IMPROVING THE BREAD QUALITY OF SUNI-BUG DAMAGED WHEAT FLOURS BY SOURDOUGH BREADMAKING AND LIQUID RYE SOUR

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The breadmaking quality of bug-damaged wheat flours with high protease activity (HPAWF) and low protease activity (LPAWF) was attempted to be improved by using sourdough (prepared by *L. plantarum* (SD1) and *L. sanfrancissensis* (SD2)) and liquid rye sour (LRS) in this study. The effects of sourdoughs (20 and 40%) and LRS (1 and 2%) on the protease activity of the HPAWF were determined by SDS-PAGE. Protease activity of HPAWF decreased with the addition of 40% SD1, 20% SD2, and both levels of LRS (1 and 2%) compared to a control sample. The HPAWF bread samples produced with LRS (1 and 2%) had higher volume (P<0.05) and bread quality as compared to sourdough applications. LPAWF bread sample was comparable with those of 40% SD2 added sample in terms of volume and hardness (N) values (P>0.05), while SD1 addition caused quality losses. The overall results suggested that addition of 2% LRS had promising results for improving bread quality flours that were damaged by suni-bug at low levels.

Keywords: high protease activity wheat flour, sourdough, liquid rye sour, bread quality

Some heteropterous insects (*Eurygaster* spp., *Aelia* spp.) called as suni-bug or wheat bug cause preharvest damage to wheat. They attack developing wheat kernels and leave their salivary secretions containing proteolytic enzymes in the grain. Damaged wheat results in a sticky dough and bread with low volume with poor crust and crumb (KARABABA & OZAN, 1998).

The bug protease is active over a broad pH range (pH 3.0-11.0) and has maximum activity at pH 8.5. The optimum temperature of this protease is $35 \,^{\circ}$ C (SIVRI & Köksel, 2000). Heat treatment at 70 °C for 2.0–3.0 min and hot tempering applications at 70 °C for 30 min improved the quality of suni-bug damaged wheats (D*iraman* & DEMIRCI, 1997). Some natural and synthetic inhibitors, chemicals (NaCI, CaCI₂, KH₂PO₄, organic acids), or additives (L-ascorbic acid, transglutaminase, glucose oxidase) in the bread formula resulted in a decrease in the proteolytic activity; and short-time fermentation and low temperature processing improved baking quality (DIRAMAN et al., 1998; SIVRI & KÖKSEL, 2002; OLANCA & SIVRI ÖZAY, 2015).

Sourdough bread making is an ancient method to improve bread quality and increase the shelf-life of bread (ARENDT et al., 2007). *L. sanfranciscensis*, *L. plantarum*, and *S. cerevisiae* are the most frequently isolated species from sourdough, and they are positively related to the sensory quality of sourdough breads (GOBBETTI et al., 2005). Liquid rye sour is a kind of

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additive used in the bakery industry in order to increase the acidity of the dough and bread with a specific light sour taste and pleasant aroma (DECOCK & CAPPELLE, 2005).

In this study, sourdough as an ancient technology and liquid rye sour were firstly used in bread formula in order to increase baking quality of bug-damaged wheat flours. Sourdoughs were prepared with *L. plantarum* (SD1) and *L. sanfranciscensis* (SD2), and liquid rye sour and proteolytic activity were monitored by SDS-PAGE. The applications having low protease activity were performed for high protease activity flour HPAWF in baking studies. In addition, effects of these applications were repeated for low protease activity flour (LPAWF).

1. Materials and methods

1.1. Materials

A sound (undamaged) wheat cultivar was obtained from Field Crops Central Research Institute, Ankara, Turkey, and a suni-bug damaged wheat cultivar was obtained from the Plant Protection Research Station, Diyarbakır, Turkey. Sound wheat and suni-bug damaged wheat samples were milled in a Buhler laboratory mill (MLU 202, Uzwil, Switzerland) to obtain straight-grade flour with the yields of 65% and 60%, respectively (Approved Method 26-21, AACC International 2000). High protease activity wheat flour (HPAWF) and low protease activity wheat flour (LPAWF) samples were prepared by blending of 50% sound wheat flour (SWF) + 50% suni-bug damaged flour and 75% SWF + 25% suni-bug damaged flour, respectively. *L. sanfranciscensis* (CIP 103252) and *L. plantarum* (CIP 102021) were purchased from the Pasteur Institute (France) and used for preparing the sourdough. Liquid rye sour (LRS) was donated by Ireks Food Industry (Turkey).

