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ORIGINAL PAPER

Development of sourdough bread from roll-milled and stone-ground soft (*Triticum aestivum*) wheat flours milled to different extraction rates

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Abstract

The aims of the present study are: (i) to verify the influence of different flour extraction rates and milling procedures on bread quality, (ii) to optimize the bread-making process by using different percentage and time of fermentation of three spontaneously developed type I sourdoughs. These latter were prepared with a whole-meal wheat flour blend (S_A), a type 0 wheat flour blend (S_B) both obtained by steel roll milling, and a type 2 wheat flour blend obtained by stone grinding (S_C). The pH, total titratable acidity (TTA), and stability of the microbiota of the three sourdoughs were assessed before baking trials. TTA, specific volume, weight, crumb core moisture, texture, and global liking of sourdough bread, in comparison to control bread made with commercial baker's yeast, were determined. Moisture, texture, and global liking of bread were also evaluated during 6 days of storage. S_A was characterized by a significantly higher pH and TTA values than S_B and S_C. Differences in the LAB-to-yeast ratio were registered among the three sourdoughs although no differences were seen in terms of the dominant microbial community. Concerning breads, although the type 0 roll-milled wheat flour showed better dough rheological performance compared to whole-meal wheat flour and stone-ground wheat flour, sourdough fermentation positively affected the specific volume, texture, and global liking of bread manufactured with stone-ground wheat flour. Overall, for an efficient use of sourdough and bread quality improvement, optimal conditions need to be found by tailoring sourdough to the type of flour used.

Keywords Flour extraction rate · Milling procedure · Sourdough · Bread-making · Bread quality

Introduction

Bread-making is mainly based on the use of low-extraction-rate soft wheat (*Triticum aestivum*) flour, from which, during milling, bran and germ are progressively excluded, thus leading to flour and leavened baked products that are poorer in terms of nutrients compared to goods manufactured with whole-meal flour [1–3]. More specifically, the modern grain milling process is a highly efficient process performed with steel rolls: the grain is scraped by high-speed rollers layer upon layer and then the flour released from the endosperm is separated by sifters into different grades or streams based on fineness, whereas the bran and germ are completely removed [2–4]. The various sieved components can be subsequently re-integrated to enable the production of a variety of flours to be sold for diverse baking purposes [5, 6]. In the last several decades, grain milling with steel rolls has almost completely replaced the ancient milling method, relying on use of millstones [3, 4]. In stone grinding, all whole-grain components (bran, germ, and the amidaceous endosperm) are ground in a single pass through and between two horizontal, round millstones, retaining and integrating wheat germ oil [4]. If conditions of slow grinding and low temperature are applied, the wheat germ is not exposed to overheating, hence being preserved from the consequent oxidation of the fat from the germ and the destruction of vitamins, polyphenols, total fiber, and carotenoids [4, 7, 8]. Furthermore, stone grinding of small amounts of grain enables a better distribution of fat, resulting in a minimization of flour spoilage [9],

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whereas the larger particle size of stone-ground flour limits nutritive losses due to oxygen exposure [10]. Stone grinding can be followed by refining, but the latter process never produces refined type 00 flour and always type 0, 1, or 2 flour. Stone-ground flour is also believed by some bakers to be nutritionally superior to steel roll-milled flour and to have a better baking capacity, being characterized by an irregular granulometry, an ivory-hued color with flecks of cream and beige, and a sweet, nutty flavor [3, 4, 7, 11]. To the best of our knowledge, published studies that analytically compared the bread-making potential of flour blends milled with these two methods are, to date, very limited [4].

Concerning leavening agents, the use of commercial baker's yeast, at least at an industrial level, has almost replaced the traditional use of sourdough. Sourdough bread has a wider range of properties compared to most commercially available yeast-started bread, such as a prolonged shelf-life [12], a lower glycemic index [1], higher contents of B vitamins, essential minerals, and bioactive compounds, uniquely balanced protein and fatty acid contents [1, 13], easier digestion [1, 14], a higher mold resistance [3, 15], and a tangy and distinctive flavor [3, 13, 16]. Though sourdough undoubtedly represents a valid alternative to baker's yeast for manufacturing high-quality bread and other leavened products [13, 16–19], its successful large-scale utilization still presents technical difficulties, such as the careful control of both the amount of sourdough to be used (to limit excessive bread acidity and sour flavor formation) and the fermentation time required for optimal increases in dough volume [29]. In line with this, the leavening strategy applied is crucial for the optimization of bread dough formulations and bread-making processes as recently reported by Verdonck et al. [21]. Overall, as major consequences of the large-scale use of low-extraction-rate flours and baker's yeast, the shelf-life, nutritional value, and organoleptic properties of bread have decreased dramatically, with considerable implications in terms of product waste and, hence, economic losses [22]. To the author's knowledge, up until now, very few studies have investigated the baking capacity of stone-ground vs. roll-milled soft wheat flour [4, 23] and the use of sourdough as a leavening agent of stone-ground wheat flour is still very limited [8].

In a previous study, three mature firm sourdoughs were spontaneously developed by using wheat flour with different extraction rates and milling procedures at an artisan bakery and they were mainly characterized from a microbiological point of view [2]. Based on the above premises, in the present study, the same sourdoughs are used to optimize the sourdough bread-making process with the final aim of improving bread quality. The pH, TTA, and stability of the microbiota of the three long-term-propagated sourdoughs are also assessed before baking trials. Finally, total titratable acidity (TTA), specific volume, weight, crumb core moisture, texture, and global liking of sourdough bread, in comparison to control bread produced with commercial bakers' yeast, are determined. Moisture, texture, and global liking of bread were also evaluated during 6 days of storage.

