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The presence of D-fagomine in the human diet from buckwheat-based foodstuffs

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ABSTRACT

Buckwheat (*Fagopyrum esculentum* Moench) groats contain the iminosugar D-fagomine as a minor component that might contribute to the alleged health benefits of this pseudo-cereal. This study presents analysis of D-fagomine in buckwheat-based foodstuffs by liquid chromatography coupled to mass spectrometry and an estimation of its presence in the human diet based on a published population-based cross-sectional nutrition survey. D-Fagomine is present in common buckwheat-based foodstuffs in amounts ranging from 1 to 25 mg/kg or mg/L, it is stable during boiling, baking, frying and fermentation, and it is biosynthesised upon sprouting. The estimated total intake of D-fagomine resulting from a diet that includes such foodstuffs would be between 3 and 17 mg per day (mean for both genders; range from P5 to P95). A diet rich in buckwheat products would provide a daily amount of D-fagomine that may in part explain the beneficial properties traditionally attributed to buckwheat consumption.

1. Introduction

Buckwheat is mainly produced in Russia, China, Ukraine, France and the USA, with 1.9 million hectares of the crop harvested and 1.6 million tonnes produced in 2010 (FAO, 2010). The produce is mainly used for food and livestock feed. Common buckwheat was domesticated and first cultivated in inland South East Asia, possibly as long ago as 6000 BC, and from there it spread to Central Asia and Tibet, and then to the Middle East and Europe (Ohnishi, 1998). Different evidence suggests that buckwheat was introduced early on into areas of present-day Germany, France and Portugal by Slavs and Scythians (Jahns, 2007). Buckwheat was later introduced into North America by Europeans. Nowadays, buckwheat flour is used to prepare different foodstuffs including noodles (Japanese soba, Korean makguksu and Italian pizzoccheri), groats (Polish Kasha), pancakes (French crêpes de Bretagne, Slavic blinis and North American ployes), boiled flour (Italian polenta, and Slovenian and Croatian žganci), fried dough (Spanish farinetes de fajol from Catalonia), beer, cookies, bread and a myriad of other foodstuffs. Buckwheat is considered a healthy food because it is a source of lysine-rich proteins of the globulin kind, which makes it suitable for both general consumers and coeliacs, and because it contains functional components such as polyphenols (rutin and flavanols) and fagopyritols that may help to regulate blood sugar and prevent insulin resistance (Ortmeyer, Larner, & Hansen, 1995; Steadman et al., 2000; Watanabe, 1998; Wijngaard & Arendt, 2006). One such component is D-fagomine; first isolated from seeds of buckwheat in 1974 (Koyama & Sakamura, 1974).

D-Fagomine (Fig. 1) is an iminocyclitol, also referred to as an iminosugar. As D-fagomine and other iminosugars are intestinal glycosidase inhibitors, they have the potential to modulate postprandial blood glucose concentration (Asano, Oseki, Tomioka, Kizu, & Matsui, 1994). Therefore, they are useful in reducing the risks of developing insulin resistance and of becoming overweight. While most iminosugars are only found in exotic plant sources used for ornamental and/or medicinal purposes, D-fagomine is the only iminosugar known to be part of a traditional food source (Asano et al., 2005). Together with other iminosugars, such as 1-deoxinojirimicin (DNJ), D-fagomine is also found in edible mulberry extracts (Asano et al., 2001). We recently reported that D-fagomine performs a double action during its transit along the intestinal tract. First, it effectively delays sucrose and starch breakdown with the consequence of lowering blood glucose concentration; and second, it selectively agglutinates fimbriated *Entero bacteriaceae*, such as *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, and

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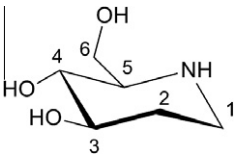

Fig. 1. Chemical structure of D-fagomine.

promotes the adhesion of probiotic bacteria to the intestinal mucosa (Gómez et al., 2012). This activity suggests that D-fagomine may contribute significantly to the healthy properties associated with buckwheat and makes D-fagomine a particularly interesting new candidate dietary and functional food ingredient. D-Fagomine has been quantified in buckwheat and other sources but to the best of our knowledge never determined in foodstuffs (Asano, Nash, Molyneux, & Fleet, 2000; Koyama, & Sakamura, 1974; Ruiz-Matute, Hernández-Hernández, Rodríguez-Sánchez, Sanz, & Martínez-Castro, 2011). Because the previously available analytical procedures did not separate isomeric species such as 3,4-di-*epi*-fagomine and therefore may have overestimated the amount of D-fagomine, we proposed a new procedure that conveniently separates D-fagomine from its main diastereomers by analytical cation-exchange chromatography coupled to mass spectrometry (Amézqueta et al., 2012). Here we report the application of this new procedure to the determination of D-fagomine in foodstuffs. We also study the stability of D-fagomine during the elaboration of different buckwheat-based foodstuffs and determine its presence in the final products. The data is then used to estimate the daily intake of D-fagomine by a population with a diet that incorporates buckwheat-based products as the main source of carbohydrates.

