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ORIGINAL PAPER

Sourdough fermentation as a tool for the manufacture of low-glycemic index white wheat bread enriched in dietary fibre

M. De Angelis · N. Damiano · C. G. Rizzello · A. Cassone · R. Di Cagno · Marco Gobbetti

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Abstract Three *Lactobacillus* strains were selected and used together as sourdough starter. Sourdough performances were evaluated for 30 days. Three breads were manufactured: wheat sourdough bread (WSB), WSB enriched with oat and rye fibres (WSB-DF) and wheat yeasted bread (WYB) fermented with baker's yeast alone. WSB-DF and WSB showed higher specific volume and lower firmness than WYB. Sensory analysis showed that WSB-DF and WSB were preferred due to acidulous smell, taste and aroma. Compared to WYB and WSB, WSB-DF had high level of dietary fibre (DF). WYB was used as the control to estimate the hydrolysis index (HI = 100). WSB-DF had values of HI lower than WSB (59 vs. 86%). As estimated on 20 volunteers, the value of GI for WSB-DF was ca. 41%. WSB-DF bread manufactured at industrial plant combined low-GI with physiologically significant supply of DF and high standard structure and sensory features.

Keywords Bread · Dietary fibre · GI · Sourdough

Abbreviations

GI	Glycemic index
WSB	Wheat sourdough bread
WYB	Wheat flour fermented with baker's yeast
WSB-DF	Wheat flour enriched with oat and rye fibres and fermented with defined multi-species sourdough starter
HI	Hydrolysis index
DF	Dietary fibre
IAUC	Incremental area under the blood glucose curve
CVD	Cardiovascular disease

M. De Angelis · C. G. Rizzello · A. Cassone · R. Di Cagno · M. Gobbetti (✉)
Department of Plant Protection and Applied Microbiology, University of Bari, Via G. Amendola 165/a, 70126 Bari, Italy
e-mail: gobbetti@agr.uniba.it

N. Damiano
Gruppo Novelli S.r.l., 05100 Terni, Italy

Introduction

Consumption of foods with low-glycemic index (GI) and naturally rich and/or enriched in cereal fibres is recommended to prevent several diseases such as diabetes, risk factors for heart disease, also characterised as the metabolic syndrome (Syndrome X) [1–4]. Evidence has been accumulating that a low-GI diet might protect against development of obesity [5], colon and breast cancer [6]. The mechanisms of protection of the low-GI diet may derive from: (1) slow release of carbohydrates in the upper gastrointestinal tract; (2) lowering of the insulin demand [7]; and (3) generally high concentration of indigestible carbohydrates such as dietary fibre (DF) and resistance starch (RS), which, in turn, increase the fermentative activity in the colon level. This fermentative activity increases the synthesis of short chain fatty acids (SCFAs) such as acetic, propionic and butyric acids. SCFAs provide energy to colonocytes, and stimulate Na^+ and water absorption from the large intestine, even under diarrheic conditions. Propionic acid is considered the moderator of the hepatic glucose [8] and lipid metabolism [9]. Besides, high DF content associated with low-GI foods may increase the metabolic merits of the low-GI diet. In this connection, it is noteworthy that there is a shortage of commercial low-GI products on the market and that most cereal foods have a low content in DF. The shortage of low-GI

products limits possibilities to significantly lower dietary GI. In particular, there is a need to develop low-GI bread products rich in DF. When DF is used in breads, it is necessary to make adjustments in various process parameters in order to obtain high quality which is acceptable for the majority of the consumers.

One option to improve quality of breads contained DF is the use of sourdough biotechnology [10]. In addition, sourdough fermentation or the direct addition of acetic, propionic and lactic acids during bread making decreased the postprandial blood glucose and insulin responses [for review see 2, 11]. Previously, sourdough lactobacilli were selected for decreasing the GI of wheat bread made with a mixture (1:1) of wheat flour and wholemeal flour enriched with oat fibre (5%) [12]. Lactobacilli were used as starter cultures [12], but no studies have considered the traditional use of the sourdough fermentation as a tool for decreasing the GI of the white wheat bread enriched in DF. Reproducible and constant activity of the sourdough lactobacilli is indispensable to achieve the optimal quality of sourdough bread, also including the level of available carbohydrates [13, 14].

This study aimed at manufacturing low-GI white bread enriched in DF by: (1) selection of sourdough lactic acid bacteria for some technological traits to obtained a stable multi-species sourdough starter; (2) set up of a sourdough biotechnology for production of bread enriched in DF with good nutritional and sensory properties; and (3) in vitro and in vivo determination of the GI of the selected white bread enriched in DF.