Materials and methods

Flours

Two batches of three soft wheat (*Triticum aestivum*) flours from modern Italian varieties (Bolero, Sagittario, Taylor, and Bologna) designed for bread making, referred to as "F_A", "F_B", and "F_C", were used in this study. Whole-meal flour blend F_A and type 0 flour blend F_B were obtained by steel roll milling as has previously been elucidated by Taccari et al. [2], whereas type 2 flour blend F_C was obtained by stone grinding using a Type A1200 commercial stone mill (Osttiroler Getreidemühlen Green, Austria) as detailed by Cardinali et al. [8]. Flours underwent chemical, farinograph, alveograph, and micro-visco-amylograph tests as reported by Taccari et al. [2]. The flours' phytate contents were determined as previously described [2].

Sourdough preparation, maintenance, and chemical and microbiological analyses

Three type I sourdoughs (namely "S_A-b", "S_B-b", and "S_C-b") were previously prepared and propagated daily for 20 days (mature sourdoughs) with three flours F_A, F_B, and F_C, respectively, at an artisan bakery as described by Taccari et al. [2]. The sourdoughs were maintained in sealed glass bottles at low temperature (4 °C) and refreshed weekly following the formula and the back-slopping procedure reported by Taccari et al. [2] for approximately 120 days (long-term propagated sourdoughs). One week before the bread-making trials, the three sourdoughs were refreshed daily using the back-slopping procedure [2].

At the end of the 120 days' propagation, the sourdoughs (hereafter referred to as "S_A", "S_B", and "S_C") were subjected to chemical and microbiological analyses to check their stability, namely through the (i) measurement of pH and TTA; (ii) enumeration of presumptive lactic acid bacteria and yeasts; and (iii) PCR-DGGE profiling using the methods proposed by Taccari et al. [2].

Bread-making trials

Sourdough bread was manufactured at a local artisan bakery ("Il Biroccio", Filottrano, Italy) according to the formulations and fermentation times reported in Table 1 and chosen on the basis of the results of previous laboratory tests in terms of dough volume increase on a graduated cylinder

Table 1 Bread dough formulas.

Sourdough bread	Sourdough bread ingredients (g/100 g of bread dough)				Fermentation time
	Sourdough	Flour blend	Water	Bakers' yeast	
B_A formula I	S_A-b, 30 g	F_A, 42.50 g	27.50 g	–	3 h
B_A formula II	S_A-b, 30 g	F_A, 42.50 g	27.50 g	0.5 g	3 h
B_A formula III	S_A-b, 10 g	F_A, 55.30 g	34.70 g	–	7 h
B_A control	–	F_A, 60 g	33.30 g	2.0 g	2.5 h
B_B formula I	S_B-b, 20 g	F_B, 48.75 g	31.20 g	–	4 h
B_B formula II	S_B-b, 20 g	F_B, 48.75 g	31.20 g	0.5 g	4 h
B_B formula III	S_B-b, 20 g	F_B, 48.75 g	31.20 g	–	6 h
B_B control	–	F_B, 60 g	33.30 g	2.0 g	2.5 h
B_C formula I	S_C-b, 30 g	F_C, 42.50 g	27.50 g	–	3 h
B_C formula II	S_C-b, 30 g	F_C, 42.50 g	27.50 g	0.5 g	3 h
B_C formula III	S_C-b, 10 g	F_C, 55.35 g	34.70 g	–	6 h
B_C control	–	F_C, 60 g	33.30 g	2.0 g	2.5 h

All the bread doughs weighed 500 g. Water was added to reach a constant dough yield (DY) of 162

(data not shown) following the procedure proposed by Zannini et al. [24]. Control bread was prepared by adding 2% (w/w) baker's yeast ("Maestro®, Lievito", AB Mauri Italy S.p.A) to reach a final yeast cell density of ca. 10^8 cfu g^{-1} for the bread dough [25]. Sourdough and control bread loaves were manufactured by mixing all the ingredients for 7 min with a spiral mixer. Bread dough was divided into 500 g loaves and placed in bread molds and allowed to ferment again for 60 min at 35 °C. The bread dough was baked in an electric oven (Mod. Ecomondial, Mondial Forni, Verona, Italy) at 220 °C for an initial 10 min and at 200 °C for another 35 min. Sourdough and control bread loaves were prepared in triplicate. They were left to cool at room temperature and further subjected to physico-chemical, textural, and sensory analyses.

Determination of bread weight, specific volume, and TTA

After 30 min of cooling, sourdough and control bread loaves were weighed and subjected to volume determination by rapeseed displacement method AACC 10–05.01 [26]. For each bread loaf, specific volume was calculated as the ratio between loaf volume and weight. TTA was determined as previously described [2].

Determination of bread moisture and texture

Cooled experimental and control bread loaves were packaged in sealed polyethylene bags and evaluated for moisture content and crumb texture at regular intervals (5, 24, and 96 h after baking) while in storage at room temperature.

Moisture content was assessed following the procedure detailed by Mariotti, Pagani, and Lucisano [27].

