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Umami Ingredient: Flavor enhancer from shiitake (*Lentinula edodes)* byproducts

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ABSTRACT

An alternative use of shiitake stipes, usually treated as waste, was proposed for the production of a powder ingredient, rich in umami compounds, aiming its application in food. The extraction of umami compounds was optimized through the Response Surface Methodology (RSM), in order to obtain an extract with high umami taste intensity. From the optimized condition, a comparative analysis of shiitake stipes dehydration method was performed. Stipes were dehydrated by hot air drying (HD) and freeze drying (FD), submitted to extraction and the umami compounds in the extracts were compared. The comparative analysis showed that the 5′ - nucleotides are more sensitive to prolonged heating, while the release of free amino acids (FAA) was favored by hot air drying. The HD samples extract showed higher Equivalent Umami Concentration (EUC). The spray drying of the HD samples extract allowed the production of a newly powder ingredient rich in umami compounds (Umami Ingredient) that can be applied in diverse food matrices. Due to the presence of umami compounds, Umami Ingredient can be a potential alternative to help in the process of sodium reduction by enhancing food flavor.

1. Introduction

World-famous, mushrooms are known for their unique flavor. This flavor comes from the mixture of various elements present in these fungi, including soluble sugars, free amino acids (FAA), 5′- nucleotides, peptides and organic acids, which contribute to taste perception, and volatile compounds that contribute to aroma (Chen et al., 2015; Dermiki, Phanphensophon, Mottram, & Methven, 2013; Kong et al., 2019). Among the taste components stand out those linked to umami taste, as edible mushrooms are considered good sources of umami compounds (Sun et al., 2020; Zhang, Venkitasamy, Pan, & Wang, 2013).

Discovered by Kikunae Ikeda in 1908, umami (fifth basic taste) is described as delicious or savory (Yamaguchi & Ninomiya, 2000). The umami taste is mainly attributed to the presence of $_{L}$ - glutamic acid ($_{L}$ - Glu) and its salt, monosodium glutamate (MSG). Besides these, $_{L}$ - aspartic acid ($_{L}$ - Asp) and 5′- nucleotides, including 5′- guanosine monophosphate (5′- GMP), 5′- inosine monophosphate (5′- IMP), 5′- adenosine monophosphate (5′- AMP) and 5′- xanthosine monophosphate (5′- XMP) also provide umami taste. The 5′- nucleotides combined with $_{L}$ - Glu and $_{L}$ - Asp have synergistic effect on umami taste perception (Dermiki et al., 2013; Yamaguchi, 1991). More recently, some peptides like Gly-Cys-Gly (GCG), Glu-Pro-Glu (EPE) and Cys-Met (CM) have also been reported as umami-enhancing substances (Kong et al., 2019).

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Mushrooms umami compounds have aroused the interest of the scientific community in recent years (Chen et al., 2015; Kong et al., 2019; Phat, Moon, & Lee, 2016; Rotola-Pukkila, Yang, & Hopia, 2019). Studies indicate the potential of using mushrooms as umami ingredients to enhance food flavor (Mattar et al., 2018; Wong et al., 2017; Zhang et al., 2013). The shiitake (*Lentinula edodes*) is the main edible mushroom cultivated worldwide (Royse, Baars, & Tan, 2017), being known, mainly, for its typical flavor and aroma (Dermiki et al., 2013; Tian, Zhao, Huang, Zeng, & Zheng, 2016). Although shiitake is among the most studied edible species, many of these studies on mushrooms umami compounds evaluate only the pileus, and few studies evaluate mushroom by products (stipes) (Chen et al., 2015; Cho, Choi, & Kim, 2010). In general, stipes are not used for commercial purposes, probably because they do not have the sensory characteristics suited to taste of consumers (Li et al., 2018), which may be related to the firmer texture, due to the high fiber content (Li et al., 2018). Finding alternatives for the use of umami compounds (and/or nutrients) present in the mushroom stipes is an economic and environmental issue, since stipes represent 25 to 33% of mushrooms fresh weight, and are usually used for activities of low economic value, such as animal feed and composting (Chou, Sheih, & Fang, 2013).

Umami compounds can play an important role in the process of reducing sodium in foods, since they enhance the perception of salty taste (Mojet, Heidema, & Christ-Hazelhof, 2004; Yamaguchi & Takahashi, 1984), avoiding loss in sensory quality and consequent rejection of food products. MSG is the most widely used umami compound to enhance food flavor. However, consumers consider food without added MSG to be safer and healthier (Radam, Yacob, Bee, & Selamat, 2010) - although this is only linked to consumer perception, and not to a proven fact, as MSG is scientifically recognized as safe (Beyreuther et al., 2007; Henry-Unaeze, 2017) – it can interfere with the positioning of added MSG products on the market. In view of this situation, the use of umami compounds naturally present in mushrooms as flavor enhancers is an alternative to satisfy the demand for savory and reduced sodium foods with fewer synthetic additives.

The aim of this study was to extract the umami compounds present in shiitake (*Lentinula edodes*) stipes to develop a newly mushroom based powder ingredient, rich in umami compounds. This new ingredient can be a potential alternative to be applied in reduced sodium foods, helping to maintain the products' flavor.

