

QUANTIFICATION OF DAIDZEIN, GENISTEIN AND FATTY ACIDS IN SOYBEANS AND SOY SPROUTS, AND SOME BIOACTIVITY STUDIES

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We used reversed-phase high performance liquid chromatography (HPLC) to analyze the amounts of daidzein and genistein, well-known isoflavonoid aglycones, in methanolic extracts (80%) prepared from soy sprouts and also two types of soybeans (*Soja hispida* L.), one purchased at the local market and the other one cultivated in Turkey. Some commercially sold preparations containing either soy extract or soy isoflavones were also analyzed by HPLC for their daidzein and genistein content. Three oils obtained from the same soybean and soy sprout samples were analyzed for fatty acids by capillary gas chromatography-mass spectrometry (GC-MS). Several in vitro biological activities of the soybean oils were examined, including anticholinesterase, antioxidant, antibacterial, antifungal and antiviral activity. The soy sprouts were much richer in genistein (232.1 µg/g) and daidzein (177.0 µg/g) than the soybean samples. The cultivated soybean sample also showed higher genistein (3.771 µg/g) and daidzein (3.366 µg/g) levels than the soybean sample of market origin (2.971 µg/g and 2.579 µg/g, respectively). The soybean oils were found to be quite rich in essential fatty acids, and the soy sprout oil also contained essential fatty acids in appreciable amounts. The soybean oil of market origin had a notable antiviral effect against *Herpes simplex* as well as antifungal activity against *Candida albicans* at 8 µg/ml.

Key words: *Soja hispida*, soybean oil, soy sprout, daidzein, genistein, fatty acids, bioactivity.

INTRODUCTION

Isoflavones have been extensively investigated for their potential health effects in areas including cardiovascular disease, cancer, osteoporosis, menopausal symptoms and cognitive function (Messina et al., 1994; Anderson and Garner, 1997). Daidzein and genistein, which are isoflavone aglycones having a 3-phenylchroman skeleton, are mainly found in soybeans (*Soja hispida* L.) and soy products (Penalvo et al., 2004; Murphy and Barr, 2005) as well as in other species of the Fabaceae family (Liggins et al., 2000; Umphress et al., 2005). A variety of commercial preparations of soy extracts or soy isoflavones are in increasing demand in the market worldwide as nutritional supplements or phytotherapeutics.

Soja hispida L. (syn. *Glycine max* L.) of the Fabaceae family, usually referred to as soybean, is

also a popular foodstuff and crop plant, used especially in traditional cooking by South Asian people. There is evidence that soybean consumption has a range of beneficial health effects such as cancer prevention, reduced risk of osteoporosis, a valuable role in chronic renal disease, and protection against some cardiovascular disorders (Grainge et al., 2001; Blair et al., 2002).

This study examines the content of daidzein and genistein in two samples of soybean, one purchased at random at the market and the other cultivated, as well as in soy sprouts produced in Turkey along with four soy-containing commercial preparations. In addition, the fatty acid composition of oils from the same samples were determined by capillary gas chromatography-mass spectrometry (GC-MS). The anticholinesterase, antioxidant, antibacterial, antifungal and antiviral activities of the same soybean oils were also tested.

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TABLE 1. Oil yields of soybeans and soy sprouts of Turkish origin

| Soybean oils | Dry weight (g) | Oil amount (g) | Yield % (w/w) |
|----------------|----------------|----------------|---------------|
| Culture origin | 50.1 | 17.6 | 35.1 |
| Market origin | 20.9 | 7.2 | 34.5 |
| Soy sprouts | 6.46 | 0.03 | 0.46 |

MATERIALS AND METHODS

PLANT MATERIALS

Soybean samples (Macon soybean with white flowers; Germplasm Center, U.S.A.) from culture were obtained from the Faculty of Agriculture, Çukurova University, Adana (Turkey) in 2003. Another soybean sample of unknown origin was bought at random from the local market in Ankara (Turkey) in 2003. The soy sprouts were the product of the Ünlüsoy Company (Turkey), obtained in April 2003.