The crumb texture was measured according to AACC method 74–09.01 [28] using a CT3-4500 texture analyzer (Brookfield Engineering Laboratories INC, Middleboro MA, USA) equipped with a 36 mm diameter bread probe (mod. TA-AACC36). Briefly, bread loaves were center sliced (25 mm thick) with an electric bread knife. Bread slices were positioned between the load cell and the base table fixture of the instrument. A 4500 g load cell was used. The probe compressed the crumb to a 40% compression limit (10 mm compression depth) at a speed of 100 mm min^{-1}. The analysis was performed in triplicate.

Bread sensory analyses

The sensory analysis of bread loaves was carried out 5, 24, and 96 h after baking by performing a small-scale consumer sensory test as proposed by Svensson [29] and applied by Mariotti et al. [30] and Cardinali et al. [8]. Briefly, nine non-trained panelists (four males and five females, non-smokers, age: 30–55) were chosen among the personnel of the bakery where the experimental bread was produced. The sensory evaluation was performed as proposed by Resurreccion [31]. The degree of global liking was ranked using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely) [32]. Results were expressed as the means of three independent experiments ($n = 3$).

Statistical analysis

All the data referring to the chemical and rheological properties of flours, long-term propagated sourdoughs (pH, TTA, microbial counts), and bread just after baking (TTA, weight, specific volume) were analyzed by one-way ANOVA. When the ANOVA indicated a significant difference, a Tukey's

HSD test at a 95% confidence level was performed for mean separation. Data assessed during the bread's shelf-life (crumb core moisture, crumb texture, and global liking) were analyzed using multivariate analysis of variance (MANOVA) for repeated measures according to the Wilks' lambda test to determine the effect of flour over time. Statistical analysis was performed using JMP 9 (SAS Institute Inc., Cary, NC).

Results and discussion

Flour analyses

The results of chemical and conventional rheological analyses of flours F_A, F_B, F_C used for the propagation of sourdoughs, leavening, and bread-making trials are shown in Table 2. F_A showed a significantly higher ash content and a greatly longer dough development time while both F_A and F_C showed higher water absorption. These features may be linked to the higher wheat fiber content of whole-meal wheat flour (F_A) and of stone-ground wheat flour (F_C) that may be detrimental to gluten networks and, thus, bread quality [2, 3, 33]. The values of water absorption were in accordance with those already reported for stone-ground wheat flour by Cardinali et al. [8] (63.75% ± 2.9) and for roll-milled whole-meal flour reported by Kihlberg et al. [34] (62.5%). The ash content value of F_C was in accordance with Italian legislation for type 2 flour that indicates a maximum ash content of 0.95 100 g^{-1} flour, while the higher ash content value of F_A was in line with those reported by Prabhasankar and Rao [35] for whole-wheat flour and ranged from 1.5 ± 0.03 and 1.6 ± 0.02. The longer dough development time of F_A was in line with a study by Kihlberg et al. [35], reporting a value of 15.0 min for roll-milled whole-meal flour. All of the flours showed high protein content, indicating a good bread-making capacity [8, 25]. The content of phytic acid, which acts as an antinutritional factor by reducing mineral bioavailability, was also estimated for the studied flours and progressively increased in the order from F_B to F_C and, finally, to F_A as also previously reported [2]. These data were expected since phytic acid content is higher in whole-meal flour compared to low-extraction-rate (refined) flour. Indeed, in refined flours, the milling of grain removes nutrients and phytate as well. By contrast, in whole-meal flour (F_A), the external part of the endosperm, the aleurone, which retains the majority of nutrients and phytic acid, is not removed [3, 33, 36, 37].

Chemical and microbiological traits of the long-term propagated sourdough

Before bread-making trials, the three sourdoughs were maintained in laboratory conditions by using the back-slopping technique for 120 days. Therefore, pH, TTA, and viable counts of presumptive lactic acid bacteria and yeasts of the three long-term propagated sourdoughs were assayed in order to verify the sourdoughs' stability (Table 3). By comparing these data with those previously obtained by Taccari et al. [2] on mature sourdough propagated for 20 days,

Table 2 Chemical and rheological traits of wholemeal (referred to as "F_A") and type 0 (referred to as "F_B") and type 2 (referred to as "F_C") flour blends obtained by steel roller milling (F_A and F_B) and stone grounding (F_C) of *Triticum aestivum* grains

	Flour blend		
	F_A	F_B	F_C
Type of flour	Wholemeal	Type 0	Type 2
Milling	Steel roller milling	Steel roller milling	Stone grounding
Ash (%)	1.77 ± 0.00^{a}	0.62 ± 0.00^{b}	0.89 ± 0.16^{b}
Moisture (%)	13.00 ± 0.42^{a}	13.25 ± 0.07^{a}	12.05 ± 0.35^{a}
Protein (%)	14.15 ± 0.64^{a}	11.60 ± 0.29^{a}	13.23 ± 1.38^{a}
P/L	5.70 ± 0.43^{a}	0.47 ± 0.13^{b}	0.63 ± 0.21^{b}
W (10^{-4} J)	165.00 ± 1.41^{a}	193.00 ± 15.56^{a}	198.50 ± 17.68^{a}
Consistency (UF)	513.00 ± 8.49^{a}	492.50 ± 4.95^{a}	502.00 ± 7.07^{a}
Water absorption (%)	68.40 ± 0.85^{a}	57.15 ± 1.63^{b}	63.90 ± 0.85^{a}
Dough development time (min)	10.30 ± 0.71^{a}	1.35 ± 0.07^{b}	4.55 ± 1.34^{b}
Dough stability (min)	8.15 ± 1.63^{a}	8.50 ± 0.71^{a}	6.50 ± 2.83^{a}
Mixing tolerance index after 10 min (BU)	16.00 ± 2.83^{a}	37.50 ± 6.36^{a}	44.50 ± 23.33^{a}
Mixing tolerance index after 12 min (BU)	65.00 ± 14.14^{a}	52.00 ± 7.07^{a}	69.00 ± 21.21^{a}
Maximum viscosity (BU)	754.00 ± 60.81^{a}	843.50 ± 79.90^{a}	1013.00 ± 90.51^{a}
Falling number (sec)	394.00 ± 22.63^{a}	381.50 ± 118.09^{a}	436.50 ± 16.26^{a}
Phytic acid (g 100 g^{-1})	0.78 ± 0.10^{a}	0.10 ± 0.02^{b}	0.20 ± 0.02^{b}

