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Full Length Research Paper

Biochemical and molecular analysis of soybean seed from Turkey

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We aimed molecular analysis of soybean by lecithin specific primer pairs LE5/LE6. Genomic DNA was extracted from soybean by CTAB method and EZ1 nucleic acid isolation system. A sensitive qualitative detection method for soybean, using the polymerase chain reaction was developed with E5/LE6 primers, produced a 195 bp product. However, the antioxidant activity of methanolic extract of soybean from Turkey by using DPPH and ABTS radical scavenging assays, showed higher antioxidant activity with 53.19±0.87% and 45.10±0.32%, respectively. Fatty acid compositions and several phenolic acids and flavonoids of soybean extract were analysed by gas and high performenace chromatography. Data showed that, the main compounds of the extract were eriodictyol, naringenin and linoleic acid.

Key words: Antioxidant activity, fatty acid, flavonoid, soybean, molecular marker.

INTRODUCTION

Many legumes such as lotus, medicago, pisum, glycine, phaselous, and vigna have begun to draw much attention through recent genomic and phylogenetic studies (Shoemaker et al., 2006, Young and Shoemaker, 2006). Since the crop legumes are economically important products, researchers have also focused on them (Shoemaker et al., 2006). Legumes play an important role in traditional diets of many regions in the world, because they have low amount of fat and are excellent sources of protein, dietary fiber, certain micronutrients and phytochemicals. One of the main protein sources of legumes is known as soybean. The main compound of soybean, lecithin, has been extracted from soybean oil which is a natural emulsifier and lubricant used in many food industries such as shortening, margarine, cooking oil, and salad dressings processes. In recent years, it has also been used in pharmaceuticals and protective coatings. There are also many studies on clinical application of soybean (De Lemos, 2001; Symolon et al., 2004; Messina et al., 2006).

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Antioxidant activity and chemical compounds such as polyphenolics and flavonoids which protect the human tissue from free radicals released from organs, thereby reducing oxidative stres (Steinmetz and Potter, 1996; Fritz et al., 2003; Lee et al., 2005; Prakash et al., 2007). Soybeans have large amount of isoflavones which exhibit strong antioxidant activity. Soybeans contain an impressive array of phytochemicals, biologically active components, the most interesting of which are known as isoflavones. Isoflavones such as genistein and daidzein in the soybean seed are being studied in relation to the relief of certain menopausal symptoms, cancer prevention, slowing or reversing osteoporosis and reducing the risk of heart disease (Steinmetz and Potter, 1996; Lee et al., 2008). Besides functioning as antioxidants, many isoflavones have been shown to interact with animal and human estrogen receptors, causing several effects in the body similar to those caused by the hormone estrogen (Ho et al., 2002). Soy isoflavones also produce non-hormonal effects which influences signal transduction in the cell. Soyfoods and isoflavones have received considerable attention for their potential role in preventing and treating cancer and osteoporosis. Vegetable protein sources like soybeans, canola and maize gluten are good

alternatives to fish meal rich lecithin.

Lecithins are carbohydrate-binding proteins or glycoproteins that occur widely in plants, animals and microorganisms. Lecithin was first found in the seeds of *Ricinus communis* (Euphorbiaceae) about one hundred years ago. Since then, numerous plant lecithins have been isolated from the seeds of leguminous and gramineous plants. Although lecithins have been extensively studied with respect to carbohydrate binding specificity and potential utility for the isolation and characterization of glycoconjugates, the physiological role of lectins in plant is not yet well understood (Pusztai, 1991). Lectins are sugar-binding proteins which are highly specific for their sugar moieties. They typically play a role in biological recognition phenomena involving cells and proteins. For example, some viruses use lectins to attach themselves to the cells of the host organism during infection. Lectins have potential use in cancer treatment strategies due to the fact that, lectins present on the surface of tumor cells are capable of binding exogenous carbohydrate-containing molecules and internalize them by endocytosis (Ogawara et al., 1985). For this purpose, an increasing number of investigation has been carried out, to find antioxidative products that can be used on human diet. As a comparison of natural food additives, synthetic antioxidants have possible toxic effect. Therefore, not only natural antioxidant products were used in pharmaceutical technology but also in food industry as an alternative for food additives.

