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Production of a yeast-free *focaccia* with reduced salt content using a selected *Leuconostoc citreum* strain and seawater

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ABSTRACT

A biotechnological protocol to produce a *focaccia* (a typical Italian flat bread) without bakers' yeast addition and with reduced salt was developed, to meet the current needs of the consumer. Based on its leavening capability, the *Leuconostoc citreum* strain C2.27 was selected to be used as a starter instead of the baker's yeast and inoculated in a liquid sourdough (type-II) for the production of the "yeast-free" *focaccia*. The addition of different NaCl concentrations and the replacement of the salt with food grade seawater were evaluated, and the capability of the selected strain to affect technological, nutritional and sensory features of the *focaccia* investigated. A significant improvement of the nutritional characteristics of the *focaccia* was observed compared to the control (leavened with bakers' yeast and added with NaCl 1.5 g/100 g) using 0.7 g/100 g of salt in the form of NaCl or seawater. Besides the reduced Na content (66% lower than the control), *focaccia* with seawater also showed a higher content of Ca^{2+} and Mg^{2+} (ca. 36% and 53%, respectively), and the lowest predicted glycemic index compared to the other experimental *focaccia*.

1. Introduction

Currently, consumer interest towards healthy foods is increasing, also supported by the World Health Organization recommendations (WHO, 2015). Baked goods are staple foods that present many issues, such as high glycemic response, low biological value of proteins, high salt and fat content, deficiency in functional compounds such as fibres and polyphenols, and presence of ingredients causing hypersensitivity reactions (e.g. gluten, wheat proteins and baker's yeast) (www.allergome.org). In particular, a high salt intake is recognized as a critical health issue (European Commission Directorate, 2014; Farquhar, Edwards, Jurkovitz, & Weintraub, 2015) and baker's yeast is considered potentially correlated with hypersensitivity reactions (Lied, Lillestøl, Valeur, & Berstad, 2010), autoimmune diseases (Muratori et al., 2003; Rinaldi, Perricone, Blank, Perricone, & Shoenfeld, 2013), and obesity (Salamati, Martins, & Kulseng, 2014). Therefore, different strategies have been recently developed to improve the nutritional/functional characteristics of bakery products. Among these, it is largely demonstrated that sourdough fermentation decreases the glycemic response of baked goods and increases the protein digestibility and the bioavailability of dietary fibres, minerals and phytochemicals (Gobbetti et al., 2019; Gobbetti, Rizzello, Di Cagno, & De Angelis, 2014; Montemurro, Pontonio, Gobbetti, & Rizzello, 2019; Siepmann, Ripari, Waszczynskyj, & Spier, 2018). Additional functional/nutritional advantages of the sourdough applications are those related to synthesis of bioactive compounds, irritable bowel syndrome diet management, and the exploitation of the non-wheat flours (legumes and pseudo-cereals) (Gobbetti et al., 2019). Moreover, a (type-II) sourdough application (De Bellis et al., 2019) allowed to develop a leavened baked good (*puccia* bread) free from baker's yeast applying the selected starter strain *Leuconostoc citreum* C2.27, even if salt affected the technological properties of the starter decreasing its leavening ability. Although salt replacement in baked goods is difficult because of its crucial effect on the sensory properties (Avramenko, Tyler, Scanlon, Hucl, & Nickerson, 2018), the use of sourdough was proposed for decreasing salt addition without affecting sensory properties thanks to both lactic acid bacteria (LAB)

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acidification and proteolysis which showed a masking effect on salt reduction (Gobbetti et al., 2019). Overall, many other strategies have been proposed to reduce the sodium chloride content of foods, such as the use of taste enhancers (amino acids, monosodium glutamate, lactates, yeast products, soy based ingredients) and replacers (KCl, spices) (Silow, Axel, Zannini, & Arendt, 2016). Recently, the use of seawater as ingredient has aroused interest and curiosity by companies and consumers and its use as a sodium chloride replacement has also been proposed (Barbanisi et al., 2019).

In the present study, a protocol based on sourdough biotechnology was developed, aiming at producing a baked good without baker's yeast addition and characterized by a reduced content of NaCl, also evaluating the use of food grade seawater. In particular, the biotechnological protocol was designed for the production of *focaccia*, a flat bread largely consumed in Italy and the Mediterranean area (Pasqualone, Delcuratolo, & Gomes, 2011).

2. Materials and methods

2.1. Characterization and selection of lactic acid bacteria

Aiming at selecting a proper starter for dough leavening instead of baker's yeast, 18 LAB strains (Table 1) were included in this study. The strains were singly inoculated in doughs and, during fermentation at 30 °C for 8 h, the volume increase (ΔV, mL) and the pH drop (ΔpH) were monitored as described by De Bellis et al. (2019). Three independent replicates of the experiments were carried out.

2.2. Catabolic profile of L. citreum C2.27

The carbon-source utilization profile of the selected *L. citreum* C2.27 (LMG S-27391, ITEM 17404) was determined by Biolog System (Biolog, Inc., Hayward, CA, United States) using Biolog AN plates. The strain was grown in de Man Rogosa Sharpe (MRS) broth (Oxoid, Basingstoke, UK) and Wheat Flour Hydrolyzed (WFH) (Siragusa et al., 2014) for 24 h and the cells were washed and then inoculated according to the manufacturer's instructions. The results were analyzed using the MicroLog3 (Biolog).

Table 1
Lactic acid bacteria strains included in the study.

Species	Strain[a]	Isolation source	Culture medium
Lb. sakei	CTR-L1	Dough	MRS
Lb. curvatus	CTR-L2	Dough	MRS
Lb. curvatus	CTR-L6	Dough	MRS
Lb. rossiae	A2.12	Dough	mMRS
Lb. plantarum	A3.11	Dough	MRS
Lb. plantarum	A5.5	Dough	MRS
Lb. plantarum	A4.1	Dough	MRS
Lb. plantarum	P4.6b	Dough	MRS
Lb. paraplantarum	P4.2	Dough	MRS
Lb .paraplantarum	P4.4	Dough	MRS
Lb. paracasei	P4.5	Dough	MRS
Lb. sanfranciscensis	A5.8	Dough	mMRS
Lb. brevis	P4.1	Dough	MRS
W. confusa	P2·C6	Dough	MRS
W. confusa	C5.7	Durum wheat semolina	MRS
L. mesenteroides	A3.23	Dough	MRS
L. pseudomesenteroides	CTR-L5	Dough	MRS
L. citreum	C2.27	Durum wheat semolina	MRS

Lb: *Lactobacillus*; *L*: *Leuconostoc*; *W*: *Weissella*.

[a] All the strains belonged to the Culture Collection of the Institute of Sciences of Food Production of National Research Council (ISPA-CNR, http://server.ispa.cnr.it/ITEM/Collection/) (De Bellis et al., 2019). Cell cultures were routinely propagated in de Man Rogosa Sharpe (MRS) broth (Oxoid Ltd, Basingstoke, UK), except those of the species *Lb. rossiae* and *Lb. sanfranciscensis*, which were cultivated in modified MRS (mMRS) broth [containing 1 g/100 mL maltose, 5 mL/100 mL fresh yeast extract, pH 5.6].

