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Effect of Production Parameters on Release of Phenolic Content of Peanut Press Cake Fermented with *A. oryzae* and *A. awamori*

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Abstract

The aim of this study was to know the effect of various parameters viz. temperature, time, inoculum age, inoculum size, carbon and nitrogen source on release of phenolic content by *A. oryzae* and *A. awamori* during solid state fermentation of peanut press cake. Peanut press cakes (PPC) a byproduct which is obtained after oil extraction from peanut seeds. Results obtained from the study showed significant increase in total phenolic content of PPC at 30 °C with 5thday of inoculum age, 1×10^{6} spore/ml as inoculum size with 1% starch, 2% glucose, 4% peptone and 1% sodium nitrate when fermented with *A. oryzae*. Whereas, PPC samples fermented with *A. awamori* showed highest phenolic content on 6thday of inoculum and1×10^{7}spore/ml as inoculum size with 4% starch, 3% glucose, 2% peptone and 2% of sodium nitrate. The phenolic content calculated after the optimization of these parameters was found approximately two fold higher i.e. 119.11±1.81 µM/g GAE than the phenolic content obtained without optimization of these parameters i.e. 60.73±2.01 µM/g GAE of PPC samples fermented with *A. oryzae*. Similarly fermentation with *A. awamori* also showed enhanced phenolic content i.e.107.34±3.11 µM/g GAE under optimized condition as compared to the phenolic content obtained before optimization i.e. 58.89±2.71 µM/g GAE.

Keywords
A. oryzae; *A. awamori*; SSF; TPC; PPC.

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a major source of edible oil and protein meal, therefore considered to be highly valuable in human and animal nutrition (1). India is the second largest producer of peanut. Peanut is grown mostly in six states namely Andhra Pradesh, Gujarat, Tamil Nadu, Karnataka, Maharashtra and Rajasthan. Peanut press cakes (PPC) are major byproduct obtained during oil processing of peanuts (2). India is one of the world's leading oilseeds producers. Total production currently stands at over 25 million tons per annum while the exports account for over 4.3 million tons of oil cake – valued at US$ 800 million annually. Peanut seeds are rich source of various micro and macro nutrients along with functional properties (3) like emulsification, foaming, water absorption properties

etc. are well known and reported by several authors (4-6).

Solid-state fermentation (SSF) is a process that used solid substrate as surface for the growth of microbes and their product formation in the absence of free floating water. SSF are used for special economic interest of countries with plenty of biomass and agro-industrial residues. Several important factors must be considered for the development of a successful bioprocess under SSF conditions. Some of the most important include the selection of a suitable microbial strain and the solid support to be used. A variety of microorganisms, including fungi (7), yeasts and bacteria may be used in SSF processes.

In present study, GRAS (generally recognized as safe) fungal strain i.e. *Aspergillus oryzae* and *Aspergillus awamori* were used for fermentation of peanut press cake. Solid state fermentation was carried out to increase the phenolic content of different substrates like agro-industrial waste, cereals and pulses (8, 9). Many biochemical changes occur during fermentation, so fermentation has been used to improve or altered ratio of nutritive and antinutritive components of plants, which affect product properties such as bioactivity and digestibility (10, 11). Polyphenols are aromatic compounds formed from the metabolism of shikimic acid and / or that of a polyacetyl. Structurally, they fall into different families including anthocyanins, coumarins, lignins, flavonoids, tannins, quinones, acids and phenols (12, 13). Antioxidant is a substance that has the ability to delay the oxidation of a substrate by inhibiting the initiation or propagation of oxidizing chain reactions caused by free radicals (14). Antioxidants arrest oxidative deterioration of lipids so is used to preserve food quality. Regarding safety concerns, plant-based antioxidants are now preferred to the synthetic ones (15-17). The aim of the current study was to examine the total phenolic content of fermented peanut press cake and to know the effect of different production parameters like temperature, carbon sources, nitrogen sources, inoculum age and inoculum size on release of phenolic compounds.

MATERIALS AND METHODS

Peanut (HNG 10) was obtained from Pilimandori, Fatehabad, Haryana (India). *Aspergillus oryzae* (MTCC 3107) and *Aspergillus awamori* (MTCC 548) were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. These fungal strains are generally recognized as safe (GRAS). Potato dextrose agar and Czapek-dox medium were procured from Sigma Aldrich Co. (St. Louis, USA) (Mumbai, India). All chemicals used were of AR grade. Triple distilled water and acid washed glassware were used throughout the experiments.

