

## Effect of Commercial Dried Sourdoughs on Structural Characteristics of Wheat Bread

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This study investigated the effect of the addition of three commercial dried sourdoughs containing different percentages of *Saccharomyces cerevisiae* (25-30%), on structural characteristics of wheat bread. Rheology, texture and physicochemical characteristics were assessed for both dough and final bread. Control samples were prepared using common dried bakery's yeast (100% *S. cerevisiae*). The rheological parameters complex viscosity ( $\eta$ ), linear viscoelastic region (LVR), storage and loss modulus ( $G'$  and  $G''$ ) and angular tangent ( $\tan\delta$ ) were evaluated on the doughs through rheological tests carried out both in rotational and oscillatory mode. Final breads were characterised for the texture profile (hardness, springiness, cohesiveness and resilience). Moisture content and pH were monitored during the process. The leavening agents (dried sourdoughs and bakery's yeast) were characterized for the content of total lactic acid bacteria and total yeasts. Rheological analysis highlighted a pseudo-plastic behaviour for all the doughs with the elastic component prevalent on the viscous one ( $G' > G''$ ), without substantial differences among samples before proofing. After 3 h leavening, storage and loss moduli and complex viscosity were lower than control for all the sourdough samples. Microbial evaluation showed no vital lactic acid bacteria in the dried sourdough that, contrary to the other two, did not give acidification during leavening and produced a final bread with texture properties comparable to those of the control.

### 1. Introduction

The use of sourdough in breadmaking is probably one of the oldest known biotechnological processes for cereal-based food production (Chavan & Chavan, 2011, Papisidero et al., 2014). Sourdough is defined as a dough made of flour and water fermented by yeast and lactic acid bacteria (LAB) and used as a leavening agent in bakery production (Siepmann et al., 2018). The use of sourdough may improve the final product shelf-life due to its higher acidity (Poutanen et al., 2009), antifungal (Manini et al., 2016) and antimicrobial activity (Neysens & De Vuyst, 2005). Moreover, there is considerable consensus regarding the positive effects conferred on the product by its use, including improvements in bread volume and crumb structure (Clarke et al., 2004) and better sensory and aroma profile (Corsetti, 2012). From a technological point of view, sourdough can be classified into three types. Type I is a dough made of water and flour which is continuously "backslopped" using a portion of previous dough as inoculum. Type I sourdough is usually produced as a firm dough exploiting the microbial flora present in the previous dough to carry out a spontaneous fermentation at room temperature (20–30 °C) (Nionelli et al., 2014). Type II is an industrial sourdough in which specific starter cultures (with a typical proportion 100:1 of LAB to yeast) are employed to improve fermentation, even though they can inhibit the growth of autochthonous microbiota. The addition of starter cultures acidifies the dough faster than in the traditional sourdough, therefore the most often LAB used are acid-tolerant, as *L. amylovorus*, *L. panis*, *L. pontis*, and *L. reuteri* (Siepmann et al., 2018). This type of sourdough is usually liquid to be easily pumpable in industrial pipeline. The production process involves a single fermentation step of 15–24 h (De Vuyst et al., 2014). Due to the addition of the starter cultures, the fermentation process is carried out at higher temperatures, above 30 °C (Gobbetti, 1998), to allow a fast and high acidification that can inhibit the growth of natural yeast. Therefore, in sourdough type II, baker's yeast can be added at the end of the fermentation

process (De Vuyst et al., 2016). Type III is obtained from type II sourdough through a drying process. On industrial scale, dehydration is preferably performed by drum dry or spray dry techniques. Sourdoughs type II and III require the addition of bakery's yeast as leavening agents to activate the fermentation process even though this has a flattening effect on the aromatic profile of the final products. In the bakery sector, Type III is preferentially used than the other two because it guarantees higher quality and standardization of final products. Moreover, the powder occupies less volume and facilitates handle and storage condition. However, the selection of starter culture is the critical step of the entire process. The selected strain should be resistant to drying and maintain its activity during the rehydration and fermentation steps. In the last years, the use of type III sourdoughs has greatly increased not only at the industrial level but also at household level.

