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Organochlorine Contaminants and Quality of Olive Oil Collected from Olive Oil Growers along the Croatian Adriatic Coast

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Abstract In this study we assessed 48 samples of virgin olive oil collected along the Croatian Adriatic coast for quality control, and for the presence of residues of seven organochlorine pesticides and 17 congeners of polychlorinated biphenyls (PCBs). Organochlorine pesticide levels ranged between below the limit of determination and 3.7 ng g^{-1} of oil, while PCBs ranged between below the limit of determination and 1.8 ng g^{-1} of oil. A larger problem than the presence of organochlorine compounds was that the seven tested oils (out of 48) did not meet some quality standards.

Keywords PCBs · OCPs · Olive oil · Quality parameters

Virgin olive oil is obtained from the fruit of the olive tree (*Olea europaea* L.) using only a cold press without heat or chemicals. The juice of this fruit is an oil that is ready for human consumption and possesses unique sensory characteristics and nutritional properties (Inarejos-García et al. 2009). Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are chemicals of anthropogenic origin that belong to the group of persistent organic pollutants (POPs). Due to the worldwide public concern about their presence and persistence in the environment and adverse outcomes in wildlife, the use of PCBs and OCPs has been banned or restricted in many industrial countries since 1970s and 1980s. Despite the ban/restriction, they are still found in the air, water, soil, animals, and humans. The distribution of organochlorines in the air is significant because air is directly exposed to different pollution sources. These pollutants tend to accumulate in epicuticular wax of foliage while root uptake is not significant for compounds with octanol-water partitioning coefficients larger than 3. The log K_{ow} for PCBs and OCPs exceeds 5.0 except for HCHs (log $K_{ow} \approx 4$).

Olive tree blossoms in May. The fruit matures on the tree for about 5 months and in that period the waxy surface of olive fruit is in contact with PCBs and OCPs from the surrounding air. As organochlorine have a preference for accumulation in lipophilic media, this makes olive fruit interesting as a matrix for determining organochlorine pollution. From this point of view, the aim of this study was to analyse olive oil for human consumption produced at the Croatian Adriatic coast for the presence of most relevant PCBs and OCPs. In addition, the upward trend in production and consumption of olive oil has been recorded in Croatia. There are a number of small producers of olive oil in Croatian Adriatic coast, every second or third household produces their own olive oil. These oils are usually not controlled. Following, quality of 48 samples of virgin olive oil produced by local olive oil growers from Croatia Adriatic coast were determined.

Materials and Methods

Samples: 48 samples of virgin olive oil produced by local olive oil growers from Croatia (regions Dalmatia and Istria) were collected in dark glass bottles during December 2008 and January 2009. The samples were transported to the laboratory and stored at 4°C for a short time until

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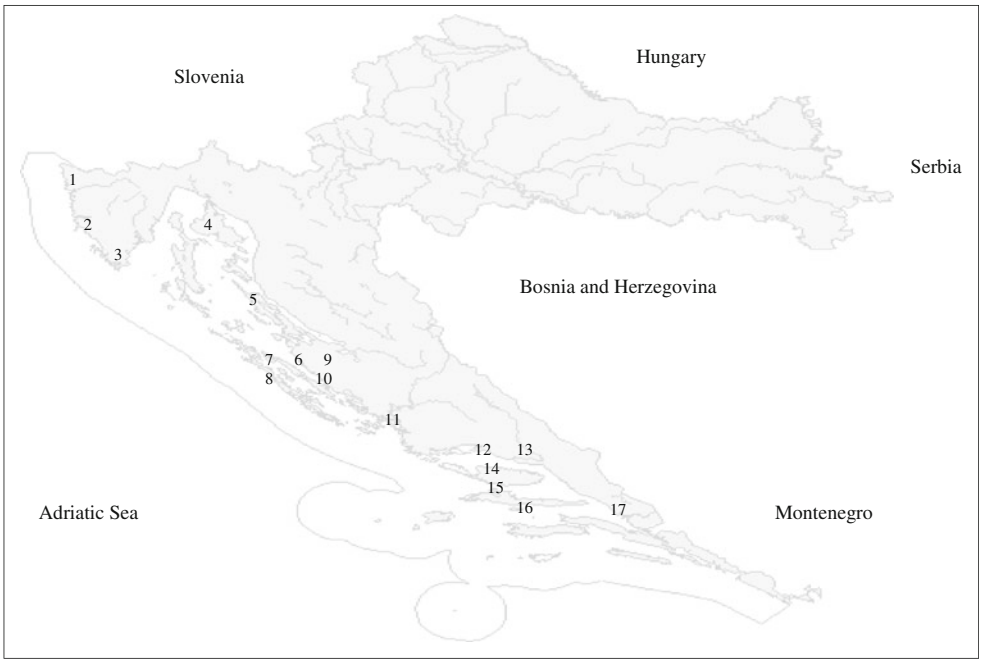

Fig. 1 Map of Croatia with marked sites of olive oil growers

analysis. We analysed the samples for free fatty acid content expressed as percentage of oleic acid (according to the method described in ISO 660 2009), peroxide content expressed as mmol O_2/kg (according to the method described in ISO 3960 2007), and organoleptic properties (according to the method described in European Communities Regulation 1991). Figure 1 shows all 17 locations of olive oil growers (48 olive oil growers in total). The locations numbered on the map are also referred to in Table 1.

