JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Influence of Dietary Genistein Levels on Tissue Genistein Deposition and on the Physical, Chemical, and Sensory Quality of Rainbow Trout, *Oncorhynchus mykiss*

NATASHA D'SOUZA,[†] DENISE I. SKONBERG,*[,†] MARY E. CAMIRE,[†] KELLY E. GUTHRIE,[†] JEFFREY MALISON,[‡] AND LUCIENNE LIMA[‡]

Department of Food Science and Human Nutrition, University of Maine, Orono, Maine 04469, and University of Wisconsin−Madison Aquaculture Program, Department of Animal Sciences, University of Wisconsin−Madison, Madison, Wisconsin 53706

Genistein, the primary isoflavone in soybean, is one of the chemical components responsible for some of the off-flavors associated with soy-based foods. The potential effects of genistein on the sensory and chemical quality of fish muscle may affect the full utilization of soybean meal as an alternative protein in aquaculture diets. Fingerling trout fed commercial diets containing 0, 500, 1000, or 3000 ppm pure genistein were analyzed after 6 and 12 months of feeding. Genistein was extracted by enzymatic digestions in Tris buffer and quantified by high-performance liquid chromatography. Moisture, fat, protein, ash, and tristimulus color of the fillets were determined. The extent of lipid oxidation occurring in fillets harvested after 12 months of feeding was studied by measurements of thiobarbituric acid reactive substances (TBARS) after 4 and 8 days of refrigerated storage at 4 °C. Triangle tests were performed to determine if there were any detectable sensory differences. A dietary genistein content of 3000 ppm led to the deposition of approximately 5.4 pmol of genistein/mg of fillet. Triangle test panelists were unable to detect any significant ($p \leq 0.05$) differences between the fillets from trout fed the 0 and 3000 ppm genistein concentrations. Moisture, ash, and protein content were influenced by time of harvest, while color was unaffected. TBARS levels on days 4 and 8 were significantly ($p < 0.05$) higher in the fillets from the 0 ppm genistein level than in fillets from fish fed dietary genistein.

KEYWORDS: Genistein; isoflavones; soy; quality; *Oncorhynchus mykiss*; high-performance liquid chromatography

INTRODUCTION

The increase in the United States consumption of seafood from 11.2 pounds per person in 1910 to 15.6 pounds per person in 2002 (*1*) has placed tremendous pressure on traditional fisheries. Nonetheless, many traditional fish stocks have been fully exploited (*1*), thus limiting the amount of fish meal, the primary protein component in aquaculture diets for salmonids. To keep up with the increased demand for seafood, aquaculture production must continue to grow thereby necessitating a suitable alternate dietary protein source. Soybean meal is a logical solution because it is consistently available in the United States and in several South American countries, economically attractive, a source of high-quality protein, and sufficiently palatable to most fish species. Soy has been increasingly investigated for its potential use in fish diets (*2*−*6*). Its isoflavone components, primarily genistein and daidzein, are reported to have anticancer and antiaging effects and to help prevent cardiovascular disease (*7*, *8*), primarily because of their antioxidative activity. Moreover, genistein can exert both estrogenic and antiestrogenic activities because of the structural similarities between soy isoflavones and estrogens (*9*, *10*).

Flesh quality of fish is strongly influenced by diet. Carotenoids, lipid sources, antioxidants, and other dietary components can affect color (*11*, *12*), fatty acid profile (*13*, *14*), texture (*15*, *16*), and flavor (*17*, *18*) of farm-raised fish. Similarly, the absorption of feed components such as isoflavones in the fish muscle may offer additional benefits to consumers. Lin et al. (*19*) showed that genistein deposited in the eggs of Japanese quail could increase the appeal of eggs and also increase human consumption of isoflavones. Dietary genistein supplementation was found to decrease lipid oxidation in the liver of hamsters, after 5 weeks of feeding; however, lipid oxidation in the muscle was not measured (*20*). Neither of the studies reported any sensory evaluation of the edible product. The off-flavors associated with soy and soy-based foods have been attributed

* To whom correspondence should be addressed. E-mail: denise.skonberg@umit.maine.edu. Telephone: 207-581-1639. Fax: 207-581-1636.
[†] University of Maine.
[‡] University of Wisconsin−Madison.

10.1021/jf0484024 CCC: $30.25
Published on Web 04/02/2005

Table 1. Proximate Composition of Rainbow Trout Diet[a]

percent crude protein	40	percent crude fiber	3
percent crude fat	10	percent ash	12

[a] Data obtained from Nelson and Sons, Inc., UT.

to lipid oxidation and isoflavones, particularly genistein, which is known to have a bitter taste (*21*), and other chemical components of soy such as saponins, ketones, and aldehydes (*21*−*23*). Lipid concentrations in the muscle of cobia fed soy-based diets were found to significantly increase with an increase in soybean meal, while muscle ash and protein varied without a definite trend (*24*). The estrogen-like effects of genistein (*6*, *9*) may affect proximate composition of fish muscle; however, the effects of individual soy components on proximate composition of fish have not been reported. Dietary genistein may therefore have positive or negative effects on the sensory and chemical quality of fish muscle, which may effect the full utilization of soy in fish feed.

