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PHYSICOCHEMICAL CHANGES OF SHIITAKE STIPE BREAD AND SILVER EAR BREAD DURING STORAGE

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Key words: Shiitake stipe; silver ear, bread, WAXD.

ABSTRACT

Shiitake [*Lentinula edodes* (Berk.) Pegler] stipe and silver ear (*Tremella fuciformis* Berkeley) were used to substitute 5% of wheat flour to make bread. No difference among fresh white, shiitake stipe and silver ear breads was found in moisture contents. Total plate counts of breads were as high as 2.92 log CFU/g dry weight at day 0 and 3.69 log CFU/g after 12 days of storage at 4°C. The melting endothermic peaks of breads reached 105.2°C to 111.7°C. The range of thermal decomposition enthalpies were between 116.6 J/g and 158.4 J/g. Three wide-angle X-ray diffraction (WAXD) patterns were similar. An electron microscopic examination revealed that the morphology of the bread products was aggregated flakes with a few porosities. In the presence of shiitake stipe flour or silver ear flour, the natural crystallinity and morphology of bread did not obviously change after 12 days of storage. Overall, shiitake stipe and silver ear could be incorporated into bread to provide its beneficial health effects.

I. INTRODUCTION

Shiitake [*Lentinula edodes* (Berkeley) Pegler] is a traditionally eaten mushroom in Japan, Korea, Taiwan and China [8]. Silver ear (*Tremella fuciformis* Berkeley) is a translucent white jelly fungus [10]. These mushrooms are highly valued as a centrepiece of Asian cooking. In addition, both shiitake and silver ear are traditional Chinese medicines and found to be medically active in several therapeutic effects such as anti-inflammatory and hyperlipidemia [9]. These mushrooms are commonly consumed as food and nutritional supplement; and they are also regarded as health foods because their beneficial effects.

Bread is a staple food world-wide and it is mainly made of wheat flour, water, salt and yeast. Other food ingredients such as yam [4] and buckwheat [6] have been included in bread formulation to replace part of wheat flour and to increase the diversity, nutritional value and product appeal of the bread. The qualities of shiitake stipe bread and silver ear bread including taste components and proximate composition were studied previously [7, 10]. However, the quality changes of these breads during storage were unknown. Accordingly, we examined the quality changes, including moisture contents and microbial count, of a bread with 5% wheat flour substituted by shiitake stipe and silver ear flours and stored at 4°C. The physicochemical properties of breads were also studied using differential scanning calorimetry (DSC), X-ray diffraction patterns and scanning electron microscopy (SEM).

II. MATERIALS AND METHODS

1. Materials and Bread Making

The high gluten wheat flour, bread improver, milk powder, salt, shortening, sugar and yeast in the formulations of bread were purchased from Tzong-Hsin Food Co., Taichung, Taiwan. Air-dried shiitake stipes and silver ears were purchased from Save & Safe supermarket, Taichung, Taiwan and ground into a coarse powder (60 mesh) using a RT-34 pulverising machine (Rong Tsong Precision Technology CO., Taichung, Taiwan). The ingredients for bread making were weighed according to the formula proportions listed in Table 1.

Dough was prepared using a straight dough method and white bread, shiitake stipe bread and silver ear bread were prepared as described [7]. After baking, three loaves from each bread formulation were freeze-dried and ground into a coarse powder (60 mesh) for thermal and X-ray diffraction analysis and SEM examination. The other loaves of breads were then individually put into plastic bags, sealed and stored at 4°C. At days 0, 3, 6, 9 and 12, three loaves from each bread formulation were randomly taken for moisture determination and microbial enumeration. In addition, at day 12, three

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Table 1. The formulations of breads.

Ingredient (%)	White bread	Shiitake stipe bread	Silver ear bread
Wheat flour	100	95	95
Shiitake stipe flour	0	5	0
Silver ear flour	0	0	5
Improver	1	1	1
Milk powder	4	4	4
Salt	1	1	1
Shortening	4	4	4
Sugar	8	8	8
Yeast	1.4	1.4	1.4
Water	64	69	69
Total	183.4	188.4	188.4

Table 2. Moisture content and total plate counts in breads during storage at 4°C for 12 days.

