6 *Egypt. J. Bot.*, **Vol**. **56,** No.1 pp. 81-96 (2016)

Alkali Pretreated Rice Straw as an Inexpensive Substrate for Single-Cell Protein Production by *Saccharomyces cerevisiae*

Sh.M. Husseiny[1*], M.S. El-Gamal[2], A.I.S. Ahmed[3], A.M. Abd El-rhman[2] and H.M.H. Khashaba[3]

[1]*Botany department, Faculty of Women for Art, Science & Education, Ain Shams University,* [2]*Botany and Microbiology Department, Faculty of Science, Al-Azhar University and* [3]*Special Food and Nutrition Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.*

AN INVESTIGATION concerning the bioconversion of an alkali-treated rice straw to single cell protein under fermentation conditions by *Saccharomyces cerevisiae* was done. The optimal environmental and nutritional conditions, which resulted in the highest protein production by *S. cerevisiae* can be summarized as following; incubation temperature, 30°C; incubation periods, 48 h; pH of 4.5; inoculum size 1 ml/100ml from a heavy spore suspension of $95x10^7$ Colony Forming Unit (CFU) /ml, 2 g % rice straw concentrations; $NH_4H_2PO_4$ as best nitrogen source; carbon source 2.5 g % of lactose. The highest yields of single cell protein were obtained under static condition. High contents of nutritional compounds such as protein, mineral, carbohydrate, lipid, vitamins and amino acids were determined in the produced yeast biomass. These results show that *Saccharomyces cerevisiae* could be suitable for single cell protein production from cheap waste products.

Keywords: Single Cell Protein, Rice straw, *Saccharomyces cerevisiae*

Technically, single cell protein (SCP) is the manufacture of cell mass using microorganisms by culturing on abundantly available wastes. Algae, fungi and bacteria are the chief sources of the microbial protein that can be utilized as SCP (Anupama and Ravindra, 2000). It refers to the dried cells of microorganisms such as yeast, bacteria, fungi and algae which grow in large-scale culture systems for use as protein sources in human food or animal feed (Prado-Rubio *et al*., 2010 and Zepka *et al*., 2010). Not only proteins but also free amino acids, lipids, carbohydrates, vitamins and minerals are often included in the single cell protein term (Zheng *et al*., 2005; Rajoka *et al*., 2006; Gao *et al*., 2007; Zhang *et al*., 2008 and Rasoul-Amini *et al*., 2009). In particular protein supply possesses a problem since essential amino acids can not be replaced. One possible solution to this problem is SCP production. Much interest has been focused on the potential of converting agriculture, industrial, municipal or forestry wastes to microbial protein.

[*]Corresponding author: Husseinymoussa@women.asu.edu.eg,Mobile :0020201222867353

The production of the microbial biomass is achieved either by a submerged or solid state fermentation (SSF) process. After fermentation, biomass harvested and may be used as a protein source or subjected to processing steps like washing, cell disruption, protein extraction and purification. In general, the high production rates and protein yields as well as ease of production control make SCP more attractive as a protein source compared with conventional plant and animal sources (Xiao *et al*., 2009).

Microorganisms have high protein content and short growth times, leading to rapid biomass production, which can be continuous and independent from the environmental conditions. The use of fungi, especially yeasts, for SCP production is more convenient, as they can be easily propagated using cheap raw materials and easily harvested due to their bigger cell sizes and flocculation abilities. Moreover, they contain lower amounts of nucleic acids than bacteria (Ravindra, 2000). The main categories of their use at present were as source of single-cell protein for application in waste treatment, as feedstock for biofuel production and as a source of biochemical components of industrial interest Zepka *et al*. (2010).

Many raw materials have been studied as substrates for the production of SCP. In many cases, these raw materials have been hydrolyzed by physical, chemical and enzymatic methods before use (Becker, 2007 and Gao *et al*., 2007) . Increasing concern about pollution that occurs from agricultural and industrial wastes has stimulated interest in converting waste materials into commercially valuable products, especially SCP (Ugwuanyi, 2008; Prado-Rubio *et al*., 2010 and Silva *et al*., 2011).

Rice straw is a by-product of rice production, and a great bio-resource as raw biomass material for manufacturing value-adding protein for animal feedstock, which has been paid more and more attention (Zheng *et al*., 2005).

In our research, studies had been aimed primarily to the use of agro-industrial wastes for the production of (SCP) with resultant protection of environment. Rice straw was chosen as fermentation substrate for this study, firstly, it is considered as a typical representative of agro-industrial wastes, which is an essential and readily available commodity in the developing countries. Secondly, they are universal and commonly used agricultural crops with essential elements for pollutions and are readily available in Egypt. Thus, degradation of these wastes requires the selection of microorganisms which are able to assimilate them. The cultivation of microbial biomass as single cell protein (SCP) has been studied.

Materails and Methods

Yeast Species

Saccharomyces cerevisiae was obtained from Botany and Microbiology Department, Faculty of science, Al-Azhar University, Cairo, Egypt.

