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An integrated technological approach to the selection of lactic acid bacteria of flour origin for sourdough production

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ABSTRACT

Several lactic acid bacteria (LAB) were evaluated *in situ* for their potential in sourdough fermentation. The strains belonged to *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus sanfranciscensis*, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides* and *Weissella cibaria*. LAB were used, in individual inocula, to carry out the fermentation of γ-ray treated (sterile) flour and untreated commercial flour, in order to evaluate their performances both in the absence and presence of the native microbiota of flour. The pH and total titratable acidity (TTA) showed a strong and fast acidification of the experimental sourdough determined by *W. cibaria* and *Ln. citreum* strains. All strains were followed during fermentation by plate count. Randomly amplified polymorphic DNA (RAPD)-PCR analysis applied on the colonies isolated from the highest dilution of samples confirmed the dominance of the added strains in all sourdoughs prepared with sterile and non-sterile flour. The analysis of organic acids, performed by high-performance liquid chromatography (HPLC), confirmed that some *W. cibaria* and *Ln. citreum* strains showed an optimal fermentation quotient. The volatile organic compound (VOC) composition resulting from the gas chromatography coupled with mass spectrometry (GC/MS) analysis of sourdough headspace recognised 51 chemical compounds including acids, alcohols, aldehydes, esters, ketones, lactones, acetate, alkane, and phenol, most of which are of LAB origin and are relevant for the final bread. After baking, the breads were evaluated for the height of the central slices, colour of crust and crumb, hardness and number and distribution of alveolus. The combination of these results indicated that strains *Ln. citreum* PON10079 and PON10080 and *W. cibaria* PON10030 and PON10032 are suitable cultures to use in industrial production.

1. Introduction

The traditional biotechnology for the production of bread and other leavened baked products is based on sourdough, which is a complex microbial ecosystem mainly constituted by lactic acid bacteria (LAB) and yeasts (Vogel et al., 1999). Although LAB are primarily involved in the development of the sensory characteristics of sourdough, several by-products generated by yeasts during fermentation contribute to the improvement of the organoleptic complexity of the resulting doughs (Valmorri, Tofalo, Settanni, Corsetti, & Suzzi, 2010). Thus, a successful sourdough fermentation is pivotal to providing the final products with the right attributes.

The typical LAB responsible for the acid production in sourdoughs belong to the genus *Lactobacillus* (Corsetti & Settanni, 2007). Sourdough lactobacilli include obligately and facultatively heterofermentative and obligately homofermentative species (Hammes & Vogel, 1995), but, contrary to the majority of other fermented food productions, the most defining role of these LAB in sourdough is played by the obligately heterofermentive strains. This is due to the production of acetic acid in addition to the lactic acid from the fermentation of carbohydrates, important for the development of the right fermentation quotient (FQ = lactic/acetic acid molar ratio). Acetic acid strongly contributes to the aroma and the structure of the final products (Corsetti & Settanni, 2007). Furthermore, heterofermentative LAB partly contribute to the process of dough leavening (Gobbetti et al., 1995).

Other species belonging to *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Weissella* are commonly detected in sourdoughs (Corsetti & Settanni, 2007), albeit at levels lower than those registered for lactobacilli. During the first stages of sourdough preparation, different non-*Lactobacillus* species have been found to prepare the environment for the establishment of the typical species (e.g. *Lactobacillus sanfranciscensis*) of mature sourdoughs (Corsetti, Settanni, Valmorri, Mastrangelo, & Suzzi, 2007). These species are primarily sourced from cereals or flours (Alfonzo et al., 2013; Corsetti, Settanni, Chaves-López, et al., 2007). Starting from flour mixed with water, the selection of the non-*Lactobacillus* species occurs under the first repeated propagations (refreshments) of the mass, but when lactobacilli dominate their levels generally decrease at about 2 – 3 orders of magnitude (Corsetti, Settanni, Valmorri, et al., 2007). However, some

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Table 1
pH of the experimental sourdoughs.

Strains	Sterile flour						Non sterile flour					
	T_0	2 h	4 h	6 h	8 h	21 h	T_0	2 h	4 h	6 h	8 h	21 h
Control SFD	5.95 ± 0.13	5.80 ± 0.06	5.80 ± 0.03	5.77 ± 0.01	5.76 ± 0.02	5.74 ± 0.02	–	–	–	–	–	–
Control nSFD	–	–	–	–	–	–	6.11 ± 0.05	5.89 ± 0.04	5.89 ± 0.04	5.89 ± 0.04	5.64 ± 0.02	5.08 ± 0.05
Control wnSFD	–	–	–	–	–	–	6.12 ± 0.05	5.94 ± 0.11	5.93 ± 0.08	5.93 ± 0.09	5.90 ± 0.06	5.28 ± 0.07
Lb. plantarum PON100274	5.74 ± 0.01aA	5.71 ± 0.01aA	5.70 ± 0.02aA	5.68 ± 0.01aA	5.58 ± 0.02aA	3.75 ± 0.0aA	6.05 ± 0.00bB	5.94 ± 0.02bB	5.88 ± 0.09bB	5.71 ± 0.14aA	5.35 ± 0.02aA	3.59 ± 0.02aA
Lb. sanfranciscensis LMG 17498^T	5.81 ± 0.01aA	5.74 ± 0.00 aA	5.74 ± 0.00 aA	5.74 ± 0.01 aA	5.72 ± 0.00 aA	3.70 ± 0.00 aA	6.07 ± 0.01bB	5.91 ± 0.02 aA	5.91 ± 0.01 aA	5.80 ± 0.00 aA	5.49 ± 0.00 aA	3.66 ± 0.01 aA
Lb. sanfranciscensis PON100100	5.75 ± 0.00aA	5.75 ± 0.01aA	5.75 ± 0.02aA	5.74 ± 0.04aA	5.70 ± 0.01aA	3.81 ± 0.03 aA	6.10 ± 0.04bB	5.90 ± 0.05bB	5.89 ± 0.05bB	5.84 ± 0.09aA	5.50 ± 0.03bB	3.81 ± 0.11aA
Lb. sanfranciscensis PON100336	5.71 ± 0.11aA	5.70 ± 0.06 aA	5.70 ± 0.03aA	5.70 ± 0.07 aA	5.70 ± 0.06aA	3.80 ± 0.04aA	6.06 ± 0.02bB	5.90 ± 0.07bA	5.89 ± 0.08bA	5.85 ± 0.11aA	5.47 ± 0.10bB	3.74 ± 0.10aA
Lb. sakei PON10098	5.79 ± 0.06aA	5.71 ± 0.01aA	5.71 ± 0.02 aA	5.63 ± 0.11aA	5.13 ± 0.05aA	3.80 ± 0.12 aA	6.09 ± 0.03bB	5.93 ± 0.09bB	5.90 ± 0.06bB	5.47 ± 0.03aA	4.39 ± 0.12bB	3.75 ± 0.04aA
Ln. citreum PON10021	5.78 ± 0.01aA	5.72 ± 0.03aA	5.71 ± 0.05aA	5.68 ± 0.02aA	5.64 ± 0.01aA	3.80 ± 0.03aA	6.06 ± 0.01bB	5.87 ± 0.02bB	5.83 ± 0.06bA	5.76 ± 0.11aA	5.51 ± 0.16aA	3.69 ± 0.03aA
Ln. citreum PON10079	5.77 ± 0.03aA	5.70 ± 0.03aA	5.69 ± 0.04aA	5.57 ± 0.02aA	4.93 ± 0.11aA	3.82 ± 0.01aA	6.06 ± 0.00bB	5.89 ± 0.05 bB	5.82 ± 0.09bA	5.18 ± 0.05bB	4.34 ± 0.06bB	3.89 ± 0.10aA
Ln. citreum PON10080	5.74 ± 0.01aA	5.69 ± 0.08aA	5.65 ± 0.07aA	5.38 ± 0.03aA	4.70 ± 0.03aA	3.90 ± 0.04aA	6.04 ± 0.03bB	5.90 ± 0.06bB	5.86 ± 0.08bB	5.33 ± 0.15aA	4.41 ± 0.07aA	3.97 ± 0.05aA
Ln. mesenteroides PON10031	5.80 ± 0.02aA	5.73 ± 0.03aA	5.72 ± 0.02aA	5.65 ± 0.04aA	5.29 ± 0.04aA	3.96 ± 0.02aA	6.07 ± 0.02aA	5.90 ± 0.07bB	5.72 ± 0.10aA	5.08 ± 0.06bB	4.36 ± 0.09bB	3.95 ± 0.01aA
Ln. pseudomesenteroides PON10024	5.77 ± 0.04aA	5.77 ± 0.03aA	5.77 ± 0.06aA	5.69 ± 0.04aA	5.46 ± 0.02aA	3.96 ± 0.03aA	6.13 ± 0.10bB	5.92 ± 0.08bA	5.81 ± 0.09aA	5.36 ± 0.06aA	4.91 ± 0.07aA	4.14 ± 0.07aA
Ln. pseudomesenteroides PON100315	5.77 ± 0.03aA	5.73 ± 0.03aA	5.72 ± 0.00aA	5.62 ± 0.08aA	5.29 ± 0.03aA	3.78 ± 0.03aA	6.06 ± 0.01bB	5.88 ± 0.04bB	5.82 ± 0.12aA	5.43 ± 0.05aA	4.64 ± 0.09bB	3.87 ± 0.02bB
W. cibaria PON10030	5.77 ± 0.04aA	5.77 ± 0.06aA	5.72 ± 0.01aA	5.46 ± 0.30aA	4.67 ± 0.03aA	3.97 ± 0.03aA	6.06 ± 0.02bB	5.92 ± 0.06bB	5.75 ± 0.13aA	5.19 ± 0.05aA	4.35 ± 0.08bA	3.95 ± 0.02aA
W. cibaria PON10032	5.80 ± 0.02aA	5.78 ± 0.05aA	5.71 ± 0.09aA	5.38 ± 0.05aA	4.72 ± 0.03aA	3.87 ± 0.06aA	6.05 ± 0.00bB	5.91 ± 0.09bA	5.74 ± 0.09aA	5.22 ± 0.07aA	4.37 ± 0.14bA	3.96 ± 0.05bA
W. cibaria PON100337	5.79 ± 0.04aA	5.76 ± 0.06aA	5.69 ± 0.01aA	5.44 ± 0.03aA	4.66 ± 0.03aA	3.75 ± 0.01aA	6.09 ± 0.05bB	5.91 ± 0.05bB	5.86 ± 0.07bB	5.47 ± 0.07aA	4.44 ± 0.04aA	3.91 ± 0.01bB
Statistical significance[a]	***	**	***	**	**	***	NS	**	NS	***	***	***

