Quality of Shiitake Stipe Steamed Bun

Yu-Hsiu TSENG[1], Joan-Hwa YANG[2], Chi-En LEE[3] and Jeng-Leun MAU[3]

[1]Department of Culinary Arts, National Kaohsiung University of Hospitality and Tourism, Kaohsiung, Taiwan, ROC; [2]Department of Restaurant and Institutional Management, Shih Chien University, Taipei, Taiwan, ROC; [3]Department of Food Science and Biotechnology, National Chung-Hsing University, Taichung, Taiwan, ROC

Abstract

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Shiitake [*Lentinula edodes* (Berk.) Pegler] stipe was incorporated into steamed bun. Quality attributes including the specific volume, colour and sensory evaluation, and taste components in shiitake stipe steamed buns were analysed and compared with those of white steamed buns. With 2% and 5% additions of shiitake stipe flour, specific volumes of steamed buns increased by 5.8% and 5.0%, respectively. White steamed buns contained more reducing sugar, fat, and protein whereas fiber and soluble polysaccharide contents were higher in shiitake stipe steamed buns. Furthermore, shiitake stipe steamed buns contained more total soluble sugars and total free amino acids. Shiitake stipe steamed buns showed lower lightness and whiteness index values and became browner with more shiitake stipe flour added. On a seven-point hedonic scale, all sensory results were 4.07–5.80. Overall, shiitake stipe could be added into steamed bun formula to provide its beneficial health effects.

Keywords: shiitake stipe; *Lentinula edodes*; steamed bun; specific volume; free amino acid; 5'-nucleotide; colour; sensory evaluation

Shiitake mushroom [*Lentinula edodes* (Berkeley) Pegler], also called forest mushroom and shingku (fragrant mushrooms), is a traditional delicacy in Japan, Korea, Taiwan, and China (Stamets 1993). Shiitake is commonly used as food in Oriental countries, but also as a traditional Chinese medicine. In fact, shiitake has been found to have several therapeutic effects including the anti-inflammatory and anti-tumour activities, blood pressure regulation, effects on hypercholesterolemia, hyperlipidemia, cardiovascular disorders, and chronic bronchitis (Wasser & Weis 1999). The stipe of shiitake is usually discarded due to its tough texture. In addition, Yen and Mau (2007) isolated 36.72% of crude chitin from air-dried shiitake stipes. After drying and grinding, shiitake stipes become a valuable ingredient rich in fungal chitin.

Chitin possesses many beneficially biological properties such as biocompatibility, biodegradability, haemostatic activity, and woundhealing property (Fleet & Phaff 1981; Farkas 1990). Chitin can be used as an antimicrobial, emulsifying, thickening, and stabilising agent in food industry (Shahidi *et al.* 1999). Furthermore, chitin is always made from crustacean and, therefore; it is unacceptable for vegetarians. Therefore, shiitake or shiitake stipes can be added to food as a supplement to extend and broaden its consumption and provide beneficial health effects through various food products.

Steamed buns are mainly made of wheat flour, water, and yeast and are consumed all over the Chinese world. Many food ingredients, other than those mentioned above, have been included in steamed buns formulation to increase their diversity, nutritional value, and product appeal. Accordingly, the objectives of this research were to make shiitake stipe steamed buns, to evaluate the influence of shiitake stipe flour on steamed buns quality attributes including the specific volume, colour and sensory evaluation. The taste components including proximate composition, soluble sugars, free amino acids, and 5'-nucleotides in shiitake stipe steamed buns were also studied.

MATERIALS AND METHODS

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 buns were purchased from a local food ingredient company. Air-dried shiitake stipe was purchased from a local market in Taichung City, Taiwan and ground into a coarse powder (120 mesh) using a mill (RETSCH ultracentrifugal mill and sieving machine, Haan, Germany). The raw materials for steamed buns making were weighed according to the formula proportions are listed in Table 1.

Steamed buns making. First of all, shiitake stipe flour was mixed with twice the amount of water (4 ml, 10 ml, and 20 ml of water used for 2 g, 5 g, and 10 g shiitake stipe flour, respectively). Then yeast was dissolved in the rest of water at 28°C and the dry ingredients were mixed. The shortening was melt and added to the dry ingredients in the liquid phase, together with the dissolved yeast. All the ingredients were mixed 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, this was rolled into smooth pastry using a Bench Dough Roller (Dai Lih). The pastry was then rolled up and cut into several buns of 20–25 g each. The buns were placed into an incubator (Yeong Soon Co., Taichung, Taiwan) at 35°C and 65% RH for the fermentation of 12–15 minutes.

