Determination of a Group of Amino Acids in the Bioproteins Resulting from Bioremediation of Remains of Marine Life

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Abstract
The research was conducted by isolating some micro organisms from the marine environment, and using it in remediation the remains of marine life. These organisms were characterized by their ability to secrete a disintegrated enzymatic spectrum of compounds in these residues and to build their protein-rich biomass through their metabolism. The results of the study showed the highest percentage of total protein in fermented biomass by Micrococcus variants which reached to (42.11%). An increase in the ratio of amino acids in this mass was notable, especially lysine (7.01 g / 100 g bioprotein) and methionine (1.96 g / 100 g bioprotein). A comparison between the protein produced from these organisms in the present study and the traditional vegetable and animal protein was performed. It was observed that the ratios of the amino acids studied were higher than those in the vegetable protein, which allowed it to be a real protein substitute in economic animals feed.

Key words: *Bioprotein, bioremediation and amino acids.*

I. INTRODUCTION

Poor countries (third world countries) suffer from food shortages and many problems such as famines, food security and diseases resulting from food shortages. The WHO confirmed that more than 12 million people die every year because of it [1], [26]. Therefore, the eye turned to trying to find cheap food sources and developed many technologies for this, including the technology of production of single-cell protein, which is an environmentally friendly product because this technology depends on the growth of a micro-organism on the residues remains, which helps in recycling it. These organisms are characterized by high protein content in addition to carbohydrates, vitamins and amino acids especially methionine and lysine which are limited in animal and vegetable food [2].

The single-cell protein contains a balanced ratio of essential amino acids in addition to containing the ratios of nitrogen necessary for the manufacture of non-essential amino acids, which are manufactured by the micro organisms itself. Essential amino acids are necessary if we know that human and animal bodies do not manufacture them, but must be obtained through food. They play a role in the growth, tissue building and immunity [3].

The fungi contain ratios of protein ranging from 35-50% and low percentages of nucleic acids. Where the fungal protein considers easy to extract and use in food and can be digested. Fungi can also treat waste without the need for the initial processing because of its ability to secrete a wide spectrum of enzymes [4]. Yeast also contains high levels of amino acids, especially tyronine, lysine and tryptophan, and has the ability to synthesize amino acids from inorganic acids and sulfur added in the form of salts in the medium and get energy and carbon from the waste [5]. The fungal protein was produced from the rice residues using the fungus *Trichoderma harzianum*, with the highest percentage of raw protein (49.50%) and the net protein (32.00%) with the content of 16 amino acids with a high percentage of lysine and a low percentage of vinyl alanine and methionine [6]. The protein produced by the fungus *Aspergillus niger* was 30.4% and contained a high level of lysine [7]. A study to determine the amino acids of the single-cell protein produced by *Aspergillus terreus* showed at least 16 amino acids, including essential and non-essential amino acids, and were compared with the FAO / WHO ratios [8], [25].

The single-cell protein resulting from some microorganisms suffers from a lack in the methionine and tryptophan acids, the essential amino acids of animals and humans. The experiment to eliminate the problem of the shortage of some sulfuric acid was successful by adding different concentrations of sulfur in the growth plant of these organisms. For example, the mass of *Candida tropicalis* was 41% higher than the original sulfuric acid [9].

The production of a single-cell protein produced from several cultured cultures together is a method of enriching the biochemical structure through the synthesis of protein and amino acids and thus doubling the proportions of these compounds. This is observed in mixed colonies such as *Trichoderma reesei* and *Kluyveromyces marxiannus* compared to a single colony of *T. reesei*, where amino acids is higher than FAO standards [10]. Amino acids play important biotic roles. For example, lysine is essential for the construction of immunoglobulin, and arginine plays an important role in the activity of large macrophages that eliminate germs. Heistidine plays an important role in the production of red and white blood cells and the activity of a group of enzymes. Valine is also one of the necessary essential amino acids to build protein and bind amino acids to each other and enter the hemoglobin structure with tyronine, and decrease one of them causes several negative effects on the immune system [3].

In this study, remains of marine life were collected, including crustaceans (shrimps and crabs) accumulated on the coast and from the residues as a prepared meal for the purpose of bioremediation using microorganisms isolated from the marine environment adapted to the accumulated remains. These organisms are characterized by their ability to build their protein-rich biomass through their metabolism. Some amino acids are estimated and compared to their proportions in animal and vegetable protein.

The goals of the present research are to:

1. Production of a single cell protein from some microorganisms.
2. Enrichment the remains of marine life with proteins by bioremediation it with these organisms, and the estimation of amino acids and compare them with their proportions in vegetable and animal protein.

The importance of the research comes from the high efficiency in transforming the substrate from matter-less to a high-quality amino acid protein that can replace the traditional vegetable and animal protein.

