Title: Free amino acids and 5'-nucleotides in Finnish forest mushrooms

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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.

Highlights

- The taste compounds of four Nordic mushroom species were analyzed
- MSG-like amino acids were among five major FAAs in all studied species
- *L. camphoratus* had the highest content of umami amino acids
- Contents of umami enhancing nucleotides were low in all studied species

Keywords: amino acids, 5'-nucleotides, umami, mushrooms

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)

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 amount 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 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, 2002; Ikeda, 1909). 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 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, Ma, & Song, 2001; Mau, Lin, Chen, Wu, & Peng, 1998; 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 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 fraction of it is picked mainly for home use and only 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 the comparison with literature could

be done (*C. cibarius* and *B. edulis*). Also species the taste properties of which have not been measured before (*L. camphoratus* and *C. tubaeformis*) were chosen.

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 unspecified amount of water (under 20, 27 and 26 % relatively) and adenosine 5'-monophosphate sodium salt also in 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 kilograms) were collected during mid-August of 2016 from south-western coast of Finland and bought from a local market. Porcinis (3.4 kilograms), curry milkcaps (0.4 kilograms) and a quarter of funnel chanterelles (1.0 kilograms) were collected during early or mid-September of 2016 from south-west coast of Finland. Rest of the funnel chanterelles (2.7 kilograms) were bought during early September of 2016 from mushroom pickers in Kainuu region in eastern Finland. The samples were cleaned with a brush and cut to pieces (width 1 cm) within 36 hours of collection. The samples were vacuum packed and cooked at 80°C for 10 minutes. The samples were cooled in water (room temperature) for 2 minutes and in ice water (5–9 °C) for 5 minutes and then frozen at -20 °C. Frozen samples were cut to 0.5 cm pieces, pooled, and put back to 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 weighted in small plastic containers in batches of about 30 grams and freeze-dried in vacuum at -40 °C for 27–29 hours. 8–9 batches of 30 grams were freeze-dried at the same time. Freeze-dried mushroom samples were ground using mortar

and pestle until fine powder was reached. The samples were weighted 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 60–140 g/kg. Thus, the species in this study fit to these margins except for *L. camphoratus*, which has 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 freezer before analysis.

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

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 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, & Šmit (2010).

Freeze-dried and ground samples (ca. 0.5 grams) were weighted 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). Samples were centrifuged at 2525g with Heraeus (Hanau, Germany) Biofuge primo centrifuge for 10 min and the supernatant was collected in 50 ml measuring flasks. The treatment was repeated three times for each sample. During the second repetition, 15 ml of water was added, and during third repetition 10 ml. The collected supernatants were mixed and the measuring flask was filled with water to a volume of 50 ml. For the amino acid and nucleotides/nucleoside analyses, the sample solutions were diluted with water in ratios 1:5 or 1:4 respectively. The solutions were finally filtered with a 0.20-µm RC syringe filter. Five repetitions of each mushroom species were prepared for both FAA and nucleotide/nucleoside analyses.

The validation of the extraction method was studied by spiking experiments and residual extraction for both amino acids and nucleotides/nucleosides. Spiking was carried out by adding standard solution to the sample before the first repetition of the extraction method. In the case of the nucleotides/nucleosides, 1 ml of each standard stock solution (500 mg/l) was added to reach a final concentration of 2.5 mg/l. In the case of the amino acids, the final added standard concentration was 5 µmol/l (for amino acids originating from liquid standard) or 10 µmol/l (solid amino acid standards and glutamic and aspartic acid). The spiking was carried out using samples of *C. tubaeformis* and replicated three times for both compound groups. To calculate recovery, three samples without standard addition were prepared. The recovery percent was calculated by subtracting the FAA/nucleotide/nucleoside contents of samples without standard addition from concentrations of samples with spiking and dividing it then with the concentration of added standard and multiplying it with 100.

