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Research Article

Quality of Enokitake Supplemented Steamed Bun

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Abstract

Enokitake [*Flammulina velutipes* (Curtis: Fries) Sing.] was incorporated into steamed bun. Quality attributes including specific volume, color and sensory evaluation, and taste components in enokitake supplemented steamed bun were analyzed and compared with those in white steamed bun. Specific volumes were 4.00, 3.45, 3.28 and 2.98 cm^3/g for white steamed bun, for 2%, 5% and 10% enokitake supplemented steamed buns, respectively. All steamed buns had a comparable profile in proximate composition. Enokitake supplemented steamed buns contained more total soluble sugars and total free amino acids. Enokitake supplemented steamed buns showed lower lightness and became browner as more enokitake flour added. Although their sensory results were lower than those of white steamed bun, 2% and 5% enokitake supplemented steamed buns were moderately acceptable. Overall, enokitake could be incorporated into steamed bun to provide its beneficial health effects.

Keywords: Enokitake; *Flammulina velutipes*; Steamed Bun; Specific Volume; Free Amino Acid; 5'-Nucleotide; Color; Sensory Evaluation

1. Introduction

Enokitake [*Flammulina velutipes* (Curtis: Fries) Sing.], also called winter mushroom and golden mushroom, is notable for its abnormal feature of small caps and long stipes (Yang et al., 2001). It is popular in Taiwan and highly valued as a centerpiece of Taiwanese cooking. In addition, enokitake is found to be medically active in several therapeutic effects including anti- inflammation, anti-tumor, blood pressure regulation, hypercholesterolemia, hyperlipidemia, cardiovascular disorders, and chronic bronchitis (Chang and Wasser, 2012).

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Mushrooms have become attractive as functional foods and a source of physiologically beneficial substances such as polysaccharide and fungal chitin (Yen and Mau, 2007; Wasser, 2010). Mushrooms polysaccharides are shown to be effective in anti-inflammation and anti-tumor effects. Besides, chitin possesses many beneficially biological properties such as biocompatibility, biodegradability, hemostatic activity, and wound-healing property (Farkas, 1990). Chitin can be used as an antimicrobial, emulsifying, thickening and stabilizing agent in food industry (Shahidi et al., 1999). However, chitin is always made from crustacean and therefore; it is unacceptable for vegetarian. Therefore, enokitake can be added to food as a supplement to extend and broaden its consumption and provide the beneficial health effects through various food products.

Steamed bun is mainly made of wheat flour, water and yeast and it is consumed all over the Chinese world. Many food ingredients, such as silver ear (Tsai et al., 2010) and shiitake stipe (Tseng et al., 2011), other than those mentioned above, have been included in steamed bun formulation to increase its diversity, nutritional value and product appeal. Accordingly, the objectives of this research were to make enokitake supplemented steamed bun, to evaluate the influence of enokitake flour on steamed bun quality attributes including specific volume, color and sensory evaluation. The taste components including proximate composition, soluble sugars, free amino acids and 5'-nucleotides in enokitake supplemented steamed bun were also studied.

2. Materials and Methods

2.1. Materials

The all-purpose wheat flour, yeast, baking powder, soybean powder, sugar, shortening, sour dough, emulsifier and dough conditioner used in the formula of steamed bun were purchased from a local food ingredient company. Air-dried enokitake was purchased from a local market in Taichung City, Taiwan and ground into a coarse powder (0.43 μm) using a mill (RT-30HS, Rong Tsong Precision Technology Co., Taichung, Taiwan). The raw materials for steamed bun making were weighed according to the formula proportions listed in Table 1.

2.2 Steamed Bun Making

First of all, enokitake flour was mixed with an equal amount of water (2, 5 and 10 mL of water used for 2, 5 and 10 g enokitake flour per 100 g wheat flour) to form a enokitake paste. Then yeast was dissolved in the rest of water at 28°C and was mixed with dry ingredients to form the main paste. Shortening was heated to melt and added into the main paste. The enokitake paste and the main paste were mixed together using a mixer (Dai Lih Machinery Factory, Taichung, Taiwan) at low speed for 2 min, followed by 6 min of mixing at high speed. After complete mixing of the dough, it was rolled into a smooth pastry using a Bench Dough Roller (Dai Lih). The pastry was then rolled up and cut into several buns; each weighed 20-25 g. Buns were placed in the incubator (Yeong Soon Co., Taichung, Taiwan) at 35°C and 65% relative humidity for the fermentation of 12-15 min.

