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Phytochemical Compositions, Antioxidant Properties, and Colon Cancer Antiproliferation Effects of Turkish and Oregon Hazelnut

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ABSTRACT

Roasted and raw Turkish and Oregon hazelnuts were examined. Whole nuts, skins, and skinless nuts of both hazelnut varieties were tested for fat contents, fatty acid profiles. Hazelnut and other byproducts were extracted with 5% acetone and examined for total phenolic contents (TPC), antioxidant activities against the peroxyl (ORAC) and DPPH radicals, and were also administered in vitro to the human colon cancer HT-29 cell line to determine antiproliferative effects. The Turkish hazelnuts contained over 65% total oil while the Oregon roasted variety contained 43.8%. The primary fatty acid in both was oleic acid (18:1n-9) comprising 76.7 g/100g oil in the Oregon variety and 83.3 g/100g oil in the Turkish variety. The TPC were 91.4 and 102.16 μg gallic acid equivalents/g sample for the Turkish roasted hazelnut skin and Oregon roasted hazelnut skin respectively, at least 30-folds as high as the hazelnut without skin. Turkish roasted hazelnut skin had the highest ORAC value of 1166.27 Trolox equivalents (TE) μmol/g sample (TE μmol/g), it is 38 times as high as the Oregon roasted hazelnut no skin which as a value of 30.2 TE μmol/g sample. The range of ED_{50} of DPPH$^{\bullet}$ is from 118.22 to 0.075 mg sample equivalents/mL among the samples, Oregon roasted hazelnut skin and Turkish raw hazelnut no skin exhibit the weakest and strongest ability to reduce DPPH$^{\bullet}$ respectively. At 6 mg/mL media Oregon roasted hazelnut skin extract significantly inhibited the growth of the HT-29 cells by 96 h following 4 days of treatment, and no effect was seen from the Turkish roasted skinned hazelnut extract. The Turkish raw hazelnut had significantly higher antioxidant activities compared to the Oregon roasted variety which may be explained by chemical changes during heating or possibly the total oil to flour ratio.

Keywords*: *Hazelnut*, *Antioxidant*, *Free Radical Scavenging*, *ORAC*, *DPPH*, *TPC*, *HT-29

1. Introduction

The total worldwide production of in shell hazelnut in 2009 was 765,666 tonnes, and 500,000 tonnes was supplied by Turkey. Other countries with significant hazelnut production in 2009 include Italy, the United States, and Spain that produced 104,900, 42,640, and 10,500 tonnes, respectively [1].

Hazelnut seed primarily consists of oil which generally comprises about 60% of the total weight but has been shown to contain over 75% oil and oil contents can vary greatly from year to year harvests [2]. Because of its high fatty acid composition, hazelnut is a very rich source of energy providing approximately 6 to 6.5 kcals/g fresh seed [3]. Recently, the understanding of the relationship between dietary fatty acids and their effect on health has been growing significantly, and the public is aware of its importance now more than ever.

The consumption of specific fatty acids such as omega-3 fatty acids and oleic acid may provide health benefits. Increasing the intake of dietary omega-3 fatty acids such as EPA (20:5n-3) and DHA (22:6n-3) may reduce the risk of several diseases including arteriosclerosis, cancer, autoimmune disorders, and hypertension [4-6]. A previous studie from Tey *et al.*, showed that the consumption of hazelnut in the diet can improve the lipoprotein profile and α-tocopherol concentration in human subjects having mild hypercholesterolemia [7]. Oleic acid (18:1n-9) has been associated with lowering the risk of heart disease. Several previous studies demonstrated that diets containing high levels of oleic acid lowered LDL and total cho-

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lesterol when experimentally replaced for saturated fat [8], and in 2004, the FDA published a qualified health claim for olive oil stating that the daily consumption of 2 tablespoons of olive oil (70% - 75% oleic acid) in place of saturated fat without increasing total calories can possibly decrease one's overall risk for heart disease due to its high content of monounsaturated fat.

