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Comparison of antiatherogenic properties of lipids obtained from wild and cultured sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)

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Abstract

The aggregatory properties of lipid fractions, obtained from wild and cultured specimens of sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) were studied. The total lipids were extracted and separated into polar and neutral lipids. The polar lipids and neutral lipids were further separated by thin layer chromatography and the fractions obtained were tested for their ability to aggregate platelets or inhibit the platelet-activating factor (PAF)-induced aggregation. The aggregatory properties of the lipid fractions were dependent on the fish species and whether the fish samples were wild or cultured. The lipid fractions extracted from wild gilthead sea bream showed strong aggregatory properties, while the lipid fractions extracted from farmed fish showed strong inhibitory activities. The aggregatory properties of total lipids were mainly attributed to polar lipids while the PAF antagonistic activity was attributed to neutral lipids. The biological activities of such fractions with respect to their aggregatory properties may explain, in part, the protective role of fish consumption against cardiovascular diseases.

Keywords: Sea bass; Sea Bream; Aquaculture; Platelet-activating factor; Atheromatosis.

1. Introduction

The most common cause of mortality in industrialized countries is heart disease and stroke. For this reason, the prevention of atherosclerosis, is a major objective of modern medical investigation. Atherosclerosis is a chronic inflammatory condition caused by cholesterol deposition in the intima of large and medium arteries (Ross, 1993) that can lead to an acute clinical event through plaque rapture and thrombosis (Demopoulos, Karantonis, & Antonopoulou, 2003) (foam cell formation). Many theories have been advanced for the explanation of the mechanism of atherosclerosis. The "response-to-injury" hypothesis presents atherosclerosis as a chronic inflammatory response to injury of the endothelium, which leads to complex cellular and molecular interactions among endothelial cells, smooth muscle cells and several blood cell components (Chan, 1998; Ross, 1993, 1995). On the other hand, the "oxidative modification" hypothesis of atherosclerosis suggests that the oxidative modification of low-density lipoprotein (LDL) in the artery wall play a significant role in the development of atherosclerosis (Steinberg, 1997; Witztum & Steinberg, 1991). Binding of LDL to proteoglycans traps LDL in the arterial intima and allows lipid oxidation (Witztum, 1994). Oxidatively modified low-density lipoproteins (ox-LDLs) become cytotoxic to endothelial cells, chemotactic for monocytes and exhibit an increased affinity for their scavenger receptor, thus stimulating the

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formation of foam cells loaded with cholesterol esters (Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989).

Platelet activating factor (PAF) (1-*O*-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine) (Demopoulos, Pinckard, & Hanahan, 1979) is a crucial inflammatory phospholipid mediator that is implicated in the mechanism of atherogenesis (Demopoulos et al., 2003). According to this mechanism, PAF is produced during LDL oxidation (Liapikos et al., 1994) and causes in situ inflammation. It is also known that PAF is a compound of atheromatic plaque and is essential for the activation of leukocytes and their binding in the endothelial cells (Mueller et al., 1995). Furthermore, evidence of the implication of PAF in atherogenesis is provided by studies in animals, which indicates that PAF antagonists have protective action against atherosclerosis (Feliste, Perret, Braqueta, & Chap, 1989; Subbanagounder, Leitinger, Shih, Faull, & Berliner, 1999), while PAF-acetylhydrolases (PAF-AHs), the main enzymes responsible for the degradation of PAF, are active in native LDL but converted inactive forms in ox-LDL, leading to higher levels of PAF (Liapikos et al., 1994). Therefore, the presence of PAF-like molecules or PAF antagonists in various foodstuffs is very important in terms of nutritional value. Previous studies from our laboratory on lipid fractions obtained from traditional foods of the Mediterranean diet, such as olive oil (Koussissis et al., 1993), honey (Koussissis et al., 1994), milk and yogurt (Antonopoulou, Semidalas, Koussissis, & Demopoulos, 1996) and red wine (Fragopoulou, Nomikos, Antonopoulou, Mitsopoulou, & Demopoulos, 2000), have demonstrated the existence of compounds with both PAF-like and anti-PAF activities in washed rabbit platelets.

Several epidemiological studies have demonstrated the protective role of fish and fish oil consumption against coronary heart diseases (Kris-Etherton, Harris, & Appel, 2003). The nutritional benefits of fish consumption were mainly attributed to the effects of $\omega - 3$ polyunsaturated fatty acids, which are thought to have several potentially cardioprotective actions along with their antithrombotic action (Din, Newby, & Flapan, 2004). However, the effects of $\omega - 3$ fatty acids on platelet function and thrombosis are controversial, suggesting that other substances, apart from $\omega - 3$ fatty acids, may be responsible for the antithrombotic properties of marine fish (Kristensen, Iversen, & Schmidt, 2001; Mori, Beilin, Burke, Morris, & Ritchie, 1997). Recent researches in our laboratory have been conducted on lipid fractions of fish, showing the existence of compounds with both aggregatory and antiaggregatory activity toward platelets (Nomikos, Karantonis, Skarvelis, & Zabetakis, in press; Panayiotou et al., 2000; Rementzis, Antonopoulou, & Demopoulos, 1997).