ANALYSIS OF ISOFLAVONES

Preparation of Extracts for HPLC Analysis

The soybean samples of culture and market origin were weighed (21.2 g and 37.6 g, respectively), ground in a mechanical grinder to fine powder, and macerated in 80% methanol for 24 h at room temperature. The methanol phases were filtered the next day and evaporated in a vacuum to obtain extracts. Fresh soy sprouts (200 g) were chopped, dried in the shade at room temperature, weighed (10.9 g) and subjected to the same extraction procedure. The amounts and yield percentages of the extracts are given in Table 1.

Commercial Supplements

The products producers of the four analyzed commercial soy-based supplements are Nature Made® tablets, Natural Nutrition® tablets, Phytosoya® capsules and Isoflavin® tablets (Tab. 2).

Chemicals

Standard daidzein was purchased from Sigma-Aldrich (EC 207-635-4, 25 mg, St. Louis, U.S.A.). Genistein used as standard was from Gynogen® (Microgen Pharmaceutical Company, Istanbul, Turkey), which contains 50 mg genistein per tablet; it was extracted using 80% methanol, filtered and evaporated in a vacuum, and checked by HPLC. All the solvents used were of analytical grade (Merck).

TABLE 2. Listed ingredients in soy-based supplements analyzed

| Brand name and item | Producer | Listed Ingredients |
|---------------------------|----------------------------------|---|
| Nature Made® tablet | Nature Made Nutritional Products | Vitamin D (200 IU) Vitamin K (40 mcg) Calcium (500 mg) Magnesium (50 mg) Zinc (5 mg) Copper (1 mg) Manganese (1.5 mg) Soy isoflavones (25 mg) Boron (150 mcg) |
| Natural Nutrition® tablet | Fingerprint Botanicals | Soy extract (10.6 g) Isoflavone (genistein) (60 mg) <i>Cimicifuga racemosa</i> (500 mg) Vitamin D3 (100 IU) Calcium (80 mg) Magnesium (40 mg) |
| Phytosoya® capsule | Arkomédika | Total isoflavone (17.5 mg) |
| Isoflavin® tablet | | Genistein (29.8 mg) Daidzein (7.8 mg) Glycitein (2.4 mg) |

HPLC Conditions

The analysis was performed with a LC system consisting of an HP Agilent 1100 series quaternary pump with degasser and photodiode array detector. Samples were injected with an HP Agilent 1100 autosamplers with a thermostatted column compartment on an ACE-5 C18 column (5 µ, 250 mm; 4.6 mm) at 40°C. The system was controlled and data analyses were performed with Agilent ChemStation. All the calculations for quantitative analysis were performed with external standardization by measurement of peak areas. A mobile phase consisting of formic acid (0.2% v/v-Solvent A):acetonitrile (Solvent B) was chosen to achieve maximum separation and sensitivity (Kledjus et al., 2004). Standard stock solutions contained 808 µg/ml daidzein and 1000 µg/ml genistein in HPLC-grade methanol. To establish the linear detection range for each standard, individual standard stock solutions were prepared in mobile phase in 10 ml measuring flasks. Aliquots of these solutions were diluted and analyzed to determine method linearity. Calibration ranges were 0.1263–202 µg/ml for daidzein and 0.07813–125 µg/ml for genistein. Triplicates of 10 µl injections were made for each standard solution. The limit of detection (LOD) was established at a signal-to-noise ratio (S/N) of 3. The limit of quantification (LOQ) was established at S/N 10. (Tab. 3).