Conventional steaming was performed for 7.5 min to 8 min in a steaming basket with a boiling wok under it. Afterwards, the steamed buns were taken out of the basket, cooled to room temperature for 30 min and weighed. The specific volume (cm^3/g) was calculated as the steamed bun volume divided by the weight of the 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 coarse powder (60 mesh) for further analysis.

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 14.091, 14.103, 14.093, 14.111 and 14.108, respectively (AOAC 1990). The nitrogen conversion factor used for the crude protein calculation was 5.70 for wheat flour, white steamed buns and shiitake stipe steamed buns (AOAC 1990), and 4.38 for shiitake stipe flour (Crisan & Sands 1978). The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat, fiber, and

Table 1. The formulations of steamed buns

Ingredient (g or ml)	White steamed bun	Shiitake stipe steamed bun		
		2%	5%	10%
Wheat flour	100	100	100	100
Shiitake stipe 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	54	60	70
Total	177.1	183.1	192.1	207.1

protein from 100% of dry matter. Total 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. Total reducing sugars were calculated based on the calibration curve of glucose.

Soluble sugar assay. Soluble sugars were extracted and analysed as described by Ajlouni *et al.* (1995). 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 evaporated using a rotary evaporator at 40°C and was redissolved in deionised water to a final volume of 10 ml. The aqueous extract was passed through a Millex-HV filter unit (13 mm, Millipore, Billerica, 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 5 µ NH_2 100A column (4.6 × 250 mm, 5 µm, Phenomenex Inc., Torrance, USA). The mobile phase was acetonitrile (LC grade, Tedia Co., Fairfield, USA)/deionised water, 85:15 (v/v) at a flow rate of 1.0 ml/min. Each sugar was identified using the authentic sugar (Sigma Chemical Co., St. Louis, USA) and quantified by means of the calibration curve of the authentic compound.

Free amino acid assay. Free amino acids were analysed according to the method of Mau *et al.* (1997). Each flour or steamed bun powder (500 mg) was shaken with 50 ml of 0.1N 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 derivatisation, and then immediately injected onto HPLC.

The HPLC system was the same as that 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 – 50mM sodium acetate (pH 5.7) containing 0.5% tetrahydofuran; B – deionised 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 minutes. The flow rate was 1.2 ml/minutes. Each amino acid was identified using the authentic amino acid (Sigma) and quantified by means of the calibration curve of the authentic compound.

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

The HPLC system was the same as that 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 0.5M 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 using the calibration curve of the authentic compound.

Colour measurement. The reflective surface colour of steamed buns was measured using a Σ80 Color Measuring System (Nippon Denshoku Inc., Tokyo, Japan) and L^*, a^*, and b^* values were recorded. A standard white plate (X = 91.98, Y = 93.97 and Z = 110.41) was used to standardise the instrument. Each steamed bun sample was individually measured in triplicate. The whiteness index (WI) was calculated based on the following equation (Hsu *et al.* 2003):

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

Sensory evaluation. The sensory evaluation was carried out on the samples within 24 h after steamed buns making. The samples served were sliced (1.5 cm thick) and evaluated in the class of the National Taichung Agricultural Senior High School, Taichung City, Taiwan. In total, 30 panellists major in food processing with the age ranged from 15–20 years completed the questionnaire. The sensory attributes of the steamed bun, including colour, flavour, texture, and overall appearance were measured using a seven-point hedonic scale with

1, 4, and 7 representing extremely dislike, neither like nor dislike and, extremely like, respectively.

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, USA, 2000). Duncan's multiple range tests were used to determine the difference among means at the level of 0.05.

RESULTS AND DISCUSSION

Proximate composition

Shiitake stipe flour contained less moisture than wheat flour (Table 2). Wheat flour had higher carbohydrate, reducing sugar, and protein contents whereas shiitake stipe flour had higher ash and fiber contents. The content of soluble polysaccharides was represented as the difference obtained by substracting the reducing sugar content from the carbohydrate content. Soluble polysaccharides were thought to be the biologically active component in mushrooms (Wasser & Weis 1999), and their contents were 5.53% and 21.04% in wheat flour and shiitake stipe flour, respectively. Since 2%, 5%, or 10% of shiitake stipe flour was added into the steamed bun formula, the proximate composition, especially the soluble polysaccharide content, would be affected expectedly.

