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ORIGINAL ARTICLE

Optimization of enzymatic hydrolysis of *Pleurotus ostreatus* derived proteins through RSM and evaluation of nutritional and functional qualities of mushroom protein hydrolysates

Otimização da hidrólise enzimática de proteínas derivadas de P. ostreatus *por meio de Metodologia de Superfície de Resposta e avaliação de qualidade nutricional e funcional de hidrolisados da proteína do cogumelo*

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Abstract

Pleurotus ostreatus (Jacq.) P. Kumm., the second most widely cultivated oyster mushroom was grown on paddy straw, which is cheap and readily available waste material. After harvesting and drying, nutritional, and antinutritional composition of *P. ostreatus* were estimated using the standard assay methods. Tannin and phytic acid were present in very negligible amount (0.095 ± 0.027 mg/g and 0.150 ± 0.083 mg/g, respectively), whereas oxalate and cyanide were absent in whole mushroom. In fact, *P. ostreatus* was hydrolysed with commercially available proteinase K, pepsin and trypsin with different concentrations of the enzymes (0.05%, 0.10% and 0.15%), at different temperatures (30 °C, 40 °C and 50 °C) for different time periods (60, 90 and 120 min) to get the mushroom protein hydrolysates. Degree of hydrolysis and protein content varied from 4.29 ± 1.12% to 99.42 ± 0.02% and from 0.25 ± 0.07 mg/mL to 3.22 ± 0.12 mg/mL, respectively. Maximum degree of hydrolysis and the highest protein content of protein hydrolysate was obtained when using 0.15% proteinase K, at 50 °C for 120 minutes. Mushroom protein hydrolysates thus obtained exhibited improved functional characteristics such as foaming capacity, foaming stability and emulsifying property than the unhydrolysed mushroom. Based on the result of the present study, the mushroom protein hydrolysates could be served as useful ingredient for food and nutraceutical applications.

Keywords: Antinutrient; Mushroom protein hydrolysate; Degree of hydrolysis; RSM; Functional properties.

Resumo

Pleurotus ostreatus, segundo cogumelo comestível mais cultivado, foi cultivado em palha de arroz, que é um resíduo barato e amplamente disponível. Após a colheita e secagem, a composição nutricional e antinutricional de *P. ostreatus* foi estimada utilizando os métodos de ensaio padrão. Tanino e ácido fítico foram encontrados em quantidade muito insignificantes (0,095 ± 0,027 mg/gm e 0,150 ± 0,083 mg/g, respectivamente), enquanto oxalato e cianeto estavam ausentes no cogumelo inteiro. *P. ostreatus* foi hidrolisado com proteinase K comercialmente disponível, pepsina e tripsina, com diferentes concentrações das enzimas (0,05%, 0,10% e 0,15%), em diferentes temperaturas (30 °C, 40 °C e 50 °C), para diferentes períodos de tempo (60, 90 e 120 min), a fim de se obter os hidrolisados da proteína do cogumelo. O grau de hidrólise e o teor de proteínas variaram de 4,29 ± 1,12% a 99,42 ± 0,02% e 0,25 ± 0,07 mg/mL a 3,22 ± 0,12 mg/mL, respectivamente. O grau máximo de hidrólise e o maior teor proteico foram obtidos ao utilizar 0,15% de proteinase K, a 50 °C por 120 minutos. Os hidrolisados da proteína de cogumelo, assim obtidos, apresentaram características funcionais melhoradas em comparação ao cogumelo não hidrolisado, como capacidade de espuma, estabilidade de espuma e propriedade emulsificante,. Com base no resultado do presente estudo, essa proteína de cogumelo hidrolisada pode ser utilizada como ingrediente para aplicações alimentares e nutracêuticas.

Palavras-chave: Antinutrientes; Hidrolisado de proteína de cogumelos; Grau de hidrólise; RSM; Propriedades funcionais.

1 Introduction

Pleurotus ostreatus (Jacq.) P. Kumm., the most widely cultivated oyster mushroom, has some medicinal properties due to presence of bioactive compounds. In fact, *P.* mushroom contains various high molecular weight and low molecular weight bioactive compounds such as polysaccharides including ß-glucans, terpenes, polypheols, fatty acid esters etc. (Golak-Siwulska et al., 2018); ferulic acid, coumaric acid (Gąsecka et al., 2016), pleuran etc. (Park et al., 2016). Its cultivation is lucrative because it can be easily grown on very cheaply and abundantly available lignocellulosic waste substrates. Mushrooms containing phytates, oxalate, tannins, etc. decrease the bioavailability of nutrients (Kadiri, 1990; Woldegiorgis et al., 2015). To quantify the safe limit of consumption of *P. ostreatus*, its antinutritional factors needs to be investigated.

In a study by Yuan et al., bioactive protein from *P. eryngii* (PEP) was isolated and amino acid sequence was analysed, where the authors found 17 novel unique peptides in it exhibiting anti-inflammatory properties (Yuan et al., 2017). Eighteen essential and non-essential amino acids were identified in *Hericium erinaceus* (Bull.) Pers. mushroom and the total amino acid content was 2.59 ± 0.072 (%w/w) (Sangtitanu et al., 2020).

In addition, mushroom protein has better digestibility ranged from 60% to 70% (Lavelli et al., 2018) and excellent amino acid profile as well as presence of phenolic compounds with various therapeutic properties like antidiabetic, anticarcinogenic, hepatoprotective, immunomodulatory, hypocholesteremia etc. (Chaturvedi et al., 2018). Protein hydrolysates have been identified as a mixture of low molecular weight peptides with as little as possible free amino acids produced from different sources of protein during their partial hydrolysis (McCarthy et al., 2013; Schaafsma, 2009). Hydrolysates can be produced both chemically and enzymatically. Enzymatic hydrolysis is more desirable for its better-quality product formation and eco-friendly nature, whereas chemical hydrolysis reduces protein quality and its bioactive value. In recent years, protein hydrolysates are extensively used not only as dietary supplements but also as a flavor enhancer in coffee whiteners, fortifying agents in soft drinks and juices. These can be also used as food additives, texture enhancers and pharmaceutical products (Liu & Chiang, 2008; Zheng et al., 2006). Hydrolysis parameters (time, temperature, enzyme concentration) influence proteolysis resulting in Degree of Hydrolysis (DH). The DH is the principal parameter to be used in the hydrolysis optimization to obtain desired protein hydrolysates (Sujith & Hymavathi, 2011). Goswami and Bhowal, reported about enzymatic hydrolysis of *P. ostreatus*

through 2,4,6-trinitrobenzene sulfonic acid (TNBS) but optimization through Response Surface Methodology (RSM) was not performed to establish the DH%. In addition, evaluation of functional properties of protein hydrolysates were not reported to assess the quality of the Mushroom Protein Hydrolysate (MPH) (Goswami & Bhowal, 2015). Protein hydrolysates with improved emulsion stability index, emulsion activity index, foam capacity, oil and Water Holding Capacity (WHC) along with their nutritional value are successfully been used in various food formulations (Chatterjee et al., 2015). Oil Holding Capacity (OHC) of protein hydrolysates is an important attribute which not only influences the taste but also affects their application in food industry (as example bakery) where oil absorption is essential. It is already reported earlier that higher OHC tends to avoid phase separation and assist in improving palatability and taste retention of many food products (cake, mayonnaise, salad dressing etc.). The present research focused on the analysis of nutritional and antinutritional composition of freshly cultivated *P. ostreatus*. This study aimed at optimization process to obtain protein hydrolysate ascertaining their functional properties.

2 Materials and methods

2.1 Spawn collection for the cultivation of edible oyster mushroom

Spawn of edible oyster mushroom *P. ostreatus* was collected from Vivekananda Institute of Biotechnology, Nimpith, West Bengal, in India and then properly stored at 4 °C for analysis. Proteinase K (ex. *Tritirachium album* (Type A), 30U/mg, Novozyme product), pepsin (from porcine gastric mucosa 0.7 FIP-U/mg) and trypsin (from pancreas 2000 U/G) used for enzymatic hydrolysis and Bovine Serum Albumin (BSA) used as standard in soluble protein analyze were purchased from Sigma-Aldrich Corp. (MO).

Disodium tetraboratedecahydrate, Sodium dodecylsulfate (SDS), O-Pthalaldehyde (OPA), Dithiothreitol (DTT), and L-serine were also purchased from Sigma-Aldrich. Tannic acid, sulfosalcilic acid and, sodium phytate and all other chemicals and reagents used were of analytical grade and were purchased from Merck, India.

2.2 Cultivation of mushroom

Cultivation of *P. ostreatus* was performed by the following steps, i.e., substrate preparation, spawning and also cropping and harvesting. Spawn grown on paddy was used as the inoculums.

2.3 Proximate analysis of cultivated *P. ostreatus*

Nutritional composition of dried edible oyster mushroom (moisture, protein, carbohydrate, fat and ash) was determined according to Association of Official Analytical Chemists method with slight modifications. Moisture content was determined in the hot air oven at 80 ºC to 100 °C for 24 hours. For the determination of soluble protein content, the lyophilized mushroom powder was pretreated with 0.1N NaOH solution for 45 minutes and then centrifuged at 6000X g for 15 minutes. The supernatant obtained was evaluated according to Folin-Lowry method. Fat content was determined by the Soxhlet method. Ash content was quantified by keeping the sample in muffle furnace for 6 hours at the temperature of 600 °C. Total carbohydrate content was determined by anthrone method. All the experiments were performed in triplicate and the results were the average of the three values.