2.3. Effect of salt/seawater addition on dough fermentation

In order to study the effect of the salt concentration/seawater on performances of selected starter, four doughs (500 g) inoculated with *L. citreum* C2.27 were prepared as previously described (De Bellis et al., 2019), except for adding different amounts of salt to each dough. In particular, the doughs contained 1.5 g/100 g (commonly used for *focaccia* production) (D1.5), 0.7 g/100 g (D0.7) and 0 g/100 g (D0) of salt (NaCl). In addition, a dough (DSW) was prepared adding a microbiologically pure seawater (Steralmar Srl, Bisceglie, Italy) in partial replacement of water (20 mL/100 g). This amount corresponded to a total salt concentration of 0.7 g/100 g in dough, considering that salts concentration in seawater was ca. 35 g/L (Liu et al., 2019). During fermentation (8 h at 30 °C), volume and pH were monitored at 1 h intervals. LAB enumeration was carried out by plate count on MRS agar. Three independent replicates of the tests were carried out. Kinetics of acidification and growth were modelled in agreement with the Gompertz equation as modified by Zwietering, Jongeberger, Roumbouts, and Van't Riet (1990). The experimental data were modelled by the non-linear regression procedure of the Statistica 12.0 software (Statsoft, Tulsa, USA).

2.4. Sourdough fermentation and focaccia-making test

L. citreum C2.27 was used as starter to produce the liquid sourdough and the *focaccia* without baker's yeast addition, according to a two-step protocol (De Bellis et al., 2019).

Liquid sourdough (S) (500 g) was prepared by mixing wheat flour (17 g/100 g), sterile tap water (58 mL/100 g) and starter suspension (25 mL/100 g). The starter cell number in the sourdough was ca. 8 log cfu/g and dough yield (DY) was 600. The mixture was incubated at 30 °C for 16 h.

The *focaccia* doughs (DY ca. 175) were prepared by mixing durum wheat semolina (ca. 26 g/100 g, Divella, Rutigliano, ITA), soft wheat flour type "00" (ca. 26 g/100 g; Casillo, Corato, ITA), tap water (20 mL/100 g), extra virgin olive oil (2.1 mL/100 g; Olearia Desantis, Bitonto, ITA), malt barley flour (0.54 g/100 g; Antico Molino Rosso, Buttapietra, ITA) and S (25 g/100 g). *Focaccia* doughs (DS) obtained using sourdough were prepared with 1.5 g/100 g (DS1.5), 0.7 g/100 g (DS0.7) or without salt (DS0). A dough (DSSW) with sourdough in which salt (NaCl) and tap water were replaced with seawater (Steralmar Srl, Bisceglie, ITA) was prepared. A control (DCTR), without sourdough, was prepared by using baker's yeast (2 g/100 g, corresponding to ca. 8 log cfu/g) and 1.5 g/100 g of salt. Dough portions of 180 g were placed in round non-stick pans, fermented for 6 h at 30 °C (1 h for DCTR) and baked in an electric oven (Ardes, Milano, ITA) at 190 °C for 20 min.

2.5. Sourdough and focaccia dough characterization

ΔpH, ΔV and total titratable acidity (TTA) were measured as previously described (De Bellis et al., 2019).

The concentration of free amino acids (FAA) and peptides were determined in the water/salt-soluble extracts (WSE) of doughs, to evaluate the degree of proteolysis of the native proteins of flour (Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010). For the peptide analysis, WSE were treated with trifluoroacetic acid (0.5 mg/mL) and subject to dialysis (cut-off 500 Da) to remove proteins and FAA, respectively, while for FAA the extracts were treated with sulfosalicylic acid (50 mg/mL). Peptide concentration was determined by reversed-phase fast performance liquid chromatography (RP-FPLC), as reported in Rizzello et al. (2010). FAA content was analyzed by a Biochrom30 series Amino Acid Analyzer (Biochrom Ltd, Cambridge Science Park, UK). Total FAA (TFAA, calculated as a sum of FAA) and asparagine concentrations were reported. Moreover, TFAA in malt barley flour was also evaluated.

Organic acids, glucose, fructose and phytic acid were determined on

the WSE using Megazyme kits (Megazyme, Bray, Ireland) K-DLATE and K-ACET (lactic and acetic acids, respectively), K-FRUGL and K-PHYT 05/07 following the manufacturer's instructions.

2.6. Microbiological analyses and identification of LAB and yeasts

Twenty grams of *focaccia* doughs were homogenized with 180 mL of sterile NaCl solution (0.85 g/100 mL) in a Stomacher (Seward, London, UK) for 2 min. Serial dilutions of the microbial suspensions were plated on mMRS agar (Oxoid) supplemented with 100 mg/L of cycloheximide for the determination of presumptive LAB, and on Sabouraud Dextrose Agar (SDA, Oxoid) supplemented with 200 mg/L chloramphenicol for the enumeration of yeasts and moulds. After incubation at 30 °C for 48 h and at 25 °C for 72 h, the 20% of the colonies from the countable mMRS agar and SDA plates, respectively, were randomly taken, purified and stored at −80 °C. LAB and yeast isolates were characterized by REP-PCR and identified by sequencing of 16S rRNA or 26S rRNA genes, respectively, as previously described (De Bellis et al., 2019).

2.7. Characterization of the focaccia

In vitro protein digestibility (IVPD), starch hydrolysis and predicted glycemic index (GI) were determined after sequential enzyme treatment mimicking the *in vivo* digestions in the gastro intestinal tract as previously described by Montemurro et al. (2019). The IVPD was expressed as the percentage of the total protein solubilized after the *in vitro* digestion. The hydrolysis index (HI) was calculated comparing the percentage of potentially available starch of *focaccia* to a reference wheat flour bread (HI = 100), leavened with baker's yeast. The predicted GI was calculated using the equation: GI = 0.549 × HI+39.71 (Capriles & Arêas, 2013). FAA concentrations in *focaccia* were also evaluated as described in *2.5*.

The specific volume of *focaccia* was measured by the BVM-test system (TexVol Instruments, Viken, Sweden). Instrumental Textural Profile Analysis (TPA) was carried out with a TVT-300XP Texture Analyzer (TexVol Instruments), equipped with a cylinder probe P-Cy25S using loaf of 200 g and without removing the crust as previously described by Montemurro et al. (2019).

The crumb structure was evaluated using image analysis technology for differentiating gas cells and non-cells using an Image Scanner (Amersham Pharmacia Biotech, Uppsala, Sweden (Montemurro et al., 2019).

The chromaticity co-ordinates of the *focaccia* crust and crumb (obtained by a Minolta CR-10 camera) were reported in the form of ΔL*, Δa*, Δb* and ΔE^*_{ab} representing the differences for L*, a*, b* values and the color difference between sample and reference (a white ceramic plate having L* = 94.8, a* = 0.4 and b* = 4.16) as follows:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

2.8. Ion content of focaccia and raw materials and assessment of mineral solubility

The principal ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-} and PO_4^{3-}) in *focaccia* and raw materials were analyzed by ion exchange chromatography (Dionex DX120, Dionex Corporation, Sunnyvale, CA) as reported by D'Imperio et al. (2019). The chemical analysis of water was performed with the same methods but without extraction or mineralization process, using only a filter of 0.45 μm (RC) and an appropriate dilution.

Mineral solubility was determined following the protocol of Anastasio et al. (2010). Briefly, 5 g of dry samples were treated with HCl (0.65 mol/L) in order to evaluate the amount of Na^+, K^+, Mg^{2+} and Ca^{2+} released from food matrix and potentially bioavailable.

