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Comparison of free amino acids and 5′-nucleotides between *Tuber* fermentation mycelia and natural fruiting bodies

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ABSTRACT

The profiles of free amino acids and 5′-nucleotides were first compared between *Tuber* fermentation mycelia and natural fruiting bodies. A total of 20 free amino acids and five 5′-nucleotides were identified in the *Tuber* fermentation mycelia and natural fruiting bodies. Not only the total contents of the free amino acids and 5′-nucleotides, but also the contents of umami amino acids and flavour 5′-nucleotides in the fermentation mycelia were higher than those in the fruiting bodies. By the addition of soybean flour in the fermentation media, the flavour 5′-nucleotides content in the fermentation mycelia was significantly increased, and the equivalent umami concentration of the fermentation mycelia (i.e., 608.07 g/100 g) was approximately 38.1–93.4 times higher than those of the fruiting bodies. From the viewpoint of umami taste, this work confirms the potentiality of *Tuber* fermentation mycelia as the alternative resource for its fruiting bodies.

1. Introduction

Truffles, belonging to *Tuber* genus, are a hypogeous fungus that establishes an ectomycorrhizal symbiosis with trees and shrubs. Due to its characteristic aroma and delicious taste, truffles are the precious and expensive delicacies that are widely used in the famous French and Italian cuisines. Because of the decrease in the natural production of truffles combined with the increase in worldwide demand, a new way to produce truffles on a large scale is urgently needed. By taking the Chinese truffle *Tuber sinense* as a typical example, our group have developed a novel submerged fermentation process for the production of mycelia and its bioactive metabolites for the first time (Tang, Zhu, Li, Mi, & Li, 2008). This process is considered a potential alternative resource for truffles, and it may also be helpful for other mushroom fermentations for bioactive metabolite production. Furthermore, the chemical compositions of fermentation system, i.e., volatile organic compounds (Li, Wang, & Tang, 2011), androstenol (Wang, Li, Li, & Tang, 2008), nucleosides and nucleobases (Liu, Li, Li, Wan, & Tang, 2011), and fatty acids (Tang, Li, Li, Wan, & Tang, 2011) were also investigated, and the *Tuber* fermentation system and fruiting bodies were confirmed to be partly similar in their volatile organic compounds (Li et al., 2011), androstenol (Wang et al., 2008), nucleosides and nucleobases (Liu et al., 2011), and fatty acids (Tang et al., 2011).

The water-soluble taste components, such as free amino acids and 5′-nucleotides, make important contributions to the typical mushroom flavour (Litchfield, 1967). The free amino acids impart the food taste with a smooth feeling, thereby soften a sharp taste from some substances. Therefore, the combination of free amino acids always gives rise to a unique natural flavour (Mau, 2005). In addition, monosodium glutamate (MSG)-like amino acids (i.e., aspartic acid and glutamic acid), also called umami amino acids, give the most typical mushroom taste (Yamaguchi, 1979). The 5′-nucleotides, including 5′-cytosine monophosphate (5′-CMP), 5′-uridine monophosphate (5′-UMP), 5′-adenosine monophosphate (5′-AMP), 5′-inosine monophosphate (5′-IMP), 5′-guanosine monophosphate (5′-GMP), and 5′-xanthosine monophosphate (5′-XMP), are commonly detected in mushrooms (Mau, 2005). 5′-AMP, 5′-IMP, 5′-GMP, and 5′-XMP, which also give the umami or palatable taste, were considered to be umami 5′-nucleotides (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971).

Umami taste, also called the palatable taste or the perception of satisfaction, is related to an overall flavour perception induced or enhanced by MSG, and considered to be the predominant flavour of mushrooms (Yamaguchi, 1979). Recently, researchers found the specific taste cell receptor on the tongue (mGluR4) for umami which

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proves that umami is a basic taste rather than a feeling factor (Chaudhari, Landin, & Roper, 2000). In particular, the umami taste of mushrooms can be synergistically increased by the combination of umami amino acids and umami 5′-nucleotides (Yamaguchi et al., 1971). Through the equation derived from sensory evaluation (Yamaguchi et al., 1971), the equivalent umami concentration (EUC) has often been calculated to understand the mushrooms umami-like taste characteristics (Cho, Choi, & Kim, 2010; Huang, Tsai, Lee, & Mau, 2006; Mau, Lin, Ma, & Song, 2001; Tsai, Weng, Huang, Chen, & Mau, 2006; Tsai, Wu, Huang, & Mau, 2007; Tseng, Lee, Li, & Mau, 2005). In addition, it was noteworthy that the EUC values and umami sensory intensities exhibited the same patterns in pine-mushrooms of different grades (Cho et al., 2007; Cho et al., 2010). However, to the best of our knowledge, the umami-like taste compound profiles in the *Tuber* fruiting bodies and fermentation mycelia have never been investigated before.

In this work, the profiles of free amino acids and 5′-nucleotides between the *Tuber* fermentation mycelia and natural fruiting bodies were compared. More precisely, this work includes the following four parts: (1) the comparison of free amino acids between *Tuber* fermentation mycelia and fruiting bodies; (2) the comparison of 5′-nucleotides between *Tuber* fermentation mycelia and fruiting bodies; (3) the comparison of EUC values between *Tuber* fermentation mycelia and fruiting bodies; and (4) the investigation on the effect of fermentation media on the EUC value of *Tuber* fermentation mycelia. This work will serve as a useful database for the nutritional or nutraceutical evaluation of both *Tuber* fruiting bodies and fermentation mycelia. Furthermore, it may guide *Tuber* submerged fermentation process.