The objective of this study was to determine if genistein was deposited into the muscle, if the tissue genistein levels were dependent upon dose, and to evaluate the effects of dietary genistein on the physical, chemical, and sensory quality of rainbow trout reared on diets containing 0, 500, 1000, or 3000 ppm genistein. Although rainbow trout diets may contain up to 40% soybean meal, corresponding to approximately 3000 ppm genistein, a 20−30% soybean meal inclusion is more typical. The rationale of the study was not to justify the use of genistein in fish feeds but to identify soy components that may potentially affect quality attributes of the end product.

MATERIALS AND METHODS

Experimental Treatments. A total of 395 rainbow trout fry, *Oncorhynchus mykiss*, having a mean weight of 4.5 g, were randomly distributed into 12 flow-through tanks (10−15 °C) at the University of Wisconsin−Madison Aquaculture program facility. The fish were fed one of four diets: a Silver Cup Trout (Nelson and Sons, Inc., UT) commercial feed (**Table 1**), which was incorporated with either 0, 500, 1000, or 3000 ppm genistein (Steraloids, Inc., Newport, RI). The genistein solutions were prepared in ethanol and sprayed onto the commercial feed. The feed was then placed in a hood until all ethanol evaporated and then stored in a refrigerator at 4 °C. Fish were fed to satiation once daily for 12 months. Three fish from each tank were sampled after 6 and 12 months of consuming the experimental feeds. The trout reared in this study were test animals in a larger collaborative research project conducted with the University of Wisconsin−Madison to evaluate the long-term effects of genistein on trout growth, reproduction, and quality. Hence, earlier sampling periods, i.e., prior to 6 months, were not conducted. The effects of genistein on growth and reproductive physiology will be reported elsewhere.

Fillet Preparation: Fish were gutted, cleaned, filleted, and then shipped overnight on ice to the University of Maine. Fillets for sensory evaluation were then rinsed with cold running water, put into Ziploc freezer bags, and frozen immediately at −17 °C. The remaining fillet portions from each tank were skinned and ground together in a food processor (Black and Decker Handy Chopper Plus, HC 3000) for 45 s. The ground sample was then placed in a Ziploc bag and refrigerated at 4 °C for genistein analyses, for determination of color, moisture, crude protein, ash, and fat content, and for analysis of thiobarbituric acid reactive substances. Genistein analysis was performed the next day, while proximate analyses were completed within a week of refrigerated storage.

Chemicals and Supplies. Genistein (4′,5,7-trihydroxyisoflavone) for the preparation of standard solutions, crude lipase (from porcine pancreas), protease (from *Streptomyces griseus*), and β-glucuronidase/arylsulfatase (98 800 units/mL of β-glucuronidase and 1000−5000 units/mL of arylsulfatase from *Helix pomatia*) were obtained from Sigma Chemical Co. (St. Louis, MO). Other reagents were purchased from Fisher Scientific Co. (Fairlawn, NJ). Reagents used for HPLC were all HPLC-grade. The solid-phase extraction cartridges were obtained from Phenomenex (Torrance, CA), and HPLC vials (2 mL) were from National Scientific Co. (Duluth, GA).

Genistein Extraction and Quantification. A modification of the procedure for genistein extraction from Maubach et al. (*25*) was used in this study. A 2 g portion of the ground fillet was mixed with 9 mL of Tris buffer (45 mM at pH 7.4) and homogenized for 60 s. An additional 10 mL of Tris buffer, lipase (200 mg), and protease (32 mg) were added to the homogenized mixture, vortexed, and incubated at 37 °C for 60 min. Then, 10 mL of sodium acetate buffer (pH 5.0) and 120 μL of β-glucuronidase/arylsulfatase were added to the sample and further incubated for 6 h at 37 °C. A 15 mL volume of hexane was added and then centrifuged (Beckman TJ-6 centrifuge with refrigeration unit, Palo Alto, CA) at 3000*g* for 30 min at 4 °C. The hexane layer was discarded, and the methanol-buffer layer was diluted with 15 mL of water. Solid-phase extraction columns were preconditioned with 4 mL of ethyl acetate, 4 mL of methanol, and 4 mL of water, consecutively. After sample application, the cartridges were rinsed with 10% methanol (v/v) in water and genistein was eluted with 4 mL of methanol. All ground fillet samples were extracted in duplicate per tank.

Experiments to validate the adequate performance of the extraction procedure were carried out on salmon tissue. Samples of salmon muscle weighing 2 g were injected with 100 μL of a 4000 μg/mL genistein stock solution (in methanol). The spiked muscle samples were held overnight at 4 °C to allow complete evaporation of methanol. Extractions as described above were performed the next day. Defatted soy flour was also extracted concurrently and analyzed as a quality control check sample.

