Enzyme-assisted extraction enhancing the umami taste amino acids recovery from several cultivated mushrooms

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A B S T R A C T

In this study, enzyme-assisted extraction was performed to extract umami taste and total free amino acids (FAAs) from the six different mushrooms including shiitake (Lentinus edodes), oyster (Pleurotus ostreatus), tea tree (Agrocybe aegerita) and, white, brown and portobello champignons (Agaricus bisporus).

β-Glucanase and Flavourzyme® were used as the enzymes for cell wall and proteins hydrolysis, respectively. It was found that β-glucanase treatment alone did not enhance the extraction efficiency, however in combination, β-glucanase and Flavourzyme® enhanced the extraction efficiency significantly up to 20-fold compared to conventional HCl mediated extraction, depending on the mushroom species. The optimal conditions for the enzyme treatment were: water as extraction solvent (initial pH = 7), enzyme concentration of 5% v/w each of β-glucanase and Flavourzyme®, temperature 50 °C and an incubation time of 1 h. White and brown champignons were found to be the richest source of umami taste FAAs (26.75 ± 1.07 and 25.6 ± 0.9 mg/g DM, respectively).

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1. Introduction

Umami is recognized as the fifth basic taste after sweet, salty, bitter, and sour, and is described by its pleasant savory or meaty flavors naturally present in many plant and animal based foods. In 1908, Kikunae Ikeda discovered that glutamate is the chemical basis of the pleasant taste and he created the term “umami” for this distinguishable taste (Ikeda, 1908). The sodium salt of glumatic acid (Glu), monosodium glutamate (MSG), is well-known to exhibit umami-type flavors and is widely used in the food industry (Food Standards Australia New Zealand., 2015). The salt of aspartic acid (Asp), aspartate, also imparts umami taste in foods (Mouritsen, Styrbæk, Johansen, & Mouritsen, 2014). The umami taste can also be achieved with the 5'-ribonucleotides such as inosinate and guanylate (Zhang, Venkitasamy, Pan, & Wang, 2013). In humans, the detection or perceived umami taste involves multiple receptors present in taste buds which include glutamate-selective G-protein-coupled receptors, mGluR4 and mGluR1, and the heterodimer T1R1 + T1R3 (Chaudhari, Pereira, & Roper, 2009).

The role of umami in flavor enhancement and appetite regulation is well documented (Masic & Yeomans, 2014). Since umami possesses a savory taste it has the potential to reduce dietary sodium intake (Nakagawa, Kohori, Koike, Katsuragi, & Shoji, 2014), thereby assists in regulating hypertension and cardiovascular diseases. Umami ingredients impart meaty flavor, create mouth fullness and makes food richer in taste, thus has a potential in reducing energy and fat intake (Imada, Hao, Torii, & Kimura, 2014; Miyaki, Imada, Hao, & Kimura, 2016). In addition, its pleasant taste could increase appetite and improve nutrition in malnourished and elderly people (Tomoe et al., 2009). Umami ingredients/extracts are considered the “chef’s choice” within the culinary world. Food industries are searching for natural umami (mono sodium glutamate (MSG)-like) ingredients due to the negative perceptions among consumers regarding “added MSG” label (Radam, Yacob, Bee, & Selamat, 2010).

Mushrooms are widely used as raw food, functional food, and seasoning particularly due to their complex flavor and are good a source of proteins, carbohydrates, vitamins, minerals and other nutrients (Kalač, 2013). They also possess a wide range of biological properties including antioxidant, antimicrobial, anticancer and immuno-modulating activities (Ganeshpurkar, Rai, & Jain, 2010), and are also good sources of umami substances (Phat, Moon, & Lee, 2016). A comprehensive review on umami based ingredients of edible mushrooms was published recently by Zhang et al. (2013), which clearly suggests that significant amounts of free Glu and Asp are present in a wide variety of mushrooms, and their...
extracts found to elicit umami flavor. Among many other mushrooms, champignons (Agaricus bispora), produced in three varieties: white champignons, cremini/brown champignons and Portobello), shiitake (Lentinus edodes) and oyster (Pleurotus ostrea-tus) mushrooms are the most widely cultivated edible mushrooms across the globe (Reis, Barros, Martins, & Ferreira, 2012). The tea tree mushroom (Agrocybe aegerita, also called poplar mushroom) is one of the most cultivated mushroom in China (Jiang et al., 2012) and used in several Chinese cuisines. These mushrooms have been identified as the richest source of umami substances (Li et al., 2014; Phat et al., 2016; Tsai et al., 2009) and moreover, these mushrooms have relatively short growth periods and can be cultivated easily and economically when compared to other edible mushrooms (Cotter, 2014; Reis et al., 2012).