Analysis of organochlorine compounds: Samples (about 5 g) were cold extracted with 20 mL of *n*-hexane. Extracts were cleaned up with 5 ml of 96% sulphuric acid. The clean up was repeated two more times. The solvent was evaporated to residues under a gentle stream of nitrogen. Before gas chromatography, the residues were dissolved in 1.0 mL of *n*-hexane. The following compounds were analysed: PCB congeners PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180, PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, PCB-170, PCB-189, PCB-60, and PCB-74 (altogether 17, numbered according to IUPAC)] and OCPs hexachlorobenzene (HCB), α-HCH, *β*-HCH, *γ*-HCH (α-, *β*-, *γ*-hexachlorocyclohexanes), 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (DDE), 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (DDD), and 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT). For high-resolution gas chromatography with electron capture detector(s) (HRGC/ECD) we used the ATI UNICAM 610 SERIES chromatographs with ^{63}Ni detectors. The compounds were analysed simultaneously on two capillary columns (Supelco, Bellefonte, USA): 1) 60 m × 0.25 mm, SPB-5 film thickness 0.25 μm, temperature program 100°C, then 4°C min^{-1} to 240°C, 50 min isothermally; and 2) 30 m × 0.25 mm, SPB-1701 film thickness 0.25 μm, temperature program 110°C, then 4°C min^{-1} to 240°C, 50 min isothermally. The carrier gas was nitrogen. The injector and detector temperature were 250 and 270°C, respectively, and the volume of injected sample was 5 μL. Only compounds identified on both columns were evaluated. The determination limits for the PCBs and OCPs ranged from 0.01 to 0.09 ng g^{-1} oil, and were calculated as the average of all determinations based on signal-to-noise ratio and recovery of compounds. Qualitative and quantitative analyses were done by comparison with external standards. The method recovery and reproducibility were determined by adding a known amount of all analysed compounds (at levels between 0.4 and 2.2 ng g^{-1} oil) to five aliquots of homogenised samples before extraction (method of additions). The recoveries of PCBs and OCPs were calculated after subtracting the mean levels of two non-fortified subsamples from the fortified ones. The recoveries for the PCBs and OCPs ranged from 60% to 74%, with relative standard deviation from 8% to 14%. Reagent blank was used to test for laboratory contamination, and the concentration of analytes was below the determination limit.

Results and Discussion

Organochlorines

Table 2 shows organochlorine levels found in olive oil. Among organochlorine pesticides, HCB, α-HCH, and DDE

