Food Chemistry 247 (2018) 23–28

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Free amino acids and 5′-nucleotides in Finnish forest mushrooms

Hanna Manninen[a,*], Minna Rotola-Pukkila[b], Heikki Aisala[c], Anu Hopia[b], Timo Laaksonen[a]

[a] Laboratory of Chemistry and Bioengineering, Tampere University of Technology, P.O. Box 541, FI-33101 Tampere, Finland
[b] Functional Foods Forum, Faculty of Medicine, University of Turku, FI-20014 Turun Yliopisto, Finland
[c] University of Turku, Department of Biochemistry, Food Chemistry and Food Development, FI-20014 Turku, Finland

ARTICLE INFO

Chemical compounds studied in this article:
L-Glutamic acid (PubChem CID: 33032)
L-Aspartic acid (PubChem CID: 5960)
Guanosine 5′-monophosphate (PubChem CID: 6804)
Inosine 5′-monophosphate (PubChem CID: 8582)

Keywords:
Amino acids
5′-Nucleotides
Umami
Mushrooms

ABSTRACT

Edible mushrooms are valued because of their umami taste and good nutritional values. Free amino acids, 5′-nucleotides and nucleosides were analyzed from four Nordic forest mushroom species (*Lactarius camphoratus*, *Boletus edulis*, *Cantharellus cibarius, Craterellus tubaeformis*) using high precision liquid chromatography analysis. To our knowledge, these taste components were studied for the first time from *Craterellus tubaeformis* and *Lactarius camphoratus*. The focus was on the umami amino acids and 5′-nucleotides. The free amino acid and 5′-nucleotide/nucleoside contents of studied species differed from each other. In all studied samples, umami amino acids were among five major free amino acids. The highest concentration of umami amino acids was on *L. camphoratus* whereas *B. edulis* had the highest content of sweet amino acids and *C. cibarius* had the highest content of bitter amino acids. The content of umami enhancing 5′-nucleotides were low in all studied species.

1. Introduction

Edible wild mushrooms are a highly valued food because of their pleasant taste properties. Furthermore, mushrooms are low in energy and fat contents and have high amounts of dietary fibers (Longvah & Deosthale, 1998; Manzi, Aguzzi, & Pizzoferrato, 2001). They are also great supplements of protein and essential amino acids (Longvah & Deosthale, 1998; Mattila, Salo-Väänänen, Könkö, Aro, & Jalava, 2002) and good sources of certain vitamins (vitamin B_2, niacin and folates) and minerals (K, P, Zn, Cu) (Mattila et al., 2001). Moreover high contents of vitamin D_2 and ergosterol have been found in wild forest mushrooms (Mattila, Lampi, Ronkainen, Toivo, & Piironen, 2002). Thus, edible mushrooms are a healthy addition to a diet.

Volatile compounds, especially carbonyl compounds and alcohols, such as 1-octen-3-ol and 1-octen-3-one, contribute to the aroma of mushrooms (Pyysalo & Suihko, 1976) whereas non-volatile compounds, like free amino acids, 5′-nucleotides, sugars, polyols and organic acids contribute to the taste of edible mushrooms (Beluhan & Ranogajec, 2011; Mau, 2005). Edible mushrooms have an especially rich umami taste, which makes them palatable and a potential raw material for the food spice industry (Zhang, Venkitasamy, Pan, & Wang, 2013). Umami, which is described as savory, meaty or brothy taste, was named and originally identified as the salt of L-glutamic acid by Kikunae Ikeda in 1908 (Ikeda, 1909, 2002). Umami taste is caused by the salts of two amino acids, L-glutamic acid (L-Glu) and L-aspartic acid (L-Asp), binding to umami taste receptors T1R1 + T1R3 (Nelson et al., 2002) and mGluR4 (Chaudhari, Landin, & Roper, 2000). L-glutamic acid has a much stronger umami taste than L-aspartic acid (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971). Also 5′-nucleotides 5′-ionosine monophosphate (5′-IMP), 5′-guanosine monophosphate (5′-GMP), 5′-xanthosine monophosphate (5′-XMP) and 5′-adenosine monophosphate (5′-AMP) attribute to the umami taste. 5′-nucleotides enhance the umami flavor in order 5′-GMP > 5′-IMP > 5′-XPM > 5′-AMP (Yamaguchi et al., 1971). They work in synergy with amino acids by intensifying the taste sensation by binding to the same T1R1 + T1R3 receptor as glutamate (Mouritsen & Khandelia, 2012; Zhang et al., 2013).

