Abu Dhabi Food Control Authority
Development Sector
Research & Development Division

Technical Report تقرير فني

Title العنوان	**Production of Single Cell Protein from Date Waste**				
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Duration فترة المشروع	**From:**	Jan. 2012	**To:**	Dec. 2013	

Background خلفية عن الموضوع

Single cell protein (SCP) is dried cell of microorganisms, which can be used as a protein supplement in human foods or animal feeds. The SCP is cheap and competes well with other source of protein and may provide good nutritive value. Besides its high protein content (60-82%) SCP contains fat, carbohydrates, vitamins and minerals (Asad et al, 2000; Jamel et al, 2008). SCP is also rich in essential amino acids such as lysine and methionine, which are limiting in most plant and animal foods (Mondal et al, 2012). Date syrup production result in unused by-products such as date fiber and seeds. For instance Al Foah Company, Al Ain, UAE produces about 1000 tons of unused by-products from syrup production annually. Al Farsi et al (2007) reported the composition of date fiber (by-product of date syrup) for three varieties of dates, their protein ranged between 3.6-5.2%, fat between 1.4-2.2% and carbohydrates between 81.9-83.3%. The use of such waste as a sole carbon and nitrogen source for the production of SCP by microorganisms could be simply attributed to their presence in nature on large scale and their cheap cost. Also utilization of such waste prevents pollution problems and sanitary hazards as well as creating another source of income to this sector.

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).

In this work five different fungal strains were evaluated for SCP production from date syrup waste and the growth medium condition of the selected strain was optimized for maximum production.

Problems التحديات

High waste produced during production of date products such as date syrup and dates paste which creates pollution problems.

Objectives الأهداف

- Production of single cell protein using date waste (date syrup by-product) as basic medium.
- Optimize single cell protein production parameters.
- Utilize date waste produced during syrup production.

Methods طريقة العمل

1. Date fiber

The dates fiber used in this study was procured from Al Foah Company, Al Ain, UAE. This is a by-product of date syrup production packed in polyethylene bags and stored at -30°C until used.

2. Fungal Strains

Five fungal strains were used, *Trichoderma reesei* (ATCC 13631), *Fusarium venenatum* (ATCC 20334), *Thermomyces lanuginosus* (ATCC 34626), *Aspergillus oryzae* (ATCC 14895) and *Fusarium graminearum* (ATCC 20333). All cultures were processed according to the specific directions of ATCC.

3. Culture Media

Two types of media were used for maintenance and routine subculture including:

a. Standard ATCC media for freeze dried culture as follows:
   - Potato dextrose agar (PDA) for *Trichoderma reesei and Fusarium graminearum.*
   - malt agar medium (ATCC 323) for *Fusarium venenatum*
   - malt extract agar medium (ATCC 325) for *Aspergillus oryzae*
   - peptone yeast extract glucose agar (PYG, ATCC 663) medium for *Thermomyces lanuginosus*

b. Date fiber agar (DFA) used as production medium for fungal strains.

4. Strain Selection

The five fungal strains were grown on date fibers growth media hydrated at 1:1 (w:v). The biomass and growth medium were dried at 70 ^{0}C until constant weight, ground to fine powder and analyzed for the protein content. The cultures were stored for long periods at - 36 ^{0}C.

5. Determination of Optimal Production Conditions

- Optimal nitrogen source.
- Optimal concentration of nitrogen source. The selected nitrogen source was added to the growth medium at 0, 0.2, 0.4, 0.6, 0.8 and 1.0%.
- Optimal medium weight to flask volume. The production medium which was used for strain selection was dispensed in 250 ml flasks at 45, 60, 75, and 90 gm/flask prior to sterilization and inoculation with the selected strain spore suspension, incubated and processed as described above.
- Optimal Initial pH. The pH of the date fiber growth medium was adjusted by 1N HCl at 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 prior to sterilization.

6. Protein Determination

Protein content of date fiber and SCP were determined by analyzing the powdered samples for nitrogen content in CHNSO analyzer (HEKAtech GmbH, Wegberg, Germany) and the obtained values were converted to protein by the factor 6.25.