Abbreviations: SFD, sterile flour dough; nSFD, non sterile flour dough; wnFDS, non sterile water non sterile flour; *Lb., Lactobacillus*; *Ln., Leuconostoc*; *W., Weissella*.
Results indicate mean values ± SD of four measurements (carried out in duplicate for two independent productions).
Lowercase (a,b) and uppercase (A, B) letters indicate different statistical significances according to Tukey's test at P values of <0.05 and <0.01, respectively.
[a] P value: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, not significant.

leuconostocs, pediococci and weissellas were found to coexist at the same levels of lactobacilli in sourdoughs (Robert, Gabriel, & Fontagné-Faucher, 2009).

In a previous work of ours (Alfonzo et al., 2013), 50 strains of LAB isolated from different flour samples employed to produce sourdoughs in bakeries located in the Sicily region (southern Italy) were characterised *in vitro* for their technological characteristics with 11 strains belonging to *Leuconostoc citreum*, *Leuconostoc pseudomesenteroides*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus sakei* and *Weissella cibaria* showing potential in sourdough fermentation. The present work was aimed to evaluate the technological performances (acidification kinetics, acid production, loaf height, colour formation, softness of bread and volatile compound generation) of these strains under controlled conditions. To this end, the fermentation tests were carried out at first in γ-ray treated wheat flour, in order to investigate the behaviour of each strain without any microbial competition, and then in untreated flour to monitor their role in the presence of the indigenous microbiota of flour.

2. Materials and methods

2.1. Strains and growth conditions

In this study, eleven LAB strains (*Lactobacillus plantarum* PON100274, *Lactobacillus sakei* PON10098, *Leuconostoc citreum* PON10021, PON10079 and PON10080, *Leuconostoc mesenteroides* PON10031, *Leuconostoc pseudomesenteroides* PON10024 and PON100315 and *Weissella cibaria* PON10030, PON10032 and PON100337), which were isolated from wheat samples (*Triticum durum* and *T. aestivum*) and selected as technologically relevant in sourdough fermentation (Alfonzo et al., 2013), were used. They were taken from the culture collection of the Agricultural Microbiology laboratory at the Department of Agricultural and Forest Science - University of Palermo (Palermo, Italy). In addition, two *Lactobacillus sanfranciscensis* strains (PON100100 and PON100336) belonging to the same culture collection and *Lb. sanfranciscensis* LMG 17498^T were used for comparison. *Lb. sanfranciscensis* strains were propagated in sourdough bacteria (SDB) broth (Kline & Sugihara, 1971), while all other LAB strains in MRS (Oxoid, Milan, Italy). All the strains were incubated overnight at 30 °C.

2.2. Dough production

The LAB strains were individually tested in experimental sourdoughs in order to evaluate their in *situ* performances. Overnight LAB cultures, grown in the corresponding optimal media, were centrifuged at 5000 ×*g* for 5 min, washed twice in Ringer's solution (Oxoid) and re-suspended in the same solution till reaching an optical density (OD) of ca. 1.00 which approximately corresponds to a concentration of 10^9 CFU mL^{-1}. The inocula were added to a final concentration of approximately 10^6 CFU/g in dough.

Each dough of 200 g was produced with a dough yield (weight of the dough/weight of the flour × 100) of 160 adding 75 mL of tap H_2O, containing the cell suspension, to 125 g of commercial flour (Il Molino Chiavazza, Casalgrasso, Italy). In order to exclude the interference of the native microflora, the experimental doughs were first prepared with wheat flour sterilised by γ-ray (25 kGy) treatment (Gammatom, Guanzate, Italy) and mixed with sterile tap H_2O (sterile flour doughs, SFD) and then the same doughs were made with untreated flour and non-sterile tap H_2O (non-sterile flour doughs, nSFD). Control doughs, without LAB inocula, were added to each dough production: sterile flour and sterile tap H_2O for SFD (control SFD); non-sterile flour and non-sterile tap H_2O for nSFD (control nSFD); and, in order to exclude the contribution of H_2O microflora to the dough, an additional control dough was prepared with non-sterile flour and sterile tap H_2O (control wnSFD).

Table 2
TTA of the experimental sourdoughs.