2.4 Determination of antinutritional properties

2.4.1. Oxalate

The oxalate content of the cultivated *P.ostreatus* was determined according to the method described by Day and Underwood, 1986 method. Oxalate (mg/g) was calculated from the titer value using the following Equation 1:

$$Oxalate\ mg/g = Titer\ Value \times 0.9004 \quad (1)$$

2.4.2 Tannin

The cultivated powdered mushroom (0.2 g) was weighed, mixed with 10 mL 70% acetone in a screw cap test tube and was placed in an ice bath for 10 minutes for complete extraction of tannin. The extract was filtered and 0.2mL of the supernatant was transferred to another test tube and diluted to 1mL with distilled water, in which a 20% Na_2CO_3 (2.5 mL) and 0.3mL of Folin reagent diluted with distilled water (1:2 v/v) were added. The resulting solution was thoroughly mixed and after 45 min of incubation at room temperature, the absorbance of the developing blue color solution and the standard solution was measured at 700 nm using spectrophotometer (JASCO V-630). Tannic acid was used as standard.

2.4.3 Phytic acid

Phytate content in the cultivated *P. ostreatus* was determined using the previous methods (Latta & Eskin, 1980; Vaintraub & Lapteva, 1988).

2.4.4 Cyanide

This experiment was determined according to the method of Association of Official Analytical Chemists (1980).

2.5 Preparation of mushroom protein hydrolysate

Fresh and cleaned fruiting bodies of mushrooms were cut into small pieces, freeze dried and grinded. The powdered mushroom was homogenized (REMI Motors RQ-124 A) with distilled water at a ratio of 1:2 ratio (w/v) in room temperature. The concentration of substrate was 0.5% (w/w) while preparing the mushroom protein hydrolysate. The powdered mushroom was centrifuged (REMI R-24) at 5000 rpm for 10 minutes to remove the unwanted debris. To obtain protein hydrolysates, the supernatant obtained from the previous step, was hydrolysed separately using proteinase k, pepsin and trypsin. The enzyme substrate ratio was 0.5% (w/v), 0.10% (w/v) and 0.15% (w/v), respectively. Different proteolytic enzymes were used at the same activity levels to compare their hydrolytic efficiency. Hydrolysis was carried out at the respective optimum pH of each protease (pH 8, 1.5 and 8 for proteinase k, pepsin and trypsin, respectively). The pH of the solution was maintained at the desired value using 1 M HCl or 0.1 M NaOH during hydrolysis. Here, the same activity level denotes that, three different proteolytic enzymes were used in three different concentrations separately at their optimum pH range to get maximum hydrolytic capacity and compare the same among each other at that concentrations. The pH range was maintained at the respective optimum pH of the three enzymes to get better hydrolysis at that concentration. After incubation, sample aliquots were withdrawn from each of proteolytic mixtures and were immediately put in a boiling water bath for 10 min to inactivate different proteases. The protein hydrolysates thus obtained were cooled to room temperature, centrifuged at 8000 rpm for 20 minutes to separate soluble and insoluble fractions. Finally, the soluble fraction was freeze-dried and

stored at -18 °C for further analysis. Mushroom protein hydrolysates from oyster mushroom were labeled as Mushroom Protein Hydrolysate (MPH). The pH of final MPH was 7.

2.6 Optimization of parameters affecting enzymatic hydrolysis by central composite design of response surface methodology

Different process parameters such enzyme loading (30 U/g for proteinase k, 0.7 U/g for pepsin and 2 U/g for trypsin), incubation temperature (30 °C to 50 °C), and reaction time (60 minutes to 120 minutes) during enzymatic hydrolysis by proteinase K were optimized by response surface methodology. The four levels +1, 0, -1 corresponded to lower, medium and higher value respectively (Table 1).

Table 1. Coded values of the independent variables of MPH.

Factors	Parameters	Units	Types	Low actual	High actual	Low coded (-α)	High coded (+α)
A	Enzyme loading	%	Numeric	0.05	0.15	-1	+1
B	Temperature	°C	Numeric	30	50	-1	+1
C	Time	minutes	Numeric	60	120	-1	+1

2.7 Degree of hydrolysis (DH)

DH is defined as the percent ratio of the number of peptide bonds broken to the total number of peptide bonds available for proteolytic hydrolysis. The DH of MPH using three proteases was determined spectrophotometrically by orthopthalaldehyde (OPA) method as described by Nielsen et al. (2001). The DH was determined from the following Formula 2 and h $_{tot}$ was dependent on the amino acid composition of the substrate.

$$DH = h / h_{tot} \times 100\% \quad (2)$$

h= number of hydrolyzed bonds; h $_{tot}$= total number of peptide bonds per protein equivalent.

when the raw material has not been identified and α, β value was 1.00 & 0.40 and the h_{tot} was 8.6 likewise (Nielsen et al., 2001).

Determination of h (Equation 3):

$$Serine - NH_2 = OD_{sample} - OD_{dark} \ / OD_{standard} - OD_{dark} * 0.9516 meqv \ / L * 0.1 * 100 \ X * P \quad (3)$$

where serine-NH_2= meqv serine NH_2/g protein; X= g sample; *p* = protein% in sample; 0.1 was the sample volume in liter (L). h was then: h= (serine-NH_2-β) / α meqv /g protein, where α, β value was 1.00 & 0.40 and the h_{tot} was 8.6 (Adler-Nissen, 1986).

2.8 Soluble protein content

The protein content in the MPHs with different DH values was determined by Folin-Lowry method (Lowry et al., 1951) using BSA as the standard.

2.9 Determination of functional properties

2.9.1 Determination of emulsifying properties

A mixture of 2 mL sunflower oil and 6 mL 1% (w/v) MPHs were mixed at pH 7. The mixture was thoroughly homogenized for 10 min at room temperature. Aliquot of 50 µl of emulsion was collected from the bottom of the tube immediately and after 10 min of emulsion formation and both the emulsions were diluted up to 5 mL with 1% (w/v) Sodium Dodecyl Sulphate (SDS). Absorbance of both the diluted aliquots designated as A_0 (immediately sampled) and A_{10} (sample after 10 min of emulsion formation) were measured spectrophotometrically at 500 nm. Emulsifying Activity Index (EAI) and Emulsion Stability Index (ESI) were calculated as follows (Equations 4 and 5):

$$EAI\left(m^2/g\right) = 2 \times 2.303 \times A_{500\,nm} \times protein\,weight\left(gm\right)/F \quad (4)$$

where, F= oil volume fraction.

$$ESI\left(min\right) = A_0 \times t/A \quad (5)$$

where, t= 10 minutes, A= A_0- A_{10}.

2.9.2 Determination of foaming properties

Foaming properties including Foaming Capacity (FC) and Foaming Stability (FS) of unhydrolysed and proteinase K treated protein hydrolysates obtained from *P. ostreatus* cultivated on paddy straw were determined according to Jamdar et al. (2010). The following Equations 6 and 7 were used for calculating the FC and FS:

$$FC\left(\%\right) = \left(V_t - V_0\right) \times 100 \times V_0 \quad (6)$$

$$FS\left(\%\right) = \left(V_t - V_0\right) \times 100/V_0 \quad (7)$$

2.9.3 Water holding capacity (WHC)

1 g dried oyster mushroom was taken in a centrifuge tube and mixed with 10 mL of distilled water and weighed properly. Then the slurry was stirred for 1 hr at room temperature and kept aside for 30 minutes without disturbances. It was centrifuged at 5000 rpm for 10 min, supernatant was discarded and the tube was again weighed (Equation 8).

$$Percentage\,of\,WHC = \left(W_1 - W_2\right)/W_0 \times 100\% \quad (8)$$

W_0 = Weight of the sample; W_1 = Previously weighed sample dispersed in distilled water + tube; W_2 = Final weight of sample after centrifugation + tube.

2.9.4 Oil holding capacity (OHC)

0.5 g of powdered MPHs was mixed with sunflower oil in a centrifuge tube and stirred for 30 minutes at room temperature. It was allowed to stand for 30 minutes. The mixture was centrifuged at 5000 rpm for 10 minutes, the oil was discarded and final weight of the tube was taken (Equation 9).

$$Percentage\,of\,OHC = \left(W_1 - W_2\right)/W_0 \times 100\% \quad (9)$$

W_0 = Weight of the sample; W_1 = Previously weighed sample dispersed in oil + tube; W_2 = Final weight of sample after centrifugation + tube.

2.10 Statistical analysis

All experiments were done in triplicate and standard deviation was determined. To determine the significance, the data was analysed by One-way Analysis of Variance (ANOVA) using post-hoc Tukey's Honest Significant Difference (HSD) Test. Tukey's test was performed for *p*-value determination. Values of $p \leq 0.05$ and $p \leq 0.01$were considered as significant value.