2.9. Sensory analysis of focaccia

Sensory analysis of *focaccia* was performed by a trained panel group composed of ten assessors (5 male and 5 female, mean age: 30 years, range: 25–54 years) with proven skills and previous experiences in bread, pasta and other cereal based product sensory evaluation. The sensory attributes, scored with a scale from 0 to 100 (with 100 the highest score), were discussed with the assessors during the introductory 2 h-training sessions performed on different commercial and experimental samples of *focaccia* purchased from local bakeries: color of crust and crumb, hardness, elasticity, dryness, gumminess, acidic smell, sweetness, acidic taste, bitterness, saltiness (Montemurro et al., 2019) and sapidity (Schettino, Pontonio, & Rizzello, 2019) (Table 8). Sensory evaluations were carried out in the library (Elia, 2011) of the Department of Soil, Plant and Food Science of the University of Bari, Italy. Three independent sessions including the evaluation of all *focaccia* samples (FS0, FS0.7, FSW, FS1.5, FCTR) were carried out. *Focaccia* samples were prepared in 3 different days and evaluated after 8h from baking, keeping samples at room temperature. *Focaccia* slices of 1.5 cm thick were coded (three-digit random numbers) and served in a randomized order, without removing the crust. A glass of water was used to clean the panelists mouth between each sample.

2.10. Statistical analysis

Data are presented as mean values ± standard deviations. Statistical analysis of the data was performed using Statistica 12.0 software. Data concerning chemical, microbial and sensory analyses were compared by applying a one-way ANOVA followed by Tukey's test to determine significantly different values ($P < 0.05$) while Pearson correlation coefficient ($P < 0.05$) was calculated to discuss sensory analysis results. Two-way ANOVA was used to validate sensory analysis results.

3. Results and discussion

3.1. Selection of a starter strain

Eighteen strains were singly used to ferment wheat flour doughs. Acidification capacity (ΔpH) and the volume increase (ΔV, mL) of the doughs were monitored during fermentation (Fig. 1; Supplementary Fig. 1). *L. citreum* C2.27 already reported for its good leavening capacities (De Bellis et al., 2019), caused the highest volume increase. Indeed, after 6 h, two *W. confusa* strains and *L. mesenteroides* A3.23 showed a relevant ΔV but 26% lower than *L. citreum* C2.27. Moreover, a ΔV ca. 51% lower than C2.27 was determined by *Lb. brevis* P4.1, *Lb. rossiae* A2.12, *Lb. sanfranciscensis* A5.8, and *L. pseudomesenteroides* CTR-L5. At the end of fermentation (8 h), all those strains showed a volume increase between 9% and 20.46% lower than C2.27. This strain, as well as the others that showed relevant volume variations, belong to obligate heterofermentive species, while facultative heterofermentive species (*Lb. plantarum*, *Lb. curvatus* and *Lb. paraplantarum*) showed the worst leavening capacities. Furthermore, *L. citreum* C2.27 determined a good acidification of the doughs (final ΔpH ca. 1.81), whereas the strains that showed greater ΔpH (until ca. 2.1) belonged to the above-mentioned facultative heterofermentive species. Therefore, *L. citreum* C2.27 was selected and used in further experiments.

3.2. Catabolic profile of L. citreum C2.27 and bread quality

The use of 95 carbon sources by *L. citreum* C2.27 was determined after its cultivation in both MRS and WFH using the Biolog system. The activity profile resulted affected by the composition of the growth medium, thus highlighting an initial adaptation of the strain to the substrate (Supplementary Fig. 2). Interestingly, after growth in WFH the strain used D-melibiose and higher amounts of dextrine and sucrose, while after cultivation in MRS broth the strain showed higher growth

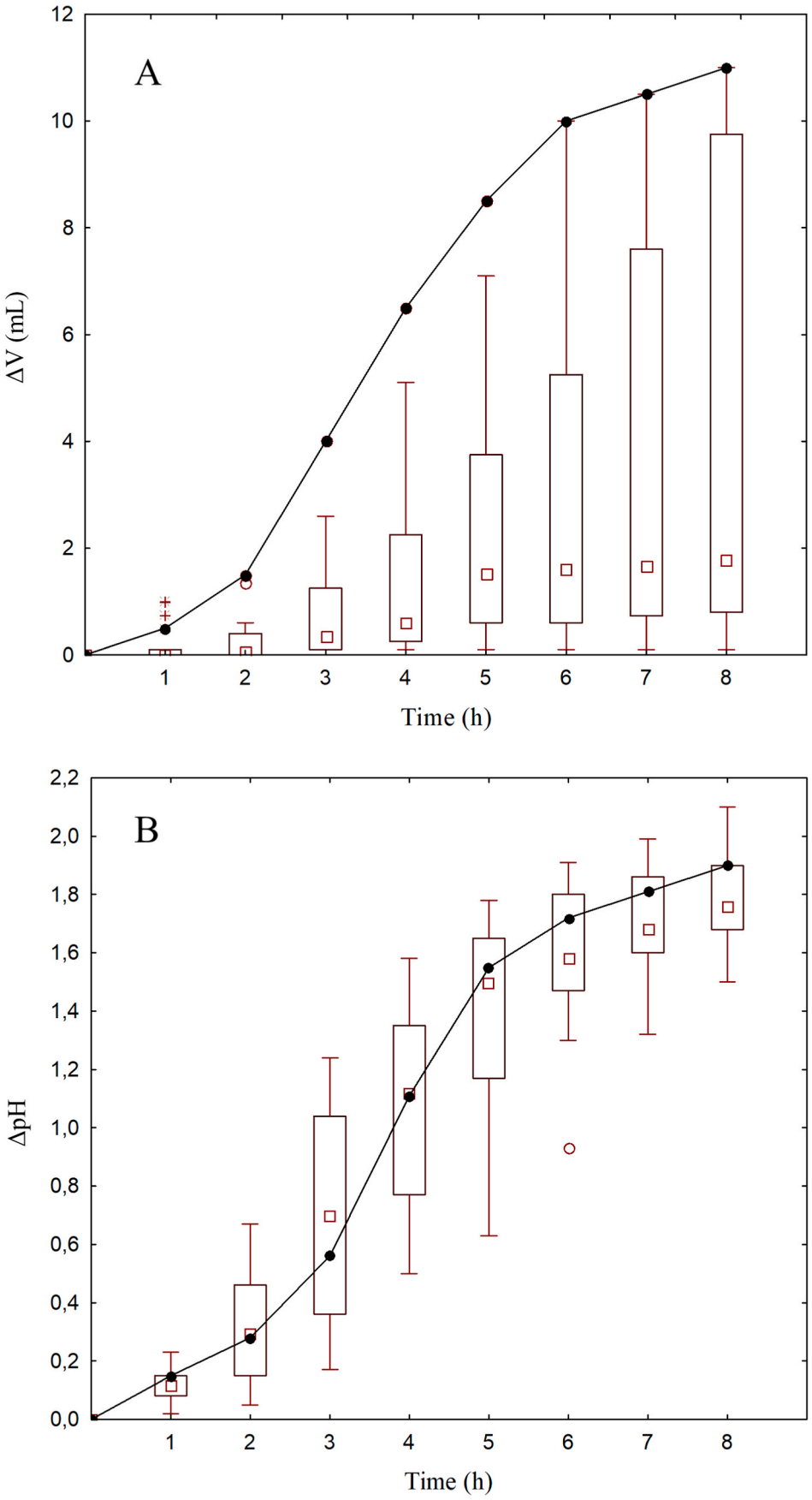

Fig. 1. Volume increase (ΔV, mL) (A) and acidification (ΔpH, pH units) (B) of doughs singly inoculated with lactic acid bacteria strains (Table 1) during 8 h of incubation at 30 °C. Aggregate data of all 18 LAB strains are shown in box plots. The top and the bottom of the box represent the 75th and 25th percentile of the data, respectively. The top and bottom of the bars represent the 5th and 95th percentile of the data, respectively. Median values (□), outliers (○) and extremes (*) are shown. The curves represent ΔV and ΔpH of doughs inoculated with *L. citreum* C2.27.

