Food Chemistry 239 (2018) 679–687

Contents lists available at ScienceDirect

Food Chemistry

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

Exploitation of alfalfa seed (*Medicago sativa* L.) flour into gluten-free rice cookies: Nutritional, antioxidant and quality characteristics

Gianluca Giuberti [a,*], Gabriele Rocchetti [a], Samantha Sigolo [a], Paola Fortunati [a], Luigi Lucini [b], Antonio Gallo [a]

[a] Institute of Food Science and Nutrition, Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29121 Piacenza, Italy
[b] Institute of Environmental and Agricultural Chemistry, Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29121 Piacenza, Italy

ARTICLE INFO

Article history:
Received 24 April 2017
Received in revised form 27 June 2017
Accepted 2 July 2017
Available online 3 July 2017

Keywords:
Alfalfa seed
Gluten-free
Starch digestibility
Antioxidant activity
Cookies

ABSTRACT

In an effort to increase the nutritional value of common gluten-free (GF) cereal-based foods, GF cookies using alfalfa seed flour (ASF), at different substitution levels to common rice flour (0% as control, 15%, 30% and 45% w/w), were produced. Crude protein, total dietary fibre, total polyunsaturated, total *n*-3 and *n*-6 fatty acid contents increased linearly ($p < 0.05$) by raising the substitution levels of rice with ASF. The hardness, the total phenolic content, the *in vitro* antioxidant capacity and the resistant starch increased linearly ($p < 0.05$), whereas the starch hydrolysis index decreased linearly ($p < 0.05$) by raising the substitution levels of rice flour with ASF. Despite the fact that ASF-substituted GF cookies had inferior sensory attributes compared to the control, the score given by the panellists remained at fairly good levels for all tested parameters, showing acceptability of the substituted GF cookies.

1. Introduction

Nowadays, the popularity of gluten-free (GF) cereal-based food products is increasing, as they not only cater to clinically diagnosed celiac patients, but also to consumers who seek to exclude gluten-containing proteins from their diet (Pellegrini & Agostoni, 2015). Therefore, the food industry has responded by improving its offer with new formulas of cereal-based GF foods. However, several indications suggested that GF-rendered foods are characterised by lower dietary fibre and resistant starch (RS) contents along with higher glycemic index (GI) than their gluten-containing equivalents (Foschia, Beraldo, & Peressini, 2017; Pellegrini & Agostoni, 2015). In addition, a lower protein content in GF compared to non-GF food products has been reported (Wu et al., 2015). Obtaining GF-rendered foods as nutritious as their gluten-containing counterparts is therefore a challenge and extensive research has been conducted to investigate the preparation of a new generation of cereal-based GF foods formulated with several value-enriched ingredients (Foschia et al., 2017). Consequently, a large number of starches and flours, in combination with non-gluten protein sources, hydrocolloids, gums, novel ingredients and technological treatments have been investigated aiming to improve the overall nutritional, functional, acceptability and technological properties of GF food categories (Jnawali, Kumar, & Tanwar, 2016).

Alfalfa (*Medicago sativa* L.) is a crop belonging to the family of Leguminosae and it represents one of the most cultivated forage legumes and one of the most economically valuable crops throughout the world (Graham & Vance, 2003). Due to its nutritive value, alfalfa (lucerne) is considered a valuable crop for livestock and can be safely consumed also by humans as edible seeds, sprouts or as protein concentrates (EFSA, 2009). Dried alfalfa seeds lack of gluten protein and, compared to refined wheat flour, they contain higher protein, fat and crude fibre contents (Ullah et al., 2016). Higher concentrations of polyunsaturated fatty acids (PUFA), especially essential fatty acids such as linoleic acid (C18:2 omega 6) and linolenic acid (C18:3 omega 3) along with higher vitamin (riboflavin, E and C) and mineral (Fe) contents have also been reported comparing dried alfalfa seeds to dried wheat seeds (Márton, Mandoki, & Csapó, 2010; Plaza, de Ancos, & Cano, 2003). In addition, the total phenolic content of alfalfa seeds was found to be comparable to that of green tea, rosemary and grape seed extracts (Bhojak et al., 2006). Lastly, even if limited human studies are present, indications suggested that adding dried alfalfa seeds to the diet on a daily basis could help to normalise serum cholesterol concentrations in patients with type II hyperlipoproteinemia due to the action of saponins (Mölgaard, von Schenck, & Olsson, 1987).