Taste properties of mushrooms have been studied from East Asian (Mau, Lin, Chen, Wu, & Peng, 1998; Mau, Lin, Ma, & Song, 2001; Tsai, Tsai, & Mau, 2008; Yang, Lin, & Mau, 2001), East African (Mdachi, Nkunya, Nyigo, & Urasa, 2004) and Southern European species (Beluhan & Ranogajec, 2011), but there is a gap in knowledge in taste properties of northern mushroom species. Umami taste of mushrooms is affected by different factors such as maturity stage and quality, storage time and conditions, species type and also the sub-strains of different species (Zhang et al., 2013). Different climate and thus different flora of

* Corresponding author.
E-mail addresses: hanna.manninen@tut.fi (H. Manninen), minna.rotola-pukkila@utu.fi (M. Rotola-Pukkila), heikki.aisala@utu.fi (H. Aisala), anu.hopia@utu.fi (A. Hopia), timo.j.laaksonen@tut.fi (T. Laaksonen).

https://doi.org/10.1016/j.foodchem.2017.12.014
Received 1 September 2017; Received in revised form 23 November 2017; Accepted 6 December 2017
Available online 07 December 2017

northern countries gives a unique breeding ground for mushroom species. Specific knowledge of their taste properties could promote industrial utilization of this great natural resource and increase common interest towards conservation of their distribution areas in northern woodlands. The annual crop of edible Finnish mushrooms is about 1200 million kilos (Salo & Lindroos, 2008). Only a fraction of it is picked mainly for home use and only a small part of it is sold (Turtiainen, Saastamoinen, Kangas, & Vaara, 2012). In a survey executed in 2011 (Turtiainen et al., 2012) it was found that chanterelles (*C. cibarius*) and milkcaps formed each about 20% of annual crop picked in Finland (23 and 21%, respectively), whereas ceps (*B. edulis)* and other boletus species formed 14% and russulas 2%. Other mushroom species, such as false morels (*Gyromitra esculenta*) and funnel chanterelles (*C. tubaeformis*), composed 40% of annual crop picked. To our knowledge, the taste properties of even some of the most common Nordic mushroom species, such as milkcaps and funnel chanterelles have not been investigated before.

In this study free amino acids and nucleotides and their corresponding nucleosides were measured from four edible Finnish forest mushroom species. 26 amino acids and 5 nucleosides were measured. The studied species were chosen so that a comparison with literature could be done (*C. cibarius* and *B. edulis*). Also, species (*L. camphoratus* and *C. tubaeformis*) were chosen, the taste properties of which have not been measured before.

2. Materials and methods

2.1. Solvents and reagents

Amino acid standards used were either 2500 μmol/l standard solutions in 0.1 M HCl (Amino acid mixture standard solution, Type H, Wako Pure Chemical Industries, Ltd. (Osaka, Japan)) or dissolved solid standards (L-asparagine monohydrate (≥99%), L-glutamine (≥99%), L-tryptophan (≥99%) and L-theanine (≥97%) from Wako pure chemicals, 4-aminobutyric acid (≥99%), beta-alanine (≥99%), L-citrulline (≥98%), L-ornitihine monohydrochloride (≥99%) and taurine (≥99%) from Sigma Aldrich, St. Louis, Missouri, USA). For spiking experiments, corresponding liquid amino acid mix from Honeywell Fluka chemicals (Morris Plains, New Jersey, USA) and solid standards of L-glutamic acid (≥99.5%) and L-aspartic acid (≥99%) from Sigma Aldrich were used. Nucleotides and nucleosides (adenosine 5′-monophosphate sodium salt (≥99%), uridine 5′-monophosphate disodium salt (≥99%), cytidine 5′-monophosphate disodium salt (≥99%), guanosine 5′-monophosphate disodium salt hydrate (≥99%), inosine 5′-monophosphate disodium salt (≥98%), inosine (≥99%), guanosine (≥98%), cytidine (≥99%), uridine (≥99%) and adenosine (≥99%)) used in this study were purchased from Sigma Aldrich. Because adenosine 5′-monophosphate sodium salt, inosine 5′-monophosphate disodium salt and guanosine 5′-monophosphate disodium salt hydrate contain an unspecified amount of water (under 20, 27 and 26% relatively) and adenosine 5′-monophosphate sodium salt also a maximum 8% of sodium, the results slightly overestimate the concentrations of these substances.

