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Quality of bread supplemented with mushroom mycelia

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ABSTRACT

Mushroom mycelia of *Antrodia camphorata*, *Agaricus blazei*, *Hericium erinaceus* and *Phellinus linteus* were used to substitute 5% of wheat flour to make bread. Bread quality, including specific volume, colour property, equivalent umami concentration (EUC), texture profile analysis, sensory evaluation and functional components, was analysed. Mycelium-supplemented bread was smaller in loaf volume and coloured, and had lower lightness and white index values. White bread contained the lowest amounts of free umami amino acids and umami 5′-nucleotides and showed the lowest EUC value. Incorporating 5% mushroom mycelia into the bread formula did not adversely affect the texture profile of the bread. However, incorporating 5% mushroom mycelia into the bread formula did lower bread's acceptability. After baking, mycelium-supplemented bread still contained substantial amounts of γ-aminobutyric acid and ergothioneine (0.23–0.86 and 0.79–2.10 mg/g dry matter, respectively). Overall, mushroom mycelium could be incorporated into bread to provide its beneficial health effects.

1. Introduction

Mushroom fruiting bodies have been used as food and food-flavouring material for centuries due to their unique and subtle flavour. Recently, mushrooms, including fruiting bodies and mycelia, have become attractive as functional foods and as a source of bioactive components (Wasser & Weis, 1999; Tseng, Yang, & Mau, 2008). In Taiwan, *Agaricus blazei* Murrill and *Hericium erinaceus* (Bulliard: Fries) Persoon were precious edible mushrooms whereas *Antrodia camphorata* (Chang & Chou) Wu, Ryvarden & Chang and *Phellinus linteus* (Berkeley & Curtis) Teng were expensive medicinal mushrooms. The cultivation of mushrooms requires a long time to produce fruiting bodies whereas the submerged culture only requires a short time to mass-produce mycelia. Accordingly, mushroom mycelia become an alternative or substitute of mushroom products (Mau, Chang, Huang, & Chen, 2004). However, mycelia are not used directly as food but could be used as food-flavouring materials and food ingredients. Therefore, the incorporation of mycelium into the formulation of different conventional foods to provide beneficial effects is of great interest.

Bread is a staple food, consumed all over the world, and is mainly made of wheat flour, water, salt and yeast. Many food ingredients, such as buckwheat (Lin, Liu, Yu, Lin, & Mau, 2009), yam (Hsu, Hurang, Chen, Weng, & Tseng, 2004) and wheat bran (Al-Saqer, Sidhu, & Al-Hooti, 2000), other than those mentioned above, have been included in bread formulation to increase its variety, nutritional value and product appeal. Besides, mushroom fruiting bodies, including shiitake stipe and silver ear, have been incorporated into bread (Lin, Tseng, Li, & Mau, 2008; Tseng, Yang, Li, & Mau, 2010).

According to the requirement in Chinese National Standards, the substitution percentage for claimed specific bread is 5% (CGPRDI, 1983). Accordingly, the objective of this research was to substitute 5% of wheat flour with mushroom mycelia of *A. blazei*, *H. erinaceus*, *A. camphorata* or *P. linteus* to make bread and to evaluate the influence of mycelia on bread quality, including specific volume, colour property, equivalent umami concentration (EUC), texture profile analysis (TPA) and sensory evaluation. The functional components, including lovastatin, γ-aminobutyric acid (GABA) and ergothioneine, in mushroom mycelium-supplemented bread, were also analysed.

2. Materials and methods

2.1. Materials

The ingredients used in the formulation of bread were high gluten wheat flour (Uni-President Enterprises Corp., Tainan, Taiwan, containing 13.6% protein and 0.43% ash, based on the moisture content of 14%), milk powder (KLIM, Nestle Taiwan Co., Taipei,

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Taiwan), sugar (Taiwan Sugar Corp., Tainan, Taiwan), salt (Taiyen Industrial Corp., Tainan, Taiwan), egg (local market, Taichung, Taiwan), yeast (Puratos Co., Bijgaarden, Belgium), bread improver {S-5000, Puratos, containing soya flour, emulsifier E472e (di-acetyltartaric esters of monoglycerides [datem]), sucrose, wheat flour and flour treatment agent E300 (ascorbic acid)} and shortening (refined oil blend, Uni-President). Freeze-dried mycelia of *A. blazei*, *A. camphorata*, *H. erinaceus* and *P. linteus* were obtained from the Biotechnology Centre, Grape King Inc., Chungli, Taiwan and were ground in a mill (RT-30HS, Rong Tsong Precision Technology Co., Taichung, Taiwan), and screened through a 0.5 mm sieve.