Important phytochemicals found in plant foods include tocopherols, carotenoids, and other antioxidant phenolic compounds. Alpha-tocopherol is a well known fat soluble vitamin antioxidant that protects unsaturated fatty acyls in lipid membranes from free radical oxidation and can stop lipid radical propagation that leads to the development of fatty streaks in the physiopathological process of arteriosclerosis. In a recent study, tocopherols including α- and γ-tocopherol were detected in tree nuts including hazelnut, macadamia, walnut, and almond, with almond containing the highest level of α-tocopherol at 31 mg/100g flesh and walnut having the highest γ-tocopherol content of 30 mg/100g flesh [9]. Carotenoids are important beneficial dietary compounds. Beta-carotene can be converted to vitamin A when needed, and other dietary carotenoids are capable of quenching free radicals and singlet oxygen which is a free radical initiator [10]. Carotenoids have been detected in several seed oils including onion, parsley, cardamom, mullein, pumpkin, milk thistle, red raspberry, blueberry, marionberry, and blueberry [11,12]. Reactive oxygen species (ROS) are the major free radical byproducts of metabolism and are implicated in many diseases including cancer, heart disease, and many others because they may oxidize important biological molecules such as nucleic acids, and proteins [13]. Dietary compounds other than antioxidant vitamins may provide a critical role in protecting against ROS induced free radical injury. Phenolic compounds are found in virtually all plant foods and many phenols can act as powerful antioxidants that may reduce free radical damage. Individual phenolic compounds with known antioxidant activities have been identified in hazelnut including gallic acid, 4-OH benzoic acid, *p*-coumaric sinaptic acid, and quercetin [14,15]. Antioxidant activities have been detected in many foods and food byproducts including fruits, vegetables, grains, seed oils, and seed flours [11,12,16-19]. Hazelnut extracted with 80% EtOH has also demonstrated antioxidant activities against ABTS, hydrogen peroxide, superoxide, $DPPH^{\bullet}$, and β-carotene linoleate system. The hazelnut extract also inhibited human LDL oxidation and DNA scission [15]. The oxygen radical absorbance capacity (ORAC) assay is a physiologically relevant antioxidant test because it measures the ability of an antioxidant system to inhibit the oxidative damage to susceptible molecules of peroxyl radicals. Previous results have shown that tree nuts have significant antioxidant activities with pecan having the highest ORAC value of 175 trolox equivalent micromoles per g whole nut compared to eight other angiosperm tree nuts [20]. Transition metals may act as radical generators in food products and biological systems by removing an electron from a molecule present. Chelating agents may bind transition metals and reduce radical-mediated oxidative damage that may prevent the deterioration of food products and molecular damage to biological systems [13]. The chelating capacity assay measures the ability of a sample to bind ferrous iron (Fe^{2+}). A recent study determined the chelating capacity of seed oils extracted with methanol and found significant chelating ability in hemp, caraway, and carrot seed oils with the carrot having the best capacity of 25.5 EDTA equivalent mg/g oil. Chelating capacity has not previously been examined in hazelnut.

Tree nuts, including hazelnut, have been examined for their ability to inhibit the proliferation of human cancer cell lines including Hep G2 (liver) and Caco-2 (colon) *in vitro* and several nuts including walnut, pecan, almond, macadamia and cashew significantly inhibited cancer cell proliferation [21]. To date, there is very limited information regarding the effect of hazelnut on the proliferation of cancer cell lines and none on the HT-29 human colon cancer line.

In the current study, Turkish raw hazelnuts and Oregon roasted hazelnuts (*Corylus avellana* L.) were examine for their total fat content and fatty acid profiles, tocopherols including α- and γ-tocopherol, and β-carotene. Defatted hazelnut flours extracted with 50% acetone were analyzed for antioxidant activities using the ORAC assay and $DPPH^{\bullet}$; they were also examined for their total phenolic content (TPC), and chelating capacity against Fe^{2+}. The extracts were also examined for their ability to inhibit the proliferation of HT-29 human colon cancer cells *in vitro*.

