LWT - Food Science and Technology 151 (2021) 112097

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Technological properties, shelf life and consumer preference of spelt-based sourdough bread using novel, selected starter cultures

Dea Korcari, Riccardo Secchiero, Monica Laureati, Alessandra Marti, Gaetano Cardone, Noemi Sofia Rabitti, Giovanni Ricci, Maria Grazia Fortina *

Università Degli Studi di Milano, Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Via Celoria 2, 20133, Milan, Italy

ARTICLE INFO

Keywords:
Spelt
Sustainable crop
Sourdough fermentation
Starter cultures
Bread quality

ABSTRACT

The goal of this work was to investigate the use of selected starter cultures to obtain a spelt-based sourdough bread with improved technological, sensory and shelf-life characteristics. Two consortia were set up, containing a yeast strain (either a commercial *Saccharomyces cerevisiae* strain or a maltose-negative *Kazachstania unispora* strain) and two strains of Lactic Acid Bacteria (LAB), belonging to *Weissella cibaria* and *Pediococcus pentosaceus* species. The ability to grow in co-culture was investigated, and no inhibitions were recorded between the LAB and yeasts, that grew in proportions deemed desirable for sourdoughs. The performance of the two consortia was assessed in a spelt-based sourdough bread, and the leavening behavior, bread volume and crumb softness, shelf life and consumer preference were assessed. The product obtained with the consortium containing *S. cerevisiae* had superior crumb texture that was maintained through 5 d of storage, and was well accepted by the consumers. Furthermore, both consortia improved the mold free shelf-life when challenged with common cereal contaminants. The data showed that selected starter cultures have a good potential in improving the quality of bakery products obtained with flours that have a poor technological performance, such as spelt, but interesting nutritional properties and sustainable cultivation.

1. Introduction

Sourdough fermentation for bread production is one of the earliest uses of bioprocesses, dating back thousands of years ago, and remains to this day one of the staple components of the human diet. Although the direct method is often used in bread production at industrial levels, the sourdough technology is still commonplace in artisanal bakeries. This practice offers a series of advantages, as several studies show the beneficial activity of Lactic Acid Bacteria (LAB) and yeasts in the production of bioactive compounds (Katina et al., 2005; Poutanen, Flander, & Katina, 2009), removal of antinutrients, such as phytate (De Angelis et al., 2003; Leenhardt, Levrat-Verny, Chanliaud, & Rémésy, 2005; Lopez et al., 2001) and improvement in texture, taste and shelf life of bread (Arendt, Ryan, & Dal Bello, 2007; Dal Bello et al., 2007; Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006).

Sourdough is a mixture of flour and water, fermented with autochthonous LAB that contribute to the aroma, taste and technological properties of the product by producing organic acids such as lactic and acetic acid. The downside of using sourdough in larger scales is the fact that the microbial composition of the sourdough may vary due to several factors, such as the time and temperature of fermentation or the chemical and microbial composition of the flour, making sourdough a difficult ingredient to standardize. A solution to this problem may be the use of well-defined starter cultures that have been selected for their desirable activities and stability. The use of sourdough is a traditional practice in many areas of the world, including Central and Eastern Europe and Scandinavia, where sourdough breads with mixed flours containing rye, barley or wheat are commonly consumed (Hammes & Gänzle, 1998). For rye, acidification achieved either chemically, or, principally, through sourdough fermentation is an essential step, that allows obtaining a desirable texture. The gluten network in rye dough is very weak, so acidification promotes water absorption by pentosans, as well as inhibits the activity of rye amylases, that can reduce loaf volume (Hammes & Gänzle, 1998; Weckx et al., 2010). Similarly, sourdough has been successfully used in gluten-free bread to improve the texture and flavor (Schober, Bean, & Boyle, 2007; Wolter, Hager, Zannini, Czerny, & Arendt, 2014).

Spelt (*T. aestivum* ssp. *spelta* (L.) is one of the oldest cereals, an

* Corresponding author.
E-mail address: grazia.fortina@unimi.it (M.G. Fortina).

https://doi.org/10.1016/j.lwt.2021.112097
Received 9 April 2021; Received in revised form 9 June 2021; Accepted 6 July 2021
Available online 8 July 2021

ancient grain which, after decades of marginal cultivation, has been upgraded for its reputation as a healthy and sustainable food. When compared to wheat (*T. aestivum* ssp. *aestivum*), spelt shows a higher content in proteins, soluble fiber and micronutrients, and lower levels of fermentable oligo- di- and monosaccharides and polyols (FODMAPs) that can lead to gastrointestinal symptoms after fermentation by the gut microbiota, as well as a better aminoacidic and lipidic profile (Escarnot, Jacquemin, Agneessens, & ; Frakolaki, Giannou, Topakas, & Tzia, 2018). Furthermore, spelt needs less inputs for its cultivation, such as pesticides and fertilizers, and can grow on marginal lands and on poorly drained and low-fertility soils, so it is a highly sustainable crop. Unfortunately, the dough obtained with spelt flour has a low elasticity, resulting in a decreased volume and increased firmness of the final product (Frakolaki, Giannou, & Tzia, 2020). Mixing spelt and wheat flour could be a valid compromise to give the bread a greater nutritional contribution, and an acceptable quality. Furthermore, the use of a microbial association consisting of LAB and yeasts, already adapted to the cereal environment, could better contribute to the rheological properties of the dough.

A number of studies are available on spelt compositional and nutritional characteristics (Arzani & Ashraf, 2017) as well as on its baking properties for breadmaking (Kulathunga, Reuhs, & Symsek, 2020). However, multidisciplinary approaches to explore the different quality aspects (e.g. microbial starters, rheological and sensory properties) of sourdough bread from ancient wheat grains are scanty (Kulathunga Reuhs & Symsek).

In this study two novel microbial consortia were set up and used to obtain spelt-based sourdough bread. The aim of this research was to investigate the activity of the mixed cultures in a guided sourdough fermentation on the technological performance, stability, consumer acceptability, and shelf life of the spelt-based bread.

2. Materials and methods

2.1. Strains and growth conditions

Two selected strains of Lactic Acid Bacteria (LAB) belonging to the species *Pediococcus pentosaceus* (strain MB33) and *Weissella cibaria* (strain CM32), and one yeast strain belonging to *Kazachstania unispora* species (strain KM11), previously isolated from natural cereal fermentations (Decimo et al., 2017; Korcari, Ricci, Quattrini, & Fortina, 2019), were studied in comparison with a *Saccharomyces cerevisiae* strain (strain SC) obtained from a commercial baker's yeast preparation. The LAB were routinely sub-cultured in De Man, Rogosa and Sharpe (MRS) broth/agar (Difco Lab., Augsburg, Germany) medium for 24–48 h at 30 °C, whereas the yeasts were grown in Yeast Peptone Dextrose (YPD) broth in the same conditions. The composition of the YPD medium is as follows: Yeast extract (10 g/L), Peptone (20 g/L), Glucose (20 g/L), pH 6.2.