7. Determination of amino acids

Amino acid content of dates fiber and SCP were determined according to the Official European Union regulation (EU, 2009).

Results النتائج

1. Strain selection

Figure 1 shows the protein content produced by these fungal strains. It clearly shows the vigorous growth of A. oryzae compared to other fungi, also the protein content produced by A. oryzae was significantly higher

than others. Similar result was found by Zadrazil et al. (1995) who used the white rot fungi on the sugarcane bagasse as substrate. Based on the obtained results (high protein, vigorous growth and heavy sporulation) A. oryzae was selected as the optimum strain for date fiber and used for optimal production condition.

2. Nitrogen source

In order to select the optimum nitrogen source for maximum microbial growth, three substances (ammonium chloride, ammonium sulfate and urea) were used as nitrogen source. Figure 2 presents the effect of each nitrogen source of protein content produced by A. oryzae after 5 days growth. It was observed that ammonium sulfate was the best source among these nitrogen sources. The possible reason may be that ammonium sulfate has some additional growth factors such as certain amino acids, minerals, and vitamins, which gave better growth results compared to other nitrogen sources (Shahzad and Rajoka, 2011). This source is rather inexpensive and easy to mix with the media.

3. Concentration of nitrogen source

The selected ammonium sulfate was used in different concentrations to determine the optimum concentration for A. oryzae growth on date fiber medium. Figure 3 shows the effect of different concentrations of ammonium sulfate on protein content produced by A. oryzae. Protein content increased with the increase of ammonium sulphate to reach to maximum content when using 1% ammonium sulfate. However, the difference between 0.8 and 1.0% concentration was insignificant.

4. Medium weight / flask volume ratios

Figure 5 presents the effect of various medium weight/flask volume ratio on protein content produced by A. oryzae. Protein content obtained from different substrate levels revealed that 75gm in 250 ml flask was the best ratio for growth. Therefore, the ratio of 75 gm/250 ml was selected as the optimal ratio. This factor is important in estimating the required media quantities according to the fermentation vessel volume.

5. Medium pH

The initial pH of the medium was sensitive to the growth of A. oryzae in date fiber medium. Different initial pH values were used to check the optimum pH value for maximum yield of the biomass. The results of present study showed (Figure 5) that biomass yield increased from pH 4 and optimum production was observed at 5.5 yielding 16.25 % of crude protein. This pH (5.5) is close to the pH of natural date fibers. Further increase in initial medium pH, a decline in protein production was observed.

6. Amino acid content

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 amino acid profile before the final product is used as a food or feed supplement. Table 1 presents the content of amino acids in date fiber and SCP of date fiber. The total content of amino acids in dates fiber was 6.02 % and 10.14 % in SCP. Glutamic acid, aspartic and proline were the major amino acids in SCP were compared. Also the essential (*) and nonessential amino acids in SCP. The results indicated that the ratio of essential amino acids to total amino acids was 46%, which indicates it is a good source of nutrition.

