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Jean-Yu Hwang
Department of Food Nutrition, Chung-Hwa University of Medical Technology, Tainan, Taiwan, R.O.C.

Wen-Chieh Sung
Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan, R.O.C., sungwilliam2001@yahoo.com.tw

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EFFECT OF MULBERRY LEES ADDITION ON THE QUALITY OF BREAD AND ANTHOCYANIN CONTENT

Jean-Yu Hwang[1] and Wen-Chieh Sung[2]

Key words: anthocyanin, mulberry lees, bread quality, farinograph, texture profile analysis.

ABSTRACT

Mulberry lees were added to bread dough at levels of 10%, 20%, and 30%. Loaves were prepared using the straight dough procedure. Bread volume, crust and crumb color, crumb texture, flavor and overall acceptability were evaluated. Anthocyanin content of crumbs was determined for 5 days storage duration. The volume of bread was decreased ($p < 0.05$) as the mulberry lees addition amount increased. Overall acceptability of 10% mulberry lees addition bread was highest among all breads. There was no significant difference in crumb texture except the firmness of 30% mulberry lees addition was higher than other groups. Addition of mulberry lees increased the anthocyanin content of crumb. Oxidation of anthocyanin occurs in crumbs during storage. Mulberry lees could be added to bread within 20% level for enhancing the nutritional quality and characteristics of bread.

I. INTRODUCTION

The most prominent leading causes of death in most of the world are chronic diseases. Factors increase the risk of developing chronic disease, such as smoking, dietary habits, physical activity, and alcohol use. Therefore, looking for inexpensive and healthy ingredients to improve dietary factor is considered an important task for baking industry. There has been increasing interest in antioxidants derived from fruits, vegetables, herbs, and beverages in recent years. Mulberry (*Morus alba L.*) has been used as medicine in traditional East Asian countries to treat fever, protect the liver, improve eyesight, strengthen joints, facilitate discharge of urine and decrease blood pressure [20]. Several studies have also shown mulberry contains important constituents, anthocyanins [8], which may reduce the incidence of heart disease through its antioxidant activity [4]. Anthocyanins, a group of phenolic compounds, are responsible for the red and blue colors of many flowers, fruits and vegetables [15]. Dietary anthocyanins have potentially beneficial effects on health, such as reducing the risk of mutagens and carcinogens [10, 16, 19]. Anthocyanin-rich fruits, extracts, and pure anthocyanins have been proved to have beneficial effects in preventing or suppressing diseased states *in vivo* [11, 17]. The potential role of mulberry also has been reported having antiviral [2], antimicrobial [6, 14], and neuroprotective effects [18].

A lot of mulberry products such as wine, liquor, juice vinegar, jam as well as baking products are commercially available in Taiwan. Mulberry lees are the leftover of mulberry wine and vinegar. They are also rich in dietary fiber and supply valuable vitamins and minerals. Our previous study reported the effects of mulberry lees addition reduced the mixing stability of dough Farinography parameters [9]. Addition of 30% mulberry lees had been proven to significantly retard the fermented rate of bread dough. Addition of 10% or 20% mulberry lees might be a good choice to the application of mulberry lees in bread making.

In this study, mulberry lees were added to bread again. Effects of adding mulberry lees on the qualities of bread, and the content of anthocyanins after storage were investigated. The results of the present study could be used for bakery industry and help the mulberry wine and vinegar maker to 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 Chen Industries Ltd. (Taipei, Taiwan).

Paper submitted 08/25/10; revised 03/23/11; accepted 05/12/11. Author for correspondence: Wen-Chieh Sung (e-mail: sungwilliam2001@yahoo.com.tw).
[1] *Department of Food Nutrition, Chung-Hwa University of Medical Technology, Tainan, Taiwan, R.O.C.*
[2] *Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.*

Table 1. Proximate compositions of toast from mulberry lees addition.

Mulberry wine lees[1] (%)	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Carbohydrate[2] (%)
0	38.1 ± 0.9^b	10.1 ± 0.1^a	3.1 ± 0.0^a	1.1 ± 0.0^a	47.6 ± 1.4^a
10	37.8 ± 1.7^b	10.6 ± 0.1^a	3.3 ± 0.0^a	0.9 ± 0.1^b	47.4 ± 2.1^a
20	37.2 ± 2.6^b	10.1 ± 0.2^a	3.1 ± 0.2^a	0.9 ± 0.1^b	48.7 ± 0.9^a
30	41.6 ± 1.5^a	10.5 ± 0.5^a	3.1 ± 0.1^a	0.8 ± 0.1^c	44.0 ± 1.8^b

[1] Mulberry lees (%) were expressed as weight of mulberry lees/weight of wheat flour × 100%.
[2] Calculated by differences. Means in the same column with different letters as superscripts are significantly different ($p < 0.05$).