2. Materials and Methods

2.1. Materials

Whole raw and roasted Turkish and Oregon hazelnuts were gifts from the Hazelnut Council, Oregon, USA. 2,2'-azobis (2-amino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). 2,2-Diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$), Folin-Ciocalteu reagent, gallic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO), β-cyclodextrin (RMCD) was purchased from Cyclolab R & D Ltd. (Budapest, Hungary). Cell culture media (McCoy's

5A Medium Modified with L-glutamine, antibiotic/antimycotic, and fetal bovine serum (FBS), 0.25% trypsin with 0.9 mM EDTA) was purchased from Invitrogen (Carlsbad, CA). HT-29 human colon cancer cells were purchased from American Type Culture Collection (Rockville, MD). All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.1.1. Extraction

Shelled hazelnuts were ground into meal, and oils were extracted using a Soxhlet apparatus with hexanes the solvent. The residual remaining following oil extraction was the flour, and flours were extracted using 10 mL 50% acetone per 1 g flour. Extracts were obtained by vortexing for 3 min 3 times at 1 h intervals then were allowed to stand overnight. The mixture was centrifuged at 500 g, and the supernatant was collected. Both oils and flours were kept under nitrogen and in the dark until analyzed.

2.1.2. Beta-Carotene

Concentrations of β-carotene were measured following a previously described method [11,22,23]. Briefly, 0.1 mL of hazelnut oil was dissolved in 0.9 mL of methanol/tetrahydrofuran (1:1, v/v) and analyzed for β-carotene using HPLC-DAD-ESI-MSMS (high performance liquid chromatography-diode array detector-electron spray ionization-tandem mass spectrometry). A TSQ quantum tandem mass spectrometer (Thermo-Finnigan, San Jose, CA) was equipped with an ESI interface and an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) with a Zorbax SB C18 column, 50 mm × 1.0 mm i.d. with a 3.5-μm particle size (Agilent Technologies, Palo Alto, CA, USA), was used to determine β-carotene. Identification was accomplished by comparing HPLC retention time and selected reactant monitoring (SRM) analyses of the sample peak with that of authorized pure β-carotene. Quantifications were conducted using the total ion counts with an external standard. Data were obtained using Xcalibur software system (Thermo-Finnigan, San Jose, CA, USA).

2.1.3. Tocopherols

The methanol/tetrahydrofuran solutions prepared for β-carotene composition were also used to quantify α- and γ-tocopherol concentrations by a previously described method [11]. HPLC with a Zorbax SB C18 column, 30 mm × 1.0 mm i.d. with a 3.5-μm particle size (Agilent Technologies, Palo Alto, CA, USA), was used to separate the tocopherols. The individual tocopherols were identified by peak retention time and selected reactant monitoring with those of the pure commercial compounds, quantification was determined using the total ion counts with external standards of the individual compounds.

2.1.4. Fatty Acid Composition

Fatty acid methyl esters (FAME) were prepared from hazelnut oil according to the previously described method [11]. Fatty acid profiles were analyzed by GC-FID using a Shimadzu GC-2010 with a FID and a Shimadzu AOC-20i autosampler (Shimadzu, Columbia, MD). The column was a Supelco 2380, 30 m × 0.25 mm i.d. with a 0.20 μm film thickness (Supelco Inc., Bellefonte, PA). Helium was the carrier gas and was set at a flow rate of 0.8 mL/min. Injection volume was 1 μL at a split ratio of 10/1. Initial oven temperature was 142˚C and increased 6˚C/min to 184˚C and held for 3 min, then 6˚C/min to 244˚C. Fatty acids were identified by retention times compared to individual commercial standard FAMEs.

