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INFLUENCE OF RAISIN STARTER SYRUP CONCENTRATIONS ON THE PROPERTIES OF SOURDOUGH AND SOURDOUGH BREAD

Jean-Yu Hwang¹ and Yung-Shin Shyu²

Key words: raisin, sourdough, toast, total titratable acidity (TTA).

ABSTRACT

The present study evaluates the effects of syrup concentrations of natural raisin microflora culture on the characteristics of sourdough and sourdough bread. The original total titratable acidity (TTA) of the starter solutions were between 10.30 and 10.45 mL, after 96 hours of incubation, the TTA of the starter solution increased to 11.30-11.85 mL. The greatest pH decrease and TTA increase occurred in the RS50 group. The microstructure of the control bread was rougher and contained more ungelatinized starch particles than bread made with sourdough. The overall acceptability and flavor of bread made using sourdough with the 150 g/L syrup solution was found to be significantly better than the control bread made using commercial yeast. The experimental results show that using a 150 g/L syrup solution and raisins as a natural starter culture helps to produce better quality bread.

I. INTRODUCTION

The production of bread can be traced back to 4,000 years B.C. Ancient Egyptians employed natural sourdough as a starter for bread production. Natural microflora of starters on fruits, flowers, leaves or cereals play an important role in bread fermentation. The raw materials of these microflora contain lactic acid bacteria (LAB), yeast, acetic bacteria, other bacteria and fungi. The industrialization of the baking process for French bread using natural sourdough was developed in the San Francisco Bay area (Simonson et al., 2003). Recently, due to the increasing consumer demand for reducing the use of additives that can decrease the healthy attributes of food has led to greater interest in natural and consumer-friendly baking

technologies (Moore et al., 2007).

Lactobacillus sanfranciscensis and *Saccharomyces exiguus* are the main cultures in San Francisco sourdough French bread. *L. sanfranciscensis* can produce large amounts of lactic acids, acetic acids, esters and alcohols. It has higher souring activity and aroma formation ability compared to other sourdough yeasts (Gobbetti and Corsetti, 1997). Acid production during sourdough fermentation increases the activities of enzymes, such as amylases, proteases (Moroni et al., 2009) and decreases lipase activity (Rieder et al., 2012). Rieder et al. (2012) reported that the lipase activity of sourdough fermented wheat germ (SFWG) is markedly lower than that found in raw wheat germ. As shown by SPME/GC/MS analysis, a very low level of volatile compounds derived from lipid oxidation was found in freeze dried sourdough fermented wheat germ during 40 days of storage (Rieder et al., 2012). Sourdough fermentation can also partially inhibit endogenous lipase activity and increase the shelf-life of wheat germ. Moreover, Rizzello et al. (2010) showed that SFWG possesses a marked antifungal activity which may extend the microbial shelf-life of leavened baked goods. No antifungal activity has been found for raw wheat germ (RWG), thus suggesting that this inhibitory activity is strictly related to sourdough fermentation since the effectiveness of many antifungal compounds (especially organic acids) have been shown to be pH dependent (Sugihara et al., 1970).

The use of sourdough improves the quality of breads made with whole grain barley flour, has a positive effect on bread volume, improves the texture, facilitates superior sensory quality and increases the shelf life of gluten free (GF) bread (Lavermicocca et al., 2000; Sangnark and Noomhorm, 2004; De Vuyst and Neysens, 2005; Schober et al., 2007). Rieder et al. (2012) found no effect or a small decrease of bread volume with the use of sourdough compared to other composite wheat breads. Crowley et al. (2002) reported that adding different amounts of sourdough, fermentation temperature, fermentation time and the firmness of optimized sourdough also affect the softness of breads (Flander et al., 2011).

