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Original article

In situ dextran synthesis by *Weissella confusa* Ck15 and *Leuconostoc pseudomesenteroides* DSM 20193 and their effect on chickpea sourdough bread

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Summary This work evaluated, for the first time, the impact of *in situ* dextran (with different branching degree) produced by *Weissella confusa* Ck15 and *Leuconostoc pseudomesenteroides* DSM 20193 strains on the technological properties of chickpea–wheat sourdough bread prepared with three levels of chickpea flour (20, 30 and 40 g/100 g). In addition *Lactiplantibacillus plantarum* F8 strain (not dextran producing) and a control without sourdough fermentation were used. Specific volume, crumb hardness and moisture content of breads were evaluated during six days of storage. At the increase of chickpea flour from 20 to 40 g/100 g in the samples, the lowest decrease in bread volume (15%) occurred when *W. confusa* Ck15 was used. Moreover, these breads showed the lowest crumb hardness at each chickpea flour percentage, 46, 80 and 98 N. Hence, *in situ* dextran synthesis by *W. confusa* Ck15 might counteract negative effects caused by gluten-free chickpea flour on technological properties of bread.

Keywords Bread, dextran, lactic acid bacteria, legumes, texture.

Introduction

The capability of some lactic acid bacteria (LAB) to produce exopolysaccharides (EPS) is of industrial importance in food, pharmaceutical and chemical sectors. In the food industry, bacterial EPS have been proposed as alternatives for plant- or seaweed-derived polysaccharides – including starch and its modified derivatives, pectins, alginates – which are currently used as thickening, stabilising, texturising and gelling agents. EPS-producing LAB are widely employed in the production of fermented foods, such as dairy products (i.e. yoghurt, cheese and fermented milk) and cereal-based goods (i.e. sourdoughs) (Broadbent *et al.*, 2003; Lynch *et al.*, 2018).

In sourdough baked products, bacterial EPS acting as hydrocolloids increase the volume and crumb softness, and delay the staling phenomenon (Ketabi *et al.*, 2008). LAB can synthesise two types of EPS: homopolysaccharides (HoPS), consisting of repeating units of one type of monosaccharide (e.g. glucose and fructose), and heteropolysaccharides (HePS) consisting of irregular repeating units (e.g. galactose and rhamnose) (Galle & Arendt, 2014; Caggianiello *et al.*, 2016). The wide structural varieties of EPS isolated from sourdough include mainly HoPS. HoPS such as glucans (dextran, mutan, alternan and reuteran) and fructans are mainly synthesised by LAB belonging to different genera such as *Weissella*, *Leuconostoc*, *Pediococcus, Limosilactobacillus* and *Fructilactobacillus,* from sucrose by extracellular enzymes glucansucrases and fructansucrase, respectively (Galle & Arendt, 2014; Zhou *et al.*, 2019; Kavitake *et al.*, 2020). Dextrans are α-glucan polymers containing consecutive α-(1 → 6) linkages in the main chain and α-(1 → 2), α-(1 → 3) or α-(1 → 4) in the branch (Bounaix *et al.*, 2009), and their use in food production has been approved in Europe since the 2000s (European Commission, 2000). Furthermore, it has been reported that dextrans characterised by a low degree of branching and a high molecular weight lead to a further improvement of the sourdough bread quality in terms of volume and crumb softness (Lacaze *et al.*, 2007). These features, overall, depend on producing strain, to some extent on fermentation conditions, and on available nutrients (Heinze *et al.*, 2006). *In situ* dextran production by LAB during fermentation might represent a suitable option for replacing hydrocolloids, thus

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favouring the production of food with a clean label. This feature could be exploited in the manufacture of leavened baked product from non-cereal or gluten-free flours, for which the use of hydrocolloids is strategic because of their poor technological quality compared to wheat flour due to the lack of gluten proteins. So far, the use of EPS-producing LAB has been proposed for improving rheological and technological features of baked products enriched in various flours. *In situ* EPS production by LAB in sourdough led to breads with softer crumb and higher specific volume using sorghum flour (0.6 to 8.0 g of EPS/kg; Galle *et al*., 2012) and pearl millet flour (3.5% dextran on dry weight) (Wang *et al*., 2019). As regard to legume flour, Wang *et al*. (2018) reported more positive effects on viscoelastic properties of dextran produced in faba bean sourdough by a *W. confusa* strain (5.2% dextran on flour basis) than by *Ln. pseudomesenteroides* DSM 20193 (3.6% dextran on flour basis) of doughs.