All strains were deposited in the culture Collection of the Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy, at −80 °C in MRS for LAB and YPD for yeasts with 15 g/100 g glycerol.

Representatives of common fungal spoilage of bread, *Aspergillus niger*, *A. flavus* and *Fusarium verticillioides* (from the Collection of the Department of Health, Animal Science and Food Safety, University of Milan, Italy) were used as target strains for the antifungal assay. Fungal strains were grown on malt extract agar (MEA) (Merck, Darmstadt, Germany) at 25 °C for 5–7 d and spore suspensions were harvested by adding 15 mL of sterile Milli-Q water and counted by flow cytometer estimation (BD Accuri C6 Flow Cytometer, BD Biosciences, Franklin Lakes, USA).

2.2. Co-cultures and microbial composition analyses

In order to study the stability of the consortia SCLAB (*S. cerevisiae* SC, *W. cibaria* CM32, *P. pentosaceus* MB33) and KULAB (*K. unispora* KM11, *W. cibaria* CM32, *P. pentosaceus* MB33), mixed cultures were grown either in MRS broth or doughs. The total yeast and LAB counts were performed in triplicate in YGC and MRS agar plates, respectively. For evaluating the growth in doughs of the two different LAB strains, a qPCR experiment was set up. For this purpose, doughs prepared with spelt flour by mixing 100% spelt flour with 52% sterile tap water and mixed for 4 min. The doughs thus obtained were inoculated with serial dilutions of an overnight-grown broth for each bacterium in a cell density range of 3–8 log cycles. For DNA extraction 1 mL of the 1:10 diluted dough sample was centrifuged at 500 rpm for 1 min, the supernatant was recovered and centrifuged at 15,000 rpm for 5 min to recover the cellular pellet, which was then used for the phenol-chloroform total DNA extraction. The DNA obtained was used for the standard curve determination. The R^2 of the curves obtained were 0.986 for *W. cibaria* and 0.98 for *P. pentosaceus*. The primers and thermal cycles are reported in Table 1. The PCR reaction was carried out in a total volume of 15 μL, containing 7.5 μL of qPCR mix (SSO Fast Supermix, BioRad, Hercules, USA), 0.36 μL of each primer (0.3 μmol/L), 1.78 μL of PCR grade water and 5 μL of DNA. The threshold level was set by the instrument (Line-Gene 9600 series, Bioer technology, Hangzhou, China), and the efficiency was calculated with formula $E = 10^{(1-/slope)} - 1$ (Rutledge & Côté, 2003). The efficiency was deemed acceptable if it fell in the range 90–110%.

2.3. Sourdough fermentation and bread preparation

Sourdoughs (Dough Yield 152) were prepared by mixing 100% spelt flour (protein content: 13 g/100 g; fiber content: 8 g/100 g; Molino Quaglia S.p.A., Padua, Italy) and 52% sterile tap water, inoculated with different microbial consortia (Table 2), by using a mixer (KitchenAid® Artisan, Model 5KSM150PS, St. Joseph, USA) equipped with a hook. Each sample was mixed for 4 min and incubated at 30 °C for 16 h, as it represents a typical combination of time-temperature used to prepare sourdough bread (Guerrini, Parenti, Angeloni, &). Sourdough bread was prepared by mixing a 1:1 mixture of wheat and spelt flour, 63% of tap water (27 °C), 30% of sourdough and 1.5% salt (all percentages are flour basis). The technological performance of the two consortia SCLAB and KULAB were compared to a control dough prepared by inoculating *S. cerevisiae* only in a dough prepared using exclusively refined wheat flour (00D; Molini Lario S.p.A.; protein: 9 g/100 g; W: 190–210*10^{-4} J; P/L: 0.6–0.8). The doughs obtained were kneaded for 5 min, maintained at rest for 10 min, divided into 250 g pieces and put into baking pans for proofing at 30 °C for 6 h, according to the rheofermentographic test, and

Table 1
Primers and thermal cycles for qPCR evaluation of *Pediococcus* and *Weissella*.

Primer	Sequence	Thermal cycles (x40 cycles)	Reference
Pediococcus spp.	F: GAACTCGTGTACGTTGAAAAGTGCTGA R: GCGTCCCTCCATTGTTCAAACAAG	94 °C × 20 s 66 °C × 20 s 72 °C × 40 s	Pfannebecker and Fröhlich (2008)
Weissella spp.	F: CGTGGGAAACCTACCTCTTA R: CCCTCAAACATCTAGCAC	94 °C × 20 s 54 °C × 20 s 72 °C × 40 s	Jang et al. (2002)

Table 2
Composition and cell density (log_{10} CFU/g) of the microbial consortia inoculated in spelt sourdough. * control sample in wheat flour.

Sourdough samples	Consortia composition	Cell density (log_{10} CFU/g)
SC	*Saccharomyces cerevisiae*	6
SCLAB	*S. cerevisiae*	6
	Pediococcus pentosaceus	6
	Weissella cibaria	5
KU	*Kazachstania unispora*	6
KULAB	*K. unispora*	6
	P. pentosaceus	6
	W. cibaria	5
SCW*	*S.cerevisiae*	6

finally baked (Self Cooking Center®, Rational International AG, Landsberg am Lech, Germany) at 190 °C for 18 min, in presence of steam during first stages of baking. After baking, the bread samples were cooled to 20 °C and samples were taken for further analyses.

2.4. Dough leavening properties and bread features

The leavening properties of the consortia were evaluated on 315 g of dough, prepared in the conditions reported in the previous section, by means of the Rheofermentometer F4 (Chopin, Tripette & Renaud, Villeneuve La Garenne Cedex, France), recording changes in dough height and production and retention of CO_2 during fermentation at 30 °C for 6 h.

The specific volume of loaves was measured in triplicate by the ratio between the apparent volume (AACC 10–05.01; AACC 2001) and its mass. In addition, the crumb and crust color were determined using a reflectance color meter (CR 210, Minolta Co., Osaka, Japan), and the results were expressed in the CIELAB color space (L*, a*, b*), as the mean ± SEM of 4 evaluations. Crumb moisture and its water activity (a_w) were determined in two separate measurements by means of the Moisture Tester MT-CA (Brabender GmbH&Co KG, Duisburg, Germany) at 130 °C for 1 h, and by a hygrometer (Novasina AG, Zurich, Switzerland) at 25 °C, respectively. At the end, crumb texture was measured in triplicate by using a Texture Analyzer TA.XT plus C (Stable Micro Systems, Surrey, UK), after 1, 2 and 5 d of storage, according to the AACC official method (AACC 74–09.01; AACC 2001).

2.5. Antifungal activity challenge test

To test the in situ antifungal activity, a challenge test as described by Black, Zannini, Curtis, and Gänzle (2013) was performed. 25 mm slices of each bread sample were inoculated with 10^2 spores of *A. niger*, *A. flavus* and *F. verticillioides*, stored in closed plastic bags with a filtered tip to ensure aerobic conditions, at room temperature for up to 7 d. The growth of the molds was recorded daily. The shelf life was expressed as the number of days before visible mold growth.