Agricultural Wastes

Rice straw was collected from Minet El-Gamh city in El-Sharkia Governorate, Egypt.

Alkali- Pretreatment

Rice straw was firstly air dried put in oven at 60°C for 24 hr and grind in electrical blender for approximately 5 min. Dried grinded rice straw wastes were treated by sodium hydroxide according to the method of Zhu *et al.* (2005). They were autoclaved at 121^{o}C for 30 min. The pretreated rice straw was allowed to cool and subsequently filtered by cloth-sheet and washed with tap water to neutral pH. It was then dried at 60^{o}C in an oven for 12 hr, and then used at 1 % (w/v) in basal medium for single cell protein production.

Cultivation Medium

Yeast Malt Glucose Peptone Agar (YMGPA) was used to cultivate and preserve *Saccharomyces cerevisiae*

Single Cell Protein Production Medium

Czapek-Dox's was prepared using a basal medium, consisted of (g/l): sodium nitrate, 3.0; potassium chloride, 0.5; magnesium sulfate, 0.5 and potassium di-hydrogen phosphate, 1.0. The pH value adjusted to 4.5-5 by citrate-phosphate buffer. The medium was dispensed in 100ml portion 250 ml Erlenmeyer flasks and supplemented with 1 g of alkali-treated rice straw.

All flasks were sterilized at 121^{o}C for 15 min. Each flask was inoculated with 1 ml of *S. cerevisiae* suspension containing (95x10^{7}) CFU/ml. They incubated at 30^{o}C for 24 hr. At the end of the incubation period, yeast cells were collected by centrifugation at 4000 rpm at 4°C for 10 min. and washed twice with distilled water. The obtained SCP was then dried at 70°C until the dry weight was constant for analysis.

Protein Determination

Extraction of Protein from Yeast Cells with Sodium Hydroxide

The extraction was achieved according to the method of Herebert *et al*. (1971) in which Twenty milliliters of 1N NaOH was added to 5g yeast biomass in 15 ml capacity glass centrifuge tubes, and left for 10 min. in boiling water bath. After alkali extraction, cooled in cold water, centrifuged for 10 min. at 4500 rpm and the supernatant subjected to protein determination. Protein content was determined by the method of (Lowry *et al*., 1951).

Some Parameters Regulating Saccharomyces cerevisiae SCP Production

The following parameters were examined for their effects on the production of *S. cerevisiae* SCP using the alkali-treated rice straw (RS1% NaOH). In each experiment the optimal conditions deduced from the previous experiments were considered. At the end of incubation period, the protein content was determined as mentioned before.

Incubation Temperature

Incubation temperatures were examined on SCP production medium by *S. cerevisiae* at different temperatures viz: 20, 25, 30, 35, 40, and 45°C for 24 hr.

Incubation Period

The influence of different incubation period on SCP production medium by *S. cerevisiae* at 30°C were applied at incubation for 0, 12, 24, 36, 48, 72 and 96 h.

Initial pH Value

The effect of different initial pH values were studied on SCP production medium by *S. cerevisiae* growing on (RS1% NaOH) at 30ºC for 48. Using citrate-phosphate buffer and phosphate buffer the pH were 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8.

Inoculum Size: Different inoculum size of heavy cell suspensions 95 x 10^7CFU/ml of the *S. cerevisiae* ranged between 0.0, 0.1, 0.3, 0.5, 1, 2, 4, 6, 8 and 10 ml were allowed to grow on RS1% NaOH at 30ºC for 48h and pH 4.5 .

Substrate Concentration: Different concentrations of RS1%NaOH (w/v) was applied at concentrations 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 g/100 ml on production medium.

Nitrogenous Source: Different nitrogen sources, peptone, yeast extract, beef extract, ammonium di-hydrogen phosphate, urea, potassium nitrate and ammonium sulphate were added to medium in equivalent to sodium nitrogen content that located into 0.3 % (w/v) of sodium nitrate except peptone, beef & yeast extract were added at 0.1% (w/v). Control was also prepared which contain 0.3% (w/v) $NaNO_3$.

Carbon Sources: Different carbon sources were added to the production medium. Control was performed without carbon source. The tested carbon sources were D-glucose, D-dextrose, D-fructose, sucrose, maltose and lactose. The carbon sources were added at an equimolecular level in 2% sucrose. Starch was added at 1% (g/v) medium.

Agitation: It was carried out by incubating the flasks containing the production media on the shaker at 150 rpm at 30°C for 48 hr. Other flasks for each one were incubated under static condition at 30°C for 48 hr.

Extraction and Nutritive Value of Saccharomyces cerevisiae SCP

S. cerevisiae was grown under optimum conditions. The cells were harvested by centrifugation at 4000 rpm at 4°C for 10 min., washed twice with distilled water and dried at 70°C until constant weight. The dried yeast cells were preserved in dry place as a bulk of cells for use, as well as, for determination of nutritional content of yeast biomass (SCP). Its nutritive value was carried out by determination of moisture content, dry weight, ash, mineral content, protein content, carbohydrate content, lipid content, vitamins and amino acids content.