Processing of peanut

Peanut seeds (HNG-10) were processed through expeller to get peanut press cakes (PPC). Peanut cakes were used as substrate which was grounded using blender (Braun AG Frankfurt A.M. Mx 32, Germany) to obtain fine powder and stored in air tight container at 4-7°C till further use.

Preparation of inoculum

The selected strains of *A. oryzae* and *A. awamori* were maintained on potato dextrose agar slants and were transferred to fresh PDA plates before starting of each experiment. The inoculated plates were incubated at 30°C for 144 h. A suspension of approximately 1×10^6 spores/ml was prepared in sterilized cellular grade water.

Production parameters

For the production process both physical as well as chemical parameters were optimized. SSF was carried out at five different temperatures i.e. 20, 25, 30, 35 and 40°C with different inoculums age i.e. 4^{th}, 5^{th}, 6^{th}, 7^{th} and 8^{th} day, inoculum size i.e. A (1×10^4), B (1×10^5), C (1×10^6), D (1×10^7) and E (1×10^8) spores/ml, respectively as physical parameters whereas glucose and starch as carbon source, peptone and sodium nitrate as nitrogen source (with different concentrations i.e. 1.0, 2.0, 3.0, 4.0 and 5 %) were taken as chemical parameters. SSF was carried out to study effect of these parameters on release of phenolic compounds during fermentation.

Conditions for solid state fermentation process

Fifty gram of grounded sample was soaked in 50 ml Czapek-dox medium in 500 ml capacity Erlenmeyer flasks and kept at 30°C for overnight. After this incubation period, remove excess of media was removed. After addition of external physical and chemical components, the substrate was autoclaved and inoculated with 5.0 ml spore suspension (1×10^6 spores/ml) of *A. oryzae*, mixed properly and incubated at 30 °C in BOD incubator (Calton, NSW-152, India) for 0, 48, 72, 96, 120 and 144 h, respectively. The non-fermented substrate was prepared without inoculation of spore suspension and considered as control. Similar conditions were set up for *A. awamori*.

Total phenolic content

Total phenolic content was calculated with the help of Folin-Ciocalteu reagent. The ethanolic extract (200 µl) of samples was mixed with 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of (7.5 %) sodium carbonate. The contents were incubated for 30 min

at room temperature to complete the reaction process. The absorbance was taken at 765 nm (Systronic 2202 UV–VIS spectrophotometer). Total phenolic content was obtained from the regression equation i.e. y = 0.0092x + 0.1053 and expressed as µM/g gallic acid equivalent.

Statistical analysis

Microsoft Excel, 2007 (Microsoft Corp., Redmond, WA, USA) formulas were applied to calculate mean and standard error mean values. Significant difference between the values was verified by one-way analysis of variance (ANOVA) and comparison between means was made by critical difference value (18).

RESULT AND DISCUSSION

Total phenolic content of PPC under solid state fermentation

Total phenolic content was determined based on conditions obtained from literature i.e. 30 °C with 5th day of inoculum and 1 × 10^6 spores/ml inoculum size (19). No additional carbon and nitrogen source was given during fermentation condition. **Figure 1** showed increased phenolic content of PPC when fermented with both the filamentous fungi i.e. *A. oryzae* and *A. awamori*. The phenolic content ranged from 31.22±1.27to 60.73±2.01 µM/g GAE during fermentation with *A. oryzae*. Whereas PPC samples fermented with *A. awamori* showed maximum phenolic content i.e. 58.89±2.71 µM/g GAE after 5th day of incubation. Increased phenolic content have been observed from different fermented substrate by different researchers (20-23), cereals (24) and pulses (25, 26).

Effect of temperature on release of phenolic content

Fermentation conditions were assessed with various parameters to obtained maximum yield of product. Among different physical parameters, temperature is one of the essential factors that affect release of phenolic compounds. Here in current study, a temperature range from 20-40 °C was studied and it was found that increase in release of bound phenolic was temperature sensitive. Total phenolic content increases with increase in temperature and found maximum i.e. 77.00±2.46 µM/g GAE, 75.44±4.14 µM/g GAE at 30 °C in PPC samples fermented with *A. oryzae* and *A. awamori*, respectively, which was higher than the non-fermented samples (32.10±1.62 µM/g GAE, 32.46±1.07 µM/g GAE). Further increase in temperature to 35 and 40 °C decreased the phenolic content **(Figure 2A).** There is a scarcity of appropriate data or literature on phenolic released from peanut press cake. However, different studies were carried out with diverse substrates to find out the effect of temperature. Several researchers have reported the effect of temperature (in the range 20–100 °C) on release of bound phenolics/antioxidants (27-30).

