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The role of oxygen in the liquid fermentation of wheat bran

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ABSTRACT

The extensive use of wheat bran as a food ingredient is limited due to its bitter taste and hard texture. To overcome these, some preprocessing methods, such as fermentation with yeast and lactic acid bacteria or enzymatic treatments have been proposed. The current work studied microbial communities, acidification, ethanol formation and metabolite profile of wheat bran fermented in either aerated or anaerobic conditions. In aerated conditions, yeasts grew better and the production of organic acids was smaller, and hence pH was higher. In anaerobic conditions, lactic acid bacteria and endogenous heterotrophic bacteria grew better. Aeration had a large effect on the sourdough metabolite profile, as analyzed by UPLC–qTOF-MS. Anaerobic conditions induced degradation of ferulic and caffeic acids, whereas the amount of sinapic acid increased. Aeration caused degradation of amino acids and hydroxycinnamic acid derivatives of polyamines. The results suggest that the control of oxygen could be used for tailoring the properties of bran sourdough.

1. Introduction

The food industry is facing a need to produce new ingredients and foods with added value, to respond to both increased consumer awareness of food healthiness and the need to exploit raw materials in an economically sustainable way. Cereal bran is of interest here, and could provide a useful base for the manufacture of foods aiming at increased delivery of health promoting compounds (Delcour, Rouau, Courtin, Poutanen, & Ranieri, 2012). However, some of the most important limits to the extensive use of bran as a food ingredient are its strong taste and hard texture. To overcome these challenges, pretreatment of bran with mechanical and bioprocessing techniques, such as fermentation with yeast and lactic acid bacteria and/or enzymatic treatments have been proposed (Anson et al., 2009; Coda et al., 2014; Delcour et al., 2012; Katina et al., 2007). Bioprocessing has been shown to improve the breadmaking properties of bran, leading to higher bread volume and improved crumb softness (Coda et al., 2014; Katina et al., 2007).

Fermentation with yeasts and lactic acid bacteria has a long history in breadmaking (Poutanen, Flander, & Katina, 2009). Due to superior sensory quality and the prolonged shelf-life of the resulting baked goods, sourdough processes have retained their importance in modern baking technology (Chavan & Chavan, 2011; De Vuyst & Neysens, 2005; Katina et al., 2005). During fermentation several changes occur in the matrix. The decrease in pH affects the activity of endogenous enzymes, such as proteases, amylases and phytases, leading to further modifications of the cereal and especially the dietary fibre (DF) matrix and its compounds. Moreover, phytochemicals of the cereal matrix can be released by the sourdough microorganisms and enzymes, and their bioavailability may, thus, be enhanced (Anson et al., 2009; Mateo Anson et al., 2011). It has been previously shown that bioprocessing of wheat bran can significantly improve the bioaccessibility of phenolic acids from wholemeal breads in the intestine and at the same time enhance the colonic release and conversion of phenolic acids to their metabolites (Anson et al., 2009). Phenolic compounds are directly related to the nutritional and sensory characteristics of food. Therefore their presence in the diet is beneficial to health, and can contribute to obtain a specific flavour (Shahidi & Naczk, 2003). Modification of flavour precursors to flavour active compounds is an example of one of the metabolite changes during sourdough fermentation (Hansen, 2005). The precursors are more intense in whole wheat flour than in refined wheat flour, which suggests that the flavour precursors are concentrated in the outer layers of the grain (Czerny, 2002). However, the phenolic constituents of the cereal matrix can also influence the growth and metabolism of lactic acid bacteria (Rodriguez et al., 2009; Sekwati-Monang, Valcheva, & Gänzle, 2012). Besides phenolic compounds, alcohols, esters,

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aldehydes and carbonyls are also an important constituent of flavour (Hansen, 2005). Amino acid degradation to odour active metabolites is one important flavour-forming reaction catalysed by yeast and lactic acid bacteria during sourdough fermentation (Czerny, 2002).

As a consequence of the industrialisation of the baking process, the demand for faster, more efficient, controllable and large-scale sourdough fermentation has resulted in the development of semi-fluid silo preparations, known as type II sourdoughs, since they are a useful tool for tailoring bread quality aiming at a product with specific properties (Carnevali, Ciati, Leporati, & Paese, 2007; De Vuyst & Neysens, 2005). However, to guarantee the reproducibility of fermentation on an industrial scale, proper selection of the starter microorganisms is required, as well as control of the fermentation pattern (Carnevali et al., 2007).

In industrial sourdough fermentations, aeration is not strictly controlled, while it is well known that the metabolism of both lactic acid bacteria and yeasts is sensitive to the amount of oxygen (Barber, OrtolÃ¡, Barber, & FernÃ¡ndez, 1992; Serrazanetti, Guerzoni, Corsetti, & Vogel, 2009). More studies are thus needed to elucidate the interactions between the starter cultures, substrate matrix and processing conditions in sourdough fermentations, and to further clarify how all these parameters can affect the quality of the final product.

The aim of the current study was to assess the role of oxygen in fermentation-induced changes during the bioprocessing of liquid wheat bran sourdough, including metabolism of the microbiota and subsequent metabolite formation.