3 Results and discussion

3.1 Cultivation of *P. ostreatus*

The productivity of edible mushrooms is dependent on genetic and biochemical factors like the species, the type of spawn, the substrates, moisture content, physiochemical conditions, etc. (Kirbag & Akyuz, 2008; Onuoha et al., 2009). *P. ostreatus* mushroom, commercially known as oyster mushroom (Sánchez, 2010), can be cultivated on a wide range of lignocellulosic waste substrates (Josiane et al., 2018; Ogundele et al., 2017; Oyetayo & Ariyo, 2013). It was reported earlier that in cultivation of *P. ostreatus*, a high percentage of the substrate could be converted to fruiting bodies and their fruiting bodies were not affected by pests and disease (Sánchez, 2009). Based on these observations it may be concluded that *P. ostreatus* can be cultivated in a simple and financially efficient manner and thus, it became an excellent choice for mushroom production. In the current study, *P. ostreatus* was successfully and economically grown on paddy straw. Paddy straw has been preferred as the substrate for its financial efficiency and abundance. The nutritional composition, as obtained through proximate analysis, of the mushroom in the current study is compatible with what has been reported by other previous studies who used costlier substrates like pineapple rind (Narh et al., 2018), banana leaves, groundnut shell, cassava and yam peel and sawdust, etc. (Peter et al., 2019) (discussed in details in Section 3.2). Moreover, the growth time for the fruiting bodies has been found to be shorter in the current study (20 days) in comparison to other studies (34 days for pineapple rind (Narh et al., 2018)) though the productivity could have been still maintained.

3.2 Proximate analysis of cultivated *P. ostreatus*

The proximate composition of dried oyster mushroom *P.ostreatus* cultivated on paddy straw was shown in Table 2. Moisture content of dried *P. ostreatus* in our study was 8.43 ± 0.05% which was in the range of the report of Oyetayo and Ariyo (9-13%) (Miles & Chang, 2004; Oyetayo & Ariyo, 2013). Ogundele et al. found that moisture content of *P. ostreatus* harvested from hardwood sawdust was higher (8.93%) than that harvested from softwood sawdust (7.88%) (Ogundele et al., 2017). The moisture content of *P. ostreatus* evaluated by Tolera and Abera was 8.45 ± 1.65%, which was similar with the experimental finding but on the other hand, Narh et al., reported higher moisture content of *P. ostreatus* ranged from 10.56 ± 0.12g/100g dw to 10.69 ± 0.02g/100g dw cultivated on composted sawdust supplemented with powdered pineapple ring (Narh et al., 2018; Tolera & Abera, 2017). Narh et al. (2018) reported that entire fruiting bodies were sundried, milled and refrigerated at -10 °C. The mushrooms were harvested on a varying concentration of powdered pineapple rind (2%, 5% and 12%). The moisture content varied from 10.56 ± 0.12% to 10.84 ± 0.04%. This might be due to different capacity of moisture absorption of the mushrooms from the environment. In sun drying, case hardening might happen and cause the outer surface dry and hard while prevents moisture escaping from the inner surface.

In our study, protein content of dried mushroom was 26.8 ± 0.40%. This result was in the same line as Tolera and Abera, (2017) and Ogundele et al., (2017) who cultivated the same strain of *P. ostreatus* on

cottonseed waste and hardwood sawdust respectively. Protein percentage of dried oyster mushroom evaluated by Narh et al. (2018) was relatively lower (20.41 ± 0.01-0.04 g/100g dw) than the experimental result. Our results showed higher protein content than the study conducted by Hoa et al. where *P. ostreatus* grown on 100% sawdust (SD) or 50% sawdust and 50% sugarcane bagasse (SB) or 80% SD & 20% SB waste or 50% SD & 50% corncob (CC) or 80% SD & 20% CC as substrate exhibited the protein content of 19.52%, 24.17%, 21.88%, 25.65% and 20.89%, respectively (Hoa et al., 2015). These findings clearly demonstrated that protein content of the mushroom which constituted of more than half of total nitrogen depended on the substrate. In addition, paddy straw mushroom contains higher percentage of essential amino acids (Ahlawat & Tewari, 2007), when compared to other plant biomass, such as softwood, paddy straw contains higher amount of hemicelluloses content and low in lignin and cellulose contents respectively (Barmina et al., 2013). The authors cultivated the mushroom on various lignocellulosic substrates like pineapple rind, sawdust, bagasse which is full of vitamins and microelements, but we cultivated mushroom only on paddy straw. The results interpreted that our mushroom was nutritionally superior to others.

Carbohydrate is the major source of energy in our body. In our study, carbohydrate content of dried *P. ostreatus* was 50.77 ± 1.36%. On the other hand, Tolera & Abera (2017) showed that carbohydrate content of *P. ostreatus* was 42.14%. Studies carried out by Ogundele et al. (2017) showed that on a dry basis, carbohydrate content of *P. ostreatus* from hardwood sawdust was lower (41.57%) when compared to that harvested from softwood sawdust (52.04%). According to Narh et al. (2018), 59.08 ± 0.02gm/100 g dw of carbohydrate was present in *P. ostreatus* grown on sawdust compost supplemented with 5% powdered pineapple rind as an organic substance which was higher than the experimental findings. As carbohydrate was found to be in considerable amount in our cultivated *P. ostreatus*, this can be attributed that it may assist in sustaining proper functioning of brain, heart, digestive system and boost immunity.

Ash content of the oyster mushroom on dry basis in this study was 5.5 ± 0.12%g/100g which was only 3% lower than the report noted by Narh et al. (2018) (8.40 ± 0.09 gm/100gm for *P. ostreatus* grown on sawdust compost supplemented with 5% powdered pineapple rind as an organic substance) whereas 5.90% of ash content was found in the *P. ostreatus* grown on 100% sawdust used as substrate which was almost similar to our findings (Hoa et al., 2015). Ash content denotes that *P. ostreatus* is full of minerals which are necessary for body building.

Fat content of our cultivated dried oyster mushroom was 2.98 ± 0.11%. Similar results were also reported by Tolera & Abera (2017). On the other hand, *P. ostreatus* cultivated on rice bran supplemented media exhibited 1% lower fat content (Narh et al., 2018). Our mushroom contains low fat, high carbohydrate and protein, so this can be successfully included as a low-calorie high protein rich food.

In this study the result of dietary fiber from the dried sample was 8.43 ± 0.05% which was significantly lower (12.87%) than reported by Tolera & Abera (2017). Comparatively, Ogundele et al. (2017) showed that crude fiber content of *P. ostreatus* harvested from hardwood sawdust was 9.59%.

The proximate composition of *P. ostreatus* grown and harvested from different substrates was variable due to difference nutritional composition of various substrates. Our findings clearly indicated that paddy straw exhibited good potential as substrate for cultivation of oyster mushroom resulting in good proximate content. High protein content of *P. ostreatus* implied that this mushroom can be included in diet to aid protein supply especially to low-income group and assist in lessening malnutrition problem.

3.3 Antinutritional properties

Some of the antinutrients studied in this research paper were oxalate, tannin, phytate and cyanide (Table 2).

3.3.1 Oxalate

Oxalate hampers the mineral absorption in the human body by binding with calcium, magnesium and iron (Ogundele et al., 2017). In our research study, oxalate was absent in the edible oyster mushroom *P. ostreatus*

cultivated on paddy straw. A previous study demonstrated that, oxalate content of *P. ostreatus* (Jacq.) of Akwa Ibom state of five different locations of Nigeria ranged from 166.9 ± 6.50 mg/g to 301.9 ± 4.90 mg/g (Godwin, 2015). The oxalate content, as reported in this study, was higher with respect to similar findings in the existing literature which might be due to the effect of climatic condition in Akwa Ibom and due to the effect of growing substrates. Since *P. ostreatus*, cultivated in this study, did not contain oxalate, it can be regarded as safe for consumption.

3.3.2 Tannin

This present study revealed that tannin content of the *P. ostreatus* was 0.095 ± 0.027 mg/g, which was within the standard safe limit (60.00 mg/100 g). Tannin content of the mushroom species evaluated by Adeduntan were of significantly low ranging from 0.035 mg/g to 0.116 mg/g (Adeduntan, 2014). According to this study, *P. sajor-caju* contained 0.080 mg/gm of tannin which was almost similar to the experimental result. According to Gaur et al., 2016 tannin content of six different edible mushroom species ranged between 0.41-0.57 mg/g which was higher than reported in the present investigation. Our study could reveal that tannin content was significantly lower than the above stated results, almost in trace quantities. It was reported that high level of tannin intake formed insoluble complexes with protein and affected the bioavailability of protein (Aletor, 1995), digestibility and palatability (Akwaowo et al., 2000; Okwu & Ndu, 2006). But recent studies demonstrated that only small quantities of tannin may be beneficial (Godwin, 2015). Tannin content is generally lower in cap and tuber than stalk. Since it is within the limit (10% on dry weight basis), it will not affect the body (Osagie et al., 1998).

3.3.3 Phytic acid

Phytates are inositol hexaphosphoric acids which can bind with calcium, zinc, magnesium, iron, hence inhibit the nutrient absorption in the body (Oly-Alawuba & Obiakor-Okeke, 2014). Our result showed that edible oyster mushroom *P. ostreatus* contained very negligible amount of phytic acid i.e. 0.15 ± 0.083 mg/g, which was far lesser than the safe limit of 22.10 mg/100g (Oly-Alawuba & Obiakor-Okeke, 2014). According to Gaur et al., *Agaricus bisporus* contained 0.11 ± 0.01 mg/g phytic acid and both *Calocybe indica* and *Macrocybe gigantea (Massee)* contained 0.19 ± 0.01 mg/g of phytic acid (Gaur et al., 2016). Woldegiorgis et al. (2015) also showed that *P. ostreatus* of Ethiopia contained 155.8 ± 12.1 mg/100 g of phytic acid (Woldegiorgis et al., 2015). Presence of 1% or more phytate content in our diet could affect mineral bioavailability. Phytate content of *P. ostreatus* considered in this study can be regarded as edible.