The residual extraction was carried out by adding 10 ml of water to the precipitate after three extraction rounds. The extraction routine (heating, ultrasound bath, centrifuging) was done once and the collected extract was diluted to 50 milliliters. The residual extraction was carried out using *B. edulis* samples and replicated three times. Residue percent was calculated by dividing the content of compounds measured after residual extraction with content of compounds measured before residual extraction and multiplying it with 100.

2.5. 5'-nucleotide and nucleoside analysis

The nucleotide/nucleoside contents were analyzed by the method modified from Ranogajec, Beluhan, & Šmit (2010). The nucleotide/nucleoside composition of samples was analyzed using UHPLC with diode array detector at wavelength 254 nm. The column used was Synergi Hydro 4u Hydro-RP 80 Å 150*3.0 mm (Phenomenex, Torrace, California, USA) with Security Cartidges AQ C18 4*2.0 mm pre-column. The solvents used were A: 20 mM phosphate buffer (pH 5.8) and 100 % MeOH. The gradient program was: 3–12 min, 0 →30 % B; 12–13.50 min, 30 % B; 13.50–16 min, 30 → 0 % B; 16 – 25 min, 0 % B with a total time of 25 minutes. The injection volume was 5 µl and the needle was washed after injection with water and 20 % ACN. The calibration curve was collected using seven different concentrations (20, 10, 5, 2.5, 2, 1, and 0.5 mg/l). 10 nucleotide/nucleoside standards were used. Stock solutions were prepared by diluting 5 mg of solid standard in 10 ml of water.

2.6. Free amino acid analysis

The free amino acid content of the samples was analyzed with HPLC with a method modified from the technical note of Shimadzu (Shimadzu Corporation). The fluorescence detector was used with excitation/emission wavelengths of 340/450 and 266/305 nm. The compounds were separated on a 100*4.6 mm Kinetex 2.6 µm C18 100Å column (Phenomenex) with a SecurityGuard ULTRA cartridge UHPLC C18 pre-column for 4.6 columns (Phenomenex) on an AJO-9000 holder.

The samples were derivatized as described on the technical note of Shimadzu. In short, the samples were derivatized with o-phthalaldehyde and 3-mercaptopropionic acid in 0.1 M borate buffer solution and 9-fluorenyl methyl chloroformate in acetonitrile. Acidic phosphate buffer (pH 2.1) was added to the solutions during derivatization. Solvents used in the gradient program were A: 20 mM phosphate buffer with pH 6.5 and B: 45/40/15 ACN/MeOH/H_2O. The gradient program used was 0–2 min, 11% B; 2–4 min, 11→17 % B; 4–5.5 min, 17→31 % B; 5.5–10 min, 31→32.5 % B; 10–12 min, 32.5→46.5 % B; 12–15.5 min, 46.5→55 % B; 15.5–16 min, 55→100 % B; 16–19.5 min, 100 % B; 19.5–20 min, 100→11 % B; 20–25 min, 11% B. The needle was washed from outside after every injection with 80 % MeOH and 20 % ACN. The temperature of the column oven was 35 degrees and injection volume 1 µl. Calibration curves were constructed using nine different concentrations (125, 50, 25, 20, 15, 10, 5, 2.5, 1 µmol/l). In total 26 standards were used. Nine amino acid standards were prepared using solid standards and the rest using the liquid standard solution. Solid standards were diluted in 0.1 M HCl to get a 5000 µmol/l stock solution. A diluted 125 µmol/l stock solution was prepared from these stock solutions by adding 0.125 ml of each stock solution from solid standards and 0.250 ml of liquid standard solution in a 5 ml measuring bottle and diluting them with water. In addition, a 250 µmol/l standard was prepared from the liquid standard solution and used in calibration. All dilutions were prepared using ultrapure water.

2.7. Statistical analysis

Differences between mushroom samples in each analyzed compound were tested with one-way analysis of variance (ANOVA) with square-, or cube-transformed data, if necessary. Tukey's HSD or Tamhane's T2 test was used for post hoc tests as directed by the tested homogeneity of variance. If the data was not normally distributed, nonparametric tests (Kruskal-Wallis and Mann-Whitney using Bonferroni correction) were used. Level of statistical significance was

set to $p<0.05$ in all tests. The tests were performed with IBM SPSS Statistics 24.0 (IBM, Corporation, Armonk, NY).