Effect of inoculum size on release of phenolic content

Replicates of the substrate were inoculated with different inoculums sizes i.e. A (1×10^4), B (1×10^5), C (1×10^6), D (1×10^7) and E (1×10^8) spores/ml. Size of inoculum as starter culture contribute a major role in fermentation process as the inoculum containing number of spores that are significantly responsible for biomass production in the fermentation process. **Figure 2B** showed the effect of inoculum sizes on release of bound phenolic content and maximum TPC was observed i.e. 136.69±11.38 µM/g GAE and 81.83±3.57 µM/g GAE with C (1×10^6) and D (1×10^7) inoculum sizes, when fermented with *A. oryzae* and *A. awamori*, respectively. The TPC was observed much higher in fermented extracts than the non-fermented extracts of peanut press cake. As various microorganisms have different optimum temperature for their growth and metabolite activities (31). Gibbons and Westby (32) studied the effects of inoculum size on solid-phase fermentation of fodder beets for biofuel production using *Saccharomyces cerevisiae* as starter culture with various concentrations of inoculum size. Effect of inoculum size of *S. cerevisiae* was also studied on wine fermentation (33) and found that 5% inoculum size resulted in rapid yeast growth and ethanol production. Jaapar *et al*. (34) also analyze various parameters such as inoculum age and size on hydrogen production by using *R. sphaeroides* and found the inoculum age of 24 h with 10 % v/v inoculum size as optimum conditions for maximum H_2 production. Various enzymes were produced by using different inoculum concentrations via solid-state fermentation with different substrates (35, 36).

Effect of inoculum age on release of phenolic content

The fermentation processes depend on the lag phase of a growth curve of inoculum, which means the time required for the cells to acclimatize with the environment. The inoculum age and its physiological condition also affect the lag phase. The inoculum age is important for the production of spores; the use of an inoculum having large number of spores would result in a longer lag phase in the successive fermentation process (37). In current study thesubstrate i.e. peanut press cake was inoculated with five different inoculums age i.e. 4th, 5th, 6th, 7th and 8th day of incubation. Results obtained from the study showed maximum TPC with 5th day of inoculum

when fermented with *A. oryzae,* whereas TPC was ranged from 47.78±1.82 to 89.19±0.94 µM/g GAE. Similarly, 6th day inoculum of *A. awamori* showed highest phenolic content i.e. 108.21±9.54 µM/g GAE and it was higher than the control or non-fermented substrate i.e. 40.63±1.46 µM/g GAE **(Figure 2C).** Jaapar *et al*. (34) also investigated the production of H_2 with different inoculum age such as 18, 24, 40 and 48 h of *R. sphaeroides*. The results showed that age of inoculum strongly associated with the growth of inoculum and production of H_2. Highest H_2 production was obtained from the inoculum age of 24 h. **Sen and Swaminathan (2004)** also carried out a study to optimize the inoculum age as well as size to obtained maximum surfactin. The optimal age and size of the inoculum at which the highest yield of surfactin obtained were 56 h and 5.5% (v/v), respectively. Similarly, other studies were also carried out to evaluate the effect of inoculum size as well as age on different substrates to enhance the yield of product by optimizing the best condition of inoculum growth (38-41)**.**

Effect of carbon and nitrogen source on release of phenolic content

The synthesis of various value added products by microorganisms are highly influenced by different factors (42)**.** Factors especially carbon and nitrogen sources with various concentrations have been remain of great interest to researchers for the design of low-cost media and to obtained high productivity (43)**.** The production costs of industrial enzymes are directly related to the cost of growth medium. Therefore, it is of great significance to optimize the conditions to enhanced product production at a very low cost. In present study, substrate was supplemented with different concentrations (1, 2, 3, 4 and 5%) of glucose and starch as additional carbon source, whereas peptone and sodium nitrate were used as additional nitrogen source. Results obtained from current investigation depicted maximum TPC level at 2% (123.41±1.80 µM/g GAE) and 3% (105.78±10.00 µM/g GAE) of glucose **(Figure 3A)** and 1% (127.03±2.08 µM/g GAE) and 4% (88.89±2.50 µM/g GAE) of starch **(Figure 3B)** as carbon source during fermentation of ethanolic extract of PPC with *A. oryzae* and *A. awamori*, respectively. Similarly, in addition of nitrogen source, the highest TPC was observed at 4% (100.42±4.25 µM/g GAE) peptone with *A. oryzae* and at 2% (112.63±2.80 µM/g GAE) peptone with *A. awamori* **(Figure 4A)** whereas, in case of $NaNO_3$, highest TPC was observed at 1% (65.93±0.38 µM/g GAE) with *A. oryzae* and at 2% (137.65±11.38 µM/g GAE) with *A. awamori* **(Figure 4B).** The amount of phenolic content of the PPC samples obtained after fermentation was higher than that of the non-fermented ones. Similarly, Vahidi *et al*. (44) observed the effect of various carbon sources such as glucose, fructose, lactose, maltose, mannitol and sucrose and concluded that the highest activity and biomass concentration were obtained in case of glucose as additional carbon source. Likewise, various studied were also carried out to know the effect of nitrogen as well as carbon source on release of secondary metabolites and other beneficial products by using different low cost substrates with variety of microorganisms (45-48)**.** Kumar *et al*. (49) also reported maximum production of lipase i.e. 3.03 U/mL using 1% w/v peptone as additional nitrogen source.