Materials and methods

Sourdoughs and lactic acid bacteria

Three sourdoughs were collected from the bakery Interpan S.p.A. Gruppo Novelli located in the Umbria region (centre of Italy). Sourdoughs were made with *Triticum aestivum* and used as natural starters for the manufacture of a typical white wheat Italian bread ("Pane di Terni", http://www.paginegolose.com/umbria/w10r305i.html). The bakery uses sourdoughs by daily propagation and the manufacture of the "Pane di Terni" included two-steps of long and short fermentation for ca. 15 and 2.20 h, respectively, at ca. 28 °C. Ten grams of sourdough were homogenised with 90 ml of sterile sodium chloride (0.9%, wt/vol) solution using a Classic Blender (PBI International, Milan, Italy). Serial dilutions were plated on SDB agar for determination of presumptive lactic acid bacteria. After incubation at 30 °C for 48 h, at least ten colonies were randomly selected from plates containing the two highest sample dilutions. Gram-positive, catalase negative, non-motile rod and cocci isolates were cultivated in SDB broth at 30 °C for 24 h and re-streaked into SDB agar. All isolates considered for further analyses could acidify the culture medium. Stock cultures were stored at −20 °C in 10% (v/v) glycerol.

DNA extraction, molecular identification and genotypic characterisation

Genomic DNA from presumptive lactic acid bacteria isolates was extracted from 2 ml samples of overnight cultures grown at 30 °C in SDB broth as described by De Angelis et al. [15]. A primer pair (Invitrogen Life Technologies, Milan, Italy), LpigF/LpigR, was used to amplify the 16S rRNA gene fragment of presumptive lactic acid bacteria. Polymerase chain reaction (PCR) amplification was performed using the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, USA). The reaction mixture and PCR conditions reported by De Angelis et al. [15] were used. The expected amplicons of ca. 1,100 bp were eluted from gel and purified by the GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Milan, Italy). DNA sequencing reactions were performed by MWG Biotech AG (Ebersberg, Germany). Taxonomic identification of strains was performed by comparing the sequences of each isolate with those reported in the Basic BLAST database (http://www.ncbi.nlm.nih.gov). Primers designed on *recA* gene were also used to discriminate between *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus paraplantarum* species [15].

Genotypic characterisation was performed by using three oligonucleotides, P1, P4 and P7 [16] to exclude clonal relatedness. The reaction mixture and PCR conditions reported by Corsetti et al. [16] were used. RAPD-PCR profiles were acquired by Gel Doc EQ System (Bio-Rad, Hercules, CA, USA) and compared using Fingerprinting II Informatix™ Software (Bio-Rad).

Strains were routinely cultivated at 30 °C for 24 h in modified MRS (mMRS) broth (Oxoid, Basingstoke, Hampshire, UK) with the addition of 5% (v/v) fresh yeast extract, 28 mM maltose and pH of 5.6.

Selection of sourdough lactic acid bacteria

Lactic acid bacteria isolated from the three sourdoughs were used for dough fermentation to select starters based on the kinetic of acidification [13]. Two-hundred grams of white wheat flour type "0" (protein N × 5.70, 11.7%, of dry matter), 70 ml of tap water and 30 ml of the cellular suspension, containing ca. 8.5 log CFU/g of individual lactic acid bacteria strains (final cell number in the dough ca. 7.5 log CFU/g), were used to prepare 300 g of dough (dough yield 150) with a continuous high-speed mixer (60×*g*, dough mixing time 5 min). Sourdough fermentation was

carried out at 30 °C for 6 h. Acidification was determined on-line by a Foodtrode electrode (Hamilton, Bonaduz, Switzerland). Fermentations were carried out in triplicate, and each dough was analysed twice. Ten grams of dough was homogenised with 90 ml of sterile sodium chloride (0.9%, wt/vol) solution using a Classic Blender (PBI International, Milan, Italy). The concentrations of lactic and acetic acids in the water extract of dough were determined by enzymatic methods (Diffchamb, Sweden).

Bread making

After cultivation in mMRS for 24 h at 30 °C, cells of the most acidifying strains (*Lactobacillus sanfranciscensis* DPPMA12, *Lactobacillus plantarum* DPPMA55 and *Lactobacillus* sp. DPPMA56) were used together to produce a defined multi-species sourdough (initial cell density of ca. 7.5 log CFU/g of dough for each strain). Sourdoughs and breads were made with three types of wheat flour, having different concentration of proteins: type "0" (protein N × 5.70, 11.7%, of dry matter); type "0" reinforced (12.6%); and type "0" Manitoba (16.2%) (Table 1). Three types of wheat flour were normally used for the manufacture of a typical white wheat Italian bread ("Pane di Terni", http://www.paginegolose.com/umbria/w10r305i.html).