2. Material and methods

2.1. Shiitake stipes samples

The shiitake stipes were collected in an industry in the state of São Paulo (Brazil) in March 2019. The samples were frozen, transported in thermal boxes with ice, and kept in a freezer (-24 °C) until use. For processing, the stipes were unfrozen and sanitized in an aqueous solution of 200 ppm sodium hypochlorite for 10 min, followed by rinsing in flowing water. Immediately after, the samples were cut (width approximately 1 cm) and blanched using water steam (Philips Walita RI9120, Blumenau, Brazil) for 3 min (Maray, Mostafa, & El-Fakhrany, 2018). Then part of the stipes was dehydrated by: 1) hot air drying at 70 ± 2 °C for 12 h in an oven (Tecnal TE 394/1, Piracicaba, Brazil) with parallel air flow renewal at constant velocity of 2.0 m/s (HD); 2) freeze drying, where the samples were frozen and freeze-dried (Liotop L101, São Carlos, Brazil, −51 °C, 0.15–0.3 mmHg) for 96 h (FD). In both cases the dehydrated material was ground in a hammer mill (Marconi MA 090, Piracicaba, Brazil), sieved (40 mesh), packed in plastic bags (polyethylene) and stored in a dry, ventilated place away from light.

2.2. Optimization of umami compounds extraction using response surface methodology (RSM)

For optimization, HD stipes were submitted to umami compounds extraction following the Central Composite Rotatable Design (CCRD), in which the independent variables were the solute:solvent ratio (w:v) and the bath temperature (Table 1). The independent variables selection and ranges were defined considering the conclusions of previous studies (Dermiki et al., 2013; Poojary, Orlien, Passamonti, & Olsen, 2017) and a preliminary test (data not shown). The variable 'extraction time' was also evaluated in the preliminary test, but it did not show significant influence on the umami taste intensity. In addition, previous research showed that the recovery of MSG-like FAA and 5′-nucleotides is not significantly influenced by extraction time (Poojary et al., 2017). Thus, the independent variable, 'extraction time', was not applied to CCRD design.

In order to apply the umami compounds of the shiitake stipes in food products, water was used as the extraction solvent. For the variable solute:solvent ratio, 1 g of solute was used with the different volumes that make up the design (Table 1). The extraction was conducted in a heated bath (Tecnal Dubnoff - NT 269, Piracicaba, Brazil) with temperature control (± 1 °C). All flasks were shaken (180 rpm) for 30 min (Poojary et al., 2017). The extracts were then filtered through a qualitative paper filter (Unifil – 501.018, pore size 4–12 μm) and frozen (−24 °C) until use. The experiments were conducted in random order to minimize the effects of uncontrolled factors.

The dependent variable was the umami taste intensity in aqueous extracts, evaluated by panelists. The sensory analysis was conducted following Stone and Sidel (2004) recommendations, with adaptations. To participate in the study, eighteen panelists were recruited, they performed a difference-from-control test with MSG solutions in

Table 1
Central composite rotatable design (CCRD) applied to umami compounds extraction from shiitake stipes and dependent variable (umami taste sensory intensity).

Experimental assay	Run order	Independent variables				Dependent variable
		Coded value		Real value		Umami taste intensity
		Volume (V - mL)	Temperature (T - °C)	Volume (V - mL)	Temperature (T - °C)	
1	2	−1	−1	27	31	4.73
2	4	1	−1	63	31	1.22
3	7	−1	1	27	63	4.14
4	6	1	1	63	63	1.68
5	12	−1.41	0	20	47	5.13
6	1	1.41	0	70	47	1.03
7	9	0	−1.41	45	24	1.40
8	3	0	1.41	45	70	3.26
9	11	0	0	45	47	2.50
10	5	0	0	45	47	1.75
11	10	0	0	45	47	1.61
12	8	0	0	45	47	1.88

different concentrations (0.2, 0.4 and 0.6% (control) w/v), in three repetitions. Fourteen panelists were pre-selected regarding their discriminative capacity (panelists' ability to discriminate different samples, $p \leq 0.30$) and reproducibility capacity (panelist's ability to repeat judgments in different sessions of analysis, $p > 0.05$).

In the next step, the pre-selected panelists defined the umami taste attribute as: 'Fifth basic taste, characteristic of a monosodium glutamate aqueous solution', as well as they defined the references of minimum (None: Water) and maximum (High: Monosodium glutamate aqueous solution 0.6%). From this, they were trained to evaluate the umami taste intensity in the samples and to use the unstructured linear intensity scale of 90 mm.

The training stage lasted seven sessions. At each session, the panelists evaluated five different samples of mushroom extract (run 5, 6, 7, 8 and 9 - Table 1), firstly three and after two samples, with 1 h break between each group in order to avoid sensory fatigue. Data resultant from the last three sessions was used for evaluation of the panelists regarding to their discriminative capacity ($p \leq 0.30$), reproducibility capacity ($p > 0.05$) and consensus with the sensory panel (Pearson's $r > 0.70$) (Damásio & Costell, 1991). For the final analysis, seven of the fourteen panelists were selected, who were again evaluated regarding their discriminative capacity ($p \leq 0.30$) and reproducibility capacity ($p > 0.05$), being necessary to exclude one more panelist. Thus, the final sensory panel consisted of six panelists, all female, aged between 19 and 32 years.