The aim of this work was to investigate the effect of the addition of three different commercial dried sourdoughs (DS) on structural characteristics of wheat bread. Rheology, texture and physicochemical characteristics were assessed for both dough and final bread. Control samples were prepared using common dried bakery's yeast (100% *Saccharomyces cerevisiae*).

## 2. Materials and Methods

### 2.1 Materials

All the ingredients were purchased from local supermarket: Manitoba flour (Molino Spadoni S.p.A.); white sugar (Zefiro, Eridania S.p.A.) and fine sea salt. Three different dried sourdoughs were purchased: DS\_A and DS\_B containing a 30% of *S. cerevisiae* and DS\_C with a lower content of yeast (25%). Commercial dried bakery's yeast (DBY) (Esselunga S.p.A) was used for control bread production.

### 2.2 Dough Kneading, Fermentation, and Baking

All the sourdoughs were used at their suggested (reported on the package) dosage of 70 g<sub>DS</sub>/kg<sub>flour</sub>, while the DBY was tested at two dosage levels: DBY\_A (70 g<sub>DBY</sub>/kg<sub>flour</sub>) and DBY\_B (20 g<sub>DBY</sub>/kg<sub>flour</sub>) respectively the suggested quantity for DS and DBY. The recipe included: 1 kg of flour, 600 g of water, 30 g of salt and 20 g of sugar. The ingredients were kneaded all together for 12 min at 48 rpm speed in a professional kneading machine (Tekno Stamp Mod. C-Line 20). The obtained doughs were divided in pieces of 110 g and shaped into round rolls, which were let leavening for 3 h at 25°C and 82% relative humidity. The rolls were then baked at 200°C for 20 min in convective mode professional oven (Wind + Stratos, Polin, Italy). The product pH was monitored before leavening ( $t_0$ ), after leavening ( $t_1$ ) and after baking ( $t_2$ ) with a pHmeter (Hanna Edge). Water activity ( $a_w$ ) was evaluated at  $t_0$  and at  $t_1$  in the doughs and at time  $t_2$  in the bread (AquaLab Devices instrument).

### 2.3 Dynamic Rheological Tests

Dynamic oscillatory tests of dough samples were carried at a constant temperature of 25°C out using both the Amplitude and the Frequency Sweep mode in a controlled-stress rheometer (MCR 302, Anton Paar, Austria) equipped with a geometrical rough plate. The plates were 25 mm in diameter with a selected 3.5 mm gap. Amplitude strain sweep test (with amplitude varying from 0.01 to 100 s<sup>-1</sup> at a constant frequency value of 1 rad s<sup>-1</sup>) was conducted to determine the linear viscoelastic region (LVR). The viscoelastic parameters, including the storage modulus ( $G'$ ), loss modulus ( $G''$ ) and loss tangent ( $\tan \delta$ ) were plotted as a function of shear strain and the LVR was determined as the region in which  $G'$  remains constant. The oscillatory frequency sweep tests were performed at a constant shear strain of 0.02% and frequency set between 100 and 0.1 rad s<sup>-1</sup>. The viscoelastic parameters, including the storage modulus ( $G'$ ), loss modulus ( $G''$ ) and loss tangent ( $\tan \delta$ ) were plotted as a function of shear strain. All the tests were carried out in triplicate.

### 2.4 Microbiological Characterisation of Leavening Agents

The three commercial dried sourdough and the bakery's yeast were analysed for the total vital LAB content and for the total vital yeast contents by plating on Rose-Bengal-Agar and RMS-Agar media at 30 °C and 37°C, respectively. Plating counting was performed after 2 and 5 days of incubation for yeasts, and after 4 days for LAB. Results were expressed as CFU (colony forming units)/g of product.

### 2.5 Texture Profile

The texture profile analysis (TPA) of breads was determined with a Texture Analyser (Perten, TVT 6700) as reported by Kadan et al. (2011) method with slight modification. Bread was cut into 30 mm thick slices which were subjected to a double compression down to 40% of their original height by a cylindrical probe of 25 mm diameter at a 3.0 mm s<sup>-1</sup> speed. A decompression step with a holding time of 30 s is applied between the two cycles to assess a partial recovery of crumb shape and the release of compression force. The instrument software computed a force versus time plot and calculated texture parameters such as hardness, cohesivity,

springiness and resilience expressing the resistance of the crumb to the penetrating plunger and representing the overall crumb firmness.