Conventionally, FAAs are extracted by agitating the mushrooms in dilute hydrochloric acid (Li et al., 2014; Phat et al., 2016; Tsai, Tsai, & Mau, 2008; Tsai et al., 2009; Tsai, Wu, Huang, & Mau, 2007). The samples are homogenized before extraction in order to achieve cell disruption thereby enhance the mass transfer of analytes into the solvent. Although, a number of researchers have used this HCl extraction of FAAs and umami taste amino acids from various mushrooms, there are no reports describing how the process of extraction can be improved or optimized. Indeed, improving the extraction of umami taste from mushrooms will reduce time and costs of production and may additional increase yield. Moreover, utilization of dilute HCl acid as an extraction solvent is not feasible in food preparations and it complicates downstream processing. In this respect, enzyme-assisted extraction would be a sustainable alternative method to improve the recovery of umami based compounds from mushrooms. Enzymes have been proven to be an environmentally friendly technique for the enhanced release of a variety of bioactives and nutraceuticals from various bio- sources (Puri, Sharma, & Barrow, 2012). Enzymes such as cellulases, pectinases and hemicellulases are often used to digest the cell wall components of the plant cell, thereby enhancing the recovery of value-added compounds. Proteases can be used to hydrolyze proteins (Merz et al., 2015a, 2015b) thereby increasing the yield of FAAs.

In this study, the potential of enzyme-assisted extraction to improve the umami taste and total FAAs from six different edible mushrooms was investigated. The industrial β-glucanase (endo-1,3(4)) containing cellulolytic activity was used to hydrolyze mushroom cell wall structures and a peptidase preparation, commercially known as Flavourzyme® containing endo- and exopeptidase activities was used for mushroom protein hydrolysis. Factors affecting the enzyme-assisted extraction process, including pH, temperature, and enzyme concentration as well extraction kinetics were investigated.

2. Materials and methods

2.1. Samples

Dried shiitake mushroom, oyster mushroom and white champignon were obtained from OSCAR A/S, Rønne, Denmark. Dried tea tree mushroom was purchased from a local market in Sichuan province, China. Brown champignon and portobello champignon were purchased in fresh form from a local super market in Copenhagen, Denmark and dried in a hot air oven at 35 °C for 2 days. The samples were ground into powder using a kitchen homogenizer.

Amino acid standards mix containing alanine (Ala), sarsosine (Ser), glycine (Gly), valine (Val), α-aminobutyric acid (ABA), β-aminoisobutyric acid (β-AIB), leucine (Leu), allo-isoleucine (αLe), 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), ornethine (Orn), lysine (Lys), glutamine (Gln), asparagine (Asn), histidine (His), hydroxylysine (Hly), tyrosine (Tyr), tryptophan (Trp), cystine (C–C), norvaline, reagents for solid phase extraction and derivatization of FAAs (included in EZ-Faast GC-FID FAA analysis kit) were purchased from Phenomenex, Værløse, Denmark. Fluorescamine (≥98%) and L-aminocaproic acid (≥99%) were purchased from Sigma–Aldrich, Munich, Germany.

Enzymes used in the present study, β-glucanase and Flavourzyme®, were gratefully donated by Novozymes ( Bagsvaerd, Denmark). According to the supplier, β-glucanase (endo-1,3(4)-) was produced from Aspergillus aculeatus and has cellulolytic activity and its declared specific enzyme activity was 100 Fungal Beta-Glucanase units (FBG)/g, while Flavourzyme® was produced from Aspergillus oryzae and contained activity of protease and its declared specific enzyme activity was 1000 Leucine Amino Pepti-dase Units (LAPU)/g.

2.3. Enzyme-assisted extraction

Extractions were carried out in batch in 100 mL screw-top Schott bottles placed in a thermostated shaking water bath (±0.1 °C). In a typical experiment, 50 mL of water or buffer solution was initially loaded into the flask along with the required volume of individual enzyme (β-glucanase or Flavourzyme®) or enzyme mixture (1:1 v/v mixture of β-glucanase and Flavourzyme®). This solution was preheated at required temperature for 15 min. The mushroom sample (1 g) was then loaded into the flask and agitated for required duration. On completion of the extraction process, stirring was stopped and the extract was filtered through a Whatman® Grade 41 filter paper under vacuum. The filtrate was subsequently stored at −20 °C until further analysis.

