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Can fermentation methods and granulometry modulate bread starch digestibility without hindering its technological quality?

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ABSTRACT

Reducing starch digestibility in bread while maintaining its quality has been a trending topic in recent decades. This study explores the effects of different fermentation methods—Direct Method (DM), Poolish (PO), and Sourdough (SD)—combined with semolina of varying granulometry (Fine Semolina (FS), ~150 μm; Coarse Semolina (CS), ~550 μm) on bread properties. A full factorial design was employed to assess the influence of these variables on dough properties (pH and total titratable acidity (TTA)), bread quality (porosity, texture profile analysis, and volume), and *in vitro* digestibility (using Englyst's method). Results indicate that SD-fermented doughs exhibited higher TTA and lower pH, leading to bread with reduced porosity and volume compared to PO and DM, and a reduced starch digestibility due to a dense crumb structure. CS produced slightly more porous bread with lower resistant starch than FS. To conclude, the fermentation method was the major factor influencing bread quality and digestibility.

1. Introduction

Durum wheat (DW) (Triticum turgidum L. subsp. durum (Desf.) Husn.) is a tetraploid cereal grain produced and consumed all over the world since ancient times (Maccaferri et al., 2019). Its production accounts for less than 7% of total wheat production and its cultivation (33.8 million tons in 2020–21) is concentrated in specific geographic regions, particularly the Mediterranean area, where it is a major cereal crop contributing significantly to food production and agricultural income (Martínez-Moreno et al., 2022). Durum wheat is used in various cereal-based products, among which pasta is the most widely manufactured and industrialized end-product. However, particularly in the Mediterranean region, it is also used in a variety of staple foods such as bread, couscous, bulgur, and different types of flatbreads and baked goods (Sissons, 2022). Durum wheat bread is gaining popularity all over the world owing to its peculiar sensorial and textural properties (Sanfilippo et al., 2023). This bread is characterized by a harder and more yellowish crust and a more compact crumb when compared to bread produced with common wheat (*Triticum aestivum*) (Bianca et al., 2023). These characteristics, together with the high water-holding capacity of the durum wheat grain, have been reported to prolong bread shelf life and preserve its sensorial features for a longer time (Rinaldi et al., 2015).

Durum wheat bread is produced mainly by reground semolina, which is milled multiple times due to the vitreous structure of the grain endosperm (Pasqualone et al., 2019). The repetitive milling process leads to durum semolina flour with a reduced particle size (< 180 μm) and consequently production of damaged starch which, in turn, promotes farinograph water absorption (Fadda et al., 2010a). During mixing and kneading, damaged starch competes for water with gluten proteins, affecting the development of a gluten network that will be weaker and, in turn, will result in bread with a compact structure and reduced volume (Fadda et al., 2010b). Besides the quality, the reduced particle size of semolina, the high surface area of the granules, and the

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holes and channels in the damaged starch granule increase the susceptibility to enzyme penetration, thus increasing starch digestibility (Wang et al., 2020). Conversely, numerous studies have shown that increasing flour particle size could have a potential role in decreasing the starch digestibility of products made with wheat endosperm flour (Bressiani et al., 2017; Lin et al., 2020). For instance, Mandalari et al. (2018) found an inverse relationship between flour particle size and starch digestibility in porridge. This was confirmed in a trial with ileostomy patients, which showed reduced glucose release following the digestion of coarser wheat flour products than after the consumption of products made with finer wheat flour (Edwards et al., 2015). Various methods have been extensively studied to decrease the starch digestibility of wheat bread. These include adding fiber-rich ingredients like bran, whole grains, and seeds, which increase digesta viscosity, slow gastric emptying, and reduce carbohydrate absorption (Agama-Acevedo et al., 2019; Scazzina et al., 2013). Additionally, using amylose-rich flour reduces starch swelling and gelatinization, delaying enzymatic hydrolysis (Arp et al., 2018).

Moreover, incorporating polyphenols or other bioactive compounds can further decrease starch digestibility by directly binding to digestive enzymes (Kan et al., 2020). However, these approaches often negatively impact bread texture and, as a result, decrease consumer acceptability. Therefore, using coarser semolina could be a promising strategy to decrease starch digestibility with minimal effects on textural properties (Tagliasco et al, 2022; Korompokis et al., 2019).

The fermentation methods can also play a role in modulating the starch digestibility of bread, although this aspect has not been thoroughly investigated in durum wheat. During fermentation, some starches, that are resistant to human digestion, can be broken down by microorganisms into simpler sugars, potentially influencing starch digestibility in various ways, depending on the fermentation method (Islam & Islam, 2024).

