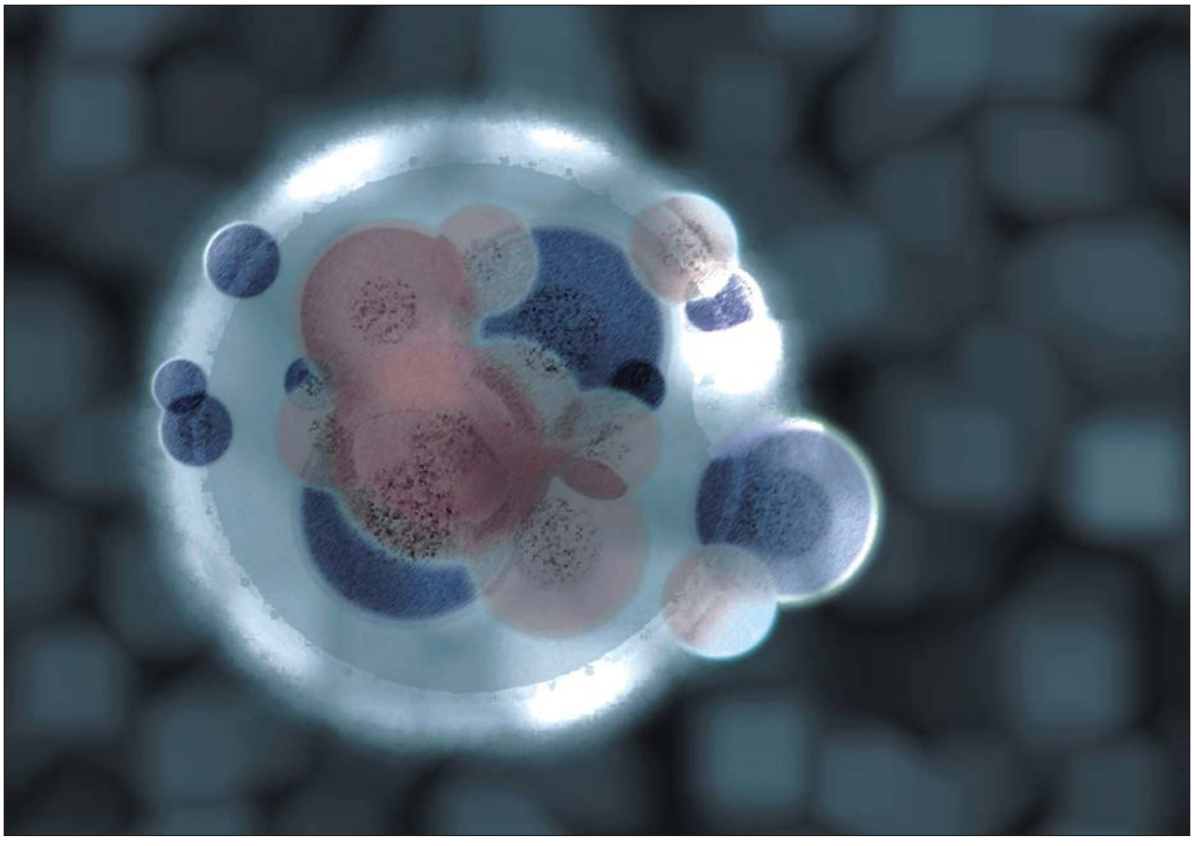

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Process of Optimization and Characterization of Protein Enrichment of Orange Wastes Through Solid State Fermentation by *Aspergillus niger* Isolate No. 5

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Abstract: The present experiment was conducted to apply Solid State Fermentation (SSF) for protein enrichment using Sweet Orange (SWO) and Orange Pulp (OPW) wastes by *Aspergillus niger* isolate No. 5, isolated from Addis Ababa city wastes. The total proteins were extracted to estimate the mycelial biomass from the seven days growth of fungal mold which have grown on orange wastes (SWO and OPW). Sodium hydroxide and phosphate extraction buffers and other growth factors i.e., moisture 60%; temperature, at 30°C; pH at 7.00 and inoculum size 10^6 spores mL^{-1} were found to be most efficient for the extraction of the proteins using the substrates by *Aspergillus niger* isolate No. 5. The maximum protein enrichment of SWO (45.56%) and OPW (47.48%) were obtained at 30°C. The highest protein enrichment 50.84% using substrate SWO was attained at pH 7. The effect of supplementation of the nitrogenous sources on the final biomass was compared with control. The result of using sodium hydroxide (NaOH) buffer has shown that 5 g of substrates, squeezed Sweet Orange Wastes (SWO) and Orange Pulp Wastes (OPW) were produced maximum protein level 23.93 and 23.59% and utilized by *Aspergillus niger* isolate No. 5 at 10^6 spores mL^{-1} inoculum (W/W) protein, respectively.