2.1.5. Oxygen Radical Absorbance Capacity (ORAC)

ORAC was determined using the protocol previously described [24]. Fluorescein was used as the fluorescent probe. The assay mixture contained 0.067 μM of fluorescein, 60 mM of AAPH, 300 μL of flour extract or 50% acetone for the reagent blank. The fluorescence of an assay mixture was recorded every minute, and the area under the curve of fluorescence vs time plot was calculated and compared against a standard curve prepared with trolox. ORAC value was expressed as trolox equivalents (TE) in μmol per gram of the fruit seed flour. Triplicate measurements were conducted.

2.1.6. DPPH$^{\bullet}$ Scavenging Activity

The stable DPPH (2,2-diphenyl-1-picryhydrazyl) radical scavenging capacities of the cold-pressed seed flour extracts were analyzed following a previously described procedure [25]. A DPPH$^{\bullet}$-50% acetone solution was freshly prepared and was mixed with 1 mL seed flour extracts at different concentrations to start the radical-antioxidant reaction. The final DPPH$^{\bullet}$ concentration was 100 μM and the final reaction volume was 2.0 mL. Absorbance readings were measured at 517 nm against a blank of 50% acetone and used to estimate the remaining radical levels according to the standard curve. The seed flour extracts were tested for their $ED_{50\text{-}DPPH}$ concentrations at 20 minutes of reaction. The $ED_{50\text{-}DPPH}$ is the concentration of extract needed to reduce 50% of the DPPH radicals under experimental conditions. Time and dose dependencies of extracts and DPPH$^{\bullet}$ reactions were demonstrated by plotting the percent of DPPH$^{\bullet}$ remaining against time for each level of the seed oil extract tested.

2.1.7. Total Phenolic Content (TPC)

The TPC of hazelnut flour extracts was estimated using

Folin & Ciocalteu's (FC) reagent [25]. Briefly, the reaction mixture contained 250 μL of freshly prepared FC reagent, 50 μL of hazelnut flour extract, 0.75 mL of 20% sodium carbonate, and 3 mL of pure water. Absorbance was read at 765 nm after 2 h of reaction at ambient temperature, and gallic acid was the standard used to calculate TPC.

2.1.8. HT-29 Colon Cancer Cell Proliferation Inhibition

HT-29 human colorectal adenocarcinoma cell line characterized by Fogh *et al.*, 1975 were propagated in T-150 flasks in Mcoy's 5A media containing 10% FBS and 1% antibiotic/antimycotic. Flasks were incubated at 37˚C in a humidified atmosphere at 5% CO_2 [26,27].

Cell proliferation was examined following a modified procedure using 12-well plates [27]. After 24 h of incubation in the control media at 37˚C in a humidified atmosphere containing 5% CO_2, cells were treated with media containing the DMSO solution of the hazelnut flour extracts at two levels, while the control cells were treated with same volume of DMSO. The two dose levels were 2.5 and 5 mg flour equivalents per mL culture media. Media and treatments were changed daily, and live cells were counted on day 1 through day 4 of treatment.

2.2. Statistical Analysis

Data were reported as mean ± standard deviation (SD). ANOVA with Tukey's post hoc test was used to identify differences among the means. Statistical significance was declared at $P < 0.05$.

3. Results and Discussion

Roasted and raw Turkish and Oregon hazelnuts were examined. Whole nuts, skins, oils, and skinless meat were examined.

Beta-Carotene. Beta-carotene was detected in both Oregon and Turkish hazelnut oil samples in approximate equal amounts (**Table 1**). The experimental values of 9.9 and 10.0 μg per 100 g oil are similar to the value of 11 μg β-carotene in 100 g of unroasted hazelnut [28]. In one previous study on 10 different nuts, including hazelnut, β-carotene was not detected in the hazelnut sample and was only detected in pistachio nuts [29]. Another study by Alasalvar *et al.* [30] did not detect a carotenoid isomer in Turkish Tombul hazelnut.

Tocopherols. Both α- and λ-tocopherol were detected in the hazelnut oil samples. The Turkish hazelnut oil contained α-tocopherol and λ-tocopherol at concentrations of 26.8 and 3.7 mg/100g oil, respectively. These values were approximately 1.3 and 15 times higher than the Oregon hazelnut oil (**Table 1**). Previous investigations have demonstrated hazelnut oil to have a range of α-tocopherol concentrations from 10.6 to 65.5 mg/100g oil [31,32], and both sample concentrations fell nearer to the lower end of this range.