3. Results and discussion

3.1. Method validation

To validate the extraction method both the content of compounds after residual extraction and the conservation of standards during extraction routine was studied. The percentage of nucleotides/nucleosides remaining after residual extraction and the percentage of added standard remaining after the extraction method are presented in Table 2. On average, the residual content was 3.4 % and with many compounds, residue was not detected at all. Added standards seem to have been well preserved. The average was 97.3 % and the lowest recovery was detected with 5'-IMP (75.4 %).

The percentage of amino acids remaining after residual extraction and percentage of added standard remaining after extraction method are presented in Table 3. Based on these results, the extraction method seems to remove amino acids from the sample material efficiently. The percentage of amino acids remaining in the precipitate was 3.7 % on average. In the majority (19/23) of the studied amino acids < 5 % was left in the precipitate after three rounds of extraction. The percentage remaining was < 10 % in all studied FAAs, except in L-methionine with 10.2 % percentage. The spiking experiments showed that the amino acids are well preserved during extraction. On average 86.1 % preservation was measured. Only differing results are L-histidine and L-cystine with 151.4 and 26.3 % recovery respectively. To conclude it can be expressed that apart from a few exceptions in both groups of compounds, the extraction method effectively removes the FAAs and nucleotides/nucleosides from the starting material and the compounds are well retained. Thus the results collected with this method are reliable.

Limit of detection (LOD) and limit of quantification (LOQ) for both amino acids and nucleotides/nucleosides were estimated based on S/N-ratio calculated with coefficients 3 and 10. LOD and LOQ values are presented in tables 2 and 3. For all nucleotides and nucleosides, the linear range was 0.5–20 mg/l and with values of $r^2 > 0.999$. For free amino acids, the linear range was 1–125 µmol/l or 1–250 µmol/l (FAAs from liquid standard) with values of $r^2 > 0.997$.

Table 2. Validation of the extraction and measurement method (nucleotides/nucleosides). The proportions of averages and standard deviations of the residual nucleotides/nucleosides compared to the original samples (n=3, number of extractions and analyzes made from freeze-dried samples of each species) and the proportions of averages and standard deviations of the standard remaining after extraction method used (n=3, number of extractions and analyzes made from freeze-dried samples of each species) are shown. Negligible stds are not shown. Limits of detection and quantification for nucleotides and nucleosides in milligrams in liter.

Compound	*Residue %*	*Recovery %*	*LOD [mg/l]*	*LOQ [mg/l]*
5'-AMP	2.5	89.7 ± 12.9	0.07	0.24
5'-CMP	1.8	88.4 ± 12.4	0.15	0.48
5'-GMP	1.2	93.1 ± 15.2	0.11	0.37
5'-IMP	n.d.	75.4 ± 5.9	0.17	0.55
5'-UMP	-	94.7± 10.9	0.13	0.44
Adenosine	8.2 ± 0.1	104.8 ± 3.9	0.04	0.15
Cytidine	n.d.	97.5 ± 9.0	0.12	0.39
Guanosine	n.d.	108.8 ± 4.3	0.03	0.11
Inosine	n.d.	108.4 ± 5.0	0.04	0.15
Uridine	n.d.	112.6 ± 7.9	0.05	0.16
Average	3.4	97.3	0.09	0.30

n.d. not detected, - not possible to quantify

Table 3. Validation of the extraction method (free amino acids). The proportions of averages and standard deviations of the residual FAAs compared to the original samples (n=3, extractions and analyzes made from freeze-dried samples of each species) and the proportions of averages and standard deviations of the standard remaining after extraction method used (n=3, extractions and analyzes made from freeze-dried samples of each species) are given. Negligible stds are not shown. Limits of detection and quantification for amino acids in micromoles in liter.

