A Proposal of Process Conditions for Obtaining Black Soymilk

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Abstract

Black soybeans are known as an herbal and health-food ingredient for hundreds of years in the Eastern Medicine, mainly because of their bioactive compounds, especially anthocyanins. However, little information is available about processing black soybean healthy food products, like soymilk or soy beverages. Preliminary studies showed that black soymilk, made from milled soybean instead of whole grain, presented higher anthocyanins content and antioxidant activity. The objective of this study was to identify the best conditions for black soymilk processing, considering cooking time and process temperature, by using a 2^2 factorial design with three central points. Temperature ranged from 80°C to 98°C and cooking time from 5 to 15 minutes. Anthocyanins (mg/100g) and isoflavones (aglycon equivalent mg/100g) were determined by HPLC systems and antioxidant activities were estimated using 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging and oxygen radical absorbance capacity (ORAC). We concluded that time and temperature were statistically significant ($p \leq 0.05$) for anthocyanins content and antioxidant activity. The 5 min cooking time at 80°C was the best black soymilk processing condition considering all the parameters evaluated in this study.

Keywords: *Black soybean, soymilk processing, antioxidant activity, anthocyanins, isoflavones*

1 Introduction

Black soybeans have been used as a health food and herbal extract for hundreds of years in Oriental medicine (XU and CHANG, 2008a). More recent studies attribute their beneficial health effects to the presence of bioactive compounds (CHENG, LIN and LIU, 2011), especially anthocyanins, present in the black seed coat (HA et al, 2009). Anthocyanins are flavonoids, red or purple color, recognized as health-promoting functional ingredients due to their antioxidant capacity (KOH, YOUN and KIM, 2011).

In general, heating processing promotes modifications in the total phenolic compounds, flavonoids, anthocyanins and the antioxidant activity (XU and CHANG, 2008b). Some studies with black soybean seeds and traditional eastern derivates, as natto and tofu, evaluated the processing conditions and their influence on bioactive compounds profile and antioxidant activity (XU and CHANG, 2008b; KIM et al, 2011). However, there is a lack of information about black soymilk and beverages processing. To better preserve black soymilk's bioactive compounds and antioxidant activity, this study aimed at identifying the best conditions for black soymilk processing, considering cooking time and process temperature, by using a 2^2 factorial design with three central points.

2 Method and Materials

2.1 Black Soybeans

Black soybean line (BRM 09-50995) was obtained from the Soybean Breeding Program for Human Consumption of Brazilian Agricultural Research Corporation (EMBRAPA), harvested in 2014. This line has black seed coat and yellow cotyledon.

2.2 Experiment Design

The combined effect of cooking time (X_1) and temperature (X_2) on the anthocyanins and isoflavones contents and estimated antioxidant activities (DPPH and ORAC methods) of black soymilk was evaluated by using a 2^2 factorial design with three central points, totaling seven randomized experiments (E1= 80°C/5 min; E2= 80°C/15 min; E3= 98°C/5 min; E4= 98°C/15 min; E5= 89°C/10 min; E6= 89°C/10 min; E7= 89°C/10 min).

Temperature (from 80°C to 98°C) and cooking time (from 5 to 15 minutes), as shown in Table 1, were selected based on literature (MORAES FILHO, 2014) and on previous studies for yellow soybeans (FELBERG et al., 2009).

Table 1. Coded levels, temperature and cooking time for 2^2 factorial design of the black soymilk processing conditions

Independent Variables	Coded levels		
	-1	0	1
Time (min)	5	10	15
Temperature (°C)	80	89	98

2.3 Black Soymilk Processing

Selected seeds were washed, dried and grounded in a hammer mill (Perten Laboratory Mill 3100) to increase surface contact. Processing was carried out by cooking milled black soybean with water (1:10; w:w), considering the four different treatments and three replicates at the central point. After cooking, the slurry was homogenized in a Waring® Blender for 2 minutes and centrifuged in a IEC Model K7165 centrifuge, containing a 150 micra nylon filter, at 4000 rpm for 5 min. The seven black soymilk samples obtained were frozen (-18 °C), lyophilized and milled to evaluate the anthocyanins and isoflavones content and estimate the antioxidant activity (DPPH and ORAC).

2.4 Methanolic Extract of Black Soymilk Samples

Methanolic extracts of black soymilk samples were obtained according to Pereira et al. (2014) and used for anthocyanin content analysis and for estimating antioxidant capacity (DPPH and ORAC). One gram of each lyophilized black soymilk sample was extracted twice with extraction solution (methanol/water/hydrochloric acid 60:39:1) in a water bath (IKA HEIZBAD HB-250) at 50°C for 1 hour, homogenizing every 10 minutes by vortexing for 5 seconds. After extraction, the mixture was centrifuged (SORVALL LEGEND Centrifuge XRT) at 12.000 RPM for 10 min at 25°C. The supernatant was completed to 50 mL (volumetric flask) with the extraction solution, filtered through a PTFE membrane CHROMAFIL Xtra – 45/25, pore size: 0.45 µM, filter-Ø: 25 mm and stored in amber bottle at –18°C. Samples were taken in duplicate.