It has been shown that sourdough fermentation can also modulate nutritional properties, such as increasing the level of various bioactive compounds, retarding starch bioavailability

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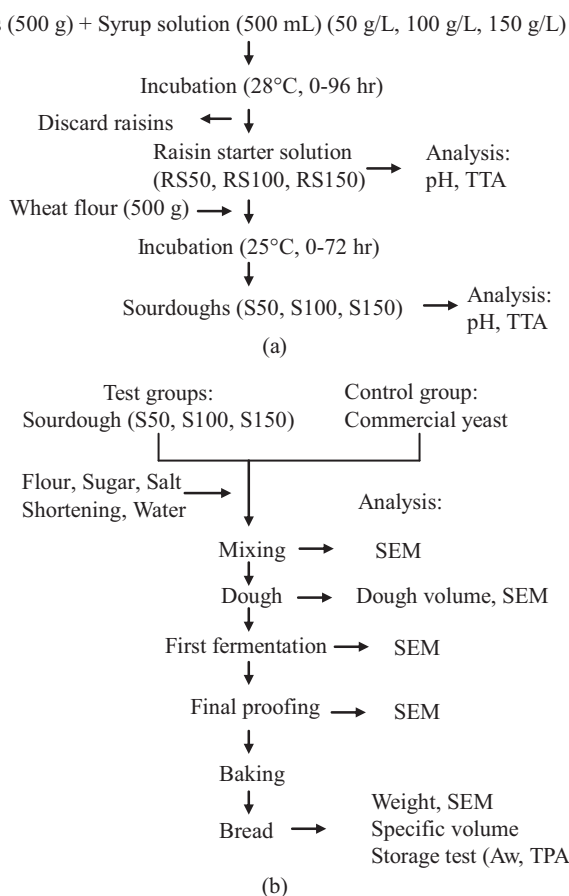


Fig. 1. Scheme for processing and analyzing the (a) sourdough and (b) bread.

and thus decreasing the glycemic index (Katina et al., 2005; Rizzello et al., 2011). The partial replacement of white wheat flour with 4% SFWG following a traditional bread formula led to improvement in the concentration of free amino acids. Phytase and antioxidant activities have also been shown to be higher in bread containing SFWG compared to traditional wheat flour bread (Rizzello et al., 2010). Rieder et al. (2012) reported that the chemical and microbial changes in sourdough depend on the flour type, amount of water, temperature, time, and type and amount of starter.

To the best of our knowledge, no published works have studied the effects of different starter syrup concentrations and natural microflora starter cultured from raisins. The objectives of this study are to examine the effects of different syrup concentrations and how they influence the properties of starter solution and sourdough, dough properties during fermentation, and hence the properties of the final product, as compared with commercial yeasts.

II. MATERIALS AND METHODS

1. Materials

Wheat flour was purchased from Uni-President Enterprises

Corp., Tainan, Taiwan. Shortening used in this study was obtained from Namchow Chemical Industrial Co. Ltd., Taoyuan, Taiwan. Instant dry yeast was produced by Yung Cheng Industries Ltd., Taipei, Taiwan. Raisins (Lion Raisins, Selma CA, USA) were purchased from a local grocery store.

2. Generation of Starter Solutions and Sourdoughs

Raisins (500 g) and various concentrations of sugar solutions (500 mL) (50 g/L, 100 g/L and 150 g/L) were incubated at 28°C for 96 hr, and then discarded. Five hundred mL of starter solution was mixed with 500 g of wheat flour and fermented at 25°C for 72 hr to obtain the sourdoughs. (Fig. 1(a))

3. Baking Formula of the Bread and Sourdough Breads

The baking formula of the control group (2,500 g), based on baker's percentage, was as follows: 100% flour (1,400 g), 8% sugar (112 g), 1.5% salt (21 g), 4% shortening (56 g), 64% water (896 g), and 1% dry yeast (14 g). The baking formula of the sourdough group (2,500 g) was as follows: sourdough (500 g), flour (1,160 g), sugar (112 g), salt (21 g), shortening (56 g), and water (651 g). All the ingredients except the shortening were combined and mixed at a moderate speed for 8 min into a dough shape. Then the shortening was added and mixed for a further 12 min to obtain optimum development. The dough was molded and fermented in a fermentation room (26°C, 75% R.H.) for complete fermentation (first fermentation). The dough was then divided into 450 g pieces, which was set aside for 10 min before molding. The molded dough was fermented in a final proofing room for a further 50 min at a temperature of 38°C and relative humidity of 85% R.H. (final proofing) and baked at 180°C. (Fig. 1(b))

4. pH Measurement

A combination electrode, standardized between pH 4.0 and 7.0 and attached to a pH/ion analyzer (Corning, Corning NY, USA), was inserted into the starter solutions to measure the pH each day.

