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Full Length Research Paper

Comparison of essential and non essential amino acids in the single cell protein (scp) of white rot fungi from wheat straw

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As the constraints related to environmental issues are becoming quite severe and stringent, it is necessary to develop optimized systems for food waste treatment. White rot fungi are ideal parameters for lignocellulosic wastes to produce microbial protein. In this study wheat straw was treated with NaOH 2% and heated at 100°C and was inoculated with *Pleurotus florida* by solid state fermentation (SSF). The crude protein was 62.8% per 100 g of dried single cell protein (SCP). The extracted protein hydrolyzed with HCl 6 Normal, and the amino acids analyzed by A-200 amino nova analyzer. The profile concentration of non essential amino acids was: Aspartic acid = 5.22, Serine = 3.6, Glutamic acid = 6.38, Prolin = 3.2, Glycine = 4.21, Alanine = 6.23, Cycteine = 1.18, Tyrosine = 2.61 g/100 g of extracted protein. The concentration of essential amino acids however has been reported. The comparison of both essential and non essential amino acids in conclusion, indicates that the ratio of essential amino acids to total amino acids was 65.6% which clearly proves the effectiveness of this fungus for enhancing the feed value of agro-residues. All experiments were carried out in triplicate and result averaged accordingly.

Key words: *Pleurotus,* microbial protein, lignocellulosic waste, animal feed, solid state fermentation.

INTRODUCTION

Single cell proteins are the dried cells of micro-organism, which are used as protein supplement in human foods or animal feeds. Most of lignocellulosic wastes have a poor balance of total amino acids compared to the needs of animals. Waste treatment techniques in general are used to alter the physical, chemical or biological characteristics of the waste to reduce its velum or toxicity and to make the waste safer for disposal (Tanaka and Matsuno, 1985). Biological treatment is commonly used to remove and degrade the hazardous constituents of wastes. Cereal straw is a major agricultural waste that can be upgraded successfully by microbial treatment (Kumar et al., 2008). During microbial process for conversion of lignocellulosic wastes into feed at least one of the three objectives must be reached: (1) An increase in the protein level (2) An increase in digestibility (3) An increase in the essential amino acids (Kamara and Zadrazil, 1988).

White rot fungi have strong decomposition ability for cellulose, hemicelluloses and lignin. Considerable work has been done on the use of the white rot fungus (Zadrazil et al., 1996). *Pleurotus* known as the oyster mushroom having unique oxidative and extracellular enzymatic ligninolytic system, which degrades lignin and opens phenyl rings (Villas-Boas et al., 2001). It is important to use microbial species able to degrade selectively the lignin in the residue and supply the culture medium with co-factors that induce selective lignin degradation (Bennet et al., 2002).

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In this study, we produced the microbial protein by *Pleurotus florida* on treated wheat straw and determined the amount of its protein. We further analyzed and investigated their amino acids by comparing their essential and non- essential amino acids.

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. After 7 to 9 days, they were separated and washed with distilled water. The spores were then added to the treated wheat.

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 prevent them from 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.

SSF substrate preparation

Wheat straw was cut into 2 cm pieces and treated with 2% NaOH and sterilized at 100°C for 30 min. The straw was washed with distilled water and dried in the oven under 80°C. The treated dried straw was moistened with Mandel's culture with 0.3 g / lit urea in conical flaks.

The flasks were autoclaved and inoculated with wheat grain based *P. Florida* inocula. 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 hand turned into a very fine powder.

Protein extraction

Protein extraction was performed by preparation of the buffer composed of: Tris HCl pH 8, Glycerol, SDS, 2-Mercaptoethanol and distilled water. Microbial protein to the amount of 0.5 g was added into the buffer and boiled for 7 to 8 min. After cooling down, the solution was centrifuged at 14000 g, and then supernatant of the solution was taken and added to acetone at 20°C to precipitate. Protein later was dissolved in Tris HCl, pH = 8 and result is concluded based on Bradford method.

