LWT - Food Science and Technology 84 (2017) 505–510

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Physicochemical and nutritional properties of a healthy snack chip developed from germinated soybeans

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ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form
19 April 2017
Accepted 11 June 2017
Available online 12 June 2017

Keywords:
Germinated soybean
Chip
Nutritional
Physicochemical
Characterization

ABSTRACT

There is a growing interest in non-genetically modified, healthy snacks. Soy snacks are available on the market but snacks from germinated soybean are limited. The objectives of this research were to germinate non-genetically modified soybeans, analyze their physicochemical and nutritional characteristics, and develop a prototype snack chip. Moisture, protein, and lipid contents of flours from soybeans germinated for 1, 3, or 5 d ranged from 2.4 to 5.3, 42.7–43.3, and 21.4–25.5 g/100 g respectively ($P > 0.05$), while lipoxygenase-1 and lipoxygenase-3 activity, and trypsin inhibitor reductions ranged from 2.8 to 17.2, 16.2–26.1, and 16.6–31.1% and estimated glycemic indices ranged from 12.1 to 19.5. These reductions and estimated glycemic indices showed significant differences among the germinated soybeans ($P < 0.05$). The flour made from 5 d germinated soybean resulted in the highest reduction in lipoxygenase-1 and lipoxygenase-3 activities, and trypsin inhibitor content. Five day germinated soybean chips were prepared with varying baking time, thickness, and amounts of baking soda. Based on fracturability, water activity, and color analysis, the optimal conditions were without baking soda, 1.25 mm thickness, and 10 min baking time. In conclusion, this prototype snack chip made from 5-day germinated soybean has the potential as a high nutritional, protein rich, low calorie healthy snack.

1. Introduction

Snacks have become an important part of the daily diet in Western cultures. Consumers with frequent access to energy-dense and low nutrient foods are more likely to be overweight and become obese or diabetic or develop nutrient deficiencies (Gillis & Bar-Or, 2003; Larson, Miller, Eisenberg, & Neumark-Sztainer, 2016; Rennie, Johnson, & Jebb, 2005). Therefore, demand for alternative healthy and good quality snacks is increasing. In general, soybeans have a high protein content (35–40 g/100 g). It has been previously shown that protein content varies little during germination (Bau, Villaume, Nicolas, & Mejean, 1997). On the other hand, lipid contents decrease during germination when the seedling starts using lipids as an energy source; a mature soybean contains about 18 g/100 g oil (Bau et al., 1997). Lipoxygenase catalyzes the oxidation of specific unsaturated fatty acids containing a 1-cis,4-cispentadiene system to oxylipins, resulting in the formation of volatile aldehydes and alcohols through the hydroperoxide lyase pathway causing off-flavors (Mandal, Dahuja, & Santha, 2014). So far six different lipoxygenase isoforms have been identified in soy with L-1, L-2, and L-3 most abundantly present, giving soy products a typical beany flavor (Mandal et al., 2014). On the other hand, trypsin inhibitors present in soy decrease the protein bioavailability and digestibility. However, during soybean germination and processing both enzymes' activities decreases (Bau, Villaume, & Mejean, 2000; Belitz, Grosch, & Schieberle, 2009; Goetz, 2012).

In this study, the physicochemical and nutritional characteristics (moisture, protein, and lipid contents, reductions of lipoxygenase activities and trypsin inhibitor content, and estimated glycemic index) of germinated soybean and the snack chips produced from germinated soybeans were evaluated.

2. Materials and methods

2.1. Materials

Soybean seeds (var. R08-4004/non-genetic modified) were

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http://dx.doi.org/10.1016/j.lwt.2017.06.020
0023-6438/

provided by the Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, U.S.A. All chemicals for analysis were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, U.S.A.) and VWR International (West Chester, PA, U.S.A.).

2.2. Soybean germination

Soybean seeds (50 g) were soaked for 1 h in de-ionized water at 37 °C after which the seeds were washed. The soaked seeds were germinated for 1, 3 or 5 d. The germination was conducted by spreading out the seeds over two layers of wet paper towels on a metal tray and covered with an additional two wet layers and a second metal tray to protect them from light. This was placed in a humidity controlled incubator (model 434304, Hotpack, Philadelphia, PA, U.S.A.) at 27 °C and 100% relative humidity. During germination, the seeds were sprayed with de-ionized water using a plant sprayer to maintain moisture. After germination, the seeds were removed and washed before drying.

2.3. Drying and grinding to flour

The germinated soybeans were dried in an oven (Equatherm 267–914, Curtin Matheson Scientific Inc., NJ, U.S.A.) for 24 h at a constant temperature of 40 °C, ground (Ika Universal Mill M20, Tekmar Company, OH, U.S.A., model A20), and passed through 60 mesh to obtain uniform particle size.

2.4. Moisture content determination

The moisture content of the samples was determined by the approved American Association of Cereal Chemists procedure [AACC] (1990) using aluminum pans and an oven (Equatherm 267–914, Curtin Matheson Scientific Inc., Houston, TX, U.S.A.) at 105–110 °C. Moisture content was calculated using the formula: Moisture (g/100 g) = (water weight/initial sample weight) x 100.

