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Jean-Yu Hwang

Department of Food Science and Technology, Chung-Hwa University of Medical Technology, Tainan, Taiwan, R.O.C.

Wen-Chieh Sung

Department of Hotel & Restaurant Management, Chia Nan University of Pharmacy and Science, Tainan, Taiwan, R.O.C.

Yung-Shin Shyu

*Department of Baking Technology and Management, Kaoushang Hospitality College, Taiwan, R.O.C.,
tristar@mail.nkhc.edu.tw*

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EFFECT OF MULBERRY LEES ADDITION ON DOUGH MIXING CHARACTERISTICS AND THE QUALITY OF MULBERRY TOAST

Jean-Yu Hwang*, Wen-Chieh Sung**, and Yung-Shin Shyu***

Key words: mulberry lees, Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC), fermentation, dough.

ABSTRACT

This study evaluated the effects of 10%-30% mulberry lees addition on the dough mixing characteristics and the quality of mulberry toast. Farinogram shows the addition of 30% mulberry lees had the shortest departure time and weakest gluten strength. Peak viscosity, pasting temperature and final viscosity of Rapid Visco Analyser pasting profiles decreased as the addition of mulberry lees increase. Dough with 10% and 20% lees addition had a similar dough expansion rate to that of the control. However, the fermentation rate of the dough with 30% mulberry lees was obviously much slower. The ice-melting onset temperature, peak temperature and enthalpy of Differential Scanning Calorimetry (DSC) decrease with the addition of mulberry lees. A scanning electron microscopy was used to study changes in structure of bread dough that had added mulberry lees and fermented for 240 minutes. Starch granules of the control dough appeared to be coated within a compact gluten matrix that retained its integrity after final proof. When the dough was added with 30% mulberry lees, the appearance of the dough altered from a loose and discontinuous protein matrix to an un-solidified and open structure after final proof. Mulberry lees could be added to bread dough at a level of less than 20% without significant reducing its bread volume and quality.

I. INTRODUCTION

High level of serum cholesterol, triglycerides, and plasma low-density lipoprotein cholesterol have been clearly identified as important risk factors to atherosclerosis and coronary heart disease. Mulberry (*Morus alba L.*) has gained much attention due to their antioxidant activity in retardation of atherosclerosis

lesion development [5-7, 10, 12, 13, 17, 19]. Anthocyanins [9], vitamin C, flavonoids [7] of mulberry could reduce the incidence of heart diseases, such as atherosclerosis, through their antioxidant activity. Mulberry fruit, leaves, bark and branches have used in Chinese medicine to treat fever, facilitate discharge of liver, protect the liver damage, strengthen joint, and lower blood pressure [26]. Mulberry fruit also could be processed into juice, soft drink, jam, and wine [16] in food industry. Mulberry lees are the straining leftover of mulberry wine. They are also rich in dietary fiber and supply valuable vitamins and minerals. Plenty of beneficial effects of dietary fiber have been extensively studied [4,23]. The deterioration in bread quality (loaf volume and absorption) is prior due to inferior mixing characteristics in now before new methods and ingredients were developed. Mixing tolerance is an important quality parameter for the baker. Overall, it may be possible to make acceptable bread from any flour by using rigidly controlled conditions. However, if bread quality deteriorates markedly with only small changes in mixing or fermentation, the baker could have major problem in maintaining a consistent product [3]. In this study, mulberry lees were added to bread dough. Effects of adding mulberry lees on dough mixing characteristics, dough expansion, microstructures, and baking test were investigated. The results of the present study could be used for bakery industry and help the mulberry wine maker for easy the problem of leftover.

II. MATERIALS AND METHODS

1. Materials

Mulberry lees, used in this study were a leftover of mulberry wine, were obtained from National Pingtung University of Science and Technology (Pingtung, Taiwan). Bread flours were purchased from Uni-President Enterprises Corporation (Tainan, Taiwan). Shortening used in this study were purchased from Namchow group (Taoyuan, Taiwan). Instant dry yeast was purchased from Yung Cheng Industries Ltd. (Taipei, Taiwan).