## 2.6 Statistical analysis

Results are reported as mean values of three replicates with their corresponding standard deviations. The influence of leavening agent on characteristics of doughs and breads was evaluated through the one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for means discrimination, at  $p \leq 0.05$  level, using statistical software SPSS® (version 21.0, SPSS Inc., Chicago, IL, USA).

## 3. Result and Discussion

### 3.1 Dynamic Rheological Tests

The obtained data of storage modulus  $G'$  (Figures 1 and 2) show trends like those typical of elastoviscous materials: as angular frequency increases up to 100 rad s<sup>-1</sup>, storage modulus slightly increases as a function of frequency. In a structured or solid-like material, the elastic modulus  $G'$  is often nearly independent of frequency. The more frequency dependent the elastic modulus is, the more fluid-like is the material.

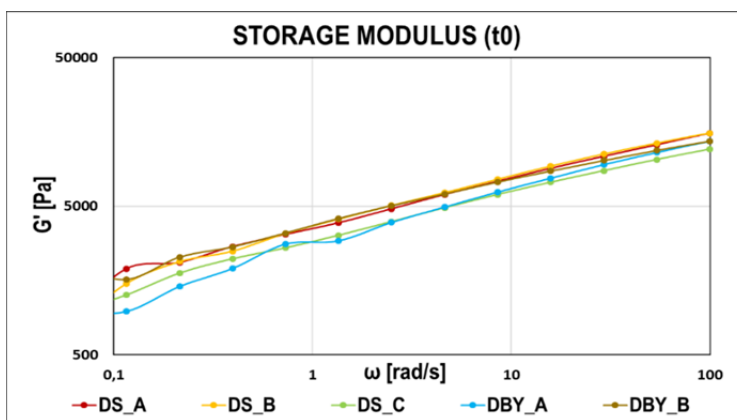


Figure 1: Storage modulus ( $G'$ ) of dough samples as a function of angular frequency ( $\omega$ ): a) before the leavening ( $t_0$ ) DS: dried sourdough; DBY: dried bakery's yeast.

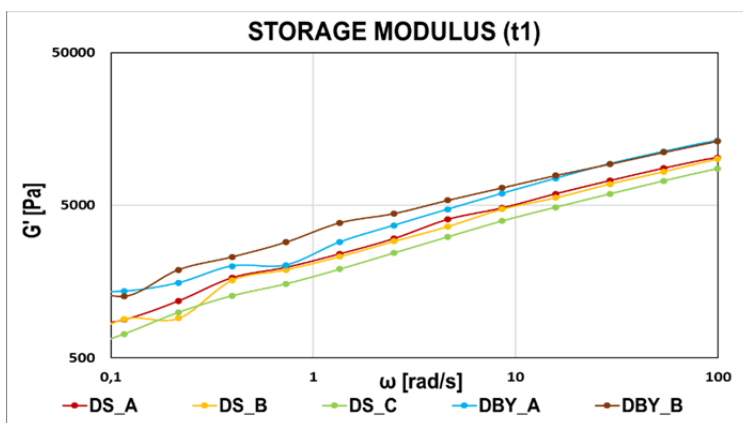


Figure 2: Storage modulus ( $G'$ ) of dough samples as a function of angular frequency ( $\omega$ ): a) after the leavening ( $t_1$ ) DS: dried sourdough; DBY: dried bakery's yeast.