1.2. Flour analyses

American Association of Cereal Chemists (AACC) Standard Methods were used for determining moisture (No: 44-15A), ash (No: 08-01), protein (No:46-30), and wet gluten contents (No: 38-11), farinograph properties (No:54-21), Zeleny sedimentation (No: 56-60A), and falling number values (No: 56-81B) (AACC, 1990). The modified sedimentation value was used to determine suni-bug damage levels (KöKSEL et al., 2002). Alveoconsistograph (Chopin, France) and Mixolab (Chopin, France) analyses were also done according to AACC Method No: 54-30A and AACC 54-60.01, respectively (AACC, 2010). Each analysis was performed as duplicate.

1.3. Inoculum preparation

All strains were stored at -25 °C in de Man Rogosa Sharpe (MRS) broth (Merck) containing 30% (v/v) glycerol as a cryoprotectant. Prior to inoculation into the bread formulation, the strains were propagated twice in 10 ml of MRS broth and incubated at 30 °C for *L. sanfranciscensis* and 37 °C for *L. plantarum* overnight. The fresh starter culture inoculum was obtained by centrifugation (9 000 × g, 10 min, 4 °C) of the second overnight culture (10⁸ CFU ml⁻¹).

1.4. Sourdough preparation

Sixty grams of flour, 110 g of sterile deionised water, and 10 ml of bacterial suspension with the cell density of 10^8 CFU ml⁻¹ were mixed to obtain faster acidification with the dough yield (DY, dough weight × 100/ flour weight) of 300. Fermentation was carried out at 37 °C 24 h for *L. plantarum* (SD1) and 30 °C 24 h for *L. sanfranciscensis* (SD2). The pH of the sourdough was measured using a Hanna Instruments 221 (Romania) device.

1.5. Sample preparation for electrophoresis

High protease activity wheat flour (HPAWF) was prepared by blending of sound (undamaged) wheat flour (50%) and suni-bug damaged wheat flour (50%). Sourdough (20% and 40%) or LRS (1 and 2%) were added to the HPAWF (50 g) and the samples were mixed for the dough developing time as determined by a farinograph. The prepared doughs were separated into two pieces. One of the dough pieces was directly lyophilised. The other piece of dough was fermented at 30 °C and 85% humidity for 115 min before lyophilisation.

1.6. Measurement of proteolytic activity

Sodium dodecyl sulphate polyacrylamid gel electrophoresis (SDS-PAGE) was used to monitor the gluten hydrolysis according to the procedure described by NG and BUSHUK (1987). Glutenin was extracted from the lyophilised dough samples according to the method described by Fu and SAPIRSTEIN (1996).

1.7. Breadmaking procedure

AACC method (No 10-11) with some modifications according to a Turkish bread formulation was used in the baking experiments (AACC, 1990). In this formulation, 100 g flour (dry basis), 25 ml 8% yeast solution, and 25 ml 6% NaCl solution were mixed (Kitchen aid, USA) with additional water as determined by a farinograph. The flour was replaced by HPAWF or LPAWF.

1.8. Bread evaluation

The bread volume was measured by using the rapeseed displacement method. Texture analysis was carried out by using a Chatillon TAPlus machine (Lloyd Instruments, UK) with a 5 kg load cell by using a 35 mm diameter aluminium probe. The test speed was 55 mm min⁻¹. Each sample was sliced to 12.5 mm thickness, and for two slices the force (N) required to compress the samples to 25% was measured. Hardness value (N) was evaluated. Colour analysis was determined using a Minolta Spectrophotometer CM-3600 d (Japan) according to the CIE L*a*b* system.

1.9. Statistical analysis

Experimental procedure used in this study was summarised in Figure 1. The effect of applications on physical quality of bread samples (1 and 2% LRS, 40% SD1, 20% SD2 to HPAWF; 2% LRS, 40% SD1 and 40% SD2 to LPAWF) was statistically evaluated by one way analyses of variance (ANOVA) using SPPS statistical software version 16.0 (2007). Some applications showing no decreasing effect on protease activity were not used for baking experiment. Duncan's test was applied to compare the mean values P<0.05.