II. MATERIALS AND METHODS

A.) Preparation of remains of marine life for biochemical remediation:

The remains of marine life were collected from the coast of Lattakia (next to the Sports City), which included the remains of crabs and shrimps husks, then washed with water, dried with a 60 ° C drying oven for 24 hours, and then grinded with an electric grinding machine to obtain small size granules. Sift with different diameters sieves to achieve a homogeneity in the size of the crushed residues. And were separated into different diameters granules ranged between 250 µm to 2 millimeters. The hydrolysis methods were used either with acid hydrolysis using HCl (1.25 N) or with alkaline hydrolysis using NaOH (1 N) [11].

B.) The chemical materials:

Hydrochloric acid (HCl), sulfuric acid (H_2SO_4), boric acid (H3BO3), agent kildal, sodium hydroxide (NaOH), biuret reagent, anhydrous sodium sulphate, monohydrogen and dichromate potassium phosphate, hydro magnesium sulfate, potassium chloride, hydrothermal sulfate, hydro zinc sulfate, sodium chloride, agar, distilled water, sea water, sodium chloride, sodium nitrate, potassium nitrate, yeast extract, meat extract, peptone, general cultures media such as Agar Nutrient, Czapeck Dox's Agar, Potato Dextrose Agar (PDA).

C.) The used devices and tools:

Kieldal device to detect the protein content (Buchi Digest system K-437), Autoclave (OT4oL ، nuvesteamArT), Shaker incubator (InfoRs), A bacterial incubator (napco), bacterial Insulation room (JSCB-1200SB), spectrophotometer (JASCO-v-630), Centrifuge (Combi 514R), microscope (motic), Hemocytometer (NEUBAUER), sensitive electronic balance (Precisa -XB22oA), oven (JANAT instRuEMents), water bath (K.F.T LaB.EouiPMENT), Grinding machine, different diameters sieves, Fridge and glass tools, and other laboratory tools.

D.) Preparation the biological Primers:

samples of beach sand containing the remains of marine life accumulated on the beach were collected, dried and planted on the PDA medium. Then incubated for a week at 28 ° C and then some fungal colonies were isolated and purified to obtain pure colonies of each fungus then classified. The following fungal species were obtained: *Mucor circinelloides*, *Trichoderma harzianum*, *Aspergillus niger* and *Aspergillus terreus.*

The suspensions of one week age colonies of these fungi were prepared, where it transferred to a physiological serum (0.9% sodium chloride). 1 ml of each suspension was taken and a number of spores or cells was performed by NEUBAUER and add the primer to prepared media (10^6 cell / mL).

E.) Production of a single cell protein from the remains of marine life in the nutrient liquid medium:

Different concentrations (0.5 and 1 g) of marine life remains were added as a powder to 100 mL of the plant medium (1 g / 1 liter distilled water): 3 ammonium sulphate, 1 (K_2HPO_4), 0.5 ($MgSO_4.7H_2O$), 0.5 potassium chloride, 0.01 ($7H_2OFeSO_4$).

Then it were shaking well. Value of pH was set at 5.5-6 for fungus with alkaline or acidic solution as needed, sealed well with cotton and aluminum foil,

sterilized at 121 ° C for 15 minutes, left to cool. Then it was injected by bio primer of each isolated fungi (10^6 cell / ml). Then put on a shaker (150 rpm) in an incubator at 28 ° C for two weeks. The biomass produced from the liquid fermentation medium was then separated by a centrifuge of 6000 rpm for a 15 minutes, dried at 60 ° C and weighed. Then added in a fridge at 4 ° C to calculate protein ratios and amino acids in them [12].

F.) Protein enrichment in the nutrient solid medium of the remains of marine life:

Micrococcus variants (Marine microorganisms decomposed into marine biota isolated from marine waters) incubated on nutrient agar for 24-hour at 30 ° C and the colony was harvested using a physiological serum (0.9% or 9 g / L NaCl). 1 ml of the suspension was taken and the number of cells was counted by NEUBAUER. The suspension 10^8 (cell / ml) were added to the media which contain of (sterile residue of shrimp residue) according to the following ratio: (2:1 w/v) (50% sterile sea water: sterile residues). It was sealed with cotton and aluminum and incubated at 30 ° C for two weeks. After incubation, dry at 60 ° C for 24 hours and store at 4 ° C for protein analysis using the Keldal method and the biuret method [13].

G.) Estimate of amino acids using spectrophotometer:

Biomass was treated with the addition of (6N HCL) and stored at 110 ° C for 24 h and then filtered for amino acid analysis using a spectrophotometer based on the standard curve determined by standard concentrations of the same acid to be measured.

1. Estimate the Tryptophan:

The determination of tryptophan is based on the Hopkins-Cole test, which results a purple compound. This is due to the interaction of the endol group in tryptophan with the glycosylic acid in a strong acid medium and the color intensity is proportional to that of the typtophan. The light absorption of the solutions is measured at 545 nm [14].