Table 1 The formulation of steamed buns

Ingredient (g or mL)	White steamed bun	Enokitake supplemented steamed bun		
		2%	5%	10%
Wheat flour	100	100	100	100
Enokitake flour	0	2	5	10
Yeast	2	2	2	2
Baking powder	1.6	1.6	1.6	1.6
Soybean powder	2	2	2	2
Sugar	8	8	8	8
Shortening	2	2	2	2
Sour dough	10	10	10	10
Emulsifier	1	1	1	1
Dough conditioner	0.5	0.5	0.5	0.5
Water	50	52	55	60
Total	177.1	181.1	187.1	197.1

Conventional steaming was performed for 7.5-8 min in a steaming basket with a boiling wok under it. Afterwards, steamed buns were taken out of the basket, cooled to room temperature for 30 min and weighed. The specific volume (cm^3/g) was the steamed bun volume divided by the weight of steamed bun. The steamed bun volume was determined by the rapeseed displacement method (AACC, 1988). Three samples from each steamed bun were freeze-dried and ground into a coarse powder (0.43 μm) for further analysis.

2.3 Proximate Analysis

The proximate compositions of flours and steamed buns, including moisture, crude ash, crude fat, crude fiber and crude protein, were determined according to the methods of AOAC (1990). The nitrogen factor used for crude protein calculation was 5.70 for wheat flour, white steamed bun and enokitake supplemented steamed buns (AOAC, 1990) and 4.38 for enokitake flour (Crisan and Sands, 1978). The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat, fiber and protein from 100%. Reducing sugars were determined using the 3,5-dinitrosalicylic acid method as described by James (1995). The absorbance of each sample solution was measured at 540 nm on a Hitachi 2001 spectrophotometer (Tokyo, Japan). Reducing sugars were calculated based on a calibration curve of glucose (Sigma Chemical Co., St. Louis, MO, USA).

2.4 Soluble Sugar Assay

Soluble sugars were extracted and analyzed as described by Mau et al. (1997). Each flour or steamed bun powder (600 mg) was extracted with 50 mL of 80% aqueous ethanol (95% pure, Taiwan Tobacco & Wine Monopoly Bureau, Taipei, Taiwan). This suspension was shaken for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The residue was washed five times with additional 25-mL portions of 80% ethanol. The combined filtrate was then rotary evaporated at 40°C and redissolved in deionized water to a final volume of 10 mL. The aqueous extract was passed through a Millex-HV filter unit (13 mm, Millipore, Billerica, MA, USA), and filtered using a 0.45-μm PVDF filter (Millipore) prior to injection onto high-performance liquid chromatograph (HPLC).

The HPLC system consisted of a Shimadzu LC-10AT *VP* pump, a Rheodyne 7725i injector, a 20-μL sample loop, a Shimadzu RID-10A detector, and a Luna 5μ NH_2 100A column (4.6 × 250 mm, 5 μm, Phenomenex Inc., Torrance, CA, USA). The mobile phase was acetonitrile (LC grade, Tedia Co., Fairfield, OH, USA)/deionized water, 85:15 (v/v) at a flow rate of 1.0 mL/min. Each sugar was identified using the authentic sugar (Sigma) and quantified by the calibration curve of the authentic compound.

2.5 Free Amino Acid Assay

Free amino acids were analyzed according to the method of Mau et al. (1997). Each flour or steamed bun powder (500 mg) was shaken with 50 mL of 100 mM HCl for 45 min at ambient temperature and filtered through Whatman No. 4 filter paper. The filtrate was then passed through a filter unit (Millipore), and filtered using a 0.45-μm PVDF non-sterile filter paper. The purified filtrate was mixed with *o*-phthalaldehyde reagent (Sigma) in an Eppendorf tube, shaken to facilitate derivatization, and then immediately injected onto HPLC.