Moisture Content and Biomass

Determination of moisture, constant weight and dry biomass was carried out according to Geary (1956)**.**

Ash and Mineral Content

Total ash determination was done according to Reith *et al*. (1948). However, the mineral content was estimated according to A.S.T.M. (2002) using Inductively Coupled Argon Plasma, ICAP 6500 Due, Thermo Scientific, England. 1000 µg/l multi-element certified standard solution, Merck, Germany was used as stock solution for instrument standardization.

Protein Content

It was determined by micro-Kjeldahl method as described by Haphries (1956) for total nitrogen determined. The total nitrogen values are multiplied by the factors (6.25) to obtain the crude protein content.

Carbohydrate Content: Extraction and determination of total soluble carbohydrates were carried out according to Umbriet *et al*., (1969).

Lipids Content: Total lipids were determined according to the A.O.A.C. (1975) using Soxhlet lipid extractor apparatus.

Vitamins Content: Vitamin C content was determined according to the A.O.A.C. (1990). Vitamin B was determined using high performance liquid chromatography system (HPLC) according to Batifoulier *et al*. (2005).

Amino Acids Content: The amino acids content was determined by amino acid analyzer {Eppendrof –LC3000} according to Block *et al*. (1958).

Results and Discussion

In the present study, the production of SCP by S. cerevisiae was performed at different incubation temperature (ranged from 30-45ºC) for 24hr. The data presented in Fig.1 showed that, the maximum SCP productivities by *S. cerevisiae* (0.3857 mg/ml) was obtained at 30ºC incubation temperature in presence of RS1%NaOH. Above or below this temperature, the protein yield was decreased gradually. These results are in agreement with the results of several workers (Paraskevopoulos *et al*., 2003 and Zhang *et al.,* 2008) who found that the maximum protein production by yeast and other organisms were obtained at 30ºC. On the other hand, the results are partially contradictory with that obtained by Anupama & Ravindra (2001) and Gao *et al*. (2007) who reported that the optimum temperature for SCP production by *Aspergillus niger* was 28ºC. Zheng *et al*. (2005) found that the optimum temperature for SCP by *Candida arborea* was 29ºC. Xiao *et al*. (2009) also recorded optimum temperature ranged from 28ºC to 32ºC for mixed fermentation by *A. niger* and *C. utilis*.

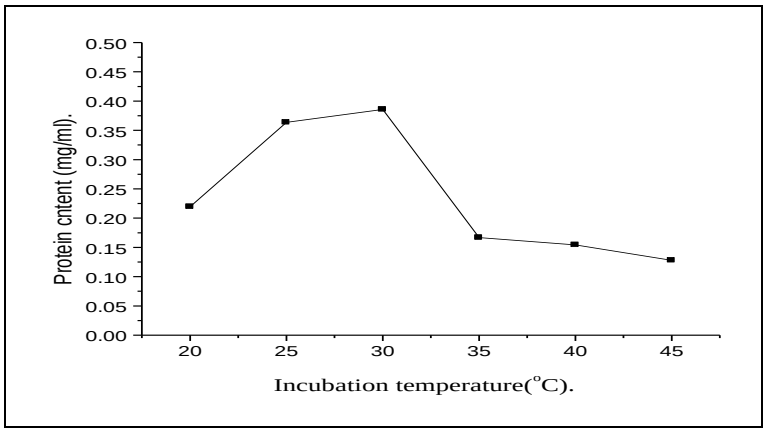

Fig. 1. Effect of different incubation temperatures on SCP productivity by *S. cerevisiae*.

Incubation Period

The results presented in Fig. 2 showed that, maximum yield of SCP was achieved after 48 hr. This result is more related with those obtained by Ghaly *et al.* (2005) who recorded protein production by yeast fermentation on acid cheese whey after 48hr. Also, Zhang *et al*. (2008) reported the same results, as maximum biomass protein production was after 48 hr by *Aspergillus oryzae*. However, Rajoka *et al*. (2006) recorded optimum fermentation period of 3 days by *Candida utilis* for protein production.

In contrast to our results, Zhang *et al*. (2008) recorded that, *Trichoderma viride* grown on winery wastewater exhibited its ability to produce biomass protein within 24 hr. Gao *et al*. (2007) reported that, optimum incubation time was 56 hr by *Cryptococcus aureus* G7a for SCP production. Silva *et al*. (2011) reported that, the optimal production for biomass production by yeast was obtained after 192 hr.