Traditionally, durum wheat bread is produced with sourdough, which results from the spontaneous fermentation of flour and water by lactic acid bacteria (LAB) (Arendt et al., 2007). This indirect method of bread-making, which involves multiple steps, is associated with numerous nutritional benefits, including the potential to lower the glycemic index of bread through the action of LAB and the production of organic acids (Scazzina et al., 2013; Gobbetti et al., 2014). However, the sourdough method has been gradually replaced by direct fermentation (using baker's yeast) due to its shorter proofing time and greater ease of use (Acquistucci et al., 2019). The direct method consists of a single fermentation where all the ingredients are mixed and left to ferment spontaneously. Another approach, the Poolish method, serves as a transitional fermentation technique between sourdough and direct fermentation. In this indirect method, a pre-fermented dough made from equal parts water and flour with added baker's yeast is fermented for at least 24 h before being mixed with fresh ingredients (Hernandez-Figueroa et al., 2022).

The choice of fermentation method, in combination with semolina of different particle sizes, could significantly influence both the textural quality of bread and its starch digestibility. Therefore, this study aims to evaluate the coupling effect of different fermentation methods and semolina with different particle sizes on durum wheat bread. In this light, a full factorial design was implemented to assess the influence of fermentation methods (Direct (DM), Poolish (PO), and Sourdough (SD)) and particle sizes of semolina on dough properties (pH and total titratable acidity (TTA)), bread quality (porosity, texture profile analysis, and volume), and *in vitro* digestibility (Englyst's method).

2. Materials and methods

2.1. Materials

"Semola per pasta'' (proteins 11.7%, fiber 2.9%, starch 68.2%, fat 1%) was kindly provided by Molino Grassi (Parma, Italy). Sourdough was kindly provided by Forno di Canolo Parmeggiani (Correggio, Parma, Italy). Enzymes used in the *in vitro* digestion [amyloglucosidase (Cat. No. A7095), invertase from baker's yeast (Cat. No. I4504), pepsin from porcine gastric mucosa (Cat. No. P700) and pancreatin from porcine pancreas (Cat. No. P7545)] were purchased from Sigma Aldrich Chemical B.V. (St Louis, MO, USA). The total starch assay kit and GOPOD assay kit were obtained from Megazyme (Bray, Ireland). All other chemicals utilized were of analytical grade.

2.2. Sample's production

For bread production, durum wheat semolina was sieved through a 400 μm mesh diameter, and only the particles $>$ 400 μm were kept for further experiments. This semolina fraction was codified as coarse semolina (CS). CS was then subjected to extensive milling to obtain fine semolina (FS) with particle sizes below 180 μm. In particular, CS was passed twice through a cryo-miller (6875D Freezer/Mill; Spex Sample Prep, Metuchen, USA) as follows: 1 min cooling, 10 min cycle for 3 cycles at 15 cps. After these cycles, the obtained semolina was sieved through a mesh of 180 μm, and the part with a particle size $>$ 180 μm was again cryomilled for 10 min at 15 cps.

2.3. Semolina characterization

Particle size distribution of CS and FS was determined using a Malvern Mastersizer 3000 (Malvern Instruments Ltd., Solihull, UK) and a Malvern Aero S powder dispenser (Malvern Instruments Ltd., Solihull, UK). Five grams of semolina were introduced into the dispenser and powered into the Mastersizer at 2.5 bar pressure and 50% feed rate. The median diameter of the particles [Dv (50)] and the volume averaged diameter D [4,3] were obtained using Mastersizer 3000 v3.81 software (Malvern Instruments Ltd., Worcestershire, UK). Measurement was determined in triplicate. Starch damage was determined according to AACC Method 76–31.01 |ICC Method No. 164, using the starch damage kit (Megazyme, Bray, Ireland). D-glucose was measured by absorbance (510 nm) with a spectrophotometer Cary 50 (Agilent Technologies, Santa Clara, USA). This analysis was performed in triplicates for both CS and FS.

2.4. Fermentation methods and dough preparation

Three fermentation methods were utilized for the dough preparation made with CS and FS flours: a Direct method (DM) and two indirect methods i.e., a pre-fermented dough made with Saccharomyces cerevisiae as a leavening agent (Poolish, PO), and a dough made with sourdough (SD). The leavening time for the DM and PO methods for the two flours (i.e., CS and FS) was determined using Risograph (National Manufacturing Co., Lincoln, NE, USA), at 30 °C with a pressure of 760 bar. Based on the literature (Sapirstein et al., 2007), the time for the fermentation of FS with direct method was set at 90 min. The total gas produced during this fermentation was quantified, and this value was used as a benchmark to standardize the proofing times for the tested samples DM and PO with FS and CS. This standardization ensured a consistent amount of CO_2 production across all dough produced with baker's yeast fermentation.