Total phenolic content of PPC under optimized condition of solid state fermentation

Using the results obtained from the study, final solid state fermentation was carried out under optimal conditions of various parameters such as temperature (30 °C), inoculum age (5th and 6thday), inoculum size (1×10^6 and 1×10^7 spores/ml), carbon source i.e. starch (1% and 4%), glucose (2% and 3%) and nitrogen source such as peptone (4% and 2%) and sodium nitrate (1% and 2%) with both GRAS fungal strains i.e. *A. oryzae* and *A. awamori*, respectively. The phenolic content calculated after the optimization of these parameters was found approximately two fold higher i.e. 119.11±1.81 µM/g GAE than the phenolic content obtained i.e. 60.73±2.01 µM/g GAE without optimization of these parameters of PPC samples fermented with *A. oryzae*. Similarly fermentation with *A. awamori* also showed enhanced value of phenolic content i.e. 107.34±3.11 µM/g GAE under optimized condition and it was also higher i.e. 58.89±2.71 µM/g GAE prior to optimization **(Figure 5).**

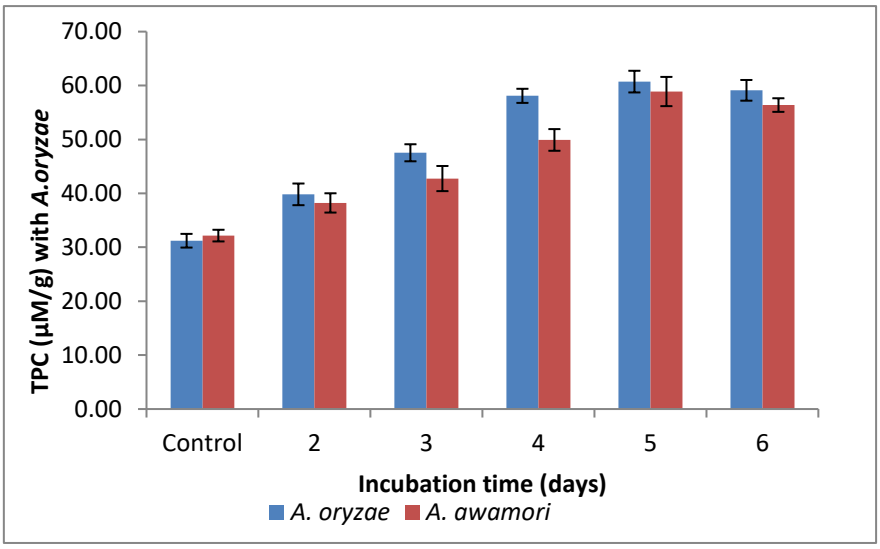
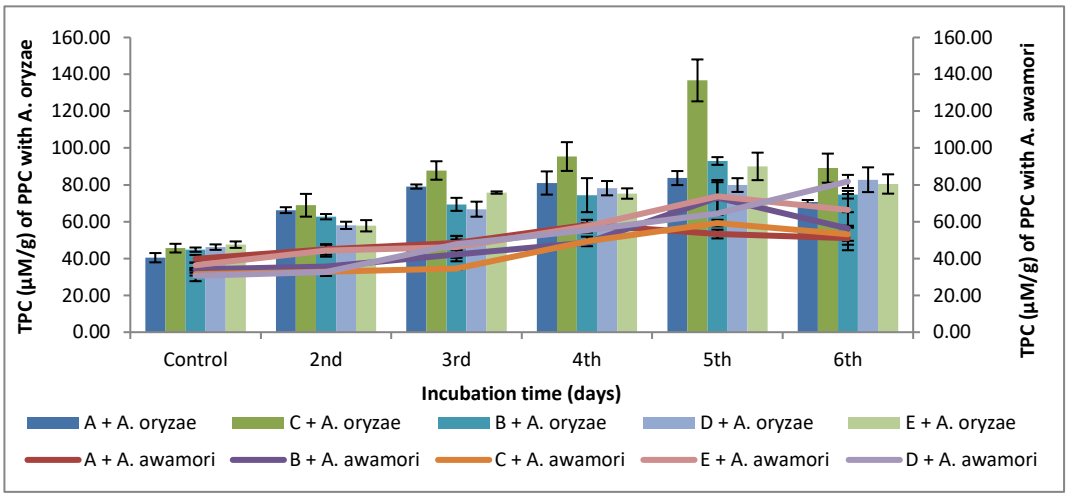
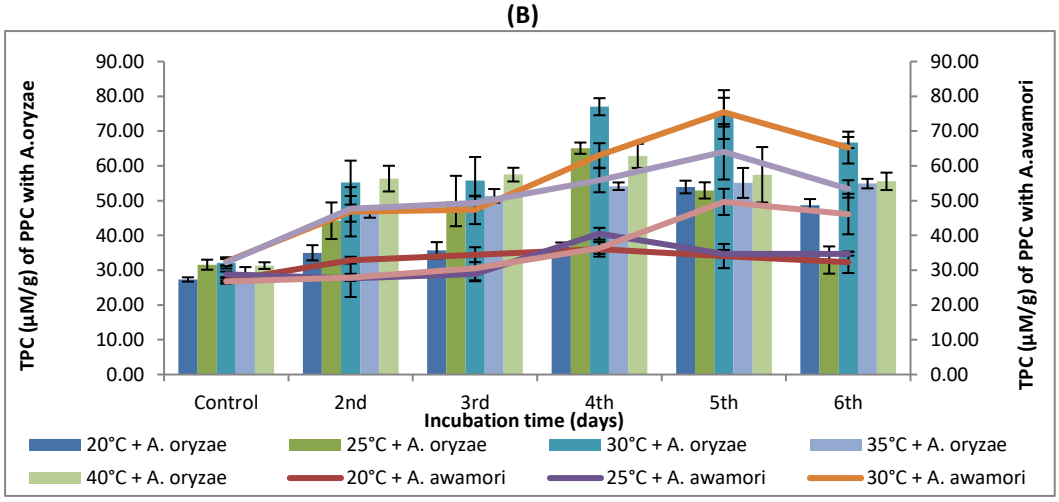