Key words: *Aspergillus niger* isolate, extraction buffers, orange wastes, protein enrichment, solid state fermentation

INTRODUCTION

The pattern of agricultural production has shifted in the world in such a manner that most of the countries around the world are not self-sufficient in their food production. In many of the developing countries, malnutrition is one of major health problems which can be avoided only by additional intake of nutrients (Ezekiel *et al*., 2010). The food processing industry generates a large amount of wastes annually including crop residues (peels, husks, cobs, shells), sugarcane baggesse, vegetable waste, cheese and whey permeates that pose serious disposal challenges (Gomez *et al*., 2005; Laufenberg *et al*., 2003). Microorganisms can utilize on a variety of inexpensive feedstock and wastes (Nasseri *et al*., 2011). To overcome the existing shortage of food and to enrich with the best and high quality of protein for those who have highly populated people and fewer amounts of protein contents of animals feed, it is important to produce use of microbial protein by Solid State Fermentation (SSF). Fermented farm byproducts can form protein-rich nutrients (Anupama and Ravindra, 2000). Advanced technologies such as Solid State Fermentation (SSF) can alleviate food deficiencies with developing nation, with enhanced nutritional and market values (Raghavarao *et al*., 2003). The SSF techmique is more suitable for fungal biomass cultivation compared to the others microbes (Pandey, 2003; Shojaosadati *et al*., 1999). In the solid state fermentation, the moisture level of the substrates and other growth factors have been considered as important factors for the growth, production and activity of microbial culture. In some regions, single cell protein could become the principal protein source that is used for domestic livestock, depending upon the population growth and the availability of plant feed protein sources. Currently, in Ethiopia there is a large and increasing quantity of citrus processing wastes are disposed of every year mainly in the form of citrus pulp wastes which is the semi solid by-product obtained after juice extraction. For this purpose, there are sufficient resources and disposable materials that can be improved through optimization and determination of protein enrichment using Sweet Orange Wastes (SWO) and Orange Pulp/peeled Wastes (OPW) exploiting *Aspergillus niger* isolate No. 5 in the country. Therefore, the objectives of this study were to investigate optimum growth conditions, inoculum potential, particle size and nitrogenous sources of SWO and OPW. This study was also planned to evaluate and determine the amount of protein produced by *A. niger* isolate No. 5 on squeezed SWO and OPW for animal feed through SSF technology.

MATERIALS AND METHODS

Sample collection and preparation of substrates: The orange peel/pulp wastes (OPW) (white and red mesocarp) was obtained after removal of the external part of the skin (orange -yellow-exocarp) and the Squeezed Sweet Orange Wastes (SWO) were obtained and purchased from local juice shops in Addis Ababa and some orange samples were collected from orange growing areas of Nazareth, Metahara, Awassa, Zeway areas of Ethiopia. The fresh peeled orange fruit wastes were collected from different juice makers shops found around Arat kilo, Addis Ababa. The orange pulp and peel were sun dried for five days. The dried orange wastes (SWO and OPW) were ground to fine particle size using mortar and pestle stored in plastic bags for subsequent laboratory experiment. The samples of orange peel and skin cut in to small pieces of 1-1.5 cm and sterilized at 121°C for 15 min. The *Aspergillus niger* isolates were used for inoculating of 10 g of orange peel and orange wastes (skin and orange). After 7 days of growth of fungal cultures on the substrates, 2 g of growth was taken and homogenized with buffers for determination of amount of protein produced by using *A. niger* isolate No. 5.

