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Tracking celiac disease-triggering peptides and whole wheat flour quality as function of germination kinetics

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ABSTRACT

Germination is already a well-accepted process by consumers with many products made from sprouted seeds or containing limited amounts of flour form sprouted grains. The present work aimed assessing the usefulness of germination in reducing gluten peptides associated with celiac disease, at the same time evaluating some technological features of the obtained germinated wheat. In the first part of the work, celiac disease (CD)-triggering peptides were tracked as a function of germination kinetics (from day 1 to day 6). Using simulated gastrointestinal digestion and liquid chromatography coupled to mass spectrometry, ten celiac disease triggering peptides were identified: seven peptides presumably involved in the adaptive immune response (TI) and three peptides mainly involved in the innate immune response (TT). All the identified peptides belonged to gliadins. TI track pattern showed three phases: the first two days displayed a significant degradation, a stability phase was observed from day 3 to day 5, and finally a drastic reduction occurred on the 6th day. For TT peptides, important degradation was exclusively observed at the 6th day. In the second part, some techno-functional features of germinated whole wheat flour were assessed to estimate its potential as an alternative to conventional flour. Functionality comparison of the non-germinated versus germinated flours revealed that germination significantly influenced solvents retention capacities as well as swelling and solubility. Thus, with a reduced amount of celiac disease triggering peptides, but also with different technological behavior compared to traditional wheat flour.

1. Introduction

Celiac disease (CD) is one of the most common autoimmune enteropathies worldwide, and its incidence is increasing, but the reasons for this are largely unclear (Shewry & Tatham, 2016). Currently, the only available treatment for CD is a strict and permanent gluten-free (GF) diet. Despite of the many progresses made in improving the organoleptic and nutritional quality of GF foods (which are much ameliorated in the last years), there are still some nutritional issues. Being GF foodstuffs based on starch, some of them are nutritionally poor and/or unbalanced particularly for what concerns minerals and vitamins (Botero-López et al., 2011; Do Nascimento, Fiates, dos Anjos, & Teixeira, 2014; Salazar et al., 2015). Therefore, fortification with legumes and pseudo cereals is commonly adopted to improve the nutritional aspect of GF foods (O'Shea, Arendt, & Gallagher, 2014). Additives and enzymes are required to build the product structure, as an alternative to gluten network. Commonly, starches are used in GF formula as principal ingredient because of their gelling, thickening, adhesion, moisture-retention, stabilizing, film forming, and texturizing properties (Houben, Höchstötter, & Becker, 2012, Höchstötter, & Becker; Horstmann, Lynch, & Arendt, 2017). Hydrocolloids are also used to act as polymeric substances that should increase the dough viscosity and gas-retaining ability (Sabanis & Tzia, 2011). Enzymes with cross-linking potential might be integrated, as well, to ensure the generation of bonds among protein chains (Heck, Faccio, Richter, & Thöny-Meyer, 2013).

GF diet is increasing considerably across consumers with medical needs as well as consumers believing that GF products are a healthier than gluten-containing products (Capriles, dos Santos, & Areas, 2016). Indeed, a recent report of Mintel (2015) revealed a gluten-free food category growth of 136% between 2013 and 2015, reaching $11 billion in the USA.

Intensive research has been made to obtain celiac-safe wheat. Genetic manipulation (e.g. transgenic wheat) allowed 97% lower gliadin content (Gil-Humanes et al., 2014). Such product is not

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marketed given the opposition to genetically manipulated products in Europe. Several processing also have been proposed, such as the use of microbial transglutaminase (Mazzeo et al., 2013), sourdough lactobacilli (Rizzello et al., 2007) and enzymes from germinating grains (Stenman et al., 2009). However, the elimination of gluten might induce several flaws in the product in terms of dough attributes, nutritional characteristics, and consumer acceptance (Naqasha, Ganib, Adil, & Masoodia, 2017). In the case of bread made from wheat flour, prefermented with a pool of selected lactobacilli and fungal proteases, gluten was degraded below 20 ppm and bread retained comparable texture and flavor to those of traditional wheat sourdough bread (Di Cagno et al., 2004; Rizzello et al., 2007). Although gluten was degraded from 6280 ppm to 1045 ppm, pasta with freeze-dried fermented semolina sourdough had better stickiness and firmness than GF pasta (De Angelis et al., 2010; Di Cagno et al., 2005). Germination enabled almost 50% reduction in gluten peptides associated with CD, and the incorporation of germinated wheat flour up to 2.5% in bread had enhanced leavening properties, dough development, specific volume and crumb softness during storage (Boukid, Prandi, Buhler, & Sforza, 2017; Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017; Marti, Cardone, Pagani, & Casiraghi, 2018). Despite its effectiveness in gluten degradation, germination received little attention. Proteases in germinating seeds are stable and highly active (Wieser & Koehler, 2012). They also act on the whole polypeptide chains, which make them more efficient in gluten degradation, whereas fungal or microbial peptidases act only near the ends of polypeptide chains (Scherf, Wieser, & Koehler, 2016). Thus, germination is a solvent free, simple, low-cost and green process that may decrease the amount of gluten peptides able to trigger an immune response in CD patients. Understanding the impact of germination kinetics on these peptides and investigating some quality features are mandatory to assess the suitability of these flours for foodstuffs-making. For this purpose, simple predictive tests such as solvent retention capacities (SRC) and swelling/solubility profiling were used. SRC is a quick and a simple method for predicting the gluten quality and overall baking functionality (Kweon, Slade, & Levine, 2011; Kweon, Slade, Levine, & Gannon, 2014). According to the AACC Method 56-11.02. (AACC International, 1999), lactic acid SRC is associated with glutenin characteristics, sodium carbonate SRC with the level of damaged starch, sucrose SRC with pentosane characteristics and water SRC with all flour constituents. Swelling and solubility also allowed to verify the attitude of starch to gelatinize at different temperatures (50–90 °C). Therefore, the present work has a dual objective. Firstly, the fate of gluten epitopes associated with CD was studied as a function of germination kinetics, and consequently immunogenic and toxic peptides were tracked (up to 6 days) in germinating durum wheat, using *in vitro* digestion and liquid chromatography coupled to mass spectrometry. SRC, swelling and solubility profiles were determined on non-germinated and germinated whole wheat flours.

