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Full Length Research Paper

Determination of the amount of tryptophan from single cell protein (SCP) of the lignocelluloses wastes

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Agricultural wastes are renewable source of energy that can be degraded by the **biological treatment methods. The utilization of these wastes in bioprocesses do not only provides alternative substrates, but also helps in solving pollution problems. White rot fungi have been used successfully in the solid state fermentation for ligninolytic enzyme production. Due to the important role of tryptophan as one of the essential amino acids in the microbial protein, it thus becomes necessary to determine the amount of tryptophan by an effective and practical method. In this study wheat straw was treated with heat at 100°C under alkaline condition. Then microbial protein was produced by cultivation of *Pleurotus florida* as white rot fungi using Solid State Fermentation. The tryptophan analysis was done by alkaline hydrolyses with $Ba(OH)_2$, 4 Normal at 110°C for 48 h of the extracted protein, then the tryptophan was detected by A-200 amino nova analyzer. The results obtained by this method showed that the amount of Tryptophan was 0.96 g/100 g of the extracted protein, indicating the fact that its feed components have a good nutritious value. The produced single cell protein can therefore be a suitable replacement for animal feed.**

Key words: Essential amino acid, microbial protein, wheat straw, *Pleurotus*, animal feed.

INTRODUCTION

Improving the efficiency of protein utilization in feeding animals with the application of amino acids will become more and more important in securing the protein supply and protecting the environment.

It is very difficult to use the lignocelluloses material effectively because the lignin surrounds the cellulose polymer and protects it from microbial attack. Many researchers have concluded that delignification by physical or chemical pretreatments can greatly enhance the susceptibility of cellulose to enzymatic hydrolysis. However, there are still many problems to be resolved both technically and economically (Sanchez, 2009). To verify this hypothesis, we selected a biological fermentation process for the production of animal feed through the growth of fungi on cereal straws. The suitability of an animal feed is based on its amino acid components. Tryptophan is one of the most important essential amino acids that are formed from proteins during digestion by the action of proteolytic enzymes. Many naturally occurring physiological substances are derived from tryptophan, such as serotonin, melanin and niacin (vitamin B_3) (Folk and Tong, 1988). Tryptophan has two important functions; firstly, a small amount of that in our diet is converted into niacin (vitamin B_3) by the liver. This conversion can help to prevent the symptoms associated with niacin deficiency when dietary intake of this vitamin is low (Sandyk, 1992). Secondly, tryptophan serves as precursor for serotonin; the neurotransmitter that helps the body regulates appetite, sleep patterns and mood. Because of its ability to raise serotonin levels, tryptophan has been used therapeutically in the treatment of a variety of conditions, most notably insomnia, depression and anxiety (Moor et al., 2000). The adequate amount of tryptophan in feed stuff portion can be used for growth development and maintenance of the animal.

The aim of the present study was to effectively convert the hemicellulose and cellulose into protein. The most rapid degraders in fungi are basidiomycetes (Bennet et al., 2002). Cultivation of white rot fungi on lignocellulosic residues is considered one of the most efficient biological

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ways for recycling them and the conversion of these materials into animal feed (Royce 1992). In this study we analyzed the tryptophan as essential amino acid of protein from the SCP that was produced by *Pleurotus florida* on wheat straw in solid state fermentation by alkaline condition.

MATERIALS AND METHODS

Cultures and maintenance

This study was carried out from 2009 to 2010 at the Alzahra University, Iran. *P. florida* was provided kindly by Dr. Mohammadi Goltapeh, E., Tarbiat Modarres University in the year of 2009. The cultures were maintained by sub-culturing potato dextrose agar (PDA) slants at 25°C. The agar media was prepared as described by Chang and Hayes (1978). 39 g of potato dextrose agar was added to 1 L of distilled water, placed in a boiling water bath to dissolve agar and later was autoclaved at 121°C for 15 min. After 7 to 9 days we added distilled water to each slant. The spores were then collected using a sterile glass rod. The estimated number of spores in the suspension was 10^7 per milliliter.

Inoculation development

Inoculants for culture were produced on boiled wheat grains supplemented with 0.2% calcium carbonate and 1.2% calcium sulfate for adjusting the pH and preventing them sticking to each other. Cultures were incubated at 25°C for three weeks in a dark place and these grains with mycelium were used as inoculants.

Preparation of Solid-state (substrate) fermentation SSF substrate

Solid-state (substrate) fermentation (SSF) has been defined as the fermentation process occurring in the absence of free water. SSF processes generally employ a natural raw material as carbon and energy source. Hence, maintenance of adequate moisture level in the solid matrix is essential for SSF processes. Solid substrates should have generally large surface area per unit volume. Smaller substrate particles provide larger surface area for microbial attack but pose difficulty in aeration/respiration due to limitation in inter-particle space available (Pandey et al., 2007).