For the DM method, all the fresh ingredients [semolina, water, dried yeast (*Saccharomyces cerevisiae*)] were simultaneously mixed, and the dough was then left to ferment for the optimum leavening time, as reported in Table 1.

For the PO method, an initial dough was prepared by manually mixing all the ingredients (i.e., flour, 50% water, and 5% dried yeast) for 5 min. The dough was then placed in a humidity-controlled chamber at 25 °C and 80% relative humidity (RH) for 24 h. After that, the pre-fermented dough (containing 20% of the total flour) was combined with fresh ingredients (flour, 1% salt, 0.5% dried yeast, and the optimum amount of water) and then left to ferment for the optimum time, as

Table 1
Formulation of durum wheat bread made with fine semolina (FS) and coarse semolina (CS) and produced with Sourdough fermentation, Direct method and Poolish method. The ingredients are expressed as a percentage of 100% flour.

	Sourdough fermentation		Direct method		Poolish method	
	FS	CS	FS	CS	FS	CS
Flour, %	100	100	100	100	100	100
Water, %	63.5	59.5	63.5	59.5	63.5	59.5
Salt, %	1	1	1	1	1	1
Yeast, %	–	–	1.2	1.2	0.5	0.5
Poolish, %	–	–	–	–	20	20
Sourdough, %	25	25	–	–	–	–
Mixing time, min	5	30	5	30	5	30
Proofing, h	24	24	1.30	1.45	2.00	2.15

indicated in Table 1.

For SD production, a spontaneously fermented sourdough was used in this study to replicate a typical domestic environment where the growth of various microorganisms is not analytically controlled. In detail, a portion of sourdough provided by an artisanal baker was refreshed by mixing it with the same amount of flour and half the amount of water. This mixture was then incubated at 30 °C, 80% RH for 4 h. The refreshed sourdough (25% of total flour) was combined with fresh ingredients (flour, 1% salt, and optimum amount of water). Unlike the DM and PO fermentations, sourdough fermentation did not produce the same level of CO_2. As a result, the fermentation period for SD was extended to 24 h.

2.5. Dough characterization: pH and total titratable acidity (TTA)

Total titratable acidity and pH were measured for all the doughs after the proofing time. Ten g of dough, previously lyophilized, was mixed with 90 mL of deionized water and tritiated with 0.1 N NaOH until the final pH of 8.5 (Balestra et al., 2015). The results were expressed as mL of 0.1 N NaOH for 10 g of dough. The pH was measured by a pH meter, (pHenomenal®, VWR international). All the analyses were repeated two times.

2.6. Breadmaking

Bread production was conducted on small-scaled puffy loaves, according to Tagliasco et al. (2022) with some modifications. All the ingredients were mixed in a 300 g farinograph (Brabender GmbH & Co KG, Duisburg, Germany) mixing bowl at 28 °C with 63 rpm rotational speed. The optimum amount of water and mixing time for the two flours (i.e., CS and FS) was determined by doing a water absorption test at 30 °C and 63 rpm in a 50 g farinograph mixing bowl (Brabender GmbH & Co KG, Duisburg, Germany) to obtain a dough consistency of 420 Farinograph Units (FU) (Renzetti et al., 2021). After mixing, the doughs were held to relax for 5 min, divided into dough pieces of 74 g, and manually molded. The molded doughs were placed in an aluminum pan and let to rest in a proofing chamber type SDCC-1P/W (Koma Koeltechnische Industrie B. V., Roermond, the Netherlands) under controlled condition (30 °C, 80% RH). Each dough was allowed to ferment for the optimized proofing time, as displayed in Table 1. After fermentation, all the loaves were baked in a customized oven at 230 °C, 65 °C dew point, 15 Hz convection, vertical top-down airflow oven for 20 min and let cool down for 1 h at room temperature. The bread production was performed three times and each time 5 puppy loaves were baked. One was stored at −20 °C for *in vitro* digestibility; the others were stored in a sealed plastic bag and analyzed the day after production.