BU: Brabender Units. Means ± standard deviations of duplicate independent experiments are shown. Means within each row followed by different letters are significantly different ($p < 0.05$)

Table 3 Biochemical properties and microbial viable counts of long-term propagated sourdoughs (120 days of propagation)

Sourdough	pH	TTA (mL NaOH)	Viable counts (Log cfu g^{-1})		
			LAB (mMRS)	LAB (mSBD)	Yeasts (WL)
S_A	4.10 ± 0.01^a	13.40 ± 0.17^a	9.35 ± 0.01^a	9.30 ± 0.02^a	9.68 ± 0.01^a
S_B	3.92 ± 0.02^b	7.80 ± 0.40^c	9.03 ± 0.04^b	9.05 ± 0.00^b	8.20 ± 0.03^b
S_C	3.92 ± 0.02^b	11.13 ± 0.23^b	9.11 ± 0.01^b	9.06 ± 0.05^c	7.84 ± 0.02^c

Data reported as mean (*n.* 2) ± SD

Different letters within each column indicate significant differences among sourdoughs ($p < 0.05$)

some evidence emerged. pH and TTA levels measured after 120 days of sourdough maintenance were stabilized around the values encountered at the end of the 20th day of continuous sourdough propagation [2], with S_A characterized by a significantly higher pH value and higher TTA than S_B and S_C. These data were previously attributed to the higher ash content of F_A compared to the other two flours under study, which affected the sourdoughs' buffering capacity [2, 30]. Furthermore, it has been reported that excessive acidity levels in sourdough and bread dough (higher than 6.7 ± 0.58 mL when 30% sourdough is used) may decrease the gas retention capacity of dough and, consequently, bread volume and crumb softness due to the degradation of gluten proteins [3, 20, 21, 38, 39].

Changes in the LAB-to-yeast ratio were also seen, passing from ca. 100:1 to 10:1 in S_B and, in S_A, passing from ca. 10:1 to 1:1 whereas, in S_C, this parameter remained stable at ca. 100:1. LAB-to-yeast ratios of 100:1 and 10:1 have already been found in 19 Italian sourdoughs used for traditional/typical breads and reported as common for sourdoughs; the only exception (with a ratio of 1:1) was recorded for Pane Casereccio di Genzano PGI sourdough [40]. The results of PCR-DGGE analysis are shown in Tables S1 and S2. By comparing the compositions of the microbiota of the three mature sourdoughs at the end of the 20 days of continuous propagation and after 120 days of maintenance, a slight evolution in the composition of both bacterial and yeast communities was seen, with the disappearance of a few species (*Weissella confusa/cibaria* in S_B; *Pediococcus pentosaceus* in S_C; *Pichia kudriavzevii* in S_A; *Kazachstania unispora/servazzii/aerobia* in both S_A and S_C) and the establishment of a core collection of species shared among all sourdoughs, stably detected in the bulks at both the highest and lowest dilutions and consisting in *Levilactobacillus brevis* (basonym *Lactobacillus brevis*), *Companilactobacillus alimentarius/paralimentarius* (basonym *Lactobacillus alimentarius/paralimentarius*), *Lactiplantibacillus plantarum/paraplantarum/pentosus* (basonym *Lactobacillus plantarum/paraplantarum/pentosus*), and *Saccharomyces cerevisiae*. Specifically, *Lpb. plantarum/paraplantarum/pentosus* became dominant after 120 propagation cycles in S_A and it replaced *Weissella confusa/cibaria* in S_B and *Pediococcus pentosaceus* in S_C, thus indicating the great competitiveness and adaptation to sourdough environments of strains of the *Lpb. plantarum* sensu *latu* group. The species *C. alimentarius* and *C. paralimentarius* as well as *Lpb. plantarum*, *Lpb. paraplantarum*, and *Lpb. pentosus* are phylogenetically closely related; therefore, apposite molecular identification methods are needed [19]. The facultatively heterofermentative *C. alimentarius/paralimentarius* is typically associated with the sourdough environment while the obligately heterofermentative *Lev. brevis* and *Lpb. plantarum* (facultatively heterofermentative) often occur in other food ecosystems, although they have been commonly identified in sourdoughs as well [19, 21]. Indeed, *Lpb. plantarum* was reported to be a LAB species characterized by a metabolic flexibility and a high tolerance towards low pH [41]. This latter consideration may also explain the feasible coexistence of *Lpb. plantarum* (which utilize all four flour carbohydrates but have a preference for glucose and fructose) and *S. cerevisiae* (maltose-positive), which was the only yeast species detected in the three long-term propagated sourdoughs under study, suggesting a stable, non-competitive association among these two species, as is often reported in the literature [19, 21]. *S. cerevisiae* is a yeast species tolerant to low pH and high osmolality; therefore, its stable persistence in the sourdoughs under study is not unusual and may also be attributed to the robustness of the autochthonous strains of *S. cerevisiae* as has also been documented by several authors [18, 19, 42].