European finfish marine culture spans a broad latitude range, from the Mediterranean basin in the south, to the north Atlantic areas bordering the Arctic Circle. Fish cultivation in marine environments is dominated in this region by three species: atlantic salmon (*Salmon salar*) (540,000 tonnes in 1999) sea bass (*D. labrax*) and gilthead sea bream (*S. aurata*) (87,000 tonnes combined in 1999) (Shields, 2001).

While production of the atlantic salmon in northern Europe accounts for the greatest tonnage of sea-reared fish, farming of ''true'' marine species is focussed on the Mediterranean region (Shields, 2001). The species that are cultured in the Mediterranean are primarily warm water species, such as sea bass and gilthead sea bream, that target the local market (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002). Sea bass is an economically important cultured fish species in the Mediterranean coastal waters (Holmer, Perez, & Duarte, 2003). Therefore, the market demand (and, as result, the price) for fresh sea bass has increased markedly over the past decade due to the desirable aroma and quality attributes of this fish (Holmer et al., 2003).

Therefore, the aim of this study is to determine and compare the biological activities of lipid fractions of wild and farmed sea bass and gilthead sea bream of the Mediterranean diet. These activities were assessed as the function of lipids to induce washed platelet aggregation or inhibit the PAF-induced aggregation.

2. Materials and methods

2.1. Reagents and instrumentation

All reagents and solvents were of analytical grade and supplied by Sigma (Gillingham, UK) and Merck (Darmstadt, Germany). Chromatographic material used for thin-layer chromatography (TLC) was silica gel G-60 (Merck). Platelet aggregation was measured in a Chrono-Log (Havertown, PA, USA) aggregometer (model 400-VS) coupled to a Chrono-Log recorder (Havertown, PA, USA).

2.2. Fish samples

Two different species of fish, namely sea bass (*D. labrax*) and Gilthead Sea Bream (*S. aurata*) were purchased from the local market. The cultured species were cultivated in Selonda marine culture, situated in Selonda bay of Sofiko, Korinthos, while the wild species were caught from the area of Messolongi in Greece. The fish were gutted and the head, skin and bones were removed. Three fish from each species were filleted and pieces from each fillet were pooled (this pooling represents each replicate). These pieces were then chopped and weighed (≈50 g), prior to homogenization. This experiment was carried out three times.

2.3. Isolation of lipid fractions

Total lipids (TL) were extracted according to the Bligh-Dyer method (Bligh & Dyer, 1959). One tenth of the TL was weighed and stored at −20 °C while the rest of it was further separated into polar lipids (PL) and neutral lipids (NL) by counter-current distribution (Galanos &

Kapoulas, 1962). The PL and NL were weighed and further separated by preparative TLC. The lipids and lipid fractions obtained were stored at −20 °C for further analysis.

2.4. Fractionation of PL and NL by thin-layer chromatography

The TLC glass plates (20 × 20 cm) were coated with silica gel G-60 and activated by heating at 130 °C for 60 min. The thickness of the TLC plates was 1.0 mm (preparative TLC). Up to 50 mg of lipids were applied to the TLC plates. An elution system consisting of chloroform:methanol:water 65:35:6 (v/v/v), was utilized for the separation of PL, while NL were separated in petroleum ether:diethyl ether:acetic acid 75:30:1 (v/v/v). The plates were stained under iodine vapours. Ten bands were appeared after the separation of NL of wild and cultured gilthead sea bass and sea bream while 10 and 9 fractions were appeared after the separation of PL of wild and cultured sea bass and gilthead sea bream, respectively. After the evaporization of iodine vapours the bands were scraped and lipids were extracted from silica gel according to the Bligh–Dyer method (Bligh & Dyer, 1959). The chloroform phase was evaporated to dryness under nitrogen and lipids were weighed, redissolved in 1 ml chloroform:methanol 1:1 (v/v) and stored at −20 °C.

2.5. Biological assay

Total lipids (TL), polar lipids (PL), neutral lipids (NL), as well as purified fractions of each lipid class by the above TLC separations were tested for their biological activity against washed rabbit platelets according to the method of Demopoulos et al. (1979). Briefly, the samples being examined and the PAF were dissolved in 2.5 mg of bovine serum albumin (BSA) per ml of saline. Various amounts of the sample being examined, ranging from 0.0012 to 0.16 mg, were added into the aggregometer cuvette and their ability to aggregate washed rabbit platelets or to inhibit PAF-induced aggregation was determined. In order to determine the aggregatory efficiency of either PAF or the samples being examined, the maximum reversible aggregation was evaluated and the 100% aggregation was determined. The plot of the percentage of the maximum reversible aggregation (ranging from 20% to 80%) versus different concentrations of the aggregatory agent was linear. From this curve, the concentration of the aggregatory agent, which induces 50% of the maximum reversible aggregation, is calculated. This value is defined as EC_{50}, namely equivalent concentration for 50% aggregation.