Hundreds of fatty acid structures are known to occur in the seed oils of various plant species. Fatty acids are classified as saturated fatty acids and unsaturated fatty acids including monounsarurated and polyunsaturated related to conjugated double bonds in fatty acids. Several fatty acids which cannot be manufactured by the body are essential to human health. The main essential fatty acids (EFAs), omega-3 and omega-6 fatty acids must be obtained from food, fish and vegetable oils. Omega-3 and omega-6 fatty acids play a crucial role in brain function, as well as normal growth and development. EFAs belong to the class of fatty acids called polyunsaturated fatty acids (PUFAs). Clinical studies suggest that omega-3 fatty acids and omega-6 fatty acids may be helpful in treating a variety of health conditions such as heart disease, diabetes, arthritis, osteoporosis, asthma, colon cancer, breast cancer and prostate cancer (Madhavi and Das, 1994; Kruger and Horrobin, 1997; Tsai et al., 1998; Simopoulos, 1999; Shils et al., 1999; Stampfer et al., 2000; Tsujikawa et al., 2000). There are a lot of reports about such an important crop, soybean, in the literature. But, any study does not exist on chemical constituents of soybean seed grown in Turkey.

The purpose of this study was to evaluate the flavonoid and phenolic contents and fatty acids of soybean seed originated from Turkey by chromatoraphic methods, high performance liquid chromatography and gas chromatography. However, antioxidant activity of the methanolic extract of soybean seed was evaluated by DPPH and ABTS scavenging ability assays. However, this study concerns an assay to investigate the fate of soy DNA fragments (120 and 195 bp) and a 180-bp fragment of the lecithin gene of soybean (*Glycine max*). For this purpose, DNA from soybean seed and fresh leaf was extracted and amplified with a lecithin specific marker, using polymerase chain reaction (Clarke and Wiseman, 2000).

MATERIALS AND METHODS

Molecular analysis

Plant material and DNA isolation

Fresh seed plantlets, as well as herbarium specimens were used in this study for DNA extraction. Herbarium samples were collected by the author in the field, gathered in herbaria. Soybean (*G. max. l.)* seeds or grains samples provided by Karadeniz Agricultural Research Institute.

Soybean seeds were germinated and then plantlets were ground to powder by liquid nitrogen treatment. Analysed samples included dried soybean seeds and fresh leaves which were used directly for DNA extraction, as they were found to yield DNAs comparable in quality and quantity to that obtained using EZ1 nucleic acid isolation analyser (Qiagen, 2007) and by the using of the CTAB method (Sambrook et al., 1989). A soybean seed bulk sample was ground to fine powder. All experiments were repeated twice under same conditions.

Polymerase chain reaction amplification

DNA extracted from soybean (*Glycine max l.*) growing in Turkey and amplified by LE5 and LE6 protein-specific primer pairs of soybean, obtained from Operon Technologies Inc. (Almeda CA, USA). The sequences of these primer pairs were 5'-TCAACGAAAACGAGTCTGGTG-3' (LE5) and 5'-GGTGGAGGCATCATAGGTAAT-3' (LE6). PCR amplification was carried out for each primer pairs. These conditions are showed as follows.

For the LE5/LE6 primer pairs, PCR were carried out in two tubes of a total volume of 25 µl containing 2 µl of 50 ng genomic DNA, 0,2 µl of 10 µmol each primer, 2,5 µl 10xPCR Buffer, 3.5 µl of 50 mmol/L $MgCl_2$, 2.5 µl of 10 mmol/L dNTP, 0.3 µl of 1U Taq DNA polymerase. The program was initiated for 5 min of denaturation, followed by 10 cycles of amplification with denaturation for 15 s 94°C, first annealing for 20 s at 55°C, and an extention at 72°C for 15 s, and followed by 40 cycle of amplification with denaturation for 1.5 min, second annealing for 1.5 min at 53°C, and an extention at 72°C for 1 min, a final elongation at 72°C for 5 min on Bio-Rad thermal cycler.

Biochemical analysis

Sample preparation

Soybean seed (50 g) were powdered and extracted with methanol for 6 h at 30°C using an orbital shaker. After filtration through a filter paper (Whatman No.1), the solvent was evaporated under vacuum to 10 ml and then dried at –50°C in a lyophiliser. The methanolic extract (yield 11%, w/w) was stored at 4°C.