Compound	*Residue (%)*	*Recovery (%)*	*LOD [µmol/l]*	*LOQ [µmol/l]*
β-Alanine	1.3 ± 0.3	87.3 ± 2.3	0.07	0.22
L-Alanine	1.5 ± 0.3	66.3 ± 5.7	0.11	0.38
γ -Aminobutyric acid	1.7 ± 0.4	95.9 ± 5.9	0.07	0.23
L-Arginine	3.2 ± 0.1	96.7 ± 6.4	0.19	0.63
L-Asparagine	1.7 ± 0.2	91.2 ± 3.4	0.07	0.24
L-Aspartic acid	1.9 ± 0.2	84.6 ± 8.5	0.10	0.34
L-Citrulline	2.4 ± 0.2	99.9 ± 3.7	0.08	0.26
L-Cystine	n.d.	26.3 ± 20.8	0.08	0.27
L-Glutamic acid	1.9 ± 0.2	73.1 ± 6.5	0.10	0.34
Glutamine	1.2 ± 0.3	94.2 ± 3.6	0.07	0.23
Glysine	2.5 ± 0.4	78.7 ± 15.3	0.19	0.63
L-Histidine	3.9 ± 0.4	151.4 ± 118.6	0.36	1.20
L-Isoleusine	6.9 ± 0.2	81.5 ± 1.9	0.17	0.56
L-Leusine	8.2 ± 0.8	81.0 ± 5.3	0.18	0.58
L-Lysine	3.8 ± 0.2	90.0 ± 2.3	0.37	1.23
L-Methionine	10.2 ± 0.8	82.7 ± 1.8	0.13	0.44
L-Ornithine	1.1 ± 0.2	100.3 ± 2.3	0.18	0.59
L-Phenylalanine	8.6 ± 0.8	83.2 ± 1.8	0.18	0.58
L-Proline	-	-	-	-
L-Serine	2.6 ± 0.3	73.3 ± 8.9	0.12	0.40
Taurine	8.0 ± 0.7	92.8 ± 2.8	0.05	0.18
L-Theanine	n.d.	99.6 ± 6.0	0.06	0.20
L-Threonine	3.4 ± 0.3	81.8 ± 3.1	0.18	0.60
L-Tryptophane	0.0 ± 0.5	91.1 ± 2.3	0.09	0.28
L-Tyrosine	4.9 ± 0.5	70.1 ± 4.9	0.10	0.33
L-Valine	4.3 ± 0.3	79.2 ± 1.4	0.12	0.39
Average	3.7	86.1	0.14	0.45

n.d. not detected, - not possible to quantify

3.2. 5’-nucleotide and nucleoside contents of the mushrooms

The nucleotide/nucleoside content of mushroom species are presented in Table 4. The contents of umami enhancing nucleotides have been divided in three ranges, low (< 1 mg/g), medium (1–5 mg/g) and high (> 5 mg/g) according to Yang et al. (2001). Accordingly, the contents of these nucleotides were low in all the studied species. Here, the contents of 5’-GMP and 5’-IMP were assumed to count towards this amount. Based on the results of statistical analysis, the mushroom species are distinctive from each other by their nucleotide/nucleoside contents.

In the literature reviewed by Zhang et al. (2013) contents of umami enhancing 5’-nucleotides (5’-GMP, 5’-IMP, 5’-XMP) of mushrooms varied between 0.38–13.88 mg/g (dw). For *B. edulis* umami enhancing nucleotide contents of 2.01 mg/g (dw) (Tsai et al., 2008) and 1.63 mg/g (dw) (Beluhan & Ranogajec, 2011) and for *C. cibarius* 0.38 mg/g (dw) (Beluhan & Ranogajec, 2011) have been reported. Based on review by Zhang et al. (2013), both *B. edulis* and *C. cibarius* have a relatively low concentration of these 5’-nucleotides compared to other mushroom species presented. Thus it can be hypothesized that also *L. camphoratus* and *C. tubaeformis* have relatively low concentration of umami enhancing 5’-nucleotides in comparison to other species.