Using legume flours in baked products is of great importance due to their nutritional quality and their distribution in the diet all of the world (Melini *et al*., 2017). For instance, chickpea flour represents a good complement of wheat flour thanks to its content of protein, dietary fibre, vitamins, minerals and its amino acid composition (Jukanti *et al*., 2012). However, legume flours exhibit some technological disadvantages mainly due to the absence of gluten and to the competition of wheat and legume proteins for water (Bresciani & Marti, 2019). Thus, the incorporation of chickpea flour in wheat dough, depending on the enrichment level, leads to doughs with decreased strength and extensibility, and to bread with decreased specific volume and crumb softness (Mohammed *et al*., 2012; Zafar *et al*., 2015). To the best of our knowledge, the use of *in situ* dextran producing LAB has been not yet investigated in chickpea sourdough for bread manufacture. Therefore, taking into consideration the potential positive effects of EPS-producing LAB in a wide variety of flours, the aim of this work was to evaluate the effect of *in situ* dextran production by *Weissella confusa* Ck15 and *Leuconostoc pseudomesenteroides* DSM 20193 strains (previously characterised by Galli *et al*., 2020 for their dextran production in chickpea sourdough), on the technological properties of chickpea–wheat sourdough bread prepared with three levels of chickpea flour (20, 30 and 40 g/100 g).

Materials and methods

Bacterial strains and culture conditions

Three LAB strains were used. *Weissella confusa* Ck15, previously isolated from chickpea sourdough spontaneous fermentation, that has been shown to produce dextran with few (2.6%) α-(1 → 3) linked branches (Galli *et al*., 2020) from sucrose. *Leuconostoc pseudomesenteroides* DSM 20193, purchased from Leibniz Institute DSMZ (Braunschweig, Germany), that has been shown to produce dextran with 6.0% of α-(1 → 3) linked branches from sucrose. *Lactiplantibacillus plantarum* F8, an EPS-negative strain belonging to the collection of Department of Agricultural, Food, Environment and Forestry (DAGRI) of the University of Florence (Italy) that was isolated from wheat sourdough. All LAB were routinely propagated in MRS broth at 30 °C (Oxoid, Basingstoke, Hampshire, England).

Ingredients for bread making

The ingredients used in this study included chickpea (*Cicer arietinum*) flour (protein 24.70 ± 0.90, fat 5.60 ± 1.10, ash 2.90 ± 0.25, moisture 11.20 ± 0.32 and carbohydrates 55.60 ± 1.20 g/100 g) commercial wheat (*Triticum aestivum* L.) flour (type '00'; COOP, Casalecchio di Reno, Bologna, Italy) (protein 12.5, fat 0.70, moisture 13.8 and carbohydrates 73.0 g/100 g); compressed baker's yeast (Zeus Iba, Florence, Italy); sucrose (Merck, Darmstadt, Germany); and salt (Italkali, Palermo, Italy). Chickpea flour was obtained by dried chickpeas, provided by a local farm (Az. Agr. Radici, Loro Ciuffenna, Arezzo, Italy) in two different periods, grounded at a mesh size of 0.5 mm by Komo Fidibus XL grinder (KoMo GmbH, Hopfgarten, Austria).

Experimental bread formulation preparation

W. confusa Ck15, *Ln. pseudomesenteroides* DSM 20193 and *Lp. plantarum* F8, propagated in MRS broth for 24 h, were recovered by centrifugation (10 000***g*** for 10 min) and washed with sterile physiological solution. Then, cells were resuspended in distilled water to obtain a cell density of about 7.0 log CFU per g and were singly used to inoculate chickpea liquid sourdoughs (dough yield of 333). For the *in situ* dextran production, 2 g/100 g of chickpea flour was substituted with sucrose, this percentage showed to enhance dextran production in this matrix, reaching 1.49 and 1.18 g/100 g in *W. confusa* Ck15 and *Ln. pseudomesenteroides* DSM 20193 sourdough, respectively (Galli *et al*., 2020). After 18 h of fermentation, chickpea sourdoughs (25% final dough) were added as ingredients for the bread production, as described in Table 1. Wheat flour was substituted with 20, 30 and 40 g/100 g of chickpea flour. For each substitution level, a control (C) sample without sourdough addition was prepared.