2. Estimate the Tyrosine:

The analysis is based on the test of xanthoprotein, which gives yellow or orange compounds by heating with netric acid in an alkaline medium. And the optical absorption of solutions is measured at a 388 nm wave [15].

3. Estimate the Arginine:

It is based on the Sakaguchi test, in which the alpha-naphthol detector is used in the presence of an oxidizing substance, sodium chlorite or hypochromite, producing a complex red-to-purple complex. The light absorption of the solutions is measured at a 501 nm wave [14].

4. Estimate the Proline:

It depends on the ninhadrine test, which produces a yellow compound, and the light absorption of the solutions is measured at a 440 nm wave [16].

5. Estimate the Glycine:

It depends on the ninhadrine test, which produces a blue compound, and the light absorption of the solutions is measured on a 567 nm wave [17].

6. Estimate the lysine:

It depends on the ninhadrine test, which produces a blue composite that measures light absorption of solutions on a 570 nm wave [16].

7. Estimate the Methionine:

Depends on the use of a nitrofriacid detector and a complex configuration that measures light absorption of solutions on a 510 nm wave [18].

III. RESULTS AND DISCUSSION:

A) Production of a single cell protein from the remains of marine life in a liquid medium:

The highest ratio of protein recorded in the fungus *Trichoderma harzianum* then *Aspergillus niger*, *Aspergillus terreus* and *Mucor circinelloides* (32.22, 30.04, 29.02 and 26.66 %) respectively. While the combined farm of *Trichoderma harzianum* and *Aspergillus niger* recorded a protein ratio of 36.44% (Table 1). These fungi are characterized by its ability to analyze the remains of crustacean marine animals, and its ability to produce the Proteinase enzyme which decomposes the protein in the waste, in addition to its ability to produce the Chitinase enzyme which disintegrates the chitin and converts it into glucose amin, which can be used as a source of carbon and nitrogen to build its biomass.

Table (1): Percentage of amino acids (g / 100 g fungal protein) in the protein produced by the studied fungi

Ratio of Protein and Amino acids (%)	*T. harzianum*	*A. niger*	*A.terreus*	*M.circinelloides*	*T. +nigerA. harzianum*
Protein Ratio	32.22	30.04	29.02	26.66	36.44
Methionine	0.52±0.09	1.53±0.53	1.79± 0.51	1.31±0.26	2.03±0.40
Lysine	4.84±0.11	5.43±1.23	5.63±0.74	6.72±3.57	4.3±1.17
Arginine	6.63± 0.66	8.44±0.71	5.48± 1.01	7.4± 1.22	6.00±0.20
Tyrosine	0.98±0.16	1.36±0.04	1.59± 0.42	1.1 ± 0.45	1.53±0.40

Glycine	1.39±0.91	3.8±1.18	5.8± 1.06	3.26 ± 1.31	7.12±0.43
Tryptophan	0.40±0.14	0.65±0.12	0.52±0.33	0.64 ± 0.25	0.67±0.29
Proline	3.29±0.62	5.69±1.07	1.96±0.31	4.95 ±2.04	4.64±1.72

B) Determination of amino acids in the single-cell protein produced by the studied fungi:

Table 1 shows concentrations of amino acids in the protein of the studied fungi. Whereas in M. circinelloides, the ratio of arginine was higher than the rest of the studied amino acids (7.4 g / 100 g fungal protein), while the lowest ratio was of tryptophan (0.64 g / 100 g fungal protein), and the A. niger and T. harzianum also had the highest arginine and least tryptophan. While A. terreus showed the highest percentage of lysine (5.63 g / 100 g fungal protein) and the least of tryptophan (0.52 g / 100 g fungal protein). The mixed farm of A. niger and T. harzianum recorded an increase in glycine (7.12 g / 100 g fungal protein) and methionine (2.03 g / 100 g fungal protein). These ratios were compared with previous studies similar to the amino acid synthesis of the protein of the studied fungi (Table 2).

Table (2): Percentage of amino acids in some fungi in reference studies

The amino acid	*T. harzianum* re. [6]	*A.terreus* Re. [8]	*A.niger* Re. [7]	*A. niger* Re. [9]	*A.niger* Re. [19]
Methionine	0.22	4.52	1.9	1.60	2.7
Lysine	21.34	18.09	8.2	6.00	9.4
Arginine	1.14	3.36	4.8	6.40	-
Tyrosine	0.51	5.08	5.6	2.10	3.1
Glycine	0.76	5.10	-	10.10	-
Tryptophan	-	-	1.6	1.40	0.9
Proline	1.85	1.89	-	5.60	-

A study conducted by re. [8] on the analysis of amino acids for single-cell protein produced from *Aspergillus terreus* showed that the protein content reached to 30%. At least 16 amino acids, including primary and non-essential amino acids, were detected and compared with ratio of FAO [26]. High levels of lysine were observed (18.09 g / 100 g fungal protein) and high levels of methionine (4.52 g / 100 g fungal protein). The ratios of arginine and glycine were high in value and importance. The fungus *T. harzianum* showed a content of 16 amino acids where the ratio of lysine was high (21.34 g / 100 g fungal protein) while the ratio of methionine was low (0.22 g / 100 g fungal protein) [6].