2. Methods

1) Physicochemical Properties of Flour and Dough Mixing

Farinographs (C.W. Brabender Instruments, Inc., South Hackensack, NJ) were run at 30°C and the bread dough was made from 300g bread flour and added water to center at 500

Paper submitted 11/22/06; accepted 03/27/07. Author for correspondence: Yung-Shin Shyu (e-mail: tristar@mail.nkmc.edu.tw).

*Department of Food Science and Technology, Chung-Hwa University of Medical Technology, Tainan, Taiwan, R.O.C.

**Department of Hotel & Restaurant Management, Chia Nan University of Pharmacy and Science, Tainan, Taiwan, R.O.C.

***Department of Baking Technology and Management, Kaoushang Hospitality College, Taiwan, R.O.C.

Brabender Units by adjusting dough water content. It is used to access flour water absorption, arriving time, departure time, stability, peak time according to AACC Method 54-21 [1]. Therefore, the amounts of water added to 0%, 10%, 20%, and 30% mulberry dough were 64%, 59%, 52%, and 46%, respectively.

The baking formula, based on baker's percentage, was as follows, 100% flour, 10% sugar, 1.5% salt, 1% salt, 1% dry yeast, 4% shortening, 0%-30% mulberry lees, and water as determined from a farinograph absorption test. All the ingredients except shortening are combined and mixed into a dough. The shortening was added after the dough was developed. Vertical mixer (Jen Dah Food machinery, Chiayi, Taiwan) attached with dough arm is used to mix experimental dough to optimum development. Dough is divided and rounded into a ball shape. The molded dough was fermented in final proof room for a further 50 minutes at a temperature of 38°C and relative humidity of 85%. The fermented dough was baked in a electric oven (Jen Dah Food machinery, Chiayi, Taiwan) at 150°C/180°C for 25 minutes. Loaf volume was measured immediately after baking by a rapeseed displacement method.

The viscoamylographs of the flours were determined using a Rapid Visco Analyzer model 3D (RVA) (Newprot Scientific Pty Ltd., Warriewood, Australia) following the method of Whalen *et al.* [25]. The RVA 3D were operated with 3.5g bread flour, 25 ml water, and 0%, 10%, 20%, and 30% mulberry lees addition, respectively. The temperature profile included a 1 min isothermal step at 50°C, a linear temperature increase to 95°C in 3.75 min, a holding step (2.5 min at 95°C), a cooling step (3.75 min) with a linear temperature decrease to 50°C. The RVA pasting parameters of peak viscosity, trough, breakdown, peak time, pasting temperature, final viscosity and setback were measured in triplicate.

2) pH Measurement

Five grams dough was added 45 grams of distilled water. It was blended and filtered with Whatman No. 1 filter paper before pH analysis (Corning Scientific Co., NY). A combination electrode, standardized between pH 4.0 and 7.0 and attached to a pH/ion analyzer (Corning Scientific Co., NY), was inserted into the strained liquid every 20 minutes.

3) Dough Expansion

Dough expansion test followed the method of Sangnark and Noomhorm [21] with slight modification. Dough after mixing was divided into 50 gram pieces and rounded. Dough samples were inserted into a 250 ml graduated cylinder. Cylinders were placed in a cabinet at a temperature of 28°C and relative humidity of 75 for 240 minutes. Dough volume was recorded every 20 minutes.

4) Thermal Characteristics

Ice melting temperature was determined using a modulated DSC 2010 differential scanning calorimeter (TA instrument, New Castle, USA) following the methods of Biliaderis *et al.* [2]. Dough samples (10 mg) were placed in aluminum crucibles and the crucible was hermetically sealed. An empty crucible was

Table 1. Effect of mulberry lees addition on dough Farinography parameters¹

	Control	10%	20%	30%
Water absorption (%)	64	59	52	46
Arrival time (min)	0.5	1.0	1.5	1.5
Peak time (min)	2.0	2.5	4.0	6.0
Departure time (min)	30.0	19.0	17.5	14.5
Mixing stability (min)	29.5	18.0	16.0	13.0

¹ All values were a mean of 2 replications with 2 sub-samples per replication.

used as reference. The ice melting temperature parameters of To (onset), Tp (peak), Tc (conclusion), and enthalpy (ΔH , J/g) were determined. The dough was heated from -50°C to 20°C at 5°C/min and carried out all experiments in triplicate.