Table 1 Bread making formulas

Ingredients	Dough				
	WSB		WSB-DF		WYB
	I	II	I	II	
Wheat flour type 0 (%)	–	57.0	–	44.6	57.0
Wheat flour cv. *Manitoba* (%)	13.50	–	13.50	–	2.0
Reinforced wheat flour (%)	31.40	–	31.40	–	4.5
Water (%)	22.50	32.12	22.50	36.18	35.62
Sourdough (%)	32.60	10	32.60	10	–
Baker's yeast (%)	–	0.88	–	0.88	0.88
Rye fibre (%)	–	–	–	5.59	–
Barley malt (%)	–	–	–	0.25	–
Sodium chloride (%)	–	–	–	0.19	–
Ascorbate/enzymes (%)	–	–	–	0.06	–
Oat fibre (%)	–	–	–	2.25	–
Temperature (°C)	20	28	20	28	28
Time of fermentation (h)	15	2. 20	15	2.20	2.20

WSB and WSB-DF were manufactured following two-steps (I and II) of fermentation. Sourdough used in step II corresponded to an aliquot of that fermented in step I

WSB wheat sourdough bread; *WSB-DF* wheat sourdough bread enriched with oat and rye fibres; *WYB* wheat yeasted bread fermented with baker's yeast alone

According to the formulas of Table 1, three breads were manufactured: wheat sourdough bread (WSB), wheat sourdough bread enriched with dietary oat and rye fibres (WSB-DF) and wheat yeasted bread (WYB) fermented with baker's yeast alone. Oat (Azelis Italia S.r.l., Milano, Italy), and rye (Lantmännen, Stockholm, Sweden) fibres preparations contained 89 and 36% of DF, respectively. The β-glucan concentrations were 23% for oat and 2.9% for rye fibres. For the manufacture of WSB and WSB-DF, long-time fermentation (Stage I) of dough I was allowed for 15 h at 20 °C. After fermentation, an aliquot (ca. 10%, w/w) of dough I was used as sourdough (natural starter) for the short-time fermentation (Stage II) of dough II at 28 °C for 2.2 h. Dough I was daily propagated for 30 days. For the manufacture of WYB, the dough was fermented for 2.2 h at 28 °C with baker's yeast alone. Baking was at ca. 220 °C for 20 min. Breads were made at industrial plant by a commercial bakery (Interpan S.p.A., Terni, Italy). Bread making was carried out in triplicate for 30 days, and each dough or bread was analysed twice. Monitoring of starter strains during sourdough propagation (30 days) was performed by using 16S gene sequencing and RAPD-PCR analysis as described above analysing doughs belonging to the dough I.

Bread characterisation

Three independent experiments were performed as described above, and the characterisation of the breads was analysed during the period of production (30 days).

Bread mass and specific volume were determined according to AACC 10-10 official method. Bread crumb grain was determined by using image analysis technology [10, 17, 18]. Images of the sliced breads were captured using an Image Scanner (Amersham Pharmacia Biotech, Uppsala, Sweden). Images were scanned full scale at 300 dpi and analysed in grey scale (0–255). Image analysis was performed using the UTHSCSA ImageTool programme (Version 2.0, University of Texas Health Science Centre, San Antonio, TX, USA, available by anonymous FTP from maxrad6.uthscsa.edu). Analysis was carried out on two sub-images of 500 × 500 pixels selected from within the bread slice. Two slices were analysed per treatment. Gas cell to total area ratio was recovered.

Laboratory acceptance panel was used to give an indication of the consumer acceptance [19]. Breads, baked the day before sensory testing, were served at room temperature and under normal (daylight) illumination. A serving of each bread, identified by code numbers, on a single tray was served to each panellist. Ten volunteers from laboratory staff evaluated each product for quality attributes: colour, flavour/aroma, elasticity, sweetness and dryness.

Acceptability of each quality attribute was rated with a score from 0 (lowest) to 10 (highest) [20].

Proteins were determined by the Kjeldahl method. Total carbohydrates and soluble sugars were determined by HPLC. Total fat, saturated fat and total DF and β-glucan were determined according to the Official methods 30-10, 58-19 and 33-05 methods, respectively (American Association of Cereal Chemistry 2003). Calories (kJ/kcal) were determined as summation of protein, lipids and carbohydrate components.

In vitro starch hydrolysis

The analysis of starch hydrolysis simulates the in vivo digestion of starch. Bread portions, contained 1 g of starch (determined in bread), were given in randomised order to ten volunteers. The analysis was performed as described by Liljeberg et al. [21]. The glucose content was measured with Enzy Plus D-Glucose kit (Diffchamb). Factor conversion from glucose to starch was 0.9. The rate of starch digestion was expressed as the percentage of potentially available starch hydrolysed at different times (30, 60, 90, 120 and 180 min). The hydrolysis curves were obtained with the equation described below, using the programme STATISTICA 6.0. Hydrolysis curves follow a first order equation: $C = C_{\infty}\,(1 - e^{-kt})$ where C is the concentration at t time, C_{∞} is the equilibrium concentration, k is the kinetic constant and t is the chosen time [12].

