Wageningen Academic Publishers

Effects of infrared treatment on tocopherols, total phenolics and antioxidant activity of soybean samples

S. Yalcin[1,2] and A. Basman[1*]

[1]Hacettepe University, Faculty of Engineering, Food Engineering Department, 06800 Beytepe, Ankara, Turkey; [2]Afyon Kocatepe University, Afyon Vocational School, Food Technology Programme, Ali Cetinkaya Campus, 03200 Afyon, Turkey; basman@hacettepe.edu.tr

Received: 4 July 2015 / Accepted: 14 August 2015

RESEARCH ARTICLE

Abstract

In this study, infrared treatment at different powers (814, 1,003, 1,208, 1,342 W) was applied to unsoaked and soaked (30 min, 45 min) soybeans (cvs. Adasoy and Nazlican) for 10 or 15 min. Effects of infrared treatment on tocopherols (α-, β+γ-, δ-tocopherol), total phenolic contents and DPPH (1,1-diphenyl 2-picrylhydrazyl) radical scavenging activity of soybeans were investigated. Infrared treated soybeans generally had higher total phenolic contents as compared to control. Total phenolic content increased as the infrared power or treatment time increased. Unsoaked soybeans had significantly higher total phenolic content as compared to soaked counterparts. Infrared treatment caused only slight changes in tocopherol contents of soybeans (except unsoaked sample treated at 1,342 W for 15 min). Minor reductions in DPPH radical scavenging activity were observed as the infrared power increased. Infrared conditions adequate for inactivation of undesirable components (trypsin inhibitor and lipoxygenase) appear to be favourable for retention of tocopherols and DPPH radical scavenging activity, especially in soaked soybeans. Increase in total phenolic contents after infrared treatment of soybean is also promising. Overall results and discussions demonstrated that the correct selection of infrared conditions is important to guarantee the quality of soybean in terms of health beneficial components and undesirable components.

Keywords: antioxidant, infrared treatment, soybean, tocopherols, total phenolics

1. Introduction

Soybean is one of the most important legumes for human consumption and the most important components of soybean are proteins (about 40%) and oil (about 20%). Soybean also contains tocopherols, isoflavones and other phenolic compounds which may contribute to the overall health benefits of soy. Phenolic compounds are of great current interest, due to their antioxidative and possible anticarcinogenic activities (Boschin and Arnoldi, 2011; Malenčić *et al.*, 2007; Sakthivelu *et al.,* 2008). Total phenolic content of soybean was reported as 2,689.13±125.63 mg gallic acid equivalents per kg (GAE/kg) by Žilić *et al.* (2014). In a study by Malenčić *et al.* (2007), total phenolics and DPPH (1,1-diphenyl 2-picrylhydrazyl) radical scavenging activities of twenty soybean genotypes were found to be between 2.70-4.88 g catechin/kg dry plant material and 22.87-48.17%, respectively.

Tocopherol is a major lipophilic antioxidant in the human diet. Although all tocopherols (α, β, γ, δ) are potent antioxidants *in vitro*, α-tocopherol is the most active in terms of vitamin E activity since it is retained in the body in preference to other tocopherols (Sattler *et al.*, 2004; Séguin *et al.*, 2009). Tocopherol also terminates the chain reaction of lipid peroxidation (Traber and Manor, 2012). Soybean is different from other legumes in terms of tocopherol content. Total tocopherol content of soybean (100 g seed) is found to be between 13.1-14.2 mg while that of chickpea is 8.67-11.3 mg, broad bean is 5.45-6.19 mg and lentil is 4.02-5.46 mg (Boschin and Arnoldi, 2011).

Besides these important components having health benefits, soybean also contains some undesirable components (trypsin inhibitor, lipoxygenase). Trypsin inhibitor reduces protein digestibility and lipoxygenase is responsible for undesirable flavour formation by the oxidation of polyunsaturated fatty acids (Iassonova *et al.*, 2009; Janssen, 1996). The undesirable components and beany flavour (occurred after long term storage of raw, wet and ground soybeans) restrict the consumption of soybean (Žilić *et al.*, 2014). Soaking, boiling, autoclaving, microwave, infrared treatment, steaming and/or roasting are used in order to improve the soybean flavour, palatability and enhance the bioavailability of bioactive compounds in soybean, by inactivating the undesirable components (Kim *et al.*, 2015; Randhir *et al.*, 2009; Shin *et al.*, 2013; Yalcin and Basman, 2015; Žilić *et al.,* 2014). However, in all these treatments, processing conditions that will not adversely alter nutritional and sensory characteristics of soybean must be investigated. Yang *et al.* (2014) reported significant reductions (expressed as a percentage of raw soybean seeds) in DPPH radical scavenging activities of yellow soybeans by roasting (-19.9), 30 min boiling (-26.55), 60 min boiling (-38.75) and microwave treatment (-28.73). Niamnuy *et al.* (2011) reported very slight differences among DPPH radical scavenging capacities of the samples dried at various drying conditions at 50, 70, 130 and 150 °C. Žilić *et al.* (2014) reported that extrusion, infrared and microwave processing (except microwave heating at 135 °C for 5 min) increased the total phenolic content of soybean. However, Boateng *et al.* (2008) reported 29% reduction in total phenolics in toasted (microwave 1,200 W, 6 min) soybeans as compared to raw soybeans. Takagi *et al.* (1999) reported 40% tocopherol loss in the coat of some soybeans with 12 min microwave roasting (2,450 MHz, 6, 12, 20 min) and <20% loss in the cotyledons and axis with 20 min roasting.