2. Materials and methods

2.1. Tuber fruiting bodies collection and fermentation mycelia culture

The fruiting bodies of *T. sinense*, *Tuber aestivum*, *Tuber indicum*, *Tuber himalayense*, and *Tuber borchii* var. *sphaerospermum* were purchased from the Kunming Rare Truffle Co. Ltd. (Yunan province, China), and the *Tuber* fruiting bodies were collected by Mr. Jian-Ming Wu, who is a very experienced wild edible truffle expert. After harvest, the truffle fruiting bodies were immediately stored in a refrigerator at −20 °C. After freeze-drying, the dried fruiting bodies were pulverised and then passed through a 250-μm stainless sieve.

The strains of *Tuber melanosporum*, *T. sinense* and *T. indicum* were provided by Mianyang Institute of Edible Fungi (Sichuan, China), and the strain of *T. aestivum* was provided by the Huazhong University of Agriculture (Hubei, China). Except otherwise mentioned, the fermentation mycelia were cultured under the following basal media: 35 g/l sucrose, 5 g/l peptone, 5 g/l yeast extract, 0.5 g/l $MgSO_4{\cdot}7H_2O$, 1 g/l KH_2PO_4, and 0.05 g/l Vitamin B_1, and the details of the culture procedure has been previously described (Tang et al., 2008). In order to investigate the effect of media on the equivalent umami concentration (EUC) of fermentation mycelia, the fermentation mycelia of *T. melanosporum* was cultured in the following three proposed media, i.e., corn media: the addition of 5 g/l corn syrup in the basal media; soybean media: the addition of 5 g/l soybean flour in the basal media; the corn and soybean media: the addition of 5 g/l soybean flour and 5 g/l corn syrup in the basal media. All the freeze-dried samples were pulverised and then subjected to pass through a 250-μm stainless sieve.

2.2. Free amino acid assay

Free amino acids were extracted and analysed as the method described by Mau, Chyau, Li, and Tseng (1997). Freeze-dried powder (500 mg) was shaken with 50 ml of 0.1 N HCl (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for 45 min at ambient temperature and filtered through filter paper. The filtrate was then filtrated by using a 0.22 μm filter prior to analysis.

The analysis of free amino acids was conducted with a Hitachi Model 835-50 high speed amino acid analyser (Hitachi, Tokyo, Japan).

2.3. 5′-Nucleotide assay

5′-Nucleotides were extracted and analysed as the method described by Taylor, Hershey, Levine, Coy, and Olivelle (1981). Freeze-dried 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 11,000×*g* for 30 min. The extraction was repeated twice. The combined supernatants were then evaporated and redissolved in deionised water to a final volume of 10 ml. The supernatant was filtrated using a 0.22 μm filter prior to HPLC analysis.

The analysis of 5′-nucleotides were preformed on Dionex Ultimate 3000 system (Dionex, USA), equipped with an on-line degasser, two solvent delivery pumps, and a diode array detector. The column used for separation was Synergi Hydro RP-18 column (250 × 4.6 mm, 4 μm, Phenomenex) fitted with a C18 guard column (Phenomenex). The optimised mobile phase was 4 mM KH_2PO4 aqueous solution, whose pH was adjusted to 2.0 by H_3PO_4. The column oven temperature was maintained at 35 °C and the flow rate at 1.0 ml min^{-1}. The detection wave was fixed at 254 nm. Each 5′-nucleotide was identified by matching its retention time with that of an authentic standard (Sigma) in the HPLC chromatogram and quantified by the calibration curve of the authentic compound.

2.4. Equivalent umami concentration (EUC)

The equivalent umami concentration [EUC, g monosodium glutamate (MSG)/100 g] is the concentration of MSG equivalent to the umami intensity given by the mixture of umami amino acids and umami 5′-nucleotides and is represented by the following addition equation (Yamaguchi et al., 1971):

$$Y = \sum a_i b_i + 1218\left(\sum a_i b_i\right)\left(\sum a_j b_j\right)$$

where Y is the EUC of the mixture in terms of g MSG/100 g; a_i is the concentration (g/100 g) of each umami amino acid [aspartic acid (Asp) or glutamic acid (Glu)]; a_j is the concentration (g/100 g) of each umami 5′-nucleotide [5′-inosine monophosphate (5′-IMP), 5′-guanosine monophosphate (5′-GMP), 5′-xanthosine monophosphate (5′-XMP) or 5′-adenosine monophosphate (5′-AMP)]; 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; 5′-GMP, 2.3; 5′-XMP, 0.61; and 5′-AMP, 0.18); and 1218 is a synergistic constant based on the concentration of g/100 g used.

2.5. Statistical analysis

The statistical data were processed and a one-way ANOVA was performed using the SPSS 16.0 software (Chicago, USA). To evaluate the difference of free amino acids and 5′-nucleotides contents in the *Tuber* samples, a post hoc analysis was performed using Tukey's test. Differences were considered significant when $p < 0.05$. Hierarchical Cluster Analysis (HCA) is a multivariate analysis technique, which is used to sort samples into groups. In our study, the results were confirmed by Hierarchical Cluster Analysis (HCA), and the Between-groups linkage cluster method, the