2.7. Bread characterization

2.7.1. Porosity determination

Digital images of the bread slices (25 mm thick) were collected with an IRIS visual analyzer VA400 (Alpha Soft, Toulouse, France), and then analyzed by Alpha M.O.S. software (Alpha Soft, Toulouse, France). The images were taken with an objective Basler 25 mm 1:2.2 lens (Basler AG, Ahrensburg, Germany) with top and bottom lighting. The "Shape Descriptor" option in the software was used to determine the total area of the breadcrumb and used again after changing the b-value to calculate the surface area of the crumb without the pores. The total surface area of the pores was calculated by subtracting the area without the pores from the total area. Porosity was calculated as the mean of nine different pictures for each bread type.

2.7.2. Moisture content and water activity (aw) measurements

The moisture content was carried out following the AACC standard method (Method, 1999). The water activity was measured using a water activity meter (AQUA LAB 4TE Decagon, Pullman, WA, USA) at 25 °C. Both analyses were carried out on the breadcrumb from the center of the loaf. The analysis was repeated four times for each bread type of each production.

2.7.3. Specific volume determination

The volume of each puppy loaf was measured according to the standard rapeseed displacement method (Method, 2009). The volume value was then divided by the weight of each loaf to obtain the specific volume (cm^3/g). The analysis was conducted at least four times for all bread samples of each production.

2.7.4. Texture profile analysis

The texture profile analysis (TPA) of bread was conducted with a TA. XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) and the data processed by Exponent software (Stable Micro Systems, Godalming, UK). Each slice of bread (20 mm) was cut in the center using a 25 mm diameter seeker. A double compression at 40% of strain was applied to the bread crumb cylinder, with a compression plate probe of 75 mm diameter, at a speed of 5 mm/s and 0.049 N trigger force (Zanoletti et al., 2017). The analysis was repeated 8 times for all types of bread for each production. The data obtained from the TPA curve were the hardness (force peak during the first compression, N), cohesiveness (ratio between the area of the second peak and the area of the first peak, dimensionless), springiness (ratio between the duration of the second compression and the first one, dimensionless) (Boukid et al., 2018).

2.8. In vitro starch digestibility

The *in vitro* starch digestion was performed according to the method described by Englyst et al. (2018). Approximately 2 g of bread was weighed and cut into uniform small pieces ($5 \times 5 \times 5$ mm^3) to simulate the mastication. The *in vitro* digestion included two phases: 30 min of gastric phase and 120 min of intestinal phase. In the first phase, the sample was incubated in a water bath for 30 min, at 37 °C and 180 rpm, after the addition of 10 mL of pepsin (≥ 250 units/mg solid)–guar solution (0.05 M HCl). During the intestinal phase, the gastric digesta was mixed with 10 mL of sodium acetate buffer (0.25 M), 5 marbles, 5 mL of enzyme mixture [pancreatin (8 x USP), invertase (≥300 U/mg solid), and amyloglucosidase (≥ 260 U/ mL)] and shaken for 120 min at 180 rpm (37 °C). Two time points were chosen to sample the digesta: after 20 min (T20) and after 120 min (T120). The enzymatic reaction was stopped with ethanol at 96% (v/v). The amount of starch digested at T20 and T120 was quantified by the GOPOD assay kit from Megazyme (Bray, Ireland). Glucose absorbance was detected at 510 nm using a Cary 60 UV–visible spectrophotometer (Agilent Technologies, Santa Clara, USA). The glucose amount was converted into starch multiplying the value by 0.9. The data were expressed as rapidly digested starch (RDS),

which is starch digested in the first 20 min of the intestinal phase; slowly digestible starch (SDS), which is the amount of glucose released between min 20 and 120, and resistant starch (RS), which is the difference between total starch (TS) and digested starch (RDS+SDS) that stands for the portion of starch that was not digested after 120 min. The amount of total starch was measured using the total starch kit purchased by Megazyme (Bray, Ireland). The digestion was performed in triplicate and the data were expressed as g of digested starch/100 g of total starch. The same batch of enzymes was used to ensure the experimental digestive conditions were consistent across all the experiments.

2.9. Statistical analysis

The results were analyzed with the software IBM SPSS Statistics 25 (IBM, Armon, USA). The normality of the data was checked by the Skewness method. All the data were normally distributed for z-value ($-1.96 < z < 1.96$) and expressed as means ± standard deviation. One-tailed *t*-test was used to compare CS and FS in the preliminary test. Multivariate analysis of variance (MANOVA) was used to evaluate the effects of fermentation methods, semolina granulometry, and their interaction on the textural properties and starch digestibility of durum wheat bread ($\alpha = 0.05$). The percentage of the total variation was computed to explain the variance of each parameter as a function of the sum squares of the main factors and their interaction. Moreover, a post hoc Tukey test ($p < 0.05$) was used to determine which samples were different from the others. The Pearson correlation coefficients between the parameters were calculated, and their significance was tested at a significance level of 0.05.

