Minufiya J. Agric. Res. Vol.37 No. 6(1):1389-1407 (2012) "http://www.mujar.net"

BIOCONVERSION OF SOME ABUNDANT FOOD INDUSTRY LIGNOCELLULOSIC WASTES TO SAFE AND NUTRITIVE PROTEINS

A. S. Ismail[(1)], A.A. El Bedawey[(2)], A.E.S. El Beltagy[(2)] and Amira A. Hassan[(1)]

[(1)] Natural and Microbial Products Chemistry, Pharmaceutical Research Division, National Research Center, Dokki, Cairo, Egypt

[(2)] Department of Food Science and Technology, Faculty of Agriculture, Minufiya University, Shibin El-Kom, Egypt

(Received: Oct. 11, 2012)

ABSTRACT: *Among ten fungal and yeast species grown on eight local food industry wastes, two strains Candia albicans NRRL YB-242 and Saccharomyces cerevisiae NRRL Y-12632 in 7-days shaken cultures were the most potent and afforded the highest safe protein yields, which were completely free of dangerous toxins and contain low nucleic acids level.*

The optimum protein production medium was formulated for the two strains and consisted of wheat bran, ammonium sulphate and glucose + manganese sulphate mixture as an additive for C. albicans strain, and of wheat bran, milk-whey and glucose + sugar beet molasses mixture as additives for the other strain S. cerevisiae. The shaken culture technique (200 rpm) for 7 days at 30°C and initial pH of 4.7 was the most preferable than solid state fermentation technique and led to the highest protein productivity by any of the two species. The suitable inoculum age was 48 h for both strains, while its size was 5 & 7.5 ml/flask, respectively.

Many trials for the maximal biomass protein extraction were performed and brought about the efficiency of sodium hydroxide (0.2-M) for protein extraction from the biomass of both strains. Evaluation of the different lyophilized protein preparation forms (culture filtrate & biomass extract) was carried out. The crude lyophilized protein preparation forms either of the filtrate or the biomass extract of C. albicans contained many important minerals, lipo and glycoprotein with low nucleic acids level (< 1.0) and were completely free of microbial toxins.

Key words: *Food industry wastes; Single cell protein; Nucleic acids; Mycotoxins; Nutritive & safe protein.*

INTRODUCTION

Huge and increasing amounts of many food industrial wastes are produced annually in Egypt, except very small quantities used in animal feeding and soil conditioning, causing very hard environmental impact, especially after burning. Increasing concern about pollution by agricultural and industrial wastes has stimulated interest in converting waste materials into commercially valuable products. Lately, very created methods were proposed for eco-friendly utilization of such food industrial wastes and the most pronounced is their biodegradation providing many plentiful products (proteins, enzymes, sugars, amino acids and others). The novelty of unwanted waste product consumption added a new economic incentive to single cell protein (SCP) production, as the idea of zero cost substrates. The SCP production benefits were thus extended from the production of food to the preservation of the environment (Litchfield, 1987).

The increasing world deficiency of protein is becoming a main problem of humankind. Intense efforts have been made to explore new, alternative and unconventional protein to alleviate the anticipated world-wide problem concerning the supply of food protein (Scrimshaw & Murray, 1995; Anupama, 2000; Ugalde & Castrillo, 2002; Mustafa, 2004; Becker, 2007 and Nasseri *et al.*, 2011). Single cell protein (refers to the dried cells of microorganisms and their secreted proteins) production is a major step in this direction. Many authors (Anupama, 2000; Ugalde & Castrillo, 2002; Mustafa,

2004; Becker, 2007 and Nasseri *et al.*, 2011) reported on different bacteria (*Cellulomonas*, *Alcaligenes*, etc.), algae (*Spirulina*, *Chlorella*, etc.), molds (*Trichoderma*, *Fusarium*, *Rhizopus*, etc.) and yeast (*Candida*, *Saccharomyces*, etc.) for SCP production.

The present work aimed at the utilization of different eight food industrial waste samples, namely, wheat bran (WB), sugar beet pulp (SBP), sugar cane bagasses (SCB), potato peels (PP), orange peels & pulp (OPP), pea pods, okra termini and jew's mallow stems (molokheya stems), for the production of safe and high nutritive protein by some fungal and yeast strains.

MATERILS AND METHODS

Materials

Microorganisms

The following fungal and yeast strains used for the protein production were obtained from the United States Department of Agriculture, Peoria, Illinois 61604, U.S.A. (NRRL): *Trichoderma viride* NRRL 6418, *T. reesei* NRRL 11460, *Chaetomium cellulolyticum* NRRL 18756, *Aspergillus terreus* NRRL 265, *Saccharomyces cerevisiae* NRRL Y-12632, *Pichia pastoris* NRRL X-11430, *Candida albicans* NRRL YB-242 and *C. tropicales* NRRL YB-366. The other two strains *A. niger* 200 and *Penicillium funiculosum* E-NRC 629 were provided from the Center of Culture Collection of the National Research Centre, Dokki, Cairo, Egypt.

Food industry wastes specimens

Eight different waste samples were collected from some food factories. Sugar beet pulp waste sample (SBP) was obtained from Delta Sugar Company, Kafr El-Sheikh, Kafr El-Sheikh governorate, Egypt. Sugar cane bagasses (SCB) were obtained from Egyptian Sugar and Integrated Industries Company, Hawamdia, Giza governorate, Egypt. Wheat bran (WB) sample was obtained from South Cairo and Giza Mills Company, Fysal, Giza governorate, Egypt. The other food industry waste samples including pea (*Pisum sativum)* pods, orange peels & pulp *(Citrus sinensis* var. Balady, OPP), okra (*Abelmoschus esculentus)* termini, Jew's mallow (Molokheya, *Corchorus olitorius*) stems and potato (*Solanum tuberosum)* peels (PP) were collected from the corresponding food industry factories in Cairo city Egypt. All samples were analyzed for their holocellulose, pectin, lignin, protein and ash contents.

Media

The following media were used in the present study and had the following composition (g/L):

Fungi maintenance and sub-culturing medium

Potato-dextrose-agar (PDA) medium (39 g/L, Merck, Germany) used for maintenance and sub-culturing of the fungal strains.

Preparation of the fungal inoculum

Fungal inoculum preparation medium composed of (g/L): glucose, 16; peptone, 1.25; yeast extract, 0.25; $MgSO_4.7H_2O$, 0.125 and K_2HPO_4, 0.25. Spore suspension of each fungal strain was prepared in PDA subculture slope which transferred to 50 ml sterilized inoculum medium and incubated in thermostatic incubator shaker (180 rpm) at 30°C for different periods, i.e. suitable for good fungal inoculum formation.

Yeast maintenance and sub-culturing medium

It contained dextrose, 40; peptone, 10; agar 20 g/L.

Subculturing and maintenance of the yeast stock cultures

The original stock cultures were subcultured on sabouraud slopes, which were then incubated at 30°C for 5 days and then stored in a refrigerator at 4°C.

Preparation of the yeast inoculum

Yeast inoculum preparation medium composed of (g/L): dextrose, 40; peptone, 10. Suspension of each yeast isolate was

prepared in saburod subculture slope, which transferred to 50 ml sterilized inoculum medium and incubated in incubation thermostatic shaker (180 rpm) at 30°C for different periods, i.e., suitable for good yeast inoculum formation.

Single cell protein production media

Eight production media were prepared according to Ismail, 1996, who formulated the multienzyme systems and protein production medium by some fungal strains utilizing Egyptian balady orange peels as follows: orange peels, 72.2; K_2HPO_4, 0.5; Mg $SO_4.7H_2O$, 0.5; $NaNO_3$, 2.5g. The other seven production media had the same aforementioned medium composition, but orange peels waste was replaced by the following food industry wastes: sugar beet pulp, 69.6; wheat bran, 67.0, sugar cane bagasses, 68.0; potato peels, 65.4; okra termini, 66.2; jew's mallow stems, 64.6; pea pods, 66.2 g.