Total Fat. The two hazelnut samples had very different total fat contents (**Table 1**). The Turkish hazelnut contained 65.7% oil which was more than 20% higher than the Oregon hazelnut. Previous studies demonstrated that hazelnuts contain from 46.7% to 76.8% oil and contain approximately 60% oil per nut on average [2,3,18,32,33].

Fatty Acid Profile. The fatty acid profiles of and Oregon and Turkish hazelnut oils demonstrated very high concentrations of oleic acid which was consistent with the literature. The Turkish hazelnut oil contained the highest amount of oleic acid (18:1n-9) at a concentration of 83.3 g/100g oil (**Table 2**). Linoleic acid was the second most prevalent fatty acid and its concentration was inversely associated to oleic acid concentration. From previous investigations, the oleic acid concentration in hazelnut oil has been in the range of 70.5% to 85.3% of total fatty acids and has been shown to be significantly and inversely related to its linoleic acid concentration [33]. Total unsaturated fatty acids for the Turkish and Oregon hazelnut oils were similar at 92.2 and 93.0 g/100g oil, respectively (**Table 2**). These results are very consistent with other studies demonstrating that total unsaturated fatty acid concentrations among many hazelnut varieties grown in many different locations have a very tight compositional range of unsaturated fatty acids from approximately 89% to 92% of the total fatty acids [3,33,34,9,2,35]. The ratio of the percent of total fat to linoleic acid from this study was consistent with previous results that found a significant negative correlation between total fat percent and linoleic acid [33]. This may indicate a negative relationship between growing temperature and total fatty acid percent considering cell membranes may increase the level of unsaturation at

Table 1. Nutritional content of hazelnut seeds.

Oil	β-Carotene (μg/100g oil)	α-Tocopherol (mg/100g oil)	γ-Tocopherol (mg/100g oil)	Total Fat %
Turkish raw	9.9	26.8	3.65	65.7
Oregon roasted	10.0	20.5	0.23	43.8

Table 2. Fatty acid profiles of hazelnut oils (g/100g oil)*.

Fatty Acid	Oregon Roasted	Turkish Raw
14:0	tr	tr
16:0	5.3 ± 0.0	5.0 ± 0.0
16:1	0.2 ± 0.0	0.1 ± 0.0
18:0	1.6 ± 0.0	2.6 ± 0.0
18:1	76.7 ± 0.0	83.3 ± 0.0
18:2	15.8 ± 0.0	8.6 ± 0.0
18:3	0.1 ± 0.0	0.1 ± 0.0
20:0	0.1 ± 0.0	0.1 ± 0.0
20:1	0.2 ± 0.0	0.2 ± 0.0
SAT	7.0	7.8
MUFA	77.1	83.6
PUFA	15.9	8.6

*Data was obtained from whole seeds and skin from samples. Data expressed as means ± standard deviations (n = 3). SAT: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; tr: trace amount.

lower temperatures to maintain membrane fluidity.

ORAC. ORAC values of the tested hazelnut samples are shown in **Table 3**. The ORAC value of the samples ranged from 1166.2 - 30.7 trolox equivalents (TE) in micromoles per g sample (TE eq μmol/g) The ORAC values of roasted Turkish hazelnut skin was significantly higher than the rest of the samples. It was 40-fold higher than Oregon roasted hazelnut without skin. Previously, 8 samples of whole hazelnut kernel were evaluated for ORAC values by testing for both lipophilic and hydrophilic antioxidant components and averaged 96.45 TE eq μmol/g whole kernel meal [20].