The final analysis of the extracts was conducted in individual booths, under white light and at a temperature of 23 °C, following the procedure performed during the training stage. The twelve samples of shiitake extract (15 mL) were presented in plastic cups, coded with three-digit random numbers and evaluated in two sessions of six samples each. This procedure was performed in three repetitions. The sample evaluation was performed in complete blocks, i.e., all the panelists evaluated all the samples. The sample presentation was monadic (one sample presented at a time) and in balanced order according to Macfie, Bratchell, Greenhoff, and Vallis (1989) to avoid bias by positional and carry over effects. The panelists received, along with the sample, a glass of water and a water biscuit to clean the entire oral cavity between the samples. This sensory test was approved by the Research Ethics Committee of ESALQ/USP (Decision 2.994.710).

From the optimized extraction condition, extracts were produced for the chemical determination (items 2.3 and 2.4) of umami compounds. In order to evaluate the effect of the dehydration method applied to the shiitake stipes prior to extraction, HD and FD samples (item 2.1) were submitted to the optimized extraction condition and their umami compounds were compared.

2.3. Free amino acids analysis

FAA were analyzed according to Wu and Meininger (2008) in a high performance liquid chromatograph (HPLC) with fluorescence detector (RF – 20 A) (HPLC-F) (Shimadzu, Kyoto, Japan), using as stationary phase a C18 column (Supelco LC-18, 250 mm × 4.6 mm, 5 μm). The mobile phase used was A: 100 mM sodium acetate (90.49%) + methanol (9%) + tetrahydrofuran (0.5%) + 6 N hydrochloric acid (0.0048%), and B: 100% methanol. The elution was conducted in gradient (LC − 10 AD pumps), as follows: 0–15 min, 14% B; 15–20 min, 14–30% B; 20–24 min, 30–35% B; 24–26 min, 35–47% B; 26–34 min, 47–50% B, 34–38 min, 50–70% B; 38–40 min, 70–100% B; 40–50 min, 100% B; 50–51 min, 14% B; 51–56 min, 14% B. The flow rate used was 1 mL/min, and the column temperature was maintained at 40 °C (CTO oven − 10 AC). All samples were filtered (0.20 μm) before injection into the system.

For the derivatization of amino acids, an OPA-Borate solution + β - mercaptoethanol (OPA + MeOH + borate buffer + β - mercaptoethanol, filtered in 0.20 μm) was used. Fluorescence detector was used to detect the amino acids with excitation at 340 nm and emission at 455 nm. Amino acids were identified by comparison with the retention time of authentic standards (Sigma Aldrich, St Louis, USA). The concentrations of analytes in the samples were determined using their calibration curves and expressed as mg free amino acid per gram of dry sample (mg FAA/g DW).

2.4. 5′- nucleotides analysis

The extracts were centrifuged at 3500 rpm (Fanem Excelsa 2206, Guarulhos, Brazil) for 20 min, 1 mL of the supernatant was filtered in a 0.22 μm membrane and packed in vials. The method proposed by Poojary et al. (2017) with minor modifications was used. The analyses were performed on a HPLC (Shimadzu, Kyoto, Japan) with diode arrangement detector (DAD, model SPD-6AV). The stationary phase used was the Agilent eclipse XDB-C18 column (250 mm × 4.6 mm; 5 μm), maintained at 25 °C. The mobile phase was composed by A: potassium phosphate buffer - KH_2PO_4, 50 mM, pH 4.8 and B: methanol, which was eluted in gradient, being: 0–5 min, 0% B; 14–22.5 min, 10% B and 23–30 min, 0% B. The flow was maintained at 0.5 mL/min. The analytes were detected and quantified at 254 nm. The identity of the analytes was confirmed by co-injection of the samples with the authentic nucleotide standards (all 5′-nucleotides standards were purchased from Sigma Aldrich, St Louis, USA, except 5′-XMP, that was purchased from Santa Cruz Biotechnology, Dallas, USA), as well as, by comparison of their UV–Vis scanning spectra. The analyte concentrations in the samples were determined with the aid of their calibration curves and expressed as mg of 5′-nucleotide per gram of dry sample (mg/g DW).

2.5. Equivalent umami concentration (EUC)

EUC is the concentration of MSG (g/100 g) equivalent to the umami taste intensity provided by the mixture of the umami amino acids (Glu and Asp) and 5′- nucleotides, which is calculated according to Eq. (1) (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971):

$$Y = \sum a_i b_i + 1218\left(\sum a_i b_i\right)\cdot\left(\sum a_j b_j\right) \quad (1)$$

where Y is the EUC of the sample (g MSG/100 g), a_i is the concentration (g/100 g) of each umami amino acid (Glu or Asp); a_j is the concentration (g/100 g) of each umami 5′- nucleotide (IMP, GMP, XMP or AMP); b_i is the relative umami concentration (RUC) for each umami amino acid in MSG (Glu, 1; Asp, 0.077); b_j is the RUC for each umami 5′- nucleotide in IMP (IMP, 1; GMP, 2.3; XMP, 0.61 and AMP, 0.18); and 1218 is the synergistic constant based on the concentration of g/100 g used.

2.6. Umami ingredient

In order to facilitate the transport, storage and application of the shiitake extract in various food products, the liquid extract with higher EUC was spray dried to obtain a powder ingredient (Umami Ingredient).