Analysis of genistein was carried out by reversed-phase HPLC using a HP 1050 series pump and auto sampler (Hewlett−Packard Inc., DE) equipped with a photodiode array detector (PDA). A guard column (Phenomenex, CA) preceded the Phenomenex (Torrance, CA) Ultremex 5 C_6 analytical column (150 × 4.5 mm, 5 μm). An isocratic mobile phase consisting of acetonitrile/water/phosphate buffer and tetrahydrofuran at a flow rate of 1 mL/min was used. The run time was set to 12 min. Injection volume was 50 μL. Elution was monitored at 262 nm. Data were collected using HP Chemstation software. Genistein was identified by comparing spectral data and retention times to that of pure genistein standards (Sigma Chemical Co., MO). Calibration curves based on peak areas, prepared by using a series of concentrations of pure genistein, were used for quantitation.

Color Analyses. Measurements of color were performed immediately on the ground fillet using a benchtop Hunter Colorscan XE (HunterLab, Reston, VA). A 0/45 angle of reflectance was used with the spec excluded. Port size was 2.00 inches, while the area of viewing was 1.00 inches. The ground homogenate was filled to a 10 mm thickness in a 2.5 inch glass cup and pushed down with a white ceramic disk. The tristimulus *L*, *a*, *b* measurements were recorded for the ground sample from each tank. The *L* variable represents lightness ($L = 0$ for black and 100 for white); *a* represents the intensity in red; and *b* represents the intensity in yellow. The values recorded were an average of three readings, each taken at a 45° angle turn.

Proximate Analyses. Protein, moisture, ash, and fat were determined using standard AOAC methods (*26*). Moisture determination was done by drying samples in an oven at 105 °C overnight. Dried samples were ground and used for determination of crude protein by the Kjeldahl method (Kjeltec Autoanalyzer, Foss Tecator, Denmark). Protein values were obtained by multiplying nitrogen by a factor of 6.25. Fat was determined by petroleum ether extraction and ash by heating at 550 °C in a muffle oven for 6 h. Proximate data were analyzed statistically on a dry-weight basis to determine the effects of dietary genistein levels or time of harvest on proximate composition.

Thiobarbituric Acid Reactive Substance (TBARS) Analysis. TBARS were determined on days 4 and 8 of refrigerated storage (4 °C) on fillets from the 12-month old trout, to observe if dietary genistein exerted any antioxidative effect. These 2 days were selected as allowing a sufficient time interval for lipid oxidation to occur and for any antioxidative effects to be noticed. The extent of lipid oxidation

was determined by measuring levels of malondialdehyde (MDA), a secondary lipid oxidation product. The thiobarbituric acid method (*27*) was used in which filtrate obtained from 4 g of ground fillet was reacted with thiobarbituric acid in a boiling water bath for 20 min and absorbance of the color complex formed was read at 530 nm.

Sensory Analysis. Sensory difference testing was conducted on fillets after 6 and 12 months of the feeding study. The fillets from the 0 and 3000 ppm diet treatments were thawed in a refrigerator at 4 °C on the day before testing was conducted. Fillets were baked in a gas oven for 15 min at 205 °C and cut into 7.5 × 5 cm portions, approximately 30 min before testing. The baked fillets were then held at room temperature for 5 min and served immediately to panelists. Testing was conducted at the Consumer Testing Center, University of Maine. A total of 24 untrained panelists, primarily University of Maine students, faculty, and staff, performed the difference tests. Triangle tests (*28*) were done in which panelists were presented three fillets (two from the same dietary treatment and one from the other dietary treatment) in randomized order. They were then asked to taste the fillets and pick the odd (different) one. The objective was to determine whether a significant number of panelists could differentiate the two samples. Fillet portions were coded with three-digit codes and served on paper plates. The sensory difference testing was done under standardized lighting, controlled temperature (22−24 °C), and in positive-air flow booths (*29*). Sensory testing of genistein-fed trout was performed with the approval of the University of Maine's Human Subjects Review Committee.

Statistical Analysis. Two-way analyses of variance (ANOVA) were performed on genistein, proximate, color, and TBARS data with the diet treatments (0, 500, 1000, and 3000 ppm) and time of harvest (6 and 12 months) as categorical variables. Calculation of standard deviations and line slope was done in Microsoft Excel (Microsoft, Redwood, WA), and ANOVA was done in SYSTAT (SYSTAT Software Inc., Point Richmond, CA). When ANOVA showed significant treatment effects, pairwise comparisons using Fisher's significant difference test were done. Statistical significance of sensory results was determined from tables for the critical number of correct responses (*28*) required for triangle tests.

RESULTS AND DISCUSSION

Genistein Analyses. Average genistein recoveries of 56.1% (±8.3%) were obtained from the spiked fish muscle, which are lower than the genistein recoveries of 70% (± 5.6%) reported by Maubach et al. (*25*) using the same method for breast tissue. Therefore, the values reported here should be considered low estimates of genistein in the muscle. However, the objective of this study is to examine whether genistein deposition occurs in fish tissues and thereafter to study the effects of genistein on quality attributes of trout fillets. Results indicate that genistein concentration in the fillets was significantly ($p < 0.05$) influenced by levels of genistein in the diet. The highest estimated level of genistein, ~5.4 pmol/mg was found in the fillets from the trout fed the 3000 ppm genistein diet (**Figure 1**). No genistein was detected in fillets from the 0 ppm genistein diet. A positive correlation (**Table 2**) was found between dietary genistein and tissue genistein levels in rainbow trout harvested at 6 months ($r = 0.93$, $p < 0.05$) and those harvested at 12 months ($r = 0.99$, $p < 0.01$). The 6 months of consuming dietary genistein resulted in the same level of genistein in the fillet as was deposited by the 12-month feeding. Analysis of variance revealed that amounts of genistein levels in fillets were not significantly different for the two time periods studied.