(ca. 50%) in the presence of lactic acid and lactic acid methyl ester. It is well-known that amylase activities in wheat sourdough release maltodextrins, maltose, and glucose during fermentation (Gänzle, 2014). *L. citreum* C2.27 was able to metabolize maltose, maltotriose, dextrin, D-glucose, D-fructose. The strain also metabolized different FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols) usually present in cereal matrices (Gobbetti et al., 2019; Menezes et al., 2018), such as D-mannitol, D-sorbitol, sucrose, D-melibiose and mannose. It is also known that the fermentable carbohydrates are quickly used in the early steps of fermentation, while carbohydrates with a higher degree of polymerization are used later. The obtained metabolic profile leads to hypothesize that a long fermentation, which is typical of sourdough, by *L. citreum* C2.27 could diminish FODMAPs levels (Gobbetti et al., 2019; Menezes et al., 2018). Moreover, the strain was unable to metabolize the evaluated amino acids as a source of energy, and genome analysis of the strain revealed the absence of genes encoding for the aminoacyl decarboxylases responsible for biogenic amine production (unpublished data) (Diana, Rafecas, & Quílez, 2014). Thus, it can be hypothesized that amino acids present or released in dough fermented with *L. citreum* C2.27 could contribute to the improvement of the sensory quality of *focaccia* without forming biogenic amines. It is also interesting to note that a recent scientific investigation (Diana et al., 2014) suggests that, in general biogenic amines could be present in bread only at very low concentration (below the limit of quantification); in fact, the European Commission does not set a maximum limit for those compounds in bread (Reg. EC no 2073/2005). The metabolic profile shown by *L. citreum* C2.27 indicated a well adaptation to the cereal environment, thus allowing potential competitiveness with the endogenous microbiota and relevant effect on the bread quality (Gänzle & Zheng, 2019).

3.3. Effect of salinity

L. citreum C2.27 showed the shortest lag phase (1.03 h) in D0, and the highest V_{max} (0.5 ΔpH/h) and A (1.83 ΔpH), while it had an opposite behavior in D1.5 (Table 2). The concentration of salt also affected the leavening (Fig. 2A). Greater volume increases were observed in doughs with lower salt concentrations. ΔV values of D0.7 and DSW were not significantly different. Bacterial load at the end of fermentation increased ca. 1.3 logarithmic cycles in D0, D0.7 and DSW, while the cell growth (Δlog cfu/g) was the lowest in D1.5 ($P < 0.05$) (Fig. 2B). As previously shown (Simonson, Salovaara, & Korhola, 2003) salt levels higher than 0.7 g/100 g markedly decreased LAB growth.

3.4. Dough characterization

The main chemical-physical characteristics of sourdough and *focaccia* doughs are reported in Table 3. The use of the sourdough caused a significantly higher ΔpH and TTA value for all DS compared to DCTR. However, TTA and lactic and acetic acids concentrations were significantly lower in DS1.5 compared to the other DS. Moreover, DS1.5 showed a ΔV value about two-fold lower than the other DS ($P < 0.05$). As expected, the use of baker's yeast caused the highest ΔV ($P < 0.05$). In DS, higher the salt concentration, lower are values of ΔV and acidification.

Table 2
Parameters of the kinetics of acidification (lag time, λ; absolute acidification rate, V_{max}; and upper asymptote, A) of doughs started with *L. citreum* C2.27 with different concentration of NaCl (0, 0.7, and 1.5 g/100 g, respectively indicated as D0, D0.7 and D1.5) or with seawater (DSW). Fermentation was carried out at 30 °C for 8 h.

	D0	D0.7	DSW	D1.5
λ (h)	1.03 ± 0.04^{d}	1.84 ± 0.03^{b}	1.47 ± 0.05^{c}	2.22 ± 0.06^{a}
V_{max} (ΔpH/h)	0.51 ± 0.02^{a}	0.44 ± 0.01^{b}	0.38 ± 0.03^{b}	0.25 ± 0.02^{c}
A (ΔpH)	1.83 ± 0.07^{a}	1.69 ± 0.06^{b}	1.63 ± 0.08^{b}	1.54 ± 0.07^{c}

Data are the means of three independent replicates ± standard deviations.
[a-d] Values referring to doughs in the same row with different letters differ significantly ($P < 0.05$).

The concentration of phytic acid in DS was about 37% lower than that found in DCTR ($P < 0.05$). As a consequence, a greater bioavailability of nutrients can be hypothesized, since phytic acid acts as an antinutritional factor by chelating proteins and minerals. The degradation of the phytic acid during LAB fermentation has been largely demonstrated (Gobbetti et al., 2014). The exploration of the genome of the *L. citreum* C2.27 did not highlight the presence of the gene coding for a phytase thus excluding its phytase activity (unpublished data), nevertheless, the acidification could reduce phytate content by enhancing the activity of flour endogenous phytase.

High amount of TFAA was found in S (742 ± 16 mg/kg); this is probably due to the low presence of yeasts (Table 3), commonly responsible for the consumption of free amino acids during fermentation (Di Cagno et al., 2014). DS showed TFAA concentration from 15% (DS0) to 34% (DSSW) higher than DCTR ($P < 0.05$). During LAB fermentation, TFAA increase due to the proteolytic activity of LAB and/or flour enzymes activated under acidic conditions (Gobbetti et al., 2014). However, the malt addition (2.83 g/kg of TFAA) explained the high TFAA concentration in DCTR (1922 ± 16 mg/kg). A higher number of peaks were observed in peptide profiles of DS0.7 and DSSW (Supplementary Fig. 3). The hydrolysis of native protein in peptides is associated to the increase of the protein digestibility and to the release of functional compounds (Gobbetti et al., 2019). Asparagine and free reducing sugars concentrations in doughs (Table 3) were evaluated as indicators of possible acrylamide content in *focaccia*, as the reaction between those compounds is considered mainly responsible for acrylamide formation (Keramat, LeBail, Prost, & Jafari, 2011). Concerning our work, the glucose and fructose concentrations were lower in DS with reduced salt or seawater addition while the highest concentrations were found in DCTR (2.86 ± 0.13 g/kg) and DS1.5 (1.43 ± 0.07 g/kg), respectively. The sum of reducing sugars resulted from 49 to 56 percent lower in DS0, DS0.7 and DSSW than in DCTR. Interestingly, the contents of asparagine and in particular of reducing sugars were lower than values detected by Nachi et al. (2018). They also found very low values of acrylamide in the resulting bread (in comparison with indicative values of acrylamide for soft bread; Reg. EC no 2158/2017), and demonstrated that the use of a sourdough inoculated with a LAB strain reduced acrylamide concentration in bread in comparison to yeast fermentation. It is also known that a low pH and the presence of divalent metal ions decrease acrylamide formation (Keramat et al., 2011). Therefore, due to the concomitant effects of different factors (i.e, low concentration of precursors, low pH and the increased concentration of divalent metal ions), a negligible risk related to the acrylamide content could be considered associated to the *focaccia* obtained in this work using DS and in particular DSSW containing a high concentration of cations (Table 5).

3.5. Microbiological analyses

At the end of fermentation, doughs were subjected to microbiological analyses (Table 3). S and DS showed LAB loads significantly higher than DCTR. Moreover, DS1.5 had a lower load than the other DS. LAB isolates from S and DS were characterized by REP-PCR. Their electrophoretic profiles were equal to that of the starter strain, which was therefore the only strain found. DCTR harboured six LAB strains belonging to *L. mesenteroides*, *Lb. rossiae*, *Lb. plantarum*, *Lb. paraplantarum*, *Lb. paracasei* (2 strains). The species identified in this study are typically associated with raw materials and doughs (De Bellis et al., 2019; De Vuyst et al., 2014).