According to Upadhyay et al. (2012) the flow properties of wheat flour-water doughs obey the power law as function of frequency ( $\omega$ ). The power law constants were then calculated from Eq(1):

$$G' = G'_0 \omega^n \quad (1)$$

Where  $G'$  represents the storage modulus (Pa),  $n$  is the power law exponent (dimensionless),  $\omega$  is the frequency (rad/s) and  $G'_0$  (Pa) is the storage modulus extrapolated to the value of initial measuring angular

frequency. The constants (Table 1) were obtained from the linear regression analysis after a logarithmic transformation of the data according to (Eq(2)).

$$\log(G') = \log(G'_0) + n \log(\omega) \quad (2)$$

The linear regression trends are in agreement with many others studies, but the found values for exponent  $n$  are in the range 0.30–0.37 (Table 1), higher than those reported in the literature in which the  $n$  value is in the range of 0.15–0.28 (Berland & Launay, 1995; Phan-Thien et al., 2000; Tanner et al., 2008; Uthayakumaran et al., 2002; Georgopoulos et al., 2004). According to Ross-Murphy (1995), the magnitude of the slope of Eq(2) provides useful indication on the material behaviour: rubbery when approaching 0; liquid flowing when approaching 2. When a 3D network is present, we expect the slope to be almost zero (Gabriele et al., 2001). In the present work, rheological analysis highlighted a pseudo-plastic behaviour in all doughs, with the elastic component being prevalent on the viscous one ( $G' > G''$ ). The storage modulus  $G'$  expresses tenacity and elasticity and the loss modulus  $G''$  represents extensibility. Consequently, the values of  $\tan \delta$ , defined as the ratio of the viscous to elastic modulus ( $G''/G'$ ), is overall lower than 1 and the material is considered to be dominantly gel or solid-like. No evident differences were observed among the samples before proofing. Moreover, the doughs obtained from DS\_A and DS\_B, with a higher content (30%) of *S. cerevisiae*, showed trends superimposable to control DBY\_A, while the dough with the DS\_C (25% of yeast) followed strictly the behaviour of the control DBY\_B. After 3 h leavening, storage and loss moduli and complex viscosity decreased for all the samples, but more markedly for those prepared with dried sourdoughs. Linear regression of  $\log(G')$  vs.  $\log(\omega)$  confirmed solid-like nature of the dough. The obtained  $n$  values were always  $< 0.4$  indicating the existence of a 3D network. Moreover, the sample prepared with sourdough showed a higher increase of power law exponent than control samples after fermentation. Greater values of  $n$  indicated a lower fraction of cross-linked material (Kokini et al., 1994). This softening effect can be related to proofing mechanisms and slight acidification promoted by LAB contained into sourdough.

*Table 1: Linear regression parameters ( $n$  and  $G'_0$ ) from application of the power law model (Eq.2) to data of Figures 1 and 2. DS: dried sourdough; DBY: dried bakery's yeast.  $R^2$ : Linear Regression coefficient. The same superscript letter under the same column indicates not significantly different means according to ANOVA and Tukey's post-hoc test.*

Dough	Before leavening			After leavening		
	$n$	$G'_0$	$R^2$	$n$	$G'_0$	$R^2$
DS_A	0,3372 ± 0,0132 <sup>ab</sup>	2263 ± 368 <sup>a</sup>	0,986	0,3275 ± 0,0126 <sup>a</sup>	3586 ± 220 <sup>a</sup>	0,993
DS_B	0,3532 ± 0,0229 <sup>a</sup>	2091 ± 67 <sup>a</sup>	0,989	0,3373 ± 0,0221 <sup>a</sup>	3454 ± 395 <sup>a</sup>	0,989
DS_C	0,3194 ± 0,0080 <sup>ab</sup>	2869 ± 230 <sup>ab</sup>	0,994	0,3589 ± 0,0048 <sup>a</sup>	1718 ± 98 <sup>c</sup>	0,995
DBY_A	0,3568 ± 0,0225 <sup>a</sup>	2759 ± 621 <sup>ab</sup>	0,985	0,3513 ± 0,0136 <sup>a</sup>	2645 ± 431 <sup>ab</sup>	0,984
DBY_B	0,2965 ± 0,0081 <sup>b</sup>	3569 ± 1200 <sup>b</sup>	0,985	0,3329 ± 0,0194 <sup>a</sup>	3075 ± 249 <sup>b</sup>	0,979