In the present study optimal conditions for the three enzyme treatments (β-glucanase, Flavourzyme® and 1:1 v/v mixture of β-glucanase and Flavourzyme®) was determined by investigating the effects of various treatment parameters on the extraction yield, taking shiitake mushroom as a model matrix. In the first set of experiments, the effect of pH was determined by varying the pH (4.0, 5.0, 6.0 and uncontrolled) of the extraction medium using citrate buffer or Milli-Q water. In this set of experiments, the extraction temperature and enzyme to substrate ratio was held constant at 50 °C and 5% v/w, respectively. In the second set, the effect of temperature was determined by varying the extraction temperature (30, 40, 50 and 60 °C), holding the pH at an optimal level and enzyme to substrate ratio at 5% v/w. In the final experiment, the optimal pH value and optimal temperature were used, while the enzyme concentration was varied from 0.1 to 5% v/w. In all experiments the extraction time was set to 1 h. Finally, extraction kinetics of the three enzyme pretreatments were investigated at the optimal conditions derived from the previous sets of experiments. In this case, samples (500 μL) were incubated at 50 °C (5% v/w enzymes, pH 7) and sampling for analysis of FAAs were taken at 0.5, 1, 1.5, 2, 3 and 5 h intervals. All experiments were carried out in triplicates. Extracts were stored at −20 °C prior to analysis.

2.4. Conventional extraction

Enzyme-assisted extraction developed in this study was compared with conventional extraction procedures previously reported in the literature (Li et al., 2014; Phat et al., 2016; Tsai et al., 2008; Tsai et al., 2009; Yang, Lin, & Mau, 2001). Briefly, a
homogenized sample was agitated in 50 mL of 0.1 M HCl for 45 min at ambient temperature, followed by filtration through a Whatman Grade 41 filter paper.

2.5. Free amino acids analysis

FAAs analysis was carried out according to the method previously described by Badawy (2012) with minor modifications. 100 μL of diluted extract was pipetted into a shell vial along with 100 μL of internal standard (norvaline, 20 nmole). The solution was aspirated gently through a cation exchange sorbent tip (solid phase extraction). The sorbent particles were then washed with 200 μL of 33% propanol. Amino acids were then eluted with 200 μL of an eluting mix consisting of 0.33 M NaOH, 80% propanol and 20% 3-picoline. Amino acids were then derivatized using 50 μL of derivatizing agent composed of 2:6:2 v/v/v mixture of propyl chloroformate/chloroform/isooctane. The resultant mixture was then vortexed for 1 min and 100 μL 90:10 v/v mixture of isooctane/chloroform was added. The solution was vortexed for a further 1 min and 100 μL 1 M HCl was added. The solution was further vortexed for an additional 1 min and allowed to separate into two phases. The upper layer containing derivatized FAAs was transferred to a GC insert for analysis.

Gas chromatographic analysis was performed using an Agilent 6890 gas chromatograph (Agilent, USA) fitted with a flame ionization detector (FID). Analytes were separated using a ZB-AAA column (10 m length × 0.25 mm ID × 0.25 μm film) and hydrogen as the carrier gas (flow rate was 1 mL/min). The injector was set at 250 °C in a split mode (1:10 ratio). Samples (3 μL) were injected using an autosampler. The temperature program was set as follows: 110–320 °C at 32 °C/min. The detector was set at 320 °C. A multiple point (n = 5) calibration curve with internal standard was plotted for quantitation of amino acids. Results are expressed as mg of FAA per g of sample (dry weight basis), and presented as mean ± standard deviation (SD) (mg/g DM).

2.6. 5’-Mononucleotide assay

A detailed experimental protocol for 5’-mononucleotide analysis is provided in the Supplementary datasheet method M11.1.

2.7. Degree of hydrolysis

A simple, rapid and convenient method was performed to determine the degree of hydrolysis (DH) based on the determination of primary amino groups using a fluorescence assay as previously described by Kastrup Dalsgaard, Holm Nielsen, and Bach Larsen (2007) with additional modifications. In a typical experiment, initially, the sample was appropriately diluted in 0.1 M borate buffer (pH 9.0). A volume of 151 μL of diluted sample was mixed with 49 μL of the fluoroscein solution (0.2 mg/mL fluorescein in water free aceton) in a well of 96-well plate. The plate was shaken immediately and the fluorescence was measured 5 min after the addition of the fluorescein in a microplate plate reader (Fluoskan Ascent, Thermo Scientific, USA) using 0.1 M borate buffer as a blank. The excitation wavelength was set at 390 nm and emission at 480 nm. Concentration of the free amino groups was calculated as 6-aminocaproic acid equivalents using an external 6-aminocaproic acid standard curve. The degree of hydrolysis was then calculated according to the following equation (Jamdar et al., 2010):

$$\%DH = \frac{A_i - A_0}{A_{max} - A_0} \times 100$$

where $A_i$ is the total amount of amino groups detected after hydrolysis performed at a particular experimental condition, $A_0$ is the total amount of amino groups present in the original sample, and $A_{max}$ is the maximum amount of the amino groups present in the completely hydrolyzed sample.

