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Quercetin properties as a functional ingredient in fish gels fed to rats

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ABSTRACT

Wistar rats were fed with surimi gels containing either sunflower oil, fish oil ($\omega 3$), and the same formulation additionally supplemented with 0.105 % quercetin ($\omega 3$ -Q). Antioxidant capacity was highest in gels with added quercetin when measured by the FRAP method, but not by the DPPH procedure. Lipid stability was not enhanced by quercetin since commercial fish oil already contains stabilizers. Quercetin modified neither rheological properties nor water holding capacity of the gels; however, it produced a large increase in yellowness (b^*). Serum lipid profile of rats was not significantly different. Total serum antioxidant capacity by FRAP was significantly increased only in the $\omega 3$ -Q group. Plasma malonaldehyde (MDA) was similar in the $\omega 3$ and $\omega 3$ -Q groups, indicating no prooxidative effect of quercetin in vivo. These results suggest that quercetin, added as a food ingredient in fish gel, is available in vivo after gelation process.

Key words: quercetin, surimi, bioavailability, lipid stability, antioxidant

INTRODUCTION

The term "Functional Foods" appeared in Japan twenty years ago to define foods that not only have nutritional value, but additionally they have potential benefits on health. They are also called fortified or enriched foods because some essential compounds are added in their composition, for example, antioxidants, omega-3 fatty acids, vitamins, minerals, fibres or probiotics.

Natural antioxidants are compounds of great interest not only for their benefits in the food itself but also because they have potential health benefits in the body. For this reason, it is necessary to focus the development of functional food from two points of view: technological and nutritional. In the food, oxidation is one of the most important processes accounting for its deterioration because it may affect food safety, color, flavor and texture. Also damage to protein can produce a modification of the functional properties of the food. Thus, preventing free radical attack and subsequent oxidative damage to foods is one of the main interests of natural antioxidants from a technological approach. Nutritionally, activity of natural antioxidants aims to the protection of molecules such as lipids, DNA, and proteins in the body, which are susceptible to free radical attack and suffer oxidation or damage (1-3).

Quercetin is a natural polyphenolic antioxidant. Polyphenols are secondary plant metabolites found as pigments in plants, being responsible for the red, blue, and yellow colors in onions, wine, tea, apples, berries, etc., having other functions such as antifungic, antibacterial, etc. (4-5). Quercetin is a flavonoid that has shown a high antioxidant activity. The antioxidant activity depends on the degree and position of

hydroxylation: All flavonoids with the 3, 4 dihydroxy configuration have antioxidant activity. Moreover, flavonoids are known to form complexes with metals, chelation occurring at the hydroxy and keto groups. In studies in vitro, quercetin exhibited a stronger antioxidant activity than some synthetic antioxidants such as BHA and BHT to stabilize fish oils (6-7) and in vivo, quercetin seems to produce health benefits such as antiinflammatory, antibacterial, antiviral and anti-hepatotoxic activity (1).

Fortification of food products with natural antioxidants (8-10) in conjunction with other flavonoids from fruit and vegetables in the diet may contribute to the overall protective activity of such antioxidants in the body. Seafood products are recognized for their nutritional quality, so they could be a good vehicle for functional ingredients. In this line, natural antioxidants added to fish and seafood products could have double protective effect in the food itself and in the consumer after its intake. Food processing technologies and storage conditions can affect antioxidants and oxidative stability of foods (11), although literature about their consequences in the organism after consumption of processed foods is scarce. Development of functional foods, thus, should focus on the technological process and nutritional properties of fortified food products. Due to the complexity of real foods, the best approach to assess the quality of processed foods is to measure antioxidant activity both in vitro on the food itself and in vivo after consumption. (12).

The aims of this work were to study the effect of quercetin on the characteristics and stability of fish gels, fortified with $\omega 3$ fatty acids; and second, to investigate quercetin bioavailability and its effect on plasma antioxidant capacity and lipid profile in rats fed three weeks with fish manufactured products enriched in quercetin.