TABLE 3. Linearity Results, Limit of Detection (LOD) and Limit of Quantification (LOQ)

| Isoflavones analyzed | λ (nm) | Equation | r^2 | LOQ ($\mu\text{g}/\text{mL}$) | LOD ($\mu\text{g}/\text{mL}$) |
|----------------------|----------------|----------------------------|--------|---------------------------------|---------------------------------|
| Genistein | 254 | $Y = 67.76221X + 136.6301$ | 0.9984 | 0.07813 | 0.02344 |
| Daidzein | 254 | $Y = 57.90179X + 112.3312$ | 0.9994 | 0.1263 | 0.03789 |

FATTY ACID ANALYSIS

Oil Extraction from Soybeans and Soy Sprouts

The soybean samples of culture and market origin were weighed (50.1 g and 20.9 g, respectively) and extracted continuously with *n*-hexane for 8 h in a Soxhlet apparatus in the presence of anhydrous Na_2SO_4 . Following extraction, the *n*-hexane phases were filtered and evaporated in a vacuum. The soy sprouts were prepared as described above and the oils were obtained. The oil amounts and yield percentages are given in Table 4.

Saponification and Methylation of Oils

The oils prepared from the two soybean samples and soy sprouts were weighed separately (450 mg) in 50 ml volumetric flasks, then saponified by adding 12 ml 0.5 N methanolic NaOH to the mixture and heating with a steam bath until the fat globules went into solution. 20 ml BF_3/MeOH (Sigma) was added to each flask and the mixtures were boiled for 2 min. After cooling down, they were made up to 50 ml with saturated NaCl solution and then transferred to separation funnels and extracted with 30 ml petroleum ether (PE) for each. The PE phases were taken and evaporated with a water bath at 60°C (Morrison and Smith, 1964). The obtained methyl esters of fatty acids were dissolved in 1 ml hexane, and 1 μl samples were injected and analyzed by GC-MS.

GC-MS Conditions

Chromatographic analysis was carried out with an Agilent 6890N Network GC system combined with an Agilent 5973 Network Mass Selective Detector (GC-MS). The capillary column used was an Agilent 19091N-136 (HP Innovax Capillary; 60.0 m \times 0.25 mm \times 0.25 μm). As mentioned in our previous publications (Orhan et al., 2007a; Şener et al., 2007), helium was used as carrier gas at a flow rate of 0.8 ml/min with 1 μl injection volume. Samples were analyzed with the column held initially at 60°C after injection during 10 min hold time; then the temperature was increased to 220°C at a 4°C/min increment and kept at 220°C for 10 min. The final temperature was increased to 240°C with a 1°C/min increment. Injection was performed in split mode (50:1). Detector and injector temperatures were 230°C and

TABLE 4. Amounts of genistein and daidzein found in soy sprouts and two soybean samples ($\mu\text{g}/\text{g}$)

| Isoflavones analyzed | Soy sprout | Soybean (Culture origin) | Soybean (Market origin) |
|----------------------|------------------|--------------------------|-------------------------|
| Genistein | 232.1 \pm 0.90 | 3.771 \pm 0.22 | 2.971 \pm 0.05 |
| Daidzein | 177.0 \pm 2.31 | 3.366 \pm 0.43 | 2.579 \pm 0.07 |

280°C, respectively. Run time was 80 min. MS scan range was (*m/z*): 35–450 atomic mass units (AMU) under electron impact (EI) ionization (70 eV). The peaks were identified using the Wiley and Nist Libraries, and comparison of the retention times (R_t) and mass spectra of standards (Sigma). Relative content of fatty acids (%) was determined using Agilent software. The results are expressed as averages of three determinations in all cases.

BIOLOGICAL ACTIVITY OF SOYBEAN OILS

AChE and BChE Inhibitory Activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were measured using the spectrophotometric method developed by Ellman et al. (1961) as described in detail in our previous publications (Orhan et al., 2004 and 2007). The experiments were done in triplicate. Galanthamine (Sigma, St. Louis, U.S.A.) was used as the reference.

DPPH Free Radical-Scavenging Assay

The antiradical activity of the soybean oils and reference (butylated hydroxyanisole, BHA) was assessed on the basis of the radical-scavenging effect of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Lee et al., 1998) as described in detail in our previous publication (Orhan et al., 2007).