3.3.4 Cyanide

As cyanide is a potential inhibitor of the respiratory chain, it is found to be very toxic at low concentration to animal (Oly-Alawuba & Obiakor-Okeke, 2014). Our results showed that, no cyanide was present in the dried *P. ostreatus* mushroom. In the study conducted by Oly-Alawuba & Obiakor-Okeke (2014) cyanide content in three different mushrooms varied from 0.198 ± 0.06 to 0.236 ± 0.04 mg/gm. Ijioma Blessing et al. (2015) reported that, HCN content in three edible mushrooms (*Temitomyces sp*., *Russula sp*. and *P. tuber-regium*) of South eastern Nigeria were ranged from 0.0019-0.13mg/100gm.

Table 2. Nutrient and antinutrient compositon of dried *P.ostreatus* cultivated on paddy straw.

Nutrients (%)		Antinutrients (mg/g)	
Moisture	5.52 ± 0.34	Oxalate	ND
Protein	26.8 ± 0.40	Tannin	0.095 ± 0.027
Carbohydrate	50.77 ± 1.36		
Fat	2.98 ± 0.11	Phytate	0.15 ± 0.083
Ash	5.5 ± 0.12	Cyanide	ND
Dietary fibre	8.43 ± 0.05		

ND = Not Detected.

3.4 Degree of hydrolysis

Determination of DH is essential to achieve desired enzymatic protein hydrolysates through optimization. Table 3 demonstrated the wide range of DH values obtained by the action of proteinase K, pepsin and trypsin respectively using different operational conditions and it was observed that DH ranged from 7.8 ± 2.06%-99.42 ± 0.02%. Among the three proteolytic enzymes, highest % DH of MPH was achieved by proteinase K followed by pepsin and trypsin. The highest % DH of MPHs observation indicated the maximum cleavage of mushroom proteins into peptides and free amino acids.

3.5 Optimization of various process parameters of degree of hydrolysis through response surface methodology

The Response Surface Methodology (RSM) is a widely used mathematical and statistical tool for modeling and analyzing a process in which the response of interest is affected by various variables and the objective of this method is to develop, improve and optimize the response. RSM evaluates an appropriate approximation relationship between input and output variables and identify the optimal operating conditions for a system under study or a region of the factor field that satisfies the operating requirements.

Different independent variables were studied for its effect upon degree of hydrolysis (DH) (%) of proteinase k, pepsin and trypsin treated MPH respectively. There were total of 10 runs were carried out for optimization of the different parameters by central composite design methodology. The quadratic polynomial diagnostic model was chosen for the elucidation of significant model based on the ANOVA statistical analysis. The following equation was derived for the analysis of the DH (%) of proteinase k, pepsin and trypsin treated MPH.

The Equation 10 for proteinase k treated MPH

$$Y_1 = 4.22061 + 89.27331A + 0.98886B + 0.14169C \quad (10)$$

The Equation 11 for pepsin treated MPH

$$Y_2 = 68.49 + 12.37A + 8.22B + 1.81C \quad (11)$$

The Equation 12 for trypsin treated MPH

$$Y_3 = 25.68 + 5.26A + 23.428B + 3.50C \quad (12)$$

where, Y_1= DH (%) of proteinase k treated MPH, Y_2= DH (%) pepsin treated MPH, Y_3 = DH (%) of trypsin treated MPH, A= Enzyme loading (%), B= Temperature (°C), C= time (minutes)

The coefficient of adjusted A response surface model was chosen for the validation of experimental design set up obtained by varying parameters affecting the DH (%) for different hydrolytic enzymes treated MPH. The lack of fit model has shown significance thereby the model is applicable for optimization parameters evaluation (Table 4).

The surface plots of two combining parameters were presented for the presentation of the optimized factors responsible for DH%. Substrate loading and enzyme loading has significant effect in enzymatic hydrolysis process. Substrate loading has achieved maximum hydrolysis efficiency for all of the three proteolytic enzymes depicted in Figures 1a, 1b, 2a, 2b and 3a, 3b. The 0.15% enzyme concentration achieved better hydrolysis efficiency for both proteinase k and pepsin, whereas trypsin showed lower hydrolysis efficacy. Effect of temperature and time assists in increment of hydrolysis rate. The optimum parameters for proteinase k enzyme were found to be 112.82 min at the temperature of 50.51 °C and the DH% was 84.06%; whereas the same amount of hydrolysis (i.e., 84.06%) was achieved at 106.54 min at the temperature of 41.9 °C in

case of pepsin. Lastly, MPHs treated by trypsin was optimized at the concentration of 0.08% after 61.61 minutes of hydrolysis and at the temperature of 41.45 °C.

Table 3. Optimization of various process parameters of DH and protein content of proteinase K, pepsin and trypsin treated MPHs (*P. ostreatus*).

Run	Enzyme loading (%w/v)	Temperature (ºC)	Time (minutes)	Degree of Hydrolysis (%) (proteinase k)	Degree of Hydrolysis (%) (pepsin)	Degree of Hydrolysis (%) (trypsin)
1	0.10	40.00	90.00	65.54	62.45	18.63
2	0.15	50.00	120.00	99.42	85.02	65.35
3	0.15	50.00	60.00	78.27	74.22	53.21
4	0.05	30.00	120.00	65.23	77.19	7.80
5	0.10	40.00	140.45	57.32	61.29	04.24
6	0.10	40.00	90.00	65.54	62.45	18.63
7	0.10	40.00	90.00	65.54	62.45	18.63
8	0.15	30.00	60.00	58.29	55.63	12.42
9	0.18	40.00	90.00	63.21	65.32	39.82
10	0.05	50.00	60.00	60.94	74.22	53.21

Table 4. Analysis of variance of the different independent variables of the response surface plots of DH (%) of proteinase k, pepsin and trypsin treated MPH.

Proteolytic enzymes	Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*P*-value
Proteinase k treated MPH	Model	691.39	3	230.44	1.95	0.2227
	Residual error	708.29	6	118.05		
	Lack of fit	708.29	4	177.07	4.66	0.0230
	Pure error	0.000	2	0.000		
	Total	1399.60	9	R^2	0.4939	
				Adjusted R^2	0.2409	
				Predicted R^2	0.1150	
Pepsin treated MPH	Model	557.79	6	92.97	1.46	0.3507
	Residual error	191.35	3	63.78		
	Lack of fit	191.35	1	191.35	1.66	0.00037
	Pure error	0.000	2	0.000		
	Total	749.19	9	R^2	0.7446	
				Adjusted R^2	0.2337	
				Predicted R^2	0.1949	
Trypsin treated MPH	Model	3378.73	3	1126.24	7.58	0.0183
	Residual error	891.54	6	148.59		
	Lack of fit	891.54	4	222.82	1.66	0.00037
	Pure error	0.000	2	0.000		
	Total	4270.27	9	R^2	0.7912	
				Adjusted R^2	0.6868	
				Predicted R^2	0.1931	

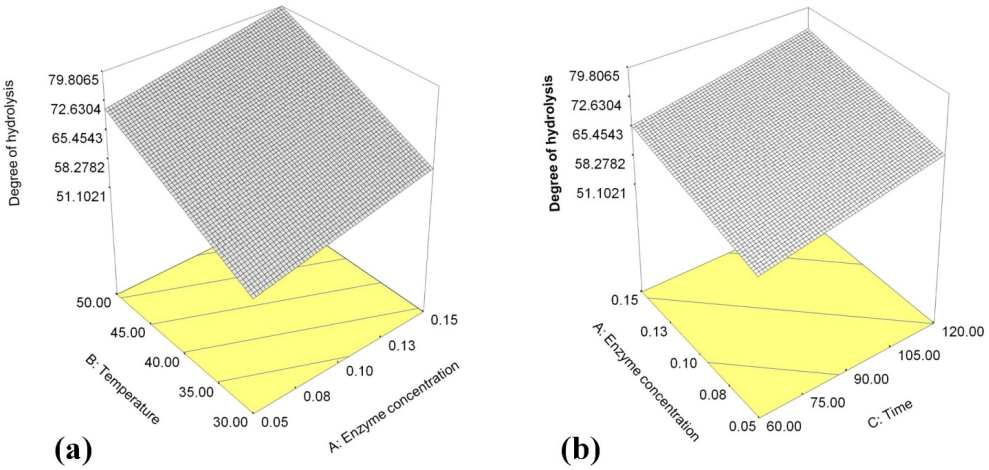

Figure 1. (a) Pictographic representation of optimization of various process parameters of DH and protein content of proteinase K treated MPHs (*P. ostreatus*) in respect of temperature; (b) Pictographic representation of optimization of various process parameters of DH and protein content of proteinase K treated MPHs (*P. ostreatus*) in respect of time.