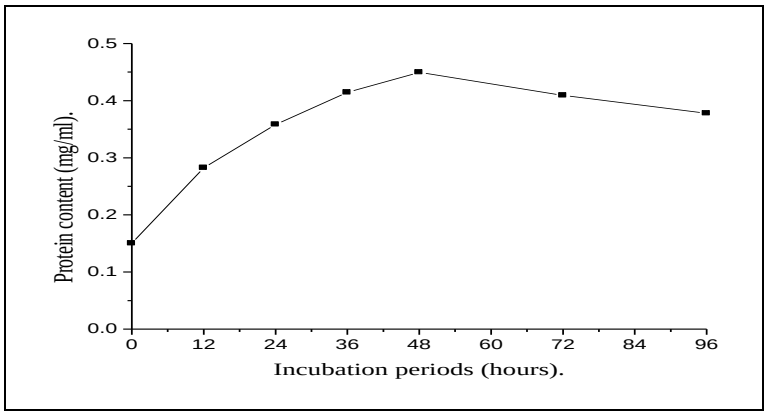

Fig. 2. Relation between different incubation periods and SCP produced by *S. cerevisiae*.

Initial pH values

Different pH values (3-8) were also tested for its effect on SCP production. It could be concluded from the results illustrated in Fig. 3 that the pH value was 4.5 was the optimum for the highest yields by *S. cerevisiae* grown on RS1%NaOH. This result is in agreement with Zhang *et al.* (2008) who reported that the maximum fungal biomass protein production was at pH 4.5 and 5.5. On the other hand, Rajoka *et al.* (2006) showed that the optimum pH for SCP from yeast and other organism was 6.

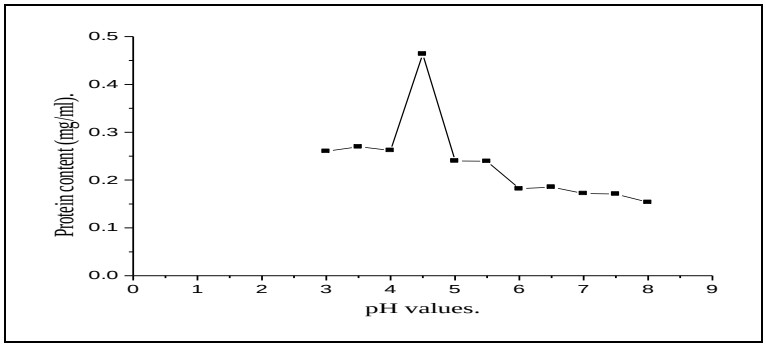

Fig. 3. Effect of different initial pH values on SCP yield by *S. cerevisiae* grown on RS1%NaOH at 30ºC.

Inoculum size

The results demonstrated that, the maximum yield of SCP was at an inoculum size of *S. cerevisiae* of 1ml/100ml production media (Fig. 4). While, Zhang *et al.* (2008) found that, the maximum fungal biomass protein production was at 2.5 % ($.5x10^8$). Zheng *et al.* (2005) reported the highest yield of biomass from *Candida arborea* at 5 % inoculum size.

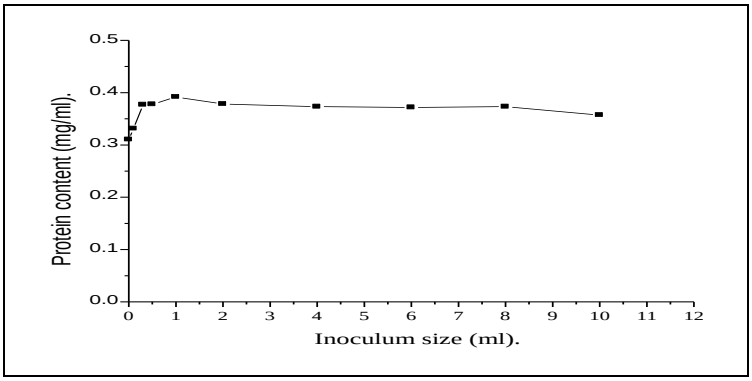

Fig. 4. Relation of inoculum size (ml) to SCP yield by *S. cerevisiae* grown on RS1%NaOH at 30ºC.

Substrate Concentration

The production of SCP reached the maximum yield in presence of 2 g of RS1%NaOH as substrate in the production media (Fig. 5). Rajoka *et al*. (2006) used 9 g % from rice polishing for SCP by *Candida utilis* and Gao *et al*. (2007) used 6 g % Jerusalem artichoke for maximum production of SCP from *Cryptococcus aureus* G7a.

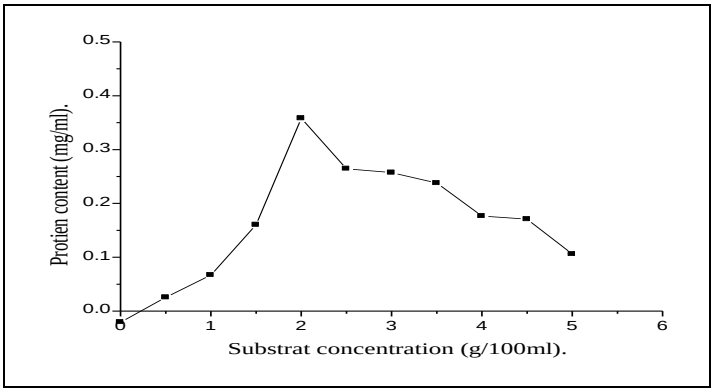

Fig. 5. Relation of substrate concentrations to SCP yields produced by *S. cerevisiae* grown on RS1%NaOH at 30ºC.

