Food Chemistry 136 (2013) 1030–1037

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Antioxidant and antihypertensive properties of liquid and solid state fermented lentils

Maria Inés Torino [a], Rocío I. Limón [b], Cristina Martínez-Villaluenga [b], Sari Mäkinen [c], Anne Pihlanto [c], Concepción Vidal-Valverde [b], Juana Frias [b,*]

[a] CCT CERELA-CONICET, Chacabuco 145, 4000 SM Tucumán, Argentina
[b] Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), Juan de la Cierva 3, 28006 Madrid, Spain
[c] MTT Biotechnology and Food Research, FIN-31600 Joikionen, Finland

ARTICLE INFO

Article history:
Received 13 June 2012
Received in revised form 30 July 2012
Accepted 5 September 2012
Available online 17 September 2012

Keywords:
Lentils
Fermentation
Functional ingredients
Antioxidant capacity
Antihypertensive compounds

ABSTRACT

The effect of liquid (LSF) and solid state fermentation (SSF) of lentils for production of water-soluble fractions with antioxidant and antihypertensive properties was studied. LSF was performed either spontaneously (NF) or by *Lactobacillus plantarum* (LP) while SSF was performed by *Bacillus subtilis* (BS). Native lactic flora in NF adapted better than *L. plantarum* to fermentative broth and BS counts increased 4.0 log CFU/g up to 48 h of SSF. LSF water-soluble fractions had higher ($P \leqslant 0.05$) free amino groups, GABA content, antioxidant and angiotensin I-converting enzyme inhibitory (ACEI) activities than SSF. In addition, GABA and ACEI activity of LSF increased in a time-dependent manner. Proteolysis by BS was limited, with slight changes in free amino groups, while GABA, total phenolic compounds and antioxidant capacity increased throughout fermentation. Higher antihypertensive potential was observed in NF (96 h) characterised by the highest GABA content (10.42 mg/g extract), ACE-inhibitory potency (expressed as IC_{50}) of 0.18 mg protein/ml and antioxidant capacity of 0.26 mmol Trolox equivalents/g extract. Therefore, water-soluble fermented lentil extracts obtained by LSF are particularly promising as functional ingredients in preventing hypertension.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Cardiovascular diseases (CVD) remain the biggest cause of deaths worldwide. More than 17 million people died from CVDs in 2008 (WHO, 2011). In terms of attributable deaths, the leading cardiovascular risk factor globally is raised blood pressure (to which 13% of global deaths are attributed) (WHO, 2009). Implementing population-wide interventions such as promoting physical activity with a healthy diet has been estimated to be a low-cost and highly feasible option to prevent and control CVD. Frequent consumption of legumes, as part of a healthy diet, has been inversely associated with CVD (Bazzano et al., 2001; Flight & Clifton, 2006). Human studies have shown that legume consumption attenuate oxidative stress, improves serum antioxidant capacity and reduces serum concentration of total and low-density lipoprotein–cholesterol, triglycerides, adhesion molecules and inflammatory biomarkers, all of which are risk factors for the development of CVD (Azadbakht et al., 2007; Crujeiras, Parra, Abete, & Martinez, 2007; Esmaillzadeh & Azadbakht, 2012; Taku et al., 2007). These protective effects of legumes against CVD have been related to their nutritional composition (Campos-Vega, Loarca-Piña, & Oomah, 2010). Legumes, besides its high protein, dietary fibre and slow-digesting carbohydrates content are good sources of phenolic compounds such as flavonoids, isoflavones and phenolic acids. Several studies demonstrated that legume proteins and fibre have lipid-lowering effects (Sirtori et al., 2012). Additionally, legume proteins are sources of hypotensive peptides with angiotensin converting-enzyme (ACE) inhibitory activity (Boye & Maltais, 2011). Flavonoids have been reported as dietary modulators of cardiovascular function by regulation of blood pressure (Galleano, Pechanova, & Fraga, 2010), oxidative stress (Cordova, Sumpio, & Sumpio, 2012; Siow & Mann, 2010) and inflammation (Pan, Lai, Dushenkov, & Ho, 2009) in the cardiovascular system.