The HPLC system was the same as for sugar analysis but included a Hitachi L-7485 fluorescence detector with fluorescence excitation at 340 nm and emission at 450 nm, and a LiChrospher 100 RP-18 column (4.6 × 250 mm, 5 μm, Merck, Darmstadt, Germany). The mobile phases were A, 50 mM sodium acetate (pH 5.7) containing 5% tetrahydofuran; B, deionized water; and C, methanol. The gradient was A:B:C 80:0:20 (v/v/v) to 33:0:67 for 0-38 min, 0:33:67 for 38-40 min, and 0:100:0 for 40-43 min. The flow rate was 1.2 mL/min. Each amino acid was identified using the authentic amino acid (Sigma) and quantified by the calibration curve of the authentic compound.

2.6 5'-Nucleotide Assay

5'-Nucleotides were extracted and analyzed as described by Taylor et al. (1981). Each flour or steamed bun powder (500 mg) was extracted with 25 mL of deionized water. This suspension was heated to boiling for 1 min, cooled, and then centrifuged at 11,800 ×*g* for 15 min. The extraction was repeated once with 20 mL of deionized water. The combined filtrate was then evaporated, and filtered prior to HPLC injection in the same manner as in soluble sugar assay.

The HPLC system was the same as for sugar analysis but included a Shimadzu UV detector and a LiChrospher 100 RP-18 column (4.6 × 250 mm, 5 μm, Merck). The mobile phase was 500 mM KH_2PO_4/H_3PO_4 (pH 4.3, Wako Pure Chemical Co., Osaka, Japan) at a flow rate of 1 mL/min and UV detection at 254 nm. Each 5'-nucleotide was identified using the authentic 5'-nucleotide (Sigma) and quantified by the calibration curve of the authentic compound.

2.7 Color Measurement

The reflective surface color of steamed buns was measured using a Σ80 Color Measuring System (Nippon Denshoku Inc., Tokyo, Japan) and *L*, *a* and *b* values were recorded. The *L* value represents lightness component on surface that the value ranges from 0 to 100 for darkness to whiteness while *a* and *b* values are chromatic components of redness (+) to greenness (-) and blueness (+) to yellowness (-), respectively. Each steamed bun sample was individually measured in triplicate. ΔE was calculated on the basis of the following equation (Hsu, 2011):

$$\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2};$$

where L_0, a_0, and b_0 are the values of white steamed buns; and L, a, and b are the values of enokitake supplemented steamed buns.

2.8 Sensory Evaluation

The sensory evaluation was carried out on the samples within 3-6 h of steamed bun making. The samples served were sliced (1.5 cm thick) and evaluated in the class of National Taichung Agricultural Senior High School, Taichung City, Taiwan. In total, 30 consumers with the age ranged from 15-20 years old completed the questionnaire. Sensory attributes of steamed bun, including color, flavor, texture, appearance and overall were measured using a seven-point hedonic scale with 1, 4 and 7 representing extremely dislike, neither like not dislike and extremely like, respectively.

2.9 Statistical Analysis

Each steamed bun formulation was steamed three times and each quality measurement was conducted in triplicate, except for the sensory evaluation (n = 30). The experimental data were 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 0.05.

3. Results and Discussion

3.1 Specific Volume

Because enokitake flour was observed to absorb more water than wheat flour, more water was added for the hydration of enokitake flour in order that the added water in the formula would not affect the hydration of wheat flour and thereby, not affecting the final volume of steamed bun. The observation is consistent with the finding of Tsai et al. (2010) and Tseng et al. (2011), in which silver ear and shiitake stipe were used. Therefore, 2%, 5% and 10% (w/w) extra water were added for 2%, 5% and 10% enokitake supplemented steamed buns, respectively. However, specific volumes (volume/weight) were 4.00, 3.45, 3.28 and 2.98 cm^3/g for white steamed bun, for 2%, 5% and 10% enokitake supplemented steamed buns, respectively. In other words, with 2%, 5% and 10% addition of enokitake flour, specific volumes decreased 13.8, 18.0 and 25.5%, respectively. It is obvious that specific volumes of steamed buns were affected by the addition of enokitake flour and with more enokitake flour added; the steamed buns became smaller as shown in Fig. 1.