Glycemic index in vivo test

Twenty healthy non-smoking volunteers, 11 women and 9 men, aged 25–54 years, with normal body mass indices (21.5 kg/m^2) and without drug therapy participated to the study. Anhydrous glucose and WSB-DF bread were used for the in vivo test. Fifty grams of available carbohydrates of bread and anhydrous glucose were consumed in a random order on separate mornings after 10–12 h overnight fast. The analysis was carried out in triplicate: each volunteer consumed the same sample during a period of 3 days. Samples were consumed over 6 min with tap water. After anhydrous glucose or bread ingestion, capillary finger-prick blood samples (3–4 drops) were taken from volunteers fasting (0 min) and after 15, 30, 45, 60, 75, 90, 105 and 120 min. Blood samples were collected into tubes and frozen at −20 °C before analysis by the glucose oxidase (EC 1.1.3.4) method [22].

The areas under the glucose-response curves for each sample were calculated geometrically, excluding beneath the fasting level. The GI was calculated by expressing the glycemic response area for the WSB-DF bread as the percentage of the mean response area of the 50 g of anhydrous glucose ingested by the same volunteers. The resulting individual GI values were averaged to obtain the GI value for WSB-DF bread.

Statistical analysis

Experimental data were subjected to analysis of variance (ANOVA) and pair-comparison of treatment means was achieved using Tukey's procedure at $P < 0.05$ using a statistical software Statistica for Windows (Statistica 6.0 per Windows 1998). For the GI in vivo test, the analyses were carried out in triplicate and the capillary finger-prick blood samples (3–4 drops) were taken from volunteers fasting (0 min) and after 15, 30, 45, 60, 75, 90, 105 and 120 min after ingestion of the sample.

Results and discussion

Lactic acid bacteria and defined multi-species sourdough starter

First, lactic acid bacteria were isolated and identified from traditional sourdoughs. The following species were identified: *L. sanfranciscensis* (15 isolates); *L. plantarum* (11); *Lactobacillus paracasei* (3); *Lactobacillus* sp. (8). Partial sequences of 16S rRNA gene of *Lactobacillus* sp. strains showed 100% of identity with *Lactobacillus* sp. CS1 (AN AJ564009). Strains showing different RAPD-PCR profiles (*L. sanfranciscensis* DPPMA5; DPPMA10; DPPMA11 and DPPMA12; *L. plantarum* DPPMA52; DPPMA55 and DPPMA57; *L. paracasei* DPPMA22 and *Lactobacillus* sp. DPPMA23, DPPMA46; DPPMA56 and DPPMA58) were used for further analyses.

Further, the above strains were selected based on the acidification activity during sourdough fermentation at 30 °C for 6 h. At the end of fermentation, all strains grew to cell densities of ca. 8.5 log CFU/g. The majority of the strains caused a lactic acidification characterised by values of ΔpH which ranged from 1.5 to 1.68. *L. sanfranciscensis* DPPMA12, *L. plantarum* DPPMA55 and *Lactobacillus* sp. DPPMA56 with ΔpH of ca. 1.85 showed the highest capacity of acidification. In agreement with the values of ΔpH, the concentration of D,L-lactic acid ranged from 7.5 to 60 mM. The capacity to synthesise acetic acid varied from 0.1 to 12 mM. Variations in the concentration of D,L-lactic and acetic acids determined the differences found for the quotient of fermentation (QF, molar ratio between D,L-lactic and acetic acids). QF is one of the fermentative parameters frequently used to link acidification and flavour. A general rule is to keep it ca. 4, which implies a considerable synthesis of acetic acid [23].

Based on the acidification activity, strains *L. sanfranciscensis* DPPMA12, *L. plantarum* DPPMA55 and *Lactobacillus* sp. DPPMA56 were selected and re-used for the manufacture of the defined multi-species sourdough starter with the capacity to rapidly acidify. *L. sanfranciscensis*

and *L. plantarum* express hetero- or homo-fermentative metabolisms and represent the most common association of lactic acid bacteria found in sourdoughs [23].

Two sourdough white wheat breads, WSB and WSB-DF, were made by using the ingredients listed in Table 1. Both breads were made by two-steps fermentation process where, after the Stage I (15 h), an aliquot (ca. 10%, w/w) of the previous sourdough was used as the starter for the Stage II (2.2 h). White flour wheat bread fermented by baker's yeast (WYB) alone was used as the control.