Lentil (*Lens culinaris*, L.) is among the oldest commodities cultivated by humans with a global consumption steadily increasing. The annual production has increased from 4 million tons (MT) in 2009 to more than 5 MT in 2010 (FAO, 2012). Unlike other legumes, lentil contains higher amounts of total phenolic compounds, saponins and condensed tannins (Campos-Vega, Loarca-Piña, & Oomah, 2010). Moreover, recent studies have shown the potential application of lentil protein hydrolysates as hypotensive ingredients containing angiotensin I-converting enzyme inhibitory peptides (Barbana & Boye, 2011; Boye, Roufik, Pesta, & Barbana, 2010). Therefore, lentils could be considered as a valuable source of cardioprotective compounds.

* Corresponding author. Tel.: +34 912587510; fax: +34 915644853.
E-mail address: frias@ictan.csic.es (J. Frias).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.09.015

Fermentation is an ancient technology for enhancing the shelf-life, nutritional and organoleptic quality of food (Doblado, Frias, Muñoz, & Vidal-Valverde, 2003). Recently, this bioprocess has been applied for the production and extraction of bioactive compounds in the food, chemical and pharmaceutical industries (Martins et al., 2011). In the last years, fermentation has been performed to increase the content of bioactive phenolic compounds in legumes, thus enhancing their antioxidant activity (Fernandez-Orozco et al., 2007; Lee, Hung, & Chou, 2008). Additionally, bioconversion of conjugate forms of phenolic compounds to their free forms during fermentation improves their health-link functionality. For instance, microbial biotransformation of isoflavones to aglycones and equol improved the antiosteoporotic and anti-inflammatory effect of fermented soymilk (Chiang & Pan, 2011; Di Cagno et al., 2010). Moreover, lactic acid bacteria have been employed to produce ACE-inhibitory peptides and γ-aminobutyric acid (GABA) in foods, both useful in the prevention and treatment of hypertension (Kono & Himeno, 2000; Matheson, Freed, & Tunnicliff, 1986; Ricci, Artacho, & Olalla, 2010). In contrast, fermentation has not been extensively applied for production of antihypertensive compounds in legumes, with the exception of soybean (Juan & Chou, 2010).

The objective of the present work was to study the efficiency of liquid (LSF) and solid state fermentation (SSF) of lentil for production of water-soluble fractions with antioxidant and antihypertensive properties. This study has addressed the use of the liquid-fraction that results from LSF, which is generally a by-product in the food industry. This fraction can be collected and concentrated as a source of soluble-containing bioactive products overcoming, at the same time, the environmental problems connected with the dumping. In addition, SSF is an economically favourable fermentation system due to its lower impact on the environment, smaller fermenter-size and, reduced downstream processing and stirring as well as lower sterilisation costs (Hölker & Lenz, 2005; Raghavarao, Ranganathan, & Karanth, 2003).

2. Materials and methods

2.1. Seeds

Lentil seeds (*Lens culinaris* var. *castellana*) were provided by Legumbres Iglesias (Salamanca, Spain). Seeds were cleaned and stored in darkness in polyethylene containers at 4–8 °C.

2.2. Selection criteria and preparation of cultures

Bacillus subtilis CECT 39^T (ATCC 6051) and *Lactobacillus plantarum* CECT 748^T (ATCC 14917) from the Spanish Type Culture Collection (CECT) were selected for SSF and LSF, respectively, based on their GRAS (Generally Recognized As Safe) status and different physiology. *L. plantarum* grows well in the conditions established in LSF (microaerophilic atmosphere, diluted medium) while *B. subtilis* performs well in the conditions established in SSF (aerobic atmosphere, concentrated medium, low water activity). Cultures were stored at −20 °C in 10% (w/v) sterile reconstituted skimmed milk containing 0.5% (w/v) yeast extract (Scharlau Chemie S.A., Barcelona, Spain), 1.0% (w/v) glucose (Sigma, St. Louis, MO) and 10% (v/v) glycerol (Sigma). *B. subtilis* was grown in Brain Hearth Infusion (BHI) broth (Conda S.A. Laboratories, Torrejón de Ardoz, Madrid, Spain) for 16 h at 30 °C. *L. plantarum* was grown in De Man, Rogosa and Sharpe (MRS) broth (Conda S.A. Laboratories) for 16 h at 37 °C.