5. Total Titratable Acidity Measurement

Total titratable acidity (TTA) was determined by 10 mL of starter solution with 90 mL of distilled water and expressed as the amount (mL) of 0.1 M NaOH to reach a pH of 8.3.

6. Dough Expansion Measurement

For the dough expansion test, we followed the method reported in Sangnark and Noomhorm (2003), with a slight modification. The dough was divided into 50 g pieces and rounded after mixing. Dough samples were inserted into a 250 mL graduated cylinder. The cylinders were placed in a cabinet at a temperature of 28°C and relative humidity of 75% for 330 min. Dough volume was recorded every 30 min.

7. Scanning Electron Microscopy (SEM) Measurement

Dough and bread samples were frozen at -50°C for 24 hr following the method reported in Kim et al. (2003) The frozen

Table 1. The pH and TTA of the raisin starter solution.

Samples		Incubation time					
		0 hr	24 hr	48 hr	72 hr	96 hr	
Raisin starter solution							
pH	RS50	4.03 ^a	3.89 ^b	3.83 ^b	3.78 ^c	3.75 ^c	
	RS100	4.03 ^a	3.91 ^b	3.83 ^b	3.81 ^b	3.79 ^b	
	RS150	4.04 ^a	4.06 ^a	3.96 ^a	3.93 ^a	3.86 ^a	
TTA (mL)	RS50	10.45 ^a	11.15 ^a	11.45 ^a	11.70 ^a	11.85 ^a	
	RS100	10.45 ^a	11.05 ^a	11.45 ^a	11.55 ^a	11.75 ^a	
	RS150	10.30 ^a	10.20 ^b	10.80 ^b	11.00 ^b	11.30 ^b	
Sourdough							
pH	S50	4.40 ^c	4.54 ^c	4.92 ^c	4.83 ^c		
	S100	4.46 ^b	4.58 ^b	4.98 ^b	4.90 ^b		
	S150	4.59 ^a	4.66 ^a	5.03 ^a	4.95 ^a		
TTA (mL)	S50	8.60 ^a	7.90 ^a	6.00 ^a	6.50 ^a		
	S100	8.30 ^b	7.70 ^a	5.70 ^a	6.10 ^b		
	S150	7.65 ^c	7.30 ^b	5.35 ^b	5.69 ^c		

¹ Values (n = 3) in the same column followed by a different letter are significantly different ($p < 0.05$).

² RS50, RS100, RS150: Raisin starter solutions made with 50 g/L, 100 g/L, 150 g/L syrup conc., respectively.

³ S50, S100, S150: Sourdough made with 50 g/L, 100 g/L, 150 g/L syrup conc., respectively.

samples were cut into $0.8 \times 0.8 \times 0.3$ cm³ pieces with a razor blade, and vacuum freeze-dried (FDU-2100; EYELA, Tokyo, Japan) for 48 hr. Each freeze-dried sample was mounted onto a brass stub using double-sided carbon conductive adhesive tape. A gold coat (20 nm) was applied using a sputter coater (no. 30800; Ladd Research, Williston VT, USA). Samples were examined at 20 kV using a Hitachi S-2500 scanning electron microscope (Tokyo, Japan).

8. Loaf Volume Measurement

Loaf volume was measured after baking by a rapeseed displacement method. Specific volume was calculated as loaf volume (cm³ loaf weight per g) (Sangnark and Noomhorm, 2004).

9. Water Activity Measurement

Five grams of the sample was placed in a water activity meter (Novasina, Lachen, Switzerland) and equilibrated for 10 min.