2.5 HPLC Analysis of Anthocyanin Content

Chromatographic analysis was performed according to Santiago et al. (2010), using a Waters® Alliance 2695 system equipped with a Waters® 2996 photodiode array detector. Anthocyanins were separated on a Thermo® Scientific C_{18} BDS (100 mm × 4.6 mm; 2.4 μm) column, using a gradient of acetonitrile and 5% aqueous formic acid as mobile phase at 1 mL/min. Column temperature was set at 30°C. Chromatograms were processed at 520 nm, using Empower® software (Waters). Anthocyanins were quantified by external standardization (SANTIAGO et al., 2010).

2.6 Antioxidant Activity

Antioxidant activity of black soymilk was estimated using Radical DPPH Scavenging Activity assay and Oxygen Radical Absorbing Capacity Assay (ORAC). DPPH assay was run according to the method described by Brand-Williams et al. (1955). Briefly, an aliquot (0.1 mL) of different dilutions of the methanolic extracts was mixed with 3.9 mL of DPPH methanolic solution; deactivation of free radicals was determined after 15 minutes of reaction by absorbance reading at 515 nm using a spectrophotometer. Antioxidant activity was expressed as the amount of extract required to reduce 50% of the initial concentration of DPPH (EC50). The ORAC assay was conducted according to Huang et al. (2002). Black soymilk extracts were diluted 7, 10, 13, 16 and 20 times in phosphate buffer pH 7.4. Initially 25 μL of sample, standard or blank solution was pipetted into a 96-well plate, added 150 μL fluorescein (61.2 nM final concentration) and kept at 37°C for 30 min. Then, 25 μL of AAPH (19.1 mM final concentration) were added to each well and the fluorescence at 528 nm was monitored for 60 min at 37°C. Quercetin was used as a reference antioxidant because of its greater chemical similarity to anthocyanins as compared to Trolox, compound commonly used as reference antioxidant in this method (HUANG et al., 2002).

2.7 Extraction and HPLC Analysis of Isoflavone Content

Extractions and analysis of isoflavone aglycones (genistein, daidzein and glycitein) and glycosides (genistin, daidzein and glycitin) from black soymilk were performed according to AOAC (2005) method 2001.10. Isoflavones identification and quantification were performed by comparison of the investigated peak retention time and UV spectrum with those of the respective standards. Results of total isoflavones were expressed as aglycon equivalents.

2.8 Statistics

Experimental design and statistical analysis were carried out by STATISTICA software, version 12.0 to Windows (StatSoft®). Analysis of variance (ANOVA) and Fisher's test (LSD) were used to verify significant differences ($p \leq 0.05$) in anthocyanins, isoflavones and antioxidant capacity assays DPPH and ORAC means among samples.

3 Results

3.1 Anthocyanin Content

Black soymilk anthocyanins content from different processing conditions ranged (mean scores) from 39.75 to 50.47 mg in 100g of lyophilized samples (Figure 1). Treatments E1 (80°C/5 min) and E3 (98°C/5 min) showed the highest anthocyanins contents as 49.75 mg/100g and 50.47 mg/100g, respectively, and no significant difference between them. Three anthocyanins were detected: delphinidin-3-glucoside, petunidin-3-glucoside and cyanidin-3-glucoside; the last one is the major anthocyanin in black soymilk.

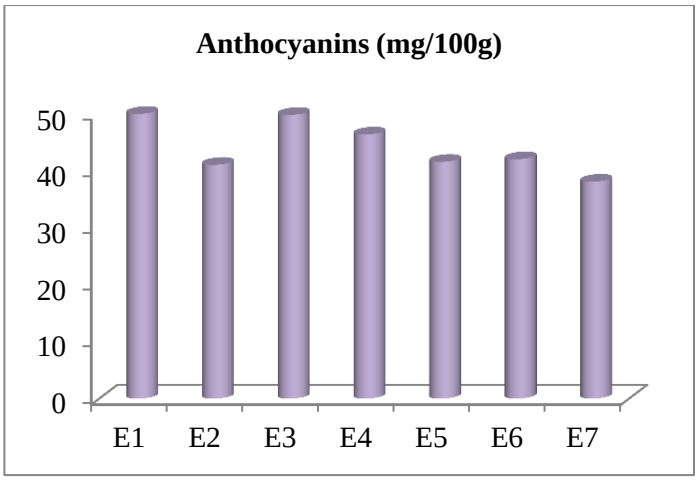

Figure 1. Anthocyanins contents (mean scores) for the seven black soymilk treatments (E1 = 80°C/5 min; E2 = 80°C/15 min; E3 = 98°C/5 min; E4 = 98°C/15 min; E5 = 89°C/10 min; E6 = 89°C/10 min; E7 = 89°C/10 min).