Cultivation

Cultivation was carried out in 250 ml Erlenmeyer flasks, each containing 50 ml of sterilized medium. Five ml of inoculum were taken from 48 h-old-culture and used for inoculating each flask. After incubation for different periods at 30°C, the culture medium from each flask was filtered off to separate the mycelium from the culture filtrate. The mycelium was dried at 50°C to stable weight and the total protein content was analyzed by Kjeldahl method (AOAC, 1980). The culture filtrate was centrifuged in a cooling centrifuge and the clear supernatant was used for soluble protein determination by the method of Lowry *et al.,* 1951.

Batch production of the crude microbial protein

The optimized production media were autoclaved for 20 min at 1.5 atm and 121°C. The autoclaved flasks were then inoculated under aseptic conditions by the optimized inoculum (age and size) of *C. albicans* or *S. cerevisiae,* respectively. The inoculated flasks were incubated in thermostatic shaker (200 rpm) at 30°C for 7 days.

Batch production of the crude protein by any of the two strains was carried out, where many cultures of each were collected at the end of fermentation process, mixed, filtered and centrifuged. The clear filtrate was frozen and lyophilized, while the residual biomass (WB residue + cell growth) was collected and applied for the protein extraction process.

Methods

Waste sample analysis

- Moisture, ash and lipid contents of different waste samples and protein forms were performed according to official methods (AOAC, 1980).
- Cellulose, hemicellulose and lignin contents were estimated following the method adopted by Mansour (1963).
- Pectic substances were determined according to Abdel-Fattah *et al.*, (1981).
- Total carbohydrates were determined as glucose, using the phenol-sulfuric acid method (Dubois *et al.*, 1956).
- Total crude protein content (T.N × 6.25) of the dried mycelium was measured by the Micro-Kjeldahl method (AOAC, 1980), while the fresh culture filtrate protein was determined by the method of Lowry *et al.,* 1951. Non-protein nitrogen and true protein were estimated according to the method described by Abdel-Fattah and Association, 1978.
- Aflatoxins in the culture medium were extracted with chloroform according the method of Bullerman (1974), while ochratoxins were extracted with chloroform according the method of van Walbeek *et al.*, 1969. Ochratoxins were detected by TLC on silica gel G following the method of Paster *et al.,* 1983.
- Determination of the nucleic acids was performed according to the proteinase K/phenol/Chloroform procedure as described by Sharma *et al.*, 2000).

Extraction of the microbial biomass protein

Extraction of the microbial biomass protein with different extracting agents

The extraction of the biomass protein was undergone with different buffers (0.1M citrate, pH 5.0; phosphate, pH 7.0 or bicarbonate, pH 10.0) at 25°C, NaOH (0.1 & 0.2M at 25 & 70 °C), NaCl (0.45 & 0.9 M) or mixture of the most effective extracting agents at 25°C.

Extraction of the microbial biomass protein with 0.2M-NaOH

The residual collected biomass protein was extracted with 0.2 M sodium hydroxide (1:20, w/v) at 70°C for 90 min. At the end of the extraction period, the sample was filtered off and centrifuged at 8000 rpm for 20 min. The clear protein supernatant was adjusted to pH 5.0 with HCl. The obtained protein solution was dialyzed against distilled water in a cellophane bag in a refrigerator until the water outside the bag gave no precipitate with 1.0% (w/v) silver nitrate solution. The protein solution was then lyophilized and the protein powder was kept in a closed vessel at 4°C.

RESULTS AND DISCUSSION

The first part of this work concerned with the evaluation of nominated eight food industry waste samples according to their percentage composition of the major components (moisture content, holocelluloses, pectin, lignin, protein and ash), which were estimated on dry basis (Table 1).

The chemical composition of SBP sample displayed high holocellulose and pectin contents (55.0 & 21%, respectively) with low lignin (3.2%) and ash (3.6%), while protein amounted to 9.0%. Sugar-cane bagasses possess the highest holocellulose (66.0) and lignin (22.0%) contents with the lowest pectin (0.75 %), protein (2.43%) and ash (8.3%) contents, compared with all the other employed wastes. Similar values of holocellulose and lignin were reported by other authors for the previously mentioned wastes (Youssif, 1996; Suhaila, 2010 and Kim & Day, 2011).

Wheat bran sample contained lower holocellulose and pectin contents (38.0 and 3.8%, respectively), with higher protein content than those in SBP. The estimated amounts of lignin and ash were found to be 4.90 and 5.37%, respectively. The obtained data was to great extent similar to those reported by Choteborska *et al.*, 2004. When potatoes were peeled, about 12% of their fresh weight was discarded (peels and trimmings) and the major component of these peels is the starch. Analysis of the PP sample declared the presence of considerable protein (10.2%), with low pectin (3.6%), while holocellulose and ash contents were 10.0 & 10.3%, respectively. During processing of orange juice, about 54% of the fresh weight of orange was separated as juice and the remaining consisted of peels, pulp and seeds. OPP composed of the highest pectin content (21.4%) and the lowest lignin (1.7%) comparing with those found in the other examined wastes. Other constitutes of OPP were as follows: holocellulose (42%), protein (11.99%) & ash (6.7%). The present OPP constituents % was in agreement with those reported by De Gregorio *et al.*, 2002.

Concerning both okra termini and pea pods, residues (after processing) were collected, dried and subjected to the % composition analysis. As far as I am aware, nothing was reported in the literature for the analysis of either okra termini or pea pods. Okra termini contain the following (%): pectin (12.55), holocellulose (44.5), protein (11.62), lignin (9.4) and ash (10.13). The values (%) for pea pods were as follows: pectin (5.40), holocellulose (50.10), protein (7.23); lignin (8.5) and ash (6.6). Finally, analysis of Jew's mallow stems shows the presence (%) of pectin (16.54), holocellulose (36.4), lignin (17.28), protein (5.22) and ash (7.2). These values are comparable with those published by Ndlovu & Afolayan, 2008, who analyzed the whole molokheya plant and evaluated its nutritive values.

Lignin in the other samples, OPP, PP, SBP and WB, was low and not exceeded than 4.9%. On the other hand, high lignin contents were found in the SCB, Jew's mallow stems and okra termini (22.00, 17.28 and 9.40%, respectively), and this may affect their biodegradation accessibility by the chosen microorganisms as studied later on. Finally, the collected samples contained variable ash contents ranging from 3.6 (in SBP) to 10.30 % (in PP).

Table 1: Percentage [(a)] composition of the food industry waste samples.

Waste sample	Moisture	Holocellulose	Pectin	Lignin	Protein	Ash
SBP	14.60	55.00	21.00	3.20	9.00	3.60
WB	10.40	38.00	3.50	4.90	12.37	5.37
SCB	11.71	66.00	0.75	22.00	2.43	8.30
PP	8.12	10.00	3.60	2.20	10.20	10.30
OPP	8.40	42.00	21.40	1.70	11.99	6.70
Okra termini	9.40	44.50	12.55	9.40	11.62	10.13
Pea pods	9.80	50.10	5.40	8.50	7.23	6.60
Jew's mallow stems	7.20	36.40	16.54	17.28	5.22	7.20

(a) Calculated on dry weight basis
SBP: Sugar beet pulp.
WB: Wheat bran.
SCB: Sugar cane bagasses
PP: Potato peels.
OPP: Orange peels & pulp.

Screening of some fungal and yeast strains for the SCP production

Ten fungal and yeast strains were tested in shaken cultures containing any of eight different food industrial wastes as the sole carbon source for the productivity of high protein yield (Table 2). Results clarified that the obtained protein depends on both the microbial strain and the waste composition.