MICROBIOLOGICAL STUDIES

For stock solution preparation, the soybean oils were dissolved in ethanol:hexane (1:1, v/v) solution using 1% Tween 80 at the final concentration of 1024 $\mu\text{g}/\text{ml}$ and sterilized by filtration (0.22 μm ; Millipore, U.S.A.). Standard antibacterial powders

of ampicillin (AMP, Fako), ofloxacin (OFX, Hoechst Marion Roussel), along with standard antifungal ketoconazole (KET, Bilim) and fluconazole (FLU, Pfizer), were prepared as follows: ampicillin was dissolved in phosphate buffer solution (pH 8.0, 0.1 mol/l), ketoconazole was dissolved in DMSO, and fluconazole and ofloxacin were dissolved in water. The stock solutions of these agents were used according to NCCLS recommendations (National Committee, 1996). Standard strains of the bacteria *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 10145), *Proteus mirabilis* (ATCC 7002), *Klebsiella pneumoniae* (RSKK 574), *Acinetobacter baumannii* (RSKK 02026), *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633) were used for determination of antibacterial activity, and a standard strain of *Candida albicans* (ATCC 10231) was employed for determination of antifungal activity. Mueller-Hinton Broth (Difco) and Mueller-Hinton Agar (Oxoid) were used for growing and diluting the bacteria. Sabouraud liquid medium (Oxoid) and Sabouraud dextrose agar (SDA) (Oxoid) were used for growing and diluting the fungus. RPMI-1640 medium with *L*-glutamine was buffered at pH 7 with 3-[*N*-morpholino]-propansulfonic acid (MOPS). Culture suspensions were prepared according to NCCLS M27-A (Özçelik et al., 2005). The bacterial suspensions used for inoculation were prepared at 10^5 cfu/ml by diluting fresh cultures at McFarland 0.5 density (10^8 cfu/ml). The fungus suspension was prepared by the spectrophotometric method of inoculum preparation at a final culture suspension of 2.5×10^3 cfu/ml (National Committee, 2002).

The microdilution method was employed for antibacterial and antifungal activity tests. Media were placed into each well of a 96-well microplate. Extract solutions at 1024 μ g/ml were added to the first row of microplates, and twofold dilutions of the compounds in the range of 512–0.25 μ g/ml were placed in the remaining wells. Culture suspensions (10 μ l) were inoculated into all the wells. The sealed microplates were incubated at 35°C for 24 h and 48 h in a humid chamber. The lowest concentration of the oils that completely inhibited macroscopic growth was determined and minimum inhibitory concentrations (MICs) were calculated (National Committee, 2002).

ANTIVIRAL ACTIVITY

Herpes simplex virus (HSV) and *Para-influenza-3 virus* (PI-3) were used to determine the antiviral activity of the soybean oils. The test viruses were obtained from the Department of Virology, Ankara University. Vero cell line (African green monkey kidney) and Madin-Darby bovine kidney (MDBK) used in this study were obtained from the Department of

Virology, Ankara University. Cells were cultured in Eagle's Minimal Essential Medium (EMEM) enriched with 10% fetal calf serum (FCS) (Biochrom, Germany), 100 mg/ml streptomycin and 100 IU/ml penicillin in a humidified atmosphere of 5% CO₂ at 37°C. The cells were harvested using trypsin solution (Bipco Life Technologies, UK). Media were placed into each of the 96 microplate wells (Greiner^R, Germany). Stock solutions of the oils were added to the first row of microplates and twofold dilutions of the compounds (512–0.25 μ g/ml) were added to the remaining wells. Twofold dilutions of each material were obtained according to Log₂ on the microplates. Acyclovir (Biofarma) and Oseltamivir (Roche) were used as the control agents. Strains of HSV and PIV titers were calculated as TCID₅₀ (Frey and Liess, 1971). Maximum cytopathogenic effect (CPE) concentrations as indicators of the antiviral activities of the extracts were determined according to the procedure described earlier (Özçelik et al., 2005).

The maximum nontoxic concentration (MNTC) of each sample was determined by the method described previously by Özçelik et al. (2005) based on alteration of cell morphology.

STATISTICAL ANALYSIS OF BIOLOGICAL DATA

Data obtained from in vitro experiments for anticholinesterase and antioxidant activity were expressed as means and standard error (\pm SEM). The significance of differences between the treatments and the control were evaluated by ANOVA; $p < 0.05$ was considered significant [* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$].