Table 1 Virgin olive oils by location: chemical and sensory analysis

Sample code	Free acidity % m/m expressed in oleic acid[a]	Peroxide value mmol O_2/kg[a]	Organoleptic assessment by panel[b]	Selling category	Negative attributes (defects)[c]	Sites of olive oil growers	Marks of areas where olive oil growers are situated
Z092	0.40	6.20	8.60	EXTRA	–	BUJE	1
Z182	0.14	4.10	8.30	EXTRA	–	ČAVAR/POREČ	2
Z214	0.15	5.40	8.60	EXTRA	–	VODNJAN/IPULA	3
Z234	0.27	5.50	8.45	EXTRA	–	PULA	
Z225	0.19	5.10	8.55	EXTRA	–	KRK	4
Z146	0.20	3.70	8.60	EXTRA	–	PAG	5
Z139	0.22	6.00	8.80	EXTRA	–	PAG	
Z004	0.19	4.70	6.50	EXTRA	–	NIN	6
Z167	0.23	3.60	5.00	VIRGIN	Fusty	PRIVLAKA/NIN	
Z029	0.43	4.90	7.90	EXTRA	–	LJUBAČ/NIN	
Z011	0.22	6.20	7.20	EXTRA	–	MURVICA/ZADAR	
Z118	0.12	4.80	7.60	EXTRA	–	ZEMUNIK/ZADAR	
Z210	0.17	5.40	6.50	EXTRA	–	PODGRADINA/ZADAR	
Z037	0.27	7.19	6.50	EXTRA	–	ZADAR	
Z199	0.22	6.30	7.90	EXTRA	–	BIBINJE/ZADAR	
Z298	0.14	3.80	5.30	VIRGIN	Fusty; muddy sediment	UGLJAN	7
Z200	0.25	6.90	3.10	LAMPANTE	Fusty; acid sour	PREKO/UGLJAN	
Z227	0.17	7.80	6.70	EXTRA	–	IŽ	
Z120	0.19	6.00	4.20	LAMPANTE	Fusty; acid sour	IŽ	
Z166	0.27	6.30	6.90	EXTRA	–	BOŽAVA/DUGI OTOK	8
Z041	0.30	5.90	8.80	EXTRA	–	BENKOVAC	9
Z032	0.52	4.20	8.05	EXTRA	–	NADIN/BENKOVAC	
Z179	0.21	4.40	6.00	VIRGIN	Fusty; acid sour	BENKOVAC	
Z110	0.17	5.50	5.50	VIRGIN	Fusty; acid sour	TURANJ/BIOGRAD	10
Z165	0.61	4.50	3.80	LAMPANTE	Fusty; acid sour	PAŠMAN/BIOGRAD	
Z235	0.25	4.50	4.00	LAMPANTE	Fusty; acid sour	PAŠMAN/BIOGRAD	
Z236	0.16	6.30	8.60	EXTRA	–	BIOGRAD	
Z237	0.16	5.80	4.35	LAMPANTE	Fusty; acid sour	BIOGRAD	
S034	0.20	3.27	8.15	EXTRA	–	BIOGRAD	
Z143	0.18	6.70	6.20	VIRGINE	Fusty	PAKOŠTANE/ BIOGRAD	
S020	0.26	2.02	6.90	EXTRA	–	KISTANJE/ŠIBENIK	11
Z135	0.32	7.60	4.40	LAMPANTE	Fusty; acid sour	TRIBUNJ/ŠIBENIK	
Z128	0.17	4.80	8.20	EXTRA	–	VODICE/ŠIBENIK	
Z131	0.15	4.50	8.60	EXTRA	–	VODICE/ŠIBENIK	
Z133	0.18	5.60	8.30	EXTRA	–	VODICE/ŠIBENIK	
S002	0.18	4.54	7.00	EXTRA	–	SKRADIN/ŠIBENIK	
Z201	0.15	4.10	8.05	EXTRA	–	ŠIBENIK	
Z224	0.14	6.60	5.00	VIRGIN	Fusty; acid sour	ŠIBENIK	
Z169	0.23	6.70	8.50	EXTRA	–	PRIMOŠTEN/ŠIBENIK	
S028	0.21	3.98	8.05	EXTRA	–	BORAJA/ŠPLIT	12
Z332	0.33	6.40	5.25	VIRGIN	–	KLIS/SPLIT	
Z127	0.17	4.70	8.70	EXTRA	–	SPLIT	
Z330	0.21	4.20	8.65	EXTRA	–	OMIŠ	13

Table 1 Virgin olive oils by location: chemical and sensory analysis

Sample code	Free acidity % m/m expressed in oleic acid[a]	Peroxide value mmol O_2/kg[a]	Organoleptic assessment by panel[b]	Selling category	Negative attributes (defects)[c]	Sites of olive oil growers	Marks of areas where olive oil growers are situated
Z144	0.16	5.50	8.35	EXTRA	–	BRAČ	14
Z219	0.25	7.10	3.10	LAMPANTE	Acid sour	HVAR	15
Z215	0.24	6.60	6.80	EXTRA	–	VELA LUKA/KORČULA	16
Z217	0.24	5.40	8.15	EXTRA	–	VELA LUKA/KORČULA	
Z152	0.31	6.30	4.70	VIRGIN	Wet wood	OPUZEN	17

[a] Results are the means of two replications

[b] Md, median value of the perceived by the nine assessors trained for virgin olive oil sensory analysis. Quality was rated using a nine-point scale from 1 (lowest quality) to 9 (best quality). VOO with score equal to or higher than 6.5 is considered of extra quality

[c] Official defects provided by International Oil Council (IOC), negative attribute more intense perceived by the tasters

Table 2 Levels of organochlorine compounds (ng g^{-1} oil) above the determination limit found in 48 olive oil samples