Breast tissue from female subjects (*25*) who took tablets containing 100 mg genistein for 5 consecutive days was found to contain 15.3 pmol of genistein/mg of tissue. Similar studies in which genistein-containing gel capsules (100 mg/day) were fed to Japanese quail (*19*) for 5 days resulted in a 3 μg genistein deposition per egg yolk. Liver levels of 7.3 pmol of total

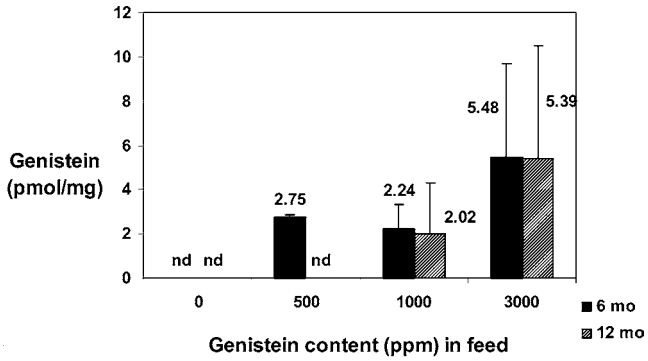

Figure 1. Mean genistein content in rainbow trout fillets. Values in chart are mean genistein content in picomoles per milligram of fillet ($n = 6$ per dietary treatment). Error bars represent ±standard deviation (SD). nd = not detected.

Table 2. Correlation between Dietary Genistein and Genistein Level Estimated in Tissues

	genistein in feed	genistein in fillets harvested at 6 months	genistein in fillets harvested at 12 months
genistein in feed[a]	1.000		
genistein in fillets harvested at 6 months[b]	0.932[c]	1.000	
genistein in fillets harvested at 12 months[b]	0.993[d]	0.889	1.000

[a] Genistein content (ppm) in feed. [b] Genistein content (pmol/mg) in fillets harvested from 6- and 12-month growth periods ($n = 4$ for all diet levels). [c] Correlation is significant at the 0.05 level (2-tailed). [d] Correlation is significant at the 0.01 level (2-tailed).

genistein/mg of tissue were obtained in studies (*30*) on female rats fed diets supplemented with 500 μg/g of genistein for 140 days. The genistein concentrations in these tissues are similar to the tissue genistein values found in our study, despite significant differences in species, tissue, length of feeding, and mode of genistein delivery. A unique feature of this study is that to the best of our knowledge this is the first published report of genistein deposition in fish muscle. It is highly probable that genistein concentration in fish tissues is dependent on the type of soy supplement ingested and on the type of tissue analyzed. The studies mentioned above reported a dose-dependent increase in genistein deposition. For both the Japanese quail (*19*) and human breast tissue (*25*) studies, the lower of two genistein doses resulted in lower amounts of genistein in the tissue. In our study too, estimated genistein deposition in trout muscle increased with dietary levels except at the 500 ppm dietary level. This may have been due to the extreme variability in final fish weight within treatments (**Table 3**), which resulted in noteworthy differences among the fillets from the 500 ppm treatment. Some fish were twice the size of others, indicating a much higher feed consumption and a correspondingly higher absolute amount of genistein consumed.

Thiobarbituric Acid Analysis. Dietary genistein had a significant lowering effect on lipid oxidation in the refrigerated fillets. TBARS levels on days 4 and 8 were significantly ($p < 0.05$) higher (**Figure 2**) in the fillets from the 0 ppm genistein level than in fillets from the other dietary treatments. This compares well with the results of Fang et al. (*20*) who showed significantly lower TBARS values in the liver of hamsters fed genistein at 200 ppm than in hamsters fed no

Table 3. Average Weight of Individual Rainbow Trout at 6 and 12 Months[a]

genistein content (ppm) in feed	average weight (g) at 6 months	average weight (g) at 12 months
0	181.0 ± 53.3	433.3 ± 136.7
500	206.5 ± 67.6	520.7 ± 173.2
1000	180.0 ± 65.4	462.7 ± 133.2
3000	170.8 ± 64.0	515.1 ± 155.6

[a] Values are average weight ± SD (n = 102 for 0 ppm diet; n = 93 for 500 ppm diet; n = 93 for 1000 ppm diet; and n = 106 for 3000 ppm diet). No statistical significant differences ($p > 0.05$) were found between mean weights for the different diet groups at either time period.