Employment of SBP as substrate, *Candida albicans* afforded the highest protein productivity (0.634 g/culture) followed by *Trichoderma reesei* and *T. viride* (0.646 and 0.626 g/culture, respectively). On the other hand, *Pichia pastoris* and *Chaetomium cellulolyticum* contributed the lowest protein productivity utilizing the same substrate. The production of SCP through SBP utilization was reported by Murad, 1985, who successfully used SBP for the production of single cell protein from *Penicillium funiculosum* 6 B and the resulting biomass contained up to 40 % of crude protein.

Application of wheat bran (WB) improved the protein productivity by *C. albicans, T. reesei*, *T. viride, Penicillium funiculosum* and *Aspergillus terreus* which amounted to 0.66, 0.623, 0.602, 0.580 & 0.571 g/culture, respectively. On the other hand, each of *Pichia pastoris* and *Chaetomium cellulolyticum* provided the lowest protein yield.

Application of the native sugar cane bagasses (SCB), which is known with its high lignin content (~ 22%), all the tested microorganisms yielded diminutive protein yields. *Candida albicans* grown on SCB yielded only 24% protein productivity that on WB. Nevertheless, *Aspergillus niger*, afforded the highest yield in 7 days shaken culture (0.247 g/culture). The poor SCP productivity by the tested microorganisms grown on native SCB in the present study may be attributed to the high cellulose crystallinity and lignin content with the rareness of the amorphous regions in the native bagasses fibers which was reported in previous studies (Hassan, 2006). Utilization of potato peels (PP) as a substrate clarified that among all the tested microorganisms *Penicillium funiculosum* utilizing PP was the most potent protein producer and afforded 0.506 g/ culture followed by *Saccharomyces cerevisiae* (0.474 g/ culture) and *Trichoderma reesei* (0.351 g/ culture). In this respect, El-Fouly *et al.*, 2001, reported on the SCP production by yeast isolates utilizing potato waste to reduce the environmental pollution with potato processing wastes.

Table 2

Table 2

Orange peels and pulp (OPP) waste was also tested as the sole carbon source for SCP production by the tested organisms. Generally, some fungal strains were more able to grow on such substrate than yeasts. Both *Penicillium funiculosum* and *Trichoderma reesei* were the highest protein producers (0.502 and 0.483 g/culture, respectively). Similar protein yields (around 0.4 g/culture) were afforded by the other yeast and yeast-like fungi strains. Nothing has yet been reported on the SCP production utilizing each of okra termini, jew's mallow stems or pea pods as substrates. Among the ten tested fungal and yeast species grown on okra termini, *Candida albicans*, *Penicillium funiculosum and C. tropicalis* produced the highest protein yields (0.636, 0.567 and 0.559 g/culture, respectively). On the other hand, *Aspergillus niger, T. viride* and *Pichia pastoris* afforded the lowest protein levels.

Similar to sugar cane bagasses, utilization of jew's mallow stems as substrate by the examined microorganisms led to lower protein yields ranged from 0.25 to 0.351 g/culture and this may be attributed to the high lignin content and high crystallinity of cellulose in such stems, as previously discussed. In 7-days-shaken cultures, the two *Trichoderma* strains *(T. reesei* and *T. viride)* were the most potent protein producers utilizing the pea pods waste (0.455 & 0.394 g/culture, respectively) followed by the other yeast-like fungi strains *C. albicans* and *C. tropicalis* (0.386 and 0.377 g/culture, respectively) while, *A. niger* afforded the lowest protein level, which amounted to 0.296 g/ culture.

Collectively, the four strains *Candida albicans, Trichoderma viride*, *Penicillium funiculosum* and *Saccharomyces cerevisiae* were the most potent organisms and afforded the highest SCP productivity in 7 days shaken culture utilizing WB or SBP wastes, respectively. The lowest protein productivities by the aforementioned strains were recorded with sugar cane bagasses, jew's mallow stems and pea pods. These findings were also reported by Pérez *et al.*, 2002 and Sánchez, 2009. In conclusion, the three native wastes wheat bran, sugar beet pulp and potato peels seemed to be the most promising substrates and led to the highest single cell protein productivity, and the more susceptibility is due to the low lignin content and poor cellulose crystallinity with the amorphous regions abundancy in these substrates.

Detection of toxins

The final protein product should not only be nutritious, but should also pass all toxicity tests to be commercialized as a safe food product. A part from the nucleic acid and all toxins that may be accumulated during the course of growth on substrates should be removed. In the present study, detection of the mycotoxins was performed in both the culture filtrates and the produced biomass of *Trichoderma viride, Penicillium funiculosum, Saccharomyces cerevisiae* and *Candida albicans* grown on SBP, WB or PP. All the tested microbial strains (Fig. 1) seemed entirely to be free from the serious known toxins (afla and ochratoxins) except the fungal strain *Trichoderma viride*, where both afla and ochratoxins were detected in its biomass of 7 days shaken culture when grown on WB or PP and in its culture filtrate only when grown on PP as the sole carbon source. Consequently, *T. viride* was excluded as SCP producer utilizing such substrates.

Endogenous nucleic acids content

Nucleic acids are necessary components of all cells, but present relatively high levels in rapidly dividing cells (Singh, 1998 and Ugalde & Castrillo, 2002). It is well known that human consumption greater than 2 g nucleic acid equivalent per day may lead to kidney stone formation and gout (Calloway, 1974 and Scrimshaw & Murray, 1995).

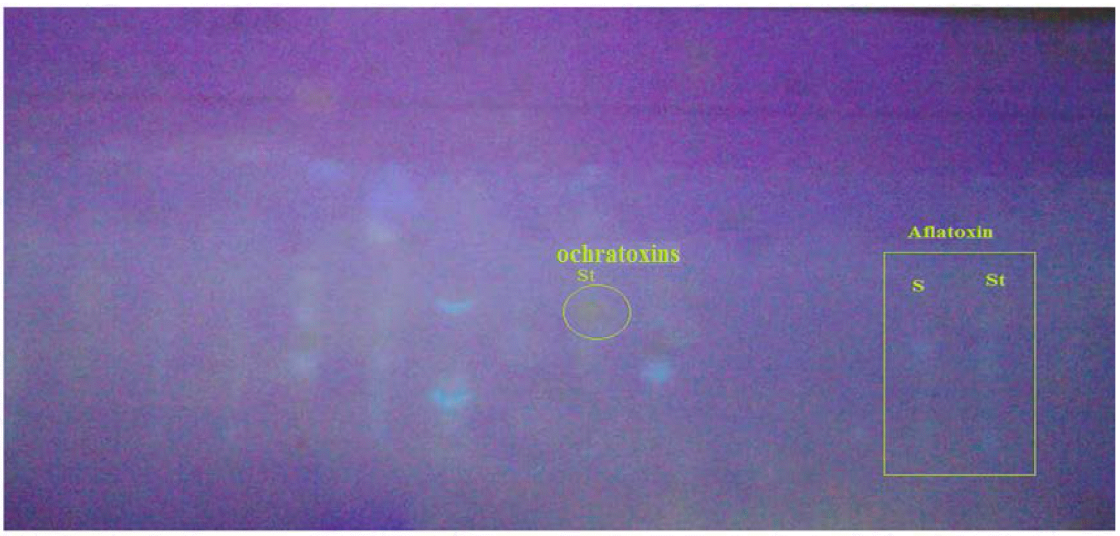

Fig. 1: Afla and ochratoxins in selected strains production media.

