

# Frozen stored barley sourdough: stability and application to wheat bread

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## Summary

The primary aim of the industrial use of sourdough is to improve bread quality and to replace additives. This study investigates the applicability of frozen stored barley sourdough in wheat bread. Sourdough was fermented with *Weissella cibaria* in the presence of sucrose and xylanase, and stored frozen for 4, 8, or 12 weeks. During storage, the number of viable cells in the sourdough declined. Softer dough and crumb were obtained after the sourdough was stored for a longer period ( $\geq 4$  weeks), while bread specific volume was unaffected by the sourdough storage time. The highest bread specific volume, shape, crumb softness, and sensory liking were obtained with 20% of the sourdough. This study demonstrates the possibility for the industrial application of frozen barley sourdough, since it can improve the quality of bread compared to the native barley flour.

**Keywords:** barley sourdough storage, blast freezing, dough consistency, *Weissella cibaria*, wheat bread quality

## Introduction

Barley is a recognized source of soluble and insoluble dietary fibres, in particular  $\beta$ -glucans and arabinoxylans, which have beneficial physiological impact, mostly expressed through their ability to decrease cholesterol and blood glucose levels (Pejcz et al., 2017). Nevertheless, despite the health benefits, barley flour is rarely used in bread making. Indeed, higher amounts of added barley flour (40%) significantly increase the bread fibre content but decrease the specific volume of bread (Trogh et al., 2004). The negative effect can be avoided by the addition of moderate amounts of whole-grain barley flour to the bread (up to 15%), which also increases the fibre content but maintains satisfactory sensory properties (Dhingra and Jood, 2004).

Several studies indicated that sourdough fermentation of barley flour positively affects volume, texture, and shelf-life of bread made from 20 to 100% barley flour (Mariotti et al., 2014; Novotni et al., 2017; Pejcz et al., 2017; Zannini et al., 2009). On the other hand, Harth et al. (2016) found that wheat breads supplemented with 20% (based on flour) of spontaneously fermented barley sourdough display a firmer texture and lower volume, but an acceptable flavour compared to wheat-based breads.

Traditional bakeries spontaneously ferment sourdoughs using microorganisms naturally present in the flour, and by applying back-slopping (inoculation with a portion of previously fermented dough). Due to the inevitable variations in raw

materials and ambient conditions in bakeries, sourdough properties, as well as the final product, alter (Lattanzi et al., 2014). Consequently, this triggered investigations into new means for the production and storage of sourdough. Sourdough can be fermented with pure cultures or defined mixtures of lactic acid bacteria (LAB) and yeast. Recently, attention has been paid to various *Weissella* strains because of their potential to synthesize exopolysaccharides (EPS) consisting of  $\alpha$ -1,6-linked glucose molecules (dextran) (Wolter et al., 2014). EPS produced by *Weissella cibaria* act as prebiotics and positively influence dough rheology by imparting increased viscosity and a softening effect, and successfully improve bread volume and texture, and retard staling (Di Cagno et al., 2006; Galle et al., 2012; Wolter et al., 2014). *W. cibaria* was found suitable for sourdough fermentation of buckwheat, quinoa, and teff flour (Wolter et al., 2014), as well as barley flour (Vrana Špoljarić et al., 2016). *W. cibaria* was also identified in spontaneous barley sourdough fermentations in bakery conditions (Harth et al., 2016). However, the effect of barley sourdough fermented with a pure culture of *W. Cibaria* on wheat bread quality was not investigated yet.

Sourdough can be preserved by back-slopping, drying, refrigeration, or freezing. Lattanzi et al. (2014) proved that frozen storage enables a partial preservation of the starter LAB for 90 days, while refrigeration and dried storage gave satisfactory results up to 30 days of storage. Although the

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leavening capacity of reactivated sourdoughs was not as high as that of fresh sourdoughs, their acidification capacity was preserved (Lattanzi et al., 2014). This indicates the frozen sourdough potential in industrial bakeries.