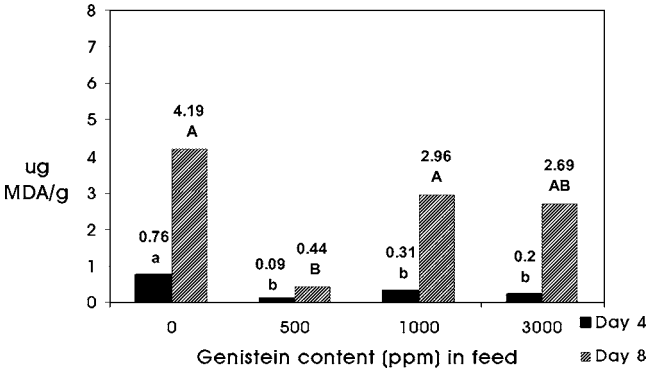

Figure 2. TBARS levels in rainbow trout fillets. Values are mean malondialdehyde in micrograms per milligram of fillet. Error bars represent ±SD (n = 6 per dietary treatment). Different letters indicate statistical difference ($p < 0.05$) among treatments on day 4 (a and b) and day 8 (A and B) based on one-way analysis of variance followed by a comparison of means with Fisher's LSD test.

genistein. TBARS levels in trout tissue were inversely related with dietary levels of genistein, particularly at the two ends of the range studied. A nonsignificant negative correlation was found between TBARS levels in the fillet and estimated tissue genistein in fillets harvested at 12 months of growth ($r = -0.496$, $p > 0.05$), indicating that TBARS levels decreased with an increase in tissue genistein levels. These results show that, although dietary genistein levels as low as 500 ppm significantly reduced the extent of lipid oxidation in the resultant fillets, the relationship between tissue genistein and fillet lipid oxidation was nonlinear.

Among other factors, the stability of the free radical generated during oxidation is key in the effectiveness of an antioxidant. Phenolic antioxidants occupy a favored status in this regard by the formation of quinones, which exhibit stability because of resonance delocalization (*31*). Genistein thus exerts antioxidative effects because of its phenolic structure. The results of this study also support the theory by Boggio et al. (*32*) who reported that storage stability of flesh could be improved by increasing the levels of natural antioxidants in flesh through feed supplementation. Other researchers have also studied the effects of dietary antioxidants on lipid oxidation and fillet quality of Atlantic salmon (*33*), turbot (*34*), and pacu (*35*). Tocopherol, which like genistein has a phenolic structure, when incorporated into the feed, protected salmon fillet (*33*) against iron ascorbate-stimulated oxidation. In iced storage, fillets of turbot (*34*) containing increased levels of α-tocopherol exhibited significantly lower ($p < 0.001$) levels of lipid oxidation and showed significantly less ($p < 0.001$) color deterioration (higher hue angle and lower chroma) than fillets containing low levels of α-tocopherol. However, an increase in ascorbic acid did not result in any detectable effects in fillet quality. Dietary tocopherol again proved to be the most effective among other antioxidants used in the study on pacu (*35*) as shown by the highest percentage of polyunsaturated fatty acids and the lowest TBARS values in the fillets. Frigg (*36*) and Scaife (*37*) both reported a reduction in lipid oxidation products in fillets from trout and salmon fed dietary tocopherols. A significant organoleptic preference for trout fillets containing the higher levels of tocopherols was also reported (*36*). Waagbo (*13*) indicated a combined effect of vitamin E and n-3 PUFA content on the flavor of salmon. Rancid flavor was significantly higher in fish raised on a high n-3 PUFA and low vitamin E diet.

The data obtained from this study suggest an antioxidant effect of dietary genistein in fish tissues. *In vitro* studies of the antioxidant activity of genistein (*38*) showed that genistein is an active scavenger of hydrogen peroxide but is less effective against other peroxidative systems. Genistein also proved effective against UVA and UVB or peroxyl-radical-induced lipid peroxidation in multilamellar liposomes. In assay studies by Lee (*39*), genistein was found to have better antioxidant capacity in human low-density lipoprotein oxidation than its glycoside but a lower antioxidant effect when compared to α-tocopherol. Although there are a number of studies describing the antioxidative effect of genistein *in vitro*, there is scant literature reporting the antioxidative effects of dietary genistein *in vivo*. However, the report by Fang et al. (*20*) indicated that hamsters fed genistein-supplemented (50 and 200 ppm) diets for 5 weeks had significantly reduced lipid oxidation in the serum, liver, and in low-density lipoproteins. Genistein supplementation significantly raised serum antioxidant capacity and decreased TBARS in the low-density lipoproteins and the liver. The antioxidative effects of genistein on hamster tissue *in vivo* corresponds well with our trout muscle TBARS data; however, a more comprehensive study is needed to prove the role of dietary genistein on lipid oxidation in fish fillets during refrigerated storage.

Color. It is well-documented that diet influences fillet color, which is a primary quality attribute for salmonids (*40*). However, to the best of our knowledge, no published research exists that specifically looks at the effects of individual soy components on fish fillet color. Preliminary results from ongoing studies in the authors' laboratory investigating the effects of diets containing whole soybean meal on the quality of rainbow trout fillets show significant effects on color. Francesco et al. (*40*) concluded that long-term feeding with plant proteins resulted in a significant color difference in rainbow trout fillets. Because in the present study no significant differences in *L*, *a*, or *b* values (**Table 4**) were found between fillets from the different diets at both time periods studied, we can conclude that genistein does not influence fillet color. There was also no effect of time of harvest on the color of the fillets.

Proximate Composition. There was a significant decrease in moisture, ash, and protein content in the fillets as fish matured. However, diet did not have a significant effect on protein, ash, fat, or moisture content of the fillets (**Table 5**).