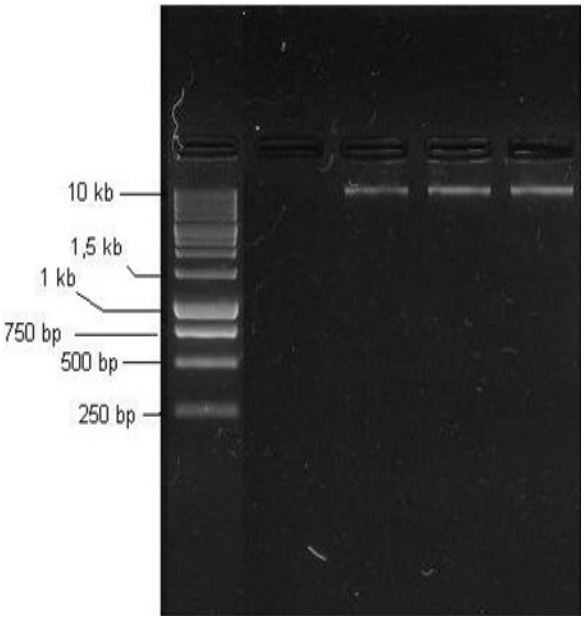

Figure 1. Genomic DNAs were loaded in a 0.7% agarose gel and separated by electrophoresis for 90 min at 50 V, then visualised by ethidium bromide staining with transillumination. Respectively, lane 1, 1 kb ladder size standard. Lane 2 negative control water blank (none DNA), Lane 3, 4 genomic DNA isolated from soybean with Bio Robot EZ1 and Lane 5 genomic DNA isolated from soybean with CTAB method.

Flavonoids and phenolics

HPLC analysis was conducted on an Shimadzu 1100 series HPLC equipment with a SIL-10AD vp atuosampler and LC-10Advp pump system, diode array detector (DAD), and an Inertsil Agilent Eclipse XDB column (240 × 4.60 mm; 5 µm particle size). Column temperature was 30°C The mobile phase components were methanol (A) and 3% (v/v) aqueous acetic acid (B). The mobile phase program was a linear gradient from 5 to 66.5% A over 75 min at 0.8 ml/min. The mobile phase program also contained a cleaning step using 100% methanol at 1 ml/min for 10 min. A series of each phenolic compunds including gallic acid, catechin, cafeic acid, epicatechin, p-coumaric acid, ferulic acid, vitexin, rutin, naringin, hesperidin, apigenin, rosmaric acid, eriodictyol, quercetin, naringenin, luteolin, apigenin and carvacrol as standards were analysed quantiatively; in order to determine phenolic and flavonoid compounds before analysis of the methanolic extract of soybean seed. 20 µl of standards and sample was injected.

Fatty acids

Gas chromatographic analysis was carried out on a Shimadzu GC 5050 gas chromatography. The chromatographic column for the analysis was a Cp Wax 52 CB capillary column (50 m / 0.32 mm, 1.2 µm). The carrier gas was helium at a flow rate of 10 psi/min. The methanolic extract of soybean seed was derived with 5% (w/v) sodium methoxide overnight. The sample was analysed with the column which was initially held at 60°C for 4 min and increased to 175°C with a 13°C/min heating ramp and then kept at 175°C for 27 min. Temperature was again increased to 215°C with a 4°C/min heating ramp and then kept at 215°C for 5 min. Finally, temperature was increased to 240°C with a 4°C/min heating ramp and the temperature was kept at 240°C for 15 min. Analysis was completed in 75 min. The injection was performed in split mode at 240°C.

Antioxidant activity

DPPH radical scavenging assay: The scavenging activity was estimated by the method described by Sanchez-Moreno (1998). According to the method, 0.5 ml of various concentrations of the extracts (0.1 to 0.5 mg /ml) in the methanol was added to 3 ml of DPPH (2,2-diphenyl-2-picrylhydrazyl) solution (0.004%) in methanol. After incubation of 30 min at room temperature in darkness, the absorbance was read against a blank at 517 nm by UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). Inhibition of the free radical by DPPH (*I*%) was calculated using the following equation described by Kartal et al. (2007):

$$I\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

where A_{blank} is the absorbance of the blank (containing all reagents except the extract or standard), and A_{sample} is the absorbance of the extract or standard. Experiments were carried out in triplicate and also butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) were used as standard antoxidants.