Strains	Sterile flour						Non sterile flour					
	T_0	2 h	4 h	6 h	8 h	21 h	T_0	2 h	4 h	6 h	8 h	21 h
Control SFD	1.40 ± 0.00	1.50 ± 0.10	1.50 ± 0.00	1.50 ± 0.20	1.50 ± 0.00	1.80 ± 0.10	–	–	–	–	–	–
Control nSFD	–	–	–	–	–	–	1.20 ± 0.00	1.40 ± 0.00	1.40 ± 0.00	1.40 ± 0.00	1.40 ± 0.00	1.80 ± 0.10
Control wnSFD	–	–	–	–	–	–	1.00 ± 0.00	1.00 ± 0.00	1.20 ± 0.00	1.20 ± 0.00	1.20 ± 0.00	1.80 ± 0.00
Lb. plantarum PON100274	1.40 ± 0.00aA	1.40 ± 0.10 aA	1.40 ± 0.20 aA	1.40 ± 0.00 aA	1.60 ± 0.00 aA	5.60 ± 0.00 aA	1.20 ± 0.10aA	1.40 ± 0.00 aA	1.40 ± 0.00 aA	1.40 ± 0.20 aA	2.00 ± 0.00 aA	7.60 ± 0.10bB
Lb. sanfranciscensis LMC 17498^T	1.50 ± 0.10 aA	1.50 ± 0.10aA	1.50 ± 0.00 aA	1.50 ± 0.20 aA	1.60 ± 0.00 aA	7.10 ± 0.10aA	1.30 ± 0.00 aA	1.30 ± 0.00bB	1.30 ± 0.00bB	1.30 ± 0.00bB	1.70 ± 0.00bB	7.20 ± 0.00bB
Lb. sanfranciscensis PON100100	1.40 ± 0.00 aA	1.40 ± 0.10 aA	1.40 ± 0.10 aA	1.40 ± 0.00 aA	1.40 ± 0.00 aA	7.00 ± 0.00 aA	1.20 ± 0.10 aA	1.40 ± 0.10	1.40 ± 0.20 aA	1.50 ± 0.10 aA	1.60 ± 0.20 aA	7.00 ± 0.10 aA
Lb. sanfranciscensis PON100336	1.40 ± 0.00 aA	1.40 ± 0.10 aA	1.40 ± 0.20 aA	1.40 ± 0.10 aA	1.40 ± 0.00 aA	5.80 ± 0.00 aA	1.30 ± 0.10 aA	1.60 ± 0.00 aA	1.60 ± 0.00 aA	1.60 ± 0.10 aA	1.70 ± 0.10 bB	9.40 ± 0.10 bB
Lb. sakei PON10098	1.60 ± 0.010aA	1.70 ± 0.00aA	1.70 ± 0.10aA	1.70 ± 0.10aA	2.00 ± 0.10aA	4.40 ± 0.20aA	1.00 ± 0.00bA	1.40 ± 0.00bB	1.40 ± 0.00bB	1.60 ± 0.00bB	4.40 ± 0.00bB	6.60 ± 0.20bB
Ln. citreum PON10021	1.00 ± 0.10aA	1.00 ± 0.00aA	1.00 ± 0.20aA	1.00 ± 0.00aA	1.00 ± 0.00aA	6.20 ± 0.00aA	0.80 ± 0.00aA	1.60 ± 0.00bB	1.60 ± 0.00bB	1.60 ± 0.00bA	2.40 ± 0.00bA	5.00 ± 0.10aA
Ln. citreum PON10079	1.20 ± 0.10aA	1.30 ± 0.00aA	1.40 ± 0.00aA	1.70 ± 0.00aA	2.30 ± 0.00aA	6.00 ± 0.00aA	1.00 ± 0.00aA	1.40 ± 0.00bB	1.40 ± 0.00aA	1.60 ± 0.00bB	7.40 ± 0.00bB	6.40 ± 0.20aA
Ln. citreum PON10080	1.40 ± 0.10aA	1.50 ± 0.20aA	1.50 ± 0.10aA	1.90 ± 0.00aA	2.40 ± 0.10aA	7.80 ± 0.10aA	1.20 ± 0.10aA	1.40 ± 0.00bB	1.40 ± 0.00bA	1.80 ± 0.00bA	5.00 ± 0.10bB	7.00 ± 0.10bA
Ln. mesenteroides PON10031	1.40 ± 0.00aA	1.50 ± 0.00aA	1.50 ± 0.00aA	1.50 ± 0.00aA	1.90 ± 0.00aA	8.00 ± 0.10aA	1.40 ± 0.00aA	1.50 ± 0.00aA	1.50 ± 0.00aA	1.70 ± 0.01bB	2.70 ± 0.10aA	6.40 ± 0.00aA
Ln. pseudomesenteroides PON10024	1.40 ± 0.00aA	1.40 ± 0.00aA	1.40 ± 0.00aA	1.40 ± 0.10aA	1.50 ± 0.20aA	5.40 ± 0.10aA	1.20 ± 0.00aA	1.40 ± 0.00aA	1.40 ± 0.10aA	1.60 ± 0.20bA	2.60 ± 0.10bA	6.20 ± 0.10aA
Ln. pseudomesenteroides PON100315	1.80 ± 0.00aA	1.80 ± 0.10aA	1.80 ± 0.20aA	1.80 ± 0.10aA	1.90 ± 0.10aA	6.00 ± 0.00aA	1.20 ± 0.00aA	1.40 ± 0.20bB	1.40 ± 0.10bB	1.60 ± 0.00bB	4.00 ± 0.00bB	7.80 ± 0.00aA
W. cibaria PON10030	1.20 ± 0.20aA	1.20 ± 0.00aA	1.20 ± 0.00aA	1.50 ± 0.00aA	2.20 ± 0.20aA	5.20 ± 0.10aA	1.20 ± 0.00aA	1.40 ± 0.00aA	1.40 ± 0.10bB	1.80 ± 0.10bB	4.60 ± 0.10bA	7.40 ± 0.20bB
W. cibaria PON10032	1.60 ± 0.10aA	1.60 ± 0.20aA	1.60 ± 0.10aA	1.70 ± 0.10aA	2.40 ± 0.00aA	6.00 ± 0.10aA	1.40 ± 0.00aA	1.40 ± 0.00bB	1.40 ± 0.00bB	1.60 ± 0.10aA	4.50 ± 0.10bB	6.20 ± 0.10aA
W. cibaria PON100337	1.40 ± 0.20aA	1.40 ± 0.10aA	1.50 ± 0.20aA	1.70 ± 0.10aA	2.40 ± 0.10aA	5.40 ± 0.00aA	1.30 ± 0.10aA	1.40 ± 0.20aA	1.40 ± 0.10bB	1.60 ± 0.20bB	4.50 ± 0.20bA	6.40 ± 0.20aA
Statistical significance[c]	***	***	***	***	***	***	*	*	**	**	***	*

Abbreviations: SFD, sterile flour dough; nSFD, non sterile flour dough; wnFDS, non sterile water non sterile flour; *Lb., Lactobacillus*; *Ln., Leuconostoc*; *W., Weissella.*
Results indicate mean values ± SD of four measurements (carried out in duplicate for two independent productions).
Lowercase (a,b) and uppercase (A, B) letters indicate different statistical significances according to Tukey's test at *P* values of <0.05 and <0.01, respectively.
[a]P value: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, not significant.

Each dough was divided in two portions: one portion of 80 g was placed in a stainless steel circular baking pan (10 cm diameter) covered with aluminium foil, incubated at 30 °C for 8 h and cooked in the industrial convection oven Modular 80012 DH (Tornati Forno S.r.l, Montelabbate, Italy), at 218 °C for 20 min; the other portion of 120 g was placed in a sterile plastic beaker covered with parafilm and incubated at 30 °C for 21 h. Both dough productions were carried out in duplicate over two consecutive weeks.

2.3. Sourdough analysis

2.3.1. pH and total titratable acidity

Sourdough fermentation was followed by pH [determined electrometrically using the pH meter BASIC 20+ (Crison Instrument S.A., Barcelona, Spain)] and total titratable acidity (TTA, determined by titration with 0.1 N NaOH and expressed in terms of mL of NaOH) tests on 5 g of each sample collected soon after mixing, at 2 h intervals for the first 8 h and then again after 21 h.

2.3.2. Microbiological analysis

The microbial loads were determined immediately after mixing and at 8 and 21 h of fermentation. Ten grams of each sample was suspended in 90 mL of Ringer's solution, homogenised in a stomacher (BagMixer® 400, Interscience, Saint Nom, France) for 2 min at the highest speed and serially diluted. Depending on the LAB inoculated as starter culture, plate counts were performed using SDB agar or MRS agar in addition to plate count agar (PCA) (Oxoid). The microbial suspensions were plated and incubated as follows: total mesophilic count (TMC) on PCA, incubated aerobically at 30 °C for 72 h; *Lb. sanfranciscensis* on SDB agar, incubated aerobically for 48 h at 30 °C, all other LAB on MRS agar, incubated anaerobically for 48 h at 30 °C. Microbiological counts were carried out in duplicate.

The presence of the microorganisms added as starter cultures was confirmed, after colony isolation from the highest dilution of sample suspensions, by microscopic inspection and randomly amplified polymorphic DNA (RAPD) analysis performed as reported by Settanni, Miceli, Francesca, and Moschetti (2012).

2.3.3. Organic acids

Lactic and acetic acid concentration was determined after 8 h of fermentation. Ten grams of each sourdough was homogenised with 90 mL distilled H_2O by stomacher and aliquots of 10 mL were added with 5 mL of 0.1 mmol/L $HClO_4$ solution. The mixtures were centrifuged at 4.000 ×*g* for 15 min at 15 °C and the supernatants were acidified to pH 3.0 ± 0.1 with 1 mmol/L $HClO_4$ and brought to the final volume of 25 mL with distilled H_2O. The solutions were left in ice for 30 min and filtered through 0.45 μm cellulose filters (Millipore). HPLC analyses were conducted as reported by Alfonzo et al. (2013). PerkinElmer software specific to the HPLC instrument (TotalChrom Workstation 2008 rev. 6.3.2) was used to acquire and process data. Analyses were carried out in triplicate and the results expressed as means ± standard deviation.

2.3.4. Volatile organic compounds

The volatile organic compounds (VOC) of the sourdoughs were determined after 8 h of fermentation. A solid phase micro extraction (SPME) isolation technique was used. Five grams of sourdough were heated to 60 °C in a vial and the headspace was collected by a DBV-Carboxen-PDMS fibers (Supelco, Bellefonte, PA) for 40 min. The SPME fibre was inserted directly into a Finnegan Trace MS for GC/MS (Agilent 6890 Series GC system, Agilent 5973 Net Work Mass Selective Detector, Milan, Italy) equipped with a DB-WAX capillary column (Agilent Technologies, 30 m, 0.250 mm i.d., film thickness 0.25 μm, part n° 122-7032) and the analyses were conducted as reported by Alfonzo et al. (2013).

Table 3
Microbial loads (Log CFU/g) of the experimental sourdoughs.