2. Methods

The baking formula, based on baker percentage, was as follows, 100% flour, 10% sugar, 1.5% salt, 1% dry yeast, 4% shortening, 0-30% mulberry lees, water as determined from a farinograph absorption test. Farinograph (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 Brabender units by adjusting dough water content (AACC 54-21) [1]. A straight dough procedure, with a 60 minutes fermentation (at 28°C, 75% relative humidity (RH)), 50 minutes proofing at 38°C (85% RH) and 25 minutes baking at 150°C/180°C (Jedah Food Machinery, Chiayi, Taiwan). Dough is divided (450 g) and rounded into a ball shape, and rested at room temperature for 20 minutes. Loaf volume was measured immediately after baking by a rapeseed displacement method. Baking loss was calculated by the following formula: (1-weight of bread/ weight of dough) * 100%. Each treatment was measured in triplicate and the average was recorded. A color meter (ZE 2000 Nippon Denshoku, Japan) was used to determine lightness (L*), red content (a*) and yellow content (b*) values on bread crust and crumb at first day. A white reference plate was used as a standard.

Sensory evaluation of baked bread followed the method of El-Adawy [3]. The trained panel consisted of 30 members selected randomly from students, laboratory staff and instructors of the department of Food Science and Technology. They were trained and instructed to evaluate the score of appearance, flavor and overall acceptability of the breads by hedonic scale method. A hedonic scale of 1-7 points (1 = dislike very much; 4 = neither like or dislike; 7 = like very much) was used. Bread was evaluated when loaves were cooled down for 3 hours and sliced into 1-cm thickness by a bread slicing machine.

Breads were sealed in 1 Kg polyethylene (PE) bags after cooling for 1 hour and held at room temperature (25°C) for further testing. Breads were sliced into $3 \times 3 \times 3$ cm^3 crumbs using a standard bread slicer after 0, 1, 3 days of storage. Hardness, springiness, cohesiveness and chewiness 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 and others [5]. Texture profile analysis (TPA) was conducted with a test speed of 5.0 mm/s. Calibration distance for the probe was 35.0 mm. The sliced crumbs were also stored at 25°C after wrapping in PE bag to prevent crumbs from drying.

Bread samples were frozen and freeze-dried in a freeze dryer. Freeze dried samples were cut into $0.8 \times 0.8 \times 0.3$ cm^3 crumbs, and mounted onto brass stubs using double sided carbon conductive adhesive tape. A gold coat (15 nm thick) was applied using a sputter coater (Giko Engineering, IB model). Samples were examined at 20 Kev using a Hitachi S-2500 Scanning Electron Microscope (Tokyo, Japan).

Anthocyanin content of acidic methanol extract from 10 g crumbs was determined by pH differential method according to the methods of Fuleki and Francis [7]. Each sample was separated and adjusted to pH 1.0 (A_1) and pH 4.5 (A_2). The absorbance at 535 nm was used to quantify the results according to the following equation: anthocyanin content (mg/ 100 g dried bread crumbs) = $((A_1\text{-}A_2)*V*340*100)/(\varepsilon*W)$. The molecular extinction coefficient (ε) of anthocyanin was determined by cyaniding to be 2.46×10^5. V and W were represented as the final volume of methanol extract and weight of the crumbs.

The data were analyzed statistically with the Statistic Analysis System (SAS) [13]. Analysis of variance (ANOVA) and Duncan test used to test the significance difference of each group. A value of $p < 0.05$ was considered significant.

III. RESULTS AND DISCUSSION

1. Chemical Composition of Breads

Table 1 lists the proximate compositions of mulberry lees addition breads. It is clear that the addition of mulberry lees had no significant effects on the crude protein and fat. The moisture of 30% mulberry lees added bread was quite higher than other samples. This was not surprising, since the mulberry lees contain 92% of moisture. As a consequence, less carbohydrate and ash content in breads were observed. The changes in proximate composition were due to the addition of mulberry lees.