Figure 1: Total phenolic content of peanut press-cake fermented with *A. oryzae* and *A. awamori* (Data are presented as means ± SEM (n=3)

(A)



(B)



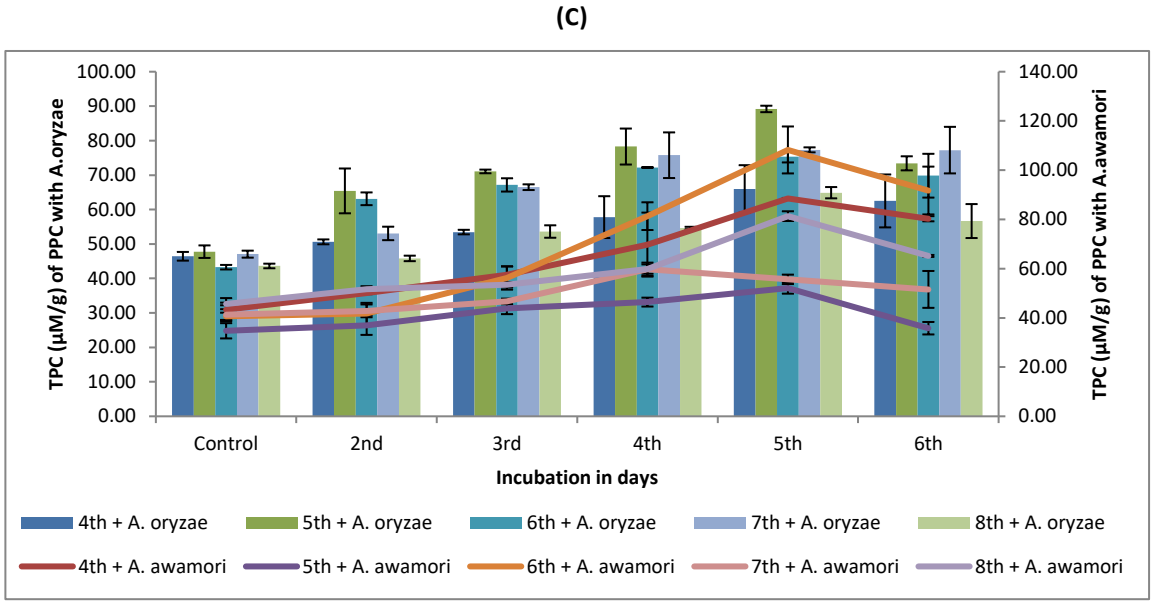
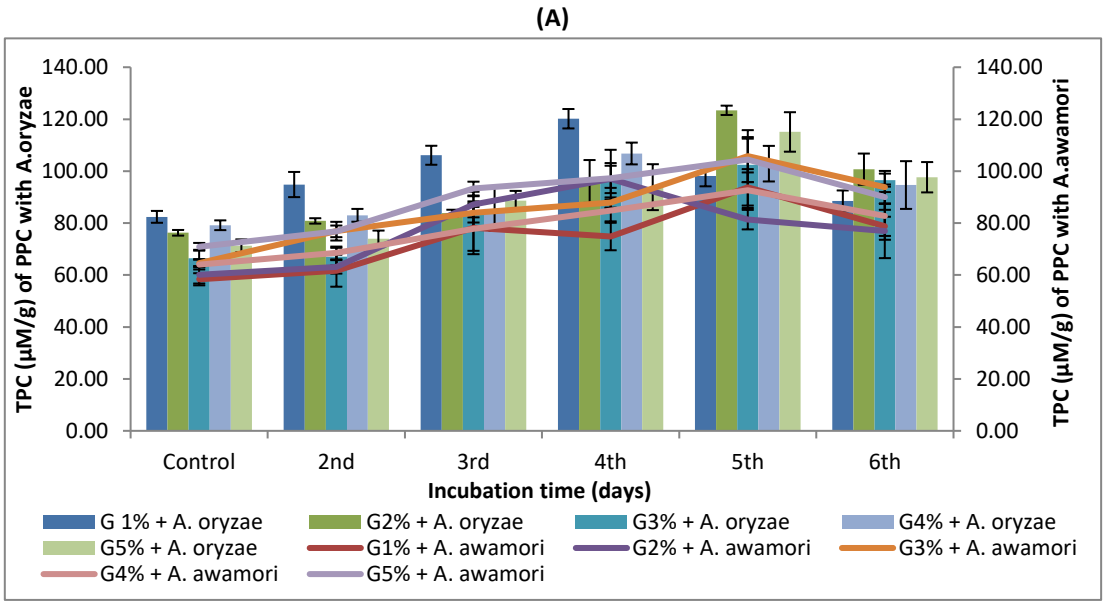


Figure2 (A) Effect of temperature (20 – 40°C), (B) inoculums size (A – E) and (C) inoculums age (4^{th} – 8^{th}) on TPC of peanut press-cake fermented with *A. oryzae* and *A. awamori* (Data are presented as means ± SEM (n=3)



(B)

