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Abstract : *Candida utilis* NRRL Y-900 was grown on horn hydrolysate for single-cell protein production. First, ram horns obtained from slaughterhouse in Erzurum were hydrolyzed by physical and chemical methods and crude horn hydrolysate (CHH) was obtained. The contents of protein, nitrogen, ash, some minerals, total sugars and amino acids of CHH were determined and it was seen that it has sufficient organic and inorganic materials to allow its use as a substrate source in the production of single–cell protein. The CHH was enriched by addition of yeast extract, glucose and KH_2PO_4. The effects of different CHH concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%) on the growth of *C. utilis* were investigated and 4% of CHH (Horn Broth=HB) was found to be optimal. The biomass yield of *C. utilis* and its protein content were found to be 6.8 g l^{-1} and 49.8% respectively. On the other hand, biomass contained 5.4% lipids, 5.94% RNA, 1.53% DNA and 9.7% ash. The biomass contained all of the essential amino acids and when compaired with FAO reference protein it showed a good profile. The results demonstrated that ram horns can be used as a substrate source in the production of single-cell protein.

Key Words: Single-cell protein; horn; fibrous proteins

Koç Boynuzu Hidrolizatından Tek Hücre Proteini Üretimi

Özet : Tek Hücre Proteini üretimi amacıyla, *Candida utilis* NRRL Y-900 boynuz hidrolizatı üzerinde üretildi. Önce Erzurum kombinasından elde edilen koç boynuzları fiziksel ve kimyasal yöntemlerle hidroliz edildi ve Ham Boynuz Hidrolizatı (HBH) elde edildi. HBH' nin protein, azot, kül, bazı mineral ve amino asit içerikleri belirlendi ve tek hücre proteini üretiminde bir substrat olarak kullanılmasına yetecek kadar organik ve inorganik maddelere sahip olduğu bulundu. HBH; maya ekstraktı, glukoz ve KH_2PO_4 ile zenginleştirildi. Farklı konsantrasyonlardaki (%1,2,3,4,5,6,7,8,9 ve 10) HBH' nin *C. utilis* NRRL Y-900 türünün üremesi üzerindeki etkileri incelendi ve % 4' lük HBH' nin (Boynuz Broth=BB) optimal olduğu bulundu. *C. utilis*' in biyomas verimi ve protein kapsamı sırasıyla 6,8 g/L ve % 49,8 olarak belirlendi. Biyomasın %5,4 lipit, %5,94 RNA, %1,53 DNA ve %9,7 kül içerdiği bulundu. Yine biyomasın bütün temel amino asitleri içerdiği ve FAO örnek proteiniyle karşılaştırıldığında iyi bir profil gösterdiği saptandı. Elde edilen sonuçlar, koç boynuzunun tek hücre proteini üretiminde bir substrat olarak kullanılabileceğini gösterdi.

Anahtar Sözcükler: Tek hücre proteini, boynuz, fibröz proteinler

Introduction

Single-cell proteins (SCP) refer to the dried cells of microorganisms. SCP's are used as protein sources in human foods or animal feeds. Many raw materials have been considered as carbon and energy sources for SCP production. In many cases, these raw materials have been hydrolyzed by physical, chemical and enzymatic methods before use (1-9).

Ram horns are significant waste products of the meat industry in Turkey. For example, the slaughterhouse in Erzurum directly discharges about 25 tons a year. Fibrous proteins such as horn, feather, nail and hair are also abundant waste products. These waste products can be converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms (10). Horns consist of α-keratin, which has a very high content of cysteine (up to 22%) and they also contain most of the common amino acids. Horns have the components of bone and blood tissues and are rich in some growth factors required by microorganisms (10-12).

The main aim of this study was to investigate the suitability of horn hydrolysate as a substrate in SCP production.

Materials and Methods

Materials

Candida utilis NRRL Y-900 was obtained from Dr. C.P. Kurtzman (1815 North University Street, Peoria, Illinois 616004, USA).

Horns were obtained from Erzurum Slaughterhouse in Turkey. The chemicals used in this study were analytical grade.