10. Texture Profile Analysis (TPA) Measurement

The pieces of bread and sourdough bread were sliced into $2.5 \times 2.5 \times 2.5$ cm³ crumbs using a standard bread slicer. The hardness and springiness of the pieces of bread were tested with a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) and a No. P/0.5S 0.5" diameter cylinder probe, according to the methods reported in Rizzello et al. (2010) and Wang and Sun (2002). The probe was allowed to place 10 mm (strain 40%) of pressure on at the crumbs in a 2-cycle test. Texture profile analysis (TPA) was conducted at a test speed of 10 mm/min. The pieces of bread were sealed in polyethylene (PE) bags after cooling and kept at room temperature (25°C) for further testing. The bread was sliced into

crumbs and hardness was recorded every day for 7 days.

11. Sensory Evaluation

The sourdough bread and control bread were served to 50 panelists for evaluation of overall scores. Fifty male and female students between the ages of 18 and 22 were participants on the panel. Panelists were instructed to evaluate each attribute using a 9-point hedonic scale, ranging from "extreme dissatisfaction" = 1 to "extreme satisfaction" = 9. A 1-to-9 hedonic scale was used for evaluating the intensity of appearance, flavor, texture, mouthfeel, and crust and crumb uniformity, together with an overall acceptability test. The pieces of bread were coded with three digits, and this information was supplied to the panelists. Each data point from sensory analysis represents an average of 50 panelists.

12. Statistical Analysis

A completely randomized block design was used, with three replications per treatment and three sub-samples per replication. Data were analyzed by analysis of variance using the SAS statistical software package. Duncan's multiple range test was used to identify differences between treatments at a significance level of $P < 0.05$.

III. RESULTS AND DISCUSSION

1. Acidification Properties of Raisin Starter Solution and Sourdough

In this study, raisins were incubated at syrup concentrations of 50 g/L, 100 g/L and 150 g/L for 0-96 hr to obtain the raisin starter solutions. The original pH of the three starter solutions were between pH 4.03 and 4.04 (Table 1), with increased in

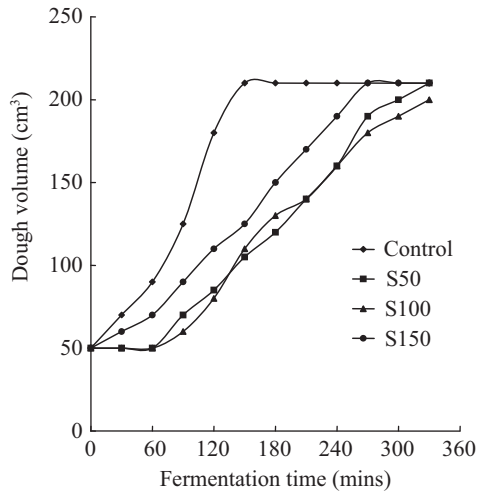


Fig. 2. Fermentation time versus dough volume of raisin starters at different syrup concentrations.

cupation time, the decreased pH value. After 96 hours of incubation, the pH of the starter solution decreased to 3.75-3.86.

The original TTAs of the three starter solutions were between 10.30 and 10.45 mL (Table 1). After 96 hours of incubation, the TTAs of the starter solutions increased to 11.30-11.85 mL. These results show that the greatest pH decrease and TTA increase occurred in the RS50 group. The final pH decreases and TTA increases of the solutions were the inverse of the levels of syrup concentration.

The association between *L. sanfranciscensis* and *S. exiguus* might not be stable when the pH drops below 3.8. Although *S. exiguus* yeast might obtain maltose at this pH, it is out the optimal growth pH range of yeast (pH 4-6) (De Vuyst and Neysens, 2005).

Raisin starter solutions were combined with wheat flour for 0-72 hr incubation to obtain sourdoughs. Our results show that the original pHs of the three sourdoughs were between pH 4.40 and 4.59. These values highly correlate with the final pHs of raisin starter solutions. During the incubation periods, the pHs of the three sourdoughs firstly increased and then decreased, and the TTAs of the sourdoughs firstly decreased and then increased.

2. Dough Volume of Fermentation

This study obtained a faster dough expansion rate from the control group. The dough volume of the control dough increased from 50 to 210 cm³ in the first 150 min (Fig. 2). However, the corresponding rate of increase in dough volume for the sourdough groups was significantly slower than that of the control. For example, the S150 group required 270 min to expand to 210 cm³ from 50 cm³. The S50 and S100 groups had the slowest fermentation rates.