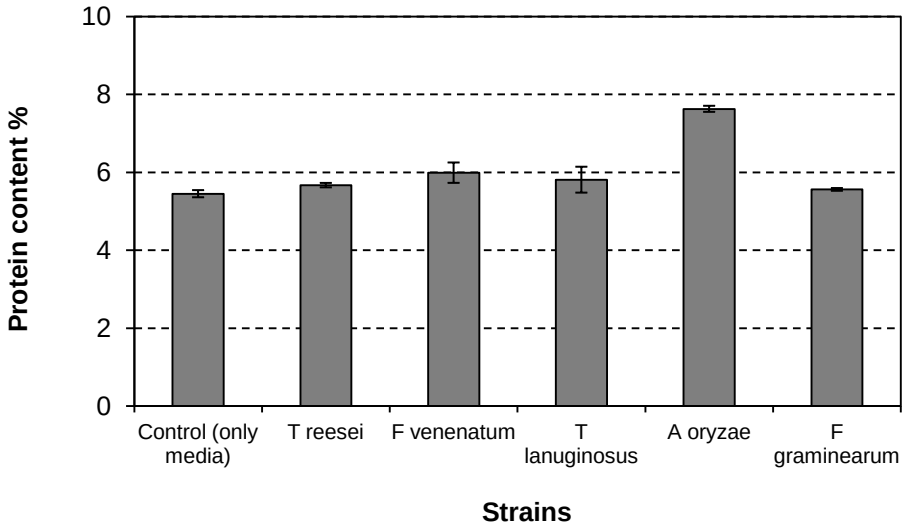

Figure 1: Protein content (%) produced by different fungal strains

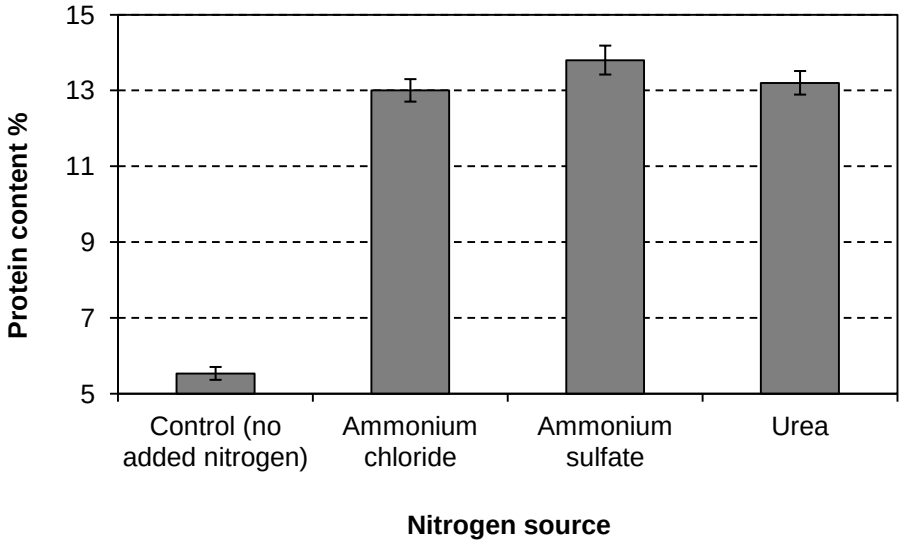

Figure 2. Effect of nitrogen source on protein content in date fiber medium after growing *Aspergillus oryzae*

Table 1. Amino acid content (%) of date fiber and single cell protein (SCP) from date fiber

Amino acids %	Date fiber	SCP
Lysine *	0.28	0.48
Methionine *	0.14	0.22
Cystine	0.11	0.19
Aspartic acid	0.64	1.01
Threonine *	0.31	0.52
Serine	0.35	0.58
Glutamic acid	0.74	1.11
Proline	0.30	0.88
Glycine	0.42	0.63
Alanine	0.43	0.68
Valine *	0.37	0.57
Isoleucine *	0.27	0.44
Leucine *	0.53	0.85
Tyrosine	0.22	0.22
Phenylalanine *	0.30	0.72
Histidine *	0.16	0.28
Arginine *	0.32	0.57
Tryptophan	0.13	0.19
Total	6.02	10.14

* The essential amino acids

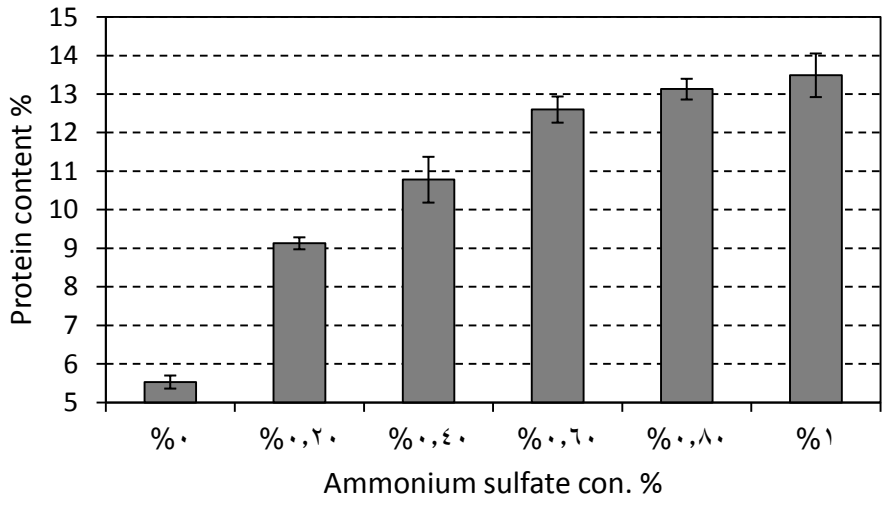

Figure 3. Effect of ammonium sulfate concentrations on protein content in date fiber medium after growing *Aspergillus oryzae*