Compound	Min	Max	PERCENTILE		
			25th	50th	75th
Organochlorine pesticides					
α-HCH	0.016	0.057	0.027	0.031	0.035
HCB	0.034	0.157	0.057	0.078	0.096
β-HCH	0	3.697	0.246	0.498	1.295
γ-HCH	0	1.031	0.094	0.346	0.476
DDE	0.048	0.44	0.064	0.077	0.113
DDT	0	0.156	0	0	0
PCBs					
PCB-28	0.079	5.49	0.538	0.734	1.571
PCB-52	0.091	1.762	0.359	0.543	0.798
PCB-101	0	0.103	0	0	0
PCB-138	0.041	0.306	0.055	0.068	0.099
PCB-153	0	6.33	0.157	0.221	0.428
PCB-180	0	0.221	0	0	0
PCB-74	0	0.064	0	0	0
PCB-60	0	0.088	0	0	0
PCB-170	0	0.111	0	0	0

were found in all samples while DDD was below the determination limit. DDT was found in three samples only. The highest level found was that of β-HCH (even though it was not present in all samples), followed by γ-HCH, DDE, α-HCH, and HCB. Polychlorinated biphenyls (PCBs) are a group of 209 congeners. Standard methods of analysis include only six indicator PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180), which are the most common in technical mixtures, environment, and animal and human tissues. However, since not all of these PCBs are toxic, we also analysed a selection of toxicologically relevant congeners, including PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, PCB-170, PCB-189, PCB-60, and PCB-74.

Among the 17 analysed congeners, only PCB-28, PCB-52, and PCB-138 were found in all samples while PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189 were below the determination limit. PCB-180 was found in 8, PCB-60 and PCB-101 in 5 samples, and PCB-74 in 1 sample only. The highest levels were found for PCB-28, followed by PCB-52, PCB-153, and PCB-138.

Overall organochlorine levels were low. As the most toxic PCB congeners were not detected in our samples, the risk related to consumption of Croatian olive oil seems to be lower than in central Italy (Guerranti et al. 2008). According to our knowledge, there are limited published data about organochlorine presence in olive oil, so, comparison is available to Italian samples only.

Contamination with PCBs and OCPs through ambient air and air-plant transfer dominates over transfer from soil into the root (Gaggi et al. 1985). Hydrophobic compounds such as PCBs and OCPs are adsorbed from the air by waxes contained in and on the green part of plant (Ockenden et al. 1998). Adsorption of organochlorine pollutants onto plant surface is usually the first step in entering the food chain. Pollutant uptake depends on many factors such as plant species, temperature, and physico-chemical properties of compounds. Olive fruits are exposed to ambient air pollution for a relatively short period of growth, which explains the relatively low organochlorine levels in olive oil samples. In addition, the olive orchard locations are far away from any pollution sources. As OCPs and PCBs use has been banned for long, it is quite likely that contaminants originate from the past or have travelled via air from distant countries where OCPs and PCBs are still in use. The dominance of above mentioned organochlorine pesticides and PCB congeners in olive oil samples indicate that contamination derives from atmosphere. These organochlorines were usually found in ambient air samples (Herceg Romanić and Krauthacker 2003) and in coniferous needle samples used for assessing ambient air levels of PCBs and OCPs (Herceg Romanić and Krauthacker 2008). In addition, organochlorine compounds were found in all parts of environment (Kaupp et al. 1996; Mössner et al. 1994; Chikuni et al. 1997). So, the presence of organochlorine compounds in olive oil in Croatia can be considered to be at the level of global environmental pollution.

Olive oil quality: European regulations EC No. 702/2007 and 640/2008 and International Olive Council standard (COI/T.15/NC No. 3/Rev. 5) have set analytical parameters to evaluate the quality of olive oil and defined minimal quality requirements for marketed oil. Commercial categories therefore include extra virgin, virgin, or lampante oil (not for consumption without further processing). Some quality standards such as free acidity, peroxide content, spectrophotometric indexes, and panel test are specific markers of genuineness aimed at safeguarding consumers' health and at fighting commercial fraud (Ferrancane et al. 2007). Seven samples out of 48 in our study do not satisfy organoleptic assessment by panel because the median of the defects is more than 3.0 and the median of the fruity attribute is almost 0 or 0, even though they were marketed and consumed locally as a food (farms, markets). That oil should be classified in the lampante virgin category (see Table 1) and they should be processed before consuming. Olive oils with defect have lower amount of healthy component, minor oil components-phenolics, associated with a lower incidence of atherosclerosis, cardiovascular disease, and certain types of cancer.

Olive oil is the main edible fat source used in the Mediterranean area, with clear health benefits (Delgado-Lista, et al. 2007). According to this, it is very important to satisfy quality standards of olive oil.

In conclusion, as discussed above, organochlorine levels in oil samples were low. However, some samples are marked as food and consumed although they do not satisfy some quality standards. This is by far a greater problem than the contamination with PCBs and OCPs we determined in them. We feel however that periodical control of contamination with organochlorines can add a new value to quality control of olive oil and to efforts to protect consumers.

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