The moisture content in shiitake stipe steamed buns was higher than that in white steamed buns. The higher was the amount of shiitake stipe flour added, the higher was the moisture content in shiitake stipe steamed buns. This is consistent with more water being used in 2%, 5%, and 10% shiitake stipe steamed bun formulae. White steamed buns contained more reducing sugar, fat, and protein whereas the fiber content in shiitake stipe steamed buns was higher with more shiitake stipe flour added. In addition, soluble polysaccharide contents (carbohydrate content – reducing sugar content) were 5.12%, 11.72%, 10.90%, and 9.04% in white steamed buns and 2%, 5%, and 10% shiitake stipe steamed buns, respectively. The soluble polysaccharide contents in shiitake stipe steamed buns were not consistent with the shiitake stipe flour added. It seems that yeast might degrade or utilise a certain amount of soluble polysaccharides during fermentation. However, higher fiber and soluble polysaccharide contents in shiitake stipe steamed buns were the consequence of shiitake stipe flour added.

Specific volumes

Because shiitake stipe flour was observed to absorb more water than wheat flour, more water was added for the hydration of shiitake stipe flour so that the added water in the formula would not affect the hydration of wheat flour and thereby not affect the final volume of steamed buns. An extra 4%, 10%, and 20% water were added for 2%, 5%, and 10% shiitake stipe steamed buns, respectively. However, the specific volumes of steamed buns (volume/weight) were 4.00 ± 0.09, 4.23 ± 0.05, 4.20 ± 0.10, and 3.69 ± 0.06 cm^3/g for white steamed buns and 2%, 5%, and

Table 2. Proximate composition of flours and steamed buns

Component[a] (%)	Wheat flour[b]	Shiitake stipe flour	White steamed bun	Shiitake stipe steamed bun		
				2%	5%	10%
Moisture	12.41 ± 0.04^E	7.79 ± 0.20^F	38.67 ± 0.28^D	39.15 ± 0.65^C	40.90 ± 0.02^B	43.54 ± 0.06^A
Dry matter	87.59 ± 0.04^B	92.21 ± 0.20^A	61.33 ± 0.28^C	60.85 ± 0.65^D	59.10 ± 0.02^E	56.46 ± 0.06^F
Carbohydrate	83.37 ± 2.34^{AB}	40.72 ± 0.33^C	81.47 ± 1.06^B	84.87 ± 2.49^A	84.12 ± 3.39^A	82.46 ± 1.22^{AB}
Reducing sugar	77.84 ± 1.04^A	19.68 ± 1.09^C	76.35 ± 0.99^A	73.15 ± 2.89^B	73.22 ± 0.27^B	73.40 ± 1.44^B
Crude ash	0.8 ± 0.00^E	3.78 ± 0.03^A	1.37 ± 0.02^B	1.01 ± 0.03^D	1.05 ± 0.04^D	1.22 ± 0.10^C
Crude fat	1.53 ± 0.03^B	1.32 ± 0.08^C	3.35 ± 0.05^A	0.65 ± 0.07^E	0.71 ± 0.01^E	0.88 ± 0.15^D
Crude fiber	3.20 ± 0.92^B	46.99 ± 2.85^A	0.91 ± 0.55^C	2.19 ± 0.29^C	2.51 ± 0.95^{BC}	3.12 ± 0.54^B
Crude protein	11.42 ± 0.19^C	7.19 ± 0.09^D	12.90 ± 0.42^A	11.28 ± 0.10^C	11.61 ± 0.22^C	12.32 ± 0.50^B

[a]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

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

10% shiitake stipe steamed buns, respectively. In other words, the specific volumes were affected by the substitution by shiitake stipe flour. With 2% and 5% additions of shiitake stipe flour, the specific volumes increased by 5.8% and 5.0%, respectively, whereas the specific volume of 10% shiitake stipe steamed buns was lower than that of white steamed buns. It seems that 2% and 5% shiitake stipe flour addition would maintain the shape of steamed buns.