Isolation of fungal isolates: The various types of fungal species were isolated from decaying wastes and composts of municipal solid wastes disposal site of Addis Ababa, Nazareth, Metahara, Awassa, Zeway areas of Ethiopia. Isolation of the fungal isolates was done by serial dilution agar plating method (Aneja, 2005). Ten grams of the collected wastes were dried and powdered aseptically homogenized with 90 mL sterile distilled water under aseptic conditions. One milliliter of soil suspension was taken from 10^1 dilution and mixed with 9 mL sterile distilled water to make 10^2 dilution. The same amount was taken from 10^2 dilution solution and used to make 10^3. This was done until 10^5 dilution factors one mL from each dilution factor (10^2 to 10^5) was taken and placed on triplicate plates of on Czapeck Dox's agar medium (CDA) by pour plate technique. Then, plates were incubated at 30°C for 5 days. Colony types were also recorded. Representative colonies were picked at random from the plates. The fungal colonies were purified on potato dextrose agar medium. The pure cultures of fungi were also kept on slants of PDA for further studied. The various isolates of *Aspergillus niger* were used for the growth and stock cultures. Stocks were maintained at 25°C and 40-60% of humidity in an incubator. Among these, only *A. niger* isolate No. 5 was selected for this study. However, *A. niger* isolates were maintained by sub culturing on Malt Extract Agar (MEA), Potato Dextrose Agar (PDA) media slants incubated for 7 days at 25°C and stored at 4°C for further study.

Identification of fungal isolates: The pure cultures were also kept on slants of PDA for identification of fungal species. They were identified according to their morphological cultural characteristics. Young, actively growing fungal isolates were picked with a sterile needle transformed on to clean glass slides and prepared for microscopic observations using lacto phenol as mountant and cotton blue as stain (Barnett and Hunter, 1972; Raper and Fennel, 1965). The slides were carefully covered with slips to exclude air bubbles. Microscopic examination of the prepared slides was carried out first using the low power objective followed by the middle power magnification objective lens for a closer examination of a selected field. Microscopic identification was based on the structures bearing the spores and on their spores. The various fungal species and isolates were identified (Barnett and Hunter, 1972; Raper and Fennel, 1965). The various isolates of *Aspergillus niger* were identified and pure cultures were preserved in PDA slants for further studies. The various fungal cultures from different areas were identified as *Aspergillus niger* (more than 10 isolates), *Penicillium* spp., *Trichoderma* spp., *Botrytis* spp. and other *Aspergillus* species. *A. niger* isolates were used for evaluation, optimization and production of maximum protein enrichment have been used to fulfill the objective of this study. *A. niger* isolate No.5 isolated from Addis Ababa city waste was selected for protein enrichment throughout this study.

Solid state fermentation (SSF): Ten grams of orange wastes (SWO and OPW), adjusted to 60% moisture content with 0.05 g of $(NH_4)_2SO_4$, autoclaved at 120°C for 15 min and taken having a capacity of in 250 mL Erlenmeyer flask in triplicate. The substrate was inoculated with 2 mL of spore suspension (10^6 spores mL^{-1}) of *A. niger* isolate No. 5. The culture was maintained in stationary conditions at 30°C for six days. The samples were taken every day from each organism and the soluble protein content and total nitrogen were measured by Folin method, modified Kjeldhal method described, respectively (Sertsu and Bekele, 2000).

Effect of moisture content on protein enrichment: The various types of *A. niger* isolates were isolated from different types of municipal wastes, composts, soils, that were collected from different parts of the country. Among

these isolates, *A. niger* No. 5 isolate has produced potential amount of protein. Therefore, *A. niger* isolate No. 5 was used for the entire experiment of this study. The various moisture levels of orange wastes were maintained to study the effect on protein enrichment. The orange wastes (SWO and OPW) in 250 mL Erlenmeyer flask were autoclaved at 121°C for 15 min and the moisture level adjusted with distilled water to obtain (40, 50 and 60%) with *A. niger* isolate No. 5. The cultures were incubated in stationary conditions at 30°C for seven days. The fermentation was carried out under the same conditions as described in the section of solid state fermentation.

Effect of temperature on protein enrichment: *Aspergillus niger* isolate No. 5 was grown at different temperatures in order to study the effect of temperature on protein enrichment of orange wastes. The culture was incubated at 25, 30 and 35°C for seven days in incubators. The protein content was quantified in order to identify the best temperature level at which highest protein content is obtained.

Effect of initial pH on protein enrichment: The effect of initial pH on protein enrichment of orange waste by *A. niger* isolate No. 5 was investigated within the range of 3-7. The pH was adjusted either with 1N NaOH or 1N HCl to pH value of 3, 4, 5 and 7 and control. The original pH of orange peel wastes initial pH with out adjustment was pH 4.29 to SWO and 4.02 to OPW was recorded.