DPPH. DPPH values were determined as $ED_{50\text{-}DPPH}$, which is the concentration of an extract required to decrease the amount of DPPH radicals to 50% of the initial concentration under experimental conditions. Oregon roasted hazelnut skin extract demonstrated greater antioxidant activity than the other samples. The $ED_{50\text{-}DPPH}$ value of the Oregon roasted hazelnut skin extract was 0.075 mg sample equivalent per ml (mg flour equiv/ml) and was approximately 235.5 times lower than that of the Oregon raw hazelnut 'without skin' extract.

A similar result was shown in the Turkish hazelnut. The $ED_{50\text{-}DPPH}$ of the Turkish roasted hazelnut skin extract was 1074.7 lower than that of the Oregon raw hazelnut without skin extract was observed (**Table 3**).

Total Phenolic Content (*TPC*)*.* TPC values showed a similar trend as compared to the ORAC test. The highest TPC value was in the skin extract of the Oregon and Turkey hazelnuts. The TPC values were 102.16 and 91.40 mg gallic acid equivalents (GAE) per g flour (mg GAE/g) respectively, and was significantly higher than the TPC of the other hazelnut extracts (**Table 3**). In a previous investigation of several tree nuts, hazelnut demonstrated a TPC of 2.91 mg GAE/g edible nut and was higher than other tested nuts including almonds, Brazil nuts, cashews, macadamias, and pines but lower than [29]. The roasted hazelnut skin samples have higher antioxidant capacities than roasted hazelnut with skin and without skin samples in both Oregon and Turkey varieties. Similar result was observed in the raw hazelnut samples.

Comparative results among ORAC, DPPH, TPC were correlated. The highest overall antioxidant capacities were observed in the roasted hazelnut skin samples extracts, and the lowest antioxidant values were seen in both raw hazelnut samples without skin. The skin showed the highest ORAC, DPPH and TPC value in both Turkey and Oregon, roasted and raw hazelnut samples. A result of a study by Monagas *et al.* 2009 showed that flavan-3-ol composition of roasted peanut, hazelnut and almond nut skins tested for total phenolic contents, flavan-3-ol found that 90% of flavan-3-ol was a combination of monomeric flavan-3-ols and hazelnuts were mainly contained B-type proanthocyanidins in the polymeric flavan-3-ols [36].

Anti-Proliferation. The effects of the hazelnut flour extracts on HT-29 colon cancer cell proliferation are shown in **Table 4**. The hazelnuts extracts are potent scavengers of free radicals and inhibit cancer cell proliferation. At 6 mg/mL media Oregon roasted hazelnut skin extract significantly inhibited the growth of the HT-29 cells by 96% and 89% of the cancer cells were inhibited by Turkish roasted hazelnut skin extract following 4 days of treatment. No effect was observed in both Turkey and Oregon roasted hazelnut with or without skin.

The Turkish hazelnut flour extract significantly inhibited the proliferation of HT-29 cells from day 2 to day 4 at 5 mg flour equivalents per ml media (mg equiv/mL) but only slightly inhibited proliferation at 2.5 mg equiv/mL (data not shown). The Oregon roasted hazelnut did not show any proliferation inhibitory effects at either concentration. The reason for the difference between the Turkish and Oregon hazelnut flour extract's effectiveness in inhibiting proliferation is not completely clear. However, previous studies have demonstrated that individual phenolic acids commonly found in plant foods, and plant food extracts with strong antioxidant activities can significantly reduce the proliferation of cancer cell growth *in vitro*, and the Turkish hazelnut had significantly

Table 3. Antioxidant properties of hazelnut flours*.