It is well known that the stability of a sourdough over time is influenced by several ecological and process parameters that quantitatively and qualitatively affect the sourdough microflora and, among these factors, flour, which acts as a source of nutrients and microorganisms, is not fully controlled during sourdough maintenance [43]. Only the intrinsically robust and most competitive microorganisms can persist within sourdough during its propagation over time despite the changing of flour batches that may introduce different autochthonous strains and nutrients depending on seasonality characterizing crops [30, 43]. It is interesting to note that no differences were seen in terms of the dominant microbial community among the three long-term propagated sourdoughs under study based on the different kinds of wheat flours obtained with different extraction rates and milling procedures. This result is in line with what was reported by Ercolini et al. [44] who found that the initial differences in microbiota among sourdoughs developed with different flours, namely rye, durum, and soft wheat flours, tend to decrease towards the establishment of a common core microbiota by increasing the number of refreshing steps, irrespective of the type of flour.

Bread characterization

Bread TTA, weight, and specific volume

Results related to TTA, weight, and specific volume of the bread formulations are shown in Table 4.

In the control group (without sourdough), loaves of bread showed the lowest TTA values. On the other hand, the addition of sourdough caused an increase in TTA values to a greater extent for bread formulations B_A and B_C, thus showing an influence of both sourdough fermentation and the different ash contents of the flours.

An increased addition of sourdough (30% versus 10%) did not lead to significantly higher TTA values for the B_A and B_C bread loaves, thus indicating that a longer fermentation time applied to bread dough with 10% sourdough may lead to the same performance as that of the 30% sourdough bread formula, in terms of bread TTA. The 20% replacement of S_B produced bread loaves B_B with not significant differences among sourdough bread loaves and control bread loaves in terms of TTA values, irrespective of whether a long or short fermentation time was used. More specifically, the TTA values of the experimental sourdough bread loaves B_B were lower than that reported by Crowley et al. [45] for wheat bread containing 20% sourdough and corresponding to 5.2 mL. In contrast, the TTA values of the sourdough bread B_C and control bread were in line with those of bread produced with stone-ground flour and 25% type I sourdough (7.25 ± 1.33) and baker's yeast (2.20 ± 0.20), respectively [8]. It has also been stated that other elements may influence bread TTA values such as different flours (type and batch) as well as different microbiota in terms of species and strains developed within sourdoughs [30]. TTA was not correlated with the specific volume of bread (correlation coefficient = -0.33 $p = 0.294$) nor with bread weight (correlation coefficient = 0.445 $p = 0.147$) or crumb texture (Table 6) (correlation coefficient = 0.226 $p = 0.481$).

Concerning weight, all the bread formulations showed a weight reduction after baking and cooling, although a higher level of weight reduction was generally seen in B_B formulations compared to B_A and B_C formulations. This result was expected since bread formulations enriched with fibers due to the presence of whole-wheat meal flour or type 2 stone-ground wheat flour are able to retain greater amounts of water as shown by analysis of flour water absorption (Table 2) and as reported by Mariotti et al. [30] and Ktenioudaki and Gallagher [33].

The positive role of sourdough on bread volume has been reported to be mainly caused by the improved gas-holding ability of gluten in mildly acidic environments, the solubilization of flour starch and pentosans, the formation of exopolysaccharides during fermentation, and the commensalism of LAB and yeasts that cause faster yeast metabolism and, thus, improve leavening when in coexistence with LAB [3, 20, 21]. Cappelli et al. [23] underlined that the specific volume of bread is the quintessential attribute of bread quality since higher values are related to greater bread quality. It is also well known that bread volume remains low in yeast-leavened whole-meal wheat bread while the addition of sourdough generally improves the specific volume of bread [3, 21]. In the present study, the specific volume of B_B bread formulations was significantly higher than those of bread formulations B_A and B_C. These data confirm the strong influence of milling properties and extraction rates on

Table 4 Total titratable acidity (TTA), weight, and specific volume of bread formulations

	TTA (mL 0.1 N NaOH)	Weight (g)	Specific volume (mL g^{-1})
B_A formula I	6.65 ± 0.49^{a}	$459.28 \pm 4.01^{a,b}$	2.18 ± 0.02^{g}
B_A formula II	$6.35 \pm 0.07^{a,b}$	451.36 ± 1.69^{b}	2.22 ± 0.00^{g}
B_A formula III	$5.65 \pm 0.07^{b,c}$	$462.51 \pm 3.15^{a,b}$	1.51 ± 0.01^{i}
B_A control	2.45 ± 0.21^{e}	$446.45 \pm 1.35^{b,c,d}$	2.24 ± 0.00^{g}
B_B formula I	3.35 ± 0.07^{d}	$432.97 \pm 4.9^{c,d}$	3.48 ± 0.02^{c}
B_B formula II	3.45 ± 0.07^{d}	404.65 ± 7.25^{e}	5.59 ± 0.05^{a}
B_B formula III	3.70 ± 0.14^{d}	431.89 ± 9.45^{d}	3.45 ± 0.04^{c}
B_B control	3.30 ± 0.00^{d}	$449.42 \pm 2.84^{b,c}$	4.44 ± 0.01^{b}
B_C formula I	$6.25 \pm 0.21^{a,b}$	$447.95 \pm 0.58^{b,c,d}$	3.34 ± 0.00^{d}
B_C formula II	5.10 ± 0.14^{c}	471.98 ± 0.77^{a}	2.97 ± 0.00^{e}
B_C formula III	$6.10 \pm 0.14^{a,b}$	454.24 ± 0.35^{b}	2.42 ± 0.00^{f}
B_C control	2.30 ± 0.14^{e}	452.93 ± 0.78^{b}	1.99 ± 0.00^{h}