### 3.2 Microbiological Characterisation of Leavening Agents

Table 2 shows that the control baker's yeast, as expected, presents a much higher content of total yeasts than the dried sourdoughs after a 2 days incubation. After 5 days of incubation, only DS\_C showed an increase in yeast content which might be correlated to a slower leavening capacity. No vital LAB were counted in DS\_C, which is in agreement with the measured constant pH after leavening (Table 3). DS\_A showed higher content in LAB than DS\_B and DBY control. In spite of lower amount of vital LAB found in DS\_B, rheological tests gave a behaviour like DS\_A. Together with the different rheological properties of the dough produced with DS\_C containing a lower amount of *S. cerevisiae* and no vital LAB, this may highlight the dominant role of yeasts in the leavening process.

The measurement of pH (Table 3) indicated a slight pH decrease after fermentation only in the samples obtained with DS\_A and DS\_B and a constant pH in the sample with DS\_C, which could agree with the LAB content of the used products. However, the pH levels are not comparable to typical data obtained for sourdoughs (pH 3.5–5) (Chavan & Chavan, 2011; Siepmann et al., 2018). The high air relative humidity of the leavening cell allowed to maintain constant the  $a_w$  of the doughs (Table 3). This parameter obviously decreased with baking. Addition of DS\_B gave a slightly higher  $a_w$  all over the process.

Table 2: Microbial characterisation of commercial dried sourdoughs (DS\_A, DS\_B, DS\_C) and Bakery's Yeast (DBY) expressed as colony forming units (CFU). ND: not detected.

Incubation time (at 30°C for bacteria, 37°C for yeasts)	Total yeasts (CFU/g)		Total LAB (CFU/g)
	2 days	5 days	4 days
DS_A	7.8·10 <sup>8</sup>	7.8·10 <sup>8</sup>	5·10 <sup>4</sup>
DS_B	9.6·10 <sup>8</sup>	9.6·10 <sup>8</sup>	3.2·10 <sup>2</sup>
DS_C	6.4·10 <sup>8</sup>	2.6·10 <sup>9</sup>	ND
DBY	6.2·10 <sup>10</sup>	6.2·10 <sup>10</sup>	8.8·10 <sup>2</sup>

Table 3: pH and water activity ( $a_w$ ) (means  $\pm$  s.d.) of dough and bread samples obtained with commercial dried sourdoughs (DS\_A, DS\_B, DS\_C) and bakery's yeast at two dosage levels (DBY\_A, DBY\_B). The same superscript letter under the same column indicates not significantly different means according to ANOVA and Tukey's post-hoc test.

	Dough before leavening		Dough after leavening		Bread	
	pH	$a_w$	pH	$a_w$	pH	$a_w$
DS_A	5.42 $\pm$ 0.02 <sup>a</sup>	0.974 $\pm$ 0.005 <sup>a</sup>	5.17 $\pm$ 0.03 <sup>a</sup>	0.970 $\pm$ 0.003 <sup>a</sup>	5.37 $\pm$ 0.03 <sup>a</sup>	0.961 $\pm$ 0.003 <sup>a</sup>
DS_B	5.44 $\pm$ 0.04 <sup>a</sup>	0.979 $\pm$ 0.003 <sup>a</sup>	5.18 $\pm$ 0.03 <sup>a</sup>	0.980 $\pm$ 0.001 <sup>c</sup>	5.37 $\pm$ 0.03 <sup>a</sup>	0.967 $\pm$ 0.004 <sup>a</sup>
DS_C	5.71 $\pm$ 0.05 <sup>c</sup>	0.977 $\pm$ 0.003 <sup>a</sup>	5.55 $\pm$ 0.02 <sup>c</sup>	0.972 $\pm$ 0.002 <sup>ab</sup>	5.60 $\pm$ 0.03 <sup>b</sup>	0.960 $\pm$ 0.006 <sup>a</sup>
DBY_A	5.56 $\pm$ 0.03 <sup>b</sup>	0.977 $\pm$ 0.002 <sup>a</sup>	5.25 $\pm$ 0.02 <sup>b</sup>	0.976 $\pm$ 0.003 <sup>bc</sup>	5.57 $\pm$ 0.02 <sup>b</sup>	0.963 $\pm$ 0.006 <sup>a</sup>
DBY_B	5.55 $\pm$ 0.05 <sup>b</sup>	0.977 $\pm$ 0.004 <sup>a</sup>	5.25 $\pm$ 0.05 <sup>b</sup>	0.976 $\pm$ 0.002 <sup>abc</sup>	5.57 $\pm$ 0.04 <sup>b</sup>	0.963 $\pm$ 0.006 <sup>a</sup>