3. Results and discussion

3.1. Semolina characterization

The particle size distribution and degree of damage starch for CS and FS are shown in Table 2. The two semolina samples had a normal particle size distribution, and they were statistically different for the diameters of 50% of the particles [Dv (50)] and the mean D [4,3]. FS had a Dv(50) diameter of less than 111.7 μm. Consistent with Pasqualone et al. (2017), who found that the size of commercial re-milled semolina was less than 180 μm for at least 70% of the particles, FS also exhibited a smaller particle size than CS. On the other hand, CS, with a D [4,3] and Dv (50) of 571.1 μm and 551.2 μm, respectively, can be defined as coarse semolina based on previously reported data (Sacchetti et al., 2011). The re-milling process of durum wheat increases the degree of starch damage. Therefore, the amount of damaged starch in FS was three times higher than that CS (Table 2). Consequentially, as shown in farinograph curves (Figure S1), FS required more water to reach the optimum consistency of the dough and a shorter mixing time (5 min) than CS. Conversely, CS needed a longer mixing time (30 min) to completely hydrate the granules and less water to reach the same dough consistency (420 FU) in alignment with previous studies (Lin et al., 2020; Pasqualone et al., 2017). Granulometry and the related starch damage content influenced the hydration rate and the ability of the flour to bind water. Indeed, the smaller the particles, the higher the amount of starch damage and consequentially the hydration rate (Liu et al., 2015).

Table 2
The diameters of 50% of the particles [Dv (50)], the mean D [4,3], the percentage of damaged starch, the optimum water, and mixing to reach 420 FU of fine semolina and coarse semolina.

	D [4,3] (μm)	Dv (50) (μm)	Damage starch on dry semolina (%)	Water absorption (mL/100 g of flour)	Mixing time (min)
FS	148.3 ± 3.2^{b}	111.7 ± 6.0^{b}	10.2 ± 0.6^{a}	63.5	5
CS	571.1 ± 8.4^{a}	551.2 ± 7.8^{a}	3.3 ± 0.1^{b}	59.5	30

The values are displayed as mean ± standard deviation (n = 3). Values sharing the same letter in the columns are not significantly different ($p < 0.05$). FS, fine semolina; CS, coarse semolina.

3.2. Dough characterization

The values of TTA and pH of proofed doughs are reported in Table 3. Regardless of granulometry, in doughs made with sourdough, TTA was significantly higher than those proofed by baker's yeast (DM and PO), and consequentially, the pH was lower. pH values were found similar among doughs made using DM and PO. Long proofing time (i.e., 24 h) and the high concentration of LAB at the end of fermentation could be the reason behind the lower pH of doughs made with sourdough (Schober & Arendt, 2003). The sourdough ecosystem mainly consists of different species of LAB, which, during the fermentation, produces a high number of organic acids causing a drop in pH (Xu et al., 2019). Statistically, the fermentation method was chiefly affected by pH and TTA, whereas the semolina granulometry had a small (~1%) but significant impact only on TTA and no effect on pH (Table S1).

3.3. Bread characterization

The characteristics of bread loaves produced with different fermentation methods and semolina particle sizes are reported in Table 4. The moisture content of bread made with SD was significantly higher than that made with PO but similar to that made with DM. As reported by Schober et al. (2003), during sourdough fermentation, the pH reduction softens the gluten network, reducing its capacity to trap CO_2, and this leads to a compact crumb structure. Consequently, the dense structure of sourdough bread may inhibit moisture diffusion within the bread crumb after baking, thereby trapping moisture within the crumb matrix. Regarding water activity, which indicates the bread matrix's capacity to bind water, no significant differences were observed among the samples.

Bread made with SD exhibited the lowest porosity and specific volume compared to those produced with baker's yeast fermentation (PO and DM). Typically, porosity and specific volume are influenced by CO_2 production during fermentation and the gluten network's ability to retain the gas (Schober & Arendt, 2003). Xu et al. (2019) highlighted the crucial role of bakery yeast in promoting CO_2 production for bread. In sourdough bread, CO_2 production is limited by the predominant presence of LAB in the sourdough ecology, which impedes and limits yeast growth. Indeed, it was not feasible to achieve the same level of CO_2 production in bread made with sourdough as in those made with baker's yeast fermentation.