Table 4. The nucleoside/nucleotide content of the studied mushroom species in mg/g (dry weight) and standard deviation (n = 5, extractions and analyzes made from freeze-dried samples of each species) is shown. Three major nucleotides/nucleosides are in bold.

	C. cibarius	*C. tubaeformis*	*B. edulis*	*L. camphoratus*
5'-AMP[d]	**0.38 ± 0.03 (A)**	**0.70 ± 0.08 (B)**	**1.39 ± 0.09 (C)**	0.08* ± 0.00 (D)
5'-CMP[c]	**0.37 ± 0.01 (A)**	**0.57 ± 0.07 (B)**	**1.87 ± 0.11 (C)**	**0.86 ± 0.03 (D)**
5'-GMP[a,b]	0.19 ± 0.03 (A)	0.11* ± 0.01 (B)	**0.60 ± 0.04 (C)**	-
5'-IMP[a,c]	0.22* ± 0.01 (A)	0.13* ± 0.01 (B)	0.35 ± 0.02 (C)	n.d.
5'-UMP[c]	0.28 ± 0.02 (A)	**0.29 ± 0.07 (A)**	-	-
Adenosine[d]	**0.37 ± 0.01 (A)**	0.10 ± 0.01 (B)	0.16 ± 0.02 (C)	**1.08 ± 0.03 (D)**
Cytidine[d]	0.05* ± 0.03 (A)	0.09* ± 0.04 (A)	n.d.	0.06* ± 0.00 (A)
Guanosine[d]	0.16 ± 0.02 (A)	0.02* ± 0.00 (B)	0.07 ± 0.05 (A)	**0.96 ± 0.06 (C)**
Inosine[c]	0.08 ± 0.01 (A)	0.05* ± 0.00 (B)	0.20 ± 0.09 (AB)	0.31 ± 0.01 (C)
Uridine[c]	0.09 ± 0.00 (A)	0.06* ± 0.00 (B)	0.13 ± 0.01 (C)	0.62 ± 0.02 (D)
Umami 5'-nucleotides	0.41	0.25	0.95	n.d.
Total nucleotides	1.43	1.81	4.21	0.94
Total nucleosides	0.75	0.32	0.56	3.06

**Results smaller than LOQ, n.d. not detected (smaller than LOD), - not possible to quantify, [a] Umami enhancing nucleotides, [b] Statistical analysis with F-values and Tukey's, [c] Statistical analysis with Brown-Forsythe and Tamhane's T2, [d] Non-parametric tests, mushrooms that are not statistically different in one row are marked with a same letter A – D.*

3.3. Free amino acids contents of the mushrooms

The amino acid contents of the studied mushroom species are presented in Table 5. The total free amino acid content varied between 14.93 and 29.54 mg/g and was the lowest in *C. tubaeformis* and the highest in *B. edulis* mushrooms. L-aspartic acid and L-glutamic acid were amongst the five major free amino acids in all studied species. Further L-arginine, L-glutamine and L-histidine were found in relatively high concentrations in all species. Based on the statistical analysis, mushrooms can be distinguished based on their amino acid profiles.

FAAs were classified into four groups, MSG-like, sweet, bitter and tasteless, based on their taste properties as described by previous publications (Beluhan & Ranogajec, 2011; Mau et al., 2001; Yang et al., 2001). Also FAAs with no reported taste properties were included to tasteless amino acids. *L. camphoratus* had the highest total umami amino acid content, whereas *B. edulis* had the highest content of sweet amino acids and *C. cibarius* the highest content of bitter amino acids.