	White bread	Shiitake stipe bread	Silver ear bread
	Moisture content^{m} (%)		
Day 0	$^{a}42.47 \pm 0.24^{A}$	$^{a}43.09 \pm 0.28^{A}$	$^{a}42.77 \pm 0.67^{A}$
Day 3	$^{a}42.02 \pm 0.11^{A}$	$^{a}43.12 \pm 0.18^{A}$	$^{ab}41.91 \pm 0.78^{A}$
Day 6	$^{a}42.00 \pm 0.26^{A}$	$^{b}41.80 \pm 0.31^{B}$	$^{b}41.75 \pm 0.32^{B}$
Day 9	$^{a}42.24 \pm 0.07^{B}$	$^{a}43.34 \pm 0.16^{A}$	$^{a}42.59 \pm 0.38^{B}$
Day 12	$^{a}41.97 \pm 0.05^{A}$	$^{b}42.20 \pm 0.04^{A}$	$^{ab}41.57 \pm 0.87^{A}$
	Total plate count (log CFU/g dry weight)		
Day 0	$^{c}2.50 \pm 0.05^{B}$	$^{c}1.68 \pm 0.11^{C}$	$^{c}2.92 \pm 0.03^{A}$
Day 3	$^{c}2.66 \pm 0.06^{B}$	$^{b}2.03 \pm 0.02^{C}$	$^{b}3.18 \pm 0.01^{A}$
Day 6	$^{b}3.40 \pm 0.17^{A}$	$^{a}2.46 \pm 0.12^{B}$	$^{ab}3.33 \pm 0.05^{A}$
Day 9	$^{b}3.48 \pm 0.02^{A}$	$^{a}2.59 \pm 0.09^{B}$	$^{a}3.59 \pm 0.20^{A}$
Day 12	$^{a}3.69 \pm 0.04^{A}$	$^{a}2.40 \pm 0.14^{C}$	$^{b}3.10 \pm 0.08^{B}$

^{m} Each value is expressed as mean ± standard error (n = 3). Means with different capital letters within a row for the same storage day are significantly different ($P < 0.05$). Means with different small letters within a column are significantly different ($P < 0.05$).

loaves were freeze-dried and prepared for X-ray diffraction analysis.

2. Moisture Determination and Microbial Enumeration

The moisture contents of stored breads were determined according to the method described in Association of Official Analytical Chemists [1]. A portion of stored bread samples (25 g) was homogenized for 2 min in a sterile Waring blender with 225 ml of sterile water. The homogenized samples were serially diluted with sterile water, and 1.0 ml aliquots of the dilutions were inoculated into plate count agar (Difco, Detroit, MI, USA) and the plates were incubated at 37°C. Microbial colonies were counted after incubation for 48 h. The bacterial counts in the bread samples were expressed as $\log_{10}$ colony forming units (CFU)/g dry weight.

3. Thermal Analysis

Thermal analysis was conducted in a Setaram DSC 121 (Setaram Co., Caluire, France). A proportion of freeze-dried sample dispersions (100-140 mg) was put into a stainless crucible (Setaram) with an aluminium O-ring and hermetically sealed by a sample-encapsulating press. These samples were heated from 30 to 350°C increasing at 5°C/min. An empty crucible was used as a reference and indium was used to calibrate the instrument. Peak enthalpy (ΔH in J/g dry weight), and onset (To), peak (Tp) and completion (Tc) temperatures were computed automatically for each bread.

4. X-ray Diffraction

The wide-angle X-ray diffraction (WAXD) analysis was applied to detect the crystallinity of fresh breads and breads stored after 12 days and their patterns were recorded using a Rigaku III diffractometer (Rigaku Corp., Tokyo, Japan) with Cu radiation (40 kV, 30 mA). Experimental data were collected at a scan rate of 1°/min with the scan angle from 2° to 40°.

5. Scanning Electron Microscopy

Three bread samples were first fixed on an SEM sample holder, dried by a critical point dryer (LADD 28000), and then coated with a thin gold layer of 3 mm by a sputter coater (JBS E5150) for conductivity. The microstructure of breads was examined using a Topcon ABT-150s scanning electron microscope (Topcon Corp., Tokyo, Japan).