2.6.1. Spray drying process: Umami Ingredient production

For powder extract (Umami Ingredient) production, some pre-tests were performed to evaluate the amount of carrier agent, maltodextrin (MD) (Ingredion DE-20), to be added to the liquid extract before drying. The concentrations of 10, 20, and 40% of solids of extract were tested, i.e. 10, 20, and 40 g of extract solids/100 g of total solids (data not shown). Each mixture was homogenized separately and dried in laboratory scale spray dryer (Mini Spray Dryer Buchi B-290, Flawil, Switzerland). The drying conditions were: inlet air temperature 170 ± 1 °C, outlet temperature 84 ± 2 °C, feed sample rate 9.4 mL/min, nozzle diameter 0.7 mm, aspiration 35 m^3/h, atomization gas flow of 600 L/min equivalent to 0.75 bar of atomization pressure, room

temperature 23 °C, room air humidity 35–45%.

For the three concentrations assessed the process yield and the efficiency in the retention of L - Glu (main umami compound) was verified. The best result was obtained with 20% concentration, in which the drying process was facilitated, in addition to presenting higher retention of L - Glu and higher yield. The use of another carrier agent, modified starch (MS) (Capsul), was also evaluated in the proportion previously tested (20 g of extract solids/100 g of total solids). Comparatively, MD and MS showed very similar performance and process yields, as well as similar flavor (data not shown). Taking into account these results, MD was chosen as the carrier agent due to its lower cost.

2.6.2. Umami Ingredient physical properties

The drying yield was determined by the ratio of the mass of solids obtained at the end of the process to the mass of solids at the beginning of the process. The moisture was determined by Karl Fischer volumetric titration (Titrando 901, Methrom Pensalab, São Paulo, Brazil), using methanol: formamide (1:1, v/v) as a solvent.

The size distribution was determined by laser scattering using a LV 950-V2 equipment (Horiba, Kyoto, Japan). The samples were dispersed in absolute ethanol. The mean diameter was expressed as the average diameter of sphere of the same volume ($D_{4.3}$) and the polydispersity was given by span index, which was calculated according to Eq. (2) (Alvim, Stein, Koury, Dantas, & Cruz, 2016).

$$SPAN = (D_{90\%} - D_{10\%})/D_{50\%} \quad (2)$$

where $D_{10\%}$, $D_{50\%}$ and $D_{90\%}$ correspond to the diameters for 10%, 50% and 90% of the cumulative size distribution.

The solubility was determined as described by Cano-Chauca, Stringheta, Ramos, and Cal-Vidal (2005). In a blender (Philips, RI2160, 550 W, Barueri, Brazil), 1.5 g (dry weight -DW) of sample were added in 150 mL of distilled water and shaken at high speed for 4 min. The solution was centrifuged at 3000 *g* for 5 min. Aliquots of 25 mL of the supernatant were transferred to previously weighed Petri dishes and dried at 105 °C for 5 h. Solubility (%) was calculated as the weight of dry solids in the supernatant represented as a percentage of the initial sample weight.

The water activity was determined in a water activity analyzer (Aqua Lab 4TE, Meter Group, Pullman, USA) at 25 ± 0.1 °C (Favaro-Trindade, Santana, Monterrey-Quintero, Trindade, & Netto, 2010).

To determine the hygroscopicity, the method described by Cai and Corke (2000), with modifications, was used. Approximately 1 g of sample was weighed and stored in a desiccator containing a saturated NaCl solution (75% RH) kept at 25 °C. After one week, the samples were weighed and the hygroscopicity was expressed as g of water absorbed by 100 g of dry solid (g H_2O/100 g DW).

The instrumental color was determined by colorimeter (Minolta Chroma Meter CR-400, Konica Minolta, NJ, USA), by reading the parameters L*, a* and b* of the CIELab system, with D65 light source.

2.6.3. Sodium content

The sodium content was determined according to the method described by AOAC (2010). The samples (1 g) were incinerated at 550 °C in muffle furnace until the ashes were obtained. These were diluted in nitric acid and ultrapure water for reading in a flame photometer (Micronal B262, São Paulo, Brazil). The equipment was calibrated with standard sodium solution.

2.6.4. Glutamic acid retention

To determine Glu retention (GR) in Umami Ingredient, this component was extracted from the powder sample (Glu quantified after processing), according to item 2.2. Glu concentration in Umami Ingredient and in liquid extract (Glu quantified before processing) was performed as described in Section 2.3. Eq. (3) was used to calculate % GR:

$$\%GR = \frac{Glu\ \ quantified\ \ after\ \ processing}{Glu\ \ quantified\ \ before\ \ processing} * 100 \quad (3)$$

2.7. Statistical analyses

The results of the experimental design were submitted to analysis of variance (ANOVA) and the significance of the experimental parameters was evaluated by the values of *F* and *P* ($p \leq 0.05$). The non-significant parameters were removed, and the mathematical model was readjusted, the lack of fit and the coefficient of determination (R^2) were verified. The response surfaces as well as the statistical analyses were performed in the Statistica 10.0 software (StatSoft Inc., USA). The other results were obtained using a completely randomized design (CRD) and the results were expressed as mean ± standard deviation. The data was submitted to exploratory analysis, checking the normality of the residues (Shapiro-Wilk test), homogeneity of variances (Brown and Forsythe's test) and the presence of outliers. The data that met the assumptions of the exploratory analysis was submitted to the *t* test ($p \leq 0.05$), the other data was analyzed using the non-parametric Wilcoxon-Mann-Whitney test ($p \leq 0.05$) to compare the results between the samples. The analyses were performed in SAS Studio software (SAS Institute, Cary, USA).