Yeasts and moulds were absent or in very low number in DS. Clearly, DCTR contained a high load of yeasts, while moulds were not detected. The yeasts isolated from DS belonged to the species *Candida glabrata*, *Wickerhamomyces anomalus*, *Cyberlindnera fabianii*, and *Rhodotorula*

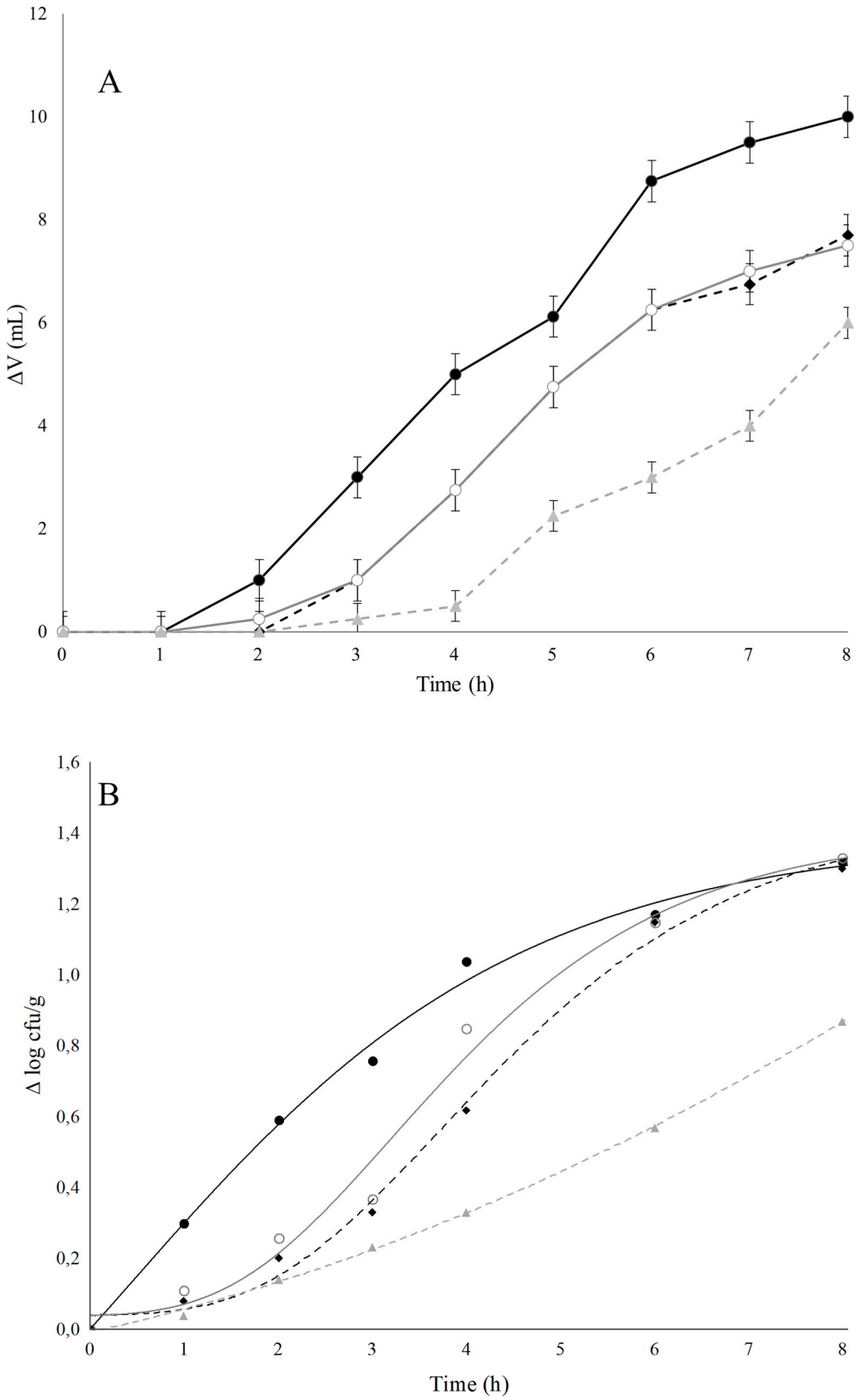

Fig. 2. Volume increase (ΔV, mL) of doughs (A) and kinetics of growth (Δlog cfu/g) of *L. citreum* C2.27 (B) in doughs with different concentration of NaCl (0, 0.7 and 1.5 g/100 g, D0—●—, D0.7– -◆- - and D1.5 - -▲- -) or with seawater (DSW—○—). The initial cell number was ca. 7 log cfu/g. Fermentation was carried out at 30 °C for 8 h. Data represent means of three independent experiments ± standard deviations.

Table 3
Chemical-physical and microbiological characteristics of liquid sourdough inoculated with *L. citreum* C2.27 (S) and *focaccia* doughs started with the liquid sourdough, without salt (DS0), with 0.7 g/100 g (DS0.7), 1.5 g/100 g salt (DS1.5) or seawater (DSSW), and *focaccia* dough made with baker's yeast (DCTR).

	S	DS0	DS0.7	DSSW	DS1.5	DCTR
LAB (log cfu/g)	9.35 ± 0.24	9.10 ± 0.01^{ab}	9.28 ± 0.22^{a}	9.18 ± 0.09^{ab}	8.86 ± 0.16^{b}	4.39 ± 0.09^{c}
Yeast (log cfu/g)	nd	nd	2.03 ± 0.30^{b}	1.81 ± 0.39^{b}	1.49 ± 0.17^{b}	8.24 ± 0.01^{a}
Moulds (log cfu/g)	1.65 ± 0.09	2.39 ± 0.36^{a}	2.29 ± 0.11^{a}	2.18±0^{a}	2.48±0^{a}	nd
pH	3.46 ± 0.16	4.19 ± 1.23^{b}	4.36 ± 0.11^{b}	4.32 ± 0.16^{b}	4.59 ± 0.13^{b}	5.40 ± 0.29^{a}
ΔpH	2.53 ± 0.16	1.23 ± 0.13^{a}	1.00 ± 0.11^{ab}	0.93 ± 0.16^{ab}	0.82 ± 0.13^{b}	0.23 ± 0.03^{c}
ΔV(mL)	–	5.25 ± 0.58^{b}	5.17 ± 1.15^{b}	5.5 ± 1.00^{b}	2.33 ± 0.29^{c}	11.25 ± 0.25^{a}
TTA (mL)	4.25 ± 0.25	8.97 ± 0.93^{a}	8.3 ± 0.69^{a}	8.03 ± 0.25^{a}	6.26 ± 0.64^{b}	3.76 ± 0.46^{c}
Lactic acid (mmol/Kg)	26.50 ± 0.01	55.19 ± 0.03^{a}	47.93 ± 0.02^{b}	42.62 ± 0.04^{c}	33.50 ± 0.01^{d}	1.90 ± 0.04^{e}
Acetic acid (mmol/Kg)	6.46 ± 0.02	28.04 ± 0.04^{c}	38.18 ± 0.01^{a}	29.03 ± 0.02^{b}	26.73 ± 0.03^{d}	nd
QF	4.10 ± 0.01	1.97 ± 0.00^{a}	1.25 ± 0.00^{c}	1.47 ± 0.00^{b}	1.25 ± 0.00^{c}	–
Phytic acid (mg/100 g)	–	19±4^{b}	15±2^{b}	17±3^{b}	17±2^{b}	27±3^{a}
Glucose (g/Kg)	–	1.11 ± 0.11^{d}	1.55 ± 0.10^{c}	1.38 ± 0.11^{cd}	2.04 ± 0.10^{b}	2.86 ± 0.13^{a}
Fructose (g/Kg)	–	0.50 ± 0.03^{c}	0.29 ± 0.03^{d}	0.31 ± 0.02^{d}	1.43 ± 0.07^{a}	0.75 ± 0.04^{b}
Asparagine (mg/Kg)	–	77.1 ± 3.5^{b}	77.9 ± 2.7^{b}	84.0 ± 3.4^{a}	82.8 ± 3.1^{a}	63.4 ± 3.2^{c}
Total Free Amino Acids (mg/Kg)	742 ± 16	2253 ± 20^{d}	2449 ± 21^{c}	2896 ± 25^{a}	2597 ± 24^{b}	1922 ± 16^{e}