3. SEM of Dough and Bread

Scanning electron micrographs (SEM) of various types of dough after mixing revealed granules of wheat starch (arrow)

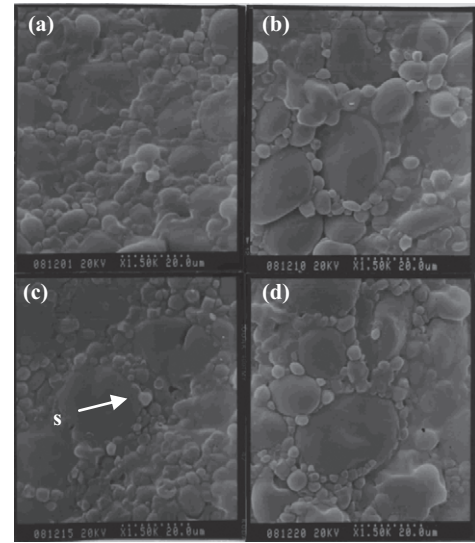


Fig. 3. Scanning electron micrographs of doughs after mixing. ((a): Control, (b): S50, (c): S100, (d): S150. 1500×)

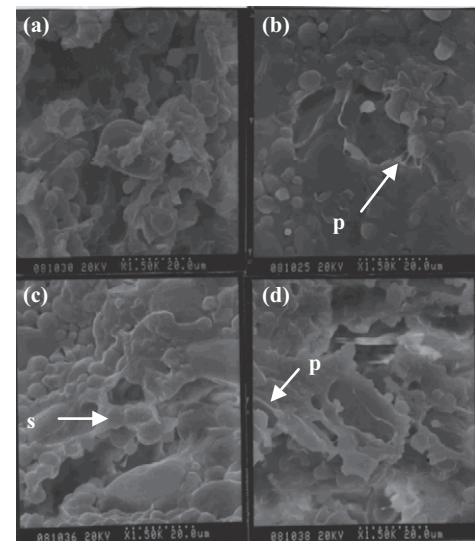


Fig. 4. Scanning electron micrographs of doughs after first fermentation. ((a): Control, (b): S50, (c): S100, (d): S150. 1500×)

uniformly dispersed in the gluten matrix (Fig. 3). All of the dough mixtures readily achieved homogeneity and adequate development of gluten into a structure with the necessary physical characteristics of pliability, elasticity and extensibility.

Fig. 4 shows SEM of various pieces of dough following fermentation. The unique film-forming and gas-retaining properties of wheat starch granules and proteins contribute to the formation of gas cells, which enlarge as fermentation proceeds (Fig. 4(a)). The formation of gas cells is less obvious in Fig. 4(b), indicating that gas production during fermentation in the S50 sourdough group was not as effective as in the other groups. Tiny bubbles of air and starch granules are clearly visible on the surface of the gluten matrix (Fig. 4(c)). The

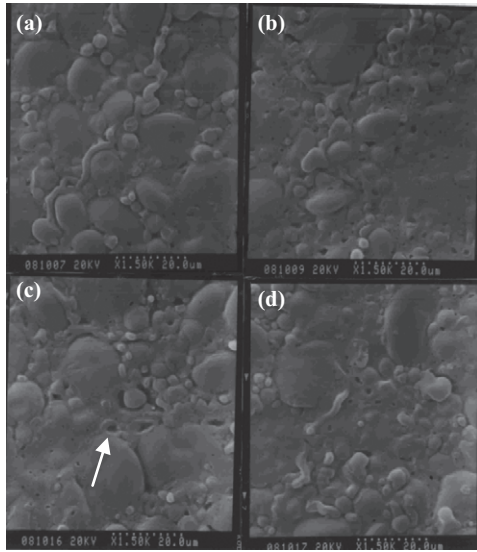


Fig. 5. Scanning electron micrographs of doughs after final proofing. ((a): Control, (b): S50, (c): S100, (d): S150. 1500 \times)

unique film-forming and enlarged gas cell-retaining properties of wheat protein are easily seen in the SEM in Fig. 4(d). A previous report proposed that the elastic protein network of denatured gluten and starch polymers forms a continuous phase. Clearly distinguished starch granules embedded in the continuous phase lead to a discontinuous phase (Gray and Bemiller, 2003).