For bread making, the ingredients were added at the same time and mixed for 10 min at 47 rpm in a twin arms mixer model RS12 (Bernardi, Villar San Costanzo, Cuneo, Italy). The doughs were divided into moulds of 100 g and placed in a proofing chamber

Table 1 Ingredients (g/100 g) for bread making. 20, 30 and 40 indicate the amount of chickpea flour in the recipe: 20, 30 and 40 g/100 g on total flour, respectively

	Bread20		Bread30		Bread40	
Ingredients	**SD**	**C**	**SD**	**C**	**SD**	**C**
Wheat flour (g)	50.6	50.6	44.3	44.3	38.0	38.0
Chickpea flour (g)	5.7	12.7	12.0	19.0	18.3	25.3
Water (mL)	16.9	34.9	16.9	34.9	16.9	34.9
Chickpea sourdough (g)	25	–	25	–	25	–
Baker's yeast (g)	0.9	0.9	0.9	0.9	0.9	0.9
Salt (g)	0.9	0.9	0.9	0.9	0.9	0.9
Dough Yield	158	158	158	158	158	158

SD: dough prepared with sourdough; C: dough prepared with only baker's yeast.

(Unipan; Alaska, Costa di Rovigo, Rovigo, Italy) at 30 °C and 88–90% relative humidity for 2 h. Finally, doughs were baked at 200 °C for 15 min in an oven (Rossella, Unox, Padua, Italy), with vapour injection in the first instants of baking.

Enumeration of lactic acid bacteria and yeasts

Ten grams of dough sample, taken at the end of leavening time, was transferred into 90 mL of sterile physiological solution and homogenised for 2 min in a Stomacher Lab Blender 400 (Seward Ltd, Worthing, West Sussex, UK). After decimal dilutions, 100 μL of these suspensions was pour-plated for cell enumeration on MRS agar for the LAB, and MYPG agar containing (in g L^{-1}): malt extract 5, yeast extract 3, meat extract 5, glucose 10 and sodium propionate (2 g L^{-1}), for the yeasts. LAB were counted after incubation for 48 h at 30 °C under anaerobic conditions using AnaeroGen Compact enzymatic kit (Oxoid Ltd, Hampshire, UK) and yeasts after incubation for 48 h at 30 °C under aerobic conditions.

pH and Total Titratable Acidity (TTA)

The pH of the dough was determined by a pHmeter (Metrohm Italiana Srl, Varese, Italy) with a food penetration probe. The total titratable acidity (TTA) was measured according to Bottani *et al.* (2018), by homogenising 10 g of dough samples with 90 mL of distilled water for 3 min. The TTA was expressed as quantity (mL) of 0.1 mol L^{-1} NaOH to reach a pH of 8.5.

Determination of organic acids

The organic acid determination of the doughs was carried out according to Galli *et al.* (2019). Samples were diluted ten times with distilled water and then filtered by Amicon® Ultra-4 Centrifugal Filters (3000 Da NMWL) (Merck Millipore, Burlington, USA) before the injection. Organic acids were determined by high-performance liquid chromatography (HPLC) analysis (Varian Inc, Palo Alto, USA). Separation was obtained with a Rezex ROA organic acid H+ column (300 × 7.8 mm; Phenomenex, Castel Maggiore, Bologna, Italy), connected to a refractive index detector (Knauer K-2301, Knauer GmbH, Berlin, Germany) and UV detector ($\lambda = 210$). Elution was performed at 65 °C with 0.0065 mol L^{-1} H_2SO_4 eluent at flow rate of 0.6 mL min^{-1}. Data were collected and analysed by using the Galaxie software (Varian Inc, Palo Alto, USA). Quantitative analysis was carried out by standard curves designed for each compound. The fermentation quotient (FQ), defined as the molar ratio between lactic and acetic acids, was calculated.

Bread characteristics

Specific volume was determined through the ratio between bread volume evaluated by seed replacement method (AACC method 10-05.01 (AACCI, 2001)) and its weight, after one day of storage. Crumb hardness was measured according to the AACC method 74-09.01 (AACCI, 2001) by using a TA.XT Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 100 N load cell and an aluminium probe with 36 mm of diameter (pre-test speed: 200 mm s^{-1}; test speed: 100 mm s^{-1}; and post-test speed: 200 mm s^{-1}). Finally, crumb moisture was determined by a Moisture Tester MT-CA (Brabender GmbH & Co., Duisburg, Germany) at 130 °C for 1 h. Crumb hardness and moisture were evaluated after one, three and six days of storage.

Statistical analysis

Each bread-making process was carried out twice. Microbial and chemical analyses were performed in triplicate and presented as the mean ± standard deviation. For each formulation, specific volume was carried out on four loaves, instead, three loaves were used for crumb hardness (i.e. two slices from each loaf, for a total of six slices); crumb moisture was determined on two slices. Data were analysed by one-way or two-way analysis of variance (ANOVA) using GraphPad Prism 6 (GraphPad Software Inc, San Diego, CA, USA). The mean comparisons were determined by Tukey's test ($P < 0.05$).