ABTS radical scavenging assay: The ability of the methanolic extract of soybean seed to scavenge ABTS was described by the method of Re et al. (1999). In this assay, ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^+$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 h before use. Absorbance of 2 mM ABTS solution in potassium persulfate was recorded at 734 nm by spectrophotometer. 0.1 ml of the extracts (0.01 to 0.5 mg/ml) was added to 1 ml of ABTS solution and absorbance change of ABTS solution was recorded after 4 min. The scavenging ability of ABTS was determined as follows:

$$I\% = [(A_{blank} - A_{sample}) / A_{blank}]_{\times} 100$$

where A_{blank} is absorbance of the blank (ABTS without extract), A_{sample} is absorbance of the extract or standard in the presence of ABTS. BHT and BHA were used as comparative standards. The samples were run in triplicate.

Statical analysis

The statistical analysis was carried out using OriginPro 7.5 software. One way ANOVA was applied to data and results were compared using Tukey test. A difference was considered to be statistically significant when the p-value is lower than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

Molecular analysis

This study was performed with primer pairs LE5/LE6 for identification of the lechitin gene of soybean. For this purpose, DNA were extracted with CTAB and EZ1 extraction method. DNAs from all extraction methods were checked for their integrity by agarose gel electrophoresis. As shown in Figure 1, genomic DNA

isolation from soybean by both extraction methods were of adequate purity and yield for applying PCR, resulting that amplifications of the expected 100 bp fragment, using the soybean specific primer pair were detected. However, the DNA obtained from both methods were of sufficient purity (A_{260}/A_{280}=1.7–2.0). Standardising the amount and quality of DNA extracted from plant material are important for further molecular methods. Especially, high DNA quality İs necessary in PCR amplification. Low quality results in inhibition of the PCR reaction. This inhibition is most likely due to a higher concentration of plant proteins and secondary components. It has been reported that, DNA extracted from processed foods and certain agricultural materials is of low quality, with available target sequences being rather short, e.g. 100 to 400 bp for soybean protein preparations.

After DNA extraction by two methods, a sensitive qualitative detection method for soybeans in foods using the polymerase chain reaction was developed. DNA samples from soybean were processed and submitted to PCR with primer pairs LE5/LE6. In all reactions, the expected product was produced by the control primer pair LE5/6. This indicated that the quantity and quality of extracted DNA was suitable for amplification of lechitin gene. In this study, the pairs of primer LE5/LE6 were used in the PCR detection of soybean genotypes sequences growing in Turkey. This primer pairs were designed for the present study and used as a specific test for the presence of soybean protein. The LE5/LE6 primer pair produces a 195 bp product which was diagnostic for the species specific to Turkey. Additionally, these primers were designed to amplify a (180 to 200 bp) portion of the lecithin gene of soybean. The results from PCR amplification from DNA extracted using these two methods were also of equivalent quality.

In summary, a rapid PCR detection method was developed for the specific detection of soybeans growing in Turkey. The methods reported in this study are simple, sensitive, and reliable for identifying a trace amount of soybean in food stuffs. We believe that the PCR method will be used to complement the protein-based detection in the future. The presence of soybean genotypes were investigated by amplifying the soy-specific lecithin gene. Lecithin primer pairs were designed to detect soybean genotypes and lectin and lecithine sequences (Chappell, 2005). This primer generates smaller amplicons and therefore have a greater opportunity of mediating amplification when degraded DNA templates such as those obtained from processed material are used. LE5/6 amplifies a 180 to 200 bp fragment of the lectin gene (Figure 2). We repeated all reactions at least two times and obtained the same DNA fingerprints, indicating high reproducibility obtained with these reactions. The results of this study, highlight a reliable and efficient way of using molecular markers from different soybean genotypes. It is desirable to characterize more DNA markers in soybean genotypes for more productive genomic studies, such as genetic mapping, marker-assisted selection, and gene discovery. Finally, the molecular markers observed in this study would be very useful for germplasm analysis, population genetic structure and phylogenetic relationships. The present study on development of the protocol for isolation of high purity DNA and optimization of the primer pairs conditions is the first report on soybean growing in Turkey.