In order to determine the inhibitory properties of the samples, various amounts of the sample being examined, ranging from 0.0012 to 0.16 mg, were added into the aggregometer cuvette and their ability to inhibit PAF-induced aggregation was determined. The platelet aggregation induced by PAF (2.5×10^{-11} M, final concentration in the cuvette) was measured as PAF-induced aggregation, in washed rabbit platelets before (considered as 0% inhibition) and after the addition of various amounts of the sample being examined. Consequently, the plot of % inhibition (ranging from 20% to 80%) versus different concentrations of the sample is linear. From this curve, the concentration of the sample, which inhibited 50% PAF-induced aggregation, is calculated. This value is defined as IC_{50} namely, inhibitory concentration for 50% inhibition.

3. Results and discussion

3.1. Total, polar and neutral lipid fractions of wild and cultured fish

The two fish species, were examined for their ability to induce washed platelet aggregation or inhibit the PAF-induced aggregation. The procedure followed for the extraction of the total lipids (TL) from the wild and cultured fish samples, along with their separation into polar lipids (PL) and neutral lipids (NL), is shown in Fig. 1.

The extraction of TL was carried out by the method of Bligh and Dyer (Bligh & Dyer, 1959), which had been satisfactorily used for the extraction of other fish lipids (Nomikos et al., 2006; Panayiotou et al., 2000; Rementzis et al., 1997).

An amount (1/10) of total lipids (TL) was stored at −20 °C in order to test its biological activity while the rest was separated into polar and neutral lipids by counter current distribution chromatography (Galanos & Kapoulas, 1962). This method allows excellent recovery of polar lipids from neutral sources. By this procedure the polar lipid fraction contained glyco- and phospholipids. The amounts of TL, PL and NL in g/50 g fish tissue are shown in Table 1.

As can be seen the content of the TL in cultured gilthead sea bream is increased compared to that of one in wild gilthead sea bream, which is in accordance with the literature (Grigorakis, Alexis, Taylor, & Hole, 2002; Grigorakis, Taylor, & Alexis, 2003). In addition, the levels of the TL in cultured sea bass are higher than the levels of the wild sea bass, which is also in accordance with the literature (Alasalvar et al., 2002; Kyrana & Lougovois, 2002). The increased amounts of total lipids in cultured gilthead sea bream and sea bass, compared with the respective wild species, is attributed to both an increase of the NL and PL. However, dealing with absolute amounts, the contribution of the PL to the increased amounts of TL in the cultured species is much higher than that of the NL. The high amounts of the total lipids in cultured fish may be attributed to the diet of the cultured fish and the confined swimming area compared to the wild fish.

3.2. Platelet aggregatory properties of lipid fractions

The extracted total lipids (TL) and polar (PL) and neutral (NL) fractions were tested for their ability to induce washed rabbit platelet aggregation or inhibit the PAF-

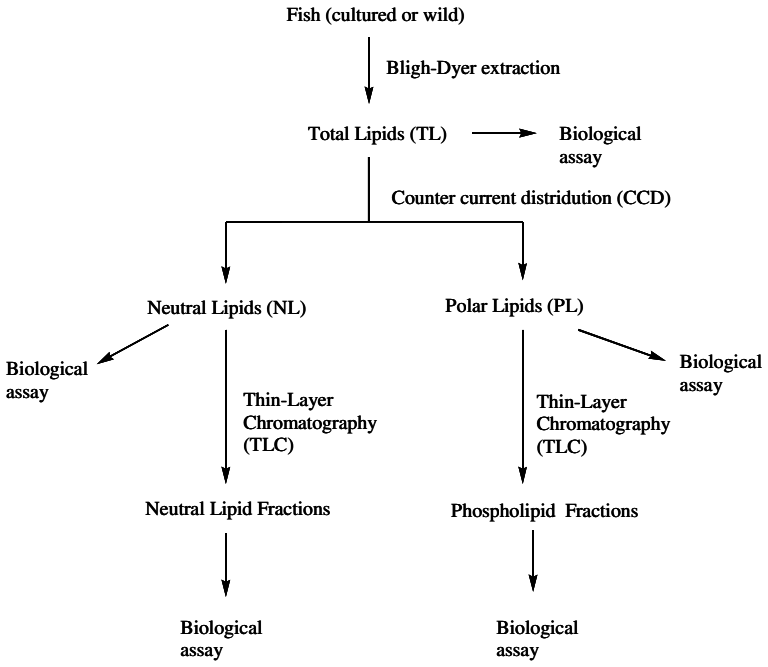

Fig. 1. Schematic diagram of the extraction and purification procedure.