Strains	Sterile flour						Non sterile flour					
	T_0		8 h		21 h		T_0		8 h		21 h	
	PCA	Specific medium	PCA	Specific medium	PCA	Specific medium	PCA	Specific medium	PCA	Specific medium	PCA	Specific medium
Control SFD	<2	MRS < 1 SDB < 2	<2	MRS < 1 SDB < 2	<2	MRS < 1 SDB < 2						
Control nSFD							3.00 ± 0.02	MRS < 1 SDB < 2	5.81 ± 0.03	MRS4.71 ± 0.03 SDB5.35 ± 0.04	6.21 ± 0.06	MRS5.77 ± 0.02 SDB6.71 ± 0.03
Control wnSFD							3.36 ± 0.04	MRS < 1 SDB < 2	6.12 ± 0.01	MRS4.47 ± 0.02 SDB5.26 ± 0.06	6.13 ± 0.05	MRS5.77 ± 0.01 SDB6.78 ± 0.03
Lb. plantarum PON100274	6.05 ± 0.05aA	6.14 ± 0.02 aA	7.15 ± 0.01aA	7.26 ± 0.08aA	8.20 ± 0.05aA	8.44 ± 0.01aA	6.62 ± 0.04aA	6.95 ± 0.02bB	7.81 ± 0.02 bB	8.50 ± 0.03bB	8.56 ± 0.03aA	8.74 ± 0.05bB
Lb. sanfranciscensis LMG 17498[T]	6.11 ± 0.01 aA	6.19 ± 0.02 aA	7.02 ± 0.02 aA	7.30 ± 0.03 aA	7.36 ± 0.01 aA	7.93 ± 0.03 aA	6.27 ± 0.02 aA	6.46 ± 0.03 aA	7.29 ± 0.01 aA	7.30 ± 0.01 aA	7.69 ± 0.02aA	7.96 ± 0.03aA
Lb. sanfranciscensis PON100100	6.44 ± 0.01aA	6.60 ± 0.0 aA	6.96 ± 0.09 aA	7.10 ± 0.01aA	7.46 ± 0.03aA	8.20 ± 0.04aA	6.25 ± 0.09aA	6.50 ± 0.05aA	6.83 ± 0.07 aA	7.34 ± 0.08aA	8.09 ± 0.07bB	8.29 ± 0.02aA
Lb. sanfranciscensis PON100336	6.23 ± 0.02aA	6.48 ± 0.03aA	6.80 ± 0.01 aA	7.20 ± 0.02aA	7.27 ± 0.04aA	7.78 ± 0.03aA	6.39 ± 0.09aA	6.52 ± 0.01aA	7.33 ± 0.03 bB	7.70 ± 0.02aA	8.43 ± 0.03bB	8.95 ± 0.07bB
Lb. sakei PON10098	6.52 ± 0.05aA	6.74 ± 0.01aA	8.64 ± 0.07 aA	8.82 ± 0.09aA	8.78 ± 0.01aA	9.11 ± 0.07aA	6.16 ± 0.05aA	6.90 ± 0.06aA	8.53 ± 0.02 bB	9.04 ± 0.02bB	8.58 ± 0.03bB	9.21 ± 0.01bB
Ln. citreum PON10021	6.59 ± 0.01aA	6.60 ± 0.02aA	7.67 ± 0.01 aA	7.78 ± 0.02aA	8.54 ± 0.03aA	8.60 ± 0.02aA	6.03 ± 0.03aA	6.11 ± 0.09aA	7.44 ± 0.06 bB	8.32 ± 0.04bB	8.94 ± 0.06bB	9.33 ± 0.05aA
Ln. citreum PON10079	6.05 ± 0.09aA	6.18 ± 0.06aA	8.07 ± 0.09 aA	8.63 ± 0.03aA	8.56 ± 0.10aA	8.96 ± 0.02aA	6.03 ± 0.07aA	6.85 ± 0.02bB	8.06 ± 0.05 aA	8.75 ± 0.01bB	8.27 ± 0.02bB	8.97 ± 0.03aA
Ln. citreum PON10080	6.17 ± 0.02aA	6.30 ± 0.02aA	8.50 ± 0.01 aA	9.10 ± 0.10aA	8.58 ± 0.07aA	9.21 ± 0.05aA	6.59 ± 0.02aA	6.80 ± 0.04aA	8.48 ± 0.04 aA	9.23 ± 0.05bB	9.34 ± 0.08bB	9.38 ± 0.03bB
Ln. mesenteroides PON10031	6.47 ± 0.01aA	6.60 ± 0.00aA	8.32 ± 0.01 aA	8.92 ± 0.04aA	8.95 ± 0.04aA	9.24 ± 0.01aA	6.33 ± 0.01aA	6.58 ± 0.03aA	8.56 ± 0.01bA	8.90 ± 0.07aA	8.83 ± 0.11aA	8.93 ± 0.01bB
Ln. pseudomesenteroides PON10024	6.17 ± 0.06aA	6.50 ± 0.03 aA	7.72 ± 0.09 aA	7.84 ± 0.02aA	7.99 ± 0.02aA	8.11 ± 0.03aA	6.28 ± 0.02aA	6.69 ± 0.03bB	8.20 ± 0.02 aA	8.83 ± 0.03bB	8.34 ± 0.05aA	9.03 ± 0.06bB
Ln. pseudomesenteroides PON100315	6.11 ± 0.07aA	6.24 ± 0.05aA	8.67 ± 0.07 aA	8.77 ± 0.04aA	8.76 ± 0.04aA	8.96 ± 0.05aA	6.55 ± 0.02aA	6.99 ± 0.01bA	8.36 ± 0.05 bB	8.79 ± 0.03aA	8.45 ± 0.01aA	8.93 ± 0.09aA
W. cibaria PON10030	6.63 ± 0.06aA	6.70 ± 0.02aA	7.92 ± 0.06aA	8.62 ± 0.01aA	8.57 ± 0.02aA	9.12 ± 0.11aA	6.20 ± 0.05bA	6.60 ± 0.05bB	8.77 ± 0.02 bB	8.81 ± 0.01bB	8.90 ± 0.03aA	8.82 ± 0.04bB
W. cibaria PON10032	6.22 ± 0.03aA	6.85 ± 0.07 aA	8.12 ± 0.02 aA	8.18 ± 0.05aA	8.31 ± 0.02aA	8.56 ± 0.08aA	6.33 ± 0.06aA	6.76 ± 0.09bB	8.60 ± 0.08 aA	9.06 ± 0.05bB	9.50 ± 0.04bA	9.72 ± 0.03bB
W. cibaria PON100337	6.41 ± 0.08aA	6.70 ± 0.07aA	8.20 ± 0.01 aA	8.83 ± 0.02	8.51 ± 0.04aA	9.17 ± 0.05aA	6.47 ± 0.04aA	6.80 ± 0.02aA	8.18 ± 0.04 aA	8.68 ± 0.02	8.58 ± 0.01aA	9.05 ± 0.01bB
Statistical significance[c]	***	***	***	***	***	***	***	***	***	***	***	***

Abbreviations: SFD, sterile flour dough; nSFD, non sterile flour dough; wnFDS, non sterile water non sterile flour; *Lb.*, *Lactobacillus*; *Ln.*, *Leuconostoc*; *W.*, *Weissella*.
Results indicate mean values ± SD of four plate counts (carried out in duplicate for two independent productions).
Lowercase (a,b) and uppercase (A, B) letters indicate different statistical significances according to Tukey's test at *P* values of <0.05 and <0.01, respectively.
[a]*P* value: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, not significant.

Table 4
Organic acids produced by LAB in sourdoughs processed with non sterile flour after 8 h of fermentation.

Strains	Lactic acid (mg/g)	Acetic acid (mg/g)	FQ
Control SFD (T_0)	0.00	0.00	
Control SFD (T_8)	1.31 ± 0.05	0.15 ± 0.01	8.73
Control wnSFD (T_0)	1.11 ± 0.03	0.17 ± 0.02	6.52
Control wnSFD (T_8)	1.31 ± 0.05	0.25 ± 0.03	5.24
Lb. plantarum PON100274	6.47 ± 0.02	0.67 ± 0.07	9.65
Lb. sanfranciscensis LMG 17498^T	1.36 ± 0.02	0.30 ± 0.04	4.53
Lb. sanfranciscensis PON100100	2.24 ± 0	0.47 ± 0.07	4.77
Lb. sanfranciscensis PON100336	4.81 ± 0.06	0.61 ± 0.02	7.88
Lb. sakei PON10098	3.96 ± 0.09	0.25 ± 0.01	15.84
Ln. citreum PON10021	2.28 ± 0.10	0.25 ± 0.03	9.12
Ln. citreum PON10079	2.45 ± 0.04	0.68 ± 0.02	3.60
Ln. citreum PON10080	3.47 ± 0.07	0.87 ± 0.13	3.99
Ln. mesenteroides PON10031	2.81 ± 0.03	0.70 ± 0.06	4.01
Ln. pseudomesenteroides PON10024	4.17 ± 0.12	0.97 ± 0.09	4.30
Ln. pseudomesenteroides PON100315	3.99 ± 0.11	1.08 ± 0.06	3.69
W. cibaria PON10030	3.31 ± 0.08	0.84 ± 0.05	3.94
W. cibaria PON10032	1.42 ± 0.02	0.46 ± 0.04	3.09
W. cibaria PON100337	2.45 ± 0.09	0.40 ± 0.02	6.12
Statistical significance[a]	***	***	n.d.