2.8. Statistical analysis

One-way ANOVA was used to determine significant differences between means using Tukey's posthoc test (P values < 0.05). Principal component analysis (PCA) was a multivariate statistical approach used to visualize differences in effects of treatment parameters on the contents of FAA. The XLSTAT (Addinsoft, USA) and Origin Pro 9.1 (OriginLab Corporation, USA) statistical software were used for statistical analyses.

3. Results and discussion

In food products, the MSG-like taste arises from the free Asp and Glu present in the matrix. Presence of these compounds in adequate concentrations improves the overall taste appeal. Preparation of extracts which are rich in umami substances can be used as cooking stock as well as food ingredients. In this study, three different sets of enzymes namely, β-glucanase, Flavourzyme® and 1:1 combination of β-glucanase and Flavourzyme® were used to improve the recovery of MSG-like FAAs from mushrooms. The β-glucanase, a multi component cellulolytic enzyme, has activities of cellulase, hemicellulase and xylanase, and is capable of hydrolyzing endo (1,3)- or (1,4)-linkages in β-d-glucans. Flavourzyme® is a proteolytic enzyme comprising of aminopeptidases, which is capable of liberating amino acids by hydrolysis of the N-terminal peptide bonds in proteins. Cell-wall degrading enzymes were employed to hydrolyze the complex polysaccharides in the cell walls (Puri et al., 2012) of mushrooms, which increases cell wall permeability and, consequently improve the extraction yields of FAAs. As mentioned, mushrooms are rich in proteins (Kalac, 2013) and in order to improve the taste-contributing FAAs contents, these proteins can be hydrolyzed using proteases as a sustainable and economical approach. In this respect, Flavourzyme® was used to hydrolyze the proteins and peptides present in all mushrooms. Flavourzyme® is generally used to produce flavorful amino acids and peptides from proteins (Berends, Appel, Eisele, Rabe, & Fischer, 2014; Merz et al., 2015a). The investigation on Flavourzyme® and the hydrolysis of plant and animal proteins, such as wheat gluten, soy proteins, red hake proteins are described in the literature (Merz et al., 2015a, 2015b). It has both endo- and exo- peptidase activities (Berends et al., 2014) suitable to yield greater amounts of FAAs. The inclusion of cellulolytic enzymes in the enzymatic hydrolysis of proteins, may allow for easier access of proteases to proteins (Passos, Yilmaz, Silva, & Coimbra, 2009), which might, in turn, improve the overall yield of FAAs.

A detailed investigation describing the effect of various extraction parameters on the recovery of umami taste FAAs are given in the following sections.

3.1. Optimization of enzyme-assisted extraction of FAAs

3.1.1. Effect of pH

The influence of pH of the medium on the extraction of MSG-like and total FAAs from shiitake mushrooms using β-glucanase, Flavourzyme® and combination of β-glucanase and Flavourzyme® is illustrated in Fig 1A–D. The pH of extraction media was varied between 4.0 and 6.0 using a buffer. An experiment was conducted without controlling the pH of the medium where Milli-Q water served as the extraction solvent (initial pH = 7.0). In this case, the pH of extraction media after the addition of sample was found to be 5.9 and it did not change significantly during the course of extraction. As seen in
Fig. 1A–D the variation in pH did not alone affect the FAAs recovery from control samples (without enzymes). The extractions with β-glucanase did not improve the extraction yield with the tested pH values. The inefficiency of β-glucanase alone in enhancing FAAs yield may be explained by the simpler structures and molecular weights of amino acids, which can easily diffuse out of the cells, wherefore cell wall disruption is not essential.

The pH of the media plays a very important role in enzyme activity as the pH influences the shape and charges of enzymes, thereby affecting its activity (Raju & Madala, 2005). However, the substrates are also important as Kao, Tsou, Kao, and Chiang (2011) observed highest Flavourzyme/C210 activity at pH 7.12 during the hydrolysis of soy protein, while Shu, Zhang, Chen, Wan, and Li (2015) used pH 6 to hydrolyze goat milk to produce casein hydrolysate using Flavourzyme® and Berends et al. (2014) used an even lower pH of 5 for wheat gluten hydrolysis. For the treatment with Flavourzyme® alone it is seen (Fig. 1C + D) that pH 6 and uncontrolled pH resulted in increased yield of the umami-like FAAs and total FAAs. Combining β-glucanase and Flavourzyme® an extra effect is observed as a significant improvement in the extraction yield was obtained in the uncontrolled pH assay resulting in the highest extraction of umami-like FAAs and total FAAs. Water was chosen as the most suitable extraction solvent in the subsequent experiments due to the higher extraction efficiency. Moreover, water is an ideal extraction solvent for food industries as it is economical and reduces costs associated with downstream processing.