Table 1
Contents of total lipids (TL), polar lipids (PL) and neutral lipids (NL) in the flesh of wild and cultured fish species

Fish species	TL (g/50 g fish tissue)	PL (g/50 g fish tissue)	NL (g/50 g fish tissue)
Cultured gilthead sea bream	0.304 ± 0.061	0.277 ± 0.056	0.027 ± 0.008
Wild gilthead sea bream	0.224 ± 0.056	0.209 ± 0.049	0.015 ± 0.006
Cultured sea bass	0.382 ± 0.078	0.353 ± 0.038	0.030 ± 0.009
Wild sea bass	0.272 ± 0.043	0.248 ± 0.041	0.024 ± 0.008

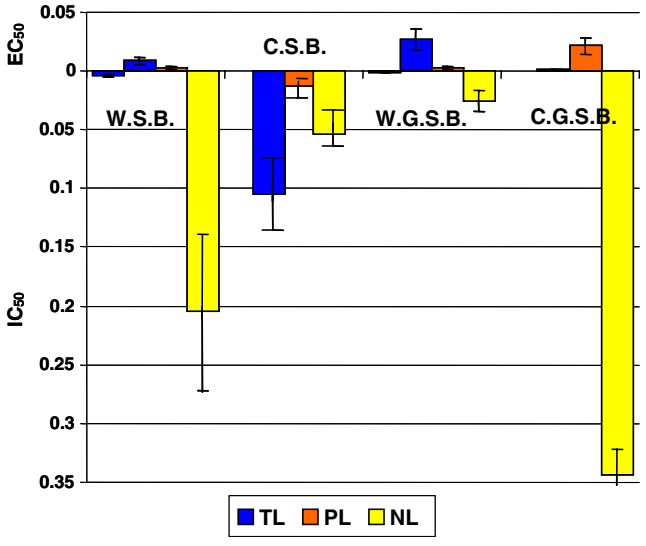

Fig. 2. Biological activity of total lipids, polar lipids and neutral lipids of wild sea bass (WSB), cultured sea bass (CSB), wild gilthead sea bream (WGSB) and cultured gilthead sea bream (CGSB).

induced platelet aggregation. The EC_{50} and IC_{50} values of each lipid fraction from both fish species, cultured and wild were expressed as a percentage of the lipid fraction and are shown in Fig. 2.

The total lipids of wild sea bass had a bimodal effect on platelets, inhibiting their PAF-induced aggregation at low concentrations and aggregating them at higher ones. The PL were responsible for the aggregatory activity of TL and the NL were responsible for the inhibitory activity of TL since they strongly aggregated platelets or inhibited PAF-induced aggregation, respectively. In contrast, the TL and both lipid fractions of cultured sea bass exhibited inhibitory properties (Fig. 2).

In addition, the TL of wild gilthead sea bream showed similar biological activity to those of wild sea bass. Specifically, the TL of wild gilthead sea bream exhibited inhibitory activity at low concentrations which is attributed to the NL and aggregatory activity at higher concentrations which is attributed to the PL. Moreover, the PL and NL of wild and cultured gilthead sea bream had the same biological activity. NL of the wild gilthead sea bream were stronger inhibitors than those ones of the cultured gilthead sea bream and PL of the wild gilthead sea bream were stronger aggregators than those ones of the cultured gilthead sea bream (Fig. 2).

3.3. Platelet aggregatory properties of polar lipid fractions

The polar lipid fractions of both fish species were further separated by preparative TLC (Fig. 3(a)) and the 10 lipid fractions obtained were tested for their ability to induce washed rabbit platelet aggregation or inhibit the PAF-induced platelet aggregation. The EC_{50} and IC_{50} values

of each polar lipid fraction, from both fish species, cultured and wild, were expressed as a percentage of the lipid fraction and are shown in Figs. 4 and 5.

Polar lipid fractions of cultured and wild sea bass exhibited the same biological activity, except for lipid fraction 1 of cultured sea bass, which showed very weak inhibitory properties, while the polar lipid fraction 1 of the wild sea bass showed aggregatory properties.

As previously mentioned, PL of the cultured sea bass exhibited inhibitory properties, that are attributed to almost all the polar lipid fractions (1, 3, 5, 6, 7, 8, 9 and 10) apart from lipid fractions 2 and 4 that exhibited aggregatory properties (Fig. 4).

Moreover, PL of the wild sea bass had aggregatory activity, due to lipid fractions 1, 2 and 4 whose aggregatory properties dominated the inhibitory activity of the rest of the lipid fractions (Fig. 4). Polar lipid fractions 2 and 4 of both cultured and wild sea bass have similar R_f values to those of PAF and phosphatidylcholine (PC), respectively. According to the literature, fish possibly contains PAF (Samples, Pool, & Lumb, 1999; Summers, al-Hassan, Thomson, Chun, & Criddle, 1991). In addition, PC does not have aggregating properties but oxidized-PC, which has a similar R_f value to that of PC, may have PAF-like activity (Tokumura, Sumida, Toujima, Kogure, & Fukuzawa, 2000).

In wild gilthead sea bream, polar lipid fraction 1 did not show any biological activity. In addition, the polar lipid fractions of cultured and wild gilthead sea bream had the same biological activities, except for lipid fraction 2 of cultured gilthead sea bream, which showed strong inhibitory properties, while the polar lipid fraction 2, of the wild gilthead sea bream showed strong aggregatory properties (Fig. 5).