Results

Dough acidification and microorganism concentrations

The results of acidification and LAB concentrations of the experimental doughs are showed in Table 2.

Table 2 Acidification parameters (pH and total titratable acidity, TTA, organic acids and fermentation quotient, FQ) and lactic acid bacteria (LAB) concentrations of the doughs at the end of fermentation. Bread20, Bread30 and Bread40 indicate the different amount of chickpea flour in the dough, 20, 30 and 40 g/100 g on total flour, respectively; Ck15: dough with *W. confusa* Ck15 sourdough; 20193: dough with *Ln. pseudomesenteroides* DSM 20193 sourdough; F8: dough with *Lp. plantarum* F8 sourdough. Results are expressed as average ± standard deviation ($n = 3$)

	Bread20 dough			Bread30 dough			Bread40 dough		
	Ck15	20193	F8	Ck15	20193	F8	Ck15	20193	F8
pH	5.11 ± 0.20^{c}	4.95 ± 0.14^{bc}	4.43 ± 0.07^{a}	5.19 ± 0.17^{c}	5.16 ± 0.26^{c}	4.61 ± 0.06^{ab}	5.26 ± 0.06^{c}	5.19 ± 0.15^{c}	4.68 ± 0.15^{ab}
TTA (mL)	5.10 ± 0.98^{a}	5.33 ± 1.11^{a}	6.43 ± 0.61^{ab}	4.47 ± 0.50^{a}	5.10 ± 0.62^{a}	6.33 ± 0.76^{ab}	5.80 ± 0.10^{a}	6.25 ± 1.33^{ab}	7.70 ± 1.04^{b}
Lactic acid (g/100 g)	0.20 ± 0.09^{a}	0.18 ± 0.04^{a}	0.43 ± 0.04^{b}	0.16 ± 0.05^{a}	0.22 ± 0.06^{a}	0.40 ± 0.04^{b}	0.23 ± 0.05^{a}	0.23 ± 0.07^{a}	0.47 ± 0.14^{b}
Acetic acid (g/100 g)	0.06 ± 0.02^{a}	0.06 ± 0.01^{a}	0.04 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.02^{a}	0.04 ± 0.01^{a}	0.06 ± 0.02^{a}	0.05 ± 0.03^{a}	0.04 ± 0.00^{a}
FQ	1.95 ± 0.31^{a}	2.34 ± 0.98^{a}	7.17 ± 0.37^{c}	1.75 ± 0.25^{a}	2.57 ± 0.81^{b}	7.62 ± 0.27^{c}	1.95 ± 0.22^{a}	2.19 ± 0.33^{a}	7.83 ± 0.82^{c}
LAB (CFU g^{-1})	$(5.90 \pm 0.49) \times 10^{8a}$	$(2.31 \pm 0.41) \times 10^{9b}$	$(1.28 \pm 0.08) \times 10^{9ab}$	$(9.63 \pm 3.29) \times 10^{8a}$	$(1.82 \pm 0.19) \times 10^{9b}$	$(1.41 \pm 0.41) \times 10^{9b}$	$(7.30 \pm 0.42) \times 10^{8b}$	$(1.93 \pm 0.46) \times 10^{9b}$	$(1.58 \pm 0.72) \times 10^{9b}$

Different letters in the same row correspond to significant differences (Tukey's test; $P < 0.05$).

Regardless of the chickpea substitution level, *Lp. plantarum* F8 was the most acidifying strain, displaying the lowest pH value and the highest lactic acid production (up to 0.43 g/100 g in Bread20 dough). In addition, these parameters were similar in *W. confusa* Ck15 and *Ln. pseudomesenteroides* DSM 20193 doughs. The highest final TTA was displayed by the dough inoculated with *Lp. plantarum* F8, in particular Bread40 dough, which, however, was not statistically different (P-value = 0.05) from Bread40 dough inoculated with *Ln. pseudomesenteroides* DSM 20193.

As regards to the organic acids, the acetic acid content did not show any significant variation neither on the base of the LAB used nor of the substitution level applied. Lactic acid amount varied depending on the LAB strain, affecting the fermentation quotient. Indeed, the FQ of the doughs inoculated with *Lp. plantarum* F8 was always higher compared to those with *W. confusa* Ck15 or *Ln. pseudomesenteroides* DSM 20193, with value up to 7.8.