Effect of inoculums dose on protein enrichment: The inoculum was prepared by scraping the spores from a week old Petri dishes into 10 mL of sterile water containing 0.02% of Tween 80%. This experiment was designed in order to determine the best load of substrate utilized by a certain amount of inoculum in a given area. Therefore, the effect of different inoculum size on protein enrichment was studied by adding spores suspension of 10^4, 10^6 and 10^8 spores mL^{-1}. Other parameters like moisture, pH and incubation temperature were kept constant.

Effect of substrate concentration on protein enrichment: Different amount of substrates with the same level of inoculum were used to investigate if there is any substrate inhibition. The modified substrate concentrations 5, 10 and 15 g of substrate were put in 250 mL Erlenmeyer flask and were given equal amount of inoculum. The final protein amount was measured, at 10^6 spores mL^{-1}.

Effect of nitrogen source: The nitrogen stock solution was prepared by adding 0.05 g the compounds $(NH_4)_2SO_4$, NH_4NO_3, $NaNO_3$ and a mixture of $(NH_4)_2SO_4$ and added 10 mL distilled water respectively, were used as nitrogen (N) sources in the medium. The media were supplemented with 1% (w/w) of the above mentioned nitrogen sources. The effect of these sources on protein enrichment was studied while other parameters are kept constant. In this experiment, urea was used as a control.

Analytical methods

Soluble protein determination: The total protein was determined by Folin method (Lowry *et al*., 1951). The following reagents were prepared for the assay: Reagent A: 2% $NaCO_3$ in 0.1 N of NaOH; Reagent B: 2% $CuSO_4.5H_2O$; Reagent C: 2% Sodium potassium tartarate or 2% trisodium citrate; Reagent D: 0.5 mL of B+0.5 mL of C in 99 mL of A (this should be prepared fresh every test experiment); Folin E: Folin Phenol reagent. Dilute Folin Phenol with distilled water to 1N. The same amount of water and folin phenol 1: 1 to change it to 1N. Hundred µL of prepared sample and 900 µL distilled water was added in a test tube in triplicate. One mL of freshly prepared reagent D was added and kept for 10 min at room temperature. Then, 0.5 mL of Folin phenol reagent was added left and kept at dark place for 30 min. After this, the samples were kept in dark place for 30 min. Immediate vortex after each addition of the reagents was recommended in order to get best result. The Optical Density (OD) was measured by spectrophotometer (Jennway 6405 UV/Vis.) at 740 nm. The standard curve for the conversion of the result was constructed using Bovine Serum Albumin (BSA).

Statistical analysis: The ANOVA was done and the means values of the treatments were calculated using the Least Significant Difference (LSD) test at values of <0.05. The statistical analysis was performed using the SPSS software (SPSS institute Inc., Cary, NC) version 13.

RESULTS

The effect of moisture content on protein enrichment: Figure 1 showed the effect of different moisture level for maximum protein enrichment in Orange Pulp Wastes (OPW) and Sweet Orange (SWO) inoculated with *Aspergillus niger* isolate No. 5 after 7 days of incubation at 30°C. The substrate SWO and OPW with 60% moisture yielded maximum protein enrichment (21.8) and (19.78%), respectively. However, the fungal isolate mycelium growth in flasks at 50% moisture content was similar to the growth of that in the flasks of 60% (visual appearance of the mycelial growth on the substrates) SWO (19.96%) and OPW (17.74%), but the protein content was less. At 40% moisture content, the protein content of SWO and

OPW were 15.60 and 15.64% respectively (Fig. 1). The amount of protein produced using the two substrates (SWO and OPW) were increased during gradual increasing of the moisture level from 40% to 60% (Fig. 1). The protein content was analyzed based on the total proteins.

Effect of different temperatures on protein enrichment: The optimum temperature for protein enrichment was found within the range of 25-35°C. The maximum protein enrichment of SWO (45.56%) and OPW (47.48%) were attained at 30°C (Fig. 2). The lowest protein enrichment of SWO (32.00%) and OPW (23.46%) has observed, at 25 and 35°C, respectively by *A. niger* isolate No. 5 (Fig. 2).

