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

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14 ## Abstract

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

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34 **Highlights**

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

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

40 Chemical compounds studied in this article

41 L-Glutamic acid (PubChem CID: 33032); L-Aspartic acid (PubChem CID: 5960), Guanosine
42 5'-monophosphate (PubChem CID: 6804), Inosine 5'-monophosphate (PubChem CID: 8582)
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44 ## 1. Introduction

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

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

68 AMP (Yamaguchi et al., 1971). They work in synergy with amino acids by intensifying the
69 taste sensation by binding to the same T1R1 + T1R3 receptor as glutamate (Mouritsen &
70 Khandelia, 2012; Zhang et al., 2013).

71 Taste properties of mushrooms have been studied from East Asian (Mau, Lin, Ma, & Song,
72 2001; Mau, Lin, Chen, Wu, & Peng, 1998; Tsai, Tsai, & Mau, 2008; Yang, Lin, & Mau, 2001),
73 East African (Mdachi, Nkunya, Nyigo, & Urasa, 2004) and Southern European species
74 (Beluhan & Ranogajec, 2011), but there is a gap in knowledge in taste properties of northern
75 mushroom species. Umami taste of mushrooms is affected by different factors such as maturity
76 stage and quality, storage time and conditions, species type and also the sub-strains of different
77 species (Zhang et al., 2013). Different climate and thus different flora of northern countries
78 gives a unique breeding ground for mushroom species. Specific knowledge of their taste
79 properties could promote industrial utilization of this great natural resource and increase
80 common interest towards conservation of their distribution areas in northern woodlands. The
81 annual crop of edible Finnish mushrooms is about 1200 million kilos (Salo & Lindroos, 2008).
82 Only fraction of it is picked mainly for home use and only small part of it is sold (Turtiainen,
83 Saastamoinen, Kangas, & Vaara, 2012). In a survey executed in 2011 (Turtiainen et al., 2012)
84 it was found that chanterelles (*C. cibarius*) and milkcaps formed each about 20 % of annual
85 crop picked in Finland (23 and 21 %, respectively), whereas ceps (*B. edulis)* and other boletus
86 species formed 14 % and russulas 2 %. Other mushroom species such as false morels
87 (*Gyromitra esculenta*) and funnel chanterelles (*C. tubaeformis*) composed 40 % of annual crop
88 picked. To our knowledge, the taste properties of even some of the most common Nordic
89 mushroom species such as milkcaps and funnel chanterelles have not been investigated before.

90 In this study free amino acids and nucleotides and their corresponding nucleosides were
91 measured from four edible Finnish forest mushroom species. 26 amino acids and 5 nucleosides
92 were measured. The studied species were chosen so that the comparison with literature could

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

95 ## 2. Materials and methods

96 ### 2.1. Solvents and reagents

97 Amino acid standards used were either 2500 µmol/l standard solutions in 0.1 M HCl (Amino
98 acid mixture standard solution, Type H, Wako Pure Chemical Industries, Ltd. (Osaka, Japan))
99 or dissolved solid standards (L-asparagine monohydrate (≥ 99 %), L-glutamine (≥ 99 %), L-
100 tryptophan (≥ 99 %) and L-theanine (≥ 97 %) from Wako pure chemicals, 4-aminobutyric acid
101 (≥ 99 %), beta-alanine (≥ 99 %), L-citrulline (≥ 98 %), L-ornitihine monohydrochloride (≥ 99
102 %), and taurine (≥ 99 %) from Sigma Aldrich, St.Louis, Missouri, USA). For spiking
103 experiments, corresponding liquid amino acid mix from Honeywell Fluka chemicals (Morris
104 Plains, New Jersey, USA) and solid standards of L-glutamic acid (≥ 99.5 %) and L-aspartic
105 acid (≥ 99 %) from Sigma Aldrich were used. Nucleotides and nucleosides (adenosine 5'-
106 monophosphate sodium salt (≥ 99 %), uridine 5'-monophosphate disodium salt (≥ 99 %),
107 cytidine 5'-monophosphate disodium salt (≥ 99 %), guanosine 5'-monophosphate disodium
108 salt hydrate (≥ 99 %), inosine 5'-monophosphate disodium salt (≥ 98 %), inosine (≥ 99 %),
109 guanosine (≥ 98 %), cytidine (≥ 99 %), uridine (≥ 99 %) and adenosine (≥ 99 %)) used in this
110 study were purchased from Sigma Aldrich. Because adenosine 5'-monophosphate sodium salt,
111 inosine 5'-monophosphate disodium salt and guanosine 5'-monophosphate disodium salt
112 hydrate contain unspecified amount of water (under 20, 27 and 26 % relatively) and adenosine
113 5'-monophosphate sodium salt also in maximum 8 % of sodium, the results slightly
114 overestimate the concentrations of these substances.