5) Texture Properties of Bread

Breads were sealed in 1Kg polyethylene (PE) bags after baking for 1 hour and held at room temperature (25°C) for further testing. Breads were sliced into 3 × 3 × 3 cm³ crumbs using a standard bread slice. Hardness of breads were tested with the TA.XT2 Texture Analyzer (Haslemere, England) and a 75 mm diameter cylinder probe according to the methods of Fiszman *et al.* [8]. Texture profile analysis (TPA) was conducted with a test speed of 5.0 mm/s. Calibration distance for the probe was 35.0 mm.

6) Scanning Electron Microscopy

Dough was examined during fermentation followed the methods of Kim *et al.* [15]. Dough samples were freeze vacuum dried at -50°C for 24 hours. Freeze-dried dough was cut with a razor blade and mounting onto brass stubs using double-sided carbon conductive adhesive tape. A gold coat (15nm thick) was then applied using a sputter coater (Giko Engineering, IB model). Samples were examined at 25 keV using a Hitachi S-2500 Scanning Electron Microscopy (Tokyo, Japan).

7) Statistical Analysis

The data were analyzed statically with the Statistic Analysis System [22]. ANOVA and Duncan's test used to test the significance difference of each group. A value of p<0.05 was considered significant.

III. RESULTS AND DISCUSSION

1. Dough Properties

Table 1 shows water absorption of farinograms decreases with the addition of mulberry lees. The moisture content of mulberry lees is 91.2%, which would be partial absorbed by bread flour before water addition and dough mixing process. The farinograms also show that the arriving time and peak time parameters increased with the addition of mulberry lees. The addition of 30% mulberry lees dough is too weak, as reflected

Table 2. Effect of mulberry lees addition on dough RVA pasting properties¹.

	Control	10%	20%	30%
Peak viscosity (RVU)	241.1 ^c	246.4 ^{bc}	249.2 ^{ab}	254.5 ^a
Trough (RVU)	164.3 ^a	157.0 ^b	151.9 ^{bc}	150.9 ^c
Breakdown (RVU)	76.7 ^d	89.4 ^c	97.2 ^b	103.6 ^a
Peak time (min)	6.3 ^a	6.3 ^a	6.3 ^a	6.2 ^a
Pasting temp (°C)	71.7 ^a	68.3 ^b	67.3 ^b	67.3 ^b
Final viscosity (RVU)	273.8 ^a	267.1 ^b	266.0 ^b	265.6 ^b
Setback (RVU)	109.5 ^a	110.1 ^a	114.1 ^a	114.7 ^a

¹ All values were a mean of 3 replications with 3 sub-samples per replication. Mean values with the different letter in the same column were significantly different ($p < 0.05$).