Infrared treatment conditions, adequate for inactivation of undesirable components of soybean, have not been tested in terms of health beneficial components (tocopherols, total phenolics, DPPH radical scavenging activity) of soybean yet. Infrared (IR) treatment has many advantages over conventional heating such as thermal efficiency, fast heating rate/response time, direct heat penetration into the product (Erdoğdu *et al.*, 2010; Sakai and Mao, 2006; Sumnu and Ozkoc, 2010). In this study, effects of infrared treatment at various powers (814, 1,003, 1,208, 1,342 W) and times (10 min, 15 min) on tocopherols, total phenolics and DPPH radical scavenging activity of unsoaked and soaked (30 min, 45 min) soybeans (cvs. Adasoy, Nazlican) were investigated. The infrared conditions used in this study were the same as the ones used for investigating the effects of IR-treatment on undesirable components (urease, trypsin inhibitor and lipoxygenase) of soybean (Yalcin and Basman, 2015). The results of the present and previous study were discussed together in order to provide a brief overview of knowledge to literature about the infrared conditions that guarantee the quality of soybean in terms of health beneficial components and undesirable components.

2. Materials and methods

Materials

Soybean samples (cvs. Adasoy, Nazlican) were obtained from Cukurova Agricultural Research Institute, Adana, Turkey. Moisture, protein and ash contents of the samples were determined according to AACC (2000).

Infrared treatment

Soybeans with uniform size (6 < x < 8 mm) were soaked in water (7:40; w/v; 30 °C) for 30 min or 45 min. Excess water on the surface was removed after soaking. The samples were allowed to rest in plastic bags at 30 °C for 5 h for the uniform distribution of water. The moisture contents of Adasoy and Nazlican samples were 40.4 and 48.5% after soaking for 30 min and 44.8 and 51.7% after soaking for 45 min, respectively. Hsu *et al.* (1983) reported that the rate of water absorption by soybeans during soaking process varied with variety and it did correlate with the size and density of the soybeans. Infrared treatment at 814, 1,003, 1,208 and 1,342 W was applied to unsoaked and soaked soybeans for 10 min or 15 min. Different IR powers and treatment times were tested in preliminary studies. Moisture content and colour of the samples were taken into account for selection of appropriate IR power and time. Surface temperatures of the soybeans were detected by an IR thermometer (MX6 Infrared Thermometer; Raytek Corporation, Santa Cruz, CA, USA). Surface temperatures of 30 and 45 min soaked samples were similar during the IR-treatment and temperatures for the samples treated for 10 min and 15 min were in the range of 60-99 and 70-120 °C, respectively. Surface temperatures of the unsoaked soybeans were higher and the temperatures for 10 min and 15 min treated samples were 90-160 and 100-170 °C, respectively.

Laboratory scale infrared equipment (Biasis Ltd. Sti., Ankara, Turkey) including a closed drying chamber fitted with twelve 150 W halogen lamps (Infrared, R125 IR; Philips, Eindhoven, the Netherlands) and two aeration channels (12 V each) was used in the study. The halogen lamps have a pronounced peak at approximately 1 µm. Infrared power can be set between 310-1,595 W by using a dimmer. The lamp system was set to a height of 20 cm from the sample tray. IR-treatment for each trial was carried out as two replicates and the samples were combined in a large batch. A resting period of 20 h at 30 °C in a fermentation cabinet after IR-treatment provided final moisture contents lower than 9%. The dried soybeans were ground (<212 µm) and used in the analyses.

Tocopherols

0.6 g sample was dissolved in 4 ml ethanol (80%) by shaking at room temperature for 1 h. Hexane (12 ml) was added to the sample extract and mixed for 1 h. Sample (1 ml) was taken from the upper hexane layer and dried under nitrogen gas flow. Dried samples were stored at -20 °C in a dark place until analysed. The extraction was carried out in duplicate. The dried sample was dissolved in 1 ml of 100% methanol and filtered through 0.22 µm syringe filter (Phenomenex, Torrance, CA, USA). α-tocopherol, β+γ-tocopherol and δ-tocopherols were quantified using reverse-phase high-performance liquid chromatography (Agilent Technologies, Santa Clara, CA, USA) with a Luna C18 column (250×4.60 mm, 5 µ, Phenomenex) fitted with a Phenomenex guard column (4×3 mm, AJO 4287; Phenomenex by using a mobile phase of 2% water and 98% methanol at a flow rate of 1 ml/min. The run time for each sample was 30 min. The tocopherols were detected at 290 nm with a photodiode array detector (G 1315B; Agilent Technologies).

Standards of α-tocopherol (Sigma T3251; Sigma-Aldrich, St Louis, MO, USA), β-tocopherol (Supelco 46401-U; Sigma-Aldrich), γ-tocopherol (Sigma T1782; Sigma-Aldrich) and δ-tocopherol (Sigma T2028; Sigma-Aldrich) were used. Stock standard solutions of each tocopherol were prepared in methanol. The working standard solution (0-10 mg/kg for α-tocopherol, 0-10 mg/kg for β+γ-tocopherol and 0-5 mg/kg for δ-tocopherol) was obtained by diluting the stock standard solution with methanol. There was no separation of β and γ tocopherols on reverse phase columns. So standards of both tocopherols were prepared together. Calibration curves were plotted using α, β+γ and δ standards and quantification of the tocopherols was done based on the resulting curves. For all standard curves, R^2 were over +0.99. Analyses were performed in duplicate. Tocopherol content was expressed in mg/kg (dry basis).

Total phenolics

Ground samples (0.5 g) were extracted with a mixture of 2 ml HCl (0.1 N), 10 ml acetonitrile and 3 ml deionised water for 5 min. Then the samples were shaken for 2 h and centrifuged at 4,500 rpm for 30 min. Extraction was carried out twice. Combined extract was dried under a flow of nitrogen gas and stored at -20 °C. Dried samples were mixed with 1 ml of 80% methanol. Total phenolic content was determined by Folin-Ciocalteu spectrophotometric method (Singleton and Rossi, 1965). Sample extract (300 µl) and 750 µl Folin–Ciocalteu's solution (diluted with deionised water, 1:10) were mixed and incubated for 5 min. Then, 750 µl Na_2CO_3 (60 g/l) was added and mixture was incubated in dark for 90 min at room temperature. The absorbance was measured at 725 nm. Total phenolic content was expressed as mg GAE/g of sample through calibration curve of gallic acid. Analyses were performed in duplicate.