115 Sodium hydroxide (≥ 99 %), boric acid (≥ 99.5 %) and potassium dihydrogen phosphate (≥ 99
116 %) used in the analysis were purchased from Merc KGaA (Darmstadt, Germany). Potassium
117 phosphate dibasic (≥ 98 %) and 3-Mercaptopropionic acid (≥ 99 %) were from Sigma Aldrich,
118 35 % HCl (35–38 %), methanol (HiPerSolv CHROMANORM® gradient for HPLC) and
119 acetonitrile (HiPerSolv CHROMANORM® Super gradient for HPLC) were from VWR
120 Chemicals (Radnor, Pennsylvania, USA), ethanol anhydr. from Yliopiston Apteekki (Helsinki,
121 Finland) and 85 % orthophosphoric acid (85–90 %), o-phthalaldehyde (≥ 98 %) and 9-
122 fluorenylmethyl chloroformate from MP Biomedicals (Santa Ana, California, USA).

123 **2.2. Samples**

124 Four species of Nordic forest mushrooms, chanterelle (*Cantharellus cibarius*), funnel
125 chanterelle (*Craterellus tubaeformis*), porcini (*Boletus edulis*) and curry milkcap (*Lactarius*
126 *camphoratus*), were studied. The chanterelles (3.3 kilograms) were collected during mid-
127 August of 2016 from south-western coast of Finland and bought from a local market. Porcinis
128 (3.4 kilograms), curry milkcaps (0.4 kilograms) and a quarter of funnel chanterelles (1.0
129 kilograms) were collected during early or mid-September of 2016 from south-west coast of
130 Finland. Rest of the funnel chanterelles (2.7 kilograms) were bought during early September
131 of 2016 from mushroom pickers in Kainuu region in eastern Finland. The samples were cleaned
132 with a brush and cut to pieces (width 1 cm) within 36 hours of collection. The samples were
133 vacuum packed and cooked at 80°C for 10 minutes. The samples were cooled in water (room
134 temperature) for 2 minutes and in ice water (5–9 °C) for 5 minutes and then frozen at -20 °C.
135 Frozen samples were cut to 0.5 cm pieces, pooled, and put back to a freezer.

136 The samples were kept in a freezer at -20 °C for 5–6 months. Samples were moved to -40 °C a
137 day before freeze-drying. The samples were weighted in small plastic containers in batches of
138 about 30 grams and freeze-dried in vacuum at -40 °C for 27–29 hours. 8–9 batches of 30 grams
139 were freeze-dried at the same time. Freeze-dried mushroom samples were ground using mortar

140 and pestle until fine powder was reached. The samples were weighted before and after freeze-
141 drying and dry matter content was calculated based on the lost weight to ensure the operation
142 of the freeze-drying method. Dry matter contents of the mushroom species are presented in
143 Table 1. The dry matter content of mushroom species varied between 77.7 and 145.2 g/kg. In
144 a review by Kalač (2013) dry matter content in mushrooms in general was estimated to be
145 between 60–140 g/kg. Thus, the species in this study fit to these margins except for *L.*
146 *camphoratus*, which has dry matter content of 145.2 g/kg on average, slightly above the range
147 given by Kalač. The samples of *L. camphoratus* were slightly dehydrated when picked which
148 could explain this difference. Additionally, the samples in our study were vacuum cooked and
149 kept in freezer before analysis.

150 *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

151 **2.3. Instrumentation**

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

157 **2.4. Extraction**

158 The same extraction method was used for the extraction of FAAs (free amino acids) and
159 nucleotides/nucleosides. The method was modified from Ranogajec, Beluhan, & Šmit (2010).

