Food Chemistry 271 (2019) 291–297

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of liquid-state fermentation on the antioxidant and functional properties of raw and roasted buckwheat flours

Henryk Zieliński, Dorota Szawara-Nowak, Natalia Bączek, Małgorzata Wronkowska*

Department of Chemistry and Biodynamic of Food, Division of Food Sciences, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, 10 Tuwima Str., 10-748 Olsztyn, Poland

ARTICLE INFO

Keywords:
Buckwheat
Fermentation
Advanced glycation end-products
Antioxidants

ABSTRACT

The influence of liquid-state fermentation (LSF) by selected lactic acid bacteria (LAB) and *Rhizopus oligosporus* fungi on the content of rutin and total phenolic compounds (TPC), antioxidant capacity measured by ABTS test, FRAP assay and photochemiluminescence technique, and the inhibitory activity against formation of fluorescent advanced glycation end-products (AGEs) *in vitro* of raw and roasted buckwheat flours was studied.

LSF caused a slight, specific LAB-dependent increase in TPC and a decrease in rutin content. Fermented raw buckwheat flours contained higher amounts of rutin and TPC with one exception when the highest increase in TPC was noted in roasted flour fermented by fungi. A LAB-dependent difference in the antioxidant capacity of buckwheat flours was noted while the inhibitory activity of fermented flours against AGEs formation was generally reduced. It can be concluded that LSF with selected LAB and fungi may improve the antioxidant and functional properties of buckwheat flours.

1. Introduction

Buckwheat is an important crop in some areas of the world which refers to any member of the *Fagopyrum* family (*Polygonaceae*). There are many species of buckwheat, however only common buckwheat (*F. esculentum*) is commonly grown. Although popular in Eastern Europe, buckwheat-based products are unknown in most of the other European countries. Moreover, increasing pressures and demand for wheat grain and recent failures in wheat crops have indicated that alternative cultivations, which may be more resistant to environmental changes and require less intensive cultivation practices, will need to be developed to ensure a more sustainable approach to the production of carbohydrate-rich staple foods. Buckwheat grain is considered as a high nutritional value pseudo-cereal grain because of its high content of vitamin B1 and B2, lysine, protein with balanced amino acid composition, flavonoids, phytosterols, soluble carbohydrates, D-chiro-inositol, fagopyritols and thiamine-binding proteins (Christa & Soral-Śmietana, 2008). Buckwheat is also rich in antioxidant compounds such as flavonoids, phenolic acids, tocopherols, reduced glutathione, inositol phosphates, and melatonin (Zieliński, Michalska, Piskuła, & Kozłowska, 2006). This pseudo-cereal contains more rutin (quercetin-3-rutinoside) than most of plants exhibiting antioxidant, anti-inflammatory and anticarcinogenic properties (Peng et al., 2008).

There is some evidence indicating that the consumption of buckwheat is associated with a wide range of biological and pharmacological activities: antioxidant capacity, as well as hypotensive, antispasmodic, hypocholesterolemic, hypoglycemic, anticancer, anti-inflammatory, and anti-glycation activities (Giménez-Bastida & Zieliński, 2015). Another reason for the development of buckwheat-based products, apart from the anticipated health benefits, is the demand from the gluten-free market. Wronkowska, Zielińska, Szawara-Nowak, Troszyńska, and Soral-Śmietana (2010), Wronkowska, Haros, and Soral-Śmietana (2013) showed that the substitution of gluten-free formula with buckwheat flour increased contents of total proteins, macro- and microelements, and improved the technological quality of bread, like loaf volume and crust porosity. Buckwheat flour significantly improved the overall sensory quality of gluten-free breads (Wronkowska et al., 2010, Wronkowska et al., 2013).