DPPH radical scavenging activity

Soy flour (100 mg) was dissolved in 80% ethanol (20 ml) for 15 min and the suspensions were centrifuged at 1660×*g* for 10 min. Supernatant fraction was collected in a volumetric flask. The precipitate was extracted with 5 ml of 80% ethanol. The total volume of the combined supernatant was adjusted to 25 ml with 80% ethanol. Antioxidant activity was determined by using the DPPH radical scavenging method (Brand-Williams *et al.*, 1995). DPPH was dissolved in 100% methanol in order to obtain a solution with a concentration of 6×10^{-5} mol/l. The sample extract (100 µl) was added to the DPPH solution (3.9 ml). After incubation in a dark place at room temperature for 30 min, the decrease in absorbance was measured at 515 nm. The DPPH solution (6×10^{-5} mol/l) was used as control for all samples. Analyses were performed in duplicate. The DPPH radical scavenging activity was calculated according to the following equation and the results were given on dry basis:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \left(1 - \frac{absorbance_{sample}}{absorbance_{control}}\right) \times 100$$

Statistical analysis

Duncan's test was used to determine the differences among main effects (soaking time, infrared power and treatment time) for tocopherols, total phenolics and DPPH radical scavenging activity. The results are given in Table 1 and 2 (e.g. for the 'soaking time 30 min' in Table 1 and 2, the average value of all samples soaked for 30 min were calculated, regardless of the infrared power (814, 1,003, 1,208, 1,342 W) and treatment time (10 min, 15 min) applied). Standard deviations were determined using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

3. Results and discussion

Protein (N×6.25, dry basis; db) and ash (%, db) contents were 39.3 and 5.15% for cv. Adasoy, 39.7 and 5.00% for cv. Nazlican, respectively.

Tocopherol content

α-, β+γ- and δ-tocopherols of the IR-treated Adasoy and Nazlican samples are presented in Figure 1-3. Multiple comparison test results of main factors showed that soaking time, infrared power or treatment time caused significant changes in α-, β+γ- and δ-tocopherol contents of Adasoy and Nazlican samples ($P<0.05$), except δ-tocopherol contents of Adasoy samples (Table 1 and 2).

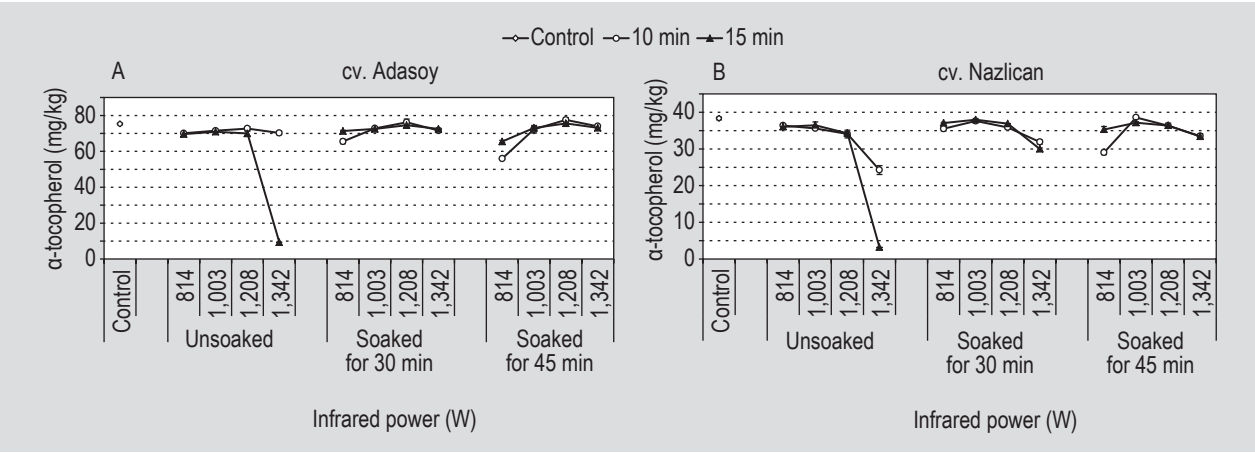

Figure 1. Effects of infrared treatment on α-tocopherol contents of (A) Adasoy and (B) Nazlican soybean samples. Values are the means of two replicates ± standard deviations.

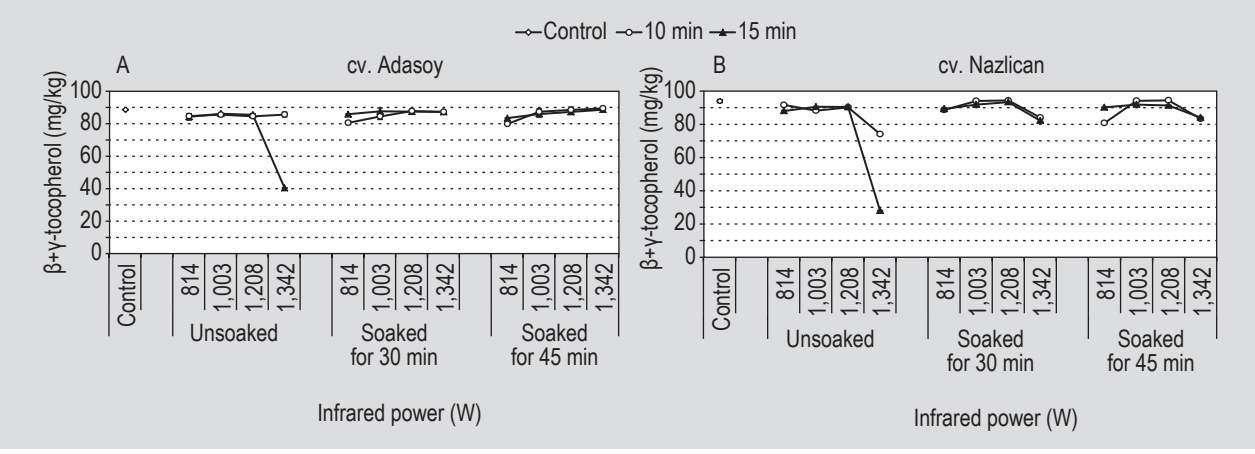

Figure 2. Effects of infrared treatment on β+γ-tocopherol contents of (A) Adasoy and (B) Nazlican soybean samples. Values are the means of two replicates ± standard deviations.