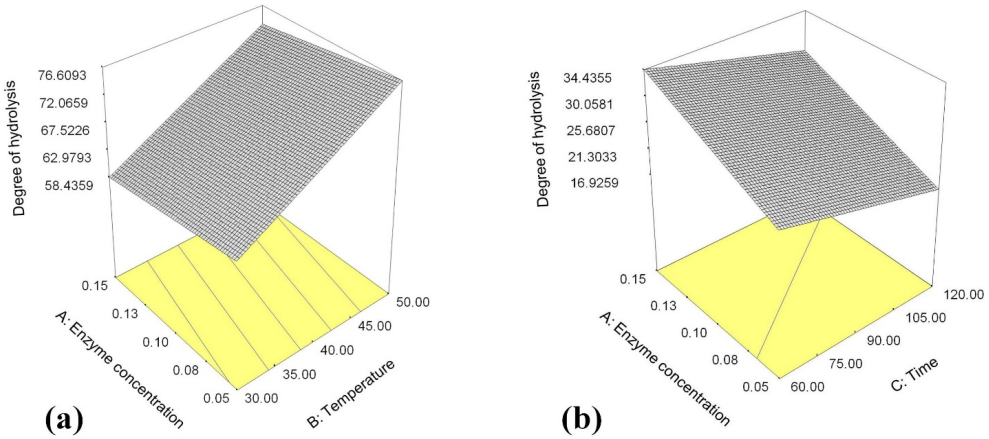

Figure 2. (a) Pictographic representation of optimization of various process parameters of DH and protein content of pepsin treated MPHs (*P. ostreatus*) in respect of temperature; (b) Pictographic representation of optimization of various process parameters of DH and protein content of pepsin treated MPHs (*P. ostreatus*) in respect of time

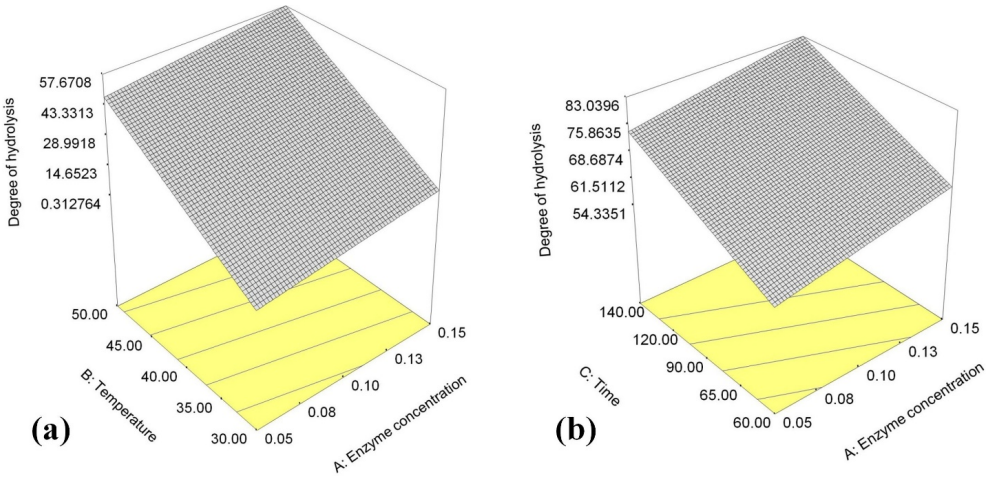

Figure 3. Pictographic representation of optimization of various process parameters of DH and protein content of trypsin treated MPHs (*P. ostreatus*) in respect of temperature; (b) Pictographic representation of optimization of various process parameters of DH and protein content of trypsin treated MPHs (*P. ostreatus*) in respect of time.

3.6 Protein content

Table 5 represented the protein content in the hydrolysates obtained using different proteolytic enzymes exhibited that it gradually increased as the enzyme concentration, temperature as well as the time of hydrolysis increased. The maximum protein content (3.22 ± 0.12 mg/mL) of MPH was obtained by proteinase K at 50 °C with the enzyme concentration of 0.15% after 120 minutes of hydrolysis followed by pepsin (2.01 ± 0.1mg/mL) and trypsin (1.65 ± 0.03mg/mL) under the same hydrolytic conditions. Thus, trypsin showed lowest protein content of 0.59 ± 0.06% mg/mL with 0.05% enzyme concentrations at 30 °C for 30 minutes of hydrolysis. It has been observed in the current study that the soluble protein content of the MPH could increase with increase in certain parameters which were DH%, concentration of certain proteolytic enzymes and temperature. Mushroom protein holds high pH and thermal stability which point out for minimal denaturation of protein upon food processing (Erjavec et al., 2012). Proteinase K is a highly active and stable endopeptidase with broad spectrum of cleaving capacity of peptide bonds, mostly after carboxyl group of N terminal of aliphatic and aromatic amino acids, also cleaves the ester and amide bonds. So, in this current study, 0.15% proteinase K was able to hydrolyse almost whole mushroom protein cleaving peptide bonds and releasing amino acids in the reaction mixtures readily available for absorption in the body. Till date, this is the first study which establishes that MPH of *P. ostreatus* could be an excellent source of nutraceuticals cum easily digestible food ingredients which can be used in food formulation. In the study of (Greeshma & Sridhar, 2018), the uncooked *Amanita* mushroom showed 32% protein solubility at pH 2 and 27% as protein solubility decreased with increasing pH till the isoelectric point. This can be useful in food formulation like carbonated beverages and infant foods. Gonzalez et al. reported that protein solubility of mushroom flour varied from 0.035 ± 0.005 and 0.51 ± 0.02 mg/ml (González et al., 2021). With the increment of pH, the solubility of protein increased because the pH rage (3-12) near to the isoelectric point at which the electrostatic force was diminished. As a result, the protein tends to precipitate.

Table 5. Optimization of various process parameters of DH and protein content of proteinase K, pepsin and trypsin treated MPHs (*P. ostreatus*).

Enzyme concentration (%)	Temp (°C)	Proteinase K					
		60 min		90 min		120 min	
		Degree of hydrolysis (%)	Protein content (mg/ml)	Degree of hydrolysis (%)	Protein content (mg/ml)	Degree of hydrolysis (%)	Protein content (mg/ml)
0.15	50	78.27 ± 1.75^{a,b}	2.32 ± 0.01^{c,d}	97.58 ± 0.98^{a,b}	3.16 ± 0.03^{c,d}	99.42 ± 0.02	3.22 ± 0.12^{c,d}
	40	60.58 ± 1.87^{a,b}	2.11 ± 0.02^{c,d}	79.03 ± 2.03^{a,b}	2.5 ± 0.02^{c,d}	98.29 ± 1.13	3.16 ± 0.07^{c,d}
	30	58.29 ± 0.86^{a,b}	2.05 ± 0.08^{c,d}	70.39 ± 1.71^{a,b}	2.19 ± 0.03^{c,d}	72.52 ± 2.18	2.11 ± 0.016^{c,d}
0.10	50	70.48 ± 1.79^{b,c}	1.44 ± 0.02^{c,d}	95.24 ± 2.07^{a,b}	2.96 ± 0.16^{a,d}	98.47 ± 0.39^{b}	2.98 ± 0.06^{a,c}
	40	64.59 ± 0.23^{b,c}	1.5 ± 0.021^{c,d}	65.54 ± 2.38^{a,b}	1.65 ± 0.03^{a,d}	66.68 ± 2.14^{b}	1.69 ± 0.07^{a,c}
	30	54.84 + 1.75^{b,c}	1.39 ± 0.56^{c,d}	60.15 ± 2.16^{a,b}	1.45 ± 0.01^{a,d}	61.36 ± 2.04^{c,d}	1.52 ± 0.32^{a,c}
0.05	50	60.94 ± 1.96^{b,c}	1.37 ± 0.01^{a,b}	66.29 ± 2.20^{b}	1.35 ± 0.02^{a,b}	77.68 ± 1.16^{c}	2.21 ± 0.10^{a,b}
	40	60.09 ± 2.11^{b,c}	1.3 ± 0.01^{a,b}	65.68 ± 1.93^{c,d}	1.32 ± 0.02^{a,d}	66.59 ± 2.02^{c}	1.41 ± 0.05^{c,d}
	30	50.08 ± 1.63^{b,c}	1.17 ± 0.04^{a,b}	62.43 ± 2.41^{c,d}	1.29 ± 0.04^{a,d}	65.23 ± 1.93^{a}	1.37 ± 0.08^{c,d}
Enzyme Concentration (%)	**Temp (°C)**	**PEPSIN**					
		60 min		**90 min**		**120 min**	
		Degree of hydrolysis (%)	**Protein content (mg/ml)**	**Degree of hydrolysis (%)**	**Protein content (mg/ml)**	**Degree of hydrolysis (%)**	**Protein content (mg/ml)**
0.15	50	74.22 ± 1.89^{c,d}	1.68 ± 0.07^{a,c}	78.16 ± 0.90^{b}	1.83 ± 0.06^{c,d}	85.02 ± 0.49^{c}	2.01 ± 0.07^{c,d}
	40	60.25 ± 1.25^{b}	1.29 ± 0.04^{a,c}	72.70 ± 1.67^{b}	1.02 ± 0.02^{c,d}	83.1 ± 1.97^{a,d}	1.52 ± 0.05^{c,d}
	30	55.63 ± 0.88^{b}	1.25 ± 0.15^{a,c}	72.62 ± 1.59^{b}	1.0 ± 0.012^{c,d}	60.46 ± 1.66^{c}	1.32 ± 0.01^{c,d}
0.10	50	64.31 ± 2.16^{b}	1.15 ± 0.01^{b}	75.01 ± 1.57^{d}	1.29 ± 0.04^{b,c}	78.37 ± 1.37^{b}	1.52 ± 0.021^{c,d}
	40	54.53 ± 1.89^{b}	0.93 ± 0.13^{b}	62.45 ± 2.23^{d}	0.81 ± 0.01^{b,c}	70.25 ± 0.05^{b}	1.45 ± 0.03^{c,d}
	30	50.72 ± 2.08^{c,d}	0.79 ± 0.05^{b}	54.52 ± 1.70^{d}	0.65 ± 0.01^{b,c}	54.72 ± 2.06^{b}	0.69 ± 0.02^{a,d}
0.05	50	62.98 ± 1.75^{a,b}	0.63 ± 0.02^{c,d}	72.26 ± 1.67^{b,c}	0.77 ± 0.05^{b,c}	77.19 ± 2.05^{d}	1.12 ± 0.01^{a,d}
	40	60.37 ± 1.80^{a,b}	0.61 ± 0.01^{c,d}	65.87 ± 0.82^{b,c}	0.64 ± 0.01^{b,c}	72.39 ± 2.20^{d}	1.09 ± 0.03^{a,d}
	30	56.57 ± 2.11^{a,b}	0.55 ± 0.05^{c,d}	61.39 ± 2.49^{b,c}	0.59 ± 0.02^{b,c}	61.46 ± 2.30^{d}	0.87 ± 0.01^{a,d}

Table 5. Continued...