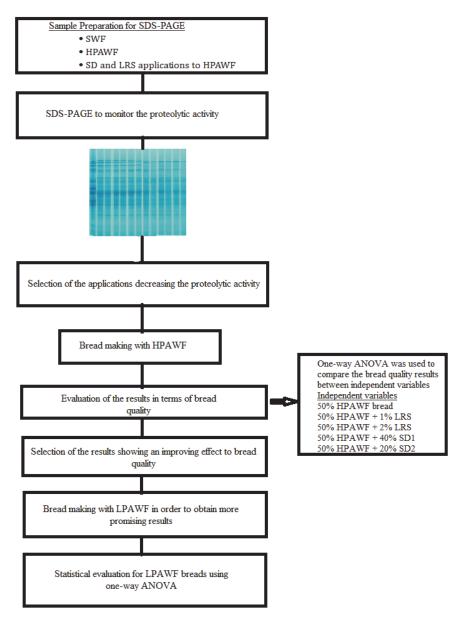


Fig. 1: Experimental procedure

2. Results and discussion

2.1. Chemical and physicochemical properties of flours

The difference between Zeleny sedimentation and modified sedimentation tests indicated that HPAWF had high protease activity (Table 1).

	Sound wheat flour (SWF)	Suni-bug damaged wheat flour		
Chemical and physicochemical properties				
Moisture (%)	12.8	11.4		
Ash content (%, db)	0.57	0.61		
Protein content*(%)	11.6	9.8		
Zeleny sedimentation value** (ml)	34	23		
Modified sedimentation value** (ml)	33	7		
Falling number*** (sec)	470	408		
Wet gluten content** (%)	32.7	Cannot be washed		
Farinograph properties				
Water absorption (%)	63.5	52.3		
Developing time (min)	2.0	1.2		
Stability (min)	1.5	1.1		
Number of kneading tolerance (BU)	50	250		
Degree of softening (BU)	100	300		
Mixolab properties				
Water absorption (%)	60.0	55.70		
Stability (min)	5.4	2.03		
C1(Nm)	1.1	1.14		
C2(Nm)	0.45	0.16		
C3(Nm)	1.94	1.30		
C4(Nm)	1.81	1.67		
C5(Nm)	2.45	2.56		
α (Nm/min)	-0.060	-0.040		
B (Nm/min)	0.476	0.124		
γ (Nm/min)	0.008	0.018		

db: dry basis

*: N×5.7; **: based on 14% moisture; ***: based on 15% moisture

C1: (1st stage, max consistency, T=30 °C) developing time, water absorption; C2: (2nd stage, max consistency, T=60 °C) protein attenuation; C3: (3rd stage, max consistency, =90 °C) starch gelatinisation; C4: (4th stage, max consistency, T=90 °C) amylase activity; C5: (5th stage, max consistency, T=50 °C) retrogradation; α , β , γ : the resistance of the dough to the kneading at 2nd, 3rd, and 4th stages

2.2. Rheological properties of flours

As expected, SWF has better rheological properties than HPAWF due to having higher water absorption, developing time, stability, lower number of kneading tolerance and degree of softening (Table 1).

C1–C5 and α , β , γ are the parameters of mixolab. C1 and C2 are related to protein quality, whereas C3, C4, and C5 are related to the starch characteristics. Slopes α , β , and γ are the indicators of protein weakening, starching speed, and enzymatic degradation. C1 represents the maximum point of the first mixing stage at 30 °C. The water absorption value of SWF and HPAWF to reach the C1 value are 60.0% and 55.7%, respectively, whereas the stability values are 5.4 and 2.0 min, respectively. At the second stage of mixolab, the dough is heated to 60 °C. Both increasing the dough temperature and kneading decrease the

consistency of the dough to C2. This decrease is related to protein weakening, and a higher difference between C1 and C2 is a sign of poor-quality flour (KOKSEL et al., 2009). Higher differences between C1 and C2 in HPAWF than SWF confirmed that HPAWF had low protein quality (Table 1).

2.3. pH values of sourdough and liquid rye sour

Incubation conditions were 24 h at 37 °C for SD1 (*L. plantarum*) and 24 h at 30 °C for SD2 (*L. sanfranciscensis*). The pH of Sourdough 1 was between 3.4-3.7, while the pH of Sourdough 2 was between 4.0-4.5. The pH of liquid rye sour was also 2.8-3.2.