Sample	[a]DPPH ED_{50} 10 min mg eq/mL	[b]ORAC (μmol TE/g pomace)	[c]TPC GAE mg/g
T. roasted w/skin	3.86f ± 0.16	71.63g ± 4.3	5.17f ± 1.38
T. roasted no skin	10.88g ± 1.03	31.73h ± 2.7	3.70fg ± 1.46
T. roasted skin	0.11a ± 0.00	1166.20a ± 16.6	91.40b ± 0.60
T. raw with skin	1.29e ± 0.00	127.80e ± 3.2	6.41f ± 0.25
T. raw no skin	118.22i ± 7.25	111.80f ± 2.5	1.00h ± 0.01
T. raw skin	0.18b ± 0.00	130.20de ± 13.5	51.23c ± 0.33
O. roasted w/skin	0.48d ± 0.02	200.97d ± 20.2	18.93e ± 0.78
O. roasted no skin	17.66h ± 4.0	30.70h ± 1.2	1.09h ± 0.01
O. roasted skin	0.08a ± 0.00	683.14b ± 36.0	102.16a ± 0.45
O. raw w/skin	0.47d ± 0.00	261.01c ± 15.3	17.97e ± 0.80
O. raw no skin	11.52g ± 0.37	36.20h ± 5.0	3.75g ± 0.07
O. raw skin	0.25c ± 0.01	170.14d ± 21.9	36.69d ± 0.63

*Values with different letters in the same column are significantly different ($P < 0.05$); [a]DPPH• scavenging capacity was analyzed for its inhibitory concentration ($DPPH_{EC50}$) which is the concentration of sample that can reduce the percent of DPPH• to 1/2 of its original concentration at a selected time (10 min). DPPH measurements are defined as mg pomace equivalents per mL (mg eq/L); [b]ORAC is the oxygen radical absorbance capacity measured as micromoles of Trolox equivalents (TE) per gram pomace (μmol TE/g); [c]TPC is Total phenolic content. TPC values are defined as the mg gallic acid equivalents per gram pomace (GAE mg/g).

Table 4. Percent antiproliferation of hazelnut extracts on HT-29 cells following 96 h of treatment.

Sample	% of Control
T. roasted w/skin	115.07 ± 13.77
T. roasted no skin	113.14 ± 11.73
T. roasted skin only	10.67 ± 2.53
T. raw with skin	47.52 ± 0.19
T. raw no skin	101.33 ± 8.88
T. roasted skin only	49.34 ± 6.38
O. roasted w/skin	96.93 ± 0.21
O. roasted no skin	95.26 ± 7.38
O. roasted skin only	4.47 ± 0.36
O. raw w/skin	5.50 ± 1.00
O. raw no skin	87.54 ± 8.34
O. raw skin only	55.15 ± 3.53

stronger antioxidant activities than the Oregon hazelnut. In a previous study of common phenolic food components, ferulic acid and coumaric acid both significantly inhibited the proliferation of Caco-2 human colon cancer cells at 1500 μmol at least in part by inhibiting cell cycle progression [37]. Silibinin is a phenolic compound isolated from milk thistle and it is well documented for its ability to inhibit the proliferation of several cancer cell models *in vitro*. A recent study of fruit seed flours determined that their extracts could significantly inhibit the proliferation of HT-29 human colon cancer cells in a dose dependent manner, and antiproliferation was positively correlated to antioxidant activity [12]. It is also possible that the roasting process may have effectively modified and inactivated chemicals responsible for antiproliferation activity in the Oregon hazelnut.

Hazelnut has the potential to be a bioactive food ingredient and increase the profits for growers as a value-adding byproduct.

4. Conclusions

Whole nuts, skins, and skinless nuts of both Turkish and Oregon hazelnuts varieties were tested for fat contents and fatty acid profiles, antioxidant capacities of ORAC, DPPH, TPC were evaluated. Hazelnut kernel contains a high concentration of total weight as oil, and may be used to increase the dietary consumption of oleic acid for those individuals looking to increase the level of mono-

unsaturated fats. The hazelnuts extracts were potent scavengers of free radicals and inhibited cancer cell proliferation. The roasted Turkish and Oregon hazelnut skin extracts had significantly higher antioxidant activities compared to the other extracted. At 6 mg/mL media Oregon roasted hazelnut skin extract significantly inhibited the growth of the HT-29 cells by 96% following 4 days of treatment, and a similar result was seen from the Turkish roasted skinned hazelnut extract with an inhibition of growth of the HT-29 cells by 89%.

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