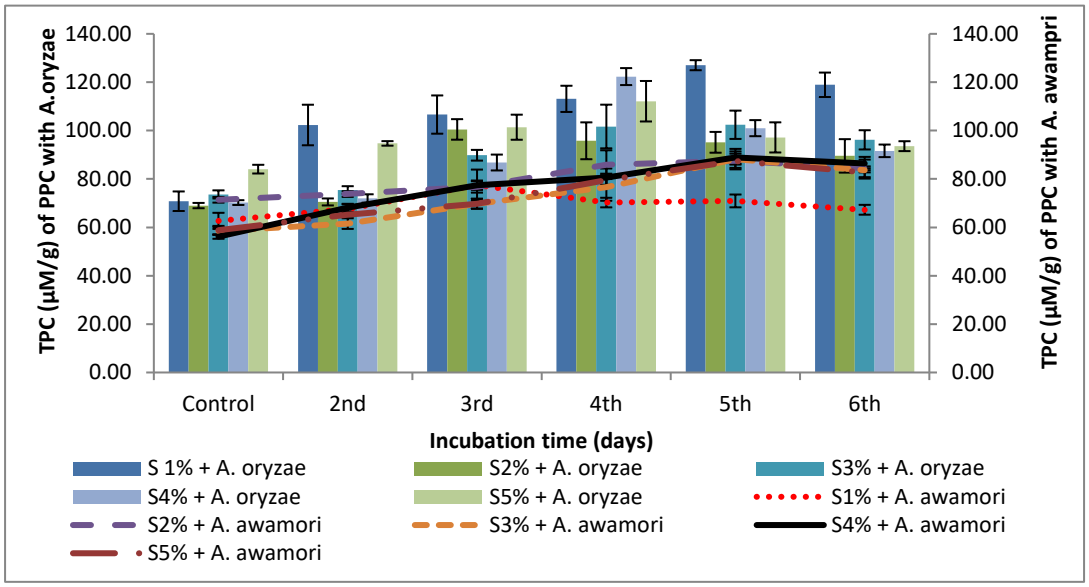
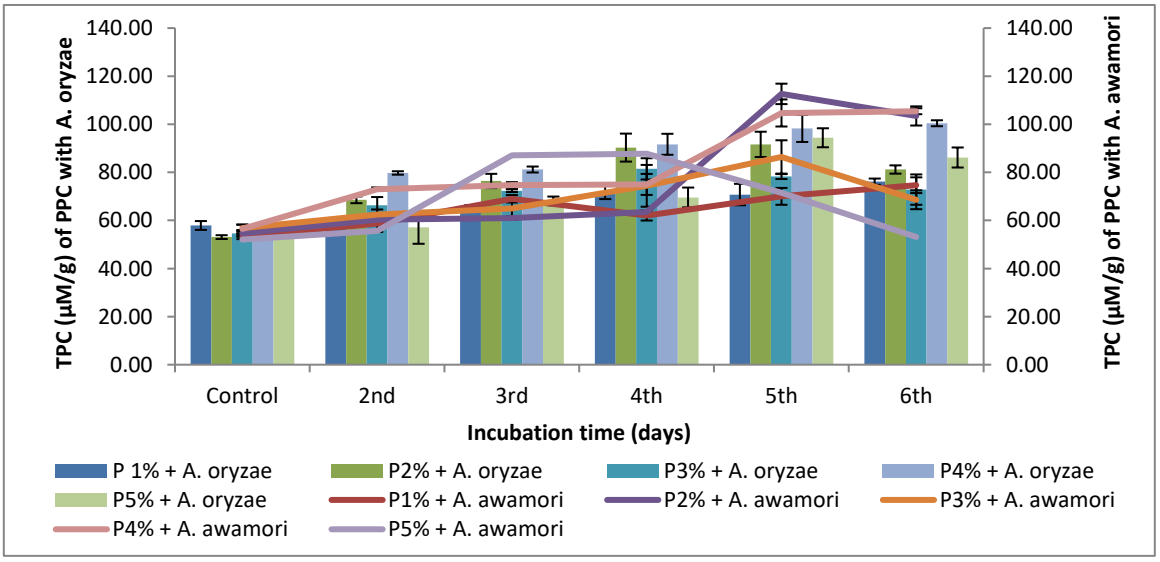


Figure 3: Effect of carbon source (A) glucose and (B) starch on TPC of peanut press-cake fermented with *A. oryzae* and *A. awamori* (Data are presented as means ± SEM (n=3)

(A)