Soluble sugars

Wheat flour contained no soluble sugars whereas the contents of fructose, glucose, and trehalose in shiitake stipe flour were 16.65, 17.52, and 7.93 mg/g, respectively. Trehalose, a major mushroom sugar (Mau *et al.* 1997), was found in shiitake stipe flour. However, trehalose was not found in shiitake stipe steamed buns. It is possible that the small amount of shiitake stipe flour added was diluted in the steamed bun formula and yeast might have used up this soluble sugar during dough fermentation for energy.

The contents of fructose were 7.35, 10.73, 11.28, and 13.46 mg/g and those of glucose were 4.09, 5.83, 6.61, and 9.28 mg/g for white steamed buns and 2%, 5%, and 10% shiitake stipe steamed buns, respectively. It seems that shiitake stipe steamed buns contained more total soluble sugars than white steamed buns and their total soluble sugar contents increased with more shiitake stipe flour added. Soluble sugars usually contribute a sweet taste (Litchfield 1967). However, only 11.44–22.74 mg/g of sugars in steamed buns would give a relatively weak sweet perception. Apparently, shiitake stipe steamed buns would taste sweeter than white steamed buns.

Table 3. Content of free amino acids[a] (mg/g dry weight) and taste components of flours and steamed buns

	Wheat flour	Shiitake stipe flour	White steamed bun	Shiitake stipe steamed bun		
				2%	5%	10%
L-Alanine	0.318 ± 0.011^{C}	1.793 ± 0.014^{A}	0.223 ± 0.017^{E}	0.271 ± 0.020^{D}	0.275 ± 0.008^{D}	0.356 ± 0.003^{B}
L-Arginine	0.062 ± 0.005^{D}	1.506 ± 0.055^{A}	0.053 ± 0.002^{D}	0.094 ± 0.010^{D}	0.128 ± 0.005^{C}	0.187 ± 0.010^{B}
L-Aspartic acid	0.334 ± 0.036^{A}	0.618 ± 0.063^{A}	0.113 ± 0.024^{D}	0.128 ± 0.012^{D}	0.159 ± 0.013^{D}	0.213 ± 0.038^{C}
L-Cysteine	0.892 ± 0.025^{B}	9.509 ± 0.277^{A}	0.222 ± 0.013^{D}	0.355 ± 0.006^{C}	0.444 ± 0.004^{C}	0.499 ± 0.058^{C}
L-Glutamic acid	0.347 ± 0.017^{B}	1.354 ± 0.148^{A}	0.276 ± 0.024^{C}	0.296 ± 0.038^{BC}	0.318 ± 0.071^{B}	0.355 ± 0.050^{B}