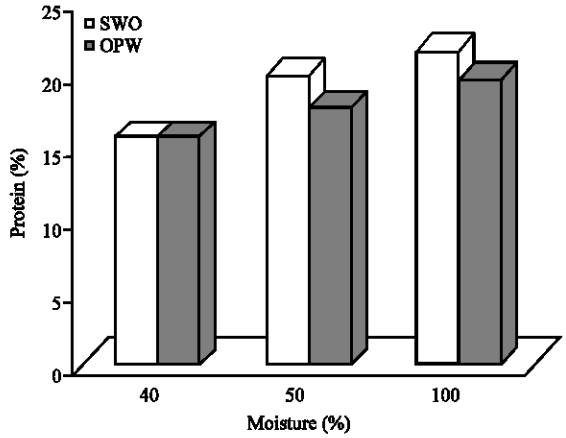

Fig.1: Effect of moisture content on protein enrichment of SWO and OPW by *A. niger* isolate No. 5 (SWO = Sweet orange, OPW = Orange pulp wastes)

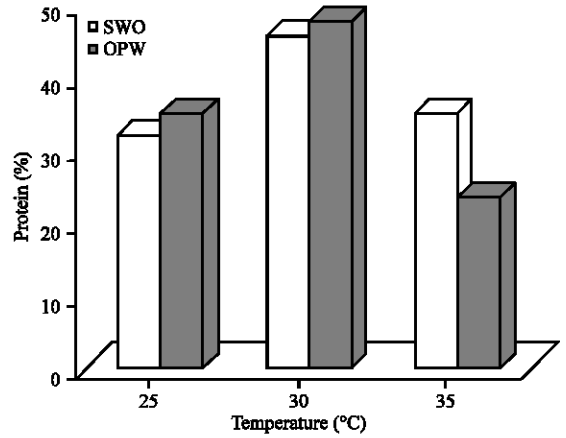

Fig. 2: Effect of temperature on protein enrichment of SWO and OPW by *A. niger* isolate No. 5 (SWO = Sweet orange, OPW = Orange pulp wastes)

Effect of initial pH on protein enrichment of orange wastes and orange yellow substrates by *A. niger* isolate No. 5: The maximum protein enrichment 50.84% using substrate SWO was attained with initial pH 7. For both substrates orange pulp wastes (OPW) and orange sweet (SWO) the optimum pH 3 and 4 respectively (Fig. 3). The lowest protein enrichment 25.12 and 26.60% has been obtained at pH 3 and 5 using substrate SWO and OPW respectively. It is evident from Fig. 3 that the initial pH 3 has adversely affected the final protein enrichment using the substrate SWO. However, at pH 3 and pH 4 of substrates SWO and OPW have obtained the same value of protein amount was produced 40.50% (Fig. 3).

Effect of inoculum size on protein enrichment: The size of inoculum was observed to be important factor influencing the growth of *A. niger* isolate No. 5 the spore suspension count was determined with the help of Heamocytometer. The inoculum potential containing spores 10^4, 10^6 and 10^8 was utilized. The result showed that 10^6 spore g^{-1} of substrates SWO and OPW produced maximum protein enrichment of 52.48% and 46.50% respectively (Fig. 4). However, the lowest protein enrichment (33.52 and 35.5%) were obtained 10^8 spores g^{-1} of substrates SWO and OPW, respectively (Fig. 4).

Effect of particle size: *Aspergillus niger* isolate No. 5 was grown on SWO and OPW of particle sizes 0.71 mm and greater than 0.71 mm mesh size. The result showed that the amount of protein yield increased with an increasing in the particle size of substrates SWO and OPW. The maximum protein yield 24.04 % (SWO) and 22.76 % (OPW) was obtained with> 0.71 mm mesh size while the optimum

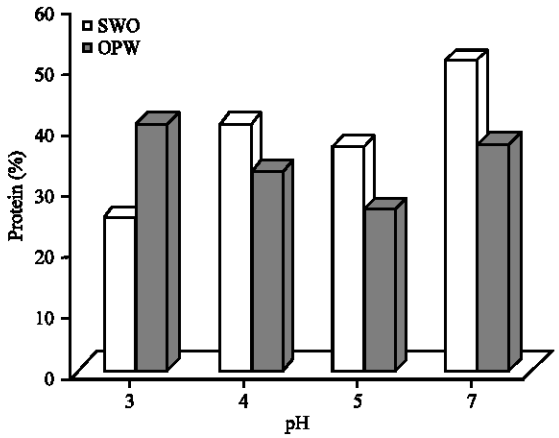

Fig. 3: Effect of pH on protein enrichment of SWO and OPW by *A. niger* isolate No. 5 (SWO = Sweet orange, OPW = Orange pulp wastes)