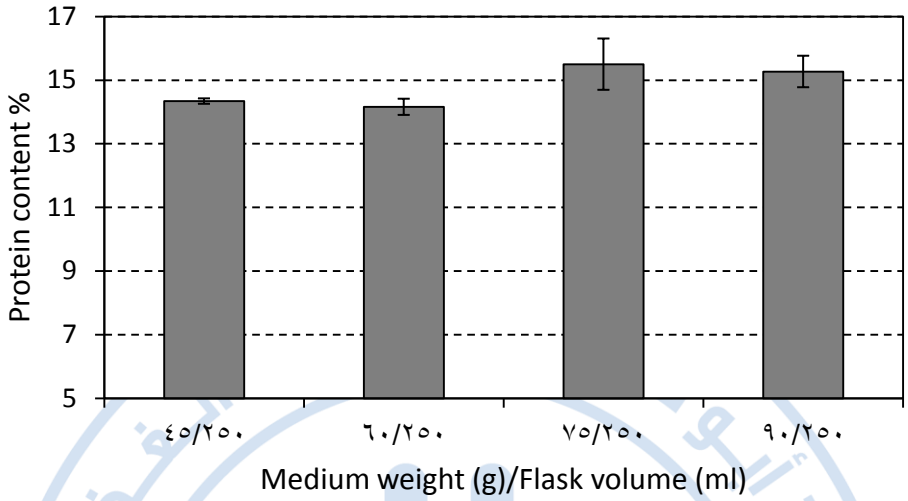

Figure 4. Effect of various medium weight / flask volume ratios on protein content in date fiber medium after growing *Aspergillus oryzae*

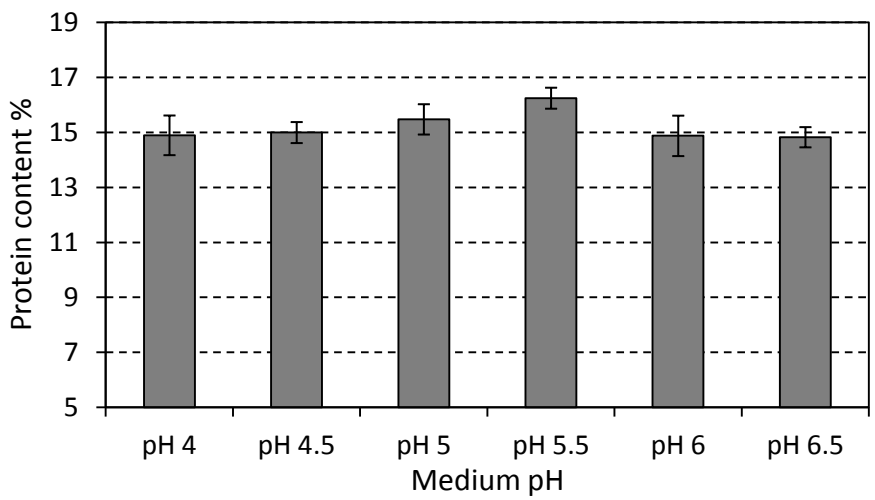

Figure 5. Effect of medium pH on protein content in date fiber medium after growing *Aspergillus oryzae*

Recommendations التوصيات

A. oryzea was selected as the optimum strain to produce SCP from date fiber. The optimum date fiber condition for SCP production was 0.8% ammonium sulfate as a nitrogen source at a ratio of 75 g media/250 ml flask and at pH 5.5. According to the successful results obtained from this study, we recommend conducting this study on a larger scale using a solid state fermenter.

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