RESULTS AND DISCUSSION

In the present study, soybean and soy sprout samples extracted with 80% methanol were analyzed to establish the amounts of daidzein and genistein by reversed-phase HPLC. Gradient elution was employed for better quantitative analysis, run for 40 min in total; daidzein and genistein appeared at 25.8 and 27.9 min, respectively. Daidzein was detected at concentrations of 3.366 μ g/g (soybean from culture), 2.579 μ g/g (soybean from market) and 177.0 μ g/g (soy sprouts); the same samples were found to contain genistein at concentrations of 3.771, 2.971, and 232.1 μ g/g, respectively (Tab. 5). Fatty acids in oils obtained from the soybean and soy sprout samples were analyzed by capillary GC-MS technique; the fatty acid components detected in both soybean oils were very similar (Tab. 6). Soybean oils of culture and market origin yielded similar amounts of palmitic acid (16:0; 11.84–11.68%) and stearic acid (18:0; 4.56–3.76%).

TABLE 5. Amounts of genistein and daidzein in some of soy-derived commercial preparations (mg per tablet or capsule)

| Isoflavones analyzed | NatureMade® | Isoflavin® | Natural Nutrition® | Phytosoya® | Gynogen® |
|----------------------|-------------|-------------|--------------------|------------|-------------|
| Genistein | 0.108±0.06 | 31.863±0.54 | 1.538±0.05 | 0.021±0.01 | 52.032±0.89 |
| Daidzein | 0.116±0.02 | 12.803±0.29 | 0.067±0.02 | 0.115±0.02 | - |

TABLE 6. Fatty acid composition of soybeans and soy sprouts of Turkish origin

| Fatty acid | Retention time (min) | Relative Composition % | | |
|------------------------------|----------------------|------------------------------|-----------------------------|-------------|
| | | Soybeans (of culture origin) | Soybeans (of market origin) | Soy sprouts |
| Saturates | | | | |
| Palmitic acid (16:0) | 48.60 | 11.84±0.38 | 11.68±0.19 | 31.13±0.90 |
| Stearic acid (18:0) | 53.58 | 4.56±0.05 | 3.76±0.003 | 1.89±0.03 |
| Behenic acid (22:0) | 70.14 | - | - | 2.27±0.36 |
| Δ9-Desaturates | | | | |
| Palmitoleic acid (16:1, n=7) | 54.43 | - | - | 3.40±1.27 |
| Vaccenic acid (18:1, n=7) | 54.48 | 0.86±0.04 | 1.24±0.07 | - |
| Oleic acid (18:1, n=9) | 55.46 | 25.28±0.24 | 30.82±0.65 | 5.79±0.52 |
| ω6-Fatty Acids | | | | |
| Linoleic acid (18:2, n=6) | 55.86 | 47.71±0.33 | 45.46±0.45 | 15.27±1.09 |
| Linolenic acid (18:3, n=6) | 58.09 | 6.53±0.13 | 4.09±0.23 | 23.57±1.25 |
| Total | | 96.78 | 97.05 | 83.32 |

Palmitic acid was markedly higher in the soy sprout oil (31.13%) than in the soybean oil samples. Vaccenic acid, a minor fatty acid, was detected in the soybean oils (18:1, n = 7), but not at all in the soy sprout oil. Oleic acid content (18:1, n = 9) was slightly higher in the soybean oil of market origin (30.82%) than in that of culture origin (25.28%). The soy sprout oil (5.79%) was rather poorer in oleic acid than the soybean oils. It is well known that the fatty acids of the Omega family, including linoleic (LA), linolenic (LNA), eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) play a vital role for human health, cannot be synthesized in the human body, and therefore must be part of dietary intake. In our study we found large amounts of linoleic acid (18:2, n = 6) in both soybean samples (47.71% and 45.46%) and in the soy sprout oil (15.27%). Importantly, the amount of linolenic acid (18:3, n = 6) found in the soy sprout oil (23.57%) was much higher than in the two soybean oils (6.53% and 4.09%). Palmitoleic acid was detected in the soy sprout oil (3.40%).