Nucleic acid contents of the most safe and potent protein producers species, *Saccharomyces cerevisiae, Candida albicans* and *Penicillium funiculosum*, were assayed after growing on WB as the sole carbon source in 7 days shaken cultures. In unrecorded data, it was indicated that the nucleic acids content of *S. cerevisiae* culture was the highest (1.21%), followed by *C. albicans* (0.9%) while of *P. funiculosum* was the lowest (0.35%). Considering the maximum permissible level of nucleic acids in human diet as 2 g/day (Scrimshaw & Murray, 1995), addition of about 165 g of *S. cerevisiae*, 220 g of *C. albicans* or 570 g of *P. funiculosum* biomass to the human diet daily will be safe. Higher addition values for animal fodder will be permissible.

Among all the fungal and the yeast species investigated, *Candida albicans*, *Saccharomyces cerevisiae* and *Penicillium funiculosum* were the most potent and afforded the highest protein productivity with the safety requirements representing the absence of toxins and low nucleic acid level. In spite of the higher protein productivity by *S. cerevisiae* grown on SBP than on WB (110% as on WB), WB seems to be more suitable according to the biomass homogeneity and absence of fibers. In addition, the extracellular proteins (filtrate) of the culture with WB were completely clear, unlike that with SBP which were viscous and turbid, and this may be due to the high protein content in SBP sample as earlier reported. Accordingly, the most promising and safe strains *S.* cerevisiae *and C. albicans* utilizing WB waste were selected for the succeeding studies.

Physiological optimization of SCP productivity

The optimization of SCP productivity by the selected strains *Candida albicans* NRRL YB-242 and *Saccharomyces cerevisiae* NRRL Y-12632 utilizing WB as substrate was studied. Accordingly, the proper WB quantity in the culture medium, nitrogen and carbon source, different additives to the culture medium, the inoculum age and size, as well the culture type were duly determined. Different quantities of WB ranged from 1-10 g/50 ml medium were applied. The total protein of *C. albicans* increased gradually to reach 0.838 g/culture at 7.5 WB g/culture and represents 3-fold that at 1.5 g/culture. On the other hand, the optimum WB concentration for *S. cerevisiae* was 5.5 g/culture, where the total protein content increased 4-fold as at 1.5 g/culture. Further increase of the substrate (WB) concentration did not improve the protein productivity. Consequently, 7.5 and 5.5 g/culture of WB will be used in the succeeding experiments for *C. albicans* and *S. cerevisiae,* respectively.

The effect of N source was conducted by the replacement of $NaNO_3$ (in the basal medium) in equal N basis by any of the biologically active organic complexes (yeast extract, soya bean powder or milk whey), or inorganic (ammonium chloride, ammonium nitrate or ammonium sulphate). For *C. albicans*, the replacement of $NaNO_3$ by any of the foregoing N sources led to varied productivity effects, whereas ammonium sulphate led to the highest protein productivity (more than 33% increase than that with $NaNO_3$). Accordingly, ammonium sulphate was selected as the proper N source in the production medium of *C. albicans* NRRL YB-242. Considring the other strain *Saccharomyces cerevisiae*, it was clarified that milk-whey led to the highest protein productivity (0.887 g protein/culture) and consequently it was selected for further studies. The optimal concentration of the N source which led to the highest protein productivity was determined. Addition of 0.4% (w/v) ammonium sulphate and 0.04% (w/v) of milk-whey (N content 2.56 %, w/w) in the production medium of both organisms in the same order were the most proper concentrations affording the highest protein productivity.

Also, the effect of some additives to the culture medium containing WB and the above selected N sources, on the productivities of protein by any of the two selected strains was studied. Among the different tested additives, glucose improved the protein productivity for both tested microorganisms. For *Candida albicans*, addition of 0.5%, w/v glucose to the culture medium increased the protein production by 11.8 % compared with 19.8 % for *Saccharomyces cerevisiae*. Other tested additives did not improve the production of protein and even led to protein productivity decrease in case of *C. albicans.* Glucose as the promising additive was added in different concentration (0.2-1.5% w/v). The highest protein productivity by *C. albicans* and *S. cerevisiae* was recorded at 1.5 & 1.0% (w/v) glucose in the production medium of the two aforementioned strains, respectively attaining 13.6 & 12.0% increase than the control, in the same order.

The effect of beet molasses (0.5 & 1.5 g/culture), also some metal ions addition to the production medium on the protein productivity was also investigated. The following metal sulphate (%, w/v); Fe^{2+} (0.056), Zn^{2+} (0.028), Cu^{2+} (0.005) or Mn^{2+} (0.168) were examined. Beet molasses (1.5 g/culture) or $MnSO_4.H_2O$ (0.168%, w/v) alone addition to *Candida albicans* production medium led to 11.8 or 17.5% protein productivity increase, respectively. On the other hand, more than 11 % protein productivity increase was recorded by the addition of 1.5 g/culture beet molasses to *Saccharomyces cerevisiae* production medium. It was noticed that, lesser improvement was recorded by the two aforementioned additives mixture to the production medium by any of the two examined organisms.

The effects of the inoculum age and size on the protein productivity by *Candida albicans* and *Saccharomyces cerevisiae* in 7-days shaken cultures were studied. Five ml (10%, v/v) of the inoculum suspension from different ages (24, 48 or 72 h) were applied for inoculation of 50 ml optimized production medium and incubated in the thermostatic shaker (200 rpm) for 7 days at 30°C. The older inocula (48h) led to the highest growth with an increase of the protein productivity, where 10.37 & 30.71% protein increase were afforded by the last-named organisms, comparing to that afforded with the 24 h-old-inoculum, in the same order. The oldest inocula (72 h) of each of the two organisms failed to improve the protein productivity, which slightly decreased. The data also revealed that the inoculum size (5-20%, v/v) had noticeable effects on the protein yield of both tested organisms. Addition of 5 ml *C. albicans* inoculum/flask led to the highest protein productivity and more than 24.7% of that with 2.5 ml inoculum. Concerning, *S. cerevisiae*, the protein level reached the maximum with 7.5 ml inoculum/flask, where the protein productivity recorded 22.34% increase than that with 2.5 ml inoculum/flask. Accordingly, 10 and 15% (v/v) inoculum were applied in the

succeeding experiments for the two organisms, in the same order.

The effect of culture type (shaken or static) on the protein productivity by either *Candida albicans* or *Saccharomyces cerevisiae* applying all the other specified conditions was also studied. The results disclosed that the protein productivity increased with the shaking speed. It was worthy to note that, for both tested organisms, the static culture afforded lower protein yields than that by shaken one, where the protein yield of the static culture was about 83% that in the shaken culture (200 rpm) of both organisms.

Conclusively, the optimized culture medium for considerable protein production by any of the chosen organisms was formulated as follows (g/L):

- *C. albicans*: WB 7.5 (g/flask); K_2HPO_4, 1.5; $MgSO_4.7H_2O$, 1.5; $(NH_4)_2SO_4$, 4.0; glucose, 15.0; $MnSO_4.H_2O$, 1.68.
- *S. cerevisiae*: WB 5.5 (g/flask); K_2HPO_4, 1.5; $MgSO_4.7H_2O$, 1.5; milk-whey, 0.4; glucose, 10.0; beet molasses, 30.0.

Both the production media were inculated with 48-h-old inoculum (10 or 15%, v/v) respectively. The inoculated flasks were incubated in thermostatic shaker (200 rpm) at 30°C for 7 days. The protein enrichment in the two cultures evaluated to 314 & 252% than that of the original WB sample (12.37%).

A sufficient protein quantity (filtrate & biomass) was prepared for each strain for the succeeding experiments. At the end of fermentation period many cultures of each were collected, mixed, filtered and centrifuged. The clear filtrate was frozen and lyophilized, while the residual biomass (WB residue + cell growth) was introduced to protein extraction process.

