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Effect of sourdough fermentation and baking process severity on dietary fibre and phenolic compounds of immature wheat flour bread

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ABSTRACT

This work aimed to study the interaction of sourdough fermentation based on a mixed culture of *Lactobacillus plantarum* 98a, *Lactobacillus sanfranciscensis* BB12, *Lactobacillus brevis* 3BHI with baking process (210–250 °C x 10–20 min) on dietary fiber and phenolic compounds content of bread obtained from immature (milky) and fully ripe flours of modern durum wheat and ancient KAMUT® khorasan wheat whole grains. The fiber in bread was analyzed by enzymatic digestion and the total dietary amount was dependent on genotype and kernel maturation stage. Although insoluble dietary fiber content ranging between 10.26 and 19.25 g/100 g dry base was generally more correlated to KAMUT® khorasan, the soluble fraction (always less than 3.83 g/100 g dry base) was associated to the durum wheat genotype. KAMUT® khorasan bread at fully ripe maturation fermented with sourdough, maximized insoluble dietary fiber content. Sourdough fermentation slightly increased the free flavonoids content while the total flavonoids (obtained by spectrophotometrically analysis) seemed mainly related to each genotype considered (ranged between 39.01 and 56.53 mg/100 g dry base) and its maturation stage. A whole chain strategy based on a combination of agronomic and processing factors was suggested to enhance specific bioactive compounds such as dietary fiber or phenolic compounds.

1. Introduction

Many scientific studies have been done on health benefits of whole grains cereals due to the fact that they are rich sources of various bioactive compounds such as dietary fiber (arabinoxylans, β-glucans, fructans, lignans), vitamins, minerals and phenolic compounds (Lattimer & Haub, 2010).

Consumption of soluble dietary fiber (SDF) reduces postprandial glucose responses after carbohydrate-rich meals, as well as lowers total and LDL cholesterol levels (Chater, Wilcox, Pearson, & Brownlee, 2015). However, the consumption of insoluble dietary fiber (IDF) and whole grains is consistently correlated with reduced risk of type 2 diabetes in large prospective cohort studies (de Munter, Hu, Spiegelman, Franz, & van Dam, 2007).

On the other hand, phenolic compounds have several protective and physiological functions in human health. They have antioxidant properties and protect against degenerative diseases like heart diseases and cancer in which reactive oxygen species i.e., superoxide anion, hydroxyl radicals and peroxyl radicals are involved (Saura-Calixto, 2011).

Whereas consumers are looking for functional and healthy foods, the food industry is looking for processes and technologies which can be used to increase the bioavailability of endogenic bioactive compounds in these foods. New ingredients or/and new formulations may enrich or fortify standard wheat bread and thereafter increase their functional characteristics. For example Benedetti et al. (2012) demonstrated that bread obtained by KAMUT® khorasan, an ancient wheat cultivar, protected rats from oxidative stress. Moreover, KAMUT® khorasan wheat bread obtained by sourdough fermentation process increased its ability to provide that protection (Gianotti et al., 2011) mainly due to its role in increasing the levels of easy-extractable phenolic compound. All those changes resulted in a diversification of gut microbiota and metabolome in healthy volunteers (Saa et al., 2014).

Depending on the kind of process and microorganism species, microbial fermentative activity in contact with dough components

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can enhance the level of several bioactive compounds having nutraceutical properties (Dey, Chakraborty, Jain, Sharma, & Kuhad, 2016; Ferri, Serrazanetti, Baldissarri, Tassoni, & Gianotti, 2016; Babini, Tagliazucchi, Martini, Dei Più & Gianotti, 2017)

The sourdough fermentation can influence the concentration and bioavailability of bioactive compounds found in whole grain cereals (Gianotti et al., 2011; Hassani, Procopio, & Becker, 2016). In fact the interactions between microorganisms and their environment generate important biotransformation of the dough ingredients whose effects can confer functional properties to the baked goods (Guerzoni et *al*., 2011). Besides the improvement of baking quality (texture, flavor, color etc.) sourdough fermentation acts both by polymerization (EPS), solubilization of healthy compounds (free ferulic acid, etc.) and consequently, by increasing their bioavailability. Finally, lactic acid bacteria (LAB) sourdough may reduce certain allergens and antinutritional phytate compounds.

Moreover thermal treatments can change the ratio between insoluble–soluble fiber, total dietary fiber content and consequently their physicochemical properties due to the changes of surface properties related to their interaction with water and oil (Elleuch et al., 2011) and, consequently, changing the effects of enzymatic digestion. On the other hand, the different polyphenol compounds may have a different thermal loss as a consequence of time and temperature during baking.