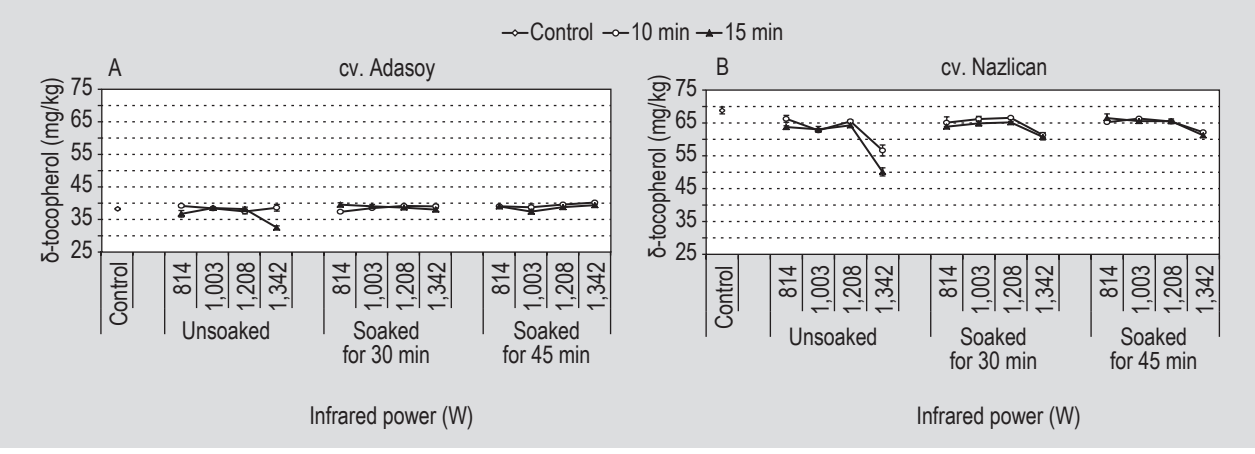

Figure 3. Effects of infrared treatment on δ-tocopherol contents of (A) Adasoy and (B) Nazlican soybean samples. Values are the means of two replicates ± standard deviations.

Table 1. Multiple comparison test results of the main factors (soaking time, infrared power, treatment time) for tocopherols, total phenolic content and DPPH radical scavenging activity of Adasoy soybean samples.[1,2,3,4,5]

Main factor	Sample	α-tocopherol (mg/kg)	β+γ-tocopherol (mg/kg)	δ-tocopherol (mg/kg)	Total phenolic content (mg GAE/g)	DPPH radical scavenging activity (%)
Soaking time (min)	Control	75.28 a	88.60 a	38.23 b	3.00 d	13.8 a
	0	63.06 d	79.60 c	37.44 c	3.36 a	12.5 c
	30	72.12 b	86.05 b	38.66 ab	3.21 b	13.2 b
	45	70.89 c	86.31 b	39.01 a	3.14 c	13.2 b
Infrared power (W)	Control	75.28 a	88.60 a	38.23 a	3.00 d	13.8 a
	814	66.34 c	83.11 c	38.47 a	3.13 c	13.5 b
	1,003	72.19 b	86.20 b	38.43 a	3.13 c	13.2 c
	1,208	74.42 a	86.86 b	38.63 a	3.34 b	12.9 d
	1,342	61.81 d	79.79 d	37.96 a	3.36 a	12.2 e
Treatment time (min)	Control	75.28 a	88.60 a	38.23 ab	3.00 c	13.8 a
	10	70.88 b	85.49 b	38.76 a	3.10 b	13.2 b
	15	66.50 c	82.49 c	37.98 b	3.38 a	12.7 c

[1] DPPH = 1,1-diphenyl 2-picrylhydrazyl; GAE = gallic acid equivalents.
[2] Values followed by the same letter in the same column for each main factor (soaking time, infrared power, treatment time) are not significantly different (P>0.05).
[3] Results are given on dry basis.
[4] 'Control' represents untreated samples.
[5] Soaking time '0 min' represents the unsoaked but infrared treated samples.

Table 2. Multiple comparison test results of the main factors (soaking time, infrared power, treatment time) for tocopherols, total phenolic content and DPPH radical scavenging activity of Nazlican soybean samples.[1,2,3,4,5]

Main factor	Sample	α-tocopherol (mg/kg)	β+γ-tocopherol (mg/kg)	δ-tocopherol (mg/kg)	Total phenolic content (mg GAE/g)	DPPH radical scavenging activity (%)
Soaking time (min)	Control	38.33 a	93.93 a	68.81 a	2.82 d	13.5 a
	0	30.03 c	80.14 d	61.54 c	3.19 a	12.1 c
	30	35.34 b	89.72 b	64.23 b	3.13 b	12.9 b
	45	34.96 b	88.76 c	64.74 b	3.08 c	12.9 b
Infrared power (W)	Control	38.33 a	93.93 a	68.81 a	2.82 d	13.5 a
	814	34.88 d	88.10 d	65.11 b	2.98 c	13.2 b
	1,003	37.23 b	91.78 c	64.79 b	3.03 b	12.8 c
	1,208	35.63 c	92.34 b	65.41 b	3.27 a	12.4 d
	1,342	26.03 e	72.60 e	58.70 c	3.26 a	12.0 e
Treatment time (min)	Control	38.33 a	93.93 a	68.81 a	2.82 c	13.5 a
	10	34.05 b	88.17 b	64.12 b	2.97 b	12.9 b
	15	32.83 c	84.25 c	62.89 c	3.30 a	12.3 c

[1] DPPH = 1,1-diphenyl 2-picrylhydrazyl; GAE = gallic acid equivalents.
[2] Values followed by the same letter in the same column for each main factor (soaking time, infrared power, treatment time) are not significantly different (P>0.05).
[3] Results are given on dry basis.
[4] 'Control' represents untreated samples.
[5] Soaking time '0 min' represents the unsoaked but infrared treated samples.