Nitrogen Sources

It is apparent from the results that the addition of nitrogen sources efficiently affects the SCP productivities by *S. cerevisiae* (Fig. 6). Addition of ammonium di-hydrogen phosphate to the production medium resulted in the highest amount of SCP. Zhang *et al*. (2008) reported that using $(NH_4)_2SO_4$ as nitrogen source give the highest yield of SCP by *A. oryzae* and *A. niger*.

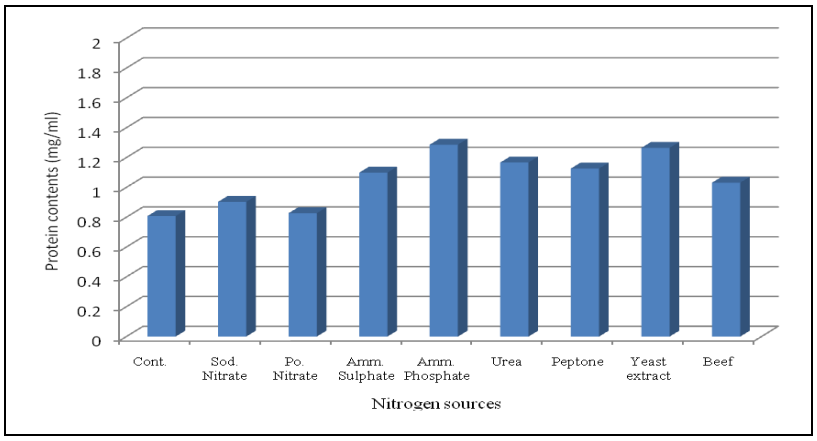

Fig. 6. Effect of different nitrogen sources on SCP productivity by *S. cerevisiae* grown on RS1%NaOH at 30ºC.

Carbon Sources

Data emphasized that the maximum yield of SCP was achieved by the addition of lactose to the production medium (Fig. 7).Camacho-Ruiz *et al.* (2003) recorded that initial sugar concentration controlling SCP production by S. *cerevisiae*. Omar (2006) showed that ribose was the best carbon sources for give the highest yield of SCP by *S. cerevisia*e. Moreover, maximum yields of biomass, its protein content and total protein were produced by *S. cerevisiae* in the presence of 1% (w/w) glucose (9.46 g/kg *orange peels*; 40.89%, w/w; 50.89%, (w/w) Hossam (2013).

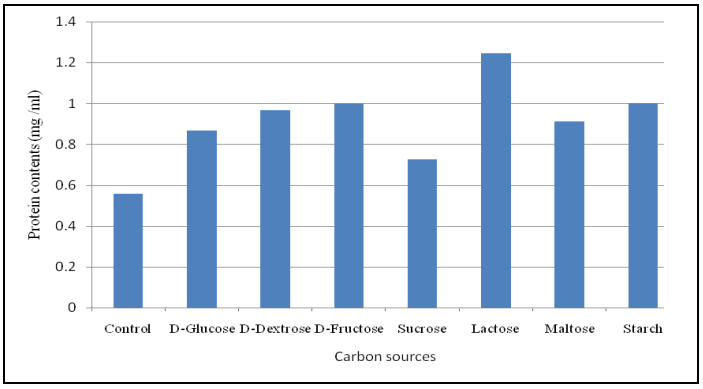

Fig. 7. Effect of carbon sources on SCP productivity by *S. cerevisiae* grown on RS1%NaOH at 30ºC.

Agitation

Results indicated that, the static condition is more favorable for protein production by yeast isolate (Fig. 8). On the contrary, Zhang *et al*. (2008) produced SCP in shaked conditions.

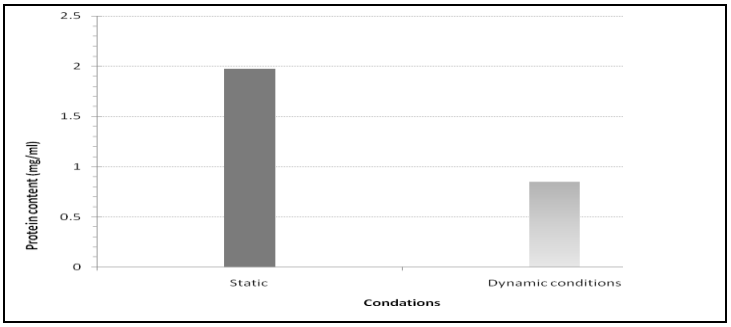

Fig. 8. Effect of agitation on SCP productivity by *S. cerevisiae* grown on RS1%NaOH at 30ºC.

At the end of parameters optimization, *S. cerevisiae* was grown under optimum conditions and by the end of incubation period, cells were harvested and weighted as mentioned before. The biomass was found to be 35 g/l

Analytical Characterization of Yeast Biomass Single Cell Protein

The composition of single cell protein obtained from the fermentation of RS1%NaOH by *S. cerevisiae* is shown in Tables 2, 3, 4 & 5. From Table 2 the moisture content of the dry cells was about 20.64 % (w/w) which higher than that reported by Zepka *et al*. (2010) who found that the dry matter of SCP by *Haloarcula* sp was 79.36%, (w/w).