To test the hypothesis that frozen stored barley sourdough can be simply thawed and directly used in bread making, we followed up the survival of EPS producing *W. cibaria* strain in the sourdough and examined the technological performance of the same sourdough in the wheat-dough system (dough consistency and physical properties of bread) during 12 weeks of storage. Sourdough was fermented in the presence of sucrose and bacterial xylanase. Di Cagno et al. (2003) already recommended the combined usage of sourdough and enzymes in the modern baking technology. In this study, xylanase was used for its ability to hydrolyse the xylan backbone of arabinoxylans, and the consequent strong impact on bread structure and functionality (Ktenioudaki et al., 2015; Ravn et al., 2016; Trogh et al., 2004). The presented study also assessed the sensory and physical impact of three addition levels of barley sourdough.

## Materials and methods

### Raw materials

Wheat flour T-850 (Žitoproizvod, Karlovac, Croatia) and wholemeal barley flour (Advent, Pula, Croatia) were purchased commercially and described beforehand (Novotni et al., 2017). We used compressed bakers' yeast for the bread making (Kvasac Ltd., Lesaffre Group, Prigorje Brdovečko, Croatia).

### Sourdough preparation, storage and characterization

The inoculum of *W. cibaria* (DSM 15787) was obtained after two successive incubations on MRS

broth (de Man, Rogosa and Sharpe, Biolife, Bolzano, Italy) for 48 h at 30 °C. Sourdough was prepared by mixing barley flour (700 g), water (1050 g in total), sucrose (70 g), bacterial xylanase (0.035 g) (AB Enzymes Veron XL), and the inoculum of *W. cibaria* (approximately 6 log colony forming units (CFU) per gram). Sourdough was fermented for 24 h at 30 °C. At the end of fermentation, the average sourdough pH was  $3.8 \pm 0.1$ . Viable lactobacilli cells in the inoculum and sourdough were enumerated in anaerobic conditions at 37 °C (ISO 15214:1998).

Sourdough portions (205 g) were frozen in a blast freezer set at -30 °C until they reached the temperature of -18 °C (approximately 55 min). After 4, 8, or 12 weeks of storage at -18 °C, the sourdough was thawed overnight at +6 °C for consequent analyses and baking.

### Bread making procedure

To investigate the effect of frozen storage (4, 8, 12 weeks), the sourdough was used in bread making at the level of 20%. Also, breads were made with different amounts of sourdough (10, 20, or 30%) stored for 8 weeks (Table 1). The control contained 15% of barley flour, which corresponds to 20% sourdough bread. Salt, sugar, and ascorbic acid were added according to the ICC Standard 131 (1980). The formulations contained compressed baker's yeast (24 g) and malt powder (2.4 g). The water amount (63%) was sufficient to obtain farinograph (Brabender OHG, Duisburg, Germany) wheat (85%) and barley (15%) flour mixture consistency of 500 Brabender Units. All the ingredients were kneaded for 2 min slowly and 10 min quickly in a farinograph bowl. After 10 min of resting, the dough was divided into 90 g pieces and proofed at 35 °C and 75% relative humidity for 45 min (Wiesheu, Affalterbach, Germany). Bread was baked in a deck oven (Wiesheu, Affalterbach, Germany) at 230 °C for 15 min with initial steaming ( $1.9 \text{ dm}^3/\text{m}^3$ ).

**Table 1.** Flour, sourdough, and water content (g) in formulations

Ingredient	Control bread	Sourdough breads		
		10%	20%	30%
Wheat flour	696	748	696	643
Water	504	426	347	269
Barley sourdough	-	136	272	407
Barley flour	104	-	-	-

### Bread characteristics

Breads were cooled for 1 h at ambient conditions before analyses were conducted in at least four replicates. The volume of the weighted sample was measured using the rapeseed displacement method (AACC 10-05.01). Bread specific volume was calculated as volume to weight ratio.

Bread shape was calculated as the ratio of height to diameter, measured with a calliper.

Crumb firmness was measured with a Texture Analyser TA.HD (Stable Micro Systems, Surrey, UK) using a 25 mm aluminium probe (AACC 74-09.01).