During Stage I, the acidification rate of the defined multi-species sourdough starter showed values of A, V_{max} and λ of 0.88 ± 0.1 units, 0.14 ± 0.02 dpH/min and 0.25 ± 0.02 h, respectively. No statistical differences ($P < 0.05$) were found between WSB and WSB-DF sourdoughs. After the Stage II, the values of pH were 4.0 ± 0.2 and 3.8 ± 0.13 for WSB and WSB-DF, respectively (Table 2). WSB and WSB-DF sourdoughs contained ca. 100 ± 3.8 and 130 ± 4.2 mM lactic acid and ca. 25 ± 3.4 and 42 ± 2.1 mM acetic acid, respectively. The QF ranged from ca. 4.0 to 3.25. The differences found in lactic and acetic acids in WSB and WSB-DF could be because the fermentation of DF by the multi-species sourdough starter [24, 25]. Alternative explanations for the increased organic acids upon addition of DF could be related to the increase of maltose and glucose levels resulting from the addition of endogenous barley malt enzymes, or the increased buffering capacity of the dough. Before baking, cell numbers of lactic acid bacteria in WSB and WSB-DF sourdoughs were ca. 9.0–9.2 log CFU/ml (Table 2). On the contrary, the final pH of WYB dough was 5.5 ± 0.14 which reflected the low decrease of pH with respect to the initial value (pH 5.8). No statistical differences ($P < 0.05$) were found for the kinetics of acidification and growth during 30 days of sourdough daily propagation. RAPD-PCR analysis showed that cell numbers of selected lactic acid bacteria strains maintained constant during daily propagation of both WSB and WSB-DF sourdoughs (data not shown).

Based on the above results, the defined multi-species sourdough starter had two main features: (1) high cell density (ca. 9.0 log CFU/g) which permit to overcome the delay in the acidification rate; and (2) constant acidifying activity during prolonged storage and/or propagation [23].

Bread characterisation

WSB, WSB-DF and WYB were analysed for specific volume, crumb grain and sensory characteristics (Table 3). The specific volume of WSB was significantly ($P < 0.05$) higher than that of WYB. The bread crumb grain was determined by image analysis (Fig. 1). Digital images were pre-processed to detect crumb cell–total area by a binary conversion (black/white pixels). The cell–total area

Table 2 Lactic acid bacteria, values of pH and concentration of organic acids of the wheat yeasted dough fermented with baker's yeast alone (WYB), wheat sourdough (WSB), wheat sourdough and enriched with oat and rye fibres (WSB-DF) at 28 °C for 2.20 h

	Dough (Stage II)		
	WYB	WSB	WSB-DF
Lactic acid bacteria (log CFU/g)	4.2 ± 0.08	9.0 ± 0.10	9.20 ± 0.07
pH	5.5 ± 0.14	4.0 ± 0.20	3.8 ± 0.13
Lactic acid (mM)	–	100.0 ± 3.80	130.0 ± 4.20
Acetic acid (mM)	–	25.0 ± 3.40	42.0 ± 2.10
Lactic acid/acetic acid (QF)	–	3.0	4.0

Data are means ± SD of three independent experiments. WSB and WSB-DF were manufactured following two-steps (I and II) of fermentation. Sourdough used in step II corresponded to an aliquot of that fermented in step I (for details see "Materials and methods")

Table 3 Characteristics of the wheat yeasted bread fermented with baker's yeast alone (WYB), wheat sourdough (WSB), wheat sourdough and enriched with oat and rye fibres (WSB-DF)

Characteristics	Bread		
	WYB	WSB	WSB-DF
Specific volume (cm^3/g)	2.18 ± 0.02	3.80 ± 0.05	3.85 ± 0.03
Crum grain cell-total area (%)	44.5 ± 1.78	50.9 ± 1.32	51.2 ± 1.56
Calories (kJ/kcal)	$1{,}062 \pm 41.2/250 \pm 8.7$	$1{,}020 \pm 15.4/250 \pm 11.7$	$978 \pm 22.5/233 \pm 6.4$
Proteins (g)	8.54 ± 0.06	8.60 ± 0.02	8.57 ± 0.09
Total carbohydrates (g)	46.50 ± 0.21	46.42 ± 2.05	46.25 ± 1.89
Total fat (g)	1.43 ± 0.47	1.45 ± 0.51	1.48 ± 0.12
Saturated fat (g)	0.28 ± 0.05	0.30 ± 0.06	0.33 ± 0.02
Total dietary fibre/β-glucan (g)	$4.10 \pm 0.18/0.0$	$4.19 \pm 0.06/0.0$	$8.13 \pm 0.15/0.8 \pm 0.02$