LAB cell density was not affected by the percentage of chickpea flour, attaining to value of 9 log CFU g^{-1} except for *W. confusa* Ck15 strain. The *W. confusa* population showed the lowest cell concentration at the end of fermentation, not exceeding 8.98 log CFU g^{-1}, highlighting a lower growth yield, that did not lead to a reduced acidification compared to *Ln. pseudomesenteroides* DSM 20193.

Bread characteristics

Specific volume

The images of bread samples and the specific volume are showed in Fig. 1. A two-way ANOVA was carried out in order to investigate the effect of both the chickpea levels and the leavening agents on the specific volume of bread (Table S1). Results pointed out that the considered factors and their interaction strongly influenced ($P < 0.0001$) this parameter.

At the lowest enrichment level (20 g/100 g), the highest specific volume was detected in the samples inoculated with *Ln. pseudomesenteroides* DSM 20193 and *Lp. plantarum* F8, and the lowest one in *W. confusa* Ck15 and control samples (Fig. 1b). The specific volume decreased as the chickpea flour content increased, except for bread with *W. confusa* Ck15 sourdough and 20 and 30 g/100 g of chickpea flour. Indeed, for this sample, no significant differences were measured in the specific volume when the enrichment level increased from 20 to 30 g/100 g. Moreover, the lowest loss of specific volume (about 15%) was measured increasing the amount of chickpea flour from 20 to 40 g/100 g.

Interestingly, loaves with the dextran producing LAB (i.e. *W. confusa* Ck15 and *Ln. pseudomesenteroides* DSM 20193) showed the highest specific

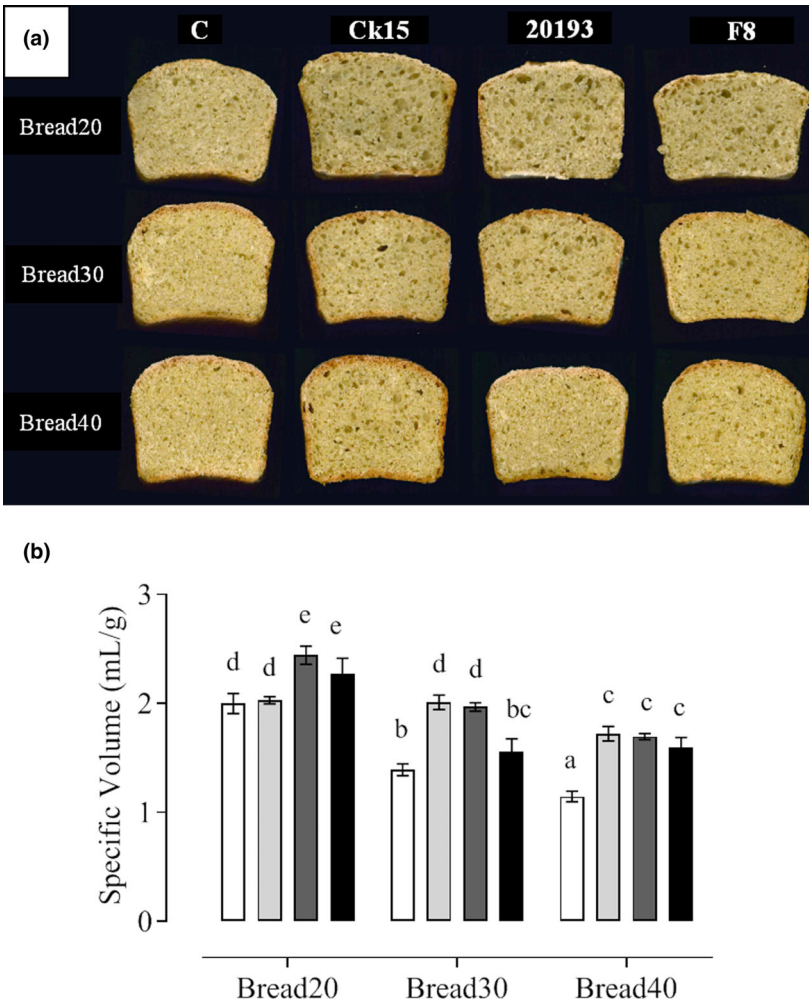

Figure 1 Slices (a) and specific volume (b) of bread. C: bread with *S. cerevisiae*; Ck15: bread with *W. confusa* Ck15; 20193: bread with *Ln. pseudomesenteroides* DSM 20193; F8: bread with *Lp. plantarum* F8. White bars: control bread; light grey bars: bread with *W. confusa* Ck15 sourdough; dark grey bars: bread with *Ln. pseudomesenteroides* DSM 20193 sourdough; black bars: bread with *Lp. plantarum* F8 sourdough. Different letters correspond to significant differences (Tukey's test; $P < 0.05$). Results are expressed as average ± standard deviation ($n = 4$). 20, 30 and 40 indicate the amount of chickpea flour in the recipe (20, 30 and 40 g/100 g, respectively).

volume among the Bread30 samples, whereas at the highest enrichment level (to 40 g/100 g), no differences among the sourdough bread samples were observed, while they showed a higher volume compared to the control bread.