Data reported as mean (n. 2) ± SD. Means within column followed by the different letter are significantly different according to Tukey's HSD Test ($p < 0.05$)

the physical features of bread [4, 30, 46]. More specifically, sourdough formulations showed specific volumes comparable to (B_A formulas I and II), higher than (B_B formula II and B_C formulas I, II, and III), or lower than (B_A formula III and B_B formulas I and III) that of control breads (baker's yeast breads). It is interesting to note that all the sourdough formulations using stone-ground flour enhanced the bread-specific volume at either 10% (long fermentation time) or 30% (short fermentation time) compared to the control bread, thus indicating that lactic acid fermentation may be a valid tool for improving the baking capacity of stone-ground flour. Specifically, the 30% addition of solely sourdough S_C produced bread with significantly higher specific volume among B_C formulations and a slightly lower numerical difference compared to B_B formulations prepared with refined wheat flour and 20% sourdough with either short or long fermentation time. On the other hand, sourdough fermentation did not improve the specific volume of B_A compared to the use of baker's yeasts, thus indicating the stronger effect of whole-meal flour fiber content that negatively influenced bread quality in terms of reduction in bread volume as already reported by Katina et al. [38]. Indeed, the use of whole-meal flour in bread making has been reported to decrease bread volume mainly due to the presence of fibers that may have a detrimental effect on the gluten network, thus decreasing gas-holding ability [30, 38]. This observation in the present study is also in line with the study of Verdonck et al. [21] that confirmed the theory that type I sourdough, containing a microbial inoculum of *Lev. brevis*, *Lpb. plantarum*, and *S. cerevisiae*, either alone or in combination with baker's yeast, cannot improve the specific volume of whole-meal bread. Concerning B_B formulations, the addition of baker's yeast to 20% sourdough S_B greatly improved B_B specific volume thus demonstrating the usefulness of combining these leavening agents for this parameter of bread quality. Indeed, baker's yeast is generally used in addition to sourdough to accelerate leavening during bread making due to its fast and strong CO_2 production ability [21]. Furthermore, the specific volume of bread was negatively correlated with weight (correlation coefficient = – 0.75, $p = 0.0009$) and with crumb texture (hardness) (Table 6) (correlation coefficient = – 0.83). This latter finding was confirmed (correlation coefficient = – 0.88, data not shown) even after 96 h of storage, thus indicating that a higher specific volume is also correlated with a reduction in the hardness of the bread during its shelf-life as previously reported [45].

Bread crumb core moisture and texture

The evolution of the bread formulations during 96 h of storage was estimated in terms of crumb core moisture and bread firmness (hardness of bread crumb).

The crumb moisture of bread formulations 5 h after baking and during storage is reported in Table 5. Multivariate analysis of variance showed that flour type had a significant effect on the moisture content of bread loaves. Although it

Table 5 Crumb moisture of the bread formulations 5 h after baking and during storage (24 h and 96 h)

Crumb moisture %				
Flour				<.0001*
Time				0.7061
Time × Flour				0.014*
	5 h	24 h	96 h	*p*
B_A formula I	45.55 ± 0.07^{ab}	44.75 ± 0.21^{ab}	45.80 ± 1.13^{ab}	n.s.
B_A formula II	46.25 ± 0.07^{a}	46.00 ± 0.28^{b}	46.55 ± 1.06^{a}	n.s.
B_A formula III	44.60 ± 0.14^{ab}	44.20 ± 0.00^{abc}	44.65 ± 0.91^{abc}	n.s.
B_A control	44.35 ± 0.35^{ab}	43.80 ± 0.56^{abc}	44.15 ± 0.77^{abcd}	n.s.
B_B formula I	44.35 ± 0.77^{ab}	42.95 ± 0.91^{bc}	42.35 ± 0.35^{cd}	n.s.
B_B formula II	43.75 ± 1.06^{b}	43.80 ± 1.13^{abc}	43.25 ± 0.35^{cd}	n.s.
B_B formula III	44.10 ± 0.70^{ab}	44.45 ± 0.07^{abc}	42.15 ± 0.07^{d}	*
B_B control	43.60 ± 0.70^{b}	44.80 ± 0.98^{ab}	42.50 ± 0.28^{cd}	n.s.
B_C formula I	39.00 ± 0.00^{c}	43.50 ± 0.28^{bc}	43.95 ± 0.21^{bcd}	*
B_C formula II	44.00 ± 0.14^{ab}	43.55 ± 0.07^{bc}	43.95 ± 0.21^{bcd}	n.s.
B_C formula III	44.00 ± 0.84^{ab}	42.80 ± 0.56^{bc}	42.70 ± 0.42^{cd}	n.s.
B_C control	43.65 ± 0.91^{b}	42.25 ± 0.49^{c}	43.10 ± 0.00^{cd}	n.s.