2.6. Assessment of consumer's acceptability

The acceptability of the four different types of spelt-based bread was assessed involving 86 regular consumers of bread (48 females and 38 males; mean age = 26.4 years; s.d. = 8.1) recruited among students and staff of the University of Milan. Although the number of subjects is somewhat small, it fulfills the requirements to perform a hedonic test (ISO 11136, 2014).

Participants were asked not to smoke, eat or drink anything, except water, for 1 h before the tasting session. The protocol was approved by the Ethics Committee of the University of Milan (n. 32/12). Written informed consent was obtained from each subject before the acceptability assessment was performed.

Subjects were invited to the sensory laboratory of the Department of Food, Nutritional and Environmental Sciences of the University of Milan and were settled in individual sensory booths.

Subjects were presented with a slice of bread for each sample and asked to express their overall liking using a 100-mm linear hedonic scale anchored at the extremes with "dislike extremely" (left of the scale, score = 0) and "like extremely" (right of the scale, score = 100). Subjects were instructed to taste a piece of each slice and to rinse their mouth with mineral water between each tasting. The evaluation took approximately 10 min.

In order to balance the effects of serving order and carry-over, samples presentation order was randomized and balanced according to William's Latin square (Macfie, Bratchell, Greenhoff, &). Samples were served at room temperature (about 20 °C) in plastic plates coded with 3-digit numbers and evaluated under white light conditions. The sample SCW (refined wheat bread inoculated with *S. cerevisiae* only) was omitted because preliminary results showed the difference this bread had with the other samples could lead the participants to underestimate the differences between the spelt-based samples.

2.7. Statistical analysis

Results are expressed as mean value ± SEM and paired comparisons were analyzed with two-tailed *t*-test (asterisks indicate significance levels: *$p \leq 0.05$; **$p < 0.01$; ***$p < 0.001$; n.s. for $p > 0.05$). Sensory data were normally distributed ($W = 0.986$, $p = 0.467$) and analyzed through mixed Analysis of Variance (ANOVA) considering subjects as a random effect and bread samples as fixed effect. Tukey's HSD test was performed after the ANOVA using XLSTAT (version 2020.5.1, AddinsoftTM, France).

Effects showing a p-value of 0.05 or lower were considered significant.

3. Results

3.1. Strains selection and co-culture growth

S. cerevisiae and *K. unispora* showed a similar growth between them when cultivated alone (7.59 log_{10} CFU/mL and 8.1 log_{10} CFU/mL, respectively) or in presence of LAB (6.26 log_{10} CFU/mL and 6.4 log_{10} CFU/mL). The growth in co-culture in MRS medium showed limited inhibition in 16 h of incubation (Fig. 1). The reduced growth of the yeasts when grown in combination with *P. pentosaceus* could be probably due to the limited nutrients in the medium, rather than inhibition, whereas the heterofermentative growth of the *W. cibaria* strain, producing different organic acids, could affect the growth of the yeasts; indeed, previous research showed that both yeast strains grow

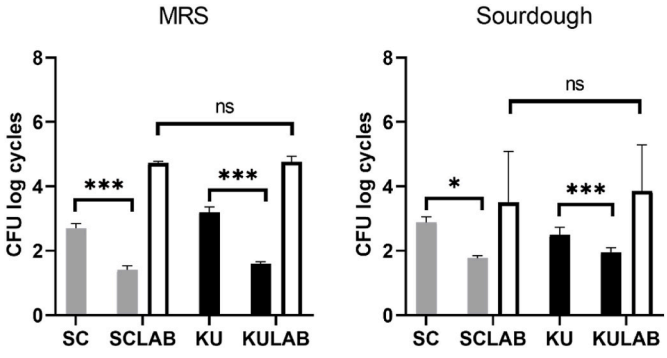


Fig. 1. Growth expressed as difference between final and initial cell density, in MRS and in spelt sourdough, of yeast strains alone or in association with LAB strains (two tailed *t*-test, asterisks indicate significance levels: *$p \leq 0.05$; ***$p \leq 0.001$) SC: *S. cerevisiae*; SCLAB: consortia of *S. cerevisiae*, *P. pentosaceus* and *W. cibaria*; KU: *K. unispora*; KULAB: consortia of *K. unispora*, *P. pentosaceus* and *W. cibaria*. ■ K. unispora ■ *S. cerevisiae* □ P. pentosaceus + *W. cibaria*. Mean ± SEM (n = 3).

efficiently in presence of lactic acid, but the growth is limited in presence of acetic acid (Korcari, Ricci, Capusoni, & Fortina, 2021). The growth of the LAB was unaffected by the presence of the yeast: the recorded growth ranged between 4.56 and 4.91 log cycles in all conditions.

Similar results were obtained when the strains were inoculated in spelt dough: the growth of the yeasts was efficient but was slightly inhibited by the LAB: whereas when inoculated alone *K. unispora* grew 2.5 log cycles, in presence of the LAB the growth resulted being 1.95 log cycles, the growth of *S. cerevisiae* was reduced from 2.88 log cycles when inoculated alone to 1.77 log cycles when used in combination with the LAB. However, the proportion between yeast cells and LAB at the end of the fermentation was 1:100, that is considered optimal for sourdough preparation. Despite the inhibitory acivity of acetic acid, the presence of the *W. cibaria* strain was deemed necessary to achieve the typical flavor and characteristics of sourdough bread, where heterofermentative LAB play a major role.

The qPCR analysis showed that the LAB grew in a similar way independently of the yeast used. During the sourdough fermentation *W. cibaria* outgrew *P. pentosaceus*, in a 4:1 proportion. During the proofing step of the bread making process, *P. pentosaceus* grew more efficiently, and the proportion between the two LAB was closer to 1:1.

3.2. Dough leavening properties

Compared to wheat dough, the presence of spelt in the formulation decreased, even though slightly, both the maximum dough height (52 *vs* 46 mm for SCW and SC, respectively) and the time to reach it (~5 *vs* ~4 h for SCW and SC, respectively) (Fig. 2). As regards gas production, the presence of spelt increased both the CO_2 produced (~1086 *vs* ~1275 mL for SCW and SC, respectively) and that one released (~43 *vs* ~133 mL for SCW and SC, respectively). When LAB were used together with *S. cerevisiae* the resulting dough required a longer time (~6 h) to reach the dough maximum height (~39 mm), but the volume of gas produced (675 mL) and released (6 mL) decreased. The worsening of leavening dough performance was even more pronounced when the *K. unispora* strain was used instead of SC. Indeed, the consortia *K. unispora* and the LAB after 6 h of fermentation reached a dough maximum height of about 27 mm and the amount of CO_2 produced was about 415 mL.