Crust colour was evaluated by a colorimeter (Spectrophotometer CH-3500 D, Konica Minolta, Milton Keynes, UK) in the CIELab system (Commission Internationale DeL'Eclairage L-a-b space). The lightness  $L^*$ , redness  $a^*$ , and yellowness  $b^*$  were determined, and the browning index (BI) was calculated according to Maskan (2001)

$BI = [100 (X - 0.31)] / 0.17$ , where  $X$  is  $X = [a^* + 1.75 L^*] / [5.645 L^* + a^* - 3.012 b^*]$ .

Sensory attributes of breads containing 10, 20, or 30% of frozen sourdough (8 weeks) were evaluated by 40 consumers (29 females and 11 males, 23 - 51 years old) on randomly coded bread quarters, within 2 h after baking. Water was provided as a palate cleanser during evaluation. Bread crust appearance, colour, aroma, taste, mouth feel, overall impression, and liking were estimated using a seven-point hedonic scale, with 1, 4, and 7 representing extremely dislike, neither like nor dislike, and extremely like, respectively.

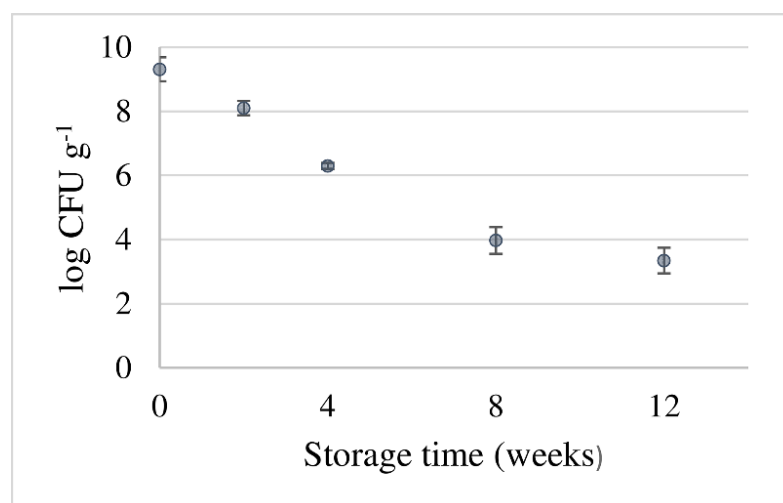
### Statistics

The results of sourdough characteristics during storage were subjected to one-way analysis of variance (ANOVA). The ANOVA, followed by the Tukey test and correlation analysis, identified the main effects of sourdough storage time and amount on dough consistency and the physical properties of bread. When estimating the effect of sourdough storage time, a control sample without sourdough was excluded from the analysis. Principal component analysis (PCA) was performed to identify the sensorial acceptability, shape, firmness, specific volume, and the browning index of breads differing in sourdough amount. Statistical analyses were performed at the significance level of  $p < 0.05$  with the Statistica 12 (StatSoft, OK, USA).

### Results and discussion

#### *Lactobacilli cell number during the frozen storage of sourdough*

The Lactobacilli cell number of the fresh sample (Fig. 1) was in agreement with previous reports for barley sourdoughs (Mariotti et al., 2014; Zannini et al., 2009), and *W. cibaria* wheat sourdough (Galle et al., 2012). Nonetheless, bacterial cell numbers significantly decreased during frozen storage ( $p = 0.002$ ) (Fig. 1). This is consistent with our previous work on sourdough in frozen storage for 4 weeks (Vrana Špoljarić et al., 2016). Still, Lattanzi et al. (2014) revealed that LAB can be partially reactivated from frozen sourdough that was stored for 90 days.