2. Materials and methods

2.1. Food samples

The commercial beer was Keks from Gbech (Girona, Spain); commercial cookies were from Can Rovira (Batet de la Selva, Spain); commercial dry noodles were from Luz de Vida – BioSpirit (Celrà, Spain) and from Nikkoku Seifun (Matsumoto, Japan); and the commercial bread was from Le pain des Fleurs – Euronat (Peaugres, France). Buckwheat flour and buckwheat groats were purchased from El Granero Integral (Madrid, Spain).

2.2. Food production

2.2.1. Cookies

Buckwheat flour (450 g), sugar (sucrose, 250 g), butter (249 g), eggs (127 g) and vanilla sugar (10 g) were thoroughly mixed. From 457 g of this mixture, 10 portions were placed on a tray and baked at 220 °C for 15 min to obtain 10 cookies (380 g total final weight).

2.2.2. Bread

Fresh yeast (25 g) was reconstituted in warm water (120 mL). Buckwheat flour (500 g), gluten (30 g) and salt (9 g) were mixed together. Then, water (180 mL), butter (19 g) and the reconstituted yeast were consecutively added to and mixed with the rest of the ingredients. The dough was then kneaded on a surface, sprinkled with buckwheat flour (18 g), put into a damp cloth and left to rise until it doubled in size (90 min). A portion of the dough (189 g) was kneaded again, shaped into a round bread form, scored and left to rise again until it doubled in size (60 min). Then, this portion of dough was put on a tray and baked for 30 min at 230 °C to obtain a loaf (170 g).

2.2.3. Farinetes

Buckwheat flour (50 g) and water (300 mL) were put into a saucepan and mixed. The temperature was increased until the mixture boiled with continuous stirring to avoid the formation of lumps. Stirring was maintained for 8 min after the mixture begun to boil. The dough (274 g) was then poured into a rectangular mould covered with plastic film and cooled in a fridge (4 °C). Once cold, the hard dough (125 g) was divided into 3 portions and fried in olive oil until crisp (90–120 s). The total weigh of the farinetes was 45 g.

2.2.4. Buckwheat sprouts

Buckwheat groats were rinsed with water, put into a container to soak in water for 2 h, then strained and left to germinate in the dark at room temperature (20–25 °C) for 3, 5 and 8 days. Twice a day, the groats were rinsed with water. Seed swelling was observed after 1 day and germination after 2 days.

2.2.5. Buckwheat beer mashed with added enzymes

Buckwheat groats (10 kg) were milled in an MXAS continuous milling/sieving device from Iberital (Sant Feliu de Llobregat, Spain). Then, a portion (3 kg) was mixed with water (9 L) at 55 °C in the presence of a cocktail of three different enzymes from Novozymes (Bagsvaerd, Denmark): BAN 240 (α-amylase, 24 g), Neutrase 0.8 (proteinase, 3 mL) and Viscoflow MG (a mixture of β-glucanase, xylanase and a wide range of carbohydrase enzymes, 0.45 g). The temperature was steadily raised to 70 °C over 30 min and the mixture was kept at 70 °C for a further 30 min. Then, the temperature was lowered to 55 °C. Next, two more enzymes were added to break the dextrins: Promoenzyme BrewQ (pululanase, 4 mL) and AMG 300 BrewQ (amyloglucosidase, 10 mL) also from Novozymes and a wort was obtained that was kept at 55 °C for 60 min. Then, the wort (7 L) was passed through a 0.5-mm mesh, the solid residue was rinsed with water (3 L) at 55 °C and the filtrate was added to the wort. An aliquot of the wort (3 L) was boiled for 15 min at 100 °C. The supernatant was separated from the sediment by decantation to obtain boiled wort (2.1 L) that was inoculated with dehydrated yeast Safale US-05 (Fermentis, Marcq-en-Baroeul, France) (0.8 g). Fermentation then took place in a thermostatic chamber at 14 °C for 10 days. The sediment was discarded and the product bottled.

2.2.6. Buckwheat beer mashed with malt

A portion of milled buckwheat groats (1.5 kg), obtained as described immediately above, was mixed with 1.2 kg milled *pale* barley malt (Mas Malta Cervecera, Sant Miquel de Balenyà, Spain) and 8.1 L water at 40 °C. The mixture was left to stand for 30 min at 40 °C. Then, the temperature was raised to 60 °C and the suspension was kept at this temperature for 30 min (to promote carbohydrate breakdown by β-amylases) after which the temperature was raised to 70 °C for a further incubation of 30 min (to promote carbohydrate breakdown by α-amylases). The wort obtained (7 L) was passed through a 0.5-mm mesh, the solid was rinsed with water (3 L) at 55 °C and the filtrate added to the wort. The final steps were exactly as described immediately above.

2.2.7. Noodle boiling

Commercial buckwheat dry noodles (16 g) were boiled in 74 g of water for 4 min, strained (37 g of water left) and allowed to cool down (35 g final weight). Another 8 g of buckwheat dry noodles were boiled in the water left from the previous operation (37 g) for 4 min, strained (7 g of water left) and allowed to cool down (20 g final weight).

2.3. Analytical reagents and solutions

A D-fagomine standard was obtained following a published method (Castillo et al., 2006; Clapés, Joglar, Castillo, & Lozano,