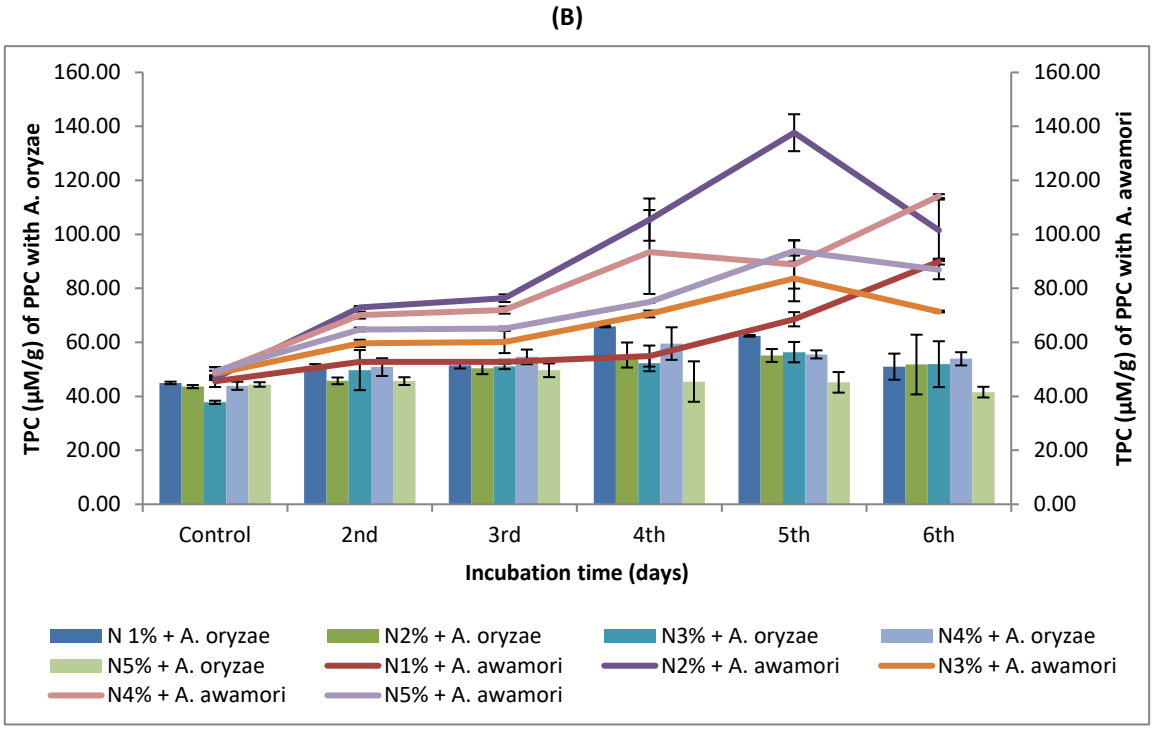


Figure 4: Effect of nitrogen source (A) peptone and (B) sodium nitrate on TPC of peanut press-cake fermented with *A. oryzae* and *A. awamori* (Data are presented as means ± SEM (n=3)

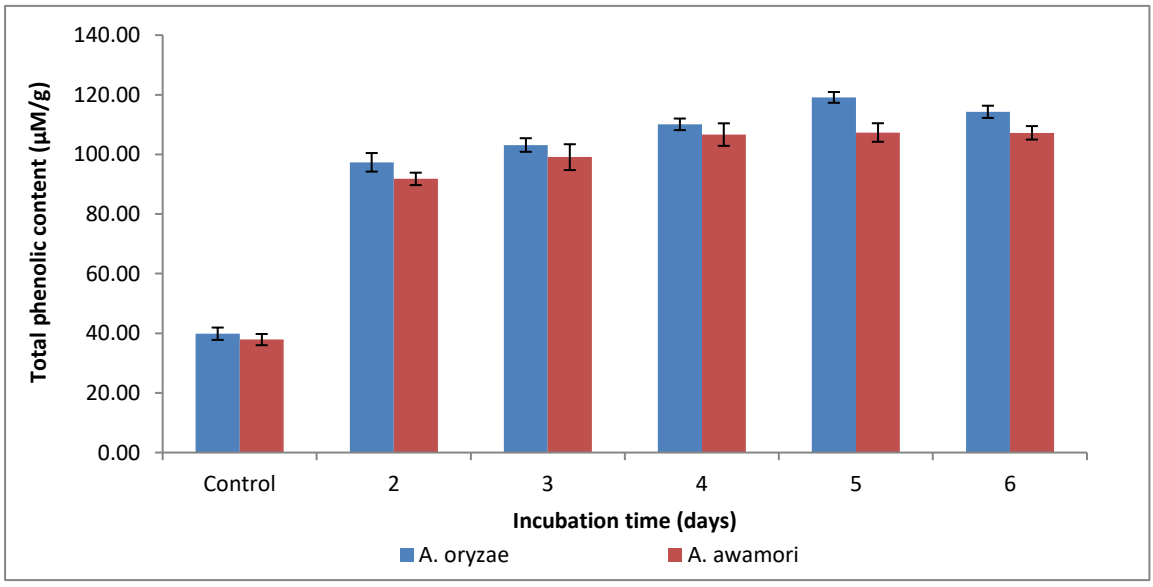


Figure 5: Total phenolic content of peanut press cake under optimized condition of fermentation process with *A. oryzae* and *A. awamori* (Data are presented as means ± SEM (n=3)

CONCLUSIONS

The synthesis of various value added products by microorganisms are highly influenced by different factors. These factors are of great interest to researchers for the design of low-cost media and to obtained high productivity with less investment. Therefore, in this study, effect of various parameters viz. temperature, time, inoculum age, inoculum size, carbon and nitrogen source on release of phenolic content during solid state fermentation of peanut

press cake with *A. oryzae* and *A. awamori* was successfully examined by calculating total phenolic content using Folin – Ciocalteu reagent method. Results obtained from the study clearly revealed that at30 °C temperature, 5th and 6thday of inoculum age, inoculum size of1×10^{6} and 1×10^{7}spores/ml with1% and 4% starch, 2% and 3% glucose, 4% and 2% peptone and 1% and 2% of sodium nitrate were observed as the best parameters for maximum release of phenolic contents from fermented PPC samples of both GRAS fungal strains i.e. *A. oryzae* and *A. awamori*, respectively, which may reduce the fermentation time as well as cost of production medium and hence increase the productivity.

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