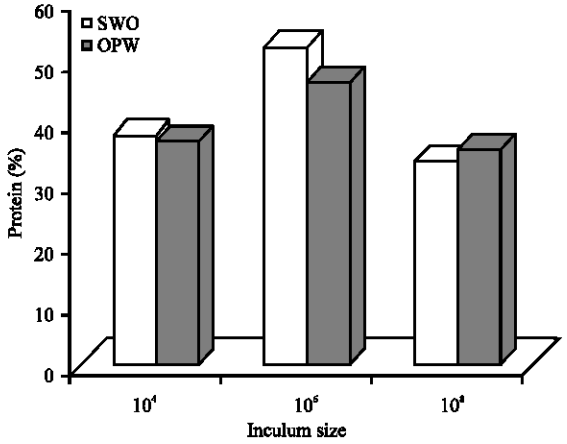

Fig. 4: Effect of inoculums size on protein enrichment of substrates SWO and OPW by *A. niger* isolate No. 5 (SWO = Sweet orange, OPW = Orange pulp wastes)

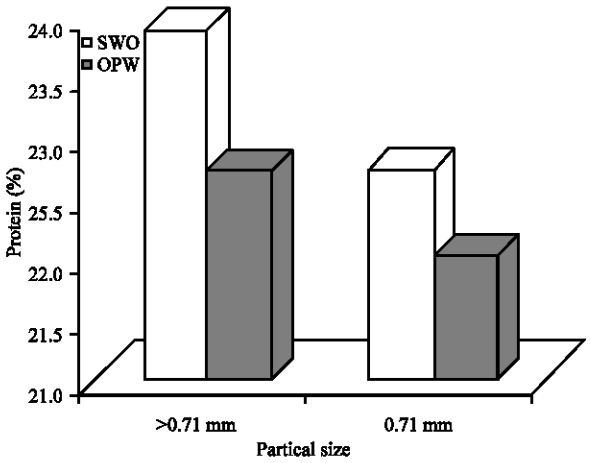

Fig. 5: Effect of particle size on protein enrichment of substrates SWO and OPW by *A. niger* isolate No-5 (SWO = Sweet orange, OPW = Orange pulp wastes)

protein yield of protein 22.73% (SWO) and 22.99% (OPW) was attained at 0.71 mm mesh size, during the investigation of this experiment (Fig. 5).

Effect of different nitrogen sources on protein enrichment by *A. niger* isolate No. 5: The study of various inorganic nutrients of $(NH_4)_2 SO_4$; NH_4NO_3 and $(NaNO_3)$ were carried out at 1% concentration of each nitrogen source after 7 days of incubation, at 30°C. A maximum protein enrichment of 23.62 and 24.04% was obtaining, using the substrates SWO and OPW with ammonium sulfate followed by sodium nitrate as the

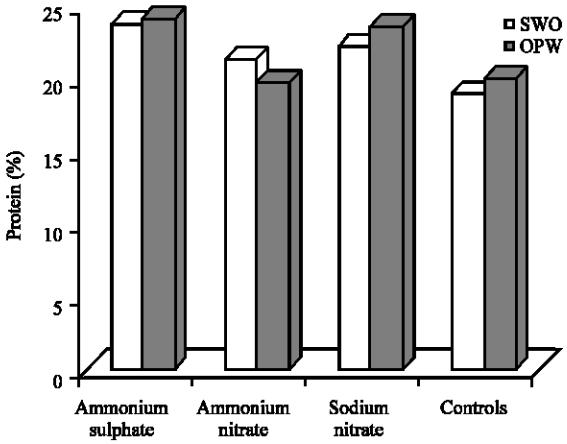

Fig. 6: Effect of nitrogenous sources on protein enrichment of substrates SWO and OPW by *A. niger* isolate No. 5 (SWO = Sweet orange, OPW = Orange pulp wastes)

nitrogen source (Fig. 6). The lowest yield of protein 21.25% (SWO) and 19.67% (OPW) was attained by ammonium nitrate. The maximum protein enrichment 18.95% and 19.96% were obtained when applied urea as a control using the substrates of SWO and OPW by *A. niger* isolate No. 5 (Fig. 6).