Enzyme Concentration (%)	Temp (°C)	TRYPSIN					
		60 min		90 min		120 min	
		Degree of hydrolysis (%)	Protein content (mg/ml)	Degree of hydrolysis (%)	Protein content (mg/ml)	Degree of hydrolysis (%)	Protein content (mg/ml)
0.15	50	53.21 ± 2.05	1.35 ± 0.026	56.48 ± 1.69^{b}	1.35 ± 0.03^{d}	65.35 ± 1.72^{d}	1.65 ± 0.03^{b,d}
	40	31.66 ± 1.73	1.05 ± 0.022	47.32 ± 1.90^{b}	1.2 ± 0.01^{d}	49.31 ± 1.71^{d}	1.25 ± 0.28^{b,d}
	30	12.42 ± 1.96	0.59 ± 0.06	16.53 ± 2.22^{b}	0.9 ± 0.33^{d}	32.33 ± 2.05^{d}	1.15 ± 0.03^{b,d}
0.10	50	21.67 ± 2.24^{b,c}	0.75 ± 0.05^{a,d}	30.41 ± 1.68^{d}	0.79 ± 0.05^{c,d}	60.64 ± 1.56^{b}	1.09 ± 0.02^{c}
	40	15.63 ± 2.04^{b,c}	0.72 ± 0.01^{a,d}	18.63 ± 2.16^{d}	0.77 ± 0.06^{c,d}	25.53 ± 1.83^{b}	0.96 ± 0.06^{c}
	30	10.31 ± 2.57^{b,c}	0.43 ± 0.04^{a,d}	15.63 ± 1.93^{d}	0.45 ± 0.08^{c,d}	24.76 ± 0.54^{b}	0.93 ± 0.17^{c}
0.05	50	7.75 ± 2.23^{c,d}	0.43 ± 0.04^{b,c}	8.38 ± 1.66^{b,c}	0.57 ± 0.04^{b,c}	9.33 ± 2.05^{a,d}	0.79 ± 0.05^{b,c}
	40	5.63 ± 1.93^{c,d}	0.34 ± 0.06^{b,c}	6.31 ± 2.57^{b,c}	0.37 ± 0.01^{b,c}	9.28 ± 1.81^{a,d}	0.64 ± 0.03^{b,c}
	30	4.29 ± 1.12^{c,d}	0.25 ± 0.07^{b,c}	4.88 ± 1.96^{b,c}	0.31 ± 0.04^{b,c}	7.8 ± 2.06^{a,d}	0.59 ± 0.06^{b,c}

Sample evaluation was done in triplicate. Values are calculated as Mean ± SD (n=3). Lowercase letters indicated significant differences ($p \leq 0.05$). Values followed by the same letter were not significantly different ($p \leq 0.05$).

3.7 Functional properties

Since MPH obtained from *P. ostreatus* using proteinase K at 0.15% concentration, at 50 °C for 120 minutes of hydrolysis period exhibited highest %DH and maximum protein content, evaluation of functional properties was carried out with this protein hydrolysate and compared those with that of unhydrolsed *P. ostreatus* (UMP).

To our knowledge, the current work for the first time evaluated the effect of DH on different functional properties of MPHs obtained from *P. ostreatus* and represented in Table 6.

3.7.1 Emulsifying properties

The EAI is the area of oil/water interface stabilized per unit weight of protein and ESI is defined as time needed to achieve a turbidity of the emulsion that is one-half of its original value (Aryee et al., 2018; Chatterjee et al., 2015). Both the EAI and ESI mainly depend on the diffusion of the peptides at oil– water interfaces which stabilize the interface and this stabilization depends on the whole ionic character of the peptides (Chatterjee et al., 2015). The emulsifying capacity, which is based on surface properties of protein hydrolysates, describes how these hydrolysates effectively lower the interfacial tension between the hydrophobic and hydrophilic component in food (Amiza et al., 2012). Emulsifying properties in terms of EAI and ESI of unhydrolysed and proteinase K treated MPH were represented in Table 6 indicating that ESI significantly increased in proteinase K treated MPH as compared to UMP. According to Taha and Ibrahim, diffusion of peptide molecules into the interfaces was facilitated by degree of hydrolysis (Taha & Ibrahim, 2002). According to Vodjdani and Whitaker, 1986, functional properties of protein hydrolysates are also influenced by the specificity of enzyme, the physical and chemical nature of intact protein, and hydrolysis conditions (Kester & Richardson, 1984). Here we have used the very specific proteolytic enzyme for hydrolysis, i.e., proteinase k, this might cause behind the good emulsifying capacity of MPHs. Hydrophilic and hydrophobic groups in the peptides stabilized oil-water interface which caused an increase in emulsifying property. Table 6 indicated that ESI significantly increased in case with Proteinase K treated MPH rather than UMP and EAI values were higher in both UMP and proteinase K treated MPH in the immediately sampled emulsion formation. This might have been due to the non-specific hydrophobic interaction and the orientation of the molecules at the oil-water interface of the formed emulsion which assisted in reducing the surface tension of the emulsion. Higher DH of hydrolysis denotes cleavage of more peptide bonds and release of free amino acids in the emulsion rendering enhanced surface4 thus causing higher functional properties.

It was also observed that for both the UMP and proteinase K treated MPH, EAI was more significant in the immediately sampled emulsion (UMP_0 and $MPHK_0$ for untreated and proteinase K treated MPHs, respectively) than 10 minutes after the emulsion formation (UMP_{10} and $MPHK_{10}$ for untreated and proteinase K treated MPHs, respectively). Similar results were also observed by Chatterjee et al., 2015 who reported that papain, pepsin and alcalase treated sesame protein hydrolysates were of improved EAI and ESI. Our results are in line with the previous findings (Amiza et al., 2012) reporting that cobia protein hydrolysate produced the most stable foam after 60 minutes of whipping with 96% hydrolysis. The foaming capability of Cobia protein hydrolysate at DH of 96% after 60 min was 113.3% and most stable among others, i.e., DH 53% and DH 71% (Amiza et al., 2012). In another study, EAI of High-pressure jet processed skim milk from 100 to 500 Mpa varied from 6.5 ± 0.7 to 8.3 ± 0.7% which was much lower than our report (Hettiarachchi et al., 2018).

3.7.2 Foaming properties

Foaming capacity and stability are indicators of whippability, hence widely demanded in food products like cake and whipping topping to develop products with better consistence and sensory property. In this study, both FS and FC of proteinase K treated MPH increased when compared with UMP. Highest FS was achieved after 10 minutes of whipping of sample extract which gradually decreased over time (Table 6). Ishara et al. evaluated functional properties of flour of button mushroom *Agaricus bisporus* and oyster mushroom *P. ostreatus* and reported that FC and FS of button flour was significantly higher than those of oyster (Ishara et al., 2018). Foaming stability of processed skimmed milk using high pressure jet from 200 to 500Mpa was approximately 55% after 24 hr (Harte et al., 2019) which was in agreement with our report of proteinase K treated MPH after 60 min of foam formation.

3.7.3 Water holding capacity (WHC)

The WHC is the quantity of water fixed per gram of sample (Bayar et al., 2017). In our study, WHC of powdered mushroom was determined to be about 88.7 ± 3.25%. According to Bayar et al. (2017) pectin extracted from *Opuntia ficus indica* (L.) Mill. through ultrasonication had 4.84 g/g of WHC. Gul et al. compared water holding capacity of kefir using different milk starter culture of which buffalo milk kefir obtained maximum WHC (77.35 ± 0.6%) among others (Gul et al., 2018). WHC which has immense importance in food industries are influenced by several factors including particle size and porosity of samples, ionic strength of the solution, pH and temperature etc. (Bayar et al., 2017; Elleuch et al., 2011). High WHC of MPH in the current study indicated that it could be used in cake preparation to avoid syneresis.

3.7.4 Oil holding capacity

The OHC of proteinase K treated MPH in the present investigation was 54.26 ± 0.21% and due to this high OHC, MPH of *P. ostreatus* may be used for the preparation of cake, mayonnaise, salad dressings especially in regard of better flavor retention, extended shelf life and preventing food from oxidative rancidity. In another study conducted by Cruz-solorio et al., they also investigated that OHC of protein concentration of 3 strains of *P. ostreatus* (PCM, POS and PCM×POM) was higher than wheat flour (Cruz-Solorio et al., 2018). Protein concentrates of these *P. ostreatus* strains showed significantly higher values than wheat flour and their corresponding wheat flour, i.e., 173.3 for protein concentrate of PCM against 122.2 for PCM flour, 214.1 for protein concentrate of POS against 125.9 for POS flour, 195.8 for protein concentrate of PCM×POS and 104.8 for PCM×POS flour; while the OHC for wheat flour was lowest, i.e., 94.9% among all of these. Maqsood et al. reported that, 180.39% of OHC of cow milk protein and for camel milk protein ranged from 161.77 to 181.12% (Maqsood et al., 2019). The OHC is a parameter having high

importance in food industries and it can be influenced by various factors as the overall charge density and the hydrophilic character of constituents (Elleuch et al., 2011).