MATERIALS AND METHODS

Material. Frozen surimi grade A from Southern Blue Whiting (*Micromesistius australius*) was used, containing 4 % sorbitol and 0.3 % sodium tripolyphosphate (Emdepes SA, Chile). Frozen blocks of surimi (10 Kg each) that had been held at -20 °C were impact-shattered, portioned into 1 Kg units, vacuum-packed (Cryovac BB4L, Duncan, SC, USA), and stored at -20 °C until needed. Moisture content of surimi was determined to be 74.96 % (13).

Gel preparation. Frozen surimi was tempered in a chilled room and placed in a refrigerated vacuum homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Germany). It was ground for 1 min at high speed. Sodium chloride (1.5 % w/w) in gel (Panreac, Montplet & Esteban S.A., Barcelona, Spain) was added and homogenized for 3 min at slow speed. Then 5 % starch (Clearam CH 20, Laisa, Barcelona, Spain), 2 % egg white (SKW Biosystems S.A., Rubí, Barcelona) were added. Finally, 0.105 % quercetin (Antrancine Q® from Altaquimica, Barcelona, Spain) and/or 8.3 % oil (ROPUFA™ '30' n-3 Food Oil, Roche Lipid Technologies, Derbyshire, DE) were added and mixed for 1 more minute. Oil was formulated for a final gel moisture (78 %) but the part of calculated water was substituted by oil. The commercial marine oils, containing large amounts of polyunsaturated fatty acids (>30 % of omega-3), already contain antioxidants to retard lipid oxidation and to decrease development of off-flavors. The amount of quercetin chosen for this study was based on an estimated average flavonol intake in the human diet, and considering a daily serving of 85 g restructured fish. The oil level was chosen to fortify the seafood gel with 2.5 % omega-3 fatty acids while retaining acceptable sensory characteristics. The homogenate was beaten slowly for 6

min under vacuum, keeping sample temperature below 10 °C, adding the quercetin in the oil phase in the last minute.

The resulting batters were stuffed into cases (Krehalon Soplaril, Barcelona, Spain) of 40 µm thickness and 3.5 cm diameter. Cases were submitted to 37 °C 30 min followed by 90 °C 50 min in a water bath. All the samples were cooled immediately and stored in a cold room at 4 °C overnight. Some samples were analyzed the day after preparation and the rest were frozen and stored at -20 °C in order to avoid modifications of the gels.

Rheological measurements. Formed gels were removed from their casings, cut into cylindrical specimens (3.5 cm diameter and 3 cm height) and briefly tempered at 20 °C. Folding test resistance of a slice (3.5 cm in diameter and 3 mm high) is folded over twice at scored 1-5 according how it breaks. The puncture test was performed with a round stainless steel probe (5 mm dia ball) penetrated gels to their breaking point on a Texture Analyzer (TA-XT2i, Texture Technologies, Corp., Scarsdale, NY, USA) at a penetration speed of 1.1 mm/s and a 5 Kg load-cell. Breaking force (g) and deformation (penetration depth, mm) were determined on at least six specimens per treatment. The compression Test: Texture Profile Analysis (TPA) was applied by a cylindrical plunger (Ø = 75 mm) adapted to a 25 Kg load cell at a deformation rate of 2 mm/s with a compression of 60 % of their height; each sample was compressed twice. The parameters determined were hardness [N], adhesiveness [J] and cohesiveness [adimensional]. Elasticity (%) was determined by stress-relaxation test after 1 min of relaxation. The percentage of relaxation was calculated as $YT = 100 (F_0 - F_1) / F_0$, where F_0 is the force registered at the onset of relaxation immediately after sample compression and F_1 is the force registered after 1 min of relaxation. Thus, 100-YT is taken as an

index of elasticity and is expressed as percentage elasticity of the gel. At least four
138 replications of all determinations were performed.