Biochemical analysis

Hundreds of unusual fatty acid structures are known to occur in the seed oils of various plant species. The biosynthetic pathways of many fatty acids are unknown or have not been well characterized. One such class consists of fatty acids with double bonds that are conjugated. This structural configuration is in contrast to that of linoleic (C18:2) and linolenic (C18:3) acids, the typical polyunsaturated fatty acids of plant seed oils, which contain double bonds that are separated by methylene (-CH_2-) groups (Cahoon et al., 1999). Especially, ω3 and ω6 PUFAs taken in diet is vital for health (Simopoulos, 1999; Tsai et al., 1998; Tsujikawa et al., 2000; Shils et al., 1999). The presence of conjugated double bonds in fatty acids markedly increases their rate of oxidation relative to polyunsaturated fatty acids with methylene-interrupted double bonds (Qi et al., 2004). This property makes seed oils, such as tung oil, that are enriched in fatty acids with conjugated double bonds well suited for use as drying agents in paints, varnishes and inks, because they require less oxygen for the polymerization reactions that occur during the drying process (Qi et al., 2004).

Ester derivatives of C8 to C20 fatty acids was prepared for determination of the temperature programmed retention indices by dilution in n-hexane. Samples diluted in *n*-hexane were analysed by gas chromatography. Internal standards were then added to each sample to aid in the standardization of retention times and the samples were analysed again. The GC analysis led to the identification and quantification of several fatty acids. The accumulation of fatty acids with polyunsaturated fatty acids (PUFAs) was detected in soybean seed. Hovewer, fatty acids with monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) were analysed in the methanolic extract of soybean seed (Table 1). Where one of the polyunsaturated fatty acid with 48.39±4.23% (w/w), linoleic acid (C 18:2), was observed the main compound of fatty acids composed of the methanolic extract, the second one is a content of palmitic acid (C16:0) with 20.14±2.11% (w/w) in the extract. In the methanolic extract of soybean, oleic acid (C18:1) was composed approximately 12.39±2.05% (w/w) of the total fatty acids. In contrast, the lowest content of fatty acids was 6.09±1.07% (w/w) stearic acid (C18:0). In comparison to the total fatty acids as saturated and unsaturated fatty

Table 1. Fatty acid composition of soybean extract as a percent of total (%).

Fatty acids	Amount (%)	Retention time (min.)
C 14:0	-	28.4
C 15:0	-	43.8
C 16:0	20.14±2.11	45.4
C 17:0	-	50.8
C 18:0	6.09±1.07	54.7
Σ SFA**	26.23	
C 18:1	12,39±2.05	56.0
Σ MUFA**	12.39	
C 18:2	48.39±4.23	58.1

*Data expressed as means ± standard deviation (n = 3). **SFAs; Saturated fatty acids, **MUFAs; Monounsaturated fatty acids, **PUFAs; Polyunsaturated fatty acids.

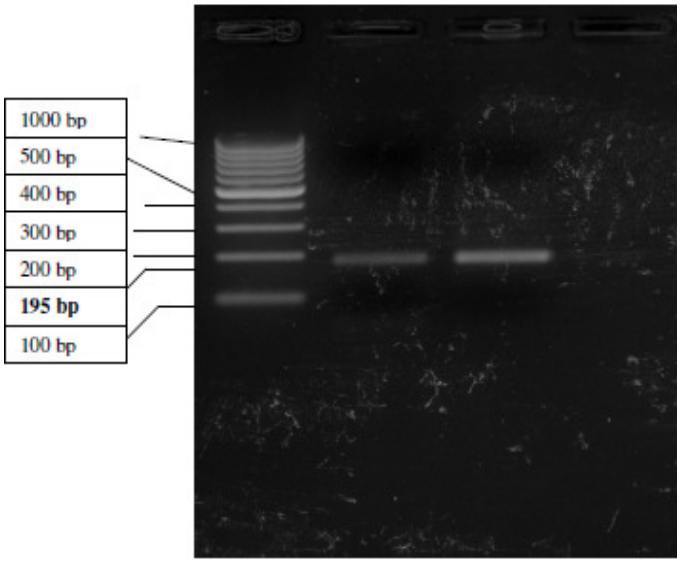

Figure 2. Agarose gel electrophoresis of LE5/LE6 gene PCR products. Respectively, Lane 1, 100 bp ladder size standard. Lane 2 and 3; plant sample genomic DNA PCR products of LE5/LE6.

acids, an approximately 5-fold decrease of PUFAs with 56.19% was also observed in amounts of MUFAs with 12.39%. Furthermore, amounts of SFAs composed of stearic acid and palmitic acid were also observed as 26.23% (w/w). As a results of analysis of fatty acids of soybean seed grown in Turkey, the main compounds of soybean is composed of EPAs such as omega-3 and omega-6-fatty acids.