Abbreviations: SFD, sterile flour dough; nSFD, non sterile flour dough; wnFDS, non sterile water non sterile flour; *Lb.*, *Lactobacillus*; *Ln.*, *Leuconostoc*; *W.*, *Weissella*; n.d., not determined.
Results indicate mean values ± SD of four measurements (carried out in duplicate for two independent productions).
P value: ***, $P \leq 0.01$.
n.d., not determined.

All compounds in the range m/z 33–495 atomic mass unit (amu) were detected by the scan mode. The identification of the individual peaks was obtained by direct comparison of their retention indices to those of authentic samples, as well as by comparing their mass spectra with the NIST/EPA/NIH Mass Spectral Library database (Version 2.0d, build 2005). The contents of the volatile compounds were expressed as relative peak areas (peak area of each compound/total area) × l00.

All solvents and reagents were purchased from WWR International (Milan, Italy). Chemical and physical determinations were performed in triplicate and the results expressed as means ± standard deviation.

2.4. Bread analysis

The bread quality attributes were evaluated after cooling at ambient temperature. After weighing, each bread was cut transversely in two halves and the height of the central slice was measured (Schober, Messerschmidt, Bean, Park, & Arendt, 2005).

Colour was measured on four points of the crust and three points of the crumb of the central slices by means of a colorimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan). The Hunter's scale parameters were determined: L*, a* and b*.

The hardness of crumb was determined by measuring its resistance in four points to the plunger (6 mm diameter stainless steel cylinder probe) of a digital penetrometer (Tr snc, Italy).

The two central slices of each loaf were scanned (Epson Perfection 4180 Photo, Seiko Epson Corp., Japan) with 350 dpi of resolution and the images were saved in TIFF format. The images were analysed with the ImageJ software (National Institutes Health, Bethesda, Md, USA). Each image was cropped to a square of 207 × 207 pixels (representing 15 × 15 mm of the slice area) and converted to grey-level image (8 bit). A binary image was obtained applying the Otsu's threshold algorithm, in order to calculate void fraction (the fraction of the total area corresponding to the bread pores), cell density (number of cells/cm^2) and mean cell area in mm^2.

2.5. Statistical analyses

Data of acidification, TTA, microbial load and organic acid concentration of sourdoughs and height, colour, hardness, void fraction, cell density and mean cell area of the resulting breads were statistically analysed using the ANOVA procedure with the software SAS 2004, version 9.1.2 (Statistical Analysis System Institute Inc., Cary, NC, USA). Differences between means were determined by Tukey's multiple-range test.

3. Results and discussion

3.1. pH, total titratable acidity and microbiological analysis of sourdoughs

The pH values registered for the experimental sourdoughs produced with sterile and non-sterile flour are reported in Table 1. Although other studies carried out to evaluate the ability of different LAB strains to act as starter cultures for sourdough products (Choi, Kim, Hwang, Kim, & Yoon, 2012; Plessas et al., 2007, 2008) tested the performances of the bacteria in untreated flour, since our study was focused on the selection of LAB, we started the screening of the strains with a sterile flour, in order to follow each culture without interference from the native microbiota within the flour.

The trials carried out with sterile flour and LAB showed an initial pH value significantly lower than the corresponding control trial (control SFD). The last dough showed only a slight pH decrease (from 5.95 to 5.74) during the 21 h of fermentation. The dough inoculated with LAB behaved similarly during the first 4 h of observation, while a different pH was registered from the 6 h onward: all *W. cibaria* strains and *Ln. citreum* PON10080 determined a lower pH than the other trials, both at 6 and 8 h, and their values were not statistically different. After 21 h of incubation, all 13 LAB dropped the pH of the sourdoughs below 4.0. Regarding the doughs produced with non-sterile flours, their pHs were all higher than 6.0 at T_0. Both control trials (with sterile and non-sterile H_2O) showed a pH decrease stronger than control dough made with sterile flour, but the value registered after 21 h was above 5.0. However, control nSFD showed a lower final pH than control wnSFD. The doughs started with non-sterile flour and LAB showed differences with the corresponding trials made with sterile flour: at 6 h, in addition to *W. cibaria* PON10030 and PON10032, *Ln. citreum* PON10079 and *Ln. mesenteroides* PON10031 displayed a fast decrease in pH value; at 8 h, the results of these four strains were superimposable with those of *W. cibaria* PON100337 and *Lb. sakei* PON10098; except *Ln. pseudomesenteroides* PON10024, all other LAB determined the decrease of pH below 4.0 after 21 h of fermentation. In general, a similar trend was observed for the LAB used as starter cultures in both conditions, with sterile and non-sterile flour. Our results are comparable with those reported by Moroni, Arendt, and Dal Bello (2001) who followed buckwheat and teff sourdoughs spontaneously fermented with lactobacilli, leuconostocs and weissellas.

TTA data (Table 2) confirmed the observations made with the pH results. After 8 h of fermentation, all weissellas and *Ln. citreum* PON10079 and PON10080 were the most acidifying strains in the presence of sterile and non-sterile flour. *Ln. citreum* strains applied in this work showed kinetics of acidification more rapid than that displayed by *Ln. citreum* HO12, of kimchii origin, tested in sourdough in conditions similar to those of our study at the same initial concentration of 10^6 CFU/g (Choi et al., 2012).

The bacterial inocula were followed during fermentation by plate counts (Table 3). All bacteria in both conditions increased their cell concentrations after 8 h. The lowest increase was registered for both *Lb. sanfranciscensis* strains evaluated, while the highest values were shown by *Ln. citreum* PON10080. After 21 h of fermentation, LAB concentrations increased by at least 1.5 orders of magnitude than T_0. No major differences were found among the concentrations in the corresponding trials carried out with sterile and non-sterile flours. Control SFD was characterised by undetectable levels of total microorganisms and LAB during the whole period of fermentation; on the contrary, control nSFD and control wnSFD, although displaying undetectable levels of LAB at T_0, showed levels of 10^4 and 10^5 CFU/g on

Table 5
Analysis of volatile organic compounds emitted from sourdoughs made from untreated wheat flour inoculated with LAB and fermented for 8 h.