6. Statistical Analysis

Each measurement was conducted in triplicate. The experimental data were expressed as mean ± standard error and subjected to an analysis of variance for a completely random design using a Statistical Analysis System (SAS Institute, Inc., Cary, NC, USA, 2000). Duncan's multiple range tests were used to determine the difference among means at the level of $\alpha = 0.05$.

III. RESULTS AND DISCUSSION

1. Moisture Contents and Microbial Counts during Storage

Shiitake stipe flour and silver ear flour were used respectively to make shiitake stipe bread and silver ear bread to substitute 5% of wheat flour [3]. No difference in the moisture contents was found among shiitake stipe, silver ear, and pure wheat breads at day 0 (Table 2). After 12 days of storage at 4°C, moisture contents of these breads were in the range of 41.57-43.34%. The moisture loss during storage was not significant (1.18-2.81%), indicating that three kinds of shiitake stipe, silver ear, and pure wheat breads had a good water holding capacity.

At day 0, total plate counts of fresh breads were as high as 2.92 log CFU/g dry weight and were in the descending order of silver ear bread > white bread > shitake stipe bread. After 12 days of storage at 4°C, total plate counts were as high

Table 3. Thermal properties of breads.

	Endotherm[n]			
	To[m] (°C)	Tp[m] (°C)	Tc[m] (°C)	ΔH[m] (J/g)
White bread	51.6 ± 0.5[A]	110.7 ± 0.4[B]	163.1 ± 0.3[C]	158.4 ± 0.1[A]
Shiitake stipe bread	51.0 ± 0.3[B]	111.7 ± 0.5[A]	166.7 ± 0.2[A]	142.8 ± 0.1[B]
Silver ear bread	51.7 ± 0.4[A]	105.2 ± 0.6[C]	164.2 ± 0.2[B]	116.6 ± 0.1[C]

[m] *To*, onset temperature; *Tp*, peak temperature; *Tc*, completion temperature; *ΔH* (J/g dry weight), peak enthalpy.

[n] Each value is expressed as mean ± standard error ($n = 3$). Means with different capital letters within a column are significantly different ($P < 0.05$).