2. Quality of Breads

Addition of mulberry lees had a significant ($p < 0.05$) effect on the value of lightness (L*), red content (a*), and yellow content (b*) values (Table 2) of bread crust and crumb, except the red content a* in crumb. Color characteristics in crust and crumb significantly ($p < 0.05$) decreased when the level of

Table 2. Effect of mulberry lees addition on the color characteristics of crust and crumb.

Mulberry lees[1] (%)	L*[2]	a*	b*	White index
Crust				
0	50.3 ± 1.0[a]	15.4 ± 0.2[a]	33.3 ± 0.6[a]	38.2 ± 0.5[a]
10	39.2 ± 1.1[b]	13.5 ± 0.3[b]	20.8 ± 1.1[b]	34.3 ± 0.7[b]
20	36.9 ± 0.5[c]	12.6 ± 0.2[c]	17.1 ± 0.4[c]	33.4 ± 0.4[b]
30	33.8 ± 1.1[d]	11.7 ± 0.5[d]	12.7 ± 0.6[d]	31.6 ± 1.0[c]
Crumb				
0	74.0 ± 2.6[a]	-1.9 ± 0.1[d]	11.2 ± 0.8[a]	71.5 ± 2.2[a]
10	57.1 ± 2.9[b]	4.6 ± 0.5[c]	8.9 ± 0.8[b]	55.9 ± 2.7[b]
20	47.0 ± 1.4[c]	7.5 ± 0.3[b]	8.1 ± 0.2[b]	45.9 ± 1.4[c]
30	40.6 ± 2.3[d]	9.5 ± 0.4[a]	7.0 ± 0.1[c]	39.4 ± 2.3[d]

[1] Mulberry lees (%) were expressed as weight of mulberry lees/weight of wheat flour × 100%.
[2] L*: 0 = black, 100 = white; a*: negative values = green, positive values = red; b*: negative values = blue, positive values = yellow. Means in the same column with different letters as superscripts are significantly different ($p < 0.05$).

Table 3. Loaf volume and sensory evaluation of bread with mulberry lees.

Mulberry lees[1] (%)	Loaf volume (ml)	Appearance[2]	Flavor	Overall acceptability
0	1966 ± 56[a]	6.5 ± 0.7[a]	6.2 ± 0.5[a]	5.4 ± 0.8[b]
10	2037 ± 28[a]	6.3 ± 1.1[a]	6.4 ± 0.6[a]	6.3 ± 0.9[a]
20	1869 ± 74[b]	4.1 ± 1.5[b]	6.6 ± 0.8[a]	5.7 ± 1.0[ab]
30	1799 ± 39[b]	3.6 ± 1.0[b]	4.9 ± 0.9[b]	5.0 ± 1.0[b]

[1] Mulberry lees (%) were expressed as weight of mulberry lees/weight of wheat flour × 100%.
[2] 1-very dislike, 4-moderate, 7-like very much. Means in the same column with different letters as superscripts are significantly different ($p < 0.05$).

mulberry lees increased. Nevertheless, red content of crumb increased as the content of mulberry lees increased.

Adding 10% mulberry lees was effective in increasing loaf volume of bread. Loaf volume decreased as the level of mulberry lees adding up more than 20% (Table 3). There was no significantly different ($p > 0.05$) in baking loss of breads among control and mulberry lees with 10-20% addition (data not shown). Our results suggest adding 10% mulberry lees might be a good choice for the application of mulberry lees in bread-making.

Overall acceptability scores of breads containing mulberry lees increased significantly ($p < 0.05$) at 10% level as compared to that of the control bread, then it decreased after further mulberry lees addition (Table 3). No significant ($p > 0.05$) differences were found in flavor (up to 20% level) and appearance (up to 10% level) between control and breads containing mulberry lees. The results show that there was a obvious increase in overall acceptability of breads containing 10-20% mulberry lees due to addition with anthocyanin and liqueur flavors, which enhancing fruit aroma and sweetness. Loaf volume and sensory properties scores of breads containing up 30% mulberry lees were decreased.

3. Scanning Electron Micrographs of Bread Crumbs

The control bread comprised irregularly larger gas cells than that of breads containing mulberry lees (Fig. 1). The gas cell revealed a smoother gluten matrix surface consisting of numerous gelatinized starches (Fig. 2). In contrast, breads of 10% mulberry lees exhibited a structure where very few ungelatinized starches were evident, and gelatinized starch were completely coated with an amorphous protein matrix (Fig. 2(b)). There were no starch particle found on the crumb with 10% mulberry lees addition. It might explain adding 10% mulberry lees was effective in increasing loaf volume of bread, due to complete gelatinization of starch in crumb. Therefore, breads containing 10% mulberry lees have finer crumb grain structure.