Crumb hardness

Crumb hardness after one, three and six days of storage is reported in Fig. 2. As for specific volume, the considered factors and their interaction were highly significant ($P < 0.0001$) at each storage time in determining crumb hardness (Table S1).

Crumb hardness increased as the level of chickpea flour substitution increased, but at different extent depending on the leavening agent. Specifically, regardless the storage time, at the lowest enrichment level (20 g/100 g), bread with *W. confusa* Ck15 and *Ln. pseudomesenteroides* DSM 20193 sourdoughs showed the lowest hardness, thus resulting softer than control and *Lp. plantarum* F8 bread samples. After six days, the hardness of Bread20 sourdough loaves increased by more than 86%. These increments were higher compared to that recorded in the C bread, due to the high initial values of hardness that characterised the C bread already after the first day of storage. In presence of 30 g/100 g of chickpea flour, after the first day of storage, the crumb hardness of bread with *W. confusa* Ck15 and *Ln. pseudomesenteroides* DSM 20193 sourdoughs was almost doubled, compared to Bread20 samples, reaching 80 and 78 N, respectively. These samples did not show statistical difference with the control sample (89 N), but they resulted softer compared to that of *Lp. plantarum* F8 (107 N).

At the highest enrichment level (40 g/100 g), *W. confusa* Ck15 bread showed the lowest crumb hardness (98 N), not statistically different (P-value = 0.05) from the C bread (102 N). Conversely, *Ln. pseudomesenteroides* DSM 20193 bread was characterised by the highest crumb hardness (135 N) among the samples.

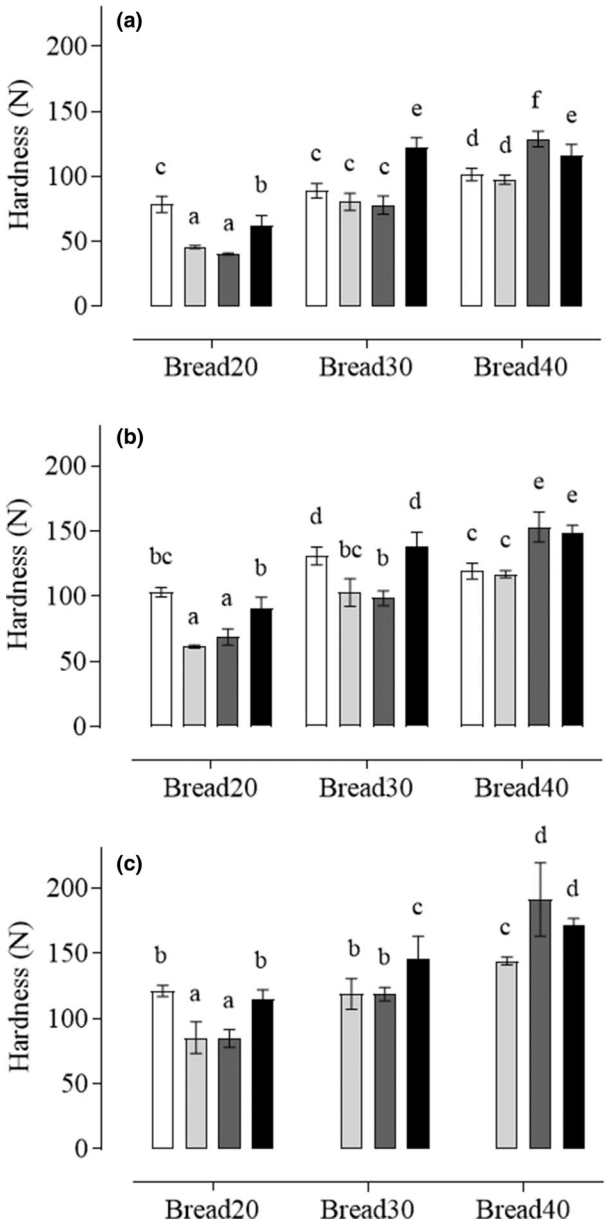

Figure 2 Crumb hardness of the bread samples after one (A), three (B) and six (C) days of storage. White bars: control bread; light grey bars: bread with *W. confusa* Ck15 sourdough; dark grey bars: bread with *Ln. pseudomesenteroides* DSM 20193 sourdough; black column: bread with *Lp. plantarum* F8 sourdough. 20, 30 and 40 indicate the amount of chickpea flour in the recipe (20, 30 and 40 g/100 g, respectively). Different letters (a-f), within the same day of storage, correspond to significant differences (Tukey's test; $P < 0.05$; $n = 6$).