This in partial agreement with Shojaosadati *et al*. (1999) who obtained dry matter 97.3 % from yeast biomass. Concerning carbohydrates content, it was found to be 30 % (w/w), which is near the value (24 % carbohydrates content) obtained by Shojaosadati *et al*. (1999) SCP by *Hansenula sp*. In view of finding of other workers, Zepka *et al*. (2010) recoded that the carbohydrate content of SCP was 64.16 %. In this work, the lipids content showed high value 5.1 % (w/w). The result was closely with the result (5.4 %) for lipid content recorded by Kurbanoulu (2001). On other hand Parajo *et al*. (1995) reported 9 % of crude lipid in SCP of yeast. With respect to crude protein produced in our study, which found to be 28%. Significant increases in protein content by yeast have been found by Ziino *et al*. (1999). Rajoka (2005) also recorded 60 % crude protein by *Candida utilis*. Gao *et al*. (2007) showed that, the content of protein in marine yeast reached 53%.

TABLE 2. Composition of yeast biomass SCP production

Component	Yeast biomass SCP
Moisture content %	20.64
Dry weight %	79.36
Ash content %	84
Protein content (mg/ml)	1.98
Crude protein (g/100g)	28
Carbohydrates content (mg/ml)	0.26
Lipids content (g/100gm)	5.05

Table 3 illustrates single cell protein composition of elements. It was detected the SCP obtained is a good source for a number of elements but cadmium was not found. These results were higher than the values which previously reported for similar products by Paraskevopoulou *et al*. (2003).

The importance of vitamins content for the quality of SCP indicated from that, vitamin C is essential for normal growth and for many physiological functions of majority of marine fishes (Chi *et al*., 2006). Therefore, vitamin C and B contents in the *S. cerevisiae* were determined.

The data in Table 4 indicated the fact that yeast cells have the capacity to synthesize vitamin C and B by direct fermentation from simple sugars. Our results also showed that the yeast cells can synthesize higher amounts of vitamin

C (32.64 mg/100g) of cell dry weight. These results were similar with Hancock *et al*. (2000) who reported that *S. cerevisiae* can synthesize a considerable amount of vitamin C under specific conditions. The results of this study also indicated that *S. cerevisiae* has high ability to synthesize vitamins B likes B1, B2, B3, B9 and B12 but not able to produce B6.

TABLE 3. Elemental analysis of some minerals of dry yeast cells S-RS1%NaOH grown under static conditions.

Parameter	Results mg/kg
	Elements
Aluminum	489.75
Boron	4.775
Cadmium	0.0
Cobalt	0.93
Chromium	46.15
Copper	79.28
Iron	1162.5
Manganese	566.25
Molybdenum	2.83
Nickel	16.98
Lead	12.93
Strontium	68.15
Vanadium	2.0
Zinc	186.68

TABLE 4. Vitamins content of dry yeast cells S-RS1% NaOH grown under static conditions.

Vitamins	mg /100g
Thiamine B1	62.096
Riboflavin B2	1.654
Nicotinic acid B3	89.051
Pyridoxine B6	-
Folic acid B9	0.700
Cobalamine B12	1.750
Vitamin C	32.64

Data recorded in Table 5 showed that, the biomass cells had 16 kind of essential amino acids, These results show that the yeast strain *S. cerevisiae* was suitable for single-cell protein production. In agreement with our results Zheng *et al*. (2005) and Rajoka *et al*. (2006) recorded that, the biomass obtained from *Candida utilis* contained all the essential amino acids.

TABLE 5. Amino acids content of dry yeast cells S-RS1%NaOH grown under static conditions.

Amino acids	g /100g
Aspartic	0.183
Threonine	0.089
Serine	0.085
Glutamic	0.238
Proline	0.083
Glycine	0.112
Alanine	0.121
Valine	0.104
Methionine	0.001
Isoleucine	0.094
Leucine	0.119
Tyrosine	0.044
Phenylalanine	0.077
Histodine	0.280
Lysine	0.093
Glutamine	1.266
Arginine	0.058

Conclusions

The results of this work shown that the yeast isolate strain can be used to produce valuable SCP from low cost agro-industrial waste. This approach could be also used to minimize the environmental pollution. However, further investigations are necessary to scale up the treatment of agro-industrial wastes and product yield. The results also suggested that rice straw wastes which selected as raw materials was suitable and could be used to produce SCP by *S. cerevisiae*.

In order to serve as a health-conserving animal feed supplement, the microbial biomass must fulfill certain nutritional prerequisites including high contents of nutritional compounds such as protein content, mineral content, carbohydrate content, lipid content, vitamins and amino acids content**.** Analysis of the nutritional quality of biomass suggests that it could be used as a source of supplemental protein for animal feed.