3.1.2. Effect of temperature

The enzymes used in this study possessed different temperature activity profiles, therefore different temperature was investigated to obtain higher recovery. Thus, the total and umami FAAs yields were investigated at different extraction temperatures (30, 40, 50 and 60 °C) at constant enzyme concentration (5% v/w). Increasing temperature did not improve the extraction yields of the control assay and the treatment with β-glucanase, while Flavourzyme® treatment improved the recovery of umami taste (Fig. 2A–C) and total FAAs (Fig. 2D). Similar results were obtained by Kao et al. (2011) and Ovissipour et al. (2009) during hydrolysis of soy proteins and Persian sturgeon proteins, respectively. The effectiveness of the two enzymes, β-glucanase and Flavourzyme®, together for extraction of total FAAs and the MSG-like amino acids is only observed to a minor degree at 50 °C and probably due to a slight enhancement in hydrolysis of the peptide bonds in proteins. Treatment at 60 °C for all assays resulted in significantly decreased yield of total and MSG-like FAAs, likely due to enzyme inactivation. In general, enzyme activity increases with temperature, however, their activity decrease at higher temperatures due to denaturation and inactivation (Daniel et al., 2010). Alternatively, a lower yield could be due to the loss of FAAs owing to degradation at higher temperatures or association with other macromolecules present in the extract. Since some amino acids have reactive amino groups, they can react with carbohydrates present in the extract at higher temperatures (Chuyen, 1998), subsequently leading to lower concentrations of FAAs in the extract. The highest MSG-like and total FAAs (18.61 ± 1.73 and 70.80 ± 1.35, respectively) were recovered at 50 °C using a combination of β-glucanase and Flavourzyme® (Fig. 2C + D). The 50 °C temperature was also used in hydrolysis of date seed flour (Ambigaipalan & Al-Khalifa, 2015), soy protein isolate (Tsou, Kao, Tseng, & Chiang, 2010), wheat gluten (Berends et al., 2014) using Flavourzyme®.

3.1.3. Effect of enzyme concentration

The umami taste FAAs (Fig. 3A–C) and total FAAs (Fig. 3D) contents of the shiitake mushrooms were measured after 1 h
enzyme-assisted extraction at 50 °C with respect to the different concentrations of enzymes used. In general, β-glucanase did not enhance the extraction yield when compared to control samples even after using the highest tested enzyme dosage (5% v/w). Commonly, increasing the enzyme concentration causes an increasing reaction rate, as more enzyme molecules are available to react with the substrate molecules (Bettelheim, Brown, Campbell, & Farrell, 2009). Apparently, this was not the case in the β-glucanase assay due to the specific action of this enzyme. However, increasing the Flavourzyme® concentration in the extraction assay significantly increased the extractability of the FAAs resulting in more than double yield of Glu and Asp at 5% v/w compared to the control. Combining β-glucanase and Flavourzyme® further enhanced the total FAAs and MSG-like FAAs yields considerably from 36.7 (0.1% v/w, each) to 71.8 mg/g DM (5% v/w, each) and 10.9 (0.1% v/w, each) to 18.4 mg/g DM (5% v/w, each) (Fig. 3), respectively.

The improved yield is caused by the higher rate of protein hydrolysis when the Flavourzyme® concentration increased. Based on these results the enzyme concentration of 5% v/w was selected as optimum for extraction of FAA and MSG-like FAAs from mushrooms. Indeed, it is assumed that at higher enzyme concentrations extraction yield would increase, but considering the production sustainability and economy higher concentrations was not assayed.

3.2. Extraction kinetics

In order to optimize the incubation time, the kinetics of release of total and MSG-like FAAs from shiitake mushrooms was investigated. The effect of the extraction time on the concentration of the individual FAAs, Asp and Glu, is shown in Supplementary datasheet Fig. S1, while Fig. 4 shows the extraction kinetics of the MSG-like and the total FAAs. The concentration of total FAAs in the control samples remained stable throughout the incubation period (Fig. 4B), however, the concentration of MSG-like FAAs decreased slightly after 2 h of incubation (Fig. 4A). Overall, β-glucanase did not enhance the extraction yield considerably in line with the previous findings (Section 3.1). A significant increase in the recovery of umami taste and total FAAs were observed after Flavourzyme® and combination of β-glucanase and Flavourzyme®, with a ≥ 2-fold increase after 1 h of incubation as compared to the control groups (Fig. 4). The enzymatic treatment did not cause significant effects on the yields of total FAAs extraction when the extraction time was further increased up to 5 h. However, a longer incubation time (>1.5 h) caused significant decrease in the recovery of umami taste FAAs, likely due to the degradation of Asp and Glu or association of these amino acids with other macromolecules such as carbohydrates at higher temperatures and prolonged reaction times.