SEM of dough after the final proofing are shown in Fig. 5. Compared to Fig. 3, the granules of wheat starches (Fig. 5) are swollen and the surface of the gluten matrix has become much smoother compared with the dough after mixing (Fig. 3). There are fewer pores on the surface of the control subsequent to the final proofing (Fig. 5(a)). Small starch granules and a protein matrix also cluster to form a tube-like structure and surround the larger starch granules. Large pores (arrow) are present on the surface of the sourdough after the final proofing (Fig. 5(b), (c), (d)); the surface is also shown to be smoother than that of the control dough following the final proofing (Fig. 5(a)). Most of the small starch granules are present as clusters on the surface of the large starch granules, and they are rather difficult to distinguish due to their spherical shape (Fig. 5(b), (c), (d)). It takes longer for natural microflora to ferment during the final proofing, thus, the starch granules swell more, leaving more pores on the dough surface after the final proofing. The microstructure of the control crumbs shows that the surfaces are rough, and ungelatinized starch particles can still be seen on them (Fig. 6(a)). Although the surfaces of crumbs in the sourdough groups are smooth, there are more cracks and large gas cells on the crumbs (Fig. 6(b), (c), (d)). However, native starch granules were not found on the surfaces of the crumbs (Fig. 6(b), (c), (d)). This phenomenon could be attributed to the longer fermentation time, which allows the starch to swell more. A previous study reported that wheat starch granules gelatinize individually and

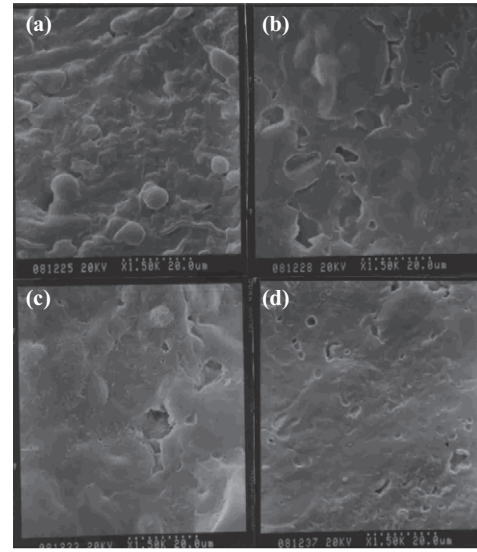


Fig. 6. Scanning electron micrographs of doughs after baking. ((a): Control, (b): S50, (c): S100, (d): S150. 1500 \times)

disrupt the cell membrane, preventing shrinkage of the loaf during cooling after baking (Kusunose et al., 1999). Larger gas cells in the control group and the S150 group (Fig. 6) show that gas production at higher syrup levels is finer for the yeast in the sourdough. The SEM of bread crumbs made using commercial yeast shows numerous ungelatinized starch particles on the surfaces.

Rieder et al. (2012) found that sourdough improves the dough structure of breads containing whole grain barley. The positive effect of sourdough on whole grain barley bread may be related to a softening effect on bran particles during fermentation resulting in less mechanical disruption of the gluten network and gas cells in the dough.

4. Weight and Specific Volume of Bread

The specific volumes of the breads were 4.90 and 4.86 cm^3/g for the control group and S150 group, respectively. Among the groups, the S50 group had the lowest specific volume (4.53 cm^3/g), whereas the bread weights of all sourdough groups were not significantly different from those of the other groups (Table 2). The weights of sourdough breads were higher than those of the control group. This could be attributed to lower water loss in sourdough bread due to low bread volume. The pattern of change in the specific volume of bread was supported, as shown in Fig. 2. Among the three sourdough groups, the S50 group had the lowest specific volume and its starter solution pH was also the lowest. Under this particular acidic environment, the leavening function of the yeast was not as good as in the case of the S150 group.