Table 6. Emulsifying properties and Foaming properties of UMP and MPHs (*P. ostreatus*).

Samples	Emulsifying Properties		Samples	Foaming Properties	
	EAI (m^2/g)	ESI (min)		FC (%)	FS (%)
UMP_0	71.05 ± 3.30	22.81 ± 1.47	UMP_{10}	390 ± 42.43**	95 ± 7.07**
			UMP_{30}	290 ± 14.14**	72.5 ± 3.53**
UMP_{10}	39.85 ± 3.69		UMP_{45}	170 ± 42.42**	37.5 ± 17.67**
			UMP_{60}	50 ± 14.14**	12.5 ± 3.53**
$MPHK_0$	28.18 ± 0.54	136 ± 36.49	$MPHK_{10}$	580 ± 14.14**	145 ± 3.53**
			$MPHK_{30}$	370 ± 14.14**	92.5 ± 3.53**
$MPHK_{10}$	25.98 ± 0.90		$MPHK_{45}$	280 ± 28.29**	70 ± 7.07**
			$MPHK_{60}$	220 ± 28.28**	55 ± 7.07**

UMP_0, UMP_{10}, UMP_{30}, UMP_{45} and UMP_{60} = Unhydrolysed mushroom immediately sampled and sampled after 10, 30, 45 and 60 min respectively. $MPHK_0$, $MPHK_{10}$, $MPHK_{30}$, $MPHK_{45}$, $MPHK_{60}$ = Proteinase K treated Mushroom protein hydrolysate with 99.42% of Degree of hydrolysis immediately sampled and sampled after 10, 30, 45 and 60 min respectively. Sample evaluation was done in triplicate. Values are calculated as Mean ± SD (n=3). ** indicates values of significantly different ($p \leq 0.01$).

4 Conclusion

The results indicated that *P. ostreatus* cultivated on paddy straw had good protein and ash content with antinutritional factors below the threshold limits. In the present study hydrolysis of this oyster mushroom using three different proteolytic enzymes yielded mushroom protein hydrolysates with favorably higher degree of hydrolysis. The entire experimental results showed that proteinase k treated mushroom protein hydrolysate contains enhanced functional properties which may be a potential option for food formulation.

References

Adeduntan, S. A. (2014). Nutritional and anti nutritional characteristics of some dominant fungi species in South Western Nigeria. *International Journal of Engineering Science*, *3*, 18-24.

Adler-Nissen, J. (1986). *Enzymic hydrolysis of food proteins*. New York: Elsevier Applied Science Publishers.

Association of Official Analytical Chemists - AOAC (1980). *Official Method of Analysis* (13th edn). Washington: Association of Official Analytical Chemists.

Ahlawat, O., & Tewari, R. (2007). *Cultivation technology of paddy straw mushroom (Volvariella volvacea)* (Vol. 36). India: National Research Centre for Mushroom.

Akwaowo, E. U., Ndon, B. A., & Etuk, E. U. (2000). Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis Hook f.*). *Food Chemistry*, *70*(2), 235-240. http://dx.doi.org/10.1016/S0308-8146(99)00207-1

Aletor, V. A. (1995). Compositional studies on edible tropical species of mushrooms. *Food Chemistry*, *54*(3), 265-268. http://dx.doi.org/10.1016/0308-8146(95)00044-J

Amiza, M., Kong, Y., & Faazaz, A. (2012). Effects of degree of hydrolysis on physicochemical properties of Cobia (*Rachycentron canadum*) frame hydrolysate. *International Food Research Journal*, *19*(1), 199-206.

Aryee, A., Agyei, D., & Udenigwe, C. (2018). Impact of processing on the chemistry and functionality of food proteins. In R. Y. Yada (Ed.), *Proteins in food processing* (pp. 27-45). Oxford: Elsevier. . http://dx.doi.org/10.1016/B978-0-08-100722-8.00003-6.

Barmina, I., Lickrastina, A., Valdmanis, R., Zake, M., Arshanitsa, A., Solodovnik, V., & Telysheva, G. (2013). Effects of biomass composition variations on gasification and combustion characteristics. *Engineering for Rural Development*, *5*, 23-24.

Bayar, N., Bouallegue, T., Achour, M., Kriaa, M., Bougatef, A., & Kammoun, R. (2017). Ultrasonic extraction of pectin from *Opuntia ficus indica cladodes* after mucilage removal: Optimization of experimental conditions and evaluation of chemical and functional properties. *Food Chemistry*, *235*, 275-282. PMid:28554636. http://dx.doi.org/10.1016/j.foodchem.2017.05.029

Chatterjee, R., Dey, T. K., Ghosh, M., & Dhar, P. (2015). Enzymatic modification of sesame seed protein, sourced from waste resource for nutraceutical application. *Food and Bioproducts Processing*, *94*, 70-81. http://dx.doi.org/10.1016/j.fbp.2015.01.007

Chaturvedi, V. K., Agarwal, S., Gupta, K. K., Ramteke, P. W., & Singh, M. (2018). Medicinal mushroom: Boon for therapeutic applications. *3 Biotech*, *8*(8), 334. http://dx.doi.org/10.1007/s13205-018-1358-0

Cruz-Solorio, A., Villanueva-Arce, R., Garín-Aguilar, M. E., Leal-Lara, H., & Valencia-del Toro, G. (2018). Functional properties of flours and protein concentrates of 3 strains of the edible mushroom *Pleurotus ostreatus. Journal of Food Science and Technology*, *55*(10), 3892-3901. PMid:30228387. http://dx.doi.org/10.1007/s13197-018-3312-x

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, *124*(2), 411-421. http://dx.doi.org/10.1016/j.foodchem.2010.06.077

Erjavec, J., Kos, J., Ravnikar, M., Dreo, T., & Sabotič, J. (2012). Proteins of higher fungi: From forest to application. *Trends in Biotechnology*, *30*(5), 259-273. PMid:22341093. http://dx.doi.org/10.1016/j.tibtech.2012.01.004

Gąsecka, M., Mleczek, M., Siwulski, M., & Niedzielski, P. (2016). Phenolic composition and antioxidant properties of *Pleurotus ostreatus* and *Pleurotus eryngii* enriched with selenium and zinc. *European Food Research and Technology*, *242*(5), 723-732. http://dx.doi.org/10.1007/s00217-015-2580-1

Gaur, T., Rao, P., & Kushwaha, K. (2016). Nutritional and anti-nutritional components of some selected edible mushroom species. *Indian Journal of Natural Products and Resources*, *7*(2), 155-161.

Godwin, O. O. (2015). Proximate and Antinutrient Content of *Pleurotus ostreatus* (*Jacq.*) P. Kumm Found In Akwa Ibom State, Nigeria.

Golak-Siwulska, I., Kałużewicz, A., Spiżewski, T., Siwulski, M., & Sobieralski, K. (2018). Bioactive compounds and medicinal properties of Oyster mushrooms (*Pleurotus sp.*). *Folia Horticulturae*, *30*(2), 191-201. http://dx.doi.org/10.2478/fhort-2018-0012

González, A., Nobre, C., Simões, L. S., Cruz, M., Loredo, A., Rodríguez-Jasso, R. M., Contreras, J., Texeira, J., & Belmares, R. (2021). Evaluation of functional and nutritional potential of a protein concentrate from *Pleurotus ostreatus* mushroom. *Food Chemistry*, *346*, 128884. PMid:33401088. http://dx.doi.org/10.1016/j.foodchem.2020.128884

Goswami, B., & Bhowal, J. (2015). Optimization of parameters for production of protein hydrolysate using edible oyster mushroom (*Pleurotus ostreatus*). *Journal of Food Processing & Technology*, *6*, 8. http://dx.doi.org/10.4172/2157-7110.S1.023

Greeshma, A., & Sridhar, K. (2018). Functional attributes of ethnically edible ectomycorrhizal wild mushroom Amanita in India. *Microbial Biosystems*, *3*(1), 34-44. http://dx.doi.org/10.21608/mb.2018.12358

Gul, O., Atalar, I., Mortas, M., & Dervisoglu, M. (2018). Rheological, textural, colour and sensorial properties of kefir produced with buffalo milk using kefir grains and starter culture: A comparison with cows' milk kefir. *International Journal of Dairy Technology*, *71*, 73-80. http://dx.doi.org/10.1111/1471-0307.12503

Harte, F. M., Martinez, M. C., & Mohan, M. S. (2019). Foaming and emulsifying properties of high pressure jet processing pasteurized milk. US Pat. No. 0,374,359. Pennsylvania: Penn State Research Foundation.

Hettiarachchi, C. A., Corzo-Martínez, M., Mohan, M. S., & Harte, F. M. (2018). Enhanced foaming and emulsifying properties of high-pressure-jet-processed skim milk. *International Dairy Journal*, *87*, 60-66. http://dx.doi.org/10.1016/j.idairyj.2018.06.004

Hoa, H. T., Wang, C.-L., & Wang, C.-H. (2015). The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, *43*(4), 423-434. PMid:26839502. http://dx.doi.org/10.5941/MYCO.2015.43.4.423

Ijioma Blessing, C., Ihediohanma Ngozi, C., Onuegbu Ngozi, C., & Okafor Damaris, C. (2015). Nutritional composition and some anti-nutritional factors of three edible mushroom species in South Eastern Nigeria. *European Journal of Food Science and Technology*, *3*(2), 57-63.