Fig. 1. Appearance of dried enokitake (top) and white steamed bun, 2%, 5% and 10% enokitake supplemented steamed buns (from left to right).

3.2 Proximate Composition

Enokitake flour contained more moisture than wheat flour (Table 2). Wheat flour was higher in carbohydrate and reducing sugar contents whereas enokitake flour was higher in ash, fat, fiber and protein contents. The major component of crude fiber is chitin, which is an important structural polysaccharide found in the fungal cell wall (Yen and Mau, 2007). The content of soluble polysaccharides was the residual content calculated by subtracting the reducing sugar content from the carbohydrate content. Soluble polysaccharides were thought to be the biologically active component in mushrooms (Wasser, 2010), and their contents were 5.53 and 36.80% for wheat flour and enokitake flour, respectively. Since 2%, 5% or 10% of enokitake flour was added into the steamed bun formula, the proximate composition, especially soluble polysaccharide, would be affected expectedly.

Moisture content in enokitake supplemented steamed buns was lower than that in white steamed bun. Generally, white steamed bun and enokitake supplemented steamed buns had a comparable profile in proximate composition due to the fact that only 2-10% of enokitake flour was added. As a result, white steamed bun contained more reducing sugar, fat and protein whereas fiber contents in enokitake supplemented steamed buns were higher with more enokitake flour added. In addition, soluble polysaccharide contents (carbohydrate content – reducing sugar content) were 5.12, 28.82, 29.54 and 31.39% for white steamed bun and for 2%, 5% and 10% enokitake supplemented steamed buns, respectively. The soluble polysaccharide contents in enokitake supplemented steamed buns were consistent with the enokitake flour added. However, higher fiber and soluble polysaccharide contents in enokitake supplemented steamed buns were the consequence of enokitake flour added.

Table 2 Proximate composition of flours and steamed buns

Component[1] (%)	Wheat flour	Enokitake flour	White steamed bun	Enokitake supplemented steamed bun		
				2%	5%	10%
Moisture	12.41 ±0.04E[2]	16.97 ±0.04D	38.67 ±0.28A	35.62 ±0.18C	35.94 ±0.26C	36.56 ±0.12B
Dry matter	87.59 ±0.04A	83.03 ±0.04B	61.33 ±0.28E	64.38 ±0.18C	64.06 ±0.26C	63.44 ±0.12D
Carbohydrate	83.37 ±0.34A	50.87 ±1.40C	81.47 ±1.06B	84.06 ±4.42A	82.84 ±3.15AB	82.14 ±2.56B
Reducing sugar	77.84 ±1.04A	14.07 ±0.55F	76.35 ±0.99B	55.24 ±0.96C	53.30 ±1.28D	50.75 ±0.09E
Crude ash	0.48 ±0.01D	9.41 ±0.08A	1.37 ±0.02C	1.39 ±0.04C	1.44 ±0.02C	1.60 ±0.03B
Crude fat	1.53 ±0.03E	3.90 ±0.03A	3.35 ±0.05B	1.70 ±0.12D	2.06 ±0.01C	1.79 ±0.07D
Crude fiber	3.20 ±0.92B	23.98 ±0.23A	0.91 ±0.55E	1.38 ±0.26DE	1.81 ±0.36CD	2.34 ±0.63C
Crude protein	11.42 ±0.19D	11.84 ±0.11A	12.90 ±0.42B	11.47 ±0.13D	11.85 ±0.33CD	12.13 ±0.26C

[1] Moisture and dry matter of flours were presented based on air-dried weight whereas those of steamed buns were presented based on fresh steamed bun weight; other components were presented on dry weight.
[2] Each value is expressed as mean ± SE (n = 3). Means with different letters within a row differ significantly (P < 0.05).