4. Texture Properties of Breads during Storage

The texture properties of crumbs containing mulberry lees (up to 20% level) were not significantly ($p > 0.05$) different from the control bread on the day of baking (Table 4). So adding mulberry lees (less than 20% level) does not affect the texture of breads. Hardness and chewiness increased, whereas the springiness and cohesiveness decreased during storage. However, texture properties of breads were not significantly ($p > 0.05$) different between the control bread and breads containing 10% mulberry lees. This indicates that staling rate of bread containing mulberry lees at 10% level was not different from the control bread. Zobel and Kulp [21] found that retrogradation correlates to loss of freshness and increases with hardness of bakery products. The addition of mulberry

Table 4. Effect of mulberry lees on the texture properties of breads during storage.

Mulberry lees[1] (%)	Hardness[2]	Springiness	Cohesiveness	Chewiness
0 day storage				
0	28.6 ± 2.95^{b}	0.83 ± 0.02^{a}	0.80 ± 0.02^{a}	19.1 ± 1.60^{b}
10	28.6 ± 3.13^{b}	0.78 ± 0.08^{a}	0.81 ± 0.00^{a}	18.0 ± 3.80^{b}
20	36.0 ± 4.46^{ab}	0.78 ± 0.04^{a}	0.75 ± 0.02^{a}	21.1 ± 3.95^{b}
30	46.0 ± 2.56^{a}	0.74 ± 0.05^{a}	0.78 ± 0.04^{a}	29.0 ± 3.90^{a}
1 day storage				
0	54.8 ± 4.05^{b}	0.76 ± 0.02^{a}	0.69 ± 0.00^{ab}	29.9 ± 3.28^{a}
10	50.8 ± 3.24^{b}	0.77 ± 0.04^{a}	0.73 ± 0.02^{a}	28.6 ± 3.02^{a}
20	54.9 ± 4.35^{b}	0.70 ± 0.20^{b}	0.59 ± 0.07^{c}	33.5 ± 2.68^{a}
30	72.9 ± 3.52^{a}	0.68 ± 0.04^{b}	0.65 ± 0.04^{bc}	32.2 ± 4.55^{a}
3 days storage				
0	64.7 ± 4.97^{c}	0.77 ± 0.02^{a}	0.65 ± 0.01^{a}	33.5 ± 5.28^{ab}
10	67.6 ± 4.30^{c}	0.77 ± 0.07^{a}	0.66 ± 0.02^{a}	35.4 ± 2.78^{ab}
20	78.3 ± 4.08^{b}	0.71 ± 0.05^{a}	0.58 ± 0.04^{b}	30.3 ± 4.62^{b}
30	88.2 ± 3.75^{a}	0.80 ± 0.10^{a}	0.59 ± 0.05^{b}	41.9 ± 8.40^{a}

[1] Mulberry lees (%) were expressed as weight of mulberry lees/weight of wheat flour × 100%.
[2] Means in the same column, within an experiment, with different letters as superscripts are significantly different ($p < 0.05$).

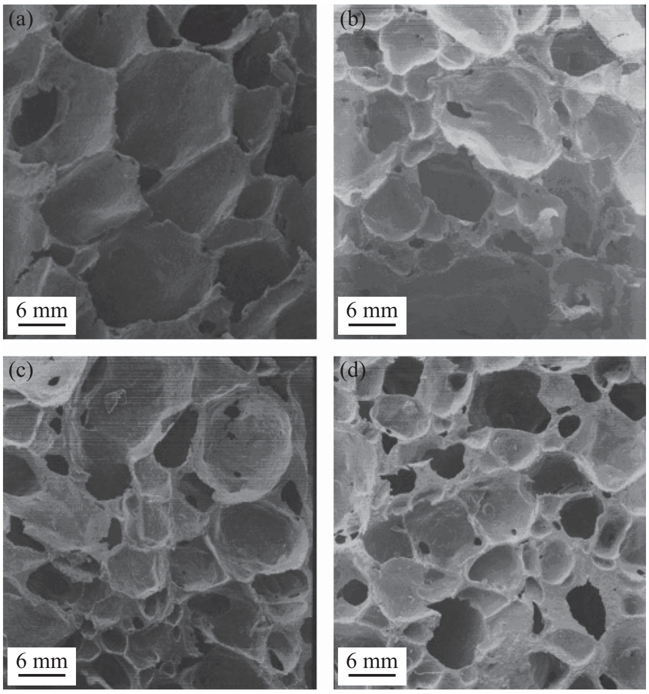

Fig. 1. Scanning electron micrographs of crumbs in (a) control bread and mulberry lees containing breads at (b) 10%, (c) 20%, and (d) 30% levels (× 25).