140 **Other protein functional properties.** *Water holding capacity* was determined
following the centrifugation method described by Montero and Gómez-Guillén (14).
142 *Color* was determined on a colorimeter (HunterLab MiniScan MS/S-4000S, Hunter
Associates Laboratory Inc., Reston, VA, USA) using the CIE Lab scale (D65/10°)
144 where b^* is the parameter that measures yellowness. Whiteness Index was defined as:
 $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (15). The result was the average of six measurements
146 taken at ambient temperature at different points on the sample.

148 **Lipid stability.** *TBARS* analysis was based on Wang and others (16) but slightly
modified in order to remove starch and other interferences from additives. A portion of
150 20 g gel was homogenized in 90 mL extract solution (7.5% trichloroacetic acid, 0.1%
propyl gallate, 0.1% EDTA) using a homogenizer (Osterizer®) at speed 5 for 1 min.
152 The homogenate was centrifuged at 6,000g for 5 min. Then supernatant was centrifuged
at 6,000g for 45 min using centrifugal filter devices of 100 K (Amicon® Ultra-15,
154 Millipore Corp). An aliquot of 2.0 mL supernatant was mixed with 2.0 mL of 80 mM
TBA solution. The mixture was incubated at 40°C for 90 min. The absorbance was read
156 at 532 nm. Results are expressed as μ moles malonaldehyde per 100 g gel.

158 **Fatty acid composition.** Total crude lipids were extracted by the Soxhlet method.
The preparation of methylesters was followed as described by Christopherson and Glass
160 (17). GC Analysis of total fatty acids: About 1 μ L of fatty methylesters (FAME) were
analyzed on an Perkin-Elmer 8500 GC with a DB 23, 60 m x 0.25 mm fused silica

column (J & W Scientific Co, Folsom, CA, USA), that was temperature programmed from 100 to 230 °C with both injector and detector temperatures set at 260-280 °C.

Identification of FAME was based on comparison of retention times to methyl ester standards (Supelco 37 component, FAME mix, Supelco, Bellefonte, PA, USA) using C9:0 (Fluka, Sigma-Aldrich, Switzerland) as the internal standard. Fatty acid composition was expressed as mg/g oil. The oxidation index, which measures the ratio of polyunsaturated fatty acids (docosahexanoic acid C22:6) to saturated fatty acids (palmitic acid C16:0), was used to indicate the oxidation of fish oil.

Antioxidant activity. *Quantification of Polyphenols* was carried out in supernatants obtained after extraction with aqueous-organic solvents of gels (18). Extractable polyphenols were analyzed by the Folin-Ciocalteu method (19) and expressed as gallic acid equivalents on a dry matter basis.

Free Radical Scavenging measurement. The antiradical capacity of the fish gel extracts and pure compounds was estimated according to the procedure reported by Brand-Williams and others (20), which was slightly modified by Sánchez-Moreno and others (21). For the preparation of food extract, gels were thawed, and the extracts were obtained by homogenizing 10 g of each gel with 50 mL of methanol in an homogenizer (Omni-mixer, Type OM, Ivan Sorvall, Inc, Norwalk Conn., USA) during 2 min (setting 6) in a bath containing water and ice. Then the extracts were filtered under suction and their capacity to scavenge the stable radical DPPH measured. EC₅₀ is the concentration of antioxidant needed to decrease by 50 % the initial DPPH concentration; reaction time (TEC₅₀) is the time needed to reach a steady state at the concentration corresponding to EC₅₀ and antiradical efficiency (AE = 1/EC₅₀.TEC₅₀) involves the potency and the reaction time.

188 *The ferric reducing/antioxidant power (FRAP)* assay was used as a measure of the
reducing ability of gels. It is based on the increase in absorbance at 595 nm of the
190 complex tripyridyltriazine (TPTZ)-Fe(II) in the presence of reducing agents. The method
of Benzie and Strain (22) with some modifications (23) was used, taking absorbance
192 readings during 30 min and expressing the results as Trolox equivalents.