Glycine	0.021 ± 0.005^{C}	0.287 ± 0.011^{A}	0.020 ± 0.003^{C}	0.031 ± 0.004^{B}	0.039 ± 0.003^{B}	0.040 ± 0.009^{B}
L-Histidine	0.211 ± 0.021^{B}	1.677 ± 0.093^{A}	0.038 ± 0.003^{C}	0.151 ± 0.017^{B}	0.173 ± 0.031^{B}	0.227 ± 0.013^{B}
L-Isoleucine	0.013 ± 0.003^{B}	0.281 ± 0.021^{A}	$0.003 \pm < 0.001^{B}$	0.008 ± 0.001^{B}	0.010 ± 0.002^{B}	0.012 ± 0.002^{B}
L-Leucine	0.033 ± 0.002^{B}	0.438 ± 0.037^{A}	0.011 ± 0.003^{B}	0.017 ± 0.008^{B}	0.022 ± 0.007^{B}	0.033 ± 0.005^{B}
L-Lysine	0.479 ± 0.017^{B}	4.047 ± 0.246^{A}	0.254 ± 0.037^{BC}	0.119 ± 0.018^{C}	0.217 ± 0.027^{C}	0.243 ± 0.037^{BC}
L-Methionine	0.063 ± 0.004^{B}	0.905 ± 0.010^{A}	0.022 ± 0.002^{D}	0.025 ± 0.008D	0.037 ± 0.001^{C}	0.046 ± 0.004^{C}
L-Phenylalanine	$0.006 \pm < 0.001^{D}$	0.114 ± 0.007^{A}	0.010 ± 0.001^{D}	0.018 ± 0.001^{CD}	0.020 ± 0.003^{C}	0.031 ± 0.008^{B}
L-Serine	0.121 ± 0.026B	0.661 ± 0.037^{A}	0.060 ± 0.004^{CD}	0.068 ± 0.002^{D}	0.083 ± 0.007^{CD}	0.108 ± 0.021^{BC}
L-Threonine	0.123 ± 0.017^{B}	0.211 ± 0.021^{A}	0.015 ± 0.002^{C}	0.023 ± 0.003^{C}	0.029 ± 0.002^{C}	0.030 ± 0.007^{C}
L-Tryptophan	0.119 ± 0.044^{B}	0.665 ± 0.025^{A}	0.042 ± 0.002^{C}	0.050 ± 0.004^{C}	0.054 ± 0.002^{C}	0.073 ± 0.002^{C}
L-Tyrosine	0.035 ± 0.005^{BC}	0.436 ± 0.007^{A}	0.016 ± 0.002^{D}	0.026 ± 0.003^{CD}	0.044 ± 0.012^{B}	0.046 ± 0.008^{B}
L-Valine	0.029 ± 0.007^{C}	0.406 ± 0.010^{A}	0.015 ± 0.004^{D}	0.024 ± 0.006^{CD}	0.038 ± 0.007^{B}	0.041 ± 0.005^{B}
Taste component[b]						
MSG	0.681 ± 0.030^{B}	1.972 ± 0.108^{A}	0.389 ± 0.014^{E}	0.424 ± 0.045^{E}	0.477 ± 0.032^{D}	0.568 ± 0.038^{C}
Sweet	0.583 ± 0.042^{F}	2.952 ± 0.126^{A}	0.318 ± 0.044^{E}	0.393 ± 0.016^{D}	0.426 ± 0.016^{C}	0.534 ± 0.041^{B}
Bitter	0.536 ± 0.025^{C}	5.992 ± 0.047^{A}	0.194 ± 0.015^{E}	0.387 ± 0.089^{D}	0.482 ± 0.057^{C}	0.650 ± 0.017^{B}
Tasteless	1.406 ± 0.026^{B}	13.992 ± 0.451^{A}	0.492 ± 0.046^{D}	0.500 ± 0.095^{D}	0.705 ± 0.030^{C}	0.788 ± 0.039^{C}
Total	3.206 ± 0.065^{B}	24.908 ± 0.348^{A}	1.393 ± 0.025^{E}	1.704 ± 0.033^{E}	2.090 ± 0.046^{D}	2.540 ± 0.065^{C}