Analysis method of amino acids

To analyze the obtained amino acids, the protein is needed to be dried first. This was done by adding the protein contained in Tris solution into the pure acetone 99% at – 20°C. The produced protein was then collected for analysis of its amino acids.

The protein was hydrolyzed by (HCl 6 Normal, 1 M, Phenol 2 µl, 2 Mercaptoethanol 2 µl) at 110°C for 48 h. Hydrolysis took place under O_2 free condition to avoid uncontrollable oxidation. The hydrolysis container was placed under vacuum. Then the German made A-200 amino nova analyzer was used for derivatization and separation steps. The same process was repeated 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

After treating the wheat straw with heat at 100°C and NaOH 2%, single cell protein (SCP) was produced by *P. florida*. Results of all amino acids analysis, obtained by this study are shown in Table 1 which indicate amount of Aspartic acid (Asp), Threonine (Thr), Serine (Ser), Glutamic acid (Glu), Prolin (Pro), Glycine (Gly), Alanine (Ala), Cysteine (Cys),Val (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe), Histidine (His), Lysine (Lys), Arginine (Arg) in grams per 100 g of extracted protein of single cell protein (SCP).

As shown Table 1, the amount of essential amino acids to non-essential amino acids was compared. Results were further compared against the total amino acids of egg and fungal species and amino acid composition of other microbial protein. Fungal species are cultured on different substrates but mostly cheap wastes, which supply the carbon and nitrogen for growth. Lignocellulosic wastes have varying composition of hemicellulose, cellulose and lignin based on the dominant component in the waste. Specific fungi can be utilized for biomass production. The biomass thus produced can be harvested and used as SCP. Zadrazil et al. (1995) used the white rot fungi on the sugarcane bagasse as substrate. The nutritious value and potency of SCP from any source is based on its composition and should be analyzed for the properties of their components such as vitamins, nitrogen, carbohydrate, fats, nucleic acids, protein concentration and amino acid profile before the final product is used as food or feed supplementation.

In this study, we determined the amount of all amino acids and compared the essential and nonessential amino acids contained in our SCP. The results indicated that the ratio of essential amino acids to total amino acids was 65.6%.This means our SCP is a good source of nutrition. High metabolic efficiency of SCP from any source is essential in order to extract the maximum possible benefits. There were no effects on metabolism or, any other harmful influence (Giesecke, 1982). Culture condition, pre-treatment of substrates, nutrient supplementation, types of fermentation processes and strain improvement can all alter the final SCP composition (Anapama and Ravinder, 2000). In this research NaOH 2% used as alkaline treatment to make a better substrate. Several fungal cultures produced doubled the amount of crude protein on alkali-treated rice straw when compared to that of untreated straw (Darmwal and Gaur, 1991). The amount of protein was 62.8% gram per 100 g of dried single cell protein (SCP). The comparison of algae, fungi and bacteria derived SCP

Table 1. The amount of all amino acids in SCP (g/100 g of protein).

Amino acid	Average
Asp(D)	5.22
Thr(T)*	0.64
Ser(S)	3.6
Glu(E)	6.38
Pro(P)	3.2
Gly(G)	4.21
Ala(A)	6.23
Cys(C)	1.18
Val(V)*	6.68
Met(M)*	2.11
Ile(I)*	7.32
Leu(L)*	6.82
Tyr(Y)	2.61
Phe(F)*	4.37
His(H)*	19.88
Lys(K)*	9.55
Arg(R)*	8.3

*The essential amino acids.

Table 2. Comparison of crude protein in SCP from algae, fungi, bacteria (g/100g dried weight).

	Algae	Fungi	Bacteria	Our SCP
Crude protein	40-60	30-70	50-83	62.8

The yield range varies with the type of substrate, the specific organism used and the maintained culture conditions. Data obtained from Anupama and Ravindra (2000).

produced the amount of crude protein with our result is shown on Table 2 and this indicates that the percentage of our protein composition is in a good range.