Traditional cereal fermented foods are made of various kinds of cereals or pseudo-cereals, and they are widespread around the world, mainly in developing countries. The fermentation process of the cereal matrix, which leads to degradation of antinutrients, increases the nutritional value and availability of minerals, proteins and carbohydrates (Simwaka, Chamba, Huiming, Masamba, & Luo, 2017). The type of cereal or pseudo-cereal and its chemical composition play a key role in the fermentation process. Based on the source of microbes involved in the fermentation process, there are natural or inoculated fermentations. In addition, fermentation can be divided into solid-state fermentation

* Corresponding author.
E-mail address: m.wronkowska@pan.olsztyn.pl (M. Wronkowska).

https://doi.org/10.1016/j.foodchem.2018.07.182
Received 27 November 2017; Received in revised form 17 April 2018; Accepted 25 July 2018
Available online 26 July 2018
0308-8146/

(SSF) and liquid-state fermentation (LSF) according to the water content in the system. Lactic acid bacteria (LAB) are the main bacteria responsible for the fermentation of cereals or pseudo-cereals. The lactic fermentation of cereals improves food quality through the development of flavour, enhancement of the nutritional value and shelf life, and by removing toxic or antinutritional compounds (Kohajdová & Karovičová, 2007). It is a result of a combination of several factors, with the production of organic acids (lactic and acetic) and pH lowering effect being the most important ones (Baráth, Halász, Németh, & Zalán, 2004).

One of the interesting fermentation processes with the key role played by the culture of strains of *Rhizopus oligosporus* mold fungi, is the tempeh type fermentation. Wronkowska, Honke, and Piskuła (2015) demonstrated that the food products manufactured from non-roasted and roasted buckwheat after fermentation with *Rhizopus oligosporus* fungi were characterized by a higher content of protein, some amino acids, macro- and microelements, and by better protein digestibility as compared to the unfermented ones. The fungi-fermented buckwheat products were positively evaluated in consumer surveys.

Little is known about the effect of LSF on other potentially beneficial traits of food such as content of phenolic compounds, antioxidant capacity and an inhibitory activity against the formation of advanced glycation end-products (AGEs). The latest are known in food as a group of heterogeneous brown and fluorescent cross-linking substances formed via Maillard reaction as well as products of protein glycation in the body. Ames (2009) showed that endogenously formed AGEs are pro-oxidative and pro-inflammatory. According to Ames (2007) it has been suggested that dietary flavonoids may serve as effective inhibitors of advanced glycation end-products (AGE) formation and contribute to the prevention of processes resulting in aging and diabetes complications. Recently, it has been shown that the inhibitory activity of extracts from buckwheat-enhanced wheat bread against AGEs formation in BSA-glucose system depended on the level of buckwheat substitution in bread formula, which clearly indicates the beneficial role of buckwheat flour (Szawara-Nowak, Koutsidis, Wiczkowski, & Zieliński, 2014).

Therefore, the aim of this study was to investigate the effect of liquid-state fermentation by fourteen strains of lactic acid bacteria and one strain of filamentous fungus on contents of phenolic compounds and rutin (quercetin-3-rutinoside), and antioxidant capacity of raw and roasted buckwheat flours, and to verify functional properties of the analyzed flours with special attention paid to their inhibitory activity against advanced glycation end-products formation.

2. Material and methods

2.1. Chemicals

n-Hexane, acetonitrile, and methanol (HPLC-grade) were provided by Merck (Darmstad, Germany). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium azide, bovine serum albumin (BSA), D-glucose, and methylglyoxal (MGO) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). PCL ACW (Antioxidant Capacity of Water-soluble substances) and PCL ACL (Antioxidant Capacity of Lipid-soluble substances) kits for PCL assay were from Analytik Jena AG (Jena, Germany). All other reagents of reagent-grade quality were from POCh, Gliwice, Poland. Water was purified with a Mili-Q-system (Milipore, Bedford, USA).