**Fig. 1.** The effect of sourdough frozen storage time on viable lactobacilli number

**Table 2.** Maximum dough consistency in the farinograph for the control and the samples differing in sourdough amount and storage time

Sourdough storage (weeks)	Sourdough amount (%)	Consistency (BU)
-	0	480 <sup>a</sup> ±6
0	20	420 <sup>b</sup> ±12
2	20	430 <sup>b</sup> ±9
4	20	365 <sup>d</sup> ±2
8	10	400 <sup>bc</sup> ±2
8	20	375 <sup>cd</sup> ±12
8	30	330 <sup>e</sup> ±0
12	20	370 <sup>cd</sup> ±26

<sup>a-e</sup> Means with a different letter significantly differ according to the Tukey test

### Dough consistency

The consistency of dough has an important role in its behaviour during proving and baking. The addition of water was kept constant in all formulations, and dough consistency was recorded during mixing. Dough consistency (Table 2) significantly decreased upon adding the sourdough. Moreover, it was dependent on both the sourdough amount and the storage time ( $p < 0.001$ ). It inversely correlated ( $p < 0.05$ ) with the sourdough amount ( $r = -0.91$ ) and sourdough storage ( $r = -0.71$ ). Our results agree with previous studies that found a significant decrease in dough strength and elasticity of *W. cibaria* fermented samples, in comparison to the unfermented control (Galle et al., 2012; Wolter et al., 2014).

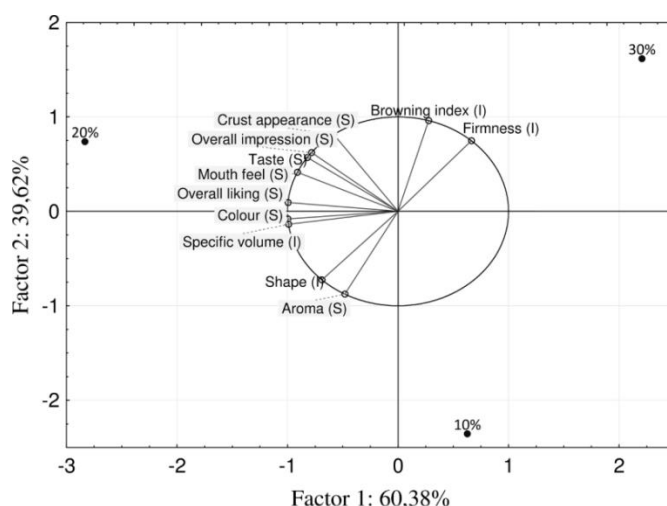
The freezing process *per se* did not influence dough consistency, since the consistency of dough with 20% of fresh sourdough or sourdough that was in frozen storage for 2 weeks were not significantly different. However, a significant drop in dough consistency occurred after adding the sourdough that was in frozen storage for 4 weeks. Also, a decrease in sourdough consistency during storage was observed visually. Dough syringing during cold storage is a phenomenon caused by the decrease in the water binding ability of the dough, due to freezing and subsequent thawing (Selomulyo and Zhou, 2007). Gys et al. (2003) showed that the loss of water holding capacity of the dough and the subsequent increase in dough syringing is caused by the degradation of arabinoxylans caused by endogenous xylanases. Bacterial xylanase that we added during sourdough fermentation to solubilise water insoluble arabinoxylans might be the main factor behind the observed reduction in consistency. Further research

on frozen storage conditions, e.g. temperature, should address the issue of syringing.

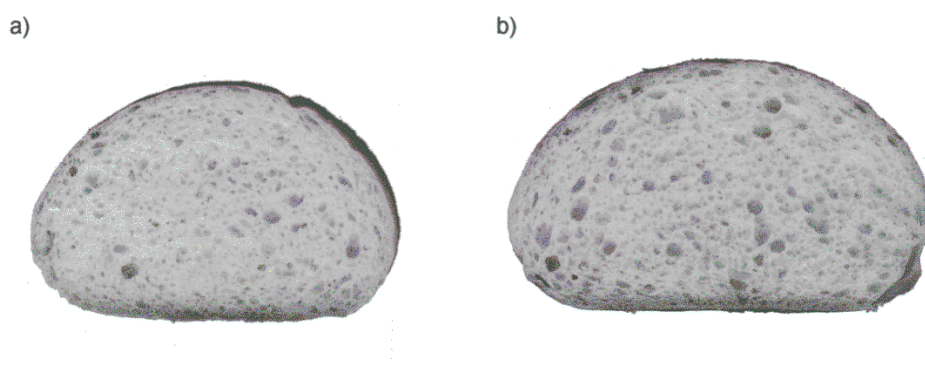
### The influence of sourdough amount on bread quality and liking