Chemical compounds[a]	Sourdoughs[b]																	
	SFD (T_0)	SFD (T_8)	wnSFD (T_0)	wnSFD (T_8)	10030	10032	10079	10080	100337	100336	100100	10098	10031	100274	100315	10021	10024	17498
Ethanol	0.00	0.00	0.00	0.00	87.95	178.75	93.55	88.51	93.72	43.40	37.68	0.00	194.62	0.00	141.68	0.00	122.39	0.00
Hexanal	1.05	1.74	0.36	1.00	0.35	1.01	0.43	0.31	0.49	1.62	0.46	0.98	1.07	0.74	0.25	0.43	0.19	0.62
3-Methyl-1-butanolacetate	0.00	0.00	0.00	0.00	0.00	0.18	0.06	0.33	1.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00
3-Methyl-1-butanol	3.43	2.42	1.65	1.82	0.90	2.65	3.73	10.76	33.86	12.33	10.14	0.61	2.41	1.96	0.61	1.93	15.88	0.27
1-Pentanol	0.79	0.76	0.82	0.30	0.53	1.11	1.12	1.12	0.91	1.63	1.09	1.34	1.04	0.65	0.78	0.73	0.98	0.33
2-Butanone-3-Hydroxy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.95	0.00	0.68	0.00	0.00
Tridecane	0.43	0.23	0.89	0.20	0.00	0.41	0.00	0.27	0.00	0.45	0.18	0.10	0.00	0.07	0.82	0.00	0.00	0.00
2-Heptenal	0.48	0.24	0.22	0.29	0.41	0.18	0.52	0.28	0.36	0.45	0.38	0.41	0.44	0.14	0.25	0.49	0.26	0.22
Ethyl lactate	0.00	0.00	0.00	0.00	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.22	0.00	3.21	0.00	0.88	0.00
1-Hexanol	5.52	4.11	5.46	4.05	9.27	15.01	11.38	10.93	11.99	14.13	9.26	5.99	15.18	7.25	6.62	4.91	8.29	2.34
Nonanal	1.20	0.37	0.14	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.10	0.00	0.47
2-Octenal	0.72	0.44	0.09	0.28	0.90	0.89	0.37	0.34	0.34	0.45	0.36	0.64	0.80	0.60	0.33	0.00	0.52	0.35
2-Pentanol	0.04	0.06	0.07	0.09	0.06	0.19	0.10	0.09	0.14	0.08	0.08	0.07	0.10	0.09	0.07	0.08	0.11	0.03
Ethyl octanoate	0.18	0.00	0.03	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.02	0.19	0.00
Acetic acid	0.24	0.30	0.60	0.25	7.74	5.11	14.66	11.56	10.57	1.93	1.51	1.21	17.91	0.84	17.16	0.95	7.40	0.05
1-Octen-3-ol	1.96	1.42	1.17	1.40	1.30	1.87	1.61	1.47	2.04	2.41	1.73	1.43	2.26	1.39	1.81	1.45	1.73	0.82
1-Heptanol	0.75	0.50	0.48	0.49	1.07	1.51	1.18	1.03	1.32	1.02	0.99	0.53	1.10	0.77	1.03	0.58	0.95	0.23
6-Methyl-5-hepten-2-ol	0.19	0.09	0.16	0.13	0.20	0.23	0.17	0.17	0.20	0.24	0.14	0.12	0.16	0.12	0.19	0.10	0.14	0.03
2-Ethylhexanol	0.97	0.46	0.87	0.61	0.45	0.87	0.52	0.46	0.38	0.66	0.67	0.00	0.58	0.57	0.53	0.61	0.49	0.21
Benzaldehyde	2.63	2.18	1.71	2.49	3.25	0.91	3.29	5.08	1.92	1.47	1.93	1.26	2.14	3.50	3.04	2.11	2.15	1.84
2-Hepten-1-ol	0.00	0.06	0.00	0.00	0.47	0.73	1.02	0.62	0.92	0.44	0.27	0.90	0.76	0.00	0.71	0.00	0.56	0.03
2-Nonenal	0.98	1.27	0.39	1.49	1.42	0.34	0.68	0.74	0.54	0.57	0.63	0.00	0.42	1.20	0.91	0.00	1.06	0.79
Propanoic acid	0.11	3.05	0.10	0.85	0.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.36	0.86	0.71	0.00
Ethyl-2-Hydroxyhexanoate	0.00	0.00	0.00	0.00	0.00	0.04	0.08	0.04	0.17	0.00	0.04	0.00	0.00	0.00	0.10	0.00	0.03	0.00
3,5-Octadien-2-one	0.14	0.11	0.05	0.14	0.11	0.15	0.04	0.04	0.10	0.17	0.10	0.06	0.00	0.08	0.09	0.18	0.04	0.12
1-Octanol	0.92	0.58	0.43	0.59	0.69	1.07	0.70	0.42	0.65	0.70	0.71	0.47	0.79	0.59	0.86	0.60	0.59	0.38
g-Butyrolactone	0.10	0.10	0.10	0.05	0.08	0.14	0.12	0.18	0.22	0.18	0.19	0.17	0.13	0.16	0.08	0.04	0.07	0.03

2-Octen-1-ol	0.00	1.86	0.00	0.31	0.57	0.60	0.00	0.73	0.80	0.28	0.22	0.00	0.35	0.00	0.69	0.26	0.67	0.00
3-Nonen-1-ol	0.11	1.61	0.00	0.70	0.68	0.90	1.67	0.59	0.78	0.27	0.20	0.64	1.53	0.00	0.76	0.60	0.64	0.18
Dodecanal	0.00	0.00	0.00	0.00	0.00	0.13	0.25	0.48	2.43	0.84	0.95	0.34	0.00	0.00	0.00	0.00	0.00	0.00
2-Methylbenzaldehyde	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.49	0.19	0.21	0.17	0.07	0.00	0.00	0.00	0.00	0.00
2-Nonen-1-ol	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	0.61	0.31	0.30	0.00	0.47	0.00	0.00	0.00	0.00	0.00
Phenylmethylacetate	0.39	0.00	0.00	0.00	1.01	0.00	2.17	2.86	1.25	0.00	0.00	0.00	0.00	0.24	0.78	0.00	0.82	0.00
Pentanoic acid	0.00	0.00	0.00	0.00	0.16	0.27	0.21	0.09	0.13	0.12	0.07	0.20	0.31	0.12	0.21	0.05	0.14	0.00
2,4-Decadienal	0.19	0.13	0.04	0.00	0.42	0.69	0.21	0.07	0.13	0.16	0.10	0.12	0.63	0.24	0.37	0.00	0.08	0.12
Methylnaphthalene	0.00	0.03	0.00	0.00	0.07	0.06	3.99	3.51	0.08	0.05	0.04	7.05	0.00	0.00	0.00	0.00	0.00	0.00
Hexanoic acid	0.00	0.00	0.00	0.00	5.18	5.40	2.78	2.29	2.46	6.53	4.71	0.00	7.69	2.89	5.45	9.44	4.31	0.00
Benzyl alcohol	22.38	16.02	11.24	9.71	57.10	15.74	64.37	121.52	24.69	8.19	25.82	10.14	10.33	67.33	76.01	19.74	48.47	14.28
Phenylethyl Alcohol	0.58	0.23	0.10	0.18	0.24	0.39	0.33	0.30	0.35	0.32	0.35	0.29	0.20	0.41	0.27	0.19	0.17	0.33
Tridecanal	0.00	0.00	0.00	0.00	0.00	0.46	0.00	0.22	0.94	0.00	0.21	0.19	0.00	0.00	0.00	0.00	0.00	0.00
1,4-Butanediol	0.04	0.05	0.07	0.00	0.09	0.29	0.13	0.13	0.22	0.22	0.10	0.10	0.12	0.07	0.11	0.00	0.03	0.00
2-Ethylhexanoic acid	0.00	0.04	0.00	0.00	0.05	0.28	0.02	0.19	0.19	0.12	0.01	0.14	0.19	0.17	0.10	0.05	0.07	0.00
Phenol	0.15	0.23	0.20	0.20	0.28	0.38	0.20	0.23	0.29	0.24	0.24	0.22	0.54	0.27	0.26	0.19	0.15	0.09
g-Nonalactone	0.31	0.32	0.36	0.32	0.36	0.49	0.29	0.27	0.33	0.51	0.33	0.29	0.38	0.30	0.45	0.40	0.35	0.13
p-Phthalaldehyde	0.12	0.12	0.24	0.12	0.13	0.31	0.29	0.30	0.38	0.41	0.38	0.23	0.33	0.24	0.15	0.12	0.07	0.00
Isophthalaldehyde	0.09	0.10	0.13	0.06	0.23	0.20	0.26	0.29	0.36	0.39	0.38	0.24	0.19	0.20	0.11	0.05	0.04	0.26
Pentadecanal	0.05	0.00	0.13	0.00	0.00	0.11	0.13	0.14	1.18	0.28	0.36	0.08	0.11	0.00	0.14	0.09	0.04	0.00
Ethylhexadecanoate	0.00	0.00	0.00	0.00	0.09	0.12	0.04	0.03	0.09	0.00	0.14	0.00	0.12	0.00	0.05	0.04	0.09	0.00
Diethylphthalate	0.54	0.04	0.06	0.00	1.16	0.13	0.12	0.13	1.38	0.28	0.24	0.11	0.00	0.08	0.09	0.00	0.00	0.22
Benzoic acid	0.04	0.10	0.00	0.42	0.11	0.00	0.06	0.09	0.11	0.04	0.10	0.14	0.11	0.10	0.04	0.04	0.00	0.00
Benzophenone	0.24	0.10	0.07	0.18	0.14	0.11	0.09	0.14	0.14	0.11	0.10	0.18	0.22	0.10	0.19	0.08	0.00	0.05

Results indicate mean values of four measurements (carried out in duplicate for two independent productions) and are expressed as relative peak areas (peak area of each compound/total area) × l00.

[a] The chemicals are shown following their retention time.

[b] Sourdoughs: SFD, sterile flour dough; wnFDS, non sterile water non sterile flour; *Lb. plantarum* PON100274; *Lb. sanfranciscensis* LMG 17498^{T}; *Lb. sanfranciscensis* PON100100; *Lb. sanfranciscensis* PON100336; *Lb. sakei* PON10098; *Ln. citreum* PON10021; *Ln. citreum* PON10079; *Ln. citreum* PON10080; *Ln. mesenteroides* PON10031; *Ln. pseudomesenteroides* PON10024; *Ln. pseudomesenteroides* PON100315; *W. cibaria* PON10030; *W. cibaria* PON10032; *W. cibaria* PON100337.