The amount of umami amino acids was 3.57–8.04 mg/g in studied species. With exception of *B. edulis*, all of the studied mushrooms had higher content of L-Glu than L-Asp. Based on the classification presented in the literature (Yang et al., 2001), all studied species except *C. tubaeformis* had medium concentrations of MSG-like amino acids (5–20 mg/g). In literature, Beluhan & Ranogajec (2011) reported L-glutamic acid contents of 39.09 mg/g for *B. edulis* and 29.99 mg/g for *C. cibarius* measured from freeze-dried fresh samples. Compared to other species studied in the literature reviewed by Zhang et al. (2013), both *C. cibarius* and *B. edulis* are relatively high in MSG-like amino acids. Based on our results *L. camphoratus* has higher content of these amino acids than *C. cibarius* or *B. edulis*, thus it can be expected to have high concentration in relation to other species too.

Measured concentrations are however in lower level compared to contents reported by Beluhan & Ranogajec (2011). This difference can be explained both by the differences in samples and by the differences in pre-processing methods. For example, Tsai et al. (2008) reported L-glutamic acid concentration as low as 0.59 mg/g from air-dried *B. edulis* samples. It has to be noted that our samples had been sous vide cooked and freeze-dried before analysis. In the literature it has been noted that cooking methods (Li et al., 2011), preservation methods (Liu et al., 2014) and post-harvest storage (Tseng & Mau, 1999) change the concentrations of amino acids and nucleotides/nucleosides. Also, the grade of the mushrooms have an effect on the amino acid and nucleotide/nucleoside contents (Cho, Choi, & Kim, 2006, 2010). Therefore,

342 the differences in pre-processing have an influence on our results and in the precision of
343 comparison.

344 *Table 5. The amino acid content of the studied mushroom species in mg/g (dry weight) and standard deviation (n = 5,*
345 *extractions and analyzes made from freeze-dried samples of each species) is shown. The amino acids are classified based on*
346 *their taste properties. The five major amino acids are are in bold.*

	C. cibarius	*C. tubaeformis*	*B. edulis*	*L. camphoratus*
MSG-like				
L-Aspartic acid[c]	**1.29 ± 0.08 (A)**	**1.65 ± 0.15 (A)**	**2.85 ± 0.44 (B)**	**3.35± 0.26 (B)**
L-Glutamic acid[c]	**3.78 ± 0.24 (A)**	**1.92 ± 0.21 (B)**	**2.62 ± 0.40 (B)**	**4.69 ± 0.36 (C)**
Total	5.08	3.57	5.47	8.04
Sweet				
L-Alanine[c]	0.65 ± 0.02 (A)	0.66 ± 0.11 (A)	**6.67 ± 0.92 (B)**	0.98 ± 0.11 (C)
Glysine[c]	0.21 ± 0.02 (A)	0.36 ± 0.03 (B)	1.29 ± 0.21 (C)	0.26 ± 0.01 (D)
L-Serine[c]	0.53 ± 0.03 (A)	0.39 ± 0.03 (B)	1.53 ± 0.23 (C)	0.62 ± 0.04 (D)
L-Threonine[a, d]	0.47 ± 0.02 (A)	0.28 ± 0.02 (B)	0.90 ± 0.12 (C)	0.58 ± 0.03 (D)
Total	1.86	1.68	10.38	2.43
Bitter				
L-Arginine[c]	**4.47 ± 0.46 (A)**	**1.15 ± 0.13 (BC)**	**1.41 ± 0.20 (C)**	**0.92 ± 0.08 (B)**
L-Histidine[a, b]	**1.13 ± 0.08 (A)**	**1.07 ± 0.10 (A)**	0.77 ± 0.14 (B)	**1.73 ± 0.12 (C)**
L-Isoleucine[a, d]	0.23 ± 0.01 (A)	0.32 ± 0.03 (B)	0.31 ± 0.03 (BC)	0.27 ± 0.01 (C)
L-Leucine[a, b]	0.46 ± 0.02 (A)	0.42 ± 0.03 (AB)	0.37 ± 0.05 (B)	0.53 ± 0.03 (C)
L-Methionine[a, b]	0.14 ± 0.00 (A)	0.14 ± 0.00 (A)	0.23 ± 0.03 (B)	0.26 ± 0.01 (B)
L-Phenylalanine[a, b]	0.25 ± 0.01 (A)	0.40 ± 0.03 (B)	0.30 ± 0.03 (C)	0.60 ± 0.03 (D)
L- Tryptophan[a, b]	0.32 ± 0.04 (A)	0.46 ± 0.05 (B)	0.48 ± 0.09 (B)	0.09 ± 0.03 (C)
L-Tyrosine[c]	0.43 ± 0.03 (A)	**1.98 ± 0.19 (B)**	0.69 ± 0.09 (C)	0.35 ± 0.01 (D)
L-Valine[a, d]	0.31 ± 0.02 (A)	0.32 ± 0.03 (AC)	0.54 ± 0.07 (B)	0.37 ± 0.02 (C)
Total	7.75	6.28	5.09	5.11