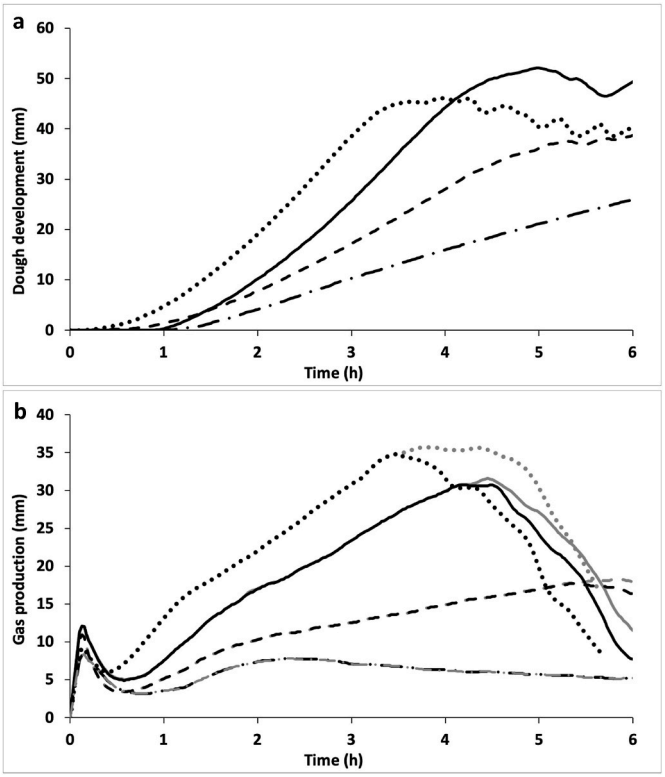


Fig. 2. Effects of SCW (solid lines), SC (dotted lines), SCLAB (dash lines), KULAB (dash-dot lines) on (a) dough development and on (b) gas production (grey lines) and retention (black lines). SCW: *S. cerevisiae* in wheat sourdough; SC: *S. cerevisiae* in spelt sourdough; SCLAB: consortia of *S. cerevisiae*, *P. pentosaceus* and *W. cibaria* in spelt sourdough; KULAB: consortia of *K. unispora*, *P. pentosaceus* and *W. cibaria* in spelt sourdough.

3.3. Bread quality

The presence of spelt flour did not cause significant ($p > 0.05$) changes in terms of either specific volume (Fig. 3) and crumb softness (Fig. 4) compared to bread produced by refined wheat flour alone. On the other hand, the combination between *S. cerevisiae* and LAB led to a decrease in specific volume (−10%). The decrease in specific volume was more evident (−30%) when *S. cerevisiae* was replaced with *K. unispora* in combination with LAB.

As regards the crumb firmness, the presence of LAB decreased this parameter up to 48 h of storage, when compared to bread leavened with *S. cerevisiae* only (Fig. 4). Based on this parameter the worst performing consortium was *K. unispora* and the LAB, as the sample reached the highest crumb firmness already after 24 h of storage.

The relative humidity and water activity of the samples did not differ significantly between the samples, and decreased as the storage time increased (data not shown). For this reason, the differences in crumb firmness between the samples are more likely due to the different leavening ability of the strains used.

From the color analysis performed on the samples (Fig. 3), it emerged that the presence of spelt, with the same leavening agent used (i.e., *S. cerevisiae*), did not significantly affect the crust color of the bread. In contrast, the related crumb became darker (i.e., decrease in L* index) and redder (i.e., increase in a* index) when spelt was used, while no change was observed in terms of yellowness (b* index). In addition, when the LAB were added, the crust resulted both less red and yellow. Unlike the consortia composed of *S. cerevisiae* and LAB, the crust of the sample containing *K. unispora* and the LAB did not differ significantly from the latter.

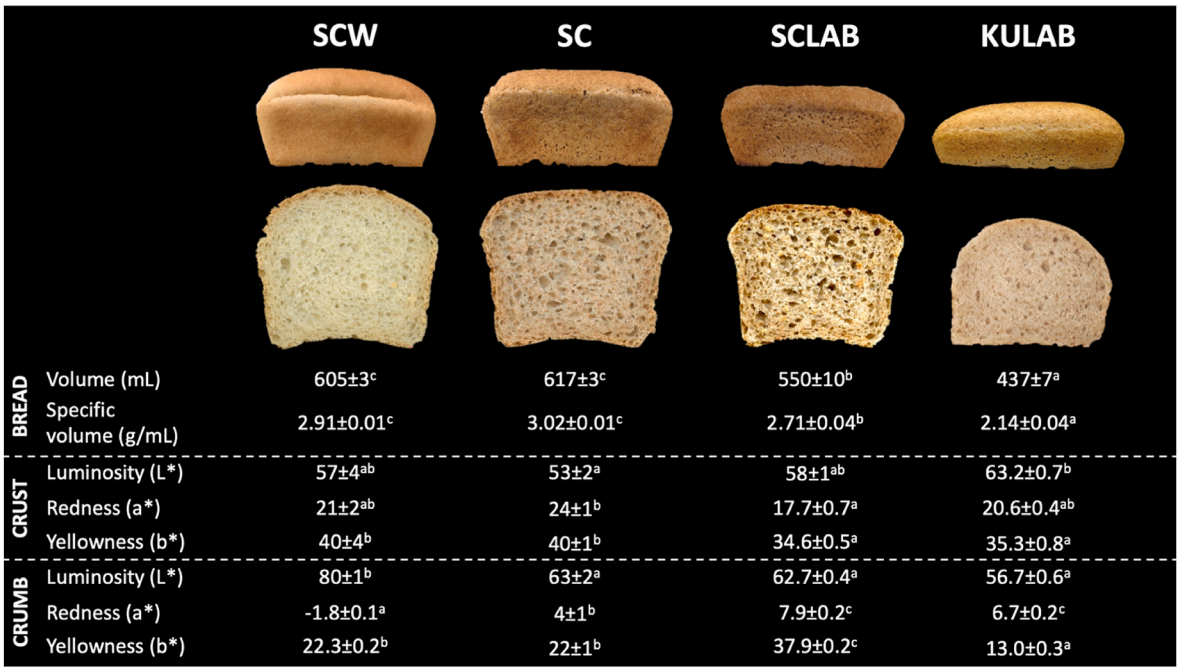
As regards crumb color, regardless of the leavening agent, the luminosity did not differ significantly among the samples containing spelt flour. The presence of LAB led to an increase in crumb redness compared to the sample containing *S. cerevisiae* only, whereas a different trend was observed in terms of yellowness. Indeed, this index significantly increased and decreased when LAB were used together *S. cerevisiae* and *K. unispora*, respectively (Fig. 3).

3.4. Antifungal activity

The antifungal activity of the consortia towards common contaminants of cereals and bakery products was assessed, after preliminary indications of an *in-vitro* antifungal activity exerted by the two strains of LAB used (Quattrini, Korcari, Ricci, & Fortina, 2019). The challenge study showed that the consortia containing the LAB, independently of the yeast used, extended the shelf life of the bread inoculated with *F. verticillioides* by 24 h, as well as inhibited the growth and sporification of the sample inoculated with *A. flavus* (Fig. 5). No inhibition was exerted towards *A. niger*.