The PCA was performed for easier comparison of breads differing in the added sourdough amounts. Two principal components (PC), with *eigen* values 6.64 and 4.36, explained the total variability (Fig. 2). PC1, explaining 60.38% of the variance, was highly negatively correlated to liking of the bread colour, overall liking, specific volume, liking of bread texture, taste, and overall impression. PC2, explaining 39.62% of variance, was positively correlated to the browning index, crumb firmness, liking of crust appearance, while it was negatively correlated to shape and aroma liking.

Bread with of 20% of added sourdough was highly scored for crust appearance, colour, mouth feel, taste, and overall impression, and had a big specific volume. Thus, the overall liking was the highest for bread with 20% of sourdough, while bread with 30% of sourdough was the least liked, due to the highest browning index and crumb firmness. In accordance to our results, Torrieri et al. (2014) reported that the crust of bread with 30% of sourdough is darker than in 20% sourdough bread samples. In agreement with our results, Wolter et al. (2014) indicated the increased preference score for wheat bread with 20% of *W. cibaria* sourdough. Settani et al. (2013) highlighted the accountability of *W. cibaria* sourdough for the generation of volatile compounds (alcohols, esters, and diacetyl) in wheat bread. Due to the best properties, the 20% addition level (Fig. 3) was chosen in the following experiments on sourdough storage time. The physical properties of breads are discussed below.



**Fig. 2.** Bi-plot of bread characteristics determined for bread samples with different amount of added sourdough. The (S) denotes the characteristic determined by sensory analysis and the (I) the instrumentally determined characteristics