In order to monitor the progress of hydrolysis, DH was calculated based on the fluorescamine assay. Proteases like Flavourzyme® will hydrolyze the peptide bonds releasing free amino groups, which react and can quantitatively be measured by fluorescence spectroscopy (Kastrup Dalsgaard et al., 2007). The DH value is then calculated based on the amine concentrations in hydrolyzed and control samples (Jamdar et al., 2010). As seen in Supplementary datasheet Fig. S2, the hydrolysis progressed rapidly during the first 1–2 h incubation and then the rate decreased due to a lower extent of hydrolysis, thereby lowering the DH. The observation was similar to typical hydrolysis curves previously
reported by Bougatef et al. (2009), Jamdar et al. (2010), Klompong, Benjakul, Kantachote, and Shahidi (2007) for the hydrolysis of different proteins. At the beginning of incubation, the availability of excess protein substrate allowed a rapid hydrolysis leading to a higher DH value, while the rate subsequently decreased due to the loss of substrates. A higher DH value was obtained for the samples treated with mixture of β-glucanase and Flavourzyme® compared to treatment with Flavourzyme® alone. This indicates that β-glucanase assists in extraction of proteins from the cells, which was subsequently hydrolyzed by Flavourzyme®. The DH profile of the β-glucanase and Flavourzyme® assays is in accordance with the higher extraction yields of FAAs obtained when combinations of β-glucanase and Flavourzyme® were used (Figs. 1–4).

Fig. 3. Effect of enzyme concentration (%v/w) on extraction of (A) aspartic acid (B) glutamic acid (C) MSG-like FAAs and (D) total FAAs from shiitake mushroom. The extraction temperature, time, and initial pH were set as 50°C, 1 h and pH 7 (pH was not controlled during the reaction), respectively. Columns labeled with different letters were significantly different at P < 0.05 (Tukey’s HSD).

Fig. 4. Extraction kinetics of (A) umami taste and (B) total FAAs. The assay (5% v/w enzymes and pH 7 (uncontrolled during the reaction)) was incubated at 50°C. Symbols in the line charts labeled with different letters were significantly different at P < 0.05 (Tukey’s HSD).
3.3. Principal component analysis

PCA was conducted to assess the effects of treatment parameters on the FAAs yields. The PCA describing the changes in profiles of individual FAAs affected by different treatments is shown in Supplementary datasets Fig. S3. For the overview and focus the groups of taste-contributing amino acid a simplified PCA plot is shown in Fig. 5, which explains 99.86% (PC1 = 97.48% and PC2 = 2.38%) of the total variability. PC1 accounted for the sweet, bitter, umami taste and total FAAs, while PC2 accounted mainly tasteless FAAs. The scores in the bi-plot show a clear discrimination among all extraction methods, based on the distance of the scores in the plot. The umami, bitter and total FAAs were positively correlated with 1-glucanase and Flavourzyme® combination treatment, distributed in the positive side of PC 1 (lower right quadrant). Sweet and tasteless FAAs were located in upper right quadrant of positive PC 2 axis, correlated with Flavourzyme® treatment. Amino acids were not clustered at the treatments corresponding to the control, conventional extraction and 1-glucanase-assisted extraction indicating lower extraction efficiency at these conditions, which was consistent with results obtained in the optimization experiment (Figs. 1–4).

3.4. Enzyme-assisted extraction of total and MSG-like FAAs from 6 different mushrooms

Based on the optimization studies performed on the shiitake mushroom, an incubation temperature of 50 °C, enzyme cocktail of 1-glucanase and Flavourzyme® at 5% v/w each, water as extraction solvent (initial pH = 7.0), and incubation time of 1 h were selected as the optimal conditions to recover total and MSG-like FAAs from mushrooms. These conditions were extended to extract FAAs from various other commercial mushrooms such as oyster mushroom, tea tree mushroom, white champignon, brown champignon and portobello champignon. The FAAs and umami amino acids extracted from various mushrooms at conventional extraction method as previously described by Li et al. (2014), Phat et al. (2016), Tsai et al. (2009) and enzyme-assisted extraction method developed in the present study is shown in Table 1.