Ishara, J. R., Sila, D. N., Kenji, G. M., & Buzera, A. K. (2018). Nutritional and functional properties of mushroom (*Agaricus bisporus* & *Pleurotus ostreatus*) and their blends with maize flour. *American Journal of Food Science and Technology*, *6*(1), 33-41. http://dx.doi.org/10.12691/ajfst-6-1-6

Jamdar, S., Rajalakshmi, V., Pednekar, M., Juan, F., Yardi, V., & Sharma, A. (2010). Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. *Food Chemistry*, *121*(1), 178-184. http://dx.doi.org/10.1016/j.foodchem.2009.12.027

Josiane, M., Estelle, M., & Francis, N. (2018). Effect of substrates on nutritional composition and functional properties of *Pleurotus ostreatus. Current Research in Agricultural Sciences*, *5*(1), 15-22.

Kadiri, M. (1990). *Physiological studies on some Nigerian mushrooms*. Nigeria: University of Ibadan.

Kester, J., & Richardson, T. (1984). Modification of whey proteins to improve functionality. *Journal of Dairy Science*, *67*(11), 2757-2774. http://dx.doi.org/10.3168/jds.S0022-0302(84)81633-1

Kirbag, S., & Akyuz, M. (2008). Effect of various agro-residues on growing periods, yield and biological efficiency of *Pleurotus eryngii. Journal of Food Agriculture and Environment*, *6*, 402-405.

Latta, M., & Eskin, M. (1980). A simple and rapid colorimetric method for phytate determination. *Journal of Agricultural and Food Chemistry*, *28*(6), 1313-1315. http://dx.doi.org/10.1021/jf60232a049

Lavelli, V., Proserpio, C., Gallotti, F., Laureati, M., & Pagliarini, E. (2018). Circular reuse of bio-resources: The role of *Pleurotus spp.* in the development of functional foods. *Food & Function*, *9*(3), 1353-1372. PMid:29480298. http://dx.doi.org/10.1039/C7FO01747B

Liu, B.-L., & Chiang, P.-S. (2008). Production of hydrolysate with antioxidative activity and functional properties by enzymatic hydrolysis of defatted sesame (*Sesamum indicum L.*). *International Journal of Applied Science and Engineering*, *6*(2), 73-83.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, *193*(1), 265-275. PMid:14907713. http://dx.doi.org/10.1016/S0021-9258(19)52451-6

Maqsood, S., Al-Dowaila, A., Mudgil, P., Kamal, H., Jobe, B., & Hassan, H. M. (2019). Comparative characterization of protein and lipid fractions from camel and cow milk, their functionality, antioxidant and antihypertensive properties upon simulated gastro-intestinal digestion. *Food Chemistry*, *279*, 328-338. PMid:30611498. http://dx.doi.org/10.1016/j.foodchem.2018.12.011

McCarthy, A. L., O'Callaghan, Y. C., & O'Brien, N. M. (2013). Protein hydrolysates from agricultural crops: Bioactivity and potential for functional food development. *Agriculture*, *3*(1), 112-130. http://dx.doi.org/10.3390/agriculture3010112

Miles, P. G., & Chang, S.-T. (2004). *Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact.* Boca Raton: CRC Press. . http://dx.doi.org/10.1201/9780203492086.

Narh, M. D. L., Addo, P., Dzomeku, M., & Obodai, M. (2018). Bioprospecting of powdered pineapple rind as an organic supplement of composted sawdust for *Pleurotus ostreatus* mushroom cultivation. *Food Science & Nutrition*, *6*(2), 280-286. PMid:29564093. http://dx.doi.org/10.1002/fsn3.551

Nielsen, P., Petersen, D., & Dambmann, C. (2001). Improved method for determining food protein degree of hydrolysis. *Journal of Food Science*, *66*(5), 642-646. http://dx.doi.org/10.1111/j.1365-2621.2001.tb04614.x

Ogundele, G., Salawu, S., Abdulraheem, I., & Bamidele, O. (2017). Nutritional composition of oyster mushroom (*Pleurotus ostreatus*) grown on softwood (*Daniella oliveri*) sawdust and hardwood (*Anogeissus leiocarpus*) sawdust. *Current Journal of Applied Science and Technology*, *20*(1), 1-7.

Okwu, D., & Ndu, C. (2006). Evaluation of the phytonutrients, mineral and vitamin contents of some varieties of yam (*Dioscorea sp.*). *International Journal of Molecular Medicine and Advanced Sciences*, *2*(2), 199-203.

Oly-Alawuba, N., & Obiakor-Okeke, P. (2014). Antinutrient profile of three mushroom varieties consumed in Amaifeke, Orlu, Imo state. *Food Science and Quality Management*, *32*, 1-5.

Onuoha, C., Uchechi, U., & Onuoha, B. (2009). Cultivation of *Pleurotus pulmonarius* (mushroom) using some agrowaste materials. *Agricultural Journal*, *4*(2), 109-112.

Osagie, A. U., Eka, O. U., & Igodan, V. O. (1998). *Nutritional quality of plant foods*. Benin: Post Harvest Research Unit, Dept. of Biochemistry, University of Benin.

Oyetayo, O. V., & Ariyo, O. O. (2013). Micro and macronutrient properties of *Pleurotus ostreatus* (*Jacq: Fries*) cultivated on different wood substrates. *Jordan Journal of Biological Sciences*, *147*(898), 1-4. http://dx.doi.org/10.12816/0001537

Park, K. H., Lee, E. S., Jin, Y. I., Myung, K. S., Park, H. W., Park, C. G., Kong, W. S., & Kim, Y. O. (2016). Inhibitory effect of Panax ginseng and *Pleurotus ostreatus* complex on expression of cytokine genes induced by extract of *Dermatophagoides pteronissinus* in human monocytic THP-1 and EoL-1 cells. *Journal of Mushroom*, *14*(4), 155-161. http://dx.doi.org/10.14480/JM.2016.14.4.155

Peter, O. E., Peter, G. R., Obele, I. I., Owuna, G., Danladi, M. M., Obiekieze, S., & Akwashiki, O. (2019). Utilization of some agro-wastes for cultivation of *Pleurotus ostreatus* (Oyster Mushroom) in Keffi Nigeria. *Environmental Microbiology*, *5*(2), 60-69.

Sánchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology Advances*, *27*(2), 185-194. PMid:19100826. http://dx.doi.org/10.1016/j.biotechadv.2008.11.001

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology*, *85*(5), 1321-1337. PMid:19956947. http://dx.doi.org/10.1007/s00253-009-2343-7

Sangtitanu, T., Sangtanoo, P., Srimongkol, P., Saisavoey, T., Reamtong, O., & Karnchanatat, A. (2020). Peptides obtained from edible mushrooms: *Hericium erinaceus* offers the ability to scavenge free radicals and induce apoptosis in lung cancer cells in humans. *Food & Function*, *11*(6), 4927-4939. PMid:32432266. http://dx.doi.org/10.1039/D0FO00227E

Schaafsma, G. (2009). Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition. *European Journal of Clinical Nutrition*, *63*(10), 1161-1168. PMid:19623200. http://dx.doi.org/10.1038/ejcn.2009.56

Sujith, P., & Hymavathi, T. (2011). Recent developments with debittering of protein hydrolysates. *Asian Journal of Food and Agro-Industry*, *4*(6), 365-381.

Taha, F., & Ibrahim, M. (2002). Effect of degree of hydrolysis on the functional properties of some oilseed proteins. *Grasas y Aceites*, *53*(3), 273-281. http://dx.doi.org/10.3989/gya.2002.v53.i3.317

Tolera, K. D., & Abera, S. (2017). Nutritional quality of Oyster Mushroom (*Pleurotus ostreatus*) as affected by osmotic pretreatments and drying methods. *Food Science & Nutrition*, *5*(5), 989-996. PMid:28948016. http://dx.doi.org/10.1002/fsn3.484

Vaintraub, I. A., & Lapteva, N. A. (1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Analytical Biochemistry*, *175*(1), 227-230. PMid:3245569. http://dx.doi.org/10.1016/0003-2697(88)90382-X

Woldegiorgis, A., Abate, D., Haki, G., & Ziegler, G. (2015). Major, minor and toxic minerals and anti-nutrients composition in edible mushrooms collected from Ethiopia. *Journal of Food Processing & Technology*, *6*(3), 1.

Yuan, B., Zhao, L., Rakariyatham, K., Han, Y., Gao, Z., Muinde Kimatu, B., Hu, Q., & Xiao, H. (2017). Isolation of a novel bioactive protein from an edible mushroom *Pleurotus eryngii* and its anti-inflammatory potential. *Food & Function*, *8*(6), 2175-2183. PMid:28524200. http://dx.doi.org/10.1039/C7FO00244K

Zheng, X., Li, L., Liu, X., Wang, X., Lin, J., & Li, D. (2006). Production of hydrolysate with antioxidative activity by enzymatic hydrolysis of extruded corn gluten. *Applied Microbiology and Biotechnology*, *73*(4), 763-770. PMid:16977469. http://dx.doi.org/10.1007/s00253-006-0537-9

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