Extraction of the biomass protein

The residual biomass, after separation of the culture filtrate, contained the cell growth and the residual unfermented wheat bran containing 80.85% moisture. The crude protein content (N× 6.25) of such biomass calculated on the dry basis amounted to 27.69 and 25.76 % for *Candida albicans* NRRL YB-242 and *Saccharomyces cerevisiae* NRRL Y-12632, respectively compared to 12.37% protein in the primary wheat bran substrate. The effective use of microbial protein for human food requires liberation of cell proteins by destruction of indigestible cell walls and reduction of nucleic acid contents (Kinsella & Shetty, 1978; Parajo *et al.*, 1995 and Nasseri *et al.*, 2011).

The extraction of microbial biomass protein of *C. albicans* or *S. cerevisiae* was carried out applying different buffers (0.1M citrate, pH 5.0, phosphate, pH 7 or bicarbonate, pH 10.0) at 25°C, NaOH (0.1 & 0.2M at 25 and 70°C), NaCl (0.45 &0.9M) or mixture of the most effective extracting agents at 25°C (Table 3) . The biomass sample was well mixed with any of the different extracting agents (1:20, w/v) and the extraction process was undergone at different temperatures for 10 min. At the end of the extraction, the filtrate was separated, centrifuged and the clear supernant was used for protein estimation.

Regarding the biomass protein extracts of the two aforementioned strains, among the tested extracting agents, 0.2M-NaOH at 25°C was the most efficient and afforded the highest level of the protein extract. 0.1M citrate buffer, pH 5.0 & 0.1M phosphate buffer, pH 7.0 were the lowest effective and extracted 26.09 & 46.35% % of that by 0.2M-NaOH at 25 °C. In addition, elevation of the extracting temperature to 70°C improved the protein extraction with 0.2M-NaOH by 75.59 & 39.11% than that at 25 °C from the two strains biomass, respectively. The superiority of NaOH as protein extracting agent may be attributed to its ability to hydrolyze the microbial cell wall and hence extraction of the protein within cells. In addition treatment with NaOH at higher temperature (70°C) afforded the lowest nucleic acid content as reported by many authors (Gnan & Abodreheba, 1987; Svetkin *et al.,* 1987 and Abou-Zeid *et al.*, 1995).

Table 3

The nucleic acid contents in the most promising biomass protein extracts of *S. cerevisiae* or *C. albicans* were estimated. The data obtained (Fig. 2) revealed that the percentage of the nucleic acids within the protein extract (mg nucleic acids/mg protein extract×100) was the highest with phosphate buffer or phosphate buffer/NaCl mixture (6.40 and 6.54%, respectively). On the contrary, the nucleic acid content in the protein extract considerably decreased via extraction with either 0.1 or 0.2-M-NaOH at 70°C (1.91 & 1.62 %, respectively).

All the biomass protein extraction trials preceded in the previous experiment could not offer more than 55.93% of the total *C. albicans* biomass protein after 10 min, therefore the extension of the extraction time seemed to be obligatory. Accordingly, an extraction time- course was carried out from 10-120 minutes by the foregoing extracting agent (0.2M-NaOH) at 70°C. The maximal extraction was recorded after 90 & 120 min and amounted to 140.09 & 143.47%, respectively, that after 10 min. In addition, the extraction of *C. albicans* biomass protein at 70°C for 90 min was 20.64% higher than that by soaking at room temperature for 24h. Many successive extraction processes of the C. *albicans* biomass protein were performed repeatly using 0.2M-NaOH at 70°C each time for 90 min till all or the maximal biomass protein was extracted and protein extract yield was estimated in the usual manner (Table 4 & Fig. 3). The first extraction succeeded to yield most of the biomass protein (78.35%, recovery) of the total biomass protein. An additional 10.01% protein recovery was added by the second extract; also the third extract added 5.03% recovered protein. The fourth extract signed the end of the extraction and added 3.15% protein recovery achieving total protein recovery of 96.54% with the four extraction processes. Further extraction failed to add any other protein at all.

The present attempts carried out for protein extraction were collectively harmonized with those reported by many authors and included the microbial cell-wall disintegration to obtain the entire protein (Parajo *et al.,* 1995; Grigorova, *et al.*, 1997 and Anupama & Ravendra 2001). Chemical methods of cell rupture include the treatment of cells with detergents (surfactants), alkalis, organic solvents, or by osmotic shock. Alkali treatment disrupts the cell walls in a number of ways including the saponification of lipids. Alkali treatment is inexpensive and effective.

The batch extraction process was performed for 4 times by 0.2M-NaOH each for 90 min at 70°C and the extracted protein was adjusted to pH 6-7 with HCl. After complete homogenization with stirring of the whole extracted protein solution, the excess chloride and low molecular weight protein fractions were expelled out through a dialysis bag. The chloride free protein was lyophilized for application in all succeeding studies.

Evaluation of *Candida albicans* protein forms

The evaluation of the *C. albicans* lyophilized protein forms of the culture filtrate or the biomass extract was performed. This includes the percent chemical composition (Table 5) and the physical features of the four *C. albicans* lyophilized protein forms.

The data demonstrated near values of moisture% for the different two crude protein forms, which ranged from 8.22-8.54%. Considerable amounts of total, true & soluble (Lowery) proteins were found in both forms. The biomass extract protein was constantly the richest in all the three kinds of protein and possessed 32.05, 26.77 & 24.41%, respectively versus 22.48, 20.29 & 19.30% in the culture filtrate protein form.

The protein content in the present protein forms were in agreeable with that reported by many authors applying different protein producers. Batch SCP production from rice polishing with *C. utilis* in continuously aerated tank reactor produced biomass with protein level 32.75% (Rajoka *et al.*, 2006). On the other hand, lower protein levels (26%) were reported by *C. tropicalis* grown on salad oil manufacturing wastewater (Zheng *et al.*, 2005).

Fig 2

Table 4: Effect of repeated extraction on the maximal biomass (1g) protein yield.

Extraction (90 min, 70°C)	BM extracted protein (mg)	%
1st extract	41.55	78.35
2nd extract	5.31	10.01
3rd extract	2.67	5.03
4th extract	1.67	3.15
Total	51.2	96.54

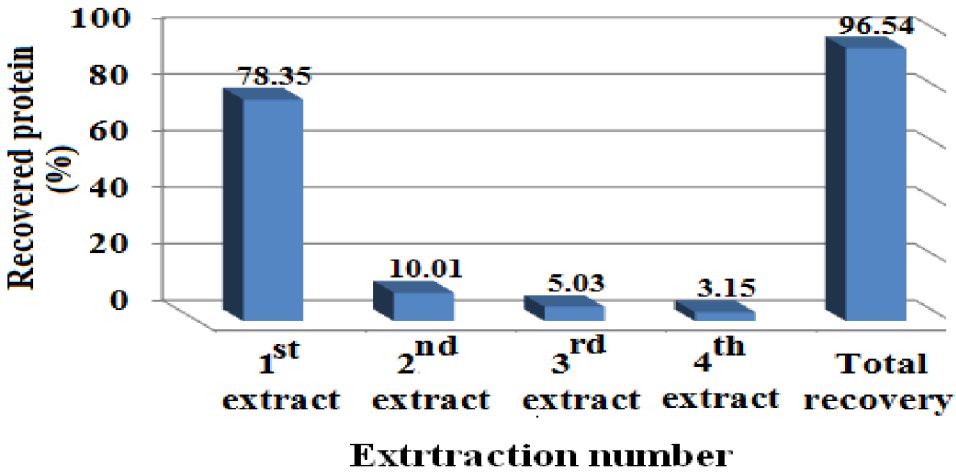

Fig. 3: Effect of repeated extraction on the maximal biomass (1g) protein yield.