Use of sourdough has been reported to either decrease (Aremero and Collar, 1996) or increase (Corsetti et al., 2000) bread volume and shelf life. Rieder et al. (2012) speculated that the effect of barley sourdough on bread volume depends on the flour characteristics. They found a small decrease in

Table 2. The baked weight, specific volume and sensory evaluation of sourdough bread.

Samples*	Weight (g)	Specific volume (cm ³ /g)	Flavor	Mouthfeel	Overall acceptability
Control	400.0 ± 0.9 ^b	4.90 ± 0.10 ^a	4.8 ± 1.5 ^b	6.0 ± 1.8 ^a	5.3 ± 0.8 ^b
S50	403.2 ± 1.9 ^a	4.53 ± 0.11 ^b	6.7 ± 0.5 ^a	5.0 ± 1.4 ^a	6.0 ± 1.4 ^b
S100	401.5 ± 2.6 ^a	4.77 ± 0.10 ^{ab}	6.8 ± 1.0 ^a	5.5 ± 1.6 ^a	5.8 ± 0.8 ^b
S150	401.0 ± 1.6 ^{ab}	4.86 ± 0.19 ^a	7.3 ± 0.8 ^a	7.0 ± 1.4 ^a	7.5 ± 1.1 ^a

¹ Values (mean ± SD, n = 3) in the same column followed by a different letter are significantly different (*p* < 0.05).

² Dislike extremely = 1; dislike very much = 2; dislike moderately = 3; dislike slightly = 4; neither like nor dislike = 5; like slightly = 6; like moderately = 7; like very much = 8; like extremely = 9.

³ S50, S100, S150: Sourdough made with 50 g/L, 100 g/L, 150 g/L syrup conc., respectively.

bread volume of wheat breads enriched with sifted barley flour sourdoughs compared to breads made with unfermented sifted barley flour. Compared to the control bread, sourdough did not increase bread volume, even though raisin microflora does increase gas production. This might be sourdough attributed to the dilution of gluten, which affects the gas-holding capacity of the dough.

5. Sensory Evaluation of Bread

Sensory testing indicates that the flavor of the S150 group bread was better than the other groups (Table 2). Bread fermented with raisin microflora received higher flavor scores (6.7-7.3), indicating it was slightly to moderately liked (Table 2). Several researchers (Lavermicocca et al., 2000; Thiele et al., 2003; Vermeulen et al., 2006; Gänzlea et al., 2007) have found similar results. It could be that the natural lactic acid bacteria and yeast in sourdough have a desirable effect on flavor. In addition to proteolysis, bacterial amino acid metabolism contributes to flavor formation during sourdough fermentation and baking.

Minor differences in mouthfeel among the pieces of bread were evaluated by panelists (*P* > 0.05). Although the control bread was softer than the tested groups, the S150 group had higher acceptability ratings than did the control, with scores of 7, meaning that it was moderately liked.

Finally, the S150 group had scores of 7.5 in overall acceptability, indicating that the quality of the S150 group bread was the best (Table 2). The quality of sourdough bread might be improved by using raisin starter solution made with 150 g/L syrup solution.

After baking, the water activity (Aw) of crumbs increased with 6 days of storage from a range of 0.92-0.93 to a range of 0.95-0.97 (Fig. 7(a)). As shown in Fig. 7(a), the water activity of crumbs increased from Aw 0.92 after baking to Aw 0.95 after 6 days of storage. The Aw of the S150 group bread was the lowest out of all the groups. S50 had the highest Aw. This could be due to its high acidity and hydrolyzed protein gluten which make it unable to hold the water excessively. The high free water made the Aw become high.

Hardness of crumbs increased with storage time, as shown in Fig. 7(b). The hardness of crumbs increased from 72.3 g on the day of baking to 153.9 g after 7 days of storage. The control group was softer than all of the sourdough bread groups.