Data are means ± SD of three independent experiments

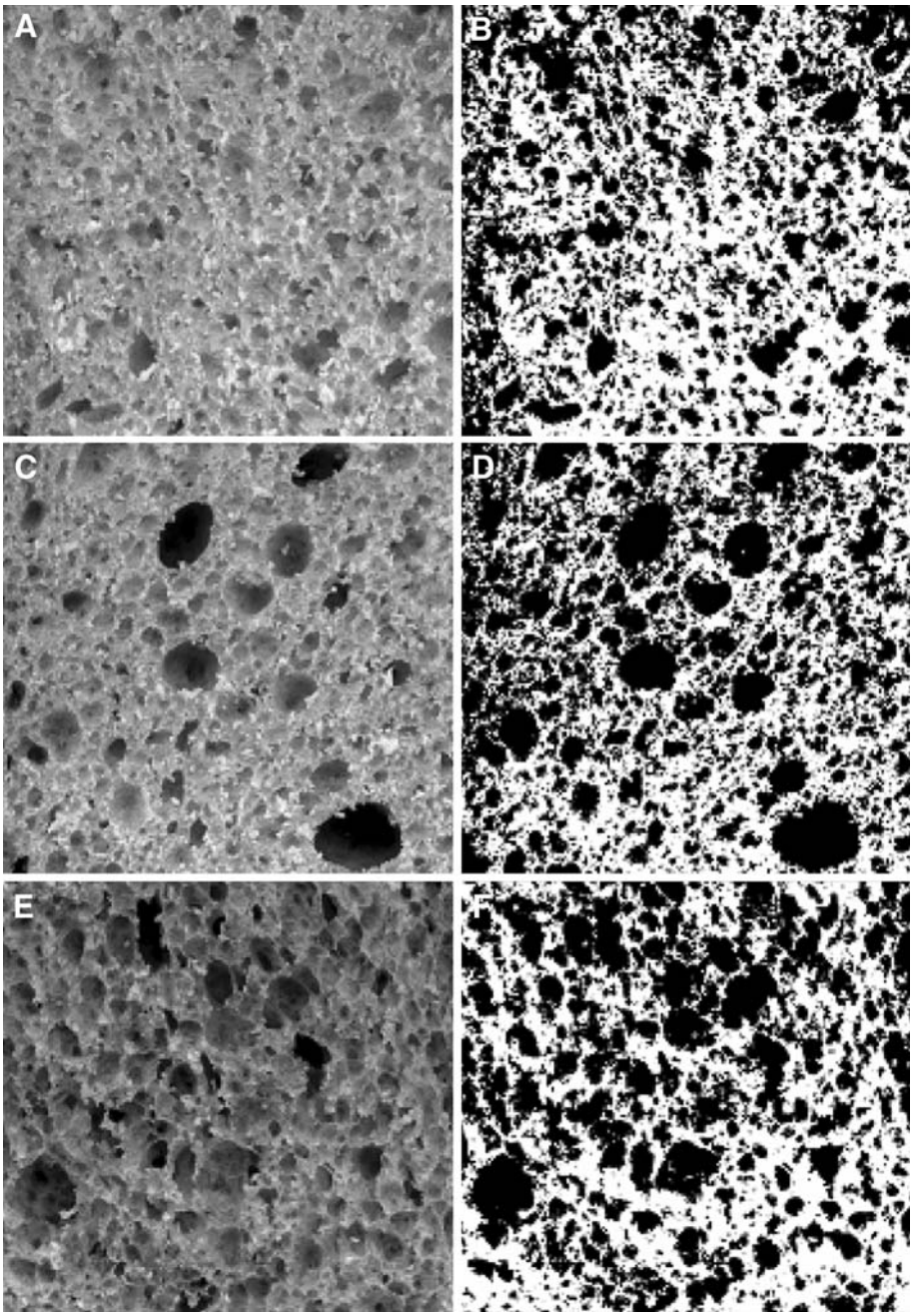

Fig. 1 Representative images of wheat yeasted bread fermented with baker's yeast alone (WYB) used as reference (**a**, **b**), wheat sourdough bread (WSB) (**c**, **d**), wheat sourdough bread enriched with oat and rye fibres (WSB-DF) (**e**, **f**). Digital images of bread showing the original grey level images (**a**, **c**, **e**) and computed binary results from grey level thresholding at the two-cluster (**b**, **d**, **f**)

of WYB (corresponding to the black pixel total area) was ca. 44%. As shown by the visual inspection of the binarised images of the breads, crumb cell detection in the field of view for WSB slices had more numerous gas cells. Indeed, a considerable variation of the cell–total area with respect to WYB was found. These effects were probably related to the highest acidity of WSB compared to WYB that provide a dough with a well-developed homogeneous gluten network that effectively contains the CO_2 produced during the final leavening phase [26]. In the doughs with kefir added, the CO_2 was released slowly creating large bubbles or open grains [17]. No statistical differences ($P < 0.05$) were observed between WSB and WSB-DF indicating that the activity of defined multi-species sourdough starter was not negatively affected by DF addition. On the contrary, the sourdough biotechnology eliminated the negative effects of the DF on the specific volume and crumb grain [10]. Except for elasticity, sweetness and dryness, WSB and WSB-DF were scored significantly ($P < 0.05$) different with respect to WYB. As expected, WSB and WSB-DF received the highest scores for acid taste and flavour. The overall perception of taste was