The retention times for α-, β+γ- and δ-tocopherols were found to be 20.2, 17.3, and 14.5 min, respectively. The α-, β+γ- and δ-tocopherol contents of Adasoy control were 75.28, 88.60 and 38.23 mg/kg, respectively, whereas the ones for Nazlican control were 38.33, 93.93 and 68.81 mg/kg, respectively. α-tocopherol content of Adasoy was higher than that of Nazlican, while β+γ-tocopherol and δ-tocopherol contents were lower than those of Nazlican. Guzman and Murphy (1986) reported that α-, γ- and δ-tocopherol contents of soybeans ranged from 10.9 to 28.4 μg/g (dry basis), 150 to 191 μg/g (dry basis), 24.6 to 72.5 μg/g (dry basis), respectively. Nishiba and Suda (1998) reported that α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherols of soybean were 36.18, 11.71, 178.35 and 89.14 μg/g, respectively.

Soaking time did not cause significant changes in α-tocopherol contents of Nazlican samples (Table 2). Significantly lower α-tocopherol contents were observed for 45 min soaked Adasoy samples as compared to those for 30 min soaked samples (Table 1). IR-treated soybeans of both cultivars were found to have lower α-tocopherol contents as compared to their controls. The lowest α-tocopherol content was observed for Adasoy and Nazlican samples treated at 1,342 W (Table 1 and 2). Infrared treatment at 1,342 W caused a marked decrease in α-tocopherol contents of unsoaked samples of Adasoy (treated for 15 min) and Nazlican (treated for 10 or15 min) (Figure 1). However, all IR-treated soybeans except these samples have comparable α-tocopherol contents with that of control (Figure 1). Soybeans of both cultivars treated for 15 min gave significantly lower α-tocopherol contents as compared to 10 min treated ones (Table 1 and 2).

Different soaking times did not cause significant changes in β+γ-tocopherol contents of Adasoy samples (Table 1). Samples of both cultivars treated for 15 min had significantly lower β+γ-tocopherol contents as compared to their respective samples treated for 10 min (Table 1 and 2). Infrared treatment caused a significant decrease in β+γ-tocopherol contents of both cultivars as compared to the controls (Table 1 and 2). The lowest β+γ-tocopherol content was observed for Adasoy and Nazlican samples treated at 1,342 W (Table 1 and 2, Figure 2). Since the surface temperatures of the unsoaked samples (90-160 °C for 10 min, 100-170 °C for 15 min) during infrared treatment were higher than those of soaked samples (60-90 °C for 10 min, 70-120 °C for 15 min), effects of infrared treatment on unsoaked samples were more pronounced (Figure 2) and a marked decrease in β+γ-tocopherol contents was determined at 1,342 W. Unsoaked Nazlican samples were more sensitive to IR-treatment and marked decrease was observed just after 10 min although it was 15 min in Adasoy (Figure 2).

In both cultivars, δ-tocopherol contents of the 30 min soaked soybeans were not significantly different than those of 45 min soaked samples (Table 1 and 2). δ-tocopherol contents of Adasoy samples treated for 10 or 15 min were not significantly different than that of control. Long treatment time (15 min) had a more pronounced effect on δ-tocopherol contents of Nazlican samples and the lowest δ-tocopherol content was obtained with the treatment time of 15 min (Table 1 and 2). Infrared treatment did not cause significant changes in δ-tocopherol contents of Adasoy samples (Table 1). However, significant decreases in δ-tocopherol contents of Nazlican samples were observed. Nazlican samples treated at 1,342 W exhibited the lowest δ-tocopherol content. δ-tocopherol contents of unsoaked Adasoy samples decreased with an IR-treatment at 1,342 W for 15 min, while those of unsoaked Nazlican samples began to decrease at 1,342 W for 10 min (Figure 3).

In our previous study (Yalcin and Basman, 2015), it was found that IR-treatment was more effective on soaked soybeans than unsoaked ones in terms of trypsin inhibitor and lipoxygenase reduction. It is worth noting that the reduction in tocopherol contents of soaked soybeans was lower as compared to that of unsoaked counterparts. Infrared conditions for complete inactivation of lipoxygenase-1 and lipoxygenase-3 (1,003 W for 10 min) and maximum trypsin inhibitor reduction (45 min soaking, 1,342 W for 15 min) caused only minor reductions in tocopherol contents. The loss in total tocopherol content was only 0.46% for Adasoy soybean and 11.18% for Nazlican soybean treated at 1,342 W for 15 min.

Lešková *et al.* (2006) reported that cooking of soaked (16 h) soybeans for 165 min caused 8% loss in tocopherol content. Yoshida and Kajimoto (1989) reported that microwave treatment (2,450 MHz) of soybeans for 6 min caused 10% reduction in tocopherols whereas 40% of the tocopherols were lost after 12 min. Yoshida *et al.* (2003) reported reductions in total tocopherol content originally present in soybean coat (25% reduction) and cotyledons (<25%) with microwave roasting for 12 min and 20 min, respectively. Moisture contents of the samples were 9.4-9.6% and the temperature of the seed increased from 25 to 100.5 °C or 123 °C after microwave roasting for 12 or 20 min, respectively. For comparison of the results of Yoshida *et al.* (2003) with the results of our study, surface temperatures of the unsoaked samples during the IR-treatment (15 min) at 814 W (101 °C) and 1,003 W (122 °C) were taken into account. Infrared treatment at 814 W for 15 min caused 5.7 and 6.6% reductions in total (α+β+γ+δ) tocopherol contents of unsoaked Adasoy (moisture contents 8.4%, db) and unsoaked Nazlican (moisture content 9.1%, db) samples while IR-treatment at 1,003 W for 15 min caused 3.2 and 5.5% reductions in unsoaked Adasoy and Nazlican samples, respectively. The reductions in total tocopherol contents of IR-treated soybeans were lower as compared

to the microwave roasted soybeans reported by Yoshida *et al.* (2003).

Total phenolics

Total phenolic contents of IR-treated soybeans are presented in Figure 4. Multiple comparison test results of main factors showed that soaking time, infrared power or treatment time caused significant changes in total phenolic contents of both soybean cultivars (P<0.05) (Table 1 and 2).