However, the potential role of alfalfa seed flour (ASF) in the formulation of GF cereal-based foods has not been fully addressed in the scientific literature. Therefore, evaluating the possible use of ASF as a value-enriched ingredient would allow for the

* Corresponding author.
E-mail address: gianluca.giuberti@unicatt.it (G. Giuberti).

http://dx.doi.org/10.1016/j.foodchem.2017.07.004
0308-8146/

development of GF baked products with improved nutritional values. Within this perspective, cookies, being one the largest categories of ready-to-eat foods worldwide consumed, could represent a potentially nutritious GF snack through the selection of ingredients. Therefore, the present study was conducted to evaluate the chemical composition, the physical and textural characteristics and the antioxidant properties of GF cookies prepared by replacing commercially available rice flour with increasing levels of ASF. Newly developed GF cookies were also evaluated for their nutritionally important starch fraction contents (rapidly digestible starch, RDS; slowly digestible starch, SDS; resistant starch, RS) along with their *in vitro* starch digestibility potential. Sensory analysis was also conducted, as the substitution of base flours in baked product formulation might impair sensory characteristics.

2. Materials and methods

2.1. Preparation of gluten-free cookies

The commercial rice flour was purchased from IpaFood s.r.l. (Roma, Italy), whereas commercially-available organic dried alfalfa seeds were acquired from Superfood Market Online Ltd (West Yorkshire, UK) and milled in a laboratory mill equipped with a 1-mm screen (Retsch grinder model ZM1; Brinkman Instruments, Rexdale, ON, Canada). All other ingredients (food grade) were acquired from local supermarkets and stored at different conditions depending on their individual requirements till further use. The chemical composition of rice flour and ASF is presented in Table 1.

For control cookies (CTR), the recipe was based on rice flour (120 g), whole egg (80 g), tap water (30 g), unsalted butter (20 g), salt (1.0 g) and GF baking powder (2.0 g). For experimental GF cookies, part of rice flour equivalent to 15%, 30% and 45% w/w was replaced with ASF to formulate 15-CK, 30-CK and 45-CK GF cookies, respectively. For all formulations, no sugar was added to limit the amount of glycemic carbohydrates. Briefly, butter was creamed, mixed with liquid ingredients and then added to dry ingredients. Materials were amalgamated with a domestic blender (Kitchen Aid, Model K5SSWH, St. Joseph, Mich., U.S.A.) for 5 min at intermediate speed to obtain homogeneous dough. The dough was laminated by a pasta roller attachment at about 0.4 cm height, allowed to rest for 30 min at 4 °C, followed by cutting with a circular mould (4 cm diameter) and baked in a household oven (RKK 66130, Rex International, Italy) at a temperature of 180 ± 4 °C for 17 ± 2 min. Once baked, all GF cookies were cooled and kept in separate airtight plastic bags at room temperature until analysis. For each recipe, three batch replicates were produced on the same day.

2.2. Chemical composition of gluten-free flours and cookies

Proximate composition analyses were performed according to AOAC (2000) for dry matter (DM; method 930.15), ash (method 942.05), crude protein (method 976.05) and crude lipid (method 954.02 without acid hydrolysis) contents. Enzymatic quantifications of total, soluble and insoluble dietary fibre (Megazyme assay kit K-INTDF 02/15, which includes RS and non-digestible oligosaccharides as a component of total dietary fibre), total starch (Megazyme assay kit K-TSTA 07/11) and free sugars (Megazyme assay kit K-SUFRG 06/14) were carried out using specific assay kits and following manufacturer's procedure. Analyses were conducted for both GF flours and GF cookie (ground through a 1-mm screen) batches.

Table 1
Chemical composition (on a dry weight basis) of gluten-free (GF) flours.

