carp reached from 176 to 386 mg squalene/kg muscle fat, which approaches the mean value of the squalene content found in all the species observed. The only exception is the quantity of squalene found in rudd muscular and visceral fat, i.e. 1537 mg/kg and 1804 mg/kg, respectively, which represents the levels comparable with those found in olive and amaranth seed oils. The accomplished experiments are the first attempt to address this issue. Subsequent research will be aimed at the correlations between the squalene content and fish size, fatness, nutrition, age, and fishing period, primarily in fish of commercial importance, which form part of the local population's diet. The present results of the squalene content determination in freshwater fish will be compared with the amount of squalene in marine fish which we intend to analyse in a further period.

Cholesterol contents ranged from 0.01% to 0.12% (average 0.07%) in fish muscle and 0.1 to 0.3% (average 0.21%) in viscera. In general, it can be said that fish viscera contained three times more cholesterol than fish muscle.

## References

Berger A., Gremaud G., Baumgartner M., Rein D., Monnard I., Kratky E., Geiger W., Burri J., Dionisi F., Allan M., Lambelet P. (2003): Cholesterol-lowering properties of amaranth grain and oil in hamsters. International Journal of Vitamine and Nutrition Research, **73**: 37–47.

Bohačenko I., Kopicová Z. (1997): Influence of polychlorinated biphenyls on the content and composi-

tion of fatty acids in fish fat. Potravinářské Vědy, **15**: 173–186.

Bohačenko I., Kopicová Z. (1999): Detection of sunflower and soybean oil adulterated with rapeseed oil. Czech Journal of Food Sciences, **17**: 182–187.

De Leonardis A., Macciola V., De Felice M. (1998): Rapid determination of squalene in virgin olive oils using gas-liquid chromatography. Italian Journal of Food Science, **10**: 75–80.

Dunford N.T. (2001): Health benefits and processing of lipid-based nutritionals. Food Technology, **55**: 38–44.

Heikki Relas (2001): Metabolism of squalene in triglyceride-rich lipoproteins in humans. [Academic Dissertation.] University of Helsinki, Helsinki.

Kayama M., Tsuchiya Y., Nevenzel J.C. (1969): The hydrocarbons of shark liver oils. Bulletin of the Japanese Society of Scientific Fisheries, **35**: 653–664.

Kohno Y., Egawa Y., Itoh S., Nagaoka S., Takahashi M., Mukai K. (1995): Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in *n*-butanol. Biochimica et Biophysica Acta, **1256**: 52–56.

Murakoshi M., Nishino H., Tokuda H., Iwashima A., Okuzumi J, Kitano H., Iwasaki R. (1992): Inhibition by squalene of the tumor-promoting activity of 12-o-tetradecanoylphorbol-13-acetate in mouse-skin carcinogenesis. International Journal of Cancer, **52**: 950–952.

Ostlund R.E. Jr., Racette S.B., Stenson W.F. (2002): Effects of trace components of dietary fat on cholesterol metabolism: phytosterols, oxysterols and squalene. Nutrition Reviews, **60**: 349–359.

O'Sullivan L., Woods J.A., O'Brien N.M. (2002): Squalene but not n-3 fatty acids protect against hydrogen peroxide-induced sister chromatid exchanges in Chinese hamster V79 CELLS. Nutrition Research, **22**: 847–857.

Pedersen A., Baumstark M.W, Arckmann P., Gylling H., Sandström B. (2000): An olive oil-rich diet results in higher concentrations of LDL cholesterol and a higher number of LDL subfraction particles than rapeseed oil and sunflower oil diets. Journal of Lipid Research, **41**: 1901–1910.

Rao Ch., Newmark H.L., Reddy B.S. (1998): Chemopreventive effect of squalene on colon cancer. Carcinogenesis, **19**: 287–290.

Shyh-Rong Fan I., Ching H.O., Fenny Lai-Fun Yeoh, Chi-Jen Lin, Te-Chang Lee (1996): Squalene inhibits sodium arsenite induced sister chromatid exchanges and micronuclei in Chinese hamster ovary-K1 cells. Mutation Research, **368**: 165–169.

Smith T.J. (2000): Squalene: potential chemopreventive agent. Expert Opinion on Investigational Drugs, **9**: 1841–1848.

Smith D., Montoro A.E., Jimenez F.P., Botet J.P, Pereperez J., Ordovas J.M. (2000): Effect of high saturated fat and cholesterol diet supplemented with squalene or β-sitosterol on lipoprotein profile in F1B hamsters. Nutrition Research, **20**: 1309–1318.

Stanley J. (2005): Olive oil may protect against breast cancer. Lipid Technology, **17**: 64–66.

Storm H.M., Oh S.Y., Kimler B.F., Norton S. (1993): Radioprotection of mice by dietary squalene. Lipids, **28**: 555–559.

Strandberg T.E., Tilvis R.S., Miettinen T.A. (1990): Metabolic variables of cholesterol during squalene feeding in humans: comparison with cholestyramine treatment. Journal of Lipid Research, **31**: 1637–1643.

Tsujimoto M. (1906): In: Kayama M., Tsuchiva Y., Nevenzel J.C. (eds) (1969): The hydrocarbons of shark liver oils. Bulletin of the Japanese Society of Scientific Fisheries, **35**: 653–664.

Zhang Z., Yeung W.K., Huang Y., Chen Z.-Y. (2002): Effect of squalene and shark liver oil on serum cholesterol level in hamsters. International Journal of Food Science and Nutrition, **53**: 411–418.

Received for publication December 20, 2006
Accepted after corrections April 19, 2007

*Corresponding author:*

RNDr. Zdenka Kopicová, CSc., Výzkumný ústav potravinářský Praha, v.v.i.,, Oddělení jakostních znaků a mikrobiálních produktů, Radiová 7, 102 31 Praha 10-Hostivař, Česká republika
tel.: + 420 296 792 300, fax: + 420 272 701 983, e-mail: zdenka.kopicova@vupp.cz