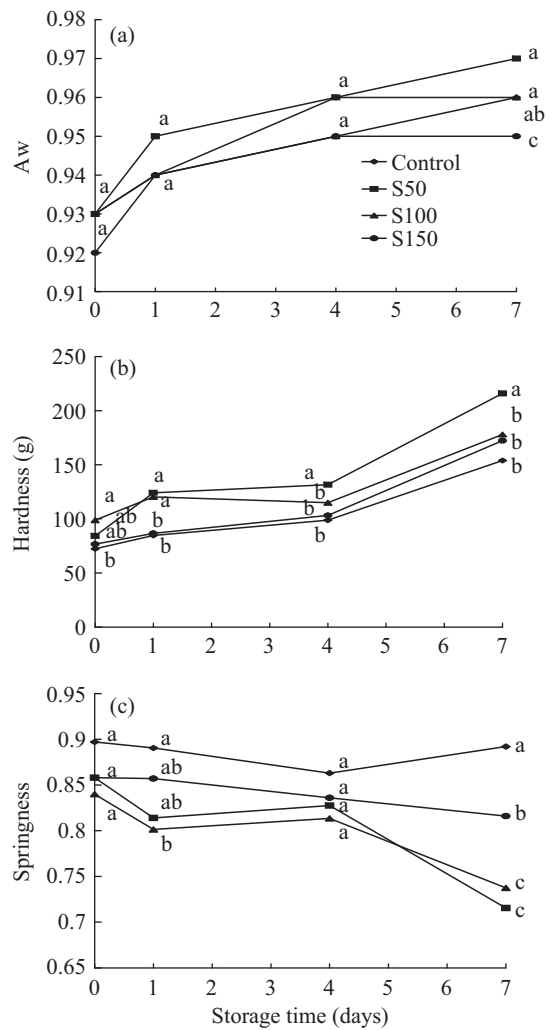


Fig. 7. Storage time versus (a) water activity, (b) hardness and (c) springiness of raisin starter bread at different syrup concentrations.

However, the S150 group had the lowest hardness among the sourdough groups.

Possibly, the gluten in the sourdough group led to partial acid hydrolysis, causing specific volumes lower than those of the control group and less softness. The S50 group had the greatest acidity and therefore the highest hardness.

Clarke et al. (2002) reported the influence of sourdough on

the specific volume and hardness of bread has been assumed to be due to the acidity-induced activation of proteolytic enzymes present in the wheat flour. Additionally, proteolysis liberates water from the gluten network, which allows increased α -amylase activity (Schwimmer, 1981), changes the water absorption of starch and/or polysaccharides, and changes the activities of other endogenous enzymes present in the dough. It might be that the high acidity of sourdough in wheat dough increases proteolysis, which, in turn, significantly decreases the volume and softness of the bread. While no detrimental effects on the quality of wheat bread have been detected when using optimized sourdough with mild acidity, the results are similar to those of Flander et al. (2007).

Springiness refers to how well a crumb physically springs back after it has been deformed during the first compression. In this study, bread springiness decreased with days of storage (Fig. 7(c)). When compared with the control (springiness value of 0.862 at the beginning), the crumb springiness of the S50 group decreased to a minimum (0.716) after 7 days of storage. The pattern of change in springiness of the S100 group bread was the same as that of the S50 group bread.

In previous studies, sourdough has been shown to produce antibacterial compounds, antifungal substances and acids; these substances can extend the shelf life of sourdough bread (Corsetti et al., 1998a; 1998b). However, the hardness of the control bread in this study was still less than that of all of the sourdough groups. The bread staling rate was not retarded by the use of sourdough, nor did the bread springiness decrease. However, overall acceptability and flavor of the breads made with sourdough were higher than those of breads made with commercial yeast.

IV. CONCLUSION

All sensory scores of bread made using sourdough were higher than those of bread made with commercial yeast. Panelists preferred bread made with sourdough, especially the S150 group. The specific volume of the bread in the S50 group was smaller than those of the control group and the S150 group. The hardness of bread made with a S50 level increased faster during storage than did the control bread. According to SEM examination, the gelatinization characteristics of sourdough bread were better than those of the control bread. However, it did not retard the bread staling during storage. It is suggested that the quality of bread might be enhanced by using raisin starter made with 150 g/L of syrup solution.

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