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Effect of *Lactobacillus brevis*-based bioingredient and bran on microbiological, physico-chemical and textural quality of yeast-leavened bread during storage

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The effects of wheat bran and of a *Lactobacillus brevis*-based bioingredient (LbBio), obtained after growth in flour-based medium, on quality of yeast-leavened wheat bread (WWB) were investigated. Bran was used in bread formulation by substituting a part (20 g/100 g) of white wheat flour (WBB), while LbBio was used instead of the water content (WWB + LbBio and WBB + LbBio). The use of LbBio in WWB resulted in the biological acidification of the dough due to lactic, phenyllactic and OH-phenyllactic acid contents determining a high fermentation quotient value and an improved bread texture and microbiological quality. Conversely, wheat bran reduced the specific volume and crumb hardness during storage at 25 °C, and affected the antibacterial ability of LbBio during 30 °C storage. Our findings demonstrated that LbBio counteracted the negative effects of bran and allowed to obtain an enriched fibre bread with specific volume and soft crumb comparable to bread without bran.

Industrial relevance: Bread is a perishable food with a short microbiological and physico-chemical shelf-life. The main microbiological alteration occurring into few days after baking is the "rope spoilage" caused by spore-forming bacteria originating from raw materials. This phenomenon, often misinterpreted as a sign of unsuccessful dough leavening and not visible from outside, is more common under industrial production conditions during the hot season causing large economic losses in the warm climates of Mediterranean countries, Africa and Australia. The use of sourdough often controls this alteration even if the industrial application of this traditional process is limited by the long leavening times. In this study, an innovative procedure for the preparation of yeast-leavened bread comprising the addition of a fermentation product from *Lactobacillus brevis* grown in a flour-based medium has been applied. The resulting fermentation product (LbBio bioingredient) acts as a sourdough acidifying the dough and improving the textural, physico-chemical and microbiological properties of the resulting bread. The application of bioingredient LbBio could represent an innovative strategy in industrial bread production to obtain acidified yeast-leavened products, thus, preventing the ropy spoilage and reducing the negative effects of bran addition.

1. Introduction

Bread is one of the principal components of the human diet, but it generally undergoes staling process and microbial contamination within few days from its production. In particular, after baking, bread can be spoiled by moulds (mainly *Penicillium* and *Aspergillus* species) and heat resistant spore-forming bacteria, naturally occurring in raw materials and foods of vegetable origin, and surviving to the cooking process (Rosenkvist & Hansen, 1995). A recurrent microbiological issue for bakery industries is represented by the ropy spoilage mainly associated to the presence of spores of *Bacillus* species in raw materials (Pepe, Blaiotta, Moschetti, Greco, & Villani, 2003; Valerio et al., 2012). The spores of these microorganisms survive in the central part of baked bread, where the temperature values reach up to 97–101 °C for some minutes. Even though the spore-forming loads are very low in flour (about 2.0 log spores/g), the baking process and the subsequent storage conditions of bread (temperature ≥ 25 °C, water activity ≥ 0.95, pH > 5) favour the germination of heat-resistant spores and their increase in total viable counts up to about 7.0 log CFU/g in bread crumb within 2 days, causing the loss of freshness and worsening of the bread quality (Rosenkvist & Hansen, 1995; Viedma, Abriouel, Omar, López, & Gálvez, 2011). As recently demonstrated (Valerio et al., 2012), flour and other raw materials (brewer yeast, improvers, etc.) used to make bread are contaminated by a great variety of spore-forming bacteria mainly belonging to the genus *Bacillus* and which include also potential toxigenic species (*Bacillus cereus* group). Recently, some authors (De Jonghe et al., 2010) demonstrated the ability of *Bacillus amyloliquefaciens* species, a common bread contaminant, to produce heat-labile cytotoxic

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substances and a heat-stable cytotoxic component. Generally, microbial contamination levels of bread higher than 5 log CFU/g are associated to the onset of spoilage process and the elevated risk of foodborne illness when the causative agent is a toxigenic species (Kramer & Gilbert, 1989).

Spore-forming bacteria are naturally occurring in soil and contaminate wheat and other cereal grain flours. In particular, bran, arising from the outer part of the grain kernels, contains a higher content of different microorganisms than those found in endosperm flours; among these microbes, the spore-forming bacteria could be related to the bread spoilage over its storage (Rosenkvist & Hansen, 1995).

In certain cases, bran has been used to replace part of the formulated flours in order to increase the dietary fibres (DF) content in the bakery products, since wheat bran typically contains approximately 45% of dietary fibre, of which about 95% is non-soluble fibre (Cornell & Hoveling, 1998; Pomeranz, 1988). Nevertheless, health claims related to the ability of wheat bran fibres to increase faecal bulk and to reduce intestinal transit time were accepted (EFSA, 2010) for labelling food high in fibres in agreement to the European Regulation (EC) No 1924/2006. Besides DF, other compounds concentrated in the outer layers of the grains, such as oligosaccharides and phytochemicals, are gaining more and more importance in improving the nutritional and functional quality of bread (Chavan & Chavan, 2011).