3. Results and discussion

3.1. Optimization of umami compounds extraction

There were significant linear and quadratic effects ($p < 0.05$) only for the 'solute: solvent ratio (w:v)' variable. The adjusted quadratic model (Eq. (4)) exhibited a coefficient of determination (R^2) of 0.87 and no significant lack of fit ($p = 0.218$). Thus, the model obtained had a good fit and is considered predictive.

$$Umami\ \ taste\ \ intensity = 2.14 - 1.47 * V + 0.58 * V^2 \quad (4)$$

Through the response surface (Fig. 1) the contribution of each variable to umami taste intensity is evident. The highest intensity of umami taste (5.13) was reached when less water was used in the extraction, i.e., using the 1:20 ratio (solute: solvent ratio (w:v)), because in this condition, umami compounds were less diluted in water, i.e., they were more concentrated, reflecting in higher intensity of the umami taste. According to Poojary et al. (2017), that optimized the extraction of umami compounds from mushrooms, the optimized extraction condition employed a 1:50 ratio (solute:solvent ratio (w:v)), nevertheless the dependent variable used in that study was the chromatographic analysis of the umami compounds present in the extract, differing from what was done in our study. This difference in results is expected because the chromatographic evaluation of umami compounds shows high sensitivity to the presence of each compound individually, whereas in the sensory evaluation all umami compounds present in the sample are evaluated simultaneously, interacting with the umami taste receptors (T1R1/T1R3, brain and taste-mGluR1, brain and taste-mGluR4) and producing nerve signals in the brain, where the characteristic umami sensation is perceived (Zhang, Sun-Waterhouse, Su, & Zhao, 2019). Poojary et al. (2017) found different optimal extraction condition (70° C, 30 min, 50 mL of water). However, it should be remembered that those authors used whole mushrooms and not only stipes for extraction. Moreover, the response was based on the chemical determination of umami compounds (FAA and 5′-nucleotides) and not on a sensory evaluation.

Thus, considering what was previously presented in the present study, to obtain an extract with high intensity of umami taste, 20 mL of water per gram of shiitake stipe (HD) during 30 min of extraction must be applied, regardless of the temperature.

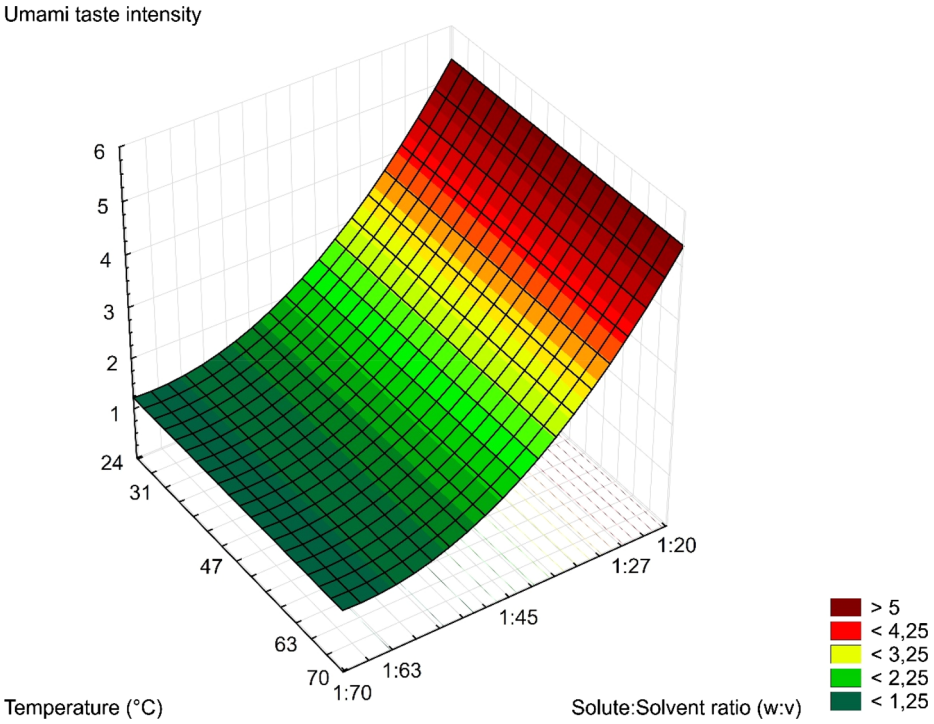

Fig. 1. Response surface showing the effect of the independent variables (temperature and solute: solvent ratio (w/v)) on the umami taste intensity of shiitake stipes extract.

3.2. Effect of dehydration method on umami compounds

Many factors influence the mushrooms taste compounds, such as the part used (stipe or pileus), the maturity stage (Chen et al., 2015; Cho et al., 2010), the conservation method (Liu et al., 2014), the type of cooking (Rotola-Pukkila et al., 2019), and the dehydration method applied (Yang et al., 2019). In the present study, it was possible to verify that the hot-air drying process made possible greater extraction of FAA (Table 2). Probably this is related to proteolysis promoted by the increase in temperature during hot-air drying, as pointed out by some authors (Xu et al., 2019; Yang et al., 2019).