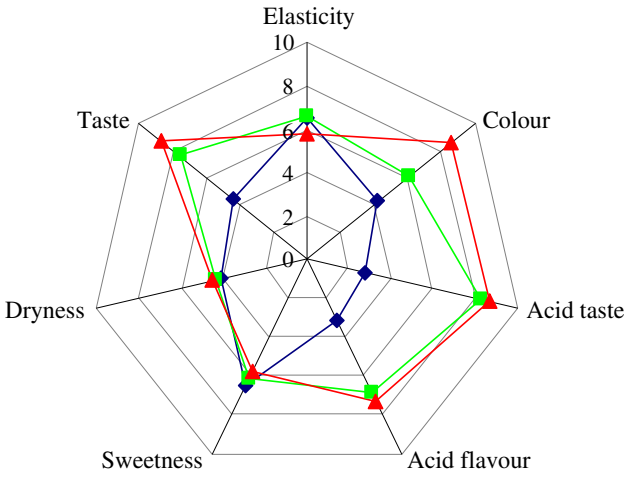

Fig. 2 Sensory analysis of wheat yeasted bread fermented with baker's yeast alone (WYB) used as reference (*open diamonds*), wheat sourdough bread (WSB) (*open squares*), wheat sourdough bread enriched with oat and rye fibres (WSB-DF) (*open triangles*). Seven attributes were used: elasticity, colour, acid taste, acid flavour, sweetness, dryness and taste. The data are the means of three independent experiments

markedly more appreciated for WSB and WSB-DF (Fig. 2). The biotechnology used could extent the shelf life of WSB and WSB-DF and their delivery to consumers in the best possible fresh conditions [10, 17, 23].

Nutritional values for 100 g of breads were showed in Table 3. Compared to WSB and WYB, the WSB-DF mainly showed high level of DF (ca. 8.13 g). High DF intake has beneficial effects on systolic blood pressure and blood lipids levels and suggests that DF intake should be increased in individuals who have diabetes mellitus to prevent complications [4]. DF regulates the rate and site of lipid and carbohydrate digestion and absorption, and thus can modify the alimentary responses to a meal [27]. Hundred grams of WSB-DF contained ca. 800 mg of β-glucan. This amount still represents the minimum effective daily sub-dose to obtain positive healthy effects [28]. In 1997, the Food and Drug Administration enacted a health claim addressing the cardiovascular benefits of oat β-glucan. Nevertheless, a large gap between dietary recommendations and daily intake of DF is currently present in the diet of a large part of the population. To enrich cereal baked goods with DF by keeping optimal sensory properties is probably the most desirable goal [3].

In vitro starch hydrolysis and glycemic index in vivo test

The rate of in vitro starch hydrolysis is considered to be a presumptive measure of the GI in healthy subjects [29]. Starch hydrolysis at 30–180 min and values of hydrolysis index (HI) are shown in Fig. 3. After 180 min, the percentage of hydrolysed starch was 51.0, 44.0 and 30.5% for WYB, WSB-DF and WSB, respectively. In agreement, the HI of WSB and WSB-DF were 86 and 59%, respectively (Fig. 3). No statistical differences ($P < 0.05$) were found for values of HI during 30 days of daily manufacture of WSB and WSB-DF. The low values of HI of sourdough fermented breads compared to WYB might be due to the decrease of pH. The lowest decrease of HI found for WSB-DF might be due to the addition of DF and to the different concentration of lactic and acetic acids [12, www.gilisting.com/2004/05/glycemic-index-of-breads.html]. Overall, the lowering of glycemia and insulinaemia by lactic acid is not attributed to the decreased gastric emptying rate [28] but to interactions between starch and gluten that limited the starch bioavailability [30, 31].

WSB-DF was selected for the in vivo determination of the blood glucose level and GI, since it was characterised by the lowest value of HI and showed structure and sensory features similar to WSB. Anhydrous glucose was used as the reference [32]. All volunteers consumed anhydrous glucose and bread according to the experimental protocol. The mean incremental blood-glucose response

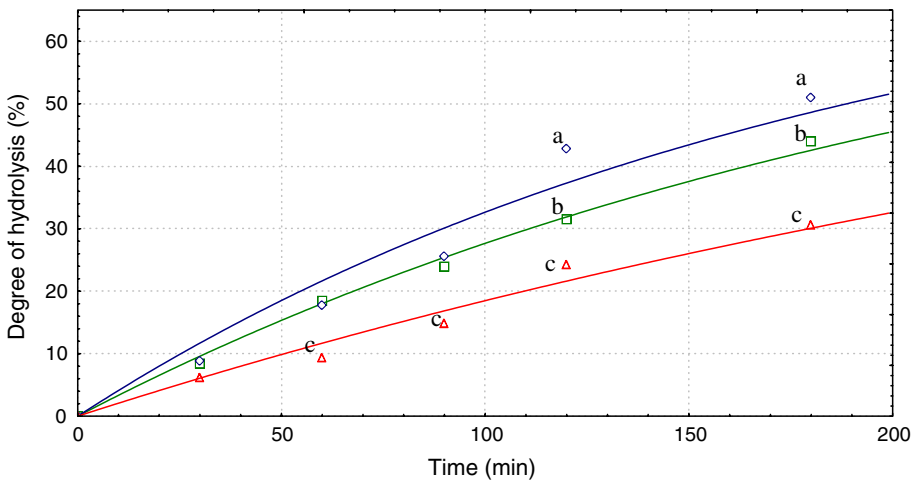

Fig. 3 Rate of starch hydrolysis following chewing, incubation with pepsin and subsequent incubation with pancreatic α-amylase in a dialysis tubing. Wheat yeasted bread fermented with baker's yeast alone (WYB) used as reference (*open diamonds*), wheat sourdough bread (WSB) (*open squares*), wheat sourdough bread enriched with oat and rye fibres (WSB-DF) (*open triangles*). Values for each time point not sharing the same letters are significantly different from each other at $P < 0.05$