Data represent means of three independent experiments ± standard deviations. $^{a\text{-}e}$ Values referring to *focaccia* doughs in the same row with different letters differ significantly ($P < 0.05$).
QF: the quotient of fermentation was determined as the molar ratio between lactic and acetic acids.
nd: not detected (<LOD, limit of detection).

babjevae (>99% identity), often associated with sourdoughs (De Vuyst et al., 2014), while *Saccharomyces cerevisiae* was absent. On the contrary a single strain of *S. cerevisiae* (100% identity) was detected in DCTR. Therefore, the results highlighted the capability of the strain *L. citreum* C2.27 to dominate the endogenous microbiota.

3.6. Focaccia characterization

Experimental *focaccia* containing sourdough (FS) and different salt concentrations (0, 0.7 and 1.5 g/100 g, respectively indicated as FS0, FS0.7 and FS1.5) or seawater (FSW) were compared with baker's yeast *focaccia* (FCTR) (Table 4). Compared to FCTR, a significant decrease (up to 18%) of the HI was observed when sourdough was used. The lowest HI value was observed for FSW (ca. 80%), corresponding to a predicted GI of 83.5 ± 1.2. Sourdough fermentation, as reported by Rizzello et al., 2010, increased the value of the *in vitro* protein digestibility (IVPD), especially when the starter strain was used in combination with 0.7 g/100 g salt concentration. The evaluation of peptides and TFAA confirmed the high degree of proteolysis in FS. As expected, FCTR showed the lowest values for peptides and TFAA, reflecting concentration found in doughs (Table 3). Several amino acids, such as Asp, Leu, Orn, Lys, Pro were at significantly higher concentrations in FSW than in other *focaccia* (Supplementary Fig. 4). Moreover, a high amount of Glu was found in FCTR. As previously described by Guerzoni, Serrazanetti, Vernocchi, and Gianotti (2013) glutamate and glutamine are synthetized from ammonium by yeast as precursors of different amino acids.

Table 4
Chemical and nutritional characteristics of *focaccia* produced using sourdough started with *L. citreum* C2.27 with different concentration of NaCl (0, 0.7 and 1.5 g/100 g, respectively indicated as FS0, FS0.7, FS1.5) or with seawater (FSW). *Focaccia* produced without sourdough and with baker's yeast (2 g/100 g) was used as a control (FCTR).

	FS0	FS0.7	FSW	FS1.5	FCTR
pH	4.4 ± 0.03^{c}	4.50 ± 0.03^{c}	4.49 ± 0.05^{c}	4.71 ± 0.10^{b}	5.82 ± 0.11^{a}
TTA (mL)	5 ± 0.2^{a}	5 ± 0.4^{a}	4 ± 0.5^{b}	3.3 ± 0.3^{b}	1.6 ± 0.3^{c}
a_w	0.96 ± 0.0^{a}	0.95 ± 0.0^{a}	0.96 ± 0.01^{a}	0.94 ± 0.0^{a}	0.95 ± 0.0^{a}
Hydrolysis index, HI (%)	81.6 ± 1.4^{c}	91.2 ± 1.5^{b}	79.8 ± 2.1^{c}	94.2 ± 1.6^{ab}	97.0 ± 1.2^{a}
Predicted Glycemic index, pGI	84.5 ± 1.5^{c}	89.8 ± 1.4^{b}	83.5 ± 1.2^{c}	91.4 ± 1.0^{ab}	93.0 ± 1.1^{a}
In vitro protein digestibility, IVPD (%)	83.0 ± 0.8^{b}	87.2 ± 1.4^{a}	82.7 ± 1.1^{b}	84.6 ± 1.9^{ab}	79.9 ± 1.5^{c}
Total Free Amino Acids (mg/Kg)	711±7^{cd}	737±7^{bc}	797±9^{a}	757±8^{b}	692±8^{d}
Peptides (mg/g)	19.18 ± 0.32^{a}	18.44 ± 0.27^{b}	18.07 ± 0.29^{c}	16.96 ± 0.28^{d}	15.11 ± 0.29^{e}

Data represent means of three independent experiments ± standard deviations. $^{a\text{-}e}$ Values referring to *focaccia* in the same row with different letters differ significantly ($P < 0.05$).

3.7. Ion content of raw materials and focaccia

The content of the principal ions in raw materials and *focaccia* is showed in Table 5. The PO_4^{3-} content in FCTR was ca. 57% lower ($P < 0.05$) than other samples. This result could be related to the greater degradation of phytic acid observed in DS (Table 3). The SO_4^{2-} concentration in FSW was significantly higher compared to the other samples, due to the anion concentration in seawater that was markedly higher than in tap water (Table 5). On the contrary, the K^+ content in the samples strictly depends on the flour used.

As expected, the higher contents of Cl^- and Na^+ were found in FCTR and in FS1.5, while they were 91 and 95% lower, respectively, in FS0. The differences between the samples were due to the amount of salt/seawater added to the dough. In FS0, the Cl^- and Na^+ content was related with raw materials used in *focaccia* making (Table 5). The addition of seawater or 0.7 g/100 g salt to doughs led to a Na^+ reduction equal to 66% in FSW and 57% in FS0.7 compared to FCTR. Based on European legislation (Reg. EC no 1924/2006), a reduction of the salt content higher than 25% (in comparison to FCTR) is necessary to attribute the "reduced salt" claim, as in the case of FSW and FS0.7. This result is very interesting considering the relation between the excessive salt daily consumption and the onset of diseases (European Commission Directorate, 2014; Farquhar et al., 2015). Indeed, the mean daily salt intake (8–12 g/d) in Europe is largely above the Recommended Daily Allowance (RDA), equal to 5 g salt/d for adults (equivalent to 2 g Na^+/d) (WHO, 2012). Therefore, there is the necessity to reduce the salt content in bread, cereals and bakery products, since they are among the main sources of salt in most national diets (European Commission Directorate, 2014).

FSW showed concentrations of Ca^{2+} and Mg^{2+} ca. 36% and 53% higher than those of the other *focaccia* ($P < 0.05$), respectively (Table 5). Ca^{2+} and Mg^{2+} values in FSW were related to the high content of these cations in seawater. The consumption of 100 g of FSW allows to intake 25 mg of Ca^{2+} and 52 mg of Mg^{2+}, equal to 3% and 14% of Daily Reference Intake (DRI) respectively (Reg. EU no 1169/2011). Because

Table 5
Content of cations and anions in tap water and seawater (mg/100 mL), flours (mg/100 g dry weight) and *focaccia* (FS0, FS0.7, FSW, FS1.5, FCTR) (mg/100 g fresh weight).