160 Freeze-dried and ground samples (ca. 0.5 grams) were weighted in centrifuge tubes. 20 ml of
161 ultrapure water was added, and the samples were carefully shaken until fully mixed. Samples
162 were heated for 1 min in boiling water (100 °C) and kept in an ultrasound bath for 10 min
163 (23°C in the beginning). Samples were centrifuged at 2525g with Heraeus (Hanau, Germany)
164 Biofuge primo centrifuge for 10 min and the supernatant was collected in 50 ml measuring
165 flasks. The treatment was repeated three times for each sample. During the second repetition,
166 15 ml of water was added, and during third repetition 10 ml. The collected supernatants were
167 mixed and the measuring flask was filled with water to a volume of 50 ml. For the amino acid
168 and nucleotides/nucleoside analyses, the sample solutions were diluted with water in ratios 1:5
169 or 1:4 respectively. The solutions were finally filtered with a 0.20-µm RC syringe filter. Five
170 repetitions of each mushroom species were prepared for both FAA and nucleotide/nucleoside
171 analyses.

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

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

190 **2.5. 5'-nucleotide and nucleoside analysis**

191 The nucleotide/nucleoside contents were analyzed by the method modified from Ranogajec,
192 Beluhan, & Šmit (2010). The nucleotide/nucleoside composition of samples was analyzed
193 using UHPLC with diode array detector at wavelength 254 nm. The column used was Synergi
194 Hydro 4u Hydro-RP 80 Å 150*3.0 mm (Phenomenex, Torrace, California, USA) with Security
195 Cartidges AQ C18 4*2.0 mm pre-column. The solvents used were A: 20 mM phosphate buffer
196 (pH 5.8) and 100 % MeOH. The gradient program was: 3–12 min, 0 →30 % B; 12–13.50 min,
197 30 % B; 13.50–16 min, 30 → 0 % B; 16 – 25 min, 0 % B with a total time of 25 minutes. The
198 injection volume was 5 µl and the needle was washed after injection with water and 20 % ACN.
199 The calibration curve was collected using seven different concentrations (20, 10, 5, 2.5, 2, 1,
200 and 0.5 mg/l). 10 nucleotide/nucleoside standards were used. Stock solutions were prepared by
201 diluting 5 mg of solid standard in 10 ml of water.

202 **2.6. Free amino acid analysis**

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

209 The samples were derivatized as described on the technical note of Shimadzu. In short, the
210 samples were derivatized with o-phthalaldehyde and 3-mercaptopropionic acid in 0.1 M borate
211 buffer solution and 9-fluorenyl methyl chloroformate in acetonitrile. Acidic phosphate buffer
212 (pH 2.1) was added to the solutions during derivatization. Solvents used in the gradient
213 program were A: 20 mM phosphate buffer with pH 6.5 and B: 45/40/15 ACN/MeOH/H_2O. The
214 gradient program used was 0–2 min, 11% B; 2–4 min, 11→17 % B; 4–5.5 min, 17→31 % B;
215 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–
216 16 min, 55→100 % B; 16–19.5 min, 100 % B; 19.5–20 min, 100→11 % B; 20–25 min, 11%
217 B. The needle was washed from outside after every injection with 80 % MeOH and 20 % ACN.
218 The temperature of the column oven was 35 degrees and injection volume 1 µl. Calibration
219 curves were constructed using nine different concentrations (125, 50, 25, 20, 15, 10, 5, 2.5, 1
220 µmol/l). In total 26 standards were used. Nine amino acid standards were prepared using solid
221 standards and the rest using the liquid standard solution. Solid standards were diluted in 0.1 M
222 HCl to get a 5000 µmol/l stock solution. A diluted 125 µmol/l stock solution was prepared
223 from these stock solutions by adding 0.125 ml of each stock solution from solid standards and
224 0.250 ml of liquid standard solution in a 5 ml measuring bottle and diluting them with water.
225 In addition, a 250 µmol/l standard was prepared from the liquid standard solution and used in
226 calibration. All dilutions were prepared using ultrapure water.