3.5. Consumer's acceptability

The mean liking scores of the bread samples are shown in Fig. 6. Bread samples were significantly different in terms of acceptability ($F_{(3,255)} = 11.1$, $p < 0.001$). With the exception of the *K. unispora* in association with LAB, all bread samples were scored higher than the 50% of the hedonic scale (corresponding to "neither liked nor disliked").



		SCW	SC	SCLAB	KULAB
BREAD	Volume (mL)	605±3^{c}	617±3^{c}	550±10^{b}	437±7^{a}
	Specific volume (g/mL)	2.91±0.01^{c}	3.02±0.01^{c}	2.71±0.04^{b}	2.14±0.04^{a}
CRUST	Luminosity (L*)	57±4^{ab}	53±2^{a}	58±1^{ab}	63.2±0.7^{b}
	Redness (a*)	21±2^{ab}	24±1^{b}	17.7±0.7^{a}	20.6±0.4^{ab}
	Yellowness (b*)	40±4^{b}	40±1^{b}	34.6±0.5^{a}	35.3±0.8^{a}
CRUMB	Luminosity (L*)	80±1^{b}	63±2^{a}	62.7±0.4^{a}	56.7±0.6^{a}
	Redness (a*)	-1.8±0.1^{a}	4±1^{b}	7.9±0.2^{c}	6.7±0.2^{c}
	Yellowness (b*)	22.3±0.2^{b}	22±1^{b}	37.9±0.2^{c}	13.0±0.3^{a}

Fig. 3. Bread and slices of bread, bread volume and specific volume and crumb and crust color. SCW: *S. cerevisiae* in wheat sourdough; SC: *S. cerevisiae* in spelt sourdough; SCLAB: consortia of *S. cerevisiae*, *P. pentosaceus* and *W. cibaria* in spelt sourdough; KULAB: consortia of *K. unispora, P. pentosaceus* and *W. cibaria* in spelt sourdough. Different letters in the same row indicate significant differences (Tukey's HSD test, $p < 0.05$). Mean ± SEM ($n = 3$ for volume and specific volume; $n = 4$ for crust and bread color). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

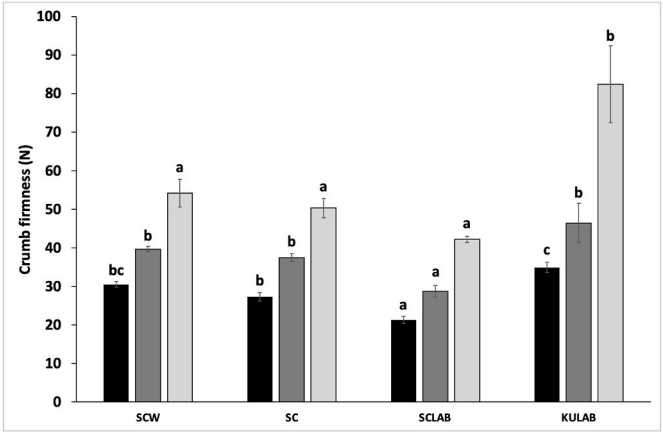


Fig. 4. Crumb firmness (N) after 1 (black bars), 2 (dark grey bars) and 5 (light grey bars) d of storage. SCW: *S. cerevisiae* in wheat sourdough; SC: *S. cerevisiae* in spelt sourdough; SCLAB: consortia of *S. cerevisiae*, *P. pentosaceus* and *W. cibaria* in spelt sourdough; KULAB consortia of *K. unispora, P. pentosaceus* and *W. cibaria* in spelt sourdough. Different letters in the same day indicate significant differences (Tukey's HSD test, $p < 0.05$). Mean ± SEM ($n = 3$).

Post-hoc comparison indicated that the *S. cerevisiae* in association with LAB and *K. unispora* samples received an acceptability score that was statistically comparable to the sample SC, whereas the sample obtained with *K. unispora* in association with LAB was statistically different and significantly less liked than the other samples.

4. Discussion

Sourdough fermentation is an ancient use of biotechnology that is gaining interest in the recent years due to the positive effects on the structure, taste, and shelf life of baked products, thanks to the activity of the LAB and yeasts (Rehman, Paterson, & Piggott, 2006). The increased demand for specialty products such as baked goods obtained from ancient grains, and the nutritional and environmental benefits that these grains have made them an important ingredient that meets both consumers' and industries' demands (Gosine & McSweeney, 2019; Kraska, Andruszczak, Gawlik-Dziki, Dziki, & Kwiecińska-Poppe, 2020; Teuber, Dolgopolova, & Nordström, 2016).

The use of selected cultures, already adapted to the specific environment, has a great potential for obtaining a product with improved quality in a controlled manner, that is a desirable feature at an industrial level. This work showed that this approach is a viable option in obtaining baked products from flours with low baking performances, such as spelt.

The microbial strains used in this research were previously selected in relation to useful physiological properties. Specifically, the strain CM32 of *W. cibaria* was chosen based on previous screenings (Quattrini et al., 2019) for its ability of producing exopolysaccharides, a high β-xylosidase activity, a high redox potential as well as the ability to inhibit the growth of *Fusarium verticillioides* and *Aspergillus flavus*. The strain *P. pentosaceus* MB33 was also chosen for the antifungal activity towards *Mucor circinelloides*, *A. flavus* and *F. verticillioides* and a good acidifying ability (Korcari et al., 2019). *K. unispora* KM11 was chosen as an alternative yeast species; in previous researches (Korcari et al., 2021) the strain showed a good leavening performance and a maltose-negative phenotype that may be advantageous in stable consortia with maltose consuming LAB (De Vuyst & Neysens, 2005).

The ability to grow in co-culture is essential for the stability of the starter cultures, and our experiments showed that *S. cerevisiae* was only slightly inhibited by the heterofermentative *W. cibaria* and the homofermentative *P. pentosaceus*. These LAB species are generally considered to be only secondary to sourdough fermentation, dominating the first steps of sourdough backslopping, as species of the old *Lactobacillus* genus, especially *Fructilactobacillus sanfranciscensis*, *Levilactobacillus brevis, Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum* dominate the sourdough environment (Oshiro, Zendo, & Nakayama, 2021). However, the positive characteristics highlighted in this research show that these alternative species are interesting and should be