3.3 Soluble Sugars

Wheat flour contained no soluble sugars whereas contents of fructose, glucose and trehalose in enokitake flour were 51.89, 8.50 and 10.40 mg/g, respectively. Trehalose, a major mushroom sugar (Mau et al., 1997), was found in enokitake flour. However, trehalose was not found in enokitake supplemented steamed buns. It is possible that small amount of enokitake flour added was diluted in the steamed bun formula and yeast might use up this soluble sugar during dough fermentation for energy.

Contents of fructose were 10.37, 11.28, 15.40 and 18.43 mg/g and those of glucose were 5.83, 12.38, 12.47 and 12.71 mg/g for white steamed bun, 2%, 5% and 10% enokitake supplemented steamed buns, respectively. It seems that enokitake supplemented steamed buns contained more total soluble sugars than white steamed bun and their total soluble sugar contents increased with more enokitake flour added. Soluble sugars usually contributed a sweet taste (Litchfield, 1967). However, only 16.20-31.14 mg/g of sugars in steamed buns would give a weak sweet perception. Apparently, enokitake supplemented steamed buns would taste sweeter than white steamed bun.

3.4 Free Amino Acids and Taste Components

Enokitake flour had a thoroughly different profile of free amino acids and its total content was about

Table 3 Content of free amino acids of flours and steamed buns

Amino acid	Content[1] (mg/g dw)					
	Wheat flour	Enokitake flour	White steamed bun	Enokitake supplemented steamed bun		
				2%	5%	10%
L-Alanine	0.318 ±0.011B	1.928 ±0.010A	0.223 ±0.017E	0.241 ±0.002D	0.249 ±0.005D	0.271 ±0.002C
L-Arginine	0.062 ±0.005B	0.295 ±0.059A	0.053 ±0.002B	0.064 ±0.004B	0.084 ±0.003B	0.099 ±0.002B
L-Aspartic acid	0.334 ±0.036B	0.591 ±0.113A	0.113 ±0.024C	0.125 ±0.002C	0.142 ±0.003C	0.169 ±0.010C
L-Cysteine	0.892 ±0.025B	31.476 ±0.318A	0.222 ±0.013C	0.454 ±0.020C	0.473 ±0.028C	0.552 ±0.005C
L-Glutamic acid	0.347 ±0.017D	3.032 ±0.012A	0.276 ±0.024E	0.323 ±0.024D	0.397 ±0.016C	0.480 ±0.015B
Glycine	0.021 ±0.005B	0.962 ±0.082A	0.020 ±0.003B	0.036 ±0.009B	0.043 ±0.004B	0.050 ±0.004B
L-Histidine	0.211 ±0.021B	6.961 ±0.752A	0.038 ±0.003B	0.126 ±0.008B	0.250 ±0.015B	0.288 ±0.023B
L-Isoleucine	0.013 ±0.003B	0.686 ±0.083A	0.003 ±0.001B	0.009 ±0.001B	0.011 ±0.002B	0.016 ±0.003B
L-Leucine	0.033 ±0.002D	0.318 ±0.007A	0.011 ±0.003F	0.019 ±0.007E	0.053 ±0.009C	0.069 ±0.009B
L-Lysine	0.479 ±0.017B	5.392 ±0.290A	0.254 ±0.037BC	0.208 ±0.011C	0.186 ±0.027C	0.252 ±0.013BC
L-Methionine	0.063 ±0.004B	1.346 ±0.039A	0.022 ±0.002B	0.033 ±0.004B	0.042 ±0.010B	0.047 ±0.001B
L-Phenylalanine	0.006 ±0.001E	0.488 ±0.006A	0.010 ±0.001E	0.035 ±0.006D	0.058 ±0.002C	0.124 ±0.003B
L-Serine	0.121 ±0.026B	1.526 ±0.118A	0.060 ±0.004D	0.085 ±0.004C	0.097 ±0.003C	0.113 ±0.018B
L-Threonine	0.123 ±0.017B	0.724 ±0.064A	0.015 ±0.002C	0.027 ±0.007C	0.032 ±0.003C	0.037 ±0.003C
L-Tryptophan	0.119 ±0.044B	1.268 ±0.045A	0.042 ±0.002D	0.064 ±0.006CD	0.087 ±0.007CD	0.100 ±0.002BC
L-Tyrosine	0.035 ±0.005D	1.289 ±0.016A	0.016 ±0.002E	0.031 ±0.002D	0.068 ±0.004C	0.087 ±0.011B
L-Valine	0.029 ±0.007C	0.984 ±0.021A	0.015 ±0.004D	0.026 ±0.001C	0.037 ±0.001BC	0.055 ±0.001B
Total	3.206 ±0.065B	59.266 ±0.874A	1.393 ±0.025F	1.906 ±0.088E	2.309 ±0.050D	2.809 ±0.057C