194 **Animal experiment.** Wistar rats bred in the animal facilities of the Facultad de
Medicina (University Complutense of Madrid) were housed in stainless steel wire-
196 bottomed cages and maintained with controlled temperature and artificial dark-light
cycle. Animals were fed a standard laboratory diet ad libitum (19 g protein, 56 g
198 carbohydrate, 3.5 g lipid and 4.5 g cellulose/100 g plus salt and vitamin mixtures) until
they reached a weight of 200 g. Animals were weighted and randomly assigned to the
200 different dietary groups. Three different groups of 10 rats each were established, the
first group received surimi gels made with sunflower oil as a control group, the second
202 group received surimi gels made with fish oil and the third one received surimi gels
made with fish oil plus quercetin. Rats were accustomed to surimi gels before starting
204 the experiment. Each rat received 30-40 g of the surimi gel daily, and was given free
access to water. All animals were submitted to this diet for three weeks, and were
206 weighted every other day. At the end of the experimental period rats were killed after an
overnight fast and trunk blood was collected. Blood samples were allowed to clot on ice
208 for 30 min, and serum was separated and stored at -80°C until assayed. All experiments
were conducted in accordance with the principles and procedures outlined in the
210 National Institutes of Health (NIH, Bethesda, MD) guide for care and use for
experimental animals.

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The ferric reducing/antioxidant power (FRAP) assay was carried out in serum from
214 experimental animals as described above. Malondialdehyde (MDA) was determined by
HPLC as the 2,4-dinitrophenylhydrazone in plasmas following the method of Mateos
216 and others (24). Also serum lipid profile was analyzed in a RA-500 autoanalyzer
(Technicon, Bayer, SA, Barcelona, Spain). Free Fatty Acids were analyzed by the
218 method of Nagele and others (25). Total Cholesterol was determined by the enzymatic
method of Siedel and others (26). Cholesterol-HDL was measured by the precipitation
220 method of Warnick and others (27). Triglycerids were determined by the enzymatic
method of Bucolo and David (28).

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Statistical analysis. One-way analysis of variance was carried out using the SPSS v
224 11.5.1 (SPSS Inc., Chicago, Illinois). The method used to discriminate among the
means was Bonferroni's multiple comparison procedure. Level of significance was set
226 at $p \leq 0.05$.

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RESULTS AND DISCUSSION

Properties of fish gels. Surimi from low fat species was selected since this is the surimi most commonly used by the industry. Surimi from high fat species is rather unstable during storage, besides, fat may interfere in gelation process and the color of the end product is darker than that obtained from low fat species.

It is known that the addition of oil can result in changes in properties of the gels (29-30). High amount of fat can be an undesirable element in gel making since it may interfere with the formation of a protein matrix and hence hinders gelation. To avoid this problem the oil was added in the last minute of homogenization after the solubilization of the protein with the salt. In addition, a certain amount of egg white and starch were added, which have been proved to improve gelation and binding properties of a raw material having poor gel forming capacity (14). Thus, despite the high oil content (8% in the final product), all gels showed good gel forming capacity evaluated on the basis of folding, puncture and compression tests. The maximum score in the folding test was obtained, and, as shown in Table 1, no significant differences in work of penetration neither in other rheological parameters, measured by TPA and stress-relaxation test, were found among the different formulations. On the other hand, taken into account that all formulations contained the same amount of oil, no significant differences were noticeable due to the type of oil used: sunflower or fish oil.

The addition of quercetin did not alter rheological properties of gels (Table 1), although it has been reported, as phenolic compound, to be potentially reactive with proteins depending of pH, phenol concentration, protein and/or phenol configuration (31-32). This could be attributed to the small amount of quercetin added and also to the

presence of a considerable amount of gelling additives such as starch and egg white. Other studies reported an increase in breaking force values in pressure-induced mackerel mince gels when 0.075 % rosemary was added, but it did not occurred in heat-induced gel (30,33).

On the contrary, added quercetin led to a significant ($p \leq 0.05$) slight decrease in water holding capacity as compared to the gel containing fish oil (Table 1).