Total phenolic contents of Adasoy and Nazlican control were 3.00±0.008 mg GAE/g and 2.82±0.007 mg GAE/g, respectively. IR-treated soybeans generally had higher total phenolic contents as compared to control. Total phenolic contents generally increased significantly as the infrared power or treatment time increased. In both cultivars, unsoaked soybeans had significantly higher total phenolic content as compared to soaked samples. Since the surface temperatures of the unsoaked samples (90-160 °C for 10 min, 100-170 °C for 15 min) during infrared treatment were found to be higher than those of soaked samples (60-90 °C for 10 min, 70-120 °C for 15 min), effects of infrared treatment on total phenolic content of unsoaked samples were more pronounced. Significantly higher values were obtained for the soybeans treated for 15 min as compared to the ones treated for 10 min (Table 1 and 2). All samples treated for 15 min had higher total phenolic content as compared to control (Figure 4). The highest total phenolic content in Adasoy (3.67±0.004 mg GAE/g) and Nazlican (3.52±0.002 mg GAE/g) was obtained for the unsoaked soybeans treated at 1,342 W for 15 min (Figure 4). The increase in total phenolic content may be derived from the increased release of phenolics from the cell matrix due to disruption of cellular constituents during thermal treatment (Dewanto *et al.*, 2002; Xu and Chang, 2008). It was reported that total free phenolics increase as the heating time and temperature increases (Dewanto *et al.*, 2002).

IR-treatment was found to be more effective on trypsin inhibitor and lipoxygenase of soaked soybeans than unsoaked ones (Yalcin and Basman, 2015). In the present study, increase in total phenolic content was found to be lower for soaked soybeans as compared to unsoaked ones. Maximum trypsin inhibitor reduction (Yalcin and Basman, 2015) was observed for 45 min soaked samples treated at 1,342 W for 15 min. After the IR-treatment at these severe conditions, total phenolic contents of Adasoy (control 3.00±0.008 mg GAE/g) and Nazlican (control 2.82±0.007 mg GAE/g) were 3.38±0.001 mg GAE/g and 3.31±0.002 mg GAE/g, respectively.

Žilić *et al.* (2014) investigated the effects of extrusion (100-140 °C for 20-30 s), infrared (100, 110, 120 and 140 °C for 40-100 s) and microwave processing (2,450 MHz, 800 W for 1-5 min, 45-135 °C) on soybean phenolic compounds. It was reported that all heat treatments (except microwave heating at 135 °C for 5 min) increased total phenolic contents. Infrared heating (at 100 °C/40 s, 110 °C/50 s, 120 °C/65 s, 140 °C/100 s) caused increases in total phenolic content. Initial total phenolic content was reported to be 2,689.13±125.63 mg GAE/kg. The highest total phenolic content was found to be 3,143.22 mg GAE/kg (at 100 °C/40 s) among IR-heated soybeans, 3,657.94 mg GAE/kg (at 140 °C/ 20-30 s) among extrusion-cooked and 3,106.54 mg GAE/kg (at 115 °C/ 3 min) among microwave-heated soybeans.

DPPH radical scavenging activity

DPPH radical scavenging activities of IR-treated soybeans are presented in Figure 5. Multiple comparison test results of main factors showed that soaking time, infrared power or treatment time caused significant changes in DPPH radical scavenging activities of Adasoy and Nazlican samples (P<0.05) (Table 1 and 2).

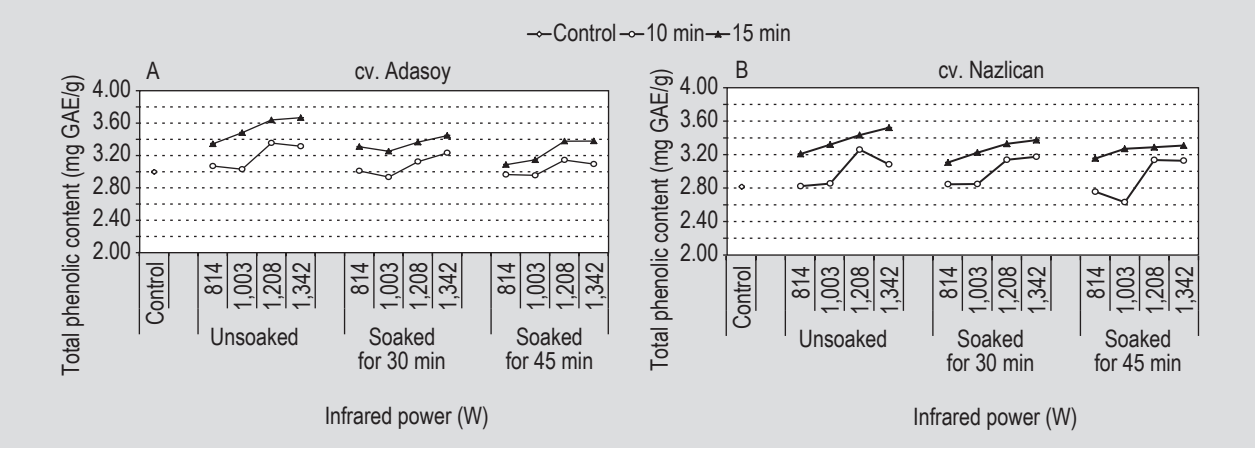

Figure 4. Effects of infrared treatment on total phenolic contents of (A) Adasoy and (B) Nazlican soybean samples. Values are the means of two replicates ± standard deviations.