Some FAA can be classified according to their taste characteristics, being divided according to the basic taste they elicit: MSG-like, sweet, bitter, and tasteless FAA. The perception of basic tastes, meanwhile, also depends on hydrophobicity, size, charge, functional groups on the side chain and chirality of the alpha carbon. The MSG-like group includes L- Aspartic and L- Glutamic acid. The amino acid group linked to sweet taste includes L- Alanine, L- Glycine, L- Serine and L- Threonine which are small and hydrophilic molecules. The FAA linked to the bitter taste are larger molecules with hydrophobic behavior, such as L- Leucine, L- Isoleucine, L- Phenylalanine, L- Tryptophan. Other molecules with intermediate properties, such as L- Methionine, L- Valine, L- Histidine and L- Arginine, elicit bitter taste too (Kawai, Sekine-Hayakawa, Okiyama, & Ninomiya, 2012).

The characteristic taste of mushrooms, umami taste or palatable taste, is mainly due to the presence of MSG-like FAA, L- Aspartic e L- Glutamic (Yang, Lin, & Mau, 2001), which were found in greater amount in the HD sample (3.07 mg/g DW). According to Yang et al. (2001), MSG-like FAA can be split into three ranges: low (< 5 mg/g), middle (5–20 mg/g), and high range (> 20 mg/g). In this study, it was observed that HD sample fits in low range, a result close to that identified by Chen et al. (2015) for samples of *L. edodes* stipe in the first stage of maturity (3.56 mg/g DW). On the other hand, the amount of MSG-like FAA found in HD was higher than that reported by Yang et al. (2019) (0.9–1.3 mg/g DW) for dried shiitake samples.

Beyond MSG-like FAA, 5′- nucleotides also influence umami taste perception, since the presence of four of the six 5′- nucleotides (5′- AMP; 5′- IMP, 5′- GMP and 5′- XMP) exerts a synergistic effect on umami taste (Dashdorj, Amna, & Hwang, 2015; Poojary et al., 2017; Yamaguchi et al., 1971). In the assessed samples among the six 5′- nucleotides evaluated, 5′ - XMP was not found and 5′ - IMP was not found in any quantifiable amount (Table 2). The amount of umami nucleotides in the HD sample was lower than in the FD sample, indicating that nucleotides are sensitive to long processes at high temperature (Li et al., 2015). 5′ - GMP and 5′ - UMP contents were higher in FD sample, whereas, 5′ - AMP and 5′ - CMP content did not differ ($p > 0.05$). The average of umami 5′ – nucleotides found in the samples was almost the same to the one found by Li et al. (2018) (3.78 mg/g DW) and higher than the amount found by Poojary et al. (2017) (1.54 mg/g DW), this difference may be related to the cultivation techniques used, part of the mushroom used, maturity stage and processing method (Zhang et al., 2013).

The combination of MSG-like FAA and umami nucleotides has synergistic effect on umami taste, a fact which was demonstrated by Yamaguchi et al. (1971). This relation was studied through sensory analysis and expressed as an equation (Eq. (1)). By evaluating the EUC of the HD and FD samples (Table 2), the influence of the dehydration process on the umami compounds is noticeable, in which the conventional hot-air drying process allowed greater recovery of these compounds, contributing to the greater EUC. In the case of the FD sample, which showed only traces of MSG-like FAA, the presence of umami nucleotides was not sufficient for its EUC to be of significant value.

According to Mau (2005), EUC values can be arranged in four levels: (1) > 1000 g MSG/100 g DW, (2) 100–1000 g MSG/100 g DW, (3) 10–100 g MSG/100 g DW, (4) < 10 g MSG/100 g DW. Hence, the HD sample fits into the third level (81.55 g MSG/100 g DW), corroborating previous studies (Chen et al., 2015; Phat et al., 2016). This result indicates that drying the shiitake stipes by the conventional method (hot-air drying) allows greater extraction of umami compounds (in the optimized condition, item 3.1).

Table 2
Free amino acids and 5′- nucleotides (mg/g DW) identified in hot-air dried (HD) and freeze-dried (FD) shiitake stipes.

	Hot-air dried (HD)	Freeze dried (FD)
Free amino acids		
L-Alanine	1.75 ± 0.12 (8.0)*A	0.02 ± 0.00 (3.0)B
L- Arginine	1.60 ± 0.06 (8.0)A	0.11 ± 0.00 (3.0)B
L- Asparagine	0.52 ± 0.02 (8.0)A	0.02 ± 0.00 (3.0)B
L- Aspartic	0.39 ± 0.02	TR
L-Citrulline	ND	ND
L- Glutamic	2.68 ± 0.09	TR
L- Glycine	0.44 ± 0.06	TR
L- Isoleucine	0.65 ± 0.03	TR
L- Leucine	0.99 ± 0.04 (8.0)A	0.01 ± 0.00 (3.0)B
L-Lysine	1.38 ± 0.25 (8.0)A	0.06 ± 0.00 (3.0)B
L-Methionine	0.10 ± 0.01	TR
L- Ornithine	5.16 ± 0.72	TR
L- Phenylalanine	0.78 ± 0.03 (8.0)A	0.01 ± 0.00 (3.0)B
L- Serine	0.41 ± 0.02 (8.0)A	0.06 ± 0.00 (3.0)B
L- Threonine	1.00 ± 0.04 (8.0)A	0.04 ± 0.00 (3.0)B
L- Tryptophan	0.24 ± 0.01	TR
L- Valine	1.05 ± 0.03 (8.0)A	0.01 ± 0.00 (3.0)B
MSG-like	3.07 ± 0.11 (8.0)A	0.00 ± 0.00 (3.0)B
5′- Nucleotides		
5′-AMP	1.46 ± 0.16a	1.73 ± 0.24 a
5′-CMP	3.80 ± 0.44a	3.92 ± 0.44 a
5′-GMP	0.96 ± 0.07b	1.31 ± 0.12a
5′-IMP	TR	TR
5′-UMP	0.69 ± 0.08b	1.19 ± 0.16a
5′-XMP	ND	ND
Umami nucleotides	2.42 ± 0.23b	3.04 ± 0.35a
Equivalent umami concentration (EUC)		
EUC (g MSG/100 g DW)	81.55 ± 6.25 (8.0)A	0.00 ± 0.00 (3.0)B