Table 1.- Characteristics of surimi gels containing the different oils with or without quercetin. Different letters (a, b, c) indicate significant differences ($p \leq 0.05$) between samples. WHC means water holding capacity and b^* is yellowness.

Surimi Gels with	Work of penetration	Hardness	Adhesiveness	Cohesiveness	Elasticity	Water holding capacity	Whiteness I	b^*
Sunflower	4967 $\pm$ 12	12503 $\pm$ 450	279 $\pm$ 55	0.73	56.4 $\pm$ 0.4	88.8 $\pm$ 1.0	70.7 $\pm$ 0.4	7.3 $\pm$ 0.2
Fish oil	4476 $\pm$ 22	12716 $\pm$ 631	281 $\pm$ 24	0.73	56.4 $\pm$ 0.5	89.2 $\pm$ 1.3	72.0 $\pm$ 0.4	9.5 $\pm$ 0.4
Fish oil + quercetin	4765 $\pm$ 31	13180 $\pm$ 997	298 $\pm$ 75	0.72	55.9 $\pm$ 0.9	86.1 $\pm$ 1.8	64.8 $\pm$ 0.3	18.3 $\pm$ 0.8

The addition of quercetin produced important changes in color of gels. In Table 1, it is shown the whiteness and the yellowness parameter (b^*). There were significant differences between formulations due to the type of oil added, showing higher yellowness the formulations with fish oil compared with the sunflower oil. The addition of quercetin to the gel containing fish oil increased the yellowness in about 50 %. This fact could be a problem for the gel acceptability by Western consumers; however, other consumers may have no such acceptability problems like Japanese, that consume a great variety of seafood products with bright colors. Chen and others (8) reported also an increase in yellowness (b^*) in pork patties because of the bright yellow color of quercetin. Overall, quercetin has been reported to improve the color stability of beef patties during chilled storage (9).

Lipid composition and stability in fish gels. In figure 1 is shown the fatty acid profile of the raw oils before addition into the surimi. Sunflower oil (Fig.1.a) contains high amount of omega-6 (linoleic acid C18:2) and omega-9 (oleic acid C18:1) fatty acids, about, 60 % and 27 %, respectively. While fish oil (Fig. 1.b) contains about 35 % of omega-3 (mainly eicosapentaenoic C20:5 and docosahexaenoic C22:6 acids).

Figure 1. Fatty acid profile as methylesters of sunflower oil (a) and fish oil (b)

It is worth noting that though the commercial fish oil used in this study contains large amounts of polyunsaturated fatty acids, it contains a mixture of antioxidants (ascorbyl

palmitate, rosemary extract and mixed tocopherols) which have commercially been added to retard lipid oxidation and to decrease development of off-flavors. On the other hand, sunflower oil also contains endogenous antioxidants (mainly α -tocopherol).

Regarding lipid stability in gel formulations, the oxidation index (Fig. 2) showed no differences between raw fish oil and after its incorporation into the surimi gel, indicating that the gelation process did not change lipid stability, probably due to the fact that commercial fish oil already contains antioxidants. However, the extra addition of quercetin produced a slight oxidation since there was a slight yet statistically significant decrease in the oxidation index (C22:6/C16:00) suggesting oxidation of the docosahexanoic acid. It is known that sometimes high amount of antioxidants can show a pro-oxidative effect, which could account for the observed results when quercetin was added to oils already containing antioxidants, where the amount of antioxidants would be higher than the usual levels in foods, around 200-700 ppm.

Figure 2.- Effect of gelation process determined by oxidation index of the raw fish oil and surimi gels containing fish oil with or without quercetin. Different letters (a, b) indicate significant differences ($p \leq 0.05$) between samples.

As reflected by the thiobarbituric acid (TBA) values (Fig.3) in gels frozen stored for five months at -20°C , the sample with lower rancidity development were the gels containing sunflower oil due to the greater stability of sunflower oil in comparison with fish oil. In the case of gels with omega-3 fatty acids, the antioxidant mixture already included in the commercial preparation seemed also to be active enough to prevent lipid oxidation or rancidity of muscle and non-muscle lipids. However, no higher protection was obtained by quercetin addition; on the contrary, the highest TBA value was found in gels containing fish oil and quercetin, which is in accordance to the results on the oxidation index shown above (Fig. 2).