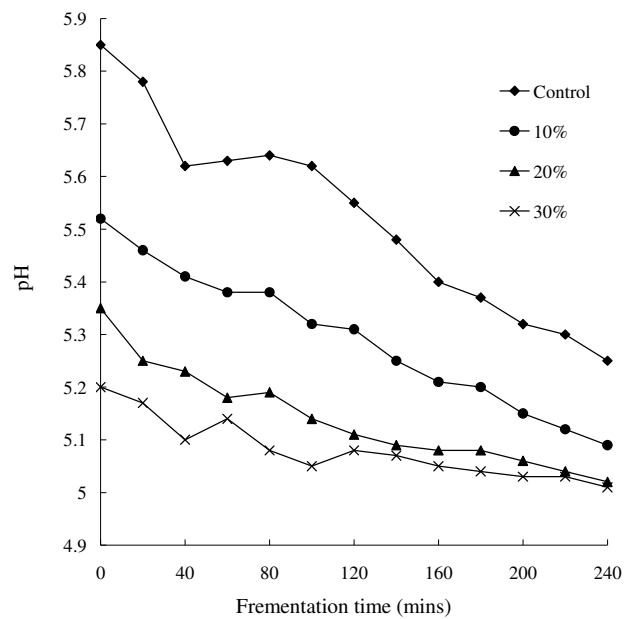
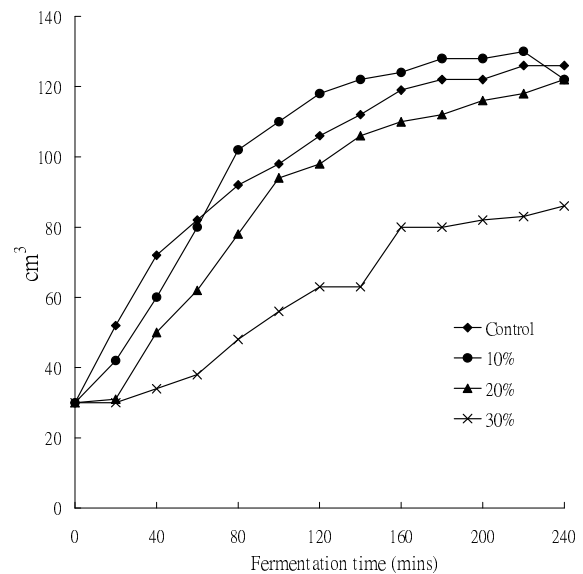
by short mixing stability and rapid departure time. Mulberry lees lack the gluten functionality will retard the development of bread dough. It is suggested the addition of water amount should decrease for making food products such as bread, cake and floured based noodles. Hamed *et al.* [11] reported sweet potato flour was added and the bread dough exhibited degrading the gluten network of bread dough. They proposed adding sweet potato flour to wheat flour will increase water absorption and decrease the peak time of farinogram. The increase water absorption as observed for sweet potato flour in their study might be due to the moisture content of sweet potato flour is less than 12%, which would competes water with bread flour.

2. Pasting Characteristics

It was found addition of mulberry lees did not significantly affect peak time of RVA, except for trough, final viscosity and peak viscosity (Table 2). Trough and final viscosity of the RVA parameters decreased with addition of mulberry lees. When 30% of mulberry lees were added into flour, the peak viscosity of flour paste would increase. This was not surprising, since the mulberry lees contain 92% of moisture. As a consequence, more water go into the interior of starch granules even when the adding amounts was 10%, induces the effect obviously. Apparently, starches of test groups swell more during gelatinization and pasting. The higher peak viscosity of test groups can be explained by higher water penetration into the starches and/or the dietary fiber of mulberry lees interacts with amylose and amylopectin to form hydrogen bonds. The addition of mulberry lees decreased the final viscosity of pasting curves by forming the hydrogen bonds between dietary fiber and starch polymers.

3. Effect of Mulberry Lees on pH Change of Fermented Dough

The pH of control group is highest (5.85). The decrease pH of control dough after fermentation was marked in comparison with that of test groups, whilst the pH of mulberry lees added dough was slightly decreased. Beyond 240 minutes of fermentation, pH of control dough decreased to 5.25 and led to a

**Fig. 1. Effect of mulberry lees addition on dough pH value during fermentation.****Fig. 2. Effect of mulberry lees addition on dough volume during fermentation.**

reduction by 10.3%. The pH of 30% mulberry lees addition decreased from 5.20 to 5.01 with a reduction of 3.7% at the end of fermentation. The pH of mulberry lees was 3.58. Thus, they also reduced the pH of 30% mulberry lees dough to 5.20 after mixing (Fig. 1). High level of mulberry lees addition will generate a lower pH dough and lower pH reduction after fermentation.

4. Effect on Dough Expansion Test of Mulberry Lees Addition

As illustrated in Fig. 2, the dough volume increase of 10%

Table 3. Effect of mulberry lees addition on dough differential scanning calorimetry (DSC) ice-melting onset temperature, peak temperature, enthalpy¹ and loaf volume².