Sensory Testing. Triangle test panelists could not detect any significant differences between the trout fillets from the 0 and 3000 ppm genistein treatments. For $N = 24$, a total of 13 correct responses were required to conclude a significant difference at $p \leq 0.05$ (*28*). However, only 8 panelists (6-month growth period) and 5 panelists (12-month growth period) correctly identified the odd fillet samples. These numbers were less than that required to confirm significance at a 5% level (**Table 6**). Matsuura et al. (*41*) suggested that genistein and daidzein may

Table 4. *L*, *a*, and *b* Values for Rainbow Trout Fillets[a]

genistein content (ppm) in feed	6 months			12 months		
	L	*a*	*b*	*L*	*a*	*b*
0	63.88 ± 1.6	2.42 ± 0.9	15.56 ± 1.5	64.18 ± 3.5	2.89 ± 0.7	16.74 ± 0.9
500	62.34 ± 1.4	2.92 ± 0.3	15.74 ± 0.6	62.46 ± 0.2	2.61 ± 0.5	15.86 ± 0.4
1000	64.99 ± 1.9	2.33 ± 0.8	15.46 ± 0.6	61.78 ± 1.0	3.77 ± 0.6	16.77 ± 0.5
3000	65.04 ± 0.6	3.16 ± 0.7	16.01 ± 0.4	63.38 ± 1.1	2.37 ± 0.2	16.40 ± 0.6

[a] Values shown are average values ± SD ($n = 3$ for each dietary treatment). Two-way analysis of variance revealed no significant ($p < 0.05$) differences between fillet color of trout fed genistein-fortified diets harvested at 6 and 12 months of growth.

Table 5. Proximate Composition of Rainbow Trout Fillets[a]

genistein content (ppm) in feed	percent moisture		percent protein[b]		percent fat[b]		percent ash[b]	
	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months
0	75.8 ± 0.8	75.8 ± 0.8	74.4 ± 1.8	73.4 ± 1.4	13.1 ± 1.0	14.8 ± 2.9	2.2 ± 0.3	1.6± 0.1
500	77.5 ± 1.9	73.9 ± 1.1	76.0 ± 2.5	71.2 ± 2.1	na[c]	na	na	na
1000	77.2 ± 1.4	75.5 ± 0.4	75.0 ± 3.7	71.7 ± 3.0	na	na	na	na
3000	76.8 ± 2.0	74.9 ± 1.3	73.0 ± 2.5	72.3 ± 2.1	14.1 ± 5.2	16.4 ± 5.8	1.9 ± 0.2	1.6 ± 0.1

[a] All values are mean ± SD ($n = 6$ per dietary treatment). [b] Percent protein, fat, and ash given on a dry weight basis. Fat and ash analyses were performed on rainbow trout fed the 0 and 3000 ppm genistein diet. [c] na = not analyzed.

Table 6. Triangle Tests on Rainbow Trout Fillets

fish pairs	number of correct responses received (out of 24)	critical number of correct responses[a]	significant difference ($p < 0.05$)
0 ppm versus 3000 ppm (6 months)	8	13	no
0 ppm versus 3000 ppm (12 months)	5	13	no

[a] Critical number of correct responses required for a statistical significance in a triangle test, adapted from Meilgaard et al. (*28*).

be responsible for the objectionable taste of soy milk. It should be noted that fluid soymilk contains 6.06 mg of genistein and 4.45 mg of daidzein per 100 g of soymilk (*42*), which are much higher than the levels found in our study. Robinson et al. (*43*) showed that genistein concentrations of less than 4.006×10^{-3} M in starch solutions were not picked up by consumers. We can conclude that, with regard to genistein in fish flesh, the estimated levels of ~5.4 pmol/mg did not result in any sensory differences between the fillets from the dietary levels tested in our study.

Dietary genistein resulted in genistein deposition in fish flesh without adversely affecting the flavor, color, or proximate composition of the fillets. However, TBARS values were lower in genistein-fed fish, suggesting that dietary genistein may improve the shelf life of fillets during refrigerated storage. The genistein levels found in trout fillets were much lower (approximately 1/100th) than that found in commercial soy foods, for example, tofu and tempeh, which contain approximately 20.6 and 24.8 mg of genistein/100 g of sample (*42*). It is therefore difficult to draw any conclusions about the potential human health benefits from these low amounts.

This study is a first step toward explaining the effects of individual soy components on some of the important quality attributes of fish. More detailed research is needed to conclude the effects of dietary genistein on the oxidative stability of fish fillets. More importantly, the results of this study are indicative only of genistein, which is one of many components of soybean. The effects of feeding soybean meal-based diets on the physical, chemical, and sensory quality of rainbow trout are currently under investigation.

ACKNOWLEDGMENT

The authors thank the United Soybean Board for funding the study. Special thanks are also due to Dr. Brian Perkins, University of Maine, for technical assistance. This manuscript is number 2792 of the University of Maine Agricultural and Forest Experiment Station.