Re. [19] recorded high ratios of lysine (9.4 g / 100 g fungal protein) and low of tryptophan (0.9 g / 100 g fungal protein). High ratios of glycine, lysine and arginine were notable [9]. And high ratios of lysine and tyrosine in a study of amino acid ratios in the fungus *A. niger* [7].

To evaluate the importance of the amino acid ratios of the fungi we obtained, it was compared with their ratios according to FAO criteria and their percentages in the traditional vegetable and animal protein (Table 3) [26].

Table (3): Percentage of amino acids (g / 100 g protein) according to FAO standards and their ratios in traditional vegetable and animal protein

The amino acid	FAO Re.[26]	Soybean Re. [20]	Egg protein Re. [21]	Prawns Re. [22]	The fish Re. [12]
Methionine	2.2	0.59	3.13	2.46	2.61
Lysine	4.2	2.37	6.13	7.19	8.66
Arginine		2.91	5.81	7.42	6.48
Tyrosine	2.8	1.35	3.11	2.80	2.22
Glycine		1.92	4.24	6.58	5.40
Tryptophan	1.00	0.57	1.61	-	1.05
Proline		2.23	3.45	4.00	3.90

Table 3 shows the low levels of amino acids in the vegetable protein below FAO standards, and the increase it in the animal protein in fish, prawns and eggs. Compared it with the protein produced from fungi in the present study, the amino acids ratios in fungi are higher than those in vegetable protein, especially methionine and lysine. Whereas the animal protein (especially fish) is the active protein containing high levels of these amino acids.

C) Protein enrichment in the solid nutrients medium from the remains of marine life

Table 4 shows protein ratios in prawn husks prior to bioremediation (24.02%). The amino acids

ratios in the prawn varied from the highest of tyrosine (5.69 g / 100 g prawn protein) to the lowest ones for methionine (0.93 g / 100 g prawn protein) [23].

As noted in the present study, the protein ratio was 18.52% in prawns before treatment and the highest ratio of tyrosine (2.85 g / 100 g prawns protein) and the lowest for methionine (0.64 g / 100 g prawns protein).

Table (4): Protein and amino acid ratios in the residues of prawn husks before and after bioremidiation

Ratio of protein and amino acids	The fermented mass of prawn residue using *Micrococcus variants*	**Residues of prawns before treatment in the present study**	Re. [24]	Re. [23]
Protein Ratio %	42.11	18.52	24.02	-
Methionine	1.96±0.84	0.64±0.13	0.57	0.93
Lysine	7.01±2.83	1.81±0.15	1.66	2.38
Arginine	3.43±0.85	2.74±0.21	-	2.70
Tyrosine	3.04±0.59	2.85±0.05	1.65	5.69
Glycine	4.26±0.63	1.31±0.45	-	2.40
Tryptophan	1.37±0.20	1.55±0.78	-	-
Proline	3.5±1.28	2.41±1.37	-	2.02

Table (4) shows that the using of agriculture on a solid nutrient medium gives a higher biomass and increases the total protein content in prawns before treatment (%18.52) to (%42.11) after its ferment. The increase in protein ratios is due to the growth and widespread spread of bacteria in the fermented substrate, which also causes an increase in the total protein (germ protein and the protein of the residues that have not yet broken down).

The percentage of amino acids in the fermented mass was high, especially methionine (1.96 g / 100 g protein), lysine (7.01 g / 100 g protein), proline (3.5 g / 100 g protein) and glycine (4.26 g / 100 g protein).

IV. CONCLUSION

- The mixed farm (*Trichoderma harzianum* and *Aspergillus niger*) recorded protein ratio reached to 36.44 %.
- The highest percentage of total protein was in fermented biomass by *Micrococcus variants* which reached to (42.11%).
- The percentage of amino acids in the fungi was higher than its values in the vegetarian protein, especially the Lysine (7.12 g/ 100 g fungal protein), and Methionine (2.03 g/ 100 g fungal protein).
- The percentage of amino acids in the fermented mass was high, especially methionine (1.96 g / 100 g protein), lysine (7.01 g / 100 g protein).

The residues of marine organisms accumulated on the beach or from food industries that can pollute the environment. These residues formed valuable raw materials that formed a medium that could be built to reach a protein-rich biomass containing a wide range of amino acids in percentages that would be a real alternative for traditional and especially vegetarian proteins.

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