Parameters	Rice flour	Alfalfa seed flour
Moisture (g water/100 g flour)	8.01 ± 0.03	6.92 ± 0.03
Total starch (%)	81.12 ± 0.10	14.71 ± 0.07
Crude protein (%)	6.93 ± 0.08	37.59 ± 0.11
Crude lipid (%)	1.11 ± 0.04	7.23 ± 0.08
Ash (%)	0.43 ± 0.02	3.74 ± 0.03
Total dietary fibre (%)	1.62 ± 0.09	26.22 ± 0.12
Soluble dietary fibre (%)	0.51 ± 0.05	6.71 ± 0.09
Insoluble dietary fibre (%)	1.11 ± 0.06	19.51 ± 0.11
Free sugars (%)	0.54 ± 0.02	2.31 ± 0.03

Data are presented as mean values ± standard deviation (n = 2, being analytical replicates).

For CTR and experimental GF cookies, the preparation of the fatty acid methyl esters (FAME) was conducted according to the direct method described by O'Fallon, Busboom, Nelson, and Gaskins (2007). The fatty acid composition of the FAME was determined using a Shimadzu 2025 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with an auto-sampler (model AOC-20 s, Shimadzu), an auto-injector (model AOC-20i, Shimadzu), a flame ionization detector, and a CP-Select CB capillary column for FAME (100 m × 0.25 mm i.d.; 0.25 μm film thickness; Chrompack, Varian, Inc., CA). The injection volume was 1 μl in split mode (split ratio 30:1) and the carrier gas was hydrogen with a constant flow of 1.5 ml/min. The injector and detector temperatures were kept at 250 °C. The column oven temperature was programmed following the procedure of Prandini, Sigolo, Tansini, Brogna, and Piva (2007) with minor modifications: 60 °C for 2 min, from 60 to 170 °C at 10 °C/min for 35 min, and from 170 to 240 °C at 4 °C/min for 9.5 min. Peak identification was possible with the aid of reference standards (Supelco 37 component FAME mix; conjugated octadecadienoic acid; Sigma Chemical Co, St. Louis, MO). Data were expressed as a percentage of total fatty acids, calculated with peak areas corrected by factors according to AOAC 963.22 method (AOAC, 2000). For each treatment, batches were analysed in triplicate.

2.3. Physical and textural characteristics of control and experimental cookies

Diameter, thickness and spread ratio were calculated as reported by Sharma, Saxena, and Riar (2016), whereas the colour of CTR and experimental GF cookies was measured on the basis of CIE L^* (lightness), a^* (redness-greenness) and b^* (yellowness-blueness) colour system using a Minolta CR410 Chroma Meter (Konica Minolta Co., Japan). Five readings were taken for each batch.

Hardness analysis was performed with a TA-XT2i Texture Analyser (Stable Micro Systems, UK) fitted with a shape blade-cutting probe. The crosshead speed was 10 mm/s, data were acquired with a resolution of 500 Hz and a 5-kg load cell was used. For each batch, 5 experimental GF cookies along with CTR were tested. Texture Export Exceed Release 2.54 (Stable Micro System) was then used to acquire the maximum peak force to snap GF cookies (hardness) expressed as fracture force (Sharma et al., 2016).

2.4. Determination of total phenolic content and antioxidant capacity

From each batch of CTR, 15-CK, 30-CK and 45-CK cookies, 3 sample replicates (1.0 g each) were extracted in a 10 ml of 1% formic acid in 80:20 methanol:H_2O solution (LCMS grade, VWR, Milan, Italy) using an Ultra-turrax (Ika T25, Staufen, Germany). Extracts were centrifuged at 7000 rpm for 10 min at 4 °C, and proteins overnight precipitated by adding 5% TCA solution (BioUltra from Sigma Aldrich, Milan, Italy) from the supernatant at −18 °C. The resulting solutions were filtered using 0.22 μm cellulose syringe filters and collected in amber vials.

The total phenolic contents (TPC) were determined colorimetrically according to the Folin-Ciocalteu assay (Rocchetti et al., 2017).