Regardless the storage time, Bread30 and Bread40 with *W. confusa* Ck15 samples had the softest crumb. After six days of storage, C bread samples with 30 and 40 g/100 g of chickpea flour were covered with moulds; hence, they were not analysed. Loaves with EPS-positive LAB showed a hardness increase not statistically (P-value = 0.05) different between the Bread30 and 40 samples, ranging from +47% to +52%. As regards the bread crumb moisture (data not shown) the lowest values were found in the control bread (<38%), while the bread from sourdough ranged between 39 and 40%. Moisture decreased in all the samples during the storage to different extents, not highlighting any effect of dextran produced by lactic acid bacteria.

Discussion

Legume flours are promising ingredients in bread making, thanks to their nutritional value. However, the incorporation of legumes leads to dough with poor technological features. Indeed, the replacement of wheat flour with legumes not only leads to the gluten dilution but also to the water competition between wheat and legume proteins, due to the high water-binding capacity of the latter (Turfani *et al.*, 2017), which would affect the optimal gluten network formation, resulting in bread with poor volume and crumb structure. In this study, the influence of chickpea sourdough containing *in situ* producing dextran strains on bread quality was investigated. The influence of EPS on bread features was evaluated using two dextran producing LAB, *W. confusa* Ck15 and *Ln. pseudomesenteroides* DSM 20193, previously characterised for their dextran production in chickpea flour (Galli *et al.*, 2020), and an EPS-negative control represented by *Lp. plantarum* F8. Sourdoughs were prepared with sucrose (2 g/100 g of flour) since this amount was found to enhance dextran production (Galli *et al.*, 2020), then used for bread manufacture.

First, acidification parameters of the doughs were determined as a fundamental feature of sourdough, strongly affecting the sensory quality and as main markers of bacterial activity. The effect of dextran on technological quality of bread (i.e. crumb texture) depends also on the acidification that, if it is strong, leads to unfolding of gluten proteins, weakening the gluten structure (Barber *et al.*, 1992). Our results indicated a higher acidification by *Lp. plantarum* F8, compared to that of the two dextran producing strains. The organic acid profile of the two EPS-positive strains did not point out any significant differences between each other (Table 2). The fermentation quotient (FQ) is a marker of a balanced production of organic acids, and it usually considered optimal between 1.5 and 4 (Spicher, 1983). The FQ of the doughs with dextran producing strains were in the range of the optimal values (~2) (Table 2). Instead, regardless the replacement level, the experimental bread samples prepared with *Lp. plantarum* F8