MRS and SDB, respectively, at 8 h and 10^5 and 10^6 CFU/g on MRS and SDB, respectively, after 21 h. Thus, the levels of LAB inoculated with the selected strains were not affected by the indigenous LAB of commercial flour and reached the typical final concentrations obtained with similar approaches (Choi et al., 2012; Moroni et al., 2001). However, the conditions of sourdough propagation might influence the interactions between LAB (Corsetti, Settanni, Valmorri, et al., 2007). Minervini, Lattanzi, De Angelis, Di Cagno, and Gobbetti (2012) reported that the LAB populations differed among sourdoughs propagated at artisan bakery and laboratory levels: *Lb. plantarum*, *Lb. sakei*, and *W. cibaria* dominated in some sourdoughs back-slopped at artisan bakeries, while *Ln. citreum* were more persistent under laboratory conditions.

3.2. Bacterial comparison

The isolates collected from MRS and SDB resulting from the plate counts performed at 8 h, at the highest dilutions of samples, were analysed at strain level by means of RAPD-PCR with primer M13. Amplified DNAs from the isolates of a given trial, together with that of the pure culture corresponding to the same trial, were loaded onto a gel in order to recognise the added bacteria through fermentation and to evaluate their contribution to the concentrations estimated. The direct comparison of the RAPD patterns (results not shown) allowed the recognition of the added cultures in both conditions of flour used. The RAPD profiles of the LAB isolated at the highest concentrations from the control doughs made with non-sterile flour (results not shown) excluded the presence of any of the 13 LAB used in the commercial flour in this study.

The use of RAPD analysis to monitor the dominance and/or the persistence of added strains is commonly applied in sourdough preparation and propagation (Ehrmann & Vogel, 2005; Minervini et al., 2010; Settanni, Massitti, Van Sinderen, & Corsetti, 2005) and our results highlighted the relevance of this technique to rapidly monitor the fermenting LAB that are responsible for the successful sourdough fermentation.

3.3. Chemical analysis of sourdoughs

Organic acid production and VOC generation were determined only for the sourdoughs obtained with the non-sterile flour, in order to evaluate the contribution of each LAB in production conditions.

The concentrations of the organic acids and the resulting FQ of the doughs are reported in Table 4. Lactic acid was in the range 1.36 – 6.47 mg/g with the lowest value shown by *Lb. sanfranciscensis* LMG 17498^T and the highest by *Lb. plantarum* PON100274. The lowest value of acetic acid was 0.25 mg/g and the highest 1.08 mg/g as registered for *Lb. sakei* PON10098 and *Ln. pseudomesenteroides* PON100315, respectively. FQ ranged between 3.09 and 15.84; the doughs started with *Ln. citreum* PON10079 and PON 10080, *Ln. pseudomesenteroides* PON100315 and *W. cibaria* PON10030 and PON10032 were characterised by a FQ comprised in the range 1.5 – 4 that is considered to affect positively the aroma profile and the structure of the final products (Spicher, 1983), but only *W. cibaria* PON10032 determined an FQ close to the optimal range of 2.0 – 2.7 suggested by Hammes and Gänzle (1998). Lactic acid can gradually account for a more elastic gluten structure. Acetic acid, produced by heterofermentative LAB, is responsible for a shorter and harder gluten (Lorenz, 1983) and shows antirope and antimould effects (Rosenquist & Hansen, 1998).

VOC composition resulting from the chromatographic analysis is reported in Table 5. In the headspace of sourdoughs, 51 compounds were identified: 5 acids, 16 alcohols, 13 aldehydes, 1 acetate, 1 alkane, 9 esters, 3 ketones, 2 lactones and 1 phenol. Several compounds, whose presence is associated with the metabolism of LAB, are able to affect the final bread since may provide unique flavours that contribute to a pleasant aroma and taste (Hansen & Hansen, 1996). Most of the compounds identified in the experimental sourdoughs are reported to be relevant for the breads processed with this technology (Hansen & Lund, 1987; Seitz, Chung, & Rengarajan, 1998), even though not all volatile compounds detected by instrumental analysis have a perceptible aroma (Meignen et al., 2001). The compounds that strongly affect bread flavour are mainly organic acids, alcohols, esters and carbonyls (Czerny & Schieberle, 2002; Kirchhoff & Schieberle, 2002).

Except in doughs started with *Lb. sakei* PON 10098, *Lb. plantarum* PON100274, *Ln. citreum* PON10021 and *Lb. sanfranciscensis* LMG 17498^T, ethanol was the VOC quantitatively most present in all other doughs. However, the amounts of ethanol revealed should not particularly affect the bread quality because the ethanol produced by baker's yeast is much higher than the level produced by LAB (Choi et al., 2012). Benzyl alcohol was the second VOC in terms of concentration, followed by 1-hexanol. Although ethanol was detected only after the 8-h fermentation period, benzyl alcohol and 1-hexanol were already present in the non-fermented doughs. Among the acid component of VOC, acetic acid was that produced at the highest level for the majority of doughs. The dough inoculated with *W. cibaria* PON10030 showed the highest concentrations of 2-nonenal and propanoic acid. *Ln. citreum* PON10079 and PON10080 determined the highest level of phenylmethylacetate. Although nonanal is reported to be present at high amounts in sourdough breads (Seitz et al., 1998), it was inversely linked to the fermentation, since it almost disappeared after 8 h. Not all sourdoughs were characterised by the increase of benzaldehyde, as commonly reported for this kind of products (Chang, Seitz, & Chambers, 1995; Seitz et al., 1998). The most noticeable differences among LAB were found for 3-methyl-1-butanol, ranging between 0.27 and 33.86, whose concentration is known to be dependent on the LAB strain performing the fermentation (Gobbetti et al., 1995).

Sourdough fermentation is essential to achieve an acceptable flavour; the comparison between chemically acidified bread and sourdough bread showed that the latter possessed a superior sensory quality (Kirchhoff & Schieberle, 2002). However, the overall aroma profile of final bread, is due to the type of dominating LAB (Corsetti & Settanni, 2007). Besides acetic acid, other compounds may play a defining role in the composition of VOCs. E.g. ethyl acetate content was higher in sourdoughs fermented with heterofermentative LAB compared to sourdoughs fermented with homofermentative LAB, while an opposite trend was observed for the content of aldehydes that was higher in sourdoughs fermented with homofermentative cultures (Lund, Hansen, & Lewis, 1989). However, due to evaporation during baking, the amounts of alcohols, esters and diacetyl in sourdough bread are much lower than in the corresponding sourdough (Hansen & Hansen, 1996; Lund et al., 1989).

3.4. Characterisation of breads

After baking, the experimental breads were subjected to several determinations. In the experimentation carried out with sterile flour (Table 6), *Ln. citreum* PON10079 and PON10080 and all *W. cibaria* strains determined a final height of breads consistently higher than control bread, while in the productions performed with non-sterile flour, the height reached with *Lb. sanfranciscensis* PON100100 was above those obtained with *Ln. citreum* and *W. cibaria* strains.

The colour of both the crust and crumb of control breads and those obtained with doughs inoculated with LAB with sterile or non-sterile flour were almost comparable, with only small differences registered for the parameters a* and b* of the crust for the sterile flour experimentation and b* of the crust for the non-sterile flour experimentation. Despite the fact that, in comparison with a non-inoculated dough the addition of LAB causes significant changes in the Hunter's scale parameters (García-Argueta et al., 2013), the colour of the final breads obtained in this study was not influenced by the different LAB strains added. However, all sourdoughs inoculated with LAB were different from the control doughs.

The hardness was greatly influenced by LAB. In general, the softness of the final breads was directly correlated with the acidification kinetics,

Table 6
Characteristics of experimental breads.