#### 4.3 Texture Profile

The following TPA parameters were calculated and reported in Table 4:

- Hardness: defined as the maximum peak force during the first compression cycle, comparable to the first bite, and often referred to as firmness;
- Springiness: linked to the height recovered by the sample during the resting time between the two compression cycles;
- Cohesiveness: the rate at which the material disintegrates under mechanical action;
- Resilience: it reflects the amount of energy that the sample needs to recover its original state.

Table 4: Texture profile of bread samples obtained with commercial dried sourdoughs (DS\_A, DS\_B, DS\_C) and bakery's yeast at two dosage levels (DBY\_A, DBY\_B). Data are reported as mean values  $\pm$  s.d. The same superscript letter under the same column indicates not significantly different means according to ANOVA and Tukey's post-hoc test.

Leavening Agent	Hardness [N]	Springiness	Cohesiveness	Resilience
DS_A	7.93 $\pm$ 2.01 <sup>a</sup>	0.96 $\pm$ 0.06 <sup>a</sup>	0.80 $\pm$ 0.06 <sup>ab</sup>	0.44 $\pm$ 0.06 <sup>ab</sup>
DS_B	7.95 $\pm$ 1.81 <sup>a</sup>	0.94 $\pm$ 0.08 <sup>a</sup>	0.81 $\pm$ 0.05 <sup>c</sup>	0.46 $\pm$ 0.05 <sup>c</sup>
DS_C	12.65 $\pm$ 1.99 <sup>b</sup>	0.94 $\pm$ 0.08 <sup>a</sup>	0.76 $\pm$ 0.07 <sup>ab</sup>	0.39 $\pm$ 0.07 <sup>a</sup>
DBY_A	14.03 $\pm$ 3.83 <sup>bc</sup>	0.97 $\pm$ 0.07 <sup>a</sup>	0.76 $\pm$ 0.07 <sup>a</sup>	0.39 $\pm$ 0.07 <sup>a</sup>
DBY_B	15.26 $\pm$ 2.30 <sup>c</sup>	0.97 $\pm$ 0.07 <sup>a</sup>	0.81 $\pm$ 0.03 <sup>c</sup>	0.42 $\pm$ 0.04 <sup>ab</sup>

The obtained results highlight how the use of a different leavening agent affected only the firmness parameter. In fact, breads prepared with bakery's yeast, at both a conventional (DB\_A) and a higher dosage level (DBY\_A) were significantly harder than sourdough bread obtained with DS\_A and DS\_B, without substantial difference due to yeast inoculum. However, the bread obtained from the sourdough DS\_C, characterised by the lack of vital LAB, showed texture properties like the control bread with DS\_A. This result may be due to a greater strength of the gluten matrix and softer and more pliable crumbs compared to the other samples.

#### 4. Conclusions

This study investigated the effect of different commercial dried sourdoughs on rheological, textural and physicochemical characteristics of wheat dough and bread in comparison to the use of commercial conventional baker's yeast. All sourdoughs showed a reduction in the rheological parameters after proofing. A slight acidification of the dough could be detected only with the use of two of the three tested dried sourdoughs which also revealed the presence of vital LAB and higher content of vital yeasts. The third dried

sourdough did not contain vital LAB and its yeast cells revealed a slower growth compared to the yeasts of both the other sourdough and baker's yeast. These characteristics could be correlated to the results obtained in the texture profile analysis. After baking, bread prepared with this dried sourdough exhibited texture properties like control bread. Higher hardness value was probably due to a greater strength development of the gluten matrix and a softer crumb compared to breads prepared with the other sourdoughs.

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