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Improved extraction methods for simultaneous recovery of umami compounds from six different mushrooms

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ABSTRACT

Given the importance of umami compounds in culinary and food industries, this study sought to develop improved methods for simultaneous extraction of various umami taste components such as free amino acids (FAAs) and 5′-mononucleotides from shiitake mushroom (*Lentinula edodes*), oyster mushroom (*Pleurotus ostreatus*), tea tree mushroom (*Agrocybe aegerita*), and white, brown and portobello champignons (*Agaricus bisporus*). An optimized HPLC method was developed for efficient analysis of 5′-mononucleotides. Using multiple response optimization approach, optimum conditions required for extraction of total FAAs, total 5′-mononucleotides and total umami compounds were determined. The highest recovery of FAAs was obtained when mushrooms were extracted at room temperature for 180 min, while the highest 5′-mononucleotides were recovered by extracting at 70 °C for 30 min. Based on the desirability function, the optimal parameters of temperature, time and solvent volume for simultaneous recovery of umami components were found to be 70 °C, 30 min and 50 mL, respectively. Using the optimized method, white champignons and tea tree mushrooms were found to be the richest source for umami components. Water was found to be a better solvent than conventionally used 0.1 M HCl for extraction of FAAs from mushrooms.

1. Introduction

Umami is the fifth basic taste, characterized by a pleasant savory flavor. It was first discovered by Professor Kikunae Ikeda and he coined the term ‘umami’, meaning delicious (Ikeda, 1908). Umaminess has been attributed mainly to the presence of monosodium glutamate (MSG), and free amino acids (FAAs), such as aspartic acid (Asp) and glutamic acid (Glu), and 5′–mononucleotides, such as 5′-guanosine monophosphate (GMP), 5′-inosine monophosphate (IMP) and 5′-xanthosine monophosphate (XMP) (Yamaguchi et al., 1971). Studies have revealed that most of these compounds act synergistically on taste enhancement (Zhang et al., 2008, 2013, 2013). Several taste receptors for the umami taste have been discovered, which includes G protein-coupled receptors, such as mGluR4, mGluR1, mGluR2 and mGluR3 for glutamate and the heterodimer T1R1/T1R3 receptors for amino acids and 5′-mononucleotides (Chaudhari et al., 2000; Kinnamon, 2009; Li et al., 2002).

Umami has received great interest in recent years due to its distinctive health and nutritional applications. Excess intake of salt (sodium chloride) is linked to hypertension and other cardiovascular diseases, and is a major global public health concern (Mugavero et al., 2014). Dietary umami compounds act as potent alternative for table salt. Studies have revealed that addition of glutamate to food preparations enhances saltiness, thus allowing significant reductions in salt usage without compromising taste (Ball et al., 2002). Umami has the potential to regulate appetite and satiety. Recent studies have revealed that the consumption of MSG preloaded foods results in less consumption of total energy and energy from sweet and high-fat foods (Imada et al., 2014; Masic and Yeomans, 2013), thus controlling obesity. Its taste appeal can also help in nutritional management of sick and elderly individuals (Mouritsen, 2012).

Mushrooms are considered a rich source of umami substances. Many recent studies have revealed that several commonly cultivated mushrooms possess a wide variety of umami substances including Asp, Glu and 5′-mononucleotides in substantial quantities (Zhang et al., 2013). Shiitake mushrooms (*Lentinus edodes*) are widely used in preparation of *Dashi,* a famous cooking stock in Japanese cuisine. Oyster mushrooms (*Pleurotus ostreatus*) and champignons (*Agaricus bisporus*) are regularly used as raw ingredients in the preparation of various dishes, as well as food stocks, replacing chicken stocks. Champignons are commercially available in three varieties, white, brown and portobello. The tea tree mushrooms (*Agrocybe aegerita*), also called willow mushrooms, have

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woody flavor and champignons (*Agaricus bisporus*) are regularly used as raw ingredients in the preparation of various dishes, as well as food stocks, replacing chicken stocks. Champignons are commercially available in three varieties, white, brown and portobello. The tea tree mushrooms (*Agrocybe aegerita*), also called willow mushrooms, have woody flavor and a meaty texture and are used in various Chinese dishes, including stew, soups, and hot pot.

In recent years, there has been a growing interest among food industries in producing natural umami formulations as there is a negative perception among consumers for using foodstuffs labeled with 'added MSG' (Radam et al., 2010). Besides, industries are looking for efficient processing conditions to extract umami components from natural sources and use them as potential food ingredients. Many previous studies have reported extraction and quantification of MSG-like amino acids and 5′-mononucleotides in separate experiments aimed at identification of these two classes of molecules in mushrooms. In nearly all cases, 0.1 M HCl was used as solvent for the extraction of MSG-like amino acids (Li et al., 2014; Phat et al., 2016; Tsai et al., 2009, 2007). However, the use of dilute HCl as an extraction solvent is not feasible in food preparations. Moreover, from the processing point of view, simultaneous extraction of these two classes of compounds in a single process is more economical and provides reliable quantitative data for further processing of food products. The present work aims at the identification and quantification of umami components from six different cultivated mushrooms including shiitake mushroom, oyster mushroom, tea tree mushroom, white champignon, brown champignon and portobello champignon. Emphasis has been placed on improvement and systematic optimization of extraction methods for simultaneous extraction of umami amino acids and nucleotides.