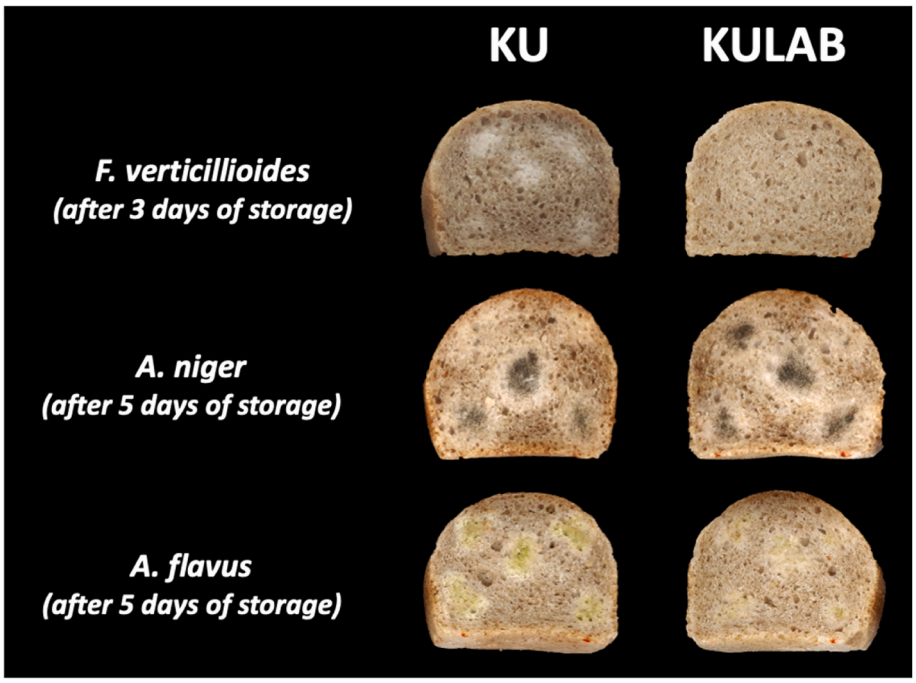


Fig. 5. Antifungal activity assay of bread obtained with *K. unispora* strain alone or in association with LAB.

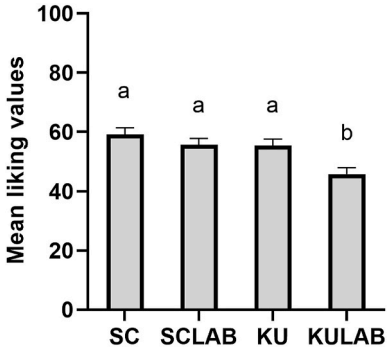


Fig. 6. Mean acceptability scores with SEM of the different bread samples. Different letters indicate significant differences ($p < 0.05$) according to post-hoc Tukey's HSD test. SC: *S. cerevisiae* in spelt sourdough; SCLAB: consortia of *S. cerevisiae*, *P. pentosaceus* and *W. cibaria* in spelt sourdough; KU: *K. unispora* in spelt sourdough; KULAB: consortia of *K. unispora, P. pentosaceus* and *W. cibaria* in spelt sourdough.

considered as adequate starters for sourdough fermentation. Specifically, the addition of *W. cibaria* and *P. pentosaceus* strains extended the shelf life, in terms of delay in fungal growth, of the spelt-enriched bread. As regards the technological properties of bread, regardless of the flour type (wheat flour alone or in presence of spelt), the use of *S. cerevisiae* alone led to the highest bread volume (Fig. 3). Combining LAB with *S. cerevisiae* decreased the dough development capability, as a result of the decrease in CO_2 production. The lower specific volume of loaves leavened by *S. cerevisiae* and LAB compared to those produced only with *S. cerevisiae* agrees with the findings by other authors (Bottani et al., 2018; Pagani, Lucisano, & Mariotti, 2008). On the contrary, other authors reported the ability of sourdough to improve the volume of the resulted bread in comparison with bread leavened by yeast only (Clarke, Schober, Angst, & Arendt, 2003; Corsetti et al., 2000). These different results might be related not only to the different leavening properties of the microorganism used, but also to the baking condition applied (e.g., temperature and leavening time).

Despite the decrease in volume, the presence of LAB enhanced the crumb softness. Such positive effect was not related to the moisture content, since no differences in this index were found among the bread samples (data not shown). Also Novotni et al. (2012) found lower crumb firmness when sourdough fermentation was used instead of yeast. According to Katina, Heiniö, Autio, and Poutanen (2006), the softer crumb texture of sourdough bread might be attributed to its lower pH compared to that leavened by *S. cerevisiae*. Previous studies showed that the presence of organic acids might cause a significant decrease in specific volume as consequence of weakening of starch and protein structure (Galal, Varriano-Marston, & Johnson, 1978; Takeda, Matsumura, & Shimizu, 2001).

The decrease in crumb luminosity after the addition of spelt flour compared to bread obtained from common wheat flour alone, could be explained by addition of spelt wholegrain flour, containing dark bran particles. The increase in crumb darkness was confirmed also by results of other studies, when spelt is used (Abdel-Aal, Hucl, Sosulski, & Bhirud, 1997; Frakolaki et al., 2018; Kohajdová & Karovicova, 2007). Instead, the increase in crumb redness in presence of LAB might be related to a more intense Maillard reaction with consequence formation of brown compounds, following the release of amino acids content as metabolism products of LAB (Winters et al., 2019).

Replacing *S. cerevisiae* with *K. unispora* resulted in a further worsening of both the dough properties (i.e., longer leavening time and lesser CO_2 production) and the bread properties (i.e., volume and specific volume). The low amount of CO_2 produced, and the consequent limited development of bread volume, might explain the highest crumb firmness achieved by this sample (Fig. 4).

On the other hand, despite the non-conventional yeast *K. unispora* did not perform as well in fermentation, it did not affect the consumer preference when it was used alone. Whereas, when *K. unispora* was used in combination with the LAB strains, the bread was significantly less preferred (Fig. 6), probably as a result of the high crumb firmness, which is reported to be a negative contributor to bread acceptance (Laureati, Giussani, & Pagliarini, 2012). Moreover, the slow leavening contributes to a higher acidity which may also lead to the lower preference observed, but the use of alternative yeasts should not be dismissed, because previous research has shown that in association with slower

fermenting LAB and at lower temperatures, they may be a viable alternative for sourdough bread production at industrial level (Häggman & Salovaara, 2008). Furthermore, long fermentations in which alternative non-*Saccharomyces* yeasts, such as *K. unispora*, are used as leavening agents, seem to have a beneficial impact on bread quality (Xu et al., 2019). It should also be considered that sour taste perception varies considerably among subjects, with consumer segments accepting and preferring products characterized by higher acidity levels (Ervina, Berget, & Almli, 2020; Törnwall et al., 2014).

5. Conclusions

In this study, two microbial consortia were studied for their ability to confer positive characteristics to a sourdough enriched with spelt flour, a low performant but sustainable and nutritional crop. The study highlights the importance of exploring the pro-technological features of non-conventional species, both yeasts and LAB, and their synergies, to obtain stable consortia that can be used at industrial level. A multidisciplinary approach was used to investigate all the aspects of the consortias' performance, with the main goal of producing a well-accepted product from a sustainable crop, with increased shelf-life and improved texture properties. This approach allows the valorization of this ancient grain that has a superior nutritional quality and requires less inputs to be cultivated compared to common wheat, overcoming the inferior technological properties through the specific design of the sourdough starters. Overall, the selected cultures exhibited potentials for future applications that deserve further studies to better understand the real role of these single or associated cultures in improvement of technological, nutritional, and sensory characteristics of bakery products.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Dea Korcari: Conceptualization, Data curation, Formal analysis. **Riccardo Secchiero:** Conceptualization, Data curation, Formal analysis. **Monica Laureati:** Supervision, Validation, Writing – review & editing. **Alessandra Marti:** Supervision, Validation, Writing – review & editing. **Gaetano Cardone:** Conceptualization, Data curation, Formal analysis. **Noemi Sofia Rabitti:** Conceptualization, Data curation, Formal analysis. **Giovanni Ricci:** Conceptualization, Data curation, Formal analysis. **Maria Grazia Fortina:** Supervision, Validation, Writing – review & editing.