The anticholinesterase, antioxidant, antibacterial, antifungal and antiviral activities of the soybean oils of culture and market origins were investigated. While the soybean oils of culture and market origins

were shown to provide 17.3 and 16.5% AChE inhibition, respectively, at 0.2 mg/ml concentration, these oils did not inhibit BChE. The soybean oils showed low antioxidant activity (27.7 and 26.3%, respectively) against DPPH radical. The oils showed negligible antibacterial activity against *P. aeruginosa*, *S. aureus*, *E. coli*, *B. subtilis* and *P. mirabilis*, and moderately inhibited the growth of *K. pneumoniae* and *A. baumannii* as compared to ampicillin (Tab. 7). The antifungal activity of the soybean oils against *C. albicans* was quite remarkable as compared to that of ketoconazole and fluconazole (Tab. 7). Interestingly, the oil obtained from the soybean sample bought at random in the local market was not active against either HSV or PI-3, whereas the oil of cultured origin showed significantly higher activity against both HSV and PI-3 as compared to acyclovir and Oseltamivir (Tab. 8).

In the literature we have not encountered any reports on the antibacterial activity of any soybean oil. Our results demonstrated that there was no difference in antibacterial properties between the soybean oils of market and culture origin. Our results showing better inhibition against *C. albicans* by soybean oils is consistent with findings from previous studies.

TABLE 7. Antibacterial and antifungal activities of soybean oils and references, expressed as minimum inhibitory concentrations (MICs) ($\mu\text{g/ml}$)

| Soybean oils | Microorganisms | | | | | | | |
|----------------|----------------|----------------------|---------------------|----------------------|---------------------|------------------|--------------------|--------------------|
| | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>P. mirabilis</i> | <i>K. pneumoniae</i> | <i>A. baumannii</i> | <i>S. aureus</i> | <i>B. subtilis</i> | <i>C. albicans</i> |
| Market origin | 64 | 128 | 64 | 16 | 16 | 128 | 128 | 8 |
| Culture origin | 64 | 128 | 64 | 16 | 16 | 128 | 128 | 8 |
| AMP | 2 | – | 2 | 2 | 2 | <0.12 | 0.12 | – |
| OFX | 0.12 | 1 | <0.12 | 0.12 | 0.12 | 0.5 | 0.5 | – |
| KET | – | – | – | – | – | – | – | 2 |
| FLU | – | – | – | – | – | – | – | 4 |

AMP – Ampicilline; OFX – Ofloxasine; KET – Ketoconazole; FLU – Fluconazole; – No activity observed.

TABLE 8. Antiviral activity of soybean oils and references

| Soybean oils | MDBK Cells | | | Vero Cells | | |
|----------------|---------------------------|------------------------------|---------|---------------------------|------------------------------|---------|
| | MNTC ($\mu\text{g/ml}$) | CPE Inhibitory Concentration | | MNTC ($\mu\text{g/ml}$) | CPE Inhibitory Concentration | |
| | | HSV | | | PI-3 | |
| | | Maximum | Minimum | | Maximum | Minimum |
| Market origin | 64 | – | – | 32 | – | – |
| Culture origin | 64 | 64 | 8 | 8 | 8 | 4 |
| Acyclovir | 16 | 16 | <0.25 | – | – | – |
| Oseltamivir | – | – | – | 32 | 32 | <0.25 |

MNTC – Maximum non-toxic concentration; CPE – Cytopathogenic effect; – No activity observed