	PO_4^{3-}	SO_4^{2-}	Cl^-	Na^+	K^+	Mg^{2+}	Ca^{2+}
Tap water	nd	6.64 ± 1.2	4.51 ± 0.03	4.07 ± 0.01	0.70 ± 0.003	0.126 ± 0.02	0.24 ± 0.07
Seawater	nd	271.0 ± 6.3	2419 ± 25.7	1064 ± 4.6	41.9 ± 5.6	124.9 ± 0.63	40.6 ± 1.16
Swf	30.6 ± 8.6	37.5 ± 4.7	135.3 ± 15.4	25.4 ± 2.3	266.4 ± 14.6	24.9 ± 1.0	31.5 ± 5.3
Dws	33.9 ± 2.2	40.8 ± 3.7	126.0 ± 7.4	23.9 ± 8.3	382.3 ± 35.6	48.7 ± 1.7	25.8 ± 1.6
FS0	140.1 ± 4.23^a	23.82 ± 1.99^b	86.5 ± 14.9^c	27.05 ± 1.66^c	187.44 ± 4.42^c	25.99 ± 1.70^b	17.66 ± 0.92^b
FS0.7	173 ± 27.25^a	54.43 ± 2.57^b	529 ± 49.56^b	258 ± 11.65^b	273 ± 20.60^a	24.56 ± 0.59^b	16.07 ± 1.01^b
FSW	127 ± 2.23^a	71.91 ± 1.38^a	444 ± 6.76^b	202 ± 3.67^b	228.76 ± 3.90^b	51.71 ± 0.79^a	25.39 ± 0.37^a
FS1.5	123 ± 8.25^a	26.76 ± 1.30^b	994 ± 49.67^a	615 ± 13.31^a	261 ± 8.35^a	22.56 ± 0.47^c	14.41 ± 1.86^b
FCTR	59.86 ± 2.34^b	24.15 ± 3.64^b	934 ± 18.27^a	598 ± 22.72^a	237 ± 1.56^b	24.95 ± 1.22^b	16.99 ± 0.34^b

Data are presented as mean values of triplicates ± standard deviation.
[a-c] Values in the same column with different letters differ significantly ($P < 0.05$).
Swf: soft wheat flour; Dws: durum wheat semolina; nd: not detected (<LOD).

the quantity of minerals in food is considered significant when 15% of the nutrient reference values is provided by 100 g of product (Reg. EU no 1169/2011), especially the Mg^{2+} amount contained in FSW was assessed as very interesting. In fact, it is well known that Mg^{2+} depletion has pathological (neurological or neuromuscular) consequences (WHO, 2004). Therefore, seawater allowed to obtain *focaccia* with both the reduction of the Na^+ intake and the fortification with beneficial minerals as Mg^{2+}.

The soluble quantity of the minerals contained in *focaccia* (mg/100 g fresh weight) is reported in Table 6. As expected, FSW presented a significantly higher soluble content of Ca^{2+}, Mg^{2+} and K^+ than the other *focaccia*, linked to the use of seawater. In contrast, FCTR had the lowest soluble amounts of Ca^{2+}, Mg^{2+} and K^+. This result could be related to the greater amount of phytic acid detected in DCTR (Table 3) and the lower quantity of PO_4^{3-} detected in FCTR (Table 5). The biotechnological protocol proposed allows the increase of Ca^{2+}, Mg^{2+} and K^+ available in chemical gastric condition from the food matrix, especially if seawater is added. At the same time FS0.7 and FSW provide a contribution of Na^+ (% DRI) up to 2.6-fold lower than F1.5 and FCTR (Table 6).

3.8. Texture, color and sensory characterization

The higher specific volume was observed in FCTR, while slightly lower value was in FS0.7. The use of 1.5 g/100 g salt decreased of ca. 41% the specific volume if compared with FCTR (Table 7). Hardness was the lowest in FCTR while, among sourdough *focaccia*, it was lower in FSW. Chewiness was similar in those two *focaccia*. FS1.5 showed the highest hardness and chewiness. The lightness (L) of crust and crumb was higher in FCTR compared to the other *focaccia* (Table 7), characterized by high concentration of TFAA available for the Maillard reaction. Compared to FCTR, the ΔE* of FS was in particular significantly different for crumb having values up to 32% higher. The cell-total of FCTR and FSW did not significantly differ, while it was the lowest for F1.5 (Table 7).

Statistical validation of sensory results was performed on the raw data of each sensory descriptor using two-way ANOVA (Supplementary Table 1). No statistically relevant effects of the replicates and judges on the sensory profiles (all the descriptors) were found, whereas samples-replicates interaction effects were observed in crust color and bitterness, probably due to cooking process made in pilot plant. Moreover, samples-judges interaction effects were found for elasticity and sapidity. Therefore, hardness perception of the panelists (Table 8) was positively correlated with the hardness of instrumental measures ($r = 0.88$, $P < 0.05$). As expected, the panel was able to detect differences in salt addition ($P < 0.05$) and a positive correlation ($r = 0.97$, $P < 0.01$) between salt content and perception was found. Moreover, a markedly negative correlation ($P < 0.01$) was found among instrumental pH decrease (Table 4) and acidic smell and taste perceptions increase ($r = -0.98$ and $r = -0.99$, respectively). The use of sourdough and seawater increased the perception of bitter taste, in fact bitterness was significantly ($P < 0.05$) higher in all FS than FCTR, probably due to the Maillard reactions in presence of higher amount of FAA and to the replacement of NaCl (Reiβner, Wendt, Zahn, & Rohm, 2019). At the same time, the use of sourdough and seawater increased the *focaccia* sapidity ($P < 0.05$), due to the release of flavoring FAA and amino acid derivatives during fermentation and to the presence of different minerals. Therefore, in addition to the sourdough, whose beneficial features are known (Gobbetti et al., 2019), also the replacement of NaCl with

Table 6
Mineral soluble amount (mg/100 g fresh weight), mineral solubility (percentage of minerals released from food matrix in chemical gastric condition), and contribution (% DRI) in *focaccia* (FS0, FS0.7, FSW, FS1.5, FCTR).

Element	DRI (mg/d)		FS0	FS0.7	FSW	FS1.5	FCTR
Na^+	2000	Soluble quantity (mg/100 g)	7.48 ± 0.16^e	146 ± 2.53^c	118 ± 1.5^d	332 ± 4.47^a	311 ± 6.69^b
		Solubility (%)	23.80 ± 6.01	56.53 ± 1.8	58.09 ± 0.81	53.98 ± 1.8	54.00 ± 2.32
		Contribution (% DRI)	0.37	7.30	5.90	16.60	15.55
K^+	2000	Soluble quantity (mg/100 g)	63.55 ± 1.21^b	65.02 ± 1.03^a	68.90 ± 0.67^a	61.56 ± 0.89^b	57.19 ± 5.14^b
		Solubility (%)	33.92 ± 0.9	23.77 ± 1.67	30.13 ± 0.81	23.61 ± 1.08	24.02 ± 2.2
		Contribution (% DRI)	3.18	3.25	3.45	3.08	2.86
Mg^{2+}	375	Soluble quantity (mg/100 g)	12.37 ± 0.17^b	13.08 ± 0.42^b	26.10 ± 2.05^a	12.46 ± 0.1^b	10.98 ± 0.84^b
		Solubility (%)	48.18 ± 2.03	53.24 ± 0.49	50.47 ± 3.82	55.24 ± 0.83	44.13 ± 4.94
		Contribution (% DRI)	3.30	3.49	6.96	3.32	2.93
Ca^{2+}	800	Soluble quantity (mg/100 g)	9.48 ± 0.65^b	10.86 ± 1.04^b	18.2 ± 0.59^a	9.62 ± 0.07^b	7.98 ± 0.57^b
		Solubility (%)	53.85 ± 11.15	67.96 ± 9.82	71.65 ± 1.46	74.84 ± 15.7	46.98 ± 3.81
		Contribution (% DRI)	1.19	1.36	2.28	1.20	1.00

DRI: Daily Reference Intakes (Reg. EU no 1169/2011).
Data are presented as mean values of triplicates ± standard deviation.
[a-e] Values in the same row with different letters differ significantly ($P < 0.05$).