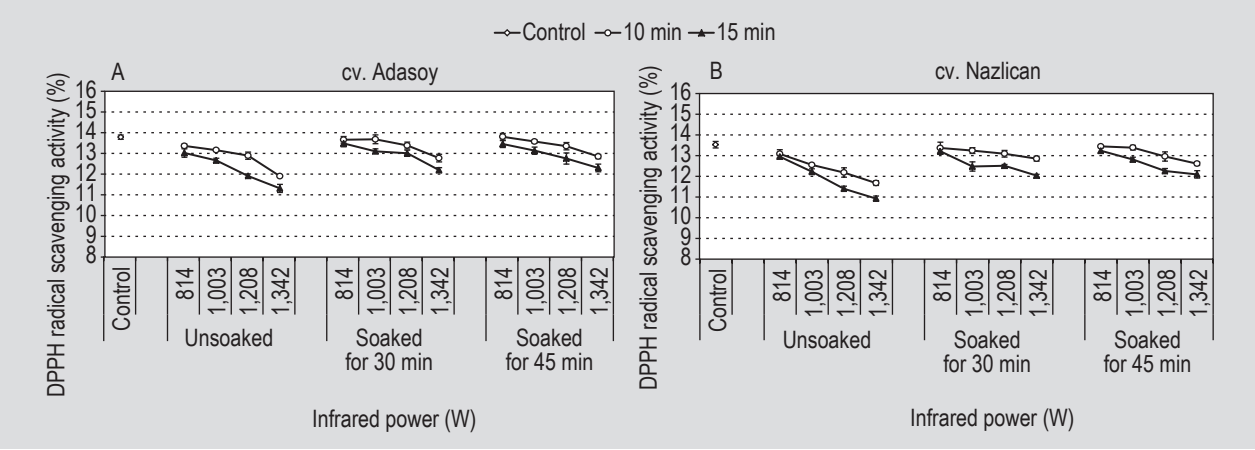

Figure 5. Effects of infrared treatment on DPPH radical scavenging activities of (A) Adasoy and (B) Nazlican soybean samples. Values are the means of two replicates ± standard deviations.

DPPH radical scavenging activities of Adasoy and Nazlican control were 13.8 and 13.5%, respectively. Infrared treatment at different powers caused significant changes in DPPH radical scavenging activities and the values decreased slightly as the infrared power increased. Significantly lower values were obtained for 15 min treated samples as compared to 10 min treated ones (Table 1 and 2). Effects of IR-treatment on trypsin inhibitor and lipoxygenase of soaked soybeans were more pronounced as compared to their unsoaked counterparts (Yalcin and Basman, 2015). Only minor reductions were observed for DPPH radical scavenging activities of all IR-treated samples. It is worth noting that the reduction for soaked soybeans was found to be lower, probably due to the lower surface temperatures of the soaked samples (60-90 °C for 10 min, 70-120 °C for 15 min) during infrared treatment as compared to unsoaked ones (90-160 °C for 10 min, 100-170 °C for 15 min).

Infrared conditions for complete inactivation of lipoxygenase-1 and lipoxygenase-3 (1,003 W for 10 min) and maximum trypsin inhibitor reduction (45 min soaking, 1,342 W for 15 min) caused only minor reductions in DPPH radical scavenging activities (Yalcin and Basman, 2015). After the IR-treatment at 1,003 W for 10 min, DPPH radical scavenging activity of cv. Adasoy decreased from 13.8% (control) to 13.2% (unsoaked), 13.7% (30 min soaked) and 13.6% (45 min soaked). For cv. Nazlican, the values decreased from 13.5% (control) to 12.6% (unsoaked), 13.2% (30 min soaked) and 13.4% (45 min soaked). DPPH radical scavenging activity of 12.3 and 12.1% were obtained for 45 min soaked Adasoy and Nazlican soybeans after the IR-treatment at 1,342 W for 15 min, respectively.

Yang *et al.* (2014) reported that DPPH radical scavenging activity of yellow soybean (16.7%) significantly decreased to 13.55% by roasting (180 °C, 20 min), 11.92% by microwaving (850 W, 3 min), 12.29% by 30 min boiling and 10.25% by 60 min boiling. For the comparison of these results with the results of our study, temperature applied during the treatment was taken into account. In our study, the highest surface temperature of 168 °C was observed for the unsoaked samples treated at 1,342 W for 15 min. DPPH radical scavenging activities of Adasoy (control 13.8%) and Nazlican (control 13.5%) decreased to 11.3 and 10.9%, respectively, in unsoaked samples treated at these IR conditions.

4. Conclusions

Beany flavour and undesirable components (trypsin inhibitor and lipoxygenase) of soybean restrict the consumption. Several processes are used in order to improve soybean flavour, palatability and enhance the bioavailability of bioactive compounds, by inactivating the undesirable components.

Infrared treatment is an efficient and energy saving technology and has obtained a great interest in the food industry, due to its advantages over conventional heating. In this study, infrared treatment conditions adequate for inactivation of undesirable components of soybean were tested in terms of health beneficial components (tocopherols, total phenolics, DPPH radical scavenging activity) of soybean. Overall results and discussions demonstrated that the correct selection of infrared conditions is important to guarantee the quality of soybean in terms of health beneficial components and undesirable components. Infrared conditions adequate for inactivation of undesirable compounds (e.g. lipoxygenase; 1,003 W for 10 min and trypsin inhibitor; 45 min soaking, 1,342 W for 15 min), appear to be favourable for retention of tocopherols and DPPH radical scavenging activity. Increase in total phenolic contents after IR-treatment of soybean is also promising. Infrared treatment has more pronounced effect on soaked soybeans in terms of undesirable components. In this respect, the results of the present study are also promising.

Acknowledgements

The authors wish to thank Hacettepe University Scientific Research Projects Coordination Unit for the financial support (project no. 08D11602003) and A. Nedim Nazlican for providing soybeans.

References

American Association of Cereal Chemists (AACC), 2000. Approved methods of the American Association of Cereal Chemists (10th Ed.). AACC, St. Paul, MN, USA.

Boateng, J., Verghese, M., Walker, L.T. and Ogutu, S., 2008. Effect of processing on antioxidant contents in selected dry beans (*Phaseolus* spp. L.). LWT-Food Science and Technology 41: 1541-1547.

Boschin, G. and Arnoldi, A., 2011. Legumes are valuable sources of tocopherols. Food Chemistry 127: 1199-1203.

Brand-Williams, W., Cuvelier, M.E. and Berset C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und Technologie 28: 25-30.

Dewanto, V., Wu, X. and Liu, R.H., 2002. Processed sweet corn has higher antioxidant activity. Journal of Agricultural and Food Chemistry 50: 4959-4964.