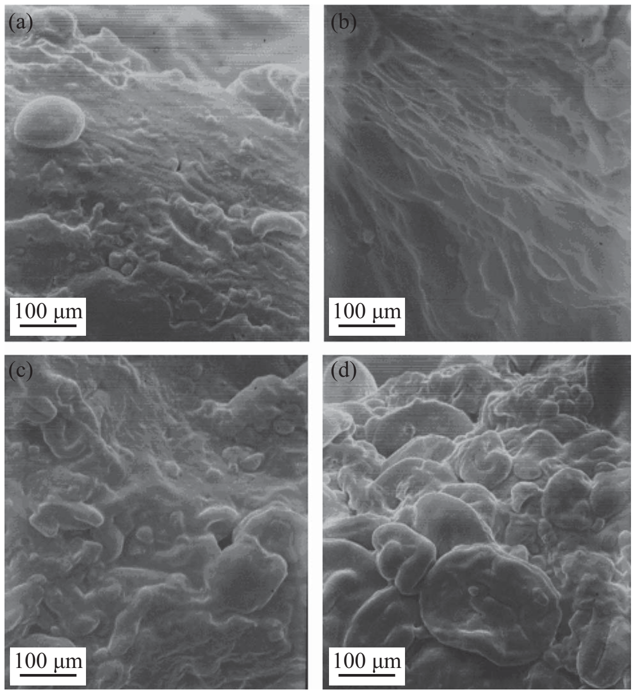

Fig. 2. Scanning electron micrographs of crumbs in (a) control bread and mulberry lees containing breads at (b) 10%, (c) 20%, and (d) 30% levels (× 1500).

lees at 10% level does not change texture properties and baking quality of breads containing mulberry lees.

5. Anthocyanin Content of Breads during Storage

Addition of mulberry lees to wheat flour increased the content of anthocyanin. The content of anthocyanin in breads increased significantly ($p < 0.05$) from 12 mg/100 g bread for control bread to 234.9 mg/100 g for 30% mulberry lees containing breads (Fig. 3). Anthocyanin content of bread containing 30% mulberry lees decreased from 234.9 mg/100 g bread to 116.3 mg/100 g bread after 5 days storage. Despite the decrease in the anthocyanins was measured, bread containing mulberry lees still exhibited higher levels of anthocyanin than that of the control bread ($p < 0.05$).

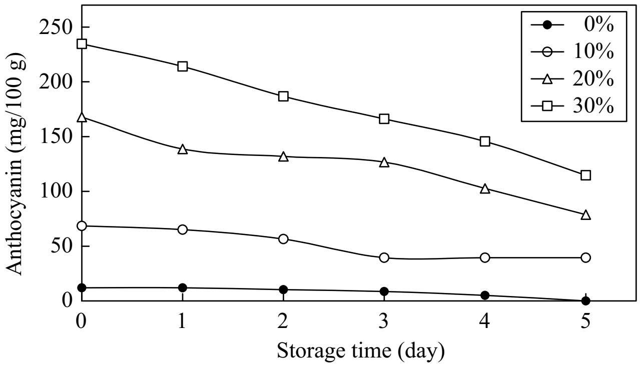

Fig. 3. Change of anthocyanin content in breads with 0-30% mulberry wine leesaddition during storage.

IV. CONCLUSION

The results show mulberry lees could be added to wheat flour at 10-20% level to increase the over acceptability without any detrimental effects on bread qualities, especially at 10% level. Furthermore, the non-toxic and non-mutagenic anthocyanin significantly increased in bread containing mulberry lees, which not only contributed to the color of bread but also exhibited potential positive therapeutic properties [12]. Therefore, adding natural anthocyanin containing mulberry lees into wheat flour for bread-making will be a beneficial application for baking industry. This could also help waste recycle and improve economic pollution.

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