LITERATURE CITED

(1) FAO. The State of World Fisheries and Aquaculture: Rome, Italy, 2002; p 112.
(2) Catacutan, M. R.; Pagador, G. E. Partial replacement of fish meal by defatted soybean meal in formulated diets for the mangrove red snapper, *Lutjanus argentimaculatus*. *Aquacult. Res*. **2004**, *35*, 299−306.
(3) Choi, S. M.; Wang, X.; Park, G. J.; Lim, S. R.; Kim, K. W.; Bai, S. C.; Shin, I. S. Dietary dehulled soybean meal as a replacement for fish meal in fingerling and growing olive flounder *Paralichthys olivaceus*. *Aquacult. Res*. **2004**, *35*, 410−418.
(4) Hardy, R. W. Alternate protein sources for salmon and trout diets. *Anim. Feed Sci. Technol*. **1996**, *59*, 71−80.
(5) Perez, H.; Lim, C.; Klesius, P. H. Nutritional value of heat-treated soybean meal for channel catfish (*Ictalurus punctatus*). *Aquaculture* **2003**, *225*, 67−82.
(6) Pollack, S. J.; Ottinger, M. A.; Sullivan, C. V.; Woods, L. C. The effects of the soy isoflavone genistein on the reproductive development of striped bass. *North Am. J. Aquacult.* **2003**, *65*, 226−234.
(7) Franke, A. A.; Custer, L. J.; Tanaka, Y. Isoflavones in human breast milk and other biological fluids. *Am. J. Clin. Nutr*. **1998**, *68* (supplement), 1466S−1473S.
(8) Ridges, L.; Sunderland, R.; Moerman, K.; Meyer, B.; Astheimer, L.; Howe, P. Cholesterol lowering benefits of soy and linseed enriched foods. *Asia Pac. J. Clin. Nutr*. **2001**, *10*, 204−211.
(9) Dixon, R. A.; Ferreira, D. Genistein. *Phytochemistry* **2002**, *60*, 205−211.