MANOVA testing the effect of flour, time and their cross interaction on crumble core moisture

P values calculated in the MANOVA according to the Wilks' Lambda test. Within each column means with different letters are significantly different according to the Tukey's HSD test ($p < 0.05$); within each row means followed by * are significantly different according to the each pair Student's *t* test ($p < 0.05$)

Table 6 Crumb texture of the bread formulation 5 h after baking and during storage (24 h and 96 h)

Crumb texture (g)				
Flour				<.0001*
Time				<.0001*
Time × Flour				<.0001*
	5 h	24 h	96 h	*p*
B_A formula I	2363.3 ± 562.0^{b}	4787.5 ± 382.9^{ab}	5014.3 ± 103.2^{a}	*
B_A formula II	2107.3 ± 390.9^{b}	3867.8 ± 282.1^{b}	4231.5 ± 402.6^{bc}	*
B_A formula III	3083.8 ± 590.4^{a}	5044.1 ± 166.7^{a}	4964.5 ± 248.4^{ab}	*
B_A control	2039.2 ± 171.3^{b}	2833.7 ± 291.6^{c}	2796.00 ± 489.0^{efg}	*
B_B formula I	377.4 ± 157.4^{def}	1719.9 ± 110.0^{d}	3010.75 ± 338.0^{de}	*
B_B formula II	124.6 ± 33.1^{f}	535.0 ± 111.9^{f}	922.0 ± 305.6^{h}	*
B_B formula III	326.3 ± 111.8^{ef}	1049.7 ± 101.9^{e}	2479.3 ± 360.4^{efg}	*
B_B control	708.9 ± 104.6^{cde}	1417.1 ± 266.8^{de}	2104.1 ± 528.6^{g}	*
B_C formula I	1024.3 ± 194.5^{cde}	1824.0 ± 61.8^{d}	2905.8 ± 334.8^{def}	*
B_C formula II	726.2 ± 59.5^{cde}	1535.8 ± 321.3^{de}	2181.8 ± 487.4^{fg}	*
B_C formula III	911.3 ± 135.3^{cde}	2807.2 ± 388.66^{c}	3636.8 ± 610.6^{cd}	*
B_C control	2483.2 ± 102.6^{b}	4685.1 ± 263.9^{a}	4163.3 ± 294.2^{c}	*

MANOVA testing the effect of flour, time and their cross interaction on crumb texture

P values calculated in the MANOVA according to the Wilks'Lambda test. Within each column means with different letters are significantly different according to the Tukey's HSD test ($p < 0.005$); within each row means followed by * are significantly different according to the each pair Student's *t* test ($p < 0.05$)

has been reported that a higher fiber content within flour may lead to moister dough and bread [30, 38], a generally similar trend in terms of bread moisture was observed in the present study irrespective of the type of flour or the leavening agents used both 5 h after baking and during storage. Indeed, during the bread's shelf-life, significant differences in crumb core moisture were found only for B_B formula III and B_C formula I where a slight decrease and an increase in moisture were observed, respectively.

Results for crumb texture analyses of the bread formulations 5 h after baking and during storage are presented in Table 6. Multivariate analysis of variance highlighted a significant effect of flour type and time on crumb texture of all bread formulations. After baking (5 h), the softness of B_A formulations differs significantly from that of B_B and B_C formulations, with B_B formula II showing the lowest firmness (highest softness) and B_A formula III showing the highest firmness (lowest softness). Overall, the highest ash content and typically high fiber content of whole-meal flour (and, to a lesser extent, of type 2 stone-ground flour) may have played a major role in increasing bread hardness being responsible for the non-elastic crumb and denser texture of bread as already reported [3, 30, 33, 38, 47]. Textural analyses highlighted the same trend already observed for the specific volume of bread: the bread that exhibited the highest softness was also characterized by the highest specific volume and the effect of sourdough on improved bread softness seems to be at least in part imputable to higher bread volume (specific volume and crumb texture are negatively correlated, correlation coefficient = – 0.83 $p = 0.0009$). More specifically, within B_C formulations, the hardness was the highest for bread leavened with baker's yeast alone (B_C control). Indeed, the B_C control exhibited a significantly higher firmness compared to all sourdough bread loaves made with roll-milled flour, which showed no significant differences among each other, irrespective of fermentation time or percentage of sourdough inoculum. This result confirms again the positive effect of sourdough on the baking capacity of stone-ground wheat flour in terms of textural improvement of bread, in addition to improvements in specific volume as detailed above. Concerning B_B formulations, formulation II (baker's yeast added with 20% sourdough S_B), as indicated above for bread volume, showed the highest improvement in terms of softness. These data are also in line with the study of Cappelli et al. [4] reporting roll milling as among the wheat-milling methods that ensure better dough rheological performance. Furthermore, B_B formula II remained the softest bread even during storage, significantly showing the lowest crumb hardness both 24 h and 96 h after baking. As was expected, all breads showed an increasing trend in hardness during storage, but different staling rates were observed and the tendency to remain soft was observed for bread loaves with higher specific volume and softness 5 h after baking.