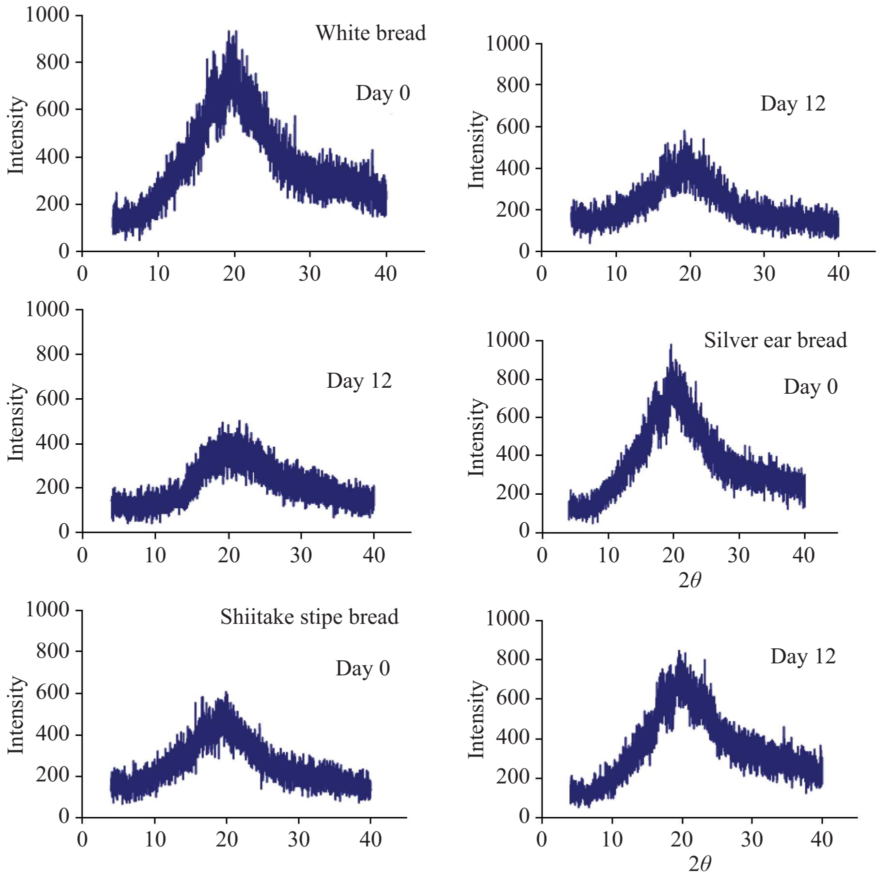


Fig. 1. The X-ray diffraction pattern of breads during storage at 4°C for 12 days.

as 3.69 log CFU/g dry weight and were in the descending order of white bread > silver ear bread > shiitake stipe bread. It seems that shiitake stipe bread showed less microbial growth during storage. However, white bread showed higher total plate counts and its total plate counts were similar to those of silver ear bread during storage. This may be attributed to a higher chitin level present in shiitake stipe, which consequently inhibit microbial growth.

Previous studies isolated fungal chitin from shiitake stipes and obtained a yield of 36.7 g chitin/100 g shiitake stipes [11]. One of the usages of chitin is an antimicrobial agent [8]. The reason for chitin as an antimicrobial agent might attribute to its monomer *N*-acetylglucosamine, which could be charged and detrimental to microbial cells. The addition of 0.2-0.5% (w/w) chitin to dough with 65% moisture caused the decrease in the water activity of bread from 0.43 to 0.38 [5]. The decrease in water activity might attribute to the better water holding capacity of chitin. The less microbial count in shiitake stipe bread might also attribute to its higher chitin content therefore better water holding capacity.

2. Thermal Analysis

Thermal properties of the three kinds of breads were analysed using DSC from 30 to 350°C. Melting endothermic peaks of breads were at 105.2-111.7°C and were in the descending order of shiitake stipe bread > white bread > silver ear bread (Table 3). The onset and completion temperatures of breads were in the range of 51.0°C to 51.7°C and 163.1°C to 166.7°C, respectively. However, the thermal decomposition enthalpies (ΔH) of breads were in the range of 116.6 J/g to 158.4 J/g dry weight and were in the descending order of white bread > shiitake stipe bread > silver ear bread. It seems that in the presence of shiitake stipe and silver ear, no obvious changes in endotherm temperatures was observed.

Previous studies reported that the endothermic peak and ΔH of shiitake stipes were 243.5°C and 33.3 J/g dry weight, respectively [13]. When shiitake stipe flour with high values of the endothermic peak and ΔH was added to replace 5% of wheat flour to make shiitake stipe bread, it resulted in lower ΔH. In addition, when 5% silver ear was added, the ΔH was even lower. Previous studies noted that the higher the enthalpy peak, and the denser the crystallinity would be [13]. Therefore, shiitake stipe bread and silver ear bread should show less dense crystallinity as evidenced by their lower ΔH. The discrepancies in the thermal analysis of the three breads could partially attribute to their different intersheet or intrasheet hydrogen-bonding systems. However, the smaller ΔH in shiitake stipe bread and silver ear bread revealed that these breads were susceptible to collapse during the thermal decomposition process.

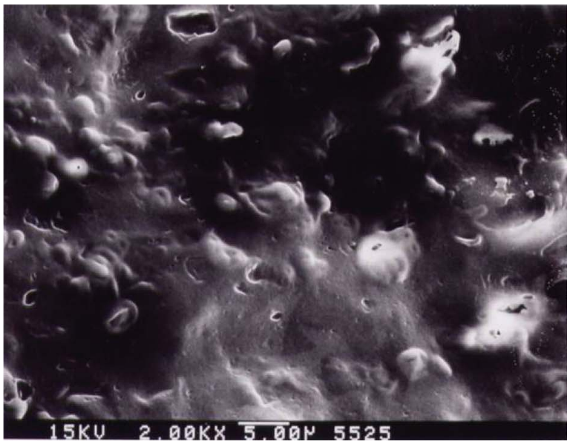
3. X-ray Diffraction Pattern

WAXD patterns of the breads were similar but that of shiitake stipe bread was obviously less intense (Fig. 1). After baking, the WAXD pattern of white bread had its characteristic crystalline peaks at 2θ = 17.45° and 20.22°. At day 0, shiitake stipe bread exhibited two crystalline reflections at 16.78° and 20.12° whereas silver ear bread showed two crystalline reflections at 17.20° and 19.62°. It seems that the replacement of 5% wheat flour with shiitake stipe flour or silver ear flour did not adversely influence the natural crystallinity of bread.