Effect of substrate dose on protein enrichment: The result of this experiment using sodium hydroxide (NaOH) has shown that 5 g of substrates SWO and OPW was produced maximum protein level 23.93 and 23.59% and utilized by *A. niger* isolate No. 5 at 10^6 spores mL^{-1} inoculum (W/W) protein, respectively (Fig. 7). The minimum protein content 15.67% (SWO) and 17.71% (OPW) was obtained with 15 g of substrates by the extraction of sodium hydroxide. Where as the result of using phosphate (Na_2HPO_4) buffer has known 10 g (SWO) and 10 g (OPW) of substrates were obtained the maximum protein enrichment 20.45 and 20.08%, respectively. However, the lowest protein enrichment 19.52 and 14.63% was obtained from 5 g of substrate SWO and OPW which was utilized by *A. niger* isolate No. 5 with the extraction of phosphate buffer (Fig. 7).

DISCUSSION

Using the substrates of Sweet Orange (SWO) and Orange Pulp Wastes (OPW) with the moisture content of 60% yielded maximum protein enrichment 21.8 and 19.78%, respectively. However, the result obtained has showed that at 40% moisture level using orange wastes KA-06 (31.7% and KC-06 (29.5%)) gave maximum protein

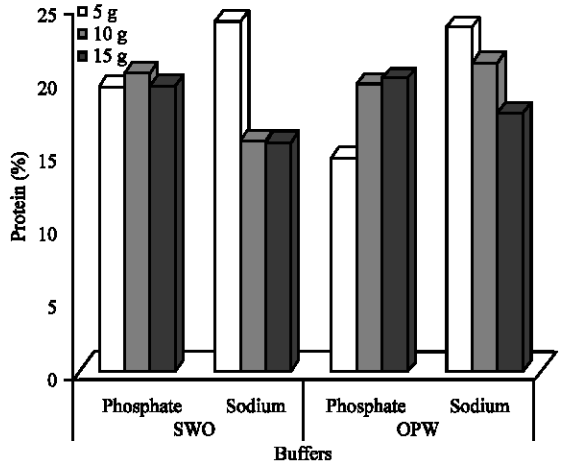

Fig. 7: Effect of substrate load using buffers on protein enrichment of substrates SWO and OPW by *A. niger* isolate No. 5 (SWO = Sweet orange, OPW = Orange pulp wastes)

production in solid state fermentation (Yalemtesfa, 2007; Yalemtesfa *et al*., 2010). In this study, the protein enrichment that was obtained is less than what was obtained (Yalemtesfa, 2007). This is most probably due to the fungal isolates' metabolic activities, isolates variations and as well as the amount, nutrient content and types of substrates that were used during the year of the investigation of the experiment.

Then highest protein enrichment SWO 45.56% and OPW 47.48% were obtained by *Aspergillus niger* isolate No. 5 after incubation, at 30°C. From this result, it is possible to conclude that for the production high protein enrichment using the substrates (SWO and OPW) in solid state fermentation needs regular and continues optimum temperatures. Similarly, it has reported that at, 28°C for *Aspergillus oryzae* on deoiled rice bran and attained 18.9% of soluble protein. It has observed that the protein content was declined at lower temperature (Shojaosadati *et al*., 1999; Rudravaram *et al*., 2006; Ravindra *et al*., 2009).

The highest protein enrichment 50.84 and 40.50% was obtained at the value of pH 7 and 3 using both substrates SWO and OPW by *A. niger* isolate No. 5, respectively. Similarly, result has obtained the maximum protein enrichment (18.90%) with initial pH 6 (Rudravaram *et al*., 2006). The highest microbial mass 29.39 and 30.50% was attained at pH value of 5.5 for KC-06 and pH 7 for KA-06, respectively (Yalemtesfa, 2007). However, it has observed on optimizing medium conditions for attaining higher production of bio-protein, with pH 4 results in 9.256 g protein/100 g *Opuntia* sp skins (Nasseri *et al*., 2011). From the result of this experiment, it has been concluded that suitable pH (3 and 7) for the growth of fungal isolate and ability of metabolized the substrates SWO and OPW showed that tends to yield the maximum amount of protein enrichment of 50.84 and 40.50% was produced by *A. niger* isolate No. 5, respectively.

During the investigation of this experiment, the amount of spore load that was used 10^6 spores mL^{-1} by *A. niger* isolate No. 5 has produced the maximum protein enrichment 52.48 (SWO) and 46.50% (OPW) when compared to the rest of spore load of 10^4 and 10^8 spores mL^{-1}. The result showed that 10^9 spores g^{-1} of deoiled rice bran produced maximum protein enrichment of 18.8% (Rudravaram *et al*., 2006). On the other hand high inoculum size will lead to competion of growth between fungal hyphal over the limited substrate (Ravinder *et al*., 2003). In the contrary the highest protein yield 39.65% by KC-06 was obtained with spore load of 10^8 spores mL^{-1} (Yalemtesfa *et al*., 2010). The results have reported that the maximum protein enrichment was obtained from 10^8 to 10^9 spores mL^{-1} (Czajkowska and Ilnicka-Olenjniczak, 1989). The optimum inoculum load at 10^6 spore mL^{-1}, scored protein yield of 30.47% (W/W) (Yalemtesfa, 2007).