Figure 3. TBA index of surimi gels containing the different oils with or without quercetin. Different letters (a, b, c) indicate significant differences ($p \leq 0.05$) between samples.

This finding agrees with the results of Pérez-Mateos and others (33) suggesting that addition of extra antioxidant amounts (green tea, rosemary) to commercial fish oil did not enhance stability of the product during storage. However, when quercetin was added as the unique antioxidant or in combination with others but at low concentration, it showed a stabilizing effect. This has been found in pork patties (8), cook-chill chicken (10); beef patties (9), ground fish (34); and fish oil (7). Karastogiannidou (10) reported that the antioxidant effect of onion, determined by TBA index, was equivalent to that of its measured quercetin content.

Antioxidant capacity of fish gels. The antioxidant capacity, measured by FRAP assay, showed the highest values in gels enriched with quercetin (Fig. 4) with no differences between the gels containing sunflower or fish oils. However, no significant differences due to quercetin addition to the gel prepared with fish oil were found when the DPPH method was used (Fig. 5), with a very low antiradical efficiency of the gel containing sunflower oil. Antioxidants can act as reducing agents, free radical scavengers and/or metal chelators. Some compounds can be good free radical scavengers whilst not showing major reducing properties (e.g. carotenoids have appreciable antiradical activity against the DPPH radical while they show no reducing power when assayed by the FRAP method (23). On the other hand, some compounds have remarkable antioxidant capacity acting at the three levels, such as the case of

quercetin, which has been shown to be a powerful antioxidant using an array of methodologies.

The results obtained in this work showed that the highest reducing and antiradical capacities (**Figs 4 and 5**) corresponded to gels containing quercetin. The lowest results were found in gels prepared with sunflower oil, which contains weaker antioxidants like tocopherols. As to the gels containing fish oil with no added quercetin, the antioxidant added as stabilizers to this commercial oil (ascorbate palmylate and tocopherols, as well as rosemary extract, rich in flavonoids and phenolic diterpens) seem to contribute to the antiradical efficiency of the gel (Fig. 5), while not showing a major ferric reducing power (Fig. 4). It should be pointed out that both methods used to measure the antioxidant capacity of gels were applied on alcoholic extracts of the samples; considering that most of the antioxidants present in the gels (tocopherols, diterpens, ascorbate palmylate) are hydrophobic compounds, the weaker antioxidant capacities found in sunflower and fish oil containing gels are to some extent expectable. Quercetin is readily soluble in alcoholic solvents such as the ones used to extract the samples for their antioxidant measurement, accounting for the higher values found in this study; also, some flavonoids present in the rosemary extract added as preservative in the commercial fish oil, are soluble in the present analytical conditions as well, which may explain the higher DPPH antiradical efficiency found in fish oil containing gels as compared with the gel containing sunflower oil. In this line, Frankel and Meyer (12) reported that the antioxidant activity in foods depends on a variety of factors, including interaction with other food components, the relative partition between phases (aqueous, lipid) and the method to determine antioxidant activity. The influence of food matrix on

the results observed in our study cannot be ruled out, since interactions of gel constituents, mainly protein, with phenolic antioxidants can be taking place.

Figure 4.- Antioxidant capacity by FRAP of surimi gels containing the different oils with or without quercetin. Different letters (a, b) indicate significant differences ($p \leq 0.05$) between samples.

Figure 5.- Antioxidant capacity by DPPH of surimi gels containing the different oils with or without quercetin.