Table 5: Chemical composition of the different *Candida albicans* lyophilized protein forms

Component \ *C. albicans* Protein	Culture filtrate	Biomass extract
Moisture	8.54	8.22
Fats	8.33	6.62
Total protein	22.48	32.05
True protein	20.29	26.77
Lowery protein	19.30	24.41
Carbohydrate	63.96	46.31
Ash	9.66	2.90
Nucleic acids	0.41	0.13
pH [b]	6.34	5.80

a: values calculated on dry weight bases.
b: pH was measured in 1.0 mg/ml protein solution in distilled H_2O

Concerning the lipids content in the different *C. albicans* protein forms, the crude filtrate possessed the highest value, which amounted to 8.33% versus 6.62% in the crude biomass extract. In this connection, Chanda & Chakrabarti (1996) reported near values (7-8%) in the biomass of three yeast species harvested after 4 days. Also, Paraskevopoulou *et al.*, 2003, found 4% lipids in SCP produced by *kefir* microflora. The lipids detected in the *C. albicans* protein pointed out the involvement of lipoproteins which possess a particular function to transport lipids (such as triacylglycerol) around the body in the blood, where all cells use the rely on fats and cholesterols as building blocks to create the multiple membranes that cells use both to control internal water content and water soluble elements and to organize their internal structure and protein enzymatic systems.

On the other hand, both protein forms contain high carbohydrate levels indicating the presence of the beneficial glycoproteins, which are known as healthy protein as the familiar P-glycoprotein, which acts as powerful pumps ejecting many hundreds of different molecules ranging in size from tens to hundreds of atoms. These include many toxic molecules, but also anticancer drugs, nevertheless the researchers found the way to block the motion needed for pumping by binding some antibodies to small regions on P-glycoprotein (Vasudevan *et al.*, 1998 and Aller *et al.*, 2009). The crude filtrate protein of *C. albicans* possessed the highest carbohydrate level (63.96%), while the biomass extract contained 46.31%. In this respect, Chanda & Chakrabarti (1996) reported lesser carbohydrate levels in the biomass harvested from three yeast species and ranged from 20.6-23.2%. Also, Shojaosadati *et al.,* (1999) found 24% carbohydrate in *Hansula sp.* SCP and the other SCP products from *Candida utilis*, *Fusarium graminearum* or *P. varioti* were carbohydrate- free.

Ash in any protein preparation indicating the presence of many minerals, the data indicated that *C. albicans* lyophilized culture filtrate had the higher ash level (9.66%) pointing out the higher mineral concentrations. These values were similar to those reported for *C. utilis* SCP (11.2%) by Shojaosadati *et al.,* (1999) and 12.95% by Rajoka *et al.*, 2006, also more or less values were reported for single cell protein by other authors (Kurbanglu, 2001 and Paraskevopoulou *et al.*, 2003).

The nucleic acids content in the *C. albicans* protein forms were determined and the data pointed out low nucleic acids level in both protein preparations (0.41 & 0.13%). Rojoka *et al.,* 2006 reported 0.275% nucleic acids in *C. utilis* SCP produced through the fermentation in an aerated 14 L fermentor. On the contrary, each of Shojaosadati *et al.,* (1999) and Kurbanglu, 2001 reported higher values (8.7 & 4.7% in *Hansula* sp. & *C. utilis NRRLY-900* SCP, respectively). All the *C. albicans* protein forms had the desirable pH values, in their aqueous solutions, which ranged from 5.8-6.34.

In conclusion, the entity of the present article aimed at the preparation of cheap and highly safe (mycotoxin-free) protein-rich fungal preparation with low nucleic acids level and highly nutritive value. The chosen fungal strain *Candida albicans* NRRL YB-242 afforded a considerable entra and extra protein yields, which totally amounted to 45.91% of the biomass dry weight after 7 days in shaken culture (200 rpm), utilizing one of the abundant food industrial wastes, i.e. wheat bran. The study included many successful created and simple methods for the perfect biomass protein extraction, dryness and storage, suitable for pilot and large-scale protein production. The diversity uses of the prepared protein may be extended to medical uses, also as an additive to some healthy foods and medical preparations after purification processes.

REFERENCES

Abdel- Fattah, A.F. and Association (1978). Utilization of Sugar-Cane bagasses. The First Technical Report, NRC, EGYPT, Grant No. FG-EG-180, USA Dept. of Agric., USA.

Abdel-Fattah, A.F., S.S. Mabrouk and A.S. Ismail (1981). Purification and some properties of *Trichodema lignorum*

polygalacturonase. Microbiol Espan., 36: 59.

Abou-Zeid, A.A., J.A. Khan and K.O. Abulnaja (1995). On methods for reduction of nucleic acids content in a single-cell protein from gas oil. Bioresource Tech., 52: 21.

Aller, S.G., J. Yu, A. Ward, Y. Weng, S. Chittaboina, R. Zhuo, P.M. Harrell, Y.T. Trinh, Q. Zhang, I.L. Urbatsch and G. Chang (2009). Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science 323: 1718.

Anupama, R.P. and P. Ravendra (2001). Studies on production of single cell protein by *Aspergillus niger* in solid state fermentation of rice bran. Br. Archives of Biology & Tech. 44: 79.

Anupama, R.P. (2000). Value-added food: single cell protein. Biotech. Advances. 18: 459.

AOAC (1980). Official Methods of Analysis, 13th ed. Association of Official Agricultural Chemists, Washington, D.C.

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

Bullerman, L.B. (1974). Screening medium and methods to detect several mycotoxins in mold cultures. Milk. Food Technolo. 37:1.

Calloway, D.H. (1974). The place of single cell protein in man's diet. In: "Single cell protein". Davies, P. editor. New York: Academic Press, pp. 129-146.

Chanda, S. and S. Chakrabarti (1996). Plant origin liquid waste: A resource for single cell production by yeast. Bioresource Technol. 57:51.

Choteborska, P.B., M.G. Palmarola-Adrados, G. Zacchi, K. Melzoch and M. Rychtera (2004). Processing of wheat bran to sugar solution, J. Food Eng. 61: 561.

De Gregorio, A., G. Mandalari, N. Arena, F. Nucita, M.M. Tripodo and R.B. Lo Curto (2002). SCP and crude pectinase production by slurry-state fermentation of lemon pulps. Bioresource Technol. 83: 89.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F.C. Smith (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350.

El-Fouly, M.Z., T.M. El-Mongy, M.R. Abo-Shady and A.B. Abd-El-Aziz (2001). Production of single cell protein (SCP) by local yeast isolates. Al-Azhar J. Microbiol. 51: 219.

Gnan, S.O. and A.O. Abodreheba (1987). Single-cell protein from methanol with *Enterobacter aerogenes*. Biotechnol. Bioengin. 29: 355.

Grigorova, D., Z. Roshkova, K. Pavlova and T. Hristozova (1997). Protein isolates from *Candida boidinii* M-363 grown on methanol. Food Research International, 30: 29.

Hassan, A.A. (2006). Microbiological studies on sugar beet wastes, M.Sc. Thesis in Food Technology, Fac. Agr., Minufiya Univ., Egypt.

Ismail, A.S. (1996). Utilization of orange peels for the production of multienzyme complex by some fungal strains. Process Biochemistry, 31: 645.

Kim, M. and D.F. Day (2011). Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. J. Ind. Microbiol. Biotechnol. 38: 803.

Kinsella, J.E. and K.J. Shetty (1978). Yeast proteins: recovery, nutritional and functional properties. Advances Exp. Medicine & Biology. 105: 797.

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

Litchfield, J.H. (1987). Microbiological and enzymic treatments for utilizing agricultural and food processing wastes, Food Biotechnol. 1: 29.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall (1951). Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265.