2. Materials and methods

2.1. Plant material and germination trials

Seeds from six durum wheat (*Triticum durum Desf.*) varieties, representative of those most widely cultivated in Italy during the 20th century, were chosen for the experiments, and they are reported in Table 1.

Germination trials were conducted on homogeneous durum wheat seeds (150 g) for six days at 25 °C. The seeds were imbibed in distilled water (450 mL) for 48 h (5 h in water, 19 h without water; twice) at humidity 100%. Then, seeds were decanted and placed on dishes (150 × 150 × 50 mm) on two layers of Whatman no.1 filter paper disks (moistened with 50 mL) and they were allowed to germinate in a growth chamber (temperature 25 °C, humidity 100%) up to 6 days in the dark, and daily washed with distilled water (100 mL, twice a day). Germination started after 1 day of steeping. Almost all the kernels germinated after two days, and consequently the sampling started since day-2 until day-6, sampling every day. Non-germinated kernels (day-0) were used as control. Germination was stopped by pouring liquid nitrogen onto the grains and then samples were crushed and lyophilized. Samples were then finely ground in a laboratory mill (Ika Werke, Staufen, Germany) and sieved (mesh size 160 μm) to obtain whole wheat flours that were stored in plastic bags at −20 °C prior to analysis. Germination trials were performed twice (n = 2) for each sample.

Table 1
Pedigree and year of release of the studied wheat genotypes (De Vita et al., 2010).

Genotypes	Pedigree	Year of release
Duilio	Cappelli × (Anhinga × Flamingo)	1984
Simeto	Capeiti 8 × Valnova	1988
Iride	Altar 84 × Ionio	1996
Svevo	Cimmyt line × Zenit	1996
Claudio	(Cimmyt selection × Durango) × (IS1938 × Grazia)	1998
Dylan	Neodur × Ulisse	2002

2.2. Gluten peptides surviving the in vitro gastrointestinal digestion

2.2.1. Simulated gastrointestinal digestion procedure

In vitro gastrointestinal digestion was conducted following the standard method of Minekus et al. (2014). In brief, 1 g of sample was incubated for 2 min with 1 mL of simulated saliva, containing amylase from porcine pancreas (75 U/mL of digesta); then, 2 mL of simulated gastric juice containing pepsin from porcine pancreas (2000 U/mL of digesta) were added and the sample was incubated for 2 h, after adjusting the pH to 3. Subsequently, 4 mL of duodenal juice containing pancreatin from porcine pancreas (100 U trypsin activity/mL of digesta) and porcine bile (10 mmol/L in the total volume) were added and the sample was incubated for 2 h at 37 °C under constant gentle mixing, after adjusting the pH to 7. For both non-germinated and germinated whole wheat flour, simulated gastrointestinal digestion was carried out twice (n = 2). Then, to inactivate the enzymes, the sample was boiled for 10 min at 95 °C. After centrifugation (3220 *g*, 4 °C, 45 min), 295 μL of each sample supernatant was added to 5 μL of internal standard solution (TQQPQQPF(d_5)PQQPQQPF(d_5)PQ).

2.2.2. UPLC/ESI-MS analysis

For the quantification of the gluten peptides generated during the digestion, UPLC-MS analysis was performed according to the method of Prandi et al. (2014). Briefly, the two independent replicates (n = 2) obtained from *in vitro* digestion were analyzed using a RP column (ACQUITY UPLC BEH 300, C18, 1.7 mm, 2.1 × 150 mm; Waters, Milford, MA, USA) equipped with a VanGuard Pre-column (ACQUITY UPLC BEH C18, 130 Å, 1.7 μm, 2.1 × 5 mm, Waters, Milford, MA, USA) in a UPLC/ESI-MS system (Acquity Ultra-performance UPLC with a single quadrupole mass spectrometer SQD, Waters Milford, MA, USA) using a gradient elution. Eluent A is a bi-distilled water solution with 0.1% formic acid (> 99%) and 0.2% acetonitrile, and eluent B is an acetonitrile solution with 0.1% formic acid (> 99%). Gradient elution was carried out as follows: 0–7 min 100% eluent A; 7–50 min from 100% to 50% eluent A; 50–52.6 min 50% eluent A; 52.6–53 min from 50% to 0% eluent A; 53–58.2 min 0% eluent A; 58.2–59 min from 0% to 100% eluent A; 59–72 min 100% eluent A. The samples were analyzed with UPLC/ ESI-MS in the Full Scan mode. Flow was 0.2 mL/min; analysis time 72 min; column temperature 35 °C; sample temperature 18 °C; injection volume 2 μL; acquisition time 7–58.2 min; ionization type was positive ions; scan range 100–2000 *m/z*; capillary voltage 3.2 kV; cone voltage 30 V; source temperature 150 °C; desolvation temperature 300 °C; cone gas flow 100 L/h; desolvation gas flow 650 L/h.