3.2 Isoflavone Content

Four isoflavones (daidzin, glycitin, genistin and daidzein) were identified in the black soymilk. Quantification was based on aglycone equivalents. The total isoflavone content ranged (mean scores) from 138.7 mg/100g (E4 = 98°C/15 min) to 148.7 mg/100g (E7 = 89°C/10 min), respectively (Figure 2).

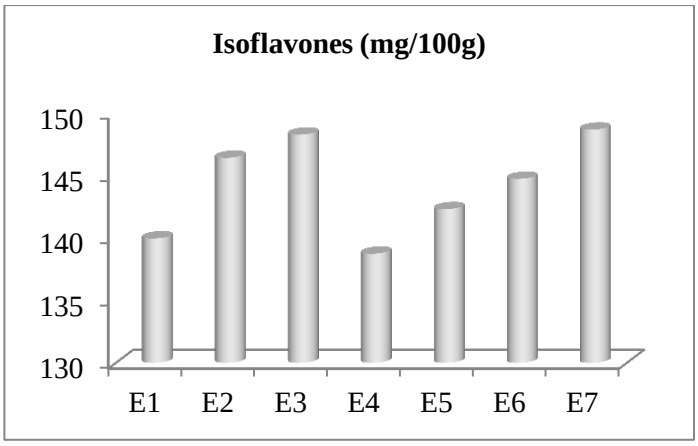

Figure 2. The total isoflavone contents (mean scores), aglycone equivalents, for the seven black soymilk treatments (E1 = 80°C/5 min; E2 = 80°C/15 min; E3 = 98°C/5 min; E4 = 98°C/15 min; E5 = 89°C/10 min; E6 = 89°C/10 min; E7 = 89°C/10 min).

3.3 Antioxidant Activity

Antioxidant activity estimated by DPPH and ORAC assays is presented in Figure 3. The EC50 of DPPH scavenging activity ranged from 0.29 mg/L (E7 = 89°C/10 min) to 0.39 mg/L (E3 = 98°C/5 min). Once DPPH assay results were expressed as EC50, lower values mean higher antioxidant activity. The ORAC values ranged from 0.31 mM (E7) to 0.46 mM (E1 = 80°C/5 min) quercetin equivalent, and E1 showed the higher antioxidant activity.

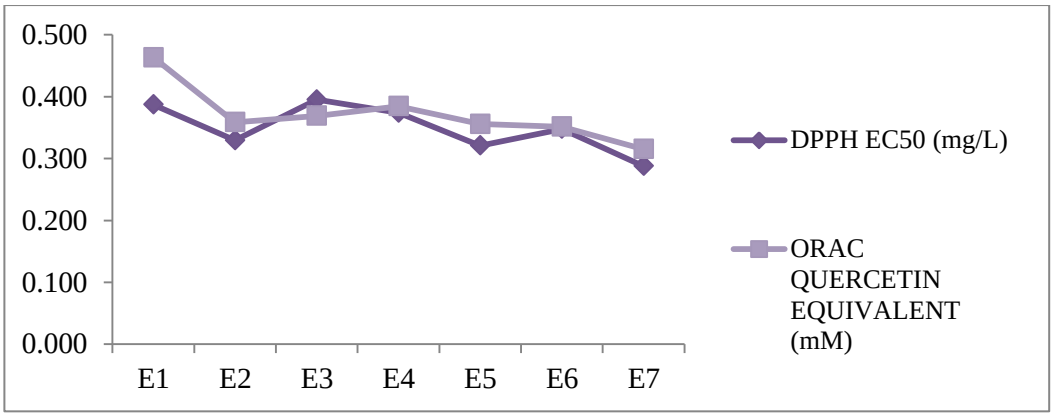

Figure 3. Antioxidant activity estimated by DPPH and ORAC assays for the seven black soymilk treatments (E1 = 80°C/5 min; E2 = 80°C/15 min; E3 = 98°C/5 min; E4 = 98°C/15 min; E5 = 89°C/10 min; E6 = 89°C/10 min; E7 = 89°C/10 min).

4 Conclusions

Considering the anthocyanins contents and ORAC assay results, the 5 min cooking time at 80°C (E1) seemed to be the best black soymilk processing condition. Further research needs to be carried out in order to evaluate pasteurization treatment and to estimate shelf life of the final product.

5 Acknowledgments

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