It has been reported that generally long fermentation times are responsible for the softest bread textures [38]. However, this assumption did not seem to be confirmed by

Table 7 Global liking of the bread formulations 5 h after baking and during storage (24 h and 96 h)

Global liking				
Flour				0.0050*
Time				<.0001*
Time × Flour				0.6514
	5 h	24 h	96 h	*p*
B_A formula I	5.80 ± 1.0^{abc}	4.05 ± 1.6^{b}	4.50 ± 1.1^{a}	*
B_A formula II	5.00 ± 1.4^{c}	4.75 ± 1.2^{ab}	4.20 ± 1.3^{a}	n.s.
B_A formula III	6.50 ± 0.7^{abc}	5.15 ± 1.1^{ab}	4.80 ± 1.4^{a}	*
B_A control	6.60 ± 0.7^{ab}	6.10 ± 1.0^{a}	4.90 ± 1.7^{a}	*
B_B formula I	6.10 ± 1.4^{abc}	5.4 ± 1.3^{ab}	4.50 ± 2.0^{a}	n.s.
B_B formula II	6.80 ± 1.1^{a}	6.00 ± 1.4^{a}	5.60 ± 1.9^{a}	n.s.
B_B formula III	5.60 ± 1.2^{abc}	5.25 ± 1.1^{ab}	5.00 ± 1.8^{a}	n.s.
B_B control	6.00 ± 1.2^{abc}	6.00 ± 1.2^{a}	5.60 ± 2.3^{a}	n.s.
B_C formula I	5.20 ± 0.9^{bc}	5.40 ± 1.0^{ab}	4.30 ± 1.2^{a}	n.s.
B_C formula II	5.40 ± 1.1^{abc}	5.00 ± 1.2^{ab}	5.20 ± 0.8^{a}	n.s.
B_C formula III	6.10 ± 0.7^{abc}	4.70 ± 1.0^{ab}	3.90 ± 0.3^{a}	*
B_C control	5.70 ± 0.8^{abc}	4.90 ± 1.3^{ab}	4.90 ± 1.0^{a}	n.s.
P value	0.0026*	0.0074*	0.2579	

MANOVA testing the effect of flour, time and their cross interaction on global liking

P values calculated in the MANOVA according to the Wilks' Lambda test

Within each column means with different letters are significantly different according to the Tukey's HSD test ($p < 0.05$); within each row means recorded at different times followed by * are significantly different according to the each pair Student's t test ($p < 0.05$)

the present data, thus indicating that for an efficient use of sourdough, optimal conditions have to be found by tailoring sourdough to the type of flour used.

Bread sensory analyses

As reported by Svensson [29], the small-scale sensory test, as that applied in the present study, is a cheap way to get indications and significant differences in liking of a product. The test groups of such sensory test involve only ten participants chosen among the regular employees of a company that are representative of the consuming population and regular users of the tested product, or at least familiar with similar products [27, 29]. Hence, this method allows the company to identify promising products in need of further evaluation [29]. Similarly to the small-scale internal consumer tests performed by Svensson [29], the nine untrained panelists involved in the present study were chosen among the employees of the bakery where the bread-making trials were performed, as also previously reported by Cardinali et al. [8] and Mariotti et al. [27]. The small-scale consumer test used was aimed at gaining insight into the experimental breads thus providing a preliminary exploration of the consumer acceptance of the sourdough bread performed by using roll-milled or stone-ground soft wheat flours milled to different extraction rates.

The global liking of bread formulations during storage is reported in Table 7. Multivariate analysis of variance showed that flour type had a significant effect on the global liking of bread loaves. To the authors' knowledge, data regarding the features of bread made with stone-ground soft wheat flour are very limited. The consumer acceptance of B_C made with 10% sourdough and 6 h of fermentation (formula III) was in line with the study of Cardinali et al. [8] using 25% type I sourdough and 5 h of fermentation (6.22 ± 0.75), thus obtaining a medium/high score of global liking and confirming the suitability of type I sourdough in bread making with stone-ground wheat flour. However, during the bread's shelf-life, a significant decrease in global liking over time was found only for B_C formula III and for B_A formulas I and III and control. Interestingly, B_B formula II (found as the bread with the highest volume and softness even during shelf-life) was also the most appreciated bread according to taste tests 5 h after baking and during the bread's shelf-life. Global liking and crumb texture (hardness) are negatively correlated (correction coefficient = – 0.64 $p < 0.0001$), thus indicating that the softness of the bread is preferred by consumers and this parameter positively influences consumer acceptance. In contrast, crumb core moisture and acidity (TTA) do not influence global liking ($p = 0.48$) (see Table 8).

Table 8 Significance probabilities of pairwise correlation among global liking and physical parameters of bread.

	Global liking	TTA	Specific volume	Weigh	crumb texture (g)	crumb core moisture %
Global liking	.					
TTA	0.1461	.				
Specific volume	0.4652	0.2941	.			
Weigh	0.1720	0.1471	**0.0050***	.		
Crumb texture (g)	**<.0001***	0.4810	**0.0009***	0.0525	.	
Crumb core moisture %	0.9572	0.9834	0.3478	0.7499	0.1835	.

Conclusion

In conclusion, the data of the present study confirmed that roll milling is a wheat-milling method that ensures better dough rheological performance compared to stone-grinding methods, although sourdough fermentation may be a valid tool for improving the baking capacity of stone-ground flour. Indeed, sourdough fermentation positively affected the specific volume, texture, and global liking of bread manufactured with stone-ground wheat flour. Overall, the highest ash and fiber contents of whole-meal flour (followed by those of stone-ground wheat flour) may have played a major role in increasing bread hardness and reducing volume. The effect of sourdough on improved softness seems to be at least in part imputable to a higher specific volume of bread. The present study confirmed that for an efficient use of sourdough, optimal conditions need to be found by tailoring sourdough to the type of flour used with the final aim of improving bread quality.

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Data availability Data are available upon request from the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subject.

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