Moreover, in SD bread the acidification reduced pH to 4, which is an optimal value for proteolytic enzyme activity. This activity might have degraded and weakened the gluten network and, thus decreased the specific volume (Barrera et al., 2007). As a result, SD bread was found

Table 3
Total titratable acidity (TTA) and pH of doughs made with fine semolina (FS) and coarse semolina (CS) and produced with Direct method, Poolish method, and Sourdough fermentation.

		TTA	pH
Sourdough fermentation	**FS**	5.0 ± 0.0^{a}	4.08 ± 0.01^{b}
	CS	5.0 ± 0.0^{a}	4.10 ± 0.02^{b}
Direct method	**FS**	2.1 ± 0.0^{b}	5.76 ± 0.02^{a}
	CS	2.6 ± 0.0^{b}	5.68 ± 0.04^{a}
Poolish method	**FS**	2.3 ± 0.0^{b}	5.74 ± 0.05^{a}
	CS	2.3 ± 0.0^{b}	5.73 ± 0.09^{a}

The values are displayed as mean ± standard deviation (n = 3). Data are presented as mean ± standard deviation of three replicates. Values sharing the same letter in the column are not significantly different. These analyses were performed on the dough.

Table 4
Moisture content, water activity (a_w), porosity (% of total crumb area), specific volume, hardness, cohesiveness, and springiness of bread made with fine semolina (FS) and coarse semolina (CS) and produced with Direct method, Poolish method, and Sourdough fermentation.

	Sourdough fermentation		Direct method		Poolish method	
	FS	CS	FS	CS	FS	CS
Moisture%	43.6 ± 1.1^{a}	43.4 ± 1.6^{a}	42.4 ± 1.9^{ab}	41.7 ± 0.8^{ab}	40.8 ± 1.9^{b}	40.3 ± 1.8^{b}
Aw	0.98 ± 0.00^{a}	0.98 ± 0.00^{a}	0.97 ± 0.00^{a}	0.98 ± 0.00^{a}	0.97 ± 0.01^{a}	0.97 ± 0.00^{a}
Porosity (%)	16.8 ± 2.5^{c}	19.1 ± 1.9^{c}	28.8 ± 3.0^{b}	36.5 ± 1.5^{a}	33.9 ± 3.9^{b}	37.7 ± 2.9^{a}
Specific volume (cm^3/g)	0.77 ± 0.06^{e}	0.92 ± 0.22^{e}	1.28 ± 0.03^{c}	1.55 ± 0.03^{a}	1.17 ± 0.07^{d}	1.42 ± 0.03^{b}
Hardness (N)	11.4 ± 1.2^{a}	9.4 ± 1.3^{b}	4.0 ± 0.9^{c}	3.3 ± 0.7^{c}	4.2 ± 1.2^{c}	3.1 ± 0.4^{c}
Cohesiveness (−)	0.85 ± 0.01^{a}	0.81 ± 0.02^{d}	0.86 ± 0.02^{a}	0.84 ± 0.02^{c}	0.86 ± 0.01^{a}	0.84 ± 0.01^{bc}
Springiness (−)	0.96 ± 0.01^{a}	0.95 ± 0.05^{a}	0.96 ± 0.10^{a}	0.97 ± 0.01^{a}	0.97 ± 0.01^{a}	0.98 ± 0.01^{a}

The values are displayed as mean ± standard deviation (n = 8). Values sharing the same letter in the row are not significantly different.

smaller and, therefore, significantly harder than those made with PO and DM, which were not significantly different from each other (Table 4). Bread hardness was also found negatively correlated to the specific volume ($r = -0.946$, $p \leq 0.05$) (Table 5). This finding supports the observation that a reduced specific volume correlates with increased hardness. However, literature reports mixed results regarding the relationship between hardness and sourdough fermentation. In agreement with our findings, several studies have reported an increase in hardness with sourdough addition (Xu et al., 2019). Conversely, Hadaegh et al. (2017) observed a decrease in hardness in bread made with sourdough. These conflicting outcomes can be attributed to varying levels of acidification resulting from different sourdough ecosystems. A significant drop in acidity could negatively affect the structure of gluten and starch by enhancing the activity of proteolytic enzymes, which reduces the formation of a stable gluten network. Bread produced with SD and coarse semolina was also the least cohesive among the analyzed samples. This is probably due to the high hardness of the bread crumb and the low adhesion of the coarse particles in the dough (Lin et al., 2020; Luo et al., 2021). However, springiness, which measures the bread's ability to return to its original height after compression, did not significantly differ among the analyzed samples (Table 4). These findings align with those reported by Fadda et al., (2010a), who observed no significant differences in springiness among bread samples proofed under varying acidification levels. Despite sourdough's impact on reducing overall bread quality, the elasticity of the crumb remained comparable to that of bread fermented with yeast. Among bread produced with baker's yeast fermentation (i.e., PO and DM), those made using PO exhibited slightly lower specific volume and porosity compared to those made with DM. It has been shown that the yeast's activity during the prolonged fermentation time (24 h) in PO can lead to the formation of peptides from partial gluten hydrolysis, which may restrict the development of an elastic gluten network (Hackenberg et al., 2017). Among bread samples fermented with yeast (DM and PO), those produced with CS exhibited a more porous, voluminous structure than those made with FS (Table 4). Damaged starch increases CO_2 production during leavening as it is more readily available for microorganisms. However, damaged starch tends to compete with gluten proteins for water. This competition can lead to damaged starch rapidly absorbing water, which interferes with the formation of disulfide bonds in gluten proteins (Fadda et al., 2010a). Similarly, the swelling of hydrated starch granules can hinder the formation of a well-structured gluten network (Hackenberg et al., 2017; Khalid et al., 2017).