The raw materials for white bread-making were weighed according to the formula proportions as follows: 100 g of wheat flour, 4 g of milk powder, 10 g of sugar, 1.5 g of salt, 52 ml of water, 8 g of egg, 1.5 g of yeast, 1 g of bread improver and 10 of g shortening, with a total of 188 g. The raw materials for mushroom mycelium-supplemented bread were weighed according to the above formula proportions, except for 95 g of wheat flour, 5 g of mycelia powder and 57 ml of water, with a total of 193 g.

2.2. Bread making and processing

Dough was prepared, using a straight dough method, on the basis of the method of Lin et al. (2008). After baking, the finished product in tin pan was taken out of the oven, cooled to room temperature for 2 h and weighed. For each type of bread, three loaves were freeze-dried and ground into a coarse powder (0.5 mm) for further analysis. The specific volume (cm^3/g) was the bread volume divided by the weight of bread and the bread volume of loaves was determined by the rapeseed displacement method (AACC, 1988). For the storage study, bread samples were sliced and packed in polypropylene bags and stored at 4 °C for 6 days. At days 0, 2, 4 and 6, six slices from each type of bread were randomly taken for TPA measurement.

2.3. Proximate analysis

The proximate composition of flours and breads, including moisture, crude ash, crude fat, crude fibre 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 crude protein calculation was 5.70. The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat, fibre and protein from 100% of dry matter.

2.4. Colour measurement

The reflective surface colour of breads was measured on crumb, using a Σ80 Colour Measuring System (Nippon Denshoku Inc., Tokyo, Japan), and *L*, *a* and *b* values were recorded. The *L* value represents lightness component on the surface, and 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 type of bread sample was individually measured in triplicate. Whiteness index (WI) was calculated on the basis of the following equation (Lin et al., 2009):

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

2.5. Assays of umami amino acids and 5′-nucleotides and equivalent umami concentration

Free umami amino acids and umami 5′-nucleotides were extracted and analysed according to the method of Mau, Chyau, Li, and Tseng (1997) and Taylor, Hershey, Levine, Coy, and Olivelle (1981), respectively. Each umami amino acid (aspartic [Asp] and glutamic acids [Glu]) and 5′-nucleotide were identified using the authentic amino acid and 5′-nucleotide (Sigma Chemical Co., St. Louis, MO, USA), respectively and then quantified by the calibration curve of the authentic compound. EUC [g monosodium glutamate (MSG) per 100 g] is the concentration of MSG equivalent to the umami intensity given by the mixture of MSG and the 5′-nucleotide and is represented by the following addition equation (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971):

$$Y = \Sigma a_i b_i + 1218(\Sigma a_i b_i)(\Sigma a_j b_j)$$

where *Y* is the EUC of the mixture in terms of g MSG/100 g; a_i is the concentration (g per 100 g) of each umami amino acid; a_j is the concentration (g per 100 g) of each umami 5′-nucleotide [5′-inosine monophosphate (5′-IMP) or 5′-guanosine monophosphate (5′-GMP)]; b_i is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077); b_j is the RUC for each umami 5′-nucleotide to 5′-IMP (5′-IMP, 1 and 5′-GMP, 2.3); 1218 is a synergistic constant, based on the concentration of g per 100 g used.

2.6. Texture profile analysis

Fresh or stored bread samples were cut into cubes with the lengths of 2.5 × 2.5 cm and all texture measurements were carried out using a TA-XA2 Texture Analyser (Stable Micro System Co., Godalming, UK) with a 25 kg load cell. The settings for TPA curve were as follows: P30C cylinder probe (30 mm diameter), pre-test speed of 2 mm/s, test speed of 2 mm/s, post speed of 2 mm/s, compression distance of 50%. Each type of bread sample was individually measured in hexplicate for every two storage days.

2.7. Sensory evaluation

The sensory evaluation was carried out on the bread samples within 3–6 h of baking. The samples served were sliced (1.5 cm thick) by a bread slicer (Rhino CM-36, Taipei, Taiwan) and evaluated in the Inventec Corp., Taichung City, Taiwan. With an age range from 25–45 years old, totally 54 untrained consumers completed the questionnaire. Sensory attributes of bread, including appearance, colour, flavour, mouthfeel 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.

2.8. Determination of lovastatin, GABA and ergothioneine

Lovastatin, GABA and ergothioneine were extracted and analysed according to the method of Chen, Ho, Hsieh, Wang, and Mau (2012). Three components were quantified by the calibration curve of the respective authentic standard (Sigma).

2.9. Statistical analysis

For each type of bread, every quality measurement was conducted in triplicate, except for TPA ($n = 6$) and sensory evaluation ($n = 54$). The experimental data were expressed as means ± 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 test was used to determine the difference among means at the level of 0.05. After multiple comparisons, the means in the following tables are followed with different letters "a–e", based on their values and statistical differences. Where a mean is followed with "ab", this mean is