In cultured gilthead sea bream, PL exhibited aggregatory activity, which is attributed to lipid fraction 4, dominating the inhibitory properties of the rest of the fractions. Finally, PL fractions of wild gilthead sea bream also showed aggregatory properties, due to lipid fractions 2 and 4 which (as previously mentioned), have R_f values similar to those of PAF and PC, respectively, and dominate the inhibitory properties of the rest of the lipid fractions (Fig. 5).

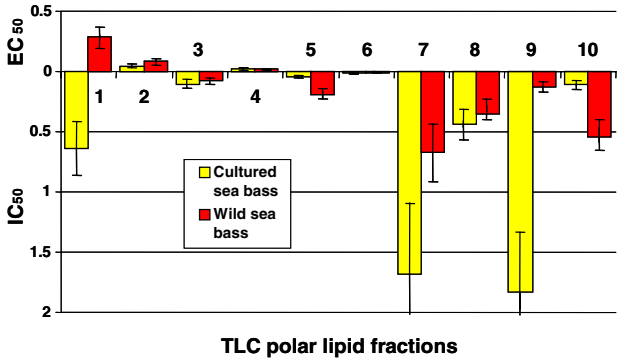

Fig. 4. Biological activities of the polar lipid fractions of cultured and wild sea bass, after separation with preparative TLC.

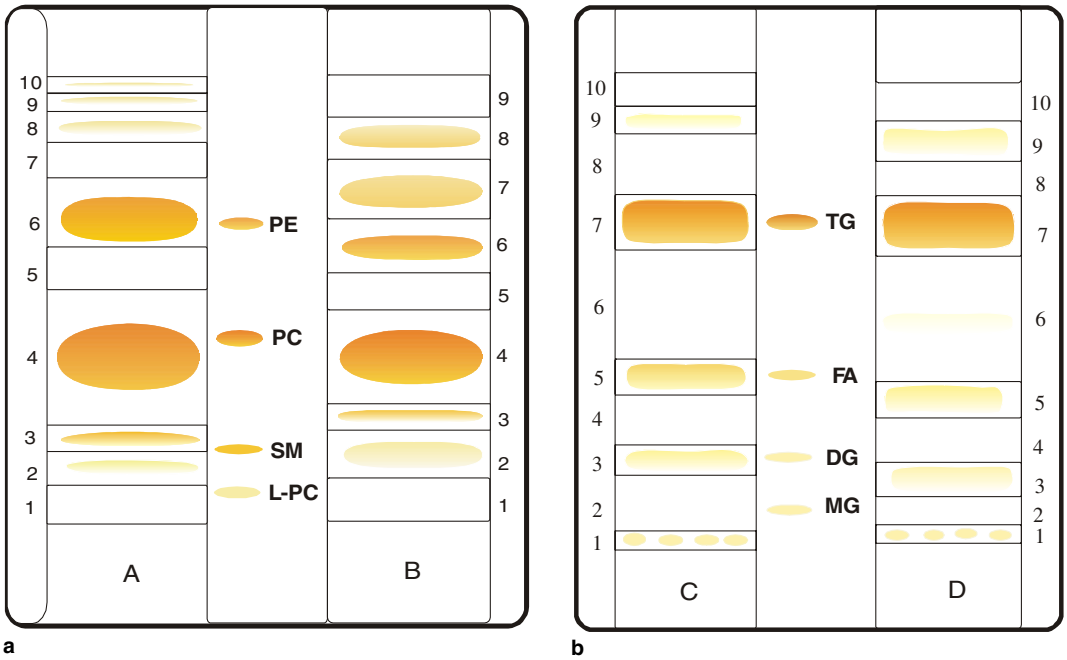

Fig. 3. (a) Typical profile of the polar lipids separation of the fish species on preparative TLC. A: wild, cultured sea bass, B: wild, cultured gilthead sea bream, L-PC: lyso-phosphatidylcholine, SM: sphingomyelin, PC: phosphatidylcholine, PE: phosphatidyl ethanolamine. (b) Typical profile of the neutral lipids separation of the fish species on preparative TLC. C: cultured gilthead sea bream, D: wild gilthead sea bream, E: wild, cultured sea bass, MG: monoglycerides, DG: diglycerides, FA: fatty acids, TG: triglycerides. The elution system used for the separation of total neutral lipids was petroleum ether:diethyl ether:acetic acid 75:30:1 (v/v/v), while the elution system used for the separation of total polar lipids was chloroform:methanol:water 65:35:6 (v/v/v).

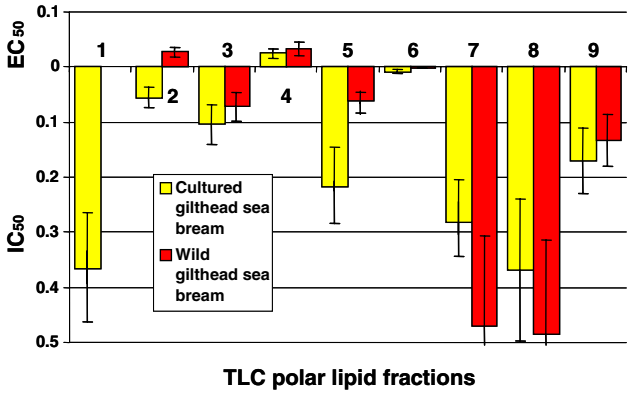

Fig. 5. Biological activities of the polar lipid fractions of cultured and wild gilthead sea bream, after separation with preparative TLC.