2. Materials and methods

2.1. Materials

Commercial wheat bran (Fazer Mills, Lahti, Finland) was ground by TurboRotor technology (Mahltechnik Görgens GmbH, Dormagen, Germany) to a median particle size of 160 μm. Enzymes used in fermentations 3 and 4 were Depol 740l (cellulose 1 nkat/ml, xylanase 10,464 nkat/ml and β-glucanase 7197 nkat/ml) (Biocatalyst Ltd., UK) and Grindamyl A1000 (α-amylase 120700 nkat/g) (Danisko, Denmark). The yeast used in all fermentations was *Kazachstania exigua* (VTT C-81116) and the lactic acid bacteria starter used in fermentations 2 and 4 was *Lactobacillus brevis* (VTT E-95612).

2.2. Bran fermentations

Four different liquid fermentations (P1 = yeast, P2 = yeast + lactic acid bacteria (LAB), P3 = yeast + enzymes and P4 = yeast + LAB + enzymes) containing bran and water in 10:90 ratio and an initial inoculum of ca. 10^6–10^7 cfu/ml for both the starter cultures was employed, were made as duplicates under two different aeration conditions. The fermentations were performed in 1 l BIOSTAT® QPlus bioreactors (Sartorius Stedim Biotech GmbH, Germany) in a controlled environment. Aeration was controlled by a flow of either nitrogen or oxygen into the bottom of the fermentor in order to create anaerobic and aerated conditions, respectively. Preparation of the sourdoughs and process parameters can be found in Supplementary material, Tables 5 and 6. Samples (285 g) were taken at 0, 12 and 24 h time points. Microbiological analyses were made from fresh samples and the rest of the samples were freeze-dried (Lyovac GT 2, Steris GmbH, Germany or Epsilon 2-25DS, Christ, Germany) and milled (Bamix, Switzerland and Retsch ZM 200, Retsch GmbH, Germany) to pass through a 0.5 mm sieve prior to further analysis.

2.3. Acidification parametres of bioprocessed bran

Acidity (pH and total titratable acids (TTA)) of bioprocessed bran was determined from a suspension containing 5 g of bran sample and 50 ml of distiled water in an automatic titrator (Mettler Toledo titrator T50). After pH measurement the suspension was titrated with 0.1 M NaOH to a final pH of 8.5. The TTA value was expressed as the amount of NaOH used in millilitres per 10 g of sample. The content of lactic- and acetic acid in samples was determined from 10 g of the sample by using specific enzymatic kits for lactic- and acetic acid (Boehringer Mannheim GmBh, Mannheim, Germany).

2.4. Microbiological analyses

Microbial growth was determined using a plate count technique. The fresh samples were diluted into Ringeŕs solution and plated as follows: yeast and moulds on yeast and mould (YM) plates (Difco Laboratories, US), lactic acid bacteria on Man, Rogosa and Sharps (MRS) plates (Oxoid, UK) and aerobic heterotrophic bacteria on plate count agar (PCA) plates (Difco Laboratories, US). YM plates were incubated in aerobic conditions at 25 °C. Chlortetracycline (0.01%) and chloramphenicol (0.01%) were added to the medium in order to prevent bacterial growth, and 0.02% Triton-X 100 (BDH, UK) was used to limit the spreading of fungal colonies. MRS plates were incubated in anaerobic conditions at 30 °C and the PCA plates in aerobic conditions at 30 °C. Both the MRS and the PCA media were supplemented with 0.001% cycloheximide (Sigma, US) to prevent fungal overgrowth of bacterial colonies. The counting of colony forming units (CFU) was performed after a minimum of 48 h of incubation. The results were expressed in CFU/g of sample. All analyses were done in duplicate.

Microbiological community analysis was done using 300 mg of the freeze-dried and ground samples by using polymerase chain reaction denaturing gel gradient electrophoresis (PCR-DGGE). The pure cultures of *K. exigua* and *L. brevis* were analysed in parallel with the samples. Genomic DNA extraction was done with a FastDNA® Spin Kit for Soil (Q-Biogene, USA) according to the manufacturer's instructions, with a modification that the samples were homogenised four times with a FastPrep-24® cell disrupter (MP Biomedicals) at 6.0 m/s for 30 s. PCR-DGGE analysis with universal bacterial primers U968-f-GC and U1401-r, amplifying a V6–V8 variable regions of the bacterial 16S rRNA gene, was essentially performed as described by Laitila, Kotaviita, Peltola, Home, and Wilhelmson (2007). The fungal-specific primers U1GC and U2 were used to amplify a region of the 28S rRNA gene. The fungal PCR reaction mixture (25 μl) contained 2 μl of the template DNA, 10 pmol of both primers U1GC and U2, 0.4 mM of deoxynucleoside triphosphate mix, 1 unit of DynaZyme hot start polymerase (Finnzymes, Finland), and reaction buffer with 2.5 mM of $MgCl_2$. The PCR program consisted of an initial denaturing at 94 °C for 10 min, followed by 40 cycles (60 s at 94 °C, 60 s at 51 °C, 45 s at 72 °C) and a final extension for 5 min at 72 °C. DGGE was performed with a DCode™ Universal Mutation Detection System (BioRad, US). Bacterial PCR products were loaded onto a 38–60% gradient of urea and formamide, and a 35–60% gradient was used for the fungal PCR products. The electrophoresis was carried out at a constant temperature of 60 °C at 200 V for 5–10 min and at 85 V for 16 h. Gels were stained with SYBRGreen (Molecular Probes, Netherlands) and documented with a GelDoc2000 system (BioRad, US). For identification, distinct bands were excised from the bacterial DGGE gels, mixed with 36 μl of sterile water and homogenised. Samples were incubated for 1 h at 80 °C and eluted overnight at 4 °C. Reamplification was done as described above. Bacterial PCR products were purified and sequenced by Macrogen Europe (Netherlands)