Each value is expressed as mean ± standard deviation (n = 5). Different capital letter following numbers within a row are significantly different by non-parametric Wilcoxon-Mann-Whitney test ($p \leq 0.05$). Different lowercase letter following numbers within a row are significantly different by test *t* ($p \leq 0.05$). TR: trace; ND: not detected. MSG-like = L- Asp + L- Glu; Umami nucleotides: 5′-GMP + 5′-IMP + 5′-XMP + 5′-AMP.

* Numbers in parentheses are mean scores used in non-parametric Wilcoxon-Mann-Whitney test.

3.3. Umami ingredient process and properties

HD extract spray drying, using maltodextrin as a carrier agent, provided a yield of 60 ± 0.03%, which is considered good for the scale used (laboratory). This result was superior to that reported by Francisco et al. (2018) and Ribeiro et al. (2015) for mushroom extract spray drying. Yield is affected by the amount of material deposited on the wall of the equipment, droplets and powder can stick on the walls and cyclone, reducing the amount of product collected at the end of the process (Wang & Langrish, 2009).

The Umami Ingredient showed low moisture and aw (Table 3), revealing the efficiency of the drying process and favoring good stability during future shelf life, since for the observed values of these properties, the microbial growth is not favored (Ross, 2007; Santana, Cano-Higuita, De Oliveira, & Telis, 2016). These results are in agreement to other studies in the literature, which used maltodextrin as a carrier agent in drying of berries extracts and mango pulp, with moisture ranging from 0.92 to 4.7% (DW) and aw from < 0.05 to 0.24 (Gagneten et al., 2019; Zotarelli, da Silva, Durigon, Hubinger, & Laurindo, 2017).

Another property linked to powder stability is the hygroscopicity. This property is closely related to the water concentration gradient between the product and the ambient air, as the higher the concentration gradient, the greater will be its hygroscopicity (Tonon, Brabet, & Hubinger, 2008). Umami Ingredient showed higher hygroscopicity than reported by Gagneten et al. (2019) in their study with berries extracts (11.8–13.14%). However, its performance was similar to that of mango powder (18.8–25.4%) studied by Zotarelli et al. (2017).

Table 3
Physicochemical properties of Umami Ingredient.

Moisture (%)	2.76 ± 0.15
Solubility (%)	99.03 ± 0.01
Aw	0.159 ± 0.001
Hygroscopicity (%)	20.77 ± 0.16
Sodium (mg Na/100 g)	83.52 ± 1.07
Glu retention (%)	97 ± 0.01
Color	
*L**	90.56 ± 0.52
*a**	4.57 ± 0.03
*b**	17.97 ± 0.07
Particle size distribution	
D_{10} (μm)	4.24 ± 0.05
D_{50} (μm)	10.33 ± 0.28
D_{90} (μm)	17.80 ± 0.81
$D_{4.3}$ (μm)	10.87 ± 0.36
Span	1.31 ± 0.05

Each value is expressed as mean ± standard deviation (n = 3).

The product presented high solubility (Table 3) since maltodextrin has high water solubility (Cano-Chauca et al., 2005) and the umami compounds in the extract are hydrophilic. This characteristic is very important for an ingredient that has the function of enhancing food flavor, since umami compounds must be released in the mouth when in contact with saliva, allowing interaction with umami taste receptors. Studies with berries extracts (Gagneten et al., 2019) and mango juice (Cano-Chauca et al., 2005) also reported high solubility (> 90%), using maltodextrin as a carrier agent.

The coloring of an ingredient is another quality parameter that should be monitored as the color of food is related to consumer acceptance (Selani et al., 2016). Umami Ingredient exhibited a high value of L* (Table 3), indicating high luminosity, favored by the white coloration of maltodextrin. This is a positive result, as dark-colored ingredients have limited application in food (Toledo et al., 2019). The product under study presented low red color intensity (a* value) and higher yellow color intensity (b* value), which was expected since the extract of shiitake stipes has brownish coloring, which can lead to changes in yellow color (Mattar et al., 2018).

Particle size distribution is an important parameter of particulate system quality as it affects its transport, storage and physical and chemical properties, changing its performance (Tontul & Topuz, 2017). D_{10}, D_{50} and D_{90}, which represent, respectively, 10%, 50%, and 90% of the volumetric diameter of the accumulated particles, presented unimodal distribution (Fig. 2), with typical polydispersity of products obtained by spray drying (represented by the Span number, Table 3). Similar values were observed in other drying studies that used resembling equipment (Alvim et al., 2016; Fadini et al., 2018). The mean diameter of the particles, $D_{4.3}$, was close to that reported by Vardanega, Muzio, Silva, Prata, and Meireles (2019) (9.00 μm) which produced microparticles of Brazilian ginseng roots by spray drying. In accordance with the literature, the particle size is affected by process conditions, liquid viscosity and carrier agent concentration (Tonon et al., 2008).