Strains	Height (mm)		Crust colour						Crumb colour						Hardness (N)		Void fraction (%)		Cell density (n. cm^{-2})		Mean cell area (mm^2)	
			L*		a*		b*		L*		a*		b*									
Experimentation with sterile flour																						
control SFD	26.00	bc	60.45	ab	1.62	ad	27.09	ac	57.70	ab	0.20	a	19.75	a	26.53	a	18.58	ef	65.60	cd	0.32	cd
Lb. plantarum PON100274	27.60	bc	61.53	ab	1.16	cd	24.29	de	55.82	ab	−0.49	ab	17.39	b	31.25	a	12.62	f	78.90	bd	0.10	f
Lb. sanfranciscensis LMG 17498^T	28.00	b	61.90	ab	1.49	ad	25.50	ce	61.69	ab	−0.09	ab	18.25	ab	15.18	ce	39.00	a	108.26	a	0.37	Bd
Lb. sanfranciscensis PON100100	28.30	b	61.68	ab	1.41	ad	25.48	ce	63.38	ab	−0.13	ab	18.43	ab	17.13	bc	39.36	a	101.95	ab	0.42	ad
Lb. sanfranciscensis PON100336	31.30	ab	62.01	ab	1.61	ad	25.62	be	60.61	ab	0.12	ab	18.03	ab	13.88	cf	38.57	a	111.70	a	0.35	Bd
Lb. sakei PON10098	22.00	c	58.57	b	1.09	d	23.82	e	56.29	ab	−0.08	ab	17.33	b	20.63	b	22.09	ce	85.99	ac	0.26	De
Ln. citreum PON10021	26.00	bc	60.91	ab	1.29	bd	25.18	ce	54.27	b	0.06	ab	18.25	ab	21.15	b	39.61	a	96.48	ab	0.42	Ad
Ln. citreum PON10079	36.20	a	62.65	ab	1.88	a	26.91	ac	64.63	ab	−0.12	ab	17.75	ab	15.25	cd	39.52	a	101.95	ab	0.40	Ad
Ln. citreum PON10080	34.00	ab	62.13	ab	1.47	ad	26.17	ae	62.58	ab	−0.31	ab	17.49	b	11.48	df	27.91	cd	78.46	bd	0.35	Bd
Ln. mesenteroides PON10031	32.00	ab	63.02	a	1.76	ab	27.90	ab	62.68	ab	−0.17	ab	17.89	ab	13.35	cf	29.63	bc	57.62	d	0.51	Ab
Ln. pseudomesenteroides PON10024	29.00	b	62.62	ab	1.46	ad	26.30	ad	61.85	ab	−0.58	b	17.51	b	15.20	ce	21.44	de	87.32	ac	0.16	Ef
Ln. pseudomesenteroides PON100315	29.50	b	60.84	ab	1.29	bd	25.80	be	60.74	ab	−0.53	b	16.51	b	10.10	f	36.69	ab	66.05	cd	0.56	A
W. cibaria PON10030	36.00	a	63.37	a	1.30	ad	27.47	ac	62.89	ab	−0.59	b	17.56	b	10.15	ef	40.25	a	90.43	ac	0.46	Ac
W. cibaria PON10032	34.20	ab	63.39	a	1.81	ab	28.32	a	65.68	a	−0.43	ab	18.49	ab	13.80	cf	42.50	a	93.31	ac	0.46	Ac
W. cibaria PON100337	35.80	a	62.39	ab	1.69	ac	26.22	ae	62.10	ab	−0.26	ab	17.26	b	10.50	df	38.72	a	92.64	ac	0.43	Ad
SEM	1.25		0.21		0.04		0.18		1.02		0.07		0.21		1.48		2.78		6.18		0.03	
Significance	**		***		***		***		**		**		**		***		***		***		***	
Experimentation with non-sterile flour																						
control nSFD	20.00	c	64.83	bd	−0.19	ab	20.50	de	65.94	ab	−1.33	ab	15.96	a	29.38	a	20.77	fg	108.60	ac	0.19	c
control wnSFD	20.00	c	63.87	cd	−0.34	b	20.58	ce	63.81	b	−1.36	ab	16.11	a	28.43	ab	15.58	g	110.82	ab	0.15	c
Lb. plantarum PON100274	24.00	c	67.53	ac	−0.13	ab	19.59	e	68.64	ab	−1.37	ab	15.66	ab	22.08	ac	14.85	g	103.94	ae	0.14	c
Lb. sanfranciscensis LMG 17498^T	30.50	b	68.32	ab	0.08	ab	22.76	ac	71.24	a	−1.47	ab	15.98	a	17.98	bd	37.52	bc	102.29	ae	0.38	bc
Lb. sanfranciscensis PON100100	36.00	a	68.66	a	0.46	a	23.53	ab	69.42	ab	−1.42	ab	14.34	ab	15.23	cd	36.43	bc	101.51	ae	0.36	c
Lb. sanfranciscensis PON100336	30.00	b	68.36	ab	0.00	ab	21.96	be	72.19	a	−1.55	ab	16.00	a	20.55	ad	39.44	ac	103.94	ae	0.39	bc
Lb. sakei PON10098	22.00	c	62.13	d	0.32	ab	20.57	ce	69.59	ab	−1.22	a	14.23	ab	20.48	ad	25.99	df	123.01	a	0.21	c
Ln. citreum PON10021	23.00	c	67.04	ac	−0.24	ab	19.56	e	68.92	ab	−1.28	a	14.88	ab	27.13	ab	22.64	eg	127.88	a	0.18	c
Ln. citreum PON10079	32.00	ab	68.36	ab	0.05	ab	24.08	ab	68.94	ab	−1.35	ab	14.04	ab	14.70	cd	43.91	ab	68.04	be	0.66	ab
Ln. citreum PON10080	33.00	ab	67.46	ac	−0.27	b	23.81	ab	68.49	ab	−1.42	ab	13.51	b	11.90	d	32.79	ce	107.49	ad	0.31	c
Ln. mesenteroides PON10031	32.00	ab	68.11	ab	−0.01	ab	22.53	ac	70.09	ab	−1.22	a	15.16	ab	16.43	cd	48.80	a	67.15	ce	0.73	a
Ln. pseudomesenteroides PON10024	31.00	ab	68.22	ab	0.02	ab	23.42	ab	65.92	ab	−1.48	ab	14.73	ab	13.95	cd	35.01	bd	97.74	ae	0.36	c
Ln. pseudomesenteroides PON100315	30.00	b	69.33	a	−0.10	ab	23.00	ac	66.45	ab	−1.28	a	14.53	ab	19.55	bd	38.55	ac	62.72	d	0.65	ab
W. cibaria PON10030	30.00	b	68.62	ab	0.08	ab	23.16	ac	70.92	a	−1.32	ab	14.87	ab	12.83	cd	42.92	ac	64.72	de	0.68	a
W. cibaria PON10032	33.00	ab	69.48	a	0.24	ab	23.00	ac	68.30	ab	−1.68	b	14.17	ab	14.23	cd	34.17	bd	112.81	a	0.30	c
W. cibaria PON100337	33.00	ab	69.26	a	0.00	ab	24.84	a	67.83	ab	−1.41	ab	14.68	ab	16.55	cd	37.75	bc	95.52	ae	0.40	bc
SEM	1.44		0.30		0.04		0.23		0.41		0.02		0.16		1.32		2.32		5.03		0.05	
Significance	**		***		***		***		***		**		*		***		***		***		***	

Abbreviations: SFD, sterile flour dough; nSFD, non sterile flour dough; wnFDS, non sterile water non sterile flour; *Lb.*, *Lactobacillus*; *Ln.*, *Leuconostoc*; *W.*, *Weissella*.
Results indicate mean values of four measurements (carried out in duplicate for two independent productions).
Data within a column followed by the same letter are not significantly different according to Tukey's test (*P* value: ***, $P \leq 0.01$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, not significant).

confirming that a soft crumb is associated with the acidification of the dough which reduces its elasticity and resistance to extension (Arendt, Ryan, & Dal Bello, 2007; Clarke, Schober, Dockery, O'Sullivan, & Arendt, 2004). The lowest values were reached by the strains *W. cibaria* PON10030 and PON100337, *Ln. pseudomesenteroides* PON100315 and *Ln. citreum* PON10080 with sterile flour. This observation was also made for *W. cibaria* PON10030 and *Ln. citreum* PON10080 in the presence of non-sterile flour. Compared to the control bread, a softer sourdough bread was obtained thanks to the action of *Ln. citreum* by Choi et al. (2012).

Recently, image analysis has been used as a quantitative tool for the assessment of crumb features (Farrera-Rebollo et al., 2012). In this study, the highest values of void fraction and cell density were registered for all *W. cibaria* strains, *Ln. citreum* PON10021 and PON10079 and *Lb. sanfranciscensis* PON100100 with sterile flour. In general, the values of void fraction and cell density displayed by the trials carried out with non-sterile flour were higher than those of the corresponding sterile flour trials. On average, cell density and mean cell area registered for the breads processed with the sterile flour showed lower values than those evaluated for the corresponding breads obtained with the non-sterile flour. Gonzales-Barron and Butler (2006) stated that slight variations in threshold led to substantial variations in crumb feature values, with cell uniformity and void fraction being more sensitive than the others. Thus, the differences estimated in this study for the different breads have to be considered consistent.

4. Conclusions

In this work, an integrated technological approach based on acidification, acid production, loaf height, colour formation, softness of bread and volatile compound generation applied on several flour LAB indicated the suitability of the strains *Ln. citreum* PON10079 and PON10080 and *W. cibaria* PON10030 and PON10032 to act as starter cultures for sourdough production.

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