Mulberry lees addition	Onset T (°C)	Peak T (°C)	Enthalpy (J/g)	Loaf volume (cm ³)
Control	-8.86	-3.76	76.86	1996 ^a
10%	-8.90	-4.30	73.89	2037 ^a
20%	-9.45	-4.78	70.45	1869 ^b
30%	-11.04	-5.25	65.25	1799 ^b

¹ All values were a mean of 2 replications with 2 sub-samples per replication.

² All values were a mean of 3 replications with 3 sub-samples per replication. Mean values with the different letter in the same column were significantly different ($p < 0.05$).

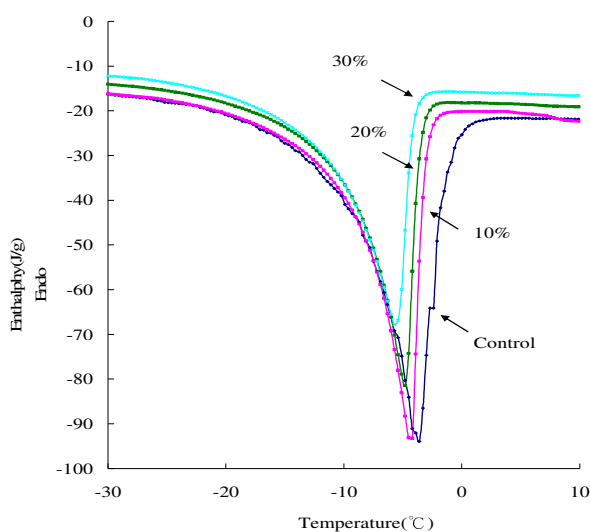


Fig. 3. Effect of mulberry lees addition on the dough ice melting temperature of Differential Scanning Calorimetry (DSC).

and 20% mulberry lees was similar to that of control group. However, the 30% addition of mulberry lees was obviously unable to retain the CO₂ which generated by the yeast. This is possibly due to it contains less gluten and high amounts of mulberry lees will retard yeast growth. Dough made from the control, 10% and 20% mulberry lees addition had a faster volume increase within the first 150 minutes than 30% mulberry lees addition. Then, all those three groups induced a slow volume increase after 150 minutes fermentation time. Optimum growth pH of yeast is pH 4.0 to pH 6.0. The pH of 30% mulberry lees dough is around pH 5.0 to pH 6.0, so the yeast still can grow well in this pH range. The results in Fig. 1 and Fig. 2 show the effect on lowering pH of mulberry lees addition is not major factor for retard dough fermentation. Dough volume of 30% mulberry lees addition (86 cm³) explaining the low fermented dough volume could be mainly attributed to the diluted gluten content of the dough, so its final volume is significantly lower than other group (122-126 cm³).

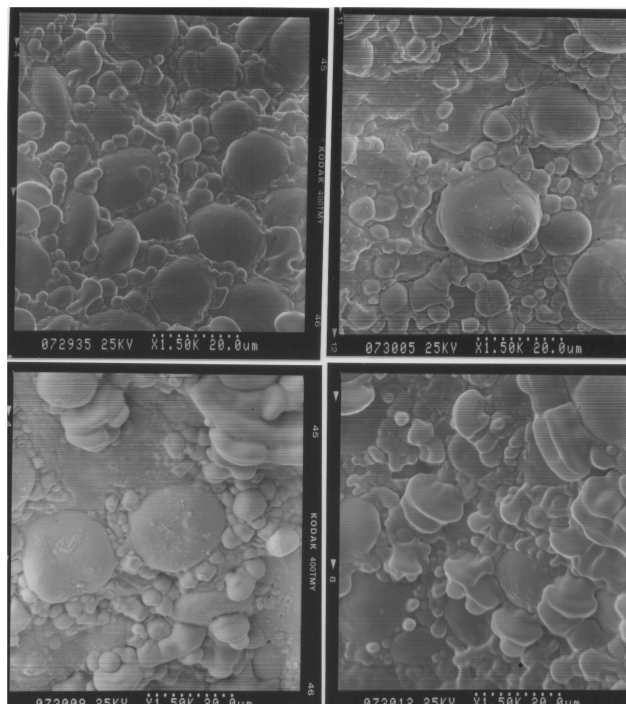


Fig. 4. Scanning electron micrographs of dough at completion mixing. (A) control dough (B) addition of 10% mulberry lees (C) addition of 20% mulberry lees (D) addition of 30% mulberry lees. (x1500)