sourdough presented a high FQ (>7), due to the greater production of lactic acid, as a consequence of its facultative heterofermentative metabolism. Leavening agent and chickpea enrichment level strongly affected the specific volume and the bread crumb hardness, up to six days of storage. Different strains led to different bread-making performances, with the *W. confusa* Ck15 and *Ln. pseudomesenteroides* DSM 20193 showing the best results in terms of specific volume and crumb hardness. A significant decrease in specific volume (Fig. 1b), together with an increase in crumb hardness (Fig. 2), was observed with the increasing amount of chickpea flour. The replacement of wheat flour with chickpea flour leads to a dilution of the gluten, causing a decrease in dough strength and extensibility (Mohammed *et al*., 2014). These negative effects were partially counteracted by sourdough with dextran, especially produced by *W. confusa* Ck15. Indeed, at 30 g/100 g replacement level, bread samples with the two dextran producing strains showed significantly higher bread specific volume compared to the other samples (Fig. 1b). However, regardless the leavening agent, at the maximum replacement level (i.e. 40 g/100 g) all the sourdough bread samples showed a higher specific volume compared to the control bread. This result might indicate that the 30 g/100 g replacement level represent the maximum limit for a marked effect of dextran in the dough structure. In any case, this aspect deserves to be further explored. Dextran has been suggested to interact with the gluten matrix by hydrogen bonds and/or steric interactions, supporting the gluten network and reinforcing the dough structure (Zhang *et al*., 2020). It is noteworthy that bread with *W. confusa* Ck15 sourdough was characterised by a constant specific volume; indeed, the value dropped to a lower extent when the chickpea flour level increased from 20 to 40 g/100 g. As already mentioned, the addition of chickpea flour boosted crumb hardness that kept increasing during the storage (Fig. 2). Interestingly, regardless the replacement level, among the bread samples with sourdough, bread with *W. confusa* Ck15 showed always the lowest hardness value, highlighting a minor staling of bread, likely due to the production of dextran. Unlike *W. confusa* Ck15, the presence of *Ln. pseudomesenteroides* DSM 20193 did not exhibit the same effect, particularly in the Bread40 sample. A better effect of dextran produced by a *W. confusa* strain compared to a *Ln. pseudomesenteroides* strain was also found in wheat–faba bean bread (Wang *et al*., 2018). The different performance of the dextran producing species relies on several factors such as EPS yield and their structure (degree of branching, molecular weight, etc.) (Zhang *et al*., 2018). Dextran produced by *W. confusa* Ck15 is characterised by a less degree of branching than those synthetised by *Ln. pseudomesenteroides* DSM 20193 (Galli *et al*., 2020). This feature seems to be positively correlated to the crumb hardness, leading to enhanced bread volume and thus crumb softness (Lacaze *et al*., 2007; Wang *et al*., 2018). Moreover, *W. confusa* Ck15 chickpea sourdough is characterised by a slightly higher dextran amount compared to *Ln. pseudomesenteroides* DSM 20193, which can further enhance its effect. Gluten–dextran interactions provide additional strength to the gas cells and hence prevent diffusion and collapse of the gas cells during proofing and baking (Bárcenas & Rosell, 2005). The minor crumb staling with *W. confusa* Ck15 sourdoughs could be ascribed to dextran that binds water in a higher extent, leading to a fewer water available for the formation of amylopectin crystallites, hence inhibiting staling process (Gray & Bemiller, 2003; Zhang *et al*., 2019). Moisture contents of loaves after six days of storage and its loss were not markedly influenced by sourdough containing dextran. Shelf life of loaves was strongly affected by the use of sourdough. Indeed, bread without sourdough inoculum were covered with moulds after six days of storage in Bread30 and Bread40 samples whereas the lower pH of sourdough bread led to a more extended microbial shelf life, corroborating the increased shelf life of sourdough products. Indeed, organic acids (mainly lactic and acetic acid), produced by LAB, diffuses over the mould cell membrane and dissociates inside the cell, releasing H+-ions that acidify the cytoplasm and stop their metabolic activities (Schnürer & Magnusson, 2005).

Conclusions

The replacement of wheat flour with different amount (from 20 to 40 g/100 g) of chickpea flour strongly affected the technological properties of bread, leading to a decrease in specific volume and an increase in crumb hardness. These effects are partially counteracted by the use of sourdough containing dextran producing LAB. In this study, the baking test indicated that the presence of sourdough with dextran producing *W. confusa* Ck15 (at 30 and 40 g/100 g replacement level) improved the quality of chickpea–wheat bread by increasing specific volume and especially decreasing crumb hardness and staling during storage. These effects were detected to a lesser extent with the incorporation of sourdough inoculated by dextran producing *Ln. pseudomesenteroides* DSM 20193, probably due to the different exopolysaccharide degree of branching. Furthermore, the use of sourdough prevented bread samples from the development of moulds, extending their shelf life. In conclusion, *W. confusa* Ck15 showed a potential application for *in situ* dextran production, and it might represent a useful approach to obtain novel baked products with improved technological features, using non-conventional flours.

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Ethical guidelines

Ethics approval was not required for this research.

Conflict of interest

None.

Author contribution

Viola Galli: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Writing-original draft (equal); Writing-review & editing (equal). **Manuel Venturi:** Conceptualization (equal); Investigation (equal); Writing-review & editing (equal). **Gaetano Cardone:** Formal analysis (supporting); Investigation (supporting); Writing-review & editing (supporting). **Niccolò Pini:** Formal analysis (supporting); Investigation (equal). **Alessandra Marti:** Writing-review & editing (equal). **Lisa Granchi:** Supervision (equal); Writing-review & editing (supporting).

Peer review

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Data availability statement

Research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Results of two-way ANOVA for the considered parameters (chickpea flour percentage and leavening agent) on specific volume of bread and on crumb hardness evaluated at different days of storage (1st, 3rd, and 6th day).