In this sense, Table 2 shows the results of the free radical scavenging capacity of comparable amounts of quercetin in the form of pure compound and after its addition to gels containing either fish oil or without oil. These results indicate that quercetin remained active after the gelation process, though its activity in gels was quite lower in comparison with the antioxidant capacity of quercetin as a pure compound. These differences, both on the EC_{50} and on the kinetic behavior resulting in a lower antiradical efficiency (AE) of quercetin extracts from gels in comparison with the pure compound could be due to the influence of the matrix, preventing a complete extraction of the antioxidant from the gel, where quercetin can be bound to protein for which polyphenols show a high affinity (4).

Additionally, these differences indicates the importance of the food matrix since the antioxidant may be entrapped in the network and distributed in the different way in the

oil-in-water emulsion system of the fish gel, thus affecting its extraction in the solvent medium. Also, partial loss of antioxidant activity after the technological process cannot be excluded. It is well known that antioxidants could be significantly lost as a consequence of processing and storage; however, quercetin has been reported to be heat-stable and processing by cooking had only small effects on its stability (35).

Table 2. Free radical scavenging parameters of quercetin as a pure compound and in gel extracts. Where EC₅₀ is the amount of sample needed to decrease by 50 % the initial DPPH• concentration; T_{EC50} is the time needed to reach the steady state at EC₅₀ concentration and AE is the antiradical efficiency

Sample	EC ₅₀ (mg / mL)	T _{EC50} (min)	AE
Pure quercetin	0.095	3.25	3.24
Quercetin from gel	0.14	11.17	0.64
Quercetin from gel containing fish oil	0.11	13.26	0.68

As expected, the concentration of phenolic based compounds present in the gel extracts, as determined by the Folin-Ciocalteu method, was higher in samples with fish oil ($\omega 3 = 0.33 \% \pm 0.03$; $\omega 3\text{-Q} = 0.60 \% \pm 0.04$ on a dry weight basis) and quite low in gels with sunflower oil ($0.066 \% \pm 0.038$ on a dry weight basis). The apparently high

polyphenolic content of fish oil may be due to the phenolics present in the rosemary extract added to the oil. These results are in agreement with the antioxidant capacity measured by FRAP (Fig. 4), where higher activity was found in gels with quercetin added and very low for sunflower oil. Nuutila and others (5) reported a correlation between antioxidant activity and the amount of phenolic compounds when pure quercetin or onion extract were used.

Bio-availability in rats. In this part of the study rats were used to study the bio-availability of quercetin when incorporated into surimi gels. In order to obtain the same nutritional composition of gels and sensorial attributes, sunflower oil was added in the formulation control. Food intake and body weight gains of rats were adequate and similar in all cases. This good tolerance indicates a very homogeneous diet and the results could be nutritionally sound.

Total serum antioxidant capacity by FRAP, was higher in the group of rats that had quercetin gels in the diet (Fig. 6). No differences in antioxidant activity were found between the gels prepared with sunflower and those with fish oil. These results reflect the absorption and subsequent bioavailability of quercetin from fish gels. As mentioned before, quercetin is one of the most potent natural antioxidants, which accounts for the increased antioxidant capacity observed in the plasma of rats fed surimi gels containing small amounts of this polyphenol in spite of the time elapsed since food intake and blood collection after an overnight fast. Quercetin can be absorbed from the stomach of rats as an aglycone, contrary to quercetin glycosides like rutin (36). Plasma concentrations of quercetin peak at 3 h postprandially, with a half life of elimination close to 17 h. Although in our experiments quercetin could not be detected in rat plasma

after HPLC-DAD analysis (data not shown), the increased FRAP activity in the plasma samples suggests the presence of this compound. Should blood samples have been taken postprandially instead of after an overnight fast, the presence of quercetin in plasma could have been detected and FRAP values likely would have been higher than the levels observed in the present experimental conditions. However, since we also aimed at studying the potential effect of surimi gels with vegetable versus fish oils, with or without added quercetin, on the levels of plasma lipids, blood samples needed to be taken in fasting animals.