5. Effect of Mulberry Lees Addition on Dough Thermal Characteristics

Differential scanning calorimetry thermograms in all samples tested showed an endothermic peak around 0°C that was attributed to ice melting [24] and was therefore used to calculate the amount of freezable water. There was considerable variation in ice-melting temperatures and enthalpy among the control and test groups. The mean ice-melting onset temperature (T_o) was -8.86 for control. The ice-melting temperature of mulberry lees addition appeared lower than that of control after differential scanning calorimeter determination (Table 3). Ice-melting peak temperature (T_p) also decline by mulberry lees addition (Fig. 3). The ΔH of ice-melting was 76.86 J/g for the control group. The addition of 30% mulberry lees decreases the ice-melting enthalpy to 65.25 J/g is postulated as a possible explanation for the amount of frozen dough of 30% mulberry lees addition has less free water than the other dough. It indicates the control dough had more freezable water associated with the melting curve than the dough containing mulberry lees (30%).

6. Effect of Mulberry Lees Addition on the Microstructure of Dough

Numerous starch granules of varying size were visible on the outer surface of gluten matrix. The control sample appears to be coated with a smooth protein film (arrow of Fig. 4A). As other researchers [14, 18, 20] have reported previously, the dough of 10% mulberry lees also exhibited numerous starch granules were coated with an amorphous protein matrix, but some small

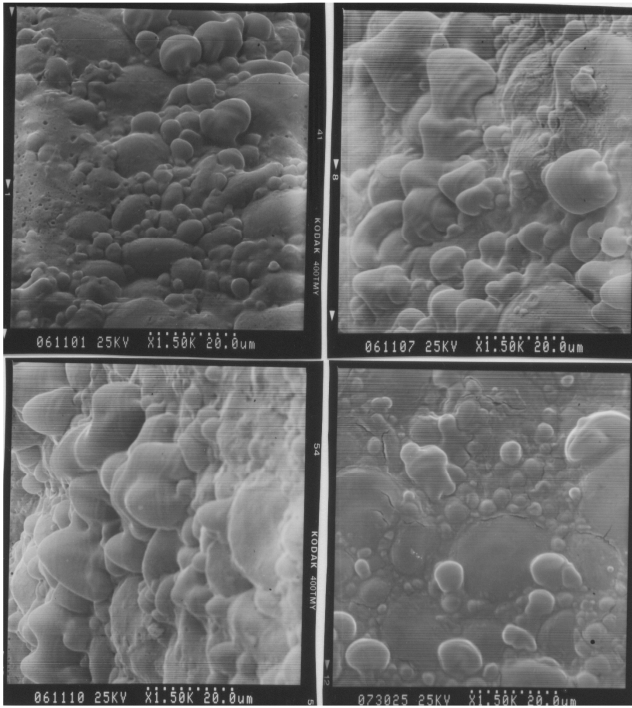


Fig. 5. Scanning electron micrographs of dough after first fermentation. (A) control dough (B) addition of 10% mulberry lees (C) addition of 20% mulberry lees (D) addition of 30% mulberry lees. (x1500)