Different substance combinations will be essential for the biological activity of different methanolic extracts of soybean seed. Analysing the different solvent extracts of palnts samples by HPLC will help to know its composition and promote biological properties (Tumbas et al., 2004). The identification of mixtures of natural products, including phenolic compounds which are attributed to

Table 2. Phenolic content of soybean extract.

Compounds	Amount (µg g^{-1})
Gallic acid	-
Catechin hydrate	-
Cafeic acid	5.6±0.49
Epicatechin	-
p-coumarin	4.2±0.27
Ferulic acid	-
Vitexin	80.9±4.67
Rutin	64.3±3.53
Naringin	112.2±5.02
Hesperidin	-
Rosmarinic acid	-
Eriodictyol	396.1±5.24
Quercetin	-
Naringenin	-
Carvacrol	-

Data expressed ($p < 0.05$) as means ± standard deviation (n = 3).

antioxidant activity of the soybean (Jin-Rui et al., 2007; Georgetti et al., 2008; Hubert et al., 2008; Malencic et al., 2008). In literature, more than 4000 phenolic compounds were identified in plant extracts. In this study, only 15 phenolic compounds were determined qualitatively and quantitatively by high performance liquid chromatography method. Phenolic compounds including carvacrol, gallic acid, ferrulic acid, epicatechin hydrate, naringenin, hesperidin, rosmarinic acid, eriodictyol, naringin, vitexin, caffeic acid, p-coumarin and rutin were identified in the methanolic extract of soybean seed. Based on the results of quantitative HPLC analysis (Table 2), it can be concluded that the highest content of phenolic acids (396.1±5.24 µg/g) was eriodictyol. The other main compounds were naringin, vitexin and rutin of which amounts were, 112.2±5.02, 80.9±4.67 and 64.3±3.53 µg/g, respectively. However, it has a little amount of caffeic acid (5.6±0.49 µg/g) and p-coumarin (4.2±0.27 µg/g). The results of qualitative HPLC analysis showed that carvacrol, gallic acid, ferulic acid, epicatechin hydrate, naringenin, hesperidin and rosmarinic acid were not detected in methanolic extract and the main flavonoid of the many plants, quercetin was not also detected.

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability or radical scavenging ability. DPPH shows a maximum absorption at 517 nm. When the methanolic extract of soybean was added to DPPH solution, its purple colour fades rapidly and rapid decrease was observed in the optical density of DPPH at 517 nm. This assay was determined by the scavenging of stable radical species of DPPH by the methanolic extract of soybean.

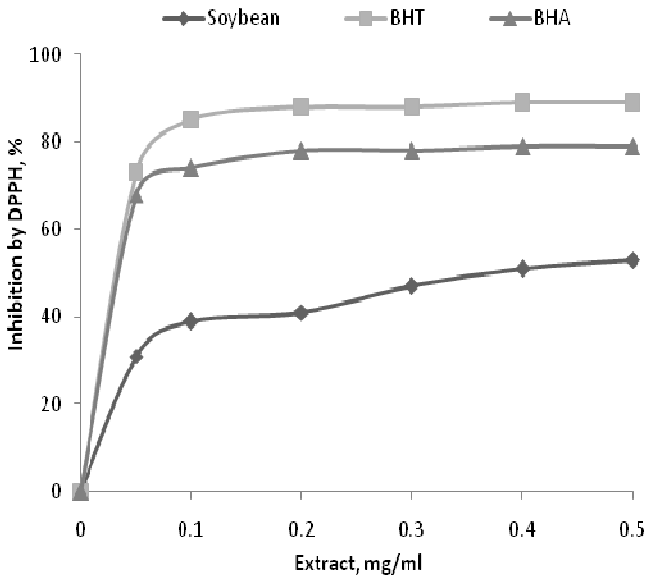

Figure 3. Inhibition ratio (%) against increasing concentration of soybean and standards, BHT and BHA, in DPPH assay.