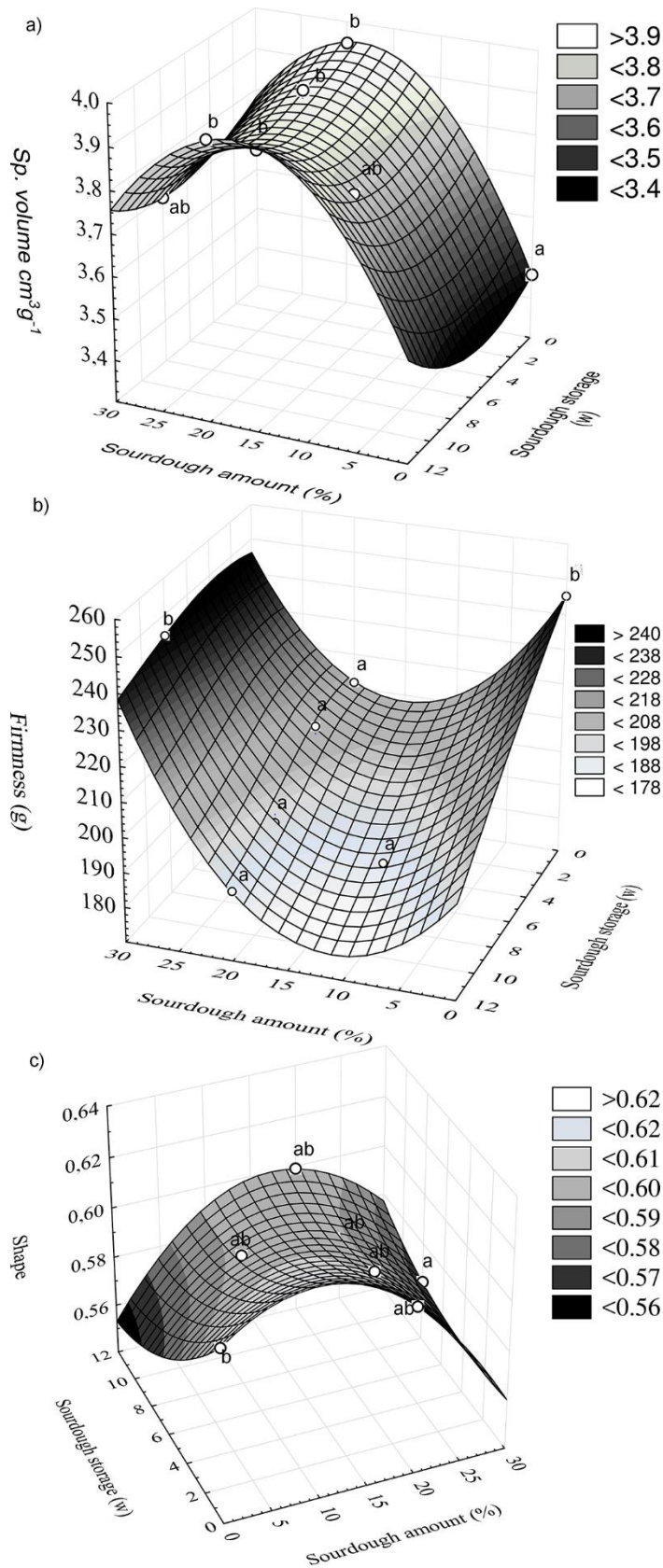


**Fig. 3.** Vertical section of a) control bread without sourdough, and b) bread with 20% of sourdough

### Physical properties of breads

Big bread volume is a crucially positive bread attribute (Wolter et al., 2014). Sourdough storage time did not affect bread specific volume, but the sourdough amount did ( $p=0.03$ ), though only breads with 20% of sourdough had significantly higher specific volume (14% on average) than the control (Fig. 4a). The improved specific volume after adding sourdough containing EPS was described in other studies; however, the reports on the extent of improvement are somewhat different. Galle et al. (2012) reported 35% higher specific volume than the control wheat bread with the incorporation of 20% of *W. cibaria* sourdough. The inconsistency between results could be attributed to the different content of EPS resulting from different conditions of sourdough fermentation: Authors used wheat flour and added 15% of sucrose. In this study, EPS content in dough was estimated to 0.2 g/kg (10% sourdough), 0.4 g/kg (20% sourdough), and 0.6 g/kg (30% sourdough), based on the previously determined 2.2 g/kg EPS in sourdough fermented under the same conditions and with the same flour

(not published). Opposite to our study, Wolter et al. (2014) found specific volume of wheat bread unaffected with the incorporation of 20% of *W. cibaria* sourdough, since no EPS synthesis was detected. Torrieri et al. (2014) observed that in the presence of EPS producing strains, bread with 30% of sourdough had higher volume than the samples with 20% of sourdough. Contrary, in our study the volume of bread with 30% of sourdough was slightly lower than in bread with 20% of sourdough, probably due to the interference of barley flour components in wheat gluten protein development (Dhingra and Jood, 2004; Trogh et al., 2004). We assume that the improved volume of the 20% sourdough bread was the result of the combined effect of EPS and soluble arabinoxylans that enhance a structure build up and interactions with the gluten network, as suggested by Tieking and Ganzle (2005) and Wang et al. (2004). Insoluble arabinoxylans suppress bread volume (Trogh et al., 2015), whereas soluble fibre retains more water and reduces the amount of water available for starch gelatinisation (Wang et al., 2004), resulting in better oven spring.



**Fig. 4.** Scatter plot with distance weighted least squares fitting for a) bread specific volume, b) crumb texture, and c) shape, depending on the sourdough amount (0-30%) and storage time (0-12 weeks). <sup>a,b</sup> Measured means with the different letter significantly differ

Further, xylanases are believed to act during the agglomeration of gluten during mixing (Wang et al., 2004). Several studies proved the benefits of using xylanase in different bread-making processes. Trogh et al. (2004) demonstrated that adding xylanase in the straight-dough process of making barley-enriched bread improves bread volume. Jacobs et al. (2008) showed that the addition of xylanase to the sponge-and-dough formula improves the loaf volume of barley fibre enriched bread. Recently, Ktenioudaki et al. (2015) significantly improved the specific volume of bread made using brewer's spent grain with the addition of xylanase, solely or in addition to sourdough. Since we added xylanase in sourdough fermentation, we believe that it mainly acted on the solubilisation of barley arabinoxylans, but its effect on gluten during mixing of bread dough is doubtful.