2.4. Effect of sourdough prepared by L. plantarum on electrophoretic patterns of glutenins

Disappearance or decreasing protein band intensities of the SDS PAGE indicate proteolytic activity. The addition of sourdough prepared by *L. plantarum* at 20% and 40% resulted in no change on the glutenin patterns of sound wheat flour (Fig. 2, lines 5 and 7). It is suggested that *L. plantarum* had no proteolytic activity on glutenins as expected (WEHRLE et al., 1999).

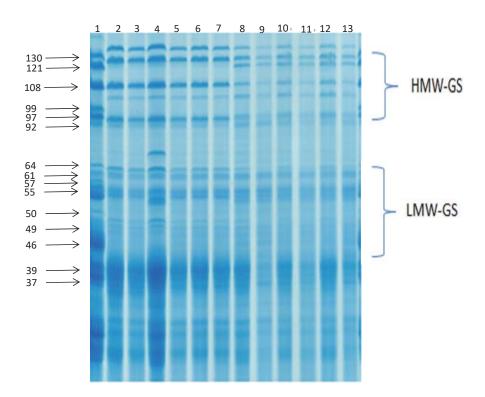


Fig. 2. Effect of SD1 on glutenin bands of SWF and HPAWF

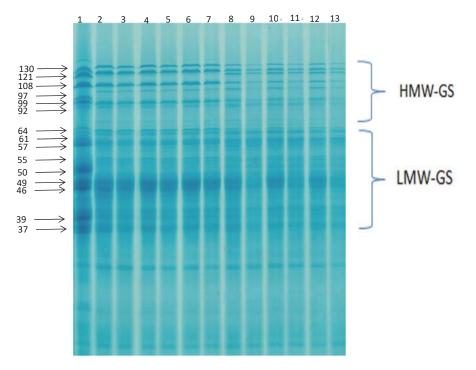
SWF: Sound wheat flour (SWF); HPAWF: high protease activity wheat flour (50% SWF+50% suni-bug damaged flour);

1: Katepwa; 2: SWF; 3: SWF after fermentation, 4: SWF +20% SD1; 5: SWF +20% SD1 after fermentation, 6: 40% SD1; 7: 40% SD1 after fermentation; 8: HPAWF; 9: HPAWF after fermentation; 10: 20% SD1 + HPAWF; 11: 20% SD1 + HPAWF after fermentation, 12: 40% SD1 + HPAWF; 13: 40% SD1 + HPAWF after fermentation

Decreasing the intensities of glutenin bands of HPAWF after 2 hours of fermentation indicated proteolytic activity (Fig. 2, lines 8 and 9). No change was observed in the intensities of glutenin bands for 20% sourdough addition, while they increased with the addition of 40% sourdough (Fig. 2, lines 9 and 13). These results demonstrated that 40% sourdough (*L. plantarum*) addition inhibited suni-bug protease.

2.5. Effect of sourdough prepared from L. sanfranciscensis on electrophoretic patterns of glutenins

Sourdough prepared with *L. sanfranciscensis* had no hydrolysing effect on glutenin bands of sound wheat flour after fermentation (Fig. 3, line 3, 5 and 7) (DI CAGNO et al. 2002, VERMEULEN et al. 2005). SDS-PAGE results of SD2 (*L. sanfranciscensis*) were similar to those of SD1 (*L. plantarum*) to some extent.





SWF: sound wheat flour (SWF); HPAWF: high protease activity wheat flour (50% SWF+50% suni-bug damaged flour);

1: Katepwa; 2: SWF; 3: SWF after fermentation; 4: 20% SD2 + HPAWF; 5: 20% SD2 + HPAWF after fermentation; 6: 40% SD2 + HPAWF; 7: 40% SD2 + HPAWF after fermentation; 8: HPAWF; 9: HPAWF after fermentation; 10: 20% SD2 + HPAWF; 11: 20% SD2 + HPAWF after fermentation; 12: 40% SD2 + HPAWF; 13: 40% SD2 + HPAWF after fermentation.

Sourdough addition at the level of 20% to HPAWF slightly increased the intensities of the glutenin bands, suggesting that proteolytic activity has been inactivated (Fig. 2, lines 9 and 11). No proteolytic activity was detected for 40% SD2 addition to HPAWF (Fig. 2, lines 9 and 13).