2.2. Buckwheat flours

Raw buckwheat flour and roasted buckwheat groats originated from Polish commercial common buckwheat (*Fagopyrum esculentum* Moench) purchased from a local industry plant (Melvit S.A., Kruki, Poland). Roasted buckwheat groats were ground in a laboratory mill equipped with screens of different diameter of holes, which resulted in obtaining roasted flour. According to the producer's declaration, contents of carbohydrate, dietary fibre, proteins and fat in buckwheat flour and roasted buckwheat groats were 62 and 69%; 2.3 and 6%; 7.2 and 13%; and 0.7 and 3% of sample dry matter, respectively.

2.3. Fermentation of raw and roasted buckwheat flours

The following fourteen selected lactic acid bacteria strains and a fungus strain were used for fermentation: *L. acidophilus* (145, La5, V); *L. casei* (LcY, 2K); *L. delbrueckii* subsp. *bulgaricus* (151, K); *L. plantarum* (W42, IB); *L. rhamnosus* (GG, 8/4, K); *L. salivarius* AWH, and *Strepcococcus thermophilus* Mk-10, all these strains originated from the Institute of Animal Reproduction and Food Research of Polish Academy of Sciences' collections, except for *Lactobacillus rhamnosus* GG which together with the filamentous fungus *Rhizopus oligosporus* 2710 was purchased from ATCC®.

Pre-treatment of raw and roasted buckwheat flours was performed before the fermentation process as described in detail by Wronkowska, Jeliński, Majkowska, and Zieliński (2018). In brief, about 50 g of flour was suspended in 950 mL of distilled water, heated at 90 °C for 45 min, then autoclaved at 121 °C/15 min and finally cooled to 37 °C.

Fermentation of buckwheat flours was carried out as follows: the 5% suspension of buckwheat flours in distilled water was inoculated with selected strains of lactic acid bacteria or fungi in the amount of 8.00 log cfu/mL or 6.00 log cfu/mL, respectively, and fermentation was performed at 37 °C for 24 h. The pre-treated buckwheat flour not subjected to the fermentation process was used as a control sample. After fermentation, the samples were freeze-dried (Christ – Epsilon 2-6D LSC plus, Germany).

2.4. Determination of contents of rutin and total phenolic compounds (TPC), and antioxidant capacity of fermented raw and roasted buckwheat flours

2.4.1. Extraction

About 100 mg of freeze-dried buckwheat fermented flours was extracted by 30 s sonication with 1 mL of a solution containing 80% MeOH. Next, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged for 5 min (5000 × *g* at 4 °C). This procedure was repeated 5 times and finally the supernatant obtained was collected into a 5-mL flask. The final extract concentration was 20 mg/mL.

2.4.2. Determination of rutin content

The HPLC system (Shimadzu, Kyoto, Japan) consisting of two pumps (LC-10 AD), a UV detector (SPD-10A) set at 330 nm, an autosampler set for 5 µL injection (SIL-10 ADVP), a column oven (CTO-10 ASVP), and a system controller (SIL-10 ADVP) was applied as described in detail by Zieliński et al. (2017). The results were expressed as milligrams of rutin per gram of sample dry matter (d.m.).

2.4.3. Determination of the total phenolic compounds (TPC) content

The buckwheat flour extracts were mixed with 0.25 mL of the Folin-Ciocalteu reagent, 0.5 mL of Na_2CO_3 solution and 4 mL of water, and left for 25 min at room temperature, and then centrifuged at 2000 × *g* for 10 min. A UV-160 1PC spectrophotometer (Shimadzu, Japan) was used to measure the absorbance at 725 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample d.m. (Zieliński et al., 2017).

2.5. Determination of the antioxidant and reducing capacity.

The antioxidant capacity against $ABTS^{\cdot+}$ was measured using a temperature-controlled spectrophotometer UV-160 1PC with CPS-Controller (Shimadzu, Japan). For measurements, the $ABTS^{\cdot+}$ solution was diluted with 80% (v/v) methanol to the absorbance of 0.70 ± 0.02 at 734 nm. The solutions of $ABTS^{\cdot+}$ (1.48 mL) and buckwheat flour extract (20 µL) were mixed for the spectrophotometric assay, then