(10) Setchell, K. D. R. Phytoestrogens: The biochemistry, physiology, and implications for human health of soy isoflavones. *Am. J. Clin. Nutr*. **1998**, *68*, 1333S−1364S.
(11) Nickell, D. C.; Bromage, N. R. The effect of timing and duration of feeding astaxanthin on the development and variation of fillet color and the efficiency of pigmentation in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **1998**, *169*, 233−246.
(12) Coral, G.; Huberman, A.; de la Lanza, G.; Monroy-Ruiz, J. Muscle pigmentation of rainbow trout (*Oncorhynchus mykiss*) fed on oil-extracted pigment from langostilla (*Pleuroncodes planipes*) compared with two commercial sources of astaxanthin. *J. Aquat. Food Prod. Technol.* **1998**, *7*, 31−46.
(13) Waagbo, R.; Sandnes, K.; Torrissen, O. J.; Sandvin, A.; Lie, O. Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of n-3 polyunsaturated fatty acids at two levels of vitamin E. *Food Chem.* **1993**, *46*, 361−366.
(14) Skonberg, D. I.; Rasco, B. A.; Dong, F. M. Fatty acid composition of salmonid muscle changes in response to a high oleic acid diet. *J. Nutr*. **1994**, *124*, 1628−1638.
(15) Røra, A. M. B.; Regost, C.; Lampe, J. Liquid holding capacity, texture, and fatty acid profile of smoked fillets of Atlantic salmon fed diets containing fish oil or soybean oil. *Food Res. Int*. **2003**, *36*, 231−239.
(16) Færgemand, J.; Rønsholdt, B.; Alsted, N.; Børresen, T. Fillet texture of rainbow trout as affected by feeding strategy, slaughtering procedure, and storage post mortem. *Water Sci. Technol*. **1995**, *31*, 225−231.
(17) Johnsen, P. B.; Dupree, H. K. Influence of feed ingredients on the flavor quality of farm-raised catfish. *Aquaculture* **1991**, *96*, 139−150
(18) Skonberg, D. I.; Rasco, B. A.; Dong, F. M. Effects of feeding high monounsaturated sunflower oil diets on sensory attributes of salmonid fillets. *J. Aquat. Food Prod. Technol*. **1993**, *2*, 117−133.
(19) Lin, F.; Wu, J.; Abdelnabi, M. A.; Ottinger, M. A.; Giusti, M. M. Effects of dose and glycosylation on the transfer of genistein into the eggs of the Japanese quail (*Coturnix japonica*). *J. Agric. Food Chem.* **2004**, *52*, 2397−2403.
(20) Fang, Y.; Chen, B.; Huang, R. S.; Lu, Y. Effect of genistein supplementation on tissue genistein and lipid peroxidation of serum, liver, and low-density lipoprotein in hamsters. *J. Nutr. Biochem.* **2004**, *15*, 142−148.
(21) Okubo, K.; Iijima, M.; Kobayashi, Y.; Yoshikoshi, M.; Uchida, T.; Kudou, S. Components responsible for the undesirable taste of soybean seeds. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 99−103.
(22) Vara-Ubol, S.; Chambers, E.; Chambers, D. H. Sensory characteristics of chemical compounds potentially associated with beany aroma in foods. *J. Sen. Stud*. **2004**, *19*, 15−26.
(23) Drewnowski, A.; Gomez-Carneros, C. Bitter taste, phytonutrients, and the consumer: A review. *Am. J. Clin. Nutr*. **2000**, *72*, 1424−1435.
(24) Chou, R. L.; Her, B. Y.; Su, M. S.; Hwang, G.; Wu, Y. H.; Chen, H. Y. Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. *Aquaculture*. **2004**, *229*, 325−333.
(25) Maubach, J.; Bracke, M. E.; Heyerick, A.; Depypere, H. T.; Serreyn, R. F.; Mareel, M. M.; de Keukeleire, D. Quantitation of soy-derived phytoestrogens in human breast tissue and biological fluids by high-performance liquid chromatography. *J. Chromatogr., B: Biomed. Sci. Appl.* **2003**, *784*, 137−144.
(26) AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists, Inc.: Arlington, VA, 1990.
(27) Raharjo, S.; Sofos, J. N.; Schmidt, G. R. Solid-phase acid extraction improves thiobarbituric acid method to determine lipid oxidation. *J. Food Sci*. **1993**, *58*, 921−924.
(28) Meilgaard, M.; Civille, G. V.; Carr, B. T., Eds. Statistical tables T8. Critical number of correct responses in a triangle test. In *Sensory Evaluation Techniques*, 3rd ed.; CRC Press: Boca Raton, FL, 1999; p 369.
(29) Eggert, J.; Zook, K., Eds. Physical requirement guidelines for sensory evaluation laboratories. In *ASTM Special Technology Publication 913*; American Society for Testing and Materials: Philadelphia, PA, 1986; Vol. 913.
(30) Chang, H. C.; Churchwell, M. I.; Delcos, K. B.; Newbold, R. R.; Doerge, D. R. Mass spectrometric determination of genistein tissue distribution in diet-exposed Sprague−Dawley rats. *J. Nutr*. **2000**, *130*, 1963−1970.
(31) Fennema, O. R. Lipids. In *Food Chemistry*, 3rd ed.; Marcel Dekker: New York, 1996, pp 276−283.
(32) Boggio, S. M.; Hardy, R. W.; Babbit, J. K.; Brannon, E. L. The influence of dietary lipid source and α-tocopheryl acetate level on product quality of rainbow trout (*Salmo gairdneri*). *Aquaculture* **1985**, *51*, 13−24.
(33) Hamre, K.; Christiansen, R.; Waagbø, R.; Maage, A.; Torstensen, B. E.; Lygren, B.; Lie, Ø.; Wathne, E.; Albrektsen, S. Antioxidant vitamins, minerals, and lipid levels in diets for Atlantic salmon (*Salmo salar*, L.): Effects on growth performance and fillet quality. *Aquacult. Nutr*. **2004**, *10*, 113−123.
(34) Ruff, N.; Fitzgerald, R. D.; Cross, T. F.; Hamre, K.; Kerry, J. P. The effect of dietary vitamin E and C level on market-size turbot (*Scophthalmus maximus*) fillet quality. *Aquacult. Nutr*. **2003**, *9*, 91−103.
(35) Sant'Ana, L. S.; Mancini-Filho, J. Influence of the addition of antioxidants *in vivo* on the fatty acid composition of fish fillets. *Food Chem.* **2000**, *68*, 175−178.
(36) Frigg, M.; Prabucki, A. L.; Ruhdel, E. U. Effect of dietary vitamin E levels on oxidative stability of trout fillets. *Aquaculture* **1990**, *84*, 145−158.
(37) Scaife, J. R.; Onibi, G. E.; Murray, I.; Fletcher, T. C.; Houlihan, D. F. Influence of α-tocopherol acetate on the short- and long-term storage properties of fillets from Atlantic salmon *Salmo salar* fed a high lipid diet. *Aquacult. Nutr*. **2000**, *6*, 65−71.
(38) Record, I. R.; Dreosti, I. E.; McInerney, J. K. The antioxidant activity of genistein *in vitro*. *J. Nutr. Biochem.* **1995**. *6*, 481−485.
(39) Lee, C. H.; Yang, L.; Xu, J. Z.; Yeung, S. Y. V.; Huang, Y.; Chen, Z. Y. Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chem.* **2005**, *90*, 735−742.
(40) Francesco, M.; Parisi, G.; Médale, F.; Lupi, P.; Kaushik, S. J.; Poli, B. M. Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **2004**, *236*, 413−429.
(41) Matsuura, N.; Obata, A.; Fukushima, D. Objectionable flavor of soy milk developed during the soaking of soybeans and its control. *J. Food Sci*. **1989**, *54*, 602−605.
(42) U.S. Department of Agriculture, Agricultural Research Service; 2002; USDA−Iowa State University Database on the Isoflavone Content of Foods; Release 1.3 Nutrient Data Laboratory Website: http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html.
(43) Robinson, K. M.; Klien, B. P.; Lee, S. Y. Utilizing the R−index measure for threshold testing in model soy isoflavone solutions. *J. Food Sci*. **2004**, *69*, SNQ1−SNQ4.

Received for review September 23, 2004. Revised manuscript received March 1, 2005. Accepted March 7, 2005.

JF0484024