References

A.O.A.C. (1975) *"Official Methods of Analysis"* (12th ed.) Washington, DC: Association of Official Analytical Chemists.

A.O.A.C. (1990) "*Official Methods of Analysis"* (15th ed.) Association of Official Analytical Chemists, Inc. USA.

A.S.T.M. (2002) American Society for Testing and Materials.

Anupama, X. and Ravindra, P. (2000) Value-added food: single cell protein. *Biotechnol. Adv*., **18**: 459–479.

Anupama, X. and Ravindra, P. (2001) Studies on production of single cell protein by *Aspergillus niger* in solid state fermentation of rice bran. *Braz. Arc. Biol. Tec*., **44**: 79-88.

Batifoulier, F., Verny, M-A. Besson, C. Demigne, C. and C. Remesy, (2005) Determination of thiamin and its phosphate esters in rat tissues analyzed as thiochromes on a RP-amide C16 column. *Journal of chromatography* **B**. **816:** 67-72.

Becker, E.W. (2007) Micro-algae as a source of protein. *Biotechnology Advances*, **25**: 207–210.

Block, R.J., Durrum, E.l. and Zwery, B. (1958) *"Manual of Paper Chromatography and Paper Electrophoresis".* 2nd ed. Academic press in C. publishers, New York.

Camacho-Ruiz, Pérez-Guerra, N. and Pérez Roses, R. (2003) Factors affecting the growth of *Saccharomyces* cerevisiae in batch culture and in solid state fermentation. *Electron. J . Environ. Agric. Food Chem*., **2** (5): [531-542]

Chi, Z., Liu, Z., Gao, L., Gong, F., Ma, C., Wang, X. and Li, H. (2006) Marine yeasts and their applications in marine culture. *J.Ocean. Univ. Chin*., **5**: 251-256.

Gao, L., Chi, Z. Sheng, J. Ni X. and Wang, L. (2007) Single - cell protein productions from Jerusalem artichoke extract by a recently isolated marine yeast *Cryptococcus* aureus G7a and its nutritive analysis. *Appl. Microbiol. Biotechnol*., **77**: 825–832.

Geary P. J. (1956) Determination of moisture in solids. B.S.I.R.A. Research Report M24.

Ghaly, A.E., Kamal, M. and Correia, L.R. (2005) Kinetic modeling of continuous submerged fermentation of cheese whey for single cell protein production. *Bioresource Technology*, **96**: 1143–1152.

Hancock, RD., Galpin, J.R. and Viola, R. (2000) Biosynthesis of L ascorbic acid (vitamin C) by *Saccharomyces cerevisiae*. *FEMS Microbial. Lett*., **186**: 245–250.

Haphries, E. (1956) Mineral component and ash analysis In: "*Modern Methods of Plant Analysis"*, Ed., K. Peach, and M.V. Tracey .156. Vol.1 Springer- Verlage, Berlin, p. 468.

Hossam S. Hamdy (2013) Production of mini-food by *Aspergillus niger*, *Rhizopus oryzae* and *Saccharomyces cerevisiae* using orange peels. *Romanian Biotechnological Letters*, **18** (1): 7929-7946.

Kurbanoulu, E.B. (2001) Production of single cell protein from ram horn hydrolysate. *Turk. J. Biol*., **25**: 371-377.

Lowry, O. H., Rosebrough, N.J. Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Bol. Chem*., 193: 265-275.

Omar, Ali M. M. I. (2006) Microbiological utilization of *Eichhorina crassipes* under solid state fermentation. *M. Sc. Thesis*, bot. & Microbiol., Dept., fac. Sci., Al-Azhar Univ., Cairo, Egypt.

Parajo, J.C., Santos, V. Dominguez, H. and Vaszquez, M. (1995) Protein concentrates from yeast cultured in wood hydrolysates. *Food Chemistry*, **53**: 157–163.

Paraskevopouloua, A., Athanasiadisa, I. Kanellakib, M. Bekatoroua, A. Blekasa, G. and Kiosseogloua, V. (2003) Functional properties of single cell protein produced by *Kefir Microflora. Food Research International,* **36**, 431–438.

Prado-Rubio, O. A., Jørgensen, J.B. and Jørgensen, S.B. (2010) Systematic model analysis for single cell protein production in a U-Loop Reactor. *Computer Aided Chemical Engineering*, **28:** 319-324.

Rajoka, M.I. (2005) Production of single cell protein through fermentation of a perennial grass grown on saline lands with *Cellulomonas biazotea*. *World Journal of Microbiology & Biotechnology* **21:** 207–211.

Rajoka, M. I., Khan, S.H. Jabbar, M.A. Awan, M.S. and Hashmi, A.S. (2006) Kinetics of batch single cell protein production from rice polishing with *Candida utilis* in continuously aerated tank reactors. *Bioresource Technology*, **97**: 1934–1941.

Rasoul-Amini, S., Ghasemi, Y. Morowvat, M.H. and Mohagheghzadeh, A. (2009) PCR amplification of 18S rRNA, single cell protein production and fatty acid evaluation of some naturally isolated microalgae. *Food Chemistry*, **116**: 129–136.