Overall, the enzyme assisted extraction enhanced the extraction yield significantly. The best results were obtained for the oyster and shiitake mushrooms, where the two enzyme mixtures enhanced the FAAs yields 7- and 4-fold, respectively, compared to the conventional extraction (Table 1). In this line, the extraction yields for the groups of taste specific amino acids increased 3- and 5-fold for the umami FAAs and up to 20-fold (bitter taste AA for oyster mushroom) compared to those obtained by the conventional method. Similar yield enhancements were observed in case of tea tree mushroom, white champignon, brown champignon and portobello champignon treated with the two-enzyme mixture of 1-glucanase and Flavourzyme®, though the total FAAs and umami FAAs yields increased only around 2-fold when compared to conventional extraction. The enzyme treatment resulted in the following order of the concentrations of MSG-like amino acids: white champignon (26.75 ± 1.0 mg/g) > brown champignon (25.6 ± 0.90 mg/g DM) > portobello champignon (21.02 ± 0.52 mg/g DM) > shiitake mushroom (18.35 ± 0.12 mg/g DM) > oyster mushroom (13.96 ± 1.11 mg/g DM) > tea tree mushroom (10.14 ± 0.12 mg/g DM). The highest total FAAs was detected in brown champignons (128.28 ± 6.62 mg/g). The presence of FAAs and MSG-like amino acids and their amounts in mushrooms depend on several factors such as mushroom species, harvesting period, storage time, as well as geographic origin (Zhang et al., 2013). To our knowledge, only conventional solvent extractions have been employed to extract umami amino acids from mushrooms using 0.1 M HCl as extraction solvent (Li et al., 2014; Phat et al., 2016; Tsai et al., 2008). Yang et al. (2011) extracted about 1.71–1.93 mg/g of MSG-like amino acids from shiitake mushrooms. The amount of these substances in oyster mushrooms was found to be 2.14 mg/g (Tsai et al., 2009), while it was ranging from 10.6 to 13.5 mg/g in champignons, based on maturity stages (Tsai et al., 2007). Li et al. (2014) and Tsai et al. (2008) extracted up to 3.62 mg/g of MSG-like amino acids from tea tree mushrooms. The yield values reported in these studies were similar to those obtained by conventional extraction performed in the present work; nevertheless, they were considerably lower when compared to enzyme-assisted extraction developed in the present work. A number of recent reports revealed that several other edible mushrooms are also a promising source for MSG-like substances [reviewed in detail in Zhang et al. (2013)]. The enzymatic treatment was found to significantly affect the extraction yield. Enzyme treatment parameters such as pH, temperature, enzyme dosage and treatment time had significant influence on the recovery of amino acids. Although the 1-glucanase treatment alone did not enhance the recovery of amino acids, the combination of 1-glucanase and Flavourzyme® enhanced the extraction yield significantly. This was indicative that cellulolytic enzyme treatment is not involved in the extraction of FAAs from the mushroom matrices, however, it aids in the transfer of proteins, which is subsequently hydrolyzed by Flavourzyme® to release FAAs.