Erdoğdu, B.S., Ekiz, I.H., Erdoğdu, F., Atungulu, G.G. and Pan, Z., 2010. Industrial applications of infrared radiation heating and economic benefits in food and agricultural processing. In: Atungulu, G.G. and Pan, Z. (eds.). Infrared heating for food and agricultural processing. CRC Press/Taylor & Francis Group, Boca Raton, FL, USA.

Guzman, G.J. and Murphy, P.A., 1986. Tocopherols of soybean seeds and soybean curd (Tofu). Journal of Agricultural and Food Chemistry 34: 791-795.

Hsu, K.H., Kim, C.J. and Wilson, L.A., 1983. Factors affecting water uptake of soybean during soaking. Cereal Chemistry 60: 208-211.

Iassonova, D.R., Johnson, L.A., Hammond, E.G. and Beattie, S.E., 2009. Evidence of an enzymatic source of off flavors in 'lipoxygenase-null' soybeans. Journal of the American Oil Chemists' Society 86: 59-64.

Janssen, M.M.T., 1996. Antinutritives. In: Vries, J. (ed.) Food safety and toxicity. CRC Press/Taylor & Francis Group, Boca Raton, FL, USA.

Kim, S.H., Yu, B.R. and Chung, I.M., 2015. Changes in the contents and profiles of selected phenolics, soyasapogenols, tocopherols, and amino acids during soybean-rice mixture cooking: electric rice cooker vs electric pressure rice cooker. Food Chemistry 176: 45-53.

Lešková, E., Kubíková, J., Kováčiková, E., Košická, M., Porubská, J. and Holčíková, K., 2006. Vitamin losses: retention during heat treatment and continual changes expressed by mathematical models. Journal of Food Composition and Analysis 19: 252-276.

Malenčić, D., Popović, M. and Miladinović, J., 2007. Phenolic content and antioxidant properties of soybean (*Glycine max* (L.) Merr.) seeds. Molecules 12: 576-581.

Niamnuy, C., Nachaisin, M., Laohavanich, J. and Devahastin, S., 2011. Evaluation of bioactive compounds and bioactivities of soybean dried by different methods and conditions. Food Chemistry 129: 899-906.

Nishiba, Y. and Suda, I., 1998. Degradation of vitamin E, vitamin C, and lutein in soybean homogenate: a comparison of normal soybean and lipoxygenase-lacking (triple-null) soybean. Journal of Agricultural and Food Chemistry 46*:* 3708-3712.

Randhir, R., Kwon, Y.I., Lin, Y.T. and Shetty, K., 2009. Effect of thermal processing on the phenolic associated health-relevant functionality of selected legume sprouts and seedlings. Journal of Food Biochemistry 33: 89-112.

Sakai, N. and Mao, W., 2006. Infrared heating. In: Sun, D.W. (ed.) Thermal food processing: new technologies and quality issues. CRC Press/Taylor & Francis Group, Boca Raton, FL, USA.

Sakthivelu, G., Akitha Devi, M.K., Giridhar, P., Rajasekaran, T., Ravishankar, G.A., Nikolova, M.T., Angelov, G.B., Todorova, R.M. and Kosturkova, G.P., 2008. Isoflavone composition, phenol content, and antioxidant activity of soybean seeds from India and Bulgaria. Journal of Agricultural and Food Chemistry 56: 2090-2095.

Sattler, S.E., Cheng, Z. and DellaPenna, D., 2004. From Arabidopsis to agriculture: engineering improved vitamin E content in soybean. Trends in Plant Science 9: 365-367.

Séguin, P., Turcotte, P., Tremblay, G., Pageau, D. and Liu, W., 2009. Tocopherols concentration and stability in early maturing soybean genotypes. Agronomy Journal 101: 1153-1159.

Shin, D.J., Kim, W. and Kim, Y., 2013. Physicochemical and sensory properties of soy bread made with germinated, steamed and roasted soy flour. Food Chemistry 141: 517-523.

Singleton, V.L. and Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology & Viticulture 16: 144-158.

Sumnu, S.G. and Ozkoc, S.O., 2010. Infrared baking and roasting. In: Atungulu, G.G. and Pan, Z. (eds.) Infrared heating for food and agricultural processing. CRC Press/Taylor & Francis Group, Boca Raton, FL, USA.

Takagi, S., Lenaga, H., Tsuchiya, C. and Yoshida, H., 1999. Microwave roasting effects on the composition of tocopherols and acyl lipids within each structural part and section of a soya bean. Journal of the Science of Food and Agriculture 79: 1155-1162.

Traber, M.G. and Manor, D., 2012. Vitamin E. Advances in Nutrition 3: 330-331.

Xu, B. and Chang, S.K.C., 2008. Total phenolics, phenolic acids, isoflavones, anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. Journal of Agricultural and Food Chemistry 56: 7165-7175.

Yalcin, S. and Basman, A., 2015. Effects of infrared treatment on urease, trypsin inhibitor and lipoxygenase activities of soybean samples. Food Chemistry 169: 203-210.

Yang, H.W., Hsu, C.K. and Yang, Y.F., 2014. Effect of thermal treatments on anti-nutritional factors and antioxidant capabilities in yellow soybeans and green-cotyledon small black soybeans. Journal of the Science of Food and Agriculture 94*:* 1794-1801.

Yoshida, H. and Kajimoto, G., 1989. Effects of microwave energy on the tocopherols of soybean seeds. Journal of Food Science 54: 1596-1600.

Yoshida, H., Matsuda, K., Hirakawa, Y. and Mizushina, Y., 2003. Roasting effects on the distribution of tocopherols and phospholipids within each structural part and section of soybeans. Journal of the American Oil Chemists' Society 80: 665-674.

Žilić, S., Ataç Mogol, B., Akıllıoğlu, G., Serpen, A., Delic, N. and Gökmen, V., 2014. Effects of extrusion, infrared and microwave processing on Maillard reaction products and phenolic compounds in soybean. Journal of the Science of Food and Agriculture 94: 45-51.