2.5. Protein content determination

A micro-Kjeldahl/nitrogen method was used to determine the soy flour protein content, described by AACC (1990). For protein digestion, soy flour (0.5 g), 7.5 mL concentrated sulfuric acid and a Kjeldahl digestion tablet (CT-37, Kelmate, Darmstadt, Germany) were mixed and heated for 2 h on a digestion heater unit (Labconco 60011, Labconco Corp., Kansas City, MO, U.S.A.). Using a nitrogen distillation unit (Labconco Rapid Still Distillation System, Labconco Corp., Kansas City, MO, U.S.A.), nitrogen from the digested sample was captured as ammoniac in a 4 g/100 mL boric acid receiver solution containing methyl red-bromocresol green as titration indicator. This solution was titrated with 0.025 mol/L HCl. Protein content was calculated on a dry basis, with 6.2 as a conversion factor: Protein (g/100 g) = (HCl volume x 0.025 × 14.007 x 6.2 × 100)/sample weight.

2.6. Lipid content determination

The Soxhlet procedure by the AACC (1990) for lipid content determination was followed. Lipid content was calculated based on triplicate analysis using the equation: Lipid (g/100 g) = (lipid weight/initial sample weight) x 100.

2.7. Lipoxygenase-1 and -3 activity determination

A modified method described by Zhu, Riaz, and Lusas (1996) was followed. A linoleic acid stock solution was prepared using 140 mg linoleic acid, 140 mg Tween 20, and 8 mL de-ionized water. To clarify the solution, 0.55 mL of 1.0 mol/L NaOH was added and the volume was diluted to 50 mL with de-ionized water. Prior to use, the linoleic acid stock solution was diluted (1:40) with sodium borate buffer (0.2 mol/L, pH 9.0) and with sodium phosphate buffer (0.2 mol/L, pH 6.5) for lipoxygenase-1 and lipoxygenase-3 activity determinations, respectively. Lipoxygenase-2 was not included due to its similar activity with lipoxygenase-1 (Ediriweera, Akiyama, & Saio, 1987). Sample solutions were prepared using 50.0 mL sodium phosphate buffer (0.2 mol/L, pH 6.5) and 1.0 g of soybean flour and stirred at room temperature (25 °C) for 2 h. Subsequently, the samples were centrifuged at 15,000 g for 30 min at 20 °C (Model J2-21, Beckman Coulter Inc., CA, U.S.A.). The supernatants (50 μL for lipoxygenase-1 and 10 μL for lipoxygenase-3) were then added with 2.5 mL substrate (linoleic acid) and mixed for 5 s. The absorbance was recorded for 5 min using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) at ambient temperature. The wavelengths for lipoxygenase-1 and lipoxygenase-3 activity determinations were 234 and 280 nm, respectively. Lipoxygenase activity (g/100 g) = (absorbance sample/absorbance control) x 100.

2.8. Trypsin inhibitor determination

A modified method described by AACC (1990) and Stauffer (1990) was followed. A 100 mesh soy flour sample (1.0 g) was mixed with 0.01 mol/L NaOH until pH was 8.4 and stirred for 3 h. Test tubes were filled with 1.4 mL of sample and diluted to 2.0 mL with de-ionized water. An additional tube was also prepared for the trypsin standard with 3.4 mL of de-ionized water. Trypsin solution containing 4 mg trypsin (Porcine pancreas, Sigma) in 200 mL 0.001 mol/L HCl (2 mL) was added to the sample/trypsin standard solution and the tubes were placed in a water bath at 37 °C. To start the reaction, 5 mL of Nα-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPA) solution (40 mg BAPA in 100 mL 0.05 mol/L Tris buffer containing $CaCl_2$, pH 8.2) was added and the reaction was stopped after 10 min incubation by adding 1 mL of acetic acid solution (30 mL glacial acetic acid in 70 mL water). The mixtures were filtered and the absorbance of the filtrates was measured at 410 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) at room temperature. Trypsin inhibitor (TI) content of the soybean was calculated as TI (mg/g soybean) = (absorbance standard − absorbance soybean sample) x dilution factor/19 (Hamerstrand, Black, & Glover, 1981). The reduction of trypsin inhibitor content was calculated by subtracting the TI content of the control determined from non-germinated soybean flours against that of the samples.

2.9. Estimated glycemic index determination

For estimated glycemic index (GI) determination, the protocol described by Goni, Garcia-Alfonso, and Saura-Calixto (1997) was used for triplicate flour samples. Flour samples (50 mg) in 10 mL KCl-HCl buffer (pH 1.5) and 0.2 mL pepsin solution (1 g pepsin from porcine gastric mucosa in 10 mL KCl-HCl buffer) were incubated in a water bath at 40 °C for 1 h for protein digestion. The digested samples were diluted to 25 mL with Tris-Maleate buffer (pH 6.9). Alpha-amylase (2.6 UI α-amylase in Tris-Maleate buffer) was added and the mixtures were incubated again in a water bath at 37 °C. Every 30 min from 0 to 3 h, an aliquot (1 mL) was taken and placed in a water bath at 100 °C for 10 min. Sodium acetate buffer (3 mL, 0.4 mol/L, pH 4.75) and amyloglucosidase (60 μL) were added and the mixture was incubated in a water bath at 60 °C for 45 min, diluted to 5 mL with de-ionized water and centrifuged at 6500 *g* for 5 min (Model 5415C, Eppendorf, NY, U.S.A.). The glucose content in the supernatants was determined by a spectrophotometric method using a glucose assay kit (Sigma, cat. #: GAGO20-1 KT) at 540 nm.