In summary, the differences observed in bread characteristics (specific volume, porosity, and hardness) were primarily influenced by the fermentation methods (Table S2). Semolina granulometry and the interaction between the fermentation method and semolina granulometry were the only parameters that significantly affected the specific volume.

3.4. *In vitro* starch digestibility

The results of *in vitro* starch digestibility are displayed in Fig. 1. Among all studied bread samples, bread made with FS and SD had the lowest RDS value and, in turn, the highest SDS and RS values, even though the values were not always statistically different. As shown in Table S2, RDS and SDS were not affected either by the fermentation method or the semolina granulometry. Table 5 shows that RDS is negatively correlated to hardness ($r = -0.882$, $p \leq 0.05$) and positively correlated to bread-specific volume ($r = 0.871$, $p \leq 0.05$). Therefore, we can hypothesize that the lower values of digestibility for bread made with FS and SD could be related to its textural properties. This bread had the highest hardness and the lowest specific volume (Table 4) and was the least digestible. Therefore, the compact structure of bread, such as hard crumbs, probably limited its physical breakdown during gastrointestinal digestion resulting in a less accessible starch (Martínez et al., 2018). Its hard texture could be attributed to the drop in pH and the increase of acidity measured during sourdough fermentation. During such a fermentation, the production of acids, such as acetic, lactic, and propionic, promotes the interaction between the starch and protein, forming a complex that could inhibit the contact between the starch and the amylolytic enzymes (Bo et al., 2017; Demirkesen-Bicak et al., 2021; Östman et al., 2002). Moreover, the amount of damaged starch due to re-milling also had a detrimental effect on the texture of the bread, limiting the development of a cohesive gluten network. In addition, it was reported that bread with low volume and high hardness is more prone to

Table 5
Pearson's correlation matrix between bread characteristics and starch digestion parameters.

	MC	aw	Springiness	Cohesiveness	Hardness	SV	RDS	SDS	RS
MC	1								
aw	0.722	1							
Springiness	−0.870*	−0.380	1						
Cohesiveness	−0.312	−0.567	0.242	1					
Hardeness	0.846*	0.599	−0.643	−0.329	1				
SV	−0.748	−0.328	0.675	0.251	−0.946**	1			
RDS	−0.669	−0.341	0.558	0.007	−0.882*	0.871*	1		
SDS	0.664	0.661	−0.490	−0.179	0.694	−0.526	−0.801	1	
RS	0.468	−0.036	−0.448	0.139	0.770	−0.895*	−0.866*	0.393	1

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level. Moisture content (MC); water activity (aw); specific volume (SV); rapidly digestible starch (RDS); slowly digestible starch (SDS); resistant starch (RS);