In Table 3, the amount of amino acids of our SCP was compared with egg, *Pleurotus ostreatus* and *Agaricus bisporus* fruit bodies.

The egg is one of the best and most inexpensive high quality protein and vitamins. It is thus frequently used as a reference for comparing the protein quality of other food (Herron and Fernandez, 2004). The amount of amino acids in the SCP from this study is nearly the same amount found in but higher in quality than *Pleurotus* and *Agaricus* that usually found in shops as a source of protein. The amount of all amino acids obtained through this study against previous researches was tabulated as follows in Table 4 and indicates that our result is almost the same as other microbial proteins treated by *Cellulomonas* sp., *Alcaligenes faecalis* and *Alfa alfa* (Han, 1974).

As shown in Table 4, the composition of various amounts of non-essential and essential amino acids of several SCPs indicates that our SCP is in a suitable range compared to others. Currently, solid state fermentation (SSF) is being used for the production of protein enriched feed (Zadrazid and Brunnret, 1981). The advantage in SSF process is the unique possibility of efficient utilization of waste as the substrate to produce commercially viable products. The process of SSF initially concentrated on enzyme production, but presently there is a worldwide interest for SCP production due to the dwindling conventional food rescores (Zadrazil et al., 1995).

Nowadays obtaining adequate information regarding the nutritious quality of the animal feed has to become a necessity of life. We already reported how important the essential amino acids are for growth in the humans and animals (Ahmadi et al., 2010). Now we would like to explain about the functions of non-essential amino acids and their deficiencies in the body.

Alanine is glycogenic, non-polar, hydrophobic and aliphatic non-essential amino acid which is important energy source for muscles. It is the primary amino acid in sugar metabolism and boosts immune system by producing antibodies; also it is a major part of connective tissue. The deficiencies in alanine are seen in the hypoglycemia and muscle breakdown and fatigue. It can

Table 3. Comparison of microbial protein with egg, *Pleurotus*, *Agaricus* and wheat straw (g/100 g protein).

Amino acid	Egg[a]	*P. ostreatus*[b]	*A. bisporus*[c]	This study	Wheat straw[d]
Asp(D)	9.1	5.0	2.2	5.2	0.13
Thr(T)	3.7	-	2.6	0.6	0.04
Ser(S)	4.6	3.6	1.4	3.6	0.05
Glu(E)	11.2	17.0	2.8	6.3	0.12
Pro(P)	2.9	2.8	1.9	3.2	0.1
Gly(G)	3.1	3.8	1.6	4.2	0.06
Ala(A)	5.2	3.3	1.3	6.2	0.11
Cys©	2.2	-	1.8	1.1	0.32
Val(V)	6.3	3.4	3.3	6.6	0.02
Met(M)	3.1	-	1.0	2.1	0.28
Ile(I)	4.8	3.6	2.0	7.3	0.14
Leu(L)	7.6	4.7	0.7	6.8	0.18
Tyr(Y)	3.3	-	1.2	2.6	0.28
Phe(F)	5.4	2.5	1.6	4.3	0.26
His(H)	2.2	4.2	3.3	19.8	0.1
Lys(K)	6.1	6.1	3.2	9.5	-
Arg(R)	5.0	-	2.6	8.3	0

[a]Data obtained from Kassis et al. (2010); [b]Data obtained from Ancona et al. (2005); [c]Data obtained from Kurbanoglu et al. (2004); [d]Data obtained from Peiji et al. (1997).

Table 4. Comparison amongst several microbial protein g/100 g extracted protein.