[a]Each value is expressed as mean ± SE (n = 3); means with different letters within a row are significantly different ($P < 0.05$)
[b]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

Free amino acids and taste components

Shiitake stipe flour had a thoroughly different profile of free amino acids and their total content was about 8-fold higher than that of wheat flour (Table 3). After steaming, total contents of free amino acids were 1.393 mg/g, 1.704 mg/g, 2.090 mg/g, and 2.504 mg/g for white steamed buns and 2%, 5%, and 10% shiitake stipe steamed buns, respectively. It seems that a higher total free amino acid content was directly the consequence of a higher amount of shiitake stipe flour added. In Table 3, free amino acids were divided into several classes on the basis of their taste characteristics, as described by Komata (1969). Aspartic and glutamic acids are monosodium glutamate-like (MSG-like) components, which give the umami taste that is the characteristic taste of MSG and 5'-nucleotides (Yamaguchi *et al.* 1971). The contents of MSG-like and sweet components in white steamed buns and shiitake stipe steamed buns were considerably low (< 1 mg/g dry weight) even though these contents were higher with higher amounts of shiitake stipe flour added. Apparently, the sweet and bitter tastes brought about by free amino acids were insignificant.

5'-Nucleotides

The contents of total and flavour 5’-nucleotides in shiitake stipe flour were much higher than those in wheat flour (Table 4). After steaming, the contents of total and flavour 5'-nucleotides in shiitake stipe steamed buns were much higher than those in white steamed buns and were higher with higher amounts of shiitake stipe flour added. Flavour 5'-nucleotides were found to be 5'-guanosine monophosphate (5'-GMP), 5'-inosine monophosphate (5'-IMP), and 5’-xanthosine monophosphate (5'-XMP) (Chen 1986). 5’-GMP gives the meaty flavour and is a flavour enhancer, much stronger than MSG (Litchfield 1967). The synergistic effect of flavour 5’-nucleotides with MSG-like components may greatly increase the umami taste of soups (Yamaguchi *et al.* 1971). Based on the contents of MSG-like components and flavour 5'-nucleotides, the contents of umami components of white steamed buns and 2%, 5%, and 10% shiitake stipe steamed buns were expected to be 0.389 + 0.021 mg/g, 0.424 + 0.061 mg/g, 0.477 + 0.094 mg/g, and 0.568 + 0.189 mg/g, respectively.

Using the equation derived from the 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, 0.439, 0.654, and 1.091 g MSG/100 g for white steamed buns and 2%, 5%, and 10% shiitake stipe steamed buns, respectively. In other words, the umami intensities of 100 g of white steamed buns and 2%, 5%, and 10% shiitake stipe steamed buns were equivalent to those given by 0.079 g, 0.439 g, 0.654 g, and 1.091 g MSG, respectively. It seems that the umami intensities of white steamed buns was considerably low and with more shiitake stipe

Table 4. Content of 5'-nucleotides[a] (mg/g dry weight) of flours and steamed buns

Nucleotide[b]	Wheat flour	Shiitake stipe flour	White steamed bun	Shiitake stipe steamed bun		
				2%	5%	10%
5'-AMP	nd	nd	0.026 ± 0.005[B]	0.046 ± 0.004[A]	0.050 ± 0.005[A]	0.056 ± 0.016[A]
5'-CMP	0.062 ± 0.008[B]	0.371 ± 0.021[A]	0.021 ± 0.003[D]	0.030 ± 0.002[CD]	0.038 ± 0.007[BC]	0.044 ± 0.004[BC]
5’-GMP	nd	0.148 ± 0.010[A]	0.004 ± < 0.001[D]	0.035 ± 0.003[C]	0.047 ± 0.007[B]	0.056 ± 0.002[B]
5'-IMP	nd	0.450 ± 0.015[A]	0.001 ± < 0.001[D]	0.013 ± 0.006[C]	0.023 ± 0.003[BC]	0.033 ± 0.003[B]
5'-UMP	0.058 ± 0.002[B]	0.438 ± 0.022[A]	0.010 ± 0.001[C]	0.020 ± 0.001[C]	0.023 ± 0.003[C]	0.025 ± 0.002[C]
5’-XMP	0.113 ± 0.004[B]	nd	0.016 ± 0.003[B]	0.013 ± 0.002[B]	0.024 ± 0.011[B]	0.100 ± 0.006[A]
Flavor 5'-nucleotides[c]	0.120 ± 0.006[C]	0.598 ± 0.023[A]	0.021 ± 0.003[F]	0.061 ± 0.003[E]	0.094 ± 0.005[D]	0.189 ± 0.011[B]
Total	0.233 ± 0.008[C]	1.407 ± 0.025[A]	0.078 ± 0.007[E]	0.157 ± 0.004[D]	0.205 ± 0.009[C]	0.314 ± 0.015[B]

[a]Each value is expressed as mean ± SE ($n = 3$); means with different letters within a row are significantly different ($P < 0.05$)
[b]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
[c]Flavor 5'-nucleotide – 5'-GMP + 5'-IMP + 5'-XMP
nd – not detected

Table 5. The colour properties of steamed buns

	Wheat flour	Shiitake stipe flour	White steamed bun	Shiitake stipe steamed bun		
				2%	5%	10%
L^*	97.75 ± 0.06^{A}	78.25 ± 0.05^{F}	84.94 ± 0.24^{B}	83.13 ± 0.41^{C}	80.89 ± 0.50^{D}	79.82 ± 0.22^{E}
a^*	–0.89 ± 0.04^{F}	5.95 ± 0.07^{A}	0.06 ± 0.02^{E}	0.37 ± 0.05^{D}	0.77 ± 0.05^{C}	1.14 ± 0.12^{B}
b^*	3.42 ± 0.04^{F}	15.76 ± 0.07^{A}	4.26 ± 0.21^{E}	6.50 ± 0.12^{D}	7.65 ± 0.14^{C}	8.98 ± 0.16^{B}
WI	95.81 ± 0.03^{A}	72.49 ± 0.04^{F}	84.34 ± 0.23^{B}	81.91 ± 0.36^{C}	79.40 ± 0.38^{D}	77.88 ± 0.16^{E}

WI (whiteness index) = $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$

Each value is expressed as mean ± SE (n = 3); mean with different letters within a column are significantly different ($P < 0.05$)

flour added, in shiitake stipe steamed buns, slight umami taste would be perceived.