2.6. Effect of liquid rye sour on electrophoretic patterns of glutenins

There was no effect of 1 and 2% liquid rye sour (LRS) addition to sound wheat flour (control) on glutenin patterns. However, the proteolytic activity of HPAWF was inhibited by LRS addition at both levels (Fig. 4, lines 9, 11, and 13).

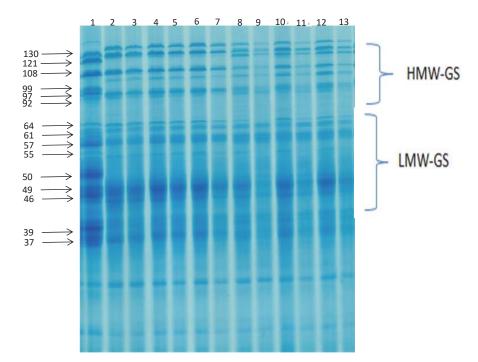


Fig. 4. Effect of LRS on glutenin bands of SWF and 50% HPAWF

SWF: sound wheat flour (SWF); HPAWF: high protease activity wheat flour (50% SWF+50% suni-bug damaged flour)

1: Katepwa; 2: SWF; 3: SWF after fermentation; 4: 1% LRS + SWF; 5: 1% LRS + SWF after fermentation; 6: 2% LRS + SWF; 7: 2% LRS + SWF after fermentation; 8: HPAWF; 9: HPAWF after fermentation; 10: 1% LRS + HPAWF; 11: 1% LRS + HPAWF after fermentation; 12: 2% LRS + HPAWF; 13: 2% LRS + HPAWF after fermentation

2.7. Effect of sourdough and liquid rye sour on bread quality

According to the SDS-PAGE results, the applications resulting low protease activity (40% SD1, 20% SD2, and 1 and 2% of LRS) were used in the baking experiments of both HPAWF and LPAWF.

Effects of addition of SD1 (40%), LRS (2%), and SD2 (20%) on volume, hardness, crumb and crust colour are shown in Table 2A. As expected, the volume of the HPAWF (317 cm³) was lower than that of control (432 cm³) (Table 2A). LRS addition (2%) increased bread volume significantly (P<0.05) (Table 2 and Fig. 5), providing higher bread volume than the other treatments (1% LRS, 40% SD1, and 20% SD2). No significant effect on volume and hardness values was observed with the addition of 40% SD1 and 20% SD2, but the bread with an additional 2% LRS was comparable with the control sample (SWF) (Table 2A).

Table 2. Quality characteristics of HPAWF and LPAWF breads ¹									
SAMPLE				Crust colour ²		Crumb colour ²			
	Volume ² (cm ³)	Specific volume ² (cm ³ g ⁻¹)	Hardness ² (N)	L*	<i>a*</i>	L*	<i>b</i> *		
A)									
SWF	432 ^a	3.21 ^a	4.55 ^c	53.00 ^d	13.01 ^a	61.11 ^{cd}	14.16 ^a		
HPAWF	317 ^d	2.45 ^{cd}	7.37 ^b	46.42 ^e	7.21 ^c	50.71^{f}	13.72 ^a		
HPAWF+ 1% LRS	351 ^c	2.67 ^c	7.38 ^b	58.66 ^{ab}	10.61 ^b	65.68 ^b	14.50 ^a		
HPAWF+ 2% LRS	388 ^b	2.95 ^b	6.81 ^b	62.48 ^a	10.08 ^b	70.93 ^a	14.50 ^a		
HPAWF+ 40% SD1	301 ^d	2.32 ^d	8.33 ^a	61.76 ^a	8.01 ^c	57.19 ^{de}	13.92 ^a		
HPAWF+ 20% SD2	307 ^d	2.39 ^d	8.87 ^a	58.75 ^{ab}	7.53 ^c	63.6 ^{bc}	13.62 ^a		
B)									
SWF	432 ^b	3.21 ^b	4.55 ^c	53.00 ^a	13.01 ^a	61.11 ^a	14.16 ^a		
LPAWF	393°	2.97 ^c	6.41 ^b	51.29 ^a	10.13 ^b	57.04 ^{ab}	13.98 ^a		
LPAWF+ 2% LRS	464 ^a	3.54 ^a	2.84 ^d	56.20 ^a	13.72 ^a	61.17 ^a	13.06 ^a		
LPAWF+ 40% SD1	357 ^d	2.72 ^d	8.72 ^a	52.57 ^a	10.74 ^b	59.32 ^{ab}	13.79 ^a		
LPAWF+ 40% SD2	394 ^c	2.97 ^c	6.56 ^b	51.83 ^a	10.42 ^b	54.93 ^b	13.67 ^a		