[1] Each value is expressed as mean ± SE (n = 3). Means with different letters within a row differ significantly ($P < 0.05$).

18-folds higher than that of wheat flour (Table 3). After steaming, total contents of free amino acids were 1.393, 1.906, 2.309 and 2.809 mg/g for white steamed bun, 2%, 5% and 10% enokitake supplemented steamed buns, respectively. It seems that higher total free amino acid content was directly the consequence of higher amount of enokitake flour added. In Table 3, free amino acids were divided into several classes on the basis of their taste characteristics (Komata, 1969). Aspartic and glutamic acids were monosodium glutamate-like (MSG-like) components, which gave the umami taste that was the characteristic taste of MSG and 5'-nucleotides (Yamaguchi et al., 1971). Contents of MSG-like and sweet components in white steamed bun and all enokitake supplemented steamed buns were considerately low (< 0.7 mg/g) (Table 4). Apparently, the sweet and bitter tastes brought by free amino acids were insignificant.

Table 4 Content of taste components of flours and steamed buns

Taste component[1]	Content[2] (mg/g dw)					
	Wheat flour	Enokitake flour	White steamed bun	Enokitake supplemented steamed bun		
				2%	5%	10%
MSG	0.681 ±0.030B	3.623 ±0.027A	0.389 ±0.014E	0.448 ±0.039D	0.539 ±0.040C	0.649 ±0.026B
Sweet	0.583 ±0.042B	5.140 ±0.012A	0.318 ±0.044F	0.389 ±0.026E	0.421 ±0.038D	0.471 ±0.048C
Bitter	0.536 ±0.025B	12.346 ±0.753A	0.194 ±0.015B	0.376 ±0.042B	0.622 ±0.062B	0.798 ±0.010B
Tasteless	1.406 ±0.026B	38.157 ±0.120A	0.492 ±0.046E	0.693 ±0.022D	0.727 ±0.070D	0.891 ±0.051C
Total	3.206 ±0.065B	59.266 ±0.874A	1.393 ±0.025F	1.906 ±0.088E	2.309 ±0.050D	2.809 ±0.057C

[1] MSG-like, monosodium glutamate-like, Asp + Glu; sweet, Ala + Gly + Ser + Thr; bitter, Arg + His + Ile + Leu + Met + Phe + Try + Val; tasteless, Lys + Tyr + Cys.
[2] Each value is expressed as mean ± SE (n = 3). Means with different letters within a row differ significantly ($P < 0.05$).

3.5 5'-Nucleotides

Contents of total and flavor 5'-nucleotides in enokitake flour were much higher than those in wheat flour (Table 5). After steaming, contents of total and flavor 5'-nucleotides in enokitake supplemented steamed bun were higher than those in white steamed bun and were higher with higher enokitake flour added. Flavor 5'-nucleotides were found to be 5'-guanosine monophosphate (5'-GMP), 5'-inosine monophosphate (5'-IMP) and 5'-xanthosine monophosphate (5'-XMP) (Mau, 2005). 5'-GMP gave the meaty flavor, and is a flavor enhancer, much stronger than MSG (Litchfield, 1967). The synergistic effect of flavor 5'-nucleotides with MSG-like components might greatly increase the umami taste of soups (Yamaguchi et al., 1971). Based on the contents of MSG-like components and flavor 5'-nucleotides, the contents of umami components of white steamed bun and 2%, 5% and 10% enokitake supplemented steamed buns were expected to be 0.389 + 0.021 mg/g, 0.448 + 0.029 mg/g, 0.539 + 0.031 mg/g and 0.649 + 0.100 mg/g.