Tasteless or no information found				
β-Alanine[b]	0.04 ± 0.01 (A)	0.14 ± 0.03 (B)	0.19 ± 0.03 (B)	0.04 ± 0.04 (A)
γ-Aminobutyric acid[d]	0.31 ± 0.02 (A)	0.26 ± 0.05 (A)	0.67 ± 0.11 (B)	0.05 ± 0.02 (C)
L-Asparagine[d]	0.29 ± 0.02 (A)	0.89 ± 0.10 (B)	0.69 ± 0.11 (C)	0.32 ± 0.04 (A)
L-Citrulline[c]	0.04 ± 0.01 (A)	0.04 ± 0.00 (A)	0.28 ± 0.04 (B)	0.07 ± 0.02 (A)
L-Cystine	n.d.	n.d.	n.d.	0.03 ± 0.02
L-Glutamine[b]	**4.74 ± 0.39 (A)**	0.69 ± 0.08 (B)	**3.87 ± 0.60 (C)**	**5.10 ± 0.44 (A)**
L-Lysine[a,d]	1.00 ± 0.10 (A)	0.53 ± 0.05 (B)	1.03 ± 0.15 (A)	0.63 ± 0.04 (B)
L- Ornithine[c]	0.84 ± 0.14 (A)	0.63 ± 0.10 (A)	1.82 ± 0.28 (B)	0.10 ± 0.01 (C)
L-Proline	-	-	-	-
Taurine[c]	n.d.	0.21 ± 0.02 (A)	0.04 ± 0.00 (B)	0.03 ± 0.00 (C)
L-Theanine	0.05 ± 0.00	n.d.	n.d.	-
Grand total	21.99	14.93	29.54	21.94

n.d. not detected (the results smaller than LOD), - not possible to quantify, [a] essential amino acids, [b] Statistical analysis with F-values and Tukey's, [c] Statistical analysis with Brown-Forsythe and Tamhane's T2, [d] Non-parametric tests, mushrooms that are not statistically different in one row are marked with a same letter A – D.

4. Conclusions

This study is to our knowledge the first one investigating the amino acid and 5'-nucleotide and nucleoside concentrations of Nordic wild edible mushrooms and the first one to measure these concentrations from the *L. camphoratus* and *C. tubaeformis*. Thus it gives important information about these commonly picked northern mushroom species that might be interesting for scientific, industrial and household use.

Based on our results, it can be concluded that both amino acid and nucleotide/nucleoside profiles were distinctive from each other in our mushroom samples. They all contained significant concentrations of umami amino acids. Thus it can be predicted that umami is a significant component of the taste profile in the studied mushrooms. The concentration of umami amino acids was the highest in *L. camphoratus*. However, especially in *C. cibarius* and

C. tubaeformis the content of bitter amino acids and in *B. edulis* sweet amino acids were high. Sensory profile of a food product is a complex phenomenon where concentration of different taste compounds are only one important factor. In addition, the interaction of different tastes and sensory factors such as smell and texture influence to each other. Therefore, further sensory examinations are needed to ensure these predictions. Furthermore, there is still a need for more comprehensive study of taste differences caused by biological variations of Nordic mushroom species encompassing both geographical and seasonal variations.

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Conflict of interest

All authors declare that there is no conflict of interest related to this article.

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