Methods

Hydrolysation Procedures of Horn: Horns were washed with deionized water and dried in an oven at 100ºC. The dry horns were cut into smaller pieces and ground with a grinder (Wiley mill, Arthur, U.S.A). The material obtained was termed horn flour (HF).

Thirty-five grams of horn flour was impregnated with 50 ml of HCl 6 N. The mixture was incubated at 80ºC for 24 h. At the end of this period, the mixture was incubated at 130ºC for 1 h by adding 100 ml deionized water. The solution was then cooled and pH adjusted to 7 with 10 N NaOH. It was filtered twice through Whatman No. 1 filter paper. The volume was completed to 400 ml with deionized water. The final clear filtrate was termed crude horn hydrolysate (CHH) and stored at 4ºC. The CHH was diluted to reduce the concentration of growth inhibitors. The various concentrations (1-9 and 10%) of the CHH was enriched by adding 0.1% yeast extract (Difco, USA), 1% glucose (Oxoid, England) and 0.1% KH_2PO_4. 3 H_2O (Difco, USA). These diluted solutions were termed horn broth (HB). The pHs of the media were adjusted to 5 with 1 N HCl and they were sterilized and used.

The control medium had the following composition in g l^{-1}: 1.0 yeast extract, 10 glucose,; 1.0 KH_2PO_4.

Inoculum: *C. utilis* was grown in the Trypticase Soy Broth (Oxoid, England) and harvested by centrifugation at 5000 rpm for 15 min. The pellet was washed twice, suspensed in 100 ml distilled water and used as inoculum for HB. An inoculum ratio of 10% (v/v) was used in all the processes.

Fermentation process: 10 ml of inoculum was transferred into 250 ml Erlenmayer flasks containing 100 ml of sterile HB medium and shaken on a rotary shaker (Rosi-1000 thermolyne, Turkey) at 160 rpm for 24, 48 and 72 hours at 30ºC.

At the end of the cultivation periods, the yeasts were harvested by centrifugation at 5000 rpm for 15 min. The pellet was washed twice with two volumes of distilled water and lyophylized.

Analysis of CHH and Biomass: Amino acid analysis of CHH and biomass were carried out after hydrolysis with 6N HCl at 110ºC for 24 h in a Biotronic LC-5001 Amino Acid Analyser (Germany). Total sugar content, dry matter and ash analysis were estimated by AOAC methods (13). Total nitrogen was estimated by the micro-Kjeldahl method. Total lipids were estimated by Folch's methods (14). The elemental composition was measured by atomic absorption spectrophotometer (UV HS-360, Germany). RNA and DNA levels were measured as described by Stewart (15).

Results and Discussion

The main chemical composition of CHH is shown in Table 1. These data show CHH to be rich in both organic and inorganic materials. In particular, it contains the substances required in microbial media such as sources of carbon, nitrogen and minerals. On the other hand, CHH is rich in amino acid. The essential amino acids are present and among them arginine (4.66 mg ml-1) is the highest. However, of all the amino acids considered, glutamic acid (8.17 mg ml-1) was the most abundant. The absence of tryptophan and proline was probably due to their degradation during the acid hydrolysis of proteins, because the hydrolysis did not allow the determination of the secondary amino acids such as proline (16).

The chemical composition of CHH is in accordance with the findings obtained from the investigation on the elemental and amino acid composition of various fibrous proteins such as nail (17,18) fish epidermis (11) and bovine hoofs (19). On the other hand, it was reported that the elemental composition of fibrous proteins is influenced by age, sex, environmental events, geographical location and subsequent metabolites (11,17-22).

Components g ml^{-100}		Amino acids mg ml^{-1}	
Nitrogen	0.881	Aspartic acid	3.90
Protein	5.500	Threonine	2.00
Dry matter	8.800	Serine	2.87
Ash	1.98	Glutamic acid	8.17
Total sugar	0.500	Glycine	5.19
Total lipids	0.300	Alanine	3.19
Mg	0.160	Cysteine	0.21
Ca	0.164	Valine	2.56
Cu	0.017	Methionine	0.41
Mn	0.036	Isoleucine	1.63
Zn	0.064	Leucine	4.02
Fe	0.123	Tyrosine	1.61
K	0.113	Phenylalanine	1.67
		Histidine	0.72
		Lysine	2.21
		Arginine	4.66
		Proline	0.0

Table 1. The main chemical composition of CHH.