Figure 6.- Total rat plasma antioxidant capacity by FRAP after intake of surimi gels containing the different oils with or without quercetin. Different letters (a, b) indicate significant differences ($p \leq 0.05$) between samples

On the other hand, the results obtained show that the apparent pro-oxidative effect of quercetin in gels containing fish oil, with a reduced C22:6/C16:0 ration (Figure 2),

would not have any major effect on plasma antioxidant activity in vivo. To check this point, malondialdehyde (MDA) was measured by HPLC in plasma as an index of lipoperoxidation in vivo (Fig. 7). MDA could not be detected in plasmas from rats consuming gels prepared with sunflower oil. On the contrary, when fish oil was used in the gel formulation, up to 0.7 nmol MDA per mL of plasma was detected, with no differences between the rats consuming gels with or without quercetin. This high MDA values may be due to the high proportion of polyunsaturated fatty acids (PUFA) in fish oil (up to 30 %, Fig 1.b), which are more susceptible to oxidation. Also, the addition of quercetin did not show any pro-oxidative effect in vivo, contrary to what was observed in the gel in vitro.

In this sense, Eder and others (37) observed that an excess vitamin E supplementation that induced prooxidation in fish oil did not show any prooxidative effect in vivo. However, in order to warrant product safety, when a functional food with antioxidant properties in the organism is looked after, it should be preferable to make fish gels with quercetin or with a similar antioxidant but with no stabilized commercial oil added.

Figure 7.- Malondialdehyde (MDA) content of plasma from rats consuming surimi gels containing the different oils with or without quercetin. Different letters (a, b) indicate significant differences ($p \leq 0.05$) between samples

As to the plasma lipid profile of $\omega 3$ and $\omega 3$ -Q fed rats, no significant differences with the control group were observed (Table 3). Total cholesterol, HDL and LDL cholesterol as well as triacylglycerides and free fatty acid levels were similar in the three animal groups in the present experimental conditions. The level of quercetin in the surimi gel consumed by the rats was probably too low to have appreciable effects in healthy animals. It has been shown that polyphenol intake can reduce plasma levels of cholesterol, specially, when these are increased as in hypercholesterolemia (38). In this sense, Yokozawa and others (39) reported a reduction of cholesterol levels which was dose-dependent (0.1-2.5 % green tea), showing reduced serum levels of all types of cholesterol except HDL cholesterol with 2.5 % polyphenol administration. It would be of interest to study the potential effect on hypercholesterolemic rats of surimi gels containing with higher levels of quercetin or $\omega 3$.

Table 3.- Plasma lipid profile in rats fed surimi gels containing the different oil and with or without quercetin. Different letters (a, b, c) indicate significant differences ($p \leq 0.05$) between samples. Ch means cholesterol, TG: triglycerides, FFA: free fatty acids, HDL-Ch; high density lipoprotein-cholesterol; LDL-Ch: low density lipoprotein-cholesterol

Diet	Total Ch (mg/dL)	TG (mg/dL)	FFA (mEq/L)	HDL-Ch (mg/dL)	LDL-Ch (mg/dL)
Sunflower	57.2 a $\pm$ 6.9	33.2 a $\pm$ 11.3	0.31 a $\pm$ 0.04	30.0 a $\pm$ 2.6	19.0 a $\pm$ 5.9
Fish oil	50.17 a $\pm$ 8.8	37.0 a $\pm$ 15.7	0.32 a $\pm$ 0.02	31.0 a $\pm$ 5.1	16.8 a $\pm$ 5.0
Fish oil + quercetin	53.5 a $\pm$ 8.1	40.2 a $\pm$ 19.2	0.32 a $\pm$ 0.03	30.0 a $\pm$ 6.5	20.2 a $\pm$ 5.7

In conclusion, quercetin addition did not alter the rheological gel characteristics, except for yellowness, and it was well tolerated by the animals. Although apparently increasing gel oxidizability, the addition of quercetin had no pro-oxidant effect in vivo, even enhancing plasma antioxidant capacity. Two different formulations of fish products should be prepared depending on the desired effect: high quercetin concentration to get a healthy effect in the organism or a lower amount of the antioxidant to get stabilization of ω 3 fatty acids in food.

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