227 **2.7. Statistical analysis**
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229 Differences between mushroom samples in each analyzed compound were tested with one-way
230 analysis of variance (ANOVA) with square-, or cube-transformed data, if necessary. Tukey's
231 HSD or Tamhane's T2 test was used for post hoc tests as directed by the tested homogeneity
232 of variance. If the data was not normally distributed, nonparametric tests (Kruskal-Wallis and
233 Mann-Whitney using Bonferroni correction) were used. Level of statistical significance was

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

236 ## 3. Results and discussion

237 ### 3.1. Method validation

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

245 The percentage of amino acids remaining after residual extraction and percentage of added
246 standard remaining after extraction method are presented in Table 3. Based on these results,
247 the extraction method seems to remove amino acids from the sample material efficiently. The
248 percentage of amino acids remaining in the precipitate was 3.7 % on average. In the majority
249 (19/23) of the studied amino acids < 5 % was left in the precipitate after three rounds of
250 extraction. The percentage remaining was < 10 % in all studied FAAs, except in L-methionine
251 with 10.2 % percentage. The spiking experiments showed that the amino acids are well
252 preserved during extraction. On average 86.1 % preservation was measured. Only differing
253 results are L-histidine and L-cystine with 151.4 and 26.3 % recovery respectively. To conclude
254 it can be expressed that apart from a few exceptions in both groups of compounds, the
255 extraction method effectively removes the FAAs and nucleotides/nucleosides from the starting
256 material and the compounds are well retained. Thus the results collected with this method are
257 reliable.

258 Limit of detection (LOD) and limit of quantification (LOQ) for both amino acids and
259 nucleotides/nucleosides were estimated based on S/N-ratio calculated with coefficients 3 and
260 10. LOD and LOQ values are presented in tables 2 and 3. For all nucleotides and nucleosides,
261 the linear range was 0.5–20 mg/l and with values of $r^2 > 0.999$. For free amino acids, the linear
262 range was 1–125 µmol/l or 1–250 µmol/l (FAAs from liquid standard) with values of $r^2 >$
263 0.997.

264 *Table 2. Validation of the extraction and measurement method (nucleotides/nucleosides). The proportions of averages and*
265 *standard deviations of the residual nucleotides/nucleosides compared to the original samples (n=3, number of extractions and*
266 *analyzes made from freeze-dried samples of each species) and the proportions of averages and standard deviations of the*
267 *standard remaining after extraction method used (n=3, number of extractions and analyzes made from freeze-dried samples*
268 *of each species) are shown. Negligible stds are not shown. Limits of detection and quantification for nucleotides and*
269 *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

270 *n.d. not detected, - not possible to quantify*
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272

273 *Table 3. Validation of the extraction method (free amino acids). The proportions of averages and standard deviations of the*
274 *residual FAAs compared to the original samples (n=3, extractions and analyzes made from freeze-dried samples of each*
275 *species) and the proportions of averages and standard deviations of the standard remaining after extraction method used*
276 *(n=3, extractions and analyzes made from freeze-dried samples of each species) are given. Negligible stds are not shown.*
277 *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

278 *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.

302 *Table 4. The nucleoside/nucleotide content of the studied mushroom species in mg/g (dry weight) and standard deviation (n =*
303 *5, extractions and analyzes made from freeze-dried samples of each species) is shown. Three major nucleotides/nucleosides*
304 *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

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

309

310 **3.3. Free amino acids contents of the mushrooms**

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

317 FAAs were classified into four groups, MSG-like, sweet, bitter and tasteless, based on their
318 taste properties as described by previous publications (Beluhan & Ranogajec, 2011; Mau et al.,
319 2001; Yang et al., 2001). Also FAAs with no reported taste properties were included to tasteless
320 amino acids. *L. camphoratus* had the highest total umami amino acid content, whereas *B. edulis*
321 had the highest content of sweet amino acids and *C. cibarius* the highest content of bitter amino
322 acids.

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

333 Measured concentrations are however in lower level compared to contents reported by Beluhan
334 & Ranogajec (2011). This difference can be explained both by the differences in samples and
335 by the differences in pre-processing methods. For example, Tsai et al. (2008) reported L-
336 glutamic acid concentration as low as 0.59 mg/g from air-dried *B. edulis* samples. It has to be
337 noted that our samples had been sous vide cooked and freeze-dried before analysis. In the
338 literature it has been noted that cooking methods (Li et al., 2011), preservation methods (Liu
339 et al., 2014) and post-harvest storage (Tseng & Mau, 1999) change the concentrations of amino
340 acids and nucleotides/nucleosides. Also, the grade of the mushrooms have an effect on the
341 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

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

350

351 ## 4. Conclusions

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

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

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

369 Acknowledgments

370 Hanna Manninen acknowledges funding from Finnish Cultural Foundation. The authors
371 acknowledge Seinäjoki University of Applied Sciences for the research facilities. Timo
372 Laaksonen acknowledges funding from the Academy of Finland (258114).

373

374 Conflict of interest

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

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