The composition of soybean oil has been investigated extensively. In addition to its fatty acids, phytosterols as well as the phospholipids phosphatidylcholine and phosphatidylethanolamine have been detected (Careri and Mangia, 2001; Jakap et al., 2002; Phillips et al., 2002; Zhang et al., 2003). Goodrum and Geller (2005) reported soybean oil to be comprised of palmitic (14.17%), stearic (5.19%), oleic (48.20%), linoleic (22.19%), linolenic (1.45%), myristic (0.56%), myristoleic (0.18%), palmitoleic (1.27%) and arachidic acids (0.28%). We did not detect the last four minor ones in our soybean samples. Cherry et al. (1985) found that the oil content of soybeans produced in southern areas of the U.S.A. was only 51% of the content of soybeans of northern origin, indicating that the fatty acid composition of soybeans is sensitive to the influence of both genotype and environment, and also found that higher ambient temperature reduced the linolenate concentration in the soybean oil. In a study of the effect of environment (e.g., locality, climatic conditions) on the fatty acid composition of soybeans growing in Egypt, Sakla et al., (1988) found differences in protein, oil, and carbohydrate content between three types of soybean. Another soybean cultivar grown in the Kashmir Valley in India was reported to be composed of myristic (1.5%),

palmitic (13.5%), stearic (6.1%), oleic (23.2%), linoleic (46.3%) and linolenic acids (9.4%) (Katiyar et al., 1989), very similar to the composition of the soybean oils analyzed here. Mohamed and Rangappa (1992) also analyzed the fatty acids of seventeen vegetable soybean genotypes growing in the U.S.A., and found 53.34% linoleic acid and 9.19% linolenic acid on average. In Grela and Günter's (1995) study, the soybean oil sample was shown to contain 53% linoleic acid, quite consistent with our results. A commercial type of soybean oil of Iranian origin was analyzed by GC-MS (Hajimahmoodi et al., 2005) and was found to contain myristic (0.1%), palmitic (11.8%), palmitoleic (0.1%), stearic (3.9%), oleic (22.9%), linoleic (51.5%), and linolenic acids (3.1%), similar to our results. Commercial soybean oil sold in China was reported to consist of palmitic (11.2%), stearic (3.7%), oleic (22.1%), linoleic (55.0%), and linolenic acids (6.8%) (Cao et al., 2005), also highly concordant with the content of our soybean samples. We also obtained interesting results on the fatty acid composition of oil from soy sprouts (Tab. 6).

There are few studies on the isoflavone content of soy sprouts (Choi and Sohn, 1998; Albertazzi and Purdie, 2002). In a study by Morton et al. (2002), daidzein and genistein were analyzed by GC-MS,

and soybean hypocotyls were found to contain higher concentrations (2.48 and 1.25 mg/g, respectively) than coarse soy grits, dragon soybeans, dehusked soybean cotyledons, soybean hulls, toasted soy flour and fine soy grits. In another study, the isoflavone profile of soy leaves was observed to differ from that of soybeans (Ho et al., 2002): soybean contained malonylgenistin in the highest amount, followed by genistin, daidzin, genistein and daidzein in decreasing order, whereas soy leaves had only trace amounts of malonylgenistin and genistin; no daidzein or genistein were detected in the soy leaves. Mazur et al. (1998) determined the isoflavone content of four kinds of soybean samples (Centennial, INIAP Bolivia, Santa Rosa, Chapman) and found that the soybean sample of Santa Rosa origin had the highest daidzein and genistein amounts (56,000 and 84,100 µg/100 g, respectively). In our study, soybean sprouts had higher amounts of genistein and daidzein (232.1 and 177.0 µg/g) than the soybean samples. The soybean samples of culture origin had slightly higher concentrations of these two isoflavones than the sample of market origin (Tab. 5).

Our results indicate that Turkish soybean contains amounts of genistein and daidzein consistent with previous reports. The soy sprouts cultivated in Turkey contained genistein and daidzein at remarkable concentrations. There was not much difference between the oils obtained from the soybeans of culture and market origin. Turkish soybean oil has been shown to be highly similar to its counterparts elsewhere in the world. To the best of our knowledge, this is the first report on the fatty acid content of soy sprouts, the anticholinesterase and antibacterial activities of soybean oil, and the genistein content and daidzein content of soybean samples and soy sprouts of Turkish origin, which are quite comparable to results from other parts of the world. These findings suggest that soy sprouts as well as soybeans are a good alternative source of essential fatty acids and isoflavones (daidzein and genistein).

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