Declaration of competing interest

No competing interest to declare.

References

Abdel-Aal, E. M., Hucl, P., Sosulski, F. W., & Bhirud, P. R. (1997). Kernel, milling and baking properties of spring-type spelt and einkorn wheats. *Journal of Cereal Science, 26*, 363–370. https://doi.org/10.1006/jcrs.1997.0139

Arendt, E. K., Ryan, L. A., & Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology, 24*, 165–174. https://doi.org/10.1016/j.fm.2006.07.011

Arzani, A., & Ashraf, M. (2017). Cultivated ancient wheats (*Triticum* spp.): A potential source of health-beneficial food products. *Comprehensive Reviews in Food Science and Food Safety, 16*, 477–488. https://doi.org/10.1111/1541-4337.12262

Black, B. A., Zannini, E., Curtis, J. M., & Gänzle, M. G. (2013). Antifungal hydroxy fatty acids produced during sourdough fermentation: Microbial and enzymatic pathways, and antifungal activity in bread. *Applied and Environmental Microbiology, 79*, 1866–1873. https://doi.org/10.1128/aem.03784-12

Bottani, M., Brasca, M., Ferraretto, A., Cardone, G., Casiraghi, M. C., Lombardi, G., & Silvetti, T. (2018). Chemical and nutritional properties of white bread leavened by lactic acid bacteria. *Journal of Functional Foods, 45*, 330–338. https://doi.org/10.1016/j.jff.2018.04.030

Clarke, C. I., Schober, T. J., Angst, E., & Arendt, E. K. (2003). Use of response surface methodology to investigate the effects of processing conditions on sourdough wheat bread quality. *European Food Research and Technology, 217*, 23–33. https://doi.org/10.1007/s00217-003-0724-1

Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., et al. (2000). Combined effect of sourdough lactic acid bacteria and additives on bread firmness and staling. *Journal of Agricultural and Food Chemistry, 48*, 3044–3051. https://doi.org/10.1021/jf990853e

Dal Bello, F., Clarke, C., Ryan, L., Ulmer, H., Schober, T., Ström, K., et al. (2007). Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. *Journal of Cereal Science, 45*, 309–318. https://doi.org/10.1016/j.jcs.2006.09.004

De Angelis, M., Gallo, G., Corbo, M. R., McSweeney, P. L., Faccia, M., Giovine, M., et al. (2003). Phytase activity in sourdough lactic acid bacteria: Purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. *International Journal of Food Microbiology, 87*, 259–270. https://doi.org/10.1016/S0168-1605(03)00072-2

De Vuyst, L., & Neysens, P. (2005). The sourdough microflora: Biodiversity and metabolic interactions. *Trends in Food Science & Technology, 16*, 43–56. https://doi.org/10.1016/j.tifs.2004.02.012

Decimo, M., Quattrini, M., Ricci, G., Fortina, M. G., Brasca, M., Silvetti, T., et al. (2017). Evaluation of microbial consortia and chemical changes in spontaneous maize bran fermentation. *AMB Express, 7*, 205. https://doi.org/10.1186/s13568-017-0506-y

Ervina, E., Berget, I., & Almli, V. L. (2020). Investigating the relationships between basic tastes sensitivities, fattiness sensitivity, and food liking in 11-year-old children. *Foods, 9*, 1315. https://doi.org/10.3390/foods9091315

Escarnot, E., Jacquemin, J. M., Agneessens, R., & Paquot, M. (2012). Comparative study of the content and profiles of macronutrients in spelt and wheat, a review. *Biotechnology, Agronomy, Society and Environment, 16*, 243–256.

Frakolaki, G., Giannou, V., Topakas, E., & Tzia, C. (2018). Chemical characterization and breadmaking potential of spelt versus wheat flour. *Journal of Cereal Science, 79*, 50–56. https://doi.org/10.1016/j.jcs.2017.08.023

Frakolaki, G., Giannou, V., & Tzia, C. (2020). The properties and breadmaking potential of freshly baked and frozen bakery products during partial replacement of wheat flour with spelt flour. *Food Science and Technology International, 26*, 485–492. https://doi.org/10.1177/1082013220905379

Galal, A. M., Varriano-Marston, E., & Johnson, J. A. (1978). Rheological dough properties as affected by organic acids and salt. *Cereal Chemistry, 55*, 683–691.

Gosine, L., & McSweeney, M. B. (2019). Consumers' attitudes towards alternative grains: A conjoint analysis study. *International Journal of Food Science and Technology, 54*, 1588–1596. https://doi.org/10.1111/ijfs.14126

Guerrini, L., Parenti, O., Angeloni, G., & Zanoni, B. (2019). The bread making process of ancient wheat: A semi-structured interview to bakers. *Journal of Cereal Science, 87*, 9–17.

Häggman, M., & Salovaara, H. (2008). Effect of fermentation rate on endogenous leavening of candida milleri in sour rye dough. *Food Research International, 41*, 266–273. https://doi.org/10.1016/j.foodres.2007.12.002

Hammes, W. P., & Gänzle, M. G. (1998). Sourdough breads and related products. In B. J. B. Wood (Ed.), *Microbiology of fermented foods*. Boston, MA: Springer. https://doi.org/10.1007/978-1-4613-0309-1_8.

ISO 11136. (2014). *Sensory analysis. Methodology. General guidance for conducting hedonic tests with consumers in a controlled area*. Geneva: International Organization for Standardization.

Jang, J., Kim, B., Lee, J., Kim, J., Jeong, G., & Han, H. (2002). Identification of *Weissella* species by the genus-specific amplified ribosomal DNA restriction analysis. *FEMS Microbiology Letters, 212*, 29–34. https://doi.org/10.1111/j.1574-6968.2002.tb11240.x

Katina, K., Arendt, E., Liukkonen, K. H., Autio, K., Flander, L., & Poutanen, K. (2005). Potential of sourdough for healthier cereal products. *Trends in Food Science & Technology, 16*, 104–112. https://doi.org/10.1016/j.tifs.2004.03.008

Katina, K., Heiniö, R. L., Autio, K., & Poutanen, K. (2006). Optimization of sourdough process for improved sensory profile and texture of wheat bread. *LWT-Food Science and Technology, 39*, 1189–1202. https://doi.org/10.1016/j.lwt.2005.08.001

Katina, K., Salmenkallio-Marttila, M., Partanen, R., Forssell, P., & Autio, K. (2006). Effects of sourdough and enzymes on staling of high-fiber wheat bread. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 39*, 479–491. https://doi.org/10.1016/j.lwt.2005.03.013

Kohajdová, Z., & Karovicova, J. (2007). *Effect of incorporation of spelt flour on the dough properties and wheat bread quality* (Vol. 14). Żywność Nauka Technologia Jakość.