Wheat straws were cut into 2 cm pieces and treated with 2% NaOH and sterilized at 100°C for 30 min. The straws were washed with distilled water and dried in the oven at 80°C. Finally 30 g of such dried straws as a substrate in 1000 ml conical flasks was moistened with Mandel's culture with 0.3 g /liter urea ($CaCl_2$ 0.3 g ,Urea 0.3 g$(NH_4)_2$ SO_4 1.4 g, KH_2 PO_4 2g $ZnSO_4$. $7H_2O$ 1.4mg $MnSO_4$. H_2O 1.6 mg, $FeSO_4$. $7H_20$ 5.0 mg/$CuCl_2$ 2.0 mg per liter).

The flasks were autoclaved at 100°C for 15 min and then inoculated with wheat grain based inocula with *P. florida*. The cultures were incubated at 25°C in a dark room for 4 weeks until the mycelium of the fungi is fully grown. The mixture was then dried at 60°C for 24 h and turned into a very fine powder.

Protein extraction

Protein extraction was performed by preparation of the sample buffer (Tris HCl PH= 8, Glycerol, SDS, 2-Mercaptoethanol, distilled water). Microbial protein was added into this buffer and boiled for 7 to 8 min. After cooling down, the solution was centrifuged at 14000 g, then supernatant of the solution was added to cold acetone (–20°C) to precipitate. Protein later was dissolved in Tris HCl and result was concluded using Bradford method.

Measurement of amino acid

To analyze the tryptophan first the extracted protein was alkaline hydrolyzed by $Ba(OH)_2$ 4N for 48 h. Then A-200 amino nova analyzer was used for derivatization and separation steps. The same process was repeated for 3 times. 32 bit control software amino control including optimized programs was used as controlling software and also acquisition and analysis software amino pick was used as chromatography software.

RESULTS AND DISCUSSION

Wheat straw treated with heat and alkaline condition and inoculated with *P. florida* produced microbial protein. After extraction of protein from this production, the determined amount of tryptophan was 0.96 g/100 g of protein. This result was compared with the amount of tryptophan found in several nutritive food sources. Tryptophan is one of the essential amino acids that our body uses to synthesis the protein it needs. It is well known for its role in the production of nervous system messengers, especially those related to relaxation, restfulness and sleep.

As an essential amino acid, dietary deficiency of tryptophan may cause the symptoms characteristic of protein deficiency, which include weight loss and impaired growth in infants and children and also may lead to low levels of serotonin (Vander, 2001). High dietary intake of tryptophan from food sources is not known to cause any symptoms of toxicity. Vitamin B_6 is necessary for the conversion of tryptophan to both niacin and serotonin; consequently a dietary deficiency of vitamin B_6 may result in low serotonin levels and/or, impaired conversation of tryptophan to niacin (Martinez et al., 2001). Vitamin B_6, vitamin C, folic acid and magnesium are necessary for metabolization of tryptophan. In addition to afore-mentioned, tyrosine and phenylalanine compete with tryptophan for absorption. Because of this, tryptophan must be taken in enough quantities to have an ensured increased level in the body.

This essential amino acid is involved in many biological functions other than the synthesis of protein associated to animal production such as growth and milk production. Two main points deserve to be kept in mind. The first one is that tryptophan appears to be a key nutrient for controlling feed intake. An adequate dietary tryptophan is particularly important for period during which appetite limits performance. The second point concerns the relationship between tryptophan and health. We showed that tryptophan requirement can be modified during period when pig's immune system is stimulated. This is the case for transition periods during which an adequate supply of tryptophan may prevent or at least limit the consequences of immune system activation on performance (Le Folch and Seve, 2007).

Table 1. The amount of Tryptophan g/100g of protein.

Essential amino acids	Egg (a)	Wheat (b)	Mushroom *Pleurotus*(a)	*C. cellulyticum* Mycelia(c)	This study SCP
Tryptophan	2.1	1.3	0.9	1.5	0.96

a: Data adabted from Bano and Srivasan (1962); b: data adapted from Abdel-Aal and Hucl(2002); c: data adapted from Peiji et al. (1997).

Tryptophan occurs naturally in nearly all foods that contain protein, but in small amount compared to the other essential amino acids (Winder et al., 2000). In Table 1 the comparison of the amount of tryptophan produced by this research with what is available in egg and mushrooms, (Bano and Srivasan (1962); Abdel-Aal and Hucl (2002); Peiji et al .,(1997)), clearly indicates that our SCP has adequate and suitable percentage of tryptophan.