3.4. Platelet aggregatory properties of neutral lipid fractions

The NL fractions of all fish species were further separated by preparative TLC (Fig. 3(b)) and the neutral lipid fractions obtained were tested for their ability to induce washed rabbit platelet aggregation or to inhibit the PAF-induced platelet aggregation. The IC_{50} values, expressed in percentage of lipid fraction, are shown in Figs. 6 and 7. All neutral lipid fractions from all fish species, either cultured or wild, exhibited inhibitory activity. Specifically, NL of cultured sea bass exhibited stronger inhibitory activity than did that of NL of wild sea bass, which was attributed to lipid fractions 1, 2, 4, 5, 6, 8 and 9. On the other hand, NL fractions of wild gilthead sea bream showed stronger inhibitory activity than that of NL of cultured gilthead sea bream, that was attributed to almost all the neutral lipid fractions of wild gilthead sea bream.

The existence of PAF antagonists in various foodstuffs is of major importance for their nutritional value, considering the importance of platelet activation and thrombosis in cardiovascular diseases. Moreover, protective intervention studies against atherogenesis have shown that only specific

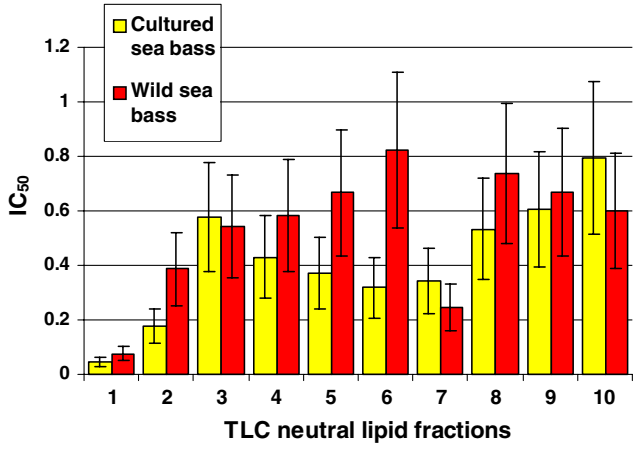

Fig. 6. Biological activities of the neutral lipid fractions of cultured and wild sea bass, after separation with preparative TLC.

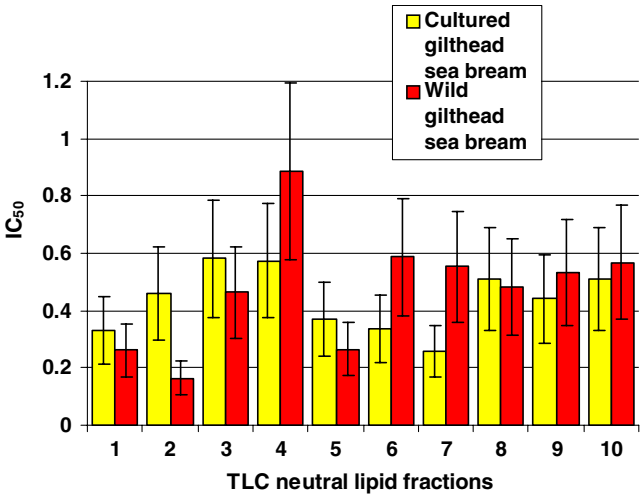

Fig. 7. Biological activities of the neutral lipid fractions of cultured and wild gilthead sea bream, after separation with preparative TLC.

PAF inhibitors (Feliste et al., 1989), olive oil polar lipids (Karantonis et al., 2004) and statins (Wierzbicki, Poston, & Ferro, 2003) are able to reduce atherogenesis in vivo. $\omega - 3$ fatty acids, even though they suppress different steps of atherogenesis are not able to reduce atherogenesis in vivo (Mayer et al., 2002). The consumption of PAF antagonists obtained from the diet is superior since they are of natural origin and therefore they imply no risks for human health as do the toxic statins.

Fish is one of the main components of the Mediterranean diet and the beneficial effects of this diet could be explained, in part, by the presence of PAF antagonists. The presence of fractions with platelet aggregating properties, such as those found in polar lipids of wild sea bass, wild and cultured gilthead sea bream, does not necessarily mean that these fractions have prothrombotic effects since they are less active than PAF. The presence of such relatively inactive molecules in the bloodstream and their binding to PAF receptors could minimize the biological actions of PAF or in other words they could be considered as PAF inhibitors.

It should be mentioned that all these lipid fractions are a mixture of lipid molecules that can potentially have aggregatory or inhibitory properties. The final activity observed depends on both the relative ability of each molecule to aggregate platelets or inhibit the PAF-induced platelet aggregation and also dependent on the relative amount of each molecule in the mixture. With this perspective, a fraction that aggregates platelet may also contain inhibitory lipid molecules or the opposite could occur.