Ravindra, A. P. (2000) Value-added food: Single cell protein, *Biotechnol. Adv*., **18**: 459–479.

Reith, J. F., Mossel, D.A.A. and de Kamer, J.H. (1948) *Anal. Chem. Acta*, **2**: 359.

Shojaosadati, S.A., Khalilzadeh, R. Jalilzadeh, A. and Sanaei, H.R. (1999) Bioconversion of molasses stillage to protein as an economic treatment of this effluent. *Resources, Conservation and Recycling*, **27**:125–138.

Silva, CF., Arcuri, S.L. Campos, C.R. Vilela, DM. Alves, J.G.L.F. and Schwan, RF. (2011) Using the residue of spirit production and bio ethanol for protein production by yeasts. *Waste Management:* **31**:108–114.

Ugwuanyi J.O. (2008) Yield and protein quality of *thermophilic Bacillus spp.* biomass related to thermophilic aerobic digestion of agricultural wastes for animal feed supplementation. *Bioresource Technology*, **99**: 3279–3290.

Umbriet, w.w., Burris, R.H. Stauffer, J.F. Cohen, P.P. Johnse, W.J. Leepage, G.A. Patler, U.R. and Scheider, W.C. (1959) "*Manometric Techniques*", a manual describing methods applicable to the study of tissue metabolism, pp. 239. Burgess publishing company.

Yang, Zhang, Yi. Gu, Jiang, Y. and Qin, B. (2009) Solid state fermentation of aquatic macrophytes for crude protein extraction. *Ecological Engineering*, **35**: 1668–1676.

Zepka, L. Q., Jacob-Lopes, E. Goldbeck, Leonor, R. Souza-Soares, A. and Queiroz, M.I. (2010) Nutritional evaluation of single-cell protein produced by *Aphanothece microscopica* Nageli. *Bioresource Technology*, **101**:7107–7111.

Zhang, Z.Y., Jin, B. Z.H. Bai and Wang, X.Y. (2008) Production of fungal biomass protein using microfungi from winery wastewater treatment. *Bioresource Technology*, **99**: 3871–3876.

Zheng, Y-G., Chen, X-L. and Zhao and Wang, Z. (2005) Microbial biomass production from rice straw hydrolysate in airlift bioreactors. *Journal of Biotechnology*, **118**: 413–420.

Zhu, S., Wu Y., Yu, Z. Zhang, X. Wang, C. Yu, F. Jin, S. Zhao, Y. Tu, S. and Xue, Y. (2005) Simultaneous saccharification and fermentation of microwave/Alkali pre-treated rice straw to ethanol. *Biosystems Engineering*, **92** (2): 229–235.

Ziino, M., R.B., Curto, R.B. Lo Salvo, F. Signorino, D. Chiofalo, B. and D. Giuffrida, D. (1999) Lipid composition of *Geotrichum candidum* single cell protein growth in continuous submerged culture. *Bioresource Technology*, **67**: 7–11.

(*Received* 16/2/ 2015;
accepted 12/4/2015)

استخدام قش الارز المعالج قلويا كبيئة رخيصة لإنتاج البروتين الحيوى بواسطة خميرة الخباز

شريف موسى حسينى[1] – ممدوح سالم الجمل[2] – على إبراهيم سيد[3] - عز الدين محمود عبد الرحمن[2] - حامد محمد حسنين خشبة [3]

[1]كلية البنات للآداب والعلوم والتربية – جامعة عين شمس و[2]كلية العلوم – جامعة الازهر و[3] قسم الاغذية الخاصة – معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية– مصر.

أجريت دراسة تتعلق بالتحويل البيولوجى لقش الارز المعالج قلوياً الى بروتين حيوى تحت ظروف التخمر بواسطة خميرة الخباز وقد اثبت الظروف البيئية والغذائية المثلى أسفرت عن أعلى انتاج للبروتين الحيوى يمكن تلخيصها على النحو التالى ، درجة التحضين 30° م ، فترة تحضين 48 ساعة ، أفضل أس هيدروجينى هو 4.5 ، حجم الحقنة الامثل كان 1 مللى من معلق كثيف من الخميرة تركيزه 95° 10^7 وحدة تكوين المستعمرة ، 2 جرام ٪ من قش الارز وامونيوم فوسفات كأفضل مصدر نيتروجين ، و 2.5 ٪ اللاكتوز كأفضل مصدر كربونى .

وقد تم الحصول على أعلى انتاجية من البروتين الحيوى تحت ظروف ساكنة . وتم تحديد محتويات عالية من المركبات الغذائية مثل البروتين، والمعادن والكربوهيدرات والدهون والفيتامينات والأحماض الأمينية في الكتلة الحيوية للخميرة المنتجة. وتشير هذه النتائج إلى أن خميرة الخباز يمكن أن تكون مناسبة لإنتاج بروتين حيوى من مخلفات رخيصة.