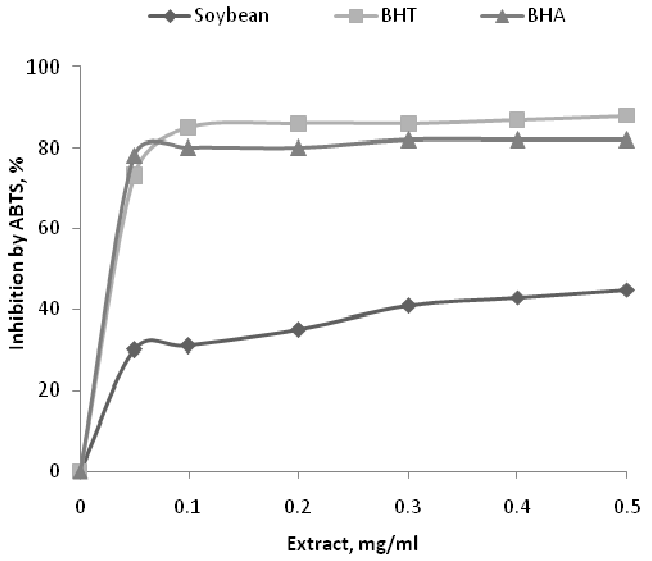

Figure 4. Inhibition ratio (%) against increasing concentration of soybean and standards, BHT nad BHA, in ABTS assay.

Figure 3 depicted that, the inhibition percentage of the methanolic extract of soybean ranged from 0.05 to 0.5 mg/ml by DPPH. Synthetic antioxidant standards, BHA and BHT, were applied to DPPH method at the same concentration range. Where inhibition percentage of soybean extract was 53.19±0.87% at 0.5 mg/ml, inhibition percentage of BHT and BHA were 94.16±1.79 and 85.46±1.31%, respectively (Table 1). Among the antioxidant compounds of soybean, rutin and caffeic acid were reported as the compounds responsible for the activity (Apak et al., 2004). Antioxidant activity of carotenoids, phenolics, and the antioxidants, determined by the decolorization of the ABTS measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm (Re et al., 1999). Figure 4 depicted that, the inhibition percentage of ABTS by the methanolic extract of soybean and synthetic antioxidant standards, BHA and BHT ranged from 0.05 to 0.5 mg/ml. In comparison with the standard antioxidants, sequence for the antioxidant activity was BHT (88.25±0.34%) > BHA (82.32±1.59%) > soybean extract (45.10±0.32%) at concentration of 0.5 mg/ml. However, all phenolic contents contibuted to a stronger antioxidant activity of the methanolic extract of soybean which are attributed to phenolic and flavonoid contents and fatty acids composition of the extract.

Conclusion

Agricultural biotechnology has opened new avenues in the development of plants for the production of food, feed, fibre, forest and other products. Soybean is a species of legume which is a plant in the family Fabaceae. It is an annual plant that has been used in China and in the other countries for 5,000 years as a food and a component of drugs.

Soy contains significant amounts of all the essential amino acids for humans, and so is a good source of protein. Soybean is the primary ingredient in many processed foods, including dairy product substitutes. The feasibility of detecting yields quality in soybean genotypes by a polymerase chain reaction (PCR) method is determined.

Therefore, polymerase chain reaction (PCR) is a sensitive method of analysing DNA. It is considered the most important method of detecting soybean in processed or raw foods. Namely, the polymerase chain reaction (PCR) is a commonly applied nucleic amplification method which is specific and sensitive enough to detect tiny amounts of organism-specific DNA sequences. Methods to verify compliance with labelling regulations are mainly based on the detection of specific DNA fragments and on the detection of newly expressed proteins by polymerase chain reaction (PCR). Although much progress has been made in the development of genetic analysis methods, such as those based on the use of PCR, the analytical technologies that can provide solutions to current technical issues in food stuffs analysis.

Furthermore, it identifies the combined work areas of analytical investigation and molecular techniques and discusses current needs and future challenges. The aim of this study was also to investigate the survival of soybean genotypes DNA fragments of the lecithin gene in specific-fish protein.

However, different types of fatty acids were analysed in the methanolic extract of soybean seed. The main fatty

acids was omega-6 fatty acid consumed in the diet from vegetable oils as linoleic acid. Data showed that soybean extract contain eriodictyol, naringin, vitexin and rutin acids in the methanolic extract of soybean as several flavonoids and phenolics. The methanolic extract of soybean exhibited high antioxidant activity by the scavenging activity of DPPH and ABTS. As discussed, this property could be largely dependent on flavonoid and phenolic contents. However, the antioxidant activity is attributed to not only total flavonoid content but also to total phenolic content and other molecules such as linolenic and linoleic acid in soybean extract. Results showed that soybean extract has a high amount of linoleic acid. Consequently, soybean seed from Turkey, is rich of phenolic and flavonoids and fatty acids, may be a source of natural antioxidants.

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