Using the equation derived from sensory evaluation (Yamaguchi et al., 1971), the equivalent umami concentration (EUC, g MSG/100 g), which is the concentration of MSG equivalent to the umami intensity given by the mixture of MSG and the 5'-nucleotide, was calculated to be 0.079, 0.111, 0.228 and 0.773 g MSG/100 g for white steamed bun and 2%, 5% and 10% enokitake supplemented teamed buns, respectively. In other words, the umami intensities of 100 g of white steamed bun and 2%, 5% and 10% enokitake supplemented steamed buns were equivalent to those given by 0.079, 0.111, 0.228 and 0.773 g MSG, respectively. It seems that the umami intensities of white steamed bun were considerately low and enokitake supplemented steamed buns would be perceived more umami taste with more enokitake flour added.

Table 5 Content of 5'-nucleotides of flours and steamed buns

5'-Nucleotide[1]	Content[3] (mg/g dw)					
	Wheat flour	Enokitake flour	White steamed bun	Enokitake supplemented steamed bun		
				2%	5%	10%
5'-AMP	nd[4]	0.079 ±0.003A	0.026 ±0.005D	0.032 ±0.002C	0.033 ±0.002C	0.041 ±0.002B
5'-CMP	0.062 ±0.008C	0.982 ±0.019A	0.021 ±0.003E	0.036 ±0.002D	0.051 ±0.001CD	0.096 ±0.005B
5'-GMP	nd	0.161 ±0.003A	0.004 ±0.001E	0.011 ±0.001C	0.007 ±0.001D	0.031 ±0.001B
5'-IMP	nd	2.887 ±0.048A	0.001 ±0.001B	nd	nd	0.002 ±0.001B
5'-UMP	0.058 ±0.002B	0.915 ±0.016A	0.010 ±0.001C	0.015 ±0.002C	0.016 ±0.004C	0.020 ±0.001C
5'-XMP	0.113 ±0.004B	1.039 ±0.379A	0.016 ±0.003B	0.018 ±0.003B	0.024 ±0.002B	0.067 ±0.003B
Flavor 5'-nucleotides[2]	0.120 ±0.006B	4.087 ±0.435A	0.021 ±0.003B	0.029 ±0.002B	0.031 ±0.002B	0.100 ±0.003B
Total	0.233 ±0.008B	6.063 ±0.472A	0.078 ±0.007B	0.112 ±0.002B	0.131 ±0.008B	0.257 ±0.005B

[1] 5'-AMP, 5'-adenosine monophosphate; 5'-CMP, 5'-cytosine monophosphate; 5'-GMP, 5'-guanosine monophosphate; 5'-IMP, 5'-inosine monophosphate; 5'-UMP, 5'-uridine monophosphate; 5'-XMP, 5'-xanthosine monophosphate.
[2] Flavor 5'-nucleotides, 5'-GMP + 5'-IMP + 5'-XMP.
[3] Each value is expressed as mean ± SE (n = 3). Means with different letters within a row differ significantly ($P < 0.05$).
[4] Not detected.

3.6 Color Properties

Enokitake flour showed lower lightness than wheat flour (Table 6). With regard to *a* and *b* values, enokitake flour showed more redness and yellowness. White steamed bun was lighter than enokitake supplemented steamed buns and with more enokitake flour added, enokitake supplemented steamed buns were less light. In addition, enokitake supplemented steamed buns became browner as more enokitake flour added. The slight brown color might be the recognition for enokitake supplemented steamed buns.