4. Conclusions

This paper demonstrates that compounds with strong anti-PAF activity are present in neutral lipids of wild gilthead sea bream and cultured sea bass and in polar lipids of cultured sea bass. It is well known that the lipids that

are of biological importance for humans are those that exhibit anti-PAF activity. However, the presence, in polar lipids of wild sea bass and gilthead sea bream, of compounds with PAF-like activity could also be beneficial as they act as weak PAF agonists and compete with PAF for common binding sites during the formation of atheromatic plaque in blood arteries, protecting them from atheromatosis generation because they act mainly as PAF inhibitors. In order to examine whether the biologically active compounds of cultured or wild fish are beneficial for humans, it is important to use the biologically active compounds of cultured and wild fish in vivo with experimental animals and to examine which compounds inhibit the formation of atheromatic plaque in blood arteries.

In addition the differences that appear between cultured and wild sea bass and gilthead sea bream may be attributed to the differences in the nutrition of cultured and wild fish. Current research in our laboratory is focussed on elucidating the structure of compounds with PAF-like and anti-PAF activities in cultured and wild sea bass and gilthead sea bream.

References

Alasalvar, C., Taylor, K. D. A., Zubcov, E., Shahidi, F., & Alexis, M. (2002). Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. *Food Chemistry, 79*, 145–150.

Antonopoulou, S., Semidalas, C. E., Koussissis, S. G., & Demopoulos, C. A. (1996). Platelet-activating factor (PAF) antagonists in foods. A study of lipids, with PAF or anti-PAF like-activity, in cow's milk and yogurt. *Journal of Agricultural and Food Chemistry, 44*, 3047–3051.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Chan, A. C. (1998). Vitamin E and atherosclerosis. *Journal of Nutrition, 128*, 1593–1596.

Demopoulos, C. A., Karantonis, H. C., & Antonopoulou, S. (2003). Platelet activating factor – a molecular link between atherosclerosis theories. *European Journal of Lipid Science and Technology, 105*, 705–716.

Demopoulos, C. A., Pinckard, R. N., & Hanahan, D. J. (1979). Platelet-activating factor. Evidence for 1-*O*-alkyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline as the active component (a new class of lipid chemical mediators). *Journal of Biological Chemistry, 254*, 9355–9358.

Din, J. N., Newby, D. E., & Flapan, A. D. (2004). Omega 3 fatty acids and cardiovascular disease – fishing for a natural treatment. *British Medical Journal, 328*, 30–35.

Feliste, R., Perret, B., Braqueta, P., & Chap, H. (1989). Protective effect of BN 52021, a specific antagonist of platelet-activating factor (PAF-acether) against diet-induced cholesteryl ester deposition in rabbitarta. *Atherosclerosis, 78*, 151–158.

Fragopoulou, E., Nomikos, T., Antonopoulou, S., Mitsopoulou, C. A., & Demopoulos, C. A. (2000). Separation of biologically active lipids from red wine. *Journal of Agricultural and Food Chemistry, 48*, 1234–1238.

Galanos, D. S., & Kapoulas, V. M. (1962). Isolation of polar lipids from triglyceride mixtures. *Journal of Lipid Research, 3*, 134–137.

Grigorakis, K., Alexis, M. N., Taylor, A. K. D., & Hole, M. (2002). Comparison of wild and cultured gilthead sea bream (*Sparus aurata*): composition, appearance and seasonal variations. *International Journal of Food Science and Technology, 37*, 477–484.

Grigorakis, K., Taylor, A. K. D., & Alexis, M. N. (2003). Organoleptic and volatile and compounds comparison of wild and cultured gilthead sea bream (*Sparus aurata*); sensory differences and possible chemical basis. *Aquaculture, 225*, 109–119.

Holmer, M., Perez, M., & Duarte, C. M. (2003). Benthic primary producers – a neglected environmental problem in Mediterranean maricultures? *Marine Pollution Bulletin, 46*, 1372–1376.

Karantonis, H. C., Tsantila, N., Antonopoulou, S., Perrea, D. N., Sokolis, D. P., Theocharis, S. E., et al. (2004). In vivo antiatherogenic properties of olive oil and its constituent lipid classes in hyperlipidemic rabbit. *Chemistry and Physics of Lipid, 130*, 59.

Koussissis, S. G., Semidalas, C. E., Antonopoulou, S., Kapoulas, V. M., Demopoulos, C. A., & Kalyvas, V. (1993). PAF antagonists in foods: isolation and identification of PAF antagonists in virgin olive oil. *Revue Francaise des Corps Gras, 40*, 323–327.

Koussissis, S. G., Semidalas, C. E., Hadzistavrou, E. C., Kalyvas, V., Antonopoulou, S., & Demopoulos, C. A. (1994). PAF antagonists in foods: isolation and identification of PAF in honey and wax. *Revue Francaise des Corps Gras, 41*, 127–132.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2003). Fish consumption fish oil, $\omega - 3$ fatty acids, and cardiovascular diseas. *Arteriosclerosis, Thrombosis and Vascular Biology, 23*, 20–31.