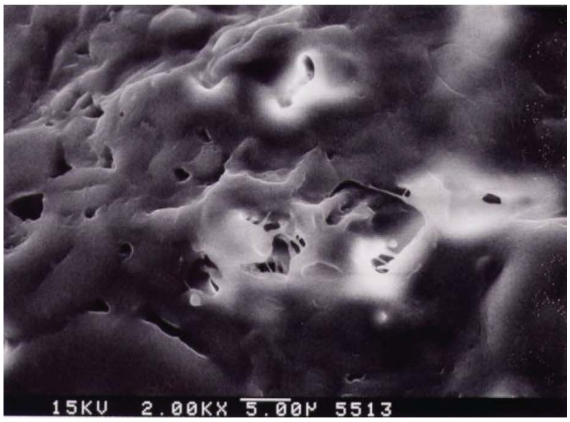
After 12 days of storage at 4°C, the intensity of the WAXD pattern of shiitake stipe bread remained the same; that of white bread decreased slightly; and that of white bread decreased significantly. At day 12, crystalline reflections were observed at 18.05°, 19.20°, 21.53° and 22.59° for white bread, 16.76°, 19.24° and 22.08° for shiitake stipe bread, and 17.53°, 18.31°, 19.54° and 23.02°. The decrease in the intensity of WAXD pattern might be related to the retrogradation of bread starch during storage [2]. Retrogradation is a reaction that takes place in gelatinized starch. This reaction can expel water from the polymer network. This is a process known as syneresis. Therefore, retrogradation is directly related to the aging of bread. Although the WAXD intensity of shiitake stipe bread was low at day 0, the addition of shiitake stipe and silver ear were capable of delaying the retrogradation of starch during storage as shown in their WAXD profiles.

4. Scanning Electron Microscopy

Under electron microscopic examination, the three breads showed aggregated flakes with a few porosities and did not exhibit microfibrillar structure (Fig. 2). Generally, the three breads exhibited comparable crystallinity. This finding was consistent with their similar characteristic peaks in WAXD patterns. Shiitake stipe bread showed the least compact but smoother structure in SEM, which was corresponding to its lowest intensity in WAXD pattern. White bread showed more compact structure in flakes and some aggregated starch crys-



White bread



Shiitake stipe bread


Silver ear bread

Fig. 2. Scanning electron micrographs of breads. Magnifications at ×2000.

talline nodes. It is obvious that the replacement of 5% wheat flour with shiitake stipe flour or silver ear flour did not significantly affect bread morphology.

IV. CONCLUSION

Shiitake stipe flour and silver ear flour were used to substitute 5% of wheat flour to make bread. No difference among white, shiitake stipe and silver ear breads was found in moisture contents at day 0. The moisture loss during storage for 12 days was not significant (1.18-2.81%) as compared to the fresh bread. Total plate counts of fresh breads were as high as 2.92 log CFU/g dry weight at day 0 and 3.69 log CFU/g after 12 days of storage at 4°C. However, shiitake stipe bread showed less microbial growth during storage than white and silver ear breads.

The melting endothermic peaks of breads reached 105.2°C to 111.7°C. The range of thermal decomposition enthalpies was between 116.6 J/g and 158.4 J/g. WAXD patterns of the breads were similar but that of shiitake stipe bread was obviously less intense. After 12 days of storage at 4°C, the intensity of the WAXD pattern of white bread decreased remarkably. The morphology of the bread products was aggregated flakes with a few porosities. Shiitake stipe bread showed the least compact but smoother structure in SEM. In the presence of shiitake stipe flour or silver ear flour, the natural crystallinity and morphology of bread did not obviously change after 12 days of storage. Overall, shiitake stipe and silver ear could be incorporated into bread to provide its beneficial health effects.

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