2. Materials and methods

2.1. Mushroom samples

Dried shiitake mushroom, oyster mushroom and white champignon were obtained from OSCAR A/S, Rønnede, Denmark. Brown champignon and portobello champignon were obtained from a local supermarket in Copenhagen, Denmark and further dried in a hot air oven (Binder, Germany) at 35 °C for 2 days and air dried for an additional 2 days. Dried tea tree mushroom was obtained from local producers in Sichuan province, China. The moisture content in the final samples was 10%. All samples were ground into fine powder using a coffee grinder before performing extraction experiments.

2.2. Chemicals

Amino acid standards mix, containing alanine (Ala), sarcosine (Sar), glycine (Gly), valine (Val), α-aminobutyric acid (ABA), β-aminoisobutyric acid (β-AIB), leucine (Leu), allo-isoleucine (aILE), isoleucine (Ile), threonine (Thr), serine (Ser), proline (Pro), aspartic acid (Asp), α-aminoadipic acid (AAA), methionine (Met), 4-hydroxyproline (Hyp), glutamic acid (Glu), phenylalanine (Phe), ornithine (Orn), lysine (Lys), glutamine (Gln), asparagine (Asn), histidine (His), hydroxylysine (Hly), tyrosine (Tyr), tryptophan (Trp), cystine (C–C), was purchased from Phenomenex, Værløse, Denmark. 5′-Mononucleotide standards used, including adenosine 5′-monophosphate disodium salt (AMP, ≥99.0%), cytidine 5′-monophosphate disodium salt (CMP, ≥99%), guanosine 5′-monophosphate disodium salt hydrate (GMP, ≥99%), inosine 5′-monophosphate disodium salt hydrate (IMP, ≥99.0%), uridine 5′-monophosphate disodium salt (UMP, ≥99%), were obtained from Sigma-Aldrich, Germany, while xanthosine 5′-monophosphate disodium salt (XMP, ≥ 98%) was obtained from Carbosynth, Compton, UK.

2.3. Extraction of umami compounds

Homogenized samples (1 g) were soaked in required volume (20, 35, or 50 mL) of Milli Q water in screw-capped flasks and agitated in a temperature controlled (± 0.1 °C) shaking water bath at varying temperatures (20, 47, or 70 °C) and times (30, 105, or 180 min), according to the experimental design (Section 2.7). For comparison, experiments with 0.1 M HCl as extraction solvent instead of water were carried out. After completion of extraction, samples were filtered through Whatman® Grade 41 filter papers under vacuum and the filtrates were stored at −20 °C until further analysis.

2.4. Free amino acids analysis

Free amino acid analysis was carried out according to Barba et al. (2017) with minor modifications. Briefly, each sample (100 µL) was mixed with norvaline solution (200 µM, 100 µL) in a shell vial and aspirated through a sorbent tip containing strong cation exchange particles. The sorbent bed was carefully washed with aqueous propanol solution (33%, 200 µL). Amino acids were then eluted using an eluting medium (200 µL) composed of 0.33 M NaOH, 80% propanol and 20% 3–picoline. The eluted amino acids were derivatized using propyl chloroformate/chloroform/isooctane solution (2:6:2 v/v/v, 50 µL). The sample was vortexed for 1 min and isooctane/chloroform solution (90:10 v/v, 100 µL) was added and vortexed again for 1 min. Hydrochloric acid (1 M, 100 µL) was then added to the sample and vortexed for 1 min. The sample was allowed to separate into two phases and the upper layer containing amino acid derivatives was transferred to an insert and placed in an autosampler vial for GC analysis. A multiple point (n = 5) internal standard calibration curve was used for quantification of amino acids. FAAs concentrations are expressed as mg of FAAs per g of dry matter sample (mg/g DM).

GC analysis was performed using an Agilent 6890 gas chromatograph (Agilent, USA) equipped with a flame ionization detector (FID). A ZB–AAA column having dimensions 10 m × 0.25 mm × 250 µm was used for separation of amino acids. Hydrogen was used as carrier gas with a flow rate of 1 mL/min. 3 µL sample was injected at 250 °C in split mode (1:10 ratio). Oven program was set as: 32 °C/min from 110 to 320 °C. The detector was maintained at 320 °C.

2.5. 5′-Mononucleotide analysis

5′-mononucleotide analysis was carried out using an Agilent 1200 HPLC (Agilent, Santa Clara, CA) equipped with a diode array detector. Each sample was mixed with an equal volume of 6% acetic acid and centrifuged at 12000*g* for 20 min. The upper layer was filtered through a 0.22-µm membrane filter and 10 µL of filtrate were used for injection. Analytes were separated using a SUPELCOSIL LC-18-T HPLC column (25 cm length × 4.6 mm internal diameter × 5 µm particle size; Sigma-Aldrich) at ambient temperature and detected at 254 nm.

A reversed-phase HPLC method was developed for separation of nucleotides. KH_2PO_4 buffer (solvent **A**) and 100% methanol (solvent **B**) were used as mobile phase for gradient mode separation. Five different buffer pH levels (4.4, 4.6, 4.8, 5.2 and 5.6) and four different buffer concentrations (10, 20, 50 and 100 mM) were tested for efficient separation of 5′-mononucleotides. In addition, several gradient modes were compared to determine the optimal condition for their separation. The optimized gradient conditions were as follows: 0–10 min 0% **B**, 28–45 min 20% **B**, 46–61 min 0% **B**. The flow rate was 0.5 mL/min. A multilevel calibration curve (n = 3) was constructed using standards for quantification of nucleotides. All analyses were performed in triplicates. 5′-Mononucleotide concentrations are expressed as mg of 5′mononucleotide per g of dry matter (mg/g DM).