Sindransky et al. (1984) have reported that tryptophan has hormone like properties in promoting protein synthesis in the rat liver by altering the permeability of the nuclear envelope and facilitating translocation of mRNA from the nucleus to the cytoplasm. Some of experiments examined the effect of dietary tryptophan concentration on growth, feed consumption and efficiency of feed utilization. It may be possible to promote muscle growth in swans by feeding concentrations of tryptophan beyond the requirements for maximal growth if a positive effect is seen on ribosomal activity resulting in an overall net protein deposition (Lin et al., 1986). Moderate excess of tryptophan may cause increasing tryptophan concentration in the blood, which can lead to elevated brain concentrations of the neurotransmitter serotonin. This may cause behavioral changes such as reduced feed intake (Fernstrom, 1985). Utilization of enough lysine and tryptophan daily meals resulted in a significant plasma cholesterol and triglyceride levels (Raja et al., 1975). Harms and Russell (2000) found out that egg weight and egg content were significantly increased by the addition of tryptophan to the basal diet. They showed that egg weight increased from 49.7 g when the diet, contained 0.12% tryptophan to 54.8 g when the diet contained 0.20% tryptophan. So the level of dietary amino acid utilized for bodily maintenance and growth, was established (Adeola, 1996). Researchers showed that in tryptophan deficiency of the basal diet graded doses of tryptophan to the basal diet resulted in improvements in weight gain, daily feed intake and feed conversion rate (Peisker, 1999).

In pig husbandry, mixing of pigs usually occurs after weaning, during transportation and after arrival at the finishing farm or slaughter house. Mixing of unfamiliar animals leads to many aggressive interaction among individuals and may negatively affect animal welfare, growth and health. A relief of the negative aspects of social stress may be obtained by dietary tryptophan can affect brain serotonin neurotransmission and can temper abnormal and aggressive behavior and susceptibility (Van Hierden et al., 2004). In pigs, the effect of tryptophan deficiency on growth is mainly associated to a reduction of appetite and feed intake (Eder et al., 2001).

Most of tryptophan degradation occurs through the complex Kynurenine pathway. Tryptophan catabolism through IDO (Indoleamine 2, 3 Dioxygenase.EC 1.13.11.42) pathway is probably involved in regulation of T cells proliferation and production of antioxidant molecules. Pigs fed with a low tryptophan diet developed a more important inflammatory response (Le Floch and Seve, 2007).

In addition to the afore-mentioned, some experiments were conducted to determine the histidine and tryptophan requirement of broiler chicks. The histidine or tryptophan deficient diet based upon corn, feather meal and soybean meal was supplemented with graded increments of either tryptophan or histidine to produce growth responses for maximal weight gain and feed efficiency (Han et al., 1991). Also amino acids mixtures deficient in lysine, methionine or tryptophan did not support the growth of young rats (Yang et al., 1968).

Henry et al. (1996) found that female swine were more sensitive than males to feed changes in the ratio of tryptophan to large neutral amino acids. Likewise, Rouvinen et al. (1999) reported behavioral effects in female, but not male, foxes subjected to the same tests after tryptophan enriched feeding. Research in other mammals indicates that behavioral responses to tryptophan supplementation may vary with age, breed and gender that can be modified by diet, exercise, social status and level of arousal (Grimmett and Sillence, 2005).

Considerable work has been done on bio delignification of cereal straws by treatment of the white rot fungus to obtain protein enriched substrate for animal feeding on solid state fermentation (Agosin et al., 1999). The amount of produced protein in the culture has a reverse relationship with the density of urea. This is because the culture has become more alkaline (Kutlu, 2000). Therefore using heat at 100°C , NaOH 2% and Urea 3 g/lit is the best condition at which the most and best quality protein was getting produced. Following these experiences, the researchers could produce more microbial protein from micro-organisms with higher nutritious quality than before.The chemical composition of SCP treated with *Pleurotus ostreatus* increased the crude protein content of the wheat straw (fazaeli et al., 2006; Zadrazil et al., 1996).

In our recent research this microbial protein was found nutritionally beneficial since as a dietary protein it contains essential amino acids which are needed for proper growth and good functionality of immunity system

of their body (Ahmadi et al., 2010). The aim of this study was to produce a high quality protein with lots of essential amino acids and by further determination of the amount of tryptophan in the single cell protein (SCP) with specific methods, we can proudly claim that it is an excellent available source of animal feed. We also proved that *P. florida* can directly grow in lignocellulosic wastes and effectively convert hemicelluloses and cellulose to a high quality protein. This feed is cheap, simple to produce and has a good and noticeable concentration of tryptophan for the better growth of animals. In this study by optimizing the conditions of fungi, substrate and environment, the nutritious value of single cell protein (SCP) was improved and the percentage of tryptophan was increased dramatically.

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