Mansour, O. (1963). Title paper and furfural from the agriculture residue. M.Sc. Thesis, Fac. Sci., Cairo Univ. Egypt.

Murad, H.A. (1985). Studies on production of fungal protein using food industry wastes. M.Sc. Thesis, Fac. Agri., Moshtohor, Zagazig Univ. Egypt.

Mustafa, O. (2004). Conversion of agricultural and industrial wastes for single cell protein production and pollution potential reduction. A review. Fresesnius Environ. Bull. 13: 693.

Nasseri, A.T., S. Rasoul-Amini, M.H. Morowvat and Y. Ghasemi (2011). Single cell protein: production and process, American J. Food Technol. 6: 103.

Ndlouv, J. and A.J. Afolayan (2008). Nutritional analysis of the south Africa wild vegetable *Corchorus olitorius* L, Asian J. Plant Sci. 7:615.

Parajo, J.C.; Santos, V.; Dominguez, H. and Vazquez, M. (1995). NH_4OH-based pretreatment for improving the nutritional quality of single-cell protein (SCP). Biochem. Biotechnol. 55: 133.

Paraskevopoulou, A., I. Athanasiadis, M. Kanellaki, A. Bekatorou, G. Blekas and V. Kiosseoglou (2003). Functional properties of single cell protein produced by *kefir* microflora. Food Research International. 36: 431.

Pérez, J., J. Muñoz-Dorado, T. De-la-Rubia and J. Martínez (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int. Microbiol. 5: 53.

Paster, N., N. Lisker and I. Chet (1983). Ochratoxin A production by *Aspergillus ochraceus* Wihelm grown under controlled atmosphere. Applied Environ. Microbiol. 45: 1136.

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

Sánchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnol. Adv. 27: 185.

Scrimshaw, N.S. and E.B. Murray (1995). Nutritional value and safety of "single cell protein, Biotechnology (2nd Edition) 221-237.

Sharma, D., R. Appa and S.M. Totey (2000). Measurement of within and between population genetic variability in quails. Br. Poult. Sci. 41: 29.

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

Singh, B.D. (1998). Biotechnology. New Delhi: Kalyani Publishers, pp. 498–510.

Suhaila, M.A. (2010). Isolation of hemicelluloses from sugar cane bagasses by alkaline extraction, Final Year Project Report, Fac. of Appl. Sci., Univ. Teknologi, MARA.

Svetkin, Y.V., L.E. Mikhalenko, R.L. Medvedovskaya, N.N. Artemenko and V.P. Mel'nik (1987). Extraction of proteins from dry biomass of ethanol-oxidizing yeast. Voprosy Khimii Khimicheskoi Tekhnologii. 85: 75.

Ugalde, U.O. and J.L. Castrillo (2002). Single-cell proteins from fungi and yeasts. Appl. Mycol. & Biotechnol. 2: 123.

Van Walbeeck, W., P.M. Scott, J. Harwing and J.W. Lawrence (1969). *Penicillium viridicatum*: a new source of ochratoxin A. Canad. J. Microbiol., 15: 1281.

Parajo, J.C., V. Santos, H. Dominguez and M. Vazquez (1995). NH_4OH-based pretreatment for improving the nutritional quality of single-cell

Vasudevan, S., T. Tsuruo and D.R. Rose (1998). Mode of binding of anti-P-glycoprotein antibody MRK-16 to its antigen. A crystallographic and molecular modeling study. J. Biol. Chem. 273: 25413

Youssif, N.O.A. (1996). Utilization of some industrial and agricultural wastes of sugar crops. Ph.D. Thesis in Food Technology, Fac. Agr. Kafr El-Sheikh, Tanta Univ., Egypt.

Zheng, S., M. Yang and Z. Yang (2005). Biomass production of yeast isolate from salad oil manufacturing wastewater. Bioresource Technol. 96: 1183.

التحويل الحيوى لبعض المخلفات اللجنوسليولوزية الوفيرة من الصناعات الغذائية الى بروتينات مأمونه عالية القيمة الغذائية

عبد المحسن صابر إسماعيل[1] ، أبو الفتح عبد القادر البديوى[2] ،
علاء الدين السيد البلتاجى[2] ، أميرة على حسن[1]

[1] قسم كيمياء المنتجات الطبيعية و الميكروبية – المركز القومى للبحوث

[2] قسم علوم وتكنولوجيا الأغذية – كلية الزراعة جامعة المنوفية

الملخص العربي

أسفر المسح على عشرة سلالات من الفطريات و الخميرة بتنميتها على ثمانية مخلفات أغذية محلية، عن إختيار كل من السلالة *Candia albicans* NRRL YB-242 والسلالة *Saccharomyces cerevisiae* NRRL Y-12632 كأقوى منتج للبروتين عالى القيمة الغذائية والمأمون (الخالى تماما من السموم) علاوة على إنخفاض محتواه من الأحماض النووية.

تم تصميم البيئة المثلى لإنتاج البروتين لكل من السلالتين حيث كانت مكوناتها كما يلى: نخالة القمح، كبريتات الأمونيوم، خليط الجلوكوز وكبريتات المنجنيز (كإضافات) وذلك للكائن *C. albicans*، ومن نخالة القمح، شرش اللبن، الجلوكوز مع مولاس بنجر السكر (كإضافات) للكائن الآخر *S. cerevisiae*. كانت تقنية المزارع المهتزة ذات عمر سبعة أيام عند 30 درجة مئوية وعند رقم هيدروجيني إبتدائى 4,7 هى الأفضل من تقنية التخمرات الصلبة، وقد أسفرت عن أعلى إنتاجية للبروتين لكل من الكائنين سابقى الذكر. و كان عمر اللقاح الأمثل هو 48 ساعة وحجمه 5، 7,5 ملى/زجاجة على التوالي.

تم إجراء محاولات عديدة للإستخلاص الأمثل لبروتين الكتلة الحيوية وأظهر محلول 0.2 جزيئى هيدروكسيد الصوديوم أعلى قدرة على إستخلاص بروتين الكتلة الحيوية لكل من الكائنين.

وقد تم التقييم الكامل للصور البروتينية المجفدة (الراشح و الكتلة الحيوية)، حيث إحتوت صور البروتين المجفدة الخام للراشح أو الكتلة الحيوية ل *Candia albicans* على الكثير من المعادن، اللليبوبروتينات و الجليكوبروتينات مع محتوى قليل من الأحماض النووية (أقل من 1.0%) مع خلوها التام من السموم الميكروبية.