Table 6 Color properties of steamed buns

	Content[3] (mg/g dw)					
	Wheat flour	Enokitake flour	White steamed bun	Enokitake supplemented steamed bun		
				2%	5%	10%
L	97.75 ±0.06A[2]	62.99 ±0.28E	84.94 ±0.24B	82.87 ±0.64C	82.09 ±0.39C	79.18 ±0.29D
a	-0.89 ±0.04F	12.09 ±0.10A	0.06 ±0.02E	0.34 ±0.05D	0.71 ±0.07C	2.21 ±0.06B
b	3.42 ±0.04F	21.25 ±0.11A	4.26 ±0.21E	6.36 ±0.13D	7.77 ±0.29C	9.33 ±0.20B
ΔE			0.00D	2.96C	4.56B	7.97A

[1] ΔE: $[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}$; L_0, a_0, b_0 are the values of white steamed bun; L, a, b are the values of enokitake supplemented steamed bun.
[2] Each value is expressed as mean ± SE (n = 3). Means with different letters within a row differ significantly ($P < 0.05$)

Hsu (2011) found that *ΔE* values of 3-6 were acceptable since only professionals could distinguish the difference. The *ΔE* value of > 6.5 was high enough for normal people to notice the color difference (Hsu, 2011). However, 2%enokitake supplemented steamed bun looked different from white bread as shown in Fig. 1 even though its *ΔE* value was only 2.96. Proper color change in bread might attract consumer's attention and affect the consumer's preference and acceptability. It seems that enokitake supplemented steamed buns showed their characteristic color quality.

3.7 Sensory Evaluation

On a seven-point hedonic scale, sensory results were 5.13-5.60, 4.30-5.07, 3.87-4.53 and 3.57-4.37 for white steamed bun and 2%, 5% and 10% enokitake supplemented steamed bun, respectively (Fig. 2). Obviously, although their sensory results were lower than those of white steamed bun, 2% and 5% enokitake supplemented steamed buns were moderately acceptable. White steamed bun showed better results in all sensory attributes than enokitake supplemented steamed buns. For all enokitake supplemented steamed buns, sensory results decreased with more enokitake flour added. The results exhibited that adding enokitake flour in the steamed bun formula did lower steamed bun acceptability. Because enokitake supplemented steamed bun was sensory evaluated without declaring its components and health functionality, the consumer's acceptability might be higher as it is labeled with enokitake added as a new functional product.

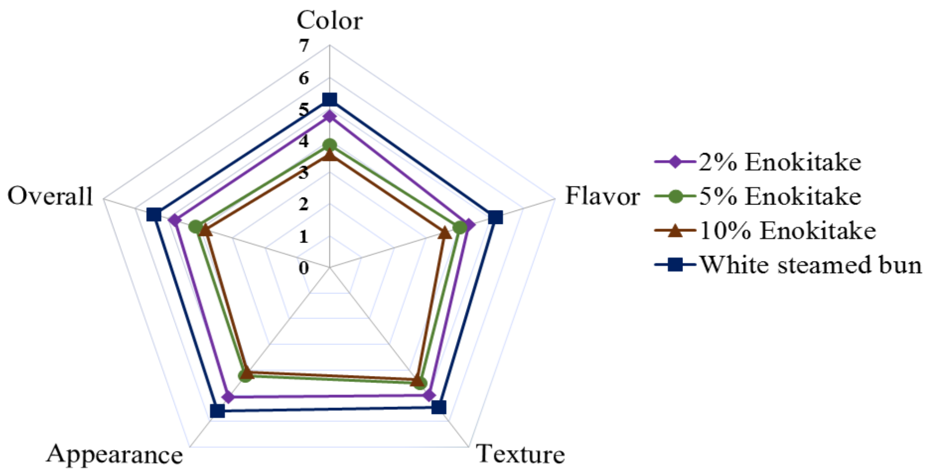

Fig. 2. Radar plot of sensory results of white steamed bun and enokitake supplemented breads.

4. Conclusion

The addition of enokitake flour in the steamed bun formula affected the specific volumes of steamed buns; and with more enokitake flour added, the steamed buns became smaller and browner. Enokitake supplemented steamed buns contained more fiber, total soluble sugars and total free amino acids than white steamed bun. Although their sensory results were lower than those of white steamed bun, 2% and 5% enokitake supplemented steamed buns were moderately acceptable. The results exhibited that adding enokitake flour in the steamed bun formula did lower steamed bun acceptability. Overall, enokitake could be incorporated into steamed bun to provide its beneficial health effects. However, to improve the consumer's acceptability of enokitake supplemented steamed bun, the modification of formula or other manipulations in steamed bun making would be another area of investigation.

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