Amino acid	*Cellulomonas sp**	*Alcaligenes faecalis**	*Alfa alfa**	*Pleurotus florida***
Asp(D)	8.3	9.1	12.5	5.2
Thr(T)	4.7	4.4	5.1	0.6
Ser(S)	4.1	3.4	5.2	3.6
Glu(E)	18.4	17.0	11.3	6.3
Pro(P)	7.5	4.4	5.1	3.2
Gly(G)	4.1	5.1	5.7	4.2
Ala(A)	8.1	8.9	6.3	6.2
Cys(C)	0.4	0.4	1.4	1.1
Val(V)	6.7	7.0	6.7	6.6
Met(M)	1.6	2.7	1.9	2.1
Ile(I)	4.1	5.4	5.5	7.3
Leu(L)	8.6	7.6	8.4	6.8
Tyr(Y)	2.4	3.0	3.7	2.6
Phe(F)	3.6	4.1	5.7	4.3
His(H)	2.9	2.5	2.5	19.8
Lys(K)	8.0	9.9	6.7	9.5
Arg(R)	6.1	4.8	5.5	8.3

*Data obtained from Han (1974); ** data obtained from this study.

also cause viral infection (Aletor et al., 2000). The alanine obtained in this study was 6.23 g/ 100 g of extracted protein from this microbial protein which in comparison whit that of egg shows we have a good result.

Aspartic acid is another non-essential amino acid and Is inter-convertible with asparagines. So they have many functions in common. It helps to protect the liver by aiding the removal of ammonia and is involved in DNA and RNA metabolism. Also it is involved in the functionality of the immune system by enhancing immunoglobulin production

and antibody formation. Aspartic acid deficiency can also lead to calcium and magnesium deficiencies (Bunchasak et al., 1998). In this study we found 8.2 g of aspartic acid in100 g of extracted protein.

Cysteine is a non-essential sulfur containing amino acid. It has a protective function against radiation, pollution, ultra violet light and other causes of increased free radical production. Cysteine is a natural detoxifier and essential for the growth and repair of skin. Also it is a major constituent of glutathione, an important three peptide made up of cysteine, glutamic acid and glycine (Huyghebaert and Pack, 1996). We detected small amount of cysteine: 1.18 g/100 g of protein.

Another acid side chain, non essential amino acid is glutamic acid which is a precursor to glutamine and GABA (two neurotransmitters) and helps to stop alcohol and sugar cravings with increased energy (Kerr and Kidd, 1999). Glycine is non-polar hydrophobic and part of the structure of hemoglobin. It is a part of the two main inhibitory neurotransmitters; the other being GABA part of cytochromes, which are enzymes involved in energy transport and it is one of the three critical glycogenic amino acids along with serine and alanine. It is also involved in glycogen production which assists in glycogen metabolism (Yamazaki et al., 1998).

In this study the amount of glutamic acid and glycine were respectively 6.38 and 4.21 g/100 of g protein of SCP, however both of them are lower than same found in egg. Proline is a hydrophilic and hydroxylic amino acid which is a critical component of cartilage and hence of joints, tendons and ligaments. It is involved in keeping heart muscle strong and works in conjunction with vitamin C in keeping skin and joints healthy (Kirchgessner, 1995).

Serine is one of the most important glycogenic amino acids and is critical in maintaining blood sugar levels. It boosts immune system by assisting the production of antibodies and immunoglobulin. Myelin sheath is the fatty acid complex that surrounds the axons of nerves and is derived from serine. One variation of serine namely phosphatidyl serine serves several important functions within the central nervous system, including development of the myelin sheath. Serine also is required for growth and maintenance of muscles (Fuller et al., 1989).

We obtained 3.2 g of proline and 3.6 g of serine per 100 g of protein in our research; both indicating that we have a good result comparing with the other fungi (Table 2).

Tyrosine in an aromatic and hydrophilic precursor to neurotransmitters dopamine, norepinephrine, epinephrine (adrenaline) and melanin. It is an effective antidepressant for norepinephrine deficient depressions and also precursor to thyroxin and growth hormone. It increases energy, improves mental clarity and concentration. The amount of tyrosine in this study was 2.61 g/100 g protein which is an optimal value.

Looking at the profile of all amino acids in this study, we proudly claim to have produced one of the best single cell protein (SCP) currently available in the world to such an extent that it can be a fully justified substitute for presently used animal feed in the market.

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