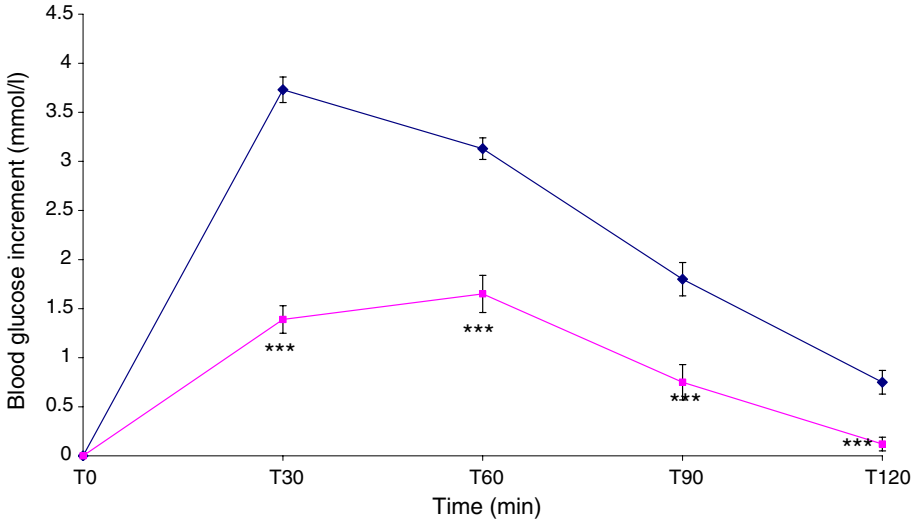

Fig. 4 Blood glucose increment (mmol/l) in healthy volunteers following ingestion of anhydrous glucose (reference) (*filled diamonds*), and wheat sourdough bread enriched with oat and rye fibres (WSB-DF) (*filled squares*). Values are means, $n = 20$. Significant differences of sourdough low-GI sourdough bread to reference are indicated as *** ($P < 0.05$)

curves during 120 min after sample ingestion are shown in Fig. 4. The GI of WSB-DF was ca. 41.1% of the reference (anhydrous glucose, GI = 100). Overall, GI of carbohydrate-based foods varied from 20 to 90 [32]. Threshold values for simple classification of foods according to their GI were defined. Three classes are commonly considered: (1) foods with low GI (values <55%); (2) foods with intermediate GI (values 55–70%); and (3) foods with high GI (values >70%). Accumulating data support the therapeutic potential of low-GI diets in diabetes and hyperlipidemia [33] as well as the preventive potential against development of diabetes and cardio vascular disease (CVD). Owing to these considerations and WHO reports [34] several carbohydrates-based foods with decreased GI are manufactured: white wheat bread enriched in β-glucan isolated from barley [35]; white wheat bread enriched in fats [36]; bread enriched with viscous DF; bread added with organic acids by chemical acidification [30] or using sourdough [37, 38]; white wheat flour and wholemeal flour bread enriched with DF and fermented by selected lactic acid bacteria [12]. The effects of baking process, freezing and toasting on glycemic response were also studied [39, 40]. As described by Björck and Liljeberg [2] and Östman et al. [30], the exchange of common high-GI bread for low-GI bread enriched with DF, as the only dietary modification, may improve the insulin economy. Cereal kernels included in bread (wheat, rye or barley) reduced the glycemic response to bread in healthy human [41] and some low-GI breads such as pumpernickel (GI = 41%) are available in the market. However, the GI of most common commercial wheat flour bread ranged between 70 and 73% [42] (revised international table of glycemic index (GI) and glycemic load (GL) 452 values; *diabetes*, http://diabetes.about.com/library/mendosagi/ngilists.htm].

Conclusion

To our knowledge, this is the first study showing white wheat flour bread with GI < 45% [42]. This bread manufactured at industrial plant combined low-GI with physiologically significant supply of DF, and high standard appearance, structure, colour and flavour. Selected sourdough may be considered as one of the biotechnological tools for the manufacture of low-GI bread. Despite of the large benefits, sourdough processes are used in the manufacture of less than 20% of the bread produced in the Central Europe, France and Italy. The protocol set up under this study may be useful to enlarge the industrial use of the sourdough biotechnology also for improving the nutritional properties of baked goods.

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