3.5. Effect of enzymes on extraction of 5'-mononucleotides

Enzyme treatments were found not to improve the extraction 5'-mononucleotides such as inosinate and guanylate from the various mushrooms. Control experiments (without using an enzyme) showed that a temperature ≥70 °C is crucial for extraction of nucleotides from mushrooms (data not shown). The tested enzymes cannot be used at such high temperature due to loss of activity, thus, the enzyme assays were not tested.
Enzyme-assisted extraction of FAA (mg/g DM) from various cultivated mushrooms.\(^A\)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Shiitake mushroom</th>
<th>Oyster mushroom</th>
<th>Tea tree mushroom</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional extraction</td>
<td>Enzyme-assisted extraction</td>
<td>Conventional extraction</td>
</tr>
<tr>
<td>Ala</td>
<td>1.07 ± 0.17 h</td>
<td>3.81 ± 0.13 f</td>
<td>2.30 ± 0.12 g</td>
</tr>
<tr>
<td>Gly</td>
<td>0.29 ± 0.00 e</td>
<td>1.55 ± 0.01 d</td>
<td>0.29 ± 0.00 e</td>
</tr>
<tr>
<td>Val</td>
<td>0.52 ± 0.02 ef</td>
<td>3.88 ± 0.12 b</td>
<td>0.35 ± 0.01 f</td>
</tr>
<tr>
<td>Leu</td>
<td>0.39 ± 0.49 g</td>
<td>4.79 ± 0.16 cd</td>
<td>0.3 ± 0.01 g</td>
</tr>
<tr>
<td>Ile</td>
<td>0.5 ± 0.01 e</td>
<td>3.84 ± 0.05 bc</td>
<td>0.45 ± 0.01 e</td>
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<tr>
<td>Thr</td>
<td>0.53 ± 0.05 d</td>
<td>3.45 ± 0.23 c</td>
<td>0.33 ± 0.01 d</td>
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<tr>
<td>Ser</td>
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<td>4.30 ± 0.01 ab</td>
<td>0.59 ± 0.02 e</td>
</tr>
<tr>
<td>Pro</td>
<td>0.30 ± 0.04 f</td>
<td>0.92 ± 0.03 ef</td>
<td>0.33 ± 0.01 f</td>
</tr>
<tr>
<td>Asn</td>
<td>0.39 ± 0.07 e</td>
<td>2.69 ± 0.28 cd</td>
<td>0.23 ± 0.01 e</td>
</tr>
<tr>
<td>Asp</td>
<td>0.76 ± 0.08 g</td>
<td>3.51 ± 0.02 d</td>
<td>1.93 ± 0.33 ef</td>
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<tr>
<td>Thr</td>
<td>ND</td>
<td>0.97 ± 0.02 d</td>
<td>ND</td>
</tr>
<tr>
<td>Glu</td>
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<td>14.85 ± 0.1 c</td>
<td>2.6 ± 0.69 f</td>
</tr>
<tr>
<td>Phe</td>
<td>0.58 ± 0.00 f</td>
<td>4.66 ± 0.13 c</td>
<td>0.53 ± 0.00 f</td>
</tr>
<tr>
<td>Gin</td>
<td>4.37 ± 0.56 e</td>
<td>7.27 ± 0.07 d</td>
<td>1.94 ± 0.07 f</td>
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<tr>
<td>Orn</td>
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<td>1.73 ± 0.07 cdef</td>
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</tr>
<tr>
<td>Lys</td>
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<td>4.82 ± 0.01 ab</td>
<td>ND</td>
</tr>
<tr>
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<td>2.11 ± 0.03 cd</td>
<td>ND</td>
</tr>
<tr>
<td>Tyr</td>
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<td>1.05 ± 0.00 de</td>
<td>1.16 ± 0.15 d</td>
</tr>
<tr>
<td>Trp</td>
<td>ND</td>
<td>1.63 ± 0.03 d</td>
<td>ND</td>
</tr>
<tr>
<td>Umani</td>
<td>3.67 ± 0.10 f</td>
<td>18.35 ± 0.12 c</td>
<td>4.54 ± 1.03 f</td>
</tr>
<tr>
<td>Sweet</td>
<td>9.6 ± 1.25 d</td>
<td>25.72 ± 0.67 c</td>
<td>6.01 ± 0.25 d</td>
</tr>
<tr>
<td>Bitter</td>
<td>3.78 ± 0.09 ef</td>
<td>21.88 ± 0.29 c</td>
<td>1.64 ± 0.04 f</td>
</tr>
<tr>
<td>Tasteless</td>
<td>1.67 ± 0.06 ef</td>
<td>5.87 ± 0.00 b</td>
<td>1.16 ± 0.15 f</td>
</tr>
<tr>
<td>Total</td>
<td>18.72 ± 2.47 f</td>
<td>71.82 ± 0.50 a</td>
<td>13.34 ± 1.47 f</td>
</tr>
</tbody>
</table>

\(^A\) Conventional extraction was performed at room temperature for 45 min using 0.1 M HCl as extraction solvent, without any enzyme treatment. Enzyme-assisted extraction was performed at optimized conditions developed in the present study (extraction was assisted by a mixture of β-glucanase and Flavourzyme\(^B\) at a concentration of 5% v/v each; water was used as extraction solvent (with initial pH 7.0); extraction temperature and time were set as 50 °C and 1 h, respectively). FAA are grouped based on their taste characteristics: umami (Asp + Glu); sweet (Thr + Ser + Gly + Ala + Pro); bitter (Val + Met + Ile + Leu + Phe + His + Arg + Trp); tasteless (Tyr + Lys). Values are represented as mean ± standard deviation (SD). SD of 0.00 indicates an SD below the significant figures shown; ND—ndetect. Means with different letters within a row are significantly different (Tukey's HSD, \(P < 0.05\)).

4. Conclusions

It has been shown that enzyme-assisted extraction is a promising method to improve the recovery of MSG-like amino acids from mushrooms. However, the type of enzyme is of crucial importance, as treatment with the cellulolytic enzyme β-glucanase did not enhance the recovery of FAAs, while treatment with protease Flavourzyme\(^B\) enhanced the umami and other taste-contributing FAAs significantly. The optimal recovery of the MSG-like FAAs from the mushrooms was obtained by extracting with a two-enzyme assay of the cell-wall degrading enzyme β-glucanase and the proteolytic enzyme Flavourzyme\(^B\). The treatment parameters pH, temperature, enzyme concentration and incubation time significantly affected the extraction yield but to different extent. Overall, increasing pH, temperature, and concentration resulted in enhanced extraction (though dependent on the actual assay), whereas conducting the incubation more than 1 h caused a loss of FAAs.
Conflict of interest

Authors have declared that no competing interests exist.

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vided by Bente Pia Danielsen and Henriette Rïïbjerg Eriksen.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.04.157.

References


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