The spray drying process using maltodextrin as an excipient, in addition to enable the drying of the liquid extract and convert it into powder, can help to protect the extract components during the process. To evaluate the efficiency in umami compounds retention, the main umami compound, L- Glu, was monitored. Umami Ingredient contained 1.81 mg L- Glu/g DW, representing 97% retention of L- Glu (Table 3) contained in the extract at the beginning of the spray drying process. This promising result shows the efficiency of the spray drying process in preserving L- Glu.

Considering the embedding of this ingredient in food products, the application of Umami Ingredient in salty foods with reduced sodium content is indicated, since the presence of umami compounds enhances the perception of salty taste (Mojet et al., 2004; Yamaguchi &

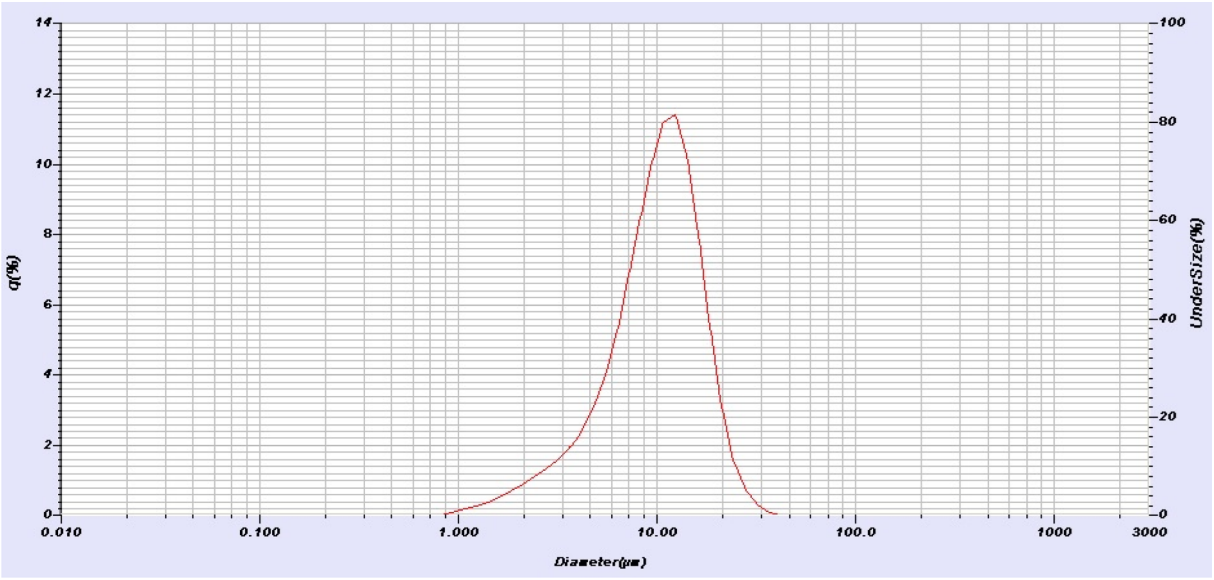

Fig. 2. Particle size distribution of spray-dried shiitake stipe extract (Umami Ingredient).

Takahashi, 1984), assisting in the preservation of flavor. In addition to concerns about excessive sodium consumption and health risks (WHO, 2014), the inclusion of Umami Ingredient could enhance food flavor, adding little sodium (83.52 mg Na/100 g) to the product. It should also be emphasized that the umami compounds present in Umami Ingredient are those naturally present in the shiitake stipes. This supports the positive perception of consumers, who consider healthier and safer non-added MSG foods and, meets the growing demand for products without MSG added (Radam et al., 2010).

4. Conclusions

It was possible to obtain an extract with umami compounds from shiitake stipes after defining optimal extraction conditions (1:20 solute: solvent ratio (w:v), i.e. 1 g of shiitake stipe (HD): 20 mL of water), 30 min of extraction, regardless of the temperature), using only water as a solvent. Hot-air drying favored the release of FAA and reduced the amount of some 5′ - nucleotides (5′ – GMP and 5′ – UMP). Spray dried extract consisted in a high $_{L}$ - Glu (main umami compound) retention product, that presented high solubility, low moisture and sodium content, in addition to light color, all suitable characteristics for a new food ingredient. The development of Umami Ingredient indicates a possibility to add value to a food byproduct. The potential of this ingredient as a flavor enhancer for low sodium foods has been assessed and results will be shown in future publications.

CRediT authorship contribution statement

Samara dos Santos Harada-Padermo: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Project administration, Writing - original draft, Writing - review & editing. **Liara Silva Dias-Faceto:** Formal analysis, Visualization, Writing - review & editing. **Miriam Mabel Selani:** Conceptualization, Resources, Funding acquisition, Writing - review & editing. **Izabela Dutra Alvim:** Investigation, Resources, Writing - review & editing. **Eny Iochevet Segal Floh:** Resources, Investigation. **Amanda Ferreira Macedo:** Investigation. **Stanislau Bogusz:** Investigation, Resources. **Carlos Tadeu dos Santos Dias:** Formal analysis. **Ana Carolina Conti-Silva:** Methodology, Writing - review & editing, Supervision. **Thais Maria Ferreira de Souza Vieira:** Funding acquisition, Methodology, Visualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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