Kristensen, S. D., Iversen, A. M., & Schmidt, E. B. (2001). $n - 3$ polyunsaturated fatty acids and coronary thrombosis. *Lipids, 36*(suppl), S79–S82.

Kyrana, V. R., & Lougovois, P. V. (2002). Sensory, chemical and biological assessment of farmed European sea bass (*Dicentrarchus labrax*) stored in melting ice. *International Journal of Food Science and Technology, 37*, 319–328.

Liapikos, T. A., Antonopoulou, S., Karabina, S. A. P., Tsoukatos, D. C., Demopoulos, C. A, & Tselepis, A. D. (1994). Platelet-activating factor formation during oxidative modification of law-density lipoprotein when PAF-acetylhydrolase has been inactivated. *Biochimica and Biophysica Acta, 1212*, 353–360.

Mayer, K., Merfels, M., Muhly-Reinholz, M., Gokorsch, S., Rosseau, S., Lohmeyer, J., et al. (2002). ω-3 fatty acids suppress monocyte adhesion to human endothelial cells: role of endothelial PAF generation. *American Journal of Physiology Heart and Circulatory Physiology, 283*, H811–H818.

Mori, T. A., Beilin, L. J., Burke, V., Morris, J., & Ritchie, J. (1997). Interactions between dietary fat, fish, and fish oil and their effects on platelet function in men at risk of cardiovascular disease. *Arteriosclerosis, Thrombosis and Vascular Biology, 17*, 279–286.

Mueller, H. W., Haught, C. A., McNatt, J. M., Cui, K., Gaskell, S. J., Johnston, D. A, et al. (1995). Measurement of platelet-activating factor in a canine model of coronary thrombosis and in endarterectomy samples from patients with advanced coronary artery disease. *Circulation Research, 77*, 54–63.

Nomikos, T., Karantonis, H. C., Skarvelis, C., Demopoulos C. A., Zabetakis, I. (2006). Platelet activating properties of lipid fractions obtained from raw and fried fish samples. *Food Chemistry, 96*, 29–35.

Panayiotou, A., Samartzis, D., Nomikos, T., Fragopoulou, E., Karantonis, H. C., Demopoulos, C. A., et al. (2000). Lipid fractions with aggregatory and antiaggregatory activity toward platelets in fresh and fried cod (*Gadus morhua*): correlation with platelet-activating factor and atherogenesis. *Journal of Agricultural and Food Chemistry, 48*(12), 6372–6379.

Rementzis, J., Antonopoulou, S., & Demopoulos, C. A. (1997). Identification and study of gangliosides from *Scomber scombrus* muscles. *Journal of Agricultural and Food Chemistry, 45*, 611–615.

Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for 1990s. *Nature, 362*, 801–809.

Ross, R. (1995). Cell biology of atherosclerosis. *Annual Review of Physiology, 57*, 791–804.

Samples, B. L., Pool, G. L., & Lumb, R. H. (1999). Subcellular localization of enzyme activities involved in the metabolism of platelet-activating factor in rainbow trout leukocytes. *Biochimica and Biophysica Acta, 1437*(3), 357–366.

Shields, R. J. (2001). Larviculture of the marine finfish in Europe. *Aquaculture, 200*, 55–88.

Steinberg, D. (1997). Low density lipoprotein oxidation and its pathobiological significance. *Journal of Biological Chemistry, 272*, 20963–20966.

Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., & Witztum, J. L. (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *New England Journal of Medicine, 320*(14), 915–924.

Subbanagounder, G., Leitinger, N., Shih, P. T., Faull, K. F., & Berliner, J. A. (1999). Evidence that phospholipid oxidation products and/or platelet-activating factor play an important role in early atherogenesis: in vitro and in vivo inhibition by WEB 2086. *Circulation Research, 85*, 311–318.

Summers, B., al-Hassan, J. M., Thomson, M., Chun, A., & Criddle, R. S (1991). Platelet activating factor (AGEPC) from epidermal secretions of the Arabian Gulf catfish, (*Arius bilineatus*), which stimulate wound healing. *Biochimica and Biophysica Acta, 1091*, 329–336.

Tokumura, A., Sumida, T., Toujima, M., Kogure, K., & Fukuzawa, K. (2000). Platelet-activating factor (PAF)-like oxidized phospholipids: relevance to atherosclerosis. *Biofactors, 13*, 29–33.

Wierzbicki, A. S., Poston, R., & Ferro, A. (2003). The lipid and non-lipid effects of statins. *Pharmacology and Therapeutics, 99*, 95–112.

Witztum, J. L. (1994). The oxidation hypothesis of atherosclerosis. *Lancet, 344*(8925), 793–795.

Witztum, J. L., & Steinberg, D. (1991). Role of oxidized low density lipoprotein in atherosclerosis. *Journal of Clinical Investigation, 88*, 1785–1792.