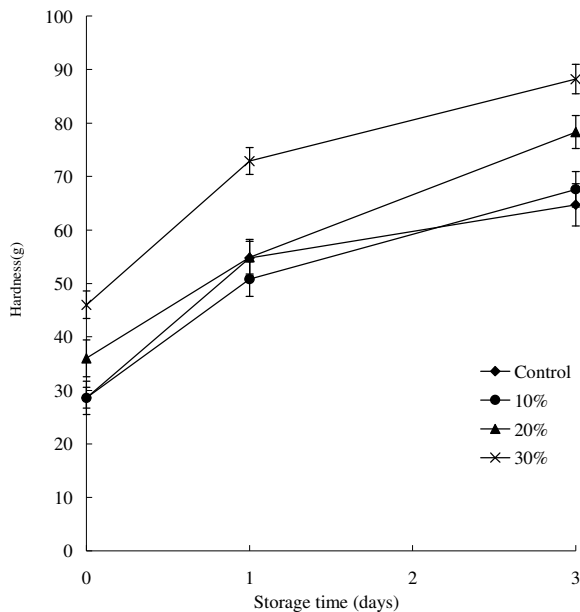


Fig. 6. Effect of mulberry lees addition on the hardness of toast.

holes and cracks were present on the surface (arrow of Fig. 4B). In contrast, Fig. 4D exhibited numerous small starch granules coated on an amorphous and less continuous protein matrix. The surface of the control dough (Fig. 5A) still appeared a smooth protein film, but the gluten matrix became less continuous and loose after fermentation. There were numerous small pores on it, which may be caused by the CO_2 generated by the yeast (arrow of Fig. 5A). Fig. 5B shows the starch slightly

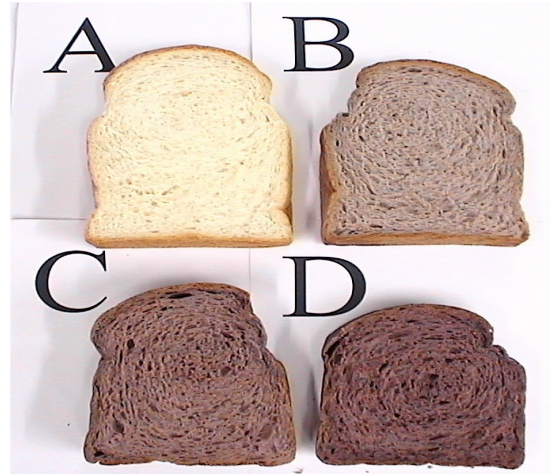


Fig. 7. Characteristics of bread made by various levels of mulberry lees addition (A) control bread (B) addition of 10% mulberry lees (C) addition of 20% mulberry lees (D) addition of 30% mulberry lees.

attached to discontinuous protein film. After first fermentation, the dough surface of the 30% mulberry lees addition became smoother than the other groups.

The texture properties of bread crumb containing mulberry lees (up to 20% level) were not significantly ($p > 0.05$) different from the control bread on the day of baking. Addition of mulberry lees (less than 20% level) does not affect the hardness of breads containing mulberry lees (Fig. 6). Addition of 10% mulberry lees was effective in increasing loaf volume of bread. Loaf volume was significantly ($p < 0.05$) decreased as the level of mulberry lees adding more than 20% (Table 3). Fig. 7 shows characteristics of bread made by various levels of mulberry lees addition. Crumb color of mulberry toast became darker and more purple with an increase in mulberry lees addition.

IV. CONCLUSION

Mulberry lees addition ranging from 0 to 30% will increase the arrival time and peak time of dough Farinography parameters. It was shown the effects of mulberry lees addition reduced the mixing stability of Farinogram. The parameters, pasting temperature, peak viscosity and final viscosity of the RVA profile significantly decreased with mulberry lees addition. Addition of 30% mulberry lees has been proven to significantly retard the fermented rate of bread dough. The addition of 10-30% mulberry lees decreases dough pH, but the decrease of pH is less than that of control sample after 240 minutes fermentation. It was shown the small spherical granules of wheat starches were slightly attached to discontinuous protein matrix of mulberry lees dough. The structure of fermented dough appeared to be quite smooth and small starch granules was not readily apparent. Many small holes were apparent on the sample of fermented control dough that might be formed by penetration of CO_2 , which was generated by yeast fermentation. But those small holes were not apparent in the protein matrix of mulberry lees added dough after first fermentation. Our results suggest addition of 10% or 20% mulberry lees might be a good choice for the application of mulberry lees in bread making.

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