First, we investigated the effect of CHH in various concentrations (1-9 and 10%) on the biomass yield. We found that the most suitable concentration for growth was 4% and the biomass yield for this application was 6.8 g l^{-1} (Figure). It was found that applications higher than 4% had an inhibitory effect. For example, the lowest biomass yield (1.8 g l^{-1}) was obtained from the application of 10% CHH (Figure). The inhibitory effect may be due to the high BOD load of CHH and presence of cell wall cations and some toxic materials in it. Similar effects have been observed from effluents with high loads of organic and inorganic materials (23). Therefore, we continued the research with 4% CHH (=HB).

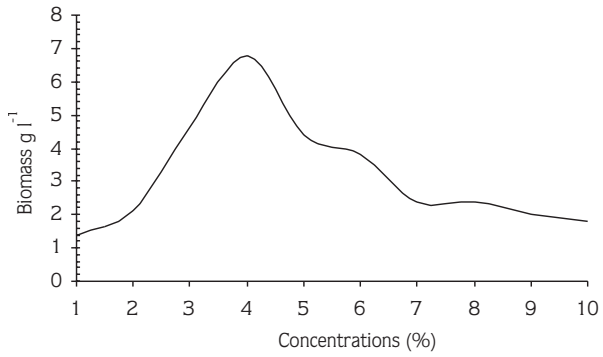

Figure. Effect of different CHH concentrations on the growth of *C. utilis* at 72 h. Fermentation conditions: Temperature: 30°C; Agitation: 160 rpm; pH: 5.

C. utilis could not grow in control medium. This result indicates that CHH contains some growth factors required by microorganisms.

Table 2 illustrates the biomass yields of yeast when grown for different periods of time in HB medium. The maximum biomass yield was obtained after 72 h.

The chemical composition of the biomass is given in Table 3. The RNA and DNA content of the biomass are 5.94% and 1.53% respectively. The RNA amount was approximately four times higher than the DNA amount and these findings are in accordance with the results of Nigam (24). On the other hand, the high RNA contents are reported to be toxic for human consumption (25,26), while harmless for most animals (27,28).

The detailed amino acid composition of *C. utilis* and FAO reference protein (5) are given in Table 4. The biomass obtained from the yeast contained all the essential amino acids. Essential

Table 2. The biomass yields from C. utilis for different periods of time.

Periods of incubation (h)	Biomass (g l^{-1})
24	3.4
48	5.6
72	6.8

Table 3. The chemical composition of SCP from *C. utilis* on a dry weight basis.

Components	g g^{-100}
Total protein	49.8
Total lipids	5.4
Ash	9.7
RNA	5.94
DNA	1.53

Table 4. Amino acid composition of SCP from *C. utilis*.

Amino acids	mg g^{-1}	FAO (mg g^{-1})
Aspartic acid	66.5	-
Threonine	34	40
Serine	36	-
Glutamic acid	90.5	-
Glycine	28	-
Alanine	46	-
Cysteine	24	20
Valine	40.5	42
Methionine	15.5	22
Isoleucine	32	42
Leucine	44	48
Tyrosine	26	-
Phenylalanine	30	28
Histidine	16	-
Lysine	76	42
Arginine	38	-
Proline	24	-

amino acid concentrations were somewhat lower than the FAO reference protein. Among the amino acids, glutamic acid was the most abundant. On the other hand, the biomass is quite rich in lysine (76 mg g^{-1}). It was reported that the potential nutritional value of SCP is determined with amounts of lysine and methionine amino acids (29,30). Therefore, the biomass obtained may be suitable for human and animal consumption.

Consequently, both the biomass yield of the microorganism (Table 2) and the protein ratio of the biomass (Table 3) were found to be similar to the results of investigations in which some other yeasts such as *Candida pseudotropicalis* CBS 607 T (29), *C. utilis*, *C. krusei* and *C. tropicalis* (31) were grown on sweet whey and vinasse medium and *C. utilis* (24) was grown on pineapple cannery effluent in batch culture, and we can suggest the growing of yeasts in continuous and semicontinuous processes on HB in order to obtain higher yields.

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