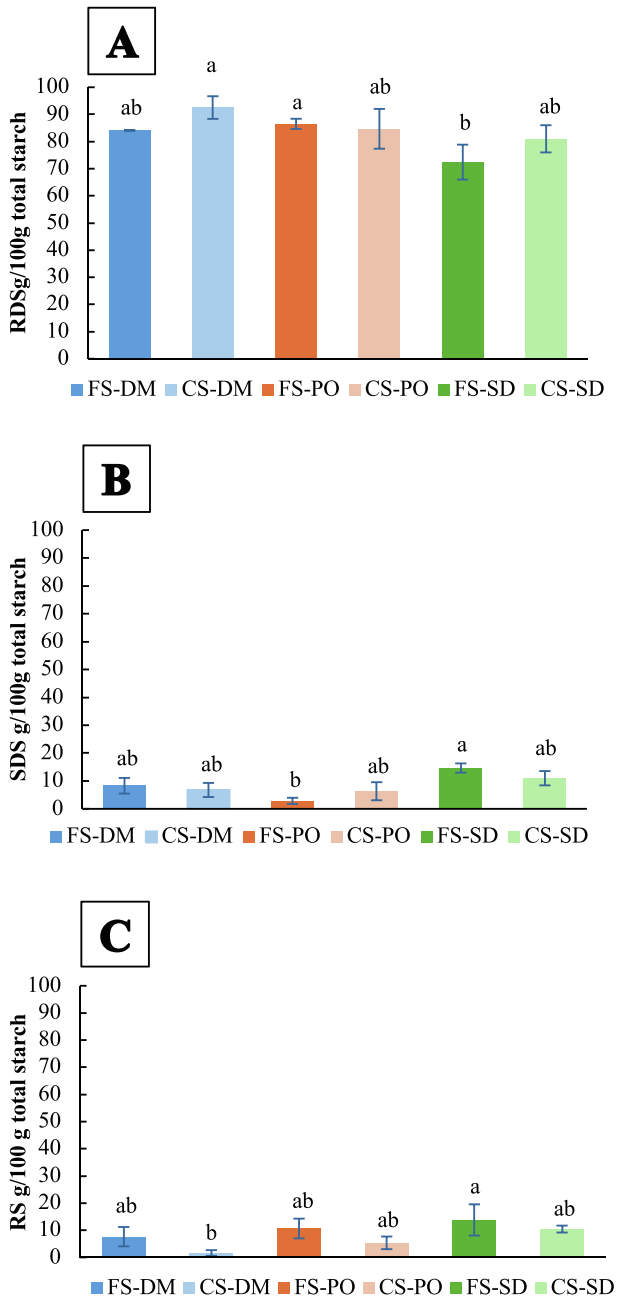

Fig. 1. (A) rapidly digestible starch (RDS), (B) slowly digestible starch (SDS), and (C) resistant starch (RS) of bread made with fine semolina and Direct method (FS-DM); coarse semolina and Direct method (CS-DM); fine semolina and Poolish method (FS-PO); coarse semolina and Poolish method (CS-PO); fine semolina and Sourdough method (FS-SD); coarse semolina and Sourdough method (CS-SD). Columns sharing the same letter are not significantly different ($p < 0.05$; Tukey's test) ($n = 9$).

form a compact bolus and more difficult to disintegrate during digestion (Gao et al., 2020). Therefore, the granulometry, *per se*, did not have any influence on starch digestibility. Consistently, as demonstrated by Korompokis et al. (2021) and Tagliasco et al. (2022), coarse granulometry did not have an effect in reducing the starch digestibility in bread, even if this effect was significantly observed in flour and porridge (Edwards et al., 2015; Korompokis et al., 2019; Mandalari et al., 2018). Probably, during bread processing, the ability of the cell wall to act as a barrier limiting the contact between starch and enzyme was lost due to the increased porosity of cell walls.

Noteworthy, MANOVA analysis showed a small but significant effect of the granulometry on RS (Table S2). The results in Fig. 1 show an overall decrease in RS for bread produced with CS. This decrease was probably linked to the higher porosity and specific volume of these breads compared to their counterpart made with FS, which led to starch being more accessible to amylase enzyme. Overall, it was not possible to find a good compromise between the technological features and reduced digestibility features. The sourdough bread samples were too hard to appreciate in terms of textural features; however, they were the only ones with slightly reduced starch digestibility.

4. Conclusion

In summary, our study indicates that fermentation methods significantly impact bread quality. Spontaneous sourdough reduced bread quality, decreasing specific volume and porosity while increasing hardness. The Poolish method yielded similar results to the Direct method, indicating minimal effect on bread texture. The use of fine semolina slightly compromised bread quality by consistently reducing specific volume regardless of the fermentation method. Notably, the combination of sourdough and fine semolina resulted in the densest and hardest bread crumb, which led to a marked reduction in rapidly digestible starch. These findings suggest a potential link between crumb structure and starch digestion that warrants further investigation.

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Marianna Tagliasco: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fatma Boukid:** Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Stefano Renzetti:** Writing – review & editing, Validation, Methodology, Data curation, Conceptualization. **Alessandra Marti:** Writing – review & editing, Validation. **Elena Bancalari:** Writing – review & editing, Validation, Methodology, Conceptualization. **Elena Vittadini:** Writing – review & editing, Validation, Methodology, Conceptualization. **Nicoletta Pellegrini:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106464.

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