The aim of this work is to study the combined effect of sourdough fermentation and baking process intensity on dietary fiber and phenolic compounds of bread formulated by flours obtained from immature flours (milky and fully ripe) of modern durum wheat and ancient KAMUT® khorasan whole grains. The sourdough fermentation was based on a mixed culture of *Lactobacillus plantarum* 98a, *Lactobacillus sanfranciscensis* BB12, *Lactobacillus brevis* 3BHI.

2. Materials and methods

2.1. Flour

KAMUT® khorasan and durum wheat grain (cv. Claudio) were obtained from the Department of Agricultural Sciences, University of Bologna (Italy). KAMUT® is a registered trademark of Kamut International, Ltd. and Kamut Enterprises of Europe, bvba. Wheat samples were grown at the same location during the same growing season and cropped according to the biodynamic agro-technique. Grains were collected at the milky (75–79 BBCH scale; 15 d after anthesis) and full ripe maturity stages (89 BBCH scale). Wheat samples were air dried until the 12 g/100 g moisture was reached and stone milled (100 g/100 g flour extraction). Wholemeal flours were characterized for the total protein content according to the Kjeldhal procedure (N x 5.7) and for the dietary fiber and phenolics content using the procedures detailed below. Both milky and fully ripe stage flour were used for two types of fermentations and baking at two different temperatures.

2.2. Strains and growth media

Lactobacillus plantarum 98a, *Lactobacillus sanfranciscensis* BB12, *Lactobacillus brevis* 3BHI and *Saccharomyces cerevisiae* LBS strain were selected on the basis of their suitability to increase the quality of bakery products (Cevoli, Gianotti, Troncoso, & Fabbri, 2015). Those strains belong to the Department of Agricultural and Food Science and Technology (DISTAL) of the University of Bologna (Italy). LAB strains were grown separately in the de Man Rogosa Sharpe (MRS) broth (Oxoid, Milan, Italy) at 37 °C for 24 h and the *Saccharomyces cerevisiae* strain was grown in the yeast extract peptone dextrose (YPD) Broth at 28 °C for 24 h. The cells have been harvested by centrifugation at 4000*g* for 10 min, and washed twice with sterile water.

2.3. Fermentation and baking processes

The industrial fermentation, considered as the reference bread samples, was based just on yeast fermentation. This control bread (conventional or standard fermentation commonly adopted for commercial bakery products), was compared to the sourdough bread based on lactic acid bacteria.

To prepare sourdough (SOUR) 600 g of KAMUT® khorasan and durum wheat flour was gently mixed with 270 mL of water, inoculated with 80 mL of each strain separately grown and incubated at 30 °C for 24 h.

The inoculums level in dough was approximately 4×10^8 CFU/g in dough for LAB and 3×10^6 CFU/g for *Saccharomyces cerevisiae*.

For the industrial fermentation (IND) which is the reference sample, the KAMUT® khorasan and durum wheat doughs were prepared with 600 g of flour and 270 mL of water inoculated with 180 mL of *Saccharomyces cerevisiae* (inoculums level 3×10^6 CFU/g) and incubated at 30 °C for 1.5 h.

The fermented doughs were baked for two different times at two different temperatures:

- Long time/high temperature (250 °C for 20 min)
- Short time/low temperature (210 °C for 10 min)

2.4. Analysis of dietary fibre

Samples of flours and bread were enzymatically digested using α-amylase, protease and amyloglucosidase, allowing the determination of IDF and SDF amounts as previously described by Di Silvestro et al. (2012) using the Megazyme assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland).

2.5. Determination of phenolic compounds

The extraction of the free (FP) and bound phenolic (BP) compounds from flours and bread was performed as previously described (Dinelli et al., 2011). The gallic acid and catechin calibration curves were used as standard for polyphenols and flavonoids, respectively.

2.6. Statistical analysis

Results were calculated from the mean of at least two replicates and expressed in g/100 g of dry base. Factorial ANOVA was used to evaluate interrelationships between the amount of all the bioactive compounds of durum wheat and KAMUT® khorasan wheat based upon the maturation stage (milky and fully ripe), fermentation (sourdough and industrial) and baking (high temperature and low temperature) in breads. These analyses were performed using "R" software, version 3.0.1.

3. Result

Results of the characterization of the whole meal flour obtained from milky and fully ripe grain are shown in Table 1. On a dry weight basis, the highest protein level was observed for the milky whole meal of KAMUT® khorasan wheat (13.4 ± 0.1 g/100 g). The difference between the two maturity stages had a lower extent in the durum wheat variety, although statistically significant. Milky grain flour evidenced also a high amount of IDF in both wheat species as compared to the fully ripe flour (Table 1). This result is