The highest protein yield 24.04 and 22.76% was obtained by *A. niger* isolate No. 5 using the sweet orange (SWO) and orange pulp wastes (OPW) with particle size of greater than 0.71 mm, respectively. This indicated that the more space and size of particle (more than 0.71mm) has allowed to fungal isolate easily to metabolize the substrates (SWO and OPW) faster and to produce high protein yield than the smaller particle size (less than 0.71 mm) of the substrates. The minimum protein yield was found at 20 mesh size (14.50%) while maximum protein (18.70%) was found at 50 mesh size (Rudravaram *et al*., 2006). It has reported that beyond 50 mesh size protein yield was decreased (Rudravaram *et al*., 2006). For cellulolytic reactions to take, place which was in turn break down into products of reaction (Mojovic *et al*., 2006). However, grinding substrate below 60 mesh size on production scale to achieve small yielded of mycelia is economically impractical (Han and Callihan, 1974).

In this study among the inorganic nitrogen source, the highest protein enrichment 23.62% and 24.04% was obtained using ammonium sulphate to supplement substrates of sweet orange (SWO) and orange wastes (OPW), respectively. The maximum protein enrichment SWO (18.95%) and OPW (19.96%) were obtained using urea as controls by *A. niger* isolate No. 5. This indicated that application of nitrogenous sources have increased the amount of protein yielded when compared to the

results of what were obtained in the controls (urea). The ammonium sulphate was found to be the best among the inorganic nitrogenous source for protein enrichment (24.30%) (Rudravaram *et al*., 2006). The result has revealed that the maximum protein enrichment using ammonium sulphate by *Chaetomium* spp. (KC-06) and *A. niger* (KA-06) was obtained 30.3 and 34.03%, respectively (Yalemtesfa *et al*., 2010). It was found that $(NH_4)_2$ SO_4 is the best among the inorganic nitrogen supplements (Oshoma and Ikenebomeh, 2005). However, it has reported that the presence of ammonium salts in the medium lower the cellulase production from *Scorpulopsis pranu* and found that $NaNO_3$ was a better nitrogen source for protein enrichment (Anupama and Ravindra, 2000).

In this study, the application of NaOH buffer extraction was given the highest protein enrichment 23.93 and 23.59% of protein yield when used 5 g substrates SWO and OPW, respectively. Whereas, Na_2HPO_4 buffer using 10 g of substrates SWO and OPW were attained the maximum protein yield 20.45 and 20.08%, respectively. This has showed that as the amount of sweet orange and orange pulp wastes increased, the protein enrichment/production has shown dramatic decline. Similar, result has obtained that 10 g of substrates was the optimum level that can be utilized by KA-06 and KC-06 inoculum to give 26.17 and 23.05% protein, respectively (Yalemtesfa, 2007). Similar result has also indicated that a protein enriched 25.5% cassava feed for cattle or pig's can be obtained by using *Trichoderma* sp. for fermenting cassava peels (Obadina *et al*., 2006). *Trichoderma viride* ATCC 36316 grew well on cassava peel fermentation for 3 to 4 days increased crude protein content of cassava peel 8-fold (4.2% to up to 37.6%) and true protein content 22-fold (1.4% to up to 31.6%) (Ezekiel *et al*., 2010). It has also given emphasis that with increase in population and worldwide protein shortage, the use of microbial biomass as food and feed, is more highlighted (Nasseri *et al*., 2011).

CONCLUSION

Among all agricultural disposal wastes possible approaches for the production of animal feed, the orange wastes from juice markers/shops, in Addis Ababa and orange growing areas, using in the solid state fermentation provides a practical way to convert the orange wastes into value added products such as protein enrichment. Therefore, the results of this study have indicated that in Solid State Fermentation (SSF), optimization of the growth factors are very important and crucial in order to obtain the maximum protein enrichment using sweet orange (SWO) and orange pulp (OPW) wastes as substrates by *Aspergillus niger* isolate No. 5.

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