Consumers prefer breads with soft crumb. The decreasing trend of crumb firmness (Fig. 4b) with sourdough storage time was detected ( $p=0.04$ ). We assume that this could be related to the observed dough softening and acetic acid evaporation during sourdough storage. The lower amount of acetic acid in sourdough is related to a softer crumb (Novotni et al., 2013). Further, crumb firmness was significantly affected by the amount of sourdough ( $p<0.001$ ). Crumb firmness decreased upon adding 10% of sourdough (-23%), and 20% of sourdough (-18% on average) compared to the control. Similarly, Corona et al. (2016) reported a 29% decrease of crumb firmness using 25% of sourdough fermented with a mixture of *W. cibaria* strains in the traditionally produced semolina bread. Much greater reduction of crumb hardness in wheat breads containing 20% of *W. cibaria* sourdough was demonstrated by Wolter et al. (2014) (-122%) and Galle et al. (2012) (3-fold decrease), who also claimed a 50% decrease with a 10% addition of sourdough. Differences could be due to the harder crumb of their control wheat breads, different substrate for sourdough fermentation, and different methodology of measuring crumb texture. Settanni et al. (2013) associated the 50% decreased hardness of *W. cibaria* sourdough bread with dough acidification, which reduces its resistance to extension. The decrease in crumb firmness observed in our 10% and 20% sourdough breads may be partly attributed to xylanase, as suggested by several studies. The decreased firmness by using xylanase was found by Corsetti et al. (2000) in the presence of *L. hilgardii* in wheat bread, Jacobs et al. (2008) for the sponge-and-dough barley-fibre enriched formula, and by Ktenioudaki et al. (2015) in brewer's spent grain breads with or without sourdough.

The firmness value of our bread containing 30% of sourdough did not differ from the control without sourdough. Different from our results, Torrieri et al. (2014) demonstrated that EPS positive sourdough has a beneficial effect when added at 30% (softer bread), while it has a detrimental effect at 20%. They pointed out the dependence of crumb texture on the size, shape, and distribution of the crumb alveoli, which are related to the gluten network behaviour during the expansion of CO<sub>2</sub> in leavening and baking. The correlation between firmness and specific volume of our breads was weak ( $r=-0.741$ ;  $p=0.057$ ). Nevertheless, our bread with 20% of sourdough had the biggest volume and the softest crumb.

Bread shape (Fig. 4c) was not significantly influenced either by sourdough storage or the amount added; though, the ratio of height to diameter decreased upon adding 30% of sourdough. Bread shape can flatten due to gluten dilution with barley flour (Dhingra and Jood, 2004), but also due to proteolysis during sourdough fermentation, and the xylanase softening effect. Since flour in our formulation had a rather low resistance to extension ratio, the dough was not capable of keeping its shape during proving and baking. These factors contributed to the flattening of the shape of the bread with 30% of sourdough. Overall, as suggested by Settanni et al. (2013), a moderate amount of *W. cibaria* sourdough has a positive effect on the physical properties of bread.

## Conclusions

This study examined the effect of adding frozen stored *W. cibaria* barley sourdough on the quality parameters of wheat bread. The important finding is that the application of 20% of sourdough, regardless of its frozen storage time, improves bread volume. Sensory liking of barley-wheat bread was the highest when 20% of sourdough was applied. Thus, whole grain barley flour could become a convenient source of fibre in bread, and sourdough could be easily applied in industrial practice, where baker's yeast is used for leavening. Further studies should address the optimal conditions of sourdough fermentation and storage for an even greater improvement of bread quality and shelf-life.

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