Table 7
Texture profile analysis of *focaccia* produced using liquid sourdough (S) started with *L. citreum* C2.27 with different concentration of NaCl (0, 0.7 and 1.5 g/100 g corresponding to FS0, FS0.7 and FS1.5) or with seawater (FSW). *Focaccia* produced without sourdough and with baker's yeast (2 g/100 g) was also used as a control (FCTR).

	FS0	FS0.7	FSW	FS1.5	FCTR
Specific volume (cm^3/g)	1.84 ± 0.09^{c}	2.21 ± 0.10^{b}	1.83 ± 0.010^{c}	1.65 ± 0.08^{d}	2.82 ± 0.11^{a}
Textural profile analysis					
Hardness (g)	7669 ± 54^{b}	7626 ± 25^{b}	5824.5 ± 23^{c}	8679.6 ± 21^{a}	3421 ± 52^{d}
Fracturability	40.9 ± 1.5^{a}	38.5 ± 1.1^{b}	39.6 ± 0.9^{b}	37.4 ± 1.4^{c}	nd
Resilience	0.6 ± 0.1^{b}	0.8 ± 0.1^{a}	0.9 ± 0.1^{a}	0.8 ± 0.1^{a}	0.9 ± 0.1^{a}
Chewiness (g)	886 ± 19^{d}	1899 ± 28^{b}	1584 ± 18^{c}	2506 ± 27^{a}	1671 ± 23^{c}
Cohesiveness	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	0.4 ± 0.1^{ab}	0.5 ± 0.1^{a}
Color analysis crust					
L*	63.2 ± 2.1^{bc}	62.5 ± 1.8^{b}	64.3 ± 0.4^{c}	66.27 ± 1.1^{ac}	68.1 ± 0.4^{a}
a*	2.18 ± 1.32^{b}	0.12 ± 0.37^{c}	−0.07 ± 0.31^{c}	−0.15 ± 0.32^{c}	4.69 ± 0.71^{a}
b*	28.77 ± 0.86^{b}	27.65 ± 0.98^{b}	28.85 ± 0.77^{b}	27.85 ± 0.81^{b}	36.35 ± 0.74^{a}
ΔE*	40.17 ± 1.71^{ab}	39.99 ± 1.34^{abc}	39.07 ± 0.91^{bc}	37.12 ± 0.78^{c}	42.19 ± 0.49^{a}
crumb					
L*	68.9 ± 4.5^{b}	65.2 ± 2.1^{b}	69.6 ± 2.5^{b}	68.9 ± 1.3^{b}	78.5 ± 1.1^{a}
a*	−1.74 ± 0.10^{b}	−1.70 ± 0.18^{b}	−2.03 ± 0.13^{c}	−0.15 ± 0.32^{a}	−2.09 ± 0.13^{c}
b*	21.37 ± 2.57^{c}	21.45 ± 0.34^{c}	21.82 ± 1.03^{c}	27.85 ± 0.17^{a}	23.18 ± 0.34^{b}
ΔE*	31.3 ± 2.41^{b}	34.34 ± 1.69^{b}	30.88 ± 2.49^{b}	37.12 ± 0.97^{a}	25.13 ± 0.62^{c}
Image analysis					
Black pixel area* (%)	25.5 ± 0.4^{b}	23.7 ± 0.3^{c}	26.9 ± 0.5^{a}	9.5 ± 0.7^{d}	27.6 ± 0.3^{a}

Data represent means of three independent experiments ± standard deviations.
[a-d] Values in the same row with different letters differ significantly ($P < 0.05$).
*The gas cell area is expressed as the percentage of black pixel to total area of the image.
nd: not detected (<LOD).

seawater showed compensative effects on salt reduction.

4. Conclusions

Because bread is a main constituent in the diet of most populations, it has been targeted by health organizations as a food staple requiring an improvement of the functional/nutritional characteristics. The development of innovative technologies is strategic for obtaining novel high quality products, increasingly requested by consumers and industry. The presented work showed the use of a liquid sourdough started with *L. citreum* C2.27 suitable to produce *focaccia* with enhanced functional and nutritional features. In fact, the strain allowed to obtain a "yeast-free" product with a reduced salt content. In addition, the replacement of salt with seawater allowed to produce an appealing innovative version of a traditional baked good, with added beneficial effect for health, due to lowest predicted glycemic index, salt reduction and Mg^{2+} fortification.

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Table 8
Sensory analysis of *focaccia* made with liquid sourdough (S), without salt (FS0), with 0.7 or 1.5 g/100 g of NaCl (FS0.7, FS1.5), or with seawater (FSW). *Focaccia* produced without sourdough and with baker's yeast was used as control (FCTR). The descriptors were crust colour (colour of the crust, from light yellow to dark brown), crumb colour (colour of the crumb, from white to yellow), hardness (the force required during the first bite, from soft to hard), elasticity (the capability of the focaccia slice to return in the original form after the first bite, from no return to total return), dryness (the humidity perception after the first bite, from humid to dry), gumminess (easiness to disintegrate bread during tasting, from easy to difficult), acidic smell (odour perceptions of acidity and pungency, from low to very high), sweetness (sweet taste, from low to high), acidic taste (acid taste, from low to high), bitterness (bitter taste, from low to high), saltiness (salty taste, from low to high) and sapidity (intensity of savory flavour perception, from low to high) using a 0 to 100 scale.

	FS0	FS0.7	FSW	FS1.5	FCTR
Crust colour	50±8^{b}	51±7^{b}	47±6^{b}	44±7^{b}	68 ± 12^{a}
Crumb colour	55±2^{a}	59±4^{a}	59±5^{a}	58±3^{a}	40±6^{b}
Hardness	37±5^{b}	36±8^{b}	30±7^{b}	53±6^{a}	24±4^{c}
Elasticity	79±8^{a}	77±9^{a}	73 ± 12^{a}	53 ± 10^{b}	78 ± 11^{a}
Dryness	57±6^{b}	57±4^{b}	56±8^{b}	61±7^{ab}	74 ± 11^{a}
Gumminess	45±7^{a}	40±7^{a}	43±9^{a}	46±8^{a}	30±8^{b}
Acidic smell	58±8^{a}	53±6^{ab}	46±6^{b}	42±5^{b}	11±5^{c}
Sweetness	58±9^{a}	32±7^{b}	30±5^{b}	32±6^{b}	35±8^{b}
Acidic taste	54±5^{a}	43±6^{ab}	49±9^{ab}	41±4^{b}	8±7^{c}
Bitterness	14±3^{b}	14±7^{ab}	30±8^{a}	23±8^{ab}	4±2^{c}
Saltiness	14±2^{c}	32±7^{b}	30±9^{b}	42±8^{ab}	50±2^{a}
Sapidity	38±7^{b}	42±6^{b}	52±5^{a}	39±4^{b}	30±4^{c}

Data represent means of three independent replicates and ten different judges' evaluations ± standard deviations.
[a-c] Values in the same row with different letters differ significantly ($P < 0.05$).

CRediT authorship contribution statement

Palmira De Bellis: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Marco Montemurro:** Investigation, Formal analysis, Visualization. **Massimiliano D'Imperio:** Investigation, Formal analysis. **Carlo Giuseppe Rizzello:** Project administration, Writing - review & editing. **Angelo Sisto:** Writing - review & editing. **Paola Lavermicocca:** Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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