Furthermore, wheat bran-based flour blend can affect the rheological properties of bread dough (Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006a) and final bread quality attributes that are consistent with reduction in volume, increase in crumb firmness, and change in flavour (Chavan & Chavan, 2011; Laurikainen, Härkönen, Autio, & Poutanen, 1998). In bread-making, these drawbacks are usually overcome by adding commercial enzyme mixtures or fermented wheat bran to the dough during bread-making (Damen et al., 2012; Katina et al., 2007; Laurikainen et al., 1998). Well studied is also the application of sourdough that, owing to fermentation by *Lactobacillus sanfranciscensis*, *Lactobacillus brevis* and *Lactobacillus plantarum*, efficiently affects bread crumb properties and controls moulds and bacterial spoilers (Coda et al., 2011; Corsetti & Settanni, 2007; Gerez, Torino, Obregozo, & Font de Valdez, 2010; Katina, Heiniö, Autio, & Poutanen, 2006b; Katina, Sauri, Alakomi, & Mattila-Sandholm, 2002; Lavermicocca et al., 2000; Niku-Paavola, Laitila, Mattila-Sandholm, & Hikara, 1999; Pepe et al., 2003; Sjögren, Magnusson, Broberg, & Schnürer, 2003; Ström, Sjögren, Broberg, & Schnürer, 2002; Valerio, De Bellis, Lonigro, Visconti, & Lavermicocca, 2008; Wang, Yan, Wang, Zhang, & Qi, 2012). Recently, some authors (Komlenić. et al., 2010) observed a modification of the rheological properties of wheat flour dough, and in particular, a reduction of bread hardness and an increase in specific volume after the addition of biological (*L. brevis* preferment and sourdough) acidifiers. In fact, the fermentation of dough with LAB enhances the level of organic acids that was reported to be involved in the reduction of dough mixing time and in a significant desirable weakening of dough (Delcour & Hoseney, 2010). The resulting bread has greater volume, lower density, softer crumb and higher slice height (Arendt, Ryan, & Dal Bello, 2007). On the other hand, a moderate dough acidification also enhanced wheat flour proteinase activities that, at the optimal pH values (3.8–4.1), influence the extensibility of gluten and the final quality of bread (Schober, Dockery, & Arendt, 2003; Thiele, Gänzle, & Vogel, 2002). The beneficial effect of LAB observed in sourdough can be obtained by the addition of LAB-derived acidifiers, even if their effects on physico-chemical, textural and microbiological quality of yeast-leavened bread formulated with wheat bran, needs to be further investigated. Thus, the aim of the current work was to apply a *L. brevis*-based bioingredient in yeast-leavened wheat bread containing bran to improve the final quality of enriched fibre bread.

2. Materials and methods

2.1. Bacterial cultures

L. brevis LMG P-25726 was isolated from sourdough and deposited in the Belgian Coordinated Collections of Microorganisms (BCCM/LGM, Gent, Belgium). For long-term storage, stock cultures were prepared by mixing 8 mL of a culture with 2 mL of Bacto glycerol (Difco, Becton Dickinson Co., Sparks, MD, USA) and freezing 1 mL portions of this mixture at −80 °C. Culture was stored frozen (−80 °C) in MRS broth (Oxoid LTD, Basinstoke Hampshire, England) supplemented with 20% Bacto glycerol (Difco) and subcultured twice before use.

2.2. LbBio bioingredient preparation

The bioingredient (LbBio) was prepared inoculating 5 mL of an overnight (37 °C, 150 rpm) *L. brevis* LMG P-25726 culture in a flour-based medium obtained by a mixture of white wheat flour (100 g), water (500 mL), demineralised whey powder W714 (5 g) (Profile™ 90, Kerry Ingredients, Listowel, Ireland) and fructose (5 g) and incubated at 37 °C, 150 rpm for 18 h. The final product was combined, instead of water amount, with ingredients of wheat bread according the formulations reported in Table 1. As a control the flour-based medium (FBM) incubated in the same conditions (37 °C, 150 rpm, 18 h) but not inoculated with the *L. brevis* strain LMG P-25726, was used.

2.3. Bread production

The bread formulation was optimised and standardised within the European Seventh Framework Programme project DREAM (Design and development of realistic food models with well characterised micro- and macro-structure and composition, http://dream.aaeuropae.org/AboutDREAM/tabid/56/Default.aspx). All ingredients were mixed and cooked in a kneading machine (Princess® Home Breadmaker, type 1936; Princess Household Appliance BV, Breda, Netherlands). Bread types were prepared according to a standard recipe as reported

Table 1
Bread formulations containing or not wheat bran and/or *Lactobacillus brevis*-based bioingredient (LbBio) and/or the flour-based medium (FBM) as control.

	Bread type[a]					
	WWB	WBB	WWB + LbBio	WBB + LbBio	WWB + FBM	WBB + FBM
Ingredient	Mass (g)					
White wheat flour	350	280	350	280	350	280
Wheat bran	–	70	–	70	–	70
Salt	6.3	6.3	6.3	6.3	6.3	6.3
Margarine	10.5	10.5	10.5	10.5	10.5	10.5
Dry yeast	5.25	5.25	5.25	5.25	5.25	5.25
Tap water	210	245	–	35	–	35
LbBio	–	–	210	210	–	–
FBM	–	–	–	–	210	210

[a] White wheat bread (WWB), wheat bran bread (WBB), white wheat bread + LbBio (WWB + LbBio), wheat bran bread + LbBio (WBB + LbBio), white wheat bread + FBM (WWB + FBM), and wheat bran bread + FBM (WBB + FBM).