Bacterial cells were propagated twice (2%, v/v) prior experimental use, recovered by centrifugation (8000 rpm for 5 min at 6 °C) and washed twice in sterile saline solution (0.90% NaCl, w/v). Obtained suspensions were used as inocula for solid or liquid fermentations.

2.3. Lentil fermentation

2.3.1. Liquid state fermentation (LSF)

LSFs were carried out in a New Brunswick 3 L BioFlo/Celligen 115 Fermentor (Eppendorf Iberica, Madrid, Spain) using lentil flour (sieved at 0.5 mm) suspended in sterile distilled water in a proportion of 200 g/L. Fermentations were carried out either spontaneously with the only microorganisms present on the seeds (natural fermentation, NF) or by inoculation of *L. plantarum* suspension (10^8 CFU/ml) at 1–2% (v/v) (LP). LSF were run for 96 h at 37 °C and 350 rpm. Samples were aseptically collected at 0, 48 and 96 h to determine changes in bacterial populations and pH. Afterwards, samples were centrifuged (10,000 rpm for 15 min at 6 °C) and supernatants were freeze-dried for further analysis. LSF was performed in triplicate. Non-fermented samples collected at 0 h were used as negative control.

2.3.2. Solid state fermentation (SSF)

SSF was carried out using cracked lentils (100 g) suspended in sterile distilled water (1:2 w/v) for 16 h at 6 °C, and subsequently autoclaved at 121 °C for 15 min. Sterile cracked seeds were homogeneously inoculated with 5% (v/w) of *B. subtilis* (10^5 CFU/g) saline suspension, vigorously mixed and aseptically distributed over Petri dishes at a ratio of 30 g, as in Fernandez-Orozco et al. (2007). A climatic chamber (Snijders-Scientific, Tiburg, Netherlands) was used to incubate the dishes for 96 h at 30 °C and 90% humidity. SSF was monitored by withdrawing samples at 0, 48 and 96 h to determine changes in bacterial populations and pH. Afterwards, the samples were autoclaved at 121 °C for 15 min and freeze-dried for further analysis. SSF was performed in triplicate. Non-fermented samples collected at 0 h were used as negative control.

2.4. Microbiological analysis

Plate counts method in appropriate agarised media was used to determine viable cells of the following microorganisms: Lactic acid bacteria (LAB) were counted in MRS agar plates after incubation in an 5% CO_2 atmosphere during 72 h; aerobic mesophilic bacteria were grown in Plate-Count Agar containing (w/v) 0.5% tryptone (Conda S.A. Laboratories), 0.25% yeast extract (Scharlau Chemie S.A.), 0.1% glucose (Sigma) and 1.5% agar (Conda S.A. Laboratories), after incubation at 30 °C during 72 h; yeasts and moulds were enumerated on sabouraud chloramphenicol agar (Scharlau Chemie S.A.) after incubation at 25 °C for 5 days; *Enterobacteriaceae* were counted in violet red bile glucose agar (VRBG, Conda S.A. Laboratories) plates incubated at 30 °C for 24 h. Coliforms were determined in violet red bile lactose agar (VRBA, Scharlau Chemie S.A.) plates incubated at 37 °C for 24 h. *B. subtilis* was enumerated in BHI broth supplemented with 1.5% (w/v) agar, plates incubated at 30 °C for 48 h. Cell counts were expressed as $\log_{10}$ CFU/ml.

2.5. Extracts preparation

LSF extracts corresponded to the recovered freeze-dried supernatants after fermentation of lentil flour. The yield after freeze-drying was ~3.5 g extract per 100 ml of supernatant. For LSF extracts, 20 mg were dissolved in 1 ml of distilled water just before analysis. For SSF, 500 mg of freeze-dried SSF-lentils were suspended in 10 ml of cold distilled water and kept overnight in continuous agitation at 4 °C. Afterwards, samples were centrifuged at 15,000 rpm for 20 min at 4 °C and supernatant was collected. The residue was then suspended in 2 ml of cold distilled water, vortexed and centrifuged in the same conditions. The supernatants were collected, fil-

Download English Version:

https://daneshyari.com/en/article/10540485

Download Persian Version:

https://daneshyari.com/article/10540485

Daneshyari.com