Colour properties

Shiitake stipe flour showed higher lightness and WI values than wheat flour (Table 5). With regard to a^*, and b^* values, shiitake stipe flour showed more redness and yellowness. White steamed buns were lighter and whiter than shiitake stipe steamed buns and with more shiitake stipe flour added, shiitake stipe steamed buns were less light and less white. In addition, shiitake stipe steamed buns became browner as more shiitake stipe flour was added. The brown colour might serve for the recognition of shiitake stipe steamed buns.

Sensory evaluation

On a seven-point hedonic scale, all sensory results were in the range of 4.07 to 5.80, indicating that four steamed buns were moderately acceptable (Table 6). No statistically significant difference between white steamed buns and shiitake stipe steamed buns was found only in flavour sensory attribute. White steamed buns showed a better colour, appearance, and overall sensory results than did shiitake stipe steamed buns. The texture was better with 2% shiitake stipe steamed buns but decreased with more shiitake stipe flour added. The results exhibited that adding shiitake stipe flour in the steamed bun formula did lower the steamed bun acceptability. Because shiitake stipe steamed buns were sensory evaluated without declaring its components and health functionality, the acceptability for consumers might possibly be higher if labelled as a new functional product with shiitake stipe added.

CONCLUSION

The addition of 2% and 5% of shiitake stipe flour in the steamed bun formula would not interfere with the specific volume of shiitake stipe steamed buns. White steamed buns contained more reducing sugar, fat, and protein whereas fiber and soluble polysaccharide contents were higher in

Table 6. Sensory evaluation of steamed buns

	White steamed bun	Shiitake stipe steamed bun		
		2%	5%	10%
Colour	5.80 ± 0.18^{A}	4.83 ± 0.21^{B}	4.37 ± 0.19^{B}	4.50 ± 0.27^{B}
Flavor	4.53 ± 0.24^{A}	4.33 ± 0.25^{A}	4.07 ± 0.24^{A}	4.53 ± 0.28^{A}
Texture	4.73 ± 0.22^{AB}	5.07 ± 0.24^{A}	4.67 ± 0.23^{AB}	4.33 ± 0.24^{B}
Appearance	5.53 ± 0.19^{A}	4.67 ± 0.20^{B}	4.50 ± 0.22^{B}	4.70 ± 0.24^{B}
Overall	5.10 ± 0.18^{A}	4.57 ± 0.20^{B}	4.30 ± 0.22^{B}	4.40 ± 0.24^{B}

Seven-point hedonic scale with 1, 4, and 7, representing extremely dislike, neither like nor dislike and extremely like, respectively.

Each value is expressed as mean ± SE (n = 30); means with different letters within a row are significantly different ($P < 0.05$)

shiitake stipe steamed buns. Furthermore, shiitake stipe steamed buns contained more total soluble sugars and total free amino acids than white steamed buns. All steamed buns contained considerably low amounts of MSG-like and sweet components of free amino acids and flavour 5'-nucleotides. Shiitake stipe flour added into the dough resulted in shiitake stipe steamed buns with lower lightness and WI values. In addition, shiitake steamed buns became browner as more shiitake stipe flour was added.

All sensory results indicated that four steamed buns were moderately acceptable. No significant difference between white steamed buns and shiitake stipe steamed buns was found only in flavour sensory attribute. The results exhibited that adding shiitake stipe flour in the steamed buns formula did lower steamed buns acceptability. Overall, shiitake stipe could be incorporated into steamed buns to provide its beneficial health effects. However, to improve the acceptability of shiitake stipe steamed buns, for consumers the modification of the formula or other manipulations in steamed buns making would be another area of investigation.

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Corresponding author:

Dr. Jeng-Leun Mau, National Chung-Hsing University, Department of Food Science and Biotechnology, 250 Kuokuang Road, Taichung 40227, Taiwan, ROC
tel.: + 886 422 854 313, e-mail: jlmau@dragon.nchu.edu.tw