Sodium hydroxide (≥99%), boric acid (≥99.5%) and potassium dihydrogen phosphate (≥99%) used in the analysis were purchased from Merc KGaA (Darmstadt, Germany). Potassium phosphate dibasic (≥98%) and 3-mercaptopropionic acid (≥99%) were from Sigma Aldrich, 35% HCl (35–38%), methanol (HiPerSolv CHROMANORM® gradient for HPLC) and acetonitrile (HiPerSolv CHROMANORM® Super gradient for HPLC) were from VWR Chemicals (Radnor, Pennsylvania, USA), ethanol anhydr. from Yliopiston Apteekki (Helsinki, Finland) and 85% orthophosphoric acid (85–90%), o-phthalaldehyde (≥98%) and 9-fluorenylmethyl chloroformate from MP Biomedicals (Santa Ana, California, USA).

2.2. Samples

Four species of Nordic forest mushrooms, chanterelle (*Cantharellus cibarius*), funnel chanterelle (*Craterellus tubaeformis*), porcini (*Boletus edulis*) and curry milkcap (*Lactarius camphoratus*), were studied. The chanterelles (3.3 kgs) were collected during mid-August of 2016 from the south-western coast of Finland and bought from a local market. Porcinis (3.4 kgs), curry milkcaps (0.4 kgs) and a quarter of funnel chanterelles (1.0 kgs) were collected during early or mid-September of 2016 from the south-west coast of Finland. The rest of the funnel chanterelles (2.7 kgs) were bought during early September of 2016 from mushroom pickers in the Kainuu region in eastern Finland. The samples were cleaned with a brush and cut to pieces (width 1 *cm*) within 36 h of collection. The samples were vacuum packed and cooked at 80 °C for 10 min. The samples were cooled in water (room temperature) for 2 min and in ice water (5–9 °C) for 5 min and then frozen at −20 °C. Frozen samples were cut to 0.5 cm pieces, pooled, and put back in a freezer.

The samples were kept in a freezer at −20 °C for 5–6 months. Samples were moved to −40 °C a day before freeze-drying. The samples were weighed in small plastic containers in batches of about 30 g and freeze-dried in vacuum at −40 °C for 27–29 h. 8–9 batches of 30 g were freeze-dried at the same time. Freeze-dried mushroom samples were ground using a mortar and pestle until a fine powder was reached. The samples were weighed before and after freeze-drying and dry matter content was calculated based on the lost weight to ensure the operation of the freeze-drying method. Dry matter contents of the mushroom species are presented in Table 1. The dry matter content of mushroom species varied between 77.7 and 145.2 g/kg. In a review by Kalač (2013) dry matter content in mushrooms in general was estimated to be between 60 and 140 g/kg. Thus, the species in this study fit to these margins except for *L. camphoratus*, which had a dry matter content of 145.2 g/kg on average, slightly above the range given by Kalač. The samples of *L. camphoratus* were slightly dehydrated when picked, which could explain this difference. Additionally, the samples in our study were vacuum cooked and kept in the freezer before analysis.

2.3. Instrumentation

The samples were analyzed with UHPLC (Nexera X2, Shimadzu, Kyoto, Japan). The apparatus used consisted of Shimadzu Nexera X2 quaternary pump (LC-30AD) combined with two degassers (DGU-20A3R, DGU-20A5R), autosampler (SIL-30AC), column oven (CTL-20AC) and detectors (diode array (SPD-M20A) and fluorescence (RF-20AXS)) connected to a computer equipped with Shimadzus LabSolutions-software (LC/GC).

2.4. Extraction

The same extraction method was used for the extraction of FAAs (free amino acids) and nucleotides/nucleosides. The method was modified from Ranogajec, Beluhan, and Šmit (2010). Freeze-dried and ground samples (ca. 0.5 g) were weighed in centrifuge tubes. 20 ml of ultrapure water was added, and the samples were carefully shaken until fully mixed. Samples were heated for 1 min in boiling water (100 °C) and kept in an ultrasound bath for 10 min (23 °C in the beginning).

Table 1
Dry matter content of mushroom species. n = number of freeze-dried samples.

	n	Dry matter ± STD [g/kg]
C. cibarius	6	80.4 ± 5.6
C. tubaeformis	10	77.7 ± 5.6
B. edulis	7	102.4 ± 4.6
L. camphoratus	5	145.2 ± 5.1