¹: Mean values followed by different letters are significantly different at P<0.05; ²: all values are average of two parellel breads; SWF: sound wheat flour; HPAWF: high protease activity wheat flour (50% SWF+50% suni-bug damaged flour); LPAWF: Low protease activity wheat flour (75% SWF+25% suni-bug damaged flour); LRS: liquid rye sour; SD1: sourdough prepared with *L. plantarum;* SD2: sourdough prepared with *L. sanfrancissensis*

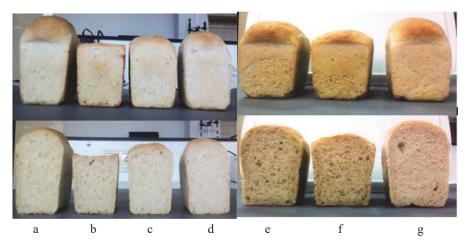


Fig. 5. External appearance and crumb structure of LRS addition to HPAWF and LPAWF breads
LRS: liquid rye sour; HPAWF: high protease activity wheat flour; LPAWF : low protease activity wheat flour a: control; b: HPAWF bread; c: HPAWF + 1% LRS bread; d: HPAWF + 2% LRS bread;
e: control; f: LPAWF bread; g: LPAWF + 2% LRS bread

LRS (2%) and SD1 (40%) additions caused increase in the L^* (Lightness) values, indicating brighter crust colour (Table 2A). a^* values of bread were lower compared to control bread. LRS addition caused significant increase in a^* values, but addition levels

(1 and 2%) had no significant effect. Other additions (40% SD1 and 20% SD2) did not affect a^* values of the bread crust (P<0.05) (Table 2A).

The L^* value of the crumb also increased with the addition of LRS and SD. Higher L^* values of the crumb were observed with increasing LRS levels (P<0.05). Highest redness (a^*) value of the crumb was measured for 40% SD1 addition, and there was no significant change in yellowness (b^*) values for any samples (P<0.05) (Table 2A).

SIVRI and KÖKSEL (2002) reported that bread additives such as ascorbic acid, DATEM and vital gluten had an improving effect on bread quality made from bug-damaged flour. However, these additives had no effect on the bread quality when the wheat was damaged at high levels. To confirm this, SD (40%) and LRS (2%) were also tried for LPAWF (Table 2B). LRS addition at the 2% level gave better results in terms of bread characteristics (Fig. 5). As compared to the control sample, higher bread volume and lower hardness values were obtained (Table 2B). L* values of the crust were comparable. Addition of 2% LRS resulted in the highest a^* value of the crust and L^* value of the crumb (Table 2B). It is well known that sourdough bread making has a positive effect on texture, shelf life, and aroma of breads, but there are also some related health issues such as gluten-free bread making (MORONI et al., 2009; TORRIERI et al., 2014). In this study, although 40% SD1 and 20% SD2 sourdough addition to HPAWF resulted in lower protease activity as expected, using sourdoughs had no detectable effect on bread quality. The effects of liquid rye sour were more obvious on bread quality for HPAWF. It can be concluded that addition of lactic acid bacteria and liquid rye sour could be a tool to improve the bread quality of suni-bug damaged wheat flours at low damage levels.

3. Conclusions

Bug damage to wheat causes serious economical losses in some countries. Many modifications were tried in the breadmaking process to overcome this problem. Decreasing the pH of the dough could be one of them, because the activity of bug protease at acidic pH is relatively low (SIVRI & KÖKSEL, 2002). This is the first report on the utilisation of sourdough, an ancient technology, in breadmaking with bug damaged wheat flour. Although SDS PAGE results showed that proteolytic activity of HPAWF decreased by addition of sourdough, no positive effect of sourdough addition was observed on the bread properties, especially volume and texture. On the other hand, liquid rye sour application had positive effect on breadmaking quality of HPAWF. It can be concluded that lactic acid bacteria and liquid rye sour can be used to improve the bread quality of suni-bug affected flours at low damage levels.

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