Korcari, D., Ricci, G., Capusoni, C., & Fortina, M. G. (2021). Physiological performance of *Kazachstania unispora* in sourdough environments. *World Journal of Microbiology and Biotechnology, 37*, 88. https://doi.org/10.1007/s11274-021-03027-0

Korcari, D., Ricci, G., Quattrini, M., & Fortina, M. G. (2019). Microbial consortia involved in fermented spelt sourdoughs: Dynamics and characterization of yeasts and lactic acid bacteria. *Letters in Applied Microbiology, 70*, 48–54. https://doi.org/10.1111/lam.13241

Kraska, P., Andruszczak, S., Gawlik-Dziki, U., Dziki, D., & Kwiecińska-Poppe, E. (2020). Wholemeal spelt bread enriched with green spelt as a source of valuable nutrients. *Processes, 8*, 389. https://doi.org/10.3390/pr8040389

Kulathunga, J., Reuhs, B. L., & Symsek, S. (2020). A review: Novel trends in hulled wheat processing for value addition. *Trends in Food Science & Technology, 106*, 232–241. https://doi.org/10.1016/j.tifs.2020.09.033

Laureati, M., Giussani, B., & Pagliarini, E. (2012). Sensory and hedonic perception of gluten-free bread: Comparison between celiac and non-celiac subjects. *Food Research International, 46*, 326–333. https://doi.org/10.1016/j.foodres.2011.12.020

Leenhardt, F., Levrat-Verny, M. A., Chanliaud, E., & Rémésy, C. (2005). Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *Journal of Agricultural and Food Chemistry, 53*, 98–102. https://doi.org/10.1021/jf049193q

Lopez, H. W., Krespine, V., Guy, C., Messager, A., Demigne, C., & Remesy, C. (2001). Prolonged fermentation of whole wheat sourdough reduces phytate level and increases soluble magnesium. *Journal of Agricultural and Food Chemistry, 49*, 2657–2662. https://doi.org/10.1021/jf001255z

Macfie, H. J., Bratchell, N., Greenhoff, K., & Vallis, L. D. (1989). Designs to balance the effect of order of presentation and first-order carrt-over effects in hall tests. *Journal of Sensory Studies, 4*. https://doi.org/10.1111/j.1745-459x.1989.tb00463.x

Novotni, D., Čukelj, N., Smerdel, B., Bituh, M., Dujmić, F., & Ćurić, D. (2012). Glycemic index and firming kinetics of partially baked frozen gluten-free bread with sourdough. *Journal of Cereal Science, 55*, 120–125. https://doi.org/10.1016/j.jcs.2011.10.008

Oshiro, M., Zendo, T., & Nakayama, J. (2021). Diversity and dynamics of sourdough lactic acid bacteriota created by a slow food fermentation system. *Journal of Bioscience and Bioengineering*. https://doi.org/10.1016/j.jbiosc.2020.11.007

Pagani, M. A., Lucisano, M., & Mariotti, M. (2008). Italian bakery. In Y. H. Hui (Ed.), *Bakery products science and technology* (pp. 527–560). Ames: Wiley Online Library. https://doi.org/10.1002/9780470277553.ch30. First.

Pfannebecker, J., & Fröhlich, J. (2008). Use of a species-specific multiplex PCR for the identification of pediococci. *International Journal of Food Microbiology, 128*, 288–296. https://doi.org/10.1016/j.ijfoodmicro.2008.08.019

Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology, 26*, 693–699. https://doi.org/10.1016/j.fm.2009.07.011

Quattrini, M., Korcari, D., Ricci, G., & Fortina, M. (2019). A polyphasic approach to characterize *Weissella cibaria* and *Weissella confusa* strains. *Journal of Applied Microbiology, 128*, 500–512. https://doi.org/10.1111/jam.14483

Rehman, S., Paterson, A., & Piggott, J. R. (2006). Flavour in sourdough breads: A review. *Trends in Food Science & Technology, 17*, 557–566. https://doi.org/10.1016/j.tifs.2006.03.006

Rutledge, R. G., & Côté, C. (2003). Mathematics of quantitative kinetic PCR and the application of standard curves. *Nucleic Acids Research, 31*. https://doi.org/10.1093/nar/gng093

Schober, T. J., Bean, S. R., & Boyle, D. L. (2007). Gluten-free sorghum bread improved by sourdough fermentation: Biochemical, rheological, and microstructural background. *Journal of Agricultural and Food Chemistry, 55*, 5137–5146. https://doi.org/10.1021/jf0704155

Takeda, K., Matsumura, Y., & Shimizu, M. (2001). Emulsifying and surface properties of wheat Gluten under acidic conditions. *Journal of Food Science, 66*, 393–399. https://doi.org/10.1111/j.1365-2621.2001.tb16116.x

Teuber, R., Dolgopolova, I., & Nordström, J. (2016). Some like it organic, some like it purple and some like it ancient: Consumer preferences and WTP for value-added attributes in whole grain bread. *Food Quality and Preference, 52*, 244–254. https://doi.org/10.1016/j.foodqual.2016.05.002

Törnwall, O., Silventoinen, K., Hiekkalinna, T., Perola, M., Tuorila, H., & Kaprio, J. (2014). Identifying flavor preference subgroups. Genetic basis and related eating behavior traits. *Appetite, 75*, 1–10.

Weckx, S., Van der Meulen, R., Maes, D., Scheirlinck, I., Huys, G., Vandamme, P., et al. (2010). Lactic acid bacteria community dynamics and metabolite production of rye sourdough fermentations share characteristics of wheat and spelt sourdough fermentations. *Food Microbiology, 27*, 1000–1008. https://doi.org/10.1016/j.fm.2010.06.005

Winters, M., Panayotides, D., Bayrak, M., Rémont, G., Viejo, C., Liu, D., & Howell, K. (2019). Defined co-cultures of yeast and bacteria modify the aroma, crumb and sensory properties of bread. *Journal of Applied Microbiology, 127*, 778–793. https://doi.org/10.1111/jam.14349

Wolter, A., Hager, A., Zannini, E., Czerny, M., & Arendt, E. K. (2014). Impact of sourdough fermented with *Lactobacillus plantarum* FST 1.7 on baking and sensory properties of gluten-free breads. *European Food Research and Technology, 239*, 1–12. https://doi.org/10.1007/s00217-014-2184-1

Xu, D., Zhang, Y., Tang, K., Hu, Y., Xu, X., & Gänzle, M. G. (2019). Effect of mixed cultures of yeast and lactobacilli on the quality of wheat sourdough bread. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.02113