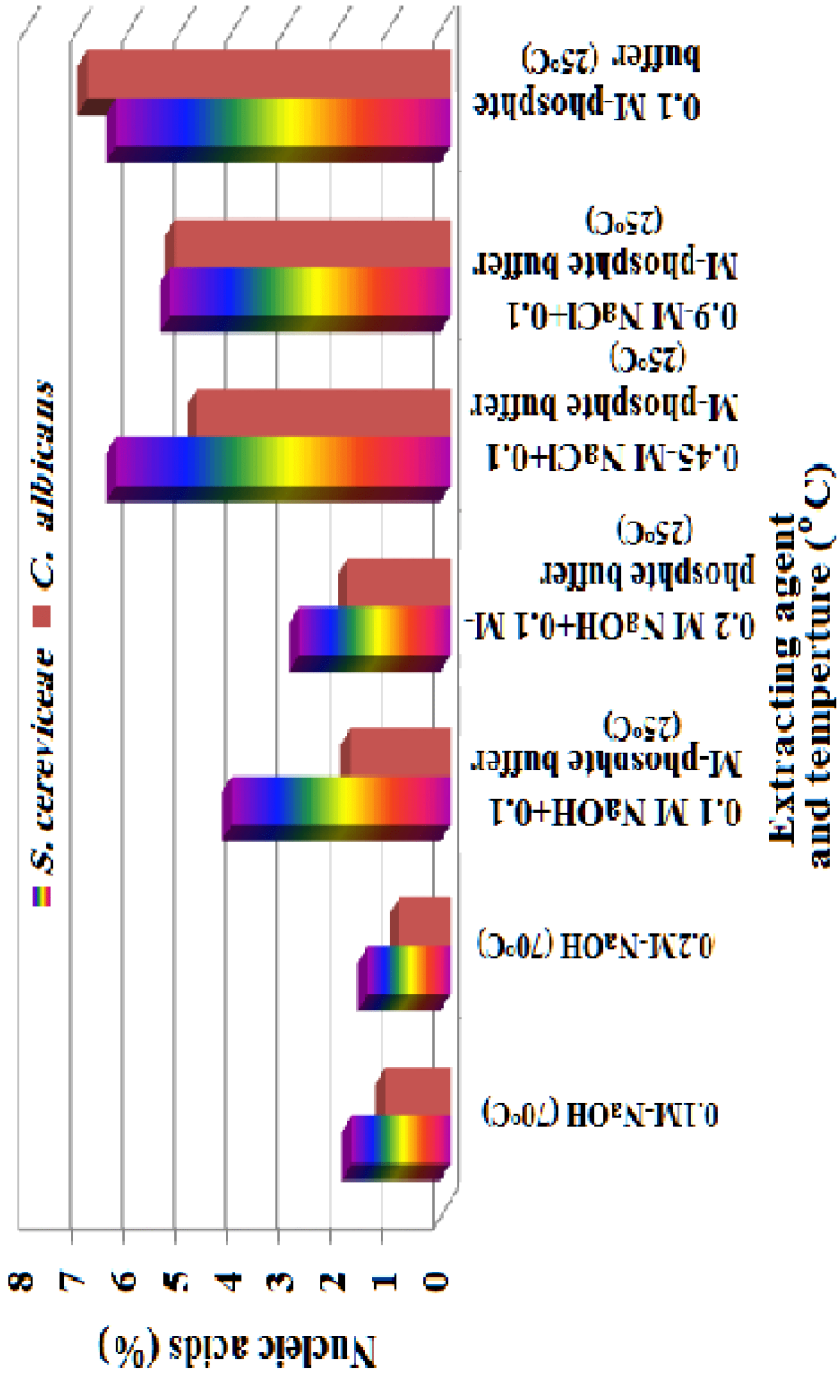

Fig. 2: Nucleic acids content (%) of the different protein extracts using different extracting agents

Table 2: Screening of some microbial strains for single cell protein production utilizing different food industry wastes in shaken cultures at 30°C.

Waste / Microorganism	Protein yield (g/culture)											
	SBP			WB			SCP			PP		
	BP [a]	SP [b]	TP [c]	BP	SP	TP	BP	SP	TP	BP	SP	TP
Aspergillus niger	0.381	0.143	0.524	0.341	0.115	0.456	0.174	0.073	0.247	0.288	0.108	0.396
A. terreus	0.356	0.136	0.492	0.397	0.160	0.557	0.062	0.087	0.149	0.288	0.152	0.440
Trichoderma viride	0.409	0.217	0.626	0.385	0.217	0.602	0.074	0.105	0.179	0.221	0.189	0.410
T. reesei	0.387	0.259	0.646	0.395	0.228	0.623	0.066	0.104	0.170	0.263	0.188	0.451
Chaetomium cellulolyticum	0.266	0.178	0.444	0.207	0.146	0.353	0.106	0.079	0.185	0.230	0.181	0.411
Penicillium funiculosum	0.336	0.183	0.519	0.397	0.178	0.575	0.114	0.077	0.191	0.292	0.214	0.506
Saccharomyces cerevisiae	0.342	0.158	0.500	0.270	0.184	0.454	0.089	0.080	0.169	0.330	0.144	0.474
Pichia pastoris	0.175	0.173	0.348	0.097	0.224	0.324	0.072	0.103	0.175	0.215	0.250	0.465
Candida tropicalis	0.297	0.201	0.498	0.418	0.148	0.566	0.079	0.081	0.16	0.270	0.149	0.419
C. albicans	0.369	0.265	0.634	0.491	0.169	0.660	0.085	0.071	0.156	0.282	0.152	0.434

Table 2: Continued.

Waste / Microorganism	Protein yield (g/culture)											
	OPP			Okra termini			jew's mallow stems			pea pods		
	BP [a]	SP [b]	TP [c]	BP	SP	TP	BP	SP	TP	BP	SP	TP
Aspergillus niger	0.159	0.197	0.356	0.231	0.088	0.319	0.185	0.058	0.243	0.170	0.126	0.296
A. terreus	0.192	0.209	0.401	0.383	0.129	0.512	0.198	0.092	0.290	0.227	0.147	0.374
Trichoderma viride	0.196	0.231	0.427	0.169	0.179	0.308	0.127	0.107	0.234	0.221	0.173	0.394
T. reesei	0.260	0.223	0.483	0.264	0.197	0.461	0.196	0.106	0.302	0.226	0.229	0.455
Chaetomium cellulolyticum	0.160	0.141	0.301	0.272	0.168	0.440	0.175	0.068	0.243	0.185	0.155	0.340
Penicillium funiculosum	0.240	0.262	0.502	0.393	0.174	0.567	0.162	0.085	0.247	0.161	0.209	0.370
Saccharomyces cerevisiae	0.152	0.250	0.402	0.313	0.182	0.495	0.160	0.124	0.284	0.144	0.206	0.350
Pichia pastoris	0.117	0.277	0.394	0.162	0.172	0.334	0.171	0.099	0.270	0.144	0.212	0.356
Candida tropicalis	0.188	0.254	0.442	0.353	0.206	0.559	0.216	0.034	0.250	0.174	0.203	0.377
C. albicans	0.190	0.215	0.405	0.472	0.164	0.636	0.265	0.086	0.351	0.176	0.210	0.386

In this and the following Tables:
a: BP = Biomass protein
b: SP = Soluble protein of the culture filtrate
c: TP = Total protein

Table 3: Extraction of *Saccharomyces cerevisiae* or *Candida albicans* biomass protein at 25 & 70 °C using different extracting agents solrents (1:20, w/v).

Extracting agent	Temperature (°C)	*Saccharomyces cerevisiae*		*Candida albicans*	
		BM Protein extract (mg) [a]	Recovered protein (%)	BM Protein extract (mg) [a]	Recovered protein (%)
0.1 M-NaOH	25	12.14	24.61	19.02	35.87
0.2 M-NaOH		14.83	30.06	20.43	38.53
0.1 M-NaOH	70	22.04	44.67	23.95	45.16
0.2 M-NaOH		26.58	53.88	28.42	53.59
0.1 M NaOH+0.1 M-phosphate buffer pH 7.0	25	7.73	15.67	17.20	32.43
0.2 M NaOH+0.1 M-phosphate buffer pH 7.0		13.09	26.54	18.62	35.11
0.45 M-NaCl		4.38	8.88	9.73	18.35
0.90 M-NaCl		4.14	8.39	10.30	19.42
0.45 M-NaCl + 0.1 M-phosphate buffer pH 7.0		5.66	11.48	11.68	22.03
0.90 M-NaCl + 0.1 M-phosphate buffer 7		6.64	13.46	11.57	21.82
0.1 M-Phosphate buffer, pH 7.0		6.09	12.35	9.47	17.86
0.1 M-Bicarbonate buffer, pH 10.0		5.88	11.91	10.13	19.10
0.1 M-Citrate buffer, pH 